VNiVERSiDAD D SALAMANCA

FACULTAD DE FARMACIA

DEPARTAMENTO DE QUÍMICA ANALÍTICA, NUTRICIÓN Y BROMATOLOGÍA

DEVELOPMENT OF FOOD BIOACTIVES THROUGH THE VALORIZATION OF OLIVE OIL PRODUCTION WASTES

DOCTORAL THESIS

Joana Filipa de Paiva dos Santos Madureira

SUPERVISORS:

Dr. Celestino Santos Buelga

Dra. Sandra Isabel Silva Damas Cabo Verde

Dra. Lillian Bouçada de Barros

Salamanca, 2023

VNiVERSiDAD D SALAMANCA

DEPARTAMENTO DE QUÍMICA ANALÍTICA, NUTRICIÓN Y BROMATOLOGÍA

Celestino Santos Buelga, Catedrático de Nutrición y Bromatología de la Universidad de Salamanca, Lillian Bouçada de Barros, Investigadora del Centro de Investigação de Montanha del Instituto Politécnico de Bragança (Bragança, Portugal), y Sandra Isabel Silva Damas Cabo Verde, Investigadora del Centro de Ciências e Tecnologias Nucleares del Instituto Superior Técnico (Lisboa, Portugal), directores del trabajo "Development of food bioactives through the valorization of olive oil production wastes", realizado por Joana Filipa de Paiva dos Santos Madureira para optar al Grado de Doctor, AUTORIZAN la presentación del mismo al considerar que se han alcanzado los objetivos inicialmente previstos.

Salamanca, 9 de mayo de 2023

SANTOS BUELGA CELESTINO - 10577196W

Firmado digitalmente por SANTOS BUELGA CELESTINO - 10577196W
Fecha: 2023.05.09 10:35:55 +02'00'

Celestino Santos-Buelga

Assinado por : **Lillian Bouçada de Barros**
Num. de Identificação: BI12058800
Data: 2023.05.09 09:39:08 +0100

Lillian Bouçada de Barros

Assinado por: **Sandra Isabel Silva Damas Cabo Verde**
Num. de Identificação: 10532207
Data: 2023.05.09 09:43:23+01'00'

Sandra Isabel Silva Damas Cabo Verde

Facultad de Farmacia. Campus Miguel de Unamuno.
37007 - Salamanca (Spain)
Tel.: +34 923 294 537

nutri@usal.es

This work was funded by the International Atomic Energy Agency (IAEA) and by the Foundation for Science and Technology (FCT, Portugal) for the PhD studentship granted to Joana Madureira (SFRH/BD/136506/2018). This work was also funded by the European Regional Development Fund (ERDF), through the projects “BIOMA” (POCI-01-0247-FEDER-046112) and “OliveBIOextract” (NORTE-01-0247-FEDER-049865) and co-funded by the P.O. FEDER of Castilla y León 2014–2020.

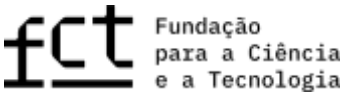


Tabla de Contenidos

LISTA DE PUBLICACIONES Y COMUNICACIONES

Esta Tesis se presenta en formato de compilación de artículos, con el fin de obtener el grado de Doctor por la Universidad de Salamanca.

El trabajo desarrollado durante la realización de la Tesis permitió la preparación de siete artículos científicos publicados en revistas indexadas en el *Journal Citation Reports* y un capítulo en un libro de referencia de una editorial internacional, así como la presentación de varias comunicaciones orales y en póster.

PEER-REVIEWED SCIENTIFIC PUBLICATIONS

[1] **Joana Madureira**, Maria Inês Dias, José Pinela, Ricardo C. Calhelha, Lillian Barros, Isabel C.F.R. Ferreira, Celestino Santos-Buelga, Fernanda M.A. Margaça, Sandra Cabo Verde (2020), "The use of gamma radiation as extractability improvement of bioactive compounds in olive wastes", *Science of the Total Environment*, 727, 138706.

doi: https://doi.org/10.1016/j.scitotenv.2020.138706 (Impact Factor 10.754 in JCR 2021, Q1 in the area of Environmental Sciences)

[2] **Joana Madureira**, Lillian Barros, Sandra Cabo Verde, Isabel C.F.R. Ferreira, Fernanda M.A. Margaça, Celestino Santos-Buelga (2020), "Ionizing radiation technologies to increase the extraction of bioactive compounds from agro-industrial residues: a review", *Journal of Agricultural and Food Chemistry*, 68, 11054−11067.

doi: https://dx.doi.org/10.1021/acs.jafc.0c04984 (Impact Factor 5.895 in JCR 2021, Q1 in the areas of Food Science and Technology, Agriculture Multidisciplinary and Chemistry Applied)

[3] **Joana Madureira**, Bruno Melgar, Celestino Santos-Buelga, Fernanda M.A. Margaça, Lillian Barros, Isabel C.F.R. Ferreira, Sandra Cabo Verde (2021), "Phenolic compounds from olive wastes: optimization of the heat-assisted extraction using response surface methodology", *Chemosensors*, 9(8), 231.

doi: https://doi.org/10.3390/chemosensors9080231 (Impact Factor 4.229 229 in JCR 2021, Q1 in the area of Instruments & Instrumentation)

[4] **Joana Madureira**, Sandra Cabo Verde, Fernanda M.A. Margaça, Celestino Santos-Buelga, Isabel C.F.R. Ferreira, Lillian Barros (2022), "Applications of bioactive compounds extracted from olive industry wastes: a review", *Comprehensive Reviews in Food Science and Food Safety*, 21: 453-476.

doi: https://doi.org/10.1111/1541-4337.12861 (Impact Factor 15.786 in JCR 2021, Q1 in the area of Food Science and Technology).

[5] **Joana Madureira**, Bianca Albuquerque, Maria Inês Dias, José Pinela, Ricardo C. Calhelha, Celestino Santos-Buelga, Fernanda M.A. Margaça, Isabel C.F.R. Ferreira, Sandra Cabo Verde, Lillian Barros (2023), "Ultrasound-assisted extraction of hydroxytyrosol and tyrosol from olive pomace treated by gamma radiation: process optimization and bioactivity assessment", *Food & Function*, 14, 3038-3050.

doi: https://doi.org/10.1039/D2FO03607J (Impact Factor 6.317 in JCR 2021, Q1 in the area of Food Science and Technology).

[6] **Joana Madureira**, Bruno Melgar, Vítor D. Alves, Margarida Moldão-Martins, Fernanda M.A. Margaça, Celestino Santos-Buelga, Lillian Barros, Sandra Cabo Verde (2023), "Effect of olive pomace extract application and packaging material on the preservation of fresh-cut 'Royal Gala' apples", *Foods*, 12(9), 1926.

doi: https://doi.org/10.3390/foods12091926 (Impact Factor 5.561 in JCR 2021, Q1 in the area of Food Science and Technology)

[7] **Joana Madureira**, Sara Gonçalves, Celestino Santos-Buelga, Fernanda M.A. Margaça, Isabel C.F.R. Ferreira, Lillian Barros, Sandra Cabo Verde (2023), "Microbiota assessment of fresh-cut apples packaged in two different films", *Microorganisms*, 11, 1157.

doi: https://doi.org/10.3390/microorganisms11051157 (Impact Factor 4.926 in JCR 2021, Q2 in the area of Microbiology).

BOOK CHAPTER OF INTERNATIONAL DISTRIBUTION

[8] **Joana Madureira**, Lillian Barros, Fernanda M.A. Margaça, Celestino Santos-Buelga, Isabel C.F.R. Ferreira, Sandra Cabo Verde (2022). "Effects of irradiation on food

bioactives". In: Jafari, S.M., Capanoglu, E. (eds) Retention of Bioactives in Food Processing. Food Bioactive Ingredients. Springer, Cham, pp 429–465, ISSN 2661-8966. doi: https://doi.org/10.1007/978-3-030-96885-4

PUBLICATIONS IN PROCESS

[9] **Joana Madureira**, Inês Gonçalves, Jéssica Cardoso, Maria Inês Dias, Pedro M.P. Santos, Fernanda M.A. Margaça, Celestino Santos-Buelga, Isabel C.F.R. Ferreira, Lillian Barros, Sandra Cabo Verde, "Effect of electron-beam radiation on the bioactive properties of olive pomace". In preparation.

ORAL COMMUNICATIONS IN NATIONAL AND INTERNATIONAL MEETINGS

[1] **Joana Madureira**, Maria Inês Dias, Lillian Barros, Celestino Santos-Buelga, Fernanda M.A. Margaça, Isabel C.F.R. Ferreira, Sandra Cabo Verde, "Effect of gamma radiation on bioactive compounds of olive wastes", 11º Encontro Nacional de Cromatografia (11ENC), Caparica, Portugal, 9-11 December, 2019.

[2] **Joana Madureira**, Bruno Melgar, Celestino Santos-Buelga, Fernanda M.A. Margaça, Isabel C.F.R. Ferreira, Lillian Barros, Sandra Cabo Verde, "Optimization of the heat-assisted extraction of bioactive compounds from olive wastes", 7 PYCheM Portuguese Young Chemists Meeting, Online, 19-21 May, 2021.

[3] **Joana Madureira**, Maria Inês Dias, José Pinela, Ricardo C. Calhelha, Lillian Barros, Celestino Santos-Buelga, Fernanda M.A. Margaça, Isabel C.F.R. Ferreira, Sandra Cabo Verde, "Improving bioactive compounds extraction from olive wastes using gamma radiation", Second International Conference on Applications of Radiation Science and Technology (ICARST-2022), Vienna, Austria, 22-26 August, 2022.

[4] **Joana Madureira**, Bruno Melgar, Bianca Albuquerque, Maria Inês Dias, José Pinela, Ricardo C. Calhelha, Fernanda M.A. Margaça, Celestino Santos-Buelga, Lillian Barros, Sandra Cabo Verde, "Optimization of ultrasound- and heat-assisted extractions of phenolic compounds from olive wastes for food applications", 8 PYCheM Portuguese Young Chemists Meeting, 17-19 May, 2023.

POSTER COMMUNICATIONS IN NATIONAL AND INTERNATIONAL MEETINGS

[1] **Joana Madureira**, Lillian Barros, Sandra Cabo Verde, Celestino Santos-Buelga, Isabel C.F.R. Ferreira, Fernanda M.A. Margaça, "Development of food bioactives

through the valorization of olive oil production wastes", Ciência 2018 - Science and Technology in Portugal Summit, Lisbon, Portugal, 2-4 July 2018.

[2] **Joana Madureira**, Lillian Barros, Sandra Cabo Verde, Celestino Santos-Buelga, Isabel C.F.R. Ferreira, Fernanda M.A. Margaça, "Impact of ionizing radiation on bioactive compounds of olive wastes", International Meeting on Radiation Processing (IMRP), Strasbourg, France, 1-5 April 2019.

[3] **Joana Madureira**, Lillian Barros, Sandra Cabo Verde, Celestino Santos-Buelga, Isabel C.F.R. Ferreira, Fernanda M.A. Margaça, "Improvement of extractability of bioactive compounds from olive wastes by gamma radiation", Ciência 2019 - Science and Technology in Portugal Summit, Lisbon, Portugal, 8-10 July 2019.

[4] **Joana Madureira**, Bruno Melgar, Celestino Santos-Buelga, Fernanda M.A. Margaça, Isabel C.F.R. Ferreira, Lillian Barros, Sandra Cabo Verde, "Recovery of bioactive compounds from olive wastes by optimization of the Heat-assisted extraction", Ciência 2021 – Encontro com a Ciência e Tecnologia em Portugal, Lisbon, Portugal, 28-30 June 2021.

ADSCRIPCIÓN DE AUTORES Y COAUTORES DE LOS ARTÍCULOS PUBLICADOS Y COMUNICACIONES

JOANA MADUREIRA

Centro de Ciências e Tecnologias Nucleares (C^2TN), Instituto Superior Técnico, Universidade de Lisboa, E.N. 10 ao km 139.7, 2695-066 Bobadela LRS, Portugal

Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

Grupo de Investigación en Polifenoles (GIP-USAL), Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno s/n, 37007 Salamanca, Spain

MARIA INÊS DIAS

Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

JOSÉ PINELA

Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

RICARDO C. CALHELHA

Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

BIANCA ALBUQUERQUE

Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

BRUNO MELGAR

Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

VITOR D. ALVES

LEAF - Linking, Landscape, Environment, Agriculture and Food - Research Center, Instituto Superior de Agronomia (ISA), Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal

MARGARIDA MOLDÃO-MARTINS

LEAF - Linking, Landscape, Environment, Agriculture and Food - Research Center, Instituto Superior de Agronomia (ISA), Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal

SARA GONÇALVES

ESTeSL - Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, Lisboa, Portugal

FERNANDA M.A. MARGAÇA

Centro de Ciências e Tecnologias Nucleares (C^2TN), Instituto Superior Técnico, Universidade de Lisboa, E.N. 10 ao km 139.7, 2695-066 Bobadela LRS, Portugal

Departamento de Engenharia e Ciências Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10, ao km 139.7, 2695-066 Loures, Portugal

ISABEL C.F.R. FERREIRA

Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

LILLIAN BARROS

Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

SANDRA CABO VERDE

Centro de Ciências e Tecnologias Nucleares (C^2TN), Instituto Superior Técnico, Universidade de Lisboa, E.N. 10 ao km 139.7, 2695-066 Bobadela LRS, Portugal

Departamento de Engenharia e Ciências Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10, ao km 139.7, 2695-066 Loures, Portugal

CELESTINO SANTOS-BUELGA

Grupo de Investigación en Polifenoles (GIP-USAL), Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno s/n, 37007 Salamanca, Spain

Unidad de Excelencia Producción, Agrícola y Medioambiente (AGRIENVIRONMENT), Parque Científico, Universidad de Salamanca, 37185 Salamanca, Spain

Agradecimientos

Em primeiro lugar, agradeço aos meus orientadores, que tanto admiro.

Ao Professor Celestino, muito obrigada por aceitar ser meu orientador, por toda a disponibilidade, dedicação, generosidade e tantos ensinamentos ao longo dos últimos anos. À Doutora Lillian Barros, minha co-orientadora, agradeço a oportunidade de fazer parte deste grupo de investigação que me deu tanto, por me transmitir tanta confiança e por ser uma inspiração para mim todos os dias. À Doutora Sandra Cabo Verde, minha co-orientadora e grande amiga, agradeço tudo e tanto que sempre fez por mim. Se estou aqui, é porque acredita sempre em mim e nas minhas capacidades. Obrigada pela dedicação, carinho e apoio. Tanta partilha científica e confiança na minha autonomia, que me continuam a mostrar estar no caminho certo. Mais do que tudo, agradeço a amizade dos três, e sei que não podia ter tido melhores pessoas ao meu lado.

Este trabalho foi realizado no Centro de Ciências e Tecnologias Nucleares do Instituto Superior Técnico e no Centro de Investigação de Montanha do Instituto Politécnico de Bragança, onde me foram disponibilizados todos os meios para a sua concretização. Agradeço a todos os meus colegas que viveram comigo este percurso, por criarem um excelente ambiente de trabalho com tanta boa disposição. Neste sentido, agradeço à Doutora Fernanda Margaça por acreditar sempre em mim e me dar as condições necessárias para usufruir desta experiência de vida, à Professora Isabel Ferreira pela oportunidade de integrar o seu grupo de investigação e me confirmar como o mundo científico é tão bonito. À Professora Margarida Moldão-Martins e ao Professor Vítor Alves do Instituto Superior de Agronomia, por me terem recebido tão bem no vosso laboratório e por toda a disponibilidade e ajuda. Aprendi muito, mas conforto é a palavra certa. Agradeço também a todos os co-autores dos meus artigos científicos, porque foram essenciais na realização deste trabalho.

Agradeço também à Fundação para a Ciência e a Tecnologia (FCT) pelo apoio financeiro através de uma Bolsa de Doutoramento (SFRH/BD/136506/2018), à UCASUL — União de Cooperativas Agrícolas do Sul pela disponibilidade e fornecimento das amostras de bagaço de azeitona e à Unidade Tecnológica de Radioesterilização da Universidade de Lisboa pela irradiação das amostras.

Porque este projeto vai muito para além do laboratório, quero agradecer às pessoas que me têm acompanhado e, em particular, às mais especiais na minha vida:

Às minhas babes Lena, Paula, Andreia, Telma e Rita, por serem minhas cúmplices e me demonstrarem amor todos os dias. Obrigada por tanta motivação e carinho, tantas

conversas e partilhas. São fundamentais na minha vida. Ao Amílcar, pela bonita amizade e compreensão, pela sinceridade e sensatez.

Ao meu amigo Pedro, por me fazer sempre acreditar que o Mundo continua a ser um lugar bonito e onde o bem e a inocência prevalecem.

Ao meu amigo Zé. Se há pessoas boas e bonitas que o Doutoramento me deu, tu foste uma delas! Tens sido essencial ao meu equilíbrio nestes anos.

Às melhores amigas do Mundo: Maria, Joana e Xana. Obrigada por me apoiarem incondicionalmente, por tornarem tudo simples e por me aquecerem sempre o coração com o vosso amor. Amigos Paulito e Marcelo, obrigada por tanta ternura e paz. Até sermos velhinhos…

À minha Isabel, que é tanto! A minha vida não seria a mesma sem as nossas conversas, sem o apoio incondicional e as palavras exatas nas horas certas. É verdade, é segurança, é amor! Ao Nuno, Mariana, Leonor e Manuel obrigada pelo carinho, abraços e força que me dão sempre.

À minha amiga Rute, o melhor que o Grupo de Químicos Jovens me deu. Obrigada por tantas conversas, compreensão e cumplicidade. És bonita e trazes mais amor à minha vida.

À Ana, que me enche a vida de gargalhadas e conversas tão boas, por se preocupar e me acompanhar sempre com tanto carinho.

À minha alma Rita, que é tudo! Obrigada por estares sempre de mão dada comigo e acreditares e viveres também este meu Mundo científico. És casa e és amor, e não saberia ter norte sem ti. Fazes-me ser melhor todos os dias!

À minha família. Aos meus pais, Manuela e Carlos, e ao meu irmão, Pedro. Não sei como sobreviveria sem vocês. Agradeço por me ensinarem a ser resiliente na conquista dos meus sonhos, por me apoiarem a segui-los e por me dissiparem as dúvidas e os receios. Obrigada do fundo do meu coração, por tanto e por tudo!

“No fundo, todos temos necessidade de dizer quem somos e o que é que estamos a fazer e a necessidade de deixar algo feito, porque esta vida não é eterna e deixar coisas feitas pode ser uma forma de eternidade.”

José Saramago

Lista de Figuras

Lista de abreviaturas – List of abbreviations

∆E	Total colour difference
*a**	Red-green colour coordinate (CIELab colour space)
AA	Ascorbic acid
AAPH	2,2'-azobis(2-amidinopropane) dihydrochloride
ABTS	2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
AGS	Gastric adenocarcinoma
AIDS	Acquired Immunodeficiency Syndrome
AIEA	Agencia Internacional de la Energía Atómica
ANOVA	Analysis of variance
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
*b**	Yellow-blue colour coordinate (CIELab colour space)
C	Chroma
C_3H_6O	Acetone
Caco-2	Colorectal adenocarcinoma cell line
CCCD	Circumscribed central composite design
CE	Catechin equivalents
CFU	Colony Forming Units
CH_2O_2	Formic acid
CH_3COOH	Acetic acid
$CHCl_3$	Chloroform
Co	Cobalt
CO_2	Carbon dioxide
COP	Crude Olive Pomace
Cs	Cesium
CUPRAC	Cupric reducing power assay

DAD	Diode array detector
D_{max}	Maximum absorbed dose
D_{min}	Minimum absorbed dose
DNA	Deoxyribonucleic acid
DNS	3,5-Dinitrosalicylic acid reagent
DPPH	Diphenyl-1-picrylhydrazyl
DUR	Dose Uniformity Ratio
dw	Dry weight
EAE	Enzyme-assisted extraction
e^-_{aq}	Hydrated electron
e-beam	Electron beam radiation
EFSA	European Food Safety Authority
EOP	Extracted Olive Pomace
ESI-MS	Electrospray ionization mass spectrometry
ESR	Electron Spin Resonance
EtOAC	Ethyl acetate
EtOH	Ethanol
EXT	Extract of olive pomace
FAO	Food and Agriculture Organization
FRAP	Ferric reducing antioxidant power
FSE	Ferrous sulphate equivalent
FTIR	Fourier transform infrared spectroscopy
g	gram
GAE	Gallic acid equivalents
GC	Gas chromatography
Gy	Gray
$H^{\bullet}$	Hydrogen radical

H_2	Diatomic hydrogen molecule
H_2O	Water
H_2O^*	Excited water molecule
H_2O^+	Ionized water molecule
H_2O_2	Hydrogen peroxide
HAdV	Human adenovirus
HAE	Heat-assisted extraction
HAT	Hydrogen atom transfer
HCl	Hydrochloric acid
HDL	High-density lipoproteins
HIV	Human immunodeficiency virus
h^o	Hue angle
$HO^\bullet$	Hydroxyl radical
HPE	High pressure extraction
HPLC	High Performance Liquid Chromatography
IAEA	International Atomic Energy Agency
IC_{50}	Half-maximal Inhibitory concentration (50%)
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
JECFI	Joint Expert Committee on Food Irradiation
kCi	Kilocurie
keV	Kiloelectron volt
kg	Kilogram
kGy	Kilogray
kHz	Kilohertz
*L**	Lightness colour coordinate (CIELab colour space)
LC-QqTOF	Liquid chromatography quadrupole time-of-flight mass spectrometry
LDL	Low-density lipoproteins

LOD	Limit of Detection
log	Logarithm
LOQ	Limit of Quantification
LPS	Lipopolysaccharide
M	Molar concentration
MAE	Microwave-assisted extraction
MAP	Modified atmosphere packaging
MBC	Minimum bactericidal concentration
MCF-7	Breast adenocarcinoma cell line
MDA	Malondialdehyde
ME	Maceration
MEA	Malt extract agar
MeOH	Methanol
MeV	Megaelectron volt
MFC	Minimum fungicidal concentration
mg	Milligram
MIC	Minimum inhibitory concentration
min	Minute
mL	Mililiter
MMM	Multifrequency multimode modulated
mmol	Milimole
MNV	Murine norovirus
MS	Mass spectrometry
MT-2	Human lymphoblastic cell line
MUFA	Monounsaturated fatty acids
NCI-H460	Lung carcinoma cell line
nm	Nanometers
NMR	Nuclear magnetic resonance

NO	Nitric oxide
O_2	Oxygen
ºC	Celsius degree
ODS	Objetivos de Desarrollo Sostenible de las Naciones Unidas
OMS	Organización Mundial de Salud
OMWW	Olive mill wastewater
OPP	Oriented polypropylene
OxHLiA	Oxidative hemolysis inhibition assay
P	Power
PAL	Phenylalanine ammonia-lyase
PBS	Phosphate-buffered saline
Pec	Pectinase
PEF	Pulsed electric field extraction
pH	Potential of hydrogen
PLA	Polylactic acid
PLE	Pressurized liquid extraction
PLP2	Non-tumour porcine liver primary culture
PMMA	Polymethyl methacrylate
PUFA	Polyunsaturated fatty acids
PVN	Paraventricular nucleus
RAW 264.7	Murine macrophage cell
RSA	Radical scavenging activity
RSM	Response surface methodology
RT	Room temperature
RR	Respiration rate
S	Solvent
SC-CO_2	Supercritical CO_2 extraction
SET	Single electron transfer

SFE	Supercritical fluid extraction
SDG	United Nations Sustainable Development Goals
SHR	Spontaneously hypertensive rat
SI	International System of Units
SLE	Superheated liquid extraction
SRB	Sulforhodamine B
t	Time
T	Temperature
TA	Titratable acidity
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TLC	Thin-layer chromatography
TPC	Total Phenolic Content
TRPA1	Transient receptor potential ankyrin 1
TRPV1	Transient receptor potential vanilloid 1
TSB	Tryptic Soy Broth
TSS	Total soluble solids
TTR	Transthyretin
UAE	Ultrasound-assisted extraction
UCASUL	União de Cooperativas Agrícolas do Sul
UFP	Unidades Formadoras de Placa
VRBA	Violet Red Blue Agar
W	Watt
WHO	World Health Organization
γ-radiation	Gamma radiation

ABSTRACT

Olive oil production is one of the most important industries in the countries of the Mediterranean region. Olive pomace is the residue generated from these industries that use a two-phase centrifugation system. These residues have harmful effects in the environment due to their high organic content and phytotoxicity. Thus, their management and disposal have raised increasing concern by the environmentalists, researchers and society in general. On the other hand, these residues present high content of bioactive compounds with putative health benefits and their recovery has aroused considerable interest for food industries in developing functional foods, due to the demonstrated antioxidant, antimicrobial, antiviral, anti-inflammatory and anticancer activities of these valuable compounds.

Ionizing radiation is a simple, modern and environment friendly technology capable to decompose complex molecules into small ones, as well as to promote an improvement in the beneficial bioactive properties of different matrices such as industrial and municipal wastewaters, agro-industrial residues, fresh fruits and aromatic and medicinal plants.

In the present work, the feasibility of using gamma radiation for improving the extractability of valuable compounds present in olive pomace samples was evaluated. Furthermore, the extraction conditions to recover the main compounds from the irradiated olive pomace were also optimized in order to find out the most promising extract to incorporate as a food preservative in fresh-cut apples and to extend their shelf life. Two types of olive pomace, Crude Olive Pomace (COP) and Extracted Olive Pomace (EOP), and different absorbed doses (5, 10, 16 and 22 kGy) were initially assayed to determine the best conditions to improve the extractability of the natural bioactives.

Hydroxytyrosol was the most abundant compound (25 ± 1 mg/g extract in EOP and 23.9 ± 0.3 mg/g extract in COP) present in the studied olive pomace extracts, followed by hydroxytyrosol-1-β-glucoside (9.8 ± 0.4 mg/g extract in EOP and 9.94 ± 0.08 mg/g extract in COP), tyrosol (5.9 ± 0.2 mg/g extract in EOP and 5.9 ± 0.4 mg/g extract in COP), luteolin-7-*O*-rutinoside (3.1 ± 0.1 mg/g extract in EOP and 4.1 ± 0.2 mg/g extract in COP), oleuropein aglycon (3.0 ± 0.3 mg/g extract in EOP and 3.1 ± 0.1 mg/g extract in COP) and verbascoside (2.29 ± 0.03 mg/g extract in EOP y 3.4 ± 0.2 mg/g extract in COP). Regarding the effect of gamma radiation, an increasing of at least 2-fold in polyphenols extractability from EOP extracts was achieved using an absorbed dose of 5 kGy, while improving the antioxidant activity of the extracts by the inhibition of lipid peroxidation and antihemolytic activity, maintaining the latter in values similar to those obtained for the

reference antioxidant, Trolox. Both extracts of COP and EOP samples presented antimicrobial activity against different studied bacterial (MIC = 20-40 mg/mL) and fungi (MIC = 40 mg/mL) strains, but EOP extracts were more effective against *Escherichia coli* and *Pseudomonas fluorescens* (MIC = 20 mg/mL). Moreover, no toxicity in non-tumor hepatic cells (PLP2) was observed for any of the extracts and gamma radiation treatment did not cause variation in the antimicrobial activity and hepatotoxicity of the extracts.

With the achieved results, EOP sample irradiated at 5 kGy was selected to optimize the extraction conditions of the main bioactive compounds, especially hydroxytyrosol and tyrosol, present in the extracts using heat-assisted extraction (HAE) and ultrasound-assisted extraction (UAE). Response Surface Methodology (RSM) was employed to explore the possible relations between different extraction variables (i.e., time – t, solvent concentration – S, temperature – T, and Power – P). In the optimal conditions obtained, UAE reduced the extraction time and the solvent consumption when compared to HAE (t = 28 min, P = 490 W and S = 7.3% ethanol for UAE; t = 120 min, T = 85 ºC and S = 76% ethanol for HAE) and led to higher extraction yields (UAE yield = 30%; HAE yield = 13.7%). Nevertheless, when comparing the bioactivities of the extracts obtained by the two processes, HAE extracts presented higher antioxidant capacity, requiring less concentration of extract to inhibit TBARS formation and oxidative hemolysis, as well as higher antibacterial and anti-inflammatory activities. Other promising results for the HAE extracts were obtained by the increased antidiabetic activity, evaluated by the inhibition in α-amylase and α-glucosidase activities, and cytotoxic effect against breast adenocarcinoma (MCF-7) cells compared to UAE extracts. In fact, the α-glucosidase inhibition of HAE extracts (IC_{50} value of 14 ± 1 mg/mL) did not differ significantly from that of the standard positive control acarbose (IC_{50} value of 11 ± 1 mg/mL).

In order to maintain the consumers' interest in healthier foods, food industries are always seeking for innovative approaches to develop new functional products, and a great attention has been given to natural additives. In this sense, extracts of irradiated olive pomace recovered using HAE optimal conditions were tested as potential substitutes to ascorbic acid in delaying the oxidation of fresh-cut apples. Besides the antioxidant capacity, the potential inhibition of microbial growth and changes produced in physicochemical characteristics (texture, weight loss and colour) were evaluated during 12 days of refrigerated storage. Furthermore, two different films, biodegradable polylactic acid (PLA) and commercial oriented polypropylene (OPP) films, were explored for packaging the fruit slices in order to assess the most adequate one to ensure the desired quality of the fruit. Fresh-cut apples with natural olive pomace extracts presented higher growth inhibition of mesophilic bacteria and filamentous fungi for 5 days of storage at 4

ºC, and no detection of coliforms was verified along the 12 days of storage, contrary to what was observed for the samples treated with ascorbic acid. In general, the olive pomace extracts preserved or improved the phenolic content and antioxidant potential of the fruits packaged in PLA bags, when compared to ascorbic acid and to OPP bags, without significant changes in their texture. On the other hand, olive pomace extracts showed lower microbial diversity than ascorbic acid samples, supporting their potential to be used as a natural antimicrobial preservative in ready-to-eat foods. A storage of 5 days in refrigerated conditions could be proposed to produce these snacks using olive pomace extracts with similar or higher quality than the ones using the commercial antioxidant.

The overall results obtained in this Thesis can be useful to fulfil the needs of the food industries in producing new ingredients for the development of functional food products (Sustainable Development Goals – SDG 2 and 3). The reuse of agro-industrial wastes to recover new compounds as natural alternatives to the synthetic preservatives contributes for the sustainability of both agro-industrial sector and environment, also promoting the circular economy concepts and the awareness of “zero waste” strategy (SDG 9).

RESUMEN

La industria del aceite de oliva es una de las más importantes en los países de la región mediterránea. El orujo de aceituna es el residuo generado en estas industrias que utilizan un sistema de centrifugado en dos fases. Estos residuos tienen efectos nocivos en el medio ambiente debido a su alto contenido orgánico y fitotoxicidad. Por ello, su gestión y eliminación son causa de una creciente preocupación para ambientalistas, investigadores y sociedad en general. Por otro lado, estos residuos aún poseen cantidades relevantes de compuestos bioactivos con potenciales beneficios para la salud, al presentar una diversidad de actividades biológicas, como pueden ser antioxidante, antimicrobiana, antiviral, antiinflamatoria o anticancerígena, por lo que su recuperación ha despertado el interés de las industrias alimentarias, que ven en ellos una oportunidad para el desarrollo de sustancias nutracéuticas y alimentos funcionales.

La radiación ionizante es una tecnología sencilla, moderna y respetuosa con el medio ambiente capaz de descomponer moléculas complejas en otras más pequeñas, así como de promover una mejora en las propiedades bioactivas beneficiosas de diferentes matrices como vertidos industriales, frutas frescas o plantas aromáticas y medicinales.

En el presente trabajo se evaluó la viabilidad del uso de radiación gamma para mejorar la extractabilidad de compuestos de interés presentes en muestras de orujo de oliva. Además, se optimizaron las condiciones de extracción para recuperar los principales bioactivos del orujo de oliva irradiado y seleccionar el extracto más prometedor para incorporar como conservante en manzanas recién cortadas con el objeto de extender su vida útil. Se partió de dos tipos de orujo de oliva, Orujo de Aceituna Crudo (COP, *crude olive pomace*) y Orujo de Oliva Extraído (EOP, *extracted olive pomace*), que se sometieron a diferentes dosis de radiación absorbidas (5, 10, 16 y 22 kGy) con el fin de encontrar las mejores condiciones de extractabilidad de los componentes bioactivos.

El hidroxitirosol fue el compuesto más abundante (25 ± 1 mg/g extracto en EOP y 23.9 ± 0.3 mg/g extracto en COP) presente en las muestras de orujo de oliva analizadas, seguido de hidroxitirosol-1-β-glucósido (9.8 ± 0.4 mg/g extracto en EOP y 9.94 ± 0.08 mg/g extracto en COP), tirosol (5.9 ± 0.2 mg/g extracto en EOP y 5.9 ± 0.4 mg/g extracto en COP), luteolina-7-*O*-rutinósido (3.1 ± 0.1 mg/g extracto en EOP y 4.1 ± 0.2 mg/g extracto en COP), oleuropeína aglicón (3.0 ± 0.3 mg/g extracto en EOP y 3.1 ± 0.1 mg/g extracto en COP) y verbascósido (2.29 ± 0.03 mg/g extracto en EOP y 3.4 ± 0.2 mg/g extracto en COP). Mediante el empleo de radiación gamma, se logró un aumento de al menos 2 veces en la extractabilidad de los polifenoles en los extractos de EOP usando

una dosis absorbida de 5 kGy, al mismo tiempo que mejoró la actividad antioxidante de los extractos en su capacidad para inhibir la peroxidación lipídica y la actividad antihemolítica, manteniendo esta última valores similares a los obtenidos con el antioxidante de referencia (Trolox).

Tanto los extractos de las muestras de COP como de EOP presentaron actividad antimicrobiana contra las cepas bacterianas (MIC = 20-40 mg/mL) y fúngicas (MIC = 40 mg/mL) estudiadas, aunque los extractos de EOP fueron más efectivos para inhibir el crecimiento de *Escherichia coli* y *Pseudomonas fluorescens* (MIC = 20 mg/mL). Además, no se observó toxicidad en células hepáticas no tumorales (PLP2) para ambos extractos y el tratamiento con radiación gamma no provocó tampoco variación ni en la actividad antimicrobiana ni en la hepatotoxicidad de los extractos.

En base a los resultados obtenidos, se seleccionó la muestra de EOP irradiada a 5 kGy para la optimización de las condiciones de extracción de los principales compuestos bioactivos presentes en los extractos, principalmente hidroxitirosol y tirosol, mediante extracción asistida por calor (HAE, *heat-assisted extraction*) y extracción asistida por ultrasonidos (UAE, *ultrasound-assisted extraction*). Se empleó la metodología de superficie de respuesta (RSM, *Response Surface Methodology*) para explorar las posibles relaciones entre diferentes variables (tiempo – t, concentración de solvente – S, temperatura – T y potencia – P). En las condiciones óptimas obtenidas, la UAE redujo el tiempo de extracción y el consumo de solvente en mayor medida que la HAE (t = 28 min, P = 490 W y S = 7,3% de etanol para UAE; t = 120 min, T = 85 ºC y S = 76% de etanol para HAE), y condujo también a mayores rendimientos de extracción (rendimiento de UAE = 30%; rendimiento de HAE = 13.7%). Sin embargo, al comparar las bioactividades de los extractos obtenidos por los dos procesos, los extractos de HAE presentaron mayor capacidad antioxidante que los de UAE, requiriendo menor concentración de extracto para inhibir la formación de TBARS y la hemólisis oxidativa, así como mayor actividad antibacteriana y antiinflamatoria. Otro resultado prometedor para los extractos de HAE fue la mayor actividad antidiabética (evaluada por la inhibición de α-amilasa y α-glucosidasa) y efecto citotóxico en células de adenocarcinoma de mama (MCF-7) en comparación con los extractos de UAE. De hecho, la inhibición de α-glucosidasa obtenida para los extractos de HAE (IC_{50} = 14 ± 1 mg/mL) no difirió significativamente de la del control positivo acarbosa (IC_{50} = 11 ± 1 mg/mL).

Las industrias alimentarias buscan constantemente enfoques innovadores para desarrollar nuevos productos funcionales que permitan mantener el interés de los consumidores en alimentos más saludables y, en este sentido, se ha prestado gran atención a los aditivos naturales. Teniendo esto en cuenta, se realizaron ensayos para

evaluar el empleo de los extractos de orujo de oliva irradiado obtenidos en las condiciones óptimas de HAE como posibles sustitutos del ácido ascórbico para retrasar la oxidación de manzanas recién cortadas. Además, se estudió el potencial de los extractos para inhibir el crecimiento microbiano, así como los posibles cambios fisicoquímicos (textura, pérdida de peso y color) que podrían producirse a lo largo de 12 días de almacenamiento en refrigeración. Se valoraron también dos recubrimientos diferentes, ácido poliláctico (PLA, *polylactic acid*) biodegradable y películas de polipropileno orientado (OPP, *oriented polypropylene*) comercial, como material para envasar piezas de fruta fresca recién cortadas, con el objeto de seleccionar el más adecuado para asegurar la calidad deseada. La adición de extractos naturales de orujo de oliva a las piezas de manzanas fue más eficaz que la de ácido ascórbico para inhibir el crecimiento de bacterias mesófilas y hongos filamentosos durante 5 días de almacenamiento a 4 ºC, y no se verificó detección de coliformes durante los 12 días de almacenamiento. En general, los extractos de orujo de oliva conservaron o mejoraron el contenido fenólico y el potencial antioxidante de los frutos envasados en bolsas de PLA, en comparación con el ácido ascórbico, sin cambios significativos en su textura. Por otro lado, los extractos de orujo de oliva mostraron una menor diversidad microbiana que las muestras en ácido ascórbico, suportando su potencial para ser utilizados como un conservante antimicrobiano natural en alimentos frescos listos para el consumo. Los resultados obtenidos permiten proponer el empleo de extractos de orujo de oliva para asegurar el almacenamiento de este tipo de aperitivo durante 5 días en condiciones de refrigeración con eficacia similar o superior a los que utilizan ácido ascórbico.

Los resultados globales obtenidos en esta Tesis pueden ser útiles para la obtención de ingredientes naturales que permitan el desarrollo de nuevos productos alimenticios saludables (Objetivos de Desarrollo Sostenible - ODS 2 y 3) capaces de satisfacer las necesidades de las industrias alimentarias y de los consumidores, a la vez que contribuyen a la sostenibilidad tanto del sector agroindustrial como del medio ambiente, promoviendo los conceptos de economía circular y “cero residuos” (ODS 9).

1. INTRODUCCIÓN

1.1. Residuos agroindustriales

Con el mundo en rápido desarrollo, las industrias agroalimentarias están generando grandes cantidades de desechos que pueden tener efectos nocivos en el medio ambiente con consecuencias sobre la salud humana y animal, mientras que, por otro lado, pueden aún contener componentes valiosos que podrían ser útiles en diferentes sectores industriales. Esta problemática ha obligado a buscar nuevos enfoques para reutilizar los residuos agroindustriales de manera más eficiente, brindando beneficios ambientales y contribuyendo a la sostenibilidad económica. Así, por ejemplo, se pueden utilizar como fertilizantes agrícolas, para la producción de biocombustibles y enzimas, la preparación de sistemas poliméricos biodegradables, compostaje o extracción de aromatizantes, conservantes y compuestos bioactivos para su empleo en alimentos (Yusuf, 2017).

1.1.1. Industria del aceite de oliva

La producción de aceite de oliva es una de las industrias más importantes para la economía de los países de la región mediterránea, en la cual se produce alrededor del 70% de aceite de oliva mundial (Dermeche et al., 2013; Roig et al., 2006).

La extracción del aceite de oliva comprende diferentes etapas, como son el lavado de la aceituna, el estrujado, el batido de las pastas resultantes y la propia extracción, que constituye la etapa básica del proceso (Roig et al., 2006; Zbakh and El Abbassi, 2012). La extracción se puede realizar por procesos discontinuos (prensado tradicional) o continuos (centrifugación). Para los procesos de centrifugación, existen dos sistemas posibles, denominados sistema trifásico y bifásico. En el sistema trifásico se genera una torta sólida y dos productos líquidos, aceite de oliva y grandes cantidades de un residuo acuoso conocido como alpechín (agua residual de almazara). En el sistema de dos fases, utilizado en las unidades modernas para reemplazar la tecnología trifásica, se usa menos agua (reducciones de alrededor del 80%) durante el proceso, lo que significa que el volumen de agua residual producida y los requisitos de energía se reducen en comparación con el sistema trifásico (Azbar et al., 2004; Dermeche et al., 2013). En el proceso bifásico, se genera una fase líquida (aceite) y un residuo semisólido, denominado alperujo, constituido por una mezcla de orujo de aceituna y alpechín (Caporaso et al., 2018; Rodrigues et al., 2015).

En Portugal, esta industria originó más de 600 000 t de residuos en el año 2020 (Muniz, 2021), cuyo vertido sin tratamiento tendría un impacto negativo en el medio ambiente.

No obstante, estos residuos también presentan un valor añadido potencial para diferentes y numerosos sectores (Figura 1):

- Tratamiento de los suelos, no solo por su bajo coste sino también por su potencial para mejorar la fertilidad del suelo y reducir los riesgos de degradación del suelo (Federici et al., 2017; Majbar et al., 2018; Regni et al., 2017);
- Biomasa para producir combustibles renovables (Abu Tayeh et al., 2014; Al-Addous et al., 2017; Al Afif & Linke, 2019; Messineo et al., 2020; Rincón, et al., 2013; Romero-García et al., 2014; Serrano et al., 2017; Valenti et al., 2017);
- Adsorbentes para el control de la contaminación del agua (Abdelhadi et al., 2017; Anastopoulos et al., 2015; Fernández-González et al., 2019; Fernando et al., 2009; Martín-Lara et al., 2013; Martinez-Garcia et al., 2006; Pagnanelli et al., 2003);
- Fabricación de ladrillos, reemplazando el agua dulce y reduciendo así el consumo de ésta (de la Casa & Castro, 2014; de la Casa et al., 2009; Eliche-Quesada et al., 2014; Mekki et al., 2006, 2008);
- Elaboración de alimentos para animales (Dunne, 2019; Estaún et al., 2014; Gerasopoulos et al., 2015; Gerasopoulos, et al., 2015; Molina-Alcaide et al., 2010; Rojas-Cano et al., 2014);
- Producción de materiales de envasado biodegradables (de Moraes Crizel et al., 2018; Lammi et al., 2018);
- Obtención de ingredientes funcionales para las industrias alimentaria, cosmética y nutracéutica (Araújo et al., 2015; Caporaso et al., 2018; Galanakis et al, 2018; Rodrigues et al., 2015, 2017; Vitali Čepo et al., 2018; Zbakh & El Abbassi, 2012).

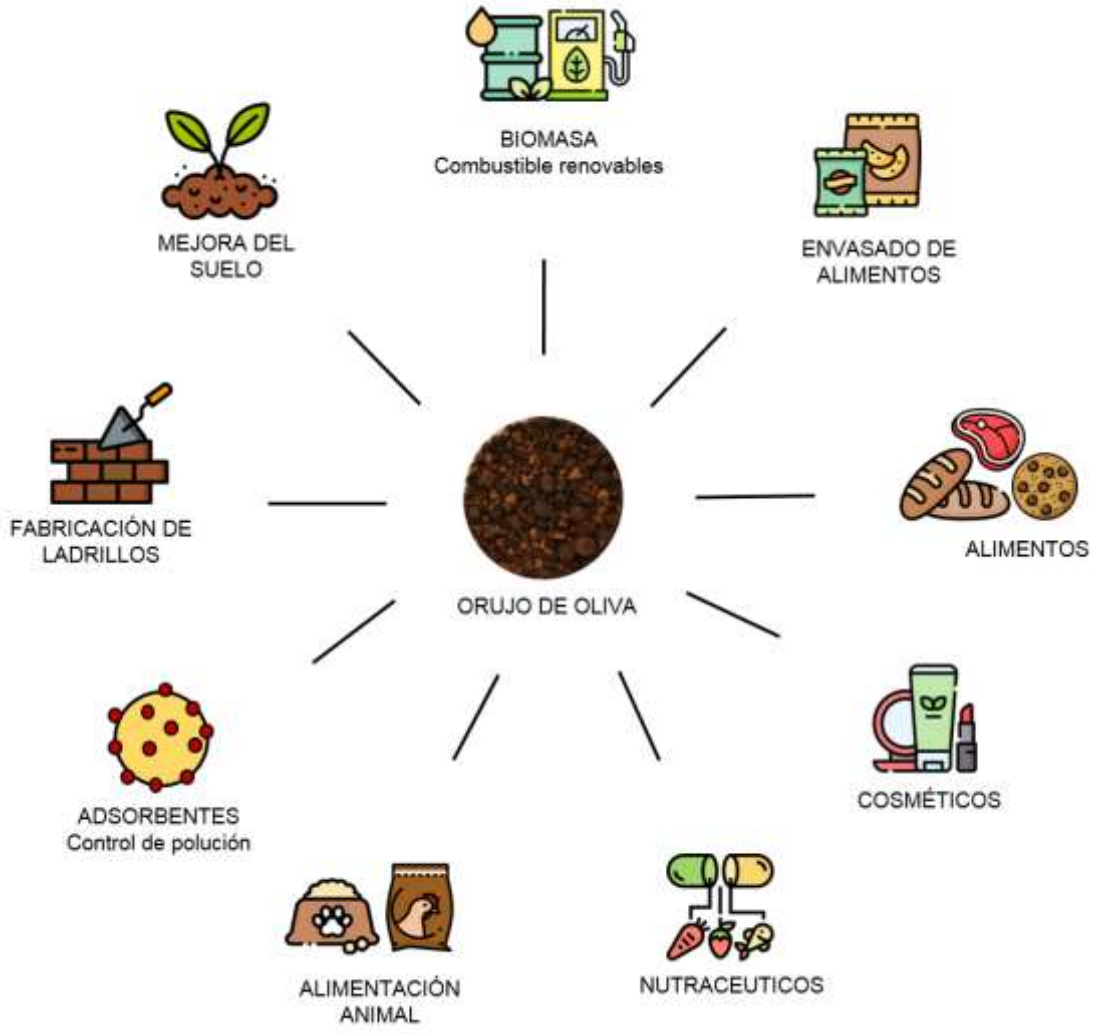


Figura 1. Aplicaciones del orujo de oliva en diferentes sectores.

1.1.2. El orujo de oliva como fuente de compuestos bioactivos

En el momento actual, la mayor parte de las almazaras, tanto en España como en Portugal, emplean el sistema bifásico de extracción. A pesar de la notable reducción que se consigue en la producción de aguas residuales en relación con el sistema trifásico, el orujo húmedo de oliva (alperujo) continúa siendo uno de los subproductos agrícolas más contaminantes en la región mediterránea (Papaioannou et al., 2013). Sin embargo, también contiene altas cantidades de sustancias con interés potencial para la industria alimentaria, como fibra dietética, azúcares, ácidos grasos, fitoesteroles, terpenoides, tocoferoles, secoiridoides y una variedad de compuestos fenólicos, incluyendo alcoholes y ácidos fenólicos, flavonoides o lignanos (El-Abbassi et al., 2012;

Nunes et al., 2018; Papaioannou et al., 2013; Yakhlef et al., 2018). Entre otras actividades, estos compuestos tienen un alto potencial como agentes antioxidantes, antiinflamatorios y antimicrobianos (Bulotta et al., 2014; Leouifoudi et al., 2014; Ribeiro et al., 2021b; Schaffer et al., 2010)

De acuerdo con la información revisada por Otero et al. (2021), durante la producción del aceite de oliva la cantidad de compuestos bioactivos extraídos que pasan a estar presentes en el aceite es muy baja, quedando la mayoría de ellos en los residuos. Por otra parte, el tipo y cantidad de compuestos fenólicos en el orujo de aceituna es variable y puede verse influido por factores diversos, como la variedad y cultivar de aceituna, la madurez del fruto, las condiciones climáticas y de crecimiento, el momento de la recolección y el proceso de extracción (Dermeche et al., 2013). Por esta razón, los datos de composición fenólica que se encuentran en la literatura no son siempre consistentes, al derivar de muestras de diferentes países y regiones, y probablemente obtenidos en diferentes períodos del año y mediante distintas metodologías analíticas. Por ejemplo, oleuropeína (81,7 – 83,9 mg/kg) y ligstrósido aglicón (27,1 – 31,1 mg/kg) fueron identificados como los compuestos más abundantes en orujos de oliva del Parque Nacional del Cilento, en Italia (Cioffi et al., 2010). En muestras de orujo de Argelia también se encontró oleuropeína (144 µg/g) como compuesto mayoritario, mientras que secoxiloganina (55 µg/g), loganina (47 µg/g) y cianidina-3-O-glucósido (39 µg/g) se detectaron por primera vez en estos residuos (Moudache et al., 2020). Malapert et al., (2018) describieron hidroxitirosol (370,7 mg/L), hidroxitirosol glucósido (165,2 mg/L) y tirosol (148,4 mg/L) como compuestos mayoritarios en orujos de aceituna procedentes de Baux-de-Provence (Francia). Igualmente, Nunes et al. (2018) observaron hidroxitirosol (83,6 mg/100 g peso fresco), como compuesto más abundante en orujo de aceituna de la región de Trás-Os-Montes (Portugal), representando el 54% de los compuestos fenólicos presentes, seguido de comselogósido (25% de fenoles totales). Hidroxitirosol fue también el compuesto fenólico mayoritario (hasta 1624,8 mg/kg de peso fresco) encontrado en extractos de muestras españolas de alperujo tratadas hidrotérrmicamente analizadas por Rubio-Senent et al. (2012), donde también se detectaron elevadas concentraciones de derivados del secoiridoide ácido elenólico (hasta 990 mg/kg), producidos durante el proceso de extracción. Además de los anteriores, una diversidad de compuestos han sido descritos en menor cantidad en orujos de oliva, como verbascósido, ácido gálico, ácido cafeico, ácido vanílico, ácido *p*-cumárico o distintos flavonoides, como rutina (quercetina-3-*O*-rutinósido), luteolina-7-*O*-rutinósido, luteolina-7-*O*-glucósido o apigenina-7-*O*-rutinósido (Cioffi et al., 2010;

Malapert et al., 2018; Peralbo-Molina et al., 2012; Rubio-Senent et al., 2013, 2012) (Figura 2).

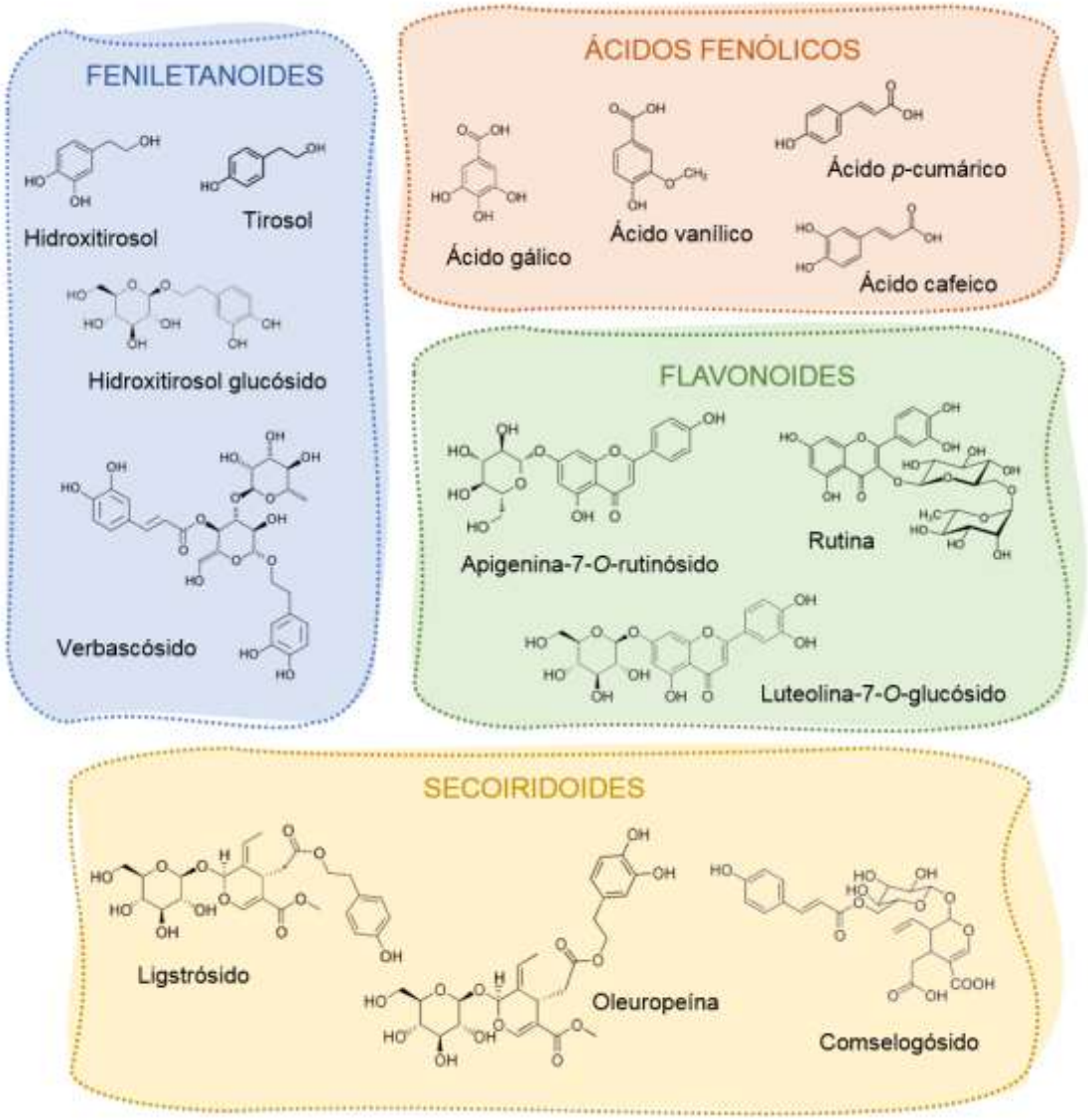


Figura 2. Estructuras químicas de los principales compuestos fenólicos del orujo de oliva.

1.1.3. Extractos de orujo de oliva como ingredientes funcionales para productos alimenticios

La preocupación de los consumidores por productos alimenticios más saludables ha conducido a una mayor búsqueda por parte de las industrias alimentarias de ingredientes naturales que puedan satisfacer esta demanda al mismo tiempo que garantizan la calidad de los alimentos. En los últimos años se han desarrollado varias metodologías para extraer compuestos bioactivos del orujo de oliva, que luego han sido incorporados a alimentos, fundamentalmente con el objetivo de aumentar la resistencia de los mismos a la oxidación, alargando así la vida útil manteniendo o mejorando sus propiedades nutricionales (Difonzo et al., 2021). Entre otros, se han utilizado en derivados de pescado (Cedola et al., 2017) y productos lácteos (Ribeiro et al., 2021a) o de panadería (Cecchi et al., 2019; Cedola et al., 2020; Cedola et al., 2019; Durante et al., 2019; Lin et al., 2017; Souza et al., 2022). Estos nuevos ingredientes también pueden proporcionar beneficios para la salud, aumentando aún más el valor añadido de los alimentos suplementados (Araújo et al., 2015; Difonzo et al., 2021; Nunes et al. 2016).

Ribeiro et al. (2021a) demostraron que la incorporación de orujo de oliva en polvo aportaba propiedades saludables a los yogures, al aumentar su capacidad antioxidante debido a la presencia de compuestos fenólicos, así como la cantidad de ácidos grasos mono- y poliinsaturados. Igualmente, hamburguesas de pescado enriquecidas con un 10% de harina de pasta seca de olivas mostraron una mejora en la actividad antioxidante, aunque los compuestos fenólicos añadidos aportaban cierto sabor amargo y picante, haciendo los productos menos agradables para el consumo (Cedola et al., 2017). Recientemente, Souza et al. (2022) incorporaron extractos de orujo de oliva en *muffins*, observando un aumento en los contenidos de fibra dietética, cenizas y lípidos, así como efectos positivos en la textura y una tonalidad más oscura, característica de estos ingredientes. Lin et al. (2017) también encontraron un aumento en los niveles de fibra dietética, asociado a un menor índice glucémico en galletas enriquecidas con extractos de orujo de oliva, en comparación con los productos tradicionales. El enriquecimiento de *taralli*, un producto de panadería típico italiano, con extractos de orujo de oliva fermentados condujo a niveles más altos de compuestos bioactivos que el producto convencional, permitiendo mantener en niveles más bajos el contenido de ácidos grasos saturados durante 90 días de almacenamiento en establecimientos minoristas (Durante et al., 2019). La incorporación de extractos de orujo de oliva en el pan también mejoró su contenido en compuestos fenólicos y su actividad antioxidante

(Cedola et al., 2020, 2019). Cecchi et al. (2019) analizaron las propiedades sensoriales y la aceptación por parte del consumidor de pasta, pan y barras de granola fortificados con orujo de oliva seco, observando una fuerte influencia sobre la apariencia de la pasta y del pan, así como un aumento en el amargor del pan y la granola, pero no de la pasta. Aun así, el 30% de los consumidores elegía la muestra enriquecida con relación al control para cada alimento, y el 50% estaba dispuesto a pagar más por los productos enriquecidos, lo que hacía este residuo interesante para añadir valor económico potencial a la industria del aceite de oliva. Además, los autores destacaban que el consumo de 63 g de pasta, 18 g de pan y 12 g de barra de granola fortificados podían ser suficientes para cumplir con la declaración de propiedades saludables relativa a los fenoles del aceite de oliva aprobada por la Autoridad Europea de Seguridad Alimentaria (EFSA) (Cecchi et al., 2019).

1.2. Tecnologías de irradiación

1.2.1. Breve introducción a la radiación ionizante

Las tecnologías de irradiación desempeñan un papel importante en el apoyo al desarrollo sostenible. Esta tecnología no requiere adición de agentes químicos o calor para inducir cambios en la materia, lo que supone menos gasto energético y hace que su huella de carbono sea menor comparada con otras tecnologías, a la vez que genera menos residuos. Todo ello hace que sea más rentable y respetuosa con el medio ambiente que las alternativas tradicionales (Ferreira et al., 2018).

Las fuentes de irradiación más comúnmente utilizadas en la industria y la agricultura son los rayos gamma y los haces de electrones acelerados (Chernyaev et al., 2019). La radiación gamma consiste en fotones emitidos por los isótopos radiactivos cobalto-60 y cesio-137, con energías de 1,17 y 1,33 MeV (^{60}Co) y 0,66 MeV (^{137}Cs). La radiación mediante haces de electrones se produce a partir de aceleradores de electrones con una energía de haz que suele oscilar entre 80 keV y 10 MeV. La principal ventaja de los rayos gamma es su gran poder de penetración, mientras que los haces de electrones implican menores tasas de irradiación, no involucran una fuente radiactiva y pueden encenderse y apagarse cuando sea necesario, lo que les convierte en una tecnología más asequible y factible para la aplicación práctica (Jiang and Iwahashi, 2020).

1.2.2. Aplicaciones de las tecnologías de irradiación

Debido a su versatilidad, las radiaciones ionizantes se utilizan actualmente para diferentes aplicaciones: esterilización de dispositivos médicos (Buchalla et al., 1995; Parsons, 2012), preparación y funcionalización de materiales híbridos que se utilizan para aplicaciones biomédicas y envasado de alimentos (Lancastre et al., 2015), descontaminación y conservación de documentos en pergamino (Nunes et al., 2013), o tratamiento y valorización de efluentes de distintas industrias (Borrely et al., 2016; Madureira et al., 2018a, 2018b, 2017). Además, la radiación ionizante tiene aplicaciones en procesado de alimentos, habiendo demostrado su eficacia para inhibir bacterias, hongos, o virus patógenos, eliminar parásitos, disminuir los brotes postcosecha en hortalizas como patatas o cebollas, prolongar la vida útil de los alimentos perecederos frescos o mejorar la capacidad de extracción de algunos compuestos químicos (Barkaoui et al., 2021a; Cardoso et al., 2019; Elias et al., 2020; Gryczka et al., 2021; Guerreiro et al., 2016; Pereira et al., 2018, 2015a, 2015b).

Guerreiro et al. (2016) y Madureira et al. (2019) demostraron que el tratamiento con haces de electrones a 3,6 kGy asociado a almacenamiento refrigerado conseguía reducir la población de bacterias mesófilas en cuatro unidades logarítmicas y eliminar hongos filamentosos y microorganismos patógenos en tomates cherry, preservando el contenido de licopeno y la actividad antioxidante, con efectos mínimos sobre los atributos sensoriales y de calidad del fruto. Además, los extractos de licopeno de los tomates cherry irradiados promovieron un efecto inhibidor sobre las células de cáncer de pulmón humano sin efecto sobre células humanas no cancerosas (Madureira et al., 2019). Resultados similares se obtuvieron para fresas (Barkaoui et al., 2021a, 2021b) y frambuesas (Elias et al., 2020) utilizando radiación gamma y haces de electrones a dosis de 2-3 kGy, garantizando la seguridad microbiana de los frutos y permitiendo prolongar al mismo tiempo su vida útil en al menos 7 días, a la vez que se obtenían mejoras en la calidad sensorial y el contenido bioactivo (Barkaoui et al., 2021a, 2021b). El tratamiento con radiación gamma fue, asimismo, capaz de reducir en dos órdenes logarítmicos el número de unidades formadoras de placa (UFP)/g de norovirus murino (MNV) y de adenovirus humano (HAdV) en fresas y frambuesas con un tratamiento a 4 kGy, y también demostró ser útil como tratamiento de desinfección (Pimenta et al., 2019).

En el caso de las plantas aromáticas y medicinales, la radiación gamma puede utilizarse como proceso de descontaminación, mejorando al mismo tiempo la extractabilidad de los compuestos bioactivos (Pereira et al., 2018, 2015b). Pereira et al. (2015a) sugirieron 1 kGy como dosis óptima a aplicar en *Ginkgo biloba* L. para mantener el perfil nutricional,

proteger algunas moléculas, como el α-tocoferol y los ácidos oxálico y málico, y aumentar la actividad antioxidante de los extractos obtenidos. La radiación gamma también se mostró eficaz para mejorar la capacidad antibacteriana de extractos de hierba luisa, hierbabuena y tomillo. (Pereira et al., 2018). Estos autores también observaron que los extractos de hierba luisa irradiados eran capaces de reducir los niveles virales de MNV y HAdV.

Gryczka et al. (2021) pusieron de manifiesto la importancia de una optimización previa de los parámetros más adecuados en el uso de tecnologías de irradiación, los cuales deben seleccionarse considerando las características de cada alimento. Los autores evaluaron el uso de haces de electrones de baja energía para descontaminar pimienta blanca y negra, concluyendo que todos los niveles de energía ensayados eran capaces de disminuir eficazmente la carga microbiana en la pimienta blanca, mientras que para la pimienta negra las energías más bajas probadas eran insuficientes para penetrar hasta la profundidad necesaria y lograr la descontaminación.

La aplicación de radiaciones ionizantes en Alimentación y Agricultura figura entre las misiones de la Agencia Internacional de Energía Atómica (AIEA) en colaboración con la Organización de las Naciones Unidas para la Agricultura y la Alimentación (FAO), que tienen como objetivo promover el uso pacífico de las tecnologías de radiación y la energía nuclear.

1.3. Hipótesis de partida

Teniendo en cuenta los antecedentes disponibles, la presente Tesis Doctoral basa sus objetivos en dos hipótesis de partida principales:

1. Las radiaciones ionizantes son capaces de aumentar la extractabilidad de los compuestos fenólicos de los orujos de aceite de oliva;
2. Los extractos recuperados de los residuos de la industria del aceite de oliva pueden utilizarse eficazmente como conservantes naturales en la funcionalización de frutas frescas recién cortadas.

1.4. Objetivos y plan de trabajo

El orujo de oliva es una fuente prometedora de compuestos bioactivos naturales que, además de su actividad antioxidante, demuestran propiedades biológicas como antimicrobianos, anticancerígenos o antiinflamatorios, pudiendo así ser utilizados en formulaciones de productos farmacéuticos y cosméticos o en la fortificación de los alimentos.

Por otra parte, las tecnologías de irradiación son procesos limpios y respetuosos con el medio ambiente que no dependen de la adición de productos químicos. Entre otras, la irradiación tiene la capacidad de degradar moléculas complejas en compuestos de bajo peso molecular, así como de mejorar la extracción y/o las propiedades beneficiosas de algunos de los compuestos químicos presentes en alimentos, desechos y plantas.

El objetivo general de esta Tesis Doctoral se centra en la obtención de bioactivos que puedan ser empleados como ingredientes alimentarios a partir del orujo de oliva utilizando las radiaciones ionizantes, en una perspectiva de tratamiento y valorización de este residuo agroindustrial. En este contexto, durante los últimos cuatro años, se abordaron los siguientes objetivos específicos para lograr este propósito general:

1) evaluar el impacto de las radiaciones ionizantes sobre el orujo de oliva, mediante el estudio de la cantidad y el perfil de los principales compuestos fenólicos de los extractos obtenidos a partir de las muestras irradiadas y evaluar sus propiedades bioactivas (actividades antioxidante, antimicrobiana, antiinflamatoria, antidiabética y antiproliferativa);

2) optimizar procesos de extracción asistida por calor y por ultrasonidos para mejorar tanto la extractabilidad de compuestos fenólicos como de las propiedades bioactivas de los extractos; y

3) explorar la capacidad del extracto de orujo de oliva obtenido empleando las condiciones más favorables para preservar la calidad de las frutas recién cortadas y comparar su eficacia como agente antioxidante y antimicrobiano con la del ácido ascórbico, el aditivo comercial utilizado con este fin por las industrias alimentarias.

El estudio se llevó a cabo utilizando dos tipos diferentes de orujo de oliva recolectados en la UCASUL – "União de Cooperativas Agrícolas do Sul" (Portugal): Orujo de Oliva sin desgrasar o Crudo (COP, *crude olive pomace*), que consiste en orujo seco antes de la extracción del aceite de orujo, y el Orujo de Oliva desgrasado o Extraído (EOP, *extracted olive pomace*), que es el residuo que queda tras la extracción del aceite de orujo.

Un esquema del plan de trabajo se muestra en la Figura 3.

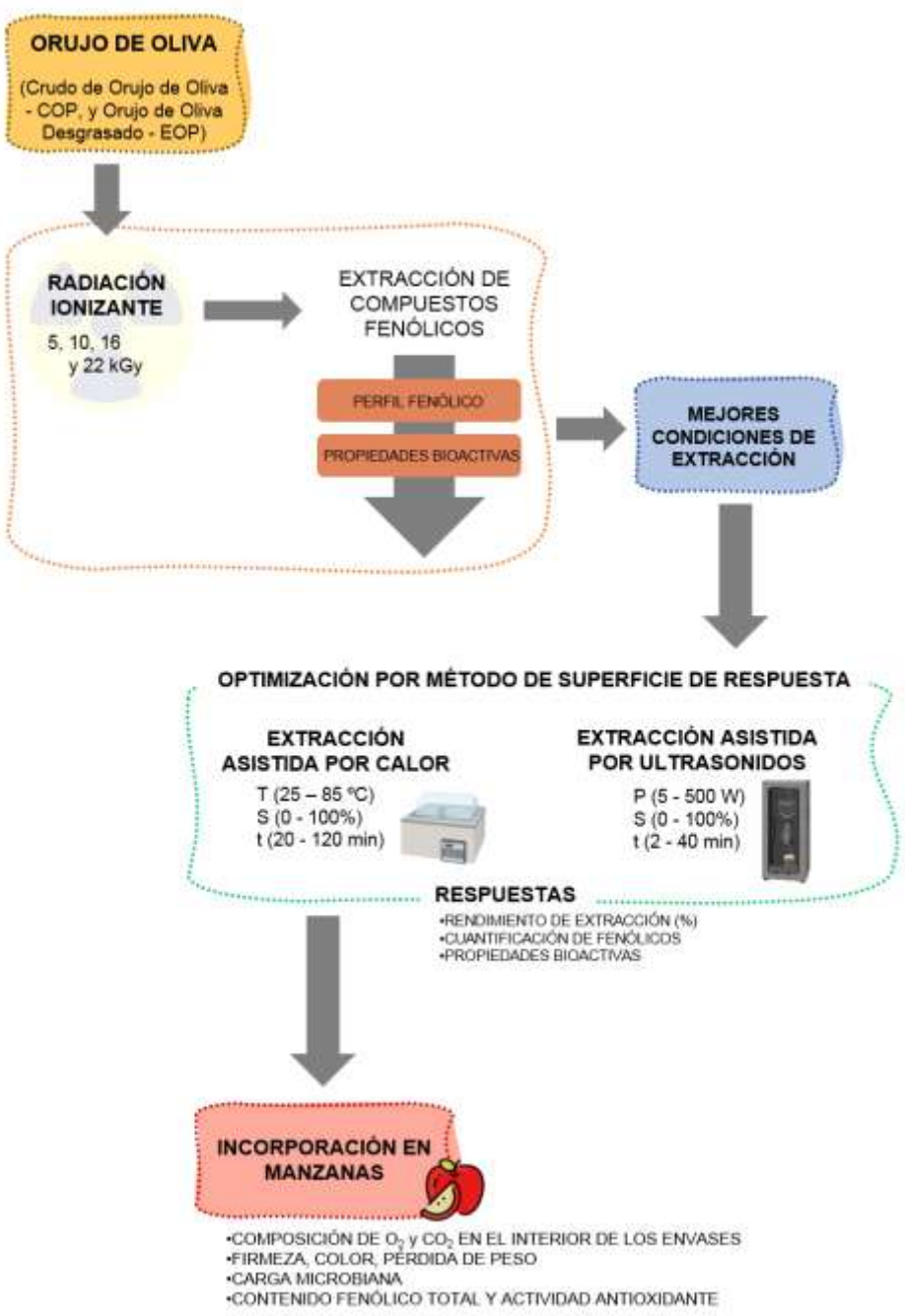


Figura 3. Diagrama esquemático del trabajo experimental desarrollado en la Tesis.

En las siguientes secciones, se presentan los trabajos a los que ha dado lugar esta Tesis Doctoral y se realiza una discusión integrada de todos ellos, para resaltar la coherencia del enfoque experimental y la relevancia de los resultados obtenidos. Finalmente, se extrae una conclusión general con los principales resultados de todo el trabajo, y se comentan brevemente las perspectivas futuras para su continuación.

Se espera que los resultados de este trabajo proporcionen impactos positivos tanto ambientales como económicos para las industrias alimentaria y del aceite de oliva.

2. PUBLICACIONES

Publicación 1. Effects of Irradiation on Food Bioactives

Joana Madureira, Lillian Barros, Fernanda M. A. Margaça, Celestino Santos-Buelga, Isabel C. F. R. Ferreira, Sandra Cabo Verde

Capítulo de libro publicado en: Jafari, S.M., Capanoglu, E. (eds) *Retention of Bioactives in Food Processing. Food Bioactive Ingredients*. Springer, Cham, pp 429–465 (2022). ISSN 2661-8966. Doi: https://doi.org/10.1007/978-3-030-96885-4

Resumen

La irradiación es una tecnología prometedora basada en la utilización de radiaciones ionizantes (rayos gamma, haces de electrones acelerados y rayos X) para el procesamiento de alimentos. Aunque su empleo en alimentos fue propuesto hace más de 100 años, solo estuvo disponible para su uso con fines comerciales a partir de la década de 1960. Se trata de un proceso no térmico, limpio y ecológico, que no implica el uso de productos químicos ni genera residuos. Es una tecnología considerada segura y eficaz por la Organización Mundial de la Salud (OMS), la Organización para la Agricultura y la Alimentación (FAO) y la Agencia Internacional de la Energía Atómica (AIEA).

Los efectos de las radiaciones ionizantes sobre los alimentos dependen del tipo de matriz, tamaño, estado, temperatura y condiciones de irradiación. Está firmemente establecido que no provoca radiactividad en los alimentos, los cuales pueden ser, además, tratados en su envase final, lo que evita la recontaminación y hace que estén disponibles de manera inmediata para su salida a la cadena de distribución.

En este capítulo, se revisan aspectos generales sobre los procesos de irradiación y se discuten los efectos de esta tecnología sobre los componentes bioactivos, así como las potenciales aplicaciones industriales de los compuestos recuperados, promoviendo así la economía circular para el desarrollo sostenible.

La revisión realizada pone de manifiesto el valor añadido de los compuestos bioactivos extraídos a partir de distintas fuentes alimentarias irradiadas, así como la necesidad de profundizar en la investigación sobre su aplicabilidad para la preparación de nuevas formulaciones en los sectores alimentario, cosmético y farmacéutico.

Chapter 14
Effects of Irradiation on Food Bioactives

Joana Madureira, Lillian Barros, Fernanda M. A. Margaça, Celestino Santos-Buelga, Isabel C. F. R. Ferreira, and Sandra Cabo Verde

14.1 Introduction

Food irradiation is a promising technology that uses ionizing radiation for food processing. Although the history of using food irradiation for several benefits has more than 100 years, only after the 1960's the concept of commercial radiation sources became available. Irradiation is a non-thermal, clean and eco-friendly process, not involving the use of chemicals or generating chemical residues. It has been considered a safe and effective technology by the World Health Organization (WHO), the Food and Agriculture Organization (FAO) and the International Atomic

J. Madureira
Centro de Ciências e Tecnologias Nucleares (C2TN), Instituto Superior Técnico, Universidade de Lisboa, Bobadela LRS, Portugal

Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal

Grupo de Investigación en Polifenoles (GIP-USAL), Facultad de Farmacia, Universidad de Salamanca, Salamanca, Spain

L. Barros · I. C. F. R. Ferreira
Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal

F. M. A. Margaça · S. Cabo Verde (✉)
Centro de Ciências e Tecnologias Nucleares (C2TN), Instituto Superior Técnico, Universidade de Lisboa, Bobadela LRS, Portugal
e-mail: sandracv@ctn.tecnico.ulisboa.pt

C. Santos-Buelga
Grupo de Investigación en Polifenoles (GIP-USAL), Facultad de Farmacia, Universidad de Salamanca, Salamanca, Spain

Unidad de Excelencia Producción, Agrícola y Medioambiente (AGRIENVIRONMENT), Parque Científico, Universidad de Salamanca, Salamanca, Spain

S. M. Jafari, E. Capanoglu (eds.), *Retention of Bioactives in Food Processing*, Food Bioactive Ingredients, https://doi.org/10.1007/978-3-030-96885-4_14

Energy Agency (IAEA). Furthermore, it is firmly established that the food does not become radioactive, can be treated in its final packaging, which avoids re-contamination of the products and makes it available for immediate distribution to food suppliers.

In this chapter, after presenting general aspects about irradiation processes, we will discuss the effects of this technology on the food bioactives, as well as the potential applications of these recovered compounds in food, cosmetic and pharmaceutical industries thus promoting the circular economy for sustainable development.

14.2 Irradiation Processes

The three forms of ionizing radiation authorized to be used in food irradiation applications are gamma rays, X-rays and electron-beam accelerators (WHO 1988).

14.2.1 Gamma Radiation

Gamma radiation is generated by photons emitted from the radioactive isotopes cobalt-60 and cesium-137, with energies of 1.17 and 1.33 MeV (^{60}Co) and 0.66 MeV (^{137}Cs).

Cobalt-60 is the most common source of gamma radiation used for food processing. There are more than 200 large-scale gamma plants operating worldwide and this number is growing. The main advantage of these radioisotopes is the high efficiency due to their penetrating power. Nevertheless, when not being used, the gamma sources have to be stored in a water pool in order to absorb the energy and protect workers. In a gamma facility, the food product can be treated in the same boxes or pallets in which they will be transported and distributed, being thus carried into the irradiator, submitted to the radiation source and taken back out again. The isotope source is constituted most often by multiple pencils in known positions (Fig. 14.1). Gamma irradiators are designed to provide an acceptable distribution of absorbed dose within the product through an arrangement of, or pathway for, products in irradiation containers around the radiation source (Fig. 14.1), which allows the products to absorb the radiation from multiple angles (Ferreira et al. 2018).

14.2.2 Electron-Beam Radiation

Electron-beam radiation is produced from electron accelerators (Fig. 14.2) and it can be used directly or, indirectly, converted to X-rays. The beam energy of e-beam accelerators is usually from 80 keV to 10 MeV.

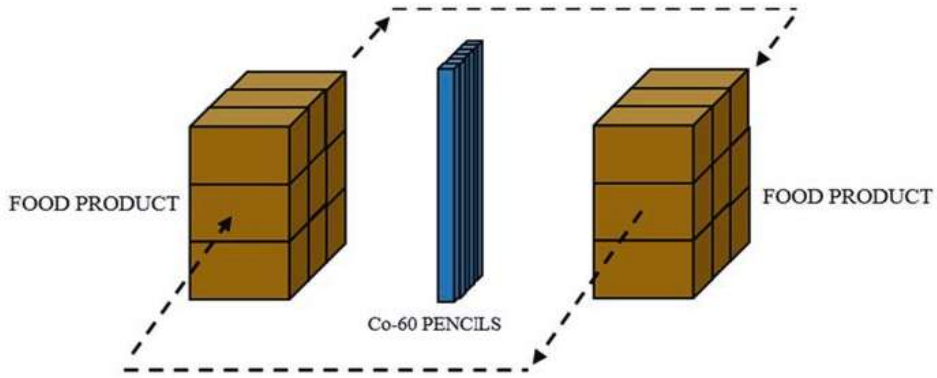


Fig. 14.1 Schematic diagram of a gamma irradiator facility

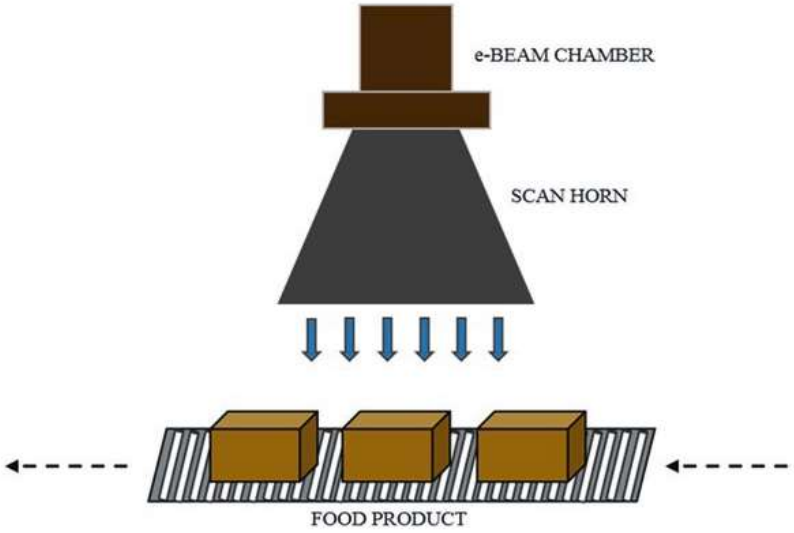


Fig. 14.2 Schematic diagram of an e-beam irradiator

The advantage of electron-beam irradiation is that the source equipment can be switched on and off depending on the necessity and do not rely on a radioactive source that radiates nonstop thus needing to be replaced after some time. In this way, the electron-beam irradiation is a more controlled and energy efficient process and it can also be considered a more environmentally acceptable alternative to ^{60}Co (Ferreira et al. 2018), yet with the disadvantages of a greater complexity of maintenance, lower dose uniformity and lower penetration in the products.

In an accelerator facility, there is a material handling system, the conveyor, that transports the food products to and from the irradiation zone in a precisely and controlled manner (Fig. 14.2) and, in some cases, moves them into and out of the facility (Miller 2005).

14.2.3 X-Rays

X-rays are generated by the same technology that produces electron-beams at or below an energy of 5 MeV, by a process known as Bremsstrahlung conversion (Miller 2005) which happens when electrons are accelerated to a metallic target (e.g. tantalum, tungsten or gold) to be converted to an X-ray steam. Nevertheless, most of the energy required to produce X-rays is lost as heat in the target, turning this process more inefficient and expensive than gamma or electron-beam radiation. Therefore, its throughput efficiency is quite low when compared to that obtained by e-beam. The photons in X-rays are similar to gamma radiation, meaning that they have very high penetration, but without using isotopes as source.

14.3 Dose Range and Dosimetry

Irradiation processing has been studied extensively worldwide and has become accepted as a proven and effective post-harvest treatment to reduce pathogenic contamination, prevent sprouting, eliminate parasites, extend the shelf-life of fresh perishable foods, and provide ready-to-eat food for astronauts and emergency rations and diets for immune-compromised patients, among other applications.

The different purposes on the use of irradiation technology depend on the absorbed radiation dose (Table 14.1), which is the accumulated energy that is transferred to the matter, i.e., absorbed energy per mass unit. The SI unit for dose is the "Gray" (Gy), defined as the absorption of one joule in a mass of 1 kg ($1\ Gy = 1\ J\ kg^{-1}$) of irradiated matter.

Dosimetry provides essential important information in radiation processing. When the food product is exposed to radiation, the absorbed doses and dose rates must be measured prior to its irradiation to determine the performance of the irradiation facility. The dosimetry system consists of the dosimeters, the measurement instruments and their associated reference standards and procedures for correct use. These systems are recommended to characterize the radiation facility for operational qualification, perform dose mappings, quality control and validation of the processes. In this way, the dosimetry systems must be calibrated to ensure that the irradiator is capable of operating and delivering the adequate doses to the product (ISO/ASTM 52303:2015). The minimum (D_{min}) and the maximum (D_{max}) absorbed doses that are applied to a food product in order to achieve the final purpose, maintaining its quality control and assurance, are based on the routine dose monitoring and regulatory limits that may be applicable. The ratio of the maximum dose received by a product stack to the minimum dose received is commonly referred to as the "Dose Uniformity Ratio" (DUR), with DUR = 1.0 as the ideal value. DUR must be optimized by changing, for example, the position of the product in relation to the source or the irradiator design. In food products with high density, the ideal DUR is difficult to achieve.

Table 14.1 Applications of food irradiation and recommended dose ranges

Purpose	Dose range (kGy)	Examples of food treated
Low doses (0.1–1 kGy)		
• Inhibition of sprouting	0.05–0.15	Potatoes, onions, garlic, ginger-root, chestnuts
• Insect disinfestation and parasite disinfection	0.15–0.50	Cereals and pulses, fresh and dried fruits, dried fish and meat, fresh pork
• Delay of physiological process (e.g., ripening)	0.50–1.0	Fresh fruits and vegetables
Medium dose (1–10 kGy)		
• Extension of shelf-life	1.0–3.0	Fresh fish, strawberries, asparagus
• Inhibition of spoilage and pathogenic microorganisms	1.0–7.0	Fresh and frozen seafood, raw or frozen poultry and meat
• Improving technological properties	2.0–7.0	Grapes (increasing juice yield), dehydrated vegetables (reduced cooking time)
High dose (10–50 kGy)		
• Industrial sterilization (in combination with mild heat)	30–50	Meat, poultry, seafood, prepared foods, sterilized hospital diets
• Decontamination of certain food additives and ingredients	10–50	Spices, enzyme preparations, natural gum

Various dosimeters can be used for food processing with a reproducible response to radiation, such as polymethyl methacrylate (PMMA) dosimeters, radiochromic dosimeters and alanine dosimeters. PMMA dosimeters can be measured in a calibrated spectrophotometer at a given specific wavelength to determine the response, the specific absorbance after irradiation and the dosimeter thickness. Alanine dosimeters can be produced in pellets or films and the concentration of free radicals formed during irradiation can be measured by Electron Spin Resonance (ESR). The American Society for Testing and Materials (ASTM) E61 'Radiation Processing' is an international group of experts who have established and maintained standard practices, methods and guides for ionizing radiation processing and dosimetry.

14.4 International Standards on Food Processing

The *Codex Alimentarius*, also known as "food code", is a compilation of all the standards, codes of practice, guidelines and recommendations of the *Codex Alimentarius* Commission, with the objective of protecting consumers' health and ensuring fair practices in the food trade. The proposed guidelines for irradiation are based on findings of the Joint Expert Committee on Food Irradiation (JECFI) composed by experts of the Food and Agriculture Organization, International Atomic Energy Agency and World Health Organization (FAO/IAEA/WHO). In 1981, WHO

published a document stating that irradiation of food at doses up to 10.0 kGy is safe and introduces no further toxicological or nutritional problem (JECFI 1981). In 1997, the FAO/IAEA/WHO Study Group on High-Dose Irradiation (JSGHDI 1999) concluded that food irradiated to any dose appropriate to achieve the intended technological objective is both safe to consume and nutritionally adequate and that no upper dose limit has to be imposed.

Food that has been irradiated must be labeled as "irradiated" or "treated with ionizing radiation" together with the Radura symbol (Fig. 14.3) and the name of the product. The word "radura" derived from radurization, a term composed of the initial letters of the word "radiation" and the terms "durus" (Latin word for "hard" or "lasting") (Maherani et al. 2016). Generally, the symbol is green and resembles a plant in a circle that illustrates the rays from the sources (Fig. 14.3).

Currently, food irradiation is approved for using in more than 50 countries worldwide for over 60 products, including Australia, Belgium, Brazil, Canada, China, India, Russia, South Africa, Thailand, USA and Vietnam. In 2015, more than 700,000 tons of foods were irradiated (Eustice 2018), including spices, meat, fresh fruits and vegetables. Based on the *Codex* standards for the Labeling of Prepackaged Foods (CAC 1985), many countries have developed their own national regulations for irradiated food labeling and in most countries there is still no requirement to use the Radura symbol. Furthermore, the list of products allowed to be irradiated differs from country to country. For instance, in the European Union, the Directive 1999/3/EC only permits the irradiation of dried aromatic herbs, spices and vegetable seasonings (European Union 1999).

Fig. 14.3 The Radura symbol

14.5 Consumers' Acceptance

Perhaps consumers' acceptance is the most difficult barrier to cross on the use of irradiation, making their perception an interesting and important point to take into consideration. Although consumers are always interested in new technologies, the truth is that they confuse irradiated food with radioactive food that can cause damage to human health or environment (Ornellas et al. 2006; Junqueira-Gonçalves et al. 2011; Roberts 2014). Actually, the main worries of the consumers include safety, nutrition, detection and labeling of irradiated food. Even so, the public would consider consuming irradiated food if their benefits are explained or if they are informed about the use of "Radura" symbol, which is believed to transmit confidence and safety. In this way, an effort on the education of consumers has to be done, providing scientific and credible information in order to familiarize them with the principles, aims and benefits of irradiated products, which may lead to change their opinion (Maherani et al. 2016).

14.6 Effects of Irradiation on Food Bioactives

Bioactive compounds are phytochemicals naturally found in fruits, vegetables or whole grains that may provide beneficial health effects, including antioxidant, anti-inflammatory, antimicrobial, anticancer and immunomodulatory activities (Kris-Etherton et al. 2002; Shashirekha et al. 2015). They include different classes of compounds, such as polyphenols, carotenoids, tocopherols, phytosterols, organosulfur compounds, fatty acids, betalains, essential oils (terpenes) and alkaloids. They have different chemical structures and solubility (hydrophilic or lipophilic), distribution in nature, range of concentrations in foods, bioavailability in the human body, sites of action, effectiveness against harmful species, specificity and biological action.

When food is exposed to ionizing radiation, some primary effects are induced in food matrices due to the presence of water molecules, via ionization and excitation, which exponentially increase by the secondary action of the free radicals formed during this process. Due to the high water contents in food, the products that are formed during water radiolysis are considered the main responsible for the potential effects on food composition. These products include several chemical species: hydrated electrons (e^-_{aq}), hydroxyl radicals ($HO^\bullet$), hydrogen radicals ($H^\bullet$), excited water molecules (H_2O^*), ionized water molecules (H_2O^+), hydrogen peroxide (H_2O_2) and diatomic hydrogen molecules (H_2) (Le Caër 2011). The occurring modifications in atoms and molecules are known as the primary or direct effects of radiation, which result in the formation of new chemical compounds and free radicals, both chemically unstable and reactive. These radical species can interact with themselves and/or continue to react with other food components, that could lead to the formation of new compounds that are not present in non-irradiated food (Ferreira et al.

2018). These new reaction products can also interact with the above-mentioned free radicals, representing the secondary or indirect chemical effects of radiation processing (Ferreira et al. 2018).

The irradiation can alter and/or improve the chemical components on food, enhance the extractability of some molecules and change its bioactivity, and these changes may depend on the irradiation conditions (water content, temperature, pH, dose and dose rate). Furthermore, the effects induced by ionizing radiation on the bioactive compounds are also dependent on the food composition. The observed increase in the extractability of compounds can be explained by changes in cellular structures, namely by the depolymerization and dissolution of the cell wall polysaccharides by irradiation (Harrison and Were 2007; Behgar et al. 2011). Thus, the improvement of total phenolic and/or flavonoid contents on irradiated samples can be related with their release from matrix structures, increasing extractability of certain molecules, but also to the degradation of larger compounds into smaller ones by irradiation. The degradation of ascorbic acid, an important vitamin found in different fruits, usually observed after food irradiation can be easily attributed to the reversible oxidation of ascorbic acid to dehydroascorbic acid that can be further hydrolyzed and oxidized irreversibly to 2,3-diketogulonic acid (Deutsch 2000). Oppositely, the increase in the antioxidant activity sometimes produced after irradiation can be associated to an enhancement in enzymes activity (e.g., phenylalanine ammonia-lyase or peroxidase). For instance, stimulation of phenylalanine ammonia-lyase activity may promote the accumulation of anthocyanins, flavonoids and other phenolic compounds (Given et al. 1988; Oufedjikh et al. 2000). It is also pertinent to mention that the increase or decrease in the extraction yield is strongly dependent on the solvents used for the extraction (Pérez et al. 2007; Khattak et al. 2008).

The effects induced by ionizing radiation on food bioactive compounds have been studied for many years and will be reviewed on this section.

14.6.1 Fruits and Vegetables

Fruits and vegetables are colorful, flavorful and nutritious components rich in bioactive compounds, such as polyphenols, carotenoids, vitamins, phytoestrogens, glucosinolates and anthocyanins. Carotenoids and anthocyanins are also important compounds as they are pigments that impart the color to fruits and vegetables.

Some studies have reported the effects of gamma and electron-beam radiation on the major compounds of berry fruits. Barkaoui et al. (2020) compared the impact of gamma and electron-beam radiation on the antioxidants of strawberries (*Fragaria* × *ananassa*), being both treatments capable of preserving the phenolic content and increasing the antioxidant activity of the fruits at 2 kGy. On the other hand, both treatments induced a significant reduction on the amount of L-ascorbic acid (vitamin C). In fact, it is documented that ascorbic acid of berry fruits is sensitive to irradiation (Hussain et al. 2012; Tezotto-Uliana et al. 2013; Elias et al. 2020) and

can be oxidized to dehydroascorbic and, consequently, to 2,3-diketogulonic acid (Deutsch 2000). Maraei and Elsawy (2017) reported an increase of phenolic and anthocyanin contents and antioxidant activity in strawberries irradiated at 600 Gy. Furthermore, gamma irradiation at 0.5 kGy was observed to increase the concentration of anthocyanins (Tezotto-Uliana et al. 2013) and doses up to 2 kGy enhanced the phenolic content and antioxidant activity (Cabo Verde et al. 2013; Guimarães et al. 2013) in raspberries (*Rubus idaeus* L.). Electron-beam radiation at 3 kGy preserved the phenolic content and antioxidant activity of raspberries (Elias et al. 2020), and the total anthocyanin content, the antioxidant activity and ascorbic acid amount in blueberries (*Vaccinium corymbosum*, cvs. Collins, Bluecrop) (Kong et al. 2014).

Different effects of ionizing radiation have been found in tomatoes (*Solanum lycopersicum* var. cerasiforme) depending on the variety and/or the harvest conditions. Guerreiro et al. (2016) reported slight increases on total phenolic content of cherry tomatoes irradiated at 3.2 kGy, while a decrease in the concentration of individual phenolic compounds was observed in round tomato (Schindler et al. 2005), not affecting the anthocyanin content (Singh et al. 2016). A decrease in the levels of lycopene, the most representative carotenoid in tomatoes, was reported with 1.5 kGy electron-beam doses (Madureira et al. 2019), which was associated to the isomerization of lycopene. On the other hand, fluctuations in the content of lycopene were observed by Loro et al. (2018) in tomatoes, with increasing and decreasing values at doses up to 1.5 kGy.

Najafabadi et al. (2017) found that gamma radiation at doses up to 2.5 kGy not only increased the content of the total phenol and anthocyanins, but also improved the amount of vitamin C in jujube (*Ziziphus jujuba* var. vulgaris) fruit, while other water-soluble vitamins, as folic acid, thiamine (B_1) and pyridoxine (B_6), decreased significantly at this absorbed dose. Concerning mangoes (*Mangifera indica* L.), the studies developed by Reyes and Cisneros-Zevallos (2007) suggested that electron-beam radiation at doses up to 3.1 kGy did not affect the total phenolic and total carotenoid contents or the antioxidant capacity even after 18 days of storage, despite the observed increase in the levels of flavonols and phenolic acids and the decrease in ascorbic acid content. Other authors reported an increase in phenolic and carotenoid compounds and a decrease in ascorbic acid concentration in stored mangoes irradiated (1–1.5 kGy) with gamma (El-Samahy et al. 2000) and electron-beam (Moreno et al. 2007) radiation. It was also verified that gamma radiation (1–2 kGy) enhanced the antioxidant properties of peach (*Prunus persica* Bausch, Cv. Elberta), due to the increase in total phenolic content via enhancement of phenylalanine ammonia-lyase (PAL) activity (Hussain et al. 2010), despite a reduction of the ascorbic acid concentration. As for irradiated chestnuts (*Castanea sativa* Mill.), it was reported that electron-beam and gamma radiation could preserve total phenolics but not flavonoid content, and increase the antioxidant potential of the fruit, at 1 and 3 kGy, respectively (Carocho et al. 2012). In another study performed by Carocho et al. (2014), 1 kGy absorbed dose was described as capable of preserving phenolic profile and antioxidant properties of chestnuts, leading to higher values of tocopherols and β-carotene bleaching inhibition.

No antioxidant activity assay exactly reflects the antioxidant capacity of the samples. In fact, it is important to assess the antioxidant potential using methods that take into account different mechanisms of action (Moharram and Youssef 2014; Shahidi and Zhong 2015), including, among others, hydrogen atom transfer (HAT), single electron transfer (SET), reducing power and metal chelation. In a study performed by Kavitha et al. (2015) different tendencies were obtained in the evaluation of antioxidant activity of *Zizyphus mauritiana* Lam. fruit using different methodologies. A significant rise was observed in total flavonoids, 1,1-diphenyl-2-picrylhydrazil (DPPH) scavenging activity and super oxide anion radical scavenging activity, while reducing power, total phenolic content and antioxidant activity measured by thiobarbituric acid reactive substances (TBARS) assay decreased with increasing irradiation doses (0.25–1.0 kGy).

In dry fruits, it was demonstrated that gamma irradiation at 5 kGy was capable of improving the contents of phenolic (>40%) and flavonoid (>56%) compounds, the DPPH scavenging activity (>18%) and the antibacterial potentials of two genotypes of Egyptian date palm fruits, *Phoenix dactylifera* L. (El-Beltagi et al. 2019). Sun dried apricots (*Prunus armeniaca* L.) also underwent a significant increase of total phenols and flavonoids, β-carotene and antioxidant activity after gamma radiation at a dose of 3 kGy (Hussain et al. 2011, 2013). Those authors also analyzed the effect of gamma radiation on individual phenolic acids and flavonoids, observing significant increases in the concentrations of gallic acid (26%), ellagic acid (24%), quercetin (26%) and apigenin (37%) induced by the treatment (Hussain et al. 2013).

The effect of gamma radiation was also evaluated in fresh green vegetables, such as watercress (*Nasturtium officinale* R. Br.) (Pinela et al. 2016, 2018), fenugreek (*Trigonella foenum–graceum* L.) and spinach (*Spinacia oleracea* L.). Gamma radiation at 5 kGy combined with modified atmosphere packaging (MAP) induced an increase on monounsaturated fatty acids (MUFA), tocopherols and total phenolic acids (Pinela et al. 2016, 2018). An increase in total phenols, flavonoids and carotenoids was observed for fenugreek and spinach at doses above 0.75 kGy, which were responsible for increasing the antioxidant activity (Hussain et al. 2016). In spinach, after 1.5 kGy irradiation dose, total phenols, flavonoids and carotenoids increased 3.7%, 15.1% and 21.7%, respectively. Concerning the irradiated fenugreek samples, increases of 2.1%, 3.3% and 8.4% in the concentrations were observed for phenols, flavonoids and carotenoids, respectively. A decrease in the ascorbic acid content and increase on dehydroascorbic acid was also verified after irradiation of both vegetables. Fan (2005) reported that irradiation up to 2 kGy increased the phenolic content and antioxidant capacity of endive (*Cichorium endiva* L), Romaine and Iceberg lettuce (*Lactuca sativa* L) further stored at 7–8 °C for 8 days. After that time, and comparing with non-irradiated samples, the phenolic content increased by 40%, 60% and 25% for Romaine and Iceberg lettuce and endive leaf tissues, respectively. Concerning the antioxidant activity, the increases were 52%, 88% and 34% for Romaine and Iceberg lettuce and endive leaf tissues, respectively. On the other hand, irradiation of baby carrots induced a reduction in the phenolic content of 20% at 1 kGy gamma radiation dose (Hirashima et al. 2013).

Table 14.2 summarizes the documented effects of irradiation on bioactive compounds in fruits and vegetables.

Table 14.2 Effects of ionizing radiation on bioactive compounds in fruits and vegetables

Fruit/vegetable	Radiation source	Applied doses	Main results	References
Fragaria × *ananassa*	γ-radiation	1, 2 and 3 kGy	Preservation of phenolic content; increase of DDPH scavenging activity at 2 kGy; degradation of L-ascorbic acid	Barkaoui et al. (2020)
	e-beam	1, 2 and 3 kGy	Preservation of phenolic compounds; increase of DPPH scavenging activity at 2 kGy; higher reducing power at 1 and 3 kGy; degradation of L-ascorbic acid	
	γ-radiation	300, 600 and 900 Gy	Increase of phenolic and anthocyanin contents and antioxidant activity at 600 Gy and during storage; degradation of ascorbic acid	Maraei and Elsawy (2017)
Rubus idaeus L.	γ-radiation	0.5, 1.0 and 2.0 kGy	Decrease of ascorbic acid levels; increase of anthocyanin content at 0.5 kGy	Tezotto-Uliana et al. (2013)
		0.5, 1.0 and 1.5 kGy	Higher values of phenolics and antioxidant activity at 1.5 kGy	Cabo Verde et al. (2013)
		0.5, 1.0, and 2.0 kGy	Increase of phenolic content and antioxidant activity at 2 kGy after 12 days of cold storage; variable tendency of ascorbic acid depending on the absorbed dose	Guimarães et al. (2013)
	e-beam	3 kGy	Preservation of phenolic content and antioxidant activity; loss of ascorbic acid	Elias et al. (2020)
Vaccinium corymbosum, cvs. Collins, Bluecrop	e-beam	0.5, 1, 2 and 3 kGy	Preservation of anthocyanins after treatment and storage; no significant difference on antioxidant activity and ascorbic acid between non-treated and treated samples, although decreasing with storage	Kong et al. (2014)
Solanum lycopersicum var. cerasiforme	γ-radiation	1.3, 3.2 and 5.7 kGy	Slight increase of phenolic content at 3.2 kGy	Guerreiro et al. (2016)
	e-beam	1.5 and 3.1 kGy	Decrease of lycopene content at 1.5 kGy and preservation of DPPH scavenging activity	Madureira et al. (2019)
Lycopersicon esculentum Mill.	γ-radiation	0.5, 0.75, 1.0, 1.5, 2.0, 3.0 and 4.0 kGy	Preservation of anthocyanins content	Singh et al. (2016)

(continued)

Table 14.2 (continued)

Fruit/vegetable	Radiation source	Applied doses	Main results	References
		0.5, 1.0 and 1.5 kGy	Preservation of ascorbic acid up to 1.5 kGy, although degradation with storage; increase of lycopene at 0.5 kGy	Loro et al. (2018)
		2, 4 and 6 kGy	Radiolytic degradation of ferulic acid, *p*-coumaric acid, rutin and naringenin	Schindler et al. (2005)
Ziziphus jujuba var. vulgaris	γ-radiation	0.5, 1.0, 2.5 and 5.0 kGy	Significant increase in total monomeric anthocyanin (~12%) and total phenolic contents (~6%) up to 2.5 kGy; significant decrease in both parameters at 5 kGy; increase of vitamin C content at 2.5 kGy	Najafabadi et al. (2017)
	e-beam	1, 1.5, and 3.1 kGy	Preservation of total phenolic content, carotenoid content and antioxidant capacity even after 18 days of storage; increase in flavonols and phenolic acids; decrease of ascorbic acid content	Reyes and Cisneros-Zevallos (2007)
Mangifera indica L.	e-beam	1.0, 1.5 and 3.0 kGy	Significant increase of phenolic (55%) and carotenoids (91%) concentrations and antioxidant activity (6%) at 1 kGy	Moreno et al. (2007)
	γ-radiation	0.5, 0.75, 1.0 and 1.5 kGy	Increase of phenolic content with increasing absorbed doses and storage time; increase in carotenoid concentrations with storage, in particular, at 1.5 kGy; slight decrease in ascorbic acid concentration with radiation	El-Samahy et al. (2000)
Prunus persica Bausch, Cv. Elberta	γ-radiation	1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 kGy	Increase of phenolic and anthocyanin contents and PAL activity with irradiation dose and storage until 21 days; reduction of ascorbic acid content with doses higher than 1.6 kGy; improvement of DPPH scavenging activity and FRAP with radiation	Hussain et al. (2010)
Castanea sativa Mill.	γ-radiation	0.5, 1 and 3 kGy	Increase of phenolics (53%), DPPH scavenging activity (71%), β-carotene bleaching inhibition (61%) and TBARS inhibition (83%) at 3 kGy; decrease in flavonoids content (65%) at 1 kGy	Carocho et al. (2012)

(continued)

14.6.2 Beverages

Table 14.2 (continued)

Fruit/vegetable	Radiation source	Applied doses	Main results	References
	e-beam	0.5, 1 and 3 kGy	Increase of phenolics (126%), DPPH scavenging activity (37%), reducing power (60%), β-carotene bleaching inhibition (67%) and TBARS inhibition (84%) at 1 kGy; decrease of flavonoids content (87%) at 1 kGy	
	γ-radiation	1 kGy	Increase of tocopherols, namely γ-tocopherol; increase of β-carotene bleaching inhibition	Carocho et al. (2014)
	e-beam	1 kGy	Increase of α- and γ-tocopherols; increase of β-carotene bleaching inhibition	
Zizyphus mauritiana Lam.	γ-radiation	0.25, 0.5, 0.75 and 1 kGy	Increase of total flavonoid and decrease of total phenolic contents at doses up to 1 kGy; increase of DPPH scavenging activity and reduction of reducing power with increasing doses	Kavitha et al. (2015)
Dried *Phoenix dactylifera* L. fruits	γ-radiation	2.5, 5.0 and 10.0 kGy	Improvement of phenolic (>40%), flavonoid (>56%) contents, DPPH scavenging activity (>18%) and antibacterial potential at 5 kGy	El-Beltagi et al. (2019)
Dried *Prunus armeniaca* L. fruits	γ-radiation	1.0, 1.5, 2.0, 2.5 and 3.0 kGy	Linear increase of β-carotene with increasing absorbed doses	Hussain et al. (2011)
		3 kGy	Significant increase of total phenolics (12%), total flavonoids (16%) and β-carotene (37%); increase in DPPH scavenging activity (23%), FRAP (14%) and β-carotene bleaching inhibition (74%); enhancement of gallic acid (26%), ellagic acid (24%), quercetin (26%) and apigenin (37%) concentrations	Hussain et al. (2013)
Nasturtium officinale R. Br.	γ-radiation	1, 2 and 5 kGy	Preservation of antioxidant activity and total flavonoids at 5 kGy; increase of MUFA, tocopherols and total phenolics at 5 kGy	Pinela et al. (2016)
Trigonella foenum–graceum L.	γ-radiation	0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 kGy	Increase of total phenols (2.1%), flavonoids (3.3%) and carotenoids (8.4%) and antioxidant potential at 1.5 kGy; loss of ascorbic acid and increase in dehydroascorbic acid after irradiation	Hussain et al. (2016)

(continued)

Table 14.2 (continued)

Fruit/vegetable	Radiation source	Applied doses	Main results	References
Spinacia oleracea L.			Increase of total phenols (3.7%), flavonoids (15.1%) and carotenoids (21.7%) and antioxidant activity after 1.5 kGy; reduction of ascorbic acid content and increase in dehydroascorbic acid after irradiation	
Cichorium endiva L.	γ-radiation	0.5, 1 and 2 kGy	Increase of phenolic content (25%) and antioxidant capacity (34%) at 2 kGy, after 8 days of storage	Fan (2005)
Romaine *Lactuca sativa* L.			Increase of phenolic content (40%) and antioxidant capacity (52%) at 2 kGy, after 8 days of storage	
Iceberg *Lactuca sativa* L.			Increase of phenolic content (60%) and antioxidant capacity (88%) at 2 kGy, after 8 days of storage	
Baby carrots	γ-radiation	0.5 and 1 kGy	20% reduction of phenolic content at 1 kGy	Hirashima et al. (2013)

Notes: PAL (Phenylalanine ammonia-lyase); DPPH (2,2-Diphenyl-1-picrylhydrazyl); FRAP (Ferric reducing power); TBARS (Thiobarbituric acid reactive substances); MUFA (Monounsaturated fatty acids)

Juices from vegetables (Song et al. 2006; Lee et al. 2009) and fruits (Girennavar et al. 2008; McDonald et al. 2013; Eissa et al. 2014; Shahbaz et al. 2014; Arjeh et al. 2015; Naresh et al. 2015) showed interesting results when subjected to radiation. In carrot (*Daucus carota* var. *sativa*) juices, after storage of 3 days, an increase in the phenolic content (1912.5 μg/mL of sample) and FRAP value (1349.7 mM FRAP/mL of sample) were observed in irradiated samples when compared to the non-irradiated and non-stored sample (1732.9 μg/mL of sample and 931.3 mM FRAP/mL, respectively, for phenolic and FRAP values) (Song et al. 2006). On the contrary, the levels of total phenolics (9121.7 μg/mL of sample) and antioxidant potential value (5776.2 mM FRAP/mL of sample) in irradiated kale (*Brassica oleracea* var. *acephala*) juice were significantly lower than the control (9635.5 μg/mL and 6251.8 mM FRAP/mL, respectively) (Song et al. 2006), and flavonoid content did not change (Jo et al. 2012). In irradiated tamarind (*Tamarindus indica* L.) juice, higher phenolic content and antioxidant activity were found at 5 kGy (Lee et al. 2009).

Low doses (up to 600 Gy) of gamma radiation caused no effect on ascorbic acid, phenolic content and antioxidant activity of orange juice (*Citrus sinensis* L. Osbeck) (McDonald et al. 2013). In watermelon (*Citrullus lanatus* cv.) and mango (*Mangifera indica* L.) juices (fresh and stored) irradiated at 3 and 5 kGy, both total phenolics and flavonoids contents were higher (15-48%) than in non-irradiated samples, although vitamin C and lycopene contents were lower (Eissa et al. 2014; Naresh et al. 2015). An enhancement in the antioxidant activity in both irradiated juices was also observed with increasing irradiation dose. Furthermore, gamma radiation

induced increases in the levels of individual phenolic acids present in mango juice, as gallic acid, chlorogenic acid and syringic acid by 3.2, 2.3 and 2.5 fold, respectively (Naresh et al. 2015). Shahbaz et al. (2014) observed a decrease in DPPH and ABTS scavenging activities probably associated with a reduction on anthocyanins and total phenolic contents of irradiated (1–2 kGy) pomegranate (*Punica granatum* L.) juice. The same observations were made by Arjeh et al. (2015) for sour cherry (*Prunus cerasus* L.) juice, with reductions in monomeric anthocyanins of more than 60% at 3 kGy after 60 days. In a study performed on grapefruit (*Citrus paradisi* Macf.) irradiated at doses ranging from 1 to 10 kGy and further juiced, electron-beam radiation was found to significantly reduce the vitamin C and lycopene contents, as well as those of nomilin (a terpenoid limonoid) and dihydroxybergamottin (a furanomcoumarin); on the other hand, naringin level was significantly higher in irradiated juice in comparison with the control sample (Girennavar et al. 2008).

Teas are the most widely consumed beverages in the world. In black tea, made from leaves of *Camellia sinensis* (L.) O. Kuntze, gamma radiation up to 2 kGy increased the total phenolic content, with lower water activity having positive influence on the obtained results (Fanaro et al. 2014). Gerolis et al. (2017) studied the effect of gamma radiation on total antioxidant capacity of green tea (*Camellia sinensis* (L.) O. Kuntze), yerba mate (*Ilex paraguariensis* A. St. Hil.) and chamomile tea (*Matricaria recutita* L.), reporting that this technology can reduce the capacity of some antioxidants and improve the capacity of others, which depends on the compounds present, solvents used and also the method of assessment. In another study developed by Janiak et al. (2017), different Bulgarian teas were studied: Mursalski tea (*Sideritis scardica* Gris), Mashterka tea (*Thymus serpyllum* L.), Good Night tea (tea mix), Staroplaninski tea (Balkan tea mix), Trakia tea (tea mix), and Mountain tea (Planinski tea mix). The authors observed improvement in tannin and phenolic contents and antioxidant capacity in some teas but not in others. Tables 14.3 and 14.4 outline the reported effects of ionizing radiation on the bioactivity of beverages.

14.6.3 Meat

Meat is widely consumed as a main source of proteins, vitamins, minerals and fatty acids. Losses of α-tocopherol and thiamine and riboflavin were observed in irradiated beef, lamb, pork and turkey with doses up to 9 kGy (Fox et al. 1995; Lakritz et al. 1995). On the other hand, significant higher levels of riboflavin and niacin were detected in pork chops and chicken breasts irradiated at the dose range 2–4 kGy (Fox et al. 1989). Fatty acids present in beef and ground beef were also significantly increased at doses up to 7 kGy (Yilmaz and Geçgel 2007; Haque et al. 2017). On Table 14.5 the reported effects of irradiation on the bioactives content of meat are collected.

Table 14.3 Effects of ionizing radiation on the bioactivity of beverages

Fruit/ vegetable beverage	Radiation source	Applied doses	Main results	References
Daucus carota var. *sativa* juice	γ-radiation	3 and 5 kGy	Significant increase of phenolic content and FRAP value in irradiated samples, even after 3 days of storage	Song et al. (2006)
Brassica oleracea var. *acephala* juice			Lower phenolic content and FRAP value in irradiated samples	
		1, 3, and 5 kGy	Slight increase of phenolic content with irradiation; no variation of flavonoid content; reduction of ascorbic acid content with irradiation	Jo et al. (2012)
Angelica keiskei Ito juice	γ-radiation	1, 3, and 5 kGy	Slight increase of phenolic content with irradiation; no variation of flavonoid content; reduction of ascorbic acid content with irradiation	Jo et al. (2012)
Tamarindus indica L. juice	γ-radiation	1, 3, and 5 kGy	Higher total phenolic content and antioxidant activity by DPPH and FRAP at 3 and 5 kGy	Lee et al. (2009)
Citrus sinensis L. Osbeck juice	γ-radiation	200, 400 and 600 Gy	No effect on ascorbic acid, phenolic content and antioxidant activity.	McDonald et al. (2013)
Citrullus lanatus cv. juice	γ-radiation	1, 3 and 5 kGy	Increase of phenolics and flavonoids contents with irradiation; reduction of vitamin C at 3 and 5 kGy; lower lycopene content at 1 kGy; enhancement of DPPH scavenging activity	Eissa et al. (2014)
Mangifera indica L. juice	γ-radiation	1, 3 and 5 kGy	Increase of total phenolic content with increasing doses; increase of total flavonoids at 3 and 5 kGy; decrease of ascorbic acid concentration with increasing doses; increase of DPPH scavenging activity with doses; enhancement in the concentrations of individual phenolic at 3 and 5 kGy: gallic acid, protocatechuic acid, syringic acid, chlorogenic acid, and rutin, and ellagic acid and *p*-coumaric acid at 5 kGy	Naresh et al. (2015)
Punica granatum L. juice	γ-radiation	0.4, 1, and 2 kGy	Decrease in total phenolic compounds, DPPH and ABTS scavenging abilities at 1 and 2 kGy	Shahbaz et al. (2014)

(continued)

Table 14.3 (continued)

Fruit/ vegetable beverage	Radiation source	Applied doses	Main results	References
Prunus cerasus L. juice	γ-radiation	0.5, 1.5, 3.0, 4.5, and 6.0 kGy	No significant difference in total phenolic content with irradiation; reduction of monomeric anthocyanins (>60%) at 3 kGy after 60 days; decrease of DPPH scavenging activity at doses >3 kGy (21%) and storage (29%); decrease in FRAP values by 20% after irradiation and 27% after storage	Arjeh et al. (2015)
Citrus paradisi Macf. juice	e-beam	1.0, 2.5, 5.0, and 10.0 kGy	Significant reduction of the vitamin C and lycopene contents at doses >2.5 kGy; preservation of β-carotene; decrease of nomilin and dihydroxybergamottin contents; increase of naringin at doses >2.5 kGy	Girennavar et al. (2008)

Notes: PAL (Phenylalanine ammonia-lyase); DPPH (2,2-Diphenyl-1-picrylhydrazyl); FRAP (Ferric reducing power); TBARS (Thiobarbituric acid reactive substances); MUFA (Monounsaturated fatty acids); ABTS (2,2′-Azinobis-(3-ethylbenzthiazolin-6-sulfonic acid)

14.6.4 Aromatic and Medicinal Plants

The effect of ionizing radiation on the phytochemicals can vary according to the plant material and the applied dose. Pereira and co-workers showed that gamma radiation at 10 kGy increased significantly (>1-fold) the content of polyphenols in ethanolic extracts and infusions of *Mentha* × *piperita* L. (peppermint), *Aloysia citrodora* Paláu (lemon verbena) and *Thymus vulgaris* L. (thyme) in comparison with the control sample (0 kGy) (Pereira et al. 2017, 2018).

In other studies, the same authors observed that an absorbed dose of 10 kGy of gamma radiation could improve the extractability of phenolic compounds (3.5–5.5 fold) and their antioxidant properties in both infusions and methanol/water extracts of *Ginkgo biloba* L. (Pereira et al. 2015a, b). An increase of 35% in total phenolic content was reported in water extracts of *Rosmarinus officinalis* L. irradiated at 30 kGy, but not in methanol or ethanol extracts (Pérez et al. 2007). The authors hypothesized that this increase could be associated with the presence of phenolic diterpenes in rosemary that result in water-soluble quinone-type compounds by gamma radiation. A previous study developed by Horváthová et al. (2007) revealed a slight increase in the total phenolic content of *Origanum vulgare* L. extracts prepared from samples irradiated at 10 kGy. Gamma irradiation of *Petroselinum crispum* (Mill.) Fuss var. neapolitanum induced a reduction in vitamin C content at 2.7 kGy, while increased total polyphenols in doses up to 2.0 kGy (Cătunescu et al. 2017). By contrast, *Salvia officinalis* L. gamma irradiated at 2 and 4 kGy demonstrated lower antioxidant capacity associated to a lower polyphenolic content (30 and 45%, respectively) (Salem et al. 2013).

Table 14.4 Effects of ionizing radiation on teas

Tea	Radiation source	Applied doses	Main results	References
Camellia sinensis L. O. Kuntze	γ-radiation	1.0, 1.5, 2.0, 2.5, 5.0, 7.5 and 10.0 kGy	Increase of total phenolic compounds at 2 kGy (for 0.183 and 0.651 water activity) or at 5 kGy (for 0.924 water activity); preservation of antioxidant activity; increase of caffeine concentration at 10 kGy	Fanaro et al. (2014)
		20 kGy	Decrease of phenolics level; decrease of flavonoids contents in methanolic extracts; no significant difference in ABTS activity between irradiated and non-irradiated samples	Gerolis et al. (2017)
Ilex paraguariensis A. St. Hil.	γ-radiation	20 kGy	Decrease of phenolics content; preservation of flavonoids content; no significant difference in ABTS activity; decrease of DPHH scavenging activity	
Matricaria recutita L.	γ-radiation	20 kGy	Preservation of phenolics content; decrease of flavonoids content in methanolic extracts; increase of β-carotene bleaching inhibition of water infusions with irradiation; decrease of DPPH scavenging activity	
Mursalski tea (*Sideritis scardica* Gris)	γ-radiation	5 kGy	Improvement of FRAP values and ABTS scavenging activity with irradiation	Janiak et al. (2017)
Mashterka tea (*Thymus serpyllum* L.)	γ-radiation		Increase of DPPH scavenging activity in irradiated samples	
Good Night tea	γ-radiation		Preservation of phenolics content and antioxidant activity	
Staroplaninski tea	γ-radiation		Increase of DPPH scavenging activity with irradiation; improvement of FRAP values and ABTS scavenging activity with irradiation	
Trakia tea	γ-radiation		Increase of total phenolics and tannins contents; improvement of FRAP values and ABTS scavenging activity with irradiation	
Mountain tea	γ-radiation		Increase of tannins content; improvement of FRAP values and ABTS scavenging activity with irradiation	

Notes: DPPH (2,2-Diphenyl-1-picrylhydrazyl); FRAP (Ferric reducing power); ABTS (2,2′-Azinobis-(3-Ethylbenzthiazolin-6-sulfonic acid)

Table 14.5 Effects of ionizing radiation on bioactive compounds of meat

Meat	Radiation source	Applied doses	Main results	References
Beef	γ-radiation	2, 4 and 6 kGy	Increase of free fatty acids with irradiation	Haque et al. (2017)
		0.24, 0.47, 0.94, 1.88, 2.81, 5.62 and 9.37 kGy	Loss of α-tocopherol	Lakritz et al. (1995)
			Reduction of thiamine and riboflavin levels with irradiation	Fox et al. (1995)
Ground beef	γ-radiation	1, 3, 5 and 7 kGy	Significant increase of *trans* fatty acids with irradiation	Yilmaz and Geçgel (2007)
Turkey	γ-radiation	0.24, 0.47, 0.94, 1.88, 2.81, 5.62 and 9.37 kGy	Loss of α-tocopherol	Lakritz et al. (1995)
			Reduction of thiamine and riboflavin levels with irradiation	Fox et al. (1995)
Pork	γ-radiation	0.24, 0.47, 0.94, 1.88, 2.81, 5.62 and 9.37 kGy	Loss of α-tocopherol	Lakritz et al. (1995)
			Reduction of thiamine and riboflavin levels with irradiation	Fox et al. (1995)
		0.5, 1.75, 3.50, 5.25 and 7.0 kGy	Loss of thiamine with increasing doses; increase of riboflavin and niacin levels at 4 kGy	Fox et al. (1989)
Lamb	γ-radiation	0.24, 0.47, 0.94, 1.88, 2.81, 5.62 and 9.37 kGy	Loss of α-tocopherol	Lakritz et al. (1995)
Chicken	γ-radiation	0.5, 1.75, 3.50, 5.25 and 7.0 kGy	Loss of thiamine with increasing doses; increase of riboflavin and niacin levels at 4 kGy	Fox et al. (1989)
		0.24, 0.47, 0.94, 1.88, 2.81, 5.62 and 9.37 kGy	Reduction of thiamine and riboflavin levels with irradiation	Fox et al. (1995)

Concerning medicinal plants, Pereira et al. (2014) observed higher contents of phenolics (107.45 mg GAE/g) and flavonoids (and 33.77 mg CE/g) in the methanolic extracts of medicinal plant borututu (*Cochlospermum angolensis* Welw.) irradiated at 10 kGy, which was correlated with an increase in the antioxidant activity. Similarly, the scavenging activity of *Amoora rohitaka* ethanolic extracts improved by 112%, while the phenolic content and reducing power of methanolic extracts was enhanced by more than 30% at 5 kGy (Rajurkar and Gaikwad 2012). A summary of the results regarding bioactive compounds in irradiated aromatic and medicinal plants is collected in Table 14.6.

14.6.5 Legumes, Cereals and Grains

Legumes are an important group of plant foodstuffs, especially in developing countries, being recognized as a cheap source of protein. Zhu et al. (2010) evaluated the effect of gamma radiation (2–10 kGy) on the phenolic compounds of three different genotypes (black, red and white) of *Oryza sativa* L. grains. The authors reported an increase of phenolic acids content in black rice extracts irradiated at 8 kGy (423.3 mg/kg) compared with the control (381.6 mg/kg), although lower doses promoted a decrease on these acids. On the other hand, irradiation at 6 kGy caused a significant increase (378.3 mg/kg) in anthocyanins content, in comparison with the control (346.6 mg/kg). Similarly, distinct effects of irradiation were observed in different genotypes of rice grains depending on the analyzed variable and absorbed doses (Shao et al. 2013).

The extraction of phenolic compounds from *Nigella sativa* L. seeds using different solvents was evaluated by Khattak et al. (2008). The authors reported that a radiation dose up to 16 kGy improved total extraction yields (3.7%, 4.2%, 9.0% and 5.6% for hexane, acetone, methanol and water, respectively), phenolic contents (2.7% in acetone extracts) and DPPH scavenging activity (10.6% and 5.4% in acetone and methanol extracts, respectively). In a study conducted by Bhat et al. (2007), the levels of total phenolics of *Mucuna pruriens* L. seeds were found to be dose-dependent, significantly increasing at doses higher than 2.5 kGy (73.4 g/kg and 116 g/kg for control and 30 kGy, respectively) while doses higher than 7.5 kGy resulted in a tannin concentration increase (2.62 g/kg for control and 5.81 g/kg for 30 kGy). Also, Siddhuraju et al. (2002) found that irradiation (2–6 kGy) after aqueous soaking increased (>1-fold) the phenolics levels in seeds of three different species of *Sesbania* (*Sesbania aculeata*, *S. rostrata* and *S. cannabina*) and one species of *Vigna* (*Vigna radiata*), attributing this increase to a higher extractability by depolymerization and dissolution of cell wall polysaccharides by irradiation.

Concerning the irradiation of soybeans (*Glycine max* (L.) Merr.), an absorbed dose of 1 kGy was enough to increase total phenolics (10%) and tannins (21.6%), which was correlated with an increase in the antioxidant activity (Štajner et al. 2007). Also, the total isoflavone content (genistein, daidzein, genistin and daidzin) increased at an applied dose of 10 kGy (666.1 mg/kg for control sample and 755.2 mg/kg for 10 kGy sample) (Popović et al. 2013). Variyar et al. (2004) observed an increase higher than onefold in aglycon content with the increase in absorbed doses (0.5–5 kGy) and consequent increase in DPPH scavenging activity, associated with the higher levels of isoflavones in treated samples. Gamma radiation at 1 kGy applied in red kidney beans (*Phaseolus vulgaris*), slightly improved phenolic content and antioxidant activity by DPPH free radical scavenging assay and inhibition in lipid peroxidation, keeping constant even after 6 months of storage (Marathe et al. 2016). Table 14.7 summarizes the effects of irradiation on the bioactive compounds in legumes, cereals and other grains.

Table 14.6 Effects of ionizing radiation on bioactive compounds of aromatic and medicinal plants

Aromatic and medicinal plant	Radiation source	Applied doses	Main results	References
Aloysia citrodora L.	γ-radiation	1, 5 and 10 kGy	Increase of verbascoside at 1 kGy	Pereira et al. (2018)
Mentha × *piperita* L.			Increase of eriodictyol-7-*O*-rutinoside, luteolin-7-*O*- rutinoside and rosmarinic acid concentrations at 5 kGy	
Thymus vulgaris L.			Increase of luteolin-7-*O*-glucuronide, luteolin-*O*-glucuronide and eriodictyol-*O*-glucuronide at 5 kGy; increase of apigenin-6,8-*C*-dihexoside and eriodictyol-7-*O*-glucuronide at 10 kGy	
Ginkgo biloba L.	γ-radiation	1 and 10 kGy	Improvement of the extractability of phenolic acids and flavonoids at 10 kGy in infusions and methanol/water extracts.	Pereira et al. (2015b)
			Increase of α-tocopherol levels at 1 kGy; increase of antioxidant activity with irradiation	Pereira et al. (2015a)
Rosmarinus officinalis L.	γ-radiation	30 kGy	Increase of total phenolic content by 35% in water extracts after treatment; increase of antioxidant activity (DPPH scavenging and reducing power) in ethanol and water extracts	Pérez et al. (2007)
Origanum vulgare L.	γ-radiation	5, 10 and 30 kGy	Significant increase of total phenolic content at 10 kGy; preservation of DPPH scavenging activity	Horváthová et al. (2007)
Salvia officinalis L.	γ-radiation	2 and 4 kGy	Decrease of total phenolics (>30%) and antioxidant activity (>11%) with irradiation	Salem et al. (2013)
Petroselinum crispum (Mill.) Fuss Var. Neapolitanum	γ-radiation	0.7, 1.4, 2.0 and 2.7 kGy	Decrease of ascorbic acid with irradiation; increase of total polyphenols at 0.7 and 2 kGy; decrease in radical scavenging activity at 2.7 kGy	Cătunescu et al. (2017)

(continued)

Table 14.6 (continued)

Aromatic and medicinal plant	Radiation source	Applied doses	Main results	References
Cochlospermum angolensis Welw.	γ-radiation	1 and 10 kGy	Increase of tocopherols at 1 kGy; increase of phenolics and flavonoids at 10 kGy; higher antioxidant activity (DPPH scavenging activity, reducing power, β-carotene bleaching inhibition and TBARS inhibition) in infusions irradiated at 10 kGy; higher antioxidant activity (DPPH scavenging activity, reducing power and TBARS inhibition) in infusions irradiated at 10 kGy	Pereira et al. (2014)
Amoora rohitaka (Rxb.) Wight & Arn	γ-radiation	1, 3 and 5 kGy	Increase of total phenolics with irradiation; increase of ABTS scavenging activity; increase of FRAP values in methanol extracts up to 5 kGy; increase of DPPH scavenging activity in aqueous and ethanol extracts	Rajurkar and Gaikwad (2012)

Notes: DPPH (2,2-Diphenyl-1-picrylhydrazyl); ABTS (2,2′-Azinobis-(3-Ethylbenzthiazolin-6-sulfonic acid)

14.6.6 Spices

Even in small quantities, spices are a potential source of natural contamination by mesophylic, sporogenic, and asporogenic bacteria, hyphomycetes, and faecal coliforms into foodstuffs where they are added (Sádecká 2007). Hence, ionizing radiation treatment is mostly used in order to eliminate the microbial contamination. Furthermore, some studies were also performed reporting the impact of this technology on the antioxidant properties of different spices.

Variyar et al. (1998) studied the effect of gamma radiation (10 kGy) in five commercially important spices: cinnamon (*Cinnamomum verum* J. Presl.), clove (*Zyzygium aromaticum*), cardamom (*Elettaria cardamomum* (L.) Maton), nutmeg and mace (*Myristica fragrans* Houtt.). In clove, the concentrations of gallic acid and syringic acid increased considerably (384.9 ± 11.6 and 33.0 ± 4.7 mg/kg dry weight, respectively) in the 10 kGy sample in comparison with the control sample (174.7 ± 7.29 and 7.9 ± 1.3 mg/kg dry weight, respectively). In irradiated nutmeg, the concentrations of some observed phenolic acids, such as syringic acid, caffeic acid + vanillic acid, gentisic acid + *p*-hydroxybenzoic acid, also increased at 10 kGy irradiation dose (five-fold, three-fold and six-fold, respectively). The increased level of these phenolic acids in clove and nutmeg could be justified by the degradation of hydrolysable tannins and consequent higher extractability of phenolic acids.

The antioxidant properties of irradiated (5–30 kGy) black pepper (*Piper nigrum* L.), clove (*Syzygium aromaticum* (L.) Merr. & L.M.Perry) and ginger (*Zingiber*

Table 14.7 Effects of ionizing radiation on bioactive compounds of legumes, cereals and grains

Legume, cereal and grain	Radiation source	Applied doses	Main results	References
Black rice	γ-radiation	2, 4, 6, 8, and 10 kGy	Increase of phenolic acids content at 8 kGy; highest level of anthocyanins at 6 kGy	Zhu et al. (2010)
		2, 4, 6, 8, and 10 kGy	Increase of antioxidant activity with doses 2–8 kGy	Shao et al. (2013)
White rice	γ-radiation	2, 4, 6, 8, and 10 kGy	Decrease of phenolic acids content with irradiation	Zhu et al. (2010)
		2, 4, 6, 8, and 10 kGy	Increase of total phenolics at 10 kGy; significant increase of antioxidant activity at 10 kGy	Shao et al. (2013)
Red rice	γ-radiation	2, 4, 6, 8, and 10 kGy	Decrease of phenolic acids content with irradiation	Zhu et al. (2010)
		2, 4, 6, 8, and 10 kGy	Enhancement of total phenolics at >6 kGy; Significant increase of antioxidant activity at 8 kGy	Shao et al. (2013)
Nigella sativa L.	γ-radiation	2, 4, 8, 10, 12 and 16 kGy	Improvement in the extraction yields (3.7%, 4.2%, 9.0% and 5.6% for hexane, acetone, methanol and water, respectively) at doses up to 16 kGy; increase by 2.7% of phenolic contents at 16 kGy in acetone extracts; enhancement of DPPH scavenging activity by 10.6% in acetone and 5.4% in methanol extracts	Khattak et al. (2008)
Mucuna pruriens L.	γ-radiation	2.5, 5.0, 7.5, 10, 15 and 30 kGy	2-Fold increase of total phenolics for doses >2.5 kGy and tannins concentrations at doses >7.5 kGy	Bhat et al. (2007)
Sesbania aculeate (Willd.) Pers.	γ-radiation	2, 4 and 6 kGy	Increase of total phenolics at 2 kGy; preservation of tannins with irradiation	Siddhuraju et al. (2002)
Sesbania rostrate Bremek. & Oberm			Increase of total phenolics at 2 kGy; preservation of tannins with irradiation	
Sesbania cannabina (Retz.) Pers.			Preservation of total phenolics tannins with irradiation	
Vigna radiate (L.) R.Wilczek			Increase of total phenolics and tannins at 2 kGy; increase of condensed tannins with irradiation	

(continued)

Table 14.7 (continued)

Legume, cereal and grain	Radiation source	Applied doses	Main results	References
Glycine max (L.) Merr.	γ-radiation	1, 2, 4, 6, 8 and 10 kGy	Highest values of phenolic compounds and tannins at 1 kGy; increase (17%) of FRAP value at 1 kGy; increase of DPPH scavenging activity with increasing irradiation doses	Štajner et al. (2007)
		1, 2, 4 and 10 kGy	Significant increase of total isoflavone content at 10 kGy; significant increase of genistein, daidzein, genistin and daidzin concentrations at 4 kGy; significant increase of total phenolic and tannin contents with irradiation; increase of DPPH scavenging activity with irradiation	Popović et al. (2013)
		0.5, 1, and 5 kGy	Decrease of total isoflavones with irradiation; increase (>1-fold) of isoflavone aglycones content with the increasing of absorbed doses; significant increase in DPPH scavenging activity with irradiation	Variyar et al. (2004)
Phaseolus vulgaris L	γ-radiation	0.25, 1.0, 5.0 and 10.0 kGy	Increase of fatty acids at 10 kGy; increase of free phenolic compounds and antioxidant activity at 1 kGy	Marathe et al. (2016)

Notes: DPPH (2,2-Diphenyl-1-picrylhydrazyl); FRAP (Ferric reducing power)

officinale Roscoe) were also evaluated (Suhaj et al. 2006; Suhaj and Horváthová 2007). The results demonstrated that DPPH scavenging activity of black pepper extracts initially decreased with increasing doses, however, after 2 months of storage, a significant increase of those values occurred due to the increase of dry matter content during the storage. On the other hand, a decrease in the reducing power of these samples was observed with increasing dose and during storage (Suhaj et al. 2006). Suhaj and Horváthová (2007) did not detect variations in phenolic compounds, antiradical activity and reducing power of irradiated clove extracts, even after 5 months of storage, except for phenolic compounds, which were observed to decrease. Contrarily, in ginger, all the parameters (phenolics, antiradical activity and reducing power) kept constant immediately after irradiation but significantly increased by 10% with storage, in particular in those irradiated at 10 and 20 kGy. In Table 14.8 the main effects of irradiation on the bioactive compounds of spices are outlined.

14.6.7 Edible Flowers

Edible flowers have been used in many culinary preparations to improve the sensorial and nutritional qualities of food, by adding color, flavor, taste and visual appeal to salads, sauces, garnish, entrees, drinks or desserts. In this way, it is important to improve the conservation and safety of these products.

Viola tricolor L. (heartseases) and *Tropaeolum majus* L. (garden nasturtium) flowers irradiated by gamma and electron beam radiation at 1 kGy demonstrated higher capacity to scavenge DPPH and to inhibit β-carotene bleaching than not irradiated samples, attributed to an increase in the levels of phenolic compounds, despite the content of anthocyanins decreased (Koike et al. 2015a, b). In addition, the authors reported no significant differences in the observed effects between gamma and electron-beam irradiation, concluding that both technologies can be used to preserve the edible flowers quality (Table 14.9).

Table 14.8 Effects of ionizing radiation on bioactive compounds of spices

Spice	Radiation source	Applied doses	Main results	References
Cinnamomum verum J.Presl	γ-radiation	10 kGy	Increase of protocatechuic acid concentration	Variyar et al. (1998)
Zyzygium aromaticum			Significant increase of gallic acid and syringic acid concentrations; decrease of *p*-coumaric acid and ferulic acid + sinapic acid concentrations	
Elettaria cardamomum (L.) Maton			Preservation of phenolic acids contents	
Myristica fragrans Houtt.			Preservation of phenolic acids concentrations	
Nutmeg			Significant increase of gentisic acid + *p*-hydroxybenzoic acid, caffeic acid + vanillic acid and syringic acid concentrations	
Piper nigrum L.	γ-radiation	5, 7.5, 10, 20, and 30 kGy	Significant decrease of DPPH scavenging activity; decrease of reducing power with irradiation	Suhaj et al. (2006)
Syzygium aromaticum (L.) Merr. & L.M.Perry	γ-radiation	5, 10, 20 and 30 kGy	Preservation of DPPH scavenging activity, reducing power and total phenols	Suhaj and Horváthová (2007)
Zingiber officinale Roscoe			Preservation of DPPH scavenging activity and reducing power with irradiation; increase of DPPH scavenging activity during storage at 10 and 20 kGy	

Notes: DPPH (2,2-Diphenyl-1-picrylhydrazyl)

Table 14.9 Effects of ionizing radiation on the bioactive content of edible flowers

Edible flower	Radiation source	Doses applied	Main results	References
Viola tricolor L.	γ-radiation	0.5, 0.8 and 1 kGy	Higher amounts of phenolic compounds at 1 kGy; increase of DPPH scavenging activity and β-carotene bleaching inhibition at 1 kGy	Koike et al. (2015a)
	e-beam	0.5, 0.8 and 1 kGy	Higher amounts of phenolic compounds at 1 kGy; highest DPPH scavenging activity and β-carotene bleaching inhibition at 1 kGy	
Tropaeolum majus L.	γ-radiation	0.5, 0.8 and 1 kGy	Increase of non-anthocyanin phenolics and decrease of anthocyanin concentrations with irradiation; decrease of DPPH scavenging activity with irradiation; increase of β-carotene bleaching inhibition at 1 kGy	Koike et al. (2015b)
	e-beam	0.5, 0.8 and 1 kGy	Increase of non-anthocyanin phenolics and decrease of anthocyanin concentrations with irradiation; decrease of DPPH scavenging activity with irradiation; enhancement of β-carotene bleaching inhibition at 1 kGy	

Notes: DPPH (2,2-Diphenyl-1-picrylhydrazyl)

14.6.8 Mushrooms

Mushrooms are one of the most perishable products and their short shelf-life is an obstacle for the distribution of the fresh product (Fernandes et al. 2013a). In fact, some studies highlighted the use of gamma irradiation as a conservation process since the results showed that this technology is effective in maintaining the chemical characteristics of *Lactarius deliciosus* L., *Boletus edulis* Bull.: Fr and *Hydnum repandum* L.: Fr (Fernandes et al. 2013a, b). Several studies were performed in order to evaluate the effect of gamma and electron-beam irradiation in the antioxidant properties of different wild and cultivated mushrooms species. Fernandes et al. (2013c) demonstrated that gamma radiation at 0.6 kGy was the most efficient method to preserve the chemical composition of fresh *Macrolepiota procera* (Scop.) Singer when compared with other processing treatments such as freezing (at −20 °C) and drying (at 30 °C). Fernandes et al. (2014) studied the effects of electron-beam irradiation on different dried wild mushrooms (*B. edulis* Bull. and *Russula delica* Fr.) on nutritional, chemical and antioxidant parameters. The antioxidant activity was improved significantly at 6 kGy for both mushroom species, attributed to the increased levels of tocopherols. The same effect was observed in the antioxidant activity for *Amanita caesarea* and *A. curtipes* irradiated at 2, 6 and 10 kGy, which was correlated with the increase in the levels of phenolic compounds (Fernandes et al. 2015). Studies in fresh samples of *Agaricus bisporus* Portobello using gamma and electron beam radiation were performed to evaluate the

effectiveness of these technologies on the conservation of this mushroom (Cardoso et al. 2019). In that work, gamma radiation treatment was associated with the increase of ergosterol, monounsaturated fatty acids and β-tocopherol, while e-beam led to higher values of polyunsaturated fatty acids.

Regarding the irradiation of truffles, total phenolics content significantly increased in *Tuber aestivum* after gamma irradiation with 1.0–2.5 kGy (Adamo et al. 2004; Tejedor-Calvo et al. 2020), although ergosterol and total sterols concentrations did not significantly change (Tejedor-Calvo et al. 2020). However, in irradiated *T. melanosporum* no significant changes were noticed in sterols even after storage of 35 days. Moreover, electron beam irradiation at 2.5 kGy enhanced the concentration of phenolic compounds up to 21 days of storage by almost three-fold (Tejedor-Calvo et al. 2019). Table 14.10 gathers the effects of irradiation treatments on the bioactive compounds of mushrooms.

14.7 Valorization of Food Bioactives: A Forthcoming Application of Food Irradiation

Although bioactive compounds are naturally present in many foods, after suitable isolation and purification, they can be used as ingredients on the development of functional foods, on cosmetics and/or on health care products.

Irradiation treatment can improve the food bioactivity, as it was highlighted in the previous sections. This potentiality could be applied to valorize other products through the improved extraction of bioactive compounds from irradiated materials and further incorporation in food, cosmetic or pharmaceutical products. To the best of our knowledge there is no documented application regarding the use of bioactives from irradiated sources in other products, but there are several encouraging outputs on the incorporation of bioactive compounds in food that will be detailed bellow.

The fortification of food through the incorporation of bioactive compounds in order to improve their quality or biological properties has been explored, especially in yogurts, meat, sausages or bread (Dall'Asta et al. 2013; Amirdivani and Baba 2014; Ribas-Agustí et al. 2014; Guiné et al. 2016; Turgut et al. 2017). Green tea (*Camellia sinensis*) infusions used during milk fermentation to produce yogurt were reported to promote the growth of beneficial yogurt bacteria and enhance the antioxidant activity of yogurt (Amirdivani and Baba 2014). Guiné et al. (2016) obtained yogurts enriched with antioxidants extracted from wine, observing an increase in antioxidant activity without affecting acidity. Chestnut flour was utilized in the formulation of functional bread (Dall'Asta et al. 2013), with a ratio of 50/50 (soft wheat/chestnut flour) leading to the highest value of antioxidant capacity in the final product. Moreover, bread produced with wheat flour fortified at 1, 2, and 3% (w/w) with dried leafy vegetable presented higher concentrations of polyphenols and higher values of antioxidant activity than the controls (Alashi et al. 2019). Other study investigated the incorporation of pomegranate peel extract at 0.5% and 1.0%

Table 14.10 Effects of ionizing radiation on mushrooms bioactive compounds

Mushroom	Radiation source	Applied doses	Main results	References
Lactarius deliciosus L.	γ-radiation	0.5 and 1.0 kGy	Reduction of total tocopherols; increase of phenolics at 0.5 kGy; increase of antioxidant activity at 0.5 kGy	Fernandes et al. (2013a)
Boletus edulis Bull.:Fr.	γ-radiation	1 and 2 kGy	Increase of δ- and γ-tocopherols at 1 kGy; increase of MUFA and decrease of PUFA at 1 kGy; preservation of total phenolics at 2 kGy; increase of TBARS formation inhibition; decrease of DPPH scavenging activity	Fernandes et al. (2013b)
Hydnum repandum L.:Fr.			Increase of δ-tocopherol at 1 kGy; increase of MUFA and decrease of PUFA at 1 kGy; higher total phenolic content at 1 kGy; increase of lipid peroxidation inhibition; decrease of DPPH scavenging activity	
Macrolepiota procera (Scop.) Singer	γ-radiation	0.6 kGy	Increase of MUFA content; enhancement of α- and γ-tocopherol contents; decrease of phenolics; increase of β-carotene bleaching inhibition and TBARS inhibition	Fernandes et al. (2013c)
Boletus edulis Bull.	e-beam	2, 6 and 10 kGy	Higher concentration of *p*-coumaric acid at 2 kGy; higher concentration of cinnamic acid at 10 kGy; increase of total tocopherols with irradiation; decrease of total phenolics with irradiation; higher DPPH scavenging activity and β-carotene bleaching inhibition at 6 kGy	Fernandes et al. (2014)
Russula delica Fr.			Higher concentrations of gallic acid and cinnamic acid at 6 kGy; higher values of total tocopherols and total phenolics at 6 kGy; higher DPPH scavenging activity and β-carotene bleaching inhibition at 6 kGy	
Amanita caesarea (Scop.) Pers.	e-beam	2, 6 and 10 kGy	Increase of *p*-hydroxybenzoic acid concentration at 2 kGy; increase of MUFA at 10 kGy; higher values of tocopherols at 10 kGy; increase of total phenolics with irradiation; increase of antioxidant activity with irradiation	Fernandes et al. (2015)
Amanita curtipes Gilbert			Increase of MUFA at 10 kGy; increase of tocopherols; increase of total phenolics at 10 kGy; increase of antioxidant activity at 10 kGy	
Agaricus bisporus Portobello	γ-radiation	1, 2 and 5 kGy	Higher amount of ergosterol at 2 kGy	Cardoso et al. (2019)

(continued)

Table 14.10 (continued)

Mushroom	Radiation source	Applied doses	Main results	References
	e-beam		Higher amount of ergosterol at 2 kGy	
Tuber aestivum	γ-radiation	1.0, 1.5 and 2.5 kGy	Increase of phenolic compounds at 1.5–2.5 kGy	Adamo et al. (2004)
		0.5, 1.0, 1.5 and 2.5 kGy	Increase of total phenolics with irradiation; preservation of total sterols.	Tejedor-Calvo et al. (2020)
Tuber melanosporum	γ-radiation	1.5 and 2.5 kGy	Decrease of total phenolic compounds at 2.5 kGy; preservation of total sterols	Tejedor-Calvo et al. (2019)
	e-beam		Increase of total phenolic compounds at 1.5 kGy; preservation of total sterols	

Notes: DPPH (2,2-Diphenyl-1-picrylhydrazyl); FRAP (Ferric reducing power); TBARS (Thiobarbituric acid reactive substances); MUFA (Monounsaturated fatty acids); PUFA (Polyunsaturated fatty acids)

concentrations in beef meatballs which was effective on retarding lipid and protein oxidations and on preventing rancid odor formation (Turgut et al. 2017). Moreover, Ribas-Agustí et al. (2014) demonstrated that it is possible to produce dry fermented sausages with natural antioxidants from grape seed and cocoa extracts without changing their sensory properties. Recently, a maceration industrial-scale extraction process was demonstrated to be effective on the recovery of bioactive compounds from unripe red grapes (cv. Sangiovese) with a high extraction yield (Fia et al. 2020). The obtained extract showed greater phenolic compound and water-soluble vitamin contents and antioxidant activity than those measured in the traditional product (called "verjuice") obtained from unripe grapes. The process was suitable to be transferred to the wine industry to produce extracts that can be used as ingredients for other industries and enhance the sustainability of the wine sector.

Concerning the pharmaceutical industry, natural bioactive compounds, in particular, omega-3 fatty acids from fish and fish oil, plant sterol esters and/or phenolic compounds from, e.g., green tea or red wine, have been combined with major hypolipidemic drugs. This co-therapy appeared to be safe and effective to prevent or treat cardiovascular diseases progression (Scolaro et al. 2018) even if further research has to be done to understand the biochemical mechanisms involved in these protective effects.

Relating to the use of food bioactive compounds in cosmetics products, there are some *in vivo* studies in the literature with positive results. In a study with 20 volunteers, it was demonstrated that a formulation containing green tea extract was able to inhibit the photoaging and tumor generation (Li et al. 2009). Also, a product developed as a combination of resveratrol, green tea polyphenols and caffeine was evaluated in a 12-week study with 16 volunteers and revealed to reduce facial redness (Ferzli et al. 2013). Black grape seed extract was also efficiently used in a water-in-oil cream as demonstrated to increase the skin elasticity, decrease

erythema effects and/or skin sebum content (Sharif et al. 2015). Nevertheless, it becomes important to continue studying the best way to recover bioactive compounds from food products and also to explain their potential added-value to the producers.

There are some patents on the application of bioactive compounds extracted from food sources in pharmaceutical and functional foods. As an example, a method to produce grape extracts with high values of Oxygen Radical Absorbance Capacity (ORAC) was patented to be added to foodstuffs and nutritional supplements as a beneficial antioxidant (Ying et al. 2011). The development of nutraceutical products from the antioxidants present in different fruits extracts and, in particular, from *Grewia asiatica* L. (phalsa) was patented based on their *in vitro* and *in vivo* antioxidant potential (Choudhary et al. 2014). Furthermore, a nutraceutical composition using resveratrol in combination with other components, e.g. genistein, lycopene, hydroxytyrosol or polyunsaturated fatty acids, was patented for delaying aging and/or for the treatment or prevention of age-related diseases in animals was patented (Raederstorff et al. 2008). Also, the production of pharmaceutical formulations to deliver biologically active compounds in a controlled manner so as to increase the bioavailability of compounds protecting them from *in vivo* degradation, has been the object of patents (Yuhua and Chien 2009). Slavko (2012) also invented a new pharmaceutical formulation that comprises silica earth, resveratrol, grape seeds extract, green tea extract and tomato powder with antioxidant effects.

14.8 Concluding Remarks

Food products are extremely rich in bioactive compounds. Due to the growing demand in society for new ingredients that can be reused on foods, cosmetics or pharmaceuticals, scientific communities are searching and developing new formulations and optimizing extraction processes. The findings presented in this chapter turn evident the potential added-value of the bioactive compounds extracted from irradiated plant and food sources. Nevertheless, further research should be performed on their applicability for the preparation of new formulations in different industries.

Acknowledgments The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES to C^{2}TN (UIDB/04349/2020), CIMO (UIDB/00690/2020) and Joana Madureira (SFRH/BD/136506/2018); Lillian Barros thanks the national funding by FCT, P.I., through the institutional scientific employment program-contract.

References

Adamo M, Capitani D, Mannina L et al (2004) Truffles decontamination treatment by ionizing radiation. Radiat Phys Chem 71:165–168. https://doi.org/10.1016/j.radphyschem.2004.03.073

Alashi AM, Taiwo KA, Oyedele DJ et al (2019) Polyphenol composition and antioxidant properties of vegetable leaf-fortified bread. J Food Biochem 43:1–9. https://doi.org/10.1111/jfbc.12625

Amirdivani S, Baba ASH (2014) Green tea yogurt: major phenolic compounds and microbial growth. J Food Sci Technol 52:4652–4660. https://doi.org/10.1007/s13197-014-1670-6

Arjeh E, Barzegar M, Ali Sahari M (2015) Effects of gamma irradiation on physicochemical properties, antioxidant and microbial activities of sour cherry juice. Radiat Phys Chem 114:18–24. https://doi.org/10.1016/j.radphyschem.2015.05.017

Barkaoui S, Madureira J, Santos PMP et al (2020) Effect of ionizing radiation and refrigeration on the antioxidants of strawberries. Food Bioprocess Technol 13:1516–1527. https://doi.org/10.1007/s11947-020-02490-1

Behgar M, Ghasemi S, Naserian A et al (2011) Gamma radiation effects on phenolics, antioxidants activity and in vitro digestion of pistachio (Pistachia vera) hull. Radiat Phys Chem 80:963–967. https://doi.org/10.1016/j.radphyschem.2011.04.016

Salem IB, Fekih S, Sghaier H et al (2013) Effect of ionising radiation on polyphenolic content and antioxidant potential of parathion-treated sage (Salvia officinalis) leaves. Food Chem 141:1398–1405. https://doi.org/10.1016/j.foodchem.2013.04.008

Bhat R, Sridhar KR, Tomita-Yokotani K (2007) Effect of ionizing radiation on antinutritional features of velvet bean seeds (Mucuna pruriens). Food Chem 103:860–866. https://doi.org/10.1016/j.foodchem.2006.09.037

Cabo Verde S, Trigo MJ, Sousa MB et al (2013) Effects of gamma radiation on raspberries: safety and quality issues. J Toxicol Environ Health A 76:291–303. https://doi.org/10.1080/15287394.2013.757256

CAC (1985) General standard for the labelling of pre-package foods. CODEX STAN 1-1985 and subsequent revisions. Food and Agriculture Organization of the UN, World Health Organization, Rome

Cardoso RVC, Fernandes Â, Barreira JCM et al (2019) Effectiveness of gamma and electron beam irradiation as preserving technologies of fresh Agaricus bisporus Portobello: a comparative study. Food Chem 278:760–766. https://doi.org/10.1016/j.foodchem.2018.11.116

Carocho M, Antonio AL, Barros L et al (2012) Comparative effects of gamma and electron beam irradiation on the antioxidant potential of Portuguese chestnuts (Castanea sativa Mill.). Food Chem Toxicol 50:3452–3455. https://doi.org/10.1016/j.fct.2012.07.041

Carocho M, Antonio AL, Barreira JCM et al (2014) Validation of Gamma and electron beam irradiation as alternative conservation technology for European Chestnuts. Food Bioprocess Technol 7:1917–1927. https://doi.org/10.1007/s11947-013-1186-5

Cătunescu GM, Rotar I, Vidican R et al (2017) Gamma radiation enhances the bioactivity of fresh parsley (Petroselinum crispum (Mill.) Fuss Var. Neapolitanum). Radiat Phys Chem 132:22–29. https://doi.org/10.1016/j.radphyschem.2016.11.010

Choudhary MI, Siddiqui J, Abbaskhan A, et al (2014) US2014/0221634 A1. Bio-active antioxidants from plant foods for nutraceutical product development

Dall'Asta C, Cirlini M, Morini E et al (2013) Effect of chestnut flour supplementation on physicochemical properties and volatiles in bread making. LWT – Food Sci Technol 53:233–239. https://doi.org/10.1016/j.lwt.2013.02.025

Deutsch JC (2000) Dehydroascorbic acid. J Chromatogr A 881:299–307. https://doi.org/10.1016/S0021-9673(00)00166-7

Eissa H, Shaheen M, Botros H (2014) Impact of γ-irradiation on aroma flavour, bio-active constituents and quality attributes of water melon juice. J Plant Pathol Microbiol 05. https://doi.org/10.4172/2157-7471.1000227

El-Beltagi HS, Aly AA, El-Desouky W (2019) Effect of Gamma irradiation on some biochemical properties, antioxidant and antimicrobial activities of Sakouti and Bondoky dry dates fruits genotypes. J Radiat Res Appl Sci 12:437–446. https://doi.org/10.1080/16878507.2019.1690799

Elias MI, Madureira J, Santos PMP et al (2020) Preservation treatment of fresh raspberries by e-beam irradiation. Innov Food Sci Emerg Technol 66:102487. https://doi.org/10.1016/j.ifset.2020.102487

El-Samahy SK, Youssef BM, Askar AA, Swailam HMM (2000) Microbiological and chemical properties of irradiated mango. J Food Saf 20:139–156

European Union (1999) Directive 1999/3/EC of the European parliament and of the council. Off J Eur Communities 66:24–25. https://doi.org/10.1016/j.jclepro.2010.02.014

Eustice RF (2018) Global status and commercial applications of food irradiation. In: Ferreira ICFRF, Antonio AL, Cabo Verde S (eds) Food irradiation technologies: concepts, applications and outcomes. Royal Society of Chemistry, pp 397–424

Fan X (2005) Antioxidant capacity of fresh-cut vegetables exposed to ionizing radiation. J Sci Food Agric 85:995–1000. https://doi.org/10.1002/jsfa.2057

Fanaro GB, Hassimotto NMA, Bastos DHM, Villavicencio ALCH (2014) Effects of γ-radiation on microbial load and antioxidant proprieties in black tea irradiated with different water activities. Radiat Phys Chem 97:217–222. https://doi.org/10.1016/j.radphyschem.2013.11.036

Fernandes Â, Antonio AL, Barreira JCM et al (2013a) Effects of Gamma irradiation on the chemical composition and antioxidant activity of lactarius deliciosus L. Wild Edible Mushroom Food Bioprocess Technol 6:2895–2903. https://doi.org/10.1007/s11947-012-0931-5

Fernandes Â, Barreira JCM, Antonio AL et al (2013b) Study of chemical changes and antioxidant activity variation induced by gamma-irradiation on wild mushrooms: Comparative study through principal component analysis. Food Res Int 54:18–25. https://doi.org/10.1016/j.foodres.2013.06.011

Fernandes Â, Barros L, Barreira JCM et al (2013c) Effects of different processing technologies on chemical and antioxidant parameters of Macrolepiota procera wild mushroom. LWT – Food Sci Technol 54:493–499. https://doi.org/10.1016/j.lwt.2013.06.027

Fernandes Â, Barreira JCM, António AL et al (2014) Feasibility of electron-beam irradiation to preserve wild dried mushrooms: effects on chemical composition and antioxidant activity. Innov Food Sci Emerg Technol 22:158–166. https://doi.org/10.1016/j.ifset.2013.12.015

Fernandes Â, Barreira JCM, António AL et al (2015) How does electron beam irradiation dose affect the chemical and antioxidant profiles of wild dried Amanita mushrooms? Food Chem 182:309–315. https://doi.org/10.1016/j.foodchem.2015.03.012

Ferreira ICFR, Antonio AL, Cabo Verde S (eds) (2018) Food irradiation technologies: Concepts, applications and outcomes. Royal Society of Chemistry

Ferzli G, Patel M, Phrsai N, Brody N (2013) Reduction of facial redness with resveratrol added to topical product containing Green Tea polyphenols and caffeine. J Drugs Dermatol 12:770–774

Fia G, Bucalossi G, Gori C et al (2020) Recovery of bioactive compounds from unripe red grapes (cv. Sangiovese) through a green extraction. Foods 9:4–7. https://doi.org/10.3390/foods9050566

Fox JB, Thayer DW, Jenkins RK et al (1989) Effect of gamma irradiation on the b vitamins of pork chops and chicken breasts. Int J Radiat Biol 55:689–703. https://doi.org/10.1080/09553008914550721

Fox JB, Lakritz L, Hampson J et al (1995) Gamma irradiation effects on Thiamin and riboflavin in Beef, Lamb, Pork, and Turkey. J Food Sci 60:596–598. https://doi.org/10.1111/j.1365-2621.1995.tb09835.x

Gerolis LGL, Lameiras FS, Krambrock K, Neves MJ (2017) Effect of gamma radiation on antioxidant capacity of green tea, yerba mate, and chamomile tea as evaluated by different methods. Radiat Phys Chem 130:177–185. https://doi.org/10.1016/j.radphyschem.2016.08.017

Girennavar B, Jayaprakasha GK, Mclin SE et al (2008) Influence of electron-beam irradiation on bioactive compounds in grapefruits (Citrus paradisi Macf.). J Agric Food Chem 56:10941–10946. https://doi.org/10.1021/jf801812h

Given NK, Venis MA, Grierson D (1988) Phenylalanine ammonia-lyase activity and anthocyanin synthesis in ripening strawberry fruit. J Plant Physiol 133:25–30. https://doi.org/10.1016/S0176-1617(88)80079-8

Guerreiro D, Madureira J, Silva T et al (2016) Post-harvest treatment of cherry tomatoes by gamma radiation: microbial and physicochemical parameters evaluation. Innov Food Sci Emerg Technol 36:1–9. https://doi.org/10.1016/j.ifset.2016.05.008

Guimarães IC, Menezes EGT, de Abreu PS et al (2013) Physicochemical and microbiological quality of raspberries (Rubus idaeus) treated with different doses of gamma irradiation. Food Sci Technol 33:316–322. https://doi.org/10.1590/s0101-20612013005000040

Guiné RPF, Rodrigues AP, Ferreira SM, Gonçalves FJ (2016) Development of Yogurts enriched with antioxidants from wine. J Culin Sci Technol 14:263–275. https://doi.org/10.1080/15428052.2015.1111180

Haque MA, Hashem MA, Hossain MM et al (2017) Effect of gamma irradiation on shelf life and quality of beef. J Meat Sci Technol 5:149–159. https://doi.org/10.3329/sja.v17i1.42768

Harrison K, Were LM (2007) Effect of gamma irradiation on total phenolic content yield and antioxidant capacity of Almond skin extracts. Food Chem 102:932–937. https://doi.org/10.1016/j.foodchem.2006.06.034

Hirashima FK, Fabbri ADT, Sagretti JMA, et al (2013) Influence of gamma irradiation on phenolic compounds of minimally processed baby carrots. In: International Nuclear Atlantic Conference (INAC). pp. 1–7

Horváthová J, Suhaj M, Polovka M et al (2007) The influence of gamma-irradiation on the formation of free radicals and antioxidant status of oregano (Origanum vulgare L.). Czech J Food Sci 25:131–143. https://doi.org/10.17221/741-CJFS

Hussain PR, Wani AM, Meena RS, Dar MA (2010) Gamma irradiation induced enhancement of phenylalanine ammonia-lyase (PAL) and antioxidant activity in peach (Prunus persica Bausch, Cv. Elberta). Radiat Phys Chem 79:982–989. https://doi.org/10.1016/j.radphyschem.2010.03.018

Hussain PR, Meena RS, Dar MA, Wani AM (2011) Gamma irradiation of sun-dried apricots (Prunus armeniaca L.) for quality maintenance and quarantine purposes. Radiat Phys Chem 80:817–827. https://doi.org/10.1016/j.radphyschem.2011.02.029

Hussain PR, Dar MA, Wani AM (2012) Effect of edible coating and gamma irradiation on inhibition of mould growth and quality retention of strawberry during refrigerated storage. Int J Food Sci Technol 47:2318–2324. https://doi.org/10.1111/j.1365-2621.2012.03105.x

Hussain PR, Chatterjee S, Variyar PS et al (2013) Bioactive compounds and antioxidant activity of gamma irradiated sun dried apricots (Prunus armeniaca L.). J Food Compos Anal 30:59–66. https://doi.org/10.1016/j.jfca.2013.02.001

Hussain PR, Suradkar P, Javaid S et al (2016) Influence of postharvest gamma irradiation treatment on the content of bioactive compounds and antioxidant activity of fenugreek (Trigonella foenum-graceum L.) and spinach (Spinacia oleracea L.) leaves. Innov Food Sci Emerg Technol 33:268–281. https://doi.org/10.1016/j.ifset.2015.11.017

ISO/ASTM 52303:2015 Guide for absorbed-dose mapping in radiation processing facilities

Janiak MA, Slavova-Kazakova A, Karamać M et al (2017) Effects of gamma-irradiation on the antioxidant potential of traditional Bulgarian teas. Nat Prod Commun 12:181–184. https://doi.org/10.1177/1934578x1701200210

JECFI (1981) Joint FAO/IAEA/WHO Expert Committee on Food Irradiation. Wholesomeness of irradiated food. Technical Report Series no. 659. Geneva, Switzerland

Jo C, Ahn DU, Lee KH (2012) Effect of gamma irradiation on microbiological, chemical, and sensory properties of fresh ashitaba and kale juices. Radiat Phys Chem 81:1076–1078. https://doi.org/10.1016/j.radphyschem.2011.11.065

JSGHDI (1999) FAO/IAEA/WHO Joint Study Group High-Dose Irradiation. Wholesomeness of food irradiated with doses above 10 kGy. Techinal Report Series no. 890. Geneva, Switzerland

Junqueira-Gonçalves MP, Galotto MJ, Valenzuela X et al (2011) Perception and view of consumers on food irradiation and the Radura symbol. Radiat Phys Chem 80:119–122. https://doi.org/10.1016/j.radphyschem.2010.08.001

Kavitha C, Kuna A, Supraja T et al (2015) Effect of gamma irradiation on antioxidant properties of ber (Zizyphus mauritiana) fruit. J Food Sci Technol 52:3123–3128. https://doi.org/10.1007/s13197-014-1359-x

Khattak KF, Simpson TJ, Ihasnullah (2008) Effect of gamma irradiation on the extraction yield, total phenolic content and free radical-scavenging activity of Nigella staiva seed. Food Chem 110:967–972. https://doi.org/10.1016/j.foodchem.2008.03.003

Koike A, Barreira JCM, Barros L et al (2015a) Edible flowers of Viola tricolor L. as a new functional food: antioxidant activity, individual phenolics and effects of gamma and electron-beam irradiation. Food Chem 179:6–14. https://doi.org/10.1016/j.foodchem.2015.01.123

Koike A, Barreira JCM, Barros L et al (2015b) Irradiation as a novel approach to improve quality of Tropaeolum majus L. flowers: benefits in phenolic profiles and antioxidant activity. Innov Food Sci Emerg Technol 30:138–144. https://doi.org/10.1016/j.ifset.2015.04.009

Kong Q, Wu A, Qi W et al (2014) Effects of electron-beam irradiation on blueberries inoculated with Escherichia coli and their nutritional quality and shelf life. Postharvest Biol Technol 95:28–35. https://doi.org/10.1016/j.postharvbio.2014.04.004

Kris-Etherton PM, Hecker KD, Bonanome A et al (2002) Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med 113:71–88. https://doi.org/10.1016/s0002-9343(01)00995-0

Lakritz L, Fox JB, Hampson J et al (1995) Effect of gamma radiation on levels of α-tocopherol in red meats and turkey. Meat Sci 41:261–271. https://doi.org/10.1016/0309-1740(94)00003-P

Le Caër S (2011) Water radiolysis: Influence of oxide surfaces on H2 production under ionizing radiation. Water 3:235–253. https://doi.org/10.3390/w3010235

Lee JW, Kim JK, Srinivasan P et al (2009) Effect of gamma irradiation on microbial analysis, antioxidant activity, sugar content and color of ready-to-use tamarind juice during storage. LWT – Food Sci Technol 42:101–105. https://doi.org/10.1016/j.lwt.2008.06.004

Li YH, Wu Y, Wei HC et al (2009) Protective effects of green tea extracts on photoaging and photommunosuppression. Skin Res Technol 15:338–345. https://doi.org/10.1111/j.1600-0846.2009.00370.x

Loro AC, Boteon VW, Spoto MHF (2018) Quality parameters of tomatoes submitted to different doses of gamma radiation. Brazilian J Food Technol 21:2017168

Madureira J, Severino A, Cojocaru M et al (2019) E-beam treatment to guarantee the safety and quality of cherry tomatoes. Innov Food Sci Emerg Technol 55:57–65. https://doi.org/10.1016/j.ifset.2019.05.013

Maherani B, Hossain F, Criado P et al (2016) World market development and consumer acceptance of irradiation technology. Foods 5:79. https://doi.org/10.3390/foods5040079

Maraei RW, Elsawy KM (2017) Chemical quality and nutrient composition of strawberry fruits treated by γ-irradiation. J Radiat Res Appl Sci 10:80–87. https://doi.org/10.1016/j.jrras.2016.12.004

Marathe SA, Deshpande R, Khamesra A et al (2016) Effect of radiation processing on nutritional, functional, sensory and antioxidant properties of red kidney beans. Radiat Phys Chem 125:1–8. https://doi.org/10.1016/j.radphyschem.2016.03.002

McDonald H, Arpaia ML, Caporaso F et al (2013) Effect of gamma irradiation treatment at phytosanitary dose levels on the quality of "Lane Late" navel oranges. Postharvest Biol Technol 86:91–99. https://doi.org/10.1016/j.postharvbio.2013.06.018

Miller RB (2005) Electronic irradiation of foods. Springer

Moharram HA, Youssef MM (2014) Methods for determining the antioxidant activity : a review. Alexandria J Food Sci Technol 11:31–42. https://doi.org/10.12816/0025348

Moreno MA, Castell-Perez ME, Gomes C et al (2007) Optimizing electron beam irradiation of "Tommy Atkins" mangoes (Mangifera Indica L.). J Food Process Eng 30:436–457. https://doi.org/10.1111/j.1745-4530.2007.00111.x

Najafabadi NS, Sahari MA, Barzegar M, Esfahani ZH (2017) Effect of gamma irradiation on some physicochemical properties and bioactive compounds of jujube (Ziziphus jujuba var vulgaris) fruit. Radiat Phys Chem 130:62–68. https://doi.org/10.1016/j.radphyschem.2016.07.002

Naresh K, Varakumar S, Variyar PS et al (2015) Enhancing antioxidant activity, microbial and sensory quality of mango (Mangifera indica L.) juice by γ-irradiation and its in vitro radioprotective potential. J Food Sci Technol 52:4054–4065. https://doi.org/10.1007/s13197-014-1502-8

Ornellas CBD, Gonçalves MPJ, Silva PR, Martins RT (2006) Atitude do consumidor frente à irradiação de alimentos. Ciência e Tecnol Aliment 26:211–213. https://doi.org/10.1590/s0101-20612006000100033

Oufedjikh H, Mahrouz M, Amiot MJ, Lacroix M (2000) Effect of γ-irradiation on phenolic compounds and phenylalanine ammonia-lyase activity during storage in relation to peel injury from peel of Citrus clementina Hort. ex. Tanaka J Agric Food Chem 48:559–565. https://doi.org/10.1021/jf9902402

Pereira C, Calhelha RC, António AL et al (2014) Effects of gamma radiation on chemical and antioxidant properties, anti-hepatocellular carcinoma activity and hepatotoxicity of borututu. Innov Food Sci Emerg Technol 26:271–277. https://doi.org/10.1016/j.ifset.2014.05.010

Pereira E, Barros L, Antonio A et al (2015a) Analytical methods applied to assess the effects of gamma irradiation on color, chemical composition and antioxidant activity of Ginkgo biloba L. Food Anal Methods 154–163. https://doi.org/10.1007/s12161-014-9883-x

Pereira E, Barros L, Dueñas M et al (2015b) Gamma irradiation improves the extractability of phenolic compounds in Ginkgo biloba L. Ind Crop Prod 74:144–149. https://doi.org/10.1016/j.indcrop.2015.04.039

Pereira E, Pimenta AI, Calhelha RC et al (2017) Infusions of gamma irradiated Aloysia citrodora L. and Mentha x piperita L.: effects on phenolic composition, cytotoxicity, antibacterial and virucidal activities. Ind Crop Prod 97:582–590. https://doi.org/10.1016/j.indcrop.2017.01.007

Pereira E, Pimenta AI, Barros L et al (2018) Effects of gamma radiation on the bioactivity of medicinal and aromatic plants: Mentha × piperita L., Thymus vulgaris L. and Aloysia citrodora Paláu as case studies. Food Funct 9:5150–5161. https://doi.org/10.1039/c8fo01558a

Pérez MB, Calderón NL, Croci CA (2007) Radiation-induced enhancement of antioxidant activity in extracts of rosemary (Rosmarinus officinalis L.). Food Chem 104:585–592. https://doi.org/10.1016/j.foodchem.2006.12.009

Pinela J, Barreira JCM, Barros L et al (2016) Suitability of gamma irradiation for preserving fresh-cut watercress quality during cold storage. Food Chem 206:50–58. https://doi.org/10.1016/j.foodchem.2016.03.050

Pinela J, Barros L, Barreira JCM et al (2018) Postharvest changes in the phenolic profile of watercress induced by post-packaging irradiation and modified atmosphere packaging. Food Chem 254:70–77. https://doi.org/10.1016/j.foodchem.2018.01.181

Popović BM, Štajner D, Mandić A et al (2013) Enhancement of antioxidant and isoflavones concentration in gamma irradiated soybean. Sci World J 2013. https://doi.org/10.1155/2013/383574

Raederstorff D, Wang-Schmidt Y, Wolfram S (2008) US2008/0262081 A1. Novel use of nutraceutical compositions comprising resveratrol

Rajurkar NS, Gaikwad KN (2012) Effect of gamma irradiation on antioxidant activity of Amoora rohitaka. J Radioanal Nucl Chem 293:409–413. https://doi.org/10.1007/s10967-012-1759-1

Reyes LF, Cisneros-Zevallos L (2007) Electron-beam ionizing radiation stress effects on mango fruit (Mangifera indica L.) antioxidant constituents before and during postharvest storage. J Agric Food Chem 55:6132–6139. https://doi.org/10.1021/jf0635661

Ribas-Agustí A, Gratacós-Cubarsí M, Sárraga C et al (2014) Stability of phenolic compounds in dry fermented sausages added with cocoa and grape seed extracts. LWT – Food Sci Technol 57:329–336. https://doi.org/10.1016/j.lwt.2013.12.046

Roberts PB (2014) Food irradiation is safe: half a century of studies. Radiat Phys Chem 105:78–82. https://doi.org/10.1016/j.radphyschem.2014.05.016

Sádecká J (2007) Irradiation of spices: a review. Czech J Food Sci 25:231–242. https://doi.org/10.17221/684-cjfs

Schindler M, Solar S, Sontag G (2005) Phenolic compounds in tomatoes. Natural variations and effect of gamma-irradiation. Eur Food Res Technol 221:439–445. https://doi.org/10.1007/s00217-005-1198-0

Scolaro B, Soo Jin Kim H, de Castro IA (2018) Bioactive compounds as an alternative for drug co-therapy: overcoming challenges in cardiovascular disease prevention. Crit Rev Food Sci Nutr 58:958–971. https://doi.org/10.1080/10408398.2016.1235546

Shahbaz HM, Ahn J-J, Akram K et al (2014) Chemical and sensory quality of fresh pomegranate fruits exposed to gamma radiation as quarantine treatment. Food Chem 145:312–318

Shahidi F, Zhong Y (2015) Measurement of antioxidant activity. J Funct Foods 18:757–781. https://doi.org/10.1016/j.jff.2015.01.047

Shao Y, Tang F, Xu F et al (2013) Effects of γ-irradiation on phenolics content, antioxidant activity and physicochemical properties of whole grain rice. Radiat Phys Chem 85:227–233. https://doi.org/10.1016/j.radphyschem.2013.01.022

Sharif A, Akhtar N, Khan MS et al (2015) Formulation and evaluation on human skin of a water-in-oil emulsion containing Muscat hamburg black grape seed extract. Int J Cosmet Sci 37:253–258. https://doi.org/10.1111/ics.12184

Shashirekha MN, Mallikarjuna SE, Rajarathnam S (2015) Status of bioactive compounds in foods, with focus on fruits and vegetables. Crit Rev Food Sci Nutr 55:1324–1339. https://doi.org/10.1080/10408398.2012.692736

Siddhuraju P, Osoniyi O, Makkar HPS, Becker K (2002) Effect of soaking and ionising radiation on various antinutritional factors of seeds from different species of an unconventional legume, Sesbania and a common legume, green gram (Vigna radiata). Food Chem 79:273–281. https://doi.org/10.1016/S0308-8146(02)00140-1

Singh A, Singh D, Singh R (2016) Shelf Life extension of tomatoes by Gamma radiation. J Radiat Sci Technol 2:17–24. https://doi.org/10.11648/j.rst.20160202.12

Slavko I (2012) EP2524691 A1. An antioxidant composition. EP Pat 0879946A2

Song HP, Kim DH, Jo C et al (2006) Effect of gamma irradiation on the microbiological quality and antioxidant activity of fresh vegetable juice. Food Microbiol 23:372–378. https://doi.org/10.1016/j.fm.2005.05.010

Štajner D, Milošević M, Popović BM (2007) Irradiation effects on phenolic content, lipid and protein oxidation and scavenger ability of soybean seeds. Int J Mol Sci 8:618–627. https://doi.org/10.3390/i8070618

Suhaj M, Horváthová J (2007) Changes in antioxidant activity induced by irradiation of clove (Syzygium aromaticum) and ginger (Zingiber officinale). J Food Nutr Res 46:112–122

Suhaj M, Rácová J, Polovka M, Brezová V (2006) Effect of γ-irradiation on antioxidant activity of black pepper (Piper nigrum L.). Food Chem 97:696–704. https://doi.org/10.1016/j.foodchem.2005.05.048

Tejedor-Calvo E, Morales D, Marco P et al (2019) Effects of combining electron-beam or gamma irradiation treatments with further storage under modified atmospheres on the bioactive compounds of Tuber melanosporum truffles. Postharvest Biol Technol 155:149–155. https://doi.org/10.1016/j.postharvbio.2019.05.022

Tejedor-Calvo E, Morales D, García-Barreda S et al (2020) Effects of gamma irradiation on the shelf-life and bioactive compounds of Tuber aestivum truffles packaged in passive modified atmosphere. Int J Food Microbiol 332:108774. https://doi.org/10.1016/j.ijfoodmicro.2020.108774

Tezotto-Uliana JV, Berno ND, Saji FRQ, Kluge RA (2013) Gamma radiation: an efficient technology to conserve the quality of fresh raspberries. Sci Hortic (Amsterdam) 164:348–352. https://doi.org/10.1016/j.scienta.2013.09.026

Turgut SS, Işıkçı F, Soyer A (2017) Antioxidant activity of pomegranate peel extract on lipid and protein oxidation in beef meatballs during frozen storage. Meat Sci 129:111–119. https://doi.org/10.1016/j.meatsci.2017.02.019

Variyar PS, Bandyopadhyay C, Thomas P (1998) Effect of gamma-irradiation on the phenolic acids of some Indian spices. Int J Food Sci Technol 33:533–537. https://doi.org/10.1046/j.1365-2621.1998.00219.x

Variyar PS, Limaye A, Sharma A (2004) Radiation-induced enhancement of antioxidant contents of soybean (Glycine max Merrill). J Agric Food Chem 52:3385–3388. https://doi.org/10.1021/jf030793j

WHO (1988) Food irradiation: a technique for preserving and improving the safety of food. World Health Organization, Geneva

Yilmaz I, Geçgel U (2007) Effects of gamma irradiation on trans fatty acid composition in ground beef. Food Control 18:635–638. https://doi.org/10.1016/j.foodcont.2006.02.009

Ying W, Xiong X, Chen J, Yang J (2011) US7959963 B2. Method for producing grape extract with high ORAC value, and grape extract so produced

Yuhua L, Chien B (2009) JP5053846B2. Pharmaceutical composition for controlled release delivery of bioactive compounds

Zhu F, Cai YZ, Bao J, Corke H (2010) Effect of γ-irradiation on phenolic compounds in rice grain. Food Chem 120:74–77. https://doi.org/10.1016/j.foodchem.2009.09.072

Publicación 2. Ionizing Radiation Technologies to Increase the Extraction of Bioactive Compounds from Agro-Industrial Residues: A Review

Joana Madureira, Lillian Barros, Sandra Cabo Verde, Fernanda M. A. Margaça, Celestino Santos-Buelga, Isabel C. F. R. Ferreira

Artículo publicado en el *Journal of Agricultural and Food Chemistry* (2020), 68, 11054−11067. Doi: https://dx.doi.org/10.1021/acs.jafc.0c04984

Resumen

Los desechos agroindustriales pueden ser naturaleza muy diversa, distinguiéndose entre residuos de campo y residuos de procesos. Pueden incluir diferentes partes de vegetales, como hojas, tallos, vainas, cáscaras, pulpa o pieles, así como subproductos del procesado industrial, como orujos resultantes de la extracción de aceites o de la elaboración de vinos, melazas, suero de leche, fangos de la producción de lana, celulosa, salvado, almidones, etc.

En un mundo en rápido desarrollo, el cada vez más elevado volumen de desechos representa un grave problema para su eliminación, con efectos nocivos para el medio ambiente y para la salud humana y animal, a la vez que supone una pérdida de biomasa, nutrientes y sustancias bioactivas, que son infrautilizados. Esta problemática impone la necesidad de encontrar estrategias para reutilizar los residuos agroindustriales valiosos de manera más eficiente, aportando, de este modo, beneficios ambientales y contribuyendo a la sostenibilidad económica. En este sentido, los residuos agroindustriales pueden encontrar utilidad como fertilizantes agrícolas, compostaje reciclado, producción de biocombustibles, obtención de enzimas, preparación de polímeros biodegradables o extracción de nutrientes, saborizantes, conservantes o compuestos bioactivos.

Actualmente, los consumidores son más conscientes y están más interesados por su alimentación, aumentando así la demanda de alimentos con características más saludables. La industria alimentaria trata de responder a este desafío a través del desarrollo de productos funcionales innovadores y con ingredientes naturales y seguros que puedan reemplazar a los sintéticos actualmente empleados, confiriendo mayor valor añadido al producto.

El objeto de esta revisión es actualizar el conocimiento acerca de la recuperación de compuestos bioactivos a partir de desechos agroindustriales, con vistas al desarrollo de alimentos funcionales/saludables. Se resume el estado actual de la extracción de estos compuestos y, por primera vez, se discute el impacto de las tecnologías de irradiación en la mejora de su extractabilidad.

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

pubs.acs.org/JAFC

Review

Ionizing Radiation Technologies to Increase the Extraction of Bioactive Compounds from Agro-Industrial Residues: A Review

Joana Madureira, Lillian Barros, Sandra Cabo Verde,* Fernanda M. A. Margaça, Celestino Santos-Buelga, and Isabel C. F. R. Ferreira*

Cite This: J. Agric. Food Chem. 2020, 68, 11054−11067

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Due to the growing demand in society for healthier foods, scientific communities are searching and developing new ingredients. In this context, agro-industrial residues, which can have a negative impact on the environment, represent a natural source for bioactive compounds and their recovery can contribute to economic and environmental sustainability. Ionizing radiation is a clean and eco-friendly technology that can be used to improve the extraction of bioactive compounds. The aim of this review, after presenting general aspects about bioactive compounds in agro-industrial residues and radiation technologies, is to focus on the effects of ionizing radiation on the extraction of bioactive compounds from these residues and related bioactive properties. Irradiated residues were demonstrated to have enhanced bioactive characteristics that turn the prepared extracts suitable for applications in food industry, resulting in high-added-value products as well as reducing adverse impacts on the environment.

KEYWORDS: *agro-industrial residues, extraction, bioactive compounds, bioactivities, ionizing radiation*

1. INTRODUCTION

The agro-industrial residues can be many and different wastes from agriculture and industry. Agriculture residues can be divided into field residues and process residues and can include leaves, stalks, seedpods, stems, molasses, husks, pulp, and peels.[1] These wastes can also comprise the byproducts of the agro-food industry such as oil cakes from oil extraction, degummed fruits and legumes, coffee dregs, milk serum, sludge from wool, cellulose, bran, starch, juice, and sugars.[2]

With the fast developing world, the high volume of wastes produced by these industries generates large quantities for disposal and represents a loss of underutilized biomass and nutrients. Although some of them can be discharged safely, others are considered to have harmful effects on the environment with consequences to human and animal health. This problem imposes the need to find strategies to reuse the valuable agro-industrial residues in a more efficient way, providing environmental benefits and contributing to the economic sustainability. The agro-industrial residues can be used as fertilizers in agriculture, biofuel and enzyme production, preparation of biodegradable polymeric systems, recycled agricultural composting and extraction of food flavoring, preservative and bioactive compounds.[3]

Nowadays, consumers are more conscious and interested in what they eat, thus increasing the demand for healthier foodstuffs.[4] In this way, the food industry tried to answer this challenge and started to develop innovative and natural functional products[5] that could be used to replace the existing synthetic ones or as new added-value ingredients. The extractable antioxidant and antimicrobial compounds from agro-industrial residues offer a more natural and safer alternative to the food industry.[6]

The purpose of this review is to provide an overview of the bioactive compounds recovery from agro-industrial wastes to develop functional/health foods. The current status of the extraction of agro-industrial bioactive compounds is summarized, and for the first time, we discuss the positive impact of the ionizing radiation technology on the extractability of these compounds.

2. EXTRACTION OF BIOACTIVE COMPOUNDS

Bioactive compounds are components naturally present in plant and food products that provide health benefits and wellness, including antioxidant, anti-inflammatory, antimicrobial, anticancer, and immunomodulatory activities.[7] Natural bioactive compounds have diverse structures and functionalities with molecules having enormous potential for the production of nutraceuticals, functional foods, and food additives.[8] With this purpose, these compounds are being intensively studied to evaluate their effects on health, showing benefic effects for cardiovascular disease, cancers, and others. They comprise compounds with different chemical structures (hydrophilic or lipophilic), distribution in nature, range of concentrations, possible site of action, effectiveness against oxidative species, specificity, and biological action. The main classes of bioactive compounds include polyphenolic compounds, carotenoids, tocopherols, phytosterols, organosulfur

Received: August 4, 2020
Revised: September 15, 2020
Accepted: September 16, 2020
Published: September 16, 2020

https://dx.doi.org/10.1021/acs.jafc.0c04984
J. Agric. Food Chem. 2020, 68, 11054−11067

compounds, fatty acids, betalains, essential oils (terpenes), and alkaloids.[7]

The extraction of these compounds can be performed by using conventional and nonconventional methods. Conventional techniques are based on solid−liquid extraction requiring the use of organic solvents, temperature, and agitation and include percolation, maceration (ME), decoction, and hydrodistillation. Some extraction variables such as type of solvent and product/solvent ratio, time and temperature of extraction, and mode of stirring are significant influencing factors on the recovery of bioactive compounds and should be considered in the process optimization.[8]

To overcome limitations of conventional methods, such as long extraction time, requirement of costly, high purity, and safe solvents, evaporation of the large amount of solvent, and/or low extraction selectivity, new and promising techniques have been introduced[10,11] such as ultrasound-assisted extraction (UAE),[12,13] pulsed electric field (PEF) extraction,[14,15] enzyme-assisted extraction (EAE),[16−18] microwave-assisted extraction (MAE),[19,20] pressurized liquid extraction (PLE),[21] supercritical fluid extraction (SFE),[22] pressurized low-polarity water extraction, and molecular distillation. M'hiri, Ioannou, Paris, Ghoul, and Boudhrioua (2016)[23] compared the efficiencies of different methods (ME, UAE, MAE, high pressure extraction (HPE), and supercritical CO_2 extraction (SC-CO_2)) for antioxidant extraction from *Citrus sinensis* (L.) Osbeck peel, evaluating selectivity, total phenol content, total and individual flavonoids, and antioxidant activity; they concluded that MAE was the extracting method to obtain higher contents of phenols, flavonoids, and individual flavonoids. Tabaraki and Ghadiri (2016)[24] also obtained the best results on the extraction of antioxidants from *Pistacia vera* L. hull using MAE when compared with UAE and conventional extraction. Vieira et al. (2017)[25] compared conventional maceration and MAE for the extraction of valuable compounds from *Juglans regia* L. leaves, also attributing to MAE better outcomes. On the other hand, UAE using acidified water−ethanol mixtures as solvent was considered the optimal technique to extract bioactive anthocyanin pigments from *Ficus carica* L. peel, when compared with heat-assisted extraction (HAE) and MAE.[26] In another study, the multifrequency multimode modulated (MMM) ultrasonic technique increased the recovery of phenolic compounds from olive pomace and its antioxidant activity when compared to the conventional extraction.[27]

In general, although the results regarding the technique of choice differ depending on the study, the assisted extraction methods showed to be more effective than the conventional ones to extract polyphenol antioxidants from agricultural byproducts.

3. ISOLATION AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS

After extraction, the obtained extracts contain complex mixtures of natural compounds that have to be isolated, purified, and further characterized. The development of efficient methods for isolation/purification/characterization of the bioactive compounds has been an important challenge for researchers, which depends on the chemical and physical characteristics of them.[28,29]

The isolation and purification of the compounds involves different chromatographic techniques, such as thin-layer chromatography (TLC), column chromatography, flash chromatography, and high-performance liquid chromatography (HPLC). TLC is a simple, cost-effective, and rapid chromatographic technique, which gives the number of components present in the mixture but can also identify the compound comparing with a standard one, and HPLC is a versatile and widely used technique for the isolation of compounds from agro-industrial residues.[30]

The structure and biological activity of the purified compounds are then determined using different methodologies. Fourier transform infrared spectroscopy (FTIR) can be considered as a fingerprinting tool due to their unique spectra of pure compounds, allowing the identification of functional groups present in a compound.[31] Furthermore, HPLC can be coupled with simple detectors, such as UV detectors, or detectors for hyphenated systems, such as UV-diode array (DAD), mass spectrometry (MS), or nuclear magnetic resonance (NMR), producing multidimensional data for online identification and dereplication purposes.[29] An overview of the employed methodologies in extraction, isolation, and characterization of bioactive compounds from residues of agro-food industries can be found in Figure 1.

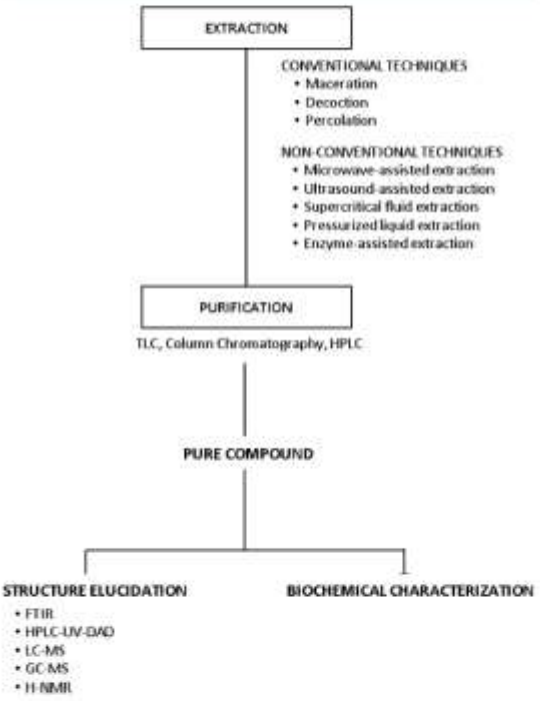


Figure 1. Methodologies used in extraction, isolation, and characterization of bioactive compounds from agro-industrial residues.

These techniques can be combined in order to provide a rapid and accurate identification of compounds, especially when the pure compound is unknown and not available. Hence, it is important to precisely select the methods to use from the extraction to the separation and purification, as this is crucial to obtain accurate results.

https://dx.doi.org/10.1021/acs.jafc.0c04984
J. Agric. Food Chem. 2020, 68, 11054−11067

Table 1. Literature Review of the Extraction of Bioactive Compounds from Agro-Industrial Residues Using Different Techniques and Conditions[a]

agro-industrial residue	extraction technique	extraction conditions					main extracted bioactive compounds	references
		solvent and proportion		temperature (°C)	time (min)	others		
sugar cane bagasse	ME	MeOH:H_2O	50:50	RT	>500		gallic acid; ferulic acid; epicatechin; quercetin derivatives; kaempferol derivatives	37
			70:30					
corn husk			100:0					
peanut husk		EtOH:H_2O	50:50					
coffee cherry husk			70:70					
			100:0					
rice bran		H_2O	100					
wheat bran								
Coffea arabica L.	filter coffee-maker	H_2O	100	90	6	defatted with petroleum ether	5-*O*-caffeoylquinic acid; 3,4-dicaffeoylquinic acid; 3-caffeoylquinic acid; 4-caffeoylquinic acid; 3,5-dicaffeoylquinic acid; 4,5-dicaffeoylquinic acid	99
Oryza sativa L. bran	ME	EtOH:H_2O	80:20	RT	20		protocatechuic acid; vanillic acid; ferulic acid; *p*-coumaric acid; α-tocopherol; γ-tocopherol; γ-tocotrienol; peonidin 3-glucoside; cyanidin 3-rutinoside; cyanidin 3-glucoside	35
wheat bran	ME	MeOH	100	RT	>500		gallic acid; caffeic acid; *p*-coumaric acid; ferulic acid	36
		MeOH:H_2O	80:20					
		EtOH	100					
		EtOH:H_2O	80:20					
		C_3H_6O:H_2O	50:50					
barley husk	ME	MeOH	100	RT	>500		gallic acid; chlorogenic acid; caffeic acid; *p*-coumaric acid; ferulic acid; benzoic acid; quercetin; kaempferol	39
		MeOH:H_2O	80:20					
		EtOH	100					
		EtOH:H_2O	80:20					
		C_3H_6O:H_2O	50:50					
Juglans regia L. leaves	ME	EtOH	0–100	42–78	30–126		3-*O*-caffeoylquinic acid; quercetin 3-*O*-glucoside; quercetin *O*-pentoside	25
	MEA	EtOH	0–100	60–180	3–37	850 W		
Araucaria angustifolia (Bertol.) Kuntze coats	ME	EtOH	100		300		protocatechuic acid; (+)-catechin; prodelphinidin B3; B-type (epi)catechin dimer; prodelphinidin B3; (−)-epicatechin	100
		EtOH:H_2O	80:20					
	ME	EtOH	38–97	21–64	15	S/L = 2–13%		101
Eucalyptus globulus Labill. bark	ME	MeOH + H_2O	100	RT	>500		gallic acid; quinic acid; catechin; chlorogenic acid; galloyl-bis(hexahydroxydiphenoyl)-glucose; galloyl-hexahydroxydiphenoyl-glucose; digalloylglucose; isorhamnetin-rhamnoside; eriodictyol	39
		MeOH:H_2O	50:50	RT	>500		eriodictyol; isorhamnetin; naringenin; methyl-ellagic acid-pentoses; methyl-ellagic acid	40
	SFE	CO_2	100	70	225	300 bar		
		CO_2:EtOH	85:15					
		CO_2:EtOAC	85:15					
		CO_2:H_2O	98:2					
Eucalyptus grandis W.Hill bark	ME	MeOH:H_2O	50:50	RT	>500		gallic acid; catechin; galloyl-bis-hexahydroxydiphenoyl-glucose; epicatechin; ellagic acid-rhamnoside; ellagic acid; digalloylglucose	41

https://dx.doi.org/10.1021/acs.jafc.0c04984
J. Agric. Food Chem. 2020, 68, 11054−11067

Table 1. continued

agro-industrial residue	extraction technique	extraction conditions				main extracted bioactive compounds	references
		solvent and proportion	temperature (°C)	time (min)	others		
Eucalyptus urograndis (E. grandis W.Hill × E. urophylla S.T.Blake) bark	ME	MeOH:H_2O	50:50	RT	>500	quinic acid; gallic acid; galloyl-bis-hexahydroxydiphenoyl-glucose; epicatechin; ellagic acid-rhamnoside; ellagic acid	41
Eucalyptus maidenii F.Muell. bark	ME	MeOH:H_2O	50:50	RT	>500	quinic acid; gallic acid; catechin; chlorogenic acid; methyl-ellagic acid pentose; myricetin-rhamnoside; ellagic acid; taxifolin	41
Quercus suber L. planks	ME	MeOH:H_2O + (C_2H_3)$_2O$	80:20	RT	>500	ellagic acid; gallic acid; protocatechuic acid; caffeic acid; esculetin; salicylic acid; eriodictyol	38
		MeOH + H_2O	100	RT	360 + 360		
			100				
Quercus suber L. wastewater						gallic acid; protocatechuic acid; vanillic acid; syringic acid; ferulic acid; ellagic acid	42
Theobroma cacao L. pod husk	ME	C_3H_6O:H_2O:CH_3COOH	70:29.5:0.5	RT	60	catechin; quercetin; (−)-epicatechin; gallic acid; coumaric acid; protocatechuic acid	43
	ME	EtOH:H_2O	80:20	40	30	protocatechuic acid; *p*-hydroxybenzoic acid; salicylic acid; kaempferol; luteolin; apigenin	44
Olea europaea L. leaves	PLE	EtOH	100	150	20	glucose acid; quinic acid; luteolin-7-*O*-glucoside; luteolin-4-*O*-glucoside; oleuropein; oleuroside; lucidumoside	52
		H_2O	100	200	20	gluconic acid; hydroxytyrosol; oleoside; elenolic acid glucoside; hydroxytyrosol acetate; luteolin-7-*O*-glucoside; oleuropein; oleuroside; luteolin	
	ME	MeOH:H_2O	80:20			secologanoside; demethyldeuropein; luteolin glucoside isomer; verbascoside; oleuropein isomer; ligstroside; oleuropein	54
	ME	H_2O	100	100	30	caffeic acid; verbascoside; oleuropein; luteolin 7-*O*-glucoside; rutin; apigenin 7-*O*-glucoside and luteolin 4′-*O*-glucoside	51
Olea europaea L. mill wastewater	ME	EtOAC	100		30	defatted with *n*-hexane	hydroxytyrosol; tyrosol; *p*-coumaric acid; anupic acid; verbascoside; hydroxytyrosol glucoside; apigenin-7-glucoside; luteolin; luteolin-7-*O*-glucoside; luteolin-7-*O*-rutinoside	49
	ME	EtOAC	100			defatted with *n*-hexane	hydroxytyrosol; gallic acid; tyrosol; *p*-coumaric acid; oleuropein aglycon	47
Olea europaea L. pomace	MMM	H_2O	100	RT	5−10	90−160 W, 20 kHz	hydroxytyrosol; comelogoside; elenolic acid derivatives; tyrosol	27
	ME	H_2O	100	40	60			
	ME	$CHCl_3$	100		>500		hydroxytyrosol; gallic acid; tyrosol; oleuropein; caffeic acid; ligstroside aglycon; oleuropein aglycon; ferulic acid; vanillic acid	45
		$CHCl_3$:MeOH	90:10					
		MeOH	100					
	PLE	EtOH:H_2O	80:20	80	10	defatted with petroleum ether	3,4-DHPEA-EDA; oleuropein aglycon; elenolic acid; hydroxytyrosol; luteolin-7-*O*-glucoside; luteolin; ligstroside derivative	53
Allium cepa L. skin	ME	MeOH:H_2O:HCl	70:29.5:0.5	35	90		quercetin-4′-*O*-glucoside; quercetin-3,4′-*O*-diglucoside	59
Lycopersicon esculentum L. processing waste	ME	MeOH	100				*p*-coumaric acid; caffeic acid; chlorogenic acid; chrysin; naringenin	60
Garcinia brasiliensis Mart. leaves	percolation	MeOH + EtOAC	100				morellaflavone-7″-*O*-glucoside; gardenia billwoniid 2a glucoside; apigenin-8-*C*-glucoside; apigenin-2″-*O*-dimytheroside-*C*-hexoside	102
Myrciaria dubia (Kunth) McVaugh peel	ME	EtOH:H_2O	80:20	RT	90		4-*O*-caffeoylquinic acid; *p*-coumaroyl hexoside; (iso)liquiritigenin-*O*-hexoside; myricetin-*O*-pentoside; myricetin-*O*-deoxyhexoside; apigenin-*O*-hexoside; ellagic acid derivatives	91
	ME	EtOH:H_2O (acidified)	80:20	RT	120		delphinidin-3-*O*-glucoside; cyanidin-3-*O*-glucoside	

https://dx.doi.org/10.1021/acs.jafc.0c04984
J. Agric. Food Chem. 2020, 68, 11054−11067

Table 1. continued

agro-industrial residue	extraction technique	extraction conditions: solvent and proportion	temperature (°C)	time (min)	others	main extracted bioactive compounds	references
Myrciaria dubia (Kunth) McVaugh seed	ME	EtOH:H_2O 80:20	RT	90		valoneic acid dilactone; di-HHDP-galloyl-glucoside; ellagic acid hexoside; ellagic acid pentoside; ellagic acid; ellagic acetyl rhamnoside	91
Myrciaria dubia (Kunth) McVaugh pulp	ME	EtOH:H_2O 80:20	RT	90		*p*-coumaroyl hexoside; ferulic acid hexoside; myricetin-*O*-hexoside; myricetin-*O*-pentoside	91
	ME	EtOH:H_2O (acidified) 80:20	RT	120		cyanidin-3-*O*-glucoside	
Carissa macrocarpa (Eckl.) A.DC. leaves	ME	EtOH:H_2O 80:20	RT	120		B-type (epi)catechin derivatives; quercetin-*O*-deoxyhexoside-*O*-deoxyhexosyl-hexoside; quercetin-3-*O*-rutinoside	103
Carissa macrocarpa (Eckl.) A.DC. stems	ME	EtOH:H_2O 80:20	RT	120		*trans*-4-*O*-caffeolyquinic acid; B-type (epi)catechin derivatives; A-type (epi) catechin trimer; quercetin-3-*O*-rutinoside	103
Carissa macrocarpa (Eckl.) A.DC. flowers	ME	EtOH:H_2O 80:20	RT	120		kaempferol-3-*O*-rutinoside; quercetin-3-*O*-rutinoside; kaempferol-*O*-deoxy-hexside-*O*-deoxyhexosyl-hexoside isomer 4; *cis*-5-*p*-coumaroylquinic acid; *cis*-4-*O*-caffeolyquinic acid	103
Actinidia deliciosa (A.Chev.) C.F.Liang and A.R.Ferguson peel	ME	EtOH:H_2O 80:20	RT	120		caffeic acid derivatives; caffeic acid hexoside; dimethyl caffeic acid hexoside; epicatechin; B-type (epi)catechin derivatives; acetyl-dimethyl caffeic acid hexoside; quercetin-3-*O*-glucoside; quercetin-3-*O*-rhamnoside	104
Actinidia deliciosa (A.Chev.) C.F.Liang and A.R.Ferguson pulp	ME	EtOH:H_2O 80:20	RT	120		caffeic acid dihexoside; caffeic acid derivative; caffeic acid hexoside; dimethyl caffeic acid hexoside; epicatechin; quercetin-3-*O*-rutinoside; kaempferol-3-*O*-rutinoside; quercetin-3-*O*-rhamnoside	104
Ficus carica L. peel	ME	EtOH 0–100 (pH 3)	20–90	5–68.8		cyanidin 3-rutinoside	26
	MAE	EtOH 0–100 (pH 3)	40–115	5–35	400 W		
	UAE	EtOH 0–100 (pH 3)	30–35	5–55	100–400 W		
Persea americana Mill. peel	ME	EtOH:H_2O 80:20	RT	120		4-*O*-caffeoylquinic acid; 5-*O*-caffeoylquinic acid; B-type (epi)catechin dimer; B-type (epi)catechin trimer; B-type (epi)catechin tetramer; quercetin-dihexoside; quercetin-pentoside-hexoside	105
Persea americana Mill. kernel	ME	EtOH:H_2O 80:20	RT	120		*cis*-3-*O*-caffeoylquinic acid; *trans*-3-*O*-caffeoylquinic acid; B-type (epi)catechin dimer; *cis*-3-*p*-coumaroylquinic acid; *cis*-5-*p*-coumaroylquinic acid; catechin; epicatechin; B-type (epi)catechin trimer	105
Rubus idaeus L. leaves	ultrasonic bath	MeOH:H_2O 70:30	RT	180		ellagic acid; rutin; caffeic acid; epigallocatechin gallate; ferulic acid catechin; 5-*O*-caffeoylquinic acid	106
Rubus fruticosus L. leaves							
Myrciaria jaboticaba (Vell.) Berg pulp	ME	EtOH:H_2O 80:20	RT	120		bis-HHDP-glucose isomer; trigalloyl-HHPD-glucose isomer; galloyl-bis-HHDP-glucose isomer; galloyl-HHDP-glucose; digalloyl-HHDP-glucose isomer; castalagin; trigalloyl-HHDP glucose; pentagalloylglucose; quercetin-2-*O*-rhamnoside	107
		EtOH:H_2O (acidified) 80:20	RT	120		delphinidin-3-*O*-glucoside; cyanidin-3-*O*-glucoside	
Citrus limon (L.) Osbeck peel	ME	MeOH + petroleum ether + EtOAC				ferulic acid; sinapic acid; *p*-coumaric acid; ascorbic acid	57
Citrus sinensis (L.) Osbeck peel	ME	MeOH + petroleum ether + EtOAC				ferulic acid; sinapic acid; *p*-coumaric acid; ascorbic acid	57
	ME	EtOH:H_2O 80:20	35	90	~125 W	eriocitrin; narirutin; naringin; hesperidin; neohesperidin; didymin; sinensetin; 3′,4′,5,5′6,7-hexamethoxyflavone; tangeretin; nobiletin	23
	UAE	EtOH:H_2O 80:20	35	90	125 W		
	MAE	EtOH:H_2O 80:20	35	0.5	170 W		
	SC-CO_2	EtOH:H_2O 80:20	35	90	22 MPa		

https://dx.doi.org/10.1021/acs.jafc.0c04984
J. Agric. Food Chem. 2020, 68, 11054−11067

Table 1. continued

agro-industrial residue	extraction technique	extraction conditions: solvent and proportion		temperature (°C)	time (min)	others	main extracted bioactive compounds	references
Citrus paradisi Macfad. peel	ME	MeOH + petroleum ether + EtOAC					ferulic acid; sinapic acid; ascorbic acid	57
Citrus paradise Changshan-huyou (*Citrus sinensis* (L.) Osbeck × *Citrus grandis* (L.) Osbeck) peel	UAE	MeOH:H_2O	80:20	RT			naringin; neohesperdin; *p*-coumaric acid; ferulic acid; sinapic acid; vanillic acid	108
Mangifera indica L. seed kernel	soxhlet	MeOH	100	35	>500	0.3 bar	3-*β*-galactopyrasonyl glucose; shikimic acid; quinic acid; galloyl glucose; galloyl diglucoside; 5-*O*-galloylquinic acid; mangiferin; methyl digallate ester; anacardic acid; ginkgoic acid; tetragalloyl glucose; pentagalloyl glucose	61
Mangifera indica L. pulp	UAE	MeOH:CH_2O_2:H_2O + MeOH:H_2O	80:2:18		30		shikimic acid-hexamalonate; shikimic acid-hexoside; gallic acid; epicatechin gallate-hexamalonate; vanillic acid; chlorogenic acid; protocatechuic acid	58
			80:20		30			
Mangifera indica L. peel	ME	MeOH	100		180	defatted with *n*-hexane	methyl gallate; gallic acid; tetra-*O*-galloyl-glucoside; penta-*O*-galloyl-glucoside; mangiferin; maclurin 3-*C*-(2-*O*-galloyl)-*β*-D-glucoside; maclurin 3-*C*-(2,3-di-*O*-galloyl)-*β*-D-glucoside; iriflophenone 3-*C*-*β*-D-glucoside; maclurin 3-*C*-*β*-D-glucoside	109
Vitis vinifera L. seed	ME	MeOH:H_2O (acidified)	80:20		360		gallic acid; caffeic acid; chlorogenic acid; *p*-coumaric acid; catechin hydrate; epicatechin; epicatechin gallate	63
Vitis vinifera L. pomace	ME	MeOH:H_2O	80:20	RT	120		*β*-type (epi)catechin derivatives; epicatechin; gallic acid; galloyl glucose; catechin; quercetin-glucuronide; laricitrin-3-*O*-galactoside; syringetin-3-*O*-galactoside; laricitrin-3-*O*-rhamnose-7-*O*-trihydroxycinnamic acid; syringetin rutinoside derivative	66
	ME	C_3H_6O-H_2O	50:50	18	60		(−)-epicatechin gallate; (+)-catechin hydrate; gallic acid; quercetin; isorhamnetin; *p*-coumaric acid; malvidin 3-*O*-glucoside; pelargonidin 3-*O*-glucoside	68
		MeOH:H_2O	70:30	18	60		(−)-epicatechin gallate; (+)-catechin hydrate; gallic acid; quercetin; isorhamnetin; malvidin 3-*O*-glucoside; pelargonidin 3-*O*-glucoside	
		Pec-H_2O	0.01:99.9	18	60		(−)-epicatechin gallate; (+)-catechin hydrate; gallic acid	
		petroleum ether	100	18	60		(−)-epicatechin gallate; quercetin; ferulic acid; *p*-coumaric acid	
	ME	MeOH (0.1% HCl)	100	RT	>500		gallic acid; syringic acid; vanillic acid; caffeic acid; quercetin; rutin; catechin; malvidin-3-*O*-glucoside; malvidin-3-*O*-acetylglucoside; malvidin-3-*O*-*p*-coumaroylglucoside	67
Vitis vinifera L. skin	ME	MeOH:H_2O (acidified)	80:20		360		caffeic acid; catechin hydrate; quercetin hydrate; rutin hydrate; *trans*-resveratrol	63
	ME	EtOH	100	RT	180	defatted with *n*-hexane	hydroxytyrosol; gallic acid; tyrosol; protocatechinic acid; vanillic acid; caffeic acid; syringic acid; *o*-coumaric acid; *p*-coumaric acid; catechin; epicatechin; cyanidin glycosides	64

[a]Notes: pressurized liquid extraction (PLE); microwave assisted extraction (MAE); supercritical CO_2 extraction (SC-CO_2); ultrasound assisted extraction (UAE); maceration extraction (ME); multifrequency multimode modulated (MMM) ultrasonic extraction; methanol (MeOH); ethyl acetate (EtOAC); water (H_2O); chloroform ($CHCl_3$); ethanol (EtOH); acetone (C_3H_6O); diethyl ether [$(C_2H_5)_2O$]; acetic acid (CH_3COOH); formic acid (CH_2O_2); hydrochloric acid (HCl); dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA); pectinase (Pec); room temperature (RT).

https://dx.doi.org/10.1021/acs.jafc.0c04984
J. Agric. Food Chem. 2020, 68, 11054−11067

4. BIOACTIVE COMPOUNDS IN AGRO-INDUSTRIAL RESIDUES

Agro-industrial residues have been explored as a source of natural bioactive compounds, such as flavonoids, phenolic acids (e.g., hydroxycinnamic and hydroxybenzoic acid derivatives), tannins, carotenoids, tocopherols, phytosterols, or arabinoxylans. These bioactive compounds exhibit a wide range of potential healthy properties including antiallergenic, antiatherogenic, anti-inflammatory, antimicrobial, anticarcinogenic, antithrombotic, antioxidant, cardioprotective, vasodilatory, or prebiotic effects, among others.[32,33] Numerous examples of the extraction of bioactive compounds from agro-industrial wastes can be found in the literature, as summarized in Table 1.

Phenolic compounds with antioxidant potential have been identified in agricultural residues, such as sugar cane bagasse, corn husk, peanut husk, coffee cherry husk, rice bran, or wheat bran.[34−37] Vijayalaxmi et al. (2015)[37] identified five compounds as the major bioactive compounds present in most of these residues: gallic acid, ferulic acid, epicatechin, quercetin, and kaempferol derivatives. Huang and Lai (2016)[35] established the profiles of bioactive compounds of outer and inner *O. sativa* L. bran from six colored rice samples, detecting protocatechuic, vanillic, ferulic, and *p*-coumaric acids as the most abundant phenolic acids present and α- and γ-tocopherols and γ-tocotrienol as the main vitamin E components. The major anthocyanins present in both black and red *O. sativa* L. were peonidin 3-glucoside, cyanidin 3-rutinoside, and cyanidin 3-glucoside.

A detailed work about the most abundant phenolic compounds in cork and pulp industrial residues was developed by S. A. O. Santos and co-workers.[38−41] The barks of *Eucalyptus globulus* Labill., *Eucalyptus grandis* W.Hill., *Eucalyptus urograndis* (*E. grandis* W.Hill. × *E. urophylla* S.T.Blake), and *Eucalyptus maidenii* F.Muell. as well as the cork from *Quercus suber* L. and its byproducts (cork powder and black condensates) are constituted by different types of phenolic compounds. Epicatechin and quercetin-glucuronide were revealed as the major phenolic compounds in *E. grandis* and *E. urograndis* bark, followed by ellagic acid-rhamnoside and ellagic acid in *E. grandis* and by galloyl-bis-hexahydroxydiphenoyl (HHDP)-glucose and gallic acid in *E. urograndis*. Catechin, chlorogenic acid, and methyl-ellagic acid-pentose were the major compounds in *E. maidenii* bark. Moreover, ellagic acid-rhamnoside, dihydroxy-isopropylchromone-hexoside, and dihydroxy-(methylpropyl)isopropylchromone-hexoside were referenced for the first time in these species.[41] Santos et al. (2011)[39] obtained the phenolic profile of *E. globulus* bark using different solvents (methanol, water, and methanol/water), with methanol/water mixtures being the most efficient to isolate polyphenols. Digalloylglucose was identified as the major compound in the methanol and methanol/water extracts, followed by isorhamnetin-rhamnoside in the methanol extract and by catechin in the methanol/water extract, whereas in the water extract catechin and galloyl-HHDP-glucose were identified as the predominant components. Other compounds were referenced for the first time as constituents of *E. globulus* bark, namely, quinic, dihydroxyphenylacetic, and caffeic acids, bis(hexahydroxydiphenoyl (HHDP))-glucose, galloyl-bis-(HHDP)-glucose, galloyl-HHDP-glucose, isorhamnetin-hexoside, quercetin-hexoside, methylellagic acid (EA)-pentose conjugate, myricetin-rhamnoside, isorhamnetin-rhamnoside, mearnsetin, phloridzin, mearnsetin-hexoside, luteolin, and a proanthocyanidin B-type dimer. In another work, Santos, Villaverde, and Silva et al. (2012)[40] analyzed the supercritical fluid extraction of phenolic compounds from *E. globulus* bark, using supercritical CO_2 alone and modified with water, ethyl acetate, and ethanol. In terms of the extraction yield and antioxidant activity of phenolic components, the best results were achieved with CO_2/EtOH, recovering much higher quantities of eriodictyol, naringenin, and isorhamentin than in the conventional solid/liquid extracts obtained before.

The major components identified in the cork from *Quercus suber* L. were ellagic acid, followed by gallic, protocatechuic, and caffeic acids and esculetin.[38] Those authors identified for the first time salicylic acid, eriodictyol, and quinic acid in cork extracts. Another byproduct from the cork industry is the effluent generated from the plank immersion on boiled water to improve characteristics such as elasticity and homogeneity and to make it flat. This wastewater also contains considerable amounts of phenolic compounds, namely, tannins and phenolic acids, such as gallic, protocatechuic, syringic, ferulic, and ellagic acids.[42]

From the cocoa and chocolate industry, the main byproducts obtained from the cocoa bean are pod husks, bean shells, and mucilage, which are recognized as important sources of bioactive compounds.[43] Phenolic compounds recovered from *Theobroma cacao* L. pod husk mostly consist of catechin, quercetin, epicatechin, and gallic, coumaric, and protocatechuic acids[43] and also flavonols (kaempferol) and flavones (linarin).[44]

The wastes generated in the olive oil industry are also important sources of phenolic compounds[27,45−54] with antiproliferative and antimicrobial properties.[46,48,51] Besides differences in the olive oil extraction technique, the phenolic composition of the olive wastes depends on the geographical areas, as they influence agricultural, varietal, and seasonal practices, e.g., the fruit variety and maturity stage.[46,49] Oleuropein is the most abundant compound present in *Olea europaea* L. leaves,[51,52,54,55] followed by verbascoside and isoverbascoside, oleoside, hydroxytyrosol (3,4-dihydroxyphenolethanol; 3,4-DHPEA), luteolin-7-*O*-glucoside, and ligstroside,[52] as well as apigenin-7-*O*-glucoside and luteolin-4′-*O*-glucoside.[51] Studies were performed to characterize the phenolic profile of olive wastes (mill wastewaters and olive cake) from four different areas of a region in Morocco,[48,49] concluding that the phenolic composition was different according to the geographical area, with hydroxytyrosol, tyrosol, *p*-coumaric acid, and hydroxytyrosol glucoside as the main compounds. El-Abbassi et al. (2012)[47] also reported hydroxytyrosol, gallic acid, and *p*-coumaric acid as the most abundant phenolic compounds in the olive mill wastewaters. Obied et al. (2008)[50] identified verbascoside as the most potent antioxidant present in Australian olive mill wastes, followed by 3,4-dihydroxyphenylethyl alcohol-deacetoxyelenolic acid dialdehyde (3,4-DHPEA-DEDA).

As mentioned above, the total phenolic content and composition recovered from the biomass also depend on the extraction procedure. The most interesting residue of the olive oil industry is the olive pomace, also known as olive cake. High amounts of oleuropein derivatives, such as elenolic acid (EA), the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA), oleuropein aglycone (3,4-DHPEA-EA), the dialdehydic form of elenolic acid linked to tyrosol (*p*-HPEA-EDA), and hydroxytyrosol, were isolated from olive

https://dx.doi.org/10.1021/acs.jafc.0c04984
J. Agric. Food Chem. 2020, 68, 11054−11067

pomaces from the region of Les Garrigues (Catalonia, Spain).[53] Cioffi et al. (2010)[45] studied the phenolic composition of virgin olive oil and olive oil pomace from the Campania region, in Italy, and found that oleuropein, oleuropein aglycone, ligstroside aglycone, hydroxytyrosol, tyrosol, and gallic acid are the most abundant phenolic compounds in both types of samples. More recently, Antónia Nunes et al. (2018)[27] compared a multifrequency multimode modulated (MMM) ultrasonic technique with a conventional solid−liquid method for the extraction of phenolic compounds from olive pomace of the Trás-os-Montes region in Portugal. The MMM technique increased the concentration of the extracted antioxidant compounds in a shorter period of time. In this sample, the major compounds identified were hydroxytyrosol > comselogoside > elenolic acid derivative > tyrosol > oleoside riboside.

Several fruits and vegetable wastes have also been reported to contain relevant amounts of phenolic compounds with high antioxidant potential. As examples, potato peel,[56] fruit peel[57] and pulp,[58] onionskin,[59] and tomato processing wastes[60] can be indicated. Gorinstein et al. (2001)[57] evaluated the antioxidant properties of some citrus fruit wastes, namely, *Citrus limon* (L.) Osbeck, *Citrus sinensis* (L.) Osbeck, and *Citrus paradise* Macfad. peels, reporting high contents of ferulic, sinapic, *p*-coumaric, caffeic, and ascorbic acids. Concerning onion wastes, Benítez et al. (2011)[59] observed that brown skin, outer and inner scales and top and bottom of *Allium cepa* L. were particularly rich in quercetin-4′-*O*-glucoside and quercetin-3,4′-*O*-diglucoside. In *Lycopersicon esculentum* L. processing wastes, mainly consisting of skin and seeds, the major compounds detected were *p*-coumaric, caffeic, and chlorogenic acids as phenolic acids and chrysin and naringenin as flavonoids.[60] Castro-Vargas et al. (2019)[61] explored the potential of the agro-industrial waste from two Colombian mango cultivars, "sugar mango" and "Tommy Atkins", as sources of bioactive phenolic compounds. The "sugar mango" kernel extracts presented the highest bioactive properties, with mangiferin and several galloyl glucosides as the most abundant compounds. Chlorogenic, gallic, vanillic, and protocatechuic acids were the major phenolic compounds found in *Mangifera indica* L. "Ataulfo" pulp,[58] which tended to increase with fruit ripening and also their contribution for antioxidant activity. More recently, Coelho et al. (2019)[62] demonstrated that mango peels could be used to produce liqueurs by alcoholic maceration. These liqueurs were revealed to have high antioxidant activity, and quantitatively, the main bioactive compounds found were flavanols (epicatechin gallate, epigallocatechin gallate), flavonols (quercetin-3-*O*-glucoside and rutin), and phenolic acids (gallic acid, *o*-coumaric acid, and syringic acid).

Wine production generates different wastes, such as stem, skin, and seeds[63,64] and pomaces.[65−68] Ribeiro et al. (2015)[67] reported the presence of high amounts of gallic, syringic, vanillic, and caffeic acids, quercetin, rutin, and catechin, in addition to anthocyanins, especially malvidin-3-*O*-glucoside, malvidin-3-*O*-acetylglucoside, and malvidin-3-*O*-*p*-coumaroyl-glucoside, in *Vitis vinifera* L. pomace. Doshi et al. (2015)[63] suggested that grape seeds, skin, and stem extracts not only have high antioxidant properties but also have the potential as insulin secretagogues, which might be useful in the treatment of type II diabetes. Gallic acid, catechin, and epicatechin were identified as major phenolic compounds in winery wastes,[64] but hydroxytyrosol, tyrosol, cyanidin glycosides, and various phenolic acids, such as caffeic, syringic, vanillic, *p*-coumaric, and *o*-coumaric acids, were also found.

5. IONIZING RADIATION TECHNOLOGIES

Radiation technologies offer versatile tools that play an important role in supporting sustainable development.[69] Ionizing radiation does not require the addition of chemicals or heat to induce changes in the matter, having a much lower carbon footprint than other technologies. In this way, it is more cost-effective and environmentally friendly than traditional alternatives, requiring less energy and generating less waste.[70]

There are different sources of ionizing radiation used in different sectors of industry and agriculture:[71,72] γ-radiation, that is constituted by photons produced from radioactive isotopes cobalt-60 (^{60}Co) and cesium-137 (^{137}Cs), and accelerators, capable of producing electron beams and X-rays. Radioisotopes are useful due to their permanent source, high efficiency associated with their penetrating power and their easy replacement. Electron-beam accelerators do not rely on a radioactive source and present the advantage that they are able to be switched on and off depending on the necessity.

Radiation technologies are safe processes becoming part of the solution for many countries to develop new tools and approaches in different areas, ranging from health and the environment to industry and infrastructure.[69]

5.1. Radiation Processing. Ionizing radiation is currently used for different applications: medical device sterilization,[73] material modification,[74] heritage preservation,[75] or wastewater treatment,[76] among others. Contrarily to ethylene oxide treatment that can leave toxic residues on treated products, ionizing radiation can be safely used to sterilize healthcare products such as syringes, surgical gloves, or tissue allografts in sealed packages, in order to reduce the microbiological contamination to acceptable levels.[77] This technology can also be applied on the preparation and functionalization of hybrid materials that are used for biomedical applications and food packaging.[74] On the other hand, Borrely et al. (2016)[76] demonstrated that electron-beam radiation was capable of reducing the color (>90% at 2.5 kGy) and toxicity of three different textile effluents and Madureira et al.[78] showed the radiolytic degradation of phenolic acids present in wastewaters from the cork industry. In addition, γ-radiation was used to decontaminate and preserve parchment documents as an alternative to the chemical (e.g., fumigants) and nonchemical (e.g., modified indoor atmospheres) treatments that induce toxicity and deterioration of the documents.[75]

Both types of irradiation have also been used for food processing and have shown to be effective in reducing pathogenic bacteria, eliminating parasites, decreasing post-harvest sprouting, and extending the shelf life of fresh perishable food.[79] Furthermore, irradiation can also be applied to enhance extraction or improve the bioactive properties of some of the chemical compounds present in food, wastes, and plants. In the present review, we will focus on the extractability and bioactive properties of agro-industrial residues.

5.2. Ionizing Radiation in Agro-Industrial Residues: Enhancement of Phenolic Compounds Extractability and Bioactive Properties. Although there is no ideal method for extracting bioactive compounds, ionizing radiation technologies have proven to improve the extraction yield and bioactive properties of agro-industrial residues. In recent years, several studies have been published regarding the extractability

https://dx.doi.org/10.1021/acs.jafc.0c04984
J. Agric. Food Chem. 2020, 68, 11054−11067

Table 2. Irradiation Effects on Phenolic Compounds and Bioactivity of Agro-Industrial Residues[a]

agro-industrial residue	extract	radiation source	doses	main results	identified compounds	references
Quercus suber L. waste-water		γ-radiation	20, 50, and 100 kGy	increase of DPPH scavenging activity in 34% at 100 kGy; increase of reducing power in 33% at 100 kGy; increase of β-carotene bleaching inhibition in 63% at 100 kGy	n.i.	79
Curcuma alismatifolia Gagnep. leaves	80% methanol	γ-radiation	10, 15, and 20 Gy	increase of total phenolics, total flavonoids, and antioxidant activity with increasing radiation doses	salicylic acid; caffeic acid; catechin; epicatechin; cinnamic acid; ellagic acid; resorcinol; rutin; naringin; quercetin; myricetin	80
Rosmarinus officinalis L. leaves	methanol; ethanol; water	γ-radiation	30 kGy	increase of the antioxidant activity of ethanol and water extracts with irradiation; increase on the total phenolic content in water extracts with irradiation	n.i.	81
Ziziphus mauritiana Lam. leaves	methanol; *n*-hexane; water	γ-radiation	2.5, 5, 7.5, 10, and 12.5 kGy	enhancement of certain phytochemicals (phenolics, flavonoids, tannins, and saponins) at doses up to 12.5 kGy; increase of DPPH scavenging activity and extraction yields of *Ziziphus mauritiana* Lam. leaf extracts with irradiation	n.i.	82
Prunus amygdalus Batsch skin	40% ethanol	γ-radiation	2–16 kGy	Blue Diamond company: increase in phenolic content at irradiation doses higher than 4.05 kGy; Campos Brothers company: increase in phenolic content at irradiation doses higher than 12.7 kGy	n.i.	83
apple pomace	84.5% methanol + 65% acetone	γ-radiation	1 and 2 kGy	more extractable individual phenolic compounds at 1 kGy; increase of total phenolic compounds and antioxidant potential at 1 kGy	quercetin-3-*O*-galactoside; quercetin-3-β-D-glucoside; quercetin-3-*O*-rhamnoside; hydroxycinnamic acid; chlorogenic acid; dihydrochalcones; phloridzin; epicatechin; epicatechin; quercetin-3-*O*-rutinoside; procyanidin B2; procyanidin B1; catechin	84
Castanea sativa Mill. skin	methanol	γ-radiation	0.27 and 0.54 kGy	enhancement of antioxidant potential of skins at 0.54 kGy	n.i.	85

[a]n.i.: not identified.

https://dx.doi.org/10.1021/acs.jafc.0c04984
J. Agric. Food Chem. 2020, 68, 11054−11067

and bioactive properties improvement of chemical compounds from irradiated food and agro-wastes, demonstrating the high potentiality of this treatment (Table 2).

A study developed by Madureira et al. (2017)[79] revealed that γ-radiation at 100 kGy was capable of improving (34−62%) the antioxidant activity of wastewaters generated during *Q. suber* L. processing. Taheri, Abdullah, Karimi, Oskoueian, and Ebrahimi (2014)[80] demonstrated that 20 Gy of γ-radiation was enough to significantly increase the total phenolics (2-fold) and total flavonoids (2-fold) contents in *Curcuma alismatifolia* Gagnep. leaves compared to the nonirradiated ones. An increase of 35% on total phenolic content was observed in water extracts of *Rosmarinus officinalis* L. leaves irradiated at 30 kGy but not in methanol or ethanol extracts.[81] The authors associated this increase with the presence of diterpenes in rosemary that could result in water-soluble quinone-type compounds by γ-radiation. For their part, Khattak and Rahman (2016)[82] showed that γ-radiation doses up to 12.5 kGy enhanced the levels of certain phytochemicals (phenolic acids, flavonoids, tannins, and saponins) and increased the DPPH scavenging activity and extraction yield of *Ziziphus mauritiana* Lam. leaves extracts.

Harrison and Were (2007)[83] reported two different irradiation levels causing the enhancement of the total phenolic content and antioxidant activity of *Prunus amygdalus* Batsch. skin extracts, although dependent on the almond skin origin. Almond skins supplied by the company Blue Diamond Growers showed an increase in phenolic content at irradiation doses higher than 4.05 kGy (about 45% compared to the control), while almond skins from Campos Brothers showed an increase in phenolic content at irradiation doses higher than 12.7 kGy (about 20% compared to the control).

Apple pomace is another interesting source of phenolic compounds. Irradiation of apple pomace flour at 1 kGy enhanced the extractability of individual and total phenolic compounds, as well as the antioxidant potential.[84] More specifically, total phenolic compounds increased from 563 ± 50 mg/100 g (0 kGy) to 661 ± 33 mg/100 g (1 kGy) and antioxidant capacity by FRAP assay increased more than 50% (from 36.19 ± 1.36 to 57 ± 1 mmol trolox equivalents/g for nonirradiated and irradiated at 1 kGy sample, respectively).

Chestnuts are also another important source of polyphenols with antioxidant potential. António et al. (2011)[85] reported an increase of the antioxidant capacity in *Castanea sativa* Mill. skins irradiated at 0.54 kGy of 64% evaluated by the β-carotene bleaching inhibition assay.

5.3. Radiation Chemistry. The effect of ionizing radiation on extraction yields can vary depending on the compounds that constitute the studied matrixes. The increase in total phenolic and flavonoid contents on irradiated samples could be due to the release of these compounds from matrix structures, increasing the extractability of certain molecules, or the degradation of larger compounds (e.g., tannins) into smaller ones by the radiolytic action of ionizing radiation.[86] The higher extractability can be explained by changes in cellular structures, namely, by the depolymerization and dissolution of the cell wall polysaccharides by irradiation.[85,87,88] Moreover, irradiation is recognized as promoting the activity of phenylalanine ammonia-lyase, responsible for the synthesis of phenolic compounds,[89] and, consequently, the enhancement of antioxidant activity.

In addition, as mentioned above, the increase or decrease on the antioxidants is strongly dependent on the solvents used for the extraction.[81,82,90]

6. APPLICATIONS OF BIOACTIVE COMPOUNDS EXTRACTED FROM AGRO-INDUSTRIAL RESIDUES

With the growing interest of consumers toward food bioactives that provide beneficial effects on human health promotion and disease risk reduction, the extracts from irradiated residues with improved properties can be incorporated in food products and contribute for the development of functional foods. Different investigations have focused on the development of functional foods through fortification, which consists of incorporating one or more bioactive compounds in order to correct or improve a potential biological activity of the food product. There are several studies concerning the incorporation of bioactive compounds from agro-industrial residues into different dietary matrixes of high importance, namely, bread, pastry, biscuits, and yogurt.[91−94] *Myrciaria dubia* (Kunth) McVaugh peel was used to fortify yogurt,[91] resulting in an enhancement in bioactive components in the new product without significantly altering its nutritional composition and fatty acid profile. *Punica granatum* L. epicarp extracts were incorporated in a typical Brazilian pastry product.[92] The new formulations showed greater antioxidant activity that remained unchanged during the 14 days of storage. At the same time, the cake prepared by new formulations presented a rose color and a soft texture, which can also be important factors for the consumer acceptance. Also, Hallabo et al.[93] substituted wheat flour of biscuits by 6% peel powders of *M. indica* L. cv. Copania, *A. cepa*, and *Solanum tuberosum* L. The obtained results demonstrated that the new prepared biscuits had higher antioxidant activity, total carotenoids, and total polyphenols than the control ones. In another study performed by Mildner-Szkudlarz et al.,[94] a new formulation for sourdough mixed rye bread was produced incorporating a maximum of 6% grape byproducts and presented an improvement in antioxidant activity due to the higher values of phenolic compounds.

There are a few patents on the application of agro-industrial residues on food products.[95] For example, a straightforward method for obtaining polyphenol extracts from white grape residues to having antioxidant and antibacterial properties was patented to be used on an industrial scale in the cosmetic, pharmaceutical, and/or food industries.[96,97] Another process that utilizes cheese/yogurt whey and fruit pomace to directly yield extruded, ready-to-eat products such as breakfast cereals, healthy snacks, protein puffs, and nutrition bars, among other food items, was also patented.[98]

The actual trend is the bioactive compounds production by extraction from residual food sources,[95] although further research on the irradiation of different agro-industrial residues should be performed to increase its applicability and to valorize the wastes by enhancing their health benefits and maintain environmental sustainability.

■ AUTHOR INFORMATION

Corresponding Authors

Sandra Cabo Verde − *Centro de Ciências e Tecnologias Nucleares (C2TN), Instituto Superior Técnico, Universidade de Lisboa, 2695-066 Bobadela LRS, Portugal;* orcid.org/0000-0002-6615-5289; Email: sandracv@ctn.tecnico.ulisboa.pt

https://dx.doi.org/10.1021/acs.jafc.0c04984
J. Agric. Food Chem. 2020, 68, 11054−11067

Isabel C. F. R. Ferreira − *Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, 5300-253 Bragança, Portugal*; Email: iferreira@ipb.pt

Authors

Joana Madureira − *Centro de Ciências e Tecnologias Nucleares (C2TN), Instituto Superior Técnico, Universidade de Lisboa, 2695-066 Bobadela LRS, Portugal; Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, 5300-253 Bragança, Portugal; Grupo de Investigación en Polifenoles (GIP-USAL), Facultad de Farmacia, Universidad de Salamanca, 37007 Salamanca, Spain*

Lillian Barros − *Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, 5300-253 Bragança, Portugal*

Fernanda M. A. Margaça − *Centro de Ciências e Tecnologias Nucleares (C2TN), Instituto Superior Técnico, Universidade de Lisboa, 2695-066 Bobadela LRS, Portugal*

Celestino Santos-Buelga − *Grupo de Investigación en Polifenoles (GIP-USAL), Facultad de Farmacia, Universidad de Salamanca, 37007 Salamanca, Spain*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jafc.0c04984

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support to C^{2}TN (UIDB/04349/2020), CIMO (UIDB/00690/2020), and J.M. (SFRH/BD/136506/2018); L.B. thanks FCT for the national funding, P.I., through the institutional scientific employment program-contract; FEDER-Interreg España-Portugal programme for financial support through the project 0377_Iberphenol_6_E.

■ REFERENCES

(1) Sadh, P. K.; Duhan, S.; Duhan, J. S. Agro-Industrial Wastes and Their Utilization Using Solid State Fermentation: A Review. *Bioresour. Bioprocess.* **2018**, *5* (1), 1−15.

(2) Bharat Helkar, P.; Sahoo, A.; Patil, N. J. Review: Food Industry By-Products Used as a Functional Food Ingredients. *Int. J. Wastes Resour.* **2016**, *6* (3), 1−6. DOI: 10.4172/2252-5211.1000248.

(3) Yusuf, M. Agro-Industrial Waste Materials and Their Recycled Value-Added Applications: Review. In *Handbook of Ecomaterials*; Martínez, L. M. T., Kharissova, O. V., Ildusovich Kharisov, B., Eds.; Springer: 2017. DOI: 10.1007/978-3-319-48281-1.

(4) Carocho, M.; Morales, P.; Ferreira, I. C. F. R. Natural Food Additives: Quo Vadis? *Trends Food Sci. Technol.* **2015**, *45*, 284−295.

(5) Faustino, M.; Veiga, M.; Sousa, P.; Costa, E. M.; Silva, S.; Pintado, M. Agro-Food Byproducts as a New Source of Natural Food Additives. *Molecules* **2019**, *24* (6), 1056.

(6) Shirahigue, L. D.; Ceccato-Antonini, S. R. Agro-Industrial Wastes as Sources of Bioactive Compounds for Food and Fermentation Industries. *Cienc. Rural* **2020**, *50* (4), No. e20190857.

(7) Vuong, Q. V. *Utilisation of Bioactive Compounds from Agricultural and Food Waste*; CRC Press: Boca Raton, FL, 2017. DOI: 10.1201/9781315151540.

(8) Azeez, S.; Narayana, C. K.; Oberoi, H. S. Extraction and Utilisation of Bioactive Compounds from Agricultural Waste. In *Utilisation of Bioactive Compounds from Agricultural and Food Waste*; Vuong, Q. V., Ed.; Taylor & Francis: Boca Raton, 2017.

(9) Santana-Méridas, O.; González-Coloma, A.; Sánchez-Vioque, R. Agricultural Residues as a Source of Bioactive Natural Products. *Phytochem. Rev.* **2012**, *11* (4), 447−466.

(10) Selvamuthukumaran, M.; Shi, J. Recent Advances in Extraction of Antioxidants from Plant By-Products Processing Industries. *Food Qual. Saf.* **2017**, *1* (1), 61−81.

(11) Fierascu, R. C.; Fierascu, I.; Avramescu, S. M.; Sieniawska, E. Recovery of Natural Antioxidants from Agro-Industrial Side Streams through Advanced Extraction Techniques. *Molecules* **2019**, *24*, 4212.

(12) Ghafoor, K.; Hui, T.; Choi, Y. H. Optimization of Ultrasonic-Assisted Extraction of Total Anthocyanins from Grape Peel Using Response Surface Methodology. *J. Food Biochem.* **2011**, *35*, 735−746.

(13) Altemimi, A.; Watson, D. G.; Choudhary, R.; Dasari, M. R.; Lightfoot, D. A. Ultrasound Assisted Extraction of Phenolic Compounds from Peaches and Pumpkins. *PLoS One* **2016**, *11* (2), e0148758.

(14) Bouras, M.; Grimi, N.; Bals, O.; Vorobiev, E. Impact of Pulsed Electric Fields on Polyphenols Extraction from Norway Spruce Bark. *Ind. Crops Prod.* **2016**, *80*, 50−58.

(15) Boussetta, N.; Grimi, N.; Vorobiev, E. Pulsed Electrical Technologies Assisted Polyphenols Extraction from Agricultural Plants and Bioresources: A Review. *Int. J. Food Process. Technol.* **2015**, *2* (1), 1−10.

(16) Mushtaq, M.; Sultana, B.; Bhatti, H. N.; Asghar, M. RSM Based Optimized Enzyme-Assisted Extraction of Antioxidant Phenolics from Underutilized Watermelon (Citrullus Lanatus Thunb.) Rind. *J. Food Sci. Technol.* **2015**, *52* (8), 5048−5056.

(17) Gómez-García, R.; Martínez-Ávila, G. C. G.; Aguilar, C. N. Enzyme-Assisted Extraction of Antioxidative Phenolics from Grape (Vitis Vinifera L.) Residues. *3 Biotech* **2012**, *2* (4), 297−300.

(18) Ranveer, R. C.; Patil, S. N.; Sahoo, A. K. Effect of Different Parameters on Enzyme-Assisted Extraction of Lycopene from Tomato Processing Waste. *Food Bioprod. Process.* **2013**, *91* (4), 370−375.

(19) Thirugnanasambandham, K.; Sivakumar, V. Microwave Assisted Extraction Process of Betalain from Dragon Fruit and Its Antioxidant Activities. *J. Saudi Soc. Agric. Sci.* **2017**, *16* (1), 41−48.

(20) Pavlić, B.; Naffati, A.; Hojan, T.; Vladić, J.; Zeković, Z.; Vidović, S. Microwave-Assisted Extraction of Wild Apple Fruit Dust—Production of Polyphenol-Rich Extracts from Filter Tea Factory by-Products. *J. Food Process Eng.* **2017**, *40* (4), e12508.

(21) Shang, Y.-F.; Hyun Cha, K.; Ha Lee, E.; Pan, C.-H.; Um, B.-H. Optimization, Bio Accessibility of Tricin and Anti-Oxidative Activity of Extract from Black Bamboo Leaves. *Free Radicals Antioxid.* **2016**, *6* (1), 64−71.

(22) Farías-Campomanes, A. M.; Rostagno, M. A.; Coaquira-Quispe, J. J.; Meireles, M. A. A. Supercritical Fluid Extraction of Polyphenols from Lees: Overall Extraction Curve, Kinetic Data and Composition of the Extracts. *Bioresour. Bioprocess* **2015**, *2*, 45.

(23) M'hiri, N.; Ioannou, I.; Paris, C.; Ghoul, M.; Boudhrioua, N. Comparison of the Efficiency of Different Extraction Methods on Antioxidants of Maltease Orange Peel. *Int. J. Food Nutr. Sci.* **2016**, *3* (2), 1−13.

(24) Tabaraki, R.; Ghadiri, F. Comparative Study of Extraction Methods for Pistachio Hull Antioxidants by Multiple Assays. *J. Appl. Chem.* **2016**, *10* (37), 19−30.

(25) Vieira, V.; Prieto, M. A.; Barros, L.; Coutinho, J. A. P.; Ferreira, O.; Ferreira, I. C. F. R. Optimization and Comparison of Maceration and Microwave Extraction Systems for the Production of Phenolic Compounds from Juglans Regia L. for the Valorization of Walnut Leaves. *Ind. Crops Prod.* **2017**, *107*, 341−352.

(26) Backes, E.; Pereira, C.; Barros, L.; Prieto, M. A.; Genena, A. K.; Barreiro, M. F.; Ferreira, I. C. F. R. Recovery of Bioactive Anthocyanin Pigments from Ficus Carica L. Peel by Heat, Microwave, and Ultrasound Based Extraction Techniques. *Food Res. Int.* **2018**, *113*, 197−209.

(27) Antónia Nunes, M.; Costa, A. S. G.; Bessada, S.; Santos, J.; Puga, H.; Alves, R. C.; Freitas, V.; Oliveira, M. B. P. P. Olive Pomace as a Valuable Source of Bioactive Compounds: A Study Regarding Its Lipid- and Water-Soluble Components. *Sci. Total Environ.* **2018**, *644*, 229−236.

(28) Zhang, Q. W.; Lin, L. G.; Ye, W. C. Techniques for Extraction and Isolation of Natural Products: A Comprehensive Review. *Chin. Med. (London, U. K.)* **2018**, *13*, 20.
(29) Brusotti, G.; Cesari, I.; Dentamaro, A.; Caccialanza, G.; Massolini, G. Isolation and Characterization of Bioactive Compounds from Plant Resources: The Role of Analysis in the Ethnopharmacological Approach. *J. Pharm. Biomed. Anal.* **2014**, *87*, 218.
(30) Fierascu, R. C.; Fierascu, I.; Avramescu, S. M.; Sieniawska, E. Recovery of Natural Antioxidants from Agro-Industrial Side Streams through Advanced Extraction Techniques. *Molecules* **2019**, *24* (23), 4212.
(31) Sasidharan, S.; Chen, Y.; Saravanan, D.; Sundram, K. M.; Latha, L. Y. Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts. *Afr. J. Tradit., Complementary Altern. Med.* **2010**, *8* (1), 1−10.
(32) Castro-Vazquez, L.; Alañón, M. E.; Rodríguez-Robledo, V.; Pérez-Coello, M. S.; Hermosin-Gutierrez, I.; Díaz-Maroto, M. C.; Jordán, J.; Galindo, M. F.; Arroyo-Jiménez, M. D. M. Bioactive Flavonoids, Antioxidant Behaviour, and Cytoprotective Effects of Dried Grapefruit Peels (Citrus Paradisi Macf.). *Oxid. Med. Cell. Longevity* **2016**, *2016*, 8915729.
(33) Babbar, N.; Oberoi, H. S.; Sandhu, S. K. Therapeutic and Nutraceutical Potential of Bioactive Compounds Extracted from Fruit Residues. *Crit. Rev. Food Sci. Nutr.* **2015**, *55* (3), 319−337.
(34) Farah, A.; Donangelo, C. M. Phenolic Compounds in Coffee. *Braz. J. Plant Physiol.* **2006**, *18* (1), 23−36.
(35) Huang, Y. P.; Lai, H. M. Bioactive Compounds and Antioxidative Activity of Colored Rice Bran. *J. Food Drug Anal.* **2016**, *24* (3), 564−574.
(36) López-Perea, P.; Guzmán-Ortiz, F. A.; Román-Gutiérrez, A. D.; Castro-Rosas, J.; Gómez-Aldapa, C. A.; Rodríguez-Marín, M. L.; Falfán-Cortés, R. N.; González-Olivares, L. G.; Torruco-Uco, J. G. Bioactive Compounds and Antioxidant Activity of Wheat Bran and Barley Husk in the Extracts with Different Polarity. *Int. J. Food Prop.* **2019**, *22* (1), 646−658.
(37) Vijayalaxmi, S.; Jayalakshmi, S. K.; Sreeramulu, K. Polyphenols from Different Agricultural Residues: Extraction, Identification and Their Antioxidant Properties. *J. Food Sci. Technol.* **2015**, *52* (5), 2761−2769.
(38) Santos, S. A. O.; Pinto, P. C. R. O.; Silvestre, A. J. D.; Neto, C. P. Chemical Composition and Antioxidant Activity of Phenolic Extracts of Cork from Quercus Suber L. *Ind. Crops Prod.* **2010**, *31* (3), 521−526.
(39) Santos, S. A. O.; Freire, C. S. R.; Domingues, M. R. M.; Silvestre, A. J. D.; Neto, C. P. Characterization of Phenolic Components in Polar Extracts of Eucalyptus Globulus Labill. Bark by High-Performance Liquid Chromatography-Mass Spectrometry. *J. Agric. Food Chem.* **2011**, *59* (17), 9386−9393.
(40) Santos, S. A. O.; Villaverde, J. J.; Silva, C. M.; Neto, C. P.; Silvestre, A. J. D. Supercritical Fluid Extraction of Phenolic Compounds from Eucalyptus Globulus Labill Bark. *J. Supercrit. Fluids* **2012**, *71*, 71−79.
(41) Santos, S. A. O.; Villaverde, J. J.; Freire, C. S. R.; Domingues, R. M. M.; Neto, C. P.; Silvestre, A. J. D. Phenolic Composition and Antioxidant Activity of Eucalyptus Grandis, E. Urograndis (E. Grandis × E. Urophylla) and E. Maidenii Bark Extracts. *Ind. Crops Prod.* **2012**, *39* (1), 120−127.
(42) Minhalma, M.; De Pinho, M. N. Tannic-Membrane Interactions on Ultrafiltration of Cork Processing Wastewaters. *Sep. Purif. Technol.* **2001**, *22*−*23*, 479−488.
(43) Valadez-Carmona, L.; Plazola-Jacinto, C. P.; Hernández-Ortega, M.; Hernández-Navarro, M. D.; Villarreal, F.; Necoechea-Mondragón, H.; Ortiz-Moreno, A.; Ceballos-Reyes, G. Effects of Microwaves, Hot Air and Freeze-Drying on the Phenolic Compounds, Antioxidant Capacity, Enzyme Activity and Microstructure of Cacao Pod Husks (Theobroma Cacao L.). *Innovative Food Sci. Emerging Technol.* **2017**, *41*, 378−386.
(44) Abdul Karim, A.; Azlan, A.; Ismail, A.; Hashim, P.; Abd Gani, S. S.; Zainudin, B. H.; Abdullah, N. A. Phenolic Composition, Antioxidant, Anti-Wrinkles and Tyrosinase Inhibitory Activities of Cocoa Pod Extract. *BMC Complementary Altern. Med.* **2014**, *14* (381), 381.
(45) Cioffi, G.; Pesca, M. S.; De Caprariis, P.; Braca, A.; Severino, L.; De Tommasi, N. Phenolic Compounds in Olive Oil and Olive Pomace from Cilento (Campania, Italy) and Their Antioxidant Activity. *Food Chem.* **2010**, *121* (1), 105−111.
(46) Yakhlef, W.; Arhab, R.; Romero, C.; Brenes, M.; de Castro, A.; Medina, E. Phenolic Composition and Antimicrobial Activity of Algerian Olive Products and By-Products. *LWT - Food Sci. Technol.* **2018**, *93* (March), 323−328.
(47) El-Abbassi, A.; Kiai, H.; Hafidi, A. Phenolic Profile and Antioxidant Activities of Olive Mill Wastewater. *Food Chem.* **2012**, *132* (1), 406−412.
(48) Leouifoudi, I.; Mbarki, M.; Tilaoui, M.; Amechrouq, A.; Mostapha, E.; Mouse, H. A.; Zyad, A. Study of the in Vitro Anticancer Activity of Moroccan Phenolic Olive Cake Extracts. *J. Pharmacogn. Phytochem.* **2014**, *2* (March), 154−165.
(49) Leouifoudi, I.; Zyad, A.; Amechrouq, A.; Oukerrou, M. A.; Mouse, H. A.; Mbarki, M. Identification and Characterisation of Phenolic Compounds Extracted from Moroccan Olive Mill Wastewater. *Food Sci. Technol.* **2014**, *34* (2), 249−257.
(50) Obied, H. K.; Prenzler, P. D.; Robards, K.; Graham, E. H. Potent Antioxidant Biophenols from Olive Mill Waste. *Food Chem.* **2008**, *111*, 171−178.
(51) Pereira, A. P.; Ferreira, I. C.; Marcelino, F.; Valentão, P.; Andrade, P. B.; Seabra, R.; Estevinho, L.; Bento, A.; Pereira, J. A. Phenolic Compounds and Antimicrobial Activity of Olive (Olea Europaea L. Cv. Cobrançosa) Leaves. *Molecules* **2007**, *12* (5), 1153−1162.
(52) Quirantes-Piné, R.; Lozano-Sánchez, J.; Herrero, M.; Ibáñez, E.; Segura-Carretero, A.; Fernández-Gutiérrez, A. HPLC-ESI-QTOF-MS as a Powerful Analytical Tool for Characterising Phenolic Compounds in Olive-Leaf Extracts. *Phytochem. Anal.* **2013**, *24* (3), 213−223.
(53) Suárez, M.; Romero, M.-P.; Ramo, T.; Macià, A.; Motilva, M.-J. Methods for Preparing Phenolic Extracts from Olive Cake for Potential Application as Food Antioxidants. *J. Agric. Food Chem.* **2009**, *57*, 1463−1472.
(54) Talhaoui, N.; Gómez-Caravaca, A. M.; León, L.; De la Rosa, R.; Segura-Carretero, A.; Fernández-Gutiérrez, A. Determination of Phenolic Compounds of "Sikitita" Olive Leaves by HPLC-DAD-TOF-MS. Comparison with Its Parents "Arbequina" and "Picual" Olive Leaves. *LWT - Food Sci. Technol.* **2014**, *58* (1), 28−34.
(55) Quirantes-Piné, R.; Zurek, G.; Barrajón-Catalán, E.; Bäßmann, C.; Micol, V.; Segura-Carretero, A.; Fernández-Gutiérrez, A. A Metabolite-Profiling Approach to Assess the Uptake and Metabolism of Phenolic Compounds from Olive Leaves in SKBR3 Cells by HPLC-ESI-QTOF-MS. *J. Pharm. Biomed. Anal.* **2013**, *72*, 121−126.
(56) Mattila, P.; Hellström, J. Phenolic Acids in Potatoes, Vegetables, and Some of Their Products. *J. Food Compos. Anal.* **2007**, *20* (3−4), 152−160.
(57) Gorinstein, S.; Martín-Belloso, O.; Park, Y. S.; Haruenkit, R.; Lojek, A.; Ďuračková, M.; Caspi, A.; Libman, I.; Trakhtenberg, S. Comparison of Some Biochemical Characteristics of Different Citrus Fruits. *Food Chem.* **2001**, *74* (3), 309−315.
(58) Palafox-Carlos, H.; Yahia, E. M.; González-Aguilar, G. A. Identification and Quantification of Major Phenolic Compounds from Mango (Mangifera Indica, Cv. Ataulfo) Fruit by HPLC-DAD-MS/MS-ESI and Their Individual Contribution to the Antioxidant Activity during Ripening. *Food Chem.* **2012**, *135* (1), 105−111.
(59) Benítez, V.; Mollá, E.; Martín-Cabrejas, M. A.; Aguilera, Y.; López-Andréu, F. J.; Cools, K.; Terry, L. A.; Esteban, R. M. Characterization of Industrial Onion Wastes (Allium Cepa L.): Dietary Fibre and Bioactive Compounds. *Plant Foods Hum. Nutr.* **2011**, *66* (1), 48−57.
(60) Kalogeropoulos, N.; Chiou, A.; Pyriochou, V.; Peristeraki, A.; Karathanos, V. T. Bioactive Phytochemicals in Industrial Tomatoes

https://dx.doi.org/10.1021/acs.jafc.0c04984
J. Agric. Food Chem. 2020, 68, 11054−11067

and Their Processing Byproducts. *LWT - Food Sci. Technol.* **2012**, *49* (2), 213−216.
(61) Castro-Vargas, H.; Ballesteros Vivas, D.; Ortega Barbosa, J.; Morantes Medina, S.; Aristizabal Gutierrez, F.; Parada-Alfonso, F. Bioactive Phenolic Compounds from the Agroindustrial Waste of Colombian Mango Cultivars 'Sugar Mango' and 'Tommy Atkins'—An Alternative for Their Use and Valorization. *Antioxidants* **2019**, *8* (2), 41.
(62) Coelho, E. M.; de Souza, M. E. A. O.; Corrêa, L. C.; Viana, A. C.; De Azevêdo, L. C.; Lima, M. D. S. Bioactive Compounds and Antioxidant Activity of Mango Peel Liqueurs (Mangifera Indica L.) Produced by Different Methods of Maceration. *Antioxidants* **2019**, *8* (4), 1−11.
(63) Doshi, P.; Adsule, P.; Banerjee, K.; Oulkar, D. Phenolic Compounds, Antioxidant Activity and Insulinotropic Effect of Extracts Prepared from Grape (Vitis Vinifera L.) Byproducts. *J. Food Sci. Technol.* **2015**, *51* (1), 181−190.
(64) Lafka, T. I.; Sinanoglou, V.; Lazos, E. S. On the Extraction and Antioxidant Activity of Phenolic Compounds from Winery Wastes. *Food Chem.* **2007**, *104* (3), 1206−1214.
(65) Iora, S. R. F.; Maciel, G. M.; Zielinski, A. A. F.; da Silva, M. V.; Pontes, P. V. d. A.; Haminiuk, C. W. I.; Granato, D. Evaluation of the Bioactive Compounds and the Antioxidant Capacity of Grape Pomace. *Int. J. Food Sci. Technol.* **2015**, *50* (1), 62−69.
(66) Peixoto, C. M.; Dias, M. I.; Alves, M. J.; Calhelha, R. C.; Barros, L.; Pinho, S. P.; Ferreira, I. C. F. R. Grape Pomace as a Source of Phenolic Compounds and Diverse Bioactive Properties. *Food Chem.* **2018**, *253*, 132−138.
(67) Ribeiro, L. F.; Ribani, R. H.; Francisco, T. M. G.; Soares, A. A.; Pontarolo, R.; Haminiuk, C. W. I. Profile of Bioactive Compounds from Grape Pomace (Vitis Vinifera and Vitis Labrusca) by Spectrophotometric, Chromatographic and Spectral Analyses. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2015**, *1007*, 72−80.
(68) Ramirez-Lopez, L. M.; DeWitt, C. A. M. Analysis of Phenolic Compounds in Commercial Dried Grape Pomace by High-Performance Liquid Chromatography Electrospray Ionization Mass Spectrometry. *Food Sci. Nutr.* **2014**, *2* (5), 470−477.
(69) Amano, Y. Radiation Technology for a More Prosperous and Sustainable Future. *IAEA Bull.* **2017**, *58* (1), 1.
(70) *Food Irradiation Technologies: Concepts, Applications and Outcomes*; Ferreira, I. C. F. R., Antonio, A. L., Cabo Verde, S., Eds.; Royal Society of Chemistry: 2018.
(71) Chernyaev, A. P.; Varzar, S. M.; Belousov, A. V.; Zheltonozhskaya, M. V.; Lykova, E. N. Prospects of Development of Radiation Technologies in Russia. *Phys. At. Nucl.* **2019**, *82* (5), 513−527.
(72) Chmielewski, A. G.; Haji-Saeid, M. Radiation Technologies: Past, Present and Future. *Radiat. Phys. Chem.* **2004**, *71* (1−2), 17−21.
(73) Buchalla, R.; Schüttler, C.; Bögl, K. W. Radiation Sterilization of Medical Devices. Effects of Ionizing Radiation on Ultra-High Molecular-Weight Polyethylene. *Radiat. Phys. Chem.* **1995**, *46* (4−6), 579−585.
(74) Lancastre, J. J. H.; Falcão, A. N.; Margaça, F. M. A.; Ferreira, L. M.; Miranda Salvado, I. M.; Casimiro, M. H.; Almásy, L.; Meiszterics, A. Influence of the Polymer Molecular Weight on the Microstructure of Hybrid Materials Prepared by γ-Irradiation. *Radiat. Phys. Chem.* **2015**, *106*, 126−129.
(75) Nunes, I.; Mesquita, N.; Cabo Verde, S.; Carolino, M. M.; Portugal, A.; Botelho, M. L. Bioburden Assessment and Gamma Radiation Inactivation Patterns in Parchment Documents. *Radiat. Phys. Chem.* **2013**, *88*, 82−89.
(76) Borrely, S. I.; Morais, A. V.; Rosa, J. M.; Badaró-pedroso, C.; Pereira, C.; Higa, M. C. Decoloration and Detoxification of Effluents by Ionizing Radiation. *Radiat. Phys. Chem.* **2016**, *124*, 198−202.
(77) Parsons, B. J. Sterilisation of Healthcare Products by Ionising Radiation: Principles and Standards. *Sterilisation of Biomaterials and Medical Devices*; Woodhead Publishing Limited: 2012; pp 56−70. DOI: 10.1533/9780857096265.56.
(78) Madureira, J.; Barros, L.; Melo, R.; Cabo Verde, S.; Ferreira, I. C. F. R.; Margaça, F. M. A. Degradation of Phenolic Acids by Gamma Radiation as Model Compounds of Cork Wastewaters. *Chem. Eng. J.* **2018**, *341*, 227−237.
(79) Madureira, J.; Pimenta, A. I.; Popescu, L.; Besleaga, A.; Dias, M. I.; Santos, P. M. P.; Melo, R.; Ferreira, I. C. F. R.; Cabo Verde, S.; Margaça, F. M. A. Effects of Gamma Radiation on Cork Wastewater: Antioxidant Activity and Toxicity. *Chemosphere* **2017**, *169*, 139−145.
(80) Taheri, S.; Abdullah, T. L.; Karimi, E.; Oskoueian, E.; Ebrahimi, M. Antioxidant Capacities and Total Phenolic Contents Enhancement with Acute Gamma Irradiation in Curcuma Alismatifolia (Zingiberaceae) Leaves. *Int. J. Mol. Sci.* **2014**, *15* (7), 13077−13090.
(81) Pérez, M. B.; Calderón, N. L.; Croci, C. A. Radiation-Induced Enhancement of Antioxidant Activity in Extracts of Rosemary (Rosmarinus Officinalis L.). *Food Chem.* **2007**, *104* (2), 585−592.
(82) Khattak, K. F.; Rahman, T. U. Effect of Gamma Irradiation on the Vitamins, Phytochemicals, Antimicrobial and Antioxidant Properties of Ziziphus Mauritiana Lam. Leaves. *Radiat. Phys. Chem.* **2016**, *127*, 243−248.
(83) Harrison, K.; Were, L. M. Effect of Gamma Irradiation on Total Phenolic Content Yield and Antioxidant Capacity of Almond Skin Extracts. *Food Chem.* **2007**, *102* (3), 932−937.
(84) Ito, V. C.; Alberti, A.; Avila, S.; Spoto, M.; Nogueira, A.; Wosiacki, G. Effects of Gamma Radiation on the Phenolic Compounds and in Vitro Antioxidant Activity of Apple Pomace Flour during Storage Using Multivariate Statistical Techniques. *Innovative Food Sci. Emerging Technol.* **2016**, *33*, 251−259.
(85) António, A. L.; Fernandes, Â.; Barreira, J. C. M.; Bento, A.; Botelho, M. L.; Ferreira, I. C. F. R. Influence of Gamma Irradiation in the Antioxidant Potential of Chestnuts (Castanea Sativa Mill.) Fruits and Skins. *Food Chem. Toxicol.* **2011**, *49* (9), 1918−1923.
(86) Hussain, P. R.; Suradkar, P.; Javaid, S.; Akram, H.; Parvez, S. Influence of Postharvest Gamma Irradiation Treatment on the Content of Bioactive Compounds and Antioxidant Activity of Fenugreek (Trigonella Foenum-Graceum L.) and Spinach (Spinacia Oleracea L.) Leaves. *Innovative Food Sci. Emerging Technol.* **2016**, *33*, 268−281.
(87) Štajner, D.; Milošević, M.; Popović, B. M. Irradiation Effects on Phenolic Content, Lipid and Protein Oxidation and Scavenger Ability of Soybean Seeds. *Int. J. Mol. Sci.* **2007**, *8* (7), 618−627.
(88) Behgar, M.; Ghasemi, S.; Naserian, A.; Borzoie, A.; Fatollahi, H. Gamma Radiation Effects on Phenolics, Antioxidants Activity and in Vitro Digestion of Pistachio (Pistachia Vera) Hull. *Radiat. Phys. Chem.* **2011**, *80* (9), 963−967.
(89) Oufedjikh, H.; Mahrouz, M.; Amiot, M. J.; Lacroix, M. Effect of γ-Irradiation on Phenolic Compounds and Phenylalanine Ammonia-Lyase Activity during Storage in Relation to Peel Injury from Peel of Citrus Clementina Hort. Ex. Tanaka. *J. Agric. Food Chem.* **2000**, *48* (2), 559−565.
(90) Kim, J. W.; Lee, B. C.; Lee, J. H.; Nam, K. C.; Lee, S. C. Effect of Electron-Beam Irradiation on the Antioxidant Activity of Extracts from Citrus Unshiu Pomaces. *Radiat. Phys. Chem.* **2008**, *77* (1), 87−91.
(91) Conceição, N.; Albuquerque, B. R.; Pereira, C.; Corrêa, R. C. G.; Lopes, C. B.; Calhelha, R. C.; Alves, M. J.; Barros, L.; C F R Ferreira, I. By-Products of Camu-Camu [Myrciaria Dubia (Kunth) McVaugh] as Promising Sources of Bioactive High Added-Value Food Ingredients: Functionalization of Yogurts. *Molecules* **2020**, *25* (1), 70.
(92) da Silva Veloso, F.; Caleja, C.; Calhelha, R. C.; Pires, T. C. S.; Alves, M. J.; Barros, L.; Genena, A. K.; Barreira, J. C. M.; Ferreira, I. C. F. R. Characterization and Application of Pomegranate Epicarp Extracts as Functional Ingredients in a Typical Brazilian Pastry Product. *Molecules* **2020**, *25* (7), 1481.
(93) Hallabo, S. A. S.; Helmy, S. A.; Elhassaneen, Y.; Shaaban, M. Utilization of Mango, Onion and Potato Peels as Sources of Bioactive Compounds in Biscuits Processing. *Biosci. Res.* **2018**, *15* (4), 3647−3657.

(94) Mildner-Szkudlarz, S.; Zawirska-Wojtasiak, R.; Szwengiel, A.; Pacyński, M. Use of Grape By-Product as a Source of Dietary Fibre and Phenolic Compounds in Sourdough Mixed Rye Bread. *Int. J. Food Sci. Technol.* **2011**, *46* (7), 1485−1493.
(95) Bonilla, J.; Vargas, F. C.; de Oliveira, T. G.; da Aparecida Makishi, G. L.; do Amaral Sobral, P. J. Recent Patents on the Application of Bioactive Compounds in Food: A Short Review. *Current Opinion in Food Science*; Elsevier Ltd: 2015; pp 1−7. DOI: 10.1016/j.cofs.2015.05.012.
(96) WO2014013122. Polyphenol Extract from White-Grape Residue. https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2014013122&recNum=201&docAn=ES201370526&queryString=(ANA:ES)&maxRec=26774 (accessed Sept 8, 2020).
(97) Lores Aguin, M.; Garcia Jares, C.; Alvarez Casas, M.; Llompart, M. Polyphenol Extract from White-Grape Residue. January 23, 2014.
(98) US20150282507A1. Extrusion of agro-food industry by-products and protein concentrates into value-added foods. https://patents.google.com/patent/US20150282507A1/en (accessed Sept 8, 2020).
(99) Bravo, J.; Juániz, I.; Monente, C.; Caemmerer, B.; Kroh, L. W.; De Peña, M. P.; Cid, C. Evaluation of Spent Coffee Obtained from the Most Common Coffeemakers as a Source of Hydrophilic Bioactive Compounds. *J. Agric. Food Chem.* **2012**, *60* (51), 12565−12573.
(100) De Freitas, T. B.; Santos, C. H. K.; da Silva, M. V.; Shirai, M. A.; Dias, M. I.; Barros, L.; Barreiro, M. F.; Ferreira, I. C. F. R.; Gonçalves, O. H.; Leimann, F. V. Antioxidants Extraction from Pinhão (Araucaria Angustifolia (Bertol.) Kuntze) Coats and Application to Zein Films. *Food Packag. Shelf Life* **2018**, *15*, 28−34.
(101) Santos, C. H. K.; Baqueta, M. R.; Coqueiro, A.; Dias, M. I.; Barros, L.; Barreiro, M. F.; Ferreira, I. C. F. R.; Gonçalves, O. H.; Bona, E.; da Silva, M. V.; Leimann, F. V. Systematic Study on the Extraction of Antioxidants from Pinhão (Araucaria Angustifolia (Bertol.) Kuntze) Coat. *Food Chem.* **2018**, *261*, 216.
(102) Zan, R. A.; Fernandes, Â.; Jedoz, S.; Oludemi, T.; Calhelha, R. C.; Pires, T. C. S. P.; Alves, M. J.; Martins, R. C. C.; Barros, L.; Ferreira, I. C. F. R. Bioactive Properties and Phytochemical Assessment of Bacupari-Anão (Garcinia Brasiliensis Mart.) Leaves Native to Rondônia, Brazil. *Food Funct.* **2018**, *9* (11), 5621−5628.
(103) Souilem, F.; Dias, M. I.; Barros, L.; Calhelha, R. C.; Alves, M. J.; Harzallah-Skhiri, F.; Ferreira, I. C. F. R. Phenolic Profile and Bioactive Properties of Carissa Macrocarpa (Eckl.) A.DC.: An in Vitro Comparative Study between Leaves, Stems, and Flowers. *Molecules* **2019**, *24* (9), 1696.
(104) Dias, M.; Caleja, C.; Pereira, C.; Calhelha, R. C.; Kostic, M.; Sokovic, M.; Tavares, D.; Baraldi, I. J.; Barros, L.; Ferreira, I. C. F. R. Chemical Composition and Bioactive Properties of Byproducts from Two Different Kiwi Varieties. *Food Res. Int.* **2020**, *127*, 108753.
(105) Melgar, B.; Dias, M. I.; Ciric, A.; Sokovic, M.; Garcia-Castello, E. M.; Rodriguez-Lopez, A. D.; Barros, L.; Ferreira, I. C. R. F. Bioactive Characterization of Persea Americana Mill. by-Products: A Rich Source of Inherent Antioxidants. *Ind. Crops Prod.* **2018**, *111*, 212−218.
(106) Pavlović, A. V.; Papetti, A.; Zagorac, D. Č. D.; Gašić, U. M.; Mišić, D. M.; Tešić, Ž. L.; Natić, M. M. Phenolics Composition of Leaf Extracts of Raspberry and Blackberry Cultivars Grown in Serbia. *Ind. Crops Prod.* **2016**, *87*, 304−314.
(107) Albuquerque, B. R.; Pereira, C.; Calhelha, R. C.; José Alves, M.; Abreu, R. M. V.; Barros, L.; Oliveira, M. B. P. P.; Ferreira, I. C. F. R. Jabuticaba Residues (Myrciaria Jaboticaba (Vell.) Berg) Are Rich Sources of Valuable Compounds with Bioactive Properties. *Food Chem.* **2020**, *309*, 125735.
(108) Xu, G.; Ye, X.; Chen, J.; Liu, D. Effect of Heat Treatment on the Phenolic Compounds and Antioxidant Capacity of Citrus Peel Extract. *J. Agric. Food Chem.* **2007**, *55*, 330−335.
(109) Barreto, J. C.; Trevisan, M. T. S.; Hull, W. E.; Erben, G.; De Brito, E. S.; Pfundstein, B.; Rtele, G. W.; Spiegelhalder, B.; Owen, R. W. Characterization and Quantitation of Polyphenolic Compounds in Bark, Kernel, Leaves, and Peel of Mango (Mangifera Indica L.). *J. Agric. Food Chem.* **2008**, *56*, 5599−5610.

Publicación 3. Applications of bioactive compounds extracted from olive industry wastes: A review

Joana Madureira, Fernanda M.A. Margaça, Celestino Santos-Buelga, Isabel C.F.R. Ferreira, Sandra Cabo Verde, Lillian Barros

Artículo publicado en *Comprehensive Reviews in Food Science and Food Safety* (2022), 21: 453-476. Doi: https://doi.org/10.1111/1541-4337.12861

Resumen

Los residuos generados durante el proceso de extracción del aceite de oliva presentan un elevado impacto negativo para el medio ambiente, aunque también contienen una variedad de compuestos bioactivos considerados beneficiosos para la salud. La gama de actividades biológicas, propiedades tecnológicas y efectos farmacológicos que pueden presentar hacen que, después de una adecuada extracción y purificación, estos compuestos se puedan utilizar, por ejemplo, como antioxidantes alimentarios o como principios activos en productos nutracéuticos y cosméticos.

En este artículo se revisan los diferentes tipos de residuos generados por la industria oleícola y sus características generales, para después enfocarse en el estudio del orujo de aceituna producido por el sistema de dos fases y explorar las aplicaciones de los principales compuestos presentes en este subproducto. En particular, se discuten las propiedades de hidroxitirosol, tirosol, oleuropeína y su aglicón y verbascósido, como compuestos bioactivos más habitualmente abundantes en el orujo de oliva. Además de su capacidad antioxidante, estos compuestos también presentan otras actividades biológicas, como antimicrobianos, anticancerígenos o antiinflamatorios, que pueden ser de gran interés para la fortificación de alimentos, elaboración de suplementos y alimentos funcionales o el desarrollo de nuevas formulaciones cosméticas o farmacéuticas.

La revisión realizada pone de manifiesto el enorme potencial del orujo de oliva para la obtención de ingredientes con características saludables y de elevado valor añadido. Es necesario, sin embargo, continuar profundizando en el conocimiento de sus propiedades, efectos y aplicaciones, así como el desarrollo de nuevos procesos de extracción verdes y eficientes, que faciliten una mayor recuperación de los compuestos de interés con el adecuado grado de pureza para su utilización industrial. De este modo, se podrá contribuir a su valorización, asegurando la rentabilidad económica y la sostenibilidad ambiental.

Received: 1 July 2021 | Revised: 20 September 2021 | Accepted: 23 September 2021

DOI: 10.1111/1541-4337.12861

COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

Comprehensive REVIEWS WILEY

Applications of bioactive compounds extracted from olive industry wastes: A review

Joana Madureira[1,2,3] | **Fernanda M. A. Margaça**[1] | **Celestino Santos-Buelga**[3,4] | **Isabel C. F. R. Ferreira**[2] | **Sandra Cabo Verde**[1] | **Lillian Barros**[2]

[1] Centro de Ciências e Tecnologias Nucleares (C2TN), Instituto Superior Técnico, Universidade de Lisboa, Loures, Portugal

[2] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal

[3] Grupo de Investigación en Polifenoles (GIP-USAL), Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno s /n, Salamanca, Spain

[4] Unidad de Excelencia Producción, Agrícola y Medioambiente (AGRIENVIRONMENT), Parque Científico, Universidad de Salamanca, Salamanca, Spain

Correspondence
Sandra Cabo Verde, Centro de Ciências e Tecnologias Nucleares (C2TN), Instituto Superior Técnico, Universidade de Lisboa, E.N. 10 ao km 139.7, 2695-066 Bobadela LRS, Portugal.
Email: sandracv@ctn.tecnico.ulisboa.pt
Lillian Barros, Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança 5300-253, Portugal.
Email: lillian@ipb.pt

Abstract

The wastes generated during the olive oil extraction process, even if presenting a negative impact for the environment, contain several bioactive compounds that have considerable health benefits. After suitable extraction and purification, these compounds can be used as food antioxidants or as active ingredients in nutraceutical and cosmetic products due to their interesting technological and pharmaceutical properties. The aim of this review, after presenting general applications of the different types of wastes generated from this industry, is to focus on the olive pomace produced by the two-phase system and to explore the challenging applications of the main individual compounds present in this waste. Hydroxytyrosol, tyrosol, oleuropein, oleuropein aglycone, and verbascoside are the most abundant bioactive compounds present in olive pomace. Besides their antioxidant activity, these compounds also demonstrated other biological properties such as antimicrobial, anticancer, or anti-inflammatory, thus being used in formulations to produce pharmaceutical and cosmetic products or in the fortification of food. Nevertheless, it is mandatory to involve both industries and researchers to create strategies to valorize these byproducts while maintaining environmental sustainability.

KEYWORDS
bioactive compounds, cosmetics, food fortification, olive wastes, pharmaceuticals

1 | INTRODUCTION

In the Mediterranean region, mainly in countries such as Spain, Italy, Portugal, Greece, Syria, Morocco, and Tunisia, the olive oil production is one of the most important industries for their economy. European Union countries produce about 69% of the world's olive oil, being the leading producer, consumer, and exporter of this product. In the last years, other countries such as Argentina, Australia, United States of America, and South Africa became olive oil producers (Dermeche et al., 2013; Roig et al., 2006) since the global consumption of olive oil has increased worldwide. As the olive oil benefits for human health are becoming widely recognized, the perspective is that of a continuous increase in its consumption (and consequent production) in the coming years.

The extraction of olive oil includes different processes such as olive washing, olive crushing, malaxing of the

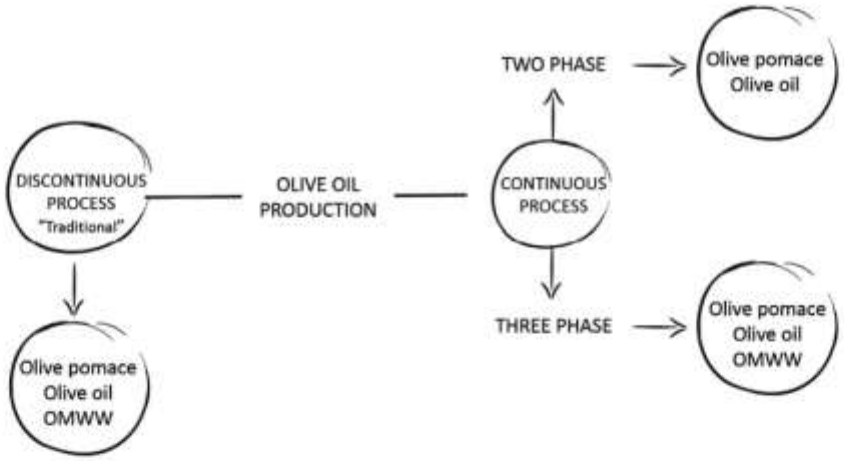


FIGURE 1 Different processes for olive oil production. OMWW, olive mill wastewater. Adapted from Rodrigues et al. (2015)

resulting pastes and the extraction itself (Roig et al., 2006; Zbakh & El Abbassi, 2012). The extraction of olive oil can be achieved through discontinuous (traditional pressing) or continuous (centrifugation) processes. Concerning the centrifugation processes, there are two possible systems, called three-phase and two-phase systems. In the three-phase system, a solid cake and two liquids, olive oil and large amounts of an aqueous liquid known as olive mill wastewater (OMWW) are generated. In the two-phase system, less water is used during the process which means that the volume of OMWW produced is reduced in comparison with the other process. In this system, besides olive oil, a semisolid residue (wet pomace or olive pomace) constituted by olive husk and OMWW is generated (Caporaso et al., 2018; Rodrigues et al., 2015). Figure 1 summarizes the processes for olive oil extraction. The two-phase system is used in the modern units replacing the three-phase technology, in order to minimize the wastewater volume and energy requirements (Dermeche et al., 2013). In fact, Azbar et al. (2004) indicated that two-phase technology saves process water by 80% and energy up to 20%. Nevertheless, its residues have a negative impact on the environment when they are discharged without treatment, due to their high toxicity and the resistance to biological degradation (Al-Khatib et al., 2009; Fiorentino et al., 2003; Khdair et al., 2019; Pavlidou et al., 2014).

Although some papers are found describing the composition of olive wastes and their applications in different areas (Bhatnagar et al., 2014; Galanakis, 2018; Rodrigues et al., 2015), there is no review that evaluated and compiled the possible applications for each of its main bioactive compounds, individually. To our knowledge, the only review describing the use of hydroxytyrosol as a functional food ingredient, not only as pure compound but also in the form of hydroxytyrosol-rich extracts, was published by Silva et al. (2020). In the present review, the applications of the different wastes generated from the olive oil industry will be described. After that, the review will focus on the solid residue (olive pomace) produced by modern two-phase system. Phenolic characterization and quantification will be discussed and the challenging applications of the individual compounds will be carefully explored.

2 | POTENTIAL APPLICATIONS OF OLIVE WASTES

Regardless of its negative environmental impacts, the potential added value of olive wastes for numerous sectors is well known. In the literature, there are a few reviews reporting the different applications for olive wastes (Dermeche et al., 2013; Roig et al., 2006).

Olive wastes have been used as soil amendment, to increase soil fertility and organic carbon stored in the agro-systems (Federici et al., 2017; Majbar et al., 2018; Regni et al., 2017). Fernández-Hernández et al. (2014) observed amended soils with higher content of nitrogen, phosphorus, potassium, and organic matter than the soil treated with inorganic fertilizer when using olive wastes composts mixed with different agro-industrial wastes applied to an olive grove in Spain. The increase of soil fertility produced an increase in the olive oil content of the fruits (Fernández-Hernández et al., 2014), although other researchers considered that recycling these wastes could promote soil phytotoxicity, considerable decline in soil germination capability, and necrosis of the olive leaves (Arvanitoyannis & Kassaveti, 2007).

Olive wastes can also be used as biomass to produce renewable fuels (Tayeh et al., 2014; Al Afif & Linke, 2019; Al-Addous et al., 2017; Messineo et al., 2020; Rincón et al., 2013; Romero-García et al., 2014; Serrano et al., 2017; Valenti et al., 2017). In a recent review paper, Messineo et al. (2020) reported the latest achievements in anaerobic digestion of olive mill residues in order to produce biofuels. In that review, the authors described not only the aspects of the process but also the existing pretreatments of olive wastes (Battista et al., 2016; Rincón et al., 2013; Siciliano et al., 2016) which are applied to induce the decomposition of the complex lignocellulosic structures before anaerobic digestion (Kumari & Singh, 2018). Serrano et al. (2017) proposed a thermal pretreatment and a subsequent phenolic recovery before the anaerobic digestion step to improve methane production. Furthermore, some chemical and physical pretreatment methods, such as ultrasonic pretreatment, basic pretreatment with sodium hydroxide, calcium carbonate, and/or hydrogen peroxide were used to optimize hydrogen and bioethanol production from olive oil production residues (Battista et al., 2016; Siciliano et al., 2016).

Other way to valorize olive wastes is to convert them into inexpensive adsorbents for water pollution control (Anastopoulos et al., 2015), in particular, heavy metals (Abdelhadi et al., 2017; Fernández-González et al., 2019; Fernando et al., 2009; Martinez-Garcia et al., 2006; Martin-Lara et al., 2013; Pagnanelli et al., 2003). Martinez-Garcia et al. (2006) observed that olive wastes maintained their adsorptive capacity over 10 cycles. Moreover, the ability of these biosorbents to adsorb several metal ions may increase their potential application on industrial wastewater treatment (Abdelhadi et al., 2017; Martinez-Garcia et al., 2006).

Other interesting and valuable use of olive mill wastes is to replace fresh water in brick manufacturing which contributes to a reduction of the water consumption (De La Casa & Castro, 2014; de la Casa et al., 2009; Eliche-Quesada et al., 2014; Mekki et al., 2006; Mekki et al., 2008). Comparing their physical properties with control products using fresh water, promising results have been obtained showing a significant increase in the volume shrinkage (10%) and the water absorption (12%), while the tensile strength remained constant (Mekki et al., 2008). Similar results were observed by de la Casa et al. (2009) also with the improvement by 33% of the dry-bending strength when compared to the control bricks.

In order to contribute to a more sustainable food production, the wastes generated from olive industry can also be transformed into animal feed (Dunne, 2019; Estaún et al., 2014; Gerasopoulos, Stagos, Kokkas, et al., 2015; Gerasopoulos, Stagos, Petrotos, et al., 2015; Molina-Alcaide et al., 2010; Rojas-Cano et al., 2014), although it is necessary to pay attention to their digestibility, palatability, and safety (Rojas-Cano et al., 2014). Rojas-Cano et al. (2014) demonstrated that the inclusion of olive soap stocks in the diet of growing crossbred Iberian pigs did not affect the apparent digestibility of nutrients or body protein accretion but increased the energy value of the diet. Also, Serra et al. (2018) found that the swine diet was improved by the inclusion of olive wastes. Lipid oxidation slowed down in the sausages despite the higher and lower contents in polyunsaturated fatty acids (PUFAs) and saturated fatty acids, respectively, compared to the controls. In another study, it was observed that feed blocks containing olive pomace could improve the quality of milk compared with a conventional diet with no impact on the milk yield and reducing the feeding costs (Molina-Alcaide et al., 2010). Similarly, for broiler chickens, the use of olive wastes extracts was effective in reducing the oxidative stress and led to higher antioxidant capacity in plasma and tissues (Gerasopoulos, Stagos, Kokkas, et al., 2015).

With increasing consumer demand for healthier food, the industry and the scientific community started to produce new functional ingredients for food and beverages. The recent pandemic situation also affected the food sector, mainly food safety and security, becoming more important in the development of sustainable and modern food systems (Galanakis, 2020; Galanakis et al., 2021). Thereby, the supplementation of consumers' diets with bioactive ingredients (vitamins, peptides, polyphenols, and lipids) can be an important key for the prevention or recovery from COVID-19 disease (Galanakis et al., 2020). The addition of OMWW and olive paste, individually or combined, to bread and pasta was assessed by Cedola et al. (2020) and Simonato et al. (2019). The results demonstrated that the enrichment of bread and pasta with OMWW slightly improved the chemical quality without compromising the sensory properties, whereas the enrichment with olive paste considerably improved both phenolic contents and antioxidant activity although the sensory acceptability was worse due to its bitter and spicy taste. The combination of the two byproducts in the fortification of bread and spaghetti increased the whole quality index, being higher for bread (Cedola, Cardinali, et al., 2020). The fortification of wheat pasta with olive pomace also enhanced the dietary fiber of the final product, while increasing its firmness and decreasing its cooking time (Simonato et al., 2019). In other study, the bread and rusks fortified with olive polyphenols (200 mg of polyphenols/kg) demonstrated higher antimicrobial activity and extended their shelf life from 10 to 15 days (Galanakis et al., 2018). Moreover, the addition of olive leaf extracts to poultry meat decreased the microbial growth and maintained both chemical quality and sensory attributes (Saleh et al., 2020), thus extending the shelf life of the meat when refrigerated for 15 days.

Olive byproducts are abundant sources of bioactive phenolic compounds (El-Abbassi et al., 2012; Madureira et al., 2020; Nunes et al., 2018; Yakhlef et al., 2018) that have promising potential as antioxidant, anti-inflammatory, and antimicrobial agents (Bulotta et al., 2014; Leouifoudi et al., 2014; Schaffer et al., 2010). Hence, the recovery of phenolic compounds from these wastes, after suitable purification, presents considerable interest for food and beverage (Araújo et al., 2015; Caporaso et al., 2018; Zbakh & El Abbassi, 2012), cosmetic (Galanakis et al., 2018; Rodrigues et al., 2017; Rodrigues et al., 2015), and nutraceutical (Vitali Čepo et al., 2018) industries, due to their interesting pharmaceutical properties. In the last years, the extracted phenolic compounds have also been used to produce biodegradable packaging materials for various types of food products to replace the synthetic ones (de Moraes Crizel et al., 2018; Lammi et al., 2018). de Moraes Crizel et al. (2018) studied the incorporation of 30% olive pomace flour in chitosan based films, protecting nuts against oxidation during 31 days. Lammi et al. (2018) developed biodegradable olive pomace-based fillers with lower stress and elongation at break and reducing costs.

3 | BIOACTIVE COMPOUNDS EXTRACTED FROM OLIVE POMACE

As previously discussed, in order to eliminate the OMWW generated in the three-phase system, the two-phase technology has emerged in the last 0 years, producing one major residue, olive pomace. Olive pomace comprises the olive husk and the OMWW, being one of the most polluting agricultural byproducts in the Mediterranean region (Papaioannou et al., 2013). Nevertheless, despite the very important polluting aspect, this waste contains high amounts of added value compounds, such as phenolic compounds, in variable amounts depending on several factors, such as olive variety and cultivar, harvest time, processing methods, and extraction process (Dermeche et al., 2013).

Cioffi et al. (2010) reported oleuropein (81.7–83.9 mg/kg of dry weight) and ligstroside aglycone (27.1–31.1 mg/kg of dry weight) as the most abundant compounds of olive pomace from Cilento National Park (Italy). In olive pomace from Algeria, oleuropein (144 mg/g of dry weight) was also detected as the major compound, and secoxyloganin (55 mg/g of dry weight), loganin (47 mg/g of dry weight), and cyanidin-3-glucoside (39 mg/g of dry weight) were observed for the first time in olive wastes (Moudache et al., 2020). On the other hand, Madureira et al. (2020) described hydroxytyrosol (25 mg/g of extract) as the main phenolic compound present in olive pomace from the region of Alentejo (Portugal), followed by hydroxytyrosol-1-β-glucoside (9.8 mg/g of extract) and tyrosol (5.9 mg/g of extract), which is consistent with the observations made by Malapert et al. (2018). These authors also described hydroxytyrosol (370.7 mg/L), hydroxytyrosol glucoside (165.2 mg/L), and tyrosol (148.4 mg/L) as the major compounds present in olive pomace from Baux-de-Provence (France). Additionally, Nunes et al. (2018) observed that hydroxytyrosol and comsegoloside represented about 79% of the total phenolics present in olive pomace from the region of Trás-Os-Montes (Portugal). On the other hand, Rubio-Senent et al. (2012) observed high quantities of 3,4-dihydroxyphenylglycol, elenolic acid derivatives, and comsegoloside besides hydroxytyrosol and tyrosol. Furthermore, verbascoside, gallic acid, caffeic acid, vanillic acid, oleuropein aglycone, *p*-coumaric acid, rutin, luteolin-7-*O*-rutinoside, and luteolin-7-*O*-glucoside were reported in lower amounts (Cioffi et al., 2010; Madureira et al., 2020; Malapert et al., 2018; Peralbo-Molina et al., 2012; Rubio-Senent et al., 2012).

Different methodologies for the extraction of these bioactive compounds from olive pomace can be performed using conventional and emerging technologies. Conventional techniques are based on solid–liquid extraction (Cardoso et al., 2005; Madureira et al., 2020; Suárez et al., 2009), but have some limitations namely long extraction times, high-energy dissipation, and low extraction selectivity and purity. In these ways, new and promising techniques were developed, such as ultrasound-assisted extraction (Goldsmith et al., 2018; Nunes et al., 2018), microwave-assisted extraction (Chanioti & Tzia, 2018; Xie et al., 2019), pressurized liquid extraction (Pavez et al., 2019), and supercritical fluid extraction (Caballero et al., 2020) in order to improve the extraction yield of phenolic compounds from olive pomace.

Table 1 describes the main phenolic compounds found in olive pomace and their MS data.

4 | APPLICATIONS OF THE MAIN BIOACTIVE COMPOUNDS EXTRACTED FROM OLIVE POMACE

The possible applications of the bioactive compounds from olive pomace in different areas, after suitable purification, can be the focus of a sustainable valorization in innovative products. This contributes not only to enhance the sustainability of the olive sector, but also for economic and environmental aspects.

Figure 2 shows the chemical structures of the bioactive compounds discussed in this review. Table 2 summarizes the major compounds present in olive pomace and their health benefits, while Table 3 outlines the existing patents on applications of these compounds and Table 4 the

TABLE 1 Main phenolic compounds identified in olive pomace and their MS data

Phenolic group	Compound	HPLC-DAD-MS(*m*/*z*)	LC-QqTOF MS/MS(*m*/*z*)	HPLC-DAD-MS(*m*/*z*)	UHPLC-DAD-MS(*m*/*z*)	LC-DAD-MS(*m*/*z*)
		Rubio-Senent, Rodríguez-Gutiérrez, Lama-Muñoz, and Fernández-Bolaños (2012)	Peralbo-Molina, Priego-Capote, and Luque De Castro (2012)	Nunes et al. (2018)	Malapert et al. (2018)	Madureira et al. (2020)
Phenyl alcohols	Hydroxytyrosol	153; 123	153.0560 (123.0450; 105.0339)	153 (123)	153	153 (123)
	Hydroxytyrosol glucoside	n.d.	315.1069 (123.0446; 153.0570)	n.d.	n.d.	315 (179, 161, 153, 135)
	Tyrosol	137	137.0608 (119.0505; 111.0098)	n.d.	137 (119)	137 (106)
Secoiridoids	Oleuropein	n.d.	539.1176 (377.1306; 307.0874; 275.0962)	n.d.	n.d.	n.d.
	3,4-DHPEA-EA	377; 307; 275; 149; 139	377.1440 (123.0446; 255.0869)	n.d.	377 (197, 153)	377 (331, 287, 179, 161, 143, 131, 119, 113, 101)
	Ligstroside	523; 361; 291; 259	n.d.	n.d.	n.d.	n.d.
	Elenolic acid	n.d.	n.d.	n.d.	241 (209, 165, 139, 127, 121, 101)	n.d.
Phenolic acids/aldehydes	Caffeic acid	n.d.	179.0352 (135.0449)	n.d.	179 (163, 135)	179 (135)
	p-coumaric acid	119	163.0403 (119.0503)	n.d.	163 (119)	n.d.
	Ferulic acid	n.d.	193.0514 (134.0377)	n.d.	n.d.	n.d.
	Gallic acid	n.d.	169.0150 (125.0246)	n.d.	n.d.	n.d.
	Vanillic acid	n.d.	167.0353 (108.0206; 123.0446)	n.d.	n.d.	n.d.
	Protocatechuic acid	109; 45	153.0193 (109.0295)	n.d.	n.d.	n.d.
	Vanillin	n.d.	n.d.	n.d.	n.d.	151 (136)
	Syringic acid	n.d.	n.d.	n.d.	n.d.	197 (153)
	Cinnamic acid	n.d.	147.0452 (147.0459)	n.d.	n.d.	n.d.
Flavonoids	Rutin	n.d.	609.1487 (301.0391)	n.d.	n.d.	n.d.
	Luteolin	n.d.	285.0420	n.d.	n.d.	n.d.
	Luteolin-7-*O*-glucoside	447; 285	447.0970 (285.0423)	n.d.	n.d.	n.d.
Phenylethanoid glycoside	Verbascoside	623; 461; 161	623.1973 (461.1744; 161.0254)	n.d.	623 (461, 477, 315)	623 (461, 315)
p-coumaric derivative	Comselogoside	535; 205; 145	n.d.	535 (491, 389, 345, 265)	535 (491, 389, 345, 307, 265, 163)	n.d.
Lignan	Pinoresinol	n.d.	357.1345	n.d.	n.d.	n.d.

n.d., not detected.

TABLE 2 Health effects reported for the main phenolic compounds present in olive pomace

Phenolic compound	Health effects	References
Hydroxytyrosol	Antioxidant, anti-inflammatory and anti-aging agent	Jeon and Choi (2018); Pérez-Bonilla et al. (2014); Takeda et al. (2014)
	Anticancer properties	Bernini et al. (2017); Imran et al. (2018); Romero et al. (2007)
	Antimicrobial properties	Medina et al. (2007)
	Protector against cardiovascular diseases	Bulotta et al. (2014; Tejada et al. (2016); Vilaplana-Pérez et al. (2014)
	Protector against high cholesterol	Illesca et al. (2019f); Tabern (2014)
	Protector against digestive disorders	Sánchez-Fidalgo et al. (2012)
	Skin protector	D'Angelo et al. (2005); Smeriglio et al. (2019)
	Prevention and treatment of osteoporosis	Hagiwara et al. (2011)
	Prevention of HIV infection and/or HIV-derived diseases	Bedoya et al. (2016); Lee-Huang et al. (2003, 2007)
Oleuropein	Antioxidant properties	Zbidi et al. (2009)
	Blood pressure lowering	Sun et al. (2017)
	Protector against cardiovascular diseases	Zhao et al. (2017)
	Neuroprotective properties	Sarbishegi et al. (2014)
	Anticancer properties	Imran et al. (2018); Ruzzolini et al. (2018); Shamshoum et al. (2017)
	Antimicrobial properties	Li et al. (2016); Liu et al. (2017)
	Skin protector	Kimura and Sumiyoshi (2009); Perugini et al. (2008)
	Hepatoprotective properties	Kim et al. (2014); Santini et al. (2020)
	Gastroprotective properties	Koc et al. (2018)
	Anti-diabetic properties	Annunziata et al. (2018); Qadir et al. (2016)
	Anti-obesity properties	Svobodova et al. (2014)
	Prevention of osteoporosis	Filip et al. (2014); Hagiwara et al. (2011)f
Tyrosol	Muscle damage inhibitor	Lee et al. (2018)
	Cholesterol efflux promotor	Berrougui et al. (2015)
	Intestine mucosa protector	Giovannini et al. (1999)
	Anti-diabetic properties	Chandramohan and Pari (2016); Zhang et al. (2019)
	Prevention of hypertension	Plotnikov et al. (2018)
	Protector against cardiovascular diseases	Samuel et al. (2008)
	Prevention of osteopenia	Puel et al. (2008)
Verbascoside	Antioxidant and anti-inflammatory properties	Cardinali et al. (2012); Chen et al. (2012); Funes et al. (2009); Funes et al. (2010); Qiao et al. (2019); Vertuani et al. (2011)
	Anticancer properties	Ma et al. (2020); Ohno et al. (2002)
	Hepatoprotective properties	Cui et al. (2018)
	Wound healing properties	de Moura Sperotto et al. (2018)
	Anti-hypertensive activity	Chen et al. (2012)
	Anti-diabetic properties	El-Marasy et al. (2020); Liu et al. (2013)
	Hypoglycemic activity	Morikawa et al. (2014)
	UV protector	Potapovich et al. (2013)
	Neuroprotective properties	Xia et al. (2018)
Oleuropein aglycone	Anti-neurodegenerative properties	Casamenti et al. (2015); Cordero et al. (2018); Grossi et al. (2013); Palazzi et al. (2018)

(Continues)

TABLE 2 (Continued)

Phenolic compound	Health effects	References
	Anticancer properties	Menendez et al. (2007)
	Lipid-lowering properties	Leenen et al. (2002)
	Anti-hyperglycemic properties	Rigacci et al. (2010)r
	Anti-obesity properties	Oi-Kano et al. (2017)
	Antimicrobial properties	Bisignano et al. (2014)

HIV, human immunodeficiency virus; UV, ultraviolet.

fortified food with the presented bioactive compounds.

As the most abundant bioactive compounds in olive pomace, hydroxytyrosol, oleuropein, and tyrosol will be discussed first in this section, followed by verbascoside and oleuropein aglycone, which are present in lower amounts in this residue but have beneficial properties and potential applications that should be explored.

4.1 | Hydroxytyrosol

Hydroxytyrosol (Figure 2), also known as 3,4-dihydroxyphenylethanol (DOPET), 3,4-dihydroxyphenolethanol (3,4-DHPEA), or 4-(2-hydroxyethyl)−1,2-benzenediol by the International Union of Pure and Applied Chemistry (IUPAC), is one of the most powerful naturally derived antioxidants with higher antioxidant potential than butylated hydroxytoluene (BHT), trolox and vitamins C and E (Pérez-Bonilla et al., 2014; Zbidi et al., 2009). Hydroxytyrosol is a main product of the hydrolysis of oleuropein (Liu et al., 2018) which occurs during the ripening of the olives, storage of the oil, and preparation of table olives, and it is responsible for the complexity and variety of the oil and olive flavors. Due to its amphipathic character, hydroxytyrosol can be found not only in olive and in olive leaf, but also in olive oil byproducts in a free form, as acetate form or as part of more complex compounds like oleacein, verbascoside, and oleuropein.

There are different patented methods to obtain purified hydroxytyrosol from products and byproducts of olive oil industry to be used in food, cosmetic, and pharmaceutical industries (Table 3). Thus, nanofiltration and reverse osmosis were patented as clean technologies to purify hydroxytyrosol (de Magalhães Nunes da Ponte et al., 2014), whereas a process using resins was patented by Fernandez-Bolaños Gusman et al. (2005).

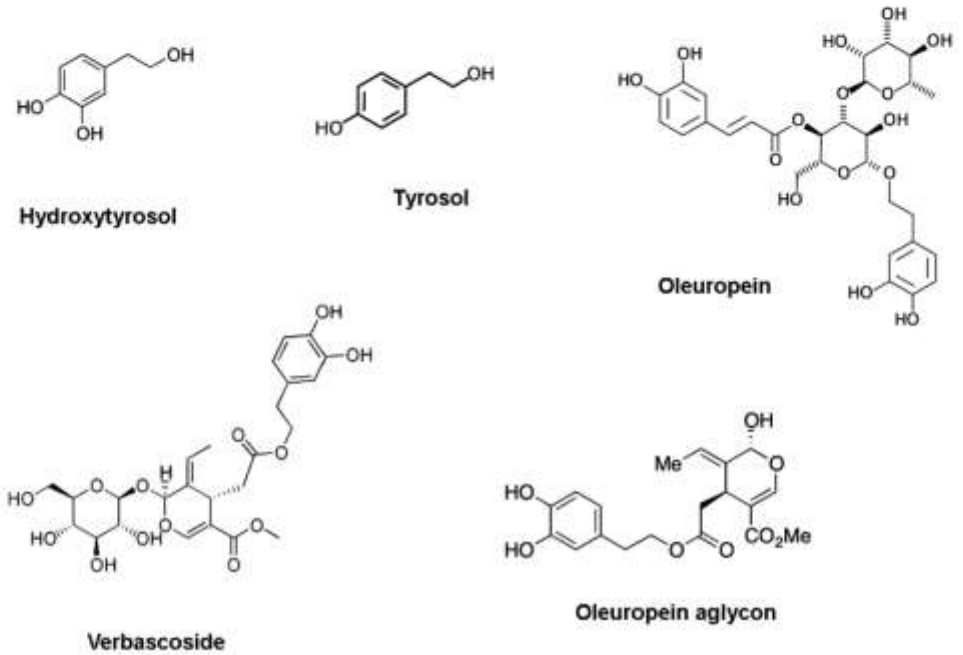


FIGURE 2 Chemical structures of some of the main phenolic compounds present in olive pomace, discussed in this review

TABLE 3 Patents on the application of phenolic compounds present in olive pomace

# Patent	Subject	Industry application	Year of publication	References
WO 2004/069218 A1	Cosmetic formulation containing verbascoside to use as a stimulant agent for the production of thermal shock proteins and as an inhibitor of collagenase and elastase and stimulant of hydroxylases.	Cosmetic industry	2004	Robin and Rolland (2004)
EP 1498131 A1	Medicinal preparation with verbascoside (5%–40%) as active ingredient of a drug used to prevent senile dementia and inhibit aggregation of blood platelets.	Pharmaceutical industry	2005	Tu et al. (2005)
WO 2007/012057 A2	Production of a hydroxytyrosol-rich formulation to treat skin inflammations, such as psoriasis, eczema, allergic dermatitis, phytohypersensitivity, dermatosis, and lichen urticatus.	Pharmaceutical industry	2007	Numano et al. (2007)
WO 2007/051829 A1	Production of a medication using oleuropein for preventing and/or treating peripheral vascular disease, particularly venous insufficiency.	Pharmaceutical industry	2007	Martin et al. (2007)
WO 2008/128629 A1	Production of a nutraceutical composition with hydroxytyrosol for regeneration and repair of cartilage injuries in joints.	Nutraceutical industry	2008	Raederstorff et al. (2008)
WO 2009/013596 A2	Process of production of food hydroxytyrosol-enriched products (fortified edible oils, fortified edible oil-containing products, and dietary supplements in the form of soft gel capsules containing fortified edible oils) to prevent or treat cardiovascular diseases, plaque build-up in the arteries, arterial hypertension, and metabolic syndrome.	Nutraceutical industry	2009	Más et al. (2009)
US 2010/0297330 A1	Production of yogurts with hydroxytyrosol to increase the antioxidant activity, preserving their organoleptic characteristics and stability for at least three months, having the role of preventing diseases.	Food industry	2010	Villanova et al. (2010)
WO 2010/070183 A1	Preparation of therapeutic compositions with oleuropein as active ingredient to induce angiogenesis and vasculogenesis.	Pharmaceutical industry	2010	Quesada-Gómez et al. (2010)
WO 2011/141611 A1	Pharmaceutical compositions containing oleuropein as the only active ingredient to heal wounds and ulcers in elderly people and/or diabetics.	Pharmaceutical industry	2011	Quesada-Gómez et al. (2011)
US 2011/0144040 A1	Compositions containing verbascoside and verbascoside-rich extracts to increase strength, muscle power, endurance, muscle protein content, and to reduce fatigue.	Nutraceutical industry	2011	Panyam and Chavanpatil (2011)
WO 2011/153647 A1	Pharmaceutical composition containing hydroxytyrosol and xanthohumol to treat asthma and rhinitis.	Pharmaceutical industry	2011	Ehrenberger and Bieberschulte (2011)

(Continues)

TABLE 3 (Continued)

# Patent	Subject	Industry application	Year of publication	References
US 2012/0260922 A1	New microbicide formulation containing hydroxytyrosol and other existing drugs to prevent sexually transmitted diseases, such as HIV-infection.	Pharmaceutical industry	2012	Gómez-Acebo et al. (2012)
US 8574635 B2	Methods and pharmaceutical compositions with oleuropein and its derivatives, to inhibit HIV-infections and also to treat obesity and type 2 diabetes, or related disorders.	Pharmaceutical industry	2013	Lee-Huang et al. (2013)
WO 2013/084249 A1	Dietary supplement containing a mixture of verbascoside and teupolioside for prevention of hypercholesterolemia. The invention is also related to the production of foodstuffs (milk, eggs, meat) with reduced cholesterol content, rich in vitamins A and E.	Nutraceutical industry	2013	Casamassima (2013)
US 2014/0011756 A1	Process of compositions production with hydroxytyrosol and oleuropein for treating HIV-associated neurological disorders, inflammation, and inflammation-associated disorders.	Pharmaceutical industry	2014	Crea (2014)
ES 2462565 A1	A dermocosmetic containing hydroxytyrosol to inhibit melanin synthesis and to reduce skin coloration, which can work as a whitening, lightening, and depigmenting agent of skin.	Cosmetic industry	2014	Codina and Monjo (2014)
WO 2016/087428 A1	Process to fortify edible oils with hydroxytyrosol to be used in all fields of nutrition.	Nutraceutical industry	2016	Bulbarello and Leuthardt (2016)
US 2017/0367995 A1	A method for production of a formulation comprising hydroxytyrosol (40%–90%) and at least one additional polyphenol to treat neurodegenerative diseases, such as Parkinson in early stage.	Pharmaceutical industry	2017	Crea (2017)
ES 2734600 A1	A pharmaceutical and nutraceutical composition using aloe vera gel (90%–99.9%) and hydroxytyrosol (0.1%–10%) for the production of a dietary supplement for the control off glycemic index and cholesterol or a medication for the treatment and/or prevention of metabolic syndrome and type 2 diabetes and as a food supplement.	Nutraceutical and Pharmaceutical industry	2019	Fernández Arche et al. (2019)
US 2019/012952 A1	Method for production of beverages containing hydroxytyrosol without changing smell and flavor.	Food industry	2019	Fuwa et al. (2019)f
US 2020/0390793 A1	Composition (food product or food supplement) with oleuropein to be administrated to treat, prevent, or reduce the progression of sarcopenia.	Nutraceutical industry	2020	Boutry et al. (2020)

HIV, human immunodeficiency virus.

TABLE 4 Fortified food with phenolic compounds present in olive pomace

Phenolic compound	Food	Benefits of fortified food	References
Hydroxytyrosol	Red and white wine	Improvement of the color for at least 6 months.	Raposo et al. (2016)
	Beer	5 g/L of hydroxytyrosol imparted a more soft and pleasant flavor and aroma.	Guglielmotti et al. (2020)
	Biscuits	Improvement of antioxidant activity, total phenol and flavonoid contents; Decrease of the levels of oxidized low-density lipoproteins in blood.	Cedola, Palermo, et al. (2020); Mateos et al. (2016)
	Sausages and meat patties	Increase of antioxidant activity and decrease of lipid and protein oxidation until 9 or 21 days of storage, maintaining color and texture; Reduction of undesirable fungi.	Balzan et al. (2017); Chaves-López et al. (2015); Muiño et al. (2017); Nieto et al., 2017a, 2017b)
	Fish patties	Delay of lipid oxidation; Preservation against pathogens, extending shelf life of the product.	Martínez et al. (2019)
	Yogurts	Improvement of the growth of lactic acid bacteria; Prevention of the spoilage during fermentation.	Georgakouli et al. (2016)
Oleuropein	Pasteurized milk	Extension of the shelf life by 60% with 7.57 mg oleuropein/100 ml milk.	Palmeri et al. (2019)
	Yogurts	Enhancement of rheological properties (higher firmness and viscosity and less syneresis).	Zoidou et al. (2017)
	Meat burgers	Delay of oxidation, increasing shelf life of the product.	Elama et al. (2017)
	Salmon burgers	Reduction of lipid oxidation.	Khemakhem et al. (2019)
Verbascoside	Sausages	Antifungal activity without compromising sensory characteristics.	Chaves-López et al. (2015)

This compound has been described as an antioxidant with many biological activities (Table 2) (Bertelli et al., 2020; Marković et al., 2019; Vilaplana-Pérez et al., 2014). There are many studies proving its antioxidant, anticancer, anti-inflammatory, and antimicrobial activities (Bernini et al., 2017; Bernini et al., 2015; Imran et al., 2018; Jeon & Choi, 2018; Medina et al., 2007; Pérez-Bonilla et al., 2014; Romero et al., 2007; Takeda et al., 2014). In addition, it was reported to act as a protector against high cholesterol blood levels (Tabern et al., 2014), metabolic diseases (Bulotta et al., 2014; Fki et al., 2020), genotoxicity, cytotoxicity and proapoptotic effects (Anter et al., 2014; Bernini et al., 2013), oxidative stress (Loru et al., 2009; Merra et al., 2014), and digestive disorders (Sánchez-Fidalgo et al., 2012). Other works have focused on the use of hydroxytyrosol in cardiovascular diseases (Bulotta et al., 2014; Tejada et al., 2016; Vilaplana-Pérez et al., 2014).

D'Angelo et al. (2005) and Smeriglio et al. (2019) demonstrated the dermatological properties of hydroxytyrosol to prevent protein damage induced by long-wavelength ultraviolet radiation in melanoma cells or to counteract atopic dermatitis, respectively. More specifically, Smeriglio et al. (2019) investigated the safety and efficacy of the Fenolia® Eudermal Cream 15 containing hydroxytyrosol in its formulation, which was demonstrated to be effective in improving the epidermal barrier effect, preventing inflammation, and repairing the skin. Actually, hydroxytyrosol is already used as an ingredient in different cosmetics, including anti-aging and lightening/whitening products. There are a few patents to produce cosmetics with hydroxytyrosol. Codina and Monjo (2014) patented a method to produce a dermocosmetic containing hydroxytyrosol to inhibit melanin synthesis, reduce skin coloration and work as a whitening, lightening, and depigmenting agent of skin. Another hydroxytyrosol-rich formulation was developed to treat skin inflammations, such as psoriasis, eczema, allergic dermatitis, photohypersensitivity dermatosis, or lichen urticatus (Numano et al., 2007).

Hydroxytyrosol has been proposed to promote fat and cholesterol metabolism, effectively protect the liver function, and cure and/or prevent the occurrence of obesity. In a study with high-fat diet fed mice, hydroxytyrosol was used as a nutritional supplement to amend dysfunctional white adipose tissue (Illesca et al., 2019). The

supplementation caused an improvement on adipocyte hypertrophy and a decrease in oxidative damage, showing it as a promising alternative to prevent obesity and its associated metabolic disturbances. A pharmaceutical or nutraceutical composition was developed and patented by Arche et al. (2019) based on an aloe vera (90–99.9%) and hydroxytyrosol (0.1%–10%) gel to be used as a dietary supplement for the control of glycemic index and high cholesterol levels or a medication for the treatment and/or prevention of metabolic syndrome and type 2 diabetes.

An interesting study by Hagiwara et al. (2011) proposed the use of hydroxytyrosol on osteoporosis' prevention and treatment, since this compound stimulated the deposition of calcium in a dose-dependent manner. Furthermore, nutraceutical and pharmaceutical compositions with hydroxytyrosol were patented for regeneration and repair of cartilage injuries in joints (Raederstorff et al., 2008).

A formulation comprising hydroxytyrosol (40%–90%) and at least one additional olive polyphenol was developed for the treatment of neurodegenerative diseases such as Parkinson in the early stages of the disease (Crea, 2017). The inventors described the administration of daily doses of capsules (72 mg olive polyphenols, including 30 mg hydroxytyrosol) to subjects for some weeks, noticing considerable improvements in their movements and reduction of the tremor after the first weeks.

The use of hydroxytyrosol to prevent human immunodeficiency virus (HIV) infection and even to palliate HIV-derived diseases has also received special attention (Bedoya et al., 2016; Lee-Huang et al., 2007; Lee-Huang et al., 2003). Bedoya et al. (2016) observed that 5-hydroxytyrosol inhibited HIV-1 infections of recombinant or wild-type viruses in human lymphoblastic cells (MT-2 cell lines) and human peripheral blood mononuclear cells (PMBCs cell lines). The combination of this compound with other drugs already used as a preventive treatment against HIV-1 infections was also tested. Additive effects were found with lamivudine and emtricitabine, whereas the combination with tenofovir seemed to be synergistic. These findings, which suggested that 5-hydroxytyrosol could be an effective and low-cost new microbicide to prevent the HIV infection with particular interest in countries with high incidence of transmission were patented by Gómez-Acebo et al. (2012). Another method for producing pharmaceutical compositions with phenolic compounds extracted from olive byproducts, including hydroxytyrosol, for treating HIV-associated neurological disorders, inflammation, and inflammation-associated conditions was patented (Crea, 2014). This composition was produced to be preferably used as a tablet formulation for oral administration.

A formulation containing hydroxytyrosol and xanthohumol as active substances in the form of an intranasal spray was also patented to treat asthma and rhinitis as well as to treat colds (Ehrenberger & Bieberschulte, 2011).

The European Food Safety Authority (EFSA) concluded that there was sufficient scientific evidence to claim several health benefits for hydroxytyrosol and related olive oil polyphenols (EFSA Panel on Dietetic Products, Nutrition & Allergies, 2011), and that no concerns exist about its safety to be used in novel food preparations under proposed uses and levels (EFSA Panel on Dietetic Products, Nutrition & Allergies, 2017). A summary on the uses of hydroxytyrosol in fortified foods is collected in Table 4. A comprehensive review on the applications of hydroxytyrosol as a functional food ingredient, either in the form of pure compound or hydroxytyrosol-rich extracts, was recently published by Silva et al. (2020). In that review, the authors described the use of this compound in edible oils, beverages, bakery products and meat, fishery, and dairy products. Several food products with higher antioxidant activity (fortified edible oils, fortified edible oil-containing products, and dietary supplements in the form of soft gel capsules containing fortified edible oils) were patented by Mas et al. (2009) for preventing or treating cardiovascular diseases, plaque build-up in the arteries, arterial hypertension, and metabolic syndrome, based on their hydroxytyrosol-rich composition. Bulbarello and Leuthardt (2016) patented capsules for oral consumption of an edible oil fortified with hydroxytyrosol (more than 250 mg hydroxytyrosol/kg fortified oil) to be used in humans and animals in all the fields of nutrition. Besides its health benefits, the fortification with hydroxytyrosol can delay the oxidation of edible oils.

Moderate consumption of red wine has been suggested to protect against cardiovascular diseases mainly due to its high content in polyphenols (Santos-Buelga & González-Manzano, 2011). When hydroxytyrosol is added to red (Raposo et al., 2016a) and white (Raposo et al., 2016b) wines to replace the use of sulfur dioxide, an intensification of color was noticed. The improvement on color was maintained even after 6 months of storage for white wines, whereas red wines were oxidized. Based on these results, the authors suggested that the combination of sulfur dioxide with hydroxytyrosol could be a suitable condition to reduce the sulfur dioxide content without compromising the wine oxidation. Recently, Guglielmotti et al. (2020) incorporated hydroxytyrosol in beer using olive leaves as an ingredient in the form of dry crumbled leaves, infusion and atomized extract, so as to explore their contribution to bitterness and antioxidant activity of beer. The ingredients were added during boiling phase of brewing, in order to promote the hydrolysis of oleuropein of the leaves to hydroxytyrosol. The results demonstrated that the

addition of 10 g/L of olive leaves imparted a sour/astringent taste and herbal aroma to the beers, whereas 5 g/L of olive left a softer and pleasant flavor and aroma in comparison to the control. Fuwa et al. (2019) studied the possibility of preparing a beverage containing hydroxytyrosol (0.5 to 50 mg/100 ml) to improve blood flow without changing smell and flavor. The authors formulated beverages comprising ethanol and/or propylene glycol (0.05% to 0.5% w/v), caffeine (10–210 mg/100 ml), and glucose and maltose. The beverages with the most favorable taste were found to be the ones with higher concentrations of caffeine (>110 mg of caffeine/100 ml) and 0.8–1.5 mg of hydroxytyrosol/100 ml. The beverages with the most favorable sense of quick sweetness were those containing 0.8–1.5 mg of hydroxytyrosol/100 ml and a ratio of glucose/maltose of 0.03–0.14.

Bakery products are also interesting products to incorporate hydroxytyrosol since they are consumed worldwide by people of different ages. Mateos et al. (2016) observed that the intake by volunteers of hydroxytyrosol-fortified biscuits (30 g biscuits providing 5.25 mg of hydroxytyrosol, after an overnight fast) could significantly decrease the levels of oxidized low-density lipoproteins (LDL) in blood, also finding that hydroxytyrosol was highly bioavailable, extensively metabolized, and rapidly eliminated. In another study by Cedola et al. (2020), the white wine used to produce the Italian biscuit "taralli" was replaced by an olive leaves extract containing hydroxytyrosol. An improvement on antioxidant activity (1.5-fold), total phenols (≥1.4-fold), and flavonoid content (4-fold) were observed, which could be responsible for the darker color of the fortified biscuits compared to the control ones.

Hydroxytyrosol has been also used in meat to improve the oxidative stability and sensory properties of lamb meat patties (Muíño et al., 2017), the nutritional profile of low-fat chicken Frankfurters (Nieto et al., 2017a) or to reduce the growth of undesirable fungi on dry fermented sausages (Chaves-López et al., 2015). Muíño et al. (2017) evaluated lamb meat patties enriched with omega 3 (fish oil) and hydroxytyrosol at different concentrations (100, 200, and 400 ppm), demonstrating that the presence of hydroxytyrosol increased the antioxidant activity of patties and decreased the lipid and protein oxidation at days 3, 6, and 9 of storage compared with control samples, while maintaining the color and texture. Hydroxytyrosol (50 ppm), in combination with olive oil and walnuts, was used to produce chicken sausages without loss of sensory attributes during storage at 4°C for 21 days. Furthermore, it was enough to maintain the color and reduce lipid and protein oxidation until 21 days (Nieto et al., 2017b; Nieto et al., 2017a). Fermented sausages added with hydroxytyrosol (100.23 ppm) showed lower lipid oxidation and volatile compounds and higher redness, as well as improved antifungal activity both in vitro and in situ (Chaves-López et al., 2015). Also, Balzan et al. (2017) prevented the lipid oxidation of pork sausages using a purified extract from the olive vegetation water containing a high amount of hydroxytyrosol.

In fishery products, hydroxytyrosol was efficient to inhibit the formation of lipid oxidation products in bulk cod liver oil (100 ppm of hydroxytyrosol), cod liver oil-in-water emulsions (100 ppm of hydroxytyrosol), and frozen minced horse mackerel (*Trachurus trachurus*) muscle (50 ppm of hydroxytyrosol) (Pazos et al., 2008); furthermore, the level of α-tocopherol and the ω-3 long-chain PUFAs was preserved. Hydroxytyrosol extracts were also able to delay the lipid oxidation and preserve fish patties against *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* contributing to extend their shelf life (Martínez et al., 2019).

The production of yoghurts that include hydroxytyrosol (in a percentage between 0.1% and 0.01%) as an additional healthy component was patented by Villanova et al. (2010). The enriched yogurts preserved their organoleptic characteristics, improved their antioxidant activity, and maintained their stability for at least 3 months. In the same way, Georgakouli et al. (2016) verified that the addition of a commercial hydroxytyrosol-rich product obtained from olive fruits (Medoliva©) in yogurts (50 mg of polyphenols) improved the growth of lactic acid bacteria and contributed to preventing spoilage during fermentation, as well as to reduce LDL cholesterol, body weight, and blood pressure.

4.2 | Oleuropein

Oleuropein belongs to a very specific group of coumarin-like compounds, called secoiridoids and consists of hydroxytyrosol, elenolic acid, and a glucose molecule (Figure 2). It is one of the main polyphenols present in olive wastes, contributing to the bitter taste of olive oil and fruit.

Oleuropein possesses numerous pharmacological benefits (Table 2) mostly related with its strong antioxidant and anti-inflammatory activities (Hassen et al., 2015; Marković et al., 2019; Nediani et al., 2019). The antioxidant activity of oleuropein is related to the presence of hydroxyl groups in its chemical structure that can donate hydrogen to prevent oxidation (Hassen et al., 2015). In fact, oleuropein and oleuropein-rich extracts were reported to have higher antioxidant activity than the synthetic antioxidant BHT (Zbidi et al., 2009). Another interesting property of oleuropein is its blood pressure-lowering effect. Sun et al. (2017) found that oleuropein can protect the paraventricular nucleus (PVN) of hypothalamus from oxidative stress, being a promising strategy both for preventing and treating hypertension. Furthermore, oleuropein has been shown to

have cardioprotective (Zhao et al., 2017), neuroprotective (Sarbishegi et al., 2014), anticancer (Imran et al., 2018; Ruzzolini et al., 2018; Shamshoum et al., 2017), and antimicrobial (Li et al., 2016; Y. Liu et al., 2017) activities. Also, it was proven to possess skin protectant (Kimura & Sumiyoshi, 2009; Perugini et al., 2008), hepatoprotective, gastroprotective, anti-diabetic and anti-obesity activities, and lipid regulating effects (Annunziata et al., 2018; Drira et al., 2011; Kim et al., 2014; Koc et al., 2018; Qadir et al., 2016; Santini et al., 2020; Svobodova et al., 2014). Based on the mentioned properties, oleuropein may be used for a variety of human disorders.

Martin et al. (2007) developed a patent on the use of oleuropein for the manufacture of a medication for preventing and/or treating a condition associated with peripheral vascular disease. A pharmaceutical composition with oleuropein (concentration between 10^{-7} and 10^{-4} M) as active ingredient was also patented to induce angiogenesis and vasculogenesis, that can be administered by oral, rectal, parenteral, intraperitoneal, intradermal, transdermal, intratracheal, intramuscular, intravenous, or inhalation (Quesada-Gómez et al., 2010). The same authors (Quesada-Gómez et al., 2011) patented a preparation containing oleuropein as the only active ingredient to be used for healing wounds and ulcers in aged and/or diabetics individuals in the form of gel, cream, or aqueous solution form.

The above mentioned medication, hydroxytyrosol based, for treating an AIDS-associated neurological disorders can also be prepared using a mixture of hydroxytyrosol and oleuropein (Crea, 2014). Lee-Huang et al. (2013) also patented a formulation able to inhibit not only the infectivity of HIV but also to treat and prevent obesity and related conditions, such as type 2 diabetes. Actually, interesting evidences have been found on the effects of oleuropein on the control of type 2 diabetes (Annunziata et al., 2018).

Oleuropein also has application in the treatment of osteoporosis. The BONOLIVE® supplement, consisting of a polyphenols mixture from olive leaf containing more than 40% of oleuropein, has been clinically proven to induce significant effects on bone health, stimulating bone building cells (osteoblasts), and improving blood lipid profiles in individuals after 12 months of treatment (Filip et al., 2014). Hagiwara et al. (2011) also reported that, as hydroxytyrosol, oleuropein may have critical effects on the formation and maintenance of bone, and that both could be used as effective medications for the treatment of osteoporosis symptoms.

Recently, Boutry et al. (2020) described a formulation to be used either as a food product or a food supplement, based on oleuropein to treat or prevent sarcopenia, indicated for elderly and/or frail individuals.

Oleuropein has been added as an active ingredient in milk and yogurt preparations for the production of novel foods with improved characteristics. Palmeri et al. (2019) observed that the addition of 5% olive leaf extract (corresponding to 7.57 mg of oleuropein/100 ml of milk) to pasteurized milk was able to extend its shelf life by 60%, thus leading to significant benefits in terms of costs linked to transport and to product returns to the dairy industry. The incorporation of oleuropein to fortify yogurts represents a major challenge for their taste and texture. Nevertheless, Zoidou et al. (2017) proved that the addition of olive leaf extract containing high amounts of oleuropein to yogurts improved their rheological properties, providing higher firmness and viscosity, and less syneresis.

Oleuropein can also be used for delaying the oxidation of meat. Elama et al. (2017) explored the possibility of using oleuropein as a natural antioxidant in frozen hamburgers to replace synthetic food antioxidants such as sodium erythorbate. Similar results were obtained for both oleuropein and sodium erythorbate, finding that a concentration of 0.5% was able to extend shelf life of hamburgers and hinder oxidation. The potential of oleuropein to preserve salmon burgers by reducing lipid oxidation during the storage at low temperatures was also demonstrated (Khemakhem et al., 2019).

4.3 | Tyrosol

Tyrosol (Figure 2), also named as 2-(4-hydroxyphenyl)-ethanol, *p*-hydroxyphenethyl alcohol, or 4-(2-hydroxyethyl)-phenol by the IUPAC, is a phenylethanoid compound naturally present in olive fruit, olive oil, and olive wastes both in their free form or as part of more complex molecules, mostly as esters of elenolic acid.

Several studies have demonstrated the potential of tyrosol as antimicrobial, anti-carcinogenic, anti-inflammatory, and antioxidant agent (Table 2). Tyrosol has been proposed to fight hypertension, atherosclerosis, coronary heart disease, chronic heart failure, insulin resistance, and obesity by modulating cluster of differentiation 14 (CD14) upregulation and inhibiting inflammation (Chang et al.,). In a study developed by Lee et al. (2018), tyrosol showed to be effective in inhibiting muscle damage from oxidative stress triggered by strenuous exercise. Tyrosol could also promote cholesterol efflux by enhancing the antiatherogenic properties of high-density lipoproteins (HDL) (Berrougui et al., 2015) and protect the intestine mucosa by preventing the oxidative damage induced by LDL, as shown in human colon adenocarcinoma cell line, Caco-2 (Giovannini et al., 1999).

Tyrosol may also play a role in the treatment of diabetes mellitus, exerting anti-inflammatory effects on

the liver and pancreas via its antioxidant activity, as observed in streptozotocin-induced diabetic rats (Chandramohan & Pari, 2016). Furthermore, the intramuscular administration of tyrosol to diabetic hind limb ischemia mice significantly enhanced the formation of blood vessels which improved the recovery of blood perfusion (Zhang et al., 2019). Those authors also reported a cytoprotective function of tyrosol against hyperglycemia-induced oxidative stress in skeletal muscle cells and an increase in their proliferation.

Plotnikov et al. (2018) described that when tyrosol was administered to young spontaneously hypertensive rats (SHRs), the development of hyperviscosity syndrome was limited, the oxygen transport capacity was improved, and the microvascular rarefaction in the cerebral cortex was eliminated. The blood viscosity increased by 16%–26% in rats treated with tyrosol and the results were similar to those obtained with pentoxyphylline, a drug used for the treatment of peripheral circulation disorders (Plotnikov et al., 2007). Samuel et al. (2008) verified that a tyrosol treatment (5 mg/kg/day for 30 days) was capable of inducing myocardial protection against ischemia-induced stress in rats, which turns important in order to develop a new drug to combat cardiovascular diseases. Moreover, Puel et al. (2008) reported that the daily consumption of tyrosol during 84 days by ovariectomized rats increased bone formation, which was associated with its antioxidant properties, thus preventing osteopenia.

In spite of its interesting properties, the hydrophilic chemical nature of tyrosol could limit its use in foods, pharmaceutical formulations, and/or cosmetics. Paulo and Santos (2020a) developed a strategy to incorporate tyrosol in alternative polymer carriers as poly(D,L-lactide-*co*-glycolide), ethylcellulose, and polycaprolactone by water-in-oil-in-water double emulsion solvent evaporation technique. The obtained results revealed that these microparticles were thermogravimetrically stable in the temperature range of 30 to 261°C. Actually, the bioaccessibility of tyrosol improved by 1.9-fold when the compound was encapsulated (Paulo & Santos, 2020b). Being an antioxidant compound, the microencapsulation may also protect tyrosol from auto-oxidation processes. Other recent study reported the impact of two different carriers for the encapsulation of tyrosol: the oligosaccharide β-cyclodextrin and the polysaccharide chitosan (Pintillo et al., 2021). The results demonstrated that the coating promoted a sustained release of tyrosol and slowed down the initial burst effect observed from the inclusion complex. Also, this compound was shown to be a ctDNA groove binder. These outcomes bring new insights concerning the encapsulation of antioxidants for further incorporation in food, pharmaceutical, cosmetic, and/or nutraceutical matrices.

4.4 | Verbascoside

Verbascoside (Figure 2), also known as acteoside, belongs to the extensive family of phenylpropanoids and is a conjugated glucoside of hydroxytyrosol and caffeic acid. This compound has been demonstrated to have antioxidant protective effects on phospholipid membranes and modulation of plasma antioxidant activity in vivo (Chen et al., 2012; Funes et al., 2009; Funes et al., 2010). Cardinali et al. (2012) reported that verbascoside has a high antioxidant capacity, acting as an effective scavenger of biologically active free radicals and an inhibitor of lipid peroxidation, so that it can be used for treating oxidative stress-related diseases, which may be interesting for application in cosmetics, nutraceuticals, or functional foods. Furthermore, antitumor, antimicrobial, anti-inflammatory, anti-thrombotic, and wound healing properties have also been described (Cui et al., 2018; de Moura Sperotto et al., 2018; Ma et al., 2020; Ohno et al., 2002; Qiao et al., 2019; Vertuani et al., 2011).

Chen et al. (2012) reported that verbascoside (10 mg/kg body weight) has anti-hypertensive activity, lowering both systolic and diastolic blood pressures in hypertensive rats. Its anti-inflammatory potential was also reported by Qiao et al. (2019) on rats with osteoarthritis, a chronic arthritis, through the inactivation of JAK/STAT signaling pathway. Vertuani et al. (2011) demonstrated the stability of verbascoside in suppositories which is an interesting pharmaceutical form to apply in treatment of inflammation of the intestinal mucosa. This compound also presents antitumor activity possibly by increasing p53 levels, as well as by inhibiting KLK expression and angiogenesis, making it a potential candidate for the treatment of advanced hepatocellular carcinoma in the clinic (Ma et al., 2020).

Hepatoprotective (Cui et al., 2018) and anti-diabetic effects (El-Marasy et al., 2020; Liu et al., 2013) have also been described and hypoglycemic activity and improved glucose tolerance were found by Morikawa et al. (2014) in starch-loaded mice.

Potapovich et al. (2013) suggested that verbascoside could inhibit inflammatory response and metabolic disorders caused by solar UV irradiation in human keratinocytes. Similarly, wound healing activity was reported, as it was demonstrated to be capable of increasing the migration of keratinocytes and inhibiting inflammation mediators (de Moura Sperotto et al., 2018).

Xia et al. (2018) described the capacity of verbascoside in attenuating oxidative stress and neuronal apoptosis in middle cerebral artery occlusion/reperfusion rats. Its administration seemed to reduce infarct volume and brain edema and improve neurological deficits, thus suggesting that it can be successfully used to treat cerebral ischemia–reperfusion injury. A review on the biological effects of

verbascoside and its potential clinical utility have been published by Alipieva et al. (2014).

Casamassima (2013) patented a dietary supplement containing verbascoside and teupolioside for prevention of hypercholesterolemia and the production of foodstuffs (particularly in meat, milk, and eggs) with reduced cholesterol content.

NuLiv Science (https://nulivscience.com/), which works on the research and development of proprietary ingredients for nutraceuticals industry, produced two products containing verbascoside: Acteolin™ and Verbasnol™. Acteolin™ is an ingredient claimed to provide benefits for cognitive and memory functions, lung, and eye health. Verbasnol™ helps boost the skin's natural ability and sweeps away the adverse effects of sunlight, oxidative stress, and dryness while maintaining skin hydration and elasticity. Robin and Rolland (2004) patented a formulation containing verbascoside to use in cosmetics as a stimulant agent for the production of thermal shock proteins and as an inhibitor of collagenase and elastase in the skin cells.

In recent years, there has been a growing interest to improve athletic performance and body composition (the ratio of lean to fat mass). Panyam and Chavanpatil (2011) patented preparations containing verbascoside or verbascoside-rich extracts to be administrated in capsule, pill, powder, edible bar, or liquid form in order to increase strength, muscle power, endurance, muscle protein content, and to reduce fatigue. Tu et al. (2005) patented a drug with verbascoside (5%–40%) as an active ingredient to prevent senile dementia and inhibit aggregation of blood platelets.

Concerning the use of this compound in food, Chaves-López et al. (2015) demonstrated that verbascoside-rich (135.20 ppm) extracts incorporated to fermented sausages had antifungal activity without inducing significant changes on sensory characteristics, thus being considered a potential alternative to synthetic antifungal compounds.

4.5 | Oleuropein aglycone

Oleuropein aglycone (Figure 2), also known as 3,4-DHPEA-EA (i.e., 3-4-DHPEA-elenolic acid mono-aldehyde, IUPAC name: methyl (2R,3Z,4S)−4-[2-[2-(3,4-dihydroxyphenyl)ethoxy]−2-oxoethyl]−3-ethylidene-2-hydroxy-3,4-dihydro-2H-pyran-5-carboxylate), is a secoiridoid formed from the deglycosylation of oleuropein, which can be produced by enzymatic, acid or acetal hydrolyses (Xu et al., 2018). Several studies referred to it as the main polyphenol present in olive oil (e.g., Cordero et al., 2018; Xu et al., 2018), but it is also abundant in olive wastes. In recent times, this compound has gained attention due to its biological activities, including anti-neurodegenerative diseases, anti-breast cancer, anti-inflammatory, anti-hyperglycemic, antioxidant, and lipid-lowering properties (Grossi et al., 2013; Menendez et al., 2007; Oi-Kano et al., 2017; Palazzi et al., 2018; Rigacci et al., 2010; Xu et al., 2018).

Oleuropein aglycone has the ability of promoting the autophagy, thus being associated with a reduction of symptoms of Alzheimer's disease and cognitive impairment (Casamenti et al., 2015; Cordero et al., 2018). Grossi et al. (2013) found that the administration of oleuropein aglycone in young/middle-aged TgCRND8 mice for 8 weeks could improve memory and animal behavior by interfering with Aβ aggregation. Oleuropein aglycone was also seen to slow down amyloid aggregation by stabilizing α-synuclein monomers, thus hampering their progression to cytototoxic amyloids, which could be helpful in the prevention of Parkinson's disease. In addition, it may also hinder the binding of α-synuclein aggregates to cell membrane components thus preventing cell oxidative damage (Palazzi et al., 2018). Leri and Bucciantini (2016) reported that oleuropein aglycone could inhibit the toxic effects of transthyretin (TTR) amyloid and be important to prevent or treat heart and liver complications. It has also been shown to be capable of preventing or delaying the progression of type 2 diabetes (Rigacci et al., 2010) and obesity (Oi-Kano et al., 2017). Oi-Kano et al. (2017) reported that it reduced the visceral fat content in high-fat diet-induced obese rats by enhancing noradrenaline secretion via β-adrenergic action following transient receptor potential ankyrin 1 (TRPA1) and vanilloid 1 (TRPV1) activation. Oleuropein aglycone was found to protect LDL in plasma against oxidation (Leenen et al., 2002) as well as to enhance the antioxidant defense system against experimental atherogenesis, which was attributed to its 3,4-dihydroxyphenyl ethanol group (Jemai et al., 2008).

Menendez et al. (2007) reported that, among the polyphenols present in olive oil, oleuropein aglycone was the most potent compound in decreasing breast cancer cells viability, by inhibiting the proteolytic processing of human epidermal growth factor receptor2 (HER2).

In a study developed by Bisignano et al. (2014), oleuropein aglycone was found to be effective against American Type Culture Collection (ATCC) and clinical isolates of *S. aureus* (minimum inhibitory concentrations [MIC] values between 125 and 250 μg/ml) and *Staphylococcus epidermidis* (MIC values between 7.81 and 62.5 μg/ml). Although further studies have to be performed in order to understand the mechanisms responsible for these results, this compound could be a potential natural antimicrobial for the treatment of skin infections.

5 | CONCLUSION

The compounds present in the wastes generated during the olive oil extraction process may have considerable health benefits and be used, after suitable purification, as food antioxidants or active ingredients in nutraceutical and cosmetic products, because they possess a range of recognized bioactivities, technological, and pharmaceutical properties. Further research on their potential applications should be granted in order to valorize them and maintain environmental sustainability. In addition, it is imperative for the development of green and efficient extraction methods to ensure higher recovery of these compounds and the cooperation between industry and researchers to generate sustainable added value to these byproducts, thus contributing to the circular economy.

ACKNOWLEDGMENTS

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES to C2TN (UIDB/04349/2020), CIMO (UIDB/00690/2020) and Joana Madureira (SFRH/BD/136506/2018). Lillian Barros thank the national funding by FCT, P.I., through the institutional scientific employment program-contract. The authors thank the European Regional Development Fund (ERDF), through the Incentive System to Research and Technological development within the Portugal2020 Competitiveness and Internationalization Operational Program for the mobilizing project "BIOMA" (POCI-01-0247-FEDER-046112). The Agroenvironment Unit is supported by *Junta de Castilla y León* (*Escalera de Excelencia* CLU-2018-04) co-funded by the P.O. FEDER of *Castilla y León* 2014–2020.

AUTHOR CONTRIBUTIONS

Joana Madureira: Data curation; investigation; visualization; writing—original draft, review, and editing. Fernanda M. A. Margaça: Funding acquisition; visualization; writing—review and editing. Celestino Santos-Buelga: Conceptualization; funding acquisition; supervision; visualization; writing—review and editing. Isabel C. F. R. Ferreira: Conceptualization; funding acquisition; project administration; writing—review and editing. Sandra Cabo Verde: Conceptualization; funding acquisition; supervision; visualization; writing—review and editing. Lillian Barros: Conceptualization; funding acquisition; supervision; writing—review and editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Sandra Cabo Verde https://orcid.org/0000-0002-6615-5289

Lillian Barros https://orcid.org/0000-0002-9050-5189

REFERENCES

Abdelhadi, S. O., Dosoretz, C. G., Rytwo, G., Gerchman, Y., & Azaizeh, H. (2017). Production of biochar from olive mill solid waste for heavy metal removal. *Bioresource Technology*, *244*, 759–767. https://doi.org/10.1016/j.biortech.2017.08.013

Al Afif, R., & Linke, B. (2019). Biogas production from three-phase olive mill solid waste in lab-scale continuously stirred tank reactor. *Energy*, *171*, 1046–1052. https://doi.org/10.1016/j.energy.2019.01.080

Al-Addous, M., Alnaief, M., Class, C., Nsair, A., Kuchta, K., & Alkasrawi, M. (2017). Technical possibilities of biogas production from olive and date waste in Jordan. *BioResources*, *12*(4), 9383–9395. https://doi.org/10.15376/biores.12.4.9383-9395

Alipieva, K., Korkina, L., Orhan, I. E., & Georgiev, M. I. (2014). Verbascoside — A review of its occurrence, (bio)synthesis and pharmacological significance. *Biotechnology Advances*, *32*(6), 1065–1076. https://doi.org/10.1016/j.biotechadv.2014.07.001

Al-Khatib, A., Aqra, F., Al-Jabari, M., Yaghi, N., Basheer, S., Sabbah, I., B. Al-Hayek, & Mosa, M. (2009). Environmental pollution resulting from olive oil production. *Bulgarian Journal of Agricultural Science*, *15*(6), 544–551.

Anastopoulos, I., Massas, I., & Ehaliotis, C. (2015). Use of residues and by-products of the olive-oil production chain for the removal of pollutants from environmental media: A review of batch biosorption approaches. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering*, *50*(7), 677–718. https://doi.org/10.1080/10934529.2015.1011964

Annunziata, G., Maisto, M., Schisano, C., Barrea, L., Ciampaglia, R., Narciso, V., Tenore, G. C., & Novellino, E. (2018). Oleuropein as a novel anti-diabetic nutraceutical. An overview. *Archives of Diabetes & Obesity*, *1*(3), 54–58. https://doi.org/10.32474/ado.2018.01.000113

Anter, J., Tasset, I., Demyda-Peyrás, S., Ranchal, I., Moreno-Millán, M., Romero-Jimenez, M., Muntané, J., de Castro, M. D. L., Muñoz-Serrano, A., & Alonso-Moraga, Á. (2014). Evaluation of potential antigenotoxic, cytotoxic and proapoptotic effects of the olive oil by-product "alperujo", hydroxytyrosol, tyrosol and verbascoside. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, *772*, 25–33. https://doi.org/10.1016/j.mrgentox.2014.07.002

Araújo, M., Pimentel, F. B., Alves, R. C., & Oliveira, M. B. P. P. (2015). Phenolic compounds from olive mill wastes: Health effects, analytical approach and application as food antioxidants. *Trends in Food Science and Technology*, *45*(2), 200–211. https://doi.org/10.1016/j.tifs.2015.06.010

Arche, M. A. F., García Gimenez, M. D., Martín, M. A., Guerreo, A. M. Q., & Martínez, J. M. Z. (2019). ES 2734600 A1. Aloe and hydroxytyrosol gel composition.

Arvanitoyannis, I. S., & Kassaveti, A. (2007). Current and potential uses of composted olive oil waste. *International Journal of Food Science and Technology*, *42*(3), 281–295. https://doi.org/10.1111/j.1365-2621.2006.01211.x

Azbar, N., Bayram, A., Filibeli, A., Muezzinoglu, A., Sengul, F., & Ozer, A. (2004). A review of waste management options in olive oil production. *Critical Reviews in Environmental Science and Technology*, *34*(3), 209–247. https://doi.org/10.1080/10643380490279932

Balzan, S., Taticchi, A., Cardazzo, B., Urbani, S., Servili, M., Di Lecce, G., Zabalza, I. B., Rodriguez-Estrada, Novelli, E., , & Fasolato, L. (2017). Effect of phenols extracted from a by-product of the oil mill on the shelf-life of raw and cooked fresh pork sausages in the absence of chemical additives. *LWT - Food Science and Technology*, *85*, 89–95. https://doi.org/10.1016/j.lwt.2017.07.001

Battista, F., Mancini, G., Ruggeri, B., & Fino, D. (2016). Selection of the best pretreatment for hydrogen and bioethanol production from olive oil waste products. *Renewable Energy*, *88*, 401–407. https://doi.org/10.1016/j.renene.2015.11.055

Bedoya, L. M., Beltrán, M., Obregón-Calderón, P., García-Pérez, J., De La Torre, H. E., González, N., Pérez-Olmeda, M., Auñón, D., Capa, L., Gómez-Acebo, E., & Alcami, J. (2016). Hydroxytyrosol: A new class of microbicide displaying broad anti-HIV-1 activity. *Aids*, *30*(18), 2767–2776. https://doi.org/10.1097/QAD.0000000000001283

Bernini, R., Carastro, I., Palmini, G., Tanini, A., Zonefrati, R., Pinelli, P., Brandi, M. L., & Romani, A. (2017). Lipophilization of hydroxytyrosol-enriched fractions from *Olea europaea* L. byproducts and evaluation of the in vitro effects on a model of colorectal cancer cells. *Journal of Agricultural and Food Chemistry*, *65*(31), 6506–6512. https://doi.org/10.1021/acs.jafc.6b05457

Bernini, R., Merendino, N., Romani, A., & Velotti, F. (2013). Naturally occurring hydroxytyrosol: Synthesis and anticancer potential. *Current Medicinal Chemistry*, *20*(5), 655–670. https://doi.org/10.2174/092986713804999367

Bernini, R., Montani, M. S. G., Merendino, N., Romani, A., & Velotti, F. (2015). Hydroxytyrosol-derived compounds: A basis for the creation of new pharmacological agents for cancer prevention and therapy. *Journal of Medicinal Chemistry*, *58*(23), 9089–9107. https://doi.org/10.1021/acs.jmedchem.5b00669

Berrougui, H., Ikhlef, S., & Khalil, A. (2015). Extra virgin olive oil polyphenols promote cholesterol efflux and improve HDL functionality. *Evidence-Based Complementary and Alternative Medicine*, *2015*, 208062. https://doi.org/10.1155/2015/208062

Bertelli, M., Kiani, A. K., Paolacci, S., Manara, E., Kurti, D., Dhuli, K., Bushati, V., Miertus, J., Pangallo, D., Baglivo, M., Beccari, T., & Michelini, S. (2020). Hydroxytyrosol: A natural compound with promising pharmacological activities. *Journal of Biotechnology*, *309*, 29–33. https://doi.org/10.1016/j.jbiotec.2019.12.016

Bhatnagar, A., Kaczala, F., Hogland, W., Marques, M., Paraskeva, C. A., Papadakis, V. G., & Sillanpää, M. (2014). Valorization of solid waste products from olive oil industry as potential adsorbents for water pollution control-a review. *Environmental Science and Pollution Research*, *21*, 268–298. https://doi.org/10.1007/s11356-013-2135-6

Bisignano, C., Filocamo, A., Ginestra, G., Giofre', S. V., Navarra, M., Romeo, R., & Mandalari, G. (2014). 3,4-DHPEA-EA from *Olea Europaea* L. is effective against standard and clinical isolates of *Staphylococcus* sp. *Annals of Clinical Microbiology and Antimicrobials*, *13*(1), 24. https://doi.org/10.1186/1476-0711-13-24

Boutry, C., Breuille, D. & Horcajada, N. (2020). US Patent 2020/0390793 A1. Compositions and methods using oleuropein or curcumin for muscle quality and/or muscle mass. Issued May 31, 2019.

Bulbarello, A. & Leuthardt, K. (2016). Patent WO 2016/087428 A1. Fortification of edible oils with hydroxytyrosol. Issued June 9, 2016.

Bulotta, S., Celano, M., Lepore, S. M., Montalcini, T., Pujia, A., & Russo, D. (2014). Beneficial effects of the olive oil phenolic components oleuropein and hydroxytyrosol: Focus on protection against cardiovascular and metabolic diseases. *Journal of Translational Medicine*, *12*, 219. https://doi.org/10.1186/s12967-014-0219-9

Caballero, A. S., Romero-Garcia, J. M., Castro, E., & Cardona, C. A. (2020). Supercritical fluid extraction for enhancing polyphenolic compounds production from olive waste extracts. *Journal of Chemical Technology and Biotechnology*, *95*(2), 356–362. https://doi.org/10.1002/jctb.5907

Caporaso, N., Formisano, D., & Genovese, A. (2018). Use of phenolic compounds from olive mill wastewater as valuable ingredients for functional foods. *Critical Reviews in Food Science and Nutrition*, *58*(16), 2829–2841. https://doi.org/10.1080/10408398.2017.1343797

Cardinali, A., Pati, S., Minervini, F., D'Antuono, I., Linsalata, V., & Lattanzio, V. (2012). Verbascoside, isoverbascoside, and their derivatives recovered from olive mill wastewater as possible food antioxidants. *Journal of Agricultural and Food Chemistry*, *60*(7), 1822–1829. https://doi.org/10.1021/jf204001p

Cardoso, S. M., Guyot, S., Marnet, N., Lopes-da-Silva, J. A., Renard, C. M. G. C., & Coimbra, M. A. (2005). Characterisation of phenolic extracts from olive pulp and olive pomace by electrospray mass spectrometry. *Journal of the Science of Food and Agriculture*, *85*(1), 21–32. https://doi.org/10.1002/jsfa.1925

Casamassima, D. (2013). Patent WO 2013/084249 A1. Dietary supplement comprising verbascoside and teupolioside for lowering LDL and increasing HDL. Issued June 13, 2013.

Casamenti, F., Grossi, C., Rigacci, S., Pantano, D., Luccarini, I., & Stefani, M. (2015). Oleuropein aglycone: A possible drug against degenerative conditions. In vivo evidence of its effectiveness against Alzheimer's disease. *Journal of Alzheimer's Disease*, *45*(3), 679–688. https://doi.org/10.3233/JAD-142850

Cedola, A., Cardinali, A., D'Antuono, I., Conte, A., & Del Nobile, M. A. (2020). Cereal foods fortified with by-products from the olive oil industry. *Food Bioscience*, *33*, 100490. https://doi.org/10.1016/j.fbio.2019.100490

Cedola, A., Palermo, C., Centonze, D., Del Nobile, M. A., & Conte, A. (2020). Characterization and bio-accessibility evaluation of olive leaf extract-enriched "Taralli. *Foods*, *9*, 1268. https://doi.org/10.3390/foods9091268

Čepo, D. V., Radić, K., Jurmanović, S., Jug, M., Rajković, M. G., Pedisić, S., Moslavac, T., & Albahari, P. (2018). Valorization of olive pomace-based nutraceuticals as antioxidants in chemical, food, and biological models. *Molecules*, *23*(8), 2070. https://doi.org/10.3390/molecules23082070

Chandramohan, R., & Pari, L. (2016). Anti-inflammatory effects of tyrosol in streptozotocin-induced diabetic Wistar rats. *Journal of Functional Foods*, *27*, 17–28. https://doi.org/10.1016/j.jff.2016.08.043

Chanioti Sofia, & Tzia Constantina. (2018). Extraction of phenolic compounds from olive pomace by using natural deep eutectic solvents and innovative extraction techniques. *Innovative Food Science & Emerging Technologies*, *48*, 228–239. https://doi.org/10.1016/j.ifset.2018.07.001

Chaves-López, C., Serio, A., Mazzarrino, G., Martuscelli, M., Scarpone, E., & Paparella, A. (2015). Control of household mycoflora

in fermented sausages using phenolic fractions from olive mill wastewaters. *International Journal of Food Microbiology*, *207*, 49–56. https://doi.org/10.1016/j.ijfoodmicro.2015.04.040

Chen, C. H., Lin, Y. S., Chien, M. Y., Hou, W. C., & Hu, M. L. (2012). Antioxidant and antihypertensive activities of acteoside and its analogs. *Botanical Studies*, *53*(4), 421–429.

Cioffi, G., Pesca, M. S., De Caprariis, P., Braca, A., Severino, L., & De Tommasi, N. (2010). Phenolic compounds in olive oil and olive pomace from Cilento (Campania, Italy) and their antioxidant activity. *Food Chemistry*, *121*(1), 105–111. https://doi.org/10.1016/j.foodchem.2009.12.013

Codina, E. G. & Monjo, E. G. (2014). Patent ES2462565 A1. Uso dermocosmético del hidroxitirosol para aclarar la piel y composiciones que lo comprenden. Issued March 4, 2015.

Cordero, J. G., García-Escudero, R., Avila, J., Gargini, R., & García-Escudero, V. (2018). Benefit of oleuropein aglycone for Alzheimer's disease by promoting autophagy. *Oxidative Medicine and Cellular Longevity*, *2018*, 5010741. https://doi.org/10.1155/2018/5010741

Crea, R. (2014). US Patent 2014/0011756 A1. Method for treatment of inflammation. Issued January 9, 2014.

Crea, R. (2017). US Patent 2017/0367995 A1. Treatment of early stage Parkinson's disease with a hydroxytyrosol-containing polyphenol formulation. Issued June 23, 2016.

Cui, Q., Pan, Y., Zhang, W., Zhang, Y., Ren, S., Wang, D., Wang, Z., Liu, X., & Xiao, W. (2018). Metabolites of dietary acteoside: Profiles, isolation, identification, and hepatoprotective capacities. *Journal of Agricultural and Food Chemistry*, *66*(11), 2660–2668. https://doi.org/10.1021/acs.jafc.7b04650

D'Angelo, S., Ingrosso, D., Migliardi, V., Sorrentino, A., Donnarumma, G., Baroni, A., Masella, L., Tufano, M. A., Zappia, M., & Galletti, P. (2005). Hydroxytyrosol, a natural antioxidant from olive oil, prevents protein damage induced by long-wave ultraviolet radiation in melanoma cells. *Free Radical Biology and Medicine*, *38*(7), 908–919. https://doi.org/10.1016/j.freeradbiomed.2004.12.015

de la Casa José A., & Castro Eulogio. (2014). Recycling of washed olive pomace ash for fired clay brick manufacturing. *Construction and Building Materials*, *61*, 320–326. https://doi.org/10.1016/j.conbuildmat.2014.03.026

de la Casa, J. A., Lorite, M., Jiménez, J., & Castro, E. (2009). Valorisation of wastewater from two-phase olive oil extraction in fired clay brick production. *Journal of Hazardous Materials*, *169*(1–3), 271–278. https://doi.org/10.1016/j.jhazmat.2009.03.095

de Magalhães Nunes da Ponte, M. L., dos Santos, J. L. C., Matias, A. A. F., Nunes, A. V. M. N., Duarte, C. M. M. & Crespo, J. P. S. G. (2014). Patent EP1910257 B1. Method of obtaining a natural hydroxytyrosol-rich concentrate from olive tree residues and subproducts using clean technologies. Issued January 1, 2014.

de Moraes Crizel Tainara, de Oliveira Ríos Alessandro, D. Alves Vitor, Bandarra Narcisa, Moldão-Martins Margarida, & Hickmann Flôres Simone. (2018). Active food packaging prepared with chitosan and olive pomace. *Food Hydrocolloids*, *74*, 139–150. https://doi.org/10.1016/j.foodhyd.2017.08.007

de Moura Sperotto, N. D., Steffens, L., Veríssimo, R. M., Henn, J. G., Péres, V. F., Vianna, P., Chies, J. A. B., Roehe, A., Saffi, J., & Moura, D. J. (2018). Wound healing and anti-inflammatory activities induced by a Plantago australis hydroethanolic extract standardized in verbascoside. *Journal of Ethnopharmacology*, *225*, 178–188. https://doi.org/10.1016/j.jep.2018.07.012

Dermeche, S., Nadour, M., Larroche, C., Moulti-Mati, F., & Michaud, P. (2013). Olive mill wastes: Biochemical characterizations and valorization strategies. *Process Biochemistry*, *48*(10), 1532–1552. https://doi.org/10.1016/j.procbio.2013.07.010

Drira, R., Chen, S., & Sakamoto, K. (2011). Oleuropein and hydroxytyrosol inhibit adipocyte differentiation in 3 T3-L1 cells. *Life Sciences*, *89*(19–20), 708–716. https://doi.org/10.1016/j.lfs.2011.08.012

Dunne, G. (2019). Transforming olive waste into animal feed. *International Journal of Clinical Nutrition & Dietetics*, *5*(142), 10–12. https://doi.org/10.15344/2456-8171/2019/142

EFSA Panel on Dietetic Products Nutrition and Allergies. (2011). Scientific Opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865), maintenance of normal blood HDL cholesterol concentrations (ID 1639), mainte. *EFSA Journal*, *9*(4), 2033. https://doi.org/10.2903/j.efsa.2011.2033

EFSA Panel on Dietetic Products Nutrition and Allergies. (2017). Safety of hydroxytyrosol as a novel food pursuant to Regulation (EC) No 258/97. *EFSA Journal*, *15*(3), e04728. https://doi.org/10.2903/j.efsa.2017.4728

Ehrenberger, K. & Bieberschulte, W. (2011). Patent WO 2011/153647 A1. Pharmaceutical composition with olive extract (hydroxytyrosol) and hop extract (xanthohumol) for intranasal application. Issued December 15, 2011.

El-Abbassi, A., Kiai, H., & Hafidi, A. (2012). Phenolic profile and antioxidant activities of olive mill wastewater. *Food Chemistry*, *132*(1), 406–412. https://doi.org/10.1016/j.foodchem.2011.11.013

Claude Elama, Mohammed Tarawa, & Fuad Al-Rimawi. (2017). Oleuropein from Olive Leaf Extract as Natural Antioxidant of Frozen Hamburger. *Journal of Food Science and Engineering*, *7*(8). https://doi.org/10.17265/2159-5828/2017.08.005

Eliche-Quesada, D., Iglesias-Godino, F. J., Pérez-Villarejo, L., & Corpas-Iglesias, F. A. (2014). Replacement of the mixing fresh water by wastewater olive oil extraction in the extrusion of ceramic bricks. *Construction and Building Materials*, *68*, 659–666. https://doi.org/10.1016/j.conbuildmat.2014.07.017

El-Marasy, S. A., El-Shenawy, S. M., Moharram, F. A., & El-Sherbeeny, N. A. (2020). Antidiabetic and antioxidant effects of acteoside from Jacaranda mimosifolia family Biognoniaceae in Streptozotocin–Nicotinamide induced diabetes in rats. *Open Access Macedonian Journal of Medical Sciences*, *8*, 125–133. https://doi.org/10.3889/OAMJMS.2020.3325

Estaún, J., Dosil, J., Al Alami, A., Gimeno, A., & de Vega, A. (2014). Effects of including olive cake in the diet on performance and rumen function of beef cattle. *Animal Production Science*, *54*(10), 1817. https://doi.org/10.1071/an14352

Federici, E., Massaccesi, L., Pezzolla, D., Fidati, L., Montalbani, E., Proietti, P., Nasini, L., Regni, L., Scargetta, S., & Gigliotti, G. (2017). Short-term modifications of soil microbial community structure and soluble organic matter chemical composition following amendment with different solid olive mill waste and their derived composts. *Applied Soil Ecology*, *119*, 234–241. https://doi.org/10.1016/j.apsoil.2017.06.014

Fernández-González, R., Martín-Lara, M. Á., Blázquez, G., Pérez, A., & Calero, M. (2019). Recovering metals from aqueous solutions by biosorption onto hydrolyzed olive cake. *Water*, *11*(12), 2519. https://doi.org/10.3390/w11122519

Fernández-Hernández, A., Roig, A., Serramiá, N., Civantos, C. G. O., & Sánchez-Monedero, M. A. (2014). Application of compost

of two-phase olive mill waste on olive grove: Effects on soil, olive fruit and olive oil quality. *Waste Management*, *34*(7), 1139–1147. https://doi.org/10.1016/j.wasman.2014.03.027

Fernando Ana, Monteiro Sofia, Pinto Filomena, & Mendes Benilde. (2009). Production of Biosorbents from Waste Olive Cake and Its Adsorption Characteristics for Zn2+ Ion. *Sustainability*, *1*(2), 277–297. https://doi.org/10.3390/su1020277

Filip, R., Possemiers Sam, Heyerick, A., Pinheiro, I., Raszewski, G., Davicco, M.-J., & Coxam V. (2015). Twelve-month consumption of a polyphenol extract from olive (Olea europaea) in a double blind, randomized trial increases serum total osteocalcin levels and improves serum lipid profiles in postmenopausal women with osteopenia. *The journal of nutrition, health & aging*, *19*(1), 77–86. https://doi.org/10.1007/s12603-014-0480-x

Fiorentino, A., Gentili, A., Isidori, M., Monaco, P., Nardelli, A., Parrella, A., & Temussi, F. (2003). Environmental effects caused by olive mill wastewaters: Toxicity comparison of low-molecular-weight phenol components. *Journal of Agricultural and Food Chemistry*, *51*(4), 1005–1009. https://doi.org/10.1021/jf020887d

Fki, I., Sayadi, S., Mahmoudi, A., Daoued, I., Marrekchi, R., & Ghorbel, H. (2020). Comparative study on beneficial effects of hydroxytyrosol- and oleuropein-rich olive leaf extracts on high-fat diet-induced lipid metabolism disturbance and liver injury in rats. *BioMed Research International*, *2021*, 1315202. https://doi.org/10.1155/2020/1315202

Funes, L., Fernández-Arroyo, S., Laporta, O., Pons, A., Roche, E., Segura-Carretero, A., A. Fernández-Gutiérrez, & Micol, V. (2009). Correlation between plasma antioxidant capacity and verbascoside levels in rats after oral administration of lemon verbena extract. *Food Chemistry*, *117*(4), 589–598. https://doi.org/10.1016/j.foodchem.2009.04.059

Funes, L., Laporta, O., Cerdán-Calero, M., & Micol, V. (2010). Effects of verbascoside, a phenylpropanoid glycoside from lemon verbena, on phospholipid model membranes. *Chemistry and Physics of Lipids*, *163*(2), 190–199. https://doi.org/10.1016/j.chemphyslip.2009.11.004

Fuwa, T., Nakao, Y. & Matsubayashi, H. (2019). US Patent 2019/012952 A1. Beverage containing hydroxytyrosol. Issued October 08, 2019.

Galanakis, Charis M. (2018). Phenols recovered from olive mill wastewater as additives in meat products. *Trends in Food Science & Technology*, *79*, 98–105. https://doi.org/10.1016/j.tifs.2018.07.010

Galanakis, Charis M. (2020). The Food Systems in the Era of the Coronavirus (COVID-19) Pandemic Crisis. *Foods*, *9*(4), 523. https://doi.org/10.3390/foods9040523

Galanakis, C. M., Aldawoud, T. M. S., Rizou, M., Rowan, N. J., & Ibrahim, S. A. (2020). Food ingredients and active compounds against the coronavirus disease (COVID-19) pandemic: A comprehensive review. *Foods*, *9*(11), 1701. https://doi.org/10.3390/foods9111701

Galanakis, Charis M., Rizou Myrto, Aldawoud, Turki M. S., Ucak Ilknur, & Rowan, Neil J. (2021). Innovations and technology disruptions in the food sector within the COVID-19 pandemic and post-lockdown era. *Trends in Food Science & Technology*, *110*, 193–200. https://doi.org/10.1016/j.tifs.2021.02.002

Galanakis, Charis M., Tsatalas Philippos, Charalambous, Zina, & Galanakis, Ioannis M. (2018). Control of microbial growth in bakery products fortified with polyphenols recovered from olive mill wastewater. *Environmental Technology & Innovation*, *10*, 1–15. https://doi.org/10.1016/j.eti.2018.01.006

Galanakis Charis M., Tsatalas Philippos, Galanakis Ioannis M. (2018). Implementation of phenols recovered from olive mill wastewater as UV booster in cosmetics. *Industrial Crops and Products*, *111*, 30–37. https://doi.org/10.1016/j.indcrop.2017.09.058

Georgakouli, K., Mpesios, A., Kouretas, D., Petrotos, K., Mitsagga, C., Giavasis, I., & Jamurtas, A. Z. (2016). The effects of an olive fruit polyphenol-enriched yogurt on body composition, blood redox status, physiological and metabolic parameters and yogurt microflora. *Nutrients*, *8*(344), 1–14. https://doi.org/10.3390/nu8060344

Gerasopoulos, K., Stagos, D., Kokkas, S., Petrotos, K., Kantas, D., Goulas, P., & Kouretas, D. (2015). Feed supplemented with byproducts from olive oil mill wastewater processing increases antioxidant capacity in broiler chickens. *Food and Chemical Toxicology*, *82*, 42–49. https://doi.org/10.1016/j.fct.2015.04.021

Gerasopoulos, K., Stagos, D., Petrotos, K., Kokkas, S., Kantas, D., Goulas, P., & Kouretas, D. (2015). Feed supplemented with polyphenolic byproduct from olive mill wastewater processing improves the redox status in blood and tissues of piglets. *Food and Chemical Toxicology*, *86*, 319–327. https://doi.org/10.1016/j.fct.2015.11.007

Giovannini, C., Straface, E., Modesti, D., Coni, E., Cantafora, A., De Vincenzi, M., Malorni, W., & Masella, R. (1999). Tyrosol, the major olive oil biophenol, protects against oxidized-LDL-induced injury in Caco-2 cells. *Journal of Nutrition*, *129*, 1269–1277. https://doi.org/10.1093/jn/129.7.1269

Goldsmith, Chloe D., Vuong, Quan V., Stathopoulos, Costas E., Roach, Paul D., Scarlett, & Christopher J. (2018). Ultrasound increases the aqueous extraction of phenolic compounds with high antioxidant activity from olive pomace. *LWT*, *89*, 284–290. https://doi.org/10.1016/j.lwt.2017.10.065

Gómez-Acebo, E., Pertejo, J. A. & Calles, D. A. (2012). US Patent 2012/0260922 A1. Topical use of hydroxytyrosol and derivatives for the prevention of HIV infection. Issued October 10, 2012.

Grossi, C., Rigacci, S., Ambrosini, S., Ed Dami, T., Luccarini, I., Traini, C., Failli, P., Berti, A., Casamenti, F., & Stefani, M. (2013). The polyphenol oleuropein aglycone protects TgCRND8 mice against AB plaque pathology. *Plos One*, *8*(8), e71702. https://doi.org/10.1371/journal.pone.0071702

Guglielmotti, M., Passaghe, P., & Buiatti, S. (2020). Use of olive (*Olea europaea* L.) leaves as beer ingredient, and their influence on beer chemical composition and antioxidant activity. *Journal of Food Science*, *85*(8), 2278–2285. https://doi.org/10.1111/1750-3841.15318

Gusman, J. F.-B., Moreno, A. H., Gutierrez, G. R., Arcos, R. R., Araujo, A. J. & Bejarano, R. G. (2005). US Patent 6849770 B2. Method for obtaining purified hydroxytyrosol from products and by-products derived from the olive tree. Issued February 01, 2005.

Hagiwara, K., Goto, T., Araki, M., Miyazaki, H., & Hagiwara, H. (2011). Olive polyphenol hydroxytyrosol prevents bone loss. *European Journal of Pharmacology*, *662*(1–3), 78–84. https://doi.org/10.1016/j.ejphar.2011.04.023

Hassen, I., Casabianca, H., & Hosni, K. (2015). Biological activities of the natural antioxidant oleuropein: Exceeding the expectation – A mini-review. *Journal of Functional Foods*, *18*, 926–940. https://doi.org/10.1016/j.jff.2014.09.001

Illesca, P., Valenzuela, R., Espinosa, A., Echeverría, F., Soto-Alarcon, S., Ortiz, M., & Videla, L. A. (2019). Hydroxytyrosol supplementation ameliorates the metabolic disturbances in white adipose tissue from mice fed a high-fat diet through

recovery of transcription factors Nrf2, SREBP-1c, PPAR-γ and NF-κB. *Biomedicine and Pharmacotherapy*, *109*, 2472–2481. https://doi.org/10.1016/j.biopha.2018.11.120

Imran, M., Nadeem, M., Gilani, S. A., Khan, S., Sajid, M. W., & Amir, R. M. (2018). Antitumor perspectives of oleuropein and its metabolite hydroxytyrosol: Recent updates. *Journal of Food Science*, *83*(7), 1781–1791. https://doi.org/10.1111/1750-3841.14198

Jemai, H., Bouaziz, M., Fki, I., El Feki, A., & Sayadi, S. (2008). Hypolipidimic and antioxidant activities of oleuropein and its hydrolysis derivative-rich extracts from Chemlali olive leaves. *Chemico-Biological Interactions*, *176*(2–3), 88–98. https://doi.org/10.1016/j.cbi.2008.08.014

Jeon Seeun, Choi Mina. (2018). Anti-inflammatory and anti-aging effects of hydroxytyrosol on human dermal fibroblasts (HDFs). *Biomedical Dermatology*, *2*(1), https://doi.org/10.1186/s41702-018-0031-x

Khdair, A. I., Abu-Rumman, G., & Khdair, S. I. (2019). Pollution estimation from olive mills wastewater in Jordan. *Heliyon*, *5*(8), e02386. https://doi.org/10.1016/j.heliyon.2019.e02386

Khemakhem, I., Fuentes, A., Lerma-Garcia, M. J., Ayadi, M. A., Bouaziz, M., & Barat, J. M. (2019). Olive leaf extracts for shelf life extension of salmon burgers. *Food Science and Technology International*, *25*(2), 91–100. https://doi.org/10.1177/1082013218795816

Kim, S. W., Hur, W., Li, T. Z., Lee, Y. K., Choi, J. E., Hong, S. W., Lyoo, K. S., You, C. R., Jung, E. S., Jung, C. K., Park, T., Um, S. J., & Yoon, S. K. (2014). Oleuropein prevents the progression of steatohepatitis to hepatic fibrosis induced by a high-fat diet in mice. *Experimental and Molecular Medicine*, *46*(4), e2. https://doi.org/10.1038/emm.2014.10

Kimura, Y., & Sumiyoshi, M. (2009). Olive leaf extract and its main component oleuropein prevent chronic ultraviolet B radiation-induced skin damage and carcinogenesis in hairless mice. *The Journal of Nutrition*, *139*(11), 2079–2086. https://doi.org/10.3945/jn.109.104992

Koc, K., Cerig, S., Ucar, S., Colak, S., Bakir, M., Erol, H. S., Yildirim, S., Hosseinigouzdagani, M., Simsek Ozek, N., Aysin, F., Fehim Kocpinar, E., Budak, H., & Geyikoglu, F. (2018). Gastroprotective effects of oleuropein and thymol on indomethacin-induced gastric ulcer in Sprague-Dawley rats. *Drug and Chemical Toxicology*, *43*(5), 441–453. https://doi.org/10.1080/01480545.2018.1530261

Kumari, D., & Singh, R. (2018). Pretreatment of lignocellulosic wastes for biofuel production: A critical review. *Renewable and Sustainable Energy Reviews*, *90*, 877–891. https://doi.org/10.1016/j.rser.2018.03.111

Lammi, S., Le Moigne, N., Djenane, D., Gontard, N., & Angellier-Coussy, H. (2018). Dry fractionation of olive pomace for the development of food packaging biocomposites. *Industrial Crops and Products*, *120*, 250–261. https://doi.org/10.1016/j.indcrop.2018.04.052

Lee Kwang Min, Hur Jinyoung, Lee Yeonmi, Yoon Bo-Ra, Choi Sang Yoon (2018). Protective Effects of Tyrosol Against Oxidative Damage in L6 Muscle Cells. *Food Science and Technology Research*, *24*(5), 943–947. https://doi.org/10.3136/fstr.24.943

Sylvia Lee-Huang, Paul L. Huang, Philip Lin Huang, Dawei Zhang, John Z. H. Zhang, Young Tae Chang, Jae Wook Lee, Ju Bao, Yongtao Sun (2013). US 8574635 B2. Compositions and methods for treating obesity, obesity related disorders and for inhibiting the infectivity of human immunodeficiency virus. Issued November 05, 2013.

Lee-Huang, S., Huang, P. L., Zhang, D., Lee, J. W., Bao, J., Sun, Y., Chang, Y. T., Zhang, J., & Huang, P. L. (2007). Discovery of small-molecule HIV-1 fusion and integrase inhibitors oleuropein and hydroxytyrosol: Part I. Integrase inhibition. *Biochemical and Biophysical Research Communications*, *354*, 872–878. https://doi.org/10.1016/j.bbrc.2007.01.071

Lee-Huang, S., Zhang, L., Huang, P. L., Chang, Y. T., & Huang, P. L. (2003). Anti-HIV activity of olive leaf extract (OLE) and modulation of host cell gene expression by HIV-1 infection and OLE treatment. *Biochemical and Biophysical Research Communications*, *307*(4), 1029–1037. https://doi.org/10.1016/S0006-291X(03)01292-0

Leenen, R., Roodenburg, A. J. C., Vissers, M. N., Schuurbiers, J. A. E., Van Putte, K. P. A. M., Wiseman, S. A., & Van de Put, F. H. M. M. (2002). Supplementation of plasma with olive oil phenols and extracts: Influence on LDL oxidation. *Journal of Agricultural and Food Chemistry*, *50*(5), 1290–1297. https://doi.org/10.1021/jf010968u

Leouifoudi, I., Mbarki, M., Tilaoui, M., Amechrouq, A., Mostapha, E., Mouse, H. A., & Zyad, A. (2014). Study of the in vitro anticancer activity of Moroccan phenolic olive cake extracts. *Journal of Pharmacognosy and Phytochemistry*, *2*(6), 154–165.

Leri, M., & Bucciantini, M. (2016). Efficacy of oleuropein aglycone in the treatment of transthyretin-amyloidosis. *Biochemistry & Molecular Biology Journal*, *2*(1), 1–6. https://doi.org/10.21767/2471-8084.100011

Li, X., Liu, Y., Jia, Q., LaMacchia, V., O'Donoghue, K., & Huang, Z. (2016). A systems biology approach to investigate the antimicrobial activity of oleuropein. *Journal of Industrial Microbiology and Biotechnology*, *43*(12), 1705–1717. https://doi.org/10.1007/s10295-016-1841-8

Liu, M., Yong, Q., & Yu, S. (2018). Efficient bioconversion of oleuropein from olive leaf extract to antioxidant hydroxytyrosol by enzymatic hydrolysis and high-temperature degradation. *Biotechnology and Applied Biochemistry*, *65*(5), 680–689. https://doi.org/10.1002/bab.1651

Liu, Y. H., Lu, Y. L., Han, C. H., & Hou, W. C. (2013). Inhibitory activities of acteoside, isoacteoside, and its structural constituents against protein glycation in vitro. *Botanical Studies*, *54*(1), 6. https://doi.org/10.1186/1999-3110-54-6

Liu, Y., McKeever, L. C., & Malik, N. S. A. (2017). Assessment of the antimicrobial activity of olive leaf extract against foodborne bacterial pathogens. *Frontiers in Microbiology*, *8*, 113. https://doi.org/10.3389/fmicb.2017.00113

Loru, D., Incani, A., Deiana, M., Corona, G., Atzeri, A., Melis, M., Rosa, A., & Dessi, M. (2009). Protective effect of hydroxytyrosol and tyrosol against oxidative stress in kidney cells. *Toxicology and Industrial Health*, *25*(5), 301–310. https://doi.org/10.1177/0748233709103028

Ma, D., Wang, J., Liu, L., Chen, M., & Wang, Z. (2020). Acteoside as a potential therapeutic option for primary hepatocellular carcinoma: A preclinical study. *BMC Cancer*, *20*(1), 936. https://doi.org/10.1186/s12885-020-07447-3

Madureira, J., Dias, M. I., Pinela, J., Calhelha, R. C., Barros, L., Santos-Buelga, C., Margaça, F. M. A., Ferreira I., & Verde, S. C. (2020). The use of gamma radiation for extractability improvement of bioactive compounds in olive oil wastes. *Science of the Total Environment*, *727*, 138706. https://doi.org/10.1016/j.scitotenv.2020.138706

Majbar Z., Lahlou, K., Ben, Abbou M., Ammar, E., Triki, A., Abid, W., Nawdali, M., Bouka, H., Taleb, M., El Haji, M., & Rais, Z. (2018). Co-composting of Olive Mill Waste and Wine-Processing Waste: An Application of Compost as Soil Amendment. *Journal of Chemistry*, *2018*, 1–9. https://doi.org/10.1155/2018/7918583

Malapert, A., Reboul, E., Loonis, M., Dangles, O., & Tomao, V. (2018). Direct and rapid profiling of biophenols in olive pomace by UHPLC-DAD-MS. *Food Analytical Methods*, *11*(4), 1001–1010. https://doi.org/10.1007/s12161-017-1064-2

Marković, A. K., Torić, J., Barbarić, M., & Brala, C. J. (2019). Hydroxytyrosol, tyrosol and derivatives and their potential effects on human health. *Molecules*, *24*(10), 2001. https://doi.org/10.3390/molecules24102001

Martin, L. C., Tirado, F. J. S. & Antoñanzas, Y. A. (2007). Patent WO 2007/051829 A1. Oleuropein for the treatment of conditions associated to the peripheral vascular disease. Issued May 10, 2007.

Martinez, L., Castillo, J., Ros, G., & Nieto, G. (2019). Antioxidant and antimicrobial activity of rosemary, pomegranate and olive extracts in fish patties. *Antioxidants*, *8*(4), 86. https://doi.org/10.3390/antiox8040086

Martinez-Garcia, G., Bachmann, Robert Th., Williams, Ceri J., Burgoyne, Andrea, Edyvean, Robert G. J. (2006). Olive oil waste as a biosorbent for heavy metals. *International Biodeterioration & Biodegradation*, *58*(3–4), 231–238. https://doi.org/10.1016/j.ibiod.2006.06.028

Martin-Lara M. A., Blázquez G., Ronda A., Pérez A., & Calero M. (2013). Development and Characterization of Biosorbents To Remove Heavy Metals from Aqueous Solutions by Chemical Treatment of Olive Stone. *Industrial & Engineering Chemistry Research*, *52*(31), 10809–10819. https://doi.org/10.1021/ie401246c

Más, J. L., Streitenberger, S., Penalver Mellado, M. & Martínez Ortiz, P. (2009). Patent WO 2009/013596 A2. Fortification of nutritional products with olive extracts containing hydroxytyrosol and hydroxytyrosol fortified nutritional products. Issued January 29, 2009.

Mateos, R., Martínez-López, S., Arévalo, G. B., Amigo-Benavent, M., Sarriá, B., & Bravo-Clemente, L. (2016). Hydroxytyrosol in functional hydroxytyrosol-enriched biscuits is highly bioavailable and decreases oxidised low density lipoprotein levels in humans. *Food Chemistry*, *205*, 248–256. https://doi.org/10.1016/j.foodchem.2016.03.011

Medina, E., Brenes, M., Romero, C., Garcia, A., & De Castro, A. (2007). Main antimicrobial compounds in table olives. *Journal of Agricultural and Food Chemistry*, *55*(24), 9817–9823. https://doi.org/10.1021/jf0719757

Mekki, H., Anderson, M., Amar, E., Skerratt, G. R., & Benzina, M. (2006). Olive oil mill waste water as a replacement for fresh water in the manufacture of fired clay bricks. *Journal of Chemical Technology & Biotechnology*, *81*, 1419–1425. https://doi.org/10.1002/jctb.1579

Mekki, H., Anderson, M., Benzina, M., & Ammar, E. (2008). Valorization of olive mill wastewater by its incorporation in building bricks. *Journal of Hazardous Materials*, *158*(2–3), 308–315. https://doi.org/10.1016/j.jhazmat.2008.01.104

Menendez, J. A., Vazquez-Martin, A., Colomer, R., Brunet, J., Carrasco-Pancorbo, A., Garcia-Villalba, R., Fernandez-Gutierrez, A.,, & Segura-Carretero, A. (2007). Olive oil's bitter principle reverses acquired autoresistance to trastuzumab (Herceptin™) in HER2-overexpressing breast cancer cells. *BMC Cancer*, *7*, 80. https://doi.org/10.1186/1471-2407-7-80

Merra, E., Calzaretti, G., Bobba, A., Storelli, M. M., & Casalino, E. (2014). Antioxidant role of hydroxytyrosol on oxidative stress in cadmium-intoxicated rats: Different effect in spleen and testes. *Drug and Chemical Toxicology*, *37*(4), 420–426. https://doi.org/10.3109/01480545.2013.878950

Messineo, A., Maniscalco, M. P., & Volpe, R. (2020). Biomethane recovery from olive mill residues through anaerobic digestion: A review of the state of the art technology. *Science of the Total Environment*, *703*, 135508. https://doi.org/10.1016/j.scitotenv.2019.135508

Molina-Alcaide, E., Morales-Garcia, E. Y., Martín-García, A. I., Salem, H. B., Nefzaoui, A., & Sanz-Sampelayo, M. R. (2010). Effects of partial replacement of concentrate with feed blocks on nutrient utilization, microbial N flow, and milk yield and composition in goats. *Journal of Dairy Science*, *93*(5), 2076–2087. https://doi.org/10.3168/jds.2009-2628

Morikawa, T., Ninomiya, K., Imamura, M., Akaki, J., Fujikura, S., Pan, Y., Yuan, D., Yoshikawa, M., Jia, A., Li, Z., & Muraoka, O. (2014). Acylated phenylethanoid glycosides, echinacoside and acteoside from *Cistanche tubulosa*, improve glucose tolerance in mice. *Journal of Natural Medicines*, *68*(3), 561–566. https://doi.org/10.1007/s11418-014-0837-9

Moudache, Messaad, Silva Filomena, Nerín Cristina, & Zaidi, Farid. (2021). Olive cake and leaf extracts as valuable sources of antioxidant and antimicrobial compounds: a comparative study. *Waste and Biomass Valorization*, *12*(3), 1431–1445. https://doi.org/10.1007/s12649-020-01080-8

Muiño, I., Diaz, M. T., Apeleo, E., Pérez-Santaescolástica, C., Rivas-Cañedo, A., Pérez, C., Cañeque, V., Lauzurica, S., & de la Fuente, J. (2017). Valorisation of an extract from olive oil waste as a natural antioxidant for reducing meat waste resulting from oxidative processes. *Journal of Cleaner Production*, *140*, 924–932. https://doi.org/10.1016/j.jclepro.2016.06.175

Nediani, C., Ruzzolini, J., Romani, A., & Calorini, L. (2019). Oleuropein, a bioactive compound from olea europaea l., as a potential preventive and therapeutic agent in non-communicable diseases. *Antioxidants*, *8*(12), 578. https://doi.org/10.3390/antiox8120578

Nieto, G., Martínez, L., Castillo, J., & Ros, G. (2017a). Effect of hydroxytyrosol, walnut and olive oil on nutritional profile of Low-Fat Chicken Frankfurters. *European Journal of Lipid Science and Technology*, *119*(9), 1600518. https://doi.org/10.1002/ejlt.201600518

Nieto, G., Martínez, L., Castillo, J., & Ros, G. (2017b). Hydroxytyrosol extracts, olive oil and walnuts as functional components in chicken sausages. *Journal of the Science of Food and Agriculture*, *97*(11), 3761–3771. https://doi.org/10.1002/jsfa.8240

Numano, F., Crea, R., Wiedemann, T. S. & Bitler, C. M. (2007). Patent WO 2007/012057 A2. Vegetation water composition for treatment of inflammatory skin conditions. Issued January 25, 2007.

Nunes, M. A., Costa, A. S. G., Bessada, S., Santos, J., Puga, H., Alves, R. C., Freitas, V., & Oliveira, M. B. P. P. (2018). Olive pomace as a valuable source of bioactive compounds: A study regarding its lipid- and water-soluble components. *Science of The Total Environment*, *644*, 229–236. https://doi.org/10.1016/j.scitotenv.2018.06.350

Ohno, T., Inoue, M., Ogihara, Y., & Saracoglu, I. (2002). Antimetastatic activity of acteoside, a phenylethanoid

glycoside. *Biological and Pharmaceutical Bulletin*, *25*(5), 666–668. https://doi.org/10.1248/bpb.25.666

Oi-Kano, Y., Iwasaki, Y., Nakamura, T., Watanabe, T., Goto, T., Kawada, T., Watanabe, K., & Iwai, K. (2017). Oleuropein aglycone enhances UCP1 expression in brown adipose tissue in high-fat-diet-induced obese rats by activating β-adrenergic signaling. *Journal of Nutritional Biochemistry*, *40*, 209–218. https://doi.org/10.1016/j.jnutbio.2016.11.009

Pagnanelli, F., Mainelli, S., Veglió, F., & Toro, L. (2003). Heavy metal removal by olive pomace: Biosorbent characterisation and equilibrium modelling. *Chemical Engineering Science*, *58*(20), 4709–4717. https://doi.org/10.1016/j.ces.2003.08.001

Palazzi, L., Bruzzone, E., Bisello, G., Leri, M., Stefani, M., Bucciantini, M., & De Laureto, P. P. (2018). Oleuropein aglycone stabilizes the monomeric α-synuclein and favours the growth of non-toxic aggregates. *Scientific Reports*, *8*(1), 833. https://doi.org/10.1038/s41598-018-26645-5

Palmeri, R., Parafati, L., Trippa, D., Siracusa, L., Arena, E., Restuccia, C., & Fallico, B. (2019). Addition of olive leaf extract (OLE) for producing fortified fresh pasteurized milk with an extended shelf life. *Antioxidants*, *8*(8), 255. https://doi.org/10.3390/antiox8080255

Panyam, J. & Chavanpatil, M. D. (2011). US Patent 2011/0144040 A1. Acteoside and Acteoside-rich plant extracts for increasing athletic performance in humans. Issued June 16, 2011.

Papaioannou, E. H., Patsios, S. I., Karabelas, A. J., & Philippopoulos, N. A. (2013). Characterization of condensates from an indirect olive oil pomace drying process: The effect of drying temperature. *Journal of Environmental Chemical Engineering*, *1*(4), 831–837. https://doi.org/10.1016/j.jece.2013.07.025

Paulo Filipa, & Santos Lúcia. (2020). Encapsulation of the Antioxidant Tyrosol and Characterization of Loaded Microparticles: an Integrative Approach on the Study of the Polymer-Carriers and Loading Contents. *Food and Bioprocess Technology*, *13*(5), 764–785. https://doi.org/10.1007/s11947-020-02407-y

Paulo, F., & Santos, L. (2020b). New insights in the in vitro release of phenolic antioxidants: The case study of the release behavior of tyrosol from tyrosol-loaded ethylcellulose microparticles during the in vitro gastrointestinal digestion. *Colloids and Surfaces B: Biointerfaces*, *196*, 111339. https://doi.org/10.1016/j.colsurfb.2020.111339

Pavez, I. C., Lozano-Sánchez, J., Borrás-Linares, I., Nuñez, H., Robert, P., & Segura-Carretero, A. (2019). Obtaining an extract rich in phenolic compounds from olive pomace by pressurized liquid extraction. *Molecules*, *24*(3108), 1–17.

Pavlidou, A., Anastasopoulou, E., Dassenakis, M., Hatzianestis, I., Paraskevopoulou, V., Simboura, N., Rousselaki, E., & Drakopoulou, P. (2014). Effects of olive oil wastes on river basins and an oligotrophic coastal marine ecosystem: A case study in Greece. *Science of the Total Environment*, *497–498*, 38–49. https://doi.org/10.1016/j.scitotenv.2014.07.088

Pazos, M., Alonso, A., Sánchez, I., & Medina, I. (2008). Hydroxytyrosol prevents oxidative deterioration in foodstuffs rich in fish lipids. *Journal of Agricultural and Food Chemistry*, *56*(9), 3334–3340. https://doi.org/10.1021/jf073403s

Peralbo-Molina, Á., Priego-Capote, F., & De Castro, M. D. L. (2012). Tentative identification of phenolic compounds in olive pomace extracts using liquid chromatography-tandem mass spectrometry with a quadrupole- quadrupole-time-of-flight mass detector. *Journal of Agricultural and Food Chemistry*, *60*(46), 11542–11550. https://doi.org/10.1021/jf302896m

Pérez-Bonilla, M., Salido, S., Van Beek, T. A., & Altarejos, J. (2014). Radical-scavenging compounds from olive tree (*Olea europaea* L.) wood. *Journal of Agricultural and Food Chemistry*, *62*(1), 144–151. https://doi.org/10.1021/jf4039981

Perugini, P., Vettor, M., Rona, C., Troisi, L., Villanova, L., Genta, I., Conti, B., & Pavanetto, F. (2008). Efficacy of oleuropein against UVB irradiation: Preliminary evaluation. *International Journal of Cosmetic Science*, *30*(2), 113–120. https://doi.org/10.1111/j.1468-2494.2008.00424.x

Pintillo, A. R. N., Konstanteli, E., Bairaktari, M. M., & Detsi, A. (2021). Encapsulation of the natural product tyrosol in carbohydrate nanosystems and study of their binding with ctDNA. *Polymers*, *13*, 87. https://doi.org/10.3390/polym13010087

Plotnikov, M. B., Aliev, O. I., Sidekhmenova, A. V., Shamanaev, A. Y., Anishchenko, A. M., Fomina, T. I., Plotnikova, T. M., & Arkhipov, A. M. (2018). Effect of p-tyrosol on hemorheological parameters and cerebral capillary network in young spontaneously hypertensive rats. *Microvascular Research*, *119*, 91–97. https://doi.org/10.1016/j.mvr.2018.04.005

Plotnikov, M. B., Chernysheva, G. A., Smol'yakova, V. I., Maslov, M. Y., Cherkashina, I. V., Krysin, A. P., Sorokina, I. V., & Tolstikova, T. G. (2007). Effect of n-tyrosol on blood viscosity and platelet aggregation. *Bulletin of Experimental Biology and Medicine*, *143*(1), 61–63. https://doi.org/10.1007/s10517-007-0017-y

Potapovich, A. I., Kostyuk, V. A., Kostyuk, T. V., De Luca, C., & Korkina, L. G. (2013). Effects of pre- and post-treatment with plant polyphenols on human keratinocyte responses to solar UV. *Inflammation Research*, *62*(8), 773–780. https://doi.org/10.1007/s00011-013-0634-z

Puel, C., Mardon, J., Agalias, A., Davicco, M. J., Lebecque, P., Mazur, A., Horcajada, M.-N., Skaltsounis, A.-L., & Coxam, V. (2008). Major phenolic compounds in olive oil modulate bone loss in an ovariectomy/inflammation experimental model. *Journal of Agricultural and Food Chemistry*, *56*(20), 9417–9422. https://doi.org/10.1021/jf801794q

Qadir, N. M., Ali, K. A., & Qader, S. W. (2016). Antidiabetic effect of oleuropein from *Olea europaea* leaf against alloxan induced type 1 diabetic in rats. *Brazilian Archives of Biology and Technology*, *59*, e16150116. https://doi.org/10.1590/1678-4324-2016150116

Qiao, Z., Tang, J., Wu, W., Tang, J., & Liu, M. (2019). Acteoside inhibits inflammatory response via JAK/STAT signaling pathway in osteoarthritic rats. *BMC Complementary and Alternative Medicine*, *19*, 264. https://doi.org/10.1186/s12906-019-2673-7

Quesada-Gómez, J. M., Mora, R. M. S. & Díaz, A. C. (2010). Patent WO 2010/070183 A1. Pharmaceutical composition comprising oleuropein for utilisation in angiogenesis and vasculogenesis induction. Issued June 24, 2010.

Quesada-Gómez, J. M., Mora, R. M. S. & Díaz, A. C. (2011). Patent WO 2011/141611 A1. Oleuropein compositions for healing wounds and ulcers in elderly people and/or diabetics. Issued November 17, 2011.

Raederstorff, D., Schwager, J. & Wertz, K. (2008). Patent WO 2008/128629 A1. Novel use of hydroxytyrosol. Issued October 30, 2008.

Raposo, R., Ruiz-Moreno, M. J., Garde-Cerdán, T., Puertas, B., Moreno-Rojas, J. M., Gonzalo-Diago, A., Guerrero, R. F., Ortiz, V., & Cantos-Villar, E. (2016). Effect of hydroxytyrosol on

quality of sulfur dioxide-free red wine. *Food Chemistry*, *192*, 25–33. https://doi.org/10.1016/j.foodchem.2015.06.085

Raposo, R., Ruiz-Moreno, M. J., Garde-Cerdán, T., Puertas, B., Moreno-Rojas, J. M., Zafrilla, P., Gonzalo-Diago, A., Guerrero, R. F., & Cantos-Villar, E. (2016). Replacement of sulfur dioxide by hydroxytyrosol in white wine: Influence on both quality parameters and sensory. *LWT - Food Science and Technology*, *65*, 214–221. https://doi.org/10.1016/j.lwt.2015.08.005

Regni, L., Nasini, L., Ilarioni, L., Brunori, A., Massaccesi, L., Agnelli, A., & Proietti, P. (2017). Long term amendment with fresh and composted solid olive mill waste on olive grove affects carbon sequestration by prunings, fruits, and soil. *Frontiers in Plant Science*, *7*, 2042. https://doi.org/10.3389/fpls.2016.02042

Rigacci, S., Guidotti, V., Bucciantini, M., Parri, M., Nediani, C., Cerbai, E., Stefani, M., & Berti, A. (2010). Oleuropein aglycone prevents cytotoxic amyloid aggregation of human amylin. *Journal of Nutritional Biochemistry*, *21*(8), 726–735. https://doi.org/10.1016/j.jnutbio.2009.04.010

Rincón, B., Bujalance, L., Fermoso, F. G., Martín, A., & Borja, R. (2013). Biochemical methane potential of two-phase olive mill solid waste: Influence of thermal pretreatment on the process kinetics. *Bioresource Technology*, *140*, 249–255. https://doi.org/10.1016/j.biortech.2013.04.090

Robin, J.-R. & Rolland, Y. (2004). Patent WO 2004/069218 A1. Use of verbascoside as a stimulant agent for the production of thermal shock proteins by the cells of the skin. Issued August 19, 2004.

Rodrigues, F., da Mota Nunes, M. A. , & Oliveira, M. B. P. P. (2017). Applications of recovered bioactive compounds in cosmetics and health care products. In *Olive mill waste: Recent advances for sustainable management* (pp. 255–274). Elsevier Inc. https://doi.org/10.1016/B978-0-12-805314-0.00012-1

Rodrigues Francisca, Pimentel, Filipa B., Oliveira, M., & Beatriz, P. P. (2015). Olive by-products: Challenge application in cosmetic industry. *Industrial Crops and Products*, *70*, 116–124. https://doi.org/10.1016/j.indcrop.2015.03.027

Roig, A., Cayuela, M. L., & Sánchez-Monedero, M. A. (2006). An overview on olive mill wastes and their valorisation methods. *Waste Management*, *26*, 960–969. https://doi.org/10.1016/j.wasman.2005.07.024

Rojas-Cano, M. L., Ruiz-Guerrero, V., Lara, L., Nieto, R., & Aguilera J. F. (2014). Digestibility and energy value of diets containing increasing proportions of olive soapstocks for Iberian crossbred pigs. *Animal Feed Science and Technology*, *191*, 83–90. https://doi.org/10.1016/j.anifeedsci.2014.01.018

Romero, C., Medina, E., Vargas, J., Brenes, M., & De Castro, A. (2007). In vitro activity of olive oil polyphenols against helicobacter pylori. *Journal of Agricultural and Food Chemistry*, *55*(3), 680–686. https://doi.org/10.1021/jf0630217

Romero-Garcia, J. M., Niño, L., Martínez-Patiño, C., Álvarez, C., Castro, E., & Negro, M. J. (2014). Biorefinery based on olive biomass. State of the art and future trends. *Bioresource Technology*, *159*, 421–432. https://doi.org/10.1016/j.biortech.2014.03.062

Rubio-Senent, F., Rodríguez-Gutiérrez, G., Lama-Muñoz, A., & Fernández-Bolaños, J. (2012). New phenolic compounds hydrothermally extracted from the olive oil byproduct alperujo and their antioxidative activities. *Journal of Agricultre and Food Chemistry*, *60*, 1175–1186. https://doi.org/10.1021/jf204223w

Ruzzolini, J., Peppicelli, S., Andreucci, E., Bianchini, F., Scardigli, A., Romani, A., La Marca, G., Nediani, C., & Calorini, L. (2018). Oleuropein, the main polyphenol of *Olea europaea* leaf extract, has an anti-cancer effect on human BRAF melanoma cells and potentiates the cytotoxicity of current chemotherapies. *Nutrients*, *10*(12), 1950. https://doi.org/10.3390/nu10121950

Saleh, E., Morshdy, A. E., El-Manakhly, E., Al-Rashed, S., Hetta, H. F., Jeandet, P., Yahia, R., El-Saber Batiha, G., & Ali, E. (2020). Effects of olive leaf extracts as natural preservative on retailed poultry meat quality. *Foods*, *9*(8), 1017. https://doi.org/10.3390/foods9081017

Samuel Samson Mathews, Thirunavukkarasu Mahesh, Penumathsa Suresh Varma, Paul Debayon, & Maulik Nilanjana. (2008). Akt/FOXO3a/SIRT1-Mediated Cardioprotection by n-Tyrosol against Ischemic Stress in Rat in Vivo Model of Myocardial Infarction: Switching Gears toward Survival and Longevity. *Journal of Agricultural and Food Chemistry*, *56*(20), 9692–9698. https://doi.org/10.1021/jf802050h

Sánchez-Fidalgo, S., De Ibargüen, L. S., Cárdeno, A., & De La Lastra, C. A. (2012). Influence of extra virgin olive oil diet enriched with hydroxytyrosol in a chronic DSS colitis model. *European Journal of Nutrition*, *51*(4), 497–506. https://doi.org/10.1007/s00394-011-0235-y

Santini, S. J., Porcu, C., Tarantino, G., Amicarelli, F., & Balsano, C. (2020). Oleuropein overrides liver damage in steatotic mice. *Journal of Functional Foods*, *65*, 103756. https://doi.org/10.1016/j.jff.2019.103756

Santos-Buelga, C., & González-Manzano, S. (2011). Wine and health relationships. A question of moderation? *Ciência e Técnica Vitivinícola*, *26*(1), 33–44.

Sarbishegi, M., Mehraein, F., & Soleimani, M. (2014). Antioxidant role of oleuropein on midbrain and dopaminergic neurons of substantia nigra in aged rats. *Iranian Biomedical Journal*, *18*(1), 16–22. https://doi.org/10.6091/ibj.1274.2013

Schaffer, S., Müller, W. E., & Eckert, G. P. (2010). Cytoprotective effects of olive mill wastewater extract and its main constituent hydroxytyrosol in PC12 cells. *Pharmacological Research*, *62*(4), 322–327. https://doi.org/10.1016/j.phrs.2010.06.004

Serra, A., Conte, G., Giovannetti, M., Casarosa, L., Agnolucci, M., Ciucci, F., Palla, M., Bulleri, E., Servili, M., & Mele, M. (2018). Olive pomace in diet limits lipid peroxidation of sausages from cinta senese swine. *European Journal of Lipid Science and Technology*, *120*(1), 1–12. https://doi.org/10.1002/ejlt.201700236

Serrano, A., Fermoso, F. G., Alonso-Fariñas, B., Rodríguez-Gutiérrez, G., Fernandez-Bolaños, J., & Borja, R. (2017). Olive mill solid waste biorefinery: High-temperature thermal pre-treatment for phenol recovery and biomethanization. *Journal of Cleaner Production*, *148*, 314–323. https://doi.org/10.1016/j.jclepro.2017.01.152

Shamshoum, H., Vlavcheski, F., & Tsiani, E. (2017). Anticancer effects of oleuropein. *Biofactors*, *43*(4), 517–528. https://doi.org/10.1002/biof.1366

Siciliano, A., Stillitano, M. A., & De Rosa, S. (2016). Biogas production from wet olive mill wastes pretreated with hydrogen peroxide in alkaline conditions. *Renewable Energy*, *85*, 903–916. https://doi.org/10.1016/j.renene.2015.07.029

Silva, A. F. R., Resende, D., Monteiro, M., Silva, A. M. S., Cardoso, S. M., & Coimbra, M. A. (2020). Application of hydroxytyrosol in the functional foods field : From ingredient to dietary supplements. *Antioxidants*, *9*(12), 1246. https://doi.org/10.3390/antiox9121246

Simonato Barbara, Trevisan Sara, Tolve Roberta, Favati Fabio, & Pasini Gabriella. (2019). Pasta fortification with olive pomace:

Effects on the technological characteristics and nutritional properties. *LWT*, *114*, 108368. https://doi.org/10.1016/j.lwt.2019.108368

Smeriglio, A., Denaro, M., Mastracci, L., Grillo, F., Cornara, L., Shirooie, S., Nabavi, S. M., & Trombetta, D. (2019). Safety and efficacy of hydroxytyrosol-based formulation on skin inflammation: in vitro evaluation on reconstructed human epidermis model. *DARU Journal of Pharmaceutical Sciences*, *27*(1), 283–293. https://doi.org/10.1007/s40199-019-00274-3

Suárez, M., Romero, M.-P., Ramo, T., Macià, A., & Motilva, M.-J. (2009). Methods for preparing phenolic extracts from olive cake for potential application as food antioxidants. *Journal of Agricultunal and Food Chemistry*, *57*, 1463–1472. https://doi.org/10.1021/jf8032254

Sun, W., Wang, X., Hou, C., Yang, L., Li, H., Guo, J., Huo, C., Wang, M., Miao, Y., Liu, J., & Kang, Y. (2017). Oleuropein improves mitochondrial function to attenuate oxidative stress by activating the Nrf2 pathway in the hypothalamic paraventricular nucleus of spontaneously hypertensive rats. *Neuropharmacology*, *113*, 556–566. https://doi.org/10.1016/j.neuropharm.2016.11.010

Svobodova, M., Andreadou, I., Skaltsounis, A. L., Kopecky, J., & Flachs, P. (2014). Oleuropein as an inhibitor of peroxisome proliferator-activated receptor gamma. *Genes and Nutrition*, *9*(1), 376. https://doi.org/10.1007/s12263-013-0376-0

Tabernero, M., Sarriá, B., Largo, C., Martínez-López, S., Madrona, A., Espartero, J. L., Bravo, L., & Mateos, R. (2014). Comparative evaluation of the metabolic effects of hydroxytyrosol and its lipophilic derivatives (hydroxytyrosyl acetate and ethyl hydroxytyrosyl ether) in hypercholesterolemic rats. *Food and Function*, *5*(7), 1556–1563. https://doi.org/10.1039/c3fo60677e

Takeda, Y., Bui, V. N., Iwasaki, K., Kobayashi, T., Ogawa, H., & Imai, K. (2014). Influence of olive-derived hydroxytyrosol on the toll-like receptor 4-dependent inflammatory response of mouse peritoneal macrophages. *Biochemical and Biophysical Research Communications*, *446*(4), 1225–1230. https://doi.org/10.1016/j.bbrc.2014.03.094

Tayeh, H., Najami, N., Dosoretz, C., Tafesh, A., & Azaizeh, H. (2014). Potential of bioethanol production from olive mill solid wastes. *Bioresource Technology*, *152*, 24–30. https://doi.org/10.1016/j.biortech.2013.10.102

Tejada, S., Pinya, S., del Mar Bibiloni, M., Tur, J. A., Pons, A., & Sureda, A. (2016). Cardioprotective effects of the polyphenol hydroxytyrosol from olive oil. *Current Drug Targets*, *18*(13), 1477–1486. https://doi.org/10.2174/1389450117666161005150650

Tu, P., Song, Z. & Lei, L. (2005). Patent EP 1498131 A1. Medicinal preparation containing phenylethanoid glycosides extracted from Cistanche tubulosa. Issued March 19, 2014.

Valenti, F., Arcidiacono, C., Chinnici, G., Cascone, G., & Porto, S. M. (2017). Quantification of olive pomace availability for biogas production by using a GIS-based model. *Biofuels, Bioproducts and Biorefining*, *11*, 784–797. https://doi.org/10.1002/bbb.1784

Vertuani, S., Beghelli, E., Scalambra, E., Malisardi, G., Copetti, S., Toso, R. D., Baldisserotto, A., & Manfredini, S. (2011). Activity and stability studies of verbascoside, a novel antioxidant, in dermocosmetic and pharmaceutical topical formulations. *Molecules*, *16*(8), 7068–7080. https://doi.org/10.3390/molecules16087068

Vilaplana-Pérez, C., Auñón, D., García-Flores, L. A., & Gil-Izquierdo, A. (2014). Hydroxytyrosol and potential uses in cardiovascular diseases, cancer, and AIDS. *Frontiers in Nutrition*, *1*, 18. https://doi.org/10.3389/fnut.2014.00018

Villanova, L., Villanova, A., Villanova, L., Merendino, A., Fasieloo, G. & Cisale, F. (2010). US Patent 2010/0297330 A1 Yoghurt containing hydroxytyrosol and other biophenols with a preventive nutritional activity beneficial to human beings. November 25, 2010.

Xia, D., Zhang, Z., & Zhao, Y. (2018). Acteoside attenuates oxidative stress and neuronal apoptosis in rats with focal cerebral ischemia-reperfusion injury. *Biological and Pharmaceutical Bulletin*, *41*(11), 1645–1651. https://doi.org/10.1248/bpb.b18-00210

Xie, P., Huang, L., Zhang, C., Deng, Y., Wang, X., & Cheng, J. (2019). Enhanced extraction of hydroxytyrosol, maslinic acid and oleanolic acid from olive pomace: Process parameters, kinetics and thermodynamics, and greenness assessment. *Food Chemistry*, *276*(16), 662–674. https://doi.org/10.1016/j.foodchem.2018.10.079

Xu, F., Li, Y., Zheng, M., Xi, X., Zhang, X., & Han, C. (2018). Structure properties, acquisition protocols, and biological activities of oleuropein aglycone. *Frontiers in Chemistry*, *6*, 239. https://doi.org/10.3389/fchem.2018.00239

Yakhlef Wahiba, Arhab Rabah, Romero Concepción, Brenes Manuel, de Castro Antonio, & Medina Eduardo. (2018). Phenolic composition and antimicrobial activity of Algerian olive products and by-products. *LWT*, *93*, 323–328. https://doi.org/10.1016/j.lwt.2018.03.044

Zbakh, H., & El Abbassi, A. (2012). Potential use of olive mill wastewater in the preparation of functional beverages: A review. *Journal of Functional Foods*, *4*(1), 53–65. https://doi.org/10.1016/j.jff.2012.01.002

Zbidi, H., Salido, S., Altarejos, J., Perez-Bonilla, M., Bartegi, A., Rosado, J. A., & Salido, G. M. (2009). Olive tree wood phenolic compounds with human platelet antiaggregant properties. *Blood Cells, Molecules, and Diseases*, *42*, 279–285. https://doi.org/10.1016/j.bcmd.2009.01.001

Zhang, J., Nugrahaningrum, D. A., Marcelina, O., Ariyanti, A. D., Wang, G., Liu, C., Wu, S., & Kasim, V. (2019). Tyrosol facilitates neovascularization by enhancing skeletal muscle cells viability and paracrine function in diabetic hindlimb ischemia mice. *Frontiers in Pharmacology*, *10*, 909. https://doi.org/10.3389/fphar.2019.00909

Zhao, Q., Bai, Y., Li, C., Yang, K., Wei, W., Li, Z., …, & Zhang, X. (2017). Oleuropein protects cardiomyocyte against apoptosis via activating the reperfusion injury salvage kinase pathway in vitro. *Evidence-Based Complementary and Alternative Medicine*, 2017, 2109018. https://doi.org/10.1155/2017/2109018

Zoidou, E., Melliou, E., Moatsou, G., & Magiatis, P. (2017). Preparation of functional yogurt enriched with olive-derived products. In N. Shah (Ed.), *Yogurt in health and disease prevention* (1st ed., pp. 203–220). Elsevier Inc. https://doi.org/10.1016/B978-0-12-805134-4.00011-0

How to cite this article: Madureira, J., Margaça, F. M. A., Santos-Buelga, C., Ferreira, I. C. F. R., Cabo Verde, S., & Barros, L. (2021). Applications of bioactive compounds extracted from olive industry wastes: A review. *Compr Rev Food Sci Food Saf*, 1–24. https://doi.org/10.1111/1541-4337.12861

Publicación 4. The use of gamma radiation for extractability improvement of bioactive compounds in olive oil wastes

Joana Madureira, Maria Inês Dias, José Pinela, Ricardo C. Calhelha, Lillian Barros, Celestino Santos-Buelga, Fernanda M.A. Margaça, Isabel C.F.R. Ferreira, Sandra Cabo Verde

Artículo publicado en *Science of the Total Environment* (2020), 727, 138706. Doi: https://doi.org/10.1016/j.scitotenv.2020.138706

Resumen

En este trabajo se evaluaron los efectos de la radiación gamma sobre la composición fenólica y las propiedades bioactivas (actividades antioxidante, antimicrobiana y hepatotoxicidad) de extractos de orujo crudo (obtenido después de la extracción del aceite virgen) y de orujo agotado (tras su extracción con disolventes para obtener aceite de orujo).

La irradiación de las muestras de orujo de oliva se realizó a temperatura ambiente en una instalación semi-industrial de Co-60 (dosis absorbidas: 5, 10, 16 y 22 kGy; tasa de dosificación: 16 kGy/h). Después, los compuestos fenólicos se extrajeron de los dos tipos de orujos utilizando etanol/agua (80:20 v/v) como disolvente y se caracterizó el perfil fenólico por HPLC-DAD-ESI/MS. Se comprobó que el tratamiento previo de los orujos con radiación gamma aumentaba al menos 2 veces la extractabilidad de los compuestos fenólicos. El hidroxitirosol fue el principal compuesto fenólico identificado en los dos extractos de orujo en concentraciones similares (24-25 mg/g). Se realizó también la evaluación de la actividad antioxidante de ambos extractos, empleando dos tipos de ensayos: capacidad para inhibir la formación de sustancias reactivas al ácido tiobarbitúrico (TBARS, *thiobarbituric acid reactive substances*) y actividad antihemolítica (OxHLIA).

Los extractos de orujo agotado fueron más eficientes en la protección de los eritrocitos contra la hemólisis inducida por oxidación, mientras que los de orujo crudo mostraron una mayor capacidad para inhibir la formación de TBARS, lo que podría estar relacionado con el mayor contenido graso de estas muestras. La aplicación de radiación gamma a una dosis de 5 kGy elevó la actividad antioxidante en el extracto de orujo agotado, manteniendo su capacidad para proteger in vitro los eritrocitos contra la hemólisis inducida por oxidación. Los extractos también mostraron actividad antimicrobiana contra bacterias Gram-positivas (*Staphylococcus aureus*, *Bacillus cereus*

y *Listeria monocytogenes*) y Gram-negativas (*Escherichia coli*, *Pseudomonas fluorescens* y *Salmonella enterica* serotype Typhimurium) y hongos (*Penicillium spinulosum* y *Aspergillus fumigatus*), y no presentaron toxicidad en células hepáticas no tumorales (PLP2), sin que el tratamiento con radiación gamma causara ninguna alteración en estas propiedades.

En conclusión, el tratamiento con radiación gamma a dosis de 5 kGy se muestra como una tecnología adecuada para mejorar la extractabilidad de los residuos producidos en la industria del aceite de oliva, contribuyendo a potenciar la extracción de compuestos fenólicos y mejorando las propiedades antioxidantes de los extractos, especialmente cuando se aplica sobre los orujos desgrasados.

Science of the Total Environment 727 (2020) 138706

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

The use of gamma radiation for extractability improvement of bioactive compounds in olive oil wastes

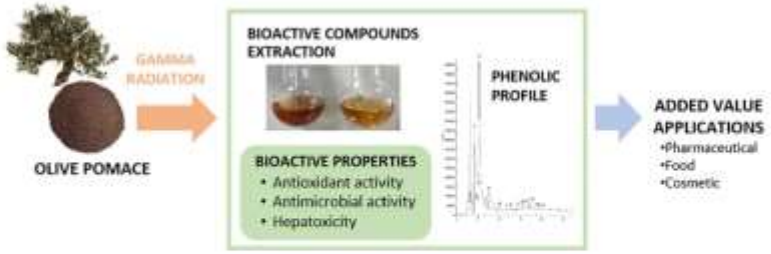
Joana Madureira [a,b,c], Maria Inês Dias [b], José Pinela [b], Ricardo C. Calhelha [b], Lillian Barros [b], Celestino Santos-Buelga [c], Fernanda M.A. Margaça [a], Isabel C.F.R. Ferreira [b,*], Sandra Cabo Verde [a,*]

[a] Centro de Ciências e Tecnologias Nucleares (C²TN), Instituto Superior Técnico, Universidade de Lisboa, E.N. 10 ao km 139.7, 2695-066 Bobadela LRS, Portugal
[b] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[c] Grupo de Investigación en Polifenoles (GIP-USAL), Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno s/n, 37007 Salamanca, Spain

HIGHLIGHTS

- Olive pomace is a source of phenolic compounds.
- Two types of olive pomace, crude and extracted, were evaluated for its bioactive potential.
- Hydroxytyrosol was the major compound in extracted and crude olive pomaces.
- Gamma radiation promoted the increase of phenolic compounds content in olive pomaces.
- Extracted olive pomace had higher bioactivity than crude olive pomace.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 11 February 2020
Received in revised form 12 April 2020
Accepted 13 April 2020
Available online 14 April 2020

Editor: Yolanda Picó

Keywords:
Olive pomace
Phenolic compounds
Bioactivity
Industrial wastes valorization
Ionizing radiation applications

ABSTRACT

Olive pomace is an environmentally detrimental waste from olive oil industry, containing large amounts of bioactive compounds that might be used by the food industry. In this work, the effects of gamma radiation on phenolic compounds and bioactive properties (antioxidant, antimicrobial activities and hepatotoxicity) of Crude Olive Pomace (COP) and Extracted Olive Pomace (EOP) extracts were evaluated. Hydroxytyrosol was the main phenolic compound identified in both olive pomace extracts (24–25 mg/g). The gamma radiation treatment of olive pomace improved at least 2-fold the extractability of phenolic compounds. Moreover, results suggested that gamma radiation at 5 kGy increased the antioxidant activity in EOP, while keeping the ability to protect erythrocytes against oxidation-induced haemolysis. Gamma radiation at 5 kGy could be a suitable technology for olive oil pomaces waste valorization, contributing to enhance extraction of phenolic compounds and bioactive properties, especially when applied on extracted material.

* Corresponding authors.
E-mail addresses: iferreira@ipb.pt (I.C.F.R. Ferreira), sandracv@ctn.tecnico.ulisboa.pt (S. Cabo Verde).

https://doi.org/10.1016/j.scitotenv.2020.138706
0048-9697/

1. Introduction

The olive oil production is an important industry, mainly of the Mediterranean region countries (Ammar et al., 2017; Suárez et al., 2010; Zorpas and Costa, 2010). In Portugal, this industry produces >600,000 t of wastes, whose discharge without treatment would have a negative impact in the environment. Due to the high content in phosphorous, potassium and organic matter, these wastes have been applied as amendment on the agricultural soils, not only because of its low cost but also for its potential to enhance soil fertility and reduce the risks of soil degradation (Federici et al., 2017; Regni et al., 2017). On the other hand, these wastes contain high amounts of organic substances (fibers, sugars, volatile fatty acids, polyalcohols, pectins and fats) and a variety of phenolic compounds such as hydroxytyrosol and tyrosol, secoiridoid derivatives, phenolic acids and flavonoids (Papaioannou et al., 2013), thus being also a promising source of valuable compounds. During the olive oil extraction process, the majority of these phenolic compounds remain in the olive pomace waste, as only 1% are found in the olive oil (Fernández-Bolaños et al., 2006).

The recovery of phenolic compounds from olive pomace have been studied through the application of several techniques, such as hydrothermal treatment (Fernández-Bolaños et al., 2002), membrane technology (Sygouni et al., 2019), centrifugation (Cioffi et al., 2010), Superheated Liquid Extraction (Peralbo-Molina et al., 2012), Pressurized Liquid Extraction (Pavez et al., 2019), Multi-frequency Multimode Modulated ultrasonic technique (Nunes et al., 2018) and solid-liquid extraction (Suárez et al., 2010, 2009; Vitali Čepo et al., 2017; Zuorro, 2014). Hydroxytyrosol has been reported as the main phenolic compound in olive pomace, being an effective natural antioxidant with beneficial properties such as cancer prevention, skin protection, anti-inflammatory activity (Fernández-Bolaños et al., 2008), and in the protection against cardiovascular diseases (Bulotta et al., 2014; Robles-Almazan et al., 2018). Other authors reported oleuropein as one of the most abundant phenolic compound in olive pomace, being the major one present in olive pomace from the Cilento National Park, in Italy (Cioffi et al., 2010). Tyrosol, verbascoside, caffeic acid, vanillin, vanillic acid, apigenin, luteolin and rutin are also found in olive pomace but in lower concentrations (Suárez et al., 2010). Verbascoside was identified as the most potent antioxidant in Australian olive mill waste extracts (Obied et al., 2008).

Nowadays, consumers prefer natural additives to be added to food products (Carocho et al., 2015) as they are considered safer than the artificial counterparts. Phenolic compounds are considered promising bioactive molecules to be used in the replacement of some of these additives. This is seen by the food industry as an opportunity to find new and more efficient natural ingredients, while also fighting to reduce the overall addition of artificial additives and producing minimally processed goods (Carocho et al., 2015). Phenolic compounds obtained from olive pomace could be suitable candidates to provide bioactive properties and bring additional value to food products, as they have been considered to lack toxicity (Bulotta et al., 2014).

Ionizing radiation is a clean and environment friendly technology. It is a physical treatment that does not rely on the addition of chemicals and have the capability of degrading complex molecules into low-molecular weight compounds. Recently, the scientific community has aroused considerable interest in the enhancement of beneficial properties by irradiation and in the ionizing radiation effects on bioactive compounds. Our previous studies have reported the use of this technology to improve phenolic content and antioxidant activity on industrial wastewater (Madureira et al., 2017), fresh fruits such as cherry tomatoes (Guerreiro et al., 2016) and raspberries (Cabo Verde et al., 2013), and dry medicinal plants (Pereira et al., 2017a), as well as its efficiency for decomposition of recalcitrant compounds (Madureira et al., 2018).

The aim of this work was to characterize the phenolic compounds profile of olive pomace waste from Portuguese olive oil industry and to assess the impact of gamma radiation on these compounds selecting the optimal radiation dose to improve their extractability. Furthermore, the extracts were evaluated in terms of their biological activity, namely antimicrobial and antioxidant properties and hepatotoxicity. To our knowledge, this study represents the first application of ionizing radiation technologies with a view to improving the recovery of phenolic compounds in order to valorize olive pomace industrial waste.

2. Material and methods

2.1. Standards and reagents

HPLC-grade acetonitrile was obtained from Fisher Scientific (Lisbon, Portugal). Methanol and formic acid were acquired from Sigma-Aldrich (St. Louis, USA) and Honeywell (Charlotte, USA), respectively. Vanillic acid (≥95%), apigenin-7-*O*-glucoside (≥99%), *p*-hydroxybenzoic acid (≥99%), caffeic acid (≥99%) and syringic acid (≥95%) were purchased from Extrasynthese (Genay, France), whereas hydroxytyrosol (≥99%) and tyrosol (≥98%) were obtained from Applichem (Darmstadt, Germany) and TCI (Tokyo, Japan), respectively. Verbascoside (≥99%), oleuropein (≥98%), trolox, ellipticine and sulforhodamine B were acquired from Sigma-Aldrich (St Louis, USA). Water was treated in a Milli-Q water purification system (Merck Millipore, USA).

2.2. Olive pomace samples

Olive pomace samples were collected, in 2018, from UCASUL - União de Cooperativas Agrícolas do Sul, located in Alentejo region, in Portugal. This is a cooperative industrial unit that receives the olive pomace produced in the olive oil mills of the region to extract olive pomace oil that has commercial value. The majority of the region mills use the two-phase process for olive oil production. Pomace enters UCASUL with high humidity (68% in average) and is first dried to 8%. UCASUL waste is the defatted or extracted olive pomace (EOP). This is considered biomass and disposed for energy production. The samples collected at UCASUL are of two different types: non-defatted or Crude Olive Pomace (COP) that consists of dried pomace before the extraction of pomace oil and defatted or Extracted Olive Pomace (EOP) that is the pomace left after the extraction of pomace oil.

2.3. Irradiation experiments

Irradiation experiments were carried out in a Co-60 semi-industrial unit (with an activity of 187 kCi) located at Technological Unit of Radiosterilization - UTR of IST, University of Lisbon. Sealed bags (10 cm × 7 cm) containing 30 g of olive pomace, COP and EOP, were irradiated at room temperature at doses ranging from 4.9 to 21.8 kGy using a dose rate of 16 kGy h^{-1}. The absorbed doses were measured by Amber Perspex routine dosimeters (Whittaker and Watts, 2001) (dose uniformity DUR = 1.03). For simplicity, the absorbed doses will be referred as 5, 10, 16 and 22 kGy. The irradiations were performed in triplicate. In order to analyze the effect of gamma radiation in olive pomace, non-irradiated (0 kGy) samples submitted to the same experimental procedure were used as control.

2.4. Phenolic compounds extraction

After irradiation, all olive pomace samples were immediately lyophilized (Heto CD8, Allerod, Denmark) and stored until used. The olive pomace extracts were prepared by a solid-liquid extraction as previously described (Pinela et al., 2016). Briefly, 1 g of freeze-dried olive pomace was stirred with a methanol:water mixture (80:20, *v*/*v*; 30 mL) for 1 h at room temperature. The supernatant was then filtered (Whatman No. 4 filter paper) and the residue was re-extracted with an additional portion of solvent (30 mL). The combined extracts were then evaporated at 40 °C under reduced pressure (rotary evaporator Büchi R-

210, Flawil, Switzerland) to remove the methanol and the aqueous phase was lyophilized to obtain dry extracts.

A schematic diagram of the procedure steps carried out for the extraction of phenolic compounds from olive pomaces and subsequent analysis of their bioactive properties is represented in Fig. 1.

2.5. Analysis of phenolic compounds

After preparation, the dry extracts (~10 mg) were dissolved in a methanol:water mixture (20:80 v/v, 2 mL) and filtered through 0.22 μm disposable LC filter disks. Then, the extracts were analyzed by HPLC-DAD-ESI/MSn (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA) using a methodology previously described by the authors (Bessada et al., 2016). Detection was performed in a Diode Array Detector (DAD) with 280, 330 and 370 nm as preference wavelengths, and in a mass spectrometer with an ESI source operating in negative mode (Linear Ion Trap LTQ XL mass spectrometer, Thermo Finnigan, San Jose, CA, USA). Data acquisition was carried out with Xcalibur® data system (Thermo Finnigan, San Jose, CA, USA).

The phenolic compounds were identified based on their chromatographic behavior and UV–vis and mass spectra, and comparison with standard compounds, when available, and data reported in the literature (Ammar et al., 2017; Cardoso et al., 2005; Obied et al., 2007). Calibration curves for quantitative analysis were prepared for each available phenolic standard based on the UV signal. For the identified phenolic compounds for which a commercial standard was not available, the quantification was performed from the calibration curve of the most similar standard. The results were expressed in mg per g of extract.

2.6. Evaluation of bioactive properties

2.6.1. Antioxidant activity

The extracts were dissolved in distilled water at a concentration of 5 mg/mL and then successive dilutions were carried out (2500 to 2 μg/mL).

The antioxidant activity was measured through the thiobarbituric acid reactive substances (TBARS) assay and anti-haemolytic activity. The TBARS assay was performed following a methodology described by Pinela et al. (2012) and the results were expressed in IC_{50} values (sample concentration providing 50% of antioxidant activity). The anti-haemolytic activity of the extracts was evaluated by the oxidative haemolysis inhibition assay (OxHLIA), as previously described by Lockowandt et al. (2019). The results were presented as IC_{50} values (extract concentration that delayed the haemolysis time for 60 and 120 min, with 50% of intact erythrocytes). In both assays, trolox was used as positive control. All determinations were performed in triplicate.

2.6.2. Hepatotoxicity

The extracts were re-dissolved in water at 8 mg/mL and further diluted in the range (400 to 1.56 μg/mL). The hepatotoxicity of olive pomace extracts was evaluated in non-tumor liver cells primary culture (PLP2) using the sulforhodamine B assay (SRB, Sigma-Aldrich, St. Louis, MO, USA) (Abreu et al., 2011). The results were expressed in GI_{50} values (sample concentration that inhibited 50% of the cell growth) and ellipticine was used as positive control. Analyses were performed in triplicate.

2.6.3. Antimicrobial activity

2.6.3.1. Antibacterial activity. The bacterial isolates used on antibacterial activity assay intend to embrace a spectrum of different types of aerobic bacteria with reported association to food outbreaks, including Gram-negative and Gram-positive, spore forming bacteria, as well as a biofilm forming bacteria due to documented difficulty to control biofilms in food environments. The antibacterial activity was evaluated using three Gram-negative bacteria: *Escherichia coli* (ATCC 8739), *Pseudomonas fluorescens* (ATCC 13525) and *Salmonella enterica* serotype Typhimurium (ATCC 14028), and three Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (SSI C1/1) and *Listeria monocytogenes* (ATCC 19111). The bacterial suspension was adjusted

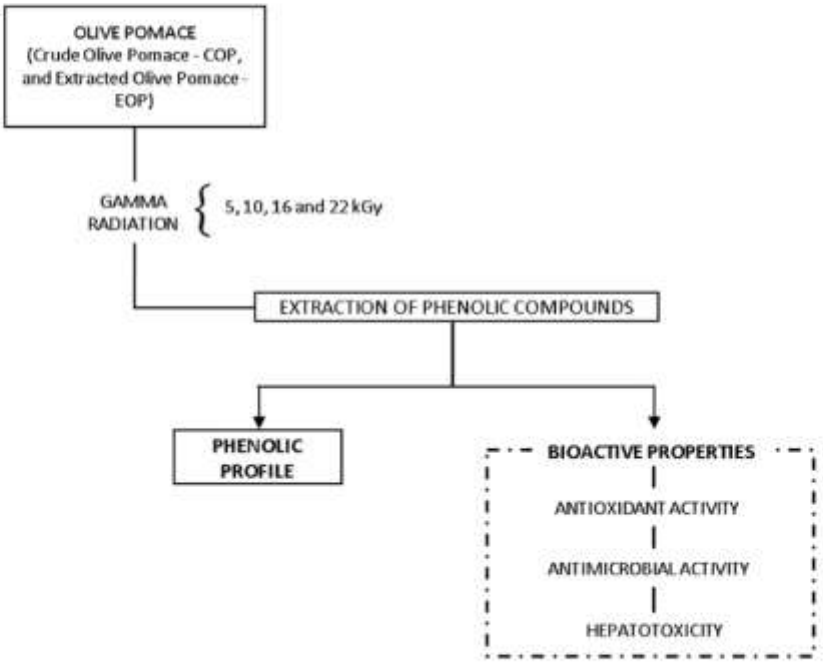


Fig. 1. Schematic diagram of the different steps of the experimental procedure for the extraction and analysis of bioactive properties of olive pomaces.

with sterile saline to a concentration of 1.0×10^5 CFU/mL. The extracts (EOP and COP) were dissolved in Tryptic Soy Broth (TSB) at a final concentration of 100 mg/mL. Then, several concentrations (10–60 mg/mL for Gram-negative bacteria and 1.25–20 mg/mL for Gram-positive bacteria) were prepared directly in the well and added 100 μL of the respective inoculum (1.0×10^4 CFU per well). The minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations were determined by the microdilution method (Sokovic et al., 2010). Streptomycin was used as a positive control. Samples were tested in triplicate and each experiment was repeated two times.

2.6.3.2. Antifungal activity. For the antifungal assays, two fungi were used: *Penicillium spinulosum* (environment isolate) and *Aspergillus fumigatus* (environment isolate). These mycotoxin producing fungi were selected since are recognized food deteriorating agents also present in food wastes. The fungal spores were washed from the surface of agar plates with sterile 0.9% saline solution. The spore suspension was adjusted with sterile saline to a concentration of 1.0×10^5 CFU/mL. The extracts (EOP and COP) were dissolved in Tryptic Soy Broth (TSB) at a final concentration of 100 mg/mL. Then, several concentrations (10–60 mg/mL) were prepared directly in the well and added 100 μL of the respective inoculum (1.0×10^4 CFU per well). Minimum inhibitory concentration (MIC) determination was performed by a serial dilution technique (Gomes Corrêa et al., 2015). Ketoconazole was used as positive control. Samples were tested in triplicate and each experiment was repeated two times.

2.7. Statistical analysis

Data results were expressed as mean ± standard deviation. In data analysis, confidence intervals for means values were estimated considering a significance level of $p < 0.05$ and the number of replicates for each assay. The differences among treatments were analyzed using the one-way analysis of variance (ANOVA) followed by Tukey's HSD test with $\alpha = 0.05$.

3. Results and discussion

Two types of olive pomace, Crude Olive Pomace (COP) and Extracted Olive Pomace (EOP), were analyzed in order to find out the best conditions to improve the extractability of its natural bioactive compounds. The extraction of phenolic compound was performed by conventional solid-liquid technique due to its feasibility on the studied substrate allied to the use of eco-friendly solvents.

3.1. Phenolic compounds characterization

The phenolic profile of olive oil pomaces was similar for non-treated and treated samples and both types of extracts (COP and EOP). Thus, to illustrate the phenolic profiles, examples of non-irradiated (control) and irradiated at 5 kGy of EOP olive pomace samples, recorded at 280 nm, are shown in Fig. 2.

Peak characteristics and tentative identities of the detected compounds are presented in Table 1. Nine phenylethanoid derivatives (peaks 1, 2, 3, 4, 5, 7, 8, 11 and 12), two phenolic acids (peak 6 and 10), one flavonoid (peak 13) and one unknown compound (peak 14) were detected in both EOP and COP samples.

Hydroxytyrosol-1-β-glucoside (peak 1), hydroxytyrosol (2), tyrosol (4), caffeic acid (6), vanillin (9), verbascoside (11), 4–hydroxyphenylacetic acid (12) and luteolin-7-*O*-rutinoside (13) were positively identified by comparison with commercial standards, all of them being previously described for olive pomace (Cardoso et al., 2005; De Marco et al., 2007; Obied et al., 2007; Suárez et al., 2010). Peaks 3 and 5 presented the same pseudomolecular ion $[M-H]^-$ at *m/z* 377, which fits the oleuropein aglycon (i.e., 3,4-(dihydroxyphenyl)ethanol elenolic acid ester, 3,4-DHPEA-EA). The presence of several oleuropein aglycon derivatives in olive oil and by-products is well reported (Obied et al., 2007; Olmo-García et al., 2018) and explained by the enzymatic loss of the glucose residue of oleuropein and further structural arrangement involving ring opening and keto-enol equilibria during olive oil processing (Obied et al., 2007). Compound 5 shows the same MS^2 fragments as reported by Cardoso et al. (2005) for the oleuropein aglycon derivative detected in olive pulp and pomace. The MS^2 fragmentation pattern of compound 3 is completely different, although its main product ions (*m/z* at 179 and 119) have also been described for oleuropein and its component elenolic acid (Ammar et al., 2017; Obied et al., 2007). All in all, an identity as oleuropein aglycon isomers has been assumed for peaks 3 and 5. Compounds 7 and 8 ($[M-H]^-$ at *m/z* 639) showed a molecular weight 16 Da higher than verbascoside and similar UV spectra to this latter. Up to four compounds with the same mass characteristics were also recovered by Cardinali et al. (2012) and Ammar et al. (2017) from *Olea europaea* by-products and identified as β-hydroxyverbascoside diastereoisomers, so that that identity was also assigned the compounds detected herein. Peak 10 presented a pseudomolecular ion $[M-H]^-$ at *m/z* 197 and a fragment ion at *m/z* 153 (−44 u; loss of a carboxyl residue), which would match syringic acid, a benzoic acid frequently found in olive fruits and by-products (Obied et al., 2007; Olmo-García et al., 2018), an identity that is given as tentative. A compound with the same pseudomolecular ion as peak 14 ($[M-H]^-$ at *m/z* 337) was detected by Ammar et al. (2017) in olive by-products and identified as ascorbyl-hexoside. Nevertheless, the late chromatographic elution of the peak and the UV spectral characteristics seem to not support that identification, so that the peak remains as unknown.

In non-irradiated samples, the individual contents of hydroxytyrosol-1-β-glucoside, hydroxytyrosol, tyrosol, oleuropein aglycon isomers 1 and 2, β-hydroxyverbascoside isomer 1, vanillin and 4-hydroxyphenylacetic acid were not significantly different ($p > 0.05$) between EOP and COP (Table 2). However, COP extracts contained significant higher levels of caffeic acid, β-hydroxyverbascoside isomer 2, syringic acid, verbascoside and luteolin-7-*O*-rutinoside. Hydroxytyrosol was the most abundant compound in both samples (25 ± 1 mg/g extract in EOP samples, and 23.9 ± 0.3 mg/g extract in COP ones), followed by hydroxytyrosol-1-β-glucoside, tyrosol, luteolin-7-*O*-rutinoside, oleuropein aglycon isomer 1 and verbascoside. Hydroxytyrosol is a phenyl alcohol for which antioxidant, anti-inflammatory and antimicrobial activities have been described (Bulotta et al., 2014; Fernández-Bolaños et al., 2008; Robles-Almazan et al., 2018). The extraction of phenolic compounds was significantly increased ($p < 0.05$) after gamma radiation in both olive pomace samples, obtaining the highest yield at 10 kGy for EOP and at 22 kGy for COP. At these doses, the concentrations of total phenolic compounds in the obtained extracts were 143 ± 4 mg/g in the EOP and 140 ± 2 mg/g in the COP ones (Table 2), representing an increase in extractable phenolic compounds of 2.4 and 2.3 fold, respectively, compared to non-irradiated samples. However, there was no significant difference between the total phenolic compounds content extracted from EOP at 10 kGy and from COP at 22 kGy. Contrary to these results, Aouidi et al. (2011) did not find that gamma radiation induced significant changes on total phenolic content in olive leaves even at doses as high as 25 kGy.

Regarding individual compounds behavior with gamma radiation, a significant decrease ($p < 0.05$) in the concentrations of some phenolics was observed at some irradiation doses in EOP samples, namely tyrosol and verbascoside at 16 kGy, 4-hydroxyphenylacetic acid and syringic acid from 10 kGy, and oleuropein aglycon isomer 2 and luteolin-7-*O*-rutinoside from 16 kGy. By contrast, the contents of vanillin and β-hydroxyverbascoside isomers 1 and 2 significantly increased in those samples at 10 kGy to decrease significantly at 16 kGy. In COP extracts, gamma radiation also led to different effects on different compounds. Thus, the concentration of oleuropein aglycon isomer 2 increased at 5 kGy to decrease significantly at 10 and to increase significantly again at 16 kGy, while vanillin significantly decreased at 10 kGy and increased

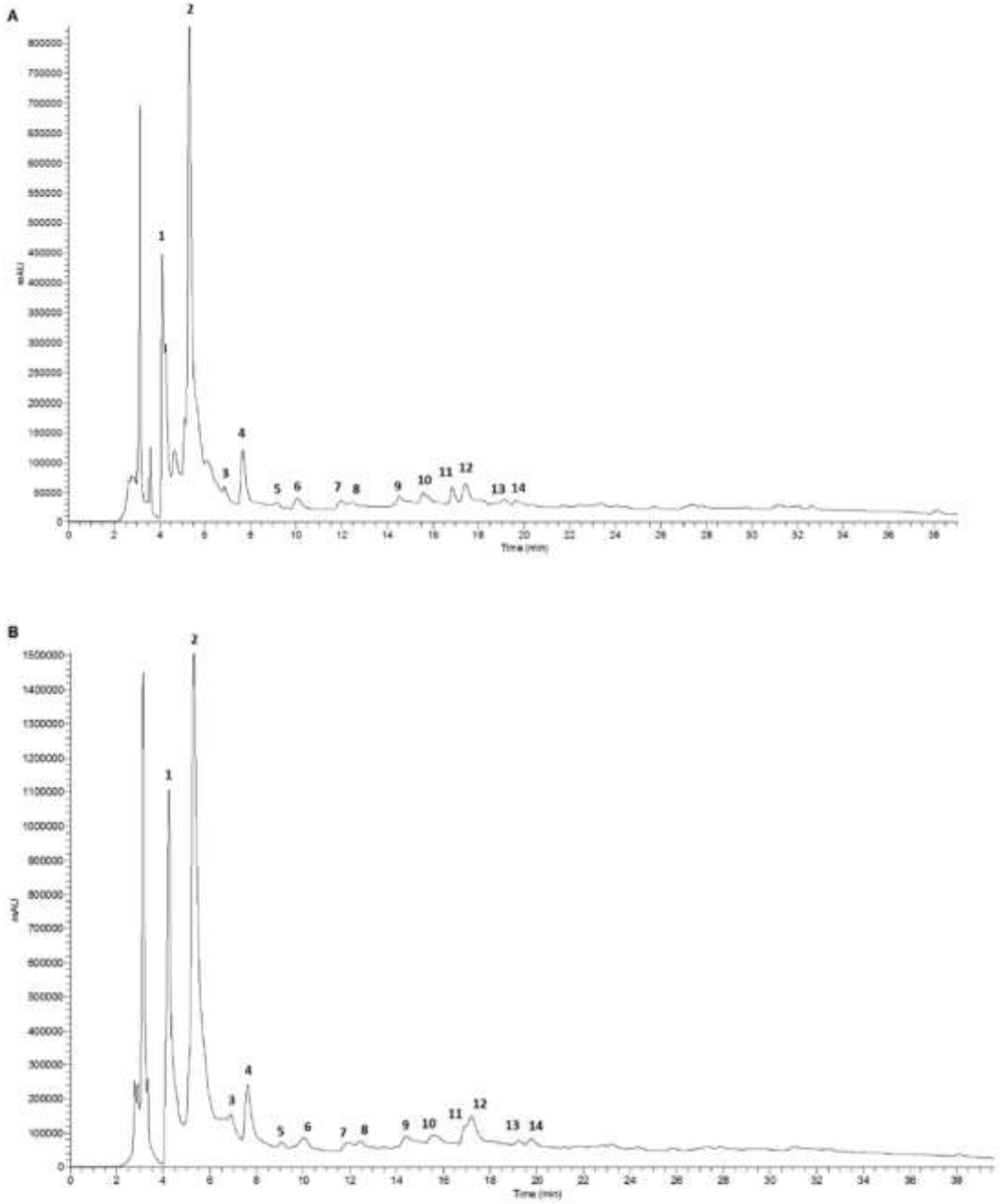


Fig. 2. Chromatographic profile of non-irradiated (A) and irradiated at 5 kGy (B) of EOP samples, recorded at 280 nm.

significantly at 16 kGy to values similar to 5 kGy. On the other hand, significant increases were produced in the concentrations of caffeic acid and oleuropein algycon isomer 1 at 16 and 22 kGy, β-hydroxyverbascoside isomers 1 and 2 at 22 kGy, and tyrosol with increasing irradiation doses. Also, the concentrations of verbascoside and luteolin-7-*O*-rutinoside increased at higher doses (16 and 22 kGy) after a significant decrease at 10 kGy, whereas a significant decrease was observed in syringic acid contents at 10 kGy comparatively to 5 kGy and higher applied doses (16 kGy and 22 kGy). As for the most abundant compound (hydroxytyrosol), a sharp increase was produced at all the applied irradiation doses in both olive pomace extracts compared to non-irradiated samples, without significant differences among the different doses, except for EOP at 16 kGy for which a punctual decrease was observed in relation to the other doses. Considering the overall results, the highest improvement (≥2 fold) in the extractability of phenolic compounds in COP was induced by irradiation at 22 kGy (Table 2). For EOP, the extractability was enhanced (≥2 fold) for the majority of the compounds after irradiation at 10 kGy, although at 5 kGy the extractability of 9 out of 13 compounds did not differ significantly from the obtained at 10 kGy. For illustrative purposes, Fig. 3 shows the

Table 1
Chromatographic and mass spectral characteristics and tentative identification of phenolic compounds in olive pomace extracts.

Peak	Rt (min)	λ_{max} (nm)	Pseudomolecular ion $[M-H]^-$ (m/z)	MS^2 (m/z)	Tentative identification
1	4.25	229, 277	315	179(3), 161(1), 153(100), 135(58)	Hydroxytyrosol-1-β-glucoside
2	5.31	281	153	123(100)	Hydroxytyrosol
3	6.90	220, 267	377	331(26), 287(42), 179(80), 161(62), 143(64), 131(25), 119(100), 113(68), 101(51)	Oleuropein aglycon isomer 1
4	7.59	220, 277	137	106(100)	Tyrosol
5	8.35	220, 267	377	197(100), 179(5), 153(20), 135(5)	Oleuropein aglycon isomer 2
6	10.02	324	179	135(100)	Caffeic acid
7	11.96	284, 326	639	621(100), 529(14), 459(5), 179(5)	β-Hydroxyverbascoside isomer 1
8	12.38	285, 327	639	621(100), 529(13), 459(5), 179(5)	β-Hydroxyverbascoside isomer 2
9	14.41	262, 295	151	136(100)	Vanillin
10	15.39	288	197	153(100)	Syringic acid
11	16.93	285, 326	623	461(50), 315(100)	Verbascoside
12	17.21	281	151	123(100), 107(5)	4-Hydroxyphenylacetic acid
13	19.24	266, 340	593	447(100), 285(95)	Luteolin-7-*O*-rutinoside
14	19.77	223, 282, 323	337	183(38), 157(100), 139(24)	Unknown

tendency of the major compounds (hydroxytyrosol-1-β-glucoside, hydroxytyrosol and tyrosol) and total phenolic compounds in EOP extracts where greater effect on their extractability was obtained with gamma irradiation.

To our knowledge, this is the first report describing the effect of gamma radiation on individual phenolic compounds of olive pomaces. Nonetheless, the influence of this technology on total phenolic composition and bioactive properties has been explored in different plant materials by other authors. Namely, Khattak et al. (2009) found that the concentration of total phenolics increased in *Nelumbo nucifera* rhizomes with the application of increasing doses of gamma radiation from 0 to 6 kGy. Further, Zhu et al. (2010) reported an increase of phenolic acids content in black rice extracts irradiated at 8 kGy, and a similar effect was observed for infusions and extracts of *Ginkgo biloba* L. (Pereira et al., 2015) and for lemon verbena and peppermint infusions (Pereira et al., 2017b) at 10 kGy irradiation dose. The increase in phenolic concentrations on irradiated samples could be related to the release of these compounds from matrix structures, increasing their extractability (Pereira et al., 2015), and/or to the radiolytic degradation of larger compounds into smaller ones (Hussain et al., 2016).

Although there are some reports in the literature concerning the recovery of phenolic compounds from olive pomace (Cioffi et al., 2010; Nunes et al., 2018; Pavez et al., 2019; Peralbo-Molina et al., 2012; Sygouni et al., 2019), most of these studies did not quantify the phenolic compounds present in the samples. Table 3 present the quantification of some compounds observed in EOP irradiated at 5 kGy in comparison with a work developed by Suárez et al. (2009) using solid-liquid extraction at atmosphere and high pressures. In this table, only the five compounds identified in both works are showed, although the total phenolic compounds content refers to all the compounds and not only to those five represented. The observed results prove that the applied methodology in this work, and especially gamma irradiation, allowed to extract higher concentrations of individual phenolic compounds than the methodologies used by Suárez et al. (2009). The total phenolic compounds concentration was higher when applying solid-liquid extraction at atmosphere pressure using ethyl acetate as solvent. Nevertheless, it is important to emphasize that these authors identified twenty-four compounds instead of our fourteen, being expectable that the total phenolic concentration is higher.

All in all, the obtained results pointed that the use of ionizing radiation can be an environmentally friendly innovative approach to improve the extractability of bioactive compounds from olive residues, helping the olive oil industry to adopt clean processes and promoting sustainable development.

3.2. Bioactive properties of olive pomace extracts

3.2.1. Antioxidant activity

The results of the antioxidant activity assays are presented in Table 4. Data are expressed as IC_{50} values, meaning the extract concentration able to provide 50% of antioxidant activity (TBARS assay) or to protect 50% of the erythrocyte population from haemolysis caused by an oxidizing agent (OxHLIA assay). The IC_{50} values in the OxHLIA assay were obtained at two times (Δt 60 min and Δt 120 min) considering that natural extracts contain different antioxidant molecules capable of interacting with each other and offering protection at different time periods. In both assays, the lower the IC_{50} values, the higher the antioxidant capacity of the olive pomace extracts.

Erythrocytes are blood cells especially susceptible to oxidation due to their high lipid content and their rich oxygen supply and presence of transition metals. Reactive oxygen species can attack the erythrocytes membrane, compromise cell integrity and induce oxidation of lipids and protein, which results in haemolysis (Lockowandt et al., 2019). Hydrophilic peroxyl radicals generated by thermal decomposition of AAPH may attack the erythrocytes membrane from the outside and the extent of haemolysis is proportional to their amount (Miki et al., 1987). Phenolic compounds can be incorporated by erythrocyte membranes, being located at the polar-unpolar interface of the membrane or entering cells (e.g., quercetin), providing antioxidant protection through the interaction with some membrane and intracellular components, such as glutathione or ascorbic acid (Alvarez-Suarez et al., 2012).

In the OxHLIA assay, the haemolysis curves for the extracts of EOP and COP at different concentrations (data not shown) revealed that higher concentrations protected the erythrocyte population from

Table 2
Quantification of phenolic compounds in EOP and COP extracts from non-irradiated and irradiated samples.

Compound	Quantification (mg/g extract)									
	EOP					COP				
	0 kGy	5 kGy	10 kGy	16 kGy	22 kGy	0 kGy	5 kGy	10 kGy	16 kGy	22 kGy
Hydroxytyrosol-1-β-glucoside[1]	9.8 ± 0.4^{d}	27 ± 1^{a,b,c}	29 ± 1^{a,b}	25 ± 1^{c}	26.6 ± 0.8^{b,c}	9.94 ± 0.08^{d}	29.3 ± 0.8^{a}	28.9 ± 0.6^{a,b}	27.0 ± 0.2^{a,b,c}	28.2 ± 0.3^{a,b}
Hydroxytyrosol[1]	25 ± 1^{d}	62 ± 1^{a}	63 ± 2^{a}	55 ± 3^{b,c}	60 ± 1^{a,b}	23.9 ± 0.3^{d}	52 ± 2^{c}	51 ± 2^{c}	51 ± 1^{c}	52.4 ± 0.8^{c}
Oleuropein aglycon isomer 1[2]	3.0 ± 0.3^{c}	8.7 ± 0.5^{a}	8.7 ± 0.6^{a}	7.4 ± 0.3^{a}	7.5 ± 0.6^{a}	3.1 ± 0.1^{c}	5.0 ± 0.2^{b}	4.9 ± 0.1^{b}	8.4 ± 0.3^{a}	8.0 ± 0.2^{a}
Tyrosol[3]	5.9 ± 0.2^{c}	14.1 ± 0.6^{a}	13.9 ± 0.3^{a}	12.2 ± 0.8^{b}	12.5 ± 0.4^{a,b}	5.9 ± 0.4^{c}	11.9 ± 0.5^{b}	12.0 ± 0.2^{b}	12.6 ± 0.6^{a,b}	14.1 ± 0.5^{a}
Oleuropein aglycon isomer 2[2]	1.80 ± 0.08^{e}	3.57 ± 0.06^{a}	3.8 ± 0.1^{a}	3.2 ± 0.1^{b}	2.80 ± 0.08^{c,d}	1.7 ± 0.1^{f}	2.54 ± 0.09^{d}	2.19 ± 0.06^{e}	2.95 ± 0.09^{b,c}	3.2 ± 0.1^{b}
Caffeic acid[4]	0.55 ± 0.01^{f}	1.36 ± 0.04^{e}	1.32 ± 0.03^{e}	1.46 0.05^{c,d}	1.62 ± 0.03^{d}	0.89 ± 0.03^{e}	1.73 ± 0.06^{c}	1.85 ± 0.07^{c}	2.0 ± 0.1^{b}	2.52 ± 0.07^{a}
β-Hydroxyverbascoside isomer 1[5]	1.70 ± 0.05^{e}	3.5 ± 0.1^{d}	4.2 ± 0.2^{c}	3.4 ± 0.1^{e}	3.41 ± 0.02^{d}	2.27 ± 0.03^{e}	4.8 ± 0.2^{a,b}	4.8 ± 0.1^{b}	4.8 ± 0.3^{b}	5.3 ± 0.2^{a}
β-Hydroxyverbascoside isomer 2[5]	1.62 ± 0.03^{e}	3.50 ± 0.06^{f}	4.4 ± 0.1^{b}	3.14 ± 0.08^{e}	3.15 ± 0.06^{e,f}	2.66 ± 0.06^{d}	4.55 ± 0.07^{b}	4.2 ± 0.1^{b}	4.6 ± 0.3^{b}	5.4 ± 0.2^{a}
Vanillin[6]	1.22 ± 0.01^{d}	2.36 ± 0.07^{b,c}	3.1 ± 0.1^{a}	2.06 ± 0.04^{c}	2.3 ± 0.1^{b,c}	1.43 ± 0.06^{d}	3.49 ± 0.07^{a}	2.5 ± 0.1^{b}	3.2 ± 0.2^{a}	3.5 ± 0.2^{a}
Syringic acid[7]	0.93 ± 0.03^{f}	3.05 ± 0.12^{a,b}	2.24 ± 0.05^{c}	1.89 ± 0.09^{d}	2.02 ± 0.12^{c,d}	1.32 ± 0.08^{e}	3.04 ± 0.08^{a,b}	2.05 ± 0.02^{c,d}	2.8 ± 0.2^{b}	3.20 ± 0.08^{a}
Verbascoside[5]	2.29 ± 0.03^{e}	3.4 ± 0.1^{d,e}	4.01 ± 0.06^{d}	3.02 ± 0.05^{e,f}	3.44 ± 0.07^{d,e}	3.4 ± 0.2^{d,e}	8.6 ± 0.3^{a}	6.5 ± 0.2^{c}	7.5 ± 0.4^{b}	9.4 ± 0.4^{a}
4-hydroxyphenylacetic acid[8]	1.60 ± 0.08^{c}	4.3 ± 0.1^{a}	3.6 ± 0.2^{b}	3.6 ± 0.1^{b}	3.7 ± 0.1^{b}	1.70 ± 0.03^{c}	nd	2.01 ± 0.04^{c}	nd	nd
Luteolin-7-O-rutinoside[9]	3.1 ± 0.1^{e}	5.1 ± 0.3^{c}	5.0 ± 0.2^{c}	4.55 ± 0.1^{d}	4.4 ± 0.1^{d}	4.1 ± 0.2^{d}	6.4 ± 0.3^{b}	2.30 ± 0.02^{f}	5.5 ± 0.2^{b,c}	7.8 ± 0.2^{a}
Unknown	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq
Total phenylethanoid derivatives	53 ± 2^{g}	129 ± 2^{a,b}	135 ± 4^{a}	116 ± 5^{c}	121 ± 2^{b,c}	54.6 ± 0.4^{d}	119 ± 3^{c}	117 ± 3^{c}	118 ± 3^{c}	126 ± 2^{a,c}
Total phenolic acids	1.48 ± 0.02^{a}	4.4 ± 0.1^{c}	3.53 ± 0.04^{d,e}	3.28 ± 0.08^{e}	3.7 ± 0.2^{c,d}	2.21 ± 0.05^{f}	4.8 ± 0.1^{b}	3.90 ± 0.05^{c}	4.7 ± 0.1^{b}	5.73 ± 0.05^{a}
Total flavonoids	3.1 ± 0.1^{e}	5.1 ± 0.3^{c}	5.0 ± 0.2^{c}	4.5 ± 0.1^{d}	4.4 ± 0.1^{d}	4.1 ± 0.2^{d}	6.4 ± 0.3^{b}	2.30 ± 0.02^{f}	5.5 ± 0.2^{c}	7.8 ± 0.2^{a}
Total phenolic compounds	57 ± 2^{f}	139 ± 2^{b,c}	143 ± 4^{a}	124 ± 6^{d,e}	129 ± 2^{b,c,e}	61.0 ± 0.5^{g}	130 ± 4^{b,d}	123 ± 3^{d,e}	128 ± 3^{b,e}	140 ± 2^{a,b,c}
Other compounds[†]	1.220 ± 0.005^{d}	2.36 ± 0.07^{b}	3.1 ± 0.1^{a}	2.06 ± 0.04^{c}	2.3 ± 0.1^{b,c}	1.43 ± 0.06^{d}	3.5 ± 0.1^{a}	2.5 ± 0.1^{b}	3.2 ± 0.2^{a}	3.5 ± 0.2^{a}

Values within a row with similar letters do not differ significantly ($p > 0.05$). nd–not detected. nq–not quantified. Calibration curves used for quantification: [1]Hydroxytyrosol ($y = 124{,}154x + 17{,}393$, $R^2 = 0.9999$), [2]Oleuropein ($y = 32{,}226x + 12{,}416$, $R^2 = 0.9999$), [3]Tyrosol ($y = 91{,}708x + 9398.5$, $R^2 = 0.9999$), [4]Caffeic acid ($y = 388{,}345x + 406{,}369$, $R^2 = 0.9939$), [5]Verbascoside ($y = 124{,}233x - 18{,}873$, $R^2 = 1$), [6]Vanillic acid ($y = 29{,}751x - 28{,}661$, $R^2 = 0.9999$), [7]Syringic acid ($y = 376{,}056x + 141{,}329$, $R^2 = 0.9995$), [8]p-hydroxybenzoic acid ($y = 208{,}604x + 173{,}056$, $R^2 = 0.9995$), [9]Apigenin-7-O-glucoside ($y = 10{,}683x - 45{,}794$, $R^2 = 0.996$).
[†] Other compounds: vanillin.

haemolysis for a longer time period. The extract of the non-irradiated EOP provided higher antihaemolytic protection (IC_{50} values of 20.0 ± 0.8 μg/mL and 35 ± 2 μg/mL, at 60 min and 120 min, respectively) than the non-irradiated COP extract (32.2 ± 0.6 μg/mL and 61 ± 2 μg/mL). These results could suggest that phenolic compounds in the EOP samples (defatted pomace) could be more bioaccessible to protect the erythrocyte membrane from the AAPH-induced oxidation due to the higher hydrophilicity of the medium. The presence of some fat content in the extracts obtained from the COP samples could somehow hamper contact between the phenolic antioxidants and the erythrocyte

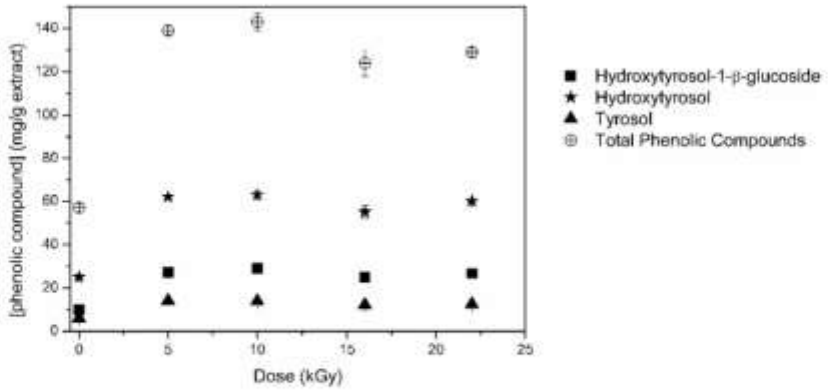


Fig. 3. Extractability of hydroxytyrosol-1-β-glucoside, hydroxytyrosol and tyrosol and total phenolic compounds in EOP extracts during the irradiation treatment.

Table 3
Comparison of the results obtained in this work with others reported in the literature (mg/g extract).

	This work	Suárez et al. (2009)	
	Extracted olive pomace, 5 kGy	Solid-liquid extraction at atmosphere pressure	Solid-liquid extraction at high pressure
Hydroxytyrosol	62	2.79	2.54
Tyrosol	14.1	0.32	0.09
Caffeic acid	1.36	0.43	0.09
Vanillin	2.36	0.06	0.05
Verbascoside	3.4	2.18	0.56
Total phenolic compounds	139	233.35	76.53

membrane or the generated radicals, thus hindering the formation of a protective barrier against the diffusion of free radicals or its elimination from the reaction system. This may justify the higher IC_{50} values of the non-defatted extracts in the OxHLIA assay.

On the other hand, since lipophilic radicals can result from the AAPH-induced oxidation of the fat present in the extracts, the haemolytic response may have been intensified. If this happened in the system, it also justifies the largest amount of COP extract (higher IC_{50} values) needed to protect the erythrocyte population from oxidation.

Interestingly, despite the increase in phenolic compounds content, gamma radiation promoted a negative effect on the antihaemolytic activity of the extracts since the IC_{50} values increased with the increase of irradiation doses. For EOP extracts, however, the differences in the IC_{50} values were not significant ($p > 0.05$) at the lower doses (i.e., 5 and 10 kGy), which did not differ from those of the non-irradiated samples and the standard antioxidant compounds, Trolox, used positive control. These findings indicated the existence of relevant antioxidant capacity in the prevention of cellular oxidative processes of the defatted pomace extracts. Similar observations were made by Lins et al. (2018) in extracts obtained from olive leaves.

Table 4
Antioxidant activity (IC_{50} values, μg/mL) and hepatotoxicity (GI_{50} values, μg/mL) of the olive pomace extracts and positive controls (trolox or ellipticine, respectively).

	Antioxidant activity[1]			Hepatotoxicity
	OxHLIA, $\Delta t = 60$ min	OxHLIA, $\Delta t = 120$ min	TBARS	PLP2
EOP samples				
0 kGy	20.0 ± 0.8^{b}	35 ± 2^{g}	49 ± 1^{a}	>400
5 kGy	20.6 ± 0.4^{b}	37 ± 1^{g}	22 ± 1^{e}	>400
10 kGy	$21.8 \pm 0.4^{g,h}$	36.2 ± 0.6^{g}	28 ± 2^{d}	>400
16 kGy	23.6 ± 0.5^{g}	$47 \pm 2^{e,f}$	38 ± 2^{c}	>400
22 kGy	27.6 ± 0.7^{f}	51 ± 2^{e}	43 ± 2^{b}	>400
COP samples				
0 kGy	32.2 ± 0.6^{e}	61 ± 2^{d}	20 ± 1^{f}	>400
5 kGy	36.1 ± 0.7^{d}	69 ± 5^{c}	19 ± 1^{f}	>400
10 kGy	56 ± 1^{c}	82 ± 2^{b}	23 ± 1^{e}	>400
16 kGy	61 ± 2^{b}	88 ± 3^{b}	24 ± 1^{e}	>400
22 kGy	68 ± 1^{a}	99 ± 2^{a}	27 ± 1^{d}	>400
Trolox	19.6 ± 0.6^{h}	$41.1 \pm 0.8^{f,g}$	5.4 ± 0.3^{g}	
Ellipticine	–	–	–	2.3 ± 0.2
Homoscedasticity[2]	0.590	0.220	0.000	
1-way ANOVA[3]	<0.001	<0.001	<0.001	

Values within a column with similar letters do not differ significantly ($p > 0.05$).
[1] The results are given as mean ± standard deviation.
[2] Homoscedasticity among samples, $p > 0.05$; heteroscedasticity among samples, $p < 0.05$.
[3] In each column, $p < 0.05$ indicates that the mean value of the evaluated parameter of at least one sample differs from the others (in this case, multiple comparison tests were performed).

Contrary to the results obtained in the OxHLIA assay, the COP extracts were more effective than the EOP ones to inhibit TBARS formation. In this assay, the reaction mechanisms occur in a lipophilic environment, where the presence of fat will not lead to the possible bioaccessibility issues mentioned for OxHLIA. However, it has been reported that thiobarbituric acid (TBA) not only reacts with malondialdehyde (MDA), which is a secondary oxidation product of lipid peroxidation, but also with other aldehydes that can be generated in the system, especially from fat-containing samples. Thus, substances that overlap with the MDA-TBA complex absorption peak are generated and may lead to an overestimation of the antioxidant capacity (Semeniuc et al., 2016). On the other hand, gamma irradiation improved the antioxidant capacity of the EOP extracts, with particular efficiency at the lowest assayed doses (5 kGy), whereas no improvement in the antioxidant effect was observed after irradiation in COP samples.

The overall results obtained in these assays suggested that gamma radiation could be a suitable technique to improve the antioxidant activity in EOP when applied at low doses (i.e., 5 kGy), at which an increase in antioxidant potential was produced as evaluated by the TBARS assay, while keeping the ability to protect erythrocytes against oxidation-induced haemolysis.

3.2.2. Hepatotoxicity

The assessment of the cytotoxicity for PLP2 (non-tumor cells) is essential considering that mammalian hepatocytes constitute a required step in the verification of toxic compounds (Abreu et al., 2011). Although the use of the PLP2 culture could not give an absolute answer about hepatotoxicity, in fact none in vitro assay can do that, it is considered a feasible method to perform preliminary hepatotoxicity studies (Abreu et al., 2011). The effects of olive pomace (EOP and COP) extracts on the growth of non-tumor liver primary cell line (PLP2) represented as the concentration that caused 50% of cell growth inhibition (GI_{50}), are also summarized in Table 4. The obtained results indicated that none of the extracts, irradiated or non-irradiated, had an inhibitory effect on the cell growth ($GI_{50} > 400$ μg/mL), suggesting no hepatotoxicity of the extracts.

3.2.3. Antimicrobial activity

Antimicrobial activity of the olive pomace extracts against three Gram-negative (*E. coli*, *S.* Typhimurium, *P. fluorescens*) and three Gram-positive bacteria (*B. cereus*, *S. aureus*, *L. monocytogenes*) and two fungi (*A. fumigatus* and *P. spinulosum*) was evaluated (Table 5).

All olive pomace extracts had similar potential antibacterial activity against the studied Gram-positive bacterial strains (MIC 20 mg/mL), whereas EOP samples showed higher antibacterial activity against Gram-negative bacteria, namely *E. coli* and *P. fluorescens* (MIC 20 mg/mL), than COP (MIC 40 mg/mL). It is to highlight the ability to inhibit the *P. fluorescens* biofilm formation after 72 h by defatted olive pomace extracts. Comparatively to COP extracts, the EOP ones presented the higher hydroxytyrosol content (Table 2), suggesting a correlation between antimicrobial activity and a rich concentration in this phenolic compound. According to a previous study, the hydroxytyrosol-rich composition sample from olive mill wastewater inhibited the growth of Gram-negative bacteria (Yangui et al., 2009). The authors attributed the effectiveness of hydroxytyrosol to its capability of chelating transition metals, lowering the reactivity of metal iron by forming an inert metal–ligand complex, reducing the bioavailability for microbial growth (Wong and Kitts, 2006; Yangui et al., 2009). In agreement with our results, Brenes et al. (2007) also observed higher sensitivity of Gram-positive than Gram-negative bacteria to olive oil polyphenols. Furthermore, the olive pomace extracts also revealed some potential antifungal against the studied fungi (MIC 40 mg/mL). In general, gamma radiation seemed to have no effect on the antimicrobial potential of the studied olive pomace extracts, but for EOP sample in the case of *A. fumigatus* (Table 5), for which the MFC value varied from 40 mg/mL in non-irradiated to ≥40 mg/mL in irradiated pomaces. Other

Table 5
Antimicrobial activity of olive pomace extracts (MIC, mg/mL).

	B. cereus	*S. aureus*	*L. monocytogenes*	*E. coli*	*S. Typhimurium*	*P. fluorescens*	*A. fumigatus*	*P. spinulosum*
					MIC (mg/mL)			
EOP samples								
0 kGy	20	20	20	20	40	20	40	40
5 kGy	20	20	20	20	40	20	40	40
10 kGy	20	20	20	20	40	20	40	40
16 kGy	20	20	20	20	40	20	40	40
22 kGy	20	20	20	20	40	20	40	40
COP samples								
0 kGy	20	20	20	40	40	40	40	40
5 kGy	20	20	20	40	40	40	40	40
10 kGy	20	20	20	40	40	40	40	40
16 kGy	20	20	20	40	40	40	40	40
22 kGy	20	20	20	40	40	40	40	40
	B. cereus	*S. aureus*	*L. monocytogenes*	*E. coli*	*S. Typhimurium*	*P. fluorescens*	*A. fumigatus*	*P. spinulosum*
			MBC (mg/mL)				MFC (mg/mL)	
EOP samples								
0 kGy	20	≥20	≥20	≥20	≥40	≥20	40	≥40
5 kGy	20	≥20	≥20	≥20	≥40	≥20	≥40	≥40
10 kGy	20	≥20	≥20	≥20	≥40	≥20	≥40	≥40
16 kGy	20	≥20	≥20	≥20	≥40	≥20	≥40	≥40
22 kGy	20	≥20	≥20	≥20	≥40	≥20	≥40	≥40
COP samples								
0 kGy	20	≥20	≥20	≥40	40	≥40	≥40	≥40
5 kGy	20	≥20	≥20	≥40	40	≥40	≥40	≥40
10 kGy	20	≥20	≥20	≥40	40	≥40	≥40	≥40
16 kGy	20	≥20	≥20	≥40	40	≥40	≥40	≥40
22 kGy	20	≥20	≥20	≥40	40	≥40	≥40	≥40

authors have demonstrated the capability of a saprophytic fungus to grow on dry olive-mill residue (Sampedro et al., 2005), which can envisage the inefficacy of pomace extracts against fungi. Furthermore, Sousa et al. (2006) observed no antifungal activity at the tested concentrations in table olive extracts.

As far as we know, this is the first report concerning the antimicrobial potential of olive pomace and the evaluation of gamma radiation effect on this activity. There are, however, some studies reporting high antimicrobial activity in table olives (Sousa et al., 2006), olive oil (Brenes et al., 2007) and olive mil wastewaters (Yakhlef et al., 2018; Yangui et al., 2009). These findings point to the possibility of using olive pomace extracts as a food preservative in order to prevent the growth of foodborne pathogens or to delay the food spoilage. Nevertheless, further studies have to be performed to identify which are the compounds responsible for this antimicrobial activity.

4. Conclusions

This work focused on the impact of gamma radiation on the phenolic composition and bioactive properties of olive pomace extracts (EOP and COP samples). HPLC-DAD-MS analyses demonstrated that olive pomace is a rich source of hydroxytyrosol, and also contains significant amounts of other phenolics, such as hydroxytyrosol-glucoside, tyrosol, syringic acid or luteolin-rutinoside. The gamma radiation treatment of olive pomace improved the extractability of phenolic compounds. The extracts of EOP olive pomaces were more efficient in protecting erythrocytes against oxidatively-induced haemolysis, while those from COP showed greater ability to inhibit TBARS formation, which might be related with fat content of the samples. In general, gamma radiation did not improve the antioxidant capacity of the olive pomaces in relation to non-irradiated ones, except in the case of TBARS inhibition of EOP samples, which was significantly increased, especially at low irradiation doses. The treatment of EOP olive pomace extracts at a dose of 5 kGy could be considered a good compromise for gamma irradiation, as it increases phenolic extractability at the same time that improves the antioxidant activity of the extracts, as evaluated by the TBARS assay, and preserves the antihaemolytic activity, maintaining it in values similar to the reference antioxidant Trolox. Both extracts presented antimicrobial activity against the studied bacterial and fungi strains, but EOP extracts were more effective against *E. coli* and *P. fluorescens*. No toxicity in non-tumor hepatic cells (PLP2) was observed, either for EOP or COP extracts. Gamma radiation treatment seemed to cause no alteration in the antimicrobial activity and hepatotoxicity of the extracts. Based on the obtained results, the olive pomace extracts could be considered a suitable alternative for the food industry to be used as food preservatives.

All in all, the observations made in the present study indicate that gamma radiation at low doses (5 kGy) could be applied as a groundbreaking technology in the valorization of olive oil by-products, with better results when extracted material is used. The olive pomace extracts could be considered a suitable alternative for the food industry to be used as food preservatives. Although, further studies are needed to evaluate the effect of gamma radiation on olive pomace individual compounds to assess its bioactivity. This work should contribute to enhance the sustainability of the agro-industrial sector considering both economic and environmental aspects.

CRediT authorship contribution statement

Joana Madureira: Investigation, Writing - original draft. **Maria Inês Dias:** Investigation. **José Pinela:** Investigation. **Ricardo C. Calhelha:** Investigation. **Lillian Barros:** Conceptualization, Methodology, Writing - review & editing, Supervision. **Celestino Santos-Buelga:** Writing - review & editing, Supervision. **Fernanda M.A. Margaça:** Writing - review & editing. **Isabel C.F.R. Ferreira:** Conceptualization, Methodology, Supervision. **Sandra Cabo Verde:** Conceptualization, Methodology, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support to C2TN [UID/Multi/04349/2019], CIMO [UID/AGR/00690/2019], J. Madureira [SFRH/BD/136506/2018]; L. Barros, J. Pinela and M.I. Dias thank the national funding by FCT, P.I., through the institutional scientific employment program-contract; FEDER-Interreg España-Portugal program for financial support through the project 0377_Iberphenol_6_E; "UCASUL - União de Cooperativas Agrícolas do Sul" agro industrial cooperative for providing the samples; to Technological Unit of Radiosterilization (University of Lisbon) for the samples irradiation; and to Molecular Materials Synthesis Laboratory (responsible Researcher Dr. Dulce Belo, C²TN) for the solvent evaporations.

References

Abreu, R.M.V., Ferreira, I.C.F.R., Calhelha, R.C., Lima, R.T., Vasconcelos, M.H., Adega, F., Chaves, R., Queiroz, M.J.R.P., 2011. Anti-hepatocellular carcinoma activity using human HepG2 cells and hepatotoxicity of 6-substituted methyl 3-aminothieno[3,2-b]pyridine-2-carboxylate derivatives: in vitro evaluation, cell cycle analysis and QSAR studies. Eur. J. Med. Chem. 46, 5800–5806. https://doi.org/10.1016/j.ejmech.2011.09.029.

Alvarez-Suarez, J.M., Giampieri, F., González-Paramás, A.M., Damiani, E., Astolfi, P., Martinez-Sanchez, G., Bompadre, S., Quiles, J.L., Santos-Buelga, C., Battino, M., 2012. Phenolics from monofloral honeys protect human erythrocyte membranes against oxidative damage. Food Chem. Toxicol. 50, 1508–1516. https://doi.org/10.1016/j.FCT.2012.01.042.

Ammar, S., Contreras, M. del M., Gargouri, B., Segura-Carretero, A., Bouaziz, M., 2017. RP-HPLC-DAD-ESI-QTOF-MS based metabolic profiling of the potential *Olea europaea* by-product "wood" and its comparison with leaf counterpart. Phytochem. Anal. 28, 217–229. https://doi.org/10.1002/pca.2664.

Aouidi, F., Ayari, S., Ferhi, H., Roussos, S., Hamdi, M., 2011. Gamma irradiation of air-dried olive leaves: effective decontamination and impact on the antioxidative properties and on phenolic compounds. Food Chem. 127, 1105–1113. https://doi.org/10.1016/j.foodchem.2011.01.109.

Bessada, S.M.F., Barreira, J.C.M., Barros, L., Ferreira, I.C.F.R., Oliveira, M.B.P.P., 2016. Phenolic profile and antioxidant activity of Coleostephus myconis (L.) Rchb.f.: an underexploited and highly disseminated species. Ind. Crop. Prod. 89, 45–51. https://doi.org/10.1016/j.indcrop.2016.04.065.

Brenes, M., Medina, E., Romero, C., De Castro, A., 2007. Antimicrobial activity of olive oil. Agro Food Ind. Hi. Tech. 18, 6–8. https://doi.org/10.1056/NEJM197206152862402.

Bulotta, S., Celano, M., Lepore, S.M., Montalcini, T., Pujia, A., Russo, D., 2014. Beneficial effects of the olive oil phenolic components oleuropein and hydroxytyrosol: focus on protection against cardiovascular and metabolic diseases. J. Transl. Med. 12, 219. https://doi.org/10.1186/s12967-014-0219-9.

Cabo Verde, S., Trigo, M.J., Sousa, M.B., Ferreira, A., Ramos, A.C., Nunes, I., Junqueira, C., Melo, R., Santos, P.M.P., Botelho, M.L., 2013. Effects of gamma radiation on raspberries: safety and quality issues. J. Toxicol. Environ. Health. A 76, 291–303. https://doi.org/10.1080/15287394.2013.757256.

Cardinali, A., Pati, S., Minervini, F., D'Antuono, I., Linsalata, V., Lattanzio, V., 2012. Verbascoside, isoverbascoside, and their derivatives recovered from olive mill wastewater as possible food antioxidants. J. Agric. Food Chem. 60, 1822–1829. https://doi.org/10.1021/jf204001p.

Cardoso, S.M., Guyot, S., Marnet, N., Lopes-da-Silva, J.A., Renard, C.M.G.C., Coimbra, M.A., 2005. Characterisation of phenolic extracts from olive pulp and olive pomace by electrospray mass spectrometry. J. Sci. Food Agric. 85, 21–32. https://doi.org/10.1002/jsfa.1925.

Carocho, M., Morales, P., Ferreira, I.C.F.R., 2015. Natural food additives: quo vadis? Trends Food Sci. Technol. 45, 284–295. https://doi.org/10.1016/j.tifs.2015.06.007.

Cioffi, G., Pesca, M.S., De Caprariis, P., Braca, A., Severino, L., De Tommasi, N., 2010. Phenolic compounds in olive oil and olive pomace from Cilento (Campania, Italy) and their antioxidant activity. Food Chem. 121, 105–111. https://doi.org/10.1016/j.foodchem.2009.12.013.

De Marco, E., Savarese, M., Paduano, A., Sacchi, R., 2007. Characterization and fractionation of phenolic compounds extracted from olive oil mill wastewaters. Food Chem. 104, 858–867. https://doi.org/10.1016/j.foodchem.2006.10.005.

Federici, E., Massaccesi, L., Pezzolla, D., Fidati, L., Montalbani, E., Proietti, P., Nasini, L., Regni, L., Scargetta, S., Gigliotti, G., 2017. Short-term modifications of soil microbial community structure and soluble organic matter chemical composition following amendment with different solid olive mill waste and their derived composts. Appl. Soil Ecol. 119, 234–241. https://doi.org/10.1016/j.apsoil.2017.06.014.

Fernández-Bolaños, J., Rodríguez, G., Rodríguez, R., Heredia, A., Guillén, R., Jiménez, A., 2002. Production in large quantities of highly purified hydroxytyrosol from liquid—solid waste of two-phase olive oil processing or "alperujo". J. Agric. Food Chem. 50, 6804–6811. https://doi.org/10.1021/jf011712r.

Fernández-Bolaños, J., Rodríguez, G., Rodríguez, R., Guillén, R., Jiménez, A., 2006. Extraction of interesting organic compounds from olive oil waste. Grasas Aceites 57, 95–106. https://doi.org/10.3989/gya.2006.v57.i1.25.

Fernández-Bolaños, J.G., Lopez, O., Fernández-Bolaños, J., Rodriguez-Gutierrez, G., 2008. Hydroxytyrosol and derivatives: isolation, synthesis, and biological properties. Curr. Org. Chem. 12, 442–463. https://doi.org/10.2174/138527208784083888.

Gomes Corrêa, R.C., Pereira de Souza, A.H., Calhelha, R.C., Barros, L., Glamoclija, J., Sokovic, M., Peralta, R.M., Bracht, A., Ferreira, I.C.F.R., 2015. Bioactive formulations prepared from fruiting bodies and sumerged culture mycelia of the Brazilian edible mushroom Pleurotus ostreatoroseus Singer. Food Funct. 6, 2155–2164. https://doi.org/10.1039/c5fo00465a.

Guerreiro, D., Madureira, J., Silva, T., Melo, R., Santos, P.M.P., Ferreira, A., Trigo, M.J., Falcão, A.N., Margaça, F.M.A., Cabo Verde, S., 2016. Post-harvest treatment of cherry tomatoes by gamma radiation: microbial and physicochemical parameters evaluation. Innov. Food Sci. Emerg. Technol. 36, 1–9. https://doi.org/10.1016/j.ifset.2016.05.008.

Hussain, P.R., Suradkar, P., Javaid, S., Akram, H., Parvez, S., 2016. Influence of postharvest gamma irradiation treatment on the content of bioactive compounds and antioxidant activity of fenugreek (Trigonella foenum-graceum L.) and spinach (*Spinacia oleracea* L.) leaves. Innov. Food Sci. Emerg. Technol. 33, 268–281. https://doi.org/10.1016/j.ifset.2015.11.017.

Khattak, K.F., Simpson, T.J., Ihasnullah, 2009. Effect of gamma irradiation on the microbial load, nutrient composition and free radical scavenging activity of *Nelumbo nucifera* rhizome. Radiat. Phys. Chem. 78, 206–212. https://doi.org/10.1016/j.radphyschem.2008.11.001.

Lins, P.G., Marina Piccoli Pugine, S., Scatolini, A.M., de Melo, M.P., 2018. In vitro antioxidant activity of olive leaf extract (*Olea europaea* L.) and its protective effect on oxidative damage in human erythrocytes. Heliyon 4. https://doi.org/10.1016/j.heliyon.2018.e00805 1–26.

Lockwandt, L., Pinela, J., Roriz, C.L., Pereira, C., Abreu, R.M.V., Calhelha, R.C., Alves, M.J., Barros, L., Bredol, M., Ferreira, I.C.F.R., 2019. Chemical features and bioactivities of cornflower (*Centaurea cyanus* L.) capitula: the blue flowers and the unexplored non-edible part. Ind. Crop. Prod. 128, 496–503. https://doi.org/10.1016/j.INDCROP.2018.11.059.

Madureira, J., Pimenta, A.I., Popescu, L., Besleaga, A., Dias, M.I., Santos, P.M.P., Melo, R., Ferreira, I.C.F.R., Cabo Verde, S., Margaça, F.M.A., 2017. Effects of gamma radiation on cork wastewater: antioxidant activity and toxicity. Chemosphere 169, 139–145. https://doi.org/10.1016/j.chemosphere.2016.11.064.

Madureira, J., Barros, L., Melo, R., Verde, S.C., Ferreira, I.C.F.R., Margaça, F.M.A., 2018. Degradation of phenolic acids by gamma radiation as model compounds of cork wastewaters. Chem. Eng. J. https://doi.org/10.1016/j.cej.2018.02.036.

Miki, M., Tamai, H., Mino, M., Yamamoto, Y., Niki, E., 1987. Free-radical chain oxidation of rat red blood cells by molecular oxygen and its inhibition by alpha-tocopherol. Arch. Biochem. Biophys. 258, 373–380.

Nunes, M.A., Costa, A.S.G., Bessada, S., Santos, J., Puga, H., Alves, R.C., Freitas, V., Oliveira, M.B.P.P., 2018. Olive pomace as a valuable source of bioactive compounds: a study regarding its lipid- and water-soluble components. Sci. Total Environ. 644, 229–236. https://doi.org/10.1016/j.scitotenv.2018.06.350.

Obied, H.K., Bedgood, D.R., Prenzler, P.D., Robards, K., 2007. Chemical screening of olive biophenol extracts by hyphenated liquid chromatography. Anal. Chim. Acta 603, 176–189. https://doi.org/10.1016/j.aca.2007.09.044.

Obied, H.K., Prenzler, P.D., Robards, K., Graham, E.H., 2008. Potent antioxidant biophenols from olive mill waste. Food Chem. 111, 171–178. https://doi.org/10.1016/j.foodchem.2008.03.058.

Olmo-García, L., Kessler, N., Neuweger, H., Wendt, K., Olmo-Peinado, J.M., Fernández-Gutiérrez, A., Baessmann, C., Carrasco-Pancorbo, A., 2018. Unravelling the distribution of secondary metabolites in *Olea europaea* L.: exhaustive characterization of eight olive-tree derived matrices by complementary platforms (LC-ESI/APCI-MS and GC-APCI-MS). Molecules 23, 1–16. https://doi.org/10.3390/molecules23102419.

Papaioannou, E.H., Patsios, S.I., Karabelas, A.J., Philippopoulos, N.A., 2013. Characterization of condensates from an indirect olive oil pomace drying process: the effect of drying temperature. J. Environ. Chem. Eng. 1, 831–837. https://doi.org/10.1016/j.jece.2013.07.025.

Pavez, I.C., Lozano-Sánchez, J., Borrás-Linares, I., Nuñez, H., Robert, P., Segura-Carretero, A., 2019. Obtaining an extract rich in phenolic compounds from olive pomace by pressurized liquid extraction. Molecules 24, 1–17.

Peralbo-Molina, Á., Priego-Capote, F., Luque De Castro, M.D., 2012. Tentative identification of phenolic compounds in olive pomace extracts using liquid chromatography-tandem mass spectrometry with a quadrupole-quadrupole-time-of-flight mass detector. J. Agric. Food Chem. 60, 11542–11550. https://doi.org/10.1021/jf302896m.

Pereira, E., Barros, L., Dueñas, M., António, A.L., Santos-Buelga, C., Ferreira, I.C.F.R., 2015. Gamma irradiation improves the extractability of phenolic compounds in *Ginkgo biloba* L. Ind. Crop. Prod. 74, 144–149. https://doi.org/10.1016/j.indcrop.2015.04.039.

Pereira, E., Barros, L., Antonio, A.L., Cabo Verde, S., Santos-Buelga, C., Ferreira, I.C.F.R., Rodrigues, P., 2017a. Is gamma radiation suitable to preserve phenolic compounds and to decontaminate mycotoxins in aromatic plants? A case-study with *Aloysia citrodora* palau. Molecules 22, 347. https://doi.org/10.3390/molecules22030347.

Pereira, E., Pimenta, A.I., Calhelha, R.C., Antonio, A.L., Barros, L., Santos-Buelga, C., Verde, S.C., Ferreira, I.C.F.R., 2017b. Infusions of gamma irradiated *Aloysia citrodora* L. and Mentha × piperita L.: effects on phenolic composition, cytotoxicity, antibacterial and virucidal activities. Ind. Crop. Prod. 97, 582–590. https://doi.org/10.1016/j.indcrop.2017.01.007.

Pinela, J., Barros, L., Dueñas, M., Carvalho, A.M., Santos-Buelga, C., Ferreira, I.C.F.R., 2012. Antioxidant activity, ascorbic acid, phenolic compounds and sugars of wild and commercial Tuberaria lignosa samples: effects of drying and oral preparation methods. Food Chem. 135, 1028–1035. https://doi.org/10.1016/j.foodchem.2012.05.038.

Pinela, J., Barreira, J.C.M., Barros, L., Verde, S.C., Antonio, A.L., Carvalho, A.M., Oliveira, M.B.P.P., Ferreira, I.C.F.R., 2016. Suitability of gamma irradiation for preserving fresh-cut watercress quality during cold storage. Food Chem. 206, 50–58. https://doi.org/10.1016/j.foodchem.2016.03.050.

Regni, L., Nasini, L., Ilarioni, L., Brunori, A., Massaccesi, L., Agnelli, A., Proietti, P., 2017. Long term amendment with fresh and composted solid olive mill waste on olive grove affects carbon sequestration by prunings, fruits, and soil. Front. Plant Sci. 7, 1–9. https://doi.org/10.3389/fpls.2016.02042.

Robles-Almazan, M., Pulido-Moran, M., Moreno-Fernandez, J., Ramirez-Tortosa, C., Rodriguez-Garcia, C., Quiles, J.L., Ramirez-Tortosa, M.C., 2018. Hydroxytyrosol: bioavailability, toxicity, and clinical applications. Food Res. Int. 105, 654–667. https://doi.org/10.1016/j.foodres.2017.11.053.

Sampedro, I., D'Annibale, A., Ocampo, J.A., Stazi, S.R., García-Romera, I., 2005. Bioconversion of olive-mill dry residue by Fusarium lateritium and subsequent impact on its phytotoxicity. Chemosphere https://doi.org/10.1016/j.chemosphere.2005.01.093.

Semeniuc, C.A., Mandrioli, M., Rodriguez-Estrada, M.T., Muste, S., Lercker, G., 2016. Thiobarbituric acid reactive substances in flavored phytosterol-enriched drinking yogurts during storage: formation and matrix interferences. Eur. Food Res. Technol. 242, 431–439. https://doi.org/10.1007/s00217-015-2554-3.

Sokovic, M., Glamoclija, J., Marina, P.D., Brkic, D., van Griensven, L.J.L.D., 2010. Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. Molecules 15, 7532–7546.

Sousa, A., Ferreira, I.C.F.R., Calhelha, R., Andrade, P.B., Valentão, P., Seabra, R., Estevinho, L., Bento, A., Pereira, J.A., 2006. Phenolics and antimicrobial activity of traditional stoned table olives "alcaparra". Bioorganic Med. Chem. 14, 8533–8538. https://doi.org/10.1016/j.bmc.2006.08.027.

Suárez, M., Romero, M.-P., Ramo, T., Macià, A., Motilva, M.-J., 2009. Methods for preparing phenolic extracts from olive cake for potential application as food antioxidants. J. Agric. Food Chem. 57, 1463–1472. https://doi.org/10.1021/jf8032254.

Suárez, M., Romero, M.-P., Motilva, M.-J., 2010. Development of a phenol-enriched olive oil with phenolic compounds from olive cake. J. Agric. Food Chem. 58, 10396–10403. https://doi.org/10.1021/jf102203x.

Sygouni, V., Pantziaros, A.G., Iakovides, I.C., Sfetsa, E., Bogdou, P.I., Christoforou, E.A., Paraskeva, C.A., 2019. Treatment of two-phase olive mill wastewater and recovery of phenolic compounds using membrane technology. Membranes (Basel) 9, 27. https://doi.org/10.3390/membranes9020027.

Vitali Čepo, D., Albahari, P., Zovko Končić, M., Radić, K., Jurmanović, S., Jug, M., 2017. Solvent extraction and chromatographic determination of polyphenols in olive pomace. Food Heal. Dis. 6, 7–14.

Whittaker, B., Watts, M.F., 2001. The influence of dose rate, ambient temperature and time on the radiation response of Harwell PMMA dosimeters. Radiat. Phys. Chem. 60, 101–110.

Wong, P.Y.Y., Kitts, D.D., 2006. Studies on the dual antioxidant and antibacterial properties of parsley (*Petroselinum crispum*) and cilantro (*Coriandrum sativum*) extracts. Food Chem. 97, 505–515. https://doi.org/10.1016/j.foodchem.2005.05.031.

Yakhlef, W., Arhab, R., Romero, C., Brenes, M., de Castro, A., Medina, E., 2018. Phenolic composition and antimicrobial activity of Algerian olive products and by-products. LWT - Food Sci. Technol. 93, 323–328. https://doi.org/10.1016/j.lwt.2018.03.044.

Yangui, T., Dhouib, A., Rhouma, A., Sayadi, S., 2009. Potential of hydroxytyrosol-rich composition from olive mill wastewater as a natural disinfectant and its effect on seeds vigour response. Food Chem. https://doi.org/10.1016/j.foodchem.2009.03.069.

Zhu, F., Cai, Y.Z., Bao, J., Corke, H., 2010. Effect of γ-irradiation on phenolic compounds in rice grain. Food Chem. 120, 74–77. https://doi.org/10.1016/j.foodchem.2009.09.072.

Zorpas, A.A., Costa, C.N., 2010. Combination of Fenton oxidation and composting for the treatment of the olive solid residue and the olive mile wastewater from the olive oil industry in Cyprus. Bioresour. Technol. 101, 7984–7987. https://doi.org/10.1016/j.biortech.2010.05.030.

Zuorro, A., 2014. Modelling of polyphenol recovery from olive pomace by response surface methodology. Int. Rev. Model. Simulations 7, 1023–1028. https://doi.org/10.15866/iremos.v7i6.4243.

Publicación 5. Phenolic Compounds from Irradiated Olive Wastes: Optimization of the Heat-Assisted Extraction Using Response Surface Methodology

Joana Madureira, Bruno Melgar, Celestino Santos-Buelga, Fernanda M. A. Margaça, Isabel C. F. R. Ferreira, Lillian Barros, Sandra Cabo Verde

Artículo publicado en *Chemosensors* (2021), 9, 231. Doi: https://doi.org/10.3390/chemosensors9080231

Resumen

En este trabajo se optimizó la extracción asistida por calor (HAE, *heat-assisted extraction*) de compuestos bioactivos de orujo de oliva mediante un diseño compuesto central circunscrito y una metodología de superficie de respuesta.

En estudios previos se había comprobado que la irradiación con rayos gamma a una dosis de 5 kGy podía mejorar al menos 2 veces la extractabilidad de los principales compuestos fenólicos del orujo de oliva (Publicación 4). Se procedió ahora a evaluar el efecto del tiempo (t), temperatura (T) y concentración de solvente (S) sobre el rendimiento en la extracción de polifenoles del orujo de oliva irradiado a 5 kGy. Para ello, utilizando cromatografía líquida de alta resolución, se cuantificaron en los extractos cuatro compuestos fenólicos bioactivos: hidroxitirosol-1-glucósido, hidroxitirosol, tirosol y ácido cafeico. Los resultados experimentales obtenidos se ajustaron a modelos teóricos para determinar las condiciones óptimas de extracción, que se establecieron en: t = 120 min, T = 85 ºC y S = 76% de etanol en agua. En estas condiciones, se obtuvo una recuperación de compuestos fenólicos totales de 19,04 ± 1,50 mg/g de peso seco de orujo y 148,88 ± 8,73 mg/g de extracto, que representaba un rendimiento de extracción de 13,7%. Estos resultados eran consistentes con el valor predicho por el modelo.

Los resultados obtenidos permiten establecer que los orujos producidos durante la extracción del aceite de oliva pueden ser fuentes valiosas de compuestos bioactivos para la recuperación de ingredientes de interés para la industria alimentaria. Así, por ejemplo, los compuestos puros aislados de los orujos podrían ser empleados como antioxidantes o conservadores en alimentos, alternativos a los aditivos alimentarios sintéticos o como ingredientes funcionales con potenciales beneficios para la salud. Se deben, sin embargo, realizar más estudios para continuar mejorando los rendimientos

de extracción y la pureza de estos y otros compuestos valiosos, como puede ser ensayar la aplicación de otras tecnologías verdes, como la extracción asistida por ultrasonidos (UAE) o por microondas (MAE).

chemosensors

MDPI

Article

Phenolic Compounds from Irradiated Olive Wastes: Optimization of the Heat-Assisted Extraction Using Response Surface Methodology

Joana Madureira [1,2,3], Bruno Melgar [2], Celestino Santos-Buelga [3,4,*], Fernanda M. A. Margaça [1], Isabel C. F. R. Ferreira [2], Lillian Barros [2] and Sandra Cabo Verde [1,*]

1 Centro de Ciências e Tecnologias Nucleares (C2TN), Instituto Superior Técnico, Universidade de Lisboa, 2695-066 Bobadela, Portugal; joanamadureira@ctn.tecnico.ulisboa.pt (J.M.); fmargaca@ctn.tecnico.ulisboa.pt (F.M.A.M.)
2 Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; bruno.melgarc@gmail.com (B.M.); iferreira@ipb.pt (I.C.F.R.F.); lillian@ipb.pt (L.B.)
3 Grupo de Investigación en Polifenoles (GIP-USAL), Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno s/n, 37007 Salamanca, Spain
4 Unidad de Excelencia Producción, Agrícola y Medioambiente (AGRIENVIRONMENT), Parque Científico, Universidad de Salamanca, 37185 Salamanca, Spain
* Correspondence: csb@usal.es (C.S.-B.); sandracv@ctn.tecnico.ulisboa.pt (S.C.V.)

Abstract: Olive pomace, an environmentally detrimental residue generated during olive oil extraction, contains bioactive compounds in demand by the food industry. To valorize this waste product a suitable yield for the extraction process is required. Heat-assisted extraction of bioactive compounds from olive pomace was optimized by a circumscribed central composite design and response surface methodology. Our previous studies indicated that irradiation could improve 2.4-fold the extractability of the main phenolic compounds from olive pomace. The effect of extraction time, temperature and solvent concentration on the yield of polyphenols from irradiated olive pomace at 5 kGy was tested. Hydroxytyrosol-1-β-glucoside, hydroxytyrosol, tyrosol and caffeic acid were quantified by High Performance Liquid Chromatography to calculate the total polyphenol content. The optimal general conditions by RSM modeling were extraction time of 120 min, temperature of 85 °C, and 76% of ethanol in water. Using these selected conditions, 19.04 ± 1.50 mg/g dry weight, 148.88 ± 8.73 mg/g extract of total polyphenols were obtained, representing a yield of 13.7%, which was consistent with the value predicted by the model. This work demonstrated the potential of residues from the olive oil industry as a suitable alternative to obtain compounds that could be used as ingredients for the food industry.

Keywords: olive pomace; bioactive compounds; ionizing radiation; heat-assisted extraction; extraction optimization

Citation: Madureira, J.; Melgar, B.; Santos-Buelga, C.; Margaça, F.M.A.; Ferreira, I.C.F.R.; Barros, L.; Cabo Verde, S. Phenolic Compounds from Irradiated Olive Wastes: Optimization of the Heat-Assisted Extraction Using Response Surface Methodology. *Chemosensors* **2021**, *9*, 231. https://doi.org/10.3390/chemosensors9080231

Academic Editor: Nicole Jaffrezic-Renault

Received: 29 June 2021
Accepted: 11 August 2021
Published: 19 August 2021

1. Introduction

The olive industry is one of the most important activities in the Mediterranean region countries, which produce 95% of the world's olive oil. This industry generates large amounts of wastes, where many potentially interesting compounds remain. Olive pomace is rich in bioactive compounds that can be divided in several classes: simple phenols (e.g., tyrosol and hydroxytyrosol) and their derivatives, benzoic and cinnamic acid derivatives (e.g., gallic acid, syringic acid, caffeic acid, p-coumaric acid, verbascoside), flavonoids (e.g., apigenin, luteolin, rutin) and secoiridoids (e.g., oleuropein and oleuropein aglycone isomers) [1–3]. Although olive oil wastes can have a negative impact to the environment when discharged without treatment, the recovery of these compounds increases the sustainability of the sector, obtaining high added-value products with low production costs and reducing

the environmental risk. In fact, society's growing demand for healthier foods challenges the scientific community to search and develop new ingredients [4]. Nowadays, with the COVID-19 pandemic situation, more efforts have to be done to develop sustainable and modern food systems. Although the lack of information in correlating the consumption of bioactive ingredients with the prevention or recovery from COVID-19 disease, it became even more imperative to have a healthier immune system which can be achieved with the supplementation of consumers' diets with vitamins, tannins, polyphenols, flavonoids, bioactive lipids and herbs [5]. In this respect, the compounds obtained from olive pomaces could be both a suitable alternative in the food industry to the use of synthetic antioxidants in order to improve the quality of foods, as well as employed in the formulation of functional foods [6].

Numerous studies have reported the extraction of bioactive compounds from olive pomace [1,3,7–9]. The extraction of these compounds can be performed by using conventional or emerging technologies, which can be advantageous since they take reduced extraction time, accelerate heat and mass transfer, increase the extraction selectivity and purity, and use safer solvents comparing to the conventional ones [10,11]. Alu'datt et al. [12] achieved the highest phenolic content extracted from olive pomace using methanol as solvent and performing the extraction for 12 h at 70 °C. Also, Vitali Čepo et al. [13] tried to optimize the extraction of hydroxytyrosol, tyrosol and oleuropein from olive pomace using simple solvent extraction and different conditions (solvent, pH, temperature and duration of extraction). The results demonstrated that the optimum conditions for phenols were 120 min at 70 °C using 60% ethanol as extraction solvent, at solvent-to-sample ratio 5:1 (v/w). Under these conditions, high recoveries of oleuropein, tyrosol and hydroxytyrosol were obtained averaging 115.14 ± 0.19, 86.05 ± 0.34 and 81.80 ± 0.41 mg/kg of fresh olive pomace, respectively. More recently, also Böhmer-Maas et al. [14] studied the extraction optimization of individual compounds from olive pomace, observing that 80% methanol, 45 °C and 180 min were the optimal conditions to recover 154.90 mg/kg dry weight of hydroxytyrosol, 1115.40 mg/kg dry weight of tyrosol, and 153.20 mg/kg dry weight of syringic acid. Zuorro [15] evaluated the effects of temperature, extraction time, solvent composition and liquid-to-solid ratio on the yield of phenolics extraction from olive pomace, showing that temperature was the most influential factor.

Ionizing radiation, a clean and environmentally friendly technology, has proven to be capable of improving phenolic extraction and antioxidant activity on industrial wastewater [16], fresh fruits such as cherry tomatoes [17], raspberries [18,19] and strawberries [20], and dried medicinal plants [21]. More recently, the potential of gamma radiation as an enhancer for phenolic compounds extraction and antioxidant capacity has been shown, with the application of low doses (5 kGy), enough to increase the extractability of the main phenolic compounds from olive pomace by 2.4-fold compared to non-irradiated samples [1].

The current study aims to explore the extraction of some phenolic compounds from olive pomace by heat-assisted extraction (HAE) using a circumscribed central composite design (CCCD) testing different conditions, namely the percentage of ethanol (0–100%), extraction times (20–120 min) and temperature (25–85 °C). The olive pomace samples used in this work were the irradiated ones for which the best results in a previous work of the authors were obtained [1]. The characterization of the individual phenolic compounds was performed, summarized and modeled by Response Surface Methodology (RSM) in order to understand the combined effects of operating variables and to maximize the responses analyzed. RSM is an efficient statistical method for optimizing processes and was originally described by Box & Wilson [22] as a statistical and mathematical tool. RSM allows a more efficient and easier presentation and interpretation of experiments compared to other methodologies. To the best of our knowledge, the optimization of tyrosol, hydroxytyrosol-1-β-glucoside and caffeic acid recovery by RSM has never been reported.

2. Materials and Methods

2.1. Standards and Reagents

HPLC-grade acetonitrile was obtained from Fisher Scientific (Lisbon, Portugal). Ethanol and formic acid were acquired from Sigma-Aldrich (St. Louis, MO, USA) and Honeywell (Charlotte, NC, USA), respectively. Caffeic acid (≥99%) was purchased from Extrasynthese (Genay, France), whereas hydroxytyrosol (≥99%) and tyrosol (≥98%) were obtained from Applichem (Darmstadt, Germany) and TCI (Tokyo, Japan), respectively. Water was treated in a Milli-Q water purification system (Merck Millipore, Burlington, MA, USA).

2.2. Olive Pomace Samples

Samples used in this work were olive pomaces collected in 2018 from UCASUL (União de Cooperativas Agrícolas do Sul, located in the Alentejo region, Alvito, Portugal) and further submitted to an irradiation-assisted extraction [1].

2.3. Irradiation Experiments

Irradiation was carried out in a Co-60 semi-industrial unit (with an activity of 187 kCi in April 2018) located at Technological Unit of Radiosterilization (UTR-IST), University of Lisbon (Portugal). Sealed bags (10 × 7 cm) containing 30 g of extracted olive pomace were irradiated at room temperature at 4.9 kGy using a dose rate of 13 kGy/h. The absorbed doses were measured by Amber Perspex routine dosimeters [23] (dose uniformity DUR = 1.2). The irradiations were performed in triplicate.

2.4. Heat-Assisted Extraction (HAE)

All irradiated olive pomace samples were immediately lyophilized (Heto CD8, Allerod, Denmark) and stored until used. Heat assisted extraction (HAE) was performed according to a methodology previously described by Pinela et al. [24], using 0.6 g of the olive pomace with 20 mL of solvent with different conditions previously defined by the RSM design (Table 1): time (t, 20 to 120 min), temperature (T, 25–85 °C) and ethanol proportion (S, 0–100%). The solid/liquid ratio (S/L) was kept constant at 30 g/L. After the extraction in a thermostatic water bath under continuous electromagnetic stirring, the samples were centrifuged (6000 rpm for 10 min at room temperature) and filtered (paper filter Whatman nº 4) and the supernatant was collected and evaporated at 35 °C under reduced pressure (rotary evaporator Büchi R-210, Flawil, Switzerland) to remove the solvent. The obtained residue was redissolved in ethanol: H_2O (20:80, v/v) and the solution was divided into two portions for HPLC-DAD and extraction yield analysis [25].

2.5. Analysed Responses

2.5.1. Extraction Yield

The residue resulting from each extraction was determined gravimetrically using crucibles. A portion (5 mL) of the redissolved extraction liquid was dried in an oven (Memmert, Schwabach, Germany) at 60 °C to evaporate the ethanol, and then at 105 °C to evaporate the water. Afterwards, the dried sample was cooled down and the residue was calculated by difference. The results were expressed in percentage (%, w/w).

Table 1. Circumscribed central composite design and experimental data for 5-level-3-factor response surface.

	Factors						Responses										
Run	X_1	X_2	X_3	X_1:t min	X_2: T °C	X_3: S %	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8	Y_9	Y_{10}	Y_{11}
1	−1	−1	−1	40.3	37.2	20.3	4.28	10.23	2.08	0.21	16.79	27.01	63.36	12.92	1.28	104.57	16.12
2	−1	−1	1	40.3	37.2	79.7	2.19	7.25	1.72	0.16	11.31	20.08	75.23	15.81	1.57	112.70	10.90
3	−1	1	−1	40.3	72.8	20.3	5.26	12.93	2.55	0.24	20.98	28.05	69.41	14.33	1.40	113.18	16.98
4	−1	1	1	40.3	72.8	79.7	3.23	11.35	2.37	0.23	17.18	23.84	88.49	18.41	1.71	132.44	13.23
5	1	−1	−1	99.7	37.2	20.3	3.98	9.61	2.09	0.19	15.87	28.34	69.60	15.13	1.40	114.47	13.81
6	1	−1	1	99.7	37.2	79.7	2.21	8.35	1.84	0.16	12.55	20.66	78.26	17.24	1.65	117.81	10.67
7	1	1	−1	99.7	72.8	20.3	5.19	12.50	2.62	0.26	20.57	27.91	66.37	13.56	1.37	109.20	18.83
8	1	1	1	99.7	72.8	79.7	3.55	12.22	2.59	0.24	18.61	25.30	91.16	19.30	1.78	137.55	13.67
9	1.68	0	0	120	55	50	4.87	12.55	2.64	0.26	20.33	29.51	73.64	14.24	1.48	118.87	17.80
10	−1.68	0	0	20	55	50	4.23	11.17	2.38	0.25	18.02	28.03	72.34	14.50	1.59	116.46	14.59
11	0	−1.68	0	70	25	50	3.46	8.81	1.79	0.16	14.22	27.38	68.68	13.51	1.25	110.82	12.63
12	0	1.68	0	70	85	50	5.04	13.22	2.91	0.25	21.42	27.23	73.01	14.94	1.37	116.55	18.49
13	0	0	−1.68	70	55	0	4.58	11.38	2.32	0.23	18.51	26.95	67.10	13.74	1.36	109.15	16.99
14	0	0	1.68	70	55	100	0.66	6.59	1.51	0.13	8.89	11.37	106.03	24.85	2.06	144.31	5.83
15	−1.68	−1.68	−1.68	20	25	0	2.86	7.13	1.42	0.14	11.54	28.68	69.86	14.28	1.33	114.15	9.96
16	−1.68	−1.68	1.68	20	25	100	0.26	1.78	0.52	0.04	2.60	9.04	62.59	18.30	1.35	91.28	2.89
17	−1.68	1.68	−1.68	20	85	0	5.09	12.43	2.46	0.23	20.21	27.84	68.06	13.44	1.26	110.60	18.28
18	−1.68	1.68	1.68	20	85	100	0.99	9.08	2.00	0.16	12.23	13.93	127.65	28.10	2.29	171.97	7.11
19	1.68	−1.68	−1.68	120	25	0	2.87	7.99	1.74	0.15	12.75	28.76	80.72	17.06	1.54	128.08	11.01
20	1.68	−1.68	1.68	120	25	100	0.32	2.57	0.67	0.05	3.61	10.97	88.03	23.02	1.72	123.74	2.93
21	1.68	1.68	−1.68	120	85	0	4.88	11.91	2.54	0.22	19.55	27.42	65.49	14.95	1.30	109.16	17.22
22	1.68	1.68	1.68	120	85	100	1.28	10.44	2.35	0.18	14.26	16.21	130.81	28.85	2.26	178.12	8.30
23	0	0	0	70	55	50	5.02	12.73	2.60	0.25	20.61	26.54	68.09	13.12	1.35	109.09	18.72
24	0	0	0	70	55	50	4.66	12.03	2.36	0.23	19.28	27.53	70.12	15.04	1.39	114.08	17.17
25	0	0	0	70	55	50	4.92	12.59	2.65	0.26	20.42	27.79	70.03	14.23	1.39	113.44	17.97
26	0	0	0	70	55	50	4.94	12.58	2.55	0.25	20.32	24.84	65.56	13.31	1.28	104.99	19.31
27	0	0	0	70	55	50	5.11	12.95	2.62	0.25	20.92	27.81	70.57	14.98	1.38	114.74	18.35
28	0	0	0	70	55	50	4.92	12.30	2.44	0.24	19.90	27.51	68.82	14.28	1.34	111.96	17.88

X_1: Time (min), X_2: Temperature (°C), X_3: Solvent concentration (% ethanol), and Y_1: Hydroxytyrosol−1-beta-glucoside (mg/g DW), Y_2: Hydroxytyrosol (mg/g DW), Y_3: Tyrosol (mg/g DW), Y_4: Caffeic acid (mg/g DW), Y_5: Total phenolic content (mg/g DW), Y_6: Hydroxytyrosol-1-beta-glucoside (mg/g Ext), Y_7: Hydroxytyrosol (mg/g Ext), Y_8: Tyrosol (mg/g Ext), Y_9: Caffeic acid (mg/g Ext), Y_{10}: Total phenolic acids (mg/g Ext), Y_{11}: Yield (%).

2.5.2. Phenolic Fingerprinting and Quantification

The phenolic fingerprinting of the extracts was determined by High Performance Liquid Chromatography (HPLC) (Prominence CBM 20-A, Shimadzu, Kyoto, Japan) with UV-DAD detector. The HPLC column was a Kinetex C18 XB-C18 (5 µm, 250 mm, 4.0 mm, Phenomenex, Torrance, CA, USA) and the detection was made at 280, 330 and 370 nm as preference wavelengths. The phytochemical molecules were analyzed using a previously described methodology [26]. Each re-dissolved solution (3 mL) was filtered through an LC filter disk (nylon filter 0.2 µm, 25 mm diameter, Whatman™, GE Healthcare, Buckinghamshire, UK). Quantitative analysis was performed using 9-level calibration curves (0.78–200 µg/mL) obtained from commercial standards of hydroxytyrosol ($y = 19203x + 8392.3$, $R^2 = 0.9999$), tyrosol ($y = 11762x - 7109.5$, $R^2 = 0.9999$) and caffeic acid ($y = 38473x - 6243.4$, $R^2 = 0.9996$). The results were expressed in mg per g of dry weight ($Y_{1–5}$) and extract ($Y_{6–10}$), and the final responses processed for all compounds were summed up to calculate total phenolic content (TPC).

2.6. Extraction Optimization by Response Surface Methodology

2.6.1. Screening Test of Factors and Level Range for Phenolic Compounds Extraction

Primary selection and evaluation of factors and levels were carried out to determine the appropriate experimental domain for the RSM design. Independent variables, which include time (t), temperature (T) and solvent percentage (S) were preliminarily tested.

2.6.2. Experimental Design

A circumscribed central composite design (CCCD) was used, consisting of 28 randomized runs with six replicates at the central point (three replicates per condition). Fixed variables in the designed experiment were defined as X_1: time (20–120 min), X_2: temperature (25–85 °C), and X_3: solvent concentration (0–100% ethanol/water). These variables were selected from a screening analysis, based on experimental data previously obtained by the research group. Each factor was tested at five different levels.

The dependent variable studied were expressed according to eleven responses (Y) format values: the total extraction yield (Y_{11}) and levels of hydroxytyrosol-1-β-glucoside (Y_1 and Y_6), hydroxytyrosol (Y_2 and Y_7), tyrosol (Y_3 and Y_8) and caffeic acid (Y_4 and Y_9) as well as the total amount resulting from the sum of the four compounds, TPC, (Y_5 and Y_{10}). To be clear, the results were expressed in mg of phenolic per g of dry weight (mg/g DW) ($Y_{1–5}$) and in mg of phenolic per g of extract residue (mg/g ext.) ($Y_{6–10}$), both used to evaluate the total phenolic purity in the extract.

The experimental data were fitted to the second-order polynomial model (Equation (1)) to obtain the regression coefficients (b) using Statgraphics Centurion XVI (StatPoint Technologies, Inc., Warrenton, VA, USA) and Design expert 12.0.1. (Stat-Ease, Inc., Minneapolis, MN, USA) software programs. The generalized second-order polynomial model used in the response surface analysis was the following:

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{j=i+1}^{k} b_{ij} X_i X_j \quad (1)$$

where Y is the dependent variable (response variable) to be modelled, b_0 is a constant coefficient (intercept); b_i, b_{ii} and b_{ij} are the coefficients of the linear, quadratic, and interactive terms, respectively; k is the number of tested variables (k = 3); X_i and X_j are the independent variables.

2.7. Statistical Analysis

The analysis of variance (ANOVA) was carried out to determine individual linear, quadratic and interaction regression coefficient as well as model significance using Statgraphics Centurion XVI software (StatPoint Technologies, Inc.), and the fitness of the polynomial equation to the responses was estimated using the coefficient of determination (R^2). The significance of all the terms of the polynomial equation was analysed statisti-

cally by computing the F value at $p < 0.05$. Design expert software was used to optimize the conditions of extraction throughout response surface methodology (RSM) with their respective 3D graphs and statistics diagnostics, such as predicted vs actual, residuals vs predicted, and run vs predicted points, which were assessed along the prior information mentioned in order to fit the model.

3. Results and Discussion

A previous study by the authors identified hydroxytyrosol, hydroxytyrosol-1-β-glucoside and tyrosol as the most abundant phenolic compounds present in olive pomace [1]. It was also verified that irradiation at 5 kGy increased the extractability of bioactive compounds from olive pomace by 2.4-fold compared to the non-irradiated ones. Based on this information, the aim of this work was to optimize the extraction of these three compounds for the 5 kGy irradiated samples. Caffeic acid was chosen to be studied in representation of phenolic acids present in olive pomace.

3.1. Single-Factor Effects for Polyphenolic Extractions

The results of 28 runs (all of them analysed in triplicate) using a circumscribed central composite design (CCCD) are given in Table 1, that includes the experimental design and the corresponding response data. The different response criteria used (yield and $Y_{1–11}$) are interesting for industrial sectors that promote the recovery of high added-value compounds from agro-industrial residues that can be used as food ingredients. The applied response criteria provide important information about the amount of olive wastes needed to achieve a certain quantity of the target compounds and their concentration in the obtained extracts.

The extraction yield ranged from 2.89 to 19.30% (Table 1). The higher value was achieved with the run 26, which combined the following conditions: t = 70 min, T = 55 °C and S = 50%. A lower yield (10.88%) was obtained by Chanioti & Tzia [27] in the ultrasound-assisted extraction of oil from olive pomace at 50 °C.

TPC (Y_5) ranged from 2.60 to 21.42 mg/g DW and the highest content was attained at the run 12. These values are higher than those reported by Böhmer-Maas et al. [14], which obtained up to 1.48 mg/g DW performing an extraction of 180 min with 80% ethanol in water solvent and at 45 °C.

3.1.1. Effect of Extraction Time on Polyphenolic Content and Extractability Yield

In order to understand the effect of time on phenolic compound extraction ($Y_{1–10}$) and yield (Y_{11}) from olive pomace, the experimental tests were carried out at different extraction times (X_1 = 20, 40.3, 70, 99.7 and 120 min), while the other two factors (X_2 = Temperature and X_3 = Solvent concentration) were adjusted according to the defined *CCCD* points presented in Table 1.

Table 2 shows the ANOVA estimated coefficients. The extractability of compounds had a small but positive trend implying that, in the range of the used extraction times, the extraction of compounds was higher when longer times were employed. Variability within the collected dataset had shown significant effect ($p < 0.05$, highlighted in green) in some of the tested phenolic compounds, but no significant repercussion on the extraction yield (grey coloured). This information is displayed at different points in the responses (Table 1) and is shown in Table 2, at the decoded optimization values section. Numerically, inflection points in the responses are mostly at the long end positive points tested (120 min), but also at some lower ones, e.g., 68 min for Y_2 and Y_5. The lowest inflection point (53 min) is located at Y_1, although this response showed no significant effect. Vitali Čepo et al. [13] and Böhmer-Maas et al. [14] also observed higher amounts of polyphenolic content and antioxidant activity in olive pomace with long extraction times, 120 min and 180 min, respectively. Furthermore, Garcia-Castello et al. [28] reported maximum total polyphenols content and antioxidant activity at 413 and 270 min, respectively, in extracts from wastes of grapefruit (*Citrus paradisi* L.)

Table 2. Statistical analysis (ANOVA) of the *CCCD* design and decoded optimization values, including response terms for building the predictive models and optimal response values for the parametric response criteria.

ANOVA Estimated Coefficients												
Term	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8	Y_9	Y_{10}	Y_{11}	
Intercept	4.75	12.28	2.53	19.81	19.56	27.29	69.50	14.02	1.40	112.22	17.56	
A-time	−0.02	0.08	0.04	0.10	0.09	0.20	1.90	0.57	0.04	2.70	−0.10	
B-Temp	0.47	1.71	0.35	2.57	2.53	0.61	5.21	0.78	0.07	6.67	1.70	
C-Solvent	−0.99	−1.11	−0.18	−2.30	−2.28	−4.22	9.38	2.71	0.17	8.04	−2.63	
AB	0.01	−0.03	0.00	−0.02	−0.02	−0.03	−1.54	−0.26	−0.02	−1.83	0.03	
AC	0.03	0.11	0.01	0.15	0.15	0.19	0.83	0.06	0.00	1.09	0.06	
BC	−0.10	0.27	0.06	0.23	0.23	0.59	5.24	0.80	0.07	6.70	−0.20	
A^2	−0.03	−0.09	−0.01	−0.12	−0.13	0.50	0.70	0.10	0.04	1.35	−0.32	
B^2	−0.14	−0.39	−0.06	−0.60	−0.59	−0.02	−0.06	0.05	−0.04	−0.07	−0.54	
C^2	−0.72	−1.11	−0.22	−2.06	−2.04	−2.90	5.51	1.85	0.10	4.56	−2.01	
R^2	0.98	0.97	0.97	0.98	0.98	0.96	0.96	0.97	0.95	0.94	0.96	
Lack of fit	0.10	0.06	0.38	0.49	0.09	0.25	0.02	0.35	0.08	0.14	0.12	

Not significant | Significant

Decoded Optimization												
Factor	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8	Y_9	Y_{10}	Y_{11}	Global
Time (min)	53	68	120	120	68	120	120	120	120	120	66	120
Temperature (°C)	84.94	84.94	84.94	72.55	84.94	81.19	84.94	84.94	84.97	84.97	84.94	84.94
Solvent (%)	26	41	45	40	36	35	1.0	1.01	1.01	1.01	28	76
Single Opt Value	5.63	14.16	2.99	0.28	22.86	30.65	127.46	28.23	2.27	174.86	20	
Global Opt Value (mg/g dry weight or mg/g extract)	3.50 ± 0.43	12.55 ± 0.99	2.75 ± 0.21	0.24 ± 0.02	19.04 ± 1.50	25.09 ± 2.45	100.77 ± 7.19	21.19 ± 1.61	1.83 ± 0.12	148.88 ± 8.73	13.70 ± 1.90	
Desirability	0.64	0.88	0.92	0.89	0.83	0.78	0.57	0.53	0.60	0.66	0.66	0.71

−1.6 | 0 | 1.6

ANOVA estimated coefficients: all numerical terms to construct second-order equations display color separated (grey: not significant, green: significant). Decoded Optimization values: separated in single response optimization values (with their respective factorial conditions exhibited numerically and colored using coded values light blue for negative extreme point −1.68 light blue to positive extreme point 1.68 dark blue), global optimization, and desirability values for each of the eleven responses. Y_1: Hydroxytyrosol-1-β-glucoside (mg/g DW), Y_2: Hydroxytyrosol (mg/g DW), Y_3: Tyrosol (mg/g DW), Y_4: Caffeic acid (mg/g DW), Y_5: Total phenolic content (mg/g DW), Y_6: Hydroxytyrosol-1-β-glucoside (mg/g Ext), Y_7: Hydroxytyrosol (mg/g Ext), Y_8: Tyrosol (mg/g Ext), Y_9: Caffeic acid (mg/g Ext), Y_{10}: Total phenolic acids (mg/g Ext), Y_{11}: Yield (%).

The range of temperature tested in this experimental design comprised five different levels (X_2 = 25, 37.2, 55, 72.8 and 85 °C). Many phenolic compounds present thermolabile characteristics and it is important to understand which temperatures are within the safe zone before occurring degradation, along with the identification of optimum recovery points.

Positive trend was also found for every analysed response with statistical significance ($p < 0.05$) (Table 2). Optimum decoded values for temperature were 85 °C in almost all responses, except for Y_4 (73 °C) and Y_6 (81 °C). It worth noting that the studied compounds seemed to be better extracted at maximum tested temperatures (85 °C). In fact, it is known that high temperatures improve the extraction efficiency [29], since it might weaken the cell membranes and hydrolyze the bonds of phenolic compounds (phenol–protein or phenol–polysaccharide) thus increasing the solubility of the compounds in the solvent [30]. The obtained results are consistent with those reported by Vitali Čepo et al. [13] who determined the higher extraction yields from olive pomace at temperatures of 70 °C and above. Also, Böhmer-Maas et al. [14] and Alu'datt et al. [12] demonstrated the higher extraction of phenolic compounds from olive pomace at higher temperatures (70 °C) using methanol as solvent.

3.1.2. Effect of Solvent Concentration on Polyphenolic Content and Extractability Yield

For solvent concentration monitoring, the whole spectrum of mixture of the two solvents were evaluated (X_3 = 0, 20.3, 50, 79.7 and 100% of ethanol in water). Besides exhibiting significant statistical differences ($p < 0.05$) in all the responses (Table 2), the employed broad ranges allowed to distinguish the maximum inflection point in the eleven different responses with strong variability among them. The results described better yield recovery on slightly polar solvents but very variable percentages within the different phenolic compounds. In fact, the optimum decoded value for the yield extraction (Y_{11}) was 28% of ethanol. The highest inflection points (100%) are located at Y_7–Y_{10}, whereas the lowest ones (26 to 45%) are located at Y_1–Y_6).

The selection of a suitable extraction solvent is one of the most important steps in assuring a successful extraction and the identification of optimal points is mandatory [31]. Thus, even using the same solvents, different percentages of combination are often reported [13,15,24,28,30], which may be due to the large diversification of polyphenolic compounds present in the different matrices. In previous studies, the ethanol percentage to obtain the optimum recoveries of compounds could also be very variable. A 60% ethanol in water was selected as optimal condition for extracting hydroxytyrosol, tyrosol and oleuropein from olive pomace by Vitali Čepo et al. [13], whereas for *Citrus paradisi* L. wastes the maximum total polyphenol content and total antioxidant activity were achieved using 20% and 50% aqueous ethanol, respectively, as extraction solvent [28]. On the other hand, for *Hibiscus sabdariffa* calyces the optimal condition to extract anthocyanins was using water (0% ethanol) [24] using HAE, probably due to the polarity of anthocyanins. Another study using methanol as solvent verified that the highest individual phenolics extracted from olive pomace was achieved at the concentration of 80% methanol [14].

Moreover, the obtained results demonstrated that the interaction between extraction temperature and ethanol concentration was statistically significant for all the studied phenolic compounds. On the other hand, the interaction between extraction time and ethanol concentration presented no significant effect for all the studied responses.

3.2. Optimization of HAE Process in Olive Pomace

3.2.1. Extracting Modelling and Analysis of Variance

In this study, a one-block experiment circumscribed central composite design (Table 2) based on RSM was used to optimize the HAE of TPC from olive pomace. For this purpose, a complete response analysis was performed with statistical determination of outliers of the raw data, followed by an ANOVA analysis (Table 2) coupled with numerical and graphical statistical diagnostics (Figures 1–5).

The increasing of the yield during HAE for longer times has been explained by several authors due to the mechanical effect of stirring coupled with the physicochemical parameters employed which causes a disruption of plant cell releasing the inner compounds embedded. Additionally, mass transfer is enhanced with small particles of the matrix analyzed due to the increment of the contact surface area between solvent and plant material [32,33].

According to the performed analysis of variance, model adjustment and the coefficients for a subsequent second order equation are shown in the first part of Table 2. As mentioned above, for the significant values of the eleven responses, the cells were colored in green, while the non-significant values were colored in grey. The coefficients $R^2 > 0.94$ for each response demonstrated the high correlation. The construction of the second order equations followed the typical notation and only significant values ($p < 0.05$, green colored) were considered. Some examples are displayed below in the Equations (2)–(4).

$$Y_1 = 4.73810 + 0.474459 * \text{Temperature} - 0.985634 * \text{Solvent} - 0.097691 * \text{Temperature} * \text{Solvent} - 0.150406 * \text{Temperature}^2 - 0.727928 * \text{Solvent}^2 \quad (2)$$

$$Y_{10} = 112.86260 + 2.70371 * \text{time} + 6.67001 * \text{Temperature} + 8.04413 * \text{Solvent} - 1.83357 * \text{time} * \text{Temperature} + 6.70288 * \text{Temperature} * \text{Solvent} + 5.33699 * \text{Solvent}^2 \quad (3)$$

$$Y_{11} = 17.46436 + 1.69988 * \text{Temperature} - 2.62870 * \text{Solvent} - 0.663910 * \text{Temperature}^2 - 2.13429 * \text{Solvent}^2 \quad (4)$$

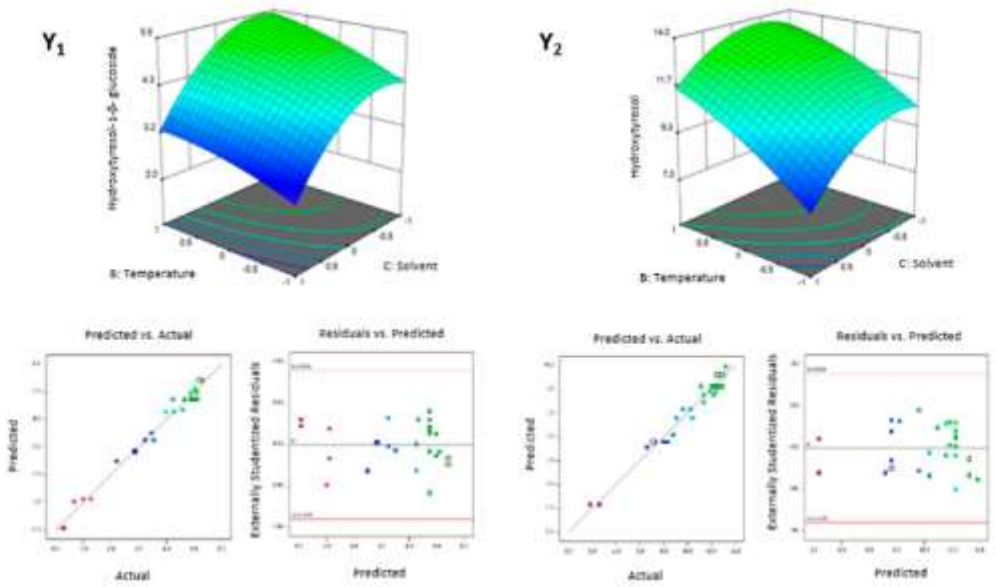


Figure 1. RSM graphs from the eleven responses with statistical diagnostic representations. Each graph displays temperature vs solvent factors, with a time factor adjusted to 0, along with two statistical diagnostics attached. On the top, the graphical representation of the adjustment between predicted vs actual points, and underneath, the graphical representation of residuals vs predicted points.

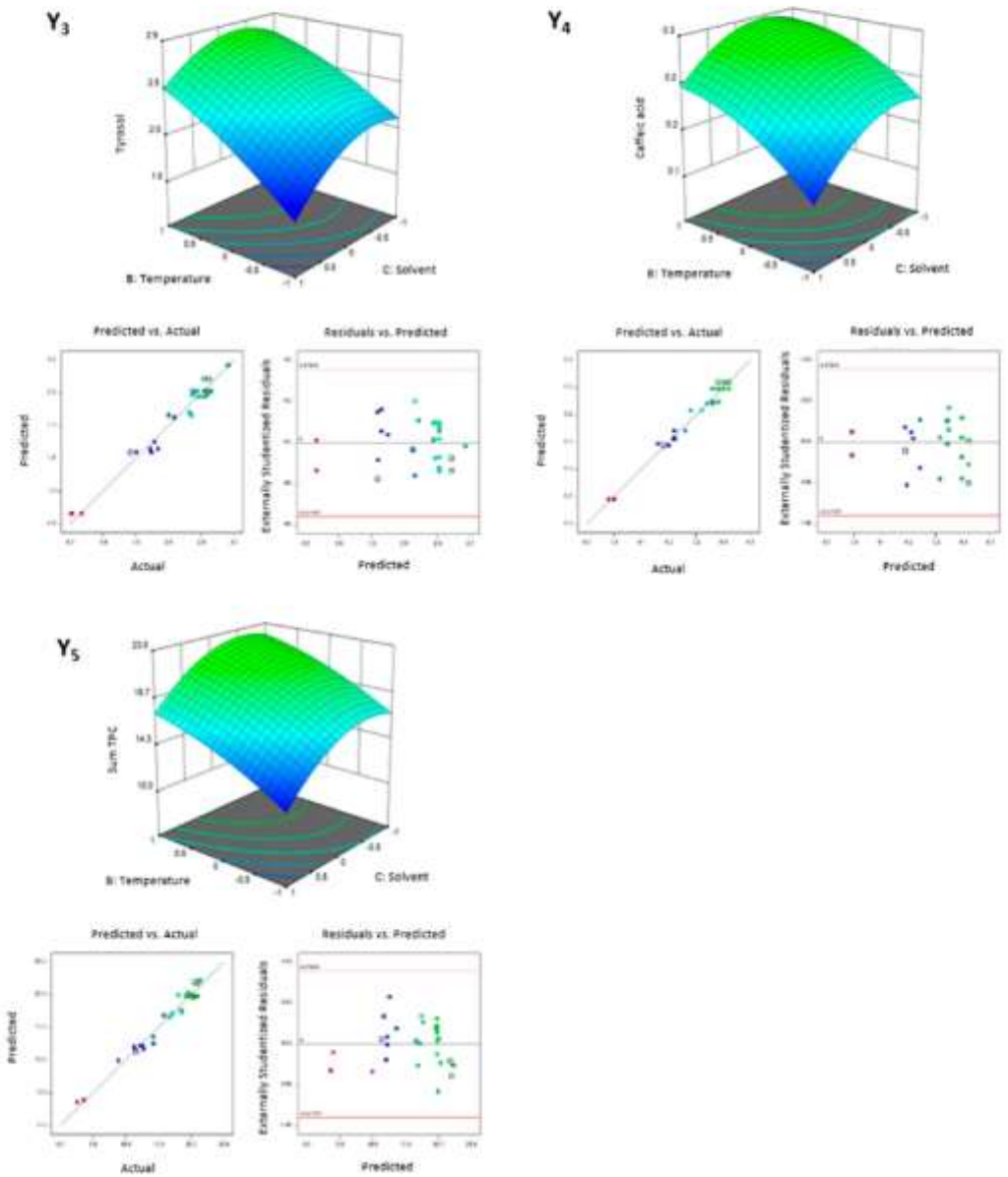


Figure 2. RSM graphs from the responses Y_3 (Tyrosol, mg/g DW), Y_4 (Caffeic acid, mg/g DW) and Y_5 (Total phenolic content, mg/g DW), with statistical diagnostic representations. Each graph displays temperature vs solvent factors, with a time factor adjusted to 0, along with two statistical diagnostics attached. On the left, the graphical representation of the adjustment between predicted vs actual points, and on the right, the graphical representation of residuals vs predicted points.

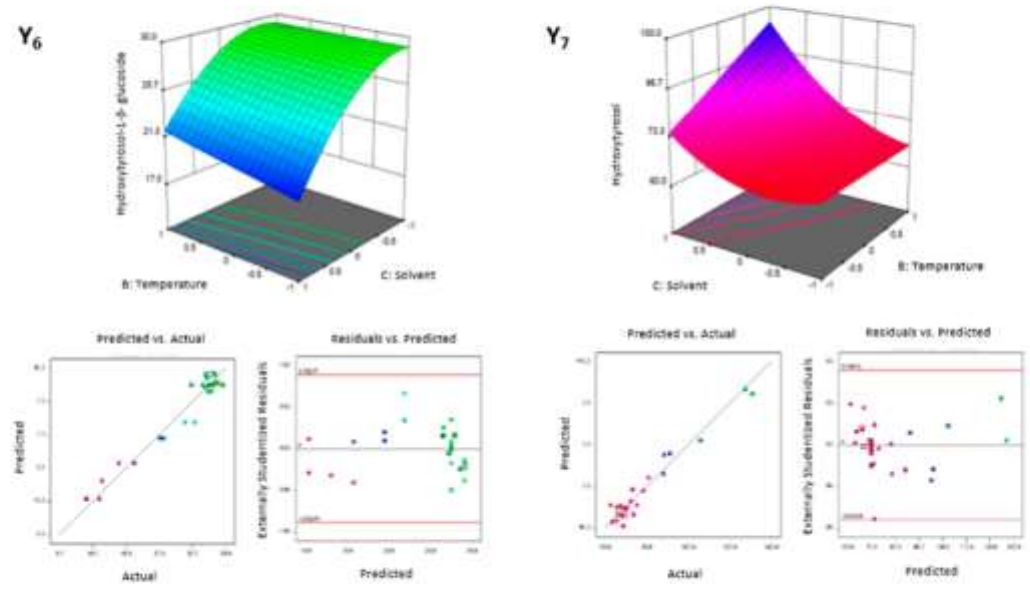


Figure 3. RSM graphs from the responses Y_6 (Hydroxytyrosol-1-beta-glucoside, mg/g Ext) and Y_7 (Hydroxytyrosol, mg/g Ext), with statistical diagnostic representations. Each graph displays temperature vs solvent factors, with a time factor adjusted to 0, along with two statistical diagnostics attached. On the left, the graphical representation of the adjustment between predicted vs actual points, and on the right, the graphical representation of residuals vs predicted points.

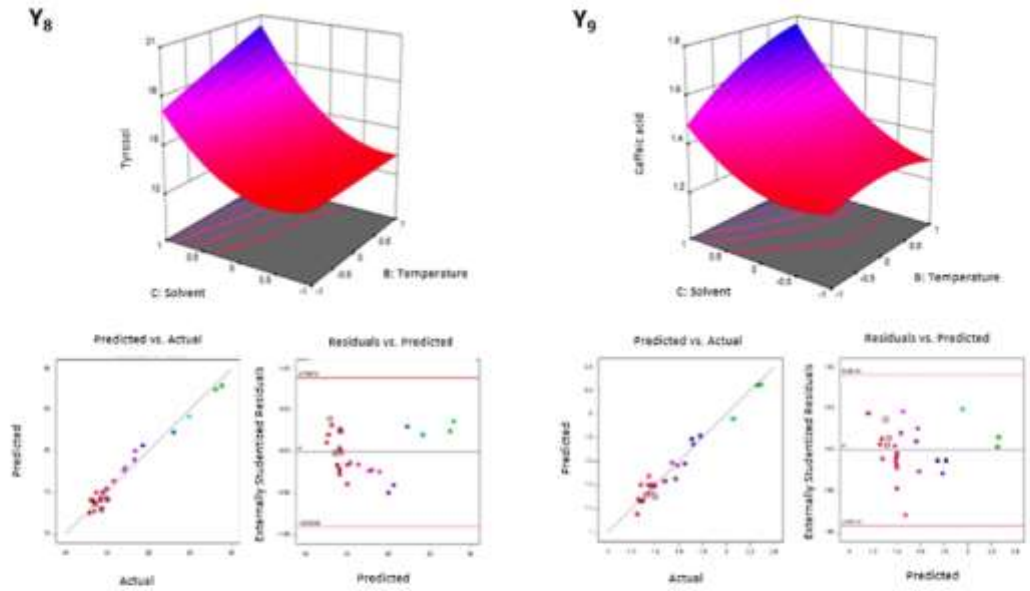


Figure 4. RSM graphs from the responses Y_8 (Tyrosol, mg/g Ext) and Y_9 (Caffeic acid, mg/g Ext), with statistical diagnostic representations. Each graph displays temperature vs solvent factors, with a time factor adjusted to 0, along with two statistical diagnostics attached. On the left, the graphical representation of the adjustment between predicted vs actual points, and on the right, the graphical representation of residuals vs predicted points.

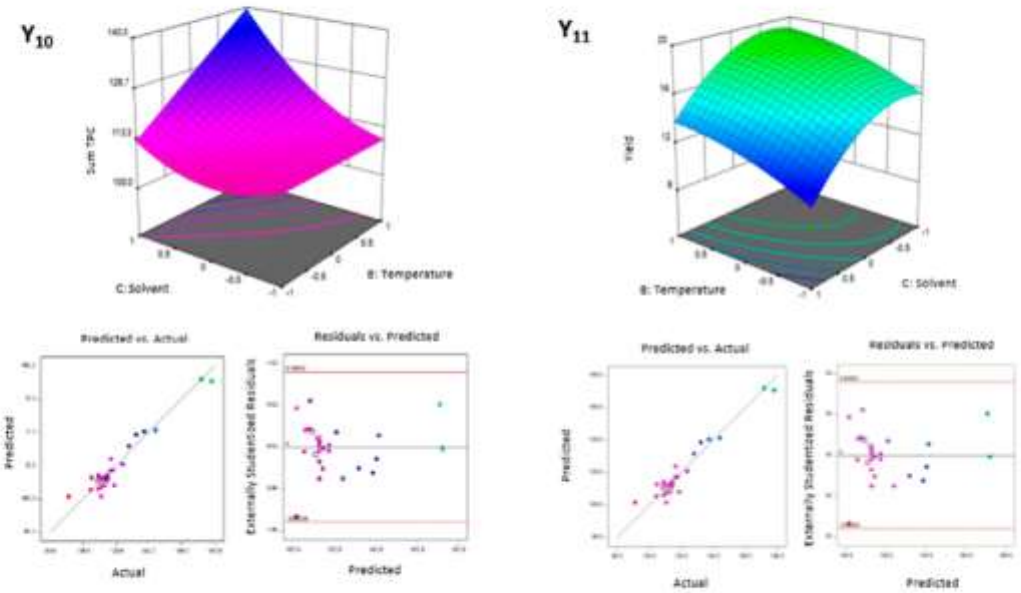


Figure 5. RSM graphs from the responses Y_{10} (Total phenolic acids, mg/g Ext) and Y_{11} (Yield, %), with statistical diagnostic representations. Each graph displays temperature vs solvent factors, with a time factor adjusted to 0, along with two statistical diagnostics attached. On the left, the graphical representation of the adjustment between predicted vs actual points, and on the right, the graphical representation of residuals vs predicted points.

3.2.2. Factorial and General Optimization

From the 28 runs (each one performed in triplicate) data acquired, the most efficient extraction conditions for maximizing each of the eleven responses using HAE were obtained by constructing 3D response surface curves with underlying contour plots (Figures 1–5) and determined by interpolation of experimental values according to the equations mentioned above.

Although ANOVA analysis becomes mandatory in understanding the patterns of the responses, 3D surface plots are the best way to visualize the effects of any independent variable on the extraction of any type of response, helping to comprehend the influence and interaction between the variables. These plots are obtained depicting two variables, temperature vs solvent, within experimental range and keeping the third variable, time, constant at zero level (Figures 1–5).

The information summarized in the eleven RSM graphs is the behavior of the responses through all the tested point, which, along with the other statistics diagnostics and mathematical equation, helps creating a net of untested points in order to give us a visual representation of data. From this representation, it becomes easy to observe that in each one of the responses, while temperature increases, the responses do it in the same manner. At the same time, it is interesting to focus on the solvent axis. Although the increments are obvious, it always presents a point of inflection that exhibits the saddle shape form on the graphs on the phenolic compound from the dry weight (Y_1–Y_5), yield response (Y_{11}) and hydroxytyrosol-1-β -glucoside from extract (Y_6). Those combinations of shapes indicate a uniform and increasing value for the factor temperature. Nevertheless, the same cannot be said for solvent factor, since this factor had the optimum point in the middle of the scale, somewhere between the factorial and central points for a big part of responses (Y_1–Y_6 and Y_{11}), while the other extract responses (Y_7–Y_{10}) highlighted at the positive factorial points analyzed. This could be explained by the polarity of the compounds as well as by yielding response. On the dry weight, other polar compounds tend to increase the interaction of the

quantified compounds, hence, different profile was found when comparing with the purity of the extract. Lastly, with their own statistics diagnostic attached, it was observed how the predicted point matched well with the actual point showing a smooth straight line. Taking into consideration the lack of fit and R^2 values in Table 2, it was possible to realize how the modeling fitted with high precision (Lack of fit < 0.05 and $R^2 > 0.94$), except in Y_7 which might be caused by an unexpected outlier in the run 16 obtaining 62.59, instead of 71.17 mg/g of extract. On the other hand, at the bottom, the residual is shown in order to describe the behavior within the runs and check that it does not exist any type of pattern which could influence the modeling.

Furthermore, a compilation of factorial terms is presented in the second part of Table 2, which takes into account the single and interaction effects, the RSM, the statistical diagnostic plots and the quadratic equation. Giving particular interest to a unique response, for example Y_2 or Y_8 (hydroxytyrosol or tyrosol), maximal optimum values of each response are displayed (14.16 mg/g DW and 28.23 mg/g extract, respectively), as well as the specific combination of the three factors time, temperature and solvent that has to be used in order to obtain the predicted values. Besides presenting the numerical values of the used decoded ranges, the cells were colored from light to strong blue to represent the coded factorial points, intending a better observation of the factorial points.

Even if the main objective of this work was to optimize the total phenolic compounds recovery from olive pomace in order to maximize the eleven responses, a combination of the three factors (time, temperature and solvent percentage) had been adjusted and displayed at the column "Global" (Table 2). Additionally, the last row of Table 2 presents the desirability values obtained from the global optimization for each response. The global factor values for maximizing the responses were obtained at 120 min (X_1), 85 °C (X_2) and 76 % of ethanol (X_3), with a global desirability of 0.71. In these conditions, it was possible to extract 25.09 ± 2.45, 100.77 ± 7.19, 21.19 ± 1.61 and 1.83 ± 0.12 mg/g of extract of hydroxytyrosol-1-β-glucoside, hydroxytyrosol, tyrosol and caffeic acid, respectively. Moreover, extraction yield (Y_{11}) of the global optimization value was 13.70 ± 1.9 %. The global results are similar to the obtained by Vitali Čepo et al. [13] which reported 120 min of time, 70 °C of temperature and 60% of ethanol as the best conditions to extract hydroxytyrosol, tyrosol and oleuropein from olive pomace. Concerning specifically the hydroxytyrosol extraction, the obtained results are higher than those found by Pavez et al. [34] using Pressurized Liquid Extraction and by Böhmer-Maas et al. [14] using maceration, where 0.258 mg/g DW and 0.154 mg/g DW, respectively, were extracted while 12.55 ± 0.99 mg/g DW were reached in the present work. Thus, olive pomace is a promising source of phenolic compounds namely hydroxytyrosol for potential use as natural preservatives.

Further studies have to be performed in order to valorize olive wastes as a source of potentially valuable food ingredients. For instance, other green technologies can be explored to maximize the extraction of bioactive compounds, such as supercritical extraction, microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) [32,35,36], or pressurized-liquid extraction (PLE) [34]. The fortification of food, especially meat [37–39], beverages [40], bakery [41–43] and dairy [44,45] products, with bioactive compounds from olive wastes not only as pure compounds but also in the form of rich extracts, was previous described due to the antioxidant ability of phenols to reduce lipid oxidation during cooking and storage or to their antimicrobial properties.

4. Conclusions

Nowadays, consumers are more interested in what they eat, thus increasing the demand for sustainable and healthier foodstuffs. The food industry tries to address this challenge developing efficient extraction methods for natural added-value ingredients from industrial by-products. In this work, a heat-assisted extraction (HAE) procedure has been optimized combining three independent variables (extraction time, temperature and solvent concentration), in order to maximize the recovery of four relevant bioactive phenolic compounds (i.e., hydroxytyrosol-1-β-glucoside, hydroxytyrosol, tyrosol and

caffeic acid) present in olive pomace, a residue generated during olive oil extraction. The achieved experimental data were fitted to theoretical models to determine the optimal extraction conditions, which were established at: t = 120 min, T = 85 °C and S = 76 % of ethanol, with an extraction yield of 13.70%, allowing to recover a total amount of 148.88 ± 8.73 mg/g extract.

This work evidenced that residues produced during olive oil extraction can be valuable sources of bioactive compounds to produce ingredients that can be used by the food industry. More studies have to be performed to further improve the recovery of these and other valuable compounds to compare with the HAE results obtained in this work, such as the application of green technologies like ultrasound-assisted extraction (UAE) or microwave-assisted extraction (MAE). The isolated pure compounds from olive wastes, as well as the obtained rich extracts can be used as preservatives in foods being suitable alternatives to synthetic food additives and/or as functional ingredients to provide consumer health benefits.

Author Contributions: Conceptualization, I.C.F.R.F., L.B. and C.S.-B.; Formal analysis, J.M.; Investigation, J.M. and B.M.; Methodology, I.C.F.R.F., L.B. and S.C.V.; Software, B.M.; Supervision, C.S.-B., L.B. and S.C.V.; Writing—original draft, J.M.; Writing—review & editing, C.S.-B., F.M.A.M., L.B. and S.C.V. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES to C2TN (UIDB/04349/2020), CIMO (UIDB/00690/2020), J.M. (SFRH/BD/136506/2018); L.B. thank the national funding by FCT, P.I., through the institutional scientific employment program-contract; and the European Regional Development Fund (ERDF) through the Regional Operational Program North 2020, within the scope of Project OliveBIOextract (NORTE-01-0247-FEDER-049865). The GIP-USAL is funded by the Strategic Research Programs for Units of Excellence (ref CLU-2018-04) and Consejería de Educación de la Junta de Castilla y León (Project SA093P20).

Acknowledgments: The authors are grateful to "UCASUL—União de Cooperativas Agrícolas do Sul" agro industrial cooperative for providing the samples, to Technological Unit of Radiosterilization (University of Lisbon) for the samples irradiation, and to Molecular Materials Synthesis Laboratory (responsible Dulce Belo, C2TN) for the solvent evaporations.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Madureira, J.; Dias, M.I.; Pinela, J.; Calhelha, R.C.; Barros, L.; Santos-Buelga, C.; Margaça, F.M.A.; Ferreira, I.C.F.R.; Cabo Verde, S. The use of gamma radiation for extractability improvement of bioactive compounds in olive oil wastes. *Sci. Total Environ.* **2020**, *727*, 138706. [CrossRef]
2. Malapert, A.; Reboul, E.; Loonis, M.; Dangles, O.; Tomao, V. Direct and Rapid Profiling of Biophenols in Olive Pomace by UHPLC-DAD-MS. *Food Anal. Methods* **2018**, *11*, 1001–1010. [CrossRef]
3. Nunes, M.A.; Costa, A.S.G.; Bessada, S.; Santos, J.; Puga, H.; Alves, R.C.; Freitas, V.; Oliveira, M.B.P.P. Olive pomace as a valuable source of bioactive compounds: A study regarding its lipid- and water-soluble components. *Sci. Total Environ.* **2018**, *644*, 229–236. [CrossRef]
4. Faustino, M.; Veiga, M.; Sousa, P.; Costa, E.M.; Silva, S.; Pintado, M. Agro-food byproducts as a new source of natural food additives. *Molecules* **2019**, *24*, 1056. [CrossRef] [PubMed]
5. Galanakis, C.M.; Aldawoud, T.M.S.; Rizou, M.; Rowan, N.J.; Ibrahim, S.A. Food Ingredients and Active Compounds against the Coronavirus Disease (COVID-19) Pandemic: A Comprehensive Review. *Foods* **2020**, *9*, 1701. [CrossRef] [PubMed]
6. De Bruno, A.; Romeo, R.; Fedele, F.L.; Sicari, A.; Piscopo, A.; Poiana, M. Antioxidant activity shown by olive pomace extracts. *J. Environ. Sci. Health Part B Pestic. Food Contam. Agric. Wastes* **2018**, *53*, 526–533. [CrossRef] [PubMed]
7. Cardoso, S.M.; Guyot, S.; Marnet, N.; Lopes-da-Silva, J.A.; Renard, C.M.G.C.; Coimbra, M.A. Characterisation of phenolic extracts from olive pulp and olive pomace by electrospray mass spectrometry. *J. Sci. Food Agric.* **2005**, *85*, 21–32. [CrossRef]
8. Fernández-Bolaños, J.; Rodríguez, G.; Rodríguez, R.; Guillén, R.; Jiménez, A. Extraction of interesting organic compounds from olive oil waste. *Grasas Aceites* **2006**, *57*, 95–106. [CrossRef]
9. Suárez, M.; Romero, M.-P.; Ramo, T.; Macià, A.; Motilva, M.-J. Methods for Preparing Phenolic Extracts from Olive Cake for Potential Application as Food Antioxidants. *J. Agric. Food Chem.* **2009**, *57*, 1463–1472. [CrossRef] [PubMed]
10. Galanakis, C.M. Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications. *Trends Food Sci. Technol.* **2012**, *26*, 68–87. [CrossRef]

11. Galanakis, C.M. Emerging technologies for the production of nutraceuticals from agricultural by-products: A viewpoint of opportunities and challenges. *Food Bioprod. Process.* **2013**, *91*, 575–579. [CrossRef]
12. Alu'datt, M.H.; Alli, I.; Ereifej, K.; Alhamad, M.; Al-Tawaha, A.R.; Rababah, T. Optimisation, characterisation and quantification of phenolic compounds in olive cake. *Food Chem.* **2010**, *123*, 117–122. [CrossRef]
13. Vitali Čepo, D.; Albahari, P.; Zovko Končić, M.; Radić, K.; Jurmanović, S.; Jug, M. Solvent Extraction and Chromatographic Determination of Polyphenols in Olive Pomace. *Food Health Dis.* **2017**, *6*, 7–14.
14. Böhmer-Maas, B.W.; Otero, D.M.; Zambiazi, R.C.; Aranha, B.C. Optimization of the extraction of phenolic compounds from olive pomace using response surface methodology. *Rev. Ceres* **2020**, *67*, 181–190. [CrossRef]
15. Zuorro, A. Modelling of polyphenol recovery from olive pomace by response surface methodology. *Int. Rev. Model. Simul.* **2014**, *7*, 1023–1028. [CrossRef]
16. Madureira, J.; Pimenta, A.I.; Popescu, L.; Besleaga, A.; Dias, M.I.; Santos, P.M.P.; Melo, R.; Ferreira, I.C.F.R.; Cabo Verde, S.; Margaça, F.M.A. Effects of gamma radiation on cork wastewater: Antioxidant activity and toxicity. *Chemosphere* **2017**, *169*, 139–145. [CrossRef]
17. Guerreiro, D.; Madureira, J.; Silva, T.; Melo, R.; Santos, P.M.P.; Ferreira, A.; Trigo, M.J.; Falcão, A.N.; Margaça, F.M.A.; Cabo Verde, S. Post-harvest treatment of cherry tomatoes by gamma radiation: Microbial and physicochemical parameters evaluation. *Innov. Food Sci. Emerg. Technol.* **2016**, *36*, 1–9. [CrossRef]
18. Cabo Verde, S.; Trigo, M.J.; Sousa, M.B.; Ferreira, A.; Ramos, A.C.; Nunes, I.; Junqueira, C.; Melo, R.; Santos, P.M.P.; Botelho, M.L. Effects of gamma radiation on raspberries: Safety and quality issues. *J. Toxicol. Environ. Health A* **2013**, *76*, 291–303. [CrossRef]
19. Elias, M.I.; Madureira, J.; Santos, P.M.P.; Carolino, M.M.; Margaça, F.M.A.; Verde, S.C. Preservation treatment of fresh raspberries by e-beam irradiation. *Innov. Food Sci. Emerg. Technol.* **2020**, *66*, 102487. [CrossRef]
20. Barkaoui, S.; Madureira, J.; Santos, P.M.P.; Margaça, F.M.A.; Miloud, N.B.; Mankai, M.; Boudhrioua, N.M.; Cabo Verde, S. Effect of Ionizing Radiation and Refrigeration on the Antioxidants of Strawberries. *Food Bioprocess Technol.* **2020**, *13*, 1516–1527. [CrossRef]
21. Pereira, E.; Barros, L.; Antonio, A.L.; Cabo Verde, S.; Santos-Buelga, C.; Ferreira, I.C.F.R.; Rodrigues, P. Is gamma radiation suitable to preserve phenolic compounds and to decontaminate mycotoxins in aromatic plants? A case-study with aloysia citrodora paláu. *Molecules* **2017**, *22*, 347. [CrossRef]
22. Box, G.E.P.; Wilson, K.B. On the Experimental Attainment of Optimum Conditions. *J. R. Stat. Soc.* **1951**, *13*, 1–45. [CrossRef]
23. Whittaker, B.; Watts, M.F. The influence of dose rate, ambient temperature and time on the radiation response of Harwell PMMA dosimeters. *Radiat. Phys. Chem.* **2001**, *60*, 101–110. [CrossRef]
24. Pinela, J.; Prieto, M.A.; Pereira, E.; Jabeur, I.; Barreiro, M.F.; Barros, L.; Ferreira, I.C.F.R. Optimization of heat- and ultrasound-assisted extraction of anthocyanins from Hibiscus sabdariffa calyces for natural food colorants. *Food Chem.* **2019**, *275*, 309–321. [CrossRef] [PubMed]
25. Primo da Silva, L.; Pereira, E.; Prieto, M.A.; Simal-Gandara, J.; Pires, T.C.S.P.; Alves, M.J.; Calhelha, R.; Barros, L.; Ferreira, I.C.F.R. Rubus ulmifolius Schott as a Novel Source of Food Colorant: Extraction optimization of coloring pigments and incorporation in a bakery product. *Molecules* **2019**, *24*, 2181. [CrossRef] [PubMed]
26. Melgar, B.; Dias, M.I.; Ciric, A.; Sokovic, M.; Garcia-Castello, E.M.; Rodriguez-Lopez, A.D.; Barros, L.; Ferreira, I.C.R.F. Bioactive characterization of Persea americana Mill. by-products: A rich source of inherent antioxidants. *Ind. Crops Prod.* **2018**, *111*, 212–218. [CrossRef]
27. Chanioti, S.; Tzia, C. Optimization of ultrasound-assisted extraction of oil from olive pomace using response surface technology: Oil recovery, unsaponifiable matter, total phenol content and antioxidant activity. *LWT Food Sci. Technol.* **2017**, *79*, 178–189. [CrossRef]
28. Garcia-Castello, E.M.; Rodriguez-Lopez, A.D.; Mayor, L.; Ballesteros, R.; Conidi, C.; Cassano, A. Optimization of conventional and ultrasound assisted extraction of flavonoids from grapefruit (*Citrus paradisi* L.) solid wastes. *LWT Food Sci. Technol.* **2015**, *64*, 1114–1122. [CrossRef]
29. Santos Felix, A.C.; Novaes, C.G.; Pires Rocha, M.; Barreto, G.E.; do Nascimento, B.B.; Giraldez Alvarez, L.D. Mixture Design and Doehlert Matrix for the Optimization of the Extraction of Phenolic Compounds from *Spondias mombin* L. Apple Bagasse Agroindustrial Residues. *Front. Chem.* **2018**, *5*, 1–8. [CrossRef]
30. Shi, J.; Yu, J.; Pohorly, J.; Young, J.C.; Bryan, M.; Wu, Y. Optimization of the extraction of polyphenols from grape seed meal by aqueous ethanol solution. *J. Food Agric. Environ.* **2003**, *1*, 42–47.
31. Belwal, T.; Chemat, F.; Venskutonis, P.R.; Cravotto, G.; Jaiswal, D.K.; Bhatt, I.D.; Devkota, H.P.; Luo, Z. Recent advances in scaling-up of non-conventional extraction techniques: Learning from successes and failures. *TrAC Trends Anal. Chem.* **2020**, *127*, 115895. [CrossRef]
32. Goldsmith, C.D.; Vuong, Q.V.; Stathopoulos, C.E.; Roach, P.D.; Scarlett, C.J. Ultrasound increases the aqueous extraction of phenolic compounds with high antioxidant activity from olive pomace. *LWT Food Sci. Technol.* **2018**, *89*, 284–290. [CrossRef]
33. Hadidi, M.; Amoli, P.I.; Jelyani, A.Z.; Kasiri, Z.; Rouhafza, A.; Ibarz, A.; Khaksar, F.B.; Tabrizi, S.T. Polysaccharides from pineapple core as a canning by-product: Extraction optimization, chemical structure, antioxidant and functional properties. *Int. J. Biol. Macromol.* **2020**, *163*, 2357–2364. [CrossRef] [PubMed]
34. Pavez, I.C.; Lozano-Sánchez, J.; Borrás-Linares, I.; Nuñez, H.; Robert, P.; Segura-Carretero, A. Obtaining an Extract Rich in Phenolic Compounds from Olive Pomace by Pressurized Liquid Extraction. *Molecules* **2019**, *24*, 3108. [CrossRef] [PubMed]

35. Irakli, M.; Chatzopoulou, P.; Ekateriniadou, L. Optimization of ultrasound-assisted extraction of phenolic compounds: Oleuropein, phenolic acids, phenolic alcohols and flavonoids from olive leaves and evaluation of its antioxidant activities. *Ind. Crops Prod.* **2018**, *124*, 382–388. [CrossRef]
36. Fu, X.; Belwal, T.; Cravotto, G.; Luo, Z. Sono-physical and sono-chemical effects of ultrasound: Primary applications in extraction and freezing operations and influence on food components. *Ultrason. Sonochem.* **2020**, *60*, 104726. [CrossRef]
37. Balzan, S.; Taticchi, A.; Cardazzo, B.; Urbani, S.; Servili, M.; Di Lecce, G.; Zabalza, I.B.; Rodriguez-Estrada, M.T.; Novelli, E.; Fasolato, L. Effect of phenols extracted from a by-product of the oil mill on the shelf-life of raw and cooked fresh pork sausages in the absence of chemical additives. *LWT Food Sci. Technol.* **2017**, *85*, 89–95. [CrossRef]
38. Chaves-López, C.; Serio, A.; Mazzarrino, G.; Martuscelli, M.; Scarpone, E.; Paparella, A. Control of household mycoflora in fermented sausages using phenolic fractions from olive mill wastewaters. *Int. J. Food Microbiol.* **2015**, *207*, 49–56. [CrossRef] [PubMed]
39. Elama, C.; Tarawa, M.; Al-Rimawi, F. Oleuropein from Olive Leaf Extract as Natural Antioxidant of Frozen Hamburger. *J. Food Sci. Eng.* **2017**, *7*, 406–412. [CrossRef]
40. Guglielmotti, M.; Passaghe, P.; Buiatti, S. Use of olive (Olea europaea L.) leaves as beer ingredient, and their influence on beer chemical composition and antioxidant activity. *J. Food Sci.* **2020**, *85*, 2278–2285. [CrossRef]
41. Cedola, A.; Cardinali, A.; D'Antuono, I.; Conte, A.; Del Nobile, M.A. Cereal foods fortified with by-products from the olive oil industry. *Food Biosci.* **2020**, *33*, 100490. [CrossRef]
42. Cedola, A.; Palermo, C.; Centonze, D.; Del Nobile, M.A.; Conte, A. Characterization and bio-accessibility evaluation of olive leaf extract-enriched "Taralli". *Foods* **2020**, *9*, 1268. [CrossRef] [PubMed]
43. Galanakis, C.M.; Tsatalas, P.; Charalambous, Z.; Galanakis, I.M. Control of microbial growth in bakery products fortified with polyphenols recovered from olive mill wastewater. *Environ. Technol. Innov.* **2018**, *10*, 1–15. [CrossRef]
44. Georgakouli, K.; Mpesios, A.; Kouretas, D.; Petrotos, K.; Mitsagga, C.; Giavasis, I.; Jamurtas, A.Z. The effects of an olive fruit polyphenol-enriched yogurt on body composition, blood redox status, physiological and metabolic parameters and yogurt microflora. *Nutrients* **2016**, *8*, 344. [CrossRef] [PubMed]
45. Palmeri, R.; Parafati, L.; Trippa, D.; Siracusa, L.; Arena, E.; Restuccia, C.; Fallico, B. Addition of olive leaf extract (OLE) for producing fortified fresh pasteurized milk with an extended shelf life. *Antioxidants* **2019**, *8*, 255. [CrossRef] [PubMed]

Publicación 6. Ultrasound-assisted extraction of hydroxytyrosol and tyrosol from olive pomace treated by gamma radiation: process optimization and bioactivity assessment

Joana Madureira, Bianca Albuquerque, Maria Inês Dias, José Pinela, Ricardo C. Calhelha, Celestino Santos-Buelga, Fernanda M.A. Margaça, Isabel C.F.R. Ferreira, Sandra Cabo Verde, Lillian Barros

Artículo publicado en *Food & Function* (2023), 14, 3038. Doi: https://doi.org/10.1039/D2FO03607J

Resumen

En este trabajo, se utilizó la extracción asistida por ultrasonidos (UAE, *ultrasound-asssited extraction*) para recuperar hidroxitirosol y tirosol de orujo de oliva irradiado.

El proceso de extracción se optimizó utilizando la metodología de superficie de respuesta, con el tiempo de tratamiento (t), la concentración de etanol (S) y la potencia ultrasónica (P) como variables independientes combinadas. Las mayores cantidades de hidroxitirosol (36 ± 2 mg/g de extracto) y tirosol (14 ± 1 mg/g de extracto) se obtuvieron después de 28 min de sonicación a 490 W utilizando etanol al 7,3% como solvente. En estas condiciones, se logró un rendimiento de extracción de 30 ± 2%.

Se evaluó, asimismo, la bioactividad del extracto obtenido en las condiciones de UAE optimizadas y se comparó con la de un extracto obtenido bajo condiciones de extracción asistida por calor (HAE) optimizadas anteriormente (Publicación 5). En comparación con la HAE, la UAE redujo el tiempo de extracción y el consumo de solvente, y también condujo a mayores rendimientos de extracción (30%, frente al 13,7%). A pesar de ello, el extracto de HAE presentó mayor actividad antioxidante requiriéndose concentraciones de extracto más bajas para inhibir la formación de TBARS y la hemólisis oxidativa, así como actividades antiinflamatoria y antibacteriana más elevadas, aunque no demostró potencial antifúngico contra *Candida albicans*. El extracto de HAE también mostró mayor efecto citotóxico frente en una línea celular de adenocarcinoma de mama (MCF-7) y mayor potencial antidiabético, evaluado por su capacidad para inhibir la α-glucosidasa, que el extracto obtenido por UAE.

Los resultados obtenidos permiten seguir profundizando en la eficacia de distintos procedimientos de extracción para la obtención de componentes bioactivos a partir de subproductos del proceso de producción de aceite de oliva, con vistas a proporcionar

información útil a la industria de cara al desarrollo de nuevos ingredientes, que pueden representar una alternativa a los aditivos sintéticos o ser empleados como nutracéuticos, cosmecéuticos o en la formulación de alimentos funcionales, contribuyendo a la sostenibilidad tanto del sector agro-industrial sector como del medio ambiente.

Food & Function

ROYAL SOCIETY OF CHEMISTRY

PAPER

Check for updates

Cite this: Food Funct., 2023, 14, 3038

Ultrasound-assisted extraction of hydroxytyrosol and tyrosol from olive pomace treated by gamma radiation: process optimization and bioactivity assessment

Joana Madureira, [a,b,c,d] Bianca Albuquerque, [b,c] Maria Inês Dias, [b,c] José Pinela, [b,c] Ricardo C. Calhelha, [b,c] Celestino Santos-Buelga, [d,e] Fernanda M. A. Margaça, [a] Isabel C. F. R. Ferreira, [b] Sandra Cabo Verde *[a] and Lillian Barros *[b,c]

Received 22nd November 2022,
Accepted 25th February 2023

DOI: 10.1039/d2fo03607j

rsc.li/food-function

Ultrasound-assisted extraction (UAE) was used to recover hydroxytyrosol and tyrosol from olive pomace, a residue generated by the olive oil industry. The extraction process was optimized using response surface methodology (RSM), with processing time, ethanol concentration and ultrasonic power as the combined independent variables. The highest amounts of hydroxytyrosol (36 ± 2 mg g^{-1} of extract) and tyrosol (14 ± 1 mg g^{-1} of extract) were obtained after 28 min of sonication at 490 W using 7.3% ethanol as the solvent. Under these global conditions, an extraction yield of 30 ± 2% was achieved. The bioactivity of the extract obtained under optimized UAE was evaluated and compared with that of an extract obtained under optimal heat-assisted extraction (HAE) conditions in a previous work of the authors. Compared to HAE, UAE reduced the extraction time and the solvent consumption, and also led to higher extraction yields (HAE yield was 13.7%). Despite this, HAE extract presented higher antioxidant, antidiabetic, anti-inflammatory and antibacterial activities and no antifungal potential against *C. albicans*. Furthermore, HAE extract also showed higher cytotoxic effects against the breast adenocarcinoma (MCF-7) cell line. These findings provide useful information for the food and pharmaceutical industries in developing new bioactive ingredients, which may represent a sustainable alternative to synthetic preservatives and/or additives.

1. Introduction

Olive pomace, although an environmentally harmful waste generated during olive oil extraction, contains considerable amounts of phenolic compounds such as hydroxytyrosol and tyrosol, oleuropein and its derivatives, phenolic acids and flavonoids[1–4] with biological properties that can have a positive impact on human health.[5–7] In recent years, the demand for natural antioxidants as safe alternatives to the synthetic ones has increased, in part due to the growing interest of consumers in healthier food. In this regard, olive wastes could be considered valuable sources of natural bioactive compounds to be added to foods as natural additives and supplements.

The development of efficient extraction processes to recover these compounds from olive pomace is an important challenge in order to obtain added-value products, maintaining the health and sustainability of the environment and industries. There are different methods for extracting phenolic compounds from olive pomace. The most used and simplest technique for this purpose is maceration; however, some emerging technologies have been developed to reduce the extraction time and the volume of solvents and to increase the extraction selectivity and purity.[8] These emerging technologies include superheated liquid extraction (SLE),[9] ultrasound-assisted extraction (UAE),[10,11] microwave-assisted extraction (MAE),[12] pressurized liquid extraction (PLE),[13] supercritical fluid extrac-

[a]Centro de Ciências e Tecnologias Nucleares (C²TN), Instituto Superior Técnico, Universidade de Lisboa, E.N. 10 ao km 139.7, 2695-066 Bobadela LRS, Portugal. E-mail: sandracv@ctn.tecnico.ulisboa.pt
[b]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal. E-mail: lillian@ipb.pt
[c]Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[d]Grupo de Investigación en Polifenoles (GIP-USAL), Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno s/n, 37007 Salamanca, Spain
[e]Unidad de Excelencia Producción, Agrícola y Medioambiente (AGRIENVIRONMENT), Parque Científico, Universidad de Salamanca, 37185 Salamanca, Spain

tion (SFE)[14] and multi-frequency multimode modulated ultrasonic technique.[4] Among these techniques, UAE is considered the simplest and most cost-effective one to use at both laboratory and industrial scale, although the use of high power levels can lead to the degradation of some compounds.

Ionizing radiation is a clean and environmentally friendly technology, that has been proved to improve phenolic extraction from and the antioxidant activity of industrial wastewater,[15] fresh fruits such as cherry tomatoes,[16] raspberries[17,18] and strawberries,[19] and dried medicinal/aromatic plants.[20] More recently, the potential of 5 kGy gamma radiation as an enhancer for phenolic compound extraction and the antioxidant capacity of olive pomace extracts was demonstrated, increasing the extractability of the main phenolic compounds by 2.4-fold in comparison with non-irradiated samples.[2]

Response surface methodology (RSM) has been proposed to provide information regarding the optimal combination of extraction factors to obtain natural antioxidants from olive pomace, using different extraction methods, such as heat-assisted extraction (HAE),[21,22] PLE[13] and UAE,[10,11,23] In fact, Goldsmith *et al.* (2018)[10] demonstrated that UAE increased the extraction of total phenolic compounds by 24% and the HPLC peak areas by 20.4%, whereas the antioxidant activity was increased by 11%. The current study aims to explore the efficiency of UAE for extracting phenylethanoids from olive pomace using a circumscribed central composite design (CCCD) testing different conditions, namely the percentage of ethanol (0–100%), extraction times (2–40 min) and ultrasonic power (5–500 W). The olive pomace samples used in this work were irradiated ones for which the best results for improving bioactive extractability were obtained in a previous work of the authors.[2] The extraction yields of the individual hydroxytyrosol and tyrosol were obtained, summarized and modeled by RSM, in order to understand the combined effects of the operating variables and to maximize the responses analyzed. Furthermore, the biological properties (antioxidant, antimicrobial, antidiabetic and anti-inflammatory activities and cytotoxicity) of olive pomace extracts obtained under UAE optimized conditions were evaluated, and compared with those of the extracts obtained under optimized conditions.[21] As far as the authors know, this is the first time that a comprehensive study of the bioactivity has been performed comparing both processes for extracting bioactive compounds from olive pomace, in the hope that the results can contribute to the development of new ingredients to be used by the food industry.

2. Materials and methods

2.1. Olive pomace samples

The samples used in this work were olive pomaces collected in November 2020 from UCASUL (União de Cooperativas Agrícolas do Sul, located in the Alentejo region, Portugal) and further submitted to a gamma radiation treatment as described by Madureira *et al.* (2020).[2]

2.2. Irradiation experiments

Irradiation was carried out in a Co-60 semi-industrial unit (with an activity of 126 kCi in March 2021) located at the Technological Unit of Radiosterilization (UTR-IST), University of Lisbon (Portugal). Sealed bags (20 cm × 10 cm) containing 100 g of extracted olive pomace were irradiated at room temperature at 5.2 ± 0.2 kGy using a dose rate of 10.4 kGy h^{-1}. The absorbed doses were measured using Amber Perspex routine dosimeters[24] (dose uniformity DUR = 1.07). The irradiations were performed in triplicate.

Fig. 1 presents a schematic diagram of the different steps carried out for the optimization of the phenolic compound extraction from irradiated olive pomace.

2.3. Experimental design for extraction optimization

A five-level circumscribed central composite design (CCCD) coupled with RSM was implemented to optimize the extraction of phenylethanoids from olive pomace. The coded and natural values of the independent variables X_A (time: t, min), X_B (power: P, W), and X_C (solvent proportion: S, % ethanol, v/v) are presented in Table 1. The 28 experimental points of the

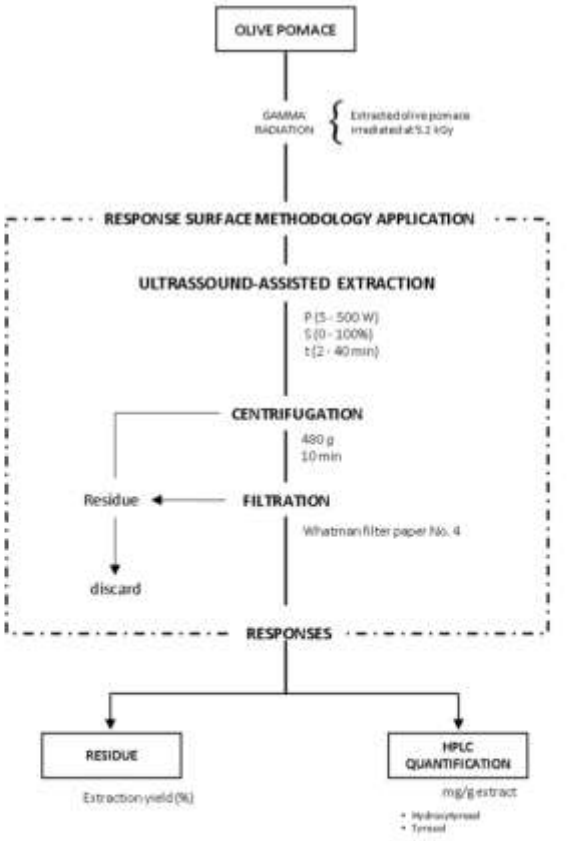


Fig. 1 Diagram of the different steps carried out for the extraction optimization of the phenolic compounds from irradiated olive pomace.

Table 1 Natural and coded values of the independent variables used in the 5-level central composite designs (CCD) implemented for optimization of ultrasound-assisted extraction using response surface methodology (RSM)

	Natural values		
Coded values (α)	Time X_A (min)	Power X_B (W)	Solvent proportion X_C (%, v/v)
−1.68	2	5	0
−1	9.7	105.3	20.3
0	21	252.5	50
1	32.3	399.7	79.7
1.68	40	500	100

CCCD design shown in Table 2 were generated using Design-Expert software, version 11 (Stat-Ease, Inc., Minneapolis, USA) by entering factor ranges in terms of alphas (α = −1.68, −1, 0, 1, 1.68), and included 6 replicates at the central point (three replicates per condition). The experimental runs were randomized to minimize the effects of unexpected variability in the observed responses.

2.4. Ultrasound-assisted extraction (UAE)

All irradiated olive pomace samples were immediately lyophilized (Heto CD8, Allerod, Denmark) and stored in dark conditions at room temperature until used. The UAE was performed using an ultrasonic system (ultrasonic homogenizer, model CY-500, Optic Ivymen Systen, Barcelona, Spain) equipped with a titanium probe and a connector for the sample temperature control, according to a methodology previously described by Albuquerque *et al.* (2020).[25] Olive pomace (0.9 g) was placed in 30 mL of solvent with different conditions previously defined by the RSM design (Table 1): time (t, 2–40 min), power (P, 5–500 W) and ethanol proportion (S, 0–100%). These experimental range conditions were selected from several screening analyses, based on experimental data previously obtained by the research group. The solid/liquid ratio was kept constant at 30 g L^{-1}, as well as temperature (T, 30–35 °C; a cold-water bath was used to control the temperature). After the extraction, the samples were centrifuged (480g for 10 min at room temperature) and filtered (paper filter; Whatman no. 4) and the supernatant was collected.

2.5. Analyzed responses

2.5.1. Extraction yield. The residue resulting from each extraction was determined gravimetrically using crucibles by the evaporation of 5 mL of supernatant (extract solution) at 100 °C for 24/48 h. The results are expressed as percentages (%, w/w).

2.5.2. Chromatographic analysis of phenolic compounds. A portion (1.5 mL) of each extract solution was filtered through 0.22 μm disposable syringe filters and analysed by HPLC–

Table 2 Experimental results obtained under the extraction conditions defined in the circumscribed central composite design (CCCD) matrix for extraction yield and contents of hydroxytyrosol (HYD), tyrosol (TYR) and both phenylethanoids (PhC) as a function of the extraction method. The natural values of the independent variables X_A (time), X_B (power) and X_C (solvent proportion) are presented in Table 1

	CCCD experimental design				Phenolic content by HPLC		
Run	X_A: t (min)	X_B: P (W)	X_C: S (%)	Residue Yield (%)	HYD (mg g^{-1} ext)	TYR (mg g^{-1} ext)	PhC (mg g^{-1} ext)
1	9.7	105.3	20.3	13.15	28 ± 1	9.9 ± 0.5	37 ± 2
2	9.7	105.3	20.3	11.46	28 ± 1	10.1 ± 0.3	38 ± 1
3	32.3	105.3	20.3	15.53	27 ± 2	9.6 ± 0.5	36 ± 2
4	32.3	105.3	20.3	12.61	27 ± 1	9.7 ± 0.3	37 ± 1
5	9.7	399.7	20.3	22.30	33 ± 1	11.8 ± 0.3	44 ± 1
6	9.7	399.7	20.3	24.14	32 ± 2	11.8 ± 0.5	44 ± 2
7	32.3	399.7	20.3	24.86	33.2 ± 0.5	12.3 ± 0.3	45 ± 1
8	32.3	399.7	20.3	23.32	33 ± 1	12.4 ± 0.1	45 ± 1
9	9.7	105.3	79.7	2.91	22 ± 1	10.9 ± 0.2	32 ± 1
10	9.7	105.3	79.7	1.66	24 ± 1	12.2 ± 0.5	36 ± 2
11	32.3	105.3	79.7	5.85	16.6 ± 0.1	8.03 ± 0.22	24.7 ± 0.3
12	32.3	105.3	79.7	2.27	18.4 ± 0.1	9.20 ± 0.03	27.6 ± 0.2
13	9.7	399.7	79.7	13.54	14.8 ± 0.3	8.1 ± 0.2	22.9 ± 0.5
14	9.7	399.7	79.7	11.14	16.6 ± 0.3	8. ± 0.1	25.2 ± 0.2
15	32.3	399.7	79.7	17.51	18.2 ± 0.6	9.53 ± 0.01	28 ± 1
16	32.3	399.7	79.7	16.96	16.4 ± 0.3	8.7 ± 0.1	25.04 ± 0.19
17	2	252.5	50	3.85	54 ± 1	21.2 ± 0.1	75 ± 1
18	40	252.5	50	15.15	27.8 ± 0.3	10.4 ± 0.4	38 ± 1
19	21	5	50	5.05	29.7 ± 0.3	12.9 ± 0.4	43 ± 1
20	21	500	50	23.34	26.9 ± 0.2	10.3 ± 0.4	37.1 ± 0.2
21	21	252.5	0	21.12	34 ± 5	11 ± 2	46 ± 7
22	21	252.5	100	0.93	5.9 ± 0.4	4.5 ± 0.4	10.5 ± 0.8
23	21	252.5	50	16.96	25.8 ± 0.1	10.7 ± 0.1	36.5 0.2
24	21	252.5	50	12.26	28.6 ± 0.5	11.5 ± 0.3	40 ± 1
25	21	252.5	50	15.18	24.7 ± 0.3	9.9 ± 0.2	34.6 ± 0.1
26	21	252.5	50	12.50	25.7 ± 0.3	10.3 ± 0.4	35.94 ± 0.04
27	21	252.5	50	19.14	24.89 ± 0.04	10.9 ± 0.2	35.8 ± 0.3
28	21	252.5	50	14.56	25.35 ± 0.08	10.80 ± 0.05	36.15 ± 0.03

DAD–ESI/MS^n (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA) using the conditions described by Bessada *et al.* (2016).[26] Quantitative analysis was performed from the peak areas recorded at 280 nm using 9-level calibration curves (1.25–80 μg mL^{-1}) obtained from commercial standards of hydroxytyrosol (y = 124154x + 17 393, R^2 = 0.999, LOD (limit of detection) = 1.22 μg mL^{-1}; LOQ (limit of quantification) = 3.68 μg mL^{-1}) and tyrosol (y = 91708x − 9398.5, R^2 = 0.999, LOD = 0.91 μg mL^{-1}; LOQ = 2.77 μg mL^{-1}). The results are expressed as mg per g of extract, and the final responses processed for all compounds were summed up to calculate phenylethanoid content (PhC).

2.6. Extraction optimization by response surface methodology

2.6.1. Response criteria and mathematical modelling. The extraction yield and levels of hydroxytyrosol (HYD) and tyrosol (TYR), as well as the total amount resulting from the sum of both compounds (TPC), were the four response variables considered to optimize the recovery of phenolic compounds from olive pomace.

The response surface models were fitted to the second-order polynomial model (eqn (1)):

$$Y = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{\substack{i=1 \\ j>1}}^{n-1} b_{ij} X_i X_j + \sum_{i=1}^{n} b_{ii} X_i^2 \qquad (1)$$

where Y is the dependent (response) variable to be modelled; X_i and X_j are the independent variables; b_0 is a constant coefficient (intercept); b_i, b_{ii} and b_{ij} are the coefficients of the linear, quadratic, and interactive terms, respectively; and n is the number of tested variables.

2.6.2. Fitting procedures and statistical analysis. Fitting procedures, coefficient estimates and statistical calculations were performed using Design-Expert software, version 11. The analyses of variance (ANOVA) was used to determine the regression coefficients and to assess the significance of the data. The coefficient of determination (R^2) and the adjusted coefficient of determination (R^2_{adj}), interpreted as the proportion of variability of the dependent variable explained by the model,[25] were used to estimate the fitness of the polynomial equation to the response. Only the statistically significant terms (p-value < 0.05) were used to fit the mathematical models. The significance of all the terms of the polynomial equations was analyzed statistically by computing the F-value at p < 0.05. The statistic lack of fit was used to evaluate the adequacy of the models. This test shows whether the model adequately describes the functional relationship between the independent variables and the obtained response. Thus, the lack of fit should be non-significant (p > 0.05). The software was also used to generate the response surface graphs.

2.7. Evaluation of the bioactive properties

The olive pomace extract obtained under the optimal conditions using UAE, as described in section 2.6, was used for evaluating the bioactive properties described below. This UAE-obtained extract was also compared with the olive pomace extract from the optimal conditions obtained using HAE.[21]

2.7.1. Antioxidant assessment. The antioxidant capacity of olive pomace was measured through the inhibition of lipid peroxidation by the decrease in thiobarbituric acid reactive substance (TBARS) formation and the oxidative hemolysis (OxHLIA) inhibition assays. Trolox was used as a positive control.

The TBARS assay measures the extract's capacity to inhibit the formation of malondialdehyde and other TBARS and was performed using porcine brain tissues as an oxidizable substrate, following the procedure previously described by Mandim *et al.* (2020).[27] The olive pomace extracts were re-dissolved in water to obtain a stock solution at 8 mg mL^{-1}, which was further diluted to obtain the range of concentrations tested (0.078–1 mg mL^{-1}). The results were expressed as the extract concentration (IC_{50}, μg mL^{-1}) that causes 50% inhibition of the oxidation process.

The OxHLIA assay measures the extract's capacity to inhibit the oxidative hemolysis and was performed using erythrocytes isolated from sheep blood collected from healthy animals, following the procedure previously described by Mandim *et al.* (2020).[27] The olive pomace extracts were re-dissolved in PBS to obtain a stock solution at 8 mg mL^{-1}, then diluted to achieve different solutions ranging from 0.078 to 0.25 mg mL^{-1}. The results were expressed as the extract concentration (IC_{50}, μg mL^{-1}) required to maintain the integrity of 50% of the erythrocyte population after 60 and 120 min.

2.7.2. Antimicrobial activity. The antimicrobial activity was assessed using three Gram-negative bacteria (*Escherichia coli* (ATCC 8739™), *Pseudomonas fluorescens* (ATCC 13525™) and *Salmonella enterica* serotype Typhimurium (ATCC 14028™)), three Gram-positive bacteria (*Staphylococcus aureus* (ATCC 6538™), *Bacillus cereus* (SSI C1/1) and *Listeria monocytogenes* (ATCC 19111™)) and a fungus (*Candida albicans* (ATCC 10231™)). The bacterial or fungal suspension was adjusted with sterile saline solution to a concentration of 1 × 10^5 CFU mL^{-1}. The extracts of olive pomace were dissolved in 10% DMSO in Tryptic Soy Broth (TSB) medium at a final concentration of 300 mg mL^{-1}. Then, several concentrations (12.5–100 mg mL^{-1}) were prepared directly in the well and 100 μL of the respective inoculum added (1 × 10^4 CFU/well). The minimum inhibitory (MIC) and minimum bactericidal (MBC) or minimum fungicidal (MFC) concentrations were determined by the microdilution method.[28] Streptomycin and ketoconazole were used as a positive control and 3% DMSO was used as negative control. Samples were tested in triplicate and each experiment was repeated three times.

2.7.3. Antidiabetic activity. The antidiabetic activity of olive pomace extracts was assessed using two different enzymes: α-amylase and α-glycosidase.

The α-amylase inhibitory activity of olive pomace extracts was measured following the method of Chen *et al.* (2020).[29] Firstly, 20.0 μL of olive pomace extracts with different concentrations (12.5–200 mg mL^{-1}, prepared in 0.1 M phosphate-

buffered saline (PBS, pH 6.9)) and 20.0 μL of α-amylase solution (1.0 U mL^{-1} in 0.1 M PBS) were mixed in a 1.5 mL centrifuge tube and incubated at 37 °C for 10 min. Then, 40.0 μL of starch solution (0.5%, w/v, in 0.1 M PBS) was added and reacted at 37 °C for another 10 min. After this, 80.0 μL of 3,5-dinitrosalicylic acid (DNS) reagent was added and the mixture was incubated in boiling water for 5 min to terminate the reaction. The absorbance was measured at 540 nm using a microplate reader (EZ Read 2000, Biochrom, Cambridge, UK). Acarbose was used as the positive control and individual blanks were prepared. The inhibition rate of α-amylase was calculated according to the following formula: α-amylase inhibition (%) = $[1 - (Abs_1 - Abs_2)/Abs_0] \times 100$, where Abs_0 is the absorbance of a mixture containing α-amylase and starch solution, Abs_1 was the absorbance of mixture containing α-amylase, starch solution and extract; and Abs_2 is the absorbance of a mixture containing extract and starch solution. The assay was performed in triplicate.

The capacity of olive pomace extracts to inhibit α-glycosidase was measured based on the methodology previously described by Silva *et al.* (2020).[30] The assay was conducted in a 96-well microplate with a reaction mixture containing 50 μL of olive pomace extract at different concentrations (3.125–100 mg mL^{-1}, prepared in 0.1 M phosphate-buffered saline (PBS, pH 6.9)) and 50 μL of yeast α-glucosidase (2 U mL^{-1} in 0.1 M PBS). The reaction mixture was then incubated for 10 min, followed by the addition of 50 μL of substrate (5 mM, *p*-nitrophenyl-α-D-glucopyranoside prepared in PBS). After 20 min of incubation at 37 °C, the release of *p*-nitrophenol was measured at 405 nm using a microplate reader (EZ Read 2000, Biochrom, Cambridge, UK). Individual blanks and a positive control (acarbose) were prepared. The results were expressed as IC_{50} values (mg mL^{-1}), which were calculated from the inhibition percentage values achieved using the formula: α-glycosidase inhibition (%) = $[(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$. The assay was performed in triplicate.

2.7.4. Cytotoxicity. The cytotoxic activity of olive pomace extracts (6.25–400 μg mL^{-1} in water) was evaluated using the sulforhodamine B assay, according to the method previously described.[31] Four human tumor cell lines (AGS (gastric adenocarcinoma), CaCo-2 (colorectal adenocarcinoma), MCF-7 (breast adenocarcinoma), and NCI-H460 (lung carcinoma)) and two non-tumor cell lines (Vero (African green monkey kidney) and PLP2 (primary pig liver culture)) were used. Ellipticine was used as a positive control. The absorbance was read at 540 nm in the Biotek ELX800 microplate reader (Bio-Tek Instruments, Inc., Winooski, VT, USA) and the results were expressed in terms of the concentration of the extract with the ability to inhibit cell growth by 50% – GI_{50} (μg mL^{-1}).

2.7.5. Anti-inflammatory activity. The capacity of olive pomace extracts (6.25–400 μg mL^{-1} in water) to inhibit the lipopolysaccharide (LPS)-induced nitric oxide (NO) production by a murine macrophage cell line (RAW 264.7) was assessed as the nitrite concentration in the culture medium.[32] The nitric oxide produced was determined by reading the absorbance at 540 nm (ELX800 Biotek microplate reader, Bio-Tek Instruments, Inc., Winooski, VT, USA). Dexamethasone was used as a positive control. The results were expressed in terms of the concentration of each extract that caused 50% inhibition of NO production – IC_{50} (μg mL^{-1}).

2.7.6. Statistical analysis. Data were expressed as mean ± standard deviation. In data analysis, confidence intervals for mean values were estimated considering a significance level of $p < 0.05$ and the number of replicates for each assay. The differences among treatments were analyzed using the one-way analysis of variance (ANOVA) followed by Tukey's HSD test with $\alpha = 0.05$.

3. Results and discussion

A previous study performed by the authors identified hydroxytyrosol and tyrosol as the most abundant phenolic compounds present in olive pomace.[2] It was also verified that gamma radiation at 5 kGy increased the extractability of bioactive compounds from olive pomace by 2.4-fold compared to the non-irradiated ones. Based on this information, the extraction of phenolic compounds from 5 kGy irradiated olive pomace by UAE was carried out by applying a RSM of three variables (*t*, *P* and *S*) in a *CCCD* with five levels of values for each variable. This multivariable approach could provide a consistent tool that minimizes experimental errors with a reduced number of tests, while optimizing the extraction conditions of the variables, according to the mathematical empirical models that predict the maximum extraction performance. After the determination of the optimal conditions of the analyzed variables, the bioactive properties of the extract were studied.

3.1. Optimization of the extraction of hydroxytyrosol and tyrosol

3.1.1. Experimental data obtained with *CCCD* design. The experimental results obtained with 28 runs of the five-level *CCCD* design matrix implemented to optimize the UAE to extract hydroxytyrosol and tyrosol from olive pomace are shown in Table 2. The extraction yield ranged from 0.93 to 24.86%. The lowest extraction yield was obtained with run 22, which combined medium *t* and *P* conditions (21 min and 252.5 W; $\alpha = 0$) with a high solvent concentration (100% ethanol, v/v; $\alpha = 1.68$), while the highest extraction yield was found with run 7, combining high *t* and *P* conditions (32.2 min and 399.7 W; $\alpha = 1$) and a low solvent concentration (20.3% ethanol, $\alpha = -1$). Concerning the phenolic compounds, hydroxytyrosol (HYD) was detected in higher quantities than tyrosol (TYR) in all the extracts, with concentrations ranging from 5.9 ± 0.4 to 54 ± 1 mg g^{-1} extract, while the TYR levels ranged from 4.5 ± 0.4 to 21.2 ± 0.1 mg g^{-1} extract. Furthermore, the highest levels of phenylethanoids (PhC; 75 ± 1 mg g^{-1} extract) were achieved with run 17, which employed lower *t* (2 min; $\alpha = -1.68$) and medium *P* (252.5 W; $\alpha = 0$) and *S* proportion (50% ethanol, v/v; $\alpha = 0$). As for extraction yield responses, higher ethanol concentrations promoted a negative effect on phenolic compound extraction by the UAE method,

as only 10.5 ± 0.8 mg PhC per g extract were obtained with run 22 (Table 2).

3.1.2. Analysis of the theoretical response surface models. RSM is a mathematical and statistical analysis tool used for optimizing processes involving one or more response variables. To elucidate how precisely the RSM can predict ideal variances is imperative to fit the models for the selected responses. In this work, the response values of Table 2 were fitted to the second-order polynomial model of eqn (1) using a non-linear least-squares procedure to develop the mathematical models (eqn (2)–(5)) and obtain the parametric values for each response criterion. The coefficients whose confidence interval value ($\alpha = 0.05$) was higher than the value of the parameter were considered statistically non-significant (Table 3) and, for this reason, were not used for the model development. The resulting models are presented below.

$$\mathrm{HYD} = 24.8800 + 0.0245P + 0.1494S - 0.0004PS - 0.0026S^2 \quad (2)$$

$$\mathrm{TYR} = 10.24513 - 0.0865t + 0.0044P + 0.0266S + 0.0003tP - 0.0002PS \quad (3)$$

$$\mathrm{PhC} = 41.5721 - 0.2772t + 0.0209P - 0.0392S + 0.0010tP - 0.0007PS \quad (4)$$

$$\mathrm{Yield} = 10.422 + 0.1209t + 0.0364P - 0.1654S \quad (5)$$

where P is ultrasonic power in W, t is the processing time in minutes and S is the ethanol concentration in % (v/v).

In mathematical terms, the sign of the parametric values determines the performance of the response: when the parametric value is positive, the response is higher at high levels whereas when it is negative, the response is lower at high levels. The higher the parametric value (in absolute terms), the more significant the weight of the corresponding variable is. The variables could be sorted in a descending order as a function of their significance in the extraction process as $S > P > t$. It was possible to observe (Table 3) that the evaluated responses were significantly affected by a linear effect, except for the variable t in HYD. Regarding the interactions between the three variables, it seemed that the more influential interactions occurred for $t \times P$ and $P \times S$, while $t \times S$ was only significant in PhC. In fact, yield was not affected by any of the variables, HYD was affected by $P \times S$, TYR by $t \times P$ and $P \times S$, and PhC by $t \times P$ and $t \times S$.

In addition, the obtained coefficients R^2_{adj} were 0.91, 0.52 and 0.88 for HYD, TYR and TPC, respectively (Table 3), indicating that the variability of each response could be explained satisfactorily for HYD and PhC and moderately for TYR by the independent variables involved in the process. All models presented a non-significant lack of fit (Table 3) ($p > 0.05$), demonstrating that the equations adequately described the effects of the variables on the evaluated responses.

In these studies, sometimes it can be difficult to not force the models to work with all the terms. To counter this situation, it is mandatory to monitor and adjust the model to significant terms or terms that display interactions, and control the statistical diagnostics. Furthermore, the compounds that are being extracted do not behave in the same way and it can be important to change the modeling within responses.

3.1.3. Effect of the independent variables on the target responses. The best way to visually describe and interpret all the effects and extraction trends is to generate 3D response surface plots that could represent the combined effects of two variables while keeping the other variable constant (Fig. 2).

The increase in the ethanol concentration affected negatively all the analyzed responses (Fig. 2), which indicated that the highest extraction of HYD and TYR was achieved for the

Table 3 Parametric results of the second-order polynomial equations for the four response value formats in terms of coded values. The parametric subscripts A, B, and C stand for the variables involving time (t), power (P) and solvent (S), respectively. Statistical analysis of the models is presented

Parameters		Yield	HYD	TYR	PhC
Effect		% (w/w)	mg g^{-1} extract	mg g^{-1} extract	mg g^{-1} extract
Intercept	b_o	13.89 ± 0.78	26.33 ± 0.58	10.12 ± 0.40	35.10 ± 1.01
Linear	b_A	1.37 ± 8.78	ns	0.03 ± 0.44	−0.36 ± 2.57
	b_B	5.36 ± 8.78	0.35 ± 0.74	0.20 ± 0.47	−0.76 ± 1.16
	b_C	−4.92 ± 8.77	−6.76 ± 0.94	−0.77 ± 0.47	−6.58 ± 1.15
Quadratic	b_{AA}	ns	ns	ns	ns
	b_{BB}	ns	ns	ns	ns
	b_{CC}	ns	−2.34 ± 1.11	ns	
Interaction	b_{AB}	ns	ns	0.58 ± 0.48	1.61 ± 1.22
	b_{AC}	ns	ns	ns	−3.16 ± 1.25
	b_{BC}	ns	−1.94 ± 1.1	−0.91 ± 0.51	ns
Statistical analysis	SM	<0.0001	<0.0001	0.0011	<0.0001
	LF	0.3966	0.0586	0.1391	0.0540
	R^2	0.8937	0.9165	0.6134	0.9041
	R^2_{adj}	0.8804	0.9125	0.5168	0.8789

HYD: hydroxytyrosol; TYR: tyrosol; PhC: phenylethanoids; SM: significance of the model; LF: lack of fit; R^2: coefficient of determination; R^2_{adj}: adjusted coefficient of determination (R^2); ns: non-significant.

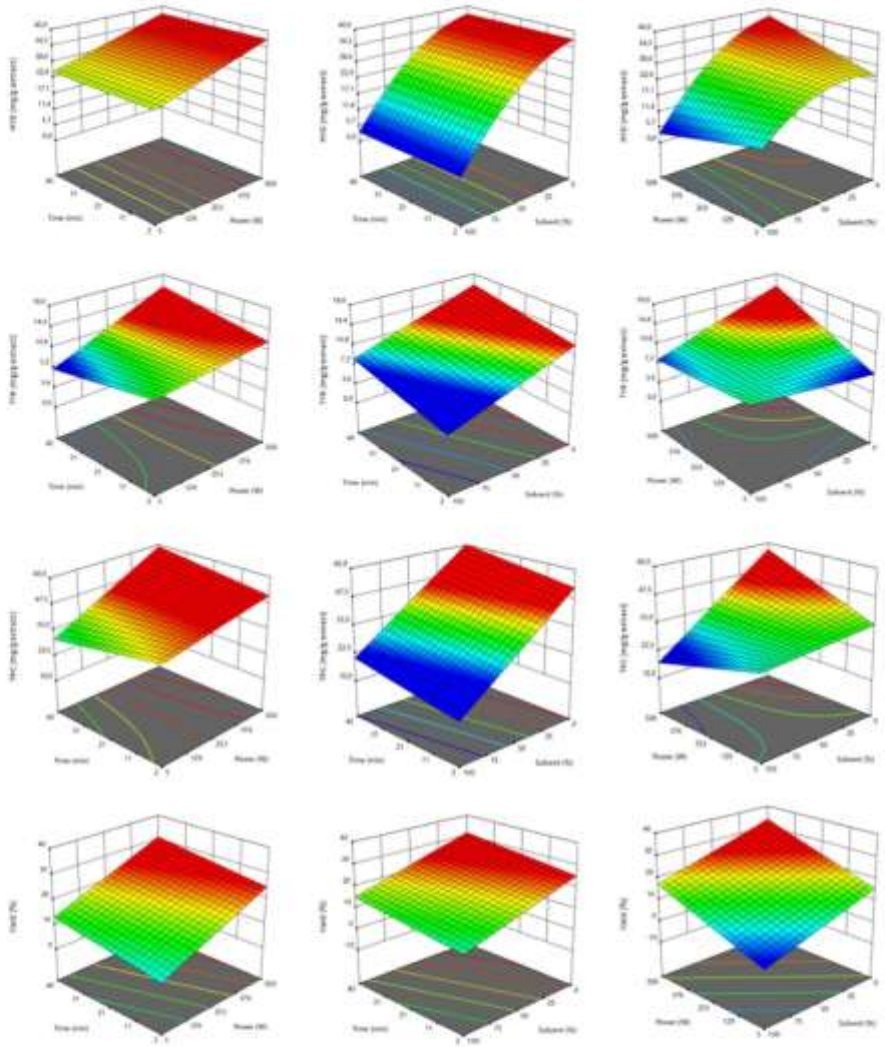


Fig. 2 Response surface graphs of the effect of the three independent extraction variables tested on the four evaluated responses: hydroxytyrosol (HYD), tyrosol (TYR) and phenylethanoids (PhC) contents and extraction yield (%). In each graph, the excluded variable was positioned at its optimal value (Table 4).

lowest ethanol concentrations. Furthermore, the extraction yield was also influenced by the ultrasound power and extraction time. The higher the ultrasound power and the extraction time, the higher the extraction yield, as can be confirmed by the positive sign obtained for these variables in contrast with the negative sign for the solvent (*i.e.* ethanol percentage) (Table 3). The response HYD was not significantly affected by the extraction time but it was favored by higher ultrasound power and lower ethanol concentrations. Both TYR and TPC responses were favored by higher ultrasound power and extraction time, and lower ethanol concentration. For these responses, a positive interaction between the two variables ($t \times P$) was also verified (Table 3). All these results supported the use of RSM as an optimization tool for the UAE optimization.

3.1.4. Optimal extraction conditions for maximizing the response criteria. After the statistical validation of the models, the optimal extraction conditions that maximize the individual and global responses were determined (Table 4).

In general, the best efficiency of the UAE process was obtained at short to medium extraction times, high ultrasound power, and low solvent percentages. Regarding the individual optimal conditions, it was possible to recover 37 ± 2 mg of hydroxytyrosol in 1 g of extract at 499 W and 13.6 min using 0% ethanol, while 15 ± 1 mg of tyrosol was obtained in 1 g of extract when applying the same ultrasound power (499 W) for

Table 4 Optimal individual and global conditions in natural values that maximize the extraction of phenolic compounds from olive pomace

Responses	Individual optimal conditions			Predictive responses	Global optimal conditions			Predictive response	Experimental responses
	t (min)	P (W)	S (%)		t (min)	P (W)	S (%)		
HYD (mg g^{-1} extract)	13.6	499	0.0	37 ± 2	28	490	7.3	36 ± 2	26 ± 1
TYR (mg g^{-1} extract)	36.4	499	3.8	15 ± 1				14 ± 1	9.7 ± 0.5
PhC (mg g^{-1} extract)	24.5	500	2.9	51 ± 3				50 ± 3	36 ± 2
Yield (%)	38.0	431	18.8	28 ± 2				30 ± 2	24 ± 1

HYD: hydroxytyrosol; TYR: tyrosol; PhC: phenylethanoids.

36.4 min and 3.8% ethanol. Similar individual optimal conditions (500 W for 24.5 min using 2.9% ethanol) were obtained for the recovery of 51 ± 3 mg of PhC in 1 g extract.

The global processing conditions that allowed simultaneous maximization of all the responses were also calculated (Table 4). The results demonstrated that 28 min of sonication at 490 W using 7.3% ethanol as the solvent were found to be the optimal conditions to recover 36 ± 2 mg of hydroxytyrosol per g of extract and 14 ± 1 mg of tyrosol per g of extract, achieving an extraction yield of 30 ± 2%.

It is important to mention that the comparison of the results from different studies can be difficult due to the variability of olive pomace samples (*e.g.*, the type of cultivation and the variety and maturation of the olives) and the different methods that are used to quantify the phenolic constituents. Furthermore, most studies in the literature did not evaluate the compounds individually, but in terms of total phenolic content and antioxidant activity. Martínez-Patiño *et al.* (2019)[11] and Gómez-Cruz *et al.* (2021)[33] selected slightly lower extraction times (15 and 12 min, respectively) to recover bioactive compounds from industrial exhausted olive pomace, although those authors used higher concentrations of ethanol (43.2%)[11] and acetone (40%).[33] On the contrary, Goldsmith *et al.* (2018)[10] optimized the extraction of phenolic compounds from olive pomace using only water as a solvent and a lower ultrasound power (250 W) than that obtained in this work, but higher extraction times (75 min). In terms of individual compounds, Xie *et al.* (2019)[12] recovered 55.1 ± 2.1 mg of hydroxytyrosol per g of olive pomace dry matter using 90% ethanol for 3 min, with an ultrasound power of 500 W. The amount of hydroxytyrosol obtained by Xie *et al.* (2019)[12] was higher than that found in the present study (10.2 ± 0.4 mg of hydroxytyrosol per g of dry weight – data not shown). Albahari *et al.* (2018)[23] reported that the addition of cyclodextrins to the extraction solvent under optimized UAE conditions enhanced the extraction of hydroxytyrosol from olive pomace when compared to the conventional methods and regular UAE, although the amounts of hydroxytyrosol obtained (523 ± 6–887 ± 13 mg kg^{-1} of olive pomace extract) were lower than the ones in the present work.

3.1.5. Experimental validation of the optimum extraction conditions. The global optimal conditions that maximize both the extraction yield and the recovery of phenolic compounds from olive pomace were then experimentally tested to analyze the model's ability to predict the experimental results (Table 4) and to obtain the phenolic-rich extracts that were used to evaluate the bioactive properties. UAE yielded 24 ± 1% and 36 ± 2 mg of PhC per g of extract, which means that the values differed by 20–28% from the predicted ones, so that the predictive capacity of the model could be considered acceptable and validated.

3.2. Evaluation of the bioactive properties

Once the optimal conditions of the analyzed variables were determined for UAE, the bioactive properties of the extract were evaluated. In a previous work, Madureira *et al.* (2021)[21] optimized the extraction of the same phenolic compounds from olive pomace using HAE and reported the highest extraction for longer extraction times (120 min), a temperature of 85 °C and 76% ethanol in water as the solvent. Comparing both techniques, UAE reduced the extraction time and the solvent consumption. Additionally, this method achieved higher extraction yields (the HAE yield was 13.7%), probably due to the structural changes in the solid sample promoted by the cavitation effects that could enhance the mass transfer. In order to determine the best conditions to obtain the extract with higher bioactivity, the extracts from the optimal conditions of both techniques (UAE and HAE) were compared in terms of antioxidant, antimicrobial, antidiabetic and anti-inflammatory capacities and cytotoxicity.

3.2.1. Antioxidant activity. Two methods were employed to evaluate the antioxidant activity of the extracts of olive pomace, OxHLIA and TBARS. The results are expressed as IC_{50} values (Table 5), meaning the extract concentration able to provide 50% of antioxidant activity (TBARS assay) or to protect 50% of the erythrocyte population from hemolysis caused by an oxidizing agent (OxHLIA assay). The IC_{50} values in the OxHLIA assay were obtained at two times (Δt 60 min and Δt 120 min) considering that natural extracts contain different antioxidant molecules capable of interacting with each other and offering protection at different time periods. In both assays, the lower the IC_{50} values, the higher the antioxidant capacity of the olive pomace extracts.

In the OxHLIA assay, the hemolysis curves for the olive pomace extracts of HAE and UAE at different concentrations (data not shown) revealed that higher concentrations protected the erythrocyte population from hemolysis for a longer period of time. The HAE extracts showed significant higher anti-

Table 5 Bioactive properties of extracts obtained under optimized HAE and UAE from olive pomace irradiated at 5 kGy

	Optimal conditions		
	HAE	UAE	Positive control
Extractability (mg g^{-1} ext)			
HYD	35.4 ± 0.6^{a}	26 ± 1^{b}	
TYR	13.8 ± 0.3^{a}	9.7 ± 0.5^{b}	
Cytotoxicity activity (GI_{50}, μg mL^{-1})			Ellipticine
AGS	238 ± 7^{a}	230 ± 11^{a}	1.23 ± 0.03^{b}
CaCo	163 ± 12^{a}	191 ± 10^{a}	1.21 ± 0.02^{b}
MCF-7	130.4 ± 10.5^{b}	249 ± 17^{a}	1.02 ± 0.02^{c}
NCI-H460	234 ± 24^{a}	226 ± 11^{a}	1.01 ± 0.01^{b}
PLP2	77 ± 4^{a}	89 ± 8^{a}	1.4 ± 0.1^{b}
VERO	184 ± 9^{a}	201 ± 20^{a}	1.41 ± 0.06^{b}
Anti-inflammatory activity (IC_{50}, μg mL^{-1})			Dexametasone
RAW 264.7	23 ± 1^{b}	168 ± 6^{a}	6.3 ± 0.4^{c}
Antioxidant activity (IC_{50}, μg mL^{-1})			Trolox
TBARS inhibition	99 ± 5^{b}	130 ± 5^{a}	5.4 ± 0.3^{c}
OxHLIA (Δt 60 min)	18.5 ± 0.5^{c}	45 ± 1^{a}	21.8 ± 0.3^{b}
(Δt 120 min)	69 ± 3^{b}	96 ± 2^{a}	43.5 ± 0.8^{c}
Antidiabetic activity (IC_{50}, mg mL^{-1})			Acarbose
α-Amylase inhibition	53 ± 10^{b}	105 ± 11^{a}	0.010 ± 0.003^{c}
α-Glucosidase inhibition	14 ± 1^{b}	26.1 ± 0.2^{a}	11 ± 1^{b}

HYD: hydroxytyrosol; TYR: tyrosol. In each row, different lowercase letters mean significant differences between average values ($p < 0.05$).

hemolytic activity (IC_{50} values of 18.5 ± 0.5 μg mL^{-1} and 69 ± 3 μg mL^{-1} at 60 and 120 min, respectively) than UAE extracts (IC_{50} values of 45 ± 1 and 96 ± 2 μg mL^{-1}) (Table 5). Furthermore, at 60 min, the IC_{50} value of HAE extract provided higher protection to the erythrocyte membranes than the positive control used, Trolox (IC_{50} value of 21.8 ± 0.3 μg mL^{-1}), demonstrating the significant antioxidant capacity of HAE extracts in the prevention of cellular oxidative processes. Nonetheless, a similar protection of the erythrocyte population (IC_{50} value of 20.6 ± 0.4 μg mL^{-1} at 60 min) in extracted olive pomace samples irradiated at 5 kGy was obtained using maceration extraction,[2] which is the same absorbed dose as applied in this work.

The HAE extracts were also more efficient in inhibiting the formation of TBARS than UAE extracts, with IC_{50} values of 99 ± 5 μg mL^{-1} and 130 ± 5 μg mL^{-1}, respectively (Table 5). Nevertheless, Trolox presented lower IC_{50} value (5.4 ± 0.3 μg mL^{-1}) than both the analyzed extracts, which could be attributed to the difference between a pure compound and the complex mixtures of olive pomace extracts.

The antioxidant activity of olive pomace extracts has usually been measured by chemical-based assays, namely for their DPPH and ABTS radical scavenging activity and ferric (FRAP) and cupric (CUPRAC) reducing power,[10,11,33–36] always obtaining the good correlation with the phenolic contents of olive pomace extracts.

3.2.2. Antimicrobial activity. The two extracts (HAE and UAE) of olive pomace were evaluated for their antimicrobial potential against three Gram-negative (*E. coli*, *S. typhimurium*, *P. fluorescens*) and three Gram-positive bacteria (*B. cereus*, *S. aureus*, *L. monocytogenes*) and one fungus (*C. albicans*).

The results showed that HAE extracts had higher antibacterial potential than UAE extracts, except for *S. typhimurium* (Table 6), which might be attributed to the higher amounts of hydroxytyrosol (35.4 ± 0.6 mg g^{-1} of extract) in HAE extracts (Table 5) that were proved to be effective against some pathogenic strains.[37] Moreover, concerning HAE extracts, Gram positive bacteria (MIC 25 mg mL^{-1}) seemed to be more sensitive than Gram negative bacteria (with the exception of *P. fluorescens*) (MIC 50 mg mL^{-1}), suggesting that the extracts could act differently on the cell wall of both bacterial types. Additionally, it is important to refer to the ability of both olive pomace extracts to inhibit the formation of *P. fluorescens* biofilm.

Regarding the antifungal potential, none of the extracts were demonstrated to have activity against *C. albicans* at the

Table 6 Antimicrobial activity of the extracts obtained under optimized HAE and UAE from olive pomace irradiated at 5 kGy

		B. cereus	*S. aureus*	*L. monocytogenes*	*E. coli*	*S. typhymurium*	*P. fluorescens*	*C. albicans*
		MIC (mg mL^{-1})						
Optimal conditions	HAE	25	25	25	50	50	25	>100
	UAE	50	50	50	100	50	50	>100
		MBC (mg mL^{-1})						MFC (mg mL^{-1})
Optimal conditions	HAE	25	100	100	100	100	25	>100
	UAE	50	>100	100	100	50	50	>100

studied concentrations (MIC > 100 mg mL^{-1}), which was also reported by Sousa *et al.* (2006)[38] in table olive extracts.

The antimicrobial potential of extracts obtained by maceration in 80% methanol from irradiated olive pomace samples was previously reported by the authors,[2] and there are also some works describing the antimicrobial activity of polyphenol extracts from olive oil,[39] olive cake[34] and olive leaves.[34,40,41] Interestingly, Moudache *et al.* (2020)[34] concluded that the extracts from olive leaves had higher antimicrobial activity than the extracts from olive cake against *S. aureus* (MIC 0.5 mg mL^{-1} and 8 mg mL^{-1} for olive leaves and olive cake, respectively) and *B. cereus* (MIC 1 mg mL^{-1} and 4 mg mL^{-1} for olive leaves and olive cake, respectively). On the other hand, in olive leaf extracts, Sweedan *et al.* (2019)[40] found MIC values of 7.5 mg mL^{-1} and 15 mg mL^{-1} against *S. aureus* and *E. coli*, respectively, while Gökmen *et al.* (2014)[41] reported MIC ≥ 32 mg mL^{-1} for *L. monocytogenes* and MIC ≥ 16 mg mL^{-1} against *B. cereus*, *S. aureus*, *E. coli* and *S. typhimurium*.

The obtained results demonstrated the potential of using irradiated olive pomace extracts as a food preservative in order to prevent the growth of foodborne pathogens together with their proved antioxidant properties.

3.2.3. Antidiabetic activity. Diabetes mellitus is caused by complete or partial deficiencies in insulin production/action and associated with various diseases such as hyperglycemia, hypertension, hyperlipidemia and cardiovascular diseases. Despite the traditional insulin therapy, demand by patients to use natural products to treat and prevent diabetes has been growing. The ability to inhibit α-amylase and α-glucosidase was reported to be effective to prevent type 2 diabetes, being useful to manage hyperglycemia.

The effectiveness of the HAE and UAE extracts in inhibiting α-amylase and α-glucosidase was expressed as IC_{50} values (Table 5). The HAE extracts presented significantly higher α-amylase (IC_{50} value of 53 ± 10 mg mL^{-1}) and α-glucosidase (IC_{50} value of 14 ± 1 mg mL^{-1}) inhibition activities than UAE extracts (IC_{50} values of 105 ± 11 and 26.1 ± 0.2 mg mL^{-1}, respectively). Furthermore, the α-glucosidase inhibition of HAE extracts (IC_{50} value of 14 ± 1 mg mL^{-1}) did not differ significantly from that for acarbose (IC_{50} value of 11 ± 1 mg mL^{-1}), the standard positive control.

As far as the authors know, this is the first study about the antidiabetic effect of olive pomace extracts, although the inhibitory activity of hydroxytyrosol and other phenolic compounds present in olive leaves and olive mill wastes was also demonstrated by Mwakalukwa *et al.* (2020)[42] and Hadrich *et al.* (2015).[43] On the other hand, extra virgin olive oils were reported to show a high digestive enzyme inhibitory activity that was associated with their phenolic composition.[44] Those authors also observed that olive oil samples with greater antioxidant effect were the most effective inhibitors of α-glucosidase.

The obtained findings revealed the existence of a relevant hypoglycemic effect, which could suggest the potential use of both extracts (and especially HAE extracts) as natural and promising antidiabetic agents.

3.2.4. Cytotoxicity and anti-inflammatory activity. The inhibitory effect of olive pomace extracts against the inflammatory response was measured using a cell-based screening bioassay to test the NO level in LPS-stimulated RAW 264.7 macrophages. Since NO has an important role in the inflammatory process, it is important to develop active formulations with natural compounds capable of inhibiting NO production. Both tested extracts showed anti-inflammatory capacity with IC_{50} values between 23 ± 1 and 168 ± 6 μg mL^{-1}, with HAE extracts presenting a much higher inhibitory activity in NO production (IC_{50} value of 23 ± 1 μg mL^{-1}) (Table 5).

There are no studies in the literature reporting the anti-inflammatory activity of extracts from irradiated olive pomace through this cell-based assay, but extra virgin olive oil was proved to have anti-inflammatory potential which was concentration dependent.[45]

The cytotoxic activity of the olive pomace extracts against four human tumor cell lines and two non-tumor cell lines was also tested. Both HAE and UAE extracts demonstrated cytotoxic activity for all the analyzed cell lines (Table 5). In absolute terms, the cell line CaCo-2 was the most susceptible to olive pomace extracts, followed by MCF-7, NCI-H460 and AGS (Table 5). Furthermore, there were no significant differences between the cytotoxic effects of both extracts, except for the breast adenocarcinoma (MCF-7) cell line, for which HAE extracts presented the highest activity (GI_{50} value of 130.4 ± 10.5 μg mL^{-1}) compared with UAE extracts (GI_{50} value of 249 ± 17 μg mL^{-1}).

The cytotoxicity of olive pomace methanol extracts against glioblastoma, breast, ovarian and pancreatic cancer cells was previously demonstrated by Goldsmith *et al.* (2018),[46] while Tezcan *et al.* (2017)[47] also showed that olive leaf extracts displayed toxicity towards glioblastoma cells.

4. Conclusions

The ultrasound-assisted extraction of phenolic compounds from olive pomace was evaluated using RSM. The results demonstrated that 28 min of sonication at 490 W using 7.3% ethanol as solvent were the conditions that maximized the extraction of hydroxytyrosol (36 ± 2 mg g^{-1} of extract) and tyrosol (14 ± 1 mg g^{-1} of extract), with a yield of 30 ± 2%. The bioactive properties of the extracts produced under the selected UAE optimal processing conditions were assessed and compared with the ones obtained using HAE. The UAE extracts presented lower bioactivity than the ones obtained by HAE. Despite reduced extraction time and solvent consumption by UAE as well as improved extraction yields in relation to HAE (yield of 13.7%), the HAE extracts showed greater antioxidant capacity, with lower extract concentrations being required to inhibit the TBARS formation and oxidative hemolysis, and also presented higher antibacterial and anti-inflammatory activities. Besides, the HAE extracts also presented higher antidiabetic potential and cytotoxic effect for the breast adenocarcinoma (MCF-7) cell line than those obtained by UAE.

According to these results, the HAE methodology was indicated to promote higher extractability and bioactivity of polyphenols from olive pomace compared with the UAE method.

The overall results in this work support the interest of food industries in developing new ingredients with bioactive potential from agro-industrial wastes, as natural alternatives to synthetic preservatives and/or additives, contributing to the sustainability of both the agro-industrial sector and the environment.

Author contributions

Conceptualization and methodology: Sandra Cabo Verde, Lillian Barros, Isabel C. F. R. Ferreira; Investigation: Joana Madureira, Bianca Albuquerque, Maria Inês Dias, José Pinela, Ricardo C. Calhelha; Writing – original draft: Joana Madureira; Writing – review & editing: Sandra Cabo Verde, José Pinela, Lillian Barros, Fernanda Margaça and Celestino Santos-Buelga; Funding acquisition: Sandra Cabo Verde, Lillian Barros and Celestino Santos-Buelga; Supervision: Sandra Cabo Verde, Lillian Barros and Celestino Santos-Buelga.

Conflicts of interest

The authors declare that they have no conflict of interest.

Acknowledgements

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES to C^{2}TN (UIDB/04349/2020), CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021); to FCT for the PhD studentships granted to J. Madureira (SFRH/BD/136506/2018) and B. Albuquerque (SFRH/BD/136370/2018). M. I. Dias, R. C. Calhelha and L. Barros thank FCT for national funding to P. I., through the institutional scientific employment program-contract. J. Pinela also thanks FCT for his contract (CEECIND/01011/2018). The authors also thank the European Regional Development Fund (ERDF), through the projects "BIOMA" (POCI-01-0247-FEDER-046112) and "OliveBIOextract" (NORTE-01-0247-FEDER-049865). The Agroenvironment Unit is supported by Junta de Castilla y León (Escalera de Excelencia CLU-2018-04) co-funded by the P. O. FEDER of Castilla y León 2014–2020. The authors are also grateful to "UCASUL – União de Cooperativas Agrícolas do Sul" agro industrial cooperative for providing the samples and to the Technological Unit of Radiosterilization (University of Lisbon) for the sample irradiation.

References

1 G. Cioffi, M. S. Pesca, P. De Caprariis, A. Braca, L. Severino and N. De Tommasi, Phenolic compounds in olive oil and olive pomace from Cilento (Campania, Italy) and their antioxidant activity, *Food Chem.*, 2010, **121**, 105–111.

2 J. Madureira, M. I. Dias, J. Pinela, R. C. Calhelha, L. Barros, C. Santos-Buelga, F. M. A. Margaça, I. C. F. R. Ferreira and S. Cabo Verde, The use of gamma radiation for extractability improvement of bioactive compounds in olive oil wastes, *Sci. Total Environ.*, 2020, **727**, 138706.

3 A. Malapert, E. Reboul, M. Loonis, O. Dangles and V. Tomao, Direct and rapid profiling of biophenols in olive pomace by UHPLC-DAD-MS, *Food Anal. Methods*, 2018, **11**, 1001–1010.

4 M. A. Nunes, A. S. G. Costa, S. Bessada, J. Santos, H. Puga, R. C. Alves, V. Freitas and M. B. P. P. Oliveira, Olive pomace as a valuable source of bioactive compounds: A study regarding its lipid- and water-soluble components, *Sci. Total Environ.*, 2018, **644**, 229–236.

5 A. K. Marković, J. Torić, M. Barbarić and C. J. Brala, Hydroxytyrosol, tyrosol and derivatives and their potential effects on human health, *Molecules*, 2019, **24**, 2001.

6 M. C. L. de las Hazas, L. Rubio, A. Macia and M. J. Motilva, Hydroxytyrosol: Emerging trends in potential therapeutic applications, *Curr. Pharm. Des.*, 2018, **24**, 2157–2179.

7 H. Shamshoum, F. Vlavcheski and E. Tsiani, Anticancer effects of oleuropein, *BioFactors*, 2017, **43**, 517–528.

8 C. M. Galanakis, Emerging technologies for the production of nutraceuticals from agricultural by-products: A viewpoint of opportunities and challenges, *Food Bioprod. Process.*, 2013, **91**, 575–579.

9 Á. Peralbo-Molina, F. Priego-Capote and M. D. Luque De Castro, Tentative identification of phenolic compounds in olive pomace extracts using liquid chromatography–tandem mass spectrometry with a quadrupole–quadrupole–time-of-flight mass detector, *J. Agric. Food Chem.*, 2012, **60**, 11542–11550.

10 C. D. Goldsmith, Q. V. Vuong, C. E. Stathopoulos, P. D. Roach and C. J. Scarlett, Ultrasound increases the aqueous extraction of phenolic compounds with high antioxidant activity from olive pomace, *LWT–Food Sci. Technol.*, 2018, **89**, 284–290.

11 J. C. Martínez-Patiño, I. Gómez-Cruz, I. Romero, B. Gullón, E. Ruiz, M. Brnčićc and E. Castro, Ultrasound-assisted extraction as a first step in a biorefinery strategy for valorisation of extracted olive pomace, *Energies*, 2019, **12**, 2679.

12 P. Xie, L. Huang, C. Zhang, Y. Deng, X. Wang and J. Cheng, Enhanced extraction of hydroxytyrosol, maslinic acid and oleanolic acid from olive pomace: Process parameters, kinetics and thermodynamics, and greenness assessment, *Food Chem.*, 2019, **276**, 662–674.

13 I. C. Pavez, J. Lozano-Sánchez, I. Borrás-Linares, H. Nuñez, P. Robert and A. Segura-Carretero, Obtaining an extract rich in phenolic compounds from olive pomace by pressurized liquid extraction, *Molecules*, 2019, **24**, 1–17.

14 A. S. Caballero, J. M. Romero-García, E. Castro and C. A. Cardona, Supercritical fluid extraction for enhancing polyphenolic compounds production from olive waste extracts, *J. Chem. Technol. Biotechnol.*, 2020, **95**, 356–362.

15 J. Madureira, A. I. Pimenta, L. Popescu, A. Besleaga, M. I. Dias, P. M. P. Santos, R. Melo, I. C. F. R. Ferreira, S. Cabo Verde and F. M. A. Margaça, Effects of gamma radiation on cork wastewater: Antioxidant activity and toxicity, *Chemosphere*, 2017, **169**, 139–145.
16 D. Guerreiro, J. Madureira, T. Silva, R. Melo, P. M. P. Santos, A. Ferreira, M. J. Trigo, A. N. Falcão, F. M. A. Margaça and S. Cabo Verde, Post-harvest treatment of cherry tomatoes by gamma radiation: Microbial and physicochemical parameters evaluation, *Innovative Food Sci. Emerging Technol.*, 2016, **36**, 1–9.
17 S. Cabo Verde, M. J. Trigo, M. B. Sousa, A. Ferreira, A. C. Ramos, I. Nunes, C. Junqueira, R. Melo, P. M. P. Santos and M. L. Botelho, Effects of gamma radiation on raspberries: Safety and quality issues, *J. Toxicol. Environ. Health, Part A*, 2013, **76**, 291–303.
18 M. I. Elias, J. Madureira, P. M. P. Santos, M. M. Carolino, F. M. A. Margaça and S. C. Verde, Preservation treatment of fresh raspberries by e-beam irradiation, *Innovative Food Sci. Emerging Technol.*, 2020, **66**, 102487.
19 S. Barkaoui, J. Madureira, P. M. P. Santos, F. M. A. Margaça, N. B. Miloud, M. Mankai, N. M. Boudhrioua and S. Cabo Verde, Effect of ionizing radiation and refrigeration on the antioxidants of strawberries, *Food Bioprocess Technol.*, 2020, **13**, 1516–1527.
20 E. Pereira, L. Barros, A. L. Antonio, S. Cabo Verde, C. Santos-Buelga, I. C. F. R. Ferreira and P. Rodrigues, Is gamma radiation suitable to preserve phenolic compounds and to decontaminate mycotoxins in aromatic plants? A case-study with *Aloysia citrodora paláu*, *Molecules*, 2017, **22**, 347.
21 J. Madureira, B. Melgar, C. Santos-Buelga, F. M. A. Margaça, I. C. F. R. Ferreira, L. Barros and S. C. Verde, Phenolic compounds from irradiated olive wastes: Optimization of the heat-assisted extraction using response surface methodology, *Chemosensors*, 2021, **9**, 231, DOI: **10.3390/chemosensors9080231**.
22 A. Zuorro, Modelling of polyphenol recovery from olive pomace by response surface methodology, *Int. Rev. Modell. Simul.*, 2014, **7**, 1023–1028.
23 P. Albahari, M. Jug, K. Radić, S. Jurmanović, M. Brnčić, S. R. Brnčić and D. Vitali Čepo, Characterization of olive pomace extract obtained by cyclodextrin-enhanced pulsed ultrasound assisted extraction, *LWT–Food Sci. Technol.*, 2018, **92**, 22–31.
24 B. Whittaker and M. F. Watts, The influence of dose rate, ambient temperature and time on the radiation response of Harwell PMMA dosimeters, *Radiat. Phys. Chem.*, 2001, **60**, 101–110.
25 B. R. Albuquerque, J. Pinela, L. Barros, M. B. P. P. Oliveira and I. C. F. R. Ferreira, Anthocyanin-rich extract of jabuticaba epicarp as a natural colorant: Optimization of heat- and ultrasound-assisted extractions and application in a bakery product, *Food Chem.*, 2020, **316**, 126364.
26 S. M. F. Bessada, J. C. M. Barreira, L. Barros, I. C. F. R. Ferreira and M. B. P. P. Oliveira, Phenolic profile and antioxidant activity of *Coleostephus myconis* (L.) Rchb. f.: An underexploited and highly disseminated species, *Ind. Crops Prod.*, 2016, **89**, 45–51.
27 F. Mandim, M. I. Dias, J. Pinela, P. Barracosa, M. Ivanov, D. Stojković, M. Soković, C. Santos-Buelga, L. Barros and I. C. F. R. Ferreira, Chemical composition and in vitro biological activities of cardoon (*Cynara cardunculus* L. var. altilis DC.) seeds as influenced by viability: Chemical prospection and bioactivity of cardoon seeds, *Food Chem.*, 2020, **323**, 126838.
28 M. Sokovic and L. J. L. D. Van Griensven, Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, *Agaricus bisporus*, *Eur. J. Plant Pathol.*, 2006, **116**, 211–224.
29 Z. Chen, C. Yin, X. Fan, K. Ma, F. Yao, R. Zhou, D. Shi, W. Cheng and H. Gao, Characterization of physicochemical and biological properties of *Schizophyllum commune* polysaccharide extracted with different methods, *Int. J. Biol. Macromol.*, 2020, **156**, 1425–1434.
30 A. R. Silva, J. Pinela, M. I. Dias, R. C. Calhelha, M. J. Alves, A. Mocan, P. A. García, L. Barros and I. C. F. R. Ferreira, Exploring the phytochemical profile of *Cytinus hypocistis* (L.) L. as a source of health-promoting biomolecules behind its in vitro bioactive and enzyme inhibitory properties, *Food Chem. Toxicol.*, 2020, **136**, 111071.
31 L. Barros, E. Pereira, R. C. Calhelha, M. Dueñas, A. M. Carvalho, C. Santos-Buelga and I. C. F. R. Ferreira, Bioactivity and chemical characterization in hydrophilic and lipophilic compounds of *Chenopodium ambrosioides* L., *J. Funct. Foods*, 2013, **5**, 1732–1740.
32 F. Sobral, A. Sampaio, S. Falcão, M. J. R. P. Queiroz, R. C. Calhelha, M. Vilas-Boas and I. C. F. R. Ferreira, Chemical characterization, antioxidant, anti-inflammatory and cytotoxic properties of bee venom collected in northeast Portugal, *Food Chem. Toxicol.*, 2016, **94**, 172–177.
33 I. Gómez-Cruz, M. D. M. Contreras, F. Carvalheiro, L. C. Duarte, L. B. Roseiro, I. Romero and E. Castro, Recovery of bioactive compounds from industrial exhausted olive pomace through ultrasound-assisted extraction, *Biology*, 2021, **10**, 1–22.
34 M. Moudache, F. Silva, C. Nerín and F. Zaidi, Olive cake and leaf extracts as valuable sources of antioxidant and antimicrobial compounds: A comparative study, *Waste Biomass Valorization*, 2020, **12**, 1431–1445.
35 B. Aliakbarian, D. Palmieri, A. A. Casazza, D. Palombo and P. Perego, Antioxidant activity and biological evaluation of olive pomace extract, *Nat. Prod. Res.*, 2012, **26**, 2280–2290.
36 H. Zhao, R. J. Avena-Bustillos and S. C. Wang, Extraction, purification and in vitro antioxidant activity evaluation of phenolic compounds in California olive pomace, *Foods*, 2022, **11**, 174, DOI: **10.3390/foods11020174**.
37 M. Ghalandari, M. Naghmachi, M. Oliverio, M. Nardi, H. R. G. Shirazi and O. Eilami, Antimicrobial effect of hydroxytyrosol, hydroxytyrosol acetate and hydroxytyrosol oleate on *Staphylococcus aureus* and *Staphylococcus epidermidis*, *Electron. J. Gen. Med.*, 2018, **15**, 4, DOI: **10.29333/ejgm/85686**.

38 A. Sousa, I. C. F. R. Ferreira, R. Calhelha, P. B. Andrade, P. Valentão, R. Seabra, L. Estevinho, A. Bento and J. A. Pereira, Phenolics and antimicrobial activity of traditional stoned table olives 'alcaparra', *Bioorg. Med. Chem.*, 2006, **14**, 8533–8538.
39 M. Brenes, E. Medina, C. Romero and A. De Castro, Antimicrobial activity of olive oil, *Agro Food Ind. Hi-Tech*, 2007, **18**, 6–8.
40 E. G. Sweedan, A. M. D. Al-haidari, A. M. Magemand and M. Abed almohsen, Antimicrobial and antibiofilm activities of alcoholic extract of olive leaves (*Olea europaea*) against pathogenic bacteria, *Biosci. Res.*, 2019, **16**, 367–374.
41 M. Gökmen, R. Kara, L. Akkaya, E. Torlak and A. Önen, Evaluation of antimicrobial activity in olive (*Olea europaea*) leaf extract, *Am. J. Microbiol.*, 2014, **5**, 37–40.
42 R. Mwakalukwa, Y. Amen, M. Nagata and K. Shimizu, Postprandial hyperglycemia lowering effect of the isolated compounds from olive mill wastes – An inhibitory activity and kinetics studies on α-glucosidase and α-amylase enzymes, *ACS Omega*, 2020, **5**, 20070–20079.
43 F. Hadrich, Z. Bouallagui, H. Junkyu, H. Isoda and S. Sayadi, The α-glucosidase and α-amylase enzyme inhibitory of hydroxytyrosol and oleuropein, *J. Oleo Sci.*, 2015, **64**, 835–843.
44 J. Collado-González, C. Grosso, P. Valentão, P. B. Andrade, F. Ferreres, T. Durand, A. Guy, J. M. Galano, A. Torrecillas and Á. Gil-Izquierdo, Inhibition of α-glucosidase and α-amylase by Spanish extra virgin olive oils: The involvement of bioactive compounds other than oleuropein and hydroxytyrosol, *Food Chem.*, 2017, **235**, 298–307.
45 Z. Almaksour, F. Boudard, M. Villareal, L. Grosmaire, C. Guzman, H. Isoda, M. Larroque and D. Margout, Anti-inflammatory and antioxidant activities of different extra virgin olive oil varieties extracts, *Arabian J. Med. Aromat. Plants*, 2017, **3**, 162–183.
46 C. D. Goldsmith, J. Sakoff, C. E. Stathopoulos, P. D. Roach and C. J. Scarlett, Cytotoxicity of methanol and aqueous olive pomace extracts towards pancreatic cancer cells in vitro, *J. Nat. Prod. Cancer Prev. Ther.*, 2018, **2**, 1–6.
47 G. Tezcan, M. O. Taskapilioglu, B. Tunca, A. Bekar, H. Demirci, H. Kocaeli, S. A. Aksoy, U. Egeli, G. Cecener and S. Tolunay, *Olea europaea* leaf extract and bevacizumab synergistically exhibit beneficial efficacy upon human glioblastoma cancer stem cells through reducing angiogenesis and invasion in vitro, *Biomed. Pharmacother.*, 2017, **90**, 713–723.

Publicación 7. Effect of olive pomace extract application and packaging material on the preservation of fresh-cut 'Royal Gala' apples

Joana Madureira, Bruno Melgar, Vitor D. Alves, Margarida Moldão-Martins, Fernanda M.A. Margaça, Celestino Santos-Buelga, Lillian Barros, Sandra Cabo Verde

Artículo publicado en *Foods* (2023), 12(9), 1926; Doi: https://doi.org/10.3390/foods12091926

Resumen

En este trabajo se utilizaron extractos de orujo de oliva como ingredientes naturales para evaluar su eficacia para retrasar el pardeamiento en piezas de manzana mínimamente procesadas.

El efecto de los extractos naturales se comparó con el de ácido ascórbico, el antioxidante comercial utilizado por la industria. Se probaron dos películas de envasado diferentes (ácido poliláctico biodegradable – PLA, y polipropileno orientado - OPP). A lo largo de 12 días de almacenamiento a 4 ºC se siguió la evolución de parámetros fisicoquímicos (firmeza, pérdida de peso y color), la composición de la atmósfera en el interior de los envases y la carga microbiana, y se analizó el contenido fenólico y la actividad antioxidante por la medida del poder reductor del ion férrico (FRAP, *Ferric Reducing Antioxidant Power*) y la capacidad de eliminación del radical DPPH (*Radical Scavenging Activity*).

Los resultados demostraron que los cortes de manzanas tratadas con extractos de orujo de oliva presentaban mayor inhibición del crecimiento de bacterias mesófilas y hongos filamentosos durante 5 días de almacenamiento a 4 ºC que las tratadas con ácido ascórbico, sin que verificara detección de coliformes durante los 12 días de almacenamiento, al contrario de lo observado empleando ácido ascórbico. En general, los extractos de orujo de oliva conservaron o mejoraron el contenido fenólico y el potencial antioxidante de los frutos, sin que se apreciaran cambios significativos en su textura, aunque con un ligero pardeamiento inicial de las muestras, que se puede asociar con el color aportado por la solución de extracto de orujo de oliva. Además, estos extractos parecían ser más efectivos cuando se combinaban con el envasado en bolsas de PLA biodegradable, de baja permeabilidad a gases, con respecto al ácido ascórbico y al envasado en bolsas de OPP convencionales.

Los resultados obtenidos permitían apoyar el potencial antimicrobiano de los extractos de orujo de oliva y su uso como antioxidante en productos mínimamente procesados en los que el color no es un factor condicionante para la evaluación y decisión del consumidor.

Las conclusiones alcanzadas pueden contribuir a una mejora en la rentabilidad y sostenibilidad de la industria del aceite de oliva, así como a reducir su impacto ambiental. Igualmente, aportan elementos a la industria alimentaria para el desarrollo de nuevos productos alimenticios con características funcionales.

foods

MDPI

Article

Effect of Olive Pomace Extract Application and Packaging Material on the Preservation of Fresh-Cut Royal Gala Apples

Joana Madureira [1,2,3,4], Bruno Melgar [2], Vítor D. Alves [5,*], Margarida Moldão-Martins [5], Fernanda M. A. Margaça [1,6], Celestino Santos-Buelga [4,7], Lillian Barros [2,3,*] and Sandra Cabo Verde [1,6]

1 Centro de Ciências e Tecnologias Nucleares (C2TN), Instituto Superior Técnico, Universidade de Lisboa, 2695-066 Loures, Portugal; joanamadureira@ctn.tecnico.ulisboa.pt (J.M.); fmargaca@ctn.tecnico.ulisboa.pt (F.M.A.M.); sandracv@ctn.tecnico.ulisboa.pt (S.C.V.)
2 Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; bruno.melgarc@ipb.pt
3 Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
4 Grupo de Investigación en Polifenoles (GIP-USAL), Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain; csb@usal.es
5 LEAF—Linking, Landscape, Environment, Agriculture and Food—Research Center, Instituto Superior de Agronomia (ISA), Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisbon, Portugal; mmoldao@isa.ulisboa.pt
6 Departamento de Engenharia e Ciências Nucleares, Instituto Superior Técnico, Universidade de Lisboa, 2695-066 Bobadela LRS, Portugal
7 Unidad de Excelencia Producción, Agrícola y Medioambiente (Agrienvironment), Parque Científico, Universidad de Salamanca, 37185 Salamanca, Spain
* Correspondence: vitoralves@isa.ulisboa.pt (V.D.A.); lillian@ipb.pt (L.B.)

Abstract: The efficiency of natural olive pomace extracts for enhancing the quality of fresh-cut apples was compared with commercial ascorbic acid and two different packaging films (biodegradable polylactic acid (PLA) and oriented polypropylene (OPP)) were tested. The composition of atmosphere inside the packages, the physicochemical parameters (firmness, weight loss and color), the microbial load, total phenolic content and antioxidant activity of fresh-cut apples were evaluated throughout 12 days of storage at 4 °C. After 12 days of refrigerated storage, a significant decrease in O_2 was promoted in PLA films, and the weight loss of the whole packaging was higher in PLA films (5.4%) than in OPP films (0.2%). Natural olive pomace extracts reduced the load of mesophilic bacteria (3.4 ± 0.1 log CFU/g and 2.4 ± 0.1 log CFU/g for OPP and PLA films, respectively) and filamentous fungi (3.3 ± 0.1 log CFU/g and 2.44 ± 0.05 log CFU/g for OPP and PLA films, respectively) growth in fresh-cut apples after five days of storage at 4 °C, and no detection of coliforms was verified throughout the 12 days of storage. In general, the olive pomace extract preserved or improved the total phenolic index and antioxidant potential of the fruit, without significant changes in their firmness. Moreover, this extract seemed to be more effective when combined with the biodegradable PLA film packaging. This work can contribute to the availability of effective natural food additives, the sustainability of the olive oil industries and the reduction of environmental impact. It can also be useful in meeting the food industries requirements to develop new functional food products.

Keywords: fresh-cut apples; storage; shelf life; natural extracts; packaging films; microbial quality

Citation: Madureira, J.; Melgar, B.; Alves, V.D.; Moldão-Martins, M.; Margaça, F.M.A.; Santos-Buelga, C.; Barros, L.; Cabo Verde, S. Effect of Olive Pomace Extract Application and Packaging Material on the Preservation of Fresh-Cut Royal Gala Apples. *Foods* **2023**, *12*, 1926. https://doi.org/10.3390/foods12091926

Academic Editor: Lubomir Lapcik

Received: 30 March 2023
Revised: 20 April 2023
Accepted: 3 May 2023
Published: 8 May 2023

1. Introduction

In recent years, changes in family lifestyles and growing health concerns among consumers have led to a growing demand for minimally processed foods, increasing market growth worldwide. Consumers are looking for fresh-like processed products, such as fruits, with high quality attributes (appearance, texture and flavor, among others) to satisfy their daily needs of antioxidants, minerals and dietary fibers [1].

Fruits, such as apples, are an excellent source of vitamins, minerals, fibers and antioxidants that are essential to reduce the risk of developing heart disease, cancer, inflammation and diabetes. In Portugal, apples are one of the most important fruit markets, with a consumption per capita that reached 30.5 kg in 2021 [2]. One of the main challenges of the fruit industries, including apple processing, is to maintain the post-harvest quality during storage, distribution and sale to the consumer in order to ensure that the fruit remains healthy and safe [3]. Minimal fruit processing can result in quality deterioration due to increased respiration rate, ethylene production and cut-surface browning, which implies water loss, softening and microbial contamination. As expected, minimally processed apples are more perishable than unprocessed whole apples, especially due to the break or elimination of natural protection systems and the susceptibility for undesirable enzymatic browning promoted by the oxidation of phenolic compounds by polyphenol oxidase, peroxidase or tyrosinase [4]. Moreover, yeast fermentation and mold spoilage can also occur on the surface of the slice [5]. Sulfites have been widely used as antibrowning agents, however, many adverse reactions to human health have been associated to their use, and they are forbidden by the Food and Drug Administration [6]. Thus, ascorbic acid, citric acid and some sulfur-containing amino acids have been introduced alone or in combination with firming or antimicrobial agents [7]. Recently, in order to replace synthetic antioxidants, natural additives from have been incorporated in food, such as meat, dairy and bakery products [8–11]. Olive wastes are considered valuable sources of natural phenolic compounds with health benefits, such as hydroxytyrosol, tyrosol, oleuropein and verbascoside [12,13], have been used as food ingredients to enrich bread, biscuits, pasta and meat [14–19]. Madureira et al. [20] and Difonzo et al. [21] reviewed the main applications of the phenolic compounds from olive pomace with high added value in food products. Lin et al. [17] used incorporated olive pomace extracts into biscuits and observed higher fiber abundance, nutritional quality and acceptability and lower calories and glycemic index. Additionally, Galanakis et al. [14] observed higher antimicrobial activity and extended shelf life of bread and rusks from 10 to 15 days when fortified with olive polyphenols (200 mg of polyphenols/kg), while the addition of olive leaf extracts to poultry meat reduced microbial growth while maintaining both chemical quality and sensory attributes, and extending the shelf life of the meat when refrigerated for 15 days [15].

The packaging is also very important to slow the respiration rate and prevent the growth of aerobic spoilage microorganisms in food products [5]. The most used food packaging materials are petroleum-based polymers such as polypropylene (PP) due to their low-cost and good barrier performances. These plastics are non-biodegradable and non-renewable, causing human and environmental risks [22]. Therefore, in recent years, special attention has been focused on biodegradable food packaging in search of alternative materials to replace these plastics. Biodegradable products are mainly produced from biopolymers, such as cellulose, chitosan, starch, polylactic acid (PLA), collagen and casein. To be used in the food industry, these materials must be non-toxic, renewable and possessing specific properties [23]. PLA is a biopolymer produced by bacterial fermentation of renewable resources such as corn or sugar beets [23], with the advantage of being compostable and biocompatible and having high mechanical strength [22].

Ionizing radiation is a clean and environmentally friendly technology that does not require the addition of chemicals. It is evidenced to be capable of enhancing the phenolic compounds extraction and antioxidant activity on industrial wastewater [24], fresh fruits such as cherry tomatoes [25], raspberries [26], and strawberries [27] and dried medicinal plants [28]. More recently, the authors demonstrated the suitability of gamma radiation at 5 kGy to improve the extractability of phenolic compounds from olive pomace by two-fold compared to the non-irradiated samples [12]. The optimized conditions to obtain extracts with higher bioactivities were also achieved using heat-assisted extraction [13]. Among other properties, these natural extracts presented higher antioxidant and antimicrobial potentials, which could indicate the possibility of using them as alternatives to synthetic food preservatives.

The aim of this work was to evaluate the use of irradiated olive pomace extracts as natural ingredients to extend shelf life of fresh-cut apples by maintaining quality attributes and increasing bioactive properties. Two different packaging films (biodegradable polylactic acid (PLA), and oriented polypropylene (OPP)) were used to compare both performances. The comparison with ascorbic acid, a commercial antioxidant used by food industries, was assessed, and physicochemical parameters (firmness, weight loss and color), atmospheric composition inside the packages and the microbial load throughout 12 days of storage at 4 °C were evaluated. Moreover, the phenolic content and antioxidant activity of the fruits were assessed throughout the 12 days of storage. As far as the authors know, this is the first study to use an extract of this agro-industrial residue as additive for fresh-cut fruits to enhance their quality. The obtained results could also represent a strategy to promote the sustainability of the olive oil industry and contribute to the promotion of a circular economy.

2. Materials and Methods

2.1. Chemicals

Methanol and ethanol were acquired from Panreac AppliChem (Darmstadt, Germany) and Honeywell (Charlotte, NC, USA), respectively. Gallic acid, iron chloride, Folin-Ciocalteau reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA), and ascorbic acid and ferrous sulphate were obtained from Merck (Darmstadt, Germany). Acetic acid, hydrochloric acid and sodium acetate were provided by Honeywell-Riedel-de Haën (Charlotte, NC, USA). Sodium carbonate was acquired from JMGS (Odivelas, Portugal), and 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) was obtained from Honeywell-Fluka (Charlotte, NC, USA). Tryptic Soy Agar (TSA), Malt Extract Agar (MEA) and Violet Red Bile Agar (VRBA) were obtained from Oxoid—Thermo Scientific (Waltham, MA, USA). Water was treated in a Milli-Q water purification system (Merck Millipore, Burlington, MA, USA).

2.2. Olive Pomace Samples and Irradiation Experiments

The samples used in this work were olive pomaces collected in November 2020 from UCASUL (União de Cooperativas Agrícolas do Sul, located in the Alentejo region, Portugal) and further subjected to gamma radiation treatment in a Co-60 semi-industrial unit (with an activity of 126 kCi in March 2021) located at Technological Unit of Radiosterilization (UTR-IST), University of Lisbon (Portugal). Sealed bags (20 cm × 10 cm) containing 100 g of extracted olive pomace were irradiated at room temperature at 5.2 ± 0.2 kGy using a dose rate of 10.4 kGy/h. The absorbed doses were measured using Amber Perspex routine dosimeters [29] (dose uniformity DUR = 1.07). The irradiation experiments were performed in triplicate.

A schematic diagram of the experimental steps performed in this research is shown in Figure 1.

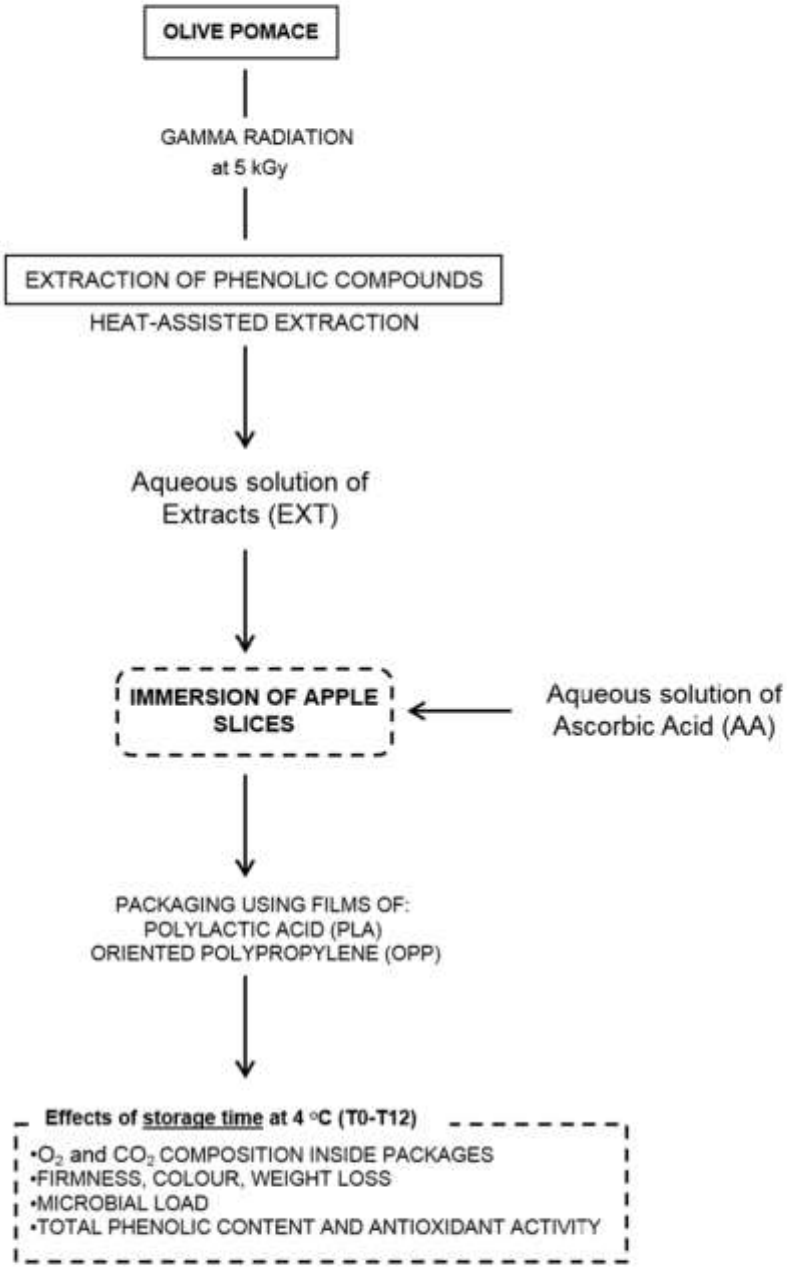


Figure 1. Schematic diagram of the experimental procedure of this work.

2.3. Olive Pomace Natural Ingredients: Phenolic Extract Preparation

After gamma radiation, the phenolic compounds from olive pomace were extracted using the optimal conditions of heat-assisted extraction explored by Madureira et al. [30]. Briefly, 7 g of olive pomace were extracted in 240 mL of 76% ethanol for 120 min at 85 °C. These extraction conditions were selected due to the improved bioactive properties obtained

for these extracts that were evaluated in a previous work of the authors [13]. The extractions were performed in triplicate.

2.4. Polylactic Acid (PLA) and Oriented Polypropylene (OPP) Films Packages

The film materials used to produce the packages for this study were polylactic acid (PLA) and oriented polypropylene (OPP).

PLA films in bag form were manufactured by the company Vegware (Edinburgh, UK). These biodegradable films were produced from corn starch and included a transparent side (thickness = 0.029 mm) and an opaque white side (thickness = 0.039 mm). The films had a permeability to O_2 of 2.97×10^{-16} m^2/s, a permeability to CO_2 of 1.22×10^{-15} m^2/s and a permeability to water vapor of 1.10×10^{-12} $mol \cdot m/m^2 \cdot s \cdot Pa$.

Conventional commercial film made of OPP was supplied by Campotec S.A (Torres Verdras, Portugal) and manufactured by Pigmea S.L. (Jaén, Spain). This film has a thickness of 0.030 mm, a permeability to O_2 of 2.68×10^{-12} m^2/s, and a permeability to water vapor of 8.75×10^{-14} $mol \cdot m/m^2 \cdot s \cdot Pa$.

Both types of film bags (10 × 10 cm) were manually constructed and further used as described below.

2.5. Preparation of Fresh-Cut Apples

Apples (Royal Gala variety) of uniform shape and size were purchased from a local supermarket in Lisbon, Portugal, and immediately stored at 4 ± 1 °C until analysis. The fruits were washed/disinfected with sodium hypochlorite solution (60 mg free chlorine/L) for 1 min and air-dried. Then, apples were cut with a disinfected kitchen knife into equal slices (8 slices per apple), and three groups of samples were prepared by immersion for one minute. The control samples were immersed in water, one experimental group was immersed in olive pomace extract solution (0.315%, *w/v*) and a second group was immersed in ascorbic acid solution (0.315%, *w/v*). A concentration of 0.315% *w/v* was applied to the samples based on preliminary experiments demonstrating that this concentration presented the highest antioxidant activity without significantly changing the color of apple slices upon application. After immersion in their respective solutions, the samples were air-dried and sealed in PLA or OPP film bags, then stored in controlled chambers at 4 °C and 85% relative humidity.

The fruits were analyzed over time (after 0, 2, 5, 7, 9 and 12 days of storage at 4 °C), in terms of surface color, weight loss, firmness, microbial load, phenolic content and antioxidant activity. The composition of the atmosphere inside the fruit packages was also measured over time. Three independent packages were analyzed for each group on each day. For physicochemical parameters (firmness, color and weight loss) and package gas composition, the control samples were fruits immersed in ascorbic acid (PLA-AA and OPP-AA). For antioxidant activity and microbial load evaluation, the control samples were apple slices with no treatment (PLA-NT and OPP-NT).

2.6. Analytical Methods

2.6.1. Soluble Solids Content, Titratable Acidity and Respiration Rate

In the initial stage of the work, a physicochemical characterization of the apple samples was performed. Total soluble solids (TSS) and titratable acidity (TA) were determined for apples using the pulp of four fruits. TSS measurements (average of six measurements) were performed with a hand-held refractometer ATAGO (Atago Co, Ltd., Tokyo, Japan), and the results were expressed as °Bx. TA was determined by titration and expressed as % of malic acid (average of three measurements). Respiration rate (RT; mmol CO_2/kg·h) was measured using a gas analyzer (Checkmate 9900, PBI Dansensor, Ringsted, Denmark), as described by Vieira, Moldão-Martins and Alves [31].

2.6.2. Composition of the Atmosphere Inside the Packages

The concentration of carbon dioxide (CO_2) and oxygen (O_2) inside the packages was measured using a gas analyzer (Checkmate 9900, PBI Dansensor, Ringsted, Denmark) [32]. An adhesive silicon septum was glued to the sampling point of the packages to prevent gas leakage during analysis. The needle of the gas analyzer was inserted through the septum, and results were expressed in percentages of O_2 and CO_2. Three packages per group were analyzed on each test day.

2.6.3. Weight Loss, Firmness and Surface Color

The physicochemical parameters were measured according to Vieira et al. [32]. The weight loss (% from the original weight) from each sealed tray was measured using an electronic balance (TC-403, Denver Instrument Company, Vernon Hills, IL, USA). The results were expressed as an average of three replicates per group for each test day.

A Texturometer (TA-XT2, Stable Micro System, Surrey, UK) with a 5 kg load cell was equipped with a flat probe (37 mm diameter) and used to evaluate the firmness of the apple slices [32]. A total of 9 slices from each package and each group (per test day) were assayed. Each fruit was positioned under the probe and compressed to 80% deformation at a speed of 1 mm/s.

A Konica Minolta CTR-300 colorimeter (Minolta, Williams Drive Ramsey, NJ, USA) was used to measure L* (lightness), a* (red–green) and b* (yellow–blue) color parameters of apple slices. The colorimeter was calibrated using a standard white plate provided by the manufacturer. The color of the samples was measured on both sides of the apple slices from each package and each group on each test day (a total of 18 measurements per package and group). Furthermore, the color of the apple slices was expressed as the hue angle (h°), total color differences (ΔE) and chroma (C) related to saturation [31], which were calculated from the parameters represented by the following Equations (1)–(5):

$$C^* = \left(a^{*2} + b^{*2}\right)^{1/2} \quad (1)$$

$$h^\circ = \arctan\left(\frac{b^*}{a^*}\right) \times \frac{180}{\Pi}, \text{ if } a^* > 0 \text{ and } b^* > 0 \quad (2)$$

$$h^\circ = \arctan\left(\frac{b^*}{a^*}\right) \times \frac{180}{\Pi} + 180, \text{ if } a^* < 0 \quad (3)$$

$$h^\circ = \arctan\left(\frac{b^*}{a^*}\right) \times \frac{180}{\Pi} + 360, \text{ if } a^* > 0 \text{ and } b^* < 0 \quad (4)$$

$$\Delta E = \left[(\Delta L)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]^{1/2} \quad (5)$$

2.6.4. Microbial Load

Apple slices from each package (~150 g) were placed in sterile stomacher bags containing 100 mL of buffered peptone water. Samples (n = 3, per each package, group and test day) were homogenized using a stomacher (Stomacher 3500; Seaward, UK) for 15 min. Serial decimal dilutions were prepared for inoculation in triplicate on Tryptic Soy Agar plates (TSA) for mesophilic microbial counts, Malt Extract Agar (MEA) plates for filamentous fungi counts and Violet Red Blue Agar (VRBA) plates for coliforms counts. TSA plates were incubated at 30 °C, MEA plates at 25 °C and VRBA plates at 37 °C. The colony numbers were counted for 7 days. The results were expressed as the log of colony-forming units per gram of fresh fruit (log CFU/g).

2.6.5. Total Phenolic Content and Antioxidant Activity

For the quantification of the total phenolic content and the antioxidant activity, extracts were obtained from freeze-dried apple slices using the same methodology described by

Madureira et al. [12], with an ethanol:water mixture (80:20 *v*/*v*) used as a solvent. The dry extracts were obtained after lyophilisation and used for all the analyses.

Total Phenolic Content (TP) was determined using the Folin-Ciocalteau method [33] using extract solutions with a concentration of 20 mg/mL. Absorbance was measured at 765 nm using a spectrophotometer (Shimadzu UV 1800, Kyoto, Japan), and the results were expressed as mg of gallic acid equivalents (GAE) per gram of extract. The assay was carried out in triplicate for each package, group and test day.

Antioxidant activity was evaluated using two assays: DPPH radical scavenging activity described by Brand-Williams, Cuvelier and Berset [34] with some modifications, and Ferric Reducing Antioxidant Power (FRAP) described by Benzie and Strain [35]. For the DPPH method, the extracts were dissolved in distilled water at a concentration of 40 mg/mL and then successive dilutions were prepared (40–1.25 mg/mL). The reduction of the DPPH radical was determined by measuring the absorption at 515 nm using an EZ Read 1200 Microplate Reader (Biochrom, Cambridge, UK). The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the following equation: $\%RSA = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract had been added and A_{DPPH} is the absorbance of the DPPH solution. Ascorbic acid was used as standard. The results were expressed in terms of IC_{50} values (mg/mL), indicating the extract concentrations that provided 50% of antioxidant activity [36]. For FRAP assay, the extracts were dissolved in distilled water at a concentration of 5 mg/mL. The reduction of the ferric ion (Fe^{3+})–ligand complex to the intensely blue-colored ferrous (Fe^{2+}) was measured at 593 nm using a spectrophotometer (Shimadzu UV 1800, Kyoto, Japan). The results were expressed as mmol of ferrous sulfate equivalent (FSE) per gram of extract. Both assays were performed in triplicate for each package, group and test day.

2.7. Statistical Analysis

Data were expressed as means ± standard error. For statistical analysis, confidence intervals for mean values were estimated considering a significance level of $p < 0.05$ and the number of replicates for each assay. The differences among treatments were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's HSD test with $\alpha = 0.05$.

2.8. Principal Components and Overlayed K-Means Clustering

The data collected from the previous analysis (included in the repository uploaded as Supplementary Materials to this work) were subjected first to mean transformation (replicate measurements) and then to data imputation using the following approaches: (1) considering the means of different observations when the data were scarce; (2) interpolations of data were performed using linear regressions; (3) one extrapolation was made using a polynomial regression employing data from similar conditions to avoid over- or under-fitting of the extrapolated value. Afterwards, the imputed dataset was used for the principal component analysis (PCA) computation considering 3 principal components represented on 2 dimensions using a conversion of the 3rd PC to factorial data. Additionally, after a first approach considering all the responses, lower variability responses (L*, a*, b*, C, firmness, DPPH and FRAP) were removed and processed again through PCA. Then, K-means clustering was overlaid on the PCA data in order to compute the optimal number of clusters relying in the average silhouette width method. The software employed was R studio version 2022.12.0 + 353. The packages and scripts are also provided in the Supplementary Materials.

3. Results and Discussion

3.1. Physicochemical Characterization of the Samples

The titratable acidity (TA) and the total soluble solids (TSS) of the apple samples were 0.23% malic acid and 12.2 °Bx, respectively, which are in accordance with the results reported in the literature for different cultivars of apples and different processing frac-

tions [37–44]. The respiration rate was assessed by evaluating the CO_2 production, which can provide essential information about the fruit's metabolic activity. In this work, the apple slices in cold storage (4 °C) showed a moderate respiration rate (11 mmol CO_2/kg·h), which significantly decreased with increasing storage time.

3.2. Composition of the Atmosphere Inside the Package

The O_2 and CO_2 contents inside the packages were recorded throughout the storage time (Figure 2).

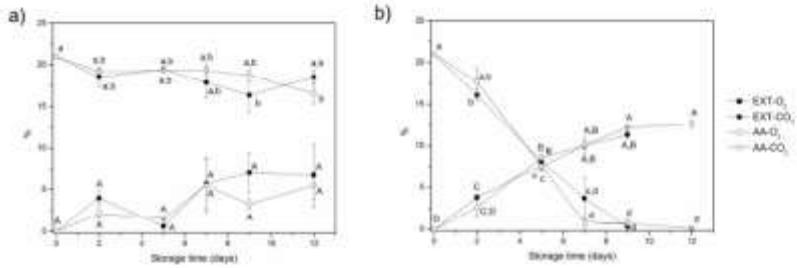


Figure 2. Variation of headspace gas composition (% O_2 and % CO_2) inside the packages of apple slices stored for 12 days at 4 °C in: (**a**) OPP films and (**b**) PLA films (OPP—oriented polypropylene; PLA—polylactic acid; EXT—extract of olive pomace; AA—ascorbic acid). Vertical bars indicate 95% confidence intervals of three replicates. Means with equal letters (uppercase letters for CO_2 and lowercase letters for O_2) are not statistically different by Tukey's test with a 5% significance level.

Considering the internal volume (81 mL) that was not occupied by the apples to be constant, the variation in the atmosphere inside the packages was created by the respiration rate of the fruits, the metabolic activity of microbial development and the permeability of the films to O_2 and CO_2, which can be affected by the temperature. As expected, a decrease in the headspace O_2 and an increase in the headspace CO_2 during the storage time were observed (Figure 2). In general, no significant differences ($p > 0.05$) were observed when comparing the samples treated with olive extracts and ascorbic acid for each film packaging: OPP (Figure 2a) or PLA (Figure 2b). Nevertheless, the atmosphere composition during storage at 4 °C varied depending on the film used. The content of O_2 inside the OPP packages did not significantly change ($p > 0.05$) during the storage time, whereas for the PLA film, a marked decrease was noticeable with storage, and it was significantly different ($p < 0.05$) from the OPP film after five days of storage. The same trend was observed for the CO_2 content. No significant differences ($p > 0.05$) were detected in CO_2 content throughout the storage time when using OPP films (Figure 2a), whereas the PLA films promoted a significant increase in CO_2 content (Figure 2b). These results revealed that PLA films are poorly permeable to gases, confirming their lower permeability to O_2 (2.97×10^{-16} m^2/s) and CO_2 (1.22×10^{-15} m^2/s) compared to OPP films (O_2 permeability: 2.68×10^{-12} m^2/s). Thus, the O_2 decrease in PLA films was mostly due to its consumption by the fruit respiration.

3.3. Weight Loss, Firmness and Surface Color

Due to their high water content (80–85%), Royal Gala apples present a high tendency to decrease in mass due to water loss caused by transpiration and respiration processes during storage. The weight loss of the whole package containing the fresh-cut apples in both package films was evaluated over 12 days of storage at 4 °C (Figure 3).

These results indicated that the percentage (%) of weight loss of the packaged apples in PLA film significantly increased ($p < 0.05$) with storage time, whereas no significant differences ($p > 0.05$) were observed in apples packaged in OPP film. After 12 days of refrigerated storage, the weight loss was higher in PLA films (5.4%) than in OPP films (0.2%) (Figure 3). These results demonstrated the higher permeability of PLA films to water, which was corroborated by the higher water vapor permeability of these biodegradable

films (1.10×10^{-12} mol·m/m^2·s·Pa) compared to OPP films (8.75×10^{-14} mol·m/m^2·s·Pa). These results are in agreement with those reported by González-Buesa et al. [45], who described a weight loss of approximately 4% in fresh-cut celery after 21 days of storage using PLA compared to non-biodegradable bags. Zhou et al. [46] also observed a higher weight loss of fresh-cut melon when using PLA containers compared to PET.

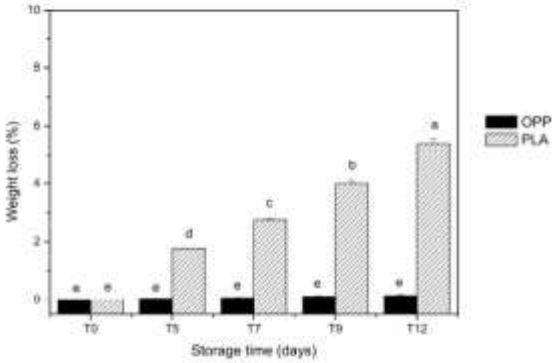


Figure 3. Changes in weight loss of packaged apple slices throughout 12 days of storage at 4 °C (OPP—oriented polypropylene; PLA—polylactic acid). Vertical bars indicate 95% confidence intervals of three replicates. Means with equal letters are not statistically different, as determined by Tukey's test with a 5% significance level. T0—immediately after packaging; T2–T12—2–12 days after packaging and storage at 4 °C.

Maintaining firmness is one of the most important physical attributes for ensuring the quality of fruits. In general, the firmness of fresh-cut apples appeared to be preserved, and no significant differences ($p > 0.05$) were observed between the firmness of apple slices stored in either package film and the tested antioxidant during storage at 4 °C (Figure 4). González-Buesa et al. [45] also reported that both PLA and petroleum-based plastic films maintained the firmness of celery during 14 days of storage.

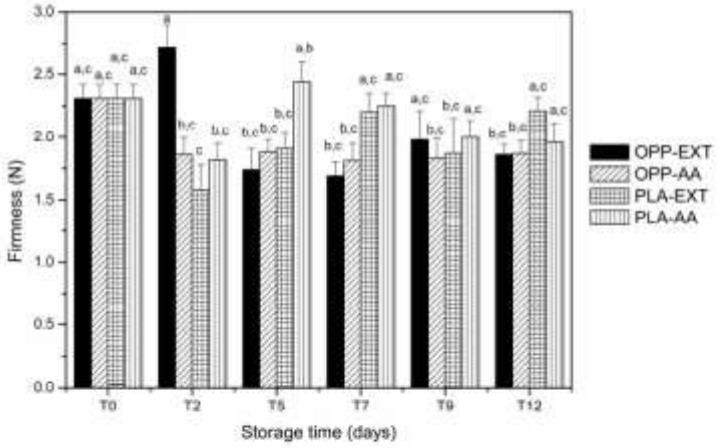


Figure 4. Changes in the firmness of packaged apple slices during 12 days of storage at 4 °C (OPP—oriented polypropylene; PLA—polylactic acid; EXT—extract of olive pomace; AA—ascorbic acid). Vertical bars indicate 95% confidence intervals of nine replicates. Means with equal letters are not statistically different, as determined by Tukey's test with a 5% significance level. T0—immediately after packaging; T2–T12—2–12 days after packaging and storage at 4 °C.

The color of the fruit is another important organoleptic factor, as it can influence customer acceptance. The color characteristics of the fresh-cut apples incorporated with olive pomace extract and ascorbic acid and packaged with PLA and OPP films were evaluated as a function of storage time using the CIEL*a*b* color space (Table 1).

Table 1. Color parameters of PLA and OPP packaged fresh-cut apples for 12 days at 4 °C.

					L*		
		T0	**T2**	**T5**	**T7**	**T9**	**T12**
OPP	EXT	$79.7 \pm 0.2^{a,A}$	$77.09 \pm 0.47^{b,B}$	$74.3 \pm 0.3^{c,C}$	$74.9 \pm 0.4^{c,C}$	$74.04 \pm 0.47^{c,C}$	$74.3 \pm 0.5^{c,C}$
	AA	$79.4 \pm 0.2^{a,A}$	$78.6 \pm 0.3^{a,b,A}$	$77.4 \pm 0.3^{b,c,B}$	$76.9 \pm 0.4^{c,B}$	$77.2 \pm 0.4^{c,B}$	$76.8 \pm 0.3^{c,B}$
PLA	EXT	$79.7 \pm 0.2^{a,A}$	$76.97 \pm 0.22^{b,c,B}$	$76.1 \pm 0.5^{c,B}$	$77.3 \pm 0.3^{b,c,A,B}$	$77.6 \pm 0.3^{b,A,B}$	$76.7 \pm 0.3^{b,c,B}$
	AA	$79.4 \pm 0.2^{a,A}$	$78.5 \pm 0.3^{a,A}$	$78.9 \pm 0.2^{a,A}$	$78.5 \pm 0.2^{a,A}$	$78.6 \pm 0.3^{a,A}$	$78.7 \pm 0.3^{a,A}$
					C		
		T0	T2	T5	T7	T9	T12
OPP	EXT	$20.9 \pm 0.2^{c,A}$	$29.1 \pm 0.6^{b,A}$	$28.6 \pm 0.4^{b,A}$	$29.4 \pm 0.5^{b,A}$	$32.2 \pm 0.5^{a,A}$	$32.1 \pm 0.4^{a,A}$
	AA	$19.7 \pm 0.2^{c,B}$	$24.9 \pm 0.5^{a,b,B}$	$22.9 \pm 0.5^{b,C}$	$24.1 \pm 0.5^{a,b,C}$	$23.5 \pm 0.5^{b,C}$	$25.3 \pm 0.4^{a,C}$
PLA	EXT	$20.9 \pm 0.2^{b,A}$	$26.6 \pm 0.5^{a,B}$	$25.8 \pm 0.5^{a,B}$	$25.8 \pm 0.4^{a,B}$	$26.1 \pm 0.3^{a,B}$	$27.01 \pm 0.40^{a,B}$
	AA	$19.7 \pm 0.2^{d,B}$	$25.4 \pm 0.5^{a,B}$	$23.2 \pm 0.4^{b,c,C}$	$22.8 \pm 0.3^{c,C}$	$23.1 \pm 0.4^{b,c,C}$	$24.5 \pm 0.4^{a,b,C}$
					h°		
		T0	T2	T5	T7	T9	T12
OPP	EXT	$99.3 \pm 0.1^{a,A}$	$87.6 \pm 0.4^{c,D}$	$89.3 \pm 0.3^{b,C}$	$89.4 \pm 0.4^{b,C}$	$89.3 \pm 0.3^{b,C}$	$87.9 \pm 0.3^{b,c,B}$
	AA	$99.6 \pm 0.3^{a,A}$	$93.4 \pm 0.4^{b,B}$	$93.5 \pm 0.4^{b,A}$	$92.7 \pm 0.4^{b,A}$	$92.6 \pm 0.4^{b,A}$	$92.7 \pm 0.3^{b,A}$
PLA	EXT	$99.3 \pm 0.1^{a,A}$	$91.6 \pm 0.3^{b,C}$	$91.5 \pm 0.3^{b,B}$	$90.7 \pm 0.2^{b,B}$	$90.7 \pm 0.3^{b,B}$	$89.5 \pm 0.4^{c,B}$
	AA	$99.6 \pm 0.3^{a,A}$	$95.8 \pm 0.4^{b,A}$	$94.7 \pm 0.3^{b,c,A}$	$93.6 \pm 0.2^{c,d,A}$	$93.5 \pm 0.3^{c,d,A}$	$93.1 \pm 0.4^{d,A}$
					ΔE		
		T0	T2	T5	T7	T9	T12
OPP	EXT	-	$11.4 \pm 0.7^{a,b,A}$	$10.3 \pm 0.5^{b,A}$	$11.8 \pm 0.4^{a,b,A}$	$11.8 \pm 0.7^{a,b,A}$	$14.1 \pm 0.7^{a,A}$
	AA	-	$5.1 \pm 0.6^{a,B}$	$4.3 \pm 0.5^{a,C}$	$5.4 \pm 0.6^{a,C,B}$	$5.2 \pm 0.5^{a,B,C}$	$6.4 \pm 0.5^{a,B,C}$
PLA	EXT	-	$7.07 \pm 0.47^{a,B}$	$7.4 \pm 0.7^{a,B}$	$6.3 \pm 0.5^{a,B}$	$6.7 \pm 0.3^{a,B}$	$8.02 \pm 0.46^{a,B}$
	AA	-	$5 \pm 1^{a,B}$	$4.2 \pm 0.5^{a,C}$	$4.01 \pm 0.30^{a,C}$	$4.7 \pm 0.6^{a,C}$	$5.5 \pm 0.5^{a,C}$

PLA—polylactic acid; OPP—oriented polypropylene; EXT—extract of olive pomace; AA—ascorbic acid; T0—immediately after packaging; T2–T12—2–12 days after packaging and storage at 4 °C. Results are presented as means ± standard error. For each color parameter, the means in each row with the same letters ($^{a-d}$) and the means in each column with the same letters ($^{A-D}$) are not significantly different ($p > 0.05$).

For all the samples prepared with olive pomace extract, L* was significantly reduced after two days (T2) of refrigeration, remaining stable throughout the storage time. However, for samples in OPP film (OPP-EXT), a decrease in L* was observed in the first five days (T5) of storage, and then remained constant. For samples prepared with ascorbic acid, no significant variation ($p > 0.05$) was observed in the fruits packaged in PLA film (PLA-AA) throughout 12 days of storage. In contrast, for the samples packaged in OPP film (OPP-AA), a slight decrease was observed only after five days (T5). After five days of refrigerated storage, significant differences ($p < 0.05$) in L* were detected between OPP and PLA in each antioxidant-treated apple. Based on the calculations, the samples at T0 presented the lowest values of Chroma (C), and refrigerated storage for two days (T2) promoted an increase of the samples' brightness (Table 1). After this, the C-values of the tested samples seemed to be preserved, although a significant increase ($p < 0.05$) was observed at T9 (32.2 ± 0.5) and T12 (32.1 ± 0.4) for olive-pomace samples in OPP film (OPP-EXT). When comparing the different treatments with antioxidant solutions and the packaging during storage, the highest values were always observed for OPP-EXT. Regarding the hue angle (h°), T0 samples had higher values, ~100°, which characterized the yellow color of the apple slices. At T2, a slight but significant decrease ($p < 0.05$) was observed in the h° of all samples, which was maintained during the storage (Table 1), which could indicate a change to slight orange colors. The lowest values were perceived for samples with olive

pomace using both films (OPP-EXT and PLA-EXT). Finally, the browning of the fruits was determined by the ΔE values obtained for each studied condition (Table 1). The results showed that after two days (T2) of refrigerated storage, an increase in the browning of the apple slices was noticed, which could be related to an increase in a* and b* values and the decrease in L*. After this, no variation in the browning of the apple slices was observed during storage (Table 1). Furthermore, no significant differences ($p > 0.05$) were observed between the packaging films when ascorbic acid was used. On the other hand, based on the significant differences ($p < 0.05$) between the browning for both films after two days of storage (7.07 ± 0.47 for PLA and 11.4 ± 0.7 for OPP) and throughout the storage time, the biodegradable PLA films seemed to be a better choice when using olive pomace extract as additive, showing that the natural extract may be more effective in packages with less O_2. Similar results were obtained by Rocha and Morais [43], who also determined that two different periods of lightness decrease over time in Jonagored apples. The first period was observed in the first three days of storage, whin L* and h° decreased and a* increased due to the consumption of substrates by polyphenol oxidase, and the second period was described between the third and seventh day of storage as a preservation of browning. Furthermore, Song et al. [47] also described a marked change of color in fresh-cut apples during the early stage of storage that was maintained thereafter. During the minimal processing operations, such as cutting, an increase in respiration in the fruits is promoted. This process can induce the activation of polyphenol oxidase enzyme, which will react with the phenolic compounds to promote a loss of the natural color [48]. Furthermore, it is important to highlight that the initial browning of the samples using natural olive pomace extract (in the first two days of cold storage) can be associated with the natural color of the extract solution.

3.4. Microbial Load

Evaluating the microbial load is also crucial for assessing the quality and safety of fresh-cut fruits. The mesophilic bacteria, filamentous fungi and coliforms populations of the packaged fresh-cut apples packaged in both films, and after the addition of the tested antioxidants, were assessed immediately after different periods, specifically after packaging (T0) and after five days (T5) and 12 days (T12) of refrigerated storage (Table 2). This evaluation aimed to evaluate the effectiveness of PLA and OPP films and the performance of the olive pomace extract in promoting fruit quality compared to the commercial antioxidant ascorbic acid and the control samples.

Table 2. Microbial load on PLA- and OPP-packaged fresh-cut apples after 12 days of storage at 4 °C.

		Mesophilic Bacteria (Log CFU/g)			Filamentous Fungi (Log CFU/g)			Coliforms (Log CFU/g)		
		T0	T5	T12	T0	T5	T12	T0	T5	T12
OPP	EXT	$3.5 \pm 0.1^{b,B}$	$3.4 \pm 0.1^{b,D}$	$5.2 \pm 0.1^{a,A}$	$2.5 \pm 0.1^{c,C}$	$3.3 \pm 0.2^{b,C}$	$5.25 \pm 0.05^{a,A}$	n.d.	n.d.	n.d.
	AA	$3.2 \pm 0.1^{c,B,C}$	$4.3 \pm 0.1^{b,B}$	$5.6 \pm 0.1^{a,A}$	$3.2 \pm 0.1^{c,B}$	$4.03 \pm 0.08^{b,B}$	$5.25 \pm 0.04^{a,A}$	n.d.	$2.0 \pm 0.3^{b,A}$	$3.1 \pm 0.1^{a,A}$
	NT	$3.3 \pm 0.1^{c,B,C}$	$5.2 \pm 0.1^{b,A}$	$5.7 \pm 0.1^{a,A}$	$2.9 \pm 0.2^{c,B,C}$	$>6^{a,A}$	$5.18 \pm 0.04^{b,A}$	2.2 ± 0.1^{a}	$2.2 \pm 0.1^{a,A}$	n.d.
PLA	EXT	$2.9 \pm 0.1^{b,B,C}$	$2.8 \pm 0.1^{c,E}$	$3.75 \pm 0.03^{a,C}$	$2.4 \pm 0.1^{b,C}$	$2.46 \pm 0.05^{b,D}$	$3.3 \pm 0.1^{a,C}$	n.d.	n.d.	n.d.
	AA	$2.9 \pm 0.1^{c,C}$	$3.90 \pm 0.06^{b,C}$	$4.2 \pm 0.1^{a,B}$	$2.8 \pm 0.1^{b,B,C}$	$3.84 \pm 0.03^{a,B}$	$4.2 \pm 0.2^{a,B}$	n.d.	$1.8 \pm 0.1^{a,A}$	$1.6 \pm 0.2^{a,B}$
	NT	$4.63 \pm 0.03^{b,A}$	$4.5 \pm 0.1^{b,B}$	$5.80 \pm 0.01^{a,A}$	$4.44 \pm 0.05^{b,A}$	$4.02 \pm 0.07^{c,B}$	$5.3 \pm 0.1^{a,A}$	n.d.	$1.5 \pm 0.1^{a,A}$	$1.3 \pm 0.3^{a,B}$

PLA—polylactic acid; OPP—oriented polypropylene; EXT—extract of olive pomace; AA—ascorbic acid; NT—non-treated samples; T0—immediately after packaging; T5—5 days after packaging and storage at 4 °C; T12—12 days after packaging and storage at 4 °C; n.d.—not detected. Results are presented as means ± standard error. For each microbial group, the means in each row with the same letters ($^{a-c}$) and means in each column with the same letters ($^{A-E}$) are not significantly different ($p > 0.05$).

The mesophilic bacteria population evaluated on the day of packaging and antioxidants addition (T0) was not significantly different ($p > 0.05$) between films, antioxidants and control samples, except for the control samples packaged in PLA film (4.63 ± 0.03 log CFU/g) (Table 2). The fresh-cut apples presented an aerobic mesophilic population ranging from 2.9 ± 0.2 log CFU/g to 4.63 ± 0.03 log CFU/g, which was in agreement with the previously reported results ranging from 2 to 4 log CFU/g [49,50]. On the other hand, the obtained counts were lower than those described by Graça et al. [51] for fresh-cut apple samples (3.3 to 8.9 log CFU/g). After storage for five and 12 days, the variation in mesophilic bacteria population in the packaged

fresh-cut apples depended on the film packaging used. In general, the PLA film was more efficient in inhibiting bacteria growth (T5 and T12), for both olive pomace extract and ascorbic acid compared to the control samples. On the other hand, the use of olive pomace extracts seemed to inhibit bacteria growth during five days of storage (T5) compared to ascorbic acid, not only for PLA film (2.4 ± 0.1 log CFU/g using olive pomace extracts and 3.90 ± 0.04 log CFU/g using ascorbic acid), but also for OPP film (3.4 ± 0.1 log CFU/g using olive pomace extracts and 4.3 ± 0.1 log CFU/g using ascorbic acid). After 12 days of refrigerated storage, a significant ($p < 0.05$) increase in the bacterial contamination of apple slices was detected (Table 2). Concerning the legislation in Portugal, the recommended limits of mesophilic bacteria in ready-to-eat foods is 6 log CFU/g. Thus, neither of the control samples complied with these limits (5.7 ± 0.1 log CFU/g for OPP film and 5.8 ± 0.0 log CFU/g for PLA film). The samples using ascorbic acid as additive and OPP film as packaging bag also had higher bacterial contamination (5.6 ± 0.1 log CFU/g). Furthermore, it was possible to conclude that the olive pomace extract was able to delay the growth of bacteria after 12 days of storage using PLA film (3.75 ± 0.03 log CFU/g).

Regarding the filamentous fungi, the counts in fresh-cut apples were between 2.4 ± 0.1 and 4.44 ± 0.05 log CFU/g (Table 2). An increase in filamentous fungi counts was observed in the stored samples, except for the sample treated with olive pomace extract and packaged in PLA film (PLA-EXT) after five days of storage (2.44 ± 0.05 log CFU/g), which did not change from the beginning of the process (2.4 ± 0.1 log CFU/g). It was evident that olive pomace extracts were more efficient in inhibiting the growth of filamentous fungi compared to the samples treated with ascorbic acid and to control samples, particularly those sealed in PLA film. The counts of filamentous fungi population in ready-to-eat foods were limited to 2.7 log CFU/g, showing that the samples stored for five days in the PLA bag using the natural extract as additive could be considered of adequate quality in this regard.

Concerning coliforms, although the concentration complied with the recommended limits (4 log CFU/g), the results indicated that olive pomace extracts were able to inhibit the growth of coliforms, unlike ascorbic acid (Table 2). In a previous study, Graça et al. [51] had observed higher levels (between 1.8 and 7.6 log CFU/g) of coliforms in fresh-cut apples.

The overall results demonstrated that the use of olive pomace extracts could be more advantageous in inhibiting microbial growth in apple slices stored in either film than the samples treated with ascorbic acid. Furthermore, evaluating the different materials, PLA also showed higher inhibition of the microbial growth than OPP, and the storage for five days in cold temperatures could be proposed to ensure the quality of the fruits. The high antimicrobial activity of these extracts had been reported before by the authors, not only against Gram-negative and Gram-positive bacteria, but also against fungi [11,12]. Botondi et al. 2015 [52] and González-Buesa [45] described the same microbial growth trends when using both PLA and conventional plastics for fresh-cut produce packaging, which suggested that the results obtained in this work could be attributed to the combined effect of olive pomace extract and PLA film. In fact, the potential of PLA films as a suitable alternative to non-biodegradable plastics in preserving the quality and safety of fresh-cut fruits and vegetables was previously well-demonstrated [45,46,52,53].

3.5. Total Phenolic Content and Antioxidant Activity

The results of total phenolic content (TP) of the extracts of fresh-cut apples using water (control), olive pomace and ascorbic acid as antioxidants and packaged in PLA and OPP films, immediately after packaging (T0) and after two (T2), five (T5), seven (T7), nine (T9) and 12 days (T12) of storage, are presented in Table 3.

Phenolic compounds are important substances with significant contributions to the nutritional and sensory quality of fruits, and they present benefits to human and animal health. The higher values of TP for all the studied samples were observed at T12; although, when compared to T0, only a slight but significant ($p < 0.05$) increase in TP for samples with ascorbic acid (OPP-AA and PLA-AA) were detected. Nevertheless, no significant differences ($p > 0.05$) were noticed between the two antioxidants (olive pomace and ascorbic

acid) and films (PLA and OPP) at T12. The overall results suggest that natural olive pomace extracts can be used as an ingredient to extend the shelf life of fresh-cut apples, particularly in terms of preserving TP during storage at refrigerated temperatures.

Table 3. Total Phenolic Content of PLA and OPP packaged fresh-cut apples for 12 days at 4 °C.

		Mg GAE/g Extract					
		T0	T2	T5	T7	T9	T12
OPP	EXT	$5.4 \pm 0.2^{a,b,c,A}$	$4.9 \pm 0.2^{b,c,A}$	$4.6 \pm 0.1^{c,A,B}$	$4.7 \pm 0.1^{b,c,A}$	$5.4 \pm 0.2^{a,b,A}$	$6.1 \pm 0.3^{a,A,B}$
	AA	$5.7 \pm 0.4^{b,A}$	$4.8 \pm 0.2^{b,c,A}$	$4.4 \pm 0.2^{c,B}$	$4.5 \pm 0.2^{c,A}$	$5.1 \pm 0.3^{b,c,A}$	$7.0 \pm 0.2^{a,A}$
	NT	$4.68 \pm 0.07^{a,b,A}$	$3.5 \pm 0.1^{c,B}$	$4.08 \pm 0.15^{b,c,B}$	$4.42 \pm 0.01^{a,b,c,A,B}$	$5.01 \pm 0.22^{a,b,A}$	$5.4 \pm 0.5^{a,B}$
PLA	EXT	$4.9 \pm 0.4^{a,b,A}$	$5.0 \pm 0.2^{a,b,A}$	$4.3 \pm 0.1^{b,c,B}$	$3.8 \pm 0.1^{c,B}$	$4.9 \pm 0.2^{a,b,A}$	$5.8 \pm 0.3^{a,B}$
	AA	$5.0 \pm 0.2^{b,A}$	$4.6 \pm 0.2^{b,A,B}$	$5.3 \pm 0.2^{b,A}$	$5.0 \pm 0.2^{b,A}$	$5.04 \pm 0.15^{b,A}$	$6.5 \pm 0.2^{a,A,B}$
	NT	$4.5 \pm 0.3^{b,c,A}$	$3.70 \pm 0.01^{d,A,B}$	$4.48 \pm 0.07^{b,c,A,B}$	$5.01 \pm 0.00^{a,b,A}$	$4.14 \pm 0.06^{c,d,A}$	$5.53 \pm 0.09^{a,B}$

PLA—polylactic acid; OPP—oriented polypropylene; EXT—extract of olive pomace; AA—ascorbic acid; NT—non-treated samples; GAE—gallic acid equivalents; T0—immediately after packaging; T2–T12—2–12 days after packaging and storage at 4 °C. Results are presented as means ± standard error. The means in each row with the same letters ($^{a–d}$) and means in each column with the same letters ($^{A–B}$) are not significantly different ($p > 0.05$).

Table 4 shows the results concerning the antioxidant activity (measured by FRAP and DPPH assays) of the extracts of apple slices packaged in PLA and OPP films using water (control), olive pomace and ascorbic acid as antioxidants. The measurements were taken immediately after packaging (T0) and after two (T2), five (T5), seven (T7), nine (T9) and 12 days (T12) of storage, at a refrigerated temperature.

Table 4. Antioxidant activity measured by FRAP and DPPH assays of PLA and OPP packaged fresh-cut apples for 12 days at 4 °C.

		mmol FSE/g Extract					
		T0	T2	T5	T7	T9	T12
OPP	EXT	$0.079 \pm 0.004^{b,A}$	$0.089 \pm 0.006^{a,b,A,B}$	$0.098 \pm 0.002^{a,B}$	$0.091 \pm 0.003^{a,b,B}$	$0.079 \pm 0.006^{b,A}$	$0.092 \pm 0.003^{a,b,B,C}$
	AA	$0.080 \pm 0.005^{c,A}$	$0.087 \pm 0.003^{b,c,A,B}$	$0.098 \pm 0.003^{a,b,B}$	$0.089 \pm 0.003^{b,c,B}$	$0.091 \pm 0.005^{a,b,c,A}$	$0.104 \pm 0.001^{a,A}$
	NT	$0.0759 \pm 0.0001^{c,A}$	$0.079 \pm 0.003^{c,A}$	$0.096 \pm 0.003^{a,B}$	$0.085 \pm 0.002^{b,B,C}$	$0.082 \pm 0.002^{b,c,A}$	$0.0862 \pm 0.0004^{b,C}$
PLA	EXT	$0.076 \pm 0.002^{b,c,A}$	$0.096 \pm 0.005^{a,A}$	$0.093 \pm 0.003^{a,b,B}$	$0.075 \pm 0.003^{c,C}$	$0.082 \pm 0.003^{a,c,A}$	$0.096 \pm 0.005^{a,A,B,C}$
	AA	$0.079 \pm 0.002^{d,A}$	$0.088 \pm 0.003^{c,d,A,B}$	$0.117 \pm 0.003^{a,A}$	$0.103 \pm 0.001^{b,A}$	$0.089 \pm 0.002^{c,A}$	$0.103 \pm 0.001^{b,A,B}$
	NT	$0.07623 \pm 0.00005^{c,A}$	$0.085 \pm 0.003^{d,B}$	$0.100 \pm 0.001^{a,B}$	$0.094 \pm 0.003^{a,b,A,B}$	$0.0683 \pm 0.0004^{c,d,A}$	$0.0890 \pm 0.0001^{b,B,C}$
		IC_{50} (mg/mL)					
		T0	T2	T5	T7	T9	T12
OPP	EXT	$6.3 \pm 0.2^{a,B}$	$4.3 \pm 0.1^{c,D}$	$4.0 \pm 0.1^{c,D}$	$5.3 \pm 0.1^{b,C}$	$4.3 \pm 0.1^{c,D}$	$6.4 \pm 0.1^{a,B,C}$
	AA	$7.8 \pm 0.2^{a,A}$	$4.5 \pm 0.1^{c,C,D}$	$5.7 \pm 0.1^{b,B}$	$5.3 \pm 0.2^{b,C}$	$7.2 \pm 0.2^{a,A}$	$5.3 \pm 0.2^{b,D}$
	NT	$7.12 \pm 0.04^{a,b,A}$	$6.2 \pm 0.1^{c,d,B}$	$5.7 \pm 0.2^{d,B}$	$6.6 \pm 0.1^{b,c,A,B}$	$4.39 \pm 0.05^{e,D}$	$7.5 \pm 0.1^{a,A}$
PLA	EXT	$7.4 \pm 0.1^{a,A}$	$4.7 \pm 0.1^{d,C}$	$6.8 \pm 0.1^{b,A}$	$7.4 \pm 0.1^{a,A}$	$5.7 \pm 0.1^{c,C}$	$7.0 \pm 0.1^{a,b,A,B}$
	AA	$5.5 \pm 0.1^{a,C}$	$4.4 \pm 0.1^{b,C,D}$	$4.1 \pm 0.1^{b,C,D}$	$5.1 \pm 0.1^{a,C}$	$4.4 \pm 0.1^{b,D}$	$5.4 \pm 0.2^{a,D}$
	NT	$7.3 \pm 0.1^{b,A}$	$8.2 \pm 0.1^{a,A}$	$6.8 \pm 0.1^{e,C}$	$6.3 \pm 0.1^{c,B}$	$6.3 \pm 0.2^{c,B}$	$5.7 \pm 0.1^{d,C,D}$

PLA—polylactic acid; OPP—oriented polypropylene; EXT—extract of olive pomace; AA—ascorbic acid; NT—non-treated samples; FES—ferrous sulfate equivalents; T0—immediately after packaging; T2–T12—2–12 days after packaging and storage at 4 °C. Results are presented as means ± standard error. For each assay, means in each row with the same letters ($^{a–e}$) and means in each column with the same letters ($^{A–D}$) are not significantly different ($p > 0.05$).

For the FRAP assay, a slight but significant ($p < 0.05$) increase was observed in the antioxidant activity of the samples at T12, except for the OPP-EXT sample (0.092 ± 0.003 mmol FSE/g extract). Nevertheless, both antioxidants demonstrated the ability to preserve or improve the antioxidant activity of the apple slices during storage. In the DPPH assay, the results were expressed as IC_{50} values, with higher values corresponding to lower antioxidant potentials (IC_{50}: extract concentration corresponding to 50% of antioxidant activity). The antioxidant activity of the samples was preserved after 12 days of storage, with the exception of the samples treated with ascorbic acid and packaged in OPP film (OPP-AA), where a significant ($p < 0.05$) increase was noticed (IC_{50} value of 5.3 ± 0.2 mg/mL). Although the highest scavenging activity was obtained at T2 for all the samples, the overall results of antioxidant activity suggest that a storage of five days would be optimal to ensure the nutritional quality of the fruits, as supported by the obtained results of the microbial quality assays.

The increase in phenolic compounds and antioxidant activity may improve the functional value of the fresh-cut fruits, which can be explained by the cutting of the fruits before immersion in the antioxidant solutions and packaging. This process induces the synthesis and accumulation of phenolic compounds in a short time as a defense response. The cell damage in the wound area can promote physiological and biochemical changes, thus increasing the metabolic reactions and inducing phenylalanine ammonia-lyase (PAL) activity, which enhances phenolic accumulation and, consequently, antioxidant activity in the apples [54].

3.6. Principal Component Analysis and Clustering

After the one variable at a time (OVAT) approach, two multivariate analysis techniques (PCA and K-means) were applied to understand the overall contribution of each part of the data collected and attached in the repository linked to this publication. The final dataset was clean and considered the means of measurement replicates while maintaining experimental replicates. Afterwards, an imputation of data was performed when necessary, and only complete cases of observations/variables were considered. Finally, the data were scaled to avoid numeric instabilities. Since the microbiological quality of the packaged fresh-cut apples was only studied for storage periods of zero, five and 12 days, and for easier interpretation of the results, the PCA analysis was performed only for these periods. In addition, some variables with lower statistical weight were removed from the analysis.

In Figure 5, a visual representation of both analyses is displayed and divided into two sections: section A shows the PCA analysis results, and section B exhibits the K-means clustering results (for numerical representation of data, please see the Supplementary Materials). The scree plot for the PCA analysis shows that the two first principal components accounted for approximately 75% of the total variance, and inclusion of the third component would increase the explained variance to approximately 90%. Therefore, by employing the loadings of the three main principal components (PC), a biplot with a two-dimensional plot representation of the PC1 and PC2 can be plotted (Figure 5(A1)). A third PC with a different marker shape was also represented, considering the negative range of numbers with a circular shape and the positive range of values with a triangular shape.

To select the optimal number of clusters for the K-means technique, the analysis relied on the silhouette method, which suggests the higher point in the average silhouette width (five in this work). Nevertheless, to further explore the possible cluster combinations, two clusters were also considered. Therefore, in the subsection plot B1 (Figure 5(B1)), the final PCA overlay data is presented in both two (circles) and five (straight lines polygons) clusters. The two K-means clusterizations suggested a separation between samples collected at day zero and day five (except for one observation of ascorbic acid treatment), emphasizing more notorious changes over longer storage times (12 days). On the other hand, when clustering with five centers, a clear separation of the treatments (EXT vs. AA) was observed for day five and, there was also a separation at day 12 based on the type of bag in which the samples were stored.

Considering plot A1 (Figure 5(A1)) as the core of the combined analysis, the variables that influenced each of the principal components could be identified. Hence, PC1 or the X-axis attributes the higher variance to the CO_2, bacteria and fungi to the right and O_2 and hue angle (h°) parameters to the left. Also, it is important to note that apple samples of the same antioxidant and polymer-bag treatments started to the left, and the time increments moved the observations to the right. On the PC2 or Y-axis, the higher positive variables spotted were bacteria, fungi and O_2, while the higher negative variables were weight loss and CO_2, demonstrating a negative correlation (please see Apples_EDA plot included in the repository) to O_2 (−0.86). It is worth noting that the reported weight loss refers to the weight loss of the samples, antioxidant, polymer bag and gases complex, and not only to apple weight loss. On the other hand, the coliforms variable represented a significant separator in the PC2 due to the absence of growth in the extract-treated samples compared to an average of 2 log CFU/g from the ascorbic acid-treated samples for samples with

storage times higher than T0. Additionally, in the PC3, it was possible to better visualize the obtained clusters in plot B1, which showed a clear difference between the different bag materials after 12 days of storage.

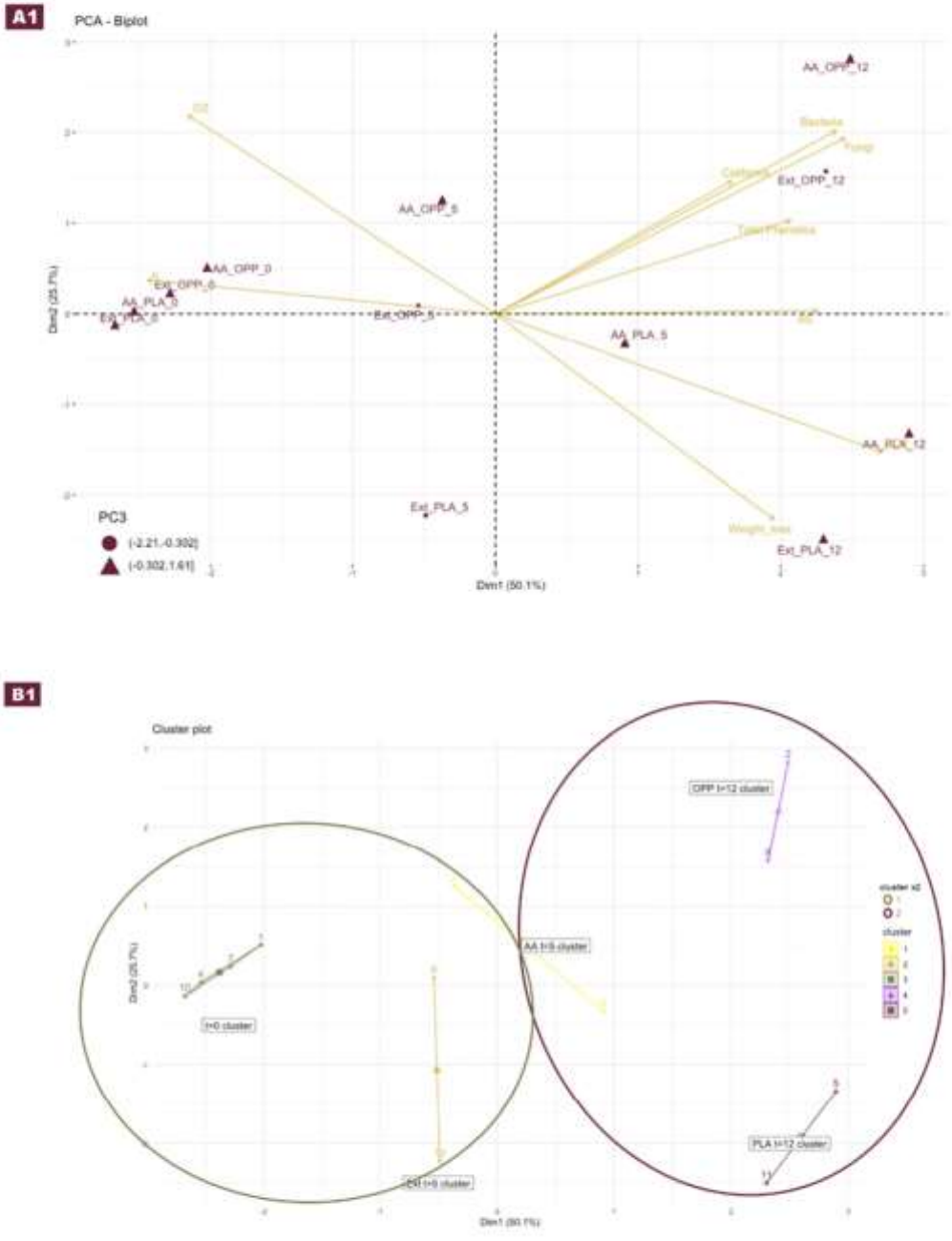


Figure 5. Principal component analysis with (**A1**) biplot visualization of the three main principal components, and K-means superimposed layer with (**B1**) cluster plot representing the K2 and K5 ranges (OPP—oriented polypropylene; PLA—polylactic acid; EXT—extract of olive pomace; AA—ascorbic acid).

4. Conclusions

The demand for fresh-cut fruits with preservation of their quality attributes has been increasing in recent years. Therefore, the food industry is always seeking out new functional products that satisfy the needs and tastes of consumers who are concerned about the choice of healthier foods. This work revealed that natural olive pomace extracts could be used as an ingredient in fresh-cut apples to increase the antioxidant activity of the fruits and to inhibit microbial growth during storage at refrigerated temperatures for at least five days. In addition, these natural extracts were demonstrated to preserve the color of the apple slices after the initial browning of the samples. This can be attributed to the natural color of the olive pomace extract solution. Concerning the packaging films used, PLA films seemed to exhibit a higher potential to preserve the quality of fresh-cut apples in combination with olive pomace extracts used as an additive compared to conventional films. Although it is also dependent on many factors related to the harvesting and postharvest handling of fresh produce, the promising results obtained in this work supported the antimicrobial potential of olive pomace extracts, suggesting that their use as an antioxidant could be more suitable for fresh-cut products in which the color is not a conditional factor for the consumer's evaluation and consumption decisions. The recovery of functional compounds from olive waste is in line with consumers' requirements for high-quality and safe processed foods. At the same time, it can reduce environmental impacts, also promoting the sustainability of the olive oil industries. Further investigation should be performed in order to understand the possible interactions between the organoleptic profile of the samples, assessed by a sensory analysis to evaluate the quality and the potential commercial acceptance of these new products. Furthermore, the possibility of incorporating these extracts into food packaging should continue to be explored to reduce food waste.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/foods12091926/s1, Figure S1: PCA and K-means Data are available online at https://github.com/Bruno-melgar/Apples (accessed on 30 March 2023).

Author Contributions: Conceptualization, V.D.A., M.M.-M., S.C.V. and L.B.; methodology, V.D.A., M.M.-M., S.C.V. and L.B.; software, B.M.; validation, B.M.; investigation, J.M.; data curation, B.M.; writing—original draft preparation, J.M. and B.M.; writing—review and editing, S.C.V., L.B., F.M.A.M., V.D.A., M.M.-M. and C.S.-B.; supervision, C.S.-B., L.B. and S.C.V.; funding acquisition, F.M.A.M., C.S.-B., L.B. and S.C.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Foundation for Science and Technology (FCT, Portugal): C^2TN (UIDB/04349/2020), CIMO (UIDB/00690/2020) and UIDP/00690/2020), LEAF (UIDP/04129/2020), SusTEC (LA/P/0007/2021), J. Madureira (SFRH/BD/136506/2018), L. Barros (institutional scientific employment program-contract); European Regional Development Fund (ERDF): projects "BIOMA" (POCI-01-0247-FEDER-046112), "OliveBIOextract" (NORTE-01-0247-FEDER-049865) and NORTE2020 (NORTE-06-3559-FSE-00018, B. Melgar contract); Junta de Castilla y León (Escalera de Excelencia CLU-2018-04) co-funded by the P.O. FEDER of Castilla y León 2014–2020: Agroenvironment Unit.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors are grateful to UCASUL—União de Cooperativas Agrícolas do Sul agro industrial cooperative for providing the samples, to Technological Unit of Radiosterilization (University of Lisbon) for the sample irradiations, and to Campotec S.A. for providing the polypropylene films.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Guarrasi, V.; Giacomazza, D.; Germanà, M.A.; Amenta, M.; San Biaggio, P.L. Monitoring the Shelf-Life of Minimally Processed Fresh-Cut Apple Slices By Physical Chemical Analysis and Electronic Nose. *Agrotechnology* **2014**, *3*, 126. [CrossRef]
2. Instituto Nacional de Estatística Consumo Humano de Frutos per Capita (Kg/Hab) Por Especie Frutícola (Balanços de Mercado). Available online: https://www.ine.pt/xportal/xmain?xpid=INE&xpgid=ine_indicadores&indOcorrCod=0000163&contexto=bd&selTab=tab2 (accessed on 20 April 2023).
3. Solís-Contreras, G.A.; Rodríguez-Guillermo, M.C.; de la Luz Reyes-Vega, M.; Aguilar, C.N.; Rebolloso-Padilla, O.N.; Corona-Flores, J.; de Abril Alexandra Soriano-Melgar, L.; Ruelas-Chacon, X. Extending Shelf-Life and Quality of Minimally Processed Golden Delicious Apples with Three Bioactive Coatings Combined with Cinnamon Essential Oil. *Foods* **2021**, *10*, 597. [CrossRef] [PubMed]
4. Singh, B.; Suri, K.; Shevkani, K.; Kaur, A.; Kaur, A.; Singh, N. Enzymatic Browning of Fruit and Vegetables: A Review. In *Enzymes in Food Technology*; Kuddus, M., Ed.; Springer: Singapore, 2018; pp. 63–78.
5. Lee, C.Y.; Smith, N.L. Minimal Processing of New York Apples. *N. Y. Food Life Sci. Bull.* **1995**, *145*, 1–11.
6. Hamdan, N.; Lee, C.H.; Wong, S.L.; Fauzi, C.E.N.C.A.; Zamri, N.M.A.; Lee, T.H. Prevention of Enzymatic Browning by Natural Extracts and Genome-Editing: A Review on Recent Progress. *Molecules* **2022**, *27*, 1101. [CrossRef]
7. Ali, H.M.; El-Gizawy, A.M.; El-Bassiouny, R.E.I.; Saleh, M.A. Browning Inhibition Mechanisms by Cysteine, Ascorbic Acid and Citric Acid, and Identifying PPO-Catechol-Cysteine Reaction Products. *J. Food Sci. Technol.* **2015**, *52*, 3651–3659. [CrossRef]
8. Grispoldi, L.; Karama, M.; El-Ashram, S.; Saraiva, C.; García-Díez, J.; Chalias, A.; De Gennis, M.; Vannuccini, A.; Poerio, G.; Torlai, P.; et al. A Study on the Application of Natural Extracts as Alternatives to Sodium Nitrite in Processed Meat. *J. Food Process. Preserv.* **2022**, *46*, e16351. [CrossRef]
9. Caleja, C.; Barros, L.; Antonio, A.L.; Oliveira, M.B.P.; Ferreira, I.C.F.R. A Comparative Study between Natural and Synthetic Antioxidants: Evaluation of Their Performance after Incorporation into Biscuits. *Food Chem.* **2017**, *216*, 342–346. [CrossRef]
10. Caleja, C.; Ribeiro, A.; Barros, L.; Barreira, J.C.M.; Antonio, A.L.; Beatriz, M.; Oliveira, P.P.; Filomena Barreiro, M.; Ferreira, I.C.F.R. Cottage Cheeses Functionalized with Fennel and Chamomile Extracts: Comparative Performance between Free and Microencapsulated Forms. *Food Chem.* **2016**, *199*, 720–726. [CrossRef]
11. Caleja, C.; Barros, L.; Antonio, A.L.; Carocho, M.; Oliveira, M.B.P.P.; Ferreira, I.C.F.R. Fortification of Yogurts with Different Antioxidant Preservatives: A Comparative Study between Natural and Synthetic Additives. *Food Chem.* **2016**, *210*, 262–268. [CrossRef]
12. Madureira, J.; Dias, M.I.; Pinela, J.; Calhelha, R.C.; Barros, L.; Santos-Buelga, C.; Margaça, F.M.A.; Ferreira, I.C.F.R.; Cabo Verde, S. The Use of Gamma Radiation for Extractability Improvement of Bioactive Compounds in Olive Oil Wastes. *Sci. Total Environ.* **2020**, *727*, 138706. [CrossRef]
13. Madureira, J.; Albuquerque, B.; Dias, M.I.; Pinela, J.; Calhelha, R.C.; Santos-Buelga, C.; Margaça, F.M.A.; Ferreira, I.C.F.R.; Cabo Verde, S.; Barros, L. Ultrasound-Assisted Extraction of Hydroxytyrosol and Tyrosol from Olive Pomace Treated by Gamma Radiation: Process Optimization and Bioactivity Assessment. *Food Funct.* **2023**, *14*, 3038–3050. [CrossRef] [PubMed]
14. Galanakis, C.M.; Tsatalas, P.; Charalambous, Z.; Galanakis, I.M. Control of Microbial Growth in Bakery Products Fortified with Polyphenols Recovered from Olive Mill Wastewater. *Environ. Technol. Innov.* **2018**, *10*, 1–15. [CrossRef]
15. Saleh, E.; Morshdy, A.E.; El-Manakhly, E.; Al-Rashed, S.; Hetta, H.F.; Jeandet, P.; Yahia, R.; El-Saber Batiha, G.; Ali, E. Effects of Olive Leaf Extracts as Natural Preservative on Retailed Poultry Meat Quality. *Foods* **2020**, *9*, 1017. [CrossRef] [PubMed]
16. Cedola, A.; Cardinali, A.; D'Antuono, I.; Conte, A.; Del Nobile, M.A. Cereal Foods Fortified with by-Products from the Olive Oil Industry. *Food Biosci.* **2020**, *33*, 100490. [CrossRef]
17. Lin, S.; Chi, W.; Hu, J.; Pan, Q.; Zheng, B.; Zeng, S. Sensory and Nutritional Properties of Chinese Olive Pomace Based High Fibre Biscuit. *Emirates J. Food Agric.* **2017**, *29*, 495–501. [CrossRef]
18. Durante, M.; Bleve, G.; Selvaggini, R.; Veneziani, G.; Servili, M.; Mita, G. Bioactive Compounds and Stability of a Typical Italian Bakery Products "Taralli" Enriched with Fermented Olive Paste. *Molecules* **2019**, *24*, 3258. [CrossRef]
19. Cecchi, L.; Schuster, N.; Flynn, D.; Bechtel, R.; Bellumori, M.; Innocenti, M.; Mulinacci, N.; Guinard, J.X. Sensory Profiling and Consumer Acceptance of Pasta, Bread, and Granola Bar Fortified with Dried Olive Pomace (Pâté): A Byproduct from Virgin Olive Oil Production. *J. Food Sci.* **2019**, *84*, 2995–3008. [CrossRef]
20. Madureira, J.; Margaça, F.M.A.; Santos-Buelga, C.; Ferreira, I.C.F.R.; Verde, S.C.; Barros, L. Applications of Bioactive Compounds Extracted from Olive Industry Wastes: A Review. *Compr. Rev. Food Sci. Food Saf.* **2022**, *21*, 453–476. [CrossRef]
21. Difonzo, G.; Troilo, M.; Squeo, G.; Pasqualone, A.; Caponio, F. Functional Compounds from Olive Pomace to Obtain High-Added Value Foods—A Review. *J. Sci. Food Agric.* **2021**, *101*, 15–26. [CrossRef]
22. Mujtaba, M.; Lipponen, J.; Ojanen, M.; Puttonen, S.; Vaittinen, H. Trends and Challenges in the Development of Bio-Based Barrier Coating Materials for Paper/Cardboard Food Packaging; a Review. *Sci. Total Environ.* **2022**, *851*, 158328. [CrossRef]
23. Motelica, L.; Ficai, D.; Ficai, A.; Oprea, O.C.; Kaya, D.A.; Andronescu, E. Biodegradable Antimicrobial Food Packaging: Trends and Perspectives. *Foods* **2020**, *9*, 1438. [CrossRef] [PubMed]
24. Madureira, J.; Pimenta, A.I.; Popescu, L.; Besleaga, A.; Dias, M.I.; Santos, P.M.P.; Melo, R.; Ferreira, I.C.F.R.; Cabo Verde, S.; Margaça, F.M.A. Effects of Gamma Radiation on Cork Wastewater: Antioxidant Activity and Toxicity. *Chemosphere* **2017**, *169*, 139–145. [CrossRef] [PubMed]

25. Guerreiro, D.; Madureira, J.; Silva, T.; Melo, R.; Santos, P.M.P.; Ferreira, A.; Trigo, M.J.; Falcão, A.N.; Margaça, F.M.A.; Cabo Verde, S. Post-Harvest Treatment of Cherry Tomatoes by Gamma Radiation: Microbial and Physicochemical Parameters Evaluation. *Innov. Food Sci. Emerg. Technol.* **2016**, *36*, 1–9. [CrossRef]
26. Elias, M.L.; Madureira, J.; Santos, P.M.P.; Carolino, M.M.; Margaça, F.M.A.; Verde, S.C. Preservation Treatment of Fresh Raspberries by E-Beam Irradiation. *Innov. Food Sci. Emerg. Technol.* **2020**, *66*, 102487. [CrossRef]
27. Barkaoui, S.; Mankai, M.; Miloud, N.B.; Kraïem, M.; Madureira, J.; Verde, S.C.; Boudhrioua, N. E-Beam Irradiation of Strawberries: Investigation of Microbiological, Physicochemical, Sensory Acceptance Properties and Bioactive Content. *Innov. Food Sci. Emerg. Technol.* **2021**, *73*, 102769. [CrossRef]
28. Pereira, E.; Barros, L.; Dueñas, M.; Antonio, A.L.; Santos-Buelga, C.; Ferreira, I.C.F.R. Gamma Irradiation Improves the Extractability of Phenolic Compounds in *Ginkgo biloba* L. *Ind. Crops Prod.* **2015**, *74*, 144–149. [CrossRef]
29. Whittaker, B.; Watts, M.F. The Influence of Dose Rate, Ambient Temperature and Time on the Radiation Response of Harwell PMMA Dosimeters. *Radiat. Phys. Chem.* **2001**, *60*, 101–110. [CrossRef]
30. Madureira, J.; Melgar, B.; Santos-Buelga, C.; Margaça, F.M.A.; Ferreira, I.C.F.R.; Barros, L.; Verde, S.C. Phenolic Compounds from Irradiated Olive Wastes: Optimization of the Heat-Assisted Extraction Using Response Surface Methodology. *Chemosensors* **2021**, *9*, 231. [CrossRef]
31. Vieira, T.M.; Moldão-Martins, M.; Alves, V.D. Composite Coatings of Chitosan and Alginate Emulsions with Olive Oil to Enhance Postharvest Quality and Shelf Life of Fresh Figs (*Ficus carica* L. Cv. 'Pingo De Mel'). *Foods* **2021**, *10*, 718. [CrossRef]
32. Vieira, T.M.; Alves, V.D.; Moldão Martins, M. Application of an Eco-Friendly Antifungal Active Package to EXtend the Shelf Life of Fresh Red Raspberry (*Rubus idaeus* L. Cv. 'Kweli'). *Foods* **2022**, *11*, 1805. [CrossRef]
33. Singleton, V.L.; Orthofer, R.R.; Lamuela-Raventós, R.M. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. *Methods Enzymol.* **1998**, *299*, 152–178. [CrossRef]
34. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of Free Radical Method to Evaluate Antioxidant Activity. *LWT-Food Sci. Technol.* **1995**, *28*, 25–30. [CrossRef]
35. Benzie, I.F.F.; Strain, J.J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Anal. Biochem.* **1996**, *239*, 70–76. [CrossRef]
36. Barkaoui, S.; Madureira, J.; Santos, P.M.P.; Margaça, F.M.A.; Miloud, N.B.; Mankai, M.; Boudhrioua, N.M.; Cabo Verde, S. Effect of Ionizing Radiation and Refrigeration on the Antioxidants of Strawberries. *Food Bioprocess Technol.* **2020**, *13*, 1516–1527. [CrossRef]
37. Jha, S.N.; Rai, D.R.; Shrama, R. Physico-Chemical Quality Parameters and Overall Quality Index of Apple during Storage. *J. Food Sci. Technol.* **2012**, *49*, 594–600. [CrossRef] [PubMed]
38. Ghafir, S.A.M.; Gadalla, S.O.; Murajei, B.N.; El-Nady, M.F. Physiological and Anatomical Comparison between Four Different Apple Cultivars under Cold-Storage Conditions. *Acta Biol. Szeged.* **2009**, *53*, 21–26.
39. Shyu, Y.S.; Chen, G.W.; Chiang, S.C.; Sung, W.C. Effect of Chitosan and Fish Gelatin Coatings on Preventing the Deterioration and Preserving the Quality of Fresh-Cut Apples. *Molecules* **2019**, *24*, 2008. [CrossRef]
40. Jan, I.; Rab, A.; Sajid, M.; Ali, A.; Shah, S.T. Response of Apple Cultivars To Different Storage Durations. *Sarhad J. Agric* **2012**, *28*, 219–225.
41. Rydzak, L.; Kobus, Z.; Nadulski, R.; Wilczyński, K.; Pecyna, A.; Santoro, F.; Sagan, A.; Starek-Wójcicka, A.; Krzywicka, M. Analysis of Selected Physicochemical Properties of Commercial Apple Juices. *Processes* **2020**, *8*, 1457. [CrossRef]
42. Muhammad, A.; Ayub, M.; Zeb, A. Physicochemical Analysis of Apple Pulp from Mashaday Variety during Storage. *Agric. Biol. J. N. Am.* **2011**, *2*, 192–196. [CrossRef]
43. Rocha, A.M.C.N.; Morais, A.M.M.B. Shelf Life of Minimally Processed Apple (Cv. Jonagored) Determined by Colour Changes. *Food Control* **2003**, *14*, 13–20. [CrossRef]
44. Rojas-Grau, M.A.; Sobrino-López, A.; Tapia, M.S.; Martín-Belloso, O. Browning Inhibition in Fresh-Cut ' Fuji ' Apple Slices by Natural Antibrowning Agents. *J. Food Sci.* **2006**, *71*, 59–65. [CrossRef]
45. González-Buesa, J.; Page, N.; Kaminski, C.; Ryser, E.T.; Beaudry, R.; Almenar, E. Effect of Non-Conventional Atmospheres and Bio-Based Packaging on the Quality and Safety of Listeria Monocytogenes-Inoculated Fresh-Cut Celery (*Apium graveolens* L.) during Storage. *Postharvest Biol. Technol.* **2014**, *93*, 29–37. [CrossRef]
46. Zhou, H.; Kawamura, S.; Koseki, S.; Kimura, T. Comparative Quality Changes of Fresh-Cut Melon in Bio-Based and Petroleum-Based Plastic Containers during Storage. *Environ. Control Biol.* **2016**, *54*, 93–99. [CrossRef]
47. Song, H.Y.; Jo, W.S.; Song, N.B.; Min, S.C.; Song, K. Bin Quality Change of Apple Slices Coated with Aloe Vera Gel during Storage. *J. Food Sci.* **2013**, *78*, C817–C822. [CrossRef]
48. Kumar, P.; Sethi, S.; Sharma, R.R.; Singh, S.; Varghese, E. Improving the Shelf Life of Fresh-Cut 'Royal Delicious' Apple with Edible Coatings and Anti-Browning Agents. *J. Food Sci. Technol.* **2018**, *55*, 3767–3778. [CrossRef] [PubMed]
49. Moreira, M.R.; Tomadoni, B.; Martín-Belloso, O.; Soliva-Fortuny, R. Preservation of Fresh-Cut Apple Quality Attributes by Pulsed Light in Combination with Gellan Gum-Based Prebiotic Edible Coatings. *LWT-Food Sci. Technol.* **2015**, *64*, 1130–1137. [CrossRef]
50. Osuga, R.; Koide, S.; Sakurai, M.; Orikasa, T.; Uemura, M. Quality and Microbial Evaluation of Fresh-Cut Apples during 10 Days of Supercooled Storage. *Food Control* **2021**, *126*, 108014. [CrossRef]
51. Graça, A.; Santo, D.; Esteves, E.; Nunes, C.; Abadias, M. Evaluation of Microbial Quality and Yeast Diversity in Fresh-Cut Apple. *Food Microbiol.* **2015**, *51*, 179–185. [CrossRef]

52. Botondi, R.; Bartoloni, S.; Baccelloni, S.; Mencarelli, F. Biodegradable PLA (Polylactic Acid) Hinged Trays Keep Quality of Fresh-Cut and Cooked Spinach. *J. Food Sci. Technol.* **2015**, *52*, 5938–5945. [CrossRef]
53. Almenar, E.; Samsudin, H.; Auras, R.; Harte, B.; Rubino, M. Postharvest Shelf Life Extension of Blueberries Using a Biodegradable Package. *Food Chem.* **2008**, *110*, 120–127. [CrossRef] [PubMed]
54. Hu, W.; Sarengaowa, W.; Guan, Y.; Feng, K. Biosynthesis of Phenolic Compounds and Antioxidant Activity in Fresh-Cut Fruits and Vegetables. *Front. Microbiol.* **2022**, *13*, 906069. [CrossRef] [PubMed]

Publicación 8. Microbiota assessment of fresh-cut apples packaged in two different films

Joana Madureira, Sara Gonçalves, Celestino Santos-Buelga, Fernanda M.A. Margaça, Isabel C.F.R. Ferreira, Lillian Barros, Sandra Cabo Verde

Artículo publicado en *Microorganisms* (2023), 11, 1157. Doi: https://doi.org/10.3390/microorganisms11051157

Resumen

En este trabajo se estudia la composición y evolución de la microbiota natural en manzanas recién cortadas durante el almacenamiento refrigerado durante 12 días.

La adición de extractos naturales de orujo de oliva como aditivo se comparó con la del antioxidante comercial utilizado por las industrias (ácido ascórbico), utilizando los materiales de envasado diferentes, una película de ácido poliláctico (PLA) biodegradable y otra convencional y comercial de polipropileno orientado (OPP).

Al cabo de 5 y 12 días de almacenamiento en refrigeración, se encontraron recuentos bacterianos más bajos en las muestras donde se utilizaban extractos de orujo de oliva y envases de PLA que en las tratadas con ácido ascórbico o envasadas en película de OPP. Las observaciones realizadas sugieren que este tipo de extractos naturales podría ser de utilidad como aditivo para retrasar el crecimiento de bacterias mesófilas en frutas.

En el tiempo de estudio, ninguna de las muestras de manzanas recién cortadas dio positivo para los principales patógenos transmitidos por los alimentos asociados con la fruta fresca. Las especies más prevalentes en las muestras analizadas fueron *Citrobacter freundii*, *Staphylococcus warneri*, *Pseudomonas oryzihabitans*, *Alcalinogenes faecalis*, *Corynebacterium jeikeium*, *Micrococcus* spp., *Pantoea aglomerans* y *Bacillus* spp, que son bacterias de origen humano y ambiental. Se observó un aumento en la diversidad microbiana a lo largo del tiempo de almacenamiento en refrigeración, mayor en las muestras donde se empleó ácido ascórbico, lo que indica la menor eficacia para inhibir el desarrollo microbiano de este aditivo, utilizado, de hecho, como antioxidante, pero no como conservante.

En conclusión, el extracto de orujo de oliva demostró potencial para ser utilizado como un conservante natural en alimentos frescos listos para consumo. Además, la combinación de los extractos de orujo con el envasado en PLA se mostró como el proceso más adecuado para obtener una menor diversidad microbiana en las muestras,

lo que también se confirmó con el cálculo de los índices de Shannon y Simpson. Los resultados obtenidos permiten aportar a la industria elementos para el desarrollo de nuevos alimentos mínimamente procesados y con características funcionales mejoradas.

microorganisms

MDPI

Article

Microbiota Assessment of Fresh-Cut Apples Packaged in Two Different Films

Joana Madureira [1,2,3,4], Sara Gonçalves [5], Celestino Santos-Buelga [4,6], Fernanda M. A. Margaça [1,7], Isabel C. F. R. Ferreira [2], Lillian Barros [2,3] and Sandra Cabo Verde [1,7,*]

[1] Centro de Ciências e Tecnologias Nucleares (C²TN), Instituto Superior Técnico, Universidade de Lisboa, EstradaNacional 10 ao km 139.7, 2695-066 Loures, Portugal; joanamadureira@ctn.tecnico.ulisboa.pt (J.M.); fmargaca@ctn.tecnico.ulisboa.pt (F.M.A.M.)
[2] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; iferreira@ipb.pt (I.C.F.R.F.); lillian@ipb.pt (L.B.)
[3] Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[4] Grupo de Investigación en Polifenoles (GIP-USAL), Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno s/n, 37007 Salamanca, Spain; csb@usal.es
[5] ESTeSL-Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, 1990-096 Lisboa, Portugal
[6] Unidad de Excelencia Producción, Agrícola y Medioambiente (AGRIENVIRONMENT), Parque Científico, Universidad de Salamanca, 37185 Salamanca, Spain
[7] Departamento de Engenharia e Ciências Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10, ao km 139.7, 2695-066 Loures, Portugal
* Correspondence: sandracv@ctn.tecnico.ulisboa.pt

Abstract: The aim of this work was to assess the natural microbiota of packed fresh-cut apples during refrigerated storage. Two different films were tested for the package, a biodegradable (PLA) film and a conventional and commercial one (OPP). Two antioxidant additives were applied, a natural olive pomace extract and the commercial ascorbic acid used by the industries. The results revealed lower bacteria counts in samples with olive pomace extract and PLA films than in those with ascorbic acid and OPP films after 5 and 12 days of storage. These findings suggest that the use of such natural extracts as additives in fruits could delay the growth of mesophilic bacteria. The characterization and identification of the bacterial isolates from fresh-cut apple samples showed that the most prevalent species were *Citrobacter freundii*, *Staphylococcus warneri*, *Pseudomonas oryzihabitans*, *Alcaligenes faecalis*, *Corynebacterium jeikeium*, *Micrococcus* spp., *Pantoea agglomerans* and *Bacillus* spp. Furthermore, an increase in the microbial diversity during the storage time at refrigerated temperatures was observed, except for the sample treated with olive pomace extract and packaged in OPP film. The highest microbial diversity was found for samples with ascorbic acid as an additive. This could indicate a negative effect of ascorbic acid on the microbial inhibition of apple slices. The natural olive pomace extract demonstrated potential as an antimicrobial additive for fresh-cut apples.

Keywords: minimally processed apple; natural extracts; packaging; natural microbiota

Citation: Madureira, J.; Gonçalves, S.; Santos-Buelga, C.; Margaça, F.M.A.; Ferreira, I.C.F.R.; Barros, L.; Cabo Verde, S. Microbiota Assessment of Fresh-Cut Apples Packaged in Two Different Films. *Microorganisms* **2023**, *11*, 1157. https://doi.org/10.3390/microorganisms11051157

Academic Editor: João Miguel F. Rocha

Received: 30 March 2023
Revised: 24 April 2023
Accepted: 27 April 2023
Published: 28 April 2023

1. Introduction

Fruits are excellent sources of nutrients, being an essential part of a healthy diet due to their nutritional qualities. The demand of consumers for minimally processed foods that preserve their safety and high quality attributes has been growing. This motivates food industries to look for innovative processes by which to manufacture these kinds of products. Ready-to-eat products, such as fresh-cut apples, are very perishable and susceptible to spoilage. This is because they can be fast oxidized and are susceptible to contamination during processing and storage [1,2]. Some of the microorganisms involved can

cause food spoilage and/or may be potentially hazardous to human health related to different illnesses. Therefore, it is important to understand their incidence and survival during the storage period [3]. Regarding the apple fruits, different apple tissues present bacterial communities that vary in diversity and abundance. On the other hand, the global core microbiome of apple fruit has been considered an important contributor to human gut microbiome, stimulating the human immune system [4].

Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and ascorbic acid, have been widely used by food production industries in food products. However, several studies have described relationships between their use and some harmful effects on health [5–8]. Engin et al. [5] reported liver toxicity associated with the use of BHT in endotoxemia settings, whereas other authors have reported the carcinogenic effect of both antioxidants in experimental animals [7,8]. Jeong et al. [6] found that high doses of BHA had endocrine-disruption effects that altered the development and functions of the reproductive systems of male and female rats including sex organ weights and sexual maturation.

In this sense, the search for natural additives to replace the synthetic antioxidants and antimicrobials as food preservatives has attracted increasing interest [9–11]. In recent years, special attention has been paid to natural additives that can be extracted from the wastes of agricultural food industries [12]. One candidate is olive pomace, the main waste generated by the olive oil industry. It is considered a rich source of bioactive compounds with putative health benefits for humans [10]. Moreover, previous studies demonstrated that olive pomace extracts provided antioxidant and antimicrobial potential against different microorganisms [13,14]. The high antidiabetic and anti-inflammatory activities of such extracts was recently reported for the first time, with a high cytotoxic effect for the breast adenocarcinoma (MCF-7) cell line [13]. In this way, these extracts could possibly be used by the industries as a new ingredient to produce functional foods with added value. Some researchers have been exploring this application [15–18]. The addition of dried olive pomace to bread, pasta and granola bars increased their total phenolic compounds [18], improving the nutritional quality of bread without compromising the product acceptability [19]. *Taralli*, a typical Italian bakery product, enriched with 20% fermented olive pomace, presented enhanced bioactive compounds content and maintained the low amounts of saturated fatty acids during storage [17]. On the other hand, Cedola et al. [16] suggested that although the enriched fish burgers with 10% dry olive pomace flour were not very acceptable due to a bitter and spicy taste, they presented high amounts of total phenolic compounds, total flavonoids and, consequently, an increase in antioxidant activity. The incorporation of olive pomace in yogurts also provided additional and essential health benefits, such as the increase in unsaturated fatty acids and the greater bioaccessibility of total phenolics after in vitro digestion [20].

Based on these observations, the aim of this work was to compare the inhibition effect on the microbial population of fresh-cut apples preserved using ascorbic acid—a commercial additive—and an olive pomace extract—a natural preservative—during 12 days of refrigerated storage in two different packaging bags composed of biodegradable polylactic acid (PLA) film and conventional oriented polypropylene (OPP) film. In our previous studies, the optimization of the extraction of olive pomace compounds was investigated. It was found that the application of ionizing radiation at 5 kGy dose to olive pomace improved the extractability of its bioactive compounds 2-fold [14], with enhanced bioactive properties [13]. Thus, the olive pomace extracts used to treat fresh-cut apples were obtained following the optimized procedure. A comprehensive characterization was performed in order to ascertain the most frequent bacteria in the samples of fresh-cut apples.

To the best of our knowledge, this study represents the first assessment of the use of olive pomace extract as a natural preservative on fresh-cut apples. We believe this work can contribute to encouraging the food industries to develop new functional foods and to promote the economic and environmental sustainability of the olive oil sector.

2. Materials and Methods

2.1. Olive Pomace Samples and Irradiation Experiments

Olive pomace samples were collected from UCASUL (União de Cooperativas Agrícolas do Sul, Alvito, Portugal). The samples were submitted to gamma radiation treatment in triplicate, using an absorbed dose of 5 kGy [14].

2.2. Olive Pomace Natural Ingredients: Phenolic Extracts Preparation

The extraction of phenolic compounds from irradiated olive pomace was performed using the optimal conditions of heat-assisted extraction obtained by Madureira et al. [21]. The selected extracts were those with the higher bioactive properties [13]. Sample extraction was performed in triplicate.

2.3. Preparation of Minimally Processed Apples

'Royal Gala' apples were purchased from a local supermarket in Lisbon (Portugal). First, they were washed, dried and cut into eight equal slices. Then, two groups of samples were prepared by immersion during 1 min in: olive pomace extract solution (EXT, 0.315%, *w*/*v*) for one group, and in ascorbic acid solution (AA, 0.315%, *w*/*v*) for the other. After that, three slices of each group were packed into biodegradable polylactic acid (PLA) (Vegware, Edinburgh, UK) and oriented polypropylene (OPP) (Campotec S.A, Torres Vedras, Portugal) film bags (10 × 10 cm) and sealed. The packs were then stored during 12 days in controlled chambers at 4 °C and 85% relative humidity. PLA biodegradable films were produced from corn starch, while the OPP films were conventional and commercial film plastic bags. Three independent packages were analyzed for each group on each day.

2.4. Characterization of Natural Mesophilic Bacteria in Fresh-Cut Apples

The fruit slices were analyzed over storage time (at 0, 5, 12 days of storage at 4 °C) for their microbial load and further phenotypic characterization. Briefly, apple slices from each package (~150 g) were homogenized in a stomacher (Stomacher 3500; Seaward, Peterlee, UK) for 15 min using 100 mL of buffered peptone water. Afterwards, serial decimal dilutions were prepared and inoculated in triplicate in Tryptic Soy Agar (TSA) plates. The plates were incubated at 30 °C for 7 days. Colony Forming Units (CFU) were counted, and the results were expressed as Log colony-forming units per gram of fresh fruit (Log CFU/g).

Bacterial isolates were phenotyped based on Bergey's Manual of Determinative Bacteriology [22]. The bacterial isolates were identified using RapID Systems (Remel, Thermo Scientific).

2.5. Statistical Analysis

Results were expressed as mean ± standard deviation. The differences between additives and packaging were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test with $\alpha = 0.05$. A significance level of $p < 0.05$ was considered. The bacterial diversity indices were estimated for bacterial species detected in fresh-cut apple samples by storage time and packaging film.

The Simpson's index of diversity (1-D) was calculated based on the following Equation (1):

$$1 - D = 1 - \left(\frac{\sum n(n-1)}{N(N-1)}\right), \quad (1)$$

where n is the total number of isolates of a certain species and N is the total number of isolates of all species [23].

The Shannon diversity index (H) was calculated by the Equation (2):

$$H = -\sum(p_i \times lnp_i), \quad (2)$$

where p_i is the proportion of the entire microbial community made up of species i [24]

3. Results and Discussion

The bacterial load present in fresh-cut apple samples was assessed immediately after packaging the samples (T0) and after 5 days (T5) and 12 days (T12) of storage at refrigerated temperatures (4 °C). This was conducted in order to establish both the efficiency of the treatment with each antioxidant solution and the effect of packaging in different films.

As expected, immediately after immersion into the antioxidant solutions and packaging (T0), no significant differences ($p > 0.05$) were observed in the bacterial concentrations of all the analyzed samples (Figure 1). The fresh-cut apples presented an aerobic bacterial mesophilic population ranging from 2.9 ± 0.2 log CFU/g to 3.5 ± 0.2 log CFU/g. This is coherent with the average levels of aerobic mesophilic bacteria ranging from 2 to 4 log CFU/g reported by other authors for fresh-cut apple samples [25,26]. On the other hand, the results found in the present work are lower than those described by Graça et al. [2] for fresh-cut apple samples that varied from 3.3 to 8.9 log CFU/g.

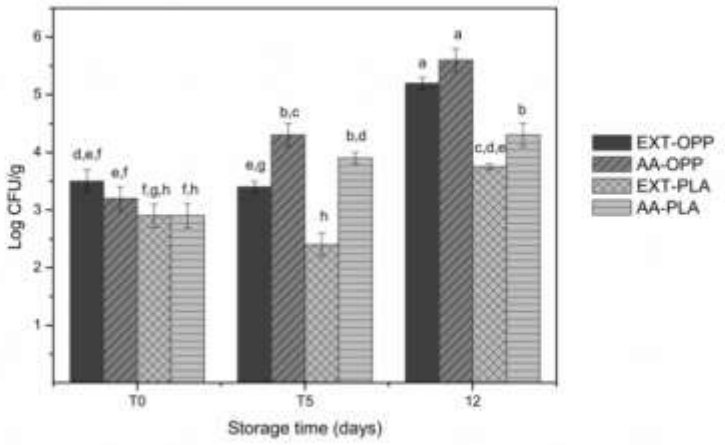


Figure 1. Aerobic mesophilic bacterial counts for fresh-cut apple samples (EXT-OPP: treated with olive pomace extracts and packaged in OPP films; AA-OPP: treated with ascorbic acid and packaged in OPP films; EXT-PLA: treated with olive pomace extracts and packaged in PLA films; AA-PLA: treated with ascorbic acid and packaged in PLA films) immediately after treatment and packaging (T0) and after 5 (T5) and 12 (T12) days of refrigerated storage. Means with equal lowercase letters are not statistically different by Tukey's test with 5% significance level.

During refrigerated storage, different trends were observed depending on the antioxidant treatment and packaging film. For samples treated with ascorbic acid solution, packed in OPP film, the mesophilic bacterial population of fresh-cut apples significantly increased ($p < 0.05$) over time. In this case (AA-OPP), the results were: 4.3 ± 0.2 log CFU/g and 5.6 ± 0.2 log CFU/g for T5 and T12, respectively. While for those packed in PLA film (AA-PLA), the results after 5 days of storage (T5) were 3.9 ± 0.1 log CFU/g, remaining roughly constant until the end of the experiment (T12): 4.3 ± 0.2 log CFU/g. For samples treated with olive pomace extract, both OPP and PLA films were able to maintain the bacterial concentrations during 5 days of refrigerated storage (3.4 ± 0.1 log CFU/g and 2.4 ± 0.2 log CFU/g, respectively). However, a significant increase was observed after 12 days of storage at 4 °C (5.2 ± 0.1 log CFU/g and 3.75 ± 0.05 log CFU/g, for OPP and PLA films, respectively).

The samples with the largest counts (Figure 1) are those with ascorbic acid as the additive and OPP film as the packaging bag (AA-OPP). These samples, with 5.6 ± 0.2 log CFU/g, are the ones closer to the Portuguese recommended limit for mesophilic bacteria in ready-to-eat foods that is 6 log CFU/g [27]. The overall results demonstrated that the lower bacteria counts were achieved for the samples using olive pomace extracts packed in PLA films. Those showed 2.4 ± 0.2 log CFU/g after 5 days and 3.75 ± 0.05 log CFU/g after 12 days of refrigerated storage. This finding highlights the combined use of the natural pomace extracts and PLA films packaging to efficiently delay the growth of mesophilic bacteria in fruits.

The quantification and identification of the bacterial isolates from fresh-cut apple samples were performed using culture-based methods (Figure 2).

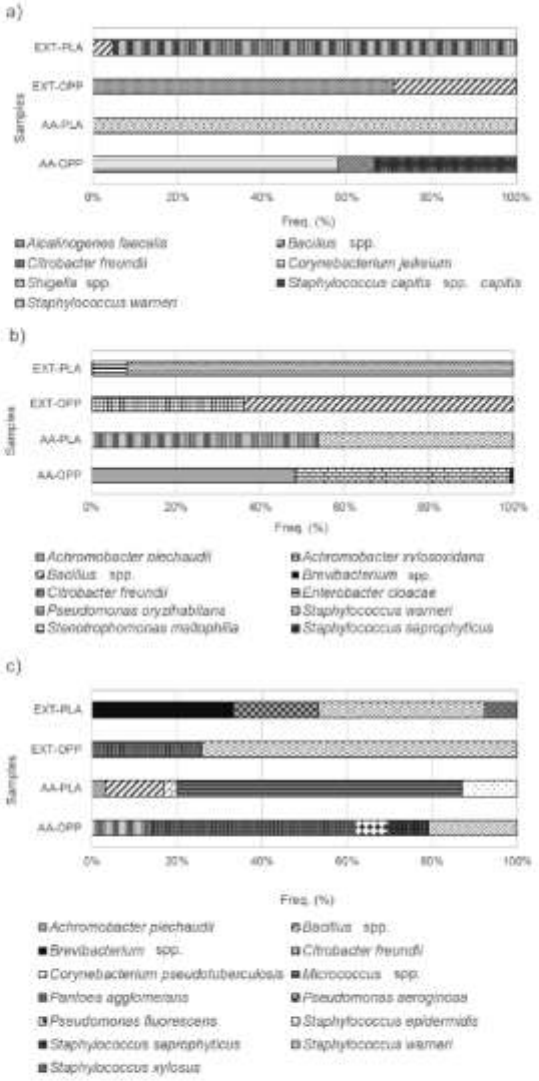


Figure 2. Relative abundances (%) of bacterial species isolated from the fresh-cut apple samples (EXT-OPP: treated with olive pomace extracts and packaged in OPP films; AA-OPP: treated with ascorbic acid and packaged in OPP films; EXT-PLA: treated with olive pomace extracts and packaged in PLA films; AA-PLA: treated with ascorbic acid and packaged in PLA films): (a) immediately

after treatment and packaging (T0), (**b**) after 5 (T5) days of refrigerated storage and (**c**) after 12 (T12) days of refrigerated storage.

Immediately after packaging (T0), the most prevalent bacterial species identified in the analyzed samples were *Citrobacter freundii* (95%) in EXT-PLA, *Alcalinogenes faecalis* (71%) in EXT-OPP, *Staphylococcus warneri* (100%) in AA-PLA and *Corynebacterium jeikeium* (58%) in AA-OPP. The first two species are mostly found in the environment, while the other two are part of the skin microbiota, and all of them could be recognized as opportunistic pathogens. *C. freundii* is a Gram-negative bacterium of the *Enterobacteriaceae* family. Although it is considered non-pathogenic when interacting with healthy individuals, it can cause a life-threatening infection that may progress into sepsis when colonizing the bloodstream [28]. *A. faecalis* is a potentially emerging pathogen for hospitalized patients and it usually causes opportunistic infections in humans such as meningitis [29], peritonitis [30] or pneumonia [31]. Moreover, its resistance to several antibiotics has been increasing, turning difficult the treatment of its infections [32,33]. *C. jeikeium* is a highly virulent pathogen and resistant to antimicrobial agents. One-third of immunocompromised patients with *C. jeikeium* infection present pulmonary lesions [34]. Additionally, the infections by this microorganism can also promote rash and skin lesions [35]. *S. warneri* is a commensal microorganism on skin flora, resistant to penicillins that rarely causes infection in healthy individuals. However, two cases were reported of urinary tract infections in a patient with liver cirrhosis [36], as well multiple subcutaneous abscesses in an immunocompetent individual [37] caused by *S. warneri* infections. This microorganism had been previously isolated from fresh apples and identified by the amplification of the 16S rRNA gene [38].

After 5 days (T5) of packaging and refrigerated storage, the most prevalent bacterial species identified in the samples were *Pseudomonas oryzihabitans* (92%) in EXT-PLA, *Bacillus* spp. (64%) in EXT-OPP, *C. freundii* (54%) and *S. warneri* (46%) in AA-PLA and *Stenotrophomonas maltophilia* (51%) and *Achromobacter piechaudii* (48%) in AA-OPP (Figure 2). The majority of *Pseudomonas* species are recognized as psychrotolerant or psychrotrophic (growing below 15 °C), justifying their prevalence on refrigerated products [39]. The abundance and persistence of *Pseudomonas* in foods could also be related to its ubiquity in the environment, and, most importantly, to its ability to form biofilm, which may increase its tolerance to adverse conditions, including several antimicrobial treatments [40]. *P. oryzihabitans* is frequently found in hospital environments. It is rarely implicated in human infections. However, it was reported as being the cause of an infection in a 72-year-old man who was apparently healthy after a total hip arthroplasty four years before [41], as well as multiple skin rashes on the scalp and neck of a 1-year-old girl from Ghana [42]. Nevertheless, *P. oryzihabitans* is easy to eradicate due to its susceptibility to antibiotic therapy. Several *Bacillus* species have been associated with a variety of infections as some of them are considered foodborne pathogens, such as *B. cereus*, *B. subtilis*, *B. anthracis* and *B. licheniformis* [43]. At the same time, *Bacillus* spp. (*B. cereus*, *B. licheniformis*, *B. subtilis*, *B. clausii*, *B. coagulans*, *B. polyfermenticus* and *B. pumilus*) are being used as probiotics due to their ability to form endospores, to replace antibiotics and to act as immune-modulators and microbiome regulators [44]. *S. maltophilia* is an environmental multiple-drug-resistant organism that has been associated with nosocomial and community-acquired infections (e.g. pneumonia, meningitis, bacteremia and endocarditis) described by Brooke [45]. *A. piechaudii* is a recently described Gram-negative bacteria usually found in soil and water with low pathogenicity. Nevertheless, a few cases of *A. piechaudii* infections have been reported, namely, bacteremia, both in a neutropenic man with hematologic malignancy [46] and in an immunocompetent host [47].

After 12 days (T12) of packaging and refrigerated storage, the most prevalent bacterial species identified in the samples were *S. warneri* in EXT-PLA (39%) and EXT-OPP (74%), *Micrococcus* spp. (67%) in AA-PLA and *Pantoea agglomerans* (48%) in AA-OPP (Figure 2). *Micrococcus* species, members of the family *Micrococcaceae*, are usually regarded as

contaminants from skin and mucous membranes, but they are not considered to be pathogenic. Nonetheless, some authors related them with some infections in immunocompromised patients, namely, pneumonia in a 26-year-old female patient with acute myeloid leukemia [48], catheter-related infection in hypertension patients [49], brain abscess in a patient with systemic lupus erythematosus [50] and native valve endocarditis in a non-Hodgkin's lymphoma patient [51], all caused by *M. luteus* infection. Infections by *P. agglomerans*, an environmental and agricultural organism of the *Enterobacteriaceae* family, are uncommon and an adequate treatment with antibiotic promotes a full recovery of the patients [52]. In spite of that, this bacteria is an opportunistic pathogen associated with wound- or hospital-acquired infections, occurring mostly in immunocompromised individuals [53–55]. *P. agglomerans* was only found at T12 and for the samples packaged in OPP films. This could indicate the lower ability of this film to control the bacteria growth owing to its permeability characteristics. Previously, Torres et al. [56] reported high viability of *P. agglomerans* in packages with low oxygen permeability. However, this is not in agreement with the results of this work since OPP films presented higher oxygen permeability (2.68×10^{-12} m²/s) when compared to PLA films (2.97×10^{-16} m²/s).

According to the literature, the bacteria frequently associated with the spoilage of minimally-processed fruit and vegetables belong to the genera *Corynebacterium*, *Pseudomonas*, *Erwinia* and other *Enterobacteriaceae* [40]. Bacterial isolates from these genera and *Enterobacteriaceae* family were isolated in the apple samples. Nevertheless, these were not the most prevalent bacteria at the end of storage period. In fact, after 12 days of refrigerated storage, the fresh-cut apples in PLA film presented *Micrococcus* spp. (AA-PLA, 67%) and *S. warneri* (EXT-PLA, 39%) as the most frequent isolate bacteria (Figure 2c).

The bacterial community in a specific environment could be characterized by the number of species present and their numerical composition, the bacterial diversity. In general, an increase in the number of detected species of aerobic mesophilic bacteria was observed in apple samples stored for 12 days (Figure 2). This can be attributed to the cutting of the apples before immersion in antioxidant solutions and packaging. This procedure increases the water activity and availability of nutrients at cut surface [57], causing tissue damages and promoting the growth of microorganisms [3]. Nonetheless, the sample with olive pomace extract as additive and packaged with OPP film (EXT-OPP) maintained the number of bacterial species during the storage period in contrast with the other samples. By contrast, the highest number of bacterial species was found in the samples using ascorbic acid as additive packaged in either film (AA-PLA and AA-OPP). This suggests that the commercial additive ascorbic acid could have a limited effect to control the microbial diversity in fruits.

A variety of indices have been developed for the comparison of the bacterial diversity [58]. Among them, the most commonly used in bacterial diversity measurements are Shannon–Weaver and Simpson diversity indices [23,24].

Simpson's index of diversity (1-D) indicates the number of different species isolated and how evenly these different species are distributed based on total detected microorganisms. The Shannon index is a quantitative indicator of the number of different bacterial species that are present in a sample, considering the uniformity in the distribution of these bacteria in these species. The Shannon diversity index takes a greater weight on species richness (number of different species present). In turn the Simpson index contemplates species evenness (the relative abundance of the different species consisting of a community) more than species richness in its estimative [58]. The calculated Simpson diversity and Shannon indices for the bacterial community detected in the fresh-cut apple samples during storage with the two different packaging films and additives are presented in Table 1.

Shannon's and Simpson's indices showed similar trends, the higher the indices' values, the higher the bacterial community diversity. The diversity indices analysis indicated an increasing trend in the bacterial community diversity of fresh-cut apples with storage time (Table 1), as mentioned above (Figure 2).

The bacterial diversity measured by the Simpson and Shannon indices showed differences among the four types of processed (package film-additive) fresh-cut apples. The lowest bacterial diversity was obtained for fresh-cut apples treated with olive pomace extract and packaged in PLA film. The samples with olive pomace extract demonstrated less bacterial diversity comparatively with the ones treated with ascorbic acid. The fresh-cut apple bacterial community composition was indicated to be influenced by the additive and film on the package. This suggests that the combination of the treatment with olive pomace extract and packaging with PLA film could be able to control the bacterial diversity in apple slices.

Table 1. Diversity indices of bacteria from fresh-cut apple samples by storage time (T0, T5 and T12) or by packaging film (OPP and PLA) and additive (ascorbic acid and olive pomace extract).

Fresh-Cut Apples [1]	Simpson's Index of Diversity (1-D)	Shannon Diversity Index (H)
T0	0.60	1.29
T5	0.71	1.56
T12	0.79	1.87
AA-PPO	0.84	1.98
AA-PLA	0.70	1.51
EXT-PPO	0.60	1.18
EXT-PLA	0.47	1.02

[1] T0: microbial community immediately after treatment and packaging; T5: microbial community after 5 days of refrigerated storage; T12: microbial community after 12 days of refrigerated storage. AA-OPP: treated with ascorbic acid and packaged in OPP films; AA-PLA: treated with ascorbic acid and packaged in PLA films; EXT-OPP: treated with olive pomace extracts and packaged in OPP films; EXT-PLA: treated with olive pomace extracts and packaged in PLA films.

None of the analyzed samples were positive for *Escherichia coli*, *Cronobacter sakazakii*, *Salmonella* spp., *Clostridium perfringens* and *Listeria monocytogenes*, the main foodborne pathogens associated with fresh fruit [3]. Previous studies also described the low or inexistent presence of foodborne microorganisms in fresh fruits [2,59]. Adi et al. [60] reported that the majority of the cultivated bacteria in apples belonged to *Bacillus*, *Curtobacterium*, *Erwinia*, *Pseudomonas* and *Xanthomonas* genera. Abdelfattah et al. [4] cited *Sphingomonas* and *Methylobacterium* as the main bacterial genera of apple microbiome. Leishman et al. [61] also reported the survival of *B. anthracis* spores in fruit juices for at least a month.

In the present study, the species *S. warneri* and the genus *Bacillus* were found to be the most common bacteria over all the analyzed periods, T0-T12 (Figure 2). López-González and co-workers [62] described the antagonistic activity of *Bacillus* species isolated from 'Royal Gala' apples against *Penicillium expansum*. This might be related to their capacity to produce lipopeptides and siderophoros. *B. mojavensis* EGE-B-5.2i and *B. thuringiensis* EGE-B-14.1i were reported to be efficient antifungal agents against *Aspergillus niger* of Turkish figs [63]. Besides being the most prevalent species found in the analyzed samples (*S. warneri*), *Staphylococcus* genus has many pathogenic species often found in the skin. Previous studies indicated that *Staphylococcus* were abundant on minimally processed fruit surfaces and its detection could indicate human cross-contamination [53]. Herein, other *Staphylococcus* species were found at frequencies lower than 33%, such as *S. capitis* ssp. *capitis*, *S. saprophyticus*, *S. xylosus* and *S. epidermidis*. Some of these species are associated with the skin microbiota and, consequently, to the human microbiome, being essential to maintaining human health and preventing diseases. For instance, *S. epidermidis* was reported as the producer of 6-N-hydroxylaminopurine (6-HAP), which can protect against neoplasia [64] and act as a stimulator of nasal epithelia to antimicrobial peptides, killing competing pathogens [65]. Recently, Adi et al. [60] suggested that some specific bacteria present in raw-eaten fruits may have beneficial properties for human health and that some

of the bacteria associated with apples can be compatible with the gastrointestinal environment.

The obtained results indicated a diverse microbial pattern for fresh-cut apples depending on the type and length of storage as influenced by a series of factors, including packaging and processing conditions.

4. Conclusions

In recent years, the growing interest in a healthy and convenient diet has led to a significant increase in the consumption of prepacked 'ready-to-eat' fruits. In this work, an assessment of the natural microbiota of apple slices treated with two different antioxidants and packed in bags made of two different films was performed during 12 days of refrigerated storage. The antioxidants were ascorbic acid and olive pomace extract, and the bags were made of a biodegradable (PLA) film and a conventional one (OPP). The results revealed lower bacteria counts in the samples using olive pomace extracts and PLA films after 5 and 12 days of storage when compared with ascorbic acid and OPP films. These findings suggest that the use of those natural extracts as additives in fruits could be efficient to delay the growth of mesophilic bacteria. The identification of the bacterial isolates from fresh-cut apple samples indicated that none of the apple samples were positive for the main foodborne pathogens associated with fresh fruit. Nevertheless, the most prevalent species of the analyzed samples were *Citrobacter freundii*, *Staphylococcus warneri*, *Pseudomonas oryzihabitans*, *Alcalinogenes faecalis*, *Corynebacterium jeikeium*, *Micrococcus* spp., *Pantoea aglomerans* and *Bacillus* spp. These are bacteria from human and environmental microbiota. It was possible to observe an increase in microbial diversity during the storage period. The highest microbial diversity was found in the samples using ascorbic acid as an additive (AA-PLA and AA-OPP). This suggests that the commercial additive, ascorbic acid, may have a limited effect on the microbial inhibition in the apple slices. As a global conclusion, it can be stated that the olive pomace extract demonstrated the potential to be used as a natural antimicrobial preservative in fresh ready-to-eat food. Furthermore, its combination with PLA film could be the most adequate process, as confirmed by Shannon's and Simpson's indices. The obtained results can assist the food industries in their efforts both to generate new functional foods and to promote the economic and environmental sustainability of the olive oil sector.

Author Contributions: Conceptualization, S.C.V.; methodology, S.C.V.; investigation, J.M. and S.G.; writing—original draft preparation, J.M.; writing—review and editing, C.S.-B., F.M.A.M., I.C.F.R.F., L.B. and S.C.V.; supervision, C.S.-B., L.B. and S.C.V.; funding acquisition, C.S.-B., L.B., S.C.V. and I.C.F.R.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Foundation for Science and Technology (FCT, Portugal): C²TN (UIDB/04349/2020), CIMO (UIDB/00690/2020) and UIDP/00690/2020), SusTEC (LA/P/0007/2021), J. Madureira (SFRH/BD/136506/2018), L. Barros (institutional scientific employment program-contract); European Regional Development Fund (ERDF): projects "BIOMA" (POCI-01-0247-FEDER-046112) and "OliveBIOextract" (NORTE-01-0247-FEDER-049865); Junta de Castilla y León (SA093P20 and CLU-2018-04) co-funded by the P.O. FEDER of Castilla y León 2014–2020: GIP-USAL.

Data Availability Statement: The datasets used/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments: The authors are grateful to "UCASUL-União de Cooperativas Agrícolas do Sul" agro industrial cooperative for providing the samples and to the Technological Unit of Radiosterilization (University of Lisbon) for the samples' irradiation.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Kabelitz, T.; Hassenberg, K. Control of apple surface microflora for fresh-cut produce by post-harvest hot-water treatment. *LWT-Food Sci. Technol.* **2018**, *98*, 492–499. https://doi.org/10.1016/j.lwt.2018.08.062.
2. Graça, A.; Santo, D.; Esteves, E.; Nunes, C.; Abadias, M. Evaluation of microbial quality and yeast diversity in fresh-cut apple. *Food Microbiol.* **2015**, *51*, 179–185. https://doi.org/10.1016/j.fm.2015.06.003.
3. Harris, L.J.; Farber, J.N.; Beuchat, L.R.; Parish, M.E.; Suslow, T.V.; Garrett, E.H.; Busta, F.F. Outbreaks Associated with Fresh Produce: Incidence, Growth, and Survival of Pathogens in Fresh and Fresh-Cut Produce. *Compr. Rev. Food Sci. Food Saf.* **2003**, *2*, 78–141.
4. Abdelfattah, A.; Freilich, S.; Bartuv, R.; Zhimo, V.Y.; Kumar, A.; Biasi, A.; Salim, S.; Feygenberg, O.; Burchard, E.; Dardick, C.; et al. Global analysis of the apple fruit microbiome: Are all apples the same? *Environ. Microbiol.* **2021**, *23*, 6038–6055. https://doi.org/10.1111/1462-2920.15469.
5. Engin, A.B.; Bukan, N.; Kurukahvecioglu, O.; Memis, L.; Engin, A. Effect of butylated hydroxytoluene (E321) pretreatment versus l -arginine on liver injury after sub-lethal dose of endotoxin administration. *Environ. Toxicol. Pharmacol.* **2011**, *32*, 457–464. https://doi.org/10.1016/j.etap.2011.08.014.
6. Jeong, S.H.; Kim, B.Y.; Kang, H.G.; Ku, H.O.; Cho, J.H. Effects of butylated hydroxyanisole on the development and functions of reproductive system in rats. *Toxicology* **2005**, *208*, 49–62. https://doi.org/10.1016/j.tox.2004.11.014.
7. Bauer, A.K.; Dwyer-Nield, L.D.; Hankin, J.A.; Murphy, R.C.; Malkinson, A.M. The lung tumor promoter, butylated hydroxytoluene (BHT), causes chronic inflammation in promotion-sensitive BALB/cByJ mice but not in promotion-resistant CXB4 mice. *Toxicology* **2001**, *169*, 1–15. https://doi.org/10.1016/S0300-483X(01)00475-9.
8. Ito, N.; Fukushima, S.; Tsuda, H. Carcinogenicity and modification of the carcinogenic response by BHA, BHT, and other antioxidants. *CRC Crit. Rev. Toxicol.* **1985**, *15*, 109–150.
9. Caleja, C.; Barros, L.; Prieto, M.A.; Bento, A.; Oliveira, M.B.P.P.; Ferreira, I.C.F.R. Development of a natural preservative obtained from male chestnut flowers: Optimization of a heat-assisted extraction technique. *Food Funct.* **2019**, *10*, 1352–1363. https://doi.org/10.1039/c8fo02234h.
10. Caleja, C.; Barros, L.; Antonio, A.L.; Oliveira, M.B.P.; Ferreira, I.C.F.R. A comparative study between natural and synthetic antioxidants: Evaluation of their performance after incorporation into biscuits. *Food Chem.* **2017**, *216*, 342–346. https://doi.org/10.1016/j.foodchem.2016.08.075.
11. Caleja, C.; Barros, L.; Antonio, A.L.; Carocho, M.; Oliveira, M.B.P.P.; Ferreira, I.C.F.R. Fortification of yogurts with different antioxidant preservatives: A comparative study between natural and synthetic additives. *Food Chem.* **2016**, *210*, 262–268. https://doi.org/10.1016/j.foodchem.2016.04.114.
12. Faustino, M.; Veiga, M.; Sousa, P.; Costa, E.M.; Silva, S.; Pintado, M. Agro-food byproducts as a new source of natural food additives. *Molecules* **2019**, *24*, 1056. https://doi.org/10.3390/molecules24061056.
13. Madureira, J.; Albuquerque, B.; Dias, M.I.; Pinela, J.; Calhelha, R.C.; Santos-Buelga, C.; Margaça, F.M.A.; Ferreira, I.C.F.R.; Cabo Verde, S.; Barros, L. Ultrasound-assisted extraction of hydroxytyrosol and tyrosol from olive pomace treated by gamma radiation: Process optimization and bioactivity assessment. *Food Funct.* **2023**, *14*, 3038–3050. https://doi.org/10.1039/d2fo03607j.
14. Madureira, J.; Dias, M.I.; Pinela, J.; Calhelha, R.C.; Barros, L.; Santos-Buelga, C.; Margaça, F.M.A.; Ferreira, I.C.F.R.; Cabo Verde, S. The use of gamma radiation for extractability improvement of bioactive compounds in olive oil wastes. *Sci. Total Environ.* **2020**, *727*, 138706. https://doi.org/10.1016/j.scitotenv.2020.138706.
15. Lin, S.; Chi, W.; Hu, J.; Pan, Q.; Zheng, B.; Zeng, S. Sensory and nutritional properties of chinese olive pomace based high fibre biscuit. *Emirates J. Food Agric.* **2017**, *29*, 495–501. https://doi.org/10.9755/ejfa.2016-12-1908.
16. Cedola, A.; Cardinali, A.; Del Nobile, M.A.; Conte, A. Fish burger enriched by olive oil industrial by-product. *Food Sci. Nutr.* **2017**, *5*, 837–844. https://doi.org/10.1002/fsn3.461.
17. Durante, M.; Bleve, G.; Selvaggini, R.; Veneziani, G.; Servili, M.; Mita, G. Bioactive compounds and stability of a typical Italian bakery products "taralli" enriched with fermented olive paste. *Molecules* **2019**, *24*, 3258. https://doi.org/10.3390/molecules24183258.
18. Cecchi, L.; Schuster, N.; Flynn, D.; Bechtel, R.; Bellumori, M.; Innocenti, M.; Mulinacci, N.; Guinard, J.X. Sensory Profiling and Consumer Acceptance of Pasta, Bread, and Granola Bar Fortified with Dried Olive Pomace (Pâté): A Byproduct from Virgin Olive Oil Production. *J. Food Sci.* **2019**, *84*, 2995–3008. https://doi.org/10.1111/1750-3841.14800.
19. Cedola, A.; Cardinali, A.; Del Nobile, M.A.; Conte, A. Enrichment of Bread with Olive Oil Industrial By-Product. *J. Agric. Sci. Technol. B* **2019**, *9*, 119–127. https://doi.org/10.17265/2161-6264/2019.02.005.
20. Ribeiro, T.B.; Bonifácio-Lopes, T.; Morais, P.; Miranda, A.; Nunes, J.; Vicente, A.A.; Pintado, M. Incorporation of olive pomace ingredients into yoghurts as a source of fibre and hydroxytyrosol: Antioxidant activity and stability throughout gastrointestinal digestion. *J. Food Eng.* **2021**, *297*, 110476. https://doi.org/10.1016/j.jfoodeng.2021.110476.
21. Madureira, J.; Melgar, B.; Santos-Buelga, C.; Margaça, F.M.A.; Ferreira, I.C.F.R.; Barros, L.; Cabo Verde, S. Phenolic compounds from irradiated olive wastes: Optimization of the heat-assisted extraction using response surface methodology. *Chemosensors* **2021**, *9*, 231. https://doi.org/10.3390/chemosensors9080231.
22. Holt, J.G.; Krieg, N.R.; Sneath, P.H.A.; Stanley, J.T.; William, S.T. *Bergey's Manual of Determinative Bacteriology*; Williams and Wilkins: Baltimore, MD, USA, 1994.
23. Simpson, E.H. Measurement of diversity. *Nature* **1949**, *163*, 688. https://doi.org/10.1038/163688A0.

24. Shannon, C.E. A Mathematical Theory of Communication. *Bell Syst. Tech. J.* **1948**, *27*, 623–656. https://doi.org/10.1002/j.1538-7305.1948.tb00917.x.
25. Moreira, M.R.; Tomadoni, B.; Martín-Belloso, O.; Soliva-Fortuny, R. Preservation of fresh-cut apple quality attributes by pulsed light in combination with gellan gum-based prebiotic edible coatings. *LWT-Food Sci. Technol.* **2015**, *64*, 1130–1137. https://doi.org/10.1016/j.lwt.2015.07.002.
26. Osuga, R.; Koide, S.; Sakurai, M.; Orikasa, T.; Uemura, M. Quality and microbial evaluation of fresh-cut apples during 10 days of supercooled storage. *Food Control* **2021**, *126*, 108014. https://doi.org/10.1016/j.foodcont.2021.108014.
27. Instituto Nacional de Saúde Doutor Ricardo Jorge. *Interpretação de Resultados de Ensaios Microbiológicos em Alimentos Prontos Para Consumo e em Superfícies do Ambiente de Preparação e Distribuição Alimentar: Valores Guia*; INSA IP: Lisbon, Portugal, 2019; ISBN 9789898794529.
28. Anderson, M.T.; Mitchell, L.A.; Zhao, L.; Mobley, H.L.T. Citrobacter freundii fitness during bloodstream infection. *Sci. Rep.* **2018**, *8*, 11792. https://doi.org/10.1038/s41598-018-30196-0.
29. García, K.C.; Duran, O.C.; Pérez, T.A.; Jiménez, Á.V.; Pérez, E.M.; Martínez-Ávila, M.C.; Yánez, T.R.; Almanza-hurtado, A. A Rare Case of Meningitis Caused by Alcaligenes faecalis in an Immunocompetent Patient. *Case Rep. Med.* **2022**, *2022*, 3–5.
30. Kahveci, A.; Asicioglu, E.; Tigen, E.; Ari, E.; Arikan, H.; Odabasi, Z.; Ozener, C. Unusual causes of peritonitis in a peritoneal dialysis patient: Alcaligenes faecalis and Pantoea agglomerans. *Ann. Clin. Microbiol. Antimicrob.* **2011**, *10*, 12. https://doi.org/10.1186/1476-0711-10-12.
31. Al-zakhari, R.; Suhail, M.; Ataallah, B.; Aljammali, S.; Grigos, A. Rare but Fatal Case of Cavitary Pneumonia Caused by Alcaligenes Faecalis. *Cureus* **2020**, *12*, e8934. https://doi.org/10.7759/cureus.8934.
32. Huang, C. Extensively drug-resistant Alcaligenes faecalis infection. *BMC Infect. Dis.* **2020**, *20*, 833.
33. Hasan, J.; Nahar, L.; Rabbani, R. Bloodstream infection with pandrug-resistant Alcaligenes faecalis treated with double-dose of tigecycline. *IDCases* **2019**, *18*, e00600. https://doi.org/10.1016/j.idcr.2019.e00600.
34. Yang, K.; Kruse, R.L.; Lin, W.V.; Musher, D.M. Corynebacteria as a cause of pulmonary infection: A case series and literature review. *Pneumonia* **2018**, *10*, 10.
35. Pardo, S.M.M.; Patel, R.H.; Ramsakal, A.; Greene, J. Disseminated Corynebacterium jeikeium Infection in Cancer Patients Case Presentation. *Cureus* **2020**, *12*, e8764. https://doi.org/10.7759/cureus.8764.
36. Kanuparthy, A.; Challa, T.; Meegada, S.; Siddamreddy, S.; Muppidi, V. Staphylococcus warneri: Skin Commensal and a Rare Cause of Urinary Tract Infection. *Cureus* **2020**, *12*, e8337. https://doi.org/10.7759/cureus.8337.
37. Ivić, I.; Karanović, J.; Pavičić-Ivelja, M. Sepsis with multiple abscesses caused by staphylococcus warneri: A case report. *Cent. Eur. J. Med.* **2013**, *8*, 45–47. https://doi.org/10.2478/s11536-012-0066-0.
38. Phukon, M.; Sahu, P.; Srinath, R.; Nithya, A.; Babu, S. Unusual Occurrence of Staphylococcus warneri as Endophyte in Fresh Fruits along with Usual Bacillus spp. *J. Food Saf.* **2013**, *33*, 102–106. https://doi.org/10.1111/jfs.12028.
39. Quintieri, L.; Fanelli, F.; Caputo, L. Antibiotic Resistant Pseudomonas Spp. Spoilers in Fresh Dairy Products: An Underestimated Risk and the Control Strategies. *Foods* **2019**, *8*, 372. https://doi.org/10.3390/FOODS8090372.
40. Sequino, G.; Valentino, V.; Torrieri, E.; De Filippis, F. Specific Microbial Communities Are Selected in Minimally-Processed Fruit and Vegetables according to the Type of Product. *Foods* **2022**, *11*, 2164. https://doi.org/10.3390/FOODS11142164/S1.
41. Panagopoulos, G.N.; Megaloikonomos, P.D.; Liontos, M.; Giannitsioti, E.; Drogari-Apiranthitou, M.; Mavrogenis, A.F.; Kontogeorgakos, V. Pseudomonas oryzihabitans Infected Total Hip Arthroplasty. *J. Bone Jt. Infect.* **2016**, *1*, 54–58. https://doi.org/10.7150/jbji.16967.
42. Owusu, M.; Owusu-Dabo, E.; Acheampong, G.; Osei, I.; Amuasi, J.; Sarpong, N.; Annan, A.; Chiang, H.; Kuo, C.; Park, S.E.; et al. Pseudomonas oryzihabitans sepsis in a 1- year-old child with multiple skin rashes: A case report. *J. Med. Case Rep.* **2017**, *11*, 10–13. https://doi.org/10.1186/s13256-017-1230-6.
43. Logan, N.A. Bacillus and relatives in foodborne illness. *J. Appl. Microbiol.* **2011**, *112*, 417–429. https://doi.org/10.1111/j.1365-2672.2011.05204.x.
44. Lee, N.-K.; Kim, W.-S.; Paik, H.-D. Bacillus strains as human probiotics : Characterization , safety, microbiome, and probiotic carrier. *Food Sci. Biotechnol.* **2019**, *28*, 1297–1305. https://doi.org/10.1007/s10068-019-00691-9.
45. Brooke, J.S. Stenotrophomonas maltophilia: An Emerging Global Opportunistic Pathogen. *Clin. Microbiol. Rev.* **2012**, *25*, 2–41. https://doi.org/10.1128/CMR.00019-11.
46. Kay, S.E.; Clark, R.A.; White, K.L.; Peel, M.M. Recurrent Achromobacter piechaudii Bacteremia in a Patient with Hematological Malignancy. *J. Clin. Microbiol.* **2001**, *39*, 808–810. https://doi.org/10.1128/JCM.39.2.808.
47. Krause, M.L.; Sohail, M.R.; Patel, R.; Wittich, C.M. Achromobacter piechaudii bloodstream infection in an immunocompetent host. *Am. J. Case Rep.* **2012**, *13*, 265–267.
48. Adang, R.; Schouten, H.; van Tiel, F.; Blijham, G. Pneumonia due to *Micrococcus* spp. in a patient with acute myeloid leukaemia. *Leukemia* **1992**, *6*, 224–226.
49. Oudiz, R.J.; Widlitz, A.; Beckmann, X.J.; Camanga, D.; Alfie, J.; Brundage, B.H.; Barst, R.J. Micrococcus-Associated Central Venous Catheter Infection in Patients With Pulmonary Arterial Hypertension. *Clin. Investig.* **2004**, *126*, 90–94.
50. Erbasan, F. Brain abscess caused by Micrococcus luteus in a patient with systemic lupus erythematosus : Case-based review. *Rheumatol. Int.* **2018**, *38*, 2323–2328. https://doi.org/10.1007/s00296-018-4182-2.
51. Ianniello, N.M.; Andrade, D.C.; Ivancic, S.; Eckardt, P.A.; Lemos Ramirez, J.C. Native valve infective endocarditis due to Micrococcus luteus in a non-Hodgkin' s lymphoma patient. *IDCases* **2019**, *18*, e00657. https://doi.org/10.1016/j.idcr.2019.e00657.

52. Dutkiewicz, J.; Mackiewicz, B.; Lemieszek, M.K.; Golec, M. Pantoea agglomerans: A mysterious bacterium of evil and good. Part III . Deleterious effects: Infections of humans, animals and plants. *Ann. Agric. Environ. Med.* **2016**, *23*, 197–205. https://doi.org/10.5604/12321966.1203878.
53. Büyükcam, A.; Tuncer, Ö.; Gür, D.; Sancak, B.; Ceyhan, M.; Cengiz, A.B.; Kara, A. Clinical and microbiological characteristics of Pantoea agglomerans infection in children. *J. Infect. Public Health* **2018**, *11*, 304–309. https://doi.org/10.1016/j.jiph.2017.07.020.
54. Tsubouchi, H.; Matsumoto, N.; Yanagi, S.; Arimura, Y. Respiratory Medicine Case Reports Successful treatment of sepsis due to Pantoea agglomerans by polymyxin B-immobilized fi ber column direct hemoperfusion therapy in a small cell lung carcinoma patient. *Respir. Med. Case Rep.* **2016**, *19*, 155–158. https://doi.org/10.1016/j.rmcr.2016.10.004.
55. Faria, N.; Costa, M.I.; Quita, R.; Dias, C.; Gomes, J.; Cruz, H.; Sucena, M. Pantoea agglomerans as a cancer hallmark in immunocompromised patients. *Rev. Patol. Respir.* **2021**, *24*, 23–25.
56. Torres, R.; Solsona, C.; Viñas, I.; Usall, J.; Plaza, P.; Teixidó, N. Optimization of packaging and storage conditions of a freeze-dried Pantoea agglomerans formulation for controlling postharvest diseases in fruit. *J. Appl. Microbiol.* **2014**, *117*, 173–184. https://doi.org/10.1111/jam.12511.
57. Olaimat, A.N.; Holley, R.A. Factors influencing the microbial safety of fresh produce: A review. *Food Microbiol.* **2012**, *32*, 1–19. https://doi.org/10.1016/j.fm.2012.04.016.
58. Kim, B.R.; Shin, J.; Guevarra, R.B.; Lee, J.H.; Kim, D.W.; Seol, K.H.; Lee, J.H.; Kim, H.B.; Isaacson, R.E. Deciphering diversity indices for a better understanding of microbial communities. *J. Microbiol. Biotechnol.* **2017**, *27*, 2089–2093. https://doi.org/10.4014/jmb.1709.09027.
59. Abadias, M.; Usall, J.; Anguera, M.; Solsona, C.; Viñas, I. Microbiological quality of fresh , minimally-processed fruit and vegetables , and sprouts from retail establishments. *Int. J. Food Microbiol.* **2008**, *123*, 121–129. https://doi.org/10.1016/j.ijfoodmicro.2007.12.013.
60. Adi, W.; Buko, A.; Kusstatscher, P.; Sinkkonen, A.; Laitinen, O.H.; Cernava, T.; Berg, G.; Virtanen, S.M.; Hy, H. Modulation of the food microbiome by apple fruit processing. *Food Microbiol.* **2022**, *108*, 104103. https://doi.org/10.1016/j.fm.2022.104103.
61. Leishman, O.N.; Johnson, M.J.; Labuza, T.P.; Diez-Gonzalez, F. Survival of Bacillus anthracis spores in fruit juices and wine. *J. Food Prot.* **2010**, *73*, 1694–1697. https://doi.org/10.4315/0362-028X-73.9.1694.
62. López-González, R.C.; Juárez-Campusano, Y.S.; Rodríguez-Chávez, J.L.; Delgado-Lamas, G.; Medrano, S.M.A.; Martínez-Peniche, R.Á.; Pacheco-Aguilar, J. ramiro Antagonistic Activity of Bacteria Isolated from Apple in Different Fruit Development Stages against Blue Mold Caused by Penicillium expansum. *Plant Pathol. J.* **2021**, *37*, 24–35.
63. Öztopuz, Ö.; Pekin, G.; Park, R.D.; Eltem, R. Isolation and Evaluation of New Antagonist Bacillus Strains for the Control of Pathogenic and Mycotoxigenic Fungi of Fig Orchards. *Appl. Biochem. Biotechnol.* **2018**, *186*, 692–711.
64. Nakatsuji, T.; Chen, T.H.; Butcher, A.M.; Trzoss, L.L.; Nam, S.J.; Shirakawa, K.T.; Zhou, W.; Oh, J.; Otto, M.; Fenical, W.; et al. A commensal strain of Staphylococcus epidermidis protects against skin neoplasia. *Sci. Adv.* **2018**, *4*, eaao4502. https://doi.org/10.1126/sciadv.aao4502.
65. Liu, Q.; Liu, Q.; Meng, H.; Lv, H.; Liu, Y.; Liu, J.; Wang, H.; He, L.; Qin, J.; Wang, Y.; et al. Staphylococcus epidermidis Contributes to Healthy Maturation of the Nasal Microbiome by Stimulating Antimicrobial Peptide Production. *Cell Host Microbe* **2020**, *27*, 68-78.e5. https://doi.org/10.1016/j.chom.2019.11.003.

3. DISCUSIÓN GLOBAL DE LOS RESULTADOS - INTEGRATED DISCUSSION OF THE RESULTS

El orujo de aceituna es un residuo de la industria del aceite de oliva cuyo desecho es causa de preocupación. No obstante, se trata de un producto rico en compuestos bioactivos con posibles beneficios para la salud humana, cuya recuperación sería de interés para la industria alimentaria por su potencial antioxidante y antimicrobiano.

Por otra parte, la irradiación es una tecnología limpia, ecológica y prometedora, que puede ser utilizada para mejorar la capacidad de extracción de compuestos bioactivos a partir de residuos agroindustriales (Harrison & Were, 2007; Ito et al., 2016; Khattak & Rahman, 2016; Pérez et al., 2007; Taheri et al., 2014), fruta o plantas medicinales (Barkaoui et al., 2021b, 2021a; Pereira et al., 2015b).

En este trabajo se evaluó la extracción de compuestos fenólicos de dos tipos de orujo procedentes de dos etapas de la producción de aceite de oliva: orujo de oliva crudo (*Crude Olive Pomace*, COP), orujo seco obtenido antes de la extracción del aceite de orujo, y orujo de oliva agotado (*Extracted Olive Pomace*, EOP), que resulta después de la extracción del aceite de orujo con disolventes. El objetivo final es aportar a la industria nuevos productos con potencial para ser utilizados como aditivos naturales o ingredientes bioactivos en la elaboración de alimentos.

El compuesto fenólico más abundante encontrado en los extractos de orujo estudiados fue el hidroxitirosol (25 ± 1 mg/g extracto en EOP y 23.9 ± 0.3 mg/g extracto en COP), seguido de hidroxitirosol-1-β-glucósido (9.8 ± 0.4 mg/g extracto en EOP y 9.94 ± 0.08 mg/g extracto en COP), tirosol (5.9 ± 0.2 mg/g extracto en EOP y 5.9 ± 0.4 mg/g extracto en COP), luteolina-7-*O*-rutinósido (3.1 ± 0.1 mg/g extracto en EOP y 4.1 ± 0.2 mg/g extracto en COP), oleuropeína aglicón (3.0 ± 0.3 mg/g extracto en EOP y 3.1 ± 0.1 mg/g extracto en COP) y verbascósido (2.29 ± 0.03 mg/g extracto en EOP y 3.4 ± 0.2 mg/g extracto en COP), que habían sido previamente descritos en la literatura en este tipo de muestras (Chanioti & Tzia, 2018; Malapert et al., 2018; Nunes M.A. et al., 2018; Suárez et al., 2010). La composición del orujo de aceituna depende de la temporada de cosecha, lo que puede influir en la reproducibilidad de los resultados de extractabilidad.

Se evaluó el efecto de las radiaciones ionizantes para mejorar la extractabilidad de estos compuestos. Los resultados obtenidos pusieron de manifiesto que la aplicación de radiación gamma a dosis de 5 kGy conseguía aumentar la cantidad de compuestos extraídos en al menos 2 veces (concentración de compuestos fenólicos totales de 57 ± 2 mg/g extracto en la muestra no irradiada y 139 ± 2 mg/g extracto en la muestra irradiada a 5 kGy), así como la actividad antioxidante de los extractos obtenidos, especialmente en los extractos de EOP en cuanto a su capacidad para inhibir la formación de sustancias reactivas a ácido tiobarbitúrico (TBARS) (IC_{50} = 49 ± 1 µg/mL

y 22 ± 1 µg/mL para las muestras no irradiada y irradiada a 5 kGy, respectivamente), manteniendo su actividad antihemolítica (IC_{50} = 20.6 ± 0.4 µg/mL) en valores similares a los del antioxidante de referencia, Trolox (IC_{50} = 19.6 ± 0.6 µg/mL). La mejora en la extracción de los compuestos fenólicos de las muestras irradiadas se atribuyó a los cambios en la estructura celular, con liberación de fracciones asociadas a polisacáridos y otros componentes de matriz, así como a la ruptura de polifenoles de mayor tamaño con liberación de otros más pequeños, por la acción radiolítica de las radiaciones ionizantes (Harrison & Were, 2007; Hussain et al., 2016).

Teniendo en cuenta estos resultados, se procedió a realizar ensayos para optimizar la extracción de los principales compuestos fenólicos en muestras de EOP irradiadas a 5 kGy, utilizando dos técnicas diferentes: extracción asistida por calor (HAE) y extracción asistida por ultrasonidos (UAE). Se aplicó una Metodología de Superficie de Respuesta (RSM) como estrategia estadística para establecer la influencia combinada de distintas variables (tiempo -t-, temperatura -T-, potencia ultrasónica -P- y tipo de solvente -S) y maximizar la respuesta. En las mejores condiciones obtenidas (t = 28 min, P = 490 W y S = etanol 7,3%, para UAE; t = 120 min, T = 85 ºC y S = etanol 76%, para HAE), la UAE redujo el tiempo de extracción y el consumo de solvente en comparación con la HAE, y también condujo a mayores rendimientos de extracción de compuestos fenólicos (30% frente a 13,7%). Este resultado se puede atribuir a los efectos de cavitación producidos por los ultrasonidos, que provocan cambios en la pared celular, facilitando así la liberación de solutos, que se encuentran más accesibles al solvente (Gómez-Cruz et al., 2021; Zhang, Lin & Ye, 2018). La temperatura del sistema UAE debe ser controlada para evitar que su aumento durante la extracción afecte la extractabilidad de los compuestos fenólicos. No obstante, a pesar del menor rendimiento de extracción de compuestos fenólicos en la HAE, los extractos obtenidos por esta técnica presentaron mayor capacidad antioxidante, medida tanto por la inhibición de la formación de TBARS (aumento de 1,3 veces) como de la hemólisis oxidativa (aumento de 2 veces), así como mayor actividad antidiabética, evaluada por la inhibición de α-amilasa (aumento de 2 veces) y de α-glucosidasa (aumento de 2 veces). Esto está de acuerdo con las observaciones de Collado-González et al. (2017), quienes relacionaron la inhibición efectiva de α-glucosidasa en muestras de aceite de oliva con una mayor actividad antioxidante. Los extractos de HAE también mostraron mayores actividades antiinflamatoria (aumento de 7 veces) y antibacteriana que los de UAE, así como mayor efecto citotóxico en una línea celular de adenocarcinoma de mama (MCF-7) (aumento de 2 veces), aunque no potencial antifúngico contra *Candida albicans*.

Las observaciones realizadas abrían la puerta a la posibilidad de utilizar estos extractos en la conservación de alimentos. Con este objetivo, se realizaron ensayos en piezas de manzanas recién cortadas, a las que se incorporó el extracto de HAE obtenido en las condiciones optimizadas, comparando su eficacia para prevenir la oxidación y retrasar el deterioro con la del ácido ascórbico, el aditivo comercial utilizado por la industria. Se estudió también la adecuación de dos materiales de envasado: polipropileno orientado comercial (OPP) y ácido poliláctico (PLA) biodegradable, para mejorar la resistencia a la oxidación y extender la duración de vida de las piezas de manzana. Se buscaba con ello comparar la eficacia de un envase alternativo sostenible que pudiera reemplazar a los polímeros tradicionales no biodegradables empleados para el envasado de alimentos, respondiendo así a la creciente preocupación ambiental de industria y consumidores.

Se puedo comprobar que el extracto obtenido a partir de orujo de oliva irradiado era capaz de inhibir al menos 1 logaritmo el crecimiento de bacterias mesófilas y hongos filamentosos durante 5 días de almacenamiento a 4 ºC de manera más eficaz que el ácido ascórbico, sin que se llegaran tampoco a detectar coliformes a lo largo de 12 días de almacenamiento, con mejores resultados cuando se combina con película PLA. Aunque incluido en la caracterización fisicoquímica, el tiempo de almacenamiento de 9 días no se consideró en la evaluación microbiológica, por lo que la eficacia del extracto de orujo de oliva sólo pudo ser establecida para 5 días. El análisis microbiológico a 9 días podría ser significativo para comprender si el extracto de orujo de oliva combinado con la película biodegradable aumenta la extensión obtenida de la vida útil de las manzanas recién cortadas. Por otra parte, la fruta tratada con los extractos envasada en bolsas de PLA mantenía el potencial antioxidante con relación a la tratada con ácido ascórbico, sin experimentar cambios significativos en su textura. Los resultados de actividad antioxidante permiten proponer un almacenamiento de 5 días para asegurar la calidad nutricional de los frutos, que puede ser avalado por los resultados obtenidos para la calidad microbiana. El pardeamiento natural de las muestras que se observó en los primeros días a temperatura de refrigeración se puede asociar con el color natural aportado por la solución de extracto de orujo de oliva, lo que sugiere que su uso como antioxidante podría ser más adecuado para productos mínimamente procesados en los que el color no es un factor condicionante para la evaluación y decisión del consumidor.

La caracterización e identificación de los aislados bacterianos de muestras de manzanas recién cortadas puso de manifiesto la ausencia de los principales patógenos transmitidos por los alimentos asociados con frutas frescas, siendo las especies más prevalentes en las muestras analizadas bacterias de origen humano y ambiental, como

Citrobacter freundii, *Staphylococcus warneri*, *Pseudomonas oryzihabitans*, *Alcalinogenes faecalis*, *Corynebacterium jeikeium*, *Micrococcus* spp., *Pantoea aglomerans* y *Bacillus* spp,. Naturalmente, se observó un aumento en la diversidad microbiana a lo largo del tiempo de almacenamiento en refrigeración, aunque la mayor diversidad se encontró en las muestras tratadas con ácido ascórbico. También se confirmó que la combinación de extractos con envases de PLA era más eficaz para conservar las manzanas ya que esas muestras presentaron menor diversidad microbiana. Estos resultados confirman lo ya conocido sobre la escasa eficacia del ácido ascórbico como conservante, al contrario que los extractos de orujo de oliva, que demostraron potencial para ser utilizados como un conservante natural en alimentos frescos listos para consumo.

En su conjunto, los resultados obtenidos avalan el interés del orujo de oliva para la obtención de compuestos fenólicos bioactivos, cuyos extractos pueden ser utilizados como aditivos naturales eficaces para mejorar la conservabilidad de piezas frescas de fruta refrigeradas, así como la viabilidad de emplear materiales biodegradables para el envasado de este tipo de productos. Todo ello sin comprometer significativamente sus atributos de calidad.

INTEGRATED DISCUSSION OF THE RESULTS

Olive pomace is an environmentally pollutant waste from the olive oil industry, although being rich in bioactive compounds with putative benefits for human health, which might be useful for food industry due to their potential as antioxidant and antimicrobial agents.

Previous works demonstrated the potential of using ionizing radiation as a clean, eco-friendly and promising technology to enhance the extractability of bioactive compounds from agro-industrial residues (Harrison & Were, 2007; Ito et al., 2016; Khattak & Rahman, 2016; Pérez et al., 2007; Taheri et al., 2014) and from fruits and medicinal plants (Barkaoui et al., 2021b, 2021a; Pereira et al., 2015b).

In this work, two types of olive pomace that corresponded to two different steps of the olive oil production: Crude Olive Pomace (COP), consisting of dried pomace before extraction of pomace oil, and Extracted Olive Pomace (EOP), resulting after solvent extraction of pomace oil, were studied for their phenolic composition and bioactive properties in order to assess their suitability for obtaining phenolic compounds to be used as bioactive ingredients by the industry.

Hydroxytyrosol was found as the most abundant compound (25 ± 1 mg/g extract in EOP and 23.9 ± 0.3 mg/g extract in COP) in the studied extracts of olive pomaces, followed by hydroxytyrosol-1-β-glucoside (9.8 ± 0.4 mg/g extract in EOP and 9.94 ± 0.08 mg/g extract in COP), tyrosol (5.9 ± 0.2 mg/g extract in EOP and 5.9 ± 0.4 mg/g extract in COP), luteolin-7-*O*-rutinoside (3.1 ± 0.1 mg/g extract in EOP and 4.1 ± 0.2 mg/g extract in COP), oleuropein aglycone (3.0 ± 0.3 mg/g extract in EOP and 3.1 ± 0.1 mg/g extract in COP), and verbascoside (2.29 ± 0.03 mg/g extract in EOP y 3.4 ± 0.2 mg/g extract in COP). These compounds were previously described in the literature as constituents of olive pomace (Chanioti & Tzia, 2018; Malapert et al., 2018; M. A. Nunes et al., 2018; Suárez et al., 2010). The composition of olive pomace was found dependent on the season harvesting which can influence the reproducibility of the extractability results.

The effect of ionizing radiation to improve the extractability of these compounds was also evaluated. The obtained results suggested that the application of gamma radiation at 5 kGy increased their extractability by at least 2-fold (total phenolic compounds concentration of 57 ± 2 mg/g extract in non-irradiated sample extract and 139 ± 2 mg/g extract in the extract of the sample irradiated 5 kGy), as well as the antioxidant activity of the prepared extracts, especially the TBARS inhibition of the EOP extracts (IC_{50} = 49 ± 1 µg/mL and 22 ± 1 µg/mL for the non-irradiated and 5 kGy irradiated samples, respectively), while the antihemolytic activity of EOP extracts was preserved (IC_{50} = 20.6 ± 0.4 µg/mL), maintaining it in values similar to the reference antioxidant, Trolox (IC_{50} =

19.6 ± 0.6 µg/mL). The increase in the phenolic compounds extractability from irradiated olive pomace samples was attributed to the changes in the cellular structure, namely by the release of fractions associated to polysaccharides and other matrix components induced by the irradiation process, as well as to the degradation of larger polyphenols into smaller ones, by the radiolytic action of ionizing radiation (Harrison & Were, 2007; Hussain et al., 2016).

Based on these results, the extraction of the main phenolic compounds present in EOP samples irradiated at 5 kGy using heat-assisted extraction (HAE) and ultrasound-assisted extraction (UAE) was optimized. With this aim, Response Surface Methodology (RSM) was applied as a statistical method to understand the combined effects of different variables (time -t-, temperature -T-, ultrasound power -P- and solvent -S) and maximizing the responses. In the optimal conditions obtained (t = 28 min, P = 490 W and S = 7.3% ethanol for UAE; t = 120 min, T = 85 ºC and S = 76% ethanol for HAE), UAE reduced the extraction time and the solvent consumption when compared to HAE, and also led to higher extraction yields of phenolic compounds (30% *vs* 13.7%). This result can be attributed to the cavitation effects produced by the ultrasounds that cause changes in the cell walls thus increasing the exposure of the solutes and consequent release of the compounds to the solvent (Gómez-Cruz et al., 2021; Zhang, Lin & Ye, 2018). The temperature of the UAE system must be controlled to avoid that its increase during extraction can affect the extractability of phenolic compounds. Nevertheless, HAE extracts comparatively to UAE extracts presented higher antioxidant capacity, measured by both the inhibition of the TBARS formation (increase by 1.3-fold) and the oxidative hemolysis (2-fold) and higher antidiabetic activity, evaluated by inhibition of α-amylase (2-fold) and α-glucosidase (2-fold). This is in agreement with the observation made by Collado-González et al. (2017) that related the effective inhibition of α-glucosidase of olive oil samples with higher antioxidant activity. In addition, HAE extracts also showed greater anti-inflammatory (7-fold) and antibacterial activities, as well as higher cytotoxic effect for breast adenocarcinoma (MCF-7) cell line (2-fold), but no antifungal potential against *Candida albicans*. These findings are very promising when thinking about the possibility of using these extracts as food preservatives for preventing oxidation and delaying spoilage.

In order to check the suitability of the HAE optimized extracts to be used as additives, they were incorporated in fresh-cut apples, and their effectiveness compared with that of ascorbic acid, the commercial additive used by the industries for this purpose. To respond to the increased environmental concerns of retailers and customers, it becomes essential the use of sustainable alternative biodegradable materials in food packaging to

replace the traditional non-biodegradable polymers. Thus, two types of bags, a PLA biodegradable film and a commercial oriented polypropylene film, were used to package the fresh-cut apples, to evaluate which of them could be more suitable to prevent the oxidation and extend the shelf life of the fresh-cut apples. It was verified that the natural extracts from irradiated olive pomace better inhibit the growth of mesophilic bacteria and filamentous fungi at least by 1 log for 5 days of storage at 4 ºC than ascorbic acid, with no detection of coliforms along the 12 days of storage, with improved performance when combined with PLA film. Although included in the physicochemical characterization, the storage time of 9 days was not evaluated in the microbiological assessment, so that the efficacy of the olive pomace extract could only be established for 5 days. The 9 days microbiological analysis could be relevant to determine if the olive pomace extract combined with the biodegradable film significantly increases the obtained extension of the fresh-cut apples' shelf life. The fruit treated with the extracts packed in PLA bags maintained the antioxidant potential compared to that treated with ascorbic acid, without experiencing significant changes in its texture. The overall results of antioxidant activity allow proposing a storage of 5 days to ensure the nutritional quality of the fruits, which can be supported by the obtained results for microbial quality. The slight browning of the samples detected in the first two days at refrigeration temperature may be associated with the natural color of the olive pomace extract solution, suggesting that its use as an antioxidant might be more suitable for minimally processed products where the color is not a conditional factor for the evaluation and decision of the consumer.

The characterization and identification of the bacterial isolates in fresh-cut apple samples indicated that none of them were positive for the main foodborne pathogens associated with fresh fruit. The most prevalent species in the analyzed samples were bacteria from human and environmental origin, such as *Citrobacter freundii*, *Staphylococcus warneri*, *Pseudomonas oryzihabitans*, *Alcalinogenes faecalis*, *Corynebacterium jeikeium*, *Micrococcus* spp., *Pantoea clusterans* and *Bacillus* spp.. As expected, an increase in microbial diversity was observed throughout the storage time at refrigeration temperature, although the highest diversity was found in the samples treated with ascorbic acid. It was also confirmed that the combination of extracts with PLA packaging was more effective in preserving apples, since they presented the lowest microbial diversity. These results supported the well-known ineffectiveness of ascorbic acid as an antimicrobial compound, in contrast to natural olive pomace extracts that have demonstrated potential to be used as a natural antimicrobial preservative in ready-to-eat fresh foods.

The overall results from incorporation studies revealed that the addition of natural olive pomace extracts to fresh-cut apples and the use of biodegradable films in the packaging of the fruits bring beneficial advantages for extending their shelf life at refrigerated storage without significantly compromising their quality attributes.

4. CONCLUSIONES Y PERSPECTIVAS - CONCLUSIONS AND PERSPECTIVES

El objetivo de la presente Tesis era profundizar en la viabilidad del orujo de oliva, un residuo de la industria del aceite de oliva, para ser utilizado como fuente para extracción de sustancias bioactivas que puedan ser empleadas como aditivos alimentarios o ingredientes en la preparación de alimentos funcionales saludables.

Como primer paso, se exploró el empleo de radiaciones ionizantes con la finalidad de proceso para mejorar la extractabilidad de compuestos fenólicos presentes en los orujos.

Los resultados obtenidos permitieron demostrar, por primera vez, que el uso de radiación gamma a bajas dosis (5 kGy) constituye una tecnología adecuada para la valorización de este residuo, al potenciar la extracción de compuestos fenólicos y de las propiedades bioactivas relacionadas.

Se optimizaron procedimientos para la extracción de los principales compuestos fenólicos de los orujos mediante extracción asistida por calor (*Heat-Assisted Extraction*, HAE) y por ultrasonidos (*Ultrasound-Assisted Extraction*, UAE). Las condiciones de extracción establecidas para la HAE dieron lugar a extractos con actividades antioxidantes, antibacterianas, antidiabéticas y antiinflamatorias, así como citotóxicas frente a líneas celulares de adenocarcinoma de mama (MCF-7), superiores a las obtenidas mediante UAE, aún cuando con este último proceso se obtenían mayores rendimientos en la extracción de compuestos fenólicos.

Los extractos de HAE obtenidos a partir de orujo de oliva irradiado se utilizaron para su incorporación en piezas de manzanas recién cortadas, que fueron envasadas en bolsas de una película biodegradable de ácido poliláctico (PLA) y de un plástico comercial (polipropileno orientado, OPP). Se pudo establecer que el envasado en PLA del producto tratado con extractos de orujo permitía conservar el contenido fenólico y la actividad antioxidante de los frutos sin provocar cambios significativos en su textura, con resultados similares o mejores a los obtenidos con el antioxidante comercial ácido ascórbico. También se observó una mayor inhibición del crecimiento microbiano con el extracto de orujo de oliva que con ácido ascórbico, durante al menos 5 días de almacenamiento en refrigeración, lo que apoya el potencial de este tipo de extractos como alternativa a ese aditivo, no solo por su capacidad antioxidante sino también por su actividad antimicrobiana. Por otro lado, se demostró que el uso de películas de ácido poliláctico biodegradable (con baja permeabilidad para los gases) presenta ventajas para el envasado de piezas de manzana tratadas con extractos de orujo de oliva, al extender su vida útil con relación a la película de polipropileno orientado comercial. Teniendo en cuenta los resultados globales de actividad antioxidante y calidad

microbiana, se podría proponer un almacenamiento de 5 días para asegurar la calidad nutricional de los frutos.

La caracterización e identificación de especies bacterianas aislados de las muestras de manzanas recién cortadas permitió establecer que las bacterias presentes eran de origen humano y ambiental, sin que se detectaran patógenos que pudieran ser transmitidos por frutos. Los extractos de orujo de oliva mostraron potencial para ser utilizados como un conservante natural en alimentos frescos listos para el consumo, ya que mostraron menor diversidad microbiana que las muestras en ácido ascórbico. También se demostró que el envasado en películas de PLA era más efectivo que el realizado en bolsas de OPP. Sin embargo, es importante mencionar que el uso de extractos de orujo de oliva parece más adecuado para su empleo en alimentos mínimamente procesados en los que el color no es un factor condicionante para la aceptación por parte del consumidor, ya que el color natural de la solución del extracto promueve un oscurecimiento inicial del producto tratado, lo que podría comprometer la elección del consumidor.

La reutilización de residuos industriales para recuperar compuestos valiosos con propiedades funcionales está en línea con los requisitos de los consumidores y la industria, cada vez más preocupados por disponer de alimentos limpios, seguros y saludables. El estudio realizado está, de este modo, alineado con los intereses de las empresas alimentarias por desarrollar nuevos ingredientes con potencial tecnológico y bioactivo a partir de residuos agroindustriales, contribuyendo a la sostenibilidad tanto del sector productivo como del medio ambiente, fomentando así la economía circular.

Como continuación de este trabajo, se espera ensayar otros procesos de extracción (por ejemplo, extracción asistida por microondas o extracción con fluidos supercríticos) para establecer si es posible llegar a condiciones que permitan preparar extractos con mayor riqueza en compuestos fenólicos y con propiedades bioactivas mejoradas a partir del orujo de oliva irradiado. Asimismo, se deben seguir explorando las posibles aplicaciones de los extractos y compuestos obtenidos en otros alimentos, ya sea como aditivos o como ingredientes funcionales (por ejemplo, otras frutas y vegetales o productos de panadería), siendo, además, necesario llevar a cabo estudios con paneles de evaluadores para verificar la calidad sensorial y aceptación por los consumidores. Por otro lado, se podría realizar un análisis en profundidad de qué compuestos individuales presentes en el orujo de oliva contribuyen a las bioactividades obtenidas para comprender la relación compuesto-bioactividad o si existe un efecto sinérgico de los compuestos.

CONCLUSIONS AND PERSPECTIVES

The objective of the present Thesis was to understand in more detail the ability of extracts of olive pomace, a residue from olive oil industry, to be used as a natural food preservative and a potential ingredient for the preparation of functional foods with increased health benefits.

As a first step, ionizing radiation was evaluated as a valorization process in order to improve the extractability of the bioactive compounds from this residue.

Besides being the first study reporting the phenolic composition of irradiated olive pomace, the obtained results proved that gamma radiation at low absorbed doses (5 kGy) is a suitable technology for the valorization of this waste, contributing to enhance the extraction of phenolic compounds and the related bioactive properties. More precisely, the optimization of the extraction of the main phenolic compounds using Heat-Assisted Extraction (HAE) provided extracts with improved antioxidant, antibacterial, antidiabetic and anti-inflammatory activities, and also cytotoxic effect against breast adenocarcinoma (MCF-7) cell lines when compared to the extracts submitted to Ultrasound-Assisted Extraction (UAE).

The use of the HAE extracts obtained from irradiated olive pomace in snacks consisting of fresh-cut apples packaged in biodegradable and commercial bag films demonstrated their capability to preserve the phenolic content and antioxidant activity of the fruits without causing significant change in their texture. A higher inhibition of the microbial growth using the olive pomace extract was also observed for at least 5 days of storage compared to ascorbic acid, supporting its suitability as an alternative to this additive, not only because of its antioxidant potential but also for its antimicrobial capacity. On the other hand, the use of biodegradable polylactic acid films (with low gas permeability) for fresh-cut apples packaging was shown to present advantages in extending their shelf life in relation to the commercial oriented polypropylene film. Taking into account the global results of antioxidant activity and microbial quality, a 5-days storage could be proposed to ensure the safety and nutritional quality of the fruits.

Finally, the characterization and identification of bacterial isolates from fresh-cut apple samples indicated that the present bacteria were from human and environmental origin, without the existence of fruit-borne pathogens. Olive pomace extracts showed potential to be used as a natural preservative in ready-to-eat fresh foods since they showed less microbial diversity than samples treated with in ascorbic acid, also confirming the advantage of using PLA film to package the fresh-cut apples. Nevertheless, it is important to mention that the use of these natural extracts as antioxidants could be more

suitable for minimally processed products in which the color is not a conditional factor for the evaluation of the consumer, since the natural color of the extract solution promotes an initial browning in the first days of storage, which may compromise consumer's choice.

Overall, the reuse of industrial wastes to recover valuable compounds with functional properties is in line with consumers and industry requirements, increasingly concerned about the choice of clean, safe and healthier foods. This comprehensive work is aligned with the interests of the food companies in developing new ingredients with technological and bioactive potential from agro-industrial wastes, contributing to the sustainability of both agro-industrial sector and environment and, therefore, promoting the circular economy.

As a continuation of this work, further optimization of the extraction conditions using other alternative processes (e.g. microwave-assisted extraction or supercritical fluid extraction) to prepare richer extracts of phenolic compounds and with enhanced bioactive properties from irradiated olive pomace should be explored. Furthermore, applications of the obtained extracts and compounds in other foods such as other fruits and vegetables or bakery products, either as additives or functional ingredients, can be investigated. Also, sensory analyses (human panelists) will be required to verify the acceptance by the consumers. On the other hand, an in-depth analysis of which individual compounds present in olive pomace contribute to the obtained bioactivities could be performed to understand the relation compound-bioactivity or if there is a synergistic effect of the compounds.

REFERENCIAS

Abdelhadi, S.O., Dosoretz, C.G., Rytwo, G., Gerchman, Y., Azaizeh, H., 2017. Production of biochar from olive mill solid waste for heavy metal removal. Bioresour. Technol. 244, 759–767. https://doi.org/10.1016/j.biortech.2017.08.013

Abu Tayeh, H., Najami, N., Dosoretz, C., Tafesh, A., Azaizeh, H., 2014. Potential of bioethanol production from olive mill solid wastes. Bioresour. Technol. 152, 24–30. https://doi.org/10.1016/j.biortech.2013.10.102

Al-Addous, M., Alnaief, M., Class, C., Nsair, A., Kuchta, K., Alkasrawi, M., 2017. Technical possibilities of biogas production from olive and date waste in Jordan. BioResources 12, 9383–9395. https://doi.org/10.15376/biores.12.4.9383-9395

Al Afif, R., Linke, B., 2019. Biogas production from three-phase olive mill solid waste in lab-scale continuously stirred tank reactor. Energy 171, 1046–1052. https://doi.org/10.1016/j.energy.2019.01.080

Anastopoulos, I., Massas, I., Ehaliotis, C., 2015. Use of residues and by-products of the olive-oil production chain for the removal of pollutants from environmental media: A review of batch biosorption approaches. J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng. 50, 677–718. https://doi.org/10.1080/10934529.2015.1011964

Araújo, M., Pimentel, F.B., Alves, R.C., Oliveira, M.B.P.P., 2015. Phenolic compounds from olive mill wastes: Health effects, analytical approach and application as food antioxidants. Trends Food Sci. Technol. 45, 200–211. https://doi.org/10.1016/j.tifs.2015.06.010

Azbar, N., Bayram, A., Filibeli, A., Muezzinoglu, A., Sengul, F., Ozer, A., 2004. A review of waste management options in olive oil production. Crit. Rev. Environ. Sci. Technol. 34, 209–247. https://doi.org/10.1080/10643380490279932

Barkaoui, S., Mankai, M., Miloud, N.B., Kraïem, M., Madureira, J., Cabo Verde, S., Boudhrioua, N., 2021a. E-beam irradiation of strawberries: Investigation of microbiological, physicochemical, sensory acceptance properties and bioactive content. Innov. Food Sci. Emerg. Technol. 73. https://doi.org/10.1016/j.ifset.2021.102769

Barkaoui, S., Mankai, M., Miloud, N.B., Kraïem, M., Madureira, J., Cabo Verde, S., Boudhrioua, N., 2021b. Effect of gamma radiation coupled to refrigeration on antioxidant capacity, sensory properties and shelf life of strawberries. LWT - Food

Sci. Technol. 150, 112088. https://doi.org/10.1016/j.lwt.2021.112088

Borrely, S.I., Morais, A. V, Rosa, J.M., Badaró-Pedroso, C., Pereira, C., Higa, M.C., 2016. Decoloration and detoxification of effluents by ionizing radiation. Radiat. Phys. Chem. 124, 1–5. https://doi.org/10.1016/j.radphyschem.2015.11.001

Buchalla, R., Schüttler, C., Bögl, K.W., 1995. Radiation sterilization of medical devices. Effects of ionizing radiation on ultra-high molecular-weight polyethylene. Radiat. Phys. Chem. 46, 579–585. https://doi.org/10.1016/0969-806X(95)00222-J

Bulotta, S., Celano, M., Lepore, S.M., Montalcini, T., Pujia, A., Russo, D., 2014. Beneficial effects of the olive oil phenolic components oleuropein and hydroxytyrosol: focus on protection against cardiovascular and metabolic diseases. J. Transl. Med. 12, 219. https://doi.org/10.1186/s12967-014-0219-9

Caporaso, N., Formisano, D., Genovese, A., 2018. Use of phenolic compounds from olive mill wastewater as valuable ingredients for functional foods. Crit. Rev. Food Sci. Nutr. 58, 2829–2841. https://doi.org/10.1080/10408398.2017.1343797

Cardoso, R.V.C., Fernandes, Â., Barreira, J.C.M., Cabo Verde, S., Antonio, A.L., Gonzaléz-Paramás, A.M., Barros, L., Ferreira, I.C.F.R., 2019. Effectiveness of gamma and electron beam irradiation as preserving technologies of fresh Agaricus bisporus Portobello: A comparative study. Food Chem. 278, 760–766. https://doi.org/10.1016/j.foodchem.2018.11.116

Cecchi, L., Schuster, N., Flynn, D., Bechtel, R., Bellumori, M., Innocenti, M., Mulinacci, N., Guinard, J.X., 2019. Sensory Profiling and Consumer Acceptance of Pasta, Bread, and Granola Bar Fortified with Dried Olive Pomace (Pâté): A Byproduct from Virgin Olive Oil Production. J. Food Sci. 84, 2995–3008. https://doi.org/10.1111/1750-3841.14800

Cedola, A., Cardinali, A., D'Antuono, I., Conte, A., Del Nobile, M.A., 2020. Cereal foods fortified with by-products from the olive oil industry. Food Biosci. 33, 100490. https://doi.org/10.1016/j.fbio.2019.100490

Cedola, A., Cardinali, A., Del Nobile, M.A., Conte, A., 2019. Enrichment of Bread with Olive Oil Industrial By-Product. J. Agric. Sci. Technol. B 9, 119–127. https://doi.org/10.17265/2161-6264/2019.02.005

Cedola, A., Cardinali, A., Del Nobile, M.A., Conte, A., 2017. Fish burger enriched by olive oil industrial by-product. Food Sci. Nutr. 5, 837–844. https://doi.org/10.1002/fsn3.461

Chanioti, S., Tzia, C., 2018. Extraction of phenolic compounds from olive pomace by

using natural deep eutectic solvents and innovative extraction techniques. Innov. Food Sci. Emerg. Technol. 48, 228–239. https://doi.org/10.1016/j.ifset.2018.07.001

Chernyaev, A.P., Varzar, S.M., Belousov, A. V., Zheltonozhskaya, M. V., Lykova, E.N., 2019. Prospects of Development of Radiation Technologies in Russia. Phys. At. Nucl. 82, 513–527. https://doi.org/10.1134/S1063778819040070

Cioffi, G., Pesca, M.S., De Caprariis, P., Braca, A., Severino, L., De Tommasi, N., 2010. Phenolic compounds in olive oil and olive pomace from Cilento (Campania, Italy) and their antioxidant activity. Food Chem. 121, 105–111. https://doi.org/10.1016/j.foodchem.2009.12.013

Collado-González, J., Grosso, C., Valentão, P., Andrade, P.B., Ferreres, F., Durand, T., Guy, A., Galano, J.M., Torrecillas, A., Gil-Izquierdo, Á., 2017. Inhibition of α-glucosidase and α-amylase by Spanish extra virgin olive oils: The involvement of bioactive compounds other than oleuropein and hydroxytyrosol. Food Chem. 235, 298–307. https://doi.org/10.1016/j.foodchem.2017.04.171

de La Casa, J.A., Castro, E., 2014. Recycling of washed olive pomace ash for fired clay brick manufacturing. Constr. Build. Mater. 61, 320–326. https://doi.org/10.1016/j.conbuildmat.2014.03.026

de la Casa, J.A., Lorite, M., Jiménez, J., Castro, E., 2009. Valorisation of wastewater from two-phase olive oil extraction in fired clay brick production. J. Hazard. Mater. 169, 271–278. https://doi.org/10.1016/j.jhazmat.2009.03.095

de Moraes Crizel, T., de Oliveira Rios, A., D. Alves, V., Bandarra, N., Moldão-Martins, M., Hickmann Flôres, S., 2018. Active food packaging prepared with chitosan and olive pomace. Food Hydrocoll. 74, 139–150. https://doi.org/10.1016/j.foodhyd.2017.08.007

Dermeche, S., Nadour, M., Larroche, C., Moulti-Mati, F., Michaud, P., 2013. Olive mill wastes: Biochemical characterizations and valorization strategies. Process Biochem. 48, 1532–1552. https://doi.org/10.1016/j.procbio.2013.07.010

Difonzo, G., Troilo, M., Squeo, G., Pasqualone, A., Caponio, F., 2021. Functional compounds from olive pomace to obtain high-added value foods – a review. J. Sci. Food Agric. 101, 15–26. https://doi.org/10.1002/jsfa.10478

Dunne, G., 2019. Transforming Olive Waste into Animal Feed. Int. J. Clin. Nutr. Diet. 5, 10–12. https://doi.org/10.15344/2456-8171/2019/142

Durante, M., Bleve, G., Selvaggini, R., Veneziani, G., Servili, M., Mita, G., 2019. Bioactive compounds and stability of a typical Italian bakery products “taralli”

enriched with fermented olive paste. Molecules 24. https://doi.org/10.3390/molecules24183258

El-Abbassi, A., Kiai, H., Hafidi, A., 2012. Phenolic profile and antioxidant activities of olive mill wastewater. Food Chem. 132, 406–412. https://doi.org/10.1016/j.foodchem.2011.11.013

Elias, M.I., Madureira, J., Santos, P.M.P., Carolino, M.M., Margaça, F.M.A., Cabo Verde, S., 2020. Preservation treatment of fresh raspberries by e-beam irradiation. Innov. Food Sci. Emerg. Technol. 66, 102487. https://doi.org/10.1016/j.ifset.2020.102487

Eliche-Quesada, D., Iglesias-Godino, F.J., Pérez-Villarejo, L., Corpas-Iglesias, F.A., 2014. Replacement of the mixing fresh water by wastewater olive oil extraction in the extrusion of ceramic bricks. Constr. Build. Mater. 68, 659–666. https://doi.org/10.1016/j.conbuildmat.2014.07.017

Estaún, J., Dosil, J., Al Alami, A., Gimeno, A., De Vega, A., 2014. Effects of including olive cake in the diet on performance and rumen function of beef cattle. Anim. Prod. Sci. 54, 1817–1821. https://doi.org/10.1071/AN14352

Federici, E., Massaccesi, L., Pezzolla, D., Fidati, L., Montalbani, E., Proietti, P., Nasini, L., Regni, L., Scargetta, S., Gigliotti, G., 2017. Short-term modifications of soil microbial community structure and soluble organic matter chemical composition following amendment with different solid olive mill waste and their derived composts. Appl. Soil Ecol. 119, 234–241. https://doi.org/10.1016/j.apsoil.2017.06.014

Fernández-González, R., Martín-Lara, M.Á., Blázquez, G., Pérez, A., Calero, M., 2019. Recovering metals from aqueous solutions by biosorption onto hydrolyzed olive cake. Water 11. https://doi.org/10.3390/w11122519

Fernando, A., Monteiro, S., Pinto, F., Mendes, B., 2009. Production of biosorbents from waste olive cake and its adsorption characteristics for Zn2+ ion. Sustainability 1, 277–297. https://doi.org/10.3390/su1020277

Ferreira, I.C.F.R., Antonio, A.L., Cabo Verde, S. (Eds.), 2018. Food Irradiation Technologies: Concepts, Applications and Outcomes. Royal Society of Chemistry.

Galanakis, C.M., Tsatalas, P., Galanakis, I.M., 2018. Implementation of phenols recovered from olive mill wastewater as UV booster in cosmetics. Ind. Crops Prod. 111, 30–37. https://doi.org/10.1016/j.indcrop.2017.09.058

Gerasopoulos, K., Stagos, D., Kokkas, S., Petrotos, K., Kantas, D., Goulas, P., Kouretas, D., 2015a. Feed supplemented with byproducts from olive oil mill wastewater

processing increases antioxidant capacity in broiler chickens. Food Chem. Toxicol. 82, 42–49. https://doi.org/10.1016/j.fct.2015.04.021

Gerasopoulos, K., Stagos, D., Petrotos, K., Kokkas, S., Kantas, D., Goulas, P., Kouretas, D., 2015b. Feed supplemented with polyphenolic byproduct from olive mill wastewater processing improves the redox status in blood and tissues of piglets. Food Chem. Toxicol. 86, 319–327. https://doi.org/10.1016/j.fct.2015.11.007

Gómez-Cruz, I., Contreras, M.D.M., Carvalheiro, F., Duarte, L.C., Roseiro, L.B., Romero, I., Castro, E., 2021. Recovery of bioactive compounds from industrial exhausted olive pomace through ultrasound-assisted extraction. Biology (Basel). 10, 1–22. https://doi.org/10.3390/biology10060514

Gryczka, U., Madureira, J., Verde, S.C., Migdał, W., Bułka, S., 2021. Determination of pepper microbial contamination for low energy e-beam irradiation. Food Microbiol. 98, 103782. https://doi.org/10.1016/j.fm.2021.103782

Guerreiro, D., Madureira, J., Silva, T., Melo, R., Santos, P.M.P., Ferreira, A., Trigo, M.J., Falcão, A.N., Margaça, F.M.A., Cabo Verde, S., 2016. Post-harvest treatment of cherry tomatoes by gamma radiation: Microbial and physicochemical parameters evaluation. Innov. Food Sci. Emerg. Technol. 36, 1–9. https://doi.org/10.1016/j.ifset.2016.05.008

Harrison, K., Were, L.M., 2007. Effect of gamma irradiation on total phenolic content yield and antioxidant capacity of Almond skin extracts. Food Chem. 102, 932–937. https://doi.org/10.1016/j.foodchem.2006.06.034

Hussain, P.R., Suradkar, P., Javaid, S., Akram, H., Parvez, S., 2016. Influence of postharvest gamma irradiation treatment on the content of bioactive compounds and antioxidant activity of fenugreek (Trigonella foenum-graceum L.) and spinach (Spinacia oleracea L.) leaves. Innov. Food Sci. Emerg. Technol. 33, 268–281. https://doi.org/10.1016/j.ifset.2015.11.017

Ito, V.C., Alberti, A., Avila, S., Spoto, M., Nogueira, A., Wosiacki, G., 2016. Effects of gamma radiation on the phenolic compounds and in vitro antioxidant activity of apple pomace flour during storage using multivariate statistical techniques. Innov. Food Sci. Emerg. Technol. 33, 251–259. https://doi.org/10.1016/j.ifset.2015.12.015

Jiang, L., Iwahashi, H., 2020. Current research on high-energy ionizing radiation for wastewater treatment and material synthesis. Environ. Prog. Sustain. Energy 39, 1–9. https://doi.org/10.1002/ep.13294

Khattak, K.F., Rahman, T.U., 2016. Effect of gamma irradiation on the vitamins,

phytochemicals, antimicrobial and antioxidant properties of Ziziphus mauritiana Lam. leaves. Radiat. Phys. Chem. 127, 243–248. https://doi.org/10.1016/j.radphyschem.2016.07.001

Lammi, S., Le Moigne, N., Djenane, D., Gontard, N., Angellier-Coussy, H., 2018. Dry fractionation of olive pomace for the development of food packaging biocomposites. Ind. Crops Prod. 120, 250–261. https://doi.org/10.1016/j.indcrop.2018.04.052

Lancastre, J.J.H., Falcão, A.N., Margaça, F.M.A., Ferreira, L.M., Miranda Salvado, I.M., Casimiro, M.H., Almásy, L., Meiszterics, A., 2015. Influence of the polymer molecular weight on the microstructure of hybrid materials prepared by γ-irradiation. Radiat. Phys. Chem. 106, 126–129. https://doi.org/10.1016/j.radphyschem.2014.06.023

Leouifoudi, I., Mbarki, M., Tilaoui, M., Amechrouq, A., Mostapha, E., Mouse, H.A., Zyad, A., 2014. Study of the in vitro anticancer activity of Moroccan phenolic olive cake extracts. J. Pharmacogn. Phytochem. 2, 154–165.

Lin, S., Chi, W., Hu, J., Pan, Q., Zheng, B., Zeng, S., 2017. Sensory and nutritional properties of chinese olive pomace based high fibre biscuit. Emirates J. Food Agric. 29, 495–501. https://doi.org/10.9755/ejfa.2016-12-1908

Madureira, J., Barros, L., Melo, R., Verde, S.C., Ferreira, I.C.F.R., Margaça, F.M.A., 2018a. Degradation of phenolic acids by gamma radiation as model compounds of cork wastewaters. Chem. Eng. J. 341, 227–237. https://doi.org/10.1016/j.cej.2018.02.036

Madureira, J., Melo, R., Pimenta, A.I., Verde, S.C., Borrely, S.I., 2018b. Evaluation of e-beam irradiation effects on the toxicity of slaughterhouse wastewaters. Environ. Technol. 39. https://doi.org/10.1080/09593330.2017.1313887

Madureira, J., Pimenta, A.I., Popescu, L., Besleaga, A., Dias, M.I., Santos, P.M.P., Melo, R., Ferreira, I.C.F.R., Cabo Verde, S., Margaça, F.M.A., 2017. Effects of gamma radiation on cork wastewater: Antioxidant activity and toxicity. Chemosphere 169, 139–145. https://doi.org/10.1016/j.chemosphere.2016.11.064

Madureira, J., Severino, A., Cojocaru, M., Garofalide, S., Santos, P.M.P., Carolino, M.M., Margaça, F.M.A., Cabo Verde, S., 2019. E-beam treatment to guarantee the safety and quality of cherry tomatoes. Innov. Food Sci. Emerg. Technol. 55, 57–65. https://doi.org/10.1016/j.ifset.2019.05.013

Majbar, Z., Lahlou, K., Ben Abbou, M., Ammar, E., Triki, A., Abid, W., Nawdali, M., Bouka, H., Taleb, M., El Haji, M., Rais, Z., 2018. Co-composting of Olive Mill Waste

and Wine-Processing Waste: An Application of Compost as Soil Amendment. J. Chem. 2018, 1–9. https://doi.org/10.1155/2018/7918583

Malapert, A., Reboul, E., Loonis, M., Dangles, O., Tomao, V., 2018. Direct and Rapid Profiling of Biophenols in Olive Pomace by UHPLC-DAD-MS. Food Anal. Methods 11, 1001–1010. https://doi.org/10.1007/s12161-017-1064-2

Martín-Lara, M.A., Blázquez, G., Ronda, A., Pérez, A., Calero, M., 2013. Development and characterization of biosorbents to remove heavy metals from aqueous solutions by chemical treatment of olive stone. Ind. Eng. Chem. Res. 52, 10809–10819. https://doi.org/10.1021/ie401246c

Martinez-Garcia, G., Bachmann, R.T., Williams, C.J., Burgoyne, A., Edyvean, R.G.J., 2006. Olive oil waste as a biosorbent for heavy metals. Int. Biodeterior. Biodegrad. 58, 231–238. https://doi.org/10.1016/j.ibiod.2006.06.028

Mekki, H., Anderson, M., Amar, E., Skerratt, G.R., Benzina, M., 2006. Olive oil mill waste water as a replacement for fresh water in the manufacture of fired clay bricks. J. Chem. Technol. Biotechnol. 81, 1419–1425. https://doi.org/10.1002/jctb

Mekki, H., Anderson, M., Benzina, M., Ammar, E., 2008. Valorization of olive mill wastewater by its incorporation in building bricks. J. Hazard. Mater. 158, 308–315. https://doi.org/10.1016/j.jhazmat.2008.01.104

Messineo, A., Maniscalco, M.P., Volpe, R., 2020. Biomethane recovery from olive mill residues through anaerobic digestion: A review of the state of the art technology. Sci. Total Environ. 703, 135508. https://doi.org/10.1016/j.scitotenv.2019.135508

Molina-Alcaide, E., Morales-García, E.Y., Martín-García, A.I., Ben Salem, H., Nefzaoui, A., Sanz-Sampelayo, M.R., 2010. Effects of partial replacement of concentrate with feed blocks on nutrient utilization, microbial N flow, and milk yield and composition in goats. J. Dairy Sci. 93, 2076–2087. https://doi.org/10.3168/jds.2009-2628

Moudache, M., Silva, F., Nerín, C., Zaidi, F., 2020. Olive cake and leaf extracts as valuable sources of antioxidant and antimicrobial compounds: a comparative study. Waste and Biomass Valorization 12, 1431–1445. https://doi.org/10.1007/s12649-020-01080-8

Muniz, F.G., 2021. Mapeamento de resíduos e subprodutos derivados da extração de azeites da região do Alentejo. Universidade de Lisboa.

Nunes, I., Mesquita, N., Cabo Verde, S., Carolino, M.M., Portugal, A., Botelho, M.L., 2013. Bioburden assessment and gamma radiation inactivation patterns in parchment documents. Radiat. Phys. Chem. 88, 82–89.

https://doi.org/10.1016/j.radphyschem.2013.03.031

Nunes, M.A., Costa, A.S.G., Bessada, S., Santos, J., Puga, H., Alves, R.C., Freitas, V., Oliveira, M.B.P.P., 2018. Olive pomace as a valuable source of bioactive compounds: A study regarding its lipid- and water-soluble components. Sci. Total Environ. 644, 229–236. https://doi.org/10.1016/j.scitotenv.2018.06.350

Nunes, M.A., Pimentel, F.B., Costa, A.S.G., Alves, R.C., Oliveira, M.B.P.P., 2016. Olive by-products for functional and food applications: Challenging opportunities to face environmental constraints. Innov. Food Sci. Emerg. Technol. 35, 139–148. https://doi.org/10.1016/j.ifset.2016.04.016

Otero, P., Garcia-Oliveira, P., Carpena, M., Barral-Martinez, M., Chamorro, F., Echave, J., Garcia-Perez, P., Cao, H., Xiao, J., Simal-Gandara, J., Prieto, M.A., 2021. Applications of by-products from the olive oil processing: Revalorization strategies based on target molecules and green extraction technologies. Trends Food Sci. Technol. 116, 1084–1104. https://doi.org/10.1016/j.tifs.2021.09.007

Pagnanelli, F., Mainelli, S., Vegliò, F., Toro, L., 2003. Heavy metal removal by olive pomace: Biosorbent characterisation and equilibrium modelling. Chem. Eng. Sci. 58, 4709–4717. https://doi.org/10.1016/j.ces.2003.08.001

Papaioannou, E.H., Patsios, S.I., Karabelas, A.J., Philippopoulos, N.A., 2013. Characterization of condensates from an indirect olive oil pomace drying process: The effect of drying temperature. J. Environ. Chem. Eng. 1, 831–837. https://doi.org/10.1016/j.jece.2013.07.025

Parsons, B.J., 2012. Sterilisation of healthcare products by ionising radiation: principles and standards, in: Sterilisation of Biomaterials and Medical Devices. Woodhead Publishing Limited, pp. 56–70. https://doi.org/10.1533/9780857096265.56

Peralbo-Molina, Á., Priego-Capote, F., Luque De Castro, M.D., 2012. Tentative identification of phenolic compounds in olive pomace extracts using liquid chromatography-tandem mass spectrometry with a quadrupole- quadrupole-time-of-flight mass detector. J. Agric. Food Chem. 60, 11542–11550. https://doi.org/10.1021/jf302896m

Pereira, E., Barros, L., Antonio, A., Bento, A., Ferreira, I.C.F.R., 2015a. Analytical Methods Applied to Assess the Effects of Gamma Irradiation on Color, Chemical Composition and Antioxidant Activity of Ginkgo biloba L. Food Anal. Methods 154–163. https://doi.org/10.1007/s12161-014-9883-x

Pereira, E., Barros, L., Dueñas, M., António, A.L., Santos-Buelga, C., Ferreira, I.C.F.R.,

2015b. Gamma irradiation improves the extractability of phenolic compounds in Ginkgo biloba L. Ind. Crops Prod. 74, 144–149. https://doi.org/10.1016/j.indcrop.2015.04.039

Pereira, E., Pimenta, A.I., Barros, L., Calhelha, R.C., Antonio, A.L., Cabo Verde, S., Ferreira, I.C.F.R., 2018. Effects of gamma radiation on the bioactivity of medicinal and aromatic plants: Mentha × piperita L., Thymus vulgaris L. and Aloysia citrodora Paláu as case studies. Food Funct. 9, 5150–5161. https://doi.org/10.1039/c8fo01558a

Pérez, M.B., Calderón, N.L., Croci, C.A., 2007. Radiation-induced enhancement of antioxidant activity in extracts of rosemary (Rosmarinus officinalis L.). Food Chem. 104, 585–592. https://doi.org/10.1016/j.foodchem.2006.12.009

Pimenta, A.I., Margaça, F.M.A., Cabo Verde, S., 2019. Virucidal activity of gamma radiation on strawberries and raspberries. Int. J. Food Microbiol. 304, 89–96. https://doi.org/10.1016/j.ijfoodmicro.2019.05.011

Regni, L., Nasini, L., Ilarioni, L., Brunori, A., Massaccesi, L., Agnelli, A., Proietti, P., 2017. Long term amendment with fresh and composted solid olive mill waste on olive grove affects carbon sequestration by prunings, fruits, and soil. Front. Plant Sci. 7, 1–9. https://doi.org/10.3389/fpls.2016.02042

Ribeiro, T.B., Bonifácio-Lopes, T., Morais, P., Miranda, A., Nunes, J., Vicente, A.A., Pintado, M., 2021a. Incorporation of olive pomace ingredients into yoghurts as a source of fibre and hydroxytyrosol: Antioxidant activity and stability throughout gastrointestinal digestion. J. Food Eng. 297. https://doi.org/10.1016/j.jfoodeng.2021.110476

Ribeiro, T.B., Oliveira, A., Coelho, M., Veiga, M., Costa, E.M., Silva, S., Nunes, J., Vicente, A.A., Pintado, M., 2021b. Are olive pomace powders a safe source of bioactives and nutrients? J. Sci. Food Agric. 101, 1963–1978. https://doi.org/10.1002/jsfa.10812

Rincón, B., Bujalance, L., Fermoso, F.G., Martín, A., Borja, R., 2013. Biochemical methane potential of two-phase olive mill solid waste: Influence of thermal pretreatment on the process kinetics. Bioresour. Technol. 140, 249–255. https://doi.org/10.1016/j.biortech.2013.04.090

Rodrigues, F., Nunes, M.A. da M., Pinto Oliveira, M.B.P., 2017. Applications of recovered bioactive compounds in cosmetics and health care products, in: Olive Mill Waste: Recent Advances for Sustainable Management. Elsevier Inc., pp. 255–274.

https://doi.org/10.1016/B978-0-12-805314-0.00012-1

Rodrigues, F., Pimentel, F.B., Oliveira, M.B.P.P., 2015. Olive by-products: Challenge application in cosmetic industry. Ind. Crops Prod. 70, 116–124. https://doi.org/10.1016/j.indcrop.2015.03.027

Roig, A., Cayuela, M.L., Sánchez-Monedero, M.A., 2006. An overview on olive mill wastes and their valorisation methods. Waste Manag. 26, 960–969. https://doi.org/10.1016/j.wasman.2005.07.024

Rojas-Cano, M.L., Ruiz-Guerrero, V., Lara, L., Nieto, R., Aguilera, J.F., 2014. Digestibility and energy value of diets containing increasing proportions of olive soapstocks for Iberian crossbred pigs. Anim. Feed Sci. Technol. 191, 83–90. https://doi.org/10.1016/j.anifeedsci.2014.01.018

Romero-García, J.M., Niño, L., Martínez-Patiño, C., Álvarez, C., Castro, E., Negro, M.J., 2014. Biorefinery based on olive biomass. State of the art and future trends. Bioresour. Technol. 159, 421–432. https://doi.org/10.1016/j.biortech.2014.03.062

Rubio-Senent, F., Rodríguez-Gutiérrez, G., Lama-Muñoz, A., Fernández-Bolaños, J., 2013. Phenolic extract obtained from steam-treated olive oil waste: Characterization and antioxidant activity. LWT - Food Sci. Technol. 54, 114–124. https://doi.org/10.1016/j.lwt.2013.04.011

Rubio-Senent, F., Rodríguez-Gutíerrez, G., Lama-Muñoz, A., Fernández-Bolaños, J., 2012. New Phenolic Compounds Hydrothermally Extracted from the Olive Oil Byproduct Alperujo and Their Antioxidative Activities. J. Agric. Food Chem. 60, 1175–1186. https://doi.org/10.1021/jf204223w

Schaffer, S., Müller, W.E., Eckert, G.P., 2010. Cytoprotective effects of olive mill wastewater extract and its main constituent hydroxytyrosol in PC12 cells. Pharmacol. Res. 62, 322–327. https://doi.org/10.1016/j.phrs.2010.06.004

Serrano, A., Fermoso, F.G., Alonso-Fariñas, B., Rodríguez-Gutierrez, G., Fernandez-Bolaños, J., Borja, R., 2017. Olive mill solid waste biorefinery: High-temperature thermal pre-treatment for phenol recovery and biomethanization. J. Clean. Prod. 148, 314–323. https://doi.org/10.1016/j.jclepro.2017.01.152

Souza, E.C. de, Cordeiro, D.A., Silva, B.S., Neves, N. de A., Schmiele, M., 2022. Development of muffin with the incorporation of olive pomace flour, extra virgin olive oil and hydrolyzed soy protein. Res. Soc. Dev. 11, e58511226012. https://doi.org/10.33448/rsd-v11i2.26012

Suárez, M., Romero, M.-P., Motilva, M.-J., 2010. Development of a Phenol-Enriched

Olive Oil with Phenolic Compounds from Olive Cake. J. Agric. Food Chem. 58, 10396–10403. https://doi.org/10.1021/jf102203x

Taheri, S., Abdullah, T.L., Karimi, E., Oskoueian, E., Ebrahimi, M., 2014. Antioxidant capacities and total phenolic contents enhancement with acute gamma irradiation in Curcuma alismatifolia (Zingiberaceae) leaves. Int. J. Mol. Sci. 15, 13077–13090. https://doi.org/10.3390/ijms150713077

Valenti, F., Arcidiacono, C., Chinnici, G., Cascone, G., Porto, S.M., 2017. Quantification of olive pomace availability for biogas production by using a GIS-based model. Biofuels, Bioprod. Biorefining 11, 784–797. https://doi.org/10.1002/bbb.1784

Vitali Čepo, D., Radić, K., Jurmanović, S., Jug, M., Rajković, M.G., Pedisić, S., Moslavac, T., Albahari, P., 2018. Valorization of olive pomace-based nutraceuticals as antioxidants in chemical, food, and biological models. Molecules 23, 2070. https://doi.org/10.3390/molecules23082070

Yakhlef, W., Arhab, R., Romero, C., Brenes, M., de Castro, A., Medina, E., 2018. Phenolic composition and antimicrobial activity of Algerian olive products and by-products. LWT - Food Sci. Technol. 93, 323–328. https://doi.org/10.1016/j.lwt.2018.03.044

Yusuf, M., 2017. Agro-Industrial Waste Materials and their Recycled Value-Added Applications: Review, in: Martínez, L.M.T., Kharissova, O.V., Ildusovich Kharisov, B. (Eds.), Handbook of Ecomaterials. Springer. https://doi.org/10.1007/978-3-319-48281-1

Zbakh, H., El Abbassi, A., 2012. Potential use of olive mill wastewater in the preparation of functional beverages: A review. J. Funct. Foods 4, 53–65. https://doi.org/10.1016/j.jff.2012.01.002

Zhang, Q.W., Lin, L.G., Ye, W.C., 2018. Techniques for extraction and isolation of natural products: A comprehensive review. Chin. Med. 13, 1–26. https://doi.org/10.1186/s13020-018-0177-x