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Ð SALAMANCA

FACULTAD DE FARMACIA

DEPARTAMENTO DE QUÍMICA ANALÍTICA, NUTRICIÓN Y BROMATOLOGÍA

Developing novel functional food formulations from natural sources of bioactives: quinoa, amaranth and potatoes

DOCTORAL THESIS

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Salamanca, 2020

This work was funded by CAPES Foundation (Coordination for the Improvement of Higher Education Personnel, Brazil) through the grant no. 99999.001423/2015-00; by the European Regional Development Fund (ERDF) through the Regional Operational Program North 2020, within the scope of Project *Mobilizador* Norte-01-0247-FEDER-024479: ValorNatural®; and by FEDER-Interreg España-Portugal programme through the projects IBERPHENOL (0377_Iberphenol_6_E) and TRANSCOLAB (0612_TRANS_CO_LAB_2_P).

AGRADECIMENTOS

Eu dedico essa tese aos meus pais, Maria e Vicente. Chegar ao final desse trabalho é a realização de um sonho muito grande que sonhamos juntos. Obrigada por me carregarem no colo em tantos momentos desse caminho em que eu pensei não ter mais forças para continuar. Obrigada por estarem ao meu lado sempre em coração e me darem todo amor e incentivo, apesar de tanta saudade. Essa tese é só mais um símbolo concreto do amor que nos une e da força da nossa família. Eu amo vocês com todo meu coração. Essa tese é de vocês dois.

Eu agradeço com todo meu coração ao melhor amigo que alguém poderia ter na vida, meu irmão Juninho. Você é minha força e de longe a minha maior inspiração. Eu aprendo todos os dias com você, te admiro demais, te amo imensamente. Obrigada por tantas vezes acreditar mais em mim do que eu mesma.

Eu agradeço ao meu cunhado Yuri e sua família - que considero minha família também – por tanto apoio, tantas palavras de incentivo, tantas conversas e recargas de afeto nessa caminhada. Muito obrigada por tanto amor.

Quero agradecer também às minhas queridas tias Lu e Fran, que são para mim como segundas mães, fontes de afeto tão importantes na minha vida. Ainda vamos comemorar da forma que mais gostamos, tomando um café da tarde maravilhoso e dando muita risada juntas.

Agradeço aos meus amigos, que são anjos na minha vida. Agradeço especialmente a Karen e Helen, irmãs que a vida me deu há 17 anos. A Stefannie, minha amiga de infância que faz parte da minha família e está conosco em todos os momentos. A Ju e Defavari, meus amigos queridos de tantos anos que dentre tantas outras coisas são os responsáveis por eu ter conseguido me matricular nesse doutorado, quando eu ainda me recuperava de uma cirurgia em 2017. A Angélica e Giorlando, meus amigos lindos que me deram a paz que eu precisava para conseguir

me formar Engenheira de Alimentos em 2013. A Cristina, Cathy, Dayana e Marta, minha família na Escócia. A Rubia, Lívia e Camila, minha família em Portugal. Muito obrigada. Cada um de vocês foi fundamental nessa caminhada. Eu amo vocês e sou grata de todo coração.

Agradeço a todos os generosos parceiros desse trabalho. Agradeço em especial a toda equipe Biochemcore, que me recebeu em Portugal e me deu todo suporte e apoio para a realização desse doutorado, onde também fiz amizades que levarei para a vida. Muito obrigada por tudo. Meus agradecimentos aos parceiros que possibilitaram a realização dessa tese, Professor Carlos Spehar (Universidade de Brasília), Professor Spyridon Petropoulos (University of Thessaly), Dr Marina Sokovic (University of Belgrade), Dr Julien Lonchamp e Dr Catriona Liddle (Queen Margaret University). Muito obrigada pelo apoio e parceria.

Finalmente, agradeço aos meus generosos orientadores, meus mestres por quem tenho profunda admiração e gratidão. Professor Celestino, muito obrigada por me aceitar como sua aluna com tanta generosidade e por seus valiosos ensinamentos em todos os trabalhos que compõe essa tese. Dr Lillian Barros, sou profundamente grata a você por sempre ter feito tudo que era possível para que esse trabalho fosse realizado, sem nunca poupar esforços. Trabalhar com você tem sido uma experiência enriquecedora e um grande aprendizado, eu tenho muito orgulho de ter sido orientada por você. Professora Isabel Ferreira, serei eternamente grata por ter prontamente me aceitado em sua equipe e assim ter me possibilitado a realização do meu sonho do doutorado. É um privilégio ter sido orientada por você. Minha admiração e gratidão por cada um de vocês, meus orientadores, é enorme e para sempre.

Muito obrigada.

Coração de estudante
Há que se cuidar da vida
Há que se cuidar do mundo
Tomar conta da amizade
Alegria e muito sonho
Espalhados no caminho
Verdes, planta e sentimento
Folhas, coração
Juventude e fé

Milton Nascimento

LIST OF PUBLICATIONS

The studies developed within this Ph.D. thesis led to the following research and review articles, copies of which are included as annexes:

1. **Shirley L. Sampaio**, Spyridon A. Petropoulos, Alexios Alexopoulos, Sandrina A. Heleno, Celestino Santos-Buelga, Lillian Barros, Isabel C.F.R. Ferreira (2020). Potato peels as sources of functional compounds for the food industry: A review. *Trends in Food Science and Technology*, 103, 118-129.
2. **Sampaio, S. L**., Fernandes, Â., Pereira, C., Calhelha, R. C., Sokovic, M., Santos-Buelga, C., Barros, L., Ferreira, I. C. F. (2020). Nutritional value, physicochemical characterization and bioactive properties of the Brazilian quinoa BRS Piabiru. *Food & Function*, 11(4), 2969-2977.
3. Petropoulos, S.A**., Sampaio, S.L**., Di Gioia, F., Tzortzakis, N., Rouphael, Y., Kyriacou, M.C., Ferreira, I. C. F. (2019). Grown to be Blue - Antioxidant Properties and Health Effects of Colored Vegetables. Part I: Root Vegetables. *Antioxidants*, 8, 617.

LIST OF PUBLICATIONS

The following research articles are currently in process:

4. **Shirley L. Sampaio**, Julien Lonchamp, Maria Inês Dias, Catriona Liddle, Spyridon A. Petropoulos, Jasmina Glamočlija, Alexios Alexopoulos, Celestino Santos-Buelga, Isabel C.F.R. Ferreira, Lillian Barros (2020). Anthocyanin-rich extracts from purple and red potatoes as natural colourants: bioactive properties, application in a soft drink formulation and sensory analysis. Food Chemistry (accepted).
5. **Shirley L. Sampaio**, João C.M. Barreira, Ângela Fernandes, Spyridon A. Petropoulos, Alexios Alexopoulos, Celestino Santos-Buelga, Isabel C.F.R. Ferreira, Lillian Barros (2020). Potato biodiversity: A linear discriminant analysis on the nutritional and physicochemical composition of fifty genotypes. Food Chemistry (under review).
6. **Shirley L. Sampaio**, Ângela Fernandes, Carla Pereira, Ricardo C. Calhelha, Marina Sokovic, Dejan Stojković, Celestino Santos-Buelga, Lillian Barros, Isabel C.F.R. Ferreira (2020). Nutritional profiling and bioactive properties of Amaranthus cruentus cv. BRS Alegria – a novel amaranth cultivar. Cereal Chemistry (in submission).
7. **Shirley L. Sampaio**, Spyridon A. Petropoulos, Maria Inês Dias, Carla Pereira, Ricardo C. Calhelha, Ângela Fernandes, Camila Leme, Celestino Santos-Buelga, Isabel C.F.R. Ferreira, Lillian Barros. Red and purple potato peels (*Solanum tuberosum* L.) are rich in bioactive compounds with antioxidant and antitumor properties. Food Chemistry (in submission).

ABSTRACT

There is an increasing interest, by the food industry and consumers, in new food products whose functions go beyond the nutritional and sensory role, having also potential benefits over the physiological functions. The effects of functional foods range from improvement of general well-being to reduction of disease risk. Recently, one of the top research areas in Food Science and Technology has been the extraction and characterisation of new natural ingredients with biological activity that can be further incorporated into a functional food, contributing to consumer's well-being. These natural ingredients should be at the same time extracted sustainably from natural sources, deliver interesting technological characteristics and provide positive biological effects.

This work aimed at the valorisation of alternative natural sources of bioactives (quinoa, amaranth and coloured potatoes) and bio-residues (potato peels) as sources of functional compounds with potential to be applied in the food industry. For this purpose, these food matrices were evaluated concerning their nutritional, physicochemical and phenolic composition, as well as their bioactive properties.

Quinoa (*Chenopodium quinoa* Willd.) and amaranth (*Amaranthus cruentus* L.) are both plants indigenous to Latin America which were credited by the Aztecs and Incas with medicinal properties. Indeed, these pseudocereals have received much attention in recent years due to their exceptional nutritional value and potential health benefits. *Chenopodium quinoa* Willd. cv. BRS Piabiru and *Amaranthus cruentus* cv. BRS Alegria are novel pseudo-cereal genotypes developed and adapted to tropical climate by the Brazilian Agriculture Research Corporation. In this work it is presented for the first time an in-depth study of these varieties on their nutritional and physicochemical composition and *in vitro* bioactive properties. The results

obtained in the present work support the potential of quinoa BRS Piabiru and amaranth BRS Alegria as nutritious food crops and as sources of functional compounds.

In addition to the pseudo-cereals quinoa and amaranth, fifty potato genotypes from twenty-four different countries of origin, four different flesh colours (yellow, purple, red and marble) and different cultivation types (Andean accessions, landraces, breeder lines and cultivated varieties) were studied in terms of their nutritional and physicochemical characteristics. The results of this work can contribute to the supply of new potato genotypes into sustainable farming systems, supporting the protection of potato biodiversity, particularly Andean accessions, landraces and coloured genotypes (red or purple flesh) which are not widely cultivated so far.

Increasing interest in anthocyanin-rich coloured potatoes as functional food from the food industry, nutritional science and consumers has recently gained the attention of potato breeders.

Purple and red coloured potatoes contain high amounts of anthocyanins depending on their cultivar. The coloured potato varieties of this study were used as sources of colouring compounds for the development of natural food colourants. For this purpose, aqueous extracts from the coloured potato varieties (three red-fleshed, three-purple fleshed, and one marble-fleshed genotype) were studied for their anthocyanin content, *in vitro* biological activities, colouring properties and their potential application in the food industry. All the extracts presented *in vitro* antioxidant, antibacterial and antifungal activities, whereas no toxic effects were detected. Finally, two selected extracts were tested as colourants in a soft drink formulation and presented suitable sensory profiles as well as high colour stability during a 30-day shelf-life when compared with the commercial colourant E163. Therefore, the tested extracts could be used as natural food colourants.

It is known that the potato processing industry is one of the largest worldwide, producing a great amount of potato peel waste that ranges from 15% to 40% of the original fresh tubers weight. With the aim of adding value to this important bio-residue, in this work we studied the potential bioactivities of the peels from ten red and purple potato varieties, original from five different countries. All varieties presented antioxidant capacity and antitumor activity, in the four tested tumor cell lines. One of the extracts also presented anti-inflammatory activity, and all extracts presented no toxic effect up to the concentration of 304 μg/mL. These results support the potential of coloured potato peels as sources of bioactive compounds for the enrichment of functional food formulations.

Overall, the results of this work contribute evidences about the nutritional and functional properties of plants that could be promoted as additional sustainable sources to current intensive crops. Furthermore, they support the extraction of plant bioactive compounds and their application as functional ingredients or natural food additives as an efficient way for the reutilization and valorisation of food processing wastes, contributing to the creation of more economically and socially sustainable productive chains.

RESUMEN

Existe un interés creciente, por parte de la industria alimentaria y los consumidores, por nuevos productos alimenticios cuyas funciones van más allá del rol nutricional y sensorial, teniendo también potenciales beneficios sobre las funciones fisiológicas. Los efectos de estos alimentos funcionales van desde la mejora del bienestar general hasta la reducción del riesgo de algunas enfermedades. En este sentido, una de las principales áreas de investigación en Ciencia y Tecnología de Alimentos está siendo la extracción y caracterización de nuevos ingredientes naturales con actividad biológica que se pueden incorporar a un alimento funcional, contribuyendo al bienestar del consumidor. Estos ingredientes naturales deben extraerse al mismo tiempo de forma sostenible de fuentes naturales, ofrecer características tecnológicas interesantes y proporcionar efectos biológicos positivos.

El objetivo de este trabajo es la valorización de alimentos (quinoa, amaranto y patatas pigmentadas) y biorresiduos (piel de patata) como fuentes naturales alternativas de compuestos bioactivos, con potencial para ser empleados en alimentos funcionales por la industria alimentaria.

La quinoa (*Chenopodium quinoa* Willd.) y el amaranto (*Amaranthus cruentus* L.) son plantas autóctonas de América Latina a las que los aztecas y incas atribuían propiedades medicinales. De hecho, estos pseudocereales han recibido mucha atención en los últimos años debido a su valor nutricional excepcional y sus posibles beneficios para la salud. *Chenopodium quinoa* Willd. cv. BRS Piabiru y *Amaranthus cruentus* L. cv. BRS Alegria son dos nuevos genotipos de estas especies adaptados al clima tropical y desarrollados por la Corporación Brasileña de Investigación Agrícola (EMBRAPA). En este trabajo se desarrolla por primera vez un estudio en profundidad sobre la composición nutricional, fisicoquímica y propiedades bioactivas *in vitro* de estas variedades. Los resultados obtenidos apoyan el potencial de la quinoa BRS

Piabiru y el amaranto BRS Alegria como cultivos alimentarios nutritivos y como fuentes de compuestos funcionales.

Además de estos pseudocereales, se estudiaron las características nutricionales y fisicoquímicas de cincuenta genotipos de patatas de veinticuatro países, con cuatro colores de pulpa diferentes (amarillo, morado, rojo y mármol), correspondientes a cuatro tipos agronómicos distintos (accesiones andinas, variedades locales, líneas reproductoras y variedades cultivadas). Se espera que los resultados obtenidos permitan la selección de nuevos genotipos de patatas para su empleo en sistemas agrícolas sostenibles, contribuyendo a la protección de la biodiversidad de la patata y, particularmente, de las accesiones andinas, las variedades locales y los genotipos coloreados (patatas rojas o moradas), de cultivo limitado hasta el momento.

El interés de la industria alimentaria, los nutricionistas y los consumidores por las patatas pigmentadas como posible alimento funcional ha atraído recientemente la atención de los cultivadores. Las patatas moradas y rojas contienen altas cantidades de antocianos, variables en función del cultivar. En el presente estudio, se exploraron las patatas coloreadas como fuentes para la extracción de este tipo de compuestos, con vistas al desarrollo de colorantes alimentarios naturales. Para ello, se estudiaron extractos acuosos de distintas variedades de patata (tres de pulpa roja, tres de pulpa púrpura y un genotipo de pulpa mármol) en cuanto a su contenido de antocianos, actividades biológicas *in vitro*, propiedades colorantes y potencial de aplicación en la industria alimentaria. Todos los extractos presentaron actividad antioxidante, antibacteriana y antifúngica *in vitro*, mientras que no se detectaron efectos tóxicos. Dos extractos seleccionados se ensayaron como colorantes en una formulación de bebida refrescante, encontrando que presentaban perfiles sensoriales adecuados, así como una alta estabilidad del color durante una vida útil de 30 días, características que eran similares o

mejores a las obtenidas con el colorante comercial E163, por lo que podrían usarse como colorantes alimentarios naturales.

La industria de transformación de la patata se encuentra entre las más importantes a nivel mundial en cantidades procesadas, produciendo una gran cantidad de desperdicios, especialmente pieles, que oscila entre el 15% y el 40% del peso original de los tubérculos frescos. Con el objetivo de agregar valor a este importante biorresiduo, en este trabajo se estudiaron las bioactividades de la piel de diez variedades de patatas rojas y moradas, originarias de cinco países diferentes. Todas las variedades presentaron capacidad antioxidante y actividad antitumoral en las cuatro líneas celulares tumorales ensayadas. Uno de los extractos también presentó actividad antiinflamatoria y ninguno de los extractos presentó efecto tóxico sobre una línea celular hepática hasta la concentración de 304 μg/mL. Estos resultados respaldan el potencial de las pieles de patatas pigmentadas como fuentes de compuestos bioactivos para el enriquecimiento de formulaciones alimentarias funcionales.

En general, los resultados de este trabajo aportan evidencias sobre el valor nutricional y funcional de cultivos alimentarios que podrían ser contemplados como fuentes alternativas y sostenibles a los cultivos de producción intensiva. Asimismo, apoyan la extracción de compuestos bioactivos vegetales y su aplicación como ingredientes funcionales o aditivos alimentarios naturales como una opción eficiente para la reutilización de residuos del procesado de alimentos, contribuyendo a la creación de cadenas de producción más sostenibles social y económicamente.

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ABBREVIATIONS

ANOVA	Analysis of variance
BHT	Butylated hydroxytoluene
CFU	Colony forming unit
CT	Cultivation type
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EMBRAPA	Brazilian Agriculture Research Corporation
ESI-MS	Electrospray ionization mass spectrometry
FAME	Fatty acids methyl ester
FC	Flesh colour
HeLa	Cervical carcinoma cell line
HepG2	Hepatocellular carcinoma cell line
HPLC	High-performance liquid chromatography
HPLC-DAD	High-performance liquid chromatography coupled to diode-array detection
HPLC-UV	High-performance liquid chromatography coupled to ultraviolet detection
IPK	Leibniz Institute of Plant Genetics and Crop Plant Research
INT	*p*-Iodonitrotetrazolium chloride
LDA	Linear discriminant analysis
LPS	Lipopolysaccharide
MA	Malt agar
MCF-7	Breast adenocarcinoma cell line
MDA	Malondialdehyde
MBC	Minimal bactericidal concentration

MFC	Minimal fungicidal concentration
MIC	Minimal inhibitory concentration
MS	Mass spectrometry
NCI-H460	Non-small cell lung cancer cell line
NO	Nitric oxide
OxHLIA	Oxidative haemolysis inhibition assay
PBS	Phosphate buffered saline
PLE	Pressurised liquid extraction
PP	Potato peels
PUFA	Polyunsaturated fatty acids
QDA	Quantitative descriptive analysis
SRB	Sulforhodamine B solution
SD	Standard deviation
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid
TSB	Tryptic soy broth
UFLC	Ultra-fast liquid chromatography

CHAPTER 1

Background

1. CHAPTER 1: Background

1.1 Alternative functional foods and additives

There is an increasing interest, by the food industry and consumers, in new food products whose functions go beyond the nutritional and sensory role, having also potential benefits over the physiological functions. The effects of functional foods range from improvement of general well-being to reduction of disease risk to treatment of illness (Bigliardi & Galati, 2013). Recently, one of the top research areas in Food Science and Technology has been the extraction and characterisation of new natural ingredients with biological activity that can be further incorporated into a functional food, contributing to consumer's well-being (Vieira da Silva, Barreira, & Oliveira, 2016). These natural ingredients should be at the same time extracted sustainably from natural sources, deliver interesting technological characteristics and provide positive biological effects.

Quinoa (*Chenopodium quinoa* Willd.) and Amaranth (*Amaranthus cruentus* L.) are both plants indigenous to Latin America which were credited by the Aztecs and Incas with medicinal properties. Indeed, these pseudocereals have received much attention in recent years due to their exceptional nutritional value and potential health benefits. Increasing research has been focused on the phytochemicals of quinoa and amaranth due to their potential role in reducing the risk of chronic disease (Tang et al., 2016). The majority of the phytochemicals in these grains are polyphenols, mainly phenolic acids and flavonoids. These compounds provide additional health benefits such as antioxidant and anti-inflammatory activities (Alvarez-Jubete, Arendt, & Gallagher, 2010). Current findings also encourage future research in identifying quinoa and amaranth bioactives related to the observed effects, to be further incorporated into functional foods (Tang & Tsao, 2017).

Quinoa and amaranth have been classified as a "future smart food" by the Food and Agriculture Organization of the United Nations (FAO), *i.e*. "foods that can bolster dietary diversification, improve micronutrient intake, enhance soil health, require fewer inputs such as chemical fertilizers, and often prove resilient to climate change and adverse farming conditions" (FAO, 2018). These pseudo-cereals have the potential of enhancing global food security for a growing world population, providing highly nutritious foods that can be grown on lands not suitable for other crops (Jarvis et al., 2017). Moreover, these plants possess a series of bioactive compounds that make them suitable for today's use in the development of functional foods, such as gluten-free bread and pasta products and beverages (Repo-Carrasco-Valencia & Vidaurre-Ruiz, 2019).

Increasing interest in anthocyanin-rich coloured potatoes as functional food for the food industry, nutritional science and consumers has recently gained the attention of potato breeders. Starchy tubers of *Solanum tuberosum* L. are a staple crop and food in many countries. Among cultivated potato varieties a huge biodiversity exists, including an increasing number of red and purple coloured cultivars. Purple and red coloured potatoes may contain high amounts of anthocyanins (up to more than 100 mg per 100 g fresh weight) depending on their cultivar (Lewis et al., 1999). The bright red and purple colours relate to nutritional benefits associated with the antioxidative capacity of anthocyanins (Oertel et al., 2017). The anthocyanin concentration in pigmented potatoes is known to vary in large ranges and correlates with the degree of pigmentation in potato flesh (Hamouz et al., 2011). Moreover, there is an interest by the food industry in anthocyanin-rich fruits and vegetables as they are bright and attractive to consumers. In this context, there is also a growing interest worldwide in the development of natural food additives, such as colourants and preservatives, as a consequence of perceived consumer preferences and the concerns about the use of synthetic compounds in food products

(Albuquerque, Pinela, Barros, Oliveira, & Ferreira, 2020; Petropoulos et al., 2019). In response to that, the food and pharmaceutical industries have been researching the use of natural matrices as sources of compounds with beneficial health effects (Wahyudi, Ramadhan, Wijaya, Ardhani, & Utami, 2020). For this purpose, the valorisation of alternative sources of bioactives (such as novel grains and wild crops) and bio-residues (that are usually discarded but can be rich in bioactive compounds) can contribute to the creation of more economically and socially sustainable productive chains (Albuquerque, Pereira, et al., 2020; Pathak, Mandavgane, Puranik, Jambhulkar, & Kulkarni, 2018). With this aim, the extraction of plant bioactive molecules has been largely explored and well established by many authors as an efficient route for the reutilization of food processing waste, especially when considering the recent technological advances in molecular separations and identifications (Wijngaard, Ballay, & Brunton, 2012). The present work focus on alternative sources of bioactives (namely quinoa, amaranth, wild and coloured potatoes) and bio-residues (namely potato peels), that will be discussed in detail in the following sections.

1.2 Quinoa

Quinoa (*Chenopodium quinoa* Willd.) is a grain-like food crop that has provided nutrition to Andean indigenous cultures for thousands of years (FAO, 2018; Graf, Rojas-silva, Rojo, Delatorre-herrera, & Balde, 2015). Usually referred to as pseudo-cereal or pseudo-grain, the plant produces seeds that can be milled into flour, with technological features that resemble those of the Gramineae family, for instance wheat (Graf et al., 2015; Vilcacundo & Hernandez-Ledesma, 2017). In addition to its excellent nutritional value, quinoa has been found to contain a high diversity of phytochemicals with recognised positive health benefits, including phytosterols, saponins, phytoecdysteroids, phenolic compounds and betanin, which may

contribute to metabolic, cardiovascular and gastrointestinal health (**Figure 1**) (FAO & CIRAD, 2015; Graf et al., 2015; Vilcacundo & Hernandez-Ledesma, 2017).

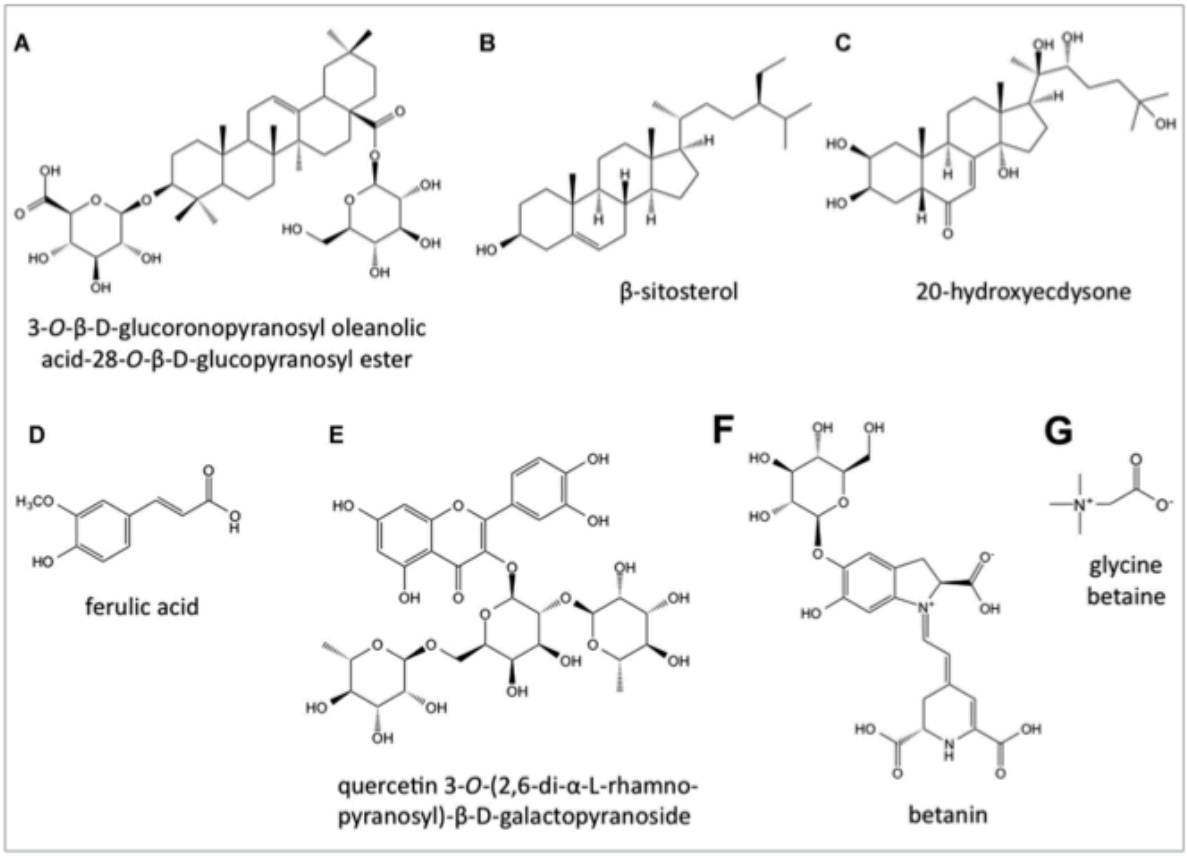


Figure 1: Representative chemical structures of the major pharmacologically active secondary metabolites present in quinoa seeds. A: triterpene saponin, B: phytosterol, C: phytoecdysteroid, D: phenolic acid, E: flavonol glycoside, F: betalain, G: glycine betaine. Source: Graf et al. (2015).

Due to its relevance as an alternative food crop, quinoa has been promoted globally, with 2013 being declared the "International Year of Quinoa" (FAO & CIRAD, 2015; FAO, 2018). As its global popularity increases, quinoa cultivation has spread to more than 70 countries, including the United States, Canada, China, India, Finland, Australia, Kenya, the United Kingdom, Japan and Brazil (Contreras-Jiménez, Torres-Vargas, & Rodríguez-García, 2019; FAO & CIRAD,

2015; FAO, 2018; Hirose, Fujita, Ishii, & Ueno, 2010). Nevertheless, the current major global producers remain the Andean countries Peru, Bolivia and Ecuador, respectively (FAOSTAT, 2020).

Consumption of seeds is the most common use of quinoa, however, the pseudo-cereal has also been traditionally used in a range of other applications (**Table 1**).

Table 1: Traditional uses of quinoa. Adapted from Vega-Gálvez et al. (2010).

Main uses	**Component implied**	**Plant organ**
Foods and drinks	Vitamins Proteins	Seeds and leaves
Animal food	Vitamins Proteins	Whole plant
Medicine	Immune system Skin applications Circulatory applications	Leaves and seeds
Repellent	Insects	Leaves and seed coat

Brazil is a stablished global grain supplier, and has placed the introduction of new crops into production systems as a high priority in research and development (FAO & CIRAD, 2015). Agricultural diversification contributes to improve income, reduce costs, improve nutrient availability, protect the soil and reduce negative environmental impacts (Spehar & Santos, 2001). The Brazilian Agricultural Research Corporation (EMBRAPA) has been working for over twenty years in the selection and adaptation of quinoa varieties to be cultivated under Brazilian tropical climate (FAO & CIRAD, 2015). BRS Piabiru **(Figure 2),** the first recommended quinoa for grain production in Brazil, is a selection of a breeding line originating from a plant population of Quito, Ecuador. The new developed genotype was tested for years

in variety trials, in Central Brazil, before being standardised for agronomic characteristics, such as rapid growth, tolerance to hydric stress, biomass production and nutrient cycle (Spehar & Santos, 2001). The Brazilian production of quinoa is expected to increase over the next years, reducing the pressure on Bolivia and Peru, where quinoa has become the sole grain crop export (FAO & CIRAD, 2015).

Figure 2: Quinoa cv. BRS Piabiru plants in a Brazilian savannah (Cerrados region) field. Author: Embrapa Cerrados.

Few studies have been performed on the chemical and technological aspects of the BRS Piabiru quinoa seeds. Palombini et al. (2013) reported the fatty acid, proximate and amino acid compositions, antioxidant activity (inhibition of DPPH assay), total phenolic content (*Folin-Ciocalteu* reagent's method), vitamin E and mineral contents. Nickel, Spanier, Botelho, Gularte, & Helbig (2016) studied the variation in the total phenolic content, antioxidant capacity (DPPH and FRAP assays) and saponin content depending on the type of processing technology. Meneguetti et al. (2011) reported the biological effects of the BRS Piabiru quinoa

extracts supplementation *in vivo* (rats), finding no hepatic nor renal toxicity. They also observed a decreased food intake, body weight, fat deposition, and blood triacylglycerol level in the supplemented groups. Moreover, three added value food applications applying the new quinoa variety have been reported to date: functional bread (Calderelli, Benassi, & Visentainer, 2010), gluten-free granola (Souza et al., 2014) and gluten free cookie (Pagamunici et al., 2014).

1.3 Amaranth

Amaranthus spp. is a food crop frequently classified as a pseudo-grain or a pseudo-cereal due to its flavour and cooking qualities (Coelho, Silva, Martins, Pinheiro, & Vicente, 2018; Food and Agriculture Organization of the United Nations, 2020). The plant belongs to the Amaranthaceae family and it is a native species to the Andean region in South America, which includes Argentina, Peru and Bolivia (Food and Agriculture Organization of the United Nations, 2020). The genus *Amaranthus* consists of more than 50 species that are cultivated for use as cereals, vegetables and ornamentals, being the amaranth grain a staple food for the Aztecs in ancient times (Arendt & Zannini, 2013).

Amaranth presents high nutritional value, above all for its balanced amino acid and dietary fibre contents (Cornejo, Novillo, Villacrés, & Rosell, 2019). Additionally, it is a gluten-free pseudo-cereal, being a suitable alternative for patients with coeliac disease or gluten intolerance (Cornejo et al., 2019). Moreover, many authors have reported the potential beneficial health effects of amaranth intake (**Table 2**). China, United States, Canada and Argentina are the main producers (Coelho et al., 2018); however, global production data is not available (FAOSTAT, 2020). Other production regions include Central America and Africa (Arendt & Zannini, 2013). Amaranth has also been classified as a "future smart food" by FAO (2018) and is also an alternative crop for agricultural diversification (Silva et al., 2019).

Table 2: Biological effects of amaranth as reported in the literature.

Beneficial biological effects of amaranth	**References**
Anti-hypertensive	(Suárez, Aphalo, Rinaldi, Añón, & Quiroga, 2020)
Antithrombotic	(Sabbione, Suárez, Añón, & Scilingo, 2018)
Hypocholesterolemic	(Coelho et al., 2018)
Antioxidant	(Coelho et al., 2018)
Anticarcinogenic	(Coelho et al., 2018)

Amaranth is also a versatile food crop; **Table 3** presents a comprehensive overview of amaranth-containing food products.

Table 3: Applications of amaranth in food products. Adapted from Arendt & Zannini (2013).

Food products	**Amaranth forms and processing**
Soup	Grain and flour
Pasta, noodles	Flour
Pancakes	Flour, whole grain, and popped grain
Breakfast cereals	Whole, popped, or sprouted-grain flour
Porridge	Popped grain in milk
Breads, rolls, muffins and many other forms of baked foods	Flour, popped grain, toasted grain, whole grain
Crepes	Flour, popped grain
Dumplings, *tostadas, tortillas, fritos* and corn pones	Flour, whole or popped grain
Cookies and crackers	Flour, whole or popped grain
Snack bars	Popped grain, toasted grain or sprouted grain
Toppings	Popped grains, flour
Beverages	Flour, popped grain

Fillers	Whole or popped grain, flour or starch
Confections	Popped grain

Amaranth stands out as an option to diversify grain cultivation in the Brazilian savannah. Although the region has experienced rapid growth in agriculture, it is based mainly on soya (Silva et al., 2019). Diversification of production systems depends on rapid growth, tolerance to hydric stress, biomass production, nutrient cycling and human and animal utilization. The amaranth species *Amaranthus caudatus*, *A. cruentus* and *A. hypochondriacus*, with light seed colour and no dormancy, present these characteristics (Spehar & Teixeira, 2003; Silva et al., 2019). They are distinguishable from the weeds *A. spinosus, A. hybridus, A. blitum and A. viridis*, with dark and dormant seeds. Their grains, with excellent protein quality, can be used in gluten-free special diets and livestock feed. Since the 1990s, research regarding the adaptability of amaranth cultivars to the Brazilian soil and tropical climate has been carried out by the Brazilian Agricultural Research Corporation (EMBRAPA) and the University of Brasilia (Spehar & Santos, 2019). *Amaranthus cruentus* L. – BRS Alegria (**Figure 3**) was the first cultivar recommended for production systems in the Brazilian savannah, originated from mass selection in the variety AM 5189 of the United States (Spehar & Teixeira, 2003).

Figure 3: *Amaranthus cruentus* BRS Alegria plants in the harvest point, evidencing the panicles. Adapted from Silva et al. (2019).

Few authors have previously studied the amaranth cultivar BRS Alegria. Tapia-Blácido et al. (2010) studied the production of flour, starch and protein concentrate from the novel amaranth grains, finding positive results particularly concerning gel formation and heat stability. Menegassi et al. (2011) also produced flour, comparing native and extruded flours obtained under mild and severe extrusion conditions. The flours produced with amaranth BRS Alegria grains exhibited high quality paste stability, low solubility in water and elastic behaviour (Menegassi et al., 2011). Palombini et al. (2013) reported the fatty acid, proximate and amino acid compositions, antioxidant activity (inhibition of DPPH assay), total phenolic content (*Folin-Ciocalteu* reagent's method), vitamin E and mineral contents. Finally, Pagamunici et al. (2014) applied the whole flour of amaranth BRS Alegria in the development of gluten-free food bars, which presented good sensory acceptance and high purchase intent.

1.4 Potatoes

1.4.1 Potato biodiversity

Potato (*Solanum tuberosum* L.) is the world's most important non-cereal food crop, cultivated in more than 150 countries and a staple for 1.3 billion people (Lutaladio, Ortiz, Haverkort, & Caldiz, 2009; Narváez-Cuenca, Peña, Restrepo-Sánchez, Kushalappa, & Mosquera, 2018; Stokstad, 2019). Its biodiversity is vast, with more than 5000 known varieties, and 200 species identified as wild (Burlingame, Mouillé, & Charrondière, 2009). Potato is the third food crop in terms of global production after rice and wheat (Romano et al., 2018), with an annual production of about 375 million tons, covering 19.2 million hectares (ha) and an average yield of 19.5 ton/ha (FAOSTAT, 2020).

Potato species is characterized by a large phenotypic diversity with great interest on the edible part of the plant (tubers), which may differ in shape, size, flesh and skin colour and texture, as well as in aspects related with chemical composition (dry matter, proteins, starch, and glycoalkaloids content) and utilisation characteristics (cooking type, susceptibility to enzymatic browning, and suitability to frying and crisping), among others (Calliope, Lobo, & Sammán, 2018; Camire, Kubow, & Donelly, 2009). This differentiation is observed not only among the more diverse wild genotypes but also between the numerous cultivated genotypes throughout the world (IBPGR, 1985).

The nutritious tubers have a great potential for reducing hunger and malnutrition, and are becoming increasingly popular in developing countries, as source of nutrients and also income for their populations (International Potato Center, 2018; Narváez-Cuenca et al., 2018; Stokstad, 2019). Potato produces more calories per hectare than rice and wheat and also stands out for its efficient water use, i.e., yielding more food per unit of water than any other major crop

(International Potato Center, 2018). This means that increasing the proportion of potato in human diet could help to alleviate pressure on water resources worldwide.

Most of the potatoes produced worldwide are traded in the form of raw commodity for fresh consumption as a traditional ingredient of many cuisines. Nevertheless, global consumption, particularly in developed countries, has been shifting from fresh tubers to processed food products such as frozen potatoes, French fries, potato crisps, dehydrated potato flakes, potato flour, and potato starch (International Potato Center, 2018). More than 7 million tons of factory-made French fries are produced per year worldwide (International Potato Center, 2018). In eastern Europe and Scandinavia, potato is also used in the distillation of alcoholic beverages, such as vodka and akvavit. Besides being an important staple in the human diet, tubers are also used as animal feed (Romano et al., 2018).

Although potato biodiversity is vast, only a limited number of varieties are currently produced and commercialized resulting in the degradation of biodiversity and the erosion of agricultural ecosystems (Bommarco, Kleijn, & Potts, 2013). Modern agricultural practices such as the replacement of local varieties with high-yielding species, along with climate change and environmental challenges, are contributing to the loss of potato biodiversity and thus the loss of valuable genes that encode nutrient biosynthetic pathways (Burlingame et al., 2009; Calliope et al., 2018). The continuous supply and introduction of new and traditional varieties into sustainable agricultural systems is a strategy to protect and increase agrobiodiversity, combat pests and diseases through tolerant or resistant genotypes, increase yields and support production in marginal lands and environments (Lutaladio et al., 2009). Moreover, the three dimensional biodiversity (ecosystems, species and within species) is essential for food security and for fighting malnutrition throughout the world (Toledo & Burlingame, 2006).

1.4.2 Coloured potatoes

Coloured root vegetable products can be alternative sources of colouring and bioactive compounds (Hossain, Rawson, Aguiló-aguayo, Brunton, & Rai, 2015; Nemś & Pęksa, 2018). Among root vegetables, potato also presents the highest genetic diversity among all cultivated species, with approximately 5000 registered varieties and a broad phenological variation in terms of flesh and skin colour.

Red and purple-fleshed potatoes are rich in phenolic compounds, particularly in anthocyanins (**Figure 4**), presenting about three times higher amounts of total phenolic compounds content than the widely consumed white- and yellow-fleshed tubers, as well as two to three times higher antioxidant activity. Anthocyanins are suitable to be used as natural colouring compounds due to their bright attractive red and purple colours and water solubility that allows for their easy incorporation into aqueous food systems (Rodriguez-Saona, Giusti, & Wrolstad, 2008; Rodríguez-Saona, Glusti, & Wrolstad, 1999). Nevertheless, they can pose problems of colour instability when they are out of their natural environment, as affected by factors such as pH, temperature, light, oxygen or interactions with other coexisting components, among others (Santos-Buelga & González-Paramás, 2019).

In their natural media anthocyanins are found as glycosylated compounds, the aglycones (or anthocyanidins) being quite unstable and rarely found in plant tissues and food. The sugar residues may be further substituted by aliphatic, hydroxybenzoic or hydroxycinnamic acids. The anthocyanin molecules found in coloured potatoes are usually acylated with *p*-coumaric or ferulic acids. The acylation of the anthocyanin molecules improves pigment stability during processing and storage (Rodriguez-Saona, Giusti, & Wrolstad, 1998). This effect is chemically explained by the stacking of the acyl groups with the pyrylium ring of the flavylium cation, which reduces the susceptibility to the nucleophile attack of water on the hydrophilic

anthocyanin molecules and the formation of a colourless pseudobase and light yellow chalcone forms (Rodriguez-Saona et al., 1998; Sasaki, Nishizaki, Ozeki, & Miyahara, 2014).

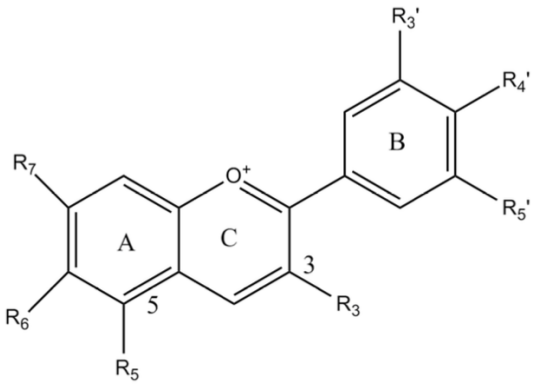
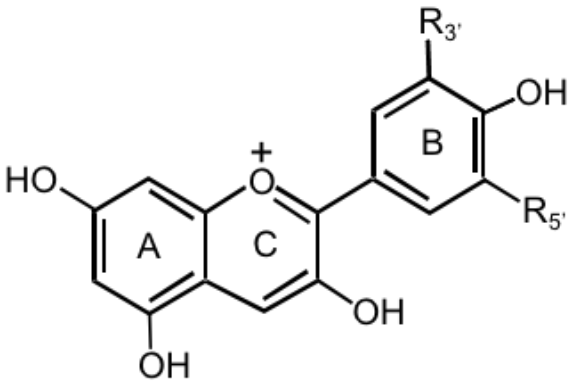


Figure 4. The core structure of anthocyanins with two aromatic benzyl rings (A and B rings) and a portion cyclized with oxygen (C ring).

Natural anthocyanins have powerful colouring capacities and at acidic pH values only small concentrations of these compounds are required to obtain the desired red, pink and purple colours in food products (Mateus & Freitas, 2009). The list of applications of anthocyanins as colouring agents is long, comprising products such as fruit preservatives (Bursać Kovačević et al., 2015), sugar confectionary (Mateus & Freitas, 2009), dairy products (Montibeller, de Lima Monteiro, Tupuna-Yerovi, Rios, & Manfroi, 2018; Pires et al., 2018), dry mixes (acid dessert mixes and drink powders) (Mateus & Freitas, 2009), frozen products (ice creams) (Mateus & Freitas, 2009), bakery products (Albuquerque et al., 2020; Da Silva et al., 2019a, 2019b) and beverages (Monteiro et al., 2017; Montibeller et al., 2018). Among all possible applications, soft drinks are suitable candidates due to their acidic pH (below 3.5) and the high solubility of anthocyanins in water (Mateus & Freitas, 2009; Montibeller et al., 2018). Nevertheless, these molecules can be affected by heat processing, hence the importance of testing the colour stability after pasteurisation, as soft drink formulations may be heat-processed to prolong the

product's safety during shelf-life. **Figure 5** presents the main anthocyanins reported in plants and food, including coloured potatoes.



	R3'	R5'
Pelargonidin	H	H
Cyanidin	OH	H
Peonidin	OCH_3	H
Delphinidin	OH	OH
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3

Figure 5. The main anthocyanidins detected in coloured potatoes.

The deep purple colour of potato flesh and skin has been associated with the presence of petunidin derivatives. Petunidin was the major anthocyanidin compound found both in the flesh and peel of purple potato varieties studied by Yin *et al.* (2016). In that study, petunidin accounted for 63-66% of the total anthocyanidin content of purple peel and flesh. The same was observed by Kita *et al.* (2013) when studying purple and red-fleshed potato cultivars, where petunidin-3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside was the major anthocyanin compound reported in the purple-fleshed varieties of Salad Blue (29.31 ± 0.73 mg/100 g dw), Valfi (43.11 ± 0.37 mg/100 g dw) and Blue Congo (36.32 ± 0.33 mg/100 g dw). Similarly, Nemš *et al.* (2015) identified petunidin-2-*O*-*p*-coumarylrutinoside-5-*O*-glucoside as the major anthocyanin present in the varieties Salad Blue (28.34 ± 9.30 mg/100 g dw), Valfi (57.77 ± 28.75 mg/100 g dw) and Blue Congo (75.97 ± 12.38 mg/100 g dw). In red-fleshed potatoes, pelargonidin acyl-glycoside derivatives appear as the main anthocyanin compounds. Kita *et al.* (2013) found pelargonidin-3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside as the major anthocyanin present in red-fleshed varieties, such as Rosalinde (15.14 ± 0.12 mg/100 g dw),

Herbie 26 (44.46 ± 0.23 mg/100 g dw) and Highland Burgundy Red (126.38 ± 0.71 mg/100 g dw). Yin *et al.* (2016) carried out an acid hydrolysis of anthocyanins to study the composition of anthocyanidins, reporting pelargonidin as the main anthocyanidin present in the red-fleshed cultivar Red Cloud No 1., with a concentration of 11.73 ± 0.16 mg/100 g fw, which corresponded to 82% of the total anthocyanidin content of the studied red potato variety. Other anthocyanin compounds reported in literature for red and purple-fleshed potatoes include delphinidin, cyanidin, peonidin and malvidin acyl-glycoside derivatives (Kita et al., 2013; Yin et al., 2016).

Kotíková *et al.* (2016) compared the carotenoid content of yellow-fleshed, white-fleshed, purple-fleshed and red-fleshed potato cultivars. Carotenoids are fat-soluble pigments that can exert antioxidant properties. Interestingly, yellow potatoes showed a much higher average total carotenoid content (26.22 μg/g dw) in comparison to the red and purple-fleshed cultivars (5.69 μg/g dw). Therefore, carotenoid pigments do not play a role in the colours of purple and red-fleshed potatoes.

Yin *et al.* (2016), when investigating ten coloured potato cultivars from China, compared the composition and antioxidant activities of their flesh and peel. The authors found potato peels were in average 15.34 times richer in anthocyanins than the flesh. The antioxidant activity of the peels extracts was also 5.75 times higher in average than the flesh extracts. In the same study, the variety Purple Cloud No.1 showed the strongest antioxidant activity in the flesh among all other varieties, also presenting the highest total content of anthocyanidins (43.38 mg/100 g fw). This correlation indicates the presence of anthocyanins as a major contributor to the antioxidant activity of coloured potatoes.

There has been an increasing interest by consumers and food producers in coloured potato varieties, due to its attractive organoleptic features (colour and taste), besides its health

beneficial chemical composition (Rytel et al., 2014). The frying process to produce coloured potato crisps can cause a degradation of anthocyanin compounds of 38-70%, with pelargonidin and malvidin acyl-glycoside derivatives being more stable during the frying process in comparison to petunidin acyl-glycoside derivatives (Kita et al., 2013). Nevertheless, despite reducing the amounts of anthocyanins when compared to raw potatoes, coloured potato crisps can present bright attractive colours, besides 2–3 times higher antioxidant activities and 40% higher contents of polyphenols than standard snacks made of traditional yellow potatoes and corn (Kita et al., 2013; Nemš et al., 2015). In a recent study, Nemś and Pęksa (2018) incorporated dried red and purple-fleshed potatoes into fried snacks, reporting a beneficial effect on the inhibition of oxidative changes in snacks lipids compared to control snacks, particularly when incorporating material from purple-fleshed potatoes of Blue Congo and Valfi varieties. Thereby, the antioxidant properties of coloured potatoes can be beneficial not only for human health, but also for the shelf life of processed food products.

1.4.3 Potato peels

Besides being one of the most commonly consumed vegetables, potato crop is also associated with one of the largest food processing sectors throughout the world (Farvin, Grejsen, & Jacobsen, 2012). In the European Union, processed potatoes worthed EUR 10 billion in 2017, which corresponds to 1.5% of the whole European food industry production value (EUROSTAT, 2019). Nevertheless, it is estimated that more than fifty percent of the total global potato production is consumed after processing, divided mainly into four types of products: frozen, dried, prepared/preserved potatoes and potato starch (EUROSTAT, 2019; International Potato Center, 2019). Generally, potato processing begins with tubers peeling, for

which methods such as steam peeling, lye peeling and abrasion have been reported, with the latter being the most used by the crisps industry (Toma, Orr, D'Appolonia, Dintzis, & Tabekhia, 1979; Wijngaard et al., 2012), as fully described in the recent review of Pathak et al. (2018). Added value processed products such as French fries, chips, hash browns, puree and frozen food account for a waste generation caused by potato peeling ranging from 15% to 40% of the original fresh weight, depending on the peeling process (Schieber, Stintzing, & Carle, 2002).

Furthermore, potato starch, flour and canning industries are also responsible for the production of large amounts of peel waste whose disposal raises great environmental concerns (Pathak et al., 2018; Schieber et al., 2002). Annually, these industries produce between 70 to 140 thousand tonnes of potato peels (PP) worldwide (Hossain et al., 2015). Most of the waste generated by potato processing industries is discarded in land-fills, with accompanying environmental consequences, or used as animal feed with a low added value in the production chain (Chohan, Aruwajoye, Sewsynker-Sukai, & Gueguim Kana, 2020; Schieber & Aranda, 2009). Recently, the use of potato processing residues as biomass sources for energy and biogas production has also been proposed (Maragkaki et al., 2016; Achinas, Li, Achinas, & Euverink, 2019; Osman, 2020).

To overcome this global issue, up-grading of this residue to added value products can therefore be of interest not only to the food industry (**Figure 6**), but also to government agencies and policy makers, decreasing the overall environmental impacts of the potato processing industry (Hossain et al., 2015). In this context, Torres et al. (2020) suggested an integrated approach for processing of discarded potatoes (irregularly shaped or very small tubers) during harvest and after storage, which may account for up to 30% of total production. Thus, this waste could be exploited for the recovery of valuable compounds, such as starch from tuber flesh, antioxidants

from skins and protein from processing wastewaters, while the second generation waste could be used for the production of potato flakes, hydrogels, biofilms, fertilizers and adsorbers (Torres et al., 2020).

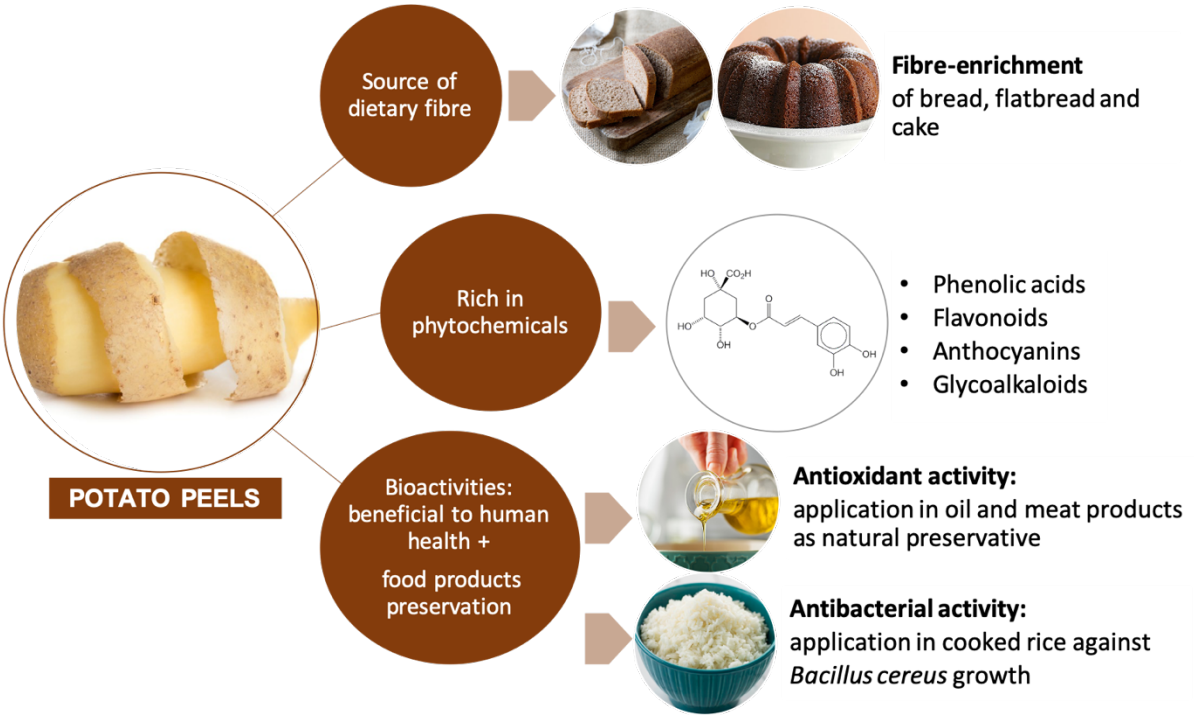


Figure 6. Potato peels and their main interesting features for the food industry.

Author: Shirley Sampaio.

The extraction of plant bioactive molecules has been largely explored and well established by many authors as an efficient route for the reutilization of food processing waste, especially when considering the recent technological advances in molecular separations and identifications (Wijngaard et al., 2012). PP can be a rich source of bioactive compounds due to their high contents in phenolic compounds with recognized health-promoting properties, such as antioxidant activity, that can also be employed in food systems to extend the shelf-life of food products (Albishi, John, Al-Khalifa, & Shahidi, 2013; Friedman et al., 2018).

1.4.3.1. Taxonomy and agronomic aspects of potato peels.

The potato skin set is affected by various factors such as the genotype which may affect the skin thickness and the strength of its adhesion to the flesh, as well as the tensile strength of skin (Bowen, Muir, & Dewar, 1996). Environmental factors also may have an impact on skin characteristics where cultivation in cold and moist soils results in thinner and weaker skin, while dry soils result in periderm with a lower number of cell layers. Agronomic factors such as nitrogen and potassium fertilization may decrease skin thickness and strength probably to the delayed maturation of crop, while the addition of micronutrients such as calcium, manganese and ferrous sulphate may increase the number of periderm cells (Wiltshire, Milne, & Peters, 2006).

From the anatomical point of view, peel or skin is the outer part of tuber periderm and a protective barrier against pest and pathogens attacks and water losses, while it serves as a wound-healing tissue of damaged epidermis (Barel & Ginzberg, 2008). Periderm consists of phellem cells (or skin), the phellogen and the phelloderm, which altogether are used to replace epidermis when the latter is damaged during tuber development, maturation and postharvest management (Barel & Ginzberg, 2008). Phellogen is a meristematic layer located at the middle section of periderm and produces phellem cells and phelloderm through outward and inward cell divisions, respectively, throughout the tuber development (Fogelman, Tanami, & Ginzberg, 2015). Phellem consists of several layers of cells with suberized cell walls after the deposit of suberin, a complex macromolecule that is responsible for the structural strength and protective properties of skin (Fogelman et al., 2015; Tanios et al., 2020). Suberin consists of two domains, an aromatic and an aliphatic one, which differentiates potato suberin polymers from other species and is responsible for the impermeable nature of PP to water, soluble compounds and gases (Schreiber, Franke, & Hartmann, 2005; Serra et al., 2010). Suberin is

naturally formed under normal conditions while its biosynthesis is triggered by wounding of any cause (mechanical damage or biotic factors) and environmental stressors (e.g. drought, salinity, etc.) (Schreiber et al., 2005; Yang & Bernards, 2006a) through the up-regulation of suberin-related genes (*StKCS20*-like, *StFAR3*, *StCYP86A22* and *StPOD72*-like genes), which are differentially expressed in skin (Vulavala et al., 2017). Periderm is further divided into native and wound periderm, where the former is naturally formed during tuber development, whereas the latter is generated only on occasions where tubers are subjected to damage and skin lesions are observed (Sabba & Lulai, 2002). Both periderm types contain a high amount of lipids, hence their hydrophobic nature, although they differ in water permeability, with wound periderm being more permeable than the native one (Schreiber et al., 2005; Yang & Bernards, 2006a). The chemical composition of periderm is evolving with tuber maturation as the cell walls of phellogen cells become thicker and stronger, while in phellem cells suberin is biosynthesized and waxes are deposited, so that altogether the periderm tissues provide the required protection for tubers from external factors (Lulai & Freeman, 2001).

The diversity in colour patterns among the tubers of various genotypes is associated with the accumulation of pigments in the periderm and the peripheral tissues of tuber cortices (Zhao et al., 2015). The skin may have red, purple and white colour, and the most common pigments isolated from tubers with coloured skin are anthocyanins (Fogelman et al., 2015). These pigments are located in the vacuoles and colour stability and saturation depend on various factors such as co-pigmentation, the interactions with metallic ions, the pH conditions of the soil and the presence of peroxidases in the vacuoles (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009).

Agronomic factors may also affect the quality and development of skin. For example, Vulavala et al. (2016) reported that Si fertilization may delay skin maturation, increase skin cell area and

upregulate the biosynthetic genes of suberin, resulting in enhanced suberization and lignification and improved resistance to skin injuries and storage ability of tubers. Skin set may be accelerated in early potato crops by applying vine killing through mechanical, physical or chemical means, allowing for earlier harvest without increased mechanical wounding incidence that immature skin could cause (Boydston, Navarre, Collins, & Chaves-Cordoba, 2018; Lulai, 2002; Renner, 1991; Waterer, 2007). Farming systems have been also reported to affect skin colour; for instance, according to Lombardo, Pandino, & Mauromicale (2017), organic farming may enhance skin colour of Arinda cultivar. Moreover, light exposure and wounding after harvest may exhibit synergistic effects on the accumulation of steroidal glycoalkaloids in tuber skins (Nie et al., 2019), while the application of nanosilica during storage may inhibit sprouting and α-solanine content, which is associated with harmful effects on human health (Zhang et al., 2018). However, glycoalkaloids content may be affected not only by post-harvest conditions but also by farming operations, growing and environmental conditions and genetic factors (Friedman, Roitman, & Kozukue, 2003; Knuthsen, Jensen, Schmidt, & Larsen, 2009; Sotelo & Serrano, 2000).

Another agronomic factor that may affect the quality of potato peels is the irrational use of pesticides and herbicides during cultivation, which may result in excessive amounts of residues of organophosphorous and other compounds that may pose animals and consumers' health to chronic cumulative risks (Quijano, Yusà, Font, & Pardo, 2016; Soliman, 2001; Tan et al., 2020; Witczak, Pohoryło, Abdel-Gawad, & Cybulski, 2018). Although these residues are in most cases accumulated in tuber peels which are discarded during domestic or industrial processing (Dong, Bian, Liu, & Guo, 2019; Keikotlhaile, Spanoghe, & Steurbaut, 2010; Soliman, 2001), the use of agrochemicals and the irrational disposal of agroindustry wastewaters may put in danger the health of animals when these peels are used as animal feed or even the human health

when the potato peels are intended for food applications (Kennedy, Garthwaite, de Boer, & Kruisselbrink, 2019; Narenderan & Meyyanathan, 2019). Therefore, the food industry is very concerned about this issue and is always seeking for advanced analytical techniques that allow for easy and accurate detection of such residuals and their fate in the food chain (Chen, Wang, Liu, & Bian, 2020; Keikotlhaile et al., 2010; Tan et al., 2020). The compliance of potato growers with regulations and best practice guides related with the proper use of agrochemicals, as well as the implementation of organic farming systems, are pivotal for the production of residual free potato tubers and peels (Kazimierczak, Srednicka-Tober, Hallmann, Kopczynska, & Zarzynska, 2019). Moreover, the management of bioconcentration factor of pesticides in root vegetables such as potato through the calculation of plant uptake and soil concentrations is of major importance for the crop safety from pesticide residuals (Hwang, Zimmerman, & Kim, 2018).

1.4.3.2. Nutritional value and chemical composition of potato peels.

PP have an important nutritional value, mainly composed of starch, dietary fibre and protein (Jeddou et al., 2017). The metabolites profile in PP peels shows a great variability depending on the genotype (Inostroza-Blancheteau et al., 2018). The most abundant macronutrients in PP are carbohydrates, accounting for 69 – 88 g/100g dw (**Table 4**). Starch is responsible for 30-52% dw of its total carbohydrates content (Arapoglou, Varzakas, Vlyssides, & Israilides, 2010; Ramaswamy, Kabel, Schols, & Gruppen, 2013). PP are also a rich source of dietary fibre (Jeddou et al., 2016), which ingestion can exert benefits to human health, such as cholesterol-lowering effect and improved diabetic control (soluble dietary fibre), besides intestinal health regulation (insoluble dietary fibre) (Jeddou et al., 2017). In a recent report, Elkahoui et al. (2018) studied the total dietary fibre content of organic and non-organic PP of the Russet

variety and described similar amounts in both farming systems (21.4% and 22.39% dw, respectively). On the other hand, Jeddou et al. (2017), employing a gravimetric enzymatic method, reported higher dietary fibre contents, focusing on the variety Spunta and a commercial unknown one. The authors found similar results for both genotypes, with the PP of Spunta and the commercial variety presenting 19.59% and 19.23% of insoluble dietary fibre, respectively, and 10.14% and 9.21% of soluble dietary fibre, respectively. An even higher total dietary fibre content of 51% was reported by Kumari *et al.* (2017) when studying PP from the Lady Claire variety. Regarding soluble sugars, the total content in PP is low, ranging around 0.9-1.0% dw (Amado, Franco, Sánchez, Zapata, & Vázquez, 2014; Arapoglou et al., 2010), while fermentable reducing sugars account for only 0.6% dw (Arapoglou et al., 2010). Some authors have reported the detection of glucose, fructose, galactose, rhamnose, arabinose and sucrose in PP, with glucose appearing as the most abundant soluble sugar (Choi, Kozukue, Kim, & Friedman, 2016; Jeddou et al., 2016). In particular, Choi et al. (2016) analysed the PP from six different Korean potato genotypes (Superior; Atlantic; Goun; K1, K20 and K30) and soluble sugars content ranged as follows: glucose 566-723 mg/100 g dw; fructose 433-683 mg/100 g dw; and sucrose 290-427 mg/100 g dw. In another study, Jeddou et al. (2016) reported the following monosaccharide composition in PP of the Spunta variety (expressed in percentages): glucose 76.25%, galactose 3.84%, rhamnose 0.51% and arabinose 0.19%. Jeddou et al. (2016) investigated the physicochemical properties of water-soluble polysaccharides from PP waste. The extracts that were rich in polysaccharides showed high water-holding and fat-binding capacities of 4.097 ± 0.537 (g H_2O/g) and 4.398 ± 0.04 (g oil/g), respectively. More recently, the same authors performed enzymatic hydrolysis of water-soluble polysaccharides from PP waste, generating low molecular weight oligosaccharides; the extracts rich in oligosaccharides showed good foaming and emulsion properties (Jeddou et al., 2018).

Protein is the second most abundant macronutrient in PP, although the content varies widely among studies, ranging from 2 to 17 g/100 dw **(Table 4).** Choi et al. (2016) studied the nutritional protein composition of PP, and reported a total crude protein concentration of 9.52-10.58 g/100 g dw, an essential amino acids content of 429-666 mg/100 g dw, a total free amino acid level of 1383-2077 mg/100 g dw, and an asparagine range of 90.4-115.8 mg/g dw.

Even among different varieties, the fat content of PP is very low, with a few authors reporting the absence of fat and others values up to 2.6 g/100 g dw **(Table 4)**. When studying a variety cultivated in China, Wu et al. (2012) detected two unsaturated fatty acids in PP, namely an omega-3 (9,10,11-trihydroxy-12(Z),15(Z)-octadecadienoic acid) and an omega-6 (9,10,11-trihydroxy-12(Z)-octadecenoic acid). Regarding other interesting components, the same authors reported the presence of quinic acid in the range of 0.63 to 0.71 mg/g dw, as quantified by UPLC–ESIMS (Wu et al., 2012); this acid has been indicated to be helpful in the treatment of diabetes, by promoting insulin secretion from pancreatic beta cells (Heikkilä, Hermant, Thevenet, & Domingo, 2019). The presence of ascorbic acid in PP has also been reported, in a concentration of 1.44 ± 0.5 mg/g dw for the Canadian cultivar Russet Burbank (Singh et al., 2011).

From the few reports available in the literature, PP appear as a rich source of nutritional compounds, particularly dietary fibre, making it a suitable candidate for incorporation in the human diet to improve foods' nutritional value. Nevertheless, there are variations in PP nutritional and chemical composition which may be attributed to various factors including varietal differences besides agronomic and environmental factors (Kumari et al., 2017).

Table 4. Nutritional value (mean values ± standard deviation) of *Solanum tuberosum* peels, as reported in the literature over the last ten years. Values are expressed in g/100 g dw.

	Potato peel variety and origin				
Proximate	**Organic Russet** United States	**Non-organic Russet** United States	**Red potato** United States	**Gold potato** United States	**Lady Rosetta** Ireland
Moisture	3.67	3.78	4.46	5.66	6.98 ± 0.05
Carbohydrates	76	71	72	70	72.53 ± 0.08
Protein	11.98	17.19	15.99	14.17	11.17 ± 0.03
Fat	1.12	1.1	0.81	1.17	2.09 ± 0.01
Ash	7.32	7.34	6.69	9.12	7.24 ± 0.02
References	(Elkahoui *et al*., 2018)	(Elkahoui *et al*., 2018)	(Elkahoui *et al*., 2018)	(Elkahoui *et al*., 2018)	(Kumari *et al.*, 2017)

	Potato peel variety and origin			
Proximate	**Lady Claire** Ireland	**Spunta** Tunisia	**Agria** Spain	**Unknown variety** Greece
Moisture	4.08 ± 0.04	7.3 ± 0.3	7.30 ± 0.23	
Carbohydrates	77.38 ± 0.65	88.0 ± 4.4	86.97 ± 0.43	68.7
Protein	12.44 ± 0.09	2.099 ± 0.105	6.47 ± 0.23	8
Fat	1.27 ± 0.38	0.733 ± 0.037	0	2.6
Ash	4.83 ± 0.13	0.906 ± 0.006	5.46 ± 0.17	6.34
References	(Kumari *et al.*, 2017)	(Jeddou *et al.*, 2016)	(Amado *et al.*, 2014)	(Arapoglou *et al.*, 2010)

1.4.3.3. Phytochemicals in potato peels.

PP are also described as being a rich source of phenolic compounds, which have been related to human health benefits, including antioxidant and antimicrobial properties (Silva-Beltrán et al., 2017). The content of phenolic compounds in PP is up to ten times higher than in potato flesh (Albishi, et al., 2013; Rytel et al., 2014; Wu et al., 2012). Additionally, some phenolic compounds present in tuber peel are only rarely detected in flesh, such as caffeic acid, coniferyl alcohol, coniferyl aldehyde, vanillin, vanillic acid, ferulic acid and *p*-coumaric acid, all of which are linked to the plant defense mechanism against pathogen attacks (Oertel et al., 2017).

Literature data show that the total amount of phenolic compounds in PP varies significantly among different varieties (Albishi et al., 2013). Nevertheless, chlorogenic acid and its isomers appear as the major molecules determined in most of the studies, followed by caffeic acid **(Table 5)**. The analysis by HPLC-DAD-ESI-MS showed that chlorogenic acid accounted for 49.3–61% of the total phenolic compounds in a study conducted by Riciputi et al. (2018). Moreover, when stored at room temperature and exposed to direct light, chlorogenic acid can be partially degraded to caffeic acid, which may explain why in some studies caffeic acid appears as the major phenolic compound, since industrial PP waste is usually stored in the open without coverage from sunlight (Wijngaard, Ballay and Brunton, 2012). Other reported phenolic compounds in PP include ferulic and *p*-coumaric acids, rutin, quercetin and catechin **(Table 5)**.

Table 5. Contents of some phytochemicals (mean values ± standard deviation) of *Solanum tuberosum* peels as reported in the literature during the last ten years

	Potato peel variety and origin						
Phenolic compounds	**Bintje** Italy (mg/g dw)	**Challenger** Italy (mg/g dw)	**Non-organic Russet** United States (µg/g dw)	**Chaqueña** Andes (µg/g fw)	**Santa María** Andes (µg/g fw)	**Organic Russet** United States (µg/g dw)	**Organic Yukon gold** United States (µg/g dw)
Chlorogenic acid	1.97 ± 0.02	1.27 ± 0.01	6422	119 ± 7.83	497 ± 33.4	7810 ± 870	1890 ± 100
Caffeic acid	0.24 ± 0.001	0.22 ± 0.002	215	17.2 ± 0.95	34.8 ± 3.17	531 ± 771	365 ± 221
Ferulic acid	0.06 ± 0.0002	0.05 ± 0.001	-	-	-	-	-
p-Coumaric acid	-	-	-	-	-	-	-
Rutin	-	-	-	-	-	-	-
Quercetin	-	-	-	-	-	-	-
Catechin	-	-	-	6.17 ± 1.07	20.0 ± 3.10	-	-
	(Riciputi *et al.*, 2018)	(Riciputi *et al.*, 2018)	(Elkahoui *et al*., 2018)	(Valiñas *et al*., 2017)	(Valiñas *et al*., 2017)	(Friedman *et al*., 2017)	(Friedman *et al*., 2017)

	Potato peel variety and origin						
Phenolic compounds	**Fianna** (acidified ethanol extract) Mexico (mg/100g dw)	**Fianna** (water extract) Mexico (mg/100g dw)	**Unknown variety** Taiwan (mg/100 g dw)	**Innovator** The Czech Republic (mg/100 g fw)	**Blaue Elise** The Czech Republic (mg/100 g fw)	**Valfi** The Czech Republic (mg/100 g fw)	**Bore Valley** The Czech Republic (mg/100 g fw)
Chlorogenic acid	346.03 ± 2.14	159.99 ± 1.05	19.5 ± 0.7	91.3 ± 1.20	248.8 ± 2.10	60.1 ± 1.60	174.3 ± 2.42
Caffeic acid	332.58 ± 3.67	56.99 ± 3.23	14.3 ± 0.73	38.0 ± 1.43	3.82 ± 0.7	103.1 ± 2.52	30.0 ± 1.56
Ferulic acid	3.29 ± 0.05	-	11.2 ± 1.8	1.83 ± 0.24	0.13 ± 0.02	0.76 ± 0.05	0.07 ± 0.001
p-Coumaric acid	-	-	10.8 ± 0.1	-	0.02 ± 0.001	0.04 ± 0.001	0.05 ± 0.004
Rutin	5.01 ± 1.03	-	0.2 ± 0.1	-	-	-	-
Quercetin	11.22 ± 0.09	2.18 ± 0.07	4.2 ± 0.3	-	-	-	-
Catechin	-	-	0.9 ± 0.1	-	-	-	-
	(Silva-Beltrán *et al*., 2017)	(Silva-Beltrán *et al*., 2017)	(Hsieh *et al*., 2016)	(Rytel *et al.*, 2014)	(Rytel *et al.,*2014)	(Rytel *et al.,*2014)	(Rytel *et al.,*2014)

	Potato peel variety and origin						
Phenolic compounds	**Blue Congo** The Czech Republic (mg/100 g fw)	**Russet** Canada (mg/100 g freeze dried sample)	**Innovator** Canada (mg/100 g freeze dried sample)	**Yellow** Canada (mg/100 g freeze dried sample)	**Purple** Canada (mg/100 g freeze dried sample)	**Unknown variety** China (mg/g dw)	**Russet Burbank** Canada (mg/g dw)
Chlorogenic acid	122.8 ± 2.32	134.9	128.9	16.9	364.9	3.87 ± 0.04	1.35 ± 0.18
Caffeic acid	15.5 ± 0.40	98.5	109.4	29.7	92	2.23 ± 0.08	1.33 ± 0.06
Ferulic acid	0.09 ± 0.008	56.9	84.8	12.5	6.9	-	0.5 ± 0.02
p-Coumaric acid	-	-	5.3	2.6	7.4	-	-
Rutin	-	-	-	-	-	-	-
Quercetin	-	-	-	-	-	-	-
Catechin	-	-	-	-	-	-	-
	(Rytel *et al.,*2014)	(Albishi *et al*., 2013)	(Albishi *et al*., 2013)	(Albishi *et al*., 2013)	(Albishi *et al*., 2013)	(Wu *et al.*, 2012)	(Singh *et al.*, 2011)

Continued Table 5

	Potato peel variety and origin							
Glycoalkaloids	**Organic Russet** United States (μg/g dw)	**Non-organic Russet** United States (μg/g dw)	**Gold potato** United States (μg/g dw)	**Red potato** United States (μg/g dw)	**Organic Russet** United States (μg/g dw)	**Conventional Russet** United States (μg/g dw)	**Organic Yukon gold** United States (μg/g dw)	**Conventional Yukon gold** United States (μg/g dw)
α-Chaconine	593	781	1301	1604	1180 ± 110	424 ± 30	2830 ± 370	670 ± 130
α-Solanine	268	347	636	572	374 ± 54	215 ± 43	750 ± 120	253 ± 44
Solanidine	-	-	-	-	-	-	-	-
Demissidine	-	-	-	-	-	-	-	-
Total glycoalkaloids	861 ± 10	1128 ± 1	1940 ± 170	2180 ± 170	1550 ± 120	639 ± 52	3580 ± 390	920 ± 140
	(Elkahoui et al., 2018)	(Elkahoui et al., 2018)	(Elkahoui et al., 2018)	(Elkahoui et al., 2018)	(Friedman *et al*., 2017)	(Friedman *et al*., 2017)	(Friedman *et al*., 2017)	(Friedman *et al*., 2017)

	Potato peel variety and origin				**Potato peel variety and origin**			
Glycoalkaloids	**Organic Red** United States (μg/g dw)	**Conventional Red** United States (μg/g dw)	**Unknown** Ireland (μg/g dw)	**Anthocyanins**	**Purple Cloud No.1** China (mg/100 g fw)	**Red Cloud No.1** China (mg/100 g fw)	**Yunnan Potato 303** China (mg/100 g fw)	**Yunnan Potato 603** China (mg/100 g fw)
α-Chaconine	610 ± 110	1297 ± 56	873	Delphinidin	2.08 ± 0.08	0.98 ± 0.04	0.49 ± 0.01	0.97 ± 0.03
α-Solanine	239 ± 34	412 ± 24	597	Cyanidin	7.17 ± 0.11	1.95 ± 0.00	1.68 ± 0.01	0.93 ± 0.00
Solanidine	-	-	374	Petunidin	177.50 ± 1.52	1.05 ± 0.49	75.37 ± 0.50	0.40 ± 0.01
Demissidine	-	-	75	Pelargonidin	3.33 ± 0.07	143.05 ± 1.04	0.93 ± 0.02	118.32 ± 0.78
Total glycoalkaloids	850 ± 120	1709 ± 61	1919	Peonidin	56.97 ± 1.17	15.34 ± 0.21	23.16 ± 0.13	7.57 ± 0.05
				Malvidin	21.71 ± 0.16	-	12.58 ± 0.06	-
	(Friedman *et al*., 2017)	(Friedman *et al*., 2017)	(Houssain *et al*., 2015)		(Yin *et al.*, 2016)	(Yin *et al.*, 2016)	(Yin *et al.*, 2016)	(Yin *et al.*, 2016)

According to the available data, the phenolic acids content in PP is in generally higher than the flavonoids content, while high-anthocyanin varieties also have higher contents of chlorogenic acid (Valiñas, Lanteri, Have, & Andreu, 2017). Hsieh et al. (2016) quantified a total phenolic acids content of 86.3 mg GAE/100 g dw in PP aqueous extracts, whereas the flavonoids content accounted for 27.5 mg GAE/100 g dw, with caffeic, *p*-coumaric, chlorogenic and ferulic acids as the most prevalent compounds (Hsieh et al., 2016). The main phenolic acids found in PP are shown in **Figure 7**.

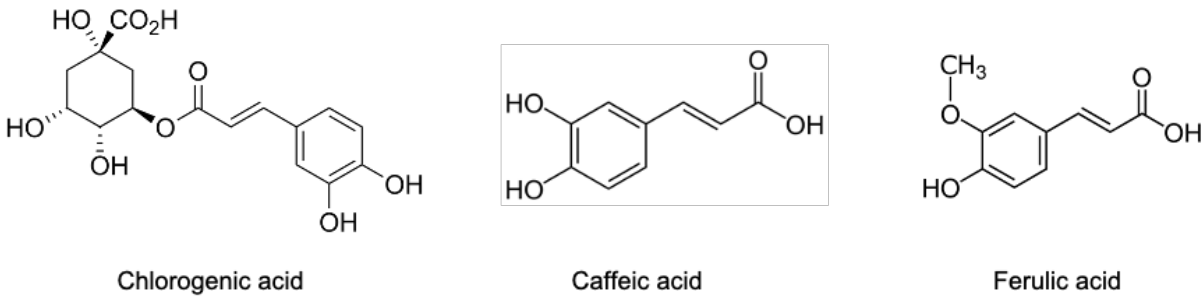


Figure 7. Main phenolic acids found in potato peels.

The difference in the phenolic compounds content among the existent studies can be in part attributed to the use of different extraction conditions, extraction solvents and methods. For instance, Silva-Beltrán et al. (2017) compared the phenolic composition of PP in aqueous and acidified ethanolic extracts, by applying maceration with constant stirring for 72 h. The acidified ethanolic extract showed higher total phenolics (14.0 mg GAE/g dw) and total flavonoids (3.3 mg quercetin equivalent/g dw) contents than the water extract (4.2 mg GAE/g dw, and 1.0 mg quercetin equivalent/g dw, respectively). The same was evidenced by Albishi et al. (2013) when studying the total content of free, esterified and bound phenolics in the peels from four different potato cultivars (Purple, Innovator, Russet and Yellow), by employing a solid-liquid extraction using methanol–acetone–water (7:7:6; v/v/v) as solvent. For the

varieties Russet and Innovator, bounded phenolics content (4.8 mg GAE/g dw and 5.3 GAE/g dw, respectively) was higher than the content in free phenolics (3.1 mg GAE/g dw and 3.2 mg GAE/g dw, respectively), which highlights the importance of including the analysis of bounded phenolics in analytical protocols in order to more accurately determinate the total phenolic compounds.

Among the different extraction techniques used to obtain these molecules, besides the traditional solid-liquid extraction, techniques such as ultrasound (Kumari et al., 2017; Paleologou, Vasiliou, Grigorakis, & Makris, 2016; Riciputi et al., 2018) and microwave-assisted treatments (Singh et al., 2011) can also improve the extraction efficiency of phenolic compounds from PP. By applying an ultrasound-assisted extraction method, Kumari et al. (2017) reported a significantly higher recovery rate of phenolic compounds compared to solid–liquid extraction process alone. These authors also compared different solvent combinations, i.e. pure distilled water, pure methanol, 80% methanol/water and 50% methanol/water (v/v). According to the results, the 80% aqueous methanol solvent was the best combination for the extraction of phenolic compounds from PP, whereas the ultrasonic treatment at 33 kHz was more effective in recovering polyphenols compared to higher frequency (42 kHz).

On the other hand, Singh et al. (2011) applied microwave-assisted extraction, combined to a response surface methodology study to optimize parameters such as extraction time, solvent concentration and microwave power. In regard to total phenolics, the maximum content (3.94 mg/g dw) was obtained when the extraction time was 15 min, the used solvent 67.33% methanol/water and the microwave power 14.67%. Optimal concentrations of caffeic (1.33 mg/g dw) and ferulic acids (0.50 mg/g dw) were achieved at 15 min, 100% methanol and 10% microwave power, whereas the maximum chlorogenic acid content (1.35 mg/g dw) was obtained at a shorter extraction time (5 min), whilst maintaining similar conditions for the rest

of the extraction parameters (100% methanol and 10% microwave power). This indicates that chlorogenic acid might be more sensitive than the other compounds to the temperature reached as a result of the microwave power levels. Overall, the authors evidenced that microwave assisted extraction exhibited a better efficiency in extracting phenolic compounds from PP than conventional methods, by comparing their results (total phenolics of 3.94 ± 0.21 mg/g dw) to previously reported literature based on solid-liquid extraction (total phenolics 1.51 ± 0.17 − 3.31 ± 0.12 mg/g dw).

As already exemplified, the solvent choice can also play a pivotal role in the efficiency of phenolic compounds extraction from PP. Solvents used include ethanol/water (Paleologou et al., 2016; Riciputi et al., 2018), methanol/water (Kumari et al., 2017), water/glycerol (Paleologou et al., 2016), acetone (Zia-ur-Rehman, Habib, & Shah, 2004) and diethyl ether (Zia-ur-Rehman et al., 2004). Nevertheless, greener solvents such as glycerol and ethanol are currently preferred over other suggested options for safety and environmental reasons.

An important class of compounds that can be obtained from PP is anthocyanins, which are responsible for the colours of purple and red-fleshed potato varieties (Albishi et al., 2013; Mori et al., 2010; Oertel et al., 2017; Rytel et al., 2014; Yin et al., 2016). Potatoes with coloured flesh usually have identically coloured skin; however, the colours of PP and flesh do not always match, as some yellow-fleshed varieties also present red or purple-coloured skin (Pathak et al., 2018).

Albishi et al. (2013) compared the content of phenolic compounds in PP of four different cultivars, three of which were yellow-fleshed, and one was purple-fleshed. The PP of the purple-fleshed genotype contained the highest amount of free (7.2 mg GAE/g dw) and esterified (4.74 mg GAE/g dw) phenolic compounds among all the four studied varieties. Moreover, in the purple potato genotype, pigments were mostly accumulated in the skin and

the outer cortices of tubers, being the content of anthocyanins in the peel 10.69 times higher than in the flesh.

Oertel et al. (2017) used UPLC coupled to UV and mass spectrometry detection to analyse the anthocyanins and polyphenol profile of potato tuber tissues (flesh and skin) from fifty-seven coloured potato cultivars. They found that the potato genotypes could be grouped according to their anthocyanin accumulation patterns. Eighteen anthocyanin profiles were identified, comprising six groups derived from red and twelve from blue and purple cultivars. Acylated pelargonidin glycosides were confirmed as the main pigments in red tubers, especially pelargonidin-3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside. For blue and purple tuber tissues, petunidin 3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside and malvidin 3-*O*-feruloylrutinoside-5-*O*-glucoside were the most abundant anthocyanins, the latter being more abundant in the skin than in the flesh. In general, a higher concentration of malvidin derivatives was detected in peels compared to flesh of purple and blue potato genotypes. The antibacterial effect of malvidin has been linked to the plant defence mechanism against pathogenic bacteria, which may explain the higher concentration in the peel as the outer barrier of tubers from external aggressions.

Yin et al. (2016) studied ten coloured potato cultivars from China (Purple Cloud No. 1, Red Cloud No. 1, Yunnan Potato 303, Yunnan Potato 603, S03-2677, S03-2685, S03-2796, S05-603, S06-277 and S06-1693), by applying an ultrasonic treatment, with 95% ethanol:1.5 M hydrochloric acid (85:15, v/v) as solvent, followed by HPLC analysis. For all the analysed cultivars, anthocyanin and phenolic content and antioxidant activity were significantly higher in the PP than in the flesh. Six anthocyanins were detected in the coloured PP, with delphinidin and peonidin being present in all the studied cultivars. The other detected anthocyanidins were petunidin, malvidin and cyanidin.

In summary, as a bio-residue of the pigmented potato processing industry, the coloured PP could be used to obtain added value extracts rich in polyphenols for further applications as natural food colouring agents or preservatives, given the antioxidant and antimicrobial properties also associated to these compounds (Yin et al., 2016).

Another group of compounds found predominantly in PP are the glycoalkaloids, which are mainly represented by the molecules α-solanine and α-chaconine (**Figure 8**) (Andreas Schieber & Aranda, 2009). The function of these compounds is to protect the plant against bacterial, fungal and insect attacks (Hossain et al., 2015). Glycoalkaloids can exert some level of toxicity to humans, but are safe for ingestion at levels of about 1-2 mg/kg of body weight (Elkahoui et al., 2018).

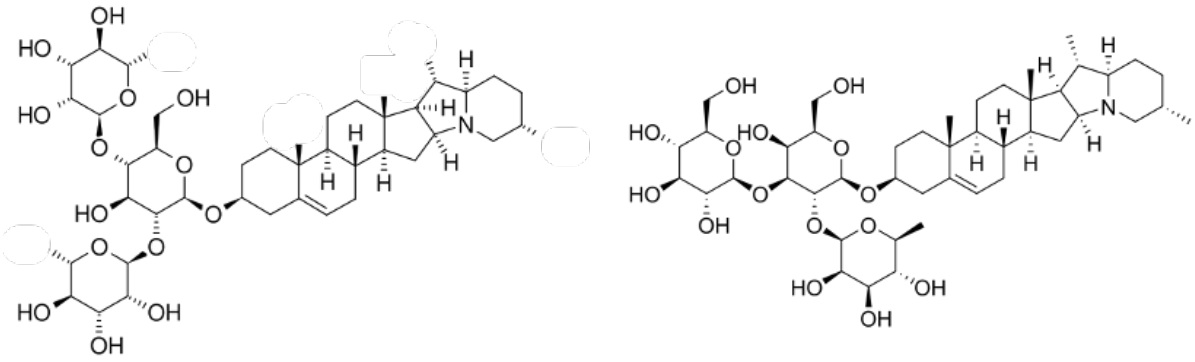


Figure 8. Main glycoalkaloids found in potato peels.

Most of the commercially available potato genotypes contain less than 10 mg/100 g fw, being 20 mg/100 g fw the suggested upper threshold (Rytel et al., 2018). Nevertheless, recent studies have indicated that these compounds may also present beneficial bioactivities to human health, such as antibacterial, anticancer, anti-inflammatory and antiobesity effects (Elkahoui et al., 2018; Friedman et al., 2018; Hossain et al., 2015). Commonly, α-chaconine is the most

abundant glycoalkaloid found in PP (**Table 5**), and it is also reported to be up to five times more bioactive than α-solanine (Friedman, Kozukue, Kim, Choi, & Mizuno, 2017).

Friedman et al. (2017) compared the α-solanine, α-chaconine and total glycoalkaloids contents in PP from organic and non-organic commercial gold, Russet and red potatoes, by applying 5% acetic acid extraction accompanied by ultrasonication. As described in **Table 5**, the PP from organic-grown gold and Russet varieties showed a higher content of glycoalkaloids (3580 and 1550 μg/g dw, respectively) compared to the non-organic samples (920 and 639 μg/g dw, respectively). For the red variety the opposite trend was observed, as the organic samples revealed lower content of glycoalkaloids (850 μg/g dw) than the conventionally grown samples (1709 μg/g dw). On the other hand, Elkahoui et al. (2018), in a study on the same varieties, found the highest glycoalkaloid contents in PP from the conventionally grown red variety (2180 μg/g dw).

Hossain et al. (2015) compared the extraction of glycoalkaloids by pressurized liquid extraction (PLE) and conventional solid-liquid extraction. The authors found that PLE was the most effective technique, with results for individual steroidal alkaloids of 597, 873, 374 and 75 μg/g dried potato peel for α-solanine, α-chaconine, solanidine (the alkaloidal aglycone) and demissidine (the solanidine dihydrogenated form), respectively. Corresponding values for solid liquid extraction were 247, 474, 224 and 36 μg/g dw, respectively (i.e., 59%, 46%, 40% and 52% lower than PLE method). The PLE parameters were also optimized by a response surface methodology, being 80°C and 89% methanol the optimal temperature/extracting solvent combination.

1.4.3.4. Bioactive properties of potato peels.

PP extracts have been shown by many studies to exert antioxidant activity, through the scavenging of several reactive oxygen species/free radicals under *in vitro* conditions. The antioxidant activity in PP extracts has been screened by different assays, such as the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging capacity (Jeddou et al., 2018; Singh et al., 2011; Singh & Rajini, 2004), reducing power (Jeddou et al., 2018; Singh & Rajini, 2004), β-carotene bleaching inhibition activity (Jeddou et al., 2018), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity (Jeddou et al., 2018), lipid peroxidation in rat liver homogenates (Singh & Rajini, 2004) and iron ion chelation (Singh & Rajini, 2004). In some studies, PP extracts demonstrated significantly higher radical scavenging activity ($p < 0.05$) than the respective flesh extracts (Albishi et al., 2013; Wu et al., 2012).

Jeddou et al. (2018) found promising results when investigating the antioxidant capacity of PP low molecular weight oligosaccharides, obtained by enzymatic hydrolysis of water-soluble polysaccharides from PP waste, by different *in vitro* methods: DPPH radical-scavenging capacity (IC_{50} = 2.5 mg/mL), reducing power (OD: 0.622 ± 0.032 at a concentration of 20 mg/mL), β-carotene bleaching inhibition activity (45.335 ± 3.653%), and ABTS radical scavenging activity (14.835 ± 0.1%). Previously, it was already shown that PP extracts had strong antioxidant activity similar to the synthetic antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), suggesting them as potential natural antioxidants for oils, fats and other food products (Zia-ur-Rehman, Habib and Shah, 2004). Indeed, in the study by Jeddou et al. (2018), oligosaccharide-rich PP extracts exhibited the same DPPH radical scavenging activity as the control BHA, both at the same concentration of 10 mg/mL (Jeddou *et al.*, 2018).

In most studies, a significant positive correlation was also found between the antioxidant activity and the phenolic content of PP extracts (Albishi et al., 2013; Singh et al., 2011; Singh & Rajini, 2004). It has also been reported that bounded and esterified phenolic compounds can contribute as much or even more than the free phenolics to the antioxidant activity of PP (Albishi et al., 2013). Moreover, PP from purple and red coloured varieties generally show higher antioxidant activity when compared to yellow-coloured varieties, which is commonly attributed to the higher content of anthocyanins present in colour-fleshed potatoes (Albishi et al., 2013).

The antioxidant activity of PP was also evidenced in an *in vivo* study carried out by Hsieh et al. (2016), who evaluated the effect of PP aqueous extracts (conventional solid-liquid extraction) on the toxicity of cholesterol oxidation products in rats. The rats fed with diets containing 2% and 3% of the PP extracts showed, after treatment, significantly increased ($p < 0.05$) liver glutathione and trolox equivalent antioxidant capacity levels, greater superoxide dismutase, catalase, and glutathione peroxidase activities, and also enhanced mRNA expression. Additionally, treated animals showed significantly decreased aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, creatinine, and cholesterol oxidation products' levels, when compared with the control rats.

PP extracts have also shown interesting microbiological and anti-parasitic potential. In a recent work, Friedman et al. (2018) investigated the *in vitro* antitrichomonad activity of PP powders prepared from commercial Russet, red, purple, and fingerling potato varieties against three pathogenic strains of trichomonads: one *Trichomonas vaginalis* strain (a sexually transmitted protozoan parasite that causes the human disease trichomoniasis) and two distinct strains of the related *Tritrichomonas foetus*, one feline and one bovine. Ethanol (1 mL, 50%)/acetic acid (0.5%) was used as solvent in a conventional solid-liquid extraction. Most of the PP samples

were active against these three trichomonad species at the concentration of 10%, w/v, with the two Russet samples being the most active against all three parasites. Additionally, and also importantly, PP did not show inhibitory activity toward several normal native vaginal bacteria species. Moreover, PP extracts have also demonstrated antibacterial activity toward *Escherichia coli* and *Salmonella Typhimurium* (Sotillo, Hadley, & Wolf-Hall, 1998).

In a study conducted by Silva-Beltrán et al. (2017), PP acidified ethanolic extracts showed antiviral effects against human enteric viruses. The PP extracts successfully inhibited the human enteric viral surrogates of Av-05 and MS2 bacteriophages. The authors performed a 3 hours incubation with PP extracts at a concentration of 5 mg/mL, which reduced the PFU/mL (plaque-forming unit per unit volume) of Av-05 and MS2 by 2.8 and 3.9 log10, respectively, in a dose-dependent manner. The results suggested that PP extract has potential to be used as an effective agent against human enteric viruses.

PP have also been studied for their anti-obesity and antidiabetic potential. For instance, the dietary supplementation of PP powder reduced weight gain in mice submitted to a high-fat diet, suggesting the potential of PP powder to serve as an anti-obesity functional food (Elkahoui et al., 2018). In this study, mice adipogenic high-fat diets (25% fat by weight) were supplemented with 10-20% PP powders for 21 days. In comparison to the control diet, the peel-containing diet induced a significant reduction in mice's weight gain by up to 73%. The positive results of PP powder as an anti-obesity agent were likely to be related to its high content of glycoalkaloids, since the weight gains of the mice were negatively correlated with both α-chaconine and α-solanine contents of the diet, as revealed by statistical analysis (Tukey test, coefficients of −0.81 and −0.80, respectively; $p < 0.05$).

There is an increasing interest among the scientific community to find out alternative treatments for type 2 diabetic patients since the commercially available drugs are reported to

cause various side effects (Arun et al., 2015). α-Glucosidase inhibitors act by retarding the liberation of glucose from dietary carbohydrates, reducing postprandial glucose levels, thus supporting the management of type 2 diabetes. In this context, Arun et al. (2015) compared the bioactivities of hexane, ethyl acetate and methanol PP extracts applying an α-glucosidase inhibition *in-vitro* assay, finding the methanolic extract as the most efficient one, exhibiting promising α-glucosidase inhibitory activity (IC_{50} value of 184.36 mg/mL).

1.4.3.5. Potato peels applications in food products.

A compilation of works found in the literature on potato peel applications in the food industry is presented in **Table 6.**

Table 6. Compilation of works found in the literature on potato peel applications in the food industry.

Application	Type of extract/product	Analyses carried out	Main findings	Reference
Bread	Fibre extracted from potato peel	Texture over shelf-life	Potato fibre was incorporated into bread (0.4 g fibre/100 g flour), reducing bread hardness over a 7 days storage period when compared to the control formulation.	Curti et al, 2016
Flatbread	Potato peel powder	Acrylamide content	5% potato peel powder was added to flour of a quinoa flatbread formulation, resulting in a significant decrease in acrylamide content (a potentially toxic compound) of baked flatbreads (from 487 to 367 μg/kg).	Crawford *et al.,* 2019
Cake	Potato peel powder	Texture and sensory analysis	Potato peel powders with high level of dietary fiber and protein were incorporated into cakes, resulting in improvement of nutritional value (higher dietary fibre content) and texture (cake hardness decrease by 30.24%). Sensory analysis results showed no statistic difference between enriched cakes and control.	Jeddou *et al.,* 2017
Soybean oil	Ethanolic potato peel extracts	Oxidative stability (peroxide, totox and *p*-anisidine indices)	Potato peel extracts were able to stabilize soybean oil under accelerated oxidation conditions (60 °C, 15 days), minimising oxidation products (peroxide, totox and *p*-anisidine indices).	Amado et al., 2014
Soybean oil	Petroleum ether potato peel extracts	Oxidative stability (free fatty acids, peroxide value and iodine value) over shelf-life	Potato peel extracts exhibited very strong antioxidant activity which was almost equal to synthetic antioxidants (BHA and BHT), retarding the development of oxidation products in soybean oil during a 60 days' storage period.	Rehman *et al.*, 2004
Soybean oil	Ethanolic potato peel extracts	Oxidative stability (peroxide, *p*-anisidine, conjugated dienes values, volatile compounds and fatty acids)	Extracts from potato peel were added to soybean oil, in the concentrations corresponding to chlorogenic acid levels of 14.01 ppm, 20.37 ppm and 31.94 ppm, and assessed under accelerated oxidation conditions. Enriched oil showed lower peroxide values, higher inhibition of hexanal production and higher overall oxidation stability compared to control untreated oil, for all tested concentrations.	Franco *et al*., 2016
Sunflower and soybean oils	Methanolic potato peel extracts	Oxidative stability (peroxide and *p*-anisidine values, conjugated dienes and conjugated trienes)	Potato peel methanolic extracts were added to sunflower and soybean oils, under accelerated oxidation conditions (70 °C, 72 hours). Results for both vegetable oils suggested a superior antioxidant activity of potato peel extracts (at the concentration of 100 ppm and 200 ppm) over synthetic antioxidants BHT and BHA at their legal limit.	Mohdaly *et al*., 2010

The first reports on the uses of PP in food applications date back from the 1970's, when Toma et al. (1979) described its use as a source of dietary fibre in bread. The authors pointed out several advantages of PP compared to wheat bran, such as mineral and total dietary fibre contents, the water-holding capacity, the lower quantity of starchy components, and the lack of phytate, which were maintained even after the baking process. They also reported increased crumb darkening and reduced loaf volume in bread which had wheat flour partially replaced by PP.

More recently, Curti et al. (2016) applied PP fibre in bread (0.4 g fibre/100 g flour) to study its ability to reduce bread stealing. The authors found that the potato fibre addition in bread increased frozen water content and resulted in a softer bread crumb over 7 days of storage. The values for crumb hardness at the end of the storage period were lower for the formulation with added PP compared to the control formulation (3.7 ± 0.6 N and 4.5 ± 0.7 N, respectively).

For their part, Crawford et al. (2019) incorporated PP in quinoa flatbreads with the aim of reducing acrylamide content, a potentially-toxic compound that is formed during thermal processing of cereals. PP powder from the Russet variety was incorporated into quinoa flour at 5%, resulting in a significant decrease of acrylamide content of the baked flatbreads compared to the control formulation (from 487 to 367 μg/kg). The results suggested that the PP supplemented quinoa flatbreads have the potential to serve as a nutritional, gluten-free, low-acrylamide functional food.

Besides bread, PP powder was also incorporated into cakes by Jeddou et al. (2017). In that study, wheat flour was substituted by 2%, 5% and 10% of PP powder of Spunta variety and a commercial unknown one. The PP powders showed high levels of dietary fibre and protein. The resulting cakes had overall improved texture, mainly due to the water binding and fat absorption capacities exhibited by PP powder. Cake hardness decreased by 30.24% for the 5% Spunta PP formulation compared with control cake. Moreover, the replacement of wheat flour

with PP powder increased dough strength and elasticity-to-extensibility ratio. A sensory analysis based on consumers' acceptance was carried out to compare fortified and control cake formulations, with results indicating no significant difference between them. In regard to nutritional value, the cake fortified with PP showed an increased soluble dietary fibre content compared to control (4.8% and 3.3%, respectively), and the same was observed for insoluble fibre content (21.4% and 15.9% respectively). Finally, the PP fortified cakes presented a darker colour compared to control, with higher a* values (red colour) and lower L* values (lightness). These changes in colour might be directly contributed by the colour attributes of PP powders, but also by a more pronounced non-enzymatic browning effect due to the sugar composition of the added PP. Other uses of PP powder include their incorporation in biscuits by substituting wheat flour which resulted in increased water absorption, dough development time and dough stability, while increasing antioxidant activities and fibre content at the same time (Hallabo, Helmy, Elhassaneen, & Shaaban, 2018).

Lipid oxidation is a free radical chain reaction that causes the reduction of shelf-life of many processed food products. With the aim of reducing rancidity and the production of toxic oxidation molecules, the addition of synthetic antioxidants to food products is one of the most effective strategies applied by the food industry. However, there is increasing concern by consumers about the safety of synthetic food additives, and a rising preference for natural alternatives (Carocho & Ferreira, 2013). Following this tendency, PP have been applied by some authors as antioxidants in the protection of soybean oil against lipid oxidation. Some measurable parameters can indicate the oxidation incidence, such as the production of peroxides (the primary products of lipid peroxidation), conjugated dienes, conjugated trienes and *p*-anisidine values.

Franco et al. (2016) found that the addition of PP ethanolic extracts to soybean oil reduced lipid oxidation indices (peroxide, conjugated dienes and *p*-anisidine values). In this study, soybean oil stability was determined under accelerated oxidation conditions (60 °C) over a 15 days storage period. Three concentrations of ethanolic extracts from PP of the Agria variety were assessed, according to chlorogenic acid levels (14.01 ppm, 20.37 ppm and 31.94 ppm). At the end of the storage period, control oil showed higher peroxide values (180.78 meq/kg oil) compared to the PP enriched oils (below 162 meq/kg oil). In regard to conjugated dienes, the oil treated with the highest concentration of PP (31.94 ppm chlorogenic acid equivalent) showed a higher inhibition percentage (52%) than the BHT-treated sample (41%). This indicates that the PP extracts showed higher ability to reduce the loss of polyunsaturated fatty acids than BHT. Finally, the *p*-anisidine values, which are related to secondary oxidation products (carbonyls), were higher in control samples than in the other treatments.

The same effect was evidenced by Amado et al. (2014), in a study on PP ethanolic extracts of Agria variety. These authors applied a response surface analysis in order to maximise antioxidant compounds extraction from PP (mainly chlorogenic and ferulic acids) and minimise oxidation indices in soybean oil. The PP extracts were able to diminish peroxide, total oxidation (totox) and *p*-anisidine indices under accelerated oxidation conditions (60 °C, 15 days). Oxidation indices ranged from 13.1 to 17.6 meq O_2/kg oil for *p*-anisidine (corresponding to 19.3% inhibition), 293.0 to 380.2 meq O_2/kg oil for totox (corresponding to 22.4% inhibition), and 139.6 to 182.1 meq O_2/kg oil for peroxide value (corresponding to 22.8% inhibition.

Similarly, Zia-ur-Rehman, Habib and Shah (2004) evaluated the ability of different PP extracts to protect a refined soybean oil from oxidation. They found that, after 60 days at 45 ºC, soybean oil containing 800, 1600 and 2400 ppm of the PP extract obtained with petroleum ether showed

lower free fatty acids (FFA) (0.176, 0.120, 0.109%, respectively) and peroxide values (POV) (40.0, 10.0, 9.0 meq/kg), and higher iodine values (IV) (69, 71, 77) than control samples (FFA 0.320%, POV 59 meq/kg, IV 58), an antioxidant efficiency nearly similar to that obtained with 200 ppm of BHA (FFA 0.102%, POV 8.0 meq/kg, IV 80) or BHT (FFA 0.078%, POV 6.0 meq/kg, IV 84). These results suggested that the addition of PP extract may be used to slow down the development of rancidity in soybean oil during storage instead of synthetic antioxidants, although the concentration of PP extract needed was about 8-12 times higher than that of BHT and BHA.

On the other hand, Mohdaly et al. (2010) compared the antioxidant properties of PP and sugar beet methanolic extracts in sunflower and soybean oils, under accelerated oxidation conditions (70 °C, 72 hours). At the end of storage time, the peroxide values of sunflower oil containing 200 ppm of PP, sugar beet pulp, *tert*-butyl hydroquinone (TBHQ), BHT and BHA were 11.9 ± 0.19, 13.8 ± 0.26, 11.0 ± 0.26, 13.4 ± 0.26 and 13.9 ± 0.24 meq/kg, respectively, whereas the control oil sample showed a much higher value of 25.8 ± 0.24 meq/kg. Regarding *p*-anisidine, maximum values of 9.10 ± 0.24, 9.40 ± 0.31, 9.42 ± 0.14, 9.36 ± 0.14, and 8.84 ± 0.14 were achieved at the end of storage for PP, sugar beet pulp, TBHQ, BHT and BHA containing samples, respectively, against 14.2 ± 0.14 reached in the control untreated sample. These results suggest a superior antioxidant activity of PP extracts over BHT and BHA, but lower than TBHQ. Similar positive results were obtained for the soybean oil samples, for which the antioxidant effect of PP extracts at 100 and 200 ppm was found to be higher than BHT and BHA at 200 ppm. Overall, the use of PP extracts in vegetable oils appears as a safe alternative of a natural antioxidant to inhibit lipid oxidation.

One study also reported the antioxidant activity of PP extracts on the storage stability of a fish-rapeseed oil mixture and a 5% oil-in-water emulsion (Koduvayur Habeebullah, Nielsen, &

Jacobsen, 2010). Ethanolic and aqueous extracts of PP from two Danish potato varieties (Sava and Bintje) were applied, and the antioxidant effect was assessed by peroxide and anisidine values, besides sensory evaluation. The obtained results showed that the PP ethanolic extract at a concentration of 2,400 mg/kg (particularly from the Sava variety) was 5.7 times more effective than BHT in reducing the peroxide value, and 9 times more effective in reducing anisidine value, whereas water extracts did not demonstrate antioxidant effect. In accordance, the results of the sensory analysis revealed that rancidity intensities were higher in oil with water extracts compared to oil enriched with ethanolic PP extracts.

Albishi et al. (2013) investigated the inhibition of oxidation in a fish meat model system by the incorporation of aqueous-methanolic-acetonic extracts from PP of four different varieties, i.e. Russet, Innovator, Purple and Yellow. The extracts were applied to retard the development of oxidative rancidity in cooked comminuted salmon over a 7-day storage period, and the results were assessed by the determination of 2-thiobarbituric acid reactive substances (TBARS). Russet, Purple, Innovator and Yellow PP extracts inhibited the formation of TBARS by 83.4%, 39.7%, 31.4% and 9.48%, respectively, at the end of the storage period.

Farvin, Grejsen and Jacobsen (2012) examined the use of PP as a source of natural antioxidants for retarding lipid and protein oxidation in another fish model, namely minced horse mackerel. The authors compared two different concentrations of PP extracts (2.4 or 4.8 g/kg) from the Sava variety, using both water and ethanol as solvents. The PP ethanolic extracts were the most effective in retarding lipid and protein oxidation, resulting in lower levels of peroxide values, volatiles and carbonyl compounds, compared to control and water extracts. For instance, at the end of the storage period, peroxide values for the addition of 2.4 and 4.8 g/kg ethanolic extracts were 0.58 and 0.41 meq/kg, whereas the control sample achieved 3.45 meq/kg, and the water extract sample an even higher peroxide value of 5.78 meq/kg. The PP ethanolic extracts also

showed a protective effect in fish meat against the loss of α-tocopherol, tryptophan and tyrosine residues. It was also found that the ethanolic extracts presented higher content of phenolic compounds than water extracts.

The suitability of PP extracts for controlling lipid oxidation in meat caused by radiation was investigated by Kanatt, Chander, Radhakrishna, & Sharma (2005). Ethanolic PP extracts were prepared from the Indian Kufri chandramukhi potato variety and added to lamb meat before irradiation (5 kGy, 4 mL of 1% PP extract in 100 g of meat). Lipid peroxidation was assessed in terms of thiobarbituric acid (TBA) number and carbonyl value. After 7 days of cold storage (0-3 °C), the TBA number was 54% lower for the meat containing PP extract in comparison to control. Similarly, the carbonyl content of PP-containing meat at the end of storage period was 30% lower than the corresponding sample not containing PP extract, demonstrating the potential of PP extracts in preventing lipid oxidation in meat products.

In another study, Mansour and Khalil (2000) assessed the potential of PP extracts (90% ethanol) in reducing lipid oxidation in ground beef patties, during cold storage (5 °C) for 12 days. The antioxidant activity of the PP extracts was assessed by TBARS, revealing 59.5% of inhibition relative to control. This activity was found to be maximum at pH ranging from 5.0 to 6.0. Moreover, a sensory analysis showed the extracts were able to control lipid oxidation and colour change of beef patties samples during cold storage, compared to control samples.

Juneja et al. (2018) studied the growth inhibition of *Bacillus cereus* in cooked rice by nine fruit and vegetable peel powders, including PP. The powders were mixed into rice at a concentration of 10% (w/w) along with four strains of *B. cereus*. Aliquots (5 g) of control cooked rice (without added peel powders) were cooled from 54.5 °C to 7.2 °C in 12, 15, 18, or 21 hours, resulting in 1.93, 2.82, 3.83, and 3.58 log cfu/g increases in *B. cereus* levels, respectively. For the rice samples added with organic gold PP powder, the results were 0.27, 0.53, 1.38 and 2.00

log cfu/g for the increase in *B. cereus* levels, under the same conditions as the control rice sample. These results demonstrated the potential of PP in retarding *B. cereus* outgrowth in food products

1.5 Objectives and working plan

The general objective of this work is the exploration of novel smart foods and the development of new food ingredients with functional activity, based on the following natural sources:

1) Brazilian quinoa (cv. BRS Piabiru);
2) Brazilian amaranth (cv. BRS Alegria);
3) Fifty potato (*Solanum tuberosum* L.) accessions from Europe, Asia, North and Latin America, including red and purple-fleshed varieties (flesh and peel).

With this aim, the following **specific objectives** were proposed:

1) to study the nutritional and physicochemical characterisation of the plant material (quinoa, amaranth and potato tubers);
2) to perform the extraction and characterisation of bioactive compounds from quinoa, amaranth and coloured potatoes flesh and peels (in particular phenolic compounds);
3) to evaluate the biological activity of the obtained extracts from quinoa, amaranth and coloured potatoes flesh and peels (antioxidant, antimicrobial, antitumor and anti-inflammatory activities);
4) to carry out the extraction and characterisation of colouring compounds from the red and purple potato tubers (anthocyanins);
5) to explore the application of the anthocyanin-based colouring extracts into food formulations;
6) to assess the stability and sensory properties of the colouring extracts.

1.5.1 Quinoa

A detailed nutritional and physicochemical characterization of the Brazilian quinoa cv. BRS Piabiru was performed and its phenolic compounds profile and bioactivity using cell-based assays (antioxidant and antimicrobial activities and cytotoxicity) has been described for the first time. This work intends to contribute to the Brazilian efforts towards the introduction of new crops into production systems, aiming at promoting agricultural diversification and contributing to reduce negative soil and environmental impacts.

1.5.2 Amaranth

A detailed chemical characterisation of the novel *Amaranthus cruentus* L. – BRS Alegria is herein presented for the first time. For this purpose, this pseudograin was evaluated in terms of macronutrients, free sugars, organic acids, tocopherols and fatty acids, as well as salt content, pH and the outer colour. Furthermore, the antioxidant, antibacterial and antifungal potentials of its hydroethanolic extract were determined. Finally, a cytotoxicity assay was employed to preliminarily assess the safety of the extracts, aiming at its potential application in food and pharmaceutical formulations. This study also intends to be a part of the Brazilian efforts towards the introduction of new crops into production systems, aiming at promoting agricultural diversification and contributing to reduce negative soil and environmental impacts.

1.5.3 Potatoes

The proposed working plan for the study of the fifty potato genotypes is summarised in **Figure 9**, and a detailed description of each study is presented below.

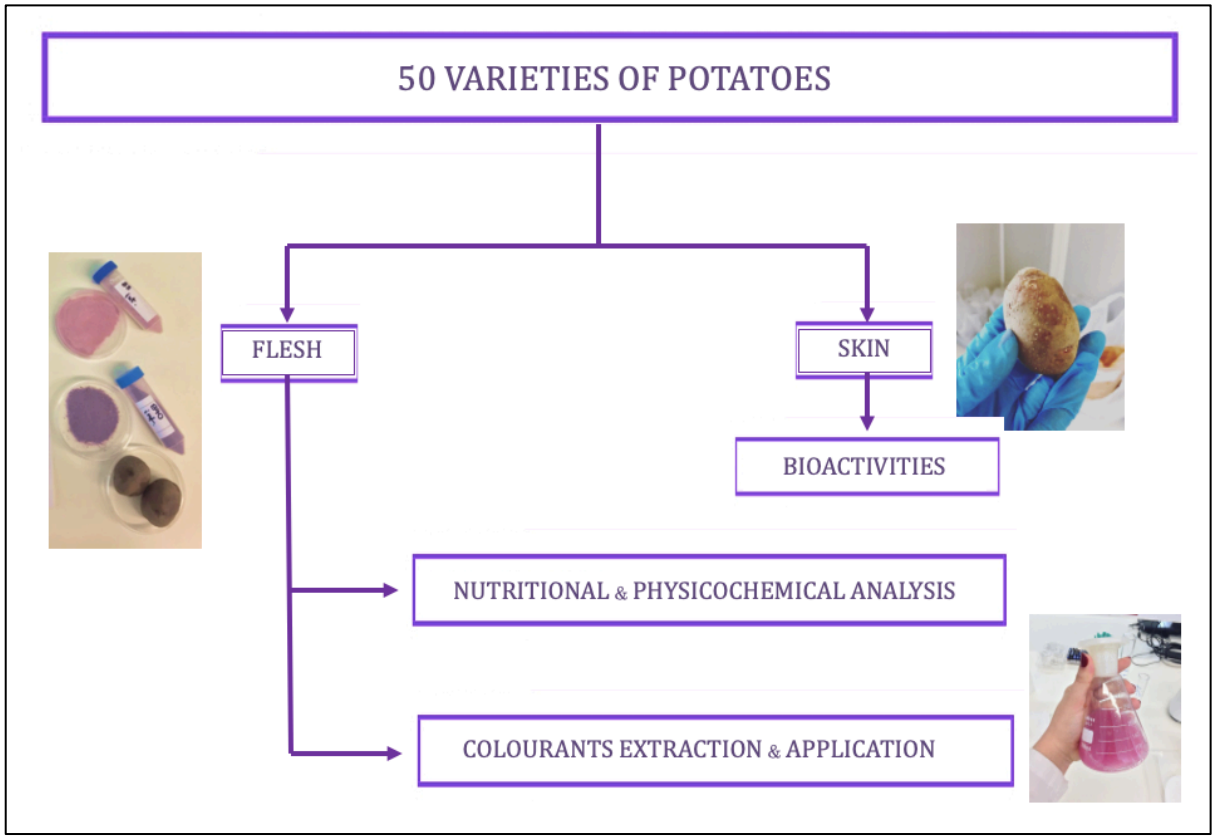


Figure 9. Working plan for the study of the fifty potato genotypes.

Nutritional and physicochemical analysis of fifty genotypes. The nutritional and physicochemical composition of potatoes depend on a number of factors, with variety being one of the most important. Several studies on the chemical composition of potatoes can be found in the literature, however the results usually relate to a limited number of genotypes, most of the time of the same area of origin. This work addresses this gap by comparing the nutritional and physicochemical characteristics of fifty potato varieties from different regions of the world. It comprises forty yellow/white-fleshed and ten coloured-fleshed genotypes (red, purple and marble-fleshed) classified into four different cultivation types (Andean accessions,

landraces, breeder lines and cultivated varieties). This characterization study aims to make available information to stimulate the commercial use of new and/or neglected varieties, ultimately improving food and nutrition security as well as promoting potato biodiversity and the sustainable reinforcement of the biodiversity of agricultural ecosystems.

Coloured potatoes. In this work an in-depth characterisation of the anthocyanin profile and the bioactivities (*in vitro* antioxidant, antibacterial and antifungal activities) of aqueous extracts from six varieties of red and purple-fleshed potatoes is performed, as well as a marble-fleshed cultivar being used for comparison purposes. Furthermore, a cytotoxicity assay to test the safety of the extracts was carried out, and then two varieties (one red and one purple) that presented the most promising results were selected for an application study by incorporating their extracts in a pasteurised soft drink model. Finally, the sensory profile of the products was assessed by quantitative descriptive analysis (QDA) and the colour stability of the produced soft drinks was monitored over a 30-day shelf-life period, comparing the performance of the extracts with the commercial colourant E163 (**Figure 10**).

Potato peels. A comprehensive assessment of the underexplored bioactive potential of ten potato peels, obtained from red and purple varieties of five different countries of origin (Chile, Germany, Austria, United Kingdom and Finland) has been carried out, with an in-depth characterisation of the phenolic composition of the peels, comprising phenolic acids, flavonoids and anthocyanins. Moreover, for the first time, the antioxidant capacity of potato peels was evaluated by applying two cell-based methods, namely the inhibition of lipid peroxidation through thiobarbituric acid reactive species (TBARS) and the oxidative haemolysis inhibition assay (OxHLIA), and the *in vitro* anti-inflammatory, antitumor and cytotoxic effect of the extracts were also assessed.

This work aims to make available valuable information to support the valorisation of this important food-industry bio-residue, particularly its underexplored potential as a natural additive in functional food formulations.

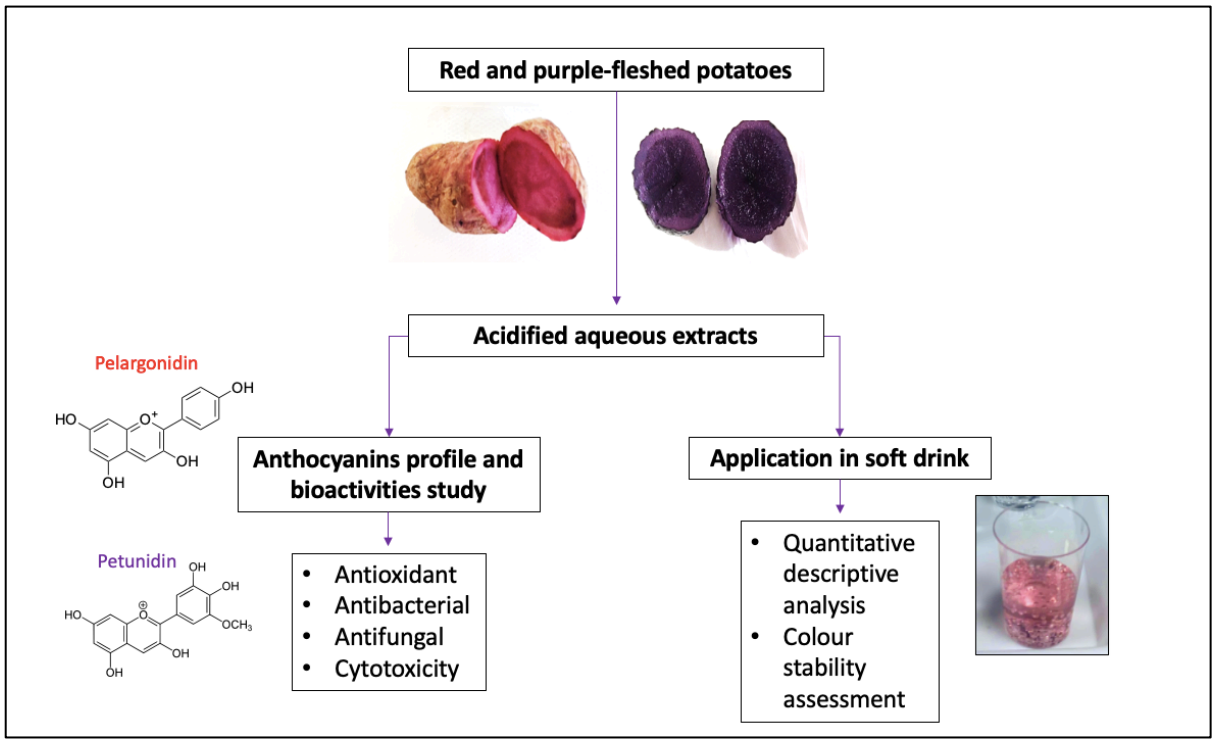


Figure 10. Working plan for the study of the coloured potato genotypes.

CHAPTER 2

Materials and Methods

2. CHAPTER 2: Materials and Methods

2.1 Standards and reagents

2.1.1 Chemical analysis

Acetonitrile, n-hexane and ethyl acetate were of HPLC grade from Fisher Scientific (Lisbon, Portugal). Fatty acids methyl ester (FAME) reference standard mixture (standard 47885-U) was purchased from Sigma-Aldrich (St. Louis, MO, USA), as well α- and δ-tocopherols, sugar and organic acid standards (oxalic acid, quinic acid, malic acid, shikimic acid, citric acid, succinic acid, and fumaric acid). Racemic tocol, 50 mg/mL, and β- and γ-tocopherols were purchased from Matreya (Pleasant Gap, PA, USA). All other general laboratory reagents were purchased from Panreac Química S.L.U. (Barcelona, Spain). Non-anthocyanin phenolic standards (apigenin-7-*O*-glucoside, caffeic acid, chlorogenic acid, hesperetin, isoliquiritigenin, isorhamnetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, naringenin, *p*-coumaric acid, quercetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside, catechin, ferulic acid, sinapic acid, syringic acid and myricetin) were from Extrasynthèse (Genay, France). Anthocyanin standards (cyanidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside; pelargonidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, malvidin-3-*O*-glucoside) were from Polyphenols (Sandnes, Norway). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

2.1.2 Antioxidant activity

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2- carboxylic acid, tris(hydroxymethyl) aminomethane (Tris) and 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.1.3 Antiproliferative activity and hepatotoxicity

Fetal bovine serum (FBS), L- glutamine, Hank's balanced salt solution (HBSS), trypsin-EDTA (ethylenediaminetetraacetic acid), penicillin/streptomycin solution (100 U/mL and 100 mg/mL, respectively), RPMI-1640 and DMEM media were purchased from Hyclone (Logan, UT, USA). Acetic acid, formic acid, ellipticine, sulforhodamine B (SRB), trypan blue, trichloroacetic acid (TCA) and Tris were purchased from Sigma-Aldrich (St. Louis, MO, USA). The Griess Reagent System Kit was purchased from Promega (Madison, WI, USA). Human tumor cell lines tested: MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma), and HepG2 (hepatocellular carcinoma) from DSMZ (Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH).

2.1.4 Antimicrobial activity

Dimethylsulfoxide (DMSO) (Merck KGaA, Germany) was used as a solvent in antimicrobial assays. The culture media Mueller Hinton broth (MHB) and tryptic soy broth (TSB) were purchased from Biomerieux (Marcy l'Etoile, France). The dye *p*- iodonitrotetrazolium chloride (INT) was purchased from Sigma-Aldrich (St Louis, MO, USA) to be used as a microbial growth indicator.

2.1.5 Anti-inflammatory activity

RAW264.7 cells were purchased from ECACC ("European Collection of Animal Cell Culture") (Salisbury, UK). DMEM medium from HyClone. Griess reagent system (Promega), Dimethyl sulfoxide (DMSO) and lipopolysaccharide (LPS) were obtained from Sigma-Aldrich Co. (Saint Louis, MO, USA).

2.2 Plant material and extracts preparation

2.2.1 Quinoa and amaranth samples

Samples of *Chenopodium quinoa* Willd. - BRS Piabiru and *Amaranthus cruentus* L. – BRS Alegria were provided by the company Harmony Bioseeds, in a partnership with the Brazilian Agricultural Research Corporation (EMBRAPA). The plants were grown in the city of Chapada Gaúcha, Minas Gerais state, Brazil. The fresh seed samples were freeze dried (-49 ºC, 0.08 bar, during 48 h, FreeZone 4.5 model 7750031, Labconco, Kansas, USA) and ground into a fine powder (20 mesh). The resulting powders were thoroughly mixed to obtain homogenized samples before analysis.

2.2.2 Hydroethanolic extracts preparation

30 mL of ethanol/water (80:20, v/v) was used to extract 1 g of freeze-dried sample. The extraction was performed twice in a magnetic stirrer plate (25 °C, 150 rpm, 1 h). The combined extracts were filtered (Whatman No. 4 paper) and vacuum-dried at 40 °C in a rotary evaporator (Büchi R-210, Flawil, Switzerland), until complete solvent removal. The obtained aqueous extracts were frozen and freeze-dried.

2.3 Potatoes

2.3.1 Potato samples

Potato seeds (tubers) from the fifty tested potato varieties (*Solanum tuberosum* L.) were purchased from the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) **(Table 7).** The genotypes were selected based on the tuber flesh and/or peel colour aiming to evaluate tubers with red and purple colours. The obtained tubers were cultivated in the experimental farm of the University of Thessaly in Velestino, Greece, according to commercial cultivation

protocols during the spring-summer growing period of 2018. Seed tubers were planted manually on 3/4/2018 with distances of 75 cm between hills and 30 cm within each hill and approximately 10 cm depth. Fertilizers were applied with base-dressing (400 kg/ha of 15-15-15 (N-P-K fertilizer) and two side-dressings (1st: 50 kg/ha of ammonium nitrate; 2nd 50 kg/ha of potassium nitrate). Irrigation was applied via a sprinkler irrigation system, while weeds and pests were controlled according to the best practice guides for the crop. Tubers were harvested manually on 27/7/2018. The fresh harvested tubers were transported to the Polytechnic Institute of Bragança, Portugal, where the samples were washed with cold water upon arrival to eliminate extraneous matter and refrigerated at 4 ºC until analysis.

Table 7. Description of the fifty studied potato genotypes: variety name, country of origin, colour of the tuber flesh and cultivation type.

Variety/accession name	Country of origin	Tubers' flesh colour	Cultivation type
Royal Andes	Chile	Yellow	*Andean accession*
UACH 0917	Chile	Purple	*Andean accession*
I-1039	India	Yellow	*Andean accession*
434.1	Peru	Yellow	*Andean accession*
Desiree	The Netherlands	Yellow	*Cultivated variety*
Odenwälder Blaue	Germany	Yellow	*Cultivated variety*
Rosemarie	Germany	Red	*Cultivated variety*
Rote Emmalie (Red Emmalie)	Germany	Red	*Cultivated variety*
Violetta (Blaue Elise)	Germany	Purple	*Cultivated variety*
Roswitha	Germany	Yellow	*Cultivated variety*
Burmania	The Netherlands	Yellow	*Cultivated variety*
Wohltmann	Germany	Yellow	*Cultivated variety*
Georgian	United States	Yellow	*na*
Rode Eersteling	The Netherlands	Yellow	*Cultivated variety*
Limba	Slovakia	Yellow	*Cultivated variety*
Peredowik	Russia	Yellow	*Cultivated variety*
Wiliya	Lithuania	Yellow	*Cultivated variety*
Ocew	Poland	Yellow	*Cultivated variety*
Rote Lötschentaler	Switzerland	Yellow	*Landrace*
R 93/25	Romania	Yellow	*Breeder's line*

Shetland Blau I	United Kingdom	Yellow	*Landrace*
Amyl	Czech Republic	Yellow	*Cultivated variety*
Cati	Romania	Yellow	*Cultivated variety*
Edzell Blue	United Kingdom	Yellow	*Cultivated variety*
Gondüzo	Hungary	Yellow	*Cultivated variety*
Hokkaiaka	Japan	Yellow	*Cultivated variety*
Montana	Germany	Yellow	*Cultivated variety*
Rosamunda	Sweden	Yellow	*Cultivated variety*
Victor	Spain	Yellow	*Cultivated variety*
Kefermarkter Blaue	Austria	Purple	*Landrace*
P 95/115	Uzbekistan	Yellow	*Breeder's line*
Ägyptische Rote	Egypt	Yellow	*na*
Atzimba	Mexico	Yellow	*Cultivated variety*
Early Ohio	United States	Yellow	*Landrace*
Red Cardinal	United Kingdom	Red	*Landrace*
Salad Blue	United Kingdom	Purple	*Cultivated variety*
Tannenzapfen	Germany	Yellow	*Landrace*
Teresa	Unknown	Yellow	*na*
Blaue aus Finnland	Finland	Purple	*Landrace*
Purple	Unknown	Purple	*na*
Creata	The Netherlands	Yellow	*Cultivated variety*
Heiderot (1977)	Germany	Yellow	*Cultivated variety*
Ijsselster	The Netherlands	Yellow	*Cultivated variety*
Lemin Punanen	Finland	Yellow	*Landrace*
Norland	USA	Yellow	*Cultivated variety*
Raudar Islenskar	Iceland	Yellow	*Landrace*
Herd Laddie	United Kingdom	Yellow	*Cultivated variety*
Shetland Black (Ellenb.)	United Kingdom	Marble	*Cultivated variety*
Geiger	Unknown	Yellow	*na*
Emma II	Germany	Yellow	*na*

2.3.2 Preparation for the nutritional and physicochemical analysis

Potato powders were prepared from the fresh tubers by hand-peeling followed by freeze-drying (-49 ºC, 0.08 bar, during 48 h, FreeZone 4.5 model 7750031, Labconco, Kansas, USA). The lyophilized samples were finally grinded into a fine powder (20 mesh) and mixed to obtain homogenized samples before analysis.

2.3.3 Acidified aqueous extraction of colouring compounds from the red and purple-fleshed varieties

Washed potato tubers were manually peeled and immediately immersed in an aqueous citric acid solution (0.5 mol/L) to prevent browning (1 g fresh tuber/2 mL citric acid solution). After the immersion in the citric acid solution, the whole peeled tubers were gently blended (commercial juice blender, Breville Blend Active model VBL134) and filtered twice (Whatman No. 4 paper). The obtained acidified aqueous extracts (pH ~3) were freeze-dried (-49 ºC, 0.08 bar, during 48 h, FreeZone 4.5 model 7750031, Labconco, Kansas, USA). All resulting extracts presented a yield of approximately 5% (mass of freeze-dried extract/mass of whole fresh potato). All steps in the extraction process were carried out in food grade facilities.

2.3.4 Hydroethanolic extraction of bioactive compounds from potato peels

A colour assessment of the samples was carried out before the peels being manually separated from the flesh. The peel samples were then freeze-dried (-49 ºC, 0.08 bar, during 48 h, FreeZone 4.5 model 7750031, Labconco, Kansas, USA) and reduced to a fine and homogeneous powder. The powdered freeze-dried peels were extracted twice with ethanol:water (80:20 v/v) under stirring for 1h and filtered (Whatman paper No. 4). The obtained solutions were evaporated under reduced pressure at 40°C (rotary evaporator Büchi R-210, Flawil, Switzerland) until total removal of the solvent. The obtained extracts were then freeze-dried.

2.4 Nutritional value and physicochemical characterisation

2.4.1 Proximate composition and energetic value

Protein, fat, carbohydrates and ash were determined following the AOAC procedures (AOAC, 2005, 2016). Crude protein content (N×6.25) was estimated by the macro-Kjeldahl method, using an automatic distillation and titration unit (model Pro-Nitro M Kjeldahl Steam Distillation System, Barcelona, Spain). This method is based on the amount of nitrogen present in a sample and relies on the destruction of all organic matter by addition of a strong acid (sulphuric acid) that retains nitrogen under the form of $(NH_4)_2SO_4$. Further addition of NaOH releases the nitrogen as NH_3 that is collected by steam distillation on a solution of 0.1N H_2SO_4; afterwards a titration with 0.1N NaOH using methyl red as an indicator is made to calculate the amount of nitrogen. Crude fat was determined using a Soxhlet apparatus by extracting a known weight of sample with petroleum ether. The ash content was determined by incineration at 550 ± 15 °C.

Total carbohydrates were calculated by difference, and total energy was calculated according to the following equation:

$$\text{Energy (kcal)} = (\text{protein mass (g)} + \text{carbohydrates mass (g)}) \times 4 + \text{fat mass (g)} \times 9$$

Equation 1. Equation for energy determination.

2.4.2 Physicochemical characterisation

Soluble sugars. Free sugars were determined by HPLC as described by Barros, Pereira et al. (2013). Dried sample powder (1.0 g) was spiked with melezitose as internal standard (IS, 5 mg/mL), and extracted with 40 mL of 80% aqueous ethanol at 80 ºC for 30 min. The resulting

suspension was centrifuged (Centurion K24OR refrigerated centrifuge, West Sussex, UK) at 15,000 g for 10 min. The supernatant was concentrated at 60 ºC under reduced pressure and defatted three times with 10 mL of ethyl ether, successively. After concentration at 40 ºC, the solid residues were dissolved in water to a final volume of 5 mL and filtered through 0.2 μm Whatman nylon filters. The HPLC equipment consisted of an integrated system with a pump (Knauer, Smartline system 1000, Berlin, Germany), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco, Easton, MD, USA) and an RI detector (Knauer Smartline 2300). Data were analysed using Clarity 2.4 Software (DataApex, Prague, Czech Republic). The chromatographic separation was achieved with a Eurospher 100-5 NH2 column (4.6 × 250 mm, 5 mm, Knauer) operating at 30 ºC (7971 R Grace oven). The mobile phase was acetonitrile/deionized water, 70:30 (v/v) at a flow rate of 1 mL/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was performed using the internal standard method (melezitose).

Organic acids. Organic acids were determined by applying a previously described methodology (Barros, Pereira, et al., 2013) and analysed using ultra-fast liquid chromatography coupled to a photodiode array detector (UFLC–DAD). Samples (approximately 2 g) were extracted by stirring with 25 mL of meta-phosphoric acid (25 ºC at 150 rpm) for 45 min and subsequently filtered through Whatman No. 4 paper. Before analysis, the sample was filtered through 0.2 μm nylon filters. The analysis was performed using a Shimadzu 20A series UFLC (Shimadzu Corporation, Kyoto, Japan). Separation was achieved on a SphereClone (Phenomenex, Torrance, CA, USA) reverse phase C18 column (5 μm, 250 mm × 4.6 mm i.d.) thermostatted at 35 ºC. The elution was performed with sulphuric acid (3.6 mM) using a flow rate of 0.8 mL/min. Detection was carried out in the DAD, using 215 nm and 245 nm (for

ascorbic acid) as preferred wavelengths. The organic acids were quantified by comparison of the area of their peaks recorded at 215 nm with calibration curves obtained from commercial standards of each compound: oxalic acid, ($y=9\times10^{6}x$; $R^2=0.99$); malic acid ($y=912441x$; $R^2=0.99$) and citric acid ($y=1\times10^{6}x$; $R^2=0.99$).

Fatty acids. Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GC–FID)/capillary column, after the extraction and derivatization procedures previously described by Barros, Pereira et al. (2013). Fatty acids (obtained after Soxhlet extraction) were methylated with 5 mL of methanol:sulphuric acid:toluene 2:1:1 (v:v:v), during at least 12 h in a bath at 50 °C and 160 rpm; then 3 mL of deionized water were added, to obtain phase separation; the FAME were recovered with 3 mL of diethyl ether by shaking in vortex, and the upper phase was passed through a micro-column of sodium sulphate anhydrous in order to eliminate the water; the sample was recovered in a vial with Teflon, and before injection the sample was filtered with 0.2 μm nylon filter from Whatman. The analysis was carried out with a DANI model GC 1000 instrument equipped with a split/splitless injector, a flame ionization detector (FID at 260 °C) and a Macherey- Nagel (Düren, Germany) column (50% cyanopropyl-methyl-50% phenylmethylpolysiloxane, 30 m × 0.32 mm i.d. × 0.25 μm df). The oven temperature programme was as follows: the initial temperature of the column was 50 °C, held for 2 min, then a 30 °C/min ramp to 125 °C, 5 °C/min ramp to 160 °C, 20 °C/min ramp to 180 °C, 3 °C/min ramp to 200 °C, 20 °C/min ramp to 220 °C and held for 15 min. The carrier gas (hydrogen) flow rate was 4.0 mL/min (0.61 bar), measured at 50 °C. Split injection (1:40) was carried out at 250 °C. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed

using the CSW 1.7 Software (DataApex, Podohradska, Czech Republic) and expressed in relative percentage of each fatty acid.

Tocopherols. Tocopherols were determined by HPLC coupled to a fluorescence detector (HPCL-FL; Knauer, Smartline system 1000, Berlin, Germany). BHT solution in hexane (10 mg/mL; 100 μL) and IS solution in hexane (tocol; 50 μg/mL; 400 μL) were added to the sample prior to the extraction procedure. The samples (approximately 500 mg) were homogenized with methanol (4 mL) by vortex mixing (1 min). Subsequently, hexane (4 mL) was added and again vortex mixed for 1 min. After that, saturated NaCl aqueous solution (2 mL) was added, the mixture was homogenized (1 min), centrifuged (5 min, 4000 g) and the clear upper layer was carefully transferred to a vial. The sample was re-extracted twice with hexane. The combined extracts were taken to dryness under a nitrogen stream, redissolved in 2 mL of n-hexane, dehydrated with anhydrous sodium sulphate, filtered through 0.2 μm nylon filters from Whatman, and transferred into a dark injection vial for the analysis. The fluorescence detector was programmed for excitation (λ_{ex}) at 290 nm and emission (λ_{em}) at 330 nm. The chromatographic separation was achieved with a Polyamide II (250 mm x 4.6 mm i.d.) normal-phase column from YMC Waters operating at 30 ºC. The mobile phase used was a mixture of n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 mL/min, and the injection volume was 20 μL. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on calibration curves obtained from commercial standards of each compound using the internal standard methodology.

NaCl. NaCl concentration was determined according to Mohr's method. Powdered samples (1 g) were dissolved in 20 mL of distilled water and filtered through a Whatman No.4 paper, five times. The pH of the final aqueous solution was then adjusted to 8.5 with sodium hydroxide,

followed by the addition of 1 mL of potassium chromate solution (5%). The mixture was titrated against $AgNO_3$ (0.05 mol/L) until the appearance of the first reddish colour (Ag_2CrO_4 precipitate). The NaCl concentration was calculated using the following equation (where 1 mL of $AgNO_3$ corresponds to 0.00292 g of NaCl):

$$\text{Salt content \%} = [(V_{titrated\ of\ AgNO3} \times 0.00292)] / [(m_{\text{sample}})] \times 100$$

Equation 2. Equation for salt content determination.

Colour. The colour measurements were taken using a Minolta spectrophotometer (model CR-400; Konica Minolta Sensing, Inc., Japan). Using illuminant C and a measuring aperture of 8 mm, the following CIELab colour space readings were measured through the computerized system: L^*: lightness from black (0) to white (100); a^*: green (−) to red (+); and b^*: blue (−) to yellow (+).

pH. The pH values of the plant material were measured using a calibrated digital pH meter (portable food and dairy pH meter HI 99161, Hanna Instruments, Woonsocket, Rhode Island, USA).

2.5 Analysis of phenolic compounds

2.5.1 In the lyophilized extracts obtained from quinoa, amaranth and potato peels

The freeze-dried extracts were dissolved in a hydroethanolic solution 80:20 (*v/v*) and filtered through a 0.22 μm nylon filter, followed by immediate characterization by high performance liquid chromatography (HPLC) analysis. Chromatographic analysis were performed in a

Dionex Ultimate 3000 UPLC (Thermo Scientific, San Jose, CA, USA) system equipped with a diode array detector coupled to a electrospray ionization mass detector (LC-DAD-ESI/MSn), a quaternary pump, an auto-sampler (kept at 5 ºC), a degasser and an automated thermostatted column compartment. Chromatographic separation was achieved with a Waters Spherisorb S3 ODS-2 C18 (3 μm, 4.6 mm × 150 mm, Waters, Milford, MA, USA) column thermostatted at 35 °C. The solvents used were: (A) 0.1% formic acid in water, (B) acetonitrile. The elution gradient established was isocratic 15% B (5 min), 15% B to 20% B (5 min), 20-25% B (10 min), 25-35% B (10 min), 35-50% B (10 min), and re-equilibration of the column, using a flow rate of 0.5 mL/min. Double online detection was carried out in the DAD using 280 and 370 nm as preferred wavelengths and in a mass spectrometer (MS) connected to HPLC system via the DAD cell outlet. MS detection was performed in negative mode, using a Linear Ion Trap LTQ XL mass spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with an ESI source. Nitrogen served as the sheath gas (50 psi); the system was operated with a spray voltage of 5 kV, a source temperature of 325 °C, a capillary voltage of -20 V. The tube lens offset was kept at a voltage of -66 V. The full scan covered the mass range from *m*/*z* 100 to 1500. The collision energy used was 35 (arbitrary units). Data acquisition was carried out with Xcalibur® data system (ThermoFinnigan, San Jose, CA, USA).

The phenolic compounds were identified by comparing their retention times, UV-vis and mass spectra with those obtained from standard compounds, when available. Otherwise, compounds were tentatively identified comparing the obtained information with available data reported in the literature. For quantitative analysis, calibration curves with known concentration (200-5 μg/mL) for each available phenolic standard: caffeic acid ($y = 388345x + 406369$, $R^2 = 0.9949$); chlorogenic acid ($y = 168823x – 161172$, $R^2 = 0.9999$), kaempferol-3-*O*-rutinoside ($y = 11117x + 30861$, $R^2 = 0.9998$), p-coumaric acid ($y = 301950x + 6966.7$, $R^2 = 0.9999$),

quercetin-3-*O*-glucoside (y = 34843x – 160173, R^2 = 0.9998), quercetin-3-*O*-rutinoside (y = 13343x + 76751, R^2 = 0.9998), were constructed based on the UV signal at 280 nm. Quantification was made from the areas of the peaks recorded at 280 nm by comparison with the calibration curve obtained from a standard of the same family. The results were expressed as μg/g of extract.

2.5.2 Analysis of anthocyanins in the lyophilized extracts from coloured potatoes

Anthocyanins were analysed in the lyophilized aqueous extracts of the different samples dissolved in water at 5 mg/mL. Chromatographic separation was achieved on an AQUA® (Phenomenex) reverse phase C_{18} column (5 μm, 150 mm × 4.6 mm i.d) column working at 35 °C. The solvents used were: (A) 0.1% trifluoroacetic acid (TFA) in water, (B) acetonitrile. The gradient elution followed these parameters: 10% B for 3 min, from 10 to 15% B for 12 min, 15% B for 5 min, from 15 to 18% B for 5 min, from 18 to 30% B for 20 min, from 30 to 35% B for 5 min, and from 35 to 10% B for 10 min. The resulting total run time was 60 minutes, followed by column reconditioning of 10 minutes, using a flow rate of 0.5 mL/min. Double online detection was carried out in the DAD using 520 nm as the preferred wavelength and in a mass spectrometer (MS) connected to HPLC system via the DAD cell outlet. MS detection was performed in positive mode, using a Linear Ion Trap LTQ XL mass spectrometer (Thermo Finnigan, San Jose, CA, USA) equipped with an ESI source. Nitrogen served as the sheath gas (50 psi); the system was operated with a spray voltage of 4.8 kV, a source temperature of 320 °C, a capillary voltage of 14 V. The tube lens offset was kept at a voltage of 75 V. The full scan covered the mass range from *m*/*z* 100 to 1500. The collision energy used was 20 (arbitrary units). Data acquisition was carried out with Xcalibur® data system (Thermo Finnigan, San Jose, CA, USA).

The identification of the anthocyanin compounds was performed using standard compounds, when available, by comparing their retention times, UV-vis and mass spectra; and also, comparing the obtained information with available data reported in the literature giving a tentative identification. Calibration curves of the available anthocyanin standards: cyanidin-3-*O*-glucoside (y = 243287x - 1E+06, R^2 = 0.995), pelargonidin-3-*O*-glucoside (y = 276117x - 480418; R^2 = 0.9979), malvidin-3-*O*-glucoside (y = 477014.9x + 38.376, R^2 = 0.999), delphinidin-3-*O*-glucoside (y = 557274x + 126.24, R^2 = 0.997) and peonidin-3-*O*-glucoside (y = 537017x - 71.469, R^2 =0.999) were constructed based on the UV signal at 520 nm to perform quantitative analysis.

For quantitative analysis, a calibration curve for each available phenolic standard was constructed based on the UV signal. For the identified phenolic compounds for which a commercial standard was not available, the quantification was performed through the calibration curve of the most similar available standard. The results were expressed as mg/g of extract.

2.6 Bioactive properties

2.6.1 Antioxidant activity

The antioxidant activity of the extracts was assessed by two cell-based methods: (1) inhibition of the production of thiobarbituric acid reactive substances (TBARS) and (2) oxidative haemolysis inhibition assay (OxHLIA).

Thiobarbituric acid reactive substances (TBARS) assay. The TBARS assay bases on the formation of a pink complex between the oxidation product malondialdehyde (MDA) and

thiobarbituric acid (TBA), and the extend of oxidation is evaluate through an absorbance measurement (**Figure 11**).

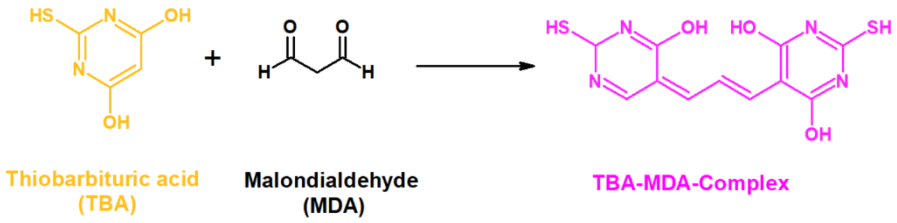


Figure 11. Formation of a pink complex through the reaction of malondialdehyde with thiobarbituric acid.

Porcine brains were obtained from a slaughterhouse and homogenised in a Tris-HCl buffer (20 mM, pH 7.4) with a stoichiometry of 1:2 (*w/v*). The brain was centrifuged for 10 min at 500 rpm, to separate the lipid solution from solid brain remnants.

The hydromethanolic extracts of the samples were re-dissolved in water to gain a stock solution of 1.25 mg/mL and then further diluted (0.625 mg/mL – 0,039 mg/mL). 0.2 mL of each concentration was added to Eppendorf's tubes in triplicate, followed by 0.1 mL of $FeSO_4$ (0.01 mM), 0.1 mL of ascorbic acid (0.1 mM), and 0.1 mL of the supernatant of the brain tissue homogenate. The test tubes were incubated at 37 °C for one hour. The reaction was stopped by the addition of 0.5 mL trichloroacetic acid (28% w/v). To visualize the extend of oxidation 0.38 mL of thiobarbituric acid (2%, w/v) was added and the mixture was heated at 80°C for 20 min. After the formation of the pink thiobarbituric acid-malondialdehyde complex (TBA-MDA), the tubes were centrifuged at 14,000 rpm for five minutes to remove the precipitated protein. The colour intensity of the complex was measured at its absorbance wavelength 532 nm. The inhibition ratio (%) was calculated using the following formula:

$$\text{Inhibition ratio (\%)} = [(A - B)/A] \times 100\%$$

Equation 3. Equation for the determination of inhibition ratio (%) in the TBARS assay.

where A and B are the absorbance of the control and the compound solution, respectively.

The extract concentration providing 50% of antioxidant activity (EC_{50}) was calculated from the graph of TBARS formation inhibition against extract concentrations. Trolox was used as a standard.

Oxidative haemolysis inhibition assay (OxHLIA). The antihaemolytic activity of the extracts was evaluated by the oxidative haemolysis inhibition assay (OxHLIA). Sheep blood samples were collected from healthy animals and centrifuged at 1000 *g* for 5 min at 10 °C. Plasma and buffy coats were discarded and erythrocytes were first washed once with NaCl (150 mM) and three times with phosphate-buffered saline (PBS, pH 7.4). The erythrocyte pellet was then resuspended in PBS at 2.8% (v/v). Using a flat bottom 48-well microplate, 200 µL of erythrocyte solution was mixed with 400 µL of either PBS solution (control), antioxidant sample dissolved in PBS, or water (for complete haemolysis). Trolox was used as positive control. After pre-incubation at 37 °C for 10 min with shaking, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH, 160 mM in PBS, 200 µL) was added and the optical density was measured at 690 nm. After that, the microplate was incubated under the same conditions and the optical density was measured every 10 min at the same wavelength for approximately 400 min. The percentage of the erythrocyte population that remained intact (*P*) was calculated according to the equation, where S_t and S_0 correspond to the optical density of the sample at *t* and 0 min, respectively, and CH_0 is the optical density of the complete haemolysis at 0 min:

$$P\ (\%) = (S_t - CH_0/S_0 - CH_0) \times 100$$

Equation 4. Equation for the determination of the erythrocyte population that remained intact in the OxHLIA assay.

The results were expressed as delayed time of haemolysis (Δt), which was calculated according to the following equation, where Ht_{50} is the 50% haemolytic time (min) graphically obtained from the haemolysis curve of each antioxidant sample concentration.

$$\Delta t\ (min) = Ht_{50}\ (sample) - Ht_{50}\ (control)$$

Equation 5. Equation for the determination of the delayed time of haemolysis in the OxHLIA assay.

The Δt values were then correlated to antioxidant sample concentrations and, from the correlation obtained, the extract concentration able to promote a Δt haemolysis delay was calculated. The results were given as IC_{50} values (μg/mL) at Δt 60 and 120 min, i.e., extract concentration required to keep 50% of the erythrocyte population intact for 60 and 120 min.

2.6.2 Anti-inflammatory activity

The extracts were dissolved in water, initially concentrated at 8 mg/mL and then further dilutions were prepared from 8 mg/mL to 0.125 mg/mL. The mouse macrophage-like cell line RAW264.7 was cultured in Dulbecco's Modified Eagle Medium **(**DMEM) supplemented with 10% heat-inactivated fetal bovine serum and glutamine at 37 °C under 5% CO_2, in humidified air. For each experiment, cells were detached with a cell scraper. Under our experimental cell density (5×10^5 cells per mL), the proportion of dead cells was less than 1%, according to Trypan blue dye exclusion tests. Cells were seeded in 96-well plates at 150 000 cells per well

and allowed to attach to the plate overnight. Then, cells were treated with the different concentrations of each of the extracts for 1 h. Dexamethasone (50 μM) was used as a positive control for the experiment. The following step was stimulation with lipopolysaccharide (LPS) (1 μg/mL) for 18 h. The effect of the tested samples in the absence of LPS was also evaluated, in order to observe if they induced changes in NO basal levels. In negative controls, no LPS was added. Both extracts and LPS were dissolved in supplemented DMEM. For the determination of nitric oxide, a Griess Reagent System kit (Promega) was used, which contains sulfanilamide and nitrite solutions. One hundred microliters of each cell culture supernatant were transferred to the plate in duplicate and mixed with sulfanilamide solutions, for 5–10 minutes each, at room temperature. The nitrite produced was determined by measuring the optical density at 515 nm, in the microplate reader referred to above, and was compared to the standard calibration curve.

2.6.3 Antibacterial activity

The following Gram-negative bacteria: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Enterobacter cloacae* (ATCC 35030), and Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240), and *Listeria monocytogenes* (NCTC 7973) were used. The microorganisms were obtained from the Mycological laboratory, Department of Plant Physiology, Institute for biological research "Siniša Stanković", University of Belgrade, Serbia. The minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations were determined by the microdilution method. Each fresh overnight culture of bacteria was adjusted spectrophotometrically to a concentration of 1×10^5 CFU m/L. The requested CFU corresponded to a bacterial suspension determined in a spectrophotometer

at 625 nm (OD625). Dilutions of inocula were cultured on solid medium to verify the absence of contamination and check the validity of each inoculum. Different solvent dilutions of the ethanolic extract were added to the wells containing 100 µL of Tryptic Soy Broth (TSB) and afterwards, 10 µL of inoculum was added to all wells. The microplates were incubated for 24 h at 37 °C. The MIC of the samples was detected following the addition of 40 µL of iodonitrotetrazolium chloride (INT) (0.2 mg m/L) and incubation at 37 °C for 30 min. The lowest concentration that produced a significant inhibition (around 50%) of the growth of the bacteria in comparison with the positive control was identified as the MIC. The minimum inhibitory concentrations (MICs) obtained from the susceptibility testing of various bacteria to the tested extracts were determined also by a colorimetric microbial viability assay based on the reduction of the INT colour and compared with a positive control for each bacterial strain. MBC was determined by serial sub-cultivation of 10 µL into microplates containing 100 µL of TSB. The lowest concentration that showed no growth after this sub-culturing was read as the MBC. Standard drugs, namely streptomycin and ampicillin, were used as positive controls. 5% dimethyl sulfoxide was used as the negative control. Samples were tested in duplicate and experiments were repeated three times.

2.6.4 Antifungal activity

For the antifungal bioassays, the following microfungi were used: *Aspergillus fumigatus* (ATCC 1022), *Aspergillus versicolor* (ATCC 11730), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus niger* (ATCC 6275), *Trichoderma viride* (IAM 5061), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112), and *Penicillium verrucosum* var. *cyclopium* (food isolate). The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research “Siniša Stanković”,

Belgrade, Serbia. The micromycetes were maintained on malt agar (MA) and the cultures were stored at 4 °C and sub-cultured once a month. The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^5 in a final volume of 100 µL per well. The inocula were stored at 4 °C for further use. Dilutions of each inoculum were cultured on solid MA to verify the absence of contamination and to check the validity of the inoculum. Minimum inhibitory concentration (MIC) determination was performed by a serial dilution technique using 96-well microtitre plates. The investigated extract was dissolved in a 5% solution of dimethyl sulfoxide and added to broth malt medium with a fungal inoculum. The microplates were incubated for 72 h at 28 °C. The lowest concentrations without visible growth (as assessed using a binocular microscope) were defined as the MICs. The minimum fungicidal concentrations (MFCs) were determined by serial sub-cultivation of 2 µL in microtiter plates containing 100 µL of malt broth per well and further incubation for 72 h at 28 °C. The lowest concentration with no visible growth was defined as the MFC, indicating 99.5% killing of the original inoculum. 5% dimethyl sulfoxide was used as a negative control, while bifonazole and ketoconazole were used as positive controls. Samples were tested in duplicate and experiments were repeated three times.

2.6.5 Antiproliferative activity

The lyophilized extracts were re-dissolved in water to obtain stock solutions of 4 mg/mL, and then submitted to further dilutions. Four human tumor cell lines were tested: MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma), and HepG2 (hepatocellular carcinoma). Sulforhodamine B assay was performed according to a procedure previously described by Barros, Pereira et al. (2013).

Each of the cell lines were plated in a 96-well plate, at an appropriate density (1.0×10^4 cells/well) and allowed to attach for 24 h. The cells were then incubated in the presence of different extract concentrations during 48 h. Afterwards, cold trichloroacetic acid (TCA 10%, 100 μL) was added in order to bind the adherent cells and further incubated for 60 min at 4ºC. After the incubation period, the plates were washed with deionized water and dried. Sulforhodamine B solution (SRB 0.1% in 1% acetic acid, 100 μL) was incorporated to each plate well, and incubated for 30 min at room temperature. The plates were washed with acetic acid (1%) in order to remove the unbound SRB and air dried. The bounded SRB was solubilised with Tris (10 mM, 200 μL) and the absorbance was measured at 540 nm using an ELX800 microplate reader (Bio-Tek Instruments, Inc; Winooski, VT, USA).

2.6.6 Hepatotoxicity

A cell culture was prepared from a freshly harvested porcine liver obtained from a local slaughterhouse. It was designated as PLP2. Briefly, the liver tissues were rinsed in Hank's balanced salt solution containing 100 U m/L penicillin and 100 μg/mL streptomycin and divided into 1×1 mm^3 explants. Some of these explants were placed in 25 cm^3 tissue flasks in DMEM supplemented with 10% fetal bovine serum, 2 mM nonessential amino acids, 100 U m/L penicillin and 100 μg/mL streptomycin and incubated at 37 °C with a humidified atmosphere containing 5% CO_2. The medium was changed every 2 days. Cultivation of the cells was continued with direct monitoring every 2–3 days using a phase contrast microscope. Before confluence, cells were sub-cultured and plated in 96-well plates at a density of 1.0×10^4 cells per well, and cultivated in DMEM medium with 10% FBS, 100 U mL penicillin and 100 μg mL streptomycin. Cells were treated for 48 h with the different diluted sample solutions and the same procedure described in the previous section for the SRB assay was followed. The

results were expressed as GI_{50} values (sample concentrations that inhibited 50% of the net cell growth). Ellipticine was used as a positive control.

2.7 Validation of the coloured potatoes' extracts as natural colourants

2.7.1 Soft drink formulation

Following the screening of the coloured potato varieties based on their anthocyanin content and bioactive properties, two extracts were selected to be applied as colouring agents in a soft drink formulation: cv. *Rosemary* (red-flesh potato variety) and cv. *Purple* (purple-fleshed potato variety). A typical raspberry flavoured soft drink formulation was prepared, composed of sparkling water, caster sugar, citric acid, sucralose and natural flavourings (raspberry and peach), in decreasing order of concentration. Three soft drink formulations were prepared with (1) the red anthocyanin-rich potato extract (*Rosemary*), (2) the purple anthocyanin-rich potato extract (*Purple*) and (3) the commercial colorant E163 (standardised in 1% anthocyanins content). The latter formulation (3) was used as control. The colourants were added to the respective formulations in order to obtain a similar concentration of anthocyanins (~8 mg/L solution), which visually matched the colour intensity of a commercial soft drink product used as reference. The commercial colorant E163 used as control in the soft drink formulations was purchased from Naturex (Derbyshire, UK), consisting of an anthocyanin glucosides' extract derived from grapes and standardised in 1% of anthocyanins content by the supplier, preserved with potassium sorbate. All soft drink samples were prepared according to good hygiene and manufacturing practices in food grade facilities.

2.7.2 Pasteurisation

The three soft drink formulations (*Rosemary*, *Purple* and *Control*) were poured into glass containers (250 mL), which were sealed and placed in a water bath at 70 °C for 20 minutes, following the *in-pack* pasteurisation method described by Ashurst (2011).

2.7.3 Colour stability and determination of pH

Following pasteurisation, the three soft drink formulations (*Rosemary*, *Purple* and *Control*) were stored at 4 °C and their colour stability was assessed at 0, 7 and 30 days of storage. A PCE colourimeter (model CSM-3; PCE Instruments, Germany) was used to measure the colour by placing 80 mL of each formulation in a quartz glass box designed for measurements carried out on liquid samples. Using a silicon photoelectric diode sensor and a measuring aperture of 8 mm, the following CIELab colour space readings were measured through the computerized system: L^*: lightness from black (0) to white (100); a^*: green (−) to red (+); b^*: blue (−) to yellow (+); C*: chroma, relative saturation; and h°: hue angle in the CIELab colour wheel. The pH of the formulated soft drinks was measured using a calibrated digital pH meter (portable food and dairy pH meter HI 99161, Hanna Instruments, Woonsocket, RI, USA). All pH measurements were carried out in triplicate. All three soft drink formulations presented a final pH of approximately 3.

2.7.4 Sensory analysis

A sensory analysis was carried out immediately after the soft drinks' production and pasteurisation (Day 0). Sessions took place in the sensory suite facility of the Scottish Centre for Food Development and Innovation (SCFDI) at Queen Margaret University (Edinburgh, UK). The test panel participants were informed about the general aim of the study and the required procedures for handling personal data. All participants gave written informed consent

prior to participation. Twelve experienced trained panelists were selected and subsequently received specific additional training on the sensory attributes relevant to the soft drink formulation developed in this project using a range of commercial products. Sensory tests were conducted under white light in each booth.

The soft drinks formulated with the two extracts obtained in the present study were compared with those of a control formulation containing the commercial colorant E163. Samples were presented to the panelists in coded clear plastic cups containing 20 mL of each formulation. Panelists were asked to cleanse their palate before the first sample and between samples using water and crackers. After training the following list of sensory attributes was generated and confirmed by the panel: colour intensity, cloudiness, sweetness, fruitiness, bitterness and sourness. A quantitative descriptive analysis (QDA) test was designed using the Compusense Cloud software (Compusense, Guelph, ON, Canada). The intensity of perception was scored for each attribute on a scale ranging from 1 (very low) to 9 (very high). Following the QDA questionnaire the panelists were asked to comment on the overall acceptability of each product based on their hedonic experience and indicate their preference among the three products.

2.8 Statistical analysis

For all experiments, three independent batch samples were used, and each sample was analysed in triplicate for every single laboratorial assay. Results are expressed as mean ± standard deviation (SD). The results were analysed using the one-way analysis of variance (ANOVA) followed by Tukey's HSD Test ($p = 0.05$). When less than three results were present in each individual analysis, t-Student test was used to determine the significant difference ($p = 0.05$).

2.8.1 For the nutritional and physicochemical characterisation of the fifty potato genotypes

An analysis of variance (ANOVA) was performed employing Tukey's HSD test (homoscedastic distributions) or Tamhane's T2 test (heteroscedastic distributions) to classify the statistical differences among potatoes accessions for each of the assayed parameters. The fulfilment of the one-way ANOVA requirements, specifically the normal distribution of the residuals and the homogeneity of variance, was tested by means of the Shapiro Wilk's and the Levene's tests, respectively. Linear discriminant analysis (LDA) was further used to identify the variables that characterized mostly each potato flesh colour (FC) or cultivation type (CT). A stepwise technique was applied, based on the Wilks' ʎ test with the usual probabilities of *F* (3.84 to enter and 2.71 to be removed) for variable selection. This procedure combines a series of forward selection and backward elimination steps, which verifies the significance of all previously included variables before adding an additional one as having discriminant ability. In this specific case, the basic purpose of the LDA was to characterize the relationship between a single categorical dependent variable (FC or CT) and the set of quantitative independent variables (all assayed parameters) to check for similarities in the potato genotypes, particularly in what concerns CT, as this may indicate specific non-cultivated genotypes to be used as alternative to cultivated ones. Through this method, it is possible to determine which of the independent variables contributed more to the differences in the average score profiles of the acorns belonging to FC or CT. To verify the significance of the canonical discriminating functions, Wilk's ʎ test was used. A leaving-one-out cross validation procedure was carried out to assess the model performance. All the mentioned statistical tests were performed considering $\alpha = 0.05$, using the Statistical Package for Social Sciences (SPSS), v. 25.0 (IBM Corp., Armonk, NY, USA).

2.8.2 For the sensory analysis of natural colourants from coloured potatoes

The sensory results were analysed using a two-way analysis of variance (ANOVA) followed by a Tukey's post-hoc Honest Significant Difference (HSD) test using the Compusense Cloud software ($p = 0.05$).

CHAPTER 3

Results and Discussion

3. CHAPTER 3: Results and Discussion

3.1 Quinoa

3.1.1 Nutritional value and physicochemical composition of quinoa

Figure 12 shows the main characteristics of BRS Piabiru quinoa and the determined nutritional value and physicochemical composition are presented in **Table 8**.

Figure 12. Brazilian quinoa BRS Piabiru and some of its most important features.

Table 8. Nutritional value (g/100g fw), energetic contribution (kcal/100 g fw) and physicochemical composition of BRS Piabiru quinoa seeds (mean ± SD).

Nutritional value		**Organic acids** (g/100 g fw)	
Moisture (%)	8.4±0.1	Oxalic acid	0.603±0.009
Proteins	17.0±0.8	Quinic acid	3.6±0.1
Lipids	6.0±0.2	Malic acid	0.41±0.03
Carbohydrates	66.5±0.5	Fumaric acid	tr
Ash	2.1±0.1	Total organic acids	4.6±0.2
Energy (kcal/100 g fw)	389±1		
Free sugars (g/100 g fw)		**Physicochemical variables**	
Fructose	0.060±0.004	NaCl (g/100 g fw)	0.39±0.02
Glucose	0.31±0.01	pH	5.65±0.01
Sucrose	0.80±0.03	Hunter scale colour parameters	
Total free sugars	1.17±0.04	*L**	78.2±0.4
		*a**	1.84±0.08
		*b**	15.2±0.1

tr - traces

The seeds showed high protein content, with a mean concentration of 17.0 g/100 g of fresh weight (fw), revealing a similar content to a previous published study on the same variety, 16.41 g/100 g fw (Palombini et al., 2013). In a recent study, Pereira et al. (2019) analysed thirty nine distinct genotypes of quinoa from Peru and Spain, with reported protein content ranging from 14.4 to 15.6 g/100 g dw. These data highlight the outstanding potential of the studied Brazilian quinoa as a source of protein for the human diet, even when compared to other quinoa varieties. Considering other major grains, quinoa presents higher protein content than wheat (12.3%), maize (8.9%), rice (7.5%) and oat (16.1%) (Graf et al., 2015). Additional features include a balanced amino acids profile, easy digestibility and the absence of gluten, being its ingestion safe for celiac disease sufferers (Graf et al., 2015; Janssen et al., 2017). Quinoa protein profile has been compared to the milk protein, as it includes reasonable amounts of all the essential amino acids, being particularly rich in lysine (Ceyhun Sezgin & Sanlier, 2019; Dakhili, Abdolalizadeh, Hosseini, Shojaee-aliabadi, & Mirmoghtadaie, 2019).

Carbohydrates appeared as the major macronutrient in the studied samples, accounting for 66.5 g/100 g fw (**Table 8**). In general, the quinoa seed is characterized by a lower content of carbohydrates than cereals like wheat, barley, maize or rice, also presenting low glycaemic index (Dakhili et al., 2019; Graf et al., 2015).

Regarding the free sugar composition, three distinct molecules were identified: two monosaccharides (fructose and glucose) and one disaccharide (sucrose) (**Table 8**). Sucrose was the most abundant one (0.80 g/100 g fw), followed by glucose (0.31 g/100 g fw) and fructose (0.06 g/100 g fw). As a low-free sugar food crop, quinoa can be classified as part of a “low fodmap diet”, which has been shown to exert beneficial impacts on irritable bowel symptoms by limiting the ingestion of readily-fermentable short-chain carbohydrates (Graf et al., 2015).

Total fat reaches a mean concentration of 6.0 g/100 g fw (**Table 8**), which is in agreement with the range of 2% to 10% described in the literature, considering distinct quinoa genotypes (Graf et al., 2015; Palombini et al., 2013; Pereira et al., 2019). The fatty acids profile was composed of 85.97% of unsaturated fatty acids, 59.94% of which polyunsaturated (PUFAs) **(Table 9)**.

The consumption of polyunsaturated fatty acids has been associated with a range of health benefits, for instance positive effects on cardiovascular disease, metabolism of prostaglandins, insulin sensitivity, immune system and cell membrane function (Filho et al., 2017; Graf et al., 2015). In the case of BRS Piabiru quinoa, the major PUFA was linoleic acid (C18:2n6, 56.70%), while monounsaturated fatty acids (MUFAs) were mostly represented by oleic acid (C18:1n9, 22.67%). The major saturated fatty acid found was palmitic (C16:0, 11.09%), which is also in agreement with the literature for other quinoa varieties (Filho et al., 2017).

Table 9. Composition of fatty acids (%) and tocopherols (mg/100 g fw) of BRS Piabiru quinoa seeds (mean±SD).

Fatty acid		**Fatty acid**	
C6:0	0.041±0.004	C18:1n9	22.67±0.04
C8:0	0.019±0.001	C18:2n6	56.70±0.03
C10:0	0.059±0.001	C18:3n6	0.092±0.004
C12:0	0.039±0.001	C18:3n3	2.74±0.01
C14:0	0.277±0.001	C20:0	0.428±0.004
C15:0	0.090±0.003	C20:1	1.50±0.08
C16:0	11.09±0.01	C20:2	0.241±0.002
C16:1	0.088±0.001	C21:0	0.053±0.001
C17:0	0.058±0.001	C22:0	0.667±0.007
C18:0	0.786±0.004	C22:1	1.78±0.01
SFA	14.03±0.01	C22:2	0.172±0.001
MUFA	26.04±0.03	C23:0	0.073±0.001
PUFA	59.94±0.05	C24:0	0.35±0.02
Tocopherols			
α-tocopherol		0.919±0.001	
γ-tocopherol		2.67±0.05	
Total tocopherols		3.59±0.05	

Caproic acid (C6:0); caprylic acid (C8:0); capric acid (C10:0); lauric acid (C12:0); myristic acid (C14:0); pentadecanoic acid (C15:0); palmitic acid (C16:0); palmitoleic acid (C16:1); margaric acid (C17:0); stearic acid (C18:0); oleic acid (C18:1n9); linoleic acid (C18:2n6); γ-linolenic acid (C18:3n6); α-linolenic acid (C18:3n3); arachidic acid (C20:0); cis-11-eicosenoic acid (C20:1); eicosadienoic acid (C20:2); heneicosanoic acid (C21:0); behenic acid (C22:0); erucic acid (C22:1); docosadienoic acid (C22:2); tricosylic acid (C23:0); lignoceric acid (C24:0). SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

Quinoa is known for containing high concentrations of antioxidants, such as tocopherols, which act as scavengers of lipid peroxyl radicals (Filho et al., 2017; Palombini et al., 2013). In this study, α- and γ- tocopherol were the main isomers found in quinoa **(Table 9)**. α-Tocopherol was found in a mean concentration of 0.919 mg/100 g fw, which was slightly lower than the concentration of 1.16 mg/100 g reported by for the same quinoa variety (Palombini et al., 2013). Those authors reported a β+γ-tocopherol concentration of 1.08 mg/100 g, which was less than half the concentration of γ-tocopherol found in the present study, 2.67 mg/100 g. Despite considering the same variety, the discrepancies found in the tocopherol content between both studies may be due to distinct climatic and soil conditions, most probably associated to the specific regions in Brazil where the crops were grown.

Four organic acids were detected, being quinic acid the major one (3.6 g/100 g fw), followed by oxalic (0.603 g/100 g fw), malic (0.41 g/100 g fw) and fumaric acids (trace amounts). Pereira et al. (2019), in a screening study on thirty-nine quinoa genotypes, also reported the presence of oxalic acid and, similarly, traces of fumaric acid, however, they did not detect quinic and malic acids. In a recent study performed by Heikkilä, Hermant, Thevenet, & Domingo (2019) in an *in vivo* mice model, it was suggested that quinic acid could be helpful in the treatment of diabetes, by promoting insulin secretion from pancreatic beta cells.

The excess of sodium intake has been shown to be strongly associated with a plethora of health disorders (Allison & Fouladkhah, 2018). This has stimulated the food industry to adopt interventions towards products development and reformulation to achieve a reduced final sodium content. The results for salt (NaCl) content in the BRS Piabiru quinoa showed a low mean value of 0.39 g/100 g fw (**Table 8**). Considering this, the herein studied quinoa could be considered for the development of new processed food products that aim, and claim, reduced salt content.

The pH value (5.65) placed the studied quinoa grains as slightly more acidic than the six commercial genotypes analysed by Pellegrini, Lucas-Gonzales, Ricci, & Fontecha (2018), which presented a pH range of 6.42 - 6.63. Regarding colour, only whole grains were measured, before any milling process was employed; therefore, all measurements corresponded to the coloured outer layer of the grains (**Figure 13**). The results showed that the grains were characterized by a high mean value of parameter *L** (78.2), which indicates high luminosity. Additionally, the low value of parameter *a** (1.84) indicates the absence of red-green intensities, and the parameter *b** value (15.2) is associated to a pale yellow colour. Escribano et al. (2017) undertook an investigation of twenty-nine distinct varieties of quinoa, including white, black, yellow and red-violet genotypes. Blanca de Junín and Inia de Salcedo were two of the varieties analysed by these authors classified as "white quinoa", with results fairly similar to the ones found in this study, *i.e.*, *L** values of 73.97 and 75.60, *a** values of 1.54 and 1.53, and *b** values of 18.14 and 19.83, respectively.

Considering these results, the great nutritional value of the BRS Piabiru quinoa, along with its physicochemical features, makes this variety an attractive food crop for direct incorporation into the human diet and in a range of food formulations, especially considering its outstanding protein content and fatty acids composition.

3.1.2 Phenolic compounds of quinoa

The analysis of phenolic compounds in the Brazilian quinoa hydroethanolic extract was performed by HPLC-DAD-ESI/MS^n. Six flavonol glycosides were detected derived from quercetin and kaempferol as concluded from their absorption and mass characteristics. Data is presented in **Table 10** together with compound quantifications. Quercetin and kaempferol 3-*O*-rutinoside (peaks 5 and 6) were positively identified by comparison with commercial

standards. Peaks 1, 2 and 3 were tentatively assigned as quercetin-3-*O*-(2″,6″-di-*O*-α-l-rhamnoside)-β-d-galactoside, quercetin-3-*O*-(2″-*O*-β-apioside-6″-*O*-α-rhamnoside)-β-galactoside and kaempferol-3-*O*-(2″,6″-di-*O*-α-rhamnoside)-β-galactoside, respectively, taking into account their previous description in quinoa samples. The absorption and mass spectra of peak 4 were the same as peak 3, being tentatively associated to kaempferol-3-*O*-(2″,6″-di-*O*-α-rhamnoside)-β-glucoside considering that glucosides elute later than the corresponding galactosides.

More than 20 phenolic compounds have been described to date for distinct varieties of quinoa seeds, in either free or conjugated forms, liberated by alkaline, acid, and/or enzymatic hydrolysis. The most abundant compounds reported are the flavonoids quercetin and kaempferol glycosides, as well as the phenolic acids vanillic acid, ferulic acid and their derivatives (Tang & Tsao, 2017; Vilcacundo & Hernandez-Ledesma, 2017). In this study, high concentrations of flavonol derivatives (quercetin and kaempferol) have been determined, which is in agreement with the literature; however, no significant amounts of phenolic acids were found. Considering that this is the first report on the identification of phenolic compounds in the quinoa BRS Piabiru genotype, this result might be explained by an actual low content, below our instrument detection limits, or inexistence of phenolic acids in this variety. Indeed, differences in phytochemical composition in natural products can be explained by both genotypic or environmental factors (Graf et al., 2015).

Table 10. Retention time (Rt), wavelengths of maximum absorption (λ_{max}), mass spectral data and quantification of the phenolic compounds tentatively identified in hydroethanolic extracts of BRS Piabiru quinoa seeds. Results are expressed as mean ± standard deviation, and the respective references for identification are presented.

Peak	Rt (min)	λmax (nm)	$[M-H]^-$ (*m*/*z*)	MS^2 (*m*/*z*)	Tentative identification	Quantification (mg/g)	References
1	14.37	352	755	760(38),301(100)	Quercetin-3-*O*-(2″,6″-di-*O*-α-l-rhamnoside)-β-d-galactoside	93.5±0.5	Gómez-Caravaca et al. (2011), Hirose et al. (2010)
2	15.51	353	741	609(100),301(80)	Quercetin-3-*O*-(2″-*O*-β-apioside-6″-*O*-α-rhamnoside)-β-galactoside	58.2±0.3	Hirose et al. (2010)
3	16.15	265,348	739	593(44),285(100)	Kaempferol-3-*O*-(2″,6″-di-*O*-α-rhamnoside)-β-galactoside	23.73±0.09	Gómez-Caravaca et al. (2011), Hirose et al. (2010)
4	16.27	265,348	739	593(44),285(100)	Kaempferol-3-*O*-(2″,6″-di-*O*-α-rhamnoside)-β-glucoside	21.5±0.1	DAD, MS Standard
5	17.53	353	609	301(100)	Quercetin-3-*O*-rutinoside	5.26±0.09	Gómez-Caravaca et al. (2011), Tang, Li, Zhang et al. (2015)
6	18.04	266,347	593	285(100)	Kaempferol-3-*O*-rutinoside	5.28±0.06	Standard
					Total phenolic compounds	207.54±0.2	

3.1.3 Bioactive properties of quinoa

Phenolic compounds have been associated with a range of biological activities due to their effects on cell-signalling and metabolism, including antioxidant, anti-inflammatory, anticancer and cardioprotective effects (Graf et al., 2015). The presence of these compounds in quinoa has been related to antidiabetic and anti-obesity properties, attributed to their α-glucosidase and pancreatic lipase inhibitory activities (Vilcacundo & Hernandez-Ledesma, 2017).

The *in vitro* antioxidant, antibacterial and antifungal properties of the quinoa hydroethanolic extracts were assessed. Additionally, cytotoxicity of the extracts was also investigated. Several studies, including clinical trials, have demonstrated the antioxidant properties of quinoa, which have been attributed to its high content on polyphenols (Dakhili et al., 2019; Graf et al., 2015; Vilcacundo & Hernandez-Ledesma, 2017). Considering that antioxidant compounds act by distinct mechanisms, in this study two different *in vitro* cell-based techniques to assess the antioxidant capacity of the BRS Piabiru quinoa hydroethanolic extract has been employed. The results of both assays are presented in **Table 11**.

Table 11. Antioxidant and cytotoxicity activities of BRS Piabiru quinoa seeds hydroethanolic extracts (mean±SD).

Cell-based antioxidant assays (IC_{50}, µg/mL)	
TBARS	764±6
OxHLIA	
Δt = 30 min	5.8±0.2
Δt = 60 min	59±1
Cytotoxicity (GI_{50} µg/mL values)	
PLP2	>400

IC_{50} values are the concentration of extract required to inhibit (delay) 50% of haemolysis for 30 min (IC_{50} (30 min)) and 60 min (IC_{50} (60 min)). GI_{50} values correspond to the sample concentration achieving 50% of growth inhibition in liver primary culture PLP2.

The Brazilian quinoa extract was effective in diminishing the production of TBAR substances, which result from lipid peroxidation-induced oxidative stress. The extract presented a mean IC_{50} value of 764 µg/mL. A protective effect against lipid peroxidation was also observed in a human dietary intervention study conducted by Carvalho & Paya (2014). A double-blind intervention was conducted on 35 women with weight excess who consumed 25 grams of quinoa flakes daily during a period of four consecutive weeks. Their results showed a significant decrease in TBAR substances in the subjects' blood samples (3.06 to 2.89 µmol/L), which suggested the efficacy of quinoa intake as an antioxidative strategy for the human diet.

The antioxidant properties of the BRS Piabiru quinoa extracts were also confirmed by OxHLIA, a cell-based assay for evaluating inhibition of free radical-induced haemolysis in sheep erythrocytes. A mean concentration of 5.8 µg/mL of extract was found to inhibit the haemolysis by 50% for 30 minutes, and 59 µg/mL for 60 minutes **(Table 11)**. To the best of our knowledge, this was the first-time that the OxHLIA assay was employed to evaluate the antioxidant properties of quinoa extracts. Other authors have also reported positive results for *in vitro,* non-cell-based, antioxidant assays of quinoa, for instance ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC) and DPPH radical scavenging assay (Escribano et al., 2017; Palombini et al., 2013; Pellegrini et al., 2018). Regarding the latter, only one study has been conducted on the same Brazilian quinoa variety herein investigated (Palombini et al., 2013), with positive although less expressive outcomes (IC_{50} average value of 313.25 µg/mL of methanolic extract).

Several antinutritional factors have been identified by other authors in different varieties of quinoa, namely saponins, phytic acid, tannins, nitrates, oxalates, and trypsin inhibitors (Filho et al., 2017; Graf et al., 2015; Vilcacundo & Hernandez-Ledesma, 2017). Nevertheless, the

hydroethanolic extract herein studied did not present toxicity against the porcine liver primary culture PLP2, once its GI_{50} value was higher than the highest tested concentration (400 µg/mL) **(Table 11).** The employment of porcine liver as an *in vitro* cytotoxicity model is justified by its similarity with the human liver, in terms of its cellular and physiological functioning (Corrêa et al., 2018). The verified absence of cytotoxicity is of interest considering the potential use of the tested preparation for food and pharmaceutical formulations, confirming the safety of the Brazilian quinoa extract for incorporation in the human diet, and other potential applications.

Meneguetti et al. (2011) investigated the biological effects of BRS Piabiru quinoa supplementation in vivo, in a rats' diet study. Those authors found that the activities of the liver enzymes aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) did not change in the supplemented groups, with values remaining at normal levels, confirming the absence of hepatoxicity. Additionally, they observed a decreased fat deposition and blood triacylglycerol levels in the supplemented groups.

The results for antimicrobial activity are shown in **Table 12**. The quinoa BRS Piabiru extract was tested against three Gram-positive and three Gram-negative bacteria, besides six fungi. The inhibitory and antibacterial activity displayed by the extract against both Gram-positive (*S. aureus*, *B. cereus* and *L. monocytogenes*) and Gram-negative (*E. coli*, *S. typhimurium* and *Enterobacter cloacae*) bacteria indicates the existence of an extensive antibiotic spectrum for their phytochemical constituents. The extract was equally active against all six tested bacteria. Besides, the quinoa extract was also effective against all six fungi tested, showing better results against *P. ochrochloron* than the fungicidal ketoconazole.

Table 12. Antimicrobial activity of BRS Piabiru quinoa seeds hydroethanolic extracts.

	Quinoa	**Streptomycin**	**Ampicillin**
Bacteria	MIC/MBC	MIC/MBC	MIC/MBC
Staphylococcus aureus	1.0/2.0	0.04/0.1	0.25/0.45
Bacillus cereus	1.0/2.0	0.1/0.2	0.25/0.4
Listeria monocytogenes	1.0/2.0	0.2/0.3	0.4/0.5
Escherichia coli	1.0/2.0	0.2/0.3	0.4/0.5
Salmonella typhimurium	1.0/2.0	0.2/0.3	0.75/1.2
Enterobacter cloacae	1.0/2.0	0.2/0.3	0.25/0.5
	Quinoa	**Ketoconazole**	**Bifonazole**
Fungi	MIC/MFC	MIC/MFC	MIC/MFC
Aspergillus fumigatus	0.5/1.0	0.25/0.5	0.15/0.2
Aspergillus ochraceus	0.5/1.0	0.2/0.5	0.1/0.2
Aspergillus niger	1.0/2.0	0.2/0.5	0.15/0.2
Penicillium funiculosum	0.5/1.0	0.2/0.5	0.2/0.25
Penicillium ochrochloron	1.0/2.0	2.5/3.5	0.2/0.25
Penicillium verrucosum var. cyclopium	0.5/1.0	0.2/0.3	0.1/0.2

The antibacterial activity of quinoa extracts was also observed by Miranda et al. (2014) studying six different quinoa seeds, grown in three distinctive geographical zones of Chile. They prepared ethanolic extracts and studied their antibacterial activity via the disk diffusion assay technique. The extracts showed antimicrobial activity in the range of 8.3 - 14.8 mm inhibition zone for *E. coli* and 8.5 - 15.0 mm inhibition zone for *S. aureus*. Those authors also reported a positive correlation between the antimicrobial activity and the total phenolic content of the extracts. The presence of flavonoids in quinoa have also been previously linked to antimicrobial activity by other authors (Tang et al., 2015), and may be responsible for the positive results found in the herein studied samples.

3.2 Amaranth

3.2.1 Nutritional value and physicochemical characterisation of amaranth

Figure 13 shows the main characteristics of *Amaranthus cruentus* L. – BRS Alegria and the determined composition of macronutrients, free sugars, organic acids, tocopherols, and fatty acids are presented in **Table 13**.

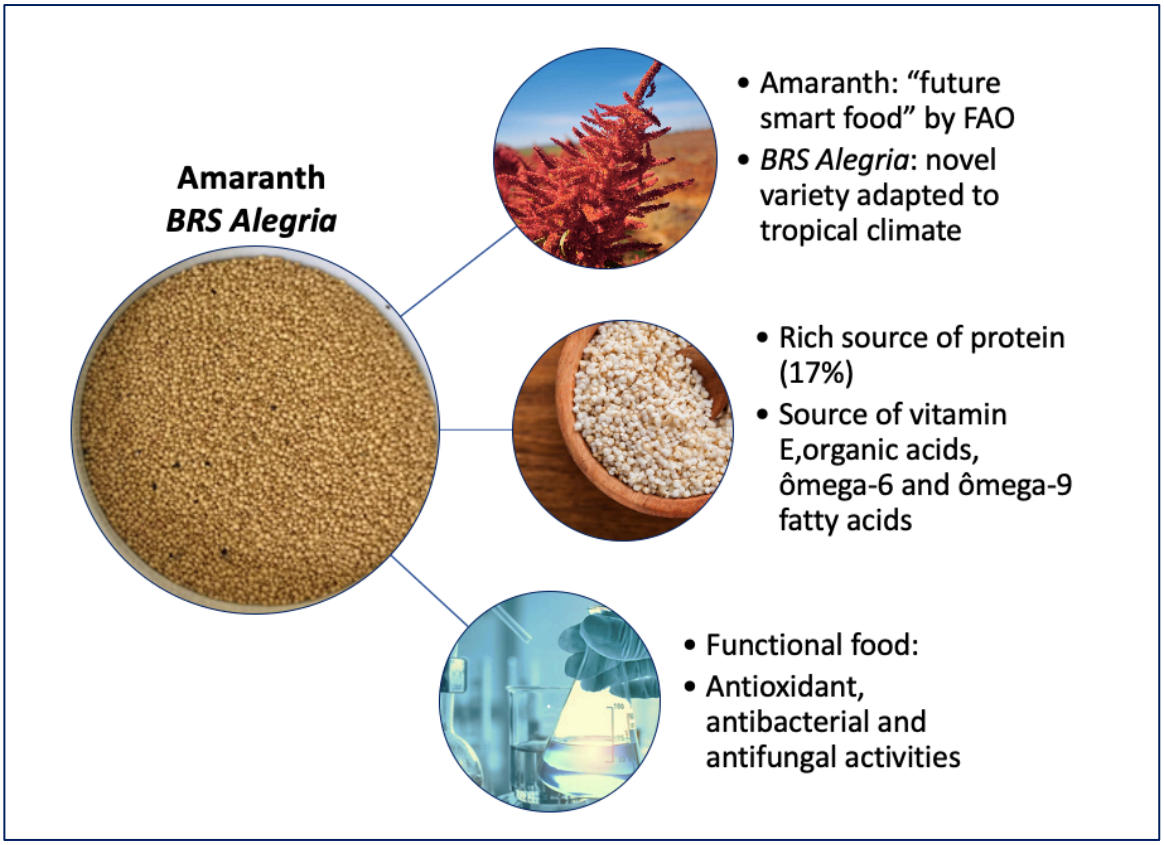


Figure 13. Brazilian pseudograin amaranth cv. BRS Alegria.

Table 13. Nutritional value, physicochemical composition, fatty acids profile and tocopherols of *Amaranthus cruentus* L. – BRS Alegria pseudograins (mean ± SD).

Nutritional value (g/100 g fw)		**Organic acids** (g/100 g fw)	
Moisture (%)	5.7±0.1	Oxalic acid	0.95±0.03
Proteins	17.2±0.3	Quinic acid	7.9±0.7
Lipids	5.1±0.2	Malic acid	0.105±0.003
Carbohydrates	67.9±0.2	Fumaric acid	traces
Ash	2.8±0.1	Total organic acids	8.9±0.8
Energy (kcal/100 g fw)	368±1		
Free sugars (g/100 g fw)		**Physicochemical variables**	
Fructose	0.10±0.02	NaCl (g/100 g fw)	0.48±0.04
Glucose	0.11±0.01	pH	6.17±0.01
Sucrose	1.06±0.08	Hunter scale colour parameters	
Raffinose	0.62±0.04	*L**	75.2±0.9
Total free sugars	1.3±0.1	*a**	3.9±0.3
		*b**	21±1
Fatty acid	**%**	**Fatty acid**	**%**
C8:0	0.01±0.01	C18:3n3	0.73±0.01
C10:0	0.04±0.01	C20:0	0.82±0.01
C12:0	0.05±0.01	C20:1	0.27±0.01
C14:0	0.26±0.01	C20:2	0.07±0.01
C15:0	0.07±0.01	C21:0	0.03±0.01
C16:0	21.28±0.01	C22:0	0.38±0.01
C16:1	0.17±0.01	C23:0	0.09±0.01
C17:0	0.11±0.01	C24:0	1.09±0.01
C18:0	3.70±0.01	**SFA**	27.91±0.01
C18:1n9	33.78±0.02	**MUFA**	34.21±0.01
C18:2n6	37.08±0.02	**PUFA**	37.88±0.02

Tocopherols (mg/100 g fw)	
α-tocopherol	0.421±0.001
γ-tocopherol	0.369±0.004
Total tocopherols	0.792±0.005

Caprylic acid (C8:0); capric acid (C10:0); lauric acid (C12:0); myristic acid (C14:0); pentadecanoic acid (C15:0); palmitic acid (C16:0); palmitoleic acid (C16:1); margaric acid (C17:0); stearic acid (C18:0); oleic acid (C18:1n9); linoleic acid (C18:2n6); α-linolenic acid (C18:3n3); arachidic acid (C20:0); cis-11-eicosenoic acid (C20:1); eicosadienoic acid (C20:2); heneicosanoic acid (C21:0); behenic acid (C22:0); tricosylic acid (C23:0); lignoceric acid (C24:0). SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

The studied amaranth samples showed high protein content (17.2 g/100 g fw), which was higher than the 14.9% reported for *Amaranthus cruentus* grains in a review by Coelho et al. (2018), comprising different cultivars. Nevertheless, Palombini et al. (2013) found an even higher protein content in a previous study on the same amaranth variety (20.9 g/100 g fw). Considering other major grains, amaranth presents higher protein content than wheat (12.3%), maize (8.9%) and rice (7.5%) (Graf et al., 2015). Additionally, amaranth has been rated above other grains in terms of protein quality due to its high content of lysine, among other essential amino acids (Coelho et al., 2018). Therefore, the amaranth cultivar herein studied is a rich plant-based source of protein and a suitable for incorporation into vegetarian diets.

Amaranth protein can also be a source of bioactive peptides, presenting benefits that go beyond their nutritional properties, such as anti-hypertensive activity. In a recent study, Suárez et al., (2020) demonstrated that amaranth peptides produced a significant decrease in the blood pressure of spontaneously hypertensive rats (from values of 200-220 mm Hg to values of 140–170 mm Hg), suggesting that amaranth protein could be used in the elaboration of functional foods for hypertensive individuals. Previously, Sabbione et al. (2018) demonstrated the potential anti-hypertensive activity of amaranth peptides applied to gluten-free cookies. Simulated gastrointestinal digestion of the product released peptides capable of exerting potential and antihypertensive activities, besides antithrombotic effect. The major macronutrients in our samples of *Amaranthus cruentus* L. – BRS Alegria were carbohydrates, accounting for 67.9 g/100 g fw. This result was slightly higher than the 64.8% total carbohydrate content reported in a previous study of the same amaranth cultivar (Palombini et al., 2013), and it was also greater than the range of 60.7-62.6 (% dry weight basis) described by Arendt and Zannini (2013) for *Amaranthus cruentus* grains of different varieties.

Soluble free sugars can only be found in small amounts in amaranth. Herein, four distinct molecules were identified: two monosaccharides (fructose and glucose), one disaccharide (sucrose) and one trisaccharide (raffinose). Sucrose was the most abundant free sugar (1.06 g/100 g fw), in agreement with Arendt and Zannini (2013) who described sucrose as the most abundant sugar for the two amaranth species *A. cruentus* and *A. caudatus,* however within a slightly greater range (1.84 to 2.17 g/100 g). In the studied Brazilian amaranth, the second most abundant sugar was raffinose (0.62 g/100 g fw), followed by glucose (0.11 g/100 g fw) and fructose (0.10 g/100 g fw).

The total fat content of amaranth can be up to three times higher than that of other cereals and presents a great variability between species (Arendt & Zannini, 2013). Several authors have related amaranth's high fatty acid content to hypocholesterolemic, antioxidant and anticarcinogenic effects (Coelho et al., 2018). The amaranth BRS Alegria presented a total fat content of 5.1 g/100 g fw. The fatty acids profile was composed of 72.1% of unsaturated fatty acids, 37.9% of which polyunsaturated (PUFAs). The major PUFA was linoleic acid (C18:2n6, 37.08%), whereas α-linolenic acid (C18:3n3) accounted for only 0.73%. Monounsaturated fatty acids (MUFAs) were mostly represented by oleic acid (C18:1n9, 33.78%). The major saturated fatty acid found was palmitic (C16:0, 21.28 %). Palombini et al. (2013) described a higher total lipid content of 8.7% for the same amaranth variety. Those authors also reported an oleic acid content of 31.33% and a linoleic acid content of 38.02%, similar to the results herein found (33.78% and 37.08, respectively).

Tocopherols are fat-soluble compounds that constitute the major isoforms of vitamin E. These compounds have been associated with a reduced risk of cancer due to their strong antioxidant properties (Gupta & Suh, 2017). In the present study, α- and γ- tocopherol were the tocopherol isomers found. α-Tocopherol was found in a mean concentration of 0.42 mg/100 g fw, which

was lower than the content of 1.15 mg/100 g reported by Palombini et al. (2013) for the same amaranth variety. Those authors also reported a β+γ-tocopherol concentration of 1.35 mg/100 g. Herein, a mean concentration of 0.369 mg/100 g was found for γ-tocopherol, whereas β-tocopherol was not detected.

Four organic acids were detected, being quinic acid the major one (7.87 g/100 g fw), followed by oxalic (0.95 g/100 g fw), malic (0.11 g/100 g fw) and fumaric acids (trace amounts). In a recent study, Heikkilä et al. (2019) suggested that quinic acid could be helpful in the treatment of diabetes, by promoting insulin secretion from pancreatic beta cells, in an *in vivo* mice model.

he results for salt (NaCl) content in the amaranth BRS Alegria showed a very low mean value of 0.48 g/100 g fw, suggesting its suitability for the development of new processed food products with low sodium content.

Regarding physical features, the Brazilian amaranth grains BRS Alegria presented a pH value of 6.17, being slightly less acidic than the herein studied Brazilian quinoa BRS Piabiru (5.65) (section 3.1.1). For the colour assessment, all measurements corresponded to the outer layer of the grains, i.e., before any milling process was employed. The amaranth grains were characterised by a high mean value of parameter L^* (75.21), which indicates high luminosity; a low value of parameter a^* (3.87), indicating the presence of red tones; and a higher parameter b^* value (20.67), which is associated to the visible yellow colour of the pseudograins outer layer **(Figure 14)**.

3.2.2 Bioactive properties of amaranth

Reactive species or free radicals can cause severe damage to the cell membrane by reacting with biomolecules such as proteins and lipids, as well as with DNA, potentially causing

carcinogenesis and degenerative processes such as cardiovascular disease (Coelho et al., 2018). Natural products with antioxidant activity might exert a positive effect when ingested by inactivating these free radicals. Considering that antioxidant compounds act by distinct mechanisms, in this study we employed two different cell-based *in vitro* techniques to assess the antioxidant capacity of the hydroethanolic extracts of *Amaranthus cruentus* L. – BRS Alegria. The results of both assays are presented in **Table 14**.

The amaranth extract was effective in diminishing the production of TBARS substances, which result from lipid peroxidation-induced oxidative stress. The extract presented a mean EC_{50} value of 2801 µg/mL. The antioxidant properties of the hydroethanolic extracts were also confirmed by OxHLIA, a cell-based assay useful to assess the inhibition of free radical-induced haemolysis in sheep erythrocytes. A mean concentration of 197 µg/mL of hydroethanolic extract was found to inhibit the haemolysis by 50% for 30 minutes. To the best of the authors' knowledge, this was the first-time that the OxHLIA assay was employed to evaluate the antioxidant properties of amaranth extracts. Other authors have also reported positive results for *in vitro,* non-cell-based, antioxidant assays of amaranth, for instance DPPH radical scavenging assay, ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC) and scavenging of ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)] assay (Alvarez-Jubete, Wijngaard, Arendt, & Gallagher, 2010; López-Mejía, López-Malo, & Palou, 2014; Orsini Delgado, Tironi, & Añón, 2011; Palombini et al., 2013; Paśko et al., 2009; Tironi & Añón, 2010). Also to the best of the authors' knowledge, only one study has been previously conducted to assess the antioxidant activity on the same amaranth variety herein investigated (Palombini et al., 2013), with also positive results (IC_{50} average value of 638.67 µg/mL of methanolic extract, employing a DPPH assay).

The results for antimicrobial activity are also shown in **Table 14**. The amaranth BRS Alegria extract was tested against three Gram-positive and three Gram-negative bacteria, besides six fungi. The inhibitory and antibacterial activities displayed by the extract against both Gram-positive (*S. aureus*, *B. cereus* and *L. monocytogenes*) and Gram-negative (*E. coli*, *S. typhimurium* and *Enterobacter cloacae*) bacteria indicate the existence of an extensive antibiotic spectrum for their phytochemical constituents. The extract was active against all six tested bacteria, presenting the highest activity against *Bacillus cereus*. Additionally, the amaranth extract was also effective against all six fungi tested, even showing better results in comparison with the fungicidal ketoconazole against *P. ochrochloron*. Other authors have also previously indicated the antifungal (Mosovska & Birosova, 2012), antibacterial (Broekaert et al., 1992; Mosovska & Birosova, 2012) and antimicrobial (Pribylova, Kralik, Pisarikova, & Pavlik, 2008) properties of amaranth, which have been linked to constituents such as bioactive peptides and phytochemicals.

Finally, the hydroethanolic extract herein studied did not present toxicity against primary porcine liver cells PLP2. As shown in **Table 14**, the GI_{50} value was higher than the highest tested concentration (400 μg/mL)**.** Considering the herein results that confirm the absence of cytotoxicity, the prepared amaranth extracts could be potentially applied in food and pharmaceutical formulations, without compromising their safety.

Table 14. Bioactivities of *Amaranthus cruentus* L. – BRS Alegria hydroethanolic extracts.

Antioxidant activity (*in* vitro cell-based) (mean ± SD)	**Citotoxicity** (GI_{50} µg/mL values)
TBARS (EC_{50}, µg/mL): 2801±72	**PLP2**: >400
OxHLIA (IC_{50}, µg/mL), Δt = 30 min: 197±10	

Antimicrobial activity (mg/mL)			
Bacteria	**Amaranth** MIC/MBC	**Streptomycin** MIC/MBC	**Ampicillin** MIC/MBC
Staphylococcus aureus	2.0/4.0	0.04/0.1	0.25/0.45
Bacillus cereus	1.0/2.0	0.1/0.2	0.25/0.4
Listeria monocytogenes	4.0/8.0	0.2/0.3	0.4/0.5
Escherichia coli	2.0/4.0	0.2/0.3	0.4/0.5
Salmonella typhimurium	4.0/8.0	0.2/0.3	0.75/1.2
Enterobacter cloacae	4.0/8.0	0.2/0.3	0.25/0.5
Fungi	**Amaranth** MIC/MFC	**Ketoconazole** MIC/MFC	**Bifonazole** MIC/MFC
Aspergillus fumigatus	1.0/2.0	0.25/0.5	0.15/0.2
Aspergillus ochraceus	1.0/2.0	0.2/0.5	0.1/0.2
Aspergillus niger	0.5/1.0	0.2/0.5	0.15/0.2
Penicillium funiculosum	1.0/2.0	0.2/0.5	0.2/0.25
Penicillium ochrochloron	1.0/2.0	2.5/3.5	0.2/0.25
Penicillium verrucosum var. cyclopium	0.5/1.0	0.2/0.3	0.1/0.2

EC_{50}: extract concentration corresponding to 50% of antioxidant activity (against lipid peroxidation). IC_{50}: values are the concentration of extract required to inhibit (delay) 50% of haemolysis for 30 min (IC_{50} (30 min)). GI_{50}: values correspond to the sample concentration achieving 50% of growth inhibition in liver primary culture PLP2. MIC: minimal inhibitory concentration (mg/mL). MBC: minimal bactericidal concentration (mg/mL). MFC: minimal fungicidal concentration (mg/mL).

3.3 Potatoes

3.3.1 Nutritional characterisation of fifty potato genotypes

Figure 14 shows examples of the different colours of the studied potato samples. The results obtained for the nutritional profile of the fifty assayed samples are collected in **Table 15**. In general, the profiles were quite similar, independently of flesh colour (FC) or cultivation type (CT). In the case of CT, the only variables with significant differences were fat (higher in landraces), protein (higher in Andean accessions) and ash (higher in landraces), nonetheless without significantly changing (p = 0.506) the energy value. Likewise, fat (higher concentrations in purple accessions) and ash (higher concentrations in red accessions) were the only nutritional parameters with significant differences among different FC. The energy and carbohydrates content remained unchanged in all the studied FC and CT.

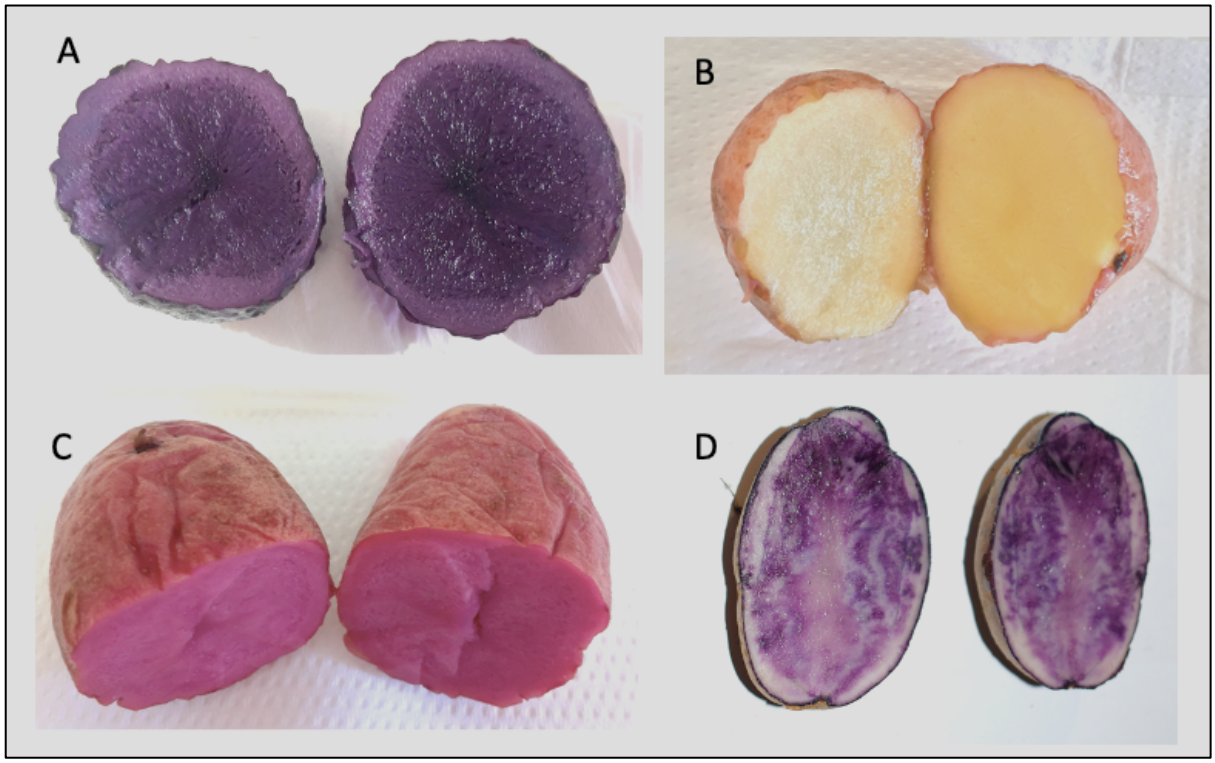


Figure 14. Examples of the different colours of the studied potato genotypes. A: UACH 0917 from Chile; B: R 93/25 from Romania; C: Red Cardinal from the United Kingdom; D: Shetland Black from the United Kingdom.

Considering all the studied fresh potato samples, moisture content ranged from 72.4 to 74.5 g/100 g fw between the different cultivation types and from 74.5 to 76.6 when the genotypes were grouped based on their tuber flesh colour. Similar values, although within a wider range (70.36 to 81.97 g/100 g fw), were reported by Calliope et al. (2018) for potato varieties from the Andean region grown in South America. Burlingame et al. (2009), in their turn, determined moisture values in a still higher range (62.68-87.0 g/100 g fw), which can be explained by the great number of varieties comprised in their literature review study on the chemical composition of potatoes. Moreover, according to Zhou, Plauborg, Kristensen, & Andersen (2017), dry mater production is correlated with the nitrogen application regimes and the mean air temperature during the stage between the end of tuber formation initiation and maturity. In the same context, Petropoulos et al. (2020) reported that nitrogen application rates may affect dry matter allocation in plant parts (shoots, leaves and tubers) in a genotype dependent manner. Therefore, differences in the moisture (or dry matter) content among the studies could be partly attributed to differences in growing conditions and fertilization practices.

Potato is not considered a rich source of protein, therefore its content was expected to be low, and this was confirmed by the results found in our study (from 1.8 to 2.1 g/100 g fw, among the cultivation types, and from 1.9 to 2.0 g/100 g fw, among the different flesh colours). Vaitkevičienė (2019) reported similar values for protein content in the flesh of coloured potato varieties (an average of 2.12 g/ 100 g fw), while Calliope et al. (2018), Burlingame et al. (2009) and Petropoulos et al. (2020) reported slightly wider ranges (from 1.93 to 4.85 g/100 g fw, 0.85-4.2 g/100 g fw and 1.4-2.6 g/100 g fw, respectively), which could be explained by differences in the factors that affect dry matter production, as mentioned before (growing conditions and nitrogen fertilization regime).

Ash content varied between 0.8 and 1.1 g/100 g fw and 0.9 and 1.3 g/100 g fw for the different cultivation types and flesh colours, respectively. Similar results for ash content were found by Calliope et al. (2018) (from 0.95 to 1.73 g/100 g fw). The observed differences in the ash content of the studied potatoes could be explained by the effect that factors such as climate, altitude and pH soil may have on dry matter allocation and the mineral composition of tubers.

Carbohydrates were the main macronutrients in concentrations, which varied from 22.0 to 24.5 g/100 g fw and 20.0 to 22.7 g/100 g fw (different CT and FC, respectively). In contrast, Calliope et al. (2018) and Petropoulos et al. (2020) found a wider range of available carbohydrates (11.87 to 24.00 g/100 g fw and 14.87 to 22.12 g/100 g fw, respectively).

Lipid content was very low, with values ranging from 0.15 to 0.32 g/100 g fw and 0.18 to 0.27 g/100 g fw (different CT and FC, respectively), whereas slightly higher ranges were reported by Burlingame et al. (2009) (0.05–0.51 g/100 g fw) and Petropoulos et al. (2020) (0.06 to 0.31 g/100 g fw). Finally, energy content ranged from 99 to 108 kcal/ 100 g fw and from 90 to 100, when considering the different cultivation types and tuber flesh colour, respectively, whereas Burlingame et al. (2009) reported a wider range among the tested Andean potatoes (57-100 kcal/100 g fw).

Table 15. Nutritional profile and physicochemical features of the studied potato genotypes in relation to cultivation type (CT) and the colour of tuber flesh (FC) (mean ± SD).

	Moisture (g/kg fw)	Fat (g/kg fw)	Protein (g/kg fw)	Ash (g/kg fw)	Carbohydrates (g/kg fw)	Energy (kcal/100 g fw)	Salt (g/kg fw)	*L**	*a**	*b**	pH
Cultivation type (CT)											
Andean accessions	724±25	1.5±0.5 b	21±4 a	8±2 b	245±29	108±11	2.6±0.3 a	61±20 b	1±8 b	26±19	6.7±0.4 a
Cultivated varieties	745±92	1.6±0.5 b	20±5 ab	10±3 ab	224±95	99±37	2.1±0.5 b	63±16 b	2±9 b	34±90	6.0±0.5 b
Landraces	745±92	3.2±0.5 a	18±2 b	11±3 a	224±48	99±20	1.9±0.4 b	55±20 b	6±10 a	19±15	5.8±0.1 b
Breeder lines	743±80	1.5±0.4 b	20±3 ab	8±1 b	225±69	99±27	2.1±0.2 b	73±1 a	-2±2 b	24±3	5.9±0.1 b
Levene's test[1]	0.152	0.006	<0.001	0.002	0.125	0.126	<0.001	<0.001	0.002	0.568	0.163
ANOVA test[2]	0.537	<0.001	0.001	<0.001	0.557	0.506	<0.001	<0.001	<0.001	0.443	<0.001
Tuber flesh colour (FC)											
Yellow	743±83	1.8±0.5 B	19±5	9±3 B	227±85	100±34	2.4±0.5	69±9 A	-1±2 D	27±6 A	6.0±0.5
Purple	749±31	2.7±0.5 A	20±3	9±2 B	218±32	98±13	2.1±0.5	25±2 D	13±2 B	-4±3 D	6.0±0.5
Red	766±19	1.9±0.5 B	20±2	13±1 A	200±19	90±8	2.3±0.3	37±2 C	31±2 A	3±3 C	5.9±0.1
Marble	764±8	2.5±0.2 AB	20±1	9±1 B	204±8	92±3	2.1±0.2	57±2 B	5±1 C	17±1 B	5.8±0.1
Levene's test[1]	0.006	<0.001	<0.001	<0.001	0.007	0.009	0.314	<0.001	<0.001	<0.001	0.065
ANOVA test[2]	0.374	<0.001	0.575	<0.001	0.272	0.309	0.816	<0.001	<0.001	<0.001	0.779

[1]*p*-values < 0.050 indicate heteroscedastic distributions; *p*-values > 0.050 indicate homoscedastic distributions.
[2]The indicated p-values were obtained from Tukey's HSD test in the case of homoscedastic distributions or Tamhane's T2, in the case of heteroscedastic distributions.
Small case letters identify parameters with significant differences among different CT. Upper case letters identify parameters with significant differences among different FC.

3.3.2 Physicochemical characterisation of fifty potato genotypes

The results for salt (NaCl) content, colour parameters and pH are also presented in **Table 15**. The Andean accessions presented significantly higher values of salt content (2.6 g/kg fw) than the other studied CT (1.9 - 2.1 g/kg fw). Nevertheless, there was no significant difference between the four tuber colours (FC) for NaCl concentration (values ranged between 2.1 - 2.4 g/kg fw). The same trend was found for the pH values, as only the Andean accessions presented significantly higher pH values (mean 6.7) than the other CT (5.8 - 6.0), and no significant difference was found among the studied FC (values ranged between 5.8 and 6.0). With exception of the Andean accessions, the pH values found in this study are in agreement with those reported by Romano et al. (2018) (5.42 – 6.25) who evaluated twenty-one commercial potato varieties grown in Italy. Similarly, Feltran, Lemos, & Vieites (2004) found values between 5.16 and 5.94 for various potato cultivars with large sized tubers cultivated in Brazil, while Yang & Achaerandio (2015) reported higher values, within the range of 5.89 and 6.00. The pH value of pulp is related to the suitable uses of tubers (table, processing, starch, universal), being negatively correlated with sugars accumulation, while values higher than 5.5 are associated with lower starch degradation due to the inactivation of phosphorylase enzyme above 5.5 values (Feltran et al., 2004).

For the quantitative colour assessment, as expected, significant differences were found between the four FC (yellow, purple, red and marble) for all measured parameters (*L**, *a** and *b*)*. The yellow-fleshed tubers presented the highest values for *L** (lightness) *and b** (yellow colour), whereas the purple-fleshed tubers presented the lowest values for the same parameters. This can be associated to the qualitative light-yellow and the deep-purple colours of the flesh of the different potato genotypes **(Figure 14)**. In their turn, the red-fleshed tubers presented the

highest *a** values, which are associated to the red colour, while the yellow tubers had the lowest ones. Among the different CTs, the breeder lines' tubers presented significantly higher *L** values, associated to lighter flesh tones. Interestingly, the landraces' samples showed significantly higher *a** values, which can be associated to a more prominent presence of red tones in tubers of this CT. According to the literature, apart from the genotypic effect on colour parameters of varieties with different flesh colour, growing conditions (location of cultivation) and cultivation system (organic *vs* conventional farming) may also affect colour parameters in potato tuber skin and flesh (Lombardo, Pandino, & Mauromicale, 2017; Yang & Achaerandio, 2015).

Three organic acids were detected in the samples, namely oxalic, malic and citric acids (**Table 16**). Citric acid was the most abundant in all FC and CT (values ranged between 5-8 g/kg fw and 4-5 g/kg fw, respectively), followed by malic and oxalic acids, respectively. The Andean accessions were the richest in citric acid (8 g/kg fw), as well as in total organic acids (12 g/kg fw), being significantly different from the other CTs. The citric acid content of the cultivated varieties was statistically equal to landraces and breeder lines cultivation types. Among the studied FC, the marble-fleshed varieties were the richest in organic acids content (11 g/kg fw), particularly in oxalic acid (2.8 g/kg fw), while red-fleshed cultivars were the richest in malic acid (3.8 g/kg fw). The citric acid content among the four studied FC was statistically equal. Besides the organic acids detected herein (citric, malic and oxalic), Burlingame et al. (2009) and Petropoulos et al. (2020) also reported the presence of ascorbic acid in potato tubers (in concentrations of up to 42 mg/100 g fw and 17 mg/100 g fw, respectively). Furthermore, earlier studies on potato tubers identified succinic, aconitic, lactic, tartaric and fumaric acid in amounts that differed among the studied cultivars (Galdón, Mesa, Elena, Rodríguez, & Romero, 2010; Wichrowska, Rogozińska, & Pawelzik, 2009; Yang & Achaerandio, 2015).

Two monosaccharides, namely fructose and glucose, and two disaccharides, sucrose and trehalose, were detected in the samples (**Table 16**). The most abundant free sugar among all CT and FC was glucose, which content was significantly higher for the purple FC and lower for the Andean accessions (400 mg/100 g fw and 200 mg/100 g fw, respectively). At the same time, the Andean accessions presented significantly higher values for sucrose and trehalose (230 mg/100 g fw and 60 mg/100 g fw, respectively) and lower amounts of fructose (200 mg/100 g fw) than the other tested FC. The total sugars content remained almost the same among all CT, whereas the purple and marble-fleshed had the highest content among the tested FC (800 and 700 mg/100 g fw) without being significantly different from each other. In agreement with our findings, Plata-Guerrero, Guerra-Hernández, & García-Villanova (2009) reported a similar profile of free sugars, where glucose was the most abundant one. In contrast, although Burlingame et al. (2009) reported the presence of glucose (15–340 mg/100 g fw), fructose (0–180 mg/100 g fw) and sucrose (80–1390 mg/100 g fw) in different potato varieties, sucrose was found to be the most abundant free sugar. Similarly, Petropoulos et al. (2020) reported the presence of the same three main sugars (sucrose, fructose and glucose) with sucrose being the most abundant one. This contradiction in the results was also found by Yang & Achaerandio (2015), who also detected differences in the profile of free sugars among eight commercial cultivars. In contrast, Wegener, Jansen, & Hans-Ulrich (2009) did not observe significant differences in reducing sugars (glucose and fructose) content between two potato groups (white/yellow-fleshed and purple-fleshed), whereas sucrose content of purple-fleshed genotypes was twice than that of white/yellow-fleshed potatoes. According to Kumar, Singh, & Kumar (2004), apart from the genotype, several pre-harvest factors such as crop (tuber) maturity, mean air temperature during cultivation, and irrigation and fertilization management may also affect free sugars content in tubers.

Table 16. Organic acids and free sugars profiles of the fifty studied potato genotypes in relation to cultivation type (CT) and the colour of tuber flesh (FC) (mean ± SD).

Cultivation type	Oxalic acid (g/kg fw)	Malic acid (g/kg fw)	Citric acid (g/kg fw)	Organic acids (g/kg fw)	Fructose (g/kg fw)	Glucose (g/kg fw)	Sucrose (g/kg fw)	Trehalose (g/kg fw)	Total free sugars (g/kg fw)
Andean accessions	1.2±0.4	2.8±0.5	8±1 a	12±1 a	2.0±0.4 c	2±1 b	2.3±0.5 a	0.6±0.2 a	7±2
Cultivated varieties	1.5±0.5	2.4±0.5	5±2 bc	9±3 b	2.5±0.4 ab	3±1 a	0.7±0.3 b	0.3±0.2 b	7±2
Landraces	1.2±0.5	2.5±0.4	5±1 c	8±1 b	2.3±0.3 bc	3±1 a	0.7±0.3 b	0.3±0.2 b	7±2
Breeder lines	1.2±0.5	2.3±0.3	6±1 b	9±1 b	2.8±0.2 a	4±1 a	0.7±0.2 b	nd	7±1
Levene's test[1]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ANOVA test[2]	0.059	0.180	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.717
Tuber flesh colour (FC)									
Yellow	1.4±0.5 B	2.2±0.5 C	5±1	9±2 B	2.4±0.5 AB	3±1 B	0.8±0.5	0.3±0.2 B	6±1 B
Purple	0.9±0.5 B	2.8±0.5 BC	5±1	9±2 B	2.8±0.5 A	4±1 A	0.7±0.5	0.4±0.2 B	8±2 A
Red	0.9±0.5 B	3.8±0.3 A	4±1	9±2 B	2.1±0.4 B	3±1 B	0.9±0.5	0.4±0.2 B	6±2 B
Marble	2.8±0.2 A	3.2±0.3 B	5±1	11±1 A	2.2±0.2 AB	3.1±0.3 B	0.9±0.1	0.6±0.2 A	7±1 AB
Levene's test[1]	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	0.001	<0.001	0.003
ANOVA test[2]	<0.001	<0.001	0.088	0.044	0.001	<0.001	0.685	0.001	<0.001

[1] *p*-values < 0.050 indicate heteroscedastic distributions; *p*-values > 0.050 indicate homoscedastic distributions.

[2] The indicated p-values were obtained from Tukey's HSD test in the case of homoscedastic distributions or Tamhane's T2, in the case of heteroscedastic distributions.

Small case letters identify parameters with significant differences among different CT. Upper case letters identify parameters with significant differences among different FC.

nd = not detected

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The fatty acids profiles are presented in **Table 17**. In total, twenty-two fatty acids were identified, however, half of them in relative percentages inferior to 1%. The majority of the detected fatty acids were saturated ones (SFA, 74-88%), followed by polyunsaturated (PUFA, 11-21%) and a small percentage of monounsaturated fatty acids (MUFA, 1.7-5.1%). In regard to the studied CT, palmitic acid was the most abundant (C16:0; 40-48%), followed by stearic acid (C18:0; 14-22%) and linolenic acid (C18:2n6; 9-17%); similar distribution was found when grouping of cultivars based on flesh colour. The Andean accessions and the coloured tubers (purple and red) were the richest in PUFA, while the yellow-fleshed and breeder lines' tubers were the richest in SFA. In agreement with our study, Petropoulos et al. (2020) reported the same fatty acids as being the most abundant ones in two potato cultivars (Spunta and Kennebeq), and a similar class classification was also observed, with SFA being the most abundant type of fatty acids. In contrast, Dobson, Griffiths, Davies, & McNicol (2004) reported linolenic acid as the most abundant fatty acid in four potato cultivars (49.2-50.2%), followed by palmitic (18.5-20.3%) and α-linoleic acid (16.5-19.5%), whereas stearic acid was detected in lower proportion (3.9-5.6%). However, these results refer to composition at 4 weeks after harvest, and according to Yang & Bernards (2006) wound induction after harvest may result in the formation of new fatty acids. In the same line, Camire, Kubow, & Donelly (2009) reported that PUFA were the most abundant fatty acids class, although this report refers to cooked (baked or boiled) tubers.

Table 17. Major fatty acids profiles[1] (relative percentage) of the fifty studied potato genotypes.

Cultivation type	C6:0	C11:0	C14:0	C16:0	C18:0	C18:1n9	C18:2n6	C20:0	C22:0	C23:0	C24:0	SFA	MUFA	PUFA
Andean accessions	0.7±0.4 b	0.8±0.4 b	1.7±0.5	46±4 ab	14±1 c	5±1 a	17±3 a	2.6±0.4	1.9±0.4 b	1.6±0.2	2.0±0.3	74±4 c	5±1 a	21±4 a
Cultivated varieties	1.2±0.5 b	3±2 a	1.9±0.5	42±6 c	20±4 b	5±2 a	12±5 b	2.5±0.5	1.8±0.5 b	2±1	2.2±0.5	81±8 b	5±2 a	14±6 b
Landraces	2.1±0.5 a	4±2 a	2.1±0.5	43±7 bc	18±3 b	5±2 a	10±5 b	2.4±0.5	2.2±0.5 b	2±1	2.1±0.5	84±7 ab	5±2 a	11±6 bc
Breeder lines	0.8±0.4 b	3.1±0.5 a	1.4±0.4	48±1 a	22±6 a	1.7±0.1 b	9±5 b	2.2±0.3	4±2 a	1.8±0.1	2.6±0.5	88±8 a	1.7±0.1 b	11±7 c
Levene's test[2]	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.024	0.001	<0.001
ANOVA test[3]	<0.001	<0.001	0.404	<0.001	<0.001	0.001	<0.001	0.154	<0.001	0.818	0.276	<0.001	0.001	<0.001
Tuber flesh colour (FC)														
Yellow	1.6±0.5A	5±2	2.0±0.5A	42±9	20±4 A	5±2	11±4 B	2.2±0.5 D	1.8±0.5 B	2.8±0.5	2.0±0.5B	83±8 A	5±2	12±5 B
Purple	0.5±0.3B	0.4±0.2	1.3±0.5B	47±6	15±2 B	4±1	15±5 A	3.1±0.5 C	2.3±0.5 AB	1.8±0.5	2.6±0.5A	77±9 AB	4±1	19±6 A
Red	nd	nd	1.2±0.5B	43±4	17±3 AB	5±1	15±3 A	3.6±0.4 B	2.5±0.4 A	2.2±0.4	2.8±0.4A	76±5 B	5±1	18±5 A
Marble	0.4±0.1B	nd	1.5±0.1B	40±1	19±1 A	5±1	13±1 AB	4.6±0.2 A	2.7±0.2 A	2.0±0.2	2.6±0.2 A	78±1 AB	5.1±0.3	16±1 AB
Levene's test[2]	<0.001	<0.001	<0.001	0.323	<0.001	<0.001	<0.001	0.073	<0.001	0.180	<0.001	<0.001	<0.001	<0.001
ANOVA test[3]	<0.001	<0.001	0.017	0.066	<0.001	0.320	<0.001	<0.001	0.003	0.881	<0.001	<0.001	0.468	<0.001

[1]Besides the tabled fatty acids C8:0, C10:0, C12:0, C13:0, C15:0, C16:1, C17:0, C17:1, C18:3n3, C21:0, C22:2, were also detected in percentages inferior to 1%.

[2]*p*-values < 0.050 indicate heteroscedastic distributions; *p*-values > 0.050 indicate homoscedastic distributions.

[3]The indicated p-values were obtained from Tukey's HSD test in the case of homoscedastic distributions or Tamhane's T2, in the case of heteroscedastic distributions.

3.3.3 Linear discriminant analysis on the nutritional and physicochemical parameters of fifty potato genotypes

Following the characterization of all individual parameters described in the former sections, the overall profiles were analysed by evaluating all differences simultaneously to verify potential linkages to the flesh colour of tubers (FC) or to the cultivation type (CT). This was achieved by linear discriminant analysis (LDA), specifically assessing the correlations among CT or FC (categorical dependent variables) and the obtained results (quantitative independent variables). The significant independent variables were selected with the stepwise method of LDA, following the Wilks' λ test. Only variables with a statistically significant classification performance (p<0.050) were maintained by the statistical model.

Starting by the effect of CT, the three defined discriminant functions included 100% (function 1: 67.5%; function 2: 26.7%; function 3: 5.8%) of the observed variance (**Figure 15**). Among the 48 considered variables, the discriminant model excluded only moisture, carbohydrates, total organic acids, fructose, glucose, total sugars, C14:0, C16:0, C18:1n9c, C18:3n3, C21:0, MUFA and PUFA, which indicate the lack of considerable differences for these parameter among different CT (i.e., these variables had no significant discriminant ability). In what concerns variables selected as being effectively discriminant, function 1 was mainly correlated with C18:2n6c, pH and protein content, all variables with significantly higher values among Andean accessions (markers projected on the positive side of function 1 axis), particularly when compared with landraces CT (markers projected on the farthest position taking Andean accessions as reference). Function 2, in turn, was more correlated with fat (highest content in landraces accessions) and sucrose (maximum value in Andean accessions). Considering the three-dimensional placement of markers corresponding to cultivated varieties and breeder lines, as well as the small percentage explained by function 3, it is obvious that these CT

presented the highest similarities, considering all assayed parameters. Actually, the corresponding markers were only separated by function 3, mostly correlated with C22:0, C12:0 and C10:0. In this first LDA, the classification performance was 100% accurate for the original grouped cases, and 99.2%, for the cross-validated grouped ones.

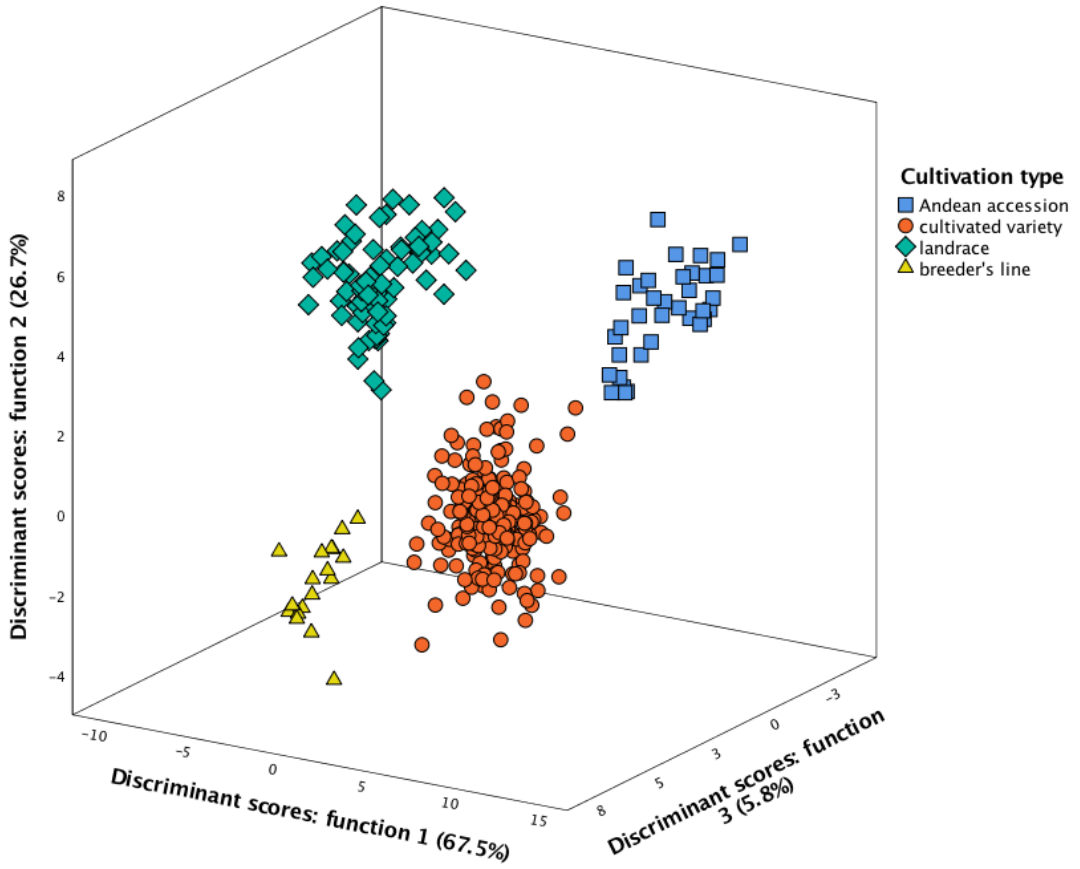


Figure 15. Spatial distribution of CT markers according to the discriminant functions coefficients. Function 1 accounted for 67.5% of the variation, function 2 accounted for 26.7%, while function 3 accounted for 5.8%.

In the case of FC, the observed variation was mainly included in function 1 (function 1: 92.8%; function 2: 5.4%; function 3: 1.8%), as represented in **Figure 16**. Considering that it was intended to identify the variables with highest differences, colour parameters were not included in the analysis, as these would certainly minimize the effect of all other observed results due to the inherent variability among the tested genotypes. Among the remaining 45 variables, the

discriminant model excluded moisture, carbohydrates, energy, citric acid, total organic acids, fructose, glucose, C8:0, C12:0, C18:0, C18:1n9c, C22:0, SFA and PUFA, indicating that these variables had not sufficient differences to be considered as having discriminant ability. In what regards variables with significant differences among the tested FC, C21:0 showed the highest correlation with function 1, which separated yellow flesh potatoes (higher C21:0 percentages) from all the other samples. Function 2, on its side, correlated mostly with C22:2, C17:1 and C16:1, separating primarily red FC from the other assayed samples. To conclude, function 3 was mostly correlated with C18:3n6, oxalic acid and C20:0, effectively separating purple and marble varieties. Overall, these last FC, which were only separated by function 3, presenting nearly the same spatial distribution according to function 1 and function 2, presented close similarity when considering all assayed parameters together. In this second LDA, the classification performance was 100% accurate either for the original grouped cases as well as for the cross-validated grouped ones.

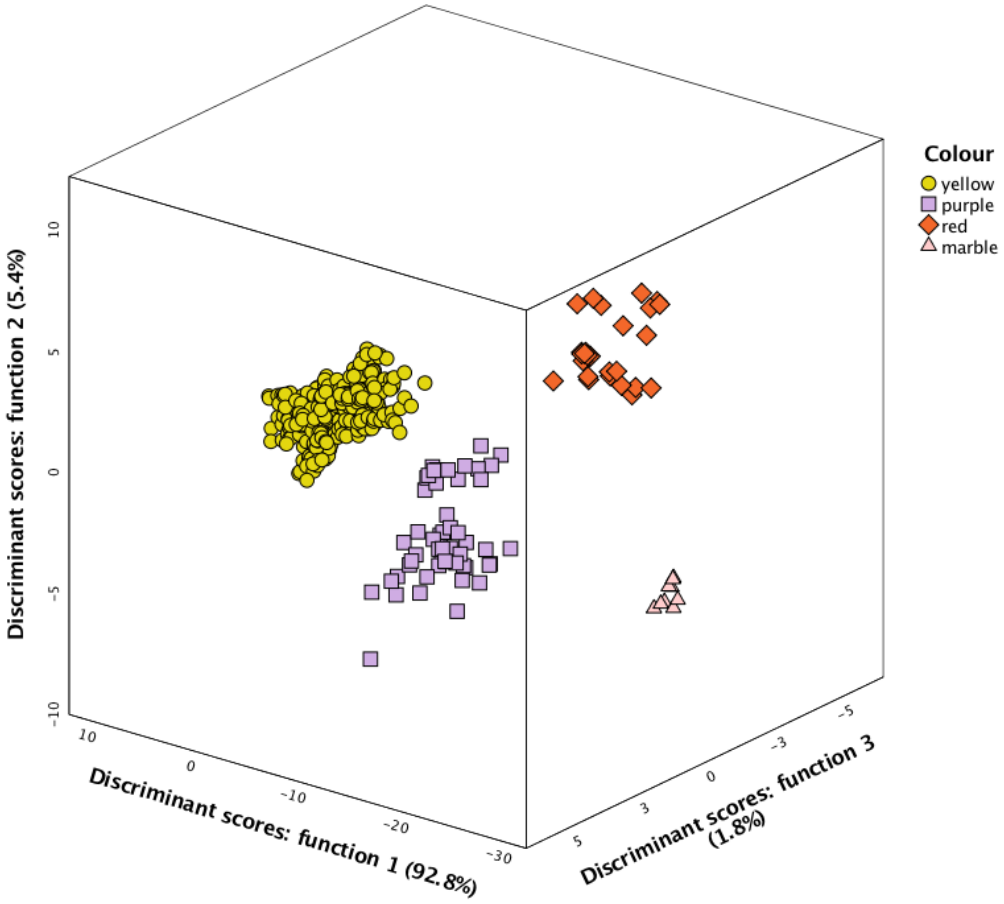


Figure 16. Spatial distribution of FC markers according to the discriminant functions coefficients. Function 1 accounted for 92.8% of the variation, function 2 accounted for 5.4%, while function 3 accounted for 1.8%.

3.4 Coloured potatoes

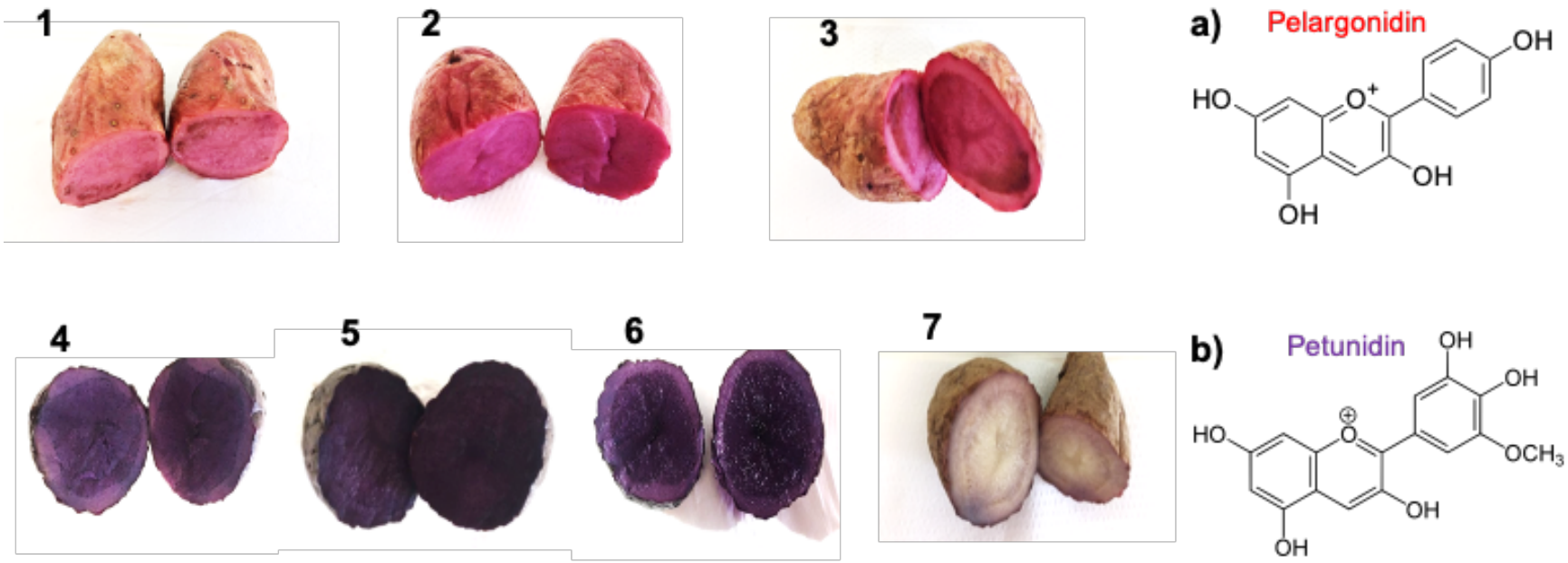


Figure 17. Fresh potato tubers of the genotypes: 1 – Rosemary; 2 - Red Emmalie; 3 - Red Cardinal; 4 – Purple; 5 – Violetta; 6 - Kefermarkter Blaue; 7 – Shetland Black, and the main class of anthocyanidins found in a) red-fleshed varieties and b) purple-fleshed varieties.

3.4.1 Anthocyanin composition of aqueous extracts from coloured potatoes

The anthocyanins profile, chromatographic characteristics, tentative identifications, and quantification of the aqueous extracts from seven coloured potato varieties (**Figure 17**) are shown in **Table 18**. All tentatively identified compounds were previously described in the literature for red and purple-fleshed potatoes (Alcalde-Eon et al., 2004; Kita, Bąkowska-Barczak, Hamouz, Kułakowska, & Lisińska, 2013; Oertel et al., 2017).

Seven peaks were detected in the red-fleshed varieties, six of which were identified as pelargonidin derivatives, based on the observation of their characteristic fragments in MS^2 spectra. **Peak 1r** ($[M]^+$ at *m*/*z* 741) was identified as pelargonidin-3-*O*-rutinoside-5-*O*-glucoside based on the HPLC-DAD-MS results and previous literature reports (Kita et al., 2013; Oertel et al., 2017), and was the major anthocyanin detected in the *Red Cardinal* extract. **Peak 2r** ($[M]^+$ at *m*/*z* 579) also showed an MS^2 fragment at *m*/*z* 271, corresponding to pelargonidin, bearing the loss of a hexose moiety (-162 u) and being assigned as pelargonidin-3-*O*-rutinoside. The remaining peaks were identified as acylated anthocyanins owing to the presence of a shoulder in the UV spectra of the compounds around 310-330 nm. **Peaks 3r**, **4r**, **5r**, **6r**, and **7r** were identified as acylated pelargonidin glycosides. Pelargonidin-3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside (**Peak 5r**) was the main compound detected in the *Red Emmalie* and *Rosemary* extracts. **Peak 3r**, with the same molecular ion, might correspond to an isomer of peak 5r having the glucoside residue located at position 7, which is expected to elute earlier than the corresponding 5-*O*-glucoside, as reported by Alcalde-Eon et al. (2004) for similar petunidin isomers in *Solanum stenotomum* tubers. In agreement with our findings, acylated pelargonidin glycosides have been previously identified by other authors as the main pigments in red potato tubers (Kita et al., 2013; Lewis et al., 1998; Oertel et al., 2017; Rodríguez-Saona et al., 1999).

All three red-fleshed varieties showed similar total anthocyanin content, and the *Red Cardinal* variety presented the highest value (886.2 ± 1.9 mg/100 g extract). Considering the extraction yield of 5% (mass of freeze-dried extract/mass of whole fresh potato), our results are in agreement with those found by Kita et al. (2013) for red-fleshed potato tubers. Moreover, Hamouz et al. (2011) also reported significant differences in total anthocyanin content among red- and purple-fleshed potato cultivars.

Regarding the purple-fleshed varieties, eight peaks were detected, seven of which were tentatively identified as petunidin derivatives (**Peaks 1p** – **6p** and **8p**) and one compound as an acylated peonidin glycoside (**Peak 7p**). The molecular ions and fragmentation patterns of peaks **4p** to **6p** were coherent with derivatives of petunidin-3-*O*-rutinoside-5-*O*-glucoside acylated with caffeic, *p*-coumaric and ferulic acids, respectively, and peak **8p** with petunidin-3-*O*-*p*-coumaroylrutinoside, all of them previously identified in purple tubers of *Solanum tuberosum* (Kita et al., 2013) and *S. stenotomum* (Alcalde-Eon et al., 2004). Similarly, the characteristics of peak **7p** coincide with those of peonidin-3-*O*-feruloylrutinoside-5-*O*-glucoside described in purple tubers of *S. stenotomum* (Alcalde-Eon et al., 2004). Peak **3p** possessed the same molecular ion $[M]^+$ at *m/z* 933 as peak **5p**, but different fragmentation pattern. Two compounds with this molecular ion were also reported by Alcalde-Eon et al. (2004) in purple tubers of *S. stenotomum* that were respectively identified as petunidin-3-*O*-*p*-coumaroylrutinoside-7-*O*-glucoside and petunidin-3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside, although in that case both compounds showed similar MS^2 fragmentation. In the present study, peak **3p** produced a main fragment at *m/z* 625, from the loss of 308 mu that could correspond either to a coumaroylglucoside or a rutinosyl residue, and a second fragment at *m/z* 479 (-146 mu) from the further loss of either a coumaroyl or a rhamnosyl residue. This fragmentation rather seems to suggest an identity where the coumaroyl would be located on a glucose moiety instead on

the rhamnose moiety, e.g., petunidin-*O*-rutinoside-*O*-*p*-coumaroylglucoside or petunidin-*O*-*p*-coumaroylglucosylrhamnoside-glucoside. Since no definite identity could be assigned this peak was just assigned as a positional isomer of peak **5p**. Finally, peaks **1p** and **2p** showed earlier retention times and their absorption spectra lacked a shoulder at 310-330 nm indicating that they were not acylated. Peak **1p** was assigned as petunidin-3-*O*-rutinoside-5-*O*-glucoside, previously identified by Kita et al. (2013) and Lewis et al. (1998) in purple tubers of *Solanum tuberosum.* The MS^2 fragmentation of peak **2p** produced ions at *m/z* 479 (-292 mu; loss of two rhamnosyl residues) and 317 (-162 mu; loss of a glucosyl residue), so that the compound could be assigned as petunidin-*O*-rutinosid*e*-*O*-rhamnoside, or alternatively to petunidin-*O*-dirhamnoside-*O*-glucoside. Petunidin-3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside (**Peak 5p**) was the main anthocyanin found in the extracts obtained from *Violetta*, *Kefermarkter Blaue* and *Shetland Black* tubers. The same compound was reported by Lewis et al. (1998) and Kita et al. (2013) and as the main anthocyanin in different varieties of purple potatoes*,* and by Alcalde-Eon et al. (2004) in purple tubers of *S. stenotomum*. In our study, among the four tested purple-fleshed genotypes, *Kefermarkter Blaue* showed the highest total anthocyanin content (533.4 ± 2.8 mg/100 g extract).

The variability in the anthocyanin content among the studied extracts could be mainly attributed to inherited differences of the potato genotypes, as all samples were subjected to the same environmental factors (growing conditions, planting location and climate), cultivation practices, processing and storage conditions. Oertel et al. (2017) reported a great diversity in polyphenol and anthocyanin profiles of red and purple-fleshed potatoes and suggested differences in the enzymatic reactions leading to the hydroxylation and methylation of B-ring of the precursor dihydroflavonols, resulting in the biosynthesis of a diverse and specific species anthocyanin backbones. In the same context, Chaves-Silva et al. (2018) attributed the

differences in the genetic regulation of biosynthetic pathways through the involvement of different structural and regulatory genes, while Lachman et al. (2012) highlighted the effect of genotype on the anthocyanin content of colour-fleshed potatoes.

Table 18. Chromatographic retention, absorption and mass spectral characteristics, tentative identification, and quantification (mg/100 g extract dw) of anthocyanins found in aqueous extracts from red and purple, potato varieties (mean ± SD).

Red flesh

Peak	Rt (min)	λ_{max} (nm)	$[M]^+$ (*m/z*)	MS^2 (*m/z*)	Tentative identification	Rosemary	Red Emmalie	Red Cardinal
1r	8.27	499	741	579(100),433(15),271(45)	Pelargonidin-3-*O*-rutinoside-5-*O*-glucoside	130 ± 1^{c}	171 ± 1^{b}	264 ± 1^{a}
2r	20.0	501	579	433(100),271(46	Pelargonidin-3-*O*-rutinoside	109 ± 1^{c}	127.9 ± 0.4^{b}	206.1 ± 0.1^{a}
3r	28.08	500	887	725(100),433(5),271(16)	Pelargonidin-3-*O*-*p*-coumaroylrutinoside-*O*-glucoside isomer	106.983 ± 0.003^{c}	116 ± 1^{b}	214 ± 1^{a}
4r	29.86	505	903	741(100),433(16),271(27)	Pelargonidin-3-*O*-caffeoylrutinoside-5-*O*-glucoside	109.7 ± 0.1	nd	nd
5r	35.34	504	887	725(100),433(10),271(13)	Pelargonidin-3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside	235 ± 1*	332 ± 1*	nd
6r	35.54	507	917	755(100),433()21,271(41)	Pelargonidin-3*p*-feruloylrutinoside-5-*O*-glucoside isomer	nd	nd	202 ± 1
7r	36.48	506	917	755(100),463(15),271(38)	Pelargonidin-3-*O*-*p*-feruloylrutinoside-5-*O*-glucoside	123.8 ± 0.3^{c}	128.9 ± 0.4^{b}	202±1^{a}
					Total anthocyanin content	**815 ± 2^{c}**	**875.6 ± 0.3^{b}**	**886 ± 2^{a}**

Purple flesh ***Marble***

Peak	Rt (min)	λ_{max} (nm)	$[M]^+$ (*m/z*)	MS^2 (*m/z*)	Tentative identification	Purple	Violetta	Kefermarkter Blaue	Shetland Black
1p	7.46	530	787	479(100),317(15)	Petunidin-3-*O*-rutinoside-5-*O*-glucoside	134.3 ± 0.1^{a}	50.57 ± 0.01^{c}	113.2 ± 0.2^{b}	125.9 ± 0.2
2p	10.04	515	771	479(100),317(16)	Petunidin-*O*-rutinoside-*O*-rhamnoside or petunidin-*O*-dirhamnoside-*O*-glucoside	nd	43 ± 1	nd	113 ± 1
3p	25.34	525	933	625(100),479(23),317(37)	Positional isomer of peak 5p	nd	43.1 ± 0.4*	104.5 ± 0.2*	102 ± 0.1
4p	27.66	529	949	625(100),479(34),317(46)	Petunidin-3-*O*-caffeoylrutinoside-5-*O*-glucoside	nd	46 ± 1*	106.5 ± 0.1*	102.0 ± 0.2
5p	33.3	530	933	771(100),479(18),317(22)	Petunidin-3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside	112.3 ± 0.1^{c}	175 ± 2^{b}	208 ± 3^{a}	158.2 ± 0.3
6p	34.69	531	963	625(100),479(10),317(30)	Petunidin-3-*O*-feruloylrutinoside-5-*O*-glucoside	101.6 ± 0.2^{b}	51.7 ± 0.3^{c}	113 ± 1^{a}	104.1 ± 0.2
7p	36.44	524	947	785(100),463(23),301(38)	Peonidin-3-*O*-feruloylrutinoside-5-*O*-glucoside	130 ± 1^{a}	64.3 ± 0.6^{c}	106.8 ± 0.2^{b}	117 ± 1
8p	39.13	529	771	625(100),479(14),317(26)	Petunidin-3-*O*-*p*-coumaroylrutinoside	nd	40.3 ± 0.1	nd	nd
					Total anthocyanin content	**478 ± 1^{c}**	**513 ± 4^{b}**	**533 ± 3^{a}**	**481 ± 1**

nd – not detected. Standard Calibration Curve used for quantification: Peonidin-3-*O*-glucoside (y = 151438x - 3E+06, R^2 = 0.9977, LOD = 0.20 µg/mL and LOQ = 0.71 µg/mL). In each column different Latin letters are significantly different according to Tukey's HSD test (p=0.05), for each potato group. *Mean statistical differences obtained by *t*-Student test.

3.4.2 Bioactive properties of aqueous extracts from coloured potatoes

The *in vitro* antioxidant, antibacterial and antifungal properties of the red and purple aqueous extracts were also evaluated, and the results are presented in **Table 19**. Additionally, a hepatotoxicity assay was carried out to assess the safety of the extracts for incorporation into food formulations.

Several studies have demonstrated the antioxidant properties of red and purple potatoes, which have been linked to their high content of anthocyanins (Jayawardana et al., 2012; Kita et al., 2013; Nemś & Pęksa, 2015, 2018). Previous studies have reported positive results for *in vitro* antioxidant assays on coloured potatoes, namely the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the ferric reducing/antioxidant power (FRAP) assays. Herein, a cell-based *in vitro* assay (inhibition of production of TBAR substances) was applied to assess the antioxidant properties of the tested colour-fleshed potato tubers.

As presented in **Table 19**, all seven studied aqueous potato extracts were effective in diminishing the production of TBAR substances, which result from lipid peroxidation-induced breakdown. Among the studied varieties, the purple-fleshed cv. *Violetta* presented the highest antioxidant capacity, as it required the lowest concentration of extract to inhibit the lipid peroxidation process by 50% (IC_{50} value = 380.1 µg/mL) compared to control (Trolox, IC_{50} value = 139±5 µg/mL). For the red-fleshed varieties, cv. *Rosemary* presented the best result (IC_{50} value = 416.9 µg/mL).

Table 19. *In-vitro* antioxidant and cytotoxicity activity (µg/mL) of aqueous extracts from red, purple and marble (yellow/purple) potato varieties (mean ± SD).

Potato varieties	Antioxidant activity (TBARS) IC_{50} values	Cytotoxicity (PLP2) GI_{50} values
Red flesh		
Rosemary	$416.9 \pm 4.9^{e,f}$	>400
Red Emmalie	669.4 ± 4.2^{a}	>400
Red Cardinal	591.79 ± 8.1^{b}	>400
Purple fleshed		
Purple	426.2 ± 2.6^{e}	>400
Violetta	380.1 ± 6.2^{f}	>400
Kefermarkter Blaue	484.5 ± 2.1^{d}	>400
Marble-fleshed (purple/yellow)		
Shetland Black	547.6 ± 6.4^{c}	>400

IC_{50}: extract concentration corresponding to 50% of antioxidant activity (against lipid peroxidation). $GI_{50:}$ values correspond to the sample concentration achieving 50% of growth inhibition in liver primary culture PLP2; maximum tested concentration: 400 µg/mL. Positive control: Trolox IC_{50} value - 139±5 µg/mL (TBARS); Ellipticine GI_{50} value - 3.2±0.7 µg/mL (PLP2). In each line different Latin letters are significantly different according to Tukey's HSD test (p=0.05).

In agreement with our findings, Kita et al. (2013) previously reported positive results for antioxidant activity assays carried out on fresh tubers of red and purple-fleshed potatoes, where for instance the purple variety *Salad Blue* showed significant antioxidant activity (ABTS: 1.56 ± 0.2 mM/100 g dw; DPPH: 0.65 ± 0.3 mM/100 g dw; FRAP: 3.17 ± 0.3 mM/100 g dw). Similarly, Nemš et al. (2015) reported a positive antioxidant activity for snacks enriched with coloured potato flour obtained from the same *Salad Blue* variety, employing *in vitro* assays (ABTS: 1.16 ± 0.07 µmol Trolox/g dw; DPPH: 0.60 ± 0.06 µmol Trolox/g dw; FRAP: 0.80 ± 0.07 µmol Trolox/g dw). Nemś and Pęksa (2018), who also incorporated dried coloured-fleshed potatoes into snacks, reported a beneficial effect on the inhibition of oxidative changes in the lipid profile compared to a control formulation over a 3-months storage period. Moreover, in a study conducted by Jayawardana et al. (2012), the addition of 2% coloured potato flakes into pork sausages suppressed lipid oxidation by 80% compared to the control. Thereby, the antioxidant properties of coloured potatoes could also have a beneficial effect in processed food products by extending their shelf-life.

In order to assess the safety of the studied extracts an *in vitro* cytotoxicity assay was performed. Porcine liver was used as a model. The results revealed that all seven aqueous extracts did not present toxicity against the porcine liver primary culture PLP2, as their GI_{50} values were higher than the highest tested concentration for all varieties (400 µg/mL) **(Table 19).** The verified absence of cytotoxicity suggests the safety of the tested extracts for their utilisation as natural food colourants.

The results for antibacterial and antifungal activities are presented in **Table 20**. All seven extracts presented relevant antibacterial and antifungal activities against all the tested bacteria and fungi strains. Cultivar *Red Cardinal* performed particularly well against bacteria, as the required MIC value of the extract against *Staphylococcus aureus* (2 mg/mL) was half the MIC

value required by the commercial preservative E211, sodium benzoate (4 mg/mL). The same variety also performed better than the preservative E224 (potassium metabisulfite) against *Bacillus cereus*, as it required half of the MIC and MBC values for this bacterium strain.

All seven aqueous extracts behaved equally or better than the preservatives E211 and E224 for all the tested fungi strains. The best results against fungi were achieved by the extract from the *Kefermarkter Blaue* variety, as its antifungal capacity exceeded both preservatives for all the tested strains. The obtained results of the antimicrobial properties indicate that all the studied extracts could be useful in retarding and/or suppressing the growth of food borne microbes, which make them suitable candidates for application in food products as natural preservatives.

Previous studies by other authors also reported positive antimicrobial activities from coloured potato extracts. For instance, Ombra et al. (2015) studied the purple-fleshed variety *Vitelotte Noire*, and found that its anthocyanin-rich extract was effective against the bacteria strains *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus cereus.* Interestingly, those authors also found that the natural bioactive compounds from purple potatoes retained their biological activity (antimicrobial, antioxidant and anti-proliferative) during their passage through the gastrointestinal tract, suggesting that the consumption of these nutrient components could be beneficial in terms of disease prevention. Similar results were reported by Bontempo et al. (2013), who tested the purple-fleshed variety *Vitelotte* and observed significant activities against *Staphylococcus aureus* and *Rhizoctonia solani*.

Table 20. Antibacterial and antifungal activities (mg/mL) of aqueous extracts from red, purple and marble-fleshed potato varieties.

	Bacteria							***Fungi***					
Potato Variety		*S.a.*	*B.c.*	*L.m.*	*E.c.*	*S.t.*	*E.c.*		*A.f*	*A.n.*	*A.v.*	*P.f.*	*P.v.c.*	*T.v.*
Red-fleshed														
Rosemary	MIC	4	2	3	3	4	3	MIC	0.5	0.5	0.5	0.35	0.5	0.25
	MBC	8	4	4	4	8	4	MFC	1	1	1	0.5	1	0.5
Red Emmalie	MIC	2	1.5	2	3	3	3	MIC	0.5	0.5	0.5	0.5	1	0.35
	MBC	4	2	4	4	4	4	MFC	1	1	1	1	2	0.5
Red Cardinal	MIC	2	1	2	1.5	3	3	MIC	1	0.5	1	0.5	0.5	0.25
	MBC	4	2	4	2	4	4	MFC	2	1	2	1	1	0.5
Purple-fleshed														
Purple	MIC	3	1	3	1.5	3	3	MIC	0.5	0.5	0.5	0.5	0.5	1
	MBC	4	2	4	2	4	4	MFC	1	1	1	1	1	2
Violetta	MIC	3	1	3	1.5	3	3	MIC	0.25	0.125	1	0.25	0.5	0.125
	MBC	4	2	4	2	4	4	MFC	0.5	0.25	2	0.5	1	0.25
Kefermarkter Blaue	MIC	6	3	3	3	6	6	MIC	0.25	0.125	0.35	0.25	0.5	0.125
	MBC	8	4	4	4	8	8	MFC	0.5	0.25	0.5	0.5	1	0.25
Marble-fleshed														
Shetland Black	MIC	4	1	3	1	4	4	MIC	1	0.5	0.5	0.75	0.5	0.25
	MBC	8	2	4	2	8	8	MFC	2	1	1	1	1	0.5
Controls														
E211	MIC	4.0	0.5	1.0	1.0	1.0	2.0	MIC	1.0	1.0	2.0	1.0	2.0	1.0
	MBC	4.0	0.5	2.0	2.0	2.0	4.0	MFC	2.0	2.0	2.0	2.0	4.0	2.0
E224	MIC	1.0	2.0	0.5	0.5	1.0	0.5	MIC	1.0	1.0	1.0	0.5	1.0	0.5
	MBC	1.0	4.0	1.0	1.0	1.0	0.5	MFC	1.0	1.0	1.0	0.5	1.0	0.5

MIC: minimal inhibitory concentration (mg/mL); MBC: minimal bactericidal concentration (mg/mL); and MFC: minimal fungicidal concentration (mg/mL). Bacterial strains: S.a. (*Staphylococcus aureus*; ATCC 6538*)*, B.c.. (*Bacillus cereus*; food isolate*)*, L.m. (*Listeria monocytogenes*; NCTC 7973), E.c. (*Escherichia coli*; ATCC 35210*),* S.t. (*Salmonella* Typhimurium; ATCC 13311), and En.cl. (*Enterobacter cloacae*; human isolate). Fungal strains: *A.f. (Aspergillus fumigatus,* human isolate*);* A.n. (*Aspergillus niger*; ATCC 6275), A.v. (*Aspergillus versicolor*; ATCC 11730), P.f. (*Penicillium funiculosum*; ATCC 36839), P.a. (*Penicillium aurantiogriseum)*; food isolate), T.v. (*Trichoderma viride*; IAM 5061). Positive controls: E211- sodium benzoate and E224 - potassium metabisulphite.

3.4.3 Application of the coloured potato extracts in soft drink formulations

Following the screening of the seven potato varieties on their anthocyanin profiles and bioactivities, two aqueous extracts were selected to be incorporated as natural colourants into soft drink formulations, namely one from the red-fleshed variety *Rosemary* and one from the purple-fleshed variety *Purple*. Then, the two soft drink formulations were assessed for their sensory attributes and stability after thermal processing (pasteurisation), in comparison with the *Control* colourant E163.

The results for the colour parameters *L** (lightness), *a** (green-redness), *b** (blue-yellowness), C* (chroma/saturation) and h° (hue) measured over time (after pasteurisation) are presented in **Figure 18**. At *Day 0* the control soft drink showed a slight distinct colouration when compared to *Rosemary* and *Purple*, presenting lower *a** and higher *b** values, which means a more intense presence of blue tones and less intensity of red. Despite all three formulations having the same concentration of anthocyanins, this difference in colour tone and intensity may be due to the distinct anthocyanin glycosides present in the two tested colouring agents and in the control E163 (grape anthocyanins). It should be noted that the colour profile of the three samples presented a great stability between *Day 0* and *Day 7* for all the analysed parameters. At *Day 30* a significant decrease in *a** values was reported for all the tested formulations, which could be related to a reduction in the intensity of the red colour. A strong positive correlation between the total anthocyanins content and the chroma parameters in purple-fleshed sweet potatoes was also observed Loypimai, Moongngarm, & Chottanom (2016). According to Ashurst (2011), in a comprehensive report on the stability and shelf life of fruit juices and soft drinks, product discoloration is a process commonly observed in almost any soft drink or fruit juice. This can be explained by a decrease in the concentration of the original anthocyanins as a result of their degradation and/or transformation in newly formed derived pigments (Santos-Buelga &

González-Paramás, 2019). The degradation of anthocyanins over time was reported by Jie et al. (2013) and Loypimai et al. (2016) in purple-fleshed sweet potato and black rice extracts, respectively. Similarly, anthocyanin decay and a discoloration process was observed by Rodriguez-Saona et al. (1998) when monitoring the anthocyanin stability of frozen red-fleshed potato tubers over a three month-period storage. Those authors attributed the observed anthocyanin content decay to possible enzymatic reactions.

According to the results herein obtained, the performances of the aqueous potato extracts over shelf-life could be considered satisfactory, as they were similar to the control commercial colourant (a grape extract containing potassium sorbate as preservative). This performance might be explained by the presence of acylated anthocyanins in potatoes. The acylation confers increased biochemical stability to anthocyanin forms, preventing the indiscriminate degradation by glycosidases, most of which are unable to act on acylated glycosides (Mateus & Freitas, 2009). Therefore, our results indicate the suitability of the red and purple potato aqueous extracts to be used as alternative natural colouring agents in beverages.

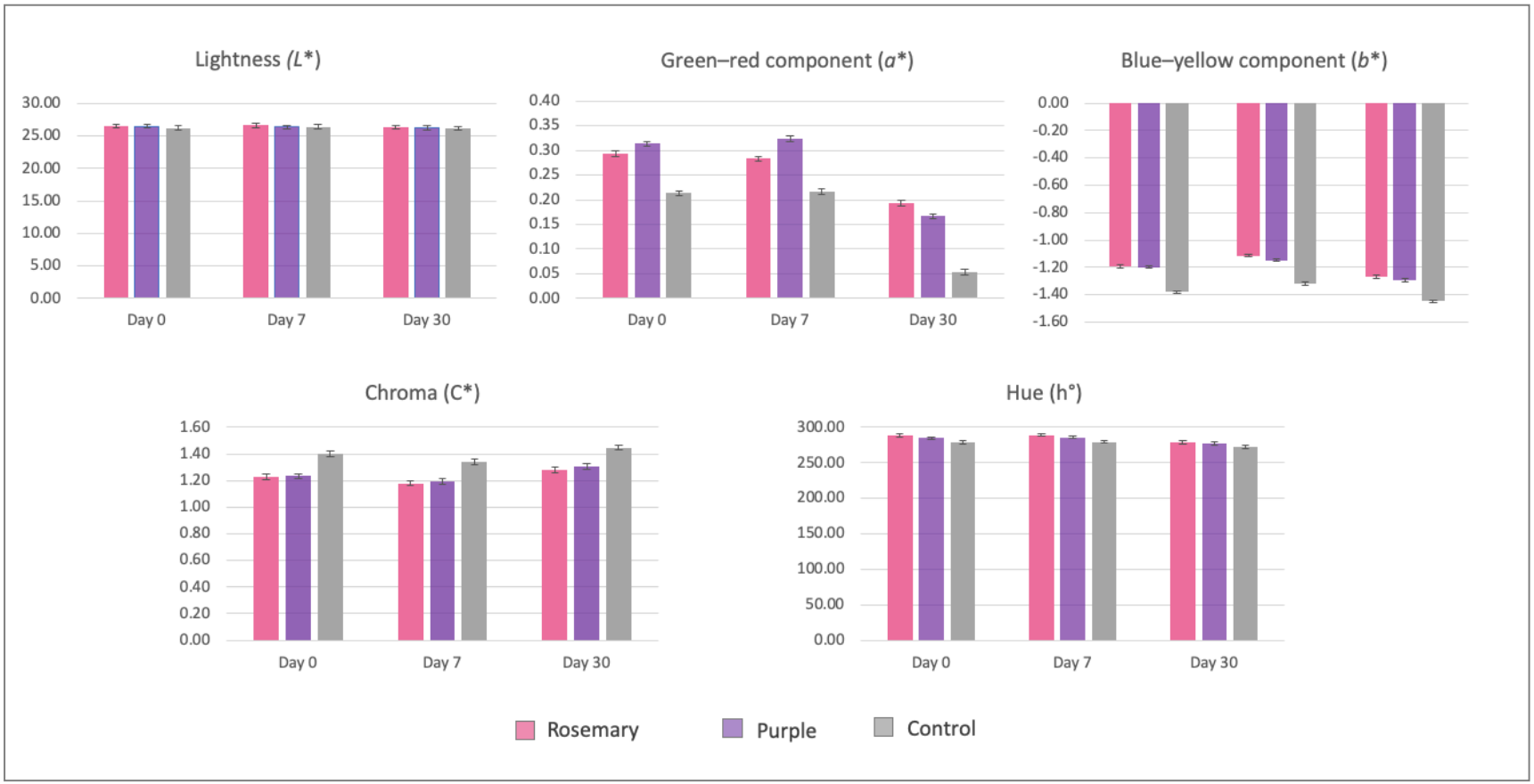


Figure 18. CIELab colour values (L*, a* and b*) and cylindrical coordinates (C* and h°) of the soft drink formulations Rosemary (red potato extract), Purple (purple potato extract) and Control (E163 commercial colouring), over a 30 days shelf-life period. L*: lightness from black (0) to white (100); a*: green (−) to red (+), b*: blue (−) to yellow (+); C*: chroma, relative saturation; and h°: angle of the hue in the CIELab colour space.

3.4.4 Sensory analysis of the coloured potato extracts in soft drink formulations

The scores obtained in the sensory analysis are shown in **Figure 19**. The *Rosemary*, *Purple* and *Control* formulations were assessed by the 12 trained panellists for the following attributes: intensity of colour, cloudiness, sweetness, fruitiness, bitterness and sourness.

Regarding visual attributes, no statistical difference was reported between the *Control* formulation and *Rosemary* for intensity of colour and cloudiness. *Purple*, however, presented a significantly cloudier solution and a significantly lighter colour than both *Rosemary* and *Control* ($p < 0.05$). *Rosemary* and the *Control* formulations were described by the panellists as presenting a light transparent pink colour while *Purple* was described as presenting a slightly opaque light pink colour.

No statistical difference was reported between the three formulations for the flavour attributes sweetness, fruitiness and bitterness. With regard to sourness, *Control* was rated significantly higher than *Rosemary* ($p < 0.05$). Nevertheless, no significant difference was found between *Purple* and *Rosemary* and between *Purple* and the *Control* for this attribute. Additionally, no off-odours or off-tastes were detected in any of the tested formulations.

At the end of the sensory session, the 12 panellists were asked to choose their preferred formulation between the three ones tested. The soft drinks formulated with *Rosemary* and *Purple* extracts performed well, with 42% of the panellists choosing *Purple* and 25% choosing *Rosemary* as their preferred one, while 33% preferred the *Control* soft drink. Interestingly, some panellists highlighted in their comments the opaqueness described in *Purple* as a positive attribute, relating it to a “natural rather than synthetic appearance”.

The results regarding the sensorial analysis and the stability over time of the red and purple-fleshed potato formulations indicate promising potential for their future application in the food industry as substitutes of synthetic colouring agents.

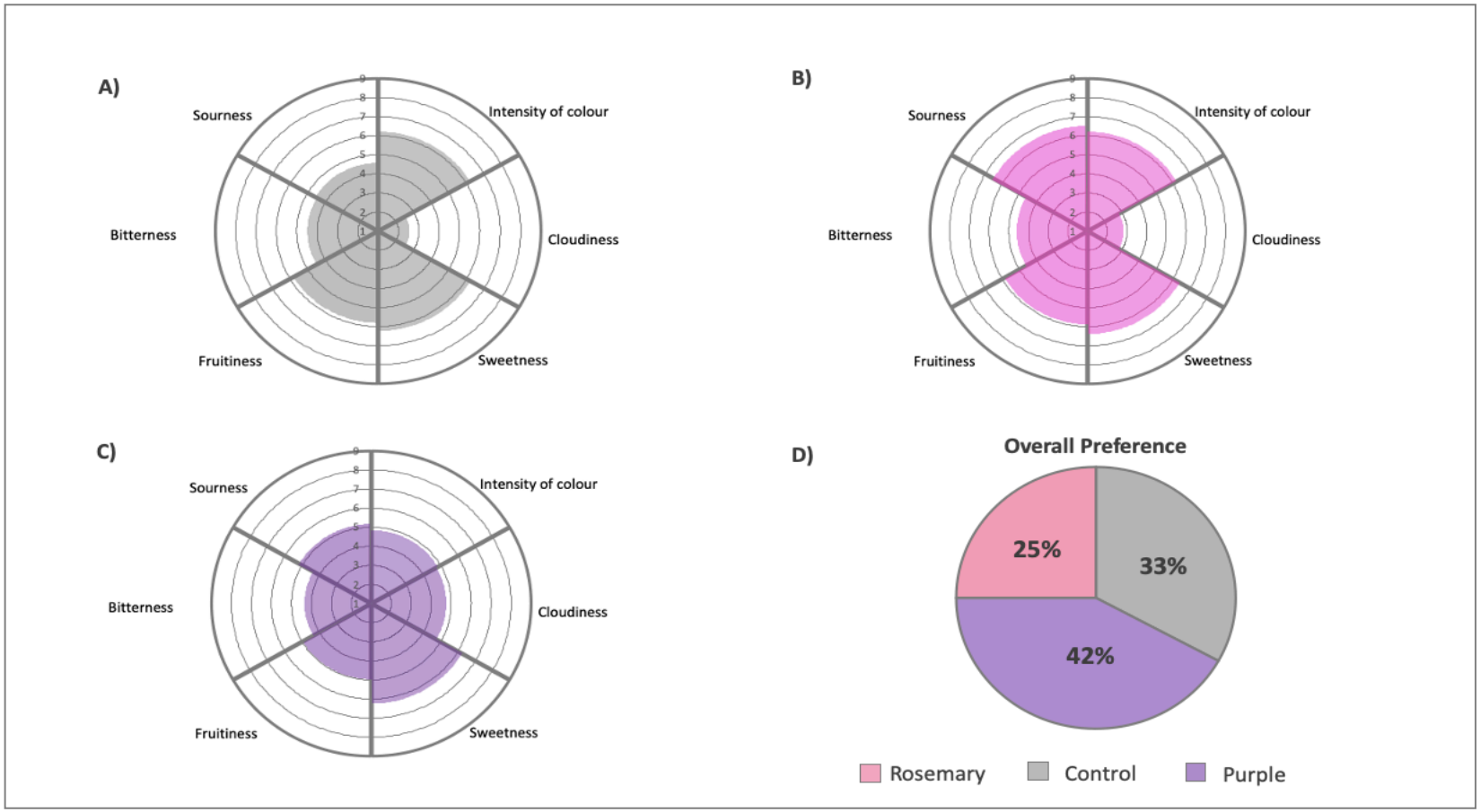


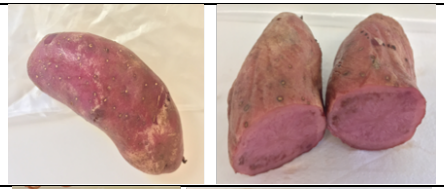
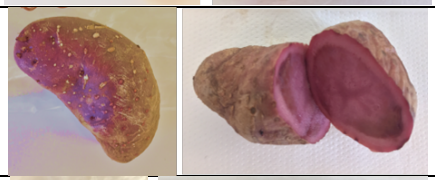
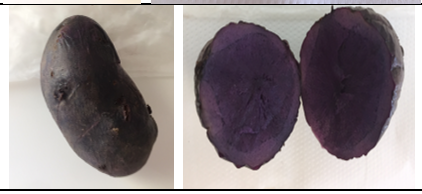
Figure 19. Sensory analysis scores (1 to 9) for the attributes: intensity of colour, cloudiness, sweetness, fruitiness, bitterness and sourness, and overall preference of the tested soft drink formulations. A) Rosemary potato extract; B) Control E163; C) Purple potato extract; D) overall preference scores.

3.5 Potato peels

3.5.1 Colour assessment

A quantitative analysis of the outer colour of the ten potato peels was carried out. The results for the Hunter colour parameters *L** (lightness), *a** (green-redness) and *b** (blue-yellowness) are presented in **Table 21**. The three red varieties presented the highest values for the parameter *a** as expected, quantitatively confirming the presence of red tones in the peels (*Rosemary*: 24.8, *Red Emmalie*: 24.4 and *Red Cardinal*: 11.7). The peels from the three tubers also presented the highest values for *b** (*Rosemary*: 10.1, *Red Emmalie*: 8.7 and *Red Cardinal*: 14.7), after only the marble (purple/yellow) variety *Shetland Black*, which presented the highest *b** values (18) among all the ten peels. This means that for this variety the presence of yellow tones was more prominent than blue. The purple varieties, in turn, presented the lowest values for *b*,* which is associated to the presence of blue tones in the peels. The *Shetland Black* variety also presented the highest values for *L**, meaning its peel had the lightest colour (presence of white tones) among the ten varieties.

Table 21: Potato variety, country of origin, cultivation type, qualitative flesh colour and quantitative peel colour (Hunter scale parameters) of the studied potato peels (mean ± SD).

Potato variety		Country of origin	Cultivation type	Flesh colour	Peel colour		
					*L**	*a**	*b**
Rosemary		Germany	Cultivated variety	Red	41±1[b]	24.8±0.4[a]	10.1±0.4[c]
Red Cardinal		United Kingdom	Landrace	Red	41±2[b]	20.1±0.7[b]	11.2±0.2[b]
Rote Emmalie (Red Emmalie)		Germany	Cultivated variety	Red	38±2[c]	24.4±0.5[a]	8.7±0.7[d]
Purple		-	-	Purple	27.3±0.9[f]	6.3±0.7[e]	3.0±0.7[f,g]

Kefermarkter Blaue		Austria	Landrace	Purple	31.2±0.5^{d}	7.3±0.3^{d}	3.3±0.3^{f,g}
UACH 0917		Chile	Andean accession	Purple	28.4±0.1^{e}	4.0±0.6^{f}	1.6±0.2^{h}
Salad Blue		United Kingdom	Cultivated variety	Purple	29.0±0.4^{e}	5.9±0.5^{e}	2.48±0.2^{g,h}
Blaue aus Finnland		Finland	Landrace	Purple	28.8±0.6^{e}	5.3±0.2^{e}	4.1±0.9^{f}
Shetland Black (Ellenb.)		United Kingdom	Cultivated variety	Marble (purple/yellow)	46±1^{a}	9.1±0.6^{c}	18±2^{a}
Violetta (Blaue Elise)		Germany	Cultivated variety	Purple	32±2^{d}	1.6±0.2^{g}	6.1±0.7^{e}

In each column different Latin letters are significantly different according to Tukey's HSD test (p=0.05), for each potato group peels.

3.5.2 Phenolic acids, flavonoids and polyamine derivatives

The phenolic acids, flavonoids and polyamine derivatives profiles, chromatographic characteristics, tentative identifications and quantification of the ten potato peels are shown in **Table 22**. Seven compounds were tentatively identified, of which three phenolic acids, three flavonoids (flavonol glycoside derivatives) and one caffeoyl-polyamine derivative, based on the observation of their characteristic fragments in MS^2 spectra and literature data (Barros, Pereira, et al., 2013; Choi et al., 2016; Friedman et al., 2017; Rommens et al., 2008; Svobodova et al., 2017; S. T. Yang, Wu, Rui, Guo, & Feng, 2015).

Peaks 1-3 were tentatively identified as phenolic acids. **Peak 1** ($[M-H]^-$ at *m*/*z* 353) was detected in all ten peels and tentatively identified as 4-*O*-caffeoylquinic acid (Svobodova et al., 2017). **Peak 2** ($[M-H]^-$ at *m*/*z* 179) was the most abundant compound in all tested varieties, with concentrations ranging from 0.42 (*Rosemary*) to 1.44 (*UACH 0917*) mg/g dw, being identified as caffeic acid by comparison with retention time, UV-vis, and chromatographic characteristics of the available commercial standard compound. **Peak 3** ($[M-H]^-$ at *m*/*z* 313) was identified as salvianolic acid F, based on its MS^2 spectra fragments previously reported in the literature (Barros, Dueñas, et al., 2013; Yang et al., 2015). **Peaks 4, 5** and **7** were identified as flavonoids, all of which kaempferol derivatives, by comparing the obtained LC-MS data with those reported by other authors (Rommens et al., 2008; Svobodova et al., 2017); their glycosylation patterns were assigned based on the mass pseudomolecular ions and mass fragmentation profiles**.** Finally, **Peak 6** ($[M-H]^-$ at *m*/*z* 472) was only detected in five potato varieties (*Purple, Kefermarkter Blaue, Salad Blue, Blaue aus Finnland* and *Violetta*) and was tentatively identified as bis(dihydrocaffeoyl) spermidine, a polyamine derivative, previously described by Svobodova et al. (2017) in the non-edible parts of *Solanum stramoniifolium* Jacq. This polyamine derivative was described as a potent oxidative inhibitor (Ponasik et al., 1995),

but also used as a molecular marker for the authentication of the origin of several potato varieties from Italy (Adamo et al., 2012). The concentration of total phenolic compounds and derivatives among the peels of the studied potato varieties ranged from 0.27 (*Red Emmalie*) to 1.76 (*UACH 0917*) mg/g dw. In accordance with our results, literature data show that the total amount of phenolic compounds in potato peels can vary significantly among different varieties.

Table 22: Chromatographic properties, tentative identification, and quantification (mg/g dw) of the non-anthocyanin phenolic compounds found in the studied potato peels (mean ± SD).

Identification					
Peak	**Rt (min)**	**λ_{max} (nm)**	**$[M-H]^-$ (*m/z*)**	**MS^2**	**Tentative identification**
1	6.87	325	353	191(19),179(53),173(100),135(5)	4-*O*-Caffeoylquinic acid
2	9.97	323	179	135(100)	Caffeic acid
3	13.66	328	313	269(100),159(5),109(5)	Salvianolic acid F
4	15.35	311	755	593(21),285(55)	Kaempferol-*O*-hexoside-*O*-deoxyhexoside-hexoside
5	19.71	317	593	285(100)	Kaempferol-*O*-deoxyhexoside-hexoside
6	20	227,290,321sh	472	350(51),308(100)	Bis(dihydrocaffeoyl)spermidine
7	21.12	324	593	285(100)	Kaempferol-3-*O*-rutinoside

Quantification										
Peak	**Rosemary**	**Red Cardinal**	**Red Emmalie**	**Purple**	**Kefermarkter Blaue**	**UACH 0917**	**Salad Blue**	**Blaue aus Finnland**	**Shetland Black**	**Violetta**
1	0.071±0.001^{e}	0.049±0.001^{h}	0.033±0.002^{i}	0.090±0.005^{d}	0.052±0.001^{g}	0.097±0.002^{c}	0.110±0.001^{b}	0.031±0.002^{j}	0.067±0.001^{f}	0.31±0.008^{a}
2	0.419±0.004^{g}	0.722±0.003^{d}	0.103±0.003^{h}	1.06±0.05^{c}	0.71±0.02^{d}	1.443±0.007^{a}	1.13±0.03^{b}	0.683±0.002^{e}	1.14±0.02^{b}	0.65±0.02^{f}
3	0.018±001^{g}	0.058±0.001^{c}	0.019±0.001^{g}	0.027±0.001^{e}	0.022±001^{f}	0.117±0.002^{a}	0.049±0.001^{d}	0.023±0.001^{f}	0.099±0.002^{b}	nd
4	0.029±001^{d}	nd	0.032±0.001^{c}	nd	nd	0.097±0.001^{b}	nd	nd	0.097±0.001^{b}	0.119±0.0001^{a}
5	nd	nd	0.024±0.001	nd	nd	nd	nd	nd	nd	nd
6	tr	tr	nd	0.26±0.01^{a}	0.211±0.004^{b}	nd	0.165±0.003^{d}	0.176±0.006^{c}	tr	0.112±0.001^{e}
7	0.036±0.001^{b}	0.054±0.001^{a}	0.054±0.001^{a}	nd	nd	nd	nd	nd	nd	nd
TPA	**0.508±0.004^{i}**	**0.829±0.003^{f}**	**0.16±0.01^{j}**	**1.18±0.05^{d}**	**0.79±0.02^{g}**	**1.657±0.007^{a}**	**1.29±0.03^{c}**	**0.737±0.001^{h}**	**1.30±0.03^{b}**	**0.96±0.02^{e}**
TF	**0.065±0.001^{d}**	**0.054±0.001^{e}**	**0.11±0.001^{b}**	**nd**	**nd**	**0.097±0.001^{c}**	**nd**	**nd**	**0.097±0.001^{c}**	**0.119±0.0001^{a}**
TPd	**tr**	**tr**	**nd**	**0.23±0.01^{a}**	**0.211±0.004^{b}**	**nd**	**0.165±0.003^{d}**	**0.176±0.006^{c}**	**tr**	**0.112±0.001^{e}**
TPCd	**0.573±0.004^{h}**	**0.882±0.003^{g}**	**0.27±0.01^{i}**	**1.44±0.04^{b}**	**0.91±0.03^{e}**	**1.755±0.007^{a}**	**1.45±0.02^{b}**	**0.913±0.005^{f}**	**1.39±0.03^{c}**	**1.19±0.02^{d}**

TPA – Total Phenolic Acids; TF – Total Flavonoids; TPd - Total Polyamine derivatives; TPCd - Total Phenolic Compounds and derivatives. tr - traces; nd – not detected. Standard Calibration Curves: chlorogenic acid (y = 168823x -161172, R^2= 0.9999, LOD = 0.20 µg/mL and LOQ = 0.68 µg/mL, peak 1); caffeic acid (y = 388345x + 406369, R^2 = 0.9939, LOD = 0.78 µg/mL and LOQ = 1.97 µg/mL, peak 2 and 6); rosmarinic acid (y = 191291x – 652903, R^2 = 0.999, LOD = 0.15 µg/mL and LOQ = 0.68 µg/mL, peak 3); and quercetin-3-*O*-rutinoside (y = 13343x + 76751, R^2 = 0.9998, LOD = 0.21 µg/mL and LOQ = 0.71 µg/mL, peaks 4, 5, and 7). In each lines different Latin letters are significantly different according to Tukey's HSD test (p=0.05), for each potato group peels.

3.5.3 Anthocyanins

The anthocyanins profile, chromatographic characteristics, tentative identifications, and quantifications for the red and purple varieties are shown in **Tables 23** and **24**, respectively. All tentatively identified compounds were previously described in the literature (Kita et al., 2013; Oertel et al., 2017; Su et al., 2016), and were tentatively identified as acylated anthocyanins, owing to the presence of a shoulder around 310-330 nm in the UV spectra. Six peaks were detected in the red varieties (*Rosemary*, *Red Cardinal* and *Red Emmalie*), comprising pelargonidin, peonidin, petunidin, malvidin and cyanidin derivatives, based on the observation of their characteristic fragments in the MS^2 spectra. **Peak 1r** ($[M]^+$ at *m*/*z* 887) was only detected in the *Rosemary* peel extract and was tentatively identified as a pelargonidin-3-*O*-*p*-coumaroylrutinoside-5-glucoside isomer, based on the HPLC-DAD-MS results and previous literature reports (Kita et al., 2013; Oertel et al., 2017). **Peak 2r** ($[M]^+$ at *m*/*z* 903), assigned as a cyanidin derivative (cyanidin 3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside), was detected in all red varieties, but not in the purple ones. **Peak 3r** ($[M]^+$ at *m*/*z* 887) might be an isomer of Peak 1r and presented the highest concentration in the *Rosemary* peel extract. **Peaks 4r** and **6r** ($[M]^+$ at *m*/*z* 917 and 947, respectively) were identified as peonidin acyl-glycosides, with **Peak 4r** being the main compound detected in the *Red Emmalie* peels. Finally, **Peak 5r** ($[M]^+$ at *m*/*z* 947) was identified as an acylated malvidin glucoside (malvidin-3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside), detected only in the *Red Emmalie* peels. All three red varieties showed very similar total anthocyanin concentration in the peels, ranging from 0.518 (*Red Emmalie*) to 0.583 mg/g dw (*Red Cardinal*).

Regarding the purple varieties, seven peaks were detected, two of which were tentatively identified as petunidin derivatives (**Peaks 1p** and **2p**), two as peonidin derivatives (**Peaks 4p**

and **6p**), two as malvidin derivatives (**Peaks 5p** and **7p**) and one as a pelargonidin derivative (**Peak 3p**). The molecular ions and fragmentation patterns of peaks **1p** and **2p** were coherent with derivatives of petunidin-3-*O*-rutinoside-5-*O*-glucoside acylated with *p*-coumaric and ferulic acids, respectively, as previously identified in coloured tubers of *Solanum tuberosum* (Kita et al., 2013). Peak **5p** possessed the same molecular ion $[M]^+$ at *m/z* 947 as peak **6p**, but different fragmentation pattern. Two compounds with this molecular ion were also reported by Kita et al. (2013), identified as malvidin-3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside and peonidin-3-*O*-feruloylrutinoside-5-*O*-glucoside, respectively, although the second appeared only in the flesh of red tubers. Petunidin-3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside (**Peak 1p**) was the main anthocyanin found in the peels of all purple varieties, except for UACH 0917 that showed malvidin-3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside (**Peak 5p**) as the main compound (0.66 mg/g dw). All purple varieties showed similar total anthocyanin content, ranging from 0.86 (*Violetta*) to 1.39 mg/g dw (*Salad Blue*).

Table 23: Chromatographic properties, tentative identification, and quantification (mg/g dw) of the anthocyanins found in the studied red potato peels (Rosemary, Red Cardinal, and Red Emmalie) (mean ± SD).

Identification					
Peak	**Retention time**	**λ_{max} (nm)**	**$[M]^+$ (*m/z*)**	**MS^2 (*m/z*)**	**Tentative identification**
1r	34.87	504	887	725(100),433(5),271(16)	Pelargonidin-3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside isomer 1
2r	36.45	519	903	449(100), 287(18)	Cyanidin 3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside
3r	38.49	505	887	725(100),433(10),271(13)	Pelargonidin-3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside isomer 2
4r	39.33	508	917	755(100),463(15),301(38)	Peonidin-3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside
5r	39.8	517	947	785(100),493(19),331(29)	Malvidin-3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside
6r	39.9	517	947	785(100),463(23),301(38)	Peonidin-3-*O*--feruloylrutinoside-5-*O*-glucoside
Quantification					
Peak	**Rosemary**		**Red Cardinal**		**Red Emmalie**
1r	0.0269±0.0005		nd		nd
2r	0.0474±0.0001[a]		0.045±0.001[b]		0.0437±0.0001[c]
3r	0.2503±0.005[a]		0.099±0.002[b]		0.0581±0.0006[d]
4r	0.248±0.004[a]		0.226±0.001[b]		0.212±0.0003[c]
5r	nd		nd		0.205±0.001
6r	nd		0.2121±0.0003		nd
TA	**0.5722±0.0003[b]**		**0.582±0.004[a]**		**0.518±0.009[c]**

TA – Total Anthocyanins. nd – not detected. Standard calibration curves: pelargonidin-3-*O*-glucoside (y = 276117x – 480418, R^2 = 0.9979, LOD = 0.24 μg/mL and LOQ = 0.76 μg/mL, peaks 1r and 3r), cyanidin-3-*O*-glucoside (y = 243287x - 1E+06, R^2 = 0.9986, LOD = 0.25 μg/mL and LOQ = 0.83 μg/mL, peak 2r), and peonidin-3-*O*-glucoside (y = 151438x - 3E+06, R^2 = 0.9965, LOD = 0.13 μg/mL and LOQ = 0.40 μg/mL, peaks 4r, 5r, and 6r). In each lines different Latin letters are significantly different according to Tukey's HSD test (p=0.05), for each potato group peels.

Table 24: Chromatographic properties, tentative identification and quantification (mg/g dw) of the anthocyanins found in the studied purple potato peels (Kefermarkter Blaue, UACH 0917, Salad Blue, Blaue aus Finnland, Shetland Black, Violetta) (mean ± SD).

***Identificat*ion**							
Peak	**Retention time**	**λ_{max} (nm)**	**$[M]^+$ (*m/z*)**	**MS^2 (*m/z*)**	**Tentative identification**		
1p	36.77	531	933	771(100),479(24),317(34)	Petunidin-3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside		
2p	37.76	531	963	801(100),479(25),317(38)	Petunidin-3-*O*-feruloylrutinoside-5-*O*-glucoside		
3p	38.59	507	887	725(100),433(10),271(13)	Pelargonidin-3-*O*-*p*-coumaroylrutinoside-5-glucoside		
4p	39.03	520	917	755(100),463(15),301(38)	Peonidin-3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside		
5p	39.21	532	947	785(100),493(19),331(29)	Malvidin-3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside		
6p	39.82	522	947	785(100),463(23),301(38)	Peonidin-3-*O*-feruloylrutinoside-5-*O*-glucoside		
7p	39.95	532	977	815(100),493(15),331(27)	Malvidin-3-*O*-feruloylrutinoside-5-*O*-glucoside		
Quantification							
Peak	**Purple**	**Kefermarkter Blaue**	**UACH 0917**	**Salad Blue**	**Blaue aus Finnland**	**Shetland Black**	**Violetta**
1p	0.843±0.001^{a}	0.65±0.01^{b}	0.282±0.004^{f}	0.66±0.004^{b}	0.59±0.007^{c}	0.298±0.007^{e}	0.3952±0.004^{d}
2p	0.233±0.005^{b}	0.168±0.002^{f}	0.0963±0.0001^{g}	0.269±0.005^{a}	0.172±0.001^{e}	0.205±0.002^{d}	0.2186±0.0001^{c}
3p	nd	nd	0.0159±0.0002^{c}	nd	nd	0.0234±0.0004^{b}	0.0387±0.00002^{a}
4p	nd	nd	nd	nd	nd	0.241±0.005	nd
5p	0.171±0.001^{d}	0.1223±0.004^{e}	0.6559±0.001^{a}	0.2466±0.0001^{b}	0.123±0.002^{e}	nd	0.211±0.001^{c}
6p	nd	nd	nd	nd	nd	0.2183±0.0002	nd
7p	0.119±0.003^{b}	0.0937±0.0014^{d}	0.2118±0.0058^{a}	0.212±0.001^{a}	0.0985±0.0004^{c}	nd	nd
TA	**1.368±0.001**^{b}	**1.04±0.02**^{d}	**1.26±0.01**^{c}	**1.39±0.01**^{a}	**0.98±0.01**^{e}	**0.99±0.02**^{e}	**0.863±0.005**^{f}

TA – Total Anthocyanins. nd – not detected. Standard calibration curves: pelargonidin-3-*O*-glucoside (y = 276117x – 480418, R^2 = 0.9979, LOD = 0.24 μg/mL and LOQ = 0.76 μg/mL, peak 3p) and peonidin-3-*O*-glucoside (y = 151438x - 3E+06, R^2 = 0.9965, LOD = 0.13 μg/mL and LOQ = 0.40 μg/mL, peaks 1p, 2p, 4, 5p, 6p, and 7p). In each lines different Latin letters are significantly different according to Tukey's HSD test (p=0.05), for each potato group peels.

Although potato peels are usually discarded, the present results show that these bio-residues are rich sources of anthocyanins and phenolic compounds. Yin et al. (2016) compared the flesh and peel of coloured potato varieties from China and found that on average the total anthocyanin contents were 15.34 times higher than of the respective flesh. Similarly, the total phenolic contents were 7.28 times higher. These results support the use of potato peels as sources of phenolic compounds with potential biological activity. It is important to note that the variability in the phenolic and anthocyanin profiles and contents of the peels herein found could be mainly attributed to inherited differences of the potato genotypes, as all samples were subjected to the same environmental factors (growing conditions, planting location and climate), transport and storage conditions.

3.5.4 Cell-based antioxidant and anti-inflammatory activities

The hydroethanolic extracts prepared from the ten potato peels were evaluated for their capacity to prevent lipid peroxidation of porcine brain tissues (TBARS assay) and haemolysis of sheep blood cells (OxHLIA assay). Additionally, the extracts' anti-inflammatory potential of inhibiting the growth of RAW 264.7 mouse macrophages was assessed. The results are presented in **Table 25**.

Table 25: Antioxidant, anti-inflammatory and anti-proliferative activities and cytotoxicity (µg/mL extract) of the studied potato peels (mean ± SD).

	Antioxidant (IC_{50} values)		**Anti-inflammatory** (IC_{50} values)	**Anti-proliferative** (GI_{50} values)				**Cytotoxicity** (GI_{50} values)
Potato varieties	**TBARS**	**OxHLIA**	**RAW 264.7**	**NCI-H460**	**HepG2**	**MCF-7**	**HeLa**	**PLP2**
Rosemary	26 ± 3^{h}	54 ± 2^{c}	141±6	69 ± 1^{e}	79 ± 4^{f}	51 ± 2^{f}	49 ± 3^{f}	304±9
Red Cardinal	187 ± 12^{c}	123 ± 7^{b}	>400	241 ± 3^{c}	305 ± 11^{d}	315 ± 10^{a}	333 ± 17^{b}	>400
Rote Emmalie (Red Emmalie)	46 ± 2^{g}	n.a.	>400	248 ± 6^{c}	301 ± 21^{d}	244 ± 17^{c}	227 ± 11^{e}	>400
Purple	114 ± 8^{f}	$16\pm1^{c,d}$	>400	268 ± 17^{b}	365 ± 18^{a}	188 ± 11^{e}	330 ± 2^{b}	>400
Kefermarkter Blaue	208 ± 12^{b}	$32\pm2^{c,d}$	>400	217 ± 12^{d}	302 ± 9^{d}	279 ± 12^{b}	313 ± 33^{c}	>400
UACH 0917	127 ± 7^{e}	$24\pm2^{c,d}$	>400	280 ± 7^{a}	319 ± 1^{c}	224 ± 10^{d}	346 ± 10^{a}	>400
Salad Blue	230 ± 6^{a}	294 ± 8^{a}	>400	281 ± 6^{a}	286 ± 12^{e}	215 ± 12^{d}	292 ± 19^{d}	>400
Blaue aus Finnland	154 ± 2^{d}	122 ± 4^{b}	>400	$276\pm2^{a,b}$	281 ± 10^{e}	281 ± 66^{b}	333 ± 12^{b}	>400
Shetland Black	190 ± 6^{c}	53 ± 2^{c}	>400	269 ± 11^{b}	343 ± 9^{b}	210 ± 10^{d}	233 ± 3^{e}	>400
Violetta (Blaue Elise)	144 ± 5^{d}	$16\pm1^{c,d}$	>400	271 ± 19^{b}	317 ± 11^{c}	216 ± 17^{d}	$335\pm11^{a,b}$	>400

IC_{50}: extract concentration corresponding to 50% of antioxidant activity against lipid peroxidation (TBARS) and haemolysis of sheep blood cells (OxHLIA; Δt = 60 min). n.a.: no activity. GI_{50} values correspond to the sample concentration achieving 50% of growth inhibition in liver primary culture PLP2; maximum tested concentration: 400 µg/mL. In each column different Latin letters are significantly different according to Tukey's HSD test (p=0.05), for each potato group peels.

All ten extracts were effective in diminishing the production of TBAR substances, however results varied greatly among varieties (IC_{50} = 26 - 240 µg/ml). The peels from the *Rosemary* tubers presented the highest antioxidant capacity, as their extracts required the lowest concentration to diminish the lipid peroxidation in 50% compared to the control Trolox (IC_{50} = 26 µg/ml), while *Salad Blue* presented the weakest activity (IC_{50} = 240 µg/ml).

Regarding the OxHLIA assay, almost all potato varieties presented positive results in retarding the erythrocytes haemolysis process (Δt = 60 minutes), with exception of *Red Emmalie*, that revealed no activity. *Violetta* and *Purple* were the varieties that presented the most prominent activity, as they required the lowest IC_{50} values (16 µg/ml for both varieties). In agreement with the results herein found in the TBARS assay, *Salad Blue* peels also presented the weakest antioxidant activity in the OxHLIA assay (IC_{50} = 294 µg/ml).

In agreement with our findings, other authors reported the antioxidant capacity of potato peel extracts in previous studies. For instance, positive results for antioxidant activity were obtained by the following assays: the DPPH radical-scavenging capacity (Jeddou et al., 2018; A. Singh et al., 2011; Singh & Rajini, 2004; Yin et al., 2016); reducing power (Jeddou et al., 2018; Singh & Rajini, 2004), β-carotene bleaching inhibition activity (Jeddou et al., 2018), ABTS radical scavenging activity (Jeddou et al., 2018), lipid peroxidation in rat liver homogenates (Singh & Rajini, 2004) and iron ion chelation (Singh & Rajini, 2004). In some studies, potato peel extracts also exerted significantly higher radical scavenging activity ($p < 0.05$) than the respective flesh extracts (Albishi et al., 2013; Wu et al., 2012).

Regarding the anti-inflammatory assay, *Rosemary* was the only variety that presented capacity to inhibit the growth of RAW 264.7 mouse macrophages (IC_{50} = 141 µg/ml). It is important to state that for the anti-inflammatory assay the maximum tested concentration of the extracts was

400 µg/ml. In comparison with other studies, the *Rosemary* extract showed greater anti-inflammatory activity than, for example, *Jaboticaba* epicarp (IC_{50} = 299 µg/ml) (Albuquerque, Pereira, et al., 2020). Wahyudi, Ramadhan, Wijaya, Ardhani, & Utami (2020) also demonstrated the anti-inflammatory properties of potato peel extracts by conducting an *in vivo* study with rats, using carrageenan-induced paw edema and diclofenac as the reference drug. Their results showed that, compared with the negative control (distilled water), treatment doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg significantly reduced the edema volume increment.

3.5.5 Anti-proliferative activity and cytotoxicity

The *in vitro* anti-proliferative properties of the coloured potato peels were investigated. Additionally, a cytotoxicity assay was carried out to assess the safety of the extracts for incorporation into food formulations. Results are presented in **Table 25**.

The inclusion of phytochemicals and functional foods in the diet has been proposed as a way of preventing the development of cancer cells (Albuquerque, Pereira, et al., 2020). In the present work, the capacity of the studied potato peels in inhibiting tumour cell growth was evaluated in four tumour cell lines. All ten hydroethanolic extracts revealed anti-proliferative activity in all tested tumour cell lines, with GI_{50} values ranging from 69 to 281 µg/ml for NCI-H460 (non-small cell lung carcinoma) cells, 79 to 365 µg/ml for HepG2 (hepatocellular carcinoma) cells, 51 to 315 µg/ml for MCF-7 (breast carcinoma) cells and 49 to 346 µg/ml for HeLa (cervical carcinoma) cells. The extract from the *Rosemary* variety presented the highest anti-proliferative capacity for all tumour cell lines, as it required the lowest concentrations to present activity in comparison with the other nine extracts. For comparison, Albuquerque et al. (2020) reported GI_{50} values within the ranges found herein for the anti-proliferative activity of *M. jaboticaba* epicarp in three tumor cell lines (HepG2 = 258 µg/ml; MCF-7 = 300 µg/ml;

HeLa = 278 µg/ml; NCI-H460 > 400 µg/ml). Reddivari, Vanamala, Chintharlapalli, Safe, & Miller (2007), in turn, also found that the anthocyanin fraction from potato extracts exhibit anticancer properties, being cytotoxic to prostate cancer cells through activation of caspase-dependent and caspase-independent pathways.

In order to assess the safety of the ten extracts herein studied, porcine liver was used as a model to assess the hepatotoxicity effects of the extracts. The results showed that the extracts did not reveal toxicity for PLP2 cells up to the maximum tested concentration (GI_{50} value > 400 µg/ml), with exception of the *Rosemary* extract. Nevertheless, the cytotoxic effect of the *Rosemary* variety was found for a high concentration of extract (GI_{50} value = 304 µg/ml). This indicates the safety of this extract to be used as a natural additive in food formulations in lower concentrations, for instance, the concentration herein found to exert antioxidant activity (IC_{50} = 26 µg/ml, TBARS assay).

4. Conclusions and Future Perspectives

The present thesis aimed to deep into the knowledge of three different food matrices, namely quinoa, amaranth and potatoes (flesh and peels), regarding their nutritional characteristics and phytochemical composition and potential beneficial healthy effects, in order to evaluate their suitability to be used either as a smart food or as natural ingredients in the preparation of functional foods with increased added value.

The **specific conclusions** obtained in the study of each food matrix are presented below.

Quinoa. The Brazilian quinoa BRS Piabiru showed a higher protein content than other previously described quinoa varieties, besides revealing a fatty acid composition with potential health benefits. Its outstanding nutritional value along with its physicochemical traits make this food crop a suitable candidate for direct incorporation in the human diet. This quinoa variety also presented a high content of quercetin and kaempferol glycoside derivatives, which might be responsible for the positive results on the seeds' bioactivities. Overall, the results of the broad characterization carried out in this study suggest the viability of exploiting the potential of this Brazilian quinoa as a functional food, supported by its relevant *in vitro* antioxidant, antibacterial and antifungal activities, with the absence of cytotoxicity. This work contributes to the growing debate on alternative sustainable and healthier foods by deriving information of a new, tropical climate adapted, quinoa genotype. This information can potentially be used by food and pharmaceutical industries in the development of new health-promoting products. This study also contributes to the FAO goal of turning quinoa into a commercial crop in Brazil, alleviating the increasing global demand pressure on Bolivia and Peru.

Amaranth. Deriving from an ancient nutritious food crop, the amaranth cultivar BRS Alegria presents an outstanding nutritional value, particularly concerning its protein (17%), fatty acid

(mainly unsaturated) and vitamin E contents. To the best of our knowledge, this is the first comprehensive report on the chemical features and bioactivities of this novel cultivar. The hydroethanolic extracts of this pseudo-grain presented satisfactory antioxidant, antibacterial and antifungal activities, being more effective than the fungicidal ketoconazole against the fungi *P. ochrochloron*. Furthermore, the extract revealed the absence of hepatotoxicity, making it a suitable candidate for incorporation in food and pharmaceutical formulations as a source of bioactive compounds. This study shows that the BRS Alegria genotype is a suitable candidate to contribute to agricultural diversification and food security, due its rich nutritional and chemical composition, along with its bioactive health-promoting properties.

Potatoes. The information obtained in this study illustrates that the vast existing biodiversity of potato reflects in differences in nutrient content of tubers. Overall, and despite the apparent similarity found when analysing all parameters individually, the obtained profiles showed to be highly correlated either with different cultivation types or different flesh colour. The characterized parameters highlighted the similarity among cultivated varieties and breeder lines, which showed slight variations only in some particular fatty acids, independently of the geographical origin or tuber flesh colour. This finding could be attributed to the higher breeding status and the lower heterogeneity in regard to specific characters that are appreciated by market and agronomic standards. Nonetheless, the low number of discriminant differences among these and the studied landraces and Andean accessions highlight their potential to be used as agronomic alternatives through breeding programs, especially participatory breeding where local farmers are highly involved. Bringing some of that ancient diversity back into cultivation could support potato biodiversity against environmental change, while supporting sustainable agrobiodiversity at the same time.

Coloured potatoes. The aqueous potato extracts tested presented high anthocyanin content and high antioxidant, antibacterial and antifungal properties. Acylated pelargonidin glycosides were the main compounds found in the red varieties and acylated petunidin glycosides in the purple ones. Additionally, no cytotoxic effect was detected in the extracts up to the maximum tested concentration (400 μg/mL), suggesting their safety to be incorporated in food formulations. The two extracts selected to be applied in a soft drink formulation showed suitable profiles in the sensory and shelf-life assessments when compared with the control commercial colourant E163. The aqueous extracts herein obtained by a simple one-step and cost-effective extraction method could be used as alternative natural food colourants, substituting synthetic colouring agents. Nevertheless, future studies in pre- and post-harvest level could support the selection of colour-fleshed potatoes with high anthocyanin content. Furthermore, the assessment of different agronomic practices may increase the concentration of anthocyanins and the added value of this important vegetable crop.

Potato peels. The potato peels studied herein presented positive results for antioxidant capacity by the two cell-based methods employed, besides antitumor activity in all tested tumor cell lines. The peels from the red variety *Rosemary* performed particularly well, as they presented the highest antitumor activity as well as the highest antioxidant activity results in the TBARS assay, among the three tested varieties. Moreover, the *Rosemary* peels were the only to reveal anti-inflammatory activity. The bioactivity exerted by the studied potato peels can be associated with their phenolic composition, herein described, composed by phenolic acids, flavonoids and anthocyanins. The ten assayed hydroethanolic extracts revealed no toxic effect up to the maximum tested concentration of 400 μg/mL, with exception of the *Rosemary* extract, that presented cytotoxicity for concentrations higher than 304 μg/mL. These results suggest the safety of the studied potato peel extracts to be used as natural food additives in functional food

formulations (in concentrations up to 304 μg/mL for the *Rosemary* extract), for added antioxidant and antitumor properties.

Future Perspectives

Taking into account the results of this work, the following future aims are proposed:

1. The optimisation of the extraction conditions, using traditional and alternative methods to prepare richer extracts of phenolic compounds.
2. The further assessment of the stability and toxicity of the prepared extracts and novel food formulations.
3. The application of the obtained natural colourants in other functional food formulations (besides soft drinks), followed by sensory and shelf-life analysis.
4. The application of the obtained extracts (particularly the extracts obtained from potato peels) as natural food preservatives in the development of novel functional formulations, followed by sensory and shelf-life analysis.

Conclusiones y perspectivas futuras

La presente tesis tuvo como objetivo profundizar en el conocimiento de tres matrices alimentarias diferentes, en particular quinoa, amaranto y patata (pulpa y piel), en cuanto a sus características nutricionales, composición fitoquímica y potenciales efectos beneficiosos para la salud, con el fin de evaluar su idoneidad para su uso como alimentos funcionales o como ingredientes naturales en la preparación de productos con mayor valor añadido.

A continuación, se presentan las conclusiones obtenidas en el estudio de cada matriz alimentaria.

Quinoa. La quinoa brasileña BRS Piabiru mostró un contenido de proteína más alto que otras variedades de quinoa descritas anteriormente, además de revelar una composición de ácidos grasos con potenciales beneficios para la salud. Su destacado valor nutricional junto con sus características fisicoquímicas hacen de este cultivo un candidato idóneo para su incorporación directa en la dieta humana. Esta variedad de quinoa también presentó un alto contenido de glucósidos de quercetina y kaempferol, que podrían ser responsables por los resultados positivos obtenidos en las bioactividades evaluadas en la semilla. En general, los resultados de la amplia caracterización realizada en esta variedad sugieren la viabilidad de explotar el potencial de la quinoa brasileña como alimento funcional, apoyado en sus relevantes actividades antioxidantes, antibacterianas y antifúngicas, determinadas *in vitro*, con ausencia de citotoxicidad.

Este trabajo contribuye al creciente debate sobre alimentos alternativos sostenibles y más saludables al aportar información sobre un nuevo genotipo de quinoa adaptado al clima tropical. Esta información puede potencialmente ser utilizada por las industrias alimentaria y farmacéutica en el desarrollo de nuevos productos para la promoción de la salud. Asimismo,

contribuye a apoyar el objetivo de la FAO de convertir la quinoa en un cultivo comercial en Brasil, aliviando la creciente presión de la demanda mundial sobre Bolivia y Perú.

Amaranto. Derivado de un antiguo cultivo nutritivo, el cultivar de amaranto BRS Alegria presenta un destacado valor nutricional, particularmente en lo que respecta a su contenido de proteínas (17%), ácidos grasos (principalmente insaturados) y vitamina E. Hasta donde se conoce, éste es el primer informe completo sobre las características químicas y bioactividad de este nuevo cultivar. Los extractos hidroetanólicos de este pseudo-grano presentaron satisfactorias actividades antioxidante, antibacteriana y antifúngica, siendo más eficaz que el fungicida ketoconazol contra el hongo *Penicillium ochrochloron*. Además, el extracto mostró ausencia de hepatotoxicidad, lo que lo convierte en un candidato adecuado para su incorporación en formulaciones alimentarias y farmacéuticas como fuente de compuestos bioactivos. Este estudio sugiere que el genotipo BRS Alegria es un candidato adecuado para contribuir a la diversificación agrícola y la seguridad alimentaria, debido a su rica composición nutricional y fitoquímica, junto con sus propiedades bioactivas.

Patatas. La información obtenida en este estudio ilustra que la vasta biodiversidad existente de la patata se refleja en diferencias en el contenido de nutrientes de los tubérculos. En general, y a pesar de la aparente similitud encontrada al analizar todos los parámetros individualmente, los perfiles obtenidos mostraron estar altamente correlacionados con los diferentes tipos de cultivo y colores de la pulpa. Los parámetros caracterizados destacaron la similitud entre las variedades cultivadas y las líneas reproductoras, las cuales mostraron ligeras variaciones solo en algunos ácidos grasos particulares, independientemente del origen geográfico o del color de la pulpa del tubérculo. Esta observación podría estar relacionada con una mayor evolución en

el estado de crianza y la menor heterogeneidad en cuanto a caracteres específicos apreciados por los estándares agronómicos y de mercado. No obstante, el bajo número de diferencias discriminantes entre las líneas reproductoras y las variedades locales y accesiones andinas estudiadas apoyan el potencial de estas últimas para ser utilizadas como alternativas agronómicas a través de programas de mejora, especialmente de tipo participativo, con amplia implicación de los agricultores locales. Recuperar parte de esa antigua diversidad podría contribuir a la lucha contra el cambio climático a través de una agrobiodiversidad sostenible.

Patatas pigmentadas. Los extractos acuosos de patata ensayados presentaron alto contenido de antocianos y destacadas propiedades antioxidantes, antibacterianas y antifúngicas. Los principales compuestos encontrados fueron acilglucósidos, de pelargonidina en las variedades rojas y de petunidina en las moradas. No se detectaron efectos citotóxicos en líneas celulares hepáticas en los extractos hasta la concentración máxima probada (400 μg / mL), lo que sugiere su seguridad para se incorporar a formulaciones alimentarias. Los dos extractos seleccionados para su aplicación en una formulación de bebida refrescante mostraron perfiles adecuados en las evaluaciones sensoriales y de vida útil en comparación con el colorante comercial E163, empleado como control. El método empleado para la preparación de los extractos es eficaz y asequible y podría usarse para su obtención con vistas a ser utilizados como colorantes alimentarios naturales alternativos a los colorantes sintéticos. No obstante, son necesarios más estudios en los niveles pre- y post-cosecha para la selección de patatas pigmentadas con alto contenido de antocianos, mediante la evaluación de diferentes prácticas agronómicas, lo que contribuiría a elevar el valor añadido de este importante cultivo vegetal.

Pieles de patatas. Los extractos de las pieles de patata estudiadas presentaron resultados positivos en cuanto a capacidad antioxidante evaluada por dos métodos utilizando sistemas celulares, así como con relación a la actividad antitumoral en todas las líneas celulares tumorales ensayadas. Las pieles de la variedad roja Rosemary ofrecieron el mejor resultado, presentando las actividades antitumoral y antioxidante en el ensayo TBARS más altas, entre las tres variedades consideradas. Además, los extractos de esta variedad fueron los únicos que revelaron actividad antiinflamatoria. La bioactividad de las pieles de patata estudiadas podría estar asociada a su composición fenólica compuesta por ácidos fenólicos, flavonoides y antocianos. Los extractos hidroetanólicos evaluados no mostraron efectos tóxicos en la línea celular utilizada hasta la concentración máxima ensayada (400 μg/mL), con excepción del extracto de pieles de la variedad Rosemary, que presentó cierta citotoxicidad, aunque a concentraciones elevadas, superiores a 304 μg/mL. Estos resultados sugieren que los extractos de piel de patata estudiados serían seguros para ser utilizados como ingredientes alimentarios naturales para incorporar propiedades antioxidantes y antitumorales en formulaciones funcionales, con la posible salvedad del extracto de Rosemary en concentraciones superiores a 304 μg/mL.

Perspectivas futuras

Teniendo en cuenta los resultados de este trabajo, se proponen los siguientes objetivos futuros de investigación y desarrollo:

1. La optimización de condiciones de extracción adecuadas para preparar extractos más ricos en compuestos fenólicos, utilizando métodos tradicionales y alternativos.

2. Profundizar en la evaluación de la estabilidad y toxicidad de los extractos preparados y su empleo en nuevas formulaciones alimentarias.

3. La aplicación de estos nuevos colorantes naturales en formulaciones alimentarias diversas (además de bebidas refrescantes) y la evaluación de su aceptabilidad sensorial y vida útil.

4. La aplicación de los extractos (particularmente de los obtenidos de piel de patata) como ingredientes naturales en el desarrollo de nuevas formulaciones funcionales, evaluando igualmente su aceptabilidad sensorial y vida útil.

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Trends in Food Science & Technology 103 (2020) 118–129

Contents lists available at ScienceDirect

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Potato peels as sources of functional compounds for the food industry: A review

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ARTICLE INFO

Keywords:
Bioactive compounds
Functional ingredients
Glycoalkaloids
Phytochemicals
Phenolic compounds
Potato peels
Solanum tuberosum L

ABSTRACT

Background: Potato (*Solanum tuberosum* L.) is the most important vegetable crop, with a global production of around 368 million tonnes and more than 5000 known varieties. Tubers are the edible part of the plant, which can be eaten in various forms e.g. boiled, cooked, fried, crisped, etc. Processing of raw tubers usually involves peeling that generates a great amount of bulky waste which is usually discarded or used as animal feed.
Scope and approach: The present review aims at deeply discussing the current knowledge on the nutritional value, chemical composition and potentially related bioactivities of potato peels. Moreover, an overview on the reutilization of this bio-residue by the food industry is presented, by discussing the reported applications/incorporations into different food matrices, along with the potential technological properties.
Key findings and conclusions: Considering the nutritional value and chemical composition of potato peels, along with the bioactivity and technological properties of their extracts, the sustainable valorization of potato processing by-products presents great interest for the food and pharmaceutical industries that could increase the overall added value and minimize the environmental impact of this food crop.

1. Introduction

Potato (*Solanum tuberosum* L.) is the most important food crop after wheat, maize and rice, being a staple for 1.3 billion people (Stokstad, 2019), with a global production of more than 368 million tonnes in 2018 (FAOSTAT, 2019). Approximately 5000 potato varieties are known, hence potato is considered the most genetically diverse crop among cultivated species (Petropoulos et al., 2019). Besides being one of the most commonly consumed vegetables, potato crop is also associated with one of the largest food processing sectors throughout the world (Farvin, Grejsen, & Jacobsen, 2012). In the European Union, processed potatoes worthed EUR 10 billion in 2017, which corresponds to 1.5% of the whole European food industry production value (EUROSTAT, 2019). Commonly, potatoes produced worldwide are consumed fresh as a traditional ingredient of many cuisines, being traded for this purpose as a raw commodity. Nevertheless, it is estimated that more than fifty percent of the total global potato production is consumed after processing, divided mainly into four types of products: frozen, dried, prepared/preserved potatoes and potato starch (EUROSTAT, 2019; International Potato Center, 2019). Generally, potato processing begins with tubers peeling, for which methods such as steam peeling, lye peeling and abrasion have been reported, with the latter being the most used by the crisps industry (Toma, Orr, D'Appolonia, Dintzis, & Tabekhia, 1979; Wijngaard, Ballay, & Brunton, 2012), as fully described in the recent review of Pathak, Mandavgane, Puranik, Jambhulkar, and Kulkarni (2018). Added value processed products such as French fries, chips, hash browns, puree and frozen food account for a waste generation caused by potato peeling ranging from 15% to 40% of the original fresh weight, depending on the peeling process (Schieber, Stintzing, & Carle, 2002). Furthermore, potato starch, flour and canning industries are also responsible for the production of large amounts of peel waste whose disposal raises great environmental concerns (Pathak et al., 2018; Schieber et al., 2002). Annually, these industries produce between 70 and 140 thousand tonnes of potato peels (PP) worldwide (Hossain, Rawson, Aguiló-Aguayo, Brunton, & Rai, 2015). Most of the waste

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https://doi.org/10.1016/j.tifs.2020.07.015
Received 18 March 2020; Received in revised form 8 June 2020; Accepted 13 July 2020
Available online 22 July 2020
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Abbreviations

PP	potato peels
GAE	gallic acid equivalent
dw	dry weight
fw	fresh weight
BHT	butylated hydroxytoluene
BHA	butylated hydroxyanisole
TBARS	2-thiobarbituric acid reactive substance

generated by potato processing industries is discarded in land-fills, with accompanying environmental consequences, or used as animal feed with a low added value in the production chain (Chohan, Aruwajoye, Sewsynker-Sukai, & Gueguim Kana, 2020; Schieber & Aranda, 2009); recently, the use of potato processing residues as biomass sources for energy and biogas production has also been proposed (Achinas, Li, Achinas, & Euverink, 2019; Maragkaki et al., 2016; Osman, 2020). To overcome this global issue, up-grading of this residue to added value products can therefore be of interest not only to the food industry, but also to government agencies and policy makers, decreasing the overall environmental impacts of the potato processing industry (Hossain et al., 2015). In this context, Torres et al. (2020) suggested an integrated approach for processing of discarded potatoes (irregularly shaped or very small tubers) during harvest and after storage, which may account for up to 30% of total production. Thus, this waste could be exploited for the recovery of valuable compounds, such as starch from tuber flesh, antioxidants from skins and protein from processing wastewaters, while the second generation waste could be used for the production of potato flakes, hydrogels, biofilms, fertilizers and adsorbers (Torres et al., 2020).

The extraction of plant bioactive molecules has been largely explored and well established by many authors as an efficient route for the reutilization of food processing waste, especially when considering the recent technological advances in molecular separations and identifications (Wijngaard et al., 2012). PP can be a rich source of bioactive compounds due to their high contents in phenolic compounds with recognized health-promoting properties, such as antioxidant activity, that can also be employed in food systems to extend the shelf-life of food products (Albishi, John, Al-Khalifa, & Shahidi, 2013; Friedman et al., 2018).

In this perspective, the present review aims at deeply discussing the current knowledge on the nutritional value, chemical composition and potentially related bioactivities of PP extracts. Moreover, a comprehensive review of the reutilization of this bio-residue by the food industry is presented, by discussing the reported applications/incorporations into food matrices to date, along with the potential technological properties of PP extracts.

2. Agronomic aspects of potato peels: taxonomy, morphology and biodiversity

Potato species is characterized by a large phenotypic diversity with great interest on the edible part of the plant (tubers), which may differ in shape, size, flesh and skin colour and texture, as well as in aspects related with chemical composition (dry matter, proteins, starch, and glycoalkaloids content) and utilisation characteristics (cooking type, susceptibility to enzymatic browning, and suitability to frying and crisping), among others (Calliope, Lobo, & Sammán, 2018; Camire, Kubow, & Donelly, 2009). This differentiation is observed not only among the more diverse wild genotypes but also between the numerous cultivated genotypes throughout the world (IBPGR, 1985).

The skin set is affected by various factors such as the genotype which may affect the skin thickness and the strength of its adhesion to the flesh, as well as the tensile strength of skin (Bowen, Muir, & Dewar, 1996). Environmental factors also may have an impact on skin characteristics where cultivation in cold and moist soils results in thinner and weaker skin, while dry soils result in periderm with a lower number of cell layers. Agronomic factors such as nitrogen and potassium fertilization may decrease skin thickness and strength probably to the delayed maturation of crop, while the addition of micronutrients such as calcium, manganese and ferrous sulphate may increase the number of periderm cells (Wiltshire, Milne, & Peters, 2006).

From the anatomical point of view, peel or skin is the outer part of tuber periderm and a protective barrier against pest and pathogens attacks and water losses, while it serves as a wound-healing tissue of damaged epidermis (Barel & Ginzberg, 2008). Periderm consists of phellem cells (or skin), the phellogen and the phelloderm, which altogether are used to replace epidermis when the latter is damaged during tuber development, maturation and postharvest management (Barel & Ginzberg, 2008). Phellogen is a meristematic layer located at the middle section of periderm and produces phellem cells and phelloderm through outward and inward cell divisions, respectively, throughout the tuber development (Fogelman, Tanami, & Ginzberg, 2015). Phellem consists of several layers of cells with suberized cell walls after the deposit of suberin, a complex macromolecule that is responsible for the structural strength and protective properties of skin (Fogelman et al., 2015; Tanios et al., 2020). Suberin consists of two domains, an aromatic and an aliphatic one, which differentiates potato suberin polymers from other species and is responsible for the impermeable nature of PP to water, soluble compounds and gases (Schreiber, Franke, & Hartmann, 2005; Serra et al., 2010).

Suberin is naturally formed under normal conditions while its biosynthesis is triggered by wounding of any cause (mechanical damage or biotic factors) and environmental stressors (e.g. drought, salinity etc) (Schreiber et al., 2005; Yang & Bernards, 2006) through the up-regulation of suberin-related genes (*StKCS20*-like, *StFAR3*, *StCYP86A22* and *StPOD72*-like genes), which are differentially expressed in skin (Vulavala et al., 2017). Periderm is further divided into native and wound periderm, where the former is naturally formed during tuber development, whereas the latter is generated only on occasions where tubers are subjected to damage and skin lesions are observed (Sabba & Lulai, 2002). Both periderm types contain a high amount of lipids, hence their hydrophobic nature, although they differ in water permeability, with wound periderm being more permeable than the native one (Schreiber et al., 2005; Yang & Bernards, 2006). The chemical composition of periderm is evolving with tuber maturation as the cell walls of phellogen cells become thicker and more strong, while in phellem cells suberin is biosynthesized and waxes are deposited, so that altogether the periderm tissues provide the required protection for tubers from external factors (Lulai & Freeman, 2001).

The diversity in colour patterns among the tubers of various genotypes is associated with the accumulation of pigments in the periderm and the peripheral tissues of tuber cortices (Zhao et al., 2015). The skin may have red, purple and white colour, and the most common pigments isolated from tubers with coloured skin are anthocyanins (pelargonidin and peonidin) (Fogelman et al., 2015). These pigments are located in the vacuoles and colour stability and saturation depend on various factors such as co-pigmentation, the interactions with metallic ions, the pH conditions of the soil and the presence of peroxidases in the vacuoles (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009).

Agronomic factors may also affect the quality and development of skin. For example, Vulavala et al. (2016) reported that Si fertilization may delay skin maturation, increase skin cell area and upregulate the biosynthetic genes of suberin, resulting in enhanced suberization and lignification and improved resistance to skin injuries and storage ability of tubers. Skin set may be accelerated in early potato crops by applying vine killing through mechanical, physical or chemical means, allowing for earlier harvest without increased mechanical wounding incidence that immature skin could cause (Boydston, Navarre, Collins, &

Chaves-Cordoba, 2018; Lulai, 2002; Renner, 1991; Waterer, 2007). Farming systems have been also reported to affect skin colour; for instance, according to Lombardo, Pandino, and Mauromicale (2017), organic farming may enhance skin colour of Arinda cultivar. Moreover, light exposure and wounding after harvest may exhibit synergistic effects on the accumulation of steroidal glycoalkaloids in tuber skins (Nie et al., 2019), while the application of nanosilica during storage may inhibit sprouting and α-solanine content, which is associated with harmful effects on human health (Zhang et al., 2018). However, glykoalkaloids content may be affected not only by post-harvest conditions but also by farming operations, growing and environmental conditions and genetic factors (Friedman, Roitman, & Kozukue, 2003; Knuthsen, Jensen, Schmidt, & Larsen, 2009; Sotelo & Serrano, 2000).

Another agronomic factor that may affect the quality of potato peels is the irrational use of pesticides and herbicides during cultivation, which may result in excessive amounts of residues of organophosphorous and other compounds that may pose animals and consumers' health to chronic cumulative risks (Quijano, Yusà, Font, & Pardo, 2016; Soliman, 2001; Tan et al., 2020; Witczak, Pohoryło, Abdel-Gawad, & Cybulski, 2018). Although these residues are in most cases accumulated in tuber peels which are discarded during domestic or industrial processing (Dong, Bian, Liu, & Guo, 2019; Keikotlhaile, Spanoghe, & Steurbaut, 2010; Soliman, 2001), the use of agrochemicals and the irrational disposal of agroindustry wastewaters may put in danger the health of animals when these peels are used as animal feed or even the human health when the potato peels are intended for food applications (see section 6) (Kennedy, Garthwaite, de Boer, & Kruisselbrink, 2019; Narenderan & Meyyanathan, 2019). Therefore, the food industry is very concerned about this issue and is always seeking for advanced analytical techniques that allow for easy and accurate detection of such residuals and their fate in the food chain (Chen, Wang, Liu, & Bian, 2020; Keikotlhaile et al., 2010; Tan et al., 2020). The compliance of potato growers with regulations and best practice guides related with the proper use of agrochemicals, as well as the implementation of organic farming systems, are pivotal for the production of residual free potato tubers and peels (Kazimierczak, Srednicka-Tober, Hallmann, Kopczynska, & Zarzynska, 2019). Moreover, the management of bioconcentration factor of pesticides in root vegetables such as potato through the calculation of plant uptake and soil concentrations is of major importance for the crop safety from pesticide residuals (Hwang, Zimmerman, & Kim, 2018).

3. Nutritional value and chemical composition of potato peels

PP have an important nutritional value, mainly composed of starch, dietary fibre and protein (Jeddou et al., 2017). The metabolites profile in PP peels shows a great variability depending on the genotype (Inostroza-Blancheteau et al., 2018).

The most abundant macronutrients in PP are carbohydrates, accounting for 69–88 g/100 g dw (Table 1). Starch is responsible for 30–52% dw of its total carbohydrates content (Arapoglou, Varzakas, Vlyssides, & Israilides, 2010; Ramaswamy, Kabel, Schols, & Gruppen, 2013). PP are also a rich source of dietary fibre (Jeddou et al., 2016), which ingestion can exert benefits to human health, such as cholesterol-lowering effect and improved diabeticcontrol (soluble dietary fibre), besides intestinal health regulation (insoluble dietary fibre) (Jeddou et al., 2017). In a recent report, Elkahoui, Bartley, Yokoyama, and Friedman (2018) studied the total dietary fibre content of organic and non-organic PP of the Russet variety and described similar amounts in both farming systems (21.4% and 22.39% dw, respectively). On the other hand, Jeddou et al. (2017), employing a gravimetric enzymatic method, reported higher dietary fibre contents, focusing on the variety Spunta and a commercial unknown one. The authors found similar results for both genotypes, with the PP of Spunta and the commercial variety presenting 19.59% and 19.23% of insoluble dietary fibre, respectively, and 10.14% and 9.21% of soluble dietary fibre, respectively. An even higher total dietary fibre content of 51% was reported by Kumari, Tiwari, Hossain, Rai, and Brunton (2017) when studying PP from the Lady Claire variety. Regarding soluble sugars, the total content in PP is low, ranging around 0.9–1.0% dw (Amado, Franco, Sánchez, Zapata, & Vázquez, 2014; Arapoglou et al., 2010), while fermentable reducing sugars account for only 0.6% dw (Arapoglou et al., 2010). Some authors have reported the detection of glucose, fructose, galactose, rhamnose, arabinose and sucrose in PP, with glucose appearing as the most abundant soluble sugar (Choi, Kozukue, Kim, & Friedman, 2016; Jeddou et al., 2016). In particular, Choi et al. (2016) analysed the PP from six different Korean potato genotypes (Superior; Atlantic; Goun; K1, K20 and K30) and soluble sugars content ranged as follows: glucose 566–723 mg/100 g dw; fructose 433–683 mg/100 g dw; and sucrose 290–427 mg/100 g dw. In another study, Jeddou et al. (2016) reported the following monosaccharide composition in PP of the Spunta variety (expressed in percentages): glucose 76.25%, galactose 3.84%, rhamnose 0.51% and arabinose 0.19%.

Jeddou et al. (2016) investigated the physicochemical properties of water-soluble polysaccharides from PP waste. The extracts that were rich in polysaccharides showed high water-holding and fat-binding capacities of 4.097 ± 0.537 (g H_2O/g) and 4.398 ± 0.04 (g oil/g), respectively. More recently, the same authors performed enzymatic hydrolysis of water-soluble polysaccharides from PP waste, generating low molecular weight oligosaccharides; the extracts rich in oligosaccharides showed good foaming and emulsion properties (Jeddou et al., 2018).

Protein is the second most abundant macronutrient in PP, although the content varies widely among studies, ranging from 2 to 17 g/100 dw (Table 1). Choi et al. (2016) studied the nutritional protein composition

Table 1
Nutritional value (mean values ± standard deviation) of *Solanum tuberosum* peels, as reported in the literature over the last ten years. Values are expressed in g/100 g dw.

Proximate	Potato peel variety and origin				
	Organic Russet United States	**Non-organic Russet** - United States	**Red potato-** United States	**Gold potato** - United States	**Lady Rosetta** - Ireland
Moisture	3.67	3.78	4.46	5.66	6.98 ± 0.05
Carbohydrates	76	71	72	70	72.53 ± 0.08
Protein	11.98	17.19	15.99	14.17	11.17 ± 0.03
Fat	1.12	1.1	0.81	1.17	2.09 ± 0.01
Ash	7.32	7.34	6.69	9.12	7.24 ± 0.02
References	Elkahoui et al. (2018)	Elkahoui et al. (2018)	Elkahoui et al. (2018)	Elkahoui et al. (2018)	Kumari et al. (2017)
Proximate	**Potato peel variety and origin**				
	Lady Claire - Ireland	**Spunta** - Tunisia	**Agria** - Spain	**Unknown variety** - Greece	
Moisture	4.08 ± 0.04	7.3 ± 0.3	7.30 ± 0.23		
Carbohydrates	77.38 ± 0.65	88.0 ± 4.4	86.97 ± 0.43	68.7	
Protein	12.44 ± 0.09	2.099 ± 0.105	6.47 ± 0.23	8	
Fat	1.27 ± 0.38	0.733 ± 0.037	0	2.6	
Ash	4.83 ± 0.13	0.906 ± 0.006	5.46 ± 0.17	6.34	
References	Kumari et al. (2017)	Jeddou et al. (2016)	Amado et al. (2014)	Arapoglou et al. (2010)	

of PP, and reported a total crude protein concentration of 9.52–10.58 g/100 g dw, an essential amino acids content of 429–666 mg/100 g dw, a total free amino acid level of 1383–2077 mg/100 g dw, and an asparagine range of 90.4–115.8 mg/g dw.

Even among different varieties, the fat content of PP is very low, with a few authors reporting the absence of fat and others values up to 2.6 g/100 g dw (Table 1). When studying a variety cultivated in China, Wu et al. (2012) detected two unsaturated fatty acids in PP, namely an omega-3 (9,10,11-trihydroxy-12(Z),15(Z)-octadecadienoic acid) and an omega-6 (9,10,11-trihydroxy-12(Z)-octadecenoic acid). Regarding other interesting components, the same authors reported the presence of quinic acid in the range of 0.63–0.71 mg/g dw, as quantified by UPLC–ESIMS (Wu et al., 2012); this acid has been indicated to be helpful in the treatment of diabetes, by promoting insulin secretion from pancreatic beta cells (Heikkilä, Hermant, Thevenet, & Domingo, 2019). The presence of ascorbic acid in PP has also been reported, in a concentration of 1.44 ± 0.5 mg/g dw for the Canadian cultivar Russet Burbank (Singh et al., 2011, pp. 2218–2232).

From the few reports available in the literature, PP appear as a rich source of nutritional compounds, particularly dietary fibre, making it a suitable candidate for incorporation in the human diet to improve foods' nutritional value. Nevertheless, there are variations in PP nutritional and chemical composition which may be attributed to various factors including varietal differences besides agronomic and environmental factors (Kumari et al., 2017, pp. 1432–1439).

4. Phytochemicals in potato peels

4.1. Phenolic compounds

PP are also described as being a rich source of phenolic compounds, which have been related to human health benefits, including antioxidant and antimicrobial properties (Silva-Beltrán et al., 2017). The content of phenolic compounds in PP is up to ten times higher than in potato flesh (Albishi et al., 2013; Rytel et al., 2014; Wu et al., 2012). Additionally, some phenolic compounds present in tuber peel are only rarely detected in flesh, such as caffeic acid, coniferyl alcohol, coniferyl aldehyde, vanillin, vanillic acid, ferulic acid and *p*-coumaric acid, all of which are linked to the plant defense mechanism against pathogen attacks (Oertel et al., 2017). Literature data show that the total amount of phenolic compounds in PP varies significantly among different varieties (Albishi et al., 2013). Nevertheless, chlorogenic acid and its isomers appear as the major molecules determined in most of the studies, followed by caffeic acid (Table 2). The analysis by HPLC-DAD-ESI-MS showed that chlorogenic acid accounted for 49.3–61% of the total phenolic compounds in a study conducted by Riciputi et al. (2018). Moreover, when stored at room temperature and exposed to direct light, chlorogenic acid can be partially degraded to caffeic acid, which may explain why in some studies caffeic acid appears as the major phenolic compound, since industrial PP waste is usually stored in the open without coverage from sunlight (Wijngaard et al., 2012). Other reported phenolic compounds in PP include ferulic and *p*-coumaric acids, rutin, quercetin and catechin (Table 2).

According to the available data, the phenolic acids content in PP is in generally higher than the flavonoids content, while high-anthocyanin varieties also have higher contents of chlorogenic acid (Valiñas, Lanteri, ten Have, & Andreu, 2017). Hsieh, Yeh, Lee, and Huang (2016) quantified a total phenolic acids content of 86.3 mg GAE/100 g dw in PP aqueous extracts, whereas the flavonoids content accounted for 27.5 mg GAE/100 g dw, with caffeic, *p*-coumaric, chlorogenic and ferulic acids as the most prevalent compounds (Hsieh et al., 2016).

The difference in the phenolic compounds content among the existent studies can be in part attributed to the use of different extraction conditions, extraction solvents and methods. For instance, Silva-Beltrán et al. (2017) compared the phenolic composition of PP in aqueous and acidified ethanolic extracts, by applying maceration with constant stirring for 72 h. The acidified ethanolic extract showed higher total phenolics (14.0 mg GAE/g dw) and total flavonoids (3.3 mg quercetin equivalent/g dw) contents than the water extract (4.2 mg GAE/g dw, and 1.0 mg quercetin equivalent/g dw, respectively). The same was evidenced by Albishi et al. (2013) when studying the total content of free, esterified and bound phenolics in the peels from four different potato cultivars (Purple, Innovator, Russet and Yellow), by employing a solid-liquid extraction using methanol–acetone–water (7:7:6; v/v/v) as solvent. For the varieties Russet and Innovator, bounded phenolics content (4.8 mg GAE/g dw and 5.3 mg GAE/g dw, respectively) was higher than the content in free phenolics (3.1 mg GAE/g dw and 3.2 mg GAE/g dw, respectively), which highlights the importance of including the analysis of bounded phenolics in analytical protocols in order to more accurately determinate the total phenolic compounds content.

Among the different extraction techniques used to obtain these molecules, besides the traditional solid-liquid extraction, techniques such as ultrasound (Kumari et al., 2017, pp. 1432–1439; Paleologou, Vasiliou, Grigorakis, & Makris, 2016; Riciputi et al., 2018) and microwave-assisted treatments (Singh et al., 2011, pp. 2218–2232) can also improve the extraction efficiency of phenolic compounds from PP. By applying an ultrasound-assisted extraction method, Kumari et al. (2017, pp. 1432–1439) reported a significantly higher recovery rate of phenolic compounds compared to solid–liquid extraction process alone. These authors also compared different solvent combinations, i.e. pure distilled water, pure methanol, 80% methanol/water and 50% methanol/water (v/v). According to the results, the 80% aqueous methanol solvent was the best combination for the extraction of phenolic compounds from PP, whereas the ultrasonic treatment at 33 kHz was more effective in recovering polyphenols compared to higher frequency (42 kHz).

On the other hand, Singh et al. (2011, pp. 2218–2232) applied microwave-assisted extraction, combined to a response surface methodology study to optimize parameters such as extraction time, solvent concentration and microwave power. In regards to total phenolics, the maximum content (3.94 mg/g dw) was obtained when the extraction time was 15 min, the used solvent 67.33% methanol/water and the microwave power 14.67%. Optimal concentrations of caffeic (1.33 mg/g dw) and ferulic acids (0.50 mg/g dw) were achieved at 15 min, 100% methanol and 10% microwave power, whereas the maximum chlorogenic acid content (1.35 mg/g dw) was obtained at a shorter extraction time (5 min), whilst maintaining similar conditions for the rest of the extraction parameters (100% methanol and 10% microwave power). This indicates that chlorogenic acid might be more sensitive than the other compounds to the temperature reached as a result of the microwave power levels. Overall, the authors evidenced that microwave assisted extraction exhibited a better efficiency in extracting phenolic compounds from PP than conventional methods, by comparing their results (total phenolics of 3.94 ± 0.21 mg/g dw) to previously reported literature based on solid-liquid extraction (total phenolics 1.51 ± 0.17 − 3.31 ± 0.12 mg/g dw).

As already exemplified, the solvent choice can also play a pivotal role in the efficiency of phenolic compounds extraction from PP. Solvents used include ethanol/water (Paleologou et al., 2016; Riciputi et al., 2018), methanol/water (Kumari et al., 2017, pp. 1432–1439), water/-glycerol (Paleologou et al., 2016), acetone (Zia-ur-Rehman, Habib, & Shah, 2004) and diethyl ether (Zia-ur-Rehman et al., 2004). Nevertheless, greener solvents such as glycerol and ethanol are currently preferred over other suggested options for safety and environmental reasons.

An important class of compounds that can be obtained from PP is anthocyanins, which are responsible for the colours of purple and red-fleshed potato varieties (Albishi et al., 2013; Mori et al., 2010; Oertel et al., 2017; Rytel et al., 2014; Yin et al., 2016). Potatoes with coloured flesh usually have identically coloured skin; however, the colours of PP and flesh do not always match, as some yellow-fleshed varieties also present red or purple-coloured skin (Pathak et al., 2018).

Table 2
Contents of some phytochemicals (mean values ± standard deviation) of *Solanum tuberosum* peels as reported in the literature during the last ten years.

Phenolic compounds	Potato peel variety and origin						
	Bintje - Italy (mg/g dw)	**Challenger** -Italy (mg/g dw)	**Non-organic Russet** -United States (μg/g dw)	**Chaquena** - Andes (μg/g fw)	**Santa María** -Andes (μg/g fw)	**Organic Russet** - United States (μg/g dw)	**Organic Yukon gold** - United States (μg/g dw)
Chlorogenic acid	1.97 ± 0.02	1.27 ± 0.01	6422	119 ± 7.83	497 ± 33.4	7810 ± 870	1890 ± 100
Caffeic acid	0.24 ± 0.001	0.22 ± 0.002	215	17.2 ± 0.95	34.8 ± 3.17	531 ± 771	365 ± 221
Ferulic acid	0.06 ± 0.0002	0.05 ± 0.001	–	–	–	–	–
p-Coumaric acid	–	–	–	–	–	–	–
Rutin	–	–	–	–	–	–	–
Quercetin	–	–	–	–	–	–	–
Catechin	–	–	–	6.17 ± 1.07	20.0 ± 3.10	–	–
	Riciputi et al. (2018)	Riciputi et al. (2018)	Elkahoui et al. (2018)	Valiñas et al. (2017)	Valiñas et al. (2017)	Friedman et al. (2017)	Friedman et al. (2017)

Phenolic compounds	**Potato peel variety and origin** **Fianna** (acidified ethanol extract) - Mexico (mg/100 g dw)	**Fianna** (water extract) - Mexico (mg/100 g dw)	**Unknown variety** - Taiwan (mg/100 g dw)	**Innovator** - The Czech Republic (mg/100 g fw)	**Blaue Elise** - The Czech Republic (mg/100 g fw)	**Valfi** - The Czech Republic (mg/100 g fw)	**Bore Valley** - The Czech Republic (mg/100 g fw)
Chlorogenic acid	346.03 ± 2.14	159.99 ± 1.05	19.5 ± 0.7	91.3 ± 1.20	248.8 ± 2.10	60.1 ± 1.60	174.3 ± 2.42
Caffeic acid	332.58 ± 3.67	56.99 ± 3.23	14.3 ± 0.73	38.0 ± 1.43	3.82 ± 0.7	103.1 ± 2.52	30.0 ± 1.56
Ferulic acid	3.29 ± 0.05	–	11.2 ± 1.8	1.83 ± 0.24	0.13 ± 0.02	0.76 ± 0.05	0.07 ± 0.001
p-Coumaric acid	–	–	10.8 ± 0.1	–	0.02 ± 0.001	0.04 ± 0.001	0.05 ± 0.004
Rutin	5.01 ± 1.03	–	0.2 ± 0.1	–	–	–	–
Quercetin	11.22 ± 0.09	2.18 ± 0.07	4.2 ± 0.3	–	–	–	–
Catechin	–	–	0.9 ± 0.1	–	–	–	–
	Silva-Beltrán et al. (2017)	Silva-Beltrán et al. (2017)	Hsieh et al. (2016)	Rytel et al. (2014)	Rytel et al. (2014)	Rytel et al. (2014)	Rytel et al. (2014)

Phenolic compounds	**Potato peel variety and origin** **Blue Congo** - The Czech Republic (mg/100 g fw)	**Russet** - Canada (mg/100 g freeze dried sample)	**Innovator** - Canada (mg/100 g freeze dried sample)	**Yellow** - Canada (mg/100 g freeze dried sample)	**Purple** - Canada (mg/100 g freeze dried sample)	**Unknown variety** - China (mg/g dw)	**Russet Burbank** - Canada (mg/g dw)
Chlorogenic acid	122.8 ± 2.32	134.9	128.9	16.9	364.9	3.87 ± 0.04	1.35 ± 0.18
Caffeic acid	15.5 ± 0.40	98.5	109.4	29.7	92	2.23 ± 0.08	1.33 ± 0.06
Ferulic acid	0.09 ± 0.008	56.9	84.8	12.5	6.9	–	0.5 ± 0.02
p-Coumaric acid	–	–	5.3	2.6	7.4	–	–
Rutin	–	–	–	–	–	–	–
Quercetin	–	–	–	–	–	–	–
Catechin	–	–	–	–	–	–	–
	Rytel et al. (2014)	Albishi et al. (2013)	Albishi et al. (2013)	Albishi et al. (2013)	Albishi et al. (2013)	Wu et al. (2012)	Singh et al. (2011)

Glycoalkaloids	**Potato peel variety and origin** **Organic Russet** - United States (μg/g dw)	**Non-organic Russet** - United States (μg/g dw)	**Gold potato** - United States (μg/g dw)	**Red potato** - United States (μg/g dw)	**Organic Russet** - United States (μg/g dw)	**Conventional Russet** - United States (μg/g dw)	**Organic Yukon gold** - United States (μg/g dw)	**Conventional Yukon gold** - United States (μg/g dw)
α-Chaconine	593	781	1301	1604	1180 ± 110	424 ± 30	2830 ± 370	670 ± 130
α-Solanine	268	347	636	572	374 ± 54	215 ± 43	750 ± 120	253 ± 44
Solanidine	–	–	–	–	–	–	–	–
Demissidine	–	–	–	–	–	–	–	–
Total glycoalkaloids	861 ± 10	1128 ± 1	1940 ± 170	2180 ± 170	1550 ± 120	639 ± 52	3580 ± 390	920 ± 140
	Elkahoui et al. (2018)	Elkahoui et al. (2018)	Elkahoui et al. (2018)	Elkahoui et al. (2018)	Friedman et al. (2017)	Friedman et al. (2017)	Friedman et al. (2017)	Friedman et al. (2017)

Glycoalkaloids	**Potato peel variety and origin** **Organic Red** - United States (μg/g dw)	**Conventional Red** - United States (μg/g dw)	**Unknown** - Ireland (μg/g dw)
α-Chaconine	610 ± 110	1297 ± 56	873
α-Solanine	239 ± 34	412 ± 24	597
Solanidine	–	–	374
Demissidine	–	–	75
Total glycoalkaloids	850 ± 120	1709 ± 61	1919
	Friedman et al. (2017)	Friedman et al. (2017)	Hossain et al. (2015)

Anthocyanins	**Potato peel variety and origin** **Purple Cloud No.1** - China (mg/100 g fw)	**Red Cloud No.1** - China (mg/100 g fw)	**Yunnan Potato 303** - China (mg/100 g fw)	**Yunnan Potato 603** - China (mg/100 g fw)
Delphinidin	2.08 ± 0.08	0.98 ± 0.04	0.49 ± 0.01	0.97 ± 0.03
Cyanidin	7.17 ± 0.11	1.95 ± 0.00	1.68 ± 0.01	0.93 ± 0.00
Petunidin	177.50 ± 1.52	1.05 ± 0.49	75.37 ± 0.50	0.40 ± 0.01
Pelargonidin	3.33 ± 0.07	143.05 ± 1.04	0.93 ± 0.02	118.32 ± 0.78
Peonidin	56.97 ± 1.17	15.34 ± 0.21	23.16 ± 0.13	7.57 ± 0.05
Malvidin	21.71 ± 0.16	–	12.58 ± 0.06	–
	Yin et al. (2016)	Yin et al. (2016)	Yin et al. (2016)	Yin et al. (2016)

Albishi et al. (2013) compared the content of phenolic compounds in PP of four different cultivars, three of which were yellow-fleshed and one was purple-fleshed. The PP of the purple-fleshed genotype contained the highest amount of free (7.2 mg GAE/g dw) and esterified (4.74 mg GAE/g dw) phenolic compounds among all the four studied varieties. Moreover, in the purple potato genotype, pigments were mostly accumulated in the skin and the outer cortices of tubers, being the content of anthocyanins in the peel 10.69 times higher than in the flesh.

Oertel et al. (2017) used UPLC coupled to UV and mass spectrometry detection to analyse the anthocyanins and polyphenol profile of potato tuber tissues (flesh and skin) from fifty-seven coloured potato cultivars. They found that the potato genotypes could be grouped according to their anthocyanin accumulation patterns. Eighteen anthocyanin profiles were identified, comprising six groups derived from red and twelve from blue and purple cultivars. Acylated pelargonidin glycosides were confirmed as the main pigments in red tubers, especially pelargonidin-3-*p*-coumaroylrutinoside-5-glucoside. For blue and purple tuber tissues, petunidin 3-*p*-coumaroylrutinoside-5-glucoside and malvidin 3-feruloylrutinoside-5-glucoside were the most abundant anthocyanins, the latter being more abundant in the skin than in the flesh. In general, a higher concentration of malvidin derivatives was detected in peels compared to flesh of purple and blue potato genotypes. The antibacterial effect of malvidin has been linked to the plant defense mechanism against pathogenic bacteria, which may explain the higher concentration in the peel as the outer barrier of tubers from external aggressions.

Yin et al. (2016) studied ten coloured potato cultivars from China (Purple Cloud No. 1, Red Cloud No. 1, Yunnan Potato 303, Yunnan Potato 603, S03-2677, S03-2685, S03-2796, S05-603, S06-277 and S06-1693), by applying an ultrasonic treatment, with 95% ethanol:1.5 M hydrochloric acid (85:15, v/v) as solvent, followed by HPLC analysis. For all the analysed cultivars, anthocyanin and phenolic content and antioxidant activity were significantly higher in the PP than in the flesh. Six anthocyanins were detected in the coloured PP, with delphinidin and peonidin being present in all the studied cultivars. The other detected anthocyanidins were petunidin, malvidin and cyanidin.

In summary, as a bio-residue of the pigmented potato processing industry, the coloured PP could be used to obtain added value extracts rich in polyphenols for further applications as natural food colouring agents or preservatives, given the antioxidant and antimicrobial properties also associated to these compounds (Yin et al., 2016).

4.2. Glycoalkaloids

Another group of compounds found predominantly in PP are the glycoalkaloids, which are mainly represented by the molecules α-solanine and α-chaconine (Andreas Schieber & Aranda, 2009). The function of these compounds is to protect the plant against bacterial, fungal and insect attacks (Hossain et al., 2015). Glycoalkaloids can exert some level of toxicity to humans, but are safe for ingestion at levels of about 1–2 mg/kg of body weight (Elkahoui et al., 2018). Most of the commercially available potato genotypes contain less than 10 mg/100 g fw, being 20 mg/100 g fw the suggested upper threshold (Rytel et al., 2018). Nevertheless, recent studies have indicated these compounds may also present beneficial bioactivities to human health, such as antibacterial, anticancer, anti-inflammatory and antiobesity effects (Elkahoui et al., 2018; Friedman et al., 2018; Hossain et al., 2015). Commonly, α-chaconine is the most abundant glycoalkaloid found in PP (Table 2), and it is also reported to be up to five times more bioactive than α-solanine (Friedman, Kozukue, Kim, Choi, & Mizuno, 2017).

Friedman et al. (2017) compared the α-solanine, α-chaconine and total glycoalkaloids contents in PP from organic and non-organic commercial gold, Russet and red potatoes, by applying 5% acetic acid extraction accompanied by ultrasonication. As described in Table 2, the PP from organic-grown gold and Russet varieties showed a higher content of glycoalkloids (3580 and 1550 μg/g dw, respectively) compared to the non-organic samples (920 and 639 μg/g dw, respectively). For the red variety the opposite trend was observed, as the organic samples revealed lower content of glycoalkaloids (850 μg/g dw) than the conventionally grown samples (1709 μg/g dw). On the other hand, Elkahoui et al. (2018), in a study on the same varieties, found the highest glycoalkaloid contents in PP from the conventionally grown red variety (2180 μg/g dw).

Hossain et al. (2015) compared the extraction of glycoalkaloids by pressurized liquid extraction (PLE) and conventional solid-liquid extraction. The authors found that PLE was the most effective technique, with results for individual steroidal alkaloids of 597, 873, 374 and 75 μg/g dried potato peel for α-solanine, α-chaconine, solanidine (the alkaloidal aglycone) and demissidine (the solanidine dihydrogenated form), respectively. Corresponding values for solid liquid extraction were 247, 474, 224 and 36 μg/g dw, respectively (i.e., 59%, 46%, 40% and 52% lower than PLE method). The PLE parameters were also optimized by a response surface methodology, being 80 °C and 89% methanol the optimal temperature/extracting solvent combination.

5. Bioactive properties

5.1. Antioxidant activity

PP extracts have been shown by many studies to exert antioxidant activity, through the scavenging of several reactive oxygen species/free radicals under *in vitro* conditions. The antioxidant activity in PP extracts has been screened by different assays, such as the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging capacity (Jeddou et al., 2018; Singh et al., 2011, pp. 2218–2232; Singh & Rajini, 2004), reducing power (Jeddou et al., 2018; Singh & Rajini, 2004), β-carotene bleaching inhibition activity (Jeddou et al., 2018), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity (Jeddou et al., 2018), lipid peroxidation in rat liver homogenates (Singh & Rajini, 2004) and iron ion chelation (Singh & Rajini, 2004). In some studies, PP extracts demonstrated significantly higher radical scavenging activity ($p < 0.05$) than the respective flesh extracts (Albishi et al., 2013; Wu et al., 2012).

Jeddou et al. (2018) found promising results when investigating the antioxidant capacity of PP low molecular weight oligosaccharides, obtained by enzymatic hydrolysis of water-soluble polysaccharides from PP waste, by different *in vitro* methods: DPPH radical-scavenging capacity (IC_{50} = 2.5 mg/mL), reducing power (OD: 0.622 ± 0.032 at a concentration of 20 mg/mL), β-carotene bleaching inhibition activity (45.335 ± 3.653%), and ABTS radical scavenging activity (14.835 ± 0.1%). Previously, it was already shown that PP extracts had strong antioxidant activity similar to the synthetic antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), suggesting them as potential natural antioxidants for oils, fats and other food products (Zia-ur-Rehman et al., 2004). Indeed, in the study by Jeddou et al. (2018), oligosaccharide-rich PP extracts exhibited the same DPPH radical scavenging activity as the control BHA, both at the same concentration of 10 mg/mL (Jeddou et al., 2018).

In most studies, a significant positive correlation was also found between the antioxidant activity and the phenolic content of PP extracts (Albishi et al., 2013; Singh et al., 2011, pp. 2218–2232; Singh & Rajini, 2004). It has also been reported that bounded and esterified phenolic compounds can contribute as much or even more than the free phenolics to the antioxidant activity of PP (Albishi et al., 2013). Moreover, PP from purple and red coloured varieties generally show higher antioxidant activity when compared to yellow-coloured varieties, which is commonly attributed to the higher content of anthocyanins present in colour-fleshed potatoes (Albishi et al., 2013).

The antioxidant activity of PP was also evidenced in an *in vivo* study carried out by Hsieh et al. (2016), who evaluated the effect of PP aqueous extracts (conventional solid-liquid extraction) on the toxicity of cholesterol oxidation products in rats. The rats fed with diets containing

2% and 3% of the PP extracts showed, after treatment, significantly increased ($p < 0.05$) liver glutathione and trolox equivalent antioxidant capacity levels, greater superoxide dismutase, catalase, and glutathione peroxidase activities, and also enhanced mRNA expression. Additionally, treated animals showed significantly decreased aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, creatinine, and cholesterol oxidation products' levels, when compared with the control rats.

5.2. Antibacterial and antiviral activities

PP extracts have also shown interesting microbiological potential. In a recent work, Friedman et al. (2018) investigated the *in vitro* anti-trichomonad activity of PP powders prepared from commercial Russet, red, purple, and fingerling potato varieties against three pathogenic strains of trichomonads: one *Trichomonas vaginalis* strain (a sexually transmitted protozoan parasite that causes the human disease trichomoniasis) and two distinct strains of the related *Tritrichomonas foetus*, one feline and one bovine. Ethanol (1 mL, 50%)/acetic acid (0.5%) was used as solvent in a conventional solid-liquid extraction. Most of the PP samples were active against these three trichomonad species at the concentration of 10%, w/v, with the two Russet samples being the most active against all three parasites. Additionally, and also importantly, PP did not show inhibitory activity toward several normal native vaginal bacteria species. Moreover, PP extracts have also demonstrated antibacterial activity toward *Eschericia coli* and *Salmonella Typhimurium* (Sotillo, Hadley, & Wolf-Hall, 1998).

In a study conducted by Silva-Beltrán et al. (2017), PP acidified ethanolic extracts showed antiviral effects against human enteric viruses. The PP extracts successfully inhibited the human enteric viral surrogates of Av-05 and MS2 bacteriophages. The authors performed a 3 h incubation with PP extracts at a concentration of 5 mg/mL, which reduced the PFU/mL (plaque-forming unit per unit volume) of Av-05 and MS2 by 2.8 and 3.9 log10, respectively, in a dose-dependent manner. The results suggested that PP extract has potential to be used as an effective agent against human enteric viruses.

5.3. Anti-obesity and antidiabetic properties

PP have also been studied for their anti-obesity and antidiabetic potential. For instance, the dietary supplementation of PP powder reduced weight gain in mice submitted to a high-fat diet, suggesting the potential of PP powder to serve as an anti-obesity functional food (Elkahoui et al., 2018). In this study, mice adipogenic high-fat diets (25% fat by weight) were supplemented with 10–20% PP powders for 21 days. In comparison to the control diet, the peel-containing diet induced a significant reduction in mice's weight gain by up to 73%. The positive results of PP powder as an anti-obesity agent were likely to be related to its high content of glycoalkaloids, since the weight gains of the mice were negatively correlated with both α-chaconine and α-solanine contents of the diet, as revealed by statistical analysis (Tukey test, coefficients of −0.81 and −0.80, respectively; $p < 0.05$).

There is an increasing interest among the scientific community to find out alternative treatments for type 2 diabetic patients since the commercially available drugs are reported to cause various side effects (Arun et al., 2015). A-glucosidase inhibitors act by retarding the liberation of glucose from dietary carbohydrates, reducing postprandial glucose levels, thus supporting the management of type 2 diabetes. In this context, Arun et al. (2015) compared the bioactivities of hexane, ethyl acetate and methanol PP extracts applying an α-glucosidase inhibition *in-vitro* assay, finding the methanolic extract as the most efficient one, exhibiting promising α-glucosidase inhibitory activity (IC_{50} value of 184.36 mg/mL).

6. Potato peels applications in food products

Table 3 presents a summary of the applications of PP in the food industry, which are illustrated in Fig. 1. These applications are discussed in depth below.

6.1. Bread, flatbread and cake

The first reports on the uses of PP in food applications date back from the 1970's, when Toma et al. (1979) described its use as a source of dietary fibre in bread. The authors pointed out several advantages of PP compared to wheat bran, such as mineral and total dietary fibre contents, the water-holding capacity, the lower quantity of starchy components, and the lack of phytate, which were maintained even after the baking process. They also reported increased crumb darkening and reduced loaf volume in bread which had wheat flour partially replaced by PP.

More recently, Curti, Carini, Diantom, and Vittadini (2016) applied PP fibre in bread (0.4 g fibre/100 g flour) to study its ability to reduce bread stealing. The authors found that the potato fibre addition in bread increased frozen water content and resulted in a softer bread crumb over 7 days of storage. The values for crumb hardness at the end of the storage period were lower for the formulation with added PP compared to the control formulation (3.7 ± 0.6 N and 4.5 ± 0.7 N, respectively).

For their part, Crawford, Kahlon, Wang, and Friedman (2019) incorporated PP in quinoa flatbreads with the aim of reducing acrylamide content, a potentially-toxic compound that is formed during thermal processing of cereals. PP powder from the Russet variety was incorporated into quinoa flour at 5%, resulting in a significant decrease of acrylamide content of the baked flatbreads compared to the control formulation (from 487 to 367 μg/kg). The results suggested that the PP supplemented quinoa flatbreads have the potential to serve as a nutritional, gluten-free, low-acrylamide functional food.

Besides bread, PP powder was also incorporated into cakes by Jeddou et al. (2017). In that study, wheat flour was substituted by 2%, 5% and 10% of PP powder of Spunta variety and a commercial unknown one. The PP powders showed high levels of dietary fibre and protein. The resulting cakes had overall improved texture, mainly due to the water binding and fat absorption capacities exhibited by PP powder. Cake hardness decreased by 30.24% for the 5% Spunta PP formulation compared with control cake. Moreover, the replacement of wheat flour with PP powder increased dough strength and elasticity-to-extensibility ratio. A sensory analysis based on consumers' acceptance was carried out to compare fortified and control cake formulations, with results indicating no significant difference between them. In regards to nutritional value, the cake fortified with PP showed an increased soluble dietary fibre content compared to control (4.8% and 3.3%, respectively), and the same was observed for insoluble fibre content (21.4% and 15.9% respectively). Finally, the PP fortified cakes presented a darker colour compared to control, with higher a* values (red colour) and lower L* values (lightness). These changes in colour might be directly contributed by the colour attributes of PP powders, but also by a more pronounced non-enzymatic browning effect due to the sugar composition of the added PP. Other uses of PP powder include their incorporation in biscuits by substituting wheat flour which resulted in increased water absorption, dough development time and dough stability, while increasing antioxidant activities and fibre content at the same time (Hallabo, Helmy, Elhassaneen, & Shaaban, 2018).

6.2. Vegetable oils, fish-rapeseed oil mixture and oil-in-water emulsions

Lipid oxidation is a free radical chain reaction that causes the reduction of shelf-life of many processed food products. With the aim of reducing rancidity and the production of toxic oxidation molecules, the addition of synthetic antioxidants to food products is one of the most effective strategies applied by the food industry. However, there is

Table 3
Compilation of works found in the literature on potato peel applications in the food industry.

Application	Type of extract/product	Analyses carried out	Main findings	Reference
Bread	Fibre extracted from potato peel	Texture over shelf-life	Potato fibre was incorporated into bread (0.4 g fibre/100 g flour), reducing bread hardness over a 7 days storage period when compared to the control formulation.	Curti et al. (2016)
Flatbread	Potato peel powder	Acrylamide content	5% potato peel powder was added to flour of a quinoa flatbread formulation, resulting in a significant decrease in acrylamide content (a potentially toxic compound) of baked flatbreads (from 487 to 367 μg/kg).	Crawford et al., 2019
Cake	Potato peel powder	Texture and sensory analysis	Potato peel powders with high level of dietary fiber and protein were incorporated into cakes, resulting in improvement of nutritional value (higher dietary fibre content) and texture (cake hardness decrease by 30.24%). Sensory analysis results showed no statical difference between enriched cakes and control.	Jeddou et al., 2017
Soybean oil	Ethanolic potato peel extracts	Oxidative stability (peroxide, totox and *p*-anisidine indices)	Potato peel extracts were able to stabilize soybean oil under accelerated oxidation conditions (60 °C, 15 days), minimising oxidation products (peroxide, totox and *p*-anisidine indices).	Amado et al. (2014)
Soybean oil	Petroleum ether potato peel extracts	Oxidative stability (free fatty acids, peroxide value and iodine value) over shelf-life	Potato peel extracts exhibited very strong antioxidant activity which was almost equal to synthetic antioxidants (BHA and BHT), retarding the development of oxidation products in soy bean oil during a 60 days' storage period.	Zia-ur-Rehman et al. (2004)
Soybean oil	Ethanolic potato peel extracts	Oxidative stability (peroxide, *p*-anisidine, conjugated dienes values, volatile compounds and fatty acids)	Extracts from potato peel were added to soybean oil, in the concentrations corresponding to chlorogenic acid levels of 14.01 ppm, 20.37 ppm and 31.94 ppm, and assessed under accelerated oxidation conditions. Enriched oil showed lower peroxide values, higher inhibition of hexanal production and higher overall oxidation stability compared to control untreated oil, for all tested concentrations.	Franco et al. (2016)
Sunflower and soybean oils	Methanolic potato peel extracts	Oxidative stability (peroxide and *p*-anisidine values, conjugated dienes and conjugated trienes)	Potato peel methanolic extracts were added to sunflower and soybean oils, under accelerated oxidation conditions (70 °C, 72 h). Results for both vegetable oils suggested a superior antioxidant activity of potato peel extracts (at the concentration of 100 ppm and 200 ppm) over synthetic antioxidants BHT and BHA at their legal limit.	Mohdaly et al. (2010)
Fish-rapeseed oil mixture and oil-in-water emulsions	Ethanolic potato peel extracts	Oxidative stability (peroxide and *p*-anisidine values) and sensory evaluation	Potato peel extracts (2,400 mg/kg) were 5.7 times more effective than BHT in reducing peroxide value, and 9 times more effective in reducing *p*-anisidine value. Sensory analysis showed that treated samples had lower rancidity scores compared to control.	Koduvayur Habeebullah et al., 2010
Cooked salmon	Aqueous methanolic–acetonic extracts	Antioxidant activity (TBARS)	Extracts rich in phenolic compounds were applied to retard the development of oxidative rancidity in fish. The extracts were effective in inhibiting the oxidation of cooked salmon in comparison with the control over a 7 day storage period.	Albishi et al. (2013)
Minced horse mackerel	Potato peels extracts with high phenolic content	Oxidative stability (peroxide values, volatiles and carbonyl compounds)	Potato peel ethanolic extracts were found to retard lipid and protein oxidation in minced horse mackerel, resulting in lower levels of peroxide values, volatiles and carbonyl compounds, compared to control and water extracts.	Farvin et al. (2012)
Irradiated lamb meat	Ethanolic potato peel extracts	Antioxidant activity (TBARS)	Potato peel ethanolic extracts (0.04%) were added to lamb meat before radiation processing, resulting in reduced lipid peroxidation after 7 days of cold storage. The antioxidant activity of the extracts applied to meat was measured in terms of TBARS, revealing inhibition rates of up to 54%.	Kanatt et al. (2005)
Beef patties	Ethanolic potato peel extracts	Antioxidant activity (TBARS) and sensory analysis	Potato peel extracts (90% ethanol) showed potent antioxidant activity (TBARS revealed 59.5% of inhibition relative to control). The addition of potato peel extracts reduced the rancid odor scores compared to control after storage (5 °C for 12 days).	Mansour and Khalil (2000)
Cooked rice	Potato peel powder	Antimicrobial activity (*Bacillus cereus* levels)	Potato peel powder was mixed into rice at the concentration of 10% (w/w), resulting in inhibition of *Bacillus cereus* outgrowth during abusive cooling regimes (from 54.5 °C to 7.2 °C in 12, 15, 18 and 21 h).	Juneja et al. (2018)

increasing concern by consumers about the safety of synthetic food additives, and a rising preference for natural alternatives (Carocho & Ferreira, 2013). Following this tendency, PP have been applied by some authors as antioxidants in the protection of soybean oil against lipid oxidation. Some measurable parameters can indicate the oxidation incidence, such as the production of peroxides (the primary products of lipid peroxidation), conjugated dienes, conjugated trienes and *p*-anisidine values.

Franco, Pateiro, Rodr, and Zapata (2016) found that the addition of PP ethanolic extracts to soybean oil reduced lipid oxidation indices (peroxide, conjugated dienes and *p*-anisidine values). In this study, soybean oil stability was determined under accelerated oxidation conditions (60 °C) over a 15 days storage period. Three concentrations of ethanolic extracts from PP of the Agria variety were assessed, according to chlorogenic acid levels (14.01 ppm, 20.37 ppm and 31.94 ppm). At the end of the storage period, control oil showed higher peroxide values

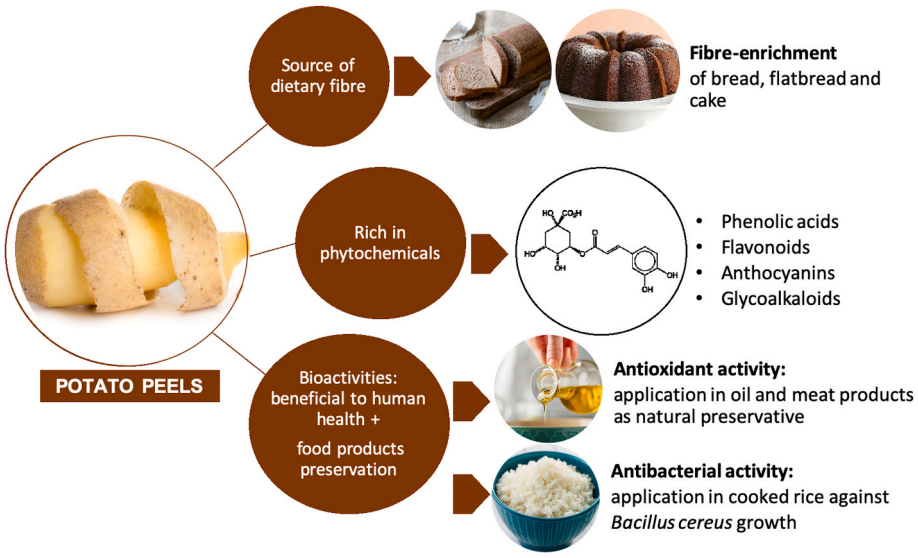

Fig. 1. Potato peels and their main interesting features for the food industry.

(180.78 meq/kg oil) compared to the PP enriched oils (below 162 meq/kg oil). In regards to conjugated dienes, the oil treated with the highest concentration of PP (31.94 ppm chlorogenic acid equivalent) showed a higher inhibition percentage (52%) than the BHT-treated sample (41%). This indicates that the PP extracts showed higher ability to reduce the loss of polyunsaturated fatty acids than BHT. Finally, the *p*-anisidine values, which are related to secondary oxidation products (carbonyls), were higher in control samples than in the other treatments.

The same effect was evidenced by Amado et al. (2014), in a study on PP ethanolic extracts of Agria variety. These authors applied a response surface analysis in order to maximise antioxidant compounds extraction from PP (mainly chlorogenic and ferulic acids) and minimize oxidation indices in soybean oil. The PP extracts were able to diminish peroxide, total oxidation (totox) and *p*-anisidine indices under accelerated oxidation conditions (60 °C, 15 days). Oxidation indices ranged from 13.1 to 17.6 meq O_2/kg oil for *p*-anisidine (corresponding to 19.3% inhibition), 293.0–380.2 meq O_2/kg oil for totox (corresponding to 22.4% inhibition), and 139.6–182.1 meq O_2/kg oil for peroxide value (corresponding to 22.8% inhibition.

Similarly, Zia-ur-Rehman et al. (2004) evaluated the ability of different PP extracts to protect a refined soybean oil from oxidation. They found that, after 60 days at 45 °C, soybean oil containing 800, 1600 and 2400 ppm of the PP extract obtained with petroleum ether showed lower free fatty acids (FFA) (0.176, 0.120, 0.109%, respectively) and peroxide values (POV) (40.0, 10.0, 9.0 meq/kg), and higher iodine values (IV) (69, 71, 77) than control samples (FFA 0.320%, POV 59 meq/kg, IV 58), an antioxidant efficiency nearly similar to that obtained with 200 ppm of BHA (FFA 0.102%, POV 8.0 meq/kg, IV 80) or BHT (FFA 0.078%, POV 6.0 meq/kg, IV 84). These results suggested that the addition of PP extract may be used to slow down the development of rancidity in soybean oil during storage instead of synthetic antioxidants, although the concentration of PP extract needed was about 8–12 times higher than that of BHT and BHA.

On the other hand, Mohdaly, Sarhan, Mahmoud, Ramadan, and Smetanska (2010) compared the antioxidant properties of PP and sugar beet methanolic extracts in sunflower and soybean oils, under accelerated oxidation conditions (70 °C, 72 h). At the end of storage time, the peroxide values of sunflower oil containing 200 ppm of PP, sugar beet pulp, *tert*-butyl hydroquinone (TBHQ), BHT and BHA were 11.9 ± 0.19, 13.8 ± 0.26, 11.0 ± 0.26, 13.4 ± 0.26 and 13.9 ± 0.24 meq/kg, respectively, whereas the control oil sample showed a much higher value of 25.8 ± 0.24 meq/kg. Regarding *p*-anisidine, maximum values of 9.10 ± 0.24, 9.40 ± 0.31, 9.42 ± 0.14, 9.36 ± 0.14, and 8.84 ± 0.14 were achieved at the end of storage for PP, sugar beet pulp, TBHQ, BHT and BHA containing samples, respectively, against 14.2 ± 0.14 reached in the control untreated sample. These results suggest a superior antioxidant activity of PP extracts over BHT and BHA, but lower than TBHQ. Similar positive results were obtained for the soybean oil samples, for which the antioxidant effect of PP extracts at 100 and 200 ppm was found to be higher than BHT and BHA at 200 ppm. Overall, the use of PP extracts in vegetable oils appears as a safe alternative of a natural antioxidant to inhibit lipid oxidation.

One study also reported the antioxidant activity of PP extracts on the storage stability of a fish-rapeseed oil mixture and a 5% oil-in-water emulsion (Koduvayur Habeebullah, Nielsen, & Jacobsen, 2010). Ethanolic and aqueous extracts of PP from two Danish potato varieties (Sava and Bintje) were applied, and the antioxidant effect was assessed by peroxide and anisidine values, besides sensory evaluation. The obtained results showed that the PP ethanolic extract at a concentration of 2,400 mg/kg (particularly from the Sava variety) was 5.7 times more effective than BHT in reducing the peroxide value, and 9 times more effective in reducing anisidine value, whereas water extracts did not demonstrate antioxidant effect. In accordance, the results of the sensory analysis revealed that rancidity intensities were higher in oil with water extracts compared to oil enriched with ethanolic PP extracts.

6.3. Cooked salmon and minced horse mackerel

Albishi et al. (2013) investigated the inhibition of oxidation in a fish meat model system by the incorporation of aqueous-methanolic-acetonic extracts from PP of four different varieties, i.e. Russet, Innovator, Purple and Yellow. The extracts were applied to retard the development of oxidative rancidity in cooked comminuted salmon over a 7-day storage period, and the results were assessed by the determination of 2-thiobarbituric acid reactive substances (TBARS). Russet, Purple, Innovator and Yellow PP extracts inhibited the formation of TBARS by 83.4%, 39.7%, 31.4% and 9.48%, respectively, at the end of the storage period.

Farvin et al. (2012) examined the use of PP as a source of natural antioxidants for retarding lipid and protein oxidation in another fish model, namely minced horse mackerel. The authors compared two different concentrations of PP extracts (2.4 or 4.8 g/kg) from the Sava variety, using both water and ethanol as solvents. The PP ethanolic extracts were the most effective in retarding lipid and protein oxidation, resulting in lower levels of peroxide values, volatiles and carbonyl

compounds, compared to control and water extracts. For instance, at the end of the storage period, peroxide values for the addition of 2.4 and 4.8 g/kg ethanolic extracts were 0.58 and 0.41 meq/kg, whereas the control sample achieved 3.45 meq/kg, and the water extract sample an even higher peroxide value of 5.78 meq/kg. The PP ethanolic extracts also showed a protective effect in fish meat against the loss of α-tocopherol, tryptophan and tyrosine residues. It was also found that the ethanolic extracts presented higher content of phenolic compounds than water extracts.

6.4. Lamb meat and beef patties

The suitability of PP extracts for controlling lipid oxidation in meat caused by radiation was investigated by Kanatt, Chander, Radhakrishna, and Sharma (2005). Ethanolic PP extracts were prepared from the Indian Kufri chandramukhi potato variety, and added to lamb meat before irradiation (5 kGy, 4 mL of 1% PP extract in 100 g of meat). Lipid peroxidation was assessed in terms of thiobarbituric acid (TBA) number and carbonyl value. After 7 days of cold storage (0–3 °C), the TBA number was 54% lower for the meat containing PP extract in comparison to control. Similarly, the carbonyl content of PP-containing meat at the end of storage period was 30% lower than the corresponding sample not containing PP extract, demonstrating the potential of PP extracts in preventing lipid oxidation in meat products.

In another study, Mansour and Khalil (2000) assessed the potential of PP extracts (90% ethanol) in reducing lipid oxidation in ground beef patties, during cold storage (5 °C) for 12 days. The antioxidant activity of the PP extracts was assessed by TBARS, revealing 59.5% of inhibition relative to control. This activity was found to be maximum at pH ranging from 5.0 to 6.0. Moreover, a sensory analysis showed the extracts were able to control lipid oxidation and colour change of beef patties samples during cold storage, compared to control samples.

6.5. Cooked rice

Juneja, Friedman, Mohr, Silverman, and Mukhopadhyay (2018) studied the growth inhibition of *Bacillus cereus* in cooked rice by nine fruit and vegetable peel powders, including PP. The powders were mixed into rice at a concentration of 10% (w/w) along with four strains of *B. cereus*. Aliquots (5 g) of control cooked rice (without added peel powders) were cooled from 54.5 °C to 7.2 °C in 12, 15, 18, or 21 h, resulting in 1.93, 2.82, 3.83, and 3.58 log cfu/g increases in *B. cereus* levels, respectively. For the rice samples added with organic gold PP powder, the results were 0.27, 0.53, 1.38 and 2.00 log cfu/g for the increase in *B. cereus* levels, under the same conditions as the control rice sample. These results demonstrated the potential of PP in retarding *B. cereus* outgrowth in food products.

7. Conclusions and future prospects

Within the context of circular economy and the increasing environmental impact of agro-industry, there is an urgent need to return byproducts and waste to the food supply chain and related industries. Potato processing industry generates a great amount of bulky waste which is usually discarded or used as animal feed, thus increasing the environmental impact and decreasing the overall added value of the crop. PP, the main by-product of this process, recently have gained the interest of the research community and the food and pharmaceutical industries due to their promising chemical composition and nutritional value. Several studies have highlighted the great potential for recovering bioactive compounds and antioxidants from PP that could be used as antimicrobial agents or as functional ingredients in food products and supplements. Considering the bulky nature of PP, future research is needed to facilitate the industrial exploitation of this promising material with efficient green protocols, while alternative uses should be also explored to increase the overall added value of this important crop.

Acknowledgements

S. Sampaio acknowledges CAPES Foundation (Ministry of Education, Brazil) for her grant (no. 99999.001423/2015-00). The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Programme PT20vd20 for financial support to CIMO (UIDB/00690/2020); L. Barros thanks to FCT, P.I., through the institutional scientific employment program-contract and Sandrina A. Heleno also thanks to FCT, P.I., through the individual scientific employment program-contract. The authors are also grateful to FEDER-Interreg España-Portugal program for financial support through the project 0377_Iberphenol_6_E.

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Cite this: DOI: 10.1039/d0fo00055h

Nutritional value, physicochemical characterization and bioactive properties of the Brazilian quinoa *BRS Piabiru*

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Received 7th January 2020,
Accepted 6th March 2020

DOI: 10.1039/d0fo00055h

rsc.li/food-function

Chenopodium quinoa Willd. is an ancient food crop that has provided nutrition to Andean populations for thousands of years. *BRS Piabiru* is a quinoa genotype developed and adapted to tropical climate by the Brazilian Agricultural Research Corporation. In this work, *BRS Piabiru* quinoa was evaluated concerning its nutritional, physicochemical and phenolic composition and also its bioactive properties. This variety showed high carbohydrate and protein contents and a low-fat level, composed of 86% of unsaturated fatty acids, 60% of which are polyunsaturated fatty acids. Four organic acids were detected, with quinic acid as the most abundant, while α- and γ-tocopherols were the vitamin E isoforms found. Quercetin and kaempferol glycosides were the main phenolic compounds in the quinoa extract, which also revealed relevant antioxidant and antimicrobial activities, with no toxic effect. These results support the potential of *BRS Piabiru* quinoa as a nutritious food crop and a source of bioactive compounds.

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1. Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a grain-like food crop that has provided nutrition to Andean indigenous cultures for thousands of years.[1,2] Usually referred to as pseudo-cereal or pseudo-grain, the plant produces seeds that can be milled into flour, with technological features that resemble those of the Gramineae family, for instance wheat.[2,3] In addition to its excellent nutritional value, quinoa has been found to contain a high content of phytochemicals with positive health benefits, including phytosterols, phytoecdysteroids, phenolic compounds and bioactive peptides, which may contribute to metabolic, cardiovascular and gastrointestinal health.[2–4]

Quinoa has been classified as a "future smart food" by the Food and Agriculture Organization of the United Nations (FAO), *i.e.* "foods that can bolster dietary diversification, improve micronutrient intake, enhance soil health, require fewer inputs such as chemical fertilizers, and often prove resilient to climate changes and adverse farming conditions".[1] This pseudo-grain has the potential to enhance global food security for a growing world population, providing highly nutritious food that can be grown on lands not suitable for other crops.[5] Due to its relevance as an alternative food crop, quinoa has been promoted globally, with 2013 being declared the "International Year of Quinoa".[1,4] As its global popularity increases, quinoa cultivation has spread to more than 70 countries, including the United States, Canada, China, India, Finland, Australia, Kenya, the United Kingdom, Japan and Brazil.[1,4,6,7] Nevertheless, current major global producers remain the Andean countries Peru, Bolivia and Ecuador, respectively.[8]

Brazil is an established global grain supplier, and has placed the introduction of new crops into production systems as a high priority in research and development.[4] Agricultural diversification contributes to improve income, reduce costs, improve nutrient availability, protect the soil and reduce negative environmental impacts.[9] The Brazilian Agricultural Research Corporation (EMBRAPA) has been working for over twenty years in the selection and adaptation of quinoa varieties to be cultivated under Brazilian tropical climate.[4] *BRS Piabiru*, the first recommended quinoa for grain production in Brazil, is a selection of a breeding line originating from a plant population of Quito, Ecuador. The newly developed genotype was tested for years in variety trials, in Central Brazil, before

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being standardised for agronomic characteristics, such as rapid growth, tolerance to hydric stress, biomass production and nutrient cycle.[9,10] The Brazilian production of quinoa is expected to increase over the next years, reducing the pressure on Bolivia and Peru, where quinoa has become the sole grain crop export.[4]

Few studies have been performed on the chemical and technological aspects of the *BRS Piabiru* quinoa seeds. Palombini *et al.*[11] reported the fatty acid, proximate and amino acid compositions, antioxidant activity (inhibition of DPPH assay), total phenolic content (*Folin–Ciocalteu* reagent method), vitamin E and mineral contents. Nickel *et al.*[12] studied the variation in the total phenolic content, antioxidant capacity (DPPH and FRAP assays) and saponin content depending on the type of processing technology. Meneguetti *et al.*[13] reported the biological effects of the *BRS Piabiru* quinoa extract supplementation *in vivo* (rats), finding no hepatic nor renal toxicity. They also observed a decreased food intake, body weight, fat deposition, and blood triacylglycerol level in the supplemented groups. Moreover, three value added food applications applying the new quinoa variety have been reported to date: functional bread,[14] gluten-free granola[15] and gluten free cookie.[16]

In this work we perform detailed nutritional and physicochemical characterization of the Brazilian quinoa *BRS Piabiru*, and describe for the first time its phenolic compound profile and bioactivity using cell-based assays (antioxidant and antimicrobial activities and cytotoxicity).

2. Materials and methods

2.1 Quinoa samples

Samples of *Chenopodium quinoa* Willd. (*BRS Piabiru* genotype) were provided by the company Harmony Bioseeds, in partnership with EMBRAPA (Fig. 1). The quinoa plant was grown in the city of Chapada Gaúcha, Minas Gerais state, Brazil. Fresh seed samples were freeze dried (−49 °C, 0.08 bar, for 48 h, FreeZone 4.5 model 7750031, Labconco, Kansas, USA) and ground into a fine powder (20 mesh). The resulting powders were thoroughly mixed to obtain homogenized samples before analysis.

2.2 Chemical characterization

2.2.1 Nutritional and energetic value. The proximate composition was determined according to AOAC procedures as previously described.[17] The crude protein content was estimated by the macro-Kjeldahl method ($N \times 6.25$) using an automatic distillation and titration unit (model Pro-Nitro M Kjeldahl Steam Distillation System, Barcelona, Spain). Crude fat was determined by extracting the powdered sample with petroleum ether, using Soxhlet apparatus. Ash contents were determined by incineration at 550 ± 15 °C. Total carbohydrates were calculated by difference according to the following equation: (g per 100 g) = 100 − ($g_{fat} + g_{protein} + g_{ash}$).

2.2.2 Determination of free sugars. Free sugars were determined by HPLC as described by the authors[17] using chromatographic equipment provided with a refraction index detector (HPLC-RI; Knauer, Smartline system 1000, Berlin, Germany). The mobile phase consisted of an acetonitrile/water mixture (70 : 30 v/v). Separation was achieved in an Eurospher 100-5 NH_2 column (4.6 × 250 mm, 5 μm, Knauer) and quantification was performed by using melezitose as the internal standard. The results were recorded and processed using Clarity 2.4 software (DataApex, Prague, Czech Republic) and expressed in g per 100 g of fresh weight (fw).

2.2.3 Determination of fatty acids. Fatty acids were determined by gas chromatography with a flame ionization detector (GC-FID), after the extraction and derivatization procedures previously described by the authors.[17] The analysis was carried

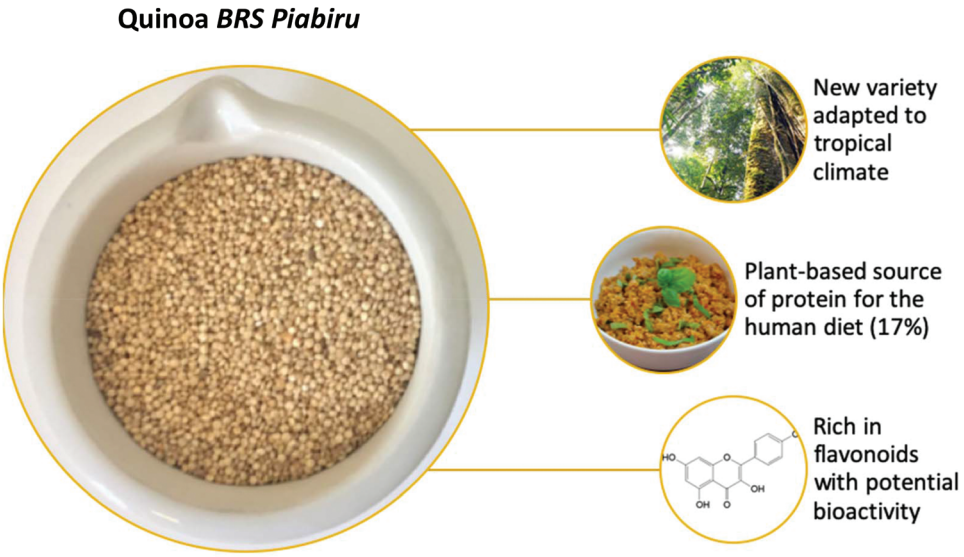

Fig. 1 Brazilian quinoa *BRS Piabiru* and some of its most important features.

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out with a DANI model GC 1000 instrument and separation was achieved using a Zebron-Kame column (30 m × 0.25 mm ID × 0.20 μm *df*, Phenomenex, Lisbon, Portugal). FAMEs were identified by comparing their retention time with standards, and the results were processed using Clarity 4.0 software (DataApex, Podohradska, Czech Republic) and expressed in relative percentage of each fatty acid.

2.2.4 Determination of tocopherols. Tocopherols were determined on the freeze-dried samples (~500 mg) using a high performance liquid chromatography system coupled to a fluorescence detector (HPCL-FL; Knauer, Smartline system 1000, Berlin, Germany), as previously described.[18] The mobile phase consisted of a hexane/ethyl acetate mixture (70 : 30 v/v) and separation was achieved using a polyamide II column (4.6 × 250 mm, 5 μm, YMC Waters, Budapest, Hungary). The quantification of the different tocopherol isoforms (α-, β-, γ- and δ-) was performed based on calibration curves constructed using authentic standards (Sigma, St Louis, MO, USA) and by using the internal standard method (Tocol, Matreya, Pleasant Gap, PA, USA). The results were recorded and processed using Clarity 2.4 software (DataApex, Prague, Czech Republic) and expressed in mg per 100 g of fw.

2.2.5 Determination of organic acids. Organic acids were determined by applying a previously described methodology and analysed using an ultra-fast liquid chromatograph coupled to a photodiode array detector.[17] The mobile phase consisted of sulphuric acid in water (3.6 mM) and separation was performed with a SphereClone reverse phase C18 column (4.6 × 250 mm, 5 μm, Phenomenex, Torrance, CA, USA). Quantification was carried out by comparison of the peak areas recorded at 215 and 240 nm, as preferred wavelengths, with calibration curves obtained from each standard compound. The results were processed using LabSolutions Multi LC-PDA software (Shimadzu Corporation, Kyoto, Japan) and expressed in g per 100 g of fresh weight (fw).

2.2.6 Determination of NaCl content. NaCl concentration was determined according to Mohr's method. Powdered samples (1 g) were dissolved in 20 mL of distilled water and filtered through Whatman No.4 paper five times. The pH of the final aqueous solution was then adjusted to 8.5 with sodium hydroxide, followed by the addition of 1 mL of potassium chromate solution (5%). The mixture was titrated against $AgNO_3$ (0.05 mol L^{-1}) until the appearance of the first reddish colour (Ag_2CrO_4 precipitate).[19] NaCl concentration was calculated using the following equation: salt content % = $[(V_{\text{titration of } AgNO_3} \times 0.00292)]/[(m_{\text{sample}})] \times 100$ (where 1 mL of $AgNO_3$ corresponds to 0.00292 g of NaCl). The results were expressed in g per 100 g of fresh weight (fw).

2.3 Physical characterization

2.3.1 pH assessment. pH was measured by blending 1 g of the powdered lyophilized sample with 20 mL of deionized water, according to the method described by Pellegrini *et al.*[20] Four measurements were undertaken using a calibrated digital pH meter (portable food and dairy pH meter HI 99161, Hanna Instruments, Woonsocket, RI, USA).

2.3.2 Colour measurement. A Minolta spectrophotometer (model CR-400; Konica Minolta Sensing, Inc., Japan) was used to measure the colour of the seeds. Using illuminant C and a diaphragm opening of 8 mm, the Hunter colour L^*, a^* and b^* values were measured through a computerized system using colour data software (Spectra Magic Nx, version CM-S100 W 2.03.0006, Konica Minolta company, Japan).

2.4 Determination of phenolic compounds and bioactivities

2.4.1 Hydroethanolic extract preparation. 30 mL of ethanol/water (80 : 20, v/v) was used to extract 1 g of freeze-dried sample. The extraction was performed twice in a magnetic stirrer plate (25 °C, 150 rpm, 1 h). The combined extracts were filtered (Whatman No. 4 paper) and vacuum-dried at 40 °C in a rotary evaporator (Büchi R-210, Flawil, Switzerland). The obtained aqueous extracts were frozen and freeze-dried.

2.4.2 Phenolic compounds. Phenolic compounds were determined by using an LC-DAD-ESI/MSn (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA), following the method described by Gonçalves *et al.*[21] The freeze-dried extracts were dissolved in ethanol/water (80 : 20, v/v), to a concentration of 10 mg mL^{-1}. Detection was performed using a DAD (280, 330, and 370 nm as preferred wavelengths) and a Linear Ion Trap LTQ XL mass spectrometer (Thermo Finnigan, San Jose, CA, USA) equipped with an ESI source working in negative mode. Identification of phenolic molecules was performed based on their retention time, UV-vis spectra and mass characteristics. Calibration curves for each phenolic standard (Extrasynthèse, Genay, France) were plotted based on the respective UV signal. The results were expressed as mg per g of extract.

2.4.3 Antioxidant activity. The antioxidant activity was assessed by two cell-based methods: (1) oxidative haemolysis inhibition assay (OxHLIA) and (2) inhibition of the production of thiobarbituric acid reactive substances (TBARS).

The OxHLIA assay was carried out as described in a previous study.[22] The results were expressed as delayed time of haemolysis (Δt), calculated as follows: Δt (min) = Ht_{50} (sample) − Ht_{50} (control), where Ht_{50} is the 50% haemolysis time (min) graphically obtained from the haemolysis curve of each sample concentration. The inhibitory concentrations of the extract able to promote a Δt haemolysis delay of 30 min ($IC_{50\ (60\ min)}$ μg mL^{-1}) and 60 min ($IC_{50\ (120\ min)}$, μg mL^{-1}) were calculated and expressed as IC_{50} values (mg mL^{-1} quinoa extract).

The method employed to measure the inhibition of production of thiobarbituric acid reactive substances (TBARS) by lipid peroxidation in brain homogenates was also described in a previous study.[23] The intensity of the pink colour resulting from the formation of the complex malondialdehyde–thiobarbituric acid (MDA-TBA) was measured at 532 nm. The inhibition ratios (%) were calculated and expressed as IC_{50} values (mg mL^{-1} quinoa extract).

2.4.4 Cytotoxicity evaluation. The quinoa extract was dissolved in water (4 mg mL^{-1}) and submitted to further dilutions. A cell culture (PLP2) was prepared using a freshly

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harvested porcine liver according to the method previously described by Abreu *et al.*[24] The sulforhodamine B assay was performed to evaluate the extract hepatotoxic potential. Ellipticine (Sigma-Aldrich, St Louis, MO, USA) was employed as a positive control and the result was expressed as GI_{50} values, which correspond to the concentration of the extract that inhibited 50% of the net cell growth.

2.4.5 Antimicrobial activity. The antimicrobial activity was evaluated according to the procedure previously described by Corrêa *et al.*[25] Three Gram (+) and three Gram (−) bacteria were tested: *Bacillus cereus* (food isolate), *Staphylococcus aureus* (ATCC 6538), *Listeria monocytogenes* (NCTC 7973), *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (human isolate) and *Salmonella typhimurium* (ATCC 13311), respectively. Additionally, six fungi were tested: *Aspergillus fumigatus* (ATCC 1022), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus niger* (ATCC 6275), *Penicillium ochrochloron* (ATCC 9112), *Penicillium funiculosum* (ATCC 36839) and *Penicillium verrucosum* var. cyclopium (food isolate). The results were expressed as the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of quinoa extracts.

2.5 Statistical analysis

Three samples of quinoa were used for each assay. All assays were carried out in triplicate ($n = 9$). The results were expressed as mean values with standard deviations (SD).

3. Results and discussion

3.1 Nutritional value and physicochemical composition

The nutritional value and physicochemical composition of *BRS Piabiru* quinoa are presented in Table 1. The seeds showed high protein content, with a mean concentration of 17.0 g per 100 g of fresh weight (fw), revealing a similar content to a previously published study on the same variety, 16.41 g per 100 g fw.[11] In a recent study, Pereira *et al.*[26] analysed thirty nine distinct genotypes of quinoa from Peru and Spain, with reported protein content ranging from 14.4 to 15.6 g per 100 g dw. These data highlight the outstanding potential of the studied Brazilian quinoa as a source of protein for the human diet, even when compared to other quinoa varieties. Considering other major grains, quinoa presents higher protein content than wheat (12.3%), maize (8.9%), rice (7.5%) and oat (16.1%).[2] Additional features include a balanced amino acid profile, easy digestibility and the absence of gluten, being its ingestion safe for celiac disease sufferers.[2,27] Quinoa protein profile has been compared to the milk protein, as it includes reasonable amounts of all the essential amino acids, being particularly rich in lysine.[28,29]

Table 1 Nutritional value (g per 100 g fw), energetic value (kcal per 100 g fw) and physicochemical composition of *BRS Piabiru* quinoa seeds (mean ± SD)

Nutritional value		Organic acids (g per 100 g fw)	
Moisture (%)	8.4 ± 0.1	Oxalic acid	0.603 ± 0.009
Proteins	17.0 ± 0.8	Quinic acid	3.6 ± 0.1
Lipids	6.0 ± 0.2	Malic acid	0.41 ± 0.03
Carbohydrates	66.5 ± 0.5	Fumaric acid	tr
Ash	2.1 ± 0.1	Total organic acids	4.6 ± 0.2
Energy (kcal per 100 g fw)	389 ± 1		
Free sugars (g per 100 g fw)		**Physicochemical variables**	
Fructose	0.060 ± 0.004	NaCl (g per 100 g fw)	0.39 ± 0.02
Glucose	0.31 ± 0.01	pH	5.65 ± 0.01
Sucrose	0.80 ± 0.03	Hunter scale colour parameters	
Total free sugars	1.17 ± 0.04	*L**	78.2 ± 0.4
		*a**	1.84 ± 0.08
		*b**	15.2 ± 0.1

tr – traces.

Carbohydrates appeared as the major macronutrient in the studied samples, accounting for 66.5 g per 100 g fw (Table 1). In general, the quinoa seed is characterized by a lower content of carbohydrates than cereals like wheat, barley, maize or rice, also presenting a low glycaemic index.[2,29]

Regarding free sugar composition, three distinct molecules were identified: two monosaccharides (fructose and glucose) and one disaccharide (sucrose) (Table 1). Sucrose was the most abundant one (0.80 g per 100 g fw), followed by glucose (0.31 g per 100 g fw) and fructose (0.06 g per 100 g fw). As a low-free sugar food crop, quinoa can be classified as part of a "low FODMAP diet", which has been shown to exert beneficial impacts on irritable bowel symptoms by limiting the ingestion of readily fermentable short-chain carbohydrates.[2]

Total fat reaches a mean concentration of 6.0 g per 100 g fw (Table 1), which is in agreement with the range of 2% to 10% described in the literature, considering distinct quinoa genotypes.[2,11,26] The fatty acid profile was composed of 85.97% of unsaturated fatty acids, 59.94% of which were polyunsaturated (PUFAs) (Table 2). The consumption of polyunsaturated fatty acids has been associated with a range of health benefits, for instance positive effects on cardiovascular disease, metabolism of prostaglandins, insulin sensitivity, the immune system and cell membrane function.[2,30] In the case of *BRS Piabiru* quinoa, the major PUFA was linoleic acid (C18:2n6, 56.70%), while monounsaturated fatty acids (MUFAs) were mostly represented by oleic acid (C18:1n9, 22.67%). The major saturated fatty acid found was palmitic (C16:0, 11.09%), which is also in agreement with the literature for other quinoa varieties.[30]

Quinoa is known for containing high concentrations of antioxidants, such as tocopherols, which act as scavengers of lipid peroxyl radicals.[11,30] In this study, α- and γ-tocopherols were the main isomers found in quinoa (Table 2). α-Tocopherol was found in a mean concentration of 0.919 mg per 100 g fw, which was slightly lower than the concentration of 1.16 mg per 100 g reported by Palombini *et al.*[11] for the same quinoa variety. Those authors reported a β + γ-tocopherol concentration of 1.08 mg per 100 g, which was less than half

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Table 2 Composition of fatty acids (%) and tocopherols (mg per 100 g fw) of *BRS Piabiru* quinoa seeds (mean ± SD)

Fatty acid		Fatty acid	
C6:0	0.041 ± 0.004	C18:1n9	22.67 ± 0.04
C8:0	0.019 ± 0.001	C18:2n6	56.70 ± 0.03
C10:0	0.059 ± 0.001	C18:3n6	0.092 ± 0.004
C12:0	0.039 ± 0.001	C18:3n3	2.74 ± 0.01
C14:0	0.277 ± 0.001	C20:0	0.428 ± 0.004
C15:0	0.090 ± 0.003	C20:1	1.50 ± 0.08
C16:0	11.09 ± 0.01	C20:2	0.241 ± 0.002
C16:1	0.088 ± 0.001	C21:0	0.053 ± 0.001
C17:0	0.058 ± 0.001	C22:0	0.667 ± 0.007
C18:0	0.786 ± 0.004	C22:1	1.78 ± 0.01
SFA	14.03 ± 0.01	C22:2	0.172 ± 0.001
MUFA	26.04 ± 0.03	C23:0	0.073 ± 0.001
PUFA	59.94 ± 0.05	C24:0	0.35 ± 0.02
Tocopherols			
α-Tocopherol	0.919 ± 0.001		
γ-Tocopherol	2.67 ± 0.05		
Total tocopherols	3.59 ± 0.05		

Caproic acid (C6:0); caprylic acid (C8:0); capric acid (C10:0); lauric acid (C12:0); myristic acid (C14:0); pentadecanoic acid (C15:0); palmitic acid (C16:0); palmitoleic acid (C16:1); margaric acid (C17:0); stearic acid (C18:0); oleic acid (C18:1n9); linoleic acid (C18:2n6); γ-linolenic acid (C18:3n6); α-linolenic acid (C18:3n3); arachidic acid (C20:0); *cis*-11-eicosenoic acid (C20:1); eicosadienoic acid (C20:2); heneicosanoic acid (C21:0); behenic acid (C22:0); erucic acid (C22:1); docosadienoic acid (C22:2); tricosylic acid (C23:0); lignoceric acid (C24:0). SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

the concentration of γ-tocopherol found in the present study, 2.67 mg per 100 g. Despite considering the same variety, the discrepancies found in the tocopherol content between both studies may be due to distinct climatic and soil conditions, most probably associated with the specific regions in Brazil where the crops were grown.

Four organic acids were detected, quinic acid being the major one (3.6 g per 100 g fw), followed by oxalic (0.603 g per 100 g fw), malic (0.41 g per 100 g fw) and fumaric acids (trace amounts). Pereira *et al.*,[26] in a screening study on thirty-nine quinoa genotypes, also reported the presence of oxalic acid and, similarly, traces of fumaric acid; however, they did not detect quinic and malic acids. In a recent study performed by Heikkilä *et al.*[32] in an *in vivo* mice model, it was suggested that quinic acid could be helpful in the treatment of diabetes, by promoting insulin secretion from pancreatic beta cells.

The excess of sodium intake has been shown to be strongly associated with a plethora of health diseases.[33] This has stimulated the food industry to adopt interventions towards product development and reformulation to achieve a reduced final sodium content. The results for salt (NaCl) content in the *BRS Piabiru* quinoa showed a low mean value of 0.39 g per 100 g fw (Table 1). Considering this, the herein studied quinoa could be considered for the development of new processed food products that aim, and claim, reduced salt content.

The pH value (5.65) placed the studied quinoa grains as slightly more acidic than the six commercial genotypes analysed by Pellegrini *et al.*,[20] which presented a pH range of 6.42–6.63. Regarding colour, only whole grains were measured, before any milling process was employed; therefore, all measurements corresponded to the coloured outer layer of the grains (Fig. 1). The results showed that the grains were characterized by a high mean value of the parameter L^* (78.2), which indicates high luminosity. Additionally, the low value of the parameter a^* (1.84) indicates the absence of red-green intensities, and the parameter b^* value (15.2) is associated with a pale yellow colour. Escribano *et al.*[34] undertook an investigation of twenty-nine distinct varieties of quinoa, including white, black, yellow and red-violet genotypes. Blanca de Junín and Inia de Salcedo were two of the varieties analysed by these authors classified as "white quinoa", with results fairly similar to the ones found in this study, *i.e.*, L^* values of 73.97 and 75.60, a^* values of 1.54 and 1.53, and b^* values of 18.14 and 19.83, respectively.

Considering these results, the great nutritional value of the *BRS Piabiru* quinoa, along with its physicochemical features, makes this variety an attractive food crop for direct incorporation into the human diet and in a range of food formulations, especially considering its outstanding protein content and fatty acid composition.

3.2 Phenolic compounds and bioactive properties

The analysis of phenolic compounds in the Brazilian quinoa hydroethanolic extract was performed by HPLC-DAD-ESI/MS^n. Six flavonol glycosides were detected, derived from quercetin and kaempferol as concluded from their absorption and mass characteristics. Data are presented in Table 3 together with compound quantifications. Quercetin and kaempferol 3-*O*-rutinoside (peaks 5 and 6) were positively identified by comparison with commercial standards. Peaks 1, 2 and 3 were tentatively assigned as quercetin 3-*O*-(2″,6″-di-*O*-α-L-rhamnoside)-β-D-galactoside, quercetin 3-*O*-(2″-*O*-β-apioside-6″-*O*-α-rhamnoside)-β-galactoside and kaempferol 3-*O*-(2″,6″-di-*O*-α-rhamnoside)-β-galactoside, respectively, taking into account their previous description in quinoa samples.[7,37] The absorption and mass spectra of peak 4 were the same as peak 3, being tentatively associated with kaempferol 3-*O*-(2″,6″-di-*O*-α-rhamnoside)-β-glucoside considering that glucosides elute later than the corresponding galactosides.

More than 20 phenolic compounds have been described to date for distinct varieties of quinoa seeds, in either free or conjugated forms, liberated by alkaline, acid, and/or enzymatic hydrolysis.[3,7,31,36,37] The most abundant compounds reported are the flavonoids quercetin and kaempferol glycosides, as well as the phenolic acids vanillic acid, ferulic acid and their derivatives.[3,38] In this study, high concentrations of flavonol derivatives (quercetin and kaempferol) have been determined, which is in agreement with the literature; however, no significant amounts of phenolic acids were found. Considering that this is the first report on the identification of phenolic compounds in the quinoa *BRS Piabiru* genotype, this result might be explained by an actual low content, below our instrument detection limits, or inexistence of phenolic acids in this variety. Indeed, differences in phytochemical composition in

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Table 3 Retention time (Rt), wavelengths of maximum absorption (λ_{max}), mass spectral data and quantification of the phenolic compounds tentatively identified in hydroethanolic extracts of *BRS Piabiru* quinoa seeds. The results are expressed as mean ± standard deviation, and the respective references for identification are presented

Peak	Rt (min)	λ_{max} (nm)	$[M-H]^-$ (m/z)	MS^2 (m/z)	Tentative identification	Quantification (mg g^{-1})	References
1	14.37	352	755	760(38), 301(100)	Quercetin 3-*O*-(2″,6″-di-*O*-α-L-rhamnoside)-β-D-galactoside	93.5 ± 0.5	Gómez-Caravaca *et al.*[37] and Hirose *et al.*[7]
2	15.51	353	741	609(100), 301(80)	Quercetin 3-*O*-(2″-*O*-β-apioside-6″-*O*-α-rhamnoside)-β-galactoside	58.2 ± 0.3	Hirose *et al.*[7]
3	16.15	265 348	739	593(44), 285(100)	Kaempferol 3-*O*-(2″,6″-di-*O*-α-rhamnoside)-β-galactoside	23.73 ± 0.09	Gómez-Caravaca *et al.*[37] and Hirose *et al.*[7]
4	16.27	265 348	739	593(44), 285(100)	Kaempferol 3-*O*-(2″,6″-di-*O*-α-rhamnoside)-β-glucoside	21.5 ± 0.1	DAD, MS
5	17.53	353	609	301(100)	Quercetin 3-*O*-rutinoside	5.26 ± 0.09	Standard, Gómez-Caravaca *et al.*[37] and Tang, Li, Zhang *et al.*[31]
6	18.04	266 347	593	285(100)	Kaempferol 3-*O*-rutinoside	5.28 ± 0.06	Standard
					Total phenolic compounds	207.54 ± 0.2	

natural products can be explained by both genotypic or environmental factors.[2]

Phenolic compounds have been associated with a range of biological activities due to their effects on cell-signalling and metabolism, including antioxidant, anti-inflammatory, anticancer and cardioprotective effects.[2] The presence of these compounds in quinoa has been related to antidiabetic and anti-obesity properties, attributed to their α-glucosidase and pancreatic lipase inhibitory activities.[3]

The *in vitro* antioxidant, antibacterial and antifungal properties of the quinoa hydroethanolic extracts were assessed. Additionally, cytotoxicity of the extracts was also investigated. Several studies, including clinical trials, have demonstrated the antioxidant properties of quinoa, which have been attributed to its high polyphenol content.[2,3,29] Considering that antioxidant compounds act by distinct mechanisms, in this study we employed two different *in vitro* cell-based techniques to assess the antioxidant capacity of the *BRS Piabiru* quinoa hydroethanolic extract. The results of both assays are presented in Table 4.

Table 4 Antioxidant and cytotoxicity activities of *BRS Piabiru* quinoa seed hydroethanolic extracts (mean ± SD)

Cell-based antioxidant assays	
TBARS (EC50 μg mL^{-1})	764 ± 6
OxHLIA (IC_{50}, μg mL^{-1})	
Δt = 30 min	5.8 ± 0.2
Δt = 60 min	59 ± 1
Cytotoxicity (GI_{50} μg mL^{-1} values)	
PLP2	>400

EC_{50}: extract concentration corresponding to 50% of antioxidant activity. Trolox EC_{50} value: 23 μg mL^{-1} (TBARS inhibition). IC_{50} values are the concentration of the extract required to inhibit (delay) 50% of haemolysis for 30 min (IC_{50} (30 min)) and 60 min (IC_{50} (60 min)). Trolox IC_{50} values: 8.8 μg mL^{-1} (OxHLIA Δt = 30 min) and 19.6 μg mL^{-1} (OxHLIA Δt = 60 min). GI_{50} values correspond to the sample concentration achieving 50% of growth inhibition in liver primary culture PLP2. GI_{50} values for ellipticine (positive control): 3 μg mL^{-1} (PLP2).

The Brazilian quinoa extract was effective in diminishing the production of TBAR substances, which result from lipid peroxidation-induced oxidative stress. The extract presented a mean IC_{50} value of 764 μg mL^{-1}. A protective effect against lipid peroxidation was also observed in a human dietary intervention study conducted by Carvalho and Paya.[39] A double-blind intervention was conducted on 35 women with excess weight who consumed 25 grams of quinoa flakes daily for a period of four consecutive weeks. Their results showed a significant decrease in TBAR substances in the subjects' blood samples (3.06 to 2.89 μmol L^{-1}), which suggested the efficacy of quinoa intake as an antioxidant strategy for the human diet.

The antioxidant properties of the *BRS Piabiru* quinoa extracts were also confirmed by OxHLIA, a cell-based assay for evaluating the inhibition of free radical-induced haemolysis in sheep erythrocytes. A mean concentration of 5.8 μg mL^{-1} of the extract was found to inhibit the haemolysis by 50% for 30 minutes and 59 μg mL^{-1} for 60 minutes (Table 4). To the best of our knowledge, this was the first time that the OxHLIA assay was employed to evaluate the antioxidant properties of quinoa extracts. Other authors have also reported positive results for *in vitro*, non-cell-based, antioxidant assays of quinoa, for instance ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC) and DPPH radical scavenging assay.[11,20,34] Regarding the latter, only one study has been conducted on the same Brazilian quinoa variety investigated herein,[11] with positive although less expressive outcomes (IC_{50} average value of 313.25 μg mL^{-1} of the methanolic extract).

Several antinutritional factors have been identified by other authors in different varieties of quinoa, namely saponins, phytic acid, tannins, nitrates, oxalates, and trypsin inhibitors.[2,3,30] Nevertheless, the hydroethanolic extract studied herein did not present toxicity against the porcine liver primary culture PLP2, once its GI_{50} value was higher than the highest tested concentration (400 μg mL^{-1}) (Table 4). The employment of the porcine liver as an *in vitro* cytotoxicity model is justified by its similarity to the human liver, in terms of its cellular and physiological functioning.[25] The verified

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Table 5 Antimicrobial activity of *BRS Piabiru* quinoa seed hydroethanolic extracts

Bacteria	Quinoa MIC/MBC	Streptomycin MIC/MBC	Ampicillin MIC/MBC
Staphylococcus aureus	1.0/2.0	0.04/0.1	0.25/0.45
Bacillus cereus	1.0/2.0	0.1/0.2	0.25/0.4
Listeria monocytogenes	1.0/2.0	0.2/0.3	0.4/0.5
Escherichia coli	1.0/2.0	0.2/0.3	0.4/0.5
Salmonella typhimurium	1.0/2.0	0.2/0.3	0.75/1.2
Enterobacter cloacae	1.0/2.0	0.2/0.3	0.25/0.5
Fungi	**Quinoa MIC/MFC**	**Ketoconazole MIC/MFC**	**Bifonazole MIC/MFC**
Aspergillus fumigatus	0.5/1.0	0.25/0.5	0.15/0.2
Aspergillus ochraceus	0.5/1.0	0.2/0.5	0.1/0.2
Aspergillus niger	1.0/2.0	0.2/0.5	0.15/0.2
Penicillium funiculosum	0.5/1.0	0.2/0.5	0.2/0.25
Penicillium ochrochloron	1.0/2.0	2.5/3.5	0.2/0.25
Penicillium verrucosum var. *cyclopium*	0.5/1.0	0.2/0.3	0.1/0.2

absence of cytotoxicity is of interest considering the potential use of the tested preparation for food and pharmaceutical formulations, confirming the safety of the Brazilian quinoa extract for incorporation in the human diet and other potential applications. Meneguetti *et al.*[13] investigated the biological effects of BRS Piabiru quinoa supplementation *in vivo*, in a rat diet study. Those authors found that the activities of the liver enzymes aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) did not change in the supplemented groups, with the values remaining at normal levels, confirming the absence of hepatotoxicity. Additionally, they observed decreased fat deposition and blood triacylglycerol levels in the supplemented groups.

The results for antimicrobial activity are shown in Table 5. The quinoa *BRS Piabiru* extract was tested against three Gram-positive and three Gram-negative bacteria, besides six fungi. The inhibitory and antibacterial activity displayed by the extract against both Gram-positive (*S. aureus*, *B. cereus* and *L. monocytogenes*) and Gram-negative (*E. coli*, *S. typhimurium* and *Enterobacter cloacae*) bacteria indicates the existence of an extensive antibiotic spectrum for their phytochemical constituents. The extract was equally active against all six tested bacteria. Besides, the quinoa extract was also effective against all six fungi tested, showing better results against *P. ochrochloron* than the fungicide ketoconazole.

The antibacterial activity of quinoa extracts was also observed by Miranda *et al.*[40] studying six different quinoa seeds, grown in three distinctive geographical zones of Chile. They prepared ethanolic extracts and studied their antibacterial activity *via* the disk diffusion assay technique. The extracts showed antimicrobial activity in the range of 8.3–14.8 mm inhibition zone for *E. coli* and 8.5–15.0 mm inhibition zone for *S. aureus*. Those authors also reported a positive correlation between the antimicrobial activity and the total phenolic content of the extracts. The presence of flavonoids in quinoa has also been previously linked to antimicrobial activity by other authors,[35] and may be responsible for the positive results found in the study herein.

4. Conclusion

The Brazilian quinoa *BRS Piabiru* presented a higher protein content than other previously described quinoa varieties, besides revealing a fatty acid composition with potential health benefits. Its outstanding nutritional value along with its physicochemical traits makes this food crop a suitable candidate for direct incorporation in the human diet. The quinoa variety also presented a high content of quercetin and kaempferol glycoside derivatives, which might be responsible for the positive results on the seeds' bioactivities. Overall, the results of the broad characterization carried out in this study suggest the viability of exploiting the Brazilian quinoa as a functional food, taking into account the verified *in vitro* antioxidant, antibacterial and antifungal activities, with the absence of cytotoxicity.

This work contributes to the growing debate on alternative sustainable and healthier foods by deriving information of a new, tropical climate adapted quinoa genotype. This information can potentially be used by the food and pharmaceutical industries in the development of new health-promoting products. This study also contributes to the FAO goal of turning quinoa into a commercial crop in Brazil, alleviating the increasing global demand pressure on Bolivia and Peru.

Conflicts of interest

The authors declare no conflict of interests.

Acknowledgements

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Programme PT2020 for financial support to CIMO (UID/AGR/00690/2019); the national funding by FCT, P.I., through the institutional scientific employment program-contract for L. Barros,

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A. Fernandes and R. Calhelha contracts; C. Pereira contract through the celebration of program-contract foreseen in No. 4, 5 and 6 of article 23° of Decree-Law No. 57/2016, of 29th August, amended by Law No. 57/2017, of 19th July; and FEDER-Interreg España-Portugal programme for financial support through the project 0377_Iberphenol_6_E. S. Sampaio thanks CAPES Foundation (Ministry of Education, Brazil) for her PhD grant no. 99999.001423/2015-00. GIP-USAL was financially supported by the Spanish Government through the project AGL2015-64522-C2-2-R.

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antioxidants

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Review

Grown to be Blue—Antioxidant Properties and Health Effects of Colored Vegetables. Part I: Root Vegetables

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Received: 16 November 2019; Accepted: 3 December 2019; Published: date

Abstract: During the last few decades, the food and beverage industry faced increasing demand for the design of new functional food products free of synthetic compounds and artificial additives. Anthocyanins are widely used as natural colorants in various food products to replenish blue color losses during processing and to add blue color to colorless products, while other compounds such as carotenoids and betalains are considered as good sources of other shades. Root vegetables are well known for their broad palette of colors, and some species, such as black carrot and beet root, are already widely used as sources of natural colorants in the food and drug industry. Ongoing research aims at identifying alternative vegetable sources with diverse functional and structural features imparting beneficial effects onto human health. The current review provides a systematic description of colored root vegetables based on their belowground edible parts, and it highlights species and/or cultivars that present atypical colors, especially those containing pigment compounds responsible for hues of blue color. Finally, the main health effects and antioxidant properties associated with the presence of coloring compounds are presented, as well as the effects that processing treatments may have on chemical composition and coloring compounds in particular.

Keywords: anthocyanins; antioxidant activity; beet root; betacyanins; cyanidin; blue potatoes; carotenoids; flavonoids; natural colorants; sweet potato

1. Introduction

Root vegetables display various colors which usually depend on the presence of three major classes of compounds, namely, flavonoids, betalains, and carotenoids, which they may define their visual appearance and consumer perception [1,2]. Anthocyanins are flavonoids responsible for the different shades of plant epidermal tissues such as purple, blue, red, and pink colors, aiming at attracting pollinators and contributing to the overall plant antioxidant mechanisms under abiotic and

biotic stress conditions [3]. They also participate in several physiological processes of the plant, including photosynthesis and plant interactions with the environment [4]. They are produced via the phenylpropanoid pathway and the conversion of leucoanthocyanidins into colored anthocyanidin and glycoside derivatives via anthocyanidin synthase and other enzymes [1,5]. The great number of anthocyanins isolated in nature so far and their high structural variation across plant species raised research interest in these compounds during the last few years in search of novel natural colorants [6,7]. The structural variation of anthocyanins is related to the substitution of hydroxyl and methoxyl groups in the B ring, glycosidic substitution at positions 3 and 5 of the A and C rings, and the possible acylation of glycosidic substitutes with aliphatic and cinnamic acids (Figures 1 and 2) [6]. These structural differences may infer significant variations in the biological activities and antioxidant properties of vegetable products. For example, Oki et al. [8] suggested that antioxidant activities of purple sweet-potato extracts from peonidin-rich cultivars were attributed to anthocyanins, whereas, in those extracts from cyanidin-rich cultivars, the antioxidant capacity was due to the phenolic compounds. Other compounds that transfuse blue color in nature are quinones, quinodes, and various alkaloids which are usually present in fungi, bacteria, and in the animal kingdom [9]. Quinones and quinodes include carbonyl groups within aromatic rings, and they also show a great variation from a structural point of view [9], while alkaloids contain nitrogen atoms and are divided into several distinct classes, including pyridine alkaloids, phenazine alkaloids, and linear tetrapyrrole and indole alkaloids, with different coloring attributes [9].

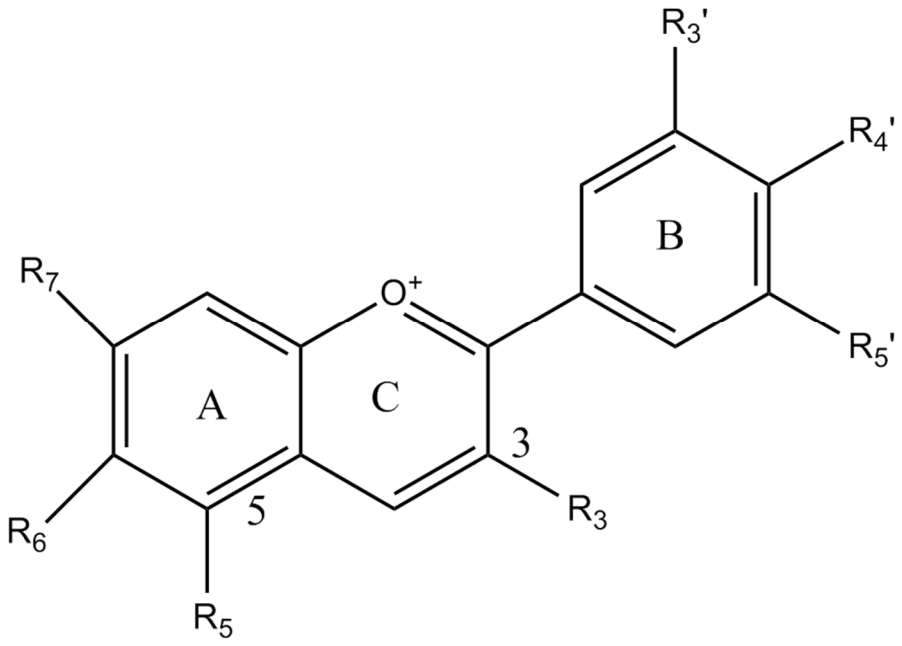


Figure 1. The core structure of anthocyanins with two aromatic benzol rings (A and B rings) and a portion cyclized with oxygen (C ring).

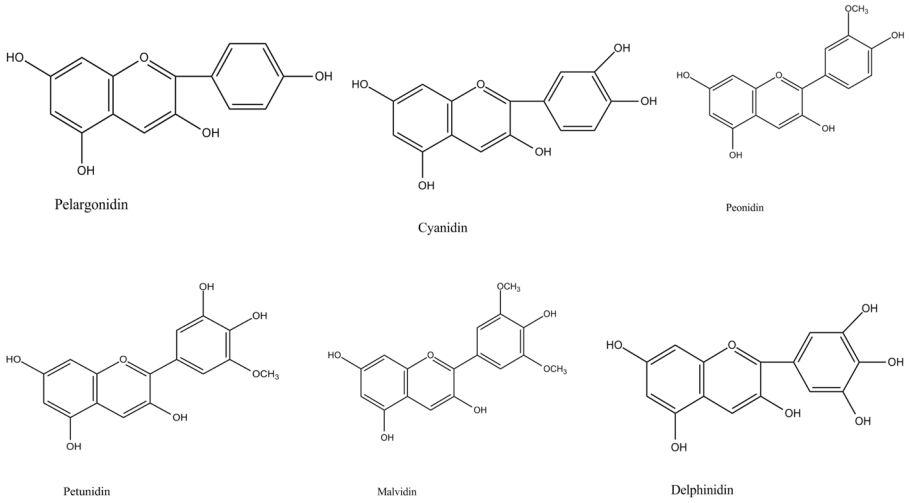


Figure 2. The main anthocyanins detected in root vegetables.

On the other hand, carotenoids are mainly responsible for yellow and orange color with several distinct compounds being detected so far in various vegetables [6,10–12], while betalains such as betacyanins and betaxanthins are also important for the violet and yellow pigmentations, respectively [13]. The main detected carotenoids are β-carotene and lycopene, which are unsaturated hydrocarbons, and they differ in terms of the β-rings, where β-carotene molecules have both ends (Figure 3), and they usually present synergistic effects [14]. Both are fat-soluble, and the number of conjugate double bonds in their structure is closely related to their superoxide inhibitory effect [15,16]. Betacyanins and betaxanthins differ in the moiety derived from betalamic acid, as towel as the fact that betaxanthins are produced from the condensation of betalamic acid with amino acids and they never show glycosidation, whereas betacyanins are the result of condensation of betalamic acid with imino compounds (Figure 4) [17,18]. Further differences are observed within each main class of betalains, namely, betacyanins and betaxanthins, with several structures identified resulting in different individual compounds with different absorption and stability capacity [19]. In particular, the various betacyanins are differentiated through the glycosyl groups attached to the *o*-position of the cyclo-dopa moiety [20], while betaxanthins are differentiated through the conjugated moiety of betalamic acid (amino acids or amines) [20]. The main pigments isolated in the various root vegetables are presented in Table 1.

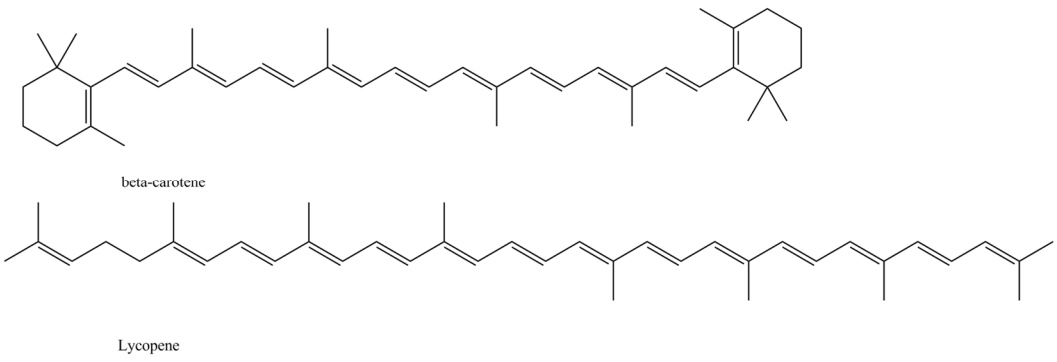
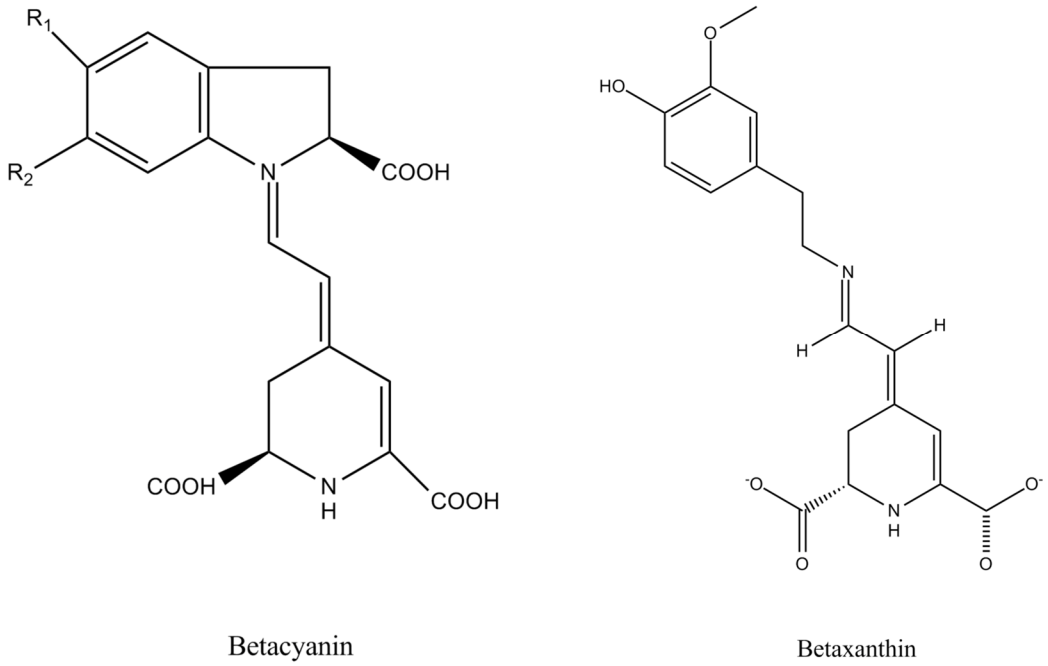


Figure 3. The main carotenoids detected in root vegetables.



Betacyanin Betaxanthin

Figure 4. The main betalains detected in root vegetables.

Table 1. The main pigments isolated in various root vegetables.

Species	Edible Part	Color	Class of Compounds	Compounds	References
Potato (*Solanum tuberosum* L.)	Tuber (stem tuber)	Purple	Petunidin derivatives	Petunidin-3-*p*-coumaroylrutinoside-5-glucoside, petunidin-2-*p*-coumarylrutinoside-5-glucoside	[21–24]
		Red	Pelargonidin, delphinidin, cyanidin, peonidin, and malvidin acyl-glycoside derivatives	Pelargonidin-3-*p*-coumaroylrutinoside-5-glucoside	[21,23]
		Purple/red	Carotenoids	Neoxanthin, violaxanthin	[25]
		Yellow	Carotenoids	Antheraxanthin	[25]
Sweet potato (*Ipomoea batatas* L. Lam.)	Tuberous root (root tuber)	Purple	Acylated anthocyanins	Cyanidin, peonidin, and pelargonidin derivatives	[26–30]
Carrot (*Daucus carota* L. ssp. *sativus* Hoffm.)	Taproot (swollen hypocotyl and root)	Purple or black	Cyanidin derivatives	Acylated cyanidin 3-xylosyl(glucosyl)galactosides with sinapic acid, ferulic acid, and coumaric acid;	[31–34,35]
				Vinylphenol and vinylguaiacol adducts of cyanidin derivatives	[31]
		Red and yellow	Carotenoids	Lycopene and β-carotene	[32,33]
Beet root (*Beta vulgaris* L.)	Root (swollen hypocotyl and root)	Purple	Betalains	Betacyanins	[39,40]
		Yellow	Betalains	Betaxanthins	[34]
				Vulgaxanthin I and betanin	[35]
Yam (*Dioscurea* sp. L.)	Tuber (stem tuber)	Purple	Cyanidin, pelargonidin, and peonidin-	Cyanidin 3-hexoside acylated with two hydroxycinnamic acids, cyanidin 3-glycoside	[36–39]

			type compounds; alatanins A–C	acylated with one hydroxycinnamic acid, cyanidin 3-glycoside acylated with one hydroxycinnamic acid, peonidin 3-glycoside acylated with one hydroxycinnamic acid, alatanin-C	
		Yellow	Carotenoids	β-Carotene	[40]
Onion (*Allium cepa* L.)	Bulb (swollen basis of leaves)	Purple	Flavonols and acylated and non-acylated cyanidin glucosides	Dihydroflavonol taxifolin and its 3-, 7-, and 4′-glucosides	[41,42]
Radish (*Raphanus sativus* L.)	Taproot (swollen root and hypocotyl)	Purple	Cyanidin glucosides	Cyanidin 3-(glucosylacyl)acylsophoroside-5-diglucosides, cyanidin 3-sophoroside-5-diglucosides, cyanidin 3-sophoroside-5-glucosides, cyanidin 3-*O*-[2-*O*-(β-glucopyranosyl)-6-*O*-(trans-feruloyl)-β-glucopyranoside]-5-*O*-[6-*O*-(malonyl)-β-glucopyranoside] cyanidin 3-[2-(glucosyl)-6-(*cis-p*-coumaroyl)-glucoside]-5-[6-(malonyl)-glucoside]	[43,44]
		Red	Anthocyanins	Pelargonidin 3-sophoroside-5-glucoside, pelargonidin 3-[2-(glucosyl)-6-(*trans-p*-coumaroyl)-glucoside]-5-glucoside, pelargonidin 3-[2-(glucosyl)-6-(*trans*-feruloyl)-glucoside]-5-glucoside, pelargonidin 3-[2-(glucosyl)-6-(*trans-p*-coumaroyl)-glucoside]-5-(6-malonylglucoside), pelargonidin 3-[2-(glucosyl)-6-(*trans*-feruloyl)-glucoside]-5-(6-malonylglucoside), 3-*O*-[2-*O*-(b-D-glucopyranosyl)-6-*O*-(trans-caffeoyl)-b-D-glucopyr-anoside]-5-*O*-(6-*O*-malonyl-b-D-glucopyranoside), pelargonidin 3-*O*-[2-*O*-(b-D-glucopyranosyl)-6-*O*-(*cis-p*-cou-maroyl)-b-D-glucopyranoside]-5-*O*-(6-*O*-malonyl-b-D-glucopyranoside	[45,46]
Kohlrabi (*Brassica oleracea* var. *gongylodes*)	Swollen epicotyl	Purple	Cyanidin and cyanidin glucoside	Cyanidin-3-diglucoside-5-glucoside, cyanidin-3-(sinapoyl)-diglucoside-5-glucoside, cyanidin 3-(feruloyl) (sinapoyl) diglucoside-5-glucoside	[47–50]
Taro (*Colocasia esculenta*)	Corm	Purple		Cyanidin and pelargonidin glucosides	[51]

The first coloring agents used in food products to improve their visual appearance were produced from natural sources; however, the high cost for the production of these coloring agents, the variation in color shades due to the inert variability in natural matrix compositions, and the increasing needs of the market resulted in the use of synthetic compounds originally derived from coal tar and then produced from petroleum and crude oil (e.g., FD&C blue No. 1 and blue No. 2) [9,52]. The consumer concerns about additives and synthetic compounds, amplified by the reports regarding the health risks and the environmental impact associated with these compounds [53–55], necessitated the shift to the root food industry dyes; recently, the food and beverage industry is

seeking natural coloring agents that could substitute synthetic dyes and coloring additives [56,57]. The colorant content of root vegetable products is associated with various health benefits including the prevention of modern chronic diseases [58–60]. However, they are often highly concentrated in the epidermal layers and skin tissues which are commonly discarded during domestic processing or in industrial applications [61–63]. For this reason, the research interest in obtaining natural pigments and bioactive compounds from agro-food waste is gaining ground within the context of circular economy and the sustainable use of natural resources [63–67]. There are also several cases where colorants can be found in high concentrations in the flesh due to the presence of pigments in parenchymal cells, increasing the antioxidant capacity and functional value of these products (e.g., potatoes, beets, carrots, and other root vegetables having colored flesh) [3]. Pigment compounds contribute to the overall antioxidant capacity in a dose-dependent and compound-specific manner [68–70], although the bioavailability and the absorption mechanisms within human body still need to be addressed [71]. Notwithstanding the genetic background of each species and/or cultivar, color attributes may be modulated by environmental factors such as the light and temperature conditions, through biotic and abiotic elicitors that may affect chemical composition, hormonal signaling, and enzymatic activities. Although not directly exposed to solar radiation, the pigmentation of root vegetables developing belowground may be indirectly modulated by the level and quality of radiation to which the aboveground plant is exposed [72]. In addition to pre-harvest factors, post-harvest conditions and processing methods may have an impact on bioavailability and biostability of natural matrices and coloring compounds [13,73,74]. Anthocyanins in particular are considered a good option as natural coloring agents due to their low toxicity and the wide range of health effects they present [75]. However, the stability and bioavailability of anthocyanins are affected by several factors (chemical structure and concentration, pH of food matrix, temperature, light, presence of co-pigments, enzymes, and metallic ions, among others), which determine the processing method specificity, and which need to be considered before using these compounds as natural coloring agents in the food industry [76,77]. Moreover, the association of structural differences with biostability and bioavailability is further reflected in the biological activities of these compounds, since, for example, acylated forms are less prone to degradation due to pH variations [14,78]. Therefore, although, for some species, there are already defined protocols for the extraction and processing requirements for obtaining natural colorants (e.g., black carrot, beet root colorants) [64,79–81], there is still a gap in the literature for other colored vegetables which could prove valuable candidates for yielding coloring agents.

The present review aims to present the main colored root vegetable crops, focusing on cultivars with colors atypical for the species. Special attention is given to blue- and purple-colored vegetables since natural colorants of these shades are less common in nature and are highly sought by the food industry, since blue shades are more difficult to replicate in food and beverages due to the susceptibility of coloring compounds to external factors (e.g., pH of the food matrix, extraction conditions). Furthermore, the main compounds responsible for uncommon colors are presented, as well as their antioxidant capacity and health-promoting effects. Finally, the effects of processing treatments on color stability are addressed. The presented information in this review was obtained from worldwide accepted databases such as Scopus, ScienceDirect, PubMed, Google Scholar, and ResearchGate, using the respective names of the studied species (both common and Latin names) and the additional terms of the main colorants and "health effects" as keywords.

2. Main Colored Root Vegetables

2.1. Potato

Potato (*Solanum tuberosum* L., Solanaceae) is the third most important food crop in the world, after wheat and rice [82]. In addition to its nutritional and calorific value, potato varieties also offer bioactive compounds with beneficial effects for human health, such as phenolic compounds and carotenoids, among others [12,23]. Several reports highlighted the beneficial effects of antioxidant-rich potatoes against various diseases, such as cardiovascular diseases [83] and various types of

cancer [84,85]. Although yellow- and white-fleshed tubers are the most commonly used ones throughout the world, potato has the highest genetic diversity among cultivated species, with approximately 5000 known varieties with broad variability in terms of flesh and skin color [86]. Red- and blue-fleshed potatoes are particularly rich in phenolic compounds, presenting about three times higher amounts of total polyphenolic content than traditional yellow-fleshed tubers, as well as two to three times higher antioxidant activity [12,23,24,87].

Acylated forms of anthocyanins were reported to be the main compounds responsible for the red and purple flesh color of potatoes [87]. In particular, the deep-purple color of potato flesh and skin is associated with the presence of petunidin derivatives, although studies on metabolite profiling revealed a genotype- and tissue-specific pattern regarding the anthocyanin composition [22]. Petunidin was the major anthocyanidin compound found both in the flesh and the peel of purple potato varieties studied by Yine et al. [21]. In this study, petunidin accounted for 63–66% of the total anthocyanidin content of purple peel and flesh. The same findings were observed by Kita et al. [23] when studying purple- and red-fleshed potato cultivars, where petunidin-3-*p*-coumaroylrutinoside-5-glucoside was the major anthocyanin compound found in the purple-fleshed varieties Salad Blue (29.31 ± 0.73 mg·100 g^{-1} dry weight (dw)), Valfi (43.11 ± 0.37 mg·100 g^{-1} dw), and Blue Congo (36.32 ± 0.33 mg·100 g^{-1} dw). Similarly, Nemś et al. [24] identified petunidin-2-*p*-coumarylrutinoside-5-glucoside as the major anthocyanin present in the cultivars Salad Blue (28.34 ± 9.30 mg·100 g^{-1} dw), Valfi (57.77 ± 28.75 mg·100 g^{-1} dw), and Blue Congo (75.97 ± 12.38 mg·100 g^{-1} dw). On the other hand, in red-fleshed potatoes, pelargonidin acyl-glycoside derivatives appear as the main anthocyanin compounds. Kita et al. [23] found pelargonidin-3-*p*-coumaroylrutinoside-5-glucoside as the major anthocyanin present in red-fleshed varieties, such as Rosalinde (15.14 ± 0.12 mg·100 g^{-1} dw), Herbie 26 (44.46 ± 0.23 mg·100 g^{-1} dw), and Highland Burgundy Red (126.38 ± 0.71 mg·100 g^{-1} dw). Yin et al. [21] carried out an acid hydrolysis of the anthocyanins, studying the composition of the aglycones (anthocyanidins), reporting pelargonidin as the main anthocyanidin present in the red-fleshed cultivar Red Cloud No. 1, with a concentration of 11.73 ± 0.16 mg·100 g^{-1} fresh weight (fw), which corresponded to 82% of the total anthocyanidin content. Other anthocyanin compounds were reported in the literature for red- and purple-fleshed potatoes, including delphinidin, cyanidin, peonidin, and malvidin acyl-glycoside derivatives [21,23]. Moreover, the simulation of domestic cooking processing and gastrointestinal digestion of *Solanum tuberosum* L. cv Vitelotte noire extracts revealed significant antimicrobial and anti-proliferative activities against *Bacillus cereus* and *Escherichia coli* in the first case (domestic cooking processes) and colon (Caco-2 and SW48) and breast cancer (MCF7, MDA-MB-231) cell lines in the latter case (gastrointestinal digestion) [88].

Carotenoids are fat-soluble pigments that can exert antioxidant properties, and they are also present in colored-flesh potatoes. According to Kotíková et al. [25] who compared the carotenoid content of yellow-fleshed, white-fleshed, purple-fleshed, and red-fleshed potato cultivars, significant differences were observed. Interestingly, yellow potatoes showed a much higher average total carotenoid content (26.22 μg·g^{-1} dw) in comparison to the red and purple-fleshed cultivars (5.69 μg·g^{-1} dw), indicating that carotenoid pigments are not highly concentrated in the flesh of purple- and red-fleshed potatoes [25].

Yin et al. [21] investigated 10 colored potato cultivars from China and compared the composition and antioxidant activities of their flesh and peel. The authors found that potato peels were on average 15.34 times richer in anthocyanins than the flesh; the antioxidant activity of the peels extracts was also 5.75 times higher on average than that of the flesh extracts [21]. In the same study, the flesh extracts of cv. Purple Cloud No.1 showed the strongest antioxidant activity among all the tested varieties, along with the highest total content of anthocyanidins (43.38 mg·100 g^{-1} fw), a correlation which indicates anthocyanins as a major contributor to the antioxidant activity of colored potatoes [21].

Recently, there was increasing interest by consumers and food producers in colored potato varieties, due to their attractive organoleptic features (color and taste) and health-promoting chemical composition [12]. The increasing interest of the market for colored potato is stimulating private and public breeding programs to release new specialty potato cultivars such as the red-skin and red-flesh

TerraRossa and AmaRosa or the purple-skin yellow-flesh cultivar Huckleberry Gold and Peter Wilcox, marketed as "Purple Sun" or "Blue Gold", which are also characterized by a higher content of anthocyanins, anthocyanidins, and other phenolic compounds [89]. The consumption of anthocyanin-rich food products such as purple-flesh potatoes is associated with the modification of the expression of various genes involved in the metabolism of lipids, inflammation, and energy homeostasis in liver and/or fat tissues [90,91]. Moreover, extracts from purple potato tubers may improve the differentiation of gut epithelia and its barrier function against gut epithelial inflammation through the activation of AMP-activated protein kinase (AMPK) and the increase of CDX2 gene [92]. Color-fleshed potatoes are an excellent source of bioactive compounds that are effective against human colon cancer cell lines (HCT-116 and HT-29); however, prolonged storage may affect their antiproliferative and pro-apoptotic activities [93]. Red- and purple-fleshed potato extracts were also effective against *tert*-butyl hydroperoxide (*t*-BHP)-induced hepatotoxicity through the recovery of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzyme activities [94]. Therefore, a market niche for colored potato-based food products was created, such as potato chips and crisps. However, the frying process to produce colored potato crisps can cause a 38–70% degradation of anthocyanin compounds, with pelargonidin and malvidin acyl-glycoside derivatives being more stable during the frying process in comparison to petunidin acyl-glycoside derivatives [23]. Nevertheless, despite the reduced contents of anthocyanins in processed compared to raw potatoes, colored potato crisps can present bright attractive colors, in addition to 2–3 times higher antioxidant activities and 40% higher contents of polyphenols than standard snacks made of commonly used yellow potatoes and corn [23,24]. Moreover, in a recent study, Nemś and Pęksa [87] incorporated dried red- and purple-fleshed potatoes into fried snacks and doughs, reporting a beneficial effect on the inhibition of oxidative changes in lipids compared to control material (yellow snacks), particularly when incorporating material from purple-fleshed potato varieties of Blue Congo and Valfi. These effects were attributed to the higher content of colored snacks in polyphenols and anthocyanins than control, with petunidin 2-*p*-coumaroyl-rutinoside-5-glucoside being the major anthocyanin present in both cultivars [87]. Other domestic cooking processes such as boiling, baking, steaming, and microwaving may also affect the anthocyanin content and antioxidant capacity of colored potatoes, with processing (steaming and microwaving) showing the best results in retaining anthocyanin content and antioxidant activity [95–99]. Thermal processing affects not only anthocyanins but also carotenoids which are heat-sensitive and may be degraded, isomerized, or oxidized after domestic cooking processes [25]. According to Qiu et al. [100], anthocyanin content decreased with prolonged drying time and high drying temperatures due to higher degradation rates and shorter half-life values compared to shorter drying procedures with lower temperatures. Therefore, the antioxidant properties of colored potatoes can be beneficial not only to human health but also to the shelf life of processed food products. Another important aspect of processed food products based on processed colored potatoes is that the various types of processing (French fries, chips, and puree) reduce the content of antinutritional factors such as the glykoalkaloids α-chaconine and α-solanine, thus increasing the overall nutritional quality of the semi-processed and final products [101].

2.2. Sweet Potato

Sweet potato (*Ipomoea batatas* (L.) Lam., Convolvulaceae) is a perennial species native to Latin America which is highly appreciated for its fleshly tuberous roots that are widely used in the food and non-food industry depending on starch content and properties [102,103]. In Japan, purple sweet-potato anthocyanins are used as ingredients in several food products and beverages [104,105]. The flesh of the roots is usually white, yellow, or orange, although several cultivars with purple-colored flesh and a high content of anthocyanins also exist [106,107]. It is the fourth most produced vegetable in the world after potato, cassava, and tomato with a total production of 113 million tons in 2017, most of which (63.8%) were produced in China [108]. The nutritional value of the edible roots consists in the richness of carbohydrates, dietary fibers, vitamins, and minerals, while several polyphenolic compounds, peptides, and carotenoids are also present in considerable amounts in the flesh [109] and

peels [67] of the tubers. The high calorific value of sweet potato roots makes the species one of the most important food crops in terms of calorific contributors to the human diet [110]. Starch is the main calorific component of sweet-potato tubers with significant variation in its structural and functional properties which depend mostly on the genotype and are not correlated with flesh color [111], although, using a proteomic approach, a recent study revealed that starch degradation may contribute to anthocyanin biosynthesis and accumulation in purple sweet-potato roots [112]. Chlorogenic acid, protocatechuic acid, salicylic acid, and caffeoylquinic acid derivatives are the main phenolic acids detected in purple sweet-potato roots and are responsible for their antioxidant capacity [41,113,114], while orange-fleshed sweet-potato cultivars are rich in provitamin A and also show significant antioxidant activity [106,115,116]. Moreover, in the study of Lebot et al. [117], the antioxidant activity of sweet-potato cultivars with purple, orange, and white flesh was correlated mostly with the presence of caffeoylquinic acid derivatives and less with total anthocyanin content, whereas, according to Oki et al. [8], the contribution of phenolic compounds in radical-scavenging activity is also dependent on the genotype. In contrast, according to the study of Kubow et al. [118], anthocyanins are responsible for the antioxidant capacity of sweet-potato tubers. In the same study, it was reported that the anthocyanin species were detected in the small intestinal and the ascending colonic vessel, depending on the sweet-potato genotype, and the antioxidant activity was increased accordingly [118]. According to the report of Meng et al. [119] who studied the digestion kinetics of sweet-potato polyphenols, the maximum release was recorded 2 h after intestinal digestion and was induced by gastric acid and pepsin [119]. Moreover, acylated anthocyanins from sweet potato are considered as complex and less susceptible to intestinal degradation [120,121], while Sun et al. [122] suggested a prebiotic-like activity of anthocyanins through the modulation of microbiota in the intestine. These results highlight the importance of unraveling the bioavailability and bioaccessibility patterns influencing the antioxidant potential of purple-fleshed sweet potatoes [118].

Acylated anthocyanins are responsible for the intense color of purple-fleshed sweet potatoes [59,123], which renders them good candidates sources for natural colorants with practical application in the food industry [124]. Moreover, peels are also a good source of natural pigments since they contain significant amounts of anthocyanins, and the exploitation of this by-product for obtaining coloring agents would increase the added value of the sweet-potato crop [67]. The total anthocyanin content and compositional profile may differ among the various genotypes, with a total of 39 different anthocyanins isolated so far [125,126]. The main anthocyanins isolated from purple sweet-potato extracts were identified as cyanidin, peonidin, and pelargonidin derivatives [26–30,103,127,128], which were effective against alcohol-induced liver injury in rats when administrated at median doses (100 mg·kg^{-1} body weight), whereas higher doses (300 mg·kg^{-1} body weight) had a pro-oxidant effect and promoted liver injury [129]. Moreover, cyanidin 3-caffeoyl-*p*-hydroxybenzoyl sophoroside-5-glucoside which was isolated from purple-fleshed sweet potatoes was shown to be effective both in vitro and in vivo in inhibiting hepatic glucose secretion and reducing blood glucose [130–132], while peonidin suppressed the excessive expression of the HER2 protein showing anticancer activities [133]. According to Luo et al. [134], cyanidin 3-caffeoyl-feruloyl sophoroside-5-glucoside and peonidin 3-dicaffeoyl sophoroside-5-glucoside were the most effective anthocyanins isolated from the purple sweet-potato cultivar Eshu No. 8. In another study, the oral administration of purple sweet-potato color attenuated cognitive deficits in domoic acid-treated mice through mitochondrial biogenesis signaling and the decrease of p47phox and gp91phox expression [135], while similar results were reported by Zhuang et al. [136], who suggested the regulation of AMPK/autophagy signaling as the mechanism of action. The same pigment was effective against neuroinflammation in mouse brain through the inhibition of mitogen-activated protein kinase (MAPK) and the activation of nuclear factor κB (NF-κB)[137], as well as against bladder cancer through the inhibition of the signaling of phosphatidylinositol-4,5-bisphosphate 3-kinase/Akt or protein kinase B (PI3K/Akt) [138]. In particular, for mitochondrial biogenesis, it was reported that anthocyanins can bind and stimulate estrogen receptor-α and then increase the expression of nuclear respiratory factor-1 (NRF-1) [139]. Anthocyanin-rich extracts from purple sweet potato were moderately effective against human colon cancer cell lines (HCT-116 and HT-29) through the inhibition of tyrosine kinase activity, whereas they

showed no effectiveness against the CCD-33Co cell line [60]. Moreover, Yoshimoto et al. reported that the antimutagenic activity of sweet-potato extracts was attributed mainly to cyanidin content (63% inhibition of mutagenicity of Trp-1 against *Salmonella tymphimurium* TA 98 at the dose of 1.5 mM) [140], while Zhao et al. suggested that anthocyanin-rich extracts from sweet potato are potent anti-aging (at the dose of 1000 mg/kg body weight), anti-hyperglycemic (at the dose of 1 g/kg body weight), and anti-tumor agents (68% tumor inhibition at the dose of 1000 mg/kg body weight) [141]. In another study, highly acylated anthocyanins showed effectiveness against hyperuricemia and kidney inflammation in allopurinol-induced hyperuricemic mice [142], while purple sweet-potato color reduced renal damage through the downregulation of vascular endothelial growth factor receptor (VEGFR2) expression [143]. The regular intake of anthocyanins is also highly associated with the prevention of various chronic liver diseases, and it can reduce lipid accumulation in liver tissues and alleviate oxidative stress and hepatic inflammation [25,95,144–149]. Other hepatoprotective effects of purple sweet potatoes include hepatic insulin resistance in high-fat diet-treated mice through the decrease of reactive oxygen species (ROS) production and the inhibition of endoplasmic reticulum (ER) liver stress (administration of purple sweet-potato color at the dose of 700 mg/kg/day) [150], through the decrease in the expression of ionized calcium-binding adapter molecule 1 (Iba1), tumor necrosis factor-α, interleukin-1β, suppressors of cytokine signaling3 (SOCS3), and galectin-3 (administration of purple sweet potato color at the dose of 500 mg/kg/day) [151], or through the inhibition of nucleotide-binding domain, leucine-rich repeat (NLR) family, pyrin domain containing 3 (NLRP3) inflammasome activation (administration of purple sweet potato color at the dose of 700 mg/kg/day) [152]. Moreover, the combinative use of black soybean and purple sweet potato (mixtures of 2:2 for black soybean and purple sweet potato) resulted in improved insulin sensitivity in streptozotocin-induced diabetic rats through the improvement of insulin and insulin receptor substrate-1 (IRS-1) expression, the increase of superoxide dismutase (SOD) levels, and reduced pancreatic necrosis [153]. In a similar study, the mixture of *Curcuma longa* L. and sweet potato (at the dose of 2–5 mg/kg body weight) showed significant immunomodulating properties in murine leukemia retrovirus-infected mice [154]. The administration of purple sweet potato to obese mice fed with a high-fat diet exhibited anti-obesity effects and attenuated gain weight [155]. Other bioactive compounds of purple sweet potatoes include alkali-soluble polysaccharides which presented anti-inflammatory properties in lipopolysaccharide (LPS)-treated macrophages (RAW 264.7) through the inhibition of nitric oxide, interleukin (IL)-6, IL-1β, and tumor necrosis factor alpha (TNF-α) and the increase of IL-10 [156], as well as anti-inflammatory effects against intestinal inflammation on dextran sulfate sodium (DSS)-induced mice [157], hepatoprotective properties [158], and immunomodulatory effects [159–161]. Non-flavonoid compounds and kaempferol derivatives are also present in sweet-potato tuber tissues, and they contribute to the overall bioactive capacity of sweet potato [28].

Processing and storage conditions are important for the chemical composition and the visual quality of sweet-potato tubers, with heating treatments and higher pH having a detrimental effect on anthocyanins and starch content and on flesh color [132,162–165]. Pretreatments such as blanching, osmotic dehydration, ultrasound-assisted dehydration, and ultrasound-assisted osmotic dehydration before microwave drying also had an impact on total phenolic and anthocyanin content of orange- and purple-fleshed sweet-potato slices [166]. Domestic cooking processes may also affect total anthocyanin and total phenolic content, with steaming suggested as the mildest process to retain the highest amount of total anthocyanins compared to fresh samples, while, at the same time, an increase in total phenolic content was observed by Phan et al. [167]. In a similar study, steaming, roasting, and boiling were suggested as the best cooking methods for retaining total phenolic, anthocyanin, and carotenoid content, respectively, in white, yellow, orange, light-purple, and deep-purple sweet-potato tubers [10].

2.3. Carrot

Carrot (*Daucus carota* L. ssp. *sativus* Hoffm.) belongs to the Apiaceae family and is a highly appreciated vegetable consumed for its edible fleshy roots. There are two cultivar groups depending on root color, namely, the carotene or western carrot (*Daucus carota* ssp. *sativus* var. *sativus*) and the

eastern or anthocyanin carrot (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.), which are widely cultivated throughout the world with an annual production of 42.8 million tons including turnips [108]. Although the orange-colored carrots are the most popular ones, a broad genetic basis exists with many other shades of root flesh (red, white, yellow, black, purple, or multi-color) which attract interest due to their nutritional value and associated health effects [168]. Recently, new genetically biofortified cultivars were developed which contain not only α- and β-carotene but also anthocyanins and lycopene [169]. In particular, for black or purple carrots, several research reports highlighted their beneficial health effects on human health, and they are widely used so far as natural sources of blue color and functional ingredients in the food industry [170][171].

Black carrots contain high amounts of mono-acylated anthocyanins which are less prone to thermal degradation, while they can retain their color at various pH values and storage conditions [172–174]. These functional and structural characteristics of colored carrot pigments make them good candidates for the extraction of natural colorant agents with practical applications in the food industry, especially in food products with low pH, in beverages and confectioneries [124,170,175]. However, despite their stability under various conditions, Espinosa-Acosta et al. [75] did not suggest their use in food models such as yoghurt and jelly, except for the case of ethanolic extracts of black carrots, which could be incorporated into jellies to increase the antioxidant activity of the final product. Moreover, Assous et al. [79] suggested the use of black-carrot pigments as coloring agents in hard candy and sweet jelly without significant differences in the sensorial profile compared to the control, while the same pigments protected sunflower oil from lipid peroxidation. The use of black-carrot extracts was also proposed for the preparation of jams and marmalades, where the main anthocyanins were slightly affected after gastric ingestion and storage at 4 °C [176], as well in co-pigmentation with other natural colorants (e.g., plum, jamun, strawberry, and pomegranate juices), to increase the color stability to heat treatments and pH variation [177]. On the other hand, red and yellow carrots are rich in carotenoids and lycopene and β-carotene in particular [32,33], which, according to Horvitz et al. [178], are both bioavailable and can provide a significant amount of these carotenoids to human diet.

The main anthocyanins detected are mostly cyanidin derivatives, and, according to Frond et al. [41], the most abundant anthocyanin identified in black-carrot extracts was cyanidin-3-(*p*-coumaroyl)-diglucoside-5-glucoside. In the study of Montilla et al. [171], the main detected anthocyanins in *Daucus carota* subsp. *sativus* var. *atrorubens* Alef. were identified as acylated cyanidin 3-xylosyl(glucosyl)galactosides with sinapic acid, ferulic acid, and coumaric acid, and significant differences were observed between genotypes (Antonina, Beta Sweet, Deep Purple, and Purple Haze) in terms of total and individual anthocyanin content. Similar results were reported in the earlier study of Kammerer et al. [179], with acylated and non-acylated cyanidin derivatives found in the highest amounts, while they also suggested significant differences between 15 different black-carrot cultivars, as well as between roots of the same cultivar. Moreover, in black-carrot juice, two more compounds were identified, namely, cyanidin-3-xylosyl-galactoside and cyanidin-3-xylosyl (feruloylglucosyl)galactoside [180], while Schwarz et al. [31] isolated four more pigments identified as vinylphenol and vinylguaiacol adducts of cyanidin derivatives which are formed during the storage of juice through the reaction of phenolic acids with anthocyanins. Regarding the health effects of anthocyanins, extracts from purple carrot were moderately effective against HCT-116 human colon cancer cell lines through the inhibition of tyrosine kinase activity, whereas they showed no effectiveness against HT-29 and CCD-33Co cell lines [60]. Yet, black-carrot crude extracts exhibited significant antioxidant, cytoprotective, and anti-angiogenic properties, indicating a synergistic effect of the various polyphenols (anthocyanins, phenolic acids, and flavonoids) contained in the root extracts [181]. Although non-digested purple carrot extract is more potent than the digested extract, Olejnik et al. [182] reported that gastrointestinal digested purple-carrot extract had intracellular ROS-inhibitory activity and protected colonic cells against oxidative stress by reducing oxidative DNA damage by 20.7%. According to Blando et al. [183], the anthocyanin-rich extracts from black carrots contained mostly anthocyanins acylated with cinnamic-acid derivatives, which exhibited anti-inflammatory activities through the reduction of the expression of endothelial inflammatory

antigens. Apart from anthocyanins, black carrots are a good source of phenolic acids, namely, chlorogenic and caffeic acids, which contribute to the overall antioxidant capacity [41,184].

Processing may affect the chemical composition and antioxidant properties of black-carrot juice, and the use of pectinase during maceration increased the total anthocyanin content, the overall antioxidant capacity, and the juice yield of enzyme-treated compared to normally pressed juice [185,186]. The use of ultrasound and mild heating (50 °C) may increase the extraction yield of anthocyanins from black-carrot pomace, especially the content of cyanidin-3-xyloside-galactoside-glucoside-ferrulic acid and cyanidin-3-xyloside-galactoside, which were detected in the highest amounts [64]. Another processing treatment which could increase the bioavailability and stability of anthocyanins in black-carrot-based food products is the microencapsulation of anthocyanin-rich extracts [76]. Moreover, wounding stress may increase anthocyanin content, chlorogenic acid in particular, thus improving the nutritional and functional value of the obtained food products [187].

2.4. Beet Root

Beet or table beet (*Beta vulgaris* L.) belongs to the Amaranthaceae family and is commonly used for its edible fleshy red roots and tender leaves. Beet roots contain betalains, a class of compounds which is further divided into betacyanins and betaxanthins [188]. The composition of betalains and the ratio of betacyanins to betaxanthins depends on tyrosine production and its conversion to betalains, with significant differences observed between red and yellow beet roots [189]. The most commonly found betacyanins are betanins which are responsible for the red vivid color of beet roots, and they are water-soluble and sensitive to prolonged heating [170]. Betanins are commercially available as color additives (E162) in powder form or as juice concentrates following vacuum evaporation [188]; however, there is a great diversity in flesh color among the beet-root genotypes with variable intensities of red color or other shades ranging from white to orange. Apart from the genotype, color intensity is also affected by growing conditions and maturity stage, storage conditions, and processing treatments [81,190,191]. Beet roots with yellow color are most abundant in betaxanthins, while betacyanins are present in lesser amounts [34]. In the study of Wettasinghe et al. [192], beet-root genotypes with diverse flesh colors exhibited significant differences in antioxidant activity and in phase II enzyme induction capacity, which is associated with cancer chemoprotective effects [192]. Moreover, Lee et al. [35] reported that betanine and betaine extracted from red- and yellow-colored beet roots were effective against HepG2 cell proliferation in a dose-dependent manner. In the same study, the main identified betalains detected in the cultivar with yellow roots (Burpee's Golden Globe) were vulgaxanthin I and betanin [35]. Vulić et al. [193] also reported that the beet-root pomace, a by-product of the beet-root juice extraction, has a high content of betalains and phenolic compounds which exhibited in vitro antiradical activities against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and in vivo antioxidant and hepatoprotective activity, suggesting that it could be used as an excellent nutraceutical resource or an ingredient of functional food products.

2.5. Yam

Yam includes various species of the *Dioscorea* genus (Dioscoreaceae), although sometimes it is confused with other root vegetables such as sweet potatoes, oca, taro, etc., which locally may be referred to as yams [194]. Tuber flesh color can be white, yellow, red, or purple depending on the cultivar, with significant differences in bioactive compound content and antioxidant properties [194,195]. Purple yam or water yam (*Dioscorea alata purpurea*) is usually cultivated in tropical and subtropical regions of the world, and its edible roots are very rich in starch and amylose [196], although a great variation in chemical composition of the edible parts of the species was reported [197]. Resistant starch from purple yam (*D. alata*) was effective against hyperlipidemia in high-fat diet-fed hamsters through the amelioration of lipid metabolism and the modulation of gut microbiota [196,198]. Moreover, extracts from roots significantly reduced blood glucose levels in Wistar rats with alloxan-induced hyperglycemia [199] or cholesterol (total and low-density lipoprotein (LDL)) and triglycerides in hypercholesterolemic rats [200], ameliorated doxorubicin (DOX)-induced

cardiotoxicity [201], showed protective effects against aniline-induced spleen toxicity [202] and in vivo anti-inflammatory activities against λ-carrageenan-induced paw edema in mice [203], and could be used as an adjuvant in bone-marrow-derived dendritic cell (DC)-based vaccines for cancer therapy [204]. *D. alata* root extracts may also alleviate cellular fibrosis through the downregulation of the transforming growth factor-beta (TGF-β)/Smad signaling pathway and the modulation of epithelial–mesenchymal transition (EMT) expression in kidneys [205]. On the other hand, according to Chan et al. [206], root extracts are also effective against CCl_4-induced liver injury and hepatic fibrosis. Other health effects include the improvement in function of large bowel and modulation of fecal microflora [207], beneficial effects in gastrointestinal function [208] and cognitive ability [209,210], and the activation of the immune system [211]. The root color of purple yam (*D. alata*) is attributed to the high content of anthocyanins which exhibit significant antibacterial activities [212], anti-inflammatory effects on trinitrobenzenesulfonic acid (TNBS)-induced colitis in mice [213], antiglycative properties [214], and antidiabetic properties [215,216]. The main detected anthocyanins in this species were identified as cyanidin, pelargonidin, and peonidin-type compounds and alatanins A–C [36]; however, the individual compound profile and the overall anthocyanin content are affected by maturity stage and the expression of the concomitant genes [37]. Apart from *D. alata*, which is considered the main purple yam, there are also cultivars of *D. trifida* or cush-cush yam which contain peonidin, cyanidin, and pelargonidin aglycones [38]. Other compounds with bioactive properties are also present, namely, phenolic acids such as ferulic, sinapic, vanillic, caffeic acid, and *p*-coumaric acid, and others, which presented immunomodulatory properties [217,218], proteins with estrogen-stimulating activities that may relieve menopausal syndrome [219], allantoin and dioscin [220], dioscorin [221], or β-sitosterol and ethyl linoleate with anti-atherosclerotic activity [222]. On the other hand, carotenoids and β-carotene in particular are responsible for the root color of yellow yam (*D. cayennensis*) [40]. Yam roots may contain antinutritional factors such as tannins and diosgenin, which also present bioactive properties. For example, antidyslipidemic effects were reported for diosgenin extracts from purple and yellow yams without affecting body weight gain [220,223], while diosgenin and furostanol glycosides and spirostanol glycosides were effective against the proliferation of various cancer cell lines (MCF-7, A 549, and Hep G2) [224].

A very common use of purple yam is the substitution of wheat flour for bakery products and food products in general without affecting the sensorial acceptance of the products by consumers [225–227], while yam flour can be used for gluten-free bakery products [228].

2.6. Onion

Onion (*Allium cepa* L., Alliaceae) is one of the most important species of the *Allium* genus, which is commonly used for its edible bulbs. There is a great number of cultivars available with a great diversity in color, which usually refers to bulb skin color, since, in most cases, the presence of pigments is limited to the outer skins of the bulb [229]. In many countries, onion bulbs are considered the main dietary source of flavonoids, a high proportion of which is attributed to the anthocyanin content [63,230]. However, most of the studies refer to red-onion cultivars which contain various polyphenols including acylated and non-acylated cyanidin glucosides, and less information is available about the profile of anthocyanins in purple onions [41,42]. The biosynthesis of anthocyanins involves the shikimate pathway and the activity of anthocyanidin synthase, which catalyzes the production of anthocyanidins, and, after further enzymatic reactions, the various anthocyanins are produced [5]. Comparing green, yellow, red, and purple onion, Benkeblia [231] observed higher total phenolic content and antioxidant properties in red and purple onion-bulb extracts. Similar results were reported by Zhang et al. [232] in a study comparing white, yellow, and red onion, with the latter showing considerably higher total anthocyanin, flavonoid, and polyphenol content, which was also correlated to high antioxidant activity measured through DPPH, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$), and fluorescence recovery after photobleaching (FRAP) assays. Bulb extracts are potent bioactive natural matrices, and, according to the study of Oboh et al. [233], extracts of purple onion were effective against angiotensin-converting enzyme, α-amylase, and α-glucosidase activity, showing significant antidiabetic and anti-hypertensive effects.

Moreover, skins of pearl onion exhibited significant anti-inflammatory properties and inhibitory effects against radical-induced DNA scission [234]. In terms of antioxidant activity, purple onions exhibited higher oxygen radical absorbance capacity (ORAC) values than white onions, which indicates a higher concentration of bioactive compounds [235]. A preliminary study conducted by Khiari et al. [236] suggested that, depending on the quality of the plant residues, onion solid waste, also constituted primarily by the outer dry layers of the bulbs, may be used to extract polyphenols with potential antioxidant activity, and the yield of total polyphenols can be optimized using ethanol extracts, with extraction time up to 6 h, while maintaining relatively low extraction temperature (40 °C gave better results than 60 °C).

2.7. Other Root Vegetables

Radish (*Raphanus sativus* L., Brassicaceae) is a cruciferous species, well known for its normally white edible fleshy hypocotyls which come in different shapes, sizes, and skin colors. Apart from white-fleshed cultivars, there are also genotypes with pink and purple hypocotyls due to the presence of pigments in the xylem [43]. Pigmentation may also change with the hypocotyl development stage [237]. Purple color implies the presence of anthocyanins, and, according to the study of Reference [44], 60 different compounds were detected and identified as cyanidin glucosides. Most of the anthocyanins are present in acylated forms of cyanidin glucosides which increase their stability, and they could be easily used as natural colorants in functional foods [7,43,238,239], while root extracts may also exhibit beneficial health effects against gastric injuries [240].

Purple kohlrabi (*Brassica oleracea* var. *gongylodes*) is another species of the Brassicaceae family with intense purple color, whose edible part is the swollen fleshy meristem. The pigments are located in the meristem skin and consist of cyanidin and cyanidin glucosides which are responsible for the strong antioxidant properties of the species [47–50]. Examining the antioxidant activity of kohlrabi ethanol and water extracts, Pak et al. [241] observed strong DPPH radical-scavenging activity, and purple kohlrabi extracts had higher antioxidant capacity compared to green kohlrabi extracts. Similarly, comparing green and red kohlrabi, Jung et al. [242] observed that the latter had double the total phenolic content, as well as a higher antioxidant (DPPH, ABTS, and peroxynitrite scavenging activity assay ($ONOO^-$)) effect compared to green kohlrabi. In the same study, red kohlrabi methanol extract had stronger anti-inflammatory, antidiabetic, and antioxidant effects than the green kohlrabi methanol extract.

Taro (*Colocasia esculenta* L.) is a root vegetable of the Araceae family with great genetic diversity in plant morphology, including the color of corm flesh, which can be white, purple, brown, or blackish [50,243,244]. The main detected anthocyanins were identified as cyanidin and pelargonidin glucosides, and they exhibit significant antioxidant and anti-inflammatory activities [51].

3. Conclusions

Root vegetables with intense and uncommon colors are very important in the human diet, not only because they increase the overall intake of health-promoting compounds, but also because they diversify the daily diet in terms of color, flavor, and chemical composition, which imparts distinct functional effects on the human body. The inclusion of such root vegetables either raw in fresh salads or in cooked dishes may increase palatability and appeal for healthier food products, although proper marketing is always an issue since consumers are usually reluctant to introduce new flavors and unconventional products that break the mold. Nevertheless, the current trends in the food and beverage market and the increased public demand for substituting synthetic compounds with natural alternatives could boost the establishment of these species and help the crossing over from niche products to widely accepted ones with diverse uses in the food industry. Further research is also needed in order to (i) identify those correlations and mechanisms of action responsible for the antioxidant properties and health effects of the pigmented vegetables, (ii) evaluate agronomic practices that will increase the bioactive capacity of the final products through the improved pigmentation, (iii) study post-harvest and processing treatments that will make these compounds less prone to degradation and easier to use in the design of functional foods and as natural coloring

agents, and (iv) define efficient extraction protocols that will allow high yields and high stability and quality of coloring agents extracted from plant sources. Finally, increasing the knowledge about the chemical composition and the health effects of individual compounds of colored root vegetables could be further exploited through breeding programs for the production of elite genotypes with increased content of coloring compounds and tailor-made health effects, as well as through plant in vitro strategies for the production of specific natural secondary metabolites and further use in the pharmaceutical and the food and beverage industries.

Author Contributions: All authors contributed equally to the writing of the original draft and final draft preparation.

Funding: This work was supported by the USDA National Institute of Food and Agriculture and Hatch Appropriations under Project #PEN04723 and Accession #1020664; The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Program PT2020 for financial support to CIMO (UID/AGR/00690/2019), to FEDER-Interreg España-Portugal programme for financial support through the project 0377_Iberphenol_6_E and TRANSCoLAB 0612_TRANS_CO_LAB_2_P, and to the European Regional Development Fund (ERDF) through the Regional Operational Program North 2020, within the scope of project Mobilizador Norte-01-0247-FEDER-024479: Valor Natural®.

Conflicts of Interest: The authors declare no conflicts of interest.

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