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Ionizing radiation applications for food preservation: effects of gamma and e-beam irradiation on physical and chemical parameters of chestnut fruits

DOCTORAL THESIS

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DÑA BEGOÑA QUINTANA ARNÉS, PROFESORA TITULAR DE UNIVERSIDAD DEL DEPARTAMENTO DE FÍSICA FUNDAMENTAL DE LA UNIVERSIDADE DE SALAMANCA,

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Aos meus pais, irmãos e irmã:
que me ensinaram o que sou e
me apoiaram incondicionalmente.

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Abstract

In Mediterranean countries chestnut fruits represent an important food product with a high economic relevance in local economy. The production of European chestnut (*Castanea sativa* Mill.) varieties in E.U. countries represents more than 100 kton, with an income for the producers of several million of euros, value that increases along the market chain. These fruits are also exported to other countries that, due to international phytosanitary laws, impose the absence of insects. Until recently, the method used for chestnuts post-harvest disinfestation was chemical fumigation that is environment aggressive and toxic for the operators.

Following the request for an urgent alternative for the agro-industry, that process and export these fruits, and considering that irradiation is a more environment friendly technology that could be used as an alternative, gamma and electron beam irradiation were tested and validated as a possible alternative. Food irradiation is already an industrial technology used for several items, nevertheless, its effects in specific food matrices should be studied and validated. Previous studies of irradiation effects in chestnuts were performed mainly in Asian varieties but in a limited number of parameters. In this research, a detailed study of the impact of gamma and electron-beam irradiation effects (dosis 0.25, 0.5, 1, 3, 6 kGy) on physical, chemical and antioxidant parameters of European chestnut fruits of *Castanea sativa* varieties (Cota, Judia and Longal from Portugal; and two varieties from Turkey and Italy), stored up to 60 days was performed.

The physical parameters evaluated were the drying rate, colour and texture; chemical analyses included determination of the nutritional profile, dry matter, ash, proteins, carbohydrates, total energy, fatty acids, sugars, organic acids, tocopherols and triacylglycerols composition; the antioxidant properties were evaluated through free radicals scavenging activity, reducing power and inhibition of lipid peroxidation inhibition, as also determination of total phenolics and flavonoids.

The effects on non-irradiated and gamma or electron-beam irradiated chestnuts were compared, as well as their interaction with storage time. Both types of irradiation showed to represent a suitable solution for chestnuts post-harvest treatment. With no exception, the storage time caused higher changes in physical, antioxidant and nutritional/chemical profiles than both irradiation types, confirming that this technology, at the applied doses, did not affected chestnut fruits quality. Qualitative changes were detected in the structure of certain fatty acid molecules, without affecting

its total content. These results were described for the first time highlighting these parameters as possible indicators of irradiation processing. In fact, the main differences found in irradiated samples were related with storage time or different assayed cultivars.

It was also analysed the irradiation feasibility and the economic impact of electron beam processing in chestnut fruits, considering that this technology could have more acceptance than gamma irradiation.

This work addressed different areas of research focusing on a technological solution of a problem proposed by the agro-industry, bringing innovation to a traditional food product. Independently of the irradiation source, chestnut variety or geographical origin, gamma and electron beam irradiation is an environmental friendly alternative technology for chestnut post-harvest treatments that can substitute the chemical fumigation also presenting a positive contribute in the economy of fruit producers.

Resumen

La castaña es un fruto típico en el sur de Europa, en las zonas montañosas de los países mediterráneos y en Asia. En los países mediterráneos de la UE representa un mercado de más de cien mil toneladas, con un ingreso de varios millones de euros sólo a nivel de producción, valor que va aumentando a lo largo de la cadena de comercialización.

Las castañas pueden ser infestadas por larvas de diferentes especies lo que causa pérdidas de ingresos para los productores y para la industria alimentaria. Las castañas exportadas deben ser tratadas posteriormente a la cosecha para eliminar los insectos y gusanos, de manera que se cumpla con las regulaciones fitosanitarias del comercio internacional. Hasta hace poco, en la desinsectación de castañas postcosecha se utilizaba un insecticida químico, el bromuro de metilo, que ha sido prohibido en la UE desde marzo de 2010 debido a su toxicidad para los operadores y para el medio ambiente. Esta decisión dejó muy pocas alternativas a la agroindustria que procesa y exporta esta fruta.

En este contexto, la eliminación de insectos en las castañas por irradiación puede ser una alternativa viable, considerando que es una tecnología respetuosa con el medio ambiente y que podría ser utilizada si el producto tratado cumple con los otros parámetros de calidad específicos para este tipo de alimentos.

Aunque la irradiación de alimentos es ya una tecnología industrial utilizada en la preservación de varios productos alimenticios, su efecto en cada matriz debe ser estudiada y validada. Cualquier transformación de los alimentos deja marcas en el producto, pero en la mayor parte de los casos constituye un requisito para comer alimentos sanos. La irradiación de alimentos puede preservar algunos componentes y degradar otros. El balance de ventajas y desventajas, en comparación con otros procesos de conservación, se debe utilizar para seleccionar o no este tipo de tecnología de procesamiento, de manera que se proporcione al consumidor un producto que cumpla con los mejores criterios de calidad.

Estudios previos de los efectos en irradiación de castañas se realizaron principalmente en las variedades asiáticas, que tienen características organolépticas distintas a las europeas, abarcando un número limitado de parámetros. En esta investigación se presenta un estudio detallado de los efectos de la radiación gamma y de electrones a dosis de 0,25, 0,50, 1, 3 y 6 kGy en las propiedades físicas (deshidratación, color, textura) y químicas (valor nutricional, cenizas, proteínas, hidratos de carbono,

azúcares, grasa, ácidos orgánicos, tocoferoles, triacilgliceroles y energía total) en castañas de origen europea (*Castanea sativa* Mill.) de distintas variedades Cota, Judia y Longal de Portugal y dos variedades de Turquía y de Italia), tras ser almacenadas durante 60 días.

Con este estudio fue posible obtener resultados de los efectos de dos tecnologías de procesamiento por irradiación y de su viabilidad. Los parámetros físico-químicos de muestras de castañas irradiadas con radiación gamma y con electrones se compararon con muestras no irradiadas, estudiando también el efecto del tiempo del almacenamiento. Las principales diferencias encontradas en muestras irradiadas están relacionadas con el tiempo de almacenamiento o con las variedades. Sin excepción, el tiempo de almacenamiento ha causado cambios mayores en estos parámetros que ambos tipos de radiación, lo que confirma que esta tecnología, a las dosis aplicadas, no afecta la alta calidad de las castañas.

Se han detectado cambios cualitativos, reordenación de la estructura de las moléculas de ácidos grasos sin afectar a su contenido total ni a sus propiedades nutricionales. Además, por primera vez, fueron identificadas como indicadores del procesamiento por irradiación, lo cual supone una alternativa a los indicadores recomendados en las normas europeas para detección de alimentos irradiados.

Los dos tipos de radiación utilizados, gamma y electrones, parecen así constituir soluciones adecuadas, independientemente de las variedades de castañas y origen geográfico, lo que es un paso importante hacia la validación de estas tecnologías en el tratamiento postcosecha en castañas.

Este trabajo ha tocado diferentes áreas de investigación con el objetivo centrado en proponer una solución tecnológica a un problema planteado por la agro-industria, trayendo innovación a un producto alimenticio tradicional en algunas regiones de Europa. Así, se incluyó también en los apéndices un breve análisis de la viabilidad económica de la irradiación; en concreto del impacto del procesamiento con electrones en el precio de las castañas, teniendo en cuenta que para los consumidores esta tecnología podría tener más aceptación que la irradiación gamma.

En resumen, se ha hecho un estudio detallado de los efectos de la radiación gamma y de electrones en los parámetros físico-químicos de castañas europeas, proponiendo una tecnología alternativa que es respetuosa con el medio ambiente y que puede tener un impacto favorable en la economía de los productores de castañas europeas, garantizando al consumidor un alimento seguro.

Resumo

A castanha é um fruto típico do sul da Europa, nas zonas montanhosas dos países mediterrânicos e na Ásia. Nos países mediterrânicos da U.E. representa um mercado de mais de cem mil toneladas e um valor comercial de alguns milhões de euros apenas no produtor, valor que aumenta ao longo de toda a cadeia de comercialização.

As castanhas podem ser infestadas por larvas de diferentes espécies, o que causa perdas de rendimento aos produtores e à indústria alimentar que processa este produto. As castanhas devem ainda ser tratadas posteriormente à colheita para eliminar insectos infestantes, bichado e gorgulho, de modo a que cumpram as normas fitossanitárias do comércio internacional. Até há pouco tempo, para este fim utilizava-se como fumigante pós-colheita o brometo de metilo, que foi proibida a sua utilização na U.E. desde Março de 2010, devido à sua toxicidade para os operadores e ser nocivo para o meio ambiente. Esta decisão deixou poucas alternativas à agro-indústria que processa e exporta este fruto. Neste contexto, a eliminação de insectos em castanhas por irradiação pode ser uma alternativa viável, considerando que é uma tecnologia amiga do ambiente e que poderia ser utilizada se o produto tratado cumprir com os outros parâmetros de qualidade específicos para este tipo de alimentos.

Ainda que a irradiação seja uma tecnologia industrial utilizada na preservação de vários produtos alimentares, o seu efeito em cada matriz deve ser estudado e validado. Qualquer transformação dos alimentos deixa marca no produto, mas na maior parte dos casos constitue um requesito para consumir alimentos saudáveis. A irradiação de alimentos pode preservar alguns componentes e degradar outros. O balanço de vantagens e desvantagens, comparativamente a outros processos de conservação, deve ser utilizado para seleccionar ou não este tipo de tecnologia de processamento, de forma a proporcionar ao consumidor um produto que cumpra os melhores critérios de qualidade.

Estudios prévios dos efeitos da irradiação em castanhas realizaram-se principalmente em variedades asiáticas, que têm características organolépticas distintas das europeias, incluindo um número limitado de parâmetros. Nesta investigação apresenta-se um estudo detalhado dos efeitos da radiação gama e de feixe de electrões nas doses de 0,25, 0,50, 1, 3 e 6 kGy nas características físicas (desidratação, cor, textura) e químicas (valor nutricional, cinzas, proteínas, hidratos de carbono, açúcares, gordura, ácidos orgânicos, tocoferois, triglicerói s e valor energético total) em castanhas

de origem europeia (*Castanea sativa* Mill.) nas variedades Cota, Judia, Longal de Portugal e em duas outras variedades provenientes da Itália e da Turquia, armazenadas ao longo de 60 dias.

Com este estudo foi possível ter resultados dos efeitos de duas tecnologias de processamento por irradiação e da sua viabilidade. Os parâmetros físico-químicos das amostras de castanhas irradiadas com radiação gama e com feixe de electrões foram comparados com amostras não irradiadas, estudando também o efeito do tempo de armazenamanento. As principais diferenças observadas nas amostras irradiadas estavam relacionadas com o tempo de armazenamento ou com as variedades. Sem excepção, o tempo de armazenamento causou maiores alterações nestes parâmetros do que os dois tipos de radiação, o que confirma que esta tecnologia, nas doses aplicadas, não afecta a alta qualidade das castanhas.

Foram detectados alterações qualitativas em algumas moléculas de ácidos gordos, reordenação na estrutura das moléculas sem afectar o seu conteúdo total nem as suas propriedades organolépticas e nutricionais. E que, pela primeira vez, foram identificadas como indicadores do processamento por irradiação, podendo ser uma alternativa aos métodos recomendados nas normas europeias para detecção de alimentos irradiados.

Os dois tipos de radiação utilizados, gama e electrões, parecem assim constituir soluções adequadas, independentemente das variedades de castanha e origem geográfica, o que é um passo importante para a validação destas tecnologias no tratamento pós-colheita de castanhas.

Este trabalho abrangeu diversas áreas de investigação com o objectivo centrado em propor uma solução tecnológica para um problema colocado pela agro-indústria, trazendo inovação a um produto alimentar tradicional em algumas regiões da Europa. Assim, incluiu-se também nos apêndices uma breve análise da viabilidade económica da irradiação, em concreto, o impacto do processamento com feixe de electrões no preço final das castanhas, por esta tecnologia ser mais aceite pelo consumidor comparativamente à irradiação gama.

Em resumo, realizou-se um estudo detalhado dos efeitos da radiação gama e feixe de electrões nos parâmetros físico-químicos de castanhas europeias, propondo uma tecnologia alternativa que é amiga do meio ambiente e que pode ter um impacto favorável na economia dos produtores de castanhas europeias, garantindo ao consumidor um alimento seguro.

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D. Published papers

A. List of papers that are part of the thesis and author's affiliations

List of papers

This thesis is presented in the format of published papers compilation to obtain the Doctor's degree by the University of Salamanca.

The presented work was based in a project with national and international collaborations (ON.2/QREN/EU nº 13968 and Eureka Idea nº 7596), obtained an award on a Food I&DT innovation fair in 2011 and was object of 14 papers, in journals and conference proceedings (10 papers in ISI journals, 2 as first author), together with 33 oral and poster communications. The author of this thesis participated in the design, implementation and final conclusions of this project, concluded in November 2013.

The thesis includes only the published papers in journals with impact factor indexed to ISI Web of Knowledge.

Published papers in journals with impact factor indexed to ISI Web of Knowledge

[1] **Amilcar L. Antonio**, Ângela Fernandes, João C.M. Barreira, Albino Bento, M. Luisa Botelho, Isabel C. F R. Ferreira. (2011). "Influence of gamma irradiation in the antioxidant potential of chestnuts *Castanea sativa* Mill.) fruits and skins". Food and Chemical Toxicology 49 (9), pp. 1918-1923. http://dx.doi.org/10.1016/j.fct.2011.02.016

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1. Ionizing radiations for food preservation

The use of ionizing radiation to preserve food started immediately after discover of this type of radiation. Röntgen x-rays discover occurred in 1895 and in 1896 H. Minsch, from Germany, proposed the use of ionizing radiation to destroy microorganisms. The first patent for food preservation was claimed in 1905 by H. Lieber in USA and J. Appleby & A.J. Banks in U.K. (Molins, 2001). In 1930, a patent was attributed in France to O. Wüst, for the use of ionizing radiation for food preservation (IAEA, 2011). In 1898, J.J. Thompson discovered the electron and in the same year Pacronotti & Procelli referred its effects in microorganisms (Molins, 2001).

Due to technical limitations, the use of these discovers did not passed immediately to a commercial phase and only fifty years later started at an industrial scale, first pushed by the governments of USA, UK, Germany and former USSR (Molins, 2001; Diehl, 2002). Recently, food irradiation is pushed by phytosanitary trade barriers, to eliminate the presence of insects, or due to health issues with contaminated food, *e.g. Salmonella* or *Listeria*, demanding new approaches to guarantee food safety, without compromise the quality of the processed product (Cabo Verde *et al.*, 2010; Antonio *et al.*, 2011a; Antonio *et al.*, 2013b).

Food preservation is a permanent defying target due to continuous growth of population, scarce of soil and health food safety aspects. Different processing technologies are currently used to preserve food (Rahman, 2007) and irradiation processing, based on the use of ionizing radiation, is used to extend the shelf-life, delay the maturation process; to decontaminate, lowering the presence of bacteria and fungi; or to sterilize food products, eliminating the microorganisms (Cabo Verde *et al.*, 2010). This process is also referred by some authors as "*cold pasteurization*", since it not increases significantly the temperature of irradiated products (Sádecká, 2007). Food components that are particular sensible to thermal treatments (*e.g.* vapour steam sterilization), like aromatic compounds in medicinal or edible plants, could be decontaminated using this technology (Sádecká, 2007; Pereira *et al.*, 2014).

Currently, three types of ionizing radiations are authorized for food irradiation processing: gamma radiation; electron beam and x-rays (E.U., 1999a). Gamma radiation comes from the spontaneous emission of the isotopes of ^{60}Co or ^{137}Cs; Electron beam (e-beam) radiation is produced by accelerating electrons till the maximum allowed energy of 10 MeV (mega electron volt), x-rays are produced by the impact of

accelerated electrons on a metallic target, with the consequent emission of radiation (photons), by a physical phenomena described as "*bremsstrahlung*", with the energy limited to 5 MeV for food irradiation applications (E.U., 1999a). The three types of radiation have different characteristics, namely depth of penetration, but all can be used for food processing, using the right configuration adapted to the type or volume of food to be processed. X-rays was the first ionizing radiation tested for food preservation, however due to the low efficiency of conversion of electrons energy to x-rays only recently, with the development of new machines, this technique regained interest (Miller, 2005).

1.1. Gamma irradiation

The first industrial gamma irradiators were built in the 1960's, in USA, and in the port of Odessa, in USSR, now Ucrania, for grain disinfestation (Nordion, 2013).

Industrial gamma irradiation plant uses the radioisotope Cobalt-60 (^{60}Co), which has a half-life of 5.3 years and decays in the stable atom of Niquel-60, emitting beta radiation, that is absorbed by the metallic sealed capsules containing ^{60}Co, emitting photons with two energies: 1.17 MeV and 1.33 MeV (1 MeV = 1.6×10^{-13} J), that are used to irradiate the material. The other authorized isotope for gamma irradiation, Cesium-137, has a half-life of 30.2 years and decays into Barium-137, emitting photons with the energy of 0.66 MeV.

In an industrial gamma plant the sources are stored in a pool, dry or with water. The products moves along a conveyor that transports automatically the boxes inside a bunker, built according radioprotection standards to guarantee the safety for the operators (IAEA, 2010), and with multiple passes to give the intended dose. After the boxes entered the bunker, gamma sources are raised to the area where the products will be irradiated (Fig. 1). The industrial plant has several redundant security systems to assure that when the sources are irradiating, up, no one is allowed to enter inside the bunker, and if this happens or on an emergency occurs, the sources automatically fall down to the pool or dry pit. The ^{60}Co sources never contact with the irradiated food, since they are encapsulated in steel rods.

The activity of the sources is measured in Becquerel, Bq, which is the number of disintegrations or emissions per second. The traditional unity for radiation activity was the Curie, Ci (1 Ci = 3.7×10^{10} Bq). A typical industrial irradiation plant has an activity of about 1 million Curie (1 MCi). The dose rate, dose per unit time, and the throughput,

processed mass per unit time, are limited by sources activity. The products will remain in front of the sources the necessary time to give the intended dose, that is expressed in Gray, Gy, that means Joule per kilogram.

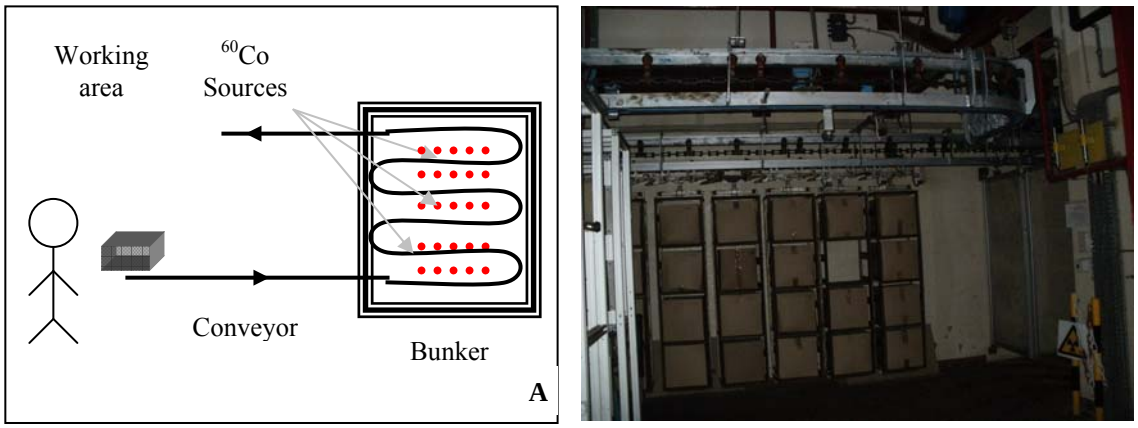


Fig. 1. Gamma irradiator simplified layout (A) and tote boxes picture (B).

The throughput of the process, mass per unit time (M/t), is given by the equation (Miller, 2005):

$$M/t = (P/D) \times F \qquad \text{(eq. 1)}$$

where M/t is expressed in kg s^{-1}; P is the power of the machine, in W; F is the efficiency of the irradiation and utilization factor of the machine (0.25 to 0.75); and D is the dose, in Gy.

The throughput, M/t, is inversely proportional to the dose delivered, D, to the product, higher dose means lower throughput:

$$M/t = (\text{const.}) \times 1/D \qquad \text{(eq. 2)}$$

The power, and consequently the throughput, is directly proportional to the activity of the sources:

$$P = (\text{const.}) \times A \times E / t \qquad \text{(eq. 3)}$$

where the effective power, P, is the energy per second delivered to the product; A is the total sources activity, in Bq, E is the mean energy per disintegration, in J, and t is the exposure time, in s.

For research on gamma irradiation there are small units of different sizes, available from several companies, *e.g.* Izotop Co., Hungary; Nordion co., Canada; or Symec Eng., India.

The experimental gamma chamber used in this work, presented in Fig. 2, is based on a machine from Graviner Company, U.K., model “Precisa 22” and adapted with a SCADA – Supervisory Control and Data Acquisition. In this chamber the

sources are inside steel rods, pneumatically commanded by the touch control panel (Fig. 2-B). This experimental chamber has four ^{60}Co sources with a total activity of 174 TBq (4.68 kCi), and with dose rates between 0.10 kGy h^{-1} and 2.60 kGy h^{-1} (in November 2013).

A B

Fig. 2. Gamma irradiation chamber (A) and sources touch control panel (B).

For food irradiation there are currently around the world about one hundred gamma irradiation plants registered in IAEA database (IAEA, 2013), that are in use for several purposes: food irradiation; and sterilization of other materials (*e.g.* medical disposables, pharmaceutical products, etc.); and increasing every year (Eustice, 2013; Kume & Todoriki, 2013).

More details about the experimental gamma chamber: characteristics; operation; and irradiation procedures, are described in the methodology for samples irradiation (Methodology - Appendix 1).

1.2. Electron-beam irradiation

Another type of ionizing radiation used for food preservation, radiation with enough energy to ionize atoms and molecules, are electrons of high energy, produced in a cathode and accelerated by an electric DC potential or by RF.

The RF accelerators are more compact allowing its use in small places, lowering installation and building costs (Lancker *et al.*, 1999). These machines can also be used to produce x-rays, using a metallic target in front of the beam, that have higher penetration depth (Auslender *et al.*, 2004; Cleland & Stichelbaut, 2013). However, the low energy conversion efficiency into x-rays (Ziaie *et al.*, 2002; Deeley, 2004) imposes some economic limitations to the use of this type of irradiation process for low valuable

food products, due to the higher price of the irradiation machine and operation costs (AAPM, 1986).

There are some self-shielded transportable systems, built by different companies (Berejka, 2004; IAEA, 2011), but due to weight limitations, radiation shielding and costs of transportation, are limited to energies of some hundreds of keV's or a few MeV's, which limits its use to applications of low penetration depth, *e.g.* waste water treatment or flue gas treatment (EBTech, 2013) and surface treatment of seeds (EVONTA, 2014).

Electron beam irradiators, available from different companies, are hardware more sophisticated than gamma irradiators, however due to several factors they are becoming more popular and being the first choice, whenever the product can be treated by low penetration radiation. And since these equipments can be used also to produce x-rays, which have a higher penetration depth, justified the increasing demand for these machines, whenever the relation operation cost and processed product price is viable.

The penetration of e-beam in food is directly proportional to the energy, and these equipments are generally set at the maximum allowed energy of 10 MeV. This limiting value is set in order to not activate the nucleus, to not induce radioactivity in the product (Miller, 2005).

The irradiated products pass in a conveyor under a vertical beam and the delivered dose is obtained adjusting the speed of the conveyor. In Fig. 3 is presented the setup used at INCT, Warsaw.

Fig. 3. Vertical beam line layout (A); chestnut fruits (B) and conveyor (C).

On an electron beam irradiation processing the main parameters are the electrons energy, which limits the depth of penetration, and the beam power, that limits the throughput of the machine.

Around the world there are several hundreds of e-beam accelerators used for different industrial applications, from which food irradiation represents only a small part (Berejka, 2009).

1.2.1. Electron-beam energy

The typical energies used on an industrial e-beam food irradiator are about 10 MeV, in order to get a good uniformity dose and versatility to irradiate different type of food products, since the penetration depth depends mainly on electrons energy.

To estimate the energy to irradiate chestnut fruits or other food products, is used the relation between the depth were the exit dose equals entrance dose (R_{opt}), and the energy for one-side and two-side irradiation, given by eq.4 and eq.5 (Sarma, 2004b):

$$E = 2.63\ R_{opt}\ \rho + 0.32 \qquad \text{one-side irradiation} \quad \text{(eq. 4)}$$

$$E = 1.19\ R_{opt}\ \rho + 0.32 \qquad \text{two-side irradiation} \quad \text{(eq. 5)}$$

where E is the energy, in MeV, R_{opt} is expressed in cm and ρ is the density, in g cm^{-3}.

The maximum efficiency is obtained when the dose on the rear surface is equals the front surface dose (Miller, 2005). For chestnut fruits, for example, considering that R_{opt} should be similar to the maximum thickness of the fruit, 2.5 cm, and considering the typical value for fruits density, about 1.2 g cm^{-3} (Antonio *et al.*, 2013a) , using eq.4 and eq.5 we get that the energy should be about 8.2 MeV for one-side irradiation and 3.9 MeV, for two-side irradiation, concluding that e-beam irradiation at 10 MeV guarantees good dose uniformity and also versatility to irradiate other type of products(Barreira *et al.*, 2012).

1.2.2. Penetration depth and irradiation geometry

In an e-beam irradiation process the absorbed dose, defined as the energy per mass (in Gray, Gy), depends on the beam current, conveyors' speed and beam geometry (Mittendorfer, 2004). Usually, the energy and beam current are kept fixed, varying the conveyors' speed to get the intended dose.

The penetration of electrons in food is limited to 5 cm or less, requiring sometimes the use of double-side irradiation: rotating the box samples; or using a double beam, one downwards and other upwards operating simultaneously, to guarantee a uniform dose inside the product.

Using the following equation for energies above 1 MeV (Sarma, 2004a):

$$x = (0.524\ E - 0.1337) / \rho \qquad \text{(eq. 6)}$$

where x is the penetration depth, in cm; E is the energy, in MeV; ρ is the density, in g cm^{-3}.

For chestnut fruits with a density of about 1.2 g cm^{-3}, we get a value of 4.3 cm for the maximum range of penetration, for a 10 MeV e-beam. The typical maximum thickness for chestnut fruits is about 2.5 cm (Antonio *et al.*, 2013a).

For chestnuts, using the typical values for fruits density, ρ = 1.2 g cm^{-3}, and for the depth of penetration the maximum thickness, x = 2.5 cm (Antonio *et al.*, 2013a), we get a surface density of z = 3 g cm^{-2}, which allows the configuration of one-side e-beam irradiation for this type of product, that is limited to 4.4 g cm^{-2} for a 10 MeV e-beam irradiatior (Miller *et al.*, 2003).

The needed beam power is related to the throughput of the machine by the equation (Miller, 2005)

$$P = D \times (M/t) / F \qquad \text{(eq. 7)}$$

where *P* is the delivered power of the e-beam, in kW; *M/t* the mass throughput, in kg s^{-1}; *D* is the absorbed dose, in kGy; and F is the efficiency of beam energy transfer.

The value for the efficiency, F, is a contribution of several factors:

$$F = F(i) \times F(j) \times F(k) \qquad \text{(eq. 8)}$$

The factor F(i) results from the non-uniform depth-dose distribution; F(j) is the over scanning to cover the edges (0.8-0.9), F(k) is the efficiency of the distribution on the conveyor (0.6-0.8), giving for F an approximate value of 0.45, or 45% (Miller, 2005).

It should be also take in account the ratio of input and output electrical energy in electron beam accelerators, which is in the range of 25% to 75% of efficiency (Berejka, 1995). For an RF accelerator the electrical efficiency is considered to be no greater than 25% (Miller, 2005).

The economic feasibility of an irradiation process depends of the throughput, processed quantity per unit time, and the impact on the cost of the product, per cubic meter or per kilogram, after being irradiated.

To determine the area throughput is used the relation

$$A/t = (M/t) \times (1/z) \qquad \text{(eq. 9)}$$

where the area throughput, A/t, is expressed in $m^2\ s^{-1}$; M/t is the mass throughput, in kg s^{-1}; and z is the areal density, in kg m^{-2}.

And the velocity for the conveyor is estimated using the relation (Miller, 2005)

$$v = (A/t) \times (1/w) \qquad \text{(eq. 10)}$$

where *v* is the velocity, in $m\ s^{-1}$ and *w* is the width of the scan, in *m*.

In grains, the irradiation setup is usually a horizontal beam in front of which the grains fall by gravity (Zakladnoi *et al.*, 1982; EVONTA, 2014). To irradiate chestnut fruits, the recommended geometry is a vertical beam and the fruits transported on a conveyor (Fig. 4). With this setup it is easier to control the velocity, guarantee the presence of only one layer of fruits and, more important, is the versatility to allow the irradiation of other fruits or materials.

1.3. Gamma *versus* electron-beam

There are several companies around the world that offer different designs for gamma and e-beam plants for food irradiation, adjusted to the needs of the product and to the requests of the final user (Berejka, 2009).

The option for a gamma or e-beam irradiator should take in account several factors: the type and dimensions of processed product; user time; maintenance costs (electrical, vacuum and cooling spare parts or ^{60}Co sources price); and electricity cost.

Comparing the two technologies for food preservation, gamma radiation has low dose rates, but high penetration, allowing the irradiation of bigger volumes. E-beam has low penetration depth, but high dose rates (dose per hour). However, in spite of significant differences in the dose rate, the throughput or processed mass per unit time could be similar for both technologies, since gamma irradiators could process bigger volumes.

In gamma plants the decay of ^{60}Co is continuous, recommending the operation all 24 h. In e-beam plants, the beam can be switched on or off when it is necessary.

The choice for which type of radiation to use depends on several parameters, namely: dose; throughput; and physical characteristics of the product to be processed.

The cost of both units for industrial use starts in 1 million Euros and could reach the value of 10 million Euros (Balaji, 2013; Cokragan, 2013; Dethier, 2013; Stein,

2013). However, if the units are operated all the year the impact on the final price of the product could be acceptable and in the order of 2-10 cents of Euro per kilogram (Morrison, 1989).

These units are sometimes dedicated also to sterilize other materials, *e.g.* clinical devices or pharmaceutical products, which could lower the impact of the installation costs in the final price of the food product.

Some authors refer also that e-beam operation is critically in shortages of electricity (Morrissey, 2002). In fact, all irradiation plants are electricity dependent, for ventilation of the irradiation area due to the production of ozone, for sources operation and for product handling.

In the European Union, the use of these technologies to process food is limited to a few countries (E.U., 2011a), mainly due to the low acceptability by the consumers for this type of processing, as will be discussed later.

In E.U. there are 16 gamma and 6 e-beam plants in 12 countries, authorized for food irradiation processing of different food products (E.U., 2011a), and a relatively recent list of 10 irradiation units in 5 non-E.U. countries, authorized to export to European market, with successive amendments, only to update the names of the new owners (E.U., 2002).

In the world, there are more than one hundred gamma and e-beam irradiation units all over the world, in about 40 countries processing different types of food (IAEA, 2013). And there are also even more irradiation units dedicated to industrial applications, such as medical devices and pharmaceutical products sterilization; materials modification; waste water and flue gas treatment (IAEA, 2004a).

Consumers' perception or acceptance of the processing technology is also taken in account in the final decision. E-beam and x-rays machines are becoming more popular, since they can easily be turned on and off, compared to the permanent emission of ^{60}Co sources, and due to the wrong association of irradiated food with gamma radiation emitted by radioisotopes and radioactive contamination (Miller *et al.*, 2003).

Regarding the feasibility of an e-beam plant for food irradiation, it is considered an intensive capital investment, mainly coming from the cost of accelerator, radiation shielding and material handling equipment (Miller, 2005). If the accelerator is integrated in the agro-industry unit other costs could be shared, *e.g.* the handling system and the building facility. And the possibility of constructing a local shield to accommodate a mobile e-beam accelerator that can be transported to other industrial

units is an issue that can also increase the depreciating rate and lower the cost of the investment (Catana *et al.*, 1995; Iacoboni *et al.*, 1998; Batskikh *et al.*, 1999).

Considering the particular case presented in this study, the Portuguese annual exportation of chestnuts is about 10 000 ton (INE, 2012) and these fruits are a seasonal product, where the throughput is not a critical parameter, since the demand from foreign markets occurs along a few months. However, an e-beam plant working only one month and in one agro-industry unit may not be economically viable, considering the increase in fruits price by the irradiation process (Appendix 2).

2. Food processing by irradiation

Food radiation processing is used almost since the presence of humans in earth, using the solar radiation to dry and preserve fruits, mushrooms, herbs or spices as a clean and environment friendly process (Khandal, 2010; Antonio *et al.*, 2012a; Fernandes *et al.*, 2012a). Non-visible and more energetic radiation, like x-rays radiation, started its application after discover of this type of radiation, to process food, first for scientific purposes and later in an industrial scale.

The irradiation technology process is based on the physics and chemistry of radiation interactions with matter (Chmielewski *et al.*, 2006). In the interaction of ionizing radiation with matter the beam looses intensity (Fig. 4), transferring its energy to the product, atoms or molecules, generating secondary charged particles.

The attenuation of radiation intensity is given by

$$I(x) = I_0 \exp(-\mu x) \qquad \text{(eq. 11)}$$

where I_0 is the intensity of the incident beam; x the absorber thickness (m); μ the absorber coefficient (m^{-1}) and I is the beam intensity after traversing the absorber material.

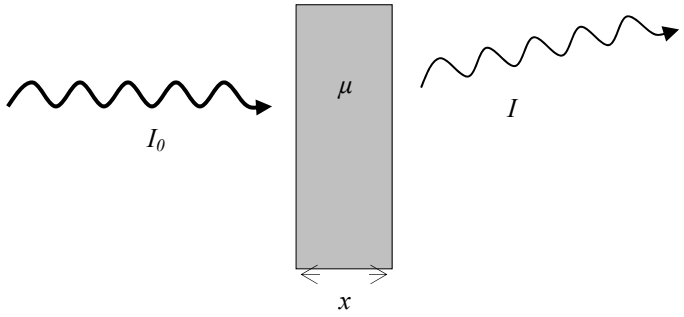


Fig. 4. Radiation attenuation.

The total linear attenuation coefficient is a contribution of different processes of radiation attenuation, coherent (Rayleigh) and incoherent (Compton) scattering, photoelectric effect, positron-electron pair production (McLaughlin *et al.*, 1989):

$$\mu = \mu_{Ry} + \mu_{Ph} + \mu_C + \mu_{PP} \qquad \text{(eq. 12)}$$

(Ry - Rayleigh scattering; Ph - Photoelectric effect; C - Compton scattering; PP - Pair Production).

Assuming Bragg additivity for the fractional composition of the compound (AAPM, 1986), the total absorption coefficient for a mixture is given by:

$$\frac{\mu}{\rho} = w_1 \frac{\mu_1}{\rho_1} + w_2 \frac{\mu_2}{\rho_2} + \cdots \qquad \text{(eq. 13)}$$

where w_i is the weight fraction of each compound, μ/ρ is the mass attenuation coefficient ($m^2\ kg^{-1}$) and ρ is the density ($kg\ m^{-3}$).

2.1. Dosimetry

The interaction of photons with matter generates secondary particles, ionized molecules and electrons, mainly by Compton scattering (Singru, 1972; McLaughlin *et al.*, 1989; IAEA, 2009). The total kinetic energy of the charged particles per unit mass is defined as *Kerma – kinetic energy released to matter* ($K = dE/dm$).

The charged particles generated by the radiation interact with the material by ionization or excitation of molecules. The total absorbed energy per mass is defined as dose, D, and is given by (McLaughlin *et al.*, 1989):

$$D = \int_0^{E\max} \Psi(E) \frac{\mu_{en}}{\rho} dE \qquad \text{(eq. 14)}$$

where $\psi(E)$ is the photon energy fluence ($J\ m^{-2}$) and μ_{en}/ρ is the mass energy-absorption coefficient ($m^2\ kg^{-1}$).

In practical applications it is assumed that the dosimeter will not affect photon fluence, otherwise should be used a correcting factor that is, however, close to 1 (f = 0.98 – 0.99) and the value for D could be estimated only with approximation, since the energy spectra is not always well known (McLaughlin *et al.*, 1989).

The total absorbed dose is expressed in Gray (Gy), absorbed energy (J) per mass (kg):

$$D = \frac{E}{m} \qquad \text{(eq. 15)}$$

In charge equilibrium the dose, D, and *Kerma*, K, have similar values (McLaughlin *et al.*, 1989). Charge equilibrium occurs when the "*total energy deposited in a region, R, by charged particles that enter from outside equals the total energy deposited outside the same region by charged particles liberated within R"* (IAEA, 2009).

The term "dose" was taken from medical applications where the irradiation was used for treatment or diagnosis (IAEA, 2011) and some food authorities, FDA - Foods and Drug Administration in USA, still keep the classification of irradiation as a "*food additive*" (web, 2013).

In food irradiation there are other important processing parameters associated with the dose: the minimum dose, D_{min}, is the value to guarantee the desired effect; the

maximum dose, D_{max}, is the value above which the food may not preserve its characteristics or the limit imposed by the legislation; and the dose uniformity ratio, DUR, is the ratio D_{min}/D_{max}.

The E.U. legislation, for example, limits to 10 *kGy* the value for the average maximum dose and the DUR factor to 3 (E.U., 1999a). The *Codex Alimentarius* in the General Standard for Irradiated Foods (Codex, 2003) refers not the average dose but only the minimum dose to "*achieve the technological purpose*" and the maximum dose "*that compromise product quality or safety*".

There is another important parameter for radiation interaction with materials, mainly in biological products the dose rate, dose per unit time. The effects on living matter or organic material depend not only on the dose but also on the dose rate. The time to kill a microorganism or the effect on a chemical reaction is dose-rate dependent (Cabo Verde *et al.*, 2010).

Since the dose rates used in industrial processing are quite high, sometimes it is not taken in account this parameter and the product comes out only with the register of the dose imparted to the product. However, this value must be part of the quality control irradiation registration, mainly on scientific research where the results should be reproducible in other experiments or facilities.

In food irradiation preservation, different dose ranges have different technological applications: for sprout inhibition (0.05-0.15 kGy); insect's disinfestation (0.15-0.5 kGy); delay of physiological processes (0.25-1.0 kGy); elimination of microorganisms (1-10 kGy); or food sterilization (10-50 kGy) (ICGFI, 1999).

In the world about 400 000 ton of food is processed by irradiation, with almost half, 186 000 ton, was to eliminate insects (Kume *et al.*, 2009). In E.U., according the last report, the total food processed by irradiation was about 8 000 ton, mainly meat products, from which about 1 200 ton were for decontamination of herbs and spices (E.U., 2011b).

2.2. Dosimetric systems

Radiation processing is dependent of a good dosimetric system. The dosimeters are a practical tool to measure the dose, the energy per mass deposited by a radiation source on a particular material, liquid, solid or gaseous, where the dose is expressed in J kg^{-1} or Gray (Gy).

The different dosimetric systems are grouped in four types: primary standard, reference standard, transfer standard and routine dosimeters (IAEA, 2002). The primary standard are maintained or regularly calibrated by a national laboratory, the reference standard dosimeters are systems with a well known response to the radiation and used in the irradiation facility to calibrate routine dosimeters, the transfer standard systems are dosimeters that allow the inter-comparison or transferring the dose form a national or international accredit laboratory to the irradiation facility. Routine dosimeters are used for continuous quality control of the irradiation process, and must be regularly calibrated against a standard or reference.

The International Atomic Energy Agency, IAEA, established a high dose dosimetry programme in 1977 (Mehta, 1998), and since then several standards were approved for industrial use of radiation processing, namely for food processing (Farrar IV, 1999).

In this work were used three types of dosimeters for food irradiation control and characterization of the irradiation facility: Ionization Chamber (primary standard), Fricke liquid solution (reference standard); and Polymethylmethacrylate (routine dosimeter), that are described in detail in Appendix 3.

The criteria to choose a dosimeter should take in account the temperature dependence, product equivalence, precision, ease to read, availability, robustness and price (McLaughlin *et al.*, 1989). Ionization Chamber and Fricke dosimeter are considered standards for absorbed dose in water (AAPM, 1986). Routine polymethylmethacrylate dosimeters main advantage is its robustness and easiness to read.

An irradiation process is preceded by the characterization of the dose rate inside the chamber. This could be done using several dosimetric systems that measures the interaction of radiation with a material (gas, liquid or solid), from which it is possible to convert the change in the value of current (Ionization Chamber detector) or colour (liquid or solid dosimeters) in dose.

In the Ionization Chamber detector is measured the current generated by the radiation ionization in a small gas volume inside the chamber, that is connected to an electrometer and is directly proportional to the dose imparted to the product.

The most popular liquid dosimeter is the Fricke solution, an aqueous ammonium ferrous sulphate solution relatively easy to prepare (ASTM, 1992). Its optical absorption, measured in UV region, changes with radiation due to the conversion of

Ferrous ions (Fe^{2+}) into Ferric ions (Fe^{3+}). This change is proportional to the irradiation dose and equivalent to the absorbed dose in water, since the dosimeter is mainly water, which is also the majority content in human tissues and food, and from that comes also its popularity.

The dose is estimated by measuring the specific absorbance at about 303 nm, for different exposure times and from a graph, the slope gives the dose rate for that position (Appendix 3).

The solid dosimeters are of different materials, *e.g.* polymethylmethacrylate with an impregnated dye that changes the colour with radiation (ICRU, 2008). The dose is estimated from a previous calibration curve, measuring the specific absorbance at a selected wavelength (ASTM, 1989). In the case of Amber Perspex dosimeters, from Harwell company, U.K. (Fig. 5-B), the specific absorbance should be measured at 603 nm in the range 1-10 kGy and at 651 nm in the range 10-30 kGy.

In Fig. 5-A is presented the dose rate contour plot in one level of the gamma irradiation chamber. To obtain good dose irradiation uniformity, and to respect the technological and legal limits imposed for D_{max}/D_{min} ratio, the samples are normally rotated during an irradiation process.

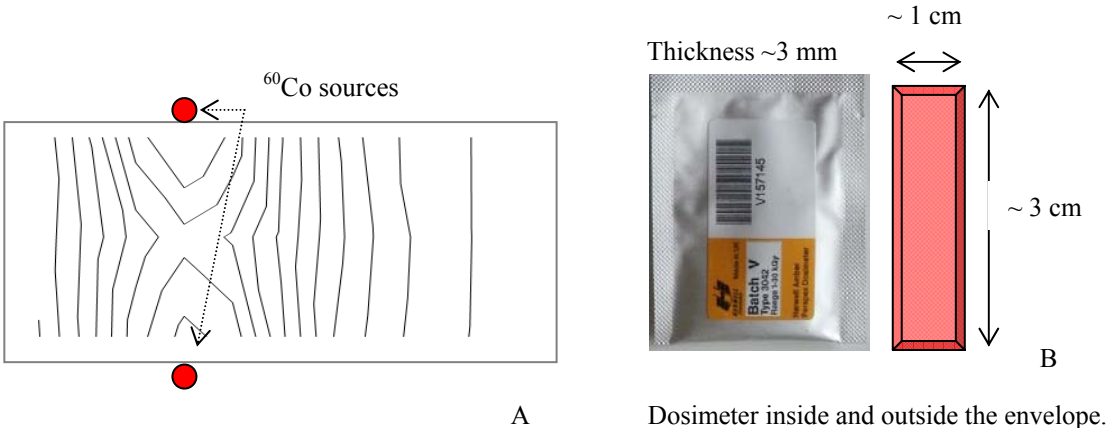


Fig. 5. Dose rate map in a gamma chamber (A); Amber Perspex dosimeters (B).

2.3. Legislation aspects and consumer concerns

The first country to regulate the use of irradiation was the Soviet Union, in 1958, followed by Canada, in 1960, for sprout inhibition, and U.S.A., in 1963, for insects' disinfestation (Nordion, 2013). In 1964, ocurred the first meeting of the Joint Comission of FAO/IAEA/WHO and in 1981 was published the report "*Wholesomeness of Irradiated Food*", after revising scientific data from several decades (WHO, 1981). This

report boosted the appearance of legislation in several countries, starting in 1983 with the *Codex Alimentarius for Food*, revised to integrate food irradiation (Codex, 2003). In 1986, the E.U. started a draft to regulate the use of these technologies, that was published only in 1999, to harmonize different country legislations, with the Directive "*on the approximation of the laws of the Member States concerning foods and food ingredients treated with ionising radiation*" (E.U., 1999a). This regulation is a transposition of the *Codex Alimentarius*, authorizing the type of radiation (gamma, e-beam, x-rays) and limiting the maximum energies for e-beam and x-rays to 10 MeV and 5 MeV, respectively.

In the same year, the Directive 1999/3/EC was issued, authorizing the irradiation of dried herbs spices and vegetables up to 10 kGy in all E.U. countries (E.U., 1999b). The irradiation of other type of foods are part of a list, authorized for each country, containing products such as fruits and vegetables; cereals and rice flour; spices and condiments; fish, shellfish; fresh meats, poultry, frog legs; raw milk camembert; gum arabic, casein/caseinates and egg white (E.U., 2013b).

2.3.1. Labeling

The legislation of several countries imposes a special labeling for irradiated food. According to United Nations organization of *Codex Alimentarius Commission*, the use of a logo "*Radura*" symbol (Fig. 6), was considered optional and a written statement obligatory (Codex, 1999, 2003).

Fig. 6. *Radura* symbol and non-commercial irradiated chestnuts.

Some countries, like USA, Canada or China, included in its legislation the symbol and the written statement as obligatory (web, 2009). In the E.U. legislation only the statement "*irradiated*" or "*treated with ionising radiation*" is required, for labeling irradiated food (E.U., 1979, 1999a).

Following the conclusions and recommendations of World Health Organization report, "*Wholesomeness of irradiated food*" and other successive reports (WHO, 1981, 1994, 1999), the expectable situation would be that countries' legislation moved to a tendency of not using the label "*Radura*", considering that Food Agencies should guarantee the quality of the food product, and not necessarily stating what kind of process was used to assure its safety, in similarity with other preservation processes, since this labeling may induce wrong information and inhibit the consumers.

2.3.2. Consumer's attitude

Even though the effects of these processing technologies on food are deeply scrutinized by the scientific community, food irradiation still remains with low acceptability by the consumers due to non-scientific reasons, namely the wrong association of irradiated food with gamma radiation emitted by radioisotopes or radioactive contamination. Food safety authorities that impose the labeling or a written statement stress the fact that "*Food irradiation has nothing to do with radioactive contamination of food resulting from a spill or an accident*" (E.U., 2013a).

Owing to this, e-beam and x-rays machines are becoming more popular, since they can be switched off when are idle (Miller, 2005). In gamma radiation, the energies emitted by ^{60}Co are about 1 MeV. These values are not enough to disturb the nuclei and to induce radioactivity in the atoms. The idea that radiation is an additive, kept inside the food after irradiation, was initially referred in some legislation, inducing a scientific misconception of a physical process that uses electromagnetic radiation (Nordion, 2013). Some food authorities, as FDA in USA, still keep the misleading classification of irradiation as a "*food additive*" (web, 2013). Another consumer concern, the formation of radiolytic or secondary products that could have health effects, was dismissed by World Health Organization report on "*Wholesomeness of irradiated food*", report of a Joint FAO/IAEA/WHO Expert Committee, and by the report "*Safety and Nutritional Adequacy of Irradiated Food*", which revised the data of more than four hundred of scientific studies (WHO, 1981, 1994). The radicals formed by radiation interaction are of short life, about 10^{-11} seconds, reacting with other components and forming stable entities (EFSA, 2011), and ionizing radiation processing generates fewer amounts of sub-products than other thermal treatments, like cooking, frozen or pasteurization (WHO, 1999).

Regarding the maximum authorized doses for food processing, a discrepancy exists between some countries. Following the recommendations of the report "*Wholesomeness of Food Irradiated with Doses above 10 kGy*", the Joint FAO/IAEA/WHO Scientific Commission considered that it is not technical necessary to impose a limit for the dose: "*... food irradiated to any dose appropriate to achieve the intended technical objective was both safe to consume and nutritionally adequate ...*" (WHO, 1999). Furthermore, the *Codex Alimentarius* transposed this conclusion, validating the use of higher doses "*... when necessary to achieve a technological purpose*." (Codex, 2003), *e.g.* for decontamination of food for immuno-compromised persons (Narvaiz, 2009), to be stored for long time under tropical conditions (Plaček, 2004), or where the sterilization conditions are a requirement, to prepare food for space missions (Song, 2009).

The Scientific Committee on Food, from European Food Safety Authority (EFSA), still keep the dose limit of 10 kGy in the regulations, however recognizes that some products, like spices, dried herbs and vegetables seasonings, may need doses up to 30 kGy for decontamination by irradiation "*...to ensure a product in a satisfactory hygienic condition*." (EFSA, 2011).

The common doses used for food processing are lower than 10 kGy. Food products irradiated at high doses, like vegetables, fruits or even dry fruits, may loose some properties, *e.g.* texture and/or colour, which have impact in the appearance of the product, limiting its acceptance by the consumer (Arvanitoyannis, 2010). Only for particular needs the applied doses are higher than this value: food sterilization, for space missions; food decontamination, for immunocompromised persons; or sterilization of canned food, to destroy all the contaminating bacteria and spores (Dauthy, 1995; WHO, 1999).

In spite of the consensus inside the scientific community about the safety of irradiated food, the non-acceptability for this type of processed food tends to persist and maybe only an education program could change this *status quo*.

3. Chestnut fruits irradiation

Chestnut tree is typical in the south of Europe, in mountain areas of Mediterranean countries, and in Asia, mainly in China. The main region production of chestnuts is Asia (85%) followed by Europe (12%), with a worldwide production of 2 million tons (FAOSTAT, 2012). In Mediterranean EU countries, this fruit represents a market of more than 100 000 ton, contributing Portugal to this quantity with an amount of 20 000 ton, exporting about 9 000 ton, that represents an income of about 20 million Euros from external market (INE, 2012).

Chestnuts are infested by larvae of different species, depending on the region of world, causing rotting and loss of incomes for the producers and for food industry (Kwon *et al.*, 2001; Kwon *et al.*, 2004). Larvae consume the product and, since there is an international market for chestnuts, the international phytosanitary regulations imposes quarantine rules whenever there's a threat of the infestants species to the local ecosystem. Quarantine treatment is an obligation for exported food products that must be post-harvest treated to eliminate insects and worms, to meet the international phytosanitary trade regulations (WTO, 1994; ICGFI, 1998).

Till recently, for post-harvest disinfestation of chestnut fruits it was used a large spectrum chemical fumigant, methyl bromide (MeBr), prohibited in EU since March 2010, due to its toxicity for the operators and for the environment (E.U., 2008). However, this decision left no or few alternatives to the agro-industry that processes and exports this fruit. Other technology in use now to meet the phytosanitary trade regulations for chestnut fruits is the hot water dip treatment, which has low efficiency and some technological problems, *e.g.* the contact of the fruit with water and low throughput, slow heating rate and long processing time, with possible damage to the flesh of some fresh fruits which may compromise fruits quality (Aegerter & Folwell, 2000; Guo *et al.*, 2011).

Post-harvest disinfestation is easily reached with fumigation but with some limitations when irradiation was used, mainly for not causing the immediately death of the larvae and the absence of trained quarantine officials for checking irradiated food (Marcotte, 1998). However, international organizations are being putting some efforts in adopting standards for phytosanitary measures, namely for the use of irradiation to prevent the introduction or spread of pests (APHIS, 1996; ISPM, 2003).

3.1. State of art

Ionizing radiation processing is an alternative to chemical fumigation that is harmful for the environment, for the operators and leaves residues in the products (Kwon *et al.*, 2004; E.U., 2008).

Irradiation appears as a safe quarantine post-harvest treatment for disinfestations, being now validated for different species of insects (IAEA, 2004b; ISPM, 2007; IDIDAS, 2012). The *Codex Alimentarius*, an international standard for good food practices, has a recommendation for the use of irradiation in disinfestations of food and agricultural products (ICGFI, 1998). This post-harvest treatment is also approved by several countries to treat different types of food, to meet the quarantine regulations during exportation (APHIS, 1996; FSANZ, 2002).

In this context, chestnut fruits insects' disinfestation by post-harvest irradiation treatment could be a feasible alternative, if the product meets other food quality parameters after processing. However, food irradiation may not be used for the preservation of all type of food, since it can produce changes such as off-flavors or texture softening in some food products (Arvanitoyannis, 2010). In this way, an irradiation process must be validated, since each type of food have different characteristics, namely size, water content or nutritional composition.

Fig. 7**.** *Castanea sativa* Mill. (varieties "Judia" and "Longal").

Fig. 8. Chestnuts damaged by worms (A) and fungi (B); and irradiated insects (C).

On European varieties of chestnut fruits (*Castanea sativa* Mill.), there's only a previous study regarding the validation of irradiation detection standards in chestnut fruits (Mangiacotti *et al.*, 2009) and nothing has been reported about the influence of ionizing radiations as a post-harvest process preservation. Previous studies on chestnut fruits irradiation effects were done mainly in Asian varieties, in *Castanea crenata* and *Castanea molissima*.

Iwata *et al.* (1959) conducted a study to determine the effect of gamma radiation on sprouting, rotting and respiration rate of *Castanea crenata* and *Castanea molissima*, where the irradiated chestnuts showed always less sprouting and rotting. Concerning respiration rate of *Castanea molissima* submitted to irradiation doses ranging from 0.10 to 0.20 kGy, showed no statistical differences in carbon dioxide release.

Guo-xin *et al.* (1980) also conducted inhibition of sprouting assays with gamma radiation on *Castanea molissima* using doses of 0.3 to 1.2 kGy, and reported no sprouting in all the irradiated samples for storage times of 86 and 108 days. Recently, Kwon *et al.* (2004) carried out a comparative assay on rotting between gamma irradiated and fumigated (methyl bromide) chestnuts (*Castanea crenata*). They reported that after 6 months of storage only the dose of 0.25 kGy had lower rotting levels when compared to the control (no treatment) and that higher doses of radiation revealed higher rotting levels when compared to the control, but all doses revealed lower rotting levels then the samples fumigated with methyl bromide.

Kwon *et al.* (2004) also compared the effects of methyl bromide and gamma irradiation on insect pests in *Castanea crenata* and determined that 100% of the pests perished in the fumigated samples and also in irradiated samples, with a dose of at least 0.5 kGy. Imamura *et al.* (2004) studied the effects of this radiation for *Castanea crenata* on the mortality of *Cydia kurkoi* (Amsel) a larvae, and reported that doses of 0.3 kGy and higher displayed a mortality rate of 100% for *C. kurokoi*.

Kwon *et al.* (2004) studied the comparative colour alteration in the internal and external flesh of chestnuts irradiated and fumigated (methyl bromide). They reported that colour parameter "*L-value*" only changed significantly at 10 kGy, but this alteration also took place for the fumigated chestnuts.

3.2. Motivation

The use of ionizing radiation is regulated and authorized by international organizations (EU – European Union, EFSA – European Food Safety Agency, IAEA – International Atomic Energy Agency, FAO – Food and Agriculture Organization, WHO – World Health Organization) for industrial radiation processing of several products: medical devices sterilization, materials modification, cultural heritage preservation and food decontamination.

The possibility of using ionizing radiation to treat foodstuff was referred in the literature one year later, in 1896, after discover of x-rays by W. C. Röntgen (Molins, 2001). Internationally, there is a code of good practices, General Standard for Irradiated Foods, to process food products with ionizing radiation (Codex, 2003). In Europe, food irradiation is used in different countries for several food products and is regulated by the Directive 1999/2/EC (E.U., 1999a). The referred codes or legislation make recommendations concerning the type of radiation authorized (gamma, x-rays, e-beam), energies (5 and 10 MeV for x-rays and e-beam, respectively) and recommended doses (in kilogray, Joule per kilogram).

Chestnut fruits are a popular nut in several countries, with a worldwide production of 2 million tons (FAOSTAT, 2011). In Mediterranean EU countries, this fruit represents a market of more than 100 000 ton, being Portugal the third producer with an amount of 20 000 ton, exporting about 25% of the production, representing an income of 15 million Euros (INE, 2012). Quarantine post-harvest treatment is an obligation for exported food products that must be post-harvest treated to eliminate insects and worms, to meet the international phytosanitary trade regulations (WTO, 1994; APHIS, 1996; ICGFI, 1998). Till recently, for post-harvest disinfestation of chestnut fruits was used a large spectrum chemical fumigant, methyl bromide (MeBr), prohibited in EU since March 2010, due to its toxicity for the operators and for the environment (E.U., 2008), following the recommendations of a scientific committee from United Nations Environmental Program, the Bromide Technical Options Committee (UNEP, 1995). However, this decision left no or few alternatives to the agro-industry that processes and exports this fruit. Other technology in use now to meet the phytosanitary trade regulations is the hot water dip treatment, which has low efficiency and some technological problems, e.g. the contact of the fruit with water and low throughput. In this context, chestnut fruits preservation by irradiation could come as

a feasible alternative if the product meets other food quality parameters after post-harvest treatment (WHO, 1981, 1994, 1999).

However, an irradiation treatment is a process that must be carefully studied for each particular material, since the results vary significantly with its atomic composition, density, radiation dose, geometry, among other factors (IAEA, 2002; Miller, 2005) Previous tests on irradiation of chestnut fruits were done only in Asian varieties (*Castanea molissima* and *Castanea crenata*) mainly for insects disinfestations validation (Imamura *et al.*, 2004; Todoriki *et al.*, 2006).

3.3. Objectives

In irradiation of chestnuts fruits little research has been done, and particularly on European varieties nothing has been reported about the effect of ionizing radiations on physical and chemical parameters of *Castanea sativa* fruits.

And based on the previous signed problems for preservation of this fruit, several steps were implemented to study the impact of ionizing radiations chestnut fruits of European varieties (*Castanea sativa* Mill.), seeking the validation of a process with interest for the agro-industry.

It was intended to get an insight in two different irradiation technologies, gamma and e-beam, as part of the research, and to characterize the effects of ionizing radiation in physical and chemical parameters of chestnut fruit samples.

The main objectives were: to characterize the effect of ionizing radiation in chestnut fruit samples of European varieties; to compare two available technologies; and, finally, propose an innovative treatment process that could replace the obsolete traditional and prohibited fumigation with methyl bromide.

For that, several activities were planned: selection and characterization of the type of samples to be handled by the proposed technology, taking into account the variety, quantity and general characteristics (size, bulk and volumetric density) of the fruits; dose validation using three independent dosimeters, to estimate the minimum dose (D_{min}); maximum dose (D_{max}); dose rate (DR); and dose uniformity ratio (DUR) in the irradiation chamber and for the irradiated product; evaluation of the effects of irradiation on physical and chemical parameters; study of the effects along storage; application of statistical tools to compare: varieties of chestnut fruits; irradiation doses; types of irradiation (gamma and electron-beam); and shelf-life times.

4. Gamma and electron-beam irradiation effects on chestnuts

Food processing technologies, gamma or electron-beam, must allow the integral maintenance of food properties to fulfill quality requirements. The effect of irradiation on food quality parameters of European varieties was validated first for gamma radiation (Antonio *et al.*, 2011a; Fernandes *et al.*, 2011a; Fernandes *et al.*, 2011b; Barreira *et al.*, 2012; Antonio *et al.*, 2012a), that were object of a review comparing the results with other authors for other varieties of chestnut fruits (Antonio *et al.*, 2012a), and more recently for electron beam radiation (Carocho *et al.*, 2012a; Carocho *et al.*, 2012b; Barreira *et al.*, 2013; Carocho *et al.*, 2013a; Carocho *et al.*, 2013b).

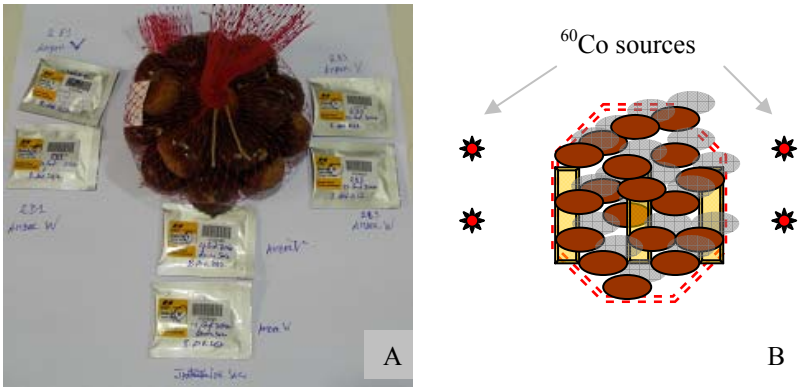


Fig. 9. Chestnuts with dosimeters (A) and relative position to ^{60}Co sources (B).

4.1. Effects on colour, texture and drying

The effects of gamma and e-beam irradiation on physical parameters, referred briefly here, were object of publication as paper proceedings and in journals not indexed to ISI Web of Knowledge. And, as so, not included in this monograph but cited in this section and in the list of references, at the end of this introduction.

Physical parameters are the first factors evaluated by the consumers, to decide to buy or not some food products. Colour, texture, physical dimensions and drying (Fig. 10) were monitored after irradiation and along storage time.

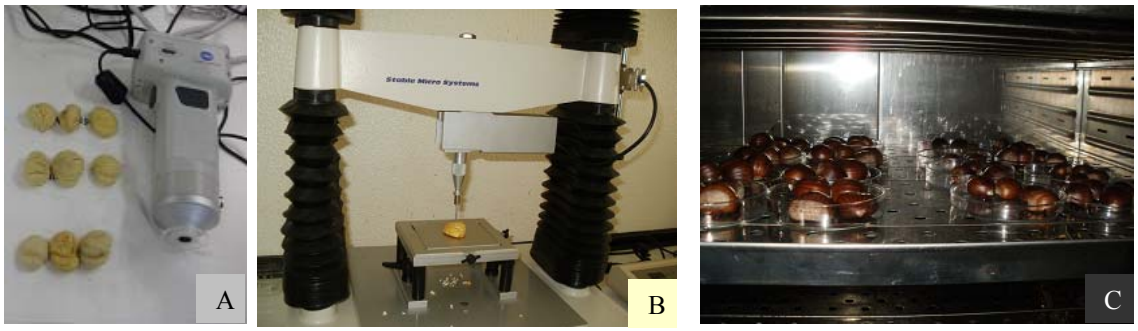


Fig. 10. Physical parameters control: colorimeter (A), texturometer (B), oven (C).

Fruits of European varieties of *Castanea sativa* and from the main country producers (Portugal, Turkey, Italy) were processed with gamma and e-beam radiation and submitted to several irradiation doses (Antonio *et al.*, 2011b; Antonio *et al.*, 2011c; Antonio *et al.*, 2012b; Antonio *et al.*, 2013a; Antonio *et al.*, 2013b).

In a first study, an industrial sample separated by size and of mixed varieties was gamma irradiated at several doses, up to 6 kGy, and stored till 30 days, the estimated commercial time between industrial processing and commercialization for fresh fruits.

For colour it were not observed significant changes for Portuguese and Turkish varieties on skins, peeled fruits and fruits interior (half-cutted), with irradiation dose and storage time, (Antonio *et al.*, 2011b; Antonio *et al.*, 2011c). Similar results were obtained with e-beam irradiation for varieties from Portugal and Italy, monitored during a long storage period, 60 days (Antonio *et al.*, 2013b).

Regarding texture, a significant softening tendency was observed only for doses higher than 3 kGy, Fig. 11 (Antonio 2013a).

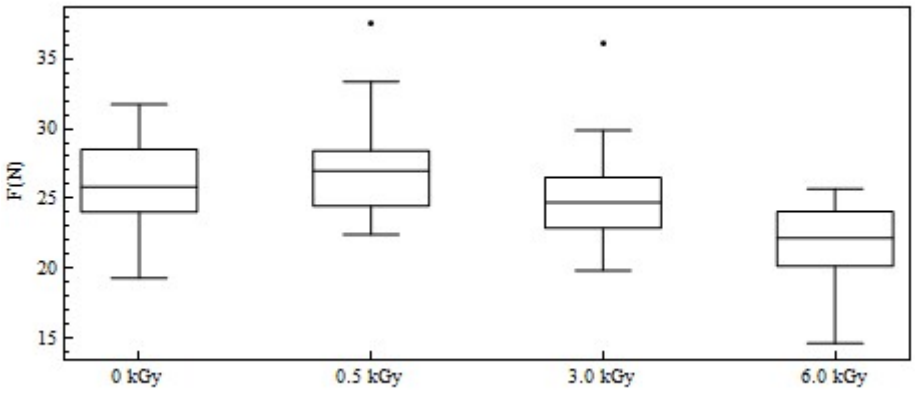


Fig. 11. Chestnuts texture variation with gamma irradiation.

This effect is also reported by other authors for irradiated foods, and explained as a radiation break of microstructure (Yu & Wang, 2007) or of tissue softening due to cell walls break (Kovács & Keresztes, 2002; Nayak *et al.*, 2007).

Another parameter that is important for the quality of fresh fruits is water loss after harvest and during storage. Based on chestnut fruit characteristics and Computed Tomography images, a compartment model was used to characterize the drying kinetics of gamma irradiated chestnut fruits, concluding that the drying curves for irradiated samples were similar to the non-irradiated chestnuts, up to 6 kGy (Antonio *et al.*, 2012b).

4.2. Effects on bioactives and nutrients

The radiation interaction with atoms may occur by three processes, schematically followed represented.

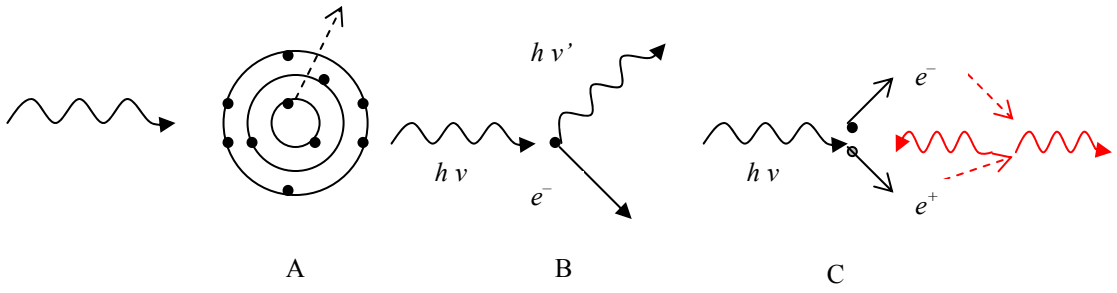


Fig. 12. Photoelectric effect (A); Compton scattering (B); Pair-production (C).

In the photoelectric effect (Fig. 12-A) an electron is ejected; in Compton scattering (Fig. 12-B) the photon transfers part of its energy to an electron; in Pair-production (Fig. 12-C) a positron and electron are generated giving two photons.

For the energies used in food irradiation Compton scattering is the dominant effect (Stewart, 2001). The passage of radiation of high-energy in food may ionize

$$M \sim\sim\sim\sim> M^{+} + e^{-}$$

and/or excite the molecules:

$$M \sim\sim\sim\sim> M^{*}$$

The analyses of gamma and e-beam irradiation effects on chestnut fruits was extended to several major and minor nutrients. Latter, the work was mainly focused in the components of each nutritional group: sugars (sucrose), fatty acids (palmitic, oleic, linoleic and linolenic acids), tocopherols (γ-tocopherol), on energetic contribution and proximate analysis (dry matter, proteins, fat, carbohydrates and ash) of chestnuts stored at 4 ^{o}C for different periods, in order to understand the possible interactions among these two main factors: the irradiation and the storage time.

In fresh nuts water represents about 50% and carbohydrates approximately 46%. In dry matter, sucrose is the main sugar, and low quantities of glucose and fructose. The oligosaccharides trehalose and raffinose were also detected. Palmitic, oleic, linoleic and linolenic acids were the most abundant fatty acids, while γ-tocopherol is the main tocopherols isoform, remotely followed by δ-tocopherol and α-tocopherol.

The total carbohydrates are calculated by difference:

$$m_c = 100 - (m_a + m_m + m_p + m_f)$$

where the mass of carbohydrates, m_c, is obtained knowing the mass of ash (m_a), moisture (m_m), proteins (m_p) and fat (m_f).

The total energy, E, was calculated with the following equation:

$$E = 4 \times (E_c + E_p) + 9\ E_f$$

Following the references, it was considered for the energy of 1 g of carbohydrates, E_c, 4 calories; for 1 g of proteins, E_p, 4 calories; and for 1 g of fat, E_f, 9 calories (Greenfield & Southgate, 2003). In the results, the total energy is expressed in traditional units, in kcal.

Tab. 1. Average composition of chestnuts (*Castanea sativa* Mill.).

Proximate composition (in g/100 g of dry weight)					
Dry matter	Fat	Proteins	Ash	Carbohydrates	Sucrose
50	2	4	2	92	20
Main fatty acids (in %)					
C16:0 - palmitic	C18:1 - oleic		C18:2 - linoleic		C18:3 - linolenic
15	25		50		8
Organic acids (mg/100 g dry matter)					
Oxalic	Quinic	Malic	Ascorbic	Citric	Fumaric
0.7	13	5	1.2	12	0.4
Tocopherols (μg/100 g de materia seca)					
α-tocopherol		γ-tocopherol		δ-tocopherol	
6		1000		40	
Total Phenols (mg GAE/g extract)			**Total Flavonoids** (mg CE/g extract)		
4			2		

Average values for non-irradiated Portuguese chestnut fruit varieties.

The average energetic value for chestnuts is about 400 kcal/100 g of dry weight (Fernandes *et al.*, 2011b). The recommended minimum daily energy intake is approximately 2 000 kcal or, in S.I. units, about 8 000 kJ (EFSA, 2013).

For the total energy in irradiated and stored chestnuts, when it was possible to separately classify the influence of one of the factors, statistical detectable differences were observed only with storage time (Barreira *et al.*, 2012; Carocho *et al.*, 2012b).

4.2.1. Water/Moisture

The main component in food is water, followed by carbohydrates, proteins and lipids; minor components include vitamins and minerals (Greenfield & Southgate, 2003).

In the case of water radiolysis, that is present in all types of food, occurs the formation of a cation:

$$H_2O \sim\sim\sim\sim> H_2O^+ + e^-$$

that dissociates in high reactive radicals:

$$H^* + OH^*$$

And they will react with other food constituents or recombine to generate the stable species H_2 and H_2O_2 or H_2O (Miller, 2005).

The yield of these processes, *G-value*, is expressed as the number of entities per 100 eV or, in S.I. units, in mol J^{-1}.

The *G-value*, in traditional units, for the high reactive species e^-, OH^*, H^* are 2.7, 2.7, 0.6, respectively, indicating its relative importance as precursors of other reactive processes (WHO, 1999). The hydrated electron, e^-, is a strong reducing agent and the hydroxyl radical, OH^*, is a powerful oxidizing agent, responsible for causing, respectively, reduction and oxidation reactions in foods.

Moisture content in food is determined by difference, weighing the samples during drying till constant weight.

Carbohydrates are a major source of energy and include sugars, starches and related compounds. These molecules are a chain of carbon, hydrogen and oxygen atoms. A major consequence of irradiation is the breaking of carbon-hydrogen bonds (C-H) and the disruption of ether linkages (– O –) (WHO, 1999). However, radiation degradation of carbohydrates is considered a complex mechanism in the presence of other food constituents, since they may exert a protective effect during irradiation (Stewart, 2001).

4.2.2. Sugars

Irradiation, in particular, is known for causing several changes in sugars, such as melting point decreases, reduction in optical rotation and browning.

Sugars are good conservation quality indicators (Kazantzis *et al.*, 2003), and in chestnuts irradiation several reports indicate the absence of marked effects in their

composition with either gamma (Iwata & Ogata, 1959; Guo-xin *et al.*, 1980; Fernandes *et al.*, 2011a; Fernandes *et al.*, 2011b) or electron beam (Carocho *et al.*, 2012b; Carocho *et al.*, 2013b). In fact, the main differences in sugars profiles resulted from storage time effect, in line with the observed for other nutritional parameters.

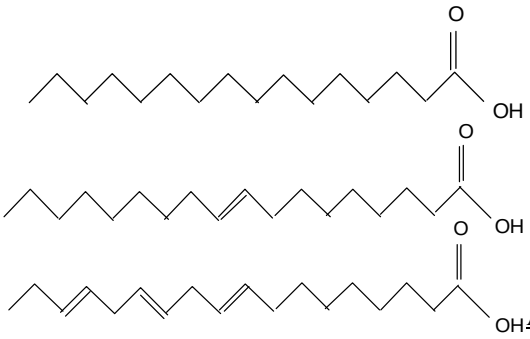
4.2.3. Lipids

Lipids are fats composed of carbon, hydrogen and oxygen. In chestnuts fat represents less than 2%.

The fatty acids are classified in saturated (SFA), with no double bonds, monounsaturated (MUFA), with one double bond, and polyunsaturated (PUFA), with more than one double bond. Unsaturated acids are more unstable than saturated fatty acids.

Regarding chestnut fruits, fatty acids profiles differences were mostly due to the effect of storage time, while irradiation treatment caused only slight alterations, either when using gamma (Fernandes *et al.*, 2011a; Fernandes *et al.*, 2011b; Barreira *et al.*, 2012; Carocho *et al.*, 2013b), as well as electron beam (Carocho *et al.*, 2012b; Carocho *et al.*, 2013a) irradiation.

Main fatty acids detected in chestnuts (Barreira *et al.*, 2012):



Palmitic acid (C16:0, saturated)
$C_{16}H_{32}O_2$ $CH_3(CH_2)_{14}COOH$

Oleic acid (C18:1, monounsaturated)
$C_{18}H_{34}O_2$ $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$

Alpha-linolenic acid (C18:3, polyunsaturated)
$C_{18}H_{30}O_2$ $CH_3(CH_2)_1CH=CH\ (CH_2)_1CH=CH(CH_2)_1CH=CH(CH_2)_7COOH$

Legend: Structure; Name (abbreviated, type); Molecular formula, Molecular structure.
Abreviated formula: Cn:m, n – number of carbons; m – number of double bonds.
Type: Saturated fatty acids, no double bonds; Unsaturated fatty acids, double bonds.

Fig. 13. Main fatty acids in chestnut fruits.

The general mechanism of lipids radiolysis involves cleavages at positions near the carbonyl group (Fig. 14) but can also occur at other locations (Stewart, 2001).

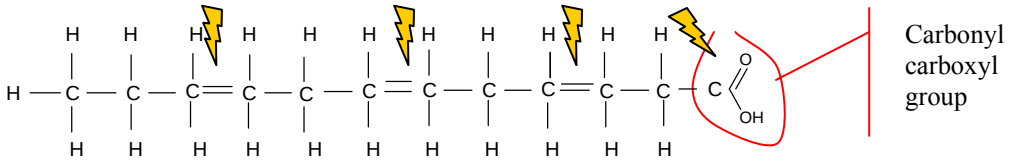


Fig. 14. Unsaturated fatty acid structure and preferential cleavage positions.

The radiolysis of natural fats is, however, more complex than presented by the models, due to the presence of a large number of fatty acids and its wide distribution (Stewart, 2001).

4.2.4. Triacylglycerols

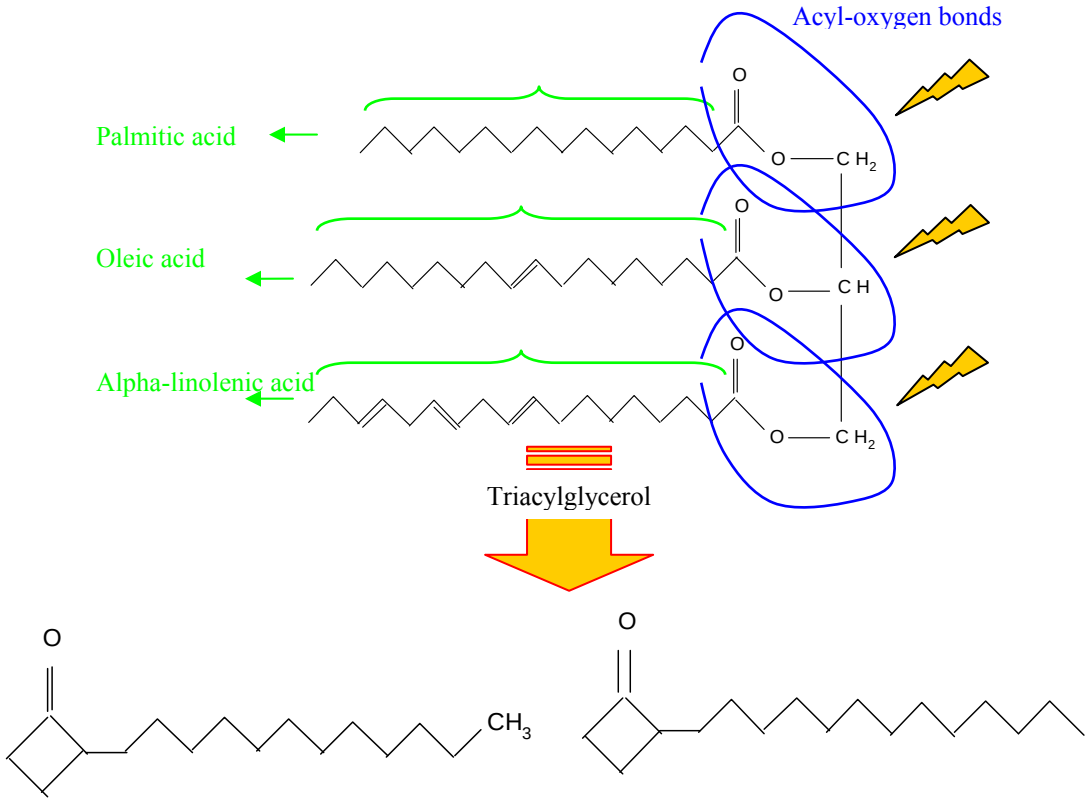
In the European standards for detecting irradiated food standard, namely in the standard EN1785: 2003 are defined methods for detection of irradiated food containing fat, based on the byproducts of triacylglycerol (TAG), the dodecylcyclobutanone (DCB) and tetradecylcyclobutanone (TCB), that are used for verification by the food authorities for detection of irradiated foods, to be labeled in accord with regulations.

The standards for the detection of irradiated food containing fat consider that during irradiation, the acyl-oxygen bond in triglycerides, or triacylglycerol, is cleaved (Fig. 15) and this reaction could result in the formation of 2-alkylcyclobutanones, containing the same number of carbon atoms as the parent fatty acid with the alkyl group located in ring position 2 (EN1785:2003).

The use of methods for detection of irradiated food products are a legal requirement and some countries, including E.U. countries, require proper labeling of irradiated foods (E.U., 1999a). To meet this requirement, some standards are used to detect whether a product was irradiated or not, based on biological, physical or chemical alterations on processed product. Presently, there are ten European standards (CEN, 2012), which have been included in the General Standard for Irradiated Foods of Codex Alimentarius (Codex, 2003). Depending on the type of food and analysis, one or more detection methods can be used, grouped into physical, chemical, biological and DNA methods (Stewart, 2001).

From the available methods for food irradiation detection, have been tested in chestnuts the DNA ("DNA Comet Assay"); ESR ("Electron Spin ressonance"); PSL ("Photostimulated Luminescence"); and TL ("Thermoluminescence") methods, by different authors, using in the experiments chestnuts subjected to gamma irradiation (Antonio *et al.*, 2012a). Of these, only the PSL method (Chung *et al.*, 2004), and the TL

method (Mangiacotti *et al.*, 2009), tested in chestnuts of Asian and European origin, respectively, have been fully validated.



DCB, 2-dodecylcyclobutanone ($C_{18}H_{34}O$) TCB, 2-tetradecylcyclobutanone ($C_{18}H_{34}O$)

Fig. 15. Triacylglycerol irradiation degradation mechanism.

Recently, we used the profile in triacylglycerols (TAG) chestnuts, measured by chromatography (HPLC - ELSD, "High Performance Liquid Chromatography - Evaporative Light Scattering Detector"), as a viable detection method, validated on chestnuts processed with gamma and electron beam radiation (Barreira *et al.*, 2013).

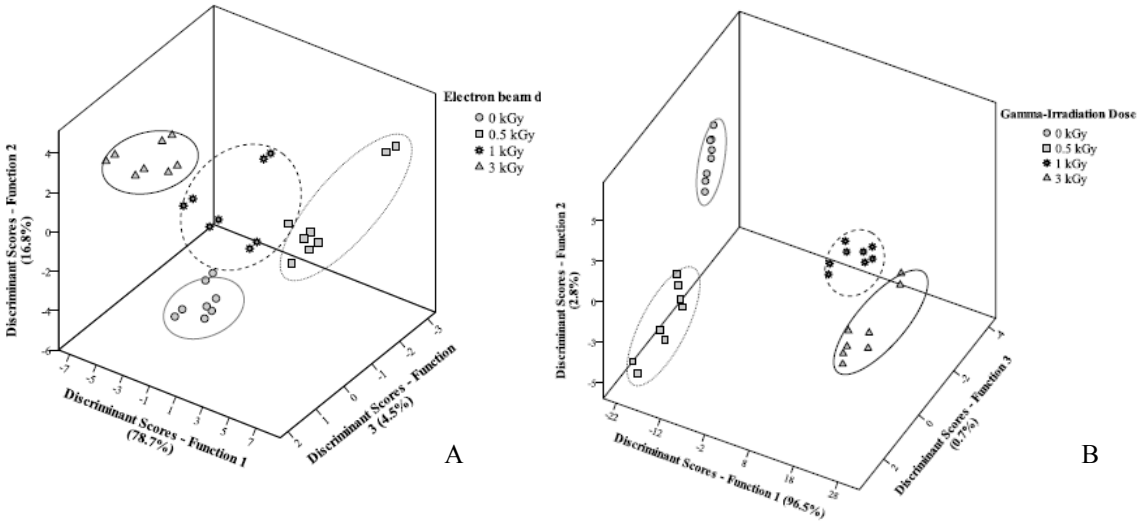


Fig. 16. Discriminant analysis of triacylglycerols profiles for chestnuts.
(A - electron beam irradiation; B – gamma irradiation).

In general, and despite that multiple comparisons could not be performed in most cases, due to the significant interaction among factors, ST×EBD and ST×GID, neither EBD nor GID seemed to induce appreciable changes in TAG profiles.

In order to obtain a more realistic idea about the influence of irradiation treatments, the results were scrutinized through a linear discriminant analysis (LDA).

The analysis was performed taking into account the applied irradiation dose (gamma, GID; electron beam, EBD) and storage time (ST).

In opposition to what could be expected from the mean values, the differences in TAG profiles allowed correct classification of 100% of the samples for the originally grouped cases either in EBD as in GID; regarding cross-validated cases, 100% of the samples were correctly classified for GID, while 96.9% (one sample irradiated with 0.5 kGy was classified as non-irradiated) were correctly classified for EBD (Fig. 16).

When evaluating triacylglycerol (TAG) composition, significant changes were detected when chestnuts were submitted to gamma or electron beam irradiation, especially for 1 and 3 kGy doses in both cases (Barreira *et al.*, 2013). However, changes in TAG profiles were mostly qualitative, which is in agreement with previous findings (Fernandes *et al.*, 2011a; Fernandes *et al.*, 2011b; Barreira *et al.*, 2012) for similar doses of irradiation, showing that the fatty acid profiles were not affected; ie a decrease of fatty acids, but a rearrangement within the glycerol molecule was observed. These changes are unlikely to affect the organoleptic characteristics of the nuts, because the fat content is below 1% (Fernandes 2011a).

In Tab. 2 are presented the validated methods for detection of irradiated chestnuts, by different authors.

Tab. 2. Validated methods for identification of irradiated chestnuts.

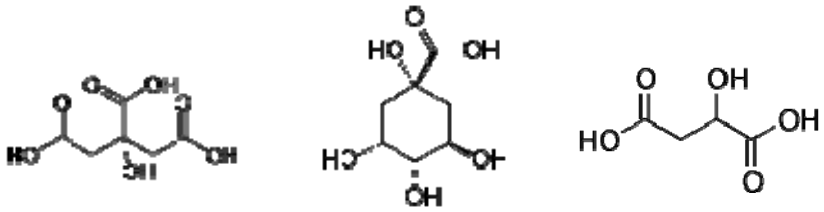
Specie	Gamma	E-beam	Reference
Castanea bungena	TL	---	Chung *et al.* (2004)
Castanea sativa	TL	---	Mangiacotti *et al.* (2009)
	TAG	TAG	Barreira *et al.* (2013)

TAG – Triacylglyceroles; TL- Thermoluminescence.
The white cells refer to studies by the author of this thesis and co-authors.
The cells in gray represent studies by other authors.

4.2.5. Organic acids

Organic acids play an important role in humans and plants metabolism, are powerful antioxidants, also used in pharmaceutical preparations. These compounds are low weight molecules with the general structure R-COOH, a carboxylic group connected to a radical (Fig. 17).

In this study, it were reported the effects of electron beam irradiation and storage time in several organic acids, namely oxalic, quinic, malic, ascorbic, citric, fumaric, succinic and shikimic acids, using Ultra-Fast Liquid Chromatography with Photodiode Array detection (UFLC-PDA).



Citric acid Quinic acid Malic acid

Fig. 17. General structure of some organic acids detected in chestnuts.

The results shown that the variance caused by the assayed irradiation doses are minimal, and do not allowed the indication of any particular tendency. Neither irradiation dose nor storage time seemed to exert high influence over organic acids profile. Concluding that, in line with previous parameters, organic acids are not greatly affected by gamma (Carocho *et al.*, 2013b) or electron-beam irradiation (Carocho *et al.*, 2013a; Carocho *et al.*, 2013b).

The maintenance of organic acid levels is a desirable feature due to their protective role against various diseases, mainly those with oxidative stress basis (Silva *et al.*, 2004a). From the conservation point of view, these are interesting results since the nature and concentration of organic acids are important factors influencing the organoleptic quality of fruit and vegetables, namely their flavor (Vaughan & Geissler, 1997), and constituting also important conservation indicators to evaluate food processing (Silva *et al.*, 2004b).

4.2.6. Proteins

Proteins are macromolecules and considered the most reliable irradiation indicators, especially due to degradation reactions such as scission of the C-N bonds in the main chain of the polypeptide (Fig. 18), and physical changes like unwinding, unfolding and aggregation (Stewart, 2001).

Nevertheless, the detail that irradiation can alter proteins does not create a significant problem from a nutritional point of view since amino acids, protected within the complex structure of the protein, may survive the process of irradiation (Stewart, 2001).

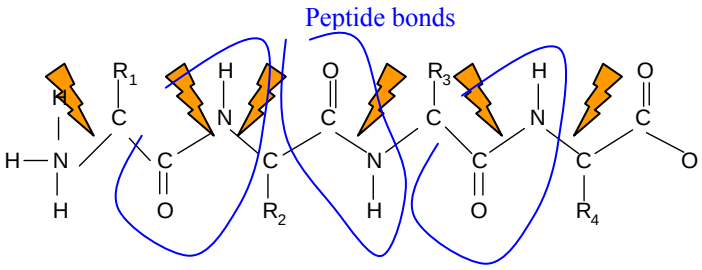


Fig. 18. Radiation scission of C-N bonds in the main chain of a polypeptide.

For protein content in chestnut fruits, the interaction among the two main factors irradiation and storage time, ST × ID, was a significant source of variation, not allowing multiple comparisons (Fernandes *et al.*, 2011b; Carocho *et al.*, 2012b). For chestnut varieties from Turkey,where this interaction was not observed, neither irradiation or storage time seems to exert a significant effect in proteins content (Barreira *et al.*, 2012).

4.2.7. Vitamins

Vitamins are a group of chemical substances that are essential in several metabolic processes. They represent a small part of food content and, as low molecules, are theoretically less prone to be affected by irradiation at low and medium doses (Miller, 2005). However, like thermal treatments, radiation processing of foods causes some loss of vitamins.

Vitamin C (ascorbic acid) is radiosensitive, is readily oxidized to dehydroascorbic acid (Stewart, 2001), Fig. 19, but this byproduct has a similar level of bioactivity (Miller, 2005).

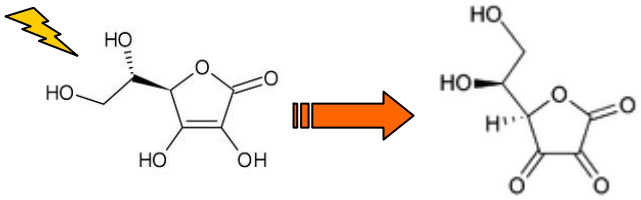
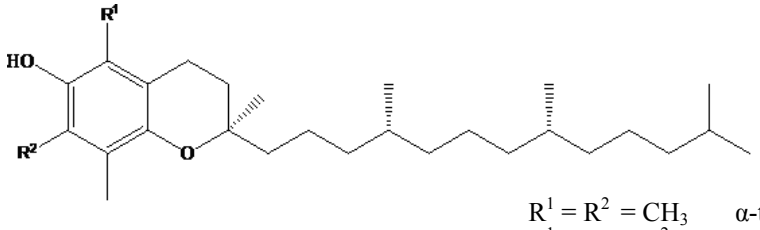


Fig. 19. Ascorbic acid irradiation degradation into dehydroascorbic acid.

Although vitamin losses generally increase with increasing radiation dose, irradiation of foods with high doses often requires processing conditions that minimize undesirable sensory effects, conditions that also contribute to a reduction in vitamin losses (WHO, 1999).

Vitamin E is a term frequently used to designate a family of related compounds, namely, tocopherols and tocotrienols, identified by a Greek letter as prefix (Fig. 20) and which are important lipophilic antioxidants, with essential effects in living systems against aging, strengthening the immune system and other positive effects for human health (Barreira *et al.*, 2009).



$R^1 = R^2 = CH_3$ α-tocoferol
$R^1 = CH_3$, $R^2 = H$ β-tocoferol
$R^1 = H$, $R^2 = CH_3$ γ-tocoferol
$R^1 = R^2 = H$ δ-tocoferol

Fig. 20. Molecular structure of tocopherols isoform.

Some bioactive compounds had also been studied in chestnuts submitted to irradiation. The tocopherols profile was studied in gamma (Fernandes *et al.*, 2011a; Fernandes *et al.*, 2011b; Barreira *et al.*, 2012; Carocho *et al.*, 2013b) and electron-beam (Carocho *et al.*, 2012b; Carocho *et al.*, 2013b) irradiated samples, revealing changes with different storage times, specially for 60 days, while irradiation exerted a protective effect in tocopherols amounts, the overall content tended to be higher in irradiated samples.

4.2.8. Total phenols and flavonoids

Phenolics consists of a hydroxyl group (—OH) bonded directly to an aromatic hydrocarbon group and flavonoids are polyphenols, a group of phenols.

These compounds are present in plants and fruits, in different forms, and are being identified as health promoters (Carocho *et al.*, 2014).

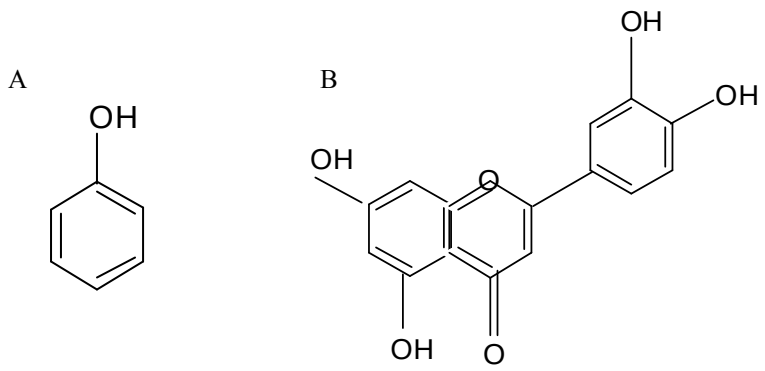


Fig. 21. Molecular structure of a phenol (A) and a flavone (polyphenol) (B).

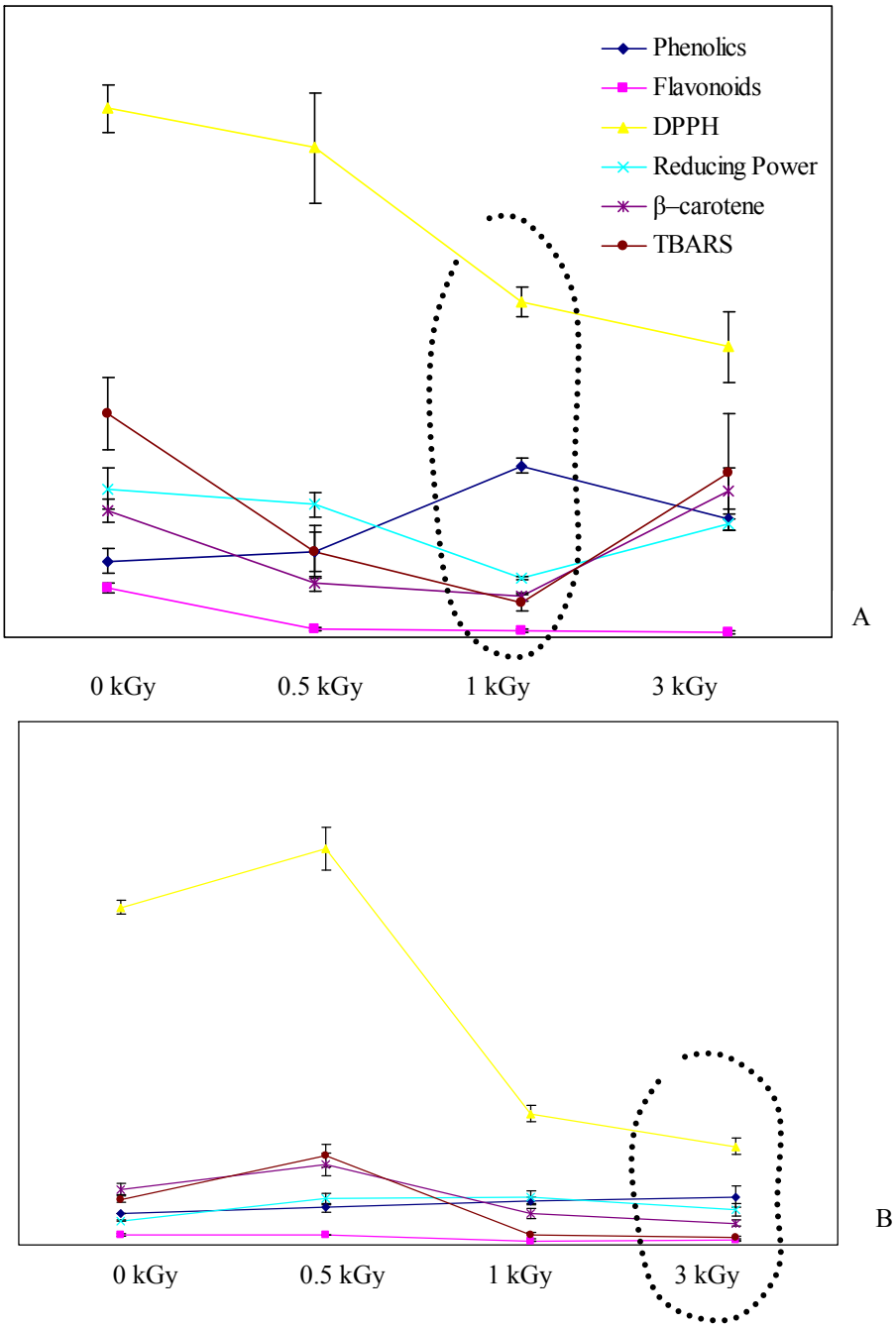


Fig. 22. Relative performance of phenolics, flavonoids and antioxidant assays. (Electron beam (A) and gamma (B) irradiations).

The effects of gamma radiation (Antonio *et al.*, 2011a; Carocho *et al.*, 2012a) and electron-beam (Carocho *et al.*, 2012a) on phenolic compounds were also studied, being verified that storage time had a much greater influence on their contents, while radiation was a minor contributor on phenolic compound changes.

Chestnuts skins (inside) and shell (exterior) present greater phenolic and flavonoid

content and lower antioxidant activity, highest EC_{50} values, than fruits (Antonio *et al.*, 2011a). And it has been verified that irradiated samples retain the total content of phenolic compounds, but not in flavonoids (Carocho *et al.*, 2012a). This could be due to the fact that phenols are smaller molecules than flavonoids (Fig. 21), which are bigger, and probably more susceptible to radiation scission.

4.2.9. Antioxidant activity

The degradation of human cells occurs by oxidative reactions. Some food components are identified as having potential to stop or delay this process, being classified as health promoters. The process of stopping cells degradation is a result of a cocktail of substances that could inhibit or stop oxidative reactions. For that, in this study several tests were perfomed to check the bioactivity of the irradiated and stored chestnut fruit extracts, evaluating the effect of gamma (Antonio *et al.*, 2011a) and e-beam (Carocho *et al.*, 2012a) irradiation on antioxidant potential.

When comparing the effects of gamma and electron beam irradiation on the antioxidant potential of Portuguese chestnuts (*Castanea sativa* Mill.), to get a perspective for the better dose in each case (Fig. 22), it was possible to conclude that the most indicated doses to maintain antioxidants content, and to increase antioxidant activity were 1 and 3 kGy for electron beam (Fig. 22A) and gamma radiation (Fig. 22B), respectively (Carocho *et al.*, 2012a).

The overall results indicate that gamma and e-beam irradiation preserve the antioxidant potential of fruits and skins (Antonio *et al.*, 2011a).

4.2.10. Minerals

Minerals content in chestnuts represent less than 1% (Nazzaro *et al.*, 2011).

It is considered that irradiation processing does not alter the minerals elements composition of food (Stewart, 2001). Otherwise, other authors reported changes in mineral content for thermal treatments, in boiling or roasting of chestnuts (Nazzaro *et al.*, 2011).

5. Summary tables

In order to gather all the information regarding radiation and its influence on various parameters chestnuts and pests, it was previously published a review of the state of art on gamma radiation (Antonio *et al.*, 2012a).

An update of that information is now presented here, to include also the effects of electron-beam irradiation in the main physicochemical parameters of chestnut fruits.

Species and doses tested by different studies are presented in Tab.3 and Tab. 4 shows a list of the studied effects of gamma radiation or electron beam ("e-beam") on physicochemical parameters of chestnut fruits, by different authors.

Previous studies on the physicochemical effects of irradiation on chestnuts were performed only in Asian varieties: *Castanea bungena*, *Castanea crenata* and *Castanea molissima*; except one study in the European species of *Castanea sativa*, only for validation of detection methods of irradiated foods. In all these studies and tests was used gamma radiation (Antonio *et al.*, 2012a).

With electron-beam, there's only one study regarding its effect on insects of Asian chestnuts (Todoriki *et al.*, 2006), and nothing has been reported about its influence on physico-chemical parameters of chestnuts of any origin.

In the study conducted and summarized in the tables, it was tested gamma radiation and electron-beam for chestnuts preservation of European origin (Portugal, Turkey, Italy) and of different varieties ("Judia", "Longal", "Cota" and "Palummina"), studying its effects on the physical (color, texture, drying rate) and chemical (bioactive and nutritional) parameters

In the validation of the two types of radiation, gamma and e-beam, for irradiation preservation of different varieties, it was found that despite the differences detected between the characteristics of some cultivars, majorly, irradiation does not caused significant alterations in the chemical parameters (Carocho *et al.*, 2013b).

Tab. 3. Irradiated chestnuts (specie, origin and doses).

Gamma Radiation		
Specie and origin	**Doses**	**Reference**
Castanea crenata Siebold & Zucc. (Japan)	0.03, 0.07, 0.12 kGy at 0.7 Gy s^{-1}	Iwata *et al.* (1959)
	0.25, 0.5, 1, 10 kGy	Kwon *et al*. (2004)
	0.05, 0.1,0.2, 0.3, 0.4, 0.5, 1 kGy at 0.40 kGy h^{-1}	Imamura *et al.* (2004)
Castanea mollissima Blume (China)	0.1, 0.15, 0.2 kGy	Iwata *et al.* (1959)
	0.3, 0.6, 0.9, 1.2 kGy	Guo-xin *et al.* (1980)
	0.25, 0.5, 1 kGy	
Castanea Bungena Blume (Korea)	0.1, 0.15, 0.25, 0.5 kGy	Chung *et al.* (2004)
Castanea sativa Miller (Portugal, Italy, Turkey)	0.15, 0.25, 0.35, 0.50, 1 kGy at 16 Gy min^{-1}	Mangiacotti *et al.* (2009)
	0.27, 0.54 kGy at 0.27 kGy h^{-1}	Antonio *et al.* (2011a, b, c)
	0.5, 1.0, 3.0, 6.0 kGy at 0.8 kGy h^{-1}	
	0.27, 0.54 kGy at 0.27 kGy h^{-1}	Fernandes *et al.* (2011a, b)
	0.25, 0.5, 1.0, 3.0 kGy	
	0.25, 0.5, 3.0, 10 kGy	Calado *et al.* (2011)
	0.5, 3.0 kGy at 1.13 kGy h^{-1}	Barreira *et al.* (2012)
	1.0, 3.0, 6.0 kGy at 2.5 kGy h^{-1}	Antonio *et al.* (2012)
	0.6, 1.1, 3.0 kGy at 0.8 kGy h^{-1}	Carocho *et al.* (2012a, b)
		Barreira *et al.* (2013)
	1.16 kGy	Carocho *et al.* (2013b)
Electron-beam		
Castanea sativa Miller (Portugal, Italy)	0.53, 0.83, 2.91, 6.10 kGy	Carocho *et al.* (2012a, b; 2013a)
		Barreira *et al.* (2013)
	1.04 kGy	Carocho *et al.* (2013b)

All the authors included in the analysis non-irradiated samples, 0 kGy (control).

The white cells refer to studies by the author of this thesis and co-authors.

The cells in gray represent studies by other authors.

Tab. 4. Studied physico-chemical and bioactive parameters in irradiated chestnuts.

Parameter	Specie	Radiation	Authors
Colour	*Castanea crenata*	gamma	Kwon *et al.* (2004)
	Castanea sativa	gamma	Antonio *et al.* (2013a)
		e-beam	Antonio *et al.* (2013b)
Texture		gamma	Antonio *et al.* (2013a)
Drying			Antonio *et al.* (2012b)
Dry matter, Ash, Fat, Protein, Carbohydrates, Sucrose, Energetic value	*Castanea sativa*	gamma	Fernandes *et al.* (2011b)
			Barreira *et al.* (2012)
		e-beam	Carocho *et al.* (2012b, 2013b)
Proteins	*Castanea mollissima*	gamma	Guo-xin *et al.* (1980)
	Castanea sativa		Fernandes *et al.* (2011b) Barreira *et al.* (2012)
		e-beam	Carocho *et al.* (2012b, 2013b)
Total Sugars	*Castanea mollissima*	gamma	Iwata *et al.* (1959)
			Guo-xin *et al.* (1980)
	Castanea sativa	gamma	Fernandes *et al.* (2011a)
		e-beam	Carocho *et al.* (2012b)
Fructose, Glucose, Raffinose		gamma	Fernandes *et al.* (2011a)
		e-beam	Carocho *et al.* (2012b)
Trehalose		gamma	Fernandes *et al.* (2011a)
Amylase, Catalase, Starch	*Castanea mollissima*	gamma	Guo-Xin *et al.*(1980)
Fatty acids	*Castanea sativa*	gamma	Fernandes *et al.* (2011a, b) Barreira *et al.* (2012) Carocho *et al.* (2013b)
Organic acids		e-beam	Carocho *et al.* (2013a, b)
Ascorbic acid	*Castanea mollissima*	gamma	Iwata *et al*. (1959)
Tocopherols	*Castanea sativa*	gamma	Fernandes *et al.* (2011a, b) Carocho *et al.* (2013b)
Triacylglycerols		gamma	Barreira *et al.* (2013)
		e-beam	
Phenolics		gamma	Antonio *et al.* (2011a)
		e-beam	Carocho *et al.* (2012a, 2013b)

The white cells refer to studies by the author of this thesis and co-authors.

The cells in gray represent studies by other authors.

6. Conclusions

Till recently, the method used for chestnuts disinfestation is chemical fumigation, but it is environment aggressive and toxic for the operators and is being banned. Irradiation is considered a more environment friendly technology, meeting the food safety requirements. And it is considered that the risk of exposure to food borne pathogens is substantially reduced with the use of irradiation (Molins, 2001).

Food irradiation may preserve some components and degrades others. However, it should be emphasized that any food processing leaves marks in the product, and that they are a requirement to eat safe food. Generally, the balance of advantages and disadvantages, in comparison to other preserving processes, should be used to select or not this type of processing technology, to provide to the consumer a product that fulfills the best criteria of quality and safety.

With this research it was possible to get an insight in irradiation processing technologies and feasibility. Both types of irradiation, gamma or e-beam, might represent suitable solutions for chestnut, postharvest treatment. The main differences found in irradiated samples are related to storage time or assayed cultivars/species. The use of irradiation for post-harvest processing does not significantly interfere with main physical and biochemical parameters. Gamma and e-beam irradiation seems not to affect the nutritional value and individual nutritional molecules in chestnuts rather than the storage time. Moreover, it protects antioxidants such as tocopherols and phenolics, and revealed higher antioxidant activity comparatively to non-irradiated samples.

The macronutrients – carbohydrates, fats, proteins and sugars - are not significantly altered in terms of nutritional value by irradiation treatment. Among the micronutrients, some of the vitamins are susceptible to irradiation to an extent very much dependent upon the composition of the food and on processing and storage conditions (WHO, 1999). Therefore, from a nutritional viewpoint, irradiated foods are substantially equivalent or superior to thermally sterilized foods (WHO, 1999). Other processing of food (curing, roasting or boiling) causes changes in nutritional composition (Gonçalves *et al.*, 2010; Nazzaro, 2011) and makes also unviable to apply the standards of irradiation detection methods (Stefanova *et al.*, 2010).

In conclusion, the biochemical parameters of non-irradiated and gamma or electron-beam irradiated chestnuts was compared, as well as its interaction with storage time. With no exception, the storage time caused higher changes in these profiles than

both irradiation types, confirming that this technology, at the applied doses, did not affect the chestnut quality.

Generally, the assayed gamma and electron-beam irradiation doses (0.5 − 6 kGy) seemed to produce less obvious effects than storage time in all of the assessed parameters.

Proper identification of irradiated food product may contribute to market confidence, as long as the consumers are aware of the safety and potential of these technologies. TAG profiles were, for the first time, identified as suitable indicators of irradiation processing in chestnuts (Barreira *et al.*, 2013) and, more recently, also validated for mushrooms (Fernandes *et al.*, 2014d).

Accordingly, irradiation might be looked up as an as practicable chestnut conservation technology, independently of the irradiation source, chestnut species and geographical origin and both types of irradiation, gamma and e-beam, seem to constitute suitable solutions for chestnut postharvest treatments, which constitute an important step toward the completion of irradiation as feasible conservation technology.

This study could also have an impact in health of users, in the protection of environment and in the economy of the fruit producers.

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C. Methodology

Appendix 1- Gamma and electron beam irradiation equipments
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Appendix 3 - Dosimetric systems and dosimetry in gamma irradiation chamber
Appendix 4 - Bioactive and nutritional parameters measurements
Appendix 5 - Statistics methodology

Appendix 1

Gamma and electron beam irradiation equipments

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An overview of the experimental gamma irradiation chamber

The experimental gamma irradiation chamber used in this work is based on a machine from Graviner Company, U.K., model "Precisa 22". In 2009 the chamber was rebuilt, recharged and adapted with a SCADA - Supervisory Control and Data Acquisition.

Fig. A1.1. Gamma chamber before rebuilt (outside and inside view).

The radioprotection barriers were built for an estimated maximum activity of 370 TBq (10 kCi) and the chamber was recharged with 8.3 kCi, in June 2009. The system has several redundant security systems, with digital control, manual keys and emergency button, to guarantee the adequate protection for the users when the sources are in the position for irradiating the samples.

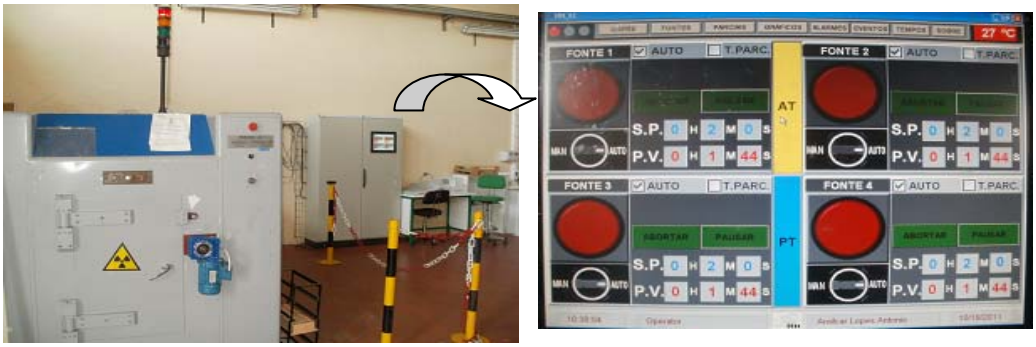


Fig. A1.2. Gamma chamber and touch panel for sources control.

The system has also three colour lights (red, yellow, green) to inform about the status of the irradiation chamber and an audible alarm to warn when the door is open.

The four ^{60}Co sources are discs with an active area of 20 mm diameter and length 30 mm, that are inside steel rods pneumatically commanded by a touch control panel (Fig. A1.2), with a total activity of 174 TBq (4.68 kCi) and with total dose rate between 0.10 kGy h^{-1} and 2.60 kGy h^{-1} (in November 2013), Fig. A1.3.

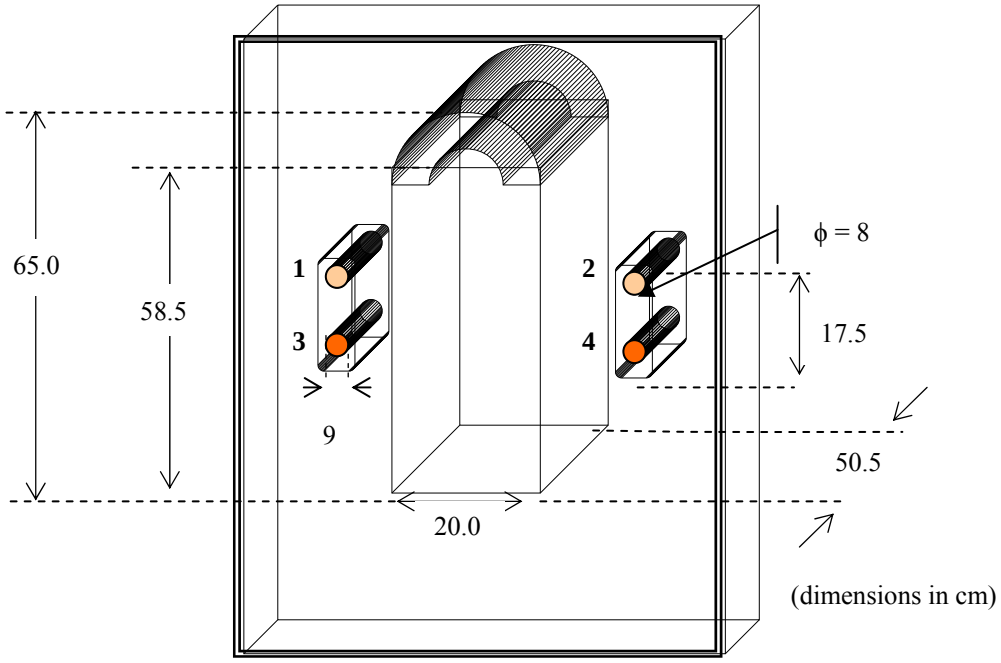


Fig. A1.3. Diagram of irradiation chamber dimensions and ^{60}Co sources position.

An aluminium support (Fig. A1.4.) was built to characterize the dose rate in four irradiation levels in the chamber.

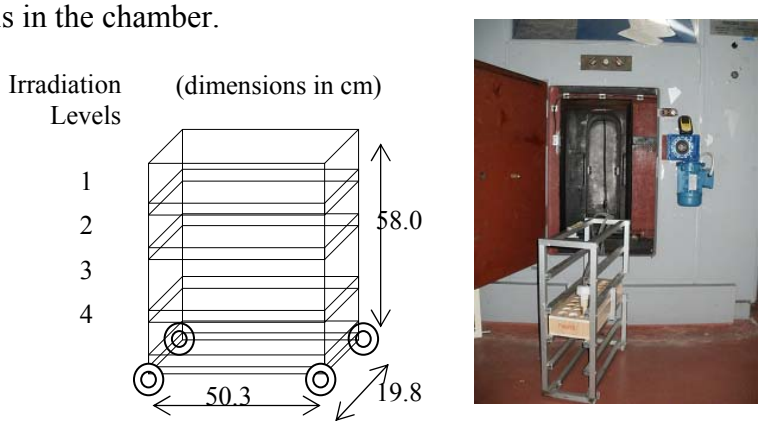


Fig. A1.4. Aluminium support: dimensions and in front of irradiation chamber.

An wood tray with 33 positions was built as support for Fricke dosimeter tubes (Fig. A1.5), to use all the available irradiation space in each level, to estimate the dose rate in each position inside the gamma irradiation chamber, for all levels.

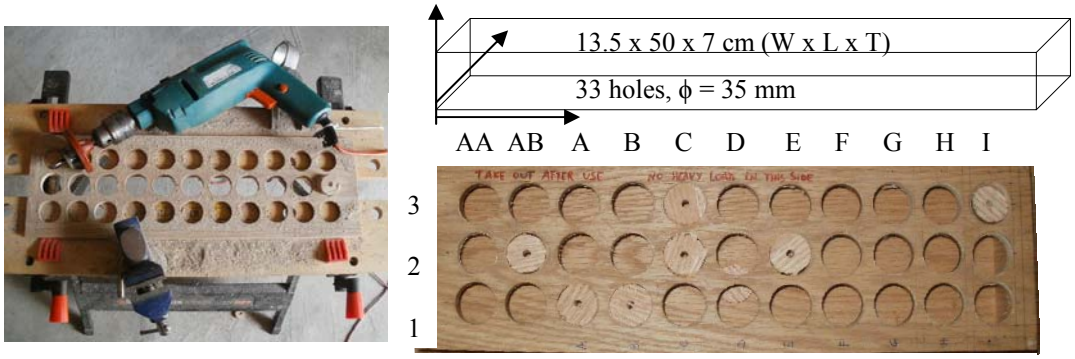
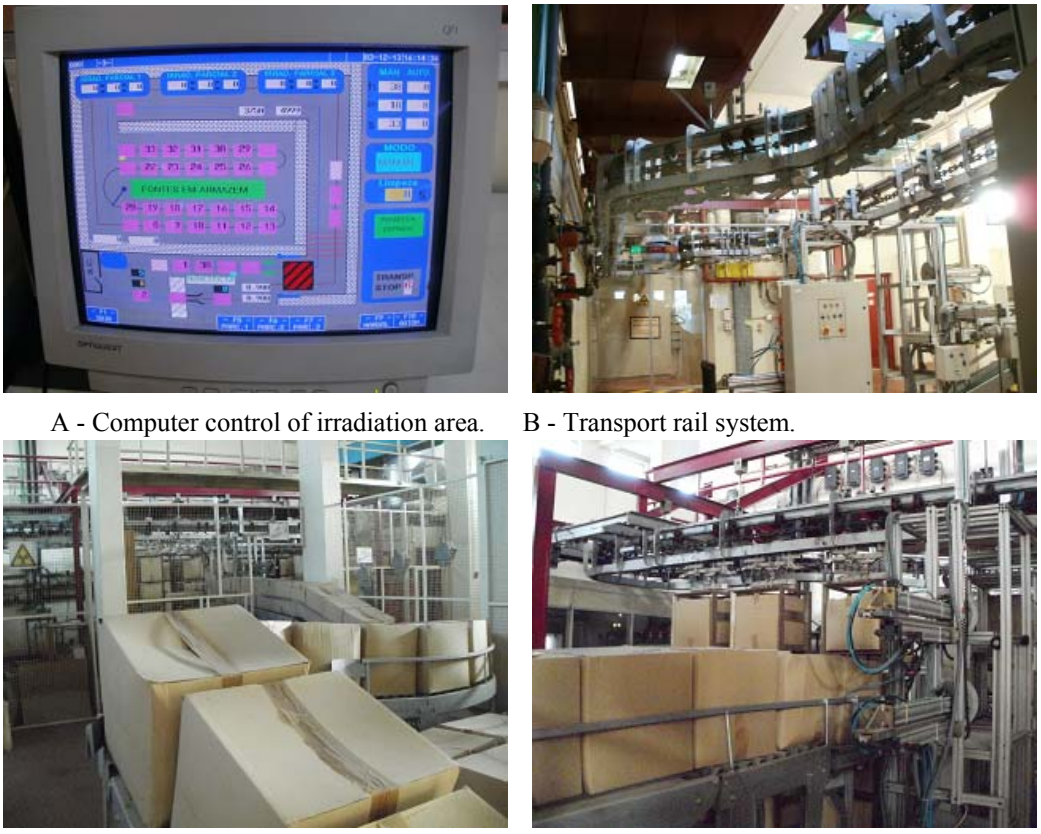


Fig. A1.5. Building a wood support for Fricke dosimeter tubes.

Industrial gamma irradiation

For gamma irradiations of the food products of these work was used only the experimental chamber. At CTN campus, Lisbon (Portugal), it is also available a semi-industrial gamma irradiator that is currently used to sterilize products for pharmaceutical industry and other non-food materials, that could be used for a scale-up of the fruits irradiation validation.

This plant allows the control of irradiation positions, the automatic transport of boxes and their interchanging to get good dose uniformity (Fig. A1.6.).



A - Computer control of irradiation area. B - Transport rail system.

C - Boxes transported in the conveyor. D - Pneumatic changing of boxes position.

Fig. A1.6 View of a gamma plant boxes entrance area and transport system.

Electron beam machine

The electron beam preliminary tests started with a linear accelerator (Linac), recently installed at that time in the CTN campus in Lisbon (Portugal). Due to technical problems of spare parts, to proceed with the work it was found an alternative at the Institute of Nuclear Chemistry and Technology (INCT) in Warsaw, Poland, where the irradiations were performed using also a Linac equipment.

To get an overview of this equipment and related preliminary work, it is presented here the main parts and first tests for the operation of the Linac equipment installed at CTN.
The electron accelerators are used for food preservation in two modes, with high energy electrons, up to 10 MeV, and for producing x-rays, up to 5 MeV.
The electron linear accelerator (Linac) installed at CTN campus, Lisbon (Portugal), is a clinical radiotherapy equipment (model GE Saturne 41, General Electric, France), adapted for research in radiation chemistry and food irradiation.
In this equipment the electrons are accelerated by RF along a wave guide, focused by a magnet and at the end curved by a bending magnet to exit the window and reach the target (Fig. A1.7.).

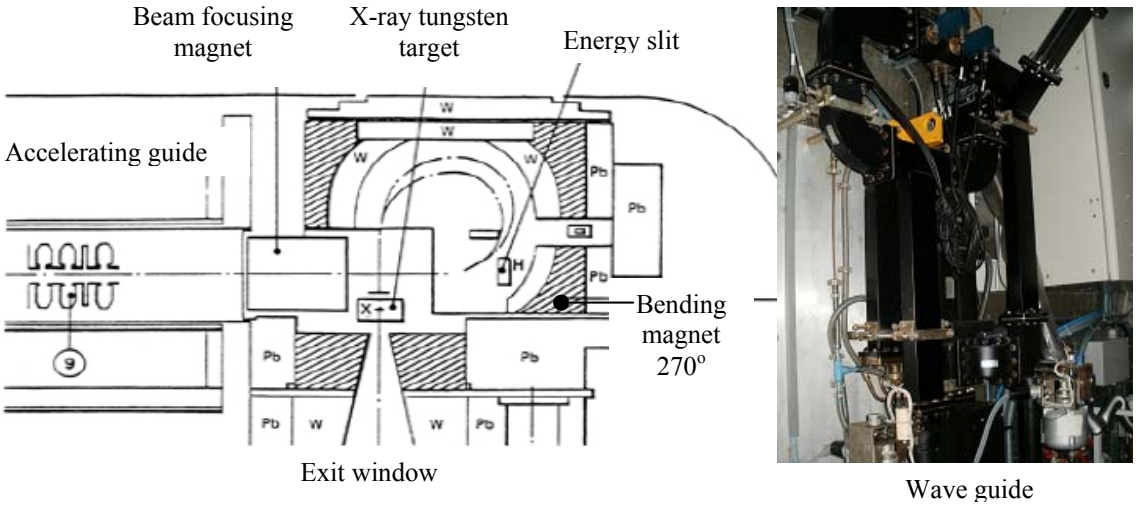


Fig. A1.7. Diagram of the main Linac components and wave guide image.

Radiofrequency (RF) produced in the magnetron is sent to the accelerator through a RF waveguide system where the electrons produced by heating a tunsgten filament (electron gun) are accelerated, focused and guided by electromagnets (Fig. A1.8.).

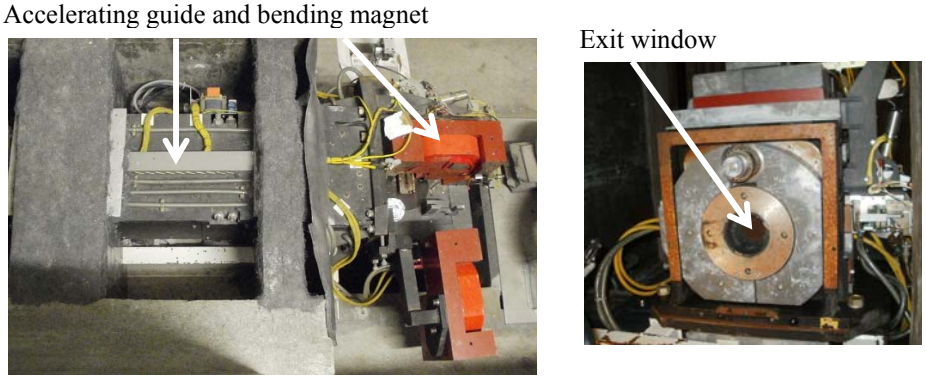


Fig. A1.8. Top view of a RF-Linac and head front view.

The equipment was installed in 2009 in a concrete shield with adequate radioprotection characteristics (Fig. A1.9.).

Fig. A1.9. E-beam bunker construction and working area during the first tests.

The Linac could irradiate with electrons at 10 MeV and with photons at 8 to 12MeV, using different configurations (Fig. A1.10.). The dose rate in e-beam mode could reach the value of 60 kGy in 5 min and 10 Gy/min in photon mode, at 1 m distance. The e-beam pulse duration is 4 μs, a repetition rate of 10-150 Hz and beam peak current of about 60 mA.

LIST OF CONFIGURATIONS

Date	Hour	Mode	Energy	Name	Comment
16/07/2009	17:54	X_HE_SI_Open	10.029997	Beam E - 10 MeV - 0.3kGy - 5 mn	0.3 kGy in 5 mn - 50mA - 2.28μs
16/07/2009	17:33	X_HE_SI_Open	9.970997	Beam E - 10 MeV - 21.3kGy - 5 mn	21.3 kGy in 5 mn - 50mA - 2.84μs
16/07/2009	17:26	X_HE_SI_Open	9.970997	Beam E - 10 MeV - 4.2kGy - 5 mn	4.2 kGy in 5 mn - 50mA - 2.8μs
16/07/2009	17:40	X_HE_SI_Open	10.029997	Beam E - 10 MeV - 42.6kGy - 5 mn	42.6kGy in 5 mn - 50mA - 2.84μs
16/07/2009	17:42	X_HE_SI_Open	10.029997	Beam E - 10 MeV - 63.9kGy - 5 mn	63.9 kGy in 5 mn - 50mA - 2.84μs
16/07/2009	11:48	X_HE_SI_Open	10.029997	Beam X - 10MeV	7.13Gy/mn - 58.6mA - 2.8μs
16/07/2009	12:12	X_HE_SI_Open	11.033001	Beam X - 11MeV	9.5GyMmn - 58.6mA - 2.8μs
16/07/2009	12:27	X_HE_SI_Open	11.977005	Beam X - 12MeV	8.7 Gy/mn - 42.4mA - 2.72μs
16/07/2009	11:30	X_HE_SI_Open	8.023990	Beam X - 8MeV	2.8Gy/mn - 50.8mA - 2.2μs
16/07/2009	11:42	X_HE_SI_Open	9.026994	Beam X - 9MeV	4.3y/mn - 54mA - 2.36μs
15/07/2009	16:16	X_HE_SI_Open	12.036001	Beam X -12MeV	6.2Gy/mn - 30mA - 2.72μs

Fig. A1.10. Print screen of irradiation configurations menu.

The first tests for the present work started in 2010, learning its operation, checking ozone formation during irradiations and doing the first dosimetric tests (Fig. A1.10.).

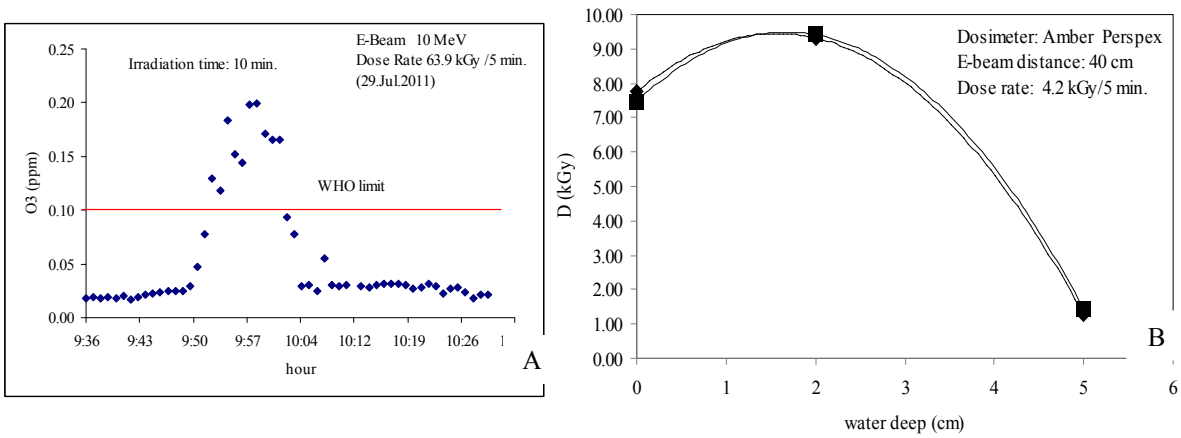


Fig. A1.11. E-beam tests: ozone concentration and dosimetry in water.

For food irradiation the e-beam and x-rays energy should be limited to 10 and 5 MeV, respectively, to not induce radioactivity in the irradiated products.

The Linac parameters are controlled by software (LabView®) allowing two modes, for users and irradiation monitoring and in maintenance mode, to adjust the hardware parameters of the machine (Fig. A1.11.).

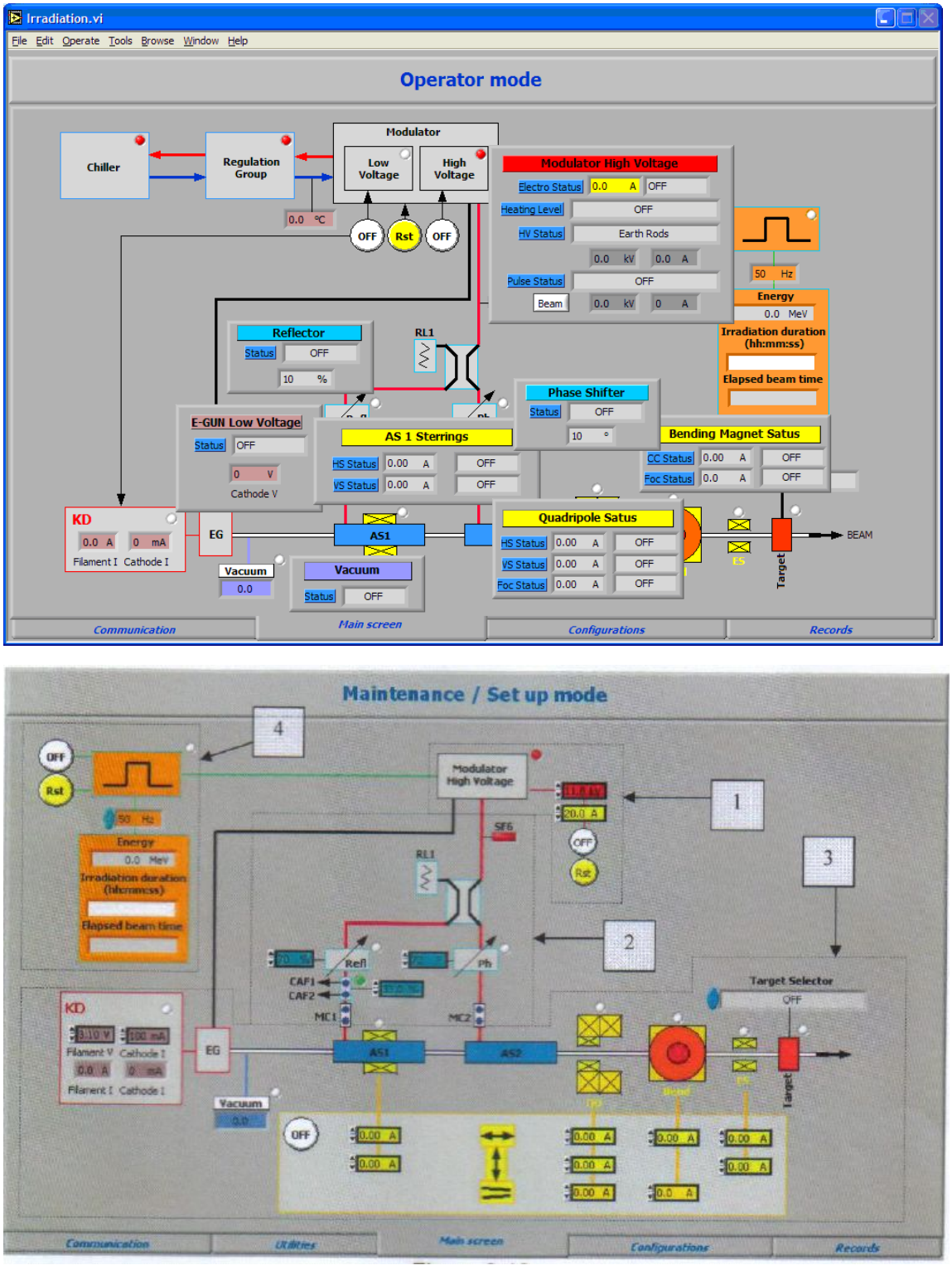


Fig. A1.12. Operator and maintenance mode main screens.

Industrial electron beam irradiations

As referred previously, due to technical problems with the equipment installed at CTN, Lisbon (Portugal), for this work the electron beam irradiations were performed using the Linac of an industrial certified sterilization e-beam plant, at Institute of Nuclear Chemistry and Technology (INCT) in Warsaw, Poland (Fig. A1.13).

The electron beam irradiator parameters used at INCT: 10 MeV energy, a pulse duration of 5.5 μs, a pulse frequency of 440 Hz, an average beam current of 1.1 mA, a scan width of 68 cm, a conveyer speed ranging from 20 to 100 cm/min, and a scan frequency of 5 Hz.

Fig. A1.13. Irradiation of chestnut fruits at INCT.
(A – chestnuts in the tray, B – tray in the conveyor,
C – irradiation area monitoring, D – dosimeters reading)

Appendix 2

Chestnut fruits production and estimated electron beam processing costs

Case 1:
Exported chestnut fruits by one agro-industrial unit (1 000 ton), operating 1 month;

Case 2:
All exported chestnut fruits (10 000 ton), operating 3 months;

Case 3:
Processing chestnuts and other fruits (aprox. 30 000 ton.), operating 1 year.

Table A2.1 – Irradiation processing impact on fruits price.

	Case 1	Case 2	Case 3
Total Yearly Cost (k€)	377	562	805
M (ton)	1 000	10 000	30 000
Throughput (ton/h)	3.5	9.5	16
Operation cost (€/ton)	380	60	27
Average fruit price (€/kg)	1.00	1.00	1.00
Processed fruit price (€/kg)	1.38	1.06	1.03
Increase in fruits price	38%	6%	3%

Table A2.2 – Hardware and building costs.

	Irradiate Chestnuts (1 industrial unit)	All Exported Chestnuts (3 months)	Chestnuts & Other Fruits (1 year)
M (ton)	1 000	10 000	30 000
P (kW)	16	52	110
E-beam cost (k€) (10^3 Log_{10} (P))	1 191	1 714	2 042
Installation (k€) (20% x E-beam Cost)	238	343	408
Shielding and Ventilation (k€) (30% x E-beam Cost)	357	514	613
Handling System (k€) (fixed estimated cost)	250	250	250
Building area (m^2)	1 000	1 000	1 000
Buildings cost (€/m^2)	600	600	600
Building cost (k€)	600	600	600
Design and Engineering (k€) 10% x (Shielding + Handling + Building)	121	136	146
Total Cost (k€)	**2 758**	**3 558**	**4 059**
E-beam price per Watt (€/W)	77	33	19

Relations adapted from R. B. Miller (2005).

Table A2.3 – Capital, labor and operation costs.

Capital Cost	Case 1	Case 2	Case 3
Interest rate (%)	8.0%	8.0%	8.0%
Useful life (years)	20	20	20
Annual amortization (k€)	281	362	413
Fixed labor costs (k€)	18	53	97
Maintenance cost (k€) (5% E-beam + 5% Handling)	72	98	115
Total Fixed Costs	371	513	625
Variable costs			
Installed power (kW) ($8 \times P_{eff}$)	124	414	882
Price per kWh (€)	0.10	0.10	0.10
Price per unit Power (€/kW)	12.40	41.40	88.20
Working hours (h)	352	1 056	1 936
Electricity cost (k€)	4	44	171
Variable Labor cost (k€) (10% Labor Cost)	2	5	10
Total Variable Cost (k€)	6	49	180
Total Yearly Cost (k€)	**377**	**562**	**805**
Operation cost per hour (€)	1 070	530	420
Capital Cost	75%	64%	51%
Electricity Cost	1%	8%	21%
Labor Cost	5%	10%	13%

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Appendix 3

Dosimetric systems and dosimetry

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Dosimetric systems

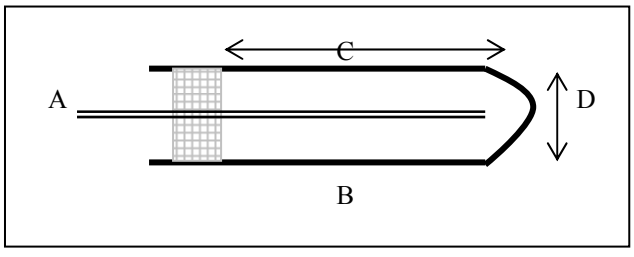
In this work were used different type of dosimetric systems, which measures the interaction of radiation in air, liquid and solid, to estimate the absorbed dose. The type of dosimeters used, calibration procedures and reading followed the standards of good practices for food irradiation (ISO/ASTM Standards and IAEA recommendations).

For gamma irradiations were used the ionization chamber, a primary standard; Fricke dosimeter, a liquid solution, as reference standard; and poly(methyl methacrylate), or PMMA, a routine dosimeter. The ionization chamber and Fricke dosimeter are considered standards for absorbed dose in water, with the adequate correction factors (AAPM, 1986). PMMA routine dosimeter main advantage is its robustness and easiness to read.

For e-beam irradiations was used also poly(methyl methacrylate), Gammachrome YR (Harwell-Dosimeters, U.K.), as a routine dosimeter and a calorimeter, as a standard dosimeter.

Ionization chamber

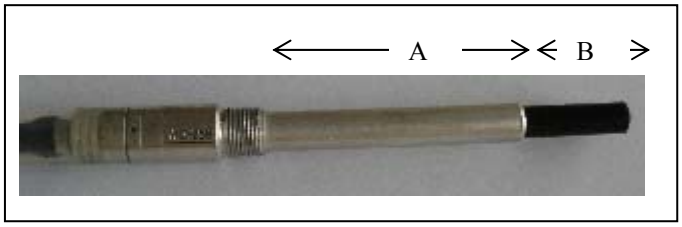
An ionization chamber (IC) is a gas-filled type detector in which a voltage difference is applied between the cathode (wall of the chamber) and anode (central wire). Generally, on a gas-filled detector the radiation transverses the chamber and will generate electrons, that moves towards the anode, and positive ions, moving towards the cathode, that are responsible for a measurable electric signal, the current, allowing the quantification of radiation. In the ionization chamber the applied voltage (~200 V to ~400 V) is adjusted in order to eliminate or minimize the recombination of charged particles with free charges or other ions of opposite charge.



A - Central Electrode; B - Outer Electrode; C - Length of Active Volume; D - Inner Diameter

Fig. A3.1. Ionization chamber detector schematic diagram.

The IC detector used in the present work (Fig. A3.2.) is air ventilated, working at ambient pressure and temperature.



(A – length 55 mm, diameter 8.5 mm; B – 25 mm, diameter 7 mm)

Fig. A3.2. Ionization Chamber.

The IC dosimeter used was standard chamber (model FC65-P, IBA Dosimetry GmbH, Germany), with an active volume of 0.65 cm^3, a length of active volume, LaV, of 23.0 mm, and an inner diameter of 6.2 mm, with graphite wall.

In an IC detector the voltage is properly adjusted in order to work in a regime where the measured current is proportional to the number of electric particles (primary electrons) generated by the radiation. The signal is measured by a digital electrometer, where the current is converted into dose (in *Gy* or *kGy*) (Fig. 3A-3). The equipment allows the registration of charge (in *C*), current (in *A*), dose (in *Gy*), and dose rate (in *Gy* s^{-1}), manually or automatically by a specific software (*Dose 1, version 1.0, from Scanditronix-Wellhöfer, Germany*).

Fig. A3.3. Electrometer.

The absorbed dose rate in the gas, $\dot{D}_g$, is given by (McLaughlin, 1989):

$$\dot{D}_g = \frac{i_s}{m} \times \frac{W}{e} \qquad \text{(eq. 1)}$$

where W is the energy absorbed by the gas, e is the electron charge, m the mass, i_s the saturation current measured by the electrometer. In dry air, W/e = 33.85 J C^{-1}.

The ionization chamber, model FC-65P, was calibrated at National Metrologic Laboratory, and the sensitivity for gamma radiation is 20.77 nC Gy^{-1} (or 48.15x10^6 Gy

C^{-1}) for the calibration factor in terms of absorbed dose to water, $N_{D,w}$, and 22.42 nC Gy^{-1} (or 44.60x10^6 Gy C^{-1}) for the calibration factor in air, N_k, During measurements, the applied voltage was set to +300 V.

From sensitivity factor, $N_{D,w}$, and for a dose rate of 2 kGy h^{-1} we may estimate the current

$$\frac{2\times10^3\ Gy}{3600\,s}\times 20.77\times\frac{10^{-9}\ C}{Gy}=11.5\times10^{9}\approx 12\ nA$$

that is in accord with the experimental measurements.

For air ventilated IC detectors, the measurements should be also corrected with ambient pressure and temperature conditions.

The correction factor, $k_{T,P}$, is given by (IAEA, 2000):

$$k_{T,P}=\frac{(273.2+T)}{(273.2+T_0)}\times\frac{P}{P_0} \qquad \text{(eq. 2)}$$

where T and P are the temperature and pressures at measurement conditions (T, P) and calibration conditions (T_0, P_0).

For FC-65P ionization chamber, the calibration conditions where T_0 = 293 K, P_0 = 1.013x10^5 Pa and the measurement conditions T = 293 K, P = 1.013x10^5 Pa. so we get for $k_{T,P}$ a small correction factor of 1.02.

Liquid chemical dosimeter

In dosimeters that use a gas cavity we have to overcome limitations such as dose rate sensitivity or saturation and the use of correction factors to estimate the absorbed dose in the irradiated material. In liquid dosimeters the correction factors used to estimate the absorbed dose, stopping power ratios, are almost equivalent to many irradiated materials, namely food. And for this type of dosimeters the volume of interaction with radiation is well known.

Dosimetry using chemical solutes (aqueous solutions) is based on reactions with these solute species formed in the radiolysis of water, radiation interaction with liquids generates other compounds that could be used for dose quantification. The radiation produces ionization and excitation of atoms and molecules along its path that are responsible for the generation of secondary substances, some with a short lifetime and others that are more stable. The latter are a mark of radiation passage and used to quantify the dose.

One of the most used and studied liquid dosimeter is the chemical solution proposed by Fricke *et al* (Fricke, 1966), recommended for the range 40 to 400 Gy if prepared according the standards (ASTM, 1992; McLaughlin, 1989).

Fricke chemical solution is a reference dosimeter which is widely used for calibration purposes and accepted as a secondary standard, since the chemical yield of sub-products is well known and the impact of radiation is easily quantified by spectrophotometric methods, in the UV region (ICRU, 1984).

The Fricke dosimeter consists of an air (or oxygen) saturated aqueous solution of ammonium ferrous sulphate, $(SO_4)_2Fe(NH_4)_26H_2O$, (0.001 mol L^{-1}), dissolved in sulphuric acid, H_2SO_4, (0.4 mol L^{-1}), with a solution of sodium chloride, NaCl, (0.001 mol L^{-1}), used to minimize the effect of impurities of organic origin. The water used to prepare the solution was triple distilled by a purifying system (Millipak®, from Merck Millipore, USA), with an activated carbon that removes dissolved organics, an UV lamp that destroys bacteria and a filter of 0.22 μm before the output.

All the glass tubes used for Fricke dosimeter were previously washed with distilled water and dried in an oven. Before filling, using a pipette and a pompette, they were rinsed twice with the non-irradiated Fricke, to eliminate the presence of impurities.

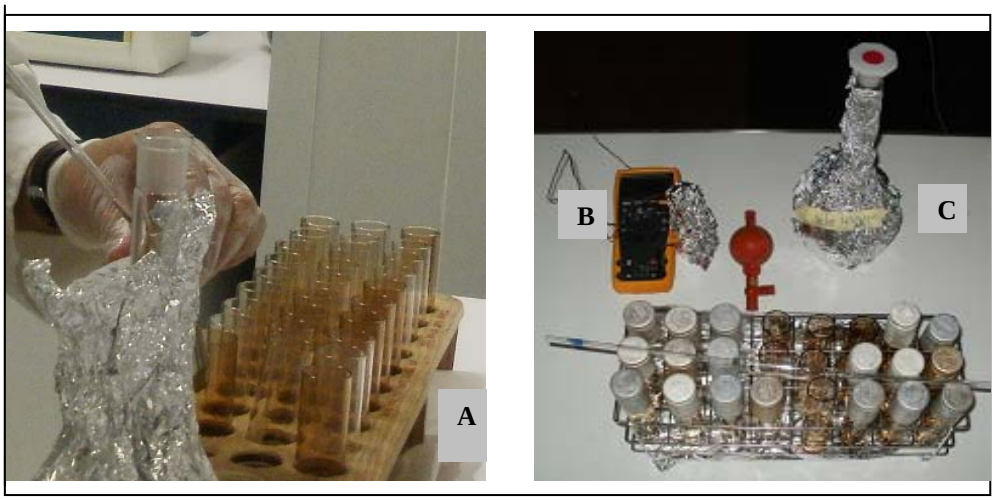


Fig. A3.4. Preparing the Fricke dosimeter.
(Filling the tubes to irradiate (A); thermocouple to read solution temperature (B) and volumetric flask wrapped with aluminium foil to protect from UV's (C))

The dosimetric solution has a tendency to oxidize when stored at room temperature and exposed to light, particularly UV light. In order to minimize these effects, the glass container was involved in aluminium foil and the solution stored in a dark room with controlled temperature.

The principle of chemical reaction is the oxidation of ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}). During irradiation, Fe^{2+} ions are converted in Fe^{3+} ions and the absorbed dose is proportional to the concentration of ferric ions in the aqueous acid solution.

The reaction mechanisms in the Fricke dosimeter are triggered by the reaction products formed in the radiolysis of water (Stewart, 2001):

$$H_2O \xrightarrow{\text{radiation}} OH^* + e^- + H^* + H_2 + H_2O_2 + H_3O^+$$

The Fricke solution is air or oxygen saturated after preparation, to have available a higher concentration of free oxygen, O_2, giving rise to the formation of hydroperoxyl radical:

$$H^* + O_2 \text{ -------} \rightarrow HO_2^*$$

The ferric ions, Fe^{3+}, are formed from ferrous ions, Fe^{2+}, by the interaction with different sub-products of water radiolysis:

$$HO_2^* + Fe^{2+} + H^+ \text{ --------} \rightarrow Fe^{3+} + H_2O_2$$

Ferric ions are also formed from the reaction of H_2O_2 and H* with Fe^{2+}

$$Fe^{2+} + H_2O_2 \text{ -----} \rightarrow Fe^{3+} + OH^- + OH^*$$

$$Fe^{2+} + OH^* \text{-----} \rightarrow Fe^{3+} + OH^-$$

The radiation chemical yield, G, of Fe^{3+} at 25 ^{o}C is 1.61×10^{-6} $mol\ J^{-1}$ The concentration of ferric ion, Fe^{3+}, formed during irradiation is measured by spectrophotometry.

The solution has two peaks, at 224 nm and 303 nm, however for experimental purposes, for dose rate measurements in different gamma chamber positions (Fig. A3.5), was used only the peak at 303 nm, since it is less sensitive to impurities (ICRU, 1984).

The optical density of the irradiated samples was measured at the peak value of the spectrum, in quartz cells of 10 mm optical path, using as reference the non-irradiated solution. The readings between irradiated solutions in the same quartz optical cell was preceded by emptying and filling it with the solution to be read twice, starting from the lowest dose, in order to avoid bias in the readings.

The estimation of the dose absorbed by the dosimetric solution, D_F, is based on the following equation (IAEA, 2002):

$$D_F = \frac{\Delta A}{\rho \varepsilon G d} \qquad \text{(eq. 3)}$$

where each symbol has the following meaning:

D_F – absorbed dose (Gy);

ΔA – absorbance difference between irradiated and non-irradiated solutions;

ρ – density of the solution ($kg\ m^{-3}$);

ε – absorption molar coefficient ($m^2\ mol^{-1}$);

G - radiation chemical yield, ($mol\ J^{-1}$);

d – optical path in the spectrophotometer cell (m);

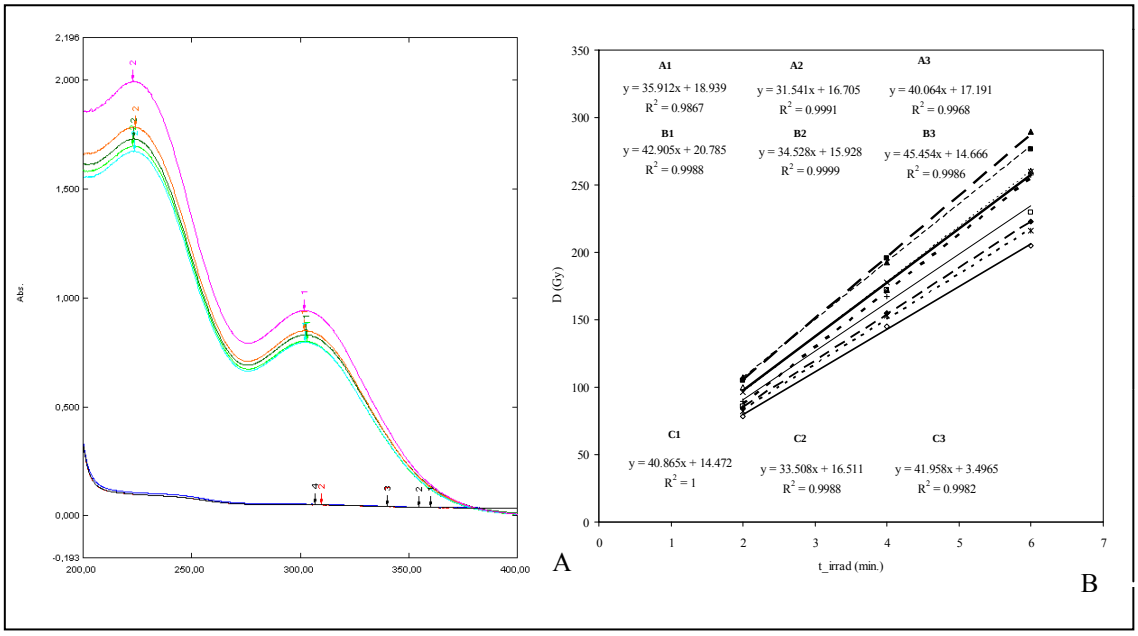


Fig. A3.5. Irradiated Fricke spectra and dose *vs.* absorbance for different positions.

The optical density of the irradiated samples is measured at the peak value of the spectrum, using as reference the non-irradiated solution.

For irradiated temperature and measurement at T = 25 ^{o}C, the value for the constants are (ASTM, 1992):

$$\rho = 1.024 \times 10^{3}\ kg\ m^{-3},\ \varepsilon \times G = 352 \times 10^{-6}\ m^{2}\ J^{-1},\ d = 1 \times 10^{-2}\ m.$$

with $\varepsilon = 219\ m^{2}\ mol^{-1}$ and $G = 1.61 \times 10^{-6}\ mol\ J^{-1}$.

To estimate the absorbed dose in water is used the following correction factors:

$$D_w = D_F\ f\ p_{wall}\ k_{dd} = \frac{\Delta A}{\rho\ \varepsilon\ G\ d}\ f\ p_{wall}\ k_{dd} \qquad \text{(eq. 4)}$$

where

D_w – is the absorbed dose in water;

D_F – is the absorbed dose in Fricke solution;

f – correction factor for the deposited dose in water compared to Fricke;

p_{wall} – correction for glass ampoules' wall;

k_{dd} – correction factor for the non-uniformity dose in the irradiated volume.

Using the values referred in literature for the correction factors (Klassen, 1999):

$$p_{wall} = 1.0001, f = 1.0032, k_{dd} = 1.0021;$$

We get,

$$D_w = 1.005 \times D_F \quad \text{(eq. 5)}$$

This correction factor, for water irradiation medium and Fricke dosimeter, shows that the absorbed radiation by the dosimeter is almost equal to the absorbed dose in water, which explains why this dosimeter is useful in clinical dosimetry and in food irradiation for dose calibration, where the irradiated targets have a density close to water.

When the temperature of irradiation and the reading of the sample is not 25 °C, the product of $\varepsilon \cdot G$ factors must be corrected, resulting the following expression to determine the absorbed dose (ASTM, 1992):

$$D_F = \frac{\Delta A}{\rho \varepsilon G d \ [1 + 0.007 \times (25 - T_a)] \times [1 + 0.0015 \times (25 - T_i)]} \quad \text{(eq. 6)}$$

where T_a is the temperature during the reading of absorbance and T_i the temperature during irradiation. ΔA is the optical absorbance difference between irradiated and non-irradiated solutions. The corrections 0.007 and 0.0015, comes from the fact that radiation chemical yield, G (Fe^{3+}), decreases with the reading temperature and with the decrease in irradiation temperature, respectively (Klassen, 1999).

In gamma irradiation (photons), the container with the dosimetric solution must be surrounded by a material with sufficient thickness to produce electron equilibrium during calibration, to ensure electronic balance in the dosimeter.

The recommended value for electronic equilibrium is 3 to 5 mm of polystyrene or acrylic since it has a atomic weight density and atomic number close to Fricke (Burlin, 1969).

In the experimental measurements was used a box of PMMA with 4 mm, to surround the dosimeters to obtain the conditions of electron equilibrium. The limited range (40-400 Gy) and the fact that it is a liquid solution are the main disadvantages for use in industrial radiation processing, particularly in food irradiation, due to the possibility of product contact with the liquid, if the ampoules or flasks break.

Fricke dosimeter is classified as a standard and is also used for calibration of other type of dosimeters, such as PMMA dosimeters.

Amber Perspex routine dosimeter

Poly(methyl methacrylate), PMMA, is a polymeric molecule $(C_5H_8O_2)_n$, a solid transparent plastic material, with a density of 1.18 g cm^{-3} that has several trade names: Lucite, Perspex, Plexiglas or Acrylic glass, and impregnated with pigments that change the colour with radiation, is used as a dosimeter for a particular dose range (ICRU, 2008).

A commercial company (Harwell-Dosimeters, U.K.) has currently available two types of these dosimeters (w1, 2013): "Red 4034", for the range 5 – 50 kGy; "Amber 3042", for the range 1 – 30 kGy, available in rectangular size, 30 x 11 mm, with a thickness of about 3 mm, in sealed sachets, to avoid humidity, hand-touch and dust, since after irradiation they are read by optical methods (Fig. A3.6). For e-beam irradiations we have also used a PMMA dosimeter, "Gammachrome YR" for the range 0.1 to 3 kGy, that has approximately the same rectangular dimensions and a thickness of about 1.7 mm, but that is not currently commercially available.

Fig. A3.6. Amber dosimeters measurement.
(Dosimeters (A), Gauge for thickness measurement (B) and spectrophotometric system (C)).

In industrial radiation processing, when it is possible, the dosimeter is chosen to have similar absorption radiation characteristics as the irradiated material. Food products have a density close to water and the stopping power ratios for water and PMMA is 1.033 (AAPM, 1995):

$$D_{water} = D_{PMMA} \times 1.033 \qquad \text{(eq. 7)}$$

The dosimeters are read after irradiation using air as reference in a double beam spectrophotometer. Amber dosimeter has two peaks, at 603 nm and 651 nm (Fig. A3.7).

Following the technical recommendations for this type of dosimeter, they should be read at peak 603 nm for the range 1 to 10 kGy and at peak 651 nm for the range 10 kGy to 30 kGy.

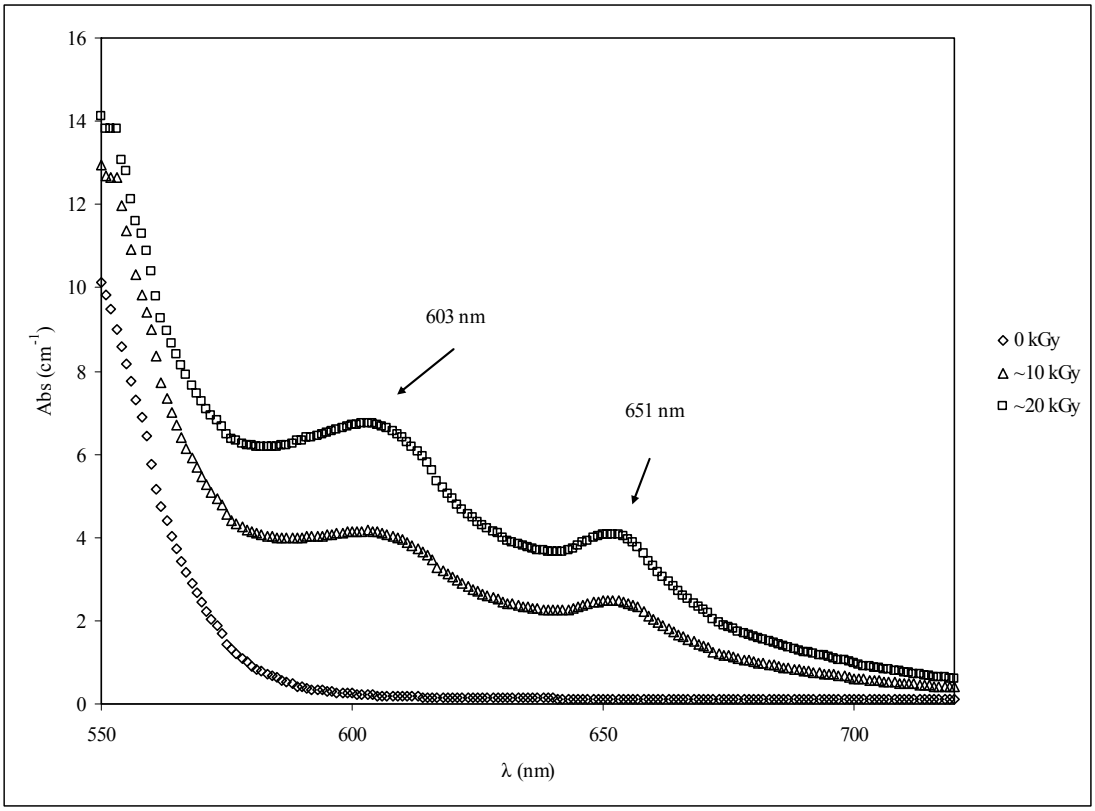


Fig. A3.7. Spectra of non-irradiated and irradiated Amber dosimeters.

PMMA dosimeters fit the requirements for a routine dosimeter in many industrial radiation processes: "product equivalence, ease to read, availability, robustness and price" (McLaughlin, 1989). However one of the main limitations for this type of dosimeters is its sensitivity to storage conditions, time and temperature. Times between 24 h and 48 h could lead to an underestimate or overestimate of irradiation dose (Watts, 1998). During calibration, the reading time after irradiation should be constant and the dosimeter should be read at a maximum time of 48 h after irradiation (Whittaker, 2001).

Amber Perspex dosimeters calibration

Amber Perspex is a trade name of PMMA commercial dosimeters that should be calibrated before use, in the conditions of the irradiation facility, against a standard or reference dosimeter.

The calibration should be done in the conditions of electronic equilibrium, surrounded by a medium with equal or similar properties. To obtain these conditions, a rectangular phantom of clear Perspex (PMMA) was built (height 70 mm, width 50 mm, thickness 10 mm), in Fig. A3-8, where it was also shown the ionization chamber, model FC-65P, used as reference, and Amber dosimeter, inside the phantom. Acrylic or PMMA is one of the recommended materials to use as a phantom (ASTM, 1989).

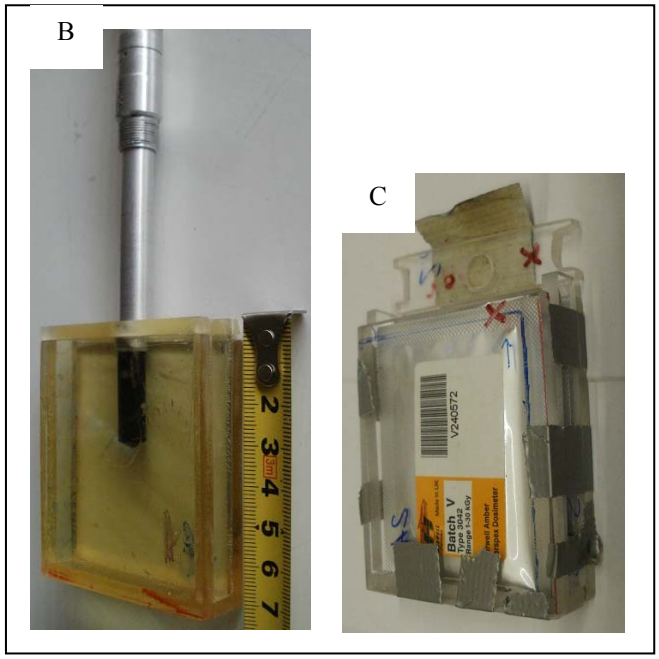


Fig. A3.8. Acrylic phantom for dosimeters calibration.
(Ionization chamber (A) and Amber dosimeters (B)).

The estimated dose was obtained multiplying the dose rate measured with the ionization chamber by the irradiation time. The absorbance was measured by spectrophotometry at 603 nm (*Shimadzu, model UV-1800, Japan*), the thickness for each dosimeter was measured with a gauge (*Mitutoyo, model no. 7360, Japan*), with an uncertainty of ± 0.01 mm. The specific absorbance was obtained dividing the absorbance by the thickness. The results were expressed as a mean of four measurements, as recommended by the standard (ASTM, 1989).

Amber dosimeter was calibrated in the dose range used in food irradiation experiments, up to *10 kGy*, and a nonlinear fitting "Dose *vs* Absorbance" was performed (*Mathematica, version 9.0, Wolfram Research Inc.*), considering the lowest polynomial order that represents the data, using the residuals plot and R-squared value to check the quality of adjusted function (Sharpe, 2009).

The fitting equation for *Amber 3042 (batch V)* is a second order polynomial function,

$$D = 0.3407 + 2.0281 \times Abs + 0.1378 \times Abs^2 \qquad \text{(eq. 8)}$$

where *Abs* is the specific absorbance (cm^{-1}) and *D* is the dose in *kGy*.

In Fig. A3.9. is represented the estimated dose and fitted curve *versus* specific absorbance.

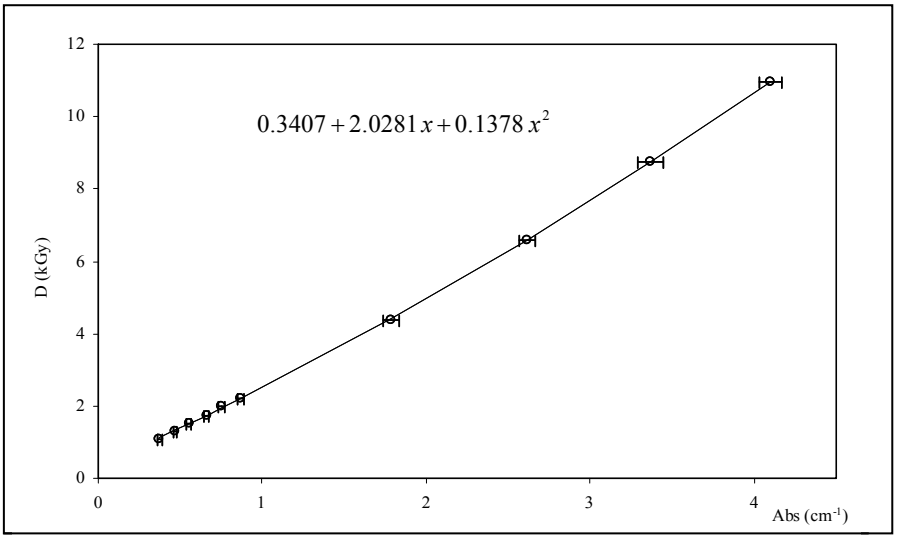


Fig. A3.9. Amber dosimeter absorbed dose and fitting curve.

Gammachrome YR dosimeter

As referred previously, the similarity between poly(methyl metacrylate) dosimeters and irradiated food is one of the main reasons for choosing this type of dosimeters, also its robustness and easy of reading.

For routine dosimetry in e-beam irradiations was used a poly(methyl metacrylate) dosimeter, Gammachrome YR (Harwell-Dosimeters, U.K.), that was recommended for the range 0.1 to 3 kGy. After irradiation the dosimeter was read using spectrophometric methods, reading the absorbance at 530 nm and the thickness to obtain the specific absorbance. Using a previous calibration curve, absorbance *versus* dose, it was obtained the estimated absorbed dose for the irradiated product.

This dosimeter it is not actually available in the market.

Following the recommendations of good practices for e-beam irradiations (ISO/ASTM ISO/ASTM51431:2005; ISO/ASTM51631:2013), it was also used a calorimeter as a standard dosimeter.

Calorimeter

For dose estimation in the electron beam irradiations it was used a graphite calorimeter, as standard dosimeter.

The calorimeter is made of a material that under e-beam irradiation the relation between absorbed dose and temperature is well defined, being previously calibrated against a primary standard.

The relation between the absorbed energy, E, mass, m, characteristics of the material (specific heat capacity, c) and temperature increase, ΔT, is given by:

$$E = m\, c\, \Delta T$$

And the absorbed dose, in Gray, is given by the equation

$$D = E / m$$

We have used a graphite calorimeter, where (ICRU, 2008)

$$c_p = 644.9 + 2.94\, T \quad (J\, kg^{-1}\, K^{-1})$$

In our case, we measured the sensor electrical resistance placed near the graphite wafer, before and after irradiation, to obtain the temperature increase during the irradiation.

The calorimeter was transported in a thermally isolated box of polystyrene foam, in the conveyor and in the same irradiation batch of the samples (Fig. A3.10). The temperature increase was measured offline, before and after irradiation, to estimate the absorbed dose following a previous calibration curve.

Fig. A3.10. Calorimeter in the e-beam conveyor and temperature reading.

Dosimetry

Gamma irradiation chamber dose mapping

A dosimetric characterization of the irradiation chamber was performed for the four levels and in each level, divided in a mesh of 33 positions, to characterize the dose rate, dose per unit time, using the support described in Appendix 1.

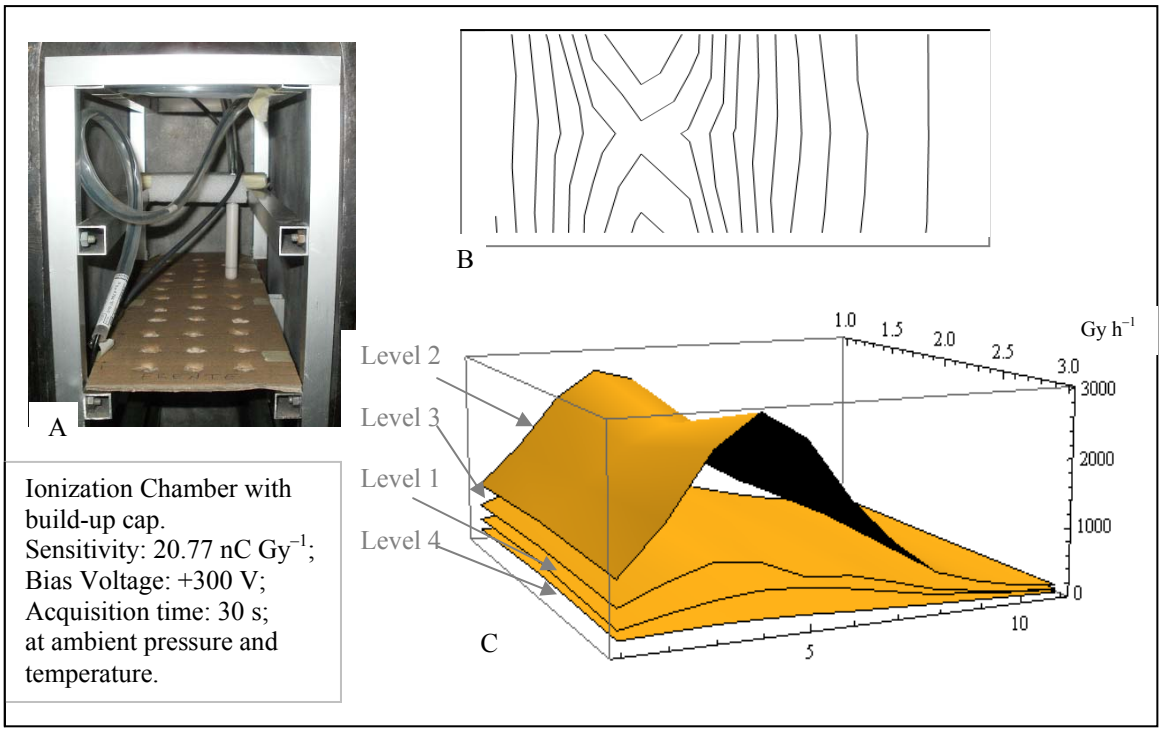


Fig. A3.11. Ionization chamber dose measurements.
(Level 2 (A); contour plot for Level 2 (B) and dose rate plot for the four levels (C).

Irradiation box dose mapping

The dose rate for each position inside the acrylic box used for fruits irradiations was measured by the three dosimetric systems: ionization chamber, Fricke dosimeter and Amber dosimeter (Fig. A3-11.).

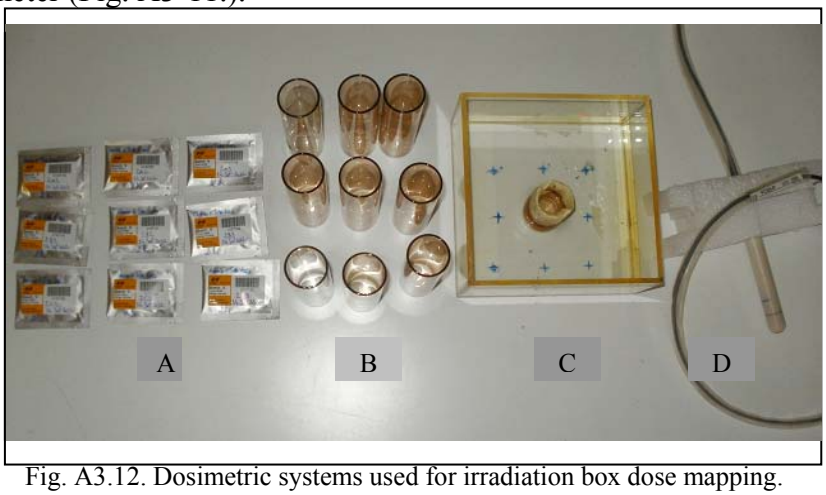


Fig. A3.12. Dosimetric systems used for irradiation box dose mapping.
(Amber perspex (A), Fricke tubes (B), irradiation acrylic box (C) and ionization chamber (D)).

Ionization chamber measurements

The ionization chamber went through all the nine positions in the irradiation box. For each position the dose rates were measured three times. In all cases the IC detector was used with the build-up cap and the sensitivity factor adjusted to $N_{D,W}$, water sensitivity.

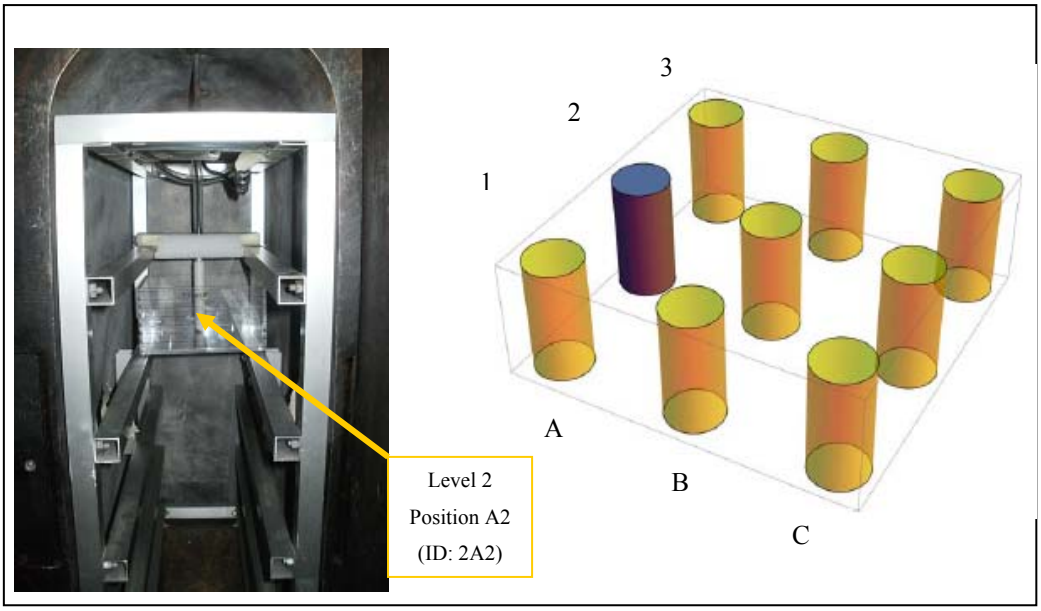


Fig. A3.13. Ionization chamber in the irradiation box and positions ID.

Amber Perspex measurements

The dosimeters are not a point and this was taken in account to choose the positions of Amber Perspex inside the irradiation box. The dosimeters were chosen in the interior top and bottom faces of irradiation box.

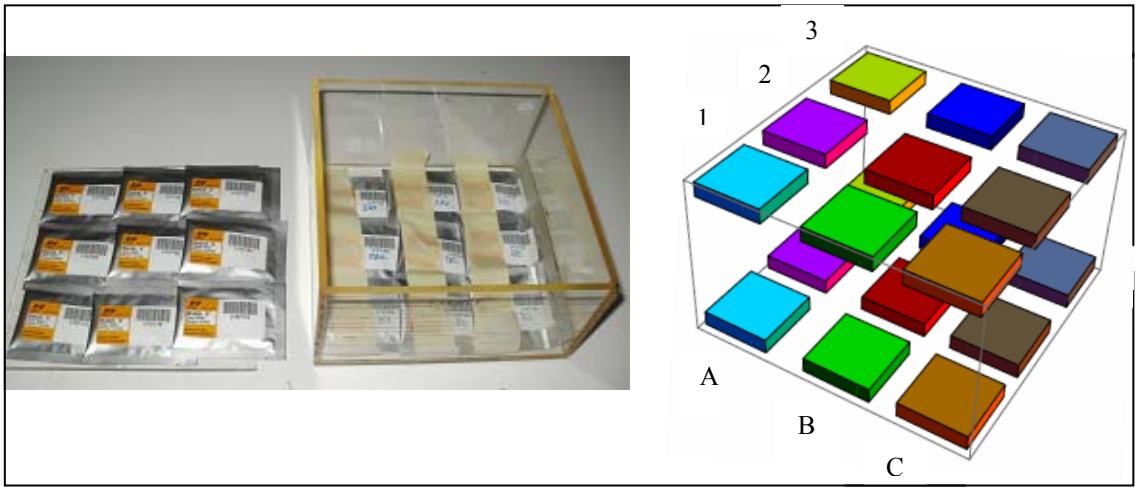


Fig. A3.14. Amber dosimeters in the top and bottom of the irradiation box.

ionization chamber measurements are already water equivalent, since they were done with the build-up cap and the sensibility factor *Nw*. Fricke measurements were

converted to absorbed dose in water using the relation, $D_w = D_{Fricke}$ x *1.005*. *Amber Perspex* absorbed dose in water was determined using the relation $D_{water} = D_{PMMA}$ x *1.033*. The results were also corrected with the decay of ^{60}Co, considering that some measurements were done in a different date.

In Fig. A3.14. is presented the contour plot, 2D and 3D, for dose rate values inside the irradiation box, measured with the ionization chamber. The dose rate profiles are similar for the three dosimetric systems.

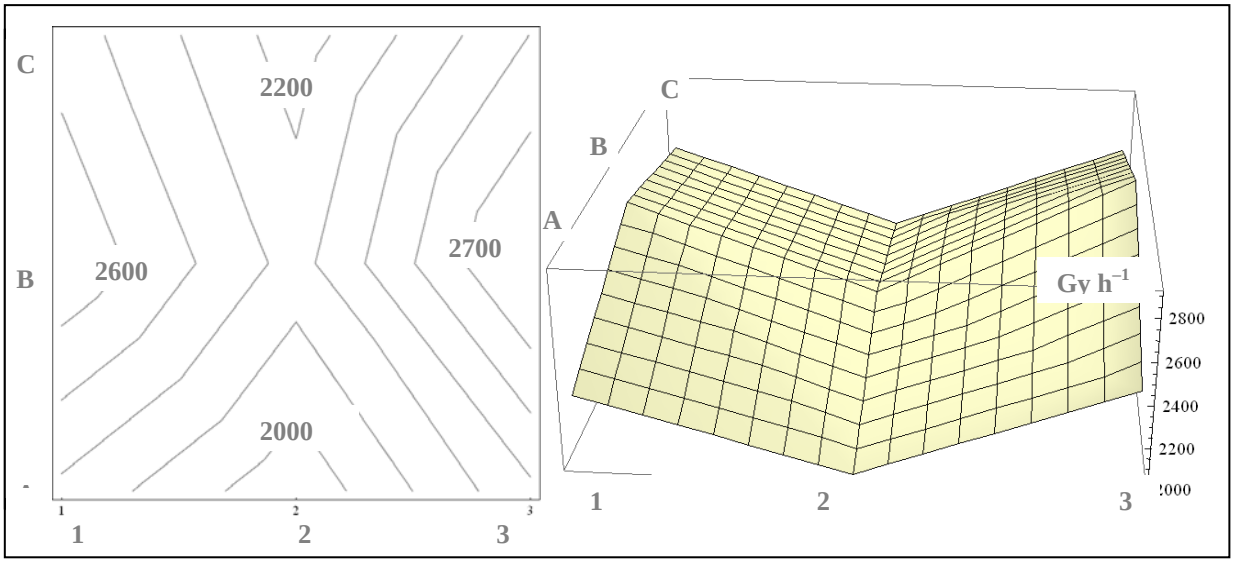


Fig. A3.15. Irradiation box dose rate map 2D and 3D.

The results indicate that to achieve a good dose uniformity ratio, low DUR value, and the samples should be rotated. This proceeding was done for the irradiated samples at half of the irradiation time.

Fruits dose validation

Food has a density similar to water, the interaction radiation mechanisms of water radiolysis are sometimes transposed to food irradiation to understand or at least give a general overview of different mechanisms involved in the interaction of radiation with the molecules that constitute the food. This fact leads to opt for water equivalent dosimeters, with a density similar to water or food, *e.g.* Fricke dosimeter or Amber Perspex $(C_5 H_8 O_2)_n$.

The dose conversion from the detector to fruit is given by (AAPM, 1986):

$$D_F = D_d \left(\frac{S}{\rho} \right)_d^F \qquad \text{(eq. 9)}$$

where D_F is the dose in the fruit; D_d the dose in the detector; $(S/\rho)^F_d$ is the detector to fruit ratio mass stopping power.

For Perspex dosimeters, the density is close to food and water, the ratio of mass stopping powers is close to one, $D_F \sim 1.033\ D_d$. The same applies for the aqueous solution Fricke dosimeter, $D_F \sim 1.005\ D_d$.

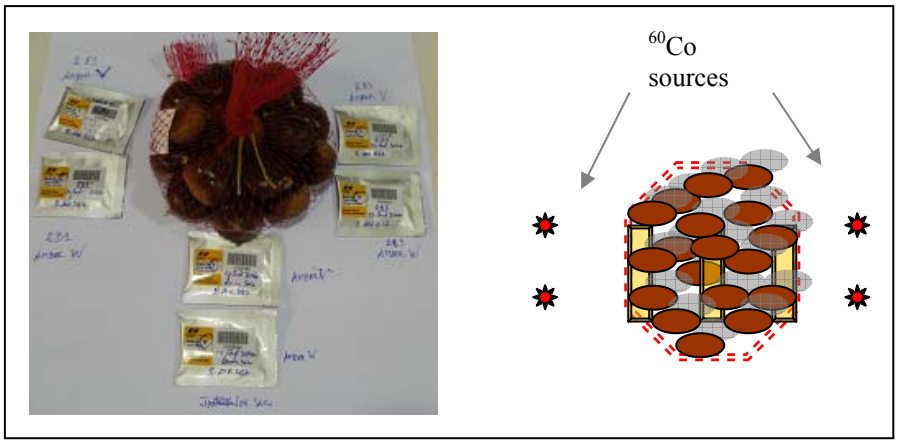


Fig. A3.16. Chestnut fruits with dosimeters and relative position to ^{60}Co sources.

Standards

ISO/ASTM51204:2004
Practice for dosimetry in gamma irradiation facilities for food processing.
ISO/ASTM51431:2005
Practice for dosimetry in electron beam and x-ray (Bremsstrahlung) irradiation facilities for food processing.
ISO/ASTM51900:2009
Guide for dosimetry in radiation research on food and agricultural products.
ISO/ASTM52116:2013
Practice for dosimetry for a self-contained dry-storage gamma irradiator.
ISO/ASTM51261:2013
Practice for calibration of routine dosimetry systems for radiation processing.
ISO/ASTM51707:2005
Guide for estimating uncertainties in dosimetry for radiation processing.
ASTM E1026:2013
Practice for using the Fricke dosimetry system
ISO/ASTM51276:2012
Practice for use of a polymethylmethacrylate dosimetry system.
ISO/ASTM51631:2013
Practice for use of calorimetric dosimetry systems for electron beam dose measurements and routine dosimeter calibration.

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Appendix 4

Bioactive and nutritional parameters

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Main methods and techniques used for sample analysis

Extraction

Use an adequate solvent, one or several times, to obtain the component for analysis. This method is normally followed by filtration and/or evaporation of the remaining solvent.

Evaporation

Eliminates the volatiles and is performed, sometimes, at low temperature and at reduced pressure, to not affect the components of the substance that are sensitive to high temperatures.

Distillation

Use of heat and a refrigerated column to separate liquid mixtures with different boiling points.

Colorimetry

Measuring the absorbance in a double beam spectrophotometer, against a blank (without sample extract).

Chromatography

Separates the components of a substance by their different flow rates in a column:

– HPLC High Performance Liquid Chromatographic system is coupled to a pump that injects the samples for analysis, diluted in a solvent or mixture of solvents (mobile phase), and flowing in different types of separation columns, adapted to the molecules to be characterized.

The characteristics of the columns are the key to get high resolution, well separated peaks, allowing the correct identification of the different substances in a mixture.

This equipment has the possibility to use different type of detectors (UV- ultraviolet, RI - refractive index, FD - fluorescence detector, DAD - diode array detector …), according to the substances that is expected to be detected, and also the possibility to adjust the temperature of the separation column.

– GC Gas chromatography: in this technique the mobile phase is a gas and is used to identify substances that can be vaporized.

Experimental procedures

Samples

These studies included samples of European chestnuts specie (*Castanea sativa* Miller) from different origins (Portugal, Turkey and Italy) and of different varieties (Longal, Judia, Cota, Palummina), that have different organoleptic and physical characteristics, namely flavour, size and texture.

After irradiation, they were stored at 4 °C for 0 days, 30 days and 60 days, and at each time point was obtained a sub-sample for analysis.

Fig. A4.1. Chestnuts in the shell and irradiated varieties "Longal" and Judia".

Samples irradiation

Gamma irradiations were performed in an experimental Co-60 research chamber, at Nuclear and Technological Institute, Lisbon, Portugal.

The electron-beam irradiations were performed at the Institute of Nuclear Chemistry and Technology, Warsaw, Poland.

For each case, an adequate dosimetric characterization was performed to estimate the absorbed dose, using standard and routine dosimeters (see Appendix 3.).

The financial support of a national research project allowed following and executing the irradiations in each institute.

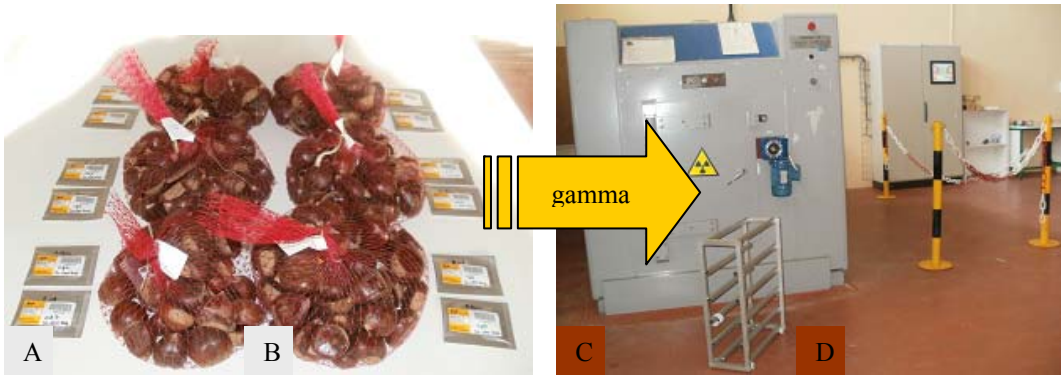

Fig. A4.2. Chestnut samples (A), dosimeters (B), Co-60 chamber (C) and aluminium support (D).

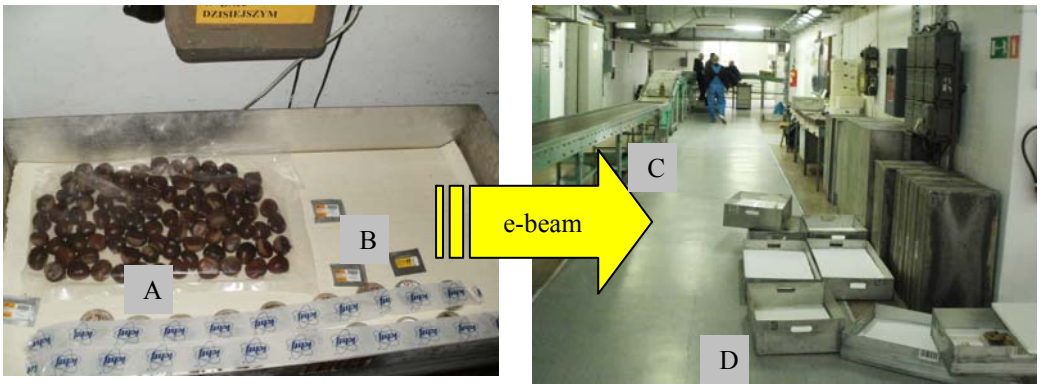

Fig. A4.3. Chestnut samples (A), dosimeters (B), e-beam conveyor (C) and aluminium trays (D).

Sample analysis

The samples were hand-peeled and the fruits separated from the outer and inner skins, to be analysed separately.

Then they were milled, mixed to obtain homogenate samples and lyophilized.

Fig. A.4.4. Chestnuts physical characterization, peeling and separated skins and fruits.

Extraction procedure

The lyophilized powder (1 g) was stirred with methanol (30 ml) at 25 ^{o}C at 150 rpm for 1 h and filtered through Whatman paper n^{o}. 4. The residue was then extracted with an additional portion of methanol (about 20 ml).

The combined methanolic extracts were evaporated under reduced pressure in a rotary evaporator (Büchi R-210; Flawil, Switzerland), re-dissolved in methanol at a defined concentration (stock solution), and stored at 4 ^{o}C for further use.

Fig. A4.5. Extraction process (A), filtration (B) and rotary evaporator for solvents (C).

Extract solutions for analysis

Successive dilutions with methanol, MeOH, were made from the stock solution to be submitted to *in vitro* assays.

The relation used for dilutions was:

$$C_1 V_1 = C_2 V_2$$

where C_1 is the initial concentration; V_1 is the initial volume (or calculated volume to be pipetted); C_2 the final concentration; and V_2 the final volume (vials volume).

For a stock solution concentration of 50 mg/ml, for example, the dilution process to fill a vial of 10 ml is represented in Fig. A4.6. For the defined concentration, an adequate volume is pipetted from the previous vial. The missing part is filled with methanol (MeOH).

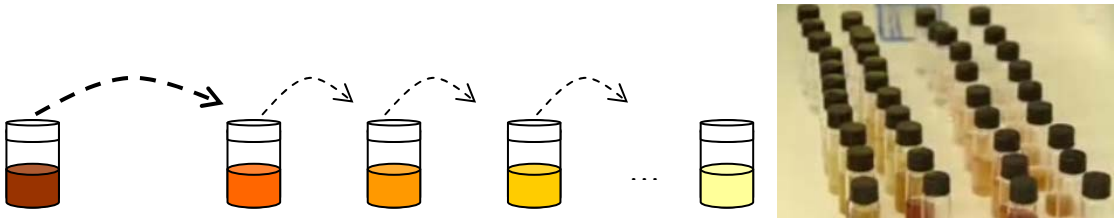

Fig. A4.6. Dilution process and vials with extracts at different concentration.

Total phenolics

In this test is used the Folin-Ciocalteu reagent, a mixture of phosphomolybdate and phosphotungstate, for colorimetric assay determination of total phenolic compounds in the extracts.

An aliquot of the extract solution (1 ml) was mixed with Folin-Ciocalteu reagent (5 ml, previously diluted with water 1:10 v/v) and sodium carbonate, Na_2CO_3, (75 g/l, 4 ml). The tubes were vortexed for 15 s and allowed to stand for 30 min. at 40 ^oC for colour development. Absorbance was measured at 765 nm (AnalytikJena 200 spectrophotometer, Jena, Germany).

Gallic acid was used in the Folin-Ciocalteau assay to calculate the standard curve and the results were expressed as mg of Gallic Acid Equivalent (GAE) per g of extract.

However, Folin-Ciocalteu reaction is considered a qualitative and limited method, since it measures phenols and other reducing substances present in the extracts. For the identification and quantification of phenols are used techniques and equipments.

Total flavonoids

An aliquot (0.5 ml) of the extract solution was mixed with distilled water (2 ml) and subsequently with $NaNO_2$ solution (5%, 0.15 ml). After 6 min, $AlCl_3$ solution (10%, 0.15 ml) was added and allowed to stand further 6 min., thereafter, NaOH solution (4%, 2 ml) was added to the mixture. Immediately, distilled water was added to bring the final volume to 5 ml. Then the mixture was properly mixed and allowed to stand for 15 min.

An isomer of Catechin, (+)Catechin, was used as reference antioxidant to calculate the standard curve, measuring the intensity of pink colour at 510 nm. The results were expressed as mg of Catechin Equivalents (CE) per g of extract.

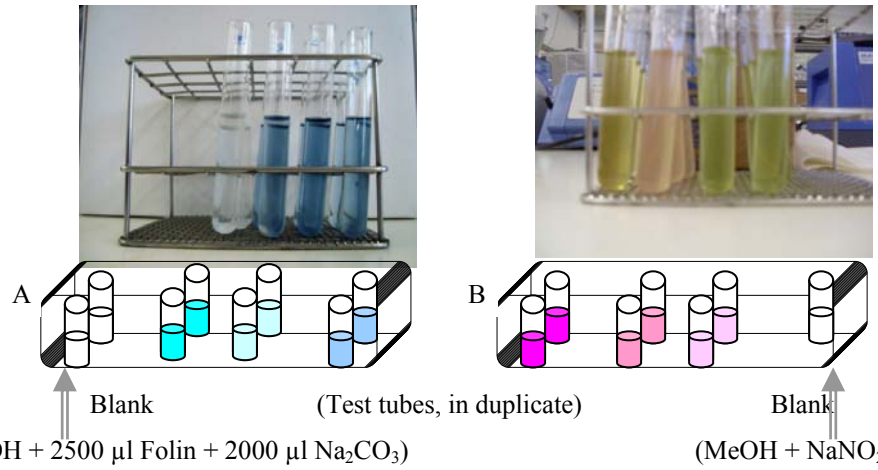

Fig. A4.7 Phenols (A) and flavonoids (B) test assays.

Antioxidant activity

Was measured by different biochemical assays: scavenging activity on DPPH radicals (measuring the decrease in DPPH radical absorption after exposure to radical scavengers); reducing power (measuring the conversion of a Fe^{3+}/ferricyanide complex to the ferrous form); inhibition of β-carotene bleaching (by neutralizing the linoleate-free radical and other free radicals formed in the system which attack the highly unsaturated β-carotene models); and TBARS assay (evaluating the decrease in thiobarbituric acid reactive substances). These tests measure *in vitro* antioxidant capacity, quantified by spectrophotometry.

The sample concentrations providing 50% of antioxidant activity or 0.5 of absorbance (EC_{50}) were calculated from the graphs of antioxidant activity percentages (DPPH, β-carotene/linoleate and TBARS assays) or absorbance at 690 nm (reducing power assay) against sample concentrations.

DPPH radical scavenging activity

This methodology measures the reaction of DPPH (2,2-diphenyl-1-picrylhydrazyl), a synthetic radical, with the antioxidant extract by colour changing from purple to light yellow. The percentage of DPPH discolouration is calculated using an ELX800 microplate reader (Bio-Tek Instruments Inc., Winooski, USA) (Fig. A4.8).

The reaction mixture in each one of the 96-wells consisted of one of the different concentrations of the extracts (30 µl) and aqueous methanolic solution (80:20 v/v, 270 µl) containing DPPH radicals ($6x10^{-5}$ mol/l). Before reading, the mixture was left to stand for 60 min. in the dark.

Fig. A4.8. Microplate reader and extracts reaction with DPPH.

The reduction of the DPPH radical with the antioxidant extract (Fig. A4.9) was determined by measuring the absorption at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation:

$$\%RSA = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$$

Where A_S is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution.

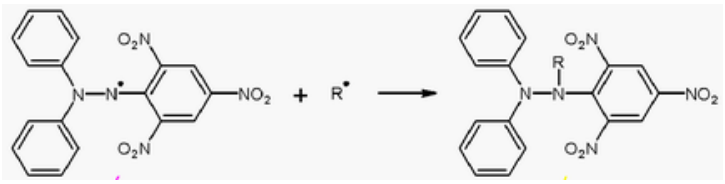

Fig. A4.9. DPPH radical reaction ("radical capture").

The extract concentration providing 50% of radicals scavenging activity (EC_{50}) was calculated from the graph of RSA percentage against extract concentration. Was used as reference Trolox, a water-soluble analogue of vitamin E, considered a standard for *in vitro* antioxidant capacity assays.

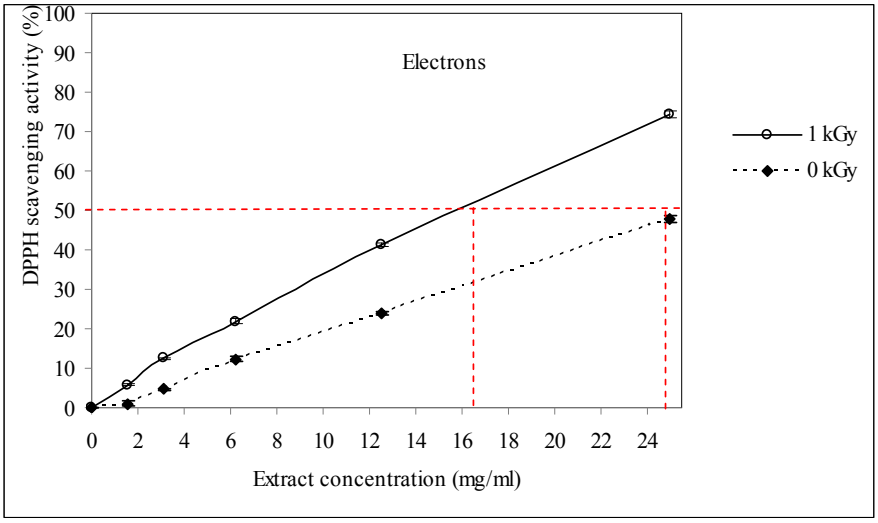

Fig. A4.10. Radical scavenging activity percentage against extract concentration.

Reducing Power

This methodology evaluated the capacity to convert Fe^{3+} into Fe^{2+}, measuring the absorbance at 690 nm using an ELX800 microplate reader (Bio-Tek Instruments Inc., Winooski, USA). In the presence of antioxidants, the yellow coloured ferrous solution changes to Prussian blue (Fig. A4.11).

The different concentrations of the extracts (0.5 ml) were mixed with sodium phosphate buffer, Na_3PO_4 (200 mmol/l, pH 6.6, 0.5 ml) and potassium ferricyanide, $K_3[Fe(CN)_6]$, (1% w/v, 0.5 ml). The mixture was incubated at 50 ^oC for 20 min, and trichloroacetic acid (10% w/v, 0.5 ml) was added. The mixture (0.8 ml) was poured in the 48-wells, as also deionised water (0.8 ml) and ferric chloride (0.1% w/v, 0.16 ml), and the absorbance was measured at 690 nm in the microplate reader.

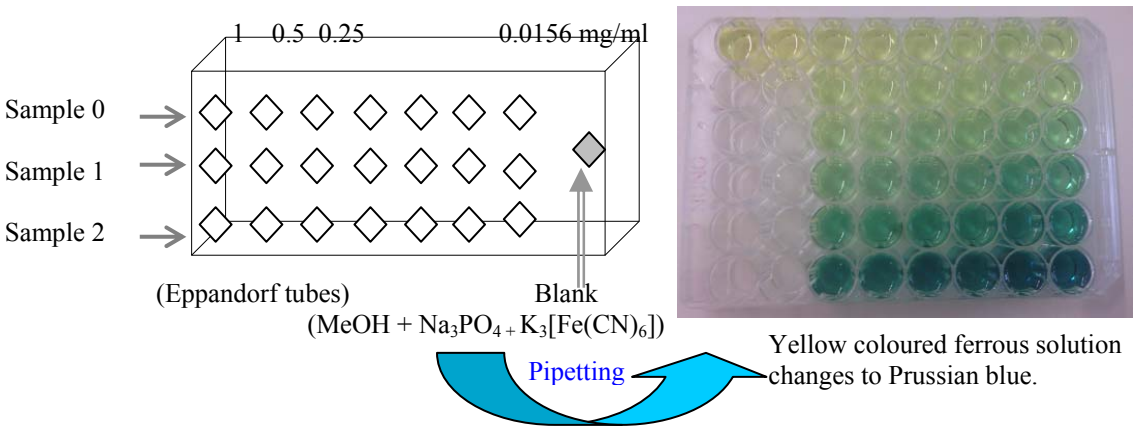

Fig. A4.11. Ferricyanide/Prussian blue assay.

The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 690 nm against extract concentration. Trolox was used as standard.

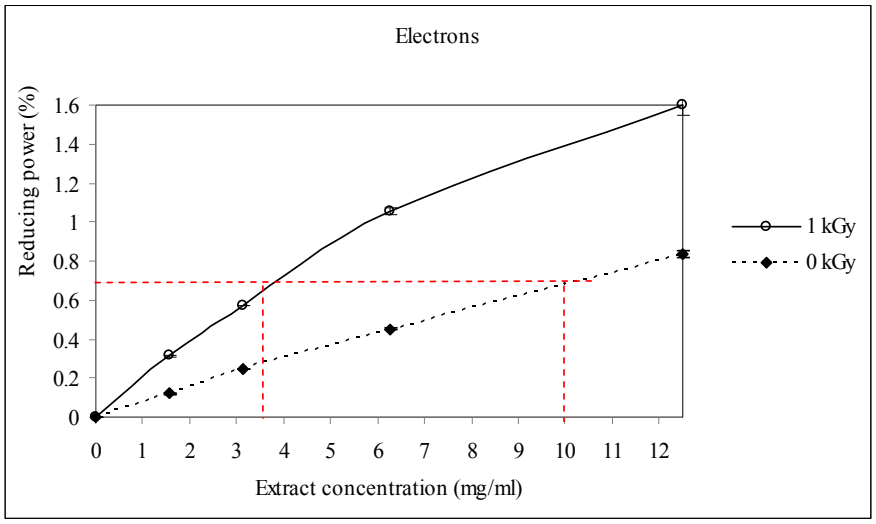

Fig. A4.12. Reducing power for irradiated and non-irradiated samples.

Inhibition of β-carotene bleaching

This capacity was evaluated though the β-carotene/linoleate assay; the neutralization of linoleate free radicals, by the antioxidants present in the sample extract, avoids β-carotene bleaching. The decolouration of orange coloured β-carotene is inversely proportional to the quantity of antioxidants in the extracts.

A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in chloroform (10 ml). Two millilitres of this solution were pipetted into a round bottom flask. After the chloroform was removed at 40 ^{o}C under vacuum, linoleic acid (40 mg), Tween® 80 emulsifier (400 mg) (Sigma-Aldrich, USA), and distilled water (100 ml) were added to the flask with vigorous shaking. Aliquots (4.8 ml) of this emulsion were transferred into different test tubes containing different concentrations of the extracts (0.2 ml). The tubes were shaken and incubated 2 h at 50 ^{o}C in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm, in a double beam spectrophotometer with a blank cell, a tube without β-carotene.

The inhibition of β-carotene bleaching, in percentage, was calculated using the following equation:

[(β-carotene absorbance after 2 h of assay) / (initial absorbance)] x *100*

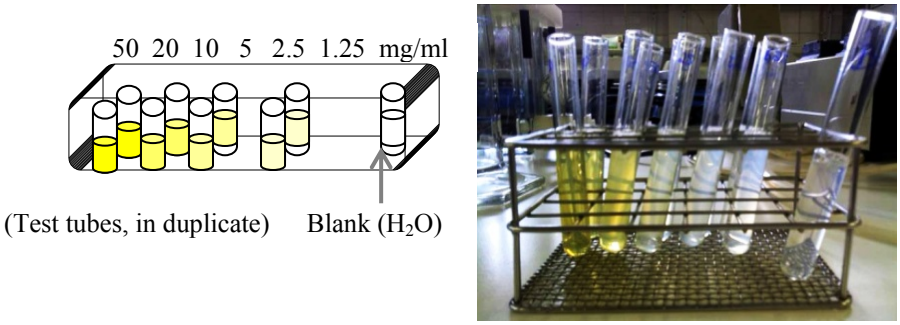

Fig. A4.13. β-carotene bleaching inhibition assay.

The extract concentration (EC) providing 50% antioxidant activity (EC_{50}) was calculated by interpolation from the graph of β-carotene bleaching inhibition percentage against extract concentration. Trolox was used as standard.

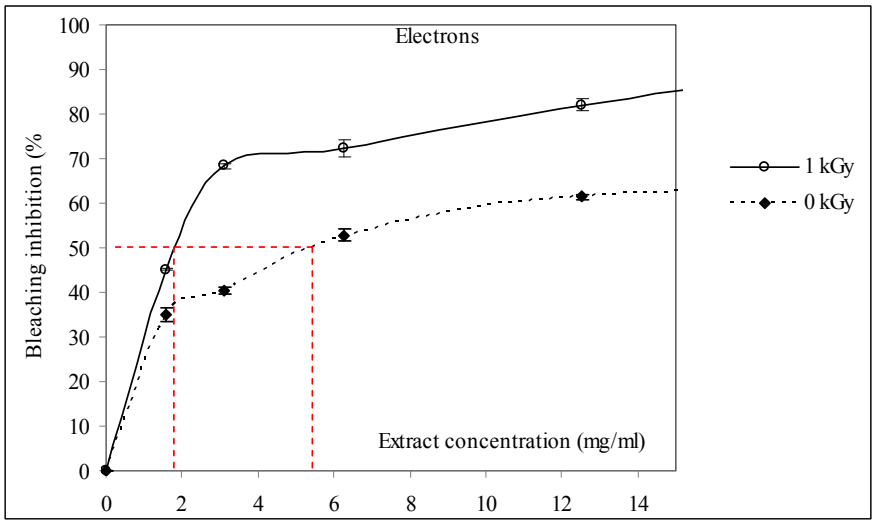

Fig. A4.14. β-carotene bleaching inhibition curve.

TBARS assay

To assess lipid peroxidation inhibition in biological material was used porcine (*Sus scrofa*) brain homogenates. It was evaluated the decreasing in thiobarbituric acid reactive substances (TBARS), that were measured by colorimetric methods.

Fig. A4.15. TBARS *in vitro* assay.

The lipid peroxidation or oxidative degradation of lipids is measured by the quantity of oxidation products that react with thiobarbituric acid to form pink compounds, quantified by spectrophotomety (Fig. A4.15).

Brains from pig (*Sus scrofa*), dissected and homogenized with Tris–HCl buffer (20 mM, pH 7.4) to produce a 1:2 (w/v) brain tissue homogenate which was centrifuged at 3000g for 10 min. An aliquot (0.1 ml) of the supernatant was incubated with the extracts (0.2 ml) in the presence of $FeSO_4$ (10 µM, 0.1 ml) and ascorbic acid (0.1 mM, 0.1 ml) at 37 ºC for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 0.5 ml), followed by thiobarbituric acid (TBA, 2%, w/v, 0.38 ml), and the mixture was then heated at 80 ºC for 20 min. After centrifugation at 3000g for 10 min to remove the precipitated protein, the colour intensity of the TBARS in the supernatant was measured by its absorbance at 532 nm.

The inhibition ratio (%) was calculated using the following formula:

$$[(A - B)/A] \times 100\%$$

where A and B were the absorbance of the control and the sample solution, respectively.

The extract concentration providing 50% lipid peroxidation inhibition (EC_{50}) was calculated from the graph of antioxidant activity percentage against extract concentration. Trolox was used as standard.

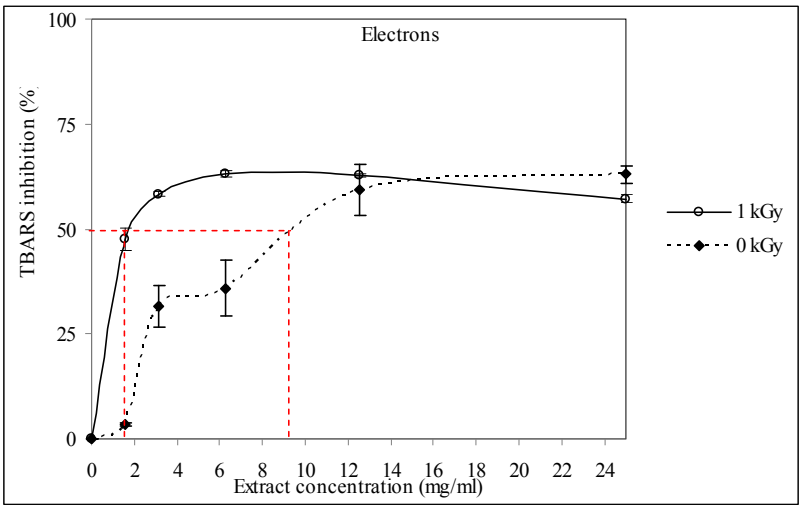

Fig. A4.16. Lipidic peroxidation inhibition curve.

Proteins

The crude protein content of the samples was estimated by the Kjeldahl method, a standard procedure that allows the estimation of nitrogen quantity in the samples.

Fig.A4.17. Digestion of the samples in sulphuric acid

Fat

The crude fat was determined by extracting a known weight of the powdered sample with petroleum ether, using a Soxhlet apparatus [Franz v. Soxhlet, 1879].

The powder of the sample, about 3 g, is putted inside paper filter and closed and the extraction procedure followed several cycles, during about 12 h.

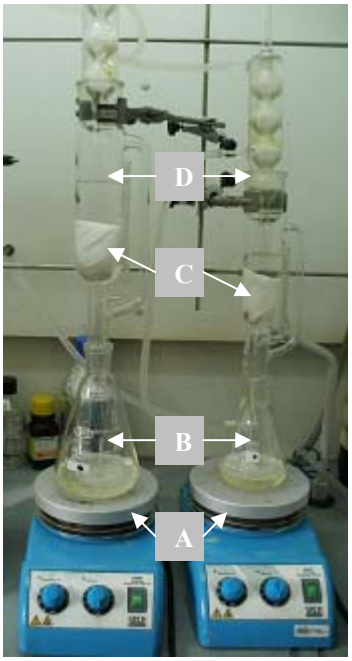

Fig. A4.18. Fat extraction of two samples in a Soxhlet.
(A – Hot plates, B – Erlenmeyer flasks,
C – Samples; D – Distillation columns)

Ash

The ash content was used only to determine the total carbohydrates by difference.

The samples were incinerated in a crucible of silica at 600 ^{o}C and the ash content measured by weight.

Fig. A4.19. Muffle for samples incineration.

Sugars, fatty acids, tocopherols, organic acids and triacylglycerols

The extraction, identification and quantification for these molecules were performed by chromatographic techniques, which were described in detail in the published papers.

It is presented in Fig. A4.20 a simplified diagram of an HPLC system and a typical chromatogram (Fig. A4.21.), where the peak position refers to a substance or molecule, identified and quantified using standards. In the separation column, different "colours" seen by the detector generates an output, an electric signal, expressed in Volt or milivolt that is registered in the data acquisition system *versus* the retention time in the column.

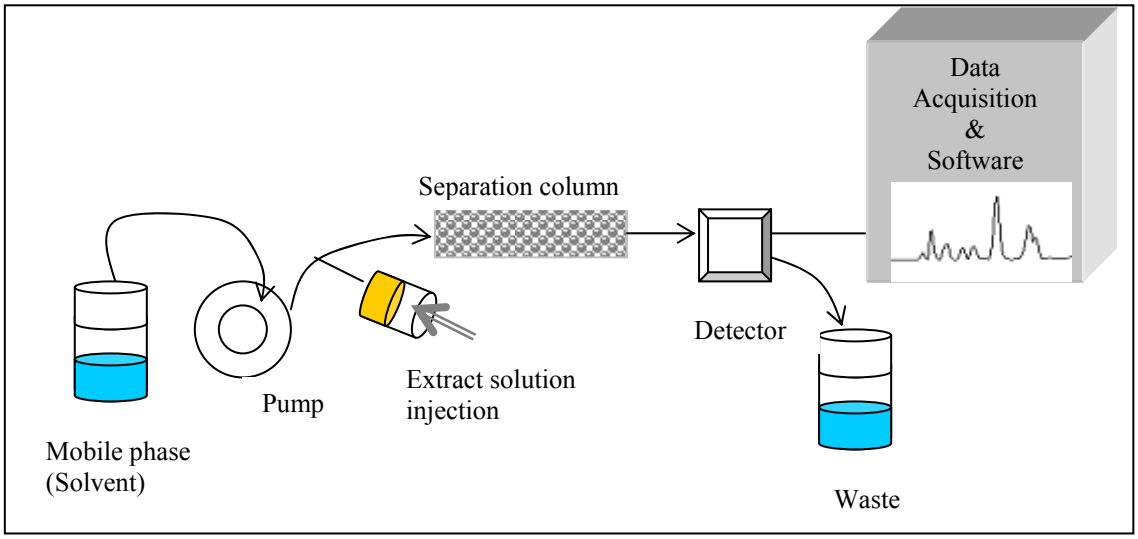

Fig. A4.20. Simplified schematic diagram of a HPLC system.

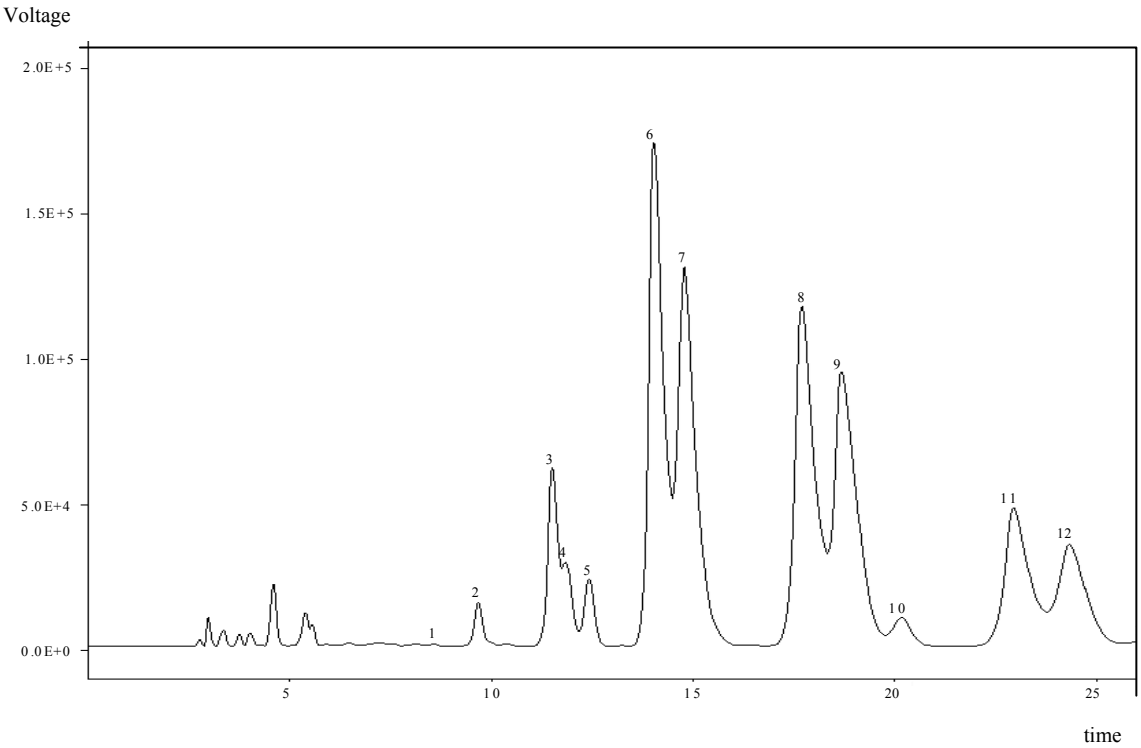

Fig. A4.21. A chromatogram for substances identification.

In Fig. A4.22 is presented the equipments used for identification and quantification of substances in irradiated and non-irradiated chestnut fruit extracts.

Fig. A4.22. Chromatographic equipments used in the experiments for compounds identification.

Reference

AOAC, 2000. Official methods of analysis of AOAC International, editor W. Horwitz (AOAC International, USA).

Appendix 5

Statistic tools and data analysis

For data analysis was used as main tool the SPSS Statistics software for Windows (IBM Corp., USA), with its integrated statistical packages. And what is referred below in this section is based mainly in SPSS users guide (IBM 2013).

To analyze the differences between groups an analysis of variance (ANOVA) with Type III sums of squares was performed, an approach that is also valid for unbalanced data and in the presence of significant interactions, using the GLM (General Linear Model) procedure of the SPSS software.

The dependent variables were analyzed using 2-way ANOVA, with the main factors ‘‘irradiation dose’’ (ID) and ‘‘storage time’’ (ST). If no statistical significant interaction was verified, the means were compared using Tukey’s test.

When a (ID x ST) interaction was detected, the two factors were evaluated simultaneously by the estimated marginal means (EMM) plots for all levels of each single factor.

Furthermore, a linear discriminant analysis (LDA) was used to assess the classification of different storage times and irradiation doses in different groups. A stepwise technique, using the Wilks’ λ method with the usual probabilities of F, 3.84 to enter and 2.71 to remove, corresponding to a p-value of 0.05 and 0.10, respectively, was applied for variable selection.

This method uses a combination of forward selection and backward elimination procedures, where before selecting a new variable to be included in the model, it is verified whether all variables previously selected remain significant. SPSS software starts the process including the variable with the smallest p-value and removing the variables where p-value is larger than the setting limits. The process stops when all the variables that meet the criteria are included (Horber 2014).

This procedure allows the identification of significant variables in each group. The model is composed of a discriminant function based on linear combinations of the predictor variables that provide the best discrimination between the groups.

To verify which canonical discriminant functions were significant, the Wilks’ λ test was applied. A leaving-one-out cross-validation (LOOCV) procedure is carried out to assess the model performance, to estimate how accurately the predictive model will perform in practice.

Leaving-one-out procedure is a sophisticated version for model validation, computing its accuracy, not including one data from the set and repeating the routine procedure for all the data (Arlot 2010).

A good model should allow a correct classification performance for the samples in the original groups ("training set"), as well in cross-validation procedure for the "test set".

Principal component analysis (PCA) was also applied to obtain the unknown patterns for the measured variables. PCA transforms the original measured variables into new uncorrelated variables called principal components. The first principal component covers as much of the variation in the data as possible. The second principal component is orthogonal to the first and covers as much of the remaining variation as possible, and so on (Pearson 1901).

The number of dimensions to keep for data analysis was evaluated by the respective eigenvalues, which should be greater than one, by the Cronbach's alpha parameter, that must be positive, and also by the total percentage of variance, that should be as higher as possible, explained by the number of components selected.

The number of dimensions considered for PCA was chosen in order to allow meaningful interpretations, and to ensure their reliability.

All the assays were carried out in triplicate, statistical tests were performed at a 5% significance level and the numerical results were expressed as mean values with standard deviation.

References

Arlot, S., Celisse, A. (2010). "A survey of cross-validation procedures for model selection." Statistics Surveys **4**: 40-79.

Horber, E. (2014). "Regression Methods." 2014, from http://www.unige.ch/ses/sococ/cl//spss/cmd/regression.methods.html.

IBM (2013). SPSS Statistics for Windows, Version 22.0. Armonk, NY, IBM Corp.

Pearson, K. (1901) "On lines and planes of closest fit to systems of points in space." Philosophical Magazine **2**, 559-572.