Effects of gamma radiation on the biological, physico-chemical, nutritional and antioxidant parameters of chestnuts - A Review

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Abstract

Gamma radiation has been used as a post-harvest food preservation process for many years. Chestnuts are a seasonal product consumed fresh or processed, and gamma irradiation emerged recently as a possible alternative technology for their post-harvest processing, to fulfil the requirements of international phytosanitary trade laws. After harvest and storage, several problems may occur, such as the presence of infestations and development of microorganisms, namely rotting and fungi. These diminish the quality and safety of the product, decreasing the yield along the production chain. In fruits, gamma irradiation treatment is for two main purposes: conservation (ripening delay) and insect disinfestation (phytosanitary treatment). In this review, the application of gamma irradiation to chestnuts is discussed, including production data, the irradiated species and the effects on biological (sprouting, rotting, respiration rate, insects, worms and fungi), physico-chemical (colour, texture, drying rate), nutritional (energetic value, proteins, sugars and fatty acids) and antioxidant (tocopherols, ascorbic acid, phenolics, flavonoids and antioxidant activity) parameters. These changes are the basis for detecting if the food product has been irradiated or not. The validation of standards used for detection of food irradiation, as applied to chestnuts, is also discussed.

Keywords: Chestnuts; gamma irradiation; biological parameters; physico-chemical parameters; irradiation detection.

1. Introduction

1.1. Chestnuts world production and main species

The chestnut tree is typically found in the south of Europe, in mountain areas of Mediterranean countries, and in Asia, mainly in China. The main region for production of chestnuts is Asia (85%), followed by Europe (12%), and they were only recently introduced in some countries of the Southern hemisphere such as Chile, Argentina, Australia and New Zealand (remaining 3%) (Pereira-Lorenzo, 2006). China leads the production of Asian varieties, with 1 620 000 tons, and for European varieties the main producer is Turkey, with 59 171 tons (**Figure 1**). In Asia there are different varieties, with the common name of Chinese chestnuts (*Castanea mollissima* Blume), Korean chestnuts (*Castanea bungena* Blume) and Japanese chestnuts (*Castanea crenata* Siebold & Zucc.), with different cultivars. In Europe, the variety produced is *Castanea sativa* Miller, with different cultivars and, like Asian varieties, with different characteristics, namely size, ability to peel and taste (Barreira et al., 2009).

1.2. Infestations in chestnuts and post-harvest treatments

Chestnuts are infested by larvae of different species, depending on the region of the world. *Curculio sikkimensis* Heller, *Curculio elephas* Gyllenhal and *Cydia splendana* Hübner are being cited as the main infestations, causing rotting and loss of income for the producers and for the food industry (Kwon et al., 2001; 2004). At harvest time, up to 20% or more of the crop could be infected by one or two of these species, and with one or several larvae (Vinghes et al., 2001). Larvae consume the product and, since there is an international market for chestnuts, the international phytosanitary regulations impose quarantine rules whenever there is a threat of the infestanting species to the local ecosystem. Several methods are used to meet quarantine safety rules; chemical fumigation with methyl bromide, a broad spectrum

fumigant, has been the most efficient method to treat stored food products, including chestnuts (Ahmed, 2001; Kwon et al., 2004). Following the Montreal Protocol (UNEP, 2006), the European Union (EU) banned its use after March 2010 and updated the decision in 2011 (EU, 2008; EU, 2011). There are also other possible post-harvest treatments, like submerging in ice, controlled atmospheres and hot water dip treatment (Kwon et al., 2001; UNEP, 1995). Hot water dip treatment is recognized as a valid treatment but with "possible damage to the flesh of some fresh fruits, which may compromise the fruit quality" (Aegerter, 2000).

To assure quarantine security, defined "in a way that the insect infestations cannot establish in an area where they do not exist" using the concept of probit-9 (mortality of 99.9968%) (Aegerter, 2000), this target was easily reached with fumigation but with some limitations when irradiation was used (Marcotte, 1998). This is mainly because it does not cause the immediate death of the larvae, and the absence of trained quarantine officials for checking irradiated food. International organizations are putting some efforts in to encourage adoption of international standards for phytosanitary measures, namely for the use of irradiation to prevent the introduction or spread of pests (ISPM, 2003). Gamma irradiation appears as a safe quarantine post-harvest treatment for disinfestations (elimination of insects), being now validated for different species of insects (IAEA, 2004; IDIDAS, 2012; ISPM 28, 2007). The *Codex Alimentarius* also has a recommendation for the use of irradiation in disinfestations of food and agricultural products (ICGFI, 1998) and this post-harvest treatment is approved by several countries to treat different food products to meet the quarantine regulations during exportation (USA Federal Register, 1996; Food Standards Australia New Zealand, 2003; USDA-APHIS, 1989), with some countries establishing a minimum dose for different classes of insects (USA Federal Register, 2006).

1.3. Gamma radiation

The possibility of using ionizing radiation to treat foodstuffs was cited in the literature in 1896, one year after the discovery of X-rays by W. C. Röntgen (Molins, 2001). Gamma radiation, more energetic than X-rays, is used from sources of radioactive isotopes, cesium-137 or cobalt-60, and it is recognized by the World Health Organization as a food preservation technique that improves food safety without altering the toxicological, biological or nutritional quality of the food (WHO, 1981). 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 (EU, 2009) and is regulated by the Directive 1999/2/EC (EU, 1999). The relevant 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). The typical doses for sprout inhibition are lower than 0.5 kGy for delaying ripening, equal or lower than 1 kGy for insect disinfestations, 3 to 5 kGy to extend shelf-life and for pathogen elimination (fungi etc.), 5 kGy or higher if the food product supports it without losing the main characteristics (EPA, 1996; EU, 2009, IAEA, 2002).

Gamma radiation can be used to treat sealed containers or large volumes of food products. Each irradiation process must be validated, since fruits have different characteristics, size, water content, nutritional composition, etc. Therefore, there are several studies testing this post-harvest treatment in chestnuts.

2. Gamma irradiation of chestnuts

Gamma irradiation of fruit has been widely studied on vegetables and fruits for many years, and the results of this sterilization treatment are very interesting. Arvanitoyannis et al. (2009) compiled all the research in vegetable and fruit irradiation and reported that it could prolong

shelf-life, had no effect on physical and organoleptic properties and was a cheap alternative to other conventional conservation methods. Regarding chestnuts, gamma radiation has been applied on them, mainly in Asian varieties (Chung et al., 2004; Imamura et al., 2004; Iwata et al., 1959; Guo-Xin et al., 1980; Kwon et al., 2004) and recently in European varieties (Antonio et al., 2011a, b, c; Barreira et al., 2012; Calado et al., 2011; Fernandes et al., 2011a, b; Mangiacotti et al., 2009) (**Table 1**). It has been already approved as a commercial technique in South Korea, for sprouting or rooting inhibition (0.25 kGy) (Chung et al., 2004) and for quarantine disinfestations (0.50 kGy) (Kwon et al., 2004). Nevertheless, it is important to evaluate the effects of gamma radiation on biological, physico-chemical, nutritional and antioxidant parameters, and also the application of standards to detect if a food product was irradiated or not.

2.1. Effects on biological parameters

Sprouting, rotting and respiration rate. 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* (**Table 2**). In the first assays, *Castanea crenata* was irradiated with 0.07 kGy, and this dose completely inhibited sprouting, even after 60 days of storage. Nevertheless, the irradiated samples had a slightly higher percentage of rotting. In the second assay, *Castanea crenata* was irradiated with different doses (0.03, 0.07 and 0.12 kGy), and the authors reported that after 10 days no rotting was visible for all the irradiation doses when compared with the 4% of rotting in the control samples. After 26 days, the rotting percentage was 6, 7, 3 and 1% for control, 0.03, 0.07 and 0.12 kGy irradiated samples, respectively. No sprouting was observed for 0.07 and 0.12 kGy, while 0.03 kGy gave 30% of sprouting after the same storage time. Regarding *Castanea molissima*, these samples were irradiated with doses of 0.1, 0.15 and 0.2 kGy, and the results were very similar to the previous assay, where

the irradiated chestnuts showed always less sprouting and rotting. Concerning the respiration rate of *Castanea molissima* submitted to irradiation doses ranging from 0.10 to 0.20 kGy, there were 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 (doses of 0.25; 0.5; 1 and 10 kGy) and fumigated (methyl bromide) chestnuts (*Castanea crenata*). They reported that after 6 months of storage only the dose of 0.25 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. Overall, the optimal irradiation dose was 0.5 kGy, but all doses revealed lower rotting levels than the samples fumigated with methyl bromide.

Insects, worms and fungi. Insects are pests that induce great losses in chestnut conservation, and a very efficient way to control them is radiation. The most important pests are *Curculio sikkimensis* (Heller) and *Dichocrocis punctiferalis* (Guenee), a coleopter and a worm, respectively (**Table 2**). Kwon et al. (2004) compared the effects of methyl bromide and gamma irradiation (doses between 0 and 10 kGy) on these pests in *Castanea crenata* and determined that 100% of the pests perished in the fumigated samples and in irradiated samples with a dose of least 0.5 kGy. Imamura et al. (2004) studied the effects of gamma irradiation for *Castanea crenata* (0.05, 0.1, 0.2, 0.3, 0.4, 0.5 and 1 kGy) on the mortality of *Cydia kurkoi* (Amsel), and *Curculio sikkimensis* (Heller). They reported that doses of 0.3 kGy and higher displayed a mortality rate of 100% for *C. kurokoi*, and, 0.4kGy and higher killed all *C. sikkimensis*.

Regarding fungi, Calado et al. (2011) studied the effect of gamma radiation (doses of 0.25 to 10 kGy) on the survival of yeasts and *Aspergillus parasiticus*, one of the most ubiquitous and toxigenic fungi. The authors reported that both yeasts and *A. parasiticus* load decreased with at least 3 kGy, and did not survive at all when irradiated with a dose of 10 kGy.

2.2. Effects on physico-chemical parameters

Colour. Although chestnuts are legally irradiated in Korea (Chung et al., 2004), the public still has reservations in consuming irradiated chestnuts due to misconceptions about the alterations that gamma rays induce in colour and texture of these fruits. Kwon at al. (2004) studied the comparative colour alteration in the internal and external flesh of chestnuts irradiated with doses of 0.25, 0.5, 1 and 10 kGy and fumigated (methyl bromide). They reported that Hunters "L" (Hunter values indicate the reflected and transmitted light colour of food; "L" represents the lightness axis; "a" represents the red-green axis and "b" the yellow blue axis- yellowness) value only changed significantly at 10 kGy, but this alteration also took place for the fumigated chestnuts, and that doses under 1 kGy did not have any effect either on Hunters "L" and "b" values. Antonio et al. (2011a) compared Portuguese and Turkish irradiated chestnuts (0.5 and 3 kGy) on the outer and inner colour, reporting that Hunters "L" and "a" values did not vary independently of the irradiation dose and storage time (0 and 30 days) for the outer flesh. Regarding Hunters "b" value, it rose during the storage time for both Portuguese and Turkish chestnuts for the control and 0.5 kGy. The highest "b" value was reported at 3 kGy for Portuguese chestnuts independently of the storage time. Regarding the inner flesh, the Turkish chestnuts reported lower values of "L" and "a" than the Portuguese ones. The authors also concluded that there were no significant differences between doses and storage time for these values. In relation to the "b" value, it seemed to increase during storage time for the Turkish fruits. Finally, they reported, after

comparing all the doses and storage times that radiation up to 3 kGy does not seem to induce yellowing of the fruit flesh (**Table 3**).

Texture. Another parameter that is important to analyze in irradiated foods is alteration in texture. Antonio et al. (2011b) compared this parameter in irradiated chestnuts (0.5, 3 and 6 kGy) during 30 days, and reported a decrease in the texture with higher doses and for 30 days of storage (**Table 3**).

Drying rate. Antonio et al. (2012) reported that chestnuts irradiated with 1, 3 and 6 kGy only had slight changes in moisture ratio and drying rates when compared to the control in a drying process at 50ºC (**Table 3**). The changes increased with increasing radiation dose.

2.3. Effects on nutritional parameters

Macronutrients and nutritional value. Fernandes et al. (2011a) studied the effects of radiation doses (0.25, 0.5, 1 and 3 kGy) and storage times of 30 and 60 days on the nutritional value of chestnuts. The authors reported that dry matter, protein and ash contents were higher for 30 days of storage time, while carbohydrates, fat and energy contents were superior in samples with no storage time. These conclusions confirm that radiation at these doses did not affect the chestnut quality. These same parameters were also studied by Barreira et al. (2012) for Turkish chestnuts (*Castanea sativa*), also submitted to gamma radiation (0.5 and 3 kGy) and storage time of 15 and 30 days. The conclusions stated that the radiation was not a source of variation in these parameters, while storage time was. Guo-Xin et al. (1980) reported a study on gamma irradiated chestnuts (*Castanea mollissima*) with doses ranging from 0.25 to 1 kGy, in which a decrease in the percentage of total proteins was observed as the dose of radiation increased (**Table 3**).

Individual sugars. Sugars are the main storage quality indicator (Kazantzis et al., 2003) and are usually during a long storage time. Iwata et al. (1959) reported that irradiation of chestnuts with doses of 0.1, 0.15 and 0.2 kGy did not significantly alter the content of reducing and total sugars, even after 14 days of storage in moist sawdust. Some years later, Guo-Xin et al. (1980) came to the same conclusion (variation between 14.53 and 20.38% of total sugars, but using doses ranging from 0.3 to 1.2 kGy). Recently, Fernandes et al. (2011a) carried out an extensive study on individual sugars, namely fructose, glucose, sucrose, trehalose and raffinose, using High Performance Liquid Chromatography coupled to a Refraction Index detector (HPLC-RI) in order to understand in detail the effect of radiation (doses of 0.27 and 0.54 kGy) on these molecules. The authors concluded that regardless of the radiation dose, no significant changes were observed in the content of sugars, but the storage time seemed to reduce the quantity of total sugars. Another study, carried out by Barreira et al. (2012) on chestnuts (*Castanea sativa*) from Turkey, reported the same findings in relation to sucrose, demonstrating that the storage time alters this parameter more than radiation.

The effect of radiation on starch quantities was also studied by Guo-Xin et al. (1980) and they reported that doses of 0.1, 0.2 and 0.3 kGy did not significantly alter the quantity of starch in chestnuts (*Castanea mollissima*). The same authors extended the study to the activity of amylase and catalase after 27 days of storage time; catalase activity decreased during storage time in the control samples and in samples irradiated with 0.1 and 0.2 kGy; furthermore, the irradiated samples also maintained a lower activity when compared with the control. Catalase is known to be a powerful endogenous antioxidant defense when combined with superoxide dismutase (Ferreira et al., 2009), and this property might be related to the increase in its activity at a dose of 30 kGy, where the radiation might have triggered oxidative stress in the chestnuts. Regarding amylase, Guo-Xin et al. (1980) reported that a dose of 0.3 kGy raised

the activity considerably after 0 days, but during the storage period the activity decreased. Doses of 0.1 and 0.2 kGy seem to maintain the activity of this enzyme relatively low when compared to the control for 21 days, but after that period, the activity increases (**Table 3**). These are encouraging results because amylase breaks down starch into simpler sugars, altering the chestnut composition, and these doses of radiation seem to reduce this phenomenon.

Individual fatty acids. Fatty acids are important in chestnuts, mainly for their nutritional quality. Fernandes et al. (2011a) examined the alterations produced by gamma irradiation (doses of 0.27 and 0.54 kGy) on chestnut storage for 0, 30 and 60 days. The authors found 17 fatty acids and another 5 in trace quantities. C14:0, C16, C16:1, C18 and C23 were higher in irradiated samples; C18:0, C20:0, C20:1, and C23:0 were favored by storage time, particularly 60 days, while C16:1, C18:1 and C24:0 decreased with storage. No major differences were detected with the increase of the radiation dose. This study is in line with another study from the same authors (Fernandes et al., 2011b), where they only quantified the main fatty acids in chestnuts: palmitic (C16:0), oleic (C18:1 cis-9), linoleic (C18:2) and linolenic acids (C18:3), using higher irradiation doses (between 0.25 and 3 kGy) for the same storage periods. Control samples had higher values of palmitic acid, but lower linoleic and linolenic acids and the radiation once again did not induce any significant alteration. Another study that corroborates these findings is the one of Barreira et al. (2012) where once again they reported that radiation doses between 0.5 and 3 kGy do not induce any differences on monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids (**Table 3**).

2.4. Effects on antioxidant parameters

Vitamins. Tocopherols are isoforms of vitamin E and have important health benefits (Benatti et al., 2004; Hensley et al., 2004). Fernandes et al. (2011a) studied these vitamers in gamma irradiated chestnuts (0.27 and 0.54 kGy) for storage periods of 0, 30 and 60 days. The authors reported that the main isoform was γ-tocopherol for all samples, followed by δ-tocopherol and finally α-tocopherol, while β-tocopherol was not detected at all. The amounts of γ, δ and total tocopherols were maintained at higher levels in the irradiated samples, revealing a higher degradation of these compounds in the control samples (non-irradiated), probably due to a higher quantity of molecular oxygen in their sample bags. Regarding storage time, the amount of tocopherols increased during the first 30 days, but then started decreasing from there onwards, implying once again that storage time had more effects on the chestnuts than radiation. These results are agreement with another study carried out by the same authors, Fernandes et al. (2011b), where they used higher doses of gamma radiation (0.25, 0.5, 1, 3 and 6 kGy), observing that even at these doses, radiation had minimal effects on γ-tocopherol. Iwata et al. (1959) reported that ascorbic acid (vitamin C) present in chestnuts seemed to be more affected by storage time (10 days) than by irradiation doses of 0.1, 0.15 and 0.2 kGy (**Table 3**).

Phenolics, flavonoids and antioxidant activity. An extensive study was carried out by Antonio et al. (2011c) regarding the effects of gamma radiation on phenolics, flavonoids and antioxidant activity on Portuguese chestnuts (*Castanea sativa*) fruits and skins. The authors used doses of 0.27 and 0.54 kGy and storage times of 0, 30 and 60 days. For all the studied parameters, they reported that storage time has a much greater influence on the variation, radiation being a minor contributor to the increases in phenolics and flavonoids during the storage period. In regard to the antioxidant activity (measured by radical scavenging activity,

reducing power and lipid peroxidation inhibition), the higher dose of radiation seemed to preserve this activity when compared to the control (**Table 3**).

2.3. Identification of irradiated chestnuts

Irradiation processing meets several food safety requirements, by lowering or eliminating the presence of biological contaminants. In this way, techniques that allow the adequate checking of whether or not a food product has been adequately irradiated are welcome.

The correct identification of an irradiated product is part of the proceedings of the *Codex Alimentarius*, to check if the product was processed in a way that fulfils the HACCP analysis (Codex, 2003). Its acceptance by the "quarantine inspectors" requires special training and regulations since irradiation, unlike fumigation, does not always cause immediate mortality of the insects or larvae, sometimes only sterilizing or dying after several days (EPA, 1996; Marcotte, 1998).

International organizations and the scientific community have undertaken standardization of the procedures to detect if a food product has been irradiated or not (CEN, 2012; IAEA, 1991).

European and international standards. Some countries and regions, EU included, impose the correct labelling of irradiated food (Arvanitoyannis, 2010; EU, 1999). Also, quarantine officials need reliable methods to check if a food product has been adequately irradiated or not.

To meet these requirements several standards are established, to detect if a product has been irradiated or not, based on some biological, physical or chemical residual alterations of the processed product.

Depending on the type of food and the parameter analysed, one or several detection methods, discussed further below, can be used, grouped in physical, chemical, biological and DNA methods (Stewart, 2001). Ten European Standards have been adopted by the *Codex Alimentarius* and are included in the Codex of General Standard for Irradiated Foods (EN 1784:1996, EN 1785:2003, EN 1786:1996, EN 1787:2000, EN 1788:2001, EN 13708:2001, EN 13751:2002, EN 13783:2001, EN 13784:2001, and EN 14569:2004) (CEN, 2012).

Techniques. Electron Paramagnetic Resonance Spectroscopy (ESR or EPR), is the base for European norms EN 1786:2001, EN 1787:2000, and EN 13708:2001. These analyses involve detection of free radicals containing unpaired electrons, which are paramagnetic in an applied external magnetic field. The limitation of the technique is the lifetime of the radicals that are more stable in solid, dry food or foodstuffs with lower water content (Stefanova, 2010).

In the thermoluminescence method (TL, EN 1788:2001), the silicate minerals isolated from the sample, in a sufficient amount, are thermally stimulated and electron-hole pairs induced by the radiation, trapped in the minerals, are released resulting in a recombination and in an emission of light that is measured as a function of temperature. The signal is compared with the re-irradiated minerals with 1 kGy and if the ratio is higher than 0.1 the material is considered irradiated. This technique is laborious, limited by the quantity of extracted minerals, and cannot provide the value of the original dose (Arvanitoyannis, 2010; Stefanova, 2010).

Photostimulated luminescence (PSL, EN 13751:2002) is based on the recombination of electron-hole pairs induced by the radiation, as for the TL technique, but in this case using optical radiation, pulsed infrared light, to excite the sample and stimulating the recombination of electrons with holes with the consequent emission of light, that is the measured signal. Contrary to TL, no mineral extraction is needed but the results of these simple and fast

techniques must be confirmed by another standard and are limited to the presence and composition of minerals (Stefanova, 2010).

DNA methods ("Comet Assay" screening method, EN 13784:2001) are based on the radiation induced damage in the DNA of the food that causes chain breakage, double-strand breaks, single-strand breaks and base damage. This technique is also useful in the case of presence of live pests in the irradiated food product (Stewart, 2001). Microgel electrophoresis techniques are used to observe and quantify the damage by microscopy. The results are compared with non-irradiated samples. This method is considered rapid, sensitive, and simple to perform and inexpensive, limited to food products that have not been submitted to other process, like cooking or freezing that causes similar damages (Stefanova, 2010).

For detection of irradiated food containing cellulose the standard is the use of ESR Spectroscopy (EN 1787, 2000). When silicate minerals from irradiated food can be isolated, the standard is the use of TL techniques (EN 1788, 2001). ESR spectroscopy is used for food containing crystalline sugar (EN 13708, 2002). In other cases, PSL spectroscopy techniques are used, regulated by the standard (EN 13751, 2002).

In general, two or more methods are simultaneously applied to check if a food product has been irradiated or not. From the different studies, Arvanitoyannis (2010) has concluded that "methods such as electron spin resonance, thermoluminescence, and DNA comet assay are the most reliable, rapid, and promising".

Validation. Since the different standards have specific particularities and limitations, they must be validated for the irradiated food product to which they are intended to be applied (**Table 4**).

ESR, TL, PSL and DNA methods were tested by Chung et al. (2004) to identify irradiated Korean chestnuts, *Castanea bungena*. The samples were gamma irradiated with 0.5 kGy and

with different detection methods; only the TL technique was adequate to distinguish irradiated from non-irradiated samples. For PSL measures, the signal was too low to distinguish the samples; with the DNA Comet method, no difference was observed between irradiated and non-irradiated samples; with ESR spectroscopy, no radiation-induced cellulose radicals were observed in the harder outer shells. The authors concluded that this low dose of radiation induces small changes that are not easily detectable by the available techniques, but TL technique could be applied to get unequivocal results to discriminate irradiated from non-irradiated samples if both shape and position of the glow curve was used.

Mangiacotti et al. (2009) tested ESR, TL and PSL standards on irradiated European chestnuts, *Castanea sativa*. Based on the presence of cellulose on the outer shell and crystalline sugar in the pulp, they tested the standards EN 1787:2000 and EN 13708:2001 for ESR spectroscopy on irradiated chestnuts. Also, due to the presence of silicates on the outer shells, the authors considered the possibility of application of standards EN 1788:2001 (TL method) and EN 13751:2002 (PSL method). The samples were gamma irradiated at different doses in the range 0.1 to 1 kGy (0.15, 0.25, 0.35, 0.50, 1 kGy) and analysed by ESR spectroscopy, TL and PSL methods. TL and PSL measures revealed low signal, low luminescence sensitivity of chestnuts. With the TL technique it was possible to correctly identify the irradiated samples even at a low dose of 0.15 kGy. The PSL signal was only just above the negative threshold for all doses, except for the lower dose of 0.15 kGy. With ESR spectroscopic methods, no radio-induced signal was observed for chestnut shell or pulp. Based on these results the authors concluded that EN Standards based on ESR technique are not useful for the correct identification of this food product. Only the PSL and TL techniques could be useful for detecting irradiated fresh chestnuts.

3. Conclusions

Food irradiation technology has been so well documented and scrutinised by the scientific community for more than half a century that some authors refuse to continue to debate the issues of food safety and wholesomeness of the processed product (Molins, 2001). It is also considered that the risk of exposure to food borne pathogens is substantially reduced with the use of irradiation (EPA, 1996). Other food processes (curing, roasting or boiling) also cause changes in nutritional composition (Gonçalves et al., 2010; Nazzaro et al., 2011) and make it non-viable to apply the standards for detection of irradiation (Stefanova et al., 2010).

The most effective method for disinfestation is chemical fumigation, but it is environmentally aggressive and can be toxic for the operators and is being banned. Irradiation is considered a more environmentally friendly technology, meeting the food safety requirements. The dose validated for quarantine disinfestation of Korean chestnuts (0.50 kGy) is also effective in sprout inhibition (Kwon et al., 2004). The results described in this review show that irradiation with gamma rays is a safe, clean and cheap alternative to methyl bromide when the concern is biological pests.

Furthermore, gamma irradiation seems not to affect the nutritional value and individual nutritional molecules (e.g. sugars, starch and fatty acids) in chestnuts, whereas the storage time does. Moreover, it protects antioxidants such as tocopherols and phenolics, and preserves higher antioxidant activity compared with non-irradiated samples.

Proper identification of the irradiated food product contributes to the confidence of the consumer. The validation of standards for irradiated food detection on chestnuts presented in this review contributes to this goal. The growing demand for chestnuts worldwide will push the processing companies to find safe, reliable, cheap and environment friendly methodologies to ensure the quality of chestnuts. In line with this, many more studies should still be carried out to overcome the fear of irradiated food by consumers worldwide, especially

European. The next steps in this field would be to find other radiation techniques (x-rays, electron beam) and verify their effects on chestnuts, as well as find a specific dose that would free the fruit from pests and contaminants as well as conserve the benefits and not alter the organoleptic and visual properties.

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Table 1. Gamma irradiated chestnut species and doses.

Species	Dose*	Reference
Castanea crenata Siebold & Zucc. (Japanese chestnuts)	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 (Chinese chestnuts)	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 (Korean chestnuts)	0.1, 0.15, 0.25, 0.5 kGy	Chung et al. (2004)
Castanea sativa Miller (European chestnuts)	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)

*Not all the authors described the used dose rate.

Table 2. Studies reporting effects of gamma radiation on biological parameters

Parammeter	Chestnut sp.	Radiation dose	Main results
Sprouting	*Castanea crenata*	0.03, 0.07, 0.12 kGy at 0.7 Gy s^{-1}	Inhibition of sprouting ever 60 days.
	Castanea mollissima	0.1, 0.15, 0.2 kGy	Inhibition of sprouting ever 60 days.
		0.3, 0.6, 0.9, 1.2 kGy	No sprouting for all irradiat samples even after 86 and 1 days.
Rotting	*Castanea mollissima*	0.1, 0.15, 0.2 kGy	Less rotting in the irradiate samples.
	Castanea crenata	0.03, 0.07, 0.12 kGy at 0.7 Gy s^{-1}	Higher percentage of rottin the irradiated samples.
		0.25, 0.5, 1, 10 kGy	Irradiated samples with low rotting percentage when compared to fumigated sam
Respiration rate	*Castanea mollissima*	0.1, 0.15, 0.2 kGy	No statistical difference bet irradiated and non-irradiate samples.
Curculio sikkimensis Heller	*Castanea crenata*	0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1 kGy at 0.40 kGy h^{-1}	100% of the pests perished fumigated and irradiated samples from 0.4kGy onwa
		0.25, 0.5, 1, 10 kGy	100% of the pest perished i fumigated and irradiated samples from 0.3kGy onwa
Dichrocis punctiferalis Guenee	*Castanea crenata*	0.25, 0.5, 1, 10 kGy	100% of the pest perished i fumigated and irradiated samples from 0.5kGy onwa
Cydia kurokoi Amsell		0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1 kGy at 0.40 kGy h^{-1}	The dose of 0.3 kGy and onwards displayed a mortal rate of 100%.
Fungi *(Aspergillus arasiticus)*	*Castanea sativa*	0.25, 0.5, 3.0, 10 kGy	For 3 kGy the microbiologi load was reduced. 100% of fungi were eradicated at 10

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

Table 3. Studies reporting effects of gamma radiation on physico-chemical, nutritional and antioxidant parameters of chestnuts.

Parameter	Chestnut sp.	Radiation dose	Main results	Reference
Colour	*Castanea crenata*	0.25, 0.5, 1, 10 kGy	Hunters values only changed at 10 kGy. No change until 1 kGy.	Kwon et al. (2004)
	Castanea sativa	0.5, 3.0, 6.0 kGy at 0.8 kGy h^{-1} (Portuguese varieties) and 0.5, 3.0 kGy at 1.13 kGy h^{-1} (Turkish varieties)	No variation for "L" and "a" Hunters values in irradiated samples. "b" Hunters values increased during storage time.	Antonio et al. (2011a)
Texture	*Castanea sativa*	0.5, 3.0, 6.0 kGy at 0.8 kGy h^{-1}	Decrease in texture for higher doses after 30 days of storage.	Antonio et al. (2011b)
Drying rate	*Castanea sativa*	1.0, 3.0, 6.0 kGy at 2.5 kGy h^{-1}	Slight changes in moisture and drying rate for irradiated samples.	Antonio et al. (2012)
Nutritional value (Dry matter Ash, Fat, Protein, Carbohydrates)	*Castanea sativa*	0.25, 0.5, 1.0, 3.0 kGy	Radiation does not affect nutritional value.	Fernandes et al. (2011b)
		0.5, 3.0 kGy at 1.13 kGy h^{-1}	Radiation was not a source of variation.	Barreira et al. (2012)
Proteins	*Castanea mollissima*	0.25, 0.5, 1 kGy	Decrease of total proteins with increase of doses.	Guo-Xin et al. (1980)
Reducing and total sugars	*Castanea mollissima*	0.1, 0.15, 0.2 kGy	No changes in reducing and total sugars in irradiated samples after 14 days.	Iwata et al. (1959)
		0.25, 0.5, 1 kGy	No changes in sugar content for irradiated samples.	Guo-Xin et al. (1980)
		0.3, 0.6, 0.9, 1.2 kGy	No changes in sugar content for irradiated samples.	
Fructose, glucose, sucrose, trehalose, raffinose, total sugars	*Castanea sativa*	0.27, 0.54 kGy at 0.27 kGy h^{-1}	No significant changes in sugar content regardless of dose. Decrease in quantity over time.	Fernandes et al. (2011a)
Sucrose	*Castanea sativa*	0.5, 3.0 kGy at 1.13 kGy h^{-1}	No significant changes in sugar content regardless of the radiation dose.	Barreira et al. (2012)

Amylase Catalase	*Castanea mollissima*	0.1, 0.2, 0.3 kGy	At 0.3 kGy high activity in amylase with decrease over time. Catalase activity decreased over storage period.	Guo-Xin et al.(1980)
Starch	*Castanea mollissima*	0.25, 0.5, 1 kGy 0.3, 0.6, 0.9, 1.2 kGy	No significant change in starch quantity for all doses.	Guo-Xin et al.(1980)
Fatty acids (including SFA, MUFA, PUFA, and total Fat)	*Castanea sativa*	0.27, 0.54 kGy at 0.27 kGy h^{-1}	Some fatty acids lowered their quantity, others raised.	Fernandes et al. (2011a)
Fatty acids (palmitic, oleic, linoleic and linolenic acids)	*Castanea sativa*	0.25, 0.5, 1.0, 3.0 kGy	Radiation did not affect the fatty acids.	Fernandes et al. (2011b)
Fatty acids (SFA, MUFA, PUFA)	*Castanea sativa*	0.5, 3.0 kGy at 1.13 kGy h^{-1}	Doses between 0.5 and 3 kGy did not alter fatty acids.	Barreira et al. (2012)
Tocopherols (α, δ, γ and total)	*Castanea sativa*	0.27, 0.54 kGy at 0.27 kGy h^{-1}	At higher doses tocopherols were maintained. Quantity decreased during storage.	Fernandes et al. (2011a)
γ-tocopherol	*Castanea sativa*	0.25, 0.5, 1.0, 3.0 kGy	Minimal effects for all doses.	Fernandes et al. (2011b)
Ascorbic acid	*Castanea mollissima*	0.1, 0.15, 0.2 kGy	Most affected by storage time than radiation.	Iwata et al. (1959)
Phenolics Flavonoids Antioxidant activity	*Castanea sativa*	0.27, 0.54 kGy at 0.27 kGy h^{-1}	Phenolics and flavonoids were more influenced by storage time than radiation. Antioxidant activity favoured with higher doses.	Antonio et al. (2011c)

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

Table 4. Identification of irradiated chestnuts.

	Method					
Species	ESR	TL	PSL	DNA	Dose and dose rate	Reference
Castanea bungena	x	v	x	x	0.1, 0.15, 0.25, 0.5 kGy	Chung et al. (2004)
Castanea sativa	x	v	y	---	0.15, 0.25, 0.35, 0.50, 1 kGy at 16 Gy min^{-1}	Mangiacotti et al. (2009)

x- tested but not validated; y- partially validated; v- tested and validated. ESR- Electron Paramagnetic Resonance Spectroscopy; TL- Thermoluminescence method; PSL- Photostimulated luminescence.

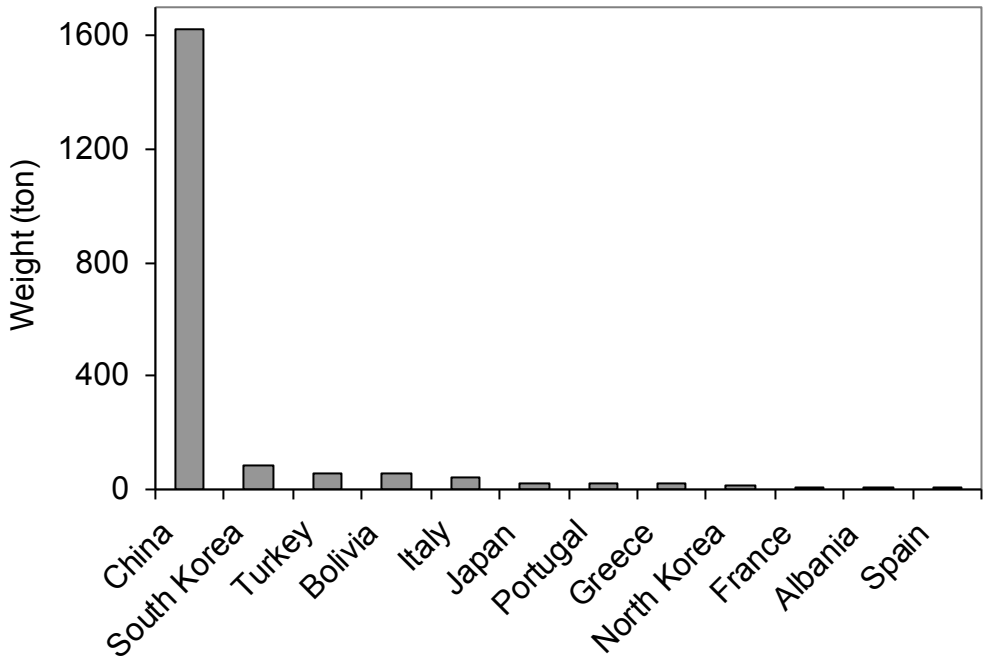

Figure 1. Chestnuts main country producers (www.faostat.com.; year 2010)