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# Effects of gamma and electron beam irradiations on the triacylglycerol profile of fresh and stored *Castanea sativa* Miller samples

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ABSTRACT

The present chestnut (*Castanea sativa* Miller) commercialization process, including distribution to novel markets, demands suitable preservation technologies. Irradiation has been considered a promising alternative to chemical fumigation (legally forbidden and harmful for human health and environment) or heat treatments (technological difficulties and low efficiency). Following previous studies on the effects of irradiation in different chemical parameters, the present work aimed to evaluate the effects of electron beam and γ-irradiation on the triacylglycerol profiles of fresh and stored chestnuts. An analysis of variance with type III sums of squares was performed using the general linear model procedure. As a classification technique, a linear discriminant analysis using the stepwise procedure was also applied. Independently of irradiation type, samples irradiated with higher doses showed higher modifications in triacylglycerol profiles. Samples irradiated with 1 and 3 kGy were clearly separated from the remaining groups in the linear discriminant analysis. The results highlight the potential of triacylglycerol profiles as indicators of chestnut irradiation. Irradiation might be recommended as a suitable method for chestnut preservation.



## 1. Introduction

Chestnut quality is measured by external factors such as color, shape, size, surface blemishes and molds, which are very important for consumer acceptance. Internal disorders may result from anatomical or physiological changes such as moisture loss, chemical conversion, discoloration, senescence, microorganism attack, cell breakdown (physiological decay) and insect injury (Upchurch et al., 1993). Weight losses due to dehydration and infestation by insects and microorganisms are the two main problems in chestnut preservation, and neither chemical fumigants, nor heat treatments, represent an effective solution (Pinto et al., 2007). Furthermore, chemical fumigation is harmful to human health and to the environment (UNEP, 2006), while heat treatments do not prevent mold growth (Jermini et al., 2006). Quality requirements demand enhanced preservation techniques for chestnuts and related products. In this context, decontamination methods based on high-energy electrons or γ-ray irradiation are being studied as alternatives. Arici et al. (2007) irradiated black cumin with 2.5–10 kGy for the purpose of microorganism elimination, while studying the effects on physico-chemical properties and on fatty acids profiles. Beneficial effects of irradiation include reduction of storage loss, shelf-life extension, and improvement of microbiological and parasitological safety of foods, while being safe to the environment. Hence, irradiation might be considered a promising preservation technology, bearing in mind that the doses applied on fresh fruit and processed fruit products are limited by the impact on their quality (Arvanitoyannis et al., 2009). Particularly, gamma-irradiation has already been applied to diverse food products such as tuber and bulb crops, stored grains, dried ingredients, meat, poultry and fish, and fruit (Farkas, 2006). It can also be applied to chestnuts which contain only 1% of fat, overcoming the production of off-odor compounds due to the radiation-induced breakdown of lipids common in high-fat-containing foods (Niyas et al., 2003).

Electron beam irradiation is also widely applied to improve food quality and safety. The use of electron accelerators as source of radiation has technological advantages such as higher throughput, wider flexibility and more potential to overcome public objections to radioactive isotopic sources (Supriya et al., 2012). Nevertheless,

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the irradiation efficacy is highly dependent on intrinsic factors of each food product, requiring continuous studies of exposure time (doses) and geometry (dose uniformity) (Belchior et al., 2007; Kim et al., 2007). The effects of radiation on chestnut composition have been progressively studied, either using electron beam treatment (Carocho et al., 2012) or γ-irradiation (Antonio et al., 2012a). Yet, the effects of these irradiations on chestnut triacylglycerol (TAG) profiles have not been evaluated. TAG profile is specific of each natural product and has been used for studying crystallization phenomena, detecting adulteration of specialty fats and oils, and recognition of oil origins, being one of the prime determinants in the study of oil oxidation (Zeb, 2012).

The evaporative light-scattering detector (ELSD) is a suitable solution for TAG analysis, since it is a mass-sensitive detector that responds to any analyte less volatile than the mobile phase. Furthermore, ELSD has a low background signal, a non-specific response (unlike a flame ionization detector), compatibility with gradient elution (dissimilarly to the refraction index detector) and with a broad range of solvents, besides having a signal independent of the degree of saturation and chain length (unlike an ultraviolet detector) (Rombaut et al., 2009). Hence, this parameter might be a good indicator of the effects of irradiation on natural food matrices. In the present study, the main purpose was to evaluate alterations in the TAG profiles of chestnuts submitted to electron beam or γ-irradiation (0, 0.5, 1 and 3 kGy), analyzed immediately after irradiation or after 30 d storage, assessing its potential use as an irradiation marker. Furthermore, the data obtained constitute complementary information to previous results regarding irradiation effects on the chemical composition and bioactivity of chestnuts (Fernandes et al., 2011a,b; Antonio et al., 2012a,b; Carocho et al., 2012).

## 2. Materials and methods

### 2.1. Standards and reagents

Triacylglycerols 1,2,3-tripalmitoylglycerol (PPP), 1,2,3-tristearoylglycerol (SSS), 1,2,3-trilinolenoylglycerol (LnLnLn), and 1,2,3-tripalmitoleoylglycerol (PoPoPo), of purity > 98%, and 1,2,3-trioleoylglycerol (OOO), 1,2,3-trilinoleoylglycerol (LLL), 1,2-dilinoleoyl-3-palmitoyl-*rac*-glycerol (PLL), 1,2-dilinoleoyl-3-oleoyl-*rac*-glycerol (OLL), 1,2-dipalmitoyl-3-oleoyl-*rac*-glycerol (PPO), 1,2-dioleoyl-3-stearoyl-*rac*-glycerol (OOS), 1-palmitoyl-2-oleoyl-3-linoleoylglycerol (POL), and 1,2-dioleoyl-3-palmitoyl-*rac*-glycerol (POO), of ≈99% purity, were purchased from Sigma (St. Louis, MO, USA). Petroleum ether was analytical grade and obtained from Fisher Scientific (Leicestershire, UK). Acetonitrile and acetone were HPLC grade and obtained from Merck (Darmstadt, Germany). The code letters used for the fatty acids are: Po, palmitoleic; L, linoleic; Ln, linolenic; M, myristic; O, oleic; P, palmitic; S, stearic.

### 2.2. Samples

The chestnut samples, previously studied for their nutritional value (Fernandes et al., 2011b; Carocho et al., 2012), were obtained in Bragança, Trás-os-Montes (Portugal). All the samples belong to the Longal cultivar from Protected Designation of Origin (PDO) "Castanha da Terra Fria". This PDO was created in 1994, with the normative decree 44/94 from February 3rd, where it is defined as the fruit obtained from *Castanea sativa*, including the varieties Longal, Judia, Amarelal, Lamela, Aveleira, Boaventura, Trigueira, Martainha and Negral (Portuguese Government Legislation, 1994: Decreto Normativo 44/94). For each irradiation procedure, chestnuts were divided into four groups: control (non-irradiated, 0 kGy), sample 1 (0.5 kGy), sample 2 (1 kGy), and sample 3 (3 kGy) with fifteen units per group. An independent control was used for each irradiation procedure (gamma irradiation was performed in Portugal, while electron beam irradiation was conducted in Poland), in order to guarantee the same conditions for all the samples avoiding a biased effect that might have been induced by potential differences among the two control samples.

### 2.3. Sample irradiation

#### 2.3.1. Electron beam radiation

The irradiation with electrons was performed at the INCT (Institute of Nuclear Chemistry and Technology), Warsaw, Poland, with an e-beam of 10 MeV of energy. Pulse duration 5.5 μs, pulse frequency 440 Hz, average beam current 1.1 mA, scan width of 68 cm, conveyer speed in the range 20–100 cm/min, scan frequency 5 Hz. The absorbed dose was 0.53, 0.83 and 2.91 kGy, with an uncertainty of 20% for two first doses and 15% for the last dose. To estimate the dose, Amber Perspex and Gammachrome YR dosimeters (from Harwell Company, U.K.) and a Graphite Calorimeter were used, depending on the dose level. The electrical resistance was read for the calorimeter dosimeter and converted according to a previous calibrated curve.

#### 2.3.2. Gamma radiation

The irradiation of the samples was performed in a Co-60 experimental chamber with four sources, a total activity of 267 TBq (6.35 kCi) in November 2011 (Precisa 22, Graviner Manufacturing Company Ltd., U.K.). After irradiation geometry dose rate estimation, using the Fricke dosimeter and the procedure described in the standards (ASTM, 1992), each group of fruit samples for irradiation was placed in a PMMA (polymethyl methacrylate) box to be irradiated at the predicted dose, at ambient atmosphere and temperature (15 °C). During the irradiation process, 4 routine dosimeters were used for each group for the higher dose to monitor the process (Amber Perspex dosimeters, from Harwell Company, U.K.). The samples were rotated up-side down (180°) at half of the time, to increase the dose uniformity. The Amber Perspex dosimeters were read in a UV-VIS Spectrophotomer (Shimadzu mini UV 1240 spectrophotometer) at 603 nm, two readings for each, to estimate the dose according to a previous calibration curve. The estimated doses after irradiation were $0.6 \pm 0.1$ kGy, $1.1 \pm 0.1$ kGy and $3 \pm 0.3$ kGy for each of the mentioned groups, respectively, at a dose rate of $0.8 \pm 0.1$ kGy $h^{-1}$.

For simplicity, from now on, in the tables and graphs we refer to the values 0, 0.5, 1 and 3 kGy, for non-irradiated and irradiated samples.

### 2.4. Triacylglycerol analysis

Before the extraction procedure, each sample was manually peeled (inner and outer skins), milled to obtain a dried powder (20 mesh) and lyophilized (Free Zone 4.5, Labconco, Kansas, MO). Each sample (50 g) was then submitted to an extraction with petroleum ether (40–60 °C) performed in Soxhlet apparatus for 1.5 h. The chromatographic analyses were carried out according to the procedure previously described (Barreira et al., 2009), with a Jasco (Tokyo, Japan) HPLC system, equipped with a PU-1580 quaternary pump and a Jasco AS-950 automatic sampler with a 10 μL loop. Detection was performed with an evaporative light-scattering detector (ELSD) (model 75-Sedere, Alfortville, France). The chromatographic separation of the compounds was achieved with a Kromasil 100 $C_{18}$ (5 μm; 250 mm × 4.6 mm) column (Teknokroma, Barcelona, Spain) operating at room temperature (≈20 °C). The mobile phase was a mixture of acetone and acetonitrile (70:30), in an isocratic mode,