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Effect of gamma irradiation and extended storage on selected chemical constituents and antioxidant activities of sliced mushroom

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ABSTRACT

Due to its exquisite odor and taste, *Boletus edulis* Bull.: Fr. mushroom is being increasingly used throughout the world. However, the availability of this product is highly restrained by its seasonality and perishability, demanding special care to keep its quality. Furthermore, mushrooms are often distributed in sliced form, and the slicing operation requires special treatments, besides augmenting the possibility of microbial contamination. Some techniques are currently applied to sliced mushrooms, but these processes had adverse effects on nutrients, odor, texture and taste, besides requiring expensive equipment. Food irradiation is being studied as an alternative technology to improve the quality and durability of determined products, having been previously applied in several mushroom species. Herein, gamma irradiation was applied to dried and sliced samples of *B. edulis*, at high doses (2, 6 and 10 kGy), and the effects on nutritional, chemical and antioxidant parameters were evaluated throughout storage (6 and 12 months). The results obtained for the chemical parameters do not advised storing *B. edulis* for such extended periods, since some relevant changes were detected, especially in samples stored for 12 months. In general, the gamma irradiation treatment did not cause further changes in the chemical composition, besides having a slightly positive effect in keeping the antioxidant activity and most of the bioactive compounds profiled in this work, contributing to maintain the potential health effects of this exquisite food, even after extended storage periods.

1. Introduction

Boletus edulis Bull.: Fr. (king bolete), is a popular edible mushroom in Europe, North America and Asia. Fresh and dried samples are available in occidental and oriental restaurants, as also in gourmet and healthy food stores. The flavor (considering its odor and taste) of the dried king, in particular, is marvelous-nutty, earthy and meaty (Tsai, Tsai, & Mau, 2008).

The availability of *B. edulis* is seasonal and, like other edible mushrooms, it is highly perishable, mainly due to the high water content (approximately 90%), high level of enzyme activity and presence of micro-flora (Jaworska & Bernaś, 2009). In fact, mushrooms need a special care to keep their quality along the marketing and distribution either in fresh, frozen and dried forms, but mostly when minimally processed, such as in the case of slicing (Iqbal, Rodrigues, Mahajan, & Kerry, 2009; Oliveira, Sousa-Gallagher, Mahajan, & Teixeira, 2012). In fact, slicing mushrooms requires special treatments to remove soil fragments and to reduce high microbial populations, in order to keep their sensorial and hygienic quality (Simón, González-Fandos, & Vázquez, 2010). This process can spread bacteria over the cut surfaces because they have a greater contact area, which amplifies spoilage problems, such as dehydration, enzymatic browning and microbial spoilage (Brennan, Port, Pulvirenti, & Gormley, 1999).

The shelf life of sliced mushrooms has been studied by the scientific community in order to find preservation and conservation alternatives. There are many methods and different processing techniques that can be applied to sliced mushrooms, such as sodium metabisulfite (Brennan et al., 1999), citric acid or hydrogen peroxide (Brennan, Port, & Gormley, 2000), chitosan coating and modified atmosphere packaging (MAP) (Kim, Ko, Lee, Park, &

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Hanna, 2006), washing and combinations of chlorine dioxide and hydrogen peroxide (Cliffe-Byrnes & O'Beirne, 2008), microwave-drying (Lombraña, Rodríguez, & Ruiz, 2010), washing with citric acid and MAP (Simón et al., 2010). However, some of these techniques can interact with specific nutrients, impair the odor and texture, produce unpleasant taste and can require expensive equipment (Junqueira et al., 2009).

Food irradiation (gamma rays or electron beam) has been applied for decades to destroy pathogenic and spoilage microorganisms without compromising the safety, nutritional and sensory quality (Inamura, Uehara, Teixeira, & Mastro, 2012). This technology is considered safe, wholesome, efficient, and reliable to obtain product improvements (Ehlermann, 2014). Our research group has been using gamma (Fernandes et al., 2013a, 2013b) and electron beam (Fernandes et al., 2014a, 2014b) irradiation in wild mushrooms (non-sliced/whole samples), concluding about its suitability to maintain the chemical profile of several species (*e.g.*, *Lactarius deliciosus* L., *Macrolepiota procera* (Scop.) Singer, *B. edulis* Bull., *Hydnum repandum* L. Fr., *Russula delica* Fr.). In the present work, gamma irradiation irradiation was applied to dried and sliced samples of *B. edulis*, at high doses (2, 6 and 10 kGy), and the effects on nutritional, chemical and antioxidant parameters were evaluated during one year of storage (6 and 12 months).

2. Materials and methods

2.1. Standards and reagents

Acetonitrile 99.9%, n-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Lab-Scan (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also other individual fatty acid isomers, tocopherol and sugar standards. Racemic tocol, 50 mg/mL, was purchased from Matreya (PA, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Standards trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and gallic acid were from Sigma (St. Louis, MO, USA). Methanol and all other chemicals were of analytical grade and obtained from common sources. Standards of phenolic compounds (protocatechuic and *p*-hydroxybenzoic acids), cinnamic acid and organic acids (oxalic acid and fumaric acid) were from Sigma Chemical Co. (St. Louis, MO, USA).

Water was treated in a Mili-Q water purification system (TGI Pure Water Systems, USA).

2.2. Samples and gamma irradiation

B. edulis dried and sliced samples were obtained in a local store (Bragança, Northeast of Portugal), in May 2013, packed in hermetic sealed polyethylene bags and labeled as "Porcini mushrooms", with the indication of species "*B. edulis*" below the commercial designation. Subsequently, the content of 10 packages (20 g each) was mixed and four individual samples were separated: control (non-irradiated, 0 kGy); sample 1 (2 kGy); sample 2 (6 kGy) and sample 3 (10 kGy).

The irradiation was performed at Gamma-Pak Sterilizasyon, Çerkezköy, Turkey. To estimate the dose during the irradiation process routine dosimeters were used (Amber Perspex batch V, Harwell Company, Oxfordshire, UK), further read by spectrophotometric methods. The routine dosimeters were previously calibrated against ceric-cerous dosimeters following the standards, with a maximum uncertainty of 4.44% (ASTM, 2015).

Before analysis, the samples were reduced to a fine dried powder (20 mesh) and mixed to obtain homogenized samples.

3. Chemical parameters

3.1. Nutritional value

Protein, fat, carbohydrates and ash were determined following the AOAC procedures (AOAC, 1995). The crude protein content ($N \times 4.38$) of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600 ± 15 °C. Total carbohydrates were calculated by difference. Energy was calculated according to the following equation: Energy (kcal) = $4 \times (g_{protein} + g_{carbohydrate}) + 9 \times (g_{fat})$.

3.2. Free sugars

Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI) after the extraction procedure described by Heleno, Barros, Sousa, Martins, and Ferreira (2009), using raffinose as internal standard (IS). The equipment consisted of an integrated system with a pump (Knauer, Smartline system 1000), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco) and a RI detector (Knauer Smartline 2300). Data were analyzed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with a Eurospher 100-5 NH_2 column (4.6 × 250 mm, 5 mm, Knauer) operating at 30 °C (7971 R Grace oven). The mobile phase was acetonitrile/deionized water, 70:30 (v/v) at a flow rate of 1 mL/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was performed using the internal standard method and sugar contents were further expressed in g per 100 g of dry weight (dw).

3.3. Fatty acids

Fatty acids were determined by gas–liquid chromatography with flame ionization detection (GC-FID), after the extraction and derivatization procedures previously described by Heleno et al. (2009). The analysis was carried out with a DANI model GC 1000 instrument equipped with a split/splitless injector, a FID at 260 °C and a Macherey–Nagel column (30 m × 0.32 mm ID × 0.25 μm d*f*). The oven temperature program was as follows: the initial temperature of the column was 50 °C, held for 2 min, then a 30 °C/min ramp to 125 °C, 5 °C/min ramp to 160 °C, 20 °C/min ramp to 180 °C, 3 °C/min ramp to 200 °C, 20 °C/min ramp to 220 °C and held for 15 min. The carrier gas (hydrogen) flow-rate was 4.0 mL/min (0.61 bar), measured at 50 °C. Split injection (1:40) was carried out at 250 °C. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using the Clarity DataApex 4.0 Software and expressed in relative percentage of each fatty acid.

3.4. Tocopherols

Tocopherols were determined after an extraction procedure previously described by Heleno, Barros, Sousa, Martins, and Ferreira (2010), using tocol as IS. The analysis was carried out in the HPLC system described above connected to a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm. The chromatographic separation was achieved with a Polyamide II normal-phase column (250 × 4.6 mm; YMC Waters) operating at 30 °C. The mobile phase used was a mixture of n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 mL/min. The compounds were identified by chromatographic comparisons