Innovative Food Science and Emerging Technologies 22 (2014) 158–166

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Feasibility of electron-beam irradiation to preserve wild dried mushrooms: Effects on chemical composition and antioxidant activity

Ângela Fernandes [a,b], João C.M. Barreira [a,b], Amilcar L. Antonio [a,c,d], M. Beatriz P.P. Oliveira [b], Anabela Martins [a], Isabel C.F.R. Ferreira [a,*]

[a] Centro de Investigação de Montanha (CIMO), ESA, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Apartado 1172, 5301-855 Bragança, Portugal
[b] REQUIMTE, Depto. de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira n.º 228, 4050-313 Porto, Portugal
[c] IST/ITN, Instituto Tecnológico e Nuclear, Estrada Nacional 10, 2686-953 Sacavém, Portugal
[d] Departamento de Física Fundamental, Universidade de Salamanca, Plaza de la Merced, 37008 Salamanca, Spain

ARTICLE INFO

Article history:
Received 20 October 2013
Accepted 21 December 2013
Available online 29 December 2013

Editor Proof Receive Date 22 January 2014

Keywords:
Wild mushrooms
Dried
Electron-beam
Chemical composition
Antioxidants

ABSTRACT

Mushrooms are highly perishable matrices and to extend time of consumption they need to be preserved. Since all the available conservation technologies present disadvantages, the combination of two different processes might minimize some of the limitations. Therefore, in the present work, electron-beam irradiation (up to 10 kGy) was applied to dried samples of *Boletus edulis* and *Russula delica*, extending previous findings using gamma- and electron-beam irradiations at lower doses (up to 6 kGy) and different wild mushroom species. The effects on nutritional, chemical and antioxidant parameters were evaluated. In general, the applied irradiation, particularly at higher doses, had significant effects on chemical profiles (protein, sugar and organic acid levels tended to decrease, while unsaturated fatty acids, tocopherols and phenolic acids presented higher levels in irradiated samples) and antioxidant activity (increased in irradiated samples). Nevertheless, the assayed doses might be considered to enhance the conservation of *B. edulis*, allowing the simultaneous achievement of disinfestation and decontamination effects.

Industrial relevance: B. edulis is among the most commercialized mushrooms worldwide. However, as all mushrooms, suffers severe conservation problems. Electron-beam irradiation (specifically at 6 kGy) proved to be a suitable technology for mushrooms conservation, since it allows disinfestation and decontamination processes without causing high changes in the chemical profiles. In *R. delica* case, differences caused by irradiation were higher, but it was also found that applying 6 kGy had the same effects of 2 kGy dose, which might be useful for disinfestation (insects elimination) and decontamination (elimination of bacteria and other microorganisms) purposes.

1. Introduction

Mushrooms are usually eaten fresh but due to their high water content, they become highly perishable and need to be preserved (Ezekiel, Sulyok, Frisvad, Somorin, Warth, Houbraken et al., 2013). When compared to vegetables, the shelf-life of mushrooms is minor, requiring special attention in their postharvest chain (Iqbal, Rodrigues, Mahajan, & Kerry, 2009). In this sense, many technologies have been applied in order to increase mushrooms shelf-life, such as drying (Ma, Haixia, Wenchai, & Zhaoshuai, 2013), freezing and cryogenic freezing (Jaworska & Bernás, 2009), modified atmosphere packaging (MAP) (Oliveira, Sousa-Gallagher, Mahajan, & Teixeira, 2012) and irradiation (Akram & Kwon, 2010; Fernandes, Barreira, Antonio, Martins, Oliveira & Ferreira, 2013a).

Drying is one of the most important processes used in preserving mushrooms fruiting bodies, removing water, so as to minimize biochemical and microbial activities (Ezekiel et al., 2013; Kumar, Singh, & Singh, 2013). Nevertheless, during the drying process, microorganisms may secrete potentially toxic metabolites and contaminate mushrooms (Ezekiel et al., 2013; Shephard, 2008).

The chemical sanitizing procedures have also inherent problems concerning residues and environmental pollution; several decontamination methods exist, but the most versatile treatment among them is the processing with ionizing radiation (Farkas, 1998). Being a cold process, food irradiation does not significantly alter physico-chemical characteristics of the treated product. It has the potential of disinfesting dried food to reduce storage losses and disinfesting fruits and vegetables to meet requirements for export trade (Loaharanu & Ahmed, 1991). Radiation decontamination of dry food, spices and herbs with doses of 3–10 kGy proved to be a viable alternative to fumigation with microbicidal gases (Farkas, 1998). The most common sources of ionizing radiation are gamma rays and electron-beam, being applied by many researchers in extending the postharvest shelf-life of mushrooms (Culleré, Ferreira, Venturini, Marco, & Blanco, 2012; Fernandes, Antonio, Oliveira, Martins, & Ferreira, 2012).

* Corresponding author. Tel.: +351 273303219; fax: +351 273325405.
E-mail address: iferreira@ipb.pt (I.C.F.R. Ferreira).

1466-8564/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ifset.2013.12.015

The safety of irradiated foods at specific doses applied for technological benefits is guaranteed by leading world health organizations (WHO, World Health Organization, 1999). Decontamination of food by ionizing radiation is a safe, efficient, environmentally clean and energy efficient process (Farkas, 1998). Many countries (Argentina, China, United Kingdom, Croatia, Belgium, Czech Republic, Poland, Serbia and Montenegro) allow the use of irradiation for fresh (1–3 kGy) and dried (1–10 kGy) mushrooms, for different technological purposes (Akram & Kwon, 2010).

In a recent study, our research group investigated and validated the effects of electron-beam irradiation (0, 0.5, 1 and 6 kGy) and storage time (0, 6 and 12 months) on nutritional and chemical parameters of dried wild *Macrolepiota procera*, concluding that this technology might act in cooperative manner, allowing benefiting from the long-lasting conservation period complied by a reduction in changes usually associated with drying treatment (Fernandes et al., 2013a). In the present work, the study was extended to different dried wild mushrooms (the worldwide appreciated *Boletus edulis* Bull. and *Russula delica* Fr.), in order to confirm the effects of electron-beam irradiation at higher doses (2, 6 and 10 kGy) on nutritional, chemical and antioxidant parameters. Despite the effectiveness verified previously for lower irradiation doses, the advisory technological limits for good irradiation practices defines that the reduction of insects (disinfestation) in food might be achieved using 1–2 kGy doses, but the elimination of bacteria and other microorganisms requires doses up to 10 kGy (Molins, 2001).

2. Materials and methods

2.1. Standards and reagents

For chemical analyses: 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, organic acids, tocopherol and sugar standards. Racemic tocol, 50 mg/mL, was purchased from Matreya (PA, USA).

For antioxidant potential analysis: 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, *p*-hydroxybenzoic and *p*-coumaric acids), cinnamic acid and organic acids (oxalic acid, quinic acid, citric acid and fumaric acid) were from Sigma Chemical Co. (St. Louis, MO, USA). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

2.2. Samples and electron-beam irradiation

B. edulis Bull. and *R. delica* Fr. wild samples were obtained in Trás-os-Montes, in the Northeast of Portugal, in November 2012, and dried at 30 °C in an oven. Subsequently, the samples were divided in four groups with six specimens of each mushroom species: control (non-irradiated, 0 kGy); sample 1 (2 kGy); sample 2 (6 kGy) and sample 3 (10 kGy), kept in polyethylene bags.

The irradiation was performed at the INCT — Institute of Nuclear Chemistry and Technology, in Warsaw, Poland. To estimate the dose during the irradiation process three types of dosimeters were used: a standard dosimeter, graphite calorimeter, and two routine dosimeters, Gammachrome YR and Amber Perspex, from Harwell Company (UK). The irradiation took place in e-beam irradiator of 10 MeV of energy with pulse duration of 5.5 μs, a pulse frequency of 440 Hz, and an average beam current of 1.1 mA; the scan width was 68 cm, the conveyer speed was settled to the range of 20–100 cm/min and the scan frequency was 5 Hz. The estimated absorbed doses were 2.5, 6.2 and 10.9 kGy, with an uncertainty of 20%. To read Amber and Gammachrome YR dosimeters, spectrophotometric methods were used at 603 nm and at 530 nm, respectively, to estimate the dose from the value of absorbance according to a previous calibration curve. For the Graphite calorimeter dosimeter the electrical resistance was read and converted in dose according to a previous calibration curve (Carocho, Barreira, Antonio, Bento, Kaluska & Ferreira, 2012). For simplicity, we refer to the irradiation doses as: 0, 2, 6 and 10 kGy.

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

2.3. Chemical parameters

2.3.1. Nutritional value

Moisture, 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: $\text{Energy (kcal)} = 4 \times (g_{\text{protein}}) + 3.75 \times (g_{\text{carbohydrate}}) + 9 \times (g_{\text{fat}})$.

2.3.2. 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 melezitose as internal standard (IS). The equipment consisted of an integrated system with a pump (Knauer, Smartline system 1000), degasser system (Smartline manager 5000), autosampler (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).

2.3.3. Fatty acids

Fatty acids were determined by gas 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 df). 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 CSW 1.7 Software (DataApex 1.7) and expressed in relative percentage of each fatty acid.

2.3.4. 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