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Combined effects of irradiation and storage time on the nutritional and chemical parameters of dried *Agaricus bisporus* Portobello mushroom flour

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Abstract: Portobello variety of *Agaricus bisporus* mushrooms, appreciated for its taste, makes it desirable to be eaten fresh and also as flour in soups and gravies. Gamma and electron-beam radiation at four doses (1, 2, 5, and 10 kGy) were used to analyze its preservation effect on Portobello mushroom flour. A proximate analysis, as well as the impact on fatty acids, tocopherols, soluble sugars, organic acids, and ergosterol profiles, were performed every 3 months, during a storage period of 1 year. Gamma rays preserved mannitol (most abundant soluble sugar) over the 12 months, while electron beam radiation preserved organic acids. No significant changes were sought for any radiation type, and the slight changes extracted from the estimated marginal means reveal a tendency for irradiation as having preserving effects of nutrients and other important molecules. Thus, both irradiation types, up to 10 kGy are suitable for preservation of *A. bisporus* Portobello flour.

KEYWORDS
Agaricus bisporus, chemical profile, electron beam, food irradiation, gamma radiation

1 | INTRODUCTION

As the consumption of fresh mushrooms grows, an increase in their production is necessary, demanding additional research to clarify the role of mushrooms in human diets and their consumption benefits (PR Newswire, 2018). In the post-harvest stage, mushrooms quickly lose quality, as indicated by the loss of moisture, discoloration, degra-dation of nutrients, and hyphae development. This high perishability is a strong disadvantage that reduces their economic value. Accordingly, there is a growing need to extend their shelf life, both in fresh and processed forms, improving yields and economic competitiveness to producers and traders, and increasing their quality for consumers (Bernaś, 2018; Cardoso et al., 2019; Kic, 2018; Taofiq et al., 2017; Zhang et al., 2018).

Irradiation technology has proven to be scientifically feasible, practical, and worthwhile for several food products (fruits and vegetables, fresh or dried; Akram et al., 2013; Cardoso et al., 2019; Fernandes et al., 2017; Ferreira et al., 2018). Gamma rays and electron-beam radiation are techniques that have been validated previously and constitute an alternative to other preservation technologies (Ferreira et al., 2018) while maintaining the chemical profile, freshness, and overall security, although the beneficial and negative effects are always dose-dependent and case-specific; hence, the importance of testing doses within current legislation on different foods (Cardoso et al., 2019; Fernandes, Barreira, et al., 2014a, 2014b). Scientific reports have shown the benefits of this technology on the physical, chemical, nutritional and bioactive properties of several edible mushroom species mushrooms (Cardoso et al., 2019; Fernandes et al., 2013b, 2015, 2017; Fernandes, Barreira, et al., 2014a, 2014b, 2014c, Fernandes et al., 2016).

The *Agaricus bisporus* (J.E. Lange) Imbach, white mushrooms, are the ones within the Agaricaceae family with the least growth time in order to have the white and soft body, which when allowed to grow for longer periods, starts to develop a brown tone that tends to intensify. These brown mushrooms are called Portobello and have a more intense and textured taste than the white ones (Bernaś, 2018; Cardoso et al., 2019; Djekic et al., 2017; Wang et al., 2018). Additionally, this variety is rich in bioactive compounds (Cardoso et al., 2019; Djekic et al., 2017; Guan et al., 2016; Teichmann et al., 2007).

Interaction of ionizing radiation with natural matrices is multifactorial, where some molecules may protect others from radiation effects, requiring case-by-case studies (Antonio et al., 2018). In this way, validating both the processing technology and the processed product is a requisite to assure the feasibility of the preservation process under validation. Still, gamma and electron-beam irradiation does not induce deep changes in fresh or dried mushrooms (Cardoso et al., 2020). Accordingly, the main objective of this work was to understand the effects of different doses (1, 2, 5, and 10 kGy) of gamma and electron-beam irradiation on nutritional and chemical parameters of Portobello mushrooms flour stored for relatively long storage periods (0, 6, and 12 months).

2 | MATERIALS AND METHODS

2.1 | Gamma and electron-beam irradiation

A. bisporus Portobello fruiting bodies were acquired in a local market of Bragança, Northeast of Portugal in June 2017, and were divided into two groups of 15 mushrooms each and further submitted to a drying process. Samples were dried at 30°C in an oven for 4 days; each group was further subdivided into five subgroups (200 g per group): Control (non-irradiated, 0 kGy); sample 1, irradiated at 1 kGy, sample 2 (2 kGy), sample 3 (5 kGy) and sample 4 (10 kGy).

Gamma irradiation was performed at the ionizing radiation facility IRIS from Centro de Ciências e Tecnologias Nucleares (Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal) in a ^{60}Co experimental chamber (model Precisa 22, Graviner Manufacturing Company Ltd., UK) with four sources and a total activity of 2.9 kCi (108 TBq, July 2017). The absorbed doses were measured by routine dosimeters (Batch X; Amber Perspex Harwell). Prior to irradiation, two dosimeters were added to each bag, and the samples were placed in line with the sources, rotated 180 degrees vertically and horizontally halfway through the irradiation to guarantee a uniform dose and to follow the recommended practices for food irradiation (dose uniformity ratio less than 3). The dosimeters were positioned according to a previous dose mapping of the irradiation chamber, and the absorbed doses were estimated using a calibration curve obtained for routine dosimeters.

The estimated absorbed doses, dose rate, and dose uniformity ratio (D_{max}/D_{min}) were, respectively, 1.2 ± 0.1, 1.9 ± 0.1, 5.0 ± 0.1, and 10.4 ± 0.3 kGy; 1.4 kGy/h and 1.3, respectively.

Electron-beam irradiation was carried out in the same facility using a LINAC equipment (Saturne 41, adapted for R&D, General Electric Co., Boston, MA, USA) with an electron beam of 10 MeV. Samples were placed at 60 cm from the beam exit and irradiated at dose rates ranging from 0.3 to 1.3 kGy/min (pulse duration 4 μs, pulse repetition frequency 20–100 Hz). The absorbed doses estimated by radiochromic film dosimeters FWT-60 (Far West Technology, Inc.) were the following: 1.0 ± 0.1, 2.0 ± 0.2, 4.7 ± 0.7, and 9.8 ± 0.1 kGy) with an uncertainty of 7% for the first dose and 10% for the other two doses. Previously to food irradiation experiments, a detailed dose mapping characterization of the radiation chamber was carried out, 60 cm of the beam, using routine radiochromic dosimeters that were calibrated against reference alanine dosimeters.

After irradiation and prior to the laboratorial analysis, the samples were ground to a fine dried powder (20 mesh) by means of an automated mill and mixed to obtain homogenized samples, and then kept in the dark at room temperature in airtight flasks until further analysis. One batch of the samples was analyzed immediately while the other two were stored for 6 and 12 months, respectively, at room temperature in the dark in airtight containers.

2.2 | Proximate composition

Carbohydrates, fat, protein, ash, and moisture were determined following AOAC (Association of Official Agricultural Chemists) procedures (AOAC, 2016). The crude protein content of the samples was determined by the macro-Kjeldahl method, the crude fat was extracted using a Soxhlet apparatus and subsequently weighed, the ash content was determined by incineration at $550 \pm 15°C$. Total carbohydrates were calculated by difference: Total carbohydrates = 100 (g of moisture + g of protein + g of fat + g of ashes); and energy was calculated according to the following equation: Energy (kcal) = $4 \times$ (g protein + g carbohydrates) + $9 \times$ (g fat).

2.3 | Chemical composition

2.3.1 | Soluble sugars

For the analysis of soluble sugars, an extraction was performed with ethanol and water, followed by a filtration (Barros, Pereira, Calhelha, et al., 2013). The identification of soluble sugars was performed on a Knauer high-performance liquid chromatograph (HPLC; Smartline system 1000, Knauer, Berlin, Germany) coupled to a refractive index detector (RI; Knauer). The compounds were identified by comparing their retention times to the ones of commercial standards. The quantification was based on the RI signal response of each standard using the internal standard (IS) methodology and raffinose as the IS. The HPLC conditions were as following: The mobile phase was a mixture of acetonitrile/water (70:30, v/v) with a flow rate of 1 ml/min. The chromatographic separation was achieved using a Eurospher 100–5 NH_2 column (5 μm, 250 × 4.6 mm, Knauer) at 35°C. Data were analyzed using Clarity 2.4 software (DataApex, Podohradska), and the results were reported in g per 100 g of dry weight (dw).

2.3.2 | Organic acids

Organic acids were extracted following a procedure previously described by the authors Pereira et al. (2015) and quantified by ultrafast liquid chromatograph (Shimadzu 20A series, Shimadzu Corp., Kyoto, Japan) coupled to photodiode array detector, using 215 nm as the preferred wavelength. Results were expressed as g/100 g dw.

2.3.3 | Fatty acids

Fatty acids were determined by gas chromatography (GC) with flame ionization detection at 260°C after extrac-

tion and derivatization procedures previously described by Heleno et al. (2009). The analysis was carried out with a DANI GC 1000 instrument (DANI Instruments, Milan, Italy) equipped with a split/splitless injector and a Zebron-Kame column (30 m × 0.25 mm ID × 0.20 μm df, Phenomenex, Torrance, CA, USA) using the methodology previously reported by Cardoso et al. (2019). The results were expressed in the relative percentage of each fatty acid.

2.3.4 | Tocopherols

Tocopherols were quantified through HPLC after extraction with hexane, methanol, and water as previously described by Reis et al. (2012), using tocol as an IS. Chromatographic separation was performed with a polyamide II normal-phase column (250 × 4.6 mm; YMC model, Waters Corp., Milford, MA, USA) operating at 35°C, and the mobile phase employed was a mixture of n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 mL/min. This analysis was done using the equipment described for the soluble sugars, although coupled to a fluorescence detector (FP-2020; Jasco), designed for excitation at 290 nm and emission at 330 nm. Quantification was based on IS methodology, and the compounds were identified by chromatographic comparisons with commercial standards. Data were evaluated using Clarity 2.4 software, and the results were expressed in mg/100 g dw.

2.3.5 | Ergosterol

Ergosterol was identified and quantified according to the methodology described by Barreira et al. (2014), after an extraction procedure previously described by Guan et al. (2016), using the HPLC described above coupled to a UV detector (Knauer Smartline 2500) and using 280 nm as the preferred wavelength (Barros et al., 2013a). The results were expressed as mg/100 g dw.

2.4 | Statistical analysis

Throughout the manuscript, all assays were carried out with three replicates, and all data are expressed as mean ± standard deviation. Samples were analyzed through a two-way analysis of variance (ANOVA) with type III sums of squares, after verifying homoscedasticity through a Levene's test. The *post-hoc* test used was either a Tukey's Honest Significant Difference (HSD) test (homoscedastic samples) or Tamhane's T2 test (non-homoscedastic samples). By employing a two-way ANOVA, it is possible to verify the

influence of each factor: Storage time (ST) or radiation dose (RD), independently of the way the other factor varies. If a significant ($p < 0.05$) interaction (ST × RD) was detected, the potential tendencies had to be extracted from the plotted estimated marginal means (EMM). Inversely, if there is no significant interaction ($p > 0.05$), each factor was classified independently using the *post-hoc* tests described above. All statistical analysis was performed using a p-value of 0.05 and the IBM SPSS software, version 25 (IBM Corp.).

3 | RESULTS AND DISCUSSION

The tabled results include nutritional, soluble sugars, and ergosterol profiles (Table 1), tocopherols and organic acids (Table 2), and fatty acids (Table 3) and are divided into two sections, the top one belonging to gamma-irradiated samples and the bottom one to those treated with the electron beam. Each section is further divided to elucidate ST (upper part) and RD (lower part) effects with the values presented for each RD including all ST periods and *vice-versa*.

In dw basis, the most abundant nutrients were carbohydrates, followed by proteins (Table 1). The fat content was very low (<1.4 g/100 g (dw), which was similar to the values presented by other fresh mushroom species (Cardoso et al., 2019). For gamma-irradiated samples, there was a significant ($p < 0.05$) interaction (ST × RD) for moisture, crude fat, proteins, and carbohydrates, meaning that both RD and ST had a significant impact on the changes registered for these nutrients. Inversely, ash and energy did not show a significant interaction, being classified individually. Ash content decreased over ST, although the significantly higher values in irradiated samples, as previously observed in irradiated foods (Khan et al., 2018). Regarding the energy values of gamma-irradiated samples, this parameter tended to increase with ST but decreased for higher RD. Considering the EMM plots, it was possible to observe that the longer the ST, the lower the fat content (Figure 1(a)), but with RDs of 5 and 10 kGy, this nutrient seems to be preserved, which is a beneficial aspect, since the loss of fat content is related to rancidification of food. Similar results were reported by Fernandes et al. (2017). Storage had a role in the reduction of fat over time, but the treatment at 5 kGy seems to preserve the integrity of these molecules during storage.

In terms of the nutritional profile of electron-beam-irradiated samples, a significant interaction was also found for ash and energy, showing that electron-beam had similar effects to those of gamma radiation, with a significant decrease of ash and an increase of energy values over ST, while a reduction of these parameters was detected with

the increase of RD. For nutrients with a significant interaction among factors, the EMM plots (Figure 1(b)), show a reduction of protein content for the stored samples, while a lower dose of irradiation (1 kGy) seems to slightly reduce this loss of protein content in stored samples. This behavior was also reported by (Fernandes et al., 2013a; Fernandes, Barreira, et al., 2014b).

In Table 1, the soluble sugars and ergosterol content are shown for both irradiation technologies. The detected sugars were fructose, mannitol, and trehalose, mannitol being the most abundant one, followed by trehalose. The same sugar profile had been identified previously in various instances for irradiated sugars Regarding gamma radiation, a non-significant interaction was found for mannitol and trehalose while mannitol was classified independently showing that while this sugar decreased over ST, the increase of RD preserved it. Trehalose did not show significant differences among ST or RD, but it can be observed that trehalose increased over the 12 months with no influence from RD. The EMM plot for total sugars, Figure 1(c), shows that the amount of these sugars reduces over time, although stored samples showed that gamma irradiation had increasing preserving capacity from 1 to 5 kGy, while the 10 kGy dose showed a better preserving potential in non-stored samples. Electron-beam-irradiated samples showed a significant interaction for all sugars with no observable tendency in the EMM.

Ergosterol was detected in samples irradiated by both technologies and showed a significant interaction with values around 239 mg/100 g for gamma irradiation and varying from 234 to 347 mg/100 g for electron beam. In a previous work by Cardoso et al. (2019), an increase in ergosterol was also recorded for irradiated samples. Overall, slight changes were detected in the nutritional and sugar profile of Portobello mushroom flour treated with gamma radiation or electron beam, clearly showing that ST had a higher impact than RD as can be observed in Figure 1.

Table 2 shows two classes of molecules detected in the mushrooms, namely, tocopherols and organic acids. Regarding tocopherols, all four isoforms were detected in gamma and electron-beam-irradiated mushrooms, with β-tocopherol showing the highest amounts and α-tocopherol the lowest. A significative interaction was found for all isoforms, not allowing to present individual classifications. Considering the EMM plots, and starting by gamma radiation, a general tendency for preservation of tocopherols was found for doses of 1 kGy with a much deeper impact resulting from ST, which tended to reduce the amount of these bioactive molecules, (Figure 2(a)). This same tendency, a decrease in the bioactive molecules over time, had also been reported previously by Fernandes et al. (2017). In the case of electron-beam-irradiated mushroom flour, ST also

TABLE 1 Centesimal composition, soluble sugars, and ergosterol quantities in gamma and electron-beam irradiated Portobello mushrooms

Gamma radiation

		Moisture (g/100 g fw)	Crude fat (g/100 g fw)	Proteins (g/100 g fw)	Ash (g/100 g fw)	Carbohydrates (g/100 g fw)	Energy (g/100 g fw)	Fructose (g/100 g dw)	Mannitol (g/100 g dw)	Trehalose (g/100 g dw)	Total sugars (g/100 g dw)	Ergosterol (mg/100 g dw)
Storage time (ST)	0 months	11 ± 2	1.4 ± 0.1	24 ± 1	9.6 ± 0.1[c]	65 ± 1	369 ± 1[a]	0.43 ± 0.05	34 ± 3[c]	0.8 ± 0.1	36 ± 3	293 ± 39
	6 months	11 ± 2	1.19 ± 0.1	21 ± 1	9.4 ± 0.1[b]	68 ± 1	368 ± 2[a, b]	0.34 ± 0.04	29 ± 3[b]	0.6 ± 0.1	30 ± 3	275 ± 14
	12 Months	12 ± 1	1.0 ± 0.1	21 ± 1	9.0 ± 0.3[a]	96 ± 2	372 ± 2[b]	0.28 ± 0.05	22 ± 2[a]	2.0 ± 0.8	21 ± 4	238 ± 30
_p_-value (_n_ = 25)	Tukey's HSD test	0.038	<0.001	<0.001	<0.001	<0.001	0.040	<0.001	<0.001	0.545	<0.001	<0.001
Radiation dose (RD)	Control	11 ± 3	1.2 ± 0.1	21 ± 2	8.5 ± 0.2[a]	69 ± 2	372 ± 2[b]	0.29 ± 0.07	24.8 ± 1.9[a]	0.4 ± 0.1	24 ± 7	248 ± 51
	1 kGy	12 ± 1	1.1 ± 0.2	21 ± 2	9.6 ± 0.3[b]	68 ± 3	367 ± 1[a]	0.33 ± 0.07	27.6 ± 1.7[a, b]	0.6 ± 0.2	28 ± 6	283 ± 42
	2 kGy	12 ± 2	1.1 ± 0.2	22 ± 2	9.5 ± 0.3[b]	67 ± 2	367 ± 2[a]	0.32 ± 0.08	29.5 ± 2.2[b]	0.5 ± 0.3	30 ± 6	272 ± 26
	5 kGy	10 ± 2	1.3 ± 0.2	22 ± 1	9.6 ± 0.2[b]	67 ± 1	368 ± 1[a]	0.39 ± 0.06	30.8 ± 1.7[b]	0.6 ± 0.3	32 ± 4	282 ± 25
	10 kGy	10 ± 1	1.3 ± 0.2	23 ± 1	9.6 ± 0.3[b]	66 ± 1	368 ± 1[a]	0.40 ± 0.06	30.3 ± 1.9[b]	0.7 ± 0.3	31 ± 7	258 ± 27
_p_-value (_n_ = 5)	Tukey's HSD test	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.452	<0.001	<0.001
ST × RD (_n_ = 50)	_p_-value	<0.001	<0.001	<0.001	0.390	<0.001	0.176	<0.001	0.326	0.443	<0.001	<0.001

Electron-beam radiation

		Moisture (g/100 g fw)	Crude fat (g/100 g fw)	Proteins (g/100 g fw)	Ash (g/100 g fw)	Carbohydrates (g/100 g fw)	Energy (g/100 g fw)	Fructose (g/100 g dw)	Mannitol (g/100 g dw)	Trehalose (g/100 g dw)	Total sugars (g/100 g dw)	Ergosterol (mg/100 g dw)
ST	0 months	11 ± 2	1.29 ± 0.03	24.4 ± 0.7	9.3 ± 0.1[c]	65.0 ± 0.9	369 ± 1[a]	0.43 ± 0.06	23 ± 3	0.88 ± 0.05	34 ± 3	347 ± 27
	6 months	10 ± 2	1.21 ± 0.03	19.7 ± 0.7	9.1 ± 0.1[b]	69.9 ± 0.9	369 ± 1[a, b]	0.38 ± 0.06	30 ± 4	0.5 ± 0.1	31 ± 4	300 ± 23
	12 months	12 ± 1	1.14 ± 0.05	19.3 ± 0.5	8.9 ± 0.1[a]	70.5 ± 0.7	370 ± 1[b]	0.30 ± 0.05	20 ± 5	0.4 ± 0.1	21 ± 5	234 ± 28
_p_-value (_n_ = 25)	Tukey's HSD test	0.007	<0.001	<0.001	<0.001	<0.001	0.045	<0.001	<0.001	<0.001	<0.001	<0.001
RD	Control	10 ± 2	1.17 ± 0.09	20 ± 2	8.5 ± 0.2[a]	70 ± 2	372 ± 1[b]	0.30 ± 0.06	21 ± 6	0.5 ± 0.2	22 ± 6	250 ± 48
	1 kGy	12 ± 2	1.23 ± 0.07	22 ± 2	9.2 ± 0.3[b]	67 ± 2	369 ± 1[a]	0.40 ± 0.06	36 ± 7	0.5 ± 0.3	27 ± 8	304 ± 66
	2 kGy	10 ± 1	1.21 ± 0.06	21 ± 2	9.2 ± 0.2[b]	68 ± 3	369 ± 1[a]	0.35 ± 0.07	27 ± 6	0.6 ± 0.2	28 ± 6	308 ± 54
	5 kGy	12 ± 1	1.22 ± 0.06	21 ± 2	9.3 ± 0.2[b]	68 ± 3	368 ± 2[a]	0.37 ± 0.03	29 ± 5	0.6 ± 0.2	30 ± 6	298 ± 33
	10 kGy	13 ± 1	1.24 ± 0.06	21 ± 3	9.2 ± 0.2[b]	69 ± 3	369 ± 1[a]	0.4 ± 0.1	33 ± 4	0.6 ± 0.2	34 ± 4	309 ± 46
_p_-value (_n_ = 5)	Tukey's HSD test	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ST × RD (_n_ = 50)	_p_-value	0.003	0.008	<0.001	0.750	<0.001	0.526	<0.001	<0.001	<0.001	<0.001	<0.001

Note: In each row, different letters mean significant statistical differences, with an overall significance value of 0.05. The presented standard deviations were calculated from results obtained under different operational conditions. Therefore, these values should not be regarded as a measure of precision rather as the range of the recorded values.

TABLE 2 Tocopherol isoforms and organic acids quantities in gamma and electron-beam-irradiated Portobello mushrooms

Gamma radiation

		α-tocopherol (mg/100 g dw)	β-tocopherol (mg/100 g dw)	γ-tocopherol (mg/100 g dw)	δ-tocopherol (mg/100 g dw)	Total tocopherols (mg/100 g dw)	Oxalic acid (g/100 g dw)	Quinic acid (g/100 g dw)	Malic acid (g/100 g dw)	Total organic acids (g/100 g dw)
ST	0 months	7.1 ± 0.2	149 ± 12	130 ± 6	65 ± 3	352 ± 20	0.88 ± 0.07	4.4 ± 0.3	4.9 ± 0.1	10.2 ± 0.4
	6 months	6.7 ± 0.3	67 ± 9	47 ± 2	51 ± 5	170 ± 14	0.82 ± 0.07	4.0 ± 0.4	4.5 ± 0.5	9.3 ± 0.9
	12 months	3.2 ± 0.6	52 ± 15	25 ± 2	19 ± 4	99 ± 21	0.67 ± 0.06	3.2 ± 0.2	3.5 ± 0.1	7.3 ± 0.4
p-value (n = 25)	Tukey's HSD test	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
RD	Control	4 ± 2	69 ± 46	63 ± 44	38 ± 21	174 ± 113	0.7 ± 0.1	3.5 ± 0.5	4.0 ± 0.6	8 ± 1
	1 kGy	5 ± 2	95 ± 50	73 ± 52	46 ± 20	219 ± 120	0.78 ± 0.08	3.9 ± 0.4	4.5 ± 0.7	9 ± 1
	2 kGy	5 ± 2	93 ± 44	66 ± 47	46 ± 20	211 ± 110	0.8 ± 0.1	4.2 ± 0.9	4.5 ± 0.7	9 ± 2
	5 kGy	5 ± 1	93 ± 45	67 ± 47	47 ± 20	213 ± 110	0.8 ± 0.1	3.8 ± 0.5	4.5 ± 0.7	9 ± 1
	10 kGy	5 ± 2	98 ± 43	67 ± 48	48 ± 20	218 ± 111	0.8 ± 0.1	3.7 ± 0.5	4.0 ± 0.7	9 ± 1
p-value (n = 5)	Tukey's HSD test	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ST × RD (n = 50)	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Electron-beam radiation

|		α-tocopherol (mg/100 g dw)	β-tocopherol (mg/100 g dw)	γ-tocopherol (mg/100 g dw)	δ-tocopherol (mg/100 g dw)	Total tocopherols (mg/100 g dw)	Oxalic acid (g/100 g dw)	Quinic acid (g/100 g dw)	Malic acid (g/100 g dw)	Total organic acids (g/100 g dw)
ST	0 months	8.3 ± 0.9	140 ± 7	119 ± 4	61 ± 3	329 ± 14	0.91 ± 0.04	4.6 ± 0.2	4.9 ± 0.2	10.4 ± 0.5[c]
	6 months	5.1 ± 0.4	70 ± 7	58 ± 6	48 ± 3	181 ± 16	0.78 ± 0.06	3.4 ± 0.2	3.6 ± 0.4	7.8 ± 0.3[b]
	12 months	3.7 ± 0.9	49 ± 14	24 ± 1	17 ± 3	93 ± 18	0.71 ± 0.09	3.2 ± 0.2	3.5 ± 0.1	7.4 ± 0.2[a]
p-value (n = 25)	Tukey's HSD test	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
RD	Control	4 ± 2	69 ± 47	60 ± 39	37 ± 20	171 ± 106	0.7 ± 0.1	3.5 ± 0.6	3.6 ± 0.8	7.7 ± 0.3[a]
	1 kGy	6 ± 2	88 ± 40	68 ± 42	43 ± 21	205 ± 104	0.7 ± 0.1	3.9 ± 0.6	4.0 ± 0.8	8.6 ± 0.6[b]
	2 kGy	6 ± 2	91 ± 41	69 ± 43	43 ± 20	210 ± 104	0.84 ± 0.1	3.7 ± 0.7	4.0 ± 0.7	8.6 ± 0.5[b]
	5 kGy	6 ± 2	91 ± 39	68 ± 43	42 ± 18	207 ± 100	0.84 ± 0.1	3.7 ± 0.7	4.1 ± 0.6	8.7 ± 0.5[b]
	10 kGy	6 ± 2	92 ± 40	69 ± 42	44 ± 20	212 ± 102	0.86 ± 0.1	3.8 ± 0.7	4.1 ± 0.6	9.0 ± 0.2[b]
p-value (n = 5)	Tukey's HSD test	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ST × RD (n = 50)	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.164

Note: In each row, different letters mean significant statistical differences, with an overall significance value of 0.05. The presented standard deviations were calculated from results obtained under different operational conditions. Therefore, these values should not be regarded as a measure of precision rather as the range of the recorded values.

TABLE 3 Individual fatty acids, saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) quantities in gamma and electron-beam irradiated Portobello mushrooms, expressed in relative percentage

Gamma radiation

		C15:0	C16:0	C18:0	C18:1	C18:2	C20:0	C22:0	C24:0	SFA	MUFA	PUFA
ST	0 months	1.4 ± 0.1	9 ± 1	4.0 ± 0.3	1.1 ± 0.1	72 ± 1	1.6 ± 0.3	1.6 ± 0.4	3.1 ± 0.2	25 ± 1	1.6 ± 0.1	72 ± 1
	6 months	1.9 ± 0.2	10 ± 1	4.5 ± 0.5	1.1 ± 0.2	74 ± 3	1.6 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	24 ± 2	1.5 ± 0.6	74 ± 3
	12 months	1.7 ± 0.1	9 ± 1	4.0 ± 0.3	0.9 ± 0.1	75 ± 1	1.7 ± 0.1	1.33 ± 0.09	1.1 ± 0.1	24 ± 1	1.1 ± 0.1	75 ± 1
p-value (n = 25)	Tukey's HSD test	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
RD	Control	1.6 ± 0.2	9.4 ± 0.4	4.0 ± 0.2	1.1 ± 0.1	74 ± 1	1.7 ± 0.1	1.6 ± 0.4	1 ± 1	24 ± 1	1.5 ± 0.3	74 ± 1
	1 kGy	1.7 ± 0.4	10.2 ± 1.2	4.2 ± 0.6	1.1 ± 0.1	74 ± 1	1.5 ± 0.3	1.3 ± 0.1	2 ± 1	25 ± 1	1.4 ± 0.3	74 ± 1
	2 kGy	1.6 ± 0.2	8.7 ± 0.5	4.0 ± 0.4	0.9 ± 0.1	75 ± 2	1.7 ± 0.1	1.5 ± 0.4	2 ± 1	23 ± 2	1.2 ± 0.3	76 ± 2
	5 kGy	1.8 ± 0.2	10.1 ± 0.9	4.6 ± 0.6	1.1 ± 0.1	72 ± 2	1.6 ± 0.1	1.2 ± 0.2	2 ± 1	26 ± 1	1.7 ± 0.6	73 ± 2
	10 kGy	1.6 ± 0.1	8.9 ± 0.5	4.1 ± 0.1	0.9 ± 0.1	75 ± 2	1.7 ± 0.1	1.4 ± 0.1	1 ± 1	24 ± 2	1.2 ± 0.2	75 ± 2
p-value (n = 5)	Tukey's HSD test	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ST × RD (n = 50)	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Electron-beam radiation

		C15:0	C16:0	C18:0	C18:1	C18:2	C20:0	C22:0	C24:0	SFA	MUFA	PUFA
ST	0 months	1.2 ± 0.1	8.1 ± 0.3	4.2 ± 0.2	1.3 ± 0.3	75 ± 1	1.8 ± 0.1	1.5 ± 0.2	1.2 ± 0.2	12 ± 1	1.7 ± 0.3	75 ± 1
	6 months	1.6 ± 0.1	9.8 ± 0.4	4.2 ± 0.1	0.4 ± 0.4	81 ± 1	1.0 ± 0.7	1.2 ± 0.1	1.0 ± 0.1	25 ± 19	0.5 ± 0.4	81 ± 1
	12 months	1.7 ± 0.2	9.7 ± 0.3	4.0 ± 0.3	0.7 ± 0.1	76 ± 1	1.6 ± 0.2	1.3 ± 0.1	1.0 ± 0.1	23 ± 1	0.9 ± 0.1	76 ± 1
p-value (n = 25)	Tukey's HSD test	<0.001	0.011	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.810	<0.001	<0.001
RD	Control	1.5 ± 0.1	9 ± 1	3 ± 2	0.9 ± 0.2	78 ± 2	1.5 ± 0.1	1.2 ± 0.2	1.0 ± 0.1	21 ± 2	1.2 ± 0.4	78 ± 2
	1 kGy	1.5 ± 0.2	9 ± 1	3 ± 2	0.9 ± 0.2	78 ± 3	1.6 ± 0.1	1.3 ± 0.1	0.9 ± 0.1	21 ± 2	1.2 ± 0.4	78 ± 2
	2 kGy	1.5 ± 0.2	9 ± 1	3 ± 2	0.6 ± 0.4	77 ± 2	1.7 ± 0.1	1.3 ± 0.1	1.0 ± 0.1	22 ± 2	0.9 ± 0.6	77 ± 2
	5 kGy	1.6 ± 0.3	9 ± 1	3 ± 2	0.6 ± 0.4	77 ± 4	1.2 ± 0.8	1.4 ± 0.2	1.3 ± 0.3	34 ± 22	0.9 ± 0.6	77 ± 4
	10 kGy	1.4 ± 0.3	9 ± 1	3 ± 2	0.9 ± 0.8	77 ± 5	1.2 ± 0.8	1.5 ± 0.3	1.2 ± 0.1	22 ± 4	1.1 ± 0.9	77 ± 5
p-value (n = 5)	Tukey's HSD test	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.017	<0.001	<0.001
ST × RD (n = 50)	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.016	<0.001	<0.001

Note: Presented standard deviations were calculated from results obtained under different operational conditions. Therefore, these values should not be regarded as a measure of precision rather as the range of the recorded values.

FIGURE 1 Estimated marginal means (EMM) plots of (a) fat (gamma radiation), (b) proteins (electron-beam radiation), and (c) total sugars (gamma radiation)
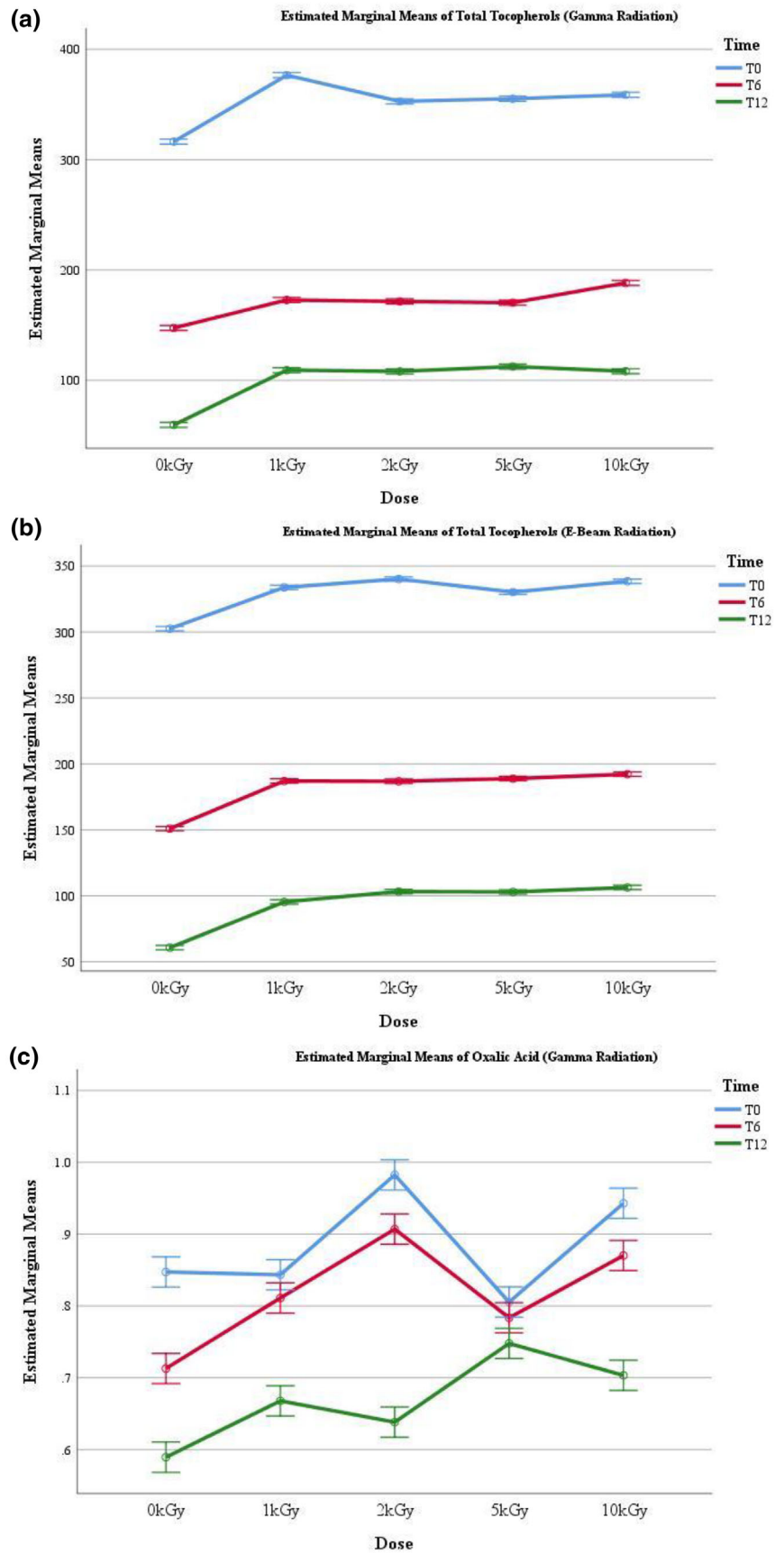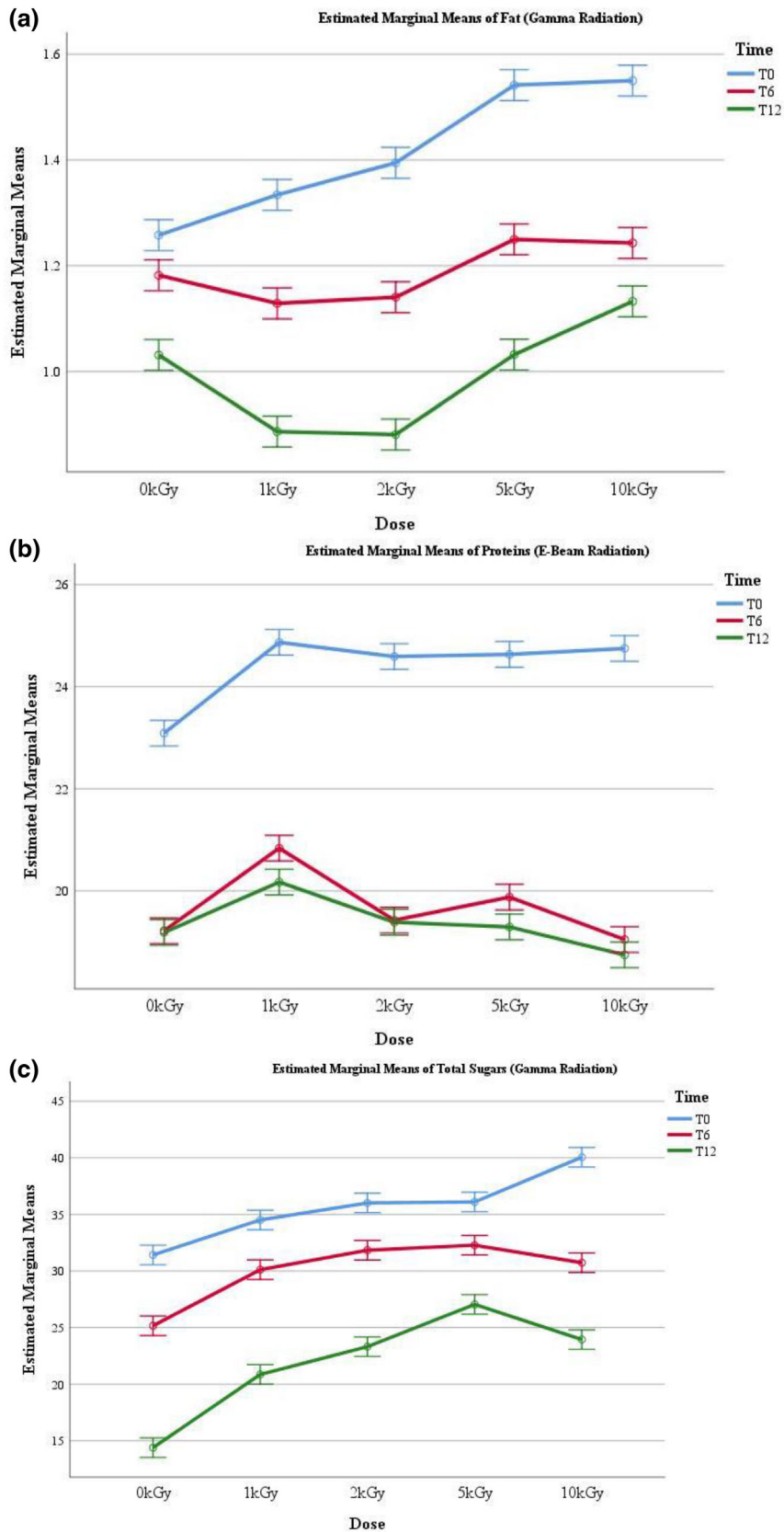
FIGURE 2 EMM plots of (a) total tocopherols (gamma radiation), (b) total tocopherols (electron-beam radiation), and (c) oxalic acid (gamma radiation)

showed a higher impact than RD. All RDs showed preserving capabilities with an increase of about 50 mg/100 g (Figure 2(b)), while ST showed a high impact as seen in the gap between non-stored samples (blue line) and stored samples (red: 6 months, green: 12 months).

Organic acids are a group of simple organic molecules with acidic properties, present in all living organisms. In the irradiated mushrooms, three organic acids were detected: Oxalic, quinic, and malic acid. Quinic and malic acid were the most abundant, practically in *ex aequo*, while oxalic acid was not detected over 1 g/100 g. For both radiation processes, a significative interaction was found for all organic acids, and thus general conclusions were sought from the EMM plots. In Figure 2(c), (oxalic acid content in gamma-irradiated Portobello), the effect of ST had a higher influence than RD, although in this case, the dose of 2 kGy seem to have protecting capabilities (for the non-stored samples and 6 months stored ones, while samples stored for 12 months showed a better response at 5 kGy). This was also verified for quinic acid (results not shown). Regarding electron-beam-irradiated mushroom flours, and in line with the observed for the gamma-irradiated samples, organic acids showed a significative interaction in all cases. Irradiated samples (independently of RD) tend to present higher organic acid contents, while a significative decrease was observed over ST. Independently of RD, electron-beam radiation preserved organic acids in Portobello mushroom flour, while long STs reduce their amounts. These are important effects to take into account for these small molecules due to their relevance in taste, aroma, and overall appreciated properties of mushrooms. Barros et al. (2014) also mentioned that 1 kGy of gamma irradiation was effective to preserve organic acids in *Macrolepiota procera* mushrooms, pointing out that irradiation could be a complementary preserving technique.

Table 3 shows the individual fatty acids found in both mushrooms, as well as the groups of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA), expressed in relative percentage. Only the eight most abundant individual fatty acids are shown, from a total of 16 identified. In both cases, C18:2 was the most abundant fatty acid with a range of 72% to 81%, followed by C16:0. Overall, PUFA showed the highest amounts, also ranging from 81% to 82%, while SFA only registered a percentage of 12% to 34%, and MUFA being the lowest group, under 2%. This high incidence of PUFA shows the health beneficial unsaturated fat found in mushroom flour, which also helps their acceptance as healthy foods. Regarding the individual fatty acids, all samples showed a significant interaction between ST and RD, meaning that even though there were very low variations

in the fatty acids, both ST and RD had an influence on the results. No tendencies could be extracted from the EMM plots, thus, concluding that due to the very low moisture, lipid peroxidation was very low, and the influence of the ST would not be drastic. Concomitantly, the influence of the different RDs did not have a great effect on the individual fatty acids.

4 | CONCLUSION

The main objective of this work was to assess the effect of different RDs on the chemical parameters of Portobello mushrooms flour (a sub-product of the fresh mushroom industry) stored at different intervals, as well as the difference between radiation sources, gamma rays, and electron beam. In this work it was possible to validate the use of both technologies with minimal effects on its main characteristics, allowing its preservation for longer times (up to 12 months) making it available to the food industry, allowing it to be incorporated into other foods, without compromising its main nutritional characteristics. Ionizing radiation using electron beam or gamma rays (up to a dose of 10 kGy) did not induce severe changes in the tested parameters, and the slight changes are all benefiting the preservation of specific nutrients like sugars and fat but also tocopherols or organic acids.

AUTHOR CONTRIBUTIONS
Formal analysis, writing-original draft: Rossana V. C. Cardoso. *Data curation, writing-review and editing*: Marcio Carocho. *Investigation*: Ângela Fernandes. *Data curation*: João C. M. Barreira. *Supervision*: Sandra Cabo Verde. *Investigation*: Pedro M. P. Santos. *Investigation, writing-review and editing*: Amilcar L. Antonio. *Supervision*: Ana M. González-Paramás. *Funding acquisition, project*

CONFLICT OF INTEREST

The authors state no conflict of interest.

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