Effects of combining electron-beam or gamma irradiation treatments with further storage under modified atmospheres on the bioactive compounds of *Tuber melanosporum* truffles

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Abbreviations: DF, dietary fibres; GC-FID-MS, Gas chromatography coupled to flame ionization detector and mass spectrometry; HPLC-DAD, High performance liquid chromatography coupled to diode array detector.

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Abstract

The effects of electron-beam or gamma irradiation (both applied at 1.5 kGy and 2.5 kGy) and subsequent storage under modified atmospheres (35 d at 4 °C) were evaluated on total carbohydrates, chitins, β-glucans, proteins, total phenols, sterols and ergocalciferol concentrations of *Tuber melanosporum* ascocarps. Irradiation procedures reduced chitin and protein concentrations and modified total phenol levels depending on the dose and irradiation type utilized. Further storage of irradiated truffles maintained the levels of all determined compounds unchanged except for the phenolic compounds and ergosterol levels that increased probably due to their yeast colonization after 21 d storage. Therefore, irradiation plus storage under modified atmosphere packings could be used as a preservation method to extent truffles quality and shelf life, but it is only recommended up to 21 d. The lower irradiation doses are encouraged to diminish protein degradation.

1. Introduction

The ascocarps or fruiting bodies of *Tuber melanosporum* are commonly known as ´black´, ´winter´ or ´Perigord´ truffles. Their particular combination of volatile compounds make them one of the most prized gourmet foods worldwide (Culleré et al., 2010). However, besides the compounds providing flavour, they also contain others with beneficial properties for human health. Truffles such as *Terfezia claveryi, Tirmania nívea, Tirmania pinoyi and Tuber aestivum* showed antioxidant, antiviral, antimicrobial, hepatoprotective, anti-mutagenic and anti-inflammatory properties. *T. melanosporum* contains bioactive compounds that also seemed to contribute to those biological activities and perhaps to others that are still not evaluated (Patel et al., 2017; Vamanu et al., 2018). Ergosterol (ergosta-5,7,22-trienol) and brassicasterol (ergosta-5,22-dienol) represented respectively 60 and 40 % of *T. melanosporum* sterols (Harki et al., 1996). Ergosterol and its derivatives are involved in a wide range of health-promoting properties, such as antioxidant (Shao, Hernandez, Kramer, Rinker & Tsao 2010), anti-inflammatory (Kuo et al., 2011), hypolipidemic (Hu et al., 2006), and hypocholesterolemic (Gil-Ramírez & Soler-Rivas, 2014) activities. *T. melanosporum* also contain bioactive polysaccharides in large quantities such as β-glucans and chitins as they are constitutive compounds from its peridium cell walls (Saidali-Savy et al., 1992). Fungal β-glucans showed antioxidative, immunomodulatory and hypocholesterolemic activities despite other less studied (Gil-Ramírez et al., 2014; Gil-Ramírez et al., 2017). Chitins are considered as dietary fibre and are precursors of chitosans with many other biological activities (Patel & Goyal, 2017).

Moreover, the black truffle contains homogentisic acid, p-hydroxybenzoic acid, 3,4-dihydroxybenzaldehyde and many other phenolic compounds that apparently showed a

protective effect against human diseases such as cancer or cardiovascular diseases (Villares et al., 2012).

On the other hand, black truffles are highly perishable mainly because they should be harvested mature to obtain the desirable organoleptic properties, and they are picked from grounds with high microbial and pests loads (Rivera et al., 2011b). Therefore, post-harvest storage of fresh black truffles to preserve pricipally their aroma is a significant concern (Rivera et al., 2010). Modified atmosphere packaging significantly extends the shelf-life of truffles reducing the weight loss, maintaining the typical hard texture, delaying the development of mycelium growth, and enabling good scores for aroma and flavour (Rivera et al., 2010b). Innovative preservation technologies such as gamma and electron-beam ionizing radiations, combined with modified atmosphere packaging, were tested with promising results since a significant reduction of the microorganisms present in the peridium was achieved (Rivera et al., 2011b). Their effects were not limited to the *T. melanosporum* surface, they could penetrate deeper and eliminate microorganisms maintaining their organoleptic characteristics almost unmodified (Rivera et al., 2011b). Similar studies were also carried out on summer truffles (*T. aestivum*) with positive effects using beta and gamma ionizing irradiations. However, the effect of these irradiation treatments on *T. melanosporum* bioactive compounds was not studied in detail. Therefore, in this work, the effect of beta and gamma rays (both at 1.5 and 2.5 kGy doses) combined with modified atmosphere was followed during 35 d at 4 °C on the levels of molecules such as chitins, β-glucans or ergosterol, with interesting biological properties.

2. Material and methods

2.1. Biological material

T. melanosporum ascocarps were collected at Sarrión woods (Teruel, Spain), identified, selected and processed as described by Rivera et al. (2011a).

2.2 Reagents

Solvents such as hexane (95%), chloroform (HPLC grade), methanol (HLPC grade), acetonitrile (HPLC grade) were obtained from LAB-SCAN (Gliwice, Poland) and absolute ethanol, sodium carbonate (Na_2CO_3) and sulfuric acid (H_2SO_4) from Panreac (Barcelona, Spain). Potassium hydroxide (KOH), ascorbic acid, 2,6-Di-tert-butyl-*p*-cresol (BHT), bovine serum albumin (BSA), acetylacetone, p-dimethylaminebenzaldehyde, HCl (37%), phenol, as well as hexadecane, ergosterol (95%), D-glucose, D-glucosamine hydrochloride, gallic acid, were purchased from Sigma-Aldrich (Madrid, Spain). All other reagents and solvents were used of analytical grade.

2.*3. Irradiation treatments and packaging for storage*

Irradiation with electron-beam and gamma rays from Cobalt-60 was carried out as described in Rivera et al. (2011b) to obtain doses of 1.5 and 2.5 kGy in both cases. After irradiation, truffles were placed in polypropylene trays (50 g per tray) heat-sealed with a microperforated film Amcor-PPlus (Amcor Flexibles, Ledbury, UK), with two microperforations of $9 \times 5 \times 10^{-5}$ m per package and stored at 4 °C during 35 d as indicated in Rivera et al. (2011b). Control non-irradiated samples were packaged and stored under the same conditions. After those 35 d, truffles were freeze-dried, powdered and stored at -20 ºC until further use.

2.4 Carbohydrates determinations

Total carbohydrate concentration of control and irradiated truffles was determined by the phenol-sulphuric acid method adapted from Dubois et al. (1956) as indicated in Morales et al. (2018). A standard curve of D-glucose was used for quantification.

Chitin content was determined according to Smiderle et al. (2017). Briefly, samples were hydrolysed with 6 M HCl at 100 °C for 2 h and adjusted to pH 10.0 afterwards they were allowed to cool down. Then, hydrolysed samples (2.5×10^{-4} L) were treated as described by Rementeria et al. (1991). Samples absorbance was determined using an Evolution 600 UV-vis (Thermo Fisher Scientific, Spain) spectrophotometer at 530 nm. A glucosamine hydrochloride standard curve was used for quantification.

The β-glucan content of the truffle samples (5×10^{-5} g) was evaluated by a β-glucan determination kit specific for mushrooms and yeasts (Megazyme®, Biocom, Barcelona, Spain) following the instructions of the user´s manual.

2.5 Total phenol and protein determination

Total protein concentration of the irradiated and control samples (10 g L^{-1}) was evaluated using the Bradford method reagents (Sigma-Aldrich, Madrid, Spain) according to the Instruction Manual. BSA was used as a standard for protein quantification.

The phenolic compounds from truffles (0.01 g) were evaluated as to their total phenol concentration determined by the Folin-Ciocalteu method according to the procedure of Ramírez-Anguiano et al. (2007). Gallic acid was used as a standard for quantification.

2.6 Ergosterol and ergocalciferol determination

Truffles irradiated with electron-beam and gamma rays were saponified using the procedure described by Gil-Ramírez et al. (2013). Afterwards, obtained unsaponified fractions (6 g L^{-1}) were injected into an Agilent HP-5ms capillary column (30 m × 0.25 mm i.d. and 0.25 µm phase thickness) from a 7890A System *GC-MS-FID* (Agilent Technologies, Santa Clara, CA). The injection, detection and temperature program were those described by Gil-Ramírez et al. (2013). Ergosterol was used as a standard and hexadecane (10% v/v) as an internal standard for ergosterol and derivative compounds quantification.

Identification and quantification of vitamin D_2 (ergocalciferol) were carried out using C_{18} Spherisorb OD52 4 x 250 mm analytical column with a 5 x 10^{-6} m particle size (Waters, Missisauga, Ontario, Canada) coupled to an HPLC system (ProStar 330, Varian, Madrid, Spain) with a photodiode array (DAD) detector (ProStar 363 module, Varian, Madrid). The unsaponified fractions (5 g L^{-1}) were dissolved in the mobile phase (95% methanol, v/v), injected (1 x 10^{-5} L) and developed isocratically under a constant flow (1 x 10^{-3} L min^{-1}).

2.7 Statistical analysis

Differences were evaluated at a 95% confidence level ($P \leq 0.05$) using a one-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison test. Statistical analysis was performed using GraphPad Prism version 5.01 (GraphPad Software, San Diego, CA).

3. Results and discussion

T. melanosporum ascocarps were submitted to different irradiation procedures and doses, and afterwards, they were stored for 35 d inside modified atmosphere packages simulating storage conditions before their commercialization or consumption.

3.1 Effect of irradiation treatments

The black truffle contained 33 % (w/w) total carbohydrates where 20.7 % of them were β-glucans, and 12.2 % were chitins, indicating that *T. melanosporum* contained more polysaccharides than other lower molecular weight sugars such as oligo- or mono-saccharides (Table 1). According to previous studies, carbohydrates levels in black truffle ranged from 8 – 30 % (Harki et al., 2006). Thus, the truffles utilized contained a high carbohydrate concentration, particularly if compared to other species such as *Tuber aestivum* that showed 5.6 % carbohydrates, *Tuber magnatum* (2.2 %) or *Tuber borchii* (3.6 %) (Saltarelli et al., 2008). Chitin content was also slightly higher than other truffle species such as *T. aestivum* (10.6 %) (Vetter & Kruzselyi, 2014) or many edible

158 mushrooms (3 – 9 %) (Gil-Ramírez & Soler-Rivas, 2014) probably because truffles
159 ascocarps show a harder texture than mushroom fruiting bodies. Moreover, β-glucan
160 levels were found within the range of many mushrooms species (5 – 48 %) (Gil-Ramírez
161 & Soler-Rivas, 2014) although higher than truffles from other genus as their dietary fibres
162 (DF) levels were 4 % in *Terfezia claveryi* (Bokhary & Parvez, 1993) and 7.4 % in *Terfezia*
163 *nívea* (Sawaya et al., 1985), and usually the fungal DF content includes mainly β-glucans
164 and chitins. Total protein and phenolic contents were higher than indicated for *T.*
165 *melanosporum* in other studies (0.87 g kg^{-1} (Saltarelli et al., 2008) and 1.20 g kg^{-1}
166 (Villares et al., 2012)), but the different environmental conditions or developmental
167 stages might be the reason for the differences.
168 When the truffle ascocarps were submitted to irradiation with electron-beam or gamma
169 rays, no significant changes were noticed in their total carbohydrates or β-glucans levels
170 compared to non-irradiated controls (Table 1). However, a marked reduction on chitin
171 levels (almost 2 fold lower) was noticed independently of the dose or type of irradiation
172 utilized. Gamma irradiation induced changes in chitins structure facilitating its extraction,
173 N-deacetylation and transformation into chitosan (Tahtat et al., 2007). They partially
174 degraded the polymers generating breaking down products of lower molecular weight
175 and higher solubility (Mahlous et al., 2007). It is believed that the carbohydrates
176 degradation occurs by breaking the glycosidic bonds, leading to the formation of lower
177 molecular mass sugars (glucose, maltose, erythrose, ribose and mannose) and formation
178 of carbonyl groups or double bonds (Xu et al., 2007). This, might explain that the total
179 carbohydrate content remained unchanged after the irradiation procedures since β-
180 glucans remained unchanged.
181 Gamma irradiation was also responsible for the partial depolymerisation of mushroom β-
182 glucans, decreasing the average molecular weight with increasing doses (from 0.5 up to

50 kGy) (Khan et al., 2015). However, with the doses applied in this work, no significant changes were observed in β-glucans content. Apparently, truffle β-glucans are resistant to these milder treatments. The application of high irradiation doses caused loss of firmness but mainly resulted from the decrease in chitin levels as noticed. The depolymerization of chitin and loss of total sugar or some combination were also pointed when higher doses were used (Jian et al., 2010).

A decrease in protein content was observed after both irradiation treatments compared to non-irradiated control. No significant differences between doses were noticed when gamma irradiation was applied but, they were significative with electron-beam irradiation. In the latter case, protein degradation was more pronounced with a higher dose. The noticed reduction could be due to proteins fragmentations, denaturalization and precipitation as noticed in other studies particularly, with high irradiation doses (Urbain, 1986). *T. melanosporum* proteins are rich in sulphur amino acids (Harki et al., 2006) forming disulphide bonds to maintain their ternary structure and they are particularly sensitive to the free radicals generated after irradiation (Ibarz, 2008). Similar results were also noticed when mushrooms (*Boletus edulis* and *Russula delica*) were submitted to electron-beam irradiation (Fernandes et al., 2014).

Irradiation also influenced phenolic compound levels. A slight increase was noticed with the lower e-beam irradiation doses dropping down below the non-irradiated levels with the higher doses utilized. When gamma irradiation was applied, only a significant decrease of total phenols was observed with the higher selected dose. Electron-beam irradiation might break down chemical bonds that bind phenolic compounds to other molecules (Alothman et al., 2009) and therefore, releasing of soluble phenols might have occurred with the lower irradiation doses. However, if the dose is high, then degradation might also take place.

Fungal sterols were resistant to the irradiation treatments as not significant changes were noticed in any of the derivatives investigated (Table 2). Ergosterol was the principal sterol found in the *Tuber* genus (as in all mushroom species), although truffles also contained 28 – 44 % brassicasterol (Harki et al., 1996). In genus such as *Terfezia* sp., brassicasterol might reach up to 98 % of total sterols reducing to small amounts their ergosterol levels (Weete et al., 1985). Ergosterol levels in non-irradiated samples were similar to previous studies (1.80 g kg^{-1}) (Villares et al., 2012). However, brassicasterol levels were lower (0.84 g kg^{-1}) (Harki et al., 1996). Other minor sterol derivatives were identified such as ergosta7,22-dienol and 19,19 cyclolanost-7-en-3-ol, but no ergocalciferol was detected. Transformation of ergosterol into vitamin D_2 takes place in the presence of UV-irradiation (Perera et al., 2003) and since truffles are usually buried close to the tree's roots associated with mycorrhizae, no vitamin D_2 was expected in control samples.

However, results indicated that other radiation types such as gamma or e-beam irradiation were not inducing ergocalciferol biosynthesis as UV-irradiation does.

3.2 Effect of storage on modified atmosphere packages after irradiations

After irradiation, truffles were packaged in modified atmospheres. The steady-state modified atmosphere conditions were reached after 2 d of storage with CO_2 and O_2 average levels of 8 % CO_2 and 11 % O_2 inside the control packages, 7 % CO_2 and 14 % O_2 for truffles treated with 1.5 kGy e-beam, 9 % CO_2 and 12 % O_2 for 1.5 kGy gamma rays, 8 % CO_2 and 13 % O_2 2.5 kGy e-beam and 11 % CO_2 and 12 % O_2 for 2.5 kGy gamma rays. A larger CO_2 accumulation and O_2 reduction were detected in the control packages showing significant differences with the rest of the batches (Rivera et al., 2011b). This fact was explained by the decrease in the respiratory activity of irradiated *T. melanosporum* being the larger decrease detected in truffles treated with the higher doses.

The stability of the main components and bioactive compounds was followed during the storage period. Total carbohydrate levels of non-irradiated samples remained constant for almost 21 d and afterwards, a 20 % reduction was noticed after 35 d (Figure 1a). In irradiated samples, carbohydrate concentrations remained stable until the end of the storage period.

When edible mushrooms were irradiated and stored, a reduction on carbohydrate levels was noticed concomitant with the storage time, fruiting bodies of *Hypsizygus marmoreus* irradiated with gamma-rays (0.8 – 2 kGy) showed a reduction of 65 % after 25 days storage under modified atmosphere (Xing et al., 2007). Similarly, irradiated *Agaricus bisporus* (1 – 2 kGy) reduced their content approx. 30 % (Duan et al., 2010) within 16 d therefore, truffles carbohydrates seemed to be less influenced by irradiation and storage than mushrooms.

The stability of carbohydrates levels in irradiated truffles could be because no degradation of their main polysaccharides (chitins (Figure 1b) and β-glucans (Figure 1c)) was noticed during the selected storage time. Similarly, the reduction noticed in non-irradiated samples could be because of the progressive decrease in chitin and β-glucan contents during the storage period. The β-glucan levels in non-irradiated truffles dropped significantly below the concentrations found in all irradiated samples indicating that irradiation, when applied even at the lower doses utilized, helped to maintain this important bioactive compound for longer storing time than without the irradiation treatment. The observed degradation could be related to the excessive microbial colonization occurring in non-irradiated truffles (particularly after 28 d). It could also be responsible (together with the water losses) of the texture depreciation and softening observed by Rivera et al. (2011b) since chitins and β-glucans are the major constituents

256 of fungal membranes and they are responsible for its structural conformation and
257 firmness.
258 Protein levels of non-irradiated truffles decreased by an approx. 23 % during the first
259 seven days of storage (Figure 2a). This initial reduction could be related with the
260 metabolic changes that truffles stimulate to survive once they are harvested and their
261 mycelia cannot obtain more nutrients where proteins, as well as complex polysaccharides
262 (particularly chitins, Figure 1b), could be utilized as an energy source. Afterwards, protein
263 levels remained almost unchanged up to 35 d (Figure 2a) which could be explained by
264 the decrease in metabolism that occurs when truffles are stored in modified atmospheres
265 with low O_2 and high CO_2 (Rivera et al., 2010).
266 Immediately after the irradiation treatments, the protein levels significantly decreased by
267 23 to 55 % despite depending on dose and irradiation type (see data in Table 1but
268 remained constant until the end of the storage. It is well-known that the irradiation
269 treatments induced protein denaturation (Urbain, 1986) so this could explain the initial
270 reduction. Moreover, irradiation treatments induce to delay the physiological processes
271 leading to senescence and decreasing the respiration rate (Benoit et al., 2010). In our
272 assay, the respiration rate (RCO_2) was lowered by 19 % and 35 % on truffles treated with
273 1.5 kGy and 2.5 kGy, respectively (Rivera et al., 2011b), which, together with the
274 inhibitory effect of the modified atmosphere packaging, could contribute to maintaining
275 the protein unchanged during storage. In irradiated samples, the irradiation treatments
276 induced protein denaturation, suggesting that many enzymes involved in the primary
277 metabolism were probably inactivated and therefore, their protein levels might remain
278 unchanged with the influence of the generated modified atmosphere.
279 These results differed from previous observations reported by Xiong et al. (2009) where
280 a gradual protein degradation of *Pleurotus nebrodensis* was noticed during 22 d storage

281 of irradiated (cobalt: 0.8 – 2 kGy) and non-irradiated samples under modified atmosphere.
282 Similarly, Jiang et al. (2010) also noticed protein degradation in irradiated (cobalt: 1 –
283 2.5 kGy) and stored *L. edodes* during 20 d. Perhaps the different shape or cell wall
284 composition of the carpophore might partially protect truffle proteins compare to
285 basidiomycetes fruiting bodies. The perforation of utilized films might also differently
286 modulate the generated atmosphere inhibiting different metabolisms because, Nazzaro et
287 al. (2007) pointed out that lower gamma irradiation doses (1.5 kGy) were more adequate
288 to preserve black truffles than higher doses (2 kGy) since lower protein degradation was
289 noticed (N_2 atmosphere storage). The following results were more in concordance with
290 data presented (Fig. 2a), where protein levels in samples irradiated with the high doses
291 were significantly lower than with milder doses.
292 Irradiation treatments induced variations on total phenol concentrations not only
293 immediately after irradiation, but also during the storage period (Figure 2b). Non-
294 irradiated truffles maintained the same phenolic compound levels during almost the 35
295 storing d. Similar behaviour was observed in electron-beam irradiated truffles when 1.5
296 kGy were applied. However, gamma irradiation and particularly electron-beam
297 irradiation with 2.5 kGy enhanced the biosynthesis of phenolic compounds up to 21 d. In
298 the latter case, an almost three fold higher concentration than its initial levels was noticed.
299 Many phenolic compounds are synthesized when organisms are under stress since they
300 are deriving from the secondary metabolism. Phenols are responsible for many beneficial
301 biological activities acting as protective molecules against irradiation, desiccation,
302 oxidation, pathogens, lack of oxygen, among others. Thus, the increase noticed in
303 irradiated truffles could be a protective answer modulated by the stronger irradiation dose.
304 Similar behaviour was observed when *T. aestivum* was gamma-irradiated with doses
305 higher than 1.5 kGy (Adamo et al., 2004) but different than those observed in *L. edodes*

306 or *A. bisporus* mushrooms treated with 1.0 kGy (gamma irradiation) and stored in
307 modified atmospheres. Edible mushrooms showed higher levels of total phenolic
308 compounds at lower doses (1.0 kGy) compared to higher doses (2.0 kGy). Jiang et al.,
309 (2010) suggest that mushrooms might be more sensitive than truffles to irradiation and
310 later storage under modified atmosphere. The activation of protective phenols might start
311 at lower doses in mushrooms being 2.0 kGy excessive inducing, therefore, phenol
312 degradation.
313 Independently of the irradiation treatment applied, truffles storage under modified
314 atmosphere packaging induced approx. 50 % reduction in ergosterol levels during the
315 first 7 days coinciding with the atmosphere adjustment until it reached the steady-state
316 (Rivera et al., 2011b). Since ergosterol is utilized as biomarker for proper mycelial growth
317 (Parsi & Górecki, 2006), this reduction might be indicated that the truffle inhibites its
318 growth probably to adapt its metabolism and respiration rate to the new modified
319 environment. However, after 21 d of storage, ergosterol levels seemed to grow slowly but
320 continuously up to the end of the storage time (Table 2). This increase was not noticed
321 for brassicasterol, suggesting that the increase noticed in ergosterol might be due to the
322 yeast and other contaminant molds that were noticed after 21 d by Rivera et al. (2011a).
323 Brassicasterol is present in truffles but not in larger concentrations in mushrooms, molds
324 or yeast, however ergosterol is also the principal sterol of the latter organisms (Tsuji &
325 Fujimoto, 2018). Thus, in this case, brassicasterol could be used as an exclusive
326 biomarker to distinguish between mycelial growth from truffle or from contaminant
327 yeasts. Its concentrations were found constant through the storage time, suggesting that
328 no further truffle growth occurred after the gases equilibrium reached in the storage bags.
329 In truffles irradiated with 2.5 kGy gamma-irradiation, a second reduction on ergosterol

levels after 21 d was also noticed, this might indicate that the ascocarp was deteriorating faster than the other truffles irradiated at lower doses or with e-beam.

4. Conclusions

Electron-beam and gamma irradiation (1.5 y 2.5 kGy) of truffles did not modify total carbohydrates, β-glucans and total sterols concentrations, but significantly reduced chitin and protein levels. Total phenol contents were modified differently depending on the irradiation treatment and the utilized dose. Further storage of irradiated truffles under modified atmosphere packages extended their shelf life since no remarkable changes were noticed in their carbohydrate, chitin, β-glucan and protein contents during 35 d while non-irradiated truffles suffered a slight reduction of carbohydrates influenced by a marked β-glucan reduction. Irradiation followed by modified atmosphere storage stimulated biosynthesis of phenolic compounds up to 21 storage days, while their levels in non-irradiated truffles remained constant during the selected storage time. Ergosterol levels decreased during the first seven storage days and afterwards increased, but it might be due to the growth of contaminant molds. Thus, the combination of irradiations plus further storage under modified atmospheres is encouraged a preservation procedure for black truffles but up to approximately 35 storage days, because after this period, irradiated and stored truffles showed higher β-glucan content than non-irradiated and stored truffles. They also showed a higher level of phenolic compounds after 21 d, and if only 1.5 kGy are utilized, a slight reduction were only noticed in the protein levels. Thus, the truffle depreciation observed by Rivera et al. (2011a) in irradiated and stored carpophores starting after 21 storage days. It was mainly due to microbial colonization rather than a deterioration of the main truffle constituents or bioactive compounds.

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525 Figures

526 Figure 1. Levels of a) total carbohydrates, b) chitins and c) β-blucans in non- and
527 irradiated *T. melanosporum* truffles stored in modified atmosphere packages at 4 ºC for
528 35 days. Values are the mean of replicates and error bars indicate standard deviations.

529 Figure 2. Levels of a) total proteins and b) total phenolic compounds in non- and
530 irradiated *T. melanosporum* truffles stored in modified atmosphere packages at 4 ºC for
531 35 days. Values are the mean of replicates and error bars indicate standard deviations.

532 Table 1. Levels of total carbohydrates, β-glucans, chitins, total proteins and total phenolic
533 compounds in non- and irradiated *T. melanosporum* truffles. Values are the mean of
534 replicates and error bars indicate standard deviations. Indicated values are w/w.

Treatment (kGy)	**Total Carbohydrates (%)**	**β-Glucans (%)**	**Chitins (%)**	**Total Proteins (%)**	**Total Phenolic compounds (g kg^{-1})**
Non-irradiated	33.00 ± 3.44^{a}	20.71 ± 0.49^{a}	12.20 ± 0.22^{a}	16.86 ± 0.19^{a}	0.76 ± 0.01^{b}
gamma 1.5	25.50 ±1.29^{a}	18.90 ± 1.26^{a}	6.11 ± 0.55^{b}	10.08 ±0.53^{c}	0.80 ± 0.02^{ab}
gamma 2.5	26.28 ±2.10^{a}	17.47 ± 1.37^{a}	5.32 ± 0.24^{b}	8.69 ± 0.77^{cd}	0.64 ± 0.00^{c}
e-beam 1.5	24.84 ±1.92^{a}	19.71 ± 0.78^{a}	5.90 ± 0.20^{b}	13.11 ± 0.44^{b}	0.87 ± 0.06^{a}
e-beam 2.5	25.32 ±0.93^{a}	19.86 ± 1.27^{a}	6.10 ± 0.45^{b}	7.62 ± 0.28^{d}	0.66 ± 0.01^{bc}

535 Different letters denote significant differences ($P \leq 0.05$) within the same column

536 Table 2. Levels of fungal sterols and derivatives in non- and irradiated *T. melanosporum*
537 truffles stored for 35 days in modified atmosphere packages at 4 ºC. Values are the mean
538 of replicates and error bars indicate standard deviations. Indicated values are w/w.

Time (days)	Treatment & doses (kGy)	Ergosterol (g kg^{-1})	Brassicasterol (g kg^{-1})	Ergosta7,22-dienol (g kg^{-1})	9,19ciclolanost-7-en-3-ol (g kg^{-1})	Ergocalciferol (g kg^{-1})	Total sterols (g kg^{-1})
0	Non-irradiated	1.55 ± 0.01^{A}	2.00 ± 0.01^{A}	0.46 ± 0.01^{A}	0.43 ± 0.01^{A}	n.d.	4.43 ± 0.04^{A}
	gamma 1.5	2.14 ± 0.47^{AB}	1.26 ± 0.25^{B}	0.51 ± 0.18^{A}	0.48 ± 0.18^{A}	n.d.	4.38 ± 1.09^{A}
	gamma 2.5	2.30 ± 0.46^{A}	1.49 ± 0.46^{AB}	0.79 ± 0.35^{A}	0.75 ± 0.31^{A}	n.d.	5.34 ± 1.60^{A}
	e-beam 1.5	1.86 ± 0.09^{A}	1.22 ± 0.16^{B}	0.85 ± 0.17^{A}	0.90 ± 0.15^{A}	n.d.	4.85 ± 0.59^{A}
	e-beam 2.5	1.45 ± 0.05^{B}	1.31 ± 0.04^{AB}	0.72 ± 0.08^{A}	0.71 ± 0.01^{A}	n.d.	4.20 ± 0.12^{A}
7	Non-irradiated	0.80 ± 0.30^{B}	0.91 ± 0.32^{B}	0.36 ± 0.18^{A}	0.34 ± 0.18^{A}	n.d.	2.42 ± 1.00^{A}
	gamma 1.5	1.21 ± 0.02^{A}	1.56 ± 0.02^{A}	0.65 ± 0.13^{A}	0.55 ± 0.13^{A}	n.d.	3.98 ± 0.31^{A}
	gamma 2.5	1.08 ±0.08^{B}	1.09 ± 0.14^{A}	0.49 ± 0.16^{A}	0.46 ± 0.17	n.d.	3.13 ± 0.56^{A}
	e-beam 1.5	1.03 ±0.05^{A}	0.81 ± 0.03^{A}	0.56 ± 0.03^{A}	0.51 ± 0.05^{A}	n.d.	2.92 ± 0.17^{A}
	e-beam 2.5	0.93 ± 0.10^{A}	0.98 ± 0.08^{A}	0.37 ± 0.06^{A}	0.36 ± 0.05	n.d.	2.36 ± 0.30^{A}
21	Non-irradiated	1.10 ± 0.15^{B}	1.00 ± 0.16^{B}	0.33 ± 0.19^{A}	0.32 ± 0.19^{A}	n.d.	2.77 ± 0.70^{A}
	gamma 1.5	1.37 ± 0.31^{AB}	1.26 ± 0.26^{A}	0.41 ± 0.06^{A}	0.43 ± 0.06^{A}	n.d.	3.47 ± 0.70^{A}
	gamma 2.5	1.91 ± 0.03^{B}	1.30 ± 0.04^{A}	0.48 ± 0.13^{A}	0.49 ± 0.11^{A}	n.d.	4.20 ± 0.32^{A}
	e-beam 1.5	1.43 ± 0.33^{A}	1.13 ± 0.28^{A}	0.63 ± 0.19^{A}	0.59 ± 0.18^{A}	n.d.	3.79 ± 1.00^{A}
	e-beam 2.5	1.14 ± 0.20^{A}	0.74 ± 0.16^{A}	0.31 ± 0.08^{A}	0.30 ± 0.08^{A}	n.d.	2.50 ± 0.53^{A}
35	Non-irradiated	1.44 ± 0.48^{AB}	0.95 ± 0.48^{B}	0.33 ± 0.09^{A}	0.36 ± 0.10^{A}	n.d.	3.08 ± 1.17^{A}
	gamma 1.5	2.12 ± 0.07^{AB}	1.50 ± 0.16^{A}	0.31 ± 0.03^{A}	0.30 ± 0.03^{A}	n.d.	4.25 ± 0.31^{A}
	gamma 2.5	1.39 ± 0.14^{B}	1.28 ± 0.07^{A}	0.77 ± 0.06^{A}	0.83 ± 0.05^{A}	n.d.	4.28 ± 0.32^{A}
	e-beam 1.5	1.78 ± 0.18^{A}	0.86 ± 0.04^{A}	0.43 ± 0.02^{A}	0.43 ± 0.01^{A}	n.d.	3.51 ± 0.27^{A}
	e-beam 2.5	1.43 ± 0.26^{A}	1.15 ± 0.22^{A}	0.63 ± 0.12^{A}	0.70 ± 0 .12^{A}	n.d.	3.92 ± 0.72^{A}

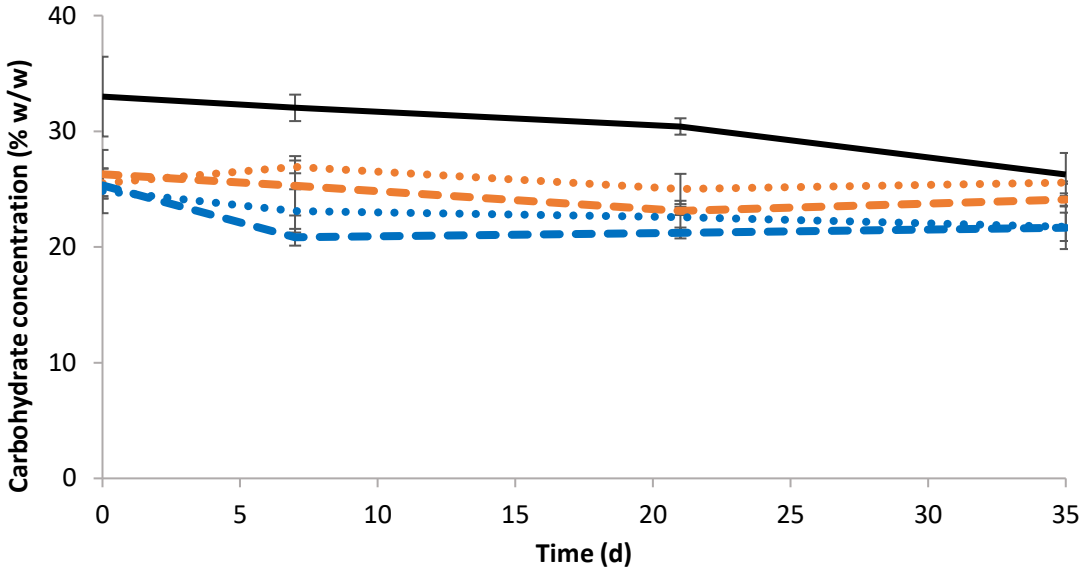
539 n.d., not detected.

[A, B] Different letters denote significant differences ($P \leq 0.05$) between different day for the same treatment.

No significant differences ($P \leq 0.05$) were found between different treatments for the same day.

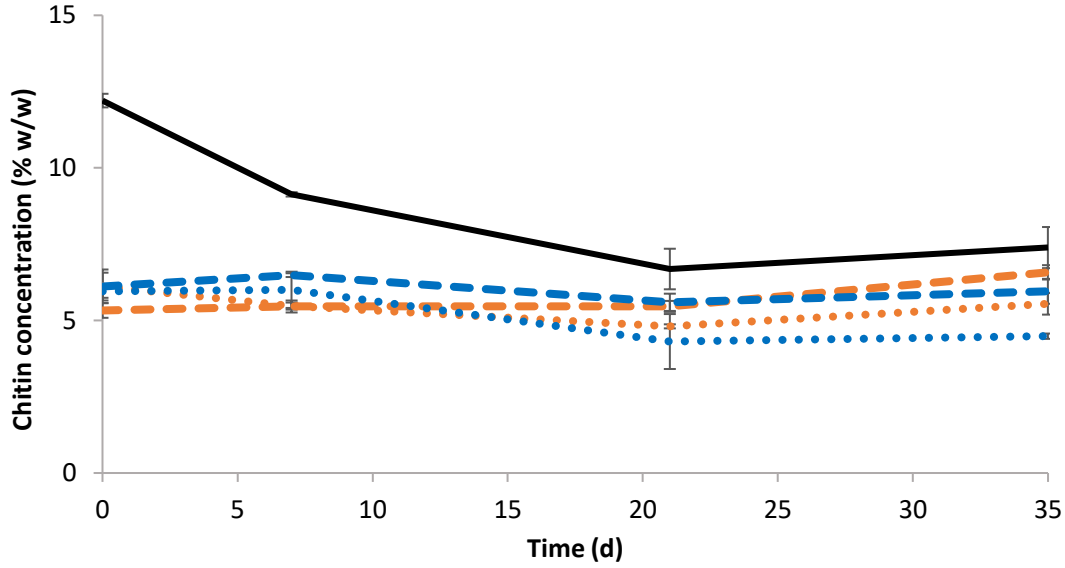
545 Figure 1

a)



546

b)



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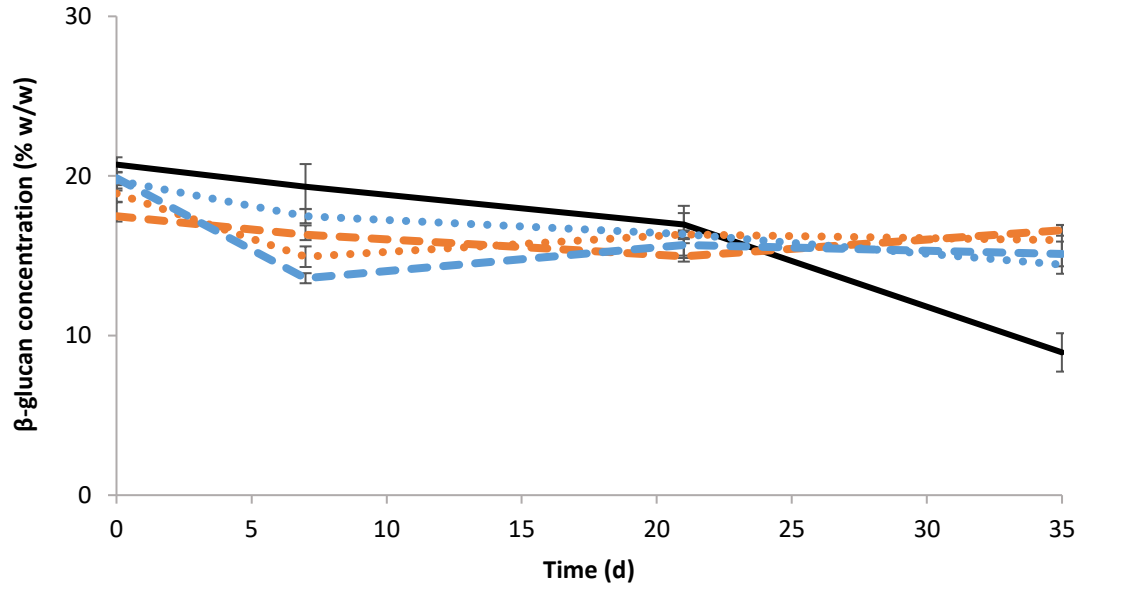
c)



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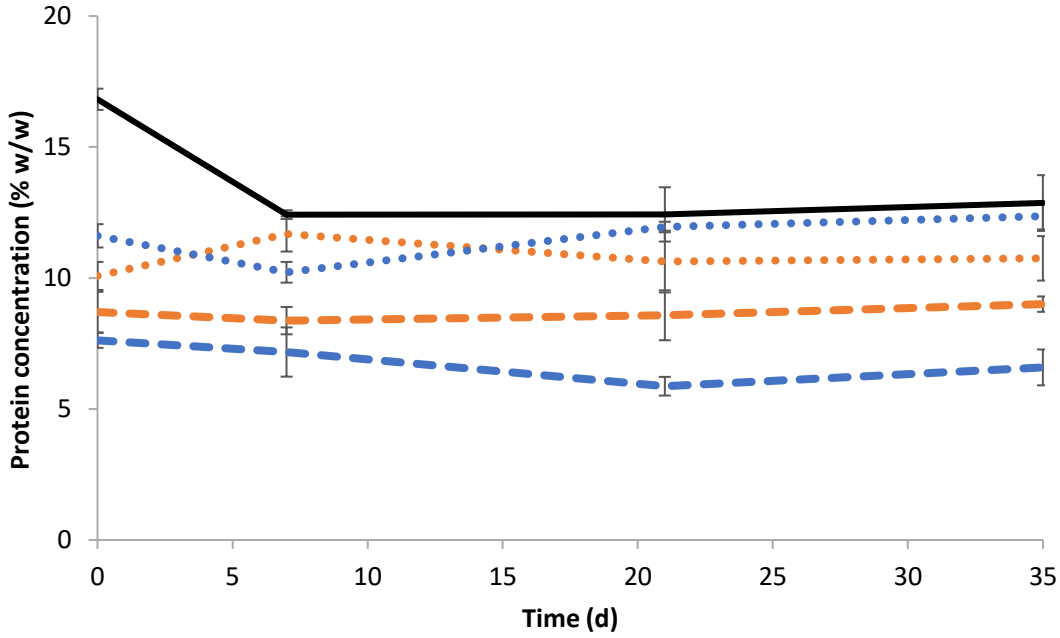
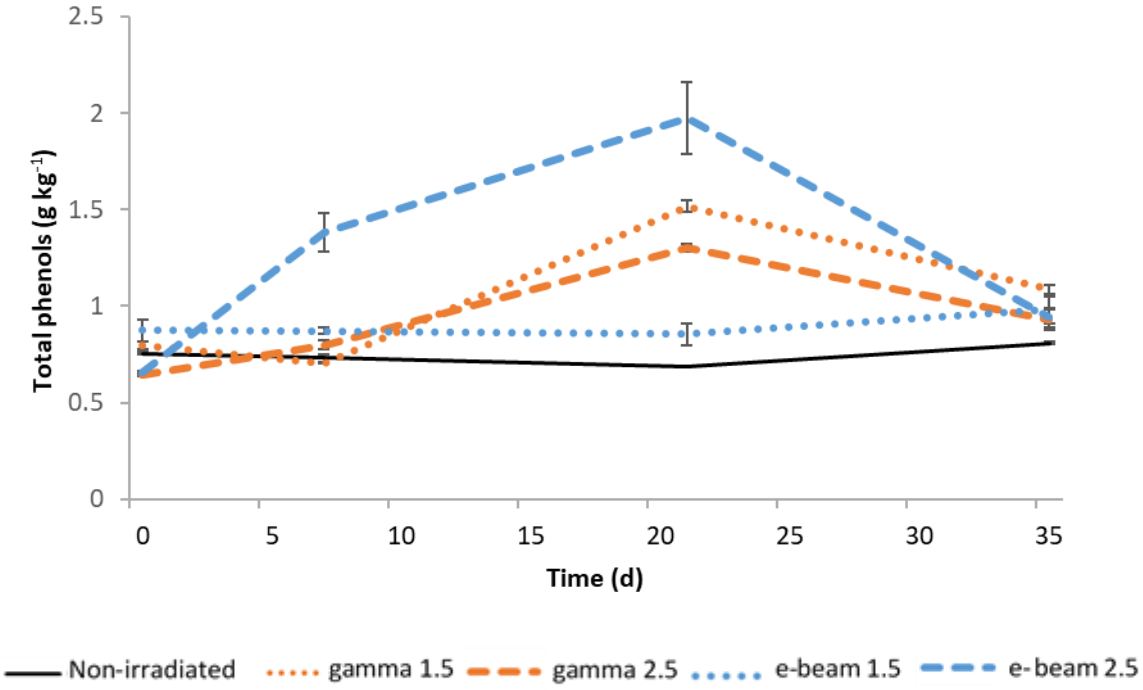
550 Figure 2

a)



551

b)


2.5
2
1.5
1
0.5
0
Total phenols (g kg-1)
0
5
10
15
20
25
30
35
Time (d)
Non-irradiated
gamma 1.5
gamma 2.5
e-beam 1.5
e-beam 2.5

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