Effects of gamma irradiation on physical parameters of *Lactarius deliciosus* wild edible mushroom

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ABSTRACT

Studies evaluating the effects of ionizing radiation in mushrooms are mostly available in cultivated species, being scarce reports on wild species, considered add-value foods. In the present work, the effects of gamma radiation dose (0, 0.5 and 1 kGy) and storage time (0 to 8 days at 5 ºC) on the physical parameters (colour, cap diameter and weight) of the wild edible mushroom *Lactarius deliciosus* were evaluated. It was observed a slight decrease in redness (*a*) with irradiation dose and a slight decrease in the cap diameter with storage time. Regarding the weight loss profiles along the 8 days of storage, the results were very similar for irradiated and non-irradiated samples. Overall, this study demonstrated that up to 1 kGy, gamma irradiation and cold storage did not affect significantly the assayed physical properties.

Keywords: Gamma irradiation; Wild mushrooms; *Lactarius deliciosus*; Colour; Cap diameter; Weight

1. Introduction

Mushrooms are highly perishable and have a limited shelf-life, frequently 1-3 days at room temperature, and tend to lose quality immediately after harvest (Akram and Kwon, 2010; Sommer et al., 2010). These motifs are an impediment to the distribution and marketing of the fresh product.

The high respiration rate, the lack of physical protection to avoid water loss and the changes due to microbial attack are often associated with mushrooms loss of quality, contributing to their deterioration through browning, cap opening, stipe elongation, cap diameter increase, weight loss and texture changes (Akram and Kwon, 2010; Singh et al., 2010; Sommer et al., 2010).

Bacteria, moulds, enzymatic activity (mainly polyphenol oxidase, PPO) and biochemical changes can cause spoilage during storage. Furthermore, the browning of mushroom cells occurs when they are subjected to forces that can disrupt cellular integrity such as vibrations, rough handling, and ageing (Beaulieu et al., 2002; Jiang et al., 2010). The most important factors determining the rate of enzymatic browning are the tissue concentrations of active PPO, pH, temperature, water activity and oxygen availability. Water loss or transpiration are important physiological processes that affect the main quality characteristics of fresh mushrooms, such as weight, appearance and texture, and these processes are dependent on surrounding temperatures and relative humidity (Pai, 2000; Singh et al., 2010).

Morphological changes, which involve exposure of the gills and sporulation, are supported by substrates which are present in the sporophore at harvest, rather than substrates of mycelial origin, as is the case of the growing sporophore. Thus the substrate expended in postharvest sporophore development, and hence respiration, is also an important factor in determining the onset of senescence; the overall decline in

respiratory activity seen after harvest is due to the exhaustion of substrates and senescence of the tissues (Singh et al., 2010).

Thus, extended shelf-life is a key factor for making any food commodity more profitable and commercially available for long periods of time at the best possible quality. The producer will benefit from the longer shelf-life to develop the market over greater distances (Akram and Kwon, 2010). A general trend in food preservation research is towards the development of preservation techniques that are less severe and therefore less damaging to food products (Gould, 1989; Minnaar et al., 1995). In this sense, there has been extensive research on finding the most appropriate technology for mushrooms preservation.

Food irradiation appears as a possible alternative for stored mushrooms in order to enhance their shelf-life as well as their safety. Particularly, gamma irradiation has been shown to be a potential tool in extending the postharvest shelf-life of fresh mushrooms (Beaulieu et al., 2002).

Studies evaluating the effects of ionizing radiation are mostly available in cultivated species with high production value such as *Agaricus bisporus* (Wani et al., 2009), *Lentinus edodes* (Jiang et al., 2010) and *Pleurotus ostreatus* (Jasinghe and Perera, 2006). Nevertheless, reports on wild species are scarce and, as far as we know, there are no studies evaluating the effects of irradiation on wild *Lactarius deliciosus* species. Moreover, it should be highlighted that wild species are considered add-value foods for commercialization.

Herein, the effects of gamma radiation doses and storage time on *Lactarius deliciosus* physical parameters (colour, cap diameter and weight) were evaluated.

2. Materials and methods

2.1. Samples and samples irradiation

Lactarius deliciosus fruiting bodies were collected in Trás-os-Montes (Northeast of Portugal) in November 2011, and divided in three groups: control (non-irradiated, 0 kGy); sample 1 (0.5 kGy) and sample 2 (1 kGy) with eighteen specimens per group. In each group, specimens in different maturity stages (distinguished by mushrooms cap diameter) were included (**Figure 1**).

The estimated dose rate for the irradiation position was obtained with Fricke dosimeter, a reference standard that provides a reliable means of absorbed doses measurement in water, based on an oxidation process of ferrous ions to ferric ions in acidic aqueous solution by ionizing radiation. The acid aqueous Fricke dosimeter solution was prepared and read following the standard procedure (ASTM, 1992). The irradiation of groups 1 and 2 was performed immediately after harvest, in a Co-60 experimental chamber with four sources, total activity 267 TBq (6.35 kCi; 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), groups 2 and 3 were placed in Poly(methyl methacrylate) (PMMA) box or acrylic glass, and irradiated at ambient atmosphere and temperature (15 ^{o}C). To monitor the process during the irradiation, 4 routine dosimeters were used for the highest dose (1 kGy) in the corners of the irradiation box (Amber Perspex dosimeters, batch V, from Harwell company, U.K.). The samples were rotated upside down (180^{o}) at half of the time, to increase the dose uniformity. The Amber Perspex dosimeters were twice read in a UV-VIS Spectrophotometer (Shimadzu mini UV 1240 spectrophotometer) at 603 nm, to estimate the dose according to a previous calibration curve. The estimated doses after irradiation

were 0.6 ± 0.1 kGy and 1.1 ± 0.1 kGy for samples 2 and 3, respectively, at a dose rate of 2.3 ± 0.1 kGy h^{-1}. For simplicity, in the text, tables and graphs we considered the values 0, 0.5 and 1 kGy, for non-irradiated and irradiated samples.

2.2. Physical parameters

2.2.1. Colour measurement

A Minolta spectrophotometer (Konica Minolta Sensing, Inc., Chroma Meter CR-400, Japan) was used to measure daily the colour in three distinct zones of the mushroom surface, being considered the average value. Using illuminant C and the diaphragm opening of 8 mm, the Hunter colour *L*, *a* and *b* values were reported through the computerized system using a colour data software Spectra Magic Nx (version CM-S100W 2.03.0006, Konica Minolta company, Japan). The instrument was calibrated to standard white tiles before analysis (Spectra Magic NX Instruction Manual, Konica Minolta Sensing, Inc. (ver 2.0), 2009, Japan).

2.2.2. Cap diameter measurement

A digital caliper with precision of 0.01 mm was used to measure daily the diameter of the mushroom cap. Minimum and maximum diameters were obtained for each sample, being considered the average value.

2.2.3. Weight loss

An electronic balance Kern ABS (Type ABS 220-4, Germany) was used to daily weighing the samples. Weight loss was determined by periodical weighing. The weight loss was calculated as:

Weight loss (%) = $[(W_i - W_s)/ W_i] \times 100$.

Where W_i = initial weight, W_s = weight at sampling period (Wani et al. 2009).

2.2.4. Temperature measurement

The samples were collected and transported in a thermal hermetic cage, putting inside a USB data logger (model LASEL – USB-2+, from Lascar Electronics Ltd., U.K.) with a sotware EasyLog USB version 5.45, for temperature, dew point and humidity monitoring during storage.

2.3. Statistical analysis

For each one of the storage times and irradiation doses, three samples were analysed, with all the assays being also carried out in triplicate. An analysis of variance (ANOVA) with Type III sums of squares was performed using the GLM (General Linear Model) procedure of the SPSS software, version 18.0 (SPSS, Inc.). All dependent variables were analysed using a 2-way ANOVA, being the main factors the "storage time (ST)" (daily, 0 to 8 days) and the "irradiation dose (ID)" (0, 0.5 and 1 kGy).

When a statistical significant interaction effect (ID×ST) was found, the two factors were evaluated simultaneously by plotting the estimated marginal means for all levels of each factor. When the mentioned interaction was not found, data were compared by multiple comparisons, using Tukey's test, whenever the homoscedasticity requirement was fulfilled.

Hierarchical cluster analysis (HCA) was used as an unsupervised learning method. HCA was applied to standardized data to investigate similarities between samples stored

for different periods or irradiated with different doses. HCA calculates the distances (or correlation) between all samples using a defined metric such as squared Euclidean distance or Chebychev distance. Hierarchical clustering is the most common approach in which clusters are formed sequentially. The most similar objects are first grouped, and these initial groups are merged according to their similarities. Eventually as the similarity decreases all subgroups are fused into a single cluster. The statistical tests were performed at a 5% significance level.

3. Results and discussion

Our previous studies assessing the potential of gamma irradiation as a suitable technique to increase natural products shelf-life were focused in nutritional and chemical parameters, or bioactive potential (Antonio et al., 2011; Barreira et al., 2012; Fernandes et al., 2011a; Fernandes et al., 2011b), as well as the way these features change along time. In general, the obtained results seemed to indicate that storage time caused more evident modifications in the evaluated parameters than the radiation dose. However, the application of gamma radiation, like any other conservation technique, demands the maintenance of the physical characteristics (like colour, shape or weight) of the targeted food product.

The results of Hunter's colour *L* (lightness), *a* (redness) and *b* (yellowness) obtained for the studied samples (Hunter and Harold, 1987) are presented in **Table 1**. *L*, depending on reflectivity of the determined surface, was used to express luminosity of the sample surface. Lower *L* indicates darkening of mushroom, while increasing *a* value shows increasing redness, and increasing *b* value suggests increasing yellowness of the samples (Du et al., 2009). The results are reported as mean value of each irradiation

dose (ID) over the different storage times (ST) as well as mean value of all ID for each ST (statistical treatment considering two factors). In this way, it is possible to evaluate the effect of irradiation without the potential influence of ST, an essential feature to consider irradiation as a potential shelf-life increasing technique. The applied multiple comparisons pointed out significant differences among 0 and 8 days, regarding ST, and samples irradiated with 1 kGy and those treated with 0.5 kGy or non-irradiated. The differences induced by ID were clearer for *a* (**Figure 2a**) and *L* (**Figure 2b**) parameters. The decrease in redness (*a*) is in accordance with previous studies assessing the effect of irradiation in mushrooms (Kim et al., 2009) or plants (Jo et al., 2003a; Jo et al., 2003b; Kim et al., 2006), and might be related with a secondary effect of the water radiolysis. It is known that this process may result in the production of chemical species such as hydrated electrons, hydroxyl radicals or hydrogen atoms that might oxidize colour compounds like carotenoids (Kim et al., 2009).

Regarding cap diameter, it was decided to analyse individually the results obtained for each ID. Despite the eighteen mushrooms selected for each ID being representative of different maturity stages (with high variation in cap diameter, **Figure 1**), there were differences among the specimens selected for each group and corresponding to the same maturity stage, which would difficult the results discussion. In general, the cap diameter tended to decrease (as can be observed by the negative slope in the equations corresponding to 0 and 1 kGy, **Figure 3**), reaching the lowest values after 8 days of storage for all doses. The high standard deviations observed for each group are related to the presence of mushrooms in different maturity stages (distinguished by the cap diameter) to accomplish a representative sample. The plotted values are the mean of the eighteen mushrooms measured in each storage period and for each irradiation dose.

Regarding the weight loss profiles along the 8 days of storage, the results were very similar for irradiated and non-irradiated samples (**Figure 4**). The observed decrease would certainly be higher if samples were not refrigerated. The storage conditions were also controlled and might be checked in **Figure 5** in which temperature, dew point and relative humidity are plotted. The peaks in temperature and dew point, that overlap the valleys in relative humidity, correspond to the measurement periods, in which samples were taken from the freezer to evaluate colour and cap diameter at room temperature. Except for these short periods, the analysis of **Figure 5** clearly indicates the homogeneity of storage temperature and humidity, allowing the attribution of any observed change in the physical parameters to the studied factors (ST and ID).

Despite the particular tendencies previously described, the results obtained for the assayed parameters seemed to indicate that neither irradiation nor cold storage, exerted significant influence. To check this assumption, a Hierarchical cluster analysis (HCA) was applied. The results obtained following HCA are shown as a dendrogram (**Figure 6**) in which well-defined clusters were not obtained. In this analysis, samples are grouped in clusters in terms of their nearness or similarity. If the differences for lightness (L), redness (a), yellowness (b), weight loss and cap diameter among samples irradiated with different doses or stored for different periods were significant, it would be expectable that a number of clusters corresponding to the naturally defined (for instance, three clusters formed for ID) would be obtained. The dendogram shows the opposite, indicating high similarity among the assayed samples, since there are mixtures of different ST or different ID in each individualized cluster. Nevertheless, the well defined evolution that some Hunter's colour parameters showed with irradiation dose should not be neglected.

Overall, this study demonstrated that up to 1 kGy, the association of gamma irradiation and cold storage did not affect the assayed physical properties.

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Table 1. Hunter's colour *L* (lightness), *a* (redness) and *b* (yellowness) of non-irradiated and irradiated *Lactarius deliciosus* samples, after different times of storage. The results are presented as mean±SD[a] (n=9, for each storage time, ST, n=24 for each irradiation dose, ID).

		L value	*a* value	*b* value
ST	0 days	43±4	12±3 a	16±2 a
	1 day	42±4	11±2 ab	15±2 ab
	2 days	40±5	10±2 abc	15±2 abc
	3 days	41±4	11±2 abc	15±2 abc
	4 days	41±4	11±2 abc	15±2 abc
	5 days	40±4	10±3 abc	15±2 bc
	6 days	39±4	10±3 bc	15±2 bc
	7 days	39±4	10±3 bc	14±2 bc
	8 days	38±4	10±3 c	14±2 c
	p-value	<0.001	<0.001	<0.001
ID	0.0 kGy	42±4	11±3 a	15±2
	0.5 kGy	40±4	11±3 a	15±2
	1.0 kGy	39±4	10±3 b	15±2
	p-value	<0.001	0.001	0.471
ST×ID	*p*-value	0.044	0.978	0.552
Levene's test	*p*-value	0.304	0.768	0.609

[a]Results are reported as mean value of each irradiation dose (ID) over the different storage times (ST) as well as mean value of all ST within each ID. Therefore, SD reflects values in those samples (under different ID or ST).

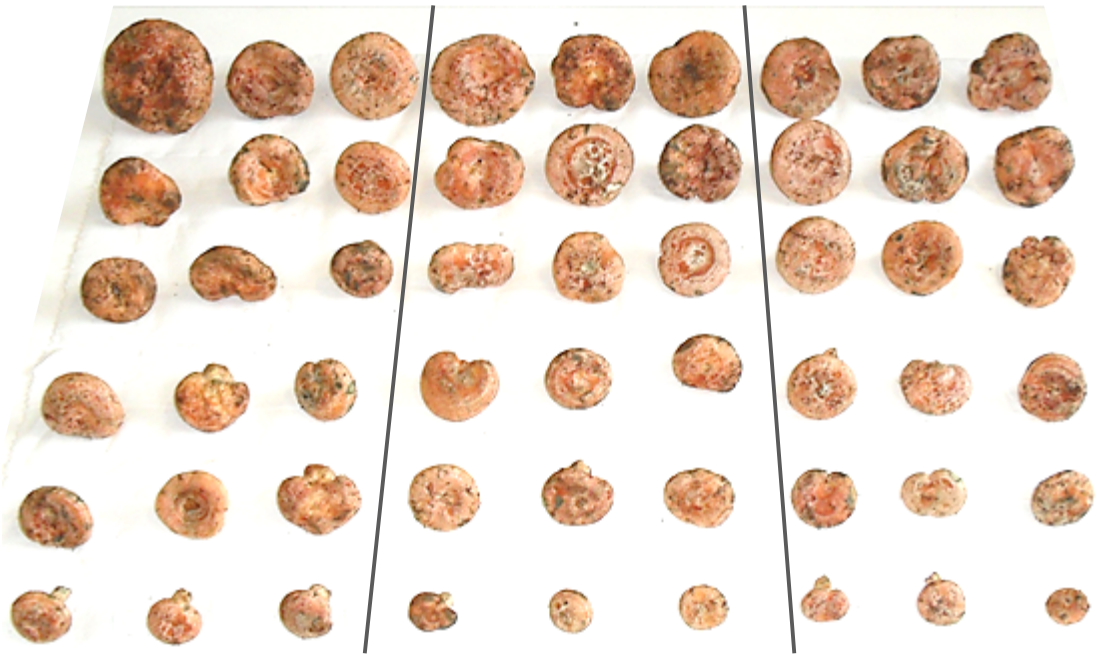
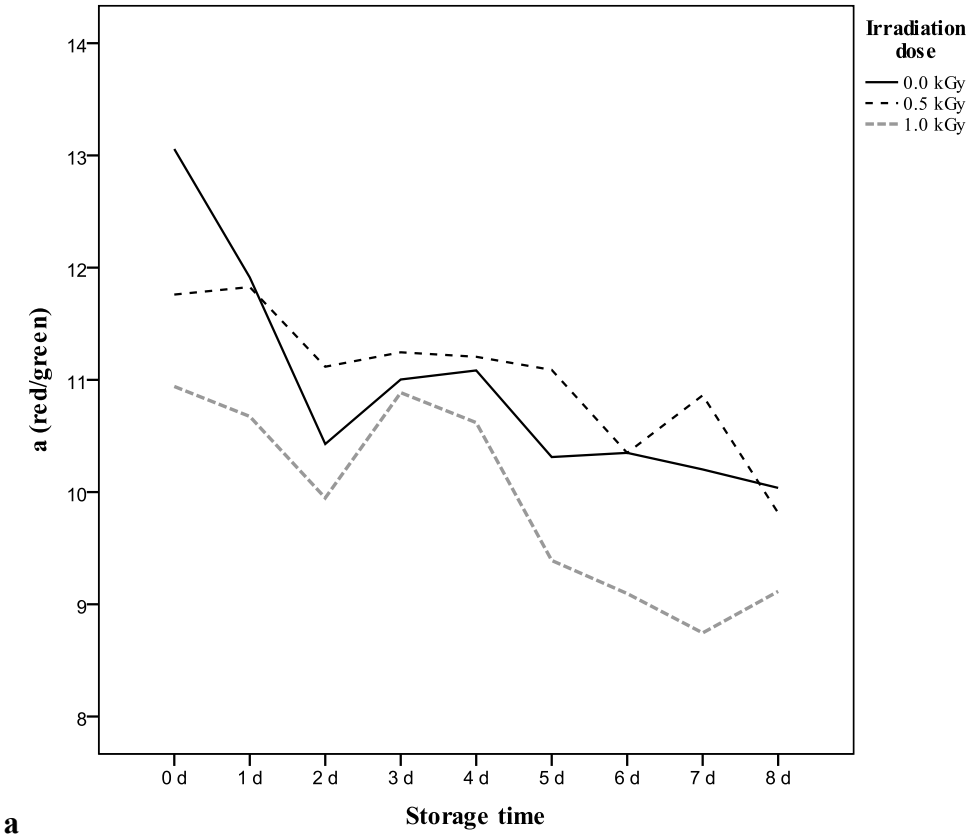


Figure 1. *Lactarius deliciosus* studied population divided in three groups: control (non-irradiated, 0 kGy), sample 1 (0.5 kGy) and sample 2 (1 kGy) with eighteen specimens per group (separated by lines). Several stages of maturity (distinguished by the cap diameter) may be found among each group. The cap diameter of the presented samples, whose picture was obtained in day 0 of storage, varied from 25 to 55 mm within each group.



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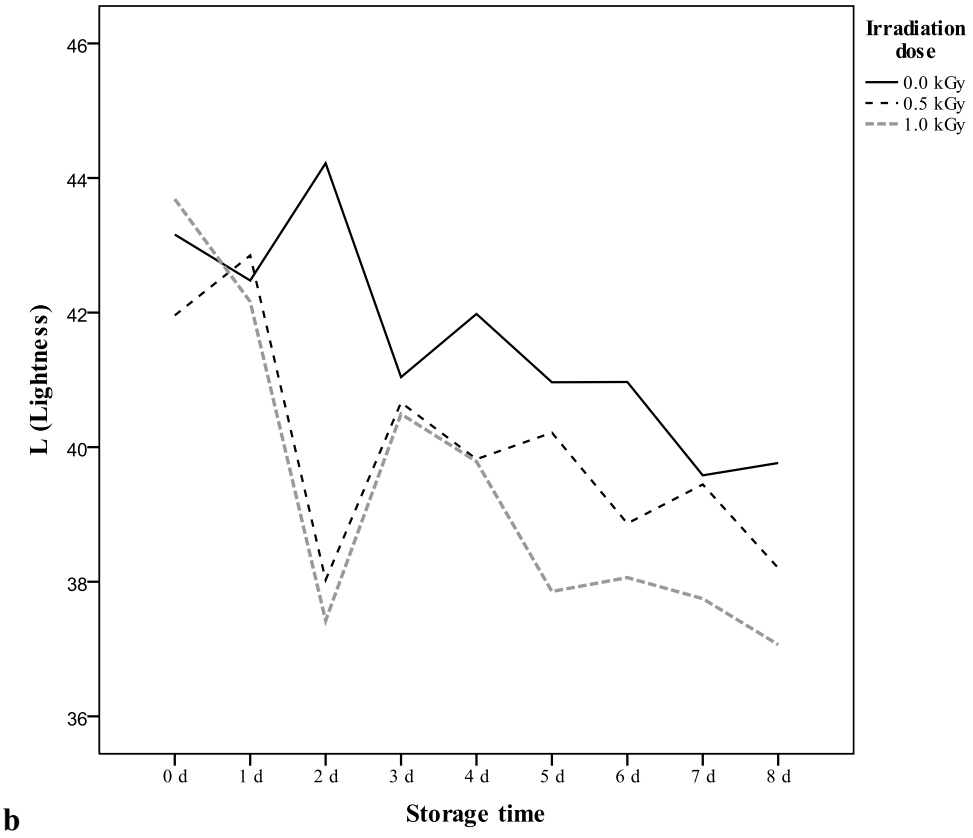


b

Figure 2. Interactions between ST (storage time) and ID (irradiation dose) effects on *Lactarius deliciosus* samples. Influence on redness (**a**) and lightness (**b**) parameters.

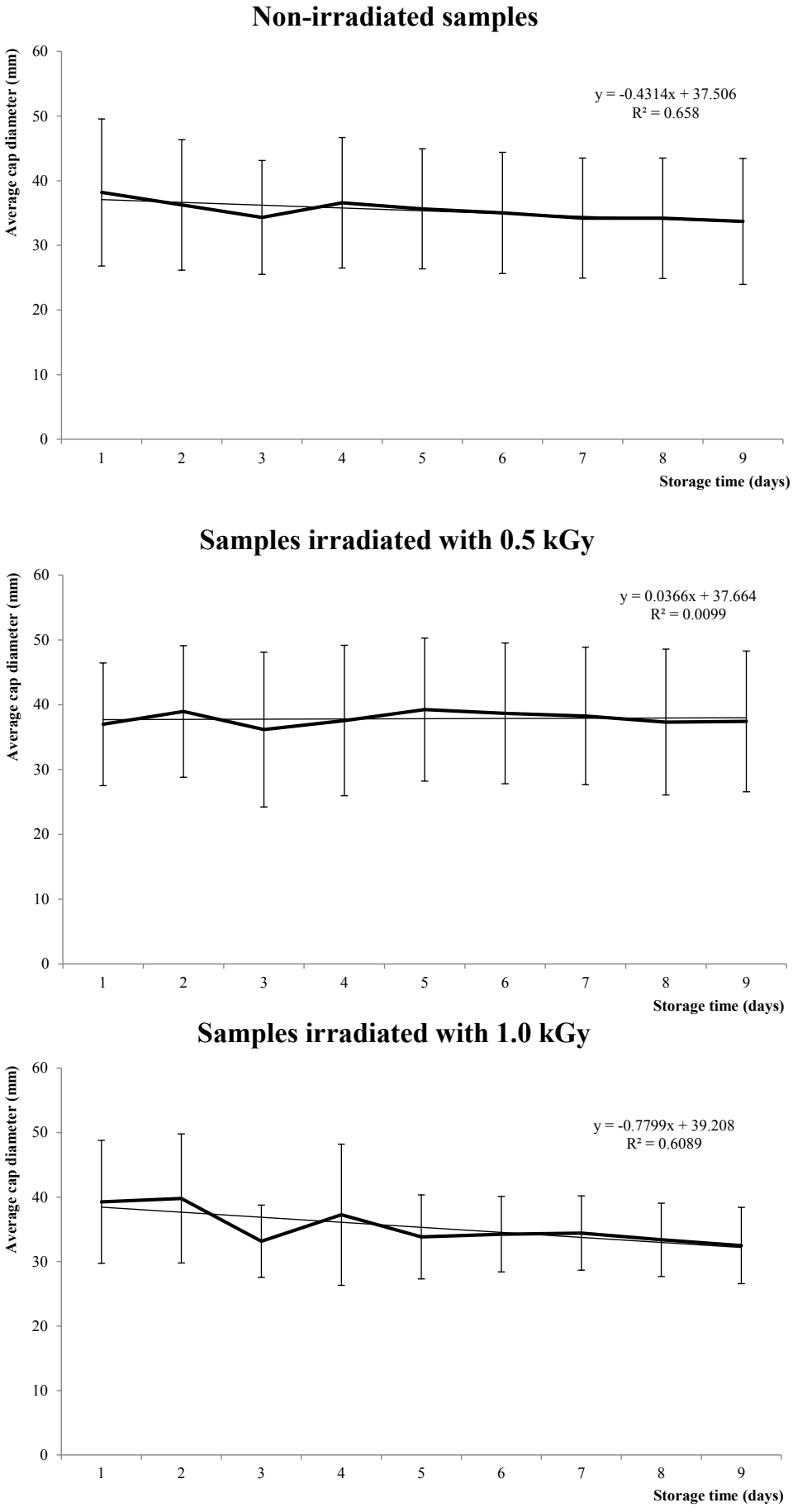


Figure 3. Variation in cap diameter along the 8 days of storage.

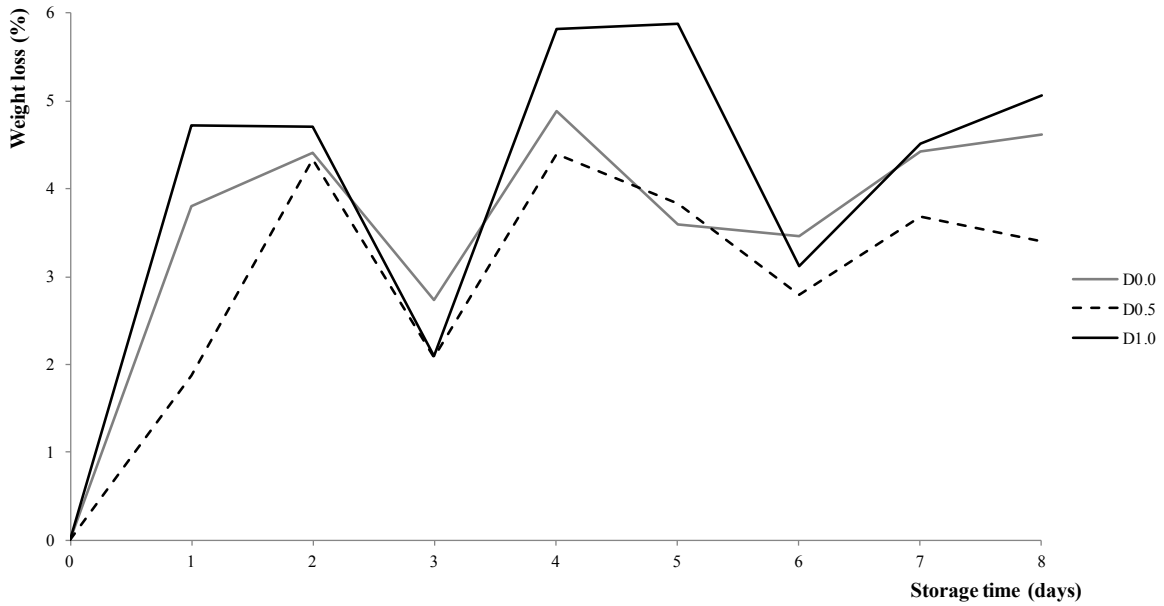
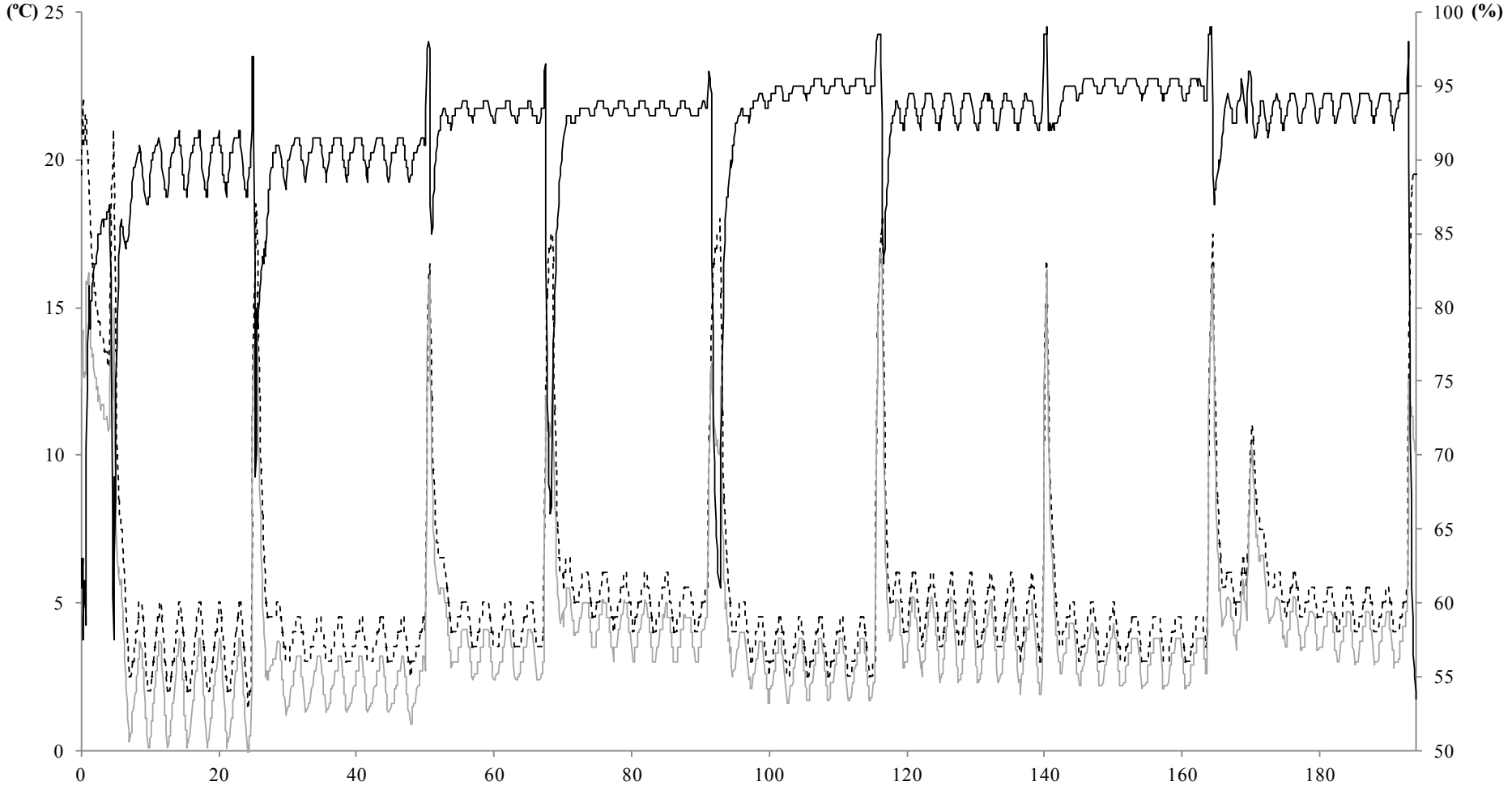


Figure 4. Weight loss profiles along the 8 days of cold storage for non-irradiated samples and samples irradiated with 0.5 or 1.0 kGy.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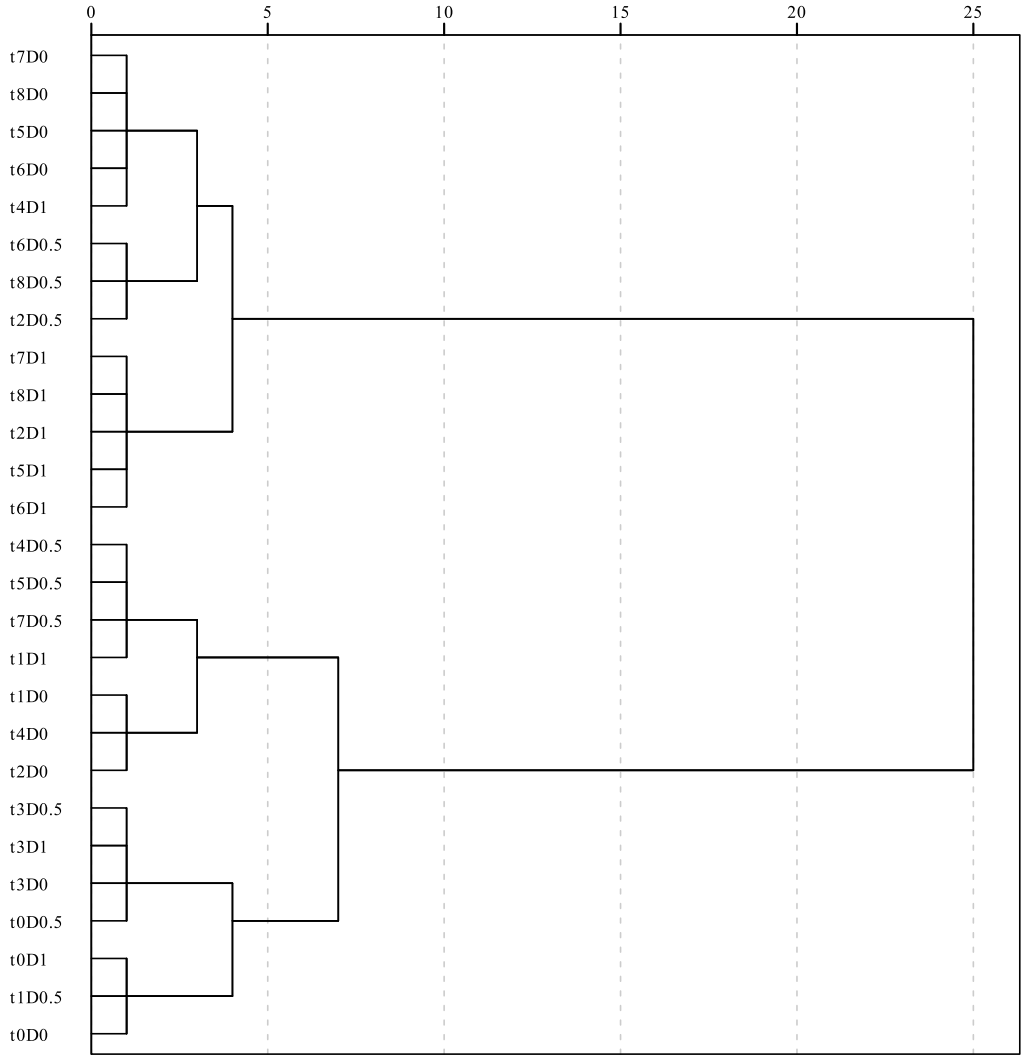

Figure 6. Dendogram obtained with Z-scores standardization results after applying Ward linkage method.