FOODSIM'2012

FREISING, GERMANY

JUNE 18-20, 2012

FRAUNHOFER INSTITUTE
FOR PROCESS ENGINEERING
AND PACKAGING IVV

Fraunhofer
IVV

EDITED BY
HORST-CHRISTIAN LANGOWSKI
AND
SVEN FRANKE

ORGANIZED BY

eti
European Technology Institute

IN COOPERATION WITH

Technische Universität München

7TH INTERNATIONAL CONFERENCE
ON
SIMULATION AND MODELLING
IN THE
FOOD AND BIO-INDUSTRY
2012

FOODSIM'2012

EDITED BY

Horst-Christian Langowski

and

Sven Franke

JUNE 18-20, 2012

FREISING, GERMANY

A Publication of EUROSIS-ETI

Printed in Ghent, Belgium

CONTENTS

FOOD CONTAMINATION AND SAFETY MEASURES

FOOD PACKAGING

COMBINED EFFECTS OF γ-IRRADIATION AND STORAGE TIMES ON SUGARS COMPOSITION OF *Lactarius deliciosus*: COMPARISON THROUGH LINEAR DISCRIMINANT ANALYSIS

Ângela Fernandes[1,2]
João C.M. Barreira[1,2]
Anabela Martins[1]
Isabel C.F.R. Ferreira[1]
[1]CIMO-ESA - Instituto Politécnico de Bragança, Portugal; Email: iferreira@ipb.pt

M. Beatriz P.P. Oliveira[2]
[2]REQUIMTE/Departamento de Ciências Químicas, Faculdade de Farmácia, Porto, Portugal

Amilcar L. Antonio[1,3]
[3]GTRPP/Unidade de Física e Aceleradores, ITN, Sacavém, Portugal.

KEYWORDS

Lactarius deliciosus, shelf-life, gamma irradiation, sugars

ABSTRACT

The effects of gamma irradiation on *Lactarius deliciosus* (L. ex Fr.) S. F. Gray sugars were evaluated in samples submitted to different storage periods (0, 4 and 8 days) at 4 °C. The irradiations were performed in a ^{60}Co experimental equipment. Changes in sugars were determined by analyzing the results obtained by high performance liquid chromatography coupled to refraction index detection (HPLC-RI) through a 2-way analysis of variance and a linear discriminant analysis. Mannitol was by far the most abundant sugar in the analyzed samples. Regarding sugars profile, storage time proved to have higher influence than irradiation dose, mainly reflected in the decrease of fructose and mannitol in stored samples.

INTRODUCTION

The special place held by mushrooms in human food is well illustrated by the statistic data regarding world production of mushrooms and truffles which in 2007 reached a volume of 3 414 392 metric tons (USDA 2009). Their global economic value is now staggering, and the reason for the rise in consumption is a combination of their value as food (Kalač 2009) and their medicinal or nutraceutical properties (Ferreira et al. 2010).

In Europe, mushrooms are highly consumed (*L. deliciosus* is among the most consumed wild species) due to their high contents of digestible proteins, carbohydrates, vitamins and fibers. Nevertheless, mushrooms are one of the most perishable products and tend to lose quality right after harvest. The short shelf-life of mushrooms (1-3 days at room temperature) is an obstacle to their distribution and marketing as fresh products. Mushrooms may suffer spoilage during storage due to bacteria, moulds, enzymatic activity, or biochemical changes. Despite the immense popularity of this food in the Northeast of Portugal (one of the European regions with the highest mushrooms biodiversity) and its increased exportation to foreign countries (particularly Spain, France and Italy), data regarding technologies to enlarge their shelf-life is still scarce. Irradiation emerges as a possible conservation technique that has been tested successfully in several food products (regulated in the European Union by Directive 1999/2/EC). Studies reporting the use of ionizing radiation on mushrooms are available mainly in cultivated species such as *Agaricus bisporus*, *Lentinus edodes* and *Pleurotus ostreatus*.

In the present study the effects of gamma irradiation on the sugars profile of *L. deliciosus* are analyzed considering samples submitted to different storage (4 °C) times (0, 4 and 8 days).

MATERIALS AND METHODS

Samples and samples irradiation

L. deliciosus specimens were collected in the Northeast of Portugal (November 2011) and divided in three groups with eighteen units per group: non irradiated samples (control), samples exposed to 0.5 kGy and samples exposed to 1.0 kGy, at a dose rate of 2.2 kGy h^{-1}.

To estimate the dose rate it was used a chemical solution sensitive to ionizing radiation, the Fricke dosimeter, prepared and measured as described by one of us (Antonio et al. 2011). Irradiations were performed on ^{60}Co gamma chamber (Precisa 22, Graviner Manufacturing Company Ltd) with four sources, and total activity of 267 TBq (7.2 kCi) in November 2010. After irradiation, mushroom samples were analyzed promptly and after 4 and 8 days of storage at 4 °C. After that time, the samples were lyophilized (FreeZone 4.5 model 7750031, Labconco), reduced to a fine dried powder (20 mesh), mixed to obtain a homogenate sample and kept at -20 °C until further analysis.

Analysis of free sugars

Free sugars were determined by HPLC-RI (Barros et al. 2007), using raffinose as internal standard (IS). The equipment consisted of an integrated system with a pump (Knauer, Smartline system 1000), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco) and a RI detector (Knauer Smartline 2300). Data were analysed 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 sugars content was further expressed in g per 100 g of dry weight (dw).

Statistical analysis

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 analyzed using a 2-way ANOVA, being the main factors the "irradiation dose (ID)" (0.0, 0.5 and 1.0 kGy) and the "storage time (ST)" (0, 4 and 8 days). Since a statistical significant interaction effect (ID×ST) was found in all tests, the two factors were evaluated simultaneously by plotting the estimated marginal means for all levels of each factor. In addition, a linear discriminant analysis (LDA) was used as a technique to classify the irradiation doses as well as the storage times according to the detected sugars profiles. A stepwise technique, using the Wilks' λ method with the usual probabilities of F (3.84 to enter and 2.71 to remove), was applied for variable selection. To verify which canonical discriminant functions were significant, the Wilks' λ test was applied. A leaving-one-out cross-validation procedure was carried out to assess the model performance. The LDA statistical analysis and the other statistical tests were performed at a 5% significance level using the SPSS software mentioned above.

RESULTS AND DISCUSSION

Mannitol is by far the major individual sugar, followed by trehalose and fructose. This is in agreement with the results reported by us in a previous study with the same mushroom species (Barros et al. 2007).

Table 1 presents the mean value of each irradiation dose (ID) over the different storage times (ST) as well as mean value of the assayed ST within each irradiation dose. The interaction effect among ST and ID was significant in all cases; thereby no multiple comparisons could be performed. Nevertheless, ST seemed to exert more evident changes in sugars profile than irradiation dose (please see LDA discussion below).

Table 1: Effect of storage time (days) and γ-irradiation doses (kGy) in sugars contents (g/100 g dw) of *Lactarius deliciosus*.

		Fructose	Mannitol	Trehalose
ST	0 days	0.18±0.03	12±2	1.4±0.5
	4 days	0.15±0.04	9±2	1.1±0.2
	8 days	0.06±0.03	8±1	1.2±0.4
	p-value	<0.001	<0.001	<0.001
ID	0.0 kGy	0.13±0.05	11±1	0.77±0.04
	0.5 kGy	0.1±0.1	8±2	1.7±0.5
	1.0 kGy	0.15±0.04	11±3	1.1±0.2
	p-value	<0.001	<0.001	<0.001
ST×ID	*p*-value	<0.001	<0.001	<0.001

The differences found for each effect (ST or ID) were reflected in the results obtained for LDA. In fact, ST conducted onto the formation of three well individualized clusters (corresponding to the three assayed periods) (Figure 2A). The model showed a very satisfactory classification performance allowing to correctly classifying 100.0% of the samples for the original groups as well as for the cross-validation procedure. Regarding ID, the discriminant scores were not clearly separated (Figure 2B), especially those belonging to non-irradiated and 1.0 kGy dose irradiated samples, proving that ST had higher influence over sugars profile of *L. deliciosus* (88.9% of the samples for the original groups and 86.1% for the cross-validation procedure correctly classified cases).

Both discriminant models defined two significant (p < 0.001 for the Wilks' λ test) functions, which explained 100.0% of the variance of the experimental data (Figure 2).

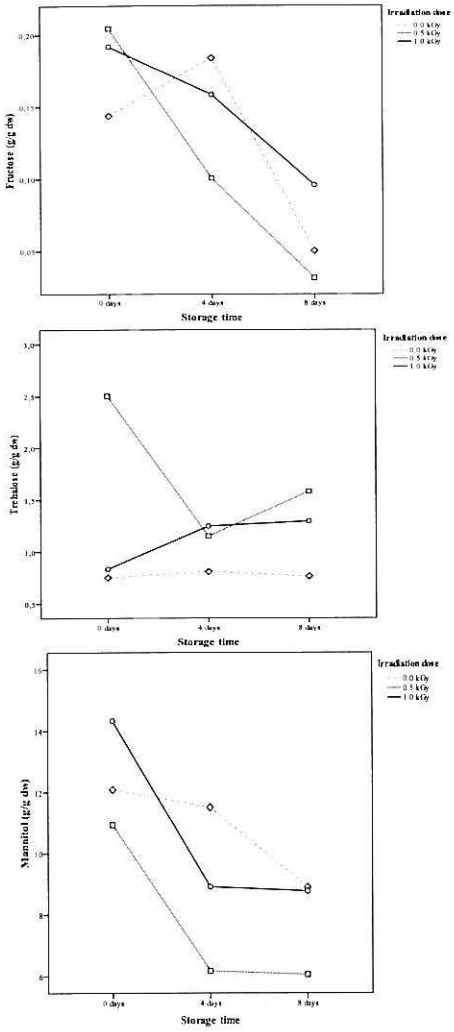


Figure 1A: Interactions between irradiation dose effects and storage times on *L. deliciosus* individual sugars.

In order to better comprehend of the evaluation of sugars profile along the time-line for each ID, the estimated marginal means obtained in the GLM are presented in Figures 1A and 1B. In these outputs, some particular tendencies could be detected: trehalose was lower for non-irradiated samples, while mannitol was lower in samples irradiated with 0.5 kGy. On the other hand, fructose and mannitol were lower after 8 days of storage. In the particular case of trehalose, a non-reducing sugar, the effect of ST is less observable due to its lower susceptibility to oxidation. Regarding ID effects, it seemed that trehalose was preserved in irradiated samples, while it decreases in non- irradiated samples.

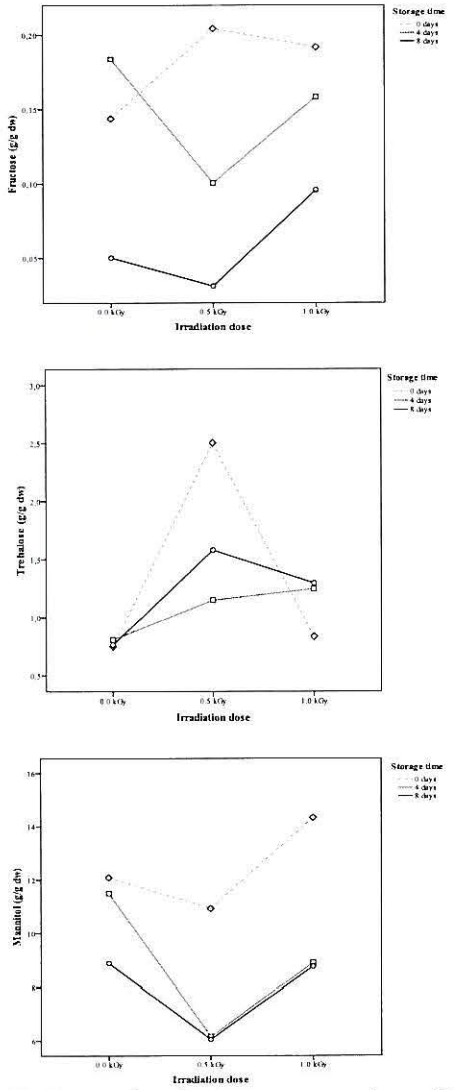
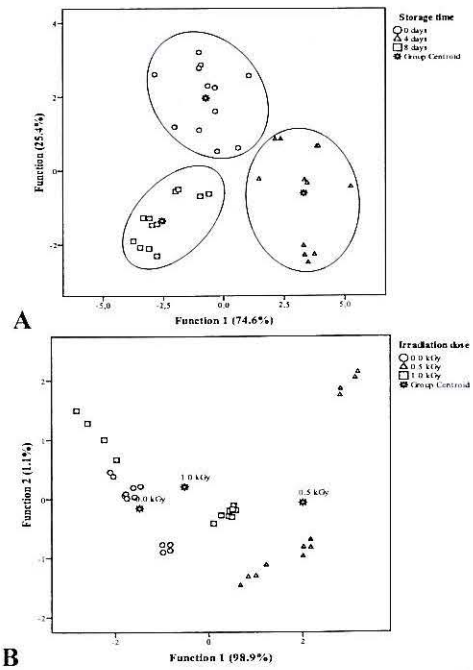


Figure 1B: Interactions between storage time effects and irradiation doses on *L. deliciosus* individual sugars.



A

B

Figure 2. Discriminant scores defined by canonical analysis of sugars profile for ST (A) and ID (B).

CONCLUSIONS

Irradiation could be an alternative to ensure the quality and extend the life of mushrooms. In fact, sugars profile is known for being a reliable indicator of adequate conservation technology. In this study, the results seemed to indicate that the effect provoked by storage time overcame the influence of irradiation, highlighting this technique as promising conservation methodology in food products. In fact, it became clear that it is easier to conclude if a mushroom sample was stored than if the same sample was irradiated, due to the dominant effects of storage over irradiation.

ACKNOWLEDGEMENTS

FCT and COMPETE/QREN/EU: Project PTDC/AGR-ALI/110062/2009 and BD/76019/2011 to A. Fernandes.

REFERENCES

USDA - Economic research service. Mushroom Industry Report 94003, 2009.

Kalač, P. Chemical composition and nutritional value of European species of wild growing mushrooms: A review. Food Chemistry, 2009, *113*, 9-16.

Ferreira, I.C.F.R., J.A. Vaz, M.H. Vasconcelos, A. Martins. Compounds from wild mushrooms with antitumor potential. Anti-cancer Agents in Medicinal Chemistry, 2010, *10*, 424-436.

Antonio, A.L., A. Fernandes, J.C.M. Barreira, A. Bento, M.L. Botelho, I.C.F.R. Ferreira. Influence of gamma irradiation in the antioxidant potential of Chestnuts (*Castanea sativa* Mill.) fruits and skins. Food and Chemical Toxicology, 2011, *49*, 1918-1923.

Barros, L., P. Baptista, D.M. Correia, J.S. Morais, I.C.F.R. Ferreira. Effects of conservation treatment and cooking on the chemical composition and antioxidant activity of Portuguese wild edible mushrooms. Journal of Agricultural and Food Chemistry, 2007, *55*, 4781-4788.

AUTHOR BIOGRAPHIES

ÂNGELA FERNANDES is working in the evaluation of the effects of ionizing radiation on mushrooms chemical composition. afeitor@ipb.pt

AMILCAR L. ANTONIO research interests are related to food irradiation. amilcar@ipb.pt

JOÃO C.M. BARREIRA research interests are related to chemical composition and bioactivity of wild mushrooms and plants. jbarreira@ipb.pt

ANABELA MARTINS research interests are in the plant and fungi biotechnology domains. amartins@ipb.pt

M. BEATRIZ P.P. OLIVEIRA research interests are related to chemical composition of food products. beatoliv@ff.up.pt

ISABEL C.F.R. FERREIRA research interests are related to chemical composition and bioactivity of wild mushrooms, plants and chestnuts. iferreira@ipb.pt