Food Structure

Volume 6 | Number 1 Article 10

1987

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A. Keresztes

E. Kovacs

FOOD MICROSTRUCTURE, Vol. 6 (1987), pp. 75-79
Scanning Microscopy International, Chicago (AMF O'Hare) , IL 60666 USA

0730-5419/87$3.00+.00

EFFECT OF IONIZING IRRADIATION AND STORAGE ON MUSHROOM ULTRASTRUCTURE II. THE STIPE AND THE UPPER PART OF THE CAP OF AGARICUS BISPORUS (LGE. IMBACH)

Á. Keresztes[1] and E. Kovács[2]

[1]Department of Plant Anatomy, Eötvös Loránd University, 1088 Budapest, Muzeum krt. 4/A, Hungary

[2]Department of Microbiology, Central Food Research Institute, 1022 Budapest, Herman Ottó u. 15, Hungary

Abstract

After having investigated the ultrastructural effects of ionizing irradiation used for shelf-life extension, on the gills of Agaricus bisporus and Pleurotus ostreatus previously, it was of interest to examine how other parts of the fruit body were affected by the same treatment. Samples were taken from the lower and upper parts of the stipe and from the upper part of the cap of the control, stored, and irradiated (2.5 kGy) then stored A.bisporus fruit bodies. Transmission electron microscopy showed that irradiated samples generally retained plasm-content, which dramatically decreased in those without irradiation by the end of storing (6 days). However, irradiation also induced autophagy and necrosis in some cells of the lower part of the stipe.

Initial paper received July 28, 1986
Manuscript received December 01, 1986
Direct inquiries to E. Kovacs
Telephone number: 36-1-152028/157341

KEY WORDS: Mushroom, Agaricus bisporus, transmission electron microscopy, ultrastructure, irradiation, growth retardation, shelf-life extension, ripening

Introduction

In the course of our earlier investigation we found that gamma-irradiation of 2.5 kGy (applied for shelf-life extension) caused a substantial degradation or a total cell necrosis in the hymenium of Agaricus bisporus or Pleurotus ostreatus, respectively (Keresztes et al. 1985). This means that irradiation inhibits spore production by destroying basidia rather than conserving their juvenile stage. Destructive but much less pronounced changes were found in the hymenophoral cells.

Irradiation apparently also affects other parts of the fruit bodies in A.bisporus, since they remain closed and do not grow at an appreciable rate when irradiated at an early stage of development. It is known that opening is caused by the elongation of stipe cells (Bonner et al. 1956, Hagimoto 1964), accompanied by cell divisions to some extent (Craig et al. 1977). The question arises as to the cause of the growth retardation of carpophores. Does irradiation treatment destroy stipe cells (as seen in the hymenium), or does it act in another way?

In this paper we demonstrate that irradiation preserved the living content of the retarded cells in the upper parts of the cap and the stipe, while some cells below the annulus showed autophagy and necrosis.

Materials and Methods

Three groups of carpophores of A.bisporus obtained from Duna MgTSz (Budapest) were used: (1) fresh control; (2) stored control (6 days), and (3) irradiated (2.5 kGy) then stored (6 days) group.

Three kinds of samples were collected from a carpophore: (a) parts from the lower part of the stipe (about halfway between the base and the annulus); (b) from the upper part of the stipe (between the pileus and the annulus), and (c) from the pileus (about halfway be-

tween the brim and the center, above the gills).

In all cases samples were cut out close to the surface. For storing and irradiation treatment, see Keresztes et al. (1985).

Fixation was carried out in 6 % (v/v) glutaraldehyde (in 0.035 M K-Na phosphate buffer, pH 7.2) for 2 hours at 4°C. After thorough washing in the above buffer samples were postfixed in 1 % (w/v) OsO_4 for 1.5 hours, dehydrated in an acetone series and embedded in Spurr's resin.

Sections were made with a Porter-Blum ultramicrotome equipped with an LKB glass knife, and after contrast-staining with uranyl acetate and lead citrate, were examined in a Tesla BS 500 electron microscope operated at 60 kV.

Results and Discussion

The lower part of the stipe

In the fresh control the cells contain smaller or larger vacuoles (Fig. 1 A). In the latter case the cytoplasm forms a layer of variable thickness and medium density. In the more frequent former case the cytoplasm is less dense and apparently less compact. This kind of plasm is termed mictoplasm (Angeli-Papa and Eymé 1978) originating from the mixing of cytoplasm and the primary vacuole. Vacuoles seen at this stage are secondary ones derived probably from plasmalemmasomes.

The cells are multinucleate (Evans 1959, Craig et al. 1979), nuclei mostly being close to each other (not shown here). At higher resolution numerous glycogen rosettes can be observed in the cyto- or mictoplasm.

Cells of the stored control markedly differ from those of the fresh control, mainly in having a thin, low-density plasm layer along the walls (Fig. 1 B). The primary or secondary status of this cannot be readily discerned. Nuclei seem to be similar to those of the fresh control.

In the irradiated then stored samples the relative plasm-content of most cells is larger than that of the stored control. The vacuoles of these cells contain numerous small vesicles (Fig. 1 C), which may be a sign of autophagy, a process that has been observed also in irradiated Pleurotus subhymenium (Keresztes et al. 1985). Among the vesicles there are electron dense fuzzy, roundish bodies of unknown nature in the vacuoles. A part of the cells contains microplasm.

Cell walls are generally thicker than in any of the controls and also local thickenings occur (Fig. 1 C). This may be the reason for the increased consistency of tissue pieces experienced at excision. We have observed an imbalance between cell wall synthesis and extension also in neutron irradiated barley leaves (Kovács et al. 1979). Nuclei seem to be similar in size, shape and number to both controls.

Among the living cells, in contrast to the controls, there are necrotized and empty cells or groups of cells (Fig. 1 C). The walls of these remained thin. These may represent the end stage of an autophagic process induced by the irradiation. We have observed a similar phenomenon in the hymenium of Pleurotus ostreatus (Keresztes et al. 1985).

The upper part of the stipe

In the fresh control the shape of the cells seems to be more variable, the mictoplasm formation is less frequent than in the lower stipe (Fig. 2 A). The cells are multinucleate here, too.

In the stored control the relative plasm-content of cells is very low (Fig. 2 B). Cells of the irradiated then stored samples retain relatively more plasm, which is generally rich in glycogen (Fig. 2 C). In most cases these cells contain several smaller vacuoles instead of a single large one and show no obvious signs of autophagy.

The upper part of the cap

A general feature of these samples is that the cell shapes are variable and irregular with large intercellular spaces (lacunae) between them. In the fresh control the plasm-content of the cells is variable but generally less than in the fresh stipe (Fig. 3 A). Mictoplasm formation occurs. We have not seen more than two nuclei per cell.

In the stored control the plasm-content decreases as in the stipe (Fig. 3 B), while the irradiated and stored samples retain the plasm-content (Fig. 3 C).

Conclusions

In all investigated parts of the A.bisporus carpophores (with the exception of the hymenium, see Keresztes et al. 1985) we found that irradiation of 2.5 kGy preserved the plasm-content to a considerable extent, which decreased to a minimal amount during storing without irradiation. This demonstrates the merit of this method of shelf-life extension for the bulk of the fruit body at the ultrastructural level.

However, irradiation induced autophagy in the lower part of the stipe. This process may progress during prolonged storing causing cell necrosis, but in such a part of the fruit body which is not generally used in food technology.

Acknowledgements

We wish to thank Mrs. P. Petrovits, Mrs.G. Gácsi, Mrs. A. Gyurek and Mrs. J.Bencze-Böcs for their valuable technical help.

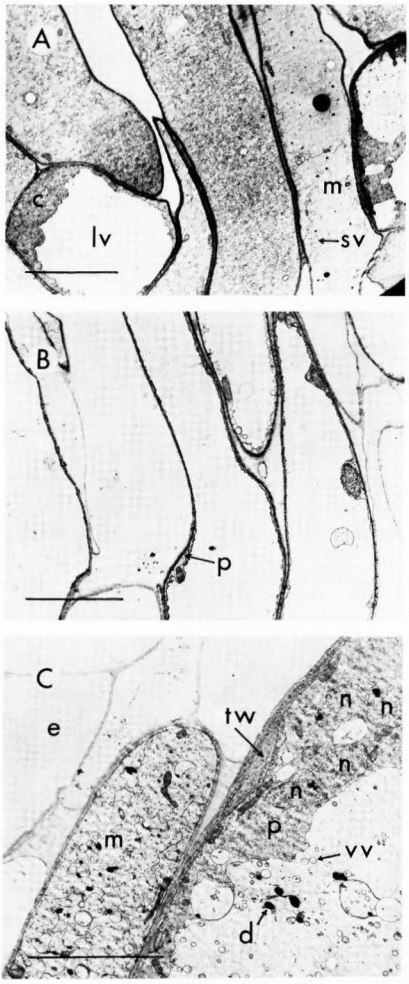

Fig. 1. Lower stipe cells. A: fresh control, B: stored control, C: irradiated then stored sample. Abbreviations: c=cytoplasm, m=mictoplasm, p=plasm, n=nucleus, tw=thickened wall, lv=large vacuole, sv=small vacuole, vv=vacuolar vesicle, d=dense body, e=empty cell. Bars equal lo µm.

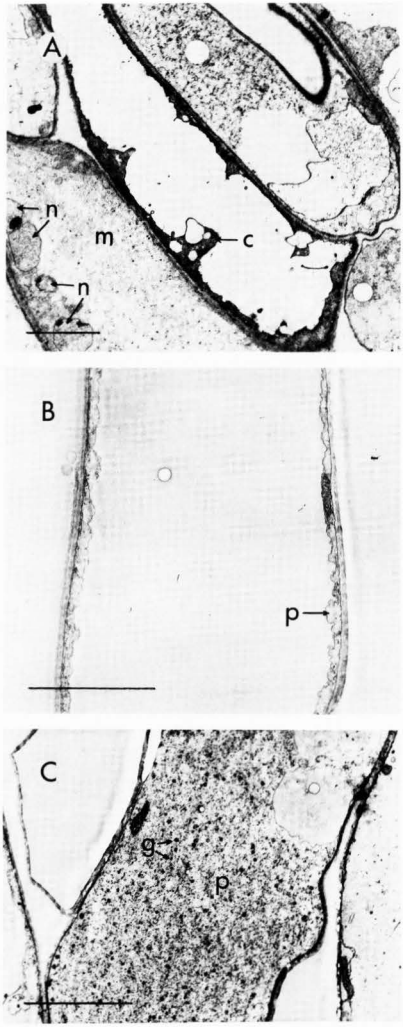

Fig.2. Upper stipe cells. A: fresh control, B: stored control, C: irradiated then stored sample.
Abbreviations: g=glycogen, for other symbols see Fig. 1. Bars equal 5 µm.

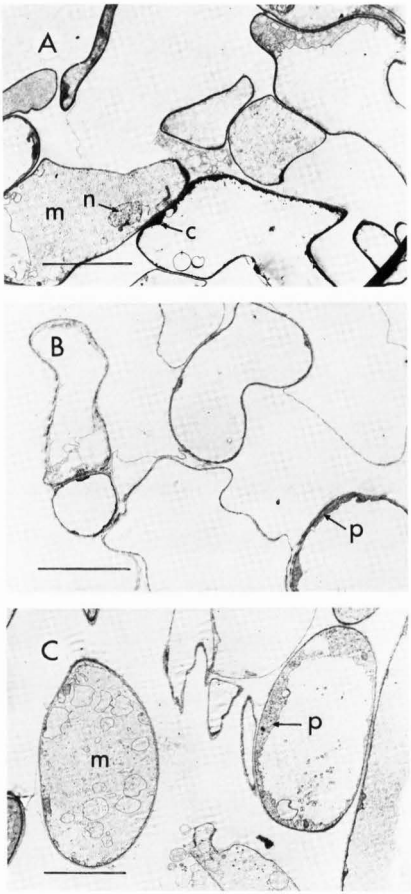

Fig. 3. Cells from the upper part of the cap. A: fresh control, B: stored control, C: irradiated then stored sample. For abbreviations see Fig. 1. Bars equal 10 µm.

References

Angeli-Papa J, Eymé J. (1978). Ultrastructural changes during development of Agaricus bisporus and Agaricus sylvicola. In: The biology and cultivation of edible mushrooms, ST Chang, WA Hayes (eds.), Academic Press, N.Y. 53-81.

Bonner JT, Kane KK, Levey RH. (1956). Studies on the mechanics of growth in the common mushroom Agaricus campestris. Mycologia 48,13-18.

Craig GD, Gull K, Wood DA, (1977). Stipe elongation in Agaricus bisporus. J. Gen. Microbiol. 102, 337-347.

Craig DJ, Newsam RJ, Gull K, Wood DA. (1979). An ultrastructural and autoradiographic study of stipe elongation in Agaricus bisporus. Protoplasma 98, 15-29.

Evans HJ. (1959). Nuclear behaviour in the cultivated mushroom. Chromosoma 10, 115-135.

Hagimoto, H. (1964). The growth of the fruit body of Agaricus bisporus (Lange) Sing. Trans. Brit. Mycol. Soc. 4, 158-164.

Keresztes Á, Kovács J, Kovács E. (1985). Effect of ionizing irradiation and storing on mushroom ultrastructure. I. The gills of Agaricus bisporus (Lange) Imbach and Pleurotus ostreatus (Jacq. ex. Fr.) Kummer. Food Microstructure 4, (2) 349-355.

Kovács V, Gyurján I, Keresztes Á, Virág E. (1979). Studies on the biological effect of fast neutrons. II. Variation of total nucleic acid content and ultrastructure in barley leaves vs. dose. Acta Biochim. et Biophys. Acad. Sci. Hung. 14, 103-109.

Discussion with Reviewers

D.L. Rinker: Shelf-life of mushrooms is not only a function of post-harvest manipulation but also reflects cultural practices, supplementation, and strain selection. How do these parameters affect shelf life of irradiated mushrooms?
Authors: Mushrooms were a commercial product of Duna MgTSz, Budapest. The strain and the cultural practices have been constant for a long time, so we could not investigate the effect of their variation on shelf-life of mushrooms.

E.M. Jasinski: What were the storage conditions that the authors used, i.e. temperature, packaging material, length of time between harvest and storage?
Authors: The samples were stored at 14-16°C, 90-95 % RH, without any packaging materials. The length of time between

harvest and storage was about 2-3 hours (spent by transportation and irradiation).

E.M. Jasinski: What was the maturity of the mushrooms at the time of harvest?
Authors: Mushroom caps were closed, the diameter of caps ranged from 4.2 to 4.9 cm. The mushrooms were picked from the second flush, which represents the best quality of mushroom.

E.M. Jasinski: Did the authors notice any bacterial degeneration of the mushroom tissue after/before storage?
Authors: No, we did not notice such signs.

E.M. Jasinski: How many of the mushrooms were tested in each of the groups? Are the pictures representative of the entire sample?
D.L. Rinker: How many samples were actually excised in order to draw the conclusions?
Authors: 50-50 carpophores were individually collected for irradiation and control. From these groups 3-3 carpophores were selected on the basis of average diameter for electron microscopy. From each sampling area on each fruit body 3-4 pieces were excised. Before the TEM investigations several thousand mushrooms had been individually measured studying the effect of irradiation on the cap opening and stipe elongation (Kovács E, Vas K. (1974). Acta Alimentaria 3, 19-25; Wahid, M. Kovács E. (1980). Acta Alimentaria 9, 357-366; Kovács E, Vörös Zs, Farkas, J. (1981). Acta Alimentaria 10, 379-388). We think the figures represent the samples.