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ULTRASTRUCTURAL STUDIES OF RAW AND PROCESSED TISSUE OF THE MAJOR CULTIVATED MUSHROOM,
AGARICUS BISPORUS

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Abstract

Commercial mushroom processors currently lose approximately 30 percent of the mushroom weight due to shrinkage during processing (blanching and canning), resulting in substantial economic losses. Microscopy was used to assess the extent and type of chemical and structural changes induced by processing mushrooms and causing shrinkage. Scanning electron microscopy revealed that the processing operations including vacuum hydration, blanching, and thermal treatment do not damage the integrity of the tissue. Light microscopy revealed that the morphology of the tissue, shape and spacing of cells, appear similar for raw and processed mushroom tissue. However, the intracellular material remained indistinct for both tissue types, and the processed tissue appeared distorted. Transmission electron microscopy revealed that commercial mushroom processing caused intracellular damage to the tissue. The heat treatment caused the coagulation of cytoplasmic material and the disruption of intracellular membranes, resulting in the loss of water holding capacity of the tissue. Therefore, shrinkage of processed mushrooms results from "denaturation" of the organelles and the associated loss of water holding capacity by those organelles.

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KEY WORDS: *Agaricus bisporus*, Ultrastructure of raw mushroom, Ultrastructure of processed mushroom, Mushroom, Canning, Transmission electron microscopy, Scanning electron microscopy, Light microscopy.

Introduction

Shrinkage, the loss of weight during processing (blanching and canning), continues to plague the mushroom processing industry. Weight losses can range as high as 30 percent, which results in substantial economic losses to the processor (Eby, 1975). Shrinkage results from a loss of water and water soluble solids from the mushroom tissue. However, the cause of this loss is not totally understood. Microscopy can be used in determining the severity of mushroom processing or in assessing the effects of processing and/or handling on the quality of mushrooms. Therefore, an ultrastructural examination of raw and processed (blanched and canned) mushroom tissue should provide insight into the causes of mushroom shrinkage.

Agaricus bisporus (Lange) Sing is the major commercially cultivated mushroom in the world (Chang, 1980). Information is available on the structure and ultrastructure of raw mushroom tissue; however, minimal information is available on the ultrastructure of processed mushroom tissue. The general ultrastructure of fungi and the ultrastructure of *Agaricus bisporus* have been investigated (Bracker, 1967; Beckett et al., 1974; Angeli-Papa and Eyme, 1978; Alexopoulos and Mims, 1979). These investigations pertain to the stipe elongation mechanism (Craig et al., 1977a; Craig et al., 1979), the cell wall structure (Novaes-Ledieu and Garcia-Mendoza, 1981), basidia and septal pore structures (Craig et al., 1977b; Flegler et al., 1976), cell membrane development (Eyme and Angeli-Couvy, 1975), the basidiospore structure (Elliott, 1977; Rast and Hollenstein, 1977), and nuclear distribution (Wang and Wu, 1974). However, the relationship of mushroom ultrastructure to processing has not been investigated.

Mushroom processing operations have two major purposes: a) to provide a sterile product and b) to minimize shrinkage of mushroom tissue, to maximize canned product yield. Various processes that limit shrinkage have been reported (Gomley, 1972; McArdle et al., 1974; Beelman and McArdle, 1975; Steinbuch, 1979). Gomley (1972) found that freezing unblanched mushrooms produced a higher quality product than freezing blanched mushrooms. Steinbuch (1979) found that vacuum packing of

unblanched frozen mushrooms showed promise in improving mushroom quality and reducing shrinkage. Beelman and McArdle (1975) found that post-harvest storage of mushrooms at elevated temperatures (12°C) for 24 hr. to 48 hr., followed by soaking (PSU-3S-Process) or vacuum hydration (SSV-Process) prior to blanching, increased the canned product yield (decreased shrinkage). Increased yields due to storage at elevated temperatures supported the hypothesis that chemical changes within the mushroom tissue caused an increase in water retention by the tissue (Beelman and McArdle, 1975). The higher storage temperatures resulted in an increase in the mushroom tissue's ability to absorb more water before processing and retain more water during processing (Beelman and McArdle, 1975). These results indicate that a loss of water holding capacity or water binding capacity of the mushroom tissue during processing causes shrinkage.

Chemical changes in the protein structure of the mushroom tissue could produce the increase in water holding capacity during storage (Beelman et al., 1973). However, the specific chemical and structural changes that occur during processing to cause shrinkage remain unknown. Therefore, this investigation uses microscopy to assess the extent and type of chemical and structural changes induced by processing (blanching and canning) mushrooms.

Materials and Methods

Samples

Raw cultivated mushrooms, *Agaricus bisporus* (Lange) Sing were obtained from The Pennsylvania State University Mushroom Research Center and the commercially processed samples were canned buttons grown and processed in Pennsylvania. Mushrooms with tightly closed veils and cap diameters ranging from 2.8-3.0 cm were selected. Seven sections of raw and processed tissue were prepared for scanning electron microscopy. These included outside surface, longitudinal, and cross-sectional cuts of the cap (pileus) and stem (stipe) and the outside surface of the gills. Five sections of raw and processed tissue were prepared for light and transmission electron microscopy. These included cross-section and longitudinal cuts of the cap and stem and cross-section of the gills.

Scanning Electron Microscopy (SEM)

The samples were prepared by a modification of the method described by Falk (1980). The sections were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.1, then dehydrated with ethanol, critical point dried with carbon dioxide (Polaron model E3000), sputter coated with gold (International Scientific Instruments model PS-2), and examined with an International Scientific Instruments ISI-60 scanning electron microscope at 10 kV. Photographs were taken with an Asahi Pentax 35 mm camera.

Light and Transmission Electron Microscopy (TEM)

Samples were prepared by a modification of the method outlined by Gabriel (1982). The tissue was fixed in 2% formaldehyde and 1% glutaraldehyde in 0.15 M cacodylate buffer, pH 7.1. The samples were treated with a vacuum for 15 min. in a vacuum oven for air removal. The tissue was transferred

to 3% glutaraldehyde in the same buffer, post fixed in 1% osmium tetroxide, and treated with 0.5% uranyl acetate in 0.1 M sodium acetate overnight. The samples were dehydrated with acetone and embedded in Spurr firm epoxy resin (Spurr, 1969). Samples were sectioned, stained with 1% toluidine blue for light microscopy or with 3% uranyl acetate in 7.5% methanol, 0.2% lead citrate (Venable and Coggeshall, 1965), and Reynolds lead stain (Reynolds, 1963) for transmission electron microscopy. A Leitz-Ortholux light microscope and an Hitachi HU11E electron microscope were used.

Results and Discussion

Typical commercial mushroom processing in the United States (Fig. 1) involves washing the mushrooms, then cold storage for up to 48 hours, and applying a vacuum hydration treatment, which completes the pre-processing steps. The remaining steps of the process include blanching, filling, brining, closing, thermal processing, cooling, and storage. The major goal of this process is for the mushroom tissue to absorb as much water as possible during soaking and vacuum hydration so that the water lost during blanching and thermal processing is mostly the absorbed water. Scanning electron, light, and transmission electron microscopy were used to determine the effects this process had on mushroom tissue.

The scanning electron micrographs of the stem, cap, and gills (Figs. 2-6) show that the absorption of water during vacuum hydration and subsequent loss of water during blanching and thermal processing does not destroy the overall integrity of the tissue. Filaments or hyphae compose the outer surface of the stem (Fig. 2) and the cap (Fig. 4). The longitudinal filaments in the raw (Fig. 2a) and processed stem (Fig. 2b) are bunched together. However, the filaments in the processed tissue appear deflated or shriveled (Fig. 2b). Monacha (1965) described the stem as a combination of wide inflated hyphae and narrow threadlike filaments. The cross-sectional view of the stem (Fig. 3) shows the two types of septate hyphae. The compact filaments of the processed tissue (Fig. 3b) with gaps between groups of filaments reveal the same distortion seen on the outside surface of the stem (Fig. 2b). Random crossing hyphae compose the cap (Fig. 4). This random crossing leaves spaces wide enough for bacteria to enter and grow in the mushroom tissue. In both cases, the filaments increase in chitin and water contents as the mushroom grows and the cells elongate (Bonner et al., 1956; Webster, 1970).

Individual club shaped basidia cover the outside surface of the gills. Rough, distorted club shaped basidia cover the processed gills (Fig. 6b) while smooth, distinct club shaped basidia cover the raw gills (Fig. 6a). Finally, the processed gills (Fig. 5b) appear more compact than the raw gills (Fig. 5a), which could be a possible indicator of shrinkage.

While certain parts of the mushroom tissue such as the surface of the gills and stem appear shriveled and distorted, the overall integrity of the tissue has not been lost as a consequence of

OUTLINE OF COMMERCIAL MUSHROOM PROCESSING OPERATION

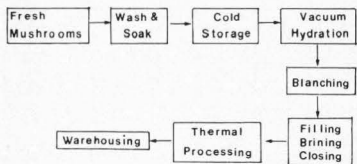


Fig. 1. Flow chart of a typical commercial mushroom processing operation.

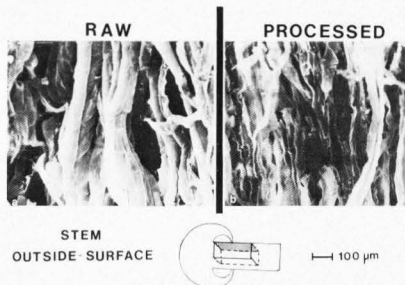


Fig. 2. Scanning electron micrograph of the outside surface of raw (a) and processed (b) mushroom stem.

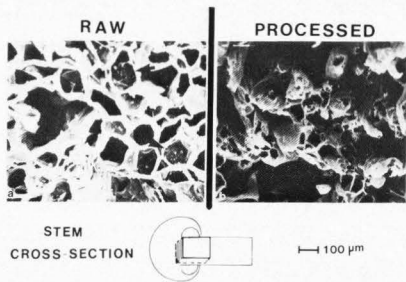


Fig. 3. SEM micrograph of the cross-section of raw (a) and processed (b) mushroom stem. Arrows indicate septa.

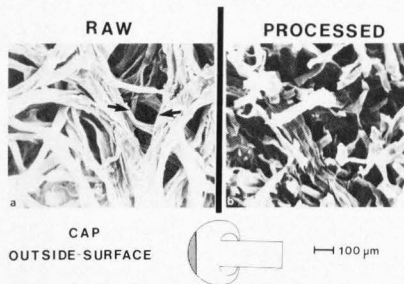


Fig. 4. SEM micrograph of the outside surface of raw (a) and processed (b) mushroom cap. Arrows indicate gaps large enough for bacteria to enter.

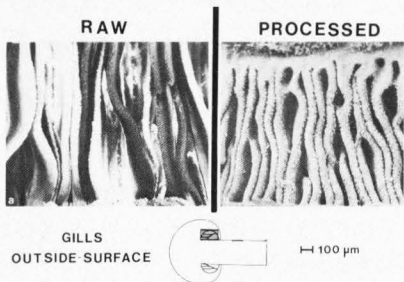


Fig. 5. SEM micrograph of the outside surface of raw (a) and processed (b) mushroom gills.

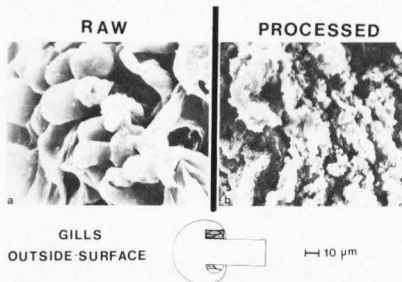


Fig. 6. SEM micrograph of the outside surface of raw (a) and processed (b) mushroom gills.

processing (Figs. 2-6). This indicates that the large amount of water forced into the mushroom tissue during vacuum hydration does not damage the external cellular structure. The loss of water from the tissue during blanching and thermal processing results in the shriveled and distorted appearance of the tissue. The second portion of this investigation used light microscopy to determine if the general morphology changed during processing.

Light microscopy revealed several characteristics present in all sections of the mushroom tissue. First, the morphology of the tissue, shape and spacing of cells, appear similar for the raw and processed mushroom tissue. Septate hyphae compose the cap and the stem with random crossing hyphae in the cap and longitudinal hyphae in the stem. The gills contain three layers of cells (Craig et al., 1977b), which include trama cells (T), subhymenial cells (SH), and hymenial cells (H) (Fig. 7). Second, the intracellular material of the raw and processed tissue remained indistinct (Fig. 7). Finally, all of the processed tissue appeared distorted although the organization remained the same as in the raw tissue (Fig. 7). The final portion of this investigation used transmission electron microscopy to determine if intracellular damage occurred as a result of mushroom processing.

The transmission electron micrographs (Figs. 8-10) revealed that commercial mushroom processing results in damage to the intracellular material (Figs. 8b,9b,10b). The raw mushroom tissue (Figs. 8a,9a,10a) contained identifiable intracellular material characteristic of basidiomycetes (Bracker, 1967; Beckett et al., 1974; Eymé and Angeli-Couvy, 1975; Craig et al., 1977a; Alexopoulos and Mims, 1979; Craig et al., 1979). The structures include cell walls (W), cellular membranes (CM), nucleus (N), nuclear membranes (NM), mitochondria (M), concentric lamellae membranes (CL), electron dense bodies (DB), lipid droplets (D), and vacuoles (V). The damaged processed mushroom tissue lacks identifiable cellular material except for remnants of the cell walls (W), cellular membranes (CM), dolipore septum (S), and vacuoles (V). The remaining intracellular material appears as clusters of precipitated electron dense material (D) throughout the cells (Figs. 8b,9b,10b).

Several of the consequences of commercial mushroom processing appear in the transmission electron micrographs (Figs. 8-10). First, as previously mentioned, the intracellular material appears as electron dense clusters rather than as distinct organelles (Figs. 8b,9b,10b). The heat treatment applied during processing caused the coagulation of cytoplasmic material such as proteins and the disruption of the compartmentalizing intracellular membranes (Figs. 8b,9b,10b). The coagulation and the disruption of intracellular membranes result in the loss of water holding capacity of the tissue so that the tissue shrinks to 80 percent of its original weight. However, the intact cell wall and coagulated material traps the remaining water in the tissue.

Two types of tissue are present in the mushroom, the stem and cap, and the gills. The stem and cap contain large vacuoles in the center of

the cells (Figs. 8a and 9a), which press the cytoplasmic material near the cell wall (Fig. 9a). Therefore, the majority of coagulated electron dense material remains near the cell wall in the processed tissue (Fig. 8b). The gills contain the greatest amount of intracellular material (Fig. 10a) due to the gills being the reproductive region of the mushroom and these regulate other functions such as stipe growth (Hagimoto and Konishi, 1972). In this case, the electron dense material generally appears in the center of the cell leaving gaps between it and the cell wall (Fig. 10b). These gaps may permit the gill tissue to lose more water than the cap or stem during processing.

Second, the outer layer of the cell wall at the tips of the basidia appear broken and separated from the rest of the cell wall (Fig. 10b, arrows). This phenomenon did not appear in any other section of the mushroom tissue. The broken cell wall may allow more water to leave the tissue.

Finally, mushroom processing causes distortion in the processed tissue (Figs. 8b,9b,10b). The distortion, most apparent in the gill tissue (Fig. 10b), appears as irregular shaped cells with gaps or channels between cells. Commercial processes allow the absorption of water into the mushroom tissue. However, whether the water is absorbed directly into the cells or into the gaps between the cells has not been determined. In any case, processing releases all of the absorbed water plus part of the original water from the tissue, causing the distortion of cells and channeling between cells seen in the processed tissue (Figs. 8b,9b,10b).

Summary and Conclusions

Commercial mushroom processing appreciably alters the intracellular organization of the mushroom tissue causing shrinkage. Scanning electron micrographs showed that processing did not destroy the surface structural features of the mushroom tissue. However, the processed tissue appeared shriveled and distorted, though intact. Light micrographs revealed similar cell types for raw and processed tissue, but the processed cells appeared distorted. Transmission electron micrographs showed that processing caused intracellular damage to the mushroom tissue. All cellular organelles became coagulated electron dense material resulting in a loss of water holding capacity of the organelles. This results in shrinkage of the tissue due to a loss of water and water soluble solids. Therefore, the loss in canned product yield of processed mushrooms results from "denaturation" of the organelles and the associated loss of water holding capacity by those organelles.

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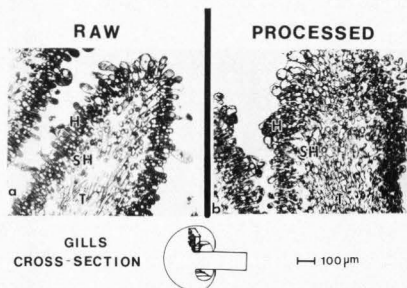


Fig. 7. Light micrograph of the cross-section of the gills for raw (a) and processed (b) mushroom. Trama (T), subhymenial (SH), and hymenial (H) cells are visualized.

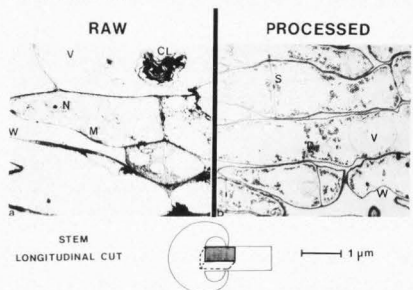


Fig. 8. Transmission electron micrograph of the longitudinal cut of the raw (a) and processed (b) mushroom stem. Nucleus (N), cell walls (W), mitochondria (M), concentric lamellae (CL), dolipore septum (S), vacuoles (V), and electron dense material (D) are observed.

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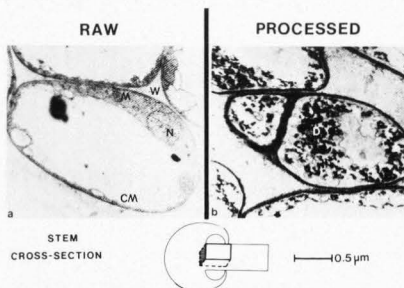


Fig. 9. TEM micrograph of the cross-section of the raw (a) and processed (b) mushroom stem. Nucleus (N), cell walls (W), cellular membranes (CM), mitochondria (M), and electron dense material (D) are observed.

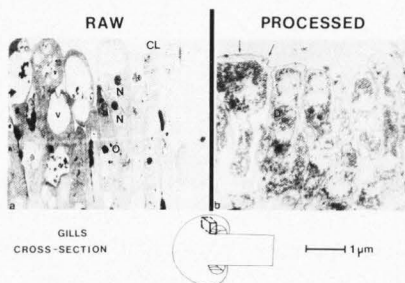


Fig. 10. TEM micrograph of the cross-section of the gills for raw (a) and processed (b) mushroom. The nucleus contains nucleus (N), lipid droplets (O), vacuoles (V), concentric lamellae (CL), and electron dense material (D). The arrows indicate partially broken cell wall.

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Discussion with Reviewers

D.A. Wood: Why were only the beginning and end stages of the process examined since the changes observed may have occurred during one of several processes?

Authors: The raw mushroom tissue was impregnated with glutaraldehyde under conditions similar to the vacuum hydration process (see Materials and Methods section). Since all cellular structures were observed in the raw tissue, the vacuum hydration process does not cause the change in the tissue. The thermal treatment accorded to the tissue during blanching is less severe than thermal processing. Also, most of the material lost from the mushroom during blanching was water impregnated into the tissue during vacuum hydration (Beelman and McArdle, 1975). Therefore, we felt that the significant change in the ultrastructure of the tissue results from thermal processing; hence, the reason for examining the beginning and end stages of the process.

D.A. Wood: Could you produce these changes merely by thermal processing?

Authors: Yes.

F. Ingrassia: In what way do the authors feel the freezing method of processing mushrooms would affect the cell ultrastructure?

Authors: Integrated Quick Blanching (IQB) and Integrated Quick Freezing (IQF) methods currently used by the frozen mushroom industry, insure the creation of small uniform ice crystals in the frozen product. Assuming minimal thermal shock during storage, we would speculate that the ultrastructure of frozen mushroom tissue would be similar to that of raw mushroom tissue. If thermal shock becomes a factor leading to the formation of large ice crystals, resulting from the migration of intracellular water to intercellular water, the rupture of cell membranes would be expected. However, we would not necessarily expect to visualize the electron dense material that was seen as a result of thermal coagulation.

D.A. Wood: What is the chemical composition of the solids lost from the mushroom tissue during processing?

Authors: The water absorbed by the mushroom tissue during vacuum hydration is the major component lost during processing (Beelman and McArdle, 1975; Steinbuch, 1978). This water contains all of the water soluble proteins originally present in the mushroom tissue (Eby, 1975). However, further chemical analysis of the solids lost from the mushroom tissue during processing was not conducted.