

Textural Changes in Mushrooms (*Agaricus bisporus*) Associated with Tissue Ultrastructure and Composition

S. ZIVANOVIC, R.W. BUESCHER, AND K.S. KIM

ABSTRACT: The objective of this study was to determine ultrastructural and compositional changes in fresh mushrooms associated with adverse changes in texture during 9 d of post-harvest storage at 12 °C. Mushroom softening (2.6N to 1.5N, puncturing force) was consistent with toughening (19.2N to 33.0N, gumminess). Protein and total carbohydrate content declined, but chitin content increased during mushroom storage. Most polysaccharides were extracted after deacetylation and depolymerization of chitin, indicating that structural glucans were mainly bound with acetylglucosamine polymers. Softening paralleled expansion of intercellular space at the pilei surface, hyphae shrinkage, central vacuole disruption, and loss of proteins ($r = 0.94$) and polysaccharides ($r = 0.84$), while toughening was associated with increased chitin content ($r = 0.95$).

Key Words: mushrooms, *A. bisporus*, texture, cell wall, storage

Introduction

THE CULTIVATION OF EDIBLE MUSHROOMS has increased continuously for several years. During 1998 to 1999, the production of *Agaricus* mushrooms, which account for more than 90% of all mushrooms grown in United States, totaled 848 million pounds (USDA 1999). More than 85% of the total crop is sold as fresh product. However, short shelf life still presents difficulties in distribution and marketing of the fresh product, and extending post-harvest storage is a constant quest.

Fresh *Agaricus* mushrooms have a shelf life of 2 to 3 d at room temperature, 8 d in modified atmosphere at 3 °C (De la Plaza and others 1995), and maximum 14 d at 2 °C in controlled atmosphere (Burton 1991). Loss of quality is caused by morphological changes due to maturation, discoloration, changes in texture and flavor, and losses of nutrients (Burton and Noble 1993; Donker and Braaksma 1997).

After harvesting, fresh mushrooms have a bright white color, closed caps, and firm texture and are easily sheared and chewed. During storage they darken, caps open exposing gills, stipes elongate, and texture becomes spongy and tough to chew and shear. Although texture is an important quality, the understanding of what causes textural changes of mushroom tissue is incomplete. Studies on mushroom softening (Beelman and others 1987; Gautam and others 1998; Rama and others 1995), toughening (Beelman and others 1987; Mac Canna and Gormley 1968), ultrastructure (Atney and Nichols 1983; Umar and Van Griensven 1997), and

composition (Hammond 1979) have been reported; however, relationships between physical and chemical changes to explain textural changes are inconclusive.

The objective of this study was to determine ultrastructural and compositional changes in fresh mushrooms related to changes in texture after harvesting.

Materials and Methods

MUSHROOMS, *AGARICUS BISPORUS* off-white F-140 hybrid, were obtained from a local grower (J&M Farms, Miami, Okla., U.S.A.). Whole fruit bodies were harvested at "closed cap" stage with pilei dia of 40 ± 5 mm and transported to the laboratory within 4 h. Stipes were trimmed to 25 ± 2 mm, and mushrooms were stored at 12 °C and 95% RH. Measurements and analyses were performed on 0, 3, 6, and 9 d.

Texture

Firmness (softening) was determined by a penetration test and toughness by compression measured by texture profile analysis using a TA.XT2 texture analyzer (Texture Technologies Corp., Scarsdale, N.Y., U.S.A.). Tests were performed with 20 replications on d 0 and with 40 replications on each sampling day during storage. One mushroom cap was used per replication for each test.

For the penetration test, a 2-mm dia probe (contact force 1N, speed 5.0mm/s) was applied for measuring force necessary to puncture the mushroom cap from the top to a 7.5-mm depth. Force for puncturing, gradient (calculated as the slope of

force before puncturing), and maximum force were recorded.

For compression of uniform disks of pilei tissue, a flat, 2-cm dia cylinder probe was used. Cylindrical disks, 11 mm in dia and 9.5 mm high, were dissected from the top of the cap (1 disk per mushroom). Compression was to 80% of original disk height; contact area was constant; time between 2 compression ("bites") was 0.20 s; and a contact force of 5N at 5 mm/s speed was used. Gumminess, hardness, and fracturability were recorded and defined as follows: Gumminess represented energy necessary for sample disintegration to a state ready for swallowing; hardness was the force required to attain a defined deformation (for example, 80% of original height); and fracturability (brittleness) was represented by the force at which the sample fractured (Stable Micro System 1993). Toughening was associated with gumminess and calculated as hardness \times cohesiveness (Bourne 1978).

Electron microscopy

For both scanning (SEM) and transmission (TEM) electron microscopy, the surface and center pieces of mushroom pilei were fixed in a modified Karnovsky's fixative, consisting of 2% glutaraldehyde and 2% paraformaldehyde in 0.05M Cacodylate buffer at pH 7.0 for 2 h at room temperature. The tissues were rinsed twice in the same buffer for 10 min each before postfixation in 1% osmium-tetroxide for 2 h at room temperature. The tissues were then dehydrated in an ethanol series followed by 2 changes of propylene oxide to

ensure complete dehydration. For SEM, tissues were critical-point-dried and sputter-coated with gold before observing with ISI-60 SEM. For TEM, tissues were embedded in Spurr's medium and sectioned with LKB ultratome. Silver/gold sections were double stained with 2% aqueous uranyl acetate and lead citrate before viewing with JEOL 100 CX TEM.

Preparation, sequential extraction, and analysis of alcohol insoluble solids (AIS)

Cap tissue (100 g) was blended for 5 min with 5 volumes of 95% ethanol, precipitated, and filtered through Whatman #42 filter paper. Residue was resuspended and extensively washed with 78% ethanol, filtered, and dried under vacuum at 50 °C. Dried AIS was weighed, ground to a fine powder by a Wiley mill (Thomas-Wiley), sieved through a mesh No. 80 screen, and stored in a dessicator. For each sampling date, 4 replications of AIS were prepared, with exception on 0 d, which consisted of 2 replicates.

The AIS samples were extracted following the modified method of Sietsma and Wessels (1981). Samples of AIS (200 mg) were hydrated, sonicated for 15 min, and extracted in a total of 15 ml of boiling deionized (d.i.) water for 2 h while stirring. Supernatants were collected after centrifuging at $13,000 \times g$ for 20 min at 4 °C, and pellets were washed twice with 2 ml of d.i. water and centrifuged. Supernatants were collected, and volumes were adjusted to 25 ml with water (water fraction). Pellets were resuspended in 15 ml of 1N NaOH and extracted at 60 °C for 20 min with occasional stirring, centrifuged, and then washed twice with water. Supernatants were combined, pH adjusted to 7.0, and brought to 25 ml volume (1N NaOH fraction). Residues were resuspended in 15 ml of 10N NaOH, boiled for 1 h while stirring, centrifuged, and pellets were washed twice with water. Volume of the supernatants was adjusted to 25 ml with water (10 N NaOH fraction). The pellet was resuspended in 5 ml of 6 N HCl, boiled for 4 h while stirring, and centrifuged. Residues were washed with water, extracted with 10 ml of 1N NaOH for 20 min at 60 °C, centrifuged, and the pellets were washed twice with water. Volume of combined supernatants was adjusted to 25 ml with water (HCl/1N NaOH fraction). Remaining residues were considered to be the nonextractable fraction.

Proteins were determined in water and 1N NaOH fractions by Coomassie blue (Bradford 1976) with BSA as standard. In AIS and in all fractions, total neutral carbohydrates were analyzed by anthrone reagent with glucose as standard (Fairbairn

1953), and chitin was measured by the Ride and Drysdale (1972) method. Total chitin in AIS was determined after acid hydrolysis (Chen and Johnson 1983). Monosaccharides in hydrolyzed alcohol insoluble solids were analyzed by GC as alditol acetates (Blakeney and others 1983).

Other measurements

Pilei dia and stipe length were measured by a digital caliper (Extech Instruments Co., Waltham, Mass., U.S.A.??). Pilei dia was determined at the widest portion of the cap and stipe length from the bottom to the cap's line.

Statistical analysis

Statistical analyses were performed with JMP and included analysis of variance ($P < 0.05$), separation of means by LSD, and determination of correlation coefficients (SAS Institute Inc. 1995).

Results and Discussion

Texture

During storage at 12 °C and 95% RH, mushrooms softened and toughened at the same time (Figure 1). Softening, as measured by force to puncture tissues, declined from 2.6 N to 1.5 N, with the most softening occurring from 3 to 6 d of storage. The same trend was noticed in the gradient and maximum force for puncturing (data not shown). Typically, for determination of mushroom softening, textural methods such as slight compression of caps (Gautam and others 1998), tissue cubes (McGarry and Burton 1994), or whole caps up to the point of biofailure (Beelman and others 1987) have been used. However, force for puncturing using a small-dia probe was observed to be a reliable method for measurement of vegetable firmness (Howard and Buescher 1990; Hudson and Buescher 1986), and our re-

sults showed that this test was reproducible and representative of mushroom firmness.

As mushrooms softened during storage, texture also became tougher. Although both changes happen at all storage temperatures (Rama and others 1995; Murr and Morris 1975a), these 2 textural characteristics have rarely been studied together. Our results showed that toughness, as measured by TPA gumminess, increased for 6 d at 12 °C from 19.2 N to 40.3 N and decreased thereafter to 33.0 N (Figure 1). Mushrooms stored for 9 d showed obvious signs of quality deterioration such as off-odor, darkening, and stickiness, which paralleled the decline of toughness. The results are consistent with previous reports that toughness increases with storage until mushroom decomposition occurs (Murr and Morris 1975a; Narvaiz 1994).

Hardness increased for the first 3 d from 95.4 N to 112.6 N ($p < 0.0001$), remained relatively constant until 6 d, and then subsequently declined to 76.8 N ($p < 0.0001$). Mean fracturability values followed similar trends as hardness. Initially, all samples exhibited fracturability, but after 3, 6, and 9 d in storage, fracturability occurred in 90%, 24%, and 0% of the samples, respectively. Changes in fracturability during storage are consistent with results from a previous study where compression of older, deteriorated samples failed to show signs of breakage (Nussinovitch and Kampf 1993).

Ultrastructure

Intercellular space between hyphae cells of surface pilei tissue enlarged during storage (Figure 2). Stretching of cells caused by cap opening (cap dia increased from 41.37 mm to 47.58 mm) and loss of turgidity caused by water loss probably accounted for enhanced intercellular space.

Hyphae from interior pilei tissue had

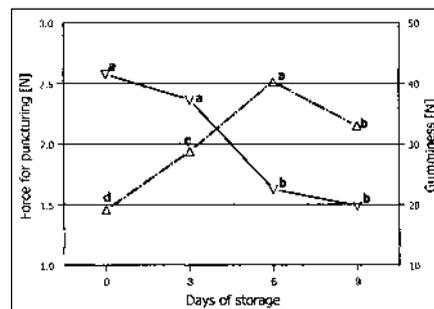


Figure 1—Softening (puncture force —●—) and toughening (gumminess —■—) of mushroom-cap tissue during storage. Mean separations by $LSD_{0.05}$; values of each textural characteristic with same letter are not significantly different.

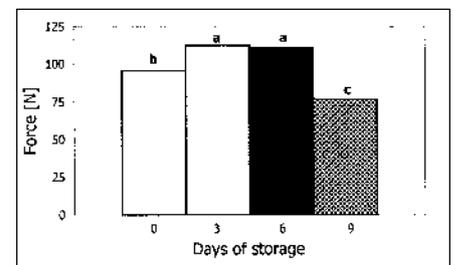


Figure 2—Hardness (force, [N], for 80% compression) of mushroom cap tissue during storage (from 0 to 9 d) at 12 °C and 95% RH. Mean separations by $LSD_{0.05}$; values of each textural characteristic with same letter are not significantly different.

larger dia than those located on the surface (Figure 3, right). Four h after harvesting, hyphae appeared as well-defined tubes, but they soon collapsed during storage, losing their structured appearance. According to Evered and Burton (1995), cell walls of collapsed cells adhered to adjacent cells leaving enlarged free space between the remaining cells. Rather than adhered traces of cell walls as previously reported, we observed aggregation of cells remaining within the intercellular space. However, even though most cells were collapsed, some hyphae still appeared intact, which does not seem to be unusual (Evered and Burton 1995; Umar and Van Griensven 1997).

Bacteria were observed by SEM in surface pilei tissue from mushrooms stored for 9 d (Figure 3). During the first 6 d of storage, while toughness increased, bacterial growth was not observed. In the samples stored for 9 d, the bacteria probably contributed to disintegration of hyphae walls resulting in loss of in toughness. Together with bacterial activity, increased activity of endogenous autolysins (Peberdy 1990; Rosenberger 1979) could have accounted for the reduction in toughness.

The ultrastructure of hyphae cells from surface pilei tissue of freshly harvested mushrooms indicated a large number of vacuoles and intact-structured cytoplasmic content (Figure 4). After 6 d in storage, organization and components within the cytoplasm was disrupted, leaving only the cell walls as a distinguishable structure. The disruption of cellular integrity undoubtedly was responsible for loss of turgidity and collapse of the hyphae cells observed by SEM.

Alcohol insoluble solids

During mushroom storage, protein and polysaccharide content declined, while chitin content increased (Figure 5). Total protein content decreased by 30.3% and 57.7% in AIS from mushrooms stored for 6 and 9 d, respectively. Proteins extracted by both water and 1N NaOH declined in AIS, although the amounts and changes in water-soluble protein were relatively small compared to those in the NaOH fraction (Figure 6). Protein degradation has been reported to be due to protease activity (Burton 1988; Murr and Morris 1975b), and the liberated amino acids are probably used in cell metabolism and chitin synthesis since no amino-acid accumulation during storage has been noted (Murr and Morris 1975b). At the beginning of our experiment, the water-soluble fraction made up 12.8% of total proteins, stayed relatively constant during storage, and at the end had increased to 18.3% (Figure 6). Since the total amount of protein de-

clined, the increase in water-soluble portion (based on total proteins) probably reflected intermediate products of proteolysis due to autolysins or bacterial activity. Degradation of proteins (total) paralleled

softening ($r = 0.94$).

Polysaccharides, excluding chitin, declined in mushrooms during storage for 6 d and then remained unchanged (Figure 5). Polysaccharides were extracted by wa-

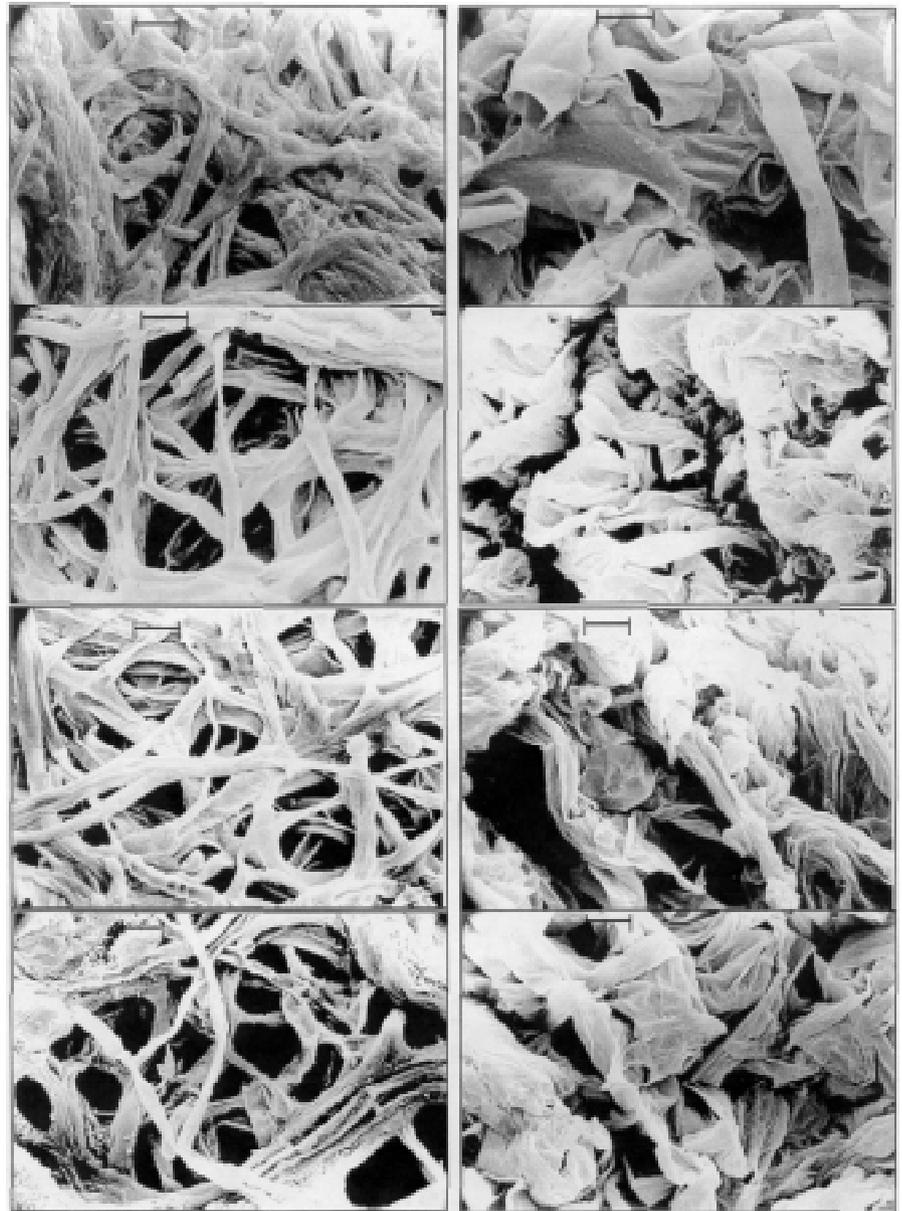


Figure 3—Scanning electron micrographs demonstrating changes in cells from pileus surface (left) and interior tissue (right) during storage of mushrooms (1st row, 0 d; 2nd row, 3 d; 3rd row, 6 d; 4th row, 9 d). Bar represents 10 μ m.

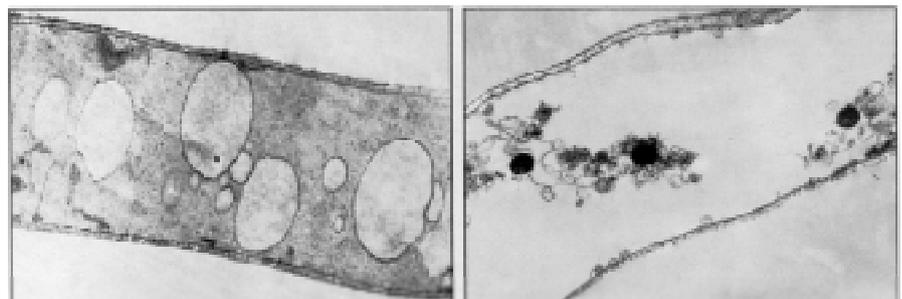


Figure 4—Transmission electron micrographs showing hyphae from the pileus surface 4 h after harvesting (left) and after 6 d of storage at 12 °C (right).

ter (glycogen and mucilage), 1N NaOH (S-glucan and mucilage), 10N NaOH, and 6N HCl followed by 1N NaOH (R-glucan) (Figure 7). Extraction with 10N NaOH was expected to deacetylate and partially depolymerize chitin, and further treatment with 6N HCl completed depolymerization resulting in liberation of bound polysaccharides (Ride and Drysdale 1972; Sietsma and Wessels 1979). Changes in polysaccharide content of the different fractions were relatively minor except for the 10N NaOH fraction. Within 3 d of storage, AIS polysaccharides extracted by 10N NaOH declined by more than 50% and then continued to decline as storage time increased. In cases of limiting conditions during development or after-harvest storage, fungal cell walls can serve as a source of carbohydrates for metabolism (Farkas 1985; Rosenberger 1979). Thus, the 10N NaOH fraction might represent wall polysaccharides that were utilized during storage.

Degradation of total polysaccharides was significantly correlated to softening ($r = 0.84$). However, a portion of polysaccharides liberated after acid depolymerization and those remaining in nonextract-

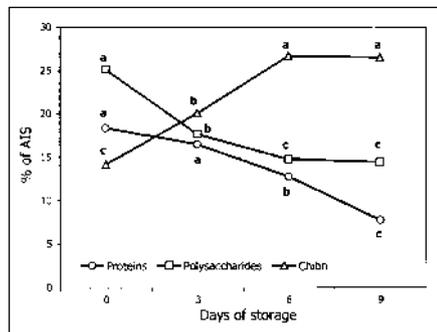


Figure 5—Chitin, protein, and polysaccharide content of alcohol insoluble solids (AIS) extracted from stored mushroom pilei tissue. Mean separations by LSD 0.05; values within components accompanied by the same letter are not significantly different.

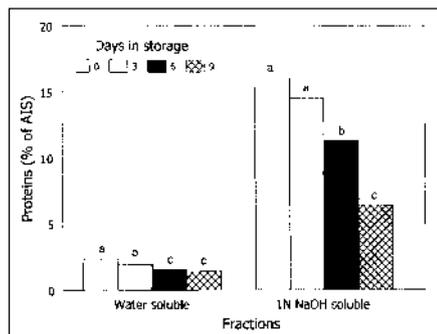


Figure 6—Changes in content and solubility of proteins in alcohol insoluble solids (AIS) from pilei tissue of stored mushrooms. Mean separations by LSD 0.05; values within fractions accompanied by the same letter are not significantly different.

Table 1—Changes in composition of polysaccharides in AIS during storage

D of storage	Monosaccharides [% of AIS]					
	fucose	ribose	xylose	mannose	galactose	glucose
0	0.25a*	0.76a	0.40a	2.01b	2.08ab	18.56a
3	0.23a	0.61b	0.44a	2.37a	2.28a	14.01b
6	0.19b	0.45c	0.44a	1.95b	1.76b	10.97c
9	0.19b	0.26d	0.43a	1.55c	1.48c	10.06c

* Mean separation by LSD0.05; values within columns followed by same letter are not significantly different.

able residue, when calculated as percentage of total polysaccharides, significantly increased with time. This increase in less soluble polysaccharides might indicate bonding between wall polysaccharides and chitin that strengthened the cell wall and caused toughening.

Hydrolyzed AIS of mushrooms contained various levels of monosaccharides in the order of glucose > mannose > galactose > ribose > xylose > fucose (Table 1). All the monosaccharides, except xylose, tended to decline during mushroom storage. Particularly large (> 50%) reductions in glucose and ribose were observed in AIS of mushrooms stored for 6 and 9 d compared to concentrations found in fresh mushrooms. The total monosaccharide content of the AIS closely represented the total amount of polysaccharides ($\pm 1.1\%$). Therefore, the decline in polysaccharides observed in AIS of stored mushrooms was primarily due to decreases in glucose content.

An increase in chitin (b-1,4-N-acetyl-D-glucosamine) content was observed during storage (Figure 8). Water and 1N NaOH extracts had less than 1% of the AIS (acetyl)glucosamine. Deacetylation and depolymerization with 10N NaOH liberated approximately 1/3, while acid depoly-

merization followed by 1N NaOH extraction solubilized about half of the total chitin content. An increased amount of (acetyl)glucosamine in the HCl/1N NaOH fraction, as well as in the residue, illustrated chitin synthesis and possibly increased intra- and intermolecular bonding. Reduced levels of chitin in the nonextractable residue might be attributed to loosening of some crystalline chitin microfibrils by activity of endogenous and/or bacterial enzymes.

Increased chitin content during the storage appeared to be associated with toughening ($r = 0.95$). Additionally, the increase in cohesiveness (data not shown) and hardness values might be attributed to increased chitin content and formation of covalent bonds between chitin and R-glucan that caused enhanced rigidity of the hyphal wall (Wessels 1986).

Conclusions

WHITE COMMON MUSHROOM *AGARICUS bisporus* simultaneously softened and toughened during post-harvest storage. Force for puncturing, representing softening, declined, while gumminess, representing toughening, increased during the first 6 d and decreased thereafter. Softening paralleled protein and polysac-

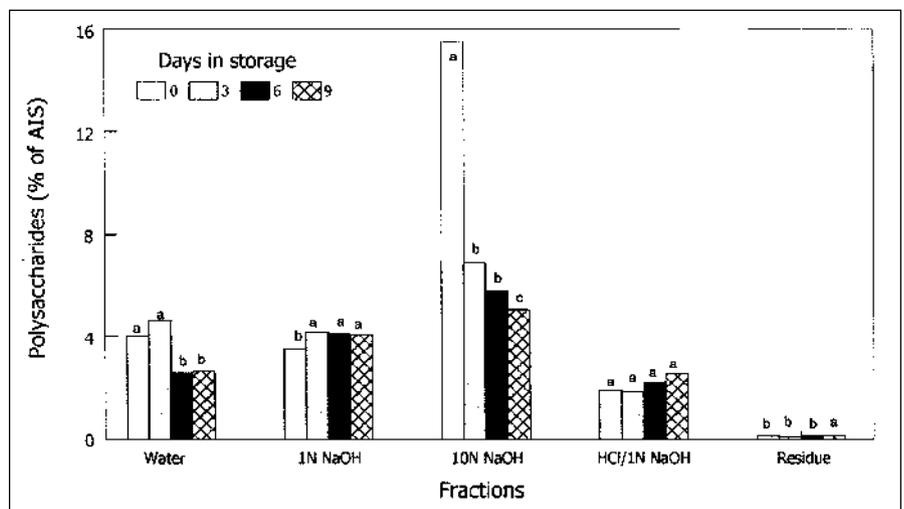


Figure 7—Changes in content and solubility of polysaccharides in alcohol insoluble solids (AIS) from stored mushroom pilei tissue. Mean separations by LSD 0.05; values within fractions accompanied by the same letter are not significantly different.

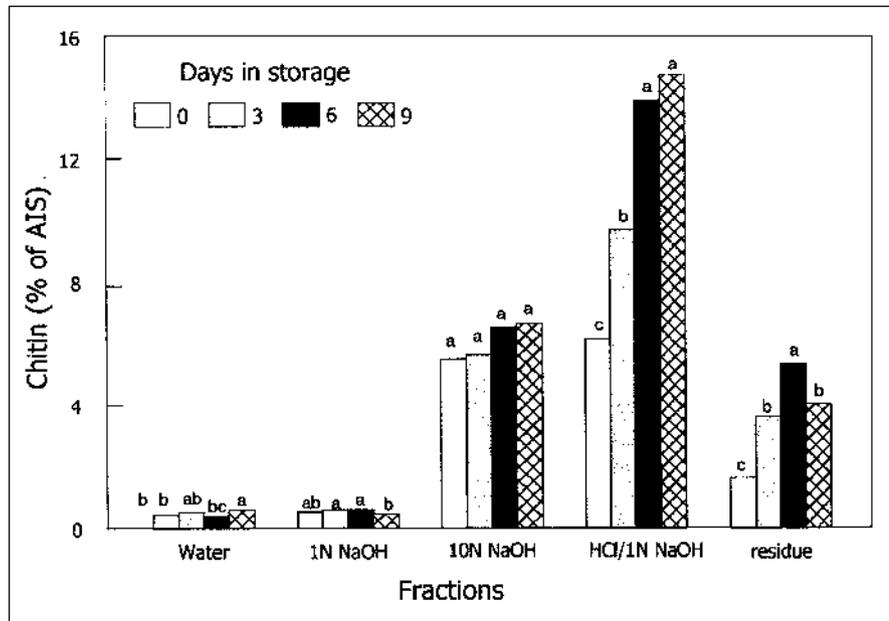


Figure 8—Changes in content and solubility of glucosamine (chitin) polysaccharides in alcohol insoluble solids (AIS) from stored mushroom pilei tissue. Mean separations by LSD 0.05; values within fractions accompanied by the same letter are not significantly different.

charide losses, hyphae shrinkage, central vacuole disruption, and expansion of intercellular space at the pilei surface, and chitin content was related to toughening. Strength of hyphae walls was evident by the fact that most of neutral polysaccharides and chitin were extracted after deacetylation and depolymerization, indicating that wall glucans were mainly bound with chitin. Glucose was the dominant monomer in wall polysaccharides, and its decline along with protein degradation coincided with softening and chitin synthesis (toughening).

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