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CHANGES IN COMPOSITION OF HARVESTED MUSHROOMS (*AGARICUS BISPORUS*)

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Key Word Index—*Agaricus bisporus*; Basidiomycetes; mushroom; storage; non-structural polysaccharides; glycogen; cell wall; chitin; urea; protein; cell wall glucan.

Abstract—Post-harvest changes in the biochemical composition of the mushroom were studied. Non-structural polysaccharide was found at levels greater than 10% dry wt in the fresh mushroom. After 4 days storage, the level had decreased to below 5% dry weight. The polysaccharide appeared to contain only glucose residues joined by α-1,4 and α-1,6 linkages. The chitin content of cell walls increased by *ca* 50% during 4 days storage, while cell wall glucan decreased. There was a large increase in urea content.

INTRODUCTION

After harvest, mushrooms have a storage life of 2–4 days at room temperature. During this time, the cap of the mushroom expands to expose the gills, which then sporulate. Changes in the texture and flavour of the mushroom also take place. Previous work has shown that respiration is high compared with other horticultural produce [1] and that it increases during the rapid development of the gills after harvest [2]. The high rate of consumption of respiratory substrate makes great demands on the resources of the mushroom. Mannitol, the most abundant carbohydrate, decreases by *ca* 50% during 4 days storage [2]. It appears that a decrease in the activity of the hexose monophosphate pathway occurs which may accommodate the change to mannitol catabolism [3]. Trehalose is also metabolized after harvest [2]. The observed breakdown of these two materials was sufficient to account for *ca* half of the post-harvest CO_2 production. Other work has suggested that there is breakdown of protein [4] and 'glycogen' (which probably included some cell wall material) [5].

The purpose of the work recorded here was to examine the levels of other possible respiratory substrates after harvest in an attempt to assess the effects of post-harvest respiration on the composition of mushrooms.

RESULTS

Non-structural polysaccharide

Hydrolysis of samples with amyloglucosidase led to the release of up to 16.3% of the dry wt as reducing sugars in freshly harvested mushrooms. Those stored for 4 days contained 8.8–12.6% non-structural polysaccharide. There was a steady fall in polysaccharide content over the storage period (Fig. 1), giving a mean decrease of 50.6 ± 7.7% (s.e.m.). Because of the considerable dry wt loss of the mushrooms during storage (*ca* 30% in 4 days) values were also calculated on the basis of the dry wt at harvest so that absolute changes in the material could

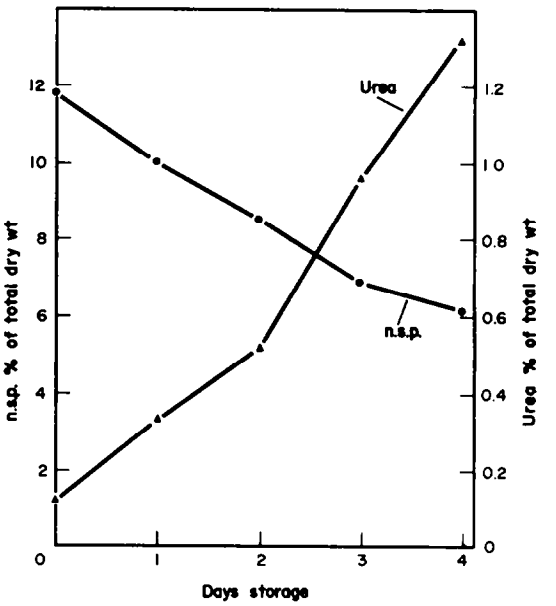

Fig. 1. Changes in non-structural polysaccharide (n.s.p) (●) and urea (▲) content of mushrooms after harvest.

Table 1. Changes in cellular components during post-harvest storage of mushrooms expressed as mg/g dry wt at harvest

Component	Day 0	2	4
Non-structural polysaccharide	118	70	45
Urea	1	4	9
Soluble protein	165	139	135
Cell wall	192	211	233
–Chitin	65	96	117
–Glucan*	84	68	63
–Protein	19	29	31

Values are the mean of 7 experiments.
*Anthrone positive material.

Table 2. Activity of amyloglucosidase against different substrates

Glycogen	110.7%
Laminarin	79.0
Cell wall (Day 0)	1.4
(Day 4)	0.7
Ethanol insoluble, hot water soluble material	49.9 (% of total non-structural polysaccharide)

Results are expressed as reducing sugars released as a percentage of dry wt of substrate.

be assessed. The loss of polysaccharide over 4 days storage was 73 mg/g dry wt (Table 1).

Previously obtained values for 'glycogen' content were lower than the present ones for non-structural polysaccharide [6]. Since the amyloglucosidase preparation used is active against β-1,3-glucans under the conditions employed here [7] (Table 2), a closer examination of the polysaccharide was initiated.

Glucose was the only monosaccharide which could be detected chromatographically in amyloglucosidase digests of the whole freeze-dried samples. Digestion of isolated cell walls with amyloglucosidase under the same conditions as those used for the freeze-dried samples released only small amounts of reducing sugar (Table 2). When the freeze-dried tissue sample, which had previously been extracted with hot 80% ethanol, was treated with boiling water for 2 hr, the extract gave values of *ca* 50% of the total non-structural polysaccharide detected in the samples, on digestion with amyloglucosidase.

Partial hydrolysis of the crude isolated polysaccharide with HCl gave disaccharides with the same R_g values as those from glycogen treated in the same way (Table 3). No β-linked disaccharides could be detected on the chromatograms. Iodine staining of the crude extracted polysaccharide [8] gave a λ_{max} at 480 nm and E_{max} of 0.12. In 25% saturated $(NH_4)_2SO_4$, the λ_{max} was 455 nm and the E_{max} increased to 0.47.

Urea, soluble proteins and free amino acids

The urea content of harvested mushrooms increased during storage until, 4 days after harvest, a mean content of 1.3% dry wt was observed (Fig. 1). The increase varied from 4- to 55-fold in different experiments. A mean increase of 8 mg urea/g dry wt was seen (Table 1).

Soluble protein content decreased slightly during storage, showing a loss of *ca* 30 mg/g dry wt over 4 days (Table 1). Two of the major components of the free amino acid pool, glutamic acid and alanine [9], decreased 75.8 and 93.6% respectively during storage.

Table 3. R_g values of disaccharides in partial HCl hydrolysates of non-structural polysaccharide

	(a)	(b)
Glucose	1.00	1.00
Maltose	0.84	—
Cellobiose	0.80	0.56
Glycogen		
– Maltose	0.84	0.69
– Isomaltose	0.69	0.51
Laminarin		
– Laminaribiose	0.86	0.73
– Gentiobiose	0.66	0.49
Non-structural polysaccharide		
–1	0.84	0.70
–2	0.69	0.51

Cellulose TLC, double development; solvents (a) Py–*n*-BuOH–H_2O (2:2:1); (b) *n*-PrOH–EtOAc–H_2O (7:1:2).

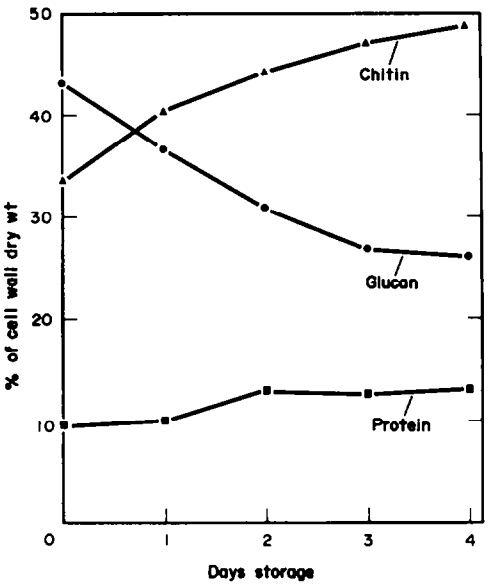

Fig. 2. Changes in the cell wall composition of mushrooms after harvest. Chitin (▲); glucan (●); protein (■).

Cell walls

The ratio of cell wall material to total dry wt increased during storage (Table 1). The mean increase in cell wall in proportion to total dry wt was 70.3 ± 8.8%.

There was also a change in the cell wall composition during storage (Fig. 2). Anthrone positive material (mainly glucans [10]) showed a decrease of 31.7 ± 9.2% as a proportion of cell wall dry wt, while chitin showed an increase of 50.0 ± 8.8%. The relative increase in chitin was due to a net synthesis of chitin during the storage period of 53 mg/g dry wt (Table 1). There was a real decrease in anthrone positive material of 21 mg/g dry wt. Cell wall protein showed an increase during the storage period (Table 1).

DISCUSSION

The post-harvest development of the mushroom must involve considerable mobilization of reserves. It has already been shown that mannitol and trehalose are probably post-harvest respiratory substrates [2]; the results reported here identify other materials that are broken down.

Of the materials studied, non-structural polysaccharide was the major component which was metabolized. The evidence from the partial hydrolysis experiments suggests that the polysaccharide is composed of glucose residues joined by α-1,4 and α-1,6 linkages. The iodine staining characteristics shown by the polysaccharide are within the range reported for glycogen from various animal sources. The large increase in E_{max} on addition of $(NH_4)_2SO_4$ is a reaction shown by glycogens [8]. The decrease in λ_{max} on addition of

$(NH_4)_2SO_4$ is unusual, however. Thus the non-structural polysaccharide was tentatively identified as glycogen.

The inclusion of significant amounts of cell wall polysaccharide in the non-structural polysaccharide estimates appears to be ruled out since the amyloglucosidase had a negligible effect on isolated cell walls. Glycogen is used as a reserve substrate in other fungi; thus its presence, and breakdown in storage may be expected.

The observation in this work of higher non-structural polysaccharide values than those seen previously [6] could be due to two factors. First, considerable variation in non-structural polysaccharide content has been observed in samples from different phases of the cropping cycle (Hammond, unpublished). Secondly, the method of analysis used here may allow the amyloglucosidase greater access to any bound non-structural polysaccharide in the cells. The previous work suggested that some of the non-structural polysaccharide in the mushroom was bound [6]; the fact that in the present experiments only 50% of the non-structural polysaccharide was extractable with hot water supports this suggestion. However, further work is necessary to confirm the occurrence of compartmentation in the non-structural polysaccharide pool, similar to that seen in yeast for example [1].

The increase in cell wall material indicates transfer of materials from the cytoplasm to allow post-harvest hyphal growth. Net synthesis of chitin after harvest was noted previously [12] and appeared to take place mainly in the stripe and probably the gills. The loss of glucan from cell walls during storage may be because it is more susceptible than chitin to autolysis; during early autolysis in *Coprinus comatus* there was greater glucanase than chitinase activity [13]. Utilization of cell wall material in periods of stress, such as fruit body formation and after harvest, has been observed in other Basidiomycetes [14, 15].

The change in cell wall composition during storage is interesting since it is similar to the increase in cell wall chitin content observed during stipe elongation in *C. macrorhizus* [16]. Although the increase in cell wall chitin content seen here was partly due to loss of glucan, it seems possible that some increase in chitin level results from the hyphal elongation which occurs during post-harvest development. No net loss of wall glucan was observed in *C. macrorhizus* [16].

Considerable breakdown of nitrogenous materials must occur to account for the increase in urea content. The urea produced over 4 days storage was equivalent to *ca* 24 mg protein/g dry wt. Murr and Morris [4] observed increased protease activity after harvest, but the present work showed little fall in protein content. It is possible that the protease mediates increased protein turnover during post-harvest development. The fall in free amino acids seen here and previously [4], together with that in protein content could account for the observed urea synthesis. However, chitin also shows net synthesis during storage. Another source of nitrogen for incorporation into chitin and urea could be the nucleic acids. Reijnders [17] reported that RNA breakdown occurred in Basidiomycetes during the later stages of fruit body growth. *C. macrorhizus* contains high levels of RNA and DNA [18], but there is no evidence of breakdown. Urea formation via purine catabolism has been demonstrated in *A. bisporus* [19].

The results presented here and previously [2] show that considerable changes in composition occur during the short post-harvest life of the mushroom. Reduction of respiration by cool storage or the use of suitable gas atmospheres [20] should be effective in retarding these changes.

EXPERIMENTAL

Mushrooms (*Agaricus bisporus* (Lange) Sing.) were grown, harvested and stored as described in ref. [3]. At the end of the experimental period, the mushrooms were either used fresh for the soluble protein determinations or sliced into liquid N_2, freeze-dried and ground to a powder.

Non-structural polysaccharide assay. Freeze-dried powder (50 mg) was heated with 10 ml 80% EtOH for 30 min at 60°. The suspension was filtered and the residue added to 5 ml H_2O. The mixture was heated at 100° for 2 hr. After cooling, 5 ml of amyloglucosidase soln (0.12 g amyloglucosidase Grade II (Sigma) in 100 ml 20 mM acetate buffer pH 4.5) was added and the mixture incubated at 50° for 18 hr in a shaking H_2O bath. After filtering, the reducing sugar content of the soln was estimated by autoanalyser using the Cu–neocuproine method [21]. Enzyme and non-enzyme blanks were included. Non-structural polysaccharide is expressed as the glucose equivalent.

Partial hydrolysis and chromatography of non-structural polysaccharide. The supernatants from the cell wall isolation (see below) were bulked and dialysed. After concn in a rotary evaporator, 2 vol. of EtOH were added. The ppt. was recovered by centrifugation and then re-dissolved at 100°. The resulting suspension was recentrifuged and the supernatant treated with 2 vol. of EtOH. After standing for 2 hr at 4° the ppt. was centrifuged. The pellet was partially hydrolysed with 11 M HCl for 1 hr at 22° [22]. Samples of laminarin and glycogen were treated in the same way. TLC of hydrolysates was carried out on pre-prepared MN 300 cellulose sheets, with glucose, maltose and cellobiose standards. Solvents used were *n*-PrOH–EtOAc–H_2O (7:1:2) and Py–*n*-BuOH–H_2O (2:2:1), double development was used in both cases. Spots were detected with alkaline $AgNO_3$ spray.

Urea assays. Freeze-dried powder (0.1 g)was added to 9 ml H_2O at 100° and heated at 100° for 10 min. After cooling, the suspension was made up to 10 ml and filtered. The filtrate (0.1 ml) was assayed for urea N with a Sigma No. 640 analytical kit.

Crude cell wall isolation. Freeze-dried powder (0.2 g) was homogenized in 20 ml 0.1 M Tris–HCl buffer (pH 8.3) and centrifuged. The pellet was resuspended in 1% NaCl soln (20 ml) and the suspension frozen. After thawing, the suspension was sonicated for 6 min with cooling using a Kerry KT100 instrument at full power. The suspension was refrozen and thawed ×3 and then centrifuged. The pellet was suspended in 1% NaCl soln, centrifuged, rewashed ×2 in H_2O and finally freeze-dried and weighed.

Cell wall glucan assays. Glucans were assayed by the anthrone method [23], using a suspension of the crude cell wall in H_2O. Glucose standards were used and the results are expressed as glucose equivalents.

Cell wall protein assays. Crude cell wall was extracted with 1 ml 0.5 M NaOH for 5 min at 100°. After cooling, the protein content of the extract was determined [24].

Chitin assays. Crude cell wall was suspended in 5 ml 6M HCl and sealed in ampoules. After hydrolysis at 100° for 12 hr, the HCl was removed by evapn at 60° in a stream of air. The residue

was dissolved in H_2O and the glucosamine content determined by the method of ref. [25]. The extent of hydrolysis was assessed by treating known quantities of chitin in the same way. Glucosamine standards were used.

Soluble protein assays. Mushrooms were sliced into cold Tris–HCl buffer (0.1 M, pH 8.6) and frozen. After thawing, the material was homogenized and made up to standard vol. with H_2O. Aliquots were taken for dry wt determinations and the homogenate was then filtered. The protein content of the filtrate was determined [24]. BSA standards were used.

Amino acid determination. Amino acids were extracted and determined as described in ref. [3].

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