Effect of Storage Temperature on Postharvest Changes in Mushrooms

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Abstract. Mushrooms [Agaricus bisporus, (Lange) Sing.] stored at 10° and 20°C showed a sigmoid pattern of growth while at 0°C growth was retarded. The postharvest growth exhibited at 10° and 20°C could be related to a decrease in free α-amino N while at 0°C there was a significant increase in the level of free α-amino N during storage. Protease activity in the tissue increased at all 3 temperatures. It is suggested that postharvest maturation of mushrooms is supported by utilization of low molecular weight nitrogenous compounds formed through increased protein degradation. Mushrooms stored at 20°C toughened and matured faster than those stored at 10° or 0°C. Increases in discoloration during storage appeared to be correlated with decreases in total phenols and with increases in o-diphenol oxidase (o-DPO) activity. The relationship of these biochemical changes to postharvest maturation of mushrooms is discussed.

Several factors contribute to postharvest quality of mushrooms. Among these are growth (stem elongation and cap opening), discoloration and changes in texture. MacCanna and Gormley (7) devised a 'quality spectrum' for mushrooms in which these factors receive the greatest emphasis and probably represent the most important factors in consumer acceptance of mushrooms.

Low temp storage has been shown to be an excellent method to retard deterioration and senescence of harvested mushrooms (2, 4, 10). Data from Lutz and Hardenburg (6) show that mushroom shelf life decreases and respiration rate increases with increasing storage temp, indicating inverse relationships of shelf life to temp.

Discoloration is a major factor contributing to quality loss of mushrooms after harvest and results from the action of o-diphenol oxidase (o-DPO) (E.C. 1.10.3.1) (polyphenoloxidase, tyrosinase) on phenolic compounds. In addition to the active form of o-DPO, the existence of an inactive or latent form of this enzyme in mushrooms has been described recently by Yamaguchi et al. (14). Therefore, o-DPO is commercially important in the storage of mushrooms since it catalyzes the first 2 steps in the formation of pigments associated with browning.

Since little is known of the postharvest behavior of mushrooms, we investigated the effect of temp on surface discoloration as related to total phenolic content and o-DPO activity. We also related the phenomenon of postharvest maturation to respiration, textural changes, and proteolytic activity.

Materials and Methods

Freshly harvested mushrooms (tan strain) were purchased and handled as described previously (8). They were sorted into lots of 20 mushrooms each and distributed to controlled temp rooms where the lots were held for up to 8 days in 20-L jars. The lots were ventilated with a humidified air stream (ca. 90%) such that respiratory CO2 would not build up to more than 0.3% in the effluent. Respiration rate was measured by the colorimetric method (1). Daily increases in pileus diameter and stipe length of mushrooms during storage were measured using vernier calipers.

Discoloration. Surface discoloration was measured with an Agtron Color Difference Meter by a method previously described (8). Results

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are expressed as percent decrease in reflectance relative to an initial reflectance of 51.4%.

Changes in α-amino N and total phenols. To determine α-amino N and total phenols, a random sample of 80-100 g of mushroom tissue was selected from each treatment at appropriate intervals, weighed and extracted with hot 95% ethanol. The extract and tissue were then homogenized in a Waring blender, filtered and the filtrate brought to a known volume with 80% ethanol.

Total phenols were determined by the method of Swain and Hillis (11) with slight modification. Folin reagent was used full strength and the samples were read against a blank at 760 nm using a Gilford spectrophotometer. Tyrosine in the range of 0-100 µg was used as a standard. The results are reported as mg eq tyrosine/100 g fresh wt.

The level of α-amino N was estimated by the method of Yemm and Cocking (15). An aliquot of the filtrate containing 0.05-3 µg α-amino N was used for assay and compared against an alanine standard with a Gilford spectrophotometer at 570 nm.

Enzyme activity. Samples of mushroom tissue were taken at appropriate intervals during storage and acetone powders prepared by a method described previously (14). A 100 mg sample of the acetone powder was homogenized in 20 ml 0.1 M phosphate buffer, pH 6.0, and centrifuged at 12,000 g and 2°C for 15 min. The latent (inactive) form of the enzyme was activated by homogenizing in buffer containing 0.1% sodium lauryl sulphate (14). Treatment with sodium lauryl sulphate resulted in recovery of total o-DPO activity. Enzyme activity in the supernatant was determined spectrophotometrically by measuring the increase in absorbance at 525 nm using substrate without enzyme as reference. Substrate consisted of 0.01 M proline and 0.01 M catechol buffered at pH 6.0. Enzyme activity at 25°C is expressed as ΔOD/min/mg protein of the extract. Latent o-DPO activity was estimated by subtracting activity in the absence of sodium lauryl sulphate from total activity. A change in OD of 0.1 was equivalent to the oxidation of 20 µmole of catechol. Protease activity in the acetone powders was measured by a method described previously (8). Enzyme protein in the extracts was estimated using the Folin phenol reagent (5).

Results

Effect of temp on growth and respiration. Growth at 0°C was negligible over an 8 day storage period whereas at 20°C growth was rapid, with a maximum rate being reached between the first and second day of storage (Fig. 1). The increase in daily growth rate at 10°C was gradual at first but became more pronounced with the maximum rate occurring on about the 4th day. The rates of cap expansion and stipe elongation seemed to increase and decrease roughly in parallel, and comparison in individual specimens showed that both ceased growing at approximately the same time. At both 10° and 20°C the pattern of respiration with time was similar to that exhibited by most fleshy fruits during ripening in that there was a rise in rate to a peak, then a subsequent decline. The respiratory peak developed about the time growth was at a maximum.

Changes in α-amino N and protease activity. After an initial lag of 2 days, protease activity at 0°C rose gradually over the next 6 days of storage (Fig. 2). Protease activity increased more than 10-fold in mushrooms stored 4 days at 20°C or 8 days at 10°C. At 20°C, free α-amino N accumulated during the first 12-hr of storage and then dropped during the remainder of the storage period. The free α-amino N also decreased at 10°C, then appeared to level off after 4 days of storage. At 0°C, there was an accumulation of α-amino N in the tissue during the 8-day storage period.

Changes in texture and discoloration. Temp and duration of holding also influenced the textural characteristics of fresh mushrooms. Mushrooms stored at 20°C matured and toughened faster than those held at 0° or 10°C (Table 1). Maturation at 0°C was strongly repressed, and consequently textural changes took place slowly.

Temperature affected the rate of discoloration in much the same way it influenced the rate of textural change. Discoloration was greatest and occurred fastest at 20°C (Table 1). The rates of discoloration at 0° and 10°C for the first few days were significantly different. After the 4th day, however, the increase in color at 10°C was only fractionally greater than at 0°C.

Effect of temp on total phenols and o-DPO. The rate of loss of phenolic compounds occurred fastest at 20°C while at 10° the change was more gradual (Fig. 3). At 0°C there was a slight decrease for the first 4 days of storage after which time the level of total phenols remained constant.

Active o-DPO increased during storage at 10° or 20°C and the rate of change in activity was related to temp (Fig. 4). Latent o-DPO activity also increased then dropped toward the end of storage. At 0°C active o-DPO remained relatively constant while the latent form increased during the first 2 days of storage then leveled off.

Discussion

After harvest mushrooms stored at 10° or 20°C showed a sigmoid type growth curve. Gruen (3) has indicated that unharvested mushrooms have a typical sigmoid growth curve and that mushrooms are generally harvested during the linear phase. In our experiments, the lag prior to resumption of maturation at 10° or 20°C suggests a buildup of metabolites necessary for continued growth. Since harvested mushrooms are no longer supported by an exogenous substrate
containing C and N, this lag period could represent a physiological
discharge to occur.

Data suggest that such systems involve the supply of low molecular
weight nitrogenous compounds. 

The wall aids in preservation of the basidiocarp and allows spore
release to occur. 

likely due to changes in the cell wall structure within the mushroom.

One feature of maturation common to many species in the Agaricales
characteristics occur is affected by temp. Those mushrooms which
be related to maturation, and the rate at which changes in these
physiological changes associated with senescence of mushrooms after
harvest. Excellent quality maintenance was achieved with 0°C and it
be seen from the data that exposure to temp above 0°C for a short
period of time can cause a rapid increase in physiological changes
associated with quality characteristics which have a bearing on
customer acceptance of fresh mushrooms. In addition, we propose
that postharvest maturation of mushrooms is supported by increased
availability of low molecular wt nitrogenous compounds brought
about by increased proteolytic activity. We believe that this increase
in proteolytic activity is a physiological adaptation to starving
conditions brought about by removal of the source of substrate
through harvesting of the mushroom.

Our results indicate that low temp significantly retards several
physiological changes associated with senescence of mushrooms after
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The rate of loss of phenolic compounds appeared to be negatively
correlated with discoloration and directly correlated with increases in
o-DPO activity. However, this correlation apparently held only for
about the first half of the storage period. Thereafter, there was a rise
in phenolic compounds at 10°C and 20°C. This rise in substrate could
not be the result of a loss of enzyme activity since o-DPO activity was
still increasing.

The effect of low temp (0°C) on o-DPO activity was apparently due
to a suppression of maturation. Yamaguchi et al. (14) have shown that
increases in o-DPO activity are associated with maturation of
mushrooms. However, postharvest treatments, such as gamma irradiation,
have been shown to have separate effects on these 2 systems (13).

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**Changes in Rooting Substances of Tomato Explants**

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**Abstract.** The rooting substances of tomato (*Lycopersicon esculentum* Mill.) explants during root regeneration were studied. The root-promoting substances of the basic, acidic, and bound ether-soluble fractions of the plant extracts after paper chromatography were determined by a tomato hypocotyl test. The basic root-promoting substances in the basal hypocotyl region changed qualitatively, while those of the acidic and bound fractions decreased with rooting. It was found that in 5-day-old explants, the highest root-promoting substances were in the bound fraction, and in the basal hypocotyl region. Histological evidence showed the presence of preformed root primordia in the rooting region of 10-day-old tomato explants.

White (15) demonstrated that excised tomato roots are capable of continued growth in aseptic culture if supplied with a mixture of inorganic salts, carbohydrates, and yeast extract. Britton et al (2) and Street (12) implicated phytohormones in the development of tomato roots. Reid (8, 9) indicated that the ability of tomato stem cuttings to regenerate roots and shoots depended upon, and varied with, the concentrations of carbohydrates and nitrogenous compounds of the cuttings. Recently, some of the root-promoting substances in tomato seedling root extracts have been shown to be auxins, and it was suggested that the auxins were concentrated in the young cotyledons (1). However, hypocotyl root regeneration of tomato explants occurs primarily at the basal 1-cm region. Therefore, it is of interest to know the extent of the alteration in the contents of these root-promoting substances in the cotyledons and root region during the course of root regeneration.

**Materials and Methods**

**Plant materials.** Seedlings of ‘Fireball’ tomato were germinated and grown in a medium of vermiculite and fine white quartz sand (1:1 v/v). The seeded flats were kept moist with tap-water and maintained at 26°C day and 20°C night temp in a growth-chamber. The seedlings received a photoperiod of 12 hr from a mixed light source of cool white fluorescent and incandescent lamps at 1,200 ft-c.

**Extraction, chromatography, and bioassay.** In experiment I, 10-day-old seedlings were defoliated by excising the primary root-system with a razor blade at the region of the transition zone. The explants were placed 3 per tube, in Pyrex culture tubes (20 x 150 mm) containing 0.5 ml of glass-distilled water and maintained in the growth-chamber. After 5 days incubation, 75 explants were divided into 150 cotyledons (250 mg), 75 midhypocotyls with apices (250 mg) and 75 defoliated 1-cm basal hypocotyl segments (200 mg). These tissues were frozen at −10°C for 24 hr, homogenized and extracted with absolute methanol at 5°C and partitioned into 3 fractions according to procedures already described (1). In experiment II, 200 explants each after 0, 2, and 4 days incubation were similarly obtained, separated into 3 sections, and extracted as mentioned above. The fresh weights were: cotyledons 2.5, 2.5, and 3.0 g; mid-hypocotyls 2.5, 2.5, and 3.0 g; and basal 1-cm hypocotyls 1.0, 1.2, and 1.5 g. The basal 1-cm hypocotyls of explants incubated for 4 days had formed visible roots, but these roots were removed before weighing and extraction.

The fractions obtained were purified by descending paper chromatography using a solvent mixture of isopropanol, ammonium hydroxide, and water (10:1:1 v/v/v), and the eluates of Rf zones from each chromatogram were bioassayed by the tomato hypocotyl test (1).

**Histology of the hypocotyl.** The basal 2.0 cm of hypocotyls of 10-day-old seedlings before rooting were cut into 0.5-cm segments and fixed for 8 hr with 10% acrolein buffered in 0.2-M sodium cacodylate at pH 7.2 (4). After dehydration in a standard ethanol and tertiary butyl alcohol series, the segments were infiltrated with Paraplast Plus, under 400 mm Hg reduced pressure, and embedded in Paraplast. Transverse and longitudinal sections 10-μ thick were made. Specimens were stained with either safranin and fast green (10), or 0.5% toluidine blue in 0.02-M benzoate buffer at pH 4.4 (11).

**Results**

Adventitious root production in 10-day-old seedlings was restricted mainly to the basal 1-cm region of the hypocotyl. Histological examination indicated 4–6 young primordia that varied in size, 3 being microscopic and located in the upper third of the region (Fig. 1-A). Although transition from root to stem was evident in the first basal 1-cm of the hypocotyl, the arrangement of primary vascular tissue resembled that of a root more than that of a stem. Depending on pattern of vascular transition in the seedling axis, root primordia were initiated acropetally at various positions in relation to the primary xylem and phloem.

Initiation and subsequent development were similar to those reported for various dicotyledon lateral roots. Like lateral roots the first anatomical evidence of root initiation in the hypocotyl was the appearance of anticlinal divisions in both pericycle and endodermis (Fig. 1-B). Histochemical tests indicated that an endodermis was present in the basal 1-cm portion of the hypocotyl (Byrne, unpublished). Repeated periclinal divisions of the pericycle and its derivatives gave rise to a 4-layered primordium bound externally by an uniseriate endodermis and internally by stelar parenchyma (Fig. 1-C). Further development of these 4 layers of pericyclic derivatives resulted in the formation of the young root primordium which consisted of an uniseriate layer of rootcap-epidermis initials, and uniseriate layer of cortex initials and an incipient vascular cylinder.