

Enzymatic Browning Control in Minimally Processed Mushrooms

ABSTRACT

Treatments to control discoloration of minimally processed mushrooms were investigated. Whole or sliced mushrooms were immersed in browning inhibitor solutions and evaluated for color change during storage. Browning was more intense in first break mushrooms than in second, and in unwashed mushrooms compared to washed. However, washing sometimes induced purple discolorations, associated with bacterial lesions. Other discolorations were induced by hypochlorite, 4-hexylresorcinol, and acidic dips. The most effective treatment was a combination of sodium erythorbate, cysteine, and EDTA at pH 5.5. Addition of preservatives to browning inhibitor dips did not improve storage life. However, dipping in 5% hydrogen peroxide prior to application of browning inhibitors significantly increased shelf-life.

Key Words: enzymatic browning, mushrooms, minimally processed, sodium erythorbate, hydrogen peroxide

INTRODUCTION

THE SHELF-LIFE of minimally processed fruits and vegetables may be limited by enzymatic browning during storage (Rolle and Chism, 1987; Sapers and Hicks, 1989a; McEvily et al., 1992). Fresh mushrooms are especially subject to this problem, developing brown, gray, black, purple, and yellow discolorations of external and cut surfaces. Such discolorations are often associated with abrasions and mechanical damage from handling, slicing, and washing but also may be related to senescence (Nichols, 1985) or to growth of spoilage bacteria such as *Pseudomonas tolaasii* (Gandy, 1985; Beelman et al., 1989). Browning in mushrooms can be retarded by storage at low temperature (Murr and Morris, 1975). Trimming of the mushroom stipe at harvest reduced browning during storage (Ajlouni et al., 1992).

Until the ban on use of sulfites on some raw fruits and vegetables (Anon., 1986), browning of mushrooms was controlled by dips in solutions of ≈ 1000 ppm of a sulfite salt (equivalent to 500 ppm SO_2) (Beelman et al., 1988). Various other chemicals such as ascorbic acid (AA) or erythorbic acid (EA), citric acid (CA), hydrogen peroxide, sodium hypochlorite, chlorine dioxide, ethylenediaminetetraacetic acid (EDTA), and calcium salts have been used as browning inhibitors in place of sulfites, but generally have not been as effective (McCord and Kilara, 1983; Pruthi et al., 1984; Kilara et al., 1984; Beelman, 1987; Markowitz and Bodine, 1989; Okereke and Beelman, 1990). However, McConnell (1991) reported that a wash containing EDTA and hydrogen peroxide was more effective than sulfite in preventing discoloration of mushrooms during storage at 12°C. Previously, we found that AA-2-phosphates, which had been used successfully to control enzymatic browning of apple and potato (Sapers et al., 1989b; Sapers and Miller, 1992), were not effective with mushrooms, probably because of high acid phosphatase activity (Sapers and Miller, 1988).

Because discoloration reactions of mushrooms are complex and depend on raw material condition; washing, cutting, handling, and packaging practices; and bacterial growth during stor-

age, efforts to control discoloration must take such factors into account. A hypothesis limited to the development of sulfite substitutes addresses only the enzymatic browning component of the problem and would not succeed by itself. Therefore, our first objective was to investigate causes of discolorations associated with minimal processing of mushrooms. A second objective was to develop effective treatments to control discoloration by appropriate browning inhibitors and/or antimicrobial agents.

MATERIALS & METHODS

Raw materials

Fresh 1st and 2nd break (flush) mushrooms were obtained in tray packs from a Kennett Square, Pennsylvania grower, chilled over ice, brought to the laboratory within several hours of harvest, and stored at 4°C until needed. In addition, 1st and 2nd break mushrooms that had been subjected to a proprietary washing procedure and corresponding unwashed mushrooms were obtained from a western Pennsylvania grower. These mushrooms were shipped to us, chilled in an insulated container containing refrigerant packs, by overnight air express.

Application of browning inhibitor treatments

Mushrooms showing no external defects were selected for treatment, and the stipes (stems) were trimmed with a sharp knife to within 1–2 cm of the pileus (cap). Between samples, the knife blade was immersed in 0.01% sodium hypochlorite solution (≈ 50 ppm Cl_2) to reduce bacterial contamination. If treatments were to be applied to cut surfaces of mushrooms (simulating slices), the washed mushrooms were cut in half along the stipe axis so treatment effects on both external and cut surfaces could be observed.

Treatments were applied as dips in aqueous solutions of various browning inhibitors and/or antimicrobials, adjusted to specific pH values with NaOH or HCl (Table 1). Samples comprising five whole mushrooms or four pairs of mushroom halves were placed between nested colanders and submerged in treatment solutions or water (controls) for 5–120 sec. Immediately after treatment, the mushrooms were drained in a colander, with the bottom of the colander blotted with paper toweling to prevent absorption of additional treatment solution. In some experiments, the weight of absorbed solution was measured. Treated whole mushrooms were packaged in plastic boxes, covered with perforated lids, or in plastic tray packs or glass crystallizing dishes (90 × 50 mm), covered with a double thickness of paper toweling, which was held in place with a rubber band. All samples were stored at 4°C.

Table 1—Components tested to control discolorations of minimally processed mushrooms

Browning inhibitors	Antimicrobials
Ascorbic acid and Na salt	Streptomycin sulfate
Erythorbic acid and Na salt	Hydrogen peroxide
Ascorbic acid-2-mono- and triphosphate	Sodium hypochlorite
Citric acid	Sodium benzoate
Cysteine	Potassium sorbate
N-acetyl cysteine	
4-Hexylresorcinol	Other variables
EDTA (disodium salt)	pH
Sodium acid pyrophosphate	Concentration
Sporix	Combinations
Sodium hexametaphosphate	Dipping time
Hydroxypropyl β -cyclodextrin	
Zinc chloride	

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Table 2—Effect of break and commercial washing treatment on browning of pileus surface^a

Sample	L* Day			a* Day			b* Day			Hue angle Day			% Reflectance at 420 nm Day		
	0	2	4	0	2	4	0	2	4	0	2	4	0	2	4
First break, not washed	86.6 ^d	84.3 ^e	83.6 ^e	2.3 ^b	2.9 ^c	3.5 ^b	16.5 ^b	18.6 ^b	20.4 ^b	82.2 ^c	81.2 ^c	80.5 ^c	43.7 ^c	38.2 ^a	35.9 ^d
Second break, not washed	87.9 ^c	86.9 ^d	85.7 ^d	2.2 ^b	2.3 ^b	2.7 ^c	14.0 ^d	15.8 ^c	17.1 ^c	81.0 ^d	81.8 ^{bc}	81.2 ^c	49.2 ^b	45.5 ^d	42.5 ^c
First break, washed	88.6 ^c	88.1 ^c	87.8 ^c	1.8 ^c	2.1 ^b	2.2 ^d	14.6 ^{cd}	15.3 ^c	16.4 ^{cd}	83.2 ^b	82.3 ^b	82.3 ^b	49.0 ^b	47.9 ^c	46.3 ^b
Second break, washed	89.8 ^b	89.6 ^b	89.0 ^b	2.1 ^b	2.1 ^b	2.1 ^d	15.0 ^c	15.4 ^c	15.9 ^d	82.2 ^c	82.4 ^b	82.4 ^b	50.7 ^b	50.1 ^b	48.7 ^b

^a Reflectance measured at top of pileus.

^{b-e} Means of 40 replicates; means within columns, followed by different superscripts, are significantly different at $p < 0.05$ by the Bonferroni LSD test.

Evaluation of treated mushrooms

Treated whole mushrooms and untreated controls were evaluated visually for severity of browning on external surfaces of the pileus and stipe, including the stipe bottom and cut edge, and presence of bacterial lesions on the pileus surface. Samples of mushroom halves were evaluated visually for presence of yellowing at cut edges and surfaces (associated with some treatments), graying of the pileus cut surface above the gills, browning of cut surfaces, especially at edges where the external surface was cut, and bacterial lesions on cut surfaces.

In addition, reflectance measurements were made with a Byk-Gardner spectrophotometer, using the C-illuminant and averaging mode, and standardizing against a white tile. Whole mushrooms were placed in an optical glass beaker, with the top of the pileus facing down, completely covering a 32 mm aperture above the sample port. In some experiments where small, localized discolorations of the pileus external surface were of interest, the affected area was placed directly over an 11 mm aperture above the sample port. Mushroom halves were placed directly over the 11 mm aperture above the sample port so that the pileus cut surface between the gills and tangential to the cut edge could be measured. Tristimulus coordinates in the CIELAB color scale and reflectance spectra were measured, and values of L*, the hue angle ($\tan^{-1} b^*/a^*$), and percent reflectance at 420 nm were used as indices of discoloration.

Scanning electron microscopy

Whole mushrooms were immersed in a solution of 1% glutaraldehyde, buffered with 0.1M sodium cacodylate (pH 7.2) and stored at 4°C. Areas of interest on the cap were excised with a stainless steel razor blade, washed in buffer solution and dehydrated in a graded series of ethanol solutions. They were critical point dried from liquid carbon dioxide, and coated with a thin layer of gold by D.C. sputtering for examination in a scanning electron microscope operating in the secondary electron imaging mode (Model 840 A, JEOL USA, Peabody, MA).

Statistical analyses

In the comparison of 1st vs 2nd break mushrooms, given a commercial wash or unwashed, 50 replicates in each group were measured individually with the spectrophotometer so that statistical analyses could be performed on all color parameters. Differences among samples were examined by the Bonferroni LSD mean separation test (Miller, 1981). In all other experiments, color measurements were made in the averaging mode, which generates values of the mean and standard deviation for the tristimulus coordinates of each set of replicates, but not for the percent reflectance at 420 nm. Consequently, statistical analyses were performed, as described above, on L*-values only. Mean values of percent reflectance at 420 nm were included in tables, without statistical analysis, because of the wide changes in this parameter in samples undergoing discoloration.

RESULTS & DISCUSSION

Effect of mushroom break and washing

The response of fresh mushrooms to treatment with browning inhibitors depends in part on their condition prior to storage. We have seen a consistent difference in the extent of browning between 1st and 2nd break mushrooms on the day that they were received at our laboratory. The 1st break mushrooms were more brown with seven out of nine lots obtained from three sources. This difference could be seen in reflectance data, measured at the pileus top surface, for sets of 50 representative mushrooms from the two breaks at 0-time (Table 2, unwashed samples). Lower values of L* and percent reflectance at 420 nm indicated

greater browning. However, the difference between breaks was not as evident in values of a*, b*, or the hue angle. During storage, the L* and percent reflectance values for both breaks declined, and the a*- and b*-values increased, as browning became more severe, but the change was somewhat greater in first break mushrooms. Effective browning inhibitor treatments must control discoloration in either first or second break mushrooms.

The difference in browning tendency between breaks may be related to composition differences or to differences in susceptibility to injury during harvesting due to environmental conditions or crop density. A study of tyrosinase activity in developing mushrooms revealed no difference between breaks (Ingebrigtsen et al., 1989). However, Burton (1988) reported greater tyrosinase activity in 1st break mushrooms than in mushrooms from breaks 2 and 3 after storage for 1–5 days at 18°C. He also reported that the phenol content was greater in 1st break mushrooms than in 2nd break.

Washing fresh mushrooms (prior to packing) to remove adhering particles of casing material would enhance appearance and suitability for use as ingredients in salads, pizza toppings, and other foods. Washing by a proprietary process consistently reduced the severity of browning over that in comparable unwashed mushrooms in six direct comparisons. The effect of washing on browning can be seen in the reflectance data for sets of 50 washed and unwashed fresh mushrooms (Table 2). The higher values of L* and percent reflectance at 420 nm of the washed mushrooms clearly showed that they were less discolored than the unwashed 1st break mushrooms initially. Differences between breaks with washed mushrooms were small. Especially note that the washed mushrooms showed little change in L* or percent reflectance at 420 nm during storage. This appears to be due to extraction of polyphenol oxidase (PPO) and/or its substrates from the mushroom surface during washing (Choi and Sapers, 1994b). Washing reduced the contents of total soluble phenols and γ -L-glutaminy-4-hydroxybenzene (GHB, a major PPO substrate in mushrooms) in mushroom skin tissue by 10–20% and also removed two of four PPO isozymes, extracted from mushroom skin and separated by native polyacrylamide gel electrophoresis.

Discolorations induced by treatments

During storage, both commercially washed mushrooms and those washed in the laboratory may develop dark-colored, sunken lesions of the pileus surface. Examination of lesions by scanning electron microscopy revealed the presence of large numbers of bacteria, subsequently shown to be *P. tolaasii* (Wells et al., 1994), in association with hyphae damaged by washing (Fig. 1). This condition occurs at the high internal humidity that results from water uptake during washing which may be as high as 8% of mushroom weight). It may be promoted by leakage of hyphae contents as a consequence of mechanical damage occurring during washing. One or two days before lesions appear, purple or gray blotches may develop on the pileus surface. Purpling of washed mushrooms also was reported by McConnell (1991). Purpling can be distinguished from typical enzymatic browning by spectrophotometry (Table 3). Mushrooms undergoing purpling and browning both showed similar decreases in L* and percent reflectance at 420 nm and increases in a* during

Table 3—Reflectance characteristics of mushrooms undergoing browning and purpling reactions during storage^a

Treatment	Mushroom	Discoloration	L*		a*		b*		Hue angle		% Reflectance at 420 nm	
			Day 0	Day 5	Day 0	Day 5	Day 0	Day 5	Day 0	Day 5	Day 0	Day 5
Not washed	1	Browning	90.8±6.8	76.6	2.7±1.2	6.9	16.9±1.7	28.5	80.8	76.4	50.1	21.6
	2	Browning	88.2±6.4	68.7	3.8±1.5	8.4	18.4±3.6	28.0	78.4	73.4	44.8	15.7
	3	Browning	87.7±2.8	77.1	3.8±0.9	6.7	20.0±2.0	27.5	79.1	76.3	41.5	23.2
Commercially washed	1	Purpling	93.8±1.2	81.4	2.0±0.9	6.1	15.1±1.0	19.3	82.4	72.6	58.3	34.9
	2	Purpling	93.1±2.5	68.1	1.7±0.8	7.3	15.3±1.3	23.0	83.5	72.4	54.9	18.6
	3	Purpling	94.2±2.0	85.8	1.5±0.8	3.7	14.9±1.5	18.5	84.1	78.8	58.2	41.5
	1	Browning	93.8±1.2	80.9	2.0±0.9	6.3	15.1±1.0	24.7	82.4	75.7	58.3	29.8
	2	Browning	93.1±2.5	68.0	1.7±0.8	9.4	15.3±1.3	26.7	83.5	70.5	54.9	16.4

^a Reflectance measurements made over 11 mm aperture. Day 0 values are means of measurements at four locations on pileus top surface; day 5 values are individual measurements at site of discoloration.

Table 4—Effective browning inhibitor combinations for mushrooms

Formulation	Sodium erythorbate (%)	Cysteine-HCl (%)	Na ₂ EDTA (ppm)	ZnCl ₂ (nM)	NaOCl (%)	pH
A	4.50	0.2	500	—	—	5.5
B	4.50	0.2	1000	—	—	5.5
C	2.25	—	500	—	—	5.5
D	4.50	—	1000	—	—	5.5
E	2.25	—	—	10	0.01	5.5
F	4.50	—	—	10	0.01	5.5

servations) while C showed least change in reflectance values. During storage, the individual mushrooms within a set differed in response to treatment, undergoing browning at different rates. This was reflected by increasing standard deviations for L* with longer storage times (data not shown). Mushrooms with the most effective treatment (C) showed the most uniform response (smallest standard deviation). By day 6, all samples developed bacterial lesions which interfered with reflectance measurements and ended storage life on day 7. With 2nd break mushrooms, treatment B showed less change than controls or treatment C, based on reflectance data and visual observations. The 2nd break mushrooms developed severe lesions by day 4 and ended storage life by day 6.

With mushroom halves (2nd break), the untreated control showed large decreases in L* and percent reflectance at 420 nm (and increases in standard deviation for L* indicating variability in browning), during storage at 4°C (Table 6). Mushrooms were highly variable in appearance with severe browning as early as day 3 of storage. Lesions developed on some control mushrooms by day 5. Reflectance data indicated that treatments were similar in effectiveness in controlling browning of the cut surface during storage. Visual observation indicated that treatments B and E were better, showing only slight or moderate browning on day 5. Treatment D showed severe browning of cut edges (outside the area measured by colorimetry), while treatment F developed severe yellow-green bacterial lesions (also outside the area measured). All samples had severe lesions and severe browning of cut edges by day 6.

Because treatment B provided the best protection to both external and cut mushroom surfaces, we carried out further trials with this combination to determine effects of varying cysteine concentration and pH. Increasing cysteine from 0.2% to 0.4% had no effect in controlling browning. Increasing the pH from 5.5 to 6.5 or 7.5 had no effect on reflectance data. However, visual observation indicated the treatment was more effective at pH 5.5 than at 6.5 or 7.5.

To determine whether treatment B had adverse effect on mushroom flavor or texture, treated and untreated whole or half mushrooms were tasted raw or after heating briefly in boiling water. No flavor or textural defects were observed. However, a slight sulfury aroma was detected in headspace above mushrooms treated with 0.4% cysteine. Consequently, we used the lower cysteine concentration in subsequent experiments.

We hypothesize that the effectiveness of treatment B was due to complementary antibrowning activity of the three individual components. Erythorbate acted both as an inhibitor of PPO and by reducing quinones to polyphenols. Cysteine forms colorless

products with quinones generated from phenolic substrates by PPO oxidation. EDTA may inhibit PPO and also stabilize erythorbate by Cu chelation (Vamos-Visyago, 1981; McEvily et al., 1992). Bacterial lesions in many treated samples demonstrated the need to combine antimicrobial treatments with browning inhibitors.

Combinations of browning inhibitors and antimicrobials

Lesion formation in washed mushrooms could be suppressed by application of streptomycin sulfate dips. Thus, we investigated the use of preservatives and other anti-microbials in combination with browning inhibitors. Concentrations of sodium benzoate or potassium sorbate as high as 0.5% in combination with cysteine, CA, or sodium ascorbate did not improve storage life over that with browning inhibitors alone (data not shown). The failure of these treatments to extend product storage was probably due to the relatively high pH of mushroom tissue, even when treated with acidic dips, which would be outside the optimum pH range for their effectiveness (Anon., 1987). Pretreating slices by dipping in 0.01% sodium hypochlorite for 20 sec prior to application of dips containing sodium ascorbate, alone or with 4-hexylresorcinol, or cysteine + citric acid + potassium sorbate did not improve product storage life. Higher concentrations of hypochlorite could not be used because of induced darkening. Stabilized chlorine dioxide has been reported to be more effective than hypochlorite in controlling bacterial growth in mushrooms (Beelman et al., 1989), and it has been used in combination with browning inhibitors (Guthrie and Beelman, 1989).

Hydrogen peroxide, an antimicrobial agent approved for use in foods, is effective against a wide range of bacteria, yeasts and fungi (Block, 1991). Pretreatment of whole mushrooms with hydrogen peroxide prior to browning inhibitors was highly effective in extending storage life (Table 7). A 30 sec dip in 5% hydrogen peroxide solution, followed by a 5 min hold to allow unreacted peroxide to be decomposed by endogenous catalase, before a 20 sec dip in combination B gave the best results, based on reflectance data. However, all products given H₂O₂ pretreatments and the browning inhibitor dip showed relatively little browning and no lesion development after 7 days at 4°C. Even after 10 days, lesion development was suppressed in those mushrooms. Those given only the browning inhibitor dip were moderately brown and had bacterial lesions after 4–8 days. Mushrooms given longer holding times after H₂O₂ treatment showed more browning, possibly due to oxidation of phenolic acids by endogenous peroxidase (Winter, 1969; Wahid, 1980; Dawley et al., 1993) or by the pseudoperoxidase activity of ox-

Table 5—Effect of browning inhibitor treatments on external surfaces of mushrooms stored at 4°C

Break	Treatment	L* ^a Day				% Reflectance at 420 nm ^c Day				Appearance ^b Day		
		0	3	4	6	0	3	4	6	3	4	6
1st	Control	88.2 ^c	87.6 ^c	85.0 ^d	83.5 ^c	48.2	44.5	39.4	35.9	SI/mod br	SI/mod br	Mod/sev br
	B	89.0 ^c	88.2 ^c	88.9 ^c	85.2 ^c	46.7	44.9	45.2	36.7	SI br	SI br	SI br +L
	C	88.8 ^c	90.5 ^c	90.2 ^c	89.1 ^c	47.9	50.0	48.7	46.0	SI br	SI br	SI br +L
	D	88.6 ^c	88.5 ^c	88.7 ^{cd}	86.6 ^c	47.2	45.6	45.1	41.3	SI br	SI/mod br	SI/mod br +L
2nd	Control	91.8 ^c	87.2 ^c	84.3 ^d	82.9 ^d	53.2	40.2	35.3	32.9	Mod/sev br	Mod/sev br	Mod/sev br +L
	B	91.3 ^c	92.0 ^c	90.7 ^c	90.6 ^c	49.3	50.8	47.8	47.6	SI/mod br	SI/mod br	SI/mod br
	C	90.7 ^c	89.3 ^c	86.5 ^{cd}	83.6 ^d	49.3	44.2	38.8	34.0	SI/sev br	SI/sev br	SI/sev br +L

^a Reflectance measured at top of pileus.

^b Slight (sl), moderate (mod), or severe (sev) browning (br); L = lesions. Range reflects variation on individual mushrooms.

^c Mean of five replicates; means within columns, followed by different superscripts, are significantly different at $p < 0.05$ by the Bonferroni LSD test.

Table 6—Effect of browning inhibitor treatments on cut surfaces of mushroom halves stored at 4°C^a

Treatment	L* ^a Day			% Reflectance at 420 nm Day			Appearance ^b Day	
	0	3	5	0	3	5	3	5
Control	96.4 ^c	91.1 ^d	86.3 ^d	68.7	52.6	42.0	SI/sev br	SI/sev br +L
A	93.9 ^{cd}	95.9 ^c	93.0 ^c	60.8	67.2	54.8	SI/sev br	SI/sev br
B	92.4 ^{ef}	96.3 ^c	94.7 ^c	57.0	66.9	57.0	SI br	SI/mod br
D	91.2 ^f	94.7 ^{cd}	93.4 ^c	51.8	59.1	54.6	SI/sev br +L	SI/sev br +L
E	95.2 ^{cd}	95.1 ^{cd}	91.8 ^{cd}	64.4	65.3	55.2	SI br	SI/mod br +L
F	93.4 ^{def}	97.4 ^c	95.2 ^c	58.6	71.8	63.0	SI/mod br	SI br + sev L

^a 2nd break; reflectance measured at cut surface of pileus.

^b Slight (sl), moderate (mod), or severe (sev) browning (br); L = lesions. Range reflects variation on individual mushrooms.

^{c-f} Mean of eight replicates; means within columns, followed by different superscripts, are significantly different at $p < 0.05$ by the Bonferroni LSD test.

Table 7—Effect of H₂O₂ dip, followed by browning inhibitor treatment, on external surfaces of mushrooms stored at 4°C^a

H ₂ O ₂ dip ^b (sec)	Equil. time (min)	Browning inhibitor dip ^c (sec)	L* ^a Day			% Reflectance at 420 nm Day			Appearance Day	
			0	4	7	0	4	7	4	7
0	0	0	93.6 ^e	90.0 ^{ef}	88.2 ^{ef}	58.3	47.0	42.5	SI/sev br	Mod/sev br
0	0	20	93.1 ^e	88.3 ^{fg}	84.2 ^g	55.0	41.9	33.8	SI/mod br +L	Mod/sev br +L
0	0	40	92.0 ^{ef}	86.4 ^g	84.9 ^{fg}	53.9	38.2	33.7	SI/mod br	Mod/sev br
30	5	20	92.1 ^{ef}	91.1 ^e	89.1 ^e	53.8	48.7	43.1	SI br	SI br
30	5	40	90.9 ^f	89.7 ^{ef}	86.5 ^{efg}	49.5	44.4	37.2	SI br	SI/mod br
30	15	20	91.4 ^{ef}	89.4 ^{ef}	86.9 ^{efg}	52.1	44.9	37.8	SI br	SI/mod br
30	15	40	92.0 ^{ef}	90.7 ^{ef}	87.2 ^{efg}	53.2	47.6	39.1	SI/mod br	SI/mod br
30	30	20	91.7 ^{ef}	89.1 ^{ef}	86.8 ^{efg}	50.5	43.5	37.9	SI br	SI/mod br
30	30	40	90.4 ^f	89.4 ^{ef}	87.1 ^{efg}	48.8	44.7	38.2	SI br	SI br

^a 2nd break; reflectance measured at top of pileus.

^b 5% H₂O₂.

^c Formulation B (0.4% cysteine, pH 5.5).

^d Slight (sl), moderate (mod) or severe (sev) browning (br); L = lesions. Range reflects variation on individual mushrooms.

^{e-g} Mean of five replicates; means within columns, followed by different superscripts, are significantly different at $p < 0.05$ by the Bonferroni LSD test.

tyrosinase, produced by reaction between tyrosinase and H₂O₂ (Jolley et al., 1974). Application of H₂O₂ pretreatment to mushrooms had no effect on flavor or texture. Similar results were obtained with 3% H₂O₂ dips (data not shown).

Pruthi et al. (1984) described the treatment of water-blanching mushrooms with 10% H₂O₂ solution for 30 min, followed by storage in a solution containing 0.25% CA and 500 ppm SO₂. They reported that the mushrooms remained "extra white" for 10–15 days at room temperature. McConnell (1991) reported that the combination of 1000 ppm EDTA and 1% H₂O₂ improved mushroom shelf-life and minimized incidence of purpling during storage at 12°C.

Our results clearly showed that a two-stage treatment entailing immersion in hydrogen peroxide solution followed by application of an effective browning inhibitor solution could inhibit enzymatic browning and bacterial spoilage. If applied with agitation to loosen and remove soil from mushroom surfaces, they would constitute an effective wash as well. Hence, such two-stage treatment may solve the problem of washing mushrooms without inducing discoloration and spoilage.

Regulatory status of treatments

According to the Code of Federal Regulations (21CFR), the components of our browning inhibitor combinations are classified as GRAS or as approved food additives for various appli-

cations. Cysteine and cysteine hydrochloride are GRAS for use as dough strengtheners (184.1271; 184.1272). Disodium EDTA and calcium disodium EDTA are permitted in numerous foods as high as 500 ppm; calcium disodium EDTA is permitted as an additive in canned mushrooms as high as 200 ppm (172.120; 172.135). H₂O₂ is GRAS for numerous specified uses in amounts sufficient for the intended purpose (184.1366).

CONCLUSIONS

MINIMALLY PROCESSED MUSHROOMS are subject to discoloration reactions and bacterial spoilage, associated with raw material condition, washing, and application of certain antimicrobial and browning inhibitor treatments. Spoilage could be delayed by dipping mushrooms in dilute hydrogen peroxide solution. Enzymatic browning of external and cut surfaces could be controlled by application of a browning inhibitor solution containing sodium erythorbate, cysteine, and EDTA. The use of these treatments in sequence, as a wash for minimally processed mushrooms, constitutes an effective method of quality improvement and shelf-life extension.

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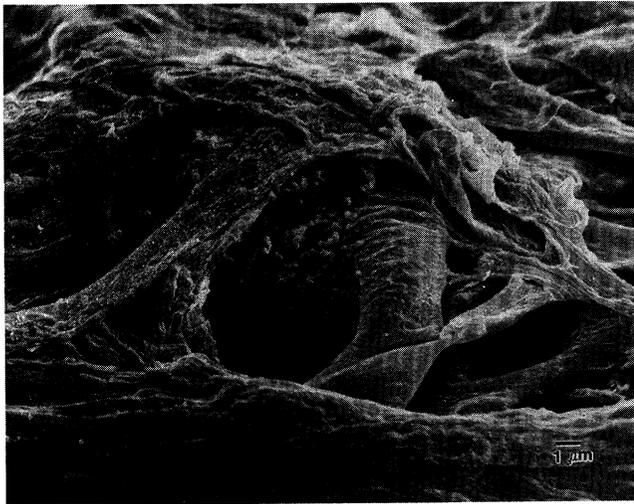


Fig. 1—Scanning electron micrograph of lesion on cap surface of washed mushroom showing flattened hyphae and attached bacterial cells in interstices.

storage. However, the purple mushrooms showed a smaller increase in b^* at comparable values of the other parameters. The purple pigment may be produced by a reaction between intermediates in the enzymatic browning of L-DOPA and sinapic acid (Choi and Sapers, 1994a). This discoloration was not prevented by dipping mushrooms 1 min in 2% AA, 500 ppm SO_2 (as sodium bisulfite), 500 ppm EDTA (calcium, disodium salt), or 1% hydrogen peroxide. Dipping in 0.5% Sporix (an acidic polyphosphate); 0.5% sodium hexametaphosphate, acidified to pH 2 with phosphoric acid; or 500 ppm streptomycin sulfate greatly reduced the incidence of lesion formation. However, purpling sometimes occurred in mushrooms treated with Sporix or the antibiotic. While these results were consistent with the bacterial origin of blotches and lesions, they did not explain the inability of browning inhibitors or antibiotics to completely suppress purpling. It may be that widespread membrane failure and occurrence of hydrolytic reactions within developing lesions provide a localized source of sinapic acid and permitted L-DOPA to undergo oxidation and further reaction, even in the presence of browning inhibitors such as sulfite. In view of the advantages of washing, modification of washing treatments to eliminate purpling and lesion formation became an important goal of our study.

Sodium hypochlorite solution is sometimes applied to mushrooms preharvest during watering to control *P. tolaasii* infection (Fletcher et al., 1986) or postharvest during washing (Beelman, 1987). In our study, application of 100 or 200 ppm NaOCl to fresh mushrooms had no effect on initial mushroom color. However, treated mushrooms showed darkening of the pileus surface within several days, as indicated by visual observation and decreasing values of L^* and reflectance at 420 nm (data not shown). Darkening apparently resulted from nonenzymatic oxidation of L-DOPA and related compounds in mushroom by hypochlorite, resulting in quinone formation and subsequent browning (Choi and Sapers, 1994b). Such darkening may limit the concentration of hypochlorite that should be applied to mushrooms.

4-Hexylresorcinol, a browning inhibitor recommended for controlling black spot in shrimp (McEvily et al., 1991), also induced darkening of mushrooms when applied as a dip at ≥ 50 ppm. At 100 ppm, darkening was evident almost immediately following treatment. At 500 ppm, 4-hexylresorcinol treatment caused yellowing of external surfaces and tissue breakdown as well as darkening.

Mushrooms treated with dips containing AA or ascorbate (i.e., 4% AA + 1% CA or 4.5% sodium ascorbate + 0.4% cysteine HCl) sometimes developed localized yellow discolorations at

edges where the external surface was cut, and occasionally on cut or external surfaces. Yellowing usually occurred after several days storage at 4°C and became more severe as mushroom condition deteriorated. Yellowing could be minimized by using less acidic dips or by reducing the amount of ascorbate in dips; however, the latter approach was limited by the need to control browning. Addition of 500 ppm streptomycin sulfate to a dip containing 4% AA + 1% CA had no effect on yellowing, indicating that bacterial growth was not involved. We hypothesize that yellowing may be related to enzymatic browning. It may represent an atypical polyphenol oxidation product, favored by conditions found in treated mushrooms. It also could be a product derived from dehydroascorbic acid produced by reaction of AA with quinones arising from polyphenol oxidation.

Sliced mushrooms frequently developed gray or black discolorations on cut surfaces above gill tissue during storage. Graying appeared to originate in the gill tissue, which contains high levels of PPO substrates such as GHB (Choi and Sapers, 1994b). When gill tissue was excised prior to storage, graying did not occur. The severity of graying increased with aging and probably reflected mushroom senescence. That would be expected to increase leakage of GHB through cell membranes and subsequent diffusion into pileus tissue where oxidation by PPO could occur (Moore and Flurkey, 1989). Graying during storage could not be diminished by treatment of cut surfaces with AA, CA, sodium acid pyrophosphate, EDTA or streptomycin sulfate. However, graying could be minimized by selecting the freshest mushrooms for minimal processing.

Effective browning inhibitor combinations for mushrooms

The application of browning inhibitors by dipping results in water absorption, which may favor the development of bacterial lesions during storage (Beelman et al., 1989). Thus, relatively brief dips were used to reduce water uptake. A dipping time of 20 sec, used for most treatments, resulted in a weight gain by whole mushrooms of about 2%; dipping for 1–2 min increased water uptake to 2.5–3%. However, if mushrooms were agitated in the dipping solution, the weight gain was as great as 8%. Because of their greater surface area, slices immersed in water for 20 sec increased in weight by 15–20%. Small variations in dipping time had relatively little effect on water absorption. Most of the weight gain occurred during the first 5–10 sec of immersion.

Various combinations of AA, EA, sodium ascorbate or erythorbate, ascorbic acid-2-mono or triphosphate, CA, cysteine, N-acetyl cysteine, 4-hexylresorcinol, EDTA, sodium acid pyrophosphate, hydroxypropyl- β -cyclodextrin, and ZnCl_2 were tested as browning inhibitors. These compounds were reported to be effective in controlling discoloration of fruits and vegetables (Sapers, 1993). They were screened by visual evaluation of treated mushrooms for extent of browning of surfaces and edges, graying above the gills, yellowing, and bacterial lesion formation. Combinations that inhibit browning by different mechanisms were considered more promising than individual compounds because of the possibility of synergistic interactions.

The most effective treatments were combinations of sodium erythorbate (or ascorbate), cysteine, and EDTA or sodium erythorbate, ZnCl_2 , and hypochlorite (Table 4). These were compared as treatments for external surfaces of whole mushrooms (Table 5) and cut surfaces of mushroom halves (Table 6). Untreated (control) whole mushrooms (1st break) showed decreases in L^* and percent reflectance at 420 nm, at the pileus top surface, and moderate to severe browning of pileus external surfaces and stipe bottom edge by day 6 of storage at 4°C (Table 5). Reflectance data paralleled visual observations except for darkening of the pileus sides, seen with some samples which was outside the field of reflectance measurements. Mushrooms given the three best treatments (B, C and D) were better in appearance than controls after 3, 4, and 6 days storage. Treatments B and C gave better results than D (based on visual ob-

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