Effects of Washing on Polyphenols and Polyphenol Oxidase in Commercial Mushrooms (*Agaricus bisporus*)

Sang W. Choi and Gerald M. Sapers*

Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 600 East Mermaid Lane, Philadelphia, Pennsylvania 19118

To explain differences in browning of skin tissue between unwashed and washed mushrooms, changes in soluble phenols, major substrates of polyphenol oxidase (PPO), and PPO isozymes during washing, as well as effects of sodium hypochlorite (NaOCl) on phenolic compounds in mushrooms were investigated. About 15% of the soluble phenols from the skin of mushrooms, mostly γ-L-glutaminyl-4-hydroxybenzene (GHB) and γ-L-glutaminyl-3,4-dihydroxybenzene (GDHB), were leached out during washing. Among four isozymes of partially purified PPO separated by native electrophoresis, the two faster migrating forms were leached out during washing. Phenolic compounds extracted from skin tissue were readily oxidized and degraded by 0.01% sodium hypochlorite. In a model system, NaOCl oxidized L-DOPA to a quinone, which turned black-brown, and degraded GHB and GDHB to unknown compounds. Darkening of washed mushrooms may be due to the reaction of a quinone derived from oxidation of L-DOPA or its derivatives by NaOCl.

Keywords: *Mushroom; washing; browning; polyphenols; polyphenol oxidase; hypochlorite*

INTRODUCTION

Recently, some mushroom producers in the United States have shown renewed interest in washing fresh whole or sliced mushrooms for the retail market. Washing is carried out to improve appearance and consumer appeal by removing casing residues from the mushroom surface. Washed mushrooms also show less enzymatic browning of the pileus surface, due to mechanical damage during harvesting and handling, than dry-packed mushrooms during the first few days of storage (Sapers et al., 1994). However, washed mushrooms generally deteriorate more rapidly than unwashed mushrooms (dry-packed) because of mechanical damage to the surface hyphae and growth of spoilage bacteria favored by water uptake during washing operations (Beelman et al., 1989; Dave, 1978; Fordyce, 1968; Sapers et al., 1994). In particular, the common spoilage bacterium *Pseudomonas tolaasii* may cause bacterial (brown) blotch, characterized by brown spots and slightly sunken lesions that develop on the pilei during storage (Dorren, 1985; Fermor, 1987). The use of chemical wash formulations, including such chemicals as calcium chloride, sodium hypochlorite, hydrogen peroxide, and other disinfectants and antibiotics, in watering mushrooms has to some extent been successful in controlling bacterial deterioration (Barden et al., 1991; Fletcher, 1978; Mau et al., 1993; Royse and Wuest, 1980; Wong and Preece, 1985a,b). While a number of factors affecting the quality of fresh mushrooms have been investigated (Beelman et al., 1989; Burton et al., 1987; Guthrie and Beelman, 1989; Hahn and Yoo, 1965; Molsberry, 1979), there are no reports of changes in mushroom composition during washing.

In this study, we investigated differences in total soluble phenol levels, levels of major polyphenol oxidase (PPO) substrates, and PPO isozyme patterns in skin tissue of commercial dry-packed and washed mushrooms (*Agaricus bisporus*). We also studied the effects of sodium hypochlorite on phenolic compounds in the skin tissue of mushrooms to develop a better understanding of atypical browning reactions at the surface of washed mushrooms.

* Author to whom correspondence should be addressed.

MATERIALS AND METHODS

Materials. Commercially dry-packed and washed mushrooms (*A. bisporus*, button stage of maturity) were supplied by a Pennsylvania mushroom grower. The mushrooms were packed with frozen gel packs in insulated containers and shipped to our laboratory within 24 h by air express. γ-L-Glutaminyl-4-hydroxybenzene (GHB) and γ-L-glutaminyl-3,4-dihydroxybenzene (GDHB), used as analytical standards, were extracted from mushroom gill tissue, following the procedure of Rast et al. (1979), and identified by UV and ^{1}H NMR spectroscopy (^{1}H NMR spectra were obtained with a JEOL GX-400 instrument with D_2O). L-DOPA and tyrosine were obtained from Sigma Chemical Co. All other chemicals were of reagent grade.

Soluble Phenols. Fresh dry-packed and washed mushrooms were freeze-dried, divided into specific tissues, dissected with a spatula, and stored in a desiccator. Soluble phenols were extracted by shaking in 80% ethanol on a steam bath at 75 °C for 1 h. After filtering, the soluble phenol levels were determined by the Folin–Ciocalteu method, as described by Singleton and Rossi (1965). Gallic acid was used as the standard.

Isolation and Quantitation of GHB, Tyrosine, GDHB, and L-DOPA from Mushrooms. Apart from minor modifications, the isolation and quantitation of major substrates of PPO in mushroom were according to the procedures of Rast et al. (1979) and Speroni and Beelman (1982), respectively.

One gram of a fine mushroom powder was blended with 50 mL of 0.1 N HCl, previously flushed with N_2 gas for 1 min. The material was rinsed from the blender with about 50 mL of 0.1 N HCl, stirred in a cold room for 24 h to facilitate extraction, and then filtered through Whatman No. 2 paper. The filtrate was evaporated to dryness below 30 °C under reduced pressure. The residue was dissolved in 20 mL of distilled water and applied to a Dowex 50W (cross-linkage, 8%; 100–200 mesh, Sigma) column (2.2 × 24 cm). The column was washed with 500 mL of water, and the adsorbed compounds were eluted with 500 mL of 2.0 N HCl and taken to dryness *in vacuo*. The residue was dissolved, diluted with

mobile phase (0.005 N NaH_2PO_4, pH 3.5), and analyzed by HPLC, as described below.

HPLC Analyses. Quantitation was done by HPLC with a Waters Chromatography Division (Millipore Corp., Milford, MA) system using a Partisil 10-SCX (5 μm packing, 4.6 mm i.d. × 250 mm) column (Whatman Inc., Clifton, NJ), a Waters 490 programmable multiwavelength detector set at 243 (GHB and GDHB) and 280 nm (tyrosine and L-DOPA), and an integrator (Hewlett-Packard Model 3390A). Elution conditions were as follows: mobile phase, 0.005 N NaH_2PO_4 (pH 3.5); flow rate, 0.6 mL/min. Under these conditions, GHB plus tyrosine and GDHB plus L-DOPA could not be resolved and were eluted as single peaks with retention times corresponding to those of authentic standards. These components could be quantified by HPLC after acid hydrolysis which eliminated GHB and GDHB from their respective peaks. Acid hydrolyses were performed according to the procedure of Brown et al. (1988). One milliliter aliquots of sample solution described above were hydrolyzed in sealed, evacuated tubes at 110 °C for 3 h with 3 mL of 5.7 M HCl.

The effect of NaOCl on phenolic compounds in mushroom was investigated by HPLC on a reversed-phase Supercosil LC-18 column (4.6 mm i.d. × 250 mm), using MeOH–H_2O (10:90) as mobile phase with the UV detector at 243 nm.

Partial Purification of Mushroom PPO. Mushroom PPO was prepared by a slight modification of the procedure reported by Ingebrigtsen et al. (1989). Fresh dry-packed and washed mushrooms were freeze-dried. Skin tissue was removed in the dry state, ground to a fine powder in liquid nitrogen, and then blended with 20 volumes (w/v) of 50 mM sodium phosphate buffer (pH 6.5) containing 1 mM ascorbic acid and 0.01% dithiothreitol, previously flushed with N_2 gas for 1 min. The homogenate was centrifuged at 12000*g* for 20 min at 4 °C. The supernatant was placed in a dialysis bag (Spectra/Por, MWCO 12 000-14 000, Spectrum Medical Industries, Inc., Houston, TX), concentrated to 2.0 mL with polyethylene glycol (PEG) 6000, and then recentrifuged at the same speed to obtain a partially purified PPO. This enzyme solution was subjected to electrophoresis.

Polyacrylamide Gel Electrophoresis. Native polyacrylamide gel electrophoresis (PAGE) was carried out in Mini-Protean II ready gels (7 × 10 cm slab gels of 1.0 mm thickness, 7.5% single percentage gel, 0.375 M Tris-HCl, pH 8.8) (Bio-Rad Laboratories, Richmond, CA) according to the "Bio-Rad Lab Instruction Manual" (Bio-Rad, 1989). Samples were made approximately 10% in glycerol before application. The electrophoretic run was at 4 °C and 100 V constant voltage. After electrophoresis, enzyme activity bands within gels were located by incubation in 50 mM sodium phosphate buffer (pH 6.5) containing 1 mM L-DOPA.

Chemical Oxidation Test. Chemical oxidation of phenolic compounds in mushrooms and of L-DOPA, GHB, and GDHB by 0.01% NaOCl (equivalent to 50 ppm chlorine) was followed by HPLC and spectrophotometry. Reaction conditions are specified in the legends to the figures. The spectrophotometric measurements were carried out at 25 °C in a Hewlett-Packard 8452A diode array spectrophotometer equipped with a thermostated cuvette holder and a recorder.

RESULTS AND DISCUSSION

Soluble Phenol Contents of Dry-Packed and Washed Mushrooms. The soluble phenol contents of dry-packed and washed mushrooms were compared (Table 1). The content of soluble phenols of washed whole mushrooms was lower than that of dry-packed mushrooms by about 10%. Most of the reduction in washed mushroom is due to the loss from skin and stipe tissues, indicating that some soluble phenols were leached out during washing. Thus, leaching of soluble phenolic compounds from skin and stipe tissues during washing may explain the lower tendency of washed mushrooms to undergo enzymatic browning during the storage prior to the onset of bacterial spoilage (Sapers et al., 1994).

Table 1. Levels of Soluble Phenols in Different Tissues of Dry-Packed and Washed Mushrooms

	soluble phenols[a]	
part	dry-packed mushroom[b]	washed mushroom[b]
whole	5.4 (0.85)[c]	4.9 (1.1)
cap	3.4 (0.6)	3.3 (0.7)
stipe	3.8 (0.4)	3.2 (0.8)
skin	7.2 (1.1)	6.1 (1.3)
gill	8.7 (1.3)	8.7 (1.7)

[a] mg/g of tissue, dry weight. [b] First break mushroom. [c] Standard error.

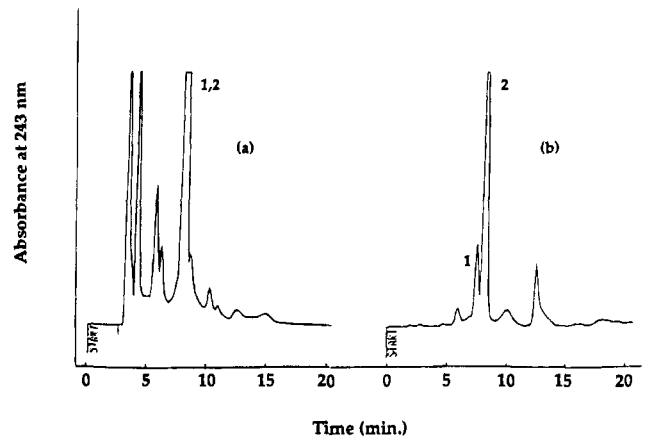

Figure 1. HPLC chromatograms of crude 0.1 N HCl extract (a) and 0.1 N HCl extract, passed through a Dowex 50W column (b) from mushrooms. Peaks: (1) GDHB and L-DOPA; (2) GHB and tyrosine. Chromatographic conditions: Partisil 10-SCX column (4.6 mm i.d. × 250 mm); mobile phase, 0.005 N NaH_2PO_4 (pH 3.5); 0.6 mL/min; UV 243 (GHB, GDHB) and 280 nm (tyrosine, L-DOPA).

Effect of Washing on Major PPO Substrates. In mushroom, several major phenolic amino acids (tyrosine, GHB, L-DOPA, and GDHB), responsible for enzymatic browning reactions, were isolated and characterized. Among them, GHB was found to be distributed in every part of the fruiting bodies at higher concentrations than other phenolic amino acids (Oka et al., 1981; Paranjpe et al., 1978; Rast et al., 1979).

Typical chromatograms of crude 0.1 N HCl extracts of mushrooms (injected directly into the liquid chromatograph) are shown in Figure 1a. As can be seen, GHB plus tyrosine and GDHB plus L-DOPA could not be resolved. However, when 0.1 N HCl extracts of mushroom were passed through a Dowex 50W column to remove interfering substances and then analyzed by HPLC, we could separately isolate GHB (tyrosine) and GDHB (L-DOPA), as shown in Figure 1b. Identification of GHB, tyrosine, L-DOPA, and GDHB was based on comparison of retention and spectral characteristics of the corresponding peaks with those of standards. It was impossible to isolate and quantify them directly because of the overlapping of GHB and tyrosine peaks and of GDHB and L-DOPA peaks. However, we could quantify them using the acid hydrolysis method. The two peaks consisted mostly of GHB and GDHB, respectively (data not shown).

The contents of GHB plus tyrosine and GDHB plus L-DOPA in fresh dry-packed and washed mushrooms (Table 2) varied considerably, probably due to varying environmental conditions during production. Generally, the contents of GHB plus tyrosine and GDHB plus L-DOPA in dry-packed mushroom were highest in gill > skin > stipe > cap, in that order. The contents of GHB plus tyrosine and GDHB plus L-DOPA in skin and

Table 2. Distribution of GHB, Tyrosine, GDHB, and L-DOPA in the Fruiting Bodies of Mushroom

	dry-packed mushroom[a]		washed mushroom	
part	GHB + tyrosine	GDHB + L-DOPA	GHB + tyrosine	GDHB + L-DOPA
whole	3.79 (0.93)[b]	0.22 (0.13)	3.40 (0.80)	0.19 (0.10)
cap	1.31 (0.6)	0.04 (0.01)	1.29 (0.3)	0.03 (0.01)
stipe	2.34 (1.2)	0.07 (0.02)	1.81 (0.7)	0.04 (0.02)
skin	5.18 (0.8)	0.14 (0.1)	4.22 (1.1)	0.07 (0.06)
gill	6.32 (1.1)	0.61 (0.4)	6.29 (1.1)	0.60 (0.3)

[a] mg/g of tissue, dry weight. [b] Standard error.

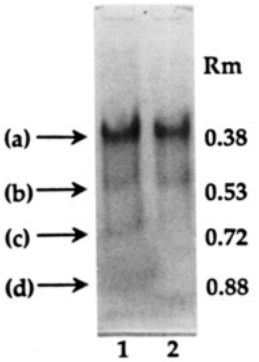

Figure 2. Electrophoretic isoenzyme pattern of partially purified PPO from skin tissue of dry-packed (lane 1) and washed (lane 2) mushrooms. After electrophoresis, enzymatic activity was located by staining with 1 mM L-DOPA.

stipe tissue of washed mushrooms were reduced by as much as 20% and 50%, respectively, compared to those of dry-packed mushrooms. This reduction is due to leaching of water-soluble phenols from skin tissue and/or oxidation and degradation of these compounds by NaOCl during washing operations, as shown in a subsequent section.

Electrophoretic Patterns of Skin Tissues in Dry-Packed and Washed Mushrooms. Mushroom tyrosinase has been examined extensively with regard to its physical and enzymatic characteristics during aging, postharvest treatments, and storage. However, no information is available on changes in PPO isozyme patterns during washing operations.

PPO isozyme patterns were examined (Figure 2) to determine whether differences in the browning tendency of skin tissue of dry-packed and washed mushrooms might be due to leaching of this enzyme during washing. Partially purified PPO from skin tissue of dry-packed and washed mushroom was subjected to native electrophoresis to examine the isozyme profiles in each tissue, using L-DOPA as the substrate to visualize the bands.

As shown in Figure 2, dry skin tissue has four isozyme bands (a–d), while washed skin tissue has only two isozyme bands with Rm 0.38 (a) and 0.53 (b). This difference in isozyme patterns suggests that two isozymes with faster migrating forms [Rm 0.72 (c) and 0.88 (d)] in mushroom skin were leached out during washing. A recent study by Dawley and Flurkey (1993) suggests that the two faster moving bands may have been laccase isozymes. A further study of substrate specificity is needed to clarify the identity of these isozymes. Thus, from the results of these analyses, we can explain the lesser tendency of washed mushroom skin to brown, compared to unwashed mushroom skin, in terms of leaching of PPO and its substrates from skin tissue during washing. This is an advantage of washing if lesion formation, caused by bacterial growth and mechanical damage during washing operations, can be overcome.

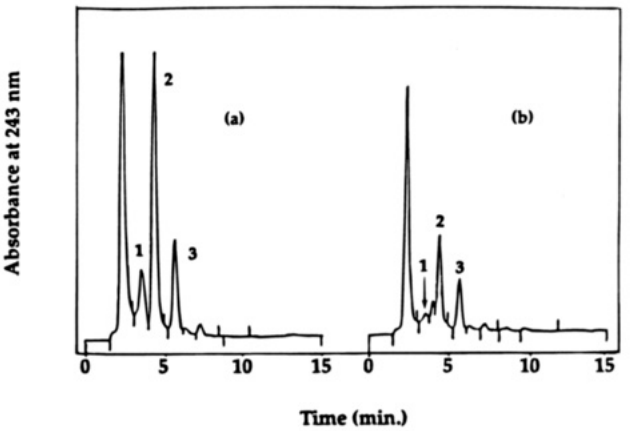

Figure 3. HPLC chromatograms of methanol extract of skin tissue of dry-packed mushroom treated with NaOCl: (a) before NaOCl treatment; (b) after NaOCl treatment. One milliliter of 0.01% NaOCl was added to 1 mL of methanol extract (1 g of mushroom skin powder/100 mL in MeOH) of mushroom. Reaction was carried out for 1 min at 25 °C, and then remaining phenolic compounds were determined by HPLC analysis. Chromatographic conditions: RP Supercosil LC-18 column (4.6 mm i.d. × 250 mm); MeOH–H_2O (10:90); 243 nm; 0.9 mL/min. Peaks: (1 and 2) polar phenolic compounds; (3) GHB and tyrosine.

Effect of Sodium Hypochlorite on the Phenolic Compounds of Mushroom. Sodium hypochlorite is used widely as a sanitizer in food processing and is also applied to mushrooms during watering and washing to control bacterial blotch and lesion formation during postharvest storage (Park et al., 1991). However, we have observed darkening at the surface of mushrooms washed with 0.01% sodium hypochlorite (Sapers et al., 1994). Therefore, an *in vitro* oxidation system was used to explain the relationship between NaOCl treatment and darkening at surfaces of washed mushrooms. The disappearance of phenolic compounds and formation of their oxidation product were monitored by HPLC and UV–visible spectroscopy. Figure 3 shows typical chromatographic profiles obtained with mixtures of phenolic compounds from mushrooms (methanol extracts of skin tissue), treated with NaOCl at 25 °C. HPLC profiles show that there was a degradation of GHB and tyrosine and also considerable depletion of more polar phenolic compounds (components 1 and 2, eluted before GHB in the reversed-phase HPLC system), suggesting that polar compounds were more susceptible to oxidation and degradation by NaOCl (Figure 3). In addition, the UV–visible spectrum of the reaction mixture showed a new absorption band at about 450 nm, indicative of browning, and the color slowly turned brown after NaOCl treatment (Figure 4). This phenomenon may be related to the surface browning induced by hypochlorite and reduction of GHB and GDHB contents in the skin tissue of mushrooms during washing operations.

Hypochlorite has been one of the most widely used and accepted chemicals for controlling brown blotch disease. However, use of sodium hypochlorite at a 50 ppm of Cl_2 level or more causes darkening of mushroom skins, which may be as unacceptable as the browning and lesions caused by the disease (Sapers et al., 1994). The applicability of this chemical is therefore limited.

Chemical Oxidation of L-DOPA, GHB, and GDHB by NaOCl. To elucidate in more detail the effects of NaOCl on the phenolic compounds of mushrooms, we investigated the chemical oxidation of major substrates

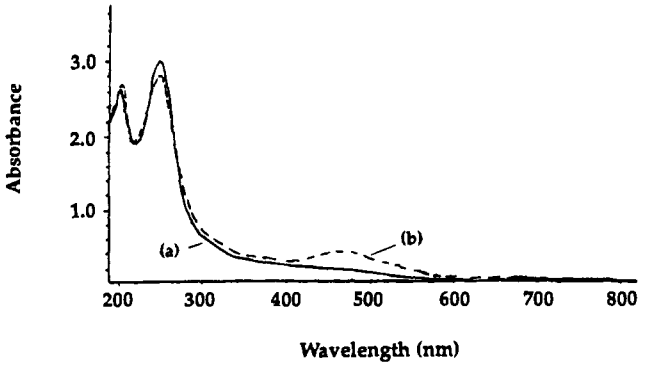

Figure 4. UV–visible absorption spectrum of methanol extracts of mushroom treated with NaOCl: (a) before NaOCl treatment; (b) after NaOCl treatment. NaOCl treatment condition is the same as in Figure 3.

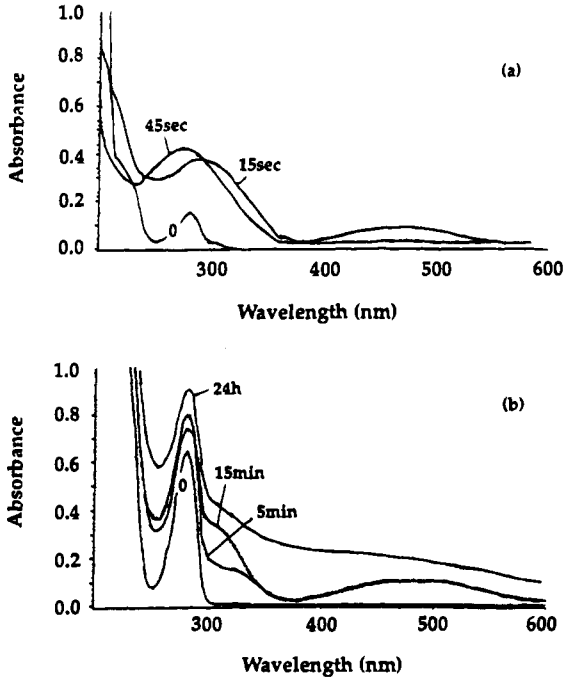

Figure 5. Spectral changes occurring during the chemical oxidation of L-DOPA by NaOCl at 25 °C. The reaction mixture included 2 mL of 0.1 mM L-DOPA (a) or 1 mM L-DOPA (b) and 2 mL of 0.01% NaOCl. The spectrum was scanned at the indicated times against a blank containing L-DOPA and distilled water.

of tyrosinase in mushrooms such as L-DOPA, GHB, and GDBH with hypochlorite.

Spectral changes occurring during reactions of L-DOPA, GHB, and GDHB with NaOCl are presented in Figures 5–7. As shown in Figure 5a, when 0.1 mM L-DOPA was incubated with NaOCl, a light brown product, characterized by peaks at 290 and 460 nm, immediately formed. After 45 s, its absorption maximum at 290 nm shifted to 270 nm and, simultaneously, the absorption maximum at 460 nm disappeared. However, when the same oxidation was carried out with 1 mM L-DOPA (Figure 5b), a spectrum with λ_{max} at 280 and 490 nm was immediately produced and remained nearly unchanged with increasing time except for the appearance of a small shoulder at 310 nm. After 24 h at 25 °C, the reaction mixture turned black-brown with a single absorption maximum at 280 nm.

In contrast, when 0.2 mM GHB was reacted with NaOCl (Figure 6), the original absorption maximum of GHB at 243 nm increased slightly within 1 min of

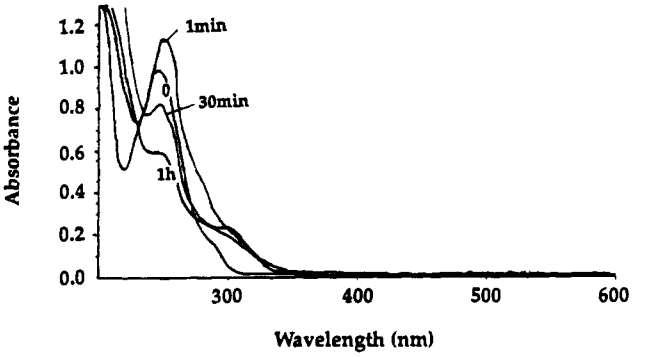

Figure 6. Spectral changes occurring during the chemical oxidation of GHB by NaOCl. The reaction mixture included 2 mL of 0.2 mM GHB and 2 mL of 0.01% NaOCl. The spectrum was scanned at the indicated times against a blank containing GHB and distilled water.

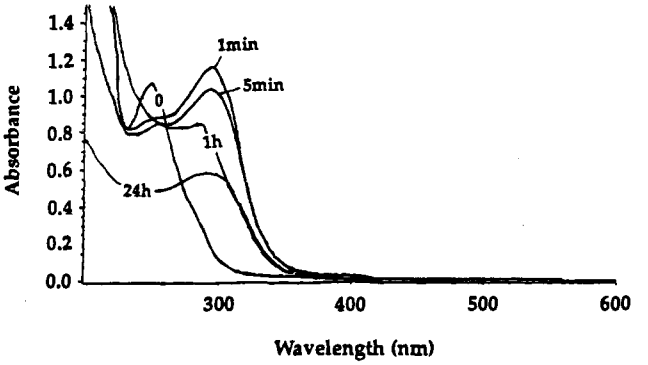

Figure 7. Spectral changes occurring during the chemical oxidation of GDHB by NaOCl. The reaction mixture included 2 mL of 0.2 mM GHB and 2 mL of 0.01% NaOCl. The spectrum was scanned at the indicated times against a blank containing GDHB and distilled water.

reaction but thereafter slowly decreased until 1 h, while a small shoulder at 300 nm appeared. In the case of the reaction of 0.2 mM GDHB with NaOCl (Figure 7), the absorption maximum at 250 nm shifted to 300 nm and then gradually disappeared. Thus, we can see a difference in chemical oxidation by NaOCl between L-DOPA and GHB or GDHB: NaOCl oxidizes L-DOPA to a quinone, which may polymerize, as indicated by increasing absorption in the UV range. However, GHB and GDHB were not only oxidized but also degraded by NaOCl. These results suggest that L-DOPA or its derivatives in mushroom might contribute to darkening at the surface of washed mushrooms in the presence of OCl^-. Further work on oxidation products formed from the degradation of GHB and its derivatives by NaOCl is needed to clarify their contribution to quality problems of washed mushroom.

ACKNOWLEDGMENT

We express our gratitude to the following companies for financial support of this research: Campbell Soup Co., Franklin Mushroom Farms, Highline Produce Ltd., J. M. Farms, Inc., Sylvan Foods, Inc., and Wilson Mushroom Co. We thank Dr. Frederick C. Miller of Sylvan Foods for providing mushrooms.

LITERATURE CITED

Barden, C. L.; Beelman, R. B.; Bartley, C. E.; Schisler, L. C. The effect of calcium chloride added to the irrigation water on quality and shelf life of harvested mushrooms. *J. Food Prot.* **1991**, *53* (9), 759–762.

Beelman, R. B.; Guthrie, B. D.; Royse, D. J. Influence of bacterial populations on postharvest deterioration of fresh mushrooms. *Mushroom Sci.* **1989**, *12*, 655–665.

Bio-Rad. "Bio-Rad Lab Instruction Manual", Bio-Rad Lab, Richmond, CA, 1989.

Brown, E. M.; Dower, H. J.; Greenberg, R. Effects of reductive alkylation on peptide retention times. *J. Chromatogr.* **1988**, *443*, 247–254.

Burton, K. S.; Frost, C. E.; Atkey, P. T. Effect of vacuum cooling on mushroom browning. *Int. J. Food Sci. Technol.* **1987**, *22*, 599–606.

Dave, B. A. Fresh mushroom treatment. U.S. Pat. 4,066,795, 1978.

Dawley, R. M.; Flurkey, W. H. Differentiation of tyrosinase and laccase using 4-hexylresorcinol, a tyrosinase inhibitor. *Phytochemistry* **1993**, *33*, 281–284.

Dorren, G. G. Bacterial and Fungal Diseases. In *The Biology and Technology of the Cultivated Mushroom*; Flegg, P. B., Spencer, D. M., Wood, D. A., Eds.; Wiley: Chichester, U.K., 1985; p 261.

Fermor, T. R. Bacterial disease of edible mushrooms and their control. In *Cultivating Edible Fungi. Developments in Crop Science*, 10th ed.; Wuest, P. J., Royse, D. J., Beelman, R. B., Eds.; Elsevier: Amsterdam, 1987; pp 361–370.

Fletcher, J. T. Terramycin for the control of bacterial blotch. *Mushroom J.* **1978**, *70*, 333–342.

Fordyce, C. Microorganisms associated with market deterioration of the cultivated mushroom, *Agaricus bisporus*. *Plant Dis. Rep.* **1968**, *52*, 712–714.

Guthrie, B. D.; Beelman, R. B. Control of bacterial deterioration in fresh washed mushrooms. *Mushroom Sci.* **1989**, *12*, 689–700.

Hahn, Y. S.; Yoo, Y. J. Preventing discoloration of mushrooms. *Kungnip Kongop Yonguso Pogo* **1965**, *15*, 85–90.

Ingebrigtsen, J.; Kang, B.; Flurkey, W. H. Tyrosinase activity and isozymes in developing mushrooms. *J. Food Sci.* **1989**, *54*, 128–131.

Mau, J.-L.; Miklus, M. B.; Beelman, R. B. The shelf life of *Agaricus* mushrooms. In *Shelf Life Studies of Foods and Beverages*; Charalambous, G., Ed.; Elsevier Science Publishers: New York, 1993; p 255.

Molsberry, C. C. Formula for preservation of mushrooms. Can. Pat. 843882, 1979.

Oka, Y.; Tsuji, H.; Ogawa, T.; Sasaoka, K. Quantitative determination of the free amino acids and their derivatives in the common edible mushroom, *Agaricus bisporus*. *J. Nutr. Sci. Vitaminol.* **1981**, *27*, 253–262.

Paranjpe, M. S.; Chen, P. K.; Jong, S. C. Phenolic and other organic compounds in morphologically different tissues of *Agaricus bisporus*. *Trans. Mycol. Soc. Jpn.* **1978**, *19*, 169–180.

Park, D. L.; Sam, M.; Rua, J. R.; Robert, F. A. Direct application of a new hypochlorite sanitizer for reducing bacterial contamination on foods. *J. Food Prot.* **1991**, *54* (12), 960–965.

Rast, D.; Stussi, H.; Zobrist, P. Self-inhibition of the *Agaricus bisporus* spore by CO_2 and or γ-glutaminyl-4-hydroxybenzene and γ-glutaminyl-3,4-benzoquinone: A biochemical analysis. *Physiol. Plant.* **1979**, *46*, 227–234.

Royse, D. J.; Wuest, P. J. Mushroom brown blotch: Effects of chlorinated water on disease intensity and bacterial populations in casing soil and on pilei. *Phytopathology* **1980**, *70* (9), 902–905.

Sapers, G. M.; Miller, R. L.; Miller, F. C.; Cooke, P. H.; Choi, S. W. Enzymatic browning control in minimally processed mushrooms. *J. Food Sci.* **1994**, in press.

Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158.

Speroni, J. J.; Beelman, R. B. High performance liquid chromatographic determination of agaritine in cultivated mushrooms. *J. Food Sci.* **1982**, *47*, 1539–1541.

Wong, W. C.; Preece, T. F. *Pseudomonas tolaasii* in cultivated mushroom (*Agaricus bisporus*) crops: effects of sodium hypochlorite on the bacterium and on blotch disease severity. *J. Appl. Bacteriol.* **1985a**, *58*, 259–267.

Wong, W. C.; Preece, J. F. *Pseudomonas tolaasii* in mushroom (*Agaricus bisporus*) crops: bactericidal effects of six disinfectants and their toxicity to mushrooms. *J. Appl. Bacteriol.*, **1985b**, *58*, 269–273.

Received for review March 7, 1994. Accepted July 26, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts,* September 1, 1994.