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Studies on the Enzymic Reduction of 1-Octen-3-one in Mushroom (*Agaricus bisporus*)

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The enzymic reduction of 1-octen-3-one into 1-octen-3-ol and 3-octanone in mushroom (*Agaricus bisporus*) was investigated by using synthetic 1-octen-3-one blended with mushrooms and the pH adjusted from 5.0 to 9.0. 1-Octen-3-ol can be converted from 1-octen-3-one, but the formation does not seem to be affected by the pH value while the formation of 3-octanone was favored at pH 8.0–9.0. It is possible there exists two enzymes that are responsible for the formation of 1-octen-3-ol and 3-octanone. However, the presence of a reduction enzyme explains only in part about the origin of 1-octen-3-ol in mushroom (*A. bisporus*).

1-Octen-3-ol, an unsaturated alcohol, occurs in many mushroom species (Cronin and Ward, 1971; Picardi and Issenberg, 1973; Pyysalo, 1976; Maga, 1981). It contributes significantly to the flavor of edible mushrooms such as *Agaricus campestris* (Tressl et al., 1980, 1982) and *Agaricus bisporus* (Wurzenberger and Grosch, 1983; Chen and Wu, 1983). Tressl et al. (1980, 1982) demonstrated that 1-octen-3-ol was formed enzymically from linoleic acid, which was shown by Holtz and Schisler (1971) to be the major fatty acid from *A. bisporus*. Enzymes involved in the pathway of formation of 1-octen-3-ol as proposed by Tressl et al. (1980) included lipoxygenase, hydroperoxide cleavage enzyme, and alcohol oxidoreductase. The activity of lipoxygenase was found in mushrooms by de Lumen et al. (1978). The activities of hydroperoxide cleavage enzyme were found in cucumbers and tomatoes (Galliard and Matthew, 1977; Wardale et al., 1978) and tea (Hatanaka et al., 1977), in which the cleavage of linoleic acid occurs via the 13- or 9-hydroperoxide. In mushroom *A. campestris*, the presence of a hydroperoxide cleavage enzyme was also proposed by Tressl et al. (1980) for the conversion of 13-hydroperoxide to 1-octen-3-ol and two 10-carbon compounds. On the contrary, Wurzenberger and Grosch (1983) proposed that a 10-peroxide might be the intermediate for the formation of 1-octen-3-ol, since they found that incubation of 13-hydroperoxide with mushroom homogenate only reduced the hydroperoxide into the corresponding 13-hydroxy fatty acid. The above two different proposals lead to two different possibilities about the formation of 1-octen-3-ol; that is, 1-octen-3-one is reduced to 1-octen-3-ol by an alcohol oxidoreductase if the proposal of Tressl et al. (1980) is followed; on the contrary, 1-octen-3-ol may be formed directly from the cleavage of 10-hydroperoxide if the proposal of Wurzenberger and Grosch (1982) is followed.

The purpose of this study was to clarify the pathway by which 1-octen-3-ol is formed. This paper presents the results of experiments in which mushrooms were blended with added 1-octen-3-one at different pH values followed by the determination of the product formed.

EXPERIMENTAL SECTION

Sample Preparation. 1-Octen-3-one was synthesized from the chromic acid oxidation of 1-octen-3-ol (97%, Fluka AG) as shown by Brown and Garg (1961). 3-Octanone (97%) was obtained from Fluka AG. Fresh mushrooms of approximately the same size (ca. 16 g) were picked daily from the local cultivation houses near Hsinchu, Taiwan. Mushrooms (250 g) were blended under room temperature for 5 min with 750 mL of distilled water, while the pH of the whole mixture was maintained at a constant value by adding 0.1 N HCl or 0.1 N NaOH solution. The pH value of the mixtures ranged from 5.0 to 9.0 with a 1.0-unit interval. The same mixtures were prepared and 160 mg of synthetic 1-octen-3-one was added during each preparation. Volatile components in each mixture were extracted for 1 h in a Likens-Nickerson apparatus (Römer and Renner, 1974). Glass-distilled pentane and ether (1:1) were used as extracting solvents. 1-Nonanol (Haarman and Reimer GmbH) was added as an internal standard. The extracted volatiles were injected directly into the gas chromatograph without further concentration.

Gas Chromatography. Gas chromatography was carried out on a Shimadzu GC-8APF equipped with dual flame ionization detectors and dual glass columns (2 m × 2.6 mm i. d.). The column packing was 10% Carbowax-20M (Varian aerograph) coated on Chromosorb W A/W DMCS (80–100 mesh, Supelco, Inc.). The oven temperature was programmed from 60 to 200 °C/min at 2 °C/min. The injector and detector temperatures were 250 °C. The

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Table I. Quantitative Changes of 3-Octanone and 1-Octen-3-ol with (A) or without (B) the Addition of 160 mg of 1-Octen-3-one; (C) Is the Net Change Due to the Addition of 1-Octen-3-one

		pH 5.0			pH 6.0			pH 7.0			pH 8.0			pH 9.0		
peak no.[a]	compound	A[b]	B[b]	C[b]	A	B	C	A	B	C	A	B	C	A	B	C
4	1-octen-3-one	94.20[c]	0.12[c]	94.08[c]	99.70	0.17	99.43	82.26	0.10	82.16	68.26	0.07	68.19	67.15	0.55	66.60
3	3-octanone	12.42	0.35	12.07	14.58	1.01	13.57	27.80	1.17	26.63	35.03	1.44	33.59	39.10	1.58	37.52
8	1-octen-3-ol	51.56	6.78	44.78	62.00	19.78	42.22	67.71	27.60	40.11	52.88	8.52	44.36	45.56	2.39	43.17

[a] Number refers to Figure 1. [b] Unit expressed on a milligram basis per 250 g of mushrooms. [c] Average of three experiments.

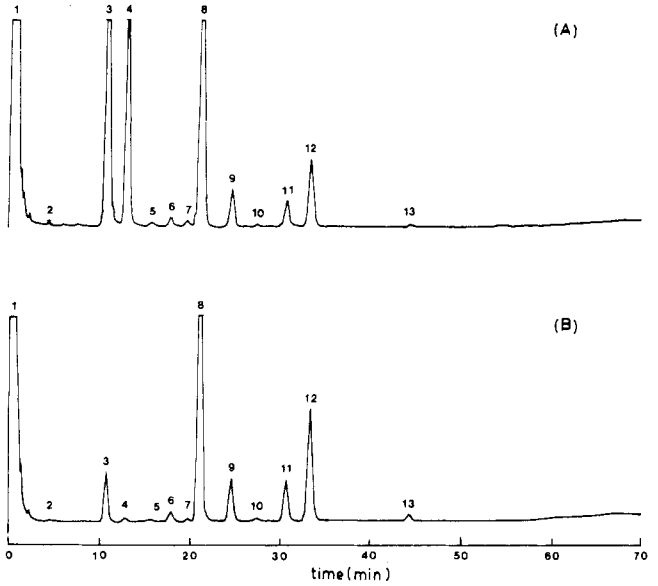

Figure 1. Gas chromatograms of volatile components of mushrooms (*A. bisporus*) formed at pH 7.0 with (A) or without (B) the addition of 1-octen-3-one (160 mg).

carrier gas was nitrogen at a flow rate of 30 mL/min. The data reported were recorded on a Hewlett-Packard 3390A integrator.

Gas Chromatography–Mass Spectrometry. A Hewlett-Packard 5985B gas chromatography–mass spectrometry system was used. Operation parameters were as follows: carrier gas, helium; ionization voltage, 70 eV; electron multiplier voltage, 2200 V; ion source temperature, 200 °C.

RESULTS AND DISCUSSION

Figure 1 shows the typical gas chromatograms of the volatiles of mushrooms (*A. bisporus*) formed at pH 7.0 with (A) or without (B) the addition of 1-octen-3-one. The identification of the volatile compounds was done according to the GC retention time of authentic samples and the mass spectra (Picardi and Issenberg, 1973; Jennings and Shibamoto, 1980; de Brauw et al., 1981; Tressl et al., 1982; Chen and Wu, 1983). Among the components found out in Figure 1, the only differences between the two chromatograms are peak 3 (3-octanone), peak 4 (1-octen-3-one), and peak 8 (1-octen-3-ol). Other components that are shown in Figure 1 are peak 1 (solvent), peak 2 (hexanal), peak 5 (1-hexanol), peak 6 (3-octanol), peak 7 (unknown), peak 9 (benzaldehyde), peak 10 (1-octanol), peak 11 (2-octen-1-ol), peak 12 (1-nonanol, internal standard), and peak 13 (benzyl alcohol). The volatile components identified in this study are similar as those reported previously (Cronin and Ward, 1971; Picardi and Issenberg, 1973; Pyysalo, 1976; Tressl et al., 1980, 1982; Wurzenberger and Grosch, 1982, 1983; Chen and Wu, 1983).

Table I shows the quantitative data of peak 3, peak 4, and peak 8 from pH 5.0 to pH 9.0, with or without the addition of 160 mg of 1-octen-3-one. Differences due to addition of 1-octen-3-one are also shown. Other volatile components that were not affected by the addition of 1-octen-3-one are not shown in Table I. The recovery at each pH value ranges from 91% (pH 8.0) to 97% (pH 6.0). The transformation of added 1-octen-3-one into 1-octen-3-ol and 3-octanone ranges from about 40% (pH 6.0) to about 60% (pH 9.0).

From the results of Figure 1 and Table I, it is clear that the added 1-octen-3-one was reduced to 1-octen-3-ol and 3-octanone. The formation of 1-octen-3-ol seemed not affected by the pH value while the formation of 3-octanone was favored at pH 8.0–9.0. In the control experiment, the formation of 1-octen-3-ol was favored at pH 6.0–7.0; this was in accordance with previous reports (de Lumen et al., 1978; Tressl et al., 1980, 1982; Wurzenberger and Grosch, 1982, 1983; Chen and Wu, 1983), while the formation of 3-octanone was also favored at pH 8.0–9.0.

In this study, 1-octen-3-one added was reduced to 1-octen-3-ol and 3-octanone; this is different from the results of Tressl et al. (1980). They postulated that 1-octen-3-ol was the major product formed by the action of an NAD(P)H-dependent alcohol oxidoreductase. In this study, it was found that the reductase of mushroom may not only reduce 1-octen-3-one to 1-octen-3-ol but also reduce the double bond of 1-octen-3-one to form 3-octanone. The above phenomenon can possibly be explained by two separate enzymes that are responsible for the formation of 1-octen-3-ol and 3-octanone or only one enzyme but the activity is affected by the pH value.

It is interesting to note that under normal conditions, the ratio of 1-octen-3-ol to 3-octanone is greater than those with added 1-octen-3-one. For example, the ratio of 1-octen-3-ol to 3-octanone of the control experiment at pH 7.0 is about 20 and the ratio of 1-octen-3-ol to 3-octanone of an additional experiment with 1-octen-3-one at pH 7.0 is only about 1.5; the apparent difference between the two experiments indicates that 1-octen-3-ol may originate by another pathway, possibly that proposed by Wurzenberger and Grosch (1982). Therefore, it is possible that the proposals of Tressl et al. (1980) about the relationship of 1-octen-3-one and 1-octen-3-ol may contribute only a minor part to the formation of 1-octen-3-ol in mushroom although there does exist a reductase system that can reduce the added 1-octen-3-one into 1-octen-3-ol and 3-octanone.

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Variations in the Occurrences of Enzymically Derived Volatile Aroma Compounds in Salt- and Freshwater Fish

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Cross-species comparisons of volatile aroma compounds from freshly harvested freshwater and saltwater fish revealed a common occurrence for hexanal, 1-octen-3-ol, 1,5-octadien-3-ol, and 2,5-octadien-1-ol in each. Freshwater fish additionally contained 1-octen-3-one and 1,5-octadien-3-one.. The eight-carbon volatile compounds contribute a distinctly pleasant, plant-like aroma to fresh fish. (*E*)-2-Hexenal, 2-octenal, 2-octen-1-ol, 2,3-octanedione, (*E*)-2-nonenal, (*E,Z*)-2,6-nonadienal, and 3,6-nonadien-1-ol were found in six of twelve freshwater fish species but were not present in the saltwater species surveyed.

The aromas of fresh fish vary considerably among species, but some common quality features for fish-like aromas are generally acknowledged. While traditional views include a major characterizing role for trimethyl- and dimethylamines (Moncrieff, 1944; Yamada, 1967; Tokunaga, 1970), volatile sulfides (Ackman et al., 1972; Tokunaga et al., 1977; Shiomi et al., 1982), and carbonyl compounds derived from classic autoxidation of lipids (Badings, 1970, 1973; Meijboom and Stroink, 1972; Ke et al., 1975; Swoboda and Peers, 1977), these compounds and their means of formation do not completely account for the various qualities of fresh fish aromas that differentiate species. Recent research has shown that the key characterizing aroma compounds in fresh whitefish (*Coregonus clupeaformis*) are comprised of hexanal and several eight- and nine-carbon carbonyls and alcohols (Josephson et al., 1983). The current investigation was conducted to extend the initial study and to determine patterns for the occurrences of volatile carbonyls and alcohols in selected species of fresh- and saltwater fish.

MATERIALS AND METHODS

Freshly harvested (4–36 h on ice) freshwater fish were obtained as gutted, unscaled samples or were caught by the authors and included whitefish (*Coregonus clupeaformis*) and smelt (*Osmerus mordax*) from Lake Michigan, Wisconsin, black crappie (*Pomoxis nigromaculatus*), bluegill (*Lepomis macrochirus*), muskellunge (*Esox masquinongy*), and perch (*Perca flavescens*) from Lake Wingra, Wisconsin, northern pike (*Esox lucius*) from Lake Winnebago, Wisconsin, ciscoe (*Coregonus artedii*) from Lake Mendota, Wisconsin, walleye pike (*Stizostedion vitreum*) and sauger (*Stizostedion canadense*) from the Wisconsin River, Wisconsin, rainbow trout (*Salmo gairdneri*) from The University of Wisconsin Aquaculture Laboratory, and live emerald shiners (*Notropis atherinoides*) from a local bait shop. Saltwater fish included ocean perch (*Sebastes marinus*), cod (*Gadus morhua*), petrole sole (*Eopsetta jordani*), and haddock (*Melanogramus aeglefinus*), and these fish were obtained from Maine as whole, unscaled fish (48–72 h on ice) via air freight through a commercial distributor (Milwaukee, WI). Edible species were eviscerated, washed, drained, vacuum packaged (720–760 mmHg) in barrier bags (Freshtuff, American Can Co., Neena, WI), and then stored at −25 °C until analyzed within 5 days.

Extracts from fish were prepared by immersing one thawed, uncooked fish in 100 mL of saturated NaCl solution, followed by agitating to recover most of the slime layer in the extract. Headspace volatiles from extracts were collected and concentrated by purging each at room temperature (21 °C) with a stream of nitrogen (100 mL/min for 3 h) onto Tenax GC as described by Steinke (1978). Capillary column gas chromatography in conjunction with mass spectrometric analyses of volatiles in ethylether extracts from Tenax GC traps was performed as described by Josephson et al. (1983). A Carbowax 20M (60 m × 0.25 mm i.d.) fused silica capillary column (J & W Scientific, Inc., Rancho Cordova, CA) operated with helium carrier gas (head pressure 10 psi, split 10 mL/min, sweep 5 mL/min) was used, and a program rate of 50 °C (5 min) to 140° C at 5 °C/min followed by a rate of 10 °C/min from 140 to 220 °C was also employed.

Identification of compounds was based on computer matching of full or partial mass spectra of compounds

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