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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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Table I. Occurrence of Volatiles in a Variety of Fish Species[a]

species	C_6-anal	C_6-(E)-2-enal	C_8-2-enal	C_8-2-(*Z*?)-en-1-ol	C_8-1-en-3-one	C_8-1-en-3-ol	C_8-(*Z*?)-1,5-dien-3-one	C_8-(*Z*?)-1,5-dien-3-ol	C_8-(*Z*,*Z*??)2,5-dien-1-ol	C_8-2,3-dione	C_9-(*E*)-2-enal	C_9-(*E*,*Z*)-2,6-dienal	C_9-(*Z*?)-6-en-1-ol	C_9-(*Z*,*Z*??)-3,6-dien-1-ol
freshwater fish														
lake white fish (*Coregonus clupeaformis*)	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ciscoe (*Coregonus artedii*)	X	X	X	X	X	X	X	X	X	X	X	X	X	X
muskellunge (*Esox masquinongy*)	X	X	X	X	X	X	X	X	X	X	X	X		X
northern pike (*Esox lucius*)	X	X	X	X	X	X	X	X	X	X	X	X		±
smelt (*Osmerus mordax*)	X	X	X	X	X	X	X	X	X	X	X	X	X	X
emerald shiner (*Notropis atherinoides*)	X	X		X	X	X		X	X	X	X	X		
walleye pike (*Stizostedion viteum*)	X				X	X	X	X	X					
sauger (*Stizostedion canadense*)	X				X	X	X	X	X					
yellow perch (*Perca flavescens*)	X				X	X	X	X	X					
rainbow trout (*Salmo gairdneri*)	X				X	X	X	X	X					
bluegill (*Lepomis macrochirus*)	X		X		X	X	X	X	X					
black crappie (*Pomoxis nigromaculatus*)	X		X		X	X	X	X	X					
saltwater fish														
cod (*Gadus morhua*)	X					X		X	X					
petrole sole (*Eopsetta jordani*)	X					X	±	X	X					
ocean perch (*Sebastes marinus*)	X					X		X						
haddock (*Melanogramus aeglefinus*)	X					X	±	X						

[a] X denotes occurrence. ± = trace; determined by odor assessment from packed column equipped with an effluent splitter.

published in "EPA/NIH Mass Spectral Data Base" (Heller and Milne, 1975, 1980) and coincidence of mass spectral patterns from isolated compounds with those of authentic compounds as well as coincidence for retention indices [I_E (Van den Dool and Kratz, 1963)].

RESULTS AND DISCUSSION

The volatile compounds that were found in the fresh freshwater fish and saltwater fish surveyed are shown in Table I. Hexanal, 1-octen-3-ol, 1,5-octadien-3-ol, and 2,5-octadien-1-ol were found widely distributed in freshwater and saltwater species, but 1-octen-3-one and 1,5-octadien-3-one were instrumentally detected only in freshwater fish. The eight-carbon alcohols were always present in higher concentrations than their corresponding vinyl ketones in freshwater fish. Trace odor occurrences were observed for 1,5-octadien-3-one (geranium leaves odor quality) at its appropriate retention index (I_E = 7.42; Carbowax 20M) in two saltwater fish (petrole sole, haddock) surveyed during packed column GC analysis, and these results were similar to those reported by Whitfield et al. (1981, 1982). These Australian workers instrumentally detected 1-octen-3-ol and (*Z*)-1,5-octadien-3-ol in seven species of prawns and one species of sand lobster, but the corresponding vinyl ketones [1-octen-3-one, (*Z*)-1,5-octadien-3-one] were detected only by their characteristic aromas at expected retention indices in just one sample.

The eight-carbon vinyl ketones and alcohols occur at concentrations above recognition thresholds in freshwater whitefish (Josephson et al., 1983), and although internal standards were not employed in quantifying the fresh fish volatiles in this study, the eight-carbon vinyl ketones (freshwater fish only) and their corresponding vinyl alcohols (fresh- and saltwater fish) appear to exist above recognition thresholds as assessed by the authors. Additionally, the nine-carbon compounds, (*E*)-2-nonenal, (*E*,*Z*)-2,6-nonadienal, and 3,6-nonadien-1-ol, were identified in six of the twelve species of freshwater fish examined, and these volatiles also exist above recognition thresholds in the species possessing them as determined by cucumber-like, melon-like odor assessments of the fresh fish. The identification of (*E*)-2-nonenal, (*E*,*Z*)-2,6-nonadienal, 6-nonen-1-ol, and 3,6-nonadien-1-ol in rainbow smelt (*Osmerus mordax*) supports the observations of Geiselman (1972), who encountered compounds in flavor isolates of this species which had aromas reminiscent of that of cucumbers. More recently, Berra et al. (1982) have identified (*E*,*Z*)-2,6-nonadienal in Australian grayling (*Prototroctes maraena*).

The saltwater species surveyed in the current investigation did not contain the nine-carbon compounds found in some freshwater fish species (Table I). However, Elliot (1946) included the term watermelon-like in the odor classification of the stages of quality for saltwater fish, and this suggests that some saltwater fish also can form the nine-carbon compounds. Notably, (*E*)-2-hexenal, 2-octenal, 2-octen-1-ol, and 2,3-octanedione were identified in all fish that also possessed the nine-carbon compounds, and this suggests a mechanistically linked pathway for these volatiles. The nine-carbon compounds appear to be associated with species that have rigid cool water temperature dependencies, and these fish also appear to be less rugged toward abrasions and handling than species that do not contain these compounds. Hexanal occurred in all fish surveyed and contributes a distinct coarse, green plant-like, aldehydic aroma note to immediately harvested finfish before blending with eight- and nine-carbon volatiles formed later (within minutes). Volatile aroma compounds in fresh whitefish were found in markedly higher concentrations in the slime and juices when compared to the tissue (Josephson et al., 1983), and all fish surveyed in this study were likewise found to exhibit these same characteristics.

In the current investigation, 1,3,5-octatriene was identified in six out of twelve species of freshwater fish and three out of four species of saltwater fish. This identification was based on a mass spectral fragmentation pattern [79 (100), 77 (54), 108 (49), 91 (38), 39 (25), 93 (24), 41 (20), 65 (18), 78 (18), 66 (17)], which compared favorably with that published for 1,3,5-octatriene by Jaenicke and Seferiadis (1975) and Juttner and Muller (1979). Notably, 1,3,5-octatriene occurred in all freshwater fish exhibiting the nine-carbon volatile compounds. Although a clear relationship between compounds has not been established yet, (3*S*)-(*Z*)-1,5-octadien-3-ol (Woolard et al., 1975) and (3*S*)-(*Z*)-1,5-undecadien-3-ol (Jaenicke et al., 1974; Kajiwara et al., 1982) have been proposed as possible precursors of the sperm-attracting hydrocarbons. These attractants include (*Z*,*Z*)-1,3,5-octatriene (fucosterratene), (*S*)-[(*Z*)-1-butenyl]-2,5-cycloheptadiene (ectocarpene), and *trans*-4-vinyl-5-(*cis*-1-butenyl)cyclopentene (multifidene), which are produced by the female gametes of the oogamous brown alga *Fucus serratus*, the isogamous brown alga *Ectocarpus siliculosus*, and the anisogamous brown alga *Cutleria multifida*, respectively.

Hydroperoxidations of fatty acids by lipoxygenases in mushrooms (Tressl et al., 1981) and cucumber and melon fruits (Galliard and Phillips, 1976; Vick and Zimmerman, 1976; Phillips et al., 1979) initiate reactions that lead to the formation of the eight-carbon and nine-carbon volatile aroma compounds, respectively, in these foods. Since the same compounds occur in fresh fish (Table I), similar enzyme-mediated pathways now appear to explain their formation in fish. Recently, German and Kinsella (1983) reported the identification of a lipoxygenase system in the gill tissue of rainbow trout (*Salmo gairdneri*), and this supports the view for an enzymic involvement in the biogenesis of lipid-derived volatiles in fresh fish.

In other recent studies employing specific lipoxygenase and fatty acid cyclooxygenase inhibitors, it has been shown that nearly complete inhibition of aroma development occurs after treatment of homogenenized emerald shiners (*N. atherinoides*) with these agents (Josephson et al., 1984), which implicates an enzymic involvement. Thus, differences in the patterns of fresh fish volatile aroma compounds found among the various species of fish appear to reflect metabolic regulation processes that involve polyunsaturated fatty acids and prostaglandins. In this context it is suggested that these processes influence regulatory activities occurring at the skin–water interface of fish.

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Registry No. C_6-anal, 66-25-1; C_8-1-en-3-ol, 3391-86-4; C_8-1,5-dien-3-ol, 83861-74-9; C_8-2,5-dien-1-ol, 83861-75-0; C_8-1-en-3-one, 4312-99-6; C_8-1,5-dien-3-one, 65213-86-7; C_6-(*E*)-2-enal, 6728-26-3; C_8-2-enal, 2363-89-5; C_8-2-en-1-ol, 22104-78-5; C_8-2,3-dienone, 585-25-1; C_9-(*E*)-2-enal, 18829-56-6; C_9-(*E*,*Z*)-2,6-dienal, 557-48-2; C_9-3,6-dien-1-ol, 76649-25-7; C_9-6-en-1-ol, 40709-05-5.

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Biogenesis of Lipid-Derived Volatile Aroma Compounds in the Emerald Shiner (*Notropis atherinoides*)

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The formation of carbonyls and alcohols that characterize the fresh fish aroma of emerald shiners (*Notropis artherinoides*) was almost completely inhibited when shiners were sacrificed and immediately exposed to acetylsalicylic acid or stannous [tin(II)] chloride (inhibitors of fatty acid cyclooxygenase and lipoxygenase, respectively). These observations are interpreted as a demonstration of the involvement of enzymic conversions of ω-6 and ω-3 fatty acids to volatile aroma compounds in fresh fish. Postulated reaction mechanisms for the enzymic conversions of prostaglandins and polyunsaturated fatty acids to volatile aroma compounds are presented.

Earlier workers generally have attributed much of the characteristics aroma of fresh fish to nonenzymic autoxidation products of highly unsaturated fatty acids in fish (Yu et al., 1961; Stansby, 1962; Mejboom and Stroink, 1972; Badings, 1973; McGill et al., 1974, 1977; Crawford et al., 1975; Ke et al., 1975; Crawford and Kretsch, 1976; Ikeda, 1979). Fish-like aromas that resemble those of oxidized fish oil also develop in oxidizing butter, soybean, and linseed oils (Forss et al., 1960; Badings, 1970; Seals and Hammond, 1970), and these occurrences have usually been interpreted as support for the view that nonenzymic processes are responsible for the aroma of fishes. However, conflicting descriptions of fishy odors, and the inability to chemically account for the different fresh aromas of various species, even when considering volatile amines (Moncrieff, 1944; Tokunaga, 1970; Yamada, 1967) and sulfur compounds (Ackman et al., 1972; Tokunaga et al., 1977; Shiomi et al., 1982), suggest that previously unrecognized reaction pathways are involved in the formation of volatile aroma compounds that characterize fresh seafoods.

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Obata et al. (1950) have reported that gauze used to wipe nearly odorless, fresh herring did not exhibit the aroma of fresh fish when it was dried rapidly. However, when a portion of the gauze was allowed to remain wet, an odor described as freshwater fish was observed. These observations suggest an enzymic involvement in the development of fresh fish aromas that was inhibited in the dried

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