Modification of Fish Oil Aroma Using a Macroalgal Lipoxygenase

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ABSTRACT: The odor of fish oil is the major factor limiting its application in food. In this study, the addition of butylated hydroxytoluene to fish oil did not significantly inhibit the generation of fishy and rancid odors. To reduce the undesirable odors, fish oil was treated with lipoxygenase (LOX) to produce volatile compounds *via* position-specific cleavage of hydroperoxides. An extract of a green marine macroalga, *Ulva conglobata,* showed a high level of 13-LOX activity and 9-LOX to a lesser extent, and produced strong green, melon-like, and fresh-fish-like flavor notes from fish oil. The LOX-modified fish oil contained 99% of the highly unsaturated fatty acids (HUFA; containing three or more double bonds) originally present, total volatile compounds increased from 3477 to 3787 ppb after LOX treatment. Compounds with strong odors accounted for about 40% of the total volatiles. Increasing the level of LOX activity used to treat fish oil produced higher concentrations of the desirable unsaturated aldehydes, ketones, and alcohols, with odors resembling fresh fish, apple, citrus, melon, fruit, and oyster. These compounds were tentatively identified as *E,Z*-2,6-nonadienal, *E*-2-hexenal, *E,E*-2,4-octadienal, *E,E*-3-5-octadien-2-one, and alcohols *E*-2-pentenol and 2-butoxyethanol. The LOX treatment also slightly increased the content of the undesirable volatile components, including sour and rancid odors, tentatively identified as acetic acid and *E,Z*- and *E,E*-2,4-decadienals.

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Since 1989, when the U.S. Food and Drug Administration approved GRAS (generally regarded as safe) status for partially and fully hydrogenated menhaden oil (1), the market share of fish oils as food has grown about 7% yearly (2). They are used in margarines, shortenings, emulsifiers, bakery products, table spreads, cooking oils (3), and foods enriched with polyunsaturated fatty acids (PUFA) (4,5). Nevertheless, they lack oxidative stability (2). Microencapsulation (6) and incorporation of α-tocopherol and other antioxidants (7) improve the oxidative and thermal stability of fish oils. Novel refining techniques have been developed to produce odorless marine oils for enriching foods or for use as nutraceuticals (4). Regardless of these advances in technology, each double bond of the PUFA in unhydrogenated fish oil is a potential site for oxidation. The odor and aftertaste of such fish oils when used as food and feed supplements can be less than desirable. If this problem can be overcome, fish oils can be used in a new array of applications.

Previous studies on model systems used partially purified lipoxygenase (LOX, EC 1.13.11.12) from fish gills incubated with PUFA, commercial fish oil, or lipid extracted from shrimp to produce compounds with a fresh-fish aroma (8). However, LOX extracted from fish was not stable during isolation. Addition of protective compounds and removal of hemoglobin from crude LOX extracts were required to maintain stability and activity (9). On the other hand, a marine green macroalga, *Enteromorpha intestinalis*, was found to have very high LOX activities, considerably greater stability than animal LOX. These algal LOX contributed to development of the desirable flavor notes of clam, oyster, algae, fresh apple, cucumber, melon, and mango (10,11).

Fish oils like tuna oil contain volatiles with very low odor thresholds, representing both normal and off odors (12). To improve the sensory quality of fish oil, the formation of the undesirable fractions should be inhibited, while the desirable fractions should be enhanced. The objective of the present study was to modify fish oil with a stable algal LOX to improve the aroma and residual taste and to retain the polyenoic acids for the physiological effects (4). Achievement of this objective will enhance the application of fish oils as food ingredients and nutritional supplement.

MATERIALS AND METHODS

Preparation of algal lipoxygenase extract. A marine green macroalga, *Ulva conglobata*, was freshly harvested from the coastal waters of Taiwan and homogenized (1:5 wt/vol) in 0.05 M potassium phosphate buffer (pH 7.5) containing 1 mM glutathione (reduced form; Sigma Chemical Co., St. Louis, MO) in a polytron (PT 3000; Kinematica, Littau, Switzerland) for 30 s and centrifuged at 20,000 × *g* for 15 min at 4°C (13–15). The supernatant was the crude LOX extract. To determine activity, algal LOX extract (0.1 mL) was diluted with 0.9 mL of 0.05 M phosphate buffer (pH 7.5) containing 0.01% Tween-20. The mixture was incubated with linoleic acid (100 μM) at 26°C

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for 5 min. LOX activity was determined by the increase in total fatty acid hydroperoxides by using a molar absorptivity of 25,000 L/mol × cm at 234 nm (16,17), measured with a spectrophotometer (Hitachi U-2000 Tokyo, Japan).

High-performance liquid chromatography (HPLC) of hydroperoxy derivatives. The hydroperoxy fatty acid products of LOX reaction were extracted with ethyl acetate and then reduced with glutathione and methylated with diazomethane. The resulting compounds were separated with a solid-phase extraction column (J&W Scientific, Folsom, CA) and then subjected to HPLC analysis (11,13,18). Normal-phase HPLC analyses were performed on a Bondclone silica column (30 cm × 3.9 mm, 10 µm; Phenomenex, Torrance, CA). An ultraviolet detector (model 490E; Waters, Milford, MA) was employed to monitor absorbance at 234 nm. The reduced hydroperoxy derivatives were eluted isocratically with a solvent system of hexane/ethanol/acetic acid (98:1.9:0.1, vol/vol/vol) at a flow rate of 1.0 mL/min. The LOX-catalyzed products, 18:2-9OOH (9-hydroperoxy octadecadienoic acid, 9-HpODE) and 18:2-13OOH (13-HpODE), were confirmed by comparison of retention times with authentic standards (Cayman, Ann Arbor, MI).

Treatment of fish oil with LOX for aroma formation. Unhydrogenated cod liver oil (10 g; H. Erhard Wagner GmbH, Hamburg, Germany, distributed by Heng-Yi Ltd., Taichung, Taiwan) and Tween 20 (0.01%) were incubated with 1,000 mL of *U. conglobata* LOX extract containing a total activity of 84,880 µmol HpODE. The reaction mixture was incubated in capped vessels with stirring in the dark at 26°C for 2 h.

Another sample of algal LOX extract was added with an inhibitor, 0.2 mM $SnCl_2$ (19,20), with constant stirring to completely solubilize $SnCl_2$, followed by addition of fish oil and incubation at 26°C for 2 h as described above.

Collection of volatile compounds. After incubation of the fish oil with the LOX extract, 15% NaCl was added to break the emulsion, and 1 mg of $C_{13}H_{28}$ (Sigma) was added as an internal standard. The volatile compounds were collected with a rotary evaporator (model R-114/C; Büchi, Uster, Switzerland) at 26°C and a liquid N_2 trap for 2 h at a vacuum of 25–35 mmHg maintained using a vacuum distillation controller (B-720, Büchi). The volatiles collected in the trap were washed with distilled water and extracted with redistilled pentane/ether (1:1, vol/vol). The extract was then washed with saturated KCl (1:1, vol/vol) to remove polar residues. The volatiles in the pentane/ether layer were stored at −20°C to remove crystallized ice. The solution was then dried over anhydrous sodium sulfate and concentrated using a spinning band distillation apparatus for solvent removal followed by gas chromatographic analysis.

Gas chromatography (GC) and GC-sniffing analysis. The aroma concentrates were analyzed using a Shimadzu GC-14A gas chromatograph (Kyoto, Japan) equipped with a Carbowax-20M fused-silica capillary column, 60 m × 0.32 mm (J&W Scientific, Folsom, CA) and a flame-ionization detector. The oven temperature was programmed from 50 to 200°C at 1.5°C/min, then held for 60 min. Both injector and detector were set at 250°C. The carrier gas was hydrogen at a flow rate of 1.5 mL/min. Effluent from the outlet of the column was split 8:1 (vol/vol) between an olfactory device (SGE, Austin, TX) and the FID. The odors of the volatile components were evaluated at the sniffing port by one or two panelists trained to be consistent in odor perception (11). GC-sniffing analyses were repeated three times.

The retention indices (RI) of the volatile components were calculated using *n*-paraffins of C_6–C_{25} (Sigma) as reference compounds (21). The data from GC-sniffing and the GC–mass spectrometry (GC–MS) were correlated based on the RI of each compound.

GC–MS. MS spectra were determined using a mass spectrometer (Hewlett-Packard 5971, Palo Alto, CA), after separation on a DB-WAX 23 fused-silica capillary column, 60 m × 0.25 mm (J&W Scientific). The oven temperature was programmed from 40 to 200°C at 1.5°C/min, then held for 30 min. Both injector and detector were set at 250°C. The carrier gas was helium at a flow rate of 1 mL/min.

Sensory evaluation. The odor of untreated and treated cod liver oil was evaluated by a sensory panel consisting of seven staff members and graduate students who showed consistency in odor description. Odor intensity was ranked from no odor, "–," or odor of increasing intensity from "+" to "++++."

RESULTS AND DISCUSSION

Ulva conglobata was chosen to prepare the LOX extract because of its high activity and its abundance in nearby coastal waters based on our previous survey (data not shown). Two isozymes, 13-LOX and 9-LOX, were identified in *U. conglobata* using linoleic acid (18:2) as substrate (Fig. 1).

Changes in PUFA. Since all LOX oxidize unsaturated fatty acids with 1,4-*cis*-*cis*-pentadienyl systems, it is expected that the volatiles generated from fish oil by LOX reactions are from PUFA (22). A quantitative comparison of the PUFA composition before and after LOX treatment is shown in Table 1.

TABLE 1
Polyunsaturated Fatty Acid Composition[a] of Cod Liver Oil Before and After Reaction with Lipoxygenase (LOX)[b]

	Before		After	
Fatty acid	mg/g oil	%	mg/g oil	Change[d] (%)
Dienoic				
18:2	6	0.8	6	0.0
HUFA	(459)	(60.4)	(454)	–1.1
18:3	3	0.4	3	0.0
20:4	18	2.4	16	–11.1
20:5	248	32.6	246	–0.8
22:6	190	25.0	189	–0.6
Unknown	88	11.6	89	+1.1
Total	760	100.0	756	–0.5

[a]Quantified based on internal standard $C_{13:0}$.
[b]A total activity of 84,880 µmol hydroperoxy octadecadienoic acid was prepared from marine green algae *Ulva conglobata*.
[c]Highly unsaturated fatty acid (number of double bonds ≥3).
[d]Increase or decrease in reference to the control.

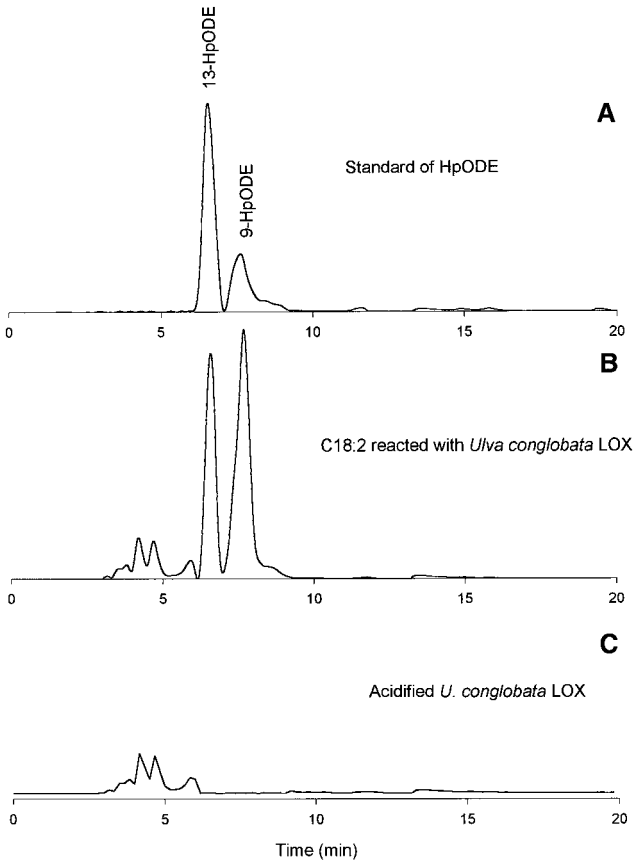

FIG. 1. Normal-phase high-performance liquid chromatogram of products, hydroperoxy octadecadienoic acid (HpODE) derived from linoleic acid treated with lipoxygenase (LOX) crude extract from *Ulva conglobata*. The presence of large peaks in B (corresponding to 13-HpODE and 9-HpODE) but absent in C indicates the presence of two LOX activities in LOX crude extract.

The unhydrogenated cod liver oil used in this study consisted of 61.2% PUFA, among which highly unsaturated fatty acids (HUFA, ≥3 double bonds) contributed 60.4% of the total fatty acids. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contributed 32.6 and 25.0%, respectively.

After LOX treatment, the total HUFA content decreased by only 1.1%, among which linolenic acid ($C_{18:3}$), EPA ($C_{20:5}$), and DHA ($C_{22:6}$) levels each declined by less than 1%. Arachidonic acid ($C_{20:4}$), although a minor component of the HUFA in fish oil, decreased by 11.1%. In actual quantity, losses of both $C_{20:4}$ and $C_{20:5}$ were about 2 mg/g oil. Arachidonic acid and EPA were the preferred substrates among the HUFA for LOX of *U. conglobata*. Fatty acid selectivity was also observed in soybean lipoxygenase (22).

Changes in undesirable volatile compounds. Fish oil can be processed to be completely odorless like vegetable oil; however, fish oil is more labile than vegetable oil, and flavor reversion to yield fish odors readily occurs because of the high content of HUFA (23). Although butylated hydroxytoluene was added to the cod liver oil by the manufacturer, the overall odor was still fishy and undesirable. GC-sniffing detected the volatiles of the fish oil, totaling 3477 ppb (Table 2). The odorous compounds contributed to 1355 ppb, of which 60.7% were undesirable.

After the algal LOX treatment, fish oil showed a slight increase (8.9%) in total volatile content as well as in odorous volatiles (2.1%), in which the undesirable odorous volatile decreased only slightly (7.2%). This minor reduction in volatile compounds indicated that in spite of the specific reactivities of LOX enzymes on the 1,4-pentadieneyl moiety of polyenoic acids, they may also catalyze the dioxygenation of allylic ketone during oxygen-deficient incubation (24,25), such as in our case.

By using a GC-sniffing technique, seven undesirable odor notes were perceived (Table 3). These included five fishy components and three rancid components, one of which was extremely rancid (RI = 1643). Six other undesirable odor components with gas-like, moldy, sour, stinkbug, metallic, and medicinal notes were detected.

E,Z-2,4-Decadienal and *E,E*-2,4-decadienal which impart rancid odors were present before LOX treatment. They have been suggested to be responsible for the fishy odor of fish oils, reversed soybean oil, and butter oil (23). The content of these two isomers increased after the LOX treatment, indicative of the cleavage of an ω-10-hydroperoxide formed from a ω-6,9-diene, e.g., 9-HpODE, either enzymatically or nonenzymatically. The increase of 2,4-heptadienal, which yielded an extremely rancid odor, indicated the cleavage of an ω-7-hydroperoxide formed from an ω-3,6-diene, e.g., 12-hydroperoxy octadecatrienoic acid. A pronounced increase in a volatile compound, of which both the RI and smell coincided with acetic acid, also resulted after LOX treatment. The mechanism of formation is not understood. A medicinal flavor note was associated with 2,6-dibutyl-4-methylphenol present at a high concentration of 441 ppb. Similarly, a medicinal odor has been associated with a high concentration of phenol present in crayfish waste (26). Since this compound was present in our fish oil before LOX treatment, it was probably carried over from raw material of the fish oil. All other compounds responsible for fishy, extremely rancid, and other undesirable odors were present at such low concentrations that they could not be identified, indicative of their extremely low odor thresholds. Since they occurred before LOX treatment, they were likely derived from autoxidation of fish oil during handling or shipping.

Increases in desirable odorous compounds. Aroma produced from LOX-treated polyenoic acids depends on the

TABLE 2
Volatile Compound Content of Fish Oil Before and After Algal LOX Treatment

Volatile compounds[a]	Before	After	Change (%)
Total volatile (ppb)	3477	3787	+8.9
Odorous volatile (ppb)	1355	1384	+2.1
Desirable	533	822	+54.2
Undesirable	822	763	–7.2
Odorous/total volatile (%)	39	42	+7.7

[a]Based on volatile compounds determined with gas chromatography–sniffing method. For abbreviation see Table 1.

TABLE 3
Undesirable Odor Compounds[a] of Cod Liver Oil Before and After Algal LOX Treatment[b]

Odor note	RI[c]	Compounds[d]	Before (ppb)[e]	After (ppb)[e]	Change[f] (%)
Fishy	1269	*	3	3	0.0
Fishy	1674	*	5	4	–20.0
Fishy	1740	*	3	6	100.0
Fishy	1746	*	2	3	50.0
Fishy	1765	*	35	7	–80.0
Rancid, extremely	1643	2,4-Heptadienal	56	58	3.6
Rancid	1819	*E,Z*-2,4-decadienal	10	16	60.0
Rancid	1837	*E,E*-2,4-decadienal	35	58	65.7
Musty	1305	*	8	4	–50.0
Metallic	1389	*	26	27	3.8
Sour	1486	Acetic acid	53	125	135.8
Stink bug	1515	*E,E*-2,4-heptadienal	144	85	–41.0
Medicinal	1911	Butyl hydroxy toluene	441	369	–16.3

[a]Volatile compounds were collected with a reduced pressure-cold trap, and detected by gas chromatography–sniffing method before and after algal LOX treatment.
[b]Cod liver oil (10 g) was reacted with 1 L of LOX extract with a total activity of 84,880 µmol hydroperoxy octadecadienoic acid (HpODE) at 26°C for 2 h.
[c]Retention index on Carbowax-20 M chromatograph of the cod liver oil, calculated according to Reference 21.
[d]Compounds were tentatively identified based on Wiley Computer Library.
[e]Quantitative determination based on $C_{13}H_{28}$ as internal standard.
[f]Increase or decrease in reference to the cod liver oil before algal LOX treatment.
*The compound was odorous but too dilute to be identified by gas chromatography–mass spectrometry (GC–MS). For abbreviation see Table 1.

specificity of volatile produced due to the positional specificity of the dioxygenation, double bond rearrangement, and cleavage of the hydroperoxides. Different endogenous or exogenous LOX present in the reaction system could result in distinctly different volatiles even with the same precursors.

A quantitative comparison of the odorous compounds between the untreated and the LOX-treated fish oil indicated a total of 53.8% increase {calculated as [(sum of ppb desirable compounds after LOX – sum of ppb desirable compounds before LOX)/(sum of ppb desirable compounds before LOX)] × 100} in all the desirable odor compounds contributing to flavor notes of grassy, apple-like, citrus, melon, almond, fruity, oyster and fresh fish-like (Table 4). Eighteen desirable aroma compounds were perceived by sniffing. Thirteen of them were tentatively identified by comparing their RI values with those listed in the Wiley Library. All the aroma compounds increased after LOX treatment. Those that increased by more than 40%, an arbitrary level, were considered to be more significant products of the treatment.

The content of *E,Z*-2,6-nonadienal, characterized as having a fresh fish-like odor, increased from 17 to 80 ppb while the content of *E,E*,-2,4-octadienal, giving an oyster flavor, in-

TABLE 4
Desirable Aroma Compounds[a] of Cod Liver Oil Before and After Algal LOX Treatment[b]

Flavor note	RI[c]	Compounds[d]	Before (ppb)[e]	After (ppb)[e]	Increase (%)[f]
Caramel	1029	*	—	11	
Green	1043	*	24	25	4.2
Grassy	1104	1-Penten-3-ol	7	13	85.7
Grassy	1173	Ethyl hexanoate	106	150	41.5
Apple	1155	*E*-2-Hexenal	50	54	8.0
Apple	1230	1-Pentanol	22	25	13.6
Citrus	1240	*E*-2-Penten-1-ol	58	74	27.6
Almond	1539	Benzaldehyde	53	62	17.0
Fruit	1549	*E,E*-3,5-Octadiene-2-one	17	25	47.0
Melon	1414	2-Butoxyethanol	99	118	19.2
Melon	1587	*E,Z*-3,5-Octadiene-2-one	7	10	42.9
Melon	1602	*E,Z*-2,4-Octadienal	3	15	400.0
Melon	1702	*	13	26	100.0
Melon	1719	*	10	24	140.0
Plant	1432	1-Octen-3-ol	5	8	60.0
Oyster	1576	*E,E*-2,4-Octadienal	44	101	129.5
Fresh fish	1592	*E,Z*-2,6-Nonadienal	17	80	370.6
Algae	1966	*	—	2	

[a–f,*]For footnotes see Table 3. For abbreviations see Tables 1 and 3.

TABLE 5
Aroma Grades Generated from Cod Liver Oil Reacted with Crude Extract of Lipoxygenase from *Ulva conglobata*

Added LOX activity[a] (μmol HpODE)	Flavor note					Overall intensity of fresh and sweet odor
	Fishy	Green	Fresh fish-like	Melon-like	Algae	
0	+	–	–	–	–	–
581	+	+	–	+	+	+
1,161	+	+	+	++	+	+++
2,322	+	+	+	++	+	+++
2,322-$SnCl_2$[b]	+	–	–	–	+	++

[a]Algal extract containing a LOX specific activity of 76 μmol HpODE/min-mg protein at a protein concentration of 0.76 mg protein/mL.
[b]LOX was inhibited by pretreatment with 0.2 mM $SnCl_2$ prior to incubation with cod liver oil.
[c]The flavor note was detected by a sensory panel of seven persons trained to give consistent flavor descriptions. For abbreviations see Tables 1 and 3.

creased from 44 to 101 ppb. Thus the treatment produced fresh seafood flavor. The content of *E,Z*-2,4-octadienal increased almost five times, together with 2-butoxy ethanol, *E,E*-3,5-octadiene-2-one, and two unidentified compounds having RI of 1702 and 1719, which had increased melon-like flavor notes (Table 4). This coincided with the finding that enhancement in overall melon aroma was associated with the increased LOX activity. When LOX activity was inhibited, the melon aroma disappeared (Table 5). The content of *E,E*-3,5-octadien-2-one, giving a fruity note, also increased, indicating that the LOX of *U. conglobata* accelerated the hydroperoxidation at 9- and 13-positions of linoleic acid (Fig. 1). The cleavage of 9-HpODE and 13-HpODE and further breakdown resulted in aldehydes and ketones of shorter chain length contributing desirable flavor notes to the modified fish oil.

The LOX preparation from algae was a crude extract. Other enzymes, e.g., hydroperoxide lyases, an important group of enzymes metabolizing fatty acid hydroperoxides and abundant in plants, might have been present in the crude extract. They can catalyze the enzymatic cleavages and formation of the aldehydes and ketones of C_6–C_9 (24,25).

An algal flavor note was detected after the LOX treatment (Table 4). The content was too low to be identified. This component did not increase with increased LOX activity nor did it disappear when the added algal LOX was inhibited by $SnCl_2$ (Table 5). It was perceived as a potent flavor compound with a long residual effect. This may serve as a unique characteristic of using LOX extracted from algae. Benzaldehyde, which has an odor of almond, was present before the LOX treatment and increased only slightly afterward. It was not a derivative from the LOX pathway.

Based on these observations, it is conceptually feasible to modify fish oil containing BHT by reacting the oil with LOX to make it smell less fishy, less rancid, less undesirable, more fruity, and more like fresh seafood. The 13- and 9-LOX extracted from *U. conglobata* were able to enhance the melon-like flavor notes significantly, in addition to other desirable odorous compounds in the algal LOX-treated fish oil. All the desirable aroma compounds seemed to have such low thresholds that the LOX modification did not decrease the HUFA content of the fish oil, thus maintaining the health benefits (4) associated with the HUFA.

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REFERENCES

1. U.S. Food and Drug Administration, Substances Affirmed as Generally Recognized as Safe: Hydrogenated and Partially Hydrogenated Menhaden Oils, *Federal Register 54*:38219–38223.
2. Lin, C.F., Flavor Chemistry of Fish Oil, in *Lipid in Food Flavors*, edited by C.-T. Ho and T.G. Hartman, *ACS Symp. Ser. 558*:208–232 (1994).
3. Anonymous, Fish Oils: Past and Present Food Uses, *J. Am. Oil Chem. Soc. 66*:1717–1726 (1989).
4. Newton, I.S., Food Enrichment with Long Chain n-3 PUFA, *INFORM 7*:169–177 (1996).
5. Bimbo, A.P., Menhaden Oil: The GRAS Petition from Hell, *Ibid. 8*:1069–1075 (1997).
6. Lin, C.C., S.Y. Lin, and L.S. Hwang, Microencapsulation of Squid Oil with Hydrophilic Macromolecules for Oxidative and Thermal Stabilization, *J. Food Sci. 60*:36–39 (1995).
7. Chang, S.S., and K. Wu, Composition of Natural Antioxidants for the Stabilization of Polyunsaturated Oils, U.S. Patent 5,077,069 (1991).
8. Pan, B.S., J.R. Tsai, L.M. Chen, and C.M. Wu, Lipoxygenase and S-Containing Amino Acids in Seafood Flavor Formation, in *Flavor and Lipid Chemistry of Seafoods*, edited by F. Shahidi and K. Cadwallader, *ACS Symp. Ser. 674*:64–84 (1997).
9. Hsu, H.H., and B.S. Pan, Effects of Protector and Hydroxyapatite Partial Purification on Stability of Lipoxygenase from Grey Mullet Gill, *J. Agric. Food Chem. 44*:741–745 (1996).
10. Kuo, J.-M., A. Hwang, and D.B. Yeh, Purification, Substrate Specificity, and Products of a Ca^{2+}-Stimulating Lipozygenase from Sea Algae *(Ulva lactuca), Ibid. 45*:2055–2060 (1997).
11. Kuo, J.-M., A. Hwang, H.H. Hsu, and B.S. Pan, Preliminary Identification of Lipoxygenase in Algae *(Enteromorpha intestinalis)* for Aroma Formation, *J. Agric. Food Chem. 44*:2073–2077 (1996).
12. Crawford, L., M.J. Kretsch, and D. Guadagni, Identification of Volatiles from Extracted Commercial Tuna Oil with a High Do-

cosahexaenoic Acid Content, *J. Sci. Food Agric. 27*:531–535 (1976).
13. German, J.B., and J.E. Kinsella, Hydroperoxide Metabolism in Trout Gill Tissue: Effect of Glutathione on Lipoxygenase Products Generated from Arachidonic Acid and Docosahexaenoic Acid, *Biochim. Biophys. Acta 879*:378–387 (1986).
14. German, J.B., and R.K. Creveling, Identification and Characterization of a 15-Lipoxygenase from Fish Gills, *J. Agric. Food Chem. 38*:2144–2147 (1990).
15. German, J.B., and R. Berger, Formation of 8,15-Dihydroxy Eicosatetraenoic Acid *via* 15- and 12-Lipoxygenase in Fish Gill, *Lipids 25*:849–853 (1990).
16. Surrey, P.K., Spectrophotometric Method for Determination of Lipoxidase Activity, *Plant Physiol. 39*:65–70 (1964).
17. Vick, B.A., and D.C. Zimmerman, Metabolism of Fatty Acid Hydroperoxide by *Chlorella pyrenoidosa*, *Ibid. 90*:125–132 (1989).
18. Kuo, J.-M., B.S. Pan, H. Zhang, and J.B. German, Identification of 12-Lipoxygenase in the Hemolymph of Tiger Shrimp (*Penaeus japonicus* Bate), *J. Agric. Food Chem. 42*:1620–1623 (1994).
19. Wallach, D.P., and V.R. Brown, A Novel Preparation of Human Platelet Lipoxygenase, *Biochim. Biophys. Acta 663*:361–372 (1981).
20. German, J.B., G.G. Bruckner, and J.E. Kinsella, Lipoxygenase in Trout Gill Tissue Acting on Arachidonic, Eicosapentaenoic and Docosahexaenoic Acids, *Ibid. 875*:12–20 (1986).
21. Schomburg, G., and G. Dielman, Identification by Mass of Retention Parameters, *J. Chromatogr. Sci. 11*:151–154 (1973).
22. Beare-Rogers, J.L., and R.G. Ackman, Reaction of Lipoxidase with Polyenoic Acids in Marine Oils, *Lipids 4*:441–443 (1969).
23. Fujimoto, K., Flavor Chemistry of Fish Oils, in *Flavor Chemistry of Lipid Foods*, edited by D.B. Min and T.H. Smouse, American Oil Chemists' Society, Champaign, pp. 190–195 (1989).
24. Grechkin, A., Recent Developments in Biochemistry of the Plant Lipoxygenase Pathway, *Prog. Lipid Res. 37*:317–352 (1998).
25. Gardner, H.W., Recent Investigation into the Lipoxygenase Pathway of Plants, *Biochim. Biophys. Acta 1084*:221–239 (1991).
26. Tanchotikul, U., and T.C.-Y. Hsieh, Volatile Flavor Components in Crayfish, *J. Food Sci. 54*:1515–1520 (1989).

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