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Identification and Sensory Evaluation of Volatile Compounds in Oxidized Porcine Liver

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Headspace solid phase microextraction (HS-SPME) was used to isolate the off-flavor volatile compounds, which are formed during the oxidation of porcine liver induced by iron. Poly(dimethylsiloxane)/divinylbenzene fiber was used in the HS-SPME. Changes in the volatile compounds of oxidized porcine liver and unsaturated fatty acids induced by iron were examined. Results showed that 1-octen-3-one (metallic), hexanol (weak metallic), 1-octen-3-ol (mushroomlike), (*E*)-2-nonenal (cardboardlike), and (*E*,*E*)-2,4-decadienal (fatty, oily) were the main contributors to the overall off-flavor of porcine liver. The results of the sensory evaluation revealed that oxidized arachidonic acid has a major impact on metallic and liverlike off-flavor and that when liverlike off-flavor is perceived, metallic is also included. Oxidized linolenic acid was the most important contributor to the objectionable fishy off-flavor. Oxidized porcine liver exhibited distinct metallic, liverlike, and weak fishy background notes. Liverlike flavor had a high correlation coefficient with odor characteristics such as metallic (0.839) and fishy (0.777). In this study, it was clearly observed that the stronger the metallic and fishy off-flavor the higher the perception of liverlike off-flavor.

KEYWORDS: Porcine liver; oxidative off-flavor; metallic; fishy; liverlike; HS-SPME

INTRODUCTION

Flavor quality is one of the most important factors to determine consumer's acceptance or preference of this characteristic meat byproduct. Several reports on flavor volatiles in liver have been made. For instance, volatile constituents of pressure-cooked pork liver were determined by simultaneous steam distillation and extraction, and a total of 179 components have been identified (*1*). Also, 108 compounds were identified in cooked sheep liver using gas chromatography−mass spectrometry (GC-MS) (*2*). The flavor of liver is not popularly accepted by consumers, so the feasibility of improving the acceptability of liver by employing different cooking methods to eliminate undesirable flavor and odor was reported (*3*, *4*). However, none of these studies mentioned above have addressed problems on liver off-flavor.

Descriptions such as fishy (*5*−*10*), painty (*11*, *12*), grassy (*12*), cardboard (*12*), and metallic (*5*, *13*) have been used to describe off-flavors and odors, and they have been compared with odors and flavors produced by the oxidation of lipids. The use of a profiling method for the sensory assessment of off-flavor could be effective to investigate the off-flavor in foods including porcine liver (PL). An odor quality assessment technique originally suggested (*14*, *15*) for use in odor description and classification studies was used.

Lipid oxidation is one of the major causes of deterioration in the quality of meat and meat products. This is not only particularly true in foods that have high lipid contents but also discernible when the lipid content is relatively low. It causes oxidative rancidity and off-flavor in uncooked and cooked meat systems. Oxidation occurs as a result of the reaction between atmospheric oxygen and unsaturated fatty acids, particularly polyenoic fatty acids (*16*). Furthermore, in lipid-rich foods, the off-flavors produced have been shown to be due largely to autoxidation of lipids producing a range of organic compounds including saturated and unsaturated aldehydes, ketones, alcohols, acids, and hydrocarbons (*17*). Because of the low odor thresholds of the majority of these compounds, the presence of volatile hydroperoxide degradation products even at low concentrations impairs the sensory properties of fat-containing food. The total of unsaturated fatty acids of PL was approximately 53%, which includes oleic acid (OA, 25.8%), linoleic acid (LA, 15.8%), linolenic acid (LLA, 0.2%), and arachidonic acid (AA, 11.2%) (*18*, *19*). To relate the problem of off-flavor development in PL to the lipid oxidation taking place during food processing and formulation variables in a more obvious way, a more detailed sensory relevance of the off-flavor compounds in oxidized PL was considered to be necessary.

Fresh PL does not exhibit strong metallic and/or fishy off-flavor. In our preliminary experiment, where blood in PL was

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almost completely removed, by inserting a cannule in the vein of the liver and running a 0.9% NaCl solution into it until the color of the PL changed and showed no sign of the presence of blood, no fishy or metallic off-flavor was felt as compared to that in the NaCl solution with blood. Also, the smell of the fresh PL was slightly different from that of the PL without blood. It was presumed that iron in the blood could have an impact on the metallic off-flavor in PL. Furthermore, we have noted the fishy and metallic off-flavor in PL caused by addition of even very low levels of ferrous chloride left at room temperature for a relatively short time. Undesirable changes in flavor, color, and even nutritive value occur as meat fats become oxidized (*20*).

The aim of the present study was to assess off-flavor of oxidized unsaturated fatty acids (AA, LLA, LA, and OA) and PL. In addition, headspace solid phase microextraction (HS-SPME) was used to identify the most important off-flavor compounds that contribute to the flavor produced during oxidation of PL.

MATERIAL AND METHODS

Materials. AA (5,8,11,14-eicosatetraenoic acid from PL, 99%) stored at −20 °C and used without further purification and ferrous chloride (tetrahydrate, $FeCl_2$, 99%) were obtained from Sigma Chemical Co. (St. Louis, MO). LLA (99%), LA (99%), OA (99%), and cyclohexanol (internal standard) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 1-Octen-3-one was provided by Takasago Int. Co. (Tokyo, Japan). PL was obtained from domestic commercial sources (Shibaura Internal Organ Co., Tokyo, Japan), frozen (−80 °C) immediately after dissection, and stored until required, when it was allowed to thaw at room temperature.

Sample Preparation. Frozen PL was homogenized. AA, LLA, LA, and OA were each dissolved in ethanol (1 mg/100 mL ethanol). Ten treatments were made, namely, OA (A) and OA + $FeCl_2$ (E), LA (B) and LA + $FeCl_2$ (F), LLA (C) and LLA + $FeCl_2$ (G), AA (D) and AA + $FeCl_2$ (H), 1 g of fresh PL (I), and 1 g of fresh PL + 10 mg of $FeCl_2$ (J). A 0.2 mL amount of the four unsaturated fatty acids solution (E−H) was each mixed with 178 μg of $FeCl_2$ (containing iron in 50 μg). The 10 samples were set up for oxidation at room temperature (20−23 °C) for 60 min, after which they were subjected to sensory evaluation and SPME.

Sensory Analysis. Sensory evaluation was performed by a nine member panel, experienced in descriptive analysis. Panelists received approximately 10 h of training during which they refined terms and decreased group standard deviation. Four PUFA (polyunsaturated fatty acids) samples (one fresh and one iron-treated) and PL samples (one fresh and one iron-treated) of 10 were submitted to panelists. Panelists were asked to identify and define odor characteristics. The odor profiling analysis of all samples was run in triplicate. All sessions were done at 20−23 °C in a sensory panel room equipped with white fluorescent lighting. The total score for each sample was used for statistical analysis. Panelists scored their odor intensity on a five point scale (0 = no smell, 1 = trace aroma, 2 = weak aroma, 3 = moderate aroma, and 4 = strong aroma) for odor in samples.

Statistical Analysis. Statistical analyses were performed by the SPSS Version 7.5J for Windows (SPSS Inc., Chicago). The nonparametric test was processed using the application of the Mann−Whitney U test (*21*), the Kruskal−Wallis test (*22*, *23*), and Pearson's correlation coefficient of analysis to the profiling data.

HS-SPME Analysis. A Supelco SPME fiber holder (manual) and a 65 μm poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB) fiber (Supelco, Inc., Bellefonte, PA) were used for the SPME method. Fiber coated with PDMS/DVB showed a higher affinity for fatty acids and effectiveness for detecting the characteristic components of lipid oxidation products such as aldehydes and alcohols in the preliminary experiment. Before use, the fiber was preconditioned in the GC injection port at 260 °C for 30 min. The sampling procedure involved placing 0.2 mL of each sample into the 4.5 mL glass vial (45 mm high and 14 mm in diameter) and sealing with a screw-top septum-containing cap. The SPME fiber was inserted through the septum, and the sample vial was placed in a water bath maintained at 37 °C. HS volatiles were purged from samples by introducing a stream of oxygen (10 mL/min for 60 min) above the surface of the sample. The Teflon-lined septum covering the vial was pierced with a SPME needle, and the fiber was exposed to each sample HS. The setting on the SPME assembly scale was adjusted to 1.0 scale unit to ensure that the fiber was positioned in the HS above the sample in exactly the same way from run to run. After the extraction by the PDMS/DVB fiber, the volatile compounds were desorbed for 2 min into the injector of a GC (Finnigan MAT, GCQ) at 240 °C.

Table 1. Odor Description Terms in Oxidized Unsaturated Fatty Acids and PL

odor characteristics			
fishy	oily (fatty)	mushroomlike	grassy (green)
metallic	rancid	earthy	woody
liverlike	meatlike	sweet	sea breeze (seaweedlike)

GC-MS. GC-MS analysis was carried out on a GCQ (Finnigan MAT) gas chromatograph interfaced with a GCQ mass spectrometer. Compounds were separated on a CP-WAX 52CB WCOT fused silica capillary column (30 m length × 0.25 mm i.d. × 0.25 μm film thickness; Chrompack, The Netherlands) under the following conditions: injection port temperature, 240 °C; oven temperature, 60 °C (4 min), and then 2 °C/min to 200 °C. The carrier gas was helium, and it was maintained at a constant flow of 1 mL/min and a linear velocity of 40 cm/s. The analysis was done in the splitless mode. MS conditions were as follows: interface temperature, 200 °C; ion source temperature, 200 °C; mass scan range, *m*/*z* 20−450. Ionization was carried out by the electron impact method at 70 eV, with a multiplier voltage of 1500 V.

Identification of Volatile Compounds. Some of the volatile compounds were identified by the comparison of their retention times and mass spectra with those of the authentic compounds, while others were tentatively identified by the comparison of their mass spectral data with those reported in the literature. The authentic compounds were used as follows: hexanal, heptanal, octanal, hexanol, (*E*)-2-octenal, acetic acid, (*E*,*E*)-2,4-heptadienal, (*E*)-2-nonenal, propionic acid, octanol, nonanol, (*E*,*E*)-2,4-decadienal, and hexanoic acid (Wako); 1-penten-3-ol, 1-octen-3-ol, and (*E*,*Z*)-2,6-nonadienal (Aldrich, Milwaukee, WI); and 1-octen-3-one (Takasago Int. Co.).

GC−Olfactometry (GC-O) Analysis. *Chromatographic Condition.* GC-O was performed using a Shimadzu GC-9A gas chromatograph (Shimadzu, Japan) equipped with a flame ionization detector (FID) and a sniffing port ODO-1 (GL-Science, Japan). Separations of the volatile compounds were done on CP-WAX 52 CB fused silica capillary column (30 m length × 0.25 mm i.d. × 0.25 μm film thickness; Chrompack, The Netherlands). The injector and detector were maintained at 240 and 250 °C, respectively. The oven temperature was programmed at 60 °C (4 min hold) and then increased to 200 °C at 2 °C/min. The sniffing port was supplied with humidified air at 30 mL/min.

Panel Condition. Three assessors experienced in sensory analysis and trained in GC-O analysis were employed. During GC-O analysis, sniffers were asked to give a description of each perceived odor, even if they did not recognize it. Two replicates of each HS-SPME sample (5 × 2) were performed. Each run lasted for 40 min to avoid fatigue.

RESULTS AND DISCUSSION

Odor Characteristics. Odor description terms were selected to cover all of the describable aspects of odor, in unsaturated fatty acids and in PL (**Table 1**). The odor description terms of the samples used were fishy, metallic, oily (fatty), rancid, liverlike, meatlike, mushroomlike, grassy (green), woody, earthy, sweet, and sea breeze (seaweedlike). For the oxidation of unsaturated fatty acids and PL with and without iron, 12 odor descriptions were awarded out of a total of 23 noted odors.

Table 2. Fishy, Metallic, and Liverlike Intensity in Unsaturated Fatty Acids and PL

	odor description term		
sample	fishy	metallic	liverlike
A: C18:1	1.33^{bc}	1.33^{bc}	1.22^{bc}
B: C18:2	2.11^{bc}	1.11^{bc}	0.67^{bc}
C: C18:3	2.00^{bc}	0.67^{c}	0.33^{bc}
D: C20:4	3.00^{ab}	3.33^{a}	2.67^{ab}
E: C18:1 + $FeCl_2$	1.11^{c}	0.89^{c}	0.22^{c}
F: C18:2 + $FeCl_2$	1.56^{bc}	2.11^{b}	1.44^{b}
G: C18:3 + $FeCl_2$	3.89^{a}	2.67^{ab}	1.22^{bc}
H: C20:4 + $FeCl_2$	2.56^{b}	3.56^{a}	3.44^{a}
I: liver	2.22^{bc}	1.89^{bc}	2.56^{ab}
J: liver + $FeCl_2$	2.56^{b}	3.44^{a}	3.44^{a}

[a−c] Means within the same column bearing the same superscript are not significantly different ($p < 0.05$).

Metallic, fishy, and liverlike were the terms most frequently used by descriptors, and these describe the overall odor of oxidized unsaturated fatty acids and PL. The 12 odor characteristics were grouped into three categories: (i) odor terms such as fishy, metallic, liverlike, and meatlike, which were generally expressed in the animal model; (ii) odor terms such as oil (fatty), rancid, and sweet, which were generally expressed in the fats and solvents model; and (iii) odor terms such as grassy, mushroomlike, woody, sea breeze (seaweedlike), and earthy, which were expressed in the plant and nature model. In conclusion, the results from odor description terms clearly showed that it was possible to represent most of the odor qualities by odor terminology, which was a positive evidence for the validity of the numerical judgment procedure used.

Sensory Evaluation. To help in the development of the procedure and to let the panelists know how their assessments were compared with others, each panelist's means and standard deviations were compared. The Kruskal−Wallis test was used to reveal the degree of significance of odor description terms among samples. Of the 12 odor description terms, fishy, metallic, and liverlike ($p < 0.05$) showed highly significant differences among the samples (**Table 2**). This showed that the samples could be considered as perceptually different, which was a necessary condition for using the odor quality assessment technique in a meaningful way. The difference in odor qualities among samples might be the influence of unsaturated fatty acids caused by the addition of metal ion, Fe^{2+}. The odor terms fishy, metallic, and liverlike have been used to describe the effects of oxidative deterioration of unsaturated fatty acids and other food products (*12*, *13*).

Using the Man−Whitney U test, the odor characteristic of sample C exhibited a weak fishy odor, but when added with Fe^{2+} (sample G), the fishy odor increased with a significant difference ($p < 0.01$). Although metallic and liverlike odor was not felt in sample C, addition of Fe^{2+} slightly increased their intensity. It is suggested that addition of Fe^{2+} in LLA contributed significantly to the increasing intensity of fishy odor. The oxidized byproduct seems to be the source of the distinct fishy odor in PL. The fishy, metallic, and liverlike odors were relatively unchanged when Fe^{2+} was added to AA. However, the aroma intensity for metallic and liverlike were 3.56 and 3.44 in sample H, which was stronger than the fishy intensity, which was 2.56. Addition of Fe^{2+} to AA plays a bigger role in the formation of metallic and liverlike intensities more than the fishy intensity. The odor intensity of metallic with and without Fe^{2+} in PL sample was 3.44 and 1.89, respectively, indicating that addition of Fe^{2+} increased the intensity of metallic. Similarly, the liverlike odor also increased in intensity with addition of Fe^{2+}; the mean intensity increased from 2.56 to 3.44. In general, addition of Fe^{2+} increased the odor intensity of metallic and liverlike odors in AA and in PL.

Table 3. Correlation Coefficient[a]

	correlation coefficient
odor characteristics	liverlike
fishy	0.777*
metallic	0.839**

[a] On the basis of the finding in **Table 2**. *Significant effect at $p < 0.05$. **At $p < 0.01$.

Table 4. HS-SPME Volatile Compounds of Oxidized Unsaturated Fatty Acids and PL (ND, Not Detected)

	estimated concentration (mg/kg)[a]					
compound	PL	OA	LA	LLA	AA	odor description[b]
hexanal[c]	0.4	ND	0.4	ND	18.1	green, grassy
1-penten-3-ol[c]	ND	ND	ND	0.7	ND	pungent
heptanal[c]	ND	0.3	ND	ND	ND	unpleasant
octanal[c]	ND	0.8	ND	ND	ND	orange peel
1-octen-3-one[c]	1.8[e]	ND	0.2	ND	3.2	metallic
(*Z*)-4-heptenal[d]	0.7[e]	ND	3.5	ND	4.0	fishy, unpleasant
hexanol[c]	0.2[e]	ND	ND	ND	ND	weak metallic
(*E*)-2-octenal[c]	1.5	ND	2.4	ND	6.4	tallowy, stale
1-octen-3-ol[c]	3.5	ND	0.3	ND	9.6	mushroomlike
(*E*,*Z*)-2,4-heptadienal[d]	ND	ND	ND	6.0	ND	fishy
acetic acid[c]	0.2[e]	2.0	ND	ND	ND	vinegar
(*E*,*E*)-2,4-heptadienal[c]	ND	ND	ND	5.1	ND	fishy
(*E*)-2-nonenal[c]	0.8[e]	ND	0.6	ND	0.3	cardboardlike
(*E*,*Z*)-3,5-octadiene-2-one[d]	ND	ND	ND	0.8	ND	fruity, fatty
propionic acid[c]	ND	ND	ND	0.3	ND	unpleasant
(*E*,*E*)-3,5-octadiene-2-one[d]	ND	ND	ND	0.7	ND	fruity, fatty
(*E*,*Z*)-2,6-nonadienal[c]	ND	ND	ND	0.4	ND	cucumber
octanol[c]	ND	0.3	ND	ND	ND	oily, walnut
(*E*,*E*)-2,4-nonadienal[d]	ND	ND	ND	ND	0.2	oily, fatty
(*E*,*Z*)-2,4-decadienal[d]	0.3[e]	ND	0.2	ND	3.5	fatty, oily
nonanol[c]	ND	0.1	ND	ND	ND	oily, walnut
(*E*,*E*)-2,4-decadienal[c]	0.3[e]	ND	0.8	ND	1.4	fatty, oily
(*E*,*Z*)-3,5-undecadien-2-one[d]	ND	ND	ND	ND	0.5	fatty, fried
hexanoic acid[c]	0.4[e]	ND	0.3	ND	2.3	fatty
(*E*,*E*)-3,5-undecadien-2-one[d]	ND	ND	ND	ND	0.3	fatty, fried

[a] Estimated concentration with respect to the internal standard. [b] Odor quality perceived at the sniffing port. [c] The compound was identified by comparing its mass spectral data and retention time with those of the reference compound. [d] The compound was tentatively identified by comparing its mass spectral data with those reported in the literature. [e] Newly identified in oxidized PL.

Correlation Coefficient of Liverlike and Odor Characteristics. In the correlation matrix for the liverlike and other odor characteristics in sample J, it can be seen that the liverlike odor has a positive correlation with metallic and fishy off-flavors. Especially, a high correlation was obtained for odor characteristics such as metallic and fishy, with correlation coefficients of 0.839 and 0.777, respectively (**Table 3**). Thus, in this study, it appears that the stronger the metallic and fishy odor the higher perception of a liverlike odor. To study the correlation between the sensory evaluation and the chemical composition of unsaturated fatty acids and PL, the volatiles were identified.

Volatile Compounds of PUFAs. Table 4 shows the volatile compounds identified by HS-SPME in each $FeCl_2$ added unsaturated fatty acids and PL at 37 °C. Twenty-five volatile components including 12 aldehydes, five alcohols, five ketones,

and three acids were identified from the oxidative unsaturated fatty acids by the HS-SPME method.

AA. Of the volatile compounds identified from autoxidized AA, seven aldehydes, six of them unsaturated, prevailed. Also, 1-octen-3-one, 1-octen-3-ol, (*E*,*Z*)-3,5-undecadien-2-one, (*E*,*E*)-3,5-undecadien-2-one, and hexanoic acid were detected. Generally, hexanal, 1-octen-3-one, 1-octen-3-ol, (*Z*)-4-heptenal, (*E*)-2-nonenal, and 2,4-decadienal were found to be widely distributed in oxidized AA. The lower threshold value (*T*) for the volatile carbonyls, especially 1-octen-3-one ($T = 0.005$ ppb), (*E*)-2-nonenal (cucumber, cardboard; $T = 0.08$ ppb), (*E*,*Z*)-2,6-nonadienal (cucumber peel; $T = 0.01$ ppb), resulted in odors stronger than their corresponding alcohols (1-octen-3-ol and 3,6-nonadien-1-ol), whose threshold values are 10 ppb (*24*).

The sample of oxidized AA exhibited a very distinct metallic, liverlike character with heavy, fishy background notes. The metallic odor was found to be associated with certain vinyl ketones, and unpleasant fishy character was found to be associated with the class of compounds containing a 2,4-dienal structure (*6*). On the basis of odor quality, 1-octen-3-one in oxidized AA was considered to contribute to overall metallic off-flavor. Forss and co-workers (*5*, *24*) were unable to identify compounds with a fishy aroma in a batch of fishy-flavored butterfat although they noted that 1-octen-3-one contributed some metallic notes also observed in the tainted butter. The flavor threshold for 1-octen-3-one has been reported at 0.005 ppb (*24*). Moreover, Hammond and Hill (*25*) reported that 1-octen-3-one accounts for the metallic flavor of oxidized milk fat.

Considerable attention has been given to (*Z*)-4-heptenal in relation to fishy flavors (*11*, *12*, *26*, *27*). When (*Z*)-4-heptenal was added to vegetable oils at a level of 0.2−0.5 ppm, it gave stale, burnt flavors. Although (*Z*)-4-heptenal has been referred to as a cold store cod compound, it did not elicit a ready recognition of fishiness when evaluated alone by individuals in the laboratory, which was in agreement with earlier observations (*28*). Instead, it contributed burnt aroma characteristics when high levels (1.0 ppm) were found in fish oil (*29*). The threshold level of (*Z*)-4-heptenal is 0.04 ppb (*27*), and it appears to complement the fishy/burnt flavor of the decatrienal when present at high concentrations (*29*). The occurrence of (*Z*)-4-heptenal in oils that had undergone some degree of oxidation provided the basis for early interpretations that this aldehyde was derived through lipid autoxidation (*30*).

(*E*,*Z*)-2,4-Decadienal and (*E*,*E*)-2,4-decadienal have detection threshold concentrations of 20 and 320 ppb in paraffin oil, respectively (*31*), and these concentrations indicate that the compounds could be influential in fish oil flavor. However, the decadienals contribute fatty, fried, aldehyde-like flavors at higher concentrations (>10 ppm) (*29*).

1-Octen-3-one and the corresponding alcohol were characterized as the compounds responsible for the metallic and mushroomlike off-odors in oxidized dairy products (*32*, *33*). Whitfield et al. (*34*, *35*) identified 1-octen-3-ol as a volatile compound important to the metallic off-flavor of deep sea prawn (*Hymenopenaeus sibogae*) and sea sand lobster (*Ibacus peronii*). Matsutake alcohol (1-octen-3-ol) from *Armillaria matsutake* (*36*) has been isolated from oxidized butter cream and held responsible for a mushroomlike flavor in dairy products (*33*). Eight carbon volatile alcohols and ketones have been found to occur in most seafoods, where they contribute distinct fresh plantlike, metallic aromas. The oxidation of unsaturated fatty acids and particularly the oxidation of AA chiefly contribute to a metallic off-flavor. These volatiles exhibited metallic and liverlike off-flavors as felt in the sensory evaluation.

LLA. In the oxidized LLA, the major compounds are 1-penten-3-ol, (*E*,*Z*)-2,4-heptadienal, (*E*,*E*)-2,4-heptadienal, (*E*,*Z*)-3,5-octadien-2-one, (*E*,*E*)-3,5-octadien-2-one, propionic acid, and (*E*,*Z*)-2,6-nonadienal. (*E*,*Z*)-2,4-Heptadienal, (*E*,*E*)- 2,4-heptadienal, (*E*,*Z*)-3,5-octadien-2-one, and (*E*,*E*)-3,5-octadien-2-one are the main volatile carbonyls derived from LLA. 1-Penten-3-ol has previously been isolated from the freshwater fish (*7*), and the characteristic fishy attribute of sardine has been reported (*37*). However, the concentration of 1-penten-3-ol in fish remains below its recognized threshold, 400 ppb (*38*); therefore, it is unlikely that this volatile contributes strongly to the characteristic aroma of freshly harvested fish. Fresh seafood flavors exhibit limited quantities of autoxidatively derived carbonyls.

The seven carbon aldehydes, (*E*,*Z*)-2,4-heptadienal ($T = 3.5$ ppb) (*26*), (*E*,*E*)-2,4-heptadienal (49 ppb) (*39*), and eight carbon volatile ketones, (*E*,*Z*)-3,5-octadien-2-one and (*E*,*E*)-3,5-octadien-2-one generally contribute to fresh seafood flavor, and fishy flavor has been associated with the autoxidative deterioration of PUFAs, particularly ω-3-type fatty acids (*40*). The fishy off-flavor of *Uroglena-Americana chryosophyceae* has been attributed to the formation of (*E*,*Z*)-2,4-heptadienal, (*E*,*Z*)-2,4-decadienal, and (*E*,*E*)-2,4-decadienal (*41*). Furthermore, (*E*,*Z*)-2,6-nonadienal, which has been isolated from autoxidized milk fat and soybean oil, exhibited a grassy flavor (*25*, *42*). Badings (*12*) isolated 3,5-octadien-2-one from oxidized linolenate and oxidized milk fat.

Ullrich and Grosch reported that (*Z*)-1,5-octadien-3-one is the most intense odorant formed by autoxidation of LLA (*43*) and (*Z*)-1,5-octadien-3-one is responsible for the "fishy" note in boiled trout (*44*, *45*). It was also found that (*Z*)-1,5-octadien-3-one and methional are responsible for the fishy off-flavor in dry spinach (*46*). Unfortunately, in this study, (*Z*)-1,5-octadien-3-one could not be identified in oxidized LLA sample by SPME.

Volatile Compounds of PL. Sensory evaluation showed that the oxidized PL exhibited very distinct metallic, fishy, and liverlike background notes. Hexanal, 1-octen-3-one, (*Z*)-4-heptenal, (*E*)-2-octenal, 1-octen-3-ol, (*E*)-2-nonenal, (*E*,*Z*)-2,4-decadienal, and (*E*,*E*)-2,4-decadienal are the main HS volatile products of PL oxidation. These compounds have also been detected throughout the present study, in oxidized AAs. GC-O of the HS-SPME sample revealed that 1-octen-3-one (metallic), hexanol (weak metallic), 1-octen-3-ol (mushroomlike), (*E*)-2-nonenal (cardboardlike), and (*E*,*E*)-2,4-decadienal (fatty, oily) are potent odorants of oxidized PL. In addition, 1-octen-3-one, (*Z*)-4-heptenal, hexanol, (*E*)-2-nonenal, (*E*,*Z*)-2,4-decadienal, and (*E*,*E*)-2,4-decadienal are newly detected volatile compounds in oxidized PL.

The fishy odor that was revealed by sensory evaluation might have been possibly due to (*E*,*Z*)-2,4-heptadienal and (*E*,*E*)-2,4-heptadienal. Although these two compounds were not identified in the oxidized PL samples, they were detected in the oxidized LLA. They were characterized to be fishy by GC-O. Their thresholds have been reported (*26*, *39*) to be low so that even if they were not detected in the GC analysis, they still could possibly contribute to the fishy off-flavor in oxidized PL.

Malodorous compounds such as (*Z*)-4-heptenal, hexanol, (*E*)-2-nonenal, propanoic acid, (*E*,*E*)-2,4-decadienal, and hexanoic acid might have an off-flavor impact on the aroma of PL. Stark and Forss (*32*) isolated and characterized 1-octen-3-one and the

corresponding alcohol as the compounds responsible, respectively, for metallic and mushroomlike off-odors in oxidized dairy products.

The unsaturated aldehydes and ketones have the lowest sensory thresholds and are usually considered the primary sources of oxidized off-flavors (*47*). The presence of 1-octen-3-one could have a significant impact on the overall off-flavor of PL. Certain fishy off-flavors in cold-stored cod (*26*, *27*), cold-stored butter (*12*), and rancid soybeans (*11*) have been reported to be caused by (*Z*)-4-heptenal. The occurrence of (*Z*)-4-heptenal in oils that had undergone some degree of oxidation provided the basis for early interpretations that this aldehyde was derived through lipid oxidation. Many investigators have attempted to characterize fishy off-flavors and identify the components responsible for such odor. Fishiness in butterfat was due to a combination of compounds that possess an oily flavor in hexanal, heptanal, and 2-hexenal and a metallic flavor in 1-octen-3-one (*32*, *48*). In the early stage of oxidation and deterioration of dairy products, metallic and fishy (like cod liver oil) taints can develop before those of rancidity (*48*, *49*). This flavor may be associated with other flavor defects such as oily, fishy, and tallowy and usually develops as a result of oxidation. The odor quality of the oxidized AA exhibited metallic character, and it could contribute to the metallic and liverlike notes of oxidized PL. The principal metallic and fishy off-flavor of PL originates from AA, LLA, and LA in PL and oxidative degradations during processing and storage of PL.

In summary, 1-octen-3-one has been found to be primarily responsible for the metallic off-flavor observed in oxidative PL, and (*E*,*E*)-2,4-heptadienal, (*E*,*Z*)-2,4-heptadienal, and (*Z*)-4-heptenal may add heavier fishy notes. Compounds causing the distinct stale and cardboardlike odors were verified as (*E*)-2-octenal and (*E*)-2-nonenal acting as overall off-flavors. The metallic and fishy off-flavor in PL was the result of an oxidative process in which some trace amounts of heavy metal ions, such as iron, would have catalyzed the oxidation of unsaturated fatty acids—AAs and LLAs—in PL. It appears that the stronger the metallic and fishy flavor, the higher the perception of liverlike off-flavor. 1-Octen-3-one was found to be the main contributor to the metallic off-flavor in oxidized PL.

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