

# Fatty Acid Profile and Aroma Compounds of Lipoyxygenase-Modified Chicken Oil

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**ABSTRACT:** Adipose fat tissue, which contributes 1.6–5.8% of total chicken carcass weight, has been underutilized by chicken processors because of its “chickenish odor.” The objective of this study was to prepare a chicken oil from which the undesirable odor notes were eliminated and in which the desirable volatile compounds were enhanced. Chicken adipose fat was dry-rendered at 140°C for 30 min and yielded 78.5% oil. Monoenoic FA constituted 55.8% of the chicken oil, and of that oleic acid constituted 92.8%. Treatment of chicken oil with an algal lipoyxygenase extracted from *Ulva* spp. at 33°C for 30 min resulted in an increase of 0.40% in total monoenoic acids, a decrease of 33.3% in total polyenoic acids, and a decrease in total FA of 0.8%. A noticeable improvement in the odor of chicken oil after lipoyxygenase treatment was observed by sensory evaluation and a GC-sniffing technique. The modified chicken oil contained more desirable volatile compounds—ethyl acetate, pentanal, 2-pentyl furan, *E*-2-heptenal, and nonanal—than the original chicken oil, provided fruity and tea-leaf aromas, and had reduced levels of the undesirable volatile compounds heptanal, 2,4-heptadienal, 2,4-nonadienal, and dodecanal. These modifications reduced the chickenish and oxidized odor notes.

Paper no. J10785 in *JAOCs* 81, 921–926 (October 2004).

**KEY WORDS:** Chicken oil, fatty acids, lipoyxygenase, odor notes, volatile compounds.

Adipose fat constitutes 1.6–5.8% (w/w) of the whole chicken carcass. In Taiwan, 10,000–37,000 tons of chicken fat is primarily disposed of each year (1).

Our preliminary studies showed that monoenoic acids constitute more than 50% of the total FA in chicken oil. For humans, an increase in dietary monoenoic acids to a ratio of saturated/monoenoic/polyenoic = 8:15:7 from a previous ratio of 10:10:10 in dietary fat (2) has been recommended by the American Heart Association (2). For this health benefit, chicken oil should be recovered for human consumption. However, the odor of chicken oil has been considered too “chickenish.” It would need to be modified before it could be considered acceptable for human use. A previous study on aroma modification of fish oil using lipoyxygenase (LOX) extracted from the marine alga *Ulva conglobata* indicated the enzymatic method had a potential for application (3).

The present study involved dry-rendering of chicken adipose fat and modifying the resultant chicken oil with a stable

algal LOX to improve the aroma. The goal was to enhance the utility of rendered chicken oil as a food ingredient and to provide the benefit that would be associated with consumption of a fat having a high monoenoic acid content. In addition, turning the fat into value-added by-products may help the poultry processing industry to reduce its waste output further.

## MATERIALS AND METHODS

**Dry-rendering of chicken oil.** The adipose fat tissue of chickens was collected from a poultry processor (Great Wall Enterprise Co., Taipei, Taiwan). The fat tissue was cut into 1 cm<sup>3</sup> pieces and dry-rendered in an oil bath (B-485; Büchi, Flawil, Switzerland) held at 120–150°C for 10–30 min. Yield was calculated as the weight of the oil obtained by dry-rendering as a percentage of that obtained by solvent extraction. Peroxide value (POV) of the rendered oil was determined following an AOAC method (4).

**Solvent extraction of chicken oil.** Chicken oil was extracted from adipose fat tissue using chloroform/methanol (2:1 vol/vol) at a fat-to-solvent ratio of 1:10 (wt/vol) according to the procedure described by Folch *et al.* (5). Hexane extraction of chicken oil was done using the same ratio of chicken fat to solvent (wt/vol) as in the method of Folch *et al.* The mixture was stirred, then set on ice for 5 min to prevent oxidation. The extraction procedure was repeated three or four times to ensure the oil was extracted completely.

**Preparation of algal LOX extract.** Freshly harvested algae (*Ulva* spp.) from the Pacific coast of northern Taiwan were homogenized with 5 vol of 0.05 M potassium phosphate buffer (pH 7.5) containing 1 mM glutathione (reduced form; Sigma, St. Louis, MO) in a polytron (PT 3000; Kinematica, Littau, Switzerland) at 4°C for 30 s and centrifuged at 20,000 × *g* for 15 min. The supernatant constituted the crude LOX extract (6).

**Treatment of chicken oil with using LOX.** Chicken oil was modified with algal LOX using the method of Hu and Pan (3). Chicken oil (10 g) and 0.01% Tween 20 were reacted with a volume of 500 mL of LOX extracted from *Ulva* spp. having a specific activity of 0.31 μmol hydroperoxy FA/mg protein-min in 0.05 M potassium phosphate buffer, pH 7.5. The reaction mixture was incubated in capped tubes with shaking at 33°C for 2 h in the dark.

**RP-HPLC of hydroperoxy derivatives.** The hydroperoxy FA products of the LOX reaction, e.g., hydroperoxyeicosatetraenoic acid (HpETE), were extracted with ethyl acetate and reduced with glutathione to hydroxyeicosatetraenoic acid (HETE), then

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methyated with diazomethane. The resulting compounds were separated on a solid-phase extraction column (J&W Scientific, Folsom, CA) and then subjected to RP-HPLC analyses. These analyses used a Lichrospher 100RP-18 column (25 × 0.4 cm, 5 µm; E. Merck KGaA, Darmstadt, Germany) equipped with a Waters pump and UV detector (Model 490E; Waters, Milford, MA). The HETE compounds were eluted isocratically by a solvent system of methanol/water (75:25 vol/vol) buffered with 50 mM ammonium acetate containing 1 mM EDTA at a flow rate of 1 mL/min (7). The LOX reaction products were identified by comparison with authentic standards of 5-, 8-, 11-, 12-, and 15-HETE (Cayman Chemical, Ann Arbor, MI).

**FA analysis.** Chicken oil (0.2 g) was saponified and esterified with methanol/boron trifluoride (4) and analyzed by GC (GC-14A; Shimadzu, Kyoto, Japan) with a DB-23 column of 60 m × 0.252 mm (J&W Scientific, Folsom, CA). The injector and FID temperatures were set at 250°C. The oven temperature was programmed from 70 to 210°C at a rate of 8°C/min. The carrier gas was hydrogen at a flow rate of 1 mL/min. The FA were identified by comparing their retention times with those of standard FA (GLC-461; Nu-Chek-Prep, Elysian, MN) and quantified using C<sub>13:0</sub> as an internal standard.

**Collection of volatile compounds.** After incubation of the chicken oil with the algal LOX, 15% NaCl was added to break the emulsion. To the chicken oil (500 mL) and the LOX-modified oil (500 mL) were added 5 ppm of benzoic acid as an internal standard to quantify the volatile compounds, which were collected for 2 h using a reduced-pressure rotavapor (R-114/C; Büchi, Flawil, Switzerland) at 140°C, 25–35 mm Hg using a vacuum distillation controller (B-720, Büchi), and a liquid N<sub>2</sub> trap, respectively. The collected volatiles were washed with distilled water and extracted with pentane/ether (1:1, vol/vol). After evaporating the solvent, the concentrate was washed with saturated KCl (1:1, vol/vol) to remove polar residues and again extracted with the same solvent. The volatiles in the pentane/ether layer were stored at –20°C to remove water in crystallized form. The solvent extract was then dried over anhydrous sodium sulfate and concentrated using a spinning band distillation apparatus followed by GC analysis.

**GC and GC-sniffing analysis.** The aroma concentrates were analyzed based on our previous method (3). A Shimadzu GC-14A gas chromatograph was equipped with a CP-Wax 52CB fused-silica capillary column (50 m × 0.53 mm i.d.; Chrompack Inc., Middleburg, The Netherlands) and FID. The oven temperature was programmed from 35 to 200°C at a rate of 1.5°C/min, then held for 70 min. Both injector and detector temperatures 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 at a ratio of 8:1 (vol/vol) between the olfactory device (SGE, Austin, TX) and the FID. One panelist evaluated the odor of the volatile components at the sniffing port; and all questionable odor descriptions were evaluated by two additional panelists; all panelists were consistent in their perceptions of odors. The analysis was repeated twice and evaluated by two panelists.

The retention indices (RI) of the volatile components were

calculated using *n*-paraffins of 6–25 carbon chain lengths (Sigma, St. Louis, MO) as reference compounds. The data from GC-sniffing and GC–MS were correlated based on the RI of each compound, which was calculated following the procedures of Schomburg and Dielmann (8).

**GC–MS analysis.** A GC/mass selective detector (MSD) (Agilent 6890 GC/5973A MSD) system was used for analyses. Each extract (1 µL) was injected in the splitless mode (injector temperature: 250°C) into a fused-silica capillary column (CP-Wax 52CB, 60 m length × 0.25 mm i.d. × 0.25 µm film thickness (Chrompack Inc.)). The oven temperature was programmed from 40 to 210°C at 2°C/min, then held for 30 min. The carrier gas was helium at a flow rate of 1 mL/min. MSD conditions were as follows: capillary direct interface temperature, 265°C; ion source temperature, 200°C; ionization energy, 70 eV; mass range, 25–400 amu; and electron multiplier voltage, 1350 V.

**Sensory evaluation.** Seven staff members and graduate students who showed consistency in odor description evaluated the odor of the chicken oils. Oily odor intensity was ranked from 1 to 9. Scores ≥5 indicated oxidized odor, and odor increased in intensity as the scores increased.

## RESULTS AND DISCUSSION

**Yield and characteristics of chicken oil.** Rendering time and temperature affected the yield, degree of oxidation, and odor intensity of chicken oil (Table 1). Maximal yield by dry-rendering was 78.5%, obtained at 140°C for 30 min; this was higher than yields obtained at 120 to 150°C for 10 to 30 min (68.5–77.5%). Solvent extraction yielded 81.0 ± 0.2 to 82.5 ± 0.3%. The POV of dry-rendered oil ranged from 0.50 ± 0.08 to 1.55 ± 0.20 meq/kg and increased with increased rendering temperature and prolonged rendering time. POV of the solvent extract was 0.35 ± 0.14 meq/kg or less, and the sensory score was 2.0. Oil rendered at 150°C had a stronger oily odor, with sensory scores of 6.0–7.0. In spite of the relatively strong oxidized odor (score of 6.5) and high POV value, rendering at 140°C for 30 min was used to obtain the maximal yield of chicken oil. Treatment of this oil with LOX was tested to modify the odor.

**Changes in the FA profile of chicken oil after algal LOX modification.** Dry-rendered chicken oil consisted of 55.8% monoenoic acids (MUFA), in which oleic acid contributed 92.8%, followed by 16:1, 20:1, and 14:1 (Table 2). Saturated FA (SFA) contributed 36.4% of the total FA. Palmitic acid (16:0) was the major SFA, being 78.4% of the total SFA, followed by 18:0 (18.8%); 14:0, 20:0, and 22:0 were all minor SFA. PUFA contributed only 7.8% in chicken oil. The major PUFA was 18:2, followed by 18:3.

Since LOX requires substrates containing a *cis,cis*-1,4-pentadiene structure, the amount of PUFA was expected to decrease after LOX modification. Treatment of chicken oil with algal LOX resulted in a decrease in PUFA content by 33.3% of the initial PUFA content; SFA increased by 4.5%; and MUFA remained almost unchanged. However, the decrease in total FA was only 0.8% (Table 2).

**TABLE 1**  
Effects of Rendering Temperature and Time on Yield, Peroxide Value (POV),  
and Odor Intensity of Chicken Oil in Comparison With Solvent Extraction

| Processing procedure                 | Heating       |               | Oil temp <sup>a</sup><br>(°C) | Yield <sup>b</sup><br>(%) | POV <sup>b</sup><br>(meq/kg) | Sensory score on<br>odor intensity <sup>d</sup> |
|--------------------------------------|---------------|---------------|-------------------------------|---------------------------|------------------------------|---|
|                                      | Temp.<br>(°C) | Time<br>(min) |                               |                           |                              |   |
| Dry rendering                        | 120           | 10            | 95.60                         | 68.5 ± 0.2                | 0.50 ± 0.08                  | 2.0   |
|                                      | 120           | 20            | 96.60                         | 72.5 ± 0.4                | 0.55 ± 0.11                  | 3.0   |
|                                      | 120           | 30            | 96.95                         | 73.0 ± 0.1                | 0.65 ± 0.07                  | 3.5   |
|                                      | 130           | 10            | 96.65                         | 74.0 ± 0.1                | 0.70 ± 0.04                  | 3.5   |
|                                      | 130           | 20            | 97.50                         | 75.3 ± 0.2                | 0.85 ± 0.12                  | 4.0   |
|                                      | 130           | 30            | 98.45                         | 77.5 ± 0.3                | 0.95 ± 0.09                  | 4.5   |
|                                      | 140           | 10            | 98.80                         | 75.5 ± 0.5                | 1.05 ± 0.04                  | 5.0   |
|                                      | 140           | 20            | 102.25                        | 76.0 ± 0.4                | 1.20 ± 0.05                  | 5.5   |
|                                      | 140           | 30            | 104.25                        | 78.5 ± 0.5                | 1.20 ± 0.16                  | 6.5   |
|                                      | 150           | 10            | 99.70                         | 73.5 ± 0.4                | 0.95 ± 0.11                  | 6.0   |
|                                      | 150           | 20            | 100.80                        | 74.0 ± 0.1                | 1.05 ± 0.14                  | 6.5   |
|                                      | 150           | 30            | 109.00                        | 74.2 ± 0.2                | 1.55 ± 0.20                  | 7.0   |
| Extraction<br>of Folch <i>et al.</i> | 15–20         | 30            | 15–20                         | 82.5 ± 0.3                | 0.30 ± 0.02                  | 2.0   |
| Hexane                               | 15–20         | 30            | 15–20                         | 81.0 ± 0.2                | 0.35 ± 0.14                  | 2.0   |

<sup>a</sup>Temperature achieved in the oil sample during rendering.

<sup>b</sup>All data were the means of triplicate determinations and are expressed as mean ± SD.

<sup>c</sup>POV, peroxide value, following the AOAC protocol (3).

<sup>d</sup>The sensory score of chicken oil odor intensity ranged from 1 to 9 (low to high); scores ≥5 represented samples that smelled oxidized.

<sup>e</sup>By the procedure in Reference 4.

Based on RP-HPLC analysis of the positions of the hydroxy derivatives of eicosatetraenoic acid (ETE), the LOX extracted from *Ulva* spp. used in this study was a mixture of three isozymes—5-, 12-, and 15-LOX—using arachidonic acid (ETE;

20:4) as substrate (Fig. 1). Considering these three isozymes, 12-HpETE (hydroperoxyeicosatetraenoic acid) was the major product; 15-HpETE was minor; and the amount of 5-HpETE formed was less than one-third of the 15-HpETE. Similar results

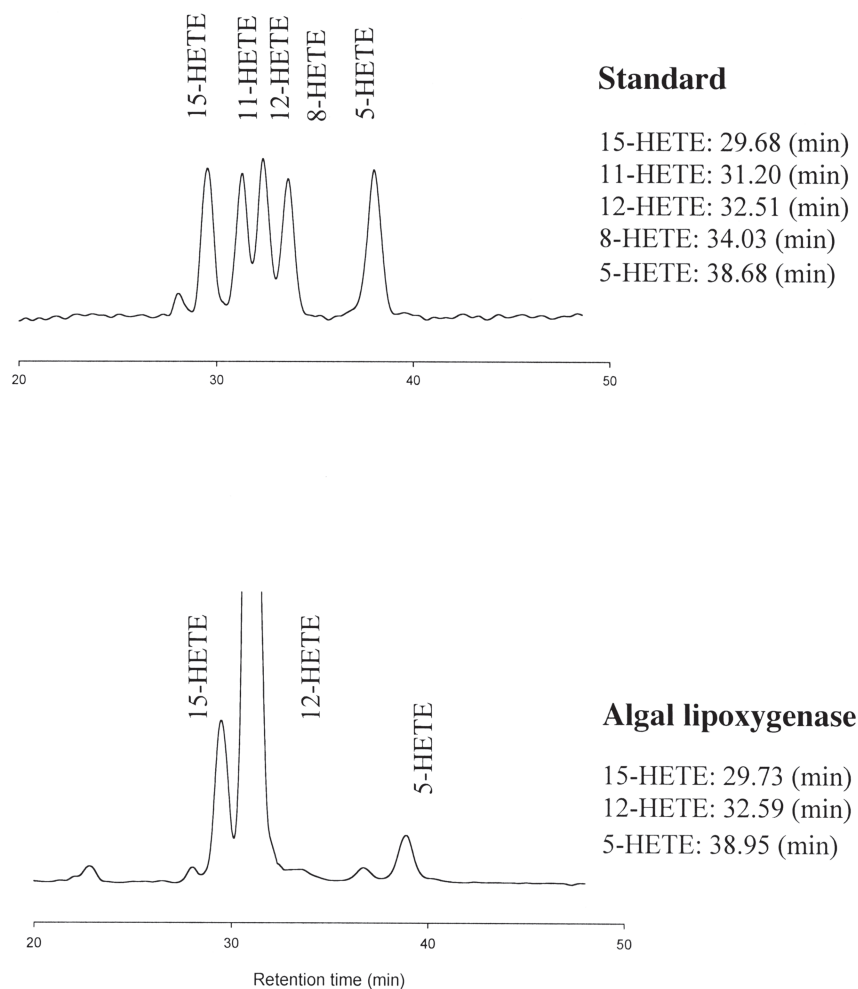
**TABLE 2**  
Changes in FA Composition of Chicken Oil Modified with Algal Lipxygenase (LOX)  
for 30 min at 33°C

| FA         | Control <sup>a</sup>  |         | LOX-modified oil <sup>b</sup> |         |          |
|------------|-----------------------|---------|-------------------------------|---------|----------|
|            | mg/g oil <sup>b</sup> | % Total | mg/g oil <sup>b</sup>         | % Total | % Change |
| SFA        | 273.43 ± 2.24)        | 36.42   | 285.66 ± 1.23                 | 38.38   | +4.47    |
| 14:0       | 4.46 ± 0.45           |         | 4.73 ± 0.21                   |         |          |
| 15:0       | ND                    |         | ND                            |         |          |
| 16:0       | 214.39 ± 4.76         |         | 224.32 ± 0.14                 |         |          |
| 18:0       | 51.32 ± 1.65          |         | 52.03 ± 0.05                  |         |          |
| 20:0       | 1.15 ± 0.18           |         | 2.21 ± 0.03                   |         |          |
| 22:0       | 2.11 ± 0.41           |         | 2.37 ± 0.12                   |         |          |
| Total MUFA | 418.54 ± 1.29         | 55.76   | 420.17 ± 1.01                 | 56.40   | +0.40    |
| 14:1       | 4.87 ± 0.34           |         | 5.17 ± 0.25                   |         |          |
| 16:1       | 20.16 ± 0.86          |         | 20.69 ± 0.12                  |         |          |
| 18:1       | 388.35 ± 2.26         |         | 389.12 ± 0.08                 |         |          |
| 20:1       | 5.16 ± 0.01           |         | 5.19 ± 0.04                   |         |          |
| Total PUFA | 58.62 ± 0.35          | 7.81    | 39.09 ± 0.54                  | 5.25    | −33.32   |
| 18:2       | 36.62 ± 1.81          |         | 25.82 ± 0.11                  |         |          |
| 18:3       | 17.91 ± 1.92          |         | 10.37 ± 0.01                  |         |          |
| 20:2       | 1.77 ± 0.14           |         | 1.45 ± 0.08                   |         |          |
| 20:3       | 1.48 ± 0.28           |         | 1.01 ± 0.12                   |         |          |
| 22:5       | 0.84 ± 0.17           |         | 0.44 ± 0.21                   |         |          |
| Total      | 750.59                | 99.99   | 744.92                        | 100.03  | −0.76    |

<sup>a</sup>All data were expressed as mean ± SD of triplicate determinations.

<sup>b</sup>Based on the internal standard tridecanoic acid (13:0).

<sup>c</sup>ND, not detectable.



**FIG. 1.** RP-HPLC chromatograms of hydroxyeicosatetraenoic acids (HETE) obtained from eicosatetraenoic acid treated with algal lipooxygenase extracted from *Ulva* spp., in reference to the standard mixture of 5-, 8-, 11-, 12-, and 15-HETE.

were found in other green macroalgae. In *U. conglobata*, 12-HpETE was the predominant product catalyzed by the algal LOX using 20:4 as substrate, whereas the 9-HpODE (hydroperoxyoctadecadienoic acid) derivative was produced in greater amount than 13-HpODE when linoleic acid was used as substrate (3,6).

**Changes in undesirable volatile compounds after LOX treatment.** Treatment of dry-rendered chicken oil with the algal extract for up to 120 min neither reduced nor intensified the oily odor initially perceived in the LOX-untreated chicken oil. LOX treatment of the dry-rendered oil did reduce the chickenish attribute, for which the odor threshold is 0.2 ppb as opposed to the 2 ppb odor threshold for the “oily odor” note evaluated in a lipid system (Table 3).

Before LOX treatment, five undesirable odor notes were perceived using the GC-sniffing technique (Table 3). The rancid and plastic odor notes disappeared after the LOX treatment. The chicken oil odor note remained but to a lesser extent, whereas the volatile compounds contributing to the oxidized and oily odor notes increased after the LOX treatment. An

overall increase in all undesirable volatile compounds of 58.1%, from a total of 920.3 to 1454.6 ppb, was observed.

However, the established odor thresholds of these compounds were very different. They also differed depending on the media system. For example, *E,E*-2,4-nonadienal has a threshold of 0.09 ppb in aqueous systems and 0.2 ppb in lipid systems (Table 3), whereas the corresponding thresholds for *E*-2-decenal are 0.3–0.4 and 2 ppb. Thus, the contribution of a 167.5% increase in *E*-2-decenal to the overall perceivable odor was much less than that of the complete disappearance of *E,E*-2,4-nonadienal owing to the 10-fold difference in odor threshold in lipid systems.

The chickenish odor, identified as *E,E*-2,4-nonadienal, was present at 33.1 ppb before LOX treatment and was undetectable afterward (Table 3). However, *E*-2-undecenal, which also smells chickenish, increased by 22.4% from 73.1 to 89.5 ppb. The oxidized odor contributed by *E,E*-2,4-heptadienal, the rancid odor of tetradecanal, and the plastic odor of heptanal were all eliminated after LOX treatment. Nevertheless, the oily odor note identified as *E*-2-decenal and the oxidized odor note of *E,Z*-2,4-decadienal increased by about 1.7- to 2.1-fold (Table 3).

**TABLE 3**  
**Changes in Undesirable Odorous Compounds of Chicken Oil Modified with Algal Lipoxigenase**

| Compound <sup>a</sup>       | RI <sup>b</sup> | Odor note   | Threshold <sup>c</sup><br>(ppb)       | Unmodified oil<br>(ppb) <sup>e</sup> | LOX-modified oil <sup>d</sup><br>(ppb) <sup>e</sup> | Change<br>(%) |
|-----------------------------|-----------------|-------------|---------------------------------------|--------------------------------------|---|---------------|
| Heptanal                    | 1187            | Plastic     | 3 <sup>a</sup> , 23 <sup>c</sup>      | 5.7                                  | —   | −100          |
| <i>E,E</i> -2,4-heptadienal | 1463            | Oxidized    | 19 <sup>c</sup>                       | 27.2                                 | —   | −100          |
| <i>E</i> -2-decenal         | 1640            | Oily        | 0.3–0.4 <sup>a</sup> , 2 <sup>c</sup> | 108.1                                | 289.2   | +167.5        |
| <i>E,E</i> -2,4-nonadienal  | 1698            | Chicken oil | 0.09 <sup>a</sup> , 0.2 <sup>c</sup>  | 33.1                                 | —   | −100          |
| <i>E</i> -2-undecenal       | 1747            | Chicken oil | ND <sup>f</sup>                       | 73.1                                 | 89.5  | +22.4         |
| <i>E,Z</i> -2,4-decadienal  | 1761            | Oxidized    | ND <sup>f</sup>                       | 204.2                                | 640.1   | +213.5        |
| <i>E,E</i> -2,4-decadienal  | 1808            | Oxidized    | 0.07 <sup>a</sup> , 0.2 <sup>c</sup>  | 419.1                                | 435.8   | +4.0          |
| Tetradecanal                | 1860            | Rancid      | 60 <sup>b</sup>                       | 49.8                                 | —   | −100          |
| Total                       |                 |             |                                       | 920.3                                | 1454.6  | +58.1         |

<sup>a</sup>Compounds were tentatively identified based on spectra of the Wiley Library.

<sup>b</sup>RI, retention index, calculated using C<sub>6</sub>–C<sub>25</sub> alkanes as references.

<sup>c</sup>Threshold in aqueous system cited from (a) <http://www.leffingwell.com/odorthre.htm>; (b) <http://www.leffingwell.com/ald1.htm>; (c) Ref. 15, in lipid systems.

<sup>d</sup>Chicken oil, 10 g, was reacted with 500 mL of lipoxigenase extract, specific activity 0.31 μmol/mg protein-min, at 33°C for 2 h.

<sup>e</sup>The volatile compounds were collected with reduced-pressure cold trap and detected with GC–MS and a GC-sniffing method. Quantitative determination based on benzoic acid as the internal standard.

<sup>f</sup>ND, not defined.

*E,E(Z)*-2,4-Decadienal comprised the major undesirable volatile of the chicken oil both before and after LOX treatment. The concentration of *E,E*-2,4-decadienal (419.1 ppb) was the highest among all undesirable odorous compounds, followed by *E,Z*-2,4-decadienal (204.2 ppb) in the dry-rendered chicken oil, and it increased significantly after LOX treatment. It was speculated that the two isomeric compounds resulted from peroxidation of C<sub>18:2</sub> and further breakdown of the hydroperoxides, i.e., 9-HpODE (9). 2,4-Decadienal has a very low detection threshold: 0.07 ppb in aqueous systems and 0.2 ppb in lipid systems (Table 3). This dienal is considered to be the primary odorant that makes chicken broth distinctively different from other meat broths (10) and is regarded as important to chicken oil odor (9). This compound has been found in the volatiles of roasted, fried, and boiled chicken (10). *E,E*-2,4-Decadienal was also the predominant volatile compound found in fresh snapper and carp (12). These aldehydes are potent odorous compounds produced by scission on either side of the alkoxy radicals of the polyunsaturated lipid molecules.

The presence of 2-undecenal was speculated to result from the 8-hydroperoxide derived from C<sub>18:2</sub> and to have formed by cleavage of the C–C bond near –COOH. *E,E*-2,4-Heptadienal was probably produced from 12-hydroperoxide and ω-7-hydroperoxide by oxidizing C<sub>18:3</sub>, followed by breaking of the ω-3,6-diene, e.g., 12-HpOTE.

*Increases in desirable volatile compounds after LOX treatment.* Untreated chicken oil contained 286.9 ppb of desirable volatile compounds (Table 4). After LOX treatment, the total content of desirable compounds increased to 1089.2 ppb, a 2.8-fold increase from the untreated oil.

The desirable volatile compounds of the five odor attributes detected included ethyl acetate (melon odor), pentanal (almond odor), 2-pentyl furan (tea leaf odor), *E*-2-heptenal (fruity odor), nonanal (green odor), and *E*-2-heptenal (melon or sweet odor) as shown in Table 4. Nonanal constituted the largest fraction of the desirable volatiles, being 150.2 ppb before LOX treatment and 323.7 ppb after; similarly, *E*-2-heptenal increased from 67.5 to 210.4 ppb, and ethyl acetate increased from 24.8 to

**TABLE 4**  
**Changes in Desirable Odorous Compounds of Chicken Oil Modified with Algal Lipoxigenase**

| Compound <sup>a</sup> | RI <sup>b</sup> | Odor note    | Threshold <sup>c</sup><br>(ppb)     | Unmodified oil<br>(ppb) | LOX-modified oil <sup>d</sup><br>(ppb) | Change<br>(%) |
|-----------------------|-----------------|--------------|-------------------------------------|-------------------------|--|---------------|
| Ethyl acetate         | 885             | Melon        | 5–5000                              | 24.8                    | 248.2                                  | +900.8        |
| Pentanal              | 978             | Almond       | 12 <sup>b</sup> , 21.9 <sup>c</sup> | 8.8                     | 90.8                                   | +931.8        |
| Limonene              | 1197            | Citrus       | 10                                  | 12.5                    | —                                      | −100          |
| 2-Pentyl furan        | 1227            | Tea leaf     | 6, 91 <sup>c</sup>                  | 23.1                    | 216.1                                  | +835.5        |
| <i>E</i> -2-Heptenal  | 1322            | Melon, sweet | 13, 63 <sup>c</sup>                 | 67.5                    | 210.4                                  | +211.7        |
| Nonanal               | 1398            | Green        | 1, 13 <sup>c</sup>                  | 150.2                   | 323.7                                  | +115.5        |
| Total                 |                 |              |                                     | 286.9                   | 1089.2                                 | +279.6        |

<sup>a</sup>Compounds were tentatively identified based on spectra from the Wiley Library.

<sup>b</sup>RI, retention index, calculated using C<sub>6</sub>–C<sub>25</sub> alkanes as references.

<sup>c</sup>Threshold in an aqueous system cited from <http://www.leffingwell.com/odorthre.htm> except (b) <http://www.leffingwell.com/ald1.htm>; (c) Ref. 15, in lipid system.

<sup>d</sup>Chicken oil (10 g) was reacted with 500 mL of lipoxigenase extract, specific activity 0.31 μmol/mg protein-min, at 33°C for 2 h.

<sup>e</sup>The volatile compounds were collected with a reduced-pressure cold trap and detected with GC–MS and the GC-sniffing method. Quantitative determination was based on benzoic acid as the internal standard.



248.2 ppb. These three compounds made up 71.8% of the total desirable odorous compounds of the treated chicken oil volatiles. Since nonanal was the major desirable odorant and showed the lowest threshold (13 ppb) in a lipid system, its increase after the LOX treatment of chicken oil was expected to bring about a major improvement in aroma.

*E*-2-Heptenal probably resulted from cleavage of a 13-hydroperoxide compound formed during the oxidation of  $C_{20:2}$ . Ethyl acetate likely resulted from cleavage of the 13-hydroperoxide derivative of  $C_{18:2}$  or from the 15-hydroperoxide compound oxidizing  $C_{20:4}$  to hexanal by autooxidation (13,14). The 9-hydroperoxide of  $C_{18:2}$  could break down to 2,4-decadienal and yield an oxidized odor (15). However, it could also produce a desirable odor by thermal breakdown of the compound to form pentanal and 2-pentyl furan (9,11). Pentanal could also be a breakdown compound of the 13-hydroperoxide of  $C_{18:2}$ . These two compounds contributed 28.2% of the total desirable volatiles of the LOX-modified chicken oil. 2-Pentyl furan also exists in vegetable oil products or foods high in oil content, giving a grassy odor. Free radicals from 9-hydroperoxide react with oxygen to form hydroxides having ethenyl moieties, resulting in 2-phenyl furan by cyclic reactions (9).

All the desirable and undesirable odorous aldehydes identified in this study were found in the summary by Ho and Chen (9) on aldehydes identified in chicken flavor. Additional volatile compounds have been identified in supercritical  $CO_2$  extracts of cooked chicken fat (16).

Thus, chicken oil obtained by dry-rendering of chicken adipose fat tissue consisted of 50.0% monoenoic acid, and 7.8% PUFA. The chickenish odor of this oil was improved after LOX treatment at 33°C for 30 min with an extract of 5-, 12-, and 15-LOX from a green marine macroalgae, *Ulva* spp. The LOX-treated oil maintained 99% of the total monoenoic acids but reduced the total PUFA content by 19.53 mg/g oil, or 33%.

The LOX treatment in this study was performed in an emulsion of oil and algal LOX extract. Immobilization of LOX is being developed to modify the chicken oil odor in order to maintain clarity of the chicken oil and to develop a working system in which the LOX can be used repeatedly.

## REFERENCES

1. Taiwan Agriculture Annual Report, Council of Agriculture, Taipei, Taiwan, 1998.
2. American Heart Association, *Physicians' Cholesterol Education Program—Pathophysiology and Treatment Rationale*, American Heart Association, Washington, DC, 1987.
3. Hu, S.P., and B.S. Pan, Modification of Fish Oil Aroma Using a Macroalgal Lipoxygenase, *J. Am. Oil Chem. Soc.* 77:343–348 (2000).
4. AOAC, *Official Methods of Analysis*, 16th edn., Association of Official Analytical Chemists, Washington, DC, 1995.
5. Folch, J., M. Lee, and G.H. Sloane Stanley, A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissue, *J. Biol. Chem.* 226:497–509 (1957).
6. Kuo, J.M., A. Huang, 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).
7. Hsieh, R.J., J.B. German, and J.E. Kinsella, Lipoxygenase in Fish Tissue: Some Properties of the 12-Lipoxygenase from Trout Gill, *Ibid.* 36:680–685 (1988).
8. Schomburg, G., and G. Dielmann, Identification by Means of Retention Parameters, *J. Chromatogr. Sci.* 11:151–158 (1973).
9. Ho, C.T., and Q. Chen, Lipid in Food Flavors: An Overview, in *Lipid in Food Flavors*, edited by C.T. Ho and T.G. Hartman, American Chemical Society, Washington, DC, 1994, pp. 1–14.
10. Gasser, U., and W. Grosch, Primary Odourants of Chicken Broth, *Z. Lebensm. Unters. Forsch.* 190:3–8 (1990).
11. Tang, J., Q.Z. Jin, G.H. Shen, C.T. Ho, and S.S. Chang, Isolation and Identification of Volatile Compounds from Fried Chicken, *J. Agric. Food Chem.* 31:1287–1292 (1983).
12. Morita, K., K. Kubota, and T. Aishima, Comparison of Aroma Characteristics of 16 Fish Species by Sensory Evaluation and Gas Chromatographic Analysis, *J. Sci. Food Agric.* 83:289–297 (2003).
13. Shahidi, F., and R.B. Pegg, Hexanal as an Indicator of the Flavor Deterioration of Meat and Meat Products, in *Lipid in Food Flavors*, edited by C.T. Ho and T.G. Hartman, American Chemical Society, Washington, DC, 1994, pp. 256–281.
14. Palamand, S.R., and R.H. Dieckmann, Biogenesis of Lipid-Derived Volatile Aroma Compounds, *J. Agric. Food Chem.* 22:503–506 (1974).
15. Meynier A., C. Genot, and G. Gandemer, Volatile Compounds of Oxidized Pork Phospholipids, *J. Am. Oil Chem. Soc.* 75:1–7 (1998).
16. Darrell, L.T., and K.L. Duane, Volatile Content and Sensory Attributes of Supercritical Carbon Dioxide Extracts of Cooked Chicken Fat, *J. Food Sci.* 60:1197–1200 (1995).

[Received January 20, 2004; accepted September 23, 2004]