# Isolation and Identification of Volatile Flavor Compounds in Commercial Oil-Free Soybean Lecithin[1]

**HEASOOK KIM, CHI-TANG HO** and **STEPHEN S. CHANG,** Department of Food Science, Cook College, New Jersey Agricultural Experiment Station, Rutgers, The State University, New Brunswick, NJ 08903

## ABSTRACT

Volatile compounds were isolated from 150 lb of commercial oil-free soybean lecithin by vacuum distillation. The isolated volatile compounds were subjected to extensive gas chromatographic fractionation, and the pure fractions thus obtained were identified by mass spectrometry. A total of 79 compounds have been identified. Most of the compounds identified can be postulated as autoxidative decomposition products of unsaturated fatty acids of phospholipids. Isophorone, an acetone-derived compound, was shown to be predominantly responsible for the undesirable flavor of oil-free soybean lecithin. Several nitrogen-containing compounds, e.g., nitriles, acetoxime and 4,5-dimethylisoxazole, were identified as unique volatile compounds from phospholipids.

## INTRODUCTION

The commercial lecithin products are predominantly soybean lecithins. Soybean lecithins are valuable as natural wetting agents, stabilizers, emulsifiers, dispersants and release agents in both food and industrial applications and for dietetic and medical applications. However, commercial oil-free soybean lecithins are known to have a characteristic objectionable odor that is difficult to remove.

Phospholipids have been shown to oxidize easily during heating and prolonged storage (1,2). Sessa et al. (3,4) reported that soybean phosphatidylcholine definitely becomes bitter and rancid when stored. Phospholipids have been shown to play a role in the oxidative deterioration that arises in the processing and storage of food products (5,6). The rate of oxidation of phospholipids was affected not only by the degree of unsaturation, but also by the nitrogen moiety. The occurrence of Maillard-type browning reaction was evidenced during the autoxidation of purified phosphatidylethanolamine and phosphatidylcholine (7). Phospholipids have also been shown to play a major role in the development of warmed-over flavor in poultry, mutton, beef and pork (8). One of the predominant odors of soy flour was characterized as lecithinlike (9). This indicates that when this flour is used in food, phospholipids can result in undesirable flavor compounds in the final product.

This paper reports the isolation and identification of volatile compounds from commercial oil-free soybean lecithin, commonly known as granular lecithin.

## EXPERIMENTAL PROCEDURES

### Isolation of the Volatile Compounds from Granular Soybean Lecithin

Commercial oil-free lecithin (containing 3% triglycerides) was used for this study. The volatile compounds were isolated by the apparatus described by Hsieh et al. (10), which used the principle of the slow evaporation of volatiles at an elevated temperature under vacuum. The oil-free lecithin was kept at 45 C during the isolation period. Twenty-four pounds of granular lecithin were used for each isolation, which lasted 1 week. A total of 6 isolations were run. The total volatile isolate collected in traps with dry ice and acetone was treated in a similar manner to that described by Herz and Chang (11). The condensate was saturated with NaCl and extracted with anhydrous diethyl ether. The ether extract was dried with anhydrous sodium sulfate and then concentrated to a final volume of 10 mL, first with a 30-plate Oldershaw column and then with a spinning band still.

### Fractionation of the Volatile Isolate

The volatile isolate of granular soybean lecithin was separated by gas chromatography (12). The chromatography was performed on a Perkin-Elmer Sigma-3 gas chromatograph equipped with a flame ionization detector (FID), fitted with a 1/8" o.d. × 12 ft steel column packed with 10% OV-351 on 60/80 mesh Supelcoport. The flow rate was 30 mL/min with a column temperature that was held at 50 C for 5 min then increased by 3 C/min to a holding temperature of 225 C. The volatile isolate was divided into 23 broad fractions. Each broad fraction was successfully collected according to the method of Thompson et al. (13). A second fractionation was performed on all 23 broad fractions using a 1/8" o.d. × 10 ft steel column packed with 10% OV-17 on 80/100 Chromosorb WHP.

### Gas Chromatography-Mass Spectroscopy Analysis

Mass spectrometry was performed on a Du Pont 21-490 mass spectrometer with a jet separator interfaced to a Varian Moduline 2700 gas chromatograph fitted with an FID detector and a 1/8" in o.d. × 12 ft steel column packed with 10% OV-101 on 80/100 Chromosorb W DMCS. The ionization voltage of the mass spectrometer was 70 eV.

### Sensory Evaluation

Three samples were prepared for sensory evaluation. A multiple comparison test was run on the samples. The commercial granular lecithin was used as control. The 2 test samples were soybean granular lecithin freshly precipitated from acetone and samples treated with isophorone at 0.06 ppm. A panel of 23 experienced judges was used to evaluate the samples. Judges were asked to smell the control sample and test samples and rate them on a scale of 1 (none) to 9 (extreme).

## RESULTS AND DISCUSSION

A total of 79 compounds were identified in the volatile compounds isolated from granular soybean lecithin (Table I). Of the 79 compounds identified, 2 are of particular interest: one is 4,5-dimethylisoxazole and the other is isophorone.

4,5-Dimethylisoxazole is of interest because it is the first oxazole or isoxazole reported as a decomposition product of lipids. 4,5-Dimethylisoxazole was identified by comparing its mass spectrum with that of the authentic compound (Figure 2). Although oxazoles are widely distributed in food flavors (14-16), isoxazole had been reported only once as being present in the volatile compounds of tomato juice (17). 4,5-Dimethylisoxazole may derive from the reaction of 1,3-dicarbonyl compound with the decomposition product of phosphatidylethanolamine.

The isophorone is of special interest because of its odor characteristics. This compound was identified in Fraction

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**TABLE I**

**Volatile Constituents Identified in Granular Soybean Lecithin**

| Fraction number[a] | Identification | Previously reported in: Soybean oil | Previously reported in: Soy protein | Relative peak size[b] |
|---|---|---|---|---|
| | Aliphatic hydrocarbons | | | |
| 1-9-1 | 2,2,4-Trimethylheptane | | | M |
| 12-14-9 | 1,1,3,5-Tetramethylcyclohexane | | | XS |
| 12-1-5 | 2-Methyl-2-butene | | | XS |
| 5-4-4 | 1-Pentene | | | XL |
| 12-15-3 | 2,5-Dimethyl-2,4-hexadiene | | | XS |
| 1-2-3 | 2-Octene | d | | XS |
| 1-6-4 | 1,3-Octadiene | | | S |
| 8-1-2 | 4-Octene | | | S |
| 4-15-5 | Limonene | | | XS |
| | Aromatic hydrocarbons | | | |
| 9-1-1 | Benzene | c | | XL |
| 1-5-3 | Toluene | | | XS |
| 1-8-1 | 1,4-Dimethylbenzene | | | L |
| 4-15-4 | 1,3,5-Trimethylbenzene | | | XS |
| 4-13-3 | Isopropylbenzene | | | XS |
| 8-13-1 | 1,2,4,5-Tetramethylbenzene | | | XS |
| 6-11-1 | 1,3-Dimethyl-5-ethylbenzene | | | XS |
| 6-12-3 | 1,2-Dimethyl-4-ethylbenzene | | | XS |
| 5-12-5 | 1,2-Diethylbenzene | | | XS |
| 5-11-7 | 1-Methyl-4-isopropylbenzene | | | S |
| 8-14-1 | 1,3-Diisopropylbenzene | | | XS |
| 3-5-5 | 1,4-Dimethyl-3-isobutylbenzene | | | XS |
| 10-6-1 | Indene | | | XS |
| 8-9-5 | 2,3-Dihydroindene (indan) | | | XS |
| 11-13-3 | 1-Methylindene | | | XS |
| 12-7-5 | Styrene | | | XS |
| 6-7-2 | α-Methylstyrene | | | XS |
| 12-17-3 | Naphthalene | | | XS |
| 8-9-4 | 1,2-Dichlorobenzene | | | XS |
| | Ketones | | | |
| 1-1-4 | 3-Methyl-2-butanone | | f | S |
| 5-4-3 | 3-Methyl-3-buten-2-one | | | XS |
| 1-2-1 | 2,3-Pentanedione | d | | XL |
| 1-5-1 | 4-Methyl-2-pentanone | | f | XS |
| 5-3-2 | 3-Penten-2-one | | | XL |
| 2 | 4-Methyl-3-penten-2-one (mesity oxide) | | | XL |
| 9-1-6 | 3-Methyl-4-penten-2-one | | | XS |
| 6-5-2 | Diacetone alcohol | | | XS |
| 1-5-2 | 4-Methyl-4-penten-2-one | | | S |
| 1-7-3 | 2-Hexanone | d | f | XL |
| 12-7-1 | 3-Hexanone | | f | XS |
| 5-3-3 | 5-Hexen-2-one | | | XS |
| 11-11-2 | 1-Acetylcyclohexene | | | XS |
| 12-14-3 | 3-Methyl-2-cyclohexen-1-one | | | XS |
| 11-13-1 | 4,4-Dimethyl-3-cyclohexen-1-one | | | S |
| 12-17-2 | 3,5,5-Trimethyl-2-cyclohexen-1-one (isophorone) | | | S |
| 3-5-5 | 2-Heptanone | c | e | M |
| 6-6-6 | 3-Hepten-2-one | | | XS |
| 12-15-9 | 6-Methyl-3,5-heptadien-2-one | | | XS |
| 12-14-8 | 2,6-Dimethyl-2,5-heptadien-4-one | | | XS |
| 5-11-6 | 2-Octanone | c | f | S |
| 4-15-3 | 3-Octanone | c | e | XS |
| 12-10-14 | 3-Octen-2-one | | e | M |
| 12-14-7 | 3,5-Octadien-2-one | | | L |
| 8-11-3 | 2-Nonanone | c | f | XS |
| 12-15-10 | 3-Nonen-2-one | | | XS |
| 9-12-7 | 2-Decanone | | f | XS |
| 6-12-3 | 2-Undecanone | | | XS |
| 9-11-2 | 2-Tridecanone | | | XS |
| 12-14-5 | Acetophenone | c | | XS |
| 9-12-11 | Isobutyl benzyl ketone | | | XS |
| | Aldehydes | | | |
| 1-7-4 | Hexanal | c | f | XL |
| 3-5-3 | 2-Methyl-2-pentenal | | f | XS |
| | Alcohols | | | |
| 4-4-2 | 1-Pentanol | c | g | XS |
| 6-6-7 | 1-Octen-3-ol | v | h | XS |
| | Esters | | | |
| 3-5-7 | n-Pentyl acetate | | i | S |
| 3-4-4 | 3-Acetoxy-1-butene | | | S |
| 1-1-7 | 1-Acetoxy-1,3-butadiene | | | XS |
| 12-5-3 | Methyl methacrylate | | | XS |

**TABLE I (Continued)**

| Fraction number[a] | Identification | Previously reported in Soybean oil | Previously reported in Soy protein | Relative peak size[b] |
|---|---|---|---|---|
| | Lactones | | | |
| 12-12-5 | 4-Hydroxybutanoic acid, lactone | | | XS |
| 4-8-3 | 3-Methyl-4-hydroxypentanoic acid, lactone | | | XS |
| | Nitriles | | | |
| 4-5-1 | n-Butyl nitrile | | | S |
| 1-1-3 | Isobutyl nitrile | | | S |
| 1-6-1 | α-Methylbutyl nitrile | | | S |
| 5-9-5 | 3-Hydroxy-4-cyano-1-butene | | | XS |
| 6-5-3 | n-Pentyl nitrile | | | XS |
| 8-10-2 | n-Hexyl nitrile | | | XS |
| | Heterocyclic compounds | | | |
| 4-14-2 | 2-Pentylfuran | c | e | XS |
| 12-15-2 | 4,5-Dimethylisoxazole | | | XS |
| | Miscellaneous compounds | | | |
| 4-19-1 | Acetoxime | | | XS |
| 4-2-1 | Chloroacetone | | | XS |

[a]The first. second and third numerals indicate the position of the GC fraction during the first, second and third chromatographies, respectively.
[b]XL = extra large; L = large; M= medium; S = small; XS = extra small.
[c]Smouse and Chang (19).
[d]Frankel (18).
[e]Hsieh et al. (10).
[f]Qvist et al. (24).
[g]Arai et al. (25).
[h]Badenhop & Wilkens (26); Cowan et al. (27).

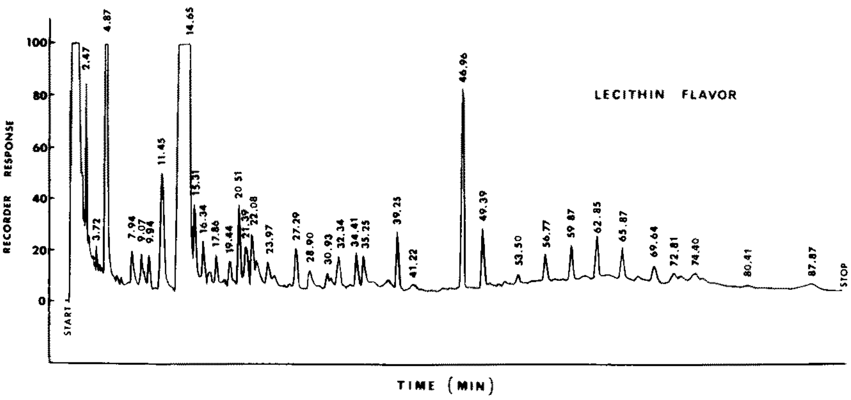


**FIG. 1. Gas chromatographic fraction of lecithin flavor.**

12 (retention time 36.4-46.0 min in Figure 1), which had a strong lecithinlike flavor. Therefore, the importance of isophorone to the overall undesirable flavor of soybean granular lecithin was studied. When 0.06 ppm of isophorone was added to the freshly prepared sample, the sensory panel could not differentiate this sample from the commercial soybean lecithin sample. However, the freshly prepared soybean granular lecithin was easily differentiated from commercial soybean granular lecithin. The sample with added isophorone was described as haylike, barnyard, branny and beany. We therefore concluded that isophorone is predominantly responsible for the characteristic undesirable flavor of granular soybean lecithin.

Isophorone was identified by comparing its mass spectrum with that of the authentic compound (Figure 3). It can be formed from the self-condensation reaction of acetone. Acetone was the solvent used for the processing of soybean granular lecithin. Aldol condensation of acetone will form mesityl oxide (Figure 4), which is the predominant component in the volatile constituents of soybean granular lecithin. Michael addition reaction between mesityl oxide and acetone will result in the formation of isophorone (Figure 4). Besides isophorone and mesityl oxide, acetoxime and chloroacetone identified in the volatiles of soybean lecithin were also considered to be derived from acetone.

The mechanism that forms hydrocarbons, ketones, aldehydes, alcohols, esters and lactones through the decomposition of hydroperoxides of unsaturated fatty esters has been reviewed (18). Furthermore, the mechanism that forms 2-pentylfuran from the autoxidative decomposition of linoleate has been established (19,20).

A large number of aldehydes have been reported in the volatile decomposition products of neutral lipids (18,19, 21). Only hexanal and 2-methyl-2-pentenal were identified in this study. The lack of aldehydes in the volatile decom-

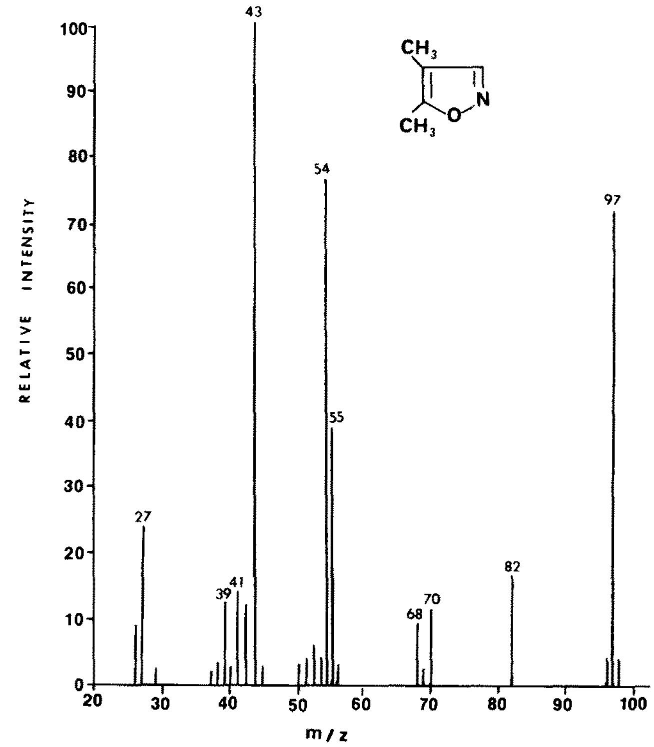


FIG. 2. Mass spectrum of 4,5-dimethylisoxazole.

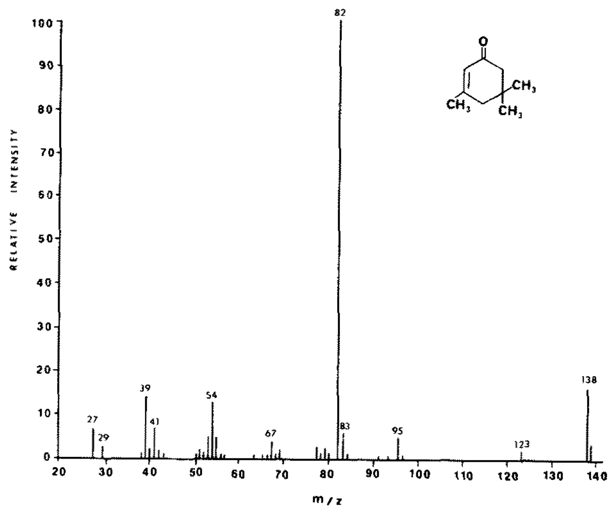


FIG. 3. Mass spectrum of isophorone.

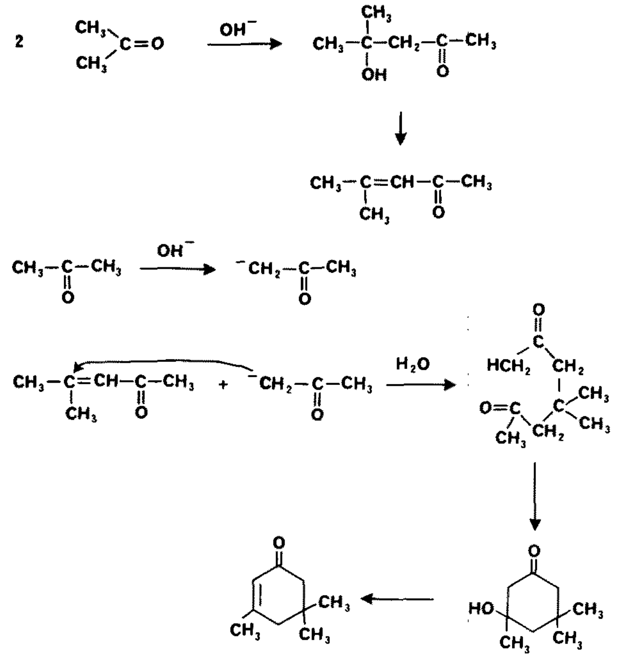

FIG. 4. Mechanism for the formation of isophorone.

position products of soybean lecithin may result from the immediate reaction of the aldehydes produced from the fatty acid moieties toward the amine group of phospholipids, especially phosphatidylethanolamine (22,23).

Six nitriles were identified in the volatile compounds of soybean lecithin. These are unique to phospholipids. The importance of nitriles to the overall flavor of soybean granular lecithin was not studied.

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