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Impact of volatiles on flavor of fresh and stored soybean oils

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Impact of volatiles on flavor of fresh and stored soybean oils

by

Jian-Wen Kao

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GENERAL INTRODUCTION

Since the 1950s, efforts to prevent cardiovascular disease have led to increased consumption of vegetable oils instead of animal fats known to contain cholesterol and high amounts of saturated fatty acids (1). But flavor deterioration of vegetable oils, especially soybean oil, is a major problem in the food oil industry.

Many researchers have studied the flavor deterioration of fats and oils (2, 3). Analytical instruments such as gas chromatography (GC), mass spectrometry (MS), and high performance liquid chromatography (HPLC) have helped researchers study off-flavor formation and the role of lipid oxidation, especially of unsaturated fatty acid (4-8). The mechanism and products of lipid oxidation, factors affecting lipid oxidation and the effects of oil processing conditions on flavor stability are well known today (9-12). Flavor reversion of soybean oil, however, is still not entirely understood. Flavor reversion is the development of offensive beany and grassy odors in soybean oil and other linolenate-containing oils at low levels of oxidation (13-28). There are many theories to explain the formation of flavor reversion but no method is available to prevent it (29). Recently, furanoid fatty acids were detected in unprocessed and refined soybean oils and were proposed to be the precursor of a potent flavor compound, 3-methylnonane-2,4-dione (MND), which might contribute to the reversion flavor of soybean oil (24, 28).

Generally, off-flavor formation in foods is associated with lipid oxidation, but fats and oils also are responsible for many desirable flavors, such as the flavor of fresh milk, creamy

flavor of cream (30) and nutty flavor in freshly deodorized vegetable oils. Today, the propensity to obesity in the U.S. population has led food companies to produce non-fat foods containing many possible fat substitutes (31). Non-fat foods, however, often lack the desirable "nutty" flavors associated with fresh and good quality vegetable oils as well as the appropriate mouthfeel.

Many researchers have identified and characterized flavor-causing compounds in oxidized soybean oil. Little work, however, has been done to evaluate the flavor compounds in freshly refined, bleached and deodorized soybean oil. The objectives of this research were: **1)** to examine the impact of 3-methylnonane-2,4-dione (MND) in flavor deterioration of soybean oil, and **2)** to identify and evaluate the "nutty" flavor associated with freshly deodorized soybean oil.

Dissertation organization

This dissertation is organized into a general introduction, a literature review, two papers, and a general conclusion. Discussed in the literature review are the history and utilization of soybeans, the mechanism of off-flavor formation in vegetable oils, and methods of flavor analysis. The first paper is entitled "The Impact of 3-methylnonane-2,4-dione (MND) on Flavor Deterioration of Soybean Oil." The second paper, entitled "Volatiles Produced during Deodorization of Soybean Oil and Their Flavor Significance," was presented as a poster session at the 1996 American Oil Chemists' Society meeting in Indianapolis, IN. Both research papers will be submitted to the Journal of the American Oil Chemists' Society.

Following the second paper is general conclusions and a list of references cited in the general introduction and literature review.

LITERATURE REVIEW

Brief history and current usage of soybean and soybean oil in the U.S.

Soybeans have long been used as nutritious foods in Asian countries because of high protein and oil content. The earliest information on soybean cultivation can be traced to 2800 B.C. (32). In the United States, soybean was first introduced as a crop to be grown to reduce the surpluses of wheat and cotton during the 1910's, and was primarily used as a forage crop. When the first soybeans were crushed in Decatur, Illinois in 1922, soybeans as a crop began their incredible growth to become the leading crop in the U.S.(29). Soybean oil accounted for about 75% of U.S. visible oil, in 1994, and was used in cooking and salad oils, mayonnaise, salad dressings, shortenings, margarines, and other products. For the same year, about 81% of soy protein produced in the United States was utilized domestically: 40% in poultry feed, 23% in swine feed, 7% in beef feed, 6% in dairy, 1% in human food and 4% in other products. The other 18% was exported (33).

Mechanisms for formation of flavors during lipid oxidation

The problem of flavor deterioration in vegetable oils, especially soybean oil which is the dominant source of edible oil, has given rise to much research on the mechanisms and origins of offensive odors. Most compounds having odor are identified as carbonyl compounds such as aldehydes and ketones, which arise mainly from oxidation of unsaturated fatty acids, such as oleate, linoleate, and linolenate, the main fatty acids in vegetable oils. Some researchers

have found that flavor compounds may come from the oxidation of minor fatty acids in the oil, such as furanoid fatty acids (28). The entire oxidation process is so complicated that many of the oxidation products are not well identified. Generally, the flavor carbonyl compounds are derived not only from the decomposition of hydroperoxides formed by the reaction of unsaturated fatty acids with oxygen, but also from the decomposition of secondary oxidation products, such as hydroperoxides, unsaturated aldehydes and ketones, dimers or other polymers of hydroperoxides, and the reaction between unsaturated fatty acids and hydroperoxides (10, 15, 34, 35). Figure 1 shows the possible pathways of flavor formation during lipid oxidation.

The formation of hydroperoxides can be categorized into two mechanisms: **1) autoxidation** (free radical mechanism) and **2) photosensitized oxidation** by participation of light and sensitizers.

1) Autoxidation (10, 11)

Autoxidation is the reaction between unsaturated fatty acids (RH) and oxygen to produce hydroperoxides with involvement of free-radical chain reactions. The free radical chain reaction can be divided into three steps: **1) initiation, 2) propagation** and **3) termination.**

1. Initiation: $RH \longrightarrow R\bullet + H\bullet$

The free radical formation in this step can take place by hydroperoxide decomposition, metal catalysis (Cu, Fe) and exposure to light (UV).

2. Propagation:

$$R\bullet + O_2 \rightleftharpoons ROO\bullet$$

$$ROO\bullet + RH \rightarrow ROOH + R\bullet$$

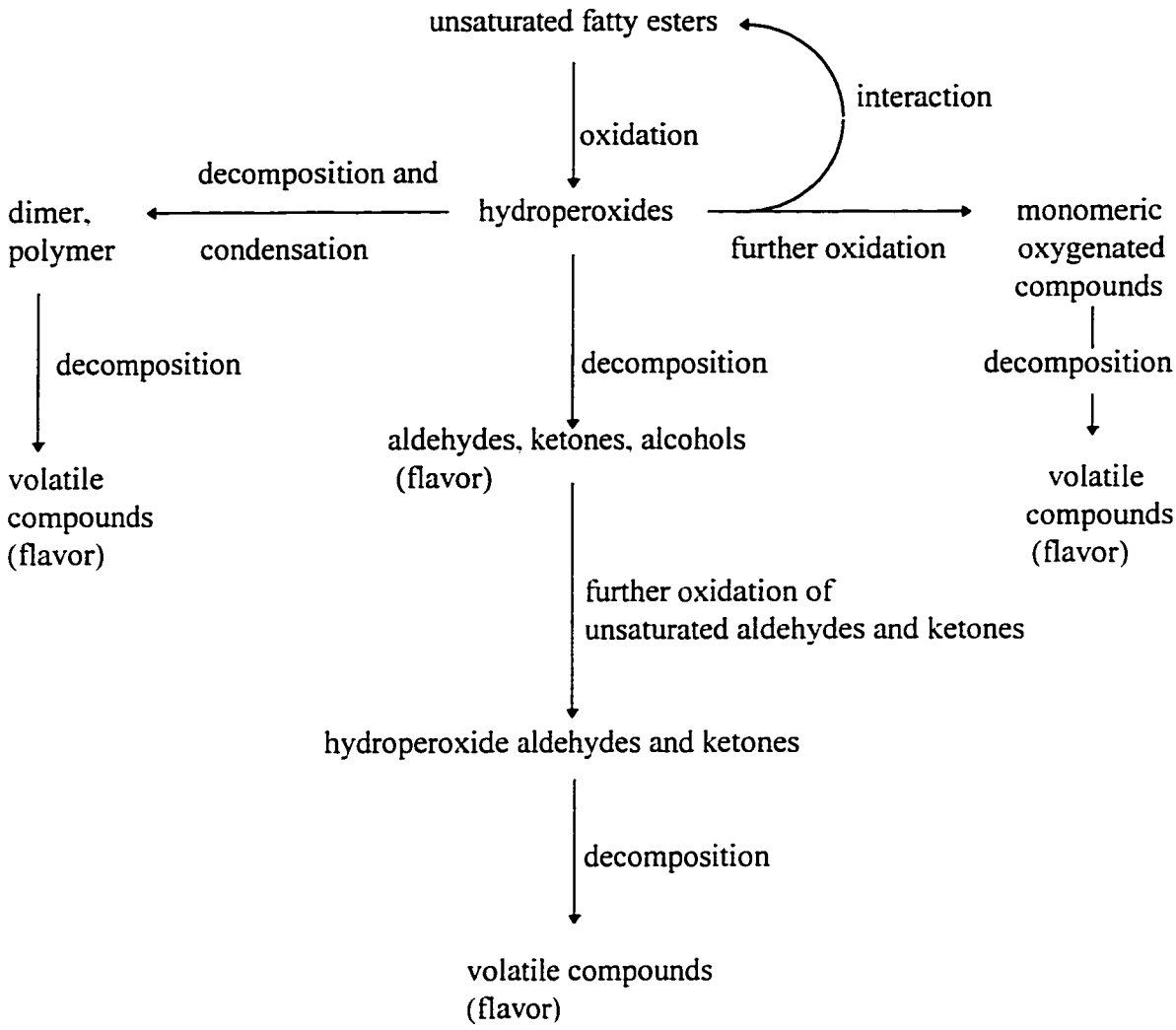

Figure 1. The possible pathways of the flavor formation during lipid oxidation

The free radical (R•) reacts with oxygen to form peroxides which then abstract the hydrogen from another fatty acid to form a new free radical. Thus, hydroperoxides are accumulated in this cyclic reaction.

3. Termination:

$$R\bullet + R\bullet \longrightarrow \text{nonradical}$$

$$R\bullet + OR\bullet \longrightarrow \text{products}$$

$$OR\bullet + OR\bullet \longrightarrow \text{(such as ketones, alcohols)}$$

The oxidation reaction is terminated when free radicals combine with other radicals to yield stable compounds.

2) Photosensitized oxidation

A mechanism, other than autoxidation, is the formation of hydroperoxides from unsaturated fatty acids (RH) by exposure to light in the presence of oxygen and a sensitizer (Sens) (9, 10, 11). There are two mechanisms proposed for photosensitized oxidation depending on the sensitizer and the formation of singlet oxygen.

Type I

$$\text{Sens} + \text{RH} + h\nu \longrightarrow \text{intermediates}$$

$$\text{intermediates} + O_2 \longrightarrow \text{products} + \text{Sens}$$

A type I reaction, without singlet oxygen, involves the reaction of unsaturated fatty acids with a sensitizer, such as riboflavin, to form intermediates during light absorption. The intermediates then react with ground state oxygen to form hydroperoxides. A type I reaction involves hydrogen abstraction from unsaturated fatty acids, and produces the same hydroperoxides as in autoxidation (9).

Type II

$$\text{Sens} \xrightarrow{h\nu} {}^1\text{Sens}^* \longrightarrow {}^3\text{Sens}^*$$

$${}^3\text{Sens}^* + {}^3\text{O}_2 \longrightarrow {}^1\text{O}_2 + \text{Sens}$$

$${}^1\text{O}_2 + \text{RH} \longrightarrow \text{ROOH}$$

In a type II reaction, oxygen is activated by an excited sensitizer to form singlet oxygen during light absorption. The oxygen inserts on either carbon of a double bond, which then shifts to yield an allylic hydroperoxide in the *trans* configuration (2). Natural pigments, such as chlorophyll in vegetable oil, can act as photosensitizers in this type of reaction (11).

Flavor reversion of soybean oil

Flavor reversion of soybean oil has been a problem for the food oil industry for over 60 years, since edible soybean oil was first produced in the 1930's (29). A refined, bleached and deodorized soybean oil, even at low oxidation conditions, produces a green, beany, weedy and hay flavor similar to the green, beany flavor of crude oil. The term, reversion, is used to describe this flavor; however, the reversion flavor is not exactly the same as in the crude oil. Perhaps a better term to describe this specific flavor is "retrogression" (29).

In early studies, linolenic acid was implicated as the precursor of the reversion flavor because this flavor mainly occurs in soybean, rapeseed, and linseed oil, all oils containing high levels of linolenic acid. Hoffmann (16) found that a green-bean odor compound, *cis*-3-hexenal, also identified by Ullrich (23), derived from oxidation of linolenic acid may contribute to reversion flavor. Dutton et al. (13) prepared low-linolenic acid (2-3%) soybean

oil by furfural extraction method and found by sensory evaluation that the low-linolenic acid oil had less reverted odor than normal soybean oil. They also conducted a storage test in which 7.5% linolenic acid was esterified into cottonseed oil. The panelists perceived the reverted flavor in the treated cottonseed oil.

Johnson et al. (15) suggested that the polymers formed during oxidation of ethyl linolenate may be the precursors of reversion compounds such as methyl ethyl ketone, propionaldehyde, and acetaldehyde. Ethyl vinyl ketone (36) and diacetyl, a buttery odor, (37) were found to be important in reverted flavor and are believed to be formed from the oxidation of linolenic acid.

2-Pentylfuran, having a beany odor, was studied as a possible contributor to the reverted flavor of soybean oil (38), but the compound was proposed to be derived from linoleic acid which is present in great amounts in nonreverted oil. Perhaps, the oxidation of linolenic acid may catalyze the oxidation of linoleic acid to produce more 2-pentylfuran. Chang et al. (21) isolated and identified 2-pentenylfurans, *cis*- and *trans*-2-(2-pentenyl)-furans and *cis*- and *trans*-2-(1-pentenyl)-furans, in reverted soybean oil. These compounds were proposed to be derived from the autoxidation of linolenic acid

Although these experiments showed that the presence of linolenic acid increased reverted reversion flavor, some researchers have suggested otherwise. Soybean oil from a low-linolenic acid (2.9%) soybean variety had no less reverted flavor than soybean oil with normal linolenic acid content (39). Sims (14) lowered the linolenic acid content of soybean

oil by hydrogenation and found no correlation between linolenic acid content and flavor stability

Many compounds have been identified in reverted soybean oil, and many theories have been postulated as the cause of flavor reversion. These include the presence of linolenic acid, isolinoleic acid, oxidative polymers, phospholipids, nonsaponifiables, multivalent metals and singlet oxygen (29). Smouse concluded that oxidation of fatty acids was the cause of flavor reversion, and only tiny amounts of oxidation were needed. Reverted flavor, he claimed, was due to the combination of compounds mainly derived from linolenic and linoleic acids. Recently, additional work has targeted identifying specific minor compounds which contribute to the reversion flavor in soybean oil because linoleic and linolenic acid contents are the same in both nonreverted and reverted oils. Therefore, reverted flavor must have another origin. Endo et al. (22, 25, 27) conducted a series of experiments to determine the impact of some specific compounds on reversion flavor in soybean oil. At first, a blended oil with the same fatty acid composition as normal soybean oil was prepared by mixing corn, safflower, and linseed oil to determine the effect of fatty acid composition on flavor reversion. The blended oil had less reversion flavor than soybean oil. Therefore, not only fatty acid composition, but also factors in soybean oil influenced the reversion flavor. In a further experiment, soybean oil was fractionated by silicic acid column chromatography into soybean oil triacylglycerides and some fractions eluted with increasing the solvent polarities. A flavor test was conducted on the soybean oil triacylglycerides alone and with each fraction added back. Soybean oil triacylglycerides with the ethyl ether/n-hexane (1:1) fraction had

the greatest reverted flavor. Unusual triacylglycerides in this fraction thought to be precursors of reverted flavor were identified as 10-oxo-8-octadecenoic acid and 10- or 9-hydroxyoctadecanoic acid.

Furanoid fatty acids, 10,13-epoxy-11-methyloctadeca-10,12-dienoic acid (I), 10,13-epoxy-11,12-dimethyloctadeca-10,12-dienoic acid (II), and 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid (III) were detected in soybean oil, wheat germ oil, rapeseed oil and corn oil by Guth et al. (28). After developing a method for quantification, they indicated that the amounts of II and III were greater in soybean oil than in corn and rapeseed oils. Furanoid fatty acid II was proposed to be quickly photoxidized to form 3-methylnonane-2,4-dione (MND), a potent compound identified in reverted soybean oil (24). Wu et al.(40) also identified this furanoid fatty acid in commercial soybean oil. The detection of MND during the storage of soybean oil under light and the flavor significance of MND evaluated in an oil/water emulsion system is presented in a later chapter.

Isolation of flavor compounds

The analysis of flavor compounds is quite difficult because these compounds exist in very low concentrations in food systems and analytical instruments have not yet been developed to have a sense of smell. Minor components often are the major contributors to odor, whereas abundant constituents sometimes are not perceived by the human nose.

Generally, the methods used to isolate flavor compounds from fats and oils utilize the volatility, solubility, and functional group of the flavor components.

Headspace method

The analysis of headspace vapors above a food seems an ideal method for flavor analysis. It is very simple, rapid and the flavor compositions are only what the nose receives. Buttery et al. (41) used this method to measure the fat autoxidation and browning aldehydes in reconstituted dehydrated potato, but only the most abundant volatiles could be detected and they may not contribute to the overall flavor.

Headspace concentration

The defect of low flavor concentration by the headspace method may be accomplished by headspace concentration. This method typically involves purging a sample with an inert gas to obtain more volatiles, and then concentrating the volatiles by either cryogenic or porous polymer trapping. Cryogenic trapping (cold trapping) is accomplished by passing the headspace volatiles through cold traps which can be cooled with dry ice/acetone or liquid nitrogen (42). The problem of this method is the co-condensation of water in the cold trap. It is necessary to do further solvent extraction prior to GC analysis. The trapping of volatiles on an adsorbent such as charcoal, Tenax, or Porapak has become the common method for concentration of volatiles without water adsorption. The adsorbed compounds can be desorbed by either thermal desorption (43) or solvent extraction (44). Table 1 shows comparisons of adsorbents in surface area and capacity (45). Although Tenax has a poor adsorption capacity which easily results in "breakthrough", it still is widely used because of

Table 1 Absorbents Used for Trapping of Organic Volatiles from Vapor Phase [a]

Absorbent	Structure	Surface Area	Absorbent Capacity[b] Ethanol	Benzene
Charcoal	Coconut carbon	1150-1250	7.9	24.7
Porapak Q	Ethyl vinyl benzene-divinyl benzene	550-650	0.18	NA[c]
Tenax GC	Diphenyl-phenylene oxide	18.6	NR[d]	0.53
XAD-4	Styrene divinyl	849	0.40	2.9
XAD-7	Acrylic ester	445	0.90	1.8
XAD-9	Sulfoxide	70	0.70	0.82

[a] From Sydor and Pietryzk (1978).
[b] Percentage of weight of adsorbent.
[c] NA, not available.
[d] NR, not retained.

its good recovery of adsorbed volatiles and its thermal stability (46). Buckholz et al. analyzed the headspace volatiles of roasted peanuts by using Tenax adsorption. The results showed that operation conditions such as purge gas flow rate and trapping time influenced the flavor profile (46).

Headspace concentration has been suitable for qualitative flavor analysis, but is not as useful for quantitative analysis. The major problems are the correlation between the amounts of flavors measured in an adsorbent and the actual amount existing in the sample (47). This problem was solved by dynamic headspace analysis with equations derived from theoretical considerations allowing the flavor concentration in a sample to be calculated from the peak area of a GC integrator (43).

Snyder et al. (48) analyzed the volatile compounds of soybean oil extracted with supercritical fluid. During the supercritical fluid extraction process, a Tenax trap was attached at the exhaust port of collection vessel to collect the volatiles. In this study CO_2 was used as an extractor and a carrier. Thermal desorption of the trapped volatiles in a GC inject port usually is followed by a cryofocusing in the first loop of a capillary column in order to obtain a high resolution peak.

Isolation of flavors by distillation

Flavor isolation from food systems by distillation takes advantage of the volatility of flavor compounds. Although much unique equipment has been designed for volatile flavor analysis (49-53), the basic apparatus includes a sample vessel, heater, high vacuum pump, and a series of cold traps and/or steam source.

Several terms are used to specify distillation methods, including high vacuum degassing, molecular distillation and steam distillation. The process commonly used in the edible oil industry to remove off-flavor components is called deodorization, a combination of high temperature, high vacuum and steam distillation.

Molecular distillation is used for isolation of flavors from a lipid sample, without steam stripping and further extraction with much solvent. This process involves the direct transfer of volatiles from lipids to the cold trap. The distance between the sample lipid and the condenser surface must be very close in order to prevent loss of volatiles (50-52).

Steam distillation is used to efficiently strip the volatiles out by introducing steam as a carrier into the lipid sample. The addition of a high vacuum apparatus can facilitate the isolation process.

Dirinck et al. (54) used a simple steam distillation method to isolate volatiles from crude palm oil. The sample oil was combined with distilled water in a flask fitted with a stirrer, and then the oil-water mixture was agitated vigorously and heated to boiling. The distillate was a dilute solution of volatiles and water. The sample needed to be extracted by an organic solvent such as pentane, diethyl ether or dichloromethane and concentrated prior to GC analysis. The major disadvantage of steam distillation is the additional steps of extraction and concentration, which might introduce impurities from the solvent and or lose the volatile compounds during concentration.

The conditions of distillation influence the efficiency of isolation. Brauyn et al. (51) examined the effect of stirring, pressure and temperature during molecular distillation. They found that stirring and high temperature increased the yield, whereas variations in pressure from 5×10^{-5} to 10^{-6} mm Hg had no effect on the yield.

Chang et al. (53) indicated the importance of selecting parameters during the isolation of reversion flavors (beany) from reverted soybean oil. If the reverted soybean oil was distilled by deodorization condition (vacuum of 0.05 mm Hg, over 200°C for 2-4 hr.), the distillate did not represent the reversion flavor. Presumably, the distillate contained not only the volatiles originally in the reverted soybean oil, but also thermal decomposition products of peroxides and free fatty acids. When the oil was properly heated at 70°C and reacted with

water vapor in an Oldershaw column for a short time. the volatiles isolated from the reverted soybean oil had a characteristic beany flavor.

Solvent extraction of steam distillate

Most flavor compounds are soluble in organic solvents such as pentane. diethyl ether. and dichloromethane. Solvent extraction is normally used on nonfat beverages or combined with steam distillation because lipids exhibit solubilities similar to those of volatiles in the organic phase.

Factors influencing the selection of a solvent include the boiling point of the flavor compounds of interest and of the solvent. the solubility of the flavors in the solvent and the purity of solvent (55). Generally, the solvent should have a lower boiling point than the flavor compounds because the solvent extract must be concentrated by evaporation of solvent prior to GC analysis. Diethyl ether and pentane are common solvents for flavor extraction since they have low boiling points. Hexane and cyclohexane are suitable for high-boiling flavor compounds.

Nonpolar solvents such as pentane. isopentane. and hexane are suitable for the extraction of flavors from alcoholic beverages because of the limited solubility of alcohol in these solvents (56, 57). Schults et al. (58) compared the extraction efficiencies of several solvents on apple essence. The results showed that isopentane and fluorocarbon had lower recoveries for the low molecular weight alcohols than did diethyl ether and liquid carbon dioxide. but the former solvents gave a higher concentration of esters and aldehydes.

As mentioned before, because the solvent extract must be concentrated for further analysis the impurities existing in a solvent will be also concentrated, causing contamination. Solvents are usually purified by a distillation apparatus equipped with a high reflux ratio column and passed through an adsorption column such as Florisil or silica gel. Diethyl ether may be reacted overnight with $LiAlH_4$ and distilled fresh each day (59).

Flavor isolation by dialysis

Flavor compounds may be extracted directly from lipid samples by dialysis methods. Volatiles are relatively smaller in molecular size than the lipid materials such as triacylglycerides, sterols, and waxes. Therefore, a suitable pore size membrane may be used to separate the flavor compounds from the lipid sample. Benkler et al. (60) isolated flavor compounds from cheddar cheese and ground beef via solvent extraction followed by dialysis with a perfluorosulfonic acid membrane in acetone-pentane mixture. Since dialysis methods are based on the molecular size, recoveries of short-chain volatiles would be expected to be better than those of long-chain compounds.

Concentration of solvent extract

Evaporation is the main method used to concentrate the solvent in an extract. Evaporation makes use of the difference in boiling point between flavor compounds and the solvent. Since the solvent extract might contain a small amount of water, it is better to remove water prior to concentration; otherwise water may become the main component after

evaporation. Generally, the water is removed from the solvent extract by adding anhydrous sodium sulfate. Buttery et al. (61) used an alternative method to remove moisture by freezing out the water. Freezing concentration can be conducted to concentrate flavor compounds in an aqueous steam distillate prior to solvent extraction by removing ice crystals frozen out of the distillate. Thus, the solvent volume is reduced for extraction. This method is not frequently used because only a minimal concentration is achieved (62).

The disadvantage of evaporative concentration is that the volatiles might be lost during evaporation by co-distillation or azeotrope formation. The losses of different compounds may not be the same in proportion, which results in quantitative error. Dirinck et al. (54) estimated the loss of flavor compounds during solvent evaporation by adding C^{14}-labeled toluene (b.p. 111°C) in dichloromethane as an internal standard. The results showed that 0.85% of toluene was lost in the first evaporation (from 200 mL to 20 mL), and 7.5% of toluene was lost in the second concentration (from 20 mL to 100 *u*l). Greater losses of lower boiling volatiles would be expected.

The simplest evaporation method is to heat the solvent extract in the vial gently with a stream of inert gas (N_2, He), provided low boiling point compounds are not of interest. A reflux column can be added on a pear-shaped flask to reduce losses of flavors with high volatility. Moshonas et al. (63, 64) concentrated the solvent extract of lemon, lime and tangerine essence by evaporation under reduced pressure. Vacuum may be used to facilitate the concentration step if the solvent extract contains relatively higher boiling flavors or thermally unstable compounds.

Concentration of flavor compounds by adsorption

Adsorption method for flavor concentration is accomplished by passing the aqueous sample or steam distillate through an adsorbent column such as charcoal, alumina, silica gel or porous polymers. Then, the concentrated compounds are eluted with a small amount of organic solvent. Vitzthum et al. (65) passed the steam distillate of coffee aroma through a glass tube packed with Porapak Q and eluted the adsorbed volatiles with diethyl ether. Heinz et al. (66) used activated coconut charcoal as an adsorbent to collect the volatiles from Bartlett pear purée. The charcoal was then freeze-dried to remove residual water and extracted with ether by utilizing a Soxhlet extractor. Problems with adsorption techniques might include contamination and selective adsorption of the adsorbent.

Fractionation of flavor compounds

The isolated flavor compounds are generally analyzed by gas chromatography; however, a high-resolution GC column may not completely separate individual components as shown by the peak overlaps of compounds. Prefractionation of isolated mixtures can simplify further analysis. Moreover, each preseparated fraction has its distinct physical and chemical properties, which provides more information on flavor research. For example, compounds containing carbonyl groups are the main source of flavor. Typically the techniques used in prefractionation include pH-based separation, adsorption chromatography, high-pressure liquid-chromatography (HPLC), and preparative GC (62).

pH based separations

Aqueous distillates of flavor compounds can be separated into acidic, neutral, and basic fractions by pH adjustment and alternative extraction between an organic solvent and an aqueous phase. The procedures of pH-based separation are as follow (55):

1. Adjust the pH of the aqueous mixture to pH 4.0 and extract with an organic solvent. The acidic and neutral compounds will go into the organic phase while the basic components still remain in the aqueous phase.

2. Change the pH of the aqueous portion to pH 10.0 and extract with organic solvent. The basic compounds will exist in the organic phase.

3. Extract the organic phase, which contains acidic and neutral compounds, with aqueous alkali at pH 10.0. The neutral compounds remain in the organic phase and acidic compounds will be in the aqueous part.

4. The pH of the aqueous phase with acidic compounds is adjusted to pH 4.0, followed by extraction with the organic solvent.

Figure 2 illustrates the scheme of pH-based separation (67).

Adsorption chromatography (68-70)

Alumina, silica gel and Florisil are commonly used to fractionate flavor compounds. Adsorption chromatography is based on the polarity of each compound and the eluting solvent. The flavor compounds are held by the adsorbent via hydrogen bonding, van der Waal's forces and ionic bonds, and are removed from the column by eluting with solvents of

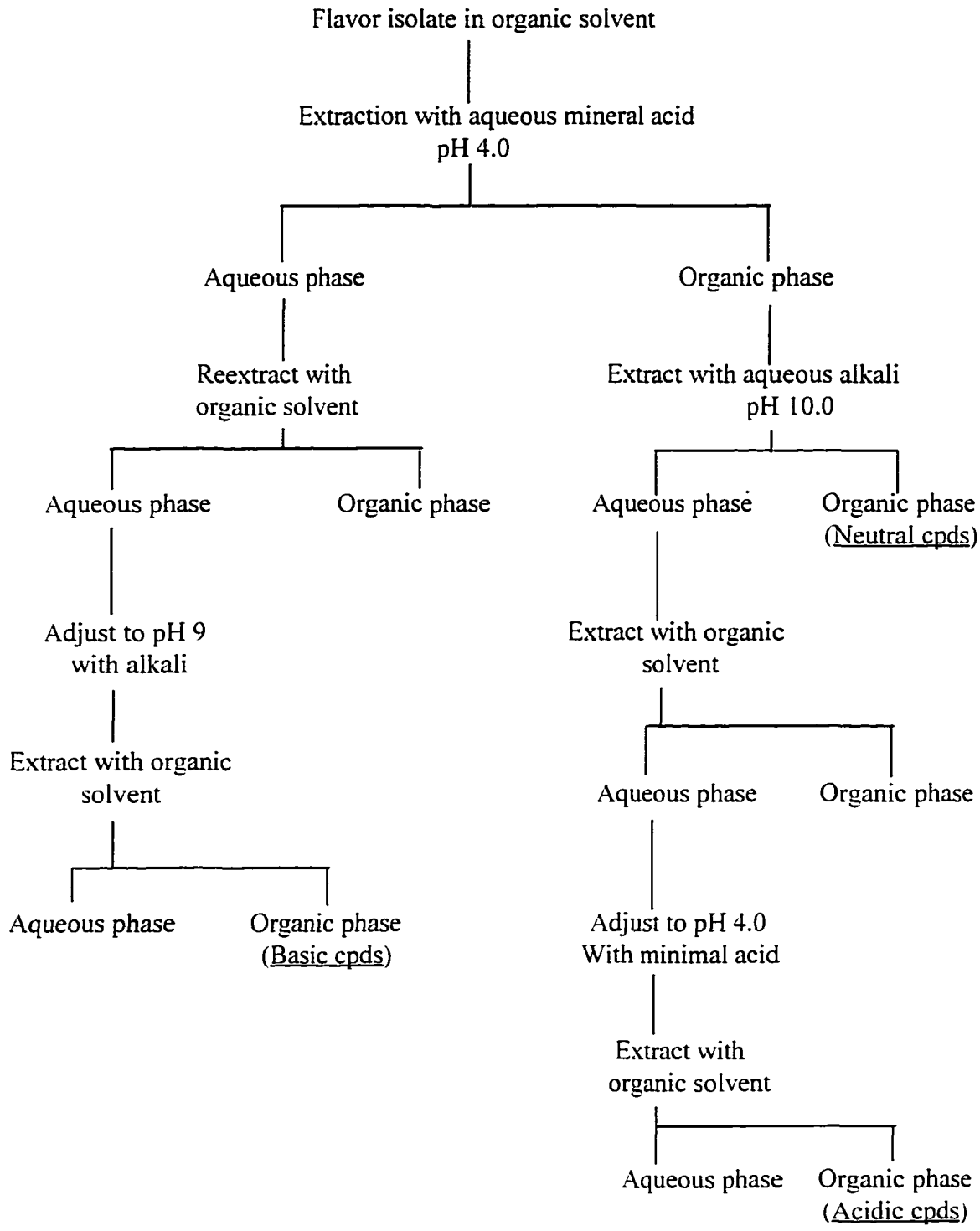

Figure 2. Fraction scheme for the separation of volatiles into acidic, neutral and basic compounds (67).

gradually increasing polarity. A series of solvents commonly used in order of increasing polarity are hexane< cyclohexane< carbon tetrachloride< benzene< chloromethane< diethyl ether< chloroform stabilized with 1% ethanol< ethyl acetate< acetone< acetonitrile< methanol< acetic acid< water (71).

Tressl et al. (68) separated hop aroma constituents by applying the aroma extract on a column filled with aluminum oxide and silica gel. Six fractions were eluted by increasing the polarity of solvents which were combined with pentane, methylene chloride, and ether in different proportions. White et al. (59) removed interfering hydrocarbons from fat and carbonyl compounds by elution with cyclohexane/ether (99:1). Buttery et al. (70) separated aroma compounds collected from cooked artichoke into hydrocarbon and oxygenated fractions by applying the volatile oils on a silica gel column and eluting with pentane and diethyl ether, respectively.

High pressure liquid chromatography (HPLC)

The HPLC is not well suited to flavor studies because it lacks resolution power; however, it may be applied to the fractionation of flavor compounds prior to GC analysis. Teitelbaum (72) used adsorption and partition columns to fractionate the flavor compounds from cocoa butter steam distillate prior to GC analysis. Guth et al. (73) also utilized HPLC to separate the distillate of oxidized soybean oil into two fractions for further analysis.

Preparative gas chromatography

Preparative GC provides the greatest fractionation power to simplify further GC analysis. Each fraction obtained from preparative GC may be rechromatographed with another packed column to get subfractions (74). The effluent from preparative GC is collected in a cold trap, which offers a solvent-free fraction and is suitable for sensory evaluation. The disadvantage of this technique is that collection steps need to be repeated in order to obtain sufficient sample for further experiments.

Separation of individual classes of volatiles by derivative formation

Isolation of individual classes takes advantage of specific functional groups in flavor compounds, and involves the formation of derivatives. Derivation often offers some advantages for isolation and quantification such as making the compounds UV detectable, able to form color, thermally stable or lower in volatility (62). Carbonyl compounds, the main sources of flavors, are commonly isolated and quantified as their hydrazone derivatives. 2,4-Dinitrophenylhydrazones (DNPHs) have been used frequently in carbonyl studies (75-78). The DNPHs are further separated by thin-layer chromatography (TLC) or column chromatography and quantified by measuring the absorbances on a spectrophotometer (75). 2,4,6-Trichlorophenylhydrazine was also used as a reagent for carbonyl derivatives, 2,4,6-trichlorophenylhydrazones (TCPHs), which can be measured by direct gas chromatography with an electron capture or a flame ionization detector (59, 79).

Identification of flavor isolates

Once the flavor compounds are isolated from a food sample, the final steps are to separate, identify, and quantify the individual compound. Gas chromatography (GC) and mass spectrometry (MS) are two powerful instruments used by flavor chemists to analyze the flavor compounds. Because flavor compounds generally exist in food systems in extremely low levels only 500 components were identified before 1963 and before GC was utilized. The invention and application of GC and MS on flavor research has resulted in thousands of compounds being identified (62).

The most important part of a GC method is the column, which decides the success or failure of sample separation. The revolution of the column from "packed column" to "fused silica capillary" enhanced the resolution power to 3000 to 5000 theoretical plates/meter, which makes it possible to separate complex flavor isolates (55). At the end of a column, a detector is connected to indicate and measure the amount of separated compound in the carrier gas. A good detector should be sensitive enough to detect trace components and respond to most of the compounds. A flame ionization detector (FID), thermal conductivity detector (TCD) or electron capture detector (ECD) are used for GC analysis; however, the human nose, a biological detector is special in flavor research since other detectors can not indicate the flavor significance of each compound. This concept will be discussed later in the section on sensory evaluation.

The FID provides excellent sensitivity and responds to most of the compounds except N_2, O_2, H_2O, and carbon disulfide (80). The TCD is a nondestructive detector which

measures thermal conductivity of gasses and responds to all compounds. However, it has a lower sensitivity than the FID and requires good temperature and flow control (80). Since it is a nondestructive detector, Dirinck et al. (54) evaluated the volatile compounds of crude palm oil by sniffing the effluent of the GC equipped with a micro thermal conductivity detector. The ECD is employed by measuring the loss of electron current captured by certain molecules. The ECD only responds to electron-adsorbing compounds such as alkyl halides, conjugated carbonyls, nitrates, and organometals, but is insensitive to hydrocarbons, ketones, and alcohols (80). Johnson et al. (79) utilized the ECD to measure carbonyl compounds by the formation of 2,4,6-trichlorophenylhydrazone derivatives.

Some selective detectors such as flame photometric (FPD) and nitrogen-phosphorus (NPD) detectors can provide more information on elemental composition to help identification of unknown peaks. The FPD is specific for sulfur compounds, whereas NPD has an enhanced response to phosphorus and nitrogen compounds (55).

The information of an unknown peak from the GC equipped with the detectors mentioned above can provide only the amount and the retention time of a compound with or without some special elements. Comparisons of responses and retention times with those of standards is helpful, but information supplied by MS is needed for true identifications. The MS is an analytical technique which provides the molecular weight and molecular structure of a compound. The combination of GC/MS has become a favorite instrument for flavor chemists to identify unknown flavor compounds.

Sensory evaluation

Once flavor compounds are identified, further challenging work involved determining the flavor significance of an individual volatile compound and the overall impact of the mixture of volatiles. Many flavor researchers have attempted to evaluate flavor significance by physical or chemical methods. However, for a true evaluation of flavor impact, one must use the only real biological detector, the human nose, since human beings are the final judges of flavor (81).

There are three fundamental types of sensory evaluation, including discriminative tests, descriptive tests, and affective tests. Discriminative tests are used to determine whether a difference exists between samples, such as the triangle test, the duo-trio test, or the two-out-of-five test. In descriptive tests, the nature and intensity of the sample is determined. Examples include scaling methods, flavor profiling, texture profiling, and quantitative descriptive analysis. Affective tests are based on the preference or acceptance of an individual person to a given sample (82).

Generally, sensory evaluation of flavor compounds is conducted by using odor or flavor tests. Panelists should be trained to have a good verbal skills to describe flavor characteristics and be familiar with the odors of compounds (82).

The odor test (one of the descriptive tests) takes advantage of the GC to separate individual volatile components. While emerging from the column, the volatiles are sniffed by a panelist and then the odor's character, intensity and retention time are recorded. During GC sniffing, the end of the column is equipped with an effluent splitter, nondestructive

detector, or even exists without a detector. The GC sniffing test produces an aromagram which is a description of the sensory response to each peak of a chromatogram (83, 84). Aromagrams and gas chromatograms produce quite different information. Often minor peaks shown on a GC have a potent odor, whereas abundant components may make no contribution to the sensory perception. The determination of aroma intensity is quite difficult during GC sniffing. Ullrich et al. (85) used aroma extract dilution analysis (AEDA) to estimate the intensity of volatiles. The AEDA is conducted by injecting a series of diluted samples until no odors are perceived.

Flavor is an overall perception combined with taste and aroma. Flavor compounds are always present in a medium such as water (86), milk (87), oil (88-90) or oil/water (43, 91, 92) and evaluated in the mouth with the tongue and olfactory system. The flavor threshold value is measured to indicate the potency of a flavor compound. A compound present at a concentration over its threshold is considered to make a direct contribution to flavor, whereas the concentration below threshold is thought to exhibit little or no effect (86). Dixon et al. (92) determined the flavor thresholds of carbonyl compounds in mineral oil/water emulsions and compared these with values in oil, water, and a milk medium reported in the literature. The results showed that the flavor thresholds were quite varied in different media. Stone et al. (91) evaluated flavor intensity of soybean oils in an emulsion system stabilized with gum acacia. An emulsion method instead of tasting oil directly avoids taste carry-over and one can evaluate more samples at a time. Since oil is often consumed in the emulsion form, such

as in a salad dressing or mayonnaise, it may be more practical to taste oil by an emulsion method.

Many researchers have worked on the correlation between GC response and flavor intensity scores by sensory evaluation. The goal would be to predict the oil quality only by instrumental analysis (93-95). The results showed that flavor intensity scores correlated well with selected volatile compounds and even with total volatiles.

THE IMPACT OF 3-METHYLNONANE-2,4-DIONE ON FLAVOR DETERIORATION OF SOYBEAN OIL

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ABSTRACT

The compound, 3-methylnonane-2,4-dione (MND), was synthesized in the laboratory and purified on a packed column (Silar 10 C). GC analysis of the purified MND on a nonpolar column (SPB-1) showed two well-separated main peaks which were presumed to represent the keto and enol forms. Between the two main peaks there was a bridge of poorly resolved components which may represent the enol form being on both the 2 and 4 positions and various hydrogen-bonding associations. The MND had an intense straw-like and fruity odor when evaluated at the outlet of a gas chromatograph. Sensory evaluation of MND in a mineral oil/water emulsion system showed that its flavor intensity increased almost imperceptibly with increased concentration (from 0.09 to 2.56 ppm), and the flavor was just above threshold. An explanation for the unusual flavor response may be that when molecularly dispersed in air, MND has an intense odor, but when dissolved in a nonpolar liquid, such as mineral oil or soybean oil, MND may exist in a form with relatively low flavor intensity. The concentrations of MND in soybean oils at various peroxide values (PVs) were obtained between 0 to 0.804 ppb which were far less than those tested in mineral

oil/water emulsion during sensory evaluation. In general, these results do not support the theory that MND contributes strongly to the reversion flavor of soybean oil.

INTRODUCTION

The compound, 3-methylnonane-2,4-dione (MND), has been found in reverted soybean oil (SBO) and is suspected of being one of the compounds contributing significantly to the reversion flavor of SBO (1, 2). It was proposed that the MND is derived from the light-induced oxidation of the furanoid fatty acid (F acid), 10,13-epoxy-11,12-dimethyloctadeca-10,12-dienoic acid, which is one of three F acids detected in soybean oil by Guth et al. (3). The other two F acids reported in SBO are 10,13-epoxy-11-methyloctadeca-10,12-dienoic acid and 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid.

The F acids are found in plants such as soybean, rapeseed (3), wheat (*Triticum aestivum*), grasses (*Poaceae spec.*), and cabbage (*Brassica oleracea spec.*) (4) as well as animals, such as northern pike (*Esox lucius*) (5, 6), cod (7), and crayfish (8). Up to eight F acids have been detected in fish (6). The various F acids differ in side-chain length and contain one or two methyl branches on the furan ring. The F acids content of SBO is comparatively low (0.016 to 0.049%) (9). In fish sources, F acids are found primarily in hepatic and testicular lipids, and their amounts vary widely depending on the season. The biological significance of the F acids is not clear.

The isolation and quantification of F acids may be accomplished by using silver ion chromatography (6, 7) or hydrogenation (8) in combination with urea fractionation. Guth et

al. (3) found no significant change in the amount of F acids before and after refining SBO. and Scrimgeour (7) also found no significant difference in the amount of F acids of crude and refined cod liver oil. Recently, Wu et al. (9) quantified the two major F acids in a number of soybean plant introductions.

In a study of MND by Guth et al. (1), the odor threshold of MND was evaluated by an olfactometric method and was reported to be very low (0.007 to 0.014 *n*g/L. in the air). The odor threshold of MND increased drastically when it was dissolved in freshly refined SBO (15 to 30 *u*g/Kg); however. the taste threshold of MND in an oil was not determined. When the MND was analyzed on a nonpolar capillary column (SE-30. SE-54) or intermediate polar column (OV-1701), it separated into two peaks. believed to be keto and enol tautomers: however, only one peak was observed on a polar column (SW-10). The objectives of the current work were to study the flavor intensity and chemical characteristics of MND synthesized in the laboratory. and the development of MND and its flavor impact during storage of SBO under light at 35°C.

MATERIALS AND METHODS

Synthesis of 3-methylnonane-2,4-dione (MND)

The synthesis of 3-methylnonane-2.4-dione (MND) was carried out by Wu (10) by using the following procedure:

1. The synthesis of methylhexanoate was accomplished by first heating and refluxing 64 g of hexanoic acid and 71 mL of methanol with 1.4 g sulfuric acid for 6 h. Distilled water (350

mL) was added to the reaction mixture, and the organic phase, methylhexanoate, (upper layer) was separated by using a separatory funnel. The organic phase was washed three times with 70 mL 5% sodium carbonate and three times with 70 mL distilled water. The organic phase was then dried with sodium sulfate and distilled. The distillate was collected between 149°C and 150°C.

2. The synthesis of nonane-2,4-dione (11) involved adding a mixture of 60 g methylhexanoate and 14 g acetone dropwise to a suspension of 30 mL cyclohexane with 9.3 g sodium hydride, and heating the mixture for 3 h under reflux. After completion of the reaction, the components boiling below 100°C were distilled from the mixture. The residue was cooled and added to a mixture of 18 g glacial acetic acid and 36 g ice. The organic phase was separated, and the remaining aqueous phase was extracted several more times with diethyl ether. The combined organic phases were washed with distilled water and dried with sodium sulfate. The nonane-2,4-dione was collected by distillation between 101°C and 103.5°C at 16mm Hg.

3. The synthesis of 3-methylnonane-2,4-dione (1) involved adding nonane-2,4-dione (4.06 g) dropwise into a solution of sodium ethylate (1.77 g) in 13 mL of ethanol at 70°C. Methyl iodide (3.69 g) was added and the mixture was refluxed for 3 h. Most of the ethanol was then distilled off, and the sodium iodide crystallized. A 0.5% solution of acetic acid in water was added until the salt crystals dissolved, and then the diketone was extracted four times with 7 mL of diethyl ether. The final compound was analyzed by a GC equipped with a flame ionization detector (Hewlett-Packard 5890 Series II, Palo Alto, CA) and GC/ MS (Hewlett-

Packard 5970 Series Mass Selective Detector. Palo Alto. CA) on a Supelco SPB-1 fused silica capillary column (30 m. 0.25 mm i.d.. 0.25 *um* film thickness) (Supelco. Bellefonte. PA) at a helium flow rate of 1.7 mL/min. The GC was held for 3 min at 30°C. programmed from 30°C to 230°C at 6°C/min. and held at 230°C for 10 min.

Isolation of 3-methylnonane-2,4-dione (MND)

Because the purity of synthesized MND was only 40%. it needed to be purified for sensory experiments. The MND was isolated by preparative gas chromatography (Varian 3700 Series Palo Alto. CA). A "T" swagelock fitting was attached to the end of a packed column (Alltech Silar 10 C. 1.83 m. 3.2 mm o.d.. 2.2 mm i.d.). The GC column effluent was split in a ratio of 1:8. with the lesser amount going to a flame ionization detector (FID) and the greater amount going to a cold trap. which was made with glass capillary tubing surrounded by dry ice. A 4% solution of the MND prepared in hexane was injected onto the column 2 µl at a time. The temperature program was held 1 min at 80°C. programmed at 6°C/min to 144°C. and held at 144°C for 2 min. At the end of each run. the oven temperature was set at 250°C for 20 min to volatilize any impurities that remained in the column. The MND eluted at an oven temperature of about 144°C. at which time the cold trap was connected to collect the sample.

When the desired amount of sample was collected from the GC. the concentrated sample was further analyzed by GC (HP 5890 series II)/MS (HP 5970 series) on a Supelco SPB-1 fused silica capillary column. The conditions were the same as described before.

Sensory evaluation of 3-methylnonane-2,4-dione (MND)

The flavor intensity of 3-methylnonane-2,4-dione (MND) was evaluated in an oil/water emulsion system (12, 13) by comparing the flavor with a blank and a series of 2-heptanone emulsion standards. A blank emulsion was prepared by blending 5.2 g of food-grade gum acacia with 800 mL of tap water for 30 sec at "whip speed" of an Osterizer blender (Oster, Inc., Milwaukee, Wisconsin). After 30 sec, 8 mL of mineral oil (Sigma, light white oil [8042-47-5]) was added and blended for 1 min. Standard emulsions (A through E) were prepared from mineral oil containing 0.25, 0.5, 1, 2, and 4×10^{-4} parts by volume of 2-heptanone (Aldrich, Milwaukee, WI) by using the procedure just described. The MND was prepared in mineral oil at concentrations of 28.4, 82.5, and 256 ppm (by volume). Each MND mineral oil stock solution was emulsified by the same method as the standards for sensory evaluation.

In a related experiment, to see if MND could be depolymerized by heating, mineral oil with 9 ppm MND was divided into two parts. One part was heated in a boiling water bath for 5 min, and another part was not heated. Both mineral oils were then diluted 100-fold with water to form emulsions containing 0.09 ppm MND. The flavor intensity of the MND emulsions with and without heat treatment were evaluated.

For sensory evaluation nine panelists were trained in separate booths with blank emulsions and emulsions containing different amounts of 2-heptanone and MND. After

each training evaluation, the panelists were informed of the identity and concentration of each sample, and panelists were allowed to reevaluate the samples to improve their accuracy.

During actual sensory sessions one emulsion containing MND and an unlabeled blank emulsion were provided along with a labeled blank and the five 2-heptanone standards. The unlabeled blank emulsion was used to monitor the accuracy of panelists. The emulsions were presented to the panelists in 266-mL plastic cups at room temperature and evaluated within 1.5 h of preparation. The panelists were instructed to swirl each emulsion before tasting it, and to rinse their mouths between samples, using the labeled blank emulsion. Saltine crackers (unsalted) also were provided for panelists to use between samples. Panelists rated the flavor intensity of unknown samples by placing a mark perpendicular to a 15-cm line to indicate their intensity relative to the standards (Figure 1). The 15-cm line was divided into four equal lengths and labeled A through E to indicate the prejudged intensity of the standard emulsions. If a sample was perceived to be weaker than A or stronger than E, it could be rated by extending the scale line in the appropriate direction (Figure 1). Each flavor intensity of MND was the average of two replications and nine measurements from panelists and calculated relative to the concentration (intensity) of 2-heptanone. The data were analyzed by standard analysis of variance. Differences in mean values among data were determined by least significant difference (LSD) at $p \leq 0.05$ (14).

Analysis of room odor characteristics of 3-methylnonane-2,4-dione (MND)

The 3-methylnonane-2,4-dione (MND) of 40% purity was analyzed for room odor at the National Center for Agricultural Utilization Research (Peoria, IL) by Kathleen Warner (15).

The room odor of MND was evaluated by placing the MND in a room-odor hood and allowing the odors to be swept into the odor room for panel evaluation. A second experiment was conducted, in which 0.2% of MND in high-oleic sunflower oil was heated to 190°C and was evaluated at 15 min and 5 h. Odors of the sample oils were evaluated in the odor room. A trained, experienced sensory analytical panel of 10 individuals evaluated the odor quality, but not intensity of all samples.

Detection of 3-methylnonane-2,4-dione (MND) during storage of commercial soybean oil

Commercial soybean oils (Wesson and Hy-Vee brands, 800mL) were deodorized at 220°C for 30 min under vacuum (0.005 mm Hg). Oil samples (27 g in 50 mL-beaker) were placed in the oven at 35°C under 1937 lux of fluorescent light. Two beakers containing oil were taken from the oven every 2 to 4 days and PVs were measured twice per beaker by using the Stamm test as modified by Hamm et al. (16).

The detection of 3-methylnonane-2,4-dione (MND) and other volatiles was conducted by headspace concentration followed by GC (HP 5890 Series II)/MS (HP 5970 Series) analysis (13). Briefly, to collect volatiles, a 4-g oil sample was placed in the volatile collection apparatus (Figure 2) which was immersed in a 50°C water bath. The helium stripped the oil of volatiles as it passed through the gas dispersion tube. The flow rate of helium was maintained at 75 mL/min by a flow controller and a rotometer. After the oil was stripped for 5 min, a Tenax trap was connected to the apparatus to collect volatiles for 30 min. The Tenax trap consisted of a Tenax TA (Alltech Associates, Deerfield, IL)-filled glass tube (3-

mm o.d.x 72-mm) previously conditioned in the inlet of a Hewlett-Packard 5890 Series II GC for over 3 h at 230°C and stored in an airtight vial until used.

To desorb the volatiles trapped in the Tenax tube, the entire Tenax trap was inserted into the GC inlet, previously adapted for this use (13), at 230°C, and the volatiles were transferred in helium at 1.7 mL/min onto a Supelco SPB-1 fused-silica capillary column (30m, 0.25mm i.d., 0.25*um* film thickness). In the first 5 min of desorption, the volatiles were condensed into the first loop of the column cooled by acetone-dry ice. After 5 min of condensation, the acetone-dry ice was removed, and the GC inlet was purged. The temperature was held at 30°C for 4 min, programmed from 30°C to 230°C at 6°C/min and held at 230°C for 10 min. The amount of MND in soybean oil was calculated from the peak area of the gas chromatogram by the method of Lee et al. (13). Although there was a small peak at the same retention time as the standard MND, this peak could not be identified by MS because of its small amount. When the volatiles were collected in a 75°C water bath for 60 min, the peak having the same retention time as MND showed the same MS pattern as MND. So, under the collection conditions of a 50°C water bath for 30 min, the peak having the same retention time as MND was assumed to be MND. The data of the amounts of MND and of PVs were the average of four measurements and the standard deviations were calculated.

RESULTS AND DISCUSSION

Gas chromatography of 3-methylnonane-2,4-dione

The gas chromatography (GC) of synthetic 3-methylnonane-2,4-dione (MND) on a SBP-1 (low polarity) capillary column resulted in two distinct and well-separated peaks joined by

a bridge of poorly resolved components. Figure 3 shows examples of these peaks under several GC conditions. The mass spectra of these two peaks and the intervening bridge were identical and similar to those reported by Guth et al. for MND (1), who also obtained a similar picture of poorly resolved peaks for MND when they chromatographed it on SE-30, SE-54 and OV-1701. Our MND preparation was estimated to be 40% pure based on GC peak areas. The MND was purified further by GC on a packed Silar 10C (polar) column. On this column the MND emerged as one peak, in agreement with the report of Guth et al. (1). A grassy, sweet aroma was noted as the MND emerged. After purification on the Silar 10C column, the MND was estimated to be about 99% pure by peak area when rechromatographed on a SPB-1 column.

Sensory evaluation of MND

Aqueous mineral oil emulsions containing 0.28, 0.85, and 2.56 ppm of MND had flavor intensities that were very similar and were rated by the panel as equal to those of 0.389, 0.323, and 0.379 ppm of 2-heptanone, respectively, in similar emulsions (Table 1). Dixon and Hammond reported that emulsions of 0.25 ppm of 2-heptanone were very near the flavor threshold (12), so intensities equal to 0.389 ppm are just slightly above threshold.

Considering the GC evidence that MND could exist in two forms and the intense odors obtained when MND was subjected to GC separations, it seemed possible that the flavor intensity of solutions of MND in nonpolar solvents such as mineral oil might become more intense when heated, so a 9 ppm solution of MND in mineral oil was heated to 100°C before

emulsification, dilution to 0.09 ppm with water and evaluation at room temperature. The emulsions made from unheated and heated MND were rated as equivalent to 0.317 and 0.280 ppm emulsions of 2-heptanone, respectively. Thus, heating to 100°C did not increase the flavor intensity perceptibly when emulsions were tasted at room temperature.

Statistical evaluation of the sensory results indicated that there was no significant difference between 0.09 ppm and 2.56 ppm MND emulsions at the 0.05 probability level (Table 1). Based on these results, MND seemed to have a fairly low flavor threshold, and its flavor intensity increased almost imperceptibly with increased concentration. If 0.09 ppm is considered the threshold, this amount is comparable to 2-octenal and several similar aldehydes (12). But since the flavor intensity of MND remained just above threshold over a 28-fold dilution, it is not clear how much more it might have been diluted before a true threshold was reached. Panelists indicated that during sensory evaluation of the emulsion they could smell the samples before tasting them, but that when tasted, the flavor intensity seemed less strong than they expected. In general, these results do not support the belief that MND is an important flavor compound in soybean oil at room temperature.

Analysis of room odor characteristics of 3-methylnonane-2,4-dione (MND)

Since MND seemed to have the most sensory impact in situations where its odor could be observed, some of our MND preparation (40% pure) was sent to Kathleen Warner at the National Center for Agricultural Utilization Research for room odor evaluations. They reported no quantitative data but reported the aroma characteristics of MND. Pure MND was

described as having an odor characteristic of hydrogenated oils. When MND was dissolved in high-oleic sunflower oil at 2000 ppm and heated to 190°C, its room odor was described as metallic, hydrogenated oil, fatty, tallowy, putty-like, fruity, and dough-like. After heating for 5 h, many of these odors had disappeared and only dough-like, cardboard-like and acrid odors were noted. Unfortunately the concentrations tested were much greater than might occur in oxidized soybean oil so it is impossible to say if the oil plays a part in room odors.

Relation of flavor response to 3-methylnonane-2,4-dione (MND) behavior

The unusual flavor response obtained is best explained by assuming that MND takes a form when molecularly dispersed in air in which it has an intense odor, but when MND is dissolved in a nonpolar liquid such as soybean oil or mineral oil, it takes a form with relatively low flavor intensity. Probably the sensory potent form favored by gaseous dispersions was in equilibrium with the sensory impotent form found in oil so that when the concentration of MND in oil was increased, the equilibrium shifted to maintain a fairly constant concentration of the sensory potent form. In this way, the flavor intensity of oil emulsions did not increase much with MND concentration.

It is not clear what relation may exist between the sensory potent and impotent forms of MND and the various peaks observed during GC on nonpolar columns. Guth et al. (1) believed, on the basis of nuclear magnetic resonance data, that in MND a keto form was favored but that when dissolved in a nonpolar solvent or when the temperature was raised, the equilibrium shifted more to an enol form. The keto and enol forms are presumed to

represent the two peaks observed in the GC traces. The bridge between the two peaks represents materials which have been in equilibrium between the two forms during the separation process and have migrated part of the time in each form.

Figure 3 indicates that the situation may be even more complex than Guth et al. suspected (1). Low temperature chromatograms in figure 3 suggest that there may be as many as six components in equilibrium with each other. These may represent the enol form being on both the 2 and 4 positions and various hydrogen-bonding associations. Figure 4 shows the proposed forms of 3-methylnonane-2,4-dione. Reversible dimerization may be occurring, although the peaks observed on GC do not seem to be far enough apart to represent dimers.

Grosch et al. (1, 2, 17, 18) have advocated identifying important flavor compounds by dilution to aroma threshold. In this method a flavor concentrate is injected into a gas chromatograph and peaks with odors are noted. More and more dilute solutions are injected until the odors one by one disappear. The last ones to disappear are regarded as the most important. This method was used to demonstrate the importance of MND. Our results indicate that this technique should be applied with care because MND does not appear to be an important flavor compound in oil at room temperature despite its odor intensity when smelled at GC outlets.

Detection of 3-methylnonane-2,4-dione (MND) and peroxide values during storage of commercial soybean oil

To relate the flavor intensity of MND to realistic amounts of MND formed in oxidizing oils, two commercial oils were stored and evaluated. The peroxide values (PVs) and the

amounts of MND of the commercial soybean oils stored at 35°C under light are shown in Table 2. The PV development over time followed a typical lipid oxidation curve. The concentrations of MND in soybean oils generally increased as the PVs increased, although there was some variability, especially in Wesson brand soybean oil stored at day 14. The MND concentrations in soybean oils at various PVs were between 0 and 0.804 ppb which were far below those tested in mineral oil during sensory evaluation. Thus, the MND could not strongly contribute to the reversion flavor of soybean oil.

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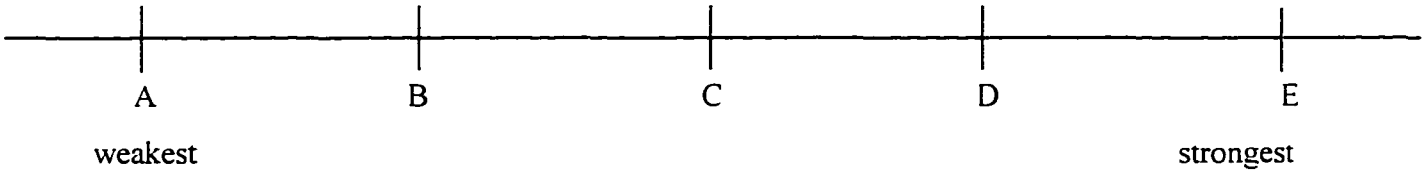
Name _____ **Date** _____

You will be given a blank emulsion, 5 standard emulsions increasing in concentration from A through E, and sample emulsions. The samples may be blanks or duplicates. You are to compare the flavor intensity of the samples with those of standards. Some of the standards and samples may be too weak for you to distinguish from the blank, others may be stronger than the strongest standard. You may place a mark to the left of A or to the right of E. For each sample place a mark across the line indicating its intensity relative to the standards. Label each mark with the appropriate 3-digit number.



Please: 1. mark a (v) on the weakest standard you can distinguish from the blank.

2. Place a (*) on samples that you cannot distinguish from the blank.

Figure 1. Questionnaire for sensory evaluation of 3-methylnonane-2,4-dione

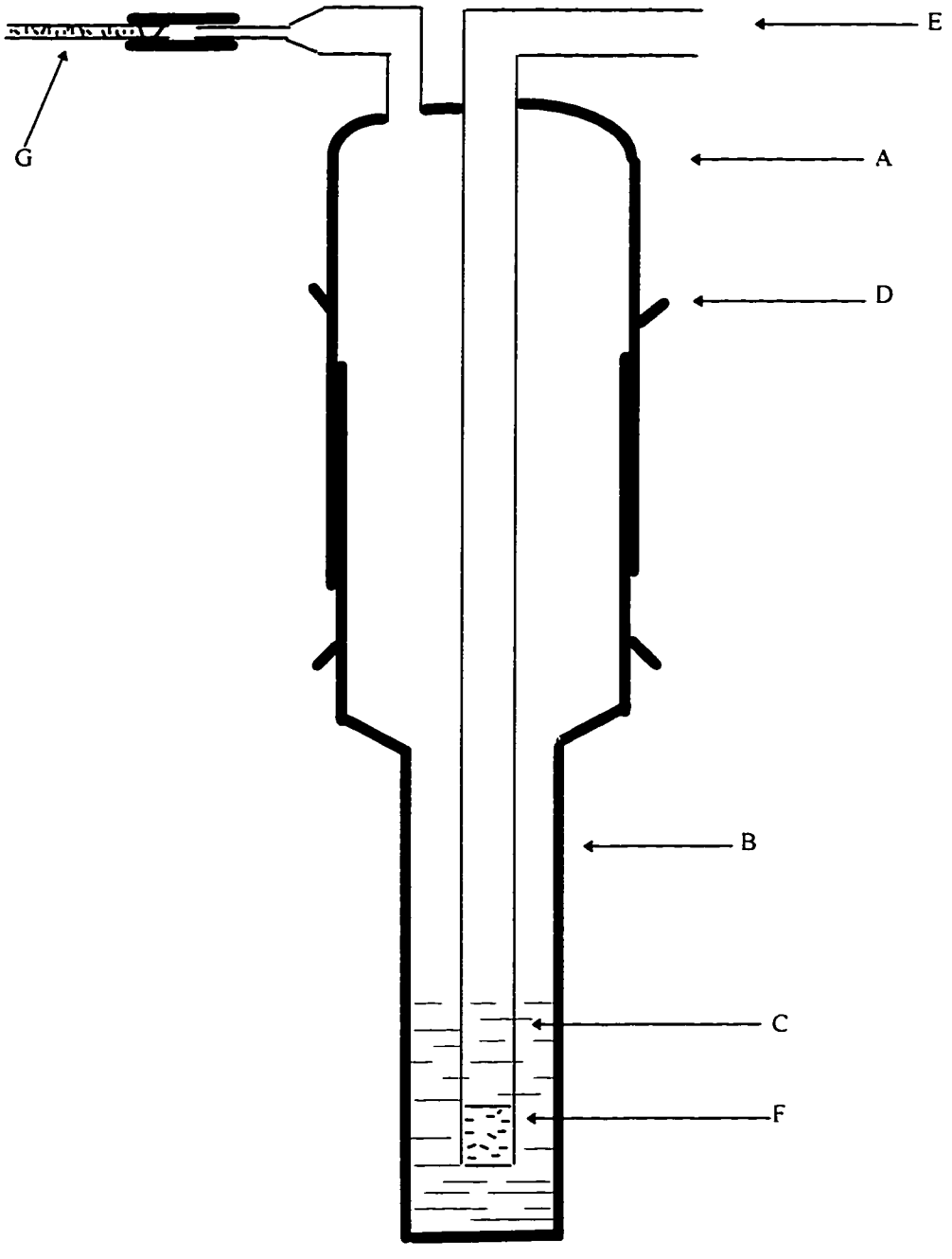


Figure 2. Glass volatiles-stripping apparatus: upper (A) and lower (B) parts jointed by a joint. After the oil sample (C) is introduced, the apparatus is closed and secured by rubber bands fastened to the projections (D). Helium gas (E) is blown into the apparatus through the dispersion tube (F) and volatiles are trapped in a tube with adsorbent, Tenax (G). Lee et al. (12).

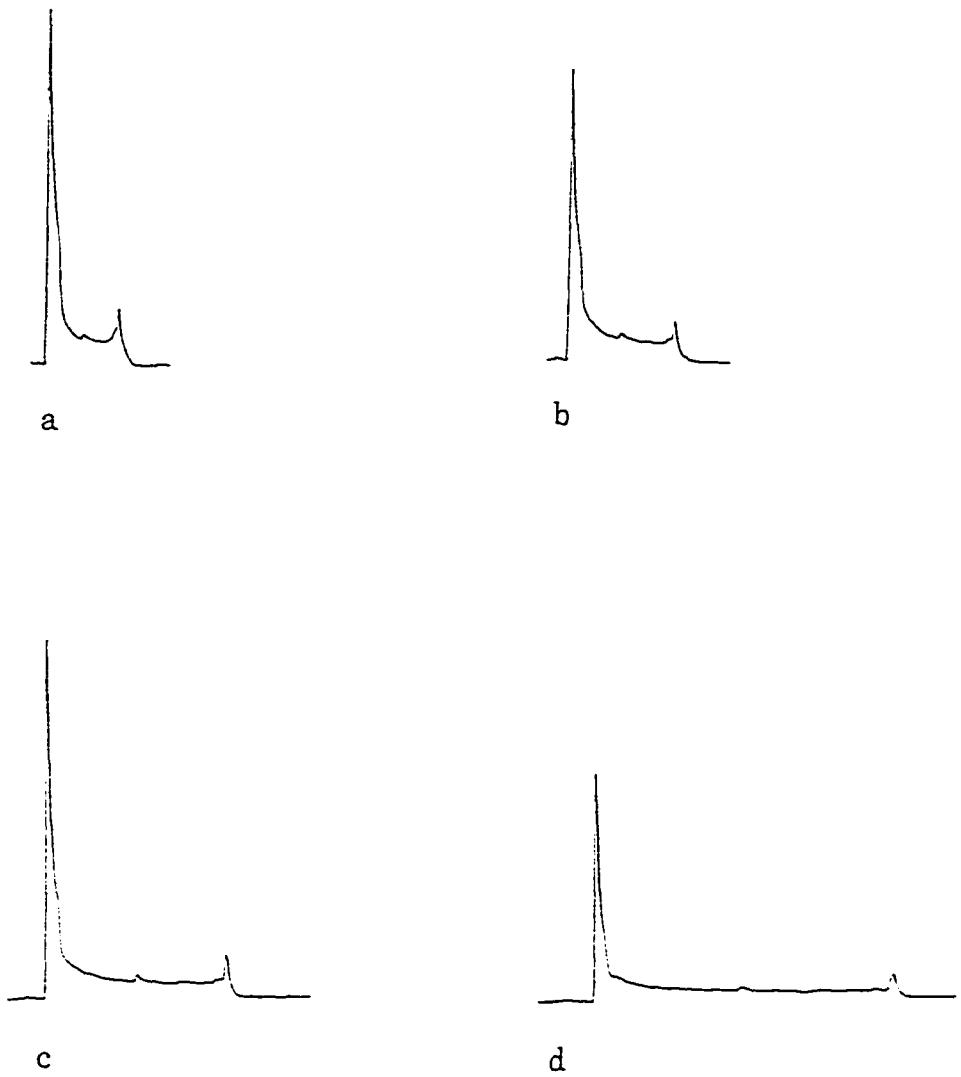


Figure 3. The gas chromatograms of 3-methylnonane-2,4-dione (MND) on a Supelco SPB-1 capillary column at various isothermal conditions: a. 110°C. b. 100°C. c. 90°C. d. 80°C.

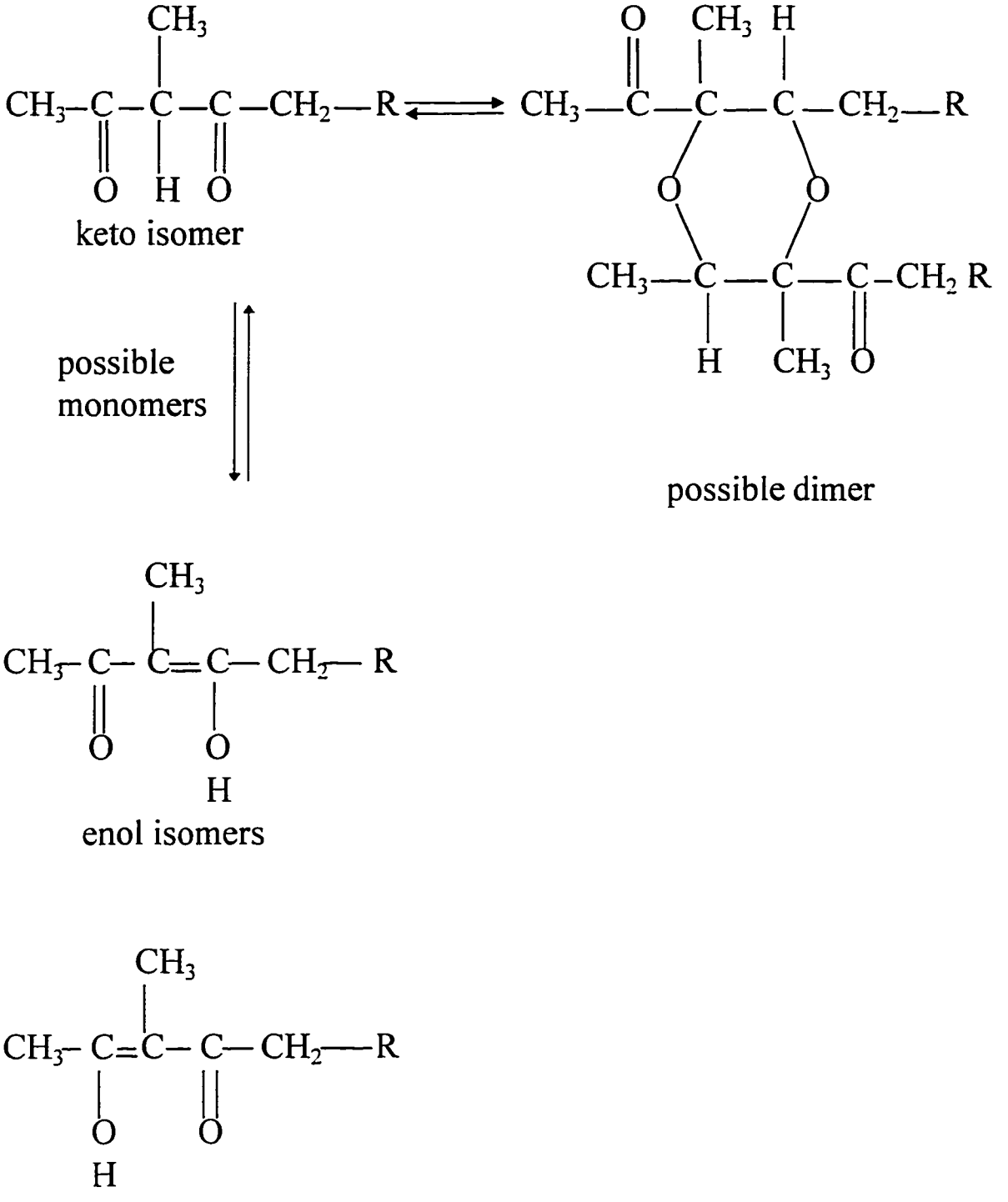


Figure 4. Proposed forms of 3-methylnonane-2,4-dione (MND)

Table 1. Flavor intensities of different concentrations of 3-methylnonane-2,4-dione (MND) emulsions represented by the intensities of 2-heptanone[a]

concentration of MND (ppm)	2.65	0.85	0.28	0.09	0.09 H[b]
intensity of MND represented by the concentration of 2-heptanone (ppm)	0.379[c]	0.323[c]	0.389[c]	0.317[c]	0.280[c]

[a] Means of two replications and nine panelists. Means followed by the same superscript are not significantly different ($p \leq 0.05$).
[b] H= heated in boiling water bath for 5 min

Table 2. The concentration of 3-methylnonane-2,4-dione (MND) in soybean oils during storage at 35°C under fluorescent light

Brand Name	Measurement	Day								
		0	2	4	6	10	14	20	24	28
Wesson	PV[a] (meq/kg)	0.1 ± 0	1.2 ± 0.1	3.4 ± 0.3	14.7 ± 0.1	40.9 ± 3.8	78.9 ± 2.3	------[b]	------	------
	MND[c](ppb)	0	0.038 ± 0.076	0.095 ± 0.09[d]	0.097 ± 0.082	0.406 ± 0.268	0.253 ± 0.109[d]	------	------	------
Hy-Vee	PV[a] (meq/kg)	0.1 ± 0	1.0 ± 0.1	2.4 ± 0.4	2.9 ± 0.5	3.3 ± 0.4	4.7 ± 0.6	12.2 ± 1.4	14.6 ± 1.1	34.8 ± 5.3
	MND[c] (ppb)	0	0	0	trace	trace	trace	0.044 ± 0.089	0.316 ± 0.234	0.804 ± 0.248

[a] Peroxide value ± standard deviation
[b] ----- : not measured
[c] MND concentration ± standard deviation. Quantities are the average of four measurements
[d] MND concentration ± standard deviation. Quantities are the average of three measurements

VOLATILES PRODUCED DURING DEODORIZATION OF SOYBEAN OIL AND THEIR FLAVOR SIGNIFICANCE

A paper to be submitted to the Journal of the American Oil Chemists' Society

Jian-Wen Kao, Earl G. Hammond and Pamela J. White

ABSTRACT

Freshly deodorized soybean oil has a characteristic nutty flavor but often yields no detectable head-space volatiles. The cause of this flavor was investigated by deodorizing soybean oil in an apparatus with a double cold trap, allowing the volatiles formed from the initial decomposition of hydroperoxides to be collected. The chief components of the deodorization volatiles were hydrocarbons, but the major flavors were carbonyls, especially heptanal and *cis*-4-heptenal. Although these components should accumulate in the deodorizing oil at some steady state concentration, none that had a high enough molecular weight to accumulate significantly in the deodorizing oil had a flavor impact that would account for the deodorized oil flavor. By using a particle detector, it was shown that small particles could be generated in the human mouth that could provide a mechanism to bring oil with nonvolatile flavor components into contact with the olfactory organ. Attempts to separate possible nonvolatile flavors in deodorized oil from triacylglycerides by chromatography on alumina or reaction with 2,4-dinitrophenylhydrazine were unsuccessful. Possibly the flavor is caused by the glycerol esters themselves.

INTRODUCTION

Freshly deodorized vegetable oils have a nutty flavor considered to be acceptable by consumers. Sensory panel members looking for oxidized flavor in vegetable oils learn to discount this flavor, but when the absolute flavor intensity of freshly deodorized oils is compared with a standard, the freshly deodorized oil is seen to have a significant flavor intensity (1), which can not be accounted for by recognized oxidized flavor volatiles. Non-fat or reduced-fat foods often lack desirable flavors associated with fresh vegetable oils. It is likely that volatile compounds do not exist in large amounts in freshly deodorized oil because of the high vacuum and temperature treatments. Little work has been done to examine the flavor compounds in freshly vegetable oil. In this study we wished to see if we could account for the flavor of freshly deodorized oil by examining the volatile flavor compounds generated during deodorization and by exploring the possible role of nonvolatile flavor compounds.

MATERIALS AND METHODS

Collection of volatiles

Freshly purchased Wesson brand soybean oil (800 g) was deodorized with steam at 235° C under vacuum (0.005 mm Hg). The volatiles that formed were collected in one cold trap (trap 1) for two hours, and then the apparatus was switched to a second cold trap (trap 2) for four hours (Figure 1).

Separation of volatiles from trap 2 into carbonyls and hydrocarbons

The volatiles in trap 2 were extracted three times with 10 mL pentane and the pentane extract was concentrated to 1.5 mL. Next, 700 μL of the pentane concentrate was applied to a 10-mm i.d. × 26-cm column filled with 10 g Florisil in pentane/ether (99:1). The Florisil was activated at 250°C overnight, and 10% of its weight of distilled water was added and allowed to equilibrate for 1 day. Hydrocarbons were eluted with 15 mL pentane/ether (99:1), and the carbonyls were eluted with 15 mL ether (2).

Instrumental analysis and GC odor evaluation

The volatiles were analyzed by gas chromatography (GC) (HP 5890 series II, Palo Alto, CA) on a Supelco SPB-1 fused silica capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness; Belletonte, PA) at a helium flow rate of 1.7 mL/min. The temperature program was held for 4 min at 30°C, raised from 30° C to 210° C at 6° C/min, and finally held at 210°C for 10 min. Peaks were detected by a flame-ionization detector (FID) held at 230°C. Volatiles were identified by mass spectrometry (MS) (HP 5970 series Mass Selective Detector, Palo Alto, CA), and when possible, by comparison of their retention times with those of known standards. Odor evaluations were accomplished by three experts who smelled individual volatiles as they issued from the GC detector port. For peaks having detectable odor, the intensity and characteristics were recorded. The GC temperature program for odor evaluation was the same as that used for GC analysis.

Measurement of aerosol particles generated in the mouth

Particles in the 0.5 to 5 μm diameter range were measured with a Met One particle detector (Met One Inc., Grants Pass OR). The counter was placed in a biological safety cabinet (Nuaire, Plymouth MN) and panelists breathed particle-free air from inside the cabinet until their breath revealed no particles (~ 1 minute). Then the panelists generated particles by smacking their lips or pulling their tongues away from the roofs of their mouths. The number of particles so generated were counted by inserting the inlet hose of the counter near or between the lips and sampling at a flow of 2.8 L/min.

Sensory evaluation of soybean oil before and after alumina fractionation

Wesson brand soybean oil (200 g) was deodorized at 220° C for 6 h under vacuum (0.005 mm Hg), and 100 g was immediately fractionated by passage through a 35-mm i.d. × 57-cm column filled with 200 g alumina in hexane (3). Triacylglycerides (TAG) and the compounds less polar than TAG were eluted with 2 L of hexane/ether (9:1). A yield of 62 g of TAG resulted after rotary evaporation of the solvent at 45° C and steam distillation at 120° C under vacuum (0.005 mm Hg) for 1 h. The TAGs with and without alumina fractionation were diluted 100-fold with water to make emulsions (4), and their flavors were evaluated by 10 panelists in a triangle test on two separate occasions. The significance was accepted at $p \leq 0.05$ (5). The samples (60 mL) were presented to the panelists in 266-mL plastic cups covered with aluminum foil and tasted at room temperature.

Synthesis of methyl 9-oxononanoate

Methyl 9-oxononanoate was prepared by the procedure of Hammond et al. for identifying positional isomers of unsaturated fatty acids (6) except the carbon disulfide was replaced by pentane. Several adaptations of the method are described. A solution of 25 mg of methyl oleate in 3 mL pentane was cooled in an acetone dry ice bath and ozone was passed through it at 20 mL/min. The presence of an excess of ozone was detected by passing the gas leaving the reaction vessel into a solution of 5 % potassium iodide in 5 % sulfuric acid containing a starch indicator, which turned blue in the presence of ozone. When ozone absorption was complete, an excess of triphenylphosphine (100 mg) was added, and the reaction mixture was allowed to warm to room temperature. The methyl 9-oxononanoate in the ending reaction mixture (1 mL) was purified by chromatography on a 10-mm i.d. × 26-cm column filled with Florisil in hexane. Hexane/ether (9:1) (100) mL was used to remove triphenylphosphine, and then ether was used to elute methyl 9-oxononanoate. The first 15 mL of elution was discarded and the next 10 mL was collected. The ether fraction containing methyl 9-oxononanoate was concentrated and rechromatographed on Florisil. The refractionated methyl 9-oxononanoate ether solution was concentrated. The aroma of methyl 9-oxononanoate was evaluated by two experts by smelling it as it exited from the GC. When the ether was evaporated, mineral oil was added to make up 1 and 10 ppm methyl 9-oxononanoate of mineral oil, and the flavor intensity and character (presence of typical nutty flavor) were compared with the emulsion prepared with plain mineral oil.

Preparation of carbonyl-free soybean oil

A 2,4-dinitrophenylhydrazine (DNPH) column was prepared according to the procedure of Schwartz et al. (7). 2,4-dinitrophenylhydrazine (DNPH) (Aldrich Chemical Company Inc., Milwaukee, MI) (0.5 g) was dissolved in 6 mL 85% phosphoric acid (Fisher Scientific, Pittsburg, PA) by grinding in a mortar and 4 mL distilled water was added to form a yellow solution. Celite (10 g) was added to the solution and ground to make a homogeneous damp. Then, the DNPH impregnated Celite was tamped in a 16-mm i.d. × 61-cm column filled with hexane.

Fresh commercial soybean oil (Wesson, 50 g) was dissolved in 60 mL hexane and applied to a 2,4-dinitrophenylhydrazine (DNPH) column. A yellowish oil was eluted with hexane (100 mL) and then passed through a column (35-mm i.d. × 57-cm) filled with 100 g alumina in hexane. The eluent (100 mL) obtained from the alumina column was passed through a new 100 g alumina column again to obtain a carbonyl- and DNPH-free, colorless soybean oil in hexane. The hexane was removed by rotary evaporation at 45°C and steam deodorization at room temperature under vacuum, and the flavor intensity and character of the carbonyl-free soybean oil were compared with freshly deodorized soybean oil twice by three expert panelists who tasted the oil directly.

Synthesis of tricaprylglyceride (TCG)

Capric acid (178 g) (Sigma Chemical Co., St. Louis, MO) and glycerol (30 g) (Sigma) were heated and refluxed with 66 mL HPLC Grade benzene (Fisher Scientific, Pittsburgh,

PA) and 3.13 g *p*-toluenesulfonic acid (Sigma) in a flask equipped with Dean-Stark trap and condenser (8). The reaction was held for 20 h and 17.3 mL of water was formed and trapped in the Dean-Stark trap. Then, the water was drained off and 50 mL of benzene was also drained away through the Dean-Stark trap. Distilled water (300 mL) was added to the reaction mixture and the organic phase (upper layer) was separated by separation funnel. The organic phase (tricaprylglyceride and a little benzene) was washed three times with 100 mL 5% sodium carbonate and three times with 100 mL distilled water. The organic phase was then dried with 23 g of sodium sulfate.

Ninety-two grams of synthesized TCG (with a little benzene) was dissolved in 100 mL hexane and passed through a 35-mm i.d.×57-cm column filled with 200 g alumina in hexane. The TCG was eluted with 600 mL of hexane/ether (9:1). After rotary evaporation at 80°C and steam distillation at 80°C under vacuum (0.005 mm Hg) for 1 h to remove hexane, ether, and benzene, 50 g of TCG was obtained. The TCG was then deodorized at 200°C under vacuum (0.005 mm Hg) for 1 h and purified again by passing through the 100 g alumina column. Twenty grams of TCG was obtained after rotary evaporation of solvent at 80°C and steam deodorization at 80°C under vacuum for 30 min. The TCG was evaluated for intensity and character by three expert panelists on two separate occasions.

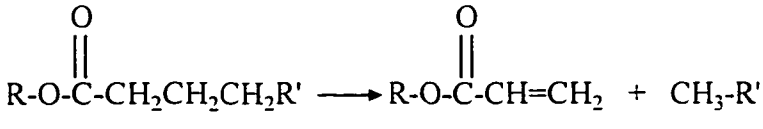
RESULTS AND DISCUSSION

Trap contents

The double cold trap on the deodorizer was designed to capture volatiles arising from previous oxidation of the oil in trap 1 and to capture volatiles produced during deodorization in trap 2. Examination of the contents of trap 2 by GC indicated that the major components were hydrocarbons but that carbonyl compounds accounted for most of the flavors. For better identification. the trap contents were separated into polar and nonpolar fractions by chromatography on a Florisil column.

Nonpolar fraction

The nonpolar fraction from the Florisil column was shown by GC/MS to be primarily unsaturated hydrocarbons varying in length from 8 to 18 carbons (Figure 2). Identification was based on the molecular weight of the largest mass peak. Compounds of less than eight carbons may have been generated but lost in the evaporation of the solvent. Probably the chains were generated by heat degradation of the carbon chains as follows:



Because 15-carbon chains were the most prevalent. seemingly scission between carbon 3 and 4 of the C_{18} chains is favored. Scission between carbon 6 and 7 or 5 and 6 also seems to favored.

Limonene was also identified in the nonpolar fraction by its MS and retention time. It was the only member of the nonpolar fraction to exhibit a noticeable aroma when smelled at the exit port of the GC. Probably the branched structure of limonene is generated from heat degradation of sterols or squalene rather than from fatty acyl groups.

Polar fraction

The polar fraction from the Florisil column consisted primarily of aldehydes (Figure 3. Table I). Of these the most prevalent were heptanal, *cis*-4-heptenal. a heptanal dimer and heptanoic acid. *Cis*-4-heptenal has been shown to arise from 2.6-nonadienal by a reversal of the aldol condensation (9), and a similar breakdown of 2-nonenal or its precursor could lead to heptanal. It is likely that the carbonyls come from the slow decomposition of the stable dialkylperoxides formed in free-radical termination reactions. The heptanal dimer is presumed to be formed by aldol condensation of the heptanal after its release from its precursor.

Many of the other compounds of trap 2 are well known oxidation products (1). The menadione. like the limonene, may come from the rearrangement of sterols or squalene or their oxidation products. 3-Methyl-2,4-nonadione has been reported as a photoxidation product of furanoid fatty esters in soybean oil (10). but a trace seemed to be generated during deodorization. Phenylpropanone. which was an important fruity flavor. may come from the degradation of linolenate.

The aromas of the heptanal and *cis*-4-heptenal were the most potent of the odors in the polar fraction. but since they are fairly volatile. not much would accumulate in the

deodorizing oil, and no compound with a low volatility was present in significant amounts or had an unusually intense aroma. Thus, although a number of volatile aromas were found in the deodorized oil, none seemed to be present in sufficient amounts or to have sufficient aroma intensity to account for the flavor of freshly deodorized soybean oil. Previous unpublished work from our laboratory also showed that contact of freshly deodorized soybean oil with human saliva did not release volatile flavor compounds. The flavor sensation of freshly deodorized oils seems to be mediated by the olfactory organ.

Flavor from aerosol particles

An alternate mechanism that might account for the flavor of freshly deodorized oil is suggested by the work of Hammond et al. (11) who reported that the aroma of swine houses was carried on particulates and that particulates enhanced aroma intensity because they were scrubbed from air streams in the nasal passage much more efficiently than molecularly dispersed odorants. This arguement is based on the observation that the olfactory organ is placed at a sharp bend in the air stream and particles in the 0.5 to 5 μM range are removed and deposited on the olfactory organ by a centrifugal effect. Thus, the load of odorants is released directly on the olfactory organ.

Using three observers, we found that several hundred particles in the 0.5 to 5 μM range could be generated by smacking ones lips and by pulling the tongue from the roof of one's mouth. Interestingly, these are precisely the conditions in which one can taste freshly deodorized oil. If one puts freshly deodorized oil in one's mouth and breathes in and out

while keeping the lips and tongue still, there is no flavor, but if one parts the lips or pulls the tongue from the roof of one's mouth while breathing in gently, the flavor is perceived.

Flavor from nonvolatile compounds

These observations encouraged us to look for nonvolatile flavor compounds in the oil. It seemed likely that the flavors would be more polar than triacylglycerols considering the nature of the flavors found in oxidized oil. Thus, we tried an experiment that might concentrate the flavors from freshly deodorized soybean oil by passing the oil through alumina columns. No difference in flavor intensity before and after treatment was perceived in the triacylglycerol fractions eluted from such columns. This treatment would eliminate mono- and di-acylglycerides or other polar compounds that might contribute to the flavor.

Aldehydes and ketones are only slightly more polar than triacylglycerols, so it seemed possible that azelaic semialdehyde esterified with glycerol, or ketoacyl groups generated by decomposition of acyl hydroperoxides esterified with glycerol could cause the flavor of freshly deodorized oils. To this end methyl azelate semialdehyde (methyl 9-oxo-nonanoate) was synthesized by ozonolysis of methyl oleate and its aroma and flavor were examined by smelling it as it exited from the GC and tasting in mineral oil solutions. Methyl 9-oxo-nonanoate had no detectable flavor or aroma. Commercial soybean oil also was passed through a 2,4-dinitrophenylhydrazine (DNPH) reaction column to convert any volatile or nonvolatile carbonyls it contained to DNPH derivatives. Then the DNPH derivatives were removed by chromatography on alumina and the oil tasted after removal of the solvent at low

temperature. The oil had a nutty flavor typical of freshly deodorized oils. Finally, tricaprylglyceride (TCG) was synthesized from glycerol and capric acid. After purification on an alumina column and steam deodorization, the flavor of TCG was perceived as none to nutty.

Thus, if there is a nonvolatile flavor component in freshly deodorized vegetable oils, it seems not to be very polar nor to be a carbonyl. One possibility that we have yet to test is that the flavor is caused simply by glycerol esters.

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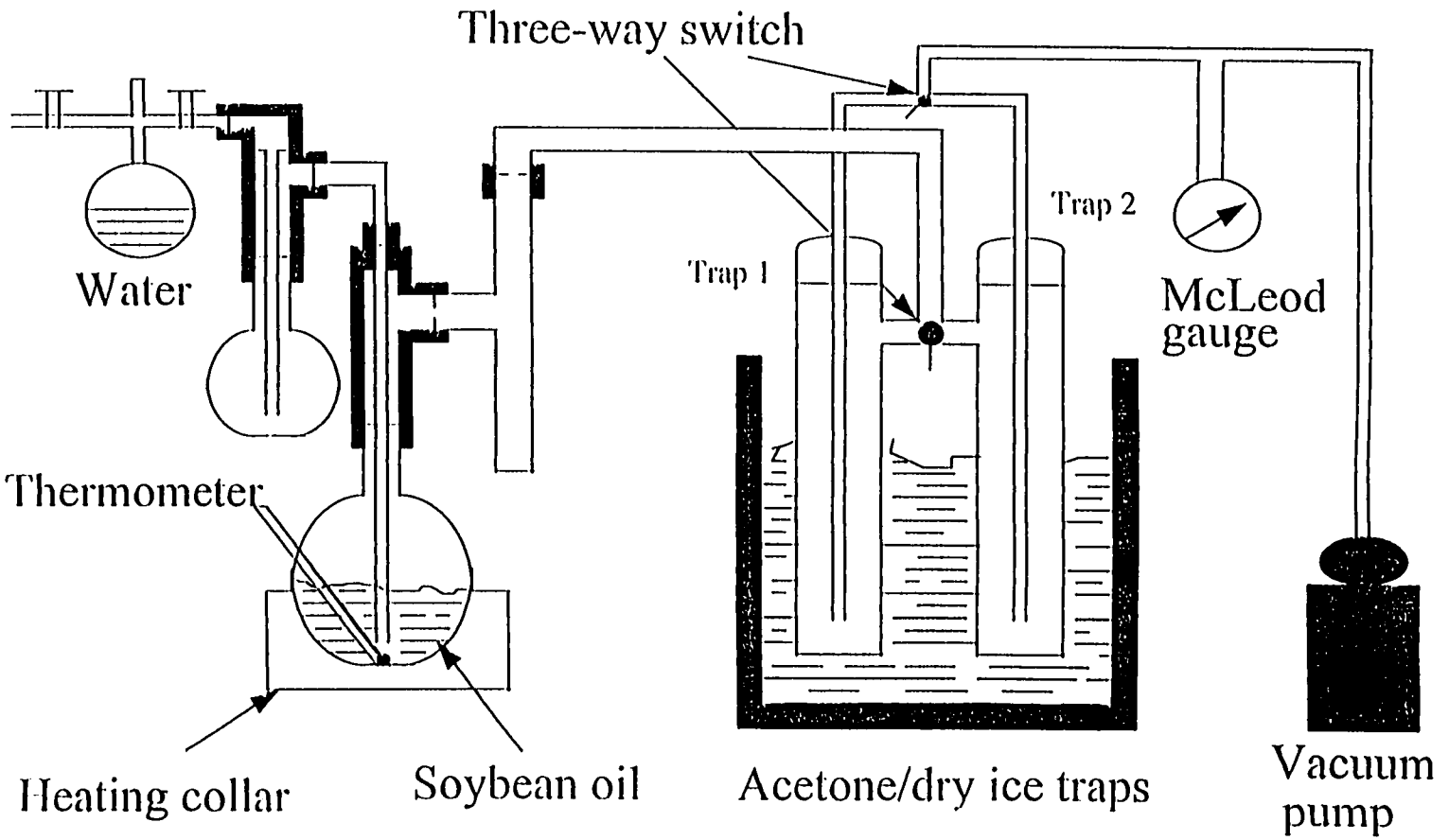

Figure 1. Diagram of deodorization apparatus

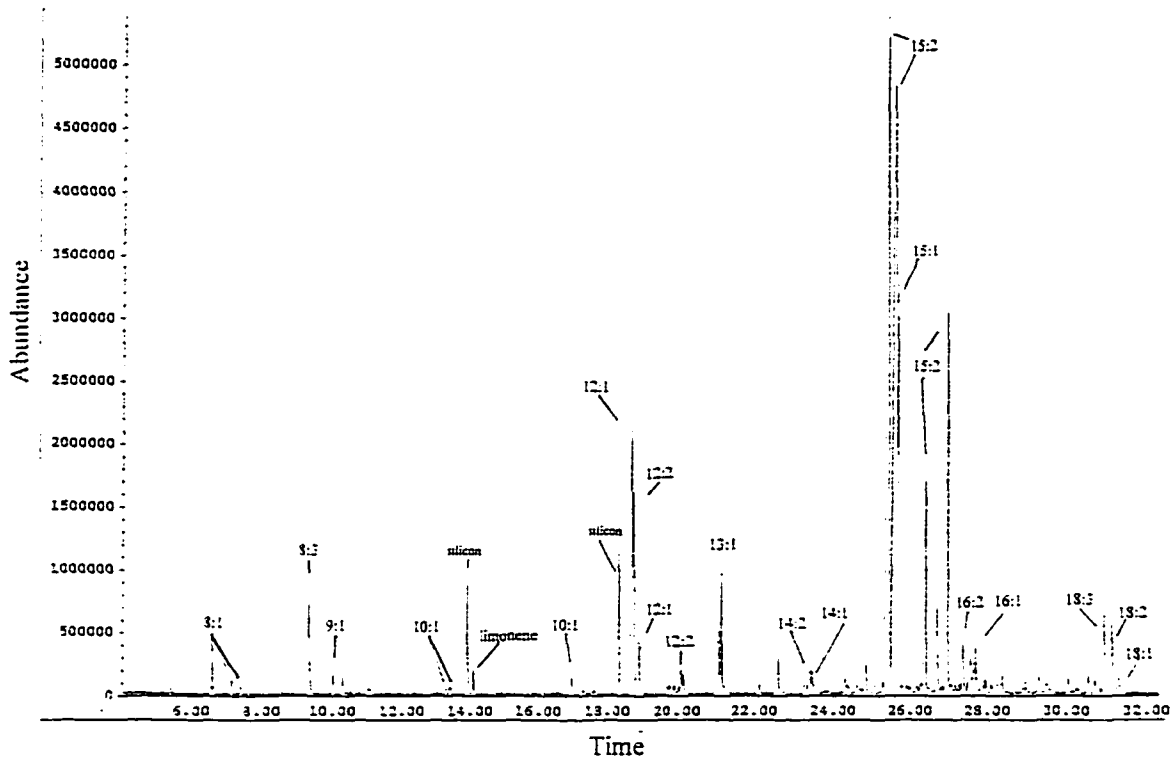

Figure 2. Hydrocarbons formed during deodorization of soybean oil

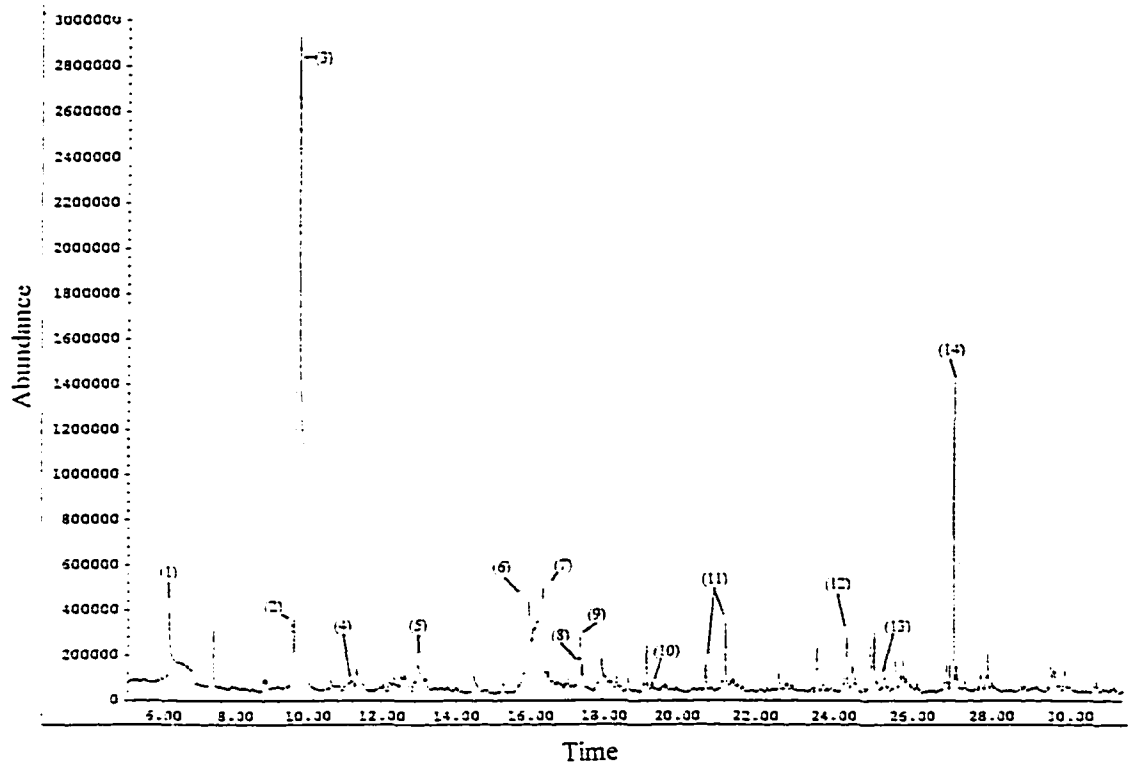

Figure 3. Carbonyls formed during deodorization of soybean oil

Table 1. Carbonyl compounds found in trap 2

NO.	Compound	Retention time (min.)	Identification MS[a]	Identification RT[b]	Characteristic odor[c]
1	hexanal	6.15	+	+	green
2	*cis*-4-heptenal	9.54	+	+	---------
3	heptanal	9.72	+	+	heptanal
4	benzaldehyde	11.21	+	+	---------
5	2,4-heptadienal	12.96	+	+	fruity
6	nonanal	15.93	+	+	slight fruity
7	heptanoic acid	16.3	+	--	---------
8	phenylpropanone	17.27	+	+	sweet fruity
9	2-nonenal	17.32	+	+	aldehyde
10	3-methyl-2,4-nonadione	19.3	+	+	licorice
11	2,4-decadienal	20.73 21.22	+	+	beany
12	decano-γ-lactone	24.3	+	+	buttery lactone
13	menadione	25.28	+	+	spicy
14	heptanal dimer	27.01	+	--	---------

[a] MS: Mass Spectrometry

[b] RT: Retention Time

[c] Odor identified within the retention time of each peak

GENERAL CONCLUSIONS

There are many theories to postulate the source of flavor reversion of soybean oil, such as the presence of linolenic acid, isolinoleic acid, oxidative polymers, phospholipids, and nonsaponifiable components; however, these compounds also exist in nonreverted oils. Specific minor compounds, such as furanoid fatty acids, which only exist in reverted oils may be precursors to reverted flavor. The compound, 3-methylnonane-2,4-dione (MND), found in reverted soybean oil has been shown to derive from a furanoid fatty acid and has an intense strawy and fruity odor. When MND was dissolved in an oil system, little flavor was noted. Perhaps reversion flavor is not due to any specific volatile component but is the combination of many volatiles derived from the oxidation of fatty acids. On the other hand, freshly deodorized soybean oil has a characteristic nutty flavor, which does not come from volatile compounds. Experiments showed that small oil particles could be generated in the human mouth, sending nonvolatile flavor compounds into the olfactory organ, but when the possible nonvolatile flavors were separated from triacylglycerides by alumina chromatography or reaction with 2,4-dinitrophenylhydrazine packed in a column, the triacylglycerides remained nutty in flavor. Possibly the flavor is caused by the triacylglycerides themselves.

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