Volatile Composition of Sunflower Oil-in-Water Emulsions during Initial Lipid Oxidation: Influence of pH

Saskia M. van Ruth,*,† Jacques P. Roozen,† Maarten A. Posthumus,† and Frans Jos H. M. Jansen‡

Department of Food Technology and Nutritional Sciences, Wageningen Agricultural University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands, and Unilever Research Vlaardingen, P.O. Box 114, 3130 AC Vlaardingen, The Netherlands

The formation of odor active compounds resulting from initial lipid oxidation in sunflower oil-in-water emulsions was examined during storage at 60 °C. The emulsions differed in initial pH, that is, pH 3 and 6. The volatile compounds were isolated under mouth conditions and were analyzed by gas chromatography/sniffing port analysis. The lipid oxidation rate was followed by the formation of conjugated hydroperoxide dienes and headspace hexanal. The initial pH affected the lipid oxidation rate in the emulsions: the formation of conjugated diene hydroperoxides and the hexanal concentration in the static headspace were increased at pH 6. Pentanal, hexanal, 3-pentanol, and 1-octen-3-one showed odor activity in the emulsions after 6 days of storage, for both pH 3 and 6. Larger amounts of odor active compounds were released from the pH 6 emulsion with extended storage. It was shown that this increased release at pH 6 was not due to increased volatility because an increase in pH diminished the static headspace concentrations of added compounds in emulsions.

Keywords: *Aroma release; emulsion; lipid oxidation; pH; sunflower oil; volatile compounds*

INTRODUCTION

Acceptability of food depends on the sensory qualities of the food, in particular its flavor. Aroma compounds contribute to the flavor of food products. These compounds are molecules with sufficiently high vapor pressures to be partially present in the gas phase. The concentration of the volatiles in the gas phase depends on their concentration, their physicochemical properties, and their interactions with other components (Landy et al., 1996). Most volatile compounds in bulk oils are compounds resulting from lipid oxidation reactions.

Oxidation of lipids is often a determining factor in the shelf life of lipid-containing foods. Lipid oxidation in bulk oils has been extensively studied (Grosch, 1987; Frankel, 1991; Mistry and Min, 1992). However, lipids are found in surfactant-stabilized dispersions in many processed foods (Mei et al., 1998). In these dispersions oxidation reactions are influenced by factors including fatty acid composition, storage conditions, and the physical state of the oil (bulk oil/emulsion). The physical state influences lipid oxidation through increased surface area (Coupland and McClements, 1996) and because antioxidants and pro-oxidants show different affinities for the lipid and water phase (Porter, 1980). Huang et al. (1996a) showed that the effectiveness of antioxidants in these heterogeneous lipid systems is very dependent on their locations in the oil and water phase (Huang et al., 1996b). Studies on the effects of pH on lipid oxidation rates in emulsions demonstrated diverse results. Some authors reported increased rates at lowered pH (Mei et al., 1998; Yamauchi et al., 1988), whereas other studies demonstrated that lipid oxidation rates decreased at lowered pH (Saunders et al., 1962; Mabrouk and Dugan, 1960).

The pH of emulsions influences not only the volatile composition but also the generation of these compounds. In the case of emulsions, in which volatile fatty acids and other ionizable compounds are important aroma compounds, the pH of the aqueous phase of an emulsion can markedly influence perception by governing the state of dissociation of these compounds (Hartwig and McDaniel, 1995; Guyot et al., 1996) and therefore their volatility. For instance, the more volatile flavorful fatty acids have p*K* values between pH 4 and 5 and are most potent below this range (Baldwin et al., 1973). Summarizing, the pH of emulsions influences the aroma profiles of emulsions through effects on aroma generation and aroma release.

The present work examines the formation of odor active compounds by lipid oxidation in sunflower oil-in-water emulsions during storage for 6 days at 60 °C. The emulsions differed in initial pH. Aroma compounds were isolated under mouth conditions and analyzed by gas chromatography/sniffing port analysis. In addition, the effect of the pH on lipid oxidation rate (aroma generation) and aroma release was studied.

MATERIALS AND METHODS

Experimental Samples. A 40% oil-in-water emulsion (40% sunflower oil, 59% deionized water, 1% Tween 60) was supplied by Unilever Research Vlaardingen (Vlaardingen, The Netherlands). The sunflower oil consisted of the following fatty acids: 6.0% 16:0, 4.3% 18:0, 23.6% C18:1, 64.3% C18:2, and 0.12% C18:3 (determined by gas chromatography of methyl esters). The sunflower oil also contained 716 mg of α-tocopherol, 26 mg of β-tocopherol, 7 mg of γ-tocopherol, and <5

* Address correspondence to this author at University College Cork, Department of Food Science and Technology, Nutritional Sciences, Cork, Ireland.

† Wageningen Agricultural University.

‡ Unilever Research Vlaardingen.

10.1021/jf990024t CCC: $18.00
Published on Web 09/14/1999

mg of δ-tocopherol per kilogram of oil (AOCS Official Method Ce 8-89, 1992). The emulsion was prepared using a homogenizer (APV Gaulin Model LAB 40-10 RBFI, APV Gaulin GmbH, Lübeck, BRD) at 150 bar for 10 min. The average particle size in the emulsion was 1.0 μm (Coulter laser measurements). The pH of the emulsions was adjusted by addition of 0.1 N HCl solution. Through the addition of the HCl solution the concentration of metals in the emulsion increased with maximal 10^{-5} ppm of Fe and 10^{-6} ppm of Cu, according to the supplier's specifications of the HCl. The emulsion samples (65 mL) were stored in glass jars (350 mL) in the dark at 60 °C for a maximum of 6 days. Duplicate samples were stored for each time measurements were conducted.

For volatility and aroma release experiments, pentanal (PolyScience, Niles, IL), hexanal (PolyScience), 3-pentanol (Aldrich, Milwaukee, WI), and 1-octen-3-one (Oxford Chemicals Ltd., Hartlepool, U.K.) were added to the fresh emulsions (0.1% v/v). The solutions were incubated for 24 h at 4 °C in the dark before being subjected to analysis. Fresh emulsions were stored under the same conditions, and the pentanal, hexanal, 3-pentanol, and 1-octen-3-one formed were determined and subtracted from the added amounts.

Analysis of Conjugated Diene Hydroperoxides. Conjugated diene hydroperoxides were measured in the oil extracted from the emulsions for each of the samples stored in duplicate. For extraction, 5 g of emulsion was added to 25 mL of methanol. After 15 min, the methanol water layer was removed and the remaining oil was used for analysis of the dienes. An aliquot of extracted oil was dissolved in 5 mL of cyclohexane in duplicate, and the absorbance was measured at 234 nm (CECIL 2020, Cecil Instruments Ltd., Cambridge, U.K.). Absorbances were calculated per milligram of oil.

Static Headspace Analysis. For static headspace gas chromatography (SHGC), 2 mL of emulsion was transferred into a 10 mL vial and incubated at 60 °C for 10 min in the headspace unit of a Carlo Erba MEGA 5300 GC (Interscience bv, Breda, The Netherlands). In volatility experiments 1 mL of emulsion and 1 mL of artificial saliva (Van Ruth et al., 1995) were combined in a vial. The GC was equipped with a DB-Wax column (J&W Scientific, Folsom, CA), 30 m length, 0.54 mm i.d., 1 μm film thickness, and a flame ionization detector (FID) at 275 °C. An initial oven temperature of 60 °C for 5 min was used, followed by a rate of 3 °C min^{-1} to 110 °C and then by 4 °C min^{-1} to 170 °C. Two replicate measurements of each stored sample were carried out.

Isolation of Volatile Compounds in the Mouth Model System. Volatile compounds were isolated in a mouth model system as described previously (Van Ruth et al., 1995). The headspace was flushed with nitrogen gas (100 mL min^{-1}), and the volatiles were trapped on Tenax TA during 2 min at 37 °C while a plunger simulated mastication.

Gas Chromatography/Sniffing Port (GC/SP) Analysis. Desorption of volatile compounds from Tenax was performed by a thermal desorption (245 °C, 5 min)/cold trap (−120 °C/260 °C) device (Carlo Erba TDAS 5000, Interscience bv, Breda, The Netherlands). Gas chromatography was carried out on a Carlo Erba MEGA 5300 (Interscience bv) equipped with a Supelcowax 10 capillary column, 60 m length, 0.25 mm i.d., 0.25 μm film thickness, and an FID at 275 °C. At the end of the column the effluent was split 1:2:2 for FID, sniffing port 1, and sniffing port 2, respectively. An initial oven temperature of 40 °C was used, followed by a rate of 2 °C min^{-1} to 92 °C and then of 6 °C min^{-1} to 272 °C.

Ten assessors were selected on the basis of their sensitivity, memory, ability to recognize odors, and availability. Prior to sniffing the effluent of the oil and emulsion samples, the assessors were trained on the technique of sniffing. Assessors used portable computers with a program in Pascal for data collection. The data were converted from the field disks into Lotus 123 software to process the raw data. Aroma descriptors were generated during preliminary GC/sniffing experiments and clustered after group sessions of the panel, resulting in a list of 19 descriptors (green; mushroom; spicy; fruity; sweet; flowers; fatty; oil; rancid; rotten; musty; chemical/glue; nuts; almond; burned; caramel; chocolate; vanilla; sharp/irritating). These descriptors and "other/I do not know" had to be used for each compound detected by the assessors at the sniffing port. Tenax tubes without adsorbed volatile compounds were used as dummy samples for determining the signal-to-noise level of the group of assessors.

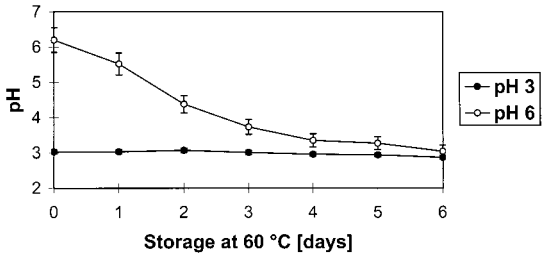

Figure 1. Change in pH of 40% sunflower oil-in-water emulsions (initial pH 3 and 6) during storage at 60 °C.

GC/Mass Spectrometry (GC/MS) Analysis. Volatile compounds were isolated as described under GC/sniffing port analysis and identified by combined GC (Varian 3400, Varian, Walnut Creek, CA) and mass spectrometry (MS; Finnigan MAT 95, Finnigan MAT, Bremen, Germany) equipped with a thermal desorption/cold trap device (TCT injector 16200, Chrompack bv, Middelburg, The Netherlands). The capillary column and oven temperature program were the same as those used in GC/SP analyses. Mass spectra were obtained with 70 eV electron impact ionization while the mass spectrometer was continuously scanning from *m*/*z* 24 to 400 at a scan speed of 0.7 s/decade (cycle time = 1.05 s).

RESULTS AND DISCUSSION

The pH of sunflower oil-in-water emulsions was adjusted to pH 3 and 6 after preparation. Although the pH differed initially, the pH of the pH 6 emulsion decreased during 6 days of storage from pH 6.2 to 3.0 (Figure 1). Huang et al. (1996b) reported a decrease in pH for a 10% corn oil-in-water emulsion as well: its pH decreased from pH 3.7 initially to pH 2.6 after 4 days of storage and, with added α-tocopherol, from pH 3.5 to 2.8. The decrease in pH might be due to the formation of several acids resulting from lipid oxidation. In GC/MS analyses acetic acid, propanoic acid, 2-methylpropanoic acid, butanoic acid, pentanoic acid, hexanoic acid, heptanoic acid, octanoic acid, and nonanoic acid were identified among the volatile compounds released from the oxidized sunflower oil-in-water emulsion in the mouth model system. The pH 3 emulsion showed a minor decrease from pH 3.0 to 2.9 during 6 days. Diminished formation of acidic compounds resulting from lipid oxidation could be responsible for this limited decrease. Furthermore, at this pH more acid is required to alter the pH. Because these acids dissociate less at pH 3, formation of these compounds will lower the pH less markedly.

The effect of the initial pH on the formation of primary lipid oxidation products was studied by following the development of conjugated diene hydroperoxides during storage (Figure 2). The hydroperoxides increased more rapidly in the pH 6 emulsion than in the pH 3 emulsion, which could be due to increased formation of hydroperoxides, as well as to decreased degradation, or a combination of both factors. The secondary lipid oxidation products, as represented by headspace hexanal (Figure 3), demonstrated a similar development over time: a more rapid increase for the pH 6 emulsion.

The odor active compounds of the pH 3 and 6 emulsions were isolated in the mouth model system and

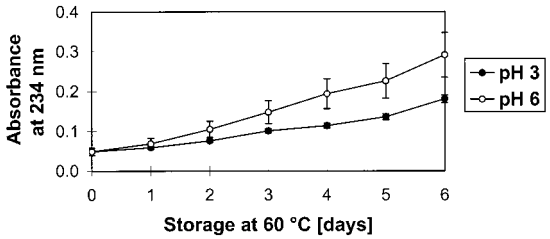

Figure 2. Formation of conjugated diene hydroperoxides in 40% sunflower oil-in-water emulsions (initial pH 3 and 6) during storage at 60 °C ($n = 4$).

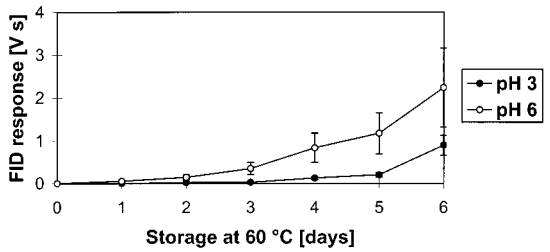

Figure 3. Formation of hexanal in 40% sunflower oil-in-water emulsions (initial pH 3 and 6) during storage at 60 °C, determined by static headspace analysis ($n = 4$).

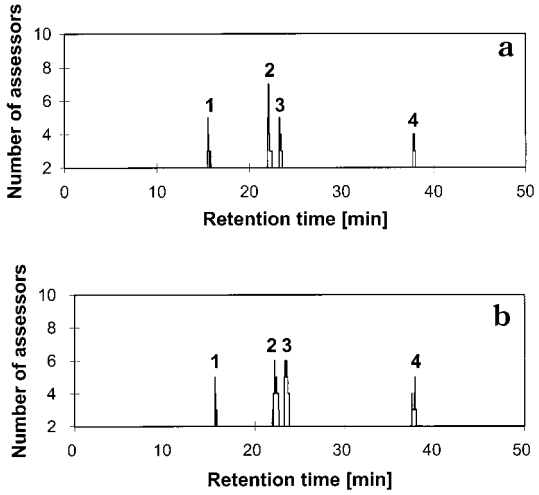

Figure 4. GC/sniffing chromatograms of the volatile compounds of 40% oil-in-water emulsions after 6 days of storage at 60 °C: (a) initial pH 3; (b) initial pH 6. Numbers in the chromatograms refer to compounds in Table 1.

Table 1. Odor Active Compounds of Stored Sunflower Oil-in-Water Emulsions (pH 3 and 6) Released in a Mouth Model System[a]

			FID peak area (V s)	
no.	compound	odor description	pH 3	pH 6
1	pentanal	green, sweet, fatty	0.073	0.136
2	hexanal	green, flowers	0.482	0.863
3	3-pentanol	chemical	0.003	0.004
4	1-octen-3-one	mushroom, musty	0.103	0.118

[a] Their odor descriptors and FID peak areas were determined by GC/SP analysis ($n = 5$).

analyzed by GC/SP after 6 days of storage. The aroma compounds were identified by GC/MS and further characterized by their retention times and by the odors described by the assessors at the sniffing port. The sniffing chromatograms of the pH 3 and 6 emulsions are presented in parts a and b of Figure 4, respectively. In Table 1, the odor active compounds are listed together with their odor descriptors and FID peak areas. GC/sniffing of dummy samples demonstrated that detection

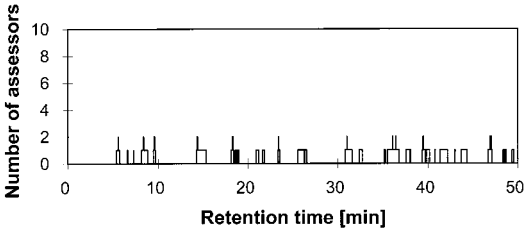

Figure 5. Sniffing chromatogram of a dummy sample.

Table 2. FID Peak Areas (V s) and Average Coefficients of Variance (CV) of Odor Active Compounds Added to Fresh Sunflower Oil-in-Water Emulsions (pH 3 and 6), Determined in Static Headspace Analysis with and without Addition of Artificial Saliva ($n = 4$)

	pH 3		pH 6	
compound	− saliva	+ saliva	− saliva	+ saliva
pentanal	0.11	0.08	0.07	0.06
hexanal	0.61	0.41	0.34	0.34
3-pentanol	2.45	1.30	1.52	0.92
1-octen-3-one	0.37	0.25	0.17	0.19
CV (%)	16.9	15.4	29.5	10.4

of an odor at the sniffing port by ≤2 of 10 assessors could be considered as "noise" (Figure 5); therefore, the perception of a compound by ≥3 assessors is considered a signal. The aroma profiles of both emulsions consisted of the same four odor active compounds: pentanal, hexanal, 3-pentanol, and 1-octen-3-one. Only these four compounds possessed detectable odors, although more volatile compounds were detected in GC/FID and GC/MS analyses. This is a relatively low number because odor activity was measured at concentration levels as present in the human mouth and the focus was on initial lipid oxidation only. Differences between the emulsions were observed in FID peak areas, showing larger peak areas for the pH 6 emulsion than for the pH 3 emulsion. These sniffing results are in agreement with headspace hexanal data, although larger differences were observed in this type of analysis. Previous work showed that the number of assessors perceiving an odor was log linearly related to the physical concentration of the compounds in the effluent (Van Ruth et al., 1996), which was in agreement with the Fechner equation (Meilgaard et al., 1991). Thus, only large differences in the concentrations in the GC effluent are likely to result in changes in the sniffing chromatograms.

The observed effect of pH comprised both aspects of aroma generation during storage and aroma release. To determine the pH effect on the release of the volatiles, the four odor active compounds were added to the fresh pH 3 and 6 emulsions. The volatilities of the compounds in both emulsions were determined by SHGC, with and without addition of artificial saliva (Table 2), and their release was studied in the mouth model system combined with GC/SP (Table 3). Generally, the release of the compounds in the mouth model system showed little difference for pH 3 and 6 emulsions, considering their coefficients of variance (CV). In SHGC the peak areas of the compounds of the pH 6 emulsion were lower than those of the pH 3 emulsion. Apparently the pH influenced their volatility, that is, the partitioning of the volatiles over the liquid and vapor phase. Similar effects of pH on the volatility of compounds were reported by Guyot et al. (1996), although the latter authors found the opposite effect for other compounds, too. Baldwin et al. (1973) stated that the flavor threshold of butyric acid was reduced from 6.1 to 0.4 when the pH was

Table 3. FID Peak Areas and Coefficients of Variance (CV) of Odor Active Compounds Added to Fresh Sunflower Oil-in-Water Emulsions (pH 3 and 6), Released in a Mouth Model System ($n = 4$)

	pH 3		pH 6	
compound	peak area (V s)	CV (%)	peak area (V s)	CV (%)
pentanal	0.40	9.6	0.36	12.4
hexanal	2.16	2.3	2.16	13.4
3-pentanol	0.38	49.7	4.44	9.4
1-octen-3-one	0.97	27.0	1.20	41.8

reduced from 6.0 to 3.2. Guyot et al. (1996) demonstrated the pH to affect the influence of emulsifiers on the volatility of compounds, which might play a role in the present work as well.

Addition of artificial saliva decreased the concentrations of the volatiles in the headspace, independent of pH, which is in agreement with previous work (Van Ruth et al., 1995). Many authors reported decreased volatility of compounds by proteins (Kim and Min, 1988; O'Keefe et al., 1991). The addition of the saliva diminished the differences in volatility of the compounds between the pH 3 and 6 emulsions. Saliva buffered the samples and equalized the pH of both emulsions. Generally, the release of the compounds from both emulsions in the mouth model system was not different (except for 3-pentanol), which was probably due to the pH-increasing effect of the added artificial saliva.

Obviously, the pH affected the volatility of the compounds present in the emulsion and therefore influenced the SHGC data of the storage study. The differences in initial pH of the emulsions hardly influenced the GC/SP data, with respect to volatility aspects, because of the buffering capacity of the artificial saliva added in the mouth model system.

Because the influence of pH on volatility (vapor concentration pH 3 > pH 6) was the opposite of the observed effect of pH during oxidation (vapor concentration pH 6 > pH 3), differences in lipid oxidation rate are expected to be responsible for the differences in volatile composition of the pH 3 and 6 emulsions. The present results are in agreement with the results of Huang et al. (1996b), who reported a study on 10% corn oil-in-water emulsions, which showed that the formation of hydroperoxides and hexanal increased with increasing pH.

The difference between the pH 3 and 6 emulsions might be due to the difference in availability of metal ions. In the presence of trace amounts of transition metals, hydroperoxides are readily decomposed to form alkoxyl radical intermediates, which can effectively propagate the free radical chain reactions (Frankel, 1991). The present results are in agreement with studies of O'Brien (1969), which showed that the decomposition of linoleic acid hydroperoxides by transition metal salts was markedly pH dependent.

In conclusion, the headspace concentrations of added odor active compounds were higher in the pH 3 sunflower oil-in-water emulsion than in the pH 6 emulsion, which influenced the headspace data in the storage study. No differences in release of added compounds were observed in the mouth model system. Therefore, differences in lipid oxidation rate are expected to be responsible for the observed differences between the aroma compositions of the pH 3 and 6 emulsions during storage.

ACKNOWLEDGMENT

We thank Jack Seijen ten Hoorn (Unilever Research Vlaardingen) for preparation of the emulsion and Annette Peltenburg (Wageningen Agricultural University) for her contribution to the analyses.

LITERATURE CITED

Baldwin, R. E.; Cloninger, M. R.; Lindsay, R. C. Flavor thresholds for fatty acids in buffered solutions. *J. Food Sci.* **1973**, *38*, 528−530.

Coupland, J. N.; McClements, D. J. Lipid oxidation in food emulsions. *Trends Food Sci. Technol.* **1996**, *7*, 83−91.

Frankel, E. N. Recent advances in lipid oxidation. *J. Sci. Food Agric.* **1991**, *54*, 495−511.

Grosch, W. Reactions of hydroperoxides—Products of low molecular weight. In *Autoxidation of Unsaturated Lipids*; Chan, H. W.-S., Ed.; Academic Press: London, U.K., 1987; pp 95−139.

Guyot, C.; Bonnafont, C.; Lesschaeve, I.; Issanchou, S.; Voilley, A.; Spinnler, H. E. Effect of fat content on odor intensity of three aroma compounds in model emulsions: δ-decalactone, diacetyl, and butyric acid. *J. Agric. Food Chem.* **1996**, *44*, 2341−2348.

Hartwig, P.; McDaniel, M. R. Flavor characteristics of lactic, malic, citric, and acetic acids at various pH levels. *J. Food Sci.* **1995**, *60*, 384−388.

Huang, S.-W.; Frankel, E. N.; Schwarz, K.; Aeschbach, R.; German, J. B. Antioxidant activity of carnosic acid and methyl carnosate in bulk oils and oil-in-water emulsions. *J. Agric. Food Chem.* **1996a**, *44*, 2951−2956.

Huang, S.-W.; Frankel, E. N.; Schwarz, K.; German, J. B. Effect of pH on antioxidant activity of α-tocopherol and trolox in oil-in-water emulsions. *J. Agric. Food Chem.* **1996b**, *44*, 2496−2502.

Kim, H.; Min, D. B. Interaction of flavour compounds with protein. In *Flavour Chemistry of Lipid Foods*; Min, D. B., Smouse, T. H., Eds.; American Oil Chemists' Society: Phoenix, AZ, 1988; pp 404−420.

Landy, P.; Courthaudon, J.-L.; Dubois, C.; Voilley, A. Effect of interface in model food emulsions on the volatility of aroma compounds. *J. Agric. Food Chem.* **1996**, *44*, 526−530.

Mabrouk, A. F.; Dugan, L. R. A kinetic study of the autoxidation of methyl linoleate and linoleic acid emulsions in the presence of sodium chloride. *J. Am. Oil Chem. Soc.* **1960**, *37*, 484−490.

Mei, L.; McClements, D. J.; Wu, J.; Decker, E. A. Iron-catalyzed lipid oxidation in emulsion as affected by surfactant, pH and NaCl. *Food Chem.* **1998**, *61*, 307−312.

Meilgaard, M.; Civille, G. V.; Carr, B. T. *Sensory Evaluation Techniques*; CRC Press: Boca Raton, FL, 1991.

Mistry, B. S.; Min, D. B. Oxidized flavor compounds in edible oils. In *Off-Flavors in Foods and Beverages*; Charalambous, G., Ed.; Elsevier: Amsterdam, The Netherlands, 1992; pp 171−209.

O'Brien, P. J. Intracellular mechanisms for the decomposition of a lipid peroxide. I. Decomposition of a lipid peroxide by metal ions, heme compounds, and nucleophiles. *Can. J. Biochem.* **1969**, *47*, 485−492.

O'Keefe, S. F.; Wilson, L. A.; Resurreccion, A. P.; Murphy, P. A. Determination of the binding of hexanal to soy glycinin and β-conglycinin in an aqueous model system using a headspace technique. *J. Agric. Food Chem.* **1991**, *39*, 1022−1028.

Porter, W. L. Recent trends in food applications of antioxidants. In *Autoxidation in Food and Biological Systems*; Simic, M. G., Karel, M., Eds.; Plenum Press: New York, 1980; pp 295−365.

Saunders, D. H.; Coleman, J. E.; Hampson, J. W.; Wells, P. A.; Riemenschneider, R. W. Autoxidation of fatty materials in emulsions. I. Pro-oxidant effect of histidine and trace

metals on the oxidation linoleate esters. *J. Am. Oil Chem. Soc.* **1962**, *39*, 434−439.

Van Ruth, S. M.; Roozen, J. P.; Cozijnsen, J. L. Changes in flavour release from rehydrated diced bell peppers (*Capsicum annuum*) by artificial saliva components in three mouth model systems. *J. Sci. Food Agric.* **1995**, *67*, 189−196.

Van Ruth, S. M.; Roozen, J. P.; Cozijnsen, J. L. Gas chromatography/sniffing port analysis evaluated for aroma release from rehydrated French beans (*Phaseolus vulgaris*). *Food Chem.* **1996**, *56*, 343−346.

Yamauchi, R.; Tatsumi, Y.; Asano, M.; Kato, K.; Ueno, Y. Effects of metal salts and fructose on the autoxidation of methyl linoleate in emulsions. *Agric. Biol. Chem.* **1988**, *52*, 849−850.

Received for review January 12, 1999. Revised manuscript received August 5, 1999. Accepted August 16, 1999.

JF990024T