Food Chemistry **50** (1994) 33–38

Enzymic and autoxidation of lipids in low fat foods: model of linoleic acid in emulsified hexadecane

J.P. Roozen,* E.N. Frankel & J.E. Kinsella

Department of Food Science and Technology, University of California, Davis, CA 95616, USA

(Received 21 April 1993; revised version received 12 june 1993; accepted 23 June 1993)

The oxidation of linoleic acid in emulsified hexadecane, which is inert to oxidation, was examined as a model for low fat foods. Static headspace gas chromatography was used to investigate the release of small amounts of volatile compounds in emulsion systems. At room temperature the initial rate of oxidation by lipoxygenase decreased in the presence of emulsion droplets. However, the total amounts of intermediate hydroperoxides formed after 4 h were similar, as well as the amounts of decomposition product hexanal formed after 22 h at 40 °C. There was no effect of droplet size of the emulsions tested. Increase of emulsion concentration from 0 to 2.4% gradually reduced the amount of hexanal released from the emulsions. The enzyme preparation used accelerated the formation of hexanal from methyl linoleate hydroperoxides prepared by autoxidation. However, hexanal was more readily formed from the linoleate hydroperoxide produced by the lipoxygenase preparation. Autoxidative decomposition of methyl linoleate at 40°C produced about the same amounts of hexanal as the corresponding linoleate containing model systems at 60°C. This study showed that lowering the fat contents of foods may increase the chances of flavour defects.

INTRODUCTION

The occurrence of lipid oxidation is a continuing problem in many areas of food processing, especially in operations like drying, separation and mixing. Products with higher lipid concentrations are naturally considered to be more prone to oxidative rancidity. However, lipids easily absorb and solubilize most of the lipophylic volatile compounds responsible for the perception of rancidity. Hence, the off-flavours are concentrated in the lipid phase, and their presence is reduced in the aqueous phases of the food or in the surrounding air (Kinsella, 1990). Therefore, diminishing the lipid content of ingredients in the preparation of low-fat foods can have an impact on the occurrence of off-flavours (Linssen, 1992). During processing and storage these flavour compounds can be formed by lipid oxidation reactions, which can be either enzymic or autoxidative in nature. In raw materials the onset of lipid oxidation is often triggered by enzymes, while autoxidation merely takes place in the final products (Gardner, 1989).

* Present address: Wageningen Agricultural University, Department of Food Science, Biotechnion, P.O. Box 8129, 6700 EV Wageningen, The Netherlands.

Food Chemistry 0308-8146/94/$07.00

One of the driving forces for the release of volatile compounds is the deviation from thermodynamic equilibrium between liquid and vapor phase. The actual partition coefficient is the ratio of the concentrations in the respective phases. Volatile compounds will be released if this coefficient is smaller than the equilibrium partition coefficient. The influence of temperature on volatility can be assessed by the Clausius–Clapeyron relationship. The addition of salts and simple sugars diminishes the bulk water content of the food, which increases the concentration of the volatile compounds and enhances their volatility (Land, 1979).

The equilibrium partition coefficient depends on the solvent properties (Reynolds & Land, 1982). There is a direct relation between the volatility of aromatic compounds and their extraction yields from water and emulsions. The more a compound is volatile, the easier it is extracted from a mixture containing lipids by an organic solvent (Le Thanh *et al.*, 1992). The release of volatile compounds from emulsions was studied by McNulty (1987). When the fraction of oil in emulsions decreases, the concentration in the oil phase increases, and thus the equilibrium concentration increases as well as the driving force for the release of volatile compounds. Rancidity problems may be consequently more likely aggravated by decreasing the lipid contents from food ingredients.

The reaction of unsaturated fats with oxygen is generally described by initiation, propagation and termination reactions. The involvement of oxygen, as a stable diradical, is unique for its relatively high concentration and low activation energy for radical propagation, which makes related taste defects difficult to prevent by lowering the temperature of storage (Grosch, 1987).

Soya bean lipoxygenase-1 (EC 1.13.11.12) is a non-heme iron dioxygenase, which catalyzes the oxidation of pentadienyl unsaturated fatty acids into their conjugated diene hydroperoxides (Iacazio *et al.*, 1990). High-performance liquid chromatography (HPLC) analyses indicated that the enzyme formed mainly 13S-hydroperoxides from linoleic acid both in an aqueous system and in reversed micelles (Shkarina *et al.*, 1992). The enzyme obeyed Michaelis–Menten kinetics in the pH range from 5.6 to 9.2 and was stable in the range 3.2–9.2 (Asbi *et al.*, 1989). Irreversible inactivation of the enzyme was caused by various peroxy acids containing a *cis,cis*-1,4-pentadiene group (Kim & Sok, 1991). The predominant volatile compound of lipoxygenases from soya is hexanal (Heimann & Franzen, 1978). A small part of the volatile compounds is formed by decomposition of hydroperoxide isomers from autoxidation, which always accompanies the enzymic reaction. A reliable indicator of non-enzymic oxygenation is sometimes found in the distribution of the different isomers (Chan & Newby, 1980). Pseudo-enzymic catalysis of the decomposition of hydroperoxides by lipoxygenase-1-isoenzyme has been described by De Groot *et al.* (1975). The oxy radical is presumed to be the source of volatiles and the reaction to be linked to the production of 285 nm UV-absorbing compounds (mainly oxooctadecadienoic acids) in the absence of oxygen.

The choice of physical or chemical analytical methods to follow progress of the oxidation reaction constitutes a separate problem. In most foods volatile compounds are present in extreme dilutions and do not interact. Under these conditions Henry's law applies and at equilibrium the partial pressure of the solute in the vapor above the solution is proportional to the solute concentration in the aqueous phase of the food (Nelson & Hoff, 1968). This relationship can be used to analyse the amount of volatiles in the food by static headspace gas chromatography.

The present study was aimed at investigating the effect of low concentrations of emulsion droplets on the enzymic and non-enzymic oxidation of linoleic acid and, on the release of volatile compounds generated. Hexadecane was chosen as an inert lipophilic component of the emulsion systems.

MATERIALS AND METHODS

Materials

Stock preparations were made for:

Tween-20 (0.3 % w/v) by dissolving 300 mg polyoxyethylene-sorbitan monolaurate (P1379, Sigma Chemical Co., St Louis, MO) in 100 ml water.

Emulsion (6 % w/v) by ultrasonic dispersing 1.5 g of *n*-hexadecane (H0255, Sigma Chemical Co., St Louis, MO) in 23.6 ml 0.3 % Tween-20 solution.

Substrate (0.056 % w/v) by ultrasonic dispersing 14 mg linoleic acid (JA 30C, Nu-Chek Prep, Inc., Elysian, MN) in 25 ml 0.3 % Tween-20 solution.

HPOD (methyl hydroperoxy octadecadienoates; 0.04 % w/v) by ultrasonic dispersing 4 mg residue of a nitrogen dried preparation in 10 ml 0.3 % Tween-20 solution. The hydroperoxides were prepared and analyzed according to Frankel *et al.* (1989). A part of the solution obtained was dried in a stream of nitrogen and dissolved in methanol. The absorbance at 234 nm was measured to find the concentration of the hydroperoxides.

Buffer (0.4 M; pH 7.5) using $NaH_2PO_4.H_2O$ and Na_2HPO_4 from Fisher Scientific (Fair Lawn, NJ).

Enzyme by dissolving 1 mg soy lipoxidase 1-B (L7395, Sigma Chemical Co, St Louis, MO) in 0.1 M sodium phosphate buffer pH 7.5.

Methods

The model system used was a modification of the conditions described by Surrey (1964) for the oxidation of linoleic acid by lipoxygenase at 20°C. Unless otherwise specified, the final concentrations of reactants and emulsions were: 0.5 mM linoleic acid (or 0.35 mM HPOD), 0.3% Tween-20, 5 ppm lipoxidase, 80 mM phosphate buffer, and emulsion series of 0.0 (control), 0.6, 1.2, 1.8, 2.4 and 3%.

Oxidation of linoleic acid by lipoxygenase was carried out in 2-ml volume cells of an oxygen uptake monitor instrument for 4 h at room temperature (±20°C). Aliquots (0.5 ml) from model systems containing the purified HPOD preparation were pipetted in 6-ml headspace bottles, which were capped and incubated for the decomposition reaction in a Gyrotary waterbath shaker (New Brunswick, Edison) at 40°C for different time intervals. The headspace bottles were also used for autoxidation experiments at 60°C, using a Lab-line Orbit environ shaker (Lab-line Instruments, Inc., Melrose Park, IL). Before and after incubation the samples were checked for conjugated diene content by differential UV-spectrophotometry of 50 μl aliquots dispersed in 3 ml water. The amounts of hexanal formed were determined by static headspace gas chromatographic analysis.

The initial rates of lipoxygenase activity were monitored with a Yellow Springs model 5300 Biological Oxygen Monitor (YSI, Yellow Springs, OH) equipped with a YSI 5331 oxygen probe and a Varian model 9176 recorder.

The amounts of hydroperoxides formed were estimated by differential absorbances (λ_{234}–λ_{260} nm) of the oxygenated fatty acids with a Shimadzu UV-160A recording spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The absorbance due to conjugated

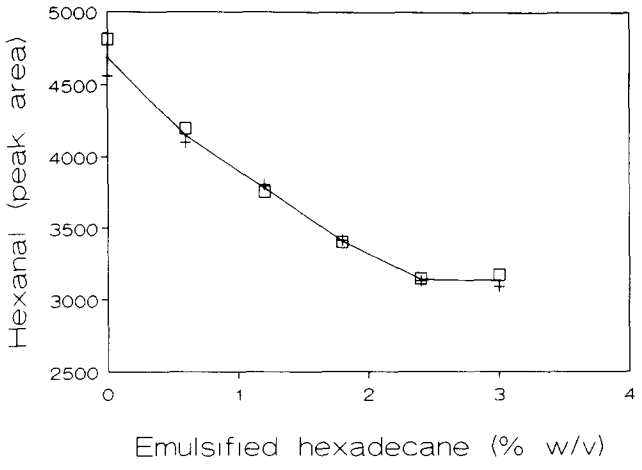

Fig. 1. Effect of emulsified hexadecane on the peak areas of 0.5 ml of 50 μM hexanal standard samples stored 22 h at 40°C, measured by static headspace gas chromatography (□, series 1; +, series 2).

diene at 234 nm was corrected for background absorbance at 260 nm. The wavelengths were taken at the maxima from the spectra and a molar extinction coefficient of 25 000 was used (Frankel, 1962).

Volatile compounds were analyzed by static headspace capillary gas chromatography of 0.5 ml samples in special headspace 6-ml bottles sealed with silicone rubber teflon caps with a crimper. The samples were incubated in a waterbath shaker for at least 0.5 h at 40°C. The bottles were then inserted into a H-6 headspace sampler at 40°C connected to the injector (180°C) of a Perkin–Elmer Sigma 3B gas chromatograph (Norwalk, CT). A capillary column, DB-1701, 30 m × 0.32 mm, 1 μm thickness (J & W Scientific, Inc., Folsom, CA) was used isothermally at 78°C with 20 ml/min flow of helium as carrier gas. The volatiles were detected by a flame ionization detector at 200°C using 140 kPa hydrogen and 280 kPa air. Before injection the bottles were pressurized in the sampler with carrier gas through a stationary injection needle for 30 s. A standard curve of peak areas for hexanal was determined at 40°C versus the emulsion concentration by adding 25 μl 1 mM hexanal in water to different emulsion concentrations (Fig. 1). This hexanal standard concentration was chosen as an average measure for the increasing concentrations (0–50 nmoles/0.5 ml) of the samples during incubations at 40°C. The peak areas for headspace hexanal were converted to concentration (nmoles/0.5 ml) using a factor derived from these data, with the factors being 187, 166, 151, 137, 126 and 125 for concentrations of 0.0, 0.6, 1.2, 1.8, 2.4 and 3.0% hexadecane.

For a droplet size experiment the stock emulsion was prepared by Dr D.J. McClements of this Department, using an Ultra Turrax J & K (IKA) and a single valve high pressure laboratory homogenizer (Tornberg & Lundh, 1978). Preparations with different droplet sizes were obtained by passing the emulsion repeatedly through the homogenizer. Droplet size distributions were measured and average sizes calculated with a laser Malvern Master Sizer (Malvern Instruments, Ltd, Malvern, Worcs, UK).

RESULTS AND DISCUSSION

A model system was made to study the enzymic and non-enzymic oxidation of linoleic acid in emulsified hexadecane, as a non-participating lipophilic component in the lipid oxidation reactions. The concentrations of the emulsions were chosen to reflect low-fat foods. The initial rates of the oxidation of linoleic acid were monitored by oxygen uptake, amounts of hydroperoxides formed estimated by diene absorbance, and production of volatile compounds determined by static headspace gas chromatography of hexanal.

Linoleic acid model

The effect of emulsion concentration on the enzymic oxidation of linoleic acid was measured by monitoring the initial rate of oxygen uptake. The absolute initial rate of the control sample was 19 nmoles oxygen/min. Linear traces were obtained during the initial 20 min corresponding to 40% oxidation of the available linoleic acid. Table 1 shows a significant effect of emulsified hexadecane on the relative initial rates of oxygen uptake at room temperature. The rates of oxygen uptake decreased to 19 and 22% for the two highest concentrations of hexadecane compared to the control sample. During the 4 h reaction time the extent of autoxidation is negligible, because the initial oxidation rate is zero in the absence of the enzyme. The availability of linoleic acid for enzymic oxidation seems to be limited by its partition between the different phases of the emulsion. It is in fact difficult to define an effective substrate concentration in the water phase, because of the micellar nature of the Tween-20/lipid substrate emulsion. Surrey (1964) found that the concentration

Table 1. Primary (20°C) and secondary (40°C) oxidation of linoleic acid by lipoxygenase in emulsified hexadecane model systems

Incubation	4 h at 20°C		22 h at 40°C	
Hexadecane (% w/v)	Rel. oxygen uptake[a] (%)	Diff. absorb.[b]	Diff. absorb.[b]	Hexanal[c] (nmole)
0·0	100	0·137	0·029	56
0·6	59	0·134	0·045	51
1·2	41	0·143	0·036	47
1·8	33	0·144	0·031	48
2·4	22	0·146	0·037	47
3·0	19	0·143	0·036	48
CV (%)[d]	14	9	7	3

[a] Relative initial rates of oxygen uptake; the absolute initial rate of oxygen uptake is 19 nmoles/min for 0·0.
[b] Differential diene absorbances at λ = 234 and 260 nm.
[c] Formation of hexanal (nmole per 0·5 ml; Fig. 1).
[d] CV (%) = overall relative standard deviation of duplicates (Anderson & Sclove, 1986).

of Tween-20 influences the activity of the enzyme. In our model systems all comparative measurements were made at constant emulsifier concentrations to correct for the possible effect of Tween-20 emulsifier on enzyme activity. The hexadecane dispersion might have changed the microenvironment of the substrate to make it less available to the enzyme.

The effects of initial oxidation rates were mostly eliminated in the measurements of hydroperoxide contents by diene absorbances after 4 h (Table 1). These data also indicate a 75% yield of hydroperoxides based on the initial amount of linoleic acid. Probably the substrate molecules available to the enzyme diffuse to the micelles according to their partition equilibrium and the same amounts of hydroperoxides are thus formed at the different emulsion concentrations after 4 h. A similar suggestion was made by Shkarina *et al.* (1992), namely that the substrate and the product of lipoxygenase are probably located in the surfactant shell of the emulsion globules, and reach the enzyme by diffusion into the aqueous phase. They also detected linoleic acid, but not its hydroperoxide, at very low concentrations in the apolar phase.

Static headspace gas chromatography of enzymic oxidation of linoleic acid in the model systems generates after a systematic air peak three main peaks related to the decomposition of hydroperoxides, including pentane, pentanal and hexanal in a peak area ratio of 5:4:100. Since this ratio remained the same throughout the study, hexanal can be considered as a reliable measure of hydroperoxide decomposition. The partitioning of hexanal between the liquid and vapor phases is affected by the presence of hexadecane, which increases the solubility of hexanal in the liquid phase (Fig. 1). This physical effect was allowed for by multiplying the mass of hexanal in the vapor phase by a factor which was dependent on the hexadecane concentration. At 2–3% levels hexanal release is about 70% compared to the control without hexadecane. These relative peak areas of volatile compounds illustrate the importance of product composition, e.g. lipid content.

The decomposition of the hydroperoxides, formed by lipoxygenase at 20°C, yielded 56 nmole of hexanal in the control sample (Table 1, 22 h at 40°C), which is 20% of the initial amount of linoleic acid and 30% of the hydroperoxides estimated spectrophotometrically. Comparing the conjugated diene spectra before and after incubation at 40°C shows a shift in the maxima of diene absorbances to lower wavelengths, probably caused by the formation of UV absorbing compounds like conjugated keto diene and polymerization products during decomposition of hydroperoxides at 40°C (De Groot *et al.*, 1975). Therefore the diene absorbance measurements may overestimate the amounts of hydroperoxides present.

Lee (1986) developed a technique to emulsify fat into small droplets, which increases the interfacial area and greatly enhances the rate of partitioning of volatile compounds. Table 2 demonstrates that droplet size, ranging from 0.45 to 1.01 μm of the hexadecane emulsion, has no significant effect on the formation of hexanal in both enzymic and non-enzymic model systems. Obviously, the rate of partitioning of hexanal is neither important for the enzymic nor for non-enzymic oxidation of linoleic acid in our model systems. Overall, the non-enzymic oxidation model systems at 60°C yielded much less hexanal (9–14 nmole) than the enzymic systems at 40°C (45–50 nmole hexanal, Tables 1 and 2). Soy lipoxygenase at neutral pH catalyzes co-oxidation of lipids by free radicals leaking from the enzyme surface. Consequently both 9- and 13-linoleic acid hydroperoxides can be detected. This radical process is likely to contribute to the increase in the formation of hexanal in the presence of the enzyme.

Table 2. Influence of droplet size of 1·2 and 2·4% (w/v) emulsified hexadecane on the formation of hexanal by enzymic (22 h at 40°C) and non-enzymic (92 h at 60°C) oxidation of linoleic acid

Size (passes)[a] (μm)	Enzymic (nmole hexanal)		Non-enzymic (nmole hexanal)	
	1·2%	2·4%	1·2%	2·4%
1·01 (1)	45	48	12	14
0·73 (3)	48	50	11	13
0·61 (5)	48	44	14	11
0·52 (8)	44	47	11	11
0·45 (12)	46	49	9	12
CV (%)[b]	5	4	17	12

[a] Passes through the homogenizer.
[b] As in Table 1.

Methyl linoleate model

Decomposition of the hydroperoxides, formed by lipoxygenase at 20°C, can either be autoxidative or catalyzed by the lipoxygenase preparation. To eliminate the effect of lipoxygenase the decomposition of a purified hydroperoxide from autoxidized methyl linoleate was followed by analyzing hexanal. The de-

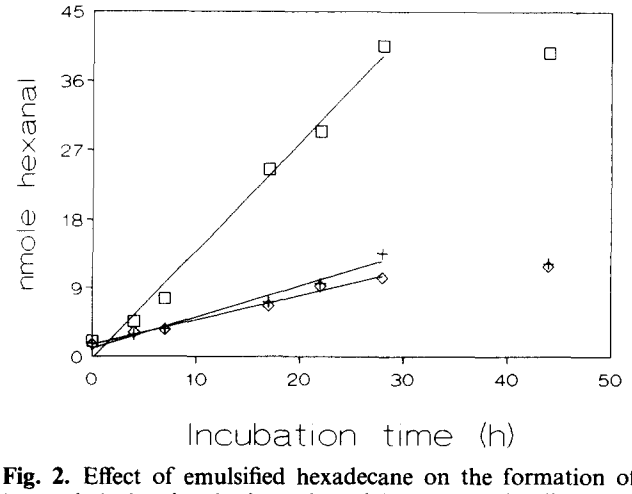

Fig. 2. Effect of emulsified hexadecane on the formation of hexanal during incubation of model systems with lipoxygenase at 40°C. Hydroperoxides used were obtained by autoxidation (□, control; +, 1.2%; ◇, 2.4% hexadecane).

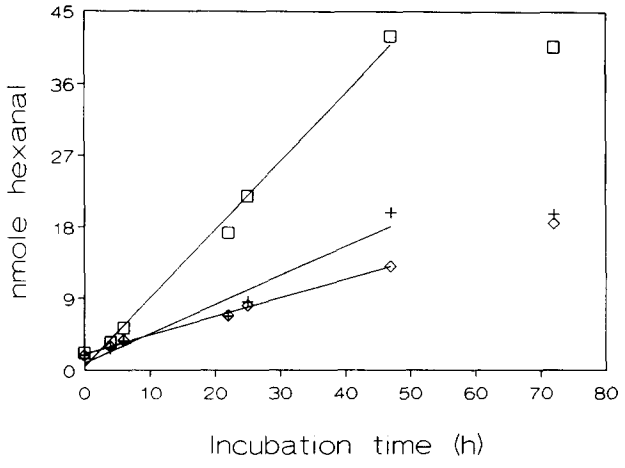

Fig. 3. Effect of emulsified hexadecane on the formation of hexanal during incubation of non-enzymic model systems; details as Fig. 2.

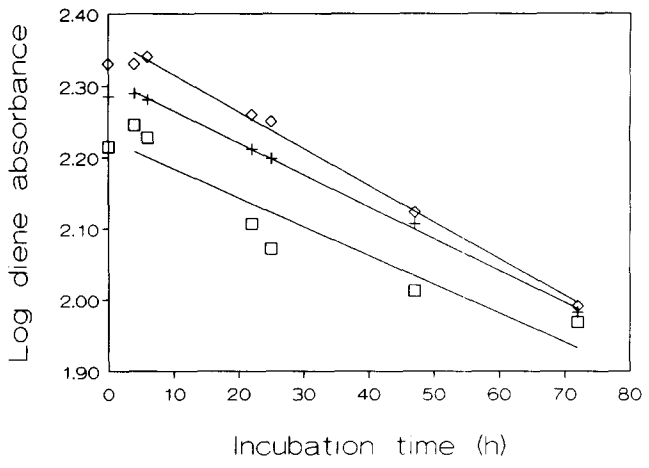

Fig. 5. Effect of emulsified hexadecane on the decomposition of hydroperoxides during incubation of non-enzymic model systems; details as Fig. 2.

composition reactions yielded 42 nmole of hexanal in a control sample with enzyme after 28 h (Fig. 2) and without enzyme after 47 h (Fig. 3). The control system containing the enzyme preparation released hexanal much faster than the system without enzyme. This result can be explained by lyase activity in the enzyme preparation. It is not clear whether this activity is connected with the lipoxygenase molecule (De Groot *et al.*, 1975) or with a separate enzyme. Gardner (1989) reported separate lyases in enzyme preparations made from soya bean seeds.

Figures 2 and 3 show also that both control samples produced about 20% less hexanal than the model in Table 1. The lower yield could be the result of a lower starting concentration of hydroperoxides, because the two models were adjusted to the same diene absorbances at the beginning of the incubation at 40°C. UV-absorbing compounds from secondary lipid oxidation were probably also present in the hydroperoxide preparation from autoxidized linoleate. The addition of emulsified hexadecane to the models with and without enzyme, slowed down the rate of formation and lowered the yield of hexanal to respectively 18 and 13 nmole (Figs 2 and 3). The slopes of the four regression curves follow the same trend and are much lower than the slopes of the control samples (Table 3). This result indicates that, in contrast to the control systems discussed above, the addition of the enzyme preparation has little effect on the rates of hexanal formation in the presence of emulsion droplets. Possibly the lipophilic methyl linoleate hydroperoxides may have migrated into an apolar phase inaccessible to the enzyme, while the partition equilibrium impairs backmigration. This explanation is supported by the similar yields of hexanal found for the autoxidation model systems of linoleic acid at 60°C (Table 2).

The log curves of the changes in diene absorbances, (Figs 4 and 5) are only in partial agreement with the curves of the formation of hexanal (Figs 2 and 3). The initial increases in diene absorbances may be due to oxidation of residual methyl linoleate in the hydroperoxide preparation used. The continuing loss of diene absorbances at the end of the curve does not correspond with the leveling of hexanal formation. The parallelism of the linear regression lines in Figs 4 and 5 denotes that the rates of decomposition are similar.

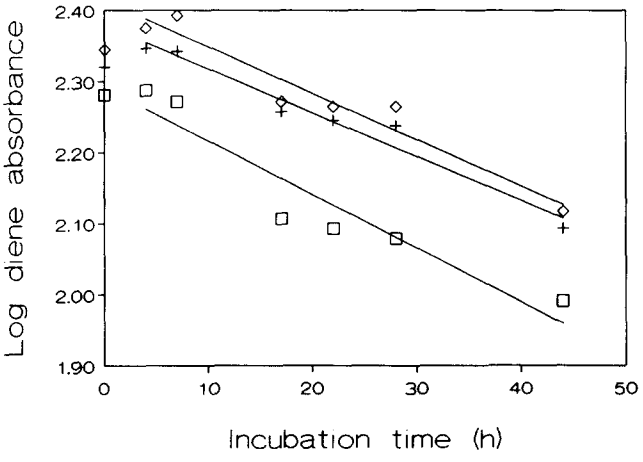

Fig. 4. Effect ot emulsified hexadecane on the decomposition of hydroperoxides during incubation of enzymic model systems; details as Fig. 2.

Table 3. Linear regression coefficients obtained from curves in Figs 2 and 3 (hexanal) and Figs 4 and 5 (diene absorbance)

	Constant	Slope	R-square
Fig 2			
Control	−0·24	1·41	0·9888
1·2%	1·10	0·41	0·9750
2·4%	1·60	0·32	0·9883
Fig. 3			
Control	0·43	0·86	0·9925
1·2%	0·86	0·37	0·9483
2·4%	2·03	0·23	0·9966
Fig. 4			
Control	2·29	−0·0075	0·8979
1·2%	2·38	−0·0062	0·9625
2·4%	2·41	−0·0065	0·9386
Fig 5.			
Control	2·22	−0·0041	0·8842
1·2%	2·31	−0·0045	0·9986
2·4%	2·37	−0·0052	0·9946

The linear regression slopes given in Table 3 demonstrate that the loss of diene absorbances is somewhat faster in the enzyme containing model systems, particularly in the control. The regression coefficients indicate that the enzyme containing model systems differ more from a log-linear decomposition reaction (first order) than the non-enzymic systems. These observations and the results of hexanal formation indicate that the enzyme is likely involved in the decomposition of methyl linoleate hydroperoxides. Furthermore, the enzyme is expected to decompose its own oxidation product (mainly 13S linoleate hydroperoxide) more easily than the hydroperoxide isomers from autoxidized methyl linoleate (Table 1 versus Fig. 2). This might be due to differences in enzyme affinity for different hydroperoxides, e.g. with a methyl ester group or with an isomeric hydroperoxide.

CONCLUSION

Increasing concentrations (0–3%) of emulsified hexadecane delayed the enzymic oxidation of linoleic acid, but the diene absorbances reached the same values after 4 h at room temperature. The hydroperoxides formed were decomposed into relatively high amounts of hexanal at 40°C. Increasing concentrations of emulsified hexadecane reduced the release of hexanal. The droplet size of hexadecane had no effect. The formation of hexanal from methyl linoleate hydroperoxides in the control system was accelerated by the enzyme, but the total amounts formed were the same as by non-enzymic decomposition of methyl linoleate hydroperoxides at 40°C. Much less hexanal was detected in these systems containing emulsified hexadecane and in samples of linoleate autoxidized at 60°C. The methyl linoleate hydroperoxides seem to be protected from enzyme decomposition by emulsified hexadecane. Therefore the rate of decomposition and the yield of hexanal are dependent only on autoxidation. The effect of vegetable oils as the emulsified apolar phase on the oxidation of linoleic acid model systems, will be the subject of another study.

ACKNOWLEDGEMENT

The authors wish to thank the Wageningen Agricultural University for an 8 months sabbatical leave of one of them (J.P.R.).

REFERENCES

Anderson, T.W. & Sclove, S.L. (1986). *The Statistical Analysis of Data*, 2nd edn. The Scientific Press, Palo Alto, CA, pp. 132–6.

Asbi, B.A., Wei, L.S. & Steinberg, M.P. (1989). Effect of pH on the kinetics of soybean lipoxygenase-1. *J. Food Sci.*, **54**, 1594–5, 1600.

Chan, H.W.-S & Newby, V.K. (1980). Hemoprotein- and transition metal ion-catalysed oxidation of linoleic acid. Selectivity of the position of oxygenation. *Biochim. Biophys. Acta*, **617**, 353–62.

De Groot, J.J.M.C., Veldink, G.A., Vliegenthart, J.F.G., Boldingh, J., Wever, R. & Van Gelder, B.F. (1975). Demonstration by EPR spectroscopy of the functional role of iron in soybean lipoxygenase-1. *Biochim. Biophys. Acta*, **377**, 71–9.

Frankel, E.N. (1962). Hydroperoxides. In *Lipids and their oxidation*, ed. H.W. Schultz, E.A. Day & R.O. Sinnhuber, AVI Publishing Co., Westport, CT, pp. 51–78.

Frankel, E.N., Hu, M.-L. & Tappel, A.L. (1989). Rapid headspace gas chromatography of hexanal as a measure of lipid peroxidation in biological samples. *Lipids*, **24**(11), 976–81.

Gardner, H.W. (1989). Soybean lipoxygenase-1 enzymically forms both 9*S*- and 13*S*-hydroperoxides from linoleic acid by a pH-dependent mechanism. *Biochim. Biophys. Acta*, **1001**, 274–81.

Grosch, W. (1987). Reactions of hydroperoxides—products of low molecular weight. In *Autoxidation of Unsaturated Lipids*, ed. H.W.-S. Chan, Academic Press, London, pp. 95–139.

Heimann, W. & Franzen, K.-H. (1978). Untersuchungen über die Entstehung flüchtiger Verbindungen aus radikalischen Reaktionszwischenstufen bei der Lipoxygenase-Reaktion. *Z. Lebensm. Unters. Forsch.*, **167**, 78–81.

Iacazio, G., Langrand, G., Baratti, J., Buono, G. & Triantaphylides, C. (1990). Preparative, enzymatic synthesis of linoleic acid (13S)-hydroperoxide using soybean lipoxygenase-1. *J. Org. Chem.*, **55**, 1690–1.

Kim, M.R. & Sok, D.-E. (1991). Irreversible inhibition of soybean lipoxygenase-1 by hydroperoxy acids as substrates. *Arch. Biochem. Biophys.*, **288**, 270–5.

Kinsella, J.E. (1990). Flavor perception and binding. *Inform*, **1**(3), 215–26.

Land, D.G. (1979). Some factors influencing the perception of flavour contributing substances in food. In *Progress in Flavour Research*, ed. D.G. Land & H. Nursten, Applied Science Publishers, London, pp. 53–66.

Lee, W.E. (1986). A suggested instrumental technique for studying dynamic flavor release from food products. *J. Food Sci.*, **51**, 249–50.

Le Thanh, M., Pham Ti, S.T. & Voilley, A. (1992). Influence of the presence of vegetable oil in emulsion on the volatility of aroma substances during the course of the extraction. *Sci. Aliment*, **12**, 587–92.

Linssen, J.P.H. (1992). Influence of polystyrene and polyethylene packaging materials on food quality. PhD thesis, Wageningen Agricultural University, The Netherlands, pp. 70–83.

McNulty, P.B. (1987). Flavour release—elusive and dynamic. In *Food Structure and Behaviour*, ed. J.M.V. Blanshard & P. Lillford, Academic Press, London.

Nelson, P.E. & Hoff, J.E. (1968). Food volatiles: gas chromatographic determination of partition coefficients in water–lipid systems. *J. Food Sci.*, **33**, 479–82.

Reynolds, J. & Land, D.G. (1982). Effects of food constituents on the vapour pressure of diacetyl. *J. Sci. Food Agric.*, **33**, 592.

Shkarina, T.N., Kuehn, H. & Scherve, T. (1992). Specificity of soybean lipoxygenase-1 in hydrated reverse micelles of sodium *bis* (2-ethylhexyl) sulfosuccinate (Aerosol OT). *Lipids*, **27**(9), 690–3.

Surrey, K. (1964). Spectrophotometric method for determination of lipoxidase activity. *Plant Physiol.*, **39**, 65–70.

Tornberg, E. & Lundh, G. (1978). Functional characterization of protein stabilized emulsions: standardized emulsifying procedure. *J. Food Sci.*, **43**, 1553–8.