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Enzymic and autoxidation of lipids in low fat foods: model of linoleic acid in emulsified triolein and vegetable oils

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To evaluate problems in novel foods with reduced fat content the linoleic acid model systems used in our previous study were applied to high oleic sunflower oil, triolein and stripped corn oil, in the presence or absence of added α-tocopherol. Initial rates of oxygen uptake by enzymic oxidation depended on the emulsion concentrations and not on their fatty acid compositions. Using static headspace gas chromatography a significant difference in the release of hexanal was detected between emulsions rich in either linoleate or oleate. The higher the linoleate content of the emulsion, the higher the conjugated diene absorbance and the amounts of hexanal produced. In the presence of α-tocopherol the diene absorbance was increased and the hexanal yields decreased, indicating that α-tocopherol retarded the decomposition of hydroperoxides. On the other hand, the antioxidant effects of α-tocopherol were gradually lost during autoxidation tests at 60°C. Therefore, large differences were observed in the amounts and compositions of volatile compounds between emulsions rich in either oleate or linoleate. Lipid concentration, type of lipids and presence of antioxidants are important factors in the oxidative formation of volatile compounds in our models. These factors are expected to impact on the flavour of low fat foods.

INTRODUCTION

Absorption of volatile oxidation compounds by triglycerides is important in foods and can markedly enhance off-flavour retention, and thus increase the threshold concentrations of flavour compounds. Because the absorption and release of volatile compounds from lipids are diffusion controlled, the concentration is influenced by the physical state of the lipid in addition to temperature (Kinsella, 1990). According to the partition coefficient relation formulated by Buttery *et al.* (1973), the release of volatile compounds from an oil/water emulsion is the same as that from a water/oil emulsion. They found this formulation to hold well for aldehyde partitioning between air and high-oleic safflower oil/water mixtures without emulsifiers. In contrast, Land (1979) found an effect of the type of emulsion on the release of dimethylsulfide, which might be due to the use of different types of emulsifiers creating different interfacial layers. However, the results of Linssen (1992) with styrene fit the ideal relationship of Buttery *et al.* (1973) with different emulsifiers and corn oil/water and water/corn oil emulsions.

Plant or animal lipids are the key constituents of food emulsions. The unsaturated fatty acid components of emulsions are particularly susceptible to autoxidation (Frankel, 1991). Kinetic studies of fat oxidation in emulsions have been carried out by Marcuse & Fredriksson (1968). Effective measures to protect lipid emulsions against oxidation include omitting oxygen, complexing transition metal catalysts, and protecting against light exposure (Allen, 1986).

Antioxidants are the most often utilized method to inhibit oxidation of lipids (Frankel, 1991). The stability of vegetable oils can also be improved by plant breeding to decrease polyunsaturated fatty acids and increase monounsaturated fatty acids, for example development of high oleic sunflower oil (Yodice, 1990). Shimada *et al.* (1992) found antioxidative properties of xanthan in a cyclodextrin emulsion of soybean oil containing α-tocopherol. The pyruvate residues of xanthan seem to chelate ferrous ions catalyzing secondary lipid oxidation reactions. The antioxidant effects of egg yolk constituents were tested in linoleate emulsions with and without added ferrous ions (Yamamoto *et al.*, 1990). In this work the antioxidant effects were lost by heating, which suggests that native protein structures

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are important for the chelating of ferrous ions. In cosmetic cream emulsions Szelag *et al.* (1990) investigated several antioxidants. Only small inhibiting effects (±15% less oxidation than in the control) were produced by α-tocopherol, propyl gallate, butylhydroxy toluene and anisole, and a mixture of 6,6–ethyliden bis(2,2,4–trimethyl)-1,2–dihydroquinoline.

In our previous study we used emulsified hexadecane as an inert lipophilic component of the model system. In this paper we investigated the role of emulsified vegetable oil on the oxidation reactions of linoleic acid and in the generation of volatile compounds. The release of these volatile compounds depends on the amounts and nature of the emulsified oils. Yields of hexanal decreased and the diene absorbances increased in the presence of α-tocopherol. Autoxidation of emulsions rich in either oleate or linoleate showed large differences in the formation of volatile compounds.

MATERIALS AND METHODS

Materials

The same stock preparations were made as described previously by Roozen *et al.* (1994), except for: *Emulsions* (6% w/v) by ultrasonic dispersing 1·5 g of resp. *n*–hexadecane (**HD**; H0255, Sigma Chemical Co, St Louis, MO); triolein stripped for 50 h with nitrogen (*TR*; 99% 18:1 and 0·5% 18:2; T235, Nu-Chek Prep, Inc., Elysian, MN); high oleic sunflower oil, containing 800 ppm natural α-tocopherol (*HO*; 79 % 18:1 and 11 % 18:2; SVO Enterprises, Eastlake, OH); stripped corn oil (*CO*; 25 % 18:1 and 60% 18:2; Eastman Kodak Co., Rochester, NY) or *CO* containing 500 ppm added DL–alpha-tocopherol (*CT*; T3251, Sigma Chemical Co, St Louis, MO) in 23·6 ml 0·3% Tween–20 solution. The oils were kept in nitrogen/cold storage to prevent oxidation.

Methods

The same model system used for the oxidation of linoleic acid and the same methods described previously by Roozen *et al.* (1994) were used to determine the initial rates of lipoxygenase activity, the amounts of hydroperoxides formed and the analysis of volatile compounds.

Unless otherwise specified, the final concentrations of reactants and emulsions were: 0·5 mM linoleic acid, 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% oil.

Oxidation of linoleic acid by lipoxygenase was carried out in 2-ml volume cells of an oxygen uptake monitor instrument for 4 h at ±20°C. Aliquots (0·5 ml) from these samples were pipetted in 6-ml headspace bottles and incubated at 4°C for 22 h. These bottles were also used for autoxidation experiments at 60°C. Before and after the incubations the samples were checked for hydroperoxide content by measuring the conjugated diene absorbance of 50 μl aliquots dispersed in 3 ml water. The amounts of hexanal formed were determined by static headspace gas chromatographic analysis.

RESULTS AND DISCUSSION

In the formulation of food emulsions, for optimum stability, we have to consider not only the fat content, but also the relative unsaturation and contribution of antioxidants. We investigated the role of emulsified vegetable oils in the enzymic and autoxidation reactions of linoleic acid in a model system. The extent of oxidation was measured by conjugated diene absorbance and hexanal release by headspace gas chromatography.

Enzymic oxidation

As in our previous study with model systems (Roozen *et al.*, 1994) the initial rates of enzymic oxidation of linoleic acid were gradually lowered by the addition of emulsified stripped corn oil with and without 500 ppm added α-tocopherol (Fig. 1). This similarity in results between corn oil emulsion and hexadecane emulsion indicates that the enzymic oxidation rate slows down by emulsion droplets independent of the nature of the apolar phase. The presence of α-tocopherol has no influence on the rate of this enzymic oxidation. Retardation of the enzymic reaction by the added emulsified oil may be caused by migration of the substrate to a more apolar phase than the enzyme containing phase and rendering the linoleic acid less available (Roozen *et al.* 1994).

The soya bean lipoxygenase–1 used is well known for oxygenation of polyenoic fatty acids containing a doubly allylic methylene to form conjugated dienoic 13–hydro-

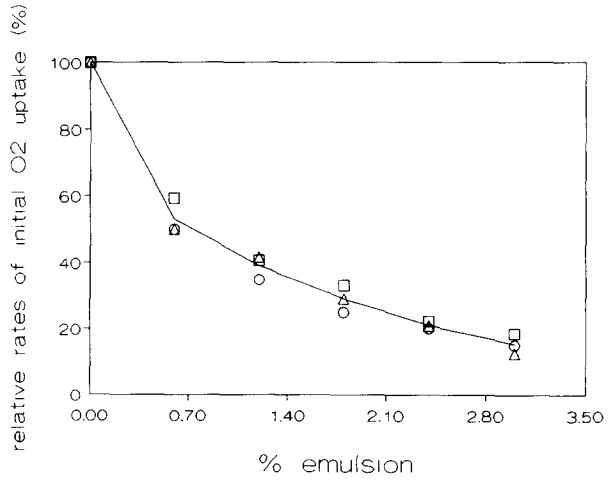

Fig. 1. Relative rates of initial oxygen uptake by enzymic oxidation of 0·5 mM linoleic acid in emulsified hexadecane (**HD**; □) and stripped corn oil with 500 ppm added α-tocopherol (**CT**; ○) and without α-tocopherol (**CO**; Δ). The initial rates of the control samples were designated 100 %.

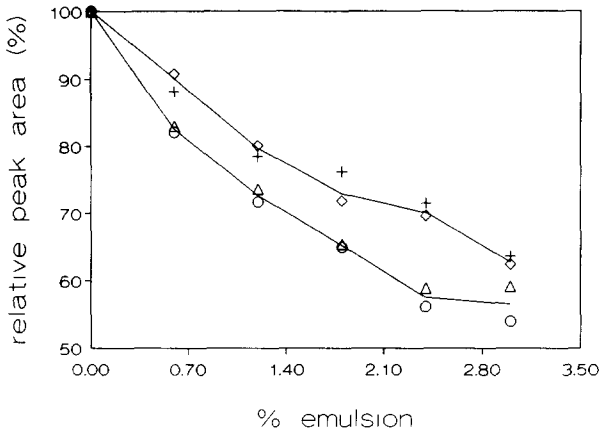

Fig. 2. Relative peak areas of 0·5 ml 50 μM hexanal standards at 40 °C. Static headspace gas chromatographic analysis of emulsified high oleic sunflower oil (**HO**, +), triolein (**TR**, ◇) and stripped corn oil with 500 ppm added α-tocopherol (**CT**; ○) and without α-tocopherol (**CO**; Δ). The peak areas of the control samples were designated 100 %.

peroxides. However, this enzyme produces almost 20% 9–hydroperoxides at neutral pHs (Gardner, 1989). This enzyme also reacts further with the 9–hydroperoxides to form oxooctadecadienoic acid (Verhagen *et al.*, 1977). The 13–hydroperoxides of linoleic acid are decomposed predominantly into hexanal, which can be monitored by static headspace gas chromatography. As demonstrated in our previous study, the peak areas of hexanal are influenced by the amount of emulsified hexadecane. In this study the peak areas presented in Fig. 2 are related to the control samples (0·0 % emulsion) in order to compensate for daily fluctuations in the response of the FID. The relative peak areas of hexanal in emulsified triolein have the same areas as in emulsified high oleic sunflower oil, but have significantly higher areas (Student-*t*: $p < 0{\cdot}05$; $n = 4$) than in emulsified corn oil with or without α-tocopherol (Fig. 2). Therefore, the nature of the emulsified oil, acting as solvent for hexanal, affects the partition of hexanal

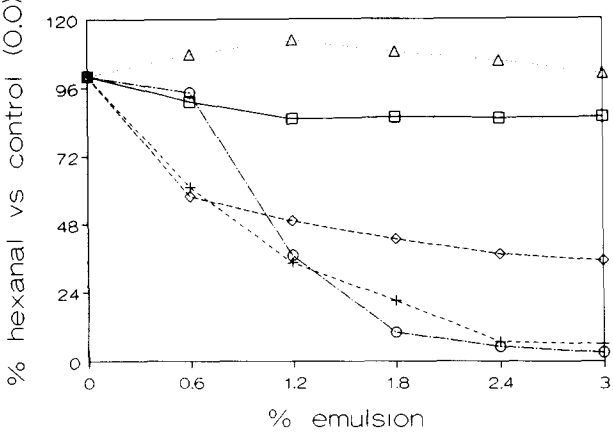

Fig. 3. Relative amounts of hexanal produced by enzymic decomposition of linoleate hydroperoxides for 22 h at 40 °C in emulsified, respectively hexadecane (**HD**; □), triolein (**TR**; ◇), high oleic sunflower oil (**HO**; +), and stripped corn oil with 500 ppm added α-tocopherol (**CT**; ○) and without α-tocopherol (**CO**; Δ).

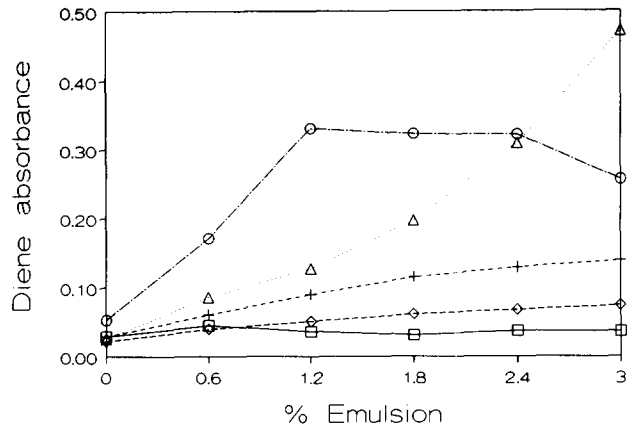

Fig. 4. Relative amounts of linoleate hydroperoxides estimated by conjugated diene absorbance measurements. Incubation and emulsion details as in Fig. 3.

between the liquid and gas phase. For this reason, standard series of hexanal should always be used to calculate the amount of hexanal formed in each of the oxidized samples.

The enzymic oxidation of linoleic acid in different amounts of emulsified oils is shown in Figures 3 and 4. Emulsified stripped corn oil produced more hexanal than the control (0·0) and the emulsified hexadecane model systems (Fig. 3). The greater amount of hexanal in the corn oil (*CO*) than the hexadecane (*HD*) system may have been generated by autoxidation of the emulsified corn oil containing linoleate. However, lipoxygenase–2 isoenzyme from soya bean might be present in our lipoxidase preparation, which oxidizes triglyceride polyunsaturated fatty acids (Christopher *et al.*, 1970). Although α-tocopherol decreased hexanal formation in the model systems (*CT*, Fig. 3), it caused a strong increase in diene absorbances (Fig. 4). The increase was not related to the interference of α-tocopherol oxidation products, because of the extreme sample dilutions before UV measurements. The increase supports our results for the initial enzymic oxidation rates, that α-tocopherol does not inhibit enzymic oxidation, but does inhibit formation of the decomposition product hexanal. Similar effects were observed between triolein (*TR*) and high oleic sunflower oil (*HO*) (Fig. 3). The presence of 800 ppm natural α-tocopherol in *HO* apparently retarded hexanal formation to the same extent as in *CT* at emulsion concentrations of 2·4 and 3·0%. In the system containing 0·6% emulsion the concentration of α-tocopherol seemed to be too low for effective antioxidant activity. At the higher emulsion concentrations the much higher diene absorbances found for *HO* compared to *TR* and *HD* may be due to cooxidation of the 11% linoleate in *HO*. However, it is more difficult to understand the lower hexanal yields in *TR* than in *HD*, because the diene absorbances are higher in *TR* than in *HD*. Perhaps *TR* favours decomposition pathways yielding secondary autoxidation products other than hexanal compared to *HD*. A lower hexanal yield was also found for an analogous model of the decomposi-

Table 1. Relative amounts (%) of hexanal formed by autoxidation of 0·5 mM linoleic acid in low fat emulsions at 60°C

Emulsified oil[a] (%)	*CO* 45h	*CT* 45h	*CT* 50h	*CT* 68h	*HO* 200h	*TR* 200h
Control	100	100	100	100	100	100
0·6	1 479	1 774	2 092	2 249	415	239
1·2	1 776	1 130	2 347	2 846	466	86
1·8	>3 500	1 631	1 956	3 310	430	47
2·4	>3 500	1 056	1 515	3 124	787	47
3·0	>3 500	671	1 141	3 104	858	53
CV(%)[b]	4	15	6	9	7	13

[a] Emulsions of corn oil (*CO* and *CT*), high oleic sunflower oil (*HO*) and triolein (*TR*). The peak areas of the control samples were designated 100%.
[b] CV = overall relative standard of deviation of two replicates (Anderson & Sclove, 1986).

tion of methyl linoleate in emulsified hexadecane (Roozen *et al.*, 1994). In both cases the substrate seems to be less available to the enzyme by its presence in a different liquid phase.

Autoxidation

The production of volatile compounds by autoxidation of the model systems without enzyme was investigated by incubation at 60°C. The hexanal yields in the control systems were designated 100% to compensate for hexanal losses during prolonged incubations, and fluctuations of the FID-GC detector (Table 1). At concentrations above 1·8% emulsified stripped corn oil (*CO*) produced excessive hexanal beyond the sensitivity range of the static headspace gas chromatographic method used. At the higher emulsion concentrations addition of α-tocopherol to the stripped corn oil (*CT*) decreased the hexanal yields markedly (more than 53 to 80%). At 0·6% emulsion α-tocopherol in the model system had no antioxidant effect. At longer incubation times hexanal yields increased at the higher emulsion concentrations. Apparently, the higher corn oil concentrations counteract the higher concentrations of α-tocopherol by increased radical production and depleting gradually the antioxidant.

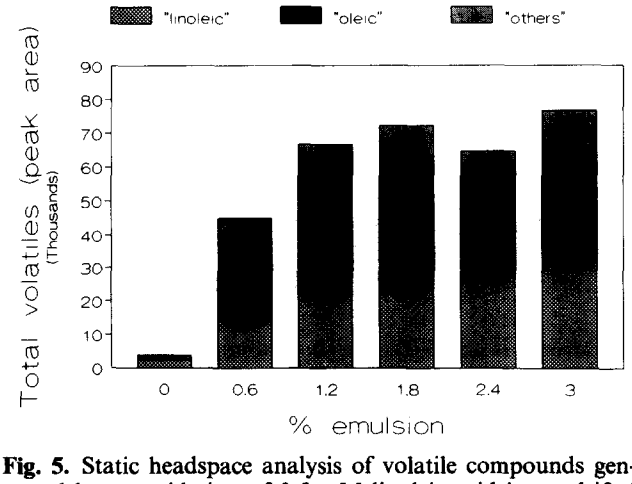

Fig. 5. Static headspace analysis of volatile compounds generated by autoxidation of 0·5 mM linoleic acid in emulsified high oleic sunflower oil (**HO**) for 200 h at 60 °C ('linoleic' products = pentane + pentanal + hexanal; 'oleic' products = heptane + octane; 'others' = mainly hexane + nonane).

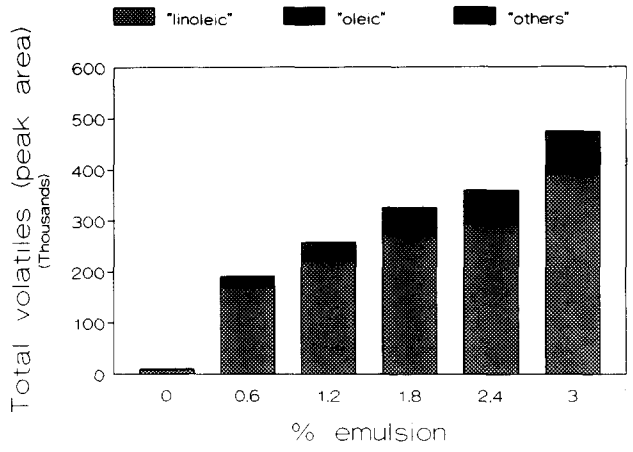

Fig. 6. As Fig. 5 in emulsified stripped corn oil with 500 ppm added α-tocopherol (**CT**) for 68 h at 60 °C.

The much lower hexanal yields in *HO* and *TR* emulsions is expected from their lower linoleate contents (11 and 0.5 %) than *CO* and *CT* (60%) (Table 1). The *HO* system contained natural α-tocopherol, which may be expected to have a similar effect in decreasing hexanal yields as added α-tocopherol in the *CT* system. The antioxidant retarded oxidation until it was consumed by the radicals formed. After 200 h incubation the highest hexanal yields were observed with the highest amounts of emulsified *HO*, when the effect of the antioxidant has almost disappeared.

Large differences were noted in the composition of volatile compounds from the autoxidation of emulsions containing mainly oleate (*HO*; Fig. 5) or mainly linoleate (*CT*; Fig. 6). Static headspace gas chromatographic analyses readily revealed that linoleic rich oils produced large amounts of pentane, pentanal and hexanal, while oleic rich oils produced large amounts of heptane and octane. These differences can be explained by variations in hydroperoxides formed from linoleate (9- and 13-OOH), compared to oleate (8-, 9-, 10- and 11-OOH) (Frankel, 1985). The differences in incubation times and total volatiles scales between Figs 5 and 6 indicate a greater production of volatile compounds in the linoleate emulsions. Figure 5 shows a similar trend as the hexanal yields discussed above (Table 1), namely a small decline in total volatile compounds at 2·4% might represent an effect of α-tocopherol. **HO** and **TR** contain respectively 79% and 99% oleate, which forms several hydrocarbons by autoxidation (Frankel, 1985).

CONCLUSION

Enzymic oxidation of linoleic acid proceeded much faster in the presence of very low concentrations of oil (< 1%) than higher concentrations (> 2%) of oil in emulsions. Incubations at 40°C caused cooxidation of

emulsions containing unsaturated triglycerides. Natural and added α-tocopherol in emulsified oil diminished the formation of volatile lipid oxidation products. However, the added antioxidant became too diluted to be effective at the low (0·6%) concentration of corn oil emulsion.

These results have practical consequences for the manufacturers of low-fat foods using raw materials with reduced fat content and thus less natural antioxidant. Similar problems arise from the addition of antioxidants, the amounts allowed of which are related to the fat content of the product.

Once the volatile lipid oxidation products have been formed, they are mostly accumulated in the lipid phase of the product. Therefore, more volatile compounds will be released from products with reduced fat contents, because of decreased fat volume as well as fat composition changes due to partial extraction of lipid components.

The results of this study have important applications in the formulation of foods containing varying amounts of different types of fats and natural tocopherols as antioxidants. Low fat foods would contribute less stabilizing antioxidants and less volatile flavour compounds would be retained. On the other hand, increasing the fat contents of food emulsions would contribute more stabilizing antioxidants and more volatile flavour compounds would be retained in the fat phase.

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