SHORT COMMUNICATION

Effect of Temperature on Linolenic Acid Loss and 18:3 Δ9-*cis*, Δ12-*cis*, Δ15-*trans* Formation in Soybean Oil

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Oil was extracted from soybeans, degummed, alkali-refined and bleached. The oil was heated at 160, 180, 200, 220 and 240°C for up to 156 h. Fatty acid methyl esters were prepared by boron trifluoride-catalyzed transesterification. Gas–liquid chromatography with a cyanopropyl CPSil88 column was used to separate and quantitate fatty acid methyl esters. Fatty acids were identified by comparison of retention times with standards and were calculated as area % and mg/g oil based on 17:0 internal standard. The rates of 18:3ω3 loss and 18:3 Δ9-*cis*, Δ12-*cis*, Δ15-*trans* (18:3*c,c,t*) formation were determined, and the activation energies were calculated from Arrhenius plots. Freshly prepared soy oil had 10.1% 18:3ω3 and no detectable 18:3*c,c,t*. Loss of 18:3ω3 followed apparent first-order kinetics. The first-order rate constants ranged from .0018 ± .00014 min^{-1} at 160°C to .083 ± .0033 min^{-1} at 240°C. The formation of 18:3*c,c,t* did not follow simple kinetics, and initial rates were estimated. The initial rates (mg per g oil per h) of 18:3*c,c,t* formation ranged from 0.0031 ± 0.0006 at 160°C to 2.4 ± .24 at 240°C. The Arrhenius activation energy for 18:3ω3 loss was 82.1 ± 7.2 kJ mol^{-1}. The apparent Arrhenius activation energy for 18:3*c,c,t* formation was 146.0 ± 13.0 kJ mol^{-1}. The results indicate that small differences in heating temperature can have a profound affect on 18:3*c,c,t* formation. Selection of appropriate deodorization conditions could limit the amount of 18:3*c,c,t* produced.

KEY WORDS: 18:3*c,c,t*, *trans* fatty acid, linolenic acid, thermodynamics.

Trans geometrical isomers of 18:3ω3 and 18:2ω6 are formed during heating of vegetable oils (1). The isomers of linolenic acid have been identified as 18:3 Δ9-*cis*, Δ12-*cis*, Δ15-*trans*, 18:3 Δ9-*trans*, Δ12-*cis*, Δ15-*cis*, 18:3 Δ9-*trans*, Δ12-*cis*, Δ15-*trans* and minor amounts of other isomers (1–3). The isomers have been reported in vegetable oils (1–3), margarine (4–7), infant formulas (8), frying oils (9) and in partially hydrogenated soybean oil (10). One of the linolenic acid isomers, 18:3 Δ9-*cis*, Δ12-*cis*, Δ15-*trans* (18:3*c,c,t*), is elongated and desaturated to omega-3 *trans* isomers of 20:5ω3 and 22:6ω3 in rats (11,12). The 20:5ω3 *trans* isomer Δ17-*trans* [eicosapentaenoic acid (EPA)] is significantly less effective than 20:5ω3 in inhibiting arachidonate-induced aggregation in human platelets (13).

Little information is available on the factors affecting 18:3*c,c,t* formation in heated oils. Laboratory-heated rapeseed oil developed noticeable levels of 18:3*c,c,t* within one hour at 230°C (1). The 18:3*c,c,t* level in soybean and rapeseed oils were initially higher after heating at 240°C compared to 200°C (14). However, the 18:3*c,c,t* and other mono-*trans* isomer levels were lower after 20 h compared to 10-h heating at 240°C (2,14). This indicates that 18:3*c,c,t* is formed during heating, then is involved in further reactions, perhaps to di- or tri-*trans* isomers, oxidized or cyclic products (3). The purpose of this work was to measure the rates of 18:3*c,c,t* formation and 18:3ω3 loss in heated soybean oil and to calculate their Arrhenius activation energies.

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EXPERIMENTAL PROCEDURES

Oil extraction and processing. Whole soybeans were obtained from a local store. Oil was extracted by blending 500 g soybeans with dichloromethane (DCM) at a ratio (wt/vol) of 1:3 for 4 min in a Waring blender under a blanket of nitrogen. The blended soybeans were filtered through Whatman No. 1 paper (Maidstone, England), and the cake was extracted again with an identical volume of DCM. The combined filtrates were evaporated in a rotary evaporator with slight (40°C) heating under vacuum. The free fatty acid content was determined by IUPAC Method No. 2.201 (15). Hexane (500 mL) was added, and the oil was dissolved. The oil-hexane micella was alkali-treated (10% excess) with 0.8N NaOH and then washed with water until neutral. After separation of the final water wash, the micella was treated with 30 g anhydrous sodium sulfate for 15 min. The sodium sulfate was removed by filtration (Whatman No. 1), silica adsorbent (10 g chromatographic-grade silica gel, 100–200 mesh; Fisher Scientific, Cincinnati, OH) was added, and the mixture was stirred for 2 h at room temperature. The silica was removed by filtration through Whatman No. 1 filter paper, and the hexane was removed by rotary evaporation. Traces of hexane were removed by slightly heating the oil while applying a vacuum (50 mm Hg) for 5 min while the oil was placed in an ultrawsonic bath. The oil was stored under nitrogen and protected from light at −20°C until used.

Heat treatment. The oil was placed in duplicate 10-mL test tubes that were cleaned with 50% (w/v) KOH in methanol, rinsed repeatedly with distilled water and oven-dried. An oil bath consisted of commercial hydrogenated vegetable shortening that was heated in a beaker on a laboratory heating plate. The oil bath was stirred, brought to temperature and equilibrated overnight. A mercury thermometer was used to monitor temperature. In this equipment, the temperature typically varied less than 1°C. The temperatures used were 160, 180, 200, 220 and 244°C. Maintenance of constant temperature at 244°C was difficult, and variations of up to 2°C were observed.

Oil samples were removed at 2-h intervals for up to 156 h. Oil was placed in plastic sample storage vials and stored at −20°C until analysis. Analysis was typically completed within 1 wk of heating.

Fatty acid analysis. A gas chromatography (GC)-14A gas chromatograph was used equipped with split/splitless injector, flame-ionization detector, Model CR5A integrator-plotter and autosampler (Shimadzu Scientific Instruments, Norcross, GA). A CPSil88 capillary column (50 m × 0.25 mm, i.d., 0.20 μm film) was used with helium carrier gas at a linear flow velocity of 25 cm/min. Column temperature was maintained at 185°C, and detector/injector temperatures were 250°C. The split ratio was 40:1.

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Fatty acid methyl esters were prepared by boron trifluoride-catalyzed transesterification (16). An internal standard (17:0 methyl ester; Nu-Chek-Prep, Elysian, MN) solution was prepared in benzene at a concentration of 2.5 mg/mL. About 55 mg heated oil was weighed into a Teflon-lined screw-capped test tube, 2 mL internal standard solution and 2 mL 6% BF_3 in methanol (Supelco, Bellefonte, PA) were added. The tube was flushed with nitrogen, capped and placed on a boiling waterbath for 60 min. After cooling, 2 mL water and 2 mL hexane were added, the tubes were vortexed for 30 s, centrifuged at low speed for 3 min and the top organic layer was placed into autosampler vials for analysis.

Fatty acids were identified by comparison of equivalent chainlengths with elaidinized linseed oil as described elsewhere (17). Fatty acids were calculated as area percent and mg/g oil.

Statistics. Linear regression was calculated with the analysis package of the Microsoft Excel 4.0 spreadsheet running on an IBM-compatible personal computer.

RESULTS AND DISCUSSION

GC analysis indicated that isomers of 18:2ω6 or 18:3ω3 were not detectable in the freshly extracted and laboratory-processed oils. The 18:3ω3 content decreased with heating time and temperature (Fig. 1). The kinetics of the reaction, determined by the method of half lives (18), was first-order. The possibility of pseudo first-order kinetics was not investigated, but given the complexity of the reactions possible for 18:3ω3 loss, it is overly simplistic to assume that the order is truly first-order without additional experimentation. The first-order kinetic constants for 18:3ω3 loss were calculated from log concentration/time plots. The rate constants ranged from .0018 min^{-1} at 160°C to .083 min^{-1} g^{-1} at 240°C (Table 1).

The change in 18:3*c,c,t* level with time in heated soybean oil is shown in Figure 2, and the initial rates of formation are presented in Table 2. The rate of 18:3*c,c,t* formation increased with temperature but followed complex kinetics. A maximum amount of 18:3*c,c,t*, 2.4% or 20.2 mg/g, was formed after 12 h at 240°C. This agrees with previous work (2,14). At 220°C, the time for maximal formation was

TABLE 1

Apparent First-Order Rate Constants for 18:3ω3 Loss in Soybean Oil

Temperature (°C)	k (min^{-1})
160	.0018 ± .0001
180	.0088 ± .0006
200	.013 ± .0002
220	.039 ± .001
240	.083 ± .003

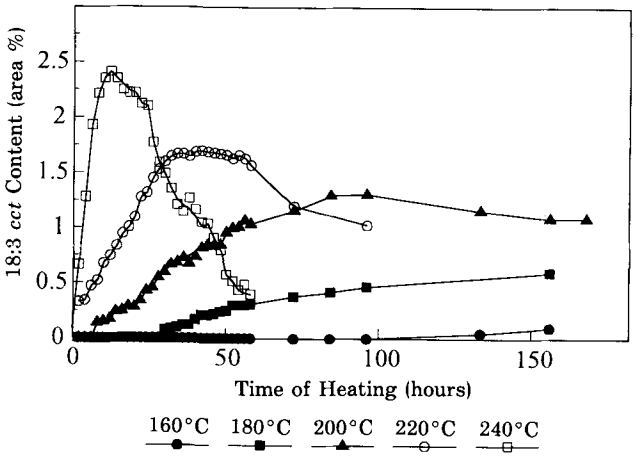

FIG. 2. Effect of temperature on 18:3*c,c,t* formation in soybean oil.

TABLE 2

Apparent Initial Rate of 18:3*c,c,t* Formation in Soybean Oil

Temperature (°C)	Rate[a]
160	0.0031 ± 0.0006
180	0.053 ± 0.003
200	0.15 ± 0.01
220	0.58 ± 0.03
240	2.4 ± 0.2

[a]mg h^{-1} g^{-1} (mean ± SE).

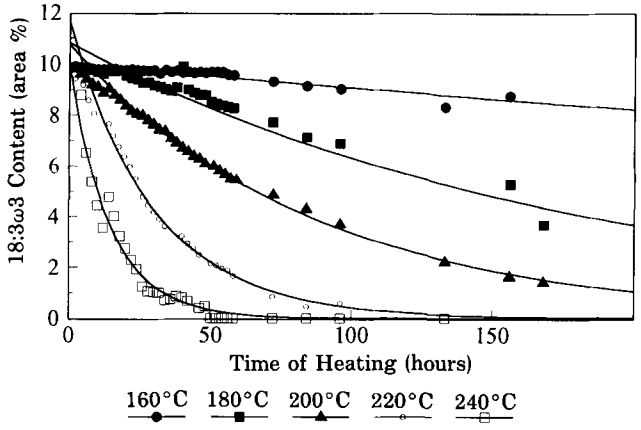

FIG. 1. Effect of temperature on 18:3ω3 loss in soybean oil.

46 h, but the level was only 1.7% (12.9 mg/g). Apparently, as heating temperature increases, a higher level of 18:3*c,c,t* is possible. This trend was also seen at 200°C, but for lower temperatures a maximum was not apparent. Because of the complex behavior observed, the linear portion of the 18:3*c,c,t* plots were used to estimate rates. The 18:3*c,c,t* formation rates were between 0.003 mg min^{-1} g^{-1} at 160°C and 2.4 mg min^{-1} g^{-1} at 240°C.

The Arrhenius plot is shown in Figure 3. The apparent activation energies (mean ± SEM) for 18:3ω3 loss and 18:3*c,c,t* formation were 82.1 ± 7.2 kJ mol^{-1} (20.3 ± 1.8 kcal mol^{-1}) and 146.0 ± 13.0 kJ mol^{-1} (34.9 ± 3.1 kcal mol^{-1}), respectively. These are in agreement with activation energies for other chemical reactions in food systems (19). The activation energies observed in this study can only be considered apparent due to the complexity of the

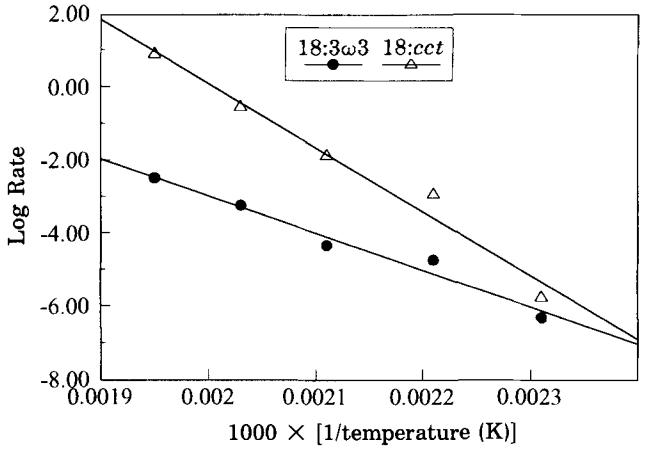

FIG. 3. Arrhenius plot of 18:3ω3 and 18:3*c,c,t* changes in soybean oil.

chemical reactions. Formation of 18:3*c,c,t* may compete with further isomerization to di- or tri-*trans* compounds, oxidation, cyclization or other reactions that would likely have different temperature dependencies (3). However, from the apparent activation energies, it is clear that 18:3*c,c,t* formation is affected more by temperature than is 18:3ω3 loss. The 18:3*c,c,t* formation accounted for between 3 and 22% of the total 18:3ω3 loss; the relative amount of *c,c,t* increased with temperature. This illustrates the greater dependence of 18:3*c,c,t* formation on temperature than 18:3ω3 loss.

Continuous deodorization of soybean oil is typically carried out at a temperature range of 204–275°C (20). Levels of 18:3*c,c,t* reported in commercially processed soybean and canola oils have recently been reported to range from 0.08 to 1.37% of the total fatty acids (3). The variation in 18:3*c,c,t* levels reported in different samples (1,3,8,9) is most likely due to differences in the deodorization time-temperature treatments of the oils. Processors could limit 18:3*c,c,t* formation by selecting longer time–lower temperature deodorization.

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