Food Chemistry **36** (1990) 295–304

Investigation by Fractional Crystallization of Classes of Compounds Formed During Frying of Vegetable Oils

M. Tasioula-Margari,[a] M. Komaitis[b] & M. G. Kontominas[a]*

[a] Laboratory of Food Chemistry and Technology, Department of Chemistry,
University of Ioannina, Ioannina 45332, Greece
[b] Laboratory of Food Chemistry, Department of Food Science,
Agricultural College of Athens, Athens 14123, Greece

(Received 19 December 1988; revised version received and accepted 3 August 1989)

ABSTRACT

In an effort to standardize a rapid and reliable method for the characterization of thermally abused vegetable oils, the technique of fractional crystallization was applied to heated samples of cottonseed and corn oil. Using this method an insoluble fraction enriched mainly in polar compounds, which form during the heating process, was obtained. These compounds, mostly of high molecular weight, are responsible for the increase in viscosity of the oil and are retained in a gas chromatographic column used for fatty acid methyl ester analysis. It was found that the above insoluble fraction formed when heated samples contained 25% polar constituents, a value which is widely accepted as the upper limit for use of heated vegetable oils.

It is proposed that the technique of fractional crystallization can be used as a method for rapid quality assessment of frying oils such as non-hydrogenated vegetable oils.

INTRODUCTION

Frying oils, used in the preparation of a variety of fried foods, are repeatedly exposed to elevated temperatures in the presence of air and moisture. Under these extreme processing conditions they undergo a series of chemical reactions, the majority of which are: oxidation, hydrolysis, thermal decomposition, polymerization and cyclization (Fritsch, 1981; Stevenson *et al.*, 1984).

---

* To whom correspondence should be addressed.

Food Chemistry 0308-8146/90/$03·50

According to recent investigations on laboratory animals fed with diets containing the products of such reactions, it was found that test animals exhibited growth retardation, increase in liver and kidney weight, etc. (Billek, 1979). These findings suggest that thermally abused vegetable oils may affect human health (Alexander, 1981; Frankel & Smith, 1984).

Methods currently being used for quality assessment of frying oils include: (a) physical methods (viscosity, smoke point, colour, refractive index, etc.), (b) chemical methods (free fatty acids, non-urea adducting fatty acids, insoluble oxidized fatty acids, total polar fraction) and (c) physical–chemical methods (GPC, HPLC, GC dimer acids, etc.) (Stevenson *et al.*, 1984).

Most of the methods mentioned require a base value for the unheated oil sample in order to make any reasonable estimation of the extent of decomposition. Other methods such as polar compounds (PC) (Walting & Wessels, 1981), GC dimers (Paradis & Nawar, 1981) GPC, etc. are based on compounds formed during thermal decomposition and have proved to be very useful in oil quality assessment.

Of all products formed during heating, the higher molecular weight compounds can be used as reliable indicators of frying oil abuse due to their low volatility and steady accumulation during the heating process.

Methods like GPC or HPLC, which determine polymeric triglycerides, are expensive in terms of equipment used and often time-consuming (Billek & Guhr, 1978). Viscosity measurement is rapid and simple, requires, however, a measurement on the initial unheated sample and does not provide information on the nature of the decomposition products formed during heating.

In the present work the technique of fractional crystallization was applied to heated samples of cottonseed and corn oil in an effort to isolate classes of altered compounds, i.e. dimers, polymers, etc., produced during the heating process. A comparison was also made between the above samples regarding: polar compounds, elution materials, dimers by GC and viscosity of oil and oil solutions.

MATERIALS AND METHODS

Cottonseed and corn oils, packed in metal containers, were purchased locally. Samples were heated to 180 ± 10°C for a total of 96 h with sequential heating and cooling cycles every 24 h and were subsequently stored at −20°C until analysis. Fractional crystallization was carried out as follows:

Oil samples of 1·00 ± 0·01 g, were weighed in a test tube of known weight and 20 ml of an acetone/methanol (9:1) mixture were added to the sample.

The test tube was sealed, the contents thoroughly mixed and placed in a freezer at 0°C. After a period of 24 h, the insoluble fraction formed was separated by filtration (1M filter) inside the freezer chamber. Under these conditions, the saturated triglycerides (SSA) precipitated (white precipitate I) and were separated from the decomposed triglycerides (yellow precipitate II) by sequential elutions with cooled acetone (−20°C) (Synouri-Vrettakou *et al.*, 1984; Tasioula-Margari & Kontominas, 1987).

Interesterification was carried out using the Morrison method (Morrison & Smith, 1964) modified as follows: a small amount of oil sample was weighed in a screw-cap test tube. To that, an appropriate volume of interesterification reagent (25% BF_3/20% hexane/55% methanol) was added so that 1 ml of the above reagent corresponded to 5 mg of oil. The test tube was then sealed and heated in a steam bath at 90°C for 45 min.

Dimer determination by GC

Dimers were determined using the method of Paradis and Nawar (1981). The analysis was carried out on a VARIAN 3700 GC equipped with a dual flame ionization detector. The column used was a 3% OV-1 made of stainless steel of dimensions 50 cm × 0·32 cm. Nitrogen was used at a flow rate of 30 ml/min. $T_{\text{inj}} = 340°\text{C}$, $T_{\text{det}} = 340°\text{C}$. Temperature program used included: $T_{\text{init}} = 220°\text{C}$, $T_{\text{final}} = 325°\text{C}$; rate = 15°C/min. The column was held at the plateau temperature of 325°C for 5 min. Interesterification was carried out as described in the previous paragraph.

Elution materials (EM)

Elution was tested on a column containing DEGS 15% on chromosorb W, 80–100 Mesh, according to the AOAC method (AOAC, 1980). $T_{\text{inj}} = 250°\text{C}$, $T_{\text{det}} = 250°\text{C}$, carrier gas: nitrogen; flow rate: 30 ml/min. Trimyristin was used as the internal standard.

Polar compounds (PC)

Polar compounds were determined according to the IUPAC method (Walting & Wessels, 1981).

Viscosity in solution (relative viscosity)

A microviscometer type AVS 300 (Schott Geräte) equipped with a Ubbelohde type microcuvette ($K = 0{\cdot}009\,533$) of capillary diameter: 0·40 mm was used for viscosity measurements in solution. Benzene, analytical grade, was used as the solvent. Temperature was maintained at 35°C ± 0·01°C using a GT 1150 type thermostat (Schott Geräte) combined with a CK 100 type cooler (Schott Geräte). The concentration of solutions examined was 10 g/litre (Tasioula-Margari & Demetropoulos, 1986).

Viscosity (kinematic viscosity)

Viscosity of the oil samples was measured at 37°C using the above viscometer.

RESULTS AND DISCUSSION

Changes in quality parameters of cottonseed and corn oils as a function of heating time are given in Tables 1 and 2, respectively.

Data show that, under similar conditions of treatment, changes in polar compounds, viscosity and weight of insoluble fractions formed, as well as change in the ratio $C_{16:0}/C_{18:2}$, are significantly greater in corn oil than in cottonseed oil. Increase in polar compounds in both oils is a lot more pronounced during the initial stages of heating as compared to the later stages where a levelling-off is observed. This is attributed to the possible degradation with time of a second fatty acid belonging to an already partly altered triglyceride, rendering it more polar. This further degradation is not 'picked up' by the method since it was already registered during degradation of the first fatty acid. The opposite trend is observed for the kinematic

TABLE 1
Changes in Quality Parameters of Cottonseed Oil and Weight of Precipitates Isolated as a Function of Heating Time

Heating time (h)	*Polar compounds (%)*	*Kinematic viscosity (cst, 37°C)*	$C_{16:0}/C_{18:2}$	*% weight of insoluble fraction*[a]	
				I	*II*
0	0·00	41·45	0·40	8·5	—
24	14·01	42·64	0·43	8·1	—
48	18·51	47·08	0·45	7·1	—
72	25·01	54·02	0·47	5·5	0·8
96	31·01	63·59	0·51	6·6	3·0

[a] Cooling temperature: 0°C. Solvent system: acetone/methanol (9:1). Cooling time: 24 h. Sample wt/solvent vol: 1:20.

viscosity which shows a significant increase in triglyceride polarity during the later stages of heating as the result of polarization of more than one fatty acid in the triglycerides.

It should be noted here that, under the present crystallization conditions, both in the cottonseed and corn oils, a white particulate precipitate (insoluble fraction I) forms, which consists of the more saturated triglycerides of the type SSA (Synouri-Vrettakou *et al.*, 1984). The same precipitate forms during heating of cottonseed oil after 24 and 48 h. However, after 72 and 96 h of heating of the same sample, a second oily (yellow in colour) precipitate (insoluble fraction II) of high density, forms. The same phenomenon occurs in corn oil in which this precipitate II forms after 24 h of heating and increases in quantity with further heating.

In Tables 1 and 2, the amount of insoluble fraction II formed is correlated with polar compounds, the ratio $C_{16:0}/C_{18:2}$ and viscosity values. One can observe that the insoluble fraction II forms when the amount of polar compounds approaches 25%, which is considered the upper limit of use of frying oils. This is true for both cottonseed and corn oils. Furthermore, the amount of the insoluble fraction II increases with increasing polar compounds. In Tables 1 and 2 it is also observed that a change of 0·11 in the ratio $C_{16:0}/C_{18:2}$ for cottonseed oil corresponds to an insoluble fraction II of 3·0% while a change of 0·10 for corn oil corresponds to an insoluble fraction II of 2·5%. It is apparent that viscosity increases with increasing amount of precipitate formed. This trend is more pronounced in corn oil. It will also show up in heated cottonseed oil after 72 and 96 h if the insoluble fraction I, composed of the more saturated SSA triglycerides, is removed after washing with cooled acetone.

TABLE 2
Changes in Quality Parameters of Corn Oil and Weight of Precipitates Isolated as a Function of Heating Time

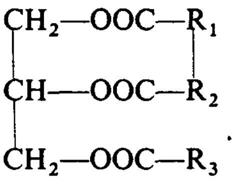
Heating time (h)	*Polar compounds (%)*	*Kinematic viscosity (cst, 37°C)*	$C_{16:0}/C_{18:2}$	*% weight of insoluble fraction*[a]	
				I	*II*
0	0·00	38·63	0·23	5·0	—
24	27·63	58·16	0·33	8·1	2·5
48	40·32	90·60	0·36	17·5	15·1
72	47·66	140·6	0·41	24·5	23·0
96	53·26	179·6	0·47	29·5	28·0

[a] Cooling temperature: 0°C. Solvent system: acetone/methanol (9:1). Cooling time: 24 h. Sample weight/solvent volume: 1:20.

Based on the above observations, analysis of both the insoluble fraction II and soluble fraction was carried out to characterize some of the classes of altered compounds which precipitate upon heating. The results of this analysis are shown in Tables 3 and 4. The amount of high molecular weight materials present in samples was measured indirectly by determination of the per cent elutable material using gas chromatography as well as by determination of the relative viscosity in solution. The amount of dimer fatty acids was determined directly by gas chromatography. An increase in viscosity values is noted in the insoluble fraction II, while viscosity values of unheated oils and soluble fractions are similar. This indicates that high molecular weight compounds, which result in viscosity increase, form and precipitate. Subsequently it is possible, using the technique of fractional crystallization under the above specified conditions, to isolate a fraction enriched in altered triglycerides which increase its viscosity, as shown by relative viscosity values.

From elution material values, it is apparent that the largest portion of triglycerides in the insoluble fraction II is retained by the GC column, while those of the soluble fraction are eluted especially during the first stages of heating. The insoluble fraction II is composed of high molecular weight compounds which result in viscosity increase and are retained by the column during GC analysis.

During the determination of dimers by GC their expected significant increase in the insoluble fraction II was not observed. This is probably due to the decomposition of most dimers of type C—O—O—C during interesterification or storage under freezing temperatures and the determination only of the remaining dimers of type C—C. The presence of large amounts of dimers in the soluble fraction (filtrate) is indicative of the presence of intramolecular bonds of the type:



$$\begin{array}{l} CH_2\text{—}OOC\text{—}R_1 \\ |\qquad\qquad\quad\;\; | \\ CH\text{——}OOC\text{—}R_2 \\ | \\ CH_2\text{—}OOC\text{—}R_3 . \end{array}$$

in large amounts.

Another very important observation is the similarity in values of fatty acids in the heated oil and in the soluble fraction, which leads to the proposition that one can use the soluble fraction instead of the heated oil sample for analysis in order to prolong the life of the GC column. This is also shown in the ratio $C_{16:0}/C_{18:2}$, values which are very similar for the heated oil and the soluble fraction.

TABLE 3
Characterization of Insoluble and Soluble Fractions in Cottonseed Oil After Heat Treatment

Heating time (*h*)	*Elution materials* (*EM*) (%)	*Dimers* (%)	*Relative viscosity* (η_r, 35°C)	*Fatty acid composition* (%)						$C_{16:0}/C_{18:2}$
				$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	
0	96·10	0	1·036	0·06	22·74	0·14	2·33	18·36	56·38	0·40
24										
unconcentrated	95·22	1·20	1·036	0·06	23·82	0·12	2·16	18·60	55·24	0·43
soluble fraction	95·01	1·20	1·037	0·01	23·36	0·10	2·97	18·64	54·53	0·42
insoluble fraction I	94·01	0·20	1·036	traces	33·12	0·10	3·96	16·86	45·47	0·72
48										
unconcentrated	92·52	—	—	—	24·46	—	2·02	19·35	54·17	0·45
soluble fraction	92·41	—	—	0·20	24·22	0·22	2·29	18·48	54·59	0·44
insoluble fraction I	90·02	—	—	0·06	33·90	0·10	3·91	17·25	44·73	0·76
72										
unconcentrated	90·74	—	—	0·12	25·07	0·15	2·10	18·89	53·23	0·47
soluble fraction	90·04	—	—	0·10	25·07	0·15	2·30	18·08	54·30	0·46
insoluble fraction II	82·83	—	—	0·12	33·85	0·17	4·04	17·61	44·21	0·77
96										
unconcentrated	85·83	2·27	1·038	0·10	26·12	0·15	2·42	20·01	51·07	0·51
soluble fraction	85·73	1·86	1·036	traces	26·70	0·09	2·70	19·92	50·51	0·52
insoluble fraction II	52·72	4·79	1·050	0·05	33·51	0·07	4·05	19·85	42·46	0·79

TABLE 4

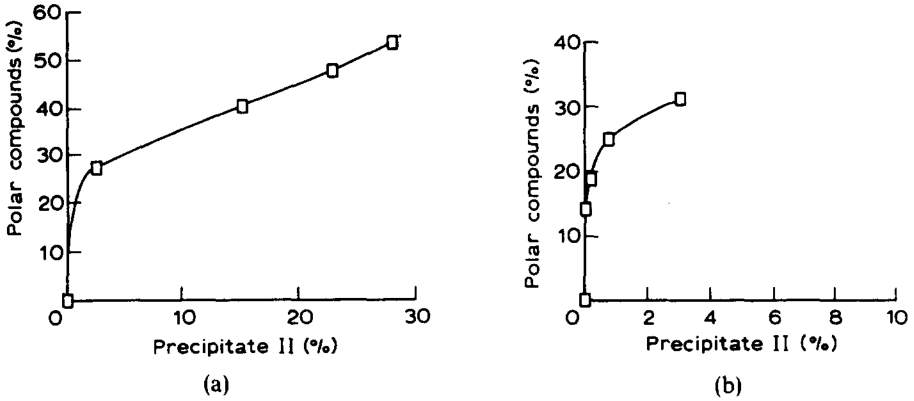
Characterization of Insoluble and Soluble Fractions in Corn Oil After Heat Treatment

Heating time (h)	*Elution materials (EM) (%)*	*Dimers (%)*	*Relative viscosity* (η_r, *35°C*)	*Fatty acid composition (%)*						$C_{16:0}/C_{18:2}$
				$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	
0	95·59	0	1·036	0·10	13·72	0·12	3·03	24·09	58·93	0·23
24										
unconcentrated	88·33	1·41	1·042	0·10	17·37	0·12	3·64	26·53	52·24	0·33
soluble fraction	84·22	1·31	1·038	0·11	17·49	0·11	3·59	26·29	52·42	0·33
insoluble fraction II	51·93	1·50	1·047	0·11	20·08	0·13	4·58	27·91	47·18	0·43
48										
unconcentrated	74·49	—[a]	—	0·11	18·53	0·11	3·80	27·09	50·36	0·37
soluble fraction	83·59	—	—	0·15	18·56	0·20	3·59	27·14	50·34	0·37
insoluble fraction II	32·47	—	—	0·12	20·07	0·10	4·04	28·35	47·53	0·42
72										
unconcentrated	68·55	—	—	0·10	19·26	0·17	3·99	28·97	47·50	0·41
soluble fraction	82·19	—	—	0·11	19·78	0·11	3·70	29·21	47·09	0·42
insoluble fraction II	30·68	—	—	0·11	21·03	0·19	4·15	30·92	43·60	0·43
96										
unconcentrated	62·97	3·48	1·050	0·10	20·73	0·13	4·17	30·52	44·35	0·47
soluble fraction	78·13	3·24	1·040	0·10	21·13	0·14	4·13	30·30	44·20	0·48
insoluble fraction II	32·93	3·55	1·079	0·11	22·53	0·12	5·39	31·03	40·81	0·55

[a] Readings were not taken.

Lastly, it should be noted that the insoluble fraction II studied in this work begins to form in cottonseed oil after 72 h of heating (0·8%) and contains 25% polar compounds while the same sample when heated for 96 h gives 3% of insoluble fraction II and contains 31% of polar compounds. In corn oil after heating for 24 h, approximately 2·5% of insoluble fraction forms, containing 27·6% polar compounds.

The amount of polar compounds which a heated oil sample may contain, while still in use, may be correlated with the formation of insoluble fraction II, which is visually detected (Fig. 1). Formation, therefore, of insoluble fraction II can be considered as the final point of use of a heated oil.



(a) (b)

Fig. 1. Correlation of the amount of polar compounds contained in a heated oil to the insoluble fraction formed. (a) Corn oil, (b) cottonseed oil.

REFERENCES

Alexander, J. C. (1981). Chemical and biological properties related to toxicity of heated fats. *J. Toxicol. Environ. Health*, 7, 125–38.

AOAC (1980). *Official Methods of Analysis*. Association of Official Analytical Chemists, Washington, DC, 28.070, p. 449.

Billek, G. (1979). Heated oils—Chemistry and nutritional aspects. *Nutr. Metab.*, **24**(1), 200–10.

Billek, G. & Guhr, G. (1978). Quality assessment of used frying fats: A comparison of four methods. *JAOCS*, **55**, 728–33.

Frankel, E. N. & Smith, E. N. (1984). Occurrence of cyclic fatty acid monomers in frying oils used for fast foods. *JAOCS*, **61**, 87–90.

Fritsch, C. W. (1981). Measurement of frying fat deterioration. *JAOCS*, **58**, 272–4.

Morrison, W. R. & Smith, L. M. (1964). Preparation of fatty acid methylesters and dimethylacetals from lipids with boron fluoride-methanol. *J. Lipid Res.*, **5**, 600–8.

Paradis, A. J. & Nawar, W. W. (1981). Gas chromatographic method for the assessment of used frying oils; Comparison with other methods. *JAOCS*, **58**, 635–8.

Stevenson, S. G., Vaisey-Genser, M. & Eskin, N. A. M. (1984). Quality control in the use of deep frying. *JAOCS*, **61**, 1102–8.

Synouri-Vrettakou, S., Komaitis, M. E. & Voudouris, E. C. (1984). Triglyceride composition of olive oil, cottonseed oil and their mixtures by low temperature crystallization and GLC, *JAOCS*, **61**, 1051–6.

Tasioula-Margari, M. & Kontominas, M. G. (1987). Unpublished data.

Tasioula-Margari, M. & Demetropoulos, J. (1986). In *Proc. 11th National Chemistry Conf.*, Greek Chemical Society, Athens, Greece, pp. 132–5.

Walting, A. C. & Wessels, H. (1981). Chromatographic separation of polar and non-polar compounds of frying fats. *J. Assoc. Off Anal. Chem.*, **64**, 1329–31.