Technical

Systematic Evaluation of Heated Fats Based on Quantitative Analytical Methods

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A simple, objective procedure for the analysis of heated or used frying fats is presented. In the proposed evaluation, based on quantitative methods, the direct analysis of the fat is combined with that of the methyl esters in order to obtain various measurements representative of the alteration proceeding from the same sample. This process permits the distinction of the three predominant types of degradation, oxidative, thermal and hydrolytic, and thus can be applied to fats of whatever level and type of alteration.

The results of application of this analytical system to palm, olive, sunflower and soybean oils both before and after heating them for 100 hr at 195 C are discussed.

At present, most edible fats are consumed after undergoing high temperatures; these fats show a broad series of chemical changes whose nutritional and toxicological importance have not yet been established. Thus, attempts to establish relationships between the fat alteration level and its physiological properties are particularly helpful in reducing present controversies.

The difficulties in finding a good definition of the fats used in the studies, in order to facilitate reproduction of the results, are demonstrated by the fact that the greater part of the nutritional and physiological works are carried out with nonheated fats, and that there are no objective criteria for discarding fats heated at high temperatures. Even the useful and accepted criterion that recommends replacing the fat when its level of polar compounds is higher than 27% cannot be considered objective, because it comes from a nonlinear correlation with the percentage of oxidized fatty acids, whose performance is not satisfactory (1). So, new possibilities should be explored to improve analysis of heated fats.

It is more and more obvious that a good definition of thermoxidized fat must be based on the quantitative analysis of the alteration products. From the qualitative point of view, this type of detailed evaluation has been the goal for a long time. The most classic processes of fractionation of the alteration compounds are based on differences in solubility (2); volatility (3-5) or differing adduction capacity with urea (6,7). Nevertheless, their replacement by chromatographic techniques has been inevitable due to the greater efficiency of separations obtained with the latter.

The analytical processes used by Artman (8) and Paulose and Chang (9) are examples of an intermediate situation where the classic processes are combined with the chromatographic while in the latest studies carried out by Ottaviani et al., on methyl esters (10), or by Gere, directly on the fat (11), the evaluation is carried out exclusively with chromatographic techniques. All these are rigorous studies whose contributions to knowledge of the principal groups of alteration compounds

TABLE 1

Chemical Characteristics of Unheated Oils

Analytical Determination		Palm oil	Olive oil	Sunflower oil	Soybean oil
FFA (% oleic acid)		0.25	0.13	0.18	0.05
PV (meq/kg)		5.7	2.2	5.0	0.5
A.O.M. (hours)		40.2	21.2	9.2	11.7
Fatty acid composition (%)	$C_{14:0}$	1.0	–	–	–
	$C_{16:0}$	38.6	10.6	7.0	11.2
	$C_{16:1}$	tr	0.9	tr	tr
	$C_{18:0}$	4.2	2.7	4.5	3.7
	$C_{18:1}$	44.5	77.0	24.6	23.1
	$C_{18:2}$	11.7	8.5	63.7	55.0
	$C_{18:3}$	tr	0.3	0.2	7.0

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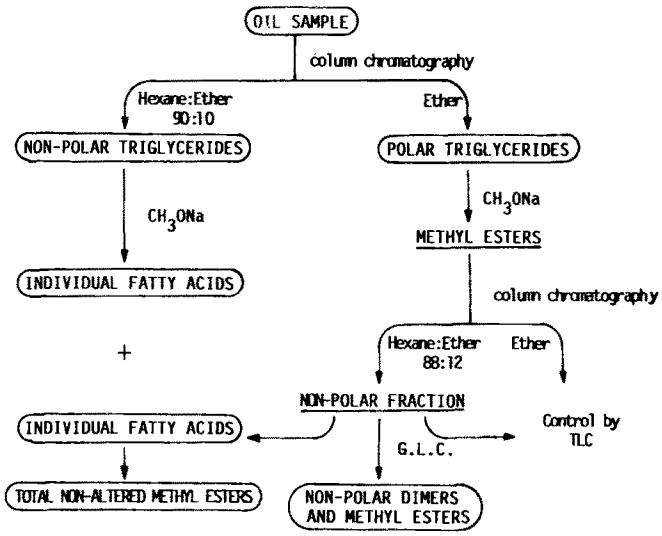

FIG. 1. Simplified scheme for the analysis of heated or used frying fats.

have been significant, although they do not give quantitative results.

It is the aim of this paper to contribute to the evaluation of the alteration level in heated fats, differentiating the three important classes of degradation implicated in the total alteration: hydrolytic, thermal and oxidative.

This study presents the results obtained by means of the application of an analytical procedure based on quantitative methods. In the proposed evaluation, the direct analysis of the fat using IUPAC polar compound determination (12) is combined with that of the methyl esters (13,14) in order to obtain various measurements representative of the alteration proceeding from the same sample.

EXPERIMENTAL

Materials. Refined palm, olive, sunflower and soybean oils were heated in a small fryer at 195 C for 100 hr in the absence of foodstuff. The samples were stored at -16 C under an atmosphere of nitrogen until needed. The chemical characteristics of the oils used are given in Table 1.

Methods. Heated and original samples were evaluated following the scheme shown in Figure 1, which permits the quantitative determination of all the parameters indicated, starting from the same sample.

This analytical process begins with a first separation of the fat sample in order to determine the percentages of nonpolar and polar triglycerides. The methyl esters were obtained from the polar triglycerides, which contain the compounds produced in the thermoxidative degradation. The methyl esters were quantitatively recovered and submitted to a second separation by silica column chromatography. The elution of the nonpolar fraction permits obtaining both the nonaltered methyl esters and nonpolar dimers, which were determined by means of gas liquid chromatography (GLC); the oxidized monomers and high molecular weight compounds were concentrated in the polar fraction. Finally, the methyl esters proceeding from the polar and nonpolar triglycerides were analyzed by GLC to determine the fatty acid composition.

Polar and nonpolar triglycerides were determined by means of silica column chromatography, following the method proposed by the IUPAC (12) with two slight modifications:

- The use of hexane:ethyl ether (90:10) for obtaining the nonpolar fraction. It has been observed that a part of the nonpolar intermolecular dimer triglycerides is eluted with hexane:ethyl ether (87:13) (13,15). In this study a sharper separation is important, because nonpolar dimers were determined in the polar fraction.
- A final elution of the sample with CH_3OH. It has been reported (1,16) that some very high polar components are not eluted from the column with ethyl ether. In these circumstances, the later use of CH_3OH improves the recovery of the sample.

The methyl esters and nonpolar dimers were determined by GLC (14) following transesterification of the polar triglyceride sample with CH_3ONa and HCl-CH_3OH. Three-tenths gram of methyl esters was eluted in a column (9 mm i.d.) containing 8 g of silica deactivated with 5% water. In a first elution with hexane:ethyl ether (88:12), a less polar fraction was obtained containing the two groups of compounds to determine, while a second elution with ethyl ether permitted recovery of the polar methyl esters. The chromatographic analysis of the nonpolar fraction was carried out in a short column (40 cm long and 1/8″ i.d.) packed with 3% SP2100 on Supelcoport 80-100 mesh using a programmed temperature of 130 to 330 C at 30 C/min without initial isotherm.

The quantitative analysis of the individual fatty acids in the nonpolar and polar triglycerides was carried out by GLC on a column 2 m long and 1/8″ i.d. packed with 15% DEGS on Supelcoport 80-100 mesh and at a temperature of 180 C. Methyl heptadecanoate was used as internal standard.

The samples were evaluated in triplicate with the aim of increasing precision in the quantitation of nonpolar dimers (14), the compounds determined with the highest error in this procedure. The standard deviation of the mean values in Table 2 for this determination ranges from 0.02 to 0.34 and corresponds to percent coefficients of variation lower than 6.7.

Alternatively, all the analytical methods described were applied, starting from separate samples of the total fat or its methyl esters, and the results compared with those obtained applying the scheme of Figure 1.

RESULTS AND DISCUSSION

Figure 2 shows a thin layer chromatogram, developed with hexane:ethyl ether:acetic acid (80:20:1), illustrating the efficiency of the principal fractionations effected following the scheme of Figure 1. The separations obtained on the column can be observed, both for the two triglyceride fractions (developments 2 and 3) and for the methyl esters proceeding from the polar triglyceride fraction (developments 5 and 6). This efficiency is

TABLE 2

Evaluation of Initial and Thermoxidized Oils, According to the Scheme of Figure 1 (wt % on fat)

Analytical Determination		Palm oil		Olive oil		Sunflower oil		Soybean oil	
		0 hr	100 hr	0 hr	100 hr	0 hr	100 hr	0 hr	100 hr
Nonpolar triglycerides		91.8	39.2	98.2	32.2	95.8	26.4	97.9	18.2
Polar triglycerides		7.8	58.4	1.7	65.8	4.0	70.8	1.9	71.3
Methyl esters of fatty acids from polar triglycerides		6.5	31.5	1.2	30.4	3.9	33.8	1.8	37.2
Methyl esters of nonpolar dimers		0.3	6.2	0.3	11.3	0.4	12.3	0.5	14.2
Methyl esters from nonpolar triglycerides	$C_{14:0}$	0.9	0.5	–	–	–	–	–	–
	$C_{16:0}$	34.8	17.6	10.4	4.8	6.7	3.1	10.4	3.2
	$C_{18:0}$	4.0	2.0	2.8	1.1	4.5	2.2	3.8	1.3
	$C_{18:1}$	40.9	16.8	76.9	24.9	23.0	10.3	23.0	7.3
	$C_{18:2}$	10.6	1.2	8.0	0.5	60.0	10.3	54.3	6.2
	$C_{18:3}$	–	–	–	–	–	–	6.4	0.2
Methyl esters from polar triglycerides	$C_{14:0}$	–	0.4	–	–	–	–	–	–
	$C_{16:0}$	2.6	17.6	0.3	5.1	0.3	3.5	0.1	5.0
	$C_{18:0}$	0.2	1.8	–	0.8	0.2	2.1	–	2.4
	$C_{18:1}$	2.7	10.5	2.0	23.5	1.0	10.9	0.2	12.1
	$C_{18:2}$	0.4	0.6	0.1	0.5	2.4	13.9	0.7	15.5
	$C_{18:3}$	–	–	–	–	–	–	–	0.4
Total methyl esters of fatty acids		97.1	69.0	100.5	61.2	98.1	56.3	98.9	53.6

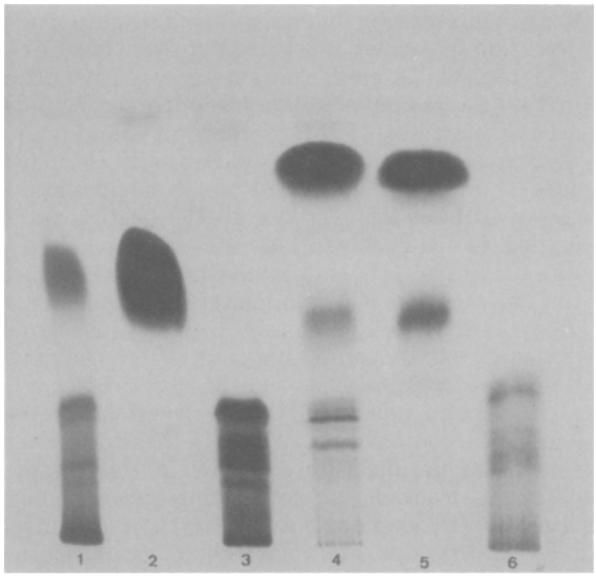

FIG. 2. Thin layer chromatography showing the efficacy of the main separations according to the procedure in Fig. 1. 1, Thermoxidized oil; 2 and 3, nonpolar triglyceridese from 1; 4, methyl esters from 3; 5 and 6, nonpolar and polar methyl esters from 4.

seen for all the samples under study, which constitutes a guarantee in the later quantifications.

It might seem that the separation of the methyl esters proceeding from the polar compounds could be eliminated without loss of information. However, the previous separation of the nonpolar fraction is very useful, as the more polar compounds are eliminated. This improves the reproducibility of the later determination by means of GLC, and avoids contamination of the column. Therefore, with the exception of the original oils, the fractionation of the methyl esters should be applied in all the thermoxidized fats, even if the amount of polar triglyceride is low, and two or three previous separations are needed.

Table 2 summarizes the results when the process was applied to samples nonheated and heated 100 hr. The great decrease in percentage of nonaltered triglycerides after heating indicates the high alteration level of the thermoxidized oils. As can be observed, this level is directly related to the initial degree of unsaturation of the oils. This first separation gives a measurement of total alteration originating in the sample, given that both the triglycerides oxidatively and thermally altered in one or more of their acyl radicals, and those which have undergone hydrolitic alteration to form free fatty acids, monoglycerides and diglycerides of greater polarity, have disappeared from the fraction of nonaltered triglycerides initially present.

Another measurement of great interest, representative of the alteration, is the total percentage of nonaltered methyl esters which can be obtained by addition of all the acids quantified individually, as seen in the final line of the table, or alternatively by addition of

TABLE 3

Evaluation of Initial and Thermoxidized Oils, Using Independent Samples (wt % on fat)

Analytical Determination		Palm oil		Olive oil		Sunflower oil		Soybean oil	
		0 hr	100 hr	0 hr	100 hr	0 hr	100 hr	0 hr	100 hr
Nonpolar triglycerides		91.8	39.2	98.2	32.2	95.8	26.4	97.9	18.2
Polar triglycerides		7.8	58.4	1.7	65.8	4.0	70.8	1.9	71.3
Nonpolar methyl esters		98.1	70.1	98.7	64.6	97.5	61.8	98.5	56.2
Polar methyl esters		1.9	28.5	1.5	33.3	2.2	36.3	1.3	39.8
Methyl ester of fatty acids		100.6	74.0	97.4	64.1	101.0	60.7	94.7	55.1
Methyl ester of nonpolar dimers		<1	6.1	<1	11.9	<1	12.7	<1	14.5
Fatty acid composition	$C_{14:0}$	1.0	1.0	–	–	–	–	–	–
	$C_{16:0}$	38.7	38.0	10.5	10.0	6.8	7.1	11.1	10.7
	$C_{18:0}$	4.2	4.2	2.6	2.7	4.4	4.5	3.7	3.6
	$C_{18:1}$	44.6	27.3	76.5	47.6	24.0	21.0	22.8	20.0
	$C_{18:2}$	11.7	1.4	8.4	0.7	62.0	25.7	54.4	20.9
	$C_{18:3}$	–	–	–	–	–	–	6.9	0.8
Total methyl esters of fatty acids		100.2	71.9	98.0	61.0	97.2	58.3	98.9	56.0

the percentages corresponding to the nonpolar triglycerides and methyl esters of fatty acids present in the polar triglycerides. This measurement evaluates the acyl radicals which have undergone oxidative or thermal degradation, as the hydrolytic alteration products form part of the nonaltered methyl esters after transesterification.

The percentage value of nonpolar dimers indicates specifically the level of thermal alteration of the oils, because it has been proved that these compounds originate in significant quantities only when the fat has undergone high temperature (14). The results obtained demonstrate equally the importance of fat unsaturation in the first step of the polymerization process.

Starting from the individual quantities of the different fatty acids in both triglyceride fractions, the real loss of all unsaturated acids can be deduced, being greater with an increase in the number of double bonds and the quantity of the sample. At the same time, this will confirm that there is no appreciable variation in the saturated acids.

The advantages of the proposed process compared with the independent application of the analytical methods to the total samples, whose results are detailed in Table 3, are summarized in the following points:

(i) The sensitivity in the determination of nonpolar dimers is increased; this permits their quantification in original samples. The fundamental difference is the distinct ratio of nonaltered methyl esters/dimers used in both determinations. In little-altered fats, this ratio is so high that when the total sample is used the dimer determination is difficult below 1%. In the conditions of the scheme, which uses only the polar triglycerides, the methyl esters/dimers ratio is made independent of the degradation level, and depends only on the type of alteration predominant, increasing by more than 10 times the sensitivity of the method.

(ii) The percentage of nonaltered methyl esters present in the polar triglycerides permits the deduction of the existence of significant hydrolytic alteration even if the free acid value is low, due to the loss of fatty acids during heating. Given that polar triglyceride molecules must have at least one of their acyl radicals altered, the existence of nonaltered methyl esters above 66% of the polar compounds is a clear indicator of the presence of mono- and diglycerides. This situation is particularly clear in the case of original palm oil; although not detected in the rest of the samples due to the heating process used, it is observable in fats used in frying.

(iii) The analytical process permits knowing separately the composition of the nonaltered and altered triglycerides. If the percentage compositions are obtained for all the samples, starting from the quantities given in Table 2, it can be observed that the nonpolar and polar triglyceride fractions are very similar in composition, which is, bearing in mind the losses of unsaturated acids in the polar triglyceride fraction, an interesting proof that the alteration takes place not only in the more unsaturated acyl groups, but also in the more unsaturated triglycerides.

(iv) This procedure brings about the concentration of the oxidized monomers, polar dimers and high molecular weight compounds, which can later be evaluated by means of liquid chromatography without the interference of less polar compounds which are in the majority. This last determination is at the moment being perfected, and its results will be communicated shortly.

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