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Results of a Collaborative Study on

DETERMINATION OF POLAR COMPOUNDS IN FRYING FATS

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RESULTS OF A COLLABORATIVE STUDY ON DETERMINATION OF POLAR COMPOUNDS IN FRYING FATS

Abstract - Results are reported of a collaborative study on the determination of polar compounds in frying fats. Polar compounds are determined to assess the deterioration of used frying fats. The essential features of the method are presented. On the basis of the results received the Commission decided to adopt the method.

INTRODUCTION

The increasing consumption of fried food products calls for the development of criteria for the evaluation of palatability of used frying fats. Health authorities in many countries are concerned with the problem.

During deep fat frying various chemical processes such as oxidation, hydrolysis, polymerization, cyclization and cracking can take place and fats decompose with the formation of volatile, monomeric, and polymeric compounds. Their amount and chemical structure depend on the fat blend, the temperature, the frying time, the frying food and to a high degree on the accessibility of oxygen.

Thermal degradation leads to thermal dimeric triglycerides, polymers and cyclic acids. Oxidative degradation may produce oxidized triglycerides containing hydroperoxide-, epoxy-, hydroxy- and keto-groups and also dimeric fatty acids or dimeric triglycerides linked by one or more oxygen atoms. Volatile degradation products are saturated and monounsaturated, hydroxy-, aldehydic-, keto- and dicarboxylic-acids; hydrocarbons; alcohols; aldehydes; ketones; and aromatic compounds. Hydrolytic degradation processes yield diglycerides and free fatty acids.

Traditional standard methods used for the analysis of frying fats include: determination of peroxide value; benzidine value; petroleum ether insoluble oxidized fatty acids; acid value; smoke point; uv-absorbance; refractive index; iodine value; viscosity; colour; and fatty acid composition. For the evaluation of the quality of used frying fats the individual criteria however are of limited significance.

Generally, the oxidized compounds are regarded as an important criterion. It is well known that using the method for the determination of petroleum ether insoluble oxidized fatty acids, the major part of the oxidized fatty acids (which is soluble in petroleum ether) is not detected. So chromatographic procedures have been developed which enable a more complete determination of the oxidized compounds. They include TLC, GLC, column chromatography and adsorption liquid chromatography. Dimeric and higher polymeric triglycerides can be determined by gel permeation chromatography.

After considering methods available for the analysis of heated fats the Working Group decided to test collaboratively three methods:

1. Column chromatographic procedure by R. Guillaumin (ref. 1) modified by M. Naudet. By this method thermooxidatively altered fatty acids in heated fats and oils are detected. They are defined as "espèces chimiques nouvelles (E.C.N.)" and comprise dimeric and oligomeric products of thermal origin and oxymonomers and oxyoligomers resulting from oxidation of the oil. Alumina (Al_2O_3) containing 20% water is used as adsorbent. The methyl esters of the sample are separated in two fractions containing unaltered and thermooxidatively altered fatty acids. They are determined gravimetrically.

2. GLC method by A. E. Waltking (ref. 2). By this procedure the degree of degradation of heated fats and oils is estimated by measuring the material not eluted in the GLC separation of the fatty acid methyl esters. The material retained on the GLC column is composed of polymers, oxidation products and the unsaponifiable matter of the sample. The procedure uses triheptadecanoin as internal standard which is inter-esterified together with the sample. The non-elution materials retained on the column are calculated from the peak areas of the internal standard and the total area of the chromatogram.

3. Column chromatographic method as proposed by A. K. Sen Gupta and G. Guhr, published by G. Guhr and J. Waibel (ref. 3). A similar procedure has been published by Ch. Gertz (ref.4). A procedure based on both these methods has recently been standardized by the Deutsche

Gesellschaft für Fettwissenschaft (DGF) (ref. 5). Frying Fats are separated by this procedure into non-polar and polar compounds using silicagel as adsorbent. Non-polar compounds are mostly unaltered triglycerides, polar compounds include polar transformation products formed during frying or heating and polar substances such as monoglycerides, diglycerides and free fatty acids which also occur in unused fats. The efficiency of the fractionation can be assessed by thin layer chromatography.

COLLABORATIVE STUDIES

The method by R. Guillaumin and M. Naudet was tested in two collaborative studies with the participation of 9 and 14 laboratories respectively. In the second collaborative study a slightly modified procedure was used. The method was generally considered to be time consuming. Because of sophisticated experimental procedures the method seemed to be more suitable for specific investigations.

The GLC procedure of A. E. Waltking was tested in one collaborative study in which 11 laboratories participated. The amount of non-elution material which was being determined seemed to be influenced by the kind of stationary phase and other parameters used in the preparation of methyl esters and in the GLC analysis. The results obtained had a high degree of deviation and this could be due to the circumstances stated above.

The column chromatographic method used by A. K. Sen Gupta and G. Guhr which gave good results was considered to be the most suitable. The collaborative study of this procedure is discussed below in detail.

Four samples were analysed by 16 laboratories from 12 countries; each sample was analysed in duplicate. Sample I was a mixture of soya bean oil and palm oil used for the production of bacon pops (frying temperature 195°C). Sample II was a mixture of soya bean oil and palm oil used for the production of potato chips (frying temperature 160-190°C). Sample III contained hardened palm oil used for the production of french fried potato chips in a continuous fryer (frying temperature 175-190°C). Sample IV was a moderately hydrogenated soya bean oil used for preparing french fried potato chips.

The draft of the method used in the collaborative study provided for the elution of the polar and the non-polar fraction through silicagel 60, particle size 0.063 - 0.2 mm (E. Merck no. 7734).

RESULTS AND DISCUSSION

Results are presented in Tables 1 and 2. Some laboratories used other types of silicagel or they used modifications of the procedure. Their results are given in Tables 1 and 2 under "Modified Procedures". The results in Table 1 were obtained when the polar compounds were determined by eluting the polar fraction. Table 2 contains data on the recovery of the sample after eluting the polar and the non-polar fraction. Table 3 gives a statistical evaluation of results based on duplicate determinations. Results received from laboratories using modified procedures were not included in the statistical evaluation.

Most laboratories using silicagel 60 (E. Merck no. 7734) received good results. When "modified procedures" were applied the laboratories 11 and 12 using silicagel from Macherey & Nagel and from G. T. Baker also obtained satisfactory results for most samples. Deviating results were obtained when A. I. Flosil (Applied Science), Florisil (a magnesium silicate) and Silicagel LS (a Czechoslovakian product) were used.

Since the results may be influenced by the type of silicagel used and since a method to check the activity of silicagel for the purpose required is not available at present, the Commission decided to define the adsorbent as "silicagel, particle size 0.063 - 0.200 mm (70-230 mesh), Merck no. 7734, or equivalent, adjusted to a water content of 5% (m/m)".

Sample IV was analysed simultaneously as Sample B in a collaborative study carried out by the DGF (ref. 5). Each of the participating 14 laboratories performed 5 determinations on the sample. The results — mean value 25.8%, reproducibility 2.2 — are in good agreement with the results of the IUPAC-study.

From the experience gained from the results the Commission decided to simplify the procedure. Accordingly the method now provides the determination of polar compounds by calculating the difference between the weight of the sample added to the column and that of the non-polar fraction eluted. The advantage is that only one fraction, which contains the non-polar compounds, is to be eluted. Besides this, direct determination of polar compounds had the shortcoming that in samples containing greater amounts of polar material the elution of polar

Table 1. Determination of Polar Compounds in Frying Fats (% m/m)

Laboratory	Sample I			Sample II			Sample III			Sample IV		
	1	2	Mean	1	2	Mean	1	2	Mean	1	2	Mean
1	7.5	8.7	8.1	7.9	7.1	7.5	11.7	12.3	12.0	26.8	23.4	25.1
2	7.6	7.7	7.7	7.2	7.2	7.2	11.2	11.1	11.2	25.5	25.8	25.7
3	21.2	20.4	20.8	18.5	23.6	21.1	27.2	37.9	32.6	53.0	45.9	49.5
4	8.0	7.9	8.0	7.1	7.0	7.1	11.3	11.3	11.3	25.4	25.4	25.4
5	7.9	7.8	7.9	6.8	7.3	7.1	11.1	13.0	12.1	25.3	25.8	25.6
6	8.6	8.5	8.6	7.2	8.1	7.7	12.2	11.9	12.1	27.7	27.2	27.5
7	7.9	7.8	7.9	7.7	7.9	7.8	11.5	11.3	11.4	26.0	26.1	26.1
8	8.1	7.7	7.9	7.1	6.7	6.9	11.7	11.5	11.6	26.1	25.9	26.0
9	7.4	8.2	7.8	7.5	7.5	7.5	10.9	10.4	10.7	24.5	26.4	25.5
10	8.0	8.1	8.1	7.3	7.5	7.4	11.6	11.4	11.5	25.9	25.7	25.8
Modified Procedures (see footnotes)												
11(a)	7.6	7.6	7.6	7.2	7.0	7.1	11.3	11.0	11.2	25.4	25.2	25.3
12(b)	10.0	8.3	9.2	7.0	7.1	7.1	11.3	11.4	11.4	28.0	25.5	26.8
13(c)	6.7	6.7	6.7	4.8	4.6	4.7	6.7	9.0	7.9	19.1	16.3	17.7
14(d)	4.4	4.5	4.5	5.6	5.6	5.6	8.9	9.9	9.4	19.0	17.6	18.3
15(e)	3.8	4.5	4.2	2.6	2.8	2.7	2.8	3.0	2.9	17.1	17.6	17.4
16(f)	4.9	6.0	5.5	5.2	5.6	5.4	3.1	3.6	3.4	6.4	6.7	6.6

Table 2. % Recovery

Laboratory	Sample I			Sample II			Sample III			Sample IV		
	1	2	Mean	1	2	Mean	1	2	Mean	1	2	Mean
1	107.0	110.3	108.7	110.6	110.3	110.5	99.9	100.3	100.1	99.5	99.9	99.7
2	99.5	99.6	99.6	99.9	99.8	99.9	99.9	99.8	99.9	99.5	100.1	99.8
3	97.9	96.8	97.4	98.7	97.0	97.9	98.0	98.0	98.0	95.2	96.9	96.1
4	99.5	99.4	99.5	99.8	99.2	99.5	99.9	99.3	99.6	99.1	98.8	99.0
5	100.5	99.8	100.2	100.4	100.0	100.2	99.9	100.1	100.0	99.9	99.5	99.7
6	96.6	97.9	97.3	101.1	98.7	99.9	96.7	99.7	98.2	98.5	99.6	99.1
7	99.7	99.7	99.7	100.8	100.5	100.7	99.7	98.9	99.3	98.9	99.7	99.3
8	99.5	98.3	98.9	99.5	99.5	99.5	99.9	99.8	99.9	99.7	99.0	99.4
9	99.2	103.0	101.1	97.9	99.2	98.6	103.0	103.0	103.0	94.2	97.1	95.7
10	99.4	99.7	99.6	99.6	99.9	99.8	99.8	99.5	99.7	98.4	99.0	98.7
Modified Procedures (see footnotes)												
11(a)	98.7	98.6	98.7	99.7	99.2	99.5	99.3	98.3	98.8	98.5	98.2	98.4
12(b)	97.3	98.7	98.0	103.4	99.0	101.2	99.8	99.4	99.6	97.6	98.2	97.9
13(c)	95.2	99.8	97.5	99.5	98.1	98.8	96.4	99.7	98.1	99.7	99.2	99.5
14(d)	99.8	99.7	99.8	97.7	101.0	99.4	100.5	100.3	100.4	99.7	100.0	99.9
15(e)	--	--	--	--	--	--	--	--	--	--	--	--
16(f)	--	--	--	--	--	--	--	--	--	--	--	--

Footnotes to Tables 1 and 2

(a) Silica gel from Macherey & Nagel, MN Kieselgel 60; 0.1 - 0.2 mm/70-140 mesh ASTM, für Säulenchromatographie was used.

(b) Silica gel from G. T. Baker Chemical Company USA, 60 - 200 mesh was used. After the elution with diethyl ether, a yellow band was eluted with ethanol.

(c) Acid treated Florisil/Merck, 0.12 - 0.18 mm was used.

(d) Silica gel from Applied Science, HI-Flosil 60/200 mesh Cat. No. 16134 was used.

(e) Silica gel LS/Spolana, Neratovice, CSSR was used. The non-polar fraction was eluted with 200 ml solvent, the polar fraction with 400 ml solvent.

(f) Silica gel Silpearl/wide pore, spheric particles/manufactured by Glass Works Kavalier Votice, CSSR, was used. The non-polar fraction was eluted with 300 ml solvent, the polar fraction with 500 ml solvent.

Table 3. Statistical Evaluation of Results

		Sample			
		I(a)	II(a)	III(a,b)	IV(a,c)
Number of laboratories		9	9	8	8
Number of results		18	18	16	16
Mean	$\bar{\bar{x}}$	8.0	7.3	11.5	25.9
Repeatability SD	s_r	0.36	0.33	0.23	0.52
Reproducibility SD	s_R	0.37	0.39	0.48	0.77
Repeatability	r (95)	1.02	0.94	0.66	1.47
Reproducibility	R (95)	1.04	1.09	1.35	2.17

Footnotes to Table 3

(a) Results of laboratory 3 rejected as outlier.

(b) Results of laboratory 5 not included on the basis of the Cochran test.

(c) Results of laboratory 1 not included on the basis of the Cochran test.

compounds might be incomplete due to small amounts of highly polar material (generally not more than 1-2%) which are not eluted under the conditions specified.

If the efficiency of the separation is to be assessed the polar and the non-polar fractions are required. The efficiency can be assessed by thin layer chromatography. The separation may also be controlled by checking the recovery of the sample bearing in mind that the elution of the polar fraction may be incomplete. A controlled separation should be carried out when working with a silicagel other than that specified in the procedure.

The method was considered to be short and easy to handle and to allow a good separation of the unaltered triglycerides from the polar material. Since the fats are separated without modification of their constituents there is no need to prepare methyl esters which may cause difficulties in the presence of oxidized material. Furthermore, there is a good correlation between the degree of degradation and the content of polar compounds.

The Commission decided to adopt the method. The full text of the procedure has been published in Pure and Applied Chemistry (ref. 6). The method will be included as no. 2.507 in the Standard Methods for the Analysis of Oils, Fats and Derivatives (ref. 7).

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REFERENCES

1. R. Guillaumin, Rev. Fr. Corps Gras 20, 285, 413, 457 (1973).
2. A. E. Waltking, J. Assoc. Off. Anal. Chem. 58, 898 (1975).
3. G. Guhr and J. Waibel, Fette. Seifen. Anstrichm. 80, 106 (1978).
4. Ch. Gertz, Lebensmittelchemie u. gerichtl. Chemie 31, 65 (1977).
5. G. Guhr, Ch. Gertz, J. Waibel & M. Arens, Fette. Seifen. Anstrichm. 83, 373 (1981).
6. IUPAC Commission on Oils, Fats and Derivatives, Pure Appl. Chem. 54, 242 (1982).
7. Standard Methods for the Analysis of Oils, Fats and Derivatives, 6th Edn. 1st Supplement, Part 4, Pure Appl. Chem. 54, 233-245 (1982).