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DETERMINATION OF 'HEXANE' RESIDUES IN OILS

Results of a collaborative study and the standardised method

Prepared for publication by

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Determination of 'hexane' residues in oils: results of a collaborative study and the standardised method

Abstract - The development, by collaborative study, of a standarized method for the determination of residual "hexane" in extracted oils is described. The procedure involves the desorption of hydrocarbons by heating the oil in a closed vial. The hydrocarbons content of the headspace is then determined by gas-liquid chromatography using a packed or capillary column and expressed in terms of hexane content. The determination of residual hexane by direct injection of oil into a pre-column was also studied, but was found to be less suitable for routine analysis.

INTRODUCTION

Hexane used industrially is, in reality, a mixture of volatile hydrocarbons. In oil-extraction plants the determination of this residual hexane in extracted oils is important in order to monitor the manufacturing process. It is also important to evaluate the risk in trasportation, due to potential dangers resulting from the presence in the oils (or fats) of an inflammable solvent which, with air, may form explosive mixtures.

The collaborative study on the determination of residual "hexane" in oils showed that the "direct injection" method and the "headspace" method give similar results, but the "headspace" method is more suitable for routine analysis.

Consequently the "headspace" method was adopted in 1985 by the Commission on Oils, Fats and Derivatives.

COLLABORATIVE STUDIES AND RESULTS

The "headspace" method studied was derived from the D.G.F. procedure (1). The technique consists of introducing the sample into a vial and crimp-sealing it with a septum. After heating the vial at 80°C for exactly 60 min, the residual hexane is then determined in the headspace by GLC using a packed or capillary column, using n-heptane (or cyclohexane) as an internal standard, following determination of the calibration factor.

The direct injection method studied was derived from the Prevot procedure (2). This technique involves direct introduction of the sample into a precolumn packed with silanized glass wool, separation on a packed GLC column heated at 70°C and determination of hexane by comparison with a calibration graph.

Two international collaborative studies were organized successively in 1984 and 1985. The study in 1984 involved 12 participants, using the "direct injection" method, to analyse three samples:

No. 1: Refined sunflower seed oil, the technical hexane content of which is negligible.

No. 2: Oil No. 1 + 12 ppm technical hexane.

No. 3: Crude rapeseed oil (pressed) + 100 ppm technical hexane.

The statistical evaluation of the results from this first study is given in Table I. For sample No. 1, 9 laboratories obtained results lower than 1 ppm, but the different ways in which the different laboratories expressed the results did not allow a statistical evaluation of the results.

During the collaborative study in 1984, one operator used the D.G.F. method (determination of hexane by GLC headspace) obtaining the following results:

Sample No. 1:	4 ppm
Sample No. 2:	10 ppm
Sample No. 3:	100 ppm

Since the "headspace" method is more suitable for routine analysis, the Commission decided to compare, in 1985, both methods.

The study in 1985 involved 19 participants who analysed six samples:

A. - Refined peanut oil, the technical hexane content of which is negligible.

B. - Sample A + 3.3 ppm technical hexane

C. - Sample A after storage + 13.2 ppm technical hexane

D. - Refined sunflower seed oil, the technical hexane content of which is negligible.

E. - Sample D + 100 ppm technical hexane

F. - Crude rapeseed oil (pressed) + 1000 ppm technical hexane

The results obtained during this second study are given in Table II (direct injection method) and Table III (headspace method) and the statistical evaluations carried out in accordance with ISO 5725, are given in Table IV (direct injection method and headspace method).

TABLE I "HEXANE" RESIDUES IN OILS - DIRECT INJECTION METHOD
STATISTICAL EVALUATION OF RESULTS: Collaborative study 1984

Sample	refined sunflower seed oil + 12 ppm hexane	crude rapeseed oil + 100 ppm hexane
Number of laboratories	12	12
Number of results	24	24
Number of laboratories used after elimination of outliers	10	12
Mean (mg/kg)	11.3	94.0
Standard deviation of repeatability (S_r)	1.0	5.0
Coefficient of repeatability variation (%)	8.8	5.3
Repeatability (2.83 x S_r)	2.8	14.1
Standard deviation of reproducibility (S_R)	4.2	17.5
Coefficient of reproducibility variation (%)	37.0	19.0
Reproducibility (2.83 x S_R)	12	49

TABLE II "HEXANE" RESIDUES IN OILS (expressed in mg/kg)
Collaborative study 1985 (Direct injection)

Laboratories	Sample A		B		C		D		E		F	
1	0	0	0	0	0	0	0	0	107	103	1002	1008
2	0	0	4.0	4.4	26.8	16.7	0	0	87	122	1065	1223
3	1.5	0.7	5.0	5.5	20.1	25.5	-	-	-	-	-	-
4	nd	nd	3.5	3.0	14.0	12.5	nd	nd	88	100	910	1030
5	0	0	2	2	14	16	0	0	112	123	1216	1236
6	1	0	3.5	6.5	15	16	8	7	80	85	610	625
7	< 2	2	2	4	14	20	< 2	2	95	105	1000	980
9	0	1.5	5.3	0	10		0	0	128	111	968	1050
10	6	4	11	12	27	22	-	-	-	-	-	-
16	-	-	-	-	-	-	6	1	102	95	792	832
18	0	0	1	1.2	12.2	13.4	-	-	-	-	-	-
19	0	0	0	0	10.7	5.6	-	-	-	-	-	-
Mean	< 0.9		3.6		15.2		< 1.9		102.7		971.3	

nd: not detectable

TABLE III " HEXANE" RESIDUES IN OILS expressed in mg/kg

Collaborative study 1985 (Headspace method)

Laboratories	Sample A		B		C		D		E		F	
1	0	0	1.5	0	14.3	14.2	0	0	108	101	951	1011
2	0	0	9	7	27	21	7	6	160	182	1857	1706
3	0.1	1.8	2.7	3.8	11.5	13.6	-	-	-	-	-	-
4	nd	nd	3	5	11	14	nd	nd	96	104	948	958
5	nd	nd	8	8	18	17	3	-	108	-	864	-
7	< 1	< 1	4	4	14	12	< 2	< 2	95	85	950	835
8	0	0	4	4	16	16	0	0	110	110	1040	1040
9	2.6	0	4.8	5.3	9.8	12.1	0	0	80	73	730	774
10	2	3	3	4	19	15	-	-	-	-	-	-
11	0	0	2	1	6	8	0	0	113	107	1089	1048
12	0	0	0	0	10	10	0	0	100	90	970	990
13	0	0	2.5	2.2	13	16	-	-	-	-	-	-
14	1	1	2	2	11	12	< 1	< 1	85	73	790	835
15	0	0	0.5	0.5	9.6	9.5	-	-	-	-	-	-
16	-	-	-	-	-	-	1.5	6	120	60	1332	1093
18	0	0	0	0	14.0	14.4	-	-	-	-	-	-
19	0	0	0	0	14.7	15.4	-	-	-	-	-	-
Mean	< 0.5		29		13.7		< 1.5		104		1047.7	

Laboratory 17 found it impossible to detect quantities below 10 ppm; its results on sample C were 12 ppm (conventional column) and 14 ppm (capillary column).

TABLE IV "HEXANE" RESIDUES IN OILS

STATISTICAL EVALUATION OF RESULTS (ISO 5725)

Collaborative study 1985

	Direct injection method				Headspace method			
	Sample				Sample			
	B	C	E	F	B	C	E	F
Number of laboratories	11	11	8	8	16	16	11	11
Number of results	22	21	16	16	32	32	21	21
Number of laboratories used after elimination of outliers	10	10	8	8	16	16	9	10
Number of results used after elimination of outliers	20	20	16	16	32	32	18	20
Mean (in mg/kg)	3.6	15.2	102.7	971.3	2.95	13.7	104	1048
Standard deviation for repeatability (S_r)	0.6	3.2	11.1	55.1	0.65	1.70	7.6	71.5
Coefficient of repeatability variation (%)	15.9	21.4	10.8	5.7	22.4	12.2	7.3	6.8
Repeatability (2.83 x S_r)	1.65	9.2	31.3	155.9	1.85	4.75	21.4	202.5
Standard deviation for reproducibility (S_R)	3.45	7.55	14.4	191.7	2.6	4.1	28.4	293.0
Coefficient of reproducibility variation (%)	95.4	49.9	14.0	19.7	89.2	29.9	27.3	28.0
Reproducibility (2.83 x S_R)	9.8	21.4	40.7	542.5	7.4	11.6	80.4	829.2
True level of hexane (in mg/kg)	3.3	13.2	100	1000	3.3	13.2	100	1000

TABLE V "HEXANE" RESIDUES IN OILS

STATISTICAL EVALUATION OF RESULTS (MODIFIED ISO)

Collaborative study 1985 (Headspace Method)

Sample	B	C	E	F
Number of laboratories	16	16	11	11
Number of results	32	32	22	21
Number of laboratories used after elimination of outliers (*)	24	30	18	19
Mean (mg/kg)	3.25	13.05	96.4	960.7
Coefficient of repeatability variation (%)	19.7	10.3	6.0	6.9
Repeatability (2.83 x S_r)	1.8	3.8	16.5	188.2
Coefficient of reproducibility variation (%)	65.1	23.5	14.0	14.9
Reproducibility (2.83 x S_R)	6.0	8.65	38.3	404.2

(*) Laboratories 12, 18 and 19, which did not detect hexane in sample B, were eliminated for calculation of B.

Laboratory 2, twice suspect in Cochran's test, is eliminated for calculation of B, C, E, and F.

Laboratory 5 is incorporated for calculation of B, C, E, and F.

The mean values are similar for both methods, and in good agreement with the true level of hexane in the samples. The reproducibility for the high levels of hexane is better with the "direct injection". If the statistical evaluation is not determined in strict accordance with ISO 5725, but is determined after the incorporation of laboratory 5, which has only given 1 result for samples B and C, and after elimination of laboratory 2 (twice suspect in Cochran's test), the reproducibility of the "headspace method" is the same or better (for the low levels) than the reproducibility of the "direct injection" method (cf. Table V).

The reproducibility appeared to be adequate for adoption of the method (given below), if the residual hexane amounts required to be determined are comparable to that in samples C, E and F. But if the residual hexane amounts are lower than 10 ppm, the reproducibility is poor, and the field of application of this method is, consequently, limited to oils and fats oils and fats containing not less than 10 ppm hexane.

2.607 *DETERMINATION OF 'HEXANE' RESIDUES IN FATS AND OILS*

1. SCOPE AND FIELD OF APPLICATION

This Standard describes a method for the determination of free volatile hydrocarbons, expressed generally in terms of hexane, remaining in animal and vegetable fats and oils after their extraction with hydrocarbon based solvents.

The method is suitable for the determination of quantities of hexane between 10 and 1500 mg per kg of fat or oil (10 to 1500 ppm).

2. DEFINITION

The "hexane" content is the quantity of all volatile hydrocarbons remaining in fats and oils following processing involving the use of solvents and which is determined by the method specified, and expressed in milligrams per kilogram (ppm).

3. PRINCIPLE

Desorption of volatile hydrocarbons by heating the sample at 80°C in a closed vessel after addition of an internal standard. After determination of the calibration factor, determination of the hydrocarbons in the headspace by gas chromatography using packed or capillary columns. Expression of the results as hexane.

4. APPARATUS

4.1 Septum vials, 20 ml capacity.

4.2 Septa and aluminium caps suitable for vials (4.1) together with crimping pliers. The septa must be resistant to oils and solvents (Note 1).

4.3 Tongs suitable for holding vials (4.1).

4.4 Syringe, of capacity 10 µl.

4.5 Syringe, of capacity 1 µl.

4.6 Syringe, of capacity 1000 µl, gas-tight.

4.7 Gas chromatograph with a flame ionisation detector and integrator/ recorder. If a capillary column is used, the apparatus shall have a 1/100 split injection system. (Note 2). Injector and detector regulated at 100°C. Column oven regulated at 50°C.

4.8 Packed column, made of steel or glass, 2 to 4 m in length, internal diameter 3.175 mm (1/8 inch), packed with 150-180 µm diatomaceous earth support, acid washed and silanised (Note 3), coated with squalane (10%) or any other phase permitting the chromatographic separation required, or, failing this,

4.9 Glass capillary column, approximately 30 m long and 0.3 mm internal diameter, coated with methylpolysiloxane (film thickness 0.2 µm).

4.10 Heating bath, equipped with clamps for holding septum vials, regulated thermostatically at 80°C; for continuous operation glycerol is recommended as the heating medium.

4.11 Shaking machine.

5. REAGENTS

5.1 Technical hexane, with a composition similar to that likely to have been used in processing or, failing this, n-hexane (Note 4).

5.2 n-Heptane, for analysis or, failing this, cyclohexane, for analysis (Note 5).

5.3 Carrier gas: hydrogen, or nitrogen, helium, etc., thoroughly dried and containing less than 10 mg/kg of oxygen.

5.4 Auxiliary gases: hydrogen 99.9% pure, containing no organic impurities; air, containing no organic impurities.

6. SAMPLING

It is essential that the sample be protected from loss of solvent residues.

7. PROCEDURE

7.1 Preparation of test sample

Prepare the test sample taking care to prevent loss of solvent residues.

7.2 Determination of the calibration factor

Weigh, to the nearest 0.01 g, 5 g of vegetable oil free from commercial hexane into each of 7 vials (4.1) and close with a septum and a cap (4.2) (Note 6). By means of the syringe (4.4 or 4.5) add solvent (5.1) to 6 of 7 vials (4.1) according to the following table (Note 7):

µl/5 g	0.5	1	2	4	7	10
mg/1000 g	67	134	268	536	938	1340

N.B. One vial will remain without the addition of solvent.

Shake vigorously, at room temperature, the six vials in the shaking machine (4.11) for 1 h.

Using the syringe (4.4), add through the septum exactly 5 µl of n-heptane (5.2) as internal standard (Note 5) to each of the 7 vials. Shake by hand for about 1 min. At intervals of about 15 min. (Note 8), place one vial at a time up to its neck in the heating bath (4.10).

Warm the gas-tight syringe (4.6) to 60°C and after a tempering time of exactly 60 min at 80°C, take from each vial (without removing it from the bath) 1000 µl of the headspace above the oil by means of the gas-tight syringe (4.6) and immediately inject into the gas chromatograph (4.7).

Calculate the calibration factor (F) from the chromatogram for the 5 g quantities of hexane-free meals "spiked" with solvent, according to the following formula:

$$F = \frac{C_s \cdot F_c}{(F_a - F_b - F_c) \cdot C_i}$$

where:

F_a is the total content of hydrocarbons including the content of internal standard, expressed as a percentage by unit area;

F_b is the content of solvent hydrocarbons of the oil sample without the addition of solvent (however, without the content of internal standard), expressed as a percentage by unit area;

F_c is the content of internal standard, expressed as a percentage by unit area;

C_i is the quantity of internal standard added, in mg per 1000 g. If 5 µl of heptane per 5 g of oil are added, C_i = 680 mg per 1000 g. (Note 9).

C_s is the quantity of hexane (5.1) added, in mg per 1000 g.

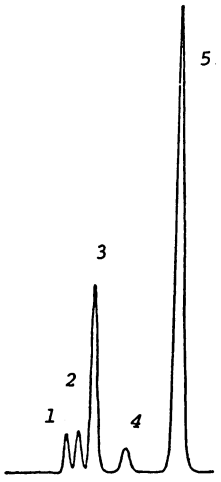
Express the results to the third decimal place.

The calibration factors of the 6 calibration samples should be approximately the same. Calculate the mean value which should be about 0.45 for heptane (Notes 10, 11, 12).

7.3 Determination of the solvent content of the sample

Weigh, to the nearest 0.01 g, 5 g of the test sample into a vial (4.1) as quickly as possible and close immediately with a septum and cap (4.2). Add 5 µl of internal standard (5.2) through the septum by means of the syringe (4.4). Shake vigorously by hand for about 1 min. and then place the vial up to its neck in the heating bath (4.10) for exactly 60 min.

Take 1000 µl from the head space by means of the gas-tight syringe (4.6), without removing the vial from the heating bath, and immediately inject into the gas chromatograph (4.7). Determine the solvent hydrocarbons content of the sample from the chromatogram (see figure 1).



1: 2-Methylpentane

2: 3-Methylpentane

3: n-Hexane

4: Methylcyclopentane

5: Cyclohexane (internal standard)
(with other retention times: n-heptane)

Figure 1 Gas chromatogram of hexane hydrocarbons from oil

7.4 Number of determinations

Carry out two determinations in rapid succession using a fresh test portion for each determination.

8. EXPRESSION OF RESULTS

The residual solvent content W, expressed in mg/kg (ppm) of the sample, is equal to:

$$W = \frac{(F_b - F_c) \cdot F \cdot C_i}{F_c}$$

where:

F_b is the total content of solvent hydrocarbons in the sample, including the content of internal standard, expressed as a percentage by unit area.

F_c is the content of internal standard in the sample, expressed as a percentage by unit area.

C_i is the quantity of internal standard added, in mg per 1000 g;

(If 5 µl of heptane per 5 g of oil are added: C_i = 680 mg per 1000 g; respectively C_i = 780 mg if cyclohexane is used.)

F is the calibration factor according to 7.2.

Report as the final result the mean of the results of the two determinations, provided the requirements for repeatability (9.1) are met. If the requirements for repeatability are not met, discard the results and carry out a further two determinations on the test sample.

9. PRECISION

The results of one interlaboratory study organized at an international level gave the statistical results shown in the **TABLE** given below.

TABLE

STATISTICAL ANALYSIS OF RESULTS FOR HEXANE IN OILS

(Duplicate determinations)

Sample**	1	2	3	4
Number of laboratories	16	16	11	11
Number of laboratories after elimination of outliers	16	16	9	10
MEAN VALUE (in ppm)	2.95	13.7	104	1048
Repeatability standard deviation (S_r)	0.65	1.70	7.6	71.5
Repeatability coefficient of variation %	22.4	12.2	7.3	6.8
REPEATABILITY VALUE r (S_r x 2.83)	1.8	4.8	21	202
Reproducibility standard deviation (S_R)	2.6	4.1	28.4	293
Reproducibility coefficient of variation %	89	30	27	28
REPRODUCIBILITY VALUE R (S_R x 2.83)	7.4	12	80	829

** Sample 1: Fresh edible peanut oil + 3.3 ppm technical hexane
Sample 2: Fresh peanut oil after storage + 13.2 ppm technical hexane
Sample 3: Crude rapeseed oil (pressed) + 100 ppm technical hexane
Sample 4: Crude rapeseed oil (pressed) + 1000 ppm technical hexane

9.1 Repeatability

When the mean of the duplicate determinations lies within the range of the mean values cited in the **TABLE** the difference between the results of the two determinations, carried out in rapid succession by the same operator, using the same apparatus for the analysis of the same test sample, should not be greater than the **repeatability value** (r), which can generally be deduced by linear interpolation from the **TABLE**.

9.2 Reproducibility

When the means of the duplicate determinations, obtained in two different laboratories using this standard method for the analysis of the same laboratory sample, lie within the range of the mean values cited in the **TABLE**, the difference between the mean results obtained by those laboratories should not be greater than the **reproducibility value** (R) (Note 13), which can generally be deduced by linear interpolation from the **TABLE**.

10. NOTES

1. The septa should be of butyl rubber or red rubber free from hydrocarbon solvent residues.

2. For series analyses a headspace gas chromatograph with automatic sample injection and tempering bath has been shown to be satisfactory. In this case manual injection is omitted.

3. Chromosorb P NAW 60 - 80 mesh is suitable.

4. For the calibration, technical extraction hexane should be used; this usually has a content of n-hexane $\geq 50\%$ and consists mainly of C_6 isomers.

5. Cyclohexane is also suitable for use as an internal standard provided that the solvent used for the extraction or calibration respectively has a negligible content of cyclohexane.

6. Freshly refined and deodorised vegetable oil, the commercial hexane content of which is negligible, is suitable for the calibration.

7. If n-hexane is added, the following table applies:

µl/5 g	0.5	1	2	4	7	10
mg/1000 g	66	132	264	528	924	1320

8. The duration of analysis depends on the time of retention of the internal standard. The samples must be placed in the heating bath in such a way that each sample is tempered for exactly 60 min.

9. If cyclohexane is used as internal standard, C_i = 780 mg per 1000 g.

10. The factor (F) so evaluated can be used for determining vial quantities of hexane less than 60 ppm. If the value of (F) found for the vial containing 0.5 µl of hexane is significantly below the mean value, this deviation is probably due to the difficulty of introducing exactly 0.5 µl and this determination must be eliminated or repeated.

11. The calibration factor for cyclohexane is normally about 0.57.

12. For quantities of hexane between 10 and 20 ppm, it is better to proceed to the calibration (7.2) under the following conditions:

- instead of "Add through the septum 5 µl of n-heptane (5.2) as internal standard", add through the septum 2 µl of n-heptane (5.2) as internal standard.
- in this case C_i = 272 mg/1000g.

13. It should be noted that the reproducibility values (R) cited in the **TABLE** apply in the particular case when the results of single determinations obtained by two laboratories are being compared. When following the method described and it is desired to compare the final results (which have been derived from the means of duplicate determinations) obtained by two laboratories, the values for (R) should be converted to the 95% probability critical difference values (CrD_{95}) applicable to the means of two determinations using the following formula:

$$CrD_{95} = \sqrt{(R^2 - r^2/2)}$$

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REFERENCES

1. Arens M. and Kroll E., **Fette Seifen Anstrich. 87** (1) 80 (1985)

2. Prevot A. and Cabeza F., **Rev. Franc. Corps Gras** 7 (1) 34 (1960)
Takagi T. and Yamasaki M., **Yukagaku 24** (6) 367 (1975)
Downes M. J., **Leatherhead Food RA Technical Circular** No. 799 (Dec. 1982)