ISSN 1018-5593

European Commission

bcr information

CHEMICAL ANALYSIS

INTERCOMPARISON OF METHODS FOR THE DETERMINATION OF TRANS FATTY ACIDS IN EDIBLE OILS AND FATS AND CERTIFICATION OF THREE MATERIALS CONTAINING TRANS FATTY ACIDS

Report
EUR 18955 EN

European Commission

bcr information

CHEMICAL ANALYSIS

INTERCOMPARISON OF METHODS FOR THE DETERMINATION OF TRANS FATTY ACIDS IN EDIBLE OILS AND FATS AND CERTIFICATION OF THREE MATERIALS CONTAINING TRANS FATTY ACIDS

Report on the First Intercomparison

F. ULBERTH([1]), M. BUCHGRABER([1]) and A. BOENKE([2])

([1]) Universität für Bodenkultur
Institut für Milchforschung und Bakteriologie
Gregor Mendel-Str. 33
1180 Wien, Austria

([2]) European Commission
DG XII-CII-3
Measurement and Testing, Infrastructure
200 Rue de la Loi
1049 Brussels, Belgium

Contract no. SMT4-CT97-2144

Directorate-General
Science, Research and Development

1999

EUR 18955 EN

EUROPEAN COMMISSION

LEGAL NOTICE

Neither the European Commission nor any person acting on behalf of the Commission is responsible for the use which might be made of the following information

A great deal of additional information on the European Union is available on the Internet.
It can be accessed through the Europa server (http://europa.eu.int).

Cataloguing data can be found at the end of this publication.

Luxembourg: Office for Official Publications of the European Communities, 1999

ISBN 92-828-2248-6

© European Communities, 1999
Reproduction is authorised provided the source is acknowledged.

Printed in Belgium

EUROPEAN COMMISSION
Edith CRESSON, Member of the Commission responsible for Research, Innovation, Education, Training and Youth
DG XII-CII-03 – Measurement &Testing, Infrastructure Programme

Contents

List of participants:

Department of Dairy Research and Bacteriology, University of Agricultural Sciences, Vienna (A), *Co-ordinator*

Leatherhead Food Research Association, Leatherhead (UK)

Scottish Crop Research Institute, Dundee (UK)

Laboratory of the Government Chemist, Teddington (UK)

Teagasc, Dairy Products Centre, Moorepark (IR)

Institute of Biochemistry and Nutrition, Danish Technical University, Lyngby (DK)

Department of Food Technology and Nutrition, University of Gent, Gent (BE)

Vandemoortele, Izegem (BE)

Unilever Research Laboratorium, Vlaardingen (NL)

Istituto Industrie Agrarie, Università degli Studii, Bologna (I)

Consejo Superior Investigaciones Scientificas, Instiuto del Frio, Madrid (ES)

Laboratory of Food Chemistry, Aristotle University, Thessaloniki (GR)

List of abbreviations

GLC	gas-liquid chromatography
HPLC	high performance liquid chromatography
TLC	thin-layer chromatography
(FT) IR	(Fourier transform) infra red
UV	ultra violet
NMR	nuclear magnetic resonance
ATR	attenuated total reflection
Sil 88	stationary phase for GLC (100 % cyanopopyl polysiloxane
BPX70	stationary phase for GLC (70 % cyanopropyl polysilphenylene siloxane)
FAME	fatty acid methyl ester
TFA	trans fatty acid
CRM	certified reference material
POV	peroxide value
AV	acid value
TBHQ	tert. butylhydroquinone
meq	milliequivalent
n_D	refractive index
r	repeatability
R	reproducibility
s_r	repeatability standard deviation
s_R	reproducibility standard deviation

Summary

The aim of the first intercomparison was twofold. Firstly, to select, stabilise and bottle suitable materials containing ca. 1 %, 5 % and 30 % total trans fatty acids (TFA) and demonstrate that packaging and storage does not affect oil composition, and secondly, to demonstrate that participating laboratories are able to achieve sufficient conformity of test results.

Stabilising oil samples with *tert.* butylhydroquinone (250 mg/kg) and packaging in inert atmosphere in amber glass ampoules resulted in homogeneous and stable lots of preliminary test materials.

Analytical precision of chromatographic methods to determine trans fatty acids (TFA) was acceptable (RSD(R) < 15 %) for analyte levels > 1 g/100 g, but for levels < 1 g/100 g precision was beyond the agreed acceptance criterion (RSD(R) > 40 %). Misidentification of peaks and inconsistent peak integration practices are considered to be the major reasons for deviating results reported by the participants.

Results obtained by chromatographic methods were in close agreement with those produced by spectroscopy (IR and NMR), thus proving that the applied chromatographic methods were not systematically biased.

The intercomparison has to be repeated with new sets of samples. Special emphasis will be placed on the correct identification of trans polyenes in order to improve precision for low levels of TFA. To facilitate correct identification, the Co-ordinator will distribute equivalent chain length data for TFA.

PART I: SELECTION AND PREPARATION OF TEST MATERIALS

Objectives

To select, stabilise and bottle suitable materials containing ca. 1 %, 5 % and 30 % total trans fatty acids (TFA) and demonstrate that packaging and storage does not affect oil composition.

Materials and methods

Three materials, i.e. a physically refined rapeseed oil (ca. 1 % TFA content), a partially hydrogenated sunflower seed oil (ca. 30 % TFA content) , and a blend of one part partially hydrogenated sunflower seed oil plus 4 parts palm oil (ca. 5 % TFA content) were selected as starting materials. All of them fulfilled the following quality criteria:

- Content of free fatty acids < 0.5 g/kg
- Peroxide value < 1.0 meq/kg
- Rancimat induction period > 10 hrs at 100 °C

Acid value (AV), peroxide value (POV) and UV absorptivities were determined according to IUPAC procedures, except that sample amounts used were down scaled to accommodate the limited sample mass (in total 5 g per ampoule).

TFA level	AV[1)]	POV[2)]	UV Absorptivity[3)] at λ			TFA (%)[4)]
			232 nm	268 nm	278 nm	
1%	0.10	0.10	3.084	0.594	0.511	0.24
5%	0.08	0.58	3.195	1.309	1.074	4.74
30%	0.06	0.20	6.052	1.157	0.941	23.46

Table 1: Selected quality criteria of the oil samples used to prepare the test materials.

[1)] g KOH necessary to neutralise free fatty acids per kg oil
[2)] meq. active O2 per kg oil
[3)] Absorptivity (1 cm, 1 %)
[4)] Content of TFA (g/100 g total FAME) by capillary GLC

Analytically determined values for the mentioned inclusion criteria of the test materials are listed in Table 1. Sample chromatograms are given in Figures 1-3.

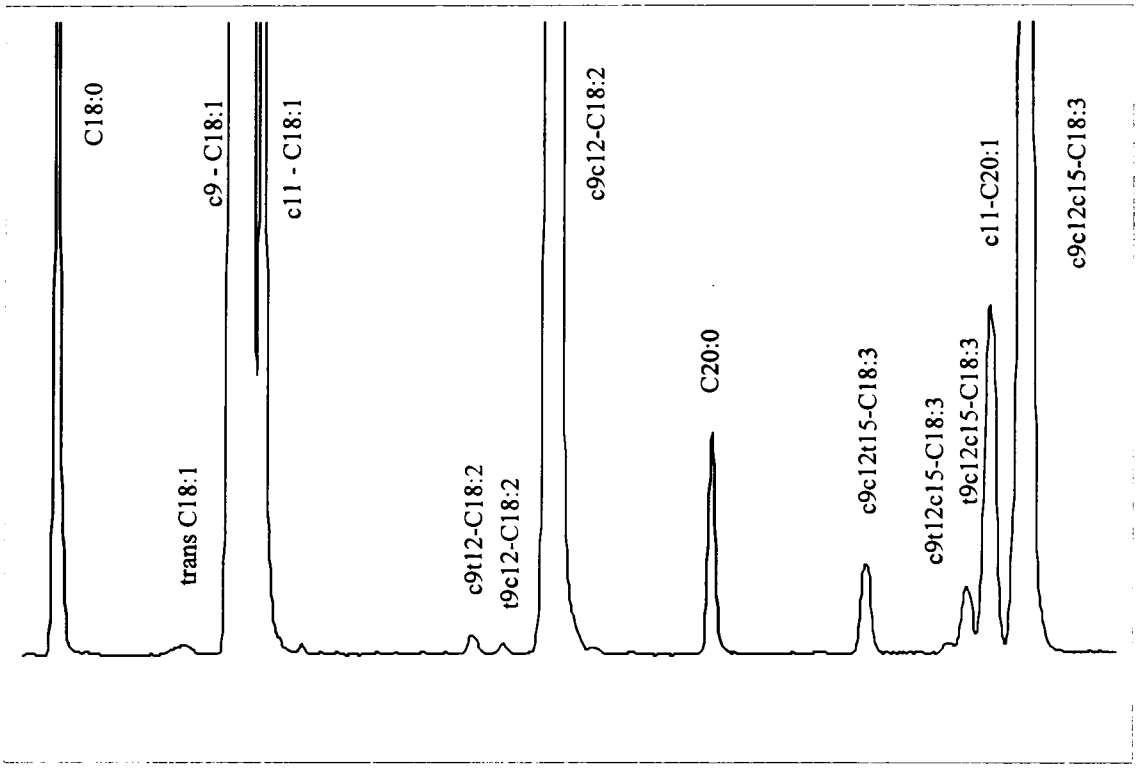


Figure 1: GLC separation of TFA in a physically refined rapeseed oil (100 m x 0.25 mm Sil-88, 178 °C)

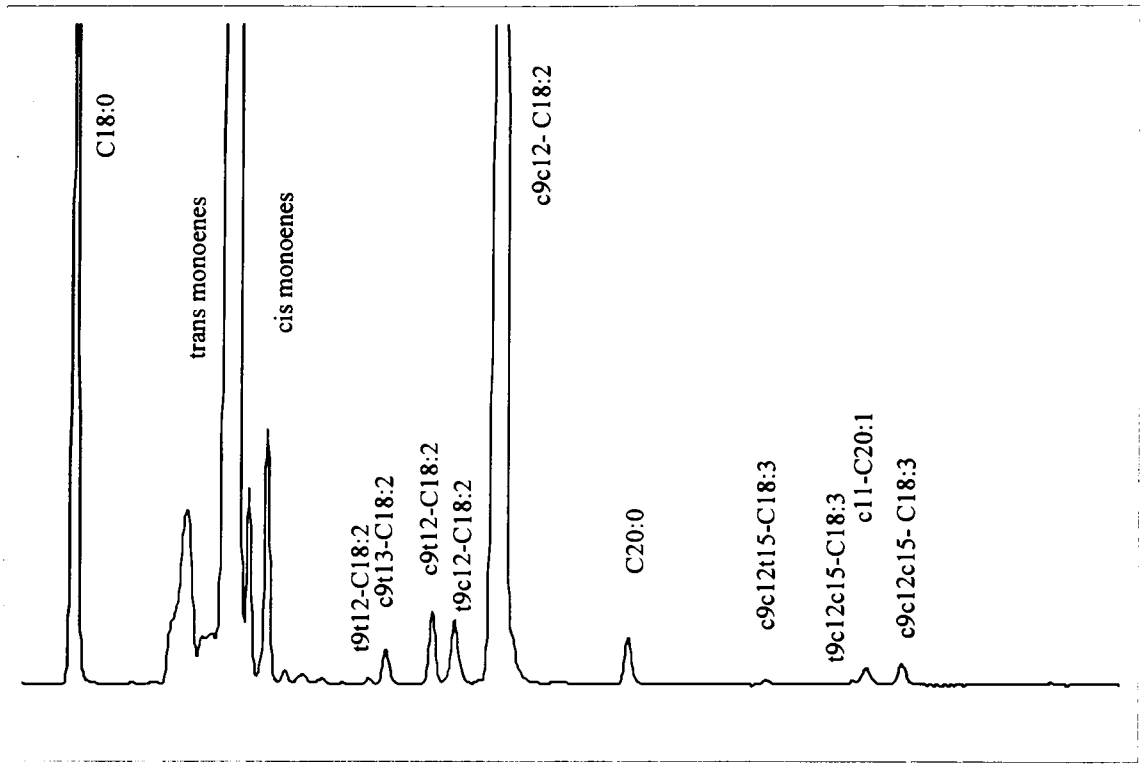


Figure 2: GLC separation of TFA in a blend of partially hydrogenated sunflower seed oil (one part) and palm oil (four parts) (100 m x 0.25 mm Sil-88, 180 °C)

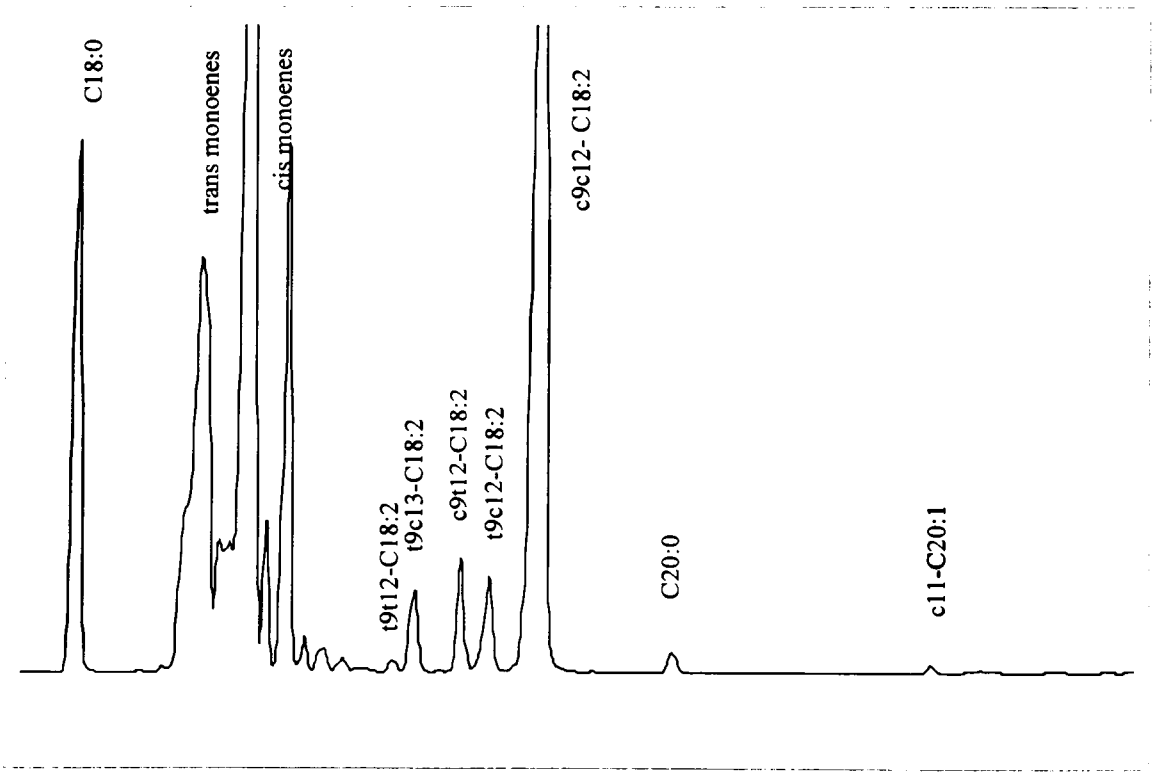


Figure 3: GLC separation of TFA in a partially hydrogenated sunflower seed oil (100 m x 0.25 mm Sil-88, 180 °C)

All materials were stabilised with 250 mg *tert.* butylhydroquinone (> 98 % by HPLC assay, Fluka Nr. 19986) per kg oil. After the addition of the antioxidant the headspace of the oil containers were flushed with nitrogen, warmed to 40 °C and the content stirred for 30 min. Five g portions were filled in amber glass ampoules by using an automatic dispensor. The headspace of the ampoules were flushed with nitrogen and subsequently flame sealed. A flow chart summarizing the preparation process is given in Figure 4.

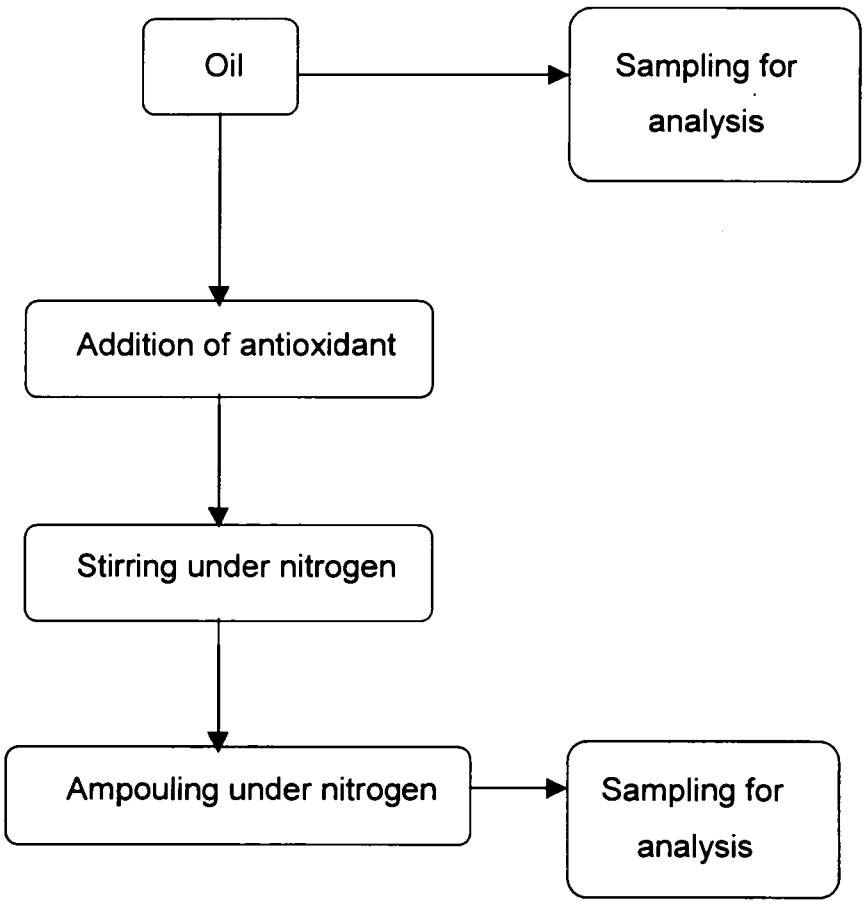


Figure 4: Preparation of preliminary test materials

Results

Homogeneity of the materials

To test whether the filling process had led to any variations in oil composition the refractive index (n_D, 40 °C) of individual ampoules was determined by means of a Carl Zeiss refractometer. The first and the last five ampoules and also every 25th ampoule of the filling sequence were taken. In addition four samples were taken at random. Determination of the n_D of these samples was done in random order. Results are tabulated in Tables 2-4.

Since no significant drift ($t_{0.95}$-test) in n_D (40 °C) and no significant difference ($F_{0.95}$-test) between units was observed the preparation and packaging process resulted in homogenous lots of test materials.

Sample (ampoule) No.	n_D at 40°C	Sample (ampoule) No.	n_D at 40°C
1	1.4644	**160**	1.4646
2	1.4646	**185**	1.4646
3	1.4646	**196**	1.4646
4	1.4646	**197**	1.4644
5	1.4646	**198**	1.4646
10	1.4646	**199**	1.4646
35	1.4646	**200**	1.4646
60	1.4645	**R***	1.4646
85	1.4646	**R***	1.4646
110	1.4646	**R***	1.4646
135	1.4646	**R***	1.4646

* samples taken at random order

Table 2: Homogeneity of the 1 % TFA material as determined by n_D (40 °C) measurements

Sample (ampoule) No.	n_D at 40°C	Sample (ampoule) No.	n_D at 40°C
1	1.4596	**160**	1.4595
2	1.4595	**185**	1.4595
3	1.4595	**196**	1.4596
4	1.4595	**197**	1.4595
5	1.4595	**198**	1.4595
10	1.4596	**199**	1.4595
35	1.4596	**200**	1.4595
60	1.4596	**R***	1.4595
85	1.4595	**R***	1.4595
110	1.4596	**R***	1.4595
135	1.4595	**R***	1.4595

* samples taken at random order

Table 3: Homogeneity of the 5 % TFA material as determined by n_D (40 °C) measurements

Sample (ampoule) No.	n_D at 40°C	Sample (ampoule) No.	n_D at 40°C
1	1.4650	**160**	1.4647
2	1.4650	**185**	1.4647
3	1.4649	**196**	1.4649
4	1.4647	**197**	1.4649
5	1.4648	**198**	1.4647
10	1.4647	**199**	1.4647
35	1.4647	**200**	1.4647
60	1.4647	**R***	1.4647
85	1.4647	**R***	1.4647
110	1.4647	**R***	1.4647
135	1.4647	**R***	1.4647

* samples taken at random order

Table 4: Homogeneity of the 30 % TFA material as determined by n_D (40 °C) measurements

Stability of the materials

To test whether increased temperatures affect the stability of the test materials part of randomly selected units throughout the prepared batch were stored at 40 °C and 70 °C. Units stored at -20 °C, 4 °C and 20 °C served as controls. These levels were regarded as potential temperatures for long-term storage of materials.

Stability was checked at regular intervals by determining AV, POV, UV absorptivity and the fatty acid profile by polar capillary GLC. Ampoules stored at 40 °C and 70 °C were sampled bi-weekly, those stored at 4 °C and 20 °C monthly, and at -20 °C bi-monthly.

No compositional change of the fatty acid profile was observed in any of the stored materials. As an example, data of the rapeseed oil, which contained substantial amounts of polyunsaturated fatty acids, are graphically presented in Figures 5-9. Data for the other materials are listed in Annex A. Even at a storage temperature of 70 °C no losses of C18:3, the most vulnerable towards oxidation, was evident.

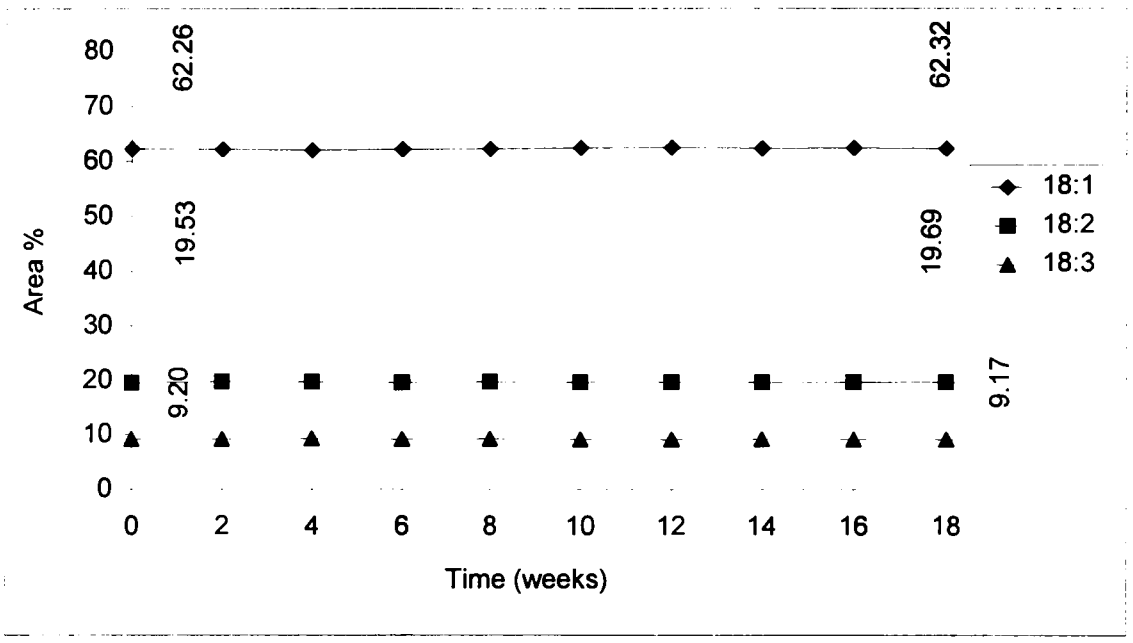


Figure 5: Changes in the fatty acid profile of the 1 % TFA sample (physically refined rapeseed oil) stored at 70 °C ($SD_{repeatability\ conditions}$=0.10 Area %).

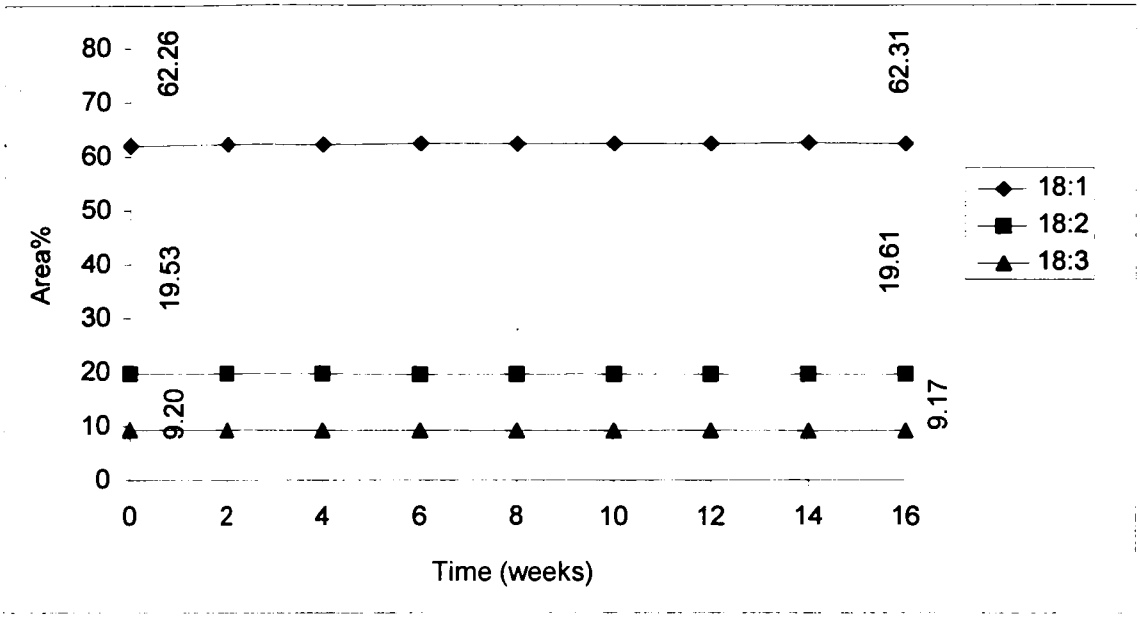


Figure 6: Changes in the fatty acid profile of the 1 % TFA sample (physically refined rapeseed oil) stored at 40 °C ($SD_{repeatability\ conditions}$=0.10 Area %).

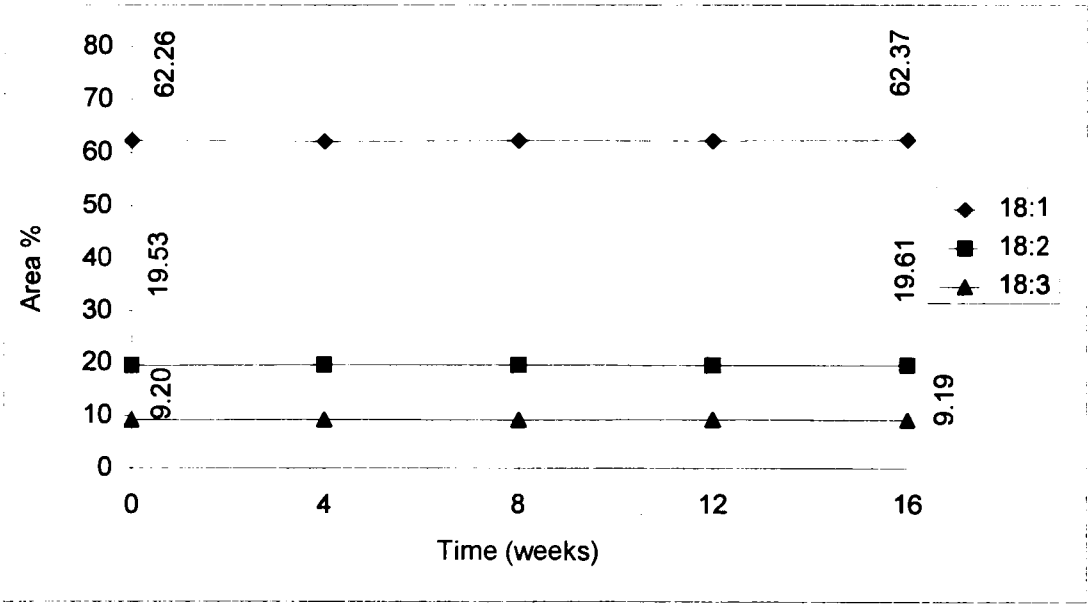


Figure 7: Changes in the fatty acid profile of the 1 % TFA sample (physically refined rapeseed oil) stored at 20 °C ($SD_{repeatability\ conditions}$=0.10 Area %).

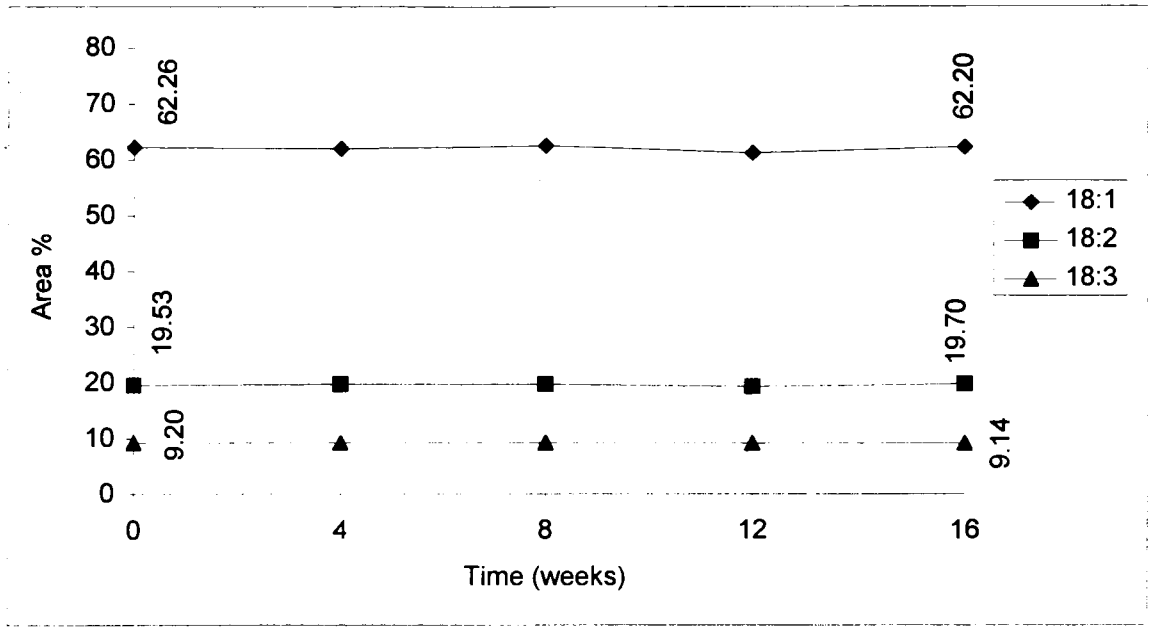


Figure 8: Changes in the fatty acid profile of the 1 % TFA sample (physically refined rapeseed oil) stored at 4 °C ($SD_{repeatability\ conditions}$=0.10 Area %).

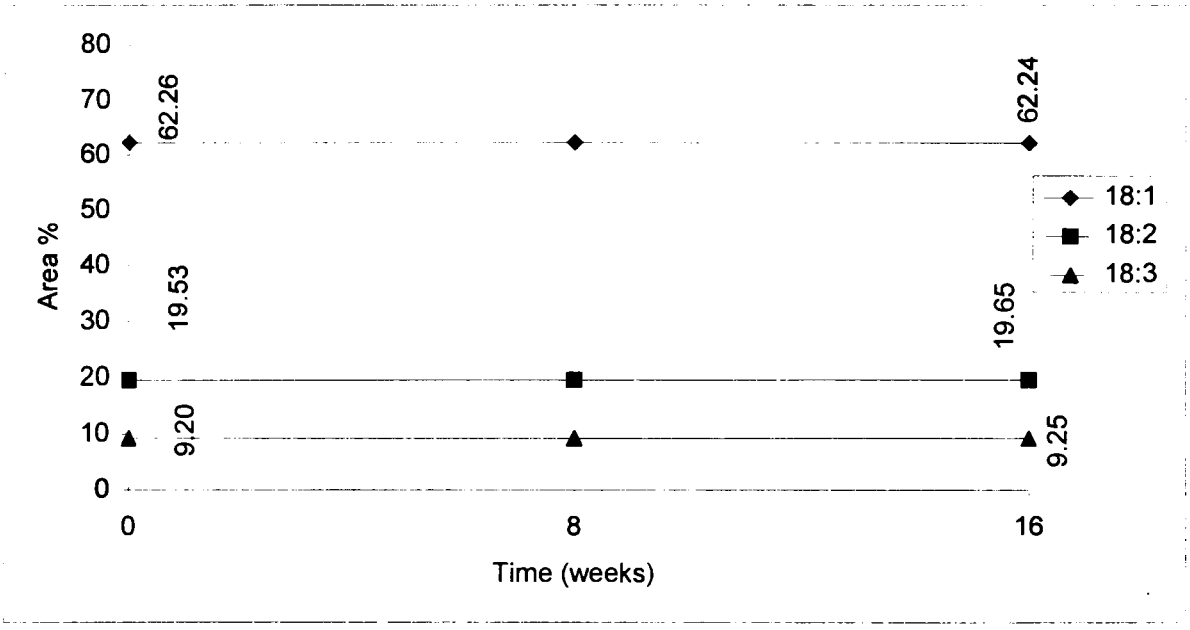


Figure 9: Changes in the fatty acid profile of the 1 % TFA sample (physically refined rapeseed oil) stored at -20 °C ($SD_{repeatability\ conditions}$=0.10 Area %).

Hydrolysis of the materials, leading to an increase in free fatty acids as monitored by AV, did not occur during oil ageing (Figures 10-12). In addition data normalized

to those obtained at -20 °C storage are given in Figures 13 and 14. Only data for rapeseed oil are presented graphically; other data are given in form of tables (Annex A).

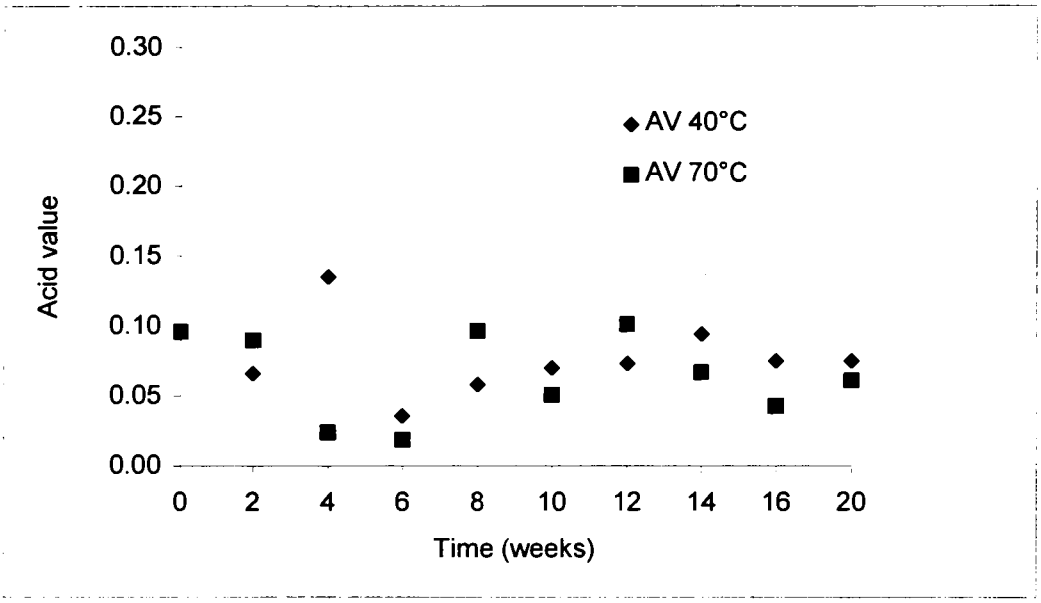


Figure 10: Acid value development in the 1 % TFA material aged at 40 °C and 70 °C ($SD_{repeatability\ conditions}$=0.05 AV).

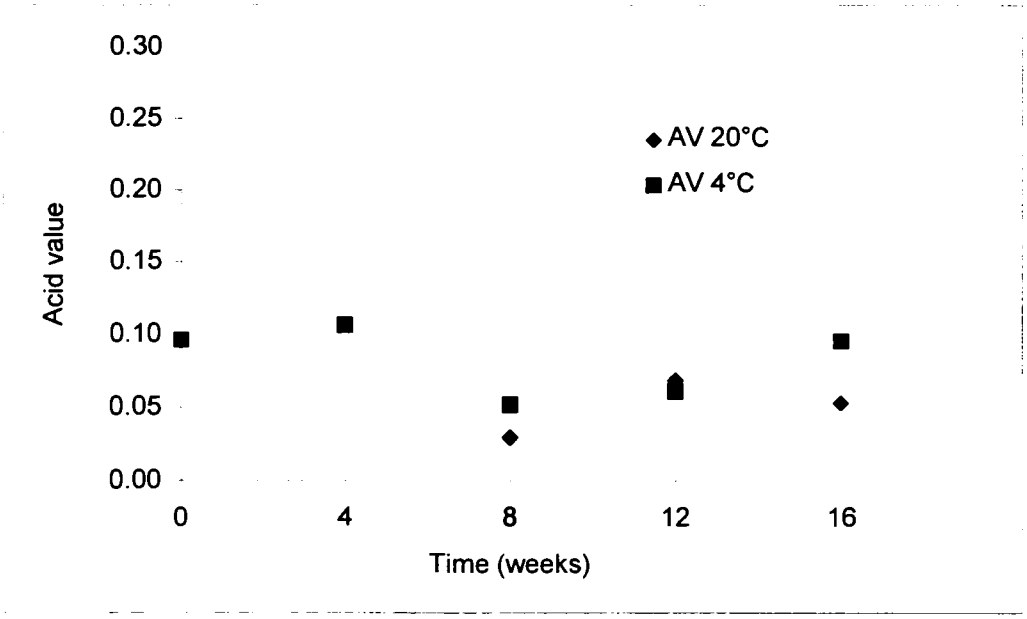


Figure 11: Acid value development in the 1 % TFA material aged at 20 °C and 4 °C ($SD_{repeatability\ conditions}$=0.05 AV).

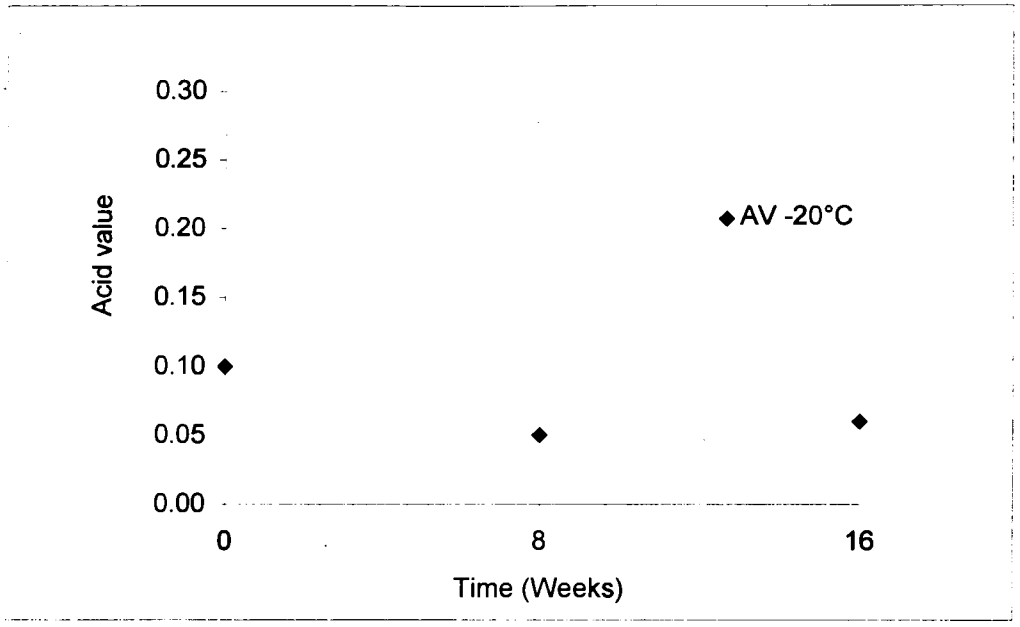


Figure 12: Acid value development in the 1 % TFA material aged at -20 °C ($SD_{repeatability\ conditions}$=0.05 AV).

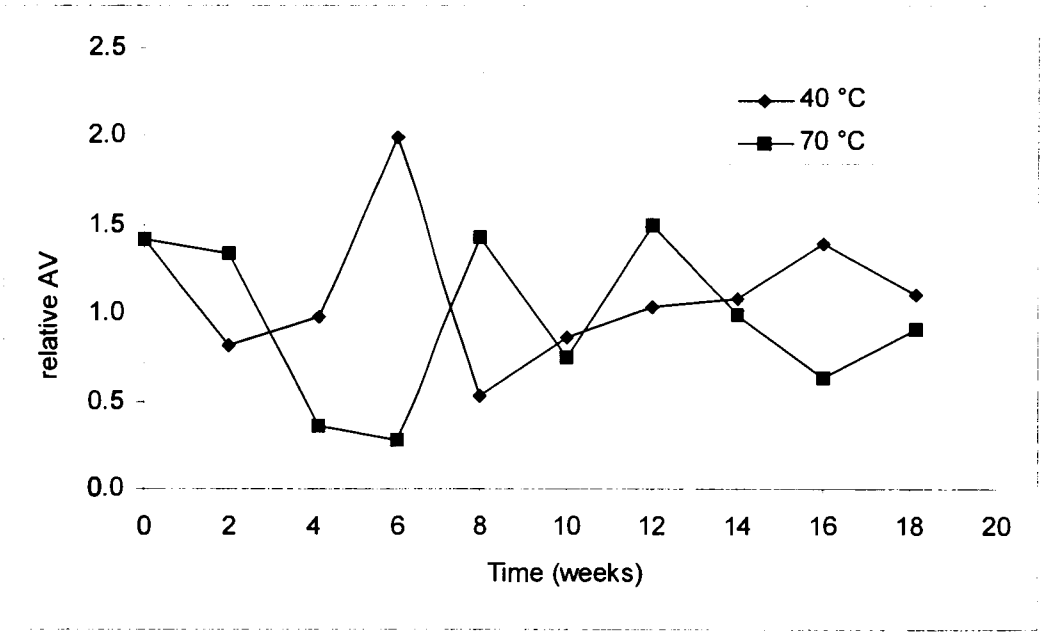


Figure 13: Relative changes of the AV in the 1 % TFA material aged at 40 °C and 70 °C (actual values normalized by those obtained at -20 °C storage).

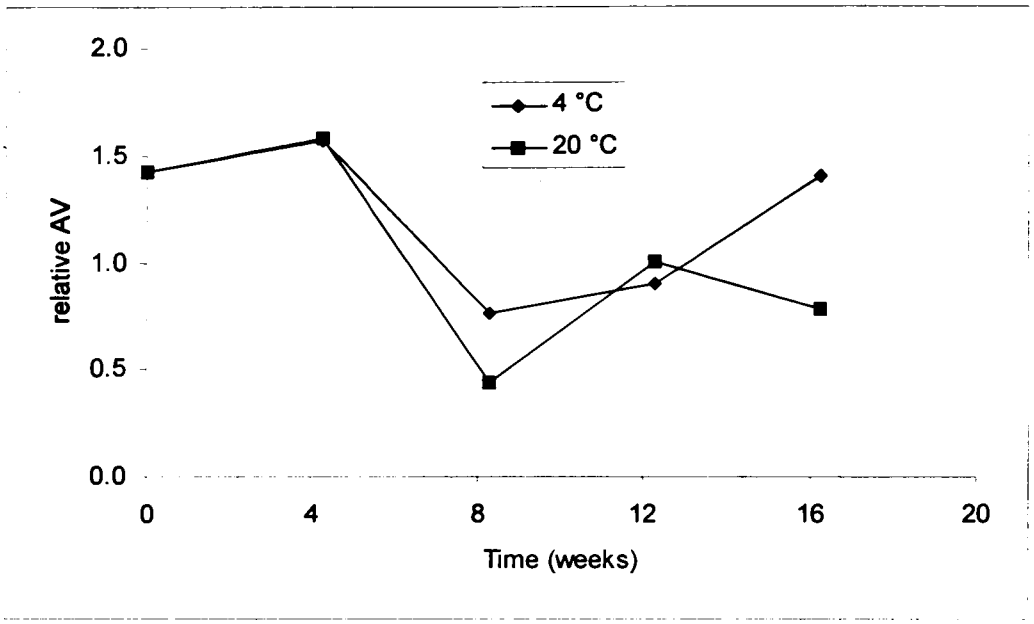


Figure 14: Relative changes of AV in the 1 % TFA material aged at 20 °C and 4 °C (actual values normalized by those obtained at -20 °C storage).

Peroxides in all units stored at 70 °C developed within two weeks but then POV started to decrease, approaching values < 0.5 meq/kg after 10 weeks (Figure 15). Units stored at 40 °C responded in the way anticipated, i.e. after an induction period of 2 weeks, POV increased to values around 2.0 meq/kg and levelled off.

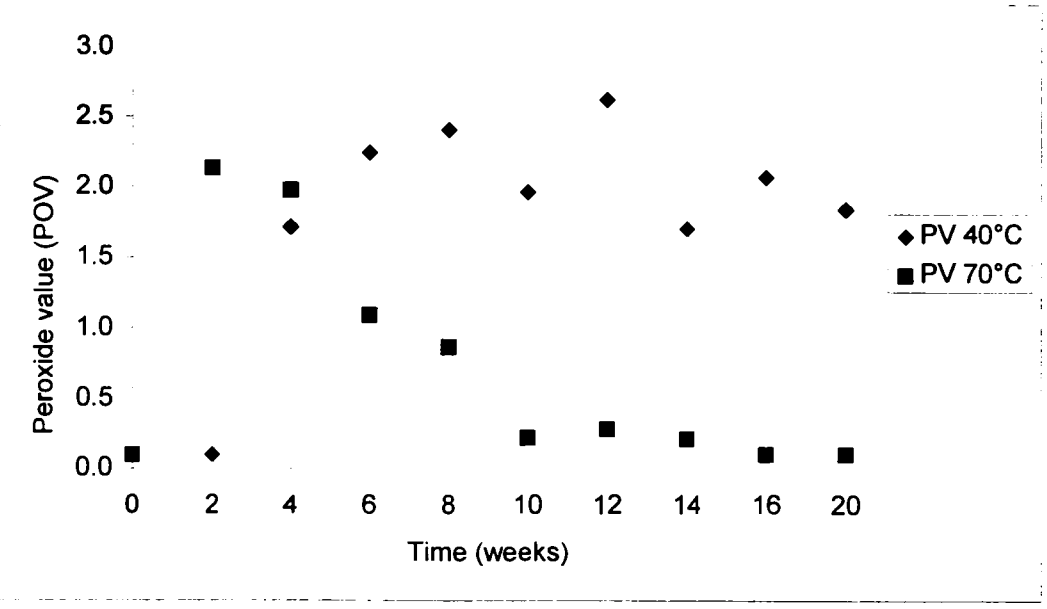


Figure 15: POV development in the 1 % TFA material aged at 40 °C and 70 °C ($SD_{repeatability\ conditions}$=0.10 POV).

At lower storage temperatures POV increased only slightly during storage (Figures 16 and 17).

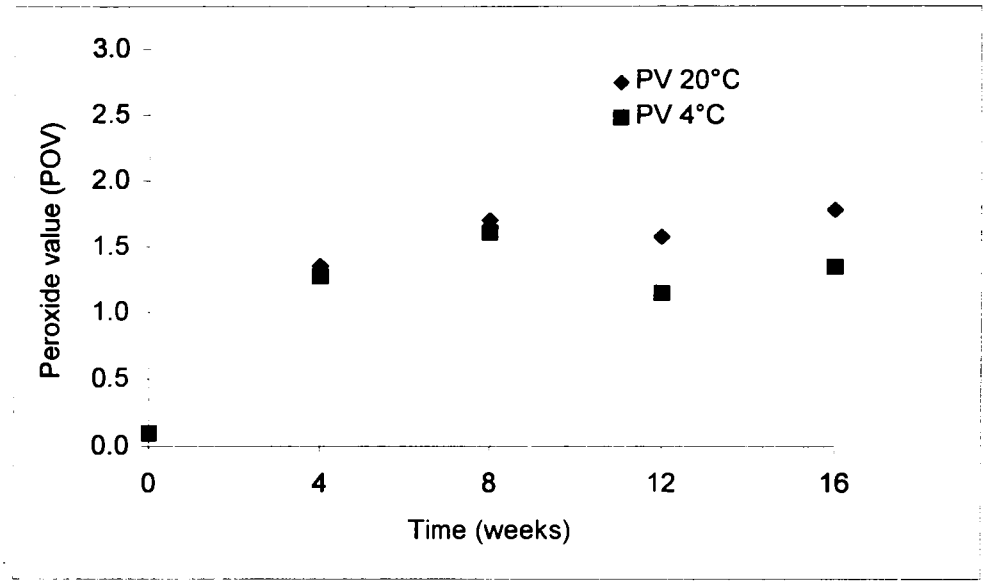


Figure 16: POV development in the 1 % TFA material aged at 20 °C and 4 °C ($SD_{repeatability\ conditions}$=0.10 POV).

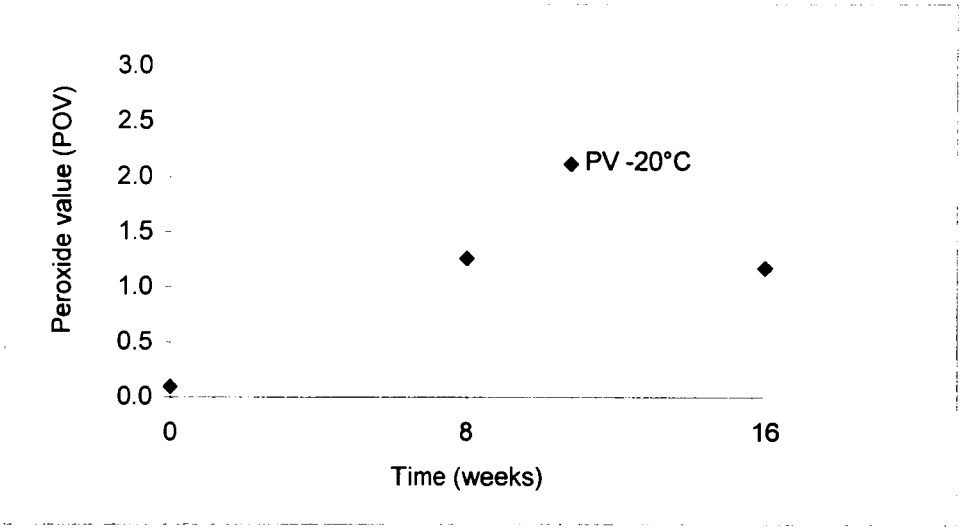


Figure 17: POV development in the 1 % TFA material aged at -20 °C ($SD_{repeatability\ conditions}$=0.10 POV).

Relative figures for POV (normalized to those stored at –20 °C) are depicted in Figures 18 and 19.

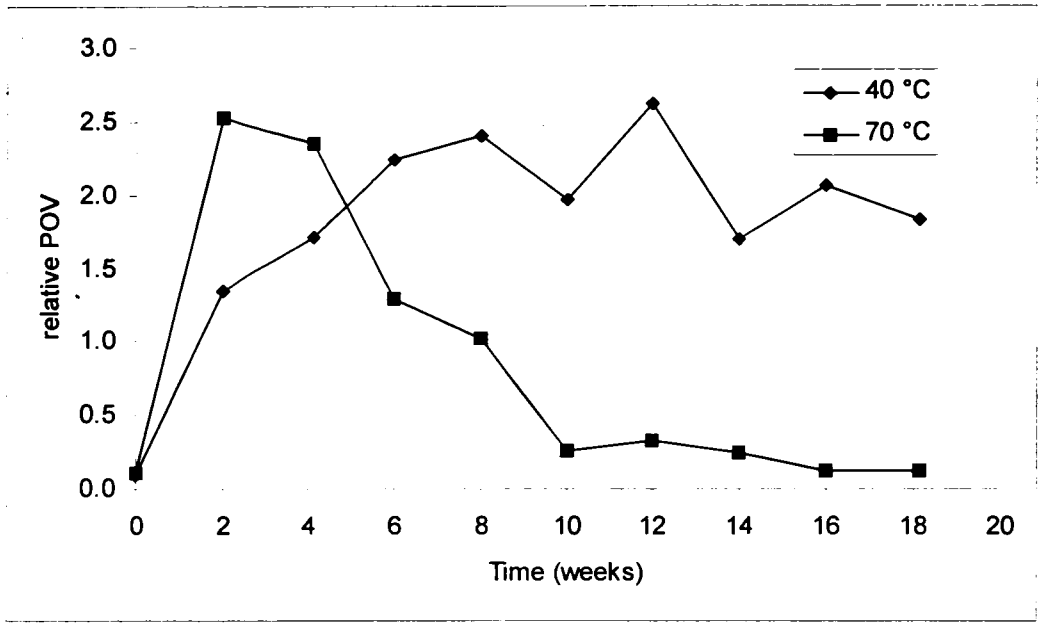


Figure 18: Relative changes of the POV in the 1 % TFA material aged at 40 °C and 70 °C (actual values normalized by those obtained at -20 °C storage).

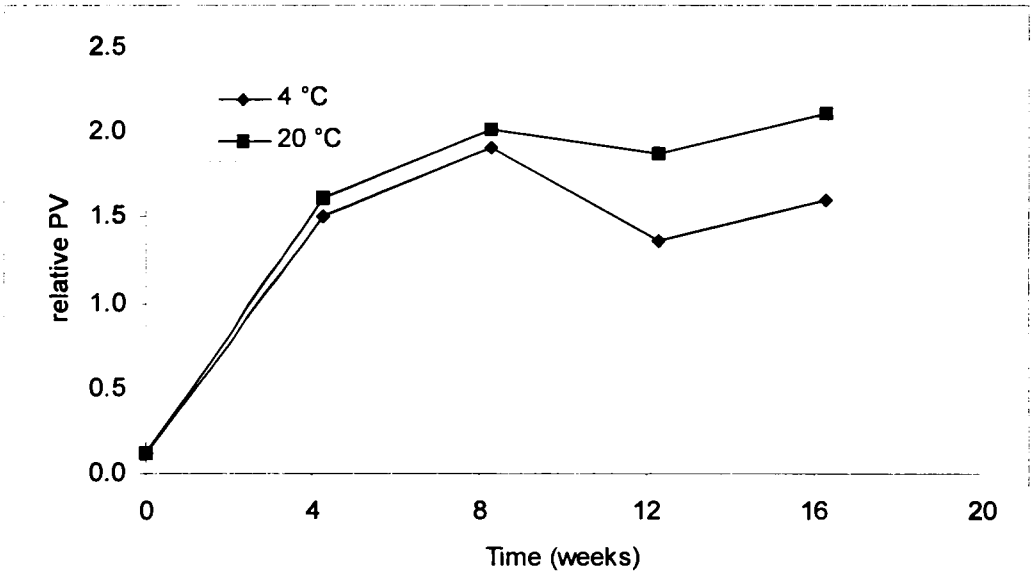


Figure 19: Relative changes of the POV in the 1 % TFA material aged at 20 °C and 4 °C (actual values normalized by those obtained at -20 °C storage).

Contrary to POV, UV measurements at λ=232 nm, 268 nm and 278 nm, which are indicative for formation of conjugated dienes (primary oxidation products) and oxo-compounds (secondary oxidation products), did neither change at elevated (Figures 20 and 21) nor at ambient (Figure 22), or sub-ambient temperatures (Figure 23 and 24). Therefore, oxidative damage of the oils stored at 70 °C is not very likely, though POV increased in an atypical manner. During autoxidation of a fat POV should develop exponentially after the induction period, but this was not seen for the units stored at 70 °C (Figure 15). A temperature induced polymerisation of peroxides is the most likely reason for the POV and UV absorptivity pattern observed.

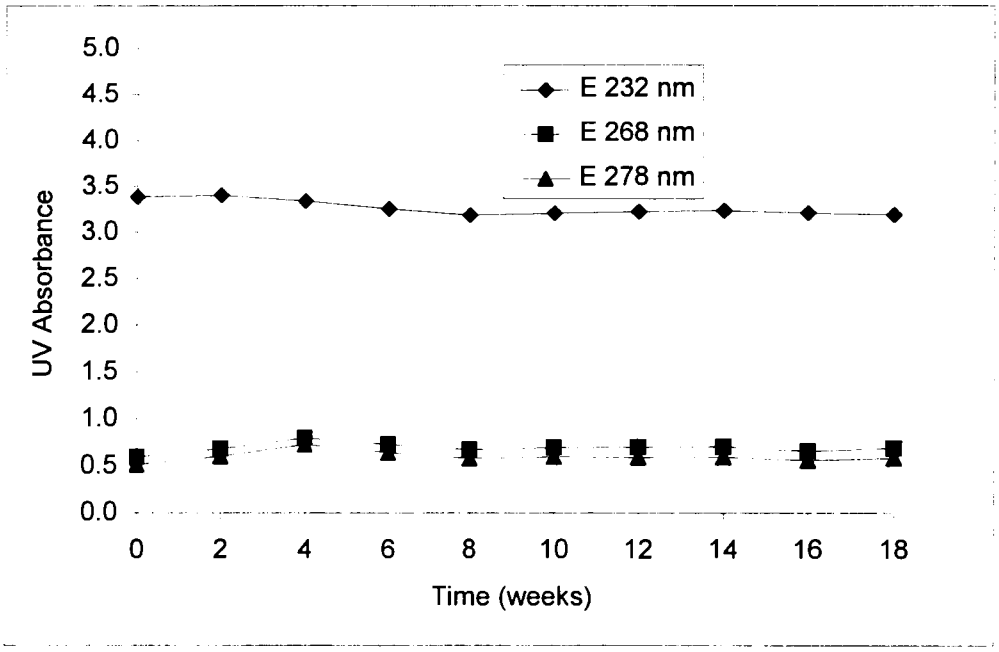


Figure 20: Development of primary and secondary oxidation products in the 1 % TFA material at 70 °C storage temperature as determined by UV measurement ($SD_{repeatability\ conditions}$=0.10 UV Absorbance).

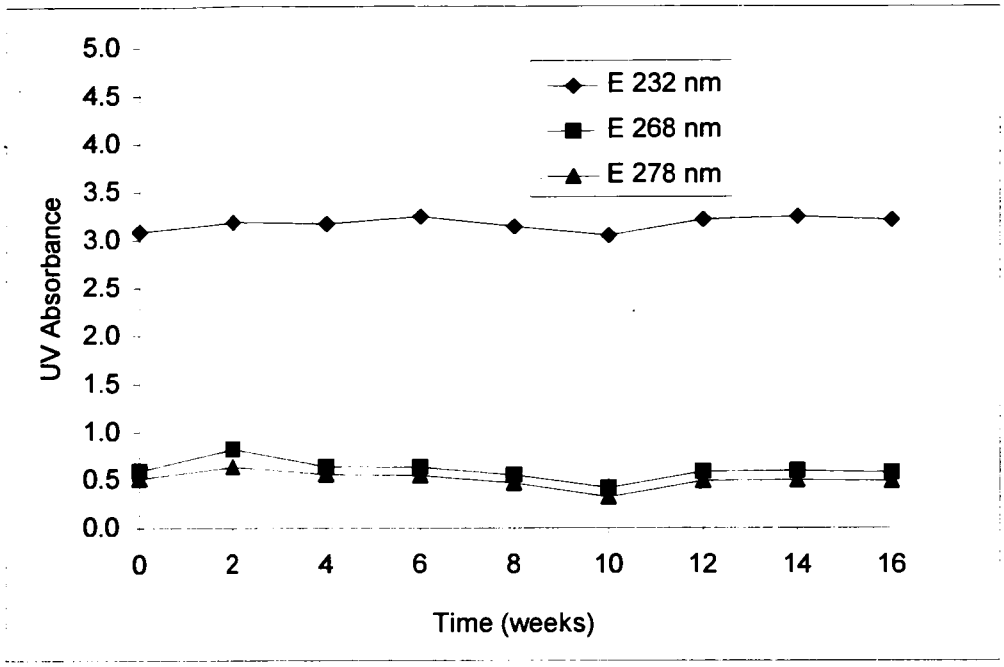


Figure 21: Development of primary and secondary oxidation products in the 1 % TFA material at 40 °C storage temperature as determined by UV measurement ($SD_{repeatability\ conditions}$=0.10 UV Absorbance).

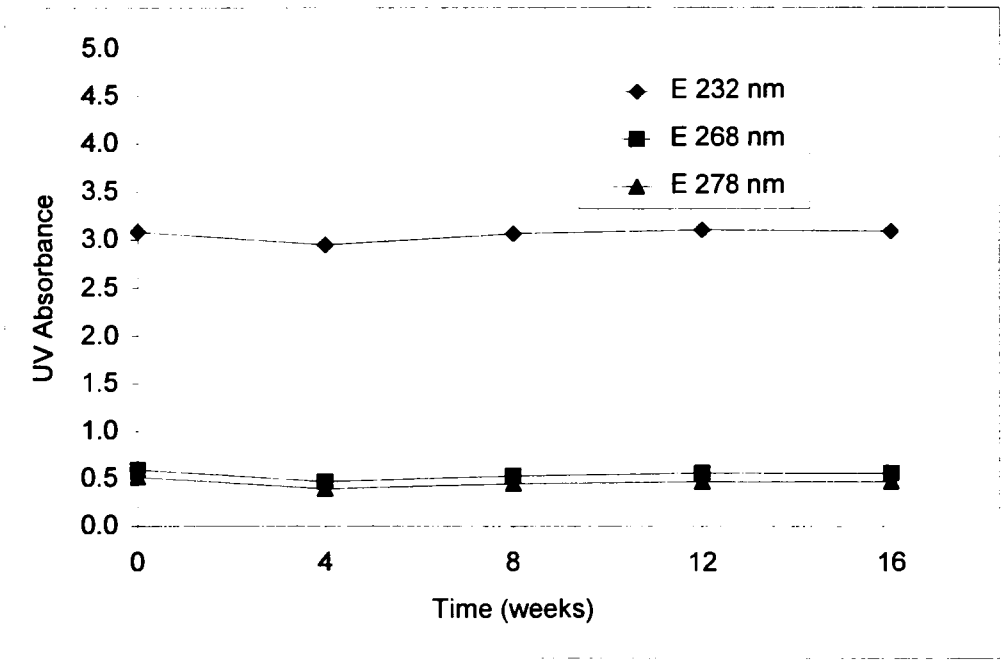


Figure 22: Development of primary and secondary oxidation products in the 1 % TFA material at 20 °C storage temperature as determined by UV measurement ($SD_{repeatability\ conditions}$=0.10 UV Absorbance).

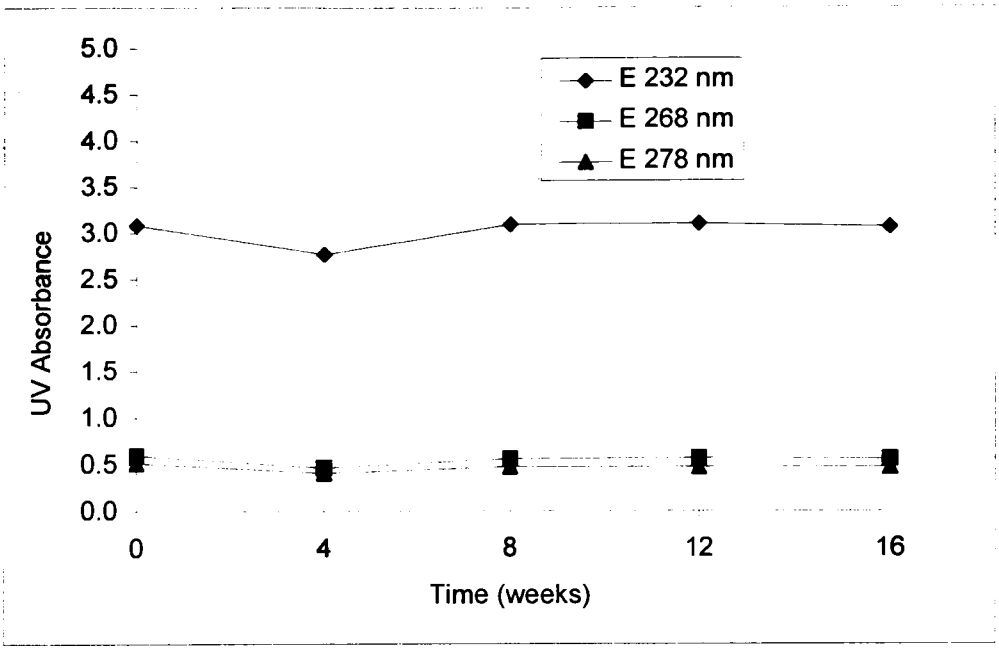


Figure 23: Development of primary and secondary oxidation products in the 1 % TFA material at 4 °C storage temperature as determined by UV measurement.

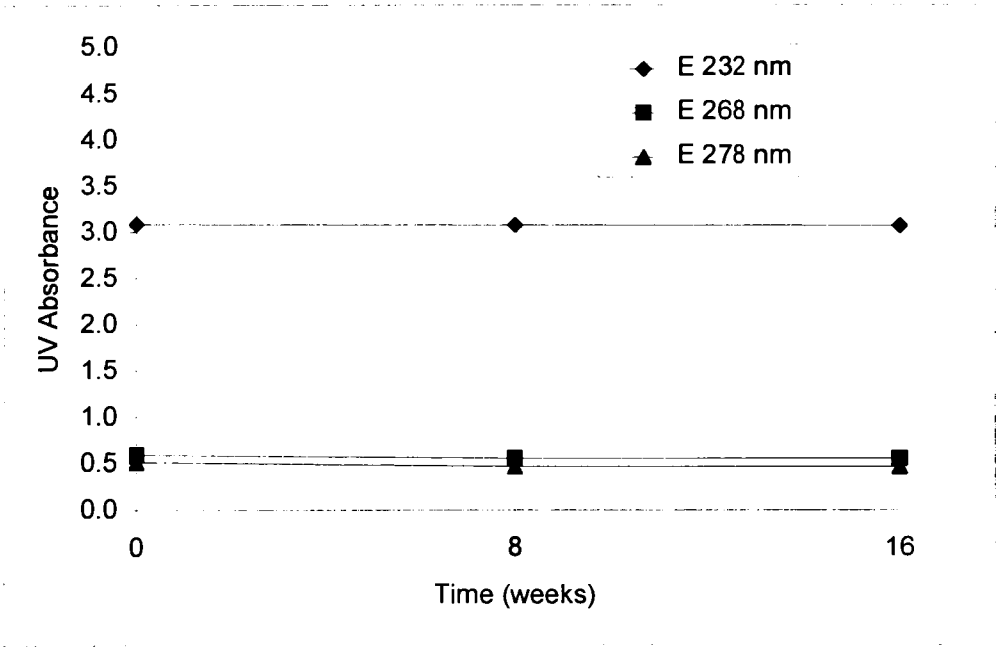


Figure 24: Development of primary and secondary oxidation products in the 1 % TFA material at -20 °C storage temperature as determined by UV measurement ($SD_{repeatability\ conditions}$=0.10 UV Absorbance).

Normalized UV data for the 1 % material are graphed in Figures 25-28, which underpin the satisfactory oxidative stability of the test materials .

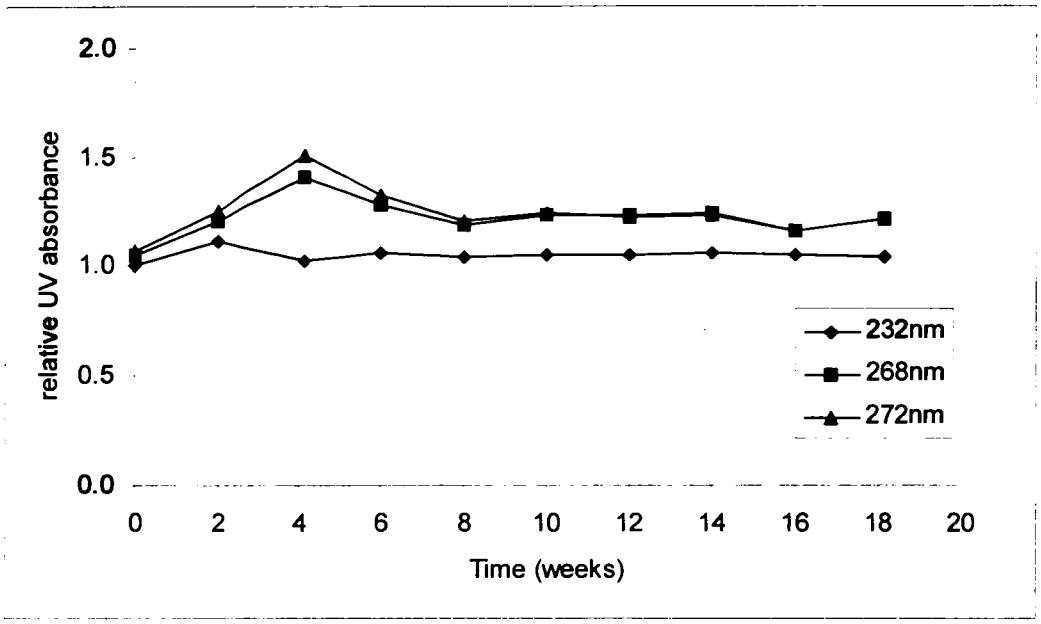


Figure 25: Relative changes of UV absorbance in the 1 % TFA material aged at 70 °C (actual values normalized by those obtained at -20 °C storage).

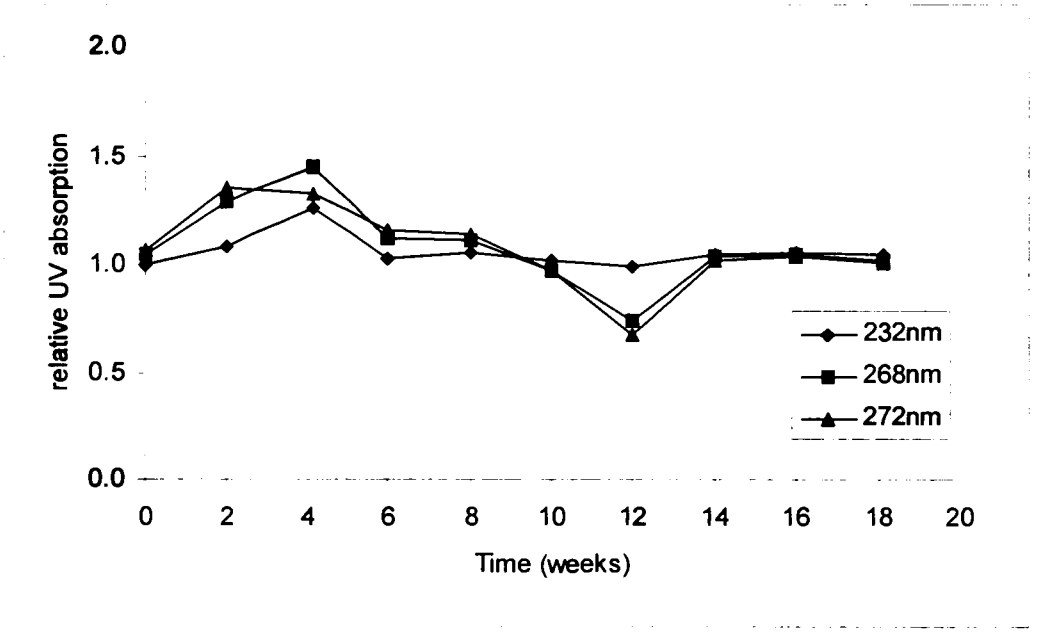


Figure 26: Relative changes of UV absorbance in the 1 % TFA material aged at 40 °C (actual values normalized by those obtained at -20 °C storage).

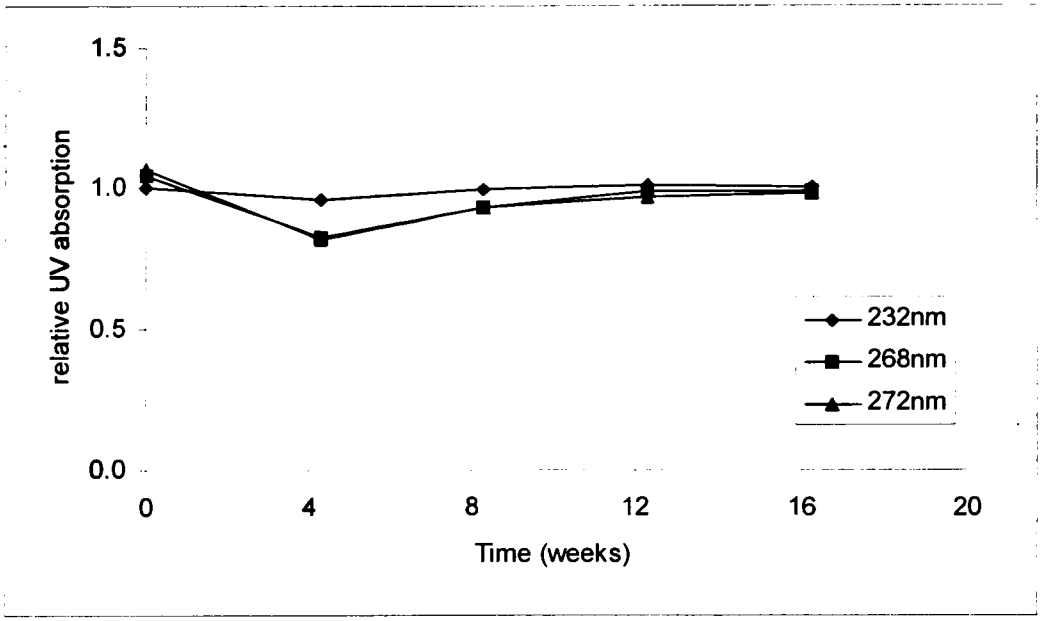


Figure 27: Relative changes of UV absorbance in the 1 % TFA material aged at 20 °C (actual values normalized by those obtained at -20 °C storage).

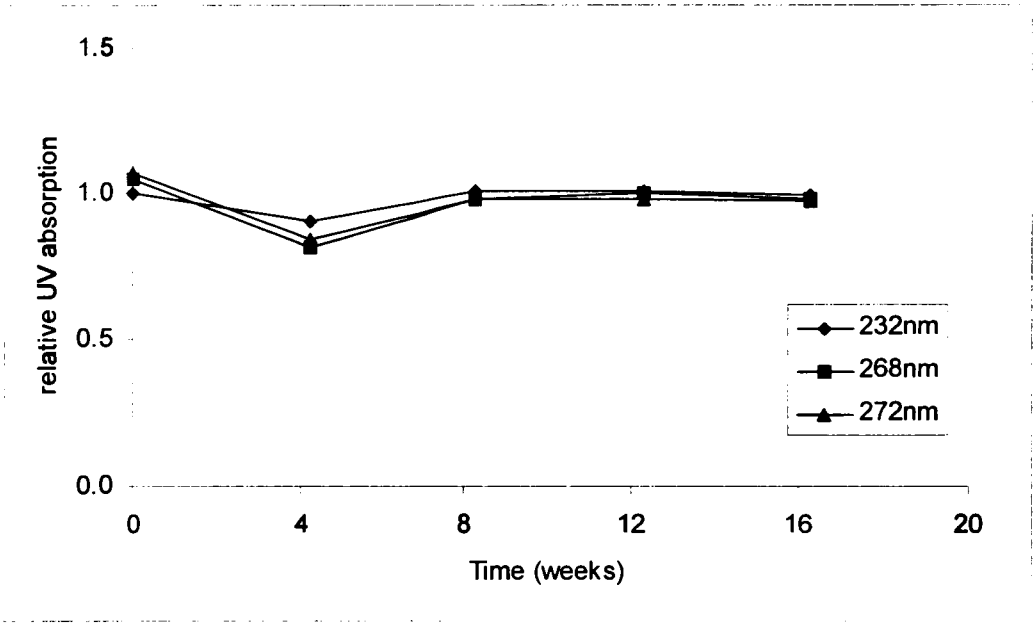


Figure 28: Relative changes of UV absorbance in the 1 % TFA material aged at 4 °C (actual values normalized by those obtained at -20 °C storage).

In conclusion, stabilisation of the oils with 250 mg *tert.* butylhydroquinone/kg and packaging in amber glass in inert atmosphere resulted in stable lots of preliminary test materials. Even after 20 weeks of storage at elevated temperatures no

deterioration was measurable. Therefore, all three materials were considered to be sufficiently stable to serve as test samples in the first intercomparison. Moreover, storage of the candidate material at 4 °C or 20 °C should result in good long-term stability.

PART II: INTERCOMPARISON OF METHODS

Objectives

To demonstrate that participating laboratories are able to achieve sufficient conformity of test results.

Methods

Participants used their own analytical methodology (Table 5) to analyse the test materials provided, after having demonstrated that the approach chosen met several performance criteria. The silver-chromatography/GLC technique had to be applied for the determination of the TFA content of the test materials containing ca. 5 % and ca. 30 %, while the TFA content of the 1 % material was determined by GC only.

Lab	Esterification	Ag-Chrom	GLC	
			Column	Conditions
1	Na-methoxide	HPLC	Sil-88, 100 m	isoth./progr.
2	BF_3/methanol	TLC	Sil-88, 50 m	progr.
3	BF_3/methanol	HPLC	BPX70, 120 m	
4	KOH/methanol	TLC	Sil-88 100m	isoth./progr.
5	BF_3/methanol	TLC	BPX70, 60 m	progr.
6	BF_3/methanol	HPLC	Sil-88, 50 m	isoth. 176°C
7	Na-methoxide	TLC	Sil-88, 100 m	isoth. 173°C
8	KOH/methanol	HPLC	BPX70, 50 m	isoth. 190°C
9	BF_3/methanol	HPLC	BPX70, 50 m	isoth./progr.
10	KOH/methanol	TLC	Sil-88, 60 m	progr.
11	Na-methoxide	HPLC	Sil-88, 100 m	isoth. 180°C
12	KOH/methanol	HPLC	Sil-88, 50 m	isoth. 184°C

Table 5: Experimental conditions used for quantification of TFA by chromatographic methods.

Silver-ion chromatography/GC

Each sample material (TFA level) had to be tested in duplicate on three separate working days. In total six independent TFA results per TFA level had to be reported. A flow-scheme detailing in principle the Ag^+-chromatography/GLC technique used for the determination of TFA is given in Figure 29.

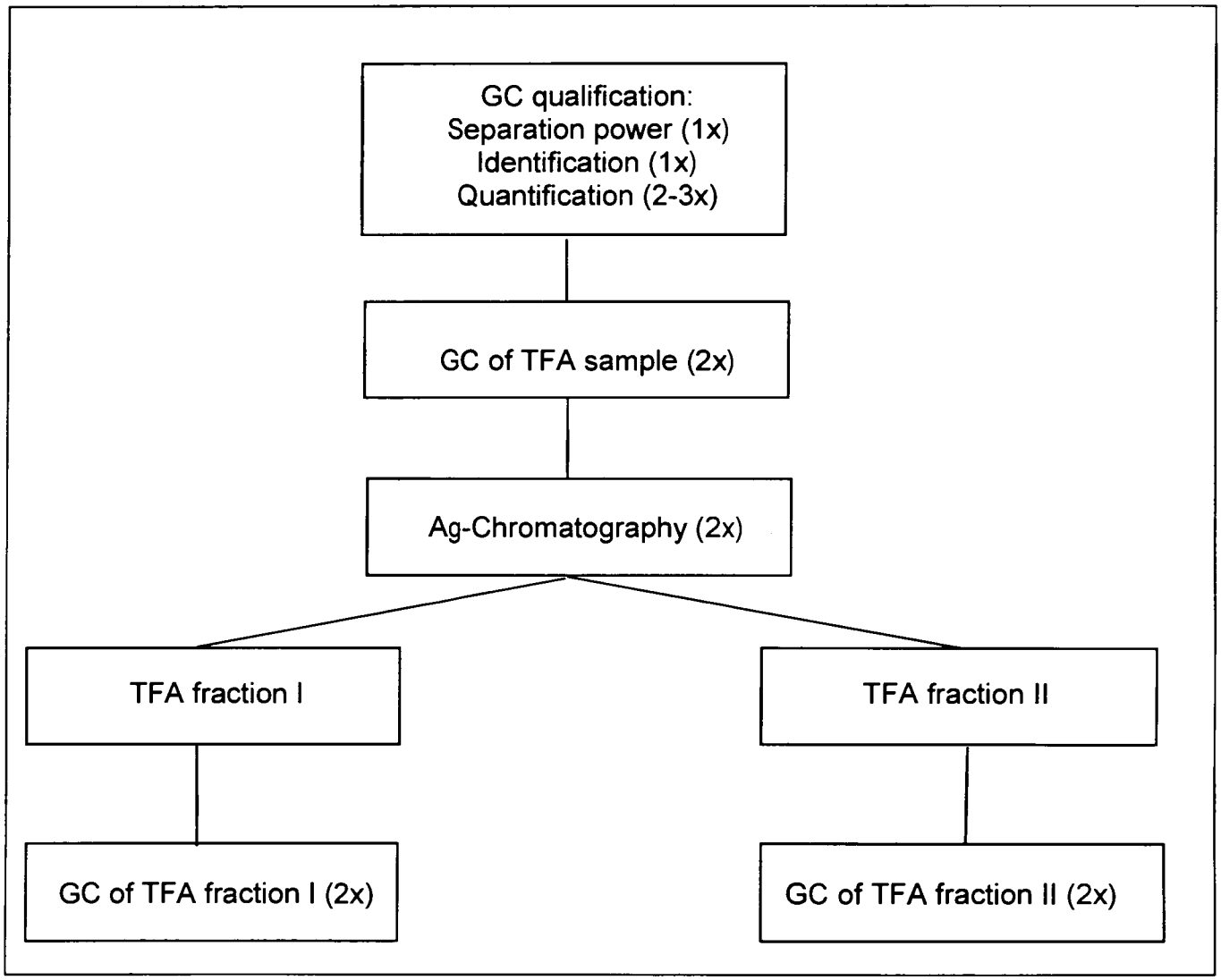


Figure 29: Schematic representation of the applied Ag^+-chromatography/GC procedure.

GC conditions:

The same analytical conditions as used in WP1 (GC operating parameters, analyte concentrations, transesterification procedure) had to be applied.

On each working day the performance of the GC system had to be checked by injecting:

- a solution containing equal amounts of the critical pairs trans-13/cis-9 C18:1 and cis-11 C20:1/all-cis C18:3 (Sil-88 column), or trans-13/cis-9 C18:1 and C20:0/all-cis C18:3 (BPX-70 column) to check the separation power (single analysis).
- a solution containing elaidinized linseed oil FAME to determine retention times of trans-containing polyunsaturates (single analysis).
- a FAME solution obtained by transesterification of CRM 162 to check accuracy (duplicates at least). On each working day a freshly derivatized sub-sample of CRM 162 has to be used.

Provided the following conditions were fulfilled, participants were entitled to proceed to the analysis of the test materials

- resolution (R) of critical pairs > 1.0
- separation of the geometrical isomers of C18:2 into 4 components (tt, ct, tc, cc) and C18:3 into 6 components (ttt, ctt+tct, ttc+cct, ctc, tcc, ccc) for the CP Sil-88 column and 7 components (ttt, ctt, tct, ttc, cct, ctc, tcc, ccc) for the BPX-70 column.
- the average concentration of individual FAME (y) of CRM 162 were within the range:

$$(\text{certified value} - 2\ SD) < y < (\text{certified value} + 2\ SD)$$

The actually found values of CRM 162 (soya/maize oil blend) had to be within the range (g/100 g total FAME) listed in Table 6.

FAME	Certified mean	Standard deviation	Range (mean±2 SD)
C16:0	10.65	0.279	10.09 - 11.21
C18:0	2.87	0.123	2.63 - 3.11
C18:1	24.14	0.392	23.36 - 24.92
C18:2	56.66	0.760	55.14 - 58.18
C18:3	4.68	0.309	4.06 - 5.30

Table 6: Certified values and ±2 SD of individual fatty acids (g/100 g of total FAME) of CRM 162.

Test materials:

On each working day a fresh ampoule of the material to be tested had to be opened, transesterified and the FAME mixture injected twice into the GC. Ampoules had to be warmed by immersion in a waterbath set to 45-50 °C until the materials were liquid. Then the contents had to be mixed by repeated inversion for not less than 30 s, the ampoule opened and the contents transferred to a clean vial, which could be tightly sealed.

The transesterification method usually applied in the laboratory of the participants had to be used for derivative formation and the obtained FAME mix separated by capillary GC using optimised operational parameters. Analytical conditions applied by the laboratories are summarised in Table 5.

FAMEs were identified by retention time matching using the elaidinized linseed oil as a reference and the contents of individual FAME had to be reported by using reporting sheets provided by the Co-ordinator.

Trans-monoenes in the 5 % and 30 % TFA samples had to be separated by silver-chromatography (two independent fractionations), and the recovered fractions

containing saturates plus trans-monoenes separated by GC (duplicate determinations). The calculation of the trans-monoene content had to be based on the content of C18:0 in the unfractionated and the fractionated sample obtained on the same working day. The mean value of C18:0 of the duplicate runs of the original FAME solution had to be used to compute the trans-monoene content.

Six independent results (duplicates obtained on three separate working days) per TFA level had to be submitted to the Co-ordinator.

Results

Before proceeding to the statistical evaluation of the intercomparison, results were checked for plausibility and missing data. Next the operational qualification tests were evaluated; one of the laboratories did not pass the resolution check and another one failed to pass the quantitation check (Table 7).

Lab	Resolution		Quantitation	
	passed	failed	passed	failed
1	x		x	
2		x	x	
3	x		x	
4	x		x	
5	x		x	
6	x		x	
7	x		x	
8	x		x	
9	x		x	
10	x		x	
11	x		x	
12	x			x

Table 7: Performance qualification of the GLC systems used by the participants.

Laboratory means for total TFA content, plotted in increasing order, are depicted in Figures 30-32, and repeatability (r) and reproducibility (R) of trans-isomers of C18:1, C18:2 and C18:3 are summarised in Tables 8-10. No lab was removed

from the data sets, and the statistical evaluation was performed as specified in ISO 5725.

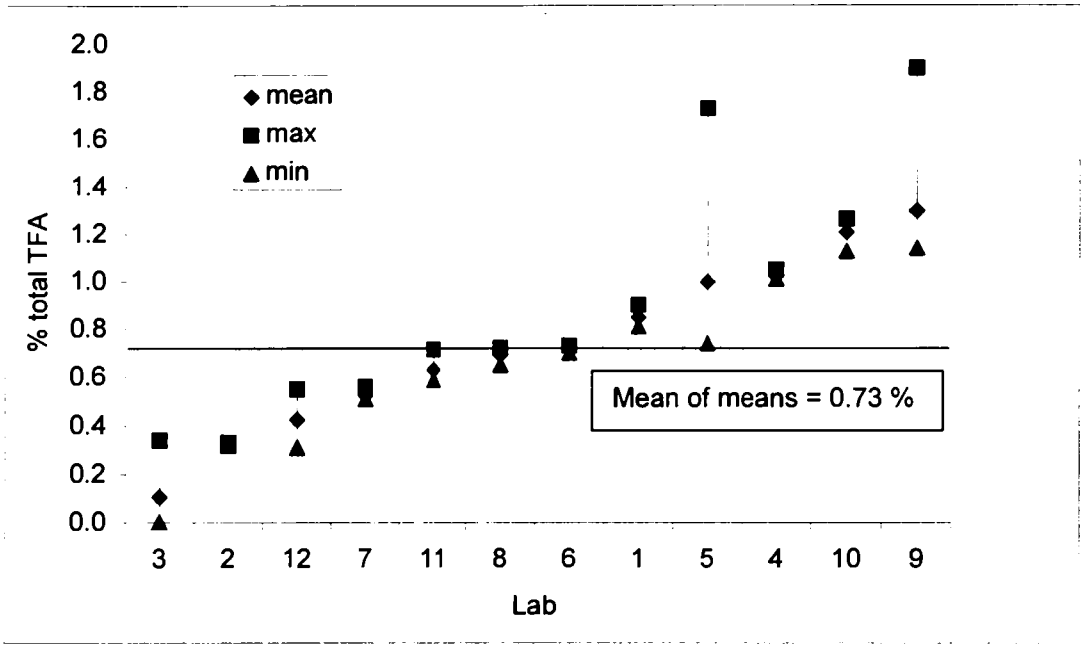


Figure 30: Laboratory means (raw data, total TFA) of the 1 % TFA sample.

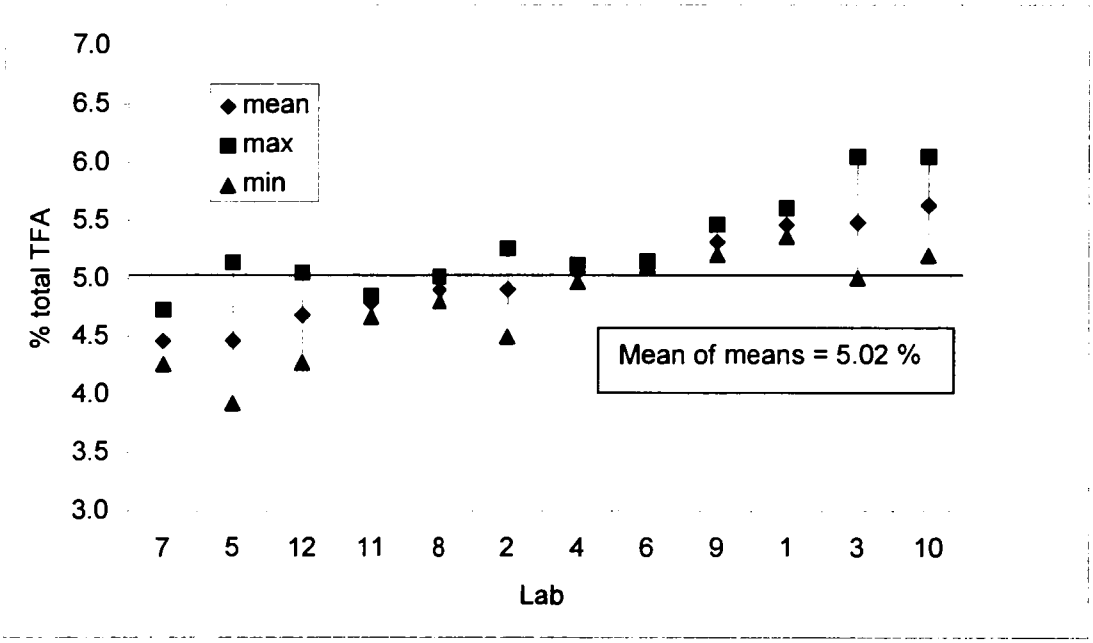


Figure 31: Laboratory means (raw data, total TFA) of the 5 % TFA sample.

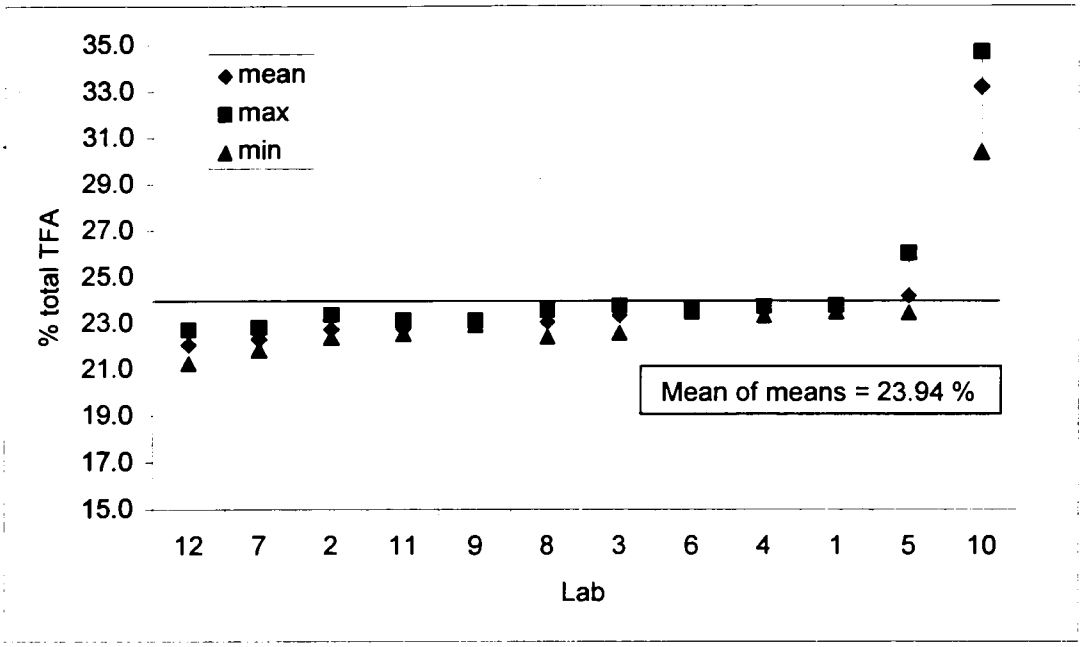


Figure 32: Laboratory means (raw data, total TFA) of the 30 % TFA sample.

	C18:1	C18:2	C18:3	Total
mean	0.09	0.11	0.53	0.73
min	0.00	0.00	0.11	0.11
max	0.82	0.35	1.02	1.30
s_r	0.02	0.08	0.12	0.16
r	0.06	0.23	0.34	0.45
RSD(r)	23	75	22	22
s_R	0.23	0.12	0.29	0.38
R	0.65	0.35	0.83	1.09
RSD(R)	247	115	55	53

Table 8: Statistical evaluation of the results submitted for the 1 % TFA sample.

	C18:1	C18:2	C18:3	Total
mean	3.75	1.15	0.12	5.02
min	3.10	0.88	0.00	4.46
max	4.70	1.40	0.46	5.63
s_r	0.22	0.10	0.04	0.25
r	0.62	0.27	0.10	0.70
RSD(r)	6	8	29	5
s_R	0.50	0.17	0.14	0.45
R	1.40	0.49	0.40	1.26
RSD(R)	13	15	116	9

Table 9: Statistical evaluation of the results submitted for the 5 % TFA sample.

	C18:1	C18:2	C18:3	Total
mean	19.92	3.96	0.06	23.94
min	17.91	3.33	0.00	22.06
max	29.22	4.96	0.34	33.18
s_r	0.66	0.19	0.03	0.60
r	1.87	0.54	0.09	1.69
RSD(r)	3	5	54	3
s_R	3.04	0.50	0.11	3.01
R	8.59	1.42	0.30	8.53
RSD(R)	15	13	179	13

Table 10: Statistical evaluation of the results submitted for the 30 % TFA sample.

To make comparisons among analyte levels easier, results were standardised by the following transformation:

$z = (\bar{x}-\bar{X})/s_R$

$\bar{x}$ ..... mean value of individual laboratory

$\bar{X}$..... overall mean value

s_R.... reproducibility standard deviation

An analytical system can be described as "well behaved" when absolute values of $z < 2$ are commonly found and values of $z > 3$ are rarely found in ring-trials (Thompson & Wood, J. AOAC Internat. (1993) 76: 926-940). Z-scores of trans C18:1, trans C18:2 and trans C18:3 contents of the three materials are plotted in Figures 33-35.

The trans-C18:1 content in all three materials were constantly over-estimated by lab 10 (z-score > 3, Figure 24). Some of the labs (4, 5, 7, 9) failed to identify traces of trans C18:2 and C18:3 correctly as judged by their increased z-scores. In general, precision was not satisfactory (RSD(R) > 40 %) for analyte levels < 1 g/100 g (trans monoenes in the 1 % TFA sample and trans polyenes in the 5 % and 30 % TFA samples), whereas higher levels were quantified with good analytical precision. Incorrect identification of trans polyenes was identified as a major source of error. Furthermore, an inconsistent reporting format of low analyte

levels (e.g. less than 0.05 %) used by some laboratories added to the high variations seen for the minor compounds.

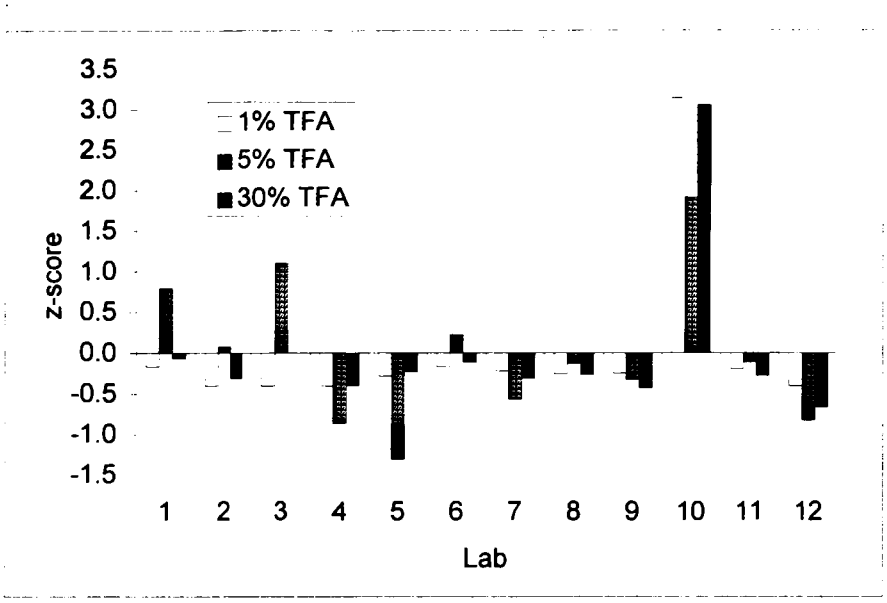


Figure 33: Performance of individual laboratories assessed by z-scores for trans C18:1.

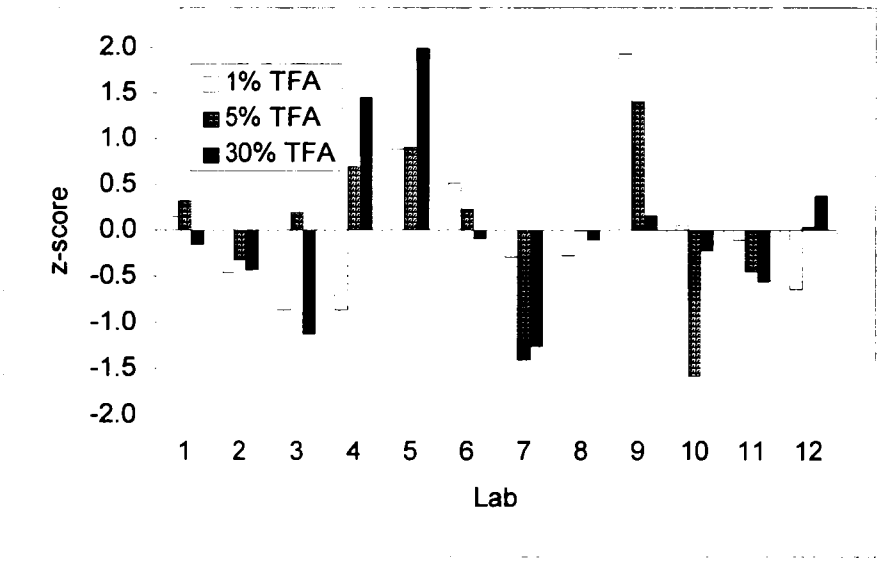


Figure 34: Performance of individual laboratories assessed by z-scores for trans C18:2.

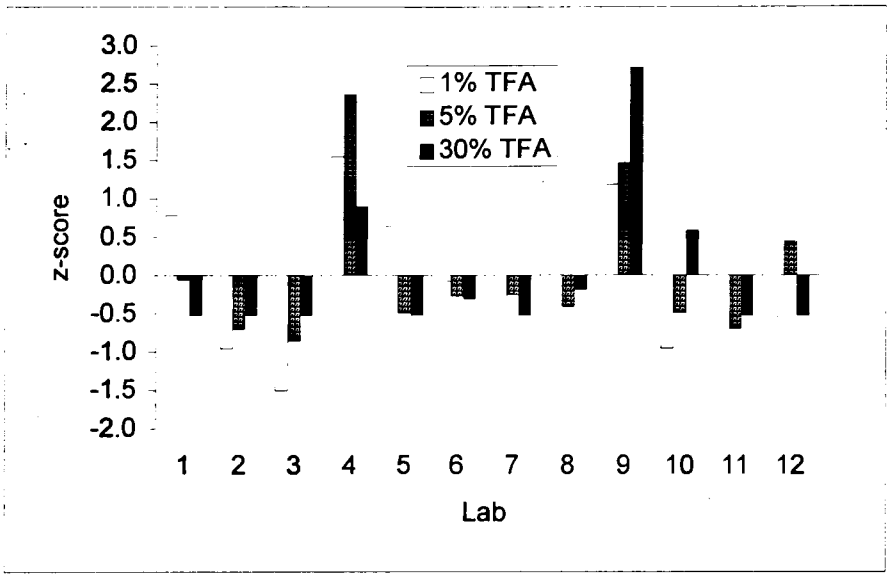


Figure 35: Performance of individual laboratories assessed by z-scores for trans C18:3.

Supportive methods to augment results obtained by chromatography

IR spectroscopy:

Those participants applying IR spectroscopy in addition to chromatographic techniques had to set up the experiments as agreed on during the last meeting, i.e. elaidic acid in form of its methyl ester or as triglyceride had to be used for instrument calibration. Either the underivatized or the derivatized sample (in form of FAME) had to be used to quantitate TFA by IR spectroscopy. Experimental conditions used by the participating laboratories are summarised in Table 11 and the experimental set up which had to be followed is outlined in Figure 36.

Lab	Measuring principle	Measured as
1	FT-IR	FAME
3	conventional IR	FAME
6	FT-IR; ATR	FAME
8	FT-IR; ATR	Triglycerides
12	FT-IR; ATR	FAME

Table 11: Experimental conditions used for TFA determination by (FT)IR.

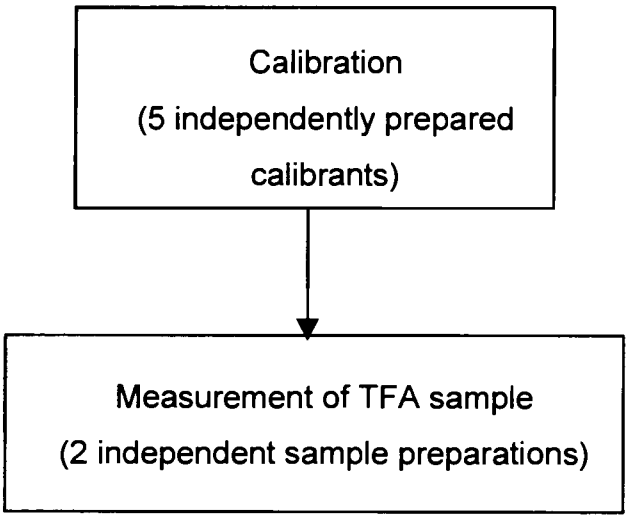


Figure 36: Experimental set up of IR measurements for the quantification of TFA.

Data submitted were checked for plausibility but no further statistical treatment according to ISO 5725 was performed, since only 4 labs participated in this exercise. Laboratory means and standard deviation values are listed in Tables 12-14.

Lab	mean	SD	RSD (%)
1	0.86	0.50	59
3	*)		
6	0.33	0.03	10
8	-0.96	0.13	14
12	0.08		
mean	0.08	0.76	980
TFA by Ag/GLC	0.73	0.36	49

*) below limit of quantitation

Table 12: Concentration of TFA in the 1 % sample determined by IR spectrometry.

Lab	mean	SD	RSD (%)
1	5.65	0.82	15
3	6.10	0.06	0.9
6	5.62	0.04	0.7
8	4.28	0.13	3
12	4.74		
mean	5.28	0.75	14
TFA by Ag/GLC	5.02	0.39	8

Table 13: Concentration of TFA in the 5 % sample determined by IR spectrometry.

Lab	mean	SD	RSD (%)
1	23.81	1.00	4
3	24.28	0.08	0.4
6	24.45	0.15	0.6
8	23.53	0.28	1
12	23.64		
mean	23.94	0.41	2
TFA by Ag/GLC	23.94	2.97	12

Table 14: Concentration of TFA in the 30 % sample determined by IR spectrometry.

In general, precision of TFA analyses was satisfactory for the medium and high level samples (RSD < 40 %) but not at the low level. IR produced unreliable

results for the low level sample, which is in general agreement with the literature. However, mean values at the medium and high level augmented the trueness of the results obtained by chromatographic methods (5.28 % by IR *vs.* 5.02 % by chromatography, and 23.94 % by IR *vs.* 23.94 % by chromatography).

NMR spectroscopy

Two laboratories conducted NMR measurements. Both reported that the limit of quantification is 3.0 mol-% (using a sample size of 100 mg/0.5 ml $CDCl_3$). Thus measurements were made with the 5 % and 30 % TFA samples. Laboratory 1 obtained results for two different sets of experimental conditions, whereas lab 2 used three sets.

Results for the 5 % TFA sample are summarised in Table 15 and for the 30 % TFA sample in Table 16. Inverse-gated proton decoupling and a 90 degree pulse (method 1 used by lab 1 and method 3 used by lab 2) produced results which were in close agreement to the results obtained by chromatography.

	Lab 1		Lab 2		
	Method 1	**Method 2**	**Method 1**	**Method 2**	**Method 3**
trans-monoenes	4.0/4.1	3.5/3.6	4.1	2.6	3.8
trans-polyenes	nd[*)]	nd	nd	nd	nd
trans-total	4.0/4.1	3.5/3.6	4.1	2.6	3.8
chromatographically obtained					
trans-monoenes		3.81			
trans-total		5.00			

[*)] not detected

Table 15: Concentration of TFA (mol-%) in the 5 % sample obtained by NMR.

	Lab 1		Lab 2		
	Method 1	**Method 2**	**Method 1**	**Method 2**	**Method 3**
trans-monoenes	13.6/19.3	15.8/16.6	30.8	17.0	19.2
trans-polyenes	2.8/4.8	4.1/4.9	2.9	4.5	3.90
trans-total	18.4/22.1	19.9/21.5	33.7	21.5	23.1
chromatographically obtained					
trans-monoenes		19.08			
trans-total		22.92			

Table 16: Concentration of TFA (mol-%) in the 30 % sample obtained by NMR.

In conclusion, TFA results obtained by spectroscopic methods agreed reasonably well and are not in contrast with those obtained by chromatographic methods (Table 17). Therefore, it is concluded that the analytical approach which will be used for the certification the TFA content in oily candidate RMs, i.e. Ag-chromatography/GLC, is not systematically biased.

	Ag/GLC	**IR**	**NMR**
1 % TFA	0.73	0.08	nd[*)]
5 % TFA	5.02	5.41	3.97
30 % TFA	23.94	23.94	21.68

[*)] not detected

Table 17: Comparison of methods for the quantification of TFA.

CONCLUSIONS

- Precision for analyte levels > 1 g/100 g was satisfactory (RSD(R) < 40 %), while it was unacceptable for analyte levels < 1 g/100 g.
- Incorrect identification of trans polyenes was a major source of error which led to deviating results.
- Results obtained by different methods (chromatography, IR, NMR) agreed sufficiently.
- Chromatographic methods were not systematically biased.

MEASURES TO BE TAKEN

- The intercomparison has to be repeated with a new set of samples. A similar test set-up will be used with the exception that per ampoule two separate transesterifications will be performed
- Identification of trans-polyenes has to be done by using equivalent chain length (ECL) values. The Co-ordinator will distribute these values for the CP Sil-88 and the BPX70 column. It is anticipated that this measure should facilitate the correct identification of trans polyenes and thus improve precision.
- Area-% values have to be reported for all peaks attributable to fatty acid methyl esters regardless of the peak size
- An electronic spreadsheet will be distributed by the Co-ordinator in order to reduce data manipulation steps and, therefore, transcription errors

ANNEX A

Stability of the test materials stored at different temperatures.

			Absorbance at (nm)					
Storage time (d)	AV	POV	232	268	278	C18:1	C18:2	C18:3
0	0.08	0.58	3.19	1.31	1.07	39.60	22.69	0.19
14	0.02	1.75	3.57	1.46	1.23	39.62	22.65	0.19
29	0.07	2.28	3.92	1.44	1.20	39.79	22.44	0.19
42	0.04	1.99	3.40	1.33	1.11	39.87	22.56	0.19
56	0.14	1.56	3.37	1.36	1.12	40.08	22.58	0.18
70	0.10	1.11	3.31	1.33	1.08	39.89	22.38	0.18
84	0.13	1.22	3.42	1.37	1.12	39.94	22.42	0.19
98	0.11	0.96	3.38	1.34	1.10	39.85	22.39	0.19
112	0.12	0.74	3.32	1.34	1.09	39.96	22.36	0.18
127	0.10	0.45	3.35	1.34	1.10	39.99	22.50	0.18

Table A1: Changes in stability parameters and the FA profile (area-%) of the 5 % TFA sample (blend of partially hydrogenated sunflower seed oil and palm oil) stored at 70 °C.

			Absorbance at (nm)					
Storage time (d)	AV	POV	232	268	278	C18:1	C18:2	C18:3
0	0.08	0.58	3.19	1.31	1.07	39.60	22.69	0.19
14	0.07	1.12	3.44	1.43	1.20	39.42	22.45	0.20
29	0.07	1.23	3.26	1.49	1.26	38.95	22.09	0.19
42	0.07	1.48	3.29	1.30	1.07	39.87	22.56	0.19
57	0.08	1.55	3.33	1.41	1.17	39.93	22.53	0.18
71	0.08	1.37	3.21	1.31	1.06	39.83	22.49	0.19
85	0.07	1.52	3.15	1.16	0.91	39.86	22.50	0.09
99	0.07	1.56	3.32	1.32	1.06	39.78	22.45	0.18
113	0.09	1.66	3.33	1.33	1.08	39.82	22.38	0.18
128	0.08	1.49	3.30	1.32	1.07	40.01	22.47	0.18

Table A2: Changes in stability parameters and the FA profile (area-%) of the 5 % TFA sample (blend of partially hydrogenated sunflower seed oil and palm oil) stored at 40 °C.

			Absorbance at (nm)					
Storage time (d)	**AV**	**POV**	**232**	**268**	**278**	**C18:1**	**C18:2**	**C18:3**
0	0.08	0.58	3.19	1.31	1.07	39.60	22.69	0.19
31	0.08	1.10	3.22	1.26	1.02	39.77	22.51	0.20
58	0.04	0.95	3.21	1.29	1.05	40.00	22.55	0.18
86	0.09	0.98	3.25	1.30	1.06	39.95	22.38	0.19
114	0.05	1.14	3.23	1.30	1.06	39.89	22.33	0.18

Table A3: Changes in stability parameters and the FA profile (area-%) of the 5 % TFA sample (blend of partially hydrogenated sunflower seed oil and palm oil) stored at 20 °C.

			Absorbance at (nm)					
Storage time (d)	**AV**	**POV**	**232**	**268**	**278**	**C18:1**	**C18:2**	**C18:3**
0	0.08	0.58	3.195	1.309	1.074	39.60	22.69	0.19
30	0.07	1.08	3.237	1.315	1.074	39.80	22.54	0.19
59	0.06	1.20	3.195	1.297	1.057	39.76	22.45	0.18
87	0.07	0.79	3.267	1.306	1.063	39.94	22.35	0.19
115	0.08	0.85	3.215	1.315	1.071	39.94	22.32	0.19

Table A4: Changes in stability parameters and the FA profile (area-%) of the 5 % TFA sample (blend of partially hydrogenated sunflower seed oil and palm oil) stored at 4 °C.

			Absorbance at (nm)					
Storage time (d)	**AV**	**POV**	**232**	**268**	**278**	**C18:1**	**C18:2**	**C18:3**
0	0.08	0.58	3.19	1.31	1.07	39.60	22.69	0.19
63	0.07	1.10	3.31	1.33	1.08	39.94	22.42	0.18
120	0.05	0.73	3.24	1.31	1.06	39.93	22.45	0.19

Table A5: Changes in stability parameters and the FA profile (area-%) of the 5 % TFA sample (blend of partially hydrogenated sunflower seed oil and palm oil) stored at -20 °C.

			Absorbance at (nm)					
Storage time (d)	AV	POV	232	268	278	C18:1	C18:2	C18:3
0	0.10	0.10	3.084	0.594	0.514	62.26	19.53	9.20
14	0.09	2.13	3.402	0.681	0.601	62.18	19.73	9.18
29	0.02	1.98	3.140	0.794	0.724	62.04	19.72	9.29
42	0.02	1.09	3.251	0.725	0.637	62.21	19.58	9.18
56	0.10	0.86	3.186	0.671	0.578	62.23	19.71	9.26
70	0.05	0.22	3.207	0.695	0.595	62.41	19.63	9.13
84	0.10	0.28	3.223	0.696	0.588	62.51	19.65	9.16
98	0.07	0.21	3.235	0.700	0.594	62.37	19.56	9.17
112	0.04	0.10	3.212	0.657	0.558	62.41	19.61	9.12
127	0.06	0.10	3.188	0.686	0.584	62.32	19.69	9.17

Table A6: Changes in stability parameters and the FA profile (area-%) of the 1 % TFA sample (physically refined rapeseed oil) stored at 70 °C.

			Absorbance at (nm)					
Storage time (d)	AV	POV	232	268	278	C18:1	C18:2	C18:3
0	0.10	0.10	3.08	0.59	0.51	62.26	19.53	9.20
14	0.06	1.35	3.34	0.73	0.65	62.01	19.69	9.27
29	0.07	1.72	3.87	0.82	0.64	62.24	19.70	9.28
42	0.13	2.24	3.17	0.64	0.56	62.16	19.70	9.20
57	0.04	2.40	3.24	0.63	0.55	62.39	19.57	9.19
71	0.06	1.96	3.14	0.55	0.47	62.36	19.57	9.13
85	0.07	2.62	3.05	0.42	0.32	62.32	19.64	9.17
99	0.07	1.70	3.21	0.59	0.49	62.27	19.57	9.21
113	0.09	2.06	3.24	0.59	0.50	62.44	19.63	9.09
128	0.07	1.84	3.21	0.58	0.49	62.31	19.61	9.17

Table A7: Changes in stability parameters and the FA profile (area-%) of the 1 % TFA sample (physically refined rapeseed oil) stored at 40 °C.

			Absorbance at (nm)					
Storage time (d)	**AV**	**POV**	**232**	**268**	**278**	**C18:1**	**C18:2**	**C18:3**
0	0.10	0.10	3.08	0.59	0.51	62.26	19.53	9.20
31	0.11	1.35	2.95	0.47	0.39	62.08	19.67	9.25
58	0.03	1.70	3.07	0.53	0.45	62.27	19.64	9.19
86	0.07	1.58	3.11	0.56	0.47	62.17	19.60	9.22
114	0.05	1.78	3.09	0.56	0.47	62.37	19.60	9.19

Table A8: Changes in stability parameters and the FA profile (area-%) of the 1 % TFA sample (physically refined rapeseed oil) stored at 20 °C.

			Absorbance at (nm)					
Storage time (d)	**AV**	**POV**	**232**	**268**	**278**	**C18:1**	**C18:2**	**C18:3**
0	0.10	0.10	3.08	0.59	0.51	62.26	19.53	9.20
31	0.11	1.27	2.77	0.46	0.41	62.00	19.74	9.21
59	0.05	1.60	3.09	0.56	0.47	62.44	19.65	9.15
87	0.06	1.14	3.10	0.57	0.47	61.15	19.24	9.10
115	0.10	1.34	3.06	0.55	0.47	62.20	19.67	9.14

Table A9: Changes in stability parameters and the FA profile (area-%) of the 1 % TFA sample (physically refined rapeseed oil) stored at 4 °C.

			Absorbance at (nm)					
Storage time (d)	**AV**	**POV**	**232**	**268**	**278**	**C18:1**	**C18:2**	**C18:3**
0	0.10	0.10	3.08	0.59	0.51	62.26	19.53	9.20
63	0.06	1.26	3.08	0.55	0.46	62.33	19.62	9.20
120	0.05	1.17	3.07	0.55	0.47	62.24	19.65	9.25

Table A10: Changes in stability parameters and the FA profile (area-%) of the 1 % TFA sample (physically refined rapeseed oil) stored at -20 °C.

			Absorbance at (nm)					
Storage time (d)	AV	POV	232	268	278	C18:1	C18:2	C18:3
0	0.06	0.20	6.05	1.16	0.94	54.88	28.13	0.00
14	0.07	1.53	6.18	1.27	1.06	54.92	28.03	0.00
29	0.01	1.66	6.26	1.18	1.17	54.42	28.06	0.02
42	0.01	1.33	5.31	1.11	0.93	54.51	28.38	0.00
56	0.05	0.97	6.02	1.17	0.95	55.35	27.64	0.01
70	0.02	0.42	5.97	1.15	0.93	55.27	27.86	0.00
84	0.12	0.48	6.07	1.19	0.97	54.95	27.95	0.00
98	0.01	0.66	6.07	1.17	0.95	55.21	27.88	0.00
112	0.03	0.20	5.74	1.13	0.90	55.24	27.93	0.00
127	0.04	0.10	6.04	1.17	0.95	55.21	27.95	0.00

Table A11: Changes in stability parameters and the FA profile (area-%) of the 30 % TFA sample (partially hydrogenated sunflower seed oil) stored at 70 °C.

			Absorbance at (nm)					
Storage time (d)	AV	POV	232	268	278	C18:1	C18:2	C18:3
0	0.06	0.20	6.05	1.16	0.94	54.88	28.13	0.00
14	0.01	0.79	6.11	1.27	1.06	55.22	28.25	0.00
30	0.01	1.03	6.20	1.05	0.99	54.40	28.31	0.00
42	0.02	1.17	6.30	1.15	0.96	54.51	28.38	0.00
57	0.03	1.30	6.06	1.22	1.00	55.18	27.89	0.00
71	0.04	1.21	5.92	1.11	0.88	55.35	27.81	0.00
85	0.04	1.38	5.84	0.96	0.74	55.05	27.97	0.00
99	0.03	1.80	6.05	1.13	0.90	54.99	27.95	0.00
113	0.04	1.36	6.02	1.14	0.91	55.04	28.11	0.00
128	0.07	1.63	6.02	1.13	0.90	55.04	28.13	0.00

Table A12: Changes in stability parameters and the FA profile (area-%) of the 30 % TFA sample (partially hydrogenated sunflower seed oil) stored at 40 °C.

			UV absorbance at (nm)					
Storage time (d)	AV	POV	232	268	278	C18:1	C18:2	C18:3
0	0.06	0.20	6.05	1.16	0.94	54.88	28.13	0.00
30	0.05	0.65	6.19	1.05	0.84	54.44	28.01	0.04
58	0.02	0.86	5.93	1.07	0.86	55.15	28.14	0.00
86	0.08	0.92	5.95	1.11	0.89	55.36	27.90	0.00
114	0.03	1.08	5.96	1.12	0.90	55.19	27.82	0.00

Table A13: Changes in stability parameters and the FA profile (area-%) of the 30 % TFA sample (partially hydrogenated sunflower seed oil) stored at 20 °C.

			UV absorbance at (nm)					
Storage time (d)	AV	POV	232	268	278	C18:1	C18:2	C18:3
0	0.06	0.20	6.05	1.16	0.94	54.88	28.13	0.00
30	0.08	0.61	5.91	1.03	0.82	56.38	27.80	0.02
59	0.03	0.91	5.91	1.10	0.89	55.44	27.82	0.01
87	0.03	0.75	5.94	1.10	0.89	55.25	28.07	0.02
115	0.06	0.75	5.92	1.11	0.89	54.98	28.16	0.03

Table A14: Changes in stability parameters and the FA profile (area-%) of the 30 % TFA sample (partially hydrogenated sunflower seed oil) stored at 4 °C.

			UV absorbance at (nm)					
Storage time (d)	AV	POV	232	268	278	C18:1	C18:2	C18:3
0	0.06	0.20	6.05	1.16	0.94	54.88	28.13	0.00
63	0.03	0.61	5.91	1.10	0.89	55.18	28.03	0.03
120	0.04	0.48	5.95	1.10	0.89	55.06	27.99	0.03

Table A15: Changes in stability parameters and the FA profile (area-%) of the 30 % TFA sample (partially hydrogenated sunflower seed oil) stored at -20 °C.

ANNEX B

Fatty acid composition of CRM162 as obtained by the participant in the intercomparison

Lab	C16:0	C18:0	C18:1	C18:2	C18:3
1	10.38 ± 0.09	2.95 ± 0.05	24.03 ± 0.03	55.75 ± 0.06	4.32 ± 0.02
2	10.48 ± 0.02	2.93 ± 0.00	24.27 ± 0.01	56.30 ± 0.03	4.43 ± 0.01
3	10.82 ± 0.13	2.91 ± 0.17	24.66 ± 0.16	57.14 ± 0.23	4.23 ± 0.13
4	10.77 ± 0.09	2.77 ± 0.02	24.00 ± 0.06	56.78 ± 0.16	4.62 ± 0.02
5	10.79 ± 0.10	2.91 ± 0.01	24.08 ± 0.02	55.96 ± 0.11	4.84 ± 0.00
6	10.46 ± 0.01	2.93 ± 0.00	24.20 ± 0.04	55.88 ± 0.04	4.80 ± 0.01
7	11.10 ± 0.11	2.81 ± 0.03	24.20 ± 0.08	56.42 ± 0.10	4.75 ±0.04
8	10.67 ± 0.11	2.85 ± 0.02	24.18 ± 0.05	56.46 ± 0.08	4.75 ± 0.02
9	10.43 ± 0.06	2.80 ± 0.08	23.96 ± 0.14	56.61 ± 0.40	4.86 ± 0.18
10	10.91 ± 0.11	2.69 ± 0.07	24.35 ± 0.23	56.31 ± 0.04	4.15 ± 0.02
11	10.24 ± 0.13	2.85 ± 0.03	24.15 ± 0.13	57.07 ± 0.25	4.65 ± 0.08
12	11.65 ± 0.46	2.84 ± 0.06	24.15 ± 0.09	56.39 ± 0.34	4.94 ± 0.17

Table B1: Fatty acid profile of CRM162 as analysed by the participants (mean value ± SD given in g/100 g total FAME).

European Commission

EUR 18955 - INTERCOMPARISON OF METHODS FOR THE DETERMINATION OF TRANS FATTY ACIDS IN EDIBLE OILS AND FATS AND CERTIFICATION OF THREE MATERIALS CONTAINING TRANS FATTY ACIDS
Report on the First Intercomparison

F. Ulberth, M. Buchgraber and A. Boenke

Luxembourg: Office for Official Publications of the European Communities

1999 54pp, num. tab., fig. - 21.0x29.7 cm

BCR information series

ISBN 92-828-2248-6

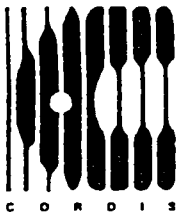
The aim of the first intercomparison was twofold. Firstly, to select, stabilise and bottle suitable materials containing ca. 1 %, 5 % and 30 % total trans fatty acids (TFA) and demonstrate that packaging and storage does not affect oil composition, and secondly, to demonstrate that participating laboratories are able to achieve sufficient conformity of test results.

Stabilising oil samples with *tert.* butylhydroquinone (250 mg/kg) and packaging in inert atmosphere in amber glass ampoules resulted in homogeneous and stable lots of preliminary test materials.

Analytical precision of chromatographic methods to determine trans fatty acids (TFA) was acceptable (RSD(R) < 15 %) for analyte levels > 1 g/100 g, but for levels < 1 g/100 g precision was beyond the agreed acceptance criterion (RSD(R) > 40 %). Misidentification of peaks and inconsistent peak integration practices are considered to be the major reasons for deviating results reported by the participants.

Results obtained by chromatographic methods were in close agreement with those produced by spectroscopy (IR and NMR), thus proving that the applied chromatographic methods were not systematically biased.

The intercomparison has to be repeated with new sets of samples. Special emphasis will be placed on the correct identification of trans polyenes in order to improve precision for low levels of TFA. To facilitate correct identification, the Co-ordinator will distribute equivalent chain length data for TFA.



The Communities research and development information service

CORDIS

A vital part of your programme's dissemination strategy

CORDIS is the information service set up under the VALUE programme to give quick and easy access to information on European Community research programmes. It is available free-of-charge online via the European Commission host organization (ECHO), and now also on a newly released CD-ROM.

CORDIS offers the European R&D community:

— a comprehensive up-to-date view of EC R&TD activities, through a set of databases and related services,

— quick and easy access to information on EC research programmes and results,

— a continuously evolving Commission service tailored to the needs of the research community and industry,

— full user support, including documentation, training and the CORDIS help desk.

The CORDIS Databases are:

R&TD-programmes – R&TD-projects – R&TD-partners – R&TD-results
R&TD-publications – R&TD-comdocuments – R&TD-acronyms – R&TD-news

Make sure your programme gains the maximum benefit from CORDIS

— Inform the CORDIS unit of your programme initiatives,

— contribute information regularly to CORDIS databases such as R&TD-news, R&TD-publications and R&TD-programmes,

— use CORDIS databases, such as R&TD-partners, in the implementation of your programme,

— consult CORDIS for up-to-date information on other programmes relevant to your activities,

— inform your programme participants about CORDIS and the importance of their contribution to the service as well as the benefits which they will derive from it,

— contribute to the evolution of CORDIS by sending your comments on the service to the CORDIS Unit.

For more information about contributing to CORDIS, contact the DG XIII CORDIS Unit

Brussels
Ms I. Vounakis
Tel. +(32) 2 299 0464
Fax +(32) 2 299 0467

Luxembourg
M. B. Niessen
Tel. +(352) 4301 33638
Fax +(352) 4301 34989

To register for online access to CORDIS, contact:

ECHO Customer Service
BP 2373
L-1023 Luxembourg
Tel. +(352) 3498 1240
Fax +(352) 3498 1248

If you are already an ECHO user, please mention your customer number.

Venta • Salg • Verkauf • Πωλήσεις • Sales • Vente • Vendita • Verkoop • Venda • Myynti • Försäljning

BELGIQUE/BELGIË

Jean De Lannoy
Avenue du Roi 202/Koningslaan 202
B-1190 Bruxelles/Brussel
Tél. (32-2) 538 43 08
Fax (32-2) 538 08 41
E-mail: jean.de.lannoy@infoboard.be
URL: http://www.jean-de-lannoy.be

La librairie européenne/De Europese Boekhandel
Rue de la Loi 244/Wetstraat 244
B-1040 Bruxelles/Brussel
Tél. (32-2) 295 26 39
Fax (32-2) 735 08 60
E-mail: mail@libeurop.be
URL: http://www.libeurop.be

Moniteur belge/Belgisch Staatsblad
Rue de Louvain 40-42/Leuvenseweg 40-42
B-1000 Bruxelles/Brussel
Tél. (32-2) 552 22 11
Fax (32-2) 511 01 84

DANMARK

J. H. Schultz Information A/S
Herstedvang 10-12
DK-2620 Albertslund
Tlf. (45) 43 63 23 00
Fax (45) 43 63 19 69
E-mail: schultz@schultz.dk
URL: http://www.schultz.dk

DEUTSCHLAND

Bundesanzeiger Verlag GmbH
Vertriebsabteilung
Amsterdamer Straße 192
D-50735 Koln
Tel. (49-221) 97 66 80
Fax (49-221) 97 66 82 78
E-Mail: vertrieb@bundesanzeiger.de
URL: http://www.bundesanzeiger.de

ΕΛΛΑΔΑ/GREECE

G. C. Eleftheroudakis SA
International Bookstore
Panepistimiou 17
GR-10564 Athina
Tel. (30-1) 331 41 80/1/2/3/4/5
Fax (30-1) 323 98 21
E-mail: elebooks@netor.gr

ESPAÑA

Boletín Oficial del Estado
Trafalgar, 27
E-28071 Madrid
Tel. (34) 915 38 21 11 (Libros)/
913 84 17 15 (Suscripciones)
Fax (34) 915 38 21 21 (Libros)/
913 84 17 14 (Suscripciones)
E-mail: clientes@com.boe.es
URL: http://www.boe.es

Mundi Prensa Libros, SA
Castelló, 37
E-28001 Madrid
Tel. (34) 914 36 37 00
Fax (34) 915 75 39 98
E-mail: libreria@mundiprensa.es
URL: http://www.mundiprensa.com

FRANCE

Journal officiel
Service des publications des CE
26, rue Desaix
F-75727 Paris Cedex 15
Tél. (33) 140 58 77 31
Fax (33) 140 58 77 00

IRELAND

Government Supplies Agency
Publications Section
4-5 Harcourt Road
Dublin 2
Tel. (353-1) 661 31 11
Fax (353-1) 475 27 60

ITALIA

Licosa SpA
Via Duca di Calabria, 1/1
Casella postale 552
I-50125 Firenze
Tel. (39-55) 64 54 15
Fax (39-55) 64 12 57
E-mail: licosa@ftbcc.it
URL: http://www.ftbcc.it/licosa

LUXEMBOURG

Messageries du livre SARL
5, rue Raiffeisen
L-2411 Luxembourg
Tel. (352) 40 10 20
Fax (352) 49 06 61
E-mail: mdl@pt.lu
URL: http://www.mdl.lu

Abonnements:

Messageries Paul Kraus
11, rue Christophe Plantin
L-2339 Luxembourg
Tel. (352) 49 98 88-8
Fax (352) 49 98 88-444
E-mail: mpk@pt.lu
URL: http://www.mpk.lu

NEDERLAND

SDU Servicecentrum Uitgevers
Christoffel Plantijnstraat 2
Postbus 20014
2500 EA Den Haag
Tel. (31-70) 378 98 80
Fax (31-70) 378 97 83
E-mail: sdu@sdu.nl
URL: http://www.sdu.nl

ÖSTERREICH

Manz'sche Verlags- und Universitätsbuchhandlung GmbH
Kohlmarkt 16
A-1014 Wien
Tel. (43-1) 53 16 11 00
Fax (43-1) 53 16 11 67
E-Mail: bestellen@manz.co.at
URL: http://www.austria.EU.net:81/manz

PORTUGAL

Distribuidora de Livros Bertrand Ld.ª
Grupo Bertrand, SA
Rua das Terras dos Vales, 4-A
Apartado 60037
P-2700 Amadora
Tel. (351-2) 495 90 50
Fax (351-2) 496 02 55

Imprensa Nacional-Casa da Moeda, EP
Rua Marquês Sá da Bandeira, 16-A
P-1050 Lisboa Codex
Tel. (351-1) 353 03 99
Fax (351-1) 353 02 94
E-mail: del.incm@mail.telepac.pt
URL: http://www.incm.pt

SUOMI/FINLAND

Akateeminen Kirjakauppa/Akademiska Bokhandeln
Keskuskatu 1/Centralgatan 1
PL/PB 128
FIN-00101 Helsinki/Helsingfors
P./tfn (358-9) 121 44 18
F./fax (358-9) 121 44 35
Sähköposti: akatilaus@stockmann.fi
URL: http://www.akateeminen.com

SVERIGE

BTJ AB
Traktorvägen 11
S-221 82 Lund
Tfn. (46-46) 18 00 00
Fax (46-46) 30 79 47
E-post: btjeu-pub@btj.se
URL: http://www.btj.se

UNITED KINGDOM

The Stationery Office Ltd
International Sales Agency
51 Nine Elms Lane
London SW8 5DR
Tel. (44-171) 873 90 90
Fax (44-171) 873 84 63
E-mail: ipaenquiries@theso.co.uk
URL: http://www.the-stationery-office.co.uk

ÍSLAND

Bokabud Larusar Blöndal
Skólavördustig, 2
IS-101 Reykjavik
Tel. (354) 551 56 50
Fax (354) 552 55 60

NORGE

Swets Norge AS
Østenjoveien 18
Boks 6512 Etterstad
N-0606 Oslo
Tel. (47-22) 97 45 00
Fax (47-22) 97 45 45

SCHWEIZ/SUISSE/SVIZZERA

Euro Info Center Schweiz
c/o OSEC
Stamplenbachstraße 85
PF 492
CH-8035 Zürich
Tel. (41-1) 365 53 15
Fax (41-1) 365 54 11
E-mail: eics@osec.ch
URL: http://www.osec.ch/eics

BÁLGARIJA

Europress Euromedia Ltd
59, blvd Vitosha
BG-1000 Sofia
Tel. (359-2) 980 37 66
Fax (359-2) 980 42 30
E-mail: Milena@mbox.cit.bg

ČESKÁ REPUBLIKA

ÚSIS
NIS-prodejna
Havelkova 22
CZ-130 00 Praha 3
Tel. (420-2) 24 23 14 86
Fax (420-2) 24 23 11 14
E-mail: nkposp@dec.nis.cz
URL: http://www.nis.cz

CYPRUS

Cyprus Chamber of Commerce and Industry
PO Box 1455
CY-1509 Nicosia
Tel. (357-2) 66 95 00
Fax (357-2) 66 10 44
E-mail: info@ccci.org.cy

EESTI

Eesti Kaubandus-Tööstuskoda (Estonian Chamber of Commerce and Industry)
Toom-Kooli 17
EE-0001 Tallinn
Tel. (372) 646 02 44
Fax (372) 646 02 45
E-mail: einfo@koda.ee
URL: http://www.koda.ee

MAGYARORSZÁG

Euro Info Service
Európa Ház
Margitsziget
PO Box 475
H-1396 Budapest 62
Tel. (36-1) 350 80 25
Fax (36-1) 350 90 32
E-mail: euroinfo@mail.matav.hu
URL: http://www.euroinfo.hu/index.htm

MALTA

Miller Distributors Ltd
Malta International Airport
PO Box 25
Luqa LQA 05
Tel. (356) 66 44 88
Fax (356) 67 67 99
E-mail: gwirth@usa.net

POLSKA

Ars Polona
Krakowskie Przedmiescie 7
Skr. pocztowa 1001
PL-00-950 Warszawa
Tel. (48-22) 826 12 01
Fax (48-22) 826 62 40
E-mail: ars_pol@bevy.hsn.com.pl

ROMÂNIA

Euromedia
Str. G-ral Berthelot Nr 41
RO-70749 Bucuresti
Tel. (40-1) 315 44 03
Fax (40-1) 315 44 03

SLOVAKIA

Centrum VTI SR
Nám. Slobody, 19
SK-81223 Bratislava
Tel. (421-7) 531 83 64
Fax (421-7) 531 83 64
E-mail: europ@tbbl.sltk.stuba.sk
URL: http://www.sltk.stuba.sk

SLOVENIA

Gospoderski Vestnik
Dunajska cesta 5
SLO-1000 Ljubljana
Tel. (386) 611 33 03 54
Fax (386) 611 33 91 28
E-mail: repansekj@gvestnik.si
URL: http://www.gvestnik.si

TURKIYE

Dünya Infotel AS
100, Yil Mahallessi 34440
TR-80050 Bagcilar-Istanbul
Tel. (90-212) 629 46 89
Fax (90-212) 629 46 27

AUSTRALIA

Hunter Publications
PO Box 404
3067 Abbotsford, Victoria
Tel. (61-3) 94 17 53 61
Fax (61-3) 94 19 71 54
E-mail: jpdavies@ozemail.com.au

CANADA

Renouf Publishing Co. Ltd
5369 Chemin Canotek Road Unit 1
K1J 9J3 Ottawa, Ontario
Tel. (1-613) 745 26 65
Fax (1-613) 745 76 60
E-mail: order.dept@renoufbooks.com
URL: http://www.renoufbooks.com

EGYPT

The Middle East Observer
41 Sherif Street
Cairo
Tel. (20-2) 393 97 32
Fax (20-2) 393 97 32

HRVATSKA

Mediatrade Ltd
Pavla Hatza 1
HR-10000 Zagreb
Tel. (385-1) 43 03 92
Fax (385-1) 43 03 92

INDIA

EBIC India
3rd Floor, Y. B. Chavan Centre
Gen. J. Bhosale Marg.
400 021 Mumbai
Tel. (91-22) 282 60 64
Fax (91-22) 285 45 64
E-mail: ebic@giasbm01.vsnl.net.in
URL: http://www.ebicindia.com

ISRAËL

ROY International
PO Box 13056
61130 Tel Aviv
Tel. (972-3) 546 14 23
Fax (972-3) 546 14 42
E-mail: royil@netvision.net.il

Sub-agent for the Palestinian Authority:

Index Information Services
PO Box 19502
Jerusalem
Tel. (972-2) 627 16 34
Fax (972-2) 627 12 19

JAPAN

PSI-Japan
Asahi Sanbancho Plaza #206
7-1 Sanbancho, Chiyoda-ku
Tokyo 102
Tel. (81-3) 32 34 69 21
Fax (81-3) 32 34 69 15
E-mail: books@psi-japan.co.jp
URL: http://www.psi-japan.com

MALAYSIA

EBIC Malaysia
Level 7, Wisma Hong Leong
18 Jalan Perak
50450 Kuala Lumpur
Tel. (60-3) 262 62 98
Fax (60-3) 262 61 98
E-mail: ebic-kl@mol.net.my

PHILIPPINES

EBIC Philippines
19th Floor, PS Bank Tower
Sen. Gil J. Puyat Ave. cor. Tindalo St.
Makati City
Metro Manilla
Tel. (63-2) 759 66 80
Fax (63-2) 759 66 90
E-mail: eccpcom@globe.com.ph
URL: http://www.eccp.com

RUSSIA

CCEC
60-letiya Oktyabrya Av. 9
117312 Moscow
Tel. (70-95) 135 52 27
Fax (70-95) 135 52 27

SOUTH AFRICA

Safto
Safto House
NO 5 Esterhyzen Street
PO Box 782 706
2146 Sandton
Tel. (27-11) 883 37 37
Fax (27-11) 883 65 69
E-mail: emailstar@ide.co.za
URL: http://www.safto.co.za

SOUTH KOREA

Information Centre for Europe (ICE)
204 Woo Sol Parktel
395-185 Seogyo Dong, Mapo Ku
121-210 Seoul
Tel. (82-2) 322 53 03
Fax (82-2) 322 53 14
E-mail: euroinfo@shinbiro.com

THAILAND

EBIC Thailand
29 Vanissa Building, 8th Floor
Soi Chidlom
Ploenchit
10330 Bangkok
Tel. (66-2) 655 06 27
Fax (66-2) 655 06 28
E-mail: ebicbkk@ksc15.th.com
URL: http://www.ebicbkk.org

UNITED STATES OF AMERICA

Bernan Associates
4611-F Assembly Drive
Lanham MD20706
Tel. (1-800) 274 44 47 (toll free telephone)
Fax (1-800) 865 34 50 (toll free fax)
E-mail: query@bernan.com
URL: http://www.bernan.com

ANDERE LÄNDER/OTHER COUNTRIES/ AUTRES PAYS

Bitte wenden Sie sich an ein Büro Ihrer Wahl / Please contact the sales office of your choice / Veuillez vous adresser au bureau de vente de votre choix

NOTICE TO THE READER

Information on European Commission publications in the areas of research and innovation can be obtained from :

- **RTD info**
  The free of charge European Commission's magazine on European research, with the focus on project results and policy issues, issued every three months in English, French and German.
  For more information and subscription, contact :
  DG XII Communication Unit, 200 rue de la Loi, B-1049 Brussels
  Fax + 32-2-295.82.20 ; e-mail : info@dg12.cec.be

15 03 CG-NA-18-955-EN-C

OFFICE FOR OFFICIAL PUBLICATIONS
OF THE EUROPEAN COMMUNITIES

L-2985 Luxembourg

ISBN 92-828-2248-6

9 789282 822487 >