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FINAL REPORT

Fatty acid composition of the oil from traditional sunflower hybrids
Seasons: 2011-12, 2012-13 and 2013-14

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1. Introduction

The fatty acid composition of sunflower oil is strongly determined by the hybrid and by the meteorological conditions during the grain filling stage. That is why the oil from different hybrids sown at the same time may present variable fatty acid composition, while the fatty acid composition of one hybrid may vary depending on the sowing date. In this sense, both night low temperature and the amount of solar light intercepted by plant leaves throughout the grain filling (Izquierdo & Aguirrezábal, 2008; Izquierdo et al, 2009; Echarte et al, 2010) increase the oleic acid percentage and decrease that of linoleic acid. Consequently, the difference in oleic acid percentage may be, for instance, of up to 40 percentage points due to temperature variations, and of up to 10 points due to variations in the amount of light intercepted. On the other hand, different traditional hybrids may respond to temperature and light to a larger or smaller extent. The release of new sunflower hybrids on the market, along with the fact that the Argentine sunflower region -presently covering latitudes from 25 to 36°S approximately- has extended to warmer areas, has resulted in the appearance of sunflower seed oils of authentic samples with a fatty acid composition beyond the ranges set up to the moment.

Consequently, it becomes necessary to explore the fatty acid composition of the oil from different sunflower hybrids presently available on the market, grown in different areas of the country.

The objective of the present work was to perform a preliminary determination of the fatty acid composition of traditional sunflower hybrids presently grown in the northern area (latitude <

30°S) of the Argentine Republic with the aim of setting the abundance range of different fatty acids in the warmer areas of sunflower production. This characterization was performed in three stages. The first (First Report, January 2013) analyzed data of sunflower hybrids sown in two locations throughout the 2011-2012 season; the second analyzed data of hybrids sown in four locations in the 2012-13 season; and the third, samples of eight sunflower hybrids from four locations, sown in the 2013-14 season.

2. Methodology

All the reported data comes from traditional sunflower hybrids of the National Network of multi-environmental **trials** of INTA. This Network is composed of a set of around thirty-five locations and sixty experiments, spread all over the region where sunflower is grown in Argentina. The INTA's professional staff and collaborators are responsible for the choice of the experimental sites where the trials are located, weed and pest control, supervision, assessment and data collecting, collection of plant material and data analysis.

The trials comply in methodology with protocols which assure the reliability of the results. The hybrids included in each trial are selected by seed supplier companies, who choose for those they consider suitable for that environment. In addition to the competence of those responsible for conducting the trials, the Network includes an External Technical Auditing, performed by independent professionals chosen with the agreement of the involved actors. The only results that are published are those of trials which attain the standard quality criteria. All the analyzed samples come from trials complying with such criteria.

2.1. Sample origin

- **First Stage (2011-12 season)**

The data reported in the First Stage is related with samples of the Network performed during 2011-2012 season in the following locations: Reconquista (Province of Santa Fe, 29°S) and Presidencia Roque Sáenz Peña (Province of Chaco, 26°S). The experimental design was in randomized complete blocks with three replications. Each plot was composed of four rows and the experimental unit (EU) was constituted by the two central rows. At stage R9 (Schneiter & Miller, 1981), all the heads of each EU were harvested and threshed. From each EU, a 30g sub-sample was taken for oil quality measurements (oil fatty acid content). In the case of the location of P. R. S. Peña, the material analyzed was a 90g sample formed by the mix, in equal parts, of achenes coming from the three replications of each hybrid. The samples represent the combination of hybrids, locations and replications detailed in Table 1.

Table 1. *Location, hybrid and replication of each sample of the First Stage. M= mix composed of achenes from the three trial replications.*

Sample	Location	Hybrid	Replication	**Sample**	Location	Hybrid	Replication
1	Reconquista	PAN 7076	I	**16**	Reconquista	ARGENSOL 40	II
2	Reconquista	PAN 7076	II	**17**	Reconquista	CACIQUE 308 CL	I
3	Reconquista	ACA 887	I	**18**	Reconquista	CACIQUE 308 CL	II
4	Reconquista	ACA 887	II	**19**	Reconquista	SPS 3120	I
5	Reconquista	DK 4045	I	**20**	Reconquista	SPS 3120	II
6	Reconquista	DK 4045	II	**21**	Reconquista	TOBSOL 261	I
7	Reconquista	DK 4065	I	**22**	Reconquista	TOBSOL 261	II
8	Reconquista	DK 4065	II	**23**	Reconquista	SY3930 CL	I

9	Reconquista	HUARPE	I	**24**	Reconquista	SY3930 CL	II
10	Reconquista	HUARPE	II	**25**	P.R.S. Peña	ARGENSOL 40	M
11	P.R.S. Peña	ACA 887	M	**26**	P.R.S. Peña	CACIQUE 308 CL	M
12	P.R.S. Peña	PAN 7076	M	**27**	P.R.S. Peña	SPS 3120	M
13	P.R.S. Peña	DK 4065	M	**28**	P.R.S. Peña	TOBSOL 261	M
14	P.R.S. Peña	DK 4045	M	**29**	P.R.S. Peña	SY3930 CL	M
15	Reconquista	ARGENSOL 40	I				

- **Second Stage (2012-13 season)**

For the Second Stage, five sunflower hybrids were sown in four locations: Sacháyoj (Province of Santiago del Estero, 26ºS), Reconquista (Province of Santa Fe, 29°S), Tostado (Province of Santa Fe, 29ºS) and Villa Ocampo (Province of Santa Fe, 28ºS). The trials were developed in the 2012-13 season following the protocols of the National Network of multi-environmental **trials** of INTA. Previous to flowering stage, the heads were covered with polyamide bags to avoid pollen contamination. The experimental design was in randomized complete blocks with three replications. Each plot was composed of four rows and the experimental unit (EU) was constituted by the two central rows. At stage R9, all the heads of each EU were harvested and threshed. A 30g sub-sample was taken from each EU for fatty acid content estimation of the oil. The samples represent the combination of hybrids, locations and replications detailed in Table 2.

Table 2*. Location, hybrid and replication of each simple of the Second Stage.*

Sample	Location	Hybrid	Replication	Sample	Location	Hybrid	Replication
1	Sacháyoj	PAN 7076	I	**25**	V. Ocampo	HUARPE	I
2	Sacháyoj	DK 4065	I	**26**	V. Ocampo	PAN 7076	II
3	Sacháyoj	ACA 887	I	**27**	V. Ocampo	DK 4065	II
5	Sacháyoj	HUARPE	I	**28**	V. Ocampo	ACA 887	II
6	Sacháyoj	PAN 7076	II	**29**	V. Ocampo	DK 4045	II
7	Sacháyoj	DK 4065	II	**30**	V. Ocampo	HUARPE	II
9	Sacháyoj	DK 4045	II	**31**	Reconquista	PAN 7076	I
10	Sacháyoj	HUARPE	II	**32**	Reconquista	DK 4065	I
11	Tostado	PAN 7076	I	**33**	Reconquista	ACA 887	I
12	Tostado	DK 4065	I	**34**	Reconquista	DK 4045	I
13	Tostado	ACA 887	I	**35**	Reconquista	HUARPE	I
14	Tostado	DK 4045	I	**36**	Reconquista	PAN 7076	II
15	Tostado	HUARPE	I	**37**	Reconquista	DK 4065	II
16	Tostado	PAN 7076	II	**38**	Reconquista	ACA 887	II
17	Tostado	DK 4065	II	**39**	Reconquista	DK 4045	II
18	Tostado	ACA 887	II	**40**	Reconquista	HUARPE	II
19	Tostado	DK 4045	II	**41**	Tostado	HUARPE	III
21	V. Ocampo	PAN 7076	I	**42**	Reconquista	ACA 887	III
22	V. Ocampo	DK 4065	I	**43**	Sacháyoj	ACA 887	III
23	V. Ocampo	ACA 887	I	**44**	Sacháyoj	DK 4045	III
24	V. Ocampo	DK 4045	I	**45**	V. Ocampo	DK 4045	III

- **Third Stage (2013-14 season)**

For the Third Stage, samples of eight hybrids from four locations were selected: La Tigra (Province of Chaco, 27°S), Las Toscas (Province of Santa Fe, 28°S), Reconquista (Province of Santa Fé, 29°S), and San Justo (Province of Santa Fe, 30°S). The trials were developed in the 2013-14 season, following the protocols of the National Network of multi-environmental **trials** of INTA. The experimental design was in randomized complete blocks with three replications. Each plot was composed of four rows and the experimental unit (EU) was constituted by the two central rows. At stage R9, all the heads of each EU were harvested and threshed. A 30g sub-sample of each EU was taken for fatty acid content estimation of the oil. The samples represent the combination of hybrids, locations and replications detailed in Table 3.

Table 3*. Location, hybrid and replication of each sample of the Third Stage*

Sample	Location	Hybrid	Replication	**Sample**	Location	Hybrid	Replication
1	Reconquista	DK4045	I	**49**	La Tigra	DK4045	I
2	Reconquista	DK4045	II	**50**	La Tigra	DK4045	II
3	Reconquista	DK4045	III	**51**	La Tigra	DK4045	III
4	Reconquista	DK4065	I	**52**	La Tigra	DK4065	I
5	Reconquista	DK4065	II	**53**	La Tigra	DK4065	II
6	Reconquista	DK4065	III	**54**	La Tigra	DK4065	III
7	Reconquista	ACA887	I	**55**	La Tigra	ACA887	I
8	Reconquista	ACA887	II	**56**	La Tigra	ACA887	II
9	Reconquista	ACA887	III	**57**	La Tigra	ACA887	III
10	Reconquista	PAN7031	I	**58**	La Tigra	PAN7031	I
11	Reconquista	PAN7031	II	**59**	La Tigra	PAN7031	II
12	Reconquista	PAN7031	III	**60**	La Tigra	PAN7031	III
13	Reconquista	MG360	I	**61**	La Tigra	MG360	I
14	Reconquista	MG360	II	**62**	La Tigra	MG360	II
15	Reconquista	MG360	III	**63**	La Tigra	MG360	III
16	Reconquista	KWSOL492	I	**64**	La Tigra	KWSOL492	I
17	Reconquista	KWSOL492	II	**65**	La Tigra	KWSOL492	II
18	Reconquista	KWSOL492	III	**66**	La Tigra	KWSOL492	III
19	Reconquista	SYN3840	I	**67***	La Tigra	SYN3840	I
20	Reconquista	SYN3840	II	**68**	La Tigra	SYN3840	II
21	Reconquista	SYN3840	III	**69**	La Tigra	SYN3840	III
22	Reconquista	ACA861	I	**70**	La Tigra	ACA861	I
23	Reconquista	ACA861	II	**71**	La Tigra	ACA861	II
24	Reconquista	ACA861	III	**72**	La Tigra	ACA861	III
25	Las Toscas	DK4045	I	**73**	San Justo	DK4045	I
26	Las Toscas	DK4045	II	**74**	San Justo	DK4045	II
27	Las Toscas	DK4045	III	**75**	San Justo	DK4045	III
28	Las Toscas	DK4065	I	**76**	San Justo	DK4065	I
29	Las Toscas	DK4065	II	**77**	San Justo	DK4065	II
30	Las Toscas	DK4065	III	**78**	San Justo	DK4065	III
31	Las Toscas	ACA887	I	**79**	San Justo	ACA887	I
32	Las Toscas	ACA887	II	**80**	San Justo	ACA887	II
33	Las Toscas	ACA887	III	**81**	San Justo	ACA887	III
34	Las Toscas	PAN7031	I	**82**	San Justo	PAN7031	I
35	Las Toscas	PAN7031	II	**83**	San Justo	PAN7031	II

36	Las Toscas	PAN7031	III	**84**	San Justo	PAN7031	III
37	Las Toscas	MG360	I	**85**	San Justo	MG360	I
38	Las Toscas	MG360	II	**86**	San Justo	MG360	II
39	Las Toscas	MG360	III	**87**	San Justo	MG360	III
40	Las Toscas	KWSOL492	I	**88**	San Justo	KWSOL492	I
41	Las Toscas	KWSOL492	II	**89**	San Justo	KWSOL492	II
42	Las Toscas	KWSOL492	III	**90**	San Justo	KWSOL492	III
43	Las Toscas	SYN3840	I	**91**	San Justo	SYN3840	I
44	Las Toscas	SYN3840	II	**92**	San Justo	SYN3840	II
45	Las Toscas	SYN3840	III	**93**	San Justo	SYN3840	III
46	Las Toscas	ACA861	I	**94**	San Justo	ACA861	I
47	Las Toscas	ACA861	II	**95**	San Justo	ACA861	II
48	Las Toscas	ACA861	III	**96**	San Justo	ACA861	III

**Sample 67 was not analyzed due to emergency problems in the plot.*

2.2. Analytic Methodology

Oil was extracted from 10-15g of grains ground using n-hexane as solvent. The sample was placed in filter paper cartridges inside soxhlet bodies in order to extract it. Extraction was done through percolation-immersion for three hours at 80ºC. After extraction, the solvent was recovered with a rotavapor with vacuum at 45ºC. The remains of the oil solvent were eliminated with a flow of N_2. The oils were kept in caramel-colored jars in an atmosphere of N_2 at 5ºC.

The fatty acids of samples of the First Stage were methylated following the technique proposed by Sukhija & Palmquist (1988). For this purpose, the oil samples dissolved in chloroform were incubated with 1 volume of methanolic acid 5% (acetyl chloride: methanol; 1:10, v/v) at 70ºC for an hour. After the addition of 4 volumes of potassium carbonate 6% (w/v), the preparations were incubated until phase separation, and the organic phase was supplemented with two volumes of chloroform. The fatty acid composition of the samples was determined through gas chromatography (GLC) with the use of Shimadzu GH-2014 (Kyoto, Japan) equipment. The injector and detector (FID) temperatures were of 250 and 275ºC respectively, whereas the temperature of the column was of 210ºC. 1 mL of sample was injected in the column (Omega wax 250, Supelco). The N_2 carrier gas was kept at a constant pressure of 100 kPa.

In order to determine the fatty acid concentration of the samples of the Second and Third Stages, protocols AOCS Ce 2-66 and Ce 1e-91(01) were followed in Shimadzu GC-2014 equipment (Kyoto, Japan).

The chromatograms obtained were acquired and processed using Shimadzu GC-solution software.

3. Results and conclusions

The percentage ranges of the fatty acids of the oil from sunflower grown in the mentioned locations are detailed on Table 4. Through all the stages of the analysis it may be observed that the oil fatty content varies, and that the analyzed hybrids may express high concentration of oleic acid. Among the analyzed hybrids, HUARPE stands out (samples 9 and 10 of the First Stage and samples 5, 10, 15, 30, 35, 40 and 41 of the Second Stage, see Appendix): it showed the highest oleic acid concentrations in both years (between 56.2 and 81.1%), a behavior comparable to that of a mid-oleic hybrid. In some particular environments, this hybrid even presented concentrations comparable to a high-oleic oil. However, even setting aside this hybrid, it may be seen that the oleic acid concentration of the analyzed hybrids varied between 28.2 and 56.4%, both years considered (Table 4).

According to these results, the ranges expressed in Table 1 of CODEX Stan 210=1999 are not valid for all traditional hybrids grown in all the locations of the Argentine sunflower region. In the northern region, oleic acid values would be higher and linoleic acid values lower, mainly due to the warm temperatures usually present at the grain filling stage.

***Table 4**. Abundance range (percentage) of each fatty acid in Sunflower seed oil, all analyzed hybrids considered.*

Fatty Acid	Abundance (%)				
	All hybrids		Excluding HUARPE hybrid		
	First Stage	Second Stage	First Stage	Second Stage	Third Stage
C 14:0	n.d.	0.0-0.1	n.d.	0.0-0.1	n.d.
C 16:0	4.3-6.0	3.8-5.8	4.3-6.0	3.9-5.8	4.3-7.1
C 18:0	2.0-6.2	1.5-4.4	2.0-6.2	1.5-4.4	1.5-4.6
C 18:1	28.2-61.1	36.8-81.1	28.2-53.4	36.8-56.4	27.4-58.2
C 18:2	29.5-62.7	9.1-54.6	38.2-62.7	33.2-54.6	32.5-61.6
C 18:3	0.0-0.1	0.0-0.1	0.0-0.1	0.0	0.0-0.1
C 20:0	0.1-0.4	0.1-0.4	0.1-0.4	0.1-0.3	0.1-0.5
C 20:1	n.d.	0.1-0.2	n.d.	0.1-0.2	0.1-0.3
C 22:0	0.5-0.9	0.4-0.9	0.5-0.9	0.4-0.8	0.4-0.9
C 22:1	0.0-0.1	n.d.-0.0	0.0-0.1	n.d.-0.0	0.0-0.2
C 24:0	0.2	0.1-0.4	0.2	0.1-0.3	0.0-0.4

The fatty acid composition of all the samples is laid out in detail in the Appendix accompanying the present report (Tables 5, 6 and 7).

Buenos Aires, May 23rd 2014

4. Bibliography

Echarte, M.M., Angeloni, P., Jaimes, F., Tognetti, J., Izquierdo N.G., Valentinuz, O., Aguirrezábal, L.A.N. 2010. Night temperature and intercepted solar radiation additively contribute to oleic acid percentage in sunflower oil. Field Crops Research 119: 27-35.

Izquierdo, N. y Aguirrezábal.L. 2008. Genetic variability of the response of fatty acid composition to temperature. Field Crops Research 106: 116-125.

Izquierdo, N.; Aguirrezábal, L.; Andrade, F.; Geroudet, C.; Pereyra Iraola, M. y Valentinuz, O. 2009. Intercepted solar radiation affects oil fatty acid composition in crop species. Field Crops Research 114: 66-74.

Schneiter, A.A.; Miller, J.F. 1981. Description of sunflower growth stages. Crop Science 21: 901–903.

Sukhija, P.; Palmquist, D.L. 1988. Rapid method for determinations of total fatty acid content and composition of feedstuffs and feces. Journal of Agriculture and Food Chemistry 36: 1202-1206.

5. Appendix

Table 5. *Percentage fatty composition of the samples analyzed in the FIRST STAGE.*

Sample	C 16:0	C 18:0	C 18:1	C 18:2	C 18:3	C 20:0	C 22:0	C 22:1	C 24:0
1	5.7	3.9	33.8	55.4	0.0	0.2	0.6	0.1	0.2
2	5.7	4.1	33.8	55.2	0.0	0.3	0.7	0.0	0.2
3	5.8	2.2	32.2	58.8	0.1	0.2	0.5	0.1	0.2
4	6.0	2.0	28.5	62.5	0.0	0.1	0.5	0.1	0.2
5	5.5	2.7	40.2	50.6	0.0	0.2	0.6	0.1	0.2
6	5.7	2.8	36.6	53.8	0.0	0.2	0.6	0.1	0.2
7	5.2	4.3	37.0	52.2	0.0	0.3	0.7	0.1	0.2
8	5.1	4.6	37.5	51.5	0.0	0.3	0.7	0.1	0.2
9	5.4	3.0	56.2	34.3	0.1	0.2	0.6	0.0	0.2
10	5.2	3.1	61.1	29.5	0.0	0.2	0.6	0.1	0.2
11	5.9	2.8	38.3	52.0	0.0	0.2	0.5	0.1	0.2
12	4.7	4.6	44.2	45.0	0.0	0.3	0.7	0.1	0.2
13	4.8	6.2	44.1	43.3	0.0	0.4	0.9	0.1	0.2
14	5.1	4.0	48.4	41.1	0.0	0.3	0.8	0.1	0.2
15	4.8	3.0	29.2	61.9	0.0	0.2	0.5	0.1	0.2
16	4.8	2.9	28.5	62.7	0.1	0.2	0.5	0.1	0.2
17	5.7	2.0	40.4	50.6	0.0	0.2	0.6	0.1	0.2
18	4.9	2.3	53.4	38.2	0.0	0.2	0.7	0.1	0.2
19	5.6	3.1	33.3	56.9	0.0	0.2	0.6	0.1	0.2
20	5.3	2.9	32.8	58.0	0.1	0.2	0.6	0.1	0.2
21	6.0	2.2	28.2	62.5	0.0	0.2	0.5	0.1	0.2
22	5.4	2.4	32.2	58.9	0.0	0.2	0.5	0.1	0.2
23	5.9	3.3	37.1	52.4	0.0	0.3	0.7	0.0	0.2
24	5.7	3.1	35.0	54.9	0.0	0.3	0.7	0.1	0.2
25	4.3	4.2	37.4	52.9	0.1	0.3	0.6	0.1	0.2
26	5.0	2.7	47.2	43.7	0.0	0.2	0.7	0.1	0.2
27	4.9	4.8	41.2	47.5	0.1	0.3	0.8	0.1	0.2
28	5.0	3.2	38.3	52.2	0.0	0.2	0.7	0.1	0.2
29	4.9	4.6	42.0	46.9	0.0	0.3	0.8	0.1	0.2

Table 6. *Percentage fatty composition of the samples analyzed in the SECOND STAGE.*

Sample	C 14:0	C 16:0	C 18:0	C 18:1	C 18:2	C 18:3	C 20:0	C 20:1	C 22:0	C 22:1	C 24:0
1	0.0	5.4	3.9	50.9	38.2	0.0	0.3	0.1	0.7	n.d.	0.2
2	0.0	4.4	4.4	56.4	33.2	0.0	0.3	0.1	0.7	n.d.	0.2
3	0.0	5.3	2.6	50.9	39.7	0.0	0.2	0.2	0.6	0.0	0.2
5	0.0	4.3	3.7	81.1	9.1	0.0	0.4	0.2	0.9	n.d.	0.3
6	0.0	5.2	4.0	54.1	34.9	0.0	0.3	0.1	0.8	n.d.	0.2
7	0.0	4.9	3.7	53.6	36.0	0.0	0.3	0.2	0.8	n.d.	0.3
9	0.0	4.4	4.4	53.5	36.1	0.0	0.3	0.1	0.7	0.0	0.2
10	0.0	4.0	3.5	80.1	10.4	0.0	0.4	0.2	0.9	n.d.	0.3
11	0.0	3.9	2.2	49.9	42.6	0.0	0.2	0.2	0.6	n.d.	0.2
12	0.0	4.0	4.0	47.4	43.0	0.0	0.3	0.1	0.8	n.d.	0.2
13	0.0	5.1	1.7	40.2	51.9	0.0	0.2	0.2	0.4	n.d.	0.2
14	0.0	4.5	2.3	51.8	40.0	0.0	0.2	0.1	0.6	n.d.	0.3
15	0.0	3.9	2.6	73.9	18.0	0.0	0.2	0.2	0.6	n.d.	0.2
16	0.0	5.0	3.1	37.3	53.0	0.0	0.2	0.2	0.7	0.0	0.3
17	0.0	3.9	3.5	49.1	42.1	0.0	0.2	0.1	0.7	n.d.	0.1
18	0.0	5.0	1.6	41.5	50.8	0.0	0.1	0.1	0.4	n.d.	0.1
19	0.0	4.2	2.8	50.2	41.5	0.0	0.2	0.1	0.6	n.d.	0.2
21	0.1	5.4	2.5	37.0	53.7	0.0	0.2	0.1	0.6	n.d.	0.2
22	0.0	4.5	3.3	46.3	44.6	0.0	0.3	0.1	0.7	n.d.	0.2
23	0.0	5.6	1.5	37.7	54.1	0.0	0.1	0.2	0.4	n.d.	0.2
24	0.0	5.0	2.1	50.6	41.0	0.0	0.2	0.1	0.6	n.d.	0.2
25	0.0	4.4	2.0	71.0	21.2	0.0	0.2	0.2	0.6	n.d.	0.2
26	0.1	5.8	2.9	36.8	53.0	n.d.	0.3	0.2	0.7	n.d.	0.2
27	0.0	4.7	3.6	46.5	43.8	0.0	0.3	0.1	0.7	n.d.	0.2
28	0.0	5.6	1.6	39.7	52.0	n.d.	0.2	0.2	0.4	n.d.	0.2
29	0.1	4.9	2.3	56.1	35.3	0.0	0.2	0.1	0.6	n.d.	0.2
30	0.0	4.5	2.2	68.7	23.4	n.d.	0.2	0.2	0.5	n.d.	0.2
31	0.0	4.8	2.9	42.7	48.0	0.0	0.2	0.2	0.7	n.d.	0.3
32	0.0	4.3	3.6	45.9	44.9	0.0	0.3	0.1	0.7	n.d.	0.1
33	0.0	5.5	1.8	41.4	50.2	0.0	0.2	0.2	0.5	n.d.	0.2
34	0.0	4.5	2.6	53.9	37.5	0.0	0.2	0.2	0.7	n.d.	0.2
35	0.0	4.2	2.8	75.2	15.7	0.1	0.3	0.2	0.8	n.d.	0.4
36	0.0	4.8	2.9	43.0	47.8	0.0	0.2	0.2	0.7	n.d.	0.3
37	0.0	4.3	3.7	48.1	42.2	0.0	0.3	0.1	0.7	n.d.	0.2
38	0.0	5.1	2.0	44.8	46.8	0.0	0.2	0.2	0.5	n.d.	0.3
39	0.1	4.8	2.3	50.6	41.0	0.0	0.2	0.2	0.6	n.d.	0.2
40	0.0	4.3	2.8	76.0	15.3	0.1	0.3	0.2	0.7	n.d.	0.2
41	0.0	3.8	3.0	73.6	18.0	0.0	0.3	0.2	0.7	0.0	0.3
42	0.0	5.6	1.5	37.0	54.6	0.0	0.1	0.2	0.4	0.0	0.2
43	0.0	5.4	2.6	49.4	41.4	0.0	0.2	0.1	0.6	0.0	0.1
44	0.0	4.4	4.3	54.7	35.1	0.0	0.3	0.1	0.8	0.0	0.2
45	0.0	4.6	2.4	55.7	36.0	0.0	0.2	0.2	0.6	n.d.	0.2

Table 7. *Percentage fatty composition of the samples analyzed in the THIRD STAGE*

Sample	**C 14:0**	**C 16:0**	**C 18:0**	**C 18:1**	**C 18:2**	**C 18:3**	**C 20:0**	**C 20:1**	**C 22:0**	**C 22:1**	**C 24:0**
1	0.0	5.9	2.9	49.6	39.7	0.1	0.5	0.3	0.8	0.2	0.0
2	0.0	5.6	2.2	53.4	37.4	0.0	0.2	0.2	0.6	0.0	0.3
3	0.0	5.6	2.3	53.7	36.9	0.0	0.2	0.2	0.7	0.0	0.3
4	0.0	5.3	3.3	43.9	45.9	0.0	0.3	0.2	0.8	0.0	0.3
5	0.0	5.3	3.2	45.9	44.0	0.0	0.3	0.2	0.8	0.0	0.2
6	0.0	5.3	3.3	43.4	46.5	0.0	0.2	0.1	0.8	0.0	0.3
7	0.0	6.0	2.1	43.8	46.8	0.0	0.2	0.2	0.6	0.0	0.2
8	0.0	5.8	1.8	43.9	47.2	0.0	0.2	0.2	0.6	0.0	0.2
9	0.0	6.2	2.1	41.3	49.1	0.0	0.2	0.2	0.6	0.0	0.3
10	0.0	6.1	1.8	43.1	47.8	0.0	0.2	0.2	0.5	0.0	0.2
11	0.0	6.1	2.6	41.0	49.0	0.0	0.2	0.2	0.6	0.0	0.2
12	0.0	6.2	2.2	39.7	50.6	0.0	0.2	0.2	0.6	0.0	0.2
13	0.0	5.6	2.3	53.2	37.6	0.0	0.2	0.2	0.6	0.0	0.3
14	0.0	5.3	3.4	44.2	45.6	0.0	0.3	0.2	0.8	0.0	0.2
15	0.0	5.6	3.1	41.1	49.0	0.0	0.2	0.2	0.6	0.0	0.2
16	0.0	6.1	1.9	43.9	46.8	0.0	0.2	0.2	0.7	0.0	0.3
17	0.0	6.5	1.7	39.9	50.6	0.0	0.2	0.2	0.6	0.0	0.3
18	0.0	6.1	1.8	46.7	43.9	0.0	0.2	0.2	0.7	0.0	0.3
19	0.0	6.2	2.6	40.8	49.0	0.0	0.2	0.2	0.6	0.0	0.3
20	0.0	5.6	2.5	41.8	48.8	0.0	0.2	0.2	0.7	0.0	0.2
21	0.0	5.6	2.5	41.3	49.3	0.0	0.2	0.2	0.7	0.0	0.3
22	0.0	6.4	1.8	38.1	52.4	0.0	0.2	0.2	0.6	0.0	0.3
23	0.0	6.8	1.8	33.8	56.6	0.0	0.2	0.2	0.5	0.0	0.3
24	0.0	6.6	1.8	35.6	54.8	0.0	0.2	0.2	0.5	0.0	0.3
25	0.0	5.8	2.1	50.8	40.0	0.0	0.2	0.2	0.6	0.0	0.2
26	0.0	5.9	2.2	49.9	40.7	0.0	0.2	0.2	0.7	0.0	0.3
27	0.0	6.0	2.3	47.1	43.3	0.0	0.2	0.2	0.6	0.0	0.3
28	0.0	5.5	3.0	44.4	45.6	0.0	0.3	0.2	0.7	0.0	0.3
29	0.0	5.2	3.2	48.7	41.2	0.0	0.3	0.2	0.8	0.0	0.3
30	0.0	5.4	3.5	46.4	43.0	0.0	0.3	0.1	0.8	0.0	0.3
31	0.0	6.4	1.7	41.8	49.0	0.0	0.1	0.2	0.5	0.0	0.3
32	0.0	6.3	1.6	42.9	48.1	0.0	0.1	0.2	0.5	0.0	0.2
33	0.0	6.1	1.9	46.2	44.7	0.0	0.2	0.2	0.6	0.0	0.2
34	0.0	6.3	2.2	38.9	51.1	0.0	0.2	0.2	0.7	0.0	0.3
35	0.0	6.4	2.3	38.8	51.1	0.0	0.2	0.2	0.6	0.0	0.3
36	0.0	6.4	2.4	39.8	49.8	0.0	0.2	0.2	0.7	0.0	0.3
37	0.0	5.6	3.0	43.5	46.6	0.0	0.2	0.2	0.7	0.0	0.3
38	0.0	6.1	2.7	36.1	53.8	0.0	0.2	0.1	0.4	0.0	0.2
39	0.0	6.3	2.9	36.2	53.3	0.0	0.3	0.2	0.6	0.0	0.2
40	0.0	6.5	1.8	46.1	44.0	0.0	0.2	0.3	0.7	0.0	0.4
41	0.0	6.8	1.5	42.5	47.8	0.1	0.2	0.3	0.5	0.0	0.2
42	0.0	6.7	1.8	48.0	42.0	0.0	0.2	0.2	0.8	0.0	0.2
43	0.0	5.7	2.6	45.2	45.2	0.0	0.3	0.2	0.6	0.0	0.2
44	0.0	5.9	2.5	41.4	48.7	0.0	0.2	0.1	0.8	0.0	0.2
45	0.0	5.7	2.6	42.6	47.7	0.0	0.2	0.1	0.8	0.0	0.3
46	0.0	6.9	1.7	37.0	53.5	0.0	0.1	0.2	0.5	0.0	0.2
47	0.0	6.7	1.7	35.5	54.8	0.0	0.2	0.1	0.6	0.0	0.3

48	0.0	7.1	1.7	32.9	57.1	0.0	0.2	0.2	0.5	0.0	0.3
49	0.0	4.8	3.2	53.6	36.9	0.0	0.3	0.2	0.8	0.0	0.3
50	0.0	4.6	3.1	54.3	36.4	0.0	0.2	0.2	0.8	0.0	0.3
51	0.0	4.6	3.1	58.2	32.5	0.0	0.2	0.2	0.8	0.0	0.3
52	0.0	4.6	4.5	44.3	44.9	0.0	0.3	0.2	0.9	0.0	0.3
53	0.0	4.5	4.6	45.2	44.0	0.0	0.3	0.2	0.9	0.0	0.3
54	0.0	4.4	4.1	49.1	40.8	0.0	0.3	0.2	0.9	0.0	0.3
55	0.0	5.3	2.5	41.5	49.2	0.0	0.2	0.2	0.7	0.0	0.2
56	0.0	5.5	2.3	39.6	51.4	0.0	0.2	0.2	0.6	0.0	0.2
57	0.0	5.4	2.4	41.2	49.7	0.0	0.2	0.2	0.7	0.0	0.2
58	0.0	4.3	2.8	50.9	40.5	0.0	0.2	0.2	0.7	0.0	0.3
59	0.0	5.2	3.3	40.2	49.8	0.0	0.2	0.2	0.8	0.0	0.3
60	0.0	5.2	3.2	41.0	49.3	0.0	0.2	0.2	0.7	0.0	0.2
61	0.0	4.8	4.2	42.3	47.1	0.0	0.3	0.2	0.8	0.0	0.2
62	0.0	4.7	4.4	45.6	43.6	0.0	0.3	0.2	0.9	0.0	0.3
63	0.0	4.7	4.2	42.4	47.4	0.0	0.2	0.1	0.7	0.0	0.3
64	0.0	5.0	3.8	38.4	51.1	0.0	0.3	0.2	0.9	0.0	0.3
65	0.0	5.2	2.6	45.3	45.2	0.0	0.3	0.2	0.9	0.0	0.3
66	0.0	5.3	2.5	47.3	43.4	0.0	0.2	0.2	0.8	0.0	0.3
68	0.0	5.1	2.4	47.8	43.0	0.0	0.2	0.3	0.9	0.0	0.2
69	0.0	5.2	3.3	39.4	50.6	0.0	0.3	0.2	0.8	0.0	0.3
70	0.0	5.5	2.8	38.4	52.0	0.0	0.2	0.2	0.7	0.0	0.2
71	0.0	5.6	2.4	36.6	54.0	0.1	0.2	0.2	0.7	0.0	0.3
72	0.0	5.2	3.1	44.2	45.9	0.0	0.2	0.2	0.7	0.0	0.2
73	0.0	5.2	2.8	57.4	33.0	0.0	0.3	0.2	0.8	0.0	0.3
74	0.0	5.2	2.6	53.9	36.9	0.0	0.2	0.1	0.6	0.0	0.2
75	0.0	5.4	2.8	48.1	42.3	0.0	0.3	0.2	0.6	0.0	0.2
76	0.0	5.2	3.7	40.9	48.6	0.0	0.3	0.2	0.8	0.0	0.2
77	0.0	5.5	3.6	35.8	53.8	0.0	0.3	0.1	0.7	0.0	0.2
78	0.0	5.4	3.2	37.7	52.4	0.0	0.3	0.2	0.7	0.0	0.1
79	0.0	5.7	2.2	39.9	51.1	0.0	0.2	0.2	0.5	0.0	0.2
80	0.0	5.6	2.1	41.7	49.5	0.0	0.2	0.2	0.6	0.0	0.1
81	0.0	5.9	2.0	41.0	50.0	0.0	0.2	0.2	0.5	0.0	0.2
82	0.0	6.2	3.0	31.2	58.5	0.0	0.2	0.2	0.6	0.0	0.1
83	0.0	6.4	3.2	27.4	61.6	0.0	0.2	0.1	0.6	0.0	0.3
84	0.0	6.0	3.0	31.5	58.2	0.0	0.2	0.1	0.6	0.0	0.3
85	0.0	5.5	3.3	37.9	52.0	0.0	0.3	0.2	0.6	0.0	0.1
86	0.0	5.4	3.8	40.4	49.0	0.0	0.3	0.2	0.8	0.0	0.2
87	0.0	5.3	3.5	42.2	47.8	0.0	0.3	0.1	0.7	0.0	0.1
88	0.0	5.8	2.1	45.2	45.5	0.0	0.2	0.2	0.7	0.0	0.2
89	0.0	6.0	2.1	40.8	49.8	0.0	0.2	0.2	0.6	0.0	0.3
90	0.0	6.0	1.9	43.8	47.2	0.0	0.2	0.2	0.6	0.0	0.2
91	0.0	5.6	3.0	35.0	55.0	0.0	0.2	0.2	0.7	0.0	0.2
92	0.0	5.8	3.0	34.3	55.4	0.0	0.3	0.1	0.7	0.0	0.3
93	0.0	5.6	2.9	35.3	55.0	0.0	0.2	0.2	0.7	0.0	0.1
94	0.0	6.3	1.8	33.9	56.9	0.0	0.1	0.2	0.4	0.0	0.3
95	0.0	6.3	1.8	33.0	57.8	0.0	0.1	0.2	0.5	0.0	0.2
96	0.0	6.0	2.1	35.9	54.9	0.0	0.2	0.2	0.5	0.0	0.2