Fatty Acids Including *trans* Content of Commercial Bakery Products Manufactured in Spain

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Fifteen samples of the most common industrial bakery products sold in Spain were analyzed for their fatty acid composition. Saturated fatty acids occurred in the largest proportions in all samples (mean = 52.8%), followed by monounsaturated (mean = 23.5%) and polyunsaturated fatty acids (mean = 17.2%). A small percentage of *trans* fatty acids, which were found in all samples, showed a mean value of 5.7%. According to their fatty acid composition, that is, their saturated, monounsaturated, and polyunsaturated fatty acid contents, the statistical analysis (discriminant analysis) showed three groups of samples, suggesting that the fat incorporated in these samples was obtained from different sources.

Keywords: *trans fatty acids; gas chromatography; bakery products; Catalonia (Spain)*

INTRODUCTION

Industrial bakery products require fats that provide special characteristics such as shortness of texture and a low melting point in the final cooked dough so that they might meld in the mouth with other components. The melting point of fats must, therefore, range between 15 and 35 °C as this gives fat plasticity to the final dough. This fat plasticity can be achieved in a number of ways, one of which is the partial hydrogenation of oils used as ingredients.

Hydrogenated oils still constitute the main source of *trans* isomers in the diet. Over the past 20 years, the effects of partially hydrogenated vegetable oils on the human diet have been discussed. In the past few years scientific evidence has shown the adverse effects of *trans* fatty acid consumption. The intake of *trans* fatty acids has been linked to several biological effects in human beings. According to several authors, *trans* fatty acid consumption has been associated with an increase in serum cholesterol levels and the risk of cardiovascular heart diseases (CHDs), and parallel to this, other researchers have reported that *trans* fatty acids decrease serum levels of HDLs (Emken, 1979, 1984; Kinsella et al., 1981; Kummerow, 1986; British Nutrition Foundation, 1987; Kris-Etherton, 1995).

Thus, it is particularly important to assess the effects of the consumption of *trans* fatty acids in the human diet. In previous research we reported on the content of *trans* fatty acids in different foods and food products in Spain. We estimated that their contribution to *trans* isomer intake in Spain was 2.4 g per person in a daily diet (Boatella et al., 1993).

Here we focus our attention on the levels of *trans* isomeric fatty acids on industrial bakery products consumed in Catalonia (Spain). Bakery products contribute minimally to the daily intake of *trans* isomers in our country (Boatella et al., 1993); however, we have chosen to analyze this kind of product because they are consumed by the majority of children in Spain. According to Decsi and Koletzko (1995) the intake of *trans* isomers by healthy children may impair desaturation and/or chain elongation of $n-6$ essential fatty acids; obviously, in this sense their consumption is not recommended in our population.

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EXPERIMENTAL PROCEDURES

Samples. Fifteen samples of industrial bakery products commercially available in Catalonia (Spain) were used in our study. Samples correspond to the main bakery products manufactured in Spain. They can be classified as follows: one Swiss cake, three Swiss rolls stuffed with chocolate, one sponge cake, three sponge cakes filled with chocolate, two doughnuts, one doughnut stuffed with chocolate, one biscuit coated with chocolate, and three cakes filled with chocolate.

Fat Extraction. Fat was extracted following a modification of the procedure proposed by Folch et al. (1957). Fifty grams of sample was homogenized in a Polytron homogenizer (Kinematica AG, Littau, Switzerland) at high speed for 2 min with 100 mL of a mixture of chloroform/methanol (2:1, v/v). The homogenized mixture was shaken in a 250-mL Erlenmeyer flask with a magnetic stirrer for a further 30 min. The mixture was filtered. The remaining solid phase was extracted again with the same volume of chloroform/methanol mixture and filtered. Liquid phases were combined in a separatory funnel. Thirty-five milliliters of saturated sodium chloride in water was added, and then the mixture was gently shaken. After phase separation, the chloroformic layer was filtered, dried with sodium sulfate, and filtered again. The chloroform was evaporated in a rotary vacuum pump. The residue was transferred to a 10-mL glass vial, and air was removed with a stream of nitrogen. The vial was sealed and maintained at −18 °C until analyses were carried out.

Analyses of Fatty Acids. Fatty acid methyl esters (FAMEs) were prepared from oil samples following the procedure proposed by Slover and Lanza (1979). Approximately 200 mg of fat was saponified with 3 mL of sodium methoxide in methanol (0.5 mol L^{-1}) for 10 min at 100 °C in a bath of boiling water. The solution was cooled to room temperature, and 2 mL of boron trifluoride/methanol complex (20%, wt) was added. The solution was heated for a further 10 min in a bath of boiling water. After cooling, 1 mL of hexane and 2.5 mL of saturated sodium chloride were added. The mixture was shaken vigorously; afterward, the organic layer was suctioned

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with a Pasteur pipet, dried with anhydrous sodium sulfate, and filtered off. Finally, the hexanic solution was transferred to a 10-mL glass vial and stored at −18 °C until analyses were carried out.

FAMEs were analyzed by gas−liquid chromatography (GLC). Samples (1 μL) were injected into the gas chromatograph (GC), a Perkin-Elmer (PE) Autosystem 8700 GC (Norwalk, CT). The GC was equipped with a 50-m CPSil-88 capillary column coated with 100% cyanopropylpolysiloxane (0.25 mm i.d., 0.20-μm film thickness) (Chrompack, Middelburg, The Netherlands). The oven was programmed as follows: 177 °C for 11.20 min, raised to 277 °C at 7 °C min^{-1}. The final oven temperature was maintained for 11 min. The injector and detector temperatures were 270 and 300 °C, respectively. Grade 4.7 helium (Air Liquide España, Madrid, Spain) was used as carrier gas (103 kPa).

FAMEs were identified by comparing their equivalent chain length to that of pure standard FAMEs (Hofstetter et al., 1965). FAMEs were quantified according to their percentage area, obtained by integration of the peak as a semiquantitative method.

Statistical Analysis. The *K*-means nonhierarchical clustering method and discriminant analysis (Fisher's method) were applied to the 15 samples using saturated, monounsaturated, polyunsaturated, and *trans* fatty acid contents as variables (Johnson and Wichern, 1988). The statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS, version 7.0) (Hispanoportuguesa, Madrid, Spain) for Microsoft Windows95 (Microsoft Iberica, Madrid, Spain).

Reagents and Standards. The solvents used (chloroform, methanol, and hexane) were all of reagent grade from Panreac (Sant Cugat del Valles, Barcelona, Spain). Both sodium sulfate and sodium chloride were of analytical grade from Panreac. Sodium methoxide in methanol was purchased from Aldrich (Alcobendas, Madrid, Spain). Boron trifluoride/methanol complex was obtained from Merck (Darmstadt, Germany).

A mixture of FAMEs that included myristic (4%), palmitic (10%), stearic (6%), oleic (25%), elaidic (10%), linoleic (34%), linoelaidic (2%), linolenic (5%), arachidic (2%), and behenic (2%) was purchased from Sigma (Alcobendas, Madrid, Spain).

RESULTS AND DISCUSSION

Twenty-three FAMEs were tentatively identified by comparing their equivalent chain lengths to those of standard FAMEs. Table 1 shows their common names, official names according to the International Union of Pure and Applied Chemistry (IUPAC), and their abbreviated names. Fatty acids were classified into four groups: saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and *trans* fatty acids (TFAs). The fatty acid contents of each sample are given in Table 2.

SFAs predominate in all samples, with a mean value of 52.8% (SD = 18.8%). In relation to the individual SFAs, hexadecanoic (palmitic) predominates (mean = 21.7%, SD = 10.7%) followed by octadecanoic (stearic) (mean = 13.6%, SD = 7.0%), dodecanoic (lauric) (mean = 10.4%, SD= 8.2%), tetradecanoic (myristic) (mean = 4,9%, SD = 3.5%), and decanoic (capric) acids (mean = 1.3%, SD = 1.0%). Eicosanoic (arachidic), docosanoic (behenic), and tetracosanoic (lignoceric) fatty acids occur in smaller amounts (<1%).

MUFAs (*cis*-monoenoic fraction) show a mean value of 23.5% (SD = 8.0%). *cis*-9-Octadecenoic acid (oleic acid) is the principal fatty acid, with a mean value of 22.2% (SD = 7.6%). *cis*-9-Hexadecenoic (palmitoleic), *cis*-11-eicosenoic, and *cis*-15-tetracosanoic (nervonic) fatty acids occur in smaller amounts. The MUFAs that appear in all samples belong to the ω−9 series.

PUFAs show a mean value of 17.2% (SD = 15.3%). *cis*,*cis*-9,12-Octadecadienoic acid (linoleic) is the principal fatty acid, with a mean value of 16.6% (SD = 15.3%). *cis*,*cis*,*cis*-6,9,12-Octadecatrienoic acid (γ-linolenic), *cis*,*cis*-11,14-eicosadienoic, and *cis*,*cis*,*cis*,*cis*-5,8,11,14-eicosatetraenoic occur in smaller amounts. All of these PUFAs belong to the ω−6 series.

Table 1. Systematic, Common, and Abbreviated Names of Fatty Acids Identified in All Samples

series	IUPAC	trivial name	abbrev
saturated	decanoic	capric	C10:0
	dodecanoic	lauric	C12:0
	tetradecanoic	miristic	C14:0
	hexadecanoic	palmitic	C16:0
	heptadecanoic	margaric	C17:0
	octadecanoic	stearic	C18:0
	eicosanoic	arachidic	C20:0
	docosanoic	behenic	C22:0
	tetracosanoic	lignoceric	C24:0
cis-monoenoic	*cis*-7-hexadecenoic		C16:1*n*−9
	cis-9-hexadecenoic	palmitoleic	C16:1*n*−7
	cis-9-octadecenoic	oleic	C18:1*n*−9
	cis-11-octadecenoic	*cis*-vaccenic	C18:1*n*−7
	cis-11-eicosenoic	gadoleic	C20:1*n*−9
	cis-tetracosenoic	nervonic	C24:1*n*−9
n−6 polyenoic	*cis*,*cis*-9,12-octadecadienoic	linoleic	C18:2*n*−6
	cis,*cis*,*cis*-6,9,12-octadecatrienoic	γ-linoleic	C18:3*n*−6
	cis,*cis*-11,14-eicosadienoic		C20:2*n*−6
	cis,*cis*,*cis*,*cis*-5,8,11,14-eicosatetraenoic	arachidonic	C20:4*n*−6
n−3 polyenoic	*cis*,*cis*,*cis*-9,12,15-octadecatrienoic	α-linolenic	C18:3*n*−3
trans-monoenoic	*trans*-9-hexadecenoic	palmitelaidic	C16:1t
	trans-9-octadecenoic	elaidic	C18:1t
trans-polyenoic	*trans*,*trans*-9,12-octadecadienoic	linoelaidic	C18:2t

TFAs show the lowest percentage in all samples (mean = 6.5%, SD = 4.2%). In relation to this fraction, *trans*-9-octadecenoic acid (elaidic) predominates (mean = 5.7%, SD = 3.8%). The TFA content observed in our samples ranges from 0.60 to 11.85%. These values are lower than those reported by Craig-Schmidt (1992), which ranged from 10 to 26% for shortening samples, with some commercial brands reaching 40%. Emken (1995) reported TFA contents for shortenings that ranged from 3 to 30% with a mean of 19.6%. He also reported some commercial brands showing a mean value of 37.4%. The same author pointed out that cookie samples showed a mean value of 18.4% and cake samples a mean value of 11.0%. Precht and Molkentin (1997) observed a C18:1t mean content in shortenings of 11.9%. Other authors have reported a decrease in TFA content in margarines and shortenings in the past few years. Also, in samples collected in Austria, Henninger and Ulberth (1996) observed a decrease from 15.7% in 1991−1992 to 6.5% in 1995. Ovesen et al. (1996), in Danish samples, observed a decrease in the C18:1t content from 9.8% in 1992 to 1.2% in 1995. Our data are more in accordance with the last values reported in European countries in recent years than those reported in the United States and Canada.

Considering the overall content for SFAs, MUFAs, PUFAs, and TFAs as variables, the $K = 3$ mean clustering analysis classifies samples into three groups. According to the clustering analysis MUFAs, PUFAs, and SFAs are the most influential of the classification variables; the TFA fraction has considerably less significance (Table 3).

A discriminant analysis (Fisher's method) identifies two discriminant functions for sample classification.

Table 2. Percentages of Fatty Acid Contents in Fat Extracted from Samples (Data Are Means of Triplicate Results)

sample[g]	fatty acids[a]																							
	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{17:0}$	$C_{18:0}$	$C_{20:0}$	$C_{22:0}$	$C_{24:0}$	SFA[b]	$C_{16:1n-9}$	$C_{16:1n-7}$	$C_{18:1n-9}$	$C_{18:1n-7}$	$C_{20:1n-9}$	$C_{24:1n-9}$	MUFA[c]	$C_{18:2n-6}$	$C_{18:3n-6}$	$C_{20:2n-6}$	$C_{20:4n-6}$	$C_{18:3n-3}$	PUFA[d]	$C_{16:1t}$	$C_{18:1t}$	$C_{18:2t}$	TFA[e]
1	0.02	0.18	0.24	8.58	0.11	6.54	0.43	0.65	0.30	17.06	0.04	0.13	30.15	1.23	0.25	0.11	31.91	41.41	0.07	0.18	tr[f]	0.25	41.91	0.01	7.45	1.66	9.12
2	0.05	0.34	0.89	35.59	0.12	7.75	0.53	0.20	0.14	45.65	0.04	0.15	33.54	1.00	0.23	tr	34.97	8.02	0.03	0.15	0.03	0.27	8.50	tr	8.74	2.15	10.89
3	0.96	8.71	3.91	27.04	0.12	10.34	0.45	0.24	0.15	51.96	0.01	0.20	27.36	0.71	0.16	0.04	28.46	10.79	0.03	0.10	0.04	0.15	11.11	tr	7.02	1.44	8.47
4	0.93	8.77	3.95	29.01	0.11	10.72	0.42	0.19	0.20	54.31	0.04	0.13	27.63	0.88	0.15	tr	28.84	6.28	0.03	0.13	0.01	0.14	6.58	0.03	8.52	1.72	10.27
5	2.88	27.40	10.95	12.67	0.08	17.77	0.30	0.17	0.08	72.32	0.07	0.21	11.84	0.48	0.10	tr	12.70	10.23	tr	tr	0.20	0.21	10.63	tr	4.17	0.17	4.34
6	1.26	11.42	5.44	27.14	0.15	27.88	0.67	0.35	0.19	74.54	0.02	0.12	10.98	0.51	0.05	tr	11.67	6.99	0.03	0.56	0.01	0.51	8.08	0.03	5.44	0.24	5.71
7	0.43	1.02	0.66	12.78	0.11	7.32	0.29	0.49	0.26	23.35	0.08	0.30	29.66	0.62	0.14	0.15	31.15	43.99	0.01	0.07	tr	0.41	44.47	0.02	0.52	0.49	1.03
8	0.20	1.61	0.73	9.66	0.10	9.58	0.44	1.03	0.39	23.75	0.04	0.13	23.80	tr	0.16	0.14	24.28	50.98	0.04	0.07	0.18	0.26	51.37	tr	0.38	0.22	0.60
9	2.12	16.05	7.47	23.42	0.10	13.57	0.34	0.13	0.11	63.37	0.05	0.18	20.24	0.55	0.18	0.03	21.36	9.18	0.12	0.07	0.12	0.61	10.10	0.02	4.85	0.30	5.17
10	2.04	16.38	7.34	13.40	0.11	13.68	0.30	0.27	0.17	53.72	0.08	0.21	17.59	1.02	0.15	0.10	19.15	13.72	0.11	0.14	0.34	0.97	15.27	0.02	11.07	0.76	11.85
11	1.97	18.93	8.01	22.21	0.11	28.59	0.44	0.17	0.09	80.55	0.02	0.14	10.08	0.34	0.06	tr	10.64	7.08	tr	tr	0.05	0.21	7.34	tr	1.16	0.31	1.47
12	0.28	1.42	1.62	47.01	0.12	5.51	0.38	0.03	0.10	56.60	0.05	0.23	28.77	2.58	0.13	0.05	31.89	9.97	0.03	0.06	0.08	0.36	10.42	0.02	0.71	0.36	1.09
13	1.30	12.63	5.40	22.44	0.10	12.35	0.38	0.22	0.10	54.94	0.03	0.11	24.93	0.92	0.19	0.01	26.20	6.69	0.05	0.14	0.08	0.40	7.36	0.02	10.79	0.70	11.50
14	1.67	16.82	6.77	12.14	0.10	16.07	0.37	0.31	0.14	54.42	0.04	0.14	15.91	0.95	0.19	tr	17.22	16.35	0.03	tr	0.10	0.86	17.34	tr	10.28	0.74	11.01
15	2.69	14.05	9.52	22.88	0.10	15.57	0.38	0.18	0.09	65.51	0.06	0.18	20.88	0.35	0.13	tr	21.59	6.92	0.09	0.12	0.13	0.45	7.71	0.01	4.91	0.27	5.19

[a] See Table 1 for nomenclature. [b] SFAs, saturated fatty acids ($C_{10:0} + C_{12:0} + C_{14:0} + C_{16:0} + C_{17:0} + C_{18:0} + C_{20:0} + C_{22:0} + C_{24:0}$). [c] MUFAs, monounsaturated fatty acids ($C_{16:1n-9} + C_{16:1n-7} + C_{18:1n-9} + C_{18:1n-7} + C_{20:1n-9} + C_{24:1n-9}$). [d] PUFAs, polyunsaturated fatty acids ($C_{18:2n-6} + C_{18:3n-6} + C_{20:2n-6} + C_{20:4n-6} + C_{18:3n-3}$). [e] TFAs, *trans* fatty acids ($C_{16:1t} + C_{18:1t} + C_{18:2t}$). [f] tr, trace amount. [g] 1, sponge cake filled with chocolate; 2, doughnut; 3, doughnut stuffed with chocolate; 5, Swiss roll stuffed with chocolate; 6, biscuit coated with chocolate; 7, sponge cake filled with chocolate; 8, sponge cake; 9, cake filled with chocolate, 10; Swiss roll stuffed with chocolate; 11, Swiss roll stuffed with chocolate; 12, sponge cake filled with chocolate; 13, Swiss cake; 14, cake filled with chocolate; 15, cake filled with chocolate.

Table 3. Results for the Analysis of Variance Cluster Analysis of Saturated, Monounsaturated, Polyunsaturated, and *trans* Fatty Acid Contents of Fat Extracted from Samples

variable[a]	cluster minimum square	DF[b]	error minimum square	DF	p[c]
MUFA	238.9	2	33.6	12	0.009
PUFA	1551.7	2	12.9	12	<0.0001
SFA	2332.2	2	24.8	12	<0.0001
TFA	51.4	2	12.0	12	0.04

[a] See Table 2 for nomenclature. [b] DF, degrees of freedom. [c] p, degree of significance.

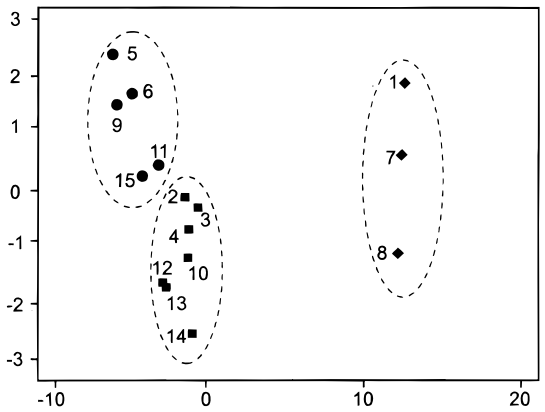

Figure 1. Plot of the values of the two discriminant scores (discriminant analysis) of industrial bakery products produced in Spain. Cases are grouped according to their fatty acid profile, SFAs, MUFAs, and PUFAs composition (group 1, samples high in PUFAs and low in MUFAs and SFAs; group 2, samples high in MUFAs and SFAs but low in PUFAS; group 3, samples high in SFAs but low in MUFAs and PUFAs) (group 1, ◆; group 2, ■; group 3, ●).

Although both discriminant functions are significant, the first of these functions accounts for 96.8% of the common variance. A plot of the group means using discriminant scores from both discriminant functions is shown in Figure 1.

On the basis of the statistical analysis, all samples could be classified into three groups: group 1, samples high in PUFAs and low in MUFAs and SFAs; group 2, samples high in MUFAs and SFAs but low in PUFAs. According to their fatty acid profiles, the fat incorporated into samples belonging to groups 1 and 2 has an animal origin. Group 3 was constituted by samples high in SFAs but low in MUFAs and PUFAs; this suggests that the fat content of samples that belong to group 3 comes from palm or coconut oil, which are both high in saturated fatty acids. All samples show a small percentage of *trans* fatty acids, mostly *trans*-9-octadecanoic (elaidic) produced during hydrogenation.

Despite the fact that there is no recommended maximum intake of *trans* fatty acids to prevent a risk of CHDs, there is a great deal of evidence that suggests a need to control the *trans* fatty acids intake (Kris-Etherton, 1995). Data from Spanish samples, as well as recent data from other countries, show a decrease in the consumption of TFAs coming from baking fats. However, we obseved that some samples are still high in these isomeric fatty acids.

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