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Original article

Trans isomer content of infant milk formulas

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Summary — Infrared (IR) spectroscopy and capillary gas chromatography (CGC) were used to investigate the *trans* isomer content of infant milk formulas. For this purpose we analysed 28 samples from the Spanish market for the total *trans* isomer content (IR-spectroscopy) and for octadecenoic (C18:1 and C18:2) isomers (CGC). Our results show that infant milk formulas have low levels of trans isomers and they comply with the EEC Proposal 85/C28/05.

***trans* isomer / infant milk formula / IR spectroscopy / capillary gas chromatography**

Résumé — Isomères *trans* dans des laits infantiles. *Le dosage des isomères* trans *dans des préparations pour allaitement des nourrissons a été mis en œuvre par spectroscopie dans l'infrarouge (IR) et par chromatographie capillaire en phase gazeuse. Pour cette étude, nous avons analysé 28 échantillons du marché espagnol pour quantifier les isomères* trans *de manière globale (spectroscopie IR) et pour isoler les différentes formes isomériques de l'acide octadécenoïque, particulier du C18:1 et C18:2. Nos résultats montrent que les laits pour nourrissons analysés ont une faible teneur en isomères* trans *et sont en accord avec la proposition de la CEE 85/C28/05.*

isomère* trans / *lait pour nourrisson / spectroscopie IR / chromatographie capillaire phase gazeuse

INTRODUCTION

Fatty acid composition of dairy products has received widespread attention (Parodi, 1983; Hachey *et al,* 1987; Koletzko *et al,* 1988). One of the reasons is the complex range of substances that arise as a result of isomerization in fatty acid unsaturation sites, which accompany hydrogenation by the rumen microbes of the unsaturated feed lipids to yield a mixture of geometrical and positional isomers, which are transmitted to the milk. Amount and type of feed can influence the microbiological population of the rumen and this can influence hydrogenation and formation of trans isomers in milk fat. In addition, seasonal feed variation results in higher unsaturated fatty acid levels in summer than in winter. Total isolated *trans* fatty acids in milkfat reported in the literature range from 2 to 11% with maximal values in the pasture feeding period due to hydrogenation and uptake of fat rich in $C_{18:2}$ and $C_{18:3}$ from fresh grass and minimal values in indoor feeding period (Deman and Deman, 1983; Sommerfeld, 1983; Zegarska and Kuzdzal-Savoie, 1988).

In the case of infant milk formulas, milkfat is totally or partially substituted by a vegetable fat in order to achieve a similar fatty acid composition to the mother's milk. This vegetable fat can be obtained by hydrogenation of unsaturated vegetable oils and in this process, *trans* isomers of fatty acids can be produced. Therefore, the total *trans* isomer content of infant milk formulas is a consequence of the milkfat composition and the *trans* fatty acid content of hydrogenated vegetable fat used as raw material in their production.

On the other hand, there is an interest among nutritional research workers and governments as to whether there is any risk to health associated with the consumption of *trans* fatty acids (Emken, 1981; Kummerow, 1986) and in this sense, the EEC proposal 85/C28/05 limits the trans fatty acid content at 8% in raw materials used in infant milk formulas.

The objective of our study was to investigate and quantify the trans isomers in several samples of formulas used in infant feeding in order to establish the general situation of these kinds of foods.

MATERIAL AND METHODS

We have analysed 28 samples of infant milk formulas from the Spanish market, including :

– adapted formulas (AD): For infants between 0 and 4–6 months (10 samples);

– follow-up formulas (FU): For infants between 4–6 and 12 months (7 samples);

– pre-term infant formulas (PT): For low birth weight infants (3 samples);

– special formulas (SP): For lactose and cow's milk protein intolerances (8 samples).

Ingredient composition was noted from labels in order to correlate results with raw materials used by manufacturers (whole milk, skimmed milk, vegetable fat, etc).

Extraction

The extraction procedure is based on the Röse-Gottlieb method (FIL 123-1985) (Lee, 1987). Weight ca 10 g to the nearest milligram into fat-extraction flask. Add 2 ml ammonium hydroxide and mix thoroughly. Add 10 ml ethyl alcohol and shake well. Add 25 ml ethyl ether to sample and shake vigorously for 1 min. Finally, add 25 ml petroleum ether and shake vigorously for 1 min. Centrifuge flask and decant ether layer. Conduct a second extraction using 5 ml ethanol and 25 ml of each ether. Combine ether extracts in round-bottom flask and rotary evaporate solvents at ca 80 °C.

Infrared spectroscopy

One gram of the fatty extract was dissolved in 10 ml of carbon disulfide and placed in a sodium chloride cell of 0.2 mm thickness in a Perkin Elmer FT-1710 infrared spectrophotometer. The measurement was taken at 966 cm^{-1}.

Results are expressed as a percentage of elaidate in the fatty extract by using a calibration curve with methyl elaidate as a standard.

Capillary gas chromatography

Methylation of the fatty extract is performed according to Slover and Lanza (1979) with the mixture BF_3/CH_3OH as a reagent.

The identification of each peak was carried out by using standard fatty acid methyl esters (Merck, Sigma and Supelco) and retention time data from bibliography (Hernandez, 1988).

Chromatographic conditions

System : Perkin Elmer Chromatograph. Sigma 300, FID. Integrator HP 3390 A; Column: Fused Silica WCOT, CP-Sil 88, 50 m x 0.25 mm ID; Oven temperature: 190 °C (isoterm); Injector temperature: 270 °C; Detector temperature: 300 °C; Sample volume: 0.15 µl; Split: 1/100; Carrier gas: He; Pressure carrier gas: 2 bar.

RESULTS AND DISCUSSION

A typical fatty acid chromatogram of infant milk formulas is shown in figure 1. Using our chromatograph conditions, satisfactory separation was obtained of $C_{16:1}$ (*cis* and *trans*), $C_{18:1}$ (*cis* and *trans*), $C_{18:2}$ *(cc, ct, tc,* tt*)* and $C_{18:3}$ *(ccc)* from other *trans*-isomers of $C_{18:3}$.

Table I shows the amount of total *trans* isomers in the different infant formulas analysed using IR spectroscopy and capillary gas chromatography (CGC). These results range between 0.16 and 4.55%, and the analysis of variance (ANOVA) shows a statistically significant correlation ($P < 0.1$) in the *trans* isomer content determinated either by the IR or CGC technique, in spite of the triglyceride interference in the IR technique (Deman and Deman, 1983).

On the other hand, the ANOVA test shows no significant difference between the trans isomer content of the 4 groups of samples (pre-term, adapted, follow-up and special).

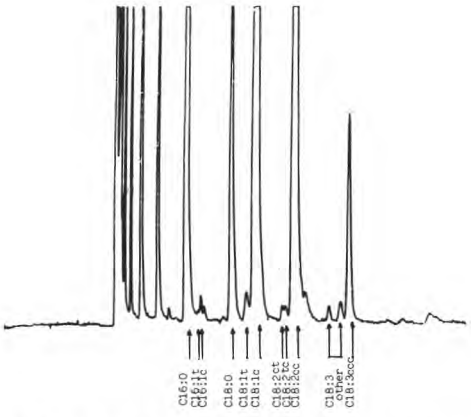

Fig 1. Typical chromatogram of fatty acid methyl esters of infant milk formula in our chromatographic conditions.
Chromatogramme typique des esters méthyliques des acides gras d'une préparation pour allaitement des nourrissons dans nos conditions chromatographiques.

Capillary gas chromatographic results are presented in table II. This table shows the total saturated fatty acids and the different percentages of unsaturated *cis* and *trans* isomers of $C_{16:1}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$. Results for $C_{18:1}$ *trans* range be-

Table I. Total *trans* isomer content by infrared and capillary gas chromatrographic techniques. *Taux d'isomères* trans *par infrarouge et par chromatographie capillaire en phase gazeuse.*

Sample No	*Type of Formula*	*IR results % trans isomers*	*CGC results % trans isomers*
01*	(PT)	3.68	2.49
02*	(PT)	2.50	2.55
03	(PT)	3.09	1.19
04*	(AD)	2.76	2.24
05*	(AD)	3.15	4.54
06	(AD)	2.15	1.52
07*	(AD)	2.88	2.54
08	(AD)	1.80	1.28
09*	(AD)	3.40	4.06
10*	(AD)	3.37	4.24
11	(AD)	1.68	1.74
12*	(AD)	4.55	4.01
13*	(AD)	2.57	2.61
14*	(FU)	4.11	3.98
15*	(FU)	2.27	2.80
16*	(FU)	2.34	3.39
17	(FU)	1.43	0.96
18	(FU)	2.30	3.58
19*	(FU)	3.15	2.14
20*	(FU)	2.21	2.59
21	(SP)	2.73	1.65
22	(SP)	2.98	1.40
23*	(SP)	1.45	2.53
24*	(SP)	4.04	2.14
25*	(SP)	1.63	0.16
26*	(SP)	3.82	2.61
27	(SP)	0.94	0.60
28	(SP)	2.37	2.06

* with whole milk as ingredient.

Table II. Capillary gas chromatographic results.
Résultats obtenus par chromatographie capillaire en phase gazeuse.

Sample No	*Type of formula*	*% total saturated*	*CGC results (relative percentages)*									
			% cis *isomers*				% trans *isomers*					
			16:1	18:1	18:2	18:3	16:1	18:1	18:2ct	18:2tc	18:2tt	Others 18:3
01*	(PT)	73.76	0.53	15.53	7.42	0.27	0.22	0.95	0.63	0.36	nd	0.33
02*	(PT)	62.23	0.24	24.01	10.85	0.12	nd	2.15	0.12	0.08	0.08	0.12
03	(PT)	62.99	0.77	27.57	7.30	0.18	nd	0.37	0.30	0.22	nd	0.30
04*	(AD)	61.67	0.53	26.07	9.10	0.39	0.19	1.38	0.18	0.08	nd	0.41
05*	(AD)	56.77	0.87	24.87	12.53	0.42	0.41	2.22	0.88	0.52	nd	0.51
06	(AD)	46.50	1.52	39.05	11.16	0.25	0.15	0.42	0.41	0.29	nd	0.25
07*	(AD)	57.68	0.28	26.71	12.59	0.20	0.10	2.10	0.11	0.12	0.11	nd
08	(AD)	42.52	1.45	44.27	10.23	0.25	0.08	0.19	0.23	0.17	nd	0.61
09*	(AD)	56.44	0.63	27.04	11.45	0.38	0.25	3.15	0.21	0.10	nd	0.35
10*	(AD)	60.01	0.62	24.18	10.48	0.47	0.21	3.12	0.19	0.11	0.05	0.56
11	(AD)	54.62	0.30	23.54	19.50	0.30	0.10	0.47	0.50	0.33	nd	0.34
12*	(AD)	59.76	0.79	23.35	11.49	0.60	0.39	1.82	0.78	0.38	0.05	0.59
13*	(AD)	56.12	0.23	27.79	13.08	0.17	0.07	2.07	0.13	0.12	nd	0.22
14*	(FU)	58.84	0.59	25.19	10.97	0.43	0.19	3.01	0.17	0.11	0.06	0.44
15*	(FU)	58.18	0.41	26.99	11.47	0.15	0.16	2.16	0.10	0.10	0.10	0.21
16*	(FU)	57.44	0.84	23.80	13.98	0.55	0.39	2.00	0.18	0.07	0.06	0.69
17	(FU)	47.80	0.28	34.74	13.14	0.08	0.10	0.23	0.25	0.16	nd	0.22
18	(FU)	55.93	0.69	27.04	12.57	0.19	0.25	2.67	0.19	0.14	0.07	0.26
19*	(FU)	57.47	1.12	32.34	6.72	0.21	0.29	0.60	0.40	0.21	0.04	0.60
20*	(FU)	56.92	0.26	27.51	12.61	0.11	0.08	1.97	0.17	0.11	0.10	0.16
21	(SP)	66.19	0.06	20.62	11.39	0.09	nd	1.45	0.09	0.11	nd	nd
22	(SP)	46.02	0.63	41.69	10.06	0.20	0.07	0.20	0.43	0.28	nd	0.42
23*	(SP)	57.03	0.87	21.56	17.57	0.44	0.36	1.05	0.04	nd	nd	1.08
24*	(SP)	56.38	0.63	28.95	11.43	0.47	0.28	0.77	0.18	0.11	0.05	0.75
25*	(SP)	73.71	0.27	11.81	13.56	0.49	0.07	0.04	0.05	nd	nd	nd
26*	(SP)	57.14	0.25	26.91	12.98	0.11	0.07	1.88	0.15	0.11	0.10	0.30
27	(SP)	43.84	0.11	26.29	27.28	1.88	nd	0.23	0.09	nd	nd	0.28
28	(SP)	53.06	0.66	26.67	17.31	0.24	0.15	0.59	0.57	0.38	nd	0.37

nd = no detectable amount; * with whole milk as ingredient.

tween 0.04 and 3.15%. These values are generally lower than those reported for human milkfat that range between 2–4% for this fatty acid (Emken, 1979). On the other hand, infant formulas that include whole cow's milk as an ingredient have higher values in *trans* isomers (average value = 1.8%) due to vaccenic acid formed in the biohydrogenation than formulas manufactured with skimmed milk (average value =

0.97%). Levels for the individual *trans* isomers of linoleic acid ($C_{18:2}$), generally provided by the vegetable fat added to the formula, were less than 1% and only 1 sample (No 18) from the group manufactured with skimmed milk as a raw material shows $C_{18:2}$ *t-t* isomers. Our results indicate that formulas manufactured with skimmed milk as an ingredient do not generally include hydrogenated vegetable fat and that the food industry complies with the EEC Proposal 85/C28/05 that limits the use of fats with a maximum of 8% *trans* isomers, and that trans isomer content of infant milk formulas is lower than in cow's milk fat and human milk fat.

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