The trans fatty acid and positional (sn-2) fatty acid composition of some Australian margarines, dairy blends and animal fats

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We have analysed the fatty acid (FA) composition including the trans fatty acid by GLC and Fourier Transform Infra-Red (FTIR) Spectrophotometry of 13 margarines, five butter/dairy blends and two animal fats (lard and dripping). The samples were purchased from supermarkets in three separate locations across Victoria: Gladstone Park (near Melbourne), Waurn Ponds (near Geelong) and Geelong city. From the FA composition, the P/S, P/(S+trans monoenoic FA), P/M(S+trans monoenoic FA) and ω6/ω3 ratios were calculated. The FA composition and trans FA content were compared with the last published analysis of Australian margarines in Sydney in 1982. The FA composition of the sn-2 position was obtained by pancreatic lipase deacylation of the whole triglycerides (TG). From this data, we estimated the per cent interesterified fat which was present in the margarines. The trans FA content of the margarines which was determined by FTIR ranged from 9.2% to 16.3% (mean of 13.1% of total FAME) (7.6 g–13.0 g trans FA/100 g sample, mean of 10.4 g/100 g sample) and from 3.2% to 4.1% (mean of 3.8%) for butter and dairy blends. Lard contained 0.4% trans FA while dripping consisted of 3.6% trans FA. The trans FA content in the margarines was similar to the values published in 1982 with the exception of four brands. The ω6/ω3 ranged from 2.5 to 363 and the P/S ranged from 1.4 to 3.3 compared with the 1982 figures where the ω6/ω3 ranged from 3 to 49 and the P/S ranged from 0.1 to 3.7. The estimated per cent interesterified fat in the margarines ranged from 25% to 100%. We estimated the total trans FA intake in the Australian diet to be between 2.7 g/head/day and 4.8 g/head/day. We also estimated that table margarines account for between 36% and 64% of the total trans FA intake in the Australian diet.

Introduction

Trans FAs are formed during the partial hydrogenation of vegetable oils[1-4] which are used for the manufacture of margarines and processed foods. Trans FA also occurs in milk fat, butter and ruminant fats such as tallow and dripping (lamb and beef fat, respectively)[1,2,4]. Trans FA may also be present in fat from non-ruminant animals that consume diets containing trans FA[1]. In recent times there has been growing concern as to the possible detrimental effects of consumption of trans FA on human health[2-9].

Dietary trans FAs have been shown to increase plasma LDL cholesterol[10-14] while decreasing plasma HDL cholesterol levels[10-15]. Plasma lipoprotein (a) which is thought to be an indicator of atherogenic risk has also been shown to rise with increasing intake of trans FA[16,17]. As a result of these observations trans monoenoic FAs, which are the major trans FA isomers found in margarines are now being considered at least as harmful as saturated fatty acids[12,14,18].

Trans FAs accumulate in a variety of tissues in different amounts with adipose tissue containing the highest level[19]. Trans FA isomers have also been detected in human breast milk and it has been suggested that these levels may be affected by the mother's diet[20,21]. Recent reports have shown that trans FA isomers of 18:3 may be metabolized to trans docosahexaenoic acid which can be detected in the brain lipids of rats fed trans 18:3[22].

The amount of trans FA in margarines depends on the extent of hydrogenation and whether or not the partially hydrogenated vegetable oil is blended with interesterified, animal or dairy fat. The main determinants in the above processes are the availability and cost of the seed oils, animal and dairy fats, in addition to the desired consistency of a particular product.

Several workers have indicated that, irrespective of fatty acid composition, the distribution of FA in the triglyceride structure effects cholesterolaemia. Thus there have been suggestions that linoleic acid is more hypocholesterolaemic[23] and stearic acid more hypercholesterolaemic[24] when present at the sn-2 position, than when esterified in positions sn-1 or sn-3. The fact that stearic acid is normally esterified at position 1 and rarely at position 2 may partly explain the apparent 'neutral' effect of stearic acid on blood cholesterol.

A Belgian research group reported that butter was less

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hypercholesterolaemic if the triglyceride FA were randomized by the technique of interesterification[25]. Butter contains a high proportion of 14:0 and 16:0 FA in the sn-2 position and the interesterification process would reduce the proportion in the 2-position, since interesterification evenly distributes the FA in each of the sn-positions in the TG. Kritchevsky et al.[26] have shown that the atherogenic effects of peanut oil for rats could be reduced if the peanut oil was interesterified.

While the mechanisms of these effects are still obscure, nevertheless the results highlight the importance of the positional distribution of FA in the TG. It has been suggested by Redgrave et al.[27] that saturated FA in the sn-2 position reduced chylomicron remnant metabolism, a process which has been implicated in atherosclerosis. Ahikari et al.[28] have been able to use two FA ratios, namely, the ratio of palmitic acid in the sn-2 position to that found in the whole triglyceride and similarly the amount of total saturated fatty acids in the sn-2 position to that found in the whole triglyceride (R1 and R2, respectively) to estimate the amount of interesterified fat added to a hydrogenated fat. Carpenter et al.[29] have been able to show that trans monoenoic FAs are concentrated in the sn-2 positions in some margarines. This observation has been attributed to polyunsaturated FAs being preferentially located in the sn-2 position of the original vegetable oil which are partly converted to trans monoenoic FA during hydrogenation.

Several analyses of Australian margarines (1976–82)[30-33] have reported values of trans FA which were lower (less than 15%) than those from other western countries notably America (10–30% in 1984)[34], Canada (10–35% in 1985)[35], Britain (4–42% in 1984)[36] and up to 50% in northern Europe where partially hydrogenated marine oils were used in the formulation of some cheaper margarines[37].

The last analysis of trans FA in Australian margarines was carried out in 1982[33]. Owing to the potential variability in margarine formulations and an upsurge in the interest in trans FA there was a need to analyse the currently produced margarines in the Australian market-place, determine the trans FA content and sn-2 FA composition and estimate the extent to which inter-esterified fats were added to partially hydrogenated vegetable oils.

Methods

(All standards and reagents were of 99.9% purity and analytical grade, respectively, unless otherwise stated).

Validation for selection of number of tubs to be analysed

Margarines from three different locations were sampled and initially two tubs were purchased from each location. Duplicate determinations of trans FA content were performed on each tub. We found that the average per cent trans FA content of the first tubs collected from the three locations (10.32 ± 0.28% for six determinations) was very similar to the average per cent trans FA content of the first and second tubs collected from the three locations (10.10 ± 0.40% for twelve determinations). From these results it was concluded that sampling three tubs, one from each location (six determinations) was sufficient.

Sample collections

After collecting one tub from each location, they were coded, dated and stored in a refrigerator at 4°C until required for analysis. Three tubs of an olive oil-based margarine manufactured in Greece (Brio brand) obtained as a gift from Unilever Australia Ltd were also analysed.

Subsampling for analysis

The top 1 cm surface layer was discarded and a core sample of approximately 2 g was taken from two locations in the tub at least 3 cm apart and placed in a 15 mL test tube with a teflon-lined screw cap.

Extraction of lipid

The sample was extracted into petroleum ether (PE) (1×10mL then 2×5 mL) with shaking/vortex mixing and centrifugation each time. Effectively all the lipid was extracted by this method as a further chloroform/methanol (2/1, v/v) re-extraction of the residue yielded no detectable lipid. The extract was transferred and made up to volume in a 25 mL standard flask.

Gravimetric determination of per cent lipid

Aliquots of 1 mL were taken from the 25 mL stock and delivered into pre-weighed glass vials. The PE was evaporated in a stream of nitrogen. The dry extract was stored overnight in a dessicator over silica gel and re-weighed the following day.

Fatty acid analysis

Saponification and methylation

A 0.5 mL aliquot from the lipid stock solution and a 1.0 mL aliquot of triheptadecanoin internal standard (13.0 mg/mL) in chloroform were taken and delivered into test tubes with teflon lined screw caps. The solvent was evaporated in a stream of nitrogen and 2 mL of potassium hydroxide in methanol KOH/MeOH (58 mg/mL) was added. The contents of the tube were flushed with nitrogen, quickly capped and placed in a fan-forced oven set at 105°C. After 10 min, 2 mL of 20% boron trifluoride in methanol (BF_3/MeOH) was added and the tube reheated for 10 min at 105°C. The fatty acid methyl esters (FAME) were then extracted into the PE. The FAME were then washed with distilled water and dried over anhydrous sodium sulphate. The FAME were chromatographed immediately or stored at −20°C until required for analysis.

Gas liquid chromatography (GLC)

The FAME were separated on a 50 m BPX70 (0.32 mm ID and 0.25 mm film thickness) bonded phase, fused silica capillary column (SGE Ringwood, Victoria, Australia), connected to a Shimadzu GC-9A chromatograph which was interfaced to a Shimadzu CR4A microprocessor integrator used for data storage and handling. The injector and FID detector temperatures were both 280°C and the linear carrier gas (Helium) flow was set to 20 cm/sec. The column oven was set at an initial temperature of 110°C for 3 min and was then increased at 1°C/min until a temperature of 170°C was reached. The rate was then increased to 5°C/min and the final temperature of 200°C was maintained for 30 min.

Total methylene interrupted trans fatty acids by FTIR
This method is based on an IUPAC official method[38]. The remaining 21.5 mL of the lipid stock was saponified by refluxing with 20 mL of KOH/MeOH (58 mg/mL) for 10 min. 20 mL of BF_3/MeOH (20%) was then added and the mixture refluxed for a further 10 min. When the mixture had cooled 20 mL of PE and 20 mL of saturated sodium chloride solution were added, the flask stoppered and the mixture shaken to extract the FAME into the PE phase. After the layers had separated the FAME were siphoned into a 50 mL round-bottomed flask and concentrated on a rotary evaporator at 35°C.

The FAME were purified by eluting through a silica sep-pak (Millipore-Waters) with 10% diethylether (DE) in PE into a pre-weighed 10 mL volumetric flask. The solvent was evaporated in a stream of nitrogen and the flask re-weighed to calculate the mass of FAME. The FAME were diluted to the mark with carbon disulphide (CS_2). A 2 mL aliquot was further diluted to 5 mL prior to measuring the IR absorbance.

A series of calibration standards made up of elaidic and stearic acid methyl esters were obtained by bulk methylation of the free fatty acids (Nu Chek Prep, Minnesota, USA) and purified by silica column chromatography using PE as the eluting solvent. The purity was rechecked by thin layer chromatography (TLC) before drying the FAME with nitrogen. The standard solutions were then used to construct a calibration curve. A CS_2-filled sodium chloride cell of 1 mm path length was used as the background blank.

The IR absorbance peak area at 970 cm^{-1} was measured on a FTIR spectrophotometer (FTS-7) (Biorad Laboratories, Digilab Division, Hercules, CA, USA) using the quantitation software (Interquant). The concentration of the total methylene interrupted trans FAME was interpolated from the calibration curve.

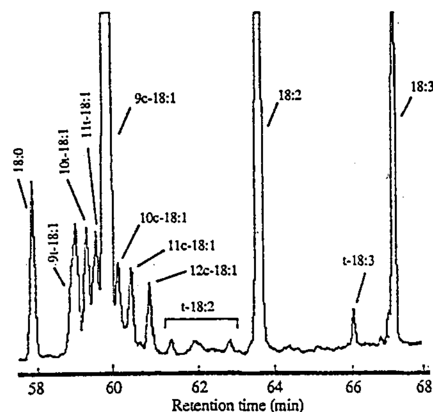
Positional (sn-2 FA) analysis of triglycerides
The purification of the TG was achieved by preparative TLC of 400 μL of the lipid stock solution on two TLC plates (200 μL on each plate). The plates were then developed in a solvent system consisting of PE/DE/glacial acetic acid (90/10/1 v/v/v). After the development the plate was sprayed with 2'7'-dichlorofluoroscein in MeOH and the TG band visualized and marked under a 360 nm UV lamp. The silica gel was then scraped off the plate and placed into a methylation tube and extracted with 10% DE in PE (3 × 5 mL). The extracts were combined and dried in a stream of nitrogen then reconstituted in 400 μL of PE.

Pancreatic lipase deacylation
All reagents used in this section were purchased from the Sigma Chemical Company (St Louis, MO, USA). This method is based on the procedure of Chacko et al.[39] Approximately 10 μL of the purified TG (10 μL/400 μL) was dried with nitrogen in a methylation tube to which 100 μL of $CaCl_2$ (22 mg/mL), 250 μL of bile salts (cholate/deoxycholate, 0.5 mg/mL) and 1 mL of pancreatic lipase (porcine, type II, crude, Sigma, 21.23 mg/mL) in Trizma buffer (Tris base, pH8). The reaction mixture was incubated at 40°C for 0.5 min then the reaction was stopped with 0.5 mL of 6M HC1. The lipase reaction was repeated using 1.0 min and 1.5 min incubations.

Table 1. The lipid content of margarines, butter/dairy blends and animal fats.

Sample	Code	%Lipid
Margarines		
Gold'n Canola	GC	85.5
Nuttlex	NUT	84.7
Miracle	M	82.9
Miracle Canola	MCAN	83.1
Meadow Lea	ML	81.1
Meadow Lea Canola	MLCAN	81.9
ETA	ETA	84.3
Flora	Flora	82.4
Daffodil	DAF	82.6
Becel	BEC	85.7
Brio	BRIO	81.0
Home Brand Margarine	HBM	83.0
Mrs McGregor's	MAC	82.6
Butter/Dairy Blends		
Western Star Butter	WSB	83.2
Western Star Country Gold	WSCG	83.0
Devondale Dairy Soft	DDS	83.1
Devondale Dairy Canola	DDC	60.9
Prefer	PRE	84.7
Animal Fat		
Lard	LAR	100
Dripping	DRIP	100

Mean (n=6).



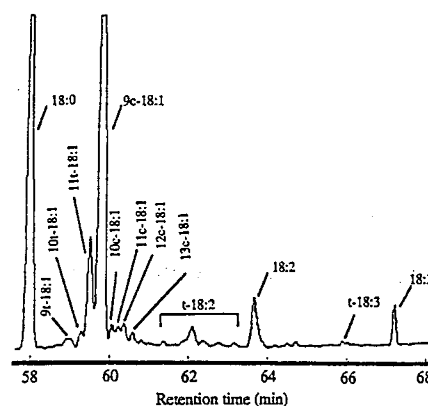


Fig. 1a. A GLC trace of a margarine (MLCAN) showing the main trans 18:1 and cis 18:1 positional isomers (tentative identification).
Fig. 1b. A GLC trace of a butter (WSB) showing differences in the amount and type of cis and trans 18:1 positional isomers.

Table 2. Fatty acid composition (% of total FA) in the sn-2 position of TG and of total margarines[a].

FA	BECEL		ML		MLCAN		M		MCAN		BRIO	
	Total FA	sn-2 FA	Total FA	sn-2 FA	Total FA	sn-2 FA	Total FA	sn-2 FA	Total FA	sn-2 FA	Total FA	sn-2 FA
10:0	nd[b]	0.60	nd	0.21	nd	0.03	nd	0.39	nd	0.08	nd	nd
12:0	nd	0.48	nd	0.17	nd	0.03	0.09	0.50	nd	nd	nd	nd
14:0	0.08	1.77	0.23	0.80	0.18	0.46	0.35	1.07	0.08	0.52	nd	nd
16:0	6.13	10.74	11.25	5.22	8.26	2.70	13.54	6.96	6.41	5.76	11.26	3.82
16:1	0.06	0.15	0.08	nd	0.16	nd	0.07	nd	0.14	0.31	0.48	nd
17:0	nd	0.86	nd	0.54	0.27	nd	nd	0.34	nd	0.66	nd	nd
17:1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
18:0	6.10	11.65	7.38	7.36	4.88	4.56	5.46	5.78	6.35	9.06	8.36	6.79
t-18:1[c]	7.51	5.67	13.20	11.77	8.83	11.18	12.11	12.84	13.22	9.74	12.78	14.14
18:1	31.00	31.33	24.59	24.33	48.32	49.06	25.18	29.84	51.47	42.34	61.19	64.90
t-18:2	0.97	1.93	0.54	0.37	0.42	0.70	0.66	0.89	1.08	1.14	nd	nd
18:2	44.29	28.82	39.05	38.10	15.51	23.96	39.48	39.00	15.75	21.78	4.90	7.53
t-18:3	0.02	0.70	0.09	0.11	0.37	0.42	0.09	nd	0.22	0.52	nd	nd
18:3	1.97	1.47	2.02	1.46	6.12	5.93	2.19	1.40	5.80	5.13	0.41	0.62
20:0	0.52	0.52	0.46	0.23	0.53	0.11	0.43	0.19	0.68	0.18	0.45	nd
t-20:1	nd	0.32	0.14	0.13	nd	0.12	0.01	nd	nd	nd	nd	nd
20:1	0.38	0.42	0.29	0.18	0.76	0.32	0.22	nd	0.78	0.32	0.17	nd
22:0	0.78	0.34	0.52	0.83	0.31	nd	0.55	0.20	0.33	0.20	nd	2.20
22:1	nd	2.03	0.02	7.90	0.14	nd	nd	1.09	nd	2.26	nd	nd
24:0	0.25	0.20	0.21	0.28	0.32	0.31	0.19	nd	0.18	nd	nd	nd
24:1	nd	nd	nd	nd	nd	nd	nd	nd	0.12	nd	nd	nd

[a] Mean (n=6)
[b] nd (not detected)
[c] t-18:1 = 9t-18:1 + 10t-18:1 + 11t-18:1.

Table 3. Fatty acid composition (% of total FA) in the sn-2 position of TG and of total margarines[a].

FA	MAC		HBM		FLORA		DAF		ETA		GC		NUT	
	Total FA	sn-2 FA	Total FA	sn-2 FA	Total FA	sn-2 FA	Total FA	sn-2 FA	Total FA	sn-2 FA	Total FA	sn-2 FA	Total FA	sn-2 FA
10:0	nd[b]	nd	0.08	0.32	nd	nd	nd	0.59	nd	nd	nd	nd	nd	nd
12:0	nd	nd	0.10	0.29	0.03	nd	0.26	0.62	0.17	0.42	nd	nd	nd	0.23
14:0	0.64	1.30	0.22	1.47	0.29	0.51	0.29	1.62	0.24	1.20	0.21	0.51	0.28	0.98
16:0	20.73	11.00	10.81	10.45	12.34	6.17	12.73	10.43	10.44	7.23	9.09	2.24	13.14	6.83
16:1	0.31	0.32	0.08	0.29	0.07	nd	0.03	0.20	0.06	nd	0.21	0.16	nd	0.20
17:0	nd	0.46	nd	0.34	nd	0.24	nd	0.77	nd	0.34	nd	0.25	nd	0.79
17:1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
18:0	5.96	12.56	7.42	8.97	5.33	6.11	4.97	10.99	7.18	8.48	5.00	3.25	5.96	7.56
t-18:1[c]	13.51	10.74	9.66	8.10	13.24	14.49	11.62	10.40	13.59	12.09	7.73	9.09	10.98	11.14
18:1	19.30	25.75	24.33	27.08	23.89	29.40	22.16	27.95	25.81	28.86	54.58	48.38	27.29	33.23
t-18:2	0.70	0.58	1.36	0.50	0.44	0.32	0.50	0.99	0.73	0.65	nd	0.22	0.67	1.09
18:2	37:48	30.42	44.65	36.17	40.81	38.45	44.02	30.36	38.63	35.05	15.11	25.62	39.92	32.77
t-18:3	0.14	1.53	nd	0.23	0.06	0.69	nd	0.61	0.08	0.26	0.28	0.58	0.11	0.57
18:3	0.15	1.06	0.74	1.76	1.84	1.48	1.44	1.25	1.42	1.51	5.71	8.50	0.11	0.64
20:0	0.42	0.33	0.39	0.36	0.65	0.18	0.46	0.47	0.49	0.31	0.58	0.10	0.34	0.29
t-20:1	0.18	0.27	nd	0.37	0.07	nd	nd	0.08	0.14	0.67	nd	0.13	nd	nd
20:1	0.20	0.34	0.13	0.40	0.22	0.55	0.18	0.34	0.31	0.27	0.97	0.18	0.11	0.66
22:0	0.17	0.25	0.61	0.26	0.54	0.16	0.57	0.29	0.52	0.18	0.28	nd	0.54	0.25
22:1	nd	3.29	0.28	2.63	0.01	1.24	nd	2.03	nd	2.48	nd	0.81	nd	2.38
24:0	0.10	nd	0.20	nd	0.19	nd	0.20	nd	0.20	nd	nd	nd	nd	0.13
24:1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.24

[a] Mean (n=6)
[b] nd (not detected)
[c] t-18:1 = 9t-18:1 + 10t-18:1 + 11t-18:1.

After extracting into DE, washing and drying as previously achieved for the FAME, the reaction mixture was examined on TLC using a developing solvent system of PE/DE/glacial acetic acid (85/15/ v/v/v). The plate was then sprayed with 10% CuS04 in 8% H_3PO4 (w/v) and charred at 140°C for 30 min.

An incubation time was selected to achieve a 50–60% hydrolysis which would provide a sufficient amount of monoglycerides (MG). If the lipase hydrolysis continues beyond the optimum time some migration of FA between sn-1, 2 and 3 positions may occur in the MG[40,41]. The optimum incubation time varied between 0.5 and 1.5 min for the various fats analysed.

Results

The measured per cent lipid of the margarines was at least what was stated on the tubs and ranged between 81% and 86% which was similar to the 1982 levels (79–84%). In the case of the dairy blends the per cent lipid ranged between 83% and 85% except for one low-fat brand. Lard and dripping each contained 100% lipid (see Table 1).

Figure 1 shows the typical chromatograms of a margarine and butter sample. It can be seen that the major trans FA and cis positional isomers are trans 18:1 positional isomers (tentatively identified as 9 trans, 10 trans and 11 trans, cis 18:1 positional isomers (tentatively identified as 9 cis, 10 cis and 11 cis), smaller amounts of trans 18:2 isomers and possibly trans 18:3 isomers. The amounts of these various isomers varied between the different brands of margarines. The FA composition of the margarines is shown in Table 2 and Table 3. The FA composition of the butter/dairy blends was more com-

Table 4. Fatty acid composition (% of total FA) in the sn-2 position of TG and of total butter/dairy blends and animal fats[a].

FA	WSB		WSCG		DDS		DDC		PRE		LAR		DRIP	
	Total FA	sn-2 FA	Total FA	sn-2 FA	Total FA	sn-2 FA	Total FA	sn-2 FA	Total FA	sn-2 FA	Total FA	sn-2 FA	Total FA	sn-2 FA
8:0	0.69	0.54	0.68	nd[b]	0.75	nd	0.45	nd	0.71	0.69	nd	nd	nd	nd
10:0	2.38	0.52	2.26	0.51	2.26	0.11	1.67	0.23	2.13	0.19	nd	0.19	nd	0.22
10:0	0.21	0.13	0.16	nd	0.16	nd	nd	nd	0.17	nd	nd	nd	nd	nd
12:0	3.11	2.84	2.95	1.55	2.67	1.54	2.12	1.59	2.60	0.90	0.08	0.33	0.15	0.22
13:0	0.07	0.12	nd	nd	0.07	0.15	nd	nd	nd	nd	nd	nd	nd	nd
14:0	11.45	17.07	9.47	8.64	8.60	9.26	7.55	9.39	9.11	28.37	1.62	2.20	4.14	2.72
14:1	0.83	0.63	0.65	0.26	0.54	0.32	0.43	0.22	0.65	0.20	nd	0.16	0.77	nd
15.0	1.10	1.47	0.88	0.74	0.79	0.80	0.66	0.80	0.90	0.80	0.13	0.29	0.58	0.28
16:0	28.84	37.33	22.56	23.00	21.39	23.42	19.17	24.97	23.81	24.17	27.48	47.70	26.52	12.55
16:1	1.23	1.70	0.89	0.83	0.81	0.88	0.82	0.90	0.99	0.96	2.02	0.87	3.12	3.27
17:0	nd	0.78	nd	0.75	nd	0.56	nd	0.77	nd	0.60	nd	1.12	nd	0.58
17:1	0.26	0.36	0.19	nd	0.17	0.20	0.21	nd	0.16	0.36	0.46	0.19	0.66	0.63
18:0	11.29	7.30	11.21	11.47	11.39	9.42	9.85	10.09	11.95	8.66	16.48	10.23	18.02	11.33
t-18:1[c]	3.35	2.60	3.09	2.88	3.36	3.34	2.34	2.39	2.71	4.23	0.34	2.24	3.10	1.64
18:1	19.58	16.84	22.69	25.78	22.70	22.98	38.46	29.20	23.67	22.46	39.20	18.66	36.92	47.18
t-18:2	0.38	1.04	0.87	0.26	0.90	1.01	0.31	0.27	1.10	1.62	nd	0.32	0.98	0.70
18:2	1.48	3.25	17.69	17.13	19.84	16.00	10.09	13.61	15.47	16.30	8.60	10.69	1.57	8.00
t-18:3	0.12	nd	nd	0.19	0.11	0.41	0.36	0.35	0.20	1.98	0.14	0.65	0.21	0.43
18:3	0.73	0.62	0.66	0.83	0.99	0.95	4.39	1.96	0.73	0.36	0.14	1.11	0.49	1.10
20:0	0.97	0.20	0.90	0.48	0.74	0.31	0.66	0.31	0.78	0.27	0.71	0.65	0.63	0.40
t-20:1	0.25	0.22	0.25	nd	0.38	nd	0.43	nd	0.34	nd	0.25	0.94	0.27	0.81
20:1	0.50	0.10	0.51	0.32	0.79	0.27	0.55	0.32	0.50	nd	2.30	0.43	0.50	0.33
22:0	0.09	0.08	0.07	0.30	0.30	0.20	0.20	0.25	0.28	0.15	nd	0.28	nd	0.25
t-22:1	0.14	nd	0.29	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
22:1	0.10	0.91	0.08	2.70	0.30	5.70	0.09	1.08	0.07	2.88	nd	3.29	nd	5.93
24:0	nd	nd	nd	nd	0.12	0.08	nd	nd	0.14	1.38	nd	nd	nd	0.17
t-24:1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
24:1	nd	nd	nd	nd	0.14	nd	nd	nd	nd	0.14	nd	nd	nd	nd

[a] Mean (n=6)
[b] nd (not detected)
[c] t-18:1 = 9t-18:1 + 10t-18:1 + 11t-18:1.

Table 5. Fatty acid ratios, trans FA content (% of total FA) and estimated per cent interesterified content of margarines[a].

	MAC	HBM	FLORA	DAF	ETA	GC	NUT	BEC	ML	MLCAN	M	MCAN	BRIO
P/S	1.35	2.29	2.20	2.33	2.09	1.39	1.98	3.34	2.05	1.49	2.03	1.55	0.26
P/(S+t-monoene)	0.91	1.54	1.31	1.46	1.22	0.92	1.28	2.17	1.23	0.93	1.28	0.8	0.16
P/M/(S+t-monoene)	(2.1/1.2/1)	(1.5/0.9/1)	(2.8/1.2/1)	(1.5/0.8/1)	(1.2/0.8/1)	(1.1/2.8/1)	(1.5/1.1/1)	(2.2/1.5/1)	(1.2/0.7/1)	(0.8/1.8/1)	(1.3/0.8/1)	(0.9/2.2/1)	(0.2/1.9/1)
ω6/ω3	250	60	22	31	27	2.7	363	22	19	2.5	18	2.7	12
% t-18:1	13.51	9.66	13.24	11.62	13.59	7.73	10.98	7.51	13.2	8.83	12.11	13.22	12.78
% t-18:2	0.70	1.36	0.44	0.50	0.73	nd	0.67	0.97	0.54	0.42	0.66	1.08	nd
% t-18:3	0.14	nd[b]	0.06	nd	0.08	0.28	0.11	0.02	0.09	0.37	0.09	0.22	nd
% t-20:1	0.18	nd	0.07	nd	0.14	nd	nd	nd	0.14	nd	0.01	nd	nd
Total t-FA-(GLC)[c]	14.53	11.02	13.81	12.12	14.54	8.01	11.76	8.50	13.97	9.62	12.87	14.52	12.78
Total t-FA-(FTIR)	15.00	11.70	15.70	14.60	14.80	10.10	12.00	9.20	12.70	11.80	14.90	16.30	11.01
R1[d]	53	97	50	82	69	25	54	90	46	33	51	90	34
R2[d]	92	113	70	132	94	42	86	196	78	56	75	117	64
~% interesterified fat added	80	100	50	85	80	25	80	100	50	30	60	100	40

[a] Mean (n=6)
[b] nd (not detected)
[c] Total t-FA-GLC = t-18:1 + t-18:2 + t-18:3 + t-20:1
[d] R1 and R2 used to interpolate the estimated percent interesterified fat added (see Adhikari et al.[30]).

Table 6. Fatty acid ratios, trans FA content (% total FA) of butter/dairy blends and animal fats[a].

	WSB	WSCG	DDS	DDC	PRE	LAR	DRIP
P/S	0.04	0.36	0.43	0.35	0.31	0.19	0.05
P/(S+trans monoene)	0.04	0.34	0.40	0.33	0.30	0.19	0.04
P/M/(S+trans monoene)	(0.04/0.4/1)	(0.3/0.5/1)	(0.4/0.5/1)	(0.3/0.9/1)	(0.3/0.5/1)	(0.2/0.9/1)	(0.04/0.8/1)
ω6/ω3	2.03	26.80	20.04	2.30	21.19	61.43	3.20
% t-18:1	3.35	3.09	3.36	2.34	2.71	0.34	3.10
% t-18:2	0.38	0.87	0.90	0.31	1.10	nd	0.98
% t-18:3	0.12	nd[b]	0.11	0.36	0.20	0.14	0.21
% t-20:1	0.25	0.25	0.38	0.43	0.34	0.25	0.27
% t-22:1	0.14	0.29	nd	nd	nd	nd	nd
Total trans FA-(GLC)[c]	4.24	4.50	4.75	3.44	4.35	0.73	4.56
Total trans FA-(FTIR)	4.10	3.80	4.10	3.20	3.6	0.42	3.6

[a] Mean (n=6)
[b] nd (not detected)
[c] Total t-FA-GLC = t-18:1 + t-18:2 + t-18:3 + t-20:1.

plex than that of the margarines in terms of having more short chain and branched FA as can be seen in Table 4. The FA composition of the animal fats was less complex than that of the dairy blends (see Table 4).

The main trans 18:1 positional isomer found in butter, dairy blends and dripping was not the 9 trans 18:1 but was tentatively identified as 11 trans 18:1 (see Figure 1) based on the separation achieved by others[42,43] and other published data showing the main positional isomers in margarine, butter and animal fat[1,2,29]. The total and individual trans FA isomer content of the margarines, dairy blends and animal fats is shown in Table 5 and Table 6.

The trans FA content determined by GLC was comparable to that obtained by FTIR, although FTIR gave slightly higher results (see Table 5 and Table 6). The trans FA content of the margarines determined by FTIR ranged from 9.2% to 16.3% (mean of 13.1% of total FAME) which was equivalent to a range of 7.6 g to 13.0 g trans FA/100 g sample (mean of 10.4 g trans FA/100 g sample). The trans FA content of the margarines determined by GLC ranged from 8.0 to 14.5% of total FAME. The trans FA content of the butter and dairy blends (FTIR) ranged from 3.2% to 4.1% (mean of 3.8% of total FAME). Lard contained 0.4% trans FA and dripping consisted of 3.6% trans FA (FTIR) (of total FAME). The trans FA content in the margarines was similar to the values published in 1982 (10–14% of total FA by GLC) with the exception of four brands which had higher trans FA values in 1992 (Becel, Mrs McGregor's, Daffodil and ETA).

The P/S ranged from 1.4 to 3.3 (see Table 5) (excluding the olive oil margarine from Greece, P/S=0.26) compared with the 1982 figures of 0.1 to 3.9. Treating trans 18:1 as a saturated FA resulted in the P/(S+trans 18:1) ranging from 0.8 to 2.2. The butter/dairy blends P/(S+trans 18:1) varied between 0.04 to 0.4 while lard and dripping had a P/(S+trans 18:1) of 0.2 and 0.04 respectively.

The ω6/ω3 ranged from 2.5 to 363 compared with the margarines analysed in 1982 which ranged from 10 to 49. For butter and the dairy blends, the ω6/ω3 varied between 2.0 and 26.8, while lard and dripping had ω6/ω3 of 61.3 and 3.2, respectively (see Table 6).

The major sn-2 FA in the margarines were 18:1ω6 and 18:2ω6, except for the Greek margarine produced by partially hydrogenating olive oil in which 18:1ω9 was the main (64.9%) sn-2 FA (see Table 2 and Table 3). In contrast to the margarines, butter and lard contained a larger proportion of saturated FA in the sn-2 position. In the case of butter the main sn-2 FA were 14:0, 16:0, 18:0 and 18:1ω9, while in the case of the lard the main sn-2 FA were 16:0, 18:0, 18:1 and 18:2. The per cent trans 18:1 in the sn-2 position in the margarines ranged from 5.7% to 14.5% (mean of 10.6% of total sn-2 FA).

In all samples, the majority of the trans 18:1 was found in the sn-2 position. In margarines this has been attributed to the preferential placement of polyunsaturated fatty acids at the sn-2 position in the original vegetable oil[29]. In comparison with butter, the dairy blends contained more 18:2ω6 and less 16:0 in the sn-2 position. In contrast to lard, dripping contained mainly 18:1 and only small quantities of 16:0 and 18:0 in the sn-2 position (see Table 4).

The estimated per cent interesterified fat in the margarines ranged from 25% to 100% (see Tables 5). The estimation of per cent added interesterified fat is achieved by calculating the amount of saturated FA in the sn-2 position and expressing it as a ratio of the saturated FA in the whole TG. This estimation is based on the assumption that vegetable oils have a very low content of saturated FA particularly in the sn-2 position and that interesterification will result in an increase in the amount of saturated FA in the sn-2 position relative to the whole TG. Although some fats contain large amounts of saturated FA, as in the case of coconut and palm oil, the saturated FA in these oils are mainly concentrated at the sn-1 and sn-3 positions with very little at the sn-2 position. It was not possible to estimate the per cent interesterified fat in the butter, dairy blends and some animal fats since these fats have a naturally higher level of saturated FA in the sn-2 position compared with vegetable oils.

Discussion

The trans FA content in the margarines was similar to the values published in 1982[36] with the exception of four brands. Higher levels of trans FA were observed in some brands of margarine compared with the previous analyses in 1982 (1992 data: Becel 9.2%, Mrs McGregor's 13.5%, Daffodil 11.6%, ETA 13.6%). In 1982 Becel contained 4% trans FA while no trans FAs were detected in the other three margarines. These results suggest that there have been changes in the formulations of these margarines with an increase in the use of partially hydrogenated vegetable oils. The highest trans FA content of margarines in Australia in 1992 (16.3% of FAME) was lower than that reported in several western countries[34,36] particularly where partially hydrogenated fish oil is used in the manufacture of cheaper margarines[37].

The P/S of Mrs McGregor's, Daffodil and ETA which were 0.3, 0.8 and 0.8 in 1982 increased to 1.4, 2.3 and 2.1, respectively, in 1992 while the ω6/ω3 increased from 11, 14 and 14 to 250, 31 and 27, respectively. This indicates that there was an increase in the amount of polyunsaturated FA in these margarines, which was mainly attributed to an increase in 18:2ω6 rather than 18:3ω3. These results may also be explained by the fact that in 1982 Mrs McGregor's margarine contained significant amounts of cholesterol (80 mg/100 g), palmitic (16:0, 22%) and stearic acids (18:0, 13%) which is suggestive of the use of animal fats, while ETA and Daffodil contained some lauric acid (2%), indicating the presence of some coconut oil. The absence of cholesterol in ETA and Daffodil in 1982 indicates that they consisted of a vegetable oil only, and the level of palmitic acid (16:0, 29% suggests that the major oil used was palm oil. The 1992 results indicate a shift away from animal blends and saturated fats such as coconut and palm oil towards the increased use of 18:2ω6-rich partially hydrogenated vegetable oils. In contrast to the above three margarines, the P/S and ω6/ω3 of Miracle, Meadow Lea, Flora and Becel had decreased since 1982. In the case of Miracle, Meadow Lea, and Flora, the decrease in P/S and ω6/ω3 was due to a decrease in 18:2ω6, a slight increase in saturated FA and a slight increase in 18:3ω3 (to

approximately 2%). In the case of Becel the decrease in P/S and ω6/ω3 was also due to a decrease in 18:2ω6 but the saturated FAs were not significantly changed. In contrast, Becel had an increased 18:1ω9 content since 1982 indicating the use of an 18:1ω9-rich oil such as canola oil. A slight increase in 18:3ω3 (to approximately 2%) in Becel supports this view.

Since the trans monoenoic FAs have been shown to behave similarly to saturated FA in adversely affecting the LDL cholesterol/HDL cholesterol quotient, expressing the P/S as P/(S+trans monoenoic FA) may be a more useful index in foods with high trans monoenoic FA content.

In 1982 there were no canola oil-based margarines, with Mother's Choice having the lowest ω6/ω3 of 10. In 1992 there were several polyunsaturated canola-oil based margarines on the market namely, Miracle Canola, Meadow Lea Canola and Gold'n Canola, which had ω6/ω3 values of 2.7, 2.5 and 2.7, respectively. The range of 18:3ω3 in the margarines in 1992 was 0.1 to 5.8% (mean 2.5%) compared with values of 0 to 3% (mean 1.5%) in 1982.

In the butter/dairy blends and dripping the main trans 18:1 isomer was tentatively identified as 11 trans 18:1 since this isomer is thought to be the main trans isomer in ruminant fats[1,2,29]. Thus, there seems to be differences between the trans 18:1 isomers produced during the biohydrogenation in the rumen and those produced during the industrial hydrogenation of vegetable oils (9, 10 and 11 trans). Most experiments which have shown trans FA to raise plasma LDL cholesterol, Lp(a) and lower plasma HDL cholesterol have used partially hydrogenated vegetable oil as the source of trans FA. In one study, an Australian group[17] used a high elaidic acid diet (9 trans 18:1) in place of a high oleic acid diet which resulted in a rise in plasma Lp(a), however, the content of other trans 18:1 positional isomers in this diet was not reported. It is not yet known to what extent the various trans 18:1 positional isomers may contribute to these adverse effects. The fact that small amounts of trans FAs were detected in lard (pig fat) was a little unexpected as pigs are a non-ruminant animal, however this result may be explained by the presence of small amounts of trans FA in the diet of pigs.

GLC provides a very good method for the separation and quantitation of the various trans FA and cis and trans FA positional isomers. Similar separations of trans FA were achieved on this BPX-70 capillary column to those obtained using a SP2560 (Supelco, USA)[42] and CP Sil88 (Chrompack, Middelburg, The Netherlands)[43] columns. GLC gave comparable results, but slightly lower results, for total trans FA (8.0–14.5%) than by FTIR (9.2-16.3%). This may be due to incomplete separation of some peaks by GLC resulting in the inclusion of some trans FA in cis FA peaks. The underestimation may also be partly due to a non-linear response particularly at smaller peak areas requiring a positive correction. Further work using a combination of silver nitrate TLC to purify the trans 18:1 positional isomers and GLC-mass spectrometry would be needed to confirm the identity of the trans 18:1 positional isomers. FTIR only provides a measure of total methylene interrupted trans FA isomers, whereas GLC is able to separate and quantitate the various trans FA isomers and their metabolites. Hence GLC is a very useful technique for scientists interested in the metabolism of these trans FA.

The presence of saturated FA in the sn-2 position may retard the clearance of chylomicrons containing saturated FA in the sn-2 position from the blood and the subsequent metabolism of the chylomicron remnants by the liver[27]. In all the margarines analysed there were significant amounts of saturated FA in the sn-2 position compared with the amount present in unhydrogenated vegetable oils. The higher amounts of saturated FA in the sn-2 position may be due to the redistribution of saturated FA from the sn-1 and sn-3 positions during the interesterification process. The hydrogenation process which decreases the 18:1ω9 content in the sn-2 position while increasing the 18:0 and trans 18:1 in the same position may potentially decrease the hypocholesterolaemic effects of these margarines: however, Nestel et al.[44] have shown that a diet containing 4% of energy as trans FA did not raise plasma LDL cholesterol or lower plasma HDL cholesterol when linoleic acid was also increased so that the ratio of linoleic acid to palmitic acid exceeded that in the national diet.

We have estimated the trans FA content of the Australian diet using the values from this paper and the 1991/92 Australian Bureau of Statistics' apparent consumption data for butter, table margarine, other margarines, whole milk, cheese and meat[45]. We then calculated a minimum and maximum value based on the lower and higher trans FA values for the table margarines. These calculations indicated a minimum value of 2.7 g/head/day and a maximum of 4.8 g/head/day. These values are similar to those reported in West Germany and the United Kingdom[46] but much lower than those reported in USA[47,48] and The Netherlands[10]. We estimated that table margarines account for between 36% and 64% of the total trans FA intake in the Australian diet.

The currently available margarines offer a better alternative in terms of P/S, ω6/ω3 and sn-2 FA than the butter/dairy blends or animal fats. As a result of the health implications of trans FA, the FA positional distribution and the continuing changes in the composition of these margarines, it is important to regularly analyse these dietary fats.

Acknowledgement — The financial support to M.P. Mansour (Junior Research Fellowship) from the Meat Research Corporation (Australia) is gratefully acknowledged.

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The trans fatty acid and positional (sn-2) fatty acid composition of some Australian margarines, dairy blends and animal fats

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Asia Pacific Journal of Clinical Nutrition 1993; 2:155–163

某些澳洲人造黃油、黃油植物油混合油和動物脂肪的反－脂肪酸和Ｓn-2 位脂肪酸的組成

摘要

我們用氣相層析（GLC）和傅立葉變換紅外綫（FTIR ）分光光度法分析了１３種人造黃油，５種黃油、植物油混合油和２種動物脂肪（猪油與牛油）的脂肪酸，包括反－脂肪酸的組成。從維多利亞州三個不同的超級市場，GLADSTONE PARK（墨爾本附近），WAURN PONDS （玆朗附近）和玆朗市（GEELONG CITY）選取樣本。從脂肪酸的組成計算了高度不飽和脂酸/飽和脂酸， 高度不飽和脂酸/（飽和脂酸＋反－單烯脂酸），高度不飽和脂酸/單不飽和脂酸（飽和脂酸＋反－單烯脂酸）和Ｗ６脂酸/Ｗ３脂酸的比值。 所得的脂肪酸組成和反－脂肪酸含量與１９８２年發表的澳洲雪梨人造黃油的結果相比較。全甘油三酯經胰酯酶脱酰基作用后可測得Ｓｎ－２位脂肪酸的組成。從這個數據，我們估計了存在于人造黃油中間酯化脂肪的百分率。用FTIR法測定人造黃油中反－脂肪酸含量在９．２％－１６．３％（平均爲總甲酯脂肪酸的１３．１％）（７．６－１３．０克反－脂肪酸/１００克樣本，平均爲１０．４克/１００克樣本）， 黃油與黃油植物油混合油的反－脂肪酸含量爲３．２％－４．１％（平均爲３．８％），猪油含反－脂肪酸０．４％， 牛油含反－脂肪酸３．６％，除四種牌子外，人造黃油的反－脂肪酸含量與１９８２年發表的數值近似。Ｗ６脂酸/Ｗ３脂酸的比值爲２．５－３６．３（１９８２年發表的數值爲３－４９）， 高度不飽和脂酸/飽和脂酸的比值爲１．４－３．３（１９８２年發表的數值爲０．１－３．７）。 估計人造黃油中間脂化脂肪爲２５％－１００％。