

# The *trans* fatty acid content of fats in some manufactured foods commonly available in New Zealand

Russell K Richardson<sup>1</sup> MSc, Bertram Y Fong<sup>1</sup> BSc and Angela M Rowan<sup>2</sup> MSc

<sup>1</sup>New Zealand Dairy Research Institute, Palmerston North

<sup>2</sup>Nutrition Consultant, Palmerston North

The *trans*-unsaturated fatty acid content of 18 foodstuffs was determined using infrared analysis for total *trans*-unsaturation and thin layer/gas chromatography for the *trans* octadecenoic acids. Infrared calibration was from mixtures of trielaidin and tristearin or methyl oleate and methyl stearate for analysis of triacylglycerols and derived fatty acid methyl esters respectively. The methyl esters were also separated by degree of unsaturation by argentation thin layer chromatography and the *trans*-monounsaturated components determined by gas chromatography. As a proportion of the extracted fat, *trans* contents ranged from margarine (14-18%) to biscuits (1-2%). All except the margarines were less than 10%. When the fat content and serving size of the foods were taken into account, the ranking of contribution to dietary *trans* fatty acids was somewhat different from that based on the *trans* content of the fats alone, pastry-based foods and margarine appearing as the major potential contributors.

**Key words:** fat, *trans* fatty acids, foods, New Zealand, margarine, biscuits, pastry, milk, meat

## Introduction

The relationship between *trans* unsaturated fatty acids and blood cholesterol was investigated over 30 years ago in various studies. Although several of these found an apparent blood-cholesterol-raising effect of dietary *trans* fatty acids<sup>1,2</sup>, little notice was taken of these results at the time. Over the last several years, there has been increased interest in the role that dietary fat plays in the development of chronic diseases such as coronary heart disease, atherosclerosis and cancer<sup>3</sup>. A number of more recent studies have been performed which have shown that dietary *trans* fatty acids raise serum cholesterol although some inconsistencies are observed<sup>4-8</sup>. It is possible that many of the human studies on *trans* fatty acid intake and heart disease risks are equivocal because of methodological limitations, including the difficulties of quantifying the consumption of *trans* fatty acids.

Small amounts of naturally occurring *trans* fatty acids are found in the fat of ruminants, occurring in meat and milk. A larger proportion of *trans* fatty acids in the human diet comes from hydrogenated vegetable and hydrogenated fish oils, used in margarine and processed fat manufacture. The chemically catalysed hydrogenation employed by the food industry produces a wider variety of *trans* isomers, often in a much higher concentration than occurs with biological processes<sup>9,10</sup>. Canadian surveys have reported the *trans* fatty acid content ranging from 10.1-49.9% in margarine<sup>11</sup> and 3.8-6.4% in butter<sup>12</sup>. These results are similar to those found in earlier Northern hemisphere studies<sup>13,14</sup>.

There is little information on the *trans* fatty acid content of New Zealand foods or on the intake of *trans* fatty acids in New Zealand. One recent study<sup>15</sup> reported the *trans* monoenoic fatty acid contents of New Zealand margarine (7.3 to 11.3%) and butter (1.6 to 2.8%) which tended to be lower than those found in other countries. In particular, the *trans* content for New Zealand butter was lower than previously reported<sup>16,17</sup>. The methodology used in the work<sup>15</sup> had the

potential to underestimate the total *trans* monoenoic acids because of incomplete separation of *cis* and *trans* isomers from total fatty acid chromatograms<sup>18,33</sup> and only partial validation of the methodology was reported.

The present study, conducted between February and April 1995, aimed to determine as accurately as possible the *trans* fatty acid content of a limited number of manufactured foods commonly available in New Zealand, by using infrared absorbance validated by chromatographic methods and hence estimate the *trans* fatty acid intake in the New Zealand diet. Foods were selected based on either the probability of containing *trans* fatty acids or their contribution to total fat intake in New Zealand<sup>19</sup>. The brand analysed for each food choice was either the largest market shareholder or one of the most popular products<sup>20</sup>.

## Methods

The American Oil Chemists' Society (AOCS) method Cd 14-61<sup>21</sup> using infrared absorption was used to quantify the total *trans* content using both the triglyceride and the methyl ester forms of the sample fats. In a separate procedure the *trans* monoene content was determined by argentation thin layer chromatography to separate the *cis* C18:1 and *trans* C18:1 isomers, followed by gas chromatography of the *trans* fatty acid methyl esters. The content of the polyunsaturated fatty acids containing one or more *trans* double bonds was estimated by difference.

Fat was extracted from the foods based on the method originally developed by Bligh & Dyer<sup>22</sup>. This method with or without variations has been widely used to extract fat from complex sample matrices, including a variety of processed

**Correspondence address:** Mr R K Richardson, Food Science Section, New Zealand Dairy Research Institute, Private Bag 11029, Palmerston North, New Zealand  
Tel: +64-6-350-4649; Fax: +64-6-356-1476  
Email: r.richardson@nzdri.org.nz

foods in a comprehensive *trans* fatty acid study of Canadian foods<sup>12</sup>. Quantitative fat recovery from the extraction procedure was determined using a margarine oil. Variation in the recovery of fat from the survey foods was estimated by repeated analysis of a selected food product (muesli bar) which was part of the *trans* fatty acid survey.

### Materials

Organic solvents, unless specified otherwise, were Analytical Reagent grade (BDH, Poole, England). Diethyl ether was redistilled prior to use. Triglyceride and methyl ester standards were obtained from Nu-Chek-Prep, Elysian, MN. Thin layer chromatography plates (20 x 20 cm, silica gel 60) were obtained from Merck, Darmstadt, Germany.

### Sample preparation

Solid foods were homogenised in a food processor before subsampling. Chocolate-containing foods were homogenised at 4°C. The pastry samples were homogenised in a semi-frozen state to avoid "doughing". Foods such as biscuits that were difficult to homogenise were ground with a pestle and mortar before mixing in the food processor. All homogenised samples were stored frozen, sealed in aluminium-foil sachets. Margarine, butter and shortening were core sampled (ca 30 g), melted at 60°C and centrifuged. The fat layer was filtered at 60°C through anhydrous sodium sulphate. The fat content of these three products was determined gravimetrically by the method of Rose-Gottlieb<sup>23</sup>.

### Fat extraction

Each homogenised sample was accurately weighed into a 250 ml glass centrifuge bottle. The sample size was calculated to provide up to 2 g of fat and no more than 16 ml of water. The moisture content was estimated from moisture tests or from the nutritional label claim. Water was added as required to a total of 16 ml.

After the addition of 40 ml of methanol and 20 ml of chloroform the sample was homogenised with a Polytron blender (Kinematica, Lucerne, Switzerland), for 1 min. A further 20 ml of chloroform and 20 ml of methanol were added and homogenised for an additional 30 s. The blender cutter was rinsed with 10 ml of chloroform into a separate beaker. The homogenised sample was filtered through a sintered glass funnel using vacuum assistance. The residue was washed using the 10 ml of chloroform rinsing collected above, and then with a further 20 ml of fresh chloroform. The combined filtrate was transferred into a clean 250 ml glass centrifuge bottle. The flask holding the original filtrate was rinsed with 10 ml of chloroform which was added to the centrifuge bottle. 10 ml of methanol and 56 ml of water were added to the filtrate, the bottle stoppered and shaken for 1 min. The contents were centrifuged at approximately 600 g for 10 min to separate the two phases. Alternatively, the bottle was left standing for a few hours to allow the two phases to separate. The top layer (water and methanol) was then aspirated to waste, avoiding transfer of any interfacial material. The bottom chloroform layer was then washed three times with water (50, 56 and 56 ml). After each wash, the bottle was centrifuged or allowed to stand, followed by removal of the aqueous layer as above. The chloroform layer was then filtered through anhydrous sodium sulphate (3 g) in a sintered glass funnel and collected in a weighed round

bottom flask. The funnel and the sodium sulphate were rinsed with 10 ml of chloroform and the combined solvent evaporated under vacuum at approximately 40°C. The flask containing the fat was dried at 102°C for 1 h and placed in a desiccator to cool for 1 h.

The optimum time required to dry the extracted fat was determined as follows. About 2 g of margarine oil was weighed into a 250 ml round bottom flask and approximately 90 ml of chloroform was added to dissolve the fat. The solvent was removed by rotary evaporator, the fat dried at 102°C for 30 min and allowed to cool (1 h) in a desiccator before weighing. The drying process was repeated three times.

### Fat methylation

Fatty acid methyl esters were prepared using an in-house method based on the procedure of Christopherson & Glass<sup>24</sup>. Direct transesterification of the anhydrous fat sample was performed using sodium methoxide/methanol reagent, prepared by dissolving clean metallic sodium in anhydrous methanol (1.15 g per 100 ml) and mixing one volume of the methanolic solution with two volumes of dry diethyl ether and two volumes of isooctane. Any unesterified fatty acids present in the fat were methylated using 14% w/v boron trifluoride in methanol (BDH).

About 500 mg of fat was weighed into a 50 ml Kimax tube. Sodium methoxide/methanol reagent (5 ml) was added to the tube, which was capped and warmed to 40°C for 5 min with gentle swirling. The solution was cooled to room temperature and 5 ml of boron trifluoride/methanol reagent added. The contents were mixed thoroughly and allowed to stand for 10 min. Hexane (20 ml) was added and mixed thoroughly. Neutralising solution (5% w/v anhydrous dipotassium hydrogen phosphate and 1.5% w/v potassium hydroxide, in water) was added (5 ml) and the contents mixed vigorously. The two phases were separated by centrifugation at approximately 600 g for 2 min. The upper hexane layer was then transferred into a 50 ml pear flask. The aqueous bottom layer was extracted with a further 5 ml of hexane, added to the pear flask. The solvent was removed by rotary evaporation under vacuum at approximately 40°C until 2-3 ml remained. The remaining solvent was evaporated at 50°C on a heating block under a gentle stream of nitrogen. (It was our practice not to prolong drying for more than 2-3 min after the solvent had been visibly removed, to minimise the loss of volatile short chain fatty acid esters.) The methyl esters were transferred by pasteur pipette to a 4 ml vial, which was nitrogen flushed, capped and stored at -18°C until required.

### Fourier transform infrared (FTIR) spectroscopy

A model 1640 FTIR spectrophotometer (Perkin Elmer, Beaconsfield, England) with a 1 mm sodium chloride window flow cell was used. Spectra were obtained from 16 scans of 2000 cm<sup>-1</sup> to 400 cm<sup>-1</sup> with a resolution of 2 cm<sup>-1</sup> and were printed on a Colour Pro Plotter, model 7440A (Hewlett-Packard, Palo Alto, CA).

The FTIR signal was calibrated with a multi-point calibration curve consisting of either trielaidin (for triglycerides analysis) or methyl elaidin (for methyl esters analysis). The total triglyceride or methyl ester weight in standard solutions was kept constant by using a *trans*-free mixture consisting of equal proportions by weight of either triolein and tristearin or methyl oleate and methyl stearate.

A total weight of 200 mg of the standard components was dissolved in carbon disulphide (BDH, spectrosol grade) and transferred quantitatively into 10 ml volumetric flasks. Solutions were made up to volume with carbon disulphide (BDH, spectrosol grade) at 20°C in a water bath and stoppered firmly. Solutions were stored at 4–6°C and warmed to room temperature (ca 20°C) before use.

Repeatability of the infra red measurements was estimated with "low" (anhydrous milkfat (AMF)) and "high" (margarine oil) *trans* C18:1 fats. Recovery was determined by spiking both the low and high *trans* fats with trielaidin at approximately 50% and 100% of the native *trans* levels. These fats were subsequently used as quality controls (QCs) for the survey.

#### Sample preparation

A sample (200 ± 1 mg) of the fat or methyl esters was prepared in a similar manner to the standards.

#### Argentation thin layer chromatography and gas chromatography (Ag-TLC/GC)

Thin layer plates were soaked in 10% w/v methanolic silver nitrate then dried and stored protected from light until required.

The methyl esters of the *trans* monoene fatty acids were separated from the *cis* isomers on the treated TLC plates and were quantified by GC. The chromatographic determination used the sample C18:0 methyl ester as an internal standard to determine the *trans* monoene methyl esters in a manner similar to that of Wolff<sup>25</sup>. However, additional work was performed to determine and correct for any evaporative losses of the methyl esters from the Ag-TLC plates.

FAME were analysed on a 15 m x 0.53 mm FFAP capillary column (Alltech Associates, NZ) using a Hewlett-Packard HP5890 gas chromatograph equipped with autosampler and flame ionisation detector (Hewlett Packard, Palo Alto, CA).

Repeatability and recovery of the chromatographic assays were determined using the same low (AMF) and high (margarine oil) *trans* C18:1 fats as used for FTIR measurements.

#### Method validation

The recovery of margarine oil from the extraction solvent mixture was 100.8 ± 1.0% (n = 4). One hour at 102°C was sufficient to evaporate residual solvent from extracted fat. The drying conditions were found to have no measurable effect on the *trans* C18:1 content, as determined by the chromatographic method (Ag-TLC/GC). Repeated fat extraction (n = 5) of muesli bar (representing a complex food matrix) gave a fat content of 19.90 ± 1.38 g/100 g.

The *trans* monoene content from Ag-TLC/GC determinations (n = 10) gave values for AMF and margarine oil of 5.49 ± 0.16% and 12.83 ± 0.39% respectively.

Absolute recovery of C18 methyl esters from Ag-TLC plates of 51% to 54% was obtained using a C23:0 methyl ester reference added to the recovered components. However, the C18:0 and C18:1 methyl esters were found to have the same relative recovery and by utilising C18:0 as an internal standard, relative recoveries (n = 7) of trielaidin added to AMF and to margarine oil were 98.9 ± 4.7% and 100.1 ± 2.7% respectively.

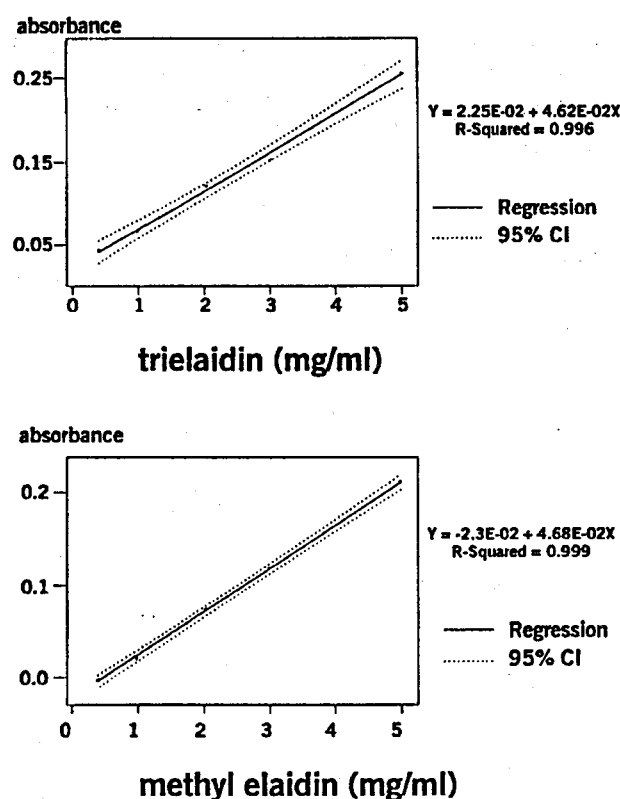
#### FTIR spectrophotometry

Recoveries (n = 4) of trielaidin added to AMF and to margarine oil were 101.2 ± 2.7% and 99.6 ± 1.9%, respectively. Recoveries (n = 4) of methyl elaidate added to the FAME of AMF and of margarine oil were 98.6 ± 3.6% and 100.0 ± 2.0% respectively.

The repeatability of the AOCS method was estimated from repeated analyses of the control substances which gave for triglycerides, AMF: 8.26 ± 0.10% (n = 4), margarine oil: 15.70 ± 0.20% (n = 5), and for methyl esters, AMF: 6.57 ± 0.43% (n = 6), margarine oil: 14.88 ± 0.34% (n = 6).

FTIR calibration curves are shown in Figure 1 and spectra of standards and sample fats in Figure 2.

Figure 1. FTIR calibration curves for *trans* fat standards.



#### Results

The results of the survey are summarised in Table 1. Total *trans* fatty acids (as elaidic acid equivalent) were determined by infrared analysis of the fat directly (AOCS-TG) or on the derived fatty acid methyl esters of the fat (AOCS-FAME) as recommended for fats containing less than 15% *trans* fatty acids<sup>21</sup>. The total fatty acid composition data were used to obtain a match with fatty acid profiles of known fats and oils, using a statistical computer program<sup>26</sup>, to provide an indication of the fat source for each product.

Estimates of the test uncertainty obtained by calculating pooled standard deviations of replicate assays of the foods were: AOCS-TG 0.41%, AOCS-FAME 0.39%, *trans* C18:1 by chromatography 0.60%. Bartlett's test for homogeneity of variance<sup>27</sup> confirmed the statistical validity of pooling the data by test method.

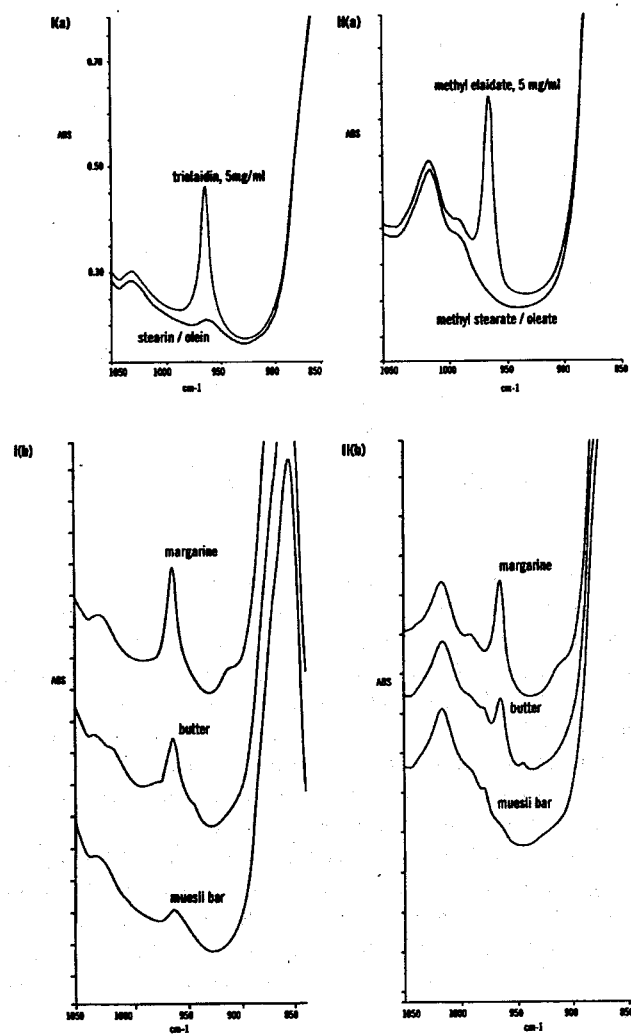
**Table 1.** Survey foods and fat results. Data are means of duplicate analyses.

Food product	Label claim: Fat type and content (g/100 g)	Analysed fat content (g/100 g product)	Total <i>trans</i> isomer, g/100 g		<i>trans</i> C18:1 (g/100 g) <sup>3</sup> Ag-TLC/GC	<sup>4</sup> Fat source
			<sup>1</sup> AOCS-TG	<sup>2</sup> AOCS-FAME		
Margarine (A)	Vegetable	81.80	17.67	14.33	13.64	Soya
Margarine (B)	Polyunsaturated/hardened	81.78	15.81	14.66	14.70	Sunflower
Commercial pastry fat (A)	*	85.14	7.88	6.56	6.38	Tallow
Commercial Pastry fat (B)	*	83.73	7.04	5.42	7.67	Lard
Commercial pastry fat (C)	*	81.69	6.13	7.02	6.69	Soya/palm
Retail pastry	Vegetable/butter	23.51	6.56	5.62	5.90	Butter/tallow
Homogenised milk	3.3	2.59	6.97	4.78	4.93	Milkfat
Reduced fat milk	1.5	1.38	6.59	5.76	5.71	Milkfat
Butter	*	82.73	8.35	6.72	6.37	Milkfat
Shortening	Fractionated beef	81.63	5.00	4.57	4.09	Tallow
Meat pie	Shortening (pastry)	13.88	4.27	3.92	3.43	Tallow/vegetable
Meat patty	Margarine/butter	24.64	4.09	4.32	3.09	Tallow
Luncheon meat	*	13.05	6.35	4.98	5.42	Lard
Muesli bar	Vegetable	19.90	1.37	0.89	1.93	Coconut/palm
Chocolate-coated biscuit	Milkfat/cocoa butter/ vegetable	26.28	0.70	1.70	3.00	Milkfat/ cocoabutter/palm
Plain sweet biscuit	Shortening	15.17	5.01	4.50	6.17	Tallow
Savoury cracker biscuit	Vegetable Shortening, 22.8	23.75	1.02	0.71	5.52	Coconut/palm
White bread	Vegetable, 1.9	1.36	2.51	2.19	2.69	Soya

<sup>1</sup> AOCS method, triglycerides; <sup>2</sup> AOCS method, fatty acid methyl esters; <sup>3</sup> As percentage of total FAME; <sup>4</sup> Inferred from fatty acid composition:

\* No information on package.

**Figure 2.** FTIR spectra of (I) triacylglycerols, (II) fatty acid methyl esters, for (a) the calibration *trans*-free synthetic fat, an elaidic acid standard, (b) fat extracted from margarine, butter, and muesli bar. (Spectra are overlaid and offset for clarity).



The two methods of infrared measurement of the fat were tested against each other for each food product, using the pooled duplicate variance. Significant differences ( $P \leq 0.05$ ) were found for several products, *viz.* milks, butter, margarines, luncheon meat, chocolate-coated biscuit, commercial and retail pastry fats. Except for commercial pastry fat C and chocolate-coated biscuit, the triglyceride measurement gave the greater result.

A comparison of *trans* C18:1 content by chromatography (Ag-TLC/GC) with the total *trans* content by infrared showed that results were consistent with literature data (Table 2), *ie* the great majority of *trans* fatty acids were C18:1 isomers. Differences were attributed to the presence of *trans* polyenoic fatty acids which are commonly 2% or less of the total fatty acids. Fat from muesli bar and the various biscuit products produced spurious infrared results which are discussed below.

## Discussion

### FTIR Calibration

The standard AOCS method allows for the use of both triglyceride and derived FAME for the quantification of total *trans* by infrared absorption. Because of interfering absorption by the acylglycerol of the triglyceride, FAME have been recommended as the preferred form when the *trans* fatty acids content is less than 15%. However, methyl esters have the disadvantage of underestimating the total *trans* fatty acids. Generally, the bias for methyl esters measurement is claimed to range from -1.5 to -3% and a positive bias of 2 to 3% is claimed to occur for *trans* fatty acids measured as triglycerides<sup>30</sup>. The methyl esters bias relative to triglycerides is therefore -3.5 to -6.0%.

Because of the inherent problems of the infrared method associated with low *trans* content, attempts have been made to improve its accuracy by the use of various techniques. Madison *et al.*<sup>30</sup> used a two-component calibration mixture consisting of methyl linoleate and methyl elaidate to increase the accuracy of the total *trans* results in the range 0.5 to 36%. This method was later recommended by the AOCS to help to remove some of the bias of the results from the standard

AOCS method. In our survey, a similar attempt to that of Madison et al. was made to increase accuracy, but using two *trans*-free components, the triglycerides of oleic (C18:1) and stearic (C18:1) acids or for methyl esters analysis a mixture of pure methyl stearate and methyl oleate. Calibration plots (Figure 1) of known levels of trielaidin or elaidic acid methyl ester revealed a small positive bias associated with triacylglycerols and a small negative bias with methyl esters. This calibration method, while theoretically eliminating bias, appeared to at least reduce the bias between the AOCS-TG and AOCS-FAME data; our results (Table 1) produced a mean bias of -2.25% *trans* (margarines) and -0.82% *trans* (all products), for methyl esters compared with triglyceride infrared measurements.

A fully hydrogenated milkfat was recently used for spectral subtraction for milkfat<sup>33</sup> for which results were shown to increase low *trans* results and remove negative data for some low *trans* samples. The standard deviation associated with the measurements was also reduced. However, it appears that this approach may be highly accurate only when the *trans*-free oil used is the same as the sample oil because the chain length of the non *trans* mixture and/or hydrogenation of *cis*-unsaturated fatty acids may cause subtle alterations to the background infrared spectrum<sup>34</sup>.

#### *Trans polyenoic fatty acids*

An estimation of the *trans* polyenoic fatty acids in this survey was based on the difference between the FTIR results and the *trans* C18:1 monoene results. Negative values resulted in cases where the infrared measurement appeared to underestimate the total *trans* fatty acids in a fat sample. Values obtained are not statistically significant, given the estimated uncertainty (standard deviation = 0.5%) but do indicate that *trans* polyenoic acids are minor components.

It is the authors' intention to determine the accuracy of the FTIR *trans* measurements in the survey, by comparing specific analyses of *trans* polyenoic fatty acids by chromatography and to report results in due course.

#### *Margarine*

Of the food products analysed in this survey, the two margarines gave the highest *trans* fatty acid content of the fat. Compared to the levels of total *trans* fatty acids determined by infrared analysis of margarines in Western countries (Table 2) these levels are relatively low. Similar results have been recently reported for four New Zealand margarines<sup>15</sup>. New Zealand food legislation requires margarines to have not less than 40% polyunsaturated fatty acid content; consequently no hard margarines with very high *trans* content are available on the New Zealand market.

#### *Dairy products*

The *trans* contents of milks and butter in the survey were at or above the maximum values reported for northern hemisphere countries (Table 2).

The total *trans* fatty acid content of milkfat has been known to show both geographical and seasonal variations<sup>28</sup>. A seasonal survey of 116 Australian milkfat samples showed that the lowest levels (4.3-4.9%) were recorded in winter, and the highest levels (6.5-7.6%) were recorded in spring-summer<sup>29</sup>. These authors considered the influence of feed on the production of *trans* fatty acids as the prime cause of the

*trans* variation. A study by Wolff<sup>25</sup> on French butter reported a similar trend, with a mean of 3.22% in autumn and a mean of 4.28% in spring. However, Gray<sup>16</sup> reported data indicating a winter maximum (7.31%) and a summer minimum (4.38%) for the Manawatu region of New Zealand. Also MacGibbon<sup>17</sup>, in a New Zealand wide survey, found a winter maximum (6.8%, measured in August-September 1987) and a summer minimum (2.6%, measured in January 1988).

The date of milk collection from which the survey butter was manufactured corresponded to the expected New Zealand seasonal maximum for *trans* content, which could explain the higher values for the butterfat compared to milkfat from the retail milks in the survey (these milks were sampled at a later time for which lower *trans* content in the milkfat would be expected).

#### *Meat Products*

The total *trans* content for processed meat products in the present survey ranged from 3.9 - 6.4%, (Table 1). Typical levels of 1.8-6.6% have been previously reported for beef fat<sup>9</sup>.

#### *Bakery Products*

For commercial pastry fat the *trans* content was in the range 5.4-7.0%. New Zealand pastry products are usually based on animal fat, rather than hydrogenated vegetable oil as is common practice overseas. This probably accounts for the relatively low *trans* levels compared with hardened pastry fats of purely vegetable origin<sup>12,14</sup>. Of the three commercial pastry fats evaluated, only pastry fat C was indicated to contain vegetable oil.

**Table 2.** Reported *trans* isomer content in margarine and butter by various authors.

Sample	Total <i>trans</i> isomer		By gas chromatography			Source	Ref
	infrared (%)	N	<i>trans</i> C18 (%)	N	<i>trans</i> polyenes (%)		
Margarine	10.1-49.9	50	10-40	50	trace-8.3	Canada, 1991	11
	11.7-50.2	7	11.7-40.8	9	trace-7.4	Canada, 1990	35
	-	-	7-31	39	0.0-5.2	USA, 1983	14
	9.2-16.3	13	7.51-13.22	13	0.94-1.36	Australia, 1993	36
	-	-	0.5-18.6	5	-	Various, 1995	37
	9-15	4	7.3-11.3	8	0.3-0.7	NZ, 1993	15
	14.3-14.7	2	13.6-14.7	2	0.0-0.7*	This study	
	3.8-6.4	2	2.9-5.6	2	0.9	Canada, 1993	12
	-	-	3.1-3.8	3	-	USA, 1983	14
	3.2-4.1	1	2.3-3.4	1	0.89-1.39	Australia, 1993	36
Butter	-	-	1.75-5.20	31	0.6-2.30	Austria, 1994	33
	-	-	3.4	1	-	USA, 1995	37
	4.27-7.64	116	-	-	-	Australia, 1971	29
	-	-	4.38-7.31	17	-	NZ, 1973	16
	-	-	2.6-6.8	55	-	NZ, 1993	17
	-	-	2.46-5.10	24	-	France, 1994	25
	-	-	1.6-2.8	2	0.2-0.4	NZ, 1993	15
	6.72	1	6.37	1	0.35*	This study	

\*From Table 1 (AOCS-FAME) - (Ag/TLC-GC)

Relatively low levels of total *trans* unsaturation were found in the fat present in the biscuits, muesli bar and bread analysed in this survey. For the biscuits and muesli bar, the chromatographic *trans* C18:1 results indicated that the infrared method underestimated the total *trans* content for these products. This problem was associated with a poorly defined *trans* peak in the infrared spectrum (Figure 2). Furthermore, two of these samples (cracker biscuit and muesli bar), appeared to contain coconut oil (Table 1), which causes a negative bias in infrared measurements apparently

associated with a high proportion of low/medium chain length fatty acids<sup>30</sup>. Indeed, negative total *trans* results have been reported with low level *trans* coconut-oil-based shortening using the standard AOCS-FAME method<sup>31</sup>.

The contribution of the survey foods to *trans* fatty acid consumption in the New Zealand diet can be estimated from the relative *trans* content per serving (Table 3). The ranking in the Table reflects the influence of "serving size" and does not necessarily imply order of importance in a particular diet with respect to *trans* fatty acid consumption.

**Table 3.** Total *trans* fatty acid content of a single serving of several New Zealand foods

Sample	Serving size <sup>38</sup> (g)	Total <i>trans</i> fatty acids per serving (g)
Meat Pie	170	0.92
Meat Patty	60	0.64
Margarine	5	0.59
Pastry	34	0.45
Shortening	10	0.37
Butter	5	0.28
Homogenised Milk	205	0.25
Reduced fat milk (1.5%)	205	0.16
Luncheon meat	25	0.16
Muesli Bar	32	0.06
Plain Sweet Biscuit	7	0.05
Chocolate-coated Biscuit	12	0.05
White Bread	30	0.01
Cracker Biscuit	3	<0.01

## Conclusions

The bias between the AOCS-TG and the AOCS-FAME methods was reduced but not eliminated by the calibration technique for a large number of the surveyed food fats. The accuracy of the FTIR measurement could not be verified in the absence of specific data (by chromatographic techniques) on *trans* polyenoic fatty acids.

Overall, New Zealand food fats do not have a *trans* fatty acid content as high as reported for some other countries. The two New Zealand manufactured margarines had a total *trans* fatty acid content of 14-18%. New Zealand butterfat has up to 6-8% total *trans* fatty acid content which is associated with a seasonal maximum. Most other food fats have 1-8% total *trans* fatty acids, consistent with the use of animal fats and/or mildly hydrogenated oils.

As concluded by previous workers, *trans*- measurements by infrared methods are subject to error particularly at low levels unless care in calibration is exercised. Accuracy is still not assured however and the more accurate approach is a chromatographic one in which complete *cis*, *trans* isomer separation is achieved.

## Acknowledgments.

Massey University Food Technology Research Centre for use of the FTIR instrument. Commercial pastry fats were supplied by Abels Ltd, New Zealand. This work was funded by the Dairy Advisory Bureau of the New Zealand Dairy Board.

## The *trans* fatty acid content of fats in some manufactured foods commonly available in New Zealand

Russell K Richardson, Bertram Y Fong and Angela M Rowan

*Asia Pacific Journal of Clinical Nutrition* (1997) Volume 6, Number 4: 239-245

# 紐西蘭常用的某些加工食品脂肪中的反式脂肪酸含量

## 摘要

作者用紅外線分析法去測定 18 種加工食品的反式不飽和脂肪酸含量，同時用薄層/氣相層析法去測定硬脂肪含量。分析三酰基甘油及其衍生物脂肪酸甲脂時，分別用三反油酸甘油酯和三硬脂酸甘油酯或甲基油酸鹽和甲基硬脂酸鹽混合物作紅外線校準。甲脂亦可用鍍銀化薄層層析法根據其不飽和程度而分離，同時反式單不飽和脂酸可用氣相層析法去測定。作為脂肪抽提物的一部分，人造黃油含反式脂肪酸 14-18%；餅干含 1-2%；除了人造黃油外，所有加工食品含反式脂肪酸均低於 10%。當把食物每次供給量及脂肪含量計算在內，發現膳食反式脂肪酸的貢獻與單一脂肪中反式脂肪酸的含量稍有不同。面制品食物和人造黃油似乎是主要的供給源。

## References

- 1 Anderson J T, Grande F & Keys A. Hydrogenated fats in the diets and lipids in serum of man. *Journal of Nutrition*, 1961, 75, 388-394.
- 2 Delongh H, Beerthuis R K, den Hartog C, Daberup L N & van der Spek P A S. The influence of some dietary fats on serum lipids in man. *Bibliotheca "Nutritio et Dieta"*, 1965, 7, 137-152.
- 3 Hunter J E. Safety and health effects of isomeric fatty acids. In: Ching Kuang Chow, ed, *Fatty acids in foods and their health implication*, Marcel Dekker, New York, 1992: 857-868.
- 4 Mensink R P & Katan M B. Effect of dietary trans fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *New England Journal of Medicine* 1990; 323: 439-445.
- 5 Mensink R P, Zock P L, Katan M B, & Hornstra H. Effect of dietary cis and trans fatty acids on serum lipoprotein [a] levels in humans. *Journal of Lipid Research* 1992; 33: 1493-1501.
- 6 Nestel P, Noakes M, Belling B, McArthur R, Clifton P, Janus E & Abbey M. Plasma lipoprotein lipid and Lp(a) with substitution of elaidic acid for oleic acid in the diet. *Journal of Lipid Research* 1992; 33: 1029-1036.
- 7 Judd J T, Clevidence B A, Muesing R A, Wittes J, Sunkin M E & Podcasy JJ. Dietary trans fatty acids: effects on plasma lipids and lipoproteins of healthy men and women. *American Journal of Clinical Nutrition* 1994; 59: 861-868.
- 8 Wood R, Kubena K & O'Brien B. Effect of butter, mono- and polyunsaturated fatty acid-enriched butter, trans fatty acid margarine, and zero trans fatty acid margarine on serum lipids and lipoproteins in healthy men. *Journal of Lipid Research* 1993; 34: 1-11.
- 9 Craig-Schmidt M C. Fatty acid isomers in foods. In: Ching Kuang Chow, ed. *Fatty acids in foods and their health implication*. Marcel Dekker, New York, 1992: 363-398.
- 10 Enig M G. Trans fatty acids - an update. *Nutrition quarterly* 1993; 17: 79-95.
- 11 Ratnayake W N M, Hollywood R & O'Grady E. Fatty acids in Canadian margarines. *Canadian Institute of Science and Technology Journal* 1991; 24: 81-86.
- 12 Ratnayake W N M, Hollywood R, O'Grady E & Pelletor G. Fatty acids in some common food items in Canada. *Journal of the American College of Nutrition* 1993; 12: 651-666.
- 13 Kochhar S P & Matsui T. Essential fatty acids and trans content of some oils, margarine and other food fats. *Food Chemistry* 1993; 13: 85-101.
- 14 Enig M G, Pallansch L A, Sampugna J & Keeney M. Fatty acid composition of fats in selected food items with emphasis on trans components. *Journal of the American Oil Chemists Society* 1983; 60: 1788-1795.
- 15 Ball M J, Hackett D & Duncan A. Trans fatty acids content of margarines, oil and blended spreads available in New Zealand. *Asia Pacific Journal of Clinical Nutrition* 1993; 2: 165-169.
- 16 Gray I K. Seasonal variation in the composition and thermal properties of New Zealand milk fat. I. Fatty-acid composition. *Journal of Dairy Research* 1973; 40: 207 - 214.
- 17 MacGibbon A K H. Milkfat and butter: seasonal changes in composition and properties. NZDRI Report MF93R09, New Zealand Dairy Research Institute, Palmerston North.
- 18 Ratnayake W N M. AOCS method Cc 1c-89 underestimates the trans-octadecenoate content in favour of the cis isomer in partially hydrogenated vegetable oils. *Journal of the Association of Official Analytical Chemists* 1992; 69: 192.
- 19 Howarth C, Parnell W, Birckbeck J, Wilson N, Russell D & Herbison B. *Life in New Zealand*. Commission report volume VI: Nutrition. University of Otago, Dunedin, 1991.
- 20 Nielsen Scantrack. *Market information digest*. AC Nielsen (NZ) Ltd, Auckland, 1994.
- 21 American Oil Chemists' Society. *Isolated trans-isomers, infrared spectrophotometric method Cd 14-61*. Official methods and recommended practices of the AOCS, 4th ed. American Oil Chemists' Society, Champaign, Illinois, 1993.
- 22 Bligh E G & Dyer W J. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 1959; 37: 911-917.
- 23 International Dairy Federation. *Determination of fat content-Rose Gottlieb method*. IDF Standard 1C:1987. International Dairy Federation, Brussels.
- 24 Christopherson S W & Glass R L. Preparation of milkfat methyl esters by alcoholysis in an essentially non-alcoholic solution. *Journal of Dairy Science* 1969; 52: 1289-1290.
- 25 Wolff R L. Contribution of trans-18:1 acids from dairy fat to European diets. *Journal of the American Oil Chemists Society* 1994; 71: 277-283.
- 26 MacGibbon A K H & van der Does Y E H. Analysis of fat mixtures. NZDRI Report MF93R14. New Zealand Dairy Research Institute, Palmerston, North.
- 27 Sacks L. *Applied statistics. A handbook of techniques*. 2nd ed. Springer Series in Statistics. New York: Springer-Verlag, 1984.
- 28 Sommerfeld M. Trans unsaturated fatty acids in natural products and processed foods. *Progress in Lipid Research* 1983; 22: 221-233.
- 29 Parodi P W & Dunstan R J. The trans unsaturation content of Queensland milkfats. *The Australian Journal of Dairy Technology* 1971; 26: 60-63.
- 30 Firestone D & LaBouliere P. Determination of isolated trans isomers by infrared spectroscopy. *Journal of the Association of Official Analytical Chemists* 1965; 48: 437-443.
- 31 Ulberth F & Haider H J. Determination of low level trans unsaturation in fats by Fourier transform infrared spectrophotometry. *Journal of Food Science* 1992; 57: 1444-1447.
- 32 Madison B L, Depalma R A and D'Alonzo R P. Accurate determination of trans isomers in shortenings and edible oils by infrared spectrophotometry. *Journal of the American Oil Chemist's Society* 1982; 59: 178-181.
- 33 Henninger M & Ulberth F. Trans fatty acids content of bovine milkfat. *Milchwissenschaft* 1994; 49: 555-558.
- 34 Huang A & Firestone D. Comparison of two infrared methods for the determination of isolated vegetable oils and derived methyl esters by differential infrared spectrophotometry. *Journal of the Association of Official Analytical Chemists* 1971; 54: 1288-1292.
- 35 Ratnayake W N M, Hollywood R, O'Grady E & Beare-Rogers J L. Determination of cis and trans-octadecenoic acid in margarine by GLC-IR spectrophotometry. *Journal of the American Oil Chemists Society* 1990; 67: 804-810.
- 36 Mansour M P & Sinclair A J. The trans fatty acids and positional (Sn-2) fatty acid composition of some Australian margarine, dairy blend and animal fats. *Asia Pacific Journal of Clinical Nutrition* 1993; 3: 155-163.
- 37 Michels K & Sacks F. Trans fatty acids in European margarines. *New England Journal of Medicine* 1995; 332: 541-542.
- 38 Burlingame B A, Milligan G C, Apimerica D E & Arthur J M. *The Concise New Zealand Food Composition Tables*. 2nd ed. New Zealand Institute for Crop and Food Research, Wellington, 1994.