TRANS UNSATURATED FATTY ACIDS:

A STUDY OF METHODOLOGY AND LEVELS IN NEW ZEALAND FOOD FATS INCLUDING MILKFAT.

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ABSTRACT

Trans fatty acids (TFAs) occur naturally in small amounts in foods such as milk, butter and tallow as a result of biohydrogenation by ruminant gut microflora. They are formed in much larger quantities during chemical hydrogenation of fats and oils. The relationship between dietary TFAs and blood cholesterol has been investigated over the last 30 years with equivocal results because of methodological limitations, including difficulties of quantifying the consumption of TFAs.

The present study was conducted to investigate the methodologies used to quantify TFAs in fat samples. Two methodologies, based on infrared spectrophotometry and argentation-thin layer chromatography/gas chromatography (Ag-TLC/GC), were optimised for TFA quantification. Improvements in the infrared methods were made using a calibration standard made up with two non-*trans* components (stearin and olein) in order to mimic the fatty acid background in the samples. Further improvements were made using a spectral subtraction technique where the non-*trans* background spectrum was subtracted from the sample spectrum using Fourier-transform infrared spectrophotometry software. Results from the improved infrared methods were compared with TFA measurements by the more detailed Ag-TLC/GC method. The spectral subtraction technique for the methyl ester samples produced results that were closest to those of the Ag-TLC/GC method. This Ag-TLC/GC method gives information about the individual *trans* isomers (C18:1 *trans* positional isomers and C18:2 and C18:3 *trans* isomers) that is not available by infrared.

The present study was also conducted to determine, as accurately as possible, the TFA content in 18 manufactured foods commonly available in New Zealand using the TFA methods mentioned above. The TFA contents in some of the foods determined by the Ag-TLC/GC method were, margarine (15.43-15.57%), butter (6.58%), milk (5.26-6.03%), meat patties (3.42%), plain sweet biscuits (3.65%) and white bread (4.41%).

Using these TFA data and the food consumption data from a *Life in New Zealand* (Horwarth *et al.,* 1991), the estimated TFA intakes in the average New Zealand diet were approximately 3.99 and 5.75 g/person/day for females and males

respectively. These figures were similar to or lower than those estimated for Northern Hemisphere countries. The predominant TFA isomer in the New Zealand diet was identified as the C18:1 Δ11t positional isomer (30-33%).

Further studies were made on the total TFA content in New Zealand milkfat. These studies indicated that the total TFA levels in New Zealand milkfat were influenced by seasonal variations, with the highest TFA content recorded in spring (September, 6.7%) and the lowest in summer (January, 5.3%). The C18:1 Δ11t isomer was found to be the predominant isomer in milkfat, making up 49-60% of the total TFA. Similar ranges were observed for several overseas butter samples. However, major differences were observed with the distribution of the C18:1 *trans* positional isomers. These differences are currently suspected to be influenced by the feed and animal husbandry methods used in some Northern Hemisphere countries, where cows are mainly grain fed in the winter months. The seasonal variation of TFAs in New Zealand butter and possible effects of feed and animal husbandry methods on the C18:1 *trans* positional isomer distribution are important factors that the New Zealand dairy industry could exploit for the production of low *trans* milkfat and/or other dairy products in which the levels of specific *trans* isomers implicated to be "harmful" to humans could be minimised.

Margarines display a *trans* isomer distribution that is quite distinct from that of butter. Unlike milkfat where the predominant *trans* isomer is C18:1 Δ11t, in margarines and hydrogenated fats and oils the positional isomers show a normal distribution around the C18:1 Δ10t-11t isomers. The predominant isomers for the margarines analysed in this study were Δ9t-Δ12t (90%) with the polyunsaturated C18:2 and C18:3 *trans* making up less than 2%. The distinct distribution of C18:1 *trans* positional isomers could serve as an additional tool for the identification of animal or hydrogenated vegetable oils used in food fats.

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NOMENCLATURE AND ABBREVIATIONS

All fatty acids mentioned in this thesis are described by their carbon number, number of unsaturated bonds and their positions. The long and complicated IUPAC names are not used. For example, octadecatrienoic acid (linolenic acid) becomes C18:3 Δ9c, 12c, 15c, where:

'Δ' (delta) indicates the double bond position from the carboxyl carbon of the fatty acid,
'c' indicates the *cis* configuration of the double bond, and
't' indicates the *trans* configuration of the double bond.

Occasionally, the 'n' system is used, where it relates to the position of the first double bond from the methyl terminal carbon of the fatty acid. For example:

C18:2 Δ9c, 12c = *cis* C18:2 (n-6)
C18:3 Δ9c, 12c, 15c = *cis* C18:3 (n-3).

In some cases, trivial names are used. Common trivial names used in this thesis are given below:

C12:0	Lauric acid
C14:0	myristic acid
C16:0	palmitic acid
C18:0	stearic acid
C18:1 Δ9c	oleic acid
C18:1 Δ9t	elaidic acid
C18:1 Δ11t	vaccenic acid
C18:2 Δ9c, 12c	linoleic acid (n-6 family)
C18:3 Δ9c, 12c, 15c	α-linolenic acid (n-3 family)
C18:3 Δ6c, 9c, 12c	γ-linolenic acid (n-6 family)

Ag-TLC	Argentation-thin layer chromatography
AMF	Anhydrous milkfat
AOCS	American Oil Chemists' Society
Conj	Conjugated
FAME	Fatty acid methyl ester
FFAP	Free fatty acid phase
FID	Flame ionisation detector
FTIR	Fourier transform infrared spectrophotometry
GC	Gas chromatography
HDL	High density lipoprotein
HATR	Horizontal attenuated transmittance reflectance
HMDS	Hexamethyldisilazane
HPLC	High performance liquid chromatography
IR	Infrared spectrophotometry
LDL	Low density lipoprotein
MARG	Margarine oil
MS	Mass spectrometry
QC	Quality control
TFA	*trans* fatty acid
TG	Triglyceride
TMCS	Trimethylchlorosilane
UV	Ultraviolet
S_{n-1}	Sample standard deviation
SS	Spectral subtraction

INTRODUCTION

Over the last several years, there has been increased interest in the role that dietary fats play in the development of chronic diseases such coronary heart disease and cancer (Gurr, 1983, 1989, 1993, 1995a, 1995b, 1996; Khosla, 1995). Generally, saturated fatty acids have been linked to these diseases. As more information becomes available, it is clear that the different fatty acids have different effects on the risk of developing these diseases. On the basis of classical studies by Keys *et al.* (1965) and Hegsted *et al.* (1965), dietary saturated fatty acids, specifically lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids, have generally been regarded as being equally cholesterol raising, whereas saturated stearic acid (C18:0) and oleic acid (C18:1) have been considered to be neutral. The polyunsaturated fatty acids (principally the C18:2 acids) are regarded as cholesterol lowering (lowers the LDL-cholesterol) but HDL-cholesterol is also lowered at very high intakes (Beynen & Katan, 1989; Khosla, 1995). In recent years, more controlled animal and human studies, utilising different dietary fat and oil blends - as a means of control for specific fatty acid levels, have begun to unravel the individual effects of these fatty acids. These studies indicated that, for normocholesterolaemic subjects when the dietary cholesterol intake is low (below 250-300 mg/day), C14:0 was the principal saturated fatty acid that raised serum cholesterol. Under such conditions, C12:0 and C16:0 appeared to be neutral in terms of their ability to influence serum cholesterol. For hypercholesterolaemic subjects (when the dietary cholesterol intake is greater than 500-600 mg/day), palmitic acid was hypercholesterolaemic. Data for lauric acid were inconclusive (Khosla, 1995). More recently, dietary TFAs have also received great interest as concerns are expressed about their increased intake and the adverse effects they have on the serum cholesterol level and other health problems such as atherosclerosis, cancer, diabetes and obesity. Additionally, the possible deleterious effect of TFAs on the foetus has recently come into focus with the demonstration of placental transfer of TFAs from mother to foetus (Koletzko, 1992). The author suggested that maternal intake of TFAs affected the birth weights of premature infants as a result of interference with essential fatty acid metabolism. Although similar concerns about the safety of TFAs in the diets of pregnant and lactating women and infants have been raised by others (Slender *et al.*, 1995), further investigations were required (Gurr, 1995a).

The evidence that these deleterious effects on human health are caused in part by the currently high intake of TFAs has led to legislative action being taken to reduce the levels of these fatty acids in hydrogenated fats, the primary source in human diets (Anon., 1995). Also, it has been suggested that these fatty acids should not be included as part of the unsaturated fatty acids in nutritional labelling, but should be considered as saturated fatty acids (Shrapnel *et al.*, 1994; Ovesen & Leth, 1995). The risk to New Zealanders of these TFA-associated diseases is unclear because little or no data are available about the level and composition of TFAs in their diets. The main objectives of this thesis were to improve and compare the two main methodologies (IR and Ag-TLC/GC) used for *trans* unsaturated fatty acid quantification in fat and oil samples and, using these techniques, to quantify TFAs in some manufactured New Zealand foods with vegetable and dairy components. The TFA data generated from this study would be used to expand the New Zealand food composition database, allowing health officials and nutritionists to better evaluate the TFA intake in the average New Zealand diet.

The seasonality of properties of New Zealand milkfat is due to pasture feeding, and hence there is a need for seasonal information of TFAs to get an accurate picture of how they may contribute to these properties. A study of TFA levels in New Zealand butter across the dairy season (1995/96) was therefore made to add to the milkfat composition database at the New Zealand Dairy Research Institute. This information would allow a better understanding of the influence of the pasture quality over the dairy season on the TFA levels in milkfat, allowing selection of products from specific times and selective blending of the milkfat obtained over the different milking periods to obtain a specific level *trans* product *etc*. Furthermore, it would allow a comparison of *trans* levels in New Zealand butter with those in some overseas butter samples.

This TFA study was therefore divided into five main objectives.

(i) Total *trans* isomer

Improvement of the American Oil Chemists' Society (AOCS, 1993a) standard method Cd 14-61 for total *trans* unsaturated fatty acid quantification by IR to allow the determination of the low levels found in milkfat and other

products.

(ii) Specific *trans* isomers:

Optimisation of the *trans* unsaturated fatty acid quantification by Ag-TLC/GC methodology to allow the determination of specific *trans* isomers in milkfat and other products.

(iii) Comparison of the New Zealand food survey TFA levels obtained using the IR and Ag-TLC/GC methods.

(iv) Estimation of the TFA intake in the New Zealand diet.

(v) Study of the TFA levels in New Zealand butters over the dairy season and in some overseas butter samples.

LITERATURE REVIEW

1 CHEMISTRY AND ORIGIN OF TFAS

Trans unsaturated fatty acids are fatty acids with double bonds that are in the *trans* geometric configuration. The results of this geometric configuration are fatty acid hydrocarbon chains that are "near straight", between the "straight" saturated fatty acids and the "bent" *cis* fatty acids (Figure 1). This difference in shape gives TFAs quite different physical and biochemical properties compared with their *cis* counterparts. In fact, because *trans* unsaturated fatty acids are "near straight", their properties are similar to those of saturated fatty acids. For example, the melting points of stearic (C18:0), elaidic (C18:1 Δ9t) and oleic (C18:1 Δ9c) acids are 69.9°C, 45-45.5°C and 12- 16°C respectively (Larsson & Quinn, 1994).

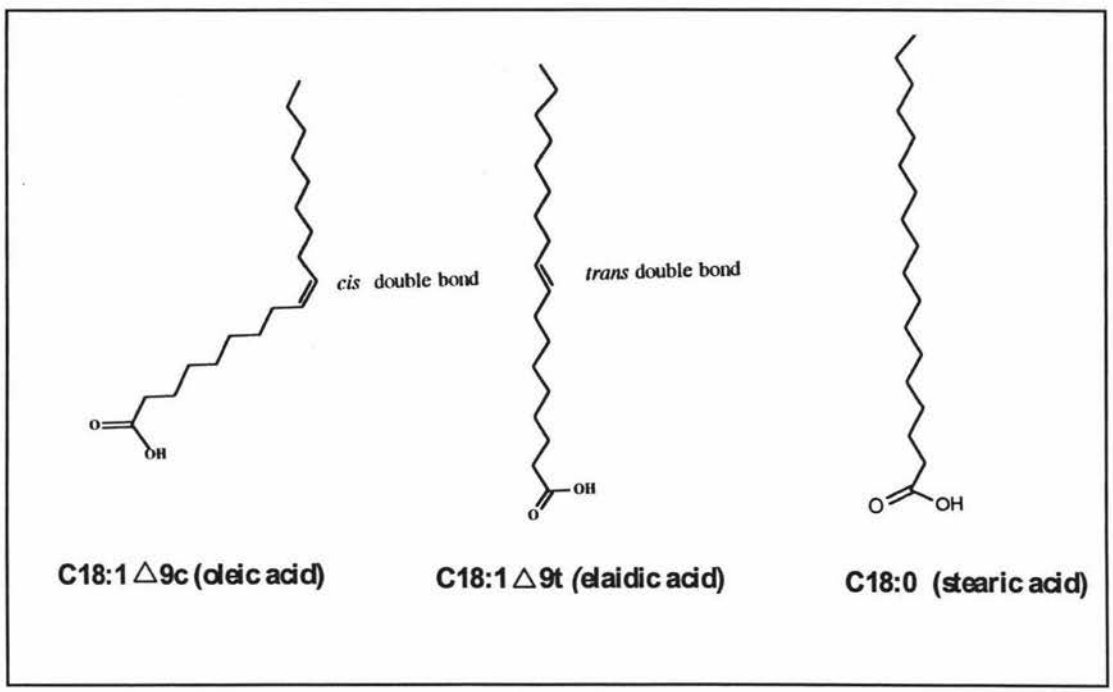

Figure 1 Geometric configuration of *cis*, *trans* unsaturation and saturated fatty acids.

Most naturally occurring unsaturated fatty acids have double bonds in the *cis* orientation. However, the *trans* orientation does occur naturally in foods. TFAs occur in ruminant fats as a result of the biohydrogenation process of dietary

polyunsaturated fat carried out by the microbial population (Craig-Schmidt, 1992; Sommerfeld, 1983). TFAs are also generated artificially from industrial partial hydrogenation of oils, mainly from vegetable and fish sources (Wolff, 1994; Bimbo, 1987). These partially hydrogenated oils are used in the manufacture of margarines, hardened fats such as shortening, and food products such as pastries and confectionery. These food products are the predominant source of these TFAs in the human diet in most western industrialised countries (Mansour & Sinclair, 1993; Ratnayake *et al.*, 1993; Enig *et al.*, 1990).

During the partial hydrogenation of these oils, apart from the generation of the *trans* geometric isomers, a whole range of positional isomers are also generated. For example, natural soya bean oil consists predominantly of palmitic, stearic, oleic, linoleic and linolenic acids. In the monoenoic fraction of the hydrogenated oil, by contrast, oleic acid may be only one of approximately 20 positional isomers (Dutton, 1979). A similar situation applies to the other polyenoic fractions. The distribution of geometric and positional isomers varies according to the hydrogenation process, the catalyst used and the composition of the original oil. A typical vegetable fat that has been partially hydrogenated may contain anywhere from 10 to 60% of its total unsaturated fatty acid as TFA isomers. The dominant *trans* isomers are mostly the C18:1 acids with double bonds at the Δ8, Δ9, Δ10, Δ11, Δ12 and Δ13 positions. In contrast, ruminant fats (such as milkfats), which have been partially hydrogenated by microorganisms in the rumen, have been reported to contain anywhere from 1 to 8% of the total fatty acids as TFA isomers, with the dominant *trans* isomer C18:1 Δ11t (vaccenic acid). The distinct distribution of the positional isomers in ruminant fat is due to the specific enzymatic hydrogenation reactions employed by the microbial flora in the rumen. Industrial partial hydrogenation, on the other hand, generally involves the use of solid nickel catalyst, pumped into an autoclave with the vegetable oils, and heated to 120°C in the presence of hydrogen gas at from 1 to 6 atmospheres. The reaction is exothermic and is controlled in a variety of ways: catalyst concentration, temperature, hydrogen pressure, rate of stirring *etc.* (Dutton, 1979).

There is a tendency for the edible oil industry to use the terms `hydrogenated', `partially hydrogenated', `hardened' and `isomerised' interchangeably. This has occasionally led to misunderstanding of the process reported in research papers.

The term `isomerisation' reflects the generic description of a change from the *cis* to the *trans* configuration but not bond migration. Such isomerised oils are only used experimentally (Enig, 1993). Commercial partial hydrogenation processes result in a change in both the bond configuration and the position of the double bond. Although both isomerisation and hydrogenation result in the formation of *trans* positional isomers, their distribution differs.

2 CURRENT HEALTH ISSUES OF TFAS

Concerns regarding the effects of dietary TFAs on serum lipids in humans were raised over the past several decades as hydrogenated oil consumption increased (Hunter, 1992). Perhaps, one of the first studies looking at the effects of the hydrogenated fats on serum lipids was conducted by Anderson *et al* (1961). These authors concluded that TFA isomers elevated serum cholesterol close to that of the average saturated fatty acid with chain length between C12:0 and C18:0. A more recent controlled study by Mensink & Kantan (1990) demonstrated clearly the elevation of total serum cholesterol and LDL levels, while reducing the HDL levels, with a diet containing significant quantities of TFAs. The saturated fatty acids were shown to increase the LDL levels but had no effect on the HDL level. However, this study has been heavily criticised by the edible oils industry (Reeves, 1991) in that the high *trans* fat used by Mensink & Katan (1990) was produced by catalytic isomerisation, not by hydrogenation under conditions typical of US margarine and shortening manufacture (in the isomerisation processes used by Mensink and Katan (1990), high oleic acid sunflower oil was treated with a catalyst to form *trans* double bonds with addition of minimal hydrogens). A similar effect of TFAs on cholesterol was reported by Willett *et al.* (1993). Because human diets are complex and subject to frequent changes, the results produced in a number of other similar studies were not always consistent (Mensink *et al.*, 1992; Nestel *et al.,* 1992; Judd *et al.,* 1994; Wood *et al.*, 1993).

Other health conditions and factors reported to be affected by dietary TFAs include cancer, diabetes, obesity and immunity. The suggestion that TFAs may be linked to cancer was made as early as 1956 by Sinclair. It was not until recently that Willett *et al.* (1993) reported a positive correlation between breast cancer and dietary intake of TFAs in 85,000 nurses. Likewise, a high correlation between

prostate cancer and dietary intake of TFAs was also found in men. Similar results were reported by Ewertz & Gill (1990), who found that the relative risk of breast cancer was increased when the fat used for frying was margarine and decreased when butter or vegetable oil was used. However, the biochemistry behind these correlations has yet to be elucidated.

The adverse effect of TFAs on insulin receptors and insulin binding has been demonstrated in primates and humans. The increased dietary TFAs were shown to affect muscle membranes in a manner that could trigger diabetes and that was worse when the subjects were obese (Enig, 1993). Furthermore, obesity research over the last several decades has pointed to the involvement of n-6 polyunsaturates in increasing fat cell numbers, whereas the n-3 fatty acids are needed to avoid weight gain. TFAs were shown to promote the adverse effects of linoleic acid, (Δ9, Δ12, an n-6 polyunsaturate) and to decrease the levels of the important n-3 fatty acids in tissues (Enig, 1993).

Reproductive studies on male animals fed TFAs also showed adverse effects, where a decrease in testosterone and an increase in abnormal sperm count were observed, and gestational problems were demonstrated with female animals (Hanis *et al.,* 1989). Preliminary lactation studies by Koletzko (1991, 1992) indicated that some of the problems observed in animal models appear to exist for humans, and that there was a relationship between TFA intake and low birth weights in infants. An altered immune response caused by TFAs has been reported in mice but these studies have not been extended to humans (Hunter, 1992).

It is clear that the safety issues of increasing TFA consumption are real and becoming more widely reported in the scientific literature.

3 METABOLISM OF TFAS

A number of researchers have investigated the metabolic properties of various *trans* isomers. It has been noted that C18:1 Δ11t is metabolised more like the saturated fatty acids (palmitic and stearic acids) and the monounsaturated fatty acid, oleic acid (Kummerow, 1979; Brisson, 1981). Historically, ruminant TFAs (predominantly C18:1 Δ11t) have been present in human diets for many centuries.

The other C18:1 *trans* isomers (Δ5t through Δ10t and Δ12t through Δ16t) would not have existed previously except in trace amounts, but they are now the dominant isomers in the partially hydrogenated vegetable fats and oils. These isomers have been reported to be metabolised more slowly in some tissues, such as heart tissues (Enig, 1993). More recently, Willett *et al.* (1993) investigated the development of heart disease relative to the dietary intake of TFAs from partially hydrogenated vegetable fats and oils from ruminant fats in 85,000 women. They reported a significant increase in the risk of heart disease associated with the TFAs from partially hydrogenated vegetable fats and oils and a non-significant increase with TFAs of ruminant origin.

The mechanism by which dietary fatty acids raise or lower serum cholesterol levels is still poorly understood. It has been suggested that regulation may be partly achieved through the stimulation or suppression of LDL receptors, but how dietary fatty acids control expression of these receptors is unknown (Grundy, 1991). Similarly, the effect of TFAs on "normal" lipid metabolism, and how they influence serum cholesterol levels, is also unclear.

The essential polyunsaturated fatty acids (C18:2 (n-6) and C18:3 (n-3)) are generally termed "good" fatty acids because they reduce the serum cholesterol level. These essential polyunsaturated fatty acids in the mammalian system are elongated and desaturated to polyunsaturated fatty acids and precursor acids for eicosanoids biosynthesis (Gurr, 1995a; Bruckner; 1992, Figure 2). These compounds have a diverse range of pathophysiological actions in the cardiovascular system and inflammatory processes. It has been implied that the excessive synthesis and/or an imbalance in the synthesis of these eiconsanoids in tissues can lead to the development of certain pathological conditions (Hwang, 1992).

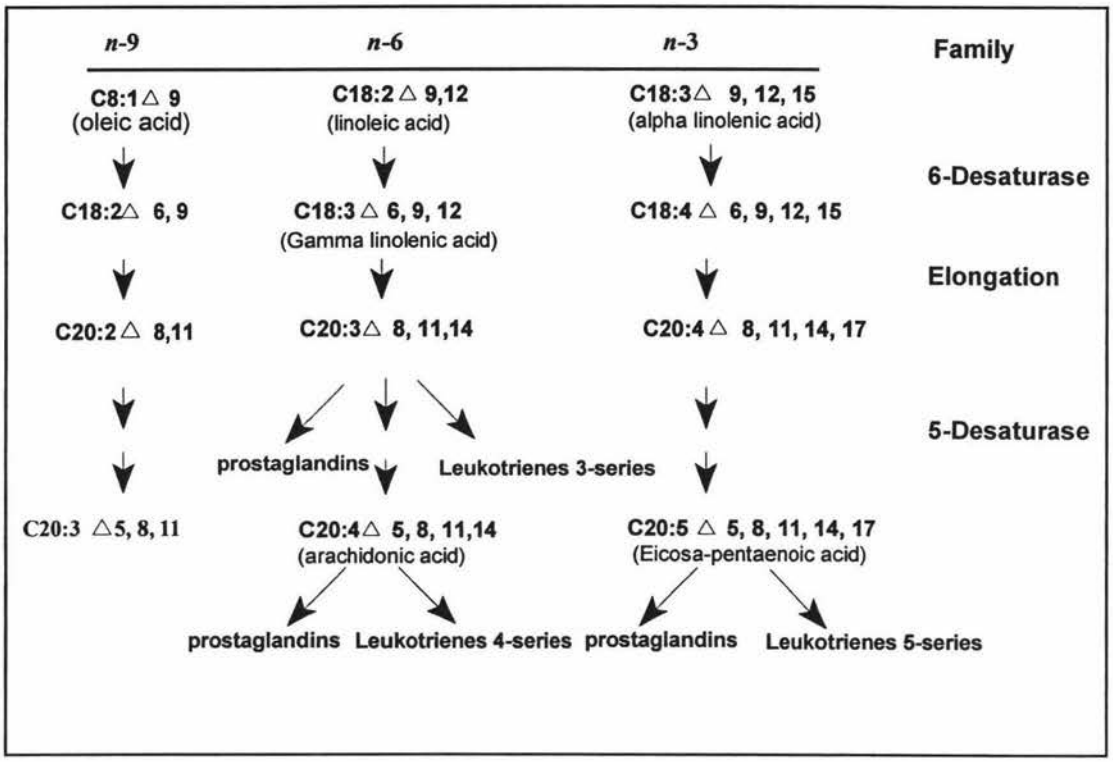

Figure 2 The metabolic pathway of the polyunsaturate families in the synthesis of eicosanoids and prostaglandins (Gurr, 1995a; Bruckner, 1992).

The possible effects of the *trans* geometric isomers of these essential fatty acids on the biosynthetic pathway of these eicosanoids and prostaglandins is not fully understood, but it has been documented that the geometric isomer C18:2 Δ9c, Δ12t can be converted to C20:4 (n-6) containing a *trans* double bond (Privett *et al.*, 1967; Anders *et al., 1975*). However, this *trans* isomer of C20:4 (n-6) inhibited the formation of prostaglandin from the all *cis* C20:4 (n-6) fatty acid. The Δ9t, Δ12c and Δ9t, Δ12t isomers of linoleic acid were also shown to inhibit the conversion of all *cis* linoleate to C20:4 (n-6) (Hwang & Kinsella, 1979; Hwang *et al.*, 1979; Hwang, 1992).

Details on the effects of linolenic acid geometric isomers on biological systems are also limited. It is clear that the production of the n-3 polyunsaturated fatty acids C22:6 (n-3) (docosahexaenoic acid, DHA) and C20:5 (n-3) (eicosa-pentaenoic acid, EPA) for incorporation into membrane phospholipids is dependent on the availability of their precursor, linolenic acid, to be desaturated by the Δ6 desaturase enzyme.

A recent study by Blond *et al.* (1995) demonstrated that the dietary *trans* isomers of C18:3 (n-3) decreased the rate of the Δ6 desaturation step in the biosynthesis of long chain n-3 polyunsaturated fatty acids (Figure 2). Earlier, one *trans* isomer, C18:3 Δ9c, 12c, 15t, was shown to be converted to the *trans* isomer of EPA, C20:5 Δ5c, 8c, 11c, 14c, 17t, and into C22:6 Δ4c, 7c, 10c, 13c, 16c, 19t and to be incorporated into various rat tissues including liver, kidney, platelet, brain and retina (Grandgirard *et al.*, 1989, 1994). The adverse effects, if any, of these *trans* isomers are unclear because they are generally present in very small amounts (<0.5%) in partially hydrogenated oils. It is, however, now well documented that the amount of the various types of eicosanoids and prostaglandins synthesised in tissues can be modulated by manipulating the composition of the dietary fatty acids (Nugteren, 1970; Hwang, 1992).

4 TFA INTAKE IN HUMAN DIET

The primary source of TFAs in the food supply is from commercial hydrogenated vegetable oils. The increasing intake of these cheaper hydrogenated fats, with longer shelf lives than the animal fats used previously, is a concern. Emken (1984) and Enig *et al.* (1990) estimated that up to 90-95% of isomeric fatty acids appearing in the diet is contributed by commercial partially hydrogenated fats. These hardened fats are found in margarine, shortening, frying fat and various processed foods. The level of total TFAs varies in these products. The levels reported for American margarine vary greatly from a few percent up to 40-50% (Enig *et al.*, 1983; Ratnayake *et al.*, 1993). Typical levels in shortening made from hydrogenated vegetable oil are reported to be in the range from 10 to 25% (Craig-Schmidt, 1992). In butter, the level varies from 3 to 8%, the variability influenced by the geography, the type of feed and season. The total TFA intakes reported for the various countries are given in Table 1.

Country	g TFA consumed/person/day	% of Fat Intake	Source
Australia	4.0-6.4 (males) 3.2-4.4 (females)	-	Noakes & Nestel (1994)
USA	12.5-15.2 (food disappearance data) 1.6-38.7 (dietary fat consumption) 11.1-27.6 (adipose tissue composition)	8	Enig *et al.* (1990)
UK	5-7 (max of 27)	24 (max)	BNFTF (1987)
Netherlands	17	12	Brussaard (1986)
W. Germany	4.5-6.5 4.1 (males) 3.4 (female)	-	Enig (1993) Steinhart & Pfalzgraf (1992)
Sweden	5.0	5	Enig (1993)
Canada	9.1	9.5	Brisson (1981)
Finland	1.7	2	Heinonen *et al.* (1992)
South Korea	0.63	-	Won & Ahn (1990)

Table 1 Published estimations of TFA consumption for a few countries. The TFA consumption as a percentage of the total fat intake is also given where available.

Data on the TFA content in New Zealand foods are sparse. Using the *Life in New Zealand* fat intake data (Horwarth *et al.*, 1991) and TFA levels in margarine reported by Ball *et al.* (1993), it has been estimated that approximately 0.5 g of TFA per day comes from margarine. Currently, more data are required, particularly from processed fat foods, in order to make a better estimate.

Because of the probable involvement of TFA isomers in the aetiology of various disorders, and a strong recommendation for them to be classified as a separate class of fatty acid in nutritional labelling (Shrapnel *et al.*, 1994; Ovesen & Leth, 1995), an accurate estimation of them in edible oils and fat products is important. The generally accepted standard methodologies for determining total TFAs are IR and GC. Unfortunately, these methods have their limitations, as do all methods. These methods were initially developed for the process control of the hydrogenation of fats in which the levels of *trans* fatty acids were relatively high. Measurement of low *trans* concentration is more difficult, requiring modification and re-optimisation.

5 METHODOLOGY

5.1 TOTAL *TRANS* CONTENT BY IR

As the isolated *trans* double bond exhibits an infrared absorption band at 965 cm^{-1} (due to the C-H out of plane deformation), the determination of TFAs by IR had been widely used in the fat and oil industry. In order to ensure an acceptable precision of this method, it has been standardised by the International Union of Pure and Applied Chemistry (1987) and the AOCS (1993a). Both standardised methods use dispersive infrared instrumentation in their applications. However, FTIR techniques have since been introduced successfully by various workers (Sleeter & Matlock, 1989). FTIR instruments offer several advantages over conventional IR equipment, namely in:

1 improved signal to noise ratios due to averaging of multiple scans;
2 high accuracy of the wavelength calibration due to a reference He-Ne laser;
3 improved speed due to use of the Michelson interferometer principle;
4 spectral manipulation by means of computer due to the digital format of the spectral data.

Although there are numerous advantages in using FTIR for TFA quantification, the principal problem of accurate low level *trans* quantification remains only partially resolved. Furthermore, inherent in the direct measurement of the fat or oil samples is the interference of acylglycerol moieties/groups because of their strong absorption in the region of the *trans* absorption band (around 965 cm^{-1}), producing *trans* values that are 2 to 3% higher (Figure 3; Firestone & Laboulier, 1965). However, this disadvantage is normally circumvented by *trans*-esterification of the fats and oils to their respective methyl esters. Unfortunately, values are 1.5-3% low (Figure 3). These bias results are caused by the nature of the baseline construction and absorption spectrum of the non *trans* component (Figure 3). Arithmetic compensation has been proposed by Huang & Firestone (1971) and was the basis of the AOAC method. Increased accuracy in the range from 0.5% to 36% *trans* was achieved by using a two-component calibration standard containing a mixture of the methyl esters of linoleate and elaidin (Madison *et al.*, 1982) for the AOCS method Cd 14-61; this eliminated the need for the arithmetic

correction. Spectral subtraction techniques have also been attempted by various authors to improve the accuracy of this infrared methodology. Ulberth & Haider (1992) attempted to increase the accuracy by subtraction of a background spectrum obtained from "cold press" *trans*-free soya bean oil for the analysis of margarine and shortening. A fully hydrogenated milkfat was recently used for spectral subtraction for milkfat (Ulberth & Henninger, 1994). Spectral subtraction results were shown to increase the total *trans* results and remove the negative data for some low *trans* samples. The standard deviations of the measurements were also reduced. However, it appears that this approach may be highly accurate only when the *trans*-free oil used in the technique is the same as the sample oil because the chain length of the non-*trans* mixture and/or hydrogenation of the *cis* unsaturated fatty acids may alter the background infrared spectrum (Huang & Firestone, 1971).

A similar spectral subtraction technique was investigated in this study using non-*trans* components of oleate and stearate.

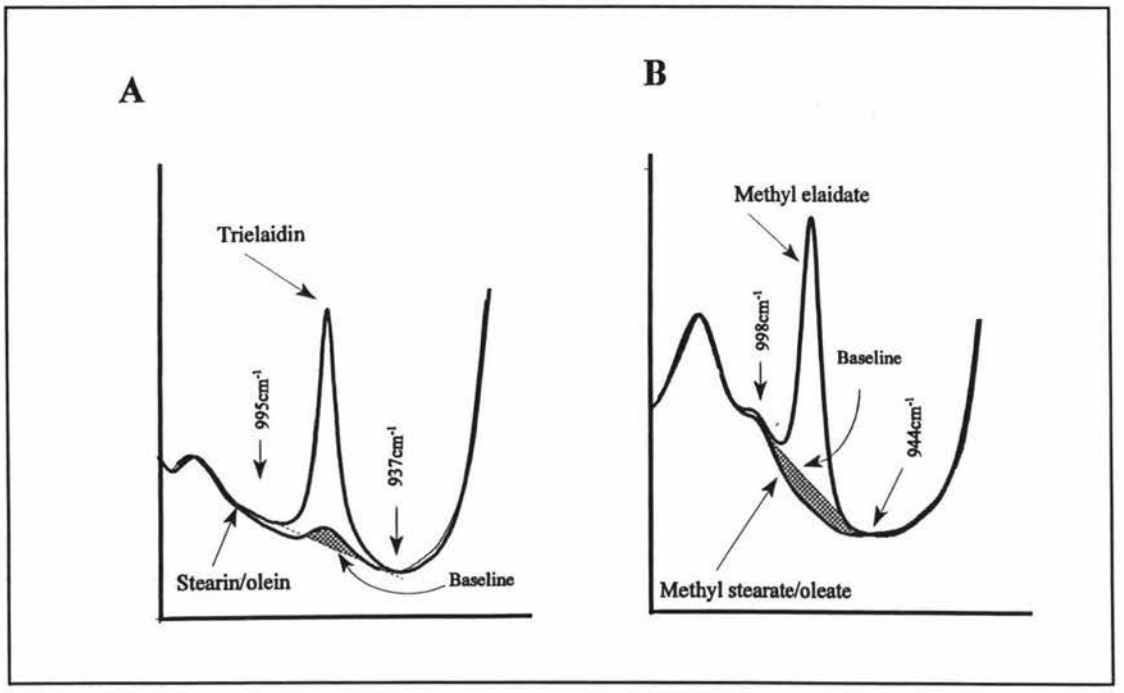

Figure 3 FTIR spectra of A: triglyceride, B: methyl ester with the respective non-*trans* background spectra. The shaded regions indicate the approximate bias generated as the result of the absorption of the non-*trans* components and the baseline boundaries specified by the AOCS Cd 14-61 method (AOCS, 1993a).

5.2 SPECIFIC TFA ISOMER DETERMINATION BY GC

Capillary GC has been used extensively for *cis/trans* isomer separation. The AOCS methods Cd 14c-94 (AOCS, 1993b) and Cd 17-85 (AOAC, 1993c) were designed to evaluate the composition of fatty acid, the level of *trans* unsaturates and *cis-cis* methylene interrupted unsaturates using a single SP234 flexible fused silica polar capillary column. These direct GC procedures were based on the assumption that C18:1 *cis* and C18:1 *trans* are completely separated on the column. However, partially hydrogenated vegetable oils (PHVO) are complex mixtures of positional and geometric isomers of C18:1, C18:2 and C18:3 fatty acids. No serious problems with overlap between the three unsaturated fatty acids were encountered with this single capillary column technique. Unfortunately, a complete resolution of C18:1 *trans* as a group from the *cis* isomers was not feasible on the capillary column SP234 or any other cyanosilicone capillary column. The consequence of this is a gross underestimation the total C18:1 *trans* values in favour of the *cis* isomer (Ratnayake & Beare-Rogers, 1990; Sampugna *et al.*, 1982). In some margarines, underestimation of the total C18:1t *trans* isomers can be as high as 32%. In the cyanosilicone columns, and perhaps in columns of similar polarity, the early eluting C18:1 *trans* isomer methyl esters with low Δ values are generally well separated from the C18:1 *cis* isomers, but the C18:1 *trans* isomers with high Δ values (Δ12t-15t) are under the C18:1 Δ9c peak, which is the major *cis* isomer in PHVO. Because PHVO may contain an appreciable amount of C18:1 *trans* isomer with high Δ values (Sampugna *et al.*, 1982), the *cis/trans* overlap should not be ignored. The level of high Δ value C18:1 positional isomers (Δ12t-16t) will vary depending on the hydrogenation condition and the source of the oil.

5.3 SEPARATION OF *CIS/TRANS* ISOMERS BY AG-TLC/GC

Prior separation of the *trans* from the *cis* fatty acids before GC analysis using Ag-TLC appears to be the most accurate and preferred method for C18:1 TFA determination. Even though it is more laborious, this technique circumvents the problem of *cis/trans* overlap experienced using the direct GC method discussed above. In Ag-TLC, the silica plates are impregnated with silver ions. Interaction between the double bonds and the silver ions serves as the basis of this separation. This interaction is dependent on three characteristics: the number of double bonds,

their geometric configuration and their position. Generally, FAMEs separate into five fractions: saturated, *trans* monoenes, *cis* monoenes, dienes and polyenes. The dienes and polyenes can be separated into the various *cis*/*trans* classes depending on the developing conditions (*e.g.* mobile phase, temperature). The TFA band is recovered from the Ag-TLC plate and analysed by GC.

The recent introduction of argentation HPLC (using silver ions bound to an ion exchange support) is an alternative to the TLC techniques. The ability to use gradient mode elution makes it a more powerful technique than TLC. Unfortunately, this system requires a mass detector for detection of the eluting fatty acids.

Although the IR and GC methods are the most extensively used for TFA quantification, GC-MS methods have also been used (Christie *et. al.,* 1987; Harvey, 1992).

5.4 IDENTIFICATION OF TFA ISOMERS BY GC-MS

GC-MS has in fact become one of the most powerful tools for the lipid analyst. As opposed to MS itself, GC-MS gives the analyst two kinds of information on a given compound: its mass spectrum and its GC retention time. Fatty acids are normally derivatised before GC-MS analysis to prevent double bond migration. A host of derivatives have been used. For example, methyl esters, pyrrolidine, picolinyl esters and other oxygenated derivatives. The fatty acid derivatives are bombarded by electrons or other ionic species causing them to ionise and fragment. The various ionic fragments produced by electron impact are separated according to mass (strictly speaking, mass/charge [m/z] ratio) in a magnetic field. A spectrum is obtained that in effect is a bar diagram showing the masses of the ions and their abundances, relative to the most abundant ion (peak base) given a value of 100%. This information, together with the retention time of the compound, provides a powerful means of identification for organic compounds. Long chain saturated fatty acids are easily identified by the characteristic prominent molecular ion in their mass spectra. For unsaturated fatty acids, definitive information on the double bond position requires the preparation of derivatives of FAMEs. For simple monoenoic fatty acids, the double bond is normally fixed by reacting with

appropriate reagents to give chemical derivatives that give distinctive fragments in the mass spectrum. The example of dimethyl disulphide derivatisation is given in Figure 4, where the molecular weight of methyl oleate increases substantially after derivatisation (from 296 to 390). The mass spectrum gives a good molecular ion where the most abundant ions represent cleavage at the carbon atoms that were originally linked by the double bond (m/z 173 and 217). This technique is generally less straight forward if there is more than one double bond in the fatty acid. Murawski & Egge (1975) reported the use of the di-trimethylsilyl derivative to determine the position of C18:2 *cis- cis* fatty acid double bonds whereas, more recently, picolinyl derivatives were used by Christie (1989a) to determine the double bond position in both C18:2 and C20:4 fatty acids. Information about the double bond configuration is not available using this technique.

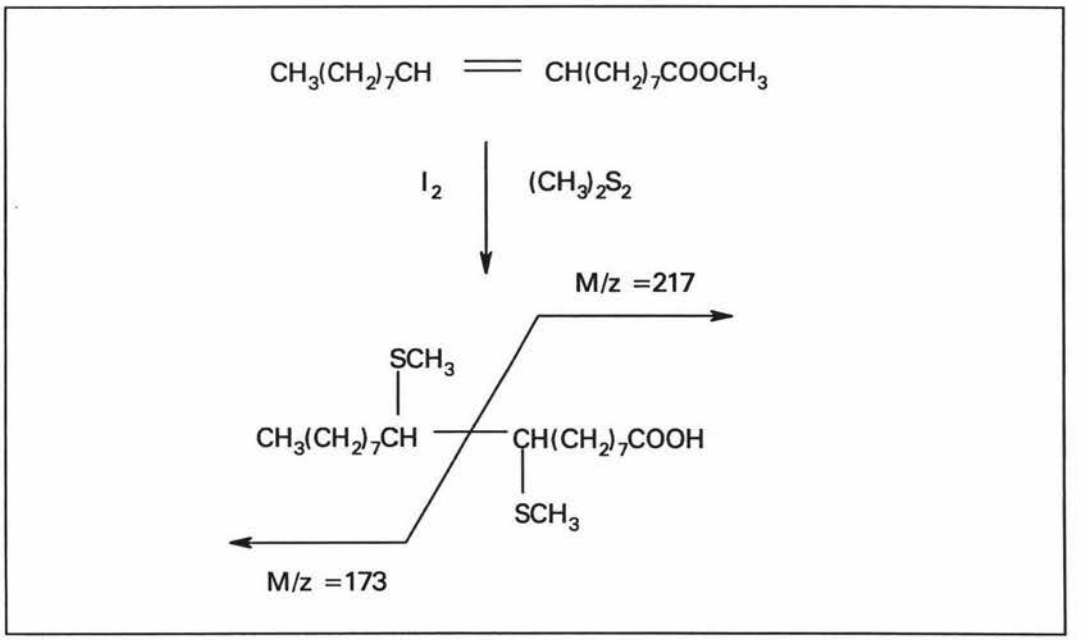

Figure 4 Reaction of dimethyl disulphide with an unsaturated ester for double bond location by MS (Christie, 1996).

6 SUMMARY

It is now fairly well recognised that TFAs are a class of fatty acid different from the typically occurring unsaturated fatty acids. Concerns about their safety are becoming more widely appreciated and reported in the scientific literature.

The levels of TFAs in many western diets are too high and are equivalent to the amounts recognised as being responsible for numerous adverse physiological effects observed in animals and humans and associated with the following chronic diseases: atherosclerosis, cancer, diabetes and obesity. Additionally, it is reported that consumption of TFAs may have undesirable effects on immune function, reproduction and lactation.

The increase risk of these disorders to New Zealanders due to dietary TFAs is unclear because little or no data are available about the TFA composition in their diets. This thesis looks into the methods to quantify TFAs and their application in a range of manufactured foods, including butters in New Zealand. Information from this study could be used to give a much better determination of the amount of TFAs consumed in the average New Zealand diet.

EXPERIMENTAL AND RESULTS

1 SAMPLES

1.1 NEW ZEALAND FOOD SURVEY SAMPLES

Several foods were selected for TFA analysis based on either the probability of their containing TFAs or their contribution to the total fat intake in the New Zealand diet (Howarth *et al.*, 1991). The brand analysed for each food choice was generally either the largest market share or one of the most popular products (Nielsen Scantrack, 1994). Table 2 lists the foods selected for analysis with the available product details. All these products were purchased (1 February 1995) from the local supermarket (Pak'n'Save, Palmerston North, New Zealand).

Sample Type	Brand	Batch Code	Fat Type Used and Content (from label)
Margarine (A),250 g tub	Meadow Lea	*	Vegetable oil
Margarine (B), 500 g tub	Miracle	*	Polyunsaturated vegetable oil, hardened vegetable oil
Commercial pastry fat (A)	Morah Nuggets	Lot 40743L17	*
Commercial pastry fat (B)	Royal Danish	Lot 40063K28	*
Commercial pastry fat (C)	Besca Pastry Gems	*	*
Pastry, 400 g pack	Ernest Adam, "Fether Flake"	3412	Vegetable oil and butter
Homogenised milk 1 L carton	Tararua	*	Milkfat; fat: 3.3 g/100 g
Reduced fat milk 1 L carton	Tararua	*	Milkfat; fat: 1.5 g/100 g
Butter, 500 g pack	Anchor	ECE26	Milkfat
Shortening, 500 g tub	Chefade	*	Fractionated beef fat

Savoury mince pie (6 per pack)	Big Ben	012 007	Pastry: shortening, Filling: beef and mutton
Meat patties, 540 g pack	Watties Steak House	*	Margarine, butter
Luncheon, sliced 255 g pack	Kiwi	*	Mutton, beef and pork
Muesli bar "Snacker Chocolate Honey Crunch", 6 bars/200 g pack	BlueBird	*	Vegetable oil
Chocolate-coated biscuit, "Tim Tam" 200 g pack	Arnott's	450	Full cream milk, cocoa, butter, vegetable oil
Plain sweet biscuit, "Superwine" 500 g twin pack	Griffin's	4342N	Shortening
Savoury cracker biscuit, "Snax" 250 g pack	Griffin's	4351D	Vegetable shortening; fat: 22.8 g/100 g
Bread, white sandwich loaf, 700 g	Nature Fresh	*	Vegetable oil; fat: 1.9 g/100 g

Table 2 Foods selected for TFA survey (* no information available).

1.2 NEW ZEALAND SEASONAL SURVEY BUTTER SAMPLES

The New Zealand seasonal survey butter samples were obtained from the New Zealand Dairy Group, Te Awamutu site, as part of the New Zealand Dairy Research Institute seasonal survey. Because TFA levels in milkfat had been reported to vary across the dairy season (Gray, 1973), six samples across the 1995/1996 dairy season were selected to cover early season (August and September), mid season (November and January) and late season (April and May). The sample details are given in Table 3.

Sample Identification Code	Sample Date
9608006-1	26 August 1995
9608006-2	26 September 1995
9608006-4	20 November 1995
9608006-6	26 January 1996
9608006-9	30 April 1996
9608006-10	1 May 1996

Table 3 New Zealand seasonal survey butter samples

1.3 OVERSEAS BUTTER SAMPLES

A number of overseas butter samples (Table 4) were kindly provided by the Food Science Section, New Zealand Dairy Research Institute.

Brand Name	Country of Origin
Devondale	Australia
Gipsland Gold - Lactic	Australia
Kerry Gold	Ireland
Lady Lee	USA
Lurpak	Denmark
Snow	Japan

Table 4 Overseas butter samples.

1.4 PARTIALLY HYDROGENATED SOYA BEAN OIL

Samples of a partially hydrogenated soya bean oil together with the parent oil (unhydrogenated) were kindly provided by the Milkfat Products Section, New Zealand Dairy Research Institute.

2 BLIGH AND DYER FAT EXTRACTION AND DETERMINATION

2.1 INTRODUCTION

Fat was extracted from the New Zealand food survey samples (except for the margarine, shortening, butter and commercial pastry fat samples) using the method developed by Bligh & Dyer (1959). This method has been widely used to extract fat from complex sample matrices (*e.g.* fish) and has been used successfully to extract fat from a wide variety of processed foods in a comprehensive TFA study of Canadian foods (Ratnayake *et al.*, 1993). This method used a mixture of chloroform and methanol in such proportions that a miscible system was formed with the water in the sample matrix. Dilution with chloroform and water separated the homogenate into two layers, the chloroform layer containing all the lipids and the methanolic layer containing all the non lipids. A purified lipid extract was obtained simply by isolating the chloroform layer. The chloroform, methanol and water before and after dilution were kept in the proportions 1:2:0.8 and 2:2:1.8 respectively. These ratios represented the total volumes present in the ternary systems, including the water present in the sample, so the sample water content had to be determined before extraction. The fat contents for the margarine, shortening, butter and commercial pastry fat samples were determined using the Roese Gottlieb fat method (New Zealand Dairy Research Institute, 1993a).

2.2 MATERIALS AND METHODS

2.2.1 Chemicals

All chemicals used were of analytical grade (BDH Chemicals, Palmerston North, New Zealand) unless otherwise specified. Water was purified through a Milli-Q water purification system (Millipore Corporation, Bedford, Massachusetts, USA).

2.2.2 Glassware preparation

All glassware used in the fat extraction procedure was cleaned by an overnight soak in a pyroneg detergent (Diversey, New Zealand Ltd., Auckland) bath, rinsed three times with tap water and three times with purified water and then drip dried.

The round bottom flasks used for weighing fats were further rinsed with hexane and dried at 102°C for 1 h. The flasks were cooled to room temperature (1 h) before labelling and weighing to four decimal places (± 0.1 mg).

2.2.3 Sample preparation

All food fat samples (Table 2, except fats and liquids) were homogenised in a food processor before subsampling. Chocolate-containing foods were homogenised at 4°C in a cool room with a precooled food processor. The pastry sample was homogenized in a semi-frozen state to avoid "doughing". Foods such as biscuits, which were difficult to homogenise, were ground with a mortar and pestle before mixing in the food processor. All homogenised samples were stored frozen, sealed in aluminium-lined sachets. The samples were warmed to room temperature before opening.

2.2.4 Fat extraction

A homogenised sample (10-15 g) was accurately weighed into a 250 mL glass centrifuge bottle. The sample size was calculated to give 1-2 g of fat with no more that 16 mL of water. Where the fat content for a food product was unknown, an estimate was made based on equivalent foods. The moisture content was determined by moisture tests (New Zealand Dairy Research Institute, 1993b) or from the nutritional label. Where the extracted sample did not contain 16 mL of water, either water was added to the sample to bring the volume up to 16 mL or sufficient sample was used to bring the total water content up to 16 mL.

The sample was homogenised with the Polytron blender (Model MODST, Biolab Scientific, New Zealand) for 1 min after the addition of 40 mL of methanol and 20 mL of chloroform. A further 20 mL of chloroform and 20 mL of methanol were added to the homogenate and homogenised for an additional 30 s.

The blender cutter was rinsed with 10 mL of chloroform, which was collected in a beaker for later use. The homogenised sample was filtered through a sintered glass funnel using vacuum assistance. The funnel was washed using the 10 mL of chloroform rinsing collected above, and then with a further 20 mL of fresh

chloroform (*i.e.* a total of 30 mL of chloroform was used).

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 and transferred to the centrifuge bottle. Twenty millilitres of methanol followed by 56 mL of water were added to the filtrate, and the bottle was stoppered and shaken for 1 min.

The contents were centrifuged (IEC, Model UV, International Equipment Co., Massachusetts, USA) at approximately 600 g. for 10 min to promote phase separation. Alternatively, the sample was allowed to sit for a few hours to allow the two phases to separate. The top layer (water and methanol) was aspirated into a collection flask, avoiding transfer of any interfacial "fluff".

The bottom organic layer was washed three times with 50, 56 and 56 mL of water. After each wash, the contents were centrifuged or allowed to sit as above to separate the two phases.

The organic layer was transferred to a separation funnel, filtered through a sintered glass funnel containing approximately 3 g of anhydrous sodium sulphate and collected in a reweighed round bottom flask. The funnel and the sodium sulphate were rinsed with 10 mL of chloroform. The solvent was evaporated under vacuum using a rotory evaporator (Buchi, Switzerland) at approximately 40°C. The round bottom flask containing the fat was dried at 102°C for 1 h. After drying, the flask was transferred to a desiccator to cool (1 h), before reweighing. The net weight of the extracted fat was determined after subtraction of the solvent blank which was carried through the extraction procedure.

Margarine, butter, shortening and commercial pastry fat samples

A core sample (approximately 30 g) was removed using a coring instrument, melted at 60°C and centrifuged. The top fat layer was filtered in a 60°C oven through filter paper containing anhydrous sodium sulphate to remove residual moisture. The fat was collected for later use. The fat content of the original sample was, however, determined by the standard Roese Gottlieb method (New Zealand Dairy

Research Institute, 1993a).

2.3 EXPERIMENTAL

2.3.1 Method validation

The fat recovery from the Bligh and Dyer fat extraction procedure was determined using a known quantity of margarine oil quality control sample. The repeatability of the fat extraction method was estimated by repeated analysis of a selected food product (muesli bar) used in the TFA food survey.

An optimum time required to dry the extracted fat of residual solvents was determined. A known amount of margarine oil quality control (MARG-QC) sample (2 g) was weighed into a reweighed 250 mL round bottom flask and approximately 90 mL of chloroform was added to dissolve the fat. The solvent was evaporated off under reduced pressure using a rotary evaporator and the fat was dried at 102°C for periods of 0.5, 1, 1.5 and 2 h. After each oven drying, the fat was placed in a desiccator to cool (1 h) before weighing. The effect of the drying time and conditions on the level of C18:1 TFAs were also determined.

2.4 RESULTS

2.4.1 Validation results for the Bligh and Dyer fat extraction method

The fat extraction repeatability data for muesli bar are shown in Table 5. A mean fat figure of 19.90 ± 1.38 g/100 g was obtained.

Muesli Bar Subsample (g)	Fat Recovered (g fat/100 g of product)
9.70	21.84
10.03	18.08
10.22	20.47
10.11	19.53
10.47	19.58
Mean ± standard deviation (S_{n-1})	19.90 ± 1.38

Table 5 Repeatability standard deviation of the Bligh and Dyer fat extraction method determined using the muesli bar sample.

The recovery of margarine oil from the extraction solvent mixture was 101 ± 1.0% (Table 6).

Original Fat Weight (g)	Fat Recovery (g)	Recovery (%)
3.05	3.10	101.6
3.12	3.05	100.0
3.10	3.09	99.7
3.00	3.12	101.7
Mean ± standard deviation (S_{n-1})		100.8 ± 1.05%

Table 6 The recovery of MARG-QC sample from the extraction solvents used in the Bligh and Dyer extraction method.

The extracted fat was dried at 102°C. One hour was sufficient to evaporate residual solvent from the fat sample (Table 7). The drying time of 1 h was shown to have no effect on the C18:1 *trans* level (Table 8).

Weight of Fat (g)	Weight of Fat (g) After Drying for			
	30 min	60 min	90 min	120 min
2.0122	2.1503	2.0322	2.0325	2.0324
2.0191	2.0818	2.0248	2.0257	2.0255

Table 7 Determination of an optimum drying time (at 102°C) for solvent present in the MARG-QC sample extracted by the Bligh and Dyer method (the 1 h drying time was selected).

	C18:1 TFAs (g/100 g fat)	
	AMF-QC	MARG-QC
No drying	5.59 ± 0.20	12.81 ± 0.23
After drying	5.49 ± 0.16	12.83 ± 0.39

Table 8 The effect of drying fat samples (AMF-QC and MARG-QC samples) at 102°C on the level of C18:1 TFA. The standard deviations were calculated from three determinations. No significant difference at the 95% confidence level was observed with both the dried and undried sample data.

2.4.2 Fat content of New Zealand food survey samples

The mean fat contents of the New Zealand food survey samples are given in Table 9.

Sample	Fat Content (g/100 g product)
Margarine A	81.80
Margarine B	81.78
Commercial pastry fat A	85.14
Commercial pastry fat B	83.73
Commercial pastry fat C	81.69
Pastry	23.51
Homogenised milk	2.59
Reduced fat milk	1.38
Butter	82.73
Shortening	81.63
Meat pie	13.88
Meat patty	24.64
Luncheon	13.05
Muesli bar	19.90
Chocolate-coated biscuit	26.28
Plain sweet biscuit	15.17
Savoury cracker biscuit	23.75
White bread	1.36

Table 9 Mean fat contents of New Zealand food survey samples.

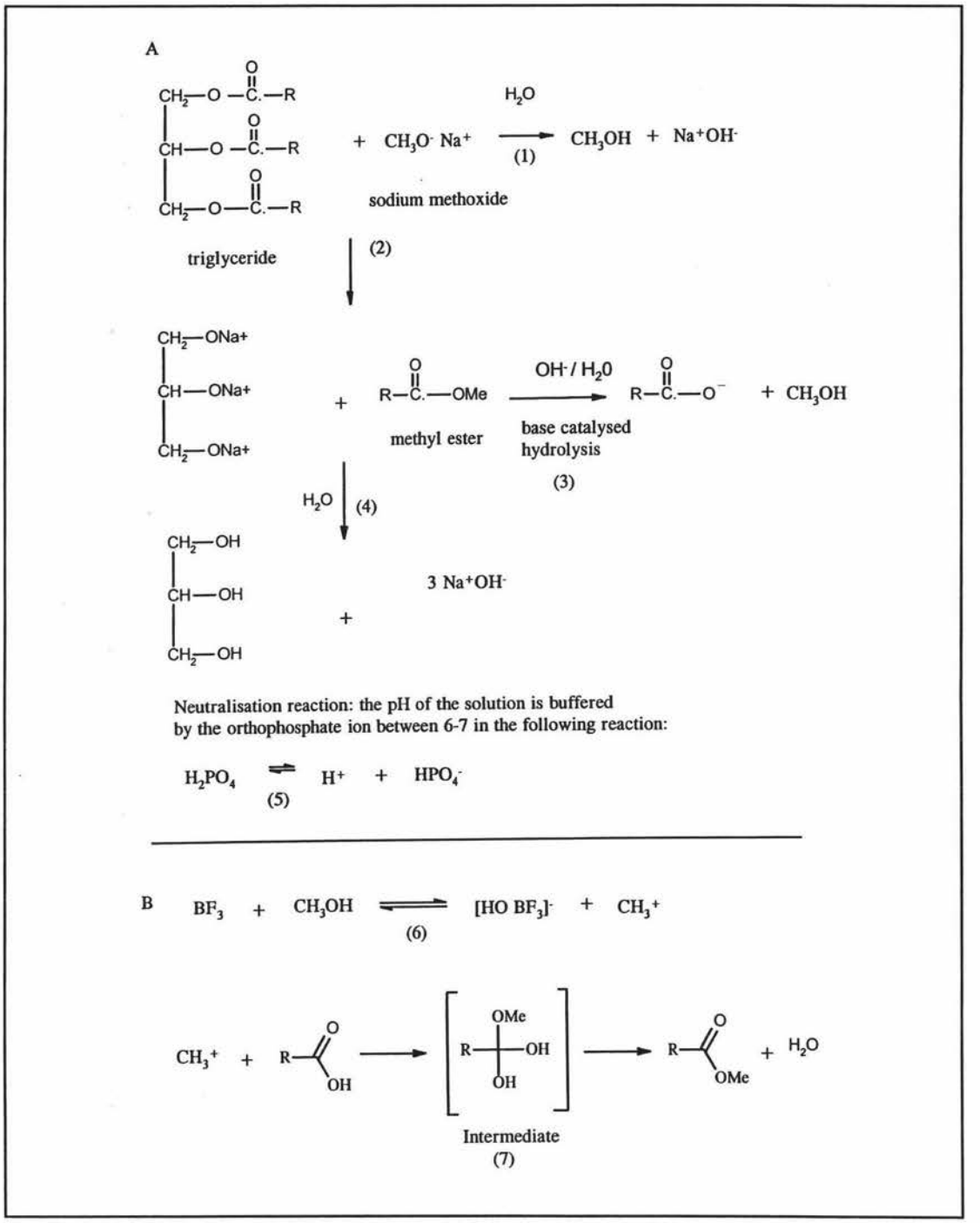

Figure 5 Preparation of FAMEs from triglycerides and free fatty acids. A. Methylation of triglyceride catalysed by sodium methoxide ($CH_3O^-Na^+$). The OH^- ion formed during the process (1 and 4) catalyses the base hydrolysis of the methyl esters (3) if not neutralised. The dihydrogen orthophosphate (5) added after the methylation reaction acts as a weak acid to neutralise the OH^- ions and buffers the solution to pH 6-7, preventing base hydrolysis of the methyl esters. B. The free fatty acids are methylated (7) by the strong electrophile (CH_3^+) generated in the BF_3 reaction (6).

3 FATTY ACID COMPOSITION DETERMINATION

3.1 INTRODUCTION

The fatty acid composition in food fats has generally been analysed by GC as ester derivatives (*e.g.* methyl, propyl or butyl esters). Esters are preferred to free acids, mainly because the acid may "associate" in pairs in the vapour phase, a long known phenomenon adversely affecting the distillation of free acids. Furthermore, acids require higher volatilisation temperatures than esters in GC analysis, and tend to absorb strongly on the stationary phase surface resulting in tailing and/or 'ghosting' peaks. Methyl esters are generally preferred over other esters mainly because of their lower boiling point, eluting from GC columns at a lower temperature than do other derivatives. In addition, their polarities are low and they can be separated by other techniques such as TLC (Christie, 1995). There are a host of simple methods available for methyl ester preparation. The chemistry of the sodium methoxide and boron trifluoride methylation procedures used in this work is described in Figure 5.

3.2 MATERIALS AND METHODS

3.2.1 Chemicals

All chemicals used were of analytical grade (BDH Chemicals, Palmerston North, New Zealand) unless otherwise specified.

3.2.2 FAME preparation

A combined methylation reaction using sodium methoxide and boron trifluoride was used in this work.

About 500 mg of fat was weighed into a 50 mL Kimax tube. Five millilitres of sodium methoxide/methanol reagent (prepared by dissolving clean metallic sodium in anhydrous methanol (1.15 g/100 mL) and mixing one volume of the methanolic solution with two volumes of dry diethyl ether and two volumes of isooctane) was added to the tube, which was then 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 was added. The contents were mixed thoroughly by vortexing and allowed to stand for 10 min. Hexane (20 mL) was added and mixed thoroughly. Neutralising solution, 5 mL (10% w/v anhydrous dipotassium hydrogen phosphate and 16% w/v sodium chloride in water) was added and the content mixed vigorously. The two phases were separated by centrifugation at approximately 600 *g* for 5 min. After centrifugation, the upper hexane layer was transferred into a 50 mL pear flask. The aqueous bottom layer was extracted with a further 5 mL of hexane. The hexane layer was added to the pear flask. An aliquot (2 mL) of the solution (approximately 20 mg FAME/mL) was reserved for TFA analysis using Ag-TLC and fatty acid composition analysis by GC. The remaining organic layer was rotary evaporated under vacuum at approximately 40°C to remove the hexane until 2-3 mL remained. The residual solvent was evaporated at 50°C on a heating block under a gentle stream of nitrogen until an oily liquid (FAMEs) remained (it was important not to prolong drying for more than 5 min after the solvent had been visibly removed, to minimise the loss of volatile esters). The FAMEs made were transferred with a pasteur pipette to a 4 mL vial, nitrogen flushed capped and stored at approximately -18°C until required. Before use, the FAMEs were reconstituted to 4-5 mg/mL in hexane. One microlitre of the FAME solution was injected into the gas chromatograph for FAME composition analysis.

3.2.3 FAME composition analysis by GC

The FAME compositions were analysed on a 15 m x 0.53 mm FFAP capillary column (Alltech Associates, New Zealand) using a Hewlett Packard HP5890 gas chromatograph (Hewlett Packard, Palo Alto, California, USA). The FAMEs were separated using a programmed temperature gradient, with an initial temperature of 50°C held for 1.5 min, before increasing to 150°C at a rate of 15°C/min. The final temperature increase to 220°C was conducted at 6°C/min, and held at that temperature for 3 min. The injector port temperature was held at 50°C during the injection of the FAME sample (1 L), while the detector temperature was held at 250°C.

The carrier gas (H_2) flowrate was set at 10 mL/min, whereas the FID gas flowrates were: H_2 at 30 ± 1.5 mL/min, air at 250 ± 5 mL/min and make-up gas (N_2) at 20 ± 5 mL/min.

3.2.4 Calculation of FAME composition

The weight percentage (g/100 g fat) of each fatty acid was calculated using the formula given in Equation 1.

Equation 1

$$\text{wt \% of fatty acid}_i = \frac{\text{peak area}_i \times \text{relative correction factor}_i}{\sum (\text{peak area}_i \times \text{relative correction factor}_i)}$$

The correction factors are given in Table 10. Calculations were performed by the electronic data processing unit.

Fatty Acid	Relative Correction Factor	Fatty Acid	Relative Correction Factor
4:0	1.374	16:0	1.000
5:0	1.208	16:1	0.992
8:0	1.125	17:0	0.993
10:0	1.075	17:1	0.985
10:1	1.063	18:0	0.986
12:0	1.042	18:1	0.979
14:0	1.018	18:2	0.972
14:1	1.009	18:3	0.965
15:0	1.008		

Table 10 Correction factors for fatty acid weight %, relative to C16:0. The derivation of the correction factors is given in Appendix 1.

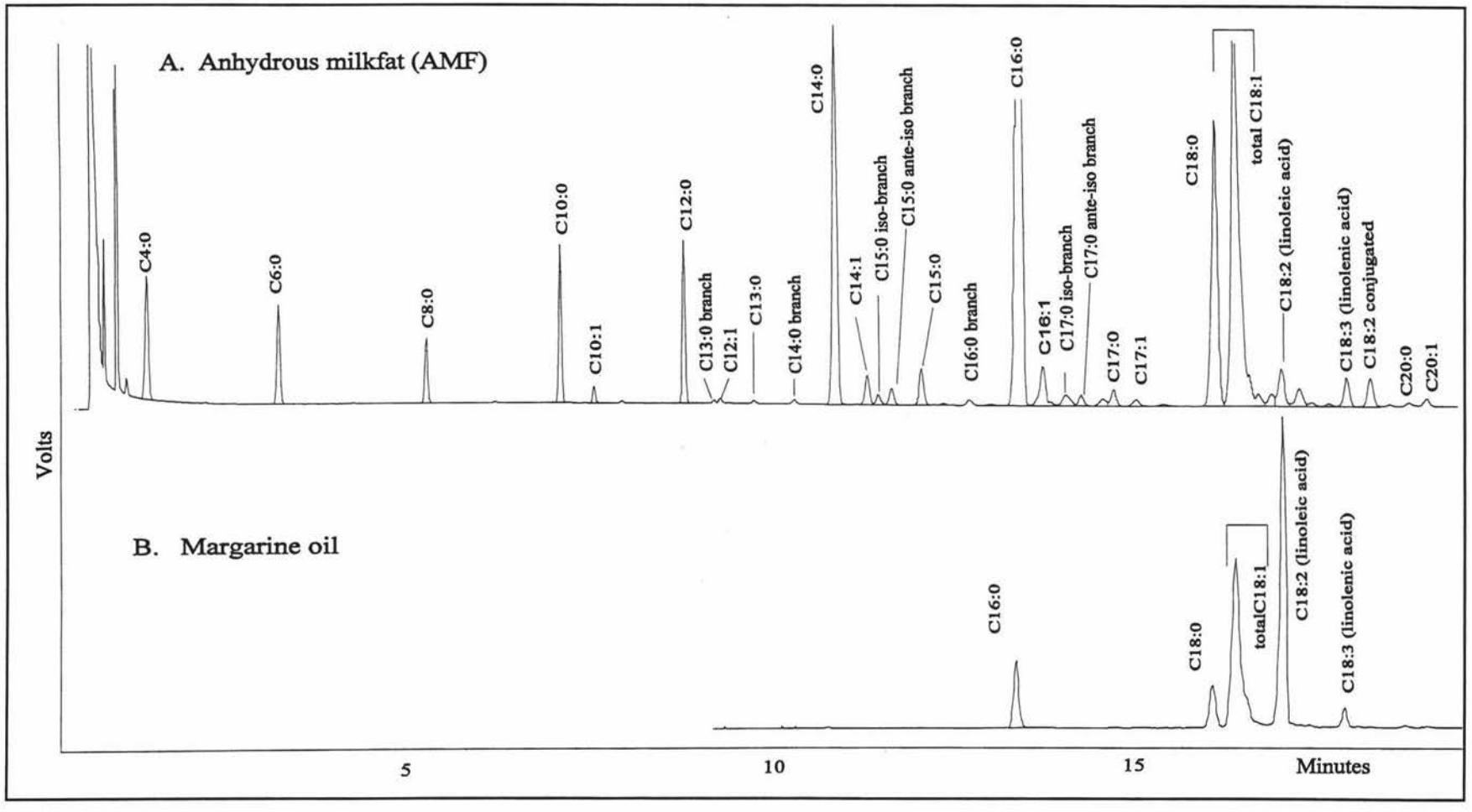

Figure 6 The FAME composition of AMF-QC (A) and MARG-QC (margarine oil) (B) samples. The analysis was carried out on an FFAP (15 m) megabore GC column using a temperature gradient (see text).

3.3 RESULTS

The fatty acid compositions of the New Zealand food survey fats, the hydrogenated soya bean oil, the New Zealand seasonal butters and the overseas butter samples are given in Tables 11, 12, 13 and 14 respectively. The fatty acid composition data from the NZ food survey samples were used to obtain a match with fatty acid profiles of known fats and oils, using a statistical computer program (MacGibbon & van der Does, 1993), to provide an indication of the fat source for each food product. Typical GC traces of the FAME composition for AMF-QC and MARG-QC are given in Figure 6.

3.4 DISCUSSION

The various fat products displayed distinctive fatty acid composition profiles that were reflective of their fat sources. Generally, milkfats consist of a range of fatty acids from short chains (C4:0) through to long chains (C20:0), which are mostly saturated. Animal depot fats, unlike the milkfats, contain only very little or no short chain fatty acids. They also contain larger amounts of monounsaturated C18:1 fatty acids. Similarly, seed oils such as sunflower, soya and palm oils do not contain short chain fatty acids. Their predominant fatty acids are unsaturated (C18:1, C18:2 and C18:3).

Seed oils are generally hardened for margarine and shortening manufacture by chemical hydrogenation. This process effectively reduces the amount of polyunsaturated fatty acids present. This is clearly evident in the hydrogenated soya bean oil sample (Table 12) used in this study, where most of the C18:2 FAMEs were hydrogenated to C18:1 FAMEs of which approximately 50% were in the *trans* configuration, a major contributor to hardness in hydrogenated products such as margarines and shortenings. Coconut oil, on the other hand, is fairly saturated and contains a large amount of C12:0 (48%; White, 1992), making products containing this oil easy to identify by their FAME compositions. Using the distinctive fatty acid profile and composition of the different fats and oils, the fat sources of the New Zealand food survey samples were predicted with reasonable accuracy (Table 11), using mathematical algorithms (MacGibbon & van der Does, 1993).

Sample	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C14:1	C15:0	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1 [1]	C18:2 [2]	C18:3 [3]	Other [4]	Fat Source [5]
Margarine (A)						0.25			12.65	0.37			4.84	33.58	44.03	1.22	3.06	Vegetable (soya)
Margarine (B)									8.71				6.30	38.10	43.46	2.46	0.97	Vegetable (sunflower)
Commercial pastry fat (A)				0.05	0.10	2.88	0.49	0.54	27.15	2.82	1.26	0.65	21.05	36.56	2.87	0.31	3.27	Animal (tallow)
Commercial pastry fat (B)					0.27	1.00			38.69	0.71			7.50	37.90	13.23	0.14	0.56	Animal (lard)
Commercial pastry fat (C)				0.05	0.13	2.53	0.26	0.42	33.90	1.82	0.84	0.36	20.76	31.99	3.03	0.56	3.35	Vegetable (soya/palm)
Pastry "Fether Flake"	0.51	0.38	0.25	0.61	0.75	4.09	0.50	0.58	25.98	2.46	1.07	0.53	19.01	32.20	6.60	0.98	3.50	Butter/tallow
Homogenised milk	3.60	2.27	1.30	2.75	3.04	10.88	1.00	1.30	29.16	1.97	0.57	0.37	11.37	22.20	1.47	0.87	5.88	Milkfat
Reduced fat milk	3.73	2.32	1.33	2.77	3.14	10.88	0.98	1.27	28.95	2.02	0.68	0.38	11.47	21.67	1.50	0.80	6.11	Milkfat
Butter	3.84	2.57	1.60	3.70	4.24	11.84	0.89	1.21	27.36	2.20	0.52	0.33	11.18	21.24	1.09	0.73	5.46	Milkfat
Shortening				0.08	0.15	3.03	0.62	0.56	23.61	3.64	1.31	0.87	16.91	41.44	3.08	0.52	4.18	Animal (tallow)
Savoury mince pie			0.13	0.12	0.68	2.56	0.26	0.40	35.60	2.18	0.83	0.43	15.48	33.00	4.75	0.51	3.07	Animal (tallow) + vegetable
Meat patties				0.08	0.08	3.08	1.03	0.47	23.77	4.47	0.97	0.94	14.81	42.76	1.76	0.75	5.03	Animal (tallow)
Luncheon				0.10	0.11	2.52	0.19	0.52	22.40	2.88	1.23	0.65	18.70	40.34	4.96	0.99	4.41	Animal (lard)
Muesli bar		0.50	5.02	4.33	44.07	17.52	0.13		11.35	0.14			9.58	4.85	2.19	0.19	0.27	Vegetable (coconut/palm)
Chocolate- coated biscuit	0.34	0.26	0.17	0.33	0.73	1.78	0.11	0.15	34.49	0.86	0.22		19.18	34.35	6.35	0.33	0.35	Milkfat/vegetable (cocoa butter/palm)
Plain sweet biscuit				0.06	0.16	3.02	0.53	0.50	23.88	3.48	1.50	0.83	18.79	38.45	5.30	0.47	3.03	Animal (tallow)
Savoury cracker biscuit		0.51	5.83	4.40	31.34	11.94			19.95	0.29			8.89	12.01	4.41	0.18	0.25	Vegetable (coconut/palm)
White bread					0.18	0.21			15.94	0.46			4.42	28.06	43.27	4.50	2.96	Vegetable (soya)

[1] Total *cis* and *trans* isomers.
[2] *cis-cis* methylene interrupted (linoleic acid).
[3] all-*cis* methylene interrupted (linolenic acid).
[4] Minor fatty acids including branched chain saturated; non all-*cis* polyunsaturated; minor saturated and monounsaturated.
[5] Inferred from fatty acid composition (MacGibbon & van der Does, 1993).

Table 11 Fatty acid composition (g/100 g fat) of extracted New Zealand food survey fats

SAMPLE	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C14:1	C15:0	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1 [1]	C18:2 [2]	C18:3 [3]	C18:2 Conj	Other[4]
Hydrogenated soya bean oil	-	-	-	-	-	-	-	-	11.6	-	-	-	5.2	73.1	3.7	-	-	6.4
Soya bean oil	-	-	-	-	-	0.1	-	-	11.0	0.1	-	-	4.0	23.4	53.2	7.8	-	0.4

Table 12 Fatty acid composition (g/100 g sample) of the hydrogenated and unhydrogenated soya bean oil samples.

Sample	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C14:1	C15:0	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1 [1]	C18:2[2]	C18:3[3]	C18:2 conj	Other[4]
Aug 95	4.11	2.49	1.49	3.19	3.50	10.10	0.54	0.99	25.19	1.46	0.61	0.34	12.94	25.43	1.32	0.95	0.94	3.90
Sep 95	3.91	2.48	1.59	3.55	3.93	11.00	0.69	1.05	25.36	1.44	0.51	0.29	12.29	23.75	1.30	0.89	1.18	4.14
Nov 95	3.70	2.39	1.47	3.33	3.83	11.83	0.88	1.22	29.30	1.48	0.56	0.28	11.70	20.22	1.13	0.87	0.94	4.21
Jan 96	3.81	2.36	1.37	2.96	3.33	11.17	0.89	1.20	31.03	1.56	0.55	0.26	11.27	20.49	1.28	0.86	1.00	5.01
Apr 96	3.46	2.11	1.28	2.68	3.19	10.85	1.13	1.32	29.68	1.96	0.50	0.30	9.94	23.17	1.24	0.85	1.30	4.36
May 96	3.43	2.10	1.26	2.73	3.22	10.91	1.14	1.33	30.02	1.97	0.50	0.30	9.84	22.87	1.27	0.81	1.27	4.36

[1] Total cis and *trans* isomers.
[2] *cis-cis* methylene interrupted (linoleic acid).
[3] all-*cis* methylene interrupted (linolenic acid).
[4] Minor fatty acids including branched chain saturated; non all-*cis* polyunsaturated; minor saturated and monounsaturated.

Table 13 New Zealand seasonal survey butter sample fatty acid compositions (g/100 g fat)

Sample	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C14:1	C15:0	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1 [1]	C18:2[2]	C18:3[3]	C18:2[4] Conj	Other[5]
New Zealand	3.41 4.11	2.10 2.49	1.26 2.49	2.73 3.55	3.19 3.93	10.10 11.83	0.54 1.14	0.99 1.33	25.19 31.03	1.44 1.97	0.50 0.61	0.28 0.34	9.84 12.94	20.22 25.43	1.24 1.32	0.81 0.95	0.94 1.30	3.90 5.01
Devondale (Australia)	3.59	2.26	1.36	2.89	3.35	10.83	1.04	1.17	27.88	1.54	0.54	0.30	11.55	23.62	1.37	0.94	1.05	4.03
GG-Lactic (Australia)	3.59	2.04	1.13	2.32	2.66	9.72	0.92	1.18	26.34	1.76	0.63	0.37	12.80	26.65	1.32	0.81	1.11	4.11
Snow (Japan)	3.63	2.27	1.36	2.96	3.44	11.46	1.00	1.19	30.47	1.62	0.61	0.34	10.65	22.04	1.79	0.47	0.54	3.56
Lady Lee (USA)	3.61	2.03	1.14	2.44	2.69	9.45	0.64	0.95	28.07	1.32	0.59	0.23	13.86	25.85	2.92	0.42	0.38	2.94
Kerry Gold (Ireland)	3.81	2.16	1.28	2.68	3.01	9.58	0.68	1.12	23.41	1.49	0.65	0.41	12.96	28.21	1.59	0.83	1.41	4.21
Lupack (Denmark)	3.76	2.21	1.29	2.76	3.57	10.63	0.85	0.85	29.74	1.67	0.48	0.24	11.23	24.54	1.97	0.34	0.49	2.84

[1] Total *cis* and *trans* isomers.
[2] *cis-cis* methylene interrupted (linoleic acid).
[3] all-*cis* methylene interrupted (linolenic acid).
[4] Conjugated linoleic acid.
[5] Minor fatty acids including branched chain saturated; non all-*cis* polyunsaturated; minor saturated and monounsaturated.

Table 14 New Zealand and overseas butter sample fatty acid compositions (g/100 g fat).

4 ARGENTATION THIN LAYER CHROMATOGRAPHY

4.1 INTRODUCTION

A major problem associated with AOCS (1993b) direct gas chromatographic analysis of C18:1 *cis/trans* fatty acids is the incomplete separation of the various *cis/trans* isomers. This leads to an underestimation of TFAs in test samples. The recognition of the limitation of the direct GC method meant that a drive to develop a more accurate C18:1 *trans* methodology was important. Ag-TLC combined with GC was the result of this, and proved to be a much more accurate and reliable method for C18:1 *trans* quantification (Lund & Jensen, 1982; Ratnayake *et al.*, 1990; Ratnayake, 1992) even though many workers had reported it to be tedious, time consuming and not robust enough to be adapted for routine use. The greater accuracy of this method was due to the prior separation of the *cis* monoenes from the *trans* monoenes using Ag-TLC, eliminating the problem of incomplete *cis/trans* resolution by the direct GC method. The underestimation of TFAs by up to 32% for some margarine samples was reported by Ratnayake (1992).

The *cis/trans* separation by Ag-TLC involves interactions of the double bonds in the alkyl chain of the FAMEs with the silver impregnated on the silica TLC plate. These interactions are of a charge-transfer type, *i.e.* the unsaturated compound acts as an electron donor and the silver ion acts as an electron acceptor (Figure 7). Two types of interactions are suggested, one involving a δ-bond, formed by an overlap of the filled π-orbital of the olefin with the free *s*-orbital of silver, and the other involving a π-bond, formed by an overlap of the vacant antibonding π-orbitals of the olefin with the filled *d*-orbitals of silver (Figure 7) (Scholfield, 1979; Strocchi & Piretti, 1968). These interactions with the silver ions are reversible.

As a result, Ag-TLC depends on three characteristics of the compound to be separated: (1) number of double bonds, (2) their geometric configuration, with *cis* forming stronger complexes with the silver ion than the corresponding *trans* and (3) their position along the alkyl chain (Nikolova-Damyanova, 1992). The unsaturated compounds move slowly on the plate, making separation on the number of double bonds readily achievable.

Gunstone *et al.* (1967) suggested that, apart from the interaction between the silver ions and olefinic groups, interactions between the silica and polar carbomethoxy groups were also significant, *i.e.* the interaction was greater for positional isomers that could adopt a conformation such that the distance between the ester group and the double bond was close to the distance between the silver ion and the silica molecules. For example, C18:1 Δ16t will interact more strongly than C18:1 Δ4t on the Ag-TLC plate, and will therefore migrate more slowly. The principles involved with silver ion chromatography are still being investigated and have been reviewed extensively by Guha & Janak (1972) and Nikolova-Damyanova (1992).

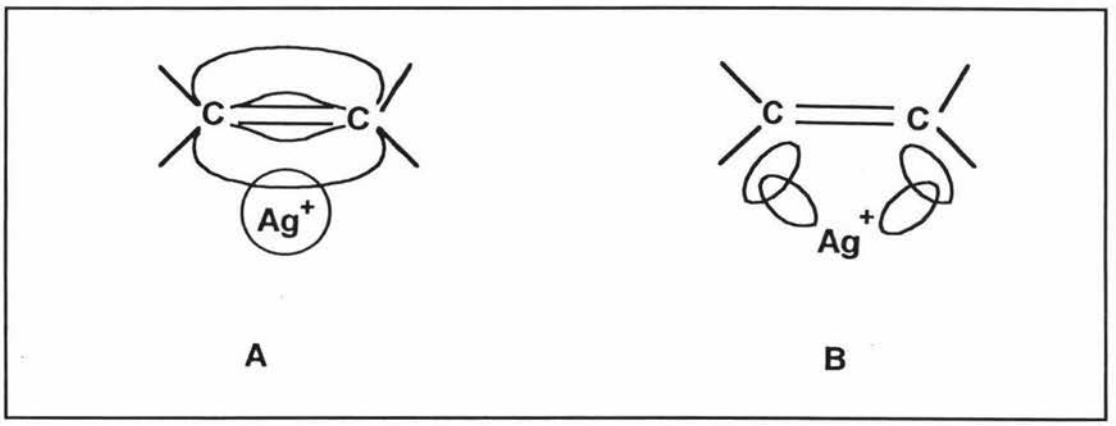

Figure 7 Proposed interaction of the silver ion with the *trans* double bond in fatty acids in Ag-TLC (Scholfield, 1979). A. δ-bond, formed by an overlap of the filled π-orbital of the olefin with the free *s*-orbital of silver. B π-bond, formed by an overlap of the vacant antibonding π-orbitals of the olefin with the filled *d*-orbitals of silver.

The general elution order of the different fatty acid groups on Ag-TLC, with least retained first, is the saturated fatty acids, followed by the *trans* monoenes, *cis* monoenes, dienes and trienes (Gunstone *et al.,* 1967; Christie, 1989b). The elution order remains the same, despite variations in the solvent composition of the mobile phase and the developing temperature. However, better resolution of the methyl esters was reported if the plates were developed at low temperatures (-5 to -25°C), as complex formation was stronger at lower temperatures, or using aromatic solvents such as toluene instead of hexane/diethyl ether (Morris *et al.*, 1967; Christie, 1989b). Using this technique, the elution of the different FAME groups (saturated, *trans* monoenes, *cis* monoenes, dienes and trienes) using various mobile phase compositions and temperatures was determined in this study.

4.2 MATERIALS AND METHODS

4.2.1 Chemicals

All chemicals were of analytical grade (BDH Chemicals, Palmerston North, New Zealand) unless otherwise specified.

4.2.2 Ag-TLC plate preparation

The Ag-TLC plates were prepared using silica gel 60 Å plates (Art. 11845, Merck, Darmstadt, Germany) impregnated with silver by immersion into a 10% w/v methanoic silver nitrate solution (15 g of $AgNO_3$ dissolved in 30 mL of distilled water, made to 150 mL with methanol). The plates were immersed for 5 min before drying in a desiccator overnight. All prepared plates were stored in the dark. On exposure to light, silver-impregnated plates darken rapidly. Whenever possible, the plates were handled under subdued incandescent flood lights (120-150 W, PAR 38).

The FAME samples (4-5 mg/mL prepared in hexane as described in Section 3.2.2) were applied in a 3 cm band on the TLC plate using a TLC applicator (A530, De Saga, Germany). The plates were developed in various mobile phase compositions until the solvent front was 1-2 cm from the top edge of the plate. The FAME bands on the plate were visualised under UV light after drying off the solvent and spraying with 2',7'-dichlorofluorescein in 95% ethanol (0.2% w/v). The FAMEs appeared as fluorescent yellow bands on a green-red background (Figure 9).

The required FAME bands were removed from the plate using a TLC plate scraper and the silica was collected in a 16 mL Kimax screw-top tube (10 mm id x 120 mm, Kimble Glass Inc., Vineland, Illinois, USA). The FAMEs were extracted twice with diethyl ether (5 mL) and each rinsing was transferred to a second 16 mL Kimax screw-top tube. The diethyl ether was evaporated off at 30°C under a dry stream of nitrogen.

The residue was redissolved in 0.1-0.2 mL of hexane. All reconstituted FAMEs were stored at 4°C until required for analysis.

4.3 EXPERIMENTAL

Various mobile phase compositions and developing temperature conditions were tested to obtain an optimised resolution of the main groups of FAMEs. These conditions are given in Table 15. For low temperature developing conditions, the TLC developing tank and mobile phase were equilibrated to that temperature before the plates were loaded.

Figure 8a & 8b	Toluene	Diethyl ether	Temperature
A	90%	10%	ambient
B	90%	10%	-15°C
C	100%	-	ambient
D	100%	-	-15°C
E	95%	5%	ambient

Table 15 Developing conditions tested for FAME separation on the Ag-TLC plate.

4.4 RESULTS

The FAME bands and their relative rates of migration (retention factor) are given in Figures 8a & 8b. An example of an Ag-TLC plate viewed under UV light is given in Figure 9.

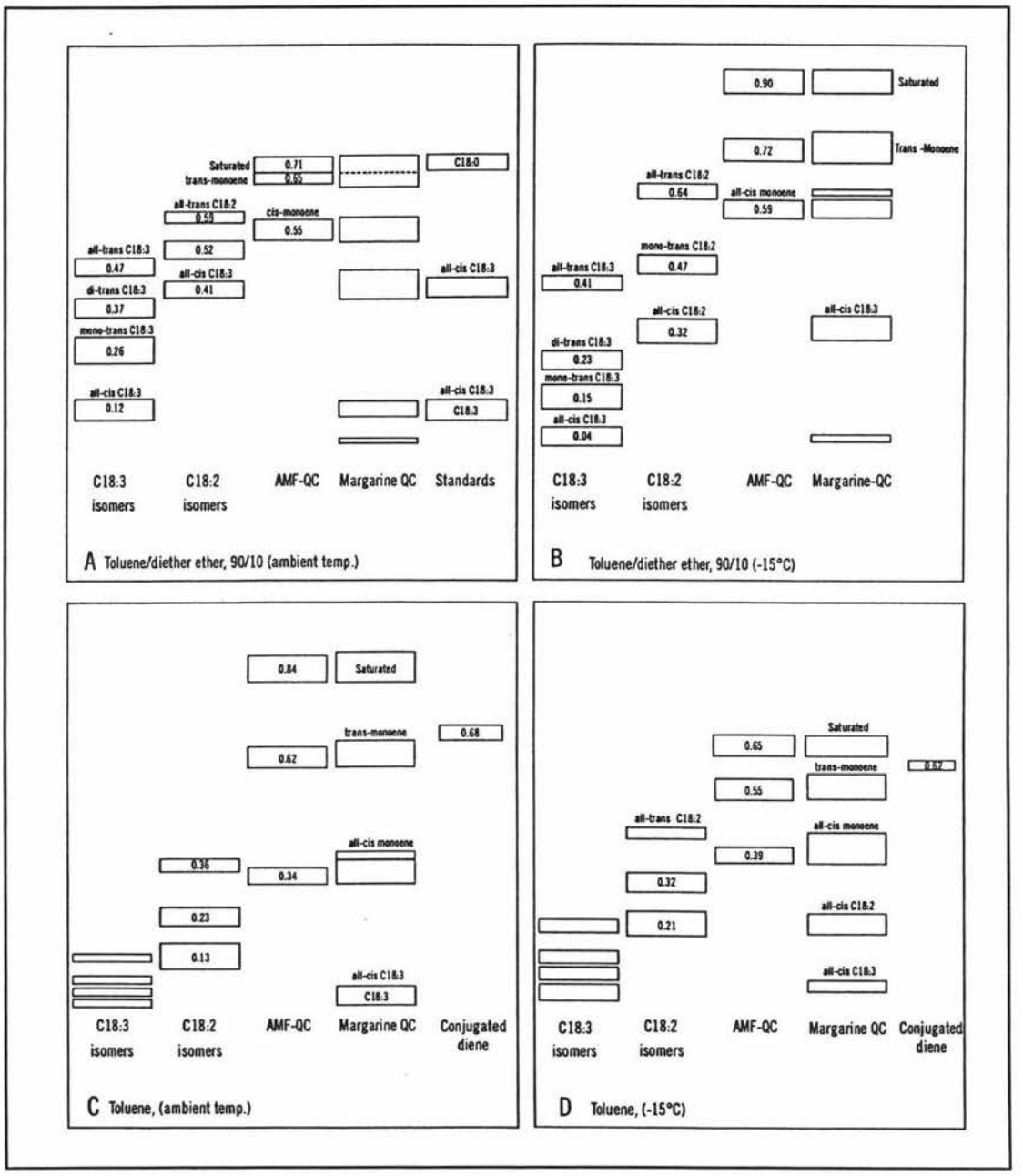

Figure 8a Ag-TLC separation of FAME groups under different mobile phase compositions and temperatures. The figures given on the bands in diagrams A-D of the Ag-TLC plates refer to the retention factor of each FAME band.

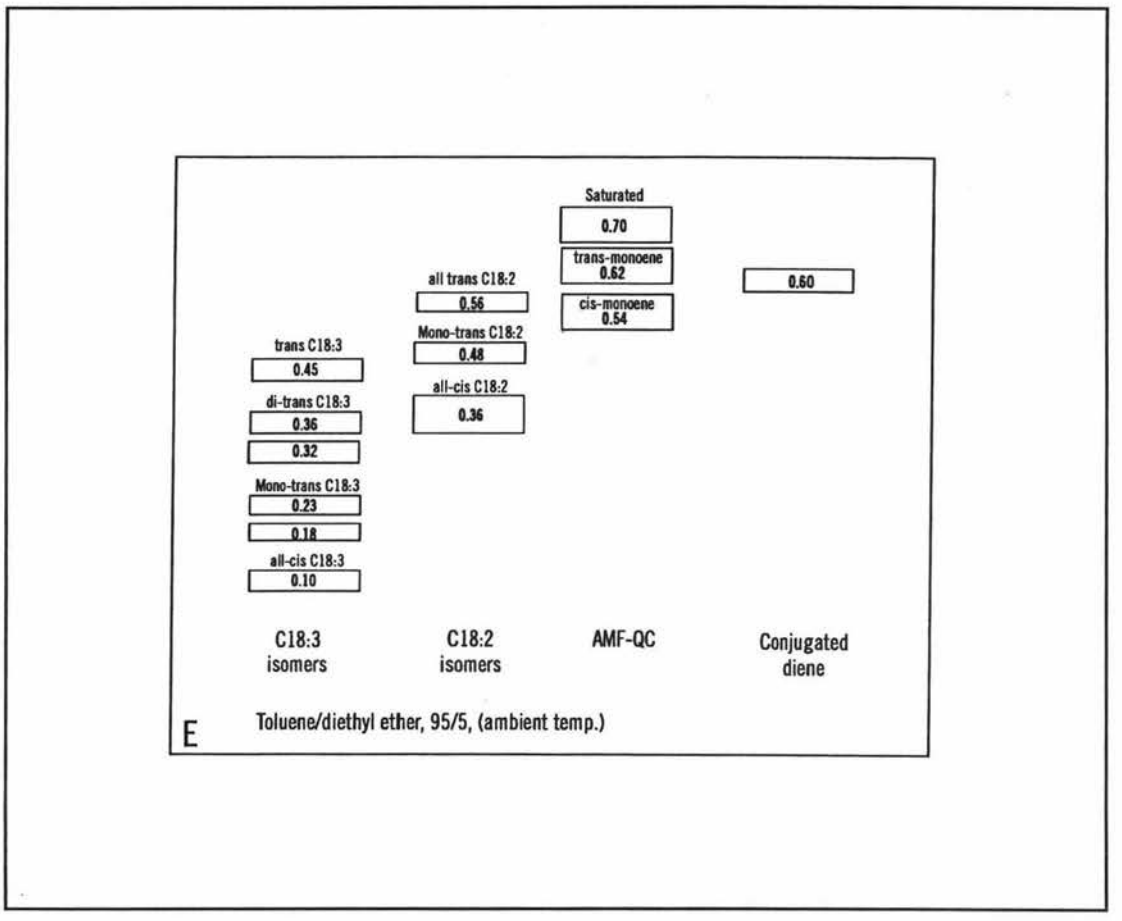

Figure 8b Ag-TLC separation of FAME goups using toluene/diethyl ether (95/5 v/v) at ambient temperature. The figures given on the bands in the diagram refer to the retention factor of each FAME band.

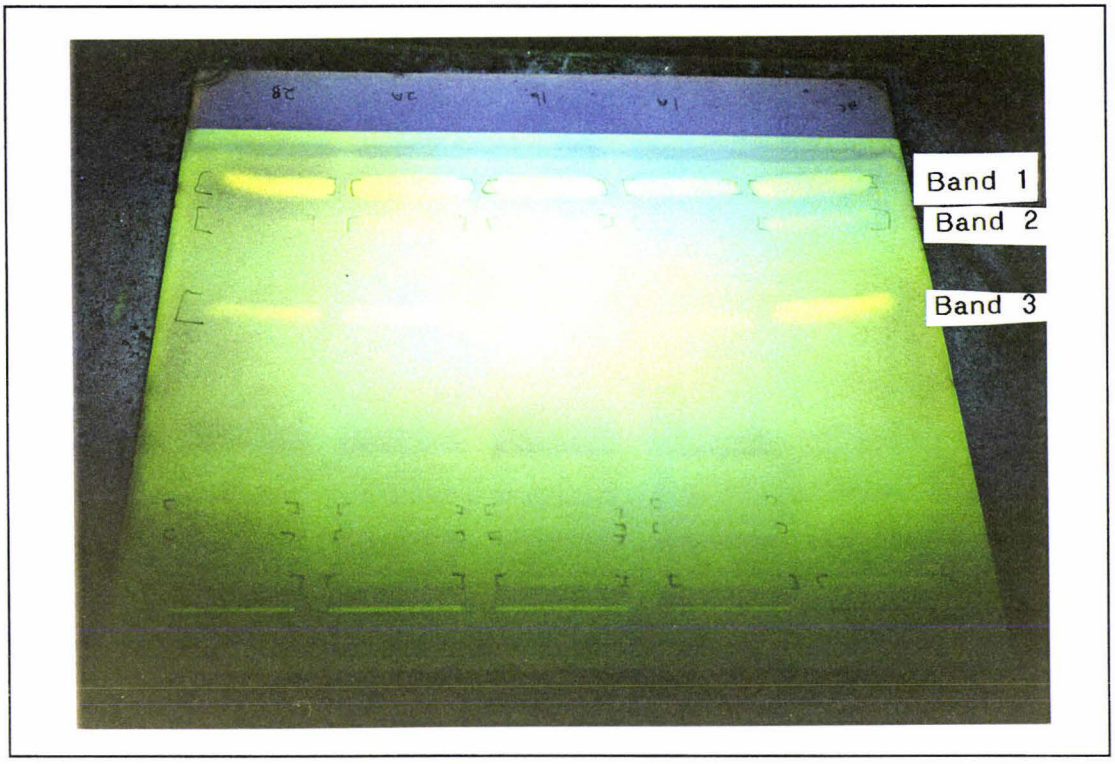

Figure 9 Stained Ag-TLC plate under UV light. Band 1: saturated FAMEs; Band 2: *trans* monoene band; Band 3: *cis* monoene band. The developing solvent was toluene at ambient temperature.

4.5 DISCUSSION

The separation of the *trans* monoene from the *cis* monoene FAMEs was achieved with all the experimented conditions tested (Table 15). However, the condition using toluene at ambient temperature was chosen because of its simplicity and convenience. These conditions were later used for the Ag-TLC/GC method for C18:1 TFA detemination.

The C18:3 and C18:2 geometric isomers were not fully resolved from each other with either the toluene or the toluene/diethyl ether mobile phase either at ambient temperature or at -15°C. However, within the individual groups, separation into the various classes was achieved. These results were similar to those reported by Strocchi & Piretti (1968) and Rakoff & Emken (1982), which they had identified to correspond to the different C18:2 and C18:3 isomer classes. The C18:2 and C18:3 classes were given as all-*cis;* mono-*cis,* mono-*trans;* all-*trans,* and all-*cis;* mono-*trans;* di-*trans;* and all-*trans* respectively. No improvement to the separation of the C18:2 geometric isomer was observed when the toluene/diethyl ether (95/5) mixture was used, but further separation was observed for the C18:3 isomers (Figure 8b). The identification of these different isomers present in the different Ag-TLC bands is discussed in later sections.

Good resolution between the C18:2 all-*trans* and *cis* monoene bands was not achieved under any of the conditions tested (Figures 8a and 8b). The C18:2 all-*trans* band migrates just ahead of the *cis* monoene band; hence extracting the C18:2 di-*trans* band could result in some cross-contamination with the *cis* monoenes.

Some workers have resorted to the use of Ag-HPLC to separate FAME groups not resolved by Ag-TLC. The increased resolving power of this techique lies with the use of a mobile phase gradient for fatty acid elution. Until recently, Ag-HPLC columns were silica-silver nitrate loaded, which had a relatively short life, due to the leaching of silver ions from the column by the polar mobile phases (Scholfield, 1979). The later use of silver ions bonded to a cation-exchange resin as HPLC columns proved to be more successful (Christie, 1987, Christie *et al.,* 1988; Ulberth & Achs, 1990). Detection was achieved using a mass detector (light

scattering). A major part of the eluent was diverted by a stream splitter at the end of the column for manual collection. No cross-contamination of fractions or contaminating silver ions was reported. Fractions were subjected to capillary GC analysis after solvent evaporation. Ag-HPLC was not used in this study, but would certainly be recommended for further studies in this area.

5 TOTAL *trans* UNSATURATED FATTY ACID DETERMINATION IN NEW ZEALAND FODD SURVEY SAMPLES BY FOURIER TRANSFORM INFRARED SPECTROPHOTOMETRY

5.1 INTRODUCTION

IR has been a traditional means of quantifying the total *trans* unsaturation in fat and oil samples. This technique takes advantage of the fact that the *trans* double bond gives rise to a characteristic absorption band at 965 cm^{-1} (10.3 μm). In order to ensure an acceptable precision of this method, it was standardised by International Union of Pure and Applied Chemistry (1987), the AOCS (1993a) and the AOAC (1995b). However, despite this, the principal problem of an accurate quantification of low TFA concentration (<15%) was not completely resolved. The recommendation of the AOCS method to improve the accuracy of the results was to use FAMEs for low *trans* samples (<15%) and triglycerides for high *trans* samples (≥15%). However, both the FAME and the triglyceride have been reported to produce bias (discussed earlier). Because of these problems associated with *trans* quantification by IR, attempts were made to improve its accuracy in this study using a spectral subtraction technique, where a non-*trans* background spectrum was subtracted from both the sample and standard spectra to obtain a difference spectrum. In addition, the calibration component was also made up in the non-*trans* background to mimic the fat sample matrix as closely as possible . The New Zealand food fat samples were analysed using these improved methods.

5.2 EXPERIMENTAL

5.2.1 AOCS Cd 14-61 vs spectral subtraction method

The total *trans* isomers in the food fat samples were measured by their infrared absorbance according to the standard AOCS method Cd 14-61 (AOCS, 1993a). The calibration standards were made up in a non-*trans* background consisting of a mixture of olein and stearin (to mimic the fat sample as closely as possible). Both the triglyceride and FAME samples were used. The results obtained by both methods (*i.e.* for the triglycerdes and FAMEs) were compared. Furthermore, a difference spectrum was generated by subtraction of the non-*trans* background

spectrum from each sample spectrum using the computerised FTIR instrument. Again both the triglyceride and FAME samples were used.

5.2.2 Method validation

The repeatabilities of the infrared total *trans* analysis methods were determined using the AMF-QC and MARG-QC samples. The recoveries after spiking both the QC samples with either trielaidin (for triglyceride analysis) or methyl elaidin (for methyl ester analysis), at approximately 50% and 100% of the native total *trans* isomer levels, were also determined.

5.3 MATERIALS AND METHODS

5.3.1 Chemicals

All chemicals were of analytical grade (BDH Chemicals, Palmerston North, New Zealand) unless otherwise specified.

5.3.2 Calibration standard solution (trielaidin and methyl elaidin) preparation

The FTIR signal was calibrated with a multi point calibration curve consisting of either trielaidin (for triglycerides) or methyl elaidin (for methyl esters). The total triglyceride or methyl ester weight in the standard solution was kept constant to 200 ± 5 mg by using the non-*trans* component mixture consisting of either triolein and tristearin (for triglycerides) or methyl olein and methyl stearin (for methyl esters).

Standards (> 99% purity, Nu Chek Prep, Minnesota, USA) were weighed to 0.1 mg accuracy as indicated in Table 16. Each component was weighed into a separate 5 mL beaker, except for trielaidin and methyl elaidin which were weighed directly into 10 mL volumetric flasks.

The non-*trans* components (tristearin or methyl stearin, triolein or methyl olein) were dissolved in carbon disulphide (CS_2) (spectrosol grade, BDH Chemicals) and transferred quantitatively into their respective 10 mL volumetric flasks. All

solutions were made up to volume with CS_2 at 20°C in a water bath and stoppered firmly.

All solutions were stored at 4-6°C and warmed to room temperature before use.

Component	Amount (mg/10 ml CS_2)					
Trielaidin or methyl elaidin	0	4	10	20	30	50
Tristearin or methyl stearin	100	98	95	90	85	75
Triolein or methyl olein	100	98	95	90	85	75
Total triglyceride or methyl ester	200	200	200	200	200	200

Table 16 Concentration of the calibration standard components

5.3.3 Sample preparation

A sample (200 ± 1 mg) of the fat (trigylceride) or FAMEs (prepared as described Section 3.2.2) was weighed into a 10 mL volumetric calibrated flask and dissolved in CS_2.

All flasks were made to volume with CS_2 in a 20°C water bath. Flasks were stoppered with a plastic stopper, mixed well and stored at 4-6°C. Solutions were warmed to room temperature before use.

5.3.4 FTIR analysis

The FTIR NaCl flow-through cell (1 mm pathlength, Perkin Elmer, Beaconfield, England) was rinsed three times with CS_2 between each standard and sample measurement and dried by sucking air through the cell with a 5 mL Luer Lok syringe (Alltech Associates, Inc., Deerfield, Illinois, USA).

The cell was filled via the Luer Lok syringe attached at the inlet port. Sample solutions were delivered into the syringe via a pasteur pipette. The plunger was slowly replaced to push the sample solution into the cell. The cell was placed at an angle to the vertical to allow air bubbles to move to the outlet at the top of the cell. The outlet port was stoppered once the solution was visible. Any residual CS_2 was wiped off before transferring the cell to the FTIR instrument (Model 1640, Perkin Elmer, Beaconfield, England). Spillage of sample into the cell's outer window was

cleaned with fresh CS_2.

All CS_2 work was conducted in a fume cupboard with the necessary safety protection.

FTIR Scanning condition

Resolution:	2.0 cm^{-1}
Scan number:	16 scans over 2 min
Wavenumber:	2000 cm^{-1} to 400 cm^{-1}

5.3.5 Spectral subtraction

The spectral substraction technique was performed using the computerised FTIR instrument. The non-*trans* background (the zero standard) was subtracted from both the sample and standard spectra by the FTIR instrument software to generate a difference spectrum.

5.3.6 Calculation

The net peak height was used to calculate the result for both the AOCS and spectral subtraction methods. The baseline for the triglyceride fat was drawn from 995 cm^{-1} to 937 cm^{-1}, whereas the methyl esters baseline was drawn from 944 cm^{-1} to 998 cm^{-1}. The final calculation were processed using a Quattro Pro spreadsheet (Version 5, Borland International Inc., Scotts Valley, California, USA).

5.3.7 Statistical analysis

A comparison of the different methods was made using the two sample t-test (at 95% confidence level) on the pooled standard deviations for all the food products obtained by each method. The two sample t-test was conducted using the Minitab statistical software package (Version 10.1, State College, Pennsylvania, USA). The validity of pooling the standard deviations was confirmed using Bartlett's test for homogenity of variance (at 95% confidence level) (Sacks, 1984).

The individual products were also compared using a generalised linear model

procedure (SAS statistical software package, Version 6.10, SAS Institute Inc., North Carolina, USA).

5.4 RESULTS

Typical calibration standard curves for both trielaidin and methyl elaidin acquired using both AOCS Cd 14-61 and spectral subtraction methods are given in Figure 10. The infrared spectra of a calibration standard solution are given in Figure 11, and spectra of MARG-QC, AMF-QC and muesli bar samples are given in Figure 12 for the IR method.

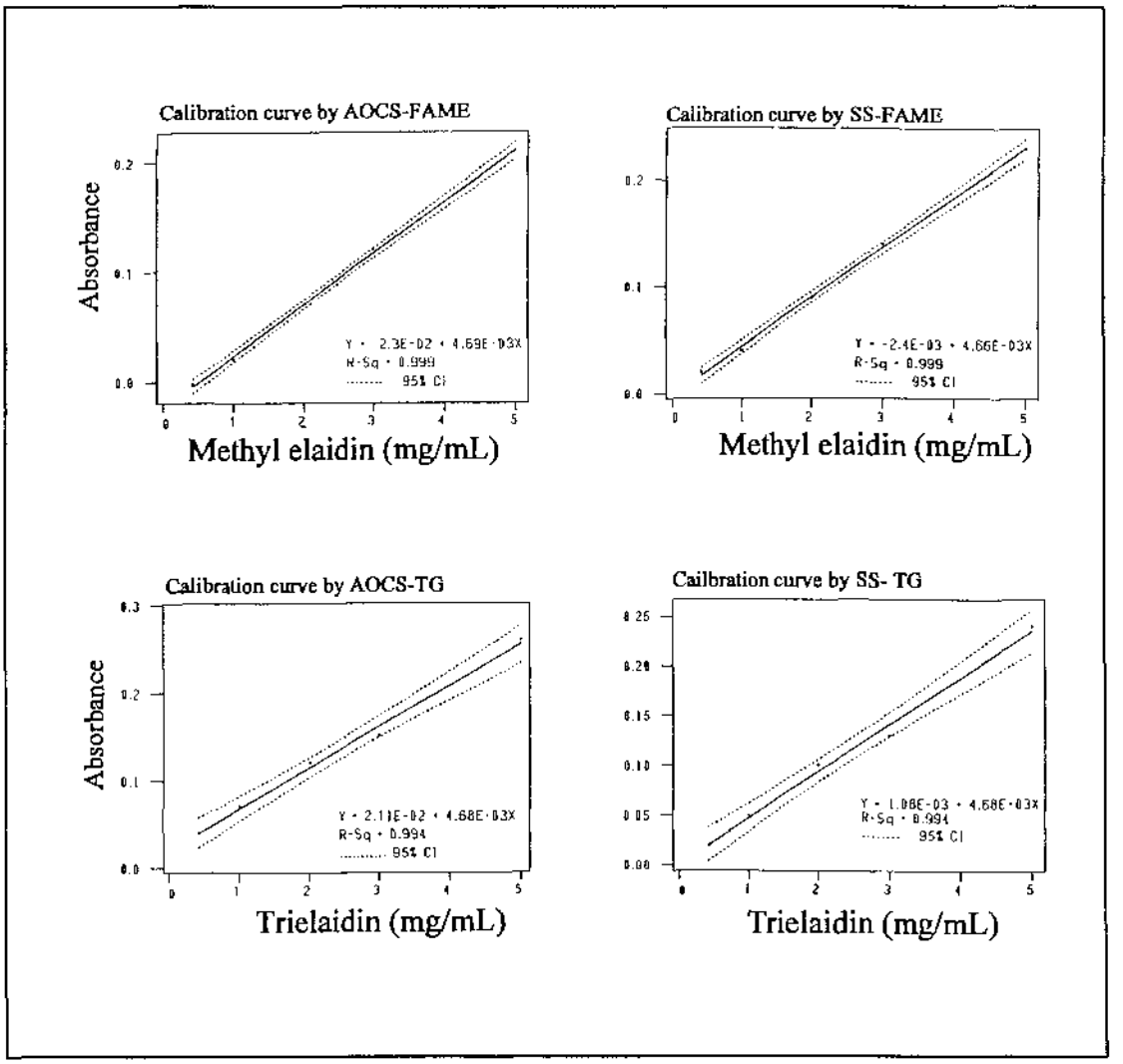

Figure 10 FTIR calibration plots for the *trans* fat standards, obtained using the AOCS Cd 14-61 and spectral subtraction methods.

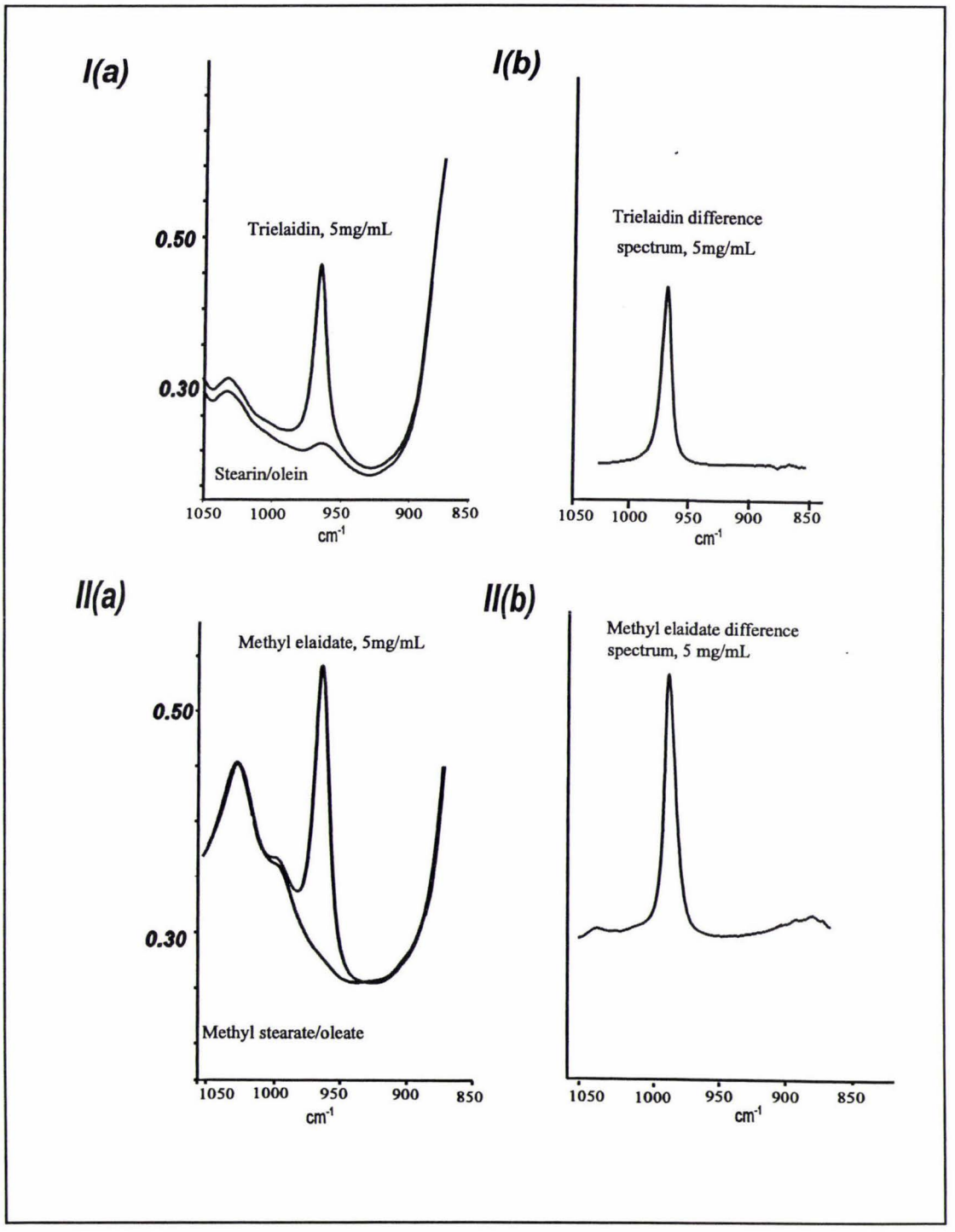

Figure 11 FTIR spectra of (I) triacyglycerols, (II) FAMEs, for (a) the calibration component, the elaidic acid standard and the non-*trans* background fat, and (b) the difference spectra obtained by the spectral subtraction of the non-*trans* background spectrum from the calibration standard spectrum. (Spectra are overlaid and offset for clarity.)

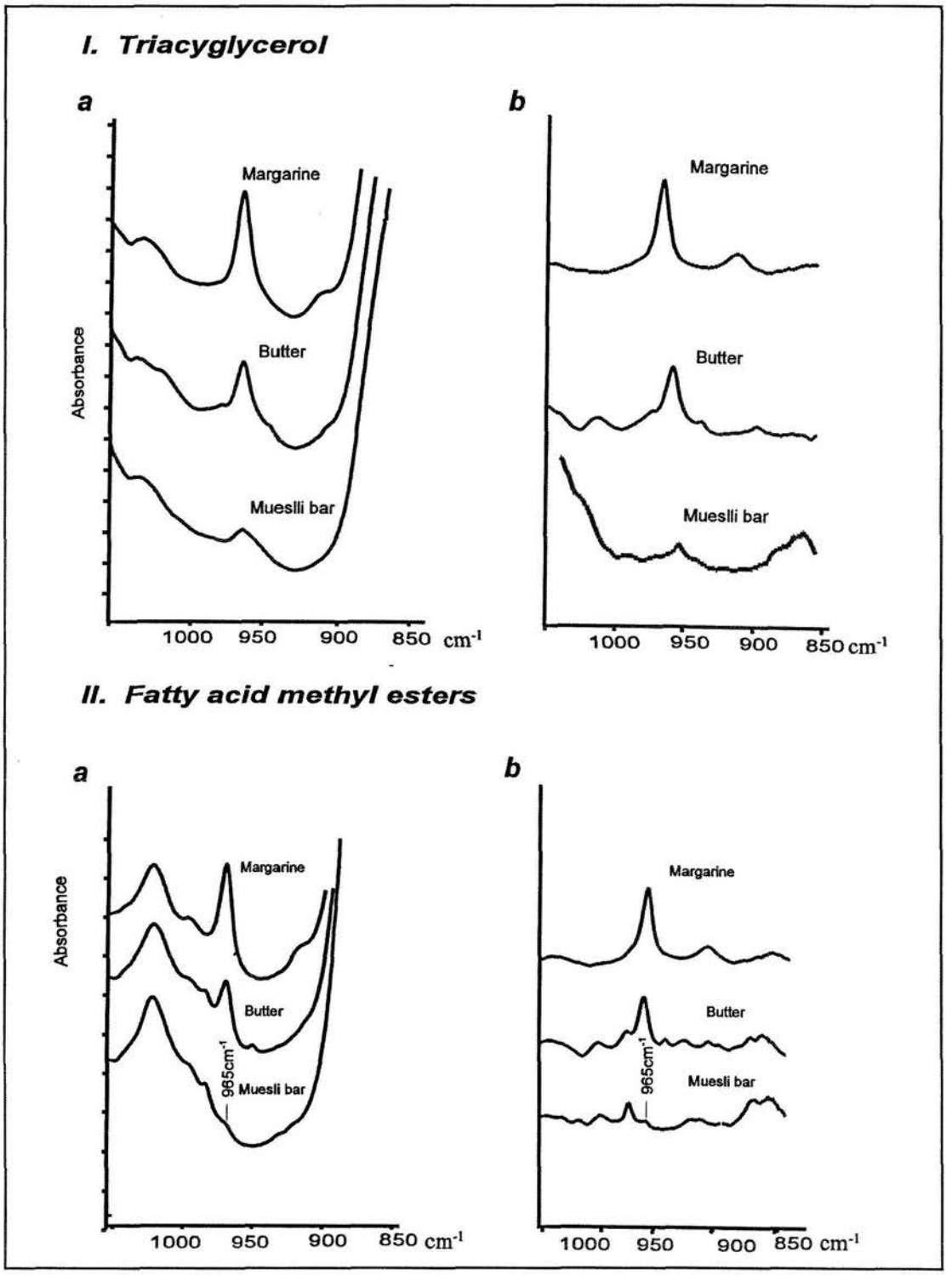

Figure 12 FTIR spectra of (I) triacyglycerols, (II) FAMEs, for (a)the fat extracted from the MARG-QC, AMF-QC and muesli bar, and (b) their respective difference spectra obtained by spectral subtraction of the non-*trans* background spectrum from the sample spectrum. (Spectra are overlaid and offset for clarity.)

5.4.1 Validation results for the FTIR methods

The repeatability standard deviations and the mean recoveries of the total *trans* methods are given in Tables 17 and 18 respectively. The replicate results are given in Tables A2.1, A2.2 and A2.3 in Appendix 2.

Sample	Total *trans* Isomer (g/100 g fat)			
	AOCS-TG	AOCS-FAME	SS-TG	SS-FAME
AMF-QC	8.26 ± 0.10	6.57 ± 0.43	8.56 ± 0.09	6.52 ± 0.92
MARG-QC	15.70 ± 0.20	14.88 ± 0.34	15.88 ± 0.19	14.71 ± 0.38

Table 17 The repeatability standard deviations of the AOCS-TG, AOCS-FAME and spectral subtraction (SS) methods determined by repeated analysis of the AMF-QC and MARG-QC samples. The results are given with ± standard deviation.

Sample	Recovery (%)			
	AOCS-TG	AOCS-FAME	SS-TG	SS-FAME
AMF-QC	101.2 ± 2.7	99.2 ±1.1	98.6 ±3.6	100.5 ± 3.7
MARG-QC	99.6 ± 1.9	98.1 ±0.8	100.0 ±2.0	101.5 ± 3.7

Table 18 The percentage recovery after spiking the AMF-QC and MARG-QC samples with trielaidin as determined by AOCS and spectral subtraction methods. The percentage recoveries are given with ± standard deviation.

5.4.2 New Zealand food survey fat results

The total *trans* results for the New Zealand food fat survey sample fats obtained using the AOCS method and the spectral subtraction technique (both TGs and FAMEs) are given in Table 19. The replicate results are given in Table A2.4, Appendix 2.

Sample	Total *trans* Isomer (g/100 g fat)			
	AOCS-TG	AOCS-FAME	SS-TG	SS-FAME
Margarine (A)	17.67	14.33	17.70	14.39
Margarine (B)	15.81	14.66	15.85	15.85
Commercial pastry fat (A)	7.88	6.56	8.24	8.81
Commercial pastry fat (B)	7.04	5.42	7.40	7.66
Commercial pastry fat (C)	6.13	7.02	6.44	8.54
Pastry	6.56	5.62	6.56	5.65
Homogenised milk	6.97	4.78	6.87	4.81
Reduced fat milk	6.59	5.76	6.59	7.44
Butter	8.35	6.72	8.41	6.82
Shortening	5.00	4.57	4.99	4.56
Meat pie	4.27	3.92	4.22	3.90
Meat patty	4.09	4.32	4.04	4.37
Luncheon	6.35	4.98	6.39	6.09
Muesli bar	1.37	0.89	1.27	0.98
Chocolate-coated biscuit	0.70	1.70	0.65	1.74
Plain sweet biscuit	5.01	4.50	5.06	5.61
Savoury cracker biscuit	1.02	0.71	0.97	1.07
White bread	2.51	2.19	3.42	2.33

Table 19 The total *trans* isomer results obtained using the AOCS and spectral subtraction techniques for the New Zealand food survey sample fats. The estimated test uncertainties are given by the pooled standard deviations. These are AOCS-TG: 0.41%; AOCS-FAME: 0.39%; SS-TG: 0.37% and SS-FAME: 0.78%.

5.5 DISCUSSION

5.5.1 AOCS Cd 14-61 method

The standard AOCS Cd 14-61 method allows for the use of both triglycerides and derived FAMEs for the quantification of total *trans* isomers by infrared absorption. Because of absorption by the acylglycerol of the triglyceride in the same region as the *trans* configuration, FAMEs have been recommended as the preferred form when a *trans* isomer level less than 15% is being quantified. However, methyl esters have the disadvantage of underestimating the total TFAs. Generally, the bias for methyl esters measurement is claimed to range from -1.5 to -3% and a positive bias of 2-3% is claimed to occur for TFAs measured as triglycerides (Firestone & Laboulier, 1965).

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.* (1982) 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 from 0.5 to 36%. This method was later recommended by the AOCS to help to remove some of the bias associated with the results produced from the standard AOCS Cd 14-61 method. In our study, a similar attempt to that of Madison *et al.* (1982) was made to increase accuracy, but using two *trans*-free components, the triglycerides of stearic (C18:0) and oleic (C18:1) acids, or, for methyl esters analysis, a mixture of pure methyl stearate and methyl oleate. The calibration plots for the AOCS Cd 14-61 method (Figure 11) of known levels of trielaidin or elaidic acid methyl ester revealed a small positive bias associated with triacylglycerols and a small negative bias associated 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 19) produced a mean bias of -2.25% *trans* (margarines) and -0.82% *trans* (all products), for methyl ester compared with triglyceride infrared measurements. These values are considerably less than the range (-3.5 to 6%) obtained by calculation using the above claimed biases from Firestone & Laboulier (1965). The two AOCS methods (TG and FAME) of infrared measurement for the fats 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.* milk, butter, margarine, luncheon meat, chocolate- coated biscuit, commercial and retail pastry fats. Except for commercial pastry fat C and the chocolate-coated biscuit, the triglyceride measurement gave greater results.

5.5.2 Spectral subtraction method

A fully hydrogenated milkfat was recently used for spectral subtraction (Ulberth & Henninger, 1994). The spectral subtraction results were shown to increase the low *trans* results and remove the 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 alter the background infrared spectrum (Huang & Firestone, 1971).

In our study, a similar attempt was made to increase the accuracy by using two *trans*- free components, the triglycerides of oleic (C18:1) and stearic (C18:0) acids, for native oil and fat samples. For the methyl esters analysis, a mixture of pure methyl stearate and methyl oleate was used. The results obtained by these spectral subtraction techniques are summarised in Table 19. The two sample t-test (95% confidence level) performed on the pooled total *trans* isomer data produced from each of the four methods (AOCS- FAME, SS-FAME, AOCS-TG and SS-TG) did not indicate any significant difference between them. However, because of the large range of sample types surveyed, individual products were statistically compared to give a better indication of the variability. For the methyl esters, the spectral subtraction (SS-FAME) results for seven of the 18 products (margarine B, reduced fat milk, plain sweet biscuit, luncheon and the three commercial pastry fats A, B and C) were significantly higher than their respective AOCS-FAME results. These differences ranged from 2.25% in commercial pastry fat A and B to 1.11% in luncheon and sweet biscuits. In contrast, for the triglycerides, the spectral subtraction results were found not to be significantly different (95% confidence level) from the AOCS-TG method results.

For the spectral subtraction results (SS-FAME and SS-TG), 11 of the 18 samples

were found not to be significantly different from each other (at the 95% confidence level). The samples that were significantly different were commercial pastry fat C, chocolate-coated biscuit, reduced fat milk, bread, margarine A, butter and homogenised milk. The differences varied from 0.85% in reduced fat milk to 3.3% in margarine A. The generally good agreement with 11 of the 18 products suggested that spectral subtraction made some contribution to removal of the bias associated with the AOCS-FAME method.

5.6 CONCLUSION

Methods for the determination of TFA isomers in a wide variety of foods were developed and validated. The negative 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 survey food fats.

Furthermore, the negative bias associated with the AOCS-FAME technique was eliminated or at least greatly reduced by the spectral subtraction-FAME method for a large number of the survey samples.

Total *trans* measurements by infrared methods are subjected to error, particularly at low levels unless care in calibration is exercised. The accuracy of these FTIR measurements was verified against the more accurate Ag-TLC/GC technique. These verifications are discussed in later sections.

6 DETERMINATION OF TOTAL *trans*-C18:1 POSITIONAL ISOMERS AND THEIR DISTRIBUTION BY Ag-TLC/GC

6.1 INTRODUCTION

In hydrogenated or partially hydrogenated oils and ruminant fats, the TFAs are made up of predominantly C18:1 *trans* positional isomers (>95%, Ratnayake *et al.*, 1993). Recently, there has been some evidence to suggest that the unfavourable effects of the C18:1 *trans* positional isomers may not be equal. It has been suggested that the C18:1 Δ9t isomer may be more harmful to animal biological systems that the C18:1 Δ11t isomer (Enig, 1993). The determination of the relative amounts of these positional isomers is therefore important.

Studies of the C18:1 *trans* positional isomers traditionally employed ozonolysis. Isolation of the *trans* monoene acids by either Ag-TLC or preparative gas-liquid chromatography (GLC) was required. The isolated *trans* C18:1 fraction was then submitted to ozonolysis. The resulting fragments (generally aldehydes and esters) were then analysed by GLC (Sampugna *et al.*, 1982; Scholfield, 1979; Parodi, 1976; Scholfield *et al.*, 1967). This method was long, tedious and suffered from the major drawback that the shorter fragments were volatile and could be partly lost during the experiment.

The quantification of total C18:1 TFAs by direct analysis, using GC on a very polar cyanosilicone capillary column (*e.g.* SP-2560 flexible fused silica capillary column, 100% cyanopropyl polysiloxane-PTMSil 88 capillary column), has now surpassed ozonolysis. This direct GC method is uncomplicated and therefore used by most laboratories for rapid *cis/trans* analysis (Duchateau *et al.*, 1996; Mansour & Sinclair, 1993; Ball *et al.*, 1993; Lanza & Slover, 1981). The direct GC method has been adopted in AOCS method Cd 14c-94 (1993b) and AOCS method Cd 17-85 (AOCS, 1993c). However, a complete resolution of the isomeric C18:1 fatty acids was not feasible by GC alone. In most polar columns, the elution of the C18:1 *trans* isomers overlapped with that of the *cis* isomers. The Δ12t-Δ14t components were under the major C18:1 Δ9c isomer. In spite of this *cis/trans* overlap, many workers considered the major C18:1 peak in this direct GC method to be 100% *cis* (Figure 14). As a result of this assumption, an underestimation of the total C18:1

TFA fatty acids content is common. To overcome this problem, Sampugna *et al.* (1982) proposed the use of an appropriate correction factor. However, the correction factor had to be calculated for each type of fat sample.

A more accurate and reliable method for C18:1 TFA determination required the prior separation of the *cis* and *trans* isomers using Ag-TLC. This pre-column fractionation circumvents the problem of *cis* and *trans* isomers co-eluting during the GC analytical step. The separation of the individual positional isomers is usually accomplished by capillary GC columns. As megabore columns do not have the resolving power to separate the isomers effectively, a 50 m BPX70 capillary GC column was used in this study.

Note: The total C18:1 *trans* and its positional isomer distribution could be determined in a single run using the capillary BPX70 column. This was not done in this study because the initial emphasis was on the total *trans* isomers. Coupled with time the constraint of having to complete the total *trans* survey on the New Zealand food fat samples and the unavailability of the BPX70 column during the early stages of this study, the 15 m FFAP megabore column, which was available then, was used. The samples were later re-analysed using the BPX70 capillary column for positional isomer distribution.

6.2 MATERIALS AND METHODS

6.2.1 Total C18:1 TFA by Ag-TLC/GC

The total C18:1 TFAs were determined by Ag-TLC/GC, as described by Lund & Jensen (1982), Ulberth & Henninger (1992) and Wolff (1994), with the C18:0 in the original sample used as an internal standard. The FAMEs of the samples were prepared by direct *trans* esterification with sodium methoxide/methanol and boron trifluoride/methanol as described in Section 3.2.2.

An aliquot of the FAME solution (approximately 55 μg/L) was loaded on to the

Ag-TLC plate and the fatty acids were fractionated as described in Section 4.2.2 using a mobile phase consisting of toluene/diethyl ether (9:1). The *trans* monoene and saturated fatty acid bands were extracted and reconsititued in 200 μL of hexane for total C18:1 *trans* analysis by GC. The GC analytical conditions were identical to those reported for FAME composition analysis using the 15 m FFAP GC megabore column (Section 3.2.3).

6.2.2 Calculations

The total C18:1 TFAs by Ag-TLC/GC were determined using the formula:

$$(C18{:}1\ trans\ wt\ \%) = \frac{corrected\ peak\ area\ of\ total\ C18{:}1\ TFA}{corrected\ peak\ area\ of\ C18{:}0} \times 0.993 \times C18{:}0\ (\%wt,\ original\ sample)$$

where the C18:0 was used as an internal standard. The C18:0 (%wt) in the original sample was determined from the standard FAME composition (Section 3.2.3), and the factor 0.993 was the response factor for C18:1 relative to C18:0 (Appendix 1).

6.3 EXPERIMENTAL

6.3.1 Method validation - total C18:1 *trans* isomers by Ag-TLC/GC (using the FFAP GC column)

The total C18:1 *trans* isomer determination by the Ag-TLC/GC method had to be validated using "low" AMF-QC and "high" MARG-QC samples before the method could be used routinely. The following areas of the method were looked at during the validation process.

Evaporative loss of C18:0 and C18:1 FAMEs

The chromatographic determination used the sample C18:0 methyl ester as an (endogenous) internal standard to determine the total C18:1 TFAs. Although the procedure was described earlier by Lund & Jensen (1982), Ulberth & Henninger

(1992) and Wolff (1994), additional work had to be performed to determine if evaporative losses of the C18:0 and C18:1 methyl esters from the Ag-TLC plates and during the evaporation of the organic solvents were occurring and if both fatty acids were lost at equal rates.

The evaporative loss experiments were conducted by using a standard mixture of C18:0, C18:1 Δ9t and C23:0 methyl esters. The amounts of C18:0 and C18:1 relative to the C23:0 fatty acids were therefore known and compared with that obtained:

(i) after direct GC analysis;
(ii) after Ag-TLC/GC (with C23:0 present before Ag-TLC);
(iii) after Ag-TLC/GC (with C23:0 added only after Ag-TLC in amounts equivalent to that plated in (ii) above).

Repeatability

The assay repeatability was determined with the two quality control samples - AMF-QC and MARG-QC. Both samples were analysed five to six times.

Recovery of the assay

The percentage recoveries, after spiking both the AMF-QC and MARG-QC samples with trielaidin at approximately 50% and 100% of the native *trans* level, were determined.

6.3.2 Separation and identification of C18:1 *trans* positional isomers using the BPX70 capillary column

The separation of the C18:1 *trans* positional isomers using the 15 m FFAP megabore GC column and the GC conditions described earlier was not possible. As a result, the BPX70 high resolution polar capillary column (SGE, Hewlett Packard Company, USA) with the stationary phase (polysilphenylene-siloxane) specifically designed for the analysis of FAME isomers was selected for the separation of the C18:1 *trans* positional isomers in this study. The optimisation of

the separation and the identification of these positional isomers were made using the *trans* monoene fraction isolated from the AMF-QC sample by Ag-TLC and the three standards available commercially (C8:1 Δ6t, C8:1 Δ9t and C8:1 Δ11t (> 99% purity from Nu Chek Prep, Minnesota, USA). The AMF-QC sample was selected for this work because the C18:1 *trans* positional isomer profile has been well documented (Wolff & Bayard, 1995; Sampugna *et al.*, 1982; Hay & Morrison, 1970). The three standards were spiked into the *trans* monoene fraction one at a time, to further confirm the peak identities made based on the published literature data.

The *cis* monoene band was also analysed in a similar fashion, solely to determine the degree of the *cis/trans* overlap. No attempts were made to identify or optimise their separation. It was well established that the C18:1 *9*Δc positional isomer was the predominant *cis* positional isomer present.

The capillary GC conditions used are given in Table 20. Approximately 0.05 - 0.2uL (containing 0.05-0.1 μg) of the C18:1 *trans* sample (analysed previously using the FFAP column) was loaded on to the BPX70 capillary column. The repeatability of the assay was determined using the AMF-QC and MARG-QC samples.

GC: Shimadzu I5A (Shimadzu Corporation, Kyoto, Japan) Column: SGE BPX70, 50 m x 0.22 mm id	Make up gas: N_2, 70 mL/min Carrier gas: H_2, 2.3 kg/cm^2
Injector: 250°C Detector: 250°C FID H_2: 0.4 kg/cm^2 FID air: 0.4 kg/cm^2	Temperature gradient: 170°C, 3°C/min (20 min), 220°C (5 min)

Table 20 Capillary GC conditions for positional isomer separation on the Shimadzu 15A gas chromatograph.

6.4 RESULTS AND DISCUSSION

6.4.1 Method validation - total C18:1 *trans* isomer, by Ag-TLC/GC (using the FFAP column)

(i) Repeatability

The repeatabilities of the assay, determined from 10 replicate analyses were: AMF-QC, 5.49 ± 0.16%; MARG-QC, 12.83% ± 0.39% (Table A3.1, Appendix 3).

(ii) Evaporative loss

Absolute losses of C18:0 and C18:1 methyl esters of up to 50% were found to occur, as indicated by the C23:0 methyl ester reference, added to the recovered TLC components. However, the C18:0 and C18:1 methyl esters were found to have the same relative recovery (Table 21); therefore C18:0 could be used as an internal standard to correct for the losses of C18:1 *trans* during the extraction procedure.

	C18:0 recovery (%)	C18:1 recovery (%)
Observed from GC analysis (mean of six runs)	99.3 ± 4.3	100.7 ± 5.3
Observed from Ag-TLC/GC; C23:0 added before Ag-TLC (mean of five runs)	100.6 ± 3.6	101.9 ± 6.1
Observed from Ag-TLC/GC; C23:0 added after Ag-TLC (mean of two runs)	50.6 ± 4.2	53.5 ± 2.5

Table 21 Relative recovery of C18 methyl esters from the TLC plates, using a standard component mixture of C18:0, C18:1 and C23:0 methyl esters (percentages relative to C23:0 methyl esters).

(iii) Recovery of spiked samples

By utilising C18:0 as an endogenous internal standard, the mean recoveries of trielaidin added to MARG-QC and AMF-QC samples were 98.9 ± 4.7% and 100.1 ± 2.7% respectively (Table A3.4, Appendix 3)

6.4.2 Separation and identification of C18:1 *trans* positional isomers using the BPX70 capillary column

(i) Optimisation and Identification

The most convenient way of obtaining a C18:1 *trans* positional isomer profile was by using the *trans* monoene Ag-TLC fraction from AMF (or any ruminant fat). The *trans* positional isomer profile is almost constant (Wolff & Bayard, 1995), with C18:1 Δ11t (vaccenic acid) been the major isomer in the fat. Hence this was used as a landmark to identify other isomers in this study. Together with the spiking results of C18:1 Δ6t, Δ9t, Δ11t isomers and literature data (Wolff & Bayard, 1995), the positional isomers were found to elute in the following order on the BPX70 capillary column: Δ6-9t, Δ11t, Δ12t, Δ13t and Δ14t, Δ15t and Δ16t (Figure 13). The *trans* positional isomers whose double bonds are closer to the carboxyl group have longer retention times than those whose double bonds are further away. This elution pattern was explained by Gunstone *et al.* (1967) stronger interactions with the polar stationary phase occurred when the distance between the olefinic group and the carboxyl group were closer; hence, for example, the C18:1 Δ9t isomer would elute before the C18:1 Δ16t isomer on a polar column.

In this study, the Δ6t-Δ9t isomers and the Δ13t and Δ14t isomers were not fully resolved and the Δ10t isomer appeared as a shoulder on the Δ11t isomer peak. These results were identical to those reported by Wolff & Bayard (1995) using a capillary column of similar polarity. However, these two workers also attempted to improve the peak resolution by doubling the length of their analytical column to 100 m (linking two 50 m columns together) and using isopropyl esters instead of methyl ester. They were able to improve the separation of some of the poorly resolved peaks to near baseline separation. However, the Δ6t - Δ8t and the Δ13t and Δ14t isomers remained unresolved (co-eluting). The major drawback with the increased column length was an increase in the analytical run time from 30 min to 1.3 h for the elution of the last C18:1 Δ16t isomer. The peak resolutions obtained using the 50 m BPX70 column were sufficient for quantification purposes in this study.

(ii) Repeatability of the positional isomer determination

The repeatability standard deviations for the C18:1 *trans* positional isomer determination were estimated using the quality control samples. These are given in Table 22. The replicate results are given in Table A3.2, Appendix 3.

Sample	C18:1 *trans* Positional Isomers (as % of the total C18:1 *trans)*							Total C18:1 TFAs (g/100 g fat)
	6-9t	10t	11t	12t	13t and 14t	15t	16t	
AMF-QC	2.70 (0.35)	3.29 (0.44)	54.51 (1.67)	7.12 (1.82)	18.36 (0.40)	7.12 (0.61)	6.90 (0.51)	5.49
MARG-QC	25.27 (3.18)	22.40 (0.75)	19.20 (0.69)	13.19 (0.93)	14.53 (0.93)	3.34 (0.51)	2.04 (0.27)	12.83

Table 22 Distribution of C18:1 *trans* positional isomers in AMF-QC and MARG-QCI samples. The repeatability standard deviation was determined from nine and eight determinations respectively.

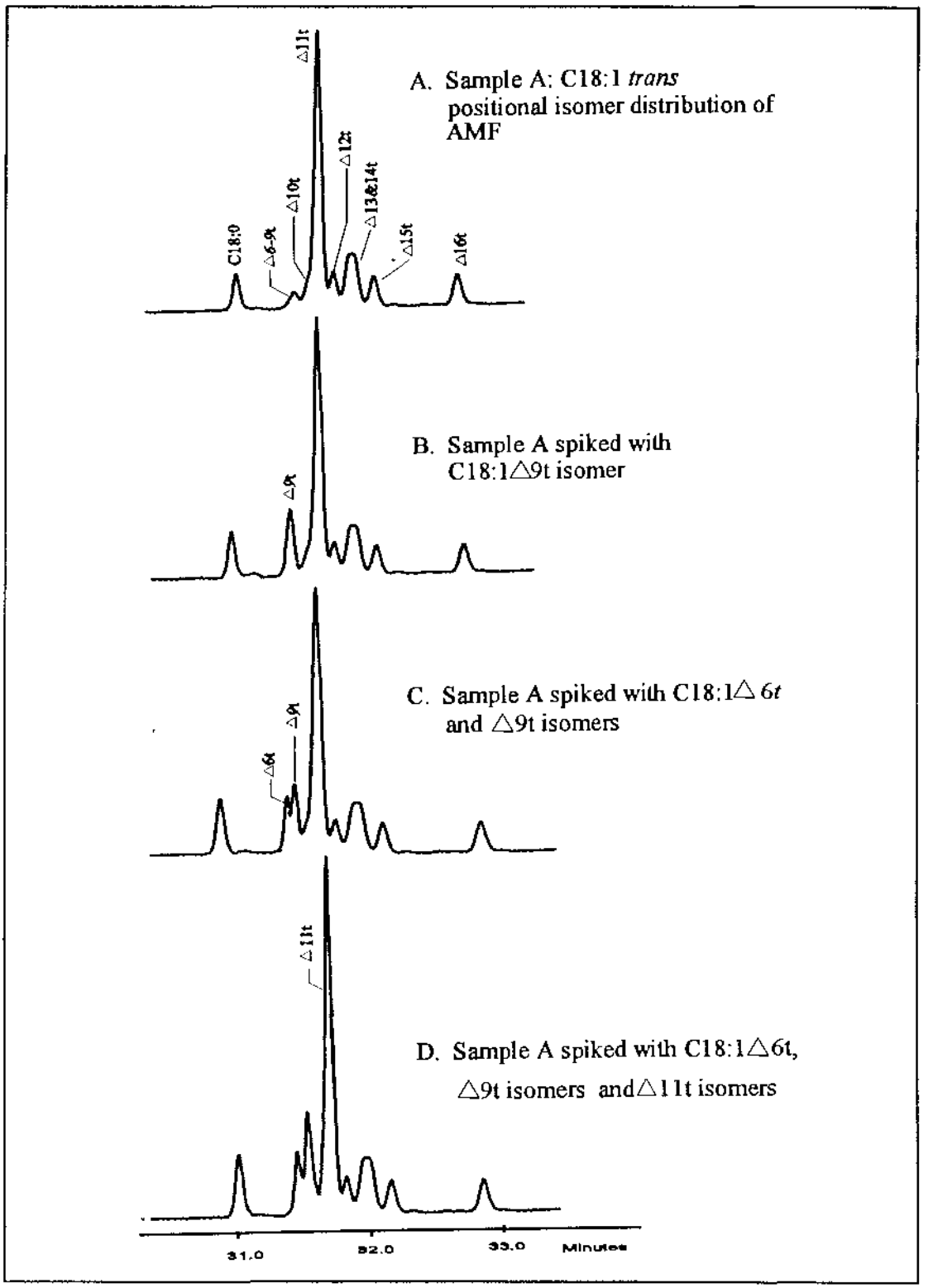

Figure 13 Separation and identification of the C18:1 *trans* positional isomers. The *trans* monoene fraction was isolated from AMF by Ag-TLC, with C18:1-Δ11t (the major positional isomer) used as a landmark to identify other isomers. Confirmation was made by spiking the AMF sample with C18:1 (b) Δ9t, (c) Δ6t & Δ9t and (d) Δ6t, Δ9t & Δ11t positional isomers.

(iii) *Cis/trans* overlap

The extent of the *cis/trans* overlap was also examined in this study by analysing using capillary GC, the *cis* and *trans* monoenoic fatty acid bands isolated by Ag-TLC. The C18:1 9Δc isomer (the predominat *cis* isomer) elutes under the 12Δt, 13Δt - Δ14t isomers (Figure 14). The degree of the overlap and the actual error incurred as a result of this overlap were not determined in this study, but an underestimation of up to 32% for margarine (with 40-60% total *trans*) was reported by Ratnayake & Beare Rogers (1990) and Ratnayake (1992), if direct GC analysis without prior *cis/trans* separation was used as the quantification method. As a result, analysts should be warned against the practice of measuring the C18:1 *trans* content in lipid directly through a single chromatographic run. The results obtained in such a way are basically erroneous and should be considered with reservation.

6.5 CONCLUSION

The Ag-TLC/GC method using a FFAP megabore column was validated to determine the total C18:1 *trans* positional isomer in fat samples. However, this FFAP column does not have the resolving power to separate the positional isomers. The 50 m BPX70 capillary column was required to separate and determine the distribution of these positional isomers. The total C18:1 TFAs and their positional isomers for the New Zealand food survey fat samples, soy bean oil samples, and the New Zealand and overseas butter samples are given in Tables 23 to 26 respectively.

It is clear that temperature was observed to greatly affect the resolution of the positional isomers. The best resolution was obtained with a temperature gradient. It may still be possible to further resolve some of the isomers under the current conditions by varying the carrier gas flowrate. Unfortunately, this was not an option with the instrument used with this research.

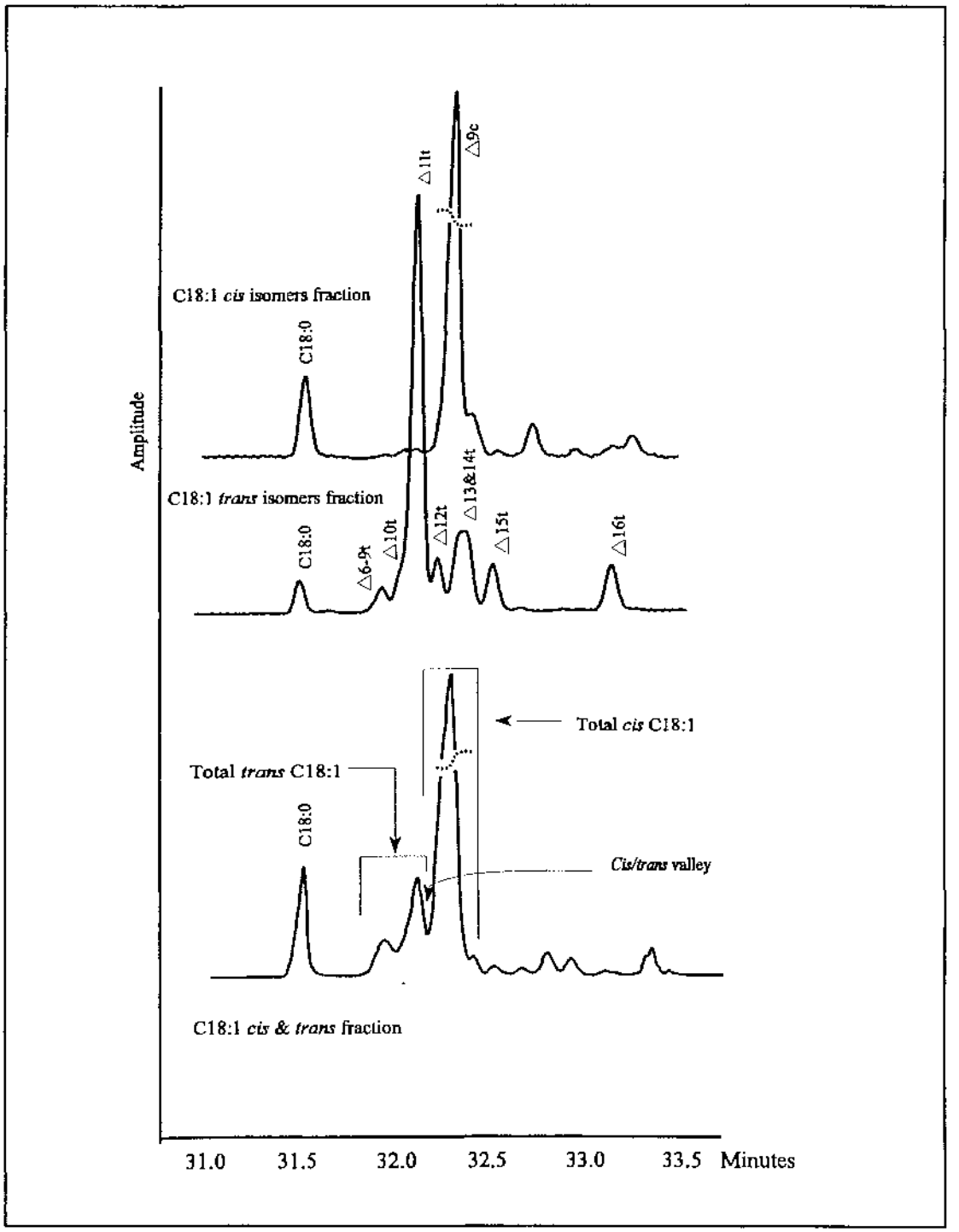

Figure 14 Overlay of the C18:1 (A) *cis* and (B) *trans* positional isomer fraction chromatograms obtained by Ag-TLC/GC, with that obtained by direct GC analysis (no prior *cis/trans* separation), using the 50 m BPX70 capillary column. The GC conditions are given in Table 20. No further identification of the *cis* positional isomers was made. The high C18:1 Δ12t - Δ14t positional isomers are shown to co-elute with the C18:1 Δ9c positional isomer.

Sample	C18:1 *trans* Positional Isomers (% of total C18:1 *trans*)							Total C18:1 *trans* (g/100 g fat)
	6t-9t	10t	11t	12t	13t-14t	15t	16t	
Margarine A	42.62	20.77	15.45	10.83	7.78	1.9	0.65	13.64
Margarine B	28.39	21.11	17.69	12.55	14.16	3.74	2.35	14.70
Commerial pastry A	26.92	13.74	34.55	6.78	10.68	3.97	3.36	6.38
Commercial pastry B	48.47	25.44	10.47	8.47	5.5	1.18	0.48	7.67
Commercial pastry C	15.4	5.92	44.86	7.67	14.62	6.4	5.12	6.69
Pastry	23.91	13.45	36.49	7.2	11.24	4.22	3.49	5.90
Homogenised milk	4.36	3.12	52.14	7.66	17.57	7.76	7.4	4.93
Reduced fat milk	5.36	3.34	54.58	6.3	16.63	6.86	6.93	5.71
Butter	4.57	3.4	53.68	7.51	16.52	8.02	6.31	6.37
Shortening	8.78	8.49	60.27	4.69	9.43	4.19	4.15	4.09
Savoury Mince pie	10.47	4.13	52.9	6.87	13.71	6.3	5.62	3.43
Meat patty	10.56	3.5	50.88	8.65	14.04	6.45	5.92	3.09
Luncheon	8.95	4.6	50.26	8.36	14.41	7.65	5.78	5.42
Muesli bar	28.52	17.81	25.18	12.05	11.13	3.68	1.63	1.93
Chocolate-coated biscuits	26.11	10.6	35.11	8.55	11.98	4.52	3.12	3.00
Plain Sweet biscuit	9.46	14.25	48.69	6.86	11.53	5.09	4.13	6.17
Savoury crackers biscuit	38.73	30.37	7.87	9.15	6.72	6.98	0.44	5.52
Bread	36.27	12.41	16.01	14.84	15.12	5.35	0.00	2.69

Table 23 C18:1 *trans* positional isomer distribution in New Zealand food survey fats. The positional isomers are given as a percentage of the total C18:1 *trans*.

C18:1 *trans* positional isomers (% of total C18:1 *trans)*							Total C18:1 *trans* (g/100 g fat)
6t-9t	10t	11t	12t	13t-14t	15t	16t	
33.5	19.8	22.6	11.4	9.1	3.4	0.20	54

Table 24 C18:1 *trans* positional isomers of hydrogenated soya bean oil

Sample Name	C18:1*trans* positional isomers (as a % of the total C18:1 *trans*)							total C18:1t (g/100g fat)
	6-9t	10t	11t	12t	13t &14t	15t	16t	
Aug 1995	3.60	2.39	56.70	6.13	17.31	6.89	7.00	6.25
Sep 1995	2.34	2.33	60.61	6.32	15.91	6.08	6.42	6.45
Nov 1995	2.01	2.75	57.77	7.10	16.26	6.82	7.29	5.0
Jan 1996	3.83	2.30	55.95	5.36	17.08	7.10	8.39	5.0
Apr 1996	4.13	3.88	48.62	6.89	23.15	6.53	6.80	5.4
May 1996	3.86	3.26	52.36	8.21	18.62	6.48	7.22	5.6

Table 25 Seasonal variation of total *trans* C18:1 isomer and their positional isomer distribution in New Zealand butter fat samples.

Sample Name	C18:1 *trans* Positional Isomers (% of total C18:1 *trans*)							Total C18:1 *trans* (g/100 g fat)
	6-9t	10t	11t	12t	13t &14t	15t	16t	Mean
New Zealand -								
Minimum	2.01	2.30	48.62	6.13	15.91	6.08	6.42	5.00
Maximum	4.13	3.88	60.61	8.21	23.15	7.10	8.39	6.45
Devondale (Australia)	4.16	2.79	53.73	6.00	17.35	7.81	8.16	4.75
GG-lactic (Australia)	4.10	2.28	50.81	17.84	14.48	4.99	5.50	4.80
Snow (Japan)	7.38	3.47	49.18	9.49	15.20	7.76	7.51	3.00
Lady Lee (USA)	7.51	12.74	25.41	12.49	22.89	10.14	8.82	6.65
Kerry Gold (Ireland)	4.35	2.14	65.39	5.33	13.04	5.58	4.17	7.00
Lurpak (Denmark)	7.90	10.12	39.06	10.58	18.51	8.65	5.17	4.00

Table 26 Total C18:1 *trans* fatty acids and their positional isomer distribution in New Zealand and some overseas butter samples. The minimum and maximum figures for the New Zealand butters were obtained from Table 25.

7 SYNTHESIS AND IDENTIFICATION OF C18:2 (LINOLEIC ACID) AND C18:3 (α-LINOLENIC ACID) GEOMETRIC ISOMERS

7.1 INTRODUCTION

The geometric isomers of C18:2 Δ9, 12 (linoleic acid) and C18:3 Δ9, 12, 15 (α-linolenic acid) fatty acids are divided into three and four different classes respectively. These are given in Table 27.

Classes	C18:2 Δ9, 12	C18:3 Δ9, 12, 15
I	all-*trans*	all-*trans*
II	*trans*, *cis*	di-*trans*, mono *cis*
III	all-*cis*	mono-*trans*, di-*cis*
IV	-	all-*cis*

Table 27 Geometric isomer classes of C18:2 Δ9, 12 (n-6 family) and C18:3 9, 12, 15 (n-3 family) fatty acids

Both these fatty acid groups are recognised as essential fatty acids for mammals because they cannot be synthesised *de novo* from endogenous precursors, and are therefore essential dietary elements. Once ingested, these acids are desaturated and elongated, primarily in the liver, into polyunsaturated C20 (eicosanoids) and C22 fatty acids, which are precursors for prostaglandin biosynthesis (Figure 2). These eicosanoids exert diverse action on the cardiovascular, reproductive, respiratory, renal, endocrine, skin, nervous and immune systems, whereas the prostaglandins are involved with platelet aggregation and vasodilation (Hwang, 1992).

The symptoms of essential fatty acid deficiency in mammals have been well characterised. For example, diminished growth, scaly dermatoses, fatty liver, inflamed epidermis, kidney degeneration, diminished skim pigmentation, increased fragility and permeability of cellular membranes, impaired reproduction and sterility (Chapkin, 1992).

Although the physiological effects of C18:2 n-6 and C18:3 n-3 *cis* isomer deficiency are known, the effects of their *trans* geometric isomers are not so clear.

It has been demonstrated that some of the geometric isomers can slow down the elongation and desaturation of the all-*cis* isomer to form the long chain C20 and C22 fatty acids, whereas in other cases, the *trans* containing long chain fatty acids inhibit the formation of prostaglandin (discussed in Literature Review, Section 3).

Quantification of the various C18:2 and C18:3 geometric isomers requires capillary GC. These geometric isomers are not available commercial; as a consequence, they had to be synthesised and identified in the laboratory before they could be used as retention time reference standards when analysing samples.

Several *cis/trans* isomerisation techniques are available for the synthesis of these geometric isomers. They are selenium-catalysed isomerisation (Strocchi & Piretti, 1968), isomerisation using p-toluenesulfinic acid (Snyder & Scholfield, 1982), alkaline isomerisation (Jamieson & Reid, 1965) and isomerisation using oxides of nitrogen (Litchfield *et al.*, 1965). Of these techniques, the oxides of nitrogen isomerisation has generally been favoured because it does not generate any positional isomers (Wolff, 1992b; Grandgirard *et al.*, 1987).

The oxides of nitrogen could be produced by a number of methods *e.g.* mercury with nitric acid, copper with nitric acid and sodium nitrite with nitric acids. The last technique was selected for use in this study because of its simplicity, where the active species, nitrogen dioxide, was produced *in situ* (Litchfield *et al.*, 1965). The sequence of reactions (Figure 15) involves nitric acid reacting with sodium nitrite to produce nitrous acid, which decomposes in the presence of the stronger nitric acid to N_2O_3 (nitrous acid anhydride). N_2O_3 then decomposes to give the free radical NO_2. After a free passage from the aqueous phase to the fatty acid phase, the free radical, NO_2, adds to a double bond, producing a freely rotating carbon-carbon single bond. The addition of the free radical NO_2 is reversible, and either a *cis* or a *trans* double bond can be formed when the nitrogen dioxide leaves. The addition of a second NO_2 molecule produces either a di-nitro or a nitro-nitrile fatty acid. Other side reactions also take place, but the reversible free radical NO_2 addition to the double bond had been suggested to produced the *cis/trans* isomerisation.

The superiority of this method lies in its ability to convert the by-product NO into additional NO_2 by the reaction: $2NO + H_2O + HNO_3 \rightleftharpoons 3HNO_3$. The relative proportion

of the geometric isomers generated by this process has been reported to be greatly influenced by temperature, nitrite concentration and incubation time (Grandgirard *et al.*, 1987). The nitrogenous by-products generated are easily removed using a silicic acid column.

The C18:2 and C18:3 geometric isomers synthesised in this study were subjected to Ag-TLC, IR and capillary GC analysis to confirm their identities.

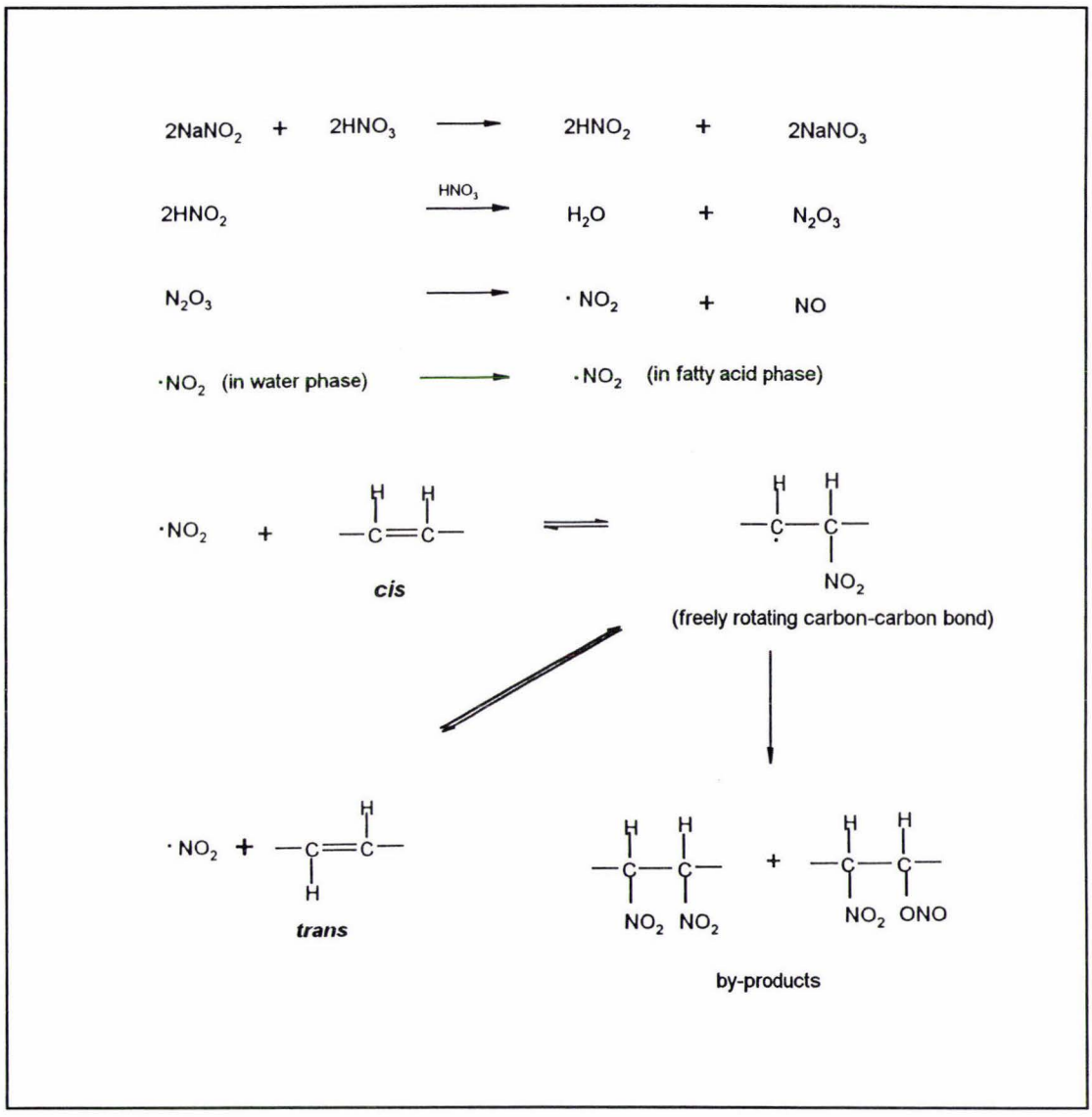

Figure 15 Nitric-acid-catalysed *cis*/*trans* isomerisation (Litchfield *et al.*, 1965). The sequence of reactions involves nitric acid reacting with sodium nitrite to produce nitrous acid, which decomposes in the presence of the stronger nitric acid to N_2O_3 (nitrous acid anhydride). N_2O_3 then decomposes to give the free radical NO_2. After a free passage from the aqueous phase to the fatty acid phase, the free radical, NO_2, adds to a double bond, producing a freely rotating carbon-carbon single bond. The addition of the free radical NO_2 is reversible, and either a *cis* or a *trans* double bond can be formed when the nitrogen dioxide leaves. The addition of a second NO_2 molecule produces either a di-nitro or a nitro-nitrile fatty acid. Other side reactions also take place, but the reversible free radical NO_2 addition to the double bond has been suggested to produce the *cis*/*trans* isomerisation.

7.2 MATERIALS AND METHODS

7.2.1 Chemicals

All chemicals used were of analytical grade unless otherwise specified.

7.2.2 *cis/trans* isomerisation

Linoleic and linolenic acid methyl ester (10 g, Sigma Chemical Company, St Louis, Missouri, USA) isomerisation was effected at 40°C for 10 min using a solution of 6 M nitric acid (0.625 mL) and 2 M sodium nitrite (1 mL) per 10 g of methyl esters. The methyl esters, after incubation, were extracted in hexane (50 mL) after the addition of 10 mL of water. The hexane layer, containing the isomerised esters, was further purified on a silicic acid column. The silicic acid (70-230 mesh, Merck, Darmstadt, Germany) column was prepared by transferring approximately 30 g of the silicic acid (rehydrated at 5% w/v) to a glass column (32 cm x 1 cm id). About 1 g of the methyl ester was fractionated at once with 200 mL of petroleum ether/diethyl ether (95/5 v/v). The solvents from the collected fractions were evaporated under reduced pressure at 40°C until only 2-3 mL remained. The residual solvents were evaporated off under a stream of nitrogen at 40°C and the isomerised products were stored under nitrogen at approximately -18°C until required.

A diluted solution of the isomerised products (0.5 mg/mL) was analysed by capillary GC (GC-15A, Shimadzu, Kyoto, Japan). Approximately 0.1-0.2 L of the solution was injected on to the capillary GC column (BPX70, 50 m x 0.22 mm id). The gas flowrate conditions for the optimised separation were identical to those used for the C18:1 *trans* positional isomer separation (Section 6.3.2).

7.2.3 Identification of C18:2 and C18:3 geometric isomers by GC, Ag-TLC and IR

The geometric isomers synthesised by nitric acid isomerisation were separated into their fatty acid classes by Ag-TLC as discussed in Section 4.3, using a mobile phase consisting of a mixture of toluene/diethyl ether (95/5 v/v). A standard solution containing C18:2 Δ9t,12t, C18:2 Δ9c,12c and C18:3 Δ9c,12c,15c (>

99% purity; Nu Chek Prep, Minnesota, USA) was also included as a reference marker.

Several Ag-TLC plates were used and the corresponding FAME bands from each plate were pooled to collect a sufficient amount of the geometric isomers for *trans* double bond measurement by IR. The FAMEs recovered from the Ag-TLC plates were reconstituted with carbon disulphide (1 mL). Each sample was scanned for 2 min with a wavelength resolution of 1 cm^{-1}. The *trans* absorbance was measured at 965 cm^{-1} (Perkin-Elmer FTIR Paragon 1000 with Spectrum software, Perkin-Elmer Corporation, Beaconsfield, Buckinghamshire, UK). After IR analyses, the actual methyl ester concentration of each solution was determined by GC to be able to determine the infrared absorbance per gram of methyl ester. Half a millilitre of the methyl ester in carbon disulphide solution was evaporated to dryness (40°C under nitrogen in a fume cupboard) and reconstituted in 100 L of C18:0 methyl ester solution (1.014 g/L) in hexane. C18:0 methyl ester solution was used as an internal standard, where the concentrations of the geometric isomers were determined using the following formula:

$$\frac{\text{corrected peak area}}{\text{corrected C18:0 peak area(internal standard)}} \; x \; wt\ \%\ of\ C18{:}0(internal\ standard)\ x\ R$$

where R is the detector response factor for C18:2 and C18:3 relative to C18:0. These are 0.986 and 0.979 for C18:2 and C18:3 respectively (Appendix 1).

Furthermore, the original geometrical isomer mixtures were also analysed and each eluting isomer was correlated to its location on the Ag-TLC plate.

The final assignment of the geometric isomer peaks in the GC trace was made by comparison with published literature data from various workers using similar polar phase capillary columns.

7.3 RESULTS AND DISCUSSION

7.3.1 *cis/trans* isomerisation using nitric acid

The capillary GC trace for the optimised separation of the various C18:2 and C18:3 geometric isomers is given in Figure 16. The relative proportions of the individual isomers are given in Table 28. Their identifications are described later.

C18:2 Geometric Isomers (%)				C18:3 Geometric Isomers (%)						
tt	ct	tc	cc	ttt	ctt	tct	ttc	ctc	tcc	ccc
4.1	10.2	11.2	74.4	1.9	4.8	5.6	20.0	12.2	12.9	42.3

Table 28 The relative proportions of the individual C18:2 and C18:3 geometric isomers as a percentage of the total C18:2 and C18:3 isomers respectively.

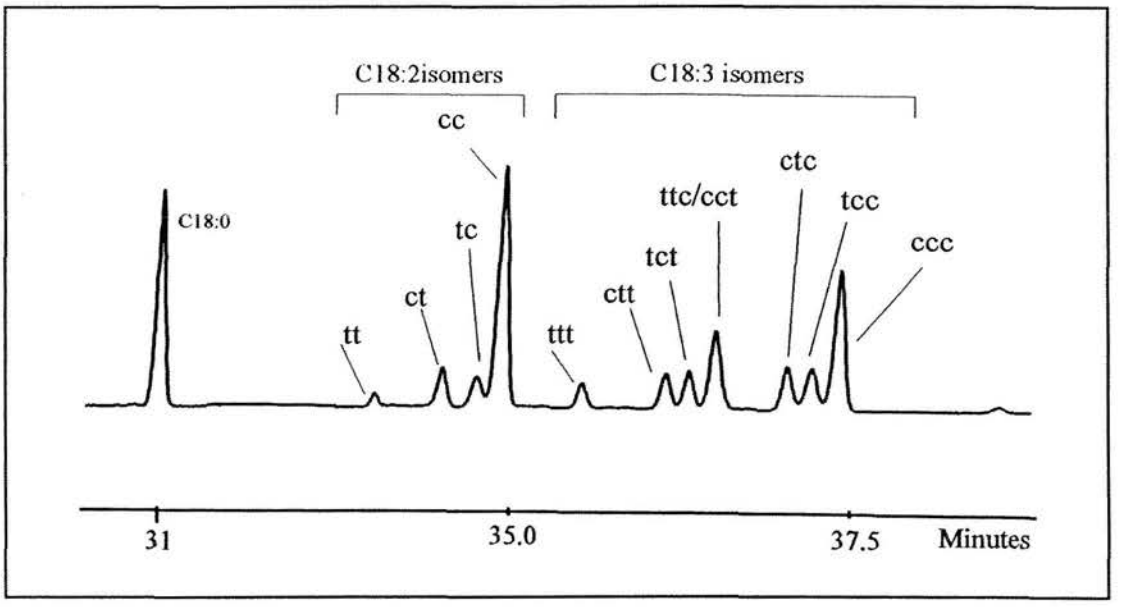

Figure 16 Gas chromatogram of the C18:2 and C18:3 geometric isomers produced by nitric acid isomerisation. The GC conditions are given in Table 20.

The relative amounts of the various isomers produced by the nitric acid isomerisation method were sufficient for use in this study. The proportions obtained were different from those reported by Grandgirard *et al.* (1987), even though the same isomerisation conditions were used. This group of workers reported a much higher amount of C18:3 ttt isomer (10%), with a lower amount of C18:3 ccc isomer (15%). The relative amount of each isomer produced by the

nitric acid isomerisation process is known to be greatly influenced by a number of conditions such as temperature, concentrations of reagents and time (Grandgirard *et al.*, 1987). Strict controls were required to maintain these conditions for reproducibility. Such stringent controls were not necessary for this study.

7.3.2 Identification of C18:2 and C18:3 geometric isomers by Ag-TLC

The C18:2 and C18:3 geometric isomers were separated into a number of bands by Ag-TLC (Figure 17). Their elution patterns were similar to those reported by Strocchi & Piretti (1968) and Rakoff & Emken (1982). From these published data and the standard solutions used, the migration order of FAME groups on an Ag-TLC plate was identified as: saturated << *trans* monoenes << *cis* monoenes << dienes << trienes. The *trans*-containing unsaturated FAMEs migrated ahead of their respective *cis* counterparts.

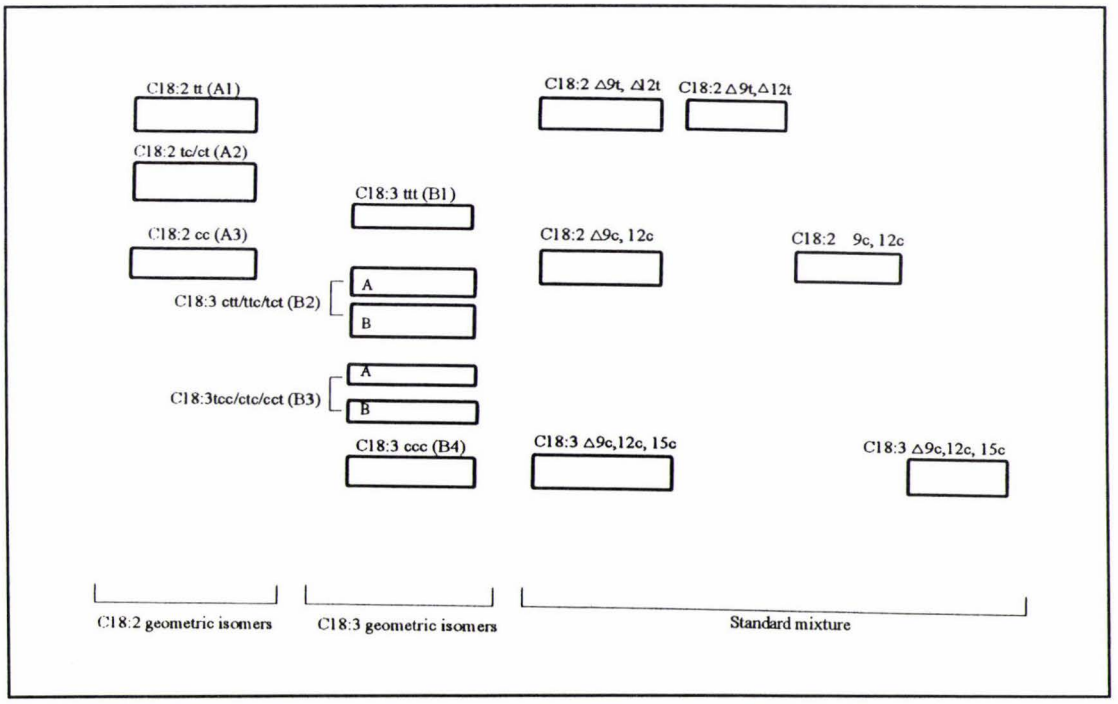

Figure 17 Ag-TLC separation using toluene/diethyl ether (95/5 v/v) of the C18:2 and C18:3 geometric isomers synthesised by nitric acid isomerisation. The identification of each band was made using published data (Strocchi & Piretti, 1968; Rakoff & Emken, 1982) and standard solutions. The assignment was made as follows: Band A1 (C18:2 tt); Band A2 (C18:2 tc/ct); Band A3 (C18:2 cc); Band B1 (C18:3 ttt); Bands B2a and B2b (C18:3 ctt; C18:3 ttc; C18:3 tct); Bands B3a and B3b (C18:3 tcc; C18:3 ctc; C18:3 cct); Band B4 (C18:3 ccc).

7.3.3 Confirmation of the presence of *trans* double bonds by IR

All of the FAME bands extracted from the Ag-TLC plate, except for Band A3 and Band B4 corresponding to the C18:2 Δ9c,12c and C18:3 Δ9c,12c,15c isomers, displayed *trans* absorbance at 965 cm^{-1} (Table 29). These IR results were consistent with the fatty acid assignment made from the Ag-TLC results (Figure 17).

Ag-TLC Band	Absorbance/g	Identification	
A1	90.9	C18:2 tt	(Class I)
A2	55.2	C18:2 ct/tc	(Class II)
A3	-	C18:2 cc	(Class III)
B1	159	C18:3 ttt	(Class I)
B2a	111	C18:3 tct	(Class II)
B2b	105	C18:3 ctt/ttc	(Class II)
B3a	47	C18:3 cct/tcc	(Class III)
B3b	46	C18:3 ctc	(Class III)
B4	-	C18:3 ccc	(Class IV)
Methyl elaidate	82	C18:1 Δ11t	

Table 29 Absolute *trans* infrared absorbance/g of fatty acids extracted from the Ag-TLC bands (from Figure 17). Absorbance conducted at 965 cm^{-1}. Samples were scanned for 2 min with a wavelength resolution of 1 cm^{-1}.

The *trans* absorbance/g also gave a good indication of the number of *trans* double bonds present. For example, the di-*trans* dienes had approximately twice the absorbance of the mono-*trans* dienes. Similar observations were made with the C18:3 geometric isomers, *i.e.* the C18:3 di-*trans* and tri-*trans* isomers had approximately two and three times the absorbance/g of the C18:3 mono-*trans* isomers respectively. Similar observations were reported by Firestone & Sheppard (1992) and Rakoff & Emken (1982).

However, it was reported that the absorbance/g of *trans* monoene C18:1 Δ11t

(elaidate) was not additive. In fact, mono-*trans* dienes have about 85% as much absorption as elaidate whereas di-*trans* dienes have about 165% (Scholfield *et al.*, 1963). The figures reported in this study, although not identical, displayed a similar trend. These were 67% and 110% respectively. The amounts of sample available for IR analysis were small; as a result, large errors were introduced (coefficients of variation of 1-9% were observed with our measurements, Table A4.1, Appendix 4). The discrepancies observed between our results and that obtained from published data may be the result of the high assay error.

7.3.4 Capillary GC identification

The GC analysis of each separated Ag-TLC band and the original geometric isomer mixtures are given in Figure 18 (for C18:2) and Figure 19 (for C18:3). Together with the Ag-TLC information, IR data and GC data published by Wolff (1992a, 1992b) using a similar polarity column (100% cyanopropyl polysiloxane film) and Rakoff & Emken (1982) and Grandgirard *et al.* (1987) using a *Silar* 10C Quadrex glass capillary column, the following elution order was concluded: C18:2 Δ9, Δ12 geometric isomers: tt < ct < cc (Figure 18) and C18:3 Δ9, Δ12, Δ15 geometric isomers: ttt < ctt < tct < ttc/cct < ctc < tcc < ccc (Figure 19).

One group of C18:3 geometric isomers, the ttc and cct, were found to co-elute under these current GC conditions but were clearly separated by Ag-TLC (Figure 19).

Identification could be made easier with a GC-FTIR instrument, where the eluting GC peaks would be subjected to IR analysis. Unfortunately this was not available in New Zealand during this work.

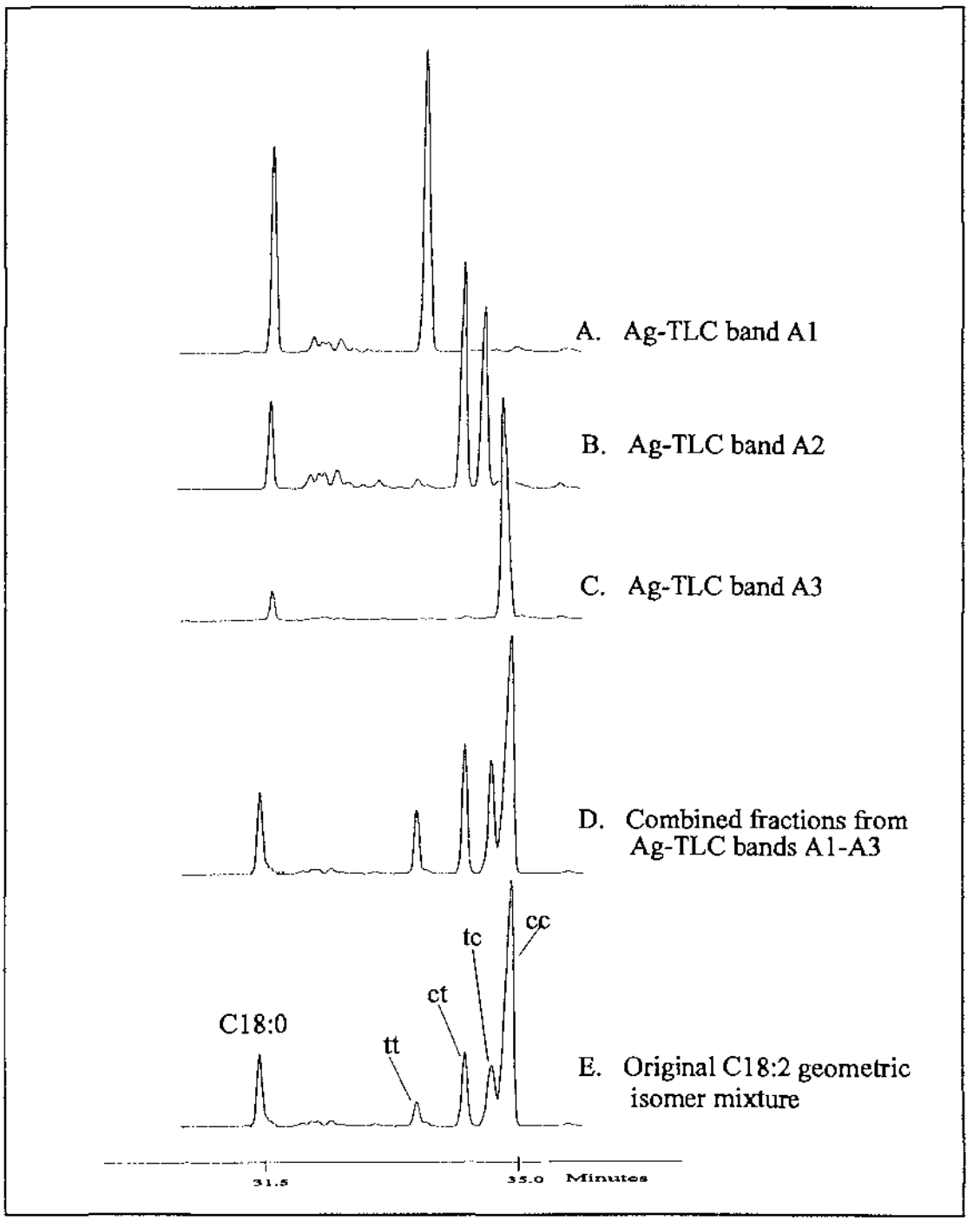

Figure 18 Gas chromatograms of the various C18:2 geometric isomers separated by Ag-TLC (from Figure 17). Each extracted band (A-C) was reconstituted in hexane and analysed using capillary GC under the conditions given in Table 20. The GC traces of the three combined bands and the original isomer mixture are given in chromatograms D and E. C18:0 was added as an internal standard for quantification purposes.

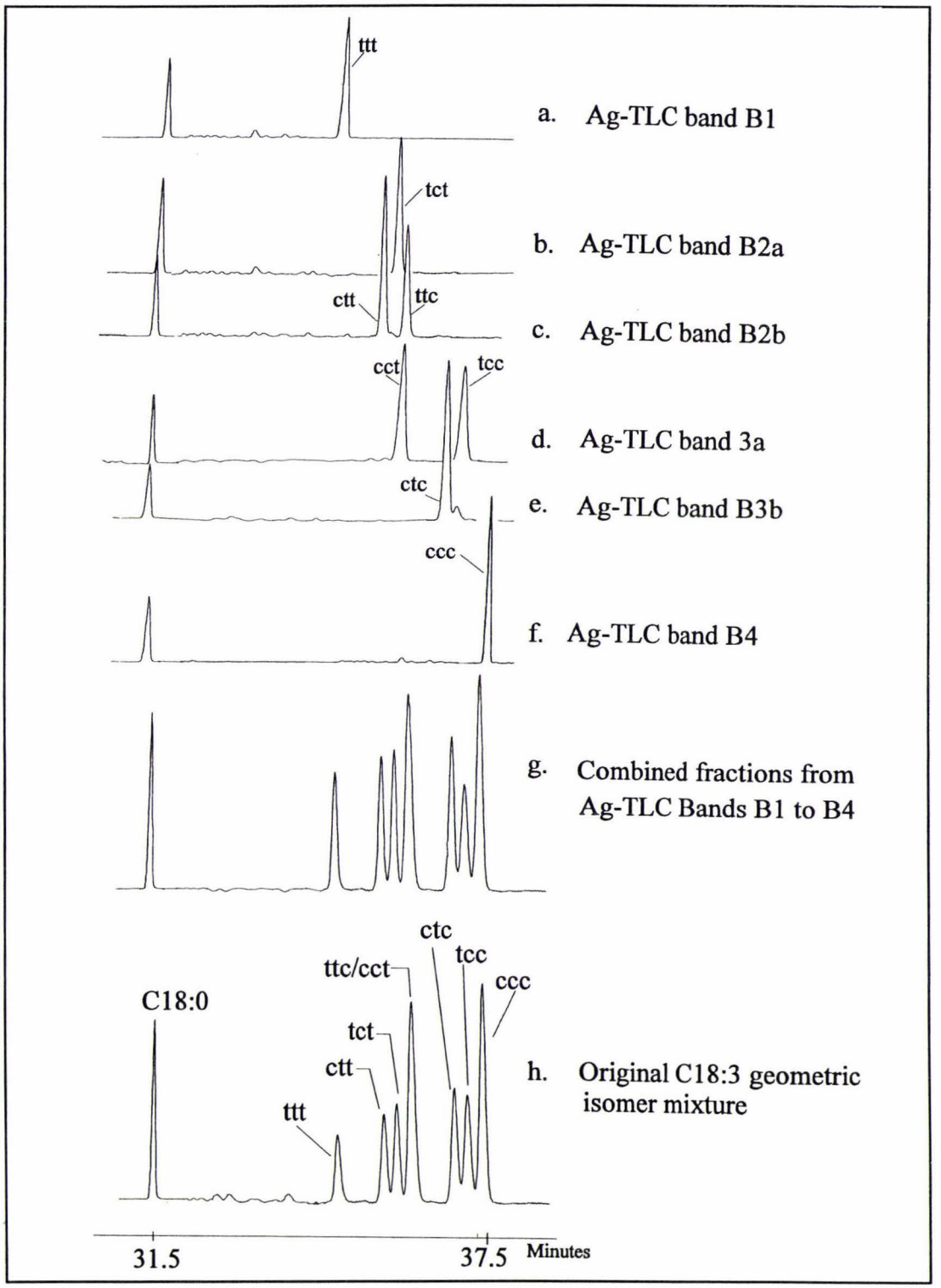

Figure 19 Gas chromatograms of the various C18:3 geometric isomers separated by Ag-TLC (from Figure 17). Each extracted band (a-f) was reconstituted in hexane and analysed using capillary GC under the conditions given in Table 20. The GC traces of the four combined bands and the original isomer mixture are given in chromatograms g and h. C18:0 was added as an internal standard for quantification purposes.

7.4 CONCLUSION

The geometric isomers of linoleic (C18:2) and linolenic acid (C18:3) were successfully synthesised using nitric acid isomerisation. The identification of the various geometric isomers was achieved using a combination of capillary GC, IR and published literature data. These isomers were later used as reference samples for peak identification (by retention time) for the identification of similar isomers in the New Zealand food survey samples, the New Zealand and overseas butters and the hydrogenated soya bean oil samples.

8 PRECONCENTRATION AND QUANTIFICATION OF C18:2 AND C18:3 GEOMETRIC ISOMERS

8.1 INTRODUCTION

The amounts of C18:2 and C18:3 *trans* geometrical isomers present in food fats are generally very low: trace levels to 4% in partially hydrogenated vegetable oils and usually less than 1% in animal or dairy fats (Ratnayake *et al.,* 1993). These low levels, particularly in animal fats, to near the detection limits of the GC analytical methods increase the uncertainties of the measured results. Preconcentration of these *trans* polyunsaturated fatty acids to a higher concentration level is therefore required. Two techniques have generally been used for obtaining enriched fractions of polyunsaturated fatty acids: Ag-TLC, as reported earlier in this study, and urea adduct formation concentration techniques.

Urea adduct formation has been reported for quantitative preconcentration of polyunsaturated fatty acids by Murawski & Egge (1975) and Christie (1989a).

Urea, when permitted to crystallise in the presence of certain long chain aliphatic compounds, forms hexagonal crystals with a channel, into which the aliphatic compounds may fit, provided they do not contain functional groups that increase their bulk, and hence they are removed from solution. Such crystals are known as urea inclusion complexes. Saturated straight chain fatty acids (or as their methyl ester derivatives) form these complexes readily. On the other hand, the double bonds of unsaturated fatty acids increase their bulk so that monoenoic fatty acids do not form complexes easily, but tend to form them more readily than dienes, which in turn form them somewhat more easily than compounds with three or more double bonds. However, fatty acids with double bonds of the *trans* configuration form complexes before the analogous compounds with *cis* double bonds (Strocchi & Bonaga, 1975).

Unfortunately this preconcentration technique is complicated by the fact that shorter chain length fatty acids (C4:0 to C8:0) do not complex as readily as do higher chain length fatty acids (Iverson & Weik, 1967). Although the technique was presented to be fairly simple and robust, the recoveries of the various C18:2

and C18:3 geometric isomers were not reported. Their recoveries were determined in this study before the technique could be used as an alternative preconcentration method over Ag-TLC/GC.

8 .2 MATERIALS AND METHODS

8.2.1 Chemicals

All chemicals used were of analytical grade unless otherwise specified.

8.2.2 Urea adduct formation preconcentration

The C18:2 and C18:3 geometric isomers (prepared as described in Section 7) were subjected to urea preconcentration. Approximately 0.5 g of the methyl esters were dissolved in methanol (5 mL) to which urea (1 g) was added. The mixture was warmed in a 70°C water bath until all the urea was in solution, and then allowed to cool to ambient temperature overnight (a minimum of 4 h was required; Christie, 1989a). The crystallised urea was filtered through a sintered Buchner funnel and then washed twice with 3 mL of methanol saturated with urea (20% w/v). The filtrate solution enriched with the polyunsaturated FAMEs was transferred quantitatively into a separation funnel (50 mL) containing 6 mL of aqueous hydrochloric acid (1% v/v). The polyunsaturated FAMEs were extracted from the aqueous solution twice with hexane (10 mL). The combined organic layers were washed twice with water (10 mL) and dried over anhydrous sodium sulphate before the solvent was removed under reduced pressure (with a rotary evaporator). The residual solvent was evaporated under a stream of nitrogen. The samples were stored under nitrogen in a freezer before capillary GC analysis.

A solution (approximately 5 mg/mL) of each group of preconcentrated geometric isomers (before and after urea preconcentration) was prepared in hexane. An aliquot (0.05-0.1 L) was injected into the capillary GC system. The capillary GC instrumentation and operating conditions were identical to those described in Sections 6 and 7.

The recoveries of the C18:2 and C18:3 geometric isomers were determined by

comparing the peak area ratios (peak area of the individual isomers relative to the peak area of their all-*cis* isomers, *e.g.* $[C18:2\ tt]_{peak\ area}/[C18:2\ cc]_{peak\ area}$), before and after urea preconcentration. A 100% recovery of the all *cis* isomers was assumed.

8.2.2 Ag-TLC/GC

Preconcentration by Ag-TLC was an alternative technique to the urea preconcentration. The Ag-TLC procedures were identical to that described earlier (Section 4). The Ag-TLC plates were developed in toluene at ambient temperature. The final methyl esters were reconstituted in 100 L of hexane. An aliquot (0.05-0.1 L) was injected into the capillary GC system. Where the analyte levels were lower, samples were reinjected with a larger injection volume (up to 0.2 L). The capillary GC instrumentation and operating conditions were identical to those described in Sections 6 & 7.

The calculation procedures were analogous to that used for the C18:1 *trans* isomers (Section 6). The C18:2 and C18:3 all-*cis* isomers were used as endogenous internal standards, to correct for any losses occurring during the extraction step. It was assumed that all the respective geometric isomers were lost at an equivalent rate. The GC formulae used were:

(i) for C18:2 geometric isomer:

$$= \frac{peak\ area\ of\ trans\ C18{:}2\ isomer}{peak\ area\ of\ C18{:}2\ cc\ (internal\ standard)} \times wt\%\ of\ C18{:}2\ cc\ (original\ sample^{*})$$

(ii) for C18:3 geometric isomer:

$$= \frac{peak\ area\ of\ trans\ C18{:}3\ isomer}{peak\ area\ of\ C18{:}3\ ccc\ (internal\ standard)} \times wt\%\ of\ C18{:}3\ ccc\ (original\ sample^{*})$$

*determined from standard FAME composition

Repeatability

The repeatability of this Ag-TLC procedure for C18:2 and C18:3 geometric isomer quantification was established using the AMF-QC and MARG-QC samples.

8.3 RESULTS

8.3.1 Urea preconcentration

The C18:2 and C18:3 geometric isomer recovery results for the urea preconcentration method are given in Tables 30 and 31 respectively. The recoveries of the various C18:2 and C18:3 geometric isomers were found to occur at different rates. A 10-40% recovery was found for the C18:2 geometric isomers, whereas the C18:3 geometric isomers recorded a recovery from 20 to 100%.

Sample	(C18:2 *trans* Isomer / all-*cis* Isomer) Peak Area Ratio		
	tt	ct	tc
Before UPC	0.09	0.26	0.26
(Standard deviation)[1]	(0)	(0)	(0.02)
After UPC	0.01	0.06	0.10
(Standard deviation)[2]	(0)	(0.01)	(0)
% Recovery	10%	23%	38%

Table 30 Effect of urea preconcentration (UPC) on the recovery of C18:2 geometric isomers. Standard deviations are given in brackets. [1]Standard deviation from two determinations, [2]Standard deviation from four determinations.

Sample	(C18:3 *trans* Isomer / all-*cis* Isomer) Peak Area Ratio					
	ttt	ctt	tct	ttc/cct	ctc	tcc
Before UPC	0.05	0.12	0.14	0.48	0.33	0.31
(Standard deviation)[1]	(0)	(0)	(0.01)	(0.01)	(0.05)	(0.01)
After UPC	0.02	0.13	0.03	0.35	0.11	0.12
(Standard deviation)[2]	(0.01)	(0.01)	(0.02)	(0.02)	(0.01)	(0.01)
% Recovery	40%	100%	21%	73%	33%	39%

Table 31 Effect of urea preconcentration (UPC) on the recovery of C18:3 geometric isomers. Standard deviations are given in brackets. [1]Standard deviation from four determinations, [2]Standard deviation from two determinations.

8.3.2 Repeatability of the Ag-TLC/GC procedure for C18:2 and C18:3 geometric isomer determination

The repeatability standard deviations determined using the AMF-QC and MARG-QC samples for the Ag-TLC method are given in Table 32.

	C18:2 Isomers (g/100 g fat)				C18:3 Isomers (g/100 g fat)						
	tt	ct	tc	cc	ttt	ctt	tct	ttc/cct	ctc	tcc	ccc
AMF-QC	0.11 (0.01)	0.04 (0.01)	0.02 (0.01)	1.58	0.03 (0.01)	0.08 (0.01)	0.01 (0.01)	0.02 (0.01)	nd	nd	1.01
MARG-QC	0.13 (0.01)	0.49 (0.04)	0.24 (0.02)	43.08	0.02 (0.01)	0.04 (0.02)	trace	0.17 (0.02)	0.04 (0.02)	0.05 (0.01)	2.64

Table 32 The repeatability standard deviations for the determination of C18:2 and C18:3 geometric isomers by the Ag-TLC/GC method. Standard deviations are given in brackets. The replicate results are given in Table A3.3, Appendix 3. nd: not detected.

8.4 DISCUSSION/CONCLUSION

A non-proportional recovery of the various C18:2 and C18:3 *trans* geometric isomers was determined for the urea adduct formation preconcentration method. As a result, this technique could not be used for the quantitative determination of these geometric isomers. The preconcentration technique using Ag-TLC was used.

This technique, although more laborious, is reliable. The large repeatability standard deviation associated with this technique is due to the extremely low level of these isomers present in the quality control samples, even after preconcentration. Under this situation, some difficulties were encountered. The C18:2 and C18:3 *trans* geometric isomer results for the New Zealand food survey fat, soya bean oil, New Zealand butter and overseas butter samples are given in Tables 33 to 36 respectively.

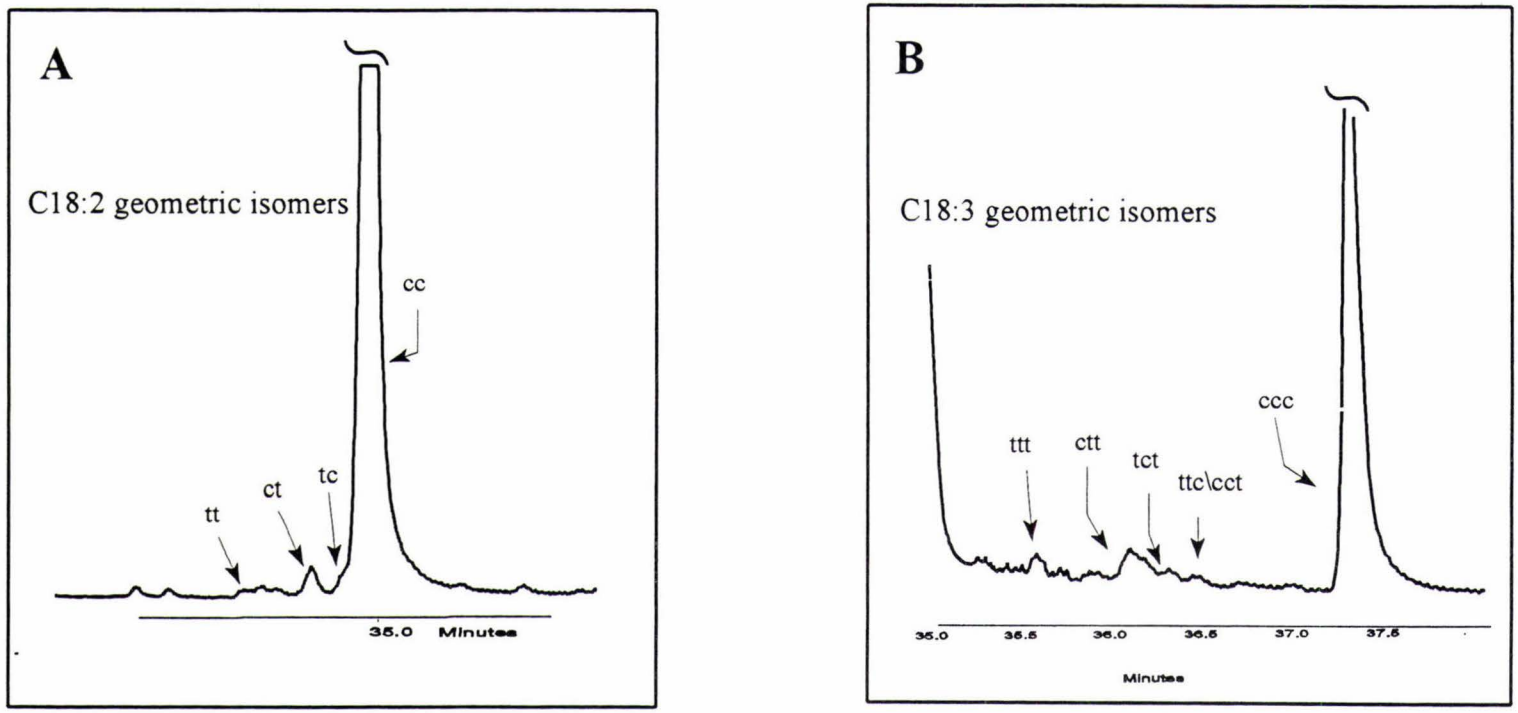

Figure 20 Gas chromatogram of A: C18:2 and B: C18:3 *trans* geometric isomers of the AMF-QC sample. The two regions were scaled to enable the low levels of the geometertic isomers to be seen.

Sample Name	Geometric Isomers of C18:2 (g/100 g fat)				Geometric Isomers of C18:3 (g/100 g fat)						
	tt	ct	tc	cc	ttt	ctt	tct	ttc/cct	ctc	tcc	ccc
Margarine A	0.20	0.55	0.28	43.03	0.03	0.38	0.05	0.26	0.05	0.13	1.22
Margarine B	0.17	0.27	0.06	43.46	0.01	0.02	nd	0.14	0.02	0.04	2.46
Commercial pastry fat A	0.08	0.10	0.02	2.87	0.02	0.02	0.06	0.02	0.01	0.01	0.31
Commercial pastry fat B	trace	0.18	0.10	13.23	trace	0.04	0.01	0.04	trace	trace	0.14
Commercial pastry fat C	0.10	0.07	0.03	3.03	trace	0.04	0.02	0.04	0.02	0.01	0.56
Pastry	0.13	0.04	0.01	6.60	0.01	0.01	0.02	0.06	0.01	0.01	0.98
Homogenised milk	0.11	0.13	nd	1.47	0.02	0.02	0.01	0.02	0.01	0.01	0.87
Reduced fat milk	0.01	0.01	0.01	1.50	0.01	0.12	nd	0.12	0.02	0.02	0.80
Butter	0.09	0.02	0.01	1.09	trace	0.04	0.01	0.02	0.01	0.01	0.73
Shortening	0.03	0.09	0.02	3.08	0.10	0.06	0.04	0.04	nd	nd	0.52
Savoury mince pie	0.08	0.09	0.01	4.75	0.02	0.04	0.03	0.04	0.02	nd	0.51
Meat patties	0.07	0.06	0.02	1.76	0.03	0.04	0.07	0.03	0.01	nd	0.75
Luncheon	0.05	0.20	nd	4.96	0.01	0.07	0.03	0.07	0.02	trace	0.99
Muesli bar	0.01	0.02	trace	2.19	trace	0.02	trace	0.01	0.01	trace	0.19
Chocolate-coated biscuits	0.47	0.09	0.05	6.35	0.01	trace	0.01	0.01	0.01	trace	0.33
Plain sweet biscuit	0.13	0.05	nd	5.30	0.01	0.06	0.01	0.08	nd	nd	0.47
Savoury cracker biscuit	0.02	0.04	nd	4.41	0.01	0.03	trace	0.01	0.01	trace	0.18
Bread	0.17	0.15	nd	43.27	0.08	0.12	0.04	0.85	0.10	0.21	4.50

Table 33 Summary of C18:2 and C18:3 geometric isomers in the New Zealand food survey fats.

Geometric Isomers of C18:2 (g/100 g fat)				
tt	ct	tc	cc	Total C18:2 geometric isomers
0.17	0.15	nd	43.27	43.59

Table 34 C18:2 geometric isomer distribution in hydrogenated soya bean oil. No C18:3 geometric isomers were detected.

Sample	C18:2 Isomers (g/100 g fat)				C18:3 Isomers (g/100 g fat)							Σ *trans* Isomers (g/100 g fat)	
	tt	ct	tc	cc	ttt	ctt	tct	ttc/cct	ctc	tcc	ccc	C18:2	C18:3
Aug 95	0.09	0.03	0.01	1.32	0.03	0.05	0.04	0.05	0.02	nd	0.95	0.13	0.19
Sep 95	0.09	0.02	0.01	1.30	0.03	0.05	0.02	0.04	0.02	nd	0.89	0.12	0.16
Nov 95	0.09	0.03	0.01	1.13	0.02	0.04	0.02	0.05	0.04	nd	0.87	0.17	0.13
Jan 96	0.08	0.03	0.01	1.28	0.03	0.04	0.01	0.03	0.02	nd	0.86	0.13	0.12
Apr 96	0.07	0.03	0.01	1.24	0.03	0.05	0.02	0.04	0.02	nd	0.85	0.16	0.11
May 96	0.08	0.02	0.02	1.27	0.02	0.04	0.01	0.03	0.02	nd	0.81	0.12	0.12

Table 35 Seasonal variations of C18:2 and C18:3 geometric isomers in New Zealand butter fat samples.

Sample Name	C18:2 Geometric Isomer (g/100 g fat)				C18:3 Geometric Isomers (g/100 g fat)							Σ *trans* C18:2 (g/100 g)	Σ *trans* C18:3 (g/100 g)
	tt	ct	tc	cc	ttt	ctt	tct	ttc/cct	ctc	tcc	ccc		
New Zealand													
- Minimum	0.07	0.02	0.01	1.13	0.02	0.04	0.01	0.03	0.02	nd	0.81	0.12	0.11
- Maximum	0.09	0.03	0.02	1.32	0.03	0.05	0.04	0.05	0.04		0.95	0.17	0.19
Devondale (Australia)	0.06	0.03	0.01	1.37	0.03	0.03	0.02	0.03	0.02	0.00	0.94	0.10	0.13
GG-lactic (Australia)	0.05	0.03	0.01	1.32	0.01	0.01	0.02	0.02	0.01	0.00	0.81	0.09	0.07
Snow (Japan)	0.01	0.08	0.00	1.79	0.01	0.01	0.03	0.01	0.01	0.00	0.47	0.09	0.07
Lady Lee (USA)	0.06	0.09	0.00	2.92	0.01	0.04	0.02	0.04	0.01	0.00	0.42	0.15	0.12
Kerry Gold (Ireland)	0.08	0.04	0.01	1.59	0.01	0.01	0.03	0.02	0.01	0.00	0.83	0.13	0.08
Lurpak (Denmark)	0.08	0.09	0.00	1.97	0.01	0.00	0.02	0.02	0.01	0.00	0.34	0.17	0.06

Table 36 Total C18:2 and C18:3 TFAs and their geometric isomer distribution in New Zealand (from seasonal survey data) and some overseas butter samples. The minimum and maximum figures for the New Zealand butters were obtained from Table 35

DISCUSSION

1 IR METHOD VS Ag-TLC/GC METHOD

The content of TFAs in fats and oils is often determined by either IR or Ag-TLC/capillary GC. The Ag-TLC/GC method is generally regarded as rather time consuming in comparison with the IR method. However, it has been considered to be a more accurate method and gives detailed information about the different fatty acids and fatty acid isomers present.

The total TFA results obtained by the four different infrared techniques were compared with those obtained by the Ag-TLC/GC method for the New Zealand foods (Table 37)

The two way ANOVA statistical analysis (Appendix 5) of the New Zealand food fats results produced by the four different infrared methods against the Ag-TLC/GC method showed that the SS-FAME and AOCS-TG and SS-TG methods gave results that were not significantly different from those of the Ag-TLC/GC method. Their respective mean results were 0.04, -0.23 and 0.155 g/100 g lower than Ag-TLC/GC results. The AOCS-FAME method on the other hand produced results that were significantly lower than the Ag-TLC/GC results by an average of -0.82 g/100 g. The greatest variations in the data were observed for the biscuit (chocolate-coated biscuit and the savoury cracker) and muesli bar samples. Both the savoury cracker and the muesli bar appeared to contain coconut oil (Table 37), which has been reported to cause a negative bias in infrared measurements, apparently associated with a high proportion of lauric acid content (Firestone & Labouliere, 1965; Deman & Deman, 1983). A similar problem was indeed reported by Ulberth & Haider (1992), where these workers encountered a negative total *trans* result with low level *trans* coconut-oil-based shortening using the AOCS Cd 14-61 method. They successfully circumvented the problem by using a *trans* free coconut fat as background for spectral subtraction.

All current infrared methods are based on the use of methyl elaidate (or trielaidin) as an external standard. However, it has been long known that the TFAs in hydrogenated oils do not absorb to the same extent as those of methyl elaidate.

Firestone (1996a/b) suggested that the actual content of TFAs in hydrogenated oils would be greater than that determined using methyl elaidate as an external

Sample	Total *trans* by Ag-TLC/GC (g/100 g fat)	Total *trans* by IR			
		AOCS-FAME	SS-FAME	AOCS-TG	SS-TG
Margarine A	15.57	14.33	14.39	17.65	17.70
Margarine B	15.43	14.66	15.85	15.81	15.85
Commercial pastry fat A	6.72	6.56	B.81	7.88	8.24
Commercial pastry fat B	8.04	5.42	7.66	7.04	7.40
Commercial pastry fat C	7.02	7.02	B.54	6.13	6.44
Pastry "Fether Flake"	6.20	5.62	5.65	6.56	6.56
Homogenised milk	5.26	4.78	4.81	6.97	6.87
Reduced fat milk	6.03	5.76	7.44	6.59	6.59
Butter	6.58	6.72	6.82	8.35	8.41
Shortening	4.47	4.57	4.56	5.00	4.99
Savoury mince pie	3.76	3.92	3.90	4.27	4.22
Meat patties	3.42	4.32	4.37	4.09	4.04
Luncheon	5.87	4.98	6.09	6.35	6.39
Muesli bar	2.00	0.89	0.98	1.37	1.27
Chocolate-coated biscuits	3.65	1.70	1.74	0.70	0.65
Plain sweet biscuits	6.51	4.50	5.61	5.01	5.06
Savoury cracker biscuits	5.64	0.71	1.07	1.02	0.97
White bread	4.41	2.19	2.33	2.51	3.42

Table 37 Comparison of total TFA results for the New Zealand food survey fats obtained using the Ag-TLC/GC method and the four different infrared methods: AOCS-FAME, SS-FAME, AOCS-TG and SS-TG.

standard, and that a FAME mixture derived from a partially hydrogenated oil might be a better external standard. This hypothesis was tested using a hydrogenated canola oil as an external standard (the actual *trans* content was determined by Ag-TLC/capillary GC). Margarines with total isolated *trans* content varying from 3 to 48% were examined by the AOAC method 994.14 (AOAC, 1995a) using both the methyl elaidate and hydrogenated canola oil external standards. The results obtained with the hydrogenated canola oil external standard were closer to the "actual *trans*" values (*e.g.* for a margarine with an "actual" *trans* value of 3.0%, the results were 2.3% with methyl elaidate standard vs 2.8% with the hydrogenated canola oil external standard). Actual values were determined by Ag-TLC/GC.

Furthermore, the IR methodologies assume that the *trans* monoene acids with double bonds in other than the Δ9 position have the same infrared absorbance as the calibrant elaidic acid (Δ9t). Studies including those in this work show that the mono-*trans* diene has about 85% as much absorption as elaidate, and that *trans, trans* diene has about 165% (Schofield, 1979). The infrared absorption maxima for the various polyunsaturated *trans* isomers have also been reported to vary (Wolff & Miwa, 1965). These workers reported that *trans* groups exhibit a characteristic absorption maximum in the 1000-909 cm^{-1} (10-11 μm) range; the most widely used IR methods of analysis for isolated *trans* double bonds are based on absorption maxima at 965 cm^{-1} (about 10.35 μm). For example; λ_{max} for *trans, trans:* 988 cm^{-1} (10.12 μm.);λ_{max} for *trans, trans, trans:* 994 cm^{-1} (10.06 μm.); λ_{max} for *cis, trans, trans:* 991 cm^{-1} (10.09 μm.).

From this study and other similar studies (Ratayanake *et al.*, 1993; Mansour & Sinclair, 1993; Lund & Jensen, 1982), the infrared method will provide a rapid method for analysis of total *trans* in fat and oil samples. It has been used widely in the fats and oils industry because it is less laborious and easily adapted for routine use in the laboratory. The Ag-TLC/GC method, although more laborious, is more accurate (not prone to errors associated with IR) and is preferred by scientists interested in the metabolism of these TFA isomers, because they do not all exert the same hazards on biological systems. The choice of methods (either IR or Ag-TLC/GC) depends on the accuracy and information required. Furthermore, the Ag-TLC/GC method does not involve the use of a highly toxic solvent, carbon disulphide. This has always been a problem with the current IR methods. The recent development of the horizontal attenuated total reflectance (HATR) technique for FTIR appears to circumvent this problem, allowing the neat sample to be analysed directly. The HATR crystal used must be transparent in the infrared region of interest and resistant to water. Zinc selenide (ZnSe) provides both these requirements and has generally been employed for *trans* analysis using this technique (Firestone, 1996a). In this technique, the infrared beam is passed though the crystal, which has a high refractive index, and undergoes total internal reflection (Figure 21). When the sample is in close contact with the surface of the crystal, the infrared beam is attenuated at the frequencies at which the sample absorbs. HATR produces vibrational bands that absorb at the same frequencies as those obtained by transmission. However, the relative intensities of the

absorption peaks in the two spectra are different. In HATR, the depth of penetration of the infrared beam into the sample increases with the wavelength, which explains the difference in the relative intensities of the absorption bands (Sadeghi-Jorabchi, 1994). Generally, this is corrected by the software algorithm accompanying the instrumentation. HATR techniques were not used in this study.

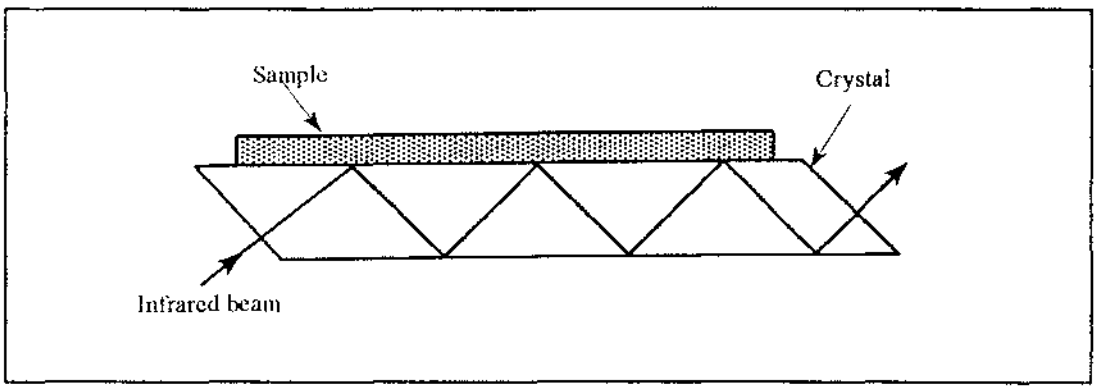

Figure 21 Horizontal attenuated total reflectance. The HATR crystal is designed to enable total internal reflection which creates an evanescent wave at the crystal surface. This wave extends into the sample held in intimate contact with the crystal. Absorption spectra can be recorded as a result. The depth of penetration of the evanescent wave into the sample decreases with an increase in the wavelength and requires correction.

2 TFAS IN NEW ZEALAND FOODS

The total TFA data (Table 37) used in this discussion were based on the standard AOCS-FAME and/or Ag-TLC/GC data. This allowed for a comparison with other published data that were based on similar methods.

Margarine

Of the food products analysed in this survey, the two margarines gave the highest TFA content in their fat (14.3-15.6%). The levels of the total TFAs determined by infrared analysis of margarines in western countries vary widely from 0 to 50%. American margarines contain from 0 to 40% (Litin & Sacks, 1993; Michels & Sacks, 1995) and up to 73% has been reported (Sommerfield, 1983). Total *trans* isomers reported for Canadian margarines were also of the same order (Table 38). In contrast, a range from 9.2 to 16.3% was reported for 13 common margarines available in Australia (Mansour & Sinclair, 1993). Similar results were reported for four New Zealand margarines (Ball *et al.*, 1993). The results produced in this study were also in the same range.

In New Zealand, the food legislation requires margarine to have not less than 40% polyunsaturated fatty acid content; consequently no highly hydrogenated margarines with very high *trans* contents are available on the New Zealand market.

Table 38 summarises the total *trans* isomer data for both margarines and butters reported by various workers.

Dairy products

The *trans* contents of milkfat in the milk and butter samples reported in this survey were 4.8-5.8 and 6.7% respectively (Table 37). These values are similar to those reported in both Australia and Northern Hemisphere countries (Table 38).

The total TFA content of milkfat is known to show both geographical and seasonal variations (Sommerfield, 1983). A seasonal survey of 116 Australian

milkfat samples showed that the lowest *trans* levels of 4.3-4.9% were recorded in winter, and the highest levels of 6.5-7.5% were recorded in spring-summer (Parodi & Dunstan, 1971). A similar seasonal study by Wolf (1994) reported, for French butters, a mean of 3.22% in autumn and a mean of 4.28% in spring. However, Gray (1973) reported data indicating a winter maximum of 7.31% and a summer minimum of 4.38% for the Manawatu region of New Zealand. MacGibbon (1993), in a New Zealand wide survey, found a spring maximum of 6.85% measured in August-September 1987, and a summer minimum of 2.6%, measured in January 1988. These seasonal variations are believed to be due to the differences in the feedstuffs, with spring and summer pastures containing more polyunsaturated fatty acids than the winter feed supply; in ruminants, *trans* unsaturated fatty acids are synthesised in the rumen by microbial hydrogenation of polyunsaturated fatty acids (Craig-Schmidt, 1992).

The butter sample analysed in the study was manufactured from milk obtained during the New Zealand spring period. The high *trans* figure measured for this sample was consistent with the seasonal trend reported by and MacGibbon (1993).

Sample	Total *trans* Isomer	Gas Chromatography		Source	Reference
		trans C18:1	*trans* Polyenes (%)		
Margarine	10.1 - 49.9 (50)	10 - 40 (50)	trace - 8.3	Canada	Ratnayake *et al.* (1991)
	11.7 - 50.2 (7)	11.7 - 40.8 (9)	trace - 7.4	Canada	Ratnayake *et al.* (1990)
	-	7 - 31 (39)	0.0 - 5.2	USA	Enig *et al.* (1983)
	9.2 - 16.3 (13)	7.51 - 13.22(13)	0.94 - 1.36	Australia	Mansour & Sinclair (1993)
	-	0.5 - 18.6 (5)	-	various	Michels & Sacks (1995)
	9 -15 (4)	7.3 - 11.3 (8)	0.3 - 0.7	New Zealand	Ball *et al.* (1993)
	6.2 - 35.5 (30)			S. Korea	Cho & Sugano (1985)
	14.3 - 14.7 (2)	13.64 - 14.70 (2)	0.73 - 1.93	New Zealand	This study
Butter	3.8 - 6.4 (2)	2.9 - 5.6 (2)	0.9	Canada	Ratnayake *et al.* (1993)
	-	3.1 - 3.8 (3)	-	USA	Enig *et al.* (1983)
	3.2 - 4.1 (1)	2.3 - 3.4 (1)	0.89 - 1.39	Australia	Mansour & Sinclair (1993)
	-	1.75 - 5.20 (31)	0.6-2.30	Austria	Henninger & Ulberth (1994)
	-	3.4 (1)	-	USA	Michels &Sacks (1995)
	4.27 - 7.64 (116)	-	-	Australia	Parodi & Dunstan (1971)
	-	4.38 - 7.31 (17)	-	New Zealand	Gray (1973)
	-	2.6 - 6.8 (55)	-	New Zealand	MacGibbon (1993)
	-	2.46 - 5.10 (24)	-	France	Wolff (1994)
	-	1.6 - 2.8 (2)	0.2 -0.4	New Zealand	Ball *et al.* (1993)
	6.72 (1)	6.37 (1)	0.21	New Zealand	This study

Table 38 Reported *trans* isomer content in margarines and butters by various workers. Numbers of samples analysed are given in parentheses.

Meat products

The total TFA contents for the processed meat products in the survey (pie, meat pattie and luncheon) were 3.8, 3.4 and 5.9% respectively (Table 37). Typical levels of 1.8-6.6% have been previously reported for beef fat in the USA (Enig *et al.*, 1983; Craig-Schmidt, 1992). Similar levels of 0.75-3.54% were also reported

recently for European beef fat (Wolff, 1995). No seasonal trend or feed related study on TFA levels in beef fat has been reported, but it is known that the *trans* level in cow's milkfat varies with the season, feed type and quality. A similar phenomenon has been hypothesised to occur with *trans* in beef fat (Wolff, 1995).

Lamb fat has been reported to contain more *trans* than beef fat (Enig *et al.*, 1983) whereas lard, because it is derived from a non-ruminant source, contains much less. The levels reported for lard or pork fat are of the order of 0.2-0.3% (Enig *et al.*, 1983). It is therefore fair to assume that, for luncheon meat, which contains beef, pork and mutton, the major sources of the TFAs present were beef and mutton fat.

Pastry fats

The TFA isomers reported for the three commercial pastry fats and for the pastry samples were in the range 6.2-8.0% (Table 37). These levels are much lower than those reported by Enig *et al.* (1983) for USA pastries and pastry crust (trace to 34.6% *trans*). The fat sources reported for these pastries and pastry crust were generally partially hydrogenated cotton seed oil, palm oil, soya bean oil, coconut oil, beef and lard fat. Similar results were reported with pastry fat for Pizza crusts in a Canadian study (22-38.8%) where partially hydrogenated vegetable oils were used in the pastry fats (Ratnayake *et al.*, 1993). Generally, pastry fats of animal or unhydrogenated vegetable oils source have much lower TFA levels, similar to those reported in milkfat or meat products (Enig *et al.*, 1983). New Zealand pastry products are usually based on animal fat rather than hydrogenated vegetable oil as is the common practice overseas (David Illingworth, 1995, personal communication). This may account for the much lower *trans* isomer level measured in the pastry fats evaluated. Only commercial pastry fat C indicated the presence of vegetable oils (Table 11).

Shortening

Total *trans* values typically reported for shortening made from hydrogenated vegetable oils range from 8 to 50%. Much lower levels are generally reported for shortening made from animal fat (Table 39). The result reported for the

shortening sample made from beef fat in this study was 4.5%.

Fat Source	Infrared	Gas Chromatography		Reference
	Total *trans*	*trans* C18:1	*trans* Polyene (C18:2 and C18:3)	
Vegetable		8.7-50.6	0.3-8.2	Enig *et al.* (1983)
Vegetable	16.6-29			Scholfield *et al.* (1967)
Animal		2.2-2.7	0.2-0.4	Ratnayake *et al.* (1993)
Animal (beef)	4.57	4.09	0.38	This study
	4.57			

Table 39 TFA levels in shortening samples of various fat sources.

Biscuits and muesli bar

Relatively low levels of total *trans* isomers were found in the fat present in the biscuits and muesli bar in this survey (biscuits: 3.7 - 6.5%, muesli bar: 2.0%). Like most processed food products, the level of *trans* present depends on the source of fat. The TFA levels reported for biscuits (cookies and crackers) made with partially hydrogenated fat available in the US and Canadian market ranges from 4 to 40%, whereas those made from animal or unhydrogenated oils generally have much lower *trans* levels (2.6-6.4%), (Enig *et. al.* 1983; Ratnayake *et al.*, 1993).

Bread

The TFA level was relatively low for the bread analysed in this food survey (4.4%). The fat for this product was of vegetable origin. Similar levels have been reported where animal or unhydrogenated vegetable oils were used (Enig *et al.*, 1983; Ratnayake *et al.*, 1993).

Partially hydrogenated soya bean oil

The TFA level (54 g/100 g fat) in the partially hydrogenated soya bean oil sample analysed in this study was reflective of the typical levels normally reported for

overseas products. This experimental oil was analysed to provide an example of an upper TFA level not generally found for New Zealand fats and oils.

3 C18:1 *trans* POSITIONAL ISOMER DISTRIBUTION IN BUTTER, MARGARINE AND OTHER PROCESSED FOOD FAT PRODUCTS

Hydrogenated vegetable oils as well as fat from ruminant animals may contain as many as 20 *cis* and *trans* positional isomers of C18:1 fatty acid. The distribution of these positional isomers in both margarines and ruminant fats has been well studied by several investigators (Scholfield *et al.,* 1967; Carpenter & Slover, 1973; Sampugna *et al.*, 1982; Hay and Morrison, 1970; Wood, 1983). Figure 22 compares the distribution of positional isomers in margarine and butter, using data from Sampugna *et al.* (1982) and Parodi (1976) respectively and those obtained for the AMF-QC and MARG-QC samples. A similar distribution was observed of the margarine and milkfat samples analysed in this study (Figure 23). Furthermore, the C18:1 *trans* positional isomer distribution pattern of beef fat (tallow) was shown to closely resemble that of milkfat in this study, and those reported by Bayard & Wolff (1996). In both butter and margarine, C18:1 TFAs with double bonds in positions Δ6-Δ16 are present. For butter, the predominant *trans* isomer is vaccenic acid (C18:1 Δ11t), making up 50-60% (Parodi, 1976). The figures observed in our study were of the same order, with 49-60% recorded for the seasonal trend studies (Table 25), whereas the homogenised milk and reduced fat milk samples recorded 52% and 55% respectively.

In a typical margarine, the positional isomers are generally normally distributed, with the Δ9-Δ12 positional isomers making up 70% to 80% of total C18:1 TFAs However, the exact distributions of the positional isomers will vary depending upon the conditions of the hydrogenation process. The distributions of the positional isomers of the MARG-QC sample and the two margarine samples (A and B) are given in Figures 22 and 23 respectively. For margarine A, a larger proportion of the positional isomers were present at the Δ6-Δ9 positions (43%) compared with margarine B, which had approximately 28%.

The distribution of the C18:1 positional isomers for the remaining New Zealand food survey fats are depicted as bar graphs in Figure 23. Because of the distinct distribution of C18:1 *trans* positional isomers in hydrogenated/partially hydrogenated and ruminant fats, the C18:1 *trans* profile can be used as a tool for identification of possible fat sources used in the various processed food products.

Although the C18:1 *cis* positional were not investigated in this study, it is well documented that oleic acid (9Δc) is the predominant *cis* isomer present. In butter, this *cis* isomer comprises of 95% of the total C18:1 *cis* fraction; in margarine, the *cis* isomers are more widely distributed amongst the Δ8-Δ12 positions, with the Δ9c isomer typically comprising approximately 60% (Parodi, 1976; Sampugna *et al.*, 1982).

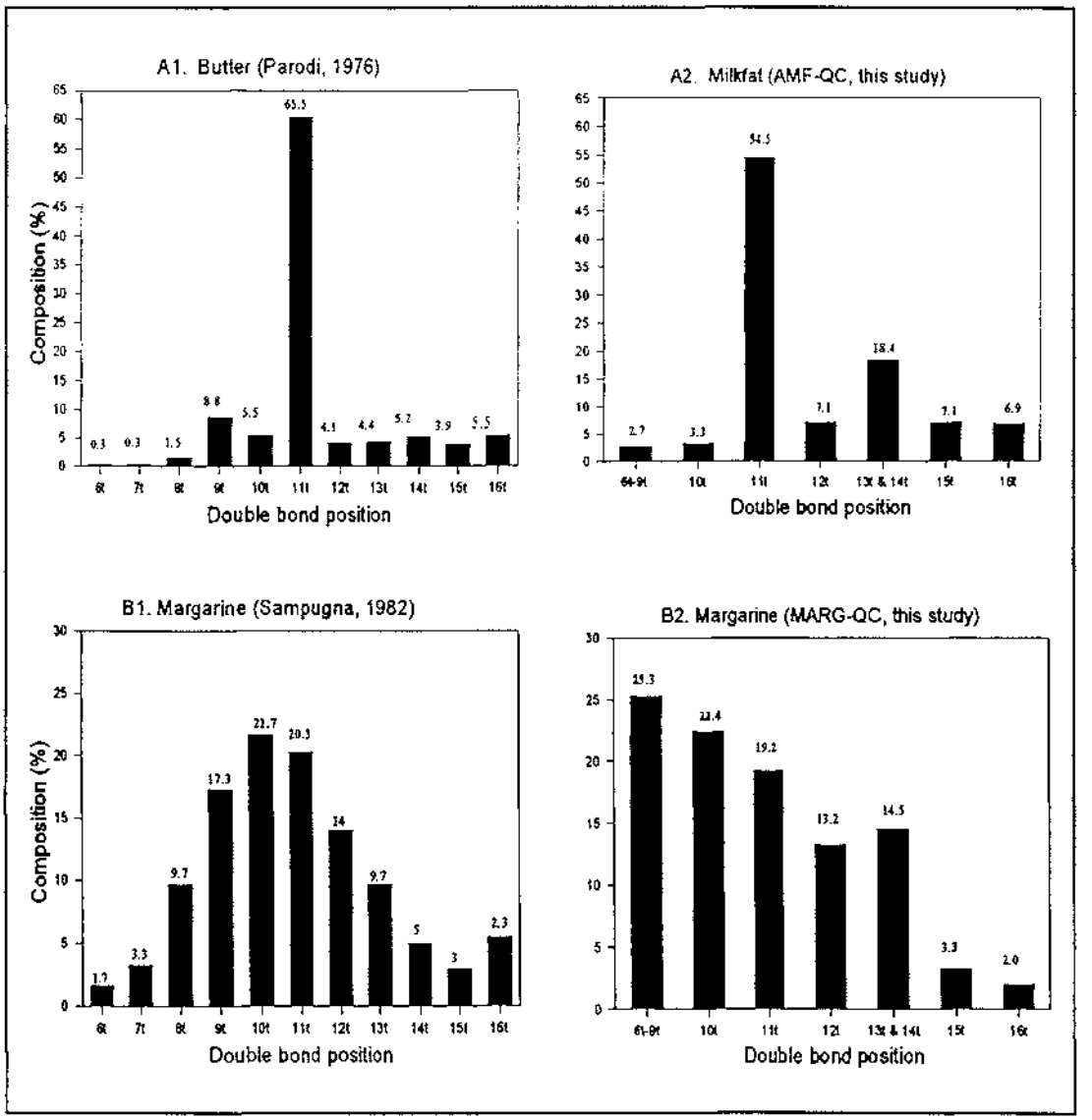

Figure 22 Distribution of C18:1 *trans* positional isomers in milkfat (A) and margarine (B). Note that the 6t-9t and 13t and 14t isomers were reported as a group as they were not separated by capillary GC in this study.

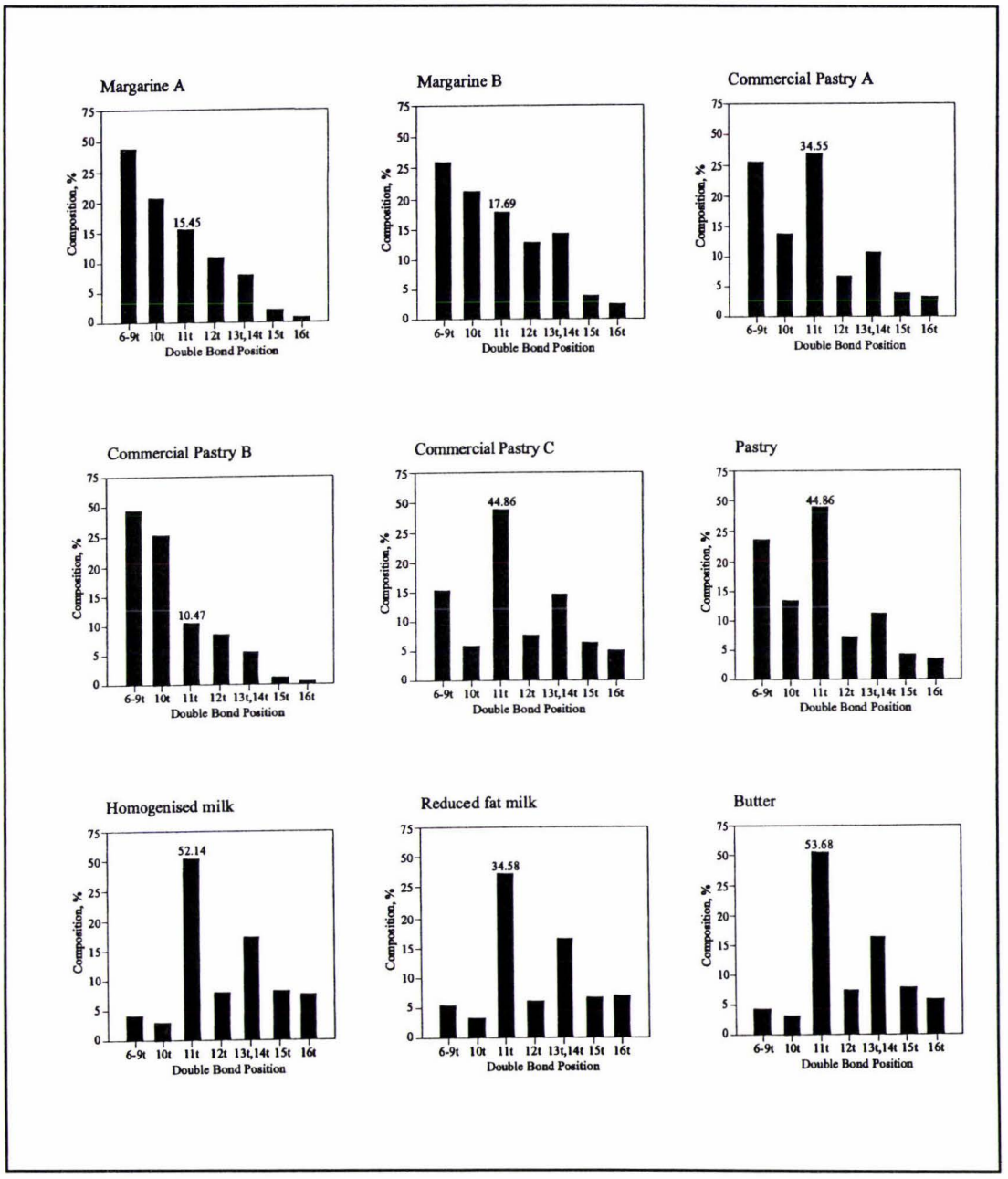

Figure 23a Distribution of C18:1 *trans* positional isomers in the New Zealand food survey fat samples.

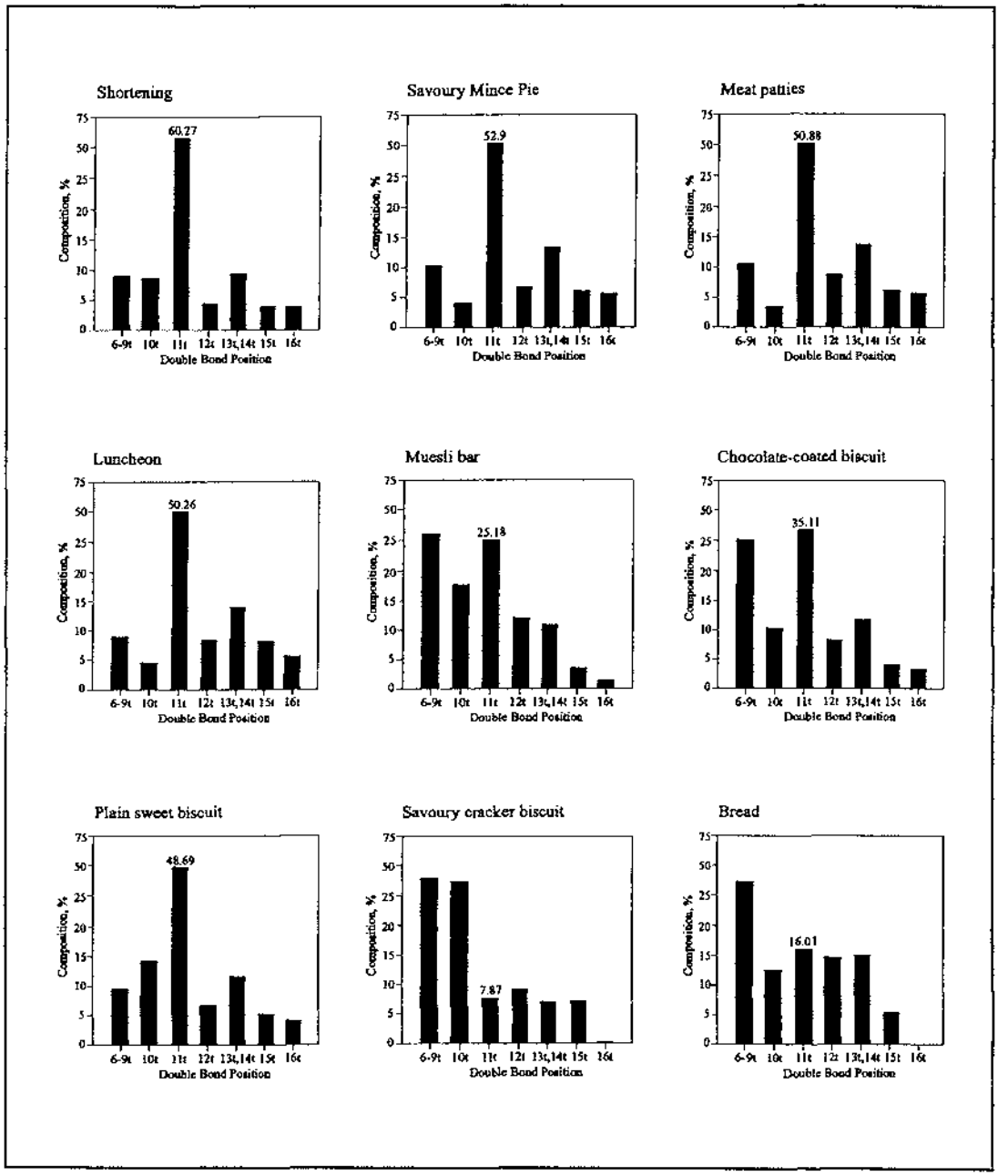

Figure 23b Distribution of C18:1 *trans* positional isomers in the New Zealand food survey fat samples.

4 POLYENOIC TFAS (C18:2 and C18:3) IN NEW ZEALAND FOOD SURVEY FATS

The total polyenoic TFA (C18:2 and C18:3) levels in the New Zealand food survey fats were generally in the lower *trans* range (0.07%-1.93%, Table 40). Unlike the C18:1 *trans* positional isomers, no distinguishing trends were observed for the various C18:2 and C18:3 geometric isomers for both butter and margarine products. This was mainly due to the very low levels of these isomers present in the food fats. The predominant contribution to the total *trans* level came from the C18:1 *trans* isomers.

Margarine

In the two margarine samples analysed in this study, the polyenoic TFAs ranged from 0.7 to 1.9%. Similar levels were reported by Mansour & Sinclair (1993) for Australian margarines, whereas Ball *et al.* (1993) reported much lower levels of 0.3-0.7% for four New Zealand margarines. In contrast, much higher levels, up to 8.3% and 5.2%, were reported for Canadian and US margarines respectively (Table 38). Generally the levels of polyenoic TFAs are dependent on the original fat source and the hydrogenation processes involved (Dutton, 1979).

Dairy fat

The polyenoic TFA level reported in some Austrian dairy fat (butter and milkfat) were in the range 0.6-2.3% (Table 38, Henninger & Ulberth, 1994). Much lower amounts were reported in Australian and Canadian milkfat, 0.89-1.39% and 0.9% respectively. Studies done by Ball *et al* (1993) showed that the levels in New Zealand butter were of the order of 0.2-0.4%. The results from this study indicate an average value of 0.2%. The total *trans* levels in milkfat have been known to vary with the dairy season and feed regime and quality of feed *etc.* (discussed in later chapters). What contribution these effects have on the polyenoic TFAs is still unclear.

Pastry fats and shortening

The pastry fat in this study recorded an average polyenoic TFAs level of 0.34%. The shortening sample recorded a level in the same region. The Canadian shortening/pastry fat made from hydrogenated fats recorded a level in the range 3.8-7.0%. In contrast, those made from unhydrogenated oils and animal fats had levels of 0.2-0.4% (Ratnayake *et al.*, 1993). Slightly higher results (0.3-8.2%) were observed with US products (Enig *et al.*, 1983).

Meat products

The average level of polyenoic TFAs reported in this study for the three meat products (mince pie, meat patty and luncheon) was 0.39%. Similar levels were reported by Sommerfield (1983) and Ratnayake lel al., (1993), from 0 to 0.5%. The contribution of polyenoic TFAs to the total *trans* level in animal fat appears to be very small.

Biscuits and muesli bar

The three biscuits and muesli bar samples analysed in this New Zealand food survey all recorded fairly low polyenoic TFAs levels (0.07% in muesli bar to 0.65% in chocolate-coated biscuits (Table 40). The levels reported in similar products by Ratnayake *et al.* (1993) were in the range 0.2-0.9% when unhydrogenated vegetable oils or animal fat was used. Much higher levels (0.3-4.6%) were reported when partially hydrogenated vegetable oil were used. Similar results were also reported by Enig *et al.* (1983) for US biscuits and cookies.

Bread

The bread sample analysed in this study indicated an unusually high contribution of polyenoic TFAs to the total *trans* level (39%), although, overall, the total *trans* levels were in the lower *trans* range (4.4%), similar to those reported in various published food surveys in the US and Canada, where unhydrogenated vegetable oils or ruminant fats were used as the fat source (Enig *et. al.*, 1983; Ratnayake

et al., 1993).

The relatively high contribution of the polyenoic TFAs was observed to come from the C18:3 ttc/cct geometric isomer (0.85%, Table 33). It is unclear at this stage if a non *trans* co-eluting peak was present. Confirmation would require other techniques such as GC-FTIR (discussed Section 7.3.4).

Sample	(g /100 g fat)			
	Total C18:1 *trans* Positional Isomers	Total C18:2 *trans* Geometric Isomers	Total C18:3 *trans* Geometric Isomers	Sum of *trans* Isomers
Margarine A	13.64	1.03	0.90	15.57
Margarine B	14.70	0.50	0.23	15.43
Commercial pastry A	6.38	0.20	0.14	6.72
Commercial pastry B	7.67	0.28	0.09	8.04
Commercial pastry C	6.69	0.20	0.13	7.02
Pastry	5.90	0.18	0.12	6.20
Homogenised milk	4.93	0.24	0.09	5.26
Reduced fat milk	5.71	0.03	0.29	6.03
Butter	6.37	0.12	0.09	6.58
Shortening	4.09	0.14	0.24	4.47
Savoury mince pie	3.43	0.18	0.15	3.76
Meat patty	3.09	0.15	0.18	3.42
Luncheon	5.42	0.25	0.20	5.87
Muesli bar	1.93	0.03	0.04	2.00
Chocolate-coated biscuit	3.00	0.61	0.04	3.65
Plain sweet biscuit	6.17	0.18	0.16	6.51
Savoury cracker biscuit	5.52	0.06	0.06	5.64
Bread	2.69	0.32	1.4	4.41

Table 40 Summary of various *trans* isomers in New Zealand food survey fat estimated using Ag-TLC/GC.

5 TFA DIETARY INTAKE IN THE NEW ZEALAND DIET

Alteration of eating patterns and changes in the economies of countries have resulted in the increased use of processed vegetable fats and oils, relative to animal fat intake. This in turn has resulted in an increased intake of TFAs. In

order to evaluate any impact of the TFAs on health, researchers must have an accurate estimate of TFA content of the diet. The lack of TFA data in the present New Zealand food composition data banks means that no estimates are currently available for TFA intake in the average New Zealand diet. The results obtained from this study were therefore used to provide an estimate for this *trans* intake. Based on the Ag-TLC/GC total TFA estimates obtained for the New Zealand food survey fats (Table 40) and information of fat intake for the various food products as determined by Howarth *et al.* (1991) for the average New Zealand diet, the total *trans* intake for a New Zealand male is 5.75 g/person/day, and the estimate for females is 3.99 g/person/day (Appendix 6). The TFA intake reported in a similar study made in the same period were 5.1 g/day and 3.1 g/day for males and females respectively (Lake & Thomson, 1996). All these estimates are similar to those reported for Australia, but are generally much less that those reported for western countries such as the USA, UK and Canada (Figure 24).

From the current *trans* intake estimates, it appears that margarine may approximately half the daily *trans* intake in both males and females (53% and 45% respectively). Butter and milk make up approximately 30-37%. These results are consistent with those reported for Australia and most countries in the EEC except France (Wolf, 1995), where ruminant fat was reported as the main *trans* contributor, making up 60% of the total C18:1 *trans* intake. Of the ruminant fat source, milkfat was responsible for a major part of the daily C18:1 *trans* consumption.

Earlier studies reported by Kummerow (1979) showed that C18:1 9Δt (elaidic acid) was oxidised more slowly by heart muscles than C18:1 11Δt or oleic acid. The C18:1 11Δt isomer and oleic acid were similar in their ability to supply the needed energy to the heart in response to stress. Brission (1981) also noted that the C18:1 11Δt isomer was metabolised more like saturated fatty acids, palmitic and stearic, and the monounsaturated oleic acids.

Almost all the estimated TFA consumption data were based on total TFAs. Little information was available on the estimated intake of the individual *cis/trans* isomers. Emken (1984) estimated that the predominantly consumed C18:1 *trans* positional isomers were the 9Δt (23%), 10Δt (22%) and 11Δt (18%) isomers. The

corresponding estimated intakes of the respective isomers were 20.9%, 12.7% and 30.% for NZ males and 18.9%, 11.7% and 33.0% for New Zealand females (Figure 25). With the recent implications made that not all the various isomeric TFAs will affect biological systems equally, it was therefore important that consumption of the various isomers could be determined, particularly for future evaluation, as more light is shed on the effect of the individual *trans* isomers.

The intakes of TFAs in the average New Zealand diet, both in amount and as a percentage of total energy from dietary fat (2.1% and 2.3% for males and females respectively), are lower than those generally reported for most western countries. The impact of similar levels of TFAs in the Australian diet has been described as minimal (Zock & Katan, 1992; Samman, 1995). Although insufficient information is available to make precise decisions on the quantities of TFA that could be safely incorporated into food diets, the general recommendation by Chisholm *et al.* (1995) was less than 10% of total energy.

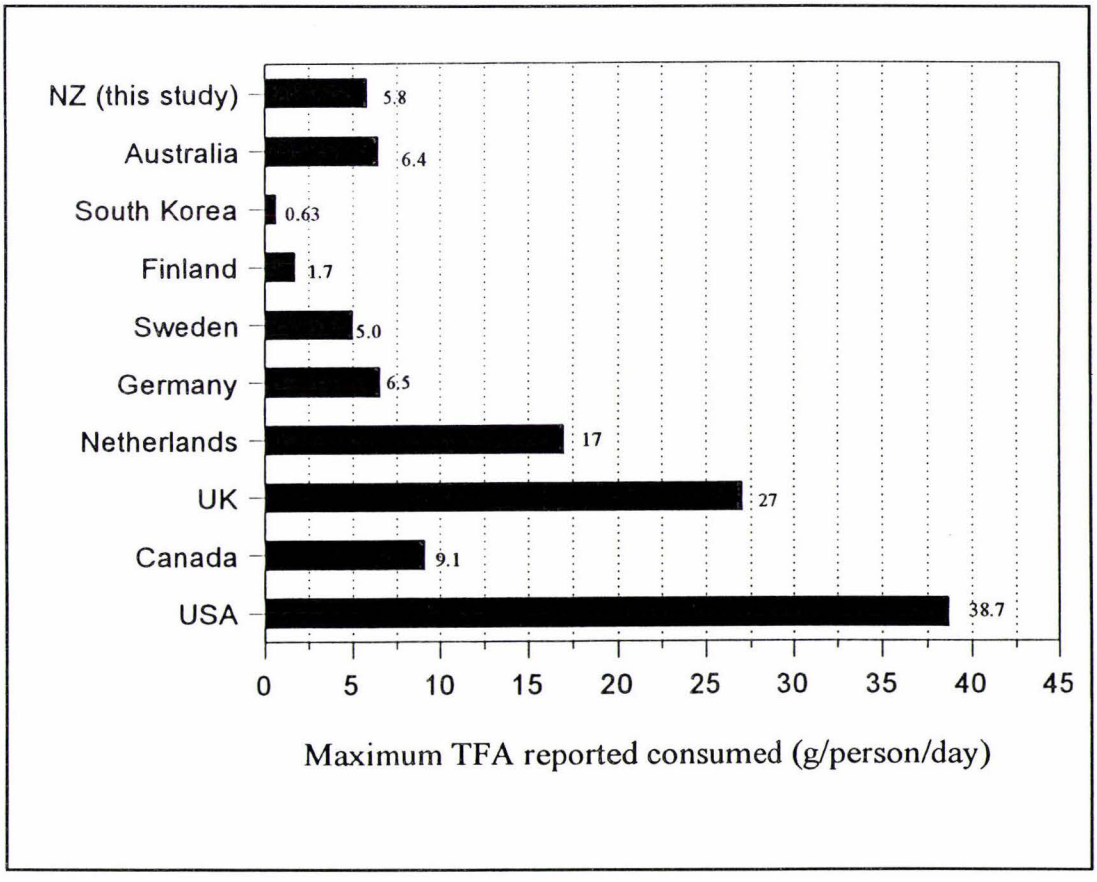

Figure 24 Total *trans* intake estimated for New Zealand compared with other countries.

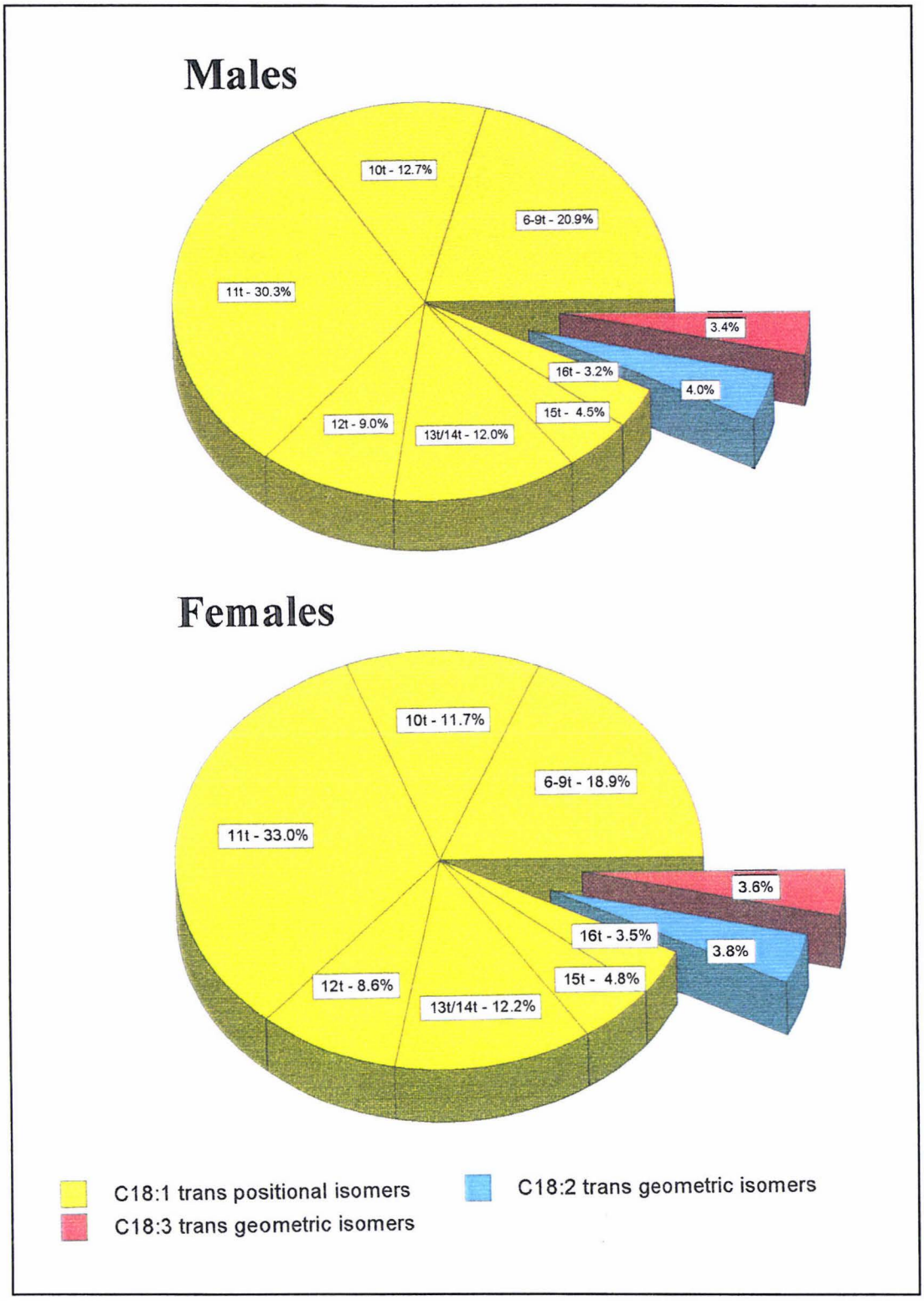

Figure 25 The estimated contribution of the various *trans* isomers to the total *trans* intake in the average New Zealand diet. Total *trans* intake for males (A) and females (B) are 5.75 and 3.99 g TFA/person/day respectively.

6 EVALUATION OF NEW ZEALAND BUTTER FAT FATTY ACID COMPOSITION SEASONAL VARIATION, WITH EMPHASIS ON TFAS

Fatty acid composition seasonal variation

The seasonal variation in the fatty acid composition of milkfat is affected by a number of factors such as composition of the feed, plane of nutrition[1] and stage of lactation of the cows. In New Zealand, dairy cows are generally grazed on pasture, normally ryegrass and clover, throughout the year, with lactation commencing in early spring, whereas cows in the Northern Hemisphere are generally fed on hay and grain in winter, and fresh grass in summer (Hawke & Taylor, 1995). Despite these complexities, studies in New Zealand (Gray, 1973; MacGibbon, 1993; this study) and overseas (Parodi & Dunstan, 1971; Wolff *et al.*, 1995) have shown that seasonal variation patterns reoccur consistently. However, the seasonal variation patterns for Southern Hemisphere countries are distinctively different from those reported for Northern Hemisphere countries.

In this study and those reported by Parodi & Dunstan (1971), Gray (1973), MacGibbon (1993) and Wolf *et. al.* (1995), a general decline in C4:0, C6:0 and C8:0 throughout the season was observed, although there was a slight rise in the amount of C4:0 in mid summer, before a final drop towards the autumn period (Figure 26). The medium chain fatty acids (C10:0, C12:0 and C14:0) increased from low levels during early spring to a maximum during early summer (Figure 26), and thereafter declined throughout the rest of the dairy season. The seasonal changes in the short chain fatty acids (C4:0 to C8:0) have been shown to be mainly influenced by lactational effects because these variations are the same as those observed for the lactational trend (Hawke & Taylor, 1995). These groups of fatty acids are synthesised in the mammary gland with little influence from pasture. Furthermore, these groups of fatty acids are generally absent from grasses (Gray, 1965).

The levels for C16:0 fatty acid were shown to rise from a low in early spring (25.2%) to a high of 31% in mid summer before a gradual decline to 29% in

[1] The extent to which food energy is partitioned towards milk production and body weight gain

autumn. The C18:0 fatty acid on the other hand showed a trend similar to that for the short chain fatty acids, with a decline throughout the season from a high of 12.9% in early spring to a low in autumn of 9.8% (Figure 26).

The total C18:1 fatty acids (*cis* and *trans*) observed in this study displayed a similar trend to C18:0 (stearic acid), but only up to November, before the level started to rise to 23% in autumn. The trend was the reverse of that observed for C16:0 fatty acid, consistent with that reported by Gray (1973) where he described a negative correlation between C16:0 acids and total C18:1 acids for New Zealand milkfat.

The seasonal trends for the C16:0, C18:0 and C18:1 fatty acids were shown not to fit into the lactational changes shown by those acids. They were suggested to be influenced mainly by dietary effects (Hawke & Taylor, 1995). Hawke (1963) reported that milkfat from cows grazed on mature summer pastures, which has less lipids than new spring and autumn growth, contains lower proportions of C18:0 and C18:1 and greater proportions of C16:0 than milkfat from cows fed with immature pastures (during spring and autumn). The immature spring/autumn pastures have much higher unsaturated fatty acid content (as the result of a higher fat content). Unsaturated fatty acids are an excellent substrate for biohydrogenation by rumen microorganisms.

The seasonal variations discussed for New Zealand were in contrast to those found in Europe (Wolff *et al.*, 1995) and in North America (Hawke & Taylor, 1995), which generally showed no consistent pattern throughout the dairy season for the C4:0 to C12:0 fatty acids. This was attributed to the practice of not confining calving to early spring, as these fatty acids are mainly influenced by lactation (Hawke & Taylor, 1995). The C14:0 to C16:0 fatty acids were lower in summer than winter and C18:0 and C18:1 were higher in summer than winter, and were the result of changes in the feeding conditions. Cows in the Northern Hemisphere are generally fed on hay and grain in winter, and fresh grass in summer. Generally, the differences between the seasonal variations in fatty acid composition in the Northern Hemisphere countries and the Southern Hemisphere countries are a consequence of different dairy husbandry practices (Hawke & Taylor, 1995).

The seasonal variations observed in this study were comparable with those in earlier studies made by Gray (1973) for New Zealand milkfat and those reported by Parodi & Dunstan (1970) for Australia, where the general patterns of dairy husbandry are similar.

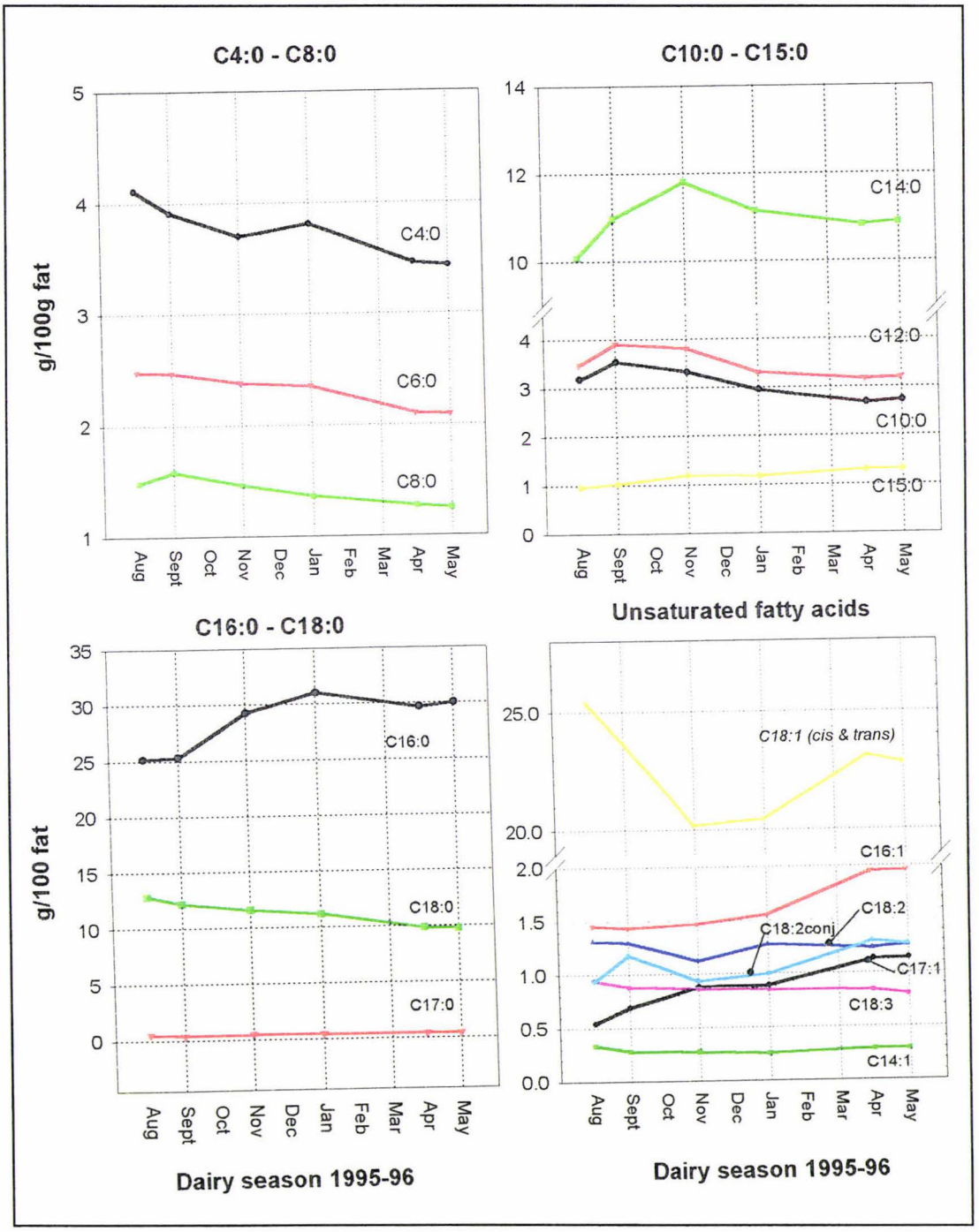

Figure 26 Seasonal variation in New Zealand milkfat fatty acid composition over the 1995/96 dairy season.

Polyunsaturated fatty acids (linoleic, linolenic and conjugated C18:2)

The polyunsaturated fatty acids in milkfat are predominantly linoleic (C18:2) and linolenic (C18:3) acids. From this study, it appeared that the levels throughout the season were almost constant (Figure 26) with a mean annual range of 1.13-1.32% for C18:2 and 0.81-0.95% for C18:3. A small decrease in the C18:3 fatty acid from spring to summer was observed, whereas the C18:2 fatty acid was relatively constant throughout the year, with a decrease in spring/early summer (Figure 26). However, these changes may be within the precision of the method.

Low levels of conjugated linoleic acid (CLA) were also found in milkfat. Despite the relative scarcity of the CLAs, some seasonal trend could be unambiguously observed (Figure 26) with low levels recorded during the early dairy season and then a gradual rise towards the late dairy season (May). Although CLAs are present in a range of foods and in milkfat in low levels (< 2%), milkfat has the highest reported concentrations (Parodi, 1994).

CLAs are normally not constituents of the cow's diet. They are the first intermediate in the biohydrogenation of linoleic acid by a linoleic acid isomerase from the rumen anaerobic bacteria, *Butyrivibrio fibrisolvens* (Parodi, 1994). The original methylene-interrupted sequence of *cis* double bonds is disrupted, with *cis/trans* isomerisation occurring as well as the migration of the double bonds along the chain.

Generally several conjugated diene isomers with double bonds in different positions are present. However, these have been collectively given the name CLA. In milkfat, the CLAs consist of essentially (90%) the Δ9c, 11t isomer (Parodi, 1994).

The biochemistry of CLA (the Δ9c, 11t) is still vague. Recent research has shown CLAs to act as an anti-cancer agent in laboratory animals and cell cultures. They were also demonstrated to reduce blood cholesterol in rabbits given high fat diets and may even reduce the progression of atherosclerosis (Ip *et al.*, 1994). The mechanism by which CLAs inhibit cancer is unknown. Research in the next few

years is likely to discover this mechanism and may pave the way for the application of CLAs in cancer therapy. However, there is abundant evidence that CLA isomers from the diet enter many body tissues. They have been detected in human adipose tissue, in milk and in the blood, where they are associated with cholesteryl esters, triacylglycerols and phospholipids. Although it is probable that most of the CLA originated from the diet, there are suggestions that small amounts might be formed in the human body by anaerobic bacteria in the human colon, and from the C18:1 Δ11t by action of fatty acid delta 9-desaturase (Parodi, 1994). C18:1 Δ11t is the predominant C18:1 *trans* positional isomer in milkfat, and hence may play an important part in future anti-cancer therapies. CLA (Δ9c, 11t isomer) have also been shown to be saturated to the C18:1 11Δt isomer by ruminant microflora.

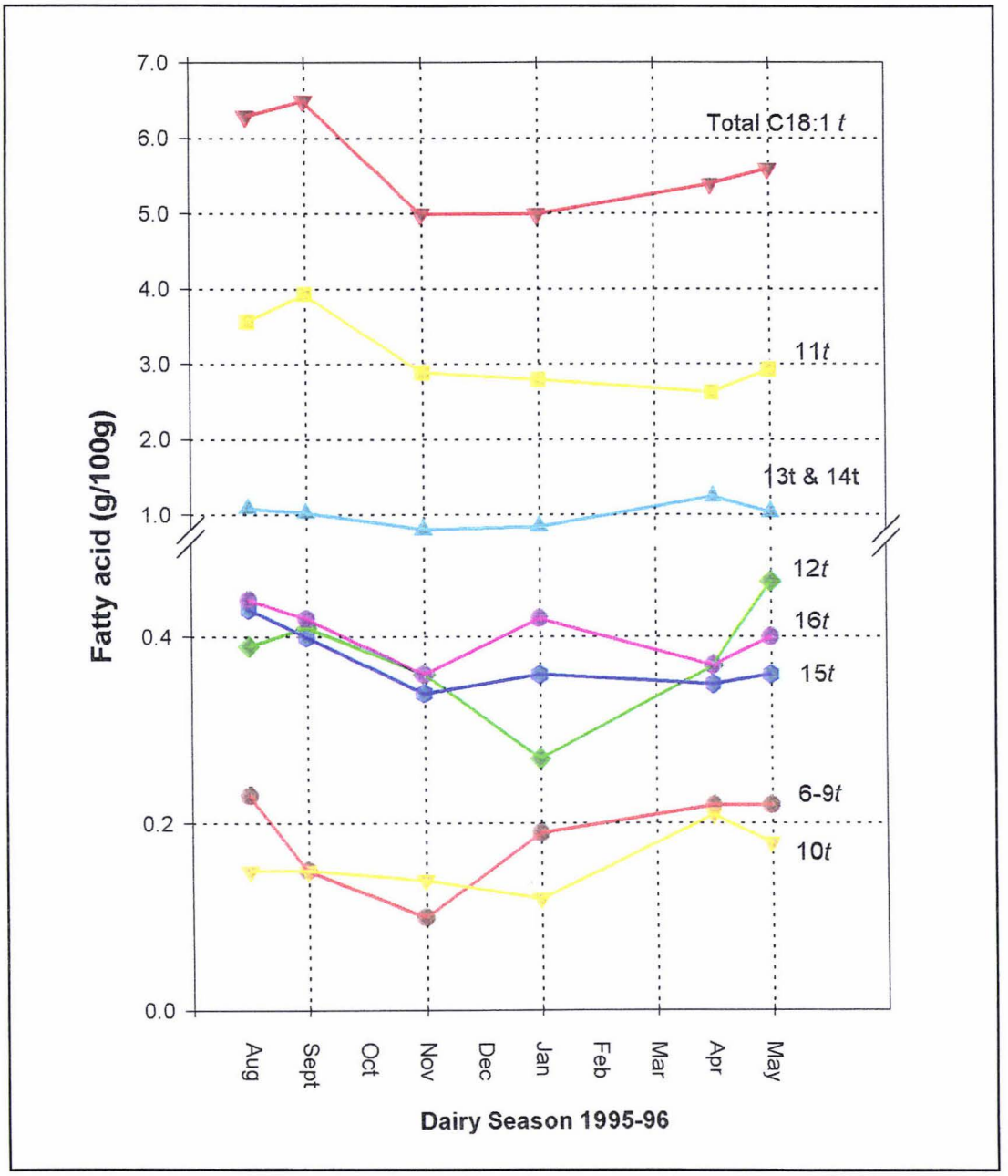

Figure 28 Distribution of the various C18:1 *trans* positional isomers in milkfat across the 1995/96 dairy season.

Correlation between the total C18:1 *trans* positional isomers and other C18 fatty acids in butter fat over the dairy season

Although it has been reported that the total TFA level in butter fat is greatly influenced by the ruminant dietary unsaturated fatty acid intake, no statistical correlation has been reported between the TFA and the other fatty acid components in the milkfat. Statistical evaluation of the current seasonal survey data indicated that a strong correlation was present between the total C18:1 isomer and both the *cis* and *trans* isomers. A good relationship was also found for the C18:1 11Δt and C18:1 15Δt with total *trans* isomers. There was no significant correlation between the TFA level and either the C18:2 or C18:3 fatty acid isomers. Similarly, no significant correlations were found between the conjugated C18:2 and the C18:1 11Δt isomers. The C18:1 11Δt positional isomer is one of the products of the saturation of conjugated C18:2 (9Δc, 11t) fatty acid by the ruminant microflora. The seasonal variations for the C18:1 *trans* positional isomers are given in Figure 28. (Correlation analysis data are given in Tables A5.2, A5.3 and A5.4, Appendix 5.)

7 COMPARISON OF NEW ZEALAND AND OVERSEAS BUTTER FAT FATTY ACID COMPOSITION, WITH EMPHASIS ON TFAS

Fatty acid composition

The fatty acid composition of milkfat is known to vary with the season and is also affected by a number of factors such as composition of diet, plane of nutrition, stage of lactation and animal husbandry and the breed of the dairy herds, as discussed earlier. In view of these complexities, it is remarkable that the average bulk milkfat fatty acid composition remains relatively constant (Hawke & Taylor, 1995).

Fatty acids in milkfat arise from two sources as discussed earlier: (i) synthesis *de novo* in the mammary gland and (ii) uptake from the circulating blood (dietary source). The fatty acid compositions from these sources are usually very different. In cows, the endogenously produced fatty acids are of carbon chain C4 to C16, while a proportion of C16 and virtually all the C18 fatty acids are from

the blood (diet) (Hawke & Taylor, 1995).

The fatty acids from *de novo* synthesis are generally subject to little variation. Likewise the extensive biohydrogenation of dietary unsaturated fatty acids and other biochemical events in the rumen considerably reduce the influence of dietary fatty acids on the milkfat fatty acid composition, leading to greater consistency (Hawke & Taylor, 1995). Despite the seasonal trend differences, the general fatty acid composition of New Zealand butters and that of overseas butters in this study also reflect this consistency (Figure 29 and Table 14).

The total TFAs (C18:1 *trans*, C18:2 *trans* and C18:3 *trans*) observed in all the overseas butters also appeared to be within the minimum and maximum *trans* values generally reported for milkfat. Likewise, the New Zealand minimum and maximum *trans* values were also similar to those reported for overseas samples. No unusually high or low *trans* values were observed. However, the influence of the factors discussed above was more prevalent when the distributions of the individual C18:1 *trans* positional isomers were examined (Figure 30). Significant differences were observed for two samples, the Lurpak (Danish) and Lady Lee (USA) butters. These samples recorded Δ11t levels of 39% and 25% respectively. The C18:1 Δ11t isomer has generally been reported to make up 50-60% of the total *trans* positional isomers (Craig-Schmidt, 1992). It is known that Northern Hemisphere countries (except Ireland; personal communication, Alastair MacGibbon) generally shift the dietary feed from fresh grass in summer to forage and concentrates (grains) in autumn and winter. In some cases, the herds are forage fed all year. These differences may account for the relatively low amount of Δ11t and much higher amount of Δ10t observed for the two butters compared with the typical trends reported for milkfat. The higher level of Δ11t observed for Ireland is the exception because the dairy herds are normally pasture fed throughout the year (similar to New Zealand and Australia). The seasonal period from which the butters were made is not known.

No published work specifically on dietary feed influence on the individual *trans* positional isomers has been reported, although Wolff (1995) indicated that the relative levels of the C18:1 11Δt isomer are related to the feed and, more precisely, the amount of grass in the feed. Wolff indicated that goats do not eat

very much grass and lower levels of 11Δt in their milk, even less than in fat from winter cow's milk. On the other hand, ewes eat grass throughout the year and the 11Δt level is as high in their milk as in spring cow's milk. Furthermore, correlation studies using data obtained for the overseas samples indicated a good correlation between conjugated C18:2 and the 11Δt isomer (Figure 30). A similar conclusion was not obtained with the New Zealand seasonal survey butter samples.

The C18:3/C18:2 ratios (Figure 31) indicated that high C18:3 levels were obtained from the New Zealand, Australian and Irish butters. This is generally indicative that cows were pasture fed. In contrast, the low C18:3/C18:2 ratios for the Japanese, American and Danish butter reflected grain feeding. Pasture-fed cows have been reported to have higher C18:3 fatty acids, and therefore higher C18:1 Δ11t, in contrast to grain-fed cows (personal communication, Alastair MaGibbon). Cows in the Northern Hemisphere do produce high levels of C18:3 fatty acid in summer, when they are moved on to fresh grass feeds.

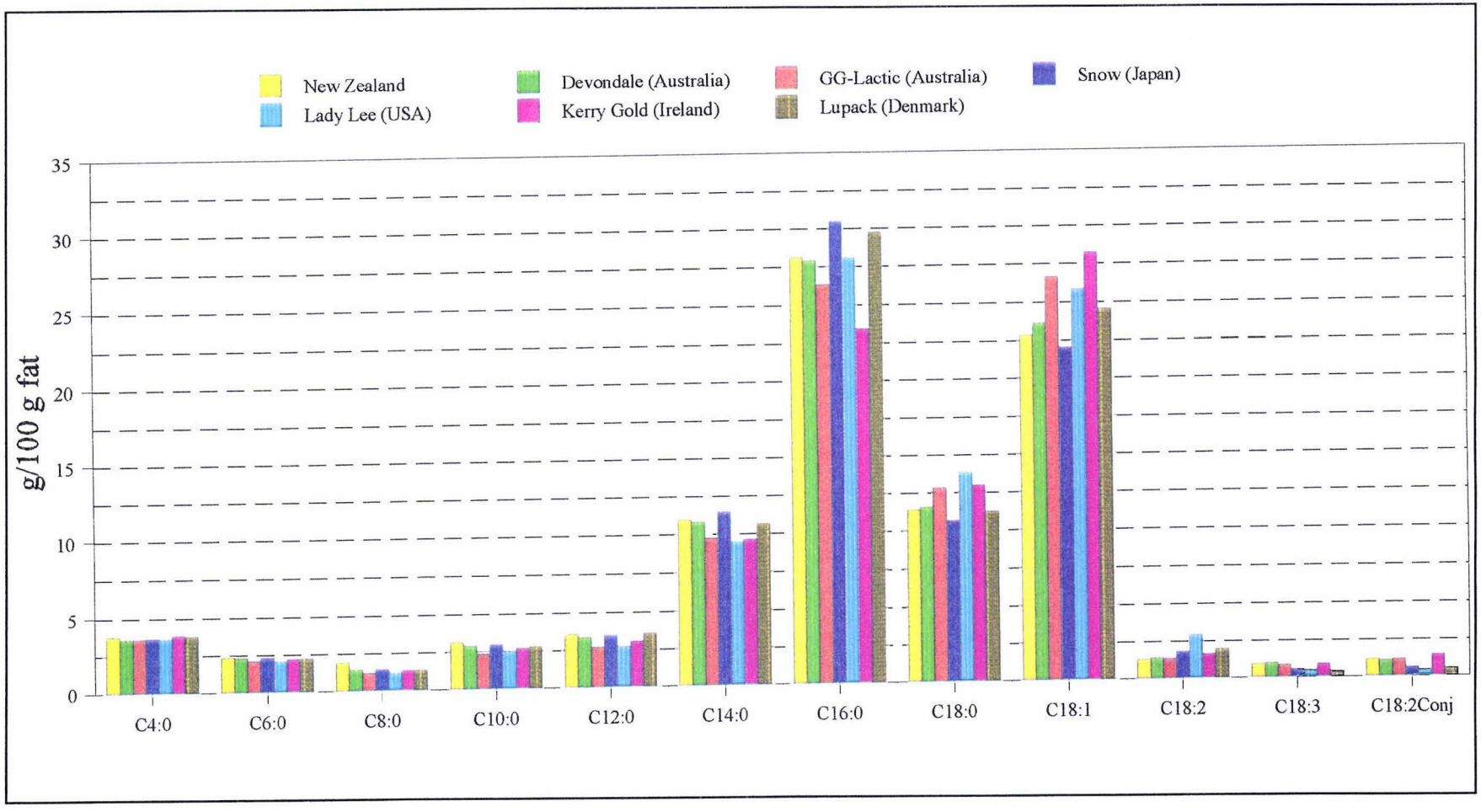

Figure 29 Fatty acid distribution for overseas and New Zealand (median used) butter fat samples. Only the major fatty acids were graphed. Full fatty acid composition data are given in Table 14.

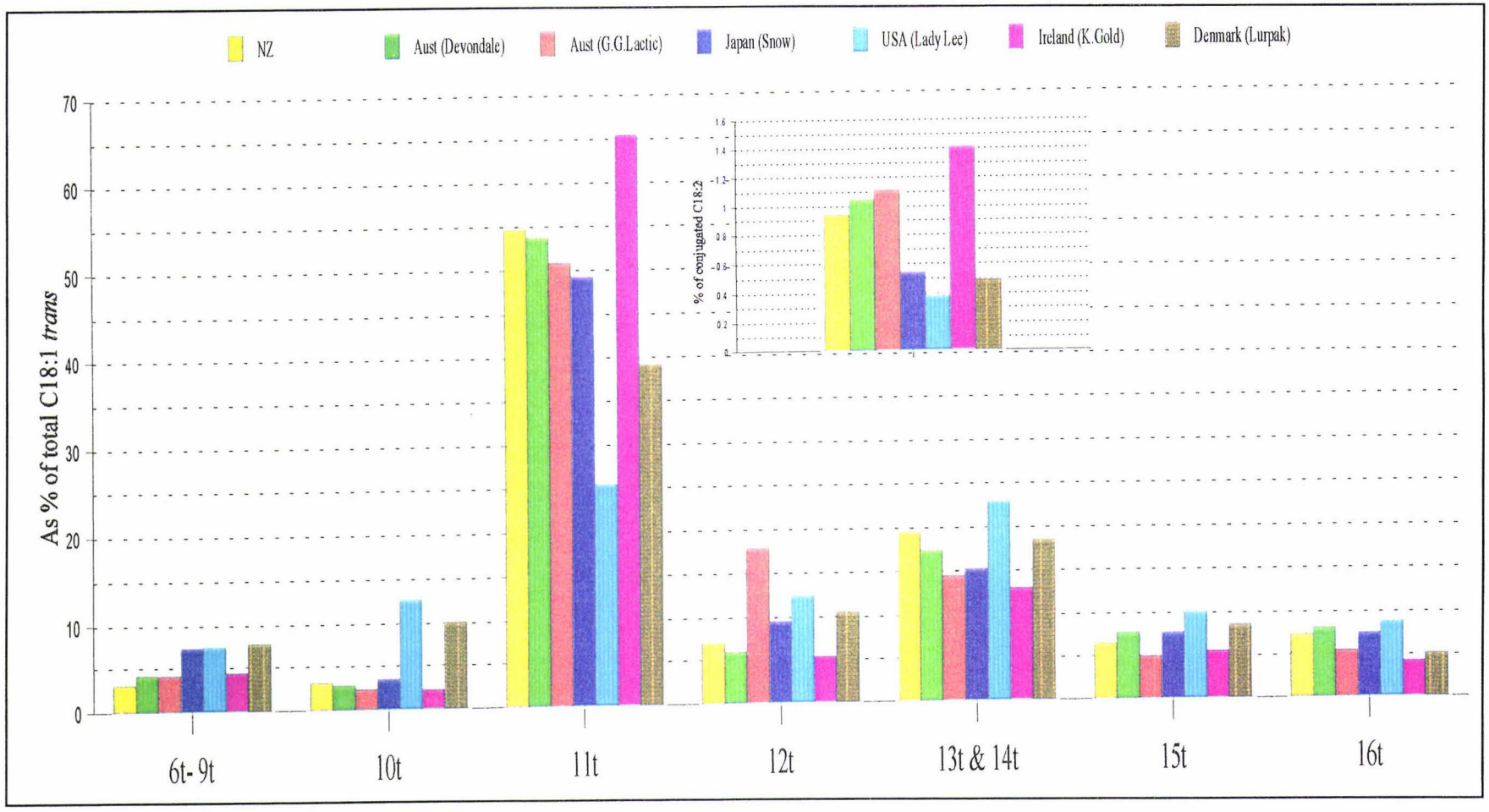

Figure 30 Distributions of the C18:1 *trans* positionl isomers and the conjugated C18:2 fatty acid in the overseas butters and the New Zealand butters (median data).

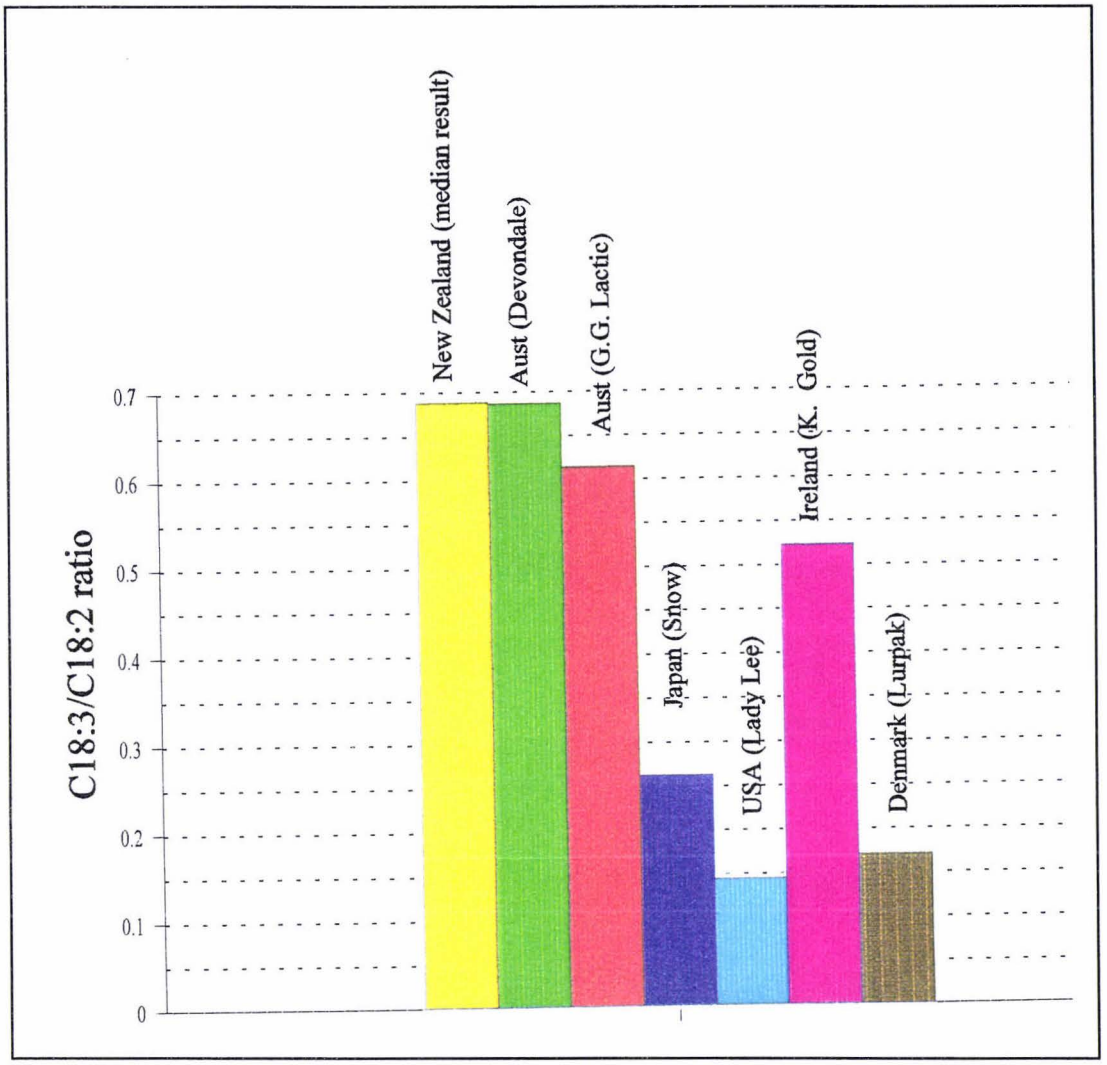

Figure 31 The C18:3/C18:2 ratios determined for the overseas and New Zealand butter (median data) samples.

CONCLUSIONS

1 METHODOLOGY

Infrared methods for the determination of TFAs in a wide variety of food fats were developed and validated. The bias between the AOCS-TG and AOCS-FAME methods was reduced but not eliminated by the calibration technique (calibrant made up in non *trans* background). The negative bias associated with the AOCS-FAME method was eliminated or at least greatly reduced for a large number of the New Zealand survey food fat samples by using the SS-FAME method. There were no significant differences between the AOCS-TG method and the SS-TG method.

All the four infrared methods produced results that were lower than those obtained using the Ag-TLC/GC method. However, of these four methods, the AOCS-FAME method was significantly different from the Ag-TLC/GC method (approximately 0.82%). Of the other three infrared methods, the SS-FAME method produced results that were closest to the Ag-TLC/GC method (0.04%), (Table A5.1, Appendix 5).

The SS-FAME will provides a rapid method for total *trans* estimation, with a reasonable accuracy. However, like all *trans* infrared quantitative methods, samples with high lauric acid content may produce biased results. The SS-FAME method would be appropriate for the laboratories where the sample matrices are consistent (*e.g.* dairy industry).

A more accurate means of *trans* quantification will require the Ag-TLC/GC method. This method is not subject to the problems, particularly for low *trans* samples and samples containing interfering components, encountered with the infrared methods, and enables the estimation of the individual *trans* isomers, information that is not available using the infrared methods. Quantification of the various individual *trans* isomers may be of importance as evidence indicates that not all *trans* isomers are equally hazardous to humans.

2 TFA LEVELS IN NEW ZEALAND FOOD FATS

Overall, New Zealand foods fats do not appear to have the high TFA contents that have been reported for other countries. Using the present study as a guide, New Zealand manufactured margarines generally have a total TFA content around 15%, whereas New Zealand milkfat has up to 6-7% TFAs. Most other foods have 1-8% TFAs, consistent with the use of animal fats and/or mildly hydrogenated oils.

3 ESTIMATED TFA INTAKE IN THE NEW ZEALAND DIET

The current estimated TFA intakes of the average New Zealand diet based on data produced from this New Zealand food fat study, are 5.75 and 3.99 g/person/day for males and females respectively. These estimated intakes are similar to or lower than those of most Northern Hemisphere countries. The predominant TFA isomer consumed was identified as the C18:1 Δ11t positional isomer, making up 30-34% of the total TFAs consumed. This isomer has been shown to be metabolised like C16:0, C18:0 and oleic acids, and serves more readily as an energy source than elaidic acid for heart muscles during stress. The relative risks for similar TFA consumption levels in the Australian diet have been described as minimal. However, risks have been reported to vary from country to country, depending on the proportions of the various *trans* isomers consumed.

4 TFAS IN NEW ZEALAND BUTTER AND MARGARINE

TFA levels in New Zealand butter were shown to be influenced by seasonal variations, with the highest recorded in spring (6.7%) and the lowest recorded in summer (5.3%); the predominant *trans* isomers were the C18:1 Δ11t fatty acid (50-60%). As a result, it is not surprising that this isomer strongly correlates to the total TFA level in milkfat. There was no evidence of any further correlation of the TFA levels in milkfat with the C18:2 and C18:3 polyunsaturated fatty acids. A similar conclusion was drawn for the conjugated C18:2 fatty acid, which has recently been shown to display anti-carcinogenic properties. The C18:2 and C18:3 *trans* geometric isomers were usually less than 0.5%.

The *trans* isomer distributions in hydrogenated or partially hydrogenated fats and oils (*e.g.* margarine) have a profile that is very distinctive compared with that of milkfat. Although the proportions of the various positional isomers differ according to the hydrogenation process and the source oils, the C18:1 *trans* positional isomers are generally reported to be distributed around the C18:1 Δ10t and Δ11t fatty acids. The C18:2 and C18:3 *trans* geometric isomers were usually less than 1% for the margarines analysed in this study.

5 OVERSEAS BUTTERS

Although the total TFA levels for both New Zealand and overseas butters appear to be within a consistent range (5-7%), the distribution of the C18:1 *trans* positional isomers is different. This has been attributed to the different animal husbandry methods used. Northern Hemisphere countries, which tend to silage feed the dairy herd, tend to have lower C18:1 Δ11t levels in their milkfat than countries where herds are pasture fed. These variations are probably due to the difference in animal feed and husbandry methods. Furthermore the conjugated C18:2 fatty acids for these overseas butters correlate with the C18:1 Δ11t isomer. This was not found with the New Zealand seasonal survey butter samples used in this study.

The manipulation and the effect of animal feed on the *trans* positional isomer distribution may prove to be an area that could be exploited by the dairy industry to produce milkfat with minimised 'harmful' TFAs, and higher levels of more desirable fatty acids.

TFAs in general have been part of the human diet ever since ruminant fat has been part of the food source. However, the distribution of the *trans* isomers were very different from that in today's chemically modified fats and oils. Furthermore, they are now present in much larger amounts. As research continues to shed light on the biological significance of the different TFA isomers, it would be important to be able to measure them accurately and to eliminate or minimise them in foods.

TECHNICAL NOTE

During the analysis of the quality control samples for *trans* polyunsaturated fatty acids, a decrease in the level of C18:2 and an increasing level of C18:0 and C18:1 isomeric fatty acids were observed (Figure 32). This apparent increase became a major concern because it was not observed in previous analyses. To rule out possibilities of `ghost' peaks, the column was baked as specified by the manufacturer (150°C for 30 min) before a standard solution of C18:2 (linoleic acid methyl ester) (> 99% purity, Nu Prep Chek, Minnesota, USA) was analysed.

The result showed a dramatic drop in the purity of the standard C18:2 solution to less than 20%. Deterioration of the standard was ruled out because the same standard analysed on a separate GC system confirmed 99% purity. Peaks with retention times corresponding to the C18:0 and C18:1 positional isomers were present in extremely high amounts (Figure 32). Hydrogenation of the sample was occurring in the system. The most likely source of the problem was the glass liner in the injector port. The port glass liner was made from borosilicate glass. The injector port glass liner was removed and cleaned with hexane, and the glasswool (silylated, Alltech Associates, Deerfield, Illinosis, USA) was also replaced as with normal routine maintenance. The extent of the hydrogenation decreased (60%), but was still unexceptable. It was postulated that the borosilicate glass lining in the injector port had worn out. The exposure of active hydroxyl groups together with hydrogen (carrier gas) and the high temperatures (250°C) had resulted in hydrogenation of the samples.

To remove the active sites, the glass liner was silylated as given in the reaction in Figure 33. Both silylating reagents, hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS), were used. The silyl derivatives were formed by replacement of the active hydrogen ion on the hydroxyl groups in the borosilicate glass liner.

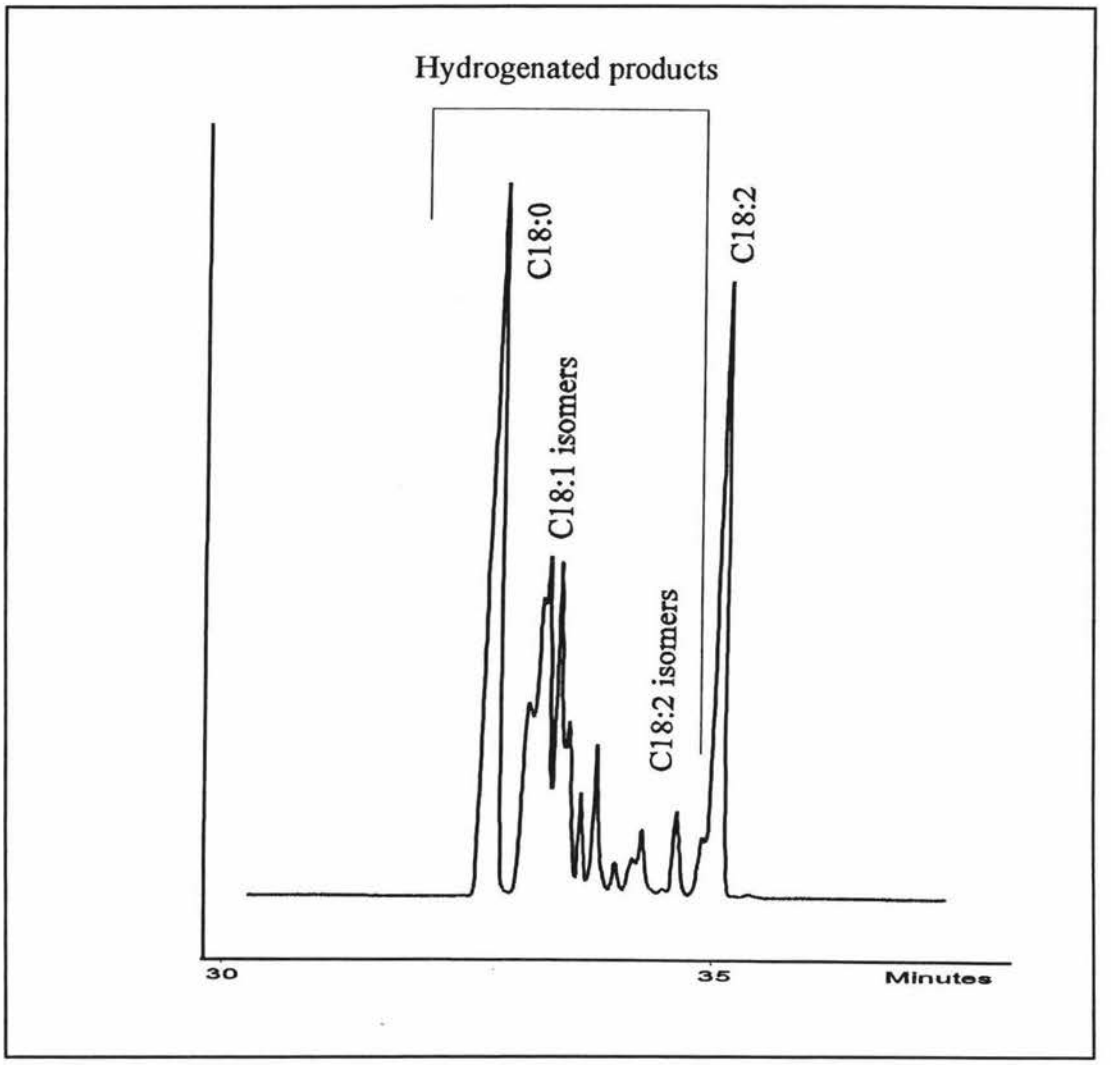

Figure 32 Decomposition of the C18:2 standard solution

After silylation, the glass liner was washed with hexane and baked at 250°C to evaporate off the residual solvents before connecting up to the capillary tubing, as silyl derivatives have been known to have an undesirable property of fouling up the FID with silica disposal.

Re-analysis of the C18:2 standard solution after silyation of the glass liner produced a purity of 99% as specified by the manufacturer (Nu Prep Check, Minnestoa, USA).

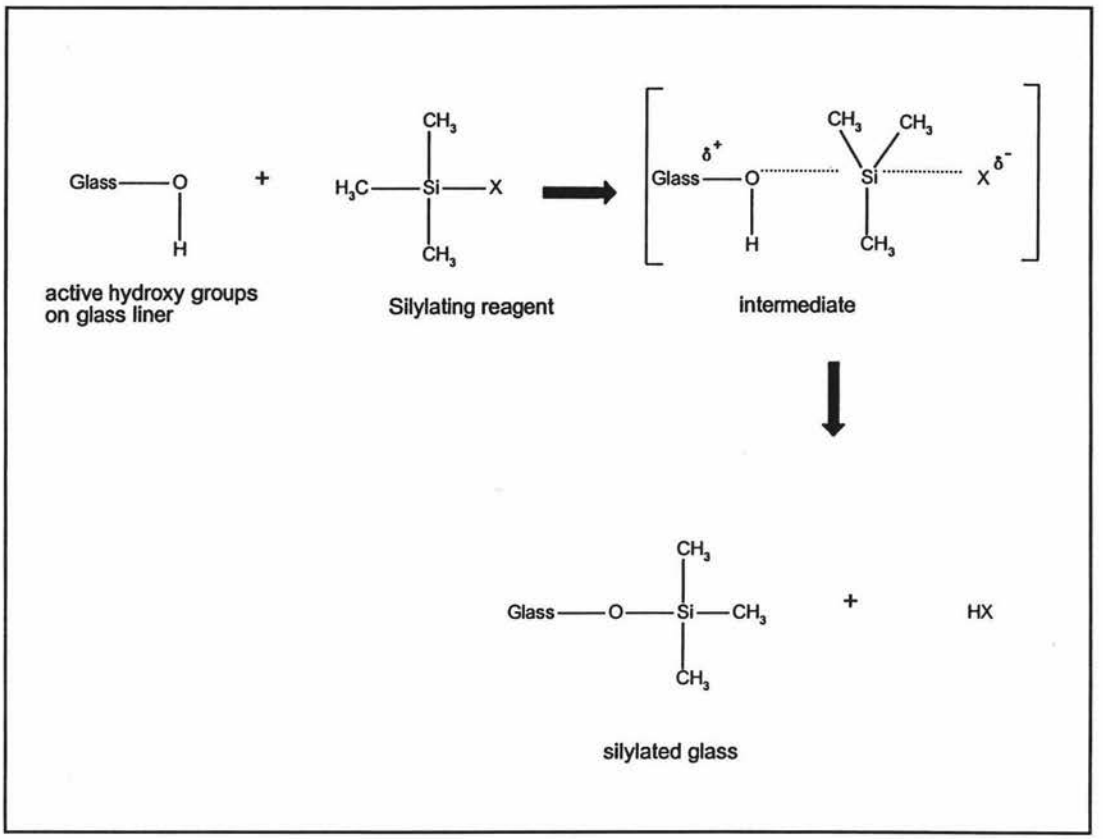

Figure 33 Silylation of the active OH groups on the borosilicate glass liner. The silyl derivatives are formed by replacement of the active hydrogen on the OH group on the borosilicate glass liner with H and X as the leaving group. TMCS was added with the HMDS to increase the silyl donor strength. The result of the reaction was the formation of a white precipitate - NH_4Cl.

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APPENDIX 1 DERIVATION OF FAME CORRECTION FACTORS (GIVEN IN TABLE 10)

The FAME correction factors corrects for (1) the FID detector response for the carboxyl and methyl ester carbons, and (2) the methly ester conversion to fatty acid. These calculations are given below:

A1.1 Calculation of response factors for FAMEs

As the carboxyl carbon of a FAME produces a negligible response in an FID, and the methyl ester carbon gives a full response, the detector response is effectively proportional to the carbon number of the corresponding fatty acid (Ackman & Sipos, 1964; Bannon *et al.,* 1986).

The "active" carbon atoms constitute a greater proportion of the longer chain FAMEs than of the short chain FAMEs. Thus the FID response is relatively higher for longer chain FAMEs than for short chain FAMEs, so that response (R) of the fatty acid of chain length (*l*) is:

$$R_i \propto \frac{n_i}{MW_{i\text{-}ME}}$$

where n_i = the carbon number of fatty acid and $Mw_{i\text{-}ME}$ = the molecular weight of the corresponding methyl ester.

Responses (R) can be calculated relative to the response of methyl palmitate (C16:0 methyl ester) as follows:

$$RR_i = \frac{R_i}{R_{C16{:}0\text{-}ME}} = \frac{n_i \times MW_{C16{:}0\text{-}ME}}{MW_i \times 16}$$

The relative response factor (*RF*) (to correct for detector response) is the inverse of the relative response (RR_i):

$$RF_i = \frac{R_{C16:0\text{-}ME}}{R_i} = \frac{16 \times MW_{i\text{-}ME}}{n_i \times MW_{C16:0\text{-}ME}}$$

Final formula corrects of the response factor for the FAMEs.

A1.2 Conversion of FAME composition to fatty acid composition

The relative proportions of FAMEs in a test mix (sample extract) differ from the relative proportions of fatty acids in the (fat) sample, because of the differences between the molecular weights of the fatty acids and those of their corresponding methyl esters.

The conversion (*C*) for the molecular weight is:

$$C = \frac{MW_{i\text{-}FA}}{MW_{i\text{-}ME}}$$

The conversion factor (CF) can be calculated relative to the conversion of palmitic acid (C16:0-FA), as follows:

$$CF_i = \frac{C_i}{C_{C16:0\text{-}FA}} = \frac{MW_{i\text{-}FA} \times MW_{C16:0\text{-}ME}}{MW_{i\text{-}ME} \times MW_{C16:0\text{-}FA}}$$

This formula convert the fatty acid methyl esters to fatty acids.

A1.3 Calculation of relative correction factors (RCF)

The overall correction factor for detector response and molecular weight conversion, relative to C16:0 is:

$$RCF_i = RF_i \times CF_i = \frac{16 \times MW_{i\text{-}ME}}{n_i \times MW_{C16:0\text{-}ME}} \times \frac{MW_{i\text{-}FA} \times MW_{C16:0\text{-}ME}}{MW_{i\text{-}ME} \times MW_{C16:0\text{-}FA}}$$

This simplifies to:

$$RCF_i = \frac{16 \times MW_{i\text{-}FA}}{MW_{C16:0\text{-}FA} \times n_i} = \frac{MW_{i\text{-}FA}}{n_i} \times 0.0624$$

The relative correction factor (RCF_i) multiplied by the normalised area percent of the FAME (from gas chromatograph) = weight pecent of the total fatty acid.

Example 1:

Fatty Acid	n	MW_{FA}	MW_{ME}	RF_i	C_i	CF_i	RCF_i
Butyric	4	88.1	102.1	1.513	0.863	0.910	1.374
Palmitic	16	256.3	270.3	1.000	0.948	1.000	1.000

Example 2:

C18:1 response factor relative to C18:0 = C18:1 RCF/C18:0 RCF = 0.979/0.986.

APPENDIX 2 VALIDATION RESULTS FOR THE FTIR METHODS

Sample	Total *trans* Isomer (g/100 g fat)			
	AOCS-TG	AOCS-FAME	SS-TG	SS-FAME
AMF-QC	8.40	6.81	8.70	6.12
	8.20	6.10	8.51	5.49
	8.21	7.01	8.52	6.32
	8.22	6.90	8.52	6.32
		6.62		6.61
		6.00		8.23
Mean ± standard deviation	8.26 ± 0.10	6.57 ± 0.43	8.56 ± 0.09	6.52 ± 0.92
MARG-QC	15.77	15.00	15.95	14.74
	15.45	15.09	15.64	14.83
	15.59	14.54	15.78	14.26
	15.97	14.62	16.16	14.35
	15.70	15.39	15.88	15.32
		14.61		14.73
Mean ± standard deviation	15.70 ± 0.20	14.88 ± 0.34	15.88 ± 0.19	14.71 ± 0.38

Table A2.1 The replicate results for the AMF-QC and MARG-QC samples used to determine the repeatability standard deviations of the AOCS-TG, AOCS-FAME and spectral subtraction (SS) infrared methods.

Sample	Added (mg)	Standard AOCS			Spectral Subtraction		
		Present (mg)	Found (mg)	Recovery (%)	Present (%)	Found (mg)	Recovery (%)
MARG-QC	17.9	28.76	46.72	100.3	28.43	46.18	99.2
	20.9	28.22	48.54	97.2	27.89	48.40	98.1
	9.0	30.29	39.45	101.8	29.93	38.71	97.6
	9.9	30.03	39.85	99.2	29.68	39.31	97.3
Mean ± standard deviation				99.6 ± 1.9			98.1 ± 0.8
AMF-QC	9.8	15.70	25.38	98.8	16.23	26.11	100.8
	12.3	15.62	27.81	99.1	16.20	28.34	98.7
	5.1	16.03	21.35	104.3	16.62	21.67	99.0
	6.2	16.00	22.36	102.6	16.59	22.68	98.2
Mean ± standard deviation				101.2 ± 2.7			99.2 ± 1.1

Table A2.2 The percentage recovery after spiking the AMF-QC and MARG-QC samples with trielaidin as determined by AOCS and spectral subtraction methods. The percentage recoveries are given with ± standard deviation, determined from the replicates.

Sample	Added (mg)	Standard AOCS			Spectral Subtraction		
		Present (mg)	Found (mg)	Recovery (%)	Present (mg)	Found (mg)	Recovery (%)
MARG-QC	22.2	25.22	47.35	99.7	25.17	47.36	100.0
	20.6	25.24	45.45	9 7.64	25.18	45.34	97.9
	12.4	25.93	38.34	100.1	25.73	38.33	101.6
	10.9	24.77	35.94	102.5	24.70	36.32	106.6
Mean ± standard deviation				100.0 ± 2.0			101.5 ± 3.7
AMF-QC	10.7	12.28	23.34	103.3	11.98	23.26	105.5
	10.4	11.91	22.14	98.4	11.60	22.06	100.5
	6.1	10.74	16.74	98.3	10.62	26.63	98.6
	7.7	10.66	17.94	94.5	10.54	18.04	97.4
Mean ± standard deviation				98.6 ± 3.6			100.5 ± 3.7

Table A2.3 The percentage recovery after spiking the AMF-QC and MARG-QC samples with methyl elaidin as determined by AOCS and spectral subtraction methods. The percentage recoveries are given with ± standard deviation, determined from the replicates.

Sample	Total *trans* Isomer (g/100 g fat)			
	AOCS-TG	AOCS-FAME	SS-TG	SS-FAME
Margarine (A)	18.25, 17.08	13.74, 14.91	18.25, 17.15	13.77, 15.01
Margarine (B)	16.53, 15.81	14.95, 14.36	16.49, 15.85	14.98. 16.67
Commercial pastry fat (A)	7.76, 8.00	6.25, 6.87	8.12, 8.36	8.50, 9.11
Commercial pastry fat (B)	7.13, 6.94	5.85, 4.99	7.49, 7.13	8.10, 7.22
Commercial pastry fat (C)	5.88, 6.38	7.16, 6.87	6.24, 6.64	8.68, 8.39
Pastry	6.57, 6.54	5.23, 6.01	6.61, 6.50	5.29, 6.01
Homogenised milk	7.32, 6.62	4.81, 4.74	7.16, 6.57	4.78, 4.83
Reduced fat milk	6.59, 6.59	6.05, 6.05, 5.52, 5.43	6.62, 6.55	8.20, 8.29, 5.61, 7.67
Butter	8.54, 8.16	6.32, 7.11	8.47, 8.34	6.52, 7.11
Shortening	5.17, 4.82	4.70, 4.43	5.21, 4.77	4.69, 4.43
Meat pie	4.54, 3.99	4.20, 3.64	4.48, 3.95	4.16, 3.64
Meat patty	3.76, 4.42	4.30, 4.34	3.70, 4.37	4.40, 4.34
Luncheon	6.27, 6.42	5.25, 4.70	6.30, 6.48	6.94, 5.24
Muesli bar	1.15, 1.59	1.11, 0.67	1.10, 1.44	1.19, 0.77
Chocolate- coated biscuit	0.72, 0.68	1.93, 1.46	0.67, 0.63	1.92, 1.56
Plain sweet biscuit	5.32, 4.70	4.37, 4.63	5.36, 4.76	6.59, 4.63
Savoury cracker biscuit	1.16, 0.88	0.67, 0.75	1.10, 0.83	1.07, -
White bread	2.54, 2.48	2.23, 2.15	3.49, 3.34	2.30, 2.35

Table A2.4 The total *trans* isomer results obtained using the AOCS and spectral subtraction techniques for the New Zealand food survey sample fats. The estimated test uncertainties are given by the pooled standard deviations. These are AOCS-TG: 0.41%; AOCS-FAME: 0.39%; SS-TG: 0.37% and SS-FAME: 0.78%.

APPENDIX 3 VALIDATION DATA FOR THE Ag-TLC/GC METHOD

Sample Number	AMF-QC	MARG-QC
	Total C18:1 *trans* positional Isomer (g/100 g fat)	
1	5.62	13.07
2	5.60	12.41
3	5.12	13.20
4	5.50	12.99
5	5.44	12.61
6	5.59	12.95
7	5.71	13.19
8	5.65	12.95
9	5.50	12.06
10	5.42	
Mean ± standard deviation	5.49 ± 0.16	12.83 ± 0.39

Table A3.1 Repeatability determination for total C18:1 *trans* positional isomer by Ag-TLC/GC methodology using AMF-QC and MARG-QC samples.

Sample Name	C18:1 *trans* Positional Isomers (% total C18:1 *trans*)						
	6-9t	10t	11t	12t	13t &14t	15t	16t
AMF-QC	2.44	3.50	55.11	6.64	18.41	6.55	7.35
	2.67	3.46	56.27	5.91	17.84	6.61	7.24
	2.02	3.84	56.44	5.92	18.10	6.54	7.14
	2.95	3.58	54.85	6.45	18.65	7.03	6.49
	2.78	2.98	55.11	7.01	18.73	7.01	6.38
	3.27	2.41	55.32	6.40	18.18	7.07	7.35
	2.68	3.68	51.48	8.36	19.00	8.48	6.32
	2.90	3.06	53.29	9.26	17.85	7.31	6.33
	2.58	3.08	52.70	8.16	18.48	7.47	7.53
	2.70 (0.35)	3.29(0.44)	54.51(1.67)	7.12(1.82)	18.36(0.40)	7.12(0.61)	6.90(0.51)
MARG-QC	21.60	23.16	20.40	13.93	15.48	3.28	2.14
	27.36	21.62	19.18	12.49	13.85	3.23	2.27
	27.26	22.65	18.51	12.78	13.89	3.08	1.84
	28.05	21.98	18.69	12.45	13.86	3.12	1.85
	21.02	23.54	19.78	14.53	15.40	3.27	2.25
	27.73	22.71	18.51	12.38	13.99	3.15	1.53
	27.45	22.14	18.87	12.53	13.77	3.04	2.20
	21.72	21.40	19.66	14.39	16.01	4.59	2.24
	25.27(3.18)	22.40(0.75)	19.20(0.69)	13.19(0.93)	14.53(0.93)	3.34(0.51)	2.04(0.27)

Table A3.2 The repeatability standard deviation of the Ag-TLC/GC method using the 50 m BPX70 capillary column for the determination of C18:1 *trans* positional isomer distribution in the AMF-QC and MARG-QC samples. Repeatability standard deviations are given in brackets.

	C18:2 Geometric Isomers (g/100 g fat)				C18:3 Geometric Isomers (g/100 g fat)							*Σtrans* Geometric Isomers (wt%)	
	tt	ct	tc	cc	ttt	ctt	tct	ttc/cct	ctc	tcc	ccc	C18:2	C18:3
AMF-QC	0.11 0.10 0.10 0.12 0.13 0.11	0.04 0.03 0.04 0.03 0.04 0.03	0.03 0.01 0.03 0.02 0.02 0.01		0.03 0.03 0.02	0.08 0.09 0.09	0.01 0.02 0.01	0.01 0.03 0.01	nd nd nd	nd nd nd		0.17 0.14 0.17 0.17 0.18 0.15	0.14 0.13 0.12
Mean	0.11 (0.01)	0.04 (0.005)	0.02 (0.01)	1.58	0.03 (0.006)	0.08 (0.006)	0.01 (0.006)	0.02 (0.01)	-	-	1.01	0.17	0.14
MARG-QC	0.13 0.14 0.14 0.15 0.11 0.13	0.49 0.55 0.48 0.48 0.44 0.52	0.29 0.23 0.25 0.22 0.24 0.24		0.03 0.01 0.01	0.04 0.02 0.05	nd nd nd	0.17 0.15 0.20	0.04 0.02 0.05	0.06 0.04 0.04		0.91 0.92 0.87 0.85 0.79 0.89	0.34 0.24 0.35
Mean	0.13 (0.01)	0.49 (0.04)	0.24 (0.02)	43.08	0.02 (0.01)	0.04 (0.02)	nd	0.17 (0.02)	0.04 (0.02)	0.05 (0.01)	2.64	0.86	0.31

Table A3.3 The repeatability of the Ag-TLC/GC method using the 50 m BPX70 capillary column for the quantification of C18:2 and C18:3 geometric isomers determined using the AMF-QC and MARG-QC samples. Standard deviations are given in brackets.

Sample	C18:1 *trans* (mg)			Recovery
	Natural	Added	Total	(%)
MARG-QC	5.38	4.95	10.29	99.4
	6.24	5.26	11.37	97.6
	6.35	5.26	11.54	98.7
	6.40	2.63	9.20	106.6
	5.45	2.48	7.76	93.2
	6.00	2.48	8.31	93.0
	5.26	2.63	7.99	103.7
Mean ± standard deviation				98.9 ± 4.7
AMF-QC	2.49	4.95	7.51	101.2
	2.32	4.95	7.35	101.4
	2.31	2.48	4.67	95.1
	2.35	1.31	3.68	101.3
	2.49	1.31	3.78	98.4
	2.52	0.66	3.17	99.5
	2.50	0.66	3.12	103.47
Mean ± standard deviation				100.1 ± 2.7

Table A3.4 Recovery of trielaidin added to the AMF-QC and MARG-QC samples, as determined by Ag-TLC/GC. Total: level of C18:1 *trans* determined after spiking the natural levels.

APPENDIX 4 IR ABSORBANCE OF C18:2 AND C18:3 GEOMETRIC ISOMERS ISOLATED BY Ag-TLC

Band	Concentr at-ion	Absorbance (height)	Absorbance /g	Postulated Isomer from GC analysis	Class and Literature
A1	175 433	0.0157 0.0398	89.7 92.0	C18:2 t,t	1 (di-*trans* diene)
A2	1650 4422	0.0897 0.2472	54.4 55.9	C18:2 ct/tc	2 (mono-*trans* diene)
A3	-	ND	ND	C18:2 cc	3 (di-*cis* diene)
C18:3 Band				C18:3 Δ9, 12, 15	
B1	340 870	0.0563 0.1322	166 152	t, t, t	1 (tri-*trans* triene)
B2a	55.4 130.6	0.0061 0.0146	110.1 112.0	t, c, t	2 (di-*trans* triene)
B2b	109 250	0.0107 0.0279	98 112	ctt/ttc	2 (di-*trans* triene)
B3a	176	0.0083	47	cct/tcc	3 (mono-*trans* triene)
B3b	56 74	0.0027 0.0033	48 44	ctc	3 (mono-*trans* triene)
B4	-	ND	-	ccc	4 (tri-*cis* triene)
Standard	0.1082 mg/ml 0.2164 mg/ml	0.0087 0.0179	80 83	C18:1	Δ11t methyl elaidate standard

Table A4.1 Infrared absorbances/g of TFA groups extracted from Ag- TLC. The pooled standard deviation was determined as 4.1 absorbance/g.

APPENDIX 5 STATISTICAL ANALYSIS

A5.1 Ag-TLC/GC vs IR method

The total *trans* fatty acid results obtained using the Ag-TLC/GC and the four different infrared methods were compared using Minitab, two way analysis of variance (ANOVA) and general linear model statistical methods (Minitab, version 11, Minitab Inc., State College, Pennsylvania, USA). This method of analysis accounts for several sources of variation, or factors at one time. In this comparison, the factors were samples and methods. A 95% confidence level was used for the analysis. Statistical analysis results are given in Table A5.1. They indicated that, of the four infrared methods, only the AOCS-FAME IR method produced results that were significantly different from the Ag-TLC/GC method. The differences for all four infrared methods were -0.82%, -0.23%, 0.04% and 0.155% for the AOCS-FAME, AOCS-TG, SS-FAME and SS-TG methods respectively.

General Linear Model; tests to see if either the method or the samples are significantly different

Products 20 (including the AMF-QC and the MARG-QC samples)

Margarine A	Margarine B	Commercial pastry fat A
Commercial pastry fat B	Commercial pastry fat C	Pastry "feather flake"
Homogenised milk	Reduced fat milk	Butter
Shortening	Savoury mince pie	Meat Patties
Luncheon	Muesli Bar	Chocolate coated biscuits
Plan sweet biscuits	Savour cracker biscuits	White bread
AMF-QC	MARG-QC	

Methods number

1. Ag-TLC/GC
2. AOCS-FAME
3. AOCS-TG
4. SS-FAME
5. SS-TG

Means for result for methods

1	6.820
2	6.005
3	6.592
4	6.863
5	6.975

Descriptive Statistics

Method No.	N	Mean*	Median	Tr Mean	StDev	SE Mean
2	20	-0.815	-0.530	-0.682	1.408	0.315
3	20	-0.228	0.115	-0.116	1.497	0.335
4	20	0.042	0.495	0.167	1.784	0.399
5	20	0.155	0.490	0.278	1.781	0.398

* average difference compared to Ag-TLC/GC method

Method No.	Min	Max	Q1	Q3
2	-4.930	0.900	-1.773	0.130
3	-4.570	2.090	-0.990	0.710
4	-4.620	2.460	-0.972	1.542
5	-4.670	2.760	-0.707	1.588

t-test of the Mean

t-test of mu = 0.000 vs mu not = 0.000

Method No.	N	Mean	StDev	SE Mean	T	P
2	20	-0.816	1.408	0.315	-2.59	0.018
3	20	-0.228	1.497	0.335	-0.68	0.50
4	20	0.042	1.784	0.399	0.11	0.92
5	20	0.155	1.781	0.398	0.39	0.70

Results

1. The t-tests indicated that on average, the methods do differ.

2. Method 2 (AOCS-FAME) produces much lower results than the other IR methods compared to the Ag-TLC/GC method.

Table A5.1 The analysis of variance (ANOVA) results for the comparison of the Ag-TLC/GC and infrared methods.

A5.2 Correlation between the C18:1 fatty acid isomers over the dairy season

The correlation analyses between the various C18:1 *trans* positional isomers and C18:0 fatty acids were examined using a two-tailed t-test at a 95% confidence level (Mendenhall & Ott, 1980). Calculations were performed using Quattro Pro spreadsheet software (Borland International, Scotts Valley, California., USA). The correlation coefficient results for the various C18 fatty acids across the dairy season are given in Tables A5.2 and A5.3. The correlation results between the C18:1 Δ11t and the conjugated C18:2 fatty acids of the New Zealand survey butter samples and the overseas butter samples are given below.

A5.3 Correlation between C18:1 Δ11t and conjugated C18:2 for New Zealand seasonal survey butter

Regression Statistics

R square : 0.0258 (no correlation)
Standard Error: 0.5495

Analysis of Variance

Regression	Sum of squares	Mean Square	F	Significance F
Regression	0.0320	0.0320	0.10605	0.7610
Residual	1.2079	0.3020		
Total	1.2400			

A5.4 Correlation between C18:1 Δ11t and conjugated C18:2 for overseas butter samples

Regression Statistics

R square : 0.8238 (good correlation)
Standard Error: 0.4969

Analysis of Variance

Regression	Sum of squares	Mean Square	F	Significance F
Regression	5.775	5.775	23.38	0.0047
Residual	1.235	0.2469		
Total	7.001			

	Total *trans* C18:1	Total *cis* C18:1	total C18:1	C18:0	C18:1 6-9Δt	C18:1 10Δt	C18:1 11Δt	C18:1 12Δt	C18:1 13-14Δt	C18:1 15Δt	C18:1 16Δt
Total *trans* C18:1	1										
Total *cis* C18:1	0.764	1									
Total C18:1	0.878	0.966	1								
C18:0	0.511	0.307	0.304	1							
C18:1 6-9Δt	-0.378	-0.413	-0.360	-0.875	1						
C18:1 10Δt	0.423	0.844	0.782	-0.134	-0.175	1					
C18:1 11Δt	0.897	0.474	0.605	0.754	-0.518	0.025	1				
C18:1 12Δt	0.382	0.171	0.331	-0.139	-0.036	0.369	0.264	1			
C18:1 13-14Δt	0.464	0.777	0.736	-0.302	0.184	0.869	0.048	0.238	1		
C18:1 15Δt	0.821	0.793	0.821	0.716	-0.670	0.404	0.764	0.016	0.267	1	
C18:1 16Δt	0.561	0.480	0.532	0.520	-0.355	0.070	0.561	-0.290	0.025	0.836	1

Table A5.2 Correlation coefficient calculated for the C18:0 and the C18:1 fatty acid groups over the dairy season (1995/96). The correlation coefficient must exceed 0.82 for the relationship to be significant at a 95% confidence level (Mendenhall & Ott, 1980).

	total c18:1 *trans*	C18:1 *cis*	C18:1 *trans*	C18:2	C18:2 *trans*	C18:3 *trans*	C18:3
C18:1 *cis*	0.767						
C18:1*trans*	0.999	0.764					
C18:2	0.668	0.666	0.686				
C18:2trans	-0.570	-0.355	-0.597	-0.848			
C18:3*trans*	0.802	0.594	0.780	0.489	-0.372		
C18:3	0.579	0.472	0.548	0.331	-0.057	0.899	
C18:2 conjugated	0.124	0.255	0.156	0.182	-0.179	-0.481	-0.635

Table A5.3 Correlation coefficient calculated for the C18:1, C18:2 and C18:3 isomeric fatty acids groups over the dairy season (1995/96). The correlation coefficient must exceed 0.80 for the relationship to be significant at a 95% confidence level (Mendenhall & Ott, 1980).

APPENDIX 6 *Trans* fatty acid intake estimation in the New Zealand Diet

TFA intake occurs primarily through the consumption of food fats. The estimation of its intake was therefore made by determining the proprotion of these various sources of food fats consumed, and the proportion of TFAs in each of these sources. Where the food fat consumption data were not available, the "apparent consumption" based on the dietaty records of food ingested were used. The food fat and "apparent consumption" data used in the calculations for TFA intake in this thesis were dervied form Horwarth *et al.* (1991) and Wilson *et al.* (1995) respectively. The "apparent consumption data" were conducted from Life in New Zealand" 24 hour diet recall survey of 1602 New Zealanders in 1991 (Russell & Wilson, 1991).

The median daily fat intake for New Zealand was 103g/day for males and 65g/day for females, contributing to approximately 38% of the total energy energy (Horwarth *et al.,* 1991). The principle sources of food fat contributing to TFA intake in the diet, taken from the Life in New Zealand survey, are given in Table A6.1. The fat source listed however, only accounts for 66% and 77% of the total dietary fat intake for males and females respectively. A number of other food items such as breakfast cereal, poultry, pasta, grains, cakes, fried potatoes, cakes *etc.,* also contributed significantly to the fat intake, but were not included in the survey because they were not expected to contribute greatly to the *trans* fatty acid intake based on scientific literature. Very few New Zealanders use margarine when frying (6%) or roasting meat (3%); the remainder use animal based fat, vegetable oil or no fats or oil (Horwarth *et al.,* 1991).

For biscuits, the fat consumption data was divided equally amongst the three products (chocolate caoted, sweet and savoury). Likewise, the "other meat" fat consumption data was divided equally between the meat patty and luncheon samples. The milk consumption data was divided according to the product disappearance data (Department of Statistics, 1991).

Total *trans* intake was calculated as: **$\sum$C18:1 t (Table A6.5) + $\sum$C18:2 t (Table A6.6) + $\sum$C18:3 t (Table A6.7)**, for both males and females.

Foods	g food consumed/day		% fat contribution/day	
	Males	Females	Males	Females
Margarine	24	14	19.4	17.7
Pastry*	17	14	3.9	5.2
Milk- homogenised	227	178	6.6	8.2
fat reduced	57	44	0.6	0.7
Butter	27	22	21.7	28.1
Shortening	5	6	4.1	7.6
Mince pie*	30	14	4.0	3.0
Meat patty*	2	1	0.5	0.5
Luncheon*	4	3	0.5	0.5
Biscuits-chocolate coated*	4	3	1.0	1.3
sweet*	7	6	1.0	1.3
savoury*	4	4	1.0	1.3
Bread	154	97	1.5	1.5

Table A6.1 The food comsumption data used to estimate the *trans* intake for the average New Zealand male and female. *Calculated from the fat consumption data. For example; the fat intake from pastry for males is 4% (4.12g fat/day) of the total fat intake of 103g fat/day. This is equivalent to 17 g pf pastry product per day (given the fat content for pastry was 0.24g/g product).

Sample	food fat content (g/g sample)	C18:1 *trans* fatty acid, g/100 g food							
		6t-9t	10t	11t	12t	13t-14t	15t	16t	∑C18:1t
Margarine A	0.82	4.75	2.31	1.73	1.21	0.87	0.21	0.07	11.13
Margarine B	0.82	3.41	2.54	2.13	1.5	1.7	0.45	0.29	12.01
Pastry fat A	0.85	1.57	0.8	2.01	0.39	0.62	0.23	0.2	5.81
Pastry fat B	0.84	3.11	1.63	0.67	0.54	0.35	0.08	0.03	6.42
Pastry fat C	0.82	0.84	0.33	2.45	0.42	0.8	0.35	0.28	5.47
Pastry	0.24	0.33	0.19	0.51	0.1	0.16	0.06	0.05	1.38
Homogenised milk	0.03	0.01	0	0.07	0.01	0.02	0.01	0.01	0.13
Reduced fat milk	0.01	0	0	0.04	0.01	0.01	0.01	0.01	0.08
Butter	0.83	0.24	0.18	2.83	0.4	0.9	0.42	0.33	5.27
Shortening	0.82	0.29	0.29	2.02	0.16	0.32	0.14	0.14	3.35
Mince pie	0.14	0.05	0.02	0.25	0.03	0.07	0.03	0.03	0.48
Meat Patty	0.25	0.08	0.03	0.39	0.07	0.11	0.05	0.04	0.76
Luncheon	0.13	0.06	0.03	0.35	0.06	0.1	0.05	0.04	0.71
Muesli bar	0.2	0.11	0.07	0.1	0.05	0.04	0.01	0.01	0.38
Chocolate biscuit	0.26	0.2	0.08	0.28	0.07	0.09	0.04	0.02	0.79
Sweet biscuit	0.15	0.09	0.13	0.46	0.06	0.11	0.05	0.04	0.93
Savoury biscuit	0.24	0.51	0.4	0.1	0.12	0.09	0.09	0	1.31
Bread	0.01	0.01	0.01	0.01	0.01	0.01	0	0	0.04

Table A6.2 The calculated C18:1 *trans* positional isomer content in the New Zealand food survey foods.

Sample	g of C18:2 fatty acid isomer /100g food				
	tt	ct	tc	cc	∑18:2
Margarine A	0.164	0.450	0.229	35.199	36.041
Margarine B	0.139	0.221	0.049	35.542	35.950
Pastry fat A	0.068	0.085	0.017	2.443	2.613
Pastry fat B	0.000	0.151	0.084	11.077	11.312
Pastry fat C	0.082	0.057	0.025	2.475	2.639
Pastry	0.031	0.009	0.002	1.552	1.594
Homogenised milk	0.003	0.003	0.000	0.038	0.044
Redued fat milk	0.000	0.000	0.000	0.021	0.021
Butter	0.074	0.017	0.008	0.902	1.001
Shortening	0.024	0.073	0.016	2.514	2.628
Mince pie	0.011	0.012	0.001	0.659	0.684
Meat patty	0.017	0.015	0.005	0.434	0.471
Luncheon	0.007	0.026	0.000	0.647	0.680
Muesli bar	0.002	0.004	0.000	0.436	0.442
Chocolate-biscuit	0.124	0.024	0.013	1.669	1.829
Sweet biscuit	0.020	0.008	0.000	0.804	0.831
Savoury biscuit	0.005	0.010	0.000	1.047	1.062
Bread	0.002	0.002	0.000	0.588	0.593

Table A6.3 The C18:2 *trans* isomer content in the New Zealand food survey foods.

Samples	g of C18:3 fatty acid isomer /100g food							
	ttt	ctt	tct	ttc\cct	ctc	tcc	ccc	∑C18:3
Margarine A	0.024	0.311	0.041	0.213	0.041	0.106	0.998	1.734
Margarine B	0.010	0.016	0.000	0.114	0.016	0.033	2.012	2.200
Pastry fat A	0.017	0.017	0.051	0.017	0.010	0.010	0.264	0.383
Pastry fat B	0.000	0.033	0.010	0.033	0.000	0.000	0.117	0.193
Pastry fat C	0.000	0.033	0.016	0.033	0.016	0.010	0.457	0.570
Pastry	0.000	0.000	0.010	0.014	0.000	0.000	0.230	0.259
Homogenisedmilk	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.025
Reduced fat milk	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.015
Butter	0.000	0.033	0.010	0.017	0.010	0.010	0.604	0.678
Shortening	0.082	0.049	0.033	0.033	0.000	0.000	0.424	0.620
Mince pie	0.000	0.010	0.000	0.010	0.000	0.000	0.071	0.092
Meat Patty	0.010	0.010	0.017	0.010	0.000	0.000	0.185	0.229
Luncheon	0.000	0.010	0.010	0.010	0.000	0.000	0.129	0.155
Muesli bar	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.046
Chocolate biscuit	0.000	0.000	0.000	0.000	0.000	0.000	0.087	0.097
Sweet biscuit	0.000	0.010	0.000	0.012	0.000	0.000	0.071	0.096
Savoury biscuit	0.000	0.010	0.000	0.000	0.000	0.000	0.043	0.057
Bread	0.000	0.000	0.000	0.012	0.000	0.000	0.061	0.080

Table A6.4 The C18:3 *trans* isomer content in the New Zealand food survey foods.

Sample	Male Food Intake g/day	*Trans* Intake (g/day) for Males								Female Food Intake g/day	*Trans* Intake (g/day) for Females							
		6t-9t	10t	11t	12t	13t-14t	15t	16t	total 18:1t		6t-9t	10t	11t	12t	13t-14t	15t	16t	total 18:1t
Margarine A	12	0.570	0.278	0.207	0.145	0.104	0.026	0.009	1.339	14	0.233	0.162	0.121	0.085	0.061	0.015	0.005	0.781
Margarine B	12	0.409	0.304	0.255	0.181	0.204	0.054	0.034	1.442	14	0.239	0.177	0.149	0.105	0.119	0.031	0.020	0.841
Pastry fat A																		
Pastry fat B																		
Pastry fat C																		
Pastry	17	0.056	0.032	0.086	0.017	0.026	0.010	0.008	0.235	14	0.046	0.026	0.071	0.014	0.022	0.008	0.007	0.194
Homogenised milk	227	0.012	0.009	0.151	0.022	0.051	0.022	0.021	0.289	178	0.010	0.007	0.118	0.018	0.040	0.018	0.017	0.227
Reduced fat milk	57	0.002	0.001	0.025	0.003	0.007	0.003	0.003	0.045	44	0.002	0.001	0.019	0.002	0.006	0.002	0.002	0.035
Butter	27	0.065	0.049	0.764	0.107	0.235	0.114	0.089	1.423	22	0.053	0.040	0.622	0.087	0.191	0.093	0.073	1.159
Shortening	5	0.015	0.014	0.101	0.008	0.016	0.007	0.007	0.167	6	0.018	0.017	0.121	0.009	0.019	0.008	0.008	0.201
Mince pie	30	0.015	0.006	0.075	0.010	0.020	0.009	0.008	0.143	14	0.007	0.003	0.035	0.005	0.009	0.004	0.004	0.076
Meat patty	2	0.002	0.001	0.008	0.001	0.002	0.001	0.001	0.015	1	0.001	0.000	0.004	0.001	0.001	0.000	0.000	0.008
Luncheon	4	0.003	0.001	0.014	0.002	0.004	0.002	0.002	0.028	3	0.002	0.001	0.011	0.002	0.003	0.002	0.001	0.021
Muesli bar																		
Chocolate-biscuit	4	0.008	0.003	0.011	0.003	0.004	0.001	0.001	0.032	3	0.006	0.003	0.008	0.002	0.003	0.001	0.001	0.024
Sweet biscuit	7	0.006	0.009	0.032	0.004	0.008	0.003	0.003	0.065	6	0.005	0.008	0.027	0.004	0.006	0.003	0.002	0.056
Savoury biscuit	4	0.020	0.016	0.004	0.005	0.004	0.004	0.000	0.053	4	0.020	0.016	0.004	0.005	0.004	0.004	0.000	0.053
Bread	154	0.021	0.007	0.009	0.008	0.009	0.003	0.000	0.056	97	0.013	0.004	0.006	0.005	0.005	0.002	0.000	0.035
SUM		**1.205**	**0.731**	**1.742**	**0.517**	**0.693**	**0.259**	**0.186**	**5.332**		**0.754**	**0.466**	**1.316**	**0.343**	**0.489**	**0.191**	**0.141**	**3.701**

Table A6.5 The calculated C18:1 *trans* positional isomer intake from the various food products for an average New Zealand male and female. These estimates were calculated based on the food consumption data given in Table A6.1 and *trans* content of these foods given in Table A6.2.

	Intake of C18:2 TFA Isomers in Males - g/day					Intake of C18:2 TFA Isomers in Females - g/day				
	tt	ct	tc	cc	∑ C18:2 *t*	tt	ct	tc	cc	∑ C18:2 *t*
Margarine A	0.020	0.054	0.027	4.224	0.101	0.011	0.031	0.016	2.464	0.059
Margarine B	0.017	0.026	0.006	4.265	0.049	0.010	0.015	0.003	2.488	0.029
Pastry fat A										
Pastry fat B										
Pastry fat C										
Pastry	0.005	0.002	0.000	0.000	0.007	0.004	0.001	0.000	0.217	0.006
Homogenised milk	0.006	0.008	0.000	0.086	0.014	0.005	0.006	0.000	0.068	0.011
Redued fat milk	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.009	0.000
Butter	0.020	0.004	0.002	0.243	0.027	0.016	0.004	0.002	0.198	0.022
Shortening	0.001	0.004	0.001	0.126	0.006	0.001	0.004	0.001	0.151	0.007
Mince pie	0.003	0.004	0.000	0.198	0.007	0.002	0.002	0.000	0.092	0.003
Meat patty	0.001	0.000	0.000	0.009	0.001	0.000	0.000	0.000	0.004	0.000
Luncheon	0.000	0.001	0.000	0.026	0.001	0.000	0.001	0.000	0.019	0.001
Muesli bar										
Chocolate-biscuit	0.005	0.001	0.001	0.067	0.006	0.004	0.001	0.000	0.050	0.005
Sweet biscuit	0.001	0.001	0.000	0.056	0.002	0.001	0.000	0.000	0.048	0.002
Savoury biscuit	0.000	0.000	0.000	0.042	0.001	0.000	0.000	0.000	0.042	0.001
Bread	0.004	0.003	0.000	0.906	0.007	0.002	0.002	0.000	0.571	0.004
SUM	**0.083**	**0.108**	**0.038**	**10.524**	**0.229**	**0.058**	**0.069**	**0.023**	**6.422**	**0.150**

Table A6.6 The calculated C18:2 *trans* positional isomer intake from the various food products for an average New Zealand male and female. These estimates were calculated based on the food consumption data given in Table A6.1 and *trans* content of these food given in Table A6.3.

	Intake of C18:3 TFA Isomer in Males - g/day								Intake of C18:3 TFA Isomer in Females - g/day							
	ttt	ctt	tct	ttc\cct	ctc	tcc	ccc	∑C18:3t	ttt	ctt	tct	ttc\cct	ctc	tcc	ccc	∑C18:3t
Margarine A	0.003	0.037	0.005	0.026	0.005	0.013	0.120	0.088	0.002	0.022	0.003	0.015	0.003	0.007	0.070	0.052
Margarine B	0.001	0.002	0.000	0.014	0.002	0.004	0.241	0.023	0.001	0.001	0.000	0.008	0.001	0.002	0.141	0.013
Pastry fat A																
Pastry fat B																
Pastry fat C																
Pastry	0.000	0.000	0.001	0.002	0.000	0.000	0.039	0.003	0.000	0.000	0.001	0.002	0.000	0.000	0.032	0.003
Homogenised milk	0.001	0.001	0.001	0.001	0.001	0.001	0.051	0.006	0.001	0.001	0.001	0.001	0.000	0.007	0.040	0.004
Reduced fat milk	0.000	0.001	0.000	0.001	0.000	0.000	0.006	0.002	0.000	0.001	0.000	0.001	0.000		0.049	0.002
Butter	0.000	0.009	0.002	0.005	0.002	0.002	0.163	0.020	0.000	0.007	0.002	0.004	0.002	0.002	0.133	0.017
Shortening	0.004	0.002	0.002	0.002	0.000	0.000	0.021	0.010	0.011	0.007	0.005	0.005	0.000	0.000	0.059	0.002
Mince pie	0.001	0.002	0.001	0.002	0.001	0.000	0.021	0.007	0.000	0.001	0.001	0.001	0.000	0.000	0.010	0.003
Meat patty	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000
Luncheon	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000
Muesli bar																
Chocolate-biscuit	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000
Sweet biscuit	0.000	0.001	0.000	0.001	0.000	0.000	0.005	0.002	0.000	0.000	0.000	0.001	0.000	0.000	0.004	0.001
Savoury biscuit	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000
Bread	0.002	0.003	0.001	0.018	0.002	0.004	0.094	0.030	0.001	0.002	0.000	0.011	0.001	0.003	0.059	0.018
SUM	**0.012**	**0.059**	**0.013**	**0.072**	**0.013**	**0.026**	**0.767**	**0.193**	**0.016**	**0.042**	**0.013**	**0.049**	**0.007**	**0.014**	**0.002**	**0.142**

Table A6.7 The calculated C18:3 *trans* positional isomer intake from the various food products for an average New Zealand male and female. These estimates were calculated based on the food consumption data given in Table A6.1 and *trans* content of these foods given in Table A6.4.