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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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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).

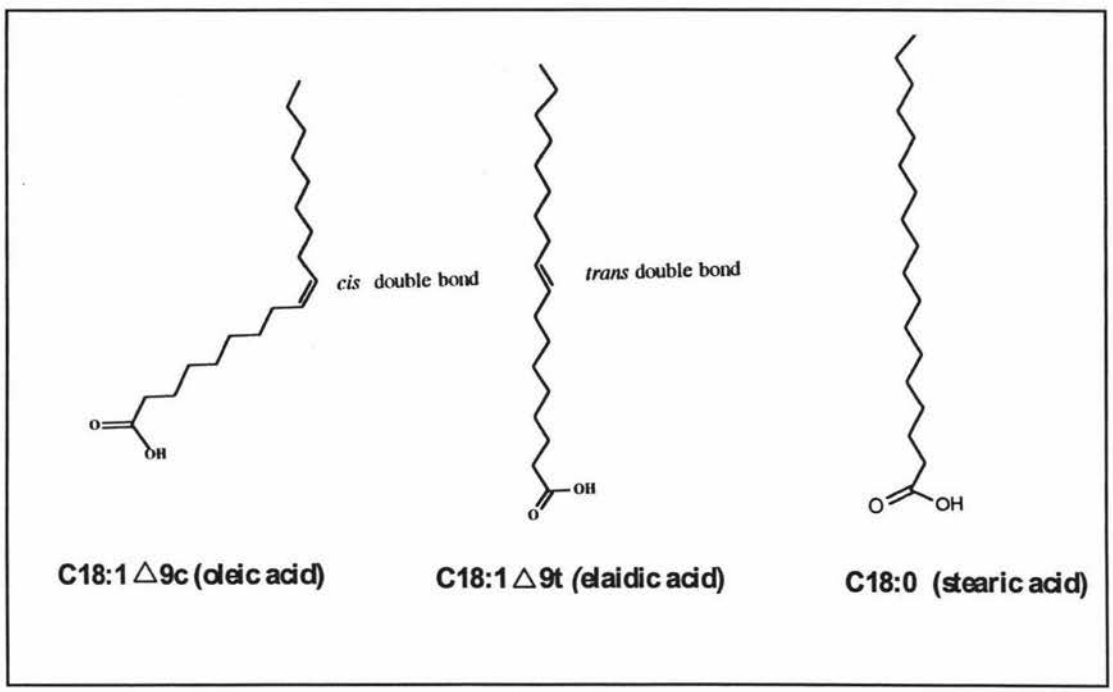

Figure 1 Geometric configuration of *cis*, *trans* unsaturation and saturated fatty acids.

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).

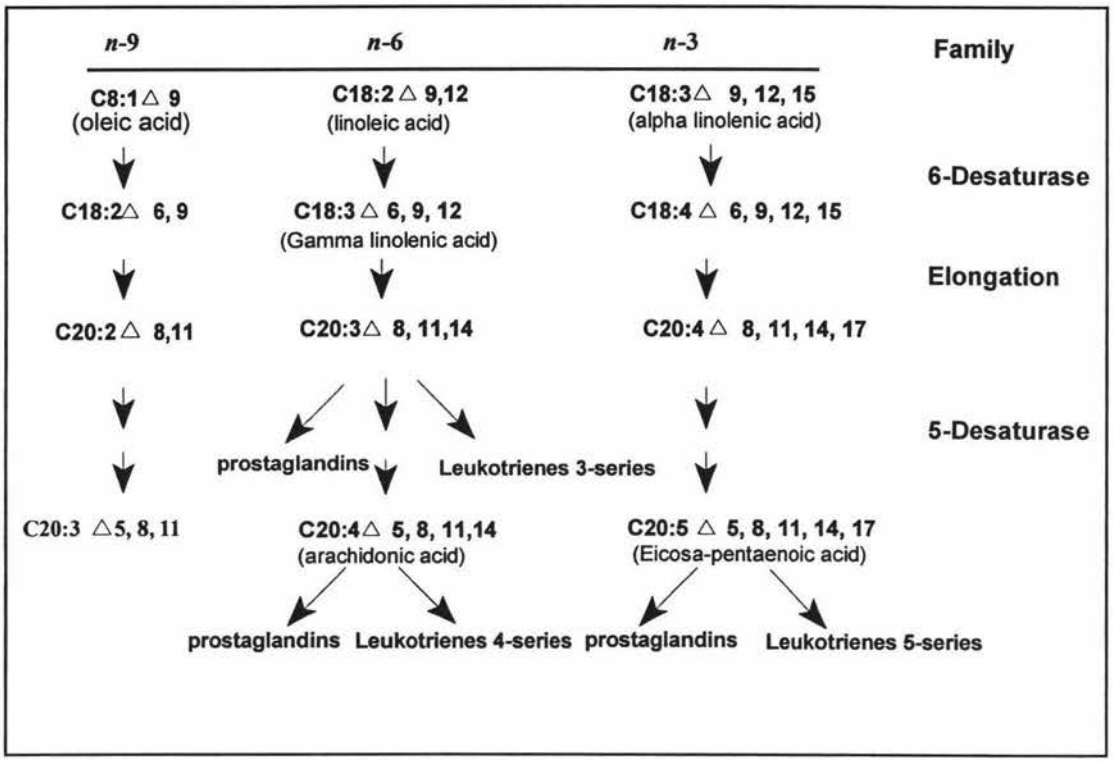

Figure 2 The metabolic pathway of the polyunsaturate families in the synthesis of eicosanoids and prostaglandins (Gurr, 1995a; Bruckner, 1992).

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.

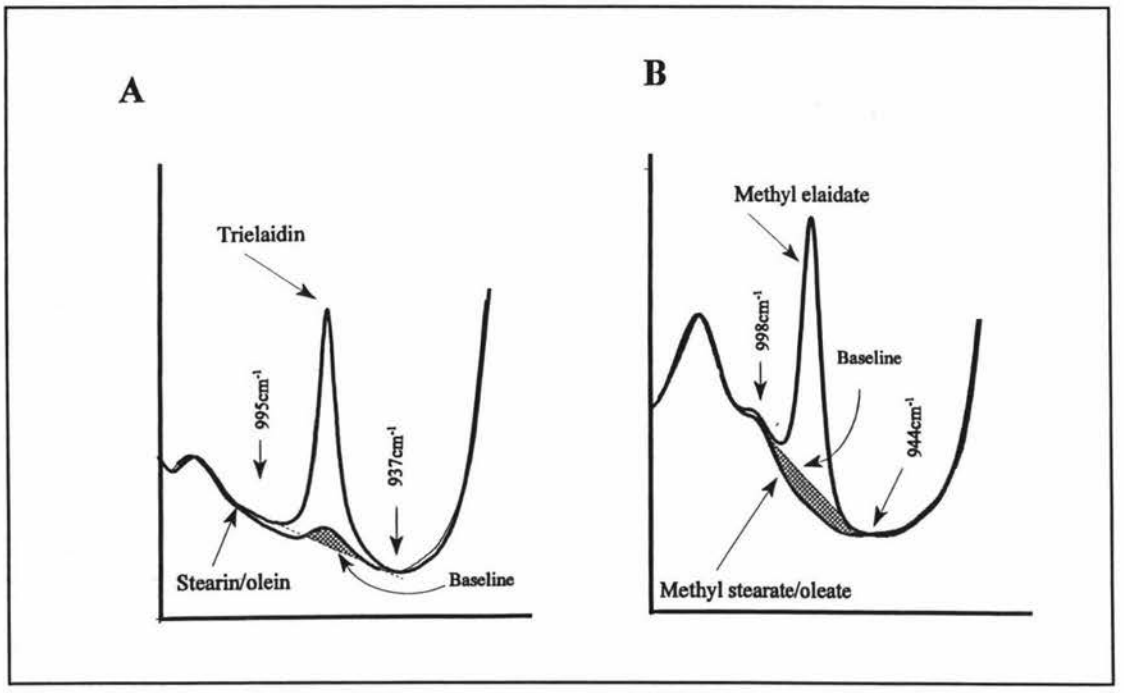

Figure 3 FTIR spectra of A: triglyceride, B: methyl ester with the respective non-*trans* background spectra. The shaded regions indicate the approximate bias generated as the result of the absorption of the non-*trans* components and the baseline boundaries specified by the AOCS Cd 14-61 method (AOCS,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

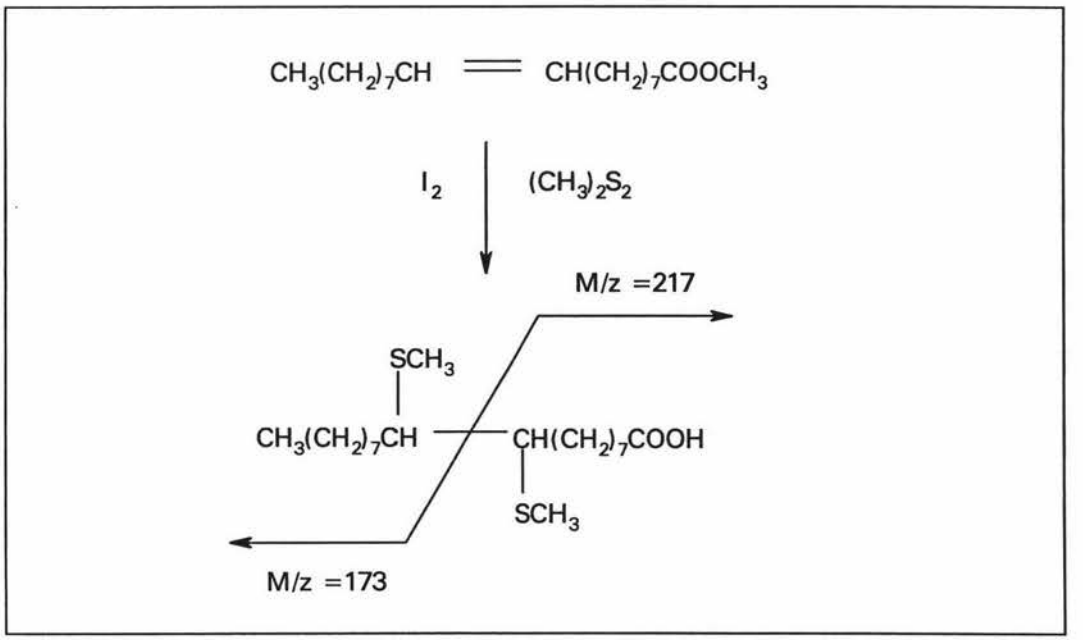

Figure 4 Reaction of dimethyl disulphide with an unsaturated ester for double bond location by MS (Christie, 1996).

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.