Original Article

Determination of *trans* fatty acid levels by FTIR in processed foods in Australia

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Health authorities around the world advise 'limiting consumption of *trans* fatty acids', however in Australia the *trans* fatty acid (TFA) content is not required to be listed in the nutrition information panel unless a declaration or nutrient claim is made about fatty acids or cholesterol. Since there is limited knowledge about *trans* fatty acid levels in processed foods available in Australia, this study aimed to determine the levels of TFA in selected food items known to be sources of TFA from previously published studies. Food items (n=92) that contain vegetable oil and a total fat content greater than 5% were included. This criterion was used in conjunction with a review of similar studies where food items were found to contain high levels of *trans* fatty acids. Lipids were extracted using solvents. Gravimetric methods were used to determine total fat content and *trans* fatty acid levels were quantified by Attenuated Total Reflectance Fourier Transform Infrared spectroscopy. High levels of *trans* fatty acids were found in certain items in the Australian food supply, with a high degree of variability. Of the samples analysed, 13 contained greater than 1 g of *trans* fatty acids per serving size, the highest value was 8.1 g/serving. Apart from when the nutrition information panel states that the content is less than a designated low level, food labels sold in Australia do not indicate *trans* fatty acid levels. We suggested that health authorities seek ways to assist consumers to limit their intakes of *trans* fatty acids.

Key Words: *trans* fatty acids, levels in foods, ATR-FTIR analysis, FSANZ, Australian foods

INTRODUCTION

Trans fatty acids (TFA) are found in processed foods. They result from the partial industrial hydrogenation of vegetable and marine oils. Low levels of TFA are also found in the fat of ruminants, as a result of biohydrogenation by ruminant microbes of the polyunsaturated fatty acids found in animal feed (in grass, clover etc.).

TFA increase low density lipoprotein (LDL) cholesterol and lower high density lipoprotein (HDL) cholesterol, both risk factors for coronary heart disease (CHD). TFA have been found to be the most harmful macronutrient to influence the ratio of total cholesterol to HDL (Total:HDL) cholesterol, a key risk factor for CHD.[1] A 2% increase in TFA intake has been calculated to increase the risk of CHD by 23%.[2] Based on isoenergenic consumption, current evidence suggests that TFA increase the risk of CHD more than any other macronutrient.[2] However, a recent paper reported that the intake of TFA from ruminant sources was not associated with a higher risk of CHD.[3]

Dietary analysis by Food Science Australia New Zealand (FSANZ), an independent statutory agency that sets food standards for Australia and New Zealand, has estimated that Australians consume 0.6% of their daily kilojoules from TFA.[4] This is below the maximum intake of 1% recommended by the World Health Organization (WHO).[5] Any evaluation of levels of TFA in the Australian food supply is at best speculative because there is currently no comprehensive TFA data base. We have limited knowledge of TFA levels in food products and large variations have been reported within similar food items.[2,6-8]

FSANZ advise 'limiting intakes' of TFA along with saturated fat. While it is mandatory to declare saturated fat content, as a percentage of total fat content, on a Nutrition Information Panel (NIP), there is no such requirement for TFA, unless a nutrient or health claim is made regarding cholesterol or fatty acids.

The aim of this study was to determine the levels of TFA in foods readily available in Australia, selected on the basis that they could be significant sources of TFA in the food supply using an internationally recognised method utilising Fourier Transform Infrared Spectroscopy with attenuated total reflection (ATR-FTIR).[9]

MATERIALS AND METHODS

Food items were selected for analysis, based on data from previous studies which reported that major sources of TFA could be found in bakery products, fast food and frozen food, packaged snacks and fats & oils.[2,4] The food items in the above categories were selected if vegetable oil was a listed ingredient and if the fat content of the food exceeded 5%. The food items were purchased from

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retail outlets in Melbourne, Victoria, Australia. Food items were stored at an appropriate temperature (room temperature, refrigerated or frozen). Perishable food items were analysed immediately and non-perishables were analysed within their use-by date. A representative portion of individual food items was homogenised in a commercial blender (Waring brand). Triplicate amounts of the food item (typically 5g with the specific weight recorded) were extracted with 2:1 chloroform-methanol solution containing 10 mg/L butylated hydroxytoluene (BHT) antioxidant.[10]

Gravimetric methods were used to determine total fat content. In total, determination of total fat content of an individual food item was the mean of nine aliquots (triplicate aliquots of three lipid extractions).

Quantification of TFA was determined by ATR-FTIR with a ZnSe crystal.[9] Trielaidin diluted in triolein was initially used to generate calibration data on a Shimadzu IRPrestige-21 (P/N 206-72010) Fourier Transform Infrared Spectrophotometer. A pooled butter sample and a vegetable oil sample supplied by the American Oil Chemists' Society, with known TFA content, were analysed at the commencement of each analysis to ensure correct calibration. The relative standard deviation (as a %) for these two controls was 3.9% for the butter sample (n=46 separate determinations in triplicate) and 2.8% for the AOCS oil sample (n=49 separate determinations in triplicate). The TFA values for the foods are the mean of nine individual readings (3 food samples analysed in triplicate).

RESULTS

Of the 92 food items tested, 13 contained more than 1g TFA per serving (Table 1). The WHO recommends a maximum TFA intake of 1% of total energy intake which is equivalent to 2.4g of TFA on a daily intake of 8700kJ (the reference value for an Australian diet). Servings of food items which provide 40% (1g) of daily recommendations of TFA have been considered high for the purposes of this study. Table 1 includes all the food items which contain more than 1g of TFA per serving size and also lists the serving size, as recommended by the food manufacturer.

The mean (and range) of total fat content (g/100g of food) and TFA as a percentage of total fat is shown in Table 2 for similar food items (e.g. cinnamon donuts) and grouped in the designated product categories (e.g. bakery). The results show a considerable variation in the amount of TFA as a percentage of total fat among similar food items. This indicates the likely use of different fats and oils in manufacturing and preparation.

Table 2 also includes grams of TFA per 100g of food. This is important as it incorporates the amount of TFA as a percentage of total fatty acids as well as the amount of fat in a product. While some food items may not be consumed in 100g portions, this amount is used for comparative purposes for food items in this study and other Australian and international studies. The variation in the number of grams of TFA per 100g of food among similar food items highlights the difficulties in providing messages to the public about TFA in food items.

Variation in TFA content is well illustrated when considering the results for cinnamon donuts, crackers and chocolate wafer biscuits. The levels of TFA in three different brands of cinnamon donuts analysed ranged from 0.7% to 29.8 % of the total fat content. This would result in a TFA intake ranging from 0.2g to 6.6g in a 100g serving. Similarly, a 100g serving of the five crackers analysed would result in intakes of TFA ranging from 0.1g to 3.9g and for chocolate wafer biscuits, a range of 0.3g to 5.7g of TFA.

Not all food items showed large variations in TFA content as a percentage of the total fat. The highest value of TFA level was measured in microwave popcorn. Three different brands of microwave popcorn were analysed. The mean TFA level was 30.0 per cent of the fat (range of 28.8 to 30.7) and the range of TFA values was 6.0g to 8.1g per 100g serving size.

Further investigation was performed to decipher whether the declaration of hydrogenated vegetable oil in the ingredient list showed any association with grams of TFA per 100g of food item. Seven of the 92 food items declared hydrogenated vegetable oil and the range of TFA in these seven items was 0.2g to 5.7 g per 100g of food item.

Origin of ingredients was also analysed to determine whether it was associated with the amount of TFA per 100g of food item. Of the 92 food items 39 used Australian made ingredients, 22 were composed of imported ingredients and the remaining 22 were made of both Aus-

Table 1. Food items which contain more than one gram of TFA per serving size

Product	TFA in a serving of product (g)	% of WHO TFA RDI	Serving size (g)
Microwave Popcorn Butter Flavour (brand A)	8.1	338	100
Microwave Popcorn Butter Flavour (brand B)	7.6	317	100
Microwave Popcorn (brand C)	6.0	250	100
Cinnamon Donut (brand A)	3.6	141	54
Pasty	3.3	137	150
Meat Pie (brand A)	2.3	96	175
Meat Pie (brand B)	2.2	92	175
Cinnamon Donut (brand B)	1.8	75	45
Sausage Roll	1.7	71	100
Meat Pie (brand C)	1.5	63	150
Meat Pie (brand D)	1.3	54	150
Choc Crème Wafer	1.1	46	20
Fried chicken nugget plus french fries	1.0	42	231

Table 2. TFA content of 92 food products determined by ATR-FTIR

Food item (number tested)	Total fat g/100g food Mean (range)	TFA as % of total fat Mean (range)	TFA g/100g food Mean (range)
Bakery Products			
Cinnamon Donuts (3)	19.5 (13.5-23.2)	19.7 (0.7-29.8)	3.6 (0.2-6.6)
Blueberry Muffins (3)	14.7 (11.5-19.8)	2.8 (1.5-4.9)	0.4 (0.2-0.6)
Croissant (2)	19.9 (19.1-20.7)	1.6 (1.6-1.7)	0.3 (0.3-0.4)
Sweet Pastry (4)	21.7 (10.1-36.2)	4.0 (3.5-4.4)	0.8 (0.6-1.3)
Meat Pies (5)	13.8 (11.4-15.9)	8.8 (7.6-11.9)	1.2 (0.9-1.8)
Sausage Rolls / Pasties (4)	12.7 (11.0-13.7)	12.5 (6.9-17.4)	1.6 (0.8-2.2)
Croutons (2)	16.6 (6.4-6.8)	3.0 (1.6-4.4)	0.2 (0.1-0.3)
Vol au Vent / Savoury Pastry (3)	23.4 (15.9-37.2)	16.8 (11.720.4)	4.2 (1.9- 7.6)
Fast & Frozen Food			
Fries (3)	17.3 (11.7- 21.4)	2.1 (1.4-3.4)	0.3 (0.3-0.4)
Chicken Nuggets (4)	19.6 (15.0-24.7)	2.2 (1.6-3.3)	0.4 (0.3-0.5)
Combined Nuggets & Fries (3)	19.0 (13.1-22.0)	2.3 (1.6-3.3)	0.4
Pizza (5)	9.6 (5.6-17.9)	6.5 (3.0-8.5)	0.9 (0.2-2.2)
Fish Fingers (3)	8.5 (6.0-10.8)	1.7 (1.4-2.0)	0.1
Crackers (5)	18.1 (16.6-21.5)	5.4 (0.8-23.4)	0.9 (0.1-3.9)
Crisps (4)	35.3 (18.6-44.1)	1.1 (0.7-1.9)	0.3 (0.3-0.4)
Corn chips (3)	25.4 (24.1-26.2)	2.1 (1.5-2.5)	0.5 (0.4-0.6)
Choc Wafer Biscuits (4)	23.7 (19.4-27.1)	6.8 (1.0-22.2)	1.7 (0.3-5.7)
Chocolate Bars (4)	30.2 (24.5-35.1)	3.5 (1.3-7.1)	1.0 (0.4-1.7)
Breakfast Bars (3)	7.3 (6.7-7.9)	1.8 (0.6-3.5)	0.1 (0.0-0.2)
Ready Popped Popcorn (3)	14.4 (4.1-26.8)	1.2 (0.8-1.4)	0.2 (0.0-0.4)
Microwave Popcorn (3)	24.1 (19.5-26.5)	30.0 (28.8-30.7)	7.2 (6.0-8.1)
Crackers (5)	18.1 (16.6-21.5)	5.4 (0.8-23.4)	0.9 (0.1-3.9)
Crisps (4)	35.3 (18.6-44.1)	1.1 (0.7-1.9)	0.3 (0.3-0.4)
Fats & Oils			
Soft Margarines (1)	64.6	11.6	7.5
Hard Margarines (4)	90.4 (80.5-100)	3.2 (1.7-4.5)	3.0 (1.7-4.5)
Dairy Blends (3)	60.8 (52.7-66.8)	5.6 (4.6-7.4)	3.4 (2.6-4.7)
Miscellaneous			
Peanut Butter (3)	48.9 (47.2-50.3)	1.4 (0.5-3.2)	0.7 (0.2-1.6)
Chocolate Spread (3)	36.5 (26.4-49.5)	0.8 (0.5-1.0)	0.3 (0.1-0.3)
Canned Fish (4)	25.0 (19.7-29.9)	2.8 (1.9-3.9)	0.7 (0.4-1.2)

tralian and imported ingredients. Mean value of grams of TFA per 100 g of these food items were 1.6 (range: 0.0-8.1); 1.1 (0.1-5.7) and 1.3 (0.1-7.6), respectively.

DISCUSSION

The present study investigated levels of TFA in the Australian food supply. High levels of TFA (over 1g per serving) were found in over 10% of food items analysed. Some similar food items were found to exhibit a large variability in TFA content. A declaration of hydrogenated vegetable oil in the ingredient list was found not to be an indicator of high levels of TFA in a food item.

Not all serving sizes are equal, even from similar food items. For a more standardised comparison of food items analysed, Table 3 lists those foods found to contain greater than 1g of TFA per 100g of the food item (31 of the 92 food items analysed).

Of the 55 food items tested by The Australian Consumers Association in 2005, over one third were found to contain TFA levels greater then 2% of their total fat.[6] In the present study, more than half (53 of the 92) food items analysed were found to contain TFA levels greater that 2% of their total fat. Under current legislation in Denmark, a large proportion of the total number of food items analysed in this study would not be permitted to be sold, as they contained more than 2% industrially produced TFA[11]

The greatest variability in TFA content was seen in crackers, donuts and chocolate wafer biscuits. For example, analysis of five different brands of crackers showed that a 100g serving of crackers could provide between 0.1g and 3.9g of TFA, depending on which brand was consumed. A similar study conducted in Canada measured TFA variations of between 1g and 13g of TFA per 100g serving of crackers.[12]

Not all food items in the present study showed high variability. For example, the TFA content of three brands of chocolate spreads ranged from 0.1g to 0.3g TFA/100g food making them all qualify for a '*trans* free' declaration in both the USA and Canada and eligible to be sold in Denmark.

There are a number of reasons why there could be large variations of TFA levels in similar food items. Production of hydrogenated oils can result in variable content of TFA due to temperature, pressure, type and amount of catalyst used.[13] Also, manufacturers may use hydrogenated or non hydrogenated fats and oils in the food items or a variety of combinations of these in order to achieve a desired physical property, such as hardness. Finally, the availability of fats and oils will vary in different countries and regions due to availability and price.[14] The TFA con-

Table 3. Products with greater than one gram of TFA per 100g food item

Product	Grams of TFA per 100g of food item	Total grams TFA in a serving of product (serving size)†
Microwave Popcorn Butter Flavour (brand A)	8.1	8.1 (100)
Microwave Popcorn Butter Flavour (brand B)	7.6	7.6 (100)
Vol au Vent- empty cases (brand A)	7.6	0.4 (5)
Canola Oil	7.5	0.4 (5)
Cinnamon Donut (brand A)	6.6	3.6 (54)
Microwave Popcorn (brand C)	6.0	6.0 (100)
Choc Crème Wafer Biscuits	5.7	1.1 (20)
Spreadable Margarine	4.7	0.2 (5)
Cooking Margarine (brand A)	4.5	N/A‡
Cinnamon Donut (brand B)	4.0	1.8 (45)
Water Crackers Sesame Seed	3.9	0.6 (16)
Cooking Margarine (brand B)	3.5	N/A
Vol au Vents (brand B)	3.1	0.5 (16.7)
Premium Butter Blend (brand A)	3.0	0.3 (10)
Extra Soft Spreadable Butter Blend (brand B)	2.6	0.13 (5)
Vegetable Shortening	2.3	N/A
Pasty	2.2	3.3 (150)
Party Pizza (brand A)	2.2	0.5 (25)
Fetta Cheese	1.9	0.6 (30)
Meat Pie (brand A)	1.8	0.9 (50)
Sausage Roll (brand A)	1.7	0.7 (38)
Sausage Roll (brand B)	1.7	1.7 (100)
Chocolate Wafer Fingers	1.7	0.3 (17.5)
Cooking Margarine (brand C)	1.7	(100)
Pizza with the lot (brand B)	1.6	0.4 (77)
Smooth Peanut Butter	1.6	0.3 (20)
Meat Pie (brand B)	1.3	2.3 (175)
Profiterole Cases	1.3	0.1 (12)
Hazelnut Praline	1.2	0.5 (40)
Meat Pie (brand C)	1.2	2.2 (175)
Tuna in Vegetable Oil	1.2	0.8 (70)

†Serving Size (g) as per NIP
‡N/A Serving Size not given by manufacturer due to variety of applications of the product

tent for a specific brand may also vary over time due to the aforementioned reasons. For example, McDonalds in Australia has recently phased out the use of partially hydrogenated oils.[15]

The large variability of TFA levels of similar food items highlights a weakness in dietary modelling used to estimate average daily intakes of TFA for individuals and thus hides the true TFA intakes at a population level. A Canadian study, using a one day sample diet calculated minimum and maximum TFA values for each food within a category.[12] The results showed TFA intakes ranging from a low 1.4g to 25.4g a day for the same diet.

Four food items were selected to demonstrate the large variations in TFA content in the present study (Cinnamon Donut, Crackers, Chocolate wafer biscuits and Pizza; refer to Table 2). Consumption of just 100g of each of these four food items could result in a total TFA intake ranging from 0.8g (by choosing all the lowest TFA brands) to 18.4g (by choosing all the highest TFA brands). An intake of 0.8g of TFA per day is below WHO recommendations of a maximum of 1% of total energy intake, while consumption of 18.4g of TFA is almost eight times greater than WHO recommendations. Daily intake of about 5g of TFA is associated with a 25% increase in the risk of ischemic heart disease.[16]

Variability between similar food items also makes it difficult for the public to follow any advice to 'limit intakes' of TFA. These results illustrate that similar food items can contain low to high levels of TFA, but without an actual listing of TFA in the NIP, there is no way to gauge levels to 'limit intakes'. Observation from supermarket visits throughout the year has shown that a listing of TFA in the NIP of food items is only present when the product contains less then a designated low level (such as <0.5% of fat).

Variability was observed not only in the present study but also when the results were compared with other Australian data. FSANZ have estimated Australia's intake of TFA to be 0.6 per cent of total energy intake. This estimation was based on dietary modelling using TFA levels found in two Australian studies.[4] A total of 16 food items could be matched between the present study and the Australian studies used by FSANZ and our observed values were mostly higher. These higher values may be due to either: the method used in the present study which determines the total TFA content (by FTIR) with greater precision than the use of gas chromatography, or it could be a chance finding given the variability found between different brands of the same item. The present results suggest there could have been an underestimation of Australia's intake of TFA by FSANZ.

A breakdown of the food items analysed in this study was performed to see whether those made in Australia, from Australian ingredients, contained lower levels of TFA compared with imported food items. The highest and the lowest levels of TFA per 100g food item were found in food items made from Australian ingredients. Based on these results, country of origin, as stated on product packaging, cannot be used to gauge the TFA levels of a food item. A limitation of this study is the small number of food items analysed from specific imported locations (often there were only one or two food items per country).

This study measured the TFA levels of several food items which declared hydrogenated vegetable oil in the ingredient list. It was found that the TFA levels in these items were very variable, with amounts ranging between 0.2g to 5.7g of TFA per 100g food item. This variability of TFA levels in food items with a listing of hydrogenated vegetable oil could illustrate the degree of hydrogenation of the vegetable oil used.

The key findings of this study are: that [1] high levels of TFA (greater than 1g per serve) were found in some food items readily available in Melbourne retail outlets, [2] variable levels TFA were found in similar food items, [3] apart from what the manufacturers want the consumer

to know, there is no way of determining TFA levels from any information provided on food labels.

These findings show the difficulty of consumers in following advice to 'limit intakes of TFA'. In 2004 Denmark introduced legislation whereby fats and oils must contain less than 2% industrially produced TFA of the total fat.[11] Advantages of Australia & other countries adopting such a policy include:

- Significantly reducing the use of industrially produced TFA by food manufacturing companies.
- Excluding ruminant TFA, which may have health benefits.
- Requiring action by the food industry to reformulate food items, but does not require additional NIP information or education for the public about TFA.
- TFA as a percentage of fat is a relatively easy measurement using FTIR methods.
- Experience from other countries has shown that TFA can largely be replaced by unsaturated fats without increasing the cost or reducing the quality or availability of foods.[2]

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AUTHOR DISCLOSURES

Justine McCarthy, Daniel Barr, Andrew Sinclair, no conflicts of interest.

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Original Article

Determination of *trans* fatty acid levels by FTIR in processed foods in Australia

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以 FTIR 定量澳洲加工食品的反式脂肪酸含量

全球各地衛生當局都呼籲”限制反式脂肪酸的攝取”。然而在澳洲，其反式脂肪酸(TFA)的含量未被要求明列於營養標示上，除非該食品有做關於脂肪酸或膽固醇的營養宣稱。由於澳洲加工食品中反式脂酸含量的資訊有限，因此，本研究主要目的是挑選一些曾被報導的反式脂酸來源的食物項目，來決定其反式脂酸的含量。挑出的 92 項食物中，都含植物油且總脂肪含量超過 5%。這個準則是根據文獻回顧中，被提及含有高濃度反式脂肪酸的食物項目而歸納出來的。脂質使用溶劑淬取出來，以稱重法決定總脂肪含量。而反式脂肪酸是利用內反射傅立葉轉換紅外線光譜儀(ATR-FTIR)定量。在某些澳洲的食品裡發現含高量反式脂肪酸，且有高變異度。分析的樣本中，有 13 件每份含超過 1 g 的反式脂肪酸，最高有達每份 8.1 g。除了在營養標示指出含量低於訂定的低劑量外，在澳洲出售的食品標示無法指出反式脂肪酸的含量。我們建議衛生當局應尋求方法幫助消費者能夠限制其反式脂肪酸的攝取量。

關鍵字：反式脂肪酸、內反射傅立葉轉換紅外線光譜儀分析、澳紐食品科學所、澳洲食品