ANALYSIS OF *TRANS* FATTY ACID IN SOME FOODS BY ATTENUATED TOTAL REFLECTION-FOURIER TRANSFORM INFRARED SPECTROSCOPY

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Abstract: *Trans* fatty acids (TFAs) are commonly found in bakery products, shortenings, margarines and cooking oils. High consumption of a high - TFA diet has been shown to be associated with greater risk of cardiovascular disease. Due to these reported detrimental health effects of a high - TFA diet, total content of TFA in twenty four samples of bakery products and six samples of partially hydrogenated vegetable oils available at the market in Bangkok area were investigated on TFAs by using attenuated total reflection fourier transform infrared spectroscopy. The range of total TFA content expressed as grams of total TFAs per 100 gram food sample in ten groups of foods were as follows: shortening 1.84 – 3.37, butter cookie 0.25 – 5.27, margarine 1.54 – 1.89, rich butter bun 0.21 – 0.88, crispy pie 0.41 – 0.58, brownie 0.18 – 0.67, croissant 0.14 – 0.83, cake cream roll 0.16 – 0.73, cracker ND - 0.15, and sandwich chocolate cookie ND - 0.14. The mean TFAs value in all selected foods ranged from 0.14 -2.43 g/100 g food while the highest amounts of TFA were found in butter cookie (5.07 g/100 g). The US Department of Agriculture recommends that the consumption of TFAs should be kept below 1 percent of total energy intake (2.2 g/day). Therefore, consumers should realize such health and avoid high TFAs - containing products.

Keywords: *Trans* fatty acid, Fourier transform infrared spectroscopy, Bakery products, partially hydrogenated vegetable oil

INTRODUCTION: *Trans* fatty acids (TFAs) are unsaturated fatty acids with at least one double bond in the *trans* configuration and has a straight chain that is similar to those structure of saturated fatty acid. TFAs are found in two major sources, natural and industrial. In natural source, TFAs originate from milk fat and tissue fat of ruminants such as cows, goat and sheep. Bacteria in their stomach can be producing TFAs by a biological hydrogenation process. Industrial TFAs are mainly generated from vegetable oil polyunsaturated fatty acids, either during partial hydrogenation or during refining process[1)].

Trans fatty acids are found in various animal products, in partially hydrogenated oils and foods made from partially hydrogenated oils. Therefore, people who consume these foods may put themselves at elevated risk of cardiovascular disease. This type of fat consumption can lead to an increased level of serum low - density lipoprotein cholesterol and triglyceride, and a decreased level of high density lipoprotein cholesterol[2-4)]. In addition, TFAs also promote systemic inflammatory responses in healthy persons[5)].

The two common quantitative methods for total TFAs analysis in food are based on gas chromatography (GC) and infrared spectroscopy (IR)[6)]. During GC analysis fatty acid in samples needs to be converted into its corresponding volatile methyl esters prior separation in a very long capillary column (100 m) which is coated with highly polar stationary phases. GC takes a long time for analysis. Another major drawback of the GC analysis is the overlapping of sample peaks. Such a problem can be solved by prior fractionation of a sample of *cis* and *trans* isomers with silver-ion thin layer chromatography (Ag-TLC), or reversed phase high performance liquid chromatography (HPLC). In addition, lack of chemical standards of all TFA isomers is also problematic to GC analysis, while the excessive processing during partial hydrogenation, heating and oxidation may lead to the formation of many *trans* containing fatty acid isomers[7-8)]. Therefore, the GC method may not be the most appropriate choice for the routine determination of TFAs for labeling purposes.

Another alternative method, infrared spectroscopy (IR) is a specific and rapid analytical

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method for the determination of total TFAs. The quantitative analysis of total TFAs by the IR method is based on the C-H out-of-plane deformation band observed at wavenumber 966 cm^{-1}, which is uniquely characteristic of isolated *trans* double bonds, regardless of the chain length or the position of the isolated *trans* double bond[9].

The purpose of this study was to determine the total TFA content in various kinds of bakery products and partially hydrogenated vegetable oils which were produced and distributed in Thailand during September 2007 and February 2008. These results can be basis of the measuring or regulating for controlling the amounts of TFAs in these kinds of foods in Thailand. Additionally, as for labeling purposes, the study intends to propose the IR method as fast in routine TFAs determination instead of slow, such as the GC technique which is currently used in Thailand**.**

MATERIALS AND METHODS:

Materials

TFAs were determined by a Fourier transform infrared spectrometer (FTIR) (Perkin Elmer Spectrum One FTIR, USA) and Zinc selenide crystal (ZnSe trough plate 45°, Perkin-Elmer, USA) attenuated total reflection infrared cell (ATR).

Fatty acid standards; Trielaidin [1,2,3, tris(*trans*-9-octadecanoate)] and Triolein [1,2,3, tris(*cis*-9-octadecanoate)] with purity of ≥ 99% were purchased from Sigma-Aldrich (St. Louis, MO, USA). *n*-Hexane was supplied by Carlo Erba (Rodano, Italy). Petroleum ether was purchased from J. T. Baker Chemicals Co. (Phillipsburg, NJ, USA). Acetone was purchased from Chromanorm (Paris, France) and Methanol was obtained from BDH (Poole, England).

Methods

Sampling

Some kinds of bakery products including butter cookie, sandwich chocolate cookie, cracker, brownie, cake cream roll, rich butter bun, crispy pie, croissant and partially hydrogenated vegetable oils, margarine and shortening were randomly selected in order to investigate TFA content. Three different brands of each type of food were purchased from local markets in Bangkok during September 2007 and February 2008.

Samples were crushed into small pieces and then homogenized again and stored in a polyethylene bag at 4°C until use. After extraction, the fat was weighted and frozen at -10°C until analysis (within four days after extraction)

Fat extraction from bakery products

In order to determine the optimum extraction conditions required for obtaining high lipid yield, three parameters (extraction time, extraction solvent and ultrasound intensity levels) were investigated. Extractions were carried out according to the following procedure: four grams of each sample and beads were placed in a 250 ml round bottom flask with 60 ml of solvent. The sample-solvent suspension was immersed into the ultrasonic bath at 40% and 80% intensity ultrasound level for 60, 90, 120 and 150 minutes. After extraction, the mixture was filtered through filter paper, Whatman no.42. If the filtrate was cloudy it should be centrifuged for 10 minutes at 2000 rpm. The filtrate was evaporated by rotary evaporator and the extract was dried in a vacuum desiccator for 90 minutes. The best condition which gave the highest lipid yield was further utilized to extract fat from other bakery products.

Fat extraction of partially hydrogenated vegetable oil

All samples were extracted with petroleum ether by modifying the procedure of Bligh and Dyer[10]. Briefly, 2.5 g of sample was placed in a 50 ml centrifuge glass tube and mixed with 15 ml of petroleum ether for 2 minutes on vortex. Then 10 ml of petroleum ether was added and the mixture was shaken vigorously for 2 minutes. Nine milliliters of distilled water were added and the mixture was vortexed again for 2 minutes. The layers were separated by centrifugation for 10 minutes at 2000 rpm. The upper layer was transferred to a 125 ml pear-shaped flask. The lower layer was extracted two times with 25 ml of petroleum ether by centrifugation for 10 minutes at 2000 rpm. The petroleum ether phase was added to the first extract. The filtrate was evaporated by rotary evaporator and the residue was dried in a vacuum desiccator for 90 minutes.

TFA content determination by AOAC Official Method 2000.10. Attenuated total reflection fourier transform infrared Spectroscopy (ATR-FTIR)[6].

The operation parameters of FTIR were set up according to the manufacturer's instructions for using a zinc selenide ATR cell with the following parameters: resolution of 4 cm^{-1} in the spectral range of 1050 – 900 cm^{-1}, 64 scans. The test portion was filled to cover the horizontal surface of the crystal. The ATR cell must be maintained at a constant temperature of 65 ± 2 °C to ensure that the sample fully melted. A single beam spectrum collection of air was used as the reference (background). The single-beam spectrum of the test portion was collected against that of the reference background and converted into absorbance. To improve sensitivity and accuracy, a new ATR-FTIR procedure that measures the height of the negative second derivative of the *trans* absorption band relative to air was used[9]. After each analysis, the ATR cell was cleaned with acetone.

RESULTS:

Optimization of fat extraction conditions from bakery products

Effect of ultrasound intensity levels on extracted lipid yield

The effect of ultrasonic intensity levels on extracted lipid yield by *n*-hexane was found that extracted lipid yield at 80% intensity was higher than those at 40% intensity level but not significant. Some studies showed that low-intensity ultrasound does not alter the physical or chemical properties of the material through which the ultrasonic wave propagates. On the other hand, high ultrasonic intensity will induce a bubble of cavitations that generates intense pressures, shear and temperature which can produce physical, chemical and mechanical effects[11]. Thus, the 40% ultrasonic intensity level was chosen for lipid extraction.

Effect of extraction time on extracted lipid yield

Butter cookie and butter cake, the representative of bakery products with low and medium moisture content, respectively were chosen. The finding indicated that extracted lipid yield involved with increasing extraction times and reached a maximum value at 120 minutes. Thus, 120 minutes at 40% ultrasonic intensity was favorable for fat extraction from bakery products.

Effect of different solvents on extracted lipid yield

The food sample was extracted at 40% ultrasonic intensity for 120 minutes in different solvents [*n*-hexane, *n*-hexane:acetone (4:1 v/v), *n*-hexane:acetone:petroleum ether (4:1:1 v/v) and *n*-hexane:acetone:methanol (4:1:1.5 v/v)]. The data show that *n*-hexane obtained fat from butter cookie at a higher amount than other solvent mixtures. Therefore, *n*-hexane was selected as the extraction solvent.

In this study, the optimal conditions for fat extraction from bakery products were obtained by using *n*-hexane as a solvent, ultrasonic intensity level at 40% and 120 minutes for extraction time.

Determination of total fat content in selected foods

The values for total fat content of each type of food (3 brands) were expressed as range and mean ± standard deviations (Table 1). In the group of bakery products, the highest amount of average total fat content was found in butter cookie (255.15 ± 28.40 mg/g food) and the lowest was found in rich butter bun (144.50 ± 56.79 mg/g food). In the other group, average total fat content of shortening (985.75 ± 6.93 mg/g food) was higher than that of margarine (837.03 ± 11.03 mg/g food).

Determination of total TFA content in selected foods

The total TFA content of the selected foods was determined by the ATR-FTIR, AOAC Official Method 2000.10. The fingerprint range (1050-900 cm^{-1}) contained the C-H out of plane deformation band (966 cm^{-1}) that is uniquely characteristic of isolated double bonds with *trans* configuration. The height of the negative second derivative of the *trans* absorption band at 966 cm^{-1} was integrated between the fixed limits of 990 and 945 cm^{-1} by using the FTIR software. Table 1 shows the TFA content found in selected foods which ranged

from 0.74 to 17.53% of total fat extracted or 0.14 – 5.07 g/100 g food.

The highest average amount of TFAs (g/100 g food) was found in shortening, followed by butter cookie, margarine, rich butter bun, crispy pie, brownie, croissant, cake cream roll, cracker and sandwich, chocolate cookie respectively.

DISCUSSION: Partial hydrogenated vegetable oil has been attractively used in food industry due to the enhance palatability of bakery products; however such oil may contain high TFAs. Therefore in this study, food containing partial hydrogenated vegetable oil and bakery product were chosen for analysis the TFA content. Several methods are available for determining total TFA content such as gas chromatography (GC) or infrared (IR) absorption spectroscopy. In this study, the IR procedure based on attenuated total reflection (ATR) fourier transform infrared (FTIR) spectroscopy was selected to determine total TFA content in selected foods. The ATR-FTIR procedure offers several advantages over the GC procedure. The analysis time is shorter than GC (about 5 minutes per analysis) and calculates TFA content from a linear regression equation. Small quantities of test samples are required. The need for weighing and quantitatively diluting test samples with solvent is eliminated with ATR-FTIR[12].

The ATR-FTIR method works well for fats containing more than 5% TFA (Milosevic et al, 2004). However, to improve sensitivity and accuracy, a new ATR-FTIR procedure that measuring the height of the negative second derivative of the *trans* absorption band at 966 cm^{-1} relative to air was recently proposed. The second derivative of an absorbance spectrum enhanced the resolution of IR bands, and made it possible to note the small shifts in IR band position and the presence of interferences[9]. Miolsevic *et al.*[7] compared the GC method with the new ATR-FTIR method that employs the negative second derivative to determination of low levels (0.5 – 5%) of TFA. It was found that the negative second derivative ATR-FTIR method is capable of determining a low level of TFA content. Therefore, the negative second derivative ATR-FTIR method was used for determining total TFA content in some Thai foods.

In this study, average total TFA content of shortening was higher than that of margarine since shortening is 100% fat, made from partially hydrogenated vegetable oil, without any added flavor. In contrast, margarine made from partially hydrogenated vegetable oil is often mixed with skimmed milk, salt, antioxidant, emulsifiers and other flavors. The present study demonstrated that TFA content in the butter cookie group varied widely (Table 1). Each 100 g of butter cookie contained 0.26 - 5.07 g of TFAs. There are several explanations for the variability of TFA content of foods within a food category. Firstly, the manufacturing condition of hydrogenated oils can result in variable content of TFAs. Such conditions include temperature, hydrogenation pressure, type and amount of catalyst and agitation. Secondly, food processing may use

Table 1. Total fat and total TFA content in selected foods.

Products	Total fat content (mg/g food)		Total TFA content			
	Range	Mean ± SD	% of total fat		g/ 100 g food	
			Range	Mean ± SD	Range	Mean ± SD
Shortening (n=3)	978.2 - 993.7	985.7 ± 6.93	1.85 - 3.42	2.46 ± 0.84	1.84 - 3.37	2.43 ± 0.82
Margarine (n=3)	826.9 - 850.8	837.0 ± 11.03	1.85 - 2.22	2.07 ± 0.19	1.54 - 1.89	1.73 ± 0.17
Butter cookie (n=3)	226.3 - 289.1	255.1 ± 28.40	1.02 - 17.53	7.63 ± 8.73	0.26 - 5.07	2.10 ± 2.59
Crispy pie (n=3)	205.3 - 236.2	221.4 ± 13.92	1.76 - 2.59	2.31 ± 0.48	0.42 - 0.58	0.51 ± 0.08
Sandwich chocolate cookie (n=3)	186.5 - 265.7	213.8 ± 40.27	ND - 0.74	0.74	ND - 0.14	0.14
Croissant (n=3)	62.9 - 316.7	196.4 ± 144.00	0.93 - 3.97	2.37 ± 1.53	0.14 - 0.83	0.42 ± 0.36
Cracker (n=3)	183.2 - 199.1	191.4 ± 7.16	ND - 0.74	0.74	ND - 0.14	0.14
Cake cream roll (n=3)	160.1 - 183.7	173.9 ± 11.04	0.93 - 3.97	2.03 ± 1.68	0.16 - 0.73	0.36 ± 0.32
Brownie (n=3)	62.5 - 192.3	146.5 ± 65.14	2.86 - 3.51	3.23 ± 0.33	0.18 - 0.68	0.49 ± 0.27
Rich butter bun (n=3)	93.5 - 215.6	144.5 ± 56.79	2.22 - 6.65	4.31 ± 2.22	0.21 - 0.88	0.64 ± 0.37

ND = Non-detected at the level of traces (TFA <0.5% of total fat)

single hydrogenated or non-hydrogenated fats or other oils or combinations of them to achieve the desired final product characteristics[13].

From the above results (Table 1), total TFA content does not well correlated with total fat content as found sandwich chocolate cookie which contained the lowest level of TFAs, but had the third highest total fat content in the bakery products group. Therefore, the way to identify total TFA content in foods is to read the TFA content on the nutrition label of foods or examine the ingredients list to see if it includes partially hydrogenated oils.

Nowadays, the food industry is trying to reduce TFAs in their products and simultaneously preserve the structural and palatable characteristics of the food product. They are currently developing technology for use in food and edible oil industries to reduce or eliminate TFA content, including: first, modification of the chemical hydrogenation process to produce partially hydrogenated fats with low TFA content such as electrocatalytic hydrogenation, precious catalyst hydrogenation and supercritical fluid state hydrogenation[14]. Second, modification of the fatty acid composition of oil seed through plant breeding and genetic engineering techniques. Other methods include interesterification of mixed fats, use of tropical oils (palm kernel oil, and coconut oil) and use of fractionated tropical oils[15]. Some of these technologies have been available to the food industry for some time. However, alternate methods to hydrogenation could be more costly than conventional hydrogenation. Therefore, new approaches may not easily replace conventional hydrogenated vegetable oils. In the meantime, consumers should avoid or limit foods that contain partially hydrogenated vegetable oils.

At present in Thailand, there is no legal regulation to enforce the manufacturer to label or declare TFA content on the product produced. With such a label, consumers could be more aware and avoid risk of excessive intake of TFAs. Therefore, the author strongly urges the health authorities to raise consumer awareness through different means of regulation. In this study, food samples were collected from supermarkets and popular bakery stores in Bangkok only. In future studies, food samples should be collected from a variety of locations to provide more information to represent the variety in the Thai marketplace. In order to estimate TFAs intake and support decision-making regarding risk management, there is a need to continue to assess the content of TFAs in the various foods in Thailand.

CONCLUSION: The average total TFA content of bakery products and partially hydrogenated vegetable oil of Thailand were low in this study. However, consumers should be aware of the adverse consequences of TFA consumption and avoid TFAs - containing products. In order to reduce the intake of TFAs, we recommend that the government and health care providers should advice consumer about how to avoid the main foods containing TFAs.

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REFERENCES:

1. Pfeuffer M, Schrezenmeir J. 2006. Impact of *trans* fatty acids of ruminant origin compared with those from partially hydrogenated vegetable oils on CHD risk. Int Dairy J 16: 1383–88.

2. Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ, Willett WC. 2006. *Trans* fatty acids and cardiovascular disease. New Engl J Med 354(15): 1601-13.

3. Lichtenstein AH, Jauhiainen M, McGladdery S, Ausman LM, Jalbert SM, Bach MV, et al. 2001. Impact of hydrogenated fat on high density lipoprotein subfractions and metabolism. J Lipid Res 42: 597-604.

4. Dyerberg J, Christensen JH, Eskesen D, Astrup A, Stender S. 2006. *Trans*, and n-3 polyunsaturated fatty acids and vascular function-A yin yang situation?. Atherosclerosis Suppl 7: 33-35.

5. Mozaffarian D, Pischon T, Hankinson SE, Rifai N, Joshipura K, Willett WC, et al. 2004. Dietary intake of *trans* fatty acids and systemic inflammation in women. Am J Clin Nutr 79: 606 –12.

6. Association of Official Analytical Chemists (AOAC). 2000. Official Method of Analysis of the Association of official analytical chemists. 17th ed.

Washington D.C.: The Association of Official Analytical Chemists.

7. Milosevic V, Kocak A. 2004. Analyzing *trans* fats in edible oils and fats using single- reflection ATR-FTIR. Am Lab (June): 30-34.

8. Destaillats F, Golay PA, Joffre F, Wispelaere MD, Huga B, Giuffrida F, *et al.* 2007. Comparison of available analytical methods to measure *trans*-octadecenoic acid isomeric profile and content by gas–liquid chromatography in milk fat. J Chromatogr. A 1145: 222–28.

9. Mossoba MM, Milosevic V, Milosevic M, Kramer JKG, Azizian H. 2007. Determination of total *trans* fats and oils by infrared spectroscopy for regulatory compliance. Anal Bioanal Chem 389: 87–92.

10. Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37, 911-17.

11. Ma Y, Ye X, Hao Y, Xu G, Xu G, Lin D. 2008. Ultrasound-assisted extraction of hesperidin from Penggan (Citrus reticulata) peel. Ultrason Sonochem 15: 227-32.

12. Al LH, Angyal G, Weaver CM, Rader JI, Mossoba MM. 1996. Determination of total *trans* fatty acids in foods: comparison of capillary-column gas chromatography and single-bounce horizontal attenuated total reflection infrared spectroscopy. J Am Oil Chem Soc 73(12): 1699-705.

13. Innis SM, Green TJ, Halsey TK. 1999. Variability in the trans fatty acid content of foods within a food category: Implications for estimation of dietary trans fatty acid intakes. J Am Coll of Nutr 18(3): 255–60.

14. Jang ES, Jung MY, Min DM. 2005. Hydrogenation for low *trans* and high conjugated fatty acids. CRFSFS 1: 22-30.

15. Tarrago-trani MT, Phillips KM, Lemar LE, Holden JM. 2006. New and existing oils and fats used in products with reduced *trans*-fatty acid content. J Am Diet Assoc 106: 867-80.