ANALYSIS OF *TRANS* FATTY ACID CONTENT IN DEEP FRIED FOODS, MILK AND DAIRY PRODUCTS BY ATTENUATED TOTAL REFLECTION – FOURIER TRANSFORM INFRARED SPECTROSCOPY

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ABSTRACT: The unsaturated fatty acid which has double bond in the *trans* configuration is called *trans* fatty acid (TFA). TFAs are found mostly in partially hydrogenated vegetable oil which are commonly used in food process, especially in deep fried foods. It is because of high stability during deep-frying. Moreover, naturally occurring TFAs are found in milk and lipid tissues of ruminant. Several researchers showed that high consumption of diet containing high TFAs were associated with greater risk of cardiovascular disease. Therefore, this study was conducted to investigate the TFA content in fifteen samples of deep fried foods and eighteen samples of milk and dairy products which were available in Bangkok area between February 2009 to September 2009, using attenuated total reflection fourier transform infrared spectroscopy. The total TFA content in this study expressed as grams of TFA content per 100 grams of food sample in eleven groups of foods were as follows: fried chicken from well-known fast food 0.08 – 0.14, fried chicken from street vender 0.01 – 0.02, french fries 0.05 – 0.10, deep fried dough stick (pa-tong-koh) 0.01 - 0.15, deep fried banana (kluay-kag) ND – 0.02, pasteurized milk 0.13 – 0.24, UHT milk 0.09 – 0.25, ice-cream 0.04 – 1.00, whipping cream 1.54 – 2.26, cheese 1.38 – 2.14 and butter 2.06 – 6.99. The mean TFA values in all selected foods ranged from 0.01 – 6.99 g/100 g food. In milk and dairy products, the highest content of TFAs was found in the selected brand of butter (6.99 g/100 g food). From the present study, deep fried food category exhibited low TFA content. However, consumers should avoid or limit consuming this food because it contains high fat and may have some harmful compounds, which occurs during frying.

Keywords: *Trans* fatty acid, Fourier transform infrared spectroscopy, deep fried foods, milk products, dairy products

INTRODUCTION

The unsaturation of fatty acids in vegetable and animal kingdoms generally have *cis* configuration. In this form, the hydrogen atoms bond to the unsaturated carbons geometrically on the same side, while the other form, called *trans*, bond to unsaturated carbons on opposite sides [1]. These fatty acids are found in two major sources, natural and industrial sources. In natural source, TFAs originate from milk fat and tissue fat of ruminants such as cow, goat and sheep. Bacteria in their stomach can produce TFA by biological hydrogenation process. Industrial TFAs are mainly generated from vegetable oil polyunsaturated fatty acids, either during partial hydrogenation or during refining process [2].

The concern about the intake of foods containing high TFA amounts has grown in the recent years which is mainly due to the hazardous effects of these lipids on plasma lipoproteins. Highly consumption of TFAs containing food may increase low density lipoprotein (LDL-C) and lipoprotein a (Lp[a]) levels and decrease the levels of high density lipoprotein (HDL-C). This condition contributes to increase the LDL-C/HDL-C ratio, which is considered to an important indicator of the risk of development of cardiovascular diseases [3-7]. In addition, TFA also promote systemic inflammatory responses in healthy persons [8]. Moreover, TFA may affect human fetal growth and infant development [9-11].

Infrared spectroscopic method (IR) is a specific and rapid analytical method for the determination of total TFA. The quantitative of total TFA by infrared spectroscopic method is base on band observed at wave number 966 cm^{-1}, which is the unique characteristic of isolated *trans* double bonds, regardless of the chain length or the position of the isolated *trans* double bond [12]. The official new attenuated total reflection–fourier transform infrared (ATR-FTIR) spectroscopy method can be conveniently applied for determination of the total *trans* configuration content of fats in vast majority of food products which contains more than 5 % *trans* fat, as percent of total fat. In addition, it was recently reported that by using the negative second derivative (-2D) instead of the absorption spectrum

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itself, spectral features were enhanced such that TFA levels as low as 0.5 % could be readily measured [13].

The purpose of this study was to determine the TFA content in some deep fried foods, milk and dairy products which are distributed in Thailand during February 2009 to September 2009 by ATR-FTIR method. The results can be the basic for setting up the limit of TFA content in these kinds of foods in Thailand. Additionally, for labeling purposes, the study intended to propose the fast routine IR method for TFA determination instead of the time consumed GC technique which currently use in Thailand.

MATERIALS AND METHODS

Materials 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, ethyl ether and ammonium hydroxide were purchased from J. T. Baker Chemicals Co. (Phillipsburg, NJ, USA) and anhydrous sodium sulfate was obtained from Merck (Darmstadt, Germany). 95% Ethanol was obtained from BDH (Poole, England).

Methods

Sample selection: Some kinds of deep fried foods including deep fried dough stick and deep fried banana were purchased from 3 different places street venders. French fries were bought from 3 well-known fast foods shops. Fried chicken was taken from 3 well-known fast food shops and 3 others from 3 street venders in different places. Likewise milk and dairy products were selected from 3 difference brands. All of these samples were purchased in Thailand during February 2009 to September 2009 and the places from where we purchased were recorded.

Sample preparation: For deep fried foods, samples were crushed into small pieces and then homogenized again and stored in polyethylene bag at 4°C until use. After extraction, the fat was weighed and frozen at -10°C until analysis (within 1 day after extraction), for fried chicken breast, de-boned was done before chopping (meat and skin parts used). Milk and dairy products were prepared according to AOAC official method 925.21 [14]. Briefly, test sample was thawed to 20°C in water bath and was mixed with mechanical blender until homogeneous.

Fat Extraction

(I) Extraction of deep fried foods

To find out the optimum extraction conditions required for obtaining high lipid yield, two parameters including extraction time and ultrasonic intensity levels were investigated. Each condition was done in tripicated. The obtained results were compared with the values declared in the nutrition fact label of the products (90 – 110 % of label amount). The appropriate condition was further utilized to extract fat from other deep fried foods.

Four grams of each sample and beads were placed in a 250 milliliters round bottom flask containing 60 milliliters of *n*-hexane. The sample-solvent suspension was immersed into the ultrasonic bath using at 20%, 40%, 60%, 80% and 100% ultrasonic intensity levels for 120 minutes. After extraction, the mixture was filtered through filter paper, Whatman no.42. If the filtrate was cloudy it should be centrifuge at 2000 rpm for 10 minutes. The filtrate was evaporated by rotary evaporator and the extract was dried in vacuum desiccator for 90 minute. Finally, chose the intensity that obtained the highest lipid yield. The selected ultrasonic intensity level was used for extraction at 30, 60, 90, 120 and 150 minutes to select the appropriate time which gave the highest lipid yield.

(II) Extraction of milk and dairy products

All samples were extracted by Roese-Gottlieb method [14]. In brief, ten grams of samples were weighed into a tube and added 1.5 milliliters of NH_4OH solution. Then both of these were mixed thoroughly and after that 10 milliliters of 95% ethanol were added and the mixtures were mixed properly. Twenty five milliliters of ethyl ether was added, stopped with stopper and was shaken very vigorously for 1 minute. Twenty five milliliters of petroleum ether was added and again repeated vigorous shaking and waited until upper layer is practically clear. The upper solution was decanted into 125 milliliters pear-shaped flask. The remaining in lower layer tube was repeated extraction twice and 15 milliliters of each solvent was used in each time and distilled water (5-10 milliliters) was added, if necessary. The ether phase was added to the first extract. The filtrate was evaporated by a rotary evaporator and the residue was dried in vacuum desiccator for 90 minutes.

Determination of *trans* fatty acid content of deep fried foods, milk and dairy products

The operational parameters of FTIR was set up according to the manufacturer's for using a zinc selenide ATR cell with following parameters: resolution of 4 cm^{-1} in the spectral range of 1050 – 900 cm^{-1}, 64 scan. Fatty acids were filled to cover the horizontal surface of the crystal. To ensure that the sample was fully melted the ATR cell must be maintained a constant temperature of 65 ± 2 °C. Single beam spectrum collected of air was used as reference (background). The single-beam spectrum of the test portion was collected against that of the reference background and convert 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. Each repeated fat

Table 1 Total fat contents of deep fried foods, milk and dairy products.

Products (n=3)	Total fat content (g/100g food)		*Trans* fatty acid content			
			g/ 100 g food		% of total fat	
	Range [a]	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Deep fried foods						
Fried chicken from well-known fast food shop	20.25 – 21.04	20.73 ± 4.20	0.08 – 0.14	0.11 ± 0.03	0.40 – 0.66	0.51 ± 0.13
Fried chicken from street vender	10.52 – 23.58	15.00 ± 74.33	0.01 – 0.02	0.01 ± 0.01	0.09 – 0.13	0.12 ± 0.02
French fries	11.27 – 15.74	14.12 ± 24.73	0.05 – 0.10	0.07 ± 0.03	0.36 – 0.64	0.47 ± 0.15
Deep fried dough stick	16.25 – 31.00	22.60 ± 75.84	0.01 - 0.15	0.07 ± 0.07	0.07 – 0.48	0.30 ± 0.21
Deep fried banana	15.37 – 18.34	16.98 ± 15.02	ND – 0.02	0.04	ND – 0.27	0.19
Milk and dairy products						
Pasteurized milk	2.97 – 5.41	4.11 ± 12.27	0.13 – 0.24	0.18 ± 0.06	4.08 – 4.38	4.28 ± 0.17
UHT milk	4.77 – 5.34	5.14 ± 3.18	0.09 – 0.25	0.17 ± 0.08	1.86 – 4.73	3.30 ± 1.44
Ice-cream	8.22 – 17.74	11.43 ± 54.65	0.04 – 1.00	0.37 ± 0.54	0.48 – 5.65	2.35 ± 2.86
Whipping cream	35.52 – 44.83	39.16 ± 49.78	1.54 – 2.26	1.78 ± 0.42	4.14 – 5.03	4.50 ± 0.47
Cheese	18.69 – 27.55	24.09 ± 47.36	1.38 – 2.14	1.67 ± 0.41	5.28 – 7.96	7.00 ± 1.49
Butter	77.42 – 82.48	80.12 ± 25.47	2.06 – 6.99	4.52 ± 2.47	2.49 – 9.02	5.70 ± 3.27

[a] Data are expressed as mean of triplicate extraction
ND = Non detectable at the level of traces

extract was scanned for 3 times. After each analysis, ATR cell was cleaned by rinsing with acetone.

Calculations: The % *trans* fat for test samples was calculated by substitution the value of the integrated height of the negative second derivative of *trans* band in the following equation:

$$\textit{Trans} \text{ fat as trielaidin, \%} = \frac{\text{height} - \text{intercept}}{\text{slope}}$$

RESULTS

Optimization of conditions for fat extraction from deep fried foods

The effect of ultrasonic intensity levels and extraction time on lipid yield by *n*-hexane as a solvent at 60^{o}C showed that lipid yield at 40% intensity for 120 minutes was the highest (data not shown).

Total fat contents in the selected samples

Value for total fat content of each type of food was expressed as range and mean ± standard deviations of triplicated (Table 1). In group of deep fried foods, the highest amount of average total fat content was found in deep fried dough stick from well-known fast food (22.60 ± 75.84 g/100 g food) and the lowest was found in french fries (14.12 ± 24.73 g/100 g food). For the milk and dairy products group, average total fat content of butter (80.12 ± 25.47 g/100 g food) was the highest and pasteurized milk was the lowest (4.11 ± 12.27 g/100 g food) in this group.

Trans fatty acid content of the selected foods

Table 1 showed the results of *trans* fatty acid contents in some deep fried foods, milk and dairy products. The average total *trans* fatty acid contents of deep fried foods ranged from 0.12 to 0.51% of total fat or 0.01 to 0.11 g/100 g food and milk and dairy products ranged from 2.35 to 7.00% of total fat or 0.17 to 4.52 g/100 g food. The *trans* isomers content of the same kind products that produced by different manufacturers showed different values of *trans* fatty acid level. The highest average amount of total *trans* fatty acid per 100 grams food was found in butter followed by whipping cream, cheese, ice-cream, pasteurized milk, UHT milk, fried chicken from well-known fast food fried, french fries, deep fried dough stick, deep fried banana and chicken from street vender respectively.

DISCUSSION

This study focused on two food categories including deep fried foods and milk and dairy products. In this study, the attenuated total reflection (ATR) fourier transform infrared (FTIR) spectroscopic method, which is more advantageous than the GC method was selected for determination of total TFA content in samples. The analysis time was shorter than GC (about 5 minutes per analysis) and TFA content was calculates by using a linear regression equation. Small quantities of test samples were taken. The need for weighing and quantitatively diluting test samples with solvent was eliminated with ATR-FTIR [15].

From the results of deep fried foods in this study, the total fat content did not correlate with total TFA content. The total *trans* fatty acid contents of deep fried foods ranged from non-detectable to 0.15 g/100 g food which were quite low while total fat content ranged from 10.52 – 23.58 g/100 g food. Different brands contain different amounts of fat and TFA which might be because of the use of different fats and oil source to make those products. The results showed that, the average TFA content of fried chicken from well-known fast food shops were 10-time more than fried chicken from street vender. This result may be because of the use of partially hydrogenated oils more than non-hydrogenated oils for preserving food whereas the street vender often use palm oil, which has high saturated fatty acids in frying process. Moreover, shops, which were sampled, did not reuse oil several times in frying. However, current nutrition recommendations point to a reduction of total dietary fats, including *trans* and saturated fatty acids. In addition, important nutritional compounds degrade during the process, and toxic molecules may generate either in the foodstuff or in the frying oil itself. In April 2002, Swedish scientists showed concern about fried foods when they discovered a certain high levels of acrylamide, a chemical compound that is listed by the World Health Organization (WHO) as a probable human carcinogen in these products [16]. These substances were produced by a reaction between amino acids and reducing sugars when food is heated above 120^{o}C [17]. Therefore, we may find the harmful compounds in frying foods.

Ruminant-produced TFAs are made by bacterial metabolism of polyunsaturated fatty acids in the rumen of ruminants, and consequently present in all milk and dairy products from these animals. Concentration of TFA in ruminant fat varies with the feed of the animals and with the seasons [18]. The results in this study showed that samples in this group had TFAs 0.48 – 9.02 % of total fat (0.04 – 6.99g/100 g food) which was quite variably. These results were similar to other countries. The amount of TFA in milk and butter from most European samples is generally below 5%. Australian and New Zealand butters have slightly higher proportions of TFA, at 6% and above [19-21]. TFA content in most European and American cheeses is 2 – 5% TFA. TFA contents of ice-cream samples made from dairy fats had 2.6 to 6% [22]. However, in this study, TFA content in pasteurized milk, UHT milk and butter were 4-fold higher than the results of Pinkaew [23]. When compare the TFA contents of butter with shortening and margarine which are used wide range as butter substitutes,the results of Narkwichian [24] showed that shortening and margarine, which were distributed in Thailand, had TFAs between 1.54 to 3.37 g/100g food. Those results revealed that they had TFA content less than butter in this study (2.06 – 6.99 g/100g food). However, results from epidemiological studies of intake of ruminant-produced TFA and risk of coronary heart disease (CHD) have indicated that intake ruminant TFAs is innocuous or even protective against CHD. Two prospective cohort studies have revealed an inverse association between energy-adjusted ruminant TFA intake and risk of CHD [25, 26]. However, Oomen et al. [27] found non-significant direct associations between the intake of ruminant produced TFA and risk of CHD.The absence of a higher risk of CHD associated with the intake of ruminant TFA as compared with the intake of industrially produced TFA may be because of lower levels of intake, different biologic effects of different isomers, or the presence of other factors in ruminant products which balance any effects of their TFAs [5].

CONCLUSION

The results in the present study indicated that average total TFA content of deep fried foods was less than 0.2 g/100 g food. It is low in comparison to other countries. Average total TFA of fried chicken from well-known outlet was the highest. Furthermore, in milk and dairy products category, the results showed that they had TFA 0.04 – 6.99g/100g food. Butter groups showed the highest level of average total TFA, followed by whipping cream, cheese, ice-cream, UHT milk, and pasteurized milk respectively. When TFA content per serving of selected samples was calculated, it was noticed that all samples had TFA contents lower than 0.5 grams. For US food labeling regulation, "*trans* free" or "0 gram *trans* fats" refer to the TFA content less than 0.5 grams per serving. Therefore, if consumers consume the products more than 1 serving, their TFA content might be more than the recommended Dietary Guidelines (less than 1 percent of total energy per day). Thus, consumers should be aware and limit the consumption of those high TFA content products.

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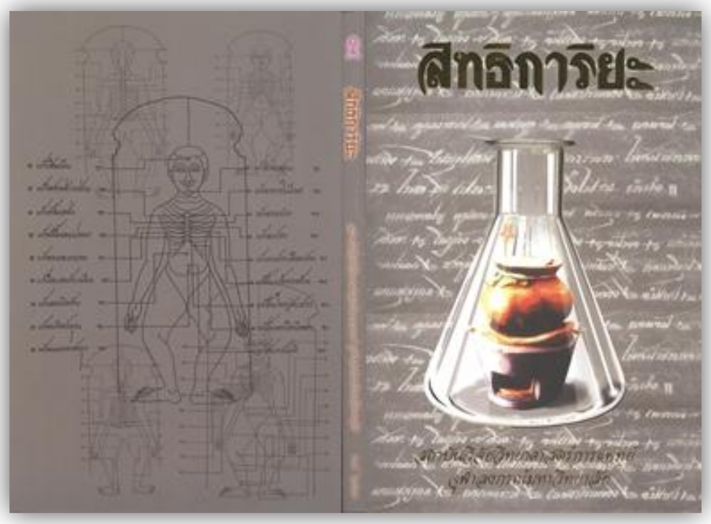
หนังสือที่น่าสนใจ

สิทธิการิยะ: รวมบทความอาศรมความคิดยาจากสมุนไพรกับการแพทย์ทางเลือก



... " **สิทธิการิยะ** มาจาก สิทธิ์ + การย์ คำ ๆ นี้ เป็นคำที่ "ผู้เป็นครู" อนุญาตหรือสั่งเอาไว้ ต้องกระทำตามจึงเกิด "ผล" ดังนั้นในคาถาต่างๆ จึงปรากฏคำๆ นี้นำหน้าเสมอ... "

... " ตำรายาไทยเก่า ๆ มักพบคำที่ขึ้นต้นว่า **"สิทธิการิยะ"** ท่านว่าให้เอาเทียนดำหนัก ๑ บาท ดอกบุนนาค ๑ ... เวลาหมอโบราณจะเขียนขึ้น ก็จะเริ่มต้นด้วยคำว่า **"สิทธิการิยะ"** แล้วจึงบอกถึงตัวยา ขนาดยา วิธีทำยา และลงท้ายด้วยขนาดรับประทานเสร็จสรรพ การเขียนตำรายาโดยขึ้นต้นด้วยคำว่าสิทธิการิยะนั้นเป็นดังคำอธิษฐาน ตามความหมายแล้ว หมายถึงอธิษฐานว่า "ขอให้กรรมนี้ จุ่งประสบสัมฤทธิผล เทอญ ... "

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153 หน้า

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