ANALYSIS OF *TRANS* FATTY ACID CONTENT IN SNACKS AND NON-DAIRY CREAMERS BY ATTENUATED TOTAL REFLECTION FOURIER TRANSFORM INFRARED SPECTROSCOPY

Waraporn Suwannakood, Chamnan Patarapanich, Linna Tongyonk*

Faculty of Phamaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

ABSTRACT: *Trans fat*s are found mostly in products which contain partially hydrogenated vegetable oils in manufacturing process such as bakery products, deep fried foods, margarine and shortening. Snacks and non-dairy creamers contain partially hydrogenated vegetable oils but *trans* fat content of them have a few report. Therefore, the aim of this study was to determine *trans* fat content in six groups of snacks including crackers, stick biscuits (Kha-Kai), potato chips, biscuits, microwave popcorns, wafers and twelve brands of non-dairy creamers. All samples were selected from supermarket in Bangkok during October 2009 to January 2010. *Trans fat* levels were determined by the attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) with negative second derivative mode. The highest of *trans* fat content found in the microwave popcorn was 6.23 g/ 100 g food (0.90-9.96 g/100 g food), following by wafer;1.25 g/ 100 g food (0.00-3.65 g/100 g food), biscuits; 0.81 g/100 g food (0.04-2.31 g/100 g food), crackers; 0.05 g/100 g food (ND-0.05 g/100 g food), potato chip; 0.01 g/100 g food (ND-0.01 g/100 g food) and stick biscuits (Kha-Kai) was not detected. Twelve brands of non-dairy creamers contained *trans* fat. *Trans* fat contents of two brands which contained soybean oil were range 10.29-14.62 g/100 g food or 0.31-0.44 g/serving size while ten brands which contained palm kernel oil were range 0.17-0.70 g/100 g food or 0.01-0.02 g/serving size. Although, the finding show that the amount of *trans* fat in samples were low however, consumers should be aware the harmful effect of *trans* fat. In the future, FDA should inquire *trans* fat contents on nutrition label and consumers should be educated about health effect of *trans* fat for minimized the cardiovascular risks associated with *trans* fat.

Keywords: *Trans* fatty acid, Fourier transform infrared spectroscopy, snacks, non-diary creamers

INTRODUCTION

Trans fatty acids (TFA) are unsaturated fatty acids with at least one double bond and the one hydrogen on opposite sides of the double bond [1]. *Trans* fats are found mostly in partially hydrogenated vegetable oil and found slightly in milk and meat of ruminants (about 5% of fatty acid) [1, 2].

Hydrogenated vegetable oil has been used in food processing because the products have long shelf life and more stability of oxidation [2, 3]. Therefore, *trans* fats could be found in shortening, margarine, bakery products, deep fried food, snacks, and non-dairy creamers [3-6].

Many countries concern about health effects of *trans* fat consumption because *trans* fat intake has been linked to varies of disease, including coronary heart disease (CHD) and diabetes type II. *Trans* fat were found to increase the level of low density lipoprotein (LDL) cholesterol and decrease the level of high density lipoprotein (HDL) cholesterol [1, 4, 7]. In addition, the consumption of *trans* fat may be involved in the risk of cancer such as colon [8, 9], prostate [10] and breast cancer [11].

* *Correspondence to: Linna Tongyonk*
E-mail: linna.t@chula.ac.th; Tel. +66 (0) 8 1205 2685

The determination of *trans* fat content was using gas chromatography (GC) and infrared (IR) spectroscopy. The GC method consists of several step to determine the total *trans* fat content while IR method used a rapid time for analyzed [12]. IR spectroscopy was used to detect TFA level and based on the C-H out of plane deformation band measured at the 966 cm^{-1} [12]. But IR method is suitable for more than 5% of *trans* fat level.

Therefore, a new ATR-FTIR method is developed for improved sensitivity and accuracy which measuring the height of the negative second derivative of *trans* fatty acid absorption band relative to air [12]. In Thailand, studies have shown *trans* fat content in some foods [13], deep fried foods, milk and milk products [5,6] but *trans* fat content in snacks and non-dairy creamers are not reported. Thus, the aim of this study was to determine the *trans* fat content in snacks by attenuated total reflection-Fourier transform infrared spectroscopy with negative second derivative mode.

MATERIALS AND METHODS

Sampling

Six groups of snacks including crackers, wafers, stick-biscuits (Kha-Kai), potato chips, biscuits and

microwave popcorns were randomly sampling, and three brands of each group were collected. Twelve brands of non-dairy creamers were selected from available brands in supermarket. All samples were purchased from supermarket in Bangkok, Thailand during October 2009 to January 2010. Each sample was analyzed in triplicate and results were reported as mean with standard deviation (mean± SD).

Reagents

Trielaidin [1,2,3 tris (trans-9-octadecanoate)] and Triolein [1,2,3 tris (cis-9-octadecanoate)] with purity ≥ 99% were purchased from Sigma Aldrich (St.Louis, MO USA). n-Hexane was purchased from Lab Scan LTD., (Dublin. Ireland). Anhydrous sodium sulfate was obtained from Merck (Darmstadt, Germany). Petroleum ether and Ethyl ether were perchased from Lab Scan LTD., (Dublin, Ireland).

Preparation of TFA ATR-FTIR calibration standards

A set of five standards covering a range of TFA level from 0.5% to 50% was prepared by trielaidin (TE) and triolein (TO), according to AOAC method 2000.10 in 2000 [14] .

Extraction of lipid

The snack samples

Total lipids from samples were extracted by n-hexane under the appropriate condition which reported by Narkwichain, 2009: 40% ultrasonic intensity level for 120 minutes [13]. Five grams of each sample were placed in 250 ml round bottom flask with 60 ml of hexane and put into the ultrasonic bath. After extraction, the sample-solvent was filtered through filter paper, Whatman no.42 and evaporated the solvent from sample by rotary evaporator. Then, the extract was dried in vacuum desiccators for 60 minutes.

The non-dairy creamer samples

Total lipids from non-dairy creamer were extracted by Roese-Gottlied method (AOAC Method 932.06 for milk powder) [13]. One gram of non-dairy creamers was weighed and filled into 10 ml beaker with 1 ml of distilled water. The sample was melted and mixed through homogenous on steam bath. Nine milliliters of distilled water and 1.25 ml of ammonium hydroxide were added into the sample which warm on steam bath. The sample mixture was transfer into Rohrig tube and added 10 ml of ethyl alcohol. After sample was so cool, first extraction, twenty five milliliters of ethyl ether were added into Rohrig tube and swirled in vertical line gently for 1 minute. Second extraction, twenty five milliliters of petroleum ether was added and swirled in vertical line gently for 1 minute again. The sample mixture was waited until separated phase. The ethyl ether phase was released into the constant weight beaker. If the sample mixture formed to emulsion, it would be treated with a little alcohol. The sample mixture was extracted two times with 15 ml of ethyl ether and 15 ml of petroleum ether. The finally extract, if the ethyl ether phase was lower the stopcock, it would be added distilled water until it was upper the stopcock. The ethyl ether phase was released into the constant weight beaker again. The filtrate was evaporated on water bath in the hood. The residue was dried in vacuum desiccators for 60 minutes. After extraction, the fat was weighted and stored at -10º C until analysis.

Determination of TFA by Attenuated Total Reflection-Fourier Transform (ATR-FTIR) Infrared Spectroscopy

The Fourier transform infrared spectrometer (Perkin Elma Spectrum One FTIR, USA) parameters was set up according to the manufacturer's recommendations for using a zinc selenide ATR cell (ZnSe through plate 45°, Perkin Elma, USA) with the following parameters: the spectral range of 1050-900 cm^{-1}, 4 cm^{-1} resolution and 64 scans. The ATR cell maintained at 65±2 º C for melted sample. The reference background was used the single beam spectrum collected of air and saved. Fifty microlitres of sample were transferred by disposable pipet to cover the surface of ATR cell. The single beam spectrum of test sample was collected against that of the reference background, and converted to absorbance and saved. After each analysis, the ATR cell was cleaned by low lint tissue paper with acetone before the other samples were repeated.

Calculations

Standard calibration curve of *trans* fatty acid content was plotted peak height of standard *trans* fatty acids *versus* % *trans* fatty acid of total fat. The linear regression equation was calculated from standard calibration curve. The % *trans* fatty acid of sample was calculated by substituting the value of the integrated height of the negative second derivative of *trans* band in the linear regression equation; % *trans* fat = 1848.4(peak height)-1.0671, $r^2 = 0.9996$ [13].

RESULTS

Total fat contents in snacks and non-dairy creamers

The total fat contents of snacks and non-dairy creamers samples were analyzed in triplicate and the values are expressed as range and mean ± SD in Table 1. The highest total fat contents was found in the microwave popcorns (22.29 to 27.04 g per 100g of food) while the lowest was found in wafers (15.62 to 22.36 g per 100g of food). In non-dairy creamer samples, total fat contents in palm kernel oil ingredient group ranged from 29.20 to 34.09 g per 100g of food while in soybean oil ingredient group ranged from 23.31 to 35.54 g per 100g food.

Table 1 Total fat contents of some selected snacks and non-dairy creamers

Products	Total fat content (g/100g food)	
	Range [a]	Mean ± SD
Cracker (n=3)	17.40-21.29	19.72±2.05
Stick biscuit (n=3)	19.71-31.92	27.45±6.74
Potato chip (n=3)	22.84-26.68	24.92±1.94
Biscuit (n=3)	15.25-22.98	18.07±4.26
Microwave popcorn (n=3)	22.29-27.04	24.95±2.43
Wafer (n=3)	15.62-22.36	18.20±3.64
Non-dairy creamer		
Palm kernel oil (n=10)	29.20-34.90	31.42±1.87
Soybean oil (n=2)	23.31-35.54	29.43±8.65

[a] All values are the mean of triplicate extraction

Table 2 The total *trans* fatty acid contents in some selected snacks and non-dairy creamers

Products	*Trans* fatty acid content			
	% of total fat		g/100 g food	
	Range	Mean ± SD	Range	Mean ± SD
Cracker (n=3)	ND-0.23	0.23	ND-0.05	0.05
Stick biscuit (n=3)	ND	ND	ND	ND
Potato chip (n=3)	ND-0.04	0.04	ND-0.01	0.01
Biscuit (n=3)	0.23-15.14	5.26±8.56	0.04-2.31	0.81±1.30
Microwave popcorn (n=3)	3.33-39.04	25.84±19.60	0.90-9.96	6.23±4.74
Wafer (n=3)	ND*-23.37	23.37	0.00-3.65	1.25±2.08
Non-dairy creamer				
Palm kernel oil (n=10)	0.57-2.35	0.92±0.57	0.17-0.70	0.29±0.17
Soybean oil (n=2)	41.14-44.16	42.65±2.14	10.29-14.62	12.46±3.06

ND = Non-detected at the level traces

Table 3 The *trans* fatty acid contents per average serving size in some selected snacks and non-dairy creamers

Products	*Trans* fatty acid content in an average serving of products (g)		
	Average serving size [a] (g)	Range	Mean ± SD
Cracker (n=3)	30	ND-0.02	0.02
Stick biscuit (n=3)	36.67	ND	ND
Potato chip (n=3)	30.33	ND	ND
Biscuit (n=3)	45	0.02-1.04	0.36 ± 0.59
Microwave popcorn (n=3)	30	0.27-2.99	1.87 ± 1.42
Wafer (n=3)	28	ND-0.91	0.91
Non-dairy creamer			
Palm kernel oil (n=10)	3	0.01-0.02	0.01 ± 0.00
Soybean oil (n=2)	3	0.31-0.44	0.38 ± 0.09

ND = Non-detected at the level traces

[a] Serving size as list on nutrition label by manufacture

Total *trans* fatty acids content in snacks and non-dairy creamers

The highest *trans* fat content were found in microwave popcorns. The mean of *trans* fat content was 6.23 g per 100 g of food (0.90 to 9.96 g per 100 g food), following by wafers, biscuits, crackers, potato chips and stick biscuits (Ka-Kai), respectively (Table 2). Non-dairy creamer samples which showed large variation in *trans* fat content that depended on major ingredient. Two brands used soybean oil in manufacturing process exhibited the higher level of *trans* fat (range of 10.29 to 14.62 g per 100 g food) contents while ten brands that were used palm kernel oil in manufacturing process found the lower level of *trans* fat (range of 0.17 to 0.70 g per 100 g food) (Table 2).

Total *trans* fatty acid contents per average serving size in snacks and non-dairy creamers

The highest level per serving size of *trans* fat was found in microwave popcorns (0.27 to 2.99 g), following by wafers (ND to 0.91g), biscuits (0.02 to 1.04 g), crackers (ND to 0.02 g), while total *trans* fatty acid contents per average serving size in potato chips and stick biscuits (Ka-Kai) were undetectable (Table 3). In non-dairy creamer groups, the soybean oils products showed the highest amount of total *trans* fatty acid contents (0.31 to 0.44 g) than the palm kernel oils products (0.01 to 0.02 g) (Table 3)

DISCUSSION

The method determination of *trans* fatty acid contents are available such as gas chromatography (GC) and infrared (IR) spectroscopy. IR spectroscopy is expressed as total *trans* fat while GC is expressed as individual *trans* fat isomer. IR spectroscopy is a popular method because the analysis time of IR method is about 5 minutes per sample that shorter than GC method [15, 16]. The ATR-FTIR method is suitable for determination of more than 5 % *trans* fat content [12]. However to improve accuracy and sensitivity, a new ATR-FTIR method that measures the height of the negative second derivative of *trans* fat absorption band relative to air was recently proposed [17-19]. The results showed that the negative second derivative ATR-FTIR method was suitable for determination of fat which containing low level of *trans* fat (0.5 to 5%) [19, 20]. In this study, ATR-FTIR with negative second derivative mode was conducted to determine total *trans* fatty acid content in snacks and non-dairy creamers.

In this study, the results showed that total fat contents and total *trans* fatty acid contents of snacks and non-dairy creamers products were varied among the different brands. This might be due to the different sources of fat or oil that used in the process. In snack samples, microwave popcorns were found the highest *trans* fat content which used shortening or margarine in the manufacturing process. The margarine and shortening are major sources of *trans* fats and in study of Narkwichian [13], it was reported that *trans* fat level in the margarine ranged from 1.54 to 1.89 g/ 100 g of food and in the shortening was 1.84 to 3.37 g/ 100 g of food [13]. While in Canadian foods, content of TFA in the margarine was 14.7 to 21.0 g/100g of food and in the shortening was 30.6 to 30.9 g/ 100 g of food [20]. According to the present study, four snack products were selected to demonstrate the variation (crackers, biscuits, wafers and potato chips). The reason of variation could be different ingredients such as crackers which are unsweetened, thin and crisp. Palm oil was used in manufacturing process of crackers. In contrast, shortenings and margarine are mostly ingredients in biscuits and wafers. They are the second or the third component, after flour and sugar. Therefore, *trans* fat contents of biscuits and wafers was higher than crackers. Some study reported that the amount of *trans* fat contents in crackers were 0.4 to 4.7 g/100 g of food [19]. Martin et al reported that *trans* fat content in biscuits was 12.2% to 31.2% of total fat [21]. Potato chips and stick biscuits (Kha-Kai) were found low level of *trans* fats. The manufacturing of potato chips used different oils in different brands. The nutrition label of potato chip showed type of oil, some brand used rice bran oil in the process for reduced saturated fatty acid and some brand used palm oil in process. The type of oil in process affect to *trans* fat contents. Stick biscuits (Kha-Kai) which used palm oil in manufacturing process were found to contain equal proportions of saturated fatty acid and unsaturated fatty acid [22]. Therefore, *trans* fat content in stick-biscuits depend on the hydrogenated unsaturated fatty acid in manufacturing process.

In non-dairy creamer products, 2 brands were found high level of *trans* fat (0.31 g and 0.44 g per serving size) which used the partially hydrogenated soybean oil as major ingredient. In contrast, 10 brands were found low level of *trans* fat which used the palm kernel oil as major ingredient. The palm kernel oils were saturated fat and some time they were partially hydrogenated fat, hence the TFA content are negligible.

The average *trans* fat contents were also varied because the different brands might use different type of fat and oil in food process. The food manufacturing may use single hydrogenated fats or combination hydrogenated fats and oils, which affect *trans* fat level in foods.

However, the consumers should be consumed the products which contain *trans* fats as low as possible to minimize the cardiovascular risk and other risk associated with *trans* fatty acids.

CONCLUSION

Although, all samples contained *trans* fat contents not more than 2 grams per serving (as list in nutrition label by manufacture). If consumed *trans* fat exceed 2 grams per day as recommended by American Heart Association, *trans* fat are dangerous for the health. Therefore, TFA levels are necessary to show on the nutrition label. The Food and Drug Administration (FDA) should educate consumers for their better food selection and should provide the regulation to require the declaration for TFA content in the nutrition label.

ACKNOWLEDGEMENT

This study was supported in part by Graduate thesis grant in academia year 2010, Graduate School, Chulalongkorn University.

REFERENCES

1. Gebauer SK, Posta TL, Kris-Etherton PM. The diversity of health effects of individual *trans* fatty acid isomers. Lipids. 2007; 42: 787-99.
2. Kim Y, Himmelsbach DS, Kays SE. ATR-Fourier transform mid-infrared spectroscopy for determination of *trans* fatty acids in ground cereal products without oil extraction. J Agric Food Chem. 2007; 55: 4327-33.
3. Saunders D, Jones S, Devane GJ, Scholes P, Lake RJ, Paulin SM. *Trans* fatty acids in the New Zealand food supply. J food Compos Anal. 2008; 21: 320-5.
4. McCarthy J, Barr D, Sinclair A. Determination of *trans* fatty acid levels by FTIR in processed foods in Australia. Asia Pac J Clin Nutr. 2008; 17(3): 391-6.
5. Soonpan P. Analysis of *trans* fatty acid in deep-fried foods milk and milk products by attenuated total reflection-fourier transform infrared spectroscopy

[Master's Thesis]. Department of Food and Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University; 2010.

6. Soonpan P, Tongyonk L, Patarapanich C. Analysis of TRANS fatty acid content in deep fried foods, milk and dairy products by attenuated total reflection fourier transform infrared spectroscopy. J Health Res. 2011; 25(4): 205-9.
7. Lemaitre RN, King IB, Mozaffarian D, Sotoodehnia N, Rea TD, Kuller LH, et al. Plasma phospholipid *trans* fatty acids, fetal ischemic heart disease, and sudden cardiac death in older adults. Circulation. 2006; 114: 209-15.
8. Lisa CV, Robert CM, Jessie AS, Jane CS, Christopher FM, Joseph GI, et al. *Trans*-fatty acid consumption and its association with distal colorectal cancer in the North Carolina Colon Cancer Study II. Canc *Causes* Contr. 2010; 21: 171-80.
9. Slattery ML, Benson J, Ma KN, Schaffer D, Potter JD. *Trans*-fatty acids and colon cancer. Nutr cancer. 2001; 39(2): 170-5.
10. Chavarro JE, Stampfer MJ, Campos H, Kurth T, Willett W, Ma J. A prospective study of *trans*-fatty acid levels in blood and risk of prostate cancer. Cancer Epidem Biomar. 2008; 17(1): 95-100.
11. Teegala SM, Willett WC, Mozaffarian. Consumption and health effects of *trans* fatty acids: A review. J AOAC Int. 2009; 92(5): 1250-5.
12. Mossoba MM, Moss J, Kramer JKG. *Trans* fat labeling and level in U.S. foods: Assessment of gas chromatographic and infrared spectroscopic techniques for regulatory compliance. J AOAC Int. 2009; 92(5): 1284-97.
13. Narkwichian N. Analysis of *trans* fatty acid in some foods by attenuated total reflection-fourier transform infrared spectroscopy [Master's Thesis]. Department of Food and Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University; 2009.
14. Association of Official Analytical Chemists (AOAC). Official method of analysis of the association of official analytical chemists. 17th ed. Washington D.C.: The Association of Official Analytical Chemists; 2000.
15. Ratnayake WMN. Overview of methods for the determination of *trans* fatty acids by gas chromatography, silver-ion thin-layer chromatography, silver-ion liquid chromatography, and gas chromatography/mass spectrometry. J AOAC Int. 2004; 87(2): 523-39.
16. Ali LH, Angyal G, Weaver CM, Rader JI, Mossoba MM. 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. 1996; 73(12): 1699-705.
17. Fritsche J, Steinhart H, Mossoba MM, Yurawcz MP, Sehatb N, Ku Y. Rapid determination of *trans* fatty acids in human adipose tissue comparison of attenuated total reflection spectroscopy and gas chromatography. J Chromatogr B. 1998; 705: 177-82.
18. Mossoba MM, Yurawcz MP, McDonald R. Rapid determination of the total *trans* content of neat hydrogenated oils by attenuated total reflection spectroscopy. J Am Oil Chem Soc. 1996; 73: 1003-9.
19. Milosevic M, Milosevic V, Kramer JKG, Azizian H, Mossoba MM. Determining low levels of *trans* fatty acids in foods using an improved ATR-FTIR procedure. Lipid Tech. 2004; 16(11): 252-5.
20. Ratnayake WMN, Abbe MRL, Farnworth S, Dumais L, Gagnon C, Lampi B, Casey V. *Trans* fatty acids: current contents in Canadians foods and estimated intake levels for the Canadian population. J AOAC Int. 2009; 92(5): 1258-83.
21. Martin CA, Carapelli R, Visantainer JV, Matsushita M, Evelazio de Souza N. *Trans* fatty acid content of Brazilian biscuits. Food Chem. 2005; 93: 445-8.
22. Neo YP, Tan CH, Ariffin A. Fatty acid composition of five Malaysian biscuits (cream crackers) with special reference to *trans*-fatty acids. Asean Food J. 2007; 14(3): 197-204.