

***Trans* fatty acids in selected bakery products and its potential dietary exposure**

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Abstract

High intake of trans fatty acids (TFA) may cause adverse changes in blood lipids as shown in many epidemiological studies. The major sources of TFA in human diet come from bakery products such as cookies, cakes, muffins, breads and pastries. In this study, total fat of baked products was determined using Soxhlet method while TFA content was determined on gas chromatograph using a highly polar capillary column and flame ionization detector. Exposure to TFA from the baked product was estimated based on Food Consumption Statistics of Malaysia 2003. High level of TFA was observed in muffins (3.21-7.34 g per 100 g food) followed by pastries (2.90-6.03 g per 100 g food) and cakes (2.26-8.36 g per 100 g food) regardless of products having brand name or not. Among the TFA isomers, trans 18:1 6t was the most abundant followed by trans 18:1 9t, trans 16:1 9t, trans 18:2 and small quantities of trans 18:1 11t. Exposure to TFA based on the Malaysian Food Consumption Statistics 2003 of the baked products was ranging from 0.14-0.18 g/day. Even with the low estimated exposure, actual intake of baked products must be monitored closely as other high fat food as these products is one of main contributor to TFA in the diet following their high frequency of daily intake.

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Introduction

Trans fatty acids (TFA) are unsaturated fatty acids containing at least one double bond in the *trans* configuration. The *trans* fatty acids are formed during the process of partial hydrogenation. In the process, liquid oils are heated in the presence of metal catalyst (such as nickel) and exposed to hydrogen gas. The process is normally done to increase the melting points of fatty acids that enable liquid oils to be used in solid fats formulations such as margarines, shortenings, vanaspati and bakery fats. The new conformation of the double bonds impacts the physical properties of the fatty acids. Some double bonds become saturated by the process while others are changed to *trans* configurations or are shifted to a new position in the aliphatic chain (Koletzko and Decsi, 1997; Fauziah and Sundram, 2003). These arrangements resulted in different configurations for the fatty acids and affected the functions in the body as well as in health (Whitney and Rolfes, 2005).

According to Emken (1995), foods containing hydrogenated oils tend to contribute to TFA in the diets. TFA in human diet comes from several natural sources like meat and milk of ruminant animals in small quantities (Huang *et al.*, 2006). However,

the major source of *trans* fatty acids in human diet comes from industrial food processing by a process called as hydrogenation (Koletzko and Decsi, 1997). Examples of food rich in TFA include cakes, muffins, pastries, doughnuts, fried foods (French fries), dairy products and meats. Depending on the production technology, the contents of TFA can be very high in margarines (up to 30% of total fatty acids), biscuits and pastry products (up to 50%) made from quality raw materials (Leco Corporation, 2008). Today, there is a dramatic increase in the consumption of convenient food products (margarine, muffin, cakes, cookies and crackers, fried potatoes, chips, and snacks) everywhere including some Eastern countries such as China and Malaysia. The consumption of these fatty acids in the food industry has also been growing. According to Cheng (2003), the demand for fatty acids in the food industry has increased for three consecutive decades.

Epidemiological studies have shown that high intake of TFA can cause adverse changes in blood lipids by increasing the level of low density lipoprotein cholesterol (LDL-c) and reducing high density lipoprotein cholesterol (HDL-c). Consequently, these may lead to high risk of cardiovascular disease (CVD), developing type 2 diabetes mellitus and cancer

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(Bakker *et al.*, 1997; Oomen *et al.*, 2001; Salmeron *et al.*, 2001). The World Heart Organization (WHO) and Food and Agriculture Organization (FAO) have recommended that fats for human consumption should contain less than 1% (2-2.5 g TFA/day) of the total fat as *trans* and urged the food industry to reduce the amount of *trans* fatty acids in their products (WHO, 1993; PAHO/WHO, 2007). At the same time, the consumption of saturated fat should not exceed 10% of the total calorie intake. However, the saturated fat consumption is in the range of 11 to 13% in most countries, which exceeds the WHO recommendations. In the case of *trans* fats, the recommendation of 1% is a compromise level (not a safe level) and certainly not a healthy level. The safe or healthy level for *trans* fats is zero. But since *trans* fats have become so prevalent in the food chain today, it is not practical today to achieve zero *trans* fat, so a compromise level of 1% was set (WHO, 2003). Nevertheless, in the case of saturated fats, the recommendation of 10% is the healthy level as some saturated fats are beneficial to health in many ways (WHO, 2003).

The earliest human study on *trans* fatty acids has shown that *trans* fatty acids at 10% energy in the diet simultaneously increased LDLc and decreased HDLc thus resulting in a less desirable total/HDL-C ratio and is associated with increased risk of cardiovascular diseases (Mensink and Katan, 1990). Several studies later showed that bakery products such as cakes, cookies, pastries and biscuits are the major sources of *trans* fatty acids in the human diet (Elias and Innis, 2002). Therefore, high consumption of bakery products can lead to high intake of dietary *trans* fatty acids. A number of other studies have also found that dietary *trans* fatty acids increased the atherogenic LDL cholesterol (Institute of Medicine, 2002). Previous data have demonstrated a dose-dependent relationship between *trans* fatty acids intake and LDL/HDL ratio and, combining a number of studies, the magnitude of the effect is greater for *trans* fatty acids compared to saturated fats (Ascherio *et al.*, 1999). These studies also have shown the implication of *trans* fatty acids are not only with coronary heart disease but also increase risk and incidence of diabetes mellitus.

Today, the Malaysian bakery and confectionery industries are growing rapidly. Both industries are changing and expanding following the shift in consumers' preferences and demand (Anonymous, 2004). According to Azimah *et al.* (2013), bakery product is one of the four major food (semisolid fats and cooking oils, fast foods, fried foods, and baked products) contributed to TFA intake of Malaysian.

In this study, we aimed to provide quantitative data on distribution of TFA in selected bakery products (cakes, muffins and pastries) from samples with brand and no brand name in the local market. Besides, estimation of exposure to TFA from baked products was also determined based on Food Consumption Statistics of Malaysia, 2003. Findings of this study is hoped to stimulate consumer awareness of the main sources of TFA in their diet, and also to sensitize local bakery industries to take serious measures to reduce or eventually eliminate the TFA in their baked products. It is vital for food manufacturers to be aware of the TFA composition in their baked products by applying feasible substitutions that could provide the similar textural attributes without any adverse health effects, as the pressure of reducing and eventually eliminating TFAs in food products continues to grow rapidly in Malaysia. In addition, the data of this study can be used for future study especially in the field of nutrition.

Materials and Methods

Samples selection

A total of eighteen samples of commercially available bakery products around the area of Seri Kembangan, Selangor, Malaysia were purchased from September to October 2010. Samples included cakes, muffins, and pastries with six samples each having brand and no brand names. The types of cake included were chocolate, cheese and butter while muffins included were chocolate, walnut and blueberry muffins. The pastries selected were Danish, croissant and puff pastries. The selection of sample types was based on common and favorite bakery products among Malaysians. In this study, product with brand name refers to product with specific name while the non-brand products refer to product that is sold without any specific name and were loosely packed. By comparison, the products with brand name are sold at higher price compared to products with no brand name. The goal of such marketing strategies is to convince consumers that the brand name is substitute or proxy for quality (John and Sons, 2010). All the samples were analyzed before their respective expiration date. A convenience sampling method was used in the study to obtain the samples.

Pre-drying of samples

The extraction efficiency of lipid from foods depends on particle size and adequate grinding is very important. Therefore, the collected samples were grinded as fine as possible in a home type blender and mixed thoroughly. At the same time, aluminum

dishes were dried in an oven at 105°C. After it has cooled to room temperature in desiccators, the weights were recorded. Then, samples were put in aluminum dishes. The dishes with sample uncovered were placed in an oven (40°C) overnight for drying. The dried sample was removed from oven, cooled in desiccators and weight again until constant weight was obtained.

Fat extraction

Extraction of fat was carried out following AOAC (1990) by using Soxhlet apparatus. About ten grams of each sample was weighed accurately and transferred in an extraction thimble. The thimble was closed with cotton ball and placed in the extractor and the extractor was connected to a weighed flask containing 200 ml petroleum ether. The extractor was connected to a reflux condenser and fat was extracted under reflux, on a water or steam bath for eight hours. Solvent was removed by evaporation using rotary evaporator (Rotavapor R-200, Buchi, Switzerland) and the flask was dried in an air-oven at 40-60°C for one hour, cooled in a dessicator and re-weighed. The step of weighing was repeated until the results of two successive weighing do not differ by more than 0.1% of the mass of the test portion. The total content of fat was calculated by using a formula as follows:

$$\text{Fat (\%)} = \frac{W - W_0}{S} \times 100$$

W = Weight (g) of flask with fat

W₀ = Weight (g) of flask without fat

S = Weight (g) of sample before drying

Preparation of standard

Five single standards of TFA were purchased from Supelco, Bellefonte, USA. The TFA standards comprised of C16:1 9*t* (palmitoelaidic acid), C18:1 6*t* (petroselaidic acid), C18:1 9*t* (elaidic acid), C18:1 11*t* (vaccenic acid), C18:2 9*t* 12*t* (linoelaidic acid). All standards were readily FAMES, and prepared individually by transferring into 2 ml amber vial before injection into GC for analysis (5 µl). An internal standard of C13:0 purchased from Supelco, Bellefonte, USA was used to quantify the TFA in samples.

Preparation of fatty acid methyl esters (FAMES)

Trans fatty acids methyl esters were prepared according to method by O'Fallen *et al.* (2007). After extraction of fat from samples, the lipid phase was esterified. For this purpose, about 1 ml of internal standard was injected into 0.2 g sample of fat. Then, 2 ml of 14% BF₃ in methanol was added into the

fat sample. After that, the sample was vortex for 30 seconds and incubated in shaking incubator at 55°C for one and half hour. After cooling, the sample was shaken vigorously every 15 min for homogenization. After that, 2 ml of saturated NaHCO₃ and 3 ml of heptanes were added into the sample and centrifuge at 3000 rpm for 15 min. Later, 5 µl of supernatant was transferred into the auto sampler vial and injected into a gas chromatograph.

GC condition

The composition of TFA was analyzed by gas chromatography (Agilent Technologies, Palo Alto, Calif., USA.) equipped with a flame ionization detector (FID). The column used was capillary column HP-88 (100.0 m x 0.25 mm x 0.2 µm of film thickness) coated with cyanopropyl-polysiloxane (Agilent J & W Scientific GC Column, USA) to separate and quantify each FAMES components. Helium gas was used as the carrier gas and maintained at a constant flow rate (30 ml/min), hydrogen gas controlled at 40 ml/min and air used for flame ionization detection (FID) was held at 450 ml/min (David *et al.*, 2005). The oven was held at an initial temperature of 120°C for 1 min, then increased to 175°C for 10 min and lastly increased to 230°C for 5 min. The temperature of the injector and flame ionization detector were set at 250°C and 280°C, respectively.

Statistical analysis

All samples were analyzed one by one, in triplicate and the results were expressed as mean ± standard deviation. The results for total fat were expressed as % of total fat while results for TFA were expressed as % of total fat and % of 100 g food. T-test was used to compare mean between brand and non-brand samples while ANOVA was used to compare mean between samples in the same group with 5% significance level using SPSS 17 (Statistical Packaged for Social Sciences).

Results and Discussion

Quality of fat, besides quantity is one of the key analyses performed by the food industries for nutritional labeling as consumers today demand for reduction of total and zero *trans* fat content in food to improve health. The *trans* fatty acid composition of the selected bakery products is shown in Table 1. According to the table, generally bakery samples with brand name showed broader range of total *trans* fatty acids content (g per 100 g food) compared to their counterparts without brand name. Among the muffins, the samples with brand name had higher content of

Table 1. TFA content food and lipid of studied bakery product

Group	Type of bakery products	TFA by type (g/100 g food)					TFA by type (g/100 g fat)					Total TFA (g)	
		16:1 9t	18:1 6t	18:1 9t	18:1 11t	18:2 9t12t	16:1 9t	18:1 6t	18:1 9t	18:1 11t	18:2 9t12t	100 g food	100 g fat
Cake A	A1	0.17	2.93	2.45	ND	ND	1.30	22.42	18.75	ND	ND	5.55	42.47
	A2	0.46	1.33	0.47	ND	ND	3.70	10.83	3.82	ND	ND	2.26	18.35
	A3	0.16	2.08	1.75	ND	ND	1.36	17.34	14.54	ND	ND	3.99	33.23
	A4	0.30	1.82	0.84	ND	ND	3.45	20.76	9.60	ND	ND	2.96	33.81
B	A5	0.13	1.10	1.66	ND	ND	2.00	16.90	25.35	ND	ND	2.89	44.25
	A6	1.20	3.09	4.07	ND	ND	9.21	23.79	31.31	ND	ND	8.36	36.12
Muffin A	B1	0.30	3.99	3.05	ND	ND	2.66	35.78	27.34	ND	ND	7.34	65.78
	B2	0.22	3.16	1.37	ND	ND	2.37	34.28	14.85	ND	ND	4.75	51.50
	B3	0.37	3.09	2.49	ND	ND	4.34	36.12	29.12	ND	ND	5.95	69.58
	B4	0.24	1.20	1.77	ND	ND	3.47	28.39	25.15	ND	ND	3.21	57.01
B	B5	0.15	4.62	1.28	ND	ND	1.80	55.26	15.35	ND	ND	6.05	72.41
	B6	0.18	4.04	0.76	ND	ND	2.39	54.71	10.24	ND	ND	4.98	67.34
Pastries A	C1	0.70	2.71	1.57	0.79	0.26	9.31	36.06	20.83	10.45	3.39	6.03	80.04
	C2	0.21	1.04	1.65	ND	ND	2.62	12.70	20.13	ND	1.29	2.90	36.74
	C3	0.09	3.67	1.32	ND	ND	0.73	29.13	10.51	ND	ND	5.08	40.37
	C4	0.97	0.83	2.30	ND	0.11	11.43	9.85	16.31	ND	1.91	4.21	39.50
B	C5	0.26	1.70	1.28	ND	ND	3.74	24.79	18.53	ND	ND	3.24	47.06
	C6	0.77	1.66	1.63	ND	0.22	13.35	28.95	28.45	ND	3.84	4.28	74.59

ND: Not detected; A: baked product with brand name; B: baked product without brand name.

TFA (4.75-7.34 g per 100 g food) compared to the non-branded muffins (3.21-6.05 g per 100 g food). Similarly, pastries with brand name also had higher TFA content than the non-brand counter-parts. In contrast, cakes without brand name had wider range of TFA content (g per 100 g food) from 2.89 to 8.36 compared to cakes with brand name (2.26 to 5.55). The differences between the brand and non-brand samples might be due to factors such as differences in method of preparation as well as fat ingredients used (type and amount) (Martin *et al.*, 2005).

Generally, the trends in level of TFA isomers per 100 g fat and per 100 g food are similar where *trans* 18:1 6t was the most abundant at values ranging from 0.83-4.62 g per 100 g food (9.85-55.26 g in 100 g fat) followed by *trans* 18:1 9t (0.47-4.07 g per 100 g food) (3.82-31.31 g in 100 g fat), *trans* 16:1 9t (0.13-0.97 g per 100 g food; 2.0-11.43 g in 100 g fat), *trans* 18:2 9t12t (0.11-0.26 g per 100 g food; 1.91-3.39 in 100 g fat) and small quantities of *trans* 18:1 11t. Variability of the *trans* fatty acids type in a way indicate variability of manufacturers/ bakers choice in the amount of fat ingredient, as well as the extent of hydrogenated fat, that was used, which depends on varied process conditions (temperature, pressure, type and amount of catalysts) that affected the final fatty acids of the finished bakery products (Martin *et al.*, 2005; Karabulut *et al.*, 2007). The percentages of TFA in 100 g fat of muffins and pastries were higher compared to cakes at more than 50% of the total fat content. The higher level of TFA in muffins is ranging from 51.50% to 72.41%, (n=6), followed by pastries from 36.74% to 48.55%, (n=4 except 2 samples had 74.59% to 80.04%) and cakes from 18.35% to 44.25% (n=6). These resulted to the higher TFA content of muffins (3.21-7.34% in 100 g food) compared to pastries (2.90-6.03% in 100 g food) and cakes (2.26-5.55% in 100 g food, except A6). When compared between samples having brand name and no brand name, it is clear that regardless of the baked product having specific brand or not, the amount and type of

TFA is product specific depending on ingredients and formulation/ recipe of the baked product.

In the present study, all the studied samples were higher in TFA content compared to a study by Azimah *et al.* (2013) who reported as a narrow range of TFA content, 0.01-0.29 g/100 g food for baked products (cakes and pastries). While the TFA contents of samples of this study were from 2.2 g to 7.3 g/100 g food (18.3-65.8 g/100 g fat), exceeding the limit of 2 g TFA for every 100 g fat (Stender and Dyaberg, 2004) making them as unhealthy choice of baked products. Previously, Innis *et al.* (1999) and Carlson *et al.* (1997) reported that the wide variability of TFA in food products is following a few reasons such as (i) variability of TFA in hydrogenation process that depend on temperature, pressure, type and amount of catalyst and agitation effect during the process, (ii) use of single hydrogenated or non-hydrogenated fats or combinations of both hydrogenated or non-hydrogenated fats thus resulted to TFA profile that is product specific. Generally, based on the values obtained, it can be concluded that the studied bakery products contained relatively high amount of TFA. This finding signifies the importance of educating manufacturers/ bakers on choice of suitable fats as well as close monitoring of quality of bakery products by the relevant authority.

In order to determine dietary exposure to TFA, the estimated intake for baked products based on data from Food Consumption Statistics of Malaysia 2003 for adult population aged 18-59 years old specific for cakes (4.19 g/day) and pastry (pizza) (3.35 g/day) were used. Based on the data, it can be estimated that exposure to TFA following intake of cake is 0.18 g/day (4.33 g TFA/100 g food; mean of TFA in cakes for products with brand and no brand name) while exposure to TFA from pastry is 0.14 g/day (4.29 g TFA/100 g food; mean of TFA in pastries with brand and no brand name). Both levels of exposure were relatively low compared to values reported by Azimah *et al.* (2013) at 3.90 g/day for cakes and

0.76 g/day for pastries. The lower exposure to TFA in this study was following calculation made based on the estimated intake of food for Malaysian. While the exposure of Azimah *et al.* (2013) was based on actual intake of respondents for 3 days (2 weekdays, 1 weekend) of 2 weeks to 1 month of the current intake ($n=105$). Principally, calculation method is less accurate as compared to face to face interview using semi-qualitative Food Frequency Questionnaires (FFQ) by trained researchers. This is due to the fact that calculation based on population intake provides only a rough estimation. Besides, the food intake data of Food Consumption Statistics of Malaysia 2003 was based on frequency of habitual intake during the last one year. Diet recall in this manner may be subjected to misreporting problems (under- and over reporting issues). According to Poslusna *et al.* (2009) misreporting in dietary assessment may lead to error in estimation of nutrient intake including energy. Poslusna *et al.* (2009) have laid some of the possible ways of handling misreporting to give a more reliable data.

There are several factors that influence the intake of TFA daily. Generally, the level of fat intake including TFA is depending on lifestyle, dietary habits, socioeconomic status of the population, frequency of consumption of food products containing TFAs, strata of the population, country's food policy and analytical issues (Emken, 1995; Ismail *et al.*, 2002). In previous studies on consumption of TFA of developed countries, it has been reported that the TFA intake was between 2.1–5.4 g/day, representing around 3.2–8.2% of total fat intake, although varies greatly between countries, geographical areas and the age of the studied group (Margaret and Schmidt, 2006). In an earlier study (TRANFAIR between 1995–1996) using various dietary data (dietary surveys, frequency of consumption and analysis of food samples) that assessed the total intake of TFA in European countries, found lowest consumption of TFA (<1% energy, 2.2 g/day) corresponded to Mediterranean area, Finland and Germany and remained moderate in countries such as Belgium, Holland, Norway and Great Britain. The highest TFA intake corresponds to Iceland (Hulshof *et al.*, 1999). In contrast to European countries, the intake of TFA in US between years 1989 and 1991 was averaged as 5.3 g/day (Allison *et al.*, 1999). Since the TRANFAIR study, the level of TFA in industrially produced foods decreased markedly following efforts from industries, societal pressure to reduce TFA in fast foods.

However in other parts of the world such as India, Brazil and Costa Rica, the reported intake of TFA were high at 5–12 g/day/capita especially in the

urban area (Khor and Norhaizan, 2007). In a more recent report, the intake of TFA in Brazil remained high at 5.0 g/day (Castro *et al.*, 2009). In Asian and Pacific region, TFA intake from daily diet is less than 1 g/day with no data of specific country except Japan with estimated daily intake of 1.56 g/capita/day (0.7% of total energy) (between 1996–2000) (Ochi *et al.*, 1996; Matsuzaki *et al.*, 1998; Okamoto *et al.*, 1999; Matsuzaki *et al.*, 2000). As for Malaysia, the exposure to TFA from common food was reported at $0.399 \pm 0.22\%$ energy (0.71 ± 0.42 g/day) (Azimah *et al.*, 2013).

Based on findings of this study, it can be deduced that the potential intake of TFA due to intake of the selected bakery products is quite low at the moment but might be increasing in future. This can be a major concern as baked product might contribute to substantial amount of TFA in a diet. Mostly, for the samples analyzed in this study, the level of TFA can be considered high. Recently, baked products such as biscuit, cakes and wafer purchased in European supermarkets (capitals of Hungary, Poland, Czech Republic, France, Germany and UK) between 2005–2009 were reported to contain a broader range of TFA contents from <0.2 g to 15.3 g per 100 g products (Stender *et al.*, 2012). The same study also demonstrated that differences exist in TFA of products bought from same retailers thus indicating that the level of TFA is highly product specific. Continuous studies on TFA content in baked products especially those without specific brand name and proper packaging is highly warranted as some consumers may not pay attention or understand the importance of labeling. For Malaysia, considering the other potential food sources of TFA in the diet especially fried foods, fast food, snacks, fat ingredients (shortening, margarines, oil), introducing a legislative limit might be advantageous for a long term control of CHD besides close monitoring of baked products by the authorities.

Conclusion

As a conclusion, the results obtained from this study showed that the level of TFA contents in bakery products with and without brand names were quite high exceeded the level recommended of 2 g/100 g fat. However, the estimated intake of TFA for the selected baked products based on Malaysian Food Consumption Statistics 2003 was low at 0.14–0.18 g/day from cakes and pastries. The food manufacturer should realize the importance of TFA reduction in their baked products while the consumers should be equipped and aware on their intake of bakery products

because of its high TFA content.

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