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Trans fatty acids content of biscuits commercially available in Malaysian market and comparison with other countries

[1]Norhayati, M., [1*]Azrina, A., [1]Norhaizan, M. E. and
[2]Muhammad Rizal, R.

[1]*Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor*
[2]*Institute for Environmental and Development (LESTARI), Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor*

Abstract: The fatty acid composition and *trans* fatty acid (TFA) contents of biscuits products were determined by gas chromatography, using a highly polar 100m capillary column (HP-88) and flame ionization detection. Total TFA ranged from 0.00 – 0.52 g/100 g total fatty acids and 0.12 – 0.68 g/100 g total fatty acids for local packed and unpacked biscuits, respectively. In imported biscuits, total TFA was higher ranging from 0.03 – 3.09 g/100 g of total fatty acids. *Trans* 16:1 was the most abundant, with values ranging from 0.01% to 38% followed by *trans* 18:1 Δ11 (0.01% - 13.11%), *trans* 18:1 Δ9 (0.01% - 4.68%), *trans* 18:2 (0.23% - 2.77%) and small quantities of *trans* 18:1 Δ6. CLA, the natural TFA constituted from 0.1% to <70% TFA. These results and comparison with published data indicate that Malaysian biscuits contained considerably low proportion of *trans* fatty acids following the wide use of palm fat.

Keywords: *Trans* fatty acids, conjugated linoleic acids, fatty acids, fat, biscuits

Introduction

Trans fatty acid is defined as unsaturated fatty acids containing one or more isolated double bonds in a *trans* configuration (Food and Drug Administration, 2003). *Trans* fatty acids in human diet can comes from natural sources like meat and milk of ruminant animals in small quantities through biological hydrogenation process of unsaturated fatty acids (Huang *et al*., 2006). However, the major source of *trans* fatty acids in human diet comes from industrial food processing by a process called hydrogenation (Koletzko and Decsi, 1997). In the food industry, vegetable oils containing high unsaturated fatty acids are hydrogenated in the presence of metal catalyst like nickel and hydrogen to produce solid or semi-solid fat. Total hydrogenation of fat is rarely occurs during food processing and most often is partial hydrogenation. Through this process, the double bonds in unsaturated fatty acids are reduced and some double bonds are converted from *cis* configuration to *trans* isomer. These arrangements has resulted in different configurations for the fatty acids and affected the functions in the body as well as the overall health (Whitney and Rolfes, 2005). For the food industries, the use of solid fat such as hydrogenated fats may provide important technological roles of lipid such as differentiation in rheological properties of dough, shelf-life stability as well as improvement in sensory profile (Maache-Rezzoug *et al*., 1998; Brown, 2008).

Early human trials have shown that high *trans* fatty acids intake can cause adverse changes in lipid profile by increasing the level of low density lipoprotein cholesterol (LDL-c) and reducing high density lipoprotein cholesterol (HDL-c) simultaneously (Mensink and Katan, 1990; Zock and Katan, 1997). Consequently, these can contribute to high risk of cardiovascular diseases (CVD), developing type 2 diabetes mellitus, and cancers (Bakker *et al*., 1997; Oomen *et al*., 2001; Salmeron *et al*., 2001). Thus, the World Heart Organization (WHO) and Food and Agriculture Organization (FAO) have recommended that fats for human consumption should contain less than 1% of the total fat as *trans*, and urged the food industries to reduce the amount of *trans* fatty acids in their products (WHO, 1993; PAHO/WHO, 2007). Despite that, more studies and research are being done on analysis of *trans* fatty acids in food products in order to ensure the safety of foods being consumed.

Several studies have shown that the major source of *trans* fatty acid in human diet is bakery products which comprising of biscuits, cakes, cookies and breads (Elias and Innis, 2002). Study on food consumption patterns among Malaysian adults between October 2002 and December 2003, showed that 16.3% of local population consumed biscuits daily with total amount of 5 pieces per day (Norimah *et al*., 2008). Earlier, food consumption statistics of Malaysia 2003 estimated that intake of biscuits was at

*Corresponding author.
Email: *azrina@medic.upm.edu.my*

18.5 g/day with 88.25% prevalence of intake (MOH, 2006). These show that biscuits are highly consumed by Malaysians and would be the major sources of *trans* fatty acids. This was further supported by improvement in biscuits sales that have shown 3.4% increased in value from RM641.5 million in 2007 to RM663.4 million in 2008. The bulk improvement in biscuit sales came from consumers picking up biscuits as convenient snack (Euromonitor International, 2008). This situation can indirectly increase the risk of cardiovascular diseases due to increase intake of TFA from biscuits. The situation can be more difficult when consumers are having difficulties to identify foods containing *trans* fatty acids since it is only optional in Malaysia to put the TFA information on the nutrition labelling panel. Based on Malaysian Food Act 1983 and Food Regulations 1985, the nutrients that must be declared on nutrition labelling are energy, carbohydrate, protein, and fat (Legal Research Board, 2008; Malaysian Guide to Nutrition Labelling and Claims, 2006).

Therefore, this study was aimed to determine and compare *trans* fatty acids content in local and imported biscuits that are commonly consumed by Malaysian. This information is not only important to consumers but also useful for nutritionist and food-related professionals in delivering nutrition counselling as well as completion of data in food composition database. To our knowledge, no data have been published on the type and level of *trans* fatty acids including conjugated linoleic acids of biscuits in Malaysia.

Materials and Methods

Sample selection

A total number of twelve samples for sweet and savoury types of biscuits were purchased from grocery stores and hypermarkets around Serdang, Selangor in September to October 2009. Each type of biscuits was coded with specific alphabet of A representing the sweet type and B representing the savoury type. Eight samples of local brands and four samples of imported brands were used. Among the local brands, four samples were common and popular while, the other four were unpopular biscuits which do not have any specific brand name. The selection of samples was based on common flavor available in the local market. Then, each of the samples was grinded and kept in the oven at 50°C until complete dryness before analysis. The sampling method used in this study was convenience sampling and analyses were carried out in triplicate.

Extraction of fat

Fat extraction for gas chromatography (GC) analysis was done using petroleum ether b.p. 40ºC - 60ºC (Fisher Scientific, UK) through a Soxhlet extractor apparatus (Electrothermal, Barnstead, US). Ten grams of sample were weighed and transferred in an extraction thimble (Whatman, Maidstone, England). Then, the thimble was placed in the extractor and connected to a weighed flask containing 250 ml petroleum ether. After that, the extractor was connected to a reflux condenser and fat was extracted under reflux, on a water or steam bath for 8 hours. The solvents were removed by evaporation using rotary evaporator (Büchi Rotavapor R-200, Switzerland) and the flask was dried in an air-oven at 40-60ºC for 1 hour, cool in desiccators, and weight. The total content of fat was calculated by using a formula (AOAC, 1990).

Preparation of standards

Five single TFA standards and two CLA standards were purchased from Supelco, Bellefonte, USA. The TFA standards comprised of C16:1 9*t*, C18:1 6*t*, C18:1 9*t*, C18:1 11*t*, C18:2 9*t* 12*t* while the CLA standards were C18:2 9c 11*t* and C18:2 10c 12*t*. Individual standards of other common fatty acids were purchased from Sigma Aldrich, St. Louis, USA. All standards were readily FAMEs, and prepared individually by transferring into 2 ml amber vial before injection into GC for analysis (5 ul). Fatty acids methyl esters mix standards was prepared for simultaneous identification. An internal standard of C13:0 was used to quantify the *trans* fatty acids in samples.

Preparation of fatty acid methyl esters (FAMEs)

About 3 ml of 10% BF_3 in methanol (Fluka, Darmstadt, Germany) was weigh and added into 40 µl of the extracted fat. Then, the sample was incubated in water bath at 55°C for 1 and half hour. After cooling, the sample was shaken vigorously every 15 minutes for homogenization. Then, 2 ml of saturated $NaHCO_3$ (R&M Merkery, Essex,UK) and 3 ml of heptanes (Sigma, Steinhelm, Germany) were added into the sample and centrifuged at 1500 rpm for 10 minutes. Later, 5 µl of supernatant was transferred into the auto sampler vial and injected in the gas chromatography (O'Fallon *et al*., 2006).

Gas chromatography (GC) analysis: *Trans* fatty acids was analyzed by using HP-88 capillary column (Agilent J&W Scientific GC Column, USA) (100.0 m x 0.25 mm x 0.2 µm of film thickness) to separate and quantify each FAMEs components. The oven temperature was held at 120°C for 1 minute, then was increased to 175°C for 10 minutes, lastly was increased to 230°C for 5 minutes. Temperature for injector and detector were set at 250°C and 280°C, respectively and 5 µl of sample volume was injected with split ratio of 0:50. A carrier gas that used for the system was helium gas, 30 ml/min controlled at 40 ml/min for hydrogen gas and air used for FID were held at 450 ml/min (Frank *et al*., 2005).

Statistical analysis

The samples for *trans* fatty acids quantification were analysed one by one, in triplicate and the results were expressed as mean + SD and in range. Results for fatty acids composition were expressed as percent of total fatty acids while results for *trans* fatty acids in samples were expressed as g/100g of total fatty acids.

Results and Discussion

Fatty acids compositions of various biscuits are presented in Table 1. In general, for all analysed biscuits, saturated fatty acids (SFA) dominated the fatty acid composition having between 44% to 73% of total fatty acids being saturated except in B3 and B6. Among the SFA, palmitic acid (C16:0) presented the highest value ranging from 18.6% to 70%, followed by heptadecanoic acid (C17:0) that varied from 0.01% to 22.3%. High level of C17:0 in samples indicate the presence of animal fat especially from milkfat as this fatty acid is commonly occurs as trace component of fat and milkfat of ruminants (Hansen *et al*., 1957; Brevik *et al*., 2005).

The presence of high amounts of C16:0 indicates the use of palm oil and palm kernel oil as other sources of fat that tally with ingredients list shown in the packaging by food manufacturer for most samples of local and imported biscuits. Meanwhile, MUFA of biscuits remained relatively constant between 15% and 38% of total fatty acids except in B3. PUFA on the other hand, were relatively low at values ranging between 5 to 32% of total fatty acids except in B6. Caponio *et al*. (2006) pointed out that the low PUFA content indicates the use of concrete fats, often obtained by hydrogenation of refined vegetable oils. The content of PUFA was higher in A5 (32.5%) and B6 (55.43%) that delineate with indication of soybean oil use as declared on the label of product. Among the unsaturated fatty acids, oleic acid (C18:1) was the most predominant with values ranging from 11% to 35% followed by linoleic acid (C18:2) ranging from 3.1% to 52.3%.

As for total TFA content, the values ranging from 0.5 to 4.8% except in B3 that contained exceptionally high level of TFA at more than 50% of total fatty acids. Higher level of TFA contents were observed in local unpacked biscuits with values ranging from 0.65% to 51.6% followed by local packed biscuits (0.35% - 4.83%) and imported biscuits (0.45% - 2.9%). These results showed that locally produced biscuits contained higher level of TFA compared to imported biscuits. Low level of TFA in imported biscuits might suggest the proactive actions taken by international food manufacturers to reformulate their products to reduce TFA content as urged by various regulatory bodies internationally (IUFoST, 2004). In greater details, among the five types of TFA analyzed, *trans* 16:1 was the most abundant, with values ranging from 0.01% to 38% followed by *trans* 18:1 Δ11 (0.01% - 13.11%), *trans* 18:1 Δ9 (0.01% - 4.68%), *trans* 18:2 (0.23% - 2.77%) and small quantities of *trans* 18:1 Δ6. The variability of results showed that the amount of TFA varied among the analyzed biscuits because of the differences in fat ingredient as well as the extent of hydrogenated fat used that depends on process condition such as temperature, pressure, type and amount of catalysts that affected the fatty acids content of the biscuits (Martin *et al*., 2005; Karabulut *et al*., 2003). However, small amounts of conjugated linoleic acid (CLA) were detected in samples of this study that might be formed during hydrogenation of fats used in the biscuit products (Karabulut, 2007) as well as from milk ingredients used (Chin *et al*., 1992).

CLA can be found naturally in the fat of ruminants and dairy products in quantities averaging 0.5% to 0.7% of total fat, respectively (Mougios *et al*., 2001) in the form of two isomers (*cis*-9 *trans*-11 and *cis*-10 *trans*-12). These two isomers were detected in this study since some of the food producer claimed the used of butter, cheese and other dairies products as ingredient (Table 1). Small amounts of CLA were found in all samples ranging from 0.01% to 0.29% except in B4 and A6. These latter two samples contained high amount of CLA due to the presence of cheese in their ingredients. Among the two isomers of CLA, the most abundant was isomer *cis*-9 *trans*-11 found in all samples with values ranging from 0.01% to 1.49%. In general, CLA constituted between 0.2% to 34.3% of total TFA in local packed biscuits, 0.1% to 69.4% in local unpacked biscuits and 0.6% to 87.8% in imported biscuits. High amount of CLA in biscuits products would give some beneficial effects to human health since many studies have shown that CLA possessed different physiological effects such as reduction in body fat, anti diabetic effects, reduction in the development of atherosclerosis, enhanced bone mineralization, anti carcinogenic effects and anti

Table 1. Fatty acids composition of various biscuit products

Fatty acid	Local (Packed Biscuits) n = 4				Local (Unpacked Biscuits) n = 4				Imported Biscuits n = 4			
	A1	A2	B1	B2	A3	A4	B3	B4	A5	A6	B5	B6
C12:0	15.30	0.02	ND	0.02	19.18	2.74	0.40	4.64	0.03	4.66	1.48	0.01
C14:0	5.45	ND	1.78	0.01	6.95	0.02	0.01	0.35	0.01	13.20	0.04	0.01
C16:0	27.85	42.35	33.38	70.70	27.69	58.98	0.01	42.01	25.94	30.17	65.65	18.66
C17:0	14.22	12.61	22.26	0.15	10.20	11.41	10.92	0.29	9.72	11.68	0.25	0.01
C18:0	5.48	3.70	2.72	ND	4.34	0.01	0.01	0.23	8.73	9.27	0.06	0.02
C20:0	0.17	ND	0.01	0.01	0.16	0.01	0.02	0.24	ND	0.09	0.51	0.02
∑ SFA	**68.47**	**58.68**	**60.15**	**70.89**	**68.52**	**73.17**	**11.36**	**47.75**	**44.43**	**69.08**	**67.98**	**18.73**
C16:1	0.19	ND	ND	0.30	0.18	0.28	0.01	0.17	0.17	2.10	0.05	0.03
C16:1 t	ND	ND	ND	ND	ND	ND	38.09	1.58	ND	ND	0.03	0.01
C18:1	23.13	24.47	29.55	11.10	23.03	11.60	32.00	35.42	22.85	23.15	21.96	25.68
C18:1 6t	ND	0.04	ND	0.02	ND	0.02	0.13	0.23	ND	ND	0.04	0.01
C18:1 9t	0.02	4.68	0.01	0.01	0.02	0.01	0.01	0.56	ND	0.38	ND	0.06
C18:1 11t	0.36	0.11	0.01	ND	0.38	3.40	13.11	0.18	0.01	0.21	ND	0.05
∑ MUFA	**23.70**	**29.30**	**29.57**	**11.44**	**23.61**	**15.30**	**83.36**	**38.13**	**23.03**	**25.83**	**22.08**	**25.84**
C18:2	7.52	12.01	9.81	17.09	7.59	11.17	4.98	11.80	30.86	3.11	9.43	52.37
C18:2 t	0.25	ND	0.33	0.52	0.25	0.28	0.25	0.23	1.63	0.72	0.38	2.77
C18:3 n3	0.02	ND	0.01	ND	0.01	0.01	0.01	0.17	0.02	0.10	0.04	0.01
C18:2 9c 11t	0.02	ND	0.12	0.03	0.02	0.01	0.04	1.49	ND	1.11	0.04	0.01
C18:2 10c 12t	0.02	0.01	ND	0.04	ND	0.06	0.01	0.44	0.01	0.04	0.06	0.28
∑ PUFA	**7.83**	**12.02**	**10.27**	**17.67**	**7.87**	**11.53**	**5.28**	**14.12**	**32.54**	**5.09**	**9.95**	**55.43**
∑ TFA	**0.63**	**4.83**	**0.35**	**0.55**	**0.65**	**3.71**	**51.59**	**2.78**	**1.64**	**1.31**	**0.45**	**2.90**
∑ CLA	**0.04**	**0.01**	**0.12**	**0.07**	**0.02**	**0.07**	**0.05**	**1.93**	**0.01**	**1.15**	**0.10**	**0.29**

*Results are expressed as the percentage of total fatty acids. A representing sweet biscuits, B representing savoury biscuits

*TFA- Trans fatty acids, MUFA- Monounsaturated fatty acids, PUFA- Polyunsaturated fatty acids, CLA – Conjugated linoleic acid. ND- not detected

oxidative (Werner *et al*., 1992; Pariza *et al*., 2001; Seckin *et al*., 2005).

The levels of TFA, SFA, MUFA and PUFA of biscuits from various countries (New Zealand, Turkey, Italy, United States, Brazil and Canada) are presented in Table 2. The results showed that local biscuits from Malaysia contained relatively low TFA with values ranging from 0.00 – 0.52 g/100 g of total fatty acids and 0.12 – 0.68 g/100 g of total fatty acids for local packed and unpacked, respectively. A high proportion of *trans* fat content were found in US biscuits (sold in US in 2002) with values of 26 g/100 g of total fatty acids for cookies and 34 g/100 g of total fatty acids for crackers (Mozaffarian *et al*., 2006). Lower tendency was found in New Zealand biscuits with values ranging from 0.00 to 3.5 g/100 g total fatty acid (Saunders *et al*., 2008). Also, high levels of TFA were found in Brazil biscuits that showed 31.2 ± 2.64 and 28.2 ± 5.61 g/100 g of total fatty acids in samples labelled as A and E as reported by Martin *et al*. (2005). In biscuit samples from Canada, TFA content was in the range of 1.3 to 45.6 g/100 g of total fatty acids (Ellias and Innis, 2002).

In term of saturated fatty acid (SFA), higher levels were found in Malaysian biscuits as analysed in this study for more than 50 g/100 g fatty acids which was characterized by 27% - 70% C16:0 of total fatty acids except in B3. SFA in the studied biscuits was mainly from the wide use of palm fat in the country (O'Holohan, 1996). Similarly, Italian biscuits were also high in SFA with mean value of 50.6 ± 9.8 g/100 g of total fatty acids (Caponio *et al*., 2006) followed by Turkey cookies and crackers (48.77 ± 0.0 g/100 g of total fatty acids and 46.57 ± 0.78 g/100 g of total fatty acids, respectively) (Karabulut, 2007). The same trend was found in Canada biscuits with mean value of 43.6 ± 22.4 g/100 g of total fatty acids (Ellias and Innis, 2002). However, the SFA contents in Brazil biscuits were lower (16.9 – 39.2 g/100 g of total fatty acids) than other countries. Besides SFA, the content of monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) in biscuits from various countries were comparable with values obtained in the present study (range; 11.4%-83.4%

Table 2. Levels of total *trans* fatty acids, SFA, MUFA and PUFA of biscuits from various countries

Country/ Author	**TFA g/100g fatty acids**		**SFA g/100g fatty acids**		**MUFA g/100g fatty acids**		**PUFA g/100g fatty acids**	
	Mean ± SD	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
Malaysia, this study								
Local (Packed)	0.19 ± 0.24	0.00 – 0.52	64.54 ± 6.04	58.7 - 70.9	23.50 ± 8.5	11.4 - 29.6	11.95 ± 4.2	7.8 - 17.7
Local (Unpacked)	0.39 ± 0.30	0.12 – 0.68	50.20 ± 28.1	11.4 - 73.2	40.10 ± 30.3	15.3 - 83.4	9.70 ± 3.9	5.3 - 14.1
Imported	1.08 ± 1.47	0.03 – 3.09	50.10 ± 23.7	18.7 - 69	24.20 ± 1.9	22.1 - 25.8	25.8 ± 23.1	5.1- 55.4
New Zealand (Saunders *et al*.,2008), Biscuits & cakes	1.1 ± 1.2	ND – 3.5	NA	NA	NA	NA	NA	NA
Turkey (Karabulut, 2007),								
Cookie (filled)	3.54 ± 2.25	NA	48.77 ± 0.0	NA	36.60 ± 2.05	NA	21.54 ± 2.06	NA
Cracker	1.19 ± 0.37		46.57 ± 0.78		39.50 ± 0.75		13.93 ± 1.53	
Italy (Caponio *et al*., 2006)	0.85 + 0.27	0.01 – 8.94	50.6 ± 9.8	18.0 – 62.9	35.5 ± 6.7	25.0 – 56.9	13.9 ± 6.2	9.1 – 53.0
US (Mozaffarian *et al*., 2006)								
Cookies	26.0	NA	NA	NA	NA	NA	NA	NA
Crackers	34.0							
Brazil (Martin *et al*., 2005),								
Sample A	31.2 ± 2.64	NA	23.9 ± 1.57	NA	NA	NA	9.74 ± 1.59	NA
Sample B	13.63 ± 3.44		16.9 ± 0.77				32.2 ± 1.10	
Sample C	12.2 ± 10.3		39.2 ± 15.8				10.8 ± 3.44	
Sample D	14.0 ± 1.25		17.7 ± 3.44				33.6 ± 1.13	
Sample E	28.2 ± 5.61		22.8 ± 1.32				11.5 ± 2.74	
Canada (Ellias & Innis, 2002)	23.0 ± 13.4	1.3 – 45.6	43.6 ± 22.4	15.9 – 83.1	27.1 ± 10.6	7.3 – 83.1	7.3 ± 5.7	2.0 – 26.6

ND- Not detected, NA- Not available

and 5.3%-17.7% respectively for MUFA and PUFA). Descending order of unsaturated fatty acids content is Turkey biscuits, followed by Italy and Canada (Ellias and Innis, 2002; Caponio *et al*., 2006; Karabulut, 2007).

In general, the level of TFA in various food products including biscuits is declining since 2002 as can be observed in imported biscuits analysed in this study (0.52 to 0.74 g/100 g of total fatty acids). This might be due to actions by Food and Drug Administration's (FDA) that proposed to include *trans* fatty acid content of foods on the standard food label in 1999 and the ruling that nutrition labels for all conventional foods and supplements must indicate the content of TFA starting 1st January, 2006 (FDA, 2005). Moreover, Dietary Guideline Advisory Committee of America has made recommendations that the consumption of *trans* fatty acids must be kept below 1 percent of total energy intake (Dietary Guideline Advisory Committee, 2005) as key to healthy lifestyle. In spite of that, many countries all over the world have taken initiatives to reduce TFA content in their food products by amending new rules on food labelling and urged food manufacturer to follow the rules.

Conclusions

In conclusion, the results of the present study indicate that Malaysian biscuits contain considerably low proportion of *trans* fatty acids compared to reported values from other countries. Lower level of TFA in Malaysian biscuits was related to the wide use of palm fat as ingredient. TFA constituted less than 5% of total fatty acids except in one sample. The TFA content of samples enclosed of 0.1% to 69% CLA from milkfat. Findings of this study provides information and comparison on the content of TFA in various biscuits products in Malaysian market, which may be useful in food composition database and be used by nutritionist, food chemists or researchers.

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