

## Consequence of fatty acids profile including *trans* fat in chocolate and pastry samples

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### Abstract

Due to reported detrimental health effects of diets which are high in trans fatty acids (TFA) particularly on blood lipids, two main convenience products (chocolate and pastry) were investigated. In this regard, to determine fatty acids composition, with special emphasis on trans fat content from extracted neat fat of the products, gas chromatography coupled with mass spectroscopic technique was used. Total saturated and unsaturated fatty acid found were in the range of 63.89- 84.35 and 15.24-36.11 for chocolate samples, and similarly 40.22-53.46% and 46.56-59.77% for pastry samples. The high amount of TFA was observed in all chocolate and pastry samples varied from 4.56-8.49 and 3.92-10.17% respectively.

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## Introduction

Fats and oils of animal origin such as butter and lard are composed of saturated fatty acids (SFAs) is responsible for a number of cardiovascular diseases (Neaton and Wentworth, 1992). In order to reduce the saturated fat content of processed foods, the food industry in developed countries moves progressively from animal fat to vegetable fat sources (Tavella *et al.*, 2000).

Unsaturated fats are heart-healthy, but they have some undesirable properties, specifically in air, they can gradually become rancid. Fat oxidation is the main reason of deterioration of foods and can directly affect many quality characteristics (Maache-Rezzoug *et al.*, 1998). The shelf life of many type of food depends on several parameters including: storage temperature and humidity, availability of oxygen in the immediate environment (Nattress *et al.*, 2004). Main parameter's to control and monitor the quality of chocolate and pastry include the amount of fat and type of fatty acids especially *trans* fat contents because the quality of fats plays a very important role in food processing technology.

Chocolate contains a variety of different compounds such as saturated fat, polyphenols, sterols, diterpenes, triterpenes, aliphatic alcohols and methylxanthines (Knight, 2000). Because of its high saturated fat content, chocolate is often postulated

to have a hypercholesterolemic effect. Pastry is the name given to various kinds of baked goods e.g. small cakes, tarts and other sweet baked goods, made from ingredients such as flour, milk, butter, shortening, baking powder or eggs.

Usually vegetable oils that contain naturally occurring double bond are in *cis* configuration, prior to hydrogenation; however, some double bonds are in the *trans* configuration, so-called trans fatty acids (TFAs) (Fritsche and Steinhart, 1998). Little amount of TFAs are also formed during deodorization process under high temperature (Sherazi *et al.*, 2009). Elaidic acid (C18:1*t*9) is the major trans fatty acid found in the processed fats. The amount of TFA in the diet is of interest due to its adverse effects on human health, which include cardiovascular disease, abnormalities in infant development, coronary heart disease, diabetes and inflammation (Ascherio *et al.*, 1999).

These concerns pushed the Food and Agriculture Organization (FAO) and the World Health Organization to recommend that fats for human consumption should contain less than 4% of the total fat as *trans*.

In previous studies which are conducted for the determination of *trans* fat in various types of foods, produced/sold in Pakistan, the *trans* fat ranged in some foods were as follows: cooking and partially hydrogenated oils 0.5-1.6 and 9.1-26.5%, respectively (Sherazi *et al.*, 2009), margarines (soft

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and hard) and shortening 1.5–23.0 and 7.3–31.7%, respectively (Bhanger and Anwar, 2004), biscuits 9.4–34.9%, (Kandhro *et al.*, 2008) potato chips 0.4–22.2%, doughnut cakes 5.3–17.1%, meat-containing foods (chicken thighs) 1.9–21.3%, and fried fish 2.7–6.6% (Anwar *et al.*, 2006). In Potato chips TFA ranged 4.91–14.13% (Kandhro *et al.*, 2010). Cereal based food 2.5% to 16.3% (Mahesar *et al.*, 2010). No data has been found so far to know about fatty acid composition specially trans fat in Pakistani products such as chocolate and pastry. Having known about wide consumption of chocolates and pastry all over the country as well as fats and oil are their raw material, it was felt necessary to determine the saturated and trans fat in these products. It may lead to know about serving of fats and trans fat by consumers and a data base of fat and oil composition of products.

## Materials and Methods

### Samples

Six different chocolate and five puff pastry samples were purchased from local supermarket in Jamshoro, Pakistan, and samples were coded as Chocolate (CHOC-1 to CHOC-6), and Pastry (PAS-1 to PAS-5). The selection of the brands was based on highest vending amongst those available in the market.

### Sample preparation

Lipid extraction from the samples was carried out with hexane under the operating conditions specified in ICC Standard No. 136, and expressed as a percentage by mass of the product (ICC, 1982). Fat obtained from samples was transferred into 5 ml glass vials. The decanted samples were all frozen at -18°C until analysis.

### Determination of fatty acid composition

FAMEs were prepared using IUPAC standard method 2.301 for the determination of FAC of the chocolate and pastry samples (IUPAC, 1979).

### GC-MS conditions

The GC-MS analysis for FAMEs was performed on Agilent 6890 N gas chromatography instrument coupled with an Agilent MS-5975 inert XL mass selective detector and an Agilent autosampler 7683-B injector (Agilent Technologies, Little Fall, NY, USA). Highly polar, Rt-2560 biscyanopropyl siloxane capillary column (100m x 0.25mm i.d x 0.2µm film thickness) was used for the separation of fatty acid methyl esters. The initial temperature of 150°C was maintained for 2 min, raised to 230°C at the rate of 4°C/min, and kept at 230°C for 5 min. The split ratio

was 1:50; helium was used as a carrier gas with a flow rate of 0.8 ml/min. The injector and detector temperatures were 240 and 260°C, respectively. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV in the scan range of 50–550 m/z.

### Calculations and statistical analyses

The identification of methyl esters carried out by NIST and Willy libraries. Two samples of each brand were collected and each sample was analyzed thrice and reported as mean ± Standard Deviation.

## Results and Discussion

Applied chromatographic conditions allowed analyzing fatty acid compositions, especially trans fat composition of frequently consumed chocolate and pastry. The results of fatty acid composition of analyzed chocolate and pastry samples are shown in Tables 1 and 2 respectively.

All analyzed chocolate and pastry samples were found to contain the significant amount of saturated fatty acids (SFAs) such as, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0 and C20:0. Among SFAs C14:0, C16:0 and C18:0 were found in large quantities in all the analyzed chocolate and pastry samples. SFA contents ranged from 63.89 to 84.35% and from 40.22 to 53.46% in chocolate and pastry samples, respectively. Figure 1 show the representative chromatogram of different fatty acids of analysed oil extracted from pastry sample, chromatogram was in a good peak shape under the optimized chromatographic conditions.

Aro *et al.* (1997) has been reported the effect saturated fatty acids (SFAs) on human health, according the him SFAs with the chain length of (C12:0–C16:0) carbon atoms cause atherogenic effects, while stearic acid (C18:0) has no effect and suppose as neutral, whereas oleic (C18:1) and polyunsaturated fatty acids (PUFAs) produce a blood lipid lowering effect.

Table 1 and 2 represent the dominant fatty acid among the saturated group is palmitic acid (C16:0) and its range varied from 34.51 to 47.70 and 26.72 to 44.95% in chocolate and pastry samples respectively. The highest amount of palmitic acid e.g. 47.70% in CHOC-3 and 44.95% in PAS-5 was found. The amount of palmitic acid indicates the greater contribution of palm oil in the manufacturing of chocolate and pastry products, similarly stearic acid (C18:0) was present ranged from 17.87 to 30.41% and 6.12 to 11.52%. Whereas, caprylic acid (C8:0) was present ranging from 0.11 to 0.75% and 0.03 to 1.67%; capric acid (C10:0) was present at 0.08 to 1.89%, 0.02 to 1.14%;

Table 1. Saturated and unsaturated fatty acids composition (mean percentage FAMEs) of chocolate samples

Fatty Acids	Samples-(n=3)						
	Mean±STD	CHOC-1	CHOC-2	CHOC-3	CHOC-4	CHOC-5	CHOC-6
C8:0	0.19±0.01	0.33±0.01	0.21±0.01	0.75±0.01	0.41±0.01	0.11±0.005	
C10:0	1.81±0.05	1.67±0.05	0.08±0.001	1.08±0.05	1.59±0.05	1.89±0.06	
C12:0	0.77±0.03	0.00±0.0	0.64±0.02	0.34±0.01	0.27±0.01	0.00±0.00	
C14:0	7.03±0.25	8.69±0.25	3.94±0.15	7.73±0.25	11.05±0.45	4.18±0.15	
C15:0	0.29±0.01	0.00±0.01	0.16±0.01	0.00±0.00	0.34±0.01	0.00±0.00	
C16:0	34.51±0.55	44.60±1.25	47.70±1.25	43.14±1.25	40.71±1.25	44.86±1.25	
C16:1n9c	0.00±0.00	0.15±0.01	0.00±0.00	0.00±0.00	4.17±0.15	4.43±0.18	
C17:0	0.62±0.02	0.31±0.01	0.30±0.01	0.18±0.01	1.60±0.06	0.33±0.01	
C18:0	17.87±0.55	20.82±0.25	25.63±0.58	28.98±1.25	26.60±1.25	30.41±1.24	
C18:1n9trans	5.07±0.25	4.24±0.18	8.14±0.25	3.98±0.15	4.36±0.15	4.72±0.12	
C18:1n9c	19.17±0.36	10.69±0.15	6.86±0.25	5.93±0.25	5.89±0.12	2.26±0.05	
C18:2n9,12t-t	0.33±0.01	0.78±0.03	0.35±0.01	2.62±0.08	0.20±0.01	0.68±0.02	
C18:2n9,12c-c	1.72±0.04	3.11±0.15	1.91±0.08	3.41±0.07	0.92±0.02	0.00±0.00	
C18:2n8,11c-c	6.62±0.15	3.33±0.15	4.01±0.15	0.00±0.00	0.00±0.00	0.64±0.03	
C18:3n9,12,15c-c	2.90±0.08	0.00±0.00	0.00±0.00	0.72±0.02	0.48±0.02	0.23±0.01	
C20:0	0.80±0.02	0.39±0.01	0.00±0.00	0.38±0.01	0.00±0.00	2.57±0.12	
C20:1n11c	0.30±0.01	0.20±0.01	0.00±0.00	0.17±0.01	0.00±0.00	2.28±0.11	

Groups and ratio between the types of fatty acids from the composition of Chocolate samples

	SFA	TotalUFA	TotalMUFA	ci MUFA	TotalPUFA	cisPUFA	Total TFA	SFA/ UFA	cisMUFA+cisPUFA	cisPUFA/SFA	trans-FA/cis-FA	SFA+TFA	cisPUFA/(SFA+TFA)	cisMUFA+PUFA/SFA+TFA
	63.89	76.80	78.65	82.59	82.57	84.35								
	36.11	22.50	21.28	16.84	16.02	15.24								
	24.55	15.28	15.00	10.08	14.42	13.69								
	19.48	11.04	6.86	6.10	10.06	8.97								
	11.56	7.22	6.28	6.76	1.60	1.55								
	11.23	6.44	5.93	4.14	1.40	0.87								
	5.40	5.02	8.49	6.60	4.56	5.40								
	1.77	3.41	3.70	4.90	5.15	5.54								
	30.71	17.48	12.79	10.24	11.46	9.84								
	0.18	0.08	0.08	0.05	0.02	0.01								
	0.18	0.29	0.66	0.64	0.40	0.55								
	69.29	81.83	87.14	89.19	87.14	89.75								
	0.16	0.08	0.07	0.05	0.02	0.01								
	0.44	0.21	0.15	0.11	0.13	0.11								

Table 2. Saturated and unsaturated fatty acids composition (mean percentage FAMEs) of pastry samples

Fatty Acids	Samples-(n=3) Mean ± STD				
	PAS-1	PAS-2	PAS-3	PAS-4	PAS-5
C8:0	1.67±0.05	0.13±0.01	0.06±0.003	0.03±0.001	0.00±0.00
C10:0	1.14±0.05	0.36±0.01	0.09±0.003	0.14±0.01	0.02±0.001
C12:0	1.21±0.04	0.25±0.01	0.30±0.01	0.00±0.00	0.70±0.02
C14:0	2.63±0.12	1.37±0.05	3.38±0.12	3.56±0.15	0.37±0.01
C16:0	26.72±0.74	42.43±1.27	31.55±1.25	31.41±1.25	44.95±1.24
C18:0	6.12±0.25	6.89±0.15	11.52±0.54	10.36±0.23	7.15±0.25
C18:1n9trans	9.11±0.26	8.50±0.21	6.98±0.15	4.92±0.15	3.78±0.15
C18:1n9c	43.90±1.23	31.72±1.23	40.23±1.25	42.62±1.46	38.72±1.46
C18:2n9,12t-t	1.06±0.05	0.12±0.01	2.22±0.05	3.70±0.16	2.31±0.11
C18:2n9,12c-c	0.00±0.00	5.08±0.18	0.35±0.01	0.16±0.01	0.14±0.005
C18:2n8,11c-c	4.09±0.12	0.00±0.00	1.65±0.04	0.00±0.00	0.00±0.00
C18:3n9,12,15c-c	1.29±0.04	0.26±0.01	1.02±0.03	2.79±0.13	0.58±0.02
C20:0	0.73±0.02	1.32±0.03	0.16±0.01	0.39±0.01	0.26±0.01
C20:1n11c	0.33±0.01	1.57±0.05	0.64±0.02	0.08±0.004	1.03±0.05

Groups and ratio between the types of fatty acids from the composition of pastry samples

	SFA	TotalUFA	TotalMUFA	ci MUFA	TotalPUFA	cisPUFA	Total TFA	SFA/ UFA	cisMUFA+cisPUFA	cisPUFA/SFA	trans-FA/cis-FA	SFA+TFA	cisPUFA/(SFA+TFA)	cisMUFA+PUFA/SFA+TFA
	40.22	52.76	47.07	45.90	53.46									
	59.77	47.24	53.08	54.27	46.56									
	53.33	41.79	47.85	47.62	43.53									
	44.22	33.29	40.87	42.70	39.75									
	6.44	5.46	5.23	6.66	3.03									
	5.38	5.34	4.88	6.49	2.89									
	10.17	8.61	7.33	5.08	3.92									
	3.96	6.12	6.42	9.03	13.62									
	49.61	38.63	45.75	49.19	42.64									
	0.13	0.10	0.10	0.14	0.05									
	0.20	0.22	0.16	0.10	0.09									
	50.39	61.37	54.40	50.98	57.38									
	0.11	0.09	0.09	0.13	0.05									
	0.98	0.63	0.84	0.96	0.74									

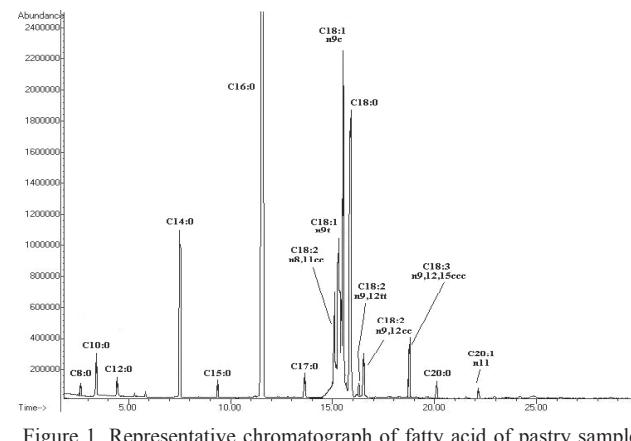


Figure 1. Representative chromatograph of fatty acid of pastry sample analysed by GC-MS

not present in the pastry samples.

Oleic acid (C18:1 *cis*-9) was the major fatty acid present amongst the unsaturated fatty acids. Oleic acid is considered to be responsible for lowering the LDL (bad) cholesterol levels (Kandhro *et al.*, 2008). Unsaturated fatty acids (UFA) ranged from 2.26 to 19.17% and 31.72 to 43.90% in chocolate and pastry samples respectively. The other members of monounsaturated fatty acids (MUFA), C16:1 *cis*-9 and C20:1 *cis*-11 was also determined in chocolate samples ranged from 0.15-4.43% and 0.17- 2.28%, respectively. While the pastry samples contained only one member of MUFA, C20:1 n11 ranged from 0.08 to 1.57%.

Polyunsaturated fatty acids (PUFAs) has major importance for biological and nutritional value; C18:2 9,12 t-t; C18:2 *cis*-9,12; C18:2 *cis*-8,11 and C18:3 *cis*-9,12,15, are the members of PUFA, ranged from 0.20-2.62; 0.92-3.41; 0.64-6.62; 0.23-2.90% determined in the chocolate samples respectively, similarly, 0.12-1.06; 2.22-5.08; 1.65-4.09; 0.26-2.79%, in pastry samples. PUFAs have beneficial effects on both normal health and chronic diseases, such as regulation of lipid levels (Mori *et al.*, 2000; Kris-Etherton *et al.*, 2002).

The major trans fat observed in all analyzed samples was elaidic acid (C18:1 *trans*-9) ranged from 3.98 to 8.14% and 3.78 to 9.11% with mean values 5.08% and 6.66% in chocolate and pastry samples respectively. Sample (CHOC-3) contained highest amount (8.14%) of elaidic acid, while among the pastry samples PAS-1 contained highest amount (9.11%). In comparison with samples of Turkish chocolates ranged at 1.85-3.68% (Karabulut, 2007), and New Zealand chocolate ranged at 0-3.1% (Saunders *et al.*, 2008). The analyzed chocolate samples contains high amount of trans fat than those of reported values, however in comparison with Austrian pastry which were ranged at flaky pastry 9.37-9.91%, Puff pastries

Table 3. Total fat content (approx. 5 g of sample), total gram/serving of fat and *trans* fat of chocolate and pastry samples

Chocolate Samples	Total fat g/100g Mean $\pm$ STD <sup>1</sup>	total fat g/serving <sup>2</sup>	Total <i>trans</i> (%)	<i>trans</i> fat g/serving <sup>3</sup>	total <i>trans</i> g/100g <sup>4</sup>
CHOC-1	15.48 $\pm$ 0.45	0.80	5.4	0.04	0.84
CHOC-2	19.45 $\pm$ 0.51	1.01	5.02	0.05	0.98
CHOC-3	25.14 $\pm$ 1.25	1.31	8.49	0.11	2.13
CHOC-4	23.54 $\pm$ 0.79	1.21	6.6	0.08	1.55
CHOC-5	29.52 $\pm$ 1.25	1.51	4.56	0.07	1.35
CHOC-6	21.42 $\pm$ 0.85	1.10	5.4	0.06	1.16
Mean Values	5.91	0.07	5.16	0.16	1.33
<b>Pastry Samples</b>					
PAS-1	36.52 $\pm$ 1.25	1.88	10.17	0.19	3.714
PAS-2	30.15 $\pm$ 1.24	1.54	8.61	0.13	2.596
PAS-3	28.45 $\pm$ 1.05	1.51	7.33	0.11	2.085
PAS-4	38.75 $\pm$ 1.35	1.99	5.08	0.10	1.969
PAS-5	29.48 $\pm$ 0.95	1.54	3.92	0.06	1.156
Mean Values	7.02	0.12	5.19	0.19	2.304

<sup>1</sup> extracted fat  $\times$ 100/weight of sample

<sup>2</sup> % of fat/100 $\times$ weight of sample

<sup>3</sup> % of trans/100 $\times$  fat g/serving

<sup>4</sup> % of trans/100 $\times$  fat g/100g

0.10 to 6.57% (Wagner *et al.*, 2008).

Many countries have implemented rules to keep controlled trans fat. Danish Government has prohibited the sale of food products containing >2% industrially produced TFA (Stender and Dyerberg, 2003). The American Governments have made rule that TFA levels should be indicated on food labels since 2003 (FDA, 2003). According to the nutritional recommendation the daily TFA intake should not exceed 2.5–3 g/day (<1% of total energy) (Wagner *et al.*, 2008).

Groups and ratio between the types of fatty acids from the composition of chocolate and pastry samples were shown in Table 1 and 2, respectively. The SFA/UFA, show the relation between two major fatty acid groups of the extracted fat. Its value varies from 1.77 to 5.54 (Chocolate) and 3.96 to 13.62 (Pastry). No one sample had a ratio less than one, which clearly indicates a high proportion of SFAs. The prevalence of unsaturated over SFAs (smaller ratio) is considered to be positive from the nutritional point of view.

Mean value indices of *cis*-MUFA+*cis*-PUFA ranged at (9.84 to 30.71 and 38.63 to 49.61) and *cis*-PUFA/SFA ranged at (0.01 to 0.18 and 0.05 to 0.14) for chocolate and pastry samples respectively. The ratio of *trans*-FA/*cis*-FA show significant degree of conversion of *cis*-form to *trans*-form, and also higher value of ratio indicates greater mixing of hydrogenated oils and presence of greater amount of *trans* (Alonso *et al.*, 2000). It appears that the ratio varies from 0.18 to 0.66 for chocolate samples. Among the pastry samples ratio varies from 0.09 to 0.22.

The saturated+TFA fraction ranged from 69.29 to 89.75% and reached a very high mean value of 84.06% in chocolates. While in pastry samples it ranged from 50.39 to 61.37% with mean value of 54.91%.

Mean value of indices of *cis*-PUFA/(SFA+TFA) and (*cis*-MUFA+*cis*-PUFA)/(SFA+TFA) commonly

used to express the nutritive value of edible oils and fats (Alonso *et al.*, 2000). The values of *cis*-PUFA/(SFA+TFA) were 0.01 and 0.16 in CHOC-6 and CHOC-1, while in pastry were at 0.05 and 0.13 in PAS-5 and PAS-4, respectively.

The level of total fat content, total fat g/serving and *trans* fat g/serving present in oil samples obtained from the chocolate and pastry samples were given in Table 3. Total fat contents of the chocolate samples ranged from 15.48–29.52% with a mean of 22.43%. While in pastry samples ranged from 28.45–38.75% with a mean of 32.67%. According to reported value of fat contents (Wagner *et al.*, 2008), of different types of pastries, like puff pastry or flaky pastry contained fat from 26.8 to 38.87% (puff pastry) and from 27.89 to 30.37% (flaky pastry). While total fat g/serving and total *trans* fats g/serving was ranged between 0.80–1.51 and 0.04–0.11 with a mean value of 1.16 and 0.07g, in chocolate and pastry samples respectively. Whereas among the pastry samples total fat g/serving and total *trans* fats g/serving ranged from 1.51 to 1.99 and 0.06 to 0.19, with mean value of 1.69 and 0.12 g respectively. The results indicate that 5.22 g of chocolate (CHOC-3) contain 1.31g of total fat and 0.11g/serving of *trans* fat (Table 3).

## Conclusion

In each of the observed categories, products with high levels of saturated and *trans* fat were found. The higher amount of saturated and TFA demonstrated the quality of samples. Further, the amount of *trans* fat was not mentioned on the label of any local manufactured chocolate and pastry as there are no regulations of saturated and TFA in the Pakistan. In order to reduce the intake of saturated and *trans* fat in foods, the general healthy eating advice provided by nutritionists should be followed.

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