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International

Journal of Health & Life Sciences

[ISSN: 2383-4390] [eISSN: 2383-4382]

Free of Charge

Pasdar Y. et al. Inter J Health Life Sci. 2016, volume 2 (number 1): page 32-41.
Classification: Nutrition and Health

You can cite this article as follows:

Pasdar Y, Bahrami G, Alghasi S, Darbandi M, Hemati Azandaryani A, Bahrami S, Rahemi P, Mirtaheri, E. Trans Fatty Acids Contents among Selected Foods in Western Iran. *Inter J Health Life Sci.* 2016, 2 (1): 32-41.

International
Journal of Health& Life Sciences
2016; 2 (1): 32-41

Trans Fatty Acids Contents among Selected Foods in Western Iran

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ARTICLE INFO
Article Type:
Original Article

Article History:
Received: 2016-06-23
Accepted: 2016-06-27
ePublished: 2016-07-15

Keywords:
Trans fatty acids
TFA
Dairy products
Folch method
Snacks
GC

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ABSTRACT

Effects of dietary fatty acids on health status depend on types and amounts of consumed fatty acids. Trans fatty acid intake is directly associated with increased risk of coronary heart disease. This study was aimed to determine the amount of fatty acids in some consumed snacks and dairy products in Kermanshah, I. R. Iran. 19 different types of foods including cakes, sweets, and dairy products were randomly purchased from all five regions of Kermanshah city. The fatty acids were extracted using the Folch method and then were analyzed using gas chromatography. The results showed that snacks, coffee mate, biscuits and cakes contained the highest levels of trans fatty acids, ranging from 6.95 % to 13.94 %. Among dairy products, trans fatty acid content of ice cream samples was remarkable. The highest levels of saturated fatty acids were observed in coffee mate, chocolate, and subsidized milk. The lowest short-chain fatty acid contents were found in ice cream and yogurt respectively. In conclusion, the results of the present study revealed that dietary trans fatty acid levels are relatively high in foods, which could be due to the hydrogenated oils used in food preparation.

Introduction

The prevalence of non-communicable chronic diseases has a direct association with lifestyle, including diets and their fat content [1]. The effect of dietary fats on health status depends on the type of fatty acids contained in foods [2]. Generally, fatty acids in the diet are divided into two main groups consisting of unsaturated and saturated fatty acids. In unsaturated fatty acids, the dual bands of carbon atoms are in *cis* and *trans* forms. Studies have shown that a diet high in saturated fatty acid (SFA) significantly reduces low density lipoprotein receptors, while a diet high in polyunsaturated fatty acid (PUFA) increases the number of these receptors. Replacing SFA with monounsaturated fatty acid (mainly oleic acid) or elimination of SFA may cause a similar decrease in plasma cholesterol, while replacing SFAs with PUFAs could cause a greater reduction in plasma cholesterol levels [3]. Further research has shown that although both saturated and *trans* fatty acids (TFAs) increase low-density lipoprotein cholesterol, TFAs also decrease cholesterol high-density lipoprotein and increase the triglyceride more than other fatty acids [4]. Industrial TFAs are directly proportional to the risk of coronary heart disease [5]. A large body of evidence suggests that the association between *trans*-fat and coronary artery disease is even more than that observed with saturated fats and carbohydrates [6]. TFAs are produced in industrial hydrogenation of edible

oils, microbial changes in unsaturated fatty acids in the body of cud-chewing animals, heating, frying in a temperature of over 180°C and deodorant of edible oils, among which industrial hydrogenation is the most important [7-8]. Through industrial hydrogenation, elaidic acid (trans isomers of oleic acid) is produced, which is a major TFA in foods [9-10]. One of the main sources of trans fats are the hydrogenated oils and fats such as solid and semi-solid oils, margarines and vegetable shortenings, cookies, crackers, snacks, fried foods, sweets, fried potato, soup powder and fast foods [11-12]. Over the past few decades, reduction of fat consumption has been highly recommended to reduce the risk of coronary artery disease [13, 14]. In addition to the listed foods, dairy products also can form 20% to 25% of the total fat intake of individuals [15]. Milk fat in a standard sample of milk normally contains 65% SFAs (mainly palmitic acid, stearic acid, and miristic acid) and 35% unsaturated fatty acids (mainly oleic acid) [16]. The composition of milk fat depends on factors such as stage of lactation, age, milking frequency, season, temperature, feed, and breed [17]. In Iran, the Institute of Standard and Industrial Research has established the Standard No. 1254 for the profile of fatty acids in the milk that can serve as a basis for comparison and the standards for these products [18].

Regarding the deleterious effects of TFAs, the U.S. Food and Drug Administration (FDA) suggests that the intake of energy through TFAs should be reduced to less than 1% of energy intake. In 2003, FDA issued a rule about TFA labeling on food packages which is actually now used in some developed countries [19]. This issue is not carried out in some countries, including Iran, which could be due to lack of sufficient information about the amount of TFAs in foods. Thus, the need for a comprehensive study in this area is evident. The purpose of this study was to determine the type and amount of fatty acids, particularly TFAs, in the dairy products and snacks.

Materials and Methods

Materials

In this study, 19 different types of foods including 13 of the most frequently consumed snacks (biscuits, rice bread, date bread, chocolate, snack, cake, chips, shortcake, Baklava, Zoolbia, Bamieh, Donuts, coffee mate) and six types of dairy products (butter, cream, cheese, sterilized milk, yogurt, subsidized milk and margarine) were selected. Three of eight different brands of butter, cream, cheese, sterilized milk, 16 brands of yogurt, 10 brands of biscuits, rice, date bread, seven brands of subsidized milk, cakes, and chips, five brands of shortcake, Baklava, Zoolbia, Bamieh, three brands of margarine and Donuts and two different brands of Coffeemate with different production dates were randomly selected and purchased from different areas of the city in at least weight of 150 g. The packaged samples were safe and without damage. The subsidized milk and yogurt could be eaten for at least two days until the expiration date; this was one week for sterilized milk, butter, cheese, ice cream and margarine. All reagents used were of analytical or HPLC grade. Double-distilled water was prepared in our laboratory.

Extraction of fatty acids

In this study, the Folch method was used for lipid extraction from samples due to the higher efficiency of this method. Then the derivation was conducted using the AOCS method by using 0.5 M methanol NaOH and 14% (W/V) BF3. After salting out performed by a saturated solution of sodium chloride, the solution was dried by nitrogen gas in 40 ºC Bain-Marie. Then 100 hexane µl added and 1 µl injected into the calibrated gas chromatography (GC) [20]. The fat content of samples were transferred to the laboratory and extracted in the same day and kept in the freezer to derive analyze by the gas chromatograph.

GC characterization

Identification and determination of fatty acids in the extracted oils was performed with the Varian GC system model CP-3800 equipped with an ion-flame detector, and CP-Sil 88 capillary column with 100 m length and 0.25 mm inside diameter. The split of the device was set to 1 to100 and the temperature of the injection environments and

the detector was set respectively to 270 and 300 ºC. The column temperature was programmed to allow sufficient time for the extraction of all fatty acids from the column. Nitrogen gas with 99.8% purity and a flow speed of 1 ml/min was used as the carrier gas. To determine the fatty acid profile, the percentage obtained for each of 19 different food brands relative to the total fatty acids present in the sample was calculated.

Statistical analysis

All obtained data have been reported as average ± standard deviations. Fatty acid compositions of different snacks were compared using analysis of variance. Statistical Package for Social Sciences (SPSS ver. 16) was used for all analyses and t-test was used to compare samples. *P* Values less than 0.05 were considered statistically significant.

Results

Elaedic Acid ($C_{18:1t}$) was found in most snacks (Fig. 1). Coffee mate showed the highest amount of $C_{18:1t}$, which was about 14% of the total fatty acids in the product. After coffee mate, biscuits contained the highest level of TFAs ($p < 0.05$). In addition to $C_{18:1t}$, this product also contains $C_{22:1t}$. The tested snacks were classified into three main categories based on the amount of the TFAs. Zoolbia, bamieh, snack, potato chips, chocolate, rice bread, date bread, and Baklava were in the first category that contained less than 3% of TFAs ($p < 0.41$). Donuts and shortcake were in the second category with range of 3 – 4% ($p < 0.05$). Cakes, biscuits, and coffee mate, due to the high rate of TFA (6 – 14%), were in the third group ($p < 0.40$).

Just like the TFAs, SFA content of biscuit was also higher than other products (Table 1). Stearic acid (C_{18}) and palmitic acid (C16) were the most SFAs in samples. Table 2 shows the amount of unsaturated fatty acids. The UK department of health (1994) has suggested that the ratio of PUFA/SFA should be at least 0.45 [21]. This issue was true just in one of the biscuit samples (1.87), and in other cases the ratio ranged from 0.16 to 0.37. Due to the high level of SFAs, the ratio of PUFA/SFA in coffee mates was low, and the average was 0.4. This ratio in all samples of rice bread, date bread, and shortcake was less than 0.45, and in one of the chocolates samples was 0.55. However, in other samples the ratio was 0.01 to 0.10. The PUFA/SFA in three tested snack samples was higher than 0.45, as well as in all cakes samples except one. According to the data, except one case, in all baklava samples, the ratio was higher than 0.45. Also, Zoolbia, Bamieh, and Donuts had PUFA/SFA ratio higher than 0.45.

Average percentages of total saturated and unsaturated fatty acids in studied dairy samples have been reported in Table 3 and 4. The highest percentage of SFA and unsaturated fatty acid in all kinds of dairy products belonged to palmitic acid and oleic acid, respectively. Sum of TFAs in margarine samples was 17.0 ± 2.6. There were 1.9 ± 2.2 of TFAs in ice-cream sample. The margarine, ice cream and cheese have short-chain fatty acids among other studied products. In all tested milk samples, the ratio of PUFA/SFA was remarkably lower than other products due to the low percentage of oleic and high percentage of palmitic acid in these products.

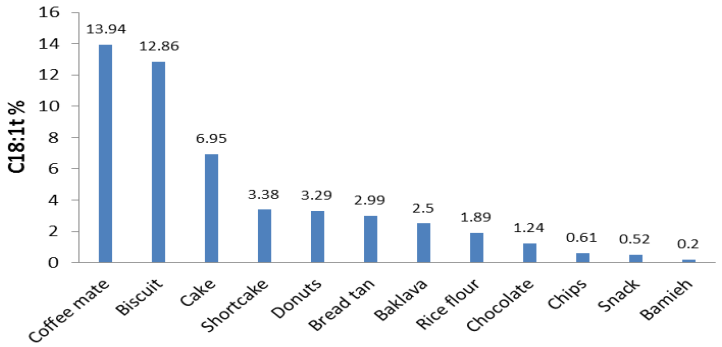

Figure 1. Percentages of TFAs were present in snacks (this value for zoolbia was not detected)

Table 1. Percentages of Saturated fatty acids were present in snacks

Sample	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
Cake	0.1±0.2	0.8±0.4	21.9±5	2.7±2.27	0.03±0.09	ND
snack	0.2±0.2	1.1±0.4	36.2±8.8	4.6±1.1	0.04±0.09	0.03±0
Chocolate	8.5±12.9	7.3±9.6	25.4±4.2	24.8±11.6	0.3±0.4	ND
Bread tan	0.05±0.07	0.7±0.2	38.3±2.9	6.9±2.8	ND	ND
Rice flour	0.3±0.5	0.9±0.3	39.7±3.3	6.8±1.4	ND	ND
Biscuit	0.05±0.07	0.6±0.1	29±8.9	8.5±2.7	0.03±0.09	ND
Coffee mate	18.7±3.2	14.7±0.8	15.4±3.8	24.6±7.8	0.1±0.2	ND
Donuts	0.05±0.04	0.6±0.2	25.9±8.01	5.03±1.1	ND	ND
Bamieh	ND	1.1±1.04	17.3±7.3	4.3±1.1	ND	ND
Zoolbia	ND	0.7±0.1	16.1±8.6	4.4±0.3	0.01±0.03	0.04±0.1
Baklava	ND	0.6±0.1	23.4±8.8	5.7±3.4	ND	ND
Shortcake	0.07±0.07	1.1±0.7	40.0±2.9	7.2±2.1	ND	ND
Chips	0.08±0.06	0.8±0.1	37.8±3.5	4.2±0.3	0.04±0.1	ND

Table 2. Percentage of polyunsaturated fatty acids in the studied snacks

Sample	$C_{16:1}$	$C_{18:1c}$	$C_{18:2}$	$C_{20:1}$	$C_{18:3}$	$C_{20:3}$
Cake	0.2±0.2	30.2±4.6	28.2±10.3	2.2±1.8	0.7±1.1	0.08±0.1
Snack	0.07±0.07	38.7±6.5	16.4±11.9	0.08±0.1	0.9±1.7	ND
Chocolate	0.08±0.1	25.7±10.2	5.4±5.8	ND	0.2±0.6	ND
Bread tan	ND	39.4±1.7	10.6±2.9	0.03±0	0.1±0.2	ND
Rice flour	0.4±1.3	37.9±1.7	10.8±2.2	0.09±0	0.1±0.2	ND
Biscuit	0.04±0.07	33.2±6.1	13.09±9.05	0.3±0.8	0.4±0.4	ND
Coffee mate	ND	8.06±7.8	2.5±0.8	ND	0.09±0.1	0.98±1.39
Donuts	0.26±0	33.8±2	27.8±6.8	1.6±2.1	0.6±0.5	0.07±0
Bamieh	ND	28.4±7.2	44.1±12.3	2.6±2.5	2.2±2.0	ND
Zoolbia	ND	26.7±7.6	46.4±14.3	3.9±2.8	ND	ND
Baklava	ND	29.6±5.2	34.1±15.3	0.02±0	2.6±1.8	ND
Shortcake	ND	36.2±2.2	10.8±2.3	ND	0.1±0.1	ND
Chips	0.02±0.04	41.7±1.0	13.5±4.4	0.4±0.5	0.1±0.1	ND

Table 3. Percentage of saturated fatty acids in the studied dairy products (mean ± SD)

	C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	total
subsidized milk	2.2±0.0	2.6±1.2	1.4±0.5	2.7±1.1	3.2±1.1	12.5±2.8	43.9±4.9	7.2±3.1	0.0	75.9±5.9
cheese	0.9±0.6	1.7±0.5	1.0±0.2	2.6±0.3	3.2±0.3	11.3±1.2	40.5±3.3	8.6±1.7	0.0	69.9±4.2
yogurt	1.6±0.6	2.0±0.5	1.3±0.2	2.5±0.4	3.0±0.5	11.9±2.0	37.9±2.1	7.5±1.8	0.0	67.9±4.1
Sterilized milk	1.8±0.3	2.2±0.2	1.3±0.1	2.5±0.5	2.9±0.6	12.0±0.8	39.6±1.98	7.6±1.5	0.0	70.1±11.0
ice cream	0.8±0.8	0.8±0.5	1.3±0.9	2.1±0.5	13.4±16.5	8.2±5.9	37.6±12.1	6.0±1.9	0.0	70.8±11.0
butter	2.3±0.2	2.6±0.3	1.6±0.3	3.7±0.5	5.5±0.1	14.6±0.1	37.3±0.3	8.0±0.1	0.0	75.8±4.6
margarine	0.00	0.00	0.00	0.00	0.09±0.0	0.4±0.2	31.4±1.1	5.6±2.3	0.0	17.0±2.6

Discussion

Based on the findings of this study, biscuits contained high amounts of elaedic acid (12.8%) which was higher than biscuits in New Zealand [22], Switzerland [7], and Spain [23]. Biscuits containing high value of $C_{22:1t}$ are commonly consumed in diet. TFAs in biscuit samples were generally higher than the samples reported by studies conducted in Argentina [24], Greece and America; and lower than studied samples in Brazil [21] and Canada [25]. Regarding SFA, examined biscuits were better than results from New Zealand and Spain, but the Brazilian biscuit has been shown the lowest contents of SFA. Studies on biscuits in Canada and Spain showed higher amounts of

Table 4. Percentage of unsaturated fatty acids in the studied dairy products (mean ± SD)

	$C_{18:1t}$	$C_{18:1c}$	Total unsaturated fatty acids	Ratio of saturated to unsaturated fatty acids	Other fatty acids
subsidized milk	0.4±0.4	13.6±6.4	13.9±6.6	0.2±0.1	10.1±5.1
cheese	0.4±0.3	26.3±3.8	27.6±4.3	0.4±0.09	2.4±1.1
yogurt	0.8±0.9	22.3±3.4	23.3±4.5	0.3±0.0	8.7±3.3
Sterilized milk	0.5±0.5	20.7±2.0	21.2±2.1	0.3±0.03	8.6±1.9
ice cream	1.9±2.2	24.1±9.4	26.6±10.9	0.4±0.1	2.9±1.3
butter	0.9±0.4	19.0±3.5	20.0±3.7	0.2±0.0	4.1±0.3
margarine	17.0±2.6	30.2±1.3	60.9±4.3	54.5±5.5	1.6±1.0

PUFAs and monounsaturated fatty acids than in our study. These differences may result from different oils used in producing biscuits. In this study, in consistent with a similar study in Brazil, the most content of SFAs was palmitic and stearic acid. After biscuits, cakes have the highest level of TFA; Cakes are given as early complementary feedings. Therefore, raw cake materials must be carefully selected for cake production. According to the results, the TFAs in analyzed cake samples in this study were more than in Canada [25] and Australia [7], and less than Spanish samples [23]. Elaedic acid in Iranian cakes was more than Swiss [7] and New Zealand [22]. It could be risky for public health, because biscuits and cakes containing high level of TFA are widely used among many population groups, especially children and adolescents, the fact reflecting the importance of this deleterious type of fatty acid. Similarly, a new study indicated that fat type has more important effect on cardiovascular disease in comparison with the amount of fat; This has been shown in several epidemiological studies as well [26].

In addition to TFA, coffee mates also contain significant amounts of SFA. The amount of the TFA and MUFA in Iranian samples was less than the Castaricaian samples [27], while the amount of the SFA in coffee mates used in Iran were even two fouled higher than the Castaricaian samples. High concentration of TFA and SFA in these coffee mates could be due to the use of hydrogenated oils such as coconut oil in their production. In fact, 100 g of coconut oil contains 86.5 g of SFAs. [28].

In margarine samples, the average value of *trans* and SFAs was reported 1.7 ± 2.6 and 17.0 ± 1.6 respectively. TFAs measured in this study were dramatically higher than that observed by Leth [29] Precht [30] and Demirbas [31]. The average of TFAs in a study by Mirzaei et al (1382) on 6 types of margarine [32] was higher than our study. The difference in results may stem from the different sampling methods and fewer sample brands in current study. Moreover, modification of margarine formulation might be another explanation for this lack of consistency between various studies. Generally, it is recommended that the dietary intake of margarine should be limited as much as possible, since they are a rich source of TFAs [17].

The average amount of lauric acid in ice cream was more than the standard range, while its linoleic acid was less than the standard. Ice cream, in comparison with other dairy products, has lower short-chain fatty acids (5%) that are unique to milk and dairy products. The TFA content of ice cream was more than other dairy products which might be as a result of using other fat sources other than milk, such as industrial hydrogenated oils in the process of making ice cream. Taking into account that ice cream is one of the most widely consumed dairy products for all ages, especially children, it's necessary to put full attention to reduce TFAs in ice creams.

The average palmitic acid (44%) in pasteurized subsidized milk was higher, and oleic acid (60/13%) and linoleic acid (zero percent) were less than the standard range [18]. Linoleic acid is the Omega-9 unsaturated fatty acid and may play a role in cardiovascular disease prevention [27]. The average percentages of total SFAs were more than Blasi et al. study in Italy [15]. The amount of palmitic acid in subsidized milk was much higher

and the amount of oleic acid was much lower than the values of Blasi. This difference may be due to animal nutrition; the use of fresh forage and diets containing unsaturated fatty acids could cause an increase in unsaturated fatty acid content of milk. The amount of milk TFA in a study by Bahrami et al. was reported 5.4% [33], which was higher than the subsidized and non-subsidized milk. This could be considered as a result of sampling in spring and summer, or more fresh forage consumption by ruminants in traditional ranching. The average amounts of lauric acid and meristic acid in butter were higher and linoleic acid was less than the standard range. The average amount of total short-chain fatty acids of butyric to capric acid in yogurt was 7.5, which was less than intraditional yogurt and milk [33].

The amount of linoleic and butyric fatty acids in cheese samples were lower than the standard range. In another study by Kinik et al. performed on 29 different types of cheese [34], the average of SFAs was reported higher than this study. The average of TFAs in Kinik samples was 0.32 % and in our samples was 0.4±0.3 %. The difference in the TFA content of foods could be partly due to the difference in fatty acid extraction methods or solvents such as benzene, cyclohexane and petrolatum used for the extraction. TFAs, mainly produced in the manufacturing process of certain foods, can lead to the production of these unsafe fatty acids in foods. Oil hydrogenation was carried out in high temperatures in food industry. Oil hydrogenation processes are not the same throughout the world; the amounts of TFAs in hydrogenated oils vary depending on different conditions such as temperature, pressure, and catalyst amount [22, 35].

Conclusion

In the present study, TFA contents of some examined food products, especially cakes, biscuits, coffee mate, and ice cream were considerably high, probably due to the use of hydrogenated and semi-hydrogenated oils in the preparation of these products. The lower price compared with liquid oils, is likely the reason for using this type of oil. It is suggested that the presence of labels displaying the exact amount of all fatty acids, especially TFAs be mandatory on food products. In addition, people should be educated to be aware of labels while they are purchasing foods. Due to the lower levels of unsaturated fatty acids in milk in comparison with the standard range and traditional products, this study put an emphasis on need for reforming the production process of such products, as well as controlling the maintenance of animals, especially reforming the diet of dairy cattle. In developed countries, there is a standard of less than one percentage of energy for TFAs. Therefore, it is highly recommended that some measurements be considered to analyze food products before marketing, to reduce or eliminate fatty acids in the Iranian diet.

Conflict of interest statement

There was no potential conflict of interest relevant to this article.

Authors' Contributions

Yahya Pasdar and Gholamreza Bahrami designed the study and managed the analyses of the fatty acids, Mitra Darbandi performed the statistical analysis and helped fist draft. Soheila Bahrami and Puneh Rahemi done laboratory analysis and managed the literature searches, Shokofeh Alghasi and Elham Mirtaheri wrote the first draft of the manuscript and Abbas Hemati Azandaryani managed the manuscript preparation and submition. All authors read and approved the final manuscript.

Acknowledgement

This work was performed in partial fulfillment of the requirements for Pharm. Ph. D in Faculty of Pharmacy, (Approval No. 91455) Kermanshah University of Medical Sciences, Kermanshah, Iran.

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