

Short Communication

Evaluation of fatty acid content of some Iranian fast foods with emphasis on trans fatty acids

Seddigheh Asgary¹, Bahar Nazari², Nizal Sarrafzadegan², Sahar Parkhideh²,
Salbali Saberi², Ahmad Esmailzadeh³, Leila Azadbakht³

¹*Isfahan Cardiovascular Research Center, WHO Collaborating Center, Applied physiology research center, Isfahan University of Medical Sciences, Isfahan, Iran*

²*Isfahan Cardiovascular Research Center, WHO Collaborating Center, Isfahan University of Medical Sciences, Isfahan, Iran*

³*Department of Nutrition, School of Public Health, Isfahan University of Medical Sciences, Isfahan, Iran*

Although the disadvantages of trans fatty acids (TFAs) are widely mentioned, limited data are available on the TFAs contents of Iranian foods, including fast foods. The aim of this study was to quantify the amounts of common fatty acids in several fast foods in Iran, with specific focus on TFAs. The most commonly consumed fast foods in Iran: sausage, calbas, hamburgers and pizzas, were randomly selected seven times from products available in supermarkets and restaurants. Each time a 10 g sample was drawn and prepared for fatty acid analysis. Total and individual fatty acids were quantified according to standard methods by gas chromatography with 60 meter capillary column and flame ionization detector. The most common saturated fatty acids in Iranian fast foods is stearic acid (C18:0) which ranged from 14.0% to 20.9%. Saturated fatty acid content in calbas was significantly higher than that found in other groups. Trans fatty acids constitute almost 23.6% to 30.6% of total fatty acids of these products. The most common TFA in these fast foods was elaidic acid (C18:1 9t). Total cis unsaturated fatty acid content of tested fast foods varied from 25.3 % (in sausage) to 46.8% (in calbas) with oleic acid (C18:1 9c) followed by linoleic acid (C18:2) being the most common fatty acids in these products. This study showed higher TFAs contents in commercially available fast foods compared to the amounts recommended by dietary guidelines in Iran. Further studies must assess the effects of these fatty acids on human health.

Key Words: trans fatty acids, saturated fatty acid, cis unsaturated fatty acid, fast food, gas chromatography

INTRODUCTION

Trans fat is the common name for a type of unsaturated fat with at least one double bond in the trans configuration.¹ A high intake of trans fatty acids (TFAs), all types of isomers, is associated with the risk of non-communicable diseases such as coronary heart disease and the metabolic syndrome.^{2,3} Trans fatty acids intake increase low density lipoprotein (LDL-C) similar to saturated fatty acids (SFA), and decrease high density lipoprotein (HDL-C) compared with SFA, resulting in a dose-dependent relationship between TFAs intake and the ratio of LDL-C: HDL-C and TC: HDL-C that is stronger than that for SFA since SFA also increases HDL-C. In addition, TFAs increases triglycerides and Lp a, when substituted for SFA.⁴ In contrast, monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) decrease plasma cholesterol concentrations in clinical studies.⁵⁻⁷

Dietary intake of TFAs is increasing in many countries as a consequence of increased intake of "fast foods".⁸ The trans fat content of fast foods based on the report from two international fast food chains in 20 countries indicated a high concentration of trans fats in their products.⁹

Fast food from major chains in most countries still contains unacceptably high levels of industrially produced TFAs. The amount of TFA varies considerably

among foods within a category, reflecting differences in the fats and oils used in the manufacturing or preparation process.¹⁰ An analysis of popular foods (fries and chicken nuggets) from fast food chains, specifically McDonald's and KFC, indicate that the TFAs content of one large serving varies greatly between countries (<1 g/serving to 24 g/serving) and even within countries (<1 g/serving to 5 g/serving). Nutritional factors inherent to fast food, such as low levels of dietary fiber, high palatability, high energy density, high fat content, high glycemic load, and high content of sugar in liquid form promote excess energy intake.¹¹ Fast food consumption contributes to the high prevalence of obesity, cardiovascular risks, inflammation, endothelial dysfunction and other chronic diseases.^{12,13}

Corresponding Author: Dr Seddigheh Asgary, Isfahan Cardiovascular Research Center, WHO Collaborating Center, Applied physiology research center, Isfahan University of Medical Sciences, Isfahan, PO. Box: 81465-1148, Iran

Tel: 0098-311-3359090; Fax: 0098-311-3373435

Email: s_asgari@crc.mui.ac.ir

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Fast food restaurants are gift of the industrial life-style which needs to be modified in accordance to the healthy food guidelines.¹²

According to the Danish Government guideline TFA content of industrial products should be limited to 2% of the total amount of fat or oil in a food product.¹⁴ Furthermore, based on the rule of the Food and Drug Administration, the appearance of TFAs in food labeling of all food products in US is necessary.¹⁵ As fast foods are one of the main source of TFA, knowing the amount of these kind of fatty acids and also other fatty acid contents of these products in Iran seems to be important. Furthermore, it is essential to obtain information regarding the fatty acid content of these products so that analysis of the relationship between these products and the prevalence of chronic diseases, as well as interpretation the mechanisms can be conducted.. The present study was undertaken to quantify the amounts of the common fatty contents of several fast foods in Iran.

MATERIALS AND METHODS

Four kinds of fast foods that are commonly consumed in Iran include: sausage (red meat, powdered milk, soy protein, oil), calbas (red meat, soy protein, oil), hamburger (red meat, soy protein, oil) and pizza (sausage, calbas, cheese, red meat, tomato). To have a representative sample of fast foods in the city, we initially asked some selected supermarkets about the usual brands people buy. We also asked this question from randomly selected group of people. After identifying these samples, each brand was randomly selected seven times from products available in supermarkets and restaurants. Each time 10 g sample was drawn and prepared for fatty acid analysis.

Total lipids were extracted by using the Folch method with chloroform: methanol (2:1 v/v).¹⁶ Ten grams of the sample was mixed with 30 ml of a chloroform-methanol (2:1, v/v) mixture. A magnetic stirrer was used for shaking this mixture in a 250 ml Erlenmeyer flask for 45 min. Then, the mixture was filtered and the solid phase was re-extracted with the same volume of extract twice. The liquid phases were mixed in a separatory funnel. Fourteen ml of saturated sodium chloride in water and 0.2 g of NaClO₄ were added, and the mixture was shaken properly. After this separation, the chloroform phase was filtered, dried with sodium sulfate and filtered again. Finally,

the extractant was dried in a N₂ current until constant weight was reached.¹⁶

Samples of the lipid extract were taken and the fatty acid components converted to their respective methyl esters. Methyl-esterification of samples was done by the BF₃-MeOH method.¹⁷ Two milliliters of 0.5 mol/L NaOH-methanol solution were added to 20 mg of extracted fat content from the samples, and the mixture was heated at 100 C° for 7 min. After cooling, 3 ml of the 14% BF₃-MeOH reagent was added, and the vessel was sealed and heated at 100 C° for 5 min. After cooling, 2 ml of hexane and 7 ml of saturated NaCl solution were added, followed by a thorough shaking. The resulting hexane layer was used for gas chromatography (GC). Two milligrams of internal standard (heptadecanoic acid) was added as a chloroform solution before esterification, and the solvent was removed under nitrogen.¹⁷ The fatty acid methyl esters were quantified using a Younglin capillary gas chromatography model 6000 equipped with flame ionization detectors and column of TR-CN100 (60 m, 0.25 mm inside diameter, 20 µm film thickness). The amount of each fatty acid was measured by **accordance** between retention time of unknown fatty acid peak and known fatty acid peak. Conditions of work were: injection temperature: 240 C°, detector temperature: 250 C°, initial temperature was 90 C°, initial time was 5 min; 150 C° for 10 min; 200 C° for 15 min and final temperature was 240 C° for 20 min. Helium used as the carrying gas, with a pressure of 20 psi and a split ratio of 20:1. Each sample was prepared three times with methyl ester..

Statistical methods

Statistical analyses was performed with SPSS statistical package (version15.0). Different superscripts (a, b, c and d) in the same row show significantly different values ($p<0.05$) as determined by one-way ANOVA followed by the Tukey post hoc test.

The ethical committee of Isfahan cardiovascular research center approved the proposal of this study.

RESULTS

The content of SFAs in each of the fast foods is presented in Table 1. Higher amounts of SFAs were found in the hamburger group compared to sausage, calbas, pizza (38.4%, 21.5%, 29.6%, 33.3% respectively, $p<0.01$).

Table 1. Saturated fatty acid (SFA) content (%) of different Iranian fast foods.

	Sausage (n=21)	Calbas (n=21)	Hamburger (n=21)	Pizza (n=21)	p-value
Total fat	27.9±2.31 [†]	26.3±5.48 [†]	35.9±4.33 [‡]	34.8±7.32 [‡]	<0.01
C4:0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	-
C8:0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	-
C10:0	0.04±0.007 [†]	0.02±0.007 [‡]	0.0±0.0 [‡]	0.002±0.001 [‡]	<0.01
C12:0	0.4±0.07 [†]	0.2±0.06 [†]	0.1±0.02 [†]	3.7±0.3 [‡]	<0.01
C14:0	0.5±0.32 [†]	1.5±0.29 [†]	3.9±0.39 [‡]	1.4±0.56 [†]	<0.01
C16:0	1.8±0.38 [†]	5.3±0.29 [†]	13.6±1.01 [‡]	8.0±0.84 [§]	<0.01
C17:0	1.4±0.28 [†]	1.5±0.34 [‡]	1.1±0.08 [‡]	0.3±0.04 [‡]	<0.01
C18:0	14.0±2.04 [†]	20.9±2.02 [‡]	19.8±1.74 [§]	19.8±0.96 [§]	<0.01
Total SFA	21.5±2.35 [†]	29.6±3.45 [†]	38.4±2.21 [‡]	33.3±4.56 [‡]	<0.01

C4:0(Butyric acid); C8:0(Caprylic acid); C10:0(Capric acid); C12:0(Lauric acid); C14:0(Myristic acid); C16:0(Palmitic acid);

C17:0(Heptadecanoic acid); C18:0(Stearic acid). [†], [‡] and [§]: Value in the same row with different superscript are significantly different ($p<0.05$.)

Table 2. Trans fatty acid (TFA) content (%) of different Iranian fast foods.

	Sausage (n=21)	Calbas (n=21)	Hamburger (n=21)	Pizza (n=21)	p-value
C16:1 t	0.4±0.16†	0.2±0.03‡	0.0±0.0‡	0.5±0.09§	<0.01
C18:1 6t	0.4±0.07†	0.1±0.05†	0.5±0.12‡	0.3±0.05†	<0.01
C18:1 9t	24.0±2.9†	21.2±1.9†	26.4±0.84‡	26.1±1.21‡	<0.01
C18:1 11t	0.1±0.04‡	0.0±0.0†	0.0±0.0†	0.3±0.04§	<0.01
C18:1 13t	0.0±0.0†	0.0±0.0†	0.0±0.0†	0.0±0.0†	<0.01
C18:2 9t,12t	1.4±0.30†	1.9±0.51†	3.7±0.32‡	0.8±0.17†	<0.01
Total TFA	26.2±2.66†	23.6±3.54‡	30.7±5.6†	28.2±4.56†	<0.01

(t:trans); C16:1 t(Palmitelaidic acid); C18:1 9t(Elaidic acid); C18:1 6t(Petroselaidic acid); C18:1 11t(trans-vaccinic acid); C18:1 13t(trans-13-octadecenoic acid); C18:2-9t 12t (Linolelaidic acid). †, ‡ and §: Value in the same row with different superscript are significantly different ($p<0.05$.)

Table 3. Unsaturated fatty acid content (%) of different Iranian fast foods.

	Sausage (n=21)	Calbas (n=21)	Hamburger (n=21)	Pizza (n=21)	p-value
C16:1	3.2±0.47†	3.4±0.44†	1.7±0.24‡	1.1±0.15‡	<0.01
C18:1 9c	34.7±1.7†	28.1±1.70‡	11.8±0.85§	23.3±1.20‡	<0.01
C18:2	12.2±0.87†	14.1±1.41†	13.6±0.51†	10.3±0.71‡	<0.01
CLA	0.03±0.007†	0.0±0.0†	0.0±0.0†	2.6±0.56‡	<0.01
C18:3	2.1±0.65†	1.1±0.20†	3.8±0.40‡	1.1±0.30†	<0.01
Total cis-unsaturated fatty acids	52.3±2.55†	46.8±6.56†	30.9±2.14‡	38.5±4.82‡	<0.01

(c:cis); C16:1(Palmitoleic acid); C18:1 9c (Oleic acid); C18:2(Linoleic acid); C18:2-c9t 11c or C18:2, t10 c12 (Conjugated linoleic acid (CLA)); C18:3(Linolenic acid). †, ‡ and §: Value in the same row with different superscript are significantly different ($p<0.05$.)

Palmitic acid (C16:0) was the most common fatty acid in this group. In all these products, stearic acid (C18:0) was the most common SFAs, except for hamburgers. Lauric acid (C12:0) was found in greater amount in pizza compared to sausage, calbas and hamburger (3.7%, 0.4%, 0.2%, 0.1%, respectively, $p<0.01$). The amount of myristic acid (C14:0) in hamburger was higher than that in the others.

Table 2 represents total and individual TFA content of different fast foods. Trans fatty acids were found in all samples, with concentration ranging from 23.6% to 30.6% of total fatty acids. The most common TFA in Iranian fast foods is elaidic acid (C18:1 9t) which is commonly presented in hamburgers and pizzas (almost 26%). Linolelaidic acid (C18:2, 9t 12t) was significantly higher in hamburger compared to sausage, calbas, pizza (3.6%, 1.4%, 1.9%, 3.6%, 0.8, respectively, $p<0.01$). Pizza had the highest amount of petroselaidic acid (C18:1 6t).

In Table 3 cis-mono and polyunsaturated fatty acid contents of different fast foods are presented. The most common unsaturated fatty acids was oleic acid (C18:1 9c) followed by linoleic acid (C18:2). Total cis unsaturated fatty acid content of calbas samples were 46.8% with oleic acid (C18:1 9c) being the most common fatty acid in these products (26.1%). Hamburger samples contain the greatest amount of linolenic acid (C18:3) overall.

DISCUSSION

Findings of our study revealed that the trans fat content of fast foods in Iran ranged from 24% in calbas to 31% in hamburger. According to our knowledge this is the first study that measures the trans fat content of the fast foods in Iran. Although, previous studies have shown that TFA consumption is detrimental to health, there is no information regarding the content of these kinds of fatty acids in

fast foods in Iran; the most probable source of trans fatty acids. Fast foods are consumed in large quantities each day. The introduction of fast-food chains and the industrial life style providing more fast-food consumption also seems to be a hallmark in the increasing prevalence of chronic diseases.¹⁸ The TFAs content of the fast foods might have an important role in these kinds of diseases.¹⁹ Based on this, the amount of TFAs in fast foods has been documented in different countries. According to the different levels of fats and oils used in manufacturing, the TFAs content of fast foods, vary greatly between countries (<1 g/serving to 24 g/serving) and even within countries (<1 g/serving to 5 g/serving).⁹ Therefore according to the guidelines, the amount of TFAs in different kinds of foods should be mentioned on the labels of these foods.²⁰

This is more important with regard to hamburgers and all fried forms of fast foods. In fried fast foods trans unsaturations started to increase at 150 °C and became much more significant from 250°C on. After heating for 20 minutes at 200, 250, and 300°C, there is an increase of 356.5%, 773.9%, and 3026.1%, respectively, in the concentration of trans isomers in relation to the initial values (0.22 mg/g).²¹

Increasing the prevalence of obesity and overweight in developing countries may be related to changing the dietary habits from traditional to westernized form.²² The high energy density of fast foods is partly brought about by a high dietary fat content,¹⁸ low levels of dietary fiber, high palatability, high energy density, high glycemic load, and high content of sugar in liquid form.⁹ Total amount of fat in the fast foods in our study reached up to 36% in hamburgers. Chemical analyses of 74 samples of fast-foods in 35 countries showed higher amount of total fat (41 to 65 g).⁹

Besides the amount of fat, the kind of fatty acids in these products is also important.^{23,24} SFAs content of fast foods in our study varied from 21.5 % to 38% in sausages and hamburger respectively.

Studies have shown that fast foods had high amounts of myristic (C14:0), palmitic (C16:0), stearic (C18:0) and trans fatty acids (C18:1).¹⁸ In most of the fast foods, the amount of stearic acid (C18:0) exceeds palmitic acid (C16:0). This might be due to the use of hydrogenated fats for the preparation of these kinds of foods. Furthermore, all of these foods have high amounts of saturated fatty acids because of its animal protein content. For example in pizza, most of the content include saturated fat which are provided from red meat, cheeses, sausages and calbas. Therefore, high proportion of stearic acid (C18:0) in these fast foods is not so surprising.

Limited evidence suggests that pro-inflammatory effects may be stronger for trans isomers of linoleic acid (trans-C18:2)²⁵ and elide acid, rather than of palmitoleic acid (trans-C16:1).²⁶⁻²⁸ Furthermore, C18:1 trans isomer comprises of approximately 80–90% of total TFA in foods.²⁹ Unfortunately it should be mentioned that most of the TFAs found in these food contain trans C18:1 isomers.

Elaidic acid (C18:1 9t) typically is the major isomer in industrial produced TFA. The concentration of the C18:1 11 trans isomer was from 0.1 % (in sausage) to 0.3 % (in pizza). Among the SFAs, stearic acid (18:0) appears to have a neutral effect on LDL-C, while lauric (12:0), myristic (14:0), and palmitic (16:0) acids are considered to be hypercholesterolemia.³⁰

In our study, stearic acid was its major SFA component, in a range of 14.0% (in sausage) to 20.9% (in calbas), and palmitic acid in a range of 1.8% (in sausage) to 13.6% (in hamburger).

Fast food outlets in Denmark and the Netherlands have been very successful at maintaining the quality of their food products while considerably reducing the their TFA content^{13,31}. In Iran the content of TFAs in fast foods can be reduced by decreasing the amount of partial hydrogenated fat usage in the preparation of fast foods.

Governmental legislation to subsidize un-hydrogenated rather than partially hydrogenated oils would be a good strategy in this regard.³²

In the interpretation of the results of this study, we should consider some limitations. First, we did not determining all fatty acids (e.g. myristelaidic acid C14:1 trans) due to budget limitation. Second, there are some biases due to measurement errors which are not completely avoidable.

In conclusion, our results indicated high amounts of TFAs in Iranian fast foods. Future studies should be done to determine the amount of total dietary intakes that comes from fast foods and the total intake of TFAs among Iranian population, as well as determining the possible effects on human health.

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AUTHOR DISCLOSURES

Authors declared no financial or personal conflicts of interest.

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Seddigheh Asgary¹, Bahar Nazari², Nizal Sarrafzadegan², Sahar Parkhideh²,
Salbali Saberi², Ahmad Esmailzadeh³, Leila Azadbakht³

¹*Isfahan Cardiovascular Research Center, WHO Collaborating Center, Applied physiology research center, Isfahan University of Medical Sciences, Isfahan, Iran*

³*Isfahan Cardiovascular Research Center, WHO Collaborating Center, Isfahan University of Medical Sciences, Isfahan, Iran*

³*Department of Nutrition, School of Public Health, Isfahan University of Medical Sciences, Isfahan, Iran*

評估某些伊朗速食品中的脂肪酸，特別是反式脂肪酸

雖然反式脂肪酸的弊害被廣泛的提及，但關於伊朗食物，包括速食品的反式脂肪酸含量的資料卻有限。本研究的目的即是定量伊朗的幾種速食中，一般脂肪酸的量，特別是反式脂肪酸。在伊朗，常吃的速食有香腸、肉餅(calbas)、漢堡、以及比薩。這些食物由超市及餐廳中隨機取樣七次，每次取得 10 g 樣本以進行脂肪酸分析。總體脂肪酸及個別脂肪酸根據標準方法，以氣相層析儀(60 米毛細管柱及火焰離子偵測器)定量。伊朗速食品中，主要的飽和脂肪酸為硬脂酸(C18:0)，含量從 14.0%到 20.9%。Calbas 中飽和脂肪酸含量顯著的高於其他食品。反式脂肪酸約佔這些產品所含總脂肪酸的 23.6%到 30.6%。這些食品中，主要的反式脂肪酸為反油酸(C18:1 9t)。這些速食中的總順式不飽和脂肪酸含量不同，從 25.3%(香腸)到 46.8%(calbas)，以油酸(C18:1 9c)含量最多，亞麻油酸(C18:2)次之。本研究顯示，與伊朗的飲食指標建議量相比，市面上可得到的速食品中有較高的反式脂肪酸含量。而進一步的研究必須要評估這些脂肪酸對人類健康的影響。

關鍵字：反式脂肪酸、飽和脂肪酸、順式不飽和脂肪酸、速食品、氣相層析