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Measurement and comparison of trans fatty acids amount in some of the vegetable oils, frying oils and animal and vegetable fats in Iran

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ABSTRACT: The role of dietary fats and oils in human nutrition is one of the most important areas of concern and investigation in the field of nutritional science. The findings of investigations on this subject have wide-ranging implications for consumers, health-care providers and nutrition educators as well as food producers, processors and distributors. New evidence concerning the benefits and risks associated with particular aspects of dietary fat. Physically, oils are liquid at room temperature, and fats are solid. Chemically, both fats and oils are composed of triglycerides. In this study, different fatty acids especially trans fatty acids in 9 samples of oil including liquid and frying oils and processed and non-processed solid fats in the Iranian market were investigated. The experiments were performed by gas chromatography. Trans fatty acids were detected from 33% to 52.8%. The highest content of trans fatty acid was identified in animal oil.

Keywords: *Dietary Fat, Gas chromatography, Oil, Trans fatty acids, Triglycerides.*

INTRODUCTION

Trans fats are a type of unsaturated fat that occur in small amounts in nature, nevertheless became widely manufactured industrially from vegetable fats for use in butter, margarine, snack food, packaged baked product and frying fast food (Vega- Lopez, 2009). In recent years, considerable attention has been paid to develop edible oil products with physicochemical functions to promote health conditions and avoid the risk of diseases (Morris & Tangney, 2014). Although trans fats are edible, consumption of trans fats has been revealed to raise the risk of coronary artery disease in particular by uplifting levels of the lipoprotein LDL (often referred to as "bad cholesterol"), lowering levels of the lipoprotein HDL (Zock, *et al.*, 1998, Farmani, 2007). Lipids with high content of saturated and trans-fatty acids are solid at room temperature and are usually referred to as ‘‘fats,’’ while lipids with higher content of unsaturated and polyunsaturated fatty acids are liquid at room temperature and are usually referred as ‘‘oils’’ (Martini, 2013). Trans-free Iranian Vanaspati through enzymatic and chemical transesterification of triple blends of fully hydrogenated soybean, rapeseed and sunflower oils was carried out (Zock, *et al.*, 1998). The result has shown free Trans Vanaspati can be produced by using enzymatic and chemical transesterification for blend-

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ing rapeseed, soybean and sunflower oil together. In this regard, study investigated the content of trans fatty acids among 134 foodstuffs such as meat product, biscuits and chocolate. The results showed that the average rate of Trans fatty acids in meat product 14.5, biscuits 0.99-17.8, chocolate 17 gram per 100 gram (Karabulut, 2006). There are two main sources of dietary trans fatty acids (TFA) in the food source, those formed during the industrial partial hydrogenation of vegetable oils (iTFA) and those formed by bio hydrogenation in ruminants (rTFA). With regards to this subject, researchers similarly studied the effect of ruminant origin compared with those from partially hydrogenated vegetable oils on CHD risk. It was reported that there were many Trans fatty acids isomer in common between these two fats. Nevertheless, there was significant deferent in amount of TFA in both sources. For instance, in hydrogenated oils and meat and dairy product it contains up to 60% and 5% of total fatty acids respectively. According to the results, TFA found in partial hydrogenation of vegetable oils are risky to human health (Pfeuffer, 2006). In an investigation, types and amount of Trans fatty acids found in edible oils of China were examined. Being reported that among 93 samples, 17 samples had contained trans fatty acid up to 2% and rest of the samples were less than this amount. With this regard, trans fatty acids in Chinese edible oils were at the lower level (Hou, 2012).

MATERIAL AND METHODS

In order to carry out this study, 9 sample of edible oils and fats such as Ladan sunflower oil, Semen Sesame Oil, Famila virgin olive oil, Bahar frying oil, Pak unprocessed butter, Shakelli unprocessed butter, Shakelli Ghee, Mahgol processed margarine, Ladan Talaie partial hydrogenation oil were purchased from supermarket. According to The global standard, trans fatty acids in oils and fats is considered zero and in this study all the result being compared with this standard (as control sample). In this study, Sodium hydroxide for breaking the bond between fatty acid and glycerol in triglycerides, Methanol, Boron trifluoride for separating the remaining fatty acids in triglycerides, Hexane used for Solubility of fatty acids isolated from the structure of triglycerides, Sodium chloride were all purchased from Merck Chemical Company.

Sampling

All of the samples were transferred to the Central Laboratory of Agricultural and Natural Resources Campus of Tehran University and stored under refrigeration during analysis.

Esterification Methods

Method described by Metcalfe, Schmitz e Pelka, 1966 (MET). Approximately, 0.04 gr pure oil was mixed with 5 ml of NaOH in methanol (2%) and heated in a bath at 100°C until the dissolution of the fat globules (10 min). Next, 2.2 mL BF3 (20%) in methanol was added and the mixture was heated in bath for another 3 min. After cooling, approximately 5.1 mL of a hexane solution was added and then approximately 1 mL of a saturated sodium chloride solution (30%) was added. The mixture was transferred to a separation funnel with 20.0 mL of petroleum ether. The funnel was vigorously stirred for 1 min and then left at rest for phase separation. The aqueous phase was discarded and the ether phase was filtered with a paper filter into a balloon. The solvent was evaporated in a bath at 60°C and the residual solvent was removed with nitrogen flow at room temperature. The methyl esters were solubilized in an heptane before injection into the gas chromatographer.

Instrumental method

In the next stage, the aforementioned extracted solution (fatty acid methyl esters) was injected into a gas chromatograph with a Flame Ionization Detector (FID). The applied instrument was Clarus 500 GC (USA) and the capillary column for GC was (CP-SIL-88) 100m*0.25mm i.d., film thickness 0.25 µm. Infusion temperature was set at 280 °C. The Detector with 250 °C and Nitrogen as gas carrier with rate of 1.5 ml/min were used. In this study, the temperature program is selected as follows: the initial temperature of oven was set at 90 °C and it was kept at this temperature for 2 min. after that the temperature increased to 230 °C and hold at this temperature for 20 min.

Identification

The fatty acid methyl esters were identified by comparison of the retention times of the sample constituents with retention times of the standards sample. Each fatty acid reaches the detector after passing through capillary column at different times and appears in the form of peaks on the monitor. The peak areas of fatty acid methyl esters were determined with the software Workstation version 5.0 (Varian). By comparing the peak area of each fatty acid in the chromatogram, the percentage of each fatty acid was determined by the sum of the column below the curve.

Statistic

Statistical comparisons were performed by using Student's t-test and SAS version 1 software and comparisons were made between them. Charts were plotted by using Excel software. Cluster Analysis was performed via SAS version 1 software and Dendrograms were schemed based on Unweighted Pair-Group method using arithmetic average (UPGMA).

RESULTS AND DISSCUTION

The results of measurements of trans fatty acids in the samples

The amount of trans fatty acids in Ladan sunflower oil, Semen Sesame Oil, Famila virgin olive oil, Bahar frying oil, Pak unprocessed butter, Shakelli unprocessed butter, Shakelli Ghee, Mahgol processed margarine, Ladan Talaie partial hydrogenation oil is shown in Table 1.

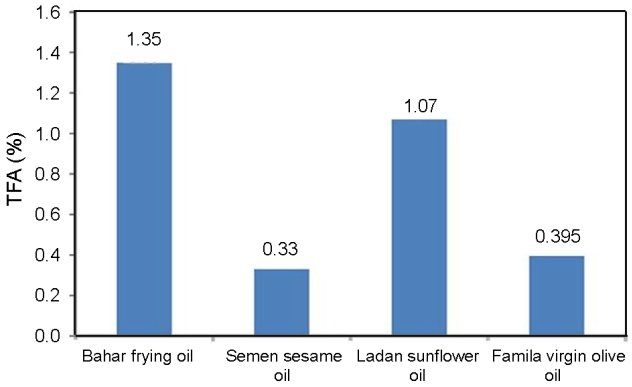

Fig. 1. Amount of trans fatty acids in vegetable oils and frying oil (%).

Comparison of the results of measuring trans fatty acids between frying oils and other oils

Differences in trans fatty acids in frying oils and other oils is presented in the Fig. 1.

Comparison of trans fatty acids in processed vegetable fats

The difference in the amount of trans fatty acids in processed vegetable fats, such as Ladan Talaie semi-hydrogenated oil and Mahgol Margarine is shown in Fig. 2.

Table 1. The percentage of trans fatty acids among the samples.

Trans Fatty Acid	Shakelli Ghee	Pak unprocessed butter	Bahar frying oil	Shakelli unprocessed butter	Mahgol processed margarine	Ladan Talaie partial hydrogenation oil	Semen Sesame oil	Ladan sunflower oil	Famila virgin olive oil
C16:1trans (Palmitelaidic acid)	0.24	0.2	0.03	0.28	0.11	0	0.02	0	0
C18:1 9-trans (Elaidic acid)	3.41	2.05	0.07	1.59	4.22	0.3	0.06	0.07	0
C18:1 11t (vaccenic acid)	2.1	1.35	0	0.96	0	0	0	0	0
C18:2 6-trans (6-linoleic acid)	0.07	0.18	1.25	0.15	0.43	0.31	0.25	0.66	0.33
C22:1 trans N9	0	0	0	0	0	0	0	0.07	0
C22:2 trans	0	0	0	0	0	0	0	0.27	0.06
Total TFA	5.82	3.78	1.35	2.98	4.76	0.61	0.33	1.07	0.39

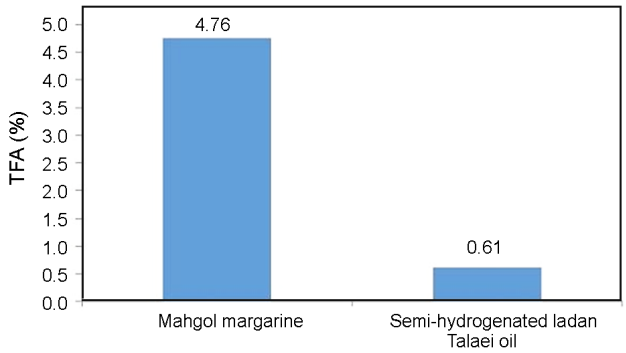

Fig. 2. The amount of trans fatty acids in Mahgol Margarine and Ladan Talaie semi-hydrogenated fat (%).

Comparison of trans fatty acid content between processed and unprocessed oil

Difference in the amount of trans fatty acids between processed fats such as (Ladan Talaie semi-hydrogenated oil and Mahgol Margarine) and unprocessed fats like (Pak Butter, Shakelli Butter, and Shakelli Ghee) is presented in Table 2.

Investigating and evaluating the amount of Trans fatty acids in Bahar frying oils and other oils

As being reported in Fig. 1, Among the oils (for salad , cooking and frying), the highest levels of trans fatty acids are found in Bahar frying oil and sunflower oil, olive oil and sesame oil respectively. Although there is no statistically significant difference between the amounts of Trans fatty acids in these samples ($p > 0.05$). the predominated trans fatty acid in Bahar frying oil and sunflower oil, Virgin olive oil and sesame oil is C18:2 6trans(Trans 6-linoleic acid) .These finding are in agreement with the findings of other researchers (Izegarska & Borejzo, 2001).

Investigating the amount of trans fatty acid in processed vegetable fats

As can be seen in Fig. 2, the amount of Trans fatty acid in Mahgol Margarine and Ladan Talaie semi-hydrogenated Fat is about 4.76 and 0.61% respectively. It is reported that the predominated Trans fatty acid in Ladan Talaie semi-hydrogenated and Mahgol Margarine are C18:2 6trans (Trans 6-linoleic acid) and C18:1 9trans (Elaidic acid) respectively.

Studying the difference between Trans fatty acids between processed and unprocessed oil

Table 2 shows that, the highest levels of trans fatty acids were found in Shakelli Ghee butter, Mahgol margarine, Pak Butter, Shakelli Butter and Ladan Talaie semi-hydrogenated oil respectively. But, there hadn't been a statistically difference between the amount of Trans fatty acids among these fats ($P > 0.05$). Among all the studied samples, the highest content of trans fatty acids were found in Shakelli Ghee butter, Mahgol margarine, Pak Butter, Shakelli Butter, Bahar frying oil, Ladan sunflower oil, Ladan Talaie semi-hydrogenated oil, Famila virgin olive oil and Semen Sesame Oil respectively. There is no statistically significant difference among the samples which get listed above in terms of content of trans fatty acids ($P > 0.05$). The result was comparable with other researcher's finding7. Additionally, the highest content of saturated fatty acids was found in Shakelli Butter, Pak Butter, Shakelli Ghee butter, Mahgol margarine, Bahar frying oil, Ladan Talaie semi-hydrogenated oil, Famila virgin olive oil, Semen Sesame Oil and Ladan sunflower oil respectively. As being reported, there has not been a statistically meaningful difference in the amount of trans fatty acids in the all oil samples ($P > 0.05$).

Table 2. Comparison of trans fatty acid content between processed and unprocessed fats.

Fatty Acids	Shakelli Ghee	Pak Butter	Shakelli Butter	Mahgol Margarine	Ladan Talaie semi-hydrogenated Fat
C16:1trans (Palmitelaidic acid)	0.24	0.2	0.28	0.11	0
C18:1 9-trans (Elaidic acid)	3.41	2.05	1.59	4.22	0.3
C18:1 11t (Vaccenic acid)	2.1	1.35	0.96	0	0
C18:2 6-trans (Trans 6-linoleic acid)	0.07	0.18	0.15	0.43	0.31
C22:1 trans N9	0	0	0	0	0

CONCLUSIONS

There are 2 main sources of dietary trans fatty acids (TFA) in the food source, those formed during the industrial partial hydrogenation of vegetable oils (iTFA) and those formed by bio hydrogenation in ruminants (rTFA). However there is a considerable similarity of trans fatty acid (TFA) isomers in fats of ruminant origin and semi hydrogenated vegetable oils (PHVOs), with many isomers in common. However, there is a substantial difference in the amount of individual TFAs in both sources. For instance, in semi-hydrogenated vegetable oils, the predominated trans fatty acid is Elaidic acid (18:1/t9), while, Vaccenic acid (18:1/t11) is main trans fatty acid found in the fat of ruminants and in dairy products. Trans fatty acid as compared to unsaturated fatty acids are risky to human body and cause harmful effects on health. The consumption of trans fatty acids ranges from 2.8 to 10 g per day can increase the risk of cardiovascular disease approximately 22%. In 1994, the UK Department of Health advised that content of trans fatty acids should not be increased to more than 5 grams per day (Roe, 2012). Among all the studied samples, the highest content of trans fatty acids were found in Shakelli Ghee butter, Mahgol margarine, Pak Butter, Shakelli Butter, Bahar frying oil, Ladan sunflower oil, Ladan Talaie semi-hydrogenated oil, Famila virgin olive oil and Semen Sesame Oil respectively. The values of trans fatty acids in all samples were reported higher than the global standard.

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