

## Determination of trans fat in traditional Pakistani breakfast and snack foods

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### Abstract

Intake of trans fat may led to cardiovascular diseases and have no nutritional advantage in the diet. Breakfast and snack foods are the major contributor of trans fat in the diet. Research was performed to determine trans fat contents and physiochemical attributes of extracted fats and oils from traditional products like poori, cake, paratha, doughnut and puff pastry which were collected from local market of Faisalabad, Pakistan. Trans fat contents were determined in term of elaidic acid through attenuated total reflection fourier transform infrared (ATR-FTIR) spectroscopy. Highest trans fat content were found in doughnuts (38.691±0.133%) followed by poori (18.480 ± 5.020%). Minimum value for trans fat was observed in puff pastry (6.190±3.080%). Overall, trans fat content ranged between 6.190±3.080 to 38.691±0.133% which shows drastically higher tans fat contents. Samples were further investigated for different quality parameters i.e. melting point, refractive index, specific gravity, free fatty acids, peroxide value, acid value, saponification value and iodine value The greatest value of melting point was found in samples of Paratha (36.1±2.1°C) and lowest value was observed in cake (31.4±0.8°C). Highest acid value and free fatty acid contents were found in poori (0.6912 ±0.12% and 0.347±0.058). Samples form poori showed highest saponification value (190.34±1.71mg KOH/g) while iodine value was highest in cake (69.921± 1.26%) and lowest in puff pastry (62.102± 1.25%).

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### Introduction

FDA's regulatory authority defines trans fat as "fatty acids that contain one or more isolated double bonds in a trans configuration" (FDA, 2003). Natural source of trans fat includes dairy and meat products. Partial hydrogenation is the major source of trans-fatty acids. Trace amounts of trans fats are also produced during the process of deodorization or refining vegetable oils (Tasan and Demirci, 2003). Elaidic acid is the chief trans fat produced during the process of hydrogenation (Karabulut, 2006; Kandhro *et al.*, 2008). It will be impossible to completely eliminate them from the diet because of its natural source. Shortening contain as much as 40 % of the trans fat compared with only 6% in natural-derived fat (Stender *et al.*, 2013).

Today, the American Heart Association (Eckel *et al.*, 2006), American Dietetic Association (Kris-Etherton *et al.*, 2007), Institute of Medicine (Institute of medicine, 2009), US Dietary Guidelines and the National Cholesterol Education Program indicate that trans fat intake should be as low as possible. The

health effect of trans fat become a protruding topic of scientific debates since 1990 when Mensink and Katan stated that Intake of Trans fat may also be associated with many diseases like inflammation, fetal growth, cancer, allergies and many other diseases. Studies demonstrated that 2% increase in daily energy intake from trans fat was associated with a 23% increase in cardiovascular disease risk (Ascherio *et al.*, 1994) .in addition, there are no known nutritional benefits over other fats (Kummerow, 2009; Backholer and Peeters, 2012). Mechanism of affecting heart health is that it adversely affect lipoprotein metabolism, presumably through increased activity of cholesteryl-ester transfer protein (Van Tol *et al.*, 1995), resulting in higher levels of LDL, very-low-density lipoprotein, triglycerides, and lipoprotein , as well as reductions in LDL size and HDL concentration (Hu *et al.*, 1997; Ascherio *et al.*, 1999). By incorporating into our cellular membranes it also affects its composition and functions (Chapman *et al.*, 1966).

Naturally, all unsaturated fatty acids in the plant oils are in the cis form (Salimon *et al.*, 2012). However, development of the hydrogenation process in the early

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20<sup>th</sup> century led to the introduction of commercial trans fats into diet. Due to This intermediate melting point, favorable texture and mouth feel it is highly desirable in food manufacturing. Further, trans fat stability, when exposed to oxygen, enhances product shelf life (Ghotra *et al.*, 2002; Khanal *et al.*, 2004). Sometime while achieving the product functionality requirements for consumer nutritive and health value is overlooked and potentially harmful substances become the part of our food system (Oomah and Mazza, 1999).

During the past 10 years, a number of alternatives to trans fats have been promulgated (Eckel *et al.*, 2006) like inter-esterification. Different countries of the world have some kind of labeling requirement, so that the consumer may not be cheated. These types of regulations are found in United States of America and Canada (FDA, 2003; Health Canada, 2003). Denmark has experienced a dramatic decline in cardiovascular disease of about 60% by progressive lowering the intake of trans fat from commercial sources. (Leth *et al.*, 2006; Stender *et al.*, 2013).

Pakistani population is using hydrogenated vegetable oil at a very high consumption rate, which is a shocking fact for public health (Bhangera and Anwar, 2004). These are used for cooking, frying and as an ingredient in bakery products such as cakes, cookies, pastries, breads and others (Osman and Aini, 1999). So, if cereal products contain TFA than is must be a dangerous fact. Current project was designed to determine the trans fatty acid (TFA) level of cereal products. FTIR Spectroscopic method was used to determine trans fat which is very less time consuming than that of G.C. (Gas chromatography) which is very beneficial for industries especially.

## Materials and Methods

### *Preparation of raw material and fat extraction*

The food samples collected were immediately homogenized and frozen until analysis. Fat extraction from food stuff were carried out by hexane extraction under the conditions specified by the International Association for Cereal Chemistry (ICC., 1982). Fat obtained from food samples was transferred to 10 mL glass vials. While reagents (analytical and GC grade) were purchased from Sigma-Aldrich (USA) and Merck (Germany) and trans fat standards were purchased from Supelco (Bellefonte).

### *Physiochemical analysis*

Samples were investigated for different quality parameters i.e. melting point, refractive index, specific gravity, free fatty acids, peroxide value, acid

value, saponification value and iodine value followed by the procedure given in AOCS (2002) Method No. Ce (1-25), Cc (7-25), Cc (10b-25), Ca (5a-40), Cd (8-53), Cd (3a-63), Cd (3-25), Cd (1b-87) respectively.

### *Determination of trans fat*

Trans fat profile was determined by attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopic method (AOAC, 2000). This method is applicable to natural fat and processed fats and oils consisting of long chain of fatty acid methyl esters or triglycerols with trans levels more than or equal 5%, as percent of total fat.

First of all trans calibration standards were prepared by using trielaidin and trans free reference fat, 0.5%,1%,5%,10%,20%,30%,40% and 50% respectively. Then ATR cell was allowed to warm up to  $\pm 2^{\circ}\text{C}$  for 30 minutes. Using disposable pipette 1 to 50  $\mu\text{l}$  of the trans free reference fat was placed on the ATR horizontal surface, while it was made sure that the ZnSe or diamond crystal were completely covered. 128-Scan single-beam FTIR spectrum was collected and saved at  $4\text{cm}^{-1}$ . ATR crystal was cleaned with low lint tissue as many times as needed to ensure absence of cross contamination. Calibration standards were melted in water bath and then using disposable pipette each standard was placed on the horizontal ATR surface making sure that the ZnSe crystals were covered completely. 128-Scan single-beam FTIR spectrum was collected and saved at  $4\text{cm}^{-1}$  resolution. This step is repeated for each of the standard. Test samples were melted in the water bath and the placed on horizontal ATR surface making sure that the surface of the ZnSe is completely covered. 128-Scan single-beam FTIR spectrum was collected and saved at  $4\text{cm}^{-1}$  resolution. This step was repeated for each of the measurement. Using the slop and intercept of the linear regression equation generated for the calibration standards, calculate the trans fat level (as percentage of total fat) for each test sample by substituting the trans band integrated into the equation:

$$\% \text{ Trans} = (\text{Area} - \text{Intercept}) / \text{Slop}$$

This equation assume that the test samples consist of trielaidin

### *Statistical analysis*

The data for each parameter was subjected to analysis of variance technique using statistical level of significance was  $P \leq 0.05$ . Means were further analyzed through Tukey's HSD Test (Steel *et al.*, 1997).

Table 1. Physical analysis of fat and oil extracted from food samples

Samples	Melting point (°C)	Refractive index	Specific gravity
Poori	34.1±1.6c	1.4637±0.001	0.9255±0.0026a
Cake	31.4±0.8d	1.4656±0.001	0.8574±0.005e
Pratha	36.1±2.1a	1.4639±0.004	0.9221±0.0134b
Doughnut	35.5±0.6b	1.4657±0.001	0.9187±0.0039c
Puff pastry	34±0.6c	1.4607±0.001	0.8629±0.0115d

## Results and Discussion

### Physiochemical analysis

Results of physical analysis i.e. melting point, refractive index and specific gravity are given in Table 1. Melting point of different samples showed great variations. It ranges from 31.4±0.8°C (Cake) to 36.1±2.1°C (Poori) which shows the proportion of saturated fatty acid, but maximum melting point was also less than human body temperature so, it means it will not pose any harmful effect on cardiac health. Results revealed non-significant differences among different samples. Maximum value for the specific gravity was observed in poori (0.9255±0.0026) followed by pratha (0.9221±0.0134) while minimum value was recorded in cake (0.8574±0.005). Results of physical analysis are confirmed by previous findings. According to Naz *et al.* (2012) melting point of hydrogenated vegetable oil ranged from 35.6°C to 37°C. The results of refractive index and specific gravity were also having same trend were found in earlier studies.

Results of the chemical analysis of the extracted fat is given in the Table 2 which indicate the maximum free fatty acid content, acid value and peroxide value were found in Poori i.e. 0.347±0.058%, 0.6912 ±0.12% and 6.23±1.89meq/kg respectively, which indicate poor keeping quality due to which it deteriorate over the period of time or it may not had withstand harsh frying and baking processes. The maximum value (0.347±0.058%) of free fatty acid was found in poori followed by 0.329±0.043% (cake). The minimum value (0.206±0.043%) was found in Puff pastry. Overall, free fatty acid of the fat extracted from cereal products ranged from 0.347±0.058% (poori) to 0.206±0.043% (puff pastry). The maximum value (6.23±1.89meq/Kg) of peroxide value was found in poori followed by 5.87±1.17meq/Kg (puff pastry). The minimum value (3.15±0.98meq/Kg) was found in paratha. Overall, free fatty acid of the fat extracted from cereal products ranged from 6.23±1.89meq/Kg (poori) to 3.15±0.98meq/Kg (paratha). The maximum value (0.6912±0.12%) of acid value was found in poori

followed by 0.654±0.09% (cake). The minimum value (0.4106 ±0.09%) was found in puff pastry. Overall, acid value of the fat extracted from cereal products ranged from 0.6912±0.12% (poori) to 0.4106 ±0.09% (puff pastry). The data collected showed grater deterioration process during processing and storage. The maximum value (190.34±1.71mg KOH/g) of saponification value was found in poori followed by 189.92±1.47mg KOH/g (paratha). The minimum value (189.92±1.47mg KOH/g) was found in cake. Overall, saponification value of the fat extracted from cereal products ranged from 190.34±1.71mg KOH/g (poori) to 189.92±1.47mg KOH/g (cake). The maximum value (69.921±1.26%) of iodine was found in cake followed by 67.061±1.35% (pratha). The minimum value (62.102±1.25%) was found in puff pastry. Overall, iodine value of the fat extracted from cereal products ranged from 69.921±1.26% (cake) to 62.102±1.25% (puff pastry). The results of chemical analysis are confirmed by previous studies. Tsuzuki *et al.* (2010) calculated peroxide value of oil which was used for frying operation in the range of 1.9 to 8 mg KOH/g. Kheiri *et al.* (1985) reported that iodine value of Pakistani vanaspati is 69g/100g. Naz *et al.* (2012) found acid value, saponification value and free fatty acid content of market oil 0.34%, 187 mg KOH/g and 0.18% respectively.

### Trans fat determination

Results of the trans fat revealed that the maximum value (38.691±0.133%) of trans fat was found in doughnut followed by 18.480 ± 5.020% (poori) (Table 2). The minimum value (6.190±3.080%) was found in puff pastry. Overall, trans fat of the fat extracted from cereal products ranged from 38.691±0.133% (doughnut) to 6.190 ± 3.080% (puff pastry). Present results for trans fat were in accordance with the values described by other scientist. Findings of Bhangar and Anwar (2004) showed that vanaspati contain trans fat in a range between 14.2 to 34.3%. Similar results were found by Jeyarani and Reddy (2005) for different brands of commercial vanaspati for their fatty acid profile through fourier transform infrared spectroscopy and they found trans fat contents

Table 2. Chemical analysis of fat and oil extracted from food samples

Samples	Free fatty acids (%)	Peroxide value (meq/Kg)	Acid value (%)	Saponification value (mg KOH/g)	Iodine value (%)	Trans fat (%)
Poori	0.347±0.058a	6.23±1.89a	0.6912±0.12a	190.34±1.71a	64.814±2.21c	18.480±5.020b
Cake	0.329±0.043b	4.16±1.96c	0.654±0.09b	185.24±0.78e	69.921±1.26a	12.020±1.780c
Pratha	0.225±0.028d	3.15±0.98e	0.4478±0.06d	189.92±1.47b	67.061±1.35b	11.010±2.050d
Doughnut	0.300±0.059c	3.24±1.18d	0.5977±0.12c	188.12±2.83c	69.65±0.86a	38.691±0.133a
Puff pastry	0.206±0.043e	5.87±1.17b	0.4106±0.09e	187.44±0.59d	62.102±1.25d	6.190±3.080e

from 5.9 to 30.0%. Similarly, Sherizi *et al.* (2008) found 26.5% trans fat in partially hydrogenated oils. However, it was quite clear that none of the sample contain trans fat in critical safe limit i.e. 2-4% set by WHO, which is a threatening dilemma to focus in future.

## Conclusions

Fat and oil is the essential ingredient which is used as shortening in the cereal based food. Health status of this ingredient directly affects the consumer health. Fatty acid profile and especially trans fat contents must be under critical limits set by national and international food safety authorities. Trans fat determination through FTIR spectroscopy is very quick, efficient and reliable method and could be adopted by food industries to protect consumer health.

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## References

- AOAC. 2002. Official Methods of Analysis. (5<sup>th</sup> edition). Washington: Association of Official Analytical Chemists.
- Ascherio, A., Hennekens, C. H., Buring, J. E., Master, C., Stampfer, M. J. and Willett, W. C. 1994. Trans-fatty acids intake and risk of myocardial infarction. *Circulation* 89: 94-101.
- Ascherio, A., Katan, M. B., Zock, P. L., Stampfer, M. J. and Willett, W. C. 1999. Trans Fatty acids and coronary heart disease. *New England Journal of Medicine* 340: 1994-1998.
- Backholer, K. and Peeters, A. 2012. Reduction of trans-Fatty Acids from Food. *Journal of American Medical Association* 308: 1858-1859.
- Bhanger, M. I., and Anwar, F. 2004. Fatty acid (FA) composition and contents of trans unsaturated FA in hydrogenated vegetable oils and blended fats from Pakistan. *Journal of American Oil Chemists Society* 81: 129-134.
- Bhanger, M. I. and Anwar, F. 2004. Fatty acid composition and contents of trans unsaturated fat in hydrogenated vegetable oils and blended fats from Pakistan. *Journal of American Oil Chemists Society* 81(2): 129-134.
- Chapman, D. and Owens, N. F. 1966. Physical studies of phospholipids. II. Monolayer studies of some synthetic 2,3-diacyl-DL-phosphatidylethanolamines and phosphatidylcholines containing trans double bonds. *Biochimica et Biophysica Acta* 120: 148-155.
- Eckel, R. H., Borra, S., Lichtenstein, A. H. and Yin-Piazza, S. Y. 2006. Understanding the complexity of trans fatty acid reduction in the American diet. In Report of the trans Fat Conference Planning Group (Eds). *Trans Fat Conference*, p. 2231-2246. American Heart Association, USA.
- FAO. 1977. Dietary fats and oils in human nutrition. Report of Food and Agriculture Organization (FAO) of the United Nations and World Health Organization (WHO). Rome
- FDA. 2003. Department of Health and Human Services. Food labeling: Trans fatty acids in nutrition labeling; nutrient content claims, and health claims; final rule. *Federal Regulations* 68(133): 41434-41506.
- Ghotra, B. S., Dyal, S. D. and Narine, S. S. 2002. Lipid shortenings: a review. *Food Research International* 35: 1015-1048.
- Health Canada. 2003. Regulations amending the food and drug regulations (nutritional labeling, nutrient content claims and health claims). *Canada Gazette*.137 (Pt II): 154-409.
- Hu, F. B., Stampfer, M. J., Manson, J. E., Rimm, E., Colditz, G. A., Rosner, B. A., Hennekens C. H. and Willett, W. C. 1997. Dietary fat intake and the risk of coronary heart disease in women. *New England Journal of Medicine* 337: 1491-1499.
- ICC. 1982. Standard methods of the international Association for Cereal Chemistry standard no: 136 ICC. Detmold, Germany.
- Jeyarani, T. and Reddy, S. Y. 2005. Physicochemical evaluation of vanaspati marketed in India. *Journal of Food Lipid* 12: 232-242.
- Kandhro, A., Sherazi, S. T. H., Mahesar, S. A., Bhanger, M. I., Talpur M. Y. and Rauf, A. 2008. GC-MS quantification of fatty acid profile including trans FA in the locally manufactured margarines of Pakistan.

Food Chemistry 109: 207-211.

- Karabulut, I. 2006. Fatty acid composition of frequently consumed foods in Turkey with special emphasis on trans fatty acids. *International Journal of Food Science and Nutrition* 32: 1-10.
- Khanal, R. C. and Dhiman, T. R. 2004. Biosynthesis of conjugated linoleic acid (CLA): A review. *Pakistan Journal of Nutrition* 3: 72-81.
- Kheiri, M. S. A. and Bangi, B. B. 1985. Palm oil products in cooking fats. *Journal of American Oil Chemists Society* 62(2): 410-416.
- Kris-Etherton, P. M. and Innis, S. 2007. Position of The American Dietetic Association and Dietitians of Canada: Dietary fatty acids. *Journal of American Dietetic Association* 107: 1599-1611.
- Kummerow, F. A. 2009. The negative effects of hydrogenated trans fats and what to do about them. *Atherosclerosis* 205: 458-465.
- Leth, T., Jensen, H. G., Mikkelsen, A. A. and Bysted, A. 2006. The effect of the regulation on trans fatty acid content in Danish food. *Atherosclerosis Supplement* 7: 53-56.
- Naz, R., Anjum, F. M., Rasool, G., Nisar, M. A., Batool, R. and Saeed, F. 2012. Total Trans Fat Content in Commercially Available Hydrogenated Vegetable Oils. *Pakistan Journal of Nutrition* 11(2): 145-149.
- Oomah, B. D., and Mazza, G. 1999. Health benefits of phytochemicals from selected Canadian crops. *Trends in Food Science and Technology* 10: 193-198.
- Salimon, J., Salih, N. and Yousif E. 2012. Living carbocationic polymerization of a vinyl ether monomer derived from soybean oil, 2-(vinylloxy)ethyl soyate. *Arab Journal of Chemistry* 5: 135-145.
- Steel, R. G. D., Torrie, J. H. and Dickey, D. 1997. Principles and procedures of statistics: a biometrical approach. In 3rd (Eds). New York: McGraw Hill Book Co. Inc.
- Internet: Food and Nutrition Research. 2008. Ruminant and industrially produced trans fatty acids: Health aspects. Download from <http://www.foodandnutritionresearch.net/index> on 10/09/2013.
- Tasan, Y. and Demirci, N. 2003. Trans FA in sunflower oil at different steps of refining. *Journal of American Oil Chemists Society* 80: 825-828.
- Van Tol, A., Zock, P. L., Gent, T. V., Scheek, L. M. and Katan, M. B. 1995. Dietary trans fatty acids increase serum cholesteryl ester transfer protein activity in man. *Atherosclerosis* 115: 129-134.