

Trans fat content in labeled and unlabelled Indian bakery products including fried snacks

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Abstract

Trans fatty acid (TFA) intake is positively associated with the risk of developing cardiovascular disease. In this context, present study provides detailed information about the total fat, fatty acid composition and trans fat content of selected labeled and unlabelled Indian bakery products (biscuits, pastries, cakes, bread, bun, puffs, rolls, burger, cutlet, samosa and noodles). Quality studies included free fatty acid value, peroxide value, p-Anisidine and totox values were performed on the extracted fat. Among the products analyzed, cake samples showed highest fat content and puff samples showed highest *trans* fat content. The major *trans* form in all samples was elaidic acid, except butter bun where vaccenic acid was the predominant TFA. Higher FFA, PV, p-Anisidine and totox values indicated the poor quality either contributed by the processing (baking) or storage deterioration. When there are regulations emphasizing on labeling the TFA content, there are arrays of unlabelled products which are not governed under any regulations. The present study shows the significance of laying regulation on such products.

Keywords

Trans fatty acid
cardiovascular disease
bakery products
fatty acid composition
quality studies

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Introduction

Trans fat comprises unsaturated fatty acids having one or more isolated double bonds in the trans geometric configuration. Trans fatty acids (TFA) occur naturally at low levels in ruminant fats and are also formed during the production of partially hydrogenated vegetable oils (Phillips *et al.*, 2010) and contribute longer shelf-life, solidity at room temperature and greater stability during high temperature frying. The major TFA formed by partial hydrogenation of vegetable oils, derived from oleic acid, is elaidic acid (C18:1 Δ 9t or 18:1 trans-9), whereas the main TFA resulting from rumen bio-hydrogenation is vaccenic acid (C18:1 Δ 11t or 18:1 trans-11) (Tardy *et al.*, 2011) and also palmitelaidic acid (C16:1 Δ 9t or 16:1 trans-9) from other ruminant fats (Mozaffarian and Cao, 2010). Trans isomers of linoleic acid (C18:2 Δ 9t Δ 12t, C18:2 Δ 9c Δ 12t and C18:2 Δ 9t Δ 12c) and linolenic acid (C18:3 Δ 9c, 12c, 15t, C18:3 Δ 9t, 12c, 15c & C18:3 Δ 9t, 12c, 15t) are also formed during deodorization of edible oils which is usually carried out at temperatures ranging from 180°C to 270°C (Ratnayake *et al.*, 1997; Martin *et al.*, 2008).

Major dietary sources of industrial TFA include bakery products (e.g., cakes, cookies and pies), deep fried and frozen foods (e.g., French fries, breaded

chicken and fish), packaged snacks (e.g., popcorn), margarines and partially hydrogenated fats directly used for cooking and also ruminant derived foods (dairy products and meat) (Micha and Mozaffarian, 2008). Numerous metabolic and epidemiological studies showed that a high intake of TFA was positively associated with the risk of developing cardiovascular disease (CVD) by increasing serum low density lipoprotein (LDL) cholesterol and decreasing high-density lipoprotein (HDL) cholesterol level relative to cis form of unsaturated fatty acids. Further, TFA showed more adverse effects than saturated fatty acid because the ratio of LDL to HDL cholesterol concentration in high TFA diet was twice higher than in high saturated diet (Mensink and Katan, 1990; Hu *et al.*, 1997). Hence the World Health Organization (WHO) recommended a TFA intake of less than 2.2g/day (1% of overall energy intake) in 2003 (WHO, 2003). Food regulation worldwide has been amended with respect to nutrition labeling and health claims on TFA. As a result, mandatory nutrition labeling of TFA came into effect in many countries such as Korea, United States, Canada, and Denmark. In 2003, the U.S. Food and Drug Administration (FDA) also passed a labeling requirement for trans fat in packaged food products, effective January 1, 2006, requiring it to be reported on the nutrition label if present at ≥ 0.5 g/serving. But allowed to be

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declared zero if <0.5g/serving (US Food and Drug Administration, 2003). India has one of the largest snack markets of the world and people consume more than 400,000 tonnes of snacks every year. Prevention of Food Adulteration Act of India, 1955 require that the foods in which hydrogenated vegetable fats or bakery shortening is used shall declare on the label that "Hydrogenated vegetable fats or bakery shortening used-contains trans fats" and a health claim of 'trans fat free' may be made where the trans fat is less than 0.2 g per serving of food. In India, about 1.1 million metric tons of vanaspati is being produced annually, and a large amount is utilized in confectionery, bakery and ready-to-eat foods (Jeyarani and Reddy, 2005) and most of these food items are unlabelled and there is no regulation to monitor the TFA content and saturated fat content in these products. According to latest recommendations, TFA in oil should not exceed 2%. However, the laboratory tests conducted by Delhi based Center for science and environment (CSE) found TFA levels to be as high as 23% in some vanaspati brands liberally consumed in India (Dhaka *et al.*, 2011). The Indian National Sample Survey Organization, India shows that consumption of beverages, processed foods, salted snacks, prepared sweets and other purchased foods, with an average consumption of 167 gm / capita/day. TFA content of Indian foods could be greater or lower than western foods but has not been systematically studied (Agrawal *et al.*, 2008).

When there are regulations emphasizing on labeling the TFA content, there are arrays of products which are not governed under any regulations which include: cakes, pastries, bun, puffs etc. In the present study labeled as well as unlabelled products (biscuits, pastries, cakes, bread, bun, puffs, rolls, burger, cutlet, samosa and noodles) has been studied for their total fat, trans fat and fatty acid composition. These data generated provide valuable information which can be utilized for laying down regulation and also point out the necessity of bringing regulation on unlabelled products.

Materials and Methods

Reagents and standards

Standards of fatty acids methyl esters (FAME) (C4:0 Methyl butyrate, C6:0 Methyl caproate, C8:0 Methyl capryllate, C10:0 Methyl caprate, C12:0 Methyl laurate, C14:0 Methyl myristate, C14:1-9c Methyl myristoleate, C14:1-9t Methyl myristelaidate, C16:0 Methyl palmitate, C16:1-11c Methyl palmitoleate, C16:1-11t Methyl palmitelaidate, C18:0 Methyl stearate, C18:1-9c Methyl oleate,

C18:1-9t Methyl elaidate, C18:1-11t Methyl vaccenate, C18:2-9, 12 Methyl linoleate (cc, ct, tc and tt isomers), C18:3-9, 12, 15 Methyl linolenate (isomeric mixture), C20:0 Methyl arachidate, C20:5 n-3 Methyl eicosapentaenoate, C22:6 n-3 Methyl docosahexanoate) were purchased from Sigma chemicals (Steinheim, Germany). High Performance Liquid Chromatography (HPLC) grade solvents were purchased from Merck (Darmstadt, Germany). All other chemicals were of laboratory grade from Spectrochem (India).

Samples for study

For the present study 11 classes of bakery food items were selected based on the market survey, which included both labeled and unlabelled products. They were biscuits, pastries, cakes, bread, bun, puffs, rolls, burger, cutlet, samosa and noodle samples. All the samples were purchased from the different bakers of the Kerala region, India, which were selected through market survey.

Fat extraction from the samples

Fat extraction was carried through Folch extraction method (Folch *et al.*, 1957). Twenty-five grams of sample was mixed with 75 ml of a 2:1 (v/v) chloroform/methanol mixture, which was shaken in a 250 ml Erlenmeyer flask by a magnetic stirrer for 45 min. Then, the mixture was filtered and the solid phase was re-extracted one or two times more, respectively, depending on the sample matrix, with the same volume of extractant. The liquid phases were combined in a separatory funnel. Thirty-five milliliters of saturated sodium chloride in water and 0.5 g of sodium perchlorate (NaClO₄) were added, and the mixture was gently shaken. After phase separation, the lower chloroform phase was filtered, dried with sodium sulfate and filtered again. Finally, the extractant was evaporated to dryness under nitrogen stream or rotary evaporator. The total time required was 150 or 270 min, respectively, depending on the sample matrix (Priego-Capote *et al.*, 2007).

Fatty acid composition

FAME was prepared by esterifying with alcoholic sulfuric acid reagent according to the International Union of Pure and Applied Chemistry (IUPAC) procedure (IUPAC, 1987). The FAME was analyzed by using Shimadzu GC 2010 fitted with a split injector (250°C) and a Flame Ionization Detector (FID 300°C). A SP-2560 Supelco column (75 m, 0.18 mm id and 0.14 µm film thickness) was utilized and operated at 200°C. Nitrogen was used as a carrier gas at a velocity

of 13.0cm/s. FAME was identified by comparison of their retention time with authentic standards, and the peaks were quantified by using digital integration according to AOCS official method Ce 1-62 (AOCS, 2003). Fatty acid levels were reported as relative proportions of the total composition.

Free fatty acids (FFA)

Acid value in terms of percentage of free fatty acids (FFA) is defined as number of milligrams of alkali (KOH) required for neutralizing 1 g of fat. FFA contents of all samples were determined using AOCS official method Ca 5a-40, and results were reported as percentage of oleic acid (AOCS, 2003).

Peroxide value (PV)

The peroxide value is defined as the amount of peroxide oxygen per 1 kilogram of fat or oil. Traditionally this was expressed in units of mill equivalents per kilogram. Peroxide values of the all samples were determined by standard AOCS official method Cd 8-53 (AOCS, 2003).

p-Anisidine value

It can be defined as 100 times the O.D measured at 350 nm in 1 cm cuvette of a solution containing 1 g of the oil in 100 ml of the solvent mixture. p-Anisidine values of all samples were determined by standard AOCS official method Cd (18) – 90 (AOCS, 2003).

Totox value

Totox value is the total oxidation value of the oil sample and it can be calculated through peroxide value and p-Anisidine value by following equation: $\text{Totox value} = 2 \times \text{Peroxide value} + \text{p-Anisidine value}$. Totox values of all samples were determined by standard AOCS official method Cg 3– 91 (AOCS, 2003).

Statistical analysis

Statistical analysis was performed using the stat comp software. All the analysis was performed in triplicate and the results are expressed as mean \pm SD.

Results and Discussion

Total fat content, Fatty acid composition and trans fat content in food samples

Labeled and unlabelled bakery products including 5 biscuits, 4 pastries, 5 cakes, 3 bread, 2 bun, 2 puffs, 2 rolls, 2 burger, 2 cutlet, 2 samosa and 2 noodle samples were selected and collected from different bakers through market survey and was analyzed for total fat, trans fat and fatty acid composition. Among the mentioned food products only biscuit samples and 2 bread items were labeled. The detailed description about the labeled products is mentioned in Table 1. Fat content from the all samples were extracted using Folch extraction method and their fatty acid composition are detailed in Table 2. In Figures 1(a) and (b) we have shown a representative chromatogram indicating the presence of elaidic acid (pastry sample) and vaccenic acid (butter bun) respectively. Figures 2 (a), (b), (c) shows total fat, TFA, SFA, MUFA and PUFA content of all the samples along with the energy (kcal) content received from fat and TFA. Each value in the table represents the average value of each category studied.

Total fat content in the samples analyzed was in the order: Cake > puff > cutlet > biscuit > pastries > samosa > roll > bun > burger > noodles > bread. In case of TFA content we observed a different pattern as shown: puff > cake > bun > pastry > bread > cutlet > samosa > roll > bun / noodles > burger. The highest total fat analyzed was $25.36 \pm 2.49\%$ (cake)

Table 1. Description of labeled food products analyzed

Product	Weight (g)	Energy(kcal)/100g	/100g of the sample		Ingredients listed on the product label
			Total fat	Trans fat	
Biscuits					
Sample 1	90	493	23.00	0	Wheat flour, sugar, Edible vegetable oil, Butter (4.2%), Cashew bits (4%), Milk solids, salts, Emulsifiers.
Sample 2	225	472	17.0	0	Wheat flour (47%), sugar, Margarine, Milk solids (4%), Liquid glucose, Salt, Raising agent (E500, E503), Emulsifier (E322).
Sample 3	180	459	14.0	-	Wheat flour (67%), sugar, vegetable oil, Milk solids (1.3%), Salt, Dough conditioner (223), Anti oxidant (319).
Sample 4	159	440	13.3	0	Wheat flour (66%), sugar, vegetable oil, Invert syrup & Liquid glucose (4.9%), Calcium, Dough conditioner (223).
Sample 5	93.75	481	18.4	-	Wheat flour (66%), Chocolate chips (27.2%), sugar, vegetable oil, Invert syrup and Liquid glucose (4.9%), Calcium, Dough conditioner (223), Antioxidant.
Bread					
Sample 1	390	300	1.30	Trace	Sugar, Wheat Flour, Acid residulant (280), Wheat flour, Yeast, salts, Emulsifier, Hydrogenated vegetable fat.
Sample 2	380	276	2.5	-	Water, Sugar, Wheat Flour, Acid residulant (280), Wheat flour, Yeast, salts, Emulsifier, Hydrogenated vegetable fat.

Table 2. Fatty acid composition of food items

Fatty acid	Biscuit	Pastry	Cake	Bread	Bun	Puff	Roll	Burger	Cutlet	Samosa	Noodles
	Weight(%)										
C6	nd ^a	nd ^a	nd ^a	nd ^a	nd ^a	0.05±0.0	0.24±0.03	nd ^a	0.45±0.05	nd ^a	nd ^a
C10	nd ^a	0.04±0.0	nd ^a	nd ^a	nd ^a	0.05±0.0	0.68±0.08	0.49±0.1	0.35±0.05	0.005±0.0	nd ^a
C12	0.43±0.02	1.33±0.2	0.72±0.16	0.02±0.0	0.13±0.01	0.71±0.08	4.6±0.3	3.68±0.28	2.4±0.1	0.045±0.0	0.08±0.0
C14	0.49±0.04	0.71±0.1	0.97±0.21	0.07±0.02	0.19±0.06	0.53±0.10	1.57±0.05	0.64±0.08	0.89±0.08	0.14±0.05	0.08±0.0
C14:1	nd ^a	0.004±0.0	0.07±0.0	nd ^a	nd ^a	nd ^a	0.01±0.0	0.02±0.0	nd ^a	0.002±0.0	0.01±0.0
C16	7.55±0.48	5.67±0.45	10.97±0.66	1.26±0.43	4.69±1.0	9.31±0.45	2.58±0.08	1.07±0.08	5.38±0.4	5.41±0.55	2.44±0.45
C16:1	0.84±0.08	0.13±0.01	0.45±0.02	0.01±0.03	0.02±0.0	0.05±0.0	0.03±0.0	0.01±0.0	0.04±0.0	0.03±0.0	0.01±0.0
C18	1.03±0.03	1.3±0.25	1.51±0.22	0.13±0.07	0.58±0.05	1.46±0.23	0.33±0.05	0.17±0.05	0.71±0.15	0.5±0.05	0.29±0.3
C18:1n	0.01±0.0	0.85±0.1	1.92±0.16	0.18±0.0	1.31±0.57	3.02±0.3	0.12±0.0	0.01±0.0	0.34±0.1	0.18±0.0	0.04±0.0
C18:1	4.98±0.32	4.39±0.5	6.98±0.76	0.87±0.01	3.06±0.2	6.42±0.4	1.98±0.05	1.32±0.1	5.31±0.65	5.46±0.25	2.16±0.35
C18:2n	nd ^a	nd ^a	0.04±0.0	0.007±0.0	0.03±0.0	0.01±0.0	nd ^a	nd ^a	0.01±0.0	0.001±0.0	nd ^a
C18:2(6n)	0.01±0.0	0.04±0.0	0.65±0.12	0.39±0.05	0.07±0.0	0.07±0.0	0.01±0.0	0.005±0.0	0.03±0.0	0.03±0.0	0.001±0.0
C18:2	1.43±0.24	1.56±0.1	0.74±0.06	0.13±0.0	0.6±0.13	0.94±0.03	0.52±0.05	1.71±0.2	1.34±0.28	1.49±0.2	0.48±0.1
C18:3	nd ^a	nd ^a	0.34±0.12	0.03±0.0	0.04±0.0	0.01±0.0	0.02±0.0	0.06±0.0	0.01±0.0	0.02±0.0	0.01±0.0
C20	0.04±0.0	nd ^a	0.004±0.0	nd ^a	nd ^a	0.04±0.0	0.01±0.0	nd ^a	0.02±0.0	0.0001±0.0	nd ^a
Fat	16.81±1.2	16.02±1.7	25.36±2.49	3.09±0.61	10.72±2.02	22.67±1.59	12.7±0.69	9.19±0.89	17.28±1.86	13.3±1.1	5.60±1.2

Each value in the table represents average (±SD) n= 3

^a not detected

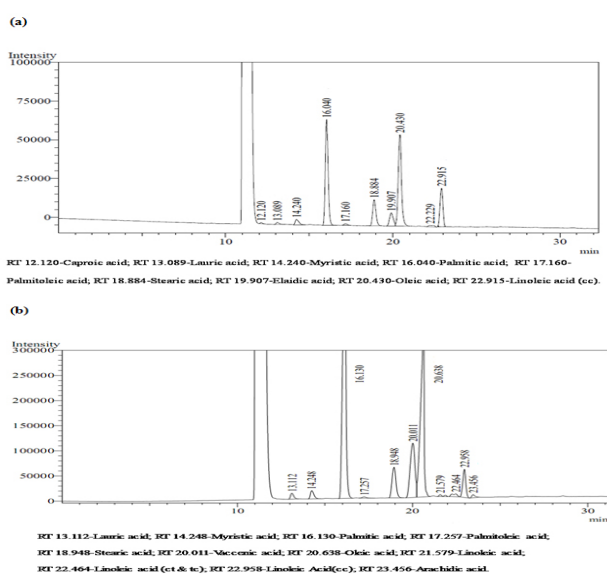


Figure 1. (a) GC of fatty acid profile in pastry (Elaidic acid is major *trans* form) (b) GC of fatty acid profile in butter bun (Vaccenic acid is major *trans* form)

and highest *trans* fat content detected was 3.09±1.5% (puff).

Quality studies

FFA, PV, p-Anisidine value and totox value of fat samples that were extracted from the selected bakery food items, were evaluated to determine the quality of the food products. The results of above mentioned parameters are detailed in Table 3. Each

value in the table represents the average value of each category studied. Biscuit samples had least FFA value (0.52±0.20%), while cake samples (2.44±0.59%) and noodle samples (2.16±0.5%) had higher FFA value. All other samples showed greater FFA values comparatively. Higher FFA value causes high acidity of the oils which was caused by the breakdown of fat after storage or use.

All samples had higher peroxide values in which bread samples showed highest PV (35.8±3.07 mEq/kg). Biscuit, samosa, burger, roll and puff samples showed higher peroxide values which corresponds to very poor quality of fat, which normally would have significant off flavors. p-Anisidine values were also higher as peroxide values. Noodle samples showed lowest p-Anisidine value (1.6±0.35). Bread, biscuits and burger samples showed higher p-Anisidine values, (bread - 18.18±2.57, biscuits - 14.59±1.78, burger - 3.1±0.35). High p-Anisidine value showed the oxidative degradation of fat in the food samples.

Totox values were also higher as PV and p-Anisidine values. Among the all samples bread samples (89.78±8.72 mEq/kg) and biscuit samples (70.62±9.52 mEq/kg) had higher totox values. TFA analysis was carried out on the selected bakery food items after extracting the fat from the samples by Folch extraction method. Folch extraction method was selected for fat extraction from the food items because of its mild working conditions – neither heat

Table 3. Quality studies of extracted fat from various bakery food items on average

Sample	FFA ^a (%Oleic acid)	PV ^b (mEq/kg)	p-Anisidine value	Totox value ^c (mEq/kg)
Biscuit	0.52±0.20	28.02±3.87	14.59±1.78	70.62±9.52
Pastry	1.29±0.38	19.68±2.25	3.53±1.15	42.87±5.65
Cake	2.44±0.59	24.98±2.61	3.8±1.06	53.78±6.27
Bread	4.17±0.26	35.8±3.07	18.18±2.57	89.78±8.72
Bun	0.83±0.07	18.1±2.27	14.45±1.76	50.65±6.29
Puff	3.27±0.09	22.7±2.3	3.5±1.5	48.9±6.07
Roll	2.6±0.735	21.6±1.4	2±0.55	43±3.35
Burger	1.54±0.43	22.5±2.45	3.1±0.35	48.1±5.25
Cutlet	2.3±0.28	20.3±1.65	2.7±0.5	43.3±6.79
Samosa	1.16±0.25	25.7±3.2	2.3±0.6	53.7±7
Noodles	2.16±0.5	14.6±1.5	1.6±0.35	30.8±3.35

Each value in the table represents average (±SD) n= 3

^a Free Fatty acid value, ^b Peroxide value, ^c Total oxidation value

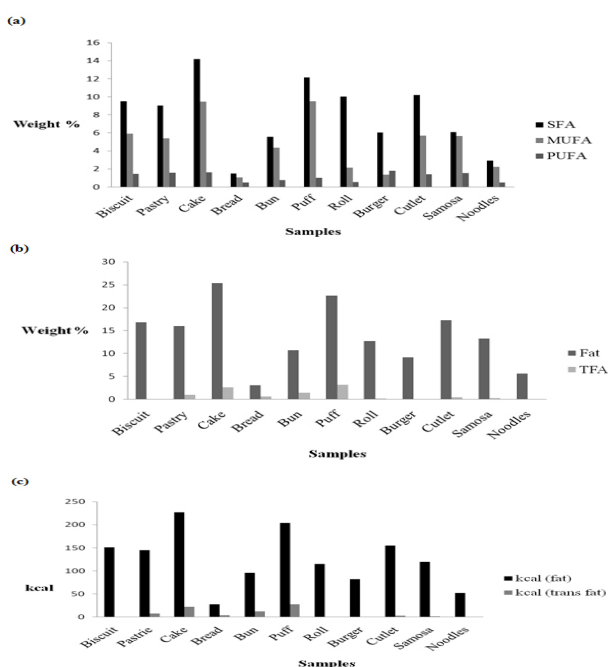


Figure 2. (a) Saturated fat, unsaturated fat and polyunsaturated fat content of food items (b) Fat content and *trans* fat content of food items (c) Energy (kcal) derived from fat and *trans* fat consumption from food items

nor high pressures are applied, which avoid potential alterations of the fat extracted (Priego-Capote *et al.*, 2007). Fat from all varieties of foods were easily extracted in less time, by using this method. GC was selected for analysis of *trans* fat because it's high sensitivity, which allows quantification of individual fatty acids, even at low concentrations (Phillips *et al.*, 2010). FAME was preferred because they are highly volatile and easy to produce, although other derivatization techniques may be used for specific purposes, notably for methods other than GC (Juaneda *et al.*, 2007). Higher length of capillary column facilitated better resolution between the eluted peaks in the chromatogram.

The difference in pattern obtained for total fat content and TFA content in the samples analyzed can be attributed to the fat / shortening / margarine that have been specifically used to contribute individual functional properties to samples. The major *trans* form found in all samples except butter bun was elaidic acid, which was originated from partially hydrogenated vegetable oil. Butter bun showed vaccenic acid as major *trans* form. During our analysis of bun samples we have selected 1 sweet bun and 1 butter bun and the average value is shown, thus the high value for total fat as well as TFA obtained for bun samples can be attributed to butter bun samples as it is different from normal sweet bun. We have observed that products with low TFA content had higher SFA content, which is another cardiovascular risk factor. The predominant saturated fatty acid found in pastry, roll and burger samples was lauric acid and palmitic acid. This showed greater versatility in the utilization of different cooking oils to prepare same food items by different bakers. Oleic acid was major monounsaturated fatty acid in most of the samples. *Trans* myristelaidic acid and *trans* palmitelaidic acid forms were not detected in any of the samples. Linoelaidic acid was detected mainly in cake, bread and cutlet samples. Linoleic acid was found as major polyunsaturated fatty acid in burger, biscuit and cake samples.

For a sedentary worker, recommended dietary allowance (RDA) of energy is 1800 kcal. In such a case if a person consumes 100 g of cake, total fat intake will be between 20.10±2.34% – 29.4±2.8%, SFA intake will be between 9.42±0.54%– 19.45±2.5% and TFA intake will be 1.53±0.2% – 3.20±1.0%. If the highest figure (29.4±2.8%) considered, the total energy obtained from fat alone will be 264.6±25.9 k.cal. in which energy contribution from SFA will be 175.05±22.5 k.cal. and that from TFA will be 23.31±0.9 k.cal. The permitted RDA from SFA is 10% i.e 180 k.cal and from TFA is 1% i.e 18 k.cal. If the same person goes with high fat diet, resultant energy may exceed the permitted level imposing the individual to many health issues. Majority of bakery food items were having *trans* fatty acids, whose values had almost crossed the limit recommended by WHO. Serving size is an important factor which contributes to total fat and TFA intake. For example one piece of puff weighing 115g, contribute TFA content of 3.02±0.5 – 3.15±0.1%. The resultant energy from TFA alone will be 27.18±4.5% – 28.35±0.9%. It is more than the limit specified by WHO i.e. <1% of the total energy should come from TFA. If the same person consumes some similar food product, TFA intake will increase further. On studying the quality

of fat in all these products we observed higher FFA, PV, p- Anisidine and totox values, indicating the poor quality either contributed by the processing (baking) or storage deterioration.

Gas chromatographic evaluation of trans fat is widely accepted method and is having high sensitivity i.e even much lower levels of TFA can be analyzed. It is achievable to get better resolution between cis and trans forms by using higher length of columns. But, simple gas chromatographic evaluation method has limitation in getting clear separation of different types of trans fatty acids in same sample, which has to be improved to avoid the underestimation of TFA content in products where both animal fats and partially hydrogenated fats are utilized during cooking. Co-elution of different isomers of same fatty acids will lead to error in the calculation of individual fatty acid levels.

When there are regulations emphasizing on labeling the TFA content, there are arrays of unlabelled products which are not governed under any regulations which include: cakes, pastries, bun, puffs, namkeens etc. Among the food items studied only biscuits samples and 2 bread samples were labeled, all the other samples were unlabelled and consumers are totally ignorant about the ingredients and quality of these products. The present study shows the significance of studying all similar products and point out the need for laying regulation on all such products. There is a need of strong food regulations especially in the developing countries to bring levels of trans fat in the processed foods to negligible levels.

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References

- AOCS. 2003. Official methods and recommended practices of the American Oil Chemist's Society. In Firestone (Ed.) 5th edition. Champaign. IL.
- Agrawal, A., Gupta, R., Varma, K. and Mathur, B. 2008. High trans fatty acid content in common Indian fast foods. *Nutrition and Food Science* 38: 564-569.
- Dhaka, V., Gulia, N., Ahlawat, K. and Khatkar, B. 2011. Trans fats – Sources, health risks and alternative approach – A review. *Journal of Food Science and Technology* 48: 534-541.
- Folch, J., Lees, M. and Stanley, G. H. S. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 226: 497-509.
- 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.
- IUPAC. 1987. Standard methods for the analysis of oils and fats and derivatives. 7th ed. London: Blackwell Scientific Publications.
- Jeyarani, T. and Reddy, S. Y. 2005. Physicochemical Evaluation of Vanaspathi Marketed in India. *Journal of Food Lipids* 12: 232-242.
- Juaneda, P., Ledoux, M. and Sebedio, J.L. 2007. Analytical methods for determination of trans fatty acid content in food. *European Journal of Lipid Science and Technology* 109: 901-917.
- Martin, C. A., de Oliveira, C. C., Visentainer, J. V., Matsushita, M. and de Souza, N. E. 2008. Optimization of the selectivity of a cyanopropyl stationary phase for the gas chromatographic analysis of trans fatty acids. *Journal of Chromatography A* 1194: 111-117.
- Mensink, R. P. and Katan, M. B. 1990. Effect of dietary trans fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *New England Journal of Medicine* 323: 439-45.
- Micha, R. and Mozaffarian, D. 2008. Trans fatty acids: effects on cardiometabolic health and implications for policy. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 79: 147-152.
- Mozaffarian, D., Cao, H., King, I. B., Lemaitre, R. N., Song, X., Siscovick, D. S. and Hotamisligil, G. S. 2010. Trans-Palmitoleic Acid, Metabolic Risk Factors, and New-Onset Diabetes in U.S. Adults. *Annals of Internal Medicine* 153: 790-799.
- Phillips, K., Ruggio, D. and Amanna, K. 2010. Optimization of Standard Gas Chromatographic Methodology for the Determination of trans Fat in Unlabeled Bakery Products. *Food Analytical Methods* 3: 277-294.
- Priego-Capote, F., Ruiz-Jiménez, J. and de Castro, M. D. L. 2007. Identification and quantification of trans fatty acids in bakery products by gas chromatography-mass spectrometry after focused microwave Soxhlet extraction. *Food Chemistry* 100: 859-867.
- Ratnayake, W. M. N., Chardigny, J. M., Wolff, R. L., Bayard, C. C., Sébédio, J. L. and Martine, L. 1997. Essential Fatty Acids and Their Trans Geometrical Isomers in Powdered and Liquid Infant Formulas Sold in Canada. *Journal of Pediatric Gastroenterology and Nutrition* 25: 400-407.
- Tardy, A.L., Morio, B., Chardigny, J.M. and Malpuech-Brugère, C. 2011. Ruminant and industrial sources of trans-fat and cardiovascular and diabetic diseases. *Nutrition Research Reviews* 24: 111-117.
- U.S. Food and Drug Administration. 2003. Food labeling: trans fatty acids in nutrition labeling, nutrient content claims, and health claims. 68. Federal Register 41433-41506
- WHO. 2003. World Health Organization, Diet, nutrition and the prevention of chronic diseases. Report of a Joint WHO/FAO Expert Consultation. WHO Technical Report Series 916.