Studies on saturated and *trans* fatty acids composition of few commercial brands of biscuits sold in Indian market

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Abstract Saturated fat and *trans* fat consumption is linked to cardiovascular disease. Considering the health implications of saturated and *trans* fats investigation was undertaken with the objective to study the fat compositions in biscuits sold in Indian market. These commercial biscuits were analysed for saturated and *trans* fatty acids using capillary GC. The results of analysis of 46 biscuit samples showed that the total fat content ranged from 9.5 to 25.0 g/100 g of biscuits. The fatty acid profile showed that, saturated fat content in biscuits ranged from 5.1 to 18.7 g/100 g. The overall range of total *trans* fat content was found to be 0.1 to 3.2 g/100 g biscuit and *cis* monounsaturated fatty acid content varied from 0.9 to 8.6 g/100 g of biscuits. The low-level *trans* fatty acid was mainly by dienes and trienes where as high-level *trans* was from monoenes of C18. Polyunsaturated fatty acids in biscuits ranged from 0.2 to 3.5 g/100 g. Biscuits of same brand on repeat analysis over a period of one year showed little variation in fat, saturated and *trans* fat content.

Keywords Saturated fat · *Trans* fatty acid · Biscuit · Cis unsaturated fatty acids · Polyunsaturated fatty acids

Introduction

Biscuits are widely consumed processed food products in India. According to Food Safety and Standards Authority of India (FSSAI), apart from maida (refined wheat flour) and sugar among major ingredients, biscuit formulation may contain vanaspati (partially hydrogenated vegetable oil) or refined

edible oil, table butter, butter, ghee, margarine or their mixture with other minor ingredients (FSSAI 2010). Food Safety and Standards Act of India specifies ash insoluble in dilute hydrochloric acid and acidity of extracted fat as quality parameters for biscuits. However, standards on nutrition quality of the fats used in the preparation of biscuits are not specified. Production of biscuits both in organized and unorganized sectors was estimated to be around 0.625 million tons during year 2003 as compared to 0.36 million tons during 1997 (FBMI 2003). The production has doubled within 6 years. The unorganized biscuit sector is estimated to have approximately 30,000 small & tiny bakeries across the country. Organized biscuit industry in India produces around 60 % of the total production, the remaining 40 % being contributed by the unorganized bakeries (FBMI 2003; Semwal et al. 1997).

It is evident that the use of refined oils for the biscuit preparation will decrease the oxidative stability and provides poor texture and shorter shelf life (O' Brien 2004; Stauffer 1996). The fats used in the manufacture of biscuits consists of both animal fat and vegetable shortenings with suitable solid fat index (SFI) and solid fat content (SFC) (Stauffer 1996; Isabel et al. 2003). The label declaration of the Indian commercial biscuits used in this study revealed the presence of either edible refined oil or hydrogenated oils as the fat ingredient. The partially hydrogenated vegetable oils (PHVO), also known as vanaspati is also being used in India for baking and frying process. Usually vanaspati is made up of vegetable oils and exist in the semi solid form at room temperature (Vandana et al. 2011; Stauffer 1996).

The consumption of saturated fatty acids (SFA) and *trans* fatty acids (TFA) is associated with the risk of increasing cardiovascular diseases. TFA increases the risk of cardiovascular diseases, by raising the LDL-cholesterol, decreasing the HDL-cholesterol, proinflammatory changes, endothelial dysfunction, and possibly by insulin resistance and displacement of essential fatty acids from membranes (O' Brien 2004;

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Stauffer 1996; Mozaffarian et al. 2006; Vandana et al. 2011). There are reports that the consumption of monounsaturated (MUFA) and polyunsaturated fatty acid (PUFA), as well as long chain omega-6 and omega-3 fatty acids is beneficial to health (O' Brien 2004; Stauffer 1996). As a consequence of this, the formation of *trans* isomers during partial hydrogenation and high-temperature physical refining has become an important risk parameter for health, as the TFA present in the fats are carried over to the food products. Countries like USA, Canada, and European Union have introduced mandatory and voluntary regulation to label TFA content in foods (Vandana et al. 2011; Jeffrey 2005). Denmark has restricted the TFA content by setting the limit as 2 % in oils and fats used for food preparation (Jeffrey 2005). The United States Food and Drug Administration (USFDA) in their final rule specify the requirement of an additional declaration of *trans* fatty acids in grams per serving just below the saturated fat content in nutrition label of foods. Those foods with less than 0.5 g TFA per serving may declare the food as containing zero grams of *trans* fat or not a significant source of *trans* fat at the bottom of label (Vandana et al. 2011; Jeffrey 2005).

As per the proposed regulation on TFA in partially hydrogenated vegetable oils (vanaspati) by FSSAI recommendation, TFA content should not exceed 10 % of total fatty acids (Vandana et al. 2011). FSSAI also recommends for mandatory labeling of TFA and SFA content of all edible oils and fats. In present situation biscuit is a hygienically packaged nutritious snack food; consumed by all strata of society which is available at very competitive prices, different volumes and tastes to people, particularly children. Though India is considered as the third largest producer of biscuits after USA and China, the per capita consumption of biscuits in India is only 2.1 kg, as compared to more than 10 kg in the USA, UK and West European countries (FBMI 2003). Biscuits in Indian market also claim to be the delivery source of many nutrients like calcium, Iron, fiber etc. The quality assessment of fats used in the preparation and the role of dietary fats on consumer health is not given importance. Also there is a lack of information about biscuits being source of individual fatty acids such as *cis*-oleic acid, linoleic acid and α - linolenic acid (ALA), which are beneficial to health, and also the hydrogenated oils used in biscuit manufacture contain such essential fatty acids. The World Health Organization (WHO) recommends that no more than 10 % of the total energy intake of humans should be from the consumption of saturated fats and no more than 1 % should be derived from TFA consumption. This is based on 2,000 kcal total daily energy derived from food per person (Vandana et al. 2011). The purpose of this study is to determine the *trans* fat and saturated fat content of biscuits consumed by Indian population. When industrially produced TFA and SFA of the low chain fatty acids pose the public health concern, present study on TFA and SFA in biscuits gives an insight to shift food composition toward healthier product formulations.

Materials and method

The biscuit samples analysed in this study were collected from 2009 to 2011, from Mysore City, Karnataka State, India. The sample weight collected was 250 g each and they were grouped into three types.

1. Plain biscuits- Glucose, Marie, Milk, Sweet and Salt, cracker type-20nos.
2. Cookies – (soft textured with high fat content)-Butter, Cashew, Coconut, Chocolate type-12 nos.
3. Cream biscuits- (sandwich type of biscuit with a layer of fat and flavored cream in between two shells)-14 nos.

These brands of biscuit samples from Mysore are available in any other city/place of India, biscuit samples sold loose were exempted from this study. The type of biscuits indicated as **a**, **b**, **c** were analysed during 2009–2010. Repeat analysis of the same brand biscuit (analysed during 2010–2011), but with different manufacturing date, is indicated as **a1**, **b1**, and **c1** in this manuscript/Tables. Milk cream **a** brand was procured and analysed one more time during 2010–2011 indicated as Milk cream **a2**.

Sample preparation and GC analysis

The extraction of fat in biscuit was first carried out by acidic hydrolysis as per the slightly modified official methods (AOAC 2005). Extracted fat was methylated to fatty acid methyl esters (FAMES) (AOCS 1998), injected three times and quantified using capillary gas chromatography. According to Nutritional Labeling Act (NLEA) of 1990 and Federal register 1993a, total fat is defined as the sum of all fatty acids obtained from total lipid extract expressed as triglycerides (Subramaniam et al. 2004; Jeffrey 2005). Prepared methyl esters were randomly checked for the impurities and completeness of esterification reaction for all types of biscuit samples by preparative thin layer chromatography, with hexane-diethyl ether (9:1, V/V) as the mobile phase (Christie 1989; Wolff et al. 2000). Total fat was estimated as sum of individual fatty acids expressed as triglyceride equivalents by multiplying with conversion factors. Saturated, *cis* monounsaturated, *trans* unsaturated (18:1 t, 18:2 t, 18:3 t) and polyunsaturated fats were calculated as sum of respective fatty acids (AOAC 2005).

The fatty acid methyl esters were extracted with hexane, and analysed using capillary column HP-88 (30mX0.25mmIDX0.20 μ m-Agilent J&W GC columns, USA) fitted into Shimadzu GC-2010, (Kyoto, Japan). Operating conditions were as follows: injector temperature 250 °C; detector temperature 270 °C; column temperature programmed at 80 °C for 2 min initially, then increased at

5 °C/min to 180 °C and held for 10 min and finally increased at 5 °C/min to 220 °C and held for 10 min. Nitrogen was used as carrier gas at a flow rate of 0.8 ml/min with a linear velocity of 27 cm/s. The fatty acids were identified by comparing their retention times with those of authentic standards (Supelco FAME mix with 37 components and Linoleic acid isomer mix, Linolenic acid isomer mix-Bellefonte, PA, USA). Also GC instrument operated under the set conditions showed area, weight relationships very close to linearity for all the fatty acids found in biscuit samples.

Statistical analysis

Data obtained was statistically analysed using Duncan's multiple range test (DMRT). The significant level was established at $p \leq 0.05$ (Duncan 1955).

Results and discussion

TFA and SFA distribution in biscuits samples

Results pertaining to fatty acid composition, especially those of TFA and SFA of 46 biscuit samples are as shown in Tables 1, 2, and 3. On analyzing the fat extracted from biscuits, the maximum TFA content was found in cookie variety of biscuit samples (Butter **b**), and maximum SFA content was found in Coconut cookie **a**. The contribution of 18:1 *t* towards TFA content in biscuit fat was maximum, and 18:2 *t* was from *cis trans* (ct) and *trans cis* (tc) isomers. The *trans trans* (tt) isomer of 18:2 was present in negligible quantity. The 18:3 *trans* isomers were mainly comprised of *trans cis trans* (tct) and *cis trans cis* (ctc) isomers. The fatty acid composition also revealed the presence of either lauric acid or palmitic acid, as predominant fatty acid among saturated fatty acids. Oleic acid represented major MUFA; where as linoleic acid was major fatty acid in PUFA. The maximum extracted fat content of 25.0 g/100 g was found in cashew cookies **b**. Marie **c** had minimum fat content of 9.5 g/100 g among all biscuits. Glucose **a1**, biscuit, sweet and salt **b** biscuits had lowest TFA 0.1 g/100 g among all the biscuits. Coconut **a** biscuit had maximum TFA + SFA.

Plain biscuits

Minimum SFA + TFA were found in Oats plain biscuits at 5.3 g/100 g. Glucose **a1** biscuit had lowest TFA and lowest SFA among all the analysed biscuit samples. Sweet and salt **a** biscuits had highest MUFA + PUFA. Salt **a** biscuit had highest TFA of 1.4 g/100 g and SFA + TFA content of this biscuit was 10.7 g/100 g. This biscuit also had maximum fat content in plain biscuit category. Four high fiber and digestive biscuits

analysed under plain biscuits. High fiber **a** biscuit had high PUFA of 3.5 g/100 g and Multi cereals, High fiber **a**, High fiber **b** biscuits covered under plain biscuits had high PUFA as compared to other biscuits.

Cookie biscuits

Cookie type of biscuits had high SFA + TFA from 8.3 to 20.3 g/100 g. Coconut cookies had high SFA-18.7 g/100 g and very low MUFA-0.9/100 g and PUFA content up to 0.2 g/100 g. Fat content of these biscuits ranged from 18.7 to 25 g/100 g. Chocolate flavour, butter and cashew **a1** cookies had maximum PUFA of 2.0 g/100 g. The butter **b** and cashew **a1** cookies had maximum MUFA content of 8.3 g/100 g of sample. Butter cookies showed fatty acid profile similar to milk fat.

Cream biscuits

Fat content of cream biscuits was similar to cookies and some high fat plain biscuits. Presence of cream fillings in between two biscuits did not increase the fat content of the biscuits. Milk cream biscuits had fatty acid compositions of lauric fats. Milk cream **a** and Milk cream **a2** biscuits had similar TFA and PUFA. Milk cream **a** biscuits had maximum SFA and TFA + SFA. Chocolate flavor **a1** had maximum MUFA 7.0 g/100 g among cream biscuits. Orange flavor **a1** biscuits had low TFA + SFA. Cardamom flavored biscuit in cream biscuit category had unusual high PUFA among all the biscuits. The PUFA content was from both linoleic acid and linolenic acid. This may be attributed to the addition of cardamom oil in the filling material (Samer et al. 2008). The consistency of the fat used for filling of cream biscuits was decided by the SFI profile of the fat. The fat for filling should have soft consistency to easily spread onto the biscuit wafers, the icing must be firm at room temperature without sliding through the sandwich wafers, and the icing should melt completely at mouth temperature (Stauffer 1996). To meet these requirements the SFI profile of filler fat should be less than 2 at 30 °C and specific density in the range of 0.8–1.1 g/ml (Stauffer 1996).

Many biscuits covered under this study showed fatty acid profile similar to lauric fat indicating the use of palm kernel fat instead of hydrogenated fat (bakery shortening) in order to reduce the TFA content (Fig. 1). This has made milk cream biscuit, butter flavored cookies to give high SFA. The fatty acid profile of Marie **a** biscuits and Milk cream **a** are as shown in Fig. 2. MUFA, PUFA present in fat is considered to be healthy, but high PUFA content alters the plasticity, sensory aspects and SFI of fat, hence these fats are not suitable in biscuit preparation.

USDA study report on cookies and crackers reported TFA content of 8 to 35 g/100 g of fat and sum of TFA + SFA of these products ranged from 39 to 58 g/100 g of fat in biscuit samples of USA (Subramaniam et al. 2004). The maximum total TFA found

Table 1 Saturated fat and *trans* fat in plain biscuits

Biscuit type	Fat (g/100 g of biscuit)	g/100 g of fat				g/100 g of product (biscuit)				
		18:1 t	18:2 t	18:3 t	Total TFA	Total TFA	SFA	MUFA	PUFA	TFA + SFA
Marie a	10.2 ^b	0.8 ^d	1.8 ^f	0.3 ^b	2.9 ^f	0.3 ^d	5.7 ^c	3.1 ^{cd}	1.0 ^c	6.0 ^d
Marie b	13.5 ^f	0.5 ^c	1.4 ^e	0.3 ^b	2.2 ^{de}	0.3 ^d	6.8 ^e	4.7 ^{gh}	1.5 ^f	7.1 ^e
Marie c	9.5 ^a	2.0 ^g	1.8 ^f	0.4 ^c	4.2 ⁱ	0.4 ^e	5.2 ^b	2.8 ^c	1.0 ^c	5.6 ^c
Marie a1	10.5 ^b	0.3 ^b	1.9 ^g	0.6 ^e	2.8 ^f	0.3 ^d	5.2 ^a	4.2 ^f	0.6 ^b	5.5 ^{ab}
Marie b1	11.2 ^a	0.0	1.1 ^c	0.5 ^d	1.6 ^c	0.2 ^c	5.5 ^b	3.8 ^c	1.5 ^f	5.7 ^{bc}
Glucose a	12.4 ^e	1.5 ^f	1.2 ^d	0.5 ^d	3.2 ^g	0.4 ^e	6.7 ^d	3.8 ^c	1.3 ^{de}	7.1 ^e
Glucose b	11.7 ^d	1.3 ^e	1.5 ^e	0.6 ^e	3.4 ^h	0.4 ^e	6.2 ^c	4.0 ^{ef}	1.3 ^{de}	6.6 ^{de}
Glucose c	11.2 ^d	2.3 ^g	1.0 ^c	0.3 ^b	3.6 ^h	0.4 ^e	7.8 ^{fg}	2.1 ^a	0.7 ^b	8.2 ^f
Glucose a1	10.9 ^{ab}	0.2 ^a	0.2 ^a	0.3 ^b	0.7 ^a	0.1 ^a	5.3 ^b	4.1 ^f	1.2 ^d	5.4 ^{ab}
Salt a	20.4 ⁱ	2.8 ^h	3.6 ⁱ	0.5 ^d	6.9 ^k	1.4 ⁱ	15.5 ^l	2.7 ^b	0.7 ^b	16.9 ^k
Salt b	19.4 ^k	2.2 ^g	4.1 ^j	0.4 ^c	6.7 ^k	1.3 ^h	13.3 ^k	3.1 ^d	1.4 ^{ef}	14.6 ^j
Sweet & salt a	22.4 ^g	0.2 ^a	0.5 ^b	0.3 ^b	1.0 ^b	0.2 ^c	10.5 ⁱ	8.6 ^j	3.0 ^h	10.7 ^h
Sweet & salt b	14.3 ^g	0.2 ^a	0.4 ^b	0.4 ^c	1.0 ^b	0.2 ^c	7.2 ^c	5.6 ⁱ	1.1 ^{cd}	7.4 ^c
Milk biscuit	14.8 ^h	2.2 ^g	1.9 ^g	0.2 ^a	4.3 ⁱ	0.6 ^g	8.2 ^g	4.3 ^{fg}	1.5 ^f	8.8 ^g
High fiber a	21.1 ^k	0.3 ^b	1.8 ^f	0.3 ^b	2.4 ^e	0.5 ^f	11.9 ^j	5.0 ^h	3.5 ⁱ	12.4 ⁱ
High fiber b	14.1 ^g	0.8 ^d	2.2 ^h	0.3 ^b	3.5 ^h	0.5 ^f	6.5 ^d	4.6 ^{gh}	2.3 ^g	7.0 ^e
Multi cereals	21.8 ^{bc}	0.3 ^b	1.2 ^d	0.5 ^d	2.0 ^d	0.4 ^e	10.4 ⁱ	8.4 ^j	2.4 ^g	10.8 ^h
Oats	10.6 ^{bc}	0.2 ^a	1.1 ^c	0.2 ^a	1.5 ^c	0.2 ^b	5.1 ^a	4.0 ^e	1.3 ^{ef}	5.3 ^a
Crackers	14.4 ^g	0.5 ^c	2.0 ^h	0.5 ^d	3.0 ^g	0.4 ^e	8.6 ^h	4.9 ^h	0.3 ^a	9.1 ^g
Coconut	14.1 ^g	0.2 ^a	1.5 ^e	0.6 ^e	2.3 ^e	0.3 ^d	7.1 ^f	5.5 ⁱ	1.0 ^c	7.4 ^f

Means followed by the same superscripts in a column are not significantly different at $p \leq 0.05$ *t-trans*, 18:1 t, 18:2 t, 18:3 t- total *trans* isomers of oleic, linoleic and linolenic acid, *SFA*-saturated fatty acid, *MUFA*-mono unsaturated fatty acid, *PUFA*-poly unsaturated fatty acid, *TFA*-total *trans* fatty acid

in butter **b** cookies in this study was 17.1 g/100 g of fat and maximum SFA found in milk cream **a** biscuit was 87.7 g/100 g of fat. The companies might have changed the fat formulations to

lower TFA, used in the preparation of biscuits. A study on TFA content from processed foods in 43 servings and 20 countries in duration of 1 year is found to be varied from country to country

Table 2 Saturated fat and *trans* fat in cookies

Biscuit type	Fat (g/100 g of biscuit)	g/100 g of fat				g/100 g of product (biscuit)				
		18:1 t	18:2 t	18:3 t	Total TFA	Total TFA	SFA	MUFA	PUFA	TFA + SFA
Butter a	22.0 ^c	1.3 ^a	3.3 ^e	1.1 ^c	5.7 ^b	1.3 ^a	16.3 ^f	3.3 ^c	0.8 ^b	17.6 ^f
Butter b	18.7 ^a	11.5 ^h	5.0 ^f	0.6 ^{ab}	17.1 ^h	3.2 ^f	5.1 ^a	8.3 ^h	1.9 ^d	8.3 ^b
Butter a1	20.3 ^c	4.2 ^{de}	0.9 ^a	1.3 ^{cd}	6.4 ^c	1.3 ^{ab}	10.3 ^b	6.5 ^f	2.0 ^e	11.6 ^c
Cashew a	23.3 ^f	2.8 ^b	2.6 ^d	1.6 ^d	7.0 ^a	1.5 ^{bc}	15.4 ^e	5.0 ^d	1.3 ^c	16.9 ^b
Cashew a1	23.1 ^f	3.0 ^c	2.2 ^c	0.8 ^b	6.0 ^b	1.4 ^b	11.1 ^c	8.3 ^h	2.1 ^e	12.5 ^a
Cashew b	25.0 ^g	3.2 ^c	1.8 ^{bc}	1.0 ^c	6.0 ^b	1.5 ^{bc}	15.5 ^g	6.1 ^f	1.7 ^d	17.0 ^g
Pista, Almond	20.6 ^c	5.6 ^f	1.8 ^{bc}	0.8 ^b	8.2 ^e	1.7 ^d	12.4 ^c	5.4 ^e	0.9 ^b	14.1 ^d
Coconut a	21.7 ^{de}	4.8 ^e	2.0 ^c	0.6 ^{ab}	7.4 ^d	1.6 ^{cd}	18.7 ^h	0.9 ^b	0.3 ^a	20.3 ^h
Coconut a1	19.7 ^b	6.8 ^g	2.5 ^d	0.8 ^b	10.1 ^g	1.7 ^d	16.8 ^{fg}	0.9 ^a	0.2 ^a	18.5 ^g
Chocolate	21.3 ^d	6.5 ^g	2.1 ^c	0.8 ^b	9.4 ^f	2.0 ^e	13.4 ^d	4.4 ^d	1.3 ^c	15.4 ^e
Pieces										
Chocolate fl.	21.1 ^d	3.9 ^{cd}	2.5 ^d	1.2 ^c	7.6 ^d	1.6 ^{cd}	10.1 ^b	7.2 ^g	2.0 ^e	11.7 ^c

Means followed by the same superscripts in a column are not significantly different at $p \leq 0.05$ *t-trans*, 18:1 t, 18:2 t, 18:3 t- total *trans* isomers of oleic, linoleic and linolenic acid, *SFA*-saturated fatty acid, *MUFA*-mono unsaturated fatty acid, *PUFA*-poly unsaturated fatty acid, *TFA*-total *trans* fatty acid, *fl.*-flavoured

Table 3 Saturated fat and *trans* fat in cream biscuits

Biscuit type	Fat (g/100 g of biscuit)	g/100 g of fat				g/100 g of product (biscuit)				
		18:1 t	18:2 t	18:3 t	Total TFA	Total TFA	SFA	MUFA	PUFA	TFA + SFA
Milk cream a	21.1 ^f	2.2 ^a	1.0 ^a	0.6 ^{ef}	3.8 ^a	0.8 ^a	18.5 ^h	0.8 ^a	0.7 ^b	19.3 ⁱ
Milk cream a1	19.3 ^d	4.9 ^f	2.1 ^{ef}	0.8 ^f	7.8 ^e	1.5 ^c	11.8 ^d	5.0 ^g	0.8 ^b	13.3 ^c
Milk cream a2	20.0 ^{ef}	2.5 ^{ab}	1.1 ^a	0.4 ^{bc}	4.0 ^a	0.8 ^a	14.6 ^f	3.6 ^e	0.8 ^b	15.4 ^f
Strawberry fl. a	19.1 ^d	4.6 ^{ef}	1.6 ^c	0.6 ^{ef}	6.8 ^c	1.3 ^d	13.7 ^e	2.8 ^d	1.1 ^c	15.0 ^f
Strawberry fl. a1	20.2 ^{ef}	3.2 ^c	1.8 ^d	0.4 ^{bc}	5.4 ^b	1.1 ^b	16.6 ^g	1.3 ^b	1.0 ^c	17.7 ^h
Cardamom fl.	21.5 ^f	4.3 ^d	2.2 ^f	0.5 ^{de}	7.0 ^c	1.5 ^e	11.0 ^d	3.7 ^e	5.1 ^f	12.5 ^d
Chocolate fl. a	15.8 ^a	5.2 ^h	1.4 ^b	1.0 ^g	7.6 ^e	1.2 ^c	8.5 ^b	4.3 ^f	1.6 ^d	9.7 ^b
Chocolate fl. a1	19.2 ^d	5.0 ^g	2.1 ^{ef}	0.7 ^f	7.8 ^e	1.5 ^e	8.4 ^b	7.0 ^j	2.1 ^e	9.9 ^c
Chocolate fl. b	17.7 ^c	5.1 ^{gh}	2.0 ^e	0.2 ^a	7.3 ^d	1.3 ^d	9.1 ^c	5.3 ⁱ	1.7 ^d	10.4 ^c
Chocolate fl. b1	15.7 ^a	4.6 ^{ef}	1.6 ^c	0.8 ^f	7.0 ^c	1.1 ^b	9.4 ^c	4.0 ^f	1.0 ^c	10.5 ^c
Pineapple fl.	19.6 ^d	5.3 ^h	1.3 ^b	1.1 ^g	7.7 ^e	1.5 ^e	14.4 ^f	2.9 ^d	0.6 ^a	15.9 ^g
Orange fl. a	21.6 ^f	2.8 ^b	2.0 ^e	0.8 ^f	5.6 ^b	1.2 ^c	17.9 ^h	1.8 ^c	0.5 ^a	19.1 ⁱ
Orange fl. a1	15.7 ^a	5.5 ^h	2.4 ^{fg}	0.4 ^{bc}	8.3 ^f	1.3 ^d	7.5 ^a	5.7 ^h	1.0 ^c	8.8 ^a
Orange fl. b	16.6 ^b	5.0 ^g	2.5 ^g	1.5 ^h	9.0 ^g	1.5 ^e	13.5 ^e	1.0 ^a	0.4 ^a	15.0 ^f

Means followed by the same superscripts in a column are not significantly different at $p \leq 0.05$ *t-trans*, 18:1 t, 18:2 t, 18:3 t- total *trans* isomers of oleic, linoleic and linolenic acid, *SFA*-saturated fatty acid, *MUFA*-mono unsaturated fatty acid, *PUFA*-poly unsaturated fatty acid, *TFA*-total *trans* fatty acid. *fl.*-flavoured

(Stender et al. 2006). It was found to be less than 1 g in Denmark and Germany to 10 g in New York city and 24 g in Hungary. The cooking oils used by the fast food manufacturers showed varied TFA content depending on the strictness of the legislation introduced by these countries (Stender et al. 2006).

During 1997, 32 brands of biscuits collected from Mysore market were analysed for moisture, fat, protein, total sugar, ash, peroxide value, free fatty acids and minerals. The brands which had very low fat levels (<5 %) invariably had very high peroxide and free fatty acid values and poor storage stability. Total fat in various biscuits covered under this study ranged between 1 to 24.6 % while total energy value ranged between 365 and 501 K calories per 100 g (Semwal et al. 1997).

The fatty acid profile of Spanish cookies and bakery products had a complex mixture of fats from animal and vegetable sources,

besides hydrogenated vegetable oils. The proportion of TFA ranged from 0.38 to 35.54 % in cookies, fat content 14.8 to 31.8 %, SFA 22.4 to 82.8 % based on fat (Isabel et al. 2003). Baked products are usually prepared using a relatively large proportion of hydrogenated fat/shortening 25–35 % in biscuits and 45 to 50 % in cookies (Stauffer 1996). The packets of biscuits in India bore a 'green dot' mark, indicating fat and other ingredients of vegetable origin were used in preparation of these biscuits.

TFA and SFA in biscuits and nutritional aspects

If biscuit manufacturer's federation of India consumption data is considered and Indian consumers' per capita consumption is 2.1 kg biscuits in a year, per day the consumption would be accounted to 5.7 g. The highest level of TFA found in biscuit was 3.2 g/100 g, considered to exist in the 5.7 g sample which gives out 0.18 g TFA on consumption. This in turn gives out energy of 1.7 kcal that equates to 0.085 % of energy. The TFA found in the biscuit samples of this study equates to an energy delivery far less than the WHO recommended level if consumption data equates to 5.7 g per day. The highest SFA found in this study was 18.7 g/100 g, gives out energy deliverance of 0.5 % per day. Even 100 g biscuit consumption a day will deliver 1.44 % and 8.41 % energy, from TFA and SFA, when maximum TFA and SFA content in biscuits as per this study is considered. This conclusion may not be accurate, as the consumption varies with different socioeconomic groups. The serving size and SFA and TFA content based on serving size also needs to be declared on the biscuits packs. Also many brands of biscuits not covered in this

Fig. 1 Distribution of saturated fatty acids in biscuit samples

Fig. 2 Enlarged partial GC chromatograms of A-Marie **a** and B-Milk cream **a** biscuits

study could have higher levels of SFA and TFA. However FSSAI allows a health claim of ‘trans fat free’ may be made where the trans fat is less than 0.2 g per serving of food and ‘saturated fat free’ may be made when SFA content does not exceed 0.1 g/100 g food (Vandana et al. 2011). Accurate per capita food consumption data of each socioeconomic group of India, epidemiological, clinical studies and analytical data altogether gives a clear estimation of link with diet and disease. Greatest concern in diseases linked to diet exists amongst children and urban population with sedentary lifestyles, who are more exposed to the health risk caused by consumption of TFA and SFA.

The edible oils supply and demand there exist a gap in India. More than 45 % of the edible oils including palm oil, vanaspati and bakery shortening consumed in India are imported to India (Dorab 2009). As India has a large number of people with diabetes and heart diseases, the quality assessment of edible oils and fats in India should be given importance (Tanuja et al. 2004; Anant 2000). The 18:2 n-6 and 18:3 n-3 fatty acids play a vital role in cholesterol reduction, when present in a ratio close to 4:1 (Stauffer 1996; Maylet et al. 2011; Russo 2009). Consumption of omega-3 fatty acids provides health benefits, while saturated and *trans* fatty acids can potentially increase the risk of cardio vascular disease (CVD). Industries also include grains and nuts, which are helpful in delivering omega fatty acids.

Biscuits could be utilized as good source of essential fatty acids for providing healthy omega-3 fatty acids. Flaxseed and soybean flour incorporated in high fiber sweet, semi sweet and

salt biscuits are available in the Indian market nowadays. Attempts to replace the hydrogenated fat and include special fat alternatives to achieve reduced TFA in end products are being made. Also research is on, to add flaxseed powder into the dough, and enhancing cookies nutrient quality as more than half the oil in flaxseed is in the form of ALA. Food and Drug Administration of USA approved the presence of flaxseed and fish oils in food as nutrient claim from May 2004 (Farhang 2007). In a research study from Jyotsna et al. (2012) cookies prepared using 5-20 % roasted flax seed incorporated in it gave 4.75 to 5.31 % ALA. A study conducted by food scientists in USA on cream filled sandwich cookies enriched with DHA and EPA found no objectionable off-flavor in the fortified cookies. The filling material inside the cookies had 400 mg of EPA and DHA derived from fish oil source and encapsulated in a matrix of starch and gelatin (Borneo et al. 2007).

Conclusion

Screening of different commercial brands of biscuits by capillary GC-FID for SFA and TFA showed presence of these fatty acids. In this study it has been observed that TFA levels in biscuits varied over a range of 0.1 to 3.2 g/100 g and SFA levels 5.1 to 18.7 g/100 g of biscuit. The products with TFA level at 0.1 to 0.5 g/100 g of biscuits may be considered as zero TFA products as these levels are insignificant. The results

revealed many samples had considerable levels of TFA and high SFA and complacency does not exist in nutrition labeling of the biscuits with respect to the fat composition. The most effective way to ensure absence of industrially produced TFA and high SFA in biscuits is to monitor the fat used in their manufacture. Fortification of omega fatty acids in biscuits would be a way to induce the general public to consume essential fatty acids along with other essential nutrients in their diet. These data will be useful for regulating consumption of saturated fat and *trans* fat through biscuits by children and health conscious consumers.

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