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Analytical Methods

GC-MS quantification of fatty acid profile including *trans* FA in the locally manufactured margarines of Pakistan

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Abstract

Ten margarine brands of Pakistan were analyzed for their fatty acid composition with emphasis on *trans* fatty acids (TFA) using GC-MS. Saturated, *cis*-monounsaturated and polyunsaturated fatty acids were present at 24.2–58.1, 5.7–35.4 and 3.8–37.4% of total fatty acids, respectively. Among the saturated fatty acids, palmitic acid (16.9–33.8%) was dominant in all analyzed margarine brands and its higher amount indicates that palm oil was a major contributor in the margarine manufacturing. Among samples tested only one contained a low level of TFA (2.2%) while the rest contained very high amounts of TFA (11.5–34.8%) which clearly shows that hydrogenated oils were used in the formulation of margarines. Fatty acid profiles demonstrated that all samples belong to the hard margarine category containing high amounts of *trans* and saturated fatty acids which is an alarming issue for the health of consumers.

Keywords: Margarine; Fatty acid profile; *Trans* fatty acids; GC-MS

1. Introduction

Margarine is a butter-like product obtained from mixtures of various edible fats and oils. Usually margarine contains appropriate ratios of hard vegetable fats from coconut, palm kernel, interesterified vegetable oils and/or hydrogenated vegetable oils. Mostly in the industrial catalytic hydrogenation process some natural fatty acids are destroyed and new artificial *trans* isomers are produced that behave similar to saturated fats. These isomers lack the essential metabolic activity of the parent compounds and inhibit the enzymatic desaturation of essential fatty acids (Dimitrios, Vasilios, & Haralambos, 2003). As a result of the economic dislocations during World War II, margarine production rose rapidly as a replacement for butter. During the 1960s margarine became viewed as the healthy alternative to butter because comparatively it was lower in saturated fat. Margarine at that time was heavily hydrogenated and was widespread in the food supply as the major source of industrially produced TFA. The curves for CHD mortality tracked fairly closely with those of TFA intake over time. Angina and myocardial infarction were unusual clinical events in the early part of the 1900s, but CHD increased rapidly and became the major cause of death by mid-twentieth century. Rates peaked in 1950–1960, and mortality has gone down by about 50% since that time. Thus, changes in TFA intake correspond roughly with the epidemic of CHD. Therefore apprehension in *trans*-fatty acids originates from their association with coronary heart disease (Willet et al., 1993; Willett, 2006). Metabolic studies have provided unequivocal evidence that TFA increase plasma concentrations of low-density lipoprotein (LDL) cholesterol and reduce concentrations of high-density lipoprotein (HDL) cholesterol relative to the parent natural fat (Ascherio & Willet, 1997). These effects of *trans* fatty acids were more harmful than saturated fatty acids. Studies also indicated that TFA

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raise lipoprotein level, an independent inherited factor of coronary heart diseases (Mensink & Katan, 1990; Sabarense & Mancini Filho, 2004). In addition to adverse effect on lipoprotein, *trans* fatty acids (TFAs) also reported to influence vascular functions, cardiac arrhythmias and SCD (Katz, 2002).

2. Materials and methods

2.1. Samples and reagents

Ten margarine samples were purchased from local supermarkets of Hyderabad, Pakistan. The choice of the brands was based on the highest consumption among those available in the market. All reagents, chemicals and solvents used were from E. Merck (Darmstadt, Germany). *Trans* and *cis* fatty acids methyl esters (FAME) standards (GLC 481-B and 607) were purchased from Nu-Check-Prep, Inc. (Elysian, MN).

2.2. Sample preparation

Approximately 1 g of margarine samples was melted in an oven at 40–50 °C to obtain the fat phase. The upper fat phase was removed after centrifugation at 448 g for 4 min (Mehmet, Ayhan, & Metin, 2003) then dried by adding anhydrous sodium sulphate to remove the moisture from margarines. Fat obtained from margarine samples was transferred into 5 ml glass vials. The decanted samples were all frozen at −18 °C until analysis.

2.3. Determination of fatty acid composition

For the determination of fatty acids composition of the margarine samples FAMEs were prepared using standard IUPAC, 1979 method 2.301. Agilent GC-MS 5975 was used with ChemStation 6890 Scale Mode software. GC-MS chromatogram obtained were compared with two libraries (NIST & Wily) which provide best information about the identification of fatty acid present in margarine samples to avoid the use of costly standards.

2.4. GC-MS conditions

The GC-MS analysis for FAMEs was performed on Agilent 6890 N gas chromatography instrument coupled with an Agilent MS-5975 inert XL mass selective detector and an Agilent autosampler 7683-B injector (Agilent Technologies, Little Fall, NY, USA). A capillary column HP-5MS (5% phenyl methylsiloxane) with dimension of 30 m × 0.25 mm i.d × 0.25 μm film thickness (Agilent Technologies, Palo Alto, CA, USA) was used for the separation of fatty acid methyl esters. The initial temperature of 150 °C was maintained for 2 min raised to 230 °C at the rate of 4 °C/min, and kept at 230 °C for 5 min. The split ratio was 1:50, and helium was used as a carrier gas with the flow rate of 0.8 ml/min. The injector and detector temperatures were 240 and 260 °C, respectively. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV in the scan range of 50–550 m/z.

2.5. Calculations and statistical analyses

Peak identification of the fatty acids in the analyzed margarine samples was carried out by the comparison with retention times and mass spectra of known standards. Standard methyl esters of palmitic, stearic, oleic and linoleic acids were used for the confirmation of GC-MS libraries result. Two samples of each brand were collected and each sample was analyzed three times. The data obtained were put into Origin 7 program and reported as mean ($n = 2 \times 3$).

3. Results and discussion

The results of fatty acid composition of analyzed margarine samples are divided into saturated and unsaturated fatty acids, shown in Tables 1 and 2, respectively. The margarine brands were coded as M-A, M-B, M-C, M-D, M-E, M-F, M-G, M-H, M-I and M-J. All analyzed margarine brands contain the significant amount of C12:0, C14:0, C16:0 and C18:0. Table 1 shows that the dominant fatty acid among the saturated group is palmitic acid (C16:0) and its range vary from 16.9 to 33.8%. The highest amount of palmitic acid (33.8%) was found in sample M-C while it was lowest 16.9% in M-D sample. The results of palmitic acid indicate the greater contribution of palm oil in the margarine manufacturing. Stearic acid (C18:0) was present at 6.1– 19.0%. Meanwhile, lauric acid (C12:0) was present at 0.1–11.2%, and myristic acid (C14:0) at 0.2–8.7%. Some odd number fatty acids like pentadecanoic acid (C15:0) and margaric acid (C17:0) were also determined in considerable amounts in some samples. Naturally these both fatty acids are not very common in the vegetable oils while almost all animal fats contain their some amount (Shoji, Kazutaka, & Masatoshi, 2005). Saturated fatty acids with the chain length of (C12:0–C16:0) carbon atoms have been reported to be atherogenic, stearic acid (C18:0) neutral, while oleic and polyunsaturated fatty acids produced a blood lipid lowering effect (Aro, Jauhiainen, Partanen, Salminen, & Mutanen, 1997; Hu et al., 1999; Yu, Etherton, & Kris-Etherton, 1995).

Table 2 includes the monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) of margarines. Among the MUFA, oleic acid (C18:1 *cis*-9) was the major fatty acid present. Oleic acid is considered to be responsible for lowering the LDL (bad) cholesterol levels. Whereas polyunsaturated fatty acids have beneficial effects on both normal health and chronic diseases, such as regulation of lipid levels (Mori et al., 2000) cardiovascular (Kris-Etherton, Harris, & Appel, 2002) and immuno functions (Hwang 2000). The highest amount of oleic acid was found in the sample M-B (34.8%) while other samples contained in the range of 4.2–19.4%. The other members of MUFA,

Table 1
Saturated fatty acids composition (mean percentage FAMEs) of margarine samples

Samples	C8:0	C10:0	C12:0	C14:0	C15:0	C16:0	C17:0	C18:0	C20:0	C22:0	C23:0
M-A	0.4	0.5	1.0	7.8	1.6	30.1	1.3	14.9	0.4	0.2	0.1
M-B	0.7	0.3	6.2	3.9	–	20.9	–	19.0	0.8	0.8	–
M-C	–	0.1	0.3	1.5	0.1	33.8	0.1	8.2	0.4	0.1	–
M-D	–	–	0.1	0.2	–	16.9	–	6.1	0.4	0.5	–
M-E	–	0.4	0.9	6.5	1.7	28.0	2.7	13.2	0.4	0.2	0.1
M-F	0.2	0.6	11.2	2.4	0.9	28.6	2.0	7.5	0.5	–	–
M-G	0.2	0.5	8.9	2.4	0.3	30.5	0.9	8.6	0.3	0.1	0.1
M-H	0.5	0.7	10.1	4.0	–	25.4	0.1	11.6	0.4	0.1	–
M-I	–	0.9	8.2	7.2	–	27.0	–	11.0	0.1	0.1	–
M-J	0.2	–	1.1	8.7	0.1	24.1	0.1	13.7	1.3	0.3	–

Table 2
Unsaturated fatty acids composition (mean percentage – FAMEs) of margarine samples

Samples	C16: 1*n*9	C18: 1*n*8	C18: 1*n*9*t*	C18: 1*n*9	C18: 2*n*10,13	C18: 2*n*8,11	C18: 2*n*9,12*t*–*t*	C18: 2*n*9,12	C18: 3*n*9,12,15	C20: 1*n*11	C20: 3*n*1,4,8*t*–*t*–*t*	C22: 1*n*13
M-A	2.3	–	24.9	5.8	1.5	1.0	1.4	2.2	0.2	0.3	0.1	1.5
M-B	0.1	–	2.2	34.8	–	9.6	–	–	–	0.5	–	–
M-C	0.2	7.5	34.7	–	–	12.3	0.1	–	–	0.1	–	–
M-D	0.1	5.7	32.6	–	35.3	–	–	0.1	2.0	–	–	–
M-E	1.9	4.9	26.5	4.2	2.3	4.3	–	0.8	–	0.5	–	0.2
M-F	0.2	1.0	10.5	14.1	–	0.1	1.0	17.3	1.3	0.1	0.1	0.6
M-G	0.1	2.2	8.1	19.4	0.2	0.1	0.2	14.1	2.0	0.7	–	–
M-H	0.1	–	11.5	13.6	0.1	–	0.2	19.1	2.0	0.1	–	0.1
M-I	–	1.1	15.0	14.7	–	–	0.5	13.7	0.5	–	–	–
M-J	2.3	3.0	28.8	10.0	0.7	3.1	1.5	–	–	0.1	–	0.6

n indicates the position of double bond.

C16:1 *cis*-9, C18:1 *cis*-8, C20:1 *cis*-11 and C22:1 *cis*-13 was also determined in the range of 0.1–2.3, 1.0–7.5, 0.1–0.7 and 0.1–1.5%, respectively. The major *trans* fatty acid observed in all margarine brands was elaidic acid (C18:1 *trans*-9) in the range of 2.2–34.7%. Out of 10, three samples (M-C and M-D and M-J) contained elaidic acid (C18:1 *trans*-9) at 28–35% (34.7, 32.6 and 28.8%), two samples (M-A and M-E) have 24.9 and 26.5%, and of four samples (M-G, M-F, M-H, M-I) ranging from 8 to 16% *trans* (8.1, 10.5, 11.5 and 15.0%), respectively. Only one (M-B) margarine sample contained 2.2% TFA which is lowest value among all analyzed margarine brands. Other *trans* fatty acids determined in the margarine samples were C18:2 *t*–*t* 9, 12 and C20:3 *t*–*t*–*t* 1, 4, 8 in the ranges of 0.1–1.5 and 0.1%, respectively. None of the analyzed sample in the present study shows less than 2% TFA, while the data of other countries indicate that each study contain few margarine samples in which there were not any TFA or had less than 1% TFA (Dimitrios et al., 2003; Jiri & Jan, 2000; Karabulut & Turan, 2006; Matsuzaki et al., 2002; Tsanev, Russeva, Rizov, & Dontcheva, 1998).

PUFA has major importance for biological and nutritional value of margarines. C18:2 *cis*10,13; C18:2 *cis* 8,11; C18:2 9,12 *t*–*t*; C18:2 *cis* 9,12, C18:3 *cis* 9,12,15, C20:3 *t*–*t*–*t* 1,4,8 are the members of PUFA determined in the margarine brands, in the range of 0.1–35.3, 0.01–12.3, 0.1–1.5, 0.1–19.1, 0.2–2.0 and 0.1%, respectively. Out of 10 samples, four (M-F, M-G, M-H and M-I) contained greater amount of linoleic acid (C18:2 *cis* 9,12) at 13.7–17.3%. In some samples like M-B, M-C and M-J it was found to be totally absent. While the samples M-D and M-E contained less than 1%. Ovesen, Torben, & Hansen (1998) divided margarine into three categories on the basis of linoleic acid (LA) (i) hard margarines with less than 20% LA (ii) semi soft margarines with 20–40% LA and (iii) soft margarines with more than 40% LA. Therefore all analyzed margarine brands were hard margarines and none of the samples was included in the semi soft or soft margarines. The presence of different isomers of oleic and linoleic acid in margarine samples identify that hydrogenated oil is used in all analyzed margarine brands. It is the only partial hydrogenation process that can change both the geometrical configuration and the double bond shift (McDonald & Mossoba, 2002).

Table 3 represents the fatty acids groups and ratio between them. The saturated/unsaturated FA shows the relation between two major fatty acid groups of the margarine fat. Its value varies from 0.32 to 1.41. Only one sample had a ratio of 0.32 while in all other samples it varied from 0.82 to 1.41 which clearly indicates a high proportion of saturated fatty acids. The prevalence of unsaturated over saturated fatty acids (smaller ratio) is considered to be positive from the nutritional point of view. The saturated + TFA fraction ranged from 54.8 to 84.5% and

Table 3
Groups and ratio between the types of fatty acids from the composition of margarine samples

Groups and ratio of FA	M-A	M-B	M-C	M-D	M-E	M-F	M-G	M-H	M-I	M-J
SFA	58.1	52.6	44.7	24.2	54.2	53.9	52.7	52.9	54.4	49.8
cis MUFA	9.9	35.4	7.7	5.7	11.8	15.9	22.3	13.9	15.8	16.0
trans MUFA	24.9	2.2	34.7	32.6	26.5	10.5	8.1	11.5	15.0	28.8
Total MUFA	34.8	37.6	42.4	38.3	38.3	26.5	30.5	25.4	30.8	44.8
cis PUFA	4.9	9.6	12.3	37.4	7.4	18.6	16.5	21.3	14.2	3.8
trans PUFA	1.5	–	0.1	–	–	1.02	0.2	0.2	0.5	1.5
Total PUFA	6.3	9.6	12.4	37.4	7.4	19.7	16.7	21.4	14.7	5.3
Total TFA	26.4	2.2	34.8	32.6	26.5	11.5	8.3	11.7	15.6	30.3
SFA + TFA	84.5	54.8	79.5	56.8	80.7	65.4	61.0	64.6	70.0	80.1
MUFA + PUFA	41.2	47.1	54.8	75.7	45.7	46.1	47.1	46.8	45.5	50.1
cis MUFA + PUFA	14.8	44.9	20.0	43.1	19.2	34.6	38.8	35.1	30.0	19.7
SFA/UFA	1.4	1.1	0.8	0.3	1.2	1.2	1.1	1.1	1.2	1.0
cis PUFA/SFA	0.1	0.2	0.3	1.6	0.1	0.4	0.3	0.4	0.3	0.1
trans/cis	1.8	0.1	1.7	0.8	1.4	0.3	0.2	0.3	0.5	1.5
cis PUFA/(SFA + TFA)	0.1	0.2	0.2	0.7	0.1	0.3	0.3	0.3	0.2	0.1
cis MUFA + PUFA/SFA + TFA	0.2	0.8	0.3	0.8	0.2	0.5	0.6	0.5	0.4	0.3

achieved a very high mean value of 69.7%. The ratio of *trans*/*cis*-FA represents the degree of formation of artificial TFA from the natural *cis* forms of unsaturated fatty acids of the margarines and the ratio varies between 0.05 and 1.79. Few samples M-B, M-F, MG and M-H had lower ratios of 0.05, 0.33, 0.21 and 0.33, respectively. While other samples had a ratio greater than 0.50 which corresponds to a higher content of TFA and showing that margarine manufactures are not serious to reduce the *trans* content in their product and still using the conventional technology in the processing and production of margarines. These results also indicate that there is great variation in the quality of local commercially available brands and even no two margarine samples had similar fatty acid profiles. The *cis*-polyunsaturated fatty acids (PUFA) which are nutritionally important were found in the range of 3.8–37.4%. The mean ratio of *cis* PUFA/SFA recommended by the British Department of Health is 0.45 (Da et al., 2002). In this study *cis* PUFA/SFA ratio ranged from 0.08 to 1.55 and the mean value was 0.36, which is less than the recommended value. The mean values of the indices most commonly used to express the nutritional value of edible fats *cis*-PUFA/SFA + TFA and *cis*-PUFA + *cis*-MUFA/SFA + TFA were 0.23 and 0.46, respectively. These values are much lower than those obtained by Alonso, Fraga, & Juarez (2000) for Spanish vegetable margarines at 1.25 and 1.92, respectively, and are slightly lower than those reported by Farooq, Bhanger, Shahid, & Bushra (2006) for Pakistani margarines at 0.25 and 0.76.

4. Conclusions

The industrial catalytic hydrogenation destroys some natural fatty acids and produces new artificial fatty acids. Therefore for the determination of fatty acid composition of margarine it is very hard to obtain all the standards of fatty acids and is difficult to identify some peaks with the conventional FID detector. In the present study GC-MS was used to achieve more accurate peak identification. Great variation in the fatty composition has been observed among different brands of analyzed Margarine. In this study, a total of ten local margarine brands were collected, nine of them were found to contain very high amounts of *trans* fatty acids (15.6–34.8%) and only one sample contained comparatively a low level of TFA (2.2%). The higher amount of TFA and saturated fatty acids demonstrated the poor quality of margarine in the market which is harmful to the health of consumers. Further the amount of *trans* fat was not mentioned on the label of any local manufactured margarine brands.

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