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GC-MS Evaluation of Fatty Acid Profile and Lipid Bioactive of Partially Hydrogenated Cooking Oil Consumed in Pakistan

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Abstract. Evaluation of fatty acid profile including *trans* fat and lipid bioactive (tocopherol and sterol contents) of most commonly used vanaspati ghee and cooking oil brands was made by gas chromatography coupled with mass spectrometer detector (GC-MSD). Among the saturated fatty acids (SFA), palmitic and stearic acid were dominant fatty acids; the mean value of SFA in ghee and oil was 44.98 and 30.83%, respectively. Mean values of monounsaturated, polyunsaturated and *trans* fatty acids in ghee were 47.51, 7.49 and 8.08%, and in oil 49.26, 19.90 and 0.91%, respectively. α-Tocopherol was the major tocopherol while campesterol, stigmasterol and sitosterol were main phytosterols in terms of their quantity.

Keywords: GC-MSD, fatty acid profile, tocopherols, phytosterols, edible fats and oils

Introduction

Vegetable oils, made up of lipids, are very common commodities in our daily life (Mikuma and Kaneko, 2010). Some vegetable oils such as olive oil or roasted sesame oil are accepted as health beneficial foods and consumer demand for these oils is increasing. Depending on the production cost and availability of plant sources, the price of some vegetable oil is changing and blended oils can be found in some markets (Park *et al*., 2010).

Fatty acids (FA) in food and biological samples were commonly analysed by gas chromatography (GC), for long (James and Martin, 1952). Characterization and determination of various kinds of saturated, mono and polyunsaturated fatty acids together with their positional and geometrical isomers have been studied using GC-FID and GC-MS methods in different samples (Bail *et al*., 2009; Tokusoglu, 2008; Brondz, 2002; Ackman, 2002; Evershed, 1996; Eder, 1995). Hyphenation of the chromatographic technique with mass spectrometry provides a selective tool for the study of FA structures. FA play important roles in biological tissues and as constituents of lipids in biological membranes influence fluidity, integrity and activities of membrane-bound enzymes. Consumption of *trans* fat may increase the risk of coronary heart disease (Vijver *et al*., 2000; Ascherio *et al*., 1999; Hu *et al*., 1997). Oils and fats have been consistently associated in both epidemiological studies and clinical feeding trials with reduced blood cholesterol levels and decreased incidence of cardiovascular disease (Li *et al*., 2007; Ostlund, 2004; Elaine and Feldman, 2002). Intake of certain fats, such as saturated fats and hydrogenated oils (vanaspati), raises the cholesterol level; alternatively vegetable oils known as poly-unsaturated fatty acids may lower the cholesterol level. This is also achieved through intake of monounsaturated fats like olive oil (Sharma, 2000).

Important part of unsaponifiable matter of fats and oils comprises of sterols (Lagarda *et al*., 2006; Cunha *et al*., 2006; Apparich and Ulberth, 2004). The qualitative analysis of sterols is very helpful when adulteration is suspected (Lognay *et al*., 1993). Plant sterols are called phytosterols, which resemble cholesterol in function and structure (Kritchevsky and Chen, 2005). Many types of phytosterols have been reported in plant species, the more abundant ones being sitosterol, stigmasterol and campesterol (Berger *et al*., 2004; Moreau *et al*., 2002), and are predominantly found in plants (Grunwald, 1975). These are present mainly in nuts, vegetable oils, seeds, cereals and beans (Mitei *et al*., 2009; De Jong *et al*., 2003, Phillips *et al*., 2002). Sterols in vegetable oils occur mainly as free sterols and esters of fatty acids. Qualitative and quantitative analysis of sterols is often used for identifying lipid mixtures when adulteration is suspected.

The present study was undertaken to evaluate the quality of vanaspati ghee and cooking oil in terms of their fatty acid profile with special reference to *trans* fat and important lipid bioactives.

Materials and Methods

Samples and reagents. Samples of vanaspati ghee and cooking oils were purchased from local supermarkets of Hyderabad, Pakistan. The choice of the brands was based on

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those most in use. All reagents, chemicals and solvents used were from E. Merck (Darmstadt, Germany).

Determination of fatty acid composition. For the determination of fatty acid composition, fatty acids methyl esters (FAMEs) were prepared using standard IUPAC method 2.301 (IUPAC, 1979).

Gas chromatography analysis of sterols. Separation of sterols was carried out after saponification of the oil sample without derivatisation according to Ramadan and Morsel (2003). Sample (250 mg) was refluxed with 5 mL of ethanolic potassium hydroxide solution (6%, w/v) and a few anti-bumping granules for 60 min. The unsaponifiables were first extracted thrice with 10 mL of petroleum ether; the extracts were combined and washed 3-times with 10 mL of neutral ethanol/water (1:1, v/v) and then dried over-night with anhydrous sodium sulphate. The extract was evaporated on a rotary evaporator at 25 °C under reduced pressure, and then ether was completely evaporated under nitrogen. Unsaponifiable residue analysis was carried out using GC-MS.

Instrumentation

The instruments used in the study included Agilent 6890 N gas chromatograph 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 model no: Agilent 19091S-433, HP-5MS (5% phenyl methylsiloxane), column length 30 m, i.d. 250 µm, film thickness 0.25 mm.

GC-MS conditions.

Temperature parameter and other conditions

Initial temperature	150 °C
Maintained for	2 min
Final temperature	230 °C
Kept for	5 min
Ramp rate	4 °C/min
Carrier gas helium flow rate	0.8 mL/min
Injector temperature	240 °C
Detector temperature	270 °C
Electron impact (EI) mode	70 eV
Scan range	50-550 *m/z*

Statistical analyses. Each GC-MS value is the mean of three replications. Values of different parameters were expressed as the mean ± standard deviation ($x \pm SD$).

Results and Discussion

Table 1 and 2 show the average saturated and unsaturated fatty acid (FA) composition expressed in percentage of total fatty acids in vanaspati ghee and cooking oil samples, coded as VG-1 to VG-7 and CO-1 to CO-7, respectively. Amongst the saturated fatty acids (SFA), palmitic acid (16:0) was predominantly present in both VG and CO samples ranging from 35.23-43.41, and 10.19-40.41%, respectively; it clearly indicated that palm oil was frequently used in the production of VG and CO. The second most abundant SFA was stearic acid (C18:0), present in the range of 4.33 to 6.33% and 2.23 to 6.17% in VG and CO, respectively. Considerable amounts of lauric (C12:0), myristic (C14:0), pentadecanoic (C15:0), margaric (C17:0), arachidic (C20:0) and docosanoic acids (C22:0) were also detected, less than 1.0%, in both types of samples. It is reported that saturated fatty acids with a chain length of C12:0-C16:0 are atherogenic, stearic acid is neutral, oleic and polyunsaturated fatty acids have a lipid lowering effects (Hu *et al*., 1999; Aro *et al*., 1997). Along with the monounsaturated fatty acids (MUFA), oleic acid (C18:1n-9 *cis*) was the principal benefactor found in the range of 33.23-44.93% and 39.34-60.59% with a mean value of 39.36% and 47.87% in VG and CO, respectively.

The other members of MUFA detected in VG and CO were palmitoleic (C16:1) and eicosenoic (C20:1n-11) acids present in less than 1% amount except only one sample CO-5 which contained 1.42% eicosenoic acid. The majority of natural vegetable oils are rich in *cis*-unsaturated fatty acid; the unsaturated constituents can be isomerized to the *trans* form during extraction and oxidative conversion during heating and by partial hydrogenation (Perez-Serradilla *et al*., 2007). Compared with *cis*-unsaturated fatty acids, structure, physical properties, chemical stability and the physiological (atherogenic) effects of *trans* fatty acids resemble those of the saturated fatty acids (Mensink and Katan, 1990). The amount of *trans* monounsaturated fatty acids in the VG and CO ranged from 2.83-15.43% and 0.18-1.48%, with the mean value of 7.89 and 0.88%, respectively. The highest value of *trans* fat was determined in VG-2 and CO-1, while the lowest level, in VG-6 and CO-5; none of the analysed samples were free from *trans* fatty acids. Minor amounts of *trans* polyunsaturated fatty acids (C18:2*n*9,12) were determined in some samples of VG and CO with the mean values 0.33 and 0.09, respectively. Normally the presence of *trans* fat in high amount in sample indicates hydrogenation process.

The fatty acid composition of the VG and CO samples are presented in Table 3. Mean value of SFA detected in VG and CO was 44.98 and 30.83% in the range of 40.88-49.44 and 13.56-47.56%, respectively. The ratio of saturated/unsaturated FA shows the relation between two major FA groups composition; its value varied from 0.69-0.98 in VG, while in CO the value varied from 0.16-0.91; these ratios indicate a higher proportion of saturated FA. Mean ratio of saturated/unsaturated in

Table 1. Saturated and unsaturated fatty acid composition (mean percentage-FAMEs) of vanaspati ghee samples

Fatty acid	Percentage composition in samples							
	VG-1	VG-2	VG-3	VG-4	VG-5	VG-6	VG-7	Average
C12:0	0.15	0.14	0.11	-	0.15	-	0.15	0.14
C14:0	0.93	0.91	0.84	0.87	0.85	0.82	0.93	0.88
C15:0	-	0.05	0.03	-	-	-	0.08	0.05
C16:0	38.79	35.80	37.72	43.41	35.23	41.24	35.40	38.23
C17:0	0.18	0.18	0.13	-	-	-	0.17	0.17
C18:0	5.61	6.33	6.09	5.16	4.33	4.33	5.65	5.36
C20:0	0.36	0.38	0.43	-	0.32	-	0.41	0.38
C22:0	-	0.03	-	-	-	-	0.17	0.10
C16:1*n*9	0.13	0.11	0.15	-	0.18	-	0.16	0.15
C18:1*n*9*t*	6.32	15.43	4.83	6.21	8.2	2.83	12.06	7.89
C18:1*n*9c	36.61	33.23	38.32	40.9	43.84	44.93	37.66	39.36
C18:2*n*9,12c	10.8	7.33	11.18	3.44	5.91	5.84	6.72	7.32
C18:2*n*9,12t	-	-	-	-	0.5	-	0.15	0.33
C18:3*n*9,12,15	-	-	-	-	0.46	-	0.1	0.28
C20:1*n*11	0.11	0.07	0.14	-	-	-	0.17	0.12

Table 2. Saturated and unsaturated fatty acid composition (mean percentage-FAMEs) of cooking oil samples

Fatty acid	Percentage composition in samples							
	CO-1	CO-2	CO-3	CO-4	CO-5	CO-6	CO-7	Average
C12:0	-	-	-	-	0.05	-	0.13	0.09
C14:0	0.23	0.54	0.46	0.87	-	0.28	0.97	0.56
C15:0	-	-	-	-	-	-	0.03	0.03
C16:0	11.82	31.62	28.76	40.41	10.19	14.92	39.22	25.28
C17:0	-	-	-	-	0.12	-	0.12	0.12
C18:0	3.56	4.49	4.72	6.28	2.54	5.23	6.17	4.71
C20:0	0.45	0.26	0.29	-	0.66	-	0.42	0.42
C16:1*n*9	0.23	-	-	-	0.32	0.27	0.15	0.24
C18:1*n*9*t*	1.48	0.96	1.32	0.79	0.18	0.63	0.77	0.88
C18:1*n*9c	51.62	48.57	50.17	41.47	60.59	43.34	39.34	47.87
C18:2*n*9,12c	29.85	13.51	13.3	10.17	20.86	30.31	12.43	18.63
C18:2*n*9,12t	-	0.04	-	-	0.14	-	0.09	0.09
C18:3*n*9,12,15	-	-	0.69	-	2.91	5.01	-	2.87
C20:1*n*11	0.75	-	0.28	-	1.42	-	0.15	0.65

VG was 0.82, while 0.50, in the CO. The British Department of Health, UK (HMSO, 1994) recommended minimum 0.45 *cis*-PUFA/SFA ratio; lower value of this ratio indicates that foods are not good for health. The analysed VG samples showed lower mean ratio 0.17, which was much lower than the recommended one, whereas higher mean value ratio of *cis*-PUFA/SFA was detected in CO at 0.94. The ratio of *trans*-FA/*cis*-FA shows significant degree of conversion of *cis*-form to *trans*-form, and also higher ratio indicates greater mixing of hydrogenated oils. The ratio varied between 0.06-0.38 with the mean value of 0.18 in VG; however, in the CO its range varied from 0.00-0.02 with the mean value of 0.01. Mean value of indices of *cis*-PUFA/(SFA+TFA) and (*cis*-MUFA +*cis*-PUFA)/(SFA+TFA), (are most commonly used to express the nutritive value of edible oils and fats (Alonso *et al*., 2000). These values of *cis* PUFA/(SFA+TFA) were 0.14 and 0.90 in VG and CO, respectively, whereas the mean value indices of (*cis* MUFA+*cis* PUFA)/(SFA+TFA) were 0.89 and 2.89 in VG and CO, respectively. Figure 1 and 2 show the representative chromatograms of different fatty acids and phytosterol, respectively. The analysed oil sample chromatogram was in a good peak shape under the optimized chromatographic conditions.

Table 4 shows the composition of phytosterol and tocopherol in the investigated VG and CO samples. Among the phytosterols, the amount of campesterol was 6.46-25.76%, while stigmasterol was determine only in two samples, VG-6 (2.80%) and VG-7 (4.73%); sitosterol was in the range of 37.65-52.38%

Table 3. Fatty acids composition of vanaspati ghee and cooking oil samples

Fatty acid	Vanaspati ghee (VG)		Cooking oils (CO)	
	Range (%)	Mean values (%)	Range (%)	Mean values (%)
SFA	40.88 - 49.44	44.98	13.56 - 47.56	30.83
UFA	50.55 - 59.09	55.00	52.43 - 86.10	69.02
Total MUFA	43.17 - 52.22	47.51	40.41 - 62.51	49.26
cis-MUFA	33.41 - 44.93	39.53	39.64 - 62.33	48.38
Total PUFA	3.43 - 11.18	7.49	10.17 - 35.32	19.90
cis-PUFA	3.44 - 11.18	7.40	10.17 - 35.32	19.86
Total TFA	2.83 - 15.43	8.08	0.32 - 1.48	0.91
SFA+TFA	49.22 - 59.25	53.06	13.88- 48.35	31.74
SFA/ UFA	0.69 - 0.98	0.82	0.16 - 0.91	0.50
cis-MUFA+cisPUFA	40.74 - 50.77	46.93	51.64 – 86.10	68.24
Cis-PUFA/SFA	0.07 - 0.25	0.17	0.21 – 1.86	0.94
trans-FA/ cis-FA	0.06 - 0.38	0.18	0.00 - 0.02	0.01
cis-PUFA/(SFA+TFA)	0.06 - 0.22	0.14	0.21 – 1.71	0.90
cis-MUFA+PUFA/SFA+TFA	0.69 - 1.03	0.89	1.07 – 6.20	2.89

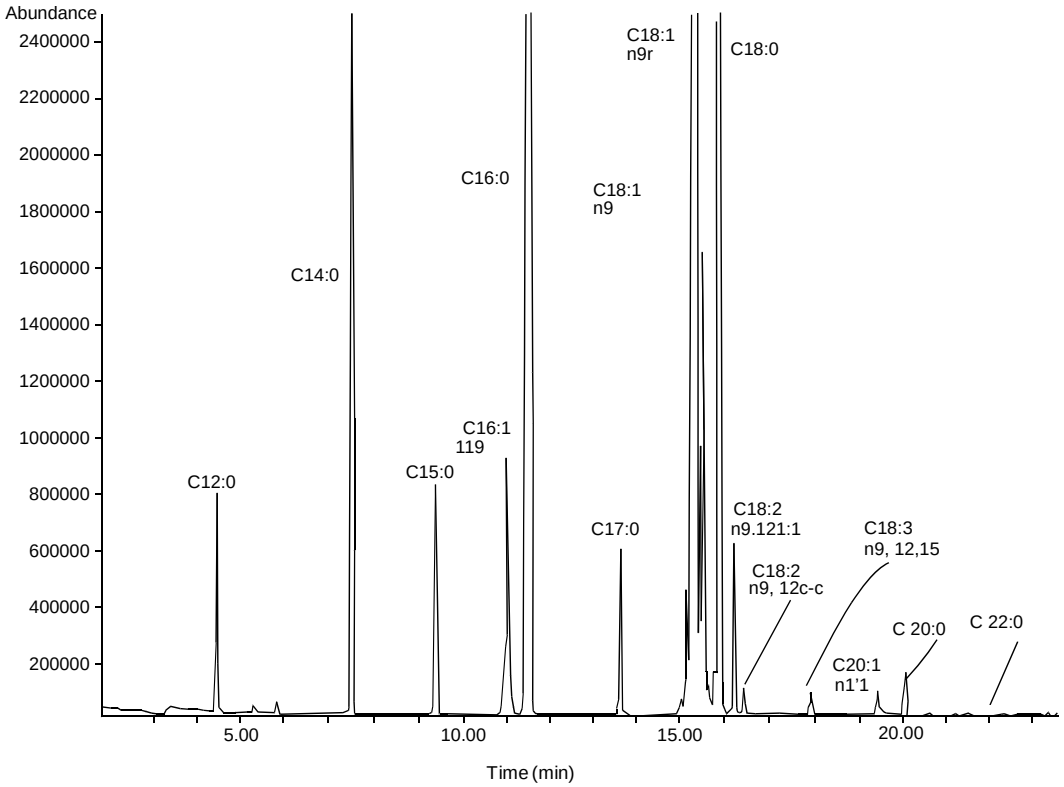

Fig. 1. Representative chromatograph of fatty acids of hydrogenated samples analysed by GC-MS.

in VG samples. In CO sample, the range of campesterol and stigmasterol were determined to be 10.11-32.62% and 4.07-69.19%, respectively, and sitosterol was found only in two samples CO-1 (39.62%) and CO-2 (53.46%). The content of tocopherols (α-tocopherol and γ-tocopherol) were also determined. α-tocopherol was in the range of 1.37-8.59% and (0.81-2.42) in VG and CO, respectively, while γ-tocopherol was present only in one sample CO-3 (1.65%). Study of phytosterols of edible oils and fats was earlier conducted previously in Pakistan (Sabir *et al*., 2003), but determined the total contents of phytosterols using spectrophotometer. In the present study, a new rapid, reliable and hyphenated

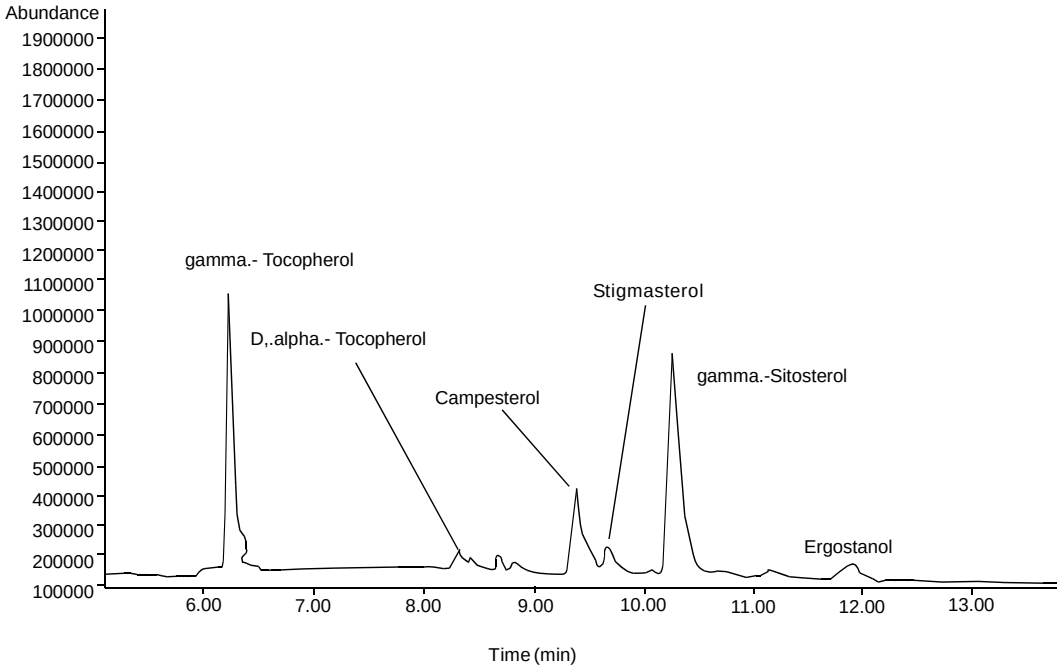

Fig. 2. Representative chromatograph of tocopherol and phytosterols analysed by GC-MS.

Table 4. Lipid bioactive composition of vanaspati ghee and cooking oil samples

Sample	gamma-Tocopherol (%)	alpha-Tocopherol (%)	Campesterol (%)	Stigmasterol (%)	Sitosterol (%)
VG-1	-	-	17.03±0.5	-	40.63±1.5
VG-2	-	2.52±0.1	20.10±0.4	-	41.05±1.25
VG-3	-	3.98±0.1	17.30±0.25	-	37.65±1.2
VG-4	-	8.59±0.3	6.46±0.2	-	52.38±1.8
VG-5	-	1.37±0.05	25.76±0.56	-	45.01±1.5
VG-6	-	1.47±0.05	23.74±0.5	2.80±0.07	-
VG-7	-	1.57±0.02	15.90±0.25	4.73±0.1	39.55±1.2
CO-1	-	1.36±0.05	16.13±0.7	-	39.62±1.25
CO-2	-	-	25.32±1.25	-	53.46±1.5
CO-3	1.65±0.05	0.81±0.03	18.34±0.75	5.78±0.2	-
CO-4	-	-	32.62±1.25	-	-
CO-5	-	-	10.11±0.5	69.19±2.5	-
CO-6	-	2.42±0.05	20.11±0.5	4.07±0.2	-
CO-7	-	2.39±0.05	23.45±0.7	45.02±1.5	-

technique (GC-MS) was used to quantify different phytosterols. It was accomplished that GC-MS has many advantages in determination of phytosterols within the limited time and accuracy.

Conclusion

The results of this study indicated presence of considerable high amounts of TFA in all the analysed samples. It is obvious that attention should be paid to the consumption of these kind of products when considering the health aspects of TFA's. On the other hand, future regulations on the nutritional labelling of foods related with TFA's, SFA's contents and phytosterols will provide better knowledge for the consumer, because both *trans* fat and saturated fat have adverse health effects. It is important to consider both types of fatty acids in evaluating the nutritional content of products including phytosterols. Using chromatographic conditions, satisfactory separation and identification of compounds were achieved. The most likely source of *trans* fatty acids and phytosterols is the vanaspati ghee (partially hydrogenated) and cooking oils.

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