Research Article

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Capillary gas chromatography method for fatty acid analysis of coconut oil

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Abstract

Gas chromatography is widely used for the fatty acid analysis of oils and fats. In this paper, a capillary gas chromatography method for fatty acid analysis of coconut oil, which can be used for other oils and fats as well, is described. The chromatogram of fatty acid methyl ester standard cock tail (C8:0 to C24:0) indicated that by following the above mentioned method, a clear separation of all saturated, mono-unsaturated and poly-unsaturated fatty acids can be achieved. The method was used to analyze the fatty acid profile of unknown samples viz., coconut oil, sunflower oil, palm oil and animal fat (butter fat) and a clear separation of the all fatty acids was obtained, clearly demonstrating that this method can be adopted for the fatty acid analysis of any other vegetable oil or fat even at very low quantities.

Key words: Capillary gas chromatography, oils, fats, coconut

Introduction

Fatty acid analysis of coconut oil is being carried out by various methods and conditions of gas chromatography using packed columns. Here a method is described using a capillary column, which gives greater precision and accuracy. The major economically important product from coconut is oil. Copra contains around 65% of oil consisting mainly of lauric acid (48%) with the balance being myristic, caprylic and palmitic acids. Coconut oil is used for various purposes i.e., edible (39.4%), toiletry (46.5%) and other industrial (14.1%) uses. For industrial use the high content of lauric acid is desirable for making high quality soaps, detergents, surfactants and shampoos. Moreover, based on the fatty acids composition of the oil, the chemical industry can produce various oleochemicals such as glycerol, fatty acid alcohols etc., from which various oleochemical derivatives can be prepared for use in fields like textile, paints, petroleum, etc., including food industry (Iyer, 2004; Naresh Kumar *et al.*, 2004 a). Coconut oil is a rich source of medium-chain-triglycerides (MCT) which are beneficial for human health and nutrition. It is also given to patients as a source of fat and also to cure fat absorption disorders. Coconut oil is used as a base in making various pharmaceutical products. In short, the market economy of the coconut and its products is controlled by coconut oil industry.

The fatty acid and triglyceride profile of coconut oil has been extensively studied (Oo and Stumpf, 1979). But research on variations in coconut oil for fatty acid composition, unsaturation to saturation ratio, etc. among varieties, hybrids and other germplasm lines has not been understood to the extent where suitable cultivars/ germplasm for specific uses can be identified. Presently the fatty acid composition of only 19 cultivars is available. Influence of climate and abiotic stresses on content and composition of fatty acids of coconut oil is not known. With this back ground, it is important to screen the elite germplasm for fatty acid composition and also to study the influence of external factors on the content and composition of fatty acids in coconut oil.

A few pioneering reports are available since 1920 on the triglyceride composition of coconut oil by fractional crystallization (Bomer and Baumann, 1920; Collin and Hildtech, 1928, Dale and Meara, 1955). Later, the use of GLC by a group of workers in France led to an extensive analysis of the triglycerides present in the oil leading to its fractionation into different groups based on their carbon

atoms from 28 to 52 (Bezard *et al.*, 1971). Thus, 79 types of triglycerides could be identified in coconut oil which represent 99.8% of the total glycerides. Even though Oo and Stumpf (1979) did the classic work on fatty acid biosynthesis in the developing endosperm of coconut in USA, only recently fatty acid and tri acyl glyceryl (TAG) composition of oils of coconut cultivars and hybrids was studied and found that the lauric acid content varied in oil samples among hybrids (Rodriguez *et al.*, 1998; Naresh Kumar and Rajagopal, 2000 and Naresh Kumar *et al.*, 2000, Marikkar *et al.*, 2004). A study on variations among 18 cultivars in the fatty acid composition revealed that coconut oil mainly contains neutral-lipids and also phospho-and glyco-lipids in lesser amounts. The study further revealed that these cultivars varied for fatty acid composition and saturated to unsaturated fatty acid ratios as well (Naresh Kumar *et al.*, 2004).

Since gas chromatography is widely used for fatty acid analysis, this paper is aimed to provide a method for fatty acid analysis of coconut oil using capillary column, which can be used for other oils and fats as well.

Materials and Methods

Copra samples

Four mature (12 months old) nuts from tagged bunches of at least six selected palms were collected. Dehusked nuts were split to make copra by oven drying at 60 °C till uniform moisture content of 6% was attained. The copra weights were taken at 6% moisture levels.

Oil percentage

Chopped copra samples (300 mg) were ground finely along with equal quantity of anhydrous sodium sulphate (for absorbing the moisture in copra). The oil extraction was made in a Soxhlet apparatus using petroleum ether (60-80 °C) as the extraction solvent. The percentage of the oil was estimated gravimetrically.

Preparation of Fatty acid methyl esters

For analysis of fatty acid profile, methyl esters of fatty acids were prepared following the method of Padua-Resurreccion and Benzon (1979) as described below. Even though several other methods are available for preparation of fatty acid methyl esters, this method was found to be simple and convenient for handling large number of samples.

Reagent : 5% HCl reagent : Added 8.3 ml of acetyl chloride drop wise to 100 ml absolute methanol. Ice jacket was used to prevent bumping due to exothermal reaction.

This reagent (2 ml) was added to 0.2 g coconut oil, taken in a 15 ml screw capped glass vial. The mixture was vortexed and incubated at 70 °C in hot air oven for 10 hours and cooled to room temperature. Five milliliters of distilled water and one ml hexane were added to the mixture and vortexed thoroughly. When two layers were separated, the hexane layer (top) was aspirated out into micro-tubes and stored for the GC analysis of methyl esters of fatty acids in hexane with out any loss. Precaution must be taken to avoid the evaporative losses of hexane, since it leads to increase in concentration of fatty acid methyl esters (FAMEs) in hexane causing problems during quantification of fatty acids. FAME in hexane (in micro tube) can be stored in fridge at 4 °C for 2 to 3 days or in glass vials with tight sealing for longer periods (months together) with out any loss of hexane (so that the FAME concentration remains the same).

Gas chromatography of FAMEs

Methyl esterified samples were diluted (40µl FAME sample+960 µl n-hexane, HPLC quality) in the sample vial, using disposable pipette tips. To avoid contamination, each sample should be taken with a fresh pipette tip. The sample vials were put in auto-injector vial tray. Methyl esterified sample (1µl) was injected into the Gas Chromatograph (GC-2010, Shimadzu, Japan), by an auto injector (AOI) and capillary column (BPX 70). The elutants were detected on Flame Ionization Detector (FID). The conditions set for analysis are presented in Table 1. The amplified signals were transferred and recorded in a computer with GC-Solutions software.

Quantitative method was followed using an external standard of mixture of fatty acids (C6-C24). Fatty acid methyl ester standards (C:6-C24; Sigma, USA), were run earlier under similar conditions of analysis. The concentrations and area of each peak was computed using a data analysis method developed using different concentrations of standard FAMEs. The data thus acquired was analyzed using the GC Post-run analysis software. By using this software the fatty acid type, concentration, area, etc of unknown samples can be detected precisely. The data were further subjected to statistical analysis in factorial RBD and CD at P=0.05 was used for comparison means.

Column conditioning: The capillary column was conditioned for at least 10 hrs prior to the use.

Table 1. The analytical conditions are set as following

Auto sampler settings			
Injection sample volume			1 ul
Terminal air gap			no
No. of rinses with solvent (Pre-run)			4
No. of rinses with solvent (Post re-run)			6
No. of rinses with sample			5
Washing volume			8 ul
Plunger suction and injection speed			High
Syringe injection speed			Low
Injection port dwell time			1 sec.
Injection port settings			
Injection mode			Split
Temperature			225 °C
Carrier gas			N_2/Air
Pressure			114.9 kPa
Total flow			68.9 mL/min
Column flow			1.29 mL/min
Linear velocity			34 cm/sec
Purge flow			3 mL/min
Split ratio			50
Column oven settings			
Initial temperature	100 °C		
Column oven temperature programme			
Equilibrium time	3 min		
Total programme time	30 min		
		Temperature	Hold time
		100 °C	1 min
		220 °C	5 min
	Rate (°C/min)	5 °C	1 min

Column Information		Detector settings/information	
Column name	: BPX-70	Detector	FID
Film thickness	: 0.25 um	Temperature	280 °C
Inner diameter	: 0.25 mm	Makeup gas	N_2/Air
Column length	: 30 m	Makeup flow	30 ml/min
Column max.temperature	:260 °C	H_2 flow	47 ml/min
		Air flow	400 ml/min
		Signal acquire	Yes
		Sampling rate	40 milli sec
		Stop time	30 min
		Delay time	0 min

Results and Discussion

The chromatogram (Fig. 1) of fatty acid methyl ester standard cock tail (C8:0 to C24:0) indicates that by following the above mentioned method, a clear seperation of all saturated, mono-unsaturated and poly-unsaturated fatty acids can be achieved in the samples. The temperature programme also is overlapped on *Ya* axis. The injected concentrations of standard cocktail were 1 mg/ml, 5 mg/ml and 10 mg/ml. These were used as the external standards. The data in Table 2 further indicates the retention times for each of compound, concentration, area percentage and many of the column performance characters.

The quantity of fatty acids injected (1µl) was around 7 to 10 mg/ml concentration. Load of the sample

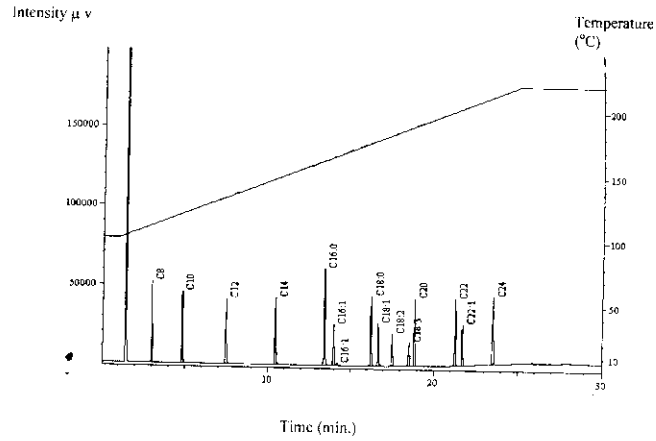

Fig. 1. The chromatogram of fatty acid methyl ester standards following the method mentioned in the paper.

concentration will be high if it is increased beyond this level. In a sample with concentrations of fatty acid methyl esters lesser than 5 mg/ml, detection of those fatty acids which are present in minute concentrations is not possible. Hence the sample loading concentrations should also be kept within certain range apart from injecting the fixed volume of sample. This method can be used for manual injection conditions as well, where the precision may vary around 2%. If one is using a packed column, the sample injection volume may have to be decreased to 0.5 µl but without diluting it with hexane. Thus the concentration required for fatty acid analysis using a packed column will be very high. The advantage of capillary colum analysis is that it is very highly discriminative with an excellent resolution. The sample requirement is also very low as compared to the packed column analysis. The column used in this analysis is thin film of 0.25µ with an internal diameter of 0.25 mm, which had advantages over the thick film narrow bore or wide bore capillary columns in terms of seperation efficacy and speed. The separation performance as indicated by a good capacity ratio range, which is in ideal range of less than 2 at lower range and beyond 10 at higher range (Table 2). The coefficient of variation for concentrations between injections from same sample was within four per cent. The column is having a bounded phase with fused silica and is categorized as very polar.

The method was used to analyze the fatty acid profile of unknown samples viz., coconut oil, sunflower oil, palm oil and animal fat (butter fat) and a clear separation of all fatty acids was obtained (Table 3). The fatty acid profile of these oils was in conformity with the ones reported in the literature for these oils and fat (Hilditch and Williams, 1964; Padua-Resurreccion and Benzon, 1979; Rao *et al.*, 1995; van der Vossen and Umali, 2001). This clearly demonstrates that this method can be

Table 2. The analysis parameters of fatty acid methyl ester standards

Peak	Compound Name	Retention Time	Area%	A/H	Concentration (mg/ml)	Theoretical Plate	Resolution	Initial Time	Final Time	Tailing Factor	Separation Factor	Capacity factor (K')
1	C8	2.978	6.6494	2.58	0.759	31939	15.61	2.90	3.16	1.14	0.00	0.53
2	C10	4.799	7.3249	3.19	0.763	53867	24.45	4.72	4.98	1.17	2.77	1.46
3	C12	7.428	7.6463	3.66	0.764	97644	29.65	7.34	7.65	1.15	1.92	2.81
4	C14	10.418	8.3578	3.89	0.764	170117	30.57	10.31	10.62	1.12	1.55	4.35
5	C16:0	13.417	12.1681	4.04	1.050	258907	29.04	13.29	13.61	1.02	1.35	5.88
6	C16:1	13.970	5.1319	3.96	0.476	294239	5.42	13.87	14.13	1.11	1.05	6.17
7	C18:0	16.221	8.8295	4.02	0.763	381471	19.25	16.11	16.40	1.04	1.17	7.33
8	C18:1	16.660	5.3311	4.04	0.477	396826	4.18	16.55	16.86	1.09	1.03	7.55
9	C18:2	17.492	3.9638	4.08	0.477	434401	7.87	17.38	17.67	1.11	1.06	7.98
10	C18:3	18.499	2.8910	4.02	0.477	492404	9.54	18.39	18.66	1.09	1.07	8.50
11	C20	18.835	8.5702	4.12	0.763	483866	3.16	18.70	19.00	1.02	1.02	8.67
12	C22	21.259	8.7540	4.09	0.764	638232	22.63	21.14	21.43	0.99	1.14	9.91
13	C22:1	21.685	5.1852	4.05	0.543	670834	4.02	21.58	21.85	1.07	1.02	10.13
14	C24	23.506	8.8789	4.18	0.764	737655	16.95	23.38	23.67	0.96	1.09	11.07

Table 3. Fatty acid profile of vegetable oils froom coconut, sunflower and oil palms and animal fat

Fatty acid	WCT	Coconut COD X WCT	WCT X COD	Sunflower oil	Palm oil	Butter fat (animal fat)
C6 (Caproic)	0.12	0.13	0.13	-	0.03	0.75
C8 (Caprylic)	5.25	4.10	4.54	0.27	0.26	0.82
C10 (Capric)	5.13	4.19	4.57	0.13	0.08	2.33
C12 (Lauric)	50.58	48.17	48.69	0.34	0.35	3.44
C14 (Myristic)	19.52	20.70	20.41	0.10	2.48	14.84
C16:0 (Palmitic)	8.12	9.89	9.46	6.26	38.22	32.44
C16:1 (Palmitoleic)	-	-	-	0.13	2.43	3.23
C18:0 (Stearic)	2.76	3.22	2.90	3.33	4.63	12.72
C18:1 (Oleic)	7.93	7.70	7.49	31.26	37.02	25.57
C18:2 (Linoleic)	1.57	1.89	1.72	56.45	9.52	1.66
C18:3 (Linolenic acid)	0.06	0.07	0.07	0.24	0.55	1.16
C20 (Arachidic)	0.06	0.08	0.09	-	0.56	0.30
C22 (Behenic)	-	-	-	-	0.44	-
C22:1 (Erucic acid)	-	-	-	0.60	2.33	0.05
C24 (Lignoceric)	0.03	-	-	-	0.35	0.28
24.8 min ret*	-	0.08	-	-	0.48	-
27.7 min ret*	-	-	0.02	-	0.17	0.07

*Standards for these fatty acids were not run

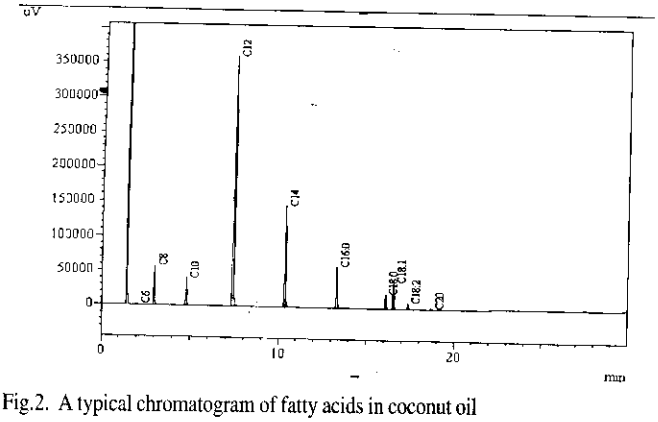

Fig.2. A typical chromatogram of fatty acids in coconut oil

adopted for the fatty acid analysis of any other vegetable oil or fat. A typical chromatogram of coconut oil fatty acids is presented in Fig.2. This method could detect not only all the reported fatty acids but also other long chain fatty acids beyond 20 °C, which were never reported in coconut oil. These fatty acids are in minute quantity thus must have evaded the detection in earlier cases.

The results indicate that the capillary gas chromatography for the analysis of fatty acids in oils and fats is more precise and sensitive. This method was used to analyze the fatty acid profiles of oil from as many as 160 cultivars and hybrids of coconut to find out for the first time the influence of season, genotype, G x E interaction, abiotic and biotic stresses, etc on oil quality (Naresh Kumar, 2005). The method described in this paper can be used for analysis for fatty acid in any oil or fat for obtaining a clear separation of fatty acid methyl esters even at very low quantities.

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