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Lipid profile of virgin coconut oil processed by different methods

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Coconut oil is used for various purposes i.e. edible (39.4 %), toiletry (46.5 %) and other industrial (14.1 %) uses (Naresh Kumar, 2007). In the recent past, the coconut oil has been used as a food ingredient in functional foods, besides being used in pharmaceuticals, nutriceuticals, cosmetics and industrial uses including biofuel. Coconut oil is a rich source of medium-chain-triglyserides (MCT) which are beneficial for human health and nutrition. Sixty three percent of coconut oil is composed of antimicrobial medium-chain fatty acids and therefore can assists the immune system in fighting against microscopic invades. Virgin coconut oil (VCO) is the purest form of coconut oil, water white in colour, contains natural vitamin E, and has not undergone any hydrolytic or atmospheric oxidation as attested by its very low free fatty acid content and peroxide values. It has a mild to intense fresh coconut scent depending on the type of process used for its production.

VCO is obtained from the fresh and mature kernel of coconut by mechanical or natural means with or without the application of heat which does not lead to alteration of the oil and its properties. It is suitable for human consumption in its natural state immediately after extraction and filtration (Bawalan, 2006). VCO greatly differs from the traditionally produced coconut oil from copra which generally undergoes chemical refining, bleaching and deodorization process to make it suitable for human consumption. RBD (refined, bleached and deodorized) coconut oil is yellow in colour and does not contain natural Vitamin E since this is removed when the oil is subjected to high temperature and various chemical processes (Bawalan, 2006).

VCO is unique among all the other vegetable oils because of its high lauric acid content. It is reported that lauric acid in coconut oil is used by the body to make the same disease fighting fatty acid derivative monolaurin that babies make from the lauric acid they get from mother's milk (Enig, 2001). The monoglyceride (monolaurin) is the substance that keeps infants away from getting viral or bacterial or protozoal infections. Such a high profile value-added product virgin coconut oil can be extracted directly from the fresh coconut meat or from coconut milk or from coconut milk residue. VCO could be produced by Hot-processing method, Natural fermentation method, Extraction from Dried Gratings (EDG) method and Centrifuging method. The choice of the technology to be adopted depends to a great extent on the scale of operation, the degree of mechanization, the amount of investment available and the market demand.

Though the fatty acid profile of coconut oil has been extensively studied (Oo and Stumpf, 1979; Naresh Kumar *et al*., 2004), composition of fatty acid in VCO produced by different processing methods has not been studied extensively.

Virgin coconut oil was produced from fully matured coconuts (11-12 months old) by three different methods namely fermentation method, hot processing method, extraction from dried gratings method using CPCRI protocols and machineries at Agro Processing Complex of Central Plantation Crops Research Institute, Kasaragod.

(i) *Fermentation method*: CPCRI has standardised the protocol for preparation of VCO by fermentation method which comprises of two stages; extraction/ preparation of coconut milk and fermentation of the milk for VCO production (Anonymous, 2010). Fresh and matured coconut was grated and the milk was extracted from the grated coconut. CPCRI has developed a coconut grating machine, a pulveriser to grate the coconut with a capacity of 250 coconuts per hour and a semi hydraulic coconut milk extractor with a capacity of 100 coconuts per hour for extracting coconut milk. The diluted coconut milk was kept in fermentation tank specially designed

for this for 16 to 20 hours under controlled atmospheric conditions at a temperature of 35-40°C and relative humidity of 75-80 %. During fermentation the oil was formed along with skim milk and cream in different layers. By carefully separating the distinct layers, the VCO was separated and filtered.

(ii) *Hot processing method*: A VCO cooker with a capacity of 125 litres per batch per 6 hrs has been designed and fabricated at CPCRI, Kasaragod for extracting virgin coconut oil from coconut milk. Fresh and matured coconut was grated and the milk was extracted from the grated coconut. CPCRI has developed a coconut grating machine, a pulveriser to mash the grated nut with a capacity of 250 coconuts per hour and a semi hydraulic milk extractor with a capacity of 100 coconuts per hour for extracting coconut milk. Virgin coconut oil was produced by heating the coconut milk in the VCO cooker developed by CPCRI under controlled temperature and constant stirring.

(iii) *Extraction from dried gratings (EDG) method*: Fresh and matured coconut was grated either by using the coconut grating machine or by a pulveriser developed by CPCRI. The gratings were dried using a unique drier developed by CPCRI where any agricultural waste could be used as fuel. Virgin coconut oil was extracted using a screw press specially designed and developed at CPCRI, Kasaragod with a capacity of 100 nuts per hour.

The fatty acid profile of virgin coconut oil was analysed by capillary gas chromatography method (Naresh Kumar, 2007) after forming methyl ester of fatty acids which was prepared by Pauda-Resurrection and Banzon (1979) method. Accordingly, five percent HCl reagent was prepared by adding 8.3 ml of acetyl chloride drop wise to 100 ml absolute methanol. Ice jacket was used to prevent bumping due to exothermal reaction. Two ml of this reagent was added to 0.2 g virgin coconut oil sample 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 of 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 without any loss.

Methyl esterified samples were diluted (40 µl FAME sample + 960 µl n-hexane, HPLC quality) in the same vial, using disposable pipette tips. The sample vials were put in auto-injector vial tray. Methyl esterified sample (1 µl) 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 amplified signals were transferred and recorded in a computer with GC solution software. The conditions set for GC analysis are presented in Table 1. The amplified signals were transferred and recorded in a computer with GC-Solutions software. The data then acquired was analysed using the GC post run analysis software.

Quantitative method was followed using an external standard mixture of fatty acids (C6-C24). Fatty

Table. 1. Test conditions of Gas Chromatography for lipid profile analysis

Auto sampler settings		
Injection sample volume		1 µ l
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 µ l
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		
Column name		BPX-70
Film thickness		0.25 µm
Inner diameter		0.25 mm
Column length		30 m
Column maximum temperature		260 C
Detector settings		
Detector		FID
Temperature		280° C
Makeup gas		N_2 / Air
Makeup flow		30 ml/min
H2 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

acid methyl ester standards (C6-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. By using this software, the fatty acid type, concentration, area etc. of these four VCO samples was detected precisely.

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

The gas chromatographic analysis of fatty acid methyl esters indicated that the virgin coconut oil mainly contains the saturated fatty acids namely C6-Caproic acid, C8-Caprylic acid, C10-Capric acid, C12-Lauric acid, C13-Tridecyclic acid, C14-Myristic acid, C16-Palmitic acid, C16-Palmitic acid, C17: 1-Heptadecanioc acid, C18: 0-Stearic acid, C18: 1-Oleic acid and C18: 2-Linoleic acid. The concentration of the lauric acid, the major component of the fatty acids in virgin coconut oil has ranged from 50.39 to 51.35 percent depending on the method of preparation. The variation in fatty acid profile of different VCO is indicated by the concentration percentage for each fatty acid in Table 2.

Table. 2. Fatty acid profile of virgin coconut oil prepared by different methods

Sl. No.	Fatty acid	Virgin Coconut Oil		
		Fermentation method (%)	Hot processing method (%)	EDG method (%)
1	C6-Caproic acid	0.14	Nil	0.11
2	C8-Caprylic acid	4.60	4.90	5.45
3	C10-Capric acid	4.54	4.96	5.42
4	C12-Lauric acid	51.09	50.39	51.35
5	C13-Tridecyclic acid	Nil	0.16	0.16
6	C14-Myristic acid	20.89	20.91	19.74
7	C16-Palmitic acid	8.70	8.54	8.09
8	C17:1-Heptadecanioc acid	0.13	0.36	0.46
9	C18:0-Stearic acid	2.64	2.53	2.49
10	C18:1-Oleic acid	6.12	6.10	5.62
11	C18:2-Linoleic acid	1.15	1.15	1.11

It could be seen from Table 2 that the highest concentration of lauric acid (51.35 %) was observed in the VCO sample produced by EDG method and the lowest lauric acid concentration of 50.39 % was observed in the VCO prepared by Hot processing method. This is probably due to the reason that in case of EDG method the VCO was extracted directly from the dried kernel and not the coconut milk. Whereas in case of hot processing method the coconut milk was extracted and heated initially to 120°C and gradually reducing to 90°C towards the end of the process to extract VCO. The coconut milk extraction process and the temperature could be the reason for the change in concentration of lauric acid in the VCO prepared by Hot process method. In EDG method, the VCO was extracted directly from freshly dried coconut gratings using the screw type expeller. This could be the reason for the higher concentration of lauric acid in the free fatty acid profile. The VCO prepared by fermentation method also yielded the lauric acid concentration to the tune of 51.09 %.

Madhavan *et al*. (2005) studied the fatty acid profile of commercial grade edible coconut oil and the result of the fatty acid profile of commercially available coconut oil is presented in Table 3. It is observed from Table 3 that the lauric acid concentration was 50.44 % which is lesser than the VCO sample produced by EDG and Fermentation method and it is on par with the hot processed VCO. The possible reason could be that the virgin coconut oil is extracted from fresh kernel where as the commercial oil is expelled from milling copra. VCO greatly differs from the traditionally produced commercial coconut oil which has to undergo chemical refining, bleaching and deodorization processes. Since the commercial coconut oil is subjected to high temperature and various chemical processes that could have affected the quality of free fatty acid and hence the lauric acid concentration was found minimum. This is inline with the findings of Naresh Kumar (2007).

Table. 3. Fatty acid profile of commercial grade edible coconut oil (Madhavan *et al.*, 2005)

Sl. No.	Fatty acid	Commercial grade edible coconut oil (%)
1	C6-Caproic acid	0.08
2	C8-Caprylic acid	4.85
3	C10-Capric acid	4.99
4	C12-Lauric acid	50.44
5	C13-Tridecyclic acid	Nil
6	C14-Myristic acid	20.94
7	C16-Palmitic acid	8.15
8	C17: 1-Heptadecanioc acid	Nil
9	C18: 0-Stearic acid	3.01
10	C18: 1-Oleic acid	5.83
11	C18: 2-Linoleic acid	1.45

It is also seen from the Tables 2 and 3 that the lauric acid concentration of the VCO and commercial coconut oil was followed by C-14 myristic acid. The good absorption of coconut oil in the skin is greatly depends on the concentration of myristic acid and hence the virgin coconut oil including coconut oil can be used as skin care tonic to keep the skin soft and smooth and promotes healthy and luxuriant hair growth (Ahmed Bavappa, 2008).

Typical chromatograms of the virgin coconut oils prepared by fermentation method, hot processing method

and EDG method are shown in Fig. 1, 2 and 3, respectively. These figures indicate that by following capillary gas chromatography method, a clear separation of all saturated, mono-unsaturated and poly-unsaturated fatty acids can be achieved in the virgin coconut oil samples.

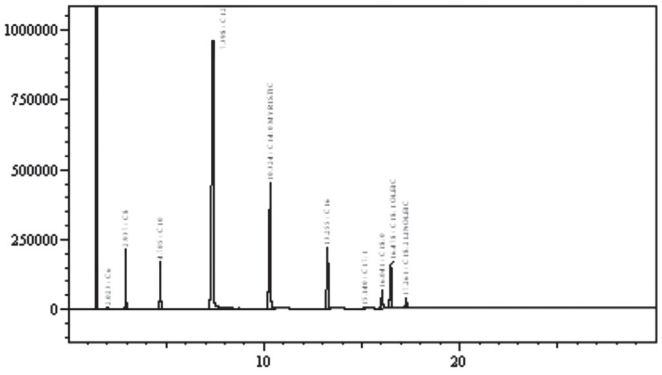

Fig. 1. A typical chromatogram of fatty acids in virgin coconut oil produced by fermentation method

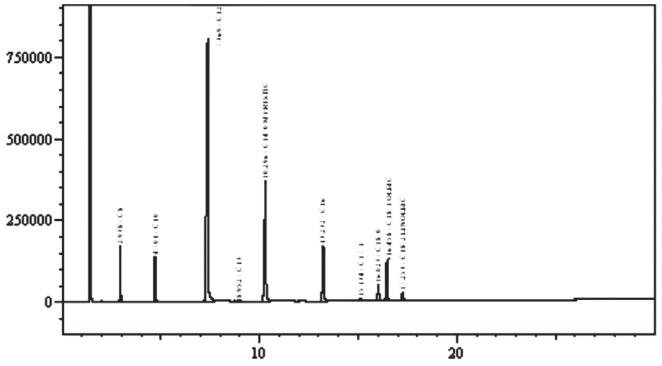

Fig. 2. A typical chromatogram of fatty acids in virgin coconut oil produced by hot processing method

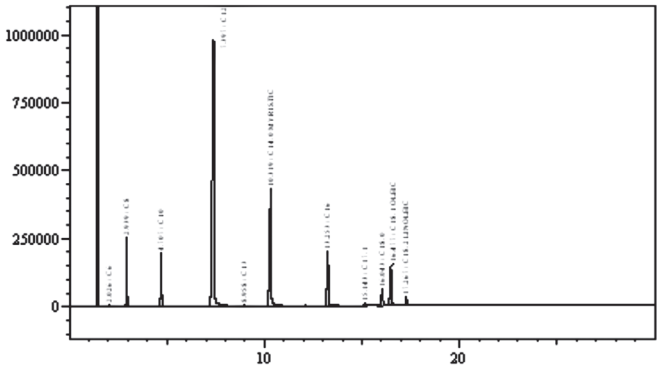

Fig. 3. A typical chromatogram of fatty acids in virgin coconut oil produced by EDG method

Results indicated that VCO has higher concentrations of short and medium chain fatty acids (C6-C12) and lower concentrations of long chain fatty acids (C13-C18) compared to the commercial grade edible coconut oil (Tables 2 and 3). The fatty acids of C6 to C12 carbon chains are the most important portions of the VCO and this constitutes around 62 per cent of the VCO. The shorter chains of C6 to C10 (about 11 % of VCO) are easily digested and absorbed in to the metabolic activities of the human body and hence these fatty acids are responsible for the fast metabolic property of VCO (Kaunitz *et al*., 1958; Kaunitz, 2001). These medium chain triacyl glycerides are directly absorbed by the intestinal cells and carried by portal circulation to liver for further oxidation and hence quick release of energy (Kaunitz and Dayrit, 1992; Enig, 1996; Blackburn *et al*., 2001). VCO, among all the other vegetable and animal oils and fats, has one of the lowest content of C14 to C18 (long chain saturated fatty acid, which is considered to be bad saturated fat) at about 30 %. VCO has the long chain unsaturated fatty acid concentration only about 8 %, of this 6 % is the good mono unsaturated long chain fatty acid of oleic acid (C18:1), which has been found to be very beneficial to the heart, anti-inflammatory and a host of other health benefits.

Based on the overall results, it can be concluded that the VCO produced by all the three methods namely fermentation, hot processing and EDG method exhibited best quality in terms of fatty acid composition. However, there is no significant change in the quality in terms of fatty acid composition among the three oils processed by different methods.

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Central Plantation Crops Research Institute,
(Indian Council of Agricultural Research)
Kasaragod - 671 124, Kerala

T. Arumuganathan,
K. Madhavan,
A.C. Mathew,
Sugada Padmanabhan