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GAS CHROMATOGRAPHIC DETERMINATION OF FATTY ACID COMPOSITION IN COCONUT OIL EXTRACTED BY PROBE SONICATION TECHNIQUE

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Coconut oil; Probe sonication; Base derivatization; GC-FID

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ABSTRACT: Coconut oil is an edible vegetable oil used in the food, pharmaceuticals, and cosmetics industries. Fatty acids are the major constituents of Coconut oil. Various methods were reported for the extraction of oil from coconut seed kernels. Extraction of coconut oil from its seed kernel using the probe sonication method is not reported in the literature. Hence, in the present study, oil was extracted using hexane and ethyl acetate. The physicochemical parameters like acid value, saponification value, refractive index, specific gravity, unsaponifiable matter, moisture content and iodine value were determined for the extracted oil. Oil samples after base-catalyzed derivatization were analyzed for the quantification of fatty acids by the GC-FID method. The percentage of oil extracted from coconut seed kernel was 31.84% and 36.32% for n-hexane and ethyl acetate extractions, respectively. Physico-Chemical parameters of the oils tested were within limits. Coconut oil obtained from n-hexane extraction contained the highest fraction of lauric acid. Oil extraction with probe sonication technique was found to be efficient as the percentage recovery of oils is in the range of 32 to 36%.

INTRODUCTION: Oils extracted from the seeds, kernels or mesocarp of the fruit of crops provide health benefits such as reducing the risk of cardiovascular diseases, reduces stomach ulcers and ulcerative colitis, boost immune system, lowers anxiety and depression, prevents osteoporosis, lowers chances of breast cancer, antioxidant property and provides omega-3 fatty acids to the body [1, 3]. Oils are chemically esters of glycerol with three molecules of fatty acids. Fatty acids are classified based on the existence and nonexistence of a double bond. Saturated fatty acids do not have a double bond. Monounsaturated fatty acids have one double bond, and polyunsaturated fatty acids have more than one double bond. Coconut oil is the main choice of vegetable oil in India and most part of Asian countries [4].

Coconut oil is an edible vegetable oil derived from the fruit of the *Cocos nucifera* L. tree belongs to the family Arecaceae. Coconut oil is extracted from coconut flakes, the endosperm portion of coconut [5]. Coconut oil is colorless to light brownish yellow in color [6] that contains 92.1 % of saturated fatty acids, 6.2 % of monounsaturated fatty acids, and 1.6 % of polyunsaturated fatty acids [7]. The high content of

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medium-chain fatty acids in the oil metabolizes differently than most other fats. Medium-chain fatty acids can increase the number of calories burned over 24 h by as much as 5%. Coconut oil boosts heart health by raise blood levels of HDL cholesterol. They can significantly reduce the appetite lead to reduced body weight. The medium-chain fatty acids in coconut oil can increase the blood concentration of ketone bodies, which can help reduce seizures in children with epilepsy. Lauric acid present in coconut oil is processed into monolaurin. That is known to boost immunity to the baby [8, 11]. Lauric acid and monolaurin can kill harmful pathogens such as bacteria, viruses, and fungi. People can apply coconut oil to their skin and hair because it works as a skin moisturizer and protects against skin damage [12, 15]. Coconut oil has high antioxidants that help fight free radicals, which is a leading natural treatment for osteoporosis [16, 17].

Coconut oil can be extracted by wet and dry process, where chilling, thawing, fermentation, enzymatic, modified kitchen method and pH method are the wet process and dehydration, centrifugation, solvent extraction, low pressure and high pressure mechanical pressing are the dry processes for the extraction of oil. But the percentage yield of oil obtained by the wet process is approximately 10-15 % lesser than the dry process [18]. The literature did not report the extraction of coconut oil from the kernel of coconut using n-hexane and ethyl acetate by probe sonication. Hence the present study was undertaken to assess whether the sonication process improves the percentage yield of the oil and the content of fatty acids in the oil.

MATERIALS AND METHODS:

Chemical and Reagents: HPLC grade n-hexane, methanol, and ethyl acetate were used for the study. All other chemicals and reagents used are of Analytical Grade obtained from Merck. Coconuts obtained from the local market was dishelmed, washed and grated to get fine particles of coconut flakes with uniform size particle.

Extraction Procedure: Twenty-five g of coconut flakes were taken in a 250 ml beaker and 100 ml of hexane added to it. The mixture was sonicated for 20 min in a Sonics Vibra cell probe sonicator. After optimization, the amplitude of sonication was set to 70% and the time interval set to 10:10. The temperature was maintained at 35 ± 1 °C. After sonication, the solvent layer was filtered out using Whatman filter paper. The coconut oil was then collected by a natural evaporation method. This procedure was repeated 10 times, and the % yield was calculated for 250 g of coconut flakes. The same process was adopted for the extraction of coconut oil by ethyl acetate solvent. The percentage yield of coconut oil obtained was calculated for both the extraction.

Determination of Physico-Chemical Properties: Food safety and standards authority of India (FSSAI) methods were used to analyze oil [19].

Acid Value: Weighed precisely 5 g of oil in a 250 ml Erlenmeyer flask, added 50 ml of the neutralized acid-free ethanol-ether mix (25+25 ml) recently neutralized with 0.1 M potassium hydroxide solution. Shaken well and titrated against 0.1 M potassium hydroxide solution utilizing phenolphthalein solution. Calculated the acid value according to the following equation:

$$\text{Acid value} = 5.61VM/W$$

Where, V= Volume of potassium hydroxide consumed (mL), M = Molarity of potassium hydroxide solution, W= Weight of coconut oil taken for analysis (g)

Saponification Value: Two g of coconut oil weighed in a 250 ml Erlenmeyer flask fitted with a reflux condenser. Twenty-five ml of 0.5 M alcoholic potassium hydroxide was added to it. The blend was refluxed on a water bath for 30 minutes. Cooled and titrated promptly with 0.5 M hydrochloric acid using phenolphthalein solution (a). Titration was rehashed by excluding the oil (blank) (b). Calculated the Saponification value according to the following equation:

$$\text{Saponification value} = 28.05\ (b-a)/W$$

Where, b = Volume in ml of hydrochloric acid required for blank (mL), A = Volume in ml of hydrochloric acid required for the sample (mL), W = Weight of coconut oil being examined (g)

Iodine Value: Five g of oil was accurately weighed in a 250 ml Erlenmeyer flask and 25 ml of carbon

tetrachloride and 25 ml of Wij's solution were added to it. The mixture was blended well and kept standing for 30 min. Then 15 ml of potassium iodide solution, followed by 100 ml of freshly boiled and cooled water, was added. The liberated iodine was titrated with standardized sodium thiosulphate solution, utilizing starch as an indicator until the blue color disappears. Blank determination was also performed without oil. Iodine value was calculated using the equation

Iodine value = 12.69 (B-S) N/W

Where, B = Volume in ml of sodium thiosulphate solution required for blank (mL), S = Volume in ml of sodium thiosulphate solution required for the sample (mL), N = Normality of the standard sodium thiosulphate solution (N), W = weight of coconut oil used for analysis (g)

Specific Gravity: Dry specific gravity bottle was filled with the oil without air bubbles. Inserted the stopper and weighed the oil at 30 °C ± 0.2 °C. In a pre-weighed specific gravity bottle, weight of water at 30 °C ± 0.2 °C was weighed.

Specific Gravity at 30 °C / 30 °C = A-B/C-B

Where, A = Weight of specific gravity bottle with oil (g), B = Weight of empty specific gravity bottle (g), C = Weight of specific gravity bottle with water (g)

Refractive Index: Refractive index was determined in Abbes Refractometer at 30 °C. After cleaning the Nicol prisms, two drops of oil were placed on the prism. Closed the prisms and allowed to stand for 1-2 min. The eyepiece micrometer was adjusted to focus the boundary between the bright and dark regions. The refractometer scale adjusted to place the cross wire of the telescope exactly on the boundary between the bright and dark regions and recorded the index of refraction.

Unsaponifiable Matter: Five g of oil was weighed precisely into a 250 ml Erlenmeyer flask, and 50 ml of alcoholic potassium hydroxide solution was added to it. The contents were boiled under reflux for 60 min. Then it was transferred to a separating funnel and washed the saponification flask first with little ethyl alcohol and then with 50 ml of water. After cooling the contents to 25 °C, 50 ml of petroleum ether was added, shaken, and permitted the layers to separate. The lower layer moved into another separating funnel and rehashed the ether extraction for another multiple time utilizing 50 ml of petroleum ether.

The merged ether portion was washed multiple times with 25 ml alcohol, followed by washing with 25 ml refined water. Ether extracts are transferred to 250 ml beaker, and the ether layer was allowed to evaporate. Then 3 ml of acetone was included and warmed on a water bath totally to expel solvent. The residue was then disintegrated in 50 ml of warm ethanol neutralized with phenolphthalein. The solution was then titrated with 0.02 N sodium hydroxide and the level of unsaponifiable value was determined by the following equation.

Unsaponifiable matter =100 (A-B)/W

Where, A = Weight of the residue (g), B = Weight of the free fatty acids in the extract (g), W = Weight of coconut oil (g)

Presence of Mineral Oil: Twenty five ml of the alcoholic KOH solution poured in a Erlenmeyer flask and added 1 ml of the oil to be tested. The contents were boiled on a water bath in a reflux condenser till the mixture becomes clear and no oily drops are found on the sides of the flask.

The flask taken out from the water-bath, transferred the contents to a wide mouthed warm test tube and carefully added 25 ml of boiling distilled water along the sides of the test tube. Shaken well the tube lightly from side to side during the addition. If Turbidity is produced it indicates the presence of mineral oil and the depth of turbidity depends on the percentage of mineral oil present.

Moisture Content: Weighed in a previously dried and tared dish about 5-10 g of oil. Loosen the lid of the dish and heated, in an oven at 105 ± 1 °C for 1 h. Removed the dish from the oven, closed the lid and heated in the oven for a further period of 1 h, cooled and weighed. Repeated this process until change in weight between two successive observations does not exceed 1 mg.

Moisture content = W1 × 100/W

Where, W1 = Loss in weight of the material on drying (g), W = Weight of coconut oil used for the analysis (g)

Extraction of Fatty Acids by Base Catalyzed Derivatization Method [20, 21]: Fifty mg of coconut oil weighed in a test tube (10 mL) and added 400 uL of 0.5 M methanolic sodium hydroxide. Heated the mixture at 50 °C for 20 sec. One mL of hexane was added to the mixture and vortexed for 30 sec. After settling, the top layer was collected in a clean tube, and to that solution, 400 µL of (2 N) methanolic hydrochloric acid was added, which was then mixed using a vortex. The top layer was used for the chromatogram analysis.

Gas Chromatography Analysis [22]: Chromatographic analysis performed in a Perkin Elmer Clarus 590 equipped with a splitless injector, flame ionization detection. Data acquisition and analysis performed in Total Chrom workstation (Perkin Elmer). Elite-5 capillary column (5 % phenyl, 95% dimethyl polysiloxane) 30 m × 0.25 mm, 0.25 µm (Perkin Elmer) was used for the analysis.

Optimized analytical conditions adopted; injector at 250 °C and detector at 260 °C. Carrier gas Nitrogen with a flow rate at 2 ml/min, Hydrogen (30 ml/min) and zero air (450 ml/min) used as auxiliary gases for the flame ionization detector. Injection of 1.0 µL in the splitless mode, the initial temperature of a column at 50 °C, isotherm at 50 °C for 1 min, with heat ratio of 10 °C/min up to 140 °C, isotherm at 140 °C for 1 min, heat ratio of 10 °C/min up to 270 °C and isotherm at 270 °C for 1 min.

RESULTS AND DİSCUSSİON: A new probe sonication technique was adopted for the extraction of oil from coconut flakes using n-hexane and ethyl acetate. The percentage yield of Coconut oil extracted from coconut flakes using n-hexane and ethyl acetate is provided in **Table 1.**

TABLE 1: PERCENTAGE YIELD OF OIL OBTAINED BY PROBE SONICATION TECHNIQUE

Description	n-Hexane	Ethyl acetate
Volume	100 mL	110 mL
Mass	79.60 g	90.81 g
% Yield of oil obtained	31.84%	36.32%

Yield of coconut oil obtained by probe sonication technique using ethyl acetate extraction (36%) and hexane (32%) was found to be high compared to the conventional extraction techniques like pressing, fermentation, heating with a high percentage oil recovery of 25%, 17%, 20% and 25% respectively [23]. Solvents n-hexane and ethyl acetate were used for extraction due to the higher solubility of coconut kernels [24, 25]. The Physico-Chemical properties of oil extracted from coconut flakes using hexane and ethyl acetate as the solvent provided in **Table 2.**

TABLE 2: PHYSICO-CHEMICAL PROPERTIES OF COCONUT OIL EXTRACTED USING DIFFERENT SOLVENTS

Parameters	n-Hexane extract	Ethyl acetate extract	Specification as per BIS[a]	Specification as per APCC[b]	Codex Alimentarius
Acid value	1.61	1.65	Max 5.0	Max 5.0	Max 6.0
Saponification value	255.25	260.86	Min 250	250-260	248-265
Iodine value	9.51	9.77	7.5-10.0	4.1-11	6.3-10.6
Specific gravity at 30 °C/30 °C	0.912	0.919	0.915-0.920	0.915-0.920	0.915-0.920
Refractive index at 30 °C	1.449	1.448	1.448-1.449	1.448 -1.449	1.447-1.450
Unsaponifiable matter	0.42%	0.46 %	Below 0.8%	0.2-0.5	≤0.2
Mineral oil	No turbidity	No turbidity	Absent	--	--
Moisture content	0.06 %	0.08 %	Maximum 1%	0.1-0.5	<0.15

A Bureau of Indian Standards; b Asian and Pacific Coconut Community

The specific gravity of the coconut oil obtained from n-hexane and ethyl acetate is 0.912 and 0.919, respectively. The refractive index of the oil from hexane and ethyl acetate extraction is 1.4485 and 1.4479, respectively. The acid value of the oil extracted using hexane and ethyl acetate is 1.61 and 1.65, respectively. Acid value determination is often used as a general indication of the condition and edibility of the oil, as an increase in acid value causes a change in flavour and odour [26]. The saponification values of the oil extracted using hexane and ethyl acetate are 255.25 and 260.86, respectively. Due to the presence of this unique composition of shorter and medium-chain fatty acids, coconut oil has a higher saponification value and does not change significantly with the method of extraction of coconut oil. It gives information concerning the character of the fatty acids of the

oil; the longer the carbon chain, the less acid is liberated per gram of oil hydrolyzed. The saponification values clearly indicate an index of mean molecular weight of the fatty acids of glycerides comprising a fat. Lower the saponification value, the larger the molecular weight of fatty acids in the glycerides and vice-versa [27]. The iodine values of the oil extracted using hexane and ethyl acetate is 9.71 and 9.77, respectively. The higher iodine value indicates the presence of more unsaturated bonds present in the oil. Iodine values above 100 are classified as drying, while those below are classified as non-drying. It can be classified as a non-drying, stable, and most edible oil with a comparison of other marketed oil. The low iodine value indicates that oils have low content of unsaturated fatty acids and may be useful in soap making [28]. The unsaponifiable matter present in oil extracted using n-hexane and ethyl acetate was 0.42% and 0.46%, respectively. This trace amount of unsaponifiable content in the oil is tocopherols and phytosterols.The absence of mineral oil in the tested samples clearly revealed the purity of the oil. The low moisture content of the oil indicates that it has a long shelf life. Moisture content is one of the major factors affecting the quality of the oil. The moisture content of the oil is of great importance for many scientific, technical, and economic reasons. Moisture content influences the taste, texture, weight, appearance, and shelf life of oils. Even a slight deviation from a defined standard can adversely impact the physical properties of oils.

An increase in moisture content causes a change in color from light brown to dark brown, a change in taste, and a decrease in percentage yield. Less than 1% of moisture content and the light yellow color of the oil confirms the extracted oil is in pure form [27].

Fatty Acid Composition Analysis: Retention times of fatty acids in coconut oils extracted using n-hexane and ethyl acetate represented in **Table 3.** Fatty acid compositions of Coconut oil extracted using n-hexane and ethyl acetate are presented in **Table 4.** Gas chromatograms of the fatty acid in coconut oil are represented in **Fig. 1** and **2.**

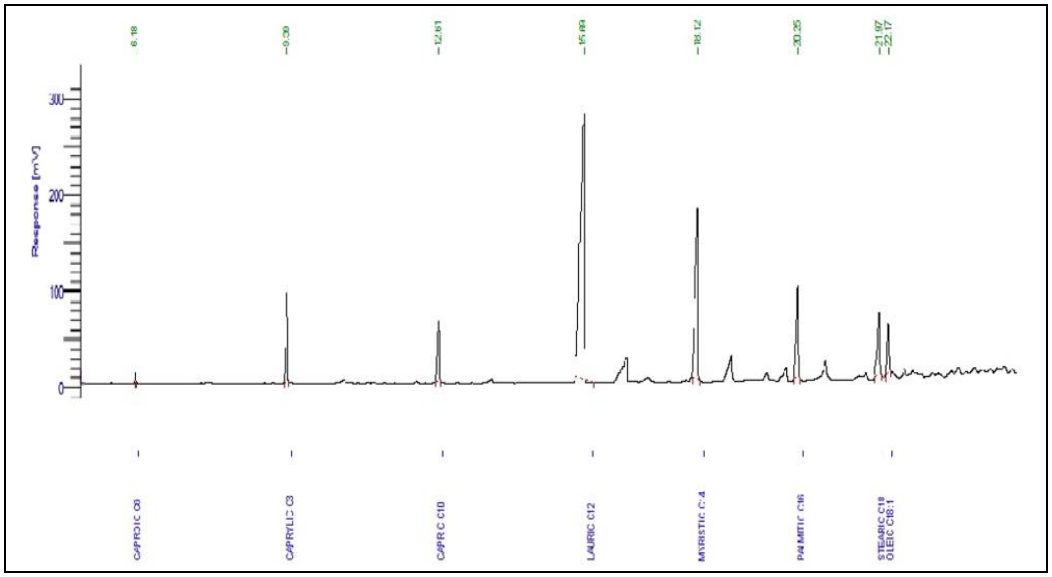

FIG. 1: GC CHROMATOGRAM OF FATTY ACID IN N-HEXANE EXTRACT OF COCONUT OIL

Coconut oil contains high amounts of saturated fatty acids as compared to other edible oils 29. This high saturated fatty acids composition protects coconut oil against oxidative rancidity, and hence it is being widely used in the food and cosmetic industry. A novel GC-FID method was developed to separate and detect the various fatty acids present in the extracted oil.GC-FID study divulged lauric acid (C12:0) is the major fatty acid present in coconut oil, and it was in correlation with the prescribed range (45.1 - 53.2%) as reported by other [22, 31]. Oil extracted using n-hexane had slightly higher lauric acid (48.56%) content than oil extracted using ethyl acetate oil (45.07%). Caprylic acid and capric acid were higher in the oil extracted using n-hexane compared to oil extracted using ethyl acetate (6.46% and 4.68%, respectively). Content of palmitic acid, stearic acid and oleic acid

was high in oil extracted using hexane (11.58%, 6.93%, and 5.75%). Caproic acid in both oils was found to contain below 0.5%. The fatty acids present in oil extracted by n-hexane and ethyl acetate were complied as per APCC and Codex Alimentarius [30].

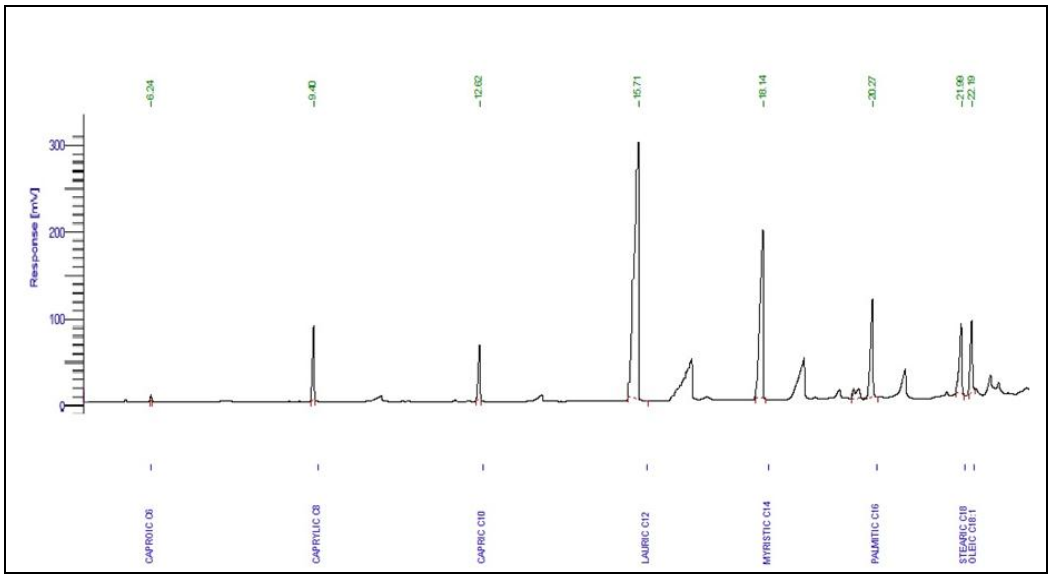

FIG. 2: GC CHROMATOGRAM OF FATTY ACID IN ETHYL ACETATE EXTRACT OF COCONUT OIL

TABLE 3: RETENTION TIME OF FATTY ACIDS IN COCONUT OIL EXTRACTED USING N-HEXANE AND ETHYL ACETATE

Fatty acid	Retention time of oil extracted from n-hexane (Min)	Retention time of oil extracted from Ethyl acetate (Min)
Caproic acid (C6:0)	6.17	6.24
Caprylic acid (C8:0)	9.39	9.40
Capric acid (C10:0)	12.61	12.62
Lauric acid (C12:0)	15.69	15.70
Myristic acid (C14:0)	18.12	18.14
Palmitic acid (C16:0)	20.24	20.26
Stearic acid (C18:0)	21.97	21.99
Oleic acid (C18:1)	22.16	22.19

TABLE 4: FATTY ACID COMPOSITION OF COCONUT OIL EXTRACTED USING N-HEXANE AND ETHYL ACETATE

Fatty acid	n-hexane extraction (%)	Ethyl acetate extraction (%)	APCC[b]	Codex Alimentarius [30]
Caproic acid	0.41	0.23	0.10-0.95	ND-0.7
Caprylic acid	6.46	4.77	4-10	4.6-10
Capric acid	4.68	3.94	4-8	5-8
Lauric acid	48.56	45.07	45-56	45.1-53.2
Myristic acid	21.36	21.74	16-21	16.8-21
Palmitic acid	8.16	11.58	7.5-10.2	7.5-10.2
Stearic acid	6.33	6.93	2-4	2-4
Oleic acid	4.03	5.75		10

B Asian and Pacific Coconut Community ND- Non-detectable, defined as ≤0.05%

CONCLUSİON: Coconut oil was extracted by probe sonication method using n-hexane and ethyl acetate solvents. The percentage yield of oil obtained from the solvents n-hexane and ethyl acetate was found to be 31.84% and 36.32%, respectively. Physico-Chemical parameters such as acid, saponification, and iodine, specific gravity, refractive index, unsaponifiable matter, mineral oil, and moisture content were within limits and complied with the standards. Base catalyzed derivatization method was used for GC-FID analysis that confirmed the presence of fatty acid analysis *viz.* caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic

acid, and oleic acid. The concentration of lauric acid in the oil was found to be high (45-48%) followed by myristic acid (20-22%). The study concludes that solvent extraction using probe sonication is thus an excellent alternative method to the traditional method as the percentage yield is high as compared with the conventional methods.

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