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Aqueous Extraction and Enzymatic Destabilization of Coconut Milk Emulsions

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Abstract Fresh and mature coconuts were subjected to deshelling, paring and disintegration. The coconut milk was extracted, treated with an enzyme (protease) at different concentrations and centrifuged, in order to separate it into coconut cream and aqueous phases. Subsequently, coconut cream was subjected to chilling (different temperatures) and thawing to ambient temperature (29 ± 2 °C) followed by centrifugation to obtain a clear virgin coconut oil (VCO). Coconut milk treated with aspartic protease at concentration of 0.02 mg/g, resulted in 90.4 ± 1.2% yield. A maximum yield of 95.3 ± 1.0% was obtained when the treatment of coconut milk with aspartic protease at concentration of 0.02 mg/g was followed by chilling (5 °C) and thawing. Physicochemical properties and fatty acid compositions were evaluated and compared with commercial coconut oil samples. It was found that the oil obtained from present study is low with respect to free fatty acids (0.31%) and peroxide value (0.81 mequiv O_2/kg) when compared with the commercial coconut oil samples. Sensory evaluation was also carried out to ensure the product acceptability.

Keywords Coconut milk · Enzyme treatment · Aqueous extraction · Protease · Destabilization · Chilling · Virgin coconut oil

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Introduction

India is one of the largest coconut producing countries in the world and around 10% of the produce goes for coconut oil production. Coconut milk is an oil-in-water emulsion, stabilized by the naturally occurring proteins (albumins and globulins) and phospholipids (lecithin and cephalin) [1], which requires extra energy to destabilize this emulsion [2]. Fresh coconut milk contains nearly 55 ± 3% aqueous, 37 ± 2% fat and 8 ± 2% protein [3]. Coconut oil is produced by either dry or wet processes. In the dry process, coconut oil is produced by expelling dry copra and in this process, oil is exposed to high temperatures during refining. Oil obtained from fresh and mature coconuts without thermal treatment and any refining is known as virgin coconut oil (VCO) [4]. Traditionally VCO is obtained by a fermentation method, where the white endosperm of freshly harvested coconuts is disintegrated, followed by milk extraction. The milk is allowed to ferment naturally for 24–36 h at ambient temperature. The fermented milk separates into two phases [oil (top) and aqueous (bottom) phases] [5]. The disadvantages of this method of extraction are low oil recovery and fermented odor in the oil, which masks the characteristic coconut flavor. VCO obtained by aqueous extraction has more beneficial effects than copra pressed oil, since it retains most of the nutritional components [6].

VCO is rich in medium chain fatty acids and contains nearly 48–53% lauric acid, having antibacterial, antifungal and antiviral properties [7]. It also contains a considerable amount of short chain fatty acids [8]. It is used in skin care products to prevent wrinkles and sagging [9]. VCO is also recognized for its quality of strengthening the structure of damaged, devitalized hair. It lubricates and softens the hair root and conditions the scalp [10]. The importance of VCO

due to its various functional properties is increasing both nationally and internationally [11].

Stringent environmental and safety restrictions imposed on the vegetable oil extraction industry have spurred interest in alternatives to solvent extraction. Aqueous extraction processing has been considered as an alternative to solvent extraction [12]. It avoids the use of organic solvent, thereby eliminating the need for its recovery by distillation [13]. The main objective of the present work was to develop a method for aqueous extraction and enzymatic destabilization of coconut milk emulsion to obtain VCO without the need of thermal treatment or fermentation.

Materials and Methods

Materials

Fresh and mature coconuts (10–12 months old) were procured from a local market. Commercial virgin coconut oil (CVCO) and commercial coconut oil (CCO) samples were procured from the market (Departmental store, Mysore). Enzyme aspartic protease (EC 3.4.23) (activity: 2,500 tyrosine units/g) of commercial grade was procured from Kaypeeyes Biotech Private Ltd., Mysore, India. The reason behind choosing aspartic protease is, the enzyme acts optimally at acidic pH and the pH of coconut milk will be around 6. In the total coconut protein, after glutamic acid, the most predominant amino acid is aspartic acid (4–5% v/v). In our preliminary experiments higher (5–7%) oil yields were observed with aspartic protease when compared to other proteases. All chemicals of analytical grade were procured from Merck Chemicals, Mumbai, India. For GC analysis, hexane (HPLC grade) was procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India and fatty acid methyl ester standards were procured from Sigma–Aldrich St. Louis, MO, USA.

Oil Extraction

Mature coconuts were manually subjected to deshelling, paring and removal of water. The white coconut kernel was disintegrated using a rotary wedge cutter (sieve size Ø 3 mm; speed 5,000 rpm; capacity 300 nuts/h; Krauss Maffei, Germany). The grated coconut kernel was subjected to expelling in a screw press (screw: Ø 24 cm; 11.5 cm pitch; and rotating at 2.5 rpm; capacity 200 kg/h; M/s. Louisville, USA) to extract coconut milk. Individual samples of freshly extracted coconut milk were treated with aspartic protease at concentrations of 0.005, 0.01, 0.015, 0.02, 0.05, and 0.5 mg/g and allowed to stand for 3 h at 37 ± 2 °C. These milk samples were centrifuged (Model No: TC-4100 D, Eltectrocraft, India) at 3,585 *g* for 10 min to separate coconut cream and aqueous phases. Individual cream samples obtained were chilled at different temperatures (20, 15, 10, and 5 °C) for 6 h and thawed to ambient temperature (29 ± 2 °C). Finally, thawed cream samples were centrifuged at 4,880*g* for 15 min to obtain clear VCO. Fat content of freshly extracted coconut milk was found to be 39.1 ± 0.9%, determined using Rose-Gottlieb AOAC method [14]. These oil extraction experiments were carried out for three different batches, with three replications for each batch.

Physicochemical Properties

The VCO samples were evaluated for moisture, specific gravity, refractive index, iodine value, Polenske value, acid value, saponification value, unsaponification matter, peroxide value according to AOAC official methods 925.10, 920.212, 921.08, 920.159, 925.41, 940.28, 920.160, 933.08, 965.33, respectively [15].

Vitamin E was estimated using the IUPAC method [16]. In this method 1.005 g of oil was taken in 25-ml flask, 4 ml of pyrogallol was added and kept in water bath and removed when it starts boiling. 4 ml of KOH was added, boiled, and cooled. 25 ml of distilled water was added and transferred to a separating funnel. Twenty milliliter of diethyl ether was added twice and ether phase was separated. Ether phase was washed until pH becomes neutral. Dried ether phase was reconstituted with 10 ml of ethyl alcohol. Further, calorimetric method was used to estimate Vitamin E. CVCO and CCO samples were also evaluated for these parameters for the purpose of comparison.

Fatty Acid Composition

VCO samples were analyzed for fatty acid composition by Gas Chromatography (GC) (Shimadzu 14 B fitted with FID) [17]. Oil samples (10 mg) were saponified using 0.5 M methanolic potassium hydroxide. Fatty acid methyl esters were prepared from the saponified mixture using boron trifluoride in methanol. These esters were extracted with hexane, dried over anhydrous sodium sulfate and analyzed using fused silica capillary column Varian: VF-1 (30 m length, 0.25 mm internal diameter and 0.25 μm film thickness, Scotland, UK). The injector and detector temperature were 230 and 240 °C, respectively. The column temperature was 220 °C and nitrogen was used as a carrier gas at a flow rate of 1 ml/min. Individual fatty acids was identified by comparing with retention time of standards. Fatty acid composition of CVCO and CCO samples were also carried out for comparison.

Free Fatty Acids

The samples of VCO (six sets) were stored in glass bottles (120 ml capacity) with a 1.5-cm head space. Oil samples were placed in dark condition at ambient temperature (29 ± 2 °C) over a period of 1 year and analyzed every 2 months for free fatty acids (FFA). Evaluation was carried out according to AOCS method Ca 5a-40 [18] and expressed in percentage FFA as lauric acid. Analysis of CVCO and CCO was also carried out for comparison.

Sensory Evaluation

Sensory odor analysis for VCO samples was carried out at the Department of Sensory Science, CFTRI. Quantitative descriptive analysis (QDA) was used for the analysis [19], where the attributes were quantified on a 15-cm structured scale. The intensity of each specific descriptor was quantified on the structured scale. In order to trace the changes in the sensory quality profile, suitable attributes (fresh, nutty, musty, rancid, ferment, soap, chemical, and metallic) specific to the oil was collected during the preliminary sessions of evaluations and scorecards were prepared. The chemical compounds used to evaluate odor attributes are as follows. For fresh, fresh coconut oil (aldehyde C-14); nutty, 2,3 dimethyl pyrazine; rancid, butyric acid; ferment, methyl thiobutyrate; chemical, phenyl acetylene; soap, detergent; metallic, hexyl amine; musty, isopropyl quinoline [20]. A trained panel of five members completed the sensory evaluation. Care was taken to avoid interference from other sources or bias. VCO, CVCO, and CCO samples were evaluated using the scorecard as per the standard conditions. Conical flasks coded with three-digit random numbers were used for the analysis to avoid bias. Oil samples were prepared in sodium citrate phosphate buffer. Oil was (10 g) placed in a conical flask and 10 ml buffer (pH 4.0–4.3) was added. Sufficient time was allowed for the headspace to develop in the flask before sniffing. Mean scores of each attribute were plotted as a bar graph.

Data Analysis

Extraction of coconut milk was carried out for three batches. Physicochemical analysis was carried out in triplicates for VCO samples obtained by the method involving enzyme treatment followed by chilling thawing (that resulted in highest yield of 95.3 ± 1.0%). Analysis was also carried out for CVCO and CCO samples. The data of all the three batches is presented in the tables and figures as mean ± standard deviation values ($n = 3$).

Results and Discussion

Enzymatic Destabilization of Oil-in-Water Emulsion

The effect of enzyme concentration on the extent or degree of destabilization of coconut milk emulsion, which in turn determines the yield of oil, is shown in Fig. 1. Oil yield increased with an increase in the enzyme concentration. Oil yield of 90.4 ± 1.2% was obtained at a enzyme concentration of 0.02 mg/g and no significant difference in yield was observed with further increases in enzyme concentration. Aspartic protease (endoprotease) was selected to destabilize the coconut milk emulsion, which hydrolyzes peptide bonds in the interior of the polypeptide chain. Thereby these exposed shorter fragments of protein/peptides decrease the emulsifying property which leads to aggregation of oil droplets. Further, these proteins/peptide fragments move towards the aqueous phase facilitating the phase separation. A high yield (97%) was observed when a mixture of enzymes namely, protease, α-amylase and pectinase, was used at 0.1% (w/v) concentration [21]. However, these researchers [21] employed higher temperatures (50 °C) during the extraction which reduces the risk of microbial contamination during the extraction process. The high cost of enzymes limits the amount that can be used in the process so an increase in reaction temperature and/or reaction time results in a decrease in production costs [21].

Chilling and Thawing

Conventionally, thermal treatment is applied to destabilize the emulsion, during which the quality of the oil is affected. To avoid such damage to the product, individual coconut cream samples were chilled at different temperatures, that is, 20, 15, 10 and 5 °C for 6 h and thawed to ambient temperature before subjecting to centrifugation to obtain VCO. The effect of chilling on the oil yield is shown in Fig. 2. Chilling

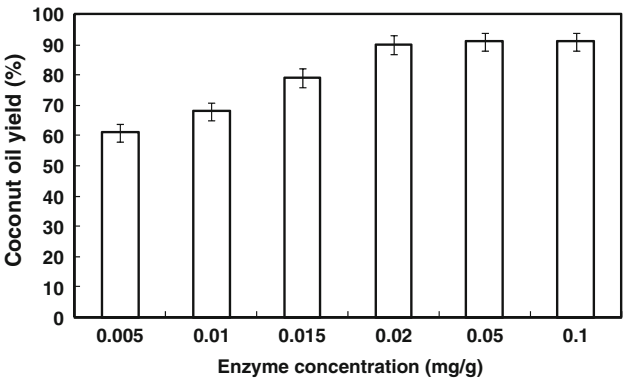

Fig. 1 Effect of enzyme concentration on destabilization of coconut milk emulsion

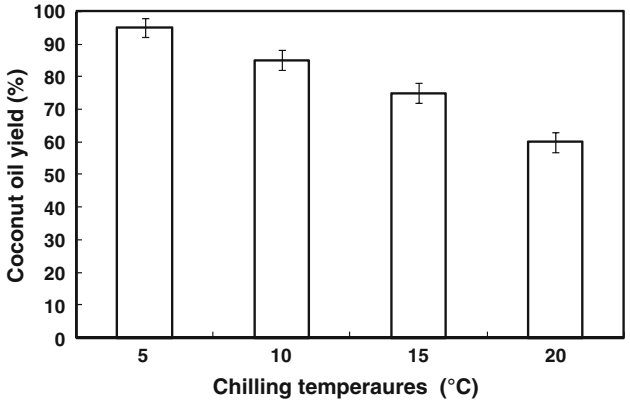

Fig. 2 Effect of chilling temperature on oil yield

at 20 and 15 °C resulted in oil yields of 64.3 ± 0.9 and 77.6 ± 0.6%, respectively. The critical temperature for demulsification of coconut milk was previously reported as 17 °C [22]. However, complete demulsification could not be observed at this temperature. Oil yield of 83.6 ± 0.6% was observed at a chilling temperature of 10 °C and a maximum yield of 95.3 ± 1.0% was achieved at 5 °C. In other words, complete demulsification was achieved only when the coconut cream was chilled at 5 °C for 6 h and thawed to ambient temperature (29 ± 2 °C). As a result of lowering the chilling temperature, the solidification of oil takes place and during thawing, oil globules lose their spherical structure and coalesce to form large droplets [22]. It can be noted chilling and thawing play an important role in complete destabilization of the enzyme treated emulsion, as packing of globules during chilling facilitates oil separation [22]. In other words, coalescence of oil globules during thawing is essential for breaking the emulsion.

Fatty Acid Composition

The fatty acid composition of VCO sample is shown in Table 1, along with that of CVCO and CCO samples. The most predominant fatty acids are of medium chain length. The dominant fatty acid was lauric acid (51.3, 49.6, and 47.1% in VCO, CVCO, and CCO samples, respectively). Lauric acid ($C_{12:0}$) accounted for 47.9% and 49.2% in VCO obtained by Marina et al. and Norulaini et al. [23, 24], respectively. Marina et al. [23] carried out the study on a commercial VCO sample. Norulaini et al. [24] employed supercritical carbon dioxide extraction for the preparation of VCO. There was no significant difference found in the content of lauric acid between VCO, CVCO and CCO samples despite variations in the extraction process. It is reported that lauric acid is a major component of tropical oils such as coconut and palm kernel fat [25]. In spite of the presence of high concentrations of saturated fatty acids, it is safe to use the oil as dietary fat since it is composed of medium chain fatty acids.

Caprylic ($C_{8:0}$) and Capric ($C_{10:0}$) accounted for 3.1 and 4.9%, respectively, in VCO when compared to 0.7 and 3.7%, respectively, for the CCO sample. The values obtained in VCO were lower compared to those values reported by Marina et al. and Norulaini et al. [23, 24]. The myristic ($C_{14:0}$) acid content in this study were slightly higher compared to the study reported by Marina et al. and Norulaini et al. [23, 24]. Palmitic ($C_{16:0}$), stearic ($C_{18:0}$) and oleic ($C_{18:1}$) acid contents in the VCO of the present study were comparable to those results reported by Marina et al. and Norulaini et al. [23, 24]. The fatty acid compositions of VCO and CCO samples were within the range of APCC standards for VCO.

Physicochemical Properties

Physicochemical properties of VCO sample in comparison with CCO are shown in Table 2. The quality of an oil is very much determined by its physicochemical properties. The moisture content in VCO is 0.15% where as in CVCO and CCO it is 0.18 and 0.27%, respectively (Table 2). Moisture content of the oil is one of the parameters which affects the shelf life. Higher moisture content adversely influences the oxidation process promoting rancidity [26].

Table 1 Fatty acid composition of oil samples

Fatty acids	Virgin coconut oil (present work) (%)	Commercial virgin coconut oil (%)	Commercial coconut oil (%)	APCC standard (%)
$C_{8:0}$ (caprylic)	3.1 ± 0.06	0.9 ± 0.04	0.7 ± 0.01	5.0–10
$C_{10:0}$ (capric)	4.9 ± 0.49	3.8 ± 0.08	3.7 ± 0.07	4.5–5.8
$C_{12:0}$ (lauric)	51.3 ± 1.68	49.5 ± 1.00	47.4 ± 0.93	43–53
$C_{14:0}$ (myristic)	22.3 ± 0.72	23.7 ± 0.58	23.5 ± 0.43	16.0–21
$C_{16:0}$ (palmitic)	8.8 ± 0.70	10.0 ± 0.19	11.1 ± 0.22	7.5–10
$C_{18:0}$ (stearic)	3.2 ± 0.36	3.8 ± 0.07	3.9 ± 0.08	2.0–10
$C_{18:1}$ (oleic)	5.7 ± 0.59	7.2 ± 0.15	8.4 ± 0.17	5.0–10
$C_{18:2}$ (linoleic)	0.7 ± 0.35	1.2 ± 0.03	2.4 ± 0.05	1.0–2.5

Values are averages ± SD from three replicate analysis

Table 2 Physicochemical properties of oil samples

Parameters	Virgin coconut oil (present work)	Commercial virgin coconut oil	Commercial coconut oil	APCC standards
Moisture (%)	0.15 ± 0.01	0.18 ± 0.02	0.27 ± 0.01	0.1–0.5
Specific gravity (30 °C)	0.92 ± 0.027	0.921 ± 0.028	0.92 ± 0.03	0.915–0.920
Refractive index	1.4486 ± 0.0434	1.4486 ± 0.0434	1.4480 ± 0.0434	1.4480–1.4492
Iodine value	3.9 ± 0.03	3.21 ± 0.02	3.92 ± 0.04	4.1–11.0
Polenske value	13.6 ± 0.3	14.1 ± 0.3	15.03 ± 0.3	13 min
Acid value	0.6 ± 0.01	0.72 ± 0.01	0.9 ± 0.02	6 max
Saponification value	253 ± 2.59	260 ± 2.67	275 ± 2.65	250–260 min
Unsaponifiable matter	0.37 ± 0.01	0.42 ± 0.01	0.61 ± 0.01	0.2–0.5
Peroxide value	0.81 ± 0.02	0.94 ± 0.02	1.6 ± 0.03	<3 meqO_2/kg
Free fatty acid (%)	0.31 ± 0.02	0.37 ± 0.02	0.45 ± 0.03	0.5
Vitamin E mg/100 ml	6.32 ± 0.13	5.4 ± 0.10	2.37 ± 0.04	–

Values are averages ± SD from three replicate analysis

Vitamin E content in VCO was found to be 6.6 mg/100 ml, whereas in CVCO and CCO it was 5.4 and 2.37 mg/100 ml, respectively. It may be noted that the Vitamin E content is higher in the VCO sample compared to CVCO and CCO samples. Several nutritional factors such as intake of Vitamin E as an antioxidant or the nature and amount of dietary fatty acids have been shown to reduce the susceptibility of LDL to lipid peroxidation in humans and laboratory animals [27]. Specific gravity and refractive index values were not much different in all three samples. The iodine value of all three samples was also not significantly different. A similar range of Iodine values for VCO samples obtained from different processes was reported by Marina et al. [23]. The low Iodine value indicates a high degree of saturation. The low degree of unsaturation leads to a high resistance to oxidative rancidity reported by Onyeike and Acheru [28]. Polenske values of all three samples were not significantly different. The acid value of oil obtained from our work was found to be 0.6% whereas, in CVCO and CCO samples it was 0.72 and 0.9%, respectively. Saponification values of all three samples were not significantly different and are comparable to the results reported by Marina et al. [23]. Peroxide values were found to be 0.81, 0.94, and 1.61 mequiv O_2/kg in VCO, CVCO and CCO samples, respectively and are within the limits of Asian and Pacific Coconut Community (APCC) standards for VCO. The peroxide values of all three samples were relatively low, indicating their stability against oxidation.

Free Fatty Acids

The free fatty acid content in VCO, CVCO, and CCO samples is shown in Fig. 3. Initially the FFA content was 0.05% in VCO, 0.1% in CVCO and 0.15% in CCO samples. These values increased to 0.12% (VCO), 0.2% (CVCO) and 0.3% (CCO) after 6 months and to 0.29% (VCO), 0.42% (CVCO) and 0.49% (CCO) after a period of 12 months. There was an increase in FFA content with respect to time, it was within the limits of APCC standards in all three samples during the storage period. Similar results with respect to FFA of VCO produced by different methods were reported by Marina et al. [23]. Further, it was observed that the FFA content in VCO was much less compared to CCO sample, because in the case of the former, thermal treatment was not applied. It is known that FFA are responsible for an undesirable flavor in oils and fats. FFA are formed during hydrolytic rancidity, caused by the hydrolysis of an ester by lipase or moisture [29].

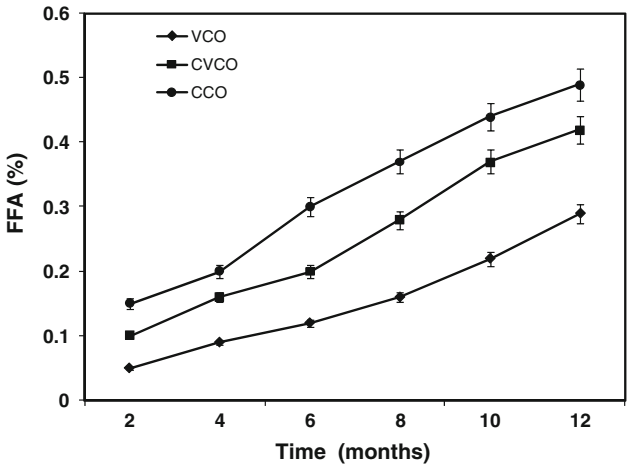

Fig. 3 Free fatty acids profiles of oils during storage

Sensory Analysis

The typical attributes specific to the coconut oil are identified and evaluated using QDA. The intensity of the each attributes was quantified as shown in Fig. 4. The CVCO and CCO had poor quality as shown by high scores for defective odor notes such as musty, rancid, fermented,

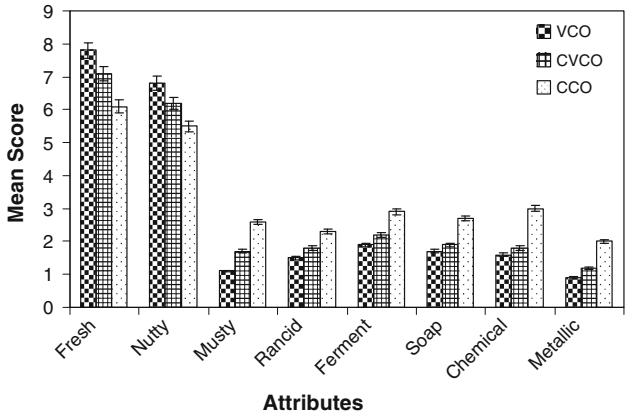

Fig. 4 Sensory profiles of oil samples

soapy, chemical, and metallic attributes and lower scores for fresh and nutty attributes. Whereas, VCO had significantly higher scores for fresh coconut, nutty odor attributes, and low intensities for musty, rancid, fermented, soapy, and metallic attributes compared to CVCO and CCO. The sensory evaluation revealed that VCO was superior to CVCO and CCO, CVCO was better than CCO.

Conclusions

Destabilization of coconut milk emulsion was achieved by enzymatic treatment, and chilling, and thawing during aqueous extraction of VCO. Protease concentration of 0.02 mg/g was found to give the best result. Chilling and thawing was found to play a significant role in the destabilization of the emulsion and consequently resulting in the highest yield (95.3 ± 1.0%). In VCO samples, lauric acid and Vitamin E were found to be higher and peroxide value lower when compared to CVCO and CCO samples. The sensory score for VCO samples showed itself to be higher in all desirable attributes compared to CVCO and CCO samples.

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