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Enzyme-Assisted Aqueous Extraction of Virgin Coconut Oil

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Abstract

This study was aimed at evaluation of the effect of enzymatic application (Viscozyme Cassava C) for extraction of Virgin Coconut Oil (VCO) from fresh coconut meat, and comparison of physico-chemical and functional properties of the extracted CVO and cold pressed oil. The parameters influencing the extraction yield included material-to-water ratio (w/v), enzyme concentration (% E/S) and incubation durations (h). The results indicated that the highest yield of VCO (19.58%), presenting 73.58% recovery of oil, from fresh coconut meat was achieved at the water ratio of 1:7, enzyme concentration 1.5% ES, and incubation time 4h. This was lower than that from cold pressing but significantly higher than the oil yield (6.92%) of sample without enzymatic treatment. The quality of VCO was determined by peroxide value (0.317 meqO_2/kg), free fatty acid value (0.393 mg KOH/g oil), iodine value (4.88 gI_2/100g oil) and total phenolic content (248.25 mg GAE/100g oil) and that was comparable with the quality of the cold-pressed oil. The VCO was rich in short-chained saturated fatty acids, with lauric acid of 46.64%, palmitic acid 8.42% and myristic acid 18.24%. Additionally, the CVO in this study contained unsaturated fatty acids in the amount of 5.57% of linoleic acid and oleic acid.

Keywords: *Enzyme Assisted Extraction; Fatty Acid Composition; Oil Quality; Total Phenolic Content; Virgin Coconut Oil*

Introduction

Coconut (*Cocos nucifera* L.) oil is being extracted from coconut meat by different methods. Virgin coconut oil (VCO) is a special product from methods that do not have to go under the refining, bleaching, deodorizing process. It is colorless, with a natural fresh coconut scent, and free from rancid odor and detectable sweet taste. VCO contains biologically active unsaponifiable and antioxidative components such as vitamin E, provitamin A, polyphenols and phytosterols. It is rich in lauric acid (a medium chain) that helps in preventing high cholesterol levels and atherosclerosis and a considerable amount of short-chain fatty acids with antimicrobial and antiviral effects. VCO is considered as the miracle oil for health and beauty. It has abundant utility in the functional foods, health foods, pharmaceuticals, nutraceuticals, infant foods and cosmetics. VCO has long been used in the medicine for skin treatments including wound healing and microbial infections. Currently, VCO is widely utilized in hair care, skin care, weight loss products, digestion, stress relief, etc. In addition, VCO stimulates metabolism, boosts energy and prevents deposition of fats, thereby helping to prevent obesity [1-3].

Unlike refined coconut oil, which is produced through dry method from copra, VCO is produced through wet method, via coconut milk. Through the wet process itself, many ways of producing coconut oil have been described as wet processing, centrifuge method, fermentation technique and enzyme-assisted extraction. Enzymatic-assisted aqueous extraction (EAAE) of oil is an emerging technology in the fat and oil industry, receiving a considerable attention from researchers recently [4]. This method is also effective method with the elimina-

tion of solvents consumption and lower cost investment compared to the conventional methods of extraction using organic solvents. These traditional processes quite often yield products of poor quality [5]. However, the use of different enzymes for VCO extraction, for example, Viscozyme Cassava C has not been reported. Viscozyme Cassava C is a multi-enzyme complex containing a broad range of cellulase activity with optimum temperature of 50°C and pH of 5.5 - 6. It was effective in cashew nut oil extraction [6].

Aim of the Study

The overall goal of this study was to investigate the effect of Viscozyme Cassava C on the VCO recovery yield, as well as to evaluate its chemical composition and quality in comparison to the oil from cold pressing method.

Materials and Methods

Materials and chemicals

Copra fruits were brought from local markets in Ben Tre province, in the 12 - 15 month maturity. The fresh copra meat without testa was hand separated, grated by grinder machine and stored at -20°C for further use.

Commercial cellulase Viscozyme Cassava C from Novozymes, Gallic acid and Folin-Ciocalteau reagent from Merck (Germany) were provided by a local agent. All chemicals, solvents for analysis were of analytical grade and supplied by local suppliers.

Oil extraction by cold pressing

The fresh copra was grated and dried at 40°C until 3.0% moisture content. The manual-pressing machine was used to obtain coconut oil (20g) from 120g of dried coconut meat. This method was conducted at room temperature, at pressure 20 MPa [7].

Enzyme assisted aqueous extraction of virgin coconut oil

The procedure for VCO obtainment was modified from [6]. 4g of ground grated copra meat was mixed with water at different ratio (1:3, 1:5, 1:7, 1:9 w/v). The mixture was homogenized and the slurry pH was adjusted to 5.5 - 6 for optimum activity of Viscozyme Cassava C. The enzyme solution was added (1% E/S) the mixture and it was incubated in water bath with constant shaking (200 rpm) at 50°C for 4h. To avoid vaporization of solvent and exposure of light, the Falcon was covered by aluminum foil. After 4h, the sample was heated at 90°C in 7 - 10 min to deactivate enzyme activity.

The sample was then cooled down (at 0°C in 30 min) for efficient recovery of oil [8] and let it settle down at room temperature before centrifuging at 10,000 rpm in 35 min. After centrifugation, the upper oil layer was firstly obtained by a Pasteur pipette. The oil part remained in emulsion phase was obtained by using hexane solvent. The total oil yield was both the upper layer oil and oil extracted from hexane; but only upper oil layer was used for physiochemical properties analysis.

The crude oil was obtained at optimum water ratio, enzyme concentration and time incubation; would be stored at 0-4 °C in refrigerator until analysis. The control sample is the sample without the use of enzyme.

The oil recovery rate in coconut meat was defined as % oil yield, based on the weight of raw material (Equation 1), and % oil recovery, based on the amount of oil obtained by Soxhlet method (Equation 2).

$$\%\ oil\ yield = \frac{weigh\ of\ extracted\ oil\ x\ 100}{weigh\ of\ raw\ material} \quad (1)$$

$$\%\ oil\ revovery = \frac{weigh\ of\ oil\ extracted\ using\ enzyme\ x\ 100}{weight\ of\ sample\ oil\ extracted\ by\ Soxhlet\ method} \quad (2)$$

Experimental design

Effect of water ratio: Different material to water ratios (1:3, 1:5, 1:7 and 1:9 w/v) were tested. The effect was evaluated in term of the oil yield. Each treatment was done in triplicate.

Effect of enzyme concentration on total oil yield: Four levels of enzyme concentration were investigated (0.5, 1, 1.5 and 2% E/S) using the best condition for water ratio as determined before.

Effect of incubation time on total oil yield: The procedure was carried as previously in different incubation times (3, 4, 5, 6h) with the best conditions for water ratio and the enzyme concentration.

Physiochemical analysis

The specific gravity of oil sample was determined by using a pycnometer (with stopper fitted with calibrated thermometer) according to method AOCS 10a-25. The free fatty acid (FFA) value of the VCO was determined by titration method using KOH solution, expressed in mg KOH/1g oil sample. The peroxide value (PV) and the Iodine value (IV) was determined by AOCS standard methods [9].

Fatty acid composition

The procedure was modified from standard AOCS method Ce 1e-9 [9]. 32 mg of VCO sample were methyl esterified. The methyl ester mixture was diluted in 1 mL n-hexane, then 2.0 micro-litter was injected into an Agilent GC 6890N, with J&W HP-5ms capillary column (12 - 60m, 0.18 - 0.32 mm internal diameter, 0.1 - 1 micrometer film thickness) and Agilent 5973N CI/EI Mass Selective Detector. Helium was used as the carrier gas at pressure of 9.53 psi. The oven temperature was programmed from 100°C at 2 min, then from 100°C to 280°C at 10°C/min and then held at 280°C for 5 min.

Total phenolic content

Total phenolic content (TPC) was determined according to the method by Gutfinger [12] with some modifications. 0.1 mL sample of oil was mixed in 75 mL distilled water with 5 mL of saturated Folin-Ciocalteu reagent. Then, 10 mL of saturated sodium carbonate solution was added and the mixture was shaken well for 1 min, let stand at room temperature for 30 min. The absorbance was read at 760 nm using a UV-VIS spectrophotometer. Gallic acid was used as a standard and TPC of the extracts was expressed as milligram of Gallic acid equivalents (mg GAE)/100 g oil.

Statistical analysis

All treatments were conducted in triplicate. Analysis of variance (ANOVA) was carried out for significant difference between means at $P < 0.05$, using standard software SPSS ver 20.0.

Results and Discussion

Effect of water ratio on total oil yield

Increasing water ratio from 1:3 to 1:9 (Figure 1), the oil recovery and oil yield increased from 38.51% to 62.62% and 10.25% to 16.67%, respectively. The highest oil recovery 62.62% and oil yield 16.67% were achieved at water ratio 1:9; however, there was no significant increase in oil recovery at water ratio 1:7 and 1:9 (60.10% and 62.62%, respectively). Thus, the water ratio at 1:7 was chosen as the best condition for following treatments.

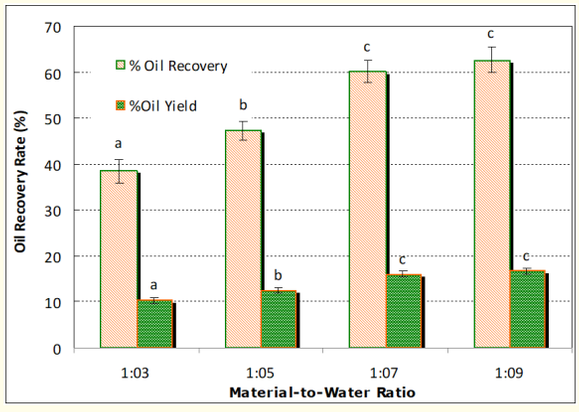

Figure 1: *% Recovery of VCO at different water ratios.*

At higher water ratio, the penetration of the enzymes is more effective, the fat globules disperse more in substrates surface area, leading to larger release of oil. However, interaction between enzyme and substrate molecules is adversely affected in very dilute suspensions 1:9, when the amount of water was too high compared to substrates. Zhang., *et al*. [11] reported that emulsified oil yield of rapeseed was enhanced with increase in use of water ratio from 1:3, 1:4 to 1:5 and slightly decreased at ratio 1:6, 1:7 and 1:8.

Effect of enzyme concentration on total oil yield

As enzyme concentration increased from 0.5% to 2% E/S, the oil recovery and oil yield increased significantly from 41.96% to 72.96% and 11.17% to 19.40%, respectively (Figure 2).

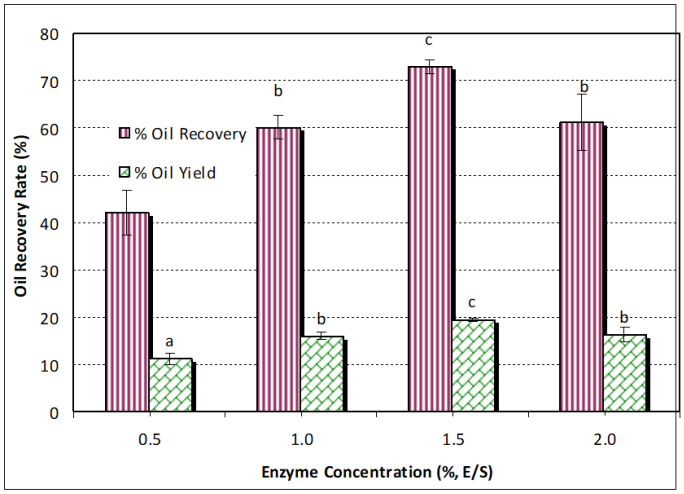

Figure 2: *% Recovery of VCO at different enzyme concentrations.*

The highest oil recovery (72.96%) and oil yield (19.40%) were achieved at 1.5% E/S and reduced to 61.06% and 16.25%, respectively, at enzyme concentration 2% E/S. The maximum recovery of rice bran oil (79%) at enzyme concentration 1% E/S was reported by Hanmounhjai., *et al* [11]. Agarwal and Bosco [4] also reported the highest coconut oil recovery (84%) at 2% E/S and the oil yield reduced when the level of enzyme (Viscozyme L) concentration increased over its optimum.

Cellulolytic enzymes helped in the break down the cellular structures to obtain a greater permeability of the cell walls, increasing extractability of oil [13]. However, too much enzyme may lead to hydrolysis of almost all the substrates during enzymatic activity. Therefore, 1.5% E/S was chosen as the best condition for conducting next treatments.

Effect of incubation time on total oil yield

With the selected water ratio and enzyme concentration, the lowest oil recovery and oil yield were 52.29% and 13.92%, respectively, at 3h of incubation time. Meanwhile, the oil recovery (72.96%) and oil yield (19.42%) were highest at 4h (Figure 3). After 5 and 6h of incubation, the oil recovery and the oil yield reduced.

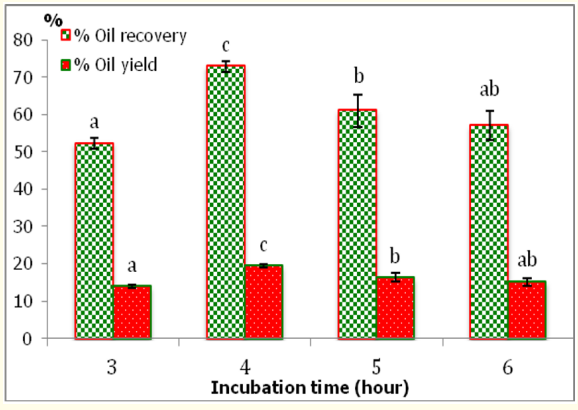

Figure 3: *% Recovery of VCO at different incubation times.*

As mentioned, the extraction yield increased when duration for enzyme incubation increased from 3h to 4h. This time was sufficient for enzymatic break-down of the cell walls, increasing the permeability of cell walls. However, the longer incubation time (5 - 6h) resulted in lower oil recovery compared to 4h. The longer time of incubation, the lower was enzyme activity over the exhausting of substrates [14]. In addition, the prolonged incubation time would glue emulsions with protein closely; thus the fat globules were difficult to release during centrifugation that led to the decreasing of extracted oil. Agarwal and Bosco [4] showed that the oil recovery increased with the increase of incubation time but it reduced significantly after 4.5 hours incubation with the use of Viscozyme L. Therefore, 4h was chosen as an appropriate incubation time for Viscozyme Cassava in this study.

The oil yields by different methods

The oil recovery and the oil yield of VCO from EAAE method were compared with cold-pressing method and the treatment without using enzyme (Figure 4). The control treatment without enzyme resulted in oil recovery of 25.99%, significantly lower than that in the EAAE (73.58%) and cold pressing method (84.43%). In the absence of enzyme, the oil was still remaining in the cell walls causing low recovery percentage [13].

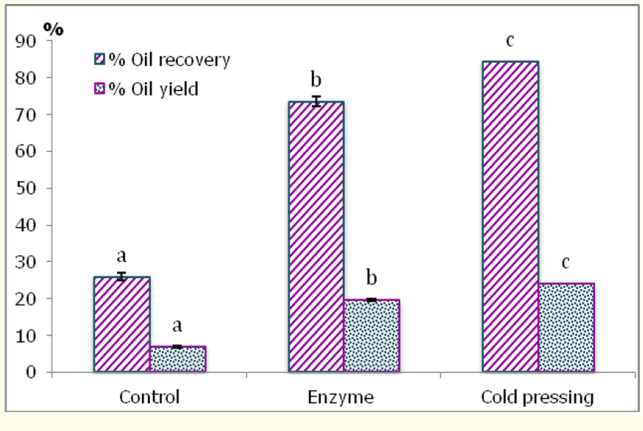

Figure 4: *Oil recovery percentage of VCO at different methods.*

For comparison, the oil recovery reached up to 90% for coconut oil, 86% for soybean and 97.7% for palm oil, and 75% for avocado oil, by using different enzymes [15]. Sharma., *et al*. [16] reported 77% oil recovery of rice bran oil by using EAAE method. Latif and Anwar [17] reported 21.40% extraction yield for sesame oil.

Physicochemical properties of VCO

In this study, the SG of EAAE oil was 0.986, higher than that from cold pressing (0.959). By comparison, the SG of sesame oil was 0.920, crambe oil 0.9078, rapeseed oil 0.9073, corn oil 0.9188, and soybean oil 0.9193 [17].

Free fatty acids value

FFA value of the oil from EAAE (0.197 mg KOH/1g oil) was lower than that of the oil from cold pressing (0.458). By comparison, the FFA values were comparable to that of oil from the chilling (0.31), fermentation (0.29) and fresh-dry (0.46) methods as reported elsewhere [18]. Exceptionally, FFA of the CVO extracted by Refining, Bleaching and Deodorizing (RBD) method was lowest (0.13) [19]. The longer duration needed to dry coconut meat prior to oil extraction was (for cold pressing and fresh-dry method), the higher rancidity, leading to increase in the FFA values. In addition, the FFA of sesame oil (0.44) was comparable [17], but the FFA of cashew oil (5.4) was much higher [20] than that of CVO. According to APCC Standard (APPC, 2007), the maximum level of FFA is 0.5 mg KOH/g.

Peroxide value

Peroxide value (PV) is an important indicator for initial oxidation by reaction of oxygen and unsaturated fatty acids, producing a quantity of hydro peroxides in oil. In this study, the PV value in the EAAE oil (0.317 meqO_2/kg) was significantly lower than that that in the CVO from cold pressing (0.917 meqO_2/kg) and fresh-dry method (0.5 - 0.8). Meanwhile, this value was higher than that in the CVO extracted RBD (0.27), wet process (0.2) and fermentation process (0.2) [18]. In general, the PV in CVO was remarkably lower than that in sesame oil [17]. According to ISO 03960:2001, the maximum level of PV for vegetable oils should be less than 40 meqO_2/kg.

Iodine value

The iodine value (IV) in the obtained VCO was lower than that in other vegetable oils, showing high degree of saturation, leading to high resistance to oxidative rancidity [19]. In this study, the IV of CVO from EAAE and cold pressing methods were 4.88 and 5.45 g I_2/100g oil, respectively, and the difference was not significantly. Those values were comparable to that from other studies: chilling (4.13), fresh-dry (4.18) and fermentation (4.3) and RBD method (4.71 g I_2/100g oil) [18]. Meanwhile, the IV value in palm, olive, corn, sunflower, peanut and soybean oils [11] were much higher, in arrange of 44 - 144 (g I_2/100g oil). According to Codex Stan (Codex Alimentarius, 2003) the limit of IV of VCO is in a range of 4 - 11 g I_2/100g oil.

Fatty acid profile

The CVO from EAAE method was rich in short-chain fatty acids, such as lauric acid (46.64%), myristic acid (18.24%) and palmitic acid (8.42%) (Figure 5). Also, the CVO had high content of total saturated fatty acid (SFA), which was more than 87.0%, comparable to that in cold pressing (88.3%), chilling (92.5%) and fermentation method (92.2%). It is noted that the fatty acid profiles of CVO from EAAE and cold-pressing methods were quite close. On the other hand, in comparison of fatty acid profiles different vegetable oils, the CVO is rich in short and medium-chain SFA, but sesame, soybean, and olive oils are rich in long- chain (C-18) unsaturated fatty acids [21]. They have completely different impacts on our body compared with both saturated and unsaturated fatty acids, so fat in VCO rarely become fat reserves and create cholesterols in blood. Therefore, not only sesame oil, olive oil or avocado oil are considered as good oil for health by the high unsaturated fatty acids, but also VCO is a miracle oil by its unique structures.

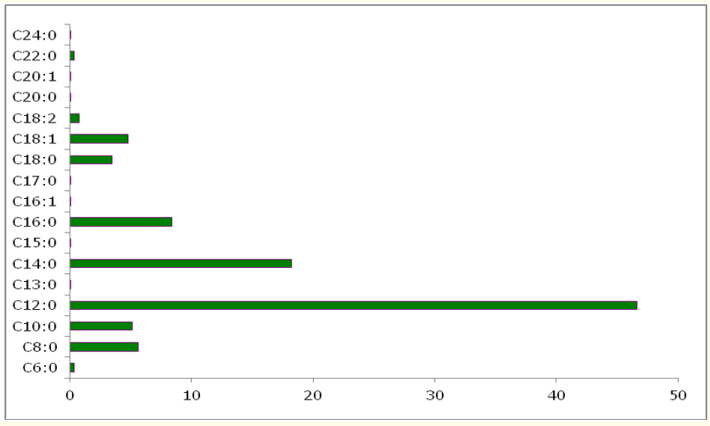

Figure 5: *The fatty acid profile of VCO from EAAE.*

Total phenolic content

The TPC in oil from EAAE was 248.25 mg GAE/100g oil; lower than in cold pressing oil (266.68 mg GAE/100g). Separation of oil from the aqueous phase in the EAAE method lead to loss of certain amount of water-soluble phenolic compounds; however, the difference was no significant. Marina., *et al*. [22] reported that the RBD method, requiring drying of coconut meat, also caused the loss in TPC in VCO. It is noted that the TPC in sunflower oil (0.0178 mg GAE/100g oil), grapeseed oil 0.059, macadamia oil 0.049 and sesame oil 2.4 [17,23-25] were remarkably lower than in the CVO.

Conclusion

Commercial enzyme Viscozyme Cassava C was applied to improve the VCO recovery. The best conditions for EAAE were determined in terms of: water ratio (1:7), enzyme concentration (1.5% E/S) and incubation time (4h), which gave the highest oil yield. The resulted oil quality was compared with that of oil from cold pressing and other methods. The quality of oil in terms of SG, FFA value, PV, IV and TPC were within a limit by the Codex STAN 19-1981 standard for vegetable oil. The VCO was rich in short and medium-chain saturated fatty acids and the fatty acid profile was close to that for the CVO from cold pressing, but the difference in quality was insignificant. The VCO from EAAE could be stable towards oil oxidation and rancidity process.

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