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Enhancing the value of oils extracted from coconut processing by-products via enzymatic extraction

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SUMMARY: The extraction of oil from coconut residue (CR) and testa (CT) using cellulasewas studied. By varying the amount of enzyme and extraction time at the enzyme's optimal pH (pH 5-5.5) and temperature (55-60ºC), the optimised conditions for extracting the oil from CR and CT were 500 U/g CR and 700U/g CT for 4 hrs. The average oil yields from the enzymatic extractions and those from hexane extraction were not statistically different according to the t-test. The properties of CR and CT oils, and oil extracted from coconut milk foulants (FO) reported in previous work were studied. CR, CT and FO had the most comparable properties to those listed in the APCC standard. Compared to virgin coconut oil (VCO), the antioxidant activities of CR and CT oil were similar but FO had ~3 times higher activity. Hence, the oils extracted from CR, CT and coconut milk foulants by cellulase-assisted extraction showed potential as high value products.

KEY-WORDS: Antioxidant activity; Cellulase; Coconut residue; Coconut testa; Oil.

RESUMEN: ***Valorización de los aceites extraídos de los subproductos del procesamiento del coco mediante extracción enzimática.*** Se estudió la extracción de aceites de residuos de coco (CR) y testa (CT) mediante celulosa. Al variar la cantidad de enzima y el tiempo de extracción a su pH óptimo (pH 5-5,5) y temperatura (55-60°C), las condiciones óptimas para la extracción de aceite de CR y CT fueron 500 U/g de CR y 700 U/g de CT durante 4 horas. Los rendimientos promedio de aceite de las extracciones enzimáticas y los de la extracción con hexano no mostraron diferencias estadísticamente significativas mediante la prueba t. Se estudiaron las propiedades de los aceites de CR y CT, así como del aceite extraído de residuos de leche de coco (FO) reportadas en trabajos previos. CR, CT y FO presentaron la mayoría de las propiedades comparables a las del estándar APCC. En comparación con el aceite de coco virgen (VCO), las actividades antioxidantes de CR y CT fueron similares, pero FO presentó una actividad aproximadamente tres veces mayor. Por lo tanto, los aceites extraídos de CR, CT y residuos de leche de coco mediante extracción asistida por celulosa mostraron potencial como productos de alto valor.

PALABRAS CLAVE: Aceite; Actividad antioxidante; Celulasa; Residuos de coco; Testa de coco.

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1. INTRODUCTION

Coconut processing is a major agro-industry in Southeast Asia, producing a variety of products such as coconut milk and coconut oil. Among the different types of coconut oil—copra oil, Refined, Bleached and Deodorized (RBD) oil and Virgin Coconut Oil (VCO)—VCO has the highest market price (Narayanankutty *et al.,* 2018, Rohman *et al.,* 2019). VCO is extracted from fresh coconut kernels using methods that generally avoid high heat and chemical refining. However, depending on the extraction technique, mild heat or enzymes may be used. The global market for VCO has been projected to reach USD 9.12 billion by 2030 (Research and Markets, 2025). While the chemical properties of VCO and RBD oil are relatively similar (Rohman., 2019), VCO has been reported to have great health benefits, including higher antioxidant capacity and vitamin E content and potential roles in preventing generative diseases (Narayanankutty *et al.,* 2018).

Coconut residue (CR) and coconut testa (CT) are by-products from VCO processing. CR is the remaining meat after milk extraction from fresh coconut meat used for VCO production (Figure 1); whereas CT is a thin brown layer between white coconut meat and shell. Based on industrial practice, CT is generally removed from the coconut meat before VCO extraction because the presence of CT results in yellowish oil; this is perceived as low-valued oil. Depending on the grade of VCO, some CT may be used together with coconut meat to produce VCO. Currently, CR and CT are underutilized, often used as animal feed or used in the production of low value cooking oil through roasting and screw-pressing by some manufacturers. However, this thermal process (150-180ºC) not only consumes significant energy but may also degrade the antioxidants present in the oil.

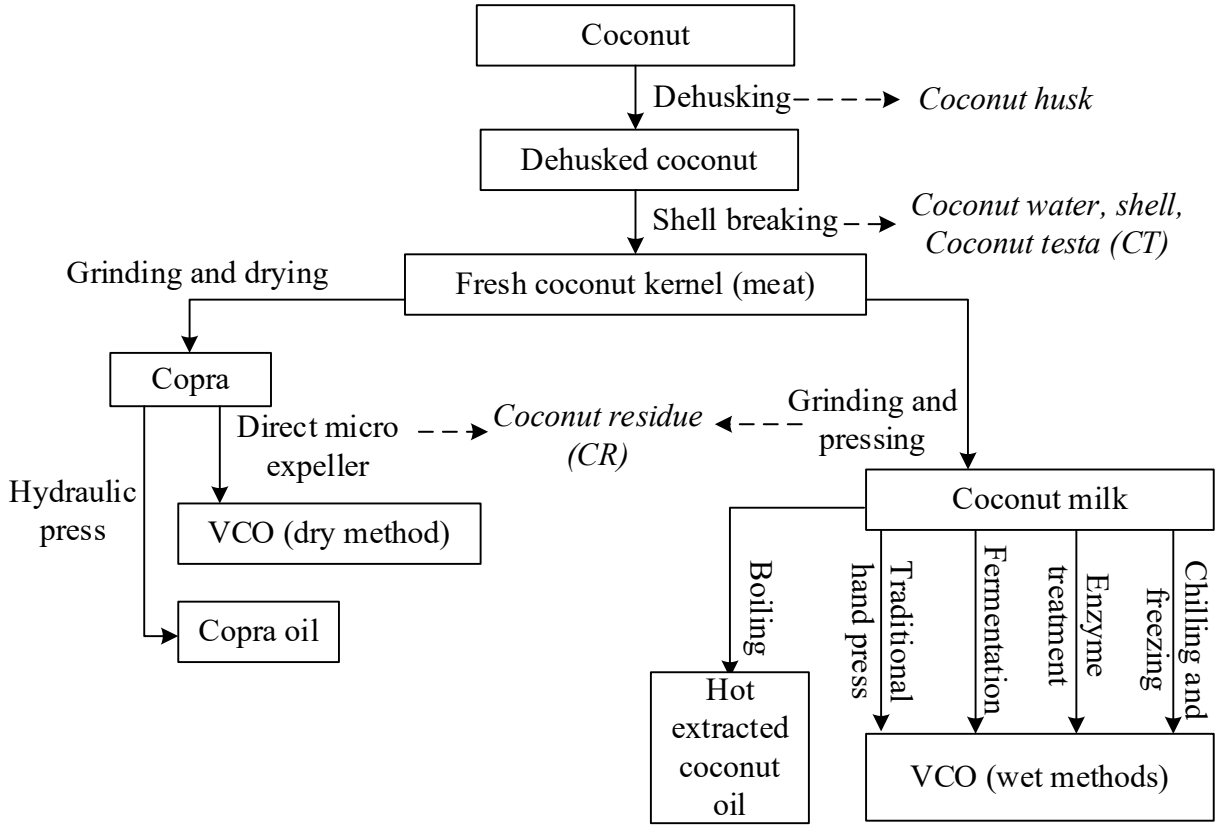

FIGURE 1. Coconut oil (copra oil and virgin coconut oil, VCO) extraction methods (dotted lines show generated by-products).

Some studies have demonstrated the potential for extracting oil from CT (CTO) using solvents. CTO obtained by solvent-extraction has been reported to have high contents of vitamin E and phenolic compounds (Kumar and Krishna, 2015; Narayanankutty *et al*., 2022). CTO extract could prevent human serum albumin

from oxidative damage (Zhang *et al.,* 2016). Hence, CTO has potential to be used in functional products with antioxidants. Although high extraction efficiency of the extraction by isopropanol has been reported (63-76%, Zhang *et al*., 2016), safety concern for the remaining isopropanol has limited the application of CTO in edible or functional products (Rohman *et al*., 2019).

To date, limited attention has been given to the application of enzymatic extraction methods for recovering oil from CR and CT, despite their potential advantages. Enzymatic-assisted extraction of compounds from plants involves the hydrolysis of plant cell walls. This assists diffusion of the extracted compounds into the extraction solvent and hence structural composition of the plant cell wall should be considered for enzyme selection (Xavier Machado *et al.,* 2021). Enzymatic extraction typically operates under less extreme conditions, e.g., lower temperature; this could preserve heat-sensitive bioactive compounds such as antioxidants. Hence, enzymatic techniques have also been used to recover bioactive compounds (Xavier Machado *et al*., 2021). An additional advantage of the enzymatic extraction is low energy consumption.

Che Man *et al*. (1996) showed that an enzyme mixture including cellulase could enhance oil recovery from coconut meat and Saikhwan *et al*. (2016) provided preliminary evidence that cellulase could be effective in extracting oil from coconut milk foulants. Hence, this study aimed to investigate the extraction of oil from coconut residue (CR) and coconut testa (CT) using cellulase, focusing on the potential of enzymatic extraction as a lower-temperature alternative to current methods. Unlike conventional roasting, which involves high temperatures of up to 180°C, enzymatic extraction operates at significantly lower temperatures (around 55°C), potentially preserving valuable oil components. Additionally, this method could be implemented using existing processing equipment, making it a practical and cost-effective option for industrial application. Only cellulase was used to simplify the process and lower operating costs as different enzymes have different optimal pH and temperature. Although kernel contained about 61% galactomannan (soluble) and only 13% cellulose (insoluble) (Balasubramaniam, 1976), after coconut milk extraction, only 2% of soluble polysaccharides remained (Hanafi *et al.,* 2022). Hence, cellulose was expected to be the major carbohydrate in CR. The properties of the oil, including antioxidant activities, were then studied to assess their potential for high-value applications.

In addition, the study explored the oil properties of coconut milk foulants (FO) formed during batch pasteurization (BP), which were previously shown to yield oil with strong antioxidant activity. The possibility of recovering usable oil from these fouling by-products was examined to reduce waste as well as enhance the value of the by-products from coconut milk processing.

2. MATERIALS AND METHODS

2.1. Selecting enzymatic extraction conditions

CR and CT were obtained from a local market. To select enzymatic extraction conditions, cellulase enzyme amount (liquid, Industrial enzyme, Iknowzyme, Thailand) and extraction time were varied, while oil yields were recorded. pH and temperature were maintained at the enzyme's optimal conditions (55-60ºC, pH 5.0-5.5, specified by the enzyme manufacturer). Preliminary experiments used enzyme amounts of 200 and 250 U/g CR with an extraction time of 6 hours. However, only a small amount of oil was obtained, significantly lower than from hexane extraction. Consequently, enzyme amounts ranging from 300 U to 700 U/g CR and extraction times of 2–6 hours were tested. The enzyme activity range selected was based on preliminary results showing 700 U/g yielded less oil than 500 U/g.

For each extraction run, 30 mL of pre-heated distilled water (~55ºC) were mixed with cellulase in a 50-mL beaker. The cellulase activity was given by the manufacturer as 13000 U/mL, so enzyme volume was adjusted to achieve the desired activity. Then 10 g CR were added and gently stirred with a glass rod to ensure full exposure to the enzyme. The beaker was covered with aluminium foil and incubated in a water bath (55-60ºC) for the specified time. Milk was then extracted from the mixture using a lever hand action juicer as reported by Saikhwan *et al.* (2022). The milk was refrigerated (4ºC, overnight) and centrifuged (10,000 rpm, 20 min, 4ºC, 50-mL tube) to replicate the cold centrifugation (Figure 1) used in some VCO production plants. Oil then was

collected using a pipette and kept for further studies. If the oil layer was too thin, samples were refrigerated for 15 minutes to solidify the oil into white pieces, which were removed with a spatula.

To select the extraction conditions for CT, the same protocol as for CR was employed. To the best of the authors' knowledge, CT cellulose content has not been reported. However, its crude polysaccharide yield is slightly higher than CR (0.91–1.24% vs. 0.73% (Mohd Nor *et al*., 2016; Gunarathne *et al*., 2024). Therefore, enzyme activities were extended to 900 U/g CT, assuming cellulose as the major polysaccharide. Each batch used 10 g CT, pre-blended by a food processor (Kenwood, 800W, max speed, 10 min) prior to extraction. After the extraction, milk was extracted and centrifuged using the same procedures used for CR. After centrifugation, 3 layers were observed: (i) oil; (ii) emulsion-like middle, and (iii) water phase (Figure 2(a)). The oil was collected using a pipette for analysis.

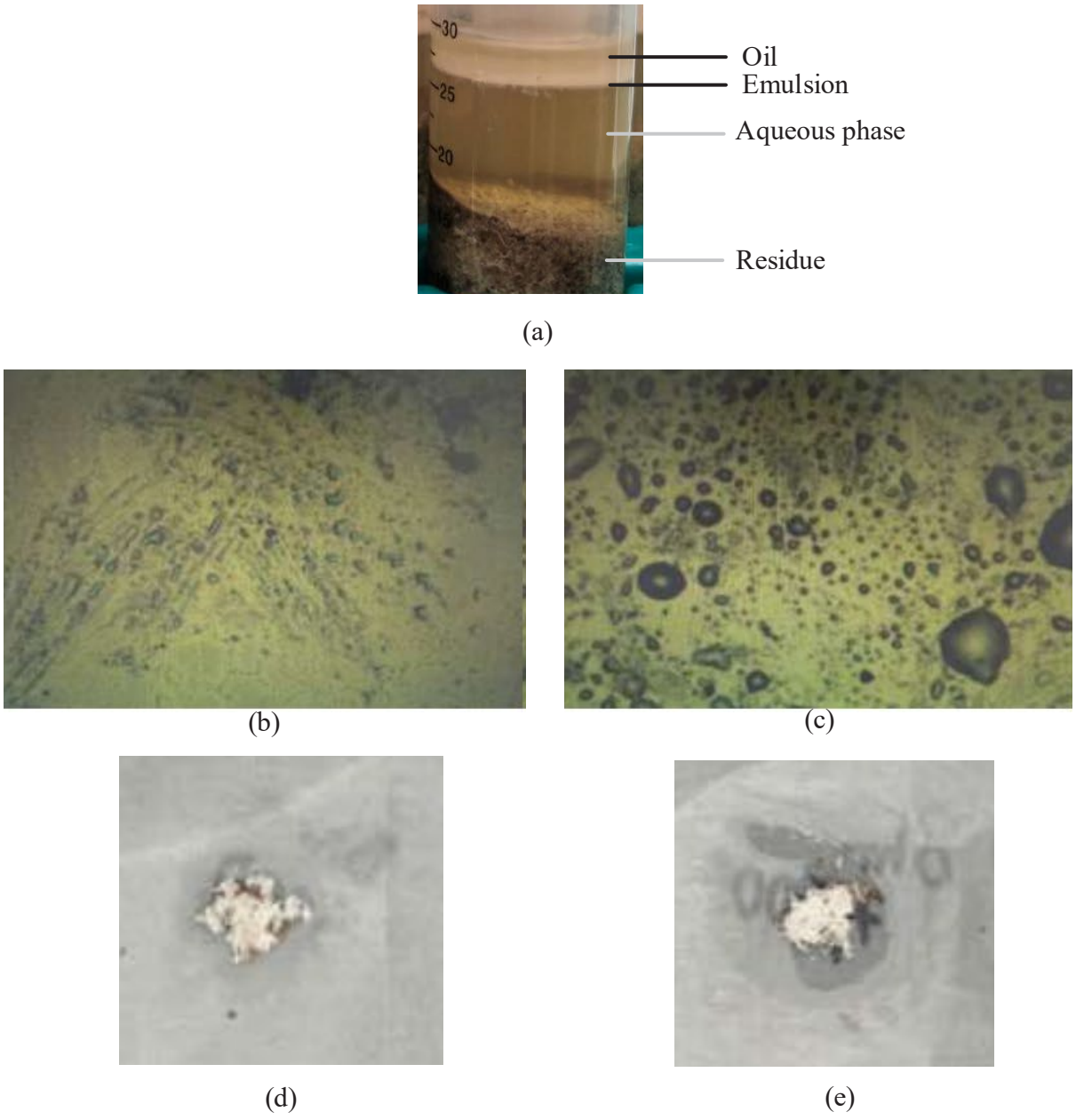

FIGURE 2. Phases obtained after centrifugation by the enzymatic extraction of CT (a), microscopic images of CR before (b) and after (c) enzymatic extraction (7000 U, 6 hr) and oil remaining in CR after enzymatic digestion for 6 hr (shown as dark areas on oil blotting paper) by 5000 U (d) and (e) 7000 U.

2.2. Preparation of other oil samples

Since the coconut breed can affect the properties of the oil obtained (Mudiyanselage and Wickramasinghe, 2023), VCO was prepared using the same batch of coconut kernels used to obtain CR and CT. Coconut milk was extracted and oil was obtained using the cold centrifugation as described in section 2.1. This oil sample is referred to as VCO_{CEN}.

Hexane (n-hexane, 95% AR, RCI Labscan) was also used to extract oil from CR, CT and BP foulants to determine oil contents. A ratio of 3 mL hexane per 1 g sample was used in three washes (Saikhwan *et al.,* 2022). The oils extracted from CR, CT, and BP foulants are referred to as CR_S, CT_S, and FO_S, respectively. Commercial oil produced by roasting CR and CT was purchased from a supermarket and referred to as CRT.

To investigate the effects of the enzymatic extraction on oil properties from coconut milk by-products, foulant oil was also studied. It was extracted using the method of Saikhwan *et al*. (2016) (0.1 g cellulase (Celluclast®, Novozyme) per 1 g foulants, pH 5.5, 50ºC, 2 h). This oil is referred to as FO.

2.3. Characterization of oil samples

The oil properties were evaluated per Asian and Pacific Coconut Community standards for VCO (Dayrit *et al*., 2007) and were used to characterize the CR and CT oils. Iodine value (IV), peroxide value (PV) and saponifiable value (SV) were determined using AOAC methods; whereas moisture content was measured using ISO 662:1998 (Fssai, 2012).

Fatty acid composition was analyzed by gas chromatography (GC). Fatty acid methyl ester (FAME) was prepared according to the IUPAC standard method 2.301 (Fssai, 2016) and FAME standards were obtained from an AccuStandard. FAME was determined on an Agilent Technologies 6890 N gas chromatography equipped with INNOWAX capillary column (30 m × 0.3 mm i.d., 0.25 µm film thickness) and a Flame Ionization Detector (FID) system. The injector and detector temperatures were set at 150 and 250°C, respectively, with helium as the carrier gas (2.3 mL/min). The GC was programmed as follows: initial temperature of 150°C, increasing to 180°C at 10°C/min, then to 200°C at 5°C/min and then held for 5 min (total runtime of 33 min). FAMEs were identified by comparison with reference standards. Because 4% unknowns in FO fatty acid profile by GC were found in a previous work (Saikhwan *et al*., 2016), the gas chromatography–mass spectrometry (GC–MS) of FO was analyzed in this work by National Nanotechnology Center, Thailand.

2.4. Antioxidant capacity of oil samples

2.4.1. Determination of radical scavenging abilities

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging and FRAP (Ferric reducing antioxidant power) assays were conducted as described by Thitipramote *et al.* (2019). For the DPPH assay, 10 µL oil were mixed with 190 µL DPPH solution and incubated in the dark (30 min). Absorbance of the mixture was then measured at 515 nm with Trolox as the standard and the activity was expressed as TEAC (mg Trolox/ g sample).

FRAP reagent was prepared from 300 mM acetate buffer, 20 mM ferric chloride solution and 10 mM TPTZ (2,4,6-Tris(2-pyridyle)-1,3,5-triazine) solution. This reagent was mixed with 10 µL oil and incubated (37°C) in the dark (15 min). The absorbance of the mixture was measured at 593 nm with Trolox as the standard.

2.4.2. Determination of total phenolic (TPC) and flavonoid contents (TFC)

TPC and TFC were determined as reported by Thitipramote *et al.* (2019). For TPC, 20 µL sample were mixed with 100 µL Folin-Ciocalteu regent (diluted tenfold), allowed to stand for 1 min and mixed with 80 µL sodium carbonate solution (7.5 % w/v). The mixture was kept at room temperature for 30 min and absorbance was measured at 765 nm with gallic acid as the standard. TPC was expressed as GAE (Gallic acid equivalent, mg GAE/ g sample).

TFC was measured by mixing 25 µL of the sample with 75 µL 95% ethanol. Then 5 µL aluminium chloride (10% w/v), 5 µL potassium acetate (1 M) and 140 µL distilled water were added. After incubating the mixture at room temperature (30 min), the absorbance was measured at 415 nm. Quercetin was used as the standard and TFC was expressed as QE (Quercetin equivalent, mg QE/ g sample).

2.4.3. Determination of vitamin E content

Because FO had higher antioxidant activity than VCO (Saikhwan *et al*., 2016), its vitamin E content was quantified using HPLC by the Institute of Nutrition, Mahidol University, Thailand following ASEANFOODS (2011). VCO_{CEN} and oil extracted from BP foulants using solvent (FO_S) were also analyzed to evaluate the effects of pasteurization on the vitamin E content.

2.5. Statistical analysis

All experiments and measurements were conducted in triplicate. Results are reported as means ± standard deviations. Student's t-test was used for two-group comparisons, while linear regression was conducted using ANOVA.

3. RESULTS AND DISCUSSION

3.1. Effects of extraction conditions

3.1.1. Oil extraction from CR

Figure 3(a) shows that 5000 U (per 10 g CR) and 4 hours gave the highest oil yield. Although this yield was slightly less than the yield from hexane extraction, the difference was not statistically significant (t-test). After each enzymatic extraction batch under the optimal conditions (10 g CR, 5000 U cellulase, 4 hours), hexane extraction of the residue showed ~0.1 g oil remaining. Given the initial total oil content of 1.22 g (from hexane extraction), the expected enzymatic extraction yield was 1.1 g. However, the actual yield was only ~0.72 g (Figure 3). This discrepancy may be due to the initial milk extraction process, where the CR was hand-pressed, potentially reducing the efficiency of subsequent oil recovery. Using a machine in a processing scale should improve the oil yield. In addition, yield would lessen through each extraction step. With only 10 g CR (containing ~1.22 oil) per batch, the yield loss appeared significant; this would be less problematic in large-scale operations.

Using 7000 U did not increase oil yield (Figure 3(a)). Post-extraction, the CR became finer and clumped together. Microscopy (2(c)) showed central voids in these clumps, where oil could be trapped inside. This feature was not observed before the extraction (Figure 2(b)). When equal amounts of CR (post-extraction with 7000 U and 5000 U) were pressed under oil blotting paper of the same weight, a larger oil-stained area was observed for the 7000 U sample (Figure 2(d-e)). Using 7000 U may break down CR more, allowing oil to be reabsorbed into the remaining solids and retained within the CR matrix. Therefore, 5000 U and 4 hr were selected for CR oil extraction.

3.1.2. Oil extraction from CT

Oil yields from CT showed large variations (Figure 3(b)). This was likely due to the presence of emulsions (Figure 2(a)). At shorter extraction times and lower enzyme activities, e.g., 2 h, at 3000 U or 5000 U, the emulsions were stable and did not mix with the oil during the oil separation. However, at longer extraction times, e.g. 3000 U for 4 or 6 hrs, emulsions destabilized and were not well separated from the extracted oil.

The variations could also come from variations associated with CT as can be seen in Figure 3(b) that large variations were also observed in the oil yields from hexane extraction. The yield from 7000 U and 4 hrs was not statistically different from the yield from the hexane extraction (student's t-test), and this condition was therefore selected.

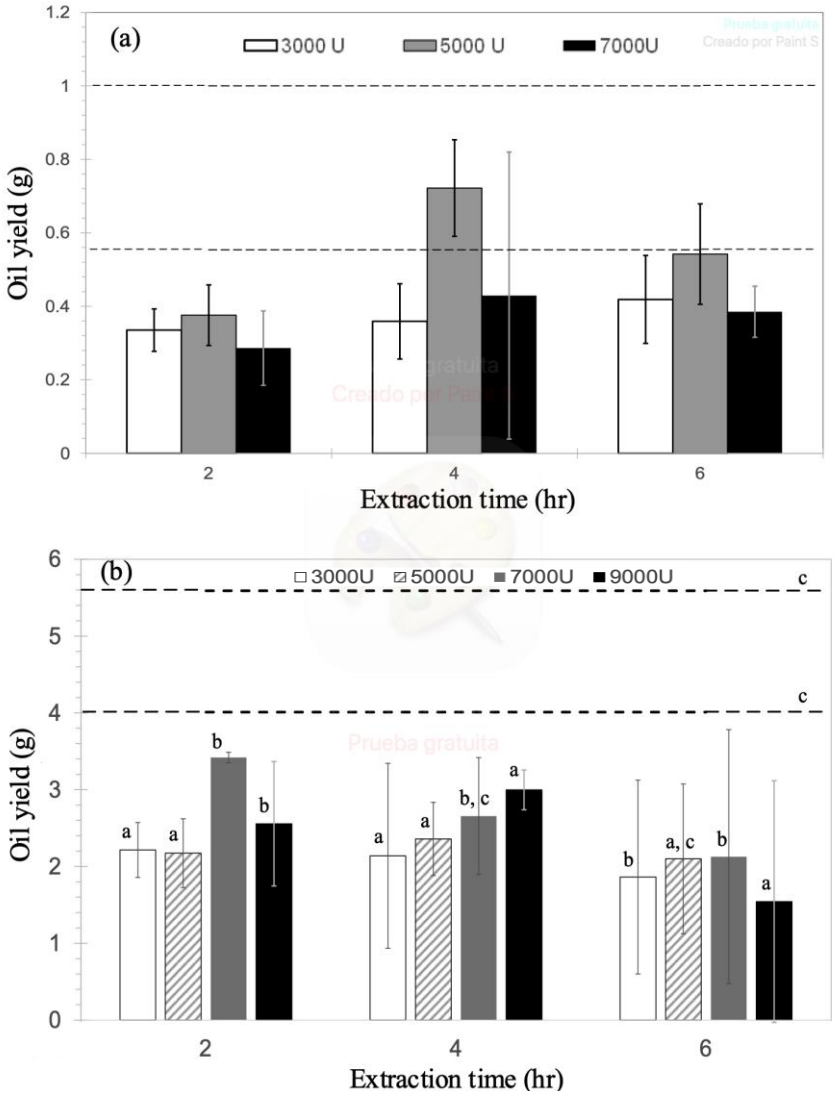

FIGURE 3. Oil yields (g) extracted from 10 g of CR (a) and 10 g of CT (b) using different enzyme activities and extraction durations. The dotted line indicates the range of oil yields (g) obtained via hexane extraction. Error bars represent standard deviations (n = 3). Bars sharing the same letter (a, b, c) denote means that are not significantly different ($p \leq 0.05$) based on a t-test.

Post-extraction oil contents in CT were also measured using hexane extraction. These values were then used to estimate expected oil yields by subtracting the post-extraction oil content from the original oil content of CT. The differences between expected and actual yields ranged from 7-100%. The largest discrepancies occurred under conditions with long extraction times and/or high enzyme doses. This aligns with the observed yield loss due to unstable emulsions under those conditions. To improve the yield obtained from the enzymatic extraction, a mean to stabilize or filter the emulsion layer should be adopted. Previous studies have shown that the destabilization of coconut milk emulsion can effectively improve oil yield using wet extraction methods (Raghavendra and Raghavarao, 2010).

3.2. Properties of CR, CT and FO oils

The oils extracted from CR, CT and FO by enzymatic extraction all had a fresh coconut aroma; The CR oil (CRO) and FO were colourless like VCO whereas the CT oil (CTO) was pale yellow. Tables 2 and 3 show the properties and fatty acid compositions of CRO, CTO and FO compared to the APCC standard for VCO. Only the APCC standard is given in the tables because it aligns with the Codex

standard as shown in Table 1. According to Table 2, IV, PV, SV and the moisture contents in FO and VCO_{CEN} were similar and within APCC standard ranges. The IV and PV of CRO and CTO were also within the standard ranges. However, the SV and moisture content in CRO and CTO exceeded the standard. Coconut oil produced by wet methods typically has higher moisture content than those from dry processes. For example, the moisture content in CRT (from a dry process) was ~1/2 of that of VCO_{CEN}. The moisture content in VCO_{CEN}, however, was ~1/10 and 1/50 of those in CRO and CTO, respectively. Although, VCO_{CEN}, CRO and CTO were all obtained using wet processes, the yields of CRO and CTO were lower than that of VCO_{CEN}. The lower yields were associated with thinner oil layers, which were often observed during oil extraction from CR. The thin oil layer made it difficult to collect the oil without also collecting water near the interphase. Extraction from a bigger batch could lessen this issue. It was also observed that oil collecting was less of an issue when extracting oil from the foulants and CT, which had larger oil yields.

TABLE 1. Codex Alimentarius standards for coconut oil and Asian and Pacific Coconut Community (APCC) for VCO (Dayrit *et al.*, 2007)

Parameter	Codex Alimentarius	APCC
Physical and chemical characteristics		
Iodine value; IV (g/100 g oil)	6.3 - 10.6	4.1 - 11.0
Peroxidation value; PV (meq/kg oil)	≤ 15	≤ 3
Saponification value; SV (mg KOH/g oil)	248 – 265	250 – 260
Moisture content % wt. max	-	0.1-0.5
% Fatty acid composition		
C6:0 (Caproic)	ND-0.7	0.4-0.6
C8:0 (Caprylic)	4.6-10.0	5.0-10.0
C10:0 (Capric)	5.0-8.0	4.5-8.0
C12:0 (Lauric)	45.1-53.2	43.0-53.0
C14:0 (Myristic)	16.8-21.0	16.0-21.0
C16:0 (Palmitic)	7.5-10.2	7.5-10.0
C18:0 (Stearic acid)	2.0-4.0	2.0-4.0
C18:1 (Oleic acid)	5.0-10.0	5.0-10.0
C18:2 (Linoleic acid)	1.0-2.5	1.0-2.5
C18:3 (Linolenic acid)	ND-0.2	
C20:0 (Arachidic acid)	ND-0.2	<0.5
C20:1 (Eicosenoic acid)	ND-0.2	
C20:2-C24:1	ND	

APCC – The Asian and Pacific Coconut Community, VCO – Virgin Coconut Oil, ND – Not Detected.

TABLE 2. Properties of CRO, CTO, FO, VCO_{CEN}, and CRT Compared to the APCC VCO Standards (Dayrit *et al.*, 2007) (Each measurement is presented as mean ± SD, n = 3)

Properties	APCC standards	VCO_{CEN}	CRO	CTO	CRT	FO
IV (g/100 g oil)	4.1 - 11.0	6.64±0.12	8.53±0.33	9.69±0.24	10.09±0.25	8.46 ± 0.22
PV (meq/kg)	≤ 3	0.398 ±0.010	2.19±0.62	1.92±0.09	ND	0.303 ±0.002
SV (mg KOH/g)	250 –260	257.73±3.21	302.5±9.36	263.5±8.92	258.33±3.44	256.4 ± 3.00
Moisture content (%wt)	≤ 0.1	0.07±0.0033	0.73±0.07	0.47±0.05	0.027±0.003	0.07 ± 0.002

APCC – The Asian and Pacific Coconut Community, CRO – oil from coconut residue by enzymatic extraction, CRT– commercial oil obtained from roasting CR and CT, CTO – oil from coconut testa by enzymatic extraction, FO – oil from coconut milk foulants formed in a batch pasteurization extracted by enzymatic extraction, IV – Iodine Value, PV – Peroxide Value, SV – Saponifiable Value, VCO – Virgin coconut oil, VCO_{CEN}– Virgin coconut oil obtained using the same batch of coconut producing coconut residue and testa, ND – Not Detected

SV increases with decreasing molecular weight (Emabu *et al*., 2022). SV also represents the amount of free fatty acids (Barret, 2018). In this case, the high SV of CRO could come from its high moisture content. Increasing free fatty acids and SV with increasing moisture content in palm oil has also been reported (Emabu *et al*., 2022).

The compositions of most fatty acids in CRO and CTO (Table 3) were within the APCC standards with palmitic acid content close to the upper limit. Nevertheless, oleic acid content was slightly higher than the upper limit. Higher palmitic acid content in oil could make the melting point higher and the oil more heat stable (Music *et al*., 2022). Palmitic acid has also been reported to play an important role in skin morphogenesis and lipid barrier formation (Atef *et al*., 2022). Various health benefits of oleic acid, such as lowering blood cholesterol levels and anticancer effects, have been reported (Lu *et al*., 2023). The acid also has penetration enhancing properties for effective skin applications of drugs or cosmetics (Atef *et al*., 2022).

TABLE 3. Fatty acid profiles of CRO, CTO, FO, VCO_{CEN} (mean ± SD, n = 3) compared to the APCC standard for VCO (Dayrit *et al*., 2007)

Fatty acid	Fatty acid composition (%)				
	APCC standard (VCO)	VCO_{CEN}	CRO	CTO	FO
C6:0	0.4 – 0.6	ND*	ND	ND	ND
C8:0	5.0 – 10.0	8.11±0.40	6.55±0.12	7.17±0.09	7.10±0.06
C10:0	4.5 – 8.0	6.62±0.14	5.38±0.05	5.89±0.03	5.88±0.08
C12:0	43.0 – 53.0	51.9±0.46	46.10±0.07	45.91±0.18	48.6±1.35
C14:0	16.0 – 21.0	18.5±0.06	20.70±0.08	19.64±0.08	17.9±0.04
C14:1	ND	ND	ND	ND	ND
C16:0	7.5 – 10.0	7.46±0.22	10.58±0.06	9.83±0.10	8.24±0.36
C18:0	2.0 – 4.0	0.54±0.93	ND	1.51±0.02	2.24±0.38
C18:1	5.0 – 10.0	6.30±1.02	10.69±0.06	10.04±0.10	5.48±0.51
C18:2	1.0 – 2.5	0.63±0.56	ND	ND	3.06±0.01
C18:3	< 0.5	ND	ND	ND	ND
C22:2	NA	ND	ND	ND	ND

APCC – The Asian and Pacific Coconut Community, CRO – oil from coconut residue by enzymatic extraction, CRT– commercial oil obtained from roasting CR and CT, CTO – oil from coconut testa by enzymatic extraction, FO – oil from coconut milk foulants formed in a batch pasteurization extracted by enzymatic extraction, VCO – Virgin coconut oil, VCO_{CEN}– Virgin coconut oil obtained using the same batch of coconut producing coconut residue and testa, ND – Not Detected

FO had a similar fatty acid composition to VCO_{CEN}. However, a slightly large content of linoleic acid was observed in FO. The oil in coconut milk foulants was from the deposition of the oil in coconut milk into denatured protein (Saikhwan *et al*., 2022) and a batch of pasteurization used coconut milk extracted from 500 g of coconut meat. Hence, the slightly larger proportion of linoleic acid was because of the accumulation of the acid from the coconut milk. Nevertheless, this highlights a potential nutritional benefit of FO. Linoleic acid is often found in olive oil and has been reported to have health benefits by reducing high cholesterol and high blood pressure (Lu *et al*., 2023).

3.3. Antioxidant activities of CRO, CTO and FO

The antioxidant activities of CRO and CTO from the enzymatic extraction were high (Table 4) and were ~10 times higher than CRT, the commercial oil obtained from CR and CT by roasting. Compared to VCO_{CEN}, CRO and CTO exhibited slightly higher DPPH scavenging activities. However, their antioxidant activities measured by FRAP assays were significantly less than those of VCO_{CEN}. The differences could be attributed to the different mechanisms involved in the two assays. The DPPH assay determines the ability to identify free radicals by a mechanism in which antioxidants act to inhibit lipid oxidation involving fast electron transfer and slow hydrogen atom transfer; whereas the FRAP assay measures the reduction in ferric ions by antioxidant molecules based on electron transfer (Christodoulou *et al*., 2022).

TABLE 4. Antioxidant capacity (per g oil) of coconut residue and testa via enzymatic and solvent extraction, compared to VCO_{CEN} and CRT (mean ± SD, n = 3).

Coconut oil samples	Antioxidant activity		Total phenolic content (mg GAE)	Flavonoid content (mg QE)	Vitamin E (mg)
	DPPH (mg TEAC)	FRAP (mg TEAC)			
CRT	ND	0.036±0.001	0.034±0.002	0.036±0.007	NA
VCO_{CEN}	0.300±0.006	0.288±0.017	0.037±0.005	0.040±0.005	0.0447[b]
CRO	0.367±0.022	0.135±0.018	0.087±0.001	0.053±0.001	NA
CR_S	ND	0.022±0.005	0.061±0.004	0.019±0.001	NA
CTO	0.326±0.008	0.055±0.004	0.077±0.002	0.050±0.001	NA
CT_S	ND	0.009±0.001	0.030±0.003	0.040±0.001	NA
FO	0.978 ± 0.58	1.70 ± 0.007	0.197 ± 0.06	6.48 ± 0.91	0.0463[b]
FO_S	0.079 ± 0.003[a]	0.935 ± 0.071[a]	0.061±0.006[a]	0.546 ± 0.015[a]	0.0086[b]
CPO	0.126±0.016[a]	0.571±0.034[a]	0.169±0.005[a]	0.132±0.009[a]	ND

[a]Saikhwan *et al*. (2016)
[b]Measurements were supplied by Institute of Nutrition, Mahidol University. CRO – oil from coconut residue by enzymatic extraction, CR_S – oil from coconut residue by solvent extraction, CTO – oil from coconut testa by enzymatic extraction, CT_S – oil from Coconut Testa by solvent extraction, VCO_{CEN}– Virgin Coconut Oil obtained using the same batch of coconut producing CR and CT, NA – Not Measured, ND – Not Detected

A regression analysis was conducted to examine the relationship between antioxidant activity (measured by DPPH and FRAP assays) and antioxidant content (measured as TPC and TFC) in $VCO_{CEN,}$ CTO and oil samples extracted from CR, CT and BP foulants by enzymatic extraction (CRO, CTO, FO) and hexane extraction (CR_s, CT_s and Fo_s). To increase the sample size, data from previous work (Oil extracted from coconut milk foulants formed continuous pasteurization (CPO) extracted by enzyme, (Saikhwan *et al.,* 2016)) were also included. However, the regression analysis showed poor correlations (low adjusted R^2 values) as presented in Table 5. The weak correlations could be due to the influence of the extraction method on both the antioxidant content and the antioxidant activity of the oils. As shown in Table 4, DPPH-scavenging activities of CRT, CR_S and CT_S were not detected and their antioxidant activities from FRAP assays were lower than those of CRO and CTO obtained using the enzymatic extraction. As the solvent used was hexane,

which is non-polar, phenolic compounds which are polar could be extracted to a lesser extent. The effect of solvent polarity on the polyphenol content in CT oil has also been reported in the literature (Arivalagan *et al.,* 2018). Lower antioxidants and antioxidant activities were also observed with FO_S compared to FO. This can be explained by the polarity of the solvent used and the findings suggest the benefits of using enzymatic extraction.

Apart from the extraction method, the temperature involved in the oil source could also affect the antioxidant activity of the obtained oil. For instance, the degradation rate of phenolic compounds in olive oil has been reported to increase significantly at 50ºC (Krichene *et al.,* 2015). This is consistent with the results of CRT; CRT had TPC similar to VCO_{CEN} but the DPPH-scavenging activity of CRT was noticeably lower. The high temperature used during the production of CRT could inactivate the phenolic compounds in CRT. CPO also had low antioxidant activity compared to VCO_{CEN}. CPO was obtained from coconut milk foulants formed during a CP so the foulants were in contact with heat (temperatures of heating surfaces ~92ºC, Saikhwan *et al*., 2022). Hence, the phenolic compounds in CPO could be inactivated during pasteurization.

Nevertheless, FO, which was also extracted from the foulants exposed to heat, had high antioxidant activity (Table 4). The activity was also higher than that of CPO. This may be explained by the physical characteristics of BP foulants. They were thicker than those formed during CP, meaning the top layers were exposed to lower temperatures (~70ºC, Saikhwan *et al*., 2022). Moreover, according to Saikhwan *et al*. (2022), oil deposition in BP foulants primarily occurred towards the end of pasteurization, indicating that most of the oil in FO was located in the upper layers, which were less exposed to heat.

Considering that some antioxidants were not obtained by the solvent extraction and some may be degraded due to the heat, the regression analysis of oil samples was also conducted excluding the data of the oils obtained by solvent extraction and CPO. The revised analysis (Table 5) showed improved adjusted R^2 values for the relationships between antioxidant activities (DPPH scavenging activity and FRAP) and the content of antioxidants (TPC and TFC). A linear relationship between TPC and TFC was also observed. It should be noted that the exclusion of some oil samples did not improve the statistical significance of the effects of TPC and TFC on the FRAP assay. According to Table 5, the *p*-value of *x*-coefficient of a regression analysis between the FRAP assay and TPC increased (less significant); whereas the *p*-value of the relationship between the FRAP assay and TFC did not change significantly. This suggests that both TPC and TFC significantly influence FRAP assay results. This is in accordance with the literature reporting that the antioxidant activities of foods and seeds measured by FRAP assays were closely related to their polyphenol contents (Xu and Chang, 2007).

TABLE 5. Regression statistics of antioxidant activities and number of antioxidants reported in Table 4.

y	*x*	*x*-coefficient	Adjusted R^2	Note
DPPH	TPC	3.52[c]	0.356	
DPPH	TPC	4.60[b]	0.910	Excluding the oils extracted by solvent and CPO
DPPH	TFC	0.125[a]	0.736	
DPPH	TFC	0.101[a]	0.990	Excluding the oils extracted by solvent and CPO
FRAP	TPC	7.04[b]	0.441	
FRAP	TPC	10.3[c]	0.736	Excluding the oils extracted by solvent and CPO
FRAP	TFC	0.728[a]	0.231	
FRAP	TFC	0.239[a]	0.976	Excluding the oils extracted by solvent and CPO
TPC	TFC	0.0192[b]	0.423	
TPC	TFC	0.02[b]	0.852	Excluding the oils extracted by solvent and CPO

a, b, c denote *p*-value less than 0.01, 0.05 and 0.1, respectively.
CPO – oil extracted by enzyme from coconut foulants formed in a continuous pasteurization process, DPPH – DPPH-scavenging activity, FRAP– FRAP assay, TFC – Total Flavonoid Content, TPC – Total Phenolic Content

It is also worth noting that FO had significantly higher DPPH scavenging activity than VCO_{CEN}. TFC of FO was also notably high. This high flavonoid content could come from the accumulation of flavonoids initially found in coconut milk during pasteurization. This assumption is in accordance with the higher flavonoid content in CPO compared to VCO_{CEN}.

However, despite the high TPC and TFC in FO, its FRAP activity was lower than expected. According to the literature, the tocopherol content in coconut oil is sensitive to processing conditions (Kumar and Krishna, 2015). To investigate whether pasteurization affected the vitamin E content in FO and CPO, the vitamin E contents in these oils and VCO_{CEN} were determined. Table 5 shows that the vitamin E contents in FO and VCO_{CEN} were similar. However, no vitamin E was detected in CPO. Vitamin E degradation increases with temperature and heat treatment time (Kuppithayanant *et al.,* 2014) so the vitamin E in CPO could be degraded during continuous pasteurization. In contrast, the similar vitamin E contents in FO and VCO_{CEN} suggest that the vitamin E in FO was less affected by heat during pasteurization. As aforementioned, BP foulants were thick and the layers at the top of the foulants were not in direct contact with heating surfaces.

4. CONCLUSIONS

Cellulase-assisted extraction of oil from CR and CT was studied. The optimal extraction conditions were 5000 U (500 U/g) for CR and 7000 U (700 U/g) for CT, with an extraction time of 4 hr (50-55ºC, pH5-5.5). After the enzymatic extraction of CT, centrifugation resulted in 3 layers with an emulsion-like interface between oil and water phases. The stability of this emulsion layer significantly affected the oil yield; therefore, destabilization or stabilization of the layer would improve the CT oil yield.

The properties of oil samples extracted from CR and CT by enzymatic extraction were investigated. The FO sample was also studied. The SV of CRO and CTO were larger than the range specified by the APCC standard. This was likely due to its high moisture content. The antioxidant activities of CRO and CTO were comparable to VCO_{CEN} and significantly higher than CRT, the current commercial product from CR and CT. Thus, using enzymatic extraction, CRO and CTO have potential as high-value products. Higher flavonoid contents were also observed in CRO and CTO.

Heat treatment was found to decrease the antioxidant activities of the oil from CP foulants but the oil from BP foulants (FO) was not affected. FO had high antioxidant activity, flavonoid content and similar vitamin E content to VCO_{CEN}. Hence, FO also has potential as a high-value product. Although enzymatic extraction gave lower yields compared to solvent extraction, the oils obtained by the enzymatic extraction demonstrated higher antioxidant activities. Therefore, there is a potential to increase the value of the oil from CR, CT and BP foulants using enzymatic extraction.

RESEARCH DATA POLICY

DATA AVAILABILITY

Raw data is presented in tables and graphs but can be provided upon request.

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DECLARATION OF COMPETING INTEREST

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AUTHORSHIP CONTRIBUTION STATEMENT

C. Puraya: Investigation, Formal analysis, Writing – some original draft. J. Somana: Formal analysis, Methodology, Writing – review & editing. Y.M.J. Chew: Formal analysis, Writing – review & editing. P. Saikhwan: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – some original draft, review & editing.

ARTIFICIAL INTELLIGENCE USAGE

Not applicable

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