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Characterization of virgin coconut oil (VCO) recovered by different techniques and fruit maturities

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Abstract

Extraction method and fruit maturity impacted on oil recovery yield and physicochemical characteristics of the extracted oils. The effects of different oil recovering techniques including conventional (i.e., fermentation and thermal cycling) and protease aided extractions on extraction yield and properties of virgin coconut oil (VCO) were observed. By using protease, the VCO with the highest extraction yield and greatest amount of unsaturated fatty acids could be produced compared to the oils given by the conventional methods. The highest contents of health promoting microconstituents, i.e., α-tocopherol and phenolic compounds, were found for the oil recovered through fermentation means. Considering on the effect of fruit maturity, the coconut at intermediate age range contained the highest oil content than those observed for young and old ages. The VCO recovered from young fruits exhibited the best characteristics for both initial quality, as indicated by the lowest free fatty acid value, and nutrition aspect, as implied by the contents of unsaturated fatty acids, α-tocopherol, and phenolic compounds.

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Introduction

Coconut oil is widely used in many industries including food, pharmaceuticals, and cosmetics due to its several advantages, such as richness in medium chain fatty acids (MCFAs) with a good digestibility and antiviral activity (Che Man and Marina, 2006; Marina *et al.*, 2009a). Coconut oil is traditionally recovered through dry extraction, and the derived oil has to be further treated through a refining, bleaching, and deodorizing (RBD) process. The RBD process affects to deteriorate oil qualities in several traits involving masking desirable natural odor, generating high content of free fatty acid (FFA), and destroying health promoting microconstituents (Nevin and Rajamohan, 2004; O'Brien, 2004; Villarino *et al.,* 2007). High content of FFA affected to shorten shelf–life and impair qualities of the oils by promoting oxidative rancidity and oil degradation when used in frying process (Che Man *et al.*, 1997; Raghavendra and Raghavarao, 2010). To conquer this drawback, wet extraction conducted by separating coconut milk from fresh coconut meat and consequently breaking the milk emulsion to liberate oil phase is a promising method. The coconut oil produced from wet extraction is called virgin coconut oil (VCO), defined as the oil obtained from fresh and mature coconuts without any chemical refining (Villarino *et al.*, 2007). Better sensory characteristic and higher nutritive value were guaranteed for VCO than the RBD treated oil, making popularity of VCO as a functional ingredient in food processing (Villarino *et al.*, 2007; Marina *et al*., 2009b). The coconut milk extracted from the endosperm of mature coconuts is present in a form of oil-in-water (O/W) emulsion, which is stabilized by proteins, i.e., globulins and albumins, and phospholipids (Tangsuphoom and Coupland, 2008). To separate oil, the coconut milk emulsion has to be destabilized by breaking down the oil–water interfacial films of the lipoproteins (Sharma *et al*., 2001). Enzymatic aided extraction is an emerging technology for oil recovery by offering many advantages compared to a conventional technique, i.e. eliminate time and solvent consumption as well as provide effective recovery yield and a good quality of the derived oils (Sharma *et al*., 2001; Marina *et al*., 2009b; Marasabessy *et al*., 2010; Jiang *et al.*, 2010). Enzymatic extraction is always conducted under mild temperature, so it is regarded as an eco-friendly process (Villarino *et al*., 2007). It has been reported

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that enzyme aided extraction could improve oil recovery yield from various plants such as *Jatropha curcas* (Marasabessy *et al*., 2010), rice bran (Sharma *et al*., 2001), and peanut (Jiang *et al*., 2010).

Physicochemical characteristics of the extracted oils play important role on their stability and utilization. Intrinsic factors, e.g., plant cultivars and maturity (Umar *et al*., 1996; Gucci *et al*., 2004; Baccouri *et al*., 2008) and extrinsic factors, e.g., extraction method (Seneviratne and Dissanayake, 2008; Marina *et al*., 2009b; Raghavendra and Raghavarao, 2010) affect the properties of oils. Baccouri *et al.* (2008) reported that oxidative stability of extra virgin olive oils recovered from different olive cultivars was differed depending on the contents of indigenous tocopherol and phenolics. By using different extraction methods, the coconut oils possessed different fatty acid profiles, thereby affecting their health benefit and storage stability (Raghavendra and Raghavarao, 2010). Fruit maturity could influence on extraction yield and properties of the derived oils (Umar *et al.*, 1996; Ryan *et al*., 2002; Gucci *et al*., 2004; Baccouri *et al*., 2008). By using olive fruits at different ripening degrees, fatty acid compositions of the oils were dissimilar (Baccouri *et al*., 2008). With different maturity stages, physiology and enzyme activity in plant cells were differed, thereby affecting the amount and profile of several microconstituents of the extracted oils, such as tocopherol, phenolics, squalene, and pigments, (Gutiérrez *et al.*, 1999; Ryan *et al*., 2002; Baccouri *et al.*, 2008). Most of the researches, reporting the effects of extraction method and fruit maturity on quality and characteristics of the extracted oils, have been done for olive oils (Gutiérrez *et al*., 1999; Caponio *et al.,* 2001; Baccouri *et al*., 2008). Regarding VCO, the data are still restricted. The present work aimed to elucidate characteristics of VCO recovered by different techniques and fruit maturities, in order to produce VCO with a good recovery yield and pleasant characteristics.

Materials and Methods

Materials

Fresh and mature coconuts at different maturities, i.e., 11, 12, and 13 months referred as young–(YCO), intermediate– (ICO), and old–coconut (OCO), respectively, and commercial VCO were purchased from a local market in Hat Yai (Songkhla, Thailand). Protease from Bacillus licheniformis with an activity of ≥ 5U/g (1 U corresponds to the amount of enzyme that sets free 1 µmol Folin-positive amino acids and peptide (as tyrosine) per min at pH 7.0 and 37°C, using casein as a substrate) and 2,2'–bipyridine were products of Sigma-Aldrich (St. Louis, MO, USA). Acetic acid, ethanol, toluene, chloroform, methanol and water HPLC grade were purchased from J.T. Baker (Center Valley, USA.). Potassium iodide, potassium hydroxide, cyclohexane, and sodium carbonate were purchased from Ajax Finechem (Auckland, New Zealand). Folin Ciocalteau's and ICl were purchased from Fluka (St. Louis, MO, USA). All the chemicals and solvents used were of analytical grade.

Preparation of VCO

Coconut milk was separated using a screw press (Fujica CM-SJ, Bangkok, Thailand). The grafted coconut meat was pressed for three times, and separated milk was pooled together by vigorously stirring for a few minutes. The chemical compositions of coconut milk were determined (AOAC, 2000). The coconut milk was destabilized by different techniques to produce VCO.

Fermentation method

The coconut milk was incubated at a controlled temperature of 30 ± 2 °C (water bath, Binder BD115, Tuttlingen, Germany) for 24, 36 and 48 h. The sample was then centrifuged (Beckman Coulter Avanti JE, California, USA) at 15,000g for 15 min to separate coconut cream and aqueous phase. To obtain clear oil, the cream phase was further centrifuged at 15,000 g for 15 min. This procedure was modified from the method of Raghavendra and Raghavarao (2010).

Thermal cycling method

The coconut milk was subjected to freeze–thaw program with various cycle numbers, according to the method described by Raghavendra and Raghavarao (2010) with some modifications. Initially, the coconut milk was freezed at -20ºC for 6 h, placed at room temperature for 30 min, and heated at 60ºC for 10 min in a water bath. This tempering program was defined as 1 cycle. The coconut milk was treated for 1–4 cycles, before centrifuging at 15,000 g for 15 min. The separated cream phase was further centrifuged at 15,000 g for 15 min to release clear oil.

Enzyme aided extraction means

Protease at different concentrations (0.05, 0.1 and 0.3 %, w/v) was introduced to the coconut milk, and oil extraction was conducted at 60 °C for various times (0, 15, 30, 60 and 120 min). The sample was then centrifuged at 15,000g for 15 min, and the cream phase was further centrifuged at 15,000 g for 15 min to release clear oil. This procedure was modified from Raghavendra and Raghavarao (2010).

Oil recovery efficiency was determined by the following equation (Mansor *et al*., 2012):

$$\% \text{ oil recovery} = \frac{(\text{weight of extracted oil/weight of coconut milk})}{(\% \text{ oil in coconut meat} / \% \text{ oil in coconut milk})} * 100$$

Peroxide value (PV)

PV was quantified according to the standard method of IUPAC (1992). The oil sample (5 g) was thoroughly mixed with a mixture of acetic acid:chloroform (3:2 v/v, 25 ml) and saturated KI solution (1 ml), before incubating in the dark for 1 h. After adding water (75 ml), the mixture was titrated with a standard solution of sodium thiosulfate (0.01 N) using a starch solution as an indicator.

Free fatty acid (FFA)

FFA was determined by a titration method (IUPAC, 1992). The oil (8 g) was mixed with ethanol (50 ml), before neutralizing with NaOH (0.01 N) using phenolphthalein as an indicator. FFA of the VCO was expressed as a percentage of lauric acid.

Saponification value (SV)

SV measurement was carried out according to the method of IUPAC (1992). The oil (2 g) was mixed with KOH solution in ethanol (0.5 N, 25 ml), before distilling for 1 h. After cooling to room temperature, the mixture was titrated with HCl (0.5 N) using phenolphthalein as an indicator.

Iodine value (IV)

IV was determined by a standard method of IUPAC (1992). The oil (1 g) was thoroughly mixed with cyclohexane (15 ml) and Wijs solution (25 ml), before incubating in the dark for 1 h. After adding with KI solution (10 % w/v, 20 ml) and water (150 ml), the mixture was titrated with a standard solution of sodium thiosulfate (0.1 N) using a starch solution as an indicator.

Total phenolic content (TPC)

TPC present in the oil sample was determined by Folin-Ciocalteau assay following the method of Arslan *et al*. (2013) with a slight modification. Briefly, the oil (5 g) was mixed with a mixture of ethanol:water (80:20 v/v, 3 ml), before centrifuging at 5000 g for 5 min. This extraction was carried out for three times and all ethanolic extracts were combined and evaporated until dryness using a rotary evaporator (Eyela N-1000, Tokyo, Japan). The dry matter was redispersed using a mixture of methanol:water (10:90 v/v, 1 ml), and added with water (8.2 ml) and Folin-Ciocalteau reagent (0.5 ml). The mixture was allowed to stand at room temperatures for 5 min, added with sodium carbonate solution (10% w/v, 1 ml), and incubated at room temperature for 60 min. The absorbance at 765 nm was then read (UV–Vis Spectrophotometer, UV-1700, Shimadzu, Kyoto, Japan). TPC was calculated using a standard curve of gallic acid (0–100 µg/100 ml) and expressed as milligrams gallic acid equivalents (GAE) per gram of oil.

Phenolic composition

The phenolic compositions present in the oil were identified by HPLC (Agilent Technologies 1200 series G1329A, Waldbronn, Germany) following the method of Arslan *et al*. (2013). The eluents were 2 % aqueous acetic acid solution (A) and methanol (B), and the gradient time program was set as following: 0 min 5 % B, 3 min 15 % B, 13 min 20 % B, 25 min 25 % B, and 32 min 30 % B. The absorbance at 240, 280, and 320 nm was read. Identification of each phenolic was determined based on a combination of retention time and spectral matching using catechin, gallic, trans-ferulic, vanillic, p-coumaric, and syringic acids as standards.

Tocopherol content

Tocopherol content present in the oils was measured according to the method described by Wong *et al.* (1998). The oil (1 g) was weighed accurately into 10 ml volumetric flask. Toluene (5 ml), 2,2'–bipyridine (0.07% w/v in 95% ethanol, 3.5 ml) and $FeCl_2$ solution (0.2% w/v in 95% ethanol, 0.5 ml) were added to the sample. The solution was then made up to 10 ml using ethanol and allowed to stand at room temperature for 1 min, before reading the absorbance at 520 nm. Blank was prepared in a same manner, but oil was omitted. Tocopherol content was determined using a standard curve of α-tocopherol (0–250 µg/kg).

Fatty acid composition

Fatty acid composition was examined according to the method of Chowdhury *et al.* (2007). The oil (50 µl) was added with KOH solution (0.5 N in methanol, 1 ml) and digested by stirring in a boiling water bath for 20 min. After cooling to room temperature, the sample was added with a mixture of HCl:methanol (4:1 v/v, 0.4 ml), deionized water (2 ml) and petroleum ether (3 ml). The distinct upper layer of methyl ester was then separated carefully and dried by nitrogen gas. The sample was redispersed using chloroform (1 ml), before introducing to GC (Agilent technologies 7890A, Wilmington, USA) equipped with a flame ionization detector. Varian's capillary column (VF-5 ms, 30 m * 0.25 mm, 0.25 µm; EZ-

GRIPTM, Wilmington USA) was used. The column was conditioned at 180°C for 2 h to attain thermal stability before use. The temperature condition was operated as following: holding at oven temperature of 150°C for 5 min, increasing to 190°C with a rate of 8 °C/min, increasing to 200°C with a rate of 2°C, and holding at 200°C for 10 min. Injection and detector temperatures were 250°C. Nitrogen was used as a carrier gas with a flow rate of 20 ml/min.

Statistical analysis

All experiments were carried out in triplicate, and the mean values with standard deviations were present. Completely Randomized Design was used. Statistical analysis of the data was performed by analysis of variance (ANOVA) using Duncan's multiple range test (SPSS for Windows, SPSS Inc., Chicago, IL, USA) at a 95 % confidence level.

Results and Discussion

Effect of extraction method on oil recovery yield and properties of VCO

Figure 1 shows VCO recovery yields provided by conventional methods of fermentation and thermal cycling techniques. From Figure 1a, the extraction yield could be improved to 74.9% by prolonging fermentation time up to 36 h (P<0.05), whereas an extended incubation time for 48 h had no further improvement effect on oil recovery yield (P>0.05). Upon fermentation, oil releasing from the coconut milk emulsion could be accomplished by gravitational force (Raghavendra and Raghavarao, 2010) and activity of airborne lactic acid bacteria (Srivastava and Semwell, 2013). Lactic acid bacteria used lactose present in the coconut milk and produced lactic acid, leading to alter acidity of the emulsion system to around pH 4, so coconut proteins were easily coagulated (Tangsuphoom and Coupland, 2008). The oil recovery yield provided by thermal cycling means was illustrated in Figure 1b. Increasing the number of freeze–thaw cycles could enhance oil liberation, and the highest yield of 79% was found after treating the coconut milk for 4 cycles (P<0.05). Lowering a temperature affected to solidify oil droplets in coconut milk, and further thawing caused a deformation of oil droplets, thereby promoting coalescence. Consequently, oil releasing from the emulsified matrix occurred (Marina *et al*., 2009b; Raghavendra and Raghavarao, 2010). Reducing temperature in a chilling step could improve oil recovery yield: the extraction yields of ca. 65, 74, and 92% were found for VCO production, after chilling the coconut milk at 20, 15, and 5°C for 6 h, respectively (Raghavendra and Raghavarao, 2010).

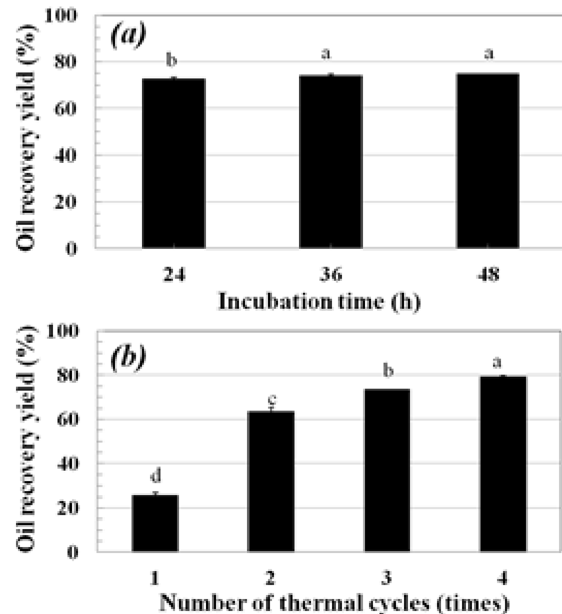

Figure 1. Oil recovery yield provided by (a) fermentation technique at different incubation times and (b) thermal cycling technique at various numbers of thermal cycles
Means with standard deviations (n=3) were shown. Different letters indicated significant difference between means (P<0.05).

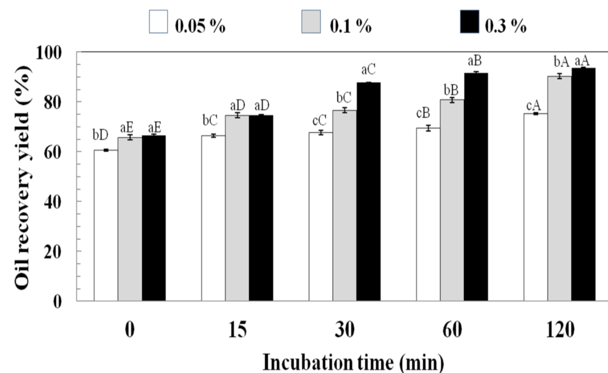

Figure 2. Oil recovery yield provided by protease aided extraction at different enzyme concentrations (0.05, 0.1, and 0.3%) and incubation times.
Means with standard deviations (n=3) were shown. Different small (capital) letters in the same incubation times (protease concentration) indicated significant difference between means (P<0.05)

Effect of enzyme aided means on VCO recovery efficiency was observed at different protease concentrations and incubation times (Figure 2). Increasing protease concentration improved oil recovery yield, especially when incubation time was increased. The highest oil yield of 93.5 % could be obtained, by using protease at the level of 0.3% and incubation time of 120 min (P<0.05). The coconut milk emulsion was partially stabilized by the coconut proteins (Tangsuphoom and Coupland,

Table 1. Fatty acid composition (g/100 g) and characteristics of the VCOs recovered by different techniques

Characteristics	Oil recovery technique			Commercial VCO	APCC standards*
	enzyme aiding	fermentation	thermal cycling		
Fatty acids					
C8:0 (caprylic)	5.81±0.71^{b}	6.69±0.31^{a}	5.93±0.12^{ab}	6.69±0.21^{a}	5.00-10.00
C10:0 (capric)	7.16±0.61^{a}	6.86±0.41^{a}	6.48±0.16^{a}	7.10±0.20^{a}	4.50-8.00
C12:0 (lauric)	50.44±0.50^{ab}	49.51±1.21^{b}	51.72±1.14^{a}	50.10±0.94^{ab}	43.00-53.00
C14:0 (myristic)	17.63±0.50^{ab}	18.28±0.48^{a}	17.80±0.26^{ab}	17.24±0.26^{b}	16.00-21.00
C16:0 (palmitic)	8.02±0.08^{b}	8.88±0.38^{a}	8.19±0.48^{b}	8.37±0.23^{ab}	7.50-10.00
C18:0 (stearic)	2.84±0.19^{a}	2.87±0.09^{a}	2.71±0.10^{ab}	2.56±0.15^{b}	2.00-4.00
C18:1 (oleic)	6.29±0.30^{a}	5.92±0.18^{ab}	5.16±0.87^{b}	5.77±0.15^{ab}	5.00-10.00
C18:2 (linoleic)	1.87±0.18^{a}	1.00±0.30^{b}	1.53±0.38^{a}	1.91±0.07^{a}	1.00-2.50
Total unsaturated fatty acids	8.16^{a}	6.92^{c}	6.68^{d}	7.68^{b}	-
PV (meq O_2/kg oil)	0.53±0.14^{b}	0.78±0.08^{a}	0.66±0.03^{ab}	0.72±0.05^{a}	Max 3
FFA (mg KOH/g oil)	0.12±0.01^{c}	0.15±0.01^{b}	0.10±0.01^{d}	0.20±0.02^{a}	Max 0.2
SV (mg KOH/g oil)	269.23±2.85^{ab}	262.77±4.66^{b}	271.72±3.35^{a}	269.05±1.61^{ab}	250 – 260 min
IV (g I_2/100g oil)	7.62±0.14^{a}	7.26±0.13^{b}	7.01±0.08^{c}	7.36±0.04^{a}	4.1 -11
TPC (mg GAE/g oil)	35.02±0.10^{b}	59.30±0.39^{a}	29.71±0.01^{d}	43.59±0.52^{c}	-
α-tocopherol (mg/g oil)	3.00±1.00^{c}	22.00±1.00^{a}	3.00±0.001^{c}	19.00±1.00^{b}	-

Means ± standard deviations (*n*=3) were shown. Different letters within a same row indicate significant difference between means ($P<0.05$).
* The Asian Pacific Coconut Community (APCC) standards (2003)

2008), so protease could enhance demulsification by hydrolyzing interior peptide bonds of the protein residues, resulting in shorten protein/peptides structures with inferior emulsifying property (Meroth *et al.*, 2003). The fractured proteins/peptide chains tended to move towards aqueous phase, thereby facilitating oil liberation from the emulsified coconut milk (Rosenthal *et al*., 1996). Moreover, proteolytic enzymes could facilitate disruption of cytoplasmic network by degrading protein molecules covering around oil bodies, leading to enhance oil separation from plant cells (Rosenthal *et al*., 1996; Jiang *et al*., 2010).

By using different oil recovery methods, physicochemical properties of the oils were affected. Some selected properties of the VCOs prepared by the conditions providing the highest recovery yields in each studied technique were examined (Table 1). The characteristics of the commercial VCO and the VCO standard of the Asian Pacific Coconut Community (APCC) were also shown in order for comparison. The major fatty acids observed for all VCOs were MCFAs, especially C12:0, which was in accordance with the previous reports (Chowdhury *et al.*, 2007; Raghavendra and Raghavarao, 2011). Compared to the VCO extracted by enzyme–aided means, the VCOs recovered by traditional techniques contained a lower amount of unsaturated fatty acids, which were monounsaturated– (MUFAs) of C18:1 and polyunsaturated fatty acids (PUFAs) of C18:2 for the oils recovered by thermal cycling and fermentation techniques, respectively ($P<0.05$). Regarding to a well recognized health benefit of unsaturated fatty acids, this result implied a better quality in term of nutritive value of the VCO recovered by protease assisted process than the counterparts derived by the traditional techniques (Reena and Lokesh, 2007). Superior nutritional value of the VCO prepared by the aid of aspartic protease compared to the commercial VCO were suggested by the higher amounts of short chain fatty acids, i.e., C8:0 and C10:0 (Raghavendra and Raghavarao, 2010). The lower PV and FFA were found for the VCO prepared by protease aided means compared to those recovered by fermentation technique ($P<0.05$), suggesting to a better initial quality of the former oil. Noted that the higher FFA (0.29–0.35 mg KOH/g) present in the VCO prepared by various methods was reported (Mansor *et al*., 2012). The highest IV was found for the oil recovered by protease aided technique ($P<0.05$), which was in agreement with the higher content of unsaturated fatty acids of the oil. The higher amount of α-tocopherol and TPC were observed for the oil prepared by fermentation means ($P<0.05$), implying to a greater nutritive value of the oil. Phenolic composition of the VCO prepared via fermentation technique was observed (Figure 3). The phenolics predominantly present in the VCO were gallic, catechin, vanillic, and p-coumaric acids. This is in correspondence with the report of Seneviratne *et al*. (2009). The unidentified signals in the HPLC profile were supposed to be oxidized and/or bound forms of phenolic compounds (Seneviratne *et al*., 2009; Arslan *et al*., 2013).

By comparing to the conventional methods, the extraction using protease could effectively

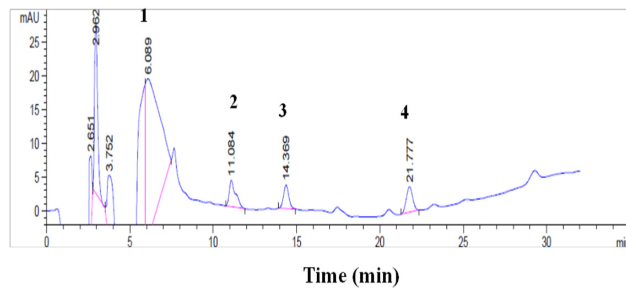

Figure 3. HPLC chromatogram of the phenolic compounds present in the VCO recovered by fermentation technique: (1) gallic acid, (2) catechin, (3) vanillic acid, and (4) *p*-coumaric acid

enhance oil recovery yield with less production time. Considering on the VCO recovered by fermentation technique, the higher amount of α-tocopherol and TPC were observed. Nonetheless, a fermented off–odor could be obviously detected for the VCO recovered through fermentation means. It was suggested that a fermented odor affected to mask a natural desirable flavor of coconut oil, thereby reducing consumer acceptability (Koh and Long, 2012). Based on economical view, better initial quality of lowered PV and FFA, and higher contents of unsaturated fatty acids of the VCO recovered by protease aided means than those observed for the oils produced by fermentation technique, the extraction using protease was selected to prepare VCO in a further study.

Effect of fruit maturity on physicochemical characteristics of VCO

With different agronomy, such as fruit ripeness degree and characteristics of plants, properties of the extracted oils were dissimilar (Robertson *et al.*, 1978; Schittenhelm, 2008; Baccouri *et al.*, 2008). Fat contents of the coconut meat with different maturities were firstly measured and reported as 33.29±0.49, 35.56±0.52, and 30.30±0.29 % for YCO, ICO, and OCO, respectively. The highest fat content of ICO (P<0.05) made it the most appropriate age range for coconut oil producing. The maximum oil content accumulated in sunflower seeds was found at 35 days after the initiation of flowering (DAF) (Robertson *et al.*, 1978). Fatty acid compositions and properties of VCOs recovered from the coconuts at different maturities were shown in Table 2. YCO provided the oil with the highest amounts of C8:0 and total unsaturated fatty acids (P<0.05). A decreasing of C8:0 and total unsaturated fatty acids with an increasing degree of maturity was observed, implying a greater health benefit of the VCO produced from YCO than the other maturities (Reena and Lokesh, 2007; Raghavendra and Raghavarao, 2010). Maturity

Table 2. Fatty acid composition (g/100 g) and characteristics of the VCOs produced from coconut fruits at different maturities

Characteristics	Fruit maturity		
	YCO	ICO	OCO
Fatty acids			
C8:0 (caprylic)	6.88±0.06[a]	5.81±0.71[b]	5.39±0.37[b]
C10:0 (capric)	6.99±0.16[a]	7.16±0.61[a]	7.15±0.23[a]
C12:0 (lauric)	51.52±0.39[a]	50.44±0.50[b]	51.81±0.13[a]
C14:0 (myristic)	17.56±0.53[a]	17.63±0.50[a]	17.49±0.52[a]
C16:0 (palmitic)	7.07±0.44[b]	8.02±0.08[a]	7.57±0.20[ab]
C18:0 (stearic)	2.38±0.19[b]	2.84±0.19[a]	2.78±0.13[a]
C18:1 (oleic)	6.53±0.25[a]	6.29±0.30[a]	6.30±0.09[a]
C18:2 (linoleic)	1.78±0.05[ab]	1.87±0.07[a]	1.51±0.24[b]
Total unsaturated fatty acids	8.31[a]	8.16[b]	7.81[c]
PV (meq O_2/kg oil)	0.11±0.03[c]	0.53±0.14[a]	0.37±0.08[b]
FFA (mg KOH/g oil)	0.09±0.01[b]	0.12±0.01[a]	0.09±0.01[b]
SV (mg KOH/g oil)	255.82±3.05[a]	255.90±2.85[a]	254.12±3.26[a]
IV (g I_2/100g oil)	7.94±0.03[a]	7.62±0.14[b]	7. 36±0.04[c]
TPC (mg GAE/g oil)	41.67±0.16[a]	35.02±0.10[b]	33.44±0.06[c]
α-tocopherol (mg/g oil)	24.00±1.00[a]	3.00±1.00[b]	3.00±1.00[b]

Means ± standard deviations (*n*=3) were shown. Different letters within a same row indicate significant difference between means (*P*<0.05).

of plants could affect fatty acid composition of the extracted oils: for sunflower seed oil, the content of C18:1 was 12% at 7 DAF, increased to 59.6% at 14 DAF, and then diminished to 31.4% at 56 DAF, whereas the content of C18:2 was 48% at 7 DAF, decreased to 23% at 14 DAF, and turned to increase to 59.2% at 56 DAF (Robertson *et al.*, 1978). Regarding extra virgin olive oil, the C18:1 content decreased gradually, whereas the C18:2 level increased as the fruit ripened (Baccouri *et al.*, 2008). C16:0 content of extra virgin olive oil diminished during the ripening process as reported by Baccouri *et al.* (2008), due to a dilution effect (Gutiérrez *et al.*, 1999). The lowest PV was observed for the oil of YCO (P<0.05), suggesting to a better initial quality of the oil. The highest PV and FFA were found for the oil of ICO (P<0.05), implying to a lower stability of the oil. This might be expected since a higher oil accumulation in the tissues of ICO. For extra virgin olive oils, the FFA content tended to increase thoroughly with degree of maturity, which was explained by an undergoing of lipase activity and more sensitivity to pathogenic infections and mechanical damage of olive fruits with later maturity (Baccouri *et al.*, 2008). Coconut fruit has a hard shell to protect oil containing parts, so different oxidative behavior with maturity degree might be supposed. The highest IV was observed for the oil extracted from YCO (P<0.05), which was in agreement with its higher content of total unsaturated fatty acids compared to the oils extracted from ICO and OCO. Interestingly, the highest contents of nutritive microconstituents, involving both of α-tocopherol and phenolic compounds, were found for the oils of YCO (P<0.05). Various compounds, i.e., phenols, tocopherols, squalene, and pigments,

were particularly higher in olive oils extracted from immature fruits compared to fully–ripened fruits (Gutiérrez *et al*., 1999; Caponio *et al.*, 2001; Baccouri *et al.*, 2008). Tocopherols and phenolic compounds were responsible for antioxidant ability (Mateos *et al*., 2003) and, therefore, better oxidative stability was found for the tocopherol and phenolic rich oils (Gutiérrez *et al.*, 1999; Baccouri *et al.*, 2008). A decrease in the oleuropein content in olives with increasing fruit ripeness was reported and attributed to phenolic degradation, caused by the increased activity of hydrolytic enzymes such as esterase at a later stage of maturity (Gutiérrez *et al.*, 1999; Ryan *et al*., 2002). However, change in the contents of microconstituents with fruit maturity could be differed, depending on other factors such as growing condition and plant cultivar (Gutiérrez *et al*., 1999; Baccouri *et al*., 2008). It has been suggested that a greater degree of phenolic synthesis in fruit, and so in the extracted oil, related to enzyme activity in plant cells, such as L-phenylalanine ammonia-lyase, whose activity was greater under higher water stress condition (Morello *et al*., 2005).

Conclusions

Extraction method and fruit maturity had impact on extraction yield and physicochemical characteristics of the derived VCO. The highest oil recovery yield was provided by protease aid extraction, and the obtained oil showed higher amount of unsaturated fatty acids than the oils extracted by fermentation and thermal cycling techniques. The VCO recovered through protease assisted means showed a greater initial quality, as suggested by the PV measurement. The highest amounts of α-tocopherol and phenolic compounds were found for the VCO prepared by fermentation technique ($P<0.05$). Phenolic compounds predominantly present in the VCO were catechin, gallic, vanillic, and p-coumaric acids. Considering on the effects of fruit maturity, the coconut at intermediate age range contained the highest oil amount than those observed for young and old ages. The VCO recovered from young fruits exhibited the best characteristics in both initial quality, as indicated by the lowest PV ($P<0.05$), and nutritive value, as implied by the highest contents of unsaturated fatty acids, α-tocopherol, and phenolic compounds ($P<0.05$). All of the observed parameters of the VCOs were within the limits of APCC standard, suggesting that the VCOs produced in the present work could be employed commercially.

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