

Physicochemical properties of virgin coconut oil extracted from different processing methods

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Abstract: Virgin Coconut Oils (VCO) were prepared from fresh-dry (grated coconut route), chilling and thawing, enzymatic and fermentation method in this study. All of the VCO produced conformed physicochemically to the standards established by the Asian and Pacific Coconut Community (APCC) and Codex Alimentarius Commission. The highest FA (fatty acid) is lauric acid in all of the VCO and ranged from 46.36 – 48.42 %, while the principal TAG (triacylglycerol) is LaLaLa (La: Lauric) with 17.94 – 19.83 % of the total TAG. Tocopherol analysis showed the presence of beta, gamma and delta tocopherols at low levels. In all, the physicochemical, FA and TAG analyses of the VCO extracted from different methods showed some significant differences, while the tocopherol content does not differ significantly among the different types of extraction methods used.

Keywords: Virgin coconut oil, FA, TAG, tocopherol analysis

Introduction

Virgin coconut oil (VCO) is defined as the oil resulting from the fresh and mature kernel of the coconut (*Cocos nucifera* L.) through mechanical and natural means, either with the use of heat or not provided that it does not lead to alteration or transformation of the oil (APCC, 2003). VCO has many advantages, which include the health benefits from the retained vitamins and antioxidants, the antimicrobial and antiviral activity from the lauric acid components and through its easy digestibility from the medium chain fatty acids (MCFA).

Apart from the above said, VCO and coconut oil have been traditionally used to enhance the beauty and promote the growth of our tresses, refine and moisturizes our skin conditions as well as being used as ailments for minor illnesses such as diarrhea and skin inflammations. Nevin and Rajamohan (2010) discovered that wound healing rate was increased in skin of rats treated with topical VCO. Lans (2007) reported that *Cocos nucifera* was also used as an “ethnomedicine” to treat gastrointestinal problems and minor cuts, injuries and swelling. The lauric acid, a medium chain fatty acid component in VCO showed potential use as anti-obesity treatment (St-

Onge and Jones, 2002; Assunção *et al.*, 2009) as it increases energy expenditure, directly absorbed and burnt as energy in the liver, resulted in early satiety and thus leading to weight loss.

VCO can be extracted from the fresh and mature kernel of the coconut meat from several methods (Bawalan and Chapman, 2006). There are no specific processing prerequisites that were established according to Marina *et al.* (2009a), however, several methods to produce VCO were found to measure up with the definition of the VCO have been reported (Bawalan and Chapman, 2006; Marina *et al.*, 2009a; Nevin and Rajamohan, 2010; Raghavendra and Raghavarao, 2010). These methods can be largely divided into wet and dry methods. In wet method, the coconut meat/kernel does not go thru drying process while in dry method, the kernel were heated under specific conditions to remove the moisture in it while preventing scorching and microbial invasion. Wet method can be further divided into chilling and thawing, fermentation, enzymatic and pH method or any of these in combination as the main aim is to destabilize the coconut milk emulsion (Raghavendra and Raghavarao, 2010). In dry method, the kernel was dried using controlled heating and subsequently pressed mechanically to obtain the oil. This current

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study revealed the effect of extraction methods of VCO on oil recovery and its physicochemical properties.

Materials and Methods

Materials

Pure and fresh coconut milk and its white meat of MAWA cultivar were obtained from a local market in Serdang, Selangor, Malaysia. Papain was obtained from Merck (Darmstadt, Germany) and yeast (*Saccharomyces cerevisiae*) was obtained from a commercial brand, Lesaffre Group, bought from a local bakery shop. All the chemicals and solvents used were of analytical grade except for chemicals used in the FA and TAG analyses, which were of HPLC grade.

Fermentation method

Fresh coconut milk was added with distilled water with 1:1 ratio. In each 1 litre of the mixture, 2.0 g of Baker's yeast (*Saccharomyces cerevisiae*) was added as an inoculum for the fermentation process. Mixture was made homogenous by mixing it rigorously. The mixture was then left to stand for 36 h at room temperature. As the layers of oil and water became separated, the upper oil layer was simply decanted. The acquired oil was prepared in triplicate and kept refrigerated until further use.

Chilling and thawing method

The chilling and thawing method was performed according to Raghavendra and Raghavarao (2010) with some modifications. Coconut milk was centrifuged at 3600 g for 10 min and the upper layer of cream was removed for chilling. Chilling was done at 5°C for 24 h and then the chilled cream was thawed slowly in water bath at 50°C to extract the oil. The oil was prepared in triplicate and kept in the refrigerator until further use.

Enzymatic treatment

The enzymatic method of VCO extraction was also prepared according to Raghavendra and Raghavarao (2010) with some modification. The milk of the coconut was mixed with papain enzyme at 0.1% (w/w) of the milk. The mixture was made into homogenous solution by stirring. It was left to stand for 3 h at 55°C as this is the optimum temperature for papain enzyme. The mixture was later centrifuged at 4900 g for 25 min to obtain the oil. The sample was prepared in triplicate and kept refrigerated afterwards until further use.

Fresh-dry process

The white meat of the coconut was shredded and dried in an aerated oven at 35°C for approximately 48 h. The coconut meat was homogeneously dried and prevented from scorching of the meat through frequent turning of the shredded meat. After drying, it was screw pressed for oil extraction. The collected oil was also filtered using Whatman filter paper no. 1 to remove some debris that escaped into the oil. The oil was prepared in triplicate and later kept in the refrigerator until further use.

Oil recovery

The determination of oil recovery was calculated according to the initial oil content in the coconut meat/milk to the oil extracted from different extraction methods elucidated above. The official AOAC Soxhlet method (AOAC, 1997) and Gerber method using Gerber butyrometer (IDF, 1981) were applied to ascertain the oil content of the coconut meat/milk. Below is the formula used for calculation of the oil recovery:-

$$\% \text{ oil extraction} = \frac{\text{Weight of oil extracted}}{\text{Weight of coconut milk/meat used}} \times 100 \quad (1)$$

$$\begin{aligned} \% \text{ oil in coconut} \\ \text{meat/milk} &= \text{measured by Gerber/Soxhlet} \quad (2) \\ \text{Oil recovery} &= (1) / (2) \end{aligned}$$

Physicochemical analyses

Saponification value (SV)

Saponification values (SV) were determined using the International Union of Pure and Applied Chemistry (IUPAC), method II.D.2 (IUPAC, 1979). 2.0 g oil samples were added with ethanolic potassium hydroxide 0.5 N and boiled for 60 minutes in a reflux condenser. The mixtures were cooled and subsequently titrated with 0.5 N hydrochloric acid until the colour of the mixtures changed from pink to the original colour. All SV determinations were carried out in triplicates.

Iodine value (IV)

The determination of Iodine Value (IV) was carried out according to the IUPAC method II.D.7 (IUPAC, 1979). Samples were reacted with the Wij's solution and left in the dark for 1 hour. Mixture was consequently titrated with sodium thiosulphate solution. IV values were determined in triplicates and expressed as gram of Iodine absorbed by 100 g of the fats (g I₂/ 100 g).

Free fattyAcids value (FFA)

Free Fatty Acid (FFA) value was ascertained using the IUPAC method II.D.1 (IUPAC, 1979). All measurements were conducted in triplicates and expressed as mg of KOH requisited to neutralize the free fatty acids in 1 g of VCO (mg KOH/g oil).

Moisture content

Determination of moisture content was based on the AOAC method (AOAC, 1997) with slight alteration. Samples were heated at 105°C in a heated and weighed crucible for at least 7 h and cooled to a room temperature and re-weighed with the samples inside until constant readings were gathered.

Viscosity and colour

The measurement of viscosity was performed using rheometer, AR G2 Rheometer (TA Instruments, US). The brightness and colour of VCOs were determined using the Hunter Lab Colorimeter, (Hunter Associates Laboratory, Inc., Virginia, USA). Both tests were carried out in triplicates.

Fatty Acid Methyl Esters (FAME) compositional analysis

Preparation of the fatty acid methyl esters were made by using the Cocks and Van Rede (1996) method with slight modification to ensure sufficient volatility for the fatty acid analysis by the GC-FID. 0.2 ml oil samples were dissolved in hexane and later added with 0.2 ml of 1M sodium methoxide. The mixtures were vortexed and FAME solution in the upper layer was collected for GC-FID analysis.

The analysis of FAME was conducted by Agilent gas chromatography (Agilent technologies 6890N, Santa Clara, CA) equipped with a flame ionization detector (FID). RESTEX 2330 column were used in this experiment (0.25 mm internal diameter, 30 m length and 0.2 µm film thickness; Restek Corp, Bellefonte, PA, USA) at a column pressure of 1.03×10^5 Pa. The column was initially set at 50°C and held for 2 min, then increased to 180°C at a rate of 5°C/min and held for another 2 min at 180°C. Subsequently, it was increased at a rate of 8°C/min to 200°C and held for 5 min at 200°C. Peak identification were compared with the standard FAMEs obtained from Sigma Chemicals, St Louis, MO. Determination were carried out in triplicates and presented as mean and standard deviation of the percentage area.

Triacylglycerol (TAG) analysis by HPLC

Reverse-phase High Performance Liquid Chromatography (HPLC), Waters (Milford, MA) coupled with Waters Refractive Index detector (Milford, MA) was utilized for TAG analysis. VCO

samples were dissolved in acetone in 1:9 v/v ratio and eluted with acetone:acetonitrile (63.5:36.5) isocratically. LiChroCART 100-RP-18 column (5 µm x 12.5 cm x 4 mm i.d.; Merck, Darmstadt) was used in this study. 10 µl samples were injected for each analysis and the TAG peak identifications were compared on the basis of retention time of TAG standards, using the Empower software (Milford, MA). Determinations were carried out in duplicates and presented as mean and standard deviations of the percentage area.

Tocopherol analysis using High Performance Liquid Chromatography (HPLC)

Tocopherol analysis was performed according to method described by Puah *et al.* (2007). 0.1 g VCO samples from each extraction were weighed and dissolved to 25 ml with n-hexane. HPLC (Waters, Milford, MA) with fluorescence detector were used with Zorbax SIL normal phase column (5 m x 150 mm x 4.6 mm ID) (Zorbax, USA). n-hexane/THF/2-propanol (500:30:2, by volume) was prepared as the mobile phase. The flow rate was established at 1.0 ml/min. Fluorescence detector was arranged to detect emission wavelength of 326 nm and excitation wavelength of 292 nm. External standards of Tocopherol set (alpha, beta, gamma and delta) was obtained from Merck, Darmstadt, Germany and used for identification of peaks from the samples. Analysis was performed in triplicates and presented as mean and standard deviations of percentage area.

Statistical analysis

Analysis of variance (ANOVA) was carried out on the results using the Minitab version 14 (Minitab Inc., State College, PA, USA). Significant differences among means were established at $p < 0.05$ using Tukeys' test. Tukeys' test was chosen because the sample size is the same for each method of extractions and we assumed that the observations are independent and there is equal variation across observations.

Results**Oil Recovery**

Oil recovery gives a quantitative measurement on the effectiveness of different method of extractions on the amount of oil produced. Table 1 showed the percentage recovery of VCO extracted from 5 different processes. From the table, it is evident that the fresh-dry method of VCO extraction recovered the most oil from the other method with $88.35 \pm 5.96\%$ oil recovery, while in 'wet' method, chilling and thawing gave the highest oil recovery ($86.62 \pm 3.63\%$) followed by fermentation and enzymatic

Table 1. Physicochemical analysis of VCO extracted from different methods^{†‡}

Analysis	Extraction method ^{†‡}				APCC standard, 2007
	Chilling	Fermentation	Fresh-dry	Enzyme	
Iodine value (g I ₂ / 100 g fats)	4.13 ± 0.02 ^a	4.30 ± 0.07 ^{b,c,d}	4.18 ± 0.04 ^{a,b}	4.26 ± 0.05 ^{a,b,c}	4.10 – 11.00
Free fatty acid (mg KOH/g oil)	0.31 ± 0.01 ^a	0.29 ± 0.02 ^{a,d}	0.46 ± 0.01 ^b	0.35 ± 0.01 ^{a,c}	0.5 max
Saponification value (mg KOH/g oil)	258.23 ± 3.09 ^a	256.73 ± 0.85 ^{a,b,d}	258.42 ± 1.41 ^{a,b}	262.72 ± 0.32 ^{a,b,c}	250 - 260 min
Moisture content (% w ₂)	0.11 ± 0.01 ^a	0.06 ± 0.00 ^d	0.04 ± 0.00 ^b	0.11 ± 0.01 ^{a,c}	0.1 - 0.5
Viscosity (Pa.s)	48.93 ± 0.31 ^a	48.73 ± 0.46 ^{c,d}	50.93 ± 0.31 ^{a,b}	48.93 ± 0.31 ^{a,c}	NA [§]

[†] Means within the same row with different superscripts are significantly different at p < 0.05.

[§] NA=not available

Table 2. Fatty acid composition of VCO produced from different methods and APCC standard FA for VCO (% area)

FA	Extraction Method ^{†‡}				APCC standard
	Chilling	Enzyme	Fermentation	Fresh-dry	
C6	0.57 ± 0.00 ^a	0.52 ± 0.00 ^c	0.57 ± 0.01 ^{a,d}	0.55 ± 0.00 ^b	0.40 – 0.60
C8	7.39 ± 0.03 ^a	6.63 ± 0.01 ^c	7.21 ± 0.13 ^{b,d}	7.23 ± 0.00 ^{a,b}	5.00 – 10.00
C10	6.15 ± 0.01 ^a	5.49 ± 0.00 ^c	6.07 ± 0.10 ^{a,d}	5.94 ± 0.01 ^b	4.50 – 8.00
C12	48.05 ± 0.11 ^a	46.36 ± 0.00 ^c	48.42 ± 0.90 ^{a,b,d}	48.07 ± 0.02 ^{a,b}	43.00 – 53.00
C14	18.45 ± 0.03 ^a	19.54 ± 0.01 ^{b,c}	18.75 ± 0.34 ^{a,d}	19.23 ± 0.00 ^b	16.00 – 21.00
C16	8.94 ± 0.05 ^a	9.94 ± 0.01 ^c	9.06 ± 0.16 ^{a,b,d}	8.91 ± 0.01 ^b	7.50 – 10.00
C18	2.96 ± 0.03 ^a	3.37 ± 0.00 ^c	3.15 ± 0.00 ^{b,d}	3.17 ± 0.09 ^b	2.00 – 4.00
C18:1	6.18 ± 0.03 ^a	6.50 ± 0.01 ^c	6.35 ± 0.01 ^d	5.79 ± 0.01 ^b	5.00 – 10.00
C18:2	1.31 ± 0.01 ^a	1.63 ± 0.00 ^c	1.36 ± 0.00 ^d	1.12 ± 0.00 ^b	1.00 – 2.50

[†] Each value represents the mean and standard deviations of triplicate determinations.

[‡] Means within the same row with different superscripts are significantly different at p < 0.05.

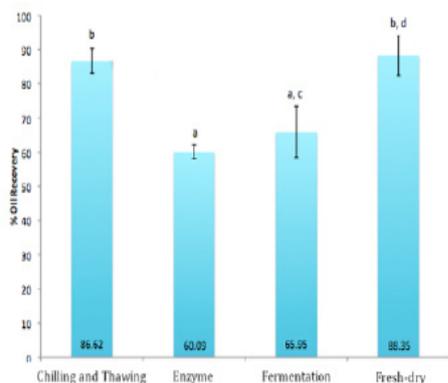


Figure 1. The oil recovery of each VCO extracted from different processes

method.

Physicochemical analysis

The physicochemical analyses performed on all the VCOs are shown in Table 1, along with the Asian Pacific Coconut Community (2003) standards. The range of IV of all the samples was 4.13 – 4.33 g I₂/100 g fats. The lowest IV was obtained from the chilling and thawing method while the highest was from fermentation method. The FFA of VCO is

expressed as lauric acid according to the nature of its fats. Overall, the FFA of all the VCOs extracted from different methods confers to the APCC (2003) standard but higher than findings from Marina *et al.* (2009b) and ranged from 0.29 – 0.46 mg KOH/1 g of fats. Fresh-dry methods resulted in the highest FFA of 0.46 ± 0.01 mg KOH/ 1 g fats for both methods, while fermentation method recorded the lowest FFA (0.29 ± 0.02 mg KOH/1 g fats).

The SV of all the samples showed high values of SV and ranged from 256.73 – 262.79 mg KOH/ g of fats. The SV gathered from this experiment alligned with the APCC and Codex Alimentarius Commission (248 – 265 mg KOH/g fats) (Codex, 2001) standards for VCO and coconut oil, respectively. The highest SV was from enzyme method followed by fresh-dry, chilling and thawing and the lowest was from fermentation method. The measurement of the moisture content in this study ranged from 0.04 – 0.11% (w/w), in which the lowest was from fresh-dry method and the highest were from chilling and thawing and enzymatic methods. The values for viscosity ranged from 48.73 - 50.93 Pa.s (Pascal second). Fresh-dry method gave the highest viscosity (48.73 ± 0.46 Pa.s) followed closely by chilling and thawing, enzymatic and fermentation methods.

Table 3. TAG composition of VCO extracted from different method (% area)

TAG ^a	Extraction method ^b			
	Chilling	Enzyme	Fermentation	Fresh-dry
CCCI	0.00 ± 0.00 ^a	0.31 ± 0.00 ^a	0.00 ± 0.00 ^{a,d}	0.44 ± 0.01 ^b
CpCpLa	0.54 ± 0.00 ^a	0.79 ± 0.01 ^c	0.41 ± 0.01 ^b	0.95 ± 0.01 ^b
CpCLa	4.11 ± 0.03 ^a	3.86 ± 0.04 ^a	3.81 ± 0.01 ^{a,d}	4.07 ± 0.03 ^{a,b}
CCLa	13.55 ± 0.01 ^a	13.36 ± 0.05 ^a	13.82 ± 0.01 ^a	12.71 ± 0.01 ^b
CLaLa	17.05 ± 0.07 ^a	17.34 ± 0.06 ^a	18.90 ± 0.01 ^a	21.10 ± 0.01 ^b
LaLaLa	22.62 ± 0.05 ^a	23.94 ± 0.01 ^a	22.21 ± 0.00 ^a	21.63 ± 0.06 ^b
LaLaM	19.83 ± 0.24 ^a	17.94 ± 0.00 ^a	19.51 ± 0.01 ^a	18.89 ± 0.01 ^b
LaLaO	1.84 ± 0.05 ^a	1.88 ± 0.00 ^a	1.72 ± 0.01 ^a	1.88 ± 0.01 ^a
LaMM	11.07 ± 0.09 ^a	10.80 ± 0.01 ^{a,b}	12.17 ± 0.01 ^a	10.77 ± 0.02 ^b
LaMO	1.07 ± 0.09 ^a	1.24 ± 0.02 ^{a,b}	1.04 ± 0.04 ^{a,b,c,d}	1.00 ± 0.00 ^{a,b}
LaMP	6.26 ± 0.04 ^a	5.94 ± 0.04 ^a	5.01 ± 0.01 ^a	4.72 ± 0.01 ^b
LaOO	0.61 ± 0.02 ^a	0.74 ± 0.00 ^a	0.57 ± 0.01 ^{a,b,c}	0.58 ± 0.02 ^b
LaPP	1.19 ± 0.02 ^a	1.64 ± 0.00 ^a	0.83 ± 0.03 ^a	1.08 ± 0.03 ^b
MOO	0.27 ± 0.01 ^a	0.22 ± 0.01 ^{a,b}	0.00 ± 0.00 ^a	0.19 ± 0.01 ^b

^aEach value represents the mean and standard deviations of duplicate determination.
^bMeans within the same row with different superscripts are significantly different at p<0.05.
^cCp, caproic; Cl, caprylic; C, capric; La, lauric; M, myristic; P, palmitic; O, oleic

Table 4. Tocopherol composition of VCO extracted from different methods (mg/kg)^{†‡}

Extraction method	Tocopherol			
	Alpha-tocopherol	Beta-tocopherol	Gamma-tocopherol	Delta-tocopherol
Chilling	ND	0.04 ± 0.00 ^a	0.01 ± 0.00 ^a	9.26 x 10 ⁻⁵ ± 0.00
Enzyme	ND	0.04 ± 0.00 ^a	0.05 ± 0.01 ^c	1.30 x 10 ⁻⁵ ± 0.00
Fermentation	ND	0.04 ± 0.00 ^a	0.05 ± 0.00 ^{c,d}	6.41 x 10 ⁻⁵ ± 0.00
Fresh-dry	ND	0.04 ± 0.01 ^a	0.01 ± 0.00 ^{a,b}	1.10 x 10 ⁻³ ± 0.00
Codex Standard for coconut oil	ND - 17	ND - 11	ND - 14	ND

[†]Each value represents the mean and standard deviations of triplicate determinations.
[‡]Means within the same column with different superscripts are significantly different at p<0.05.
[§]ND = not detected.

The color measurement study was performed by the Hunter Lab colorimeter, most importantly to check for the brightness and the yellowness of the VCO. The highest of brightness was achieved by the enzymatic treatment (92.25 ± 0.11) followed by fresh-dry (92.17 ± 0.06), fermentation (92.06 ± 0.10) and chilling and thawing method (91.91 ± 0.00). In contrast, the highest value for yellowness was from fresh-dry method (3.88 ± 0.02) followed by enzyme (1.80 ± 0.02), fermentation (1.68 ± 0.01) chilling and thawing method (0.76 ± 0.04).

Fatty Acid Methyl Esters (FAME) compositional analysis

VCO is coined a medium chain fatty acid (MCFA) oil because of the high content of MCFA, predominant with lauric acid (C12:0). This is evident in Table 2, where the total lauric acid in VCO produced from different extraction methods ranged from 46.36% to 48.42% and the total MCFA in the oil (C6, caproic;

C8, caprylic acid; C10, capric acid and C12, lauric acid) ranged from 59.02% to 62.27 % of the total FA. The highest lauric acid was obtained from the samples extracted from the fermentation process with 48.42% ± 0.90%, followed by fresh-dry, chilling, and enzyme method with 48.07 ± 0.02%, 48.05 ± 0.11% and 46.36 ± 0.00%, respectively, with only enzymatic method being significantly different from other methods.

The lowest FA in all types of VCO as well as the APCC and Codex (APCC, 2003; Marina, 2009b) standards was caproic acid (C6) with range between 0.52% (fresh-dry) to 0.57 % (chilling and thawing and fermentation). The total saturated FA was from 91.87% to 93.27% while the total unsaturated FA (mono- and di-unsaturated FA) was from 6.73% to 8.13%.

TAG analysis by reverse phase HPLC

The analysis of TAG to all the VCO samples were presented in Table 3. From the analysis, the principal TAG component was LaLaLa, (La:lauric), which ranged from 21.63 – 23.94% of the total TAG compositions, while the second highest TAG was LaLaM (M:myristic) (range 17.94 – 19.83%). The third highest was CLaLa (C:capric) with values from 17.05 – 21.10%. Unsaturated FA was mainly from oleic acids, with values of 1.72 – 1.88% for LaLaO; 1.00 – 1.24% for LAMO (O:oleic); 0.57 – 0.74% for LaOO and 0.00 – 0.27% for MOO TAG.

Tocopherol analysis by HPLC

Tocopherol analysis was performed on each extracted VCO and findings are presented in Table 4. The tocopherol from Codex Alimentarius Commission for coconut oil was also included in the table for comparison. Three types of tocopherol were detected namely, beta, gamma and delta-tocopherol. Beta-tocopherol ranged from 0.04 – 0.05 mg/kg, gamma-tocopherol ranged from 0.01 – 0.05 mg/kg, while delta-tocopherol was detected at a very low level for each VCO (1.30 x 10⁻⁵ to 1.10 x 10⁻³ mg/kg). Alpha tocopherol was not detected in our study and was set at the non-detection level to 17 mg/kg alpha tocopherol from the Codex standard (Codex, 2001).

Discussion

Oil Recovery

The fresh-dry method gave the highest oil recovery (88.35 ± 5.96 %), which could be explained that the process of destabilization of the coconut emulsion were not as effective in the ‘wet’ methods as compared to the ‘dry’ process. In wet method, chilling and thawing gave the highest percentage of oil recovery

followed by fermentation and enzymatic methods. In our study, the chilling condition was established at 5°C, as it has been shown by Raghavendra and Raghavarao (2010) that oil recovery is the highest at 5°C although Gunetileke and Laurentius (1974) initially stated that the critical temperature for optimum oil separation was at 17°C. Oil globules solidify on chilling or freezing of the VCO, and upon thawing, the oil globules coalesce, causing a higher destabilization of the coconut emulsion. This has thus made the extraction easier as compared to other wet methods. In the meanwhile, the fermentation of the coconut milk by the baker's yeast caused the release of alcohol, organic acid and CO₂ through the breakdown of glucose in the coconut milk. Alcohol and organic acid involves in the coagulation process of the proteins and thus, responsible for the destabilization of the coconut emulsion.

The use of enzyme papain in our study just managed to obtain only 60.09% oil recovery, which is the lowest in all methods. This is perhaps because of the use of single enzyme (papain), which only works on proteins and not on carbohydrates. Papain is also known scientifically as a cysteine protease, which is present in papaya fruits. It would be reasonable to hypothesize based on many other studies that the addition of other enzymes such as cellulase and carbohydrases enzymes would increase the yield furthermore. Che Man *et al.* (1996), discovered a 74% oil yield from 1% enzyme mixture of cellulase, α -amylase, polygalacturonase, and protease. Christensen (1989) also reported a very significant oil yield of more than 90% by the use of galactomannase in combination with a polysaccharide-enzyme.

Physicochemical analysis

All the measurement fulfilled the requirements established by the APCC and were aligned with the findings from Marina *et al.* (2009b). In all, the IV from all the samples was low owing to the high degree on saturation of VCO. This highlights the low likeliness of VCO to become rancid from lipid oxidation (Onyeike and Acheru, 2002). The IV would have effects on overall quality parameters such as the shelf life of VCO, appearance as well as the taste and smell. The slight differences in the values for each method of extraction could be reasoned by the different extraction methods and the titration precision on each measurement.

FFA is the measure of mg of potassium hydroxide required to neutralize the free fatty acid present in 1 g of fats. The FFA of all the VCO extracted from different methods confers to the APCC (2003) standard although slightly higher than findings from

Marina *et al.* (2009b) and ranged from 0.29 – 0.46 mg KOH/1 g of fats. These FFA are formed from the hydrolysis of an ester by lipase or moisture (Osawa *et al.*, 2007) and contributes to the off taste and aroma in fats. Fresh-dry method resulted in the highest FFA of 0.46 ± 0.01 mg KOH/ 1 g fats, which could be related to the relatively long duration taken to dry the coconut meat. The longer time taken to dry the meat prior to oil extraction could in turn causes more time for the process of rancidity that can take place, which increases the FFA as the triglycerides is broken into glycerol and FAs.

The SV basically refers to the mean molecular mass of the fats and oils and have an inverse relationship with the chain length of the FA in fats and oils. This means, the longer the average FA chain length, the smaller the SV. Again, the SV obtained from this experiments aligned with the APCC and Codex Alimentarius Commission (248 – 265 mg KOH/g fats) (Codex, 2001) standards for VCO and coconut oil, respectively. It was however found that the highest SV was from enzymatic method and the lowest was from fermentation method. In this case, as the SV would depend on the types of FA present and the FA values were fairly similar with slight differences within different extraction methods, we could conclude that the slight SV discrepancies are caused by the different method of extractions applied. We can also conclude here that all the VCO produced in each method is highly acceptable according the standards and parallel to its characteristic of having high medium chain FA.

Moisture content is another important quality characteristics for oils and fats. It is desirable to keep the moisture content low as it will increase the shelf life by preventing oxidation and rancidity processes. The high moisture content, as explained earlier, will assist in hydrolysis process (Osawa *et al.*, 2007; Lawson, 1985). The highest recorded viscosity was from fresh-dry method while the lowest was from fermentation method. Meanwhile, the objective colour measurement of the VCO samples, it was found that all VCO impart high brightness (L) and low yellowness (b). This is in accordance to the specifications of APCC (2003), which determines VCO as water clear or almost colorless (Villarino *et al.*, 2007). The highest yellowness has been expected from fresh-dry method as during the drying process, some of the coconut meat might have been over-heated, causing slight yellowish discoloration. This should be prevented in the production of VCO by frequent turning of the coconut meat. The colour measurement is important because it reflects the quality, consistency and safety of the

VCO. VCO should have a high brightness and low yellowness as well as being consistent from batch-to-batch production and from different techniques of extraction, regardless of the cultivar types and production plants.

Fatty Acid Methyl Esters (FAME) compositional analysis

VCO is predominantly made up of lauric acids. It is known that monolaurin, a monoglyceride form of lauric acid proven to possess certain antibacterial and antiviral activities (Wang *et al.*, 1993; Kabara, 1984; Enig, 1998). Although the level of monolaurin was not ascertained in this study to correlate its equivalence to lauric acid, the ingestion of lauric acid would lead to systemic breakdown of VCO in our body into lauric acid and/or monolaurin (Dayrit, 2000). Monolaurin also resembles human breast milk, which is known to benefit babies and confers immunity to the baby.

The total lauric acid all concurred with the APCC (2003) standard for VCO, Codex (2001) standard for coconut oil (45.10 – 53.20%), Marina *et al.* (2009b) and Dayrit *et al.* (2007). It can be concluded that the coconut oil (the refined-bleached-deodorized) and all VCO from any extraction method fall into these range, as the different method of extraction and the processing of these oil does not affect the FA so much. The slight differences observed can be attributed to the differences in processing methods. However, we cannot exclude the variations due to different coconut nuts that can occur within the same coconut variety, which can influence the FA of the extracted oil.

In all, the FAs determined concurred with the standard given by the APCC (2003). Although the total saturation in VCO is high, it is still deemed one of the best oil as it aids in digestion and the presence of high amount of MCFA ensures the FAs are not stored as fats, instead being used as energy (Babayán, 1987).

TAG analysis by reverse phase HPLC

The TAG analysis showed that the principal TAG component was LaLaLa, while the second highest TAG was LaLaM and the third highest was CLaLa. This is in line with the inherent nature of VCO that contains high amounts of lauric acid, thus occupying most of TAG components. It was however noted that samples from fresh-dry method has CLaLa value more than LaLaM, with values of $21.10 \pm 0.01\%$ and $18.89 \pm 0.01\%$, respectively and different from other methods which gave higher LaLaM than CLaLa.

According to Marina *et al.* (2009b), the TAG compositions showed conformity with our current

studies, suggesting that all VCOs were predominantly made of LaLaLa TAG component (22.78 – 25.84%). Nevertheless, the second most predominant TAG in her studies was CLaLa, followed closely with LaLaM, same as our fresh-dry VCO. This difference to the other three methods namely enzymatic, fermentation and chilling and thawing methods could be due to multiple factors, which include the different cultivar, geographical origin, maturity of the nuts used and the different types of extraction of the VCO.

VCO are known as a saturated oils, hence this finding again, confirmed that the presence of unsaturated TAGs was low. Oleic acid was found to be part of LaLaO, LaMO, LaOO and MOO TAGs. The corresponding values for each was 1.72 – 1.88% for LaLaO; 1.00 – 1.24% for LAMO; 0.57 - 0.74% for LaOO and 0.00 – 0.27% for MOO TAG. However, in Marina *et al.* (2009b), POO TAG was found in VCO and ranged from 0.01 – 0.44 % from the samples produced from Malaysia. It did not show up in our samples probably due to the difference in coconut variety and hybrid used (Laureles *et al.*, 2002).

Tocopherol analysis by HPLC

Vitamin E or tocopherol is an antioxidant that works at the primary level and is also a peroxy free radical scavenger. Tocopherol itself is a term that constitutes eight different subtypes namely alpha tocopherol (α -T), beta tocopherol (β -T), gamma tocopherol (γ -T), delta tocopherol (δ -T) and alpha (α -T3), beta (β -T3), gamma (γ -T3) and delta tocotrienols (δ -T3). Tocotrienols differs in terms of the presence of double bonds at the isoprenoid side chain. Tocopherol was shown to prevent oxidative damages at the tissue level *in vivo*, and would be able to prevent degenerative diseases and cancer by guarding against oxidative stress damages (Packer, 1991).

Tocopherol analysis was performed on each extracted VCO and their findings were presented in Table 4 above. The tocopherol from Codex Alimentarius Commission for coconut oil was also included in the table for comparison. There was only slight difference among our VCO samples and the Codex Standard (Codex, 2001). The coconut oils' delta-tocopherol was not detected according to Codex (2001), nevertheless, in our VCO samples, delta-tocopherol was detected at a very low level. Beta-tocopherol for each VCO was all valued at 0.04 mg/kg while gamma-tocopherol ranged from 0.01 – 0.05 mg/kg. Although the presence of beta and gamma-tocopherol were within the standards of Codex, the tocopherol level was still low as compared to other vegetable oils. By nature of VCO that has low

unsaturated FA and high saturated FA, tocopherol would not be oxidized as much compared to oils with high unsaturated FA. In addition to that, VCO were produced naturally without or with mild heating and did not go through refining, bleaching or deodorizing processes, which further preserve the tocopherol and other antioxidants in the VCO (Eitenmiller and Landen, 1999).

Beta-tocopherol was shown to not differ significantly among all the VCO samples, albeit the chilling and thawing method gave the highest result. For gamma-tocopherol, fresh-dry method and chilling and thawing method gave the lowest results with 0.01 ± 0.00 mg/kg. In fresh-dry method, we could hypothesize that the use of heat leads to oxidation of some of the tocopherol. The contribution of delta-tocopherol is the least of the three detected tocopherols with fresh-dry method giving the highest level (1.10×10^{-5} mg/kg). Nevertheless, the effect of different extraction procedures to the VCO did not differ from each other.

Conclusion

There are many methods in which we can produce VCO. The main methods were divided on the basis of whether the meat was dried (dry-method) or the coconut milk was used (wet-method). Although the process themselves are different from each other, the VCO does not vary greatly from each other and conformed to the standards given by the APCC (2003) and Codex (2001). The differences are subtle and do not have high ranges. The highest oil recovery was obtained from the fresh-dry method, nevertheless, the fermentation method resulted in a superior quality VCO if compared from others with low SV, FFA, moisture content and acceptable range of brightness and yellowness. FA and TAG as well as tocopherol analyses only gave minor differences from each method of extractions.

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