

Physico-Chemical and Antioxidant Analysis of Virgin Coconut Oil Using West African Tall Variety

Z. Ahmad^{2,a}, R. Hasham^{*,2}, N. F. Aman Nor^{1,b}, and M. R. Sarmidi^{1,c}

¹Institute of Bioproduct Development, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor Darul Ta'zim, Malaysia.

²Department of Bioprocess and Polymer Engineering, Faculty of Chemical and Energy Engineering, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor Darul Ta'zim, Malaysia.

^aZunairah_ahmad@yahoo.co.uk, ^{*}Rosnani@ibd.utm.my, ^bFarah@ibd.utm.my, ^cmroji@cheme.utm.my

Abstract - Virgin coconut oil (VCO) has been widely used traditionally in Malaysia as moisturiser and food supplement. Recently, VCO has been growing in popularity because of its better chemical and biological properties compared to coconut oil. VCO contains medium chain fatty acids of which 45-50% lauric acid. Lauric acid has been proven to become a strong anti-inflammatory and anti-microbial agent. In the present study, the physico-chemical and antioxidant properties of VCO extracted using integrated wet process (IWP) were investigated. The physico-chemical analyses were done, which comprise fatty acid content, iodine value, free fatty acid (FFA), peroxide value, total phenolic content and antioxidant activity. The properties of fatty acid (FA) in VCO were found containing predominantly lauric acid (C12), which ranges from 50.74% to 51.12%, while the free fatty acid (FFA) content is $0.13\% \pm 0.06\%$. The FFA is an indicator of quality such as off taste and aroma. The iodine value (IV) is $12.28\% \pm 0.66\%$. Meanwhile, the peroxide value (PV) is 0.87 ± 0.02 meq oxygen/kg. The total phenolic content is about 16.02 ± 0.44 mg GAE/ 100 g oil. The antioxidant activity was determined using DPPH radical-scavenging activity and the amount of VCO extract to decrease the DPPH radical concentration by 50% is 5.07 ± 0.19 mg/L. Collectively, all results obtained in this study were found comparable with the Standard APCC except for iodine value, which has a slightly higher value compared to the standard value. **Copyright © 2015 Penerbit Akademia Baru - All rights reserved.**

Keywords: Virgin coconut oil, fatty acid content, antioxidants, lauric acid, integrated wet process.

1.0 INTRODUCTION

Virgin coconut oil (VCO) is produced from the fresh coconut milk. The nutrients available in coconut milk are vitamin, antioxidant, amino acids and essential fatty acid. The coconut flesh has the most valuable component, which is oil and high total lipid content including tocotrienols. The coconut oil is highly saturated oil with medium-chain fatty acids including capric (7%), lauric (49%), myristic (18%), palmitic (9%), stearic (2%) and small percentages of unsaturated oil such as oleic (6%) and linoleic acids (2%) [1]. The virgin coconut oil is colourless and clear with the aroma of fresh coconut.

Coconut, *Cocos nucifera L* of the family *Arecaceae* (Palmae) is the source of VCO. VCO can be defined as “oil obtained from the fresh, mature kernel of coconut by mechanical or natural means, with or without the use of heat, without undergoing chemical refining, bleaching or deodorising, which does not leads to the alteration of the nature of oil” [2].

There are varieties of coconut that were considered as the source of virgin coconut oil. The popular parental for hybridisation of coconut are the West African Tall (WAT), Malayan Yellow dwarf (MYD), Rennell Island Tall (RIT), Malayan Red Dwarf (MRD), Vanuatu Tall (VVT), Malayan Green Dwarf (MGD) and the Cameroon Red Dwarf (CRD). The study done by Nor Farahiyah (2015) [6] stated that the coconut variety which produces the highest oil yield is the West African Tall (WAT) among other varieties such as Rennel Tall, Matag, MAWA, Maren and Tagnanan Tall. Various researches have revealed that the factors such as location, age of nut, time of nut harvested and the age of copra give effect on the production of oil yield [3].

The method of processing VCO can be divided into dry and wet method. In the wet method, the oil is extracted from fresh coconut meat without drying. The “coconut milk” is obtained by pressing the grated wet coconut meat. Meanwhile, the wet method includes several techniques of chilling and thawing, fermentation, enzymatic or any combination of these methods to destabilise the coconut milk emulsion [4, 5].

Integrated wet process (IWP) is the latest technique for the production of VCO that was introduced by the Institute of Bioproduct Development (IBD) [6]. In this method, the coconut milk obtained from the grated coconut meat is cooled to the desired temperature ($T= 10\text{ }^{\circ}\text{C}$). Then, it is churned to separate the water and butter oil. The butter is then melted in a water bath at $37\text{ }^{\circ}\text{C}$ and is centrifuged after that before being filtered to get the VCO [6].

VCO contains medium chain fatty acids of about 63%, which does not increase the cholesterol level in the blood. Medium chain fatty acids (MCFA) are transported directly to the liver to immediately provide energy and not deposited as fat [1]. The MCFA has been used in the clinical area of enteral and parenteral nutrition in diverse medical conditions for the treatment of patients that suffer from fat malabsorption [7]. Coconut oil is useful for patients with fat digestion problem including premature infants [8, 9]. VCO has been used traditionally for treating the skin and hair growth since centuries ago. VCO contains high saturated fatty acids, which are mostly lauric acid that has a high resistance against oxidation and inhibits rancidity due to its stability and functionality [10]. The quality characteristics of VCO can be identified through its physical and chemical properties such as iodine value, free fatty acid and peroxide value.

VCO contains lauric acid (49%), which has an antimicrobial property on certain bacteria such as *Listeria monocytogenes* and *Propionibacterium acnes* (*P. acnes*) [11, 12]. Lauric acid can be potentially used as an alternative treatment for antibiotic therapy of acne vulgaris. Lauric acid has effectively reduced the inflammation and swelling in the mouse ear model caused by *P. acnes* [11]. Monolaurin, a monoglyceride form of lauric acid also resembles human breast milk, which is known to benefit and confer immunity to babies [5]. Infant formulas derived from cow's milk are being fortified with coconut oil that contains lauric acid to protect the baby from infection [8, 9]. VCO has many benefits including high phenolic compound and better antioxidant activity compared to coconut oil [2]. The phenolic compound in VCO exhibits its antioxidant activities such as “antimutagenic”, “antiproliferative” and “anticarcinogenic” that benefits the human being [2]. In the previous study carried out by Nevin and Rajamohan (2006) [13], the effect of VCO on the antioxidant enzymes activities and lipid

peroxidation was carried out on a rat model with the results showed that VCO are effective in preventing the peroxidation of lipid and increase the level of antioxidant enzymes. A research has been conducted on the healing properties of VCO, which demonstrated that wounds treated with VCO healed much faster as indicated by a decreased time of complete epithelisation and higher level of various skin components [14].

In the current research, the physio-chemical properties of VCO such as fatty acid content, free fatty acid, iodine value, peroxide value, total phenolic content and antioxidant activity were analysed to meet the quality of standard VCO according to Standard APCC. The extraction of VCO was carried out using the integrated wet process because of the high oil quality obtained in the previous study conducted by Nor Farahiyah (2015) [6]. The integrated wet process is an efficient and quick technique to produce VCO with the highest heat stability [4].

2.0 METHODOLOGY

2.1 Material and chemicals

Coconut fruits known as *Cocos nucifera* were selected from the species of West African Tall (WAT). The harvesting cycle of the coconut from one specific location (Department of Agriculture, Batu Pahat, Johor) was recorded to be 120 days. The fresh coconut milk was processed by the local coconut suppliers in Gelang Patah, Johor. The list of solvents used in the analysis of physico-chemical properties of VCO are ethanol 99.8%, methanol, n-hexane (Qrec (Asia) Sdn. Bhd, Selangor, Malaysia), chloroform, acetic acid glacial, Wijs solution and hydrochloric acid (RCI Labscan, Bangkok, Thailand) and cyclohexane (Brightchem Sdn. Bhd., Penang, Malaysia). The reagent used in the analysis of phenolic content and antioxidant is Folin & Ciocalteu's phenol (Sigma-Aldrich Chemie GmbH, Steinheim). Gas chromatography, (GC-FID) (Perkin Elmer Clarus 500) was used as the equipment in the analysis of fatty acid composition performed by Allied Chemists Laboratory Sdn. Bhd. (ACL) located in Johor, Malaysia.

2.2 Extraction of VCO method

The extraction of VCO using integrated wet process (IWP) was performed according to the method proposed by Nur Arbainah (2012) [4] with slight modification. The extraction was repeated for three batches for the collection of VCO samples. The solid endosperm of mature coconut was de-husked and grated, which was further made into viscous slurry. The slurry was squeezed through cheesecloth to obtain coconut milk and refrigerated for 24 hours. Later, the coconut milk was stirred using mixer until coconut butter was formed. The water was separated from the coconut butter after that. Then, the coconut butter was soaked in the water bath (Mettler, USA) at 37 °C. This step was followed by the centrifugation of solution to separate the oil from aqueous layer. The obtained VCO was filtered through cheesecloth and kept in the storage at room temperature prior to be used in the next experiment.

2.3 Determination of VCO physico-chemical properties

2.3.1 Fatty acid

Fatty acid methyl esters (FAME) was separated by gas chromatography using a system of GC-FID (Perkin Elmer Clarus 500) equipped with an automatic on-column injector, a polar capillary column and a flame ionisation detector. Helium was used as the carrier gas at a flow rate of 5.4 mL/min. Retention time of fatty acid methyl esters was identified by comparing it with those of individually purified standard [13]. According to AOCS Method Ce 1-62 (1998),

approximately 50 mg of extracted oils was mixed in 0.95 ml n-hexane in a 2 ml vial. The vial was shaken to dissolve the oil. Then, 0.05 ml sodium methoxide was added to the solution using a micropipette. The solution was mixed thoroughly for 5 second with a vortex mixer. The mixture was separated into two layers; the upper layer is clear and the bottom layer is the sodium glyceroxide, which was precipitated. The clear upper layer of methyl ester was analysed by gas chromatography. The weight fractions of the FAMES were measured based on the percentage represented by the area of corresponding peak relative to sum up the area of all peaks.

2.3.2 Iodin value

The analysis of iodine value was conducted using AOCS Official Method No. Cd 1d-92 (Wijs Method) (AOCS, 2004). In the first steps, VCO that weigh approximately 3.0 g was filled into a 250 mL conical flask. Then, 20 mL cyclohexane was added into VCO to dissolve the fat. 25 mL Wijs solution (RCI Labscan, Thailand) was added into the VCO solution. The flask was completely closed by parafilm and the solution was continuously shaken for about 30 minutes. After that, 20 mL of 15% potassium iodide solution (KI) (Rhone-Poulenc Chemicals Pty. Ltd.) and 100 mL of DI water were added into the mixture. The mixture of VCO solution was titrated with 0.1 N Sodium thiosulfate solution ($\text{Na}_2\text{S}_2\text{O}_3$) (Merck KGaA, Darmstadt, Germany) until the yellow colour form has almost disappeared. Next, 2-3 drops of starch solution (Fisher Scientific, UK) were added (blue colour solution will appear) and titration was continued until the blue colour has disappeared. Volume of $\text{Na}_2\text{S}_2\text{O}_3$ is represented as S . The titration step was repeated with blank sample and the volume of $\text{Na}_2\text{S}_2\text{O}_3$ is represented as B . The IV was calculated using Equation 1 as per below:

$$\text{Iodin Value} = \frac{(B - S) \times N \text{ of } \text{Na}_2\text{S}_2\text{O}_3 \times 12.69}{\text{Weight of sample (g)}} \quad (1)$$

Where,

$B = V$ mL of $\text{Na}_2\text{S}_2\text{O}_3$ volume for blank

$S = V$ mL of $\text{Na}_2\text{S}_2\text{O}_3$ volume for sample

$N =$ Normality of $\text{Na}_2\text{S}_2\text{O}_3$

2.3.3 Free fatty acid (FFA)

Free fatty acid content was measured according to the Official Method 940.28, (AOAC, 2000). First, 7.05 g of VCO was weighed and filled in the 250 mL flask. Then, 2 ml of phenolphthalein solution and a few drop of 0.1 M Natrium Hydroxide (NaOH) (Qrec (Asia) Sdn. Bhd., Malaysia) were added into the oil. The mixture was well-mixed to produce faint pink colour. Next, 50 mL of ethanol (Qrec (Asia) Sdn. Bhd., Malaysia) was added into the solution. Then, the titration was performed with 0.25 M and vigorously shaken until permanent faint pink has appeared (persist in one minute). A volume of 0.25 M NaOH was recorded and remarked as S . The steps were repeated using a blank without the VCO. Finally, the volume of 0.25 M NaOH for blank was recorded and remarked as B . The percentage of FFA (% FFA) was calculated using Eqn. 2 as per below.

$$\% \text{ FFA} = \text{Acid value (AV)} / 1.99 \quad (2)$$

$$\text{AV} = \frac{(B-S) \text{ ml of NaOH} \times N \times 56}{\text{Weight of sample}} \quad (3)$$

Where,

B = volume of NaOH required by blank

S = volume of NaOH required by sample

N = Normality of NaOH

2.3.4 Peroxide value

The peroxide value was determined using the standard method produced by the Association of Official Analytical Chemist (AOAC, 1998). A sample of 5 g coconut oil was added with 30 mL of acetic acid-chloroform (3:2) (RCI Labscan, Thailand) and the solution was stirred until the oil has been completely dissolved. Then, 0.5 ml of saturated potassium iodine (KI) was added and stirred for about one minute. The solution was titrated with 0.01 N Na₂SO₃ until its colour changed to light yellow. The step of titrating can be skipped by adding 0.5 mL of 1% soluble starch as an indicator that gives a light blue colour, followed by titration with 0.01N Na₂SO₃ until the colour disappear. The volume of titration was recorded and peroxide value (PV) was calculated using Equation 4 as follows:

$$PV = \frac{N \times V}{W} \quad (4)$$

Where, PV unit is in mili-equivalents (meq) of peroxide O₂ per kg of oil.

V is the titre volume of Na₂SO₃ solution (0.01 N), W is the weight of coconut oil (kg) and N is the normality of Na₂SO₃ solution (0.01N).

2.3.5 Total phenolic content

Polyphenol (PF) from the test oils was extracted according to the method described by Gutfinger (1981) [15]. 10 g oil was dissolved in 50 ml hexane and successively extracted three times with 20 ml portions of 60% methanol (Qrec (Asia) Sdn. Bhd., Malaysia). Triplicate of extracts and solvent was evaporated to dry (60 °C) using a rotary evaporator (Laborota 4003) (Heidolph, Germany). The final residue obtained after evaporation was mixed in a known volume of 60% methanol. The total polyphenol content of this solution was estimated using Folin–Ciocalteu reagent [15]. An aliquot of test samples (1 mg/ mL) was mixed with 1.0 mL of Folin-Ciocalteu reagent (Sigma-Aldrich Chemie GmbH, Steinheim) (previously, it has been diluted with 10-fold of DI water). A 7.5% sodium carbonate solution (Sigma-Aldrich Chemie GmbH, Steinheim) (0.8 mL) was added and allowed to stand at room temperature for 30 minutes. The absorbance of phenolic content in methanol was read using spectrophotometer (Shimadzu UV–1800, Japan) at 765 nm. The total phenolic content was expressed as gallic acid equivalents per 100 g oil [13].

2.3.6 Antioxidant activity

DPPH radical-scavenging activity of the phenolic extracts of VCO was measured according to the method reported by Hatano *et al.* (1988) [16]. Phenolic substances were extracted by the method explained under the determination of total phenol content from VCO samples in section 2.3.5 above. Total phenolic content of the phenolic extracts measured using Folin–Ciocalteu reagent method was adjusted to the required concentrations by suitable dilutions with the same solvent system used for the extraction of phenolic substances. Each phenolic extract (300 µl) of various concentrations (25, 50, 75 and 100 mg/l) from VCO was added to a methanolic

solution of DPPH (0.3 ml, 0.8 mM) (Sigma-Aldrich Chemie GmbH, Steinheim) and the resultant mixture was vortex at 40 Hz for five minutes. After 30 minutes of incubation at room temperature in the dark, the absorbance of each reaction mixture was measured at 517 nm using spectrophotometer (Shimadzu, UV-1800, Japan). The DPPH radical scavenging activity was similarly measured for a series of 25 – 100 mg/l solutions of butylated hydroxytoluene (BHT) (Merck KGaA, Darmstadt, Germany). The inhibitory effect of DPPH radical was calculated according to the following formula:

$$\text{Inhibition (\%)} = [(A_0 - A_1 / A_0)] \times 100 \quad (5)$$

Where A_0 is the absorbance of reaction mixture with solvent system (control) and A_1 is the absorbance of reaction mixture with phenolic extract or BHT [17].

2.4 Statistical analysis

All results are presented as the average \pm standard error of the mean (SEM) of the combined data from replicate experiments. A significance of $P < 0.05$ was used to test the significant difference of all results. The statistical tests were conducted using SPSS software (IBM Corporation, United State).

3.0 RESULTS AND DISCUSSION

Table 1 shows the physical and chemical properties of VCO analysis result from three batches of integrated wet extraction process. Furthermore, the standard for VCO was also provided in the table using Asia Pacific Coconut Community (APCC) (2009) standard as comparison. All parameters are in the range of Standard APCC except for iodine value (IV), which has a slightly higher value compared to the standard value.

3.1 Iodin Value

Iodin value (IV) can be defined as the percentage by weight of which an oil or fatty acid will absorb halogens such as iodine under the test conditions [8]. The mean of IV is $12.28 \pm 0.66\%$. The iodine number could determine the amount of iodine compounds present in the oil. More unsaturated fatty acid bonds are available in oil and fat when higher iodine number is detected [20].

3.2 Free fatty acid

The free fatty acid (FFA) obtained from this study is about $0.13\% \pm 0.06\%$ which is still within the range of the APCC standard and is acceptable to be used for further experiment (see Table 1). The FFA is an indicator of oil quality such as off taste and aroma [5]. FFA is the most crucial characteristic in the VCO production and product sales [18].

3.3 Peroxide value

The peroxide value (PV) presented in Table 1 has been determined to be about 0.87 ± 0.02 meq oxygen/kg, which is below the maximum value of APCC standard. The PV could express the oxidation or rancidity level of VCO. Peroxide value gives an indication of the primary oxidation state of oil [18].

Table 1: The physical and chemical properties of VCO using West African Tall (WAT) variety.

Parameters	Integrated wet process	APCC standard
Iodin Value (%)	12.28 ± 0.66	4 - 11
Peroxide Value (meq oxygen/kg)	0.87 ± 0.02	≤ 3.0
Free fatty acid (%)	0.13 ± 0.06	≤ 0.5
Antioxidant properties:		
a) Polyphenol (mg GAE/100 g oil)	16.02 ± 0.44	
b) Antioxidant free radical scavenging activity, EC ₅₀ (mg/L)	5.07 ± 0.19	

3.4 Total phenolic content

The total phenolic content (TPC) has been detected to be about 16.02 ± 0.44 mg GAE/100 g oil in the VCO extracted using IWP. TPC in oil was effected by the processing method for example fermentation method, chilling and thawing technique and RBD (refined, bleached, deodorised) process [21]. The TPC obtained in this study is higher compared to the value obtained in the previous study by Nur Arbainah (2012) [4], which is about 4.34 ± 0.09 mg GAE/g oil using the same method of extraction. It is proven that the West African Tall (WAT) variety contributed to the higher amount of TPC in the VCO.

3.5 Antioxidant activity

VCO was able to reduce the initial DPPH radical concentration by 50% and EC₅₀ of about 5.07 ± 0.19 mg/L antioxidant capacity. The VCO contains hydrogen-donating capabilities and acts as an antioxidant [21]. The processing condition of VCO could contribute to the level of free-radical scavenging activity [21]. Previous study carried out by Marina *et al.* (2009) [21] has reported the free radical scavenging activity (EC₅₀) of VCO using fermentation method (1.24 ± 0.04 mg/ml), chilling and thawing technique (1.66 ± 0.01 mg/ml) and RBD process (3.23 ± 0.14 mg/ml). The WAT variety has been revealed to contain 0.48 ± 0.01 mg GAE/ ml, which is the highest antioxidant activity in VCO compared to other coconut varieties [23].

3.6 Fatty acid content

The fatty acid (FA) composition in VCO predominantly contains lauric acid (C12) ranging from 50.74% to 51.12%. The total lauric acid is in agreement with the APCC (2009) standard for VCO and Codex (2001) in coconut oil (45.10 – 53.20%). The lauric acid composition was effectively maximised using the integrated wet process (IWP) [19]. The fatty acid analysis is essential in providing the information regarding fatty acid distribution of fats and oil products [18]. The variations of different coconut nuts that can occur within the same coconut variety could influence the FA composition during extraction [5]. The medium chain fatty acid (C6 – C12) was ranged from 62.6% to 63.7% with long chain FA (C14 - C18) of about 29.05% ± 1.34%, and unsaturated FA (C18:1 and C18:2) of about 6.33% ± 0.12%. The linolenic acid (C18:3) cannot be detected in the sample of VCO as shown in Table 2 below.

Table 2: Fatty acid composition in VCO using West African Tall (WAT) variety.

Parameters	Fatty acid composition (%)	APCC standard (%)
Caproic acid, C6:0	0.40 ± 0.0	0.40 – 0.60
Caprylic acid, C8:0	6.05 ± 0.12	5.00 – 10.0
Capric acid, C10:0	5.77 ± 0.24	4.50 – 8.00
Lauric acid, C12:0	50.93 ± 0.19	43.0 – 53.0
Myristic acid, C14:0	19.40 ± 0.85	16.0 – 21.0
Palmitic acid, C16:0	8.17 ± 0.42	7.50 – 10.0
Stearic acid, C18:0	2.82 ± 0.07	2.00 – 4.00
Oleic acid, C18:1	5.23 ± 0.05	5.00 – 10.0
Linoleic acid, C18:2	1.10 ± 0.0	1.00 – 2.50
Linolenic acid, C18:3	Not detected	<0.5

4.0 CONCLUSION

In the present study, the physico-chemical properties (fatty acid content, free fatty acid, iodine value and peroxide value) of VCO have been shown to be comparable with the APCC standard. The total phenolic content and antioxidant capacity was available in a certain amount depending on the processing method of VCO using a specific variety of coconut such as West African Tall variety. The result obtained from this study could be used as a guideline for further study using a different variety of coconut in the processing of VCO.

REFERENCES

- [1] M. DebMandal, S. Mandal, Coconut (*Cocos nucifera* L.: Arecaceae): in health promotion and disease prevention. *Asian Pacific Journal of Tropical Medicine* 4 (3) (2011) 241–247.
- [2] A.M. Marina, Y.B. Che Man, I. Amin, Virgin coconut oil: emerging functional food oil. *Trends in Food Science & Technology* 20 (10) (2009) 481-487.
- [3] E.V. Carandang, Health Benefits of Virgin Coconut Oil Explained. *PJCS* 31 (2) (2008) 1-12.
- [4] S.A. Nur Arbainah, Lipid and Phytochemicals Profiles of Non-Heated Treated Virgin Coconut Oil, Master Dissertation, Universiti Teknologi Malaysia, Skudai, Johor, 2012.
- [5] T.S.T. Mansor, Y.B. Che Man, M. Shuhaimi, M.J. Abdul Afiq, F.K.M. Nurul, Physicochemical properties of virgin coconut oil extracted from different processing methods. *International Food Research Journal* 19 (3) (2012) 837–845.
- [6] A.N. Nor Farahiyah, Influence of churning and thawing temperatures on yield and quality of virgin coconut oil, Master Dissertation, Universiti Teknologi Malaysia, Skudai, Johor, 2015.

- [7] Y.B. Che Man, Suhardiyono, A.B. Asbi, M.N. Azudin, L.S. Wei, Aqueous enzymatic extraction of coconut oil. *Journal of the American Oil Chemists' Society* 73 (1996) 683-686.
- [8] S. Nandi, S. Gangopadhyay, S. Ghosh, Production of medium chain glycerides from coconut and palm kernel fatty acid distillates by lipase- catalyzed reaction. *Enzyme and Microbial Technology* 36 (2005) 725–728.
- [9] B. Marten, M. Pfeuffer, J. Schrezenmeir, Medium-chain triglyceride (review). *International Dairy Journal* 16 (2006) 1374–1382.
- [10] R.D. O'Brien, *Fats and Oils*, third ed., CRC Press, Taylor & Francis Group, 2009, pp. 2-52.
- [11] T. Nakatsuji, Kao, C. Mandy, J.Y. Fang, C.C. Zouboulis, Gallo, L. Richard, C.M. Huang, Acnes: Its Therapeutic Potential for Inflammatory Acne Vulgaris. *Journal Invest Dermatol* 129 (10) (2009) 2480-2488.
- [12] L.L. Wang, E.A. Johnson, Inhibition of *Listeria monocytogenes* by fatty acids and monoglycerides. *Applied and Environmental Microbiology* 58 (1992) 624-629.
- [13] K.G. Nevin, T. Rajamohan, Virgin coconut oil supplemented diet increases the antioxidant status in rats. *Food Chemistry* 99 (2) (2006) 260-266.
- [14] K.G. Nevin, T. Rajamohan, Effect of topical application of virgin coconut oil on skin components and antioxidant status during dermal wound healing in young rats. *Skin Pharmacology and Physiology* 23 (6) (2010) 290-297.
- [15] T. Gutfinger, Polyphenols in olive oil. *Journal of American Oil Chemists Society* 1 (1981) 966–968.
- [16] T. Hatano, H. Kagawa, T. Yasuhara, T. Tasuhara, T. Okuda, Two new flavonoids and other constituents in licorice root: Their relative astringency and radical-scavenging effects. *Chemical and Pharmaceutical Bulletin* 36 (1988) 2090–2097.
- [17] K.N. Seneviratne, M.S. Dissanayake, Variation of phenolic content in coconut oil extracted by two conventional methods. *International Journal of Food Science and Technology* 43 (2008) 597-602.
- [18] L. Kamariah, A. Azmi, A. Rosmawati, M.G.W. Ching, M.D. Azlina, C.P. Tan, O.M. Lai, Physico-chemical and quality characteristics of virgin coconut oil – A Malaysian survey. *Journal Tropical Agricultural and Fundamental Science* 36 (2) (2008) 1-10.
- [19] M.A. Hamid, M.R. Sarmidi, T.H. Mokhtar, W.R.W. Sulaiman, R.A. Azila, Innovative integrated wet-process for virgin coconut oil production. *Journal of Applied Sciences* 11 (13) (2011) 2467–2469.
- [20] N.A. Fakhri, H.K. Qadir, Studies on Various Physico-Chemical Characteristics of Some Vegetable Oils. *Journal of Environmental Science and Engineering* 5 (2011) 844–849.

- [21] A.M. Marina, Y.B. Che Man, S.A.H. Nazimah, I. Amin, Antioxidant capacity and phenolic acids of virgin coconut oil. *International Journal of Food Sciences and Nutrition* 60 (suppl. 2) (2009) 114-123.
- [22] APCC (Asian Pacific Coconut Community), Standards for Virgin Coconut Oil. Retrieved on February 25, 2014 from <http://www.apccsec.org/standards.htm>, 2009.
- [23] R. Arlee, S. Suanphairoch, P. Pakdeechanuan, Differences in chemical components and antioxidant-related substances in virgin coconut oil from coconut hybrids and their parents. *International Food Research Journal* 20 (5) (2013) 2103–2109.