academicJournals

Vol. 11(3) pp. 58-66, March 2017
DOI: 10.5897/AJFS2016.1493
Article Number: C9ABA7762892
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

Full Length Research Paper

Effect of extraction techniques on the quality of coconut oil

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Received 26 July, 2016; Accepted 7 December, 2016

Coconut oil (*Cocos nucifera* L.) has a unique role in the diet as an important physiologically functional food. The health and nutritional benefits that can be derived from consuming coconut oil have been recognized in many parts of the world for centuries. The aim of this study was to compare the quality parameters of coconut oil under different common extraction techniques. Six different techniques of coconut oil extraction were employed to produce virgin coconut oil (VCOs) and refined coconut oil (RCO). VCOs were produced using enzymatic, chilling and thawing, centrifugation, natural-fermentation and induced-fermentation processes. The highest oil yield (83%) was from RCO and also RCO had a significantly higher peroxide value (1.06 meq/kg oil) than VCO samples. Antioxidant activity of RCO was significantly ($p<0.5$) lower than those of VCO samples, with induced-fermentation having the highest antioxidant activity of 28.3%. Interestingly, enzymatic extraction resulted in higher quantity of short-chain triglycerides. Although, there was no method which could result significantly in high quantity of all the tested parameters, induced-fermentation showed relatively high oil yield and antioxidant activity.

Key words: Antioxidants, coconut oil, extraction, fatty acids, quality.

INTRODUCTION

Coconut oil is a vegetable oil extracted from coconut palm (*Cocos nucifera* L.). Coconut is the most extensively grown and used palm in the world with approximately 12 million hectare in cultivation (FAO, 2014) serving as a major source of income and food for about 10 million families from over 80 countries (Perera et al., 2010). In coconut oil producing countries, extraction process is still crude and usually involves the use of locally sourced equipment that gives oil with poor quality (Bawalan, 2011).

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Virgin coconut oil (VCO) is defined as the oil resulting from the fresh and mature kernel of the coconut 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, 2009).. There are no specific processing prerequisites that are established for coconut oil production (Marina et al., 2009a). However, several methods to produce VCO are found to measure up with the definition of the VCO (Marina et al., 2009a; Bawalan and Chapman, 2006; 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 through drying process, while in dry method, the kernel is 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 is dried using controlled heating and subsequently pressed mechanically to obtain the oil (APCC, 2009). The method of extraction influences the quality and grade of the oil (Amri, 2011). Moisture content, free fatty acid, peroxide value and antioxidant content are common oil quality parameters. While saponification value and fatty acid profile are identification parameters. These parameters can be used to compare oil to determine how extraction conditions impacts on quality.

VCO retains its naturally occurring phytochemicals which produce a distinctive coconut taste and smell. The oil is pure white when the oil is solidified, or crystal clear like water when liquefied. The oil contains high lauric acid (C-12) as medium chain fatty acid (MCFA). MCFAs are burned up immediately after consumption and therefore the body uses it immediately to make energy rather than store it as body fat (Enig, 1996). Coconut oil contains about 90% saturated fats, with 60% being medium chain triglycerides (MCFAs) (Nagao and Yanagita, 2010). A few clinical trials and animal studies using a formulation of MCFAs reported significant health benefits such as the reduction of body weight, inflammatory disease, metabolic syndrome and serum cholesterol concentration (Han et al., 2007). MCFAs are broken down once consumed almost immediately by enzymes in the saliva and gastric juices, without the need for pancreatic fat-digesting enzymes due to its low molecular weight (Marten et al., 2006). Apart from the MCFAs, the antioxidant profile of coconut oil also assists in the earlier mentioned health benefits of coconut oil (Marina et al., 2009c).

Despite this link between coconut oil and health benefits, the impact of the different common coconut oil extraction method on quality parameters is yet to be fully examined collectively. There is therefore, need to evaluate the common methods used in the extraction of coconut oil to determine which of the method optimizes the MCFAs and antioxidant activity of coconut oil. The current study aims to address this knowledge gap by investigating the effect of extraction techniques of coconut oil with respect to its quality parameters.

MATERIALS AND METHODS

Sample collection and preparation

Dehusked coconut fruits with the shell from one batch were purchased from the local Coles Supermarket in Bentley, Western Australia. Coconuts were de-shelled, cleaned and shredded using a Robot Coupe Blixer 4 V.V at a speed set at 3 for 5 min. All chemicals were of analytical and HPLC grade and were purchased from Sigma Aldrich, Australia.

Coconut oil extraction

Six different techniques were used to extract coconut oil. Induced fermentation (IF), natural fermentation (NF), enzyme (EV), centrifugation (CE), chilling and thawing (CH) produced VCO and other RCO. All extractions were conducted in duplicate.

Induced fermented VCO (IF VCO)

Shredded coconut meat (500 g) was mixed with water at 70°C at a ratio of 1:1. The mixture was kneaded by hand for 5 min and strained through a cheese cloth to obtain coconut milk. The coconut milk was allowed to settle for 6 h. The resulted upper layer of coconut cream was collected by decanting and inoculated with (*Lactobacillus* (*L.*) *plantarum* ATCC 14917 (5% w/w)) previously activated in MRS medium. The inoculated cream was allowed to ferment at 40°C for 10 h. After the fermentation, mixture was centrifuged at 4000 rpm (3220 ×g) using Eppendorf centrifuge 5810-R (Hamburg, Germany) for 30 min at room temperature (20°C±2) to obtain the coconut oil. Coconut oil was heated to 50°C using Contherm thermotec 2200 (Lower Hutt, New Zealand) to remove aromatic compounds, weighed, flushed with nitrogen and stored in dark brown bottles at 5°C prior to analysis.

Natural fermented VCO (NF VCO)

Shredded coconut meat (500 g) and water at 70°C at a ratio of 1:2 was kneaded by hand for 5 min. Mixture was strained through a cheese cloth to obtain coconut milk. The coconut milk was left to ferment naturally for 16 h at 40°C. Oil was separated from fermented curd by centrifuging at 4000 rpm (3220 ×g) using an Eppendorf centrifuge 5810-R (Hamburg, Germany) for 30 min at room temperature. The separated coconut oil was heated at 50°C using Contherm thermotec 2200 (Lower Hutt, New Zealand) to remove aromatic compounds. It was then weighed, flushed with nitrogen and stored in dark brown bottles at 5°C.

Enzymatically extracted VCO (EV VCO)

Shredded coconut meat (500 g) was mixed with water (1:4) and the temperature of mixture was brought to 40°C using a water bath (Grant OLS200, Cambridge, UK) (Che Man et al., 1996; Mansor et al., 2012; McGlone et al., 1986). Amylases (1%) from *Aspergillus oryzae*, pectinase (1%) from *Aspergillus niger* and proteases (1%)

from *Streptomyces griseus* purchased from Sigma-Aldrich (Australia) were added to the coconut mixture and temperature was maintained at 40°C and agitated for 3 h using a shaking water bath. After 3 h, the solution was centrifuged at 4000 rpm (3220 ×g) using Eppendorf centrifuge 5810-R (Hamburg, Germany) for 30 min at room temperature to obtain upper coconut oil layer. Coconut oil was weighed, flushed with nitrogen and stored in dark brown bottles at 5°C.

Centrifugation (CE VCO)

Coconut meat (500 g) which was grated was mixed with water (1:1) to extract the coconut milk. Centrifugation (Eppendorf centrifuge model 5810-R, Hamburg, Germany) was done twice (4000 rpm) to destabilise the oil-water emulsion for 30 min at room temperature. Initial centrifugation was to obtain the cream and the second centrifugation separated the cream into three layers (oil, cream and aqueous). The top oil layer was decanted, weighed, flushed with nitrogen and stored in dark brown bottles at 5°C prior to analysis.

Chilling and thawing (CH VCO)

Grated coconut meat (500 g) was mixed with water (1:1), hand kneaded for 5 min and filtered to extract coconut milk. Coconut milk was centrifuged at 3220 ×g for 10 min and the upper layer of cream was removed for chilling. Chilling was done at 0°C for 6 h and then the chilled cream was thawed slowly at room temperature to extract the oil (Raghavendra and Raghavarao, 2011). Centrifugation (Eppendorf centrifuge model 5810-R Hamburg, Germany) was applied (4000 rpm) for 30 min at room temperature to obtain coconut cream. Coconut cream was further centrifuged at 4000 rpm for another 30 min to produce CT VCO. Oil was weighed, flushed with nitrogen and stored in dark at 5°C prior to analysis.

Refined coconut oil (RCO)

Coconut meat (shredded, 500 g) was oven dried using Contherm thermotec 2200 (Lower Hutt, New Zealand) at 75°C (to a moisture content of 7%) Amri (2011). Oil was extracted from the dried coconut by solvent extraction using n-hexane in a Soxhlet apparatus (Buchi E-816, Flawil, Switzerland). Thermal cycle was done at 80°C for 8 h. Solvent was recovered using a rotary evaporator at 40°C under vacuum (Ixtaina et al., 2011). Solvent extracted oil was refined according to Canapi et al. (2005). The coconut oil was preheated to 80°C and 85% phosphoric acid (0.1% w/w) was added and temperature maintained at 85°C for 20 min. One percent of bleaching earth/activated carbon (10:1) was added to the oil and temperature was further adjusted to 95°C under vacuum for another 20 min. The bleaching earth was removed by filtration with aid of vacuum using Whatman No. 1. Oil was deodorized by heating under pressure and high temperature (240°C) for 1.5 h (Lindberg/Blue M™ Vacuum Oven). Oil was weighed, flushed with nitrogen and stored in dark at 5°C prior to analysis.

Determination of physiochemical parameters

Analysis for each physiochemical parameter was carried out in triplicates except otherwise stated.

Oil yield

Oil yield (%) was calculated compared to total oil content. Total oil content was determined according to the procedure Am 5-04 (AOCS, 2009). All analysis was carried out in duplicates.

$$\text{Oil Yeild (\%)} = \text{Weight of oil extracted (g)} \div \text{Total oil content (g)} \times 100$$

Moisture and volatile content

Determination of moisture and volatile content was performed using AOCS Method Ca 2b-38 (AOCS, 2009). Samples (20 g) were heated at a temperature of 110±5°C in a pre-dried beaker until cessation of rising bubbles of steam and incipient smoking. Heated samples were cooled to room temperature in a desiccator and re-weighed. The moisture and volatile content was calculated by difference.

$$\textit{Moisture and volatile (\%)} = \textit{Initial weight} - \textit{Final weight} \div \textit{Initial weight} \times 100$$

Free fatty acid (FFA)

Free fatty acid value was determined using the AOCS Official Methods Ca 5a-40 (AOCS, 2009). All measurements were expressed as the percentage of free fatty acid (as lauric).

$$FFA(Lauric\ \%) = mL\ of\ alkali \times Molarity \times 20\ \div Mass\ of\ test\ portion$$

Fatty acid composition (FAC)

Preparation of fatty acid methyl esters (FAMEs) were carried out using 12% BCl_3-Methanol according to the Sigma Aldrich method (Sigma-Aldrich, 1997) with slight modification to allow for sufficient volatility of FAMEs by the GC-FID. Toluene (5 ml) was added to 0.1 g oil to dissolve. BCl_3-methanol (10 ml) was added to the mixture and flushed with nitrogen gas. Mixture was left at 60°C for 10 min in a water bath. FAMEs were extracted twice using 20 ml of hexane. The FAMEs mixture was washed five times with water to remove any trace of BCl_3 and dried with anhydrous sodium sulphate. FAMES were filtered into 100 ml flask and made to mark with hexane.

The fatty acids from the FAMEs were analyzed according to Coorey et al. (2012). One microliter of FAMEs was injected via an auto sampler into a gas chromatography (Perkin Elmer model, Autosystem XL, USA) coupled with flame ionization detector running at 250°C and SGE forte BPX 70 capillary column (30 m × 0.32 mm × 0.25 µm) using helium as the carrier gas set at 20 ml/min with injector running at 200°C. The oven temperature was set at 80°C for 2 min and increased to 130°C (45°C/1 min) and left for 10 min. It was further increased to 172°C (2°C/1 min) for the final 6 min. Peak identification were compared with the standard FAMEs obtained from Sigma Chemicals, Australia.

$$\textit{Fatty acids (\%)} = (\textit{FA concentration} \times \textit{dilution factor} \times \textit{extraction volume}) \times 100 \div \textit{Sample weight}$$

Saponification value

Saponification value (SV) was ascertained using AOCS Official Methods Cd 3-25 (AOCS, 2009). Two grams of filtered oil was mixed with 0.5 N ethanolic potassium hydroxide and boiled under reflux for 60 min. The mixture was left to cool slightly at room temperature and subsequently titrated with 0.5 N hydrochloric acid until the colour changed from pink to colourless. A blank sample was also carried using same method without oil.

$$SV = (\textit{Volume of blank titrant} - \textit{Volume of sample titrant}) \times M(HCl) \times 56.1 \div \textit{Weight of oil}$$

Table 1. Comparison of major differences of each extraction method.

Techniques	(Coconut Meat:Water)	Inoculum/Enzyme	Temperature/Duration
IF	1:1	*L. plantarum*	40°C/10 h
NF	1:2	Nil	40°C/16 h
Enzymatic	1:4	Amylase, pectinase, protease	40°C/3 h
Centrifugation	1:1	Nil	Nil
CH	1:1	Nil	5°C/6 h
RCO	Nil	Hexane	80°C/8 h

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 thiosulphate (0.01 N) using a starch solution as an indicator.

$$PV = Volume\ of\ sample\ titrant - Volume\ of\ blank\ titrant \times N(Na_2S_2O_3) \times 1000 \div Weight\ of\ oil$$

Triglycerides (TAG)

The triglycerides were identified following AOCS official method Ce 5b-89 AOCS (2009) as modified by Cunha and Oliveira (2006). Oil (0.2 g) previously dehydrated with anhydrous sodium sulphate and filtered was dissolved in 4.0 ml of acetone and homogenized by stirring. The mixture passed through a 0.22 pm disposable LC filter disk and analyzed using a reversed-phase high performance liquid chromatography (HPLC) (Hewlett-Packard model 1100 Waldbronn, Germany) equipped with Evaporative Light Scattering (ELS) detector (Alltech 2000ES, USA). Acetone dissolved oils (10 µl) were eluted with acetone: acetonitrile using Apollo C18 (5 µm; 250 × 4.6 mm) column (Alltech Grace, USA) operating at room temperature. TAG peaks were identified by comparing retention time with TAG standards (Sigma Aldrich, Australia) and compared on the basis of retention time of TAG standards using chemstation software.

Antioxidant activity

The antioxidant activity was determined according to (Ramadan and Wahdan, 2012). Oil (10 mg) was mixed with 100 µl of toluene and 390 µl of freshly prepared toluenic-DPPH solution (10^{-4} M). Mixture was vortexed for 20 s and left at room temperature for 60 min. Decrease in absorbance of toluenic-DPPH solution with oil and without oil (control) using toluene without DPPH as blank was measured at 515 nm using a Novaspec II visible recording spectrophotometer (Pharmacia Biotech, Cambridge, England).

$$Antioxidant\ activity(\%) = Absorbance\ of\ control - Absorbance\ of\ sample \times 100 \div Absorbance\ of\ sample$$

Statistical analysis

Extraction of coconut oil was carried out in duplicates. All chemical analyses were conducted in triplicate. Analysis of variance (ANOVA) was carried out on the results using IBM SPSS version 22 (IBM Corp., NY, USA). Significant differences among means were determined at $p<0.05$ using Tukeys' test.

RESULTS AND DISCUSSION

Oil yield

The oil yield of the different techniques of extractions revealed the differences in the quantity of oil extracted. Significantly ($p < 0.05$), higher oil yield was observed from RCO compared to the different VCO methods (Table 1). Among the VCO extracted oil, centrifugation had a significantly low oil yield. The low oil yield from centrifugation may be attributed to the speed of centrifugal force used (4000 rpm) as no other means was combined to destabilize the oil-water emulsion as seen in the other methods which employed enzymes, bacteria or physical means such as chilling. Later studies may need to optimise the centrifugal force that gives maximum oil yield if employed singularly. Nour et al. (2009)suggested that the yield of oil was directly proportional to the centrifugal force used in extraction. The higher yeild of oil from RCO may be due to the use of hexane which is a non-polar solvent capable of dissolving fats coupled with prolonged exposure to heat, that is, 80°C for 8 h.

The oil yield of the enzyme assisted extraction (65.74%) was low compared to earlier studies. Che Man et al. (1996) reported that higher oil yeild (73%) using cellulase, protease, α amylase and polygalacturonase (1% w/w each) at 60°C and pH 7. McGlone et al. (1986) also reported that 80% of oil recovery using similar enzymes used in this study. The results from enzyme extraction using α-amylase (from *A. oryzae*), pectinase (from *A. niger*) and proteases (from *Streptomyces griseus*) indicated that there is need to determine the optimal temperature and pH at which the enzymes are most active in order to achieve higher oil yield. Chih et al. (2012) showed that oil yield of olive oil can be increased by enzyme treatment.

Inducing fermentation (*L. plantarum)* led to a significant increase in yield of oil ($p < 0.05$) as compared to natural fermentation (Table 2). The effect of *L. plantarum* in destabilizing coconut emulsion was also reported by Che Man et al. (1997) with oil yield (95%) compared to control (84%). This result suggests that the use of microorganisms such as *L. plantarum* in the production of VCO may lead to the optimization of the oil yield of coconut.

Table 2. Physiochemical properties of coconut oil extracted from different techniques.

Extraction method	Oil yield (%)	FFA (%)	Moisture and Volatiles (%)	Peroxide value (meq/kg oil)	Saponification value (Mg KOH/G)
EVCO	65.74±2.19^{a}	3.28±0.2^{c}	0.39± 0.01^{b}	0.43±0.04^{b}	259±0.82^{ab}
NFVCO	68.13±2.4^{a}	0.36±0.05^{a}	0.11±0.02^{a}	0.68±0.02^{a}	257±0.96^{a}
IFVCO	77.67±2.26^{b}	0.3±0^{a}	0.12±0.02^{a}	0.66±0^{a}	254±8.7^{ab}
CHVCO	69.31± 0.47^{a}	0.08±0^{b}	0.15±0.04^{a}	0.43±0.03^{b}	261±3.9^{b}
CEVCO	54.4±1.1^{d}	0.17±0.01^{ab}	0.34±003^{b}	0.34±0.07^{b}	250±9.4^{ab}
RCO	83.23±3.38^{c}	0.06±0^{d}	0.02±0.1^{c}	1.06±0.22^{c}	256±7.1^{ab}
Standards	NA	≤ 0.5^{2}	0.2^{1,2}	<15^{1}<3^{2}	248-265

Means (n=6) within the same column with different superscripts are significantly different at p <0.05. NA= not available. Standards for CODEX and APCC are coded 1 and 2, respectively.

FFA

FFA of coconut oil from different extraction is as shown in Table 2. All samples except for enzymatic extraction had FFA within the Asian and Pacific Coconut Community (APCC) Standard (APCC, 2009) for virgin coconut oil (0.5%). The relatively high FFA from enzymatic extraction could be due to the enzymatic hydrolysis of triglycerides in coconut oil resulting in the increase in FFA content. FFA are formed from the hydrolysis of an ester by lipase or moisture (Choe and Min, 2006). According to Raghavendra and Raghavarao (2011), hydrolytic rancidity could be due to hydrolysis of triglycerides of fats and oils by enzymes resulting in an increase in FFA of oil and fats.

Moisture and volatile

Moisture and volatile matter are an important determinant of oil quality (Choe and Min, 2006). It is desirable to keep the moisture content low as it will increase the shelf life by preventing oxidation and rancidity processes. High moisture content promotes hydrolytic rancidity of fats and oils (Raghavendra and Raghavarao, 2011). RCO had significantly lower ($p < 0.05$) moisture content than all VCO samples. Marina et al. (2009a) also reported that lower moisture content of RCO compared with VCO. VCO from IF, CH and NF were in accordance to APCC standards (APCC, 2009). Enzymatic and centrifugation techniques had moisture content of 0.39 and 0.34%, respectively which were both above APCC set standard of <0.2%. As separation of water and oil phase is based on the centrifugal force used (Nour et al., 2009), it may be necessary for future studies to determine the optimum centrifugation technique. The high moisture (0.39%) in coconut oil from enzymatic extraction may have led to the high FFA observed.

Peroxide value

All oil samples had peroxide values below the CODEX and APCC limit (Table 2). This indicates that samples were highly stable against oxidative rancidity. Overall, RCO had significantly higher peroxide value (1.06 ± 0.22) $p< 0.05$ compared to the VCO samples. Other studies carried out by Raghavendra and Raghavarao (2011), Dayrit et al. (2011), and Gopala Krishna et al. (2010) comparing RCO and VCO also found a higher peroxide value in RCO samples. According to Cunha and Oliveira (2006), higher degree of unsaturation in fats and oils increased the chances of oxidative rancidity. Coconut oil generally has low percentage of unsaturated fats making it relatively stable to oxidation (Gopala Krishna et al., 2010). The higher peroxide value in RCO could be due to the high temperature used in its refining. Heat has been suggested as a factor that enhances oxidative rancidity (Marina et al., 2009a). VCO methods of producing coconut oil is a better option to RCO method in controlling oxidative rancidity, has it requires the use of low heat as opposed to RCO that uses high heat.

Saponification value

Saponification value is an indication of the degree of saturation, where high values correspond to shorter chain fatty acids in the glycerol bond (Marina et al., 2009a). Coconut oil has a relatively high saponification value due to its high concentration of short and medium chain triglycerides (Gopala Krishna et al., 2010). All the oil samples had saponification value within CODEX standard of 248 to 265 mg KOH/g of oil (FAO, 2009).

Fatty acid composition

The fatty acid compositions of coconut oil from different extraction are presented in Table 3 along with CODEX standard (FAO, 2009). Coconut oil is predominantly comprised of MCFAs. MCFAs are saturated fatty acids with a carbon chain of 6 to 12 atoms. Of these MCFAs, lauric acid (C12) is predominant with antiviral and antimicrobial properties similar to monolaurin in human

Table 3. Fatty acid composition of coconut oil produced from different techniques and Codex standard.

Extraction method	C6	C8	C10	C12	C14	C16	C18	C18:1	C18:2
EVCO	0.65±0.09^{a}	8.44±0.0^{a}	7.05±0.0^{a}	47.15±0.0^{d}	18.85±0.02^{a}	8.3±0.2^{d}	2.02±0.03^{a}	6.32±0.35^{ab}	1.68±0.002^{b}
NFVCO	0.83±0.0^{b}	9.02±0.0^{d}	7.29±0.0^{b}	49.81±0.0^{c}	18.25±0.01^{b}	6.62±0.02^{ab}	2.01±0.01^{a}	5.36±0.03^{c}	1.21±0.01^{c}
IFVCO	1.02±0.0^{d}	8.22±0.0^{b}	8.38±0.0^{c}	48.94±0.00^{cd}	19.01±0.05^{c}	6.26±0.0^{c}	2.00±0.01^{a}	6.68±0.01^{a}	0.27±0.0^{d}
CHVCO	0.66±0.0^{a}	8.36±0.0^{a}	7.07±0.0^{a}	49.37±0.00^{ac}	19.36±0.01^{d}	6.87±0.0^{a}	2.56±0.054^{b}	5.27±0.02^{c}	0.49±0.01^{e}
CEVCO	0.68±0.01^{a}	8.25±0.0^{b}	6.24±0.0^{d}	50.12±0.01^{c}	19.69±0.01^{e}	6.36±0.0^{bc}	2.18±0.02^{c}	6.18±0.05^{b}	0.44±0.0^{a}
RCO	1.12±0.0^{d}	8.90±0.0^{d}	6.16±0.0^{e}	49.33±0.05^{c}	18.46±0.01^{e}	8.91±0.0^{e}	2.01±0.02^{a}	5.08±0.005^{c}	0.43±0.0^{a}
CODEX	ND-0.7	4.6-10	5.0-8.0	45.1-53.2	16.8-21.0	7.5-10.2	2.0-4.0	5.0-10	1-2.5

Means (n=6) within the same column with different superscripts are significantly different at p<0.05. ND: Not detected.

milk (Mansor et al., 2012). The lauric content of all oils was not significantly different from each other (Table 3). This indicates that extraction method does not enhance the lauric content for coconut oil. Myristic acid (C14), the next highest MCFA after lauric acid, showed similar trend of non-significant difference among methods (18.25 to 19.69%). These values obtained for Myristic acid were similar to myristic content of VCO samples reported by Mansor et al. (2012) and Marina et al. (2009c), but slightly lower than the values obtained by Raghavendra and Raghavarao (2011) which was 22.3%. Overall, all the percentage of fatty acids had their values within CODEX standard for coconut oil except for caproic acid (C6) which had values slightly higher than CODEX (<0.7) (FAO, 2009), NF, IF and RCO had values 0.83, 1.02 and 1.12%, respectively,

Antioxidant act43ivity

There have been increasing studies suggesting that consumption of food containing phenolic antioxidant may help fight against several disease (Marina et al., 2009c). These studies showed that increase in phenolic content leads to an increase in antioxidant activity (Marina et al., 2009c; Marina et al., 2009b)

The antioxidant activity of the VCO samples and RCO is as shown in Figure 1. Percentage anti-oxidative activity of free radical scavenging system (RSA) was high in oil from IF (28.29%). NF, enzymatic, and centrifugation quenched DPPH radical by 19.7, 24.23 and 23.51%, respectively. The lowest antioxidant activity from VCO extracted oil sample was from CH (17.32%).

RSA of RCO was significantly lower than VCOs with ratio of 1:4 compared with IF (Figure 1). Earlier study comparing the ability of coconut oil from different extraction methods (fermentation, CH and RCO) to quench DPPH radicals also showed that fermentation gave significantly higher RSA compared to RCO (1:3) (Marina et al., 2009b). The low antioxidant activity of RCO may be due to the exposure to high heat during the extraction process (Seneviratne et al., 2008).

Marina et al. (2009b) reported that the reason for low RSA in CH compared to other VCOs may be due to more processing steps involved. Seneviratne et al. (2008) also concluded that the possibility of slight heat employed in other processing methods may enhance RSA, but excess heat will lead to reduction in RSA as observed by oil sample form RCO method. The extent of heat required to enhance the antioxidant properties of coconut oil is not established Marina et al. (2009c). However, the reduced RSA of RCO which uses high heat above 200°C is in agreement with other reported studies by Nevin and Rajamohan (2004), Seneviratne et al. (2008), and Marina et al. (2009b).

Triacylglycerol (TAG) composition

The major triglycerides in coconut oil are the MCTs, with equivalent carbon number ranging from 32 to 42. Triglyceride composition is used to distinguish coconut oil from other lauric acid containing oils, that is, palm kernel oil due to its high composition of short chain triglycerides (C30 to C34) and lower composition of long chain triglycerides (C44 to C54) (Amri, 2011). VCO sample from enzymatic extraction was the only oil with C26 and C28 with values of 3.38 ± 0.17 and 2.11 ± 0.05% (Table 4). The major triglycerides present in VCO samples consist of 23.31 to

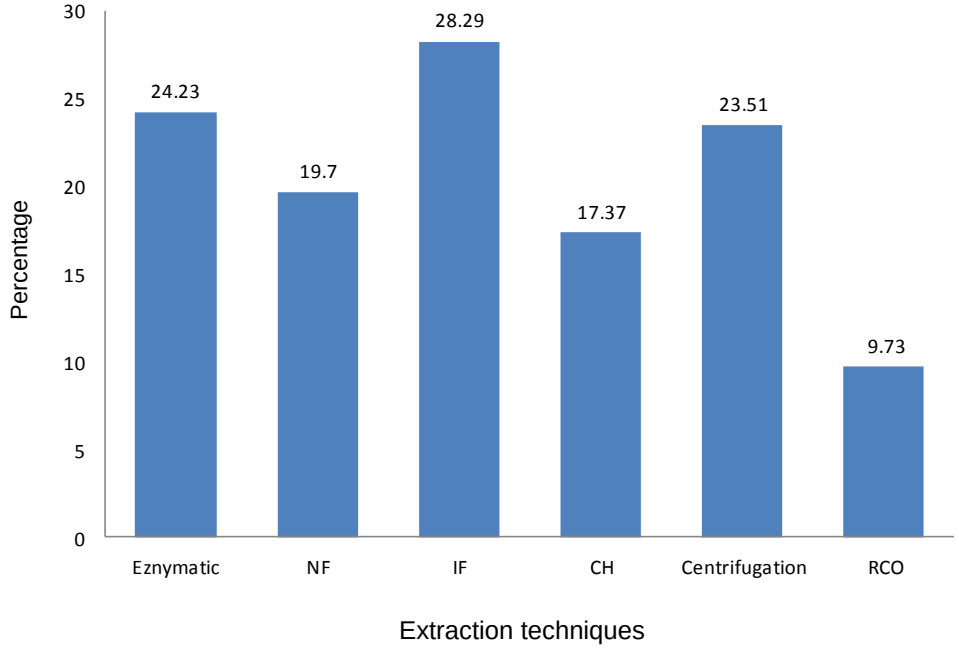

Figure 1. Antioxidant activity (%) of coconut oils extracted with different techniques.

Table 4. Triacylglycerol composition of coconut oil produced from different techniques and Codex standard.

Extraction method	C24	C26	C28	C30	C32	C34	C36	C38	C40	C42	C44	C46	C48	C50
EVCO	0.49±0.08^{ac}	3.38±0.17	2.11±0.05	3.66±0.08^{b}	15.75±0.31^{ac}	21.69±0.25^{a}	23.31±0.19^{c}	15.74±0.29^{a}	8.37±0.026^{b}	3.53±0.82^{ab}	2.36±1.33^{b}	0.63±0.07^{a}	ND	ND
NFVCO	0.78±0.1^{b}	ND	ND	3.93±0.8^{c}	19.38±1.17^{b}	21.72±0.17^{a}	26±0.51^{a}	15.87±0.11^{a}	7.8±0.14^{a}	3.4±0.57^{ab}	0.74±0.08^{a}	0.3±0.02^{a}	ND	ND
IFVCO	0.45±0.01^{c}	ND	ND	4.04±0.04^{a}	15.98±0.31^{a}	21.84±0.18^{a}	26.12±0.2^{ab}	17.18±0.16^{b}	9.31±0.11^{c}	3.89±0.5^{a}	0.92±0.03^{a}	ND	ND	ND
CHVCO	0.7±0.1^{ab}	ND	ND	3.75±0.03^{bc}	19.57±0.24^{b}	24.57±0.01^{b}	25.74±0.14^{a}	14.74±1.00^{c}	7.42±0.03^{d}	2.82±0.42^{bc}	0.24±0^{a}	ND	ND	ND
CEVCO	1.03±0.05^{d}	ND	ND	ND	21.59±0.28^{d}	23.29±0.31^{c}	27.51±0.12^{d}	16.32±0.4^{ab}	7.94±0.04^{a}	2.11±0.38^{c}	0.27±0.06^{a}	ND	ND	ND
RCO	0.65±0.1^{c}	ND	ND	ND	14.79±0.12^{c}	17.73±0.03^{d}	26.63±0.11^{b}	20.13±0.04^{d}	11.88±0.02^{e}	6.03±0.02^{d}	ND	1.57±0.05^{b}	0.52±0.02	0.14±0.02

Means (n=6) within the same column with different superscripts are significantly different at p<0.05. ND: Not detected. %RSA.

27.51% of C36, 17.73 to 24.57% of C34, 14.79 to 21.59% of C32, 14.47 to 20.13% of C38 and 7.42 to 11.88% of C40. These values were in agreement with the values of VCO samples reported by Marina et al. (2009a). Overall, RCO had higher composition of longer chain triglycerides

(C38 to C50) than shorter chain triglycerides (C24 to C34) and was the only oil sample which contained C46, C48 and C50. It indicates higher degree of unsaturation in coconut oil produced by RCO. Similar findings of higher long chain triglycerides in RCO compared to VCO was reported by (Gopala Krishna et al., 2010).

Conclusion

RCO shows more economic prospect for coconut oil extraction due to higher oil yield. The method of extraction does not significantly alter the fatty acid composition of coconut oil. Also the triglyceride of coconut oil extracted from enzymatic, centrifugation, natural fermentation, induced fermentation, chilling and thawing and refined coconut oil is relatively similar. However, with regards to oil quality, VCO is a better option for coconut oil extraction compared to RCO, with higher phenolic antioxidant activity. Of the VCO methods of extraction, induced fermentation using *L. plantarum* holds promising prospect due to its relatively high oil yield compared to other VCO methods and its higher level of antioxidant activity compared to other methods. On average, the physiochemical parameters of the coconut oil produced from different methods did not vary from set standards

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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