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Bioactive and nutritional compounds in virgin coconut oils

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ABSTRACT

Introduction: Virgin coconut oil (VCO) is very much in demand among health-conscious consumers. VCO is produced from fresh coconut milk by using centrifugation (CVCO) or fermentation (FVCO). Since the conditions used for these processes are quite different, this study aimed to investigate their effects on the contents of selected bioactive compounds that have potential health benefits. **Methods**: CVCO and FVCO were produced from the same batch of fresh coconut (*Cocos nucifera L.*) milk. CVCO was obtained by centrifuging coconut milk in three steps with vacuum evaporation, while FVCO was obtained by anaerobically fermenting coconut milk at 35°C for 16 h. The products were analysed for macronutrients, fatty acid profiles, phytosterols and phenolic compounds. Potential health benefits were determined by calculating the chance of fatty acid bioavailability and analysing antioxidant activities. **Results**: Both VCO production processes removed all hydrophilic compounds, with the remaining fat and moisture contents not significantly different at 99.90% and 0.10%, respectively. Their fatty acid profiles were 90% saturated and 60% medium chain (mainly lauric acid). The phenolic compound (originally found high in coconut milk) was present in trace amounts in the VCOs. However, phytosterols became more concentrated. Chances of medium chain fatty acid becoming more available for health benefit were at 54% and 58%, and were insignificant among both VCOs. Fermentation caused more rancidity to the product. **Conclusion**: Both centrifugation and fermentation production processes did not qualitatively and quantitatively affect the bioactive compounds of virgin coconut oil.

Keywords: Centrifugation, fermentation, medium chain fatty acid, phytosterols, virgin coconut oil

INTRODUCTION

Virgin coconut oil (VCO) that is produced in certain countries of Southeast Asia is used as a dietary supplement with the aim of reducing the risk of certain non-communicable diseases (NCDs). The demand for VCO is increasing among consumers globally. A result of market analysis indicated that the compound annual growth rate (CAGR) of global

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VCO market would be around 10% during 2017-2021 (Technavio, 2017).

The key factors of such growth are due to the increased use of natural health products among health-conscious consumers and aging population. However, the quality of VCOs available in the market varies depending on the quality of production facilities, which also affects its market price. As compared to traditional VCO produced by mechanical extraction from copra (sun-dried coconut meat), the new generation of VCO is produced by oil separation from fresh coconut milk. The oil separation process is performed under less severe or mild conditions, which results in products of better quality.

Natural or pure culture lactic acid fermentation, chilling and thawing, centrifugation, or mixed enzyme (cellulose, amylase, polygalacturonase and protease) digestion is the condition that can be used for oil separation. Due to the freshness of raw material and mild production process, these VCOs are believed to maintain the nutrient profile and potential health benefits of coconut.

Commercial VCOs are produced by centrifugation (CVCO) or fermentation (FVCO). CVCO is normally produced industrially in less than 10 minutes by separating VCO from fresh coconut milk using a high speed centrifugal machine. FVCO is produced by small scale or cottage industries using natural fermentation process which takes approximately two weeks.

The oil separation process in CVCO depends on mechanical force, while FVCO relies on the denaturation of a natural emulsifying agent. Both kinds of VCOs are widely available in the market with claims on various health benefits, such as weight and cholesterol reduction, immune system improvement, lower risk of Alzheimer, and antimicrobial growth (DebMandal & Mandal, 2011). These health benefits might be due to available bioactive compounds in the VCOs, which are also found in coconut meat and milk. Coconut meat is known to be a good source of medium chain fatty acid, especially lauric acid, which is directly metabolised into energy with no effects on blood cholesterol.

Phenolic compounds and phytosterols are antioxidants related to risk reduction of non-communicable diseases (NCDs), are also found in both coconut meat and coconut milk. The different production processes of these VCOs could have different effects on the contents and availability of these bioactive compounds. This study was aimed at investigating the effects of VCO production process, i.e. centrifugation and fermentation, on the contents of potential bioactive compounds including medium chain fatty acids, triacylglycerol composition, total phenolic content, phenolic acids and flavonoids and phytosterols.

MATERIALS AND METHODS

Coconut milk

Coconut milk was prepared in batches of three at the Theppadungporn Coconut Co., Ltd., Nakhonpathom, Thailand. The peeled coconut meat was cleaned in chlorinated water, shredded, and expressed to extract coconut milk. The coconut milk was then stored in tightly closed glass bottle at -20°C until analysis.

Virgin coconut oil (VCO)

VCOs were produced from freshly extracted coconut milk of the same batch at the Theppadungporn Coconut Co., Ltd. by using centrifugation and fermentation methods. Centrifuged virgin coconut oil (CVCO) was produced by centrifuging coconut milk in a series of three centrifugal machines (GEA, GEA Westfalia Separator Group GmbH, Oelde, Germany) then residual water

in the oil was finally removed in a vacuum evaporator (Behle Apparate & Behälterbau, H. Behle GmbH, Bielefeld, Germany). Fermented virgin coconut oil (FVCO) was produced by naturally fermenting coconut milk in closed glass jars under anaerobic condition at 35°C for 16 h. The FVCO was harvested and filtered through cheesecloth. The produced VCOs were sampled and stored in closed amber glass bottles at -20°C until analysis.

Proximate analysis

Moisture content was determined by measuring the constant weight after drying in a hot air oven (AOAC INTERNATIONAL, 2012). Total fat content was determined by extracting VCOs or hydrolysed coconut milk with petroleum ether in Soxtec system (Model HT 1043, Tecator Co., Ltd., Hoganas, Sweden) (AOAC INTERNATIONAL, 2012).

Protein content was analysed according to Kjeldahl method (AOAC INTERNATIONAL, 2012), with 6.25 as the multiplication factor for converting total nitrogen into protein content. Ash content was determined after the sample had been burnt in a muffle furnace at 550°C for 2.5 h (AOAC INTERNATIONAL, 2012). Carbohydrate was calculated by subtracting moisture, fat, protein and ash contents from 100 (FAO, 1998).

Fatty acid profile

The extracted oil from coconut milk or VCO was saponified with 0.5M KOH in methanol at 95°C, methylated into fatty acid methyl esters (FAMEs) by adding 14% Boron trifluoride in methanol (Petrović Kezić & Bolanča, 2010). FAMEs were analysed on the DB-23 capillary GC column (60 m x 0.25 mm I.D., 0.25 µm) installed in an Agilent 9860 gas chromatograph system equipped with a flame ionisation detector and a split/splitless injector (Agilent Technologies, Santa Clara, CA, USA). Methyl heptadecanoate, C17:0 was used as the internal standard and helium was the carrier gas. The Supelco™ 37 Component FAME Mix (10 mg/ml) was used as the standard (Sigma-Aldrich, MO, USA.).

Triacylglycerol (TAG) composition

Five milligrams of oil extracted from coconut milk or VCO was mixed with 2 ml of the solvent mixture of methylene chloride: isopropanol: methanol (25:10:65 v/v/v) added with 50 µg butylated hydroxytoluene (BHT)/ml. The mixture (3 µl) was analysed in the High Strength Silica (HSS) T3 column (1.8µm particle 100 x 2.1 mm id, Waters, Milford, Massachusetts, USA) equipped on the quadrupole time-of-flight (TOF) mass spectrometer (MS) (AB SCIEX, TripleTOF 5600) that was operated under the information-dependent MS/MS acquisition mode.

The gradient mobile phase consisted of acetonitrile: water containing 10 mM ammonium formate (60:40 v/v) and isopropanol: acetonitrile: water containing 10 mM ammoniumformate (90:10:5 v/v/v). The scan range of TOF/MS was m/z 70-1,200 and MS/MS was m/z 50-1,200 (Choi *et al.*, 2015). The PeakView™ software (SCIEX, MA, USA.) was used to identify triacylglycerol species and content of each triacylglycerol species was calculated as relative quantification. Moreover, the chance of medium chain fatty acid, MCFA for being on sn-1 and sn-3 in sample was calculated as:

$$\frac{\sum\left(\frac{\text{No. of MCFA on each TAG}}{3}\right)}{\text{No. of TAG in the species}} \times \text{Relative content of each TAG species}$$

Where:

No. of MCFA each TAG = number of MCFA (fatty acids that contain 8-12 carbon atoms) in each TAG species

No. of TAG in the species = total number of TAG possibly in the species

Total phenolic content, phenolic acid and flavonoids

The extract of coconut milk or VCO in 80% methanol was the sample used for analyses. Total phenolic was analysed by using Folin-Ciocalteu assay (Martin *et al.*, 2009), which was measured the absorbance at 755 nm on spectrophotometer (UV1601, Shimadzu, Kyoto, Japan). The total phenolic content was determined regarding the standard curve of gallic acid (0.04-0.20 mg/ml).

The phenolic acid and flavonoids were determined by High Performance Liquid Chromatography (HPLC) with Synergi Hydro-RP column (4 μm particle, 250 x 4.60 mm id, Phenomenex, Torrance, CA, USA) and Allsphere ODS-2 as a guard column (10 x 4.6 mm id, Alltech, Deerfield, IL, USA) using Perkin-Elmer Series 400, equipped with a Hewlett-Packard 1040A photodiode array detector.

The gradient mobile phases system consisting of acetonitrile (mobile phase A) and glacial acetic acid in deionisation water (mobile phase B) with the initial ratio 5% mobile phase A for 3 min then increased to 25% in 27 min and increased to 75% in 5 min were applied. The injection volume was 10 μl and a flow rate of 1 ml/min was used (Lee, Durst & Wrolstad, 2002).

Phenolic acid and flavonoids were detected at 260, 280 and 320 nm. The standards used included chlorogenic acid, orcinol, caffeic acid, epicatechin, caffeine, p-coumeric acid, ferulic acid, rutin, Q-3-rhamnoside, hesperetin, phloridzin, resveratrol and kaempferol.

Antioxidant activity

The antioxidant activities in the 80% methanol extract of coconut milk or VCO were determined as Oxygen Radical Absorbance Capacity (ORAC) (Huang *et al.*, 2002) and Ferric Reducing Antioxidant Power (FRAP) assays (Benzie & Strain, 1996). The ORAC was analysed on 96-well microplate reader (Synergy HT, Bio-Tek Instruments, Winooski, VT, USA.).

The ORAC value was determined regarding the Trolox standard curve of 3.125-100 μM in 75 mM phosphate buffer pH 7.4. The FRAP assay was performed on microplate reader (Synergy HT, Bio-Tek Instruments, Winooski, VT, USA). The FRAP value was determined regarding the Trolox standard curve of 7.8125-250 μM in deionised water.

Phytosterols

After being saponified with potassium hydroxide (KOH) in ethanol, coconut milk or VCO was added with 50 μl of 0.2 mg/ml Δ^7-cholesterol as internal standard. The mixture was then extracted with n-hexane. The extract was dried under nitrogen gas and dissolved in chloroform: methanol solvent (1:3 v/v).

The analysis was performed on Liquid Chromatography–Atmospheric Pressure Chemical Ionization Mass Spectrometry (LC-APCI-MS/MS) with 3 μm particle, 100 x 2 mm id Luna™ C18 (2) column (Phenomenex, Torrance, CA, USA.) operations in positive ion and selective reaction monitoring (SRM) modes. An isocratic mobile phase of acetonitrile: methanol (99:1 v/v) at flow rate 0.6 ml/min was use (Mo *et al.*, 2013). The optimum sensitivity and selectivity for quantitative analysis were established for campesterol, β-sitosterol, stigmasterol, Δ^5-avenasterol, brassicasterol, cycloartenol, β-sitostenol and campestenol.

Quality parameters of VCO

The VCO was determined for acid value (AV), free fatty acid content (calculated as lauric acid), peroxide value (PV), iodine value (IV) according to AOCS Method Cd 3d-63, AOCS Method Cd 8-53,

AOCS Method Ca 5a-40, AOCS Method Cd 1d-92, respectively (AOCS, 1998). Colour was measured in L*a*b* unit by a colorimeter (Color Flex EZ, Color global Co., Ltd., Bangkok, Thailand).

Statistical analysis

The IBM SPSS Statistics 19.0™ software (IBM Corp., Armonk, New York, USA) was used for statistical analysis and determine the significant difference at $p<0.05$. All analyses were performed in triplicate and the results were expressed as mean and standard deviation (SD). One-way ANOVA and Duncan's multiple range tests were conducted to assess difference among mean values from analyses of coconut milk and the VCOs. Student's *t*-test was used to evaluate difference between mean values from chemical analyses of the VCOs.

RESULTS AND DISCUSSION

Macronutrients analysis

Coconut milk (with no water added during the extraction process) contained approximately 60% water, 30% fat and small contents of carbohydrate, protein and ash. For the VCO production, at least 30% fat in the coconut milk was required. Protein as a natural emulsifier could stabilise the fat emulsion in coconut milk (Gonzalez, 1990), which could trouble the oil separation process in the VCO productions.

Oil was separated due to breakage of the fat emulsion in coconut milk, either caused by mechanical force (centrifugation) or protein denaturation (fermentation). Under the commercial VCO production processes, the role of protein as emulsifier in coconut milk could be overcome, which resulted in the

Table 1. Chemical compositions and fatty profiles of coconut milk, centrifuged virgin coconut oil (CVCO) and fermented virgin coconut oil (FVCO)[†]

Parameters	*Content (%)*		
	Coconut milk	*CVCO*	*FVCO*
Moisture[‡]	59.98±0.97[b]	0.11±0.01[a]	0.13±0.02[a]
Protein	3.41±0.02[b]	0.00[a]	0.00[a]
Fat	31.88±1.49[a]	99.90±0.01[b]	99.87±0.01[b]
Ash	0.97±0.08[b]	0.00[a]	0.00[a]
Carbohydrate	4.59±0.29[b]	0.00[a]	0.00[a]
Fatty acids			
C 8:0	3.59±1.24[a]	2.51±1.28[a]	2.26±1.40[a]
C 10:0	5.26±0.61[a]	4.86±0.48[a]	4.71±0.70[a]
C 12:0	47.81±1.84[a]	49.80±1.12[a]	49.48±1.12[a]
C 14:0	19.20±1.29[a]	21.27±0.99[a]	21.41±1.28[a]
C 16:0	11.16±1.70[a]	10.21±0.80[a]	10.43±0.92[a]
C 18:0	4.06±0.39[a]	3.92±0.36[a]	4.00±0.45[a]
C 18:1	7.76±0.83[b]	6.40±0.42[a]	6.66±0.54[a,b]
C 18:2	1.17±0.13[a]	1.02±0.03[a]	1.06±0.06[a]
n-6	1.17±0.13[a]	1.02±0.03[a]	1.06±0.06[a]
n-9	7.76±0.83[b]	6.40±0.41[a]	6.66±0.54[a,b]
S:M:P[§]	1:0.09:0.01	1:0.07:0.01	1:0.07:0.01

[†]Mean±SD (n=3)

[a,b]Different alphabets within the same row denote significant difference at $p<0.05$

[‡]APCC recommendations in 2009 for moisture contents of VCO was 0.1%

[§]S: Saturated fatty acid; M: Monounsaturated fatty acid; P: Polyunsaturated fatty acid

VCOs of the fat and moisture contents regarding the Asian and Pacific Coconut Community (APCC) recommendation, amended in August 2009 (Asian and Pacific Coconut Community, 2009).

There were no significant differences (p>0.05) in fat and moisture contents of the VCOs from both production processes (Table 1). Since ancient times, traditional-pressed copra-coconut oil used in Asian cuisines was the primary source of fat for the population. In contrast, VCO is presently produced from less severe processes, and is marketed as a functional food of high economic value and with claims of potential health benefits.

Fatty acid profile

The fatty acid profile of coconut milk, CVCO and FVCO were mostly not significantly different (p>0.05), except for C18:1 and n-9 fatty acids (Table 1). The VCO production processes did not affect the original fatty acid profile of the coconut milk.

Among the saturated fatty acids, medium chain fatty acid, MCFA (C8-C12) contributed the most to VCOs, of which lauric acid (C12:0) was the major fatty acid (50% of total fatty acids). The contents of MCFA were not significantly different in the VCOs derived from both processes. Compared to fatty acids >C12, MCFA (C8-C12) are metabolised more efficiently and showed less accumulation in the body (Marten, Pfeuffer & Schrezenmeir, 2006). Lauric acid, as compared to myristic acid (C14:0) and palmitic acid (C16:0), increases blood LDL-cholesterol as well as HDL-cholesterol. However lauric acid shows its ability to reduce the ratio of TC/HDL-cholesterol (Mensink, 2016).

Cohort studies reported that lauric acid consumption at 0.63% of energy intake (1.4 g/day) could reduce the risk of type 2 diabetes (Liu *et al.*, 2018). By having lauric acid at 0.7g/day, it could not reduce the risk of coronary heart disease (Zong *et al.*, 2016) and had no effect on BMI (Raatz *et al.*, 2017). Nonetheless, one should be mindful of the saturated fatty acids content in VCO. One serving of VCO as a dietary supplement (e.g. 1 tablespoon, 15 ml or 14.5 g) provides saturated fatty acids amounting to 67% of the recommendation of the World Health Organization (WHO) of <20 g/day (WHO, 2003). Positive correlation between saturated fatty acids and risk of cardiovascular disease has been shown in several studies (Mensink, 2016).

Triacylglycerol composition

The triacylglycerol (TAG) composition is used to determine the proportions of individual TAG molecular species. Based on this data, the potential TAG molecular species are shown in Table 2, Column 2. By using the value of relative content of the molecular species, the proportions (%) of MCFA (C8-12) in sn-1 and sn-3 positions was evaluated. This information is of nutritional relevance because fatty acids at the stereospecific sn-1 and sn-3 positions in the TAG have been shown to have a better chance of being hydrolysed by lipase and hence improving their bioavailability.

TAG 30:0 species means a triglyceride with 30 carbon atoms with no double bond, can be 3 ways of arrangement. The relative content of this species in the coconut milk is 7.07%, of which 6.28% of MFCA are in the sn-1 and sn-3. Table 2 indicates that up to 54-58% of MCFA in coconut milk, CVCO or FVCO were in the sn-1 and/or sn-3 positions. In comparing the products from the 2 processes, the FVCO showed higher proportions of MCFA at these positions.

MCT has been promoted for problems related to fat digestion, metabolism and utilisation (Hamosh *et al.*, 1991; Li *et al.*, 2015). This study showed that the chance of having triacylglycerol molecules as MCT in our studied coconut products was 21-24%.

Table 2. Quantity (%) of MCFA in coconut milk, centrifuged virgin coconut oil (CVCO) and fermented virgin coconut oil (FVCO) at sn-1 and/or sn-3 positions of TAGs[†]

TAG Species	*TAG composition in the species*	*MCFA (%) at sn-1 and sn-3 [Relative content of the TAG species (%)]*		
		Coconut milk	*CVCO*	*FVCO*
TAG 30:0	10:0/10:0/10:0, 8:0/10:0/12:0, 8:0/8:0/14:0	6.28±1.7^{a} [7.07±1.92^{A}]	4.34±0.30^{a} [4.51±0.70^{A}]	4.38±1.30^{a} [4.93±1.46^{A}]
TAG 32:0	10:0/10:0/12:0, 8:0/10:0/14:0, 8:0/12:0/12:0	12.12±1.29^{b} [13.63±1.45^{A}]	10.80±0.15^{a,b} [12.15±0.17^{A}]	8.94±0.60^{a} [10.54±1.35^{A}]
TAG 34:0	10:0/10:0/14:0, 10:0/12:0/12:0	9.81±0.83^{a,b} [11.77±0.99^{A,B}]	12.91±1.47^{b} [15.49±1.76^{B}]	9.31±0.56^{a} [10.70±1.34^{A}]
TAG 36:0	10:0/10:0/16:0, 10:0/12:0/14:0, 12:0/12:0/12:0	9.69±0.69^{a} [12.45±0.89^{A}]	8.22±0.40^{a*} [10.57±0.52^{A*}]	12.32±0.08^{b} [15.78±0.20^{B}]
TAG 38:0	10:0/10:0/18:0, 10:0/12:0/16:0, 10:0/14:0/14:0, 12:0/12:0/14:0	7.76±0.95^{b} [13.30±1.62^{B}]	5.36±0.42^{a*} [9.19±0.71^{A*}]	9.00±0.05^{b} [15.37±0.18^{B}]
TAG 38:1	10:0/10:0/18:1, 8:0/12:0/18:1	1.61±0.52^{a} [2.41±0.79^{A}]	1.51±0.36^{a} [2.27±0.53^{A}]	1.76±0.25^{a} [2.37±0.76^{A}]
TAG 40:0	10:0/10:0/18:0, 12:0/14:0/14:0, 10:0/14:0/16:0, 12:0/12:0/16:0	2.86±0.53^{a} [5.71±1.06^{A}]	3.88±0.36^{a*} [7.76±0.34^{A}]	5.27±0.10^{b} [10.69±0.42^{B}]
TAG 40:1	10:0/12:0/18:1, 8:0/14:0/18:1	0.70±0.24^{a} [1.41±0.47^{A}]	1.05±0.12^{a} [2.00±0.25^{A}]	0.89±0.32^{a} [1.83±0.14^{A}]
TAG 42:0	10:0/14:0/18:0, 10:0/16:0/16:0, 12:0/12:0/18:0, 12:0/14:0/16:0, 14:0/14:0/14:0	1.85±0.37^{a} [5.55±1.10^{A}]	1.91±0.01^{a*} [5.73±0.03^{A*}]	1.44±0.06^{a} [4.20±0.31^{A}]
TAG 42:1	12:0/12:0/18:1, 10:0/14:0/18:1, 8:0/16:0/18:1	1.71±0.16^{b} [3.86±0.37^{B}]	1.04±0.10^{a*} [2.34±0.23^{A*}]	1.62±0.06^{b} [3.74±0.27^{B}]
TAG 44:0	12:0/14:0/18:0, 12:0/16:0/16:0, 10:0/14:0/20:0, 14:0/14:0/16:0, 10:0/16:0/18:0	0.68±0.01^{a} [2.73±0.02^{A}]	1.05±0.51^{a} [4.20±2.05^{A}]	0.65±0.02^{a} [2.56±0.12^{A}]
TAG 44:1	12:0/12:0/20:1, 12:0/14:0/18:1, 10:0/14:0/20:1, 10:0/16:0/18:1	0.92±0.43^{a} [2.75±1.28^{A}]	0.65±0.03^{a} [1.95±0.09^{A}]	0.94±0.18^{a} [2.45±1.06^{A}]
TAG 44:2	12:0/14:0/18:2, 10:0/16:0/18:2	0.43±0.02^{a} [1.28±0.06^{A}]	0.31±0.03^{a} [0.93±0.10^{A}]	0.43±0.05^{a} [1.39±0.32^{A}]
TAG 46:0	12:0/16:0/18:0, 14:0/14:0/18:0, 14:0/16:0/16:0, 10:0/16:0/20:0, 10:0/18:0/18:0	0.18±0.00^{a} [1.10±0.02^{A}]	0.23 ± 0.02^{a} [1.37±0.10^{A}]	0.23±0.03^{a} [1.46±0.30^{A}]
TAG 46:1	12:0/16:0/18:1, 14:0/14:0/18:1, 10:0/18:0/18:1	0.37±0.04^{a} [1.68±0.19^{A}]	0.41±0.11^{a} [1.84±0.51^{A}]	0.39±0.01^{a} [1.81±0.09^{A}]
TAG 46:2	12:0/16:0/18:2, 14:0/14:0/18:2, 10:0/18:0/18:2, 10:0/18:1/18:1	0.30±0.04^{a} [1.81±0.09^{A}]	0.33±0.05^{a} [1.31±0.19^{A}]	0.40±0.06^{a} (1.41±0.50^{A}]
TAG 48:1	12:0/18:0/18:1, 14:0/16:0/18:1	0.23±0.00^{a} [1.33±0.04^{A}]	0.30±0.05^{a} [1.79±0.32^{A}]	0.25±0.01^{a} [1.57±0.14^{A}]
TAG 48:2	12:0/18:0/18:2, 12:0/18:1/18:1, 14:0/16:0/18:2	0.22±0.00^{a} [0.98±0.00 ^{A}]	0.61±0.04^{b} [2.75±0.18^{B*}]	0.24±0.02^{a} [1.07±0.16^{A}]
Total		57.72±3.34^{a} [90.26±4.70^{A}]	54.91±0.39^{a*} [88.63±2.49^{A}]	58.48±0.93^{a} [95.67±0.54^{A}]

[†]Mean±SD (n=3)

^{a,b}Different alphabets denote significant difference (p<0.05) between means within the same species (same row) for coconut milk, CVCO and FVCO

^{A,B}Different alphabets denote significant difference (p<0.05) between means within the same species (same row) for coconut milk, CVCO and FVCO

*Significant mean difference (p<0.05) within the same species (same row) between CVCO and FVCO

Table 3. Total phenolic content, phytosterols and antioxidant activity in coconut milk, centrifuged virgin coconut oil (CVCO) and fermented virgin coconut oil (FVCO)†

Parameter	*Coconut milk*	*CVCO*	*FVCO*
Total phenolic content (mg GAE/100 g)	2911.24±399.02[b]	Not detected[*]	59.44±13.40[a]
Antioxidant activity (µmole Trolox/100 g)			
FRAP	33.24±2.21[b]	0.11±0.01[a*]	0.83±0.12[a]
ORAC	362.37±42.33[b]	0.77±0.26[a*]	5.22±0.42[a]
Phytosterols (mg/100 g)			
Campesterol	2.22±0.17[a]	5.91±0.10[b*]	6.21±0.11[c]
b-sitosterol	20.30±1.72[a]	49.42±0.56[b]	51.57±3.38[b]
Stigmasterol	3.85±0.30[a]	8.50±0.28[b*]	9.20±0.43[c]
D^5-Avenasterol	6.59±0.04[a]	16.53±1.40[b*]	18.26±0.04[c]
Brassesterol	tr[a]	tr[a]	tr[a]
Cycloartenol	1.42±0.11[a]	4.97±0.63[b]	5.30±0.82[b]
b-sitostenol	1.63±0.14[a]	4.14±0.09[b]	4.18±0.32[b]
Campestenol	1.03±0.00[b]	0.65±0.09[a]	0.97±0.32[a]
Total phytosterol	36.01±2.26[a]	89.89±2.08[b*]	95.12±3.37[b]

†Mean±SD (n=3), tr = trace
[a,b,c]Different alphabets within the same row denote significant difference at $p<0.05$
[*]Significant mean difference ($p<0.05$) within the same row between CVCO and FVCO

Table 4. Physicochemical properties of centrifuged virgin coconut oil (CVCO) and fermented virgin coconut oil (FVCO)†

Parameter	*CVCO*	*FVCO*
Moisture content (%)	0.11±0.01[a]	0.13±0.02[a]
Iodine Value (g of iodine/100 g oil)	3.41±0.22[a]	4.21±0.45[a]
Peroxide value (mEq/kg oil)	0.00±0.00[a]	2.39±0.62[b]
Acid value (mg KOH/g oil)	0.08±0.00[a]	0.21±0.02[b]
Free fatty acids (mg lauric acid/g oil)	0.29±0.00[a]	0.76±0.08[b]
Colour‡:		
- L	4.83±0.30[a]	5.07±0.19[a]
- a	-0.26±0.03[a]	-0.31±0.03[a]
- b	0.26±0.04[a]	0.33±0.04[a]

†Mean±SD (n=3)
[a,b]Different alphabets within the same row denote significant difference at $p<0.05$
‡"L" represents the lightness, "a" represents green (-a) to red (+a) colour, and "b" represents blue (-b) to yellow (+b) colour

The MCFA at the sn-1 and sn-3 are partly hydrolysed in the stomach by gastric lipase, but mostly in the intestines by pancreatic and intestinal lipases. The MCFA are eventually metabolised to acetyl CoA for utilisation as energy (Marten, 2006). The position of MCFA on a triglyceride indicates potential bioavailability.

Total phenolic content, phenolic acid, flavonoids and antioxidant activity

The total phenolic content was found not as high in VCOs as in coconut milk (Table

3). Since most phenolic compounds were hydrophilic, they were unfortunately removed during the oil separation processes and are therefore not found in VCOs. FVCO tends to contain slightly higher phenolic content than CVCO. During fermentation, microorganisms digest the TAG into free fatty acids, which tend to lower the hydrophobicity of the coconut oil. The higher free fatty acid content produced in the FVCO leads to more phenolic compounds in the final product (Table 4). As a bioactive compound, polyphenol intake was associated with a 46% reduction in risk for cardiovascular disease when comparing between the highest (1,235 mg/d) and lowest (483 mg/d) quintile of intake (Tresserra-Rimbau *et al.*, 2014).

Phytosterols

Phytosterols are lipophilic, therefore found being more concentrated in the VCOs than coconut milk after being oil-separated. The contents were not significantly different between the VCOs from both production processes (Table 3).

Phytosterols are reported to reduce cholesterol absorption due to the similarity of their chemical structures (Ostlund, 2004). Phytosterols compete with cholesterol in mixing with micelles, leading to reduced cholesterol absorption in the small intestine (Mel'nikov, Seijen ten Hoorn & Eijkelenboom, 2004).

A serving (80 g) of coconut milk containing 30 mg phytosterols is close to the amount in nuts such as almond (37.78 mg / serving) and walnut (37.99 mg / serving) (Kornsteiner-Krenn, Wagner & Elmadfa, 2013). However, in the case of VCOs which are the extracts from coconut milk, one serving provides only 13.5 mg phytosterols, this amount is too low for considering its phytosterols as a bioactive compound. It has been suggested that phytosterol intake must be up to 800-1000 and 2000 mg/d for reducing 5% (Berger, Jones & Abumweis, 2004), and 10% of blood LDL-cholesterol (Normén, Frohlich & Trautwein, 2004), respectively.

Quality parameters of VCO

Differences in the production processes resulted in different contents of the quality parameters in the VCOs. Factors that related to oil quality deterioration were found to be significantly higher (p<0.05) in the FVCO (Table 4). Both VCOs had a similar iodine value which indicates no changes in the fatty acid profile during the production processes. Due to uncontrollable factors and longer production period of fermentation, the FVCO had higher acid and peroxide values due to increased hydrolytic and oxidative rancidity than CVCO (Raghavendra & Raghavarao, 2011). Differences in VCO production processes did not affect the colour of the products. The final products from both production processes passed the Asian and Pacific Coconut Community (APCC) standards for VCO (Asian and Pacific Coconut Community, 2009).

CONCLUSION

The quality of most bioactive compounds found in fresh coconut milk remains unaltered by the production processes of VCOs, except for phenolic compounds. Lacking of hydrophilic phenolic compounds in the VCOs could negatively affect their antioxidant activities. The most promising bioactive compound in VCOs was MCFA, of which lauric acid was the main contributor. The low levels of phytosterols in both VCOs were not deemed to have potential health benefits.

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Authors' contributions

Chavasit V, designed the experiment and wrote the manuscript; Ngampeerapong C, performed the experiments, analysed the data and drafted the manuscript; Durst RW, designed the methodology and analysed the data.

Conflicts of interest

None.

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