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Standards for Essential Composition and Quality Factors of Commercial Virgin Coconut Oil and its Differentiation from RBD Coconut Oil and Copra Oil

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Commercial samples of virgin coconut oil (VCO), refined, bleached and deodorized coconut oil (RBD CNO), and copra oil were analyzed using standard chemical parameters: gas chromatography (GC) of the fatty acid methyl esters (FAME), % moisture by Karl Fischer titration, % volatile matter at 120° C, % free fatty acid, iodine value, peroxide value, and microbial contamination. Principal components analysis (PCA) of the GC-FAME results indicates that the various samples cannot be differentiated by their fatty acid composition, indicating that the fatty acid profile is not affected by the processing method. No trans-fatty acid was detected in all samples down to 0.01% (w/w) detection limit. VCO can be differentiated from RBD CNO and copra oil using the following tests: % moisture by Karl Fischer, % volatile matter volatile at 120° C, and peroxide value.

Key Words: refined, bleached and deodorized coconut oil (RBD CNO), virgin coconut oil (VCO), copra oil, % fatty acid composition, iodine value, % moisture by Karl Fischer titration, % volatile matter at 120 ºC, peroxide value, principal components analysis (PCA).

INTRODUCTION

Coconut oil is a vegetable oil derived from the kernel of *Cocos nucifera* Linn. The international standards for coconut oil are set mainly by 2 organizations: the Codex Alimentarius, and the Asian and Pacific Coconut Community (APCC). The current Codex standard for coconut oil, which is based on commercial refined, bleached and deodorized coconut oil (RBD CNO), states that edible vegetable oils may be refined by alkali extraction and washing, bleaching and deodorization to remove undesirable constituents and to prolong shelf life (Codex Alimentarius 2006).

Codex gives a general definition for "virgin oils", which states that such oils should be suitable for human consumption in its natural state and may be purified by "washing with water, settling, filtering, and centrifuging only". However, no specific standard for virgin coconut oil (VCO) has been made.

In response to the specific needs of coconut producers, the APCC (2006) promulgated an interim standard for VCO. It should be noted that while APCC gives a similar definition as Codex, it has an added condition that VCO may be produced by "natural means" as long as this does not alter the oil.

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A number of papers have been written on the fatty acid composition, minor components, and physico-chemical characteristics of coconut oil. Banzon and Resurreccion (1979) reported that the manner of processing does not affect the fatty acid profile. Padolina, Lucas, and Torres (1987) wrote a comprehensive review of chemical and physical properties of coconut oil. This review included a discussion of the major and minor chemical constituents of coconut oil and various physical properties. Laureles and co-workers (2002) observed varietal differences in fatty acid composition, particularly in the C8 and C10 components. Dia and co-workers (2005) conducted a comparative physico-chemical study on VCO and RBD CNO to determine whether these could be differentiated. They prepared VCO using 3 methods and 3 types of coconut meat. Among the VCO samples, they found that although differences in chemical and quality properties were noted, these were not significant enough to affect their overall quality. For all samples, their properties were within the Codex standards for coconut oil.

In 2004, an intergovernmental technical panel drafted the interim Philippine National Standard for Virgin Coconut Oil (PNS/BAFPS 22:2004) in response to the need to specify quality standards for VCO products. PNS/BAFPS 22:2004 was based largely on Codex and APCC (Table 1). However, there remained the imperative for a standard that would differentiate VCO from RBD CNO.

In 2006, a new labeling requirement was introduced by the US Food and Drug Administration regarding the trans-fatty acid content in vegetable oils (US FDA 2007). The major source of trans-fats is partial hydrogenation and high temperature processing of polyunsaturated vegetable oils, such as corn and soya oils.

There are 2 methods used for the determination of trans-fatty acids: infrared (IR) spectroscopy and gas chromatography (GC). IR spectroscopy is simpler but it is not accurate below 5% and is subject to interferences. GC analysis of fatty acid methyl esters (FAME) is more accurate, but prior silver-ion TLC separation is recommended for oils with high levels of polyunsaturated fatty acids (LFI 2006). Because coconut oil contains less than 10% total unsaturated fats (oleic acid and linoleic acid), direct GC analysis after methylation is adequate.

In this work, commercial VCO, RBD CNO, and copra oil were analyzed to determine whether these can be differentiated using standard chemical methods of analysis. The standard methods from Codex Alimentarius were reassessed using appropriate reference compounds and internal standards. Response factors, spikes, and % recoveries were performed. The following standard methods were applied: % fatty acid profile by GC including analysis of trans-fatty acids, % moisture by Karl Fischer method, % matter volatile at 120° C by gravimetric

Table 1. Quality parameters from existing standards: Codex Alimentarius for coconut oil, APCC for virgin coconut oil, and interim Philippine National Standard for VCO (PNS / BAFPS 22:2004)

Parameter	Codex Alimentarius	APCC	PNS / BAFPS 22:2004
% Fatty acid composition			
C6:0	ND- 0.7	0.4 - 0.6	ND -0.7
C8:0	4.6- 10.0	5.0 - 10.0	4.6 - 10
C10:0	5.0- 8.0	4.5 - 8.0	5.0 - 8.0
C12:0	45.1 53.2	43.0 - 53.0	45.1 -53.2
C14:0	16.8- 21.0	16.0 - 21.0	16.8 -21
C16:0	7.5- 10.2	7.5 - 10.0	7.5 -10.2
C18:0	2.0- 4.0	2.0 - 4.0	2.0 - 4.0
C18:1	5.0- 10.0	5.0 - 10.0	5.0 - 10.0
C18:2	1.0- 2.5	1.0 - 2.5	1.0 - 2.5
C18:3	ND- 0.2		ND -0.2
C20:0	ND- 0.2	<0.5	---
C20:1	ND- 0.2		---
C20:2 – C24:1	ND		ND
Iodine value	6.3 – 10.6	4.1 – 11.00	---
% Free fatty acid	None	≤0.5%	0.20%
% Moisture, max	---	0.1 – 0.5	0.2%
% Volatile matter at 105°C, m/m	0.2%	0.2%	0.2%
Peroxide value, meq/kg oil	<15	< 3	3.0
Microbiological contamination, cfu/mL	---	<10	

method, iodine value, peroxide value, % free fatty acid (as lauric acid), and microbial contamination by colony forming units (cfu).

MATERIALS AND METHODS

Coconut oil samples

Commercial samples of VCO were provided by members of the Virgin Coconut Oil Producers and Marketers Association, Inc. (VCO Association) and were purchased commercially. Samples of RBD CNO were purchased from supermarkets and were provided by Spring Oil Co. (Malabon). Copra oil samples were provided by the Philippine Coconut Authority.

Thirty-three coconut oil samples were analyzed: commercial VCO (n=20), RBD CNO (n=10), and copra oil (n=3). Information on the samples is provided in Table 2.

Determination of % fatty acid composition and trans-fatty acids in coconut oil by GC

This procedure is based on AOAC Official Method 969.33/963.22 (AOAC 1995). The fatty acid (FA) and FAME standards used in this analysis were obtained from Sigma-Aldrich: octanoic acid (C8, 99+%), methyl octanoate (C8ME, 99%), undecanoic acid (C11, 99%), methyl undecanoate (C11ME, 99%), lauric acid (C12, 98%), methyl laurate (C12ME, 99.5%), stearic acid (C18, 99%), methyl stearate (C18ME, 99%), oleic acid (C18:1c9, 99%), and linoleic acid (C18:2c9,c12, 99+%). Standards used for trans-fatty acids were: trans-9-octadecenoic acid (C18:1t9, 99%) and trans-13-octadecenoic acid (C18:1t13, 99%).

The % fatty acid composition was determined by hydrolysis and methylation of coconut oil sample together with the C11 internal standard using the boron trifluoride method to produce the FAMEs, followed by GC analysis. One µL of FAME extract was then injected into a Shimadzu GC-14B gas chromatograph equipped with flame ionization detector. Separation was done on a DB-1 capillary column (J&W Scientific, polydimethylsiloxane, 60m x 0.25mm i.d. x 0.25 µm film thickness) with the following oven temperature program: initial temperature at 60º C, hold for 6 min; increase to 180º C at 5º C/min, hold for 2 min; increase to 210º C at 5º C/min, and increased to 230º C at 1º C/min, hold for 5 min. The injector and detector temperatures were set at 210º C and 230º C, respectively.

The relative GC response factors were obtained for C8ME, C12ME, C18:0ME, C18:1c9ME, C18:2c9,c12ME, C18:1t9ME and C18:1t13ME versus the C11ME internal standard (IS) by taking the average response factor from the methylation of 5 solutions of the respective fatty acids prepared within the expected concentration range for each fatty acid. The R^2 values of the calibration lines for the FAME standards were better than 0.99. The response factors for the other saturated FAMEs were obtained by extrapolation from the plot of carbon number versus response factor. The %FAME composition for each sample was converted to %FA composition (w/w) by molecular weight correction. Eight replicate analyses of a VCO sample gave a 1% relative standard deviation (RSD) for C12 and C14 FA and <5% RSD for the other fatty acids. GC analysis of coconut oil samples was done in duplicate.

Table 2. List of coconut oil samples used in this study. Total number of samples = 33

Classification (number of samples)	Description (number of samples)	Code
Virgin coconut oil (n=20)	The sources of nuts of the VCO samples were Batangas, Laguna, Quezon and Davao.	
	Centrifuge (n=5)	Cen1 ~ Cen5
	Expeller (n = 5)	Exp1 ~ Exp5
	Enzymatic (n=2)	Enz1, Enz2
	Fermentation with heat (n=3)	FWH1 ~ FWH3
	Fermentation without heat (n=4)	FNH1 ~ FNH4
	Settling (n=1)	Set1
RBD CNO (n=10)	Commercial brands used: Baguio, Cook Best, Magic Fry, Minola, Nutri Oil and Spring Oil	RBD1 ~ RBD10
Copra oil (n=3)	The copra oil samples were obtained from Lucena, Davao, and Zamboanga.	Cop1 ~ Cop3

Moisture content by Karl Fischer titration. This procedure is based on AOAC Official Method 984.20 (AOAC 1995). The moisture content was determined using a Metrohm 785 DMP Titrino Karl Fischer titrator. This method gave a recovery of 99-102% and a detection limit of 0.01%. Analysis of samples was done in duplicate.

Volatile matter content by gravimetric analysis. Codex (2006) stipulates a gravimetric procedure using oven heating of the oil sample at 105° C and repeated until constant weight is obtained. However, comparison of preliminary results from Karl Fischer determination indicated that a higher temperature is needed for reproducible results, and a larger amount of sample is needed to obtain a comparable detection limit for the gravimetric method. Additional determinations were carried out at 103, 114, 120, and 133° C using 5 g and 20 g sample amounts. A temperature of 120° C using 20 g oil sample was found to give the best results and was used for this study.

Heating at 120° C gave a 75% average higher loss of volatile matter compared with 105°C. Eight replicate analyses at 120° C gave a repeatability standard deviation of 0.02%. Coconut oil samples spiked with known amounts of water gave an average recovery of 106 - 111% at 120° C with a detection limit of 0.01%. Analysis of samples was done in duplicate.

% Free fatty acids as lauric acid.
This procedure is based on AOAC Official Method 940.28 (AOAC 1995). Recovery of the method was 83%, which corresponds to the difference of 1 drop of titrant. Analysis of samples was done in duplicate.

Iodine value
This procedure is based on AOAC Official Method 920.158 (AOAC 1995). The % recovery of the method was determined to be 92% and 85% for oleic acid and linoleic acid, respectively. Analysis of samples was done in duplicate.

Peroxide Value
This procedure is based on AOAC Official Method 965.33 (AOAC 1995). The minimum detectable amount was 0.1 meq/kg. Analysis was done in duplicate.

Microbial contamination
The determination of microbial contamination was carried out by the Microbiological Services Laboratory, Natural Sciences Research Institute, University of the Philippines in Diliman. The colonies appearing per plate were counted and the number of colony-forming units (cfu) per mL.

Principal Components Analysis
Principal components analysis (PCA) was performed using The Unscrambler™ (CAMO Process AS, Oslo, Norway). The data were first normalized and standardized before PCA was carried out.

RESULTS

Coconut oil samples
The VCO samples were submitted by the VCO Association as coded samples and were commercial products of the VCO producers. Six types of commercial VCO production methods were included in this study: centrifuge, expeller, enzymatic, fermentation with heat, fermentation without heat, and settling (Table 2). The number of samples analyzed roughly reflects the number of producers who use the particular method. Production conditions, such as variety and age of coconut, manner of handling, temperature, and time of processing were not controlled. RBD CNO samples were purchased from retail outlets and obtained from Spring Oil. Copra oil samples were provided by the Philippine Coconut Authority.

% Fatty acid composition
The % fatty acid composition is the most important parameter used to differentiate the various vegetable oils. The GC response factor for each FAME standard was obtained versus the IS at the expected composition level. For example, the response factor for methyl laurate was determined by averaging the response factors of 5 solutions within the range 40 to 60% versus a constant IS concentration of 5%, while the response factor for methyl stearate was determined within the range 0 to 5% against the same IS concentration of 5%. The linearity of the individual plots was better than 0.99. The response factors for the homologous saturated FAME compounds versus the IS were plotted against carbon number (Figure 1). From the plot, the response factors for the other saturated FAMEs were obtained. It should be noted that the response factors for the various saturated FAMEs vary and are applicable only to the specific GC conditions used.

The % FAME profile for each sample was determined against the IS, and the % FA composition was calculated by molecular weight correction. The % FA composition of the coconut samples generally fell within the Codex and APCC standards, except for some minor variances for C6, C8, and C10 (Table 3).

The results obtained in this study involved the use of a IS with individual response factors and molecular weight correction from % FAME to % FA composition. In

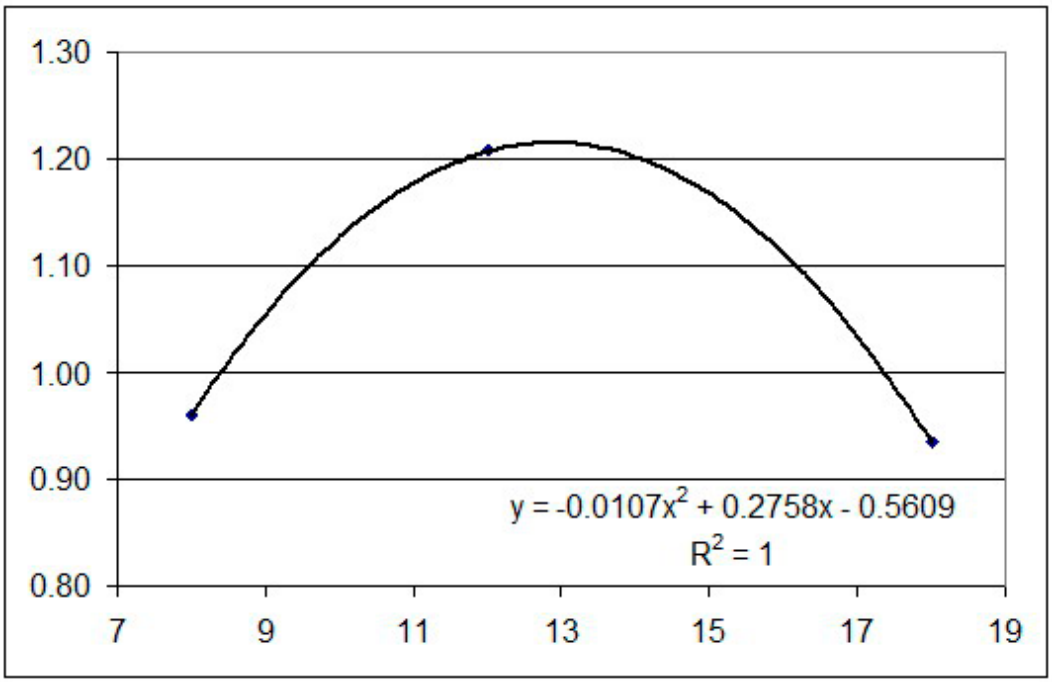

Figure 1. Plot of relative response factor of homologous saturated FAMEs versus C11ME internal standard

Table 3. Comparison of % FA profile with Codex Alimentarius and APCC. The results of the Principal Components Analysis (PCA) of the fatty acid profiles are plotted in Figure 2. The analysis for trans-fatty acids by GC using C18:1t9 and C18:1t13 as reference compounds gave a negative result down to a detection level of 0.01% for all coconut oil samples analyzed

Standard	Fatty acid, %								
	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1c9	C18:2c9,c12
Codex Alimentarius	ND ~ 0.7	4.6 ~10.0	5.0 ~ 8.0	45.1 ~ 53.2	16.8 ~ 21.0	7.5 ~ 10.2	2.0 ~ 4.0	5.0 ~ 10.0	1.0 ~ 2.5
APCC	0.4 ~ 0.6	5.0 ~10.0	4.5 ~ 8.0	43.0 ~ 53.0	16.0 ~ 21.0	7.5 ~ 10.0	2.0 ~ 4.0	5.0 ~ 10.0	1.0 ~ 2.5
Samples									
All CNO samples									
Average	0.40	7.06	5.11	48.62	17.82	8.57	3.42	7.28	1.71
Range	0.23 ~ 0.59	4.15 ~ 9.23	4.17 ~ 6.08	46.0 ~ 52.6	15.5 ~ 19.7	7.65 ~ 10.1	2.73 ~ 4.63	5.93 ~ 8.54	1.00 ~ 2.36
VCO samples									
Average	0.40	7.23	5.21	48.66	17.82	8.51	3.50	7.16	1.52
Range	0.24 ~ 0.56	4.15 ~ 9.23	4.27 ~ 6.08	46.0 ~ 52.6	16.0 ~ 19.7	7.65 ~ 10.1	2.73 ~ 4.63	5.93 ~ 8.53	1.00 ~ 2.03
RBD CNO samples									
Average	0.41	6.61	5.00	48.14	17.88	8.88	3.26	7.63	2.19
Range	0.32 ~ 0.59	5.32 ~ 8.83	4.56 ~ 6.03	46.7 ~ 49.4	16.2 ~ 19.6	7.80 ~ 9.73	2.94 ~ 3.69	7.24 ~ 8.04	1.82 ~ 2.36
Copra oil samples									
Average	0.34	6.74	4.61	49.16	17.77	8.50	3.23	7.49	2.16
Range	0.23 ~ 0.40	5.48 ~ 7.39	4.17 ~ 5.07	48.33 ~ 49.82	17.18 ~ 18.09	7.80 ~ 9.48	3.04 ~ 3.43	6.71 ~ 8.54	2.08 ~ 2.31

comparison, GC results from simple area integration and normalization gives only relative % FAME composition and results in a different profile. For example, % C12ME is up to 2% higher, while lower values are obtained for shorter and longer chain FAMEs.

Trans-fatty acids

In this study, C18:1t9 and C18:1t13 were selected as the trans-fatty acid reference compounds. Calibration solutions of the trans-fatty acids and IS were prepared down to the 0.01% level. Analysis of the coconut oil samples by GC-MS

did not detect the presence of C18:1t9 and C18:1t13 or any other mono-unsaturated C18 fatty acid, apart from C18:1c9 (oleic acid), down to the 0.01% detection limit.

Moisture content by Karl Fischer titration

Moisture is an important factor that determines the product quality of VCO. High moisture increases hydrolysis, which leads to a higher free fatty acid content and hydrolytic rancidity.

The Karl Fischer titration method is a voltammetric method that is widely used for the direct determination of water in oils (Hahn 2006). The volumetric Karl Fischer method used in this study has a detection limit of about 0.01% water (Aquastar 2007). By Karl Fischer, the moisture content of the VCO samples averaged 0.08%, with a range of 0.05 to 0.12% (Table 4). The data suggest that most commercial VCO manufacturers are able to produce VCO with moisture content below 0.1%. On the other hand, RBD CNO samples had lower average moisture content of 0.05%, with a range from 0.01 to 0.10% moisture. Copra oil gave a higher average moisture of 0.11%, with a range of 0.08 to 0.14%.

Although the respective average moisture contents of VCO, RBD CNO, and copra oil differ, there is a significant overlap in the values of individual samples.

Volatile matter

Codex and APCC specify the gravimetric method for the determination of moisture content and stipulates a maximum loss of 0.2% at 105° C. However, because this weight loss corresponds to the loss of both water and volatile organic compounds (VOCs), this is not an accurate determination of moisture content.

We sought to improve the precision and detection limit of this method and found that better results can be obtained at 120°C using a 20 g sample size, with an experimental detection limit of 0.02%.

VCO samples gave an average mass loss of 0.13%, with a range of 0.07 to 0.18%. In comparison, RBD CNO samples gave an average mass loss of 0.03% with a range of ND to 0.08%.

On the other hand, copra oil gave high average mass losses of at least 1.91% (Table 4). Some copra oil samples continued to lose weight even after 3 days of heating. For these samples, the minimum mass loss was reported. Because the mass loses were so high, this does not affect the conclusions of the analysis.

The difference between the % volatile matter and % moisture by Karl Fischer titration can be assigned to VOCs (Table 4). The VCO samples had an average % VOC of 0.04%, with a range of 0.00 to 0.08%. All of the RBD CNO samples analyzed, on the other hand, had a % VOC content of 0.00%. This means that all of the volatile matter of RBD CNO, is water, and RBD CNO contains no detectable VOC. In contrast, copra oil gave a high VOC level of 1.77%.

Taken together, the % volatile matter and % moisture by Karl Fischer can be used to differentiate VCO from both RBD CNO and copra oil. RBD CNO samples have negligible VOCs, while copra oil samples give much higher VOC levels. Therefore, VCO can be differentiated from RBD CNO and copra oil by the VOC content.

It should be noted, however, that some VCO production processes tend to give very low VOC content, particularly the centrifuge method.

% Free fatty acids as lauric acid

Free fatty acids (FFAs) are naturally present at low amounts in all vegetable oils. During extraction and storage, additional FFAs may be formed by reaction with residual water in the oil. Hydrolysis can occur by chemical or enzymatic mechanisms. Enzymatic hydrolysis (e.g., with lipases) may occur through indigenous plant enzymes or microbial contaminants. High levels of FFA are undesirable because of their unpleasant taste.

The APCC standard for free fatty acids is 0.5% as lauric acid. In this study, the VCO samples gave an average value of 0.131%, with a range of 0.037 to 0.337%. For RBD CNO samples, the FFAs were lower as expected, with an average of 0.021% and a range of 0.008 to 0.076%, while copra oil samples gave a relatively higher FFA average of 1.410% with a range of 0.660 to 2.502% (Table 4). There is some overlap in the individual values of FFA in VCO and RBD CNO.

We can conclude that the VCO and RBD CNO samples met the APCC standard for FFA of 0.5%, but copra oil did not. However, based on studies conducted by the Philippine Coconut Authority, a 0.2% FFA limit is recommended (Gonzales 2004).

Iodine value

The iodine value refers to the percentage by weight of molecular iodine, I_2, absorbed by an oil or fat and is a standard procedure for determining the amount of unsaturation in an oil sample. This test involves the addition of iodine to double bonds, although small quantities of substitution products may be formed. Vegetable oils can be differentiated by the amount of I_2 that is absorbed (Kolthoff and Stenger 1942). For coconut oil, the Codex range for iodine value is 6.3 to 10.6, while for APCC it is 4.1 to 11.0.

Table 4. Results of analyses of VCO, RBD CNO and copra oil, and comparison with Codex and APCC standards. ("NA": not analyzed)

Standard	% Moisture, Karl Fischer	% Volatile matter, (w/w)[1]	% VOCs (w/w)[2]	% FFA, as Lauric acid	Iodine value	Peroxide value, (meq/kg oil)[3]	Microbial contamination, (cfu/mL)[4]
Codex Alimentarius	-	0.2	-	-	6.3 – 10.6	15	-
APCC	-	0.2	-	<0.5%	4.1 – 11.0	≤3	≤10
Sample							
VCO							
Cen1	0.05	NA	NA	0.057	7.64	0.89	<10
Cen2	0.05	NA	NA	0.337	5.83	0.88	<10
Cen3	0.09	0.16	0.06	0.178	5.87	0.67	<10
Cen4	0.08	0.12	0.04	0.047	7.94	0.00	<10
Cen5	0.07	0.07	0.00	0.180	6.59	0.50	<10
Enz1	0.07	NA	NA	0.079	6.79	0.56	<10
Enz2	0.10	0.12	0.02	0.086	7.76	0.95	<250
Exp1	0.05	NA	NA	0.042	5.64	0.10	<250
Exp2	0.11	NA	NA	0.184	5.89	0.10	<250
Exp3	0.10	0.15	0.05	0.038	6.29	0.00	<10
Exp4	0.12	0.15	0.02	0.124	6.88	0.47	<10
Exp5	0.07	0.09	0.02	0.085	NA	0.09	NA
FNH1	0.05	NA	NA	0.207	7.56	0.48	<10
FNH2	0.10	0.18	0.08	0.180	7.47	0.73	<10
FNH3	0.08	0.14	0.06	0.129	8.07	0.00	<250
FNH4	0.10	0.13	0.03	0.037	8.09	0.86	<10
FWH1	0.09	0.13	0.04	0.093	10.34	0.00	<10
FWH2	0.07	0.12	0.04	0.164	7.99	0.67	<10
FWH3	0.08	0.12	0.04	0.211	7.30	1.86	<10
Set1	0.05	NA	NA	0.167	8.30	1.43	<10
RBD CNO							
RBD01	0.09	NA	NA	0.018	8.32	0.80	<10
RBD02	0.10	NA	NA	0.076	8.91	0.30	<10
RBD03	0.02	0.01	0.00	0.012	7.62	0.83	<10
RBD04	0.02	0.00	0.00	0.011	6.81	1.19	<10
RBD05	0.02	0.00	0.00	0.011	8.34	1.65	<10
RBD06	0.04	0.04	0.00	0.021	NA	0.35	NA
RBD07	0.07	0.08	0.00	0.030	NA	0.51	NA
RBD08	0.07	0.07	0.00	0.015	NA	0.27	NA
RBD09	0.03	0.03	0.00	0.008	NA	0.49	NA
RBD10	0.01	0.00	0.00	0.011	NA	3.39	NA
Copra							
Cop1	0.08	NA	NA	0.660	7.31	2.77	<250
Cop2	0.14	>1.91	>1.77	2.502	6.61	0.94	<250
Cop3	0.12	NA	NA	1.067	6.91	0.72	28
Average values							
All CNO samples	0.07	0.17	0.10	0.214	7.37	0.77	
VCO samples	0.08	0.13	0.04	0.131	7.28	0.56	
RBD CNO samples	0.05	0.03	0.00	0.021	8.00	0.98	
Copra oil	0.11	>1.91	>1.77	1.410	6.94	1.48	

[1] % Volatile matter: Conditions specified by Codex Alimentarius and APCC: 5 g and 105° C. Conditions used in this study: 20 g and 120° C. Detection limit: 0.01%.
[2] %VOCs were obtained by subtracting the individual values of the % Moisture (Karl Fischer) from the % Volatile matter.
[3] Peroxide value: Detection limit: 0.1 meq/kg.
[4] Count of number of samples with microbial contamination levels of <10 and <250 cfu/mL.

The iodine values obtained for the VCO samples in this study gave an average value of 7.28 and a range of 5.64 to 10.34 (Table 4). VCO samples that include the testa in its preparation gave the highest iodine value of 10.34. The iodine value of the RBD CNO samples, on the other hand, gave an average of 8.00, with a range of 6.81 to 8.91, while copra oil gave an average of 6.94, and a range of 6.61 to 7.31. There is no differentiation among the different types of coconut oil samples as far as iodine value is concerned.

All of the samples comply with APCC but some do not comply with Codex. Thus, for coconut oil, the APCC standard for iodine value is more appropriate than Codex.

The results of the iodine value can be cross-checked against the % FA acid profile obtained by GC analysis. Since the iodine value is a measure of total double bonds in an oil sample, the iodine value should be comparable with the GC results in the absence of other olefinic compounds. However, it should be noted that the % recovery for the iodine value method was determined to be 92% and 85% for oleic acid and linoleic acid, respectively. This means that the addition of iodine is incomplete and that the iodine value tends to underestimate the number of double bonds in the fatty acids present.

The number of milliequivalents of double bonds from unsaturated fatty acids per gram of oil can be calculated from the amount of C18:1 and C18:2 present in the sample (Table 5, columns 1 and 2, respectively); the sum gives the total milliequivalent double bonds per gram of sample arising from unsaturated fatty acids by GC analysis (column 3). The calculated iodine values from the GC data can be obtained by multiplying the meq double bond with twice the atomic weight of iodine (column 4).

Column 5 of Table 5 shows the experimental iodine values. The calculated total milliequivalent double bonds per gram of oil sample can be obtained by dividing this value by twice the atomic weight of iodine (column 6).

A comparison of columns 3 and 6 shows that while there is general comparability between GC analysis and the iodine value method, the iodine values are lower by an average of 15%. This discrepancy may arise from the incomplete reaction of I_2 with double bonds. On the other hand, the presence of other compounds with unsaturation will increase the iodine value relative to the GC value. Consistent with this, higher iodine values were obtained for Minola oil, which is enriched with Vitamin A, and a VCO sample that included the testa.

Peroxide value

Olefinic bonds in unsaturated fatty acids are oxidized over time or during high temperature processing resulting in the formation of hydroperoxides which leads to rancidity (Gunstone 1996). The peroxide value is based on the reaction of hydroperoxides with potassium iodide. This reaction yields molecular iodine (I_2), which is then titrated using standard solution of sodium thiosulfate. The peroxide value is expressed in meq active oxygen (peroxide) per kilogram of oil sample.

Table 5. Number of (milliequivalent double bond / gram of coconut oil) from GC and iodine value

Sample	Unsaturated FA from Gas Chromatography				Iodine value	
	meq double bond/g			(4) Iodine value (theoretical from column 3)	(5) Iodine value (exptl.)	(6) meq double bond/g (calc. from column 5)
	(1) C18:1	(2) C18:2	(3) Total meq double bond/g (exptl.)			
All CNO samples						
Average	0.22	0.10	0.33	8.34	7.30	0.29
Range	0.18 ~ 0.28	0.06 ~ 0.15	0.25 ~ 0.44	6.26 ~ 11.05	5.28 ~ 10.34	0.21 ~ 0.41
VCO samples						
Average	0.22	0.09	0.31	7.99	7.28	0.29
Range	0.18 ~ 0.27	0.06 ~ 0.12	0.25 ~ 0.38	6.26 ~ 9.76	5.64 ~ 10.34	0.22 ~ 0.41
RBD CNO samples						
Average	0.23	0.14	0.37	9.38	8.00	0.32
Range	0.22 ~ 0.25	0.11 ~ 0.15	0.33 ~ 0.40	8.48 ~ 10.25	6.81 ~ 8.91	0.27 ~ 0.35
Copra oil samples						
Average	0.24	0.14	0.38	9.54	6.94	0.27
Range	0.21 ~ 0.28	0.13 ~ 0.15	0.34 ~ 0.44	8.62 ~ 11.05	6.61 ~ 7.31	0.26 ~ 0.29

Codex gives a peroxide value limit of 15 meq/kg for virgin oils in general, while APCC specifies 3 meq /kg oil for VCO. The VCO samples in this study gave an average value of 0.56 and a range of ND to 1.86 (Table 4). The low average peroxide value indicates that commercial VCO samples do not undergo significant peroxidation during processing. The VCO sample that gave the highest peroxide value of 1.86 was processed using heat (FWH3). However, the temperature used for processing was not provided.

RBD CNO samples, on the other hand, gave an average peroxide value of 0.98 and a range of 0.27 to 3.39. The higher peroxide values are consistent with high processing temperatures. Copra oil gave the highest average peroxide value of 1.48 with a range of 0.72 to 2.77.

The peroxide values for all coconut oil samples, except for 1 RBD CNO sample that gave a relatively high peroxide value of 3.39, were well below the APCC limit of 3 meq/kg oil. These peroxide values are much lower than those allowed for polyunsaturated vegetable oils (Codex 2006). Coconut oil is the most oxidatively stable vegetable oil; oxidative rancidity is not a significant cause of degradation.

Microbial contamination

The APCC standard is <10 colony forming units/mL. Failure to meet this standard indicates that the product is of poor quality and is a potential health hazard (BFAD 2004). Fifteen out of 19 VCO samples analyzed for microbial contamination gave a result of <10 cfu/mL, and 4 samples gave a result of <250 cfu/mL. The samples that gave high cfu/mL values were roughly distributed randomly among the various VCO processes. This suggests that microbial contamination in VCO products is due to the quality of production, and not the type of process. The 5 RBD CNO samples tested gave a result of <10 cfu/mL, while all 3 copra oil samples were <250 cfu/mL (Table 4).

Principal components analysis

PCA was applied to the % FA composition data to determine whether there is a correlation between % FA composition and type of sample. The PCA scores plot (Figure 2) indicates that there is no correlation between % FA composition and type of sample. That is, % FA composition cannot be used to differentiate among the various types of coconut oil, whether VCO, RBD CNO, or copra oil. This result is consistent with the conclusions of Banzon and Resurreccion (1979).

PCA was then performed using selected chemical parameters to determine which combination of parameters gives the maximum separation of the 3 groups of samples. The PCA results indicate that the use of % moisture, % VOC and % FFA is best able to distinguish the 3 types of CNO (Figure 3). That is, these 3 parameters are the most useful for differentiating VCO from RBD CNO and copra oil. However, the spread of VCO samples in Figure 3 also suggests that the commercial VCO samples, regardless of the preparation method, vary in the characteristics of % moisture, % VOC, and % FFA.

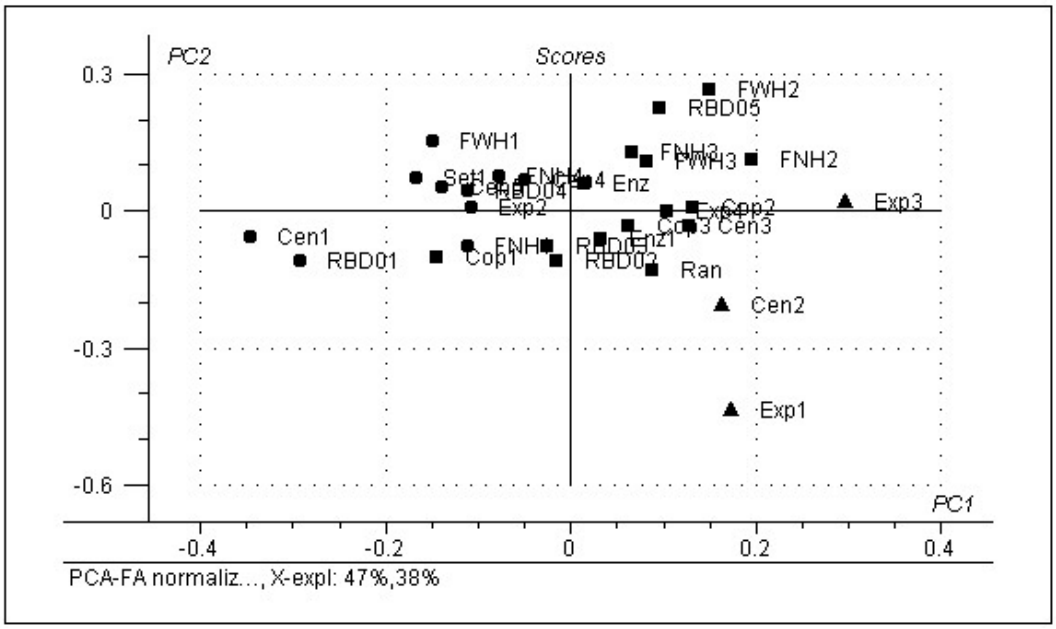

Figure 2. PCA analysis (scores plot) of VCO, RBD CNO and copra oil samples based on % fatty acid composition. (See Table 2 for sample codes.)

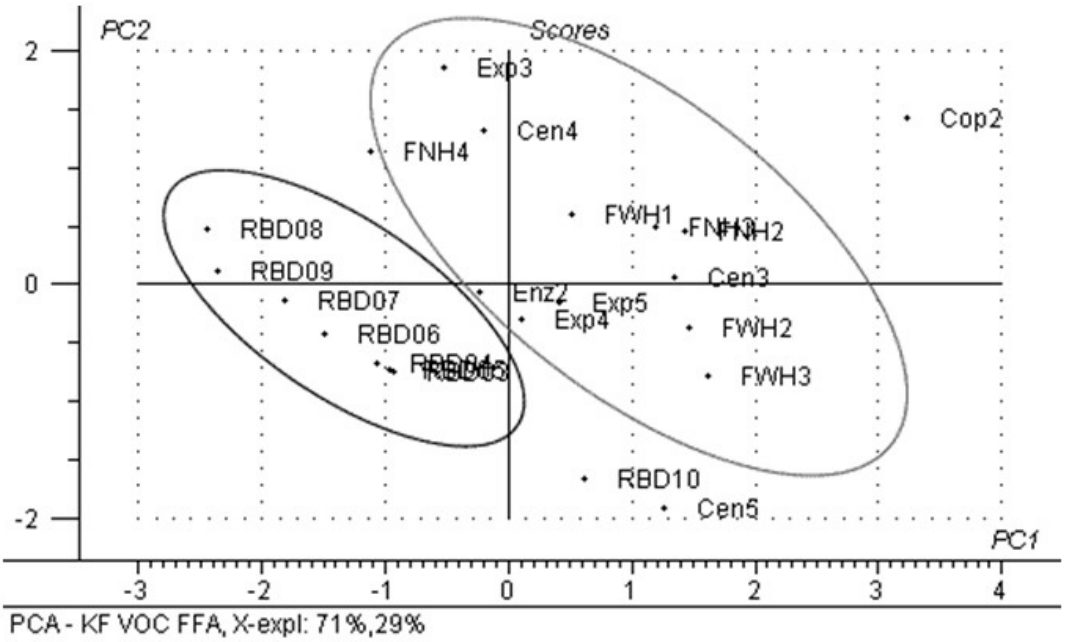

Figure 3. PCA analysis (scores plot) of VCO, RBD CNO and copra oil samples using % moisture by Karl Fischer, %VOC, %FFA give the maximum separation of the three groups of samples. RBD10 and Cen5 are outliers in their respective groups. (See Table 2 for sample codes.)

Stated differently, to attain uniformity in VCO product quality, these three parameters should be monitored. It should be noted, however, that % VOC is the difference between % volatile matter and % moisture. Adequate and reproducible amounts of volatile matter can be obtained by avoiding high heat and high vacuum; these methods should not be used to remove moisture from the oil as they also remove VOCs.

DISCUSSION

This paper sought to address the question of whether VCO products can be differentiated from RBD CNO and copra oil. To do this, the following standard analyses were conducted: % FA profile, % moisture by Karl Fischer titration, % volatile matter at 120° C, % FFA as lauric acid, iodine value, peroxide value, and microbial contamination.

Absolute % FA composition was obtained using response factors for each FAME versus an IS, followed by conversion of % FAME to % FA composition. While the % FA composition of all the coconut oil samples analyzed generally fell within the Codex and APCC standards, some minor differences were noted in the lower limits of the following FAs: C6, C8, C10, and C14. The % C12 compositions for all samples were within the Codex and APCC ranges.

However, it should be anticipated that if coconut varieties with higher lauric acid content are developed, an adjustment of the fatty acid profile may be necessary. The % FA composition cannot differentiate VCO from RBD CNO and copra oil.

The analysis for trans-fatty acids by GC using C18:1t9 and C18:1t13 as reference compounds gave a result of ND down to a detection level of 0.01% for all coconut oil samples analyzed.

The results of the iodine value test were within the Codex and APCC standards. However, this test cannot differentiate among VCO, RBD CNO and copra oil. It should be noted that because the iodine value result can be increased by additives, such as vitamin A reinforcement and the use of testa, it cannot be used as a reliable measure for unsaturated fatty acids.

The iodine value test can be replaced by a quantitative measurement of the unsaturated fatty acids by GC. The determination of total double bonds from GC analysis was generally comparable with the results from the iodine value method. But results from the iodine value tend to be lower by an average of 15% due to incomplete reaction.

The measurement of % moisture should be made more specific by the use of Karl Fischer titration. The volumetric method is of sufficient reliability and sensitivity. The results show that VCO samples can readily meet the standard for moisture of ≤0.10%.

The measurement % volatile matter can be improved by determination at 120°C instead of 105°C, and using a 20 g sample. Based on the VCO samples analyzed, a standard of 0.12 to 0.20% volatile matter is suggested.

Subtraction of % moisture from % volatile matter gives the volatile organic compounds. The VCO samples analyzed generally gave VOC content from ND to 0.08%. The VCO samples with low VOC content may have been processed using heat and/or vacuum. In comparison, RBD CNO samples gave negligible values, while copra oil had a higher value. Therefore, estimate of the VOC content from the % volatile matter and % moisture is the simplest strategy for differentiating VCO from both RBD CNO and copra oil.

PCA showed that proper control of the VCO manufacturing process is needed to maintain correct and reproducible levels of moisture, VOC, and FFA.

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