

Quality characteristics of virgin coconut oil: Comparisons with refined coconut oil*

Fabian M. Dayrit^{1,‡}, Ian Ken D. Dimzon¹, Melodina F. Valde¹,
Jaclyn Elizabeth R. Santos¹, Mark Joseph M. Garrovillas¹, and
Blanca J. Villarino²

¹Department of Chemistry, Ateneo de Manila University, Katipunan Avenue, Loyola Heights, Quezon City 1108, Philippines; ²Department of Food Science and Nutrition, College of Home Economics, University of the Philippines, Diliman, Quezon City 1108, Philippines

Abstract: Virgin coconut oil (VCO) is a vegetable oil that is extracted from fresh coconut meat and is processed using only physical and other natural means. VCO was compared to refined, bleached, and deodorized coconut oil (RCO) using standard quality parameters, ³¹P nuclear magnetic resonance (NMR) spectroscopy, and headspace solid-phase microextraction/gas chromatography mass spectrometry (SPME/GCMS). VCO tends to have higher free fatty acids (FFAs), moisture, and volatile matter and lower peroxide value than RCO. However, the range of values overlap and no single standard parameter alone can be used to differentiate VCO from RCO. Using ³¹P NMR, VCO and RCO can be distinguished in terms of the total amount of diglycerides: VCO showed an average content (w/w %) of 1.55, whereas RCO gave an average of 4.10. There was no overlap in the values found for individual VCO and RCO samples.

There are four common methods of producing VCO: expeller (EXP), centrifuge (CEN), and fermentation with and without heat. VCO products prepared using these four methods could not be differentiated using standard quality parameters. Sensory analysis showed that VCO produced by fermentation (with and without heat) could be distinguished from those produced using the EXP and CEN methods; this sensory differentiation correlated with the higher levels of acetic acid and octanoic acid in the VCO produced by fermentation.

Studies on physicochemical deterioration of VCO showed that VCO is stable to chemical and photochemical oxidation and hydrolysis. VCO is most susceptible to microbial attack, which leads to the formation of various organic acids, in particular, lactic acid. However, at moisture levels below 0.06 %, microbial action is significantly lessened.

Keywords: food chemistry; headspace solid-phase microextraction/gas chromatography mass spectrometry (SPME/GCMS); ³¹P NMR; sensory analysis; virgin coconut oil (VCO).

INTRODUCTION

Coconut oil is a vegetable oil that is derived from the kernel of *Cocos nucifera*, Linn. Commercial refined coconut oil (RCO) is extracted from dried copra, and the resulting crude oil is processed on an industrial scale by washing, bleaching, and deodorization [1]. Virgin coconut oil (VCO), on the other

*Paper based on a presentation made at the 11th Eurasia Conference on Chemical Sciences, The Dead Sea, Jordan, 6–10 October 2010. Other presentations are published in this issue, pp. 1643–1799.

[‡]Corresponding author

hand, is extracted from fresh mature coconut meat and processed using only physical or natural means [2,3]. There are four common methods of producing VCO: expeller (EXP), centrifuge (CEN), and fermentation with (FWH) and without heat (FNH). VCO is considered as a traditional product that is produced by small to medium enterprises.

Recently, there has been a growing interest in VCO as a functional food and cosmetic [4]. With the growing demand, it was important that the quality characteristics of VCO be studied and improved. This paper presents some comparisons between VCO and RCO, as well as studies that compare VCO products that are produced using different methods.

DIFFERENTIATING VCO FROM REFINED, BLEACHED, AND DEODORIZED COCONUT OIL

Table 1 compares the Codex standards for coconut oil, the Asian and Pacific Coconut Community (APCC) standards for VCO, and the range of values for RCO and VCO products tested. The results indicate that, consistent with previous reports [5,6], the manner of processing does not affect the free fatty acid (FFA) profile. Also, VCO can be differentiated from RCO using the test for % volatile matter volatile at 120 °C, % FFA and peroxide value. In particular, RCO gives off much fewer volatiles and has lower % FFA and peroxide value when compared to VCO [7].

Table 1 Standard quality parameters from Codex Alimentarius for coconut oil and APCC for VCO in comparison with the range of values from commercial RCO and VCO products. The average experimental values are given in parentheses.

Parameter	Codex standard for coconut oil [2]	APCC standard for VCO [3]	RCO products, <i>n</i> = 10 [7]	VCO products, <i>n</i> = 20 [7]
% FFA composition				
C6:0	ND–0.7	0.4–0.6	0.32–0.59 (0.41)	0.24–0.56 (0.40)
C8:0	4.6–10.0	5.0–10.0	5.32–8.83 (6.61)	4.15–9.23 (7.23)
C10:0	5.0–8.0	4.5–8.0	4.56–6.03 (5.00)	4.27–6.08 (5.21)
C12:0	45.1–53.2	43.0–53.0	46.7–49.4 (48.14)	46.0–52.6 (48.66)
C14:0	16.8–21.0	16.0–21.0	16.2–19.6 (17.88)	16.0–19.7 (17.82)
C16:0	7.5–10.2	7.5–10.0	7.80–9.73 (8.88)	7.65–10.1 (8.51)
C18:0	2.0–4.0	2.0–4.0	2.94–3.69 (3.26)	2.73–4.63 (3.50)
C18:1	5.0–10.0	5.0–10.0	7.24–8.04 (7.63)	5.93–8.53 (7.16)
C18:2	1.0–2.5	1.0–2.5	1.82–2.36 (2.19)	1.00–2.03 (1.52)
Iodine value	6.3–10.6	4.1–11.0	6.81–8.91 (8.00)	5.6–10.3 (7.28)
% FFA, as lauric acid	No standard	≤0.5	0.008–0.076 (0.021)	0.047–0.337 (0.131)

(continues on next page)

Table 1 (Continued).

Parameter	Codex standard for coconut oil [2]	APCC standard for VCO [3]	RCO products, <i>n</i> = 10 [7]	VCO products, <i>n</i> = 20 [7]
% Moisture	No standard	0.1–0.5	0.01–0.10 ^a (0.05)	0.05–0.11 ^a (0.08)
% Volatile matter	0.2 ^b	0.2 ^b	0.00–0.08 ^c (0.03)	0.07–0.18 ^c (0.13)
Peroxide value, meq/kg oil	<15	<3	0.27–3.39 (0.98)	0.00–1.86 (0.56)

^aDetermined by Karl–Fisher titration.^bMass loss at 105 °C.^cMass loss at 120 °C.

VCO contains low amounts of proteins, ranging from ND to 0.12 % (LOD = 0.01 %) while no proteins could be detected in RCO [8]. Similarly, VCO contains low amounts of antioxidants and α -tocopherol, while these are virtually absent in RCO. Various studies have measured the total antioxidants in VCO to range from nondetected (LOD = 0.017) to 0.170 mmol/100 g oil [9]. However, the differences in these values may reflect differences in coconut varieties and the inclusion of testa in the extraction.

Another method used to differentiate VCO from RCO is phosphorus-31 nuclear magnetic resonance spectroscopy (³¹P NMR) [10]. In this method, compounds with alcohol and carboxylic acid functionalities in the oil, such as monoglycerides, diglycerides, sterols, and FFAs, are converted into dioxaphospholane derivatives and subsequently analyzed by ³¹P NMR. The mono- and diglycerides could be clearly distinguished by their ³¹P chemical shift and quantified by integration (Table 2). 1-Monoglycerides were found to be higher in VCO (ave.: 0.027 %) than RCO (ave.: 0.019 %). 2-Monoglycerides were not detected in any of the samples down to a detection limit of 0.015 %.

There were more total diglycerides in RCO (ave.: 4.10 %) than VCO (ave.: 1.55 %). However, it can be seen that the relative proportion of 1,2-DG vs. 1,3-DG decreases when the sample is exposed to more heat. For example, the ratio of 1,2-DG:1,3-DG is 3:1 in VCO and almost 1:1 in RCO. This pattern has been used to measure freshness in virgin olive oil [11,12]. Total phytosterols were higher on average in VCO (0.096 %) compared with RCO (0.032 %). This result is expected since RCO is subjected to various chemical processes that can remove sterols. The FFA content as measured by ³¹P NMR was 8 times higher in VCO (0.127 %) than RCO (0.015 %). The FFA results were comparable to those obtained using the standard titration method. These results show that ³¹P NMR is a very useful method for the measurement of several parameters that give information on the quality of the VCO sample.

Table 2 1-Monoglycerides (1-MG), diglycerides, total phytosterols, and FFAs (w/w %), in VCO and RCO samples using ^{31}P NMR analysis. Ranges of values are given; the average values are indicated in parenthesis [10].

Parameter, w/w %	RCO products, <i>n</i> = 8	VCO products, <i>n</i> = 16
Monoglycerides		
1-MG	ND–0.04 (0.02)	ND–0.05 (0.03)
2-MG	ND	ND
Diglycerides		
1,2-DG	1.6–3.11 (2.13)	0.81–1.72 (1.17)
1,3-DG	1.47–2.71 (1.96)	ND–0.89 (0.37)
Total DG	3.15–5.82 (4.10)	1.14–1.93 (1.55)
Phytosterols, total	ND–0.09 (0.03)	0.07–0.16 (0.10)
FFA, by ^{31}P NMR	ND–0.04 (0.02)	ND–0.42 (0.13)
FFA, by titrimetry	0.01–0.03 (0.02)	0.02–0.33 (0.14)

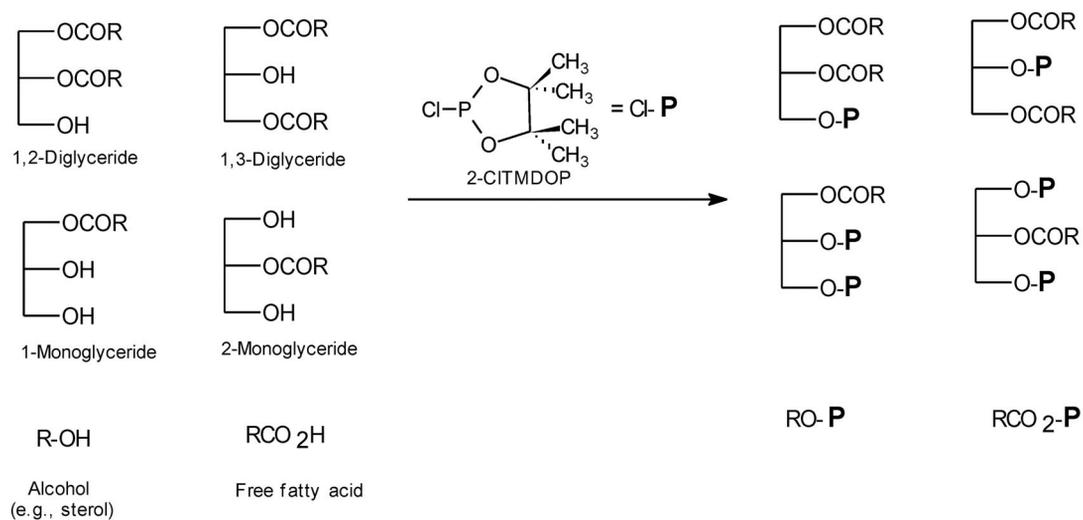


Fig. 1 Monoglycerides, diglycerides, alcohols (such as sterols), and FFAs are converted into phosphorous derivatives that can be analyzed by ^{31}P NMR using a phosphitylizing agent (2-chloro-4,4,5,5-tetramethyl-1,3-dioxo-2-phospholane, 2-CITMDOP).

DIFFERENTIATING VCO PRODUCTS ACCORDING TO METHOD OF PREPARATION

The second comparison of interest is whether VCO prepared by various methods, such as FWH, FNH, CEN, and EXP, can be differentiated based on their chemical characteristics. The CEN and fermentation methods are wet processes, while the EXP method is a dry process. Natural fermentation occurs through the action of bacteria that are naturally present [13]. Table 3 compares VCO produced by four methods using parameters that give characteristics that are affected by the method of production. The VCO produced by adventitious fermentation tended to have the highest content of volatile organic compounds (VOCs), which may be attributed to metabolites formed from microbial processes. The low VOC content of VCO produced by the CEN method may be due to the use of vacuum, which the producers use to remove moisture. The CEN and fermentation methods produced higher amounts of FFA, suggesting that these methods may have higher % FFA due to microbial hydrolysis of the vegetable oil. The VCO from these methods also gave higher peroxide values. However, the % FFA and peroxide values for all VCO products were well within the Codex and APCC standards. Because the ranges of values of the standard parameters overlapped, these parameters cannot be used to distinguish the VCO products made by various methods.

Table 3 Comparison of standard quality parameters of VCO products prepared by the EXP, CEN, and fermentation methods, with heat and without heat. The range of values is given; the average values are in parentheses.

Parameter	Type of VCO manufacturing process			
	Centrifuge	Expeller	Fermentation without heat	Fermentation with heat
% VOCs ^a	0.00–0.06 (0.03)	0.02–0.05 (0.03)	0.03–0.08 (0.06)	0.04 (0.04)
% FFA, as lauric acid	0.047–0.337 (0.155)	0.038–0.184 (0.095)	0.037–0.270 (0.154)	0.93–0.211 (0.156)
Peroxide value, meq/kg oil	0.00–0.89 (0.59)	0.00–0.47 (0.15)	0.00–0.86 (0.52)	0.00–1.86 (0.84)

^a% VOCs are obtained by subtracting the % moisture (determined by Karl–Fischer) from the % volatile matter at 120 °C.

VOLATILE ORGANIC COMPOUNDS AND OLFACTORY CHARACTERISTICS OF VCO

ASTM E1627-94 provides a standard method for the sensory analysis of edible oils [14]. It lists seven aroma attributes for coconut oil: bacony, burnt, buttery, nutty, rancid, soapy, and waxy. Sensory analysis of VCO and refined, bleached, and deodorized samples by trained panelists resulted in the generation of three additional aroma attributes—acid, cocojam, and latik—and nine attributes for appearance, taste, and flavor. The acid aroma is based on a dilute acetic acid solution, while the cocojam and latik aromas are associated with sweetish burnt/roasted coconut and sweet coagulated coconut milk, respectively [15].

The olfactory characteristics of various food products are due to VOCs released by the sample. The sampling of volatile compounds from various food products has been carried out using solid phase microextraction (SPME) followed by identification and quantification by gas chromatography-mass spectrometry (GCMS) [16]. SPME/GCMS has been used to distinguish virgin olive oil products according to their origins [17,18].

Sensory analysis was performed by a trained panel using five aroma descriptors (acid, cocojam, latik, nutty and rancid aromas). In general, VCO produced by FWH and FNH could be distinguished

from those produced by the CEN and EXP methods due to the higher acid and rancid aromas of fermentation VCO.

Headspace analysis of the VCO samples using SPME-GCMS detected the following VOCs in various amounts: ethyl acetate, acetic acid, 2-pentanone, hexanal, *n*-octane, 2-heptanone, limonene, nonanal, octanoic acid, ethyl octanoate, δ -octalactone, ethyl decanoate, δ -decalactone, and dodecanoic acid (Table 4). Quantitative analysis of the VOCs from the four types of VCO samples gave a number of trends. The compounds that are most commonly associated with coconut—lactones—were present in all coconut products. The lactone that was found in the highest amount was δ -octalactone, followed by δ -decalactone. The compounds associated with peroxidation—hexanal and nonanal—were found in low quantities. The VCO produced by fermentation had higher amounts of acetic acid. Acetic acid was not detected in the headspace of the VCO produced by the CEN method; however, this may be due to the use of vacuum drying by the producers. Low amounts of methylketones—2-pentanone and 2-heptanone—suggest that deterioration due to fungi is not a major process in these samples [19]. The VOCs which were present in the highest amounts in the headspace were the medium-chain fatty acids—octanoic acid and dodecanoic acid—which are presumably formed from the hydrolysis of the triglycerides. The longer-chain fatty acids may be present in solution, but their low volatility would make them less likely to have a significant presence in the headspace.

Table 4 Average concentration (ppm) of VOCs in commercial VCO samples produced by CEN, EXP, and FWH and FNH. VOCs were measured by SPME-GCMS. The number of samples analyzed is indicated.

Compound	Concentration in sample (ppm)			
	Centrifuge, <i>n</i> = 7	Expeller, <i>n</i> = 6	Fermentation without heat, <i>n</i> = 6	Fermentation with heat, <i>n</i> = 5
Ethyl acetate	0.25	0.22	0.67	0.34
Acetic acid	0.00	4.58	14.51	23.13
2-Pentanone	0.06	0.05	0.05	0.04
Hexanal	0.22	0.07	0.34	1.45
<i>n</i> -Octane	0.01	0.02	0.05	0.05
2-Heptanone	1.08	0.58	1.26	0.16
Limonene	0.00	1.91	0.24	0.00
C ₆ H ₁₀ O ₂ ^a	1.20	0.94	1.02	0.85
Nonanal	0.00	0.43	0.76	0.51
Octanoic acid	53.59	2.61	192.72	135.39
Ethyl octanoate	2.04	1.70	6.73	1.63
δ -Octalactone	102.05	107.81	115.48	75.11
Ethyl decanoate	5.53	6.45	9.67	7.74
δ -Decalactone	30.06	66.27	66.89	42.09
Dodecanoic acid	30.23	ND	75.40	661

^aUnidentified.

The quantitative data of the headspace analysis were combined with the sensory data to determine whether certain compounds can be correlated with aroma characteristics. It was found that samples produced by CEN and EXP methods, which are found on the left side of Fig. 2, correlated with the high levels of lactones (Fig. 3), while samples produced by FWH and FNH methods, which are found on the right side of Fig. 2, correlated with high levels of octanoic acid (Fig. 3).

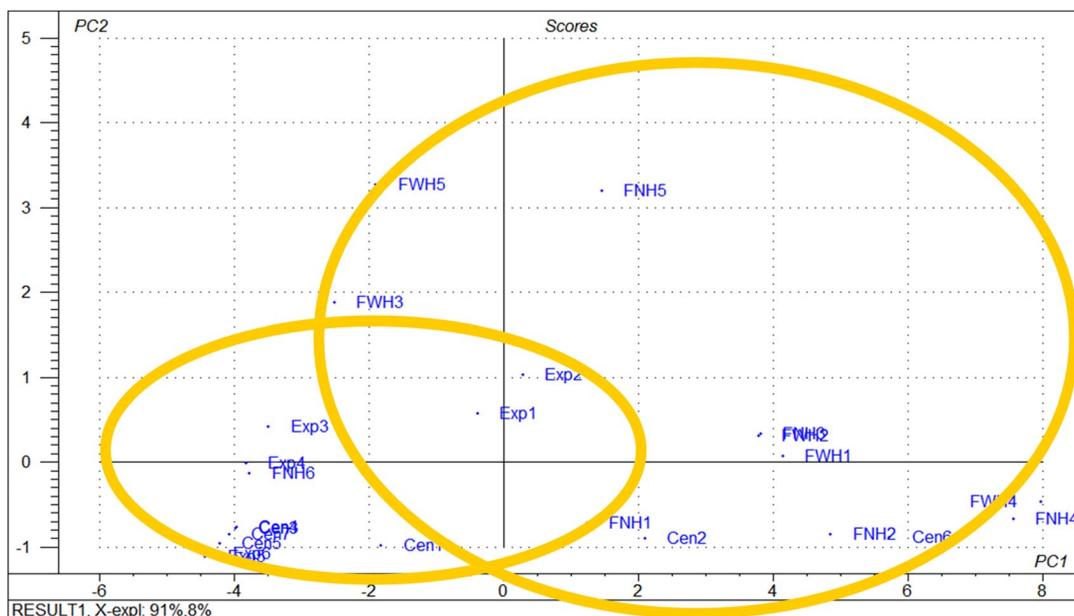


Fig. 2 Scores plot of the principal components analysis (PCA) of the SPME GCMS analysis of 24 VCO samples shows that VCO produced by FWH and FNH can be distinguished from those produced using EXP and CEN methods.

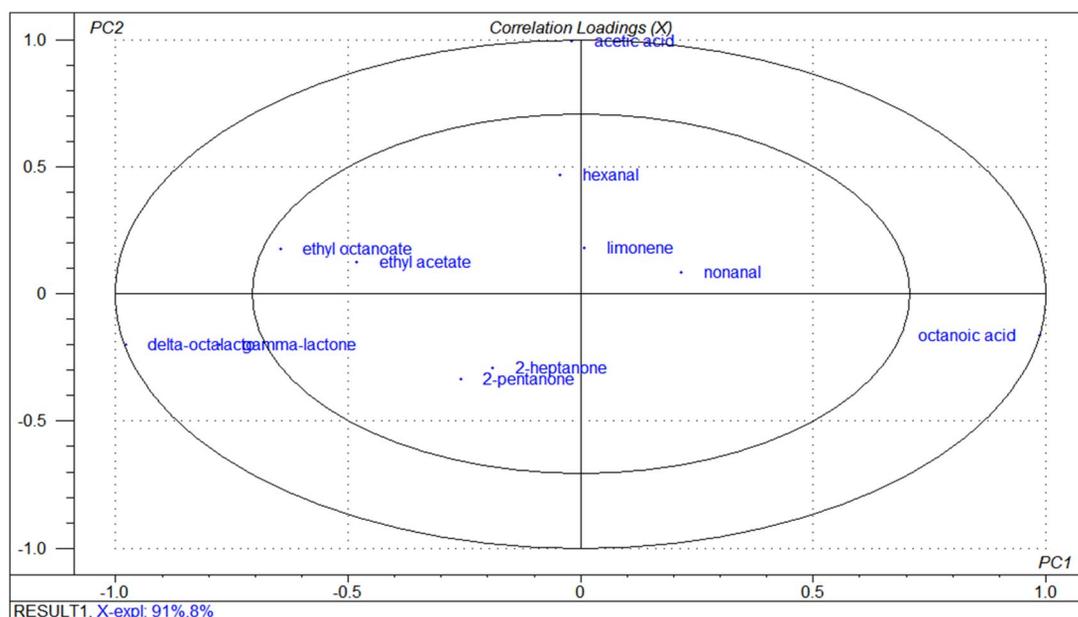


Fig. 3 Correlation loadings plot of the PCA analysis of VOCs of 24 VCO samples by headspace SPME/GCMS analysis. The outer and inner circles in the correlation loadings plot represent 100 and 50 % explained variance, respectively.

STUDIES ON THE DETERIORATION OF VCO

The deterioration of vegetable oils can occur due to chemical hydrolysis, chemical oxidation, and microbial action [20] and is promoted by enzymes, metals, heat, light, and air [21]. However, the presence of minor components or additives in the oil, such as phenols, can increase the stability of the oil. The problem of rancidity of coconut oil has been the subject of studies dating back to the early 1900s, and the possible causes have been attributed to molds, high moisture content, and exposure to light [22,23]. Although RCO is well known for its stability [24], the stability of VCO has not yet been established.

Detailed studies on the physicochemical stability of VCO showed that under ambient conditions (air, room temperature) oxidation was negligible. Oxidation of VCO was observed only in the presence of air, UV radiation, ferric ions, and high FFA content [16].

Hydrolysis of vegetable oils is promoted by the presence of moisture and leads to the formation of FFA. Chemical hydrolysis of VCO at different moisture levels (from 0.06 to 0.22 % (water saturated)) and temperatures (30, 60, and 80 °C) was carried out. Because of the low rate of hydrolysis, the standard titration method could not be used to measure FFA accurately after 2 weeks of reaction. As an alternative, the formation of FFA was measured by derivatization with 2-CITMDOP and measured using ^{31}P NMR [25]. At room temperature (30 °C) and 0.06 % moisture, the rate of FFA formation was found to be less than 0.001 $\mu\text{mol/g-hr}$ (expressed as lauric acid). Under conditions of saturated water (0.22 % moisture) at 80 °C, FFA formation occurred at a rate of 0.066 $\mu\text{mol/g-hr}$. Since the VCO quality standard sets a limit of 0.1 % FFA, the number of days for the VCO product with an initial % FFA content of 0.02 % to comply with this standard can be estimated (Table 5).

Table 5 Hydrolysis rates of VCO as measured by ^{31}P NMR. The hydrolysis reaction rate was obtained by measuring the change in concentration of FFA in VCO over 1 week at various temperatures in the presence of various amounts of moisture. The initial % FFA of the VCO sample was about 0.02 %.

% Moisture	Temperature, °C	Rate of FFA formation, $\mu\text{mol/g-hr}$, as lauric acid	Number of days to form 0.1 % FFA
0.063	30	<0.001	>197
0.063	80	0.008	26
0.084	80	0.011	19
0.22 (sat'd.)	80	0.066	3

One major cause of degradation of the VCO product that has been identified is microbial action [26]. The microbial decomposition of VCO was determined after 4 days of incubation at 37 °C by extraction, conversion into tetramethylsilyl derivatives, and analysis by GCMS. At low moisture levels (<0.06 %), VCO was stable to microbial decomposition. However, above 0.06 % moisture, there was an increase in the formation of organic acids, in particular, lactic acid, indicating that microbial action by lactic acid-producing bacteria had occurred (Fig. 4 and Table 6). Therefore, one can conclude that the most important conditions that influence the physicochemical and microbial degradation of VCO are moisture, temperature, and the presence of microorganisms. This makes the VCO made by the fermentation method most susceptible to microbially induced degradation. However, these degradation processes can be minimized if the moisture level is maintained below 0.06 %.

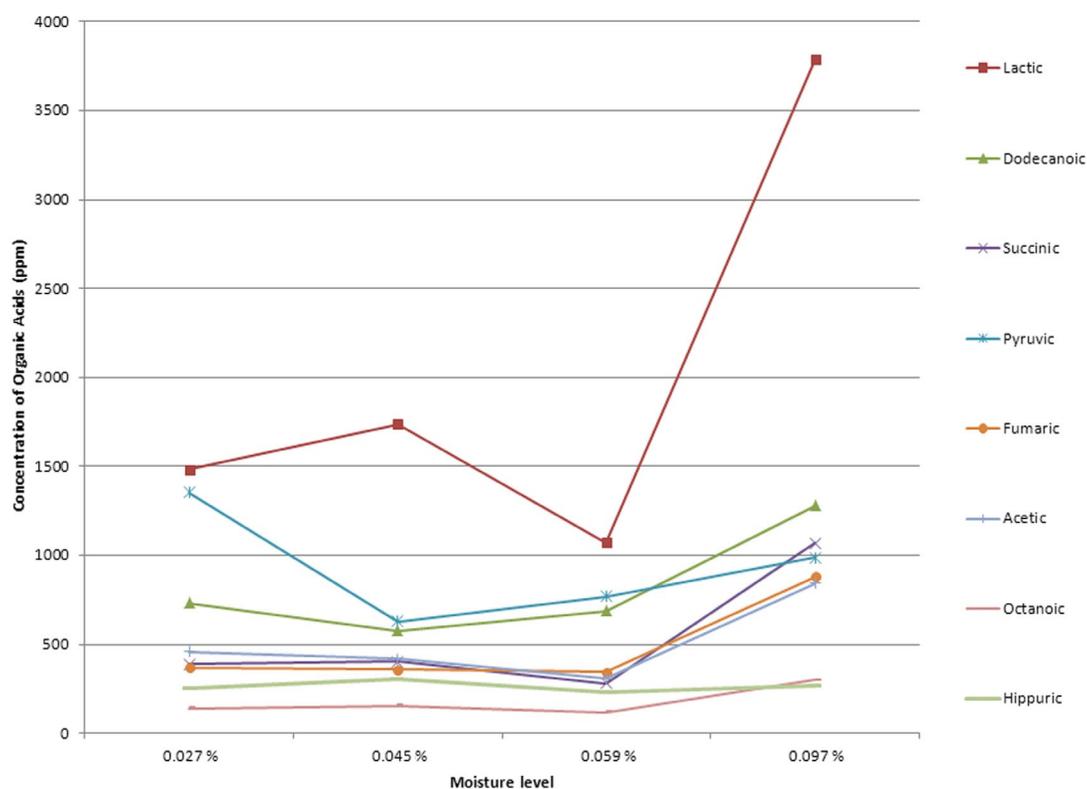


Fig. 4 One of the causes of rancidity of VCO is microbial attack. Organic acids are produced when microorganisms degrade the VCO product. This figure shows the concentrations of the organic acids in VCO samples after 4 days of incubation at 37 °C at different moisture levels.

Table 6 Concentration of organic acids in VCO after 4 days of incubation with inoculated bacteria at 37 °C in the presence of various amounts of moisture. The concentrations are in (µg/mL). The value in parenthesis is the % relative standard deviation of duplicate runs. The data are plotted in Fig. 4.

Organic acid	Moisture level			
	0.027 %	0.045 %	0.059 %	0.097 %
Lactic	1484 (±21 %)	1736 (±12 %)	1072 (±20 %)	3787 (±13 %)
Dodecanoic	731 (±4 %)	576 (±2 %)	686 (±14 %)	1280 (±1 %)
Succinic	388 (±3 %)	403 (±35 %)	282 (±11 %)	1070 (±4 %)
Pyruvic	1352 (±24 %)	629 (±22 %)	770 (±17 %)	985 (±21 %)
Fumaric	369 (±8 %)	359 (±24 %)	343 (±2 %)	881 (±1 %)
Acetic	457 (±19 %)	417 (±23 %)	305 (±11 %)	843 (±7 %)
Octanoic	140 (±3 %)	153 (±17 %)	118 (±3 %)	298 (±8 %)

CONCLUSIONS

On the average, VCO and RCO differ in terms of the average values for a number of standard quality parameters. In particular, VCO tends to have higher FFA, moisture, and volatile matter and lower peroxide value than RCO. However, the range of values overlaps and no single standard parameter alone

can be used to differentiate VCO from RCO. Using the more sophisticated method of ^{31}P NMR, VCO and RCO can be distinguished by measuring the total amount of diglycerides.

There is no overlap in the values found among VCO and RCO samples. The various VCO products, which were made using four methods of production (EXP, CEN, FWH and FNH), could not be differentiated using standard quality parameters. On the other hand, sensory analysis showed that VCO produced by fermentation (with and without heat), could be distinguished from those produced using the EXP and CEN methods due to their higher acid and rancid aromas. VCO produced by fermentation contained higher levels of acetic acid and octanoic acid.

Studies on the physicochemical deterioration of VCO showed that it is stable to chemical and photochemical oxidation and hydrolysis. However, VCO is most susceptible to microbial attack, which leads to the formation of various organic acids, in particular, lactic acid. However, at moisture levels below 0.06 %, microbial action is significantly lessened.

REFERENCES

1. F. D. Gunstone (Ed.). *Vegetable Oils in Food Technology: Composition, Properties and Uses*, Blackwell, Oxford (2002).
2. FAO/WHO. *Codex Alimentarius Stan 201: Standard for Named Vegetable Oils* (2003). Downloaded from <<http://www.codexalimentarius.net/>> on 3 February 2011.
3. Asian and Pacific Coconut Community (APCC). *Standards for Virgin Coconut Oil* (2003). Downloaded from <<http://www.apccsec.org/standards.htm>> on 3 February 2011.
4. A. Marina, Y. Che Man, I. Amin. *Trends Food Sci. Technol.* **20**, 481 (2009).
5. J. Banzon, A. Resurreccion. *Phil. J. Coco. Stud.* **IV**, 1 (1979).
6. V. P. Dia, V. V. Garcia, R. C. Mabesa, E. M. T. Mendoza. *Phil. Agric. Scientist* **88**, 462 (2005).
7. F. Dayrit, O. Buenafe, E. Chainani, I. de Vera, I. Dimzon, E. Gonzales, J. Santos. *Philipp. J. Sci.* **136**, 121 (2007).
8. I. Dimzon, M. Valde, J. Santos, M. Garrovillas, H. Dejarne, J. Remollo, F. Dayrit. *Philipp. J. Sci.* **140**, 89 (2011).
9. A. Marina, Y. Che Man, S. Nazimah, I. Amin. *J. Am. Oil Chem. Soc.* **86**, 301 (2009).
10. F. Dayrit, O. Buenafe, E. Chainani, I. de Vera. *J. Agric. Food Chem.* **56**, 5765 (2008).
11. R. Sacchi, F. Addeo, L. Paolillo. *Magn. Reson. Chem.* **35**, S133 (1997).
12. A. Spyros, A. Philippidis, P. Dais. *J. Agric. Food Chem.* **52**, 157 (2004).
13. P. C. Sanchez. *Philippine Fermented Foods: Principles and Technology*, University of the Philippines Press, Quezon City, Philippines (2008).
14. ASTM E1627 – 94 (Reapproved 2004): Standard Practice for Sensory Evaluation of Edible Oils and Fats, ASTM International, PA, USA.
15. B. Villarino, L. Dy, M. C. Lizada. *LTW* **40**, 193 (2007).
16. A. Harmon. "Solid-phase microextraction for the analysis of aromas and flavors", in *Flavor, Fragrance, and Odor Analysis*, R. Marsili (Ed.), CRC Press (2001).
17. S. Vichi, L. Pizzale, L. Conte, S. Buxaderas, E. Lopez-Tamames. *J. Agric. Food Chem.* **51**, 6572 (2003).
18. C. Benincasa, A. de Nino, N. Lombardo, E. Perri, G. Sindona, A. Tagarelli. *J. Agric. Food Chem.* **51**, 733 (2003).
19. J. L. Kinderlerer, B. Kellard. *Phytochemistry* **23**, 2847 (1984).
20. C. Ho, Q. Chen. "Lipids in food Flavors: An overview", in *Lipids in Food Flavors*, C. Ho, T. Hartman (Eds.), pp. 2–14, ACS Symposium Series No. 558, American Chemical Society, Washington, DC (1994).
21. E. R. Sherwin. *J. Am. Oil Chem. Soc.* **55**, 809 (1978).
22. H. Walker. *Philipp. J. Sci.* **1**, 117 (1906).

23. G. C. Mañalac, A. H. Soliven. *Philipp. J. Sci.* **98**, 133 (1969).
24. M. H. Gordon, I. A. Rahman. *J Am. Oil Chem. Soc.* **68**, 574 (1991).
25. A. Spyros, P. Dais. *J. Agric. Food Chem.* **48**, 802 (2000).
26. W. N. Stokoe. *Biochem. J.* **22**, 80 (1928).