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Analysis of Volatile Organic Compounds in Virgin Coconut Oil and their Sensory Attibutes

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The volatile compounds in the headspace of twenty-four commercial virgin coconut oil (VCO) samples prepared by different methods (i.e. expeller, centrifugation, and fermentation with and without heat) were analyzed by solid phase microextraction-gas chromatography mass spectrometry (SPME-GCMS). The following volatile organic compounds (VOCs) were identified: 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. Fermentation-produced samples were found to have higher levels of acetic acid and free fatty acids in the headspace compared to VCO produced using the centrifuge and expeller methods. Descriptive sensory analysis of the VCO samples by a trained panel was carried out to determine its sensory attributes and to correlate the volatile compounds that are responsible for VCO aroma. Principal components regression (PCR) of the SPME-derived analytical and sensory data indicates that lactones impart coconut-like aroma, while octanoic acid is mainly responsible for the rancid and acid aroma.

SPME-GCMS can be used to differentiate VCO produced by physical means from fermentation-produced samples and can be used as a method to monitor VCO product quality.

Key Words: principal components analysis (PCA), principal components regression (PCR), solid phase microextraction gas chromatography-mass spectrometry (SPME-GCMS), virgin coconut oil (VCO), volatile organic compounds (VOCs)

INTRODUCTION

Virgin coconut oil (VCO) is a vegetable oil that is extracted from fresh coconut meat or kernel, and can be processed using only physical or other natural means (APCC 2006). The physical means can include pressing, washing with water, settling, filtering and centrifugation, while the other natural means can include fermentation by naturally-occurring microorganisms. There are four common methods used for the commercial production of VCO, namely expelling, centrifugation and fermentation with and without heat (Dia et al. 2005). VCO produced through the expeller route involves extraction of the oil from the air-dried coconut meat using a screw-type press. The fermentation process involves separation of the oil from the aqueous portion of the coconut milk using microorganisms which are naturally present. Fermentation is carried out for 16-24 h; separation and drying of the oil may be done with or without the application of heat. The centrifuge process involves phase separation of the coconut milk using a centrifuge (Bawalan 2005).

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A descriptive sensory analysis was conducted to describe and differentiate VCO and refined, bleached and deodorized (RBD) coconut oil samples (Villarino et al. 2007).

VOCs which are responsible for the aroma attributes of vegetable oils can be identified and quantified by analysis of the headspace using methods such as static headspace, dynamic headspace (i.e. purge-and-trap), headspace sorptive extraction, direct thermal desorption, and solid phase microextraction (SPME) techniques. These techniques have been used to identify the VOCs in the headspace of virgin olive oil (VOO) (Angerosa et al. 1999; Ridolfi et al. 2002; Cavalli et al. 2003; Sanchez-Ortiz et al. 2008). More than 100 VOCs composed mainly of hydrocarbons, ketones, aldehydes, alcohols, esters and carboxylic acids were identified in the headspace of VOO using SPME (Vichi et al. 2003). Sensory analysis of VOO was then related to the presence of VOCs to determine aroma compounds and off-flavors (Morales et al. 1995; 2005; Aparicio et al. 1996).

Early studies on coconut oil have identified a number of hydrocarbons, aldehydes, alcohols, methyl ketones, and δ-lactones as significant contributors to its aroma (Allen 1965; Pai et al. 1970; Lin & Wilkins 1970). The distinct coconut odor has been shown to be due to δ-octalactone (Padolina et al. 1987; Maarse 1991). Hydrolytic rancidity in coconut oil has been attributed to the presence of free fatty acids (FFA) (Fernandez 1988), while ketonic rancidity has been linked to the presence of methyl ketones (Kinderlerer 1987).

Because VCO is produced commercially using different methods, it is of interest to compare the VOC profiles in these products and to combine this with sensory analysis in order to determine which compounds are responsible for its aroma characteristics. In this study, commercial VCO samples prepared by different methods were analyzed by SPME-GCMS in order to identify and quantify the VOCs in the headspace. The same VCO samples were subjected to sensory evaluation in order to determine the relationship between the various VOCs and the aroma attributes of the VCO samples.

Large amounts of data can be analyzed using Principal Components Analysis (PCA). PCA can be used to determine how samples in a data matrix differ from each other, which parameters or variables contribute to this difference and whether these variables are correlated or independent from each other. Through mathematical manipulation, PCA projects the original data set from a high dimensional space onto a lower dimensional space generating a PCA model, which has a smaller number of variables, called principal components (PCs) (Unscrambler 1986). PCs represent the variations present in the samples (Beebe 1998). PCA can be used to differentiate samples from each other or to determine groupings among samples (Chapman et al 2001; Marsili 2000). On the other hand, Principal Components Regression (PCR) can be used to find relationships between two different sets of data.

MARTERIALS AND METHODS

Virgin coconut oil samples

Commercial samples of virgin coconut oil were provided by members of the Virgin Coconut Oil Producers and Traders Association, Inc. (VCO Association). Twenty-four VCO samples of the following types were analyzed: centrifuge (Cen, *n* = 7), expeller process (Exp, *n* = 6), fermentation with heat (FWH, *n* = 5) and fermentation, no heat (FNH, *n* = 6).

Chemicals and materials

SPME analysis was carried out using a DVB/CAR/PDMS 50/30 µm fiber (Supelco, Bellefonte, PA). The following chemicals were used as standards: ethyl acetate (99.9 %, J.T. Baker), acetic acid (100 %, Merck), 2-pentanone (99%, Sigma-Aldrich), hexanal (98%, Aldrich), *n*-octane (98%, Aldrich), 2-heptanone (99%, Sigma-Aldrich), limonene (97%, Aldrich), nonanal (95%, Aldrich), octanoic acid (99%, Sigma), ethyl octanoate (99%, Aldrich), δ-octalactone (Soda Aromatic Co., Ltd), ethyl decanoate (99%, Aldrich), δ-decalactone (98%, Aldrich), and dodecanoic acid (98%, Aldrich). 2-Octanol (97%, Sigma) was used as internal standard (IS) for calibration of retention time and quantitation.

Headspace analysis by quantitative SPME-GCMS

This method was adapted from the analysis of virgin olive oil (Vichi et al. 2003) using a divinybenzene-carboxen-polydimethylsiloxane (DVB/Car/PDMS) fiber. The fiber was cleaned by immersing twice in acetone for 5 min and heating for 10 min in the GC injection port at 250°C. To determine the fiber exposure time, the SPME fiber was exposed to the headspace of the standard mixture (5 ppm in VCO matrix) at 40ºC for time periods of 15, 30, 60, and 90 min. Each determination was done twice.

Coconut oil (ca. 11 g) was accurately weighed with 0.1 g of 2% (w/w) internal standard solution (2-octanol in blank coconut oil and VCO matrix) in a 15-mL clear vial capped with PTFE/silicone septum (Supelco). The SPME fiber was exposed to the headspace of the sample at 40°C for 30 min, and was then immediately desorbed into the GC injector port at 260ºC while a small loop in the front portion of the GC column was immersed in liquid nitrogen. The GC temperature program was started after a 2 min desorption and column trapping period.

GCMS analysis was carried out using the Hewlett Packard 5890 Series II gas chromatograph coupled to a Finnigan MAT95 mass spectrometer. Separation was done on a DB-1 column (J&W Scientific, 60 m x 0.25 mm i.d. x 0.25 µm film thickness) with the following oven temperature program: initial temperature at 40ºC, hold for 10 min; increased to 200ºC at 3ºC/min. The injector and detector temperatures were set at 260ºC and 240ºC, respectively. MS analysis was carried out by electron ionization at 70 eV, scanning from m/z 40 to 240. Identification of the GC peaks was carried out by comparison with the 62k NIST library. The identity of the various compounds was confirmed using standards and quantification was done using 2-octanol as internal standard (IS). The method response for each compound was determined six times and the results were averaged. The analysis of coconut oil samples was done in duplicate.

The limit of detection (LOD) for each compound was determined by performing six replicate SPME analyses of a sample. The LOD for each compound was taken as three times the repeatability standard deviation for each compound, while the limit of quantification (LOQ) was taken as ten times the repeatability standard deviation for each compound (IUPAC 2002).

Descriptive analysis of oil samples

Descriptive sensory analysis of the VCO samples was carried out by the University of the Philippines-Diliman College of Home Economics. A 10-member sensory panel was trained and samples were evaluated using the Generic Descriptive Method, a combination of Quantitative Descriptive Method and Spectrum™ Analysis Method. Ten-mL samples of oil were presented to the panelists in 6-inch test tubes maintained at room temperature. The evaluation of all samples was done with 4-5 replicates. Descriptors used in sensory analysis were as follows: acid (associated with acetic acid solution), cocojam (associated with sweetish burnt/roasted coconut), *latik* (associated with cooked sweet coagulated coconut milk), nutty (associated with the 2nd layer of fresh coconut kernel with testa), and rancid (associated with old stored oil) (Villarino et al. 2007).

The aromatic sensory threshold for compounds was determined by spiking a known amount of standard compound into a VCO sample for which the background VOC concentrations were already known and presenting this to the 10-member sensory panel for evaluation.

Statistical Analysis

Means, standard deviations and standard errors were computed to analyze panel performance and sample attributes. Single-factor ANOVA ($p<0.5$) across all attributes was used to analyze performance of the individual panelists. The Duncan's Multiple Range Test (DMRT) was used to determine differences of aroma attributes of VCO samples and the Fisher's least significant difference (LSD) test was used to determine sample differences using SPME GCMS analysis. Principal component analyses (PCA) was applied on the correlation matrices generated, by using the mean values per replicate across the coconut oil attributes. Moreover, factor analysis (FA) was performed to evaluate what variables loaded on what factors and thus the patterns of correlation. All statistical analyses for the descriptive analysis of oil samples were done using SAS Enterprise Guide Version 2.0 (SAS Institute 2002).

Statistical analysis of the SPME data includes single-factor ANOVA, PCA and Principal Components Regression (PCR). PCR and PCA were performed using Unscrambler™ (CAMO Process AS, Oslo, Norway).

RESULTS AND DISCUSSION

Identification of volatile organic compounds

SPME-GCMS analysis was performed on the volatile organic compounds (Figure 1). Fourteen compounds were identified through their MS fragmentation patters. These identities were confirmed by comparison with pure standards as follows (Table 1 and Figure 2): ethyl acetate (1), acetic acid (2), 2-pentanone (3), hexanal (4), *n*-octane (5), 2-heptanone (6), limonene (7), nonanal (9), octanoic acid (10), ethyl octanoate (11), δ-octalactone (12), ethyl decanoate (13), δ-decalactone (14), and dodecanoic acid (15).

Peak 8 (retention time: 32.57 min) which could not be identified from the MS library, gave the following fragmentation data (*m/z*, %relative intensity): 114 ($P^{+\cdot}$, 2.1%), 99 (4%), 85 (11%), 71(9.4%), 70 (45%), 55(19.5%), 45(1.1%), 44(17.5%), 43 (18.4%), 42 (100%). However, the identity of peak 8 could not be determined.

Quantitative analysis of VOCs in commercial VCO

The SPME-GCMS method response factor, *K*, of each compound was determined by spiking a known amount of the compound into the VCO matrix solution containing 2-octanol at a constant concentration of 0.02%, sampling the headspace by SPME under optimized sampling conditions, and analysis by GCMS. *K* quantitatively relates the solution concentration of a compound in coconut oil with its GCMS signal. It includes effects such as the relative volatility of each compound, the efficiency of trapping by the SPME fiber, and the GCMS response factor.

The method response factor of each identified compound was determined using standards. The method response

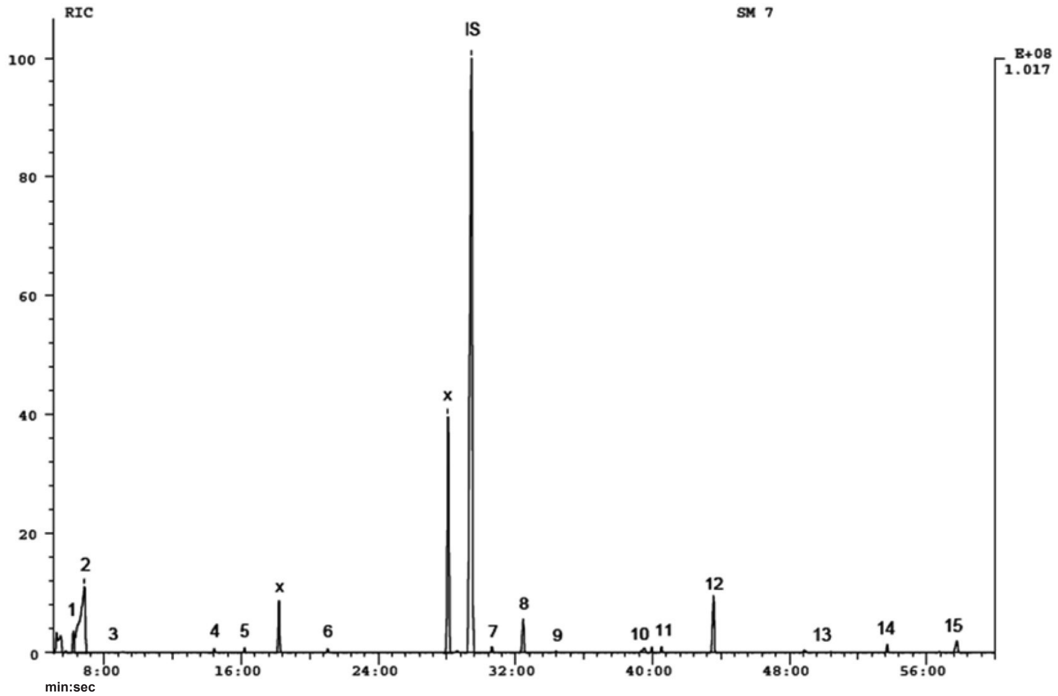

Figure 1. Typical headspace SPME GCMS chromatogram of a VCO sample (FWH2). Numbered peaks are identified in Table 1. "IS" is the internal standard (2-octanol); peaks marked "x" are background peaks which are found in the blank runs.

Table 1. Retention times, limits of detection (LOD) and limits of quantification (LOQ) of VOCs which were identified in the VCO by headspace SPME-GCMS. Peak numbers correspond to labels in Figure 1. ND: not determined.

Peak	Compound	Retention time (min)	LOD (ppm)	LOQ (ppm)
1	Ethyl acetate	6.22	0.43	1.44
2	Acetic acid	6.58	4.17	13.89
3	2-Pentanone	8.14	0.09	0.29
4	Hexanal	14.49	0.07	0.23
5	Octane	16.18	0.05	0.16
6	2-Heptanone	21.13	0.20	0.67
7	Limonene	31.35	0.07	0.24
8	Unidentified	32.57	ND	ND
9	Nonanal	35.08	0.28	0.93
10	Octanoic acid	39.33	7.83	26.10
11	Ethyl octanoate	40.53	2.04	6.79
12	δ-Octalactone	43.62	18.10	60.34
13	Ethyl decanoate	50.42	19.34	64.48
14	δ-Decalactone	53.71	70.15	233.84
15	Dodecanoic acid	57.58	5.55	18.48

factor for the unknown compound 8 was estimated using 2-heptanone which has a comparable molecular mass (114 amu) and retention time. The LOD and LOQ for each compound were then determined from SPME analysis carried out in six replicates of the same sample (Table 1). The LOD and LOQ for each compound were calculated as three times and ten times, respectively, of the standard deviation for the compound (IUPAC 2002).

The results of the quantitative VOC analyses of commercial VCO samples produced by centrifuge (Cen), expeller (Exp), fermentation without heat (FNH), and fermentation with heat (FWH)are given in Tables 2, 3, 4, and5, respectively. δ-Octalactone was the most predominant compound detected, both in terms of frequency of occurrence in the various VCO samples and quantity. Acetic acid and octanoic acid are more commonly found in VCO produced by fermentation. Dodecanoic (lauric) acid was detected in moderate to high amounts in VCO products which are produced by both physical and fermentation processes.

PCA of VCO and VOCs

Applying PCA to chemical data shows that this technique can be used to differentiate between VCO samples produced by physical means (Cen and Exp) and fermentation (FNH and FWH) (Figure 3A). The results indicate that only two principal components are needed to explain 99% of the total variance. The three compounds which have significant loadings along PC1 are δ-octalactone (-0.674) and octanoic acid (0.737).

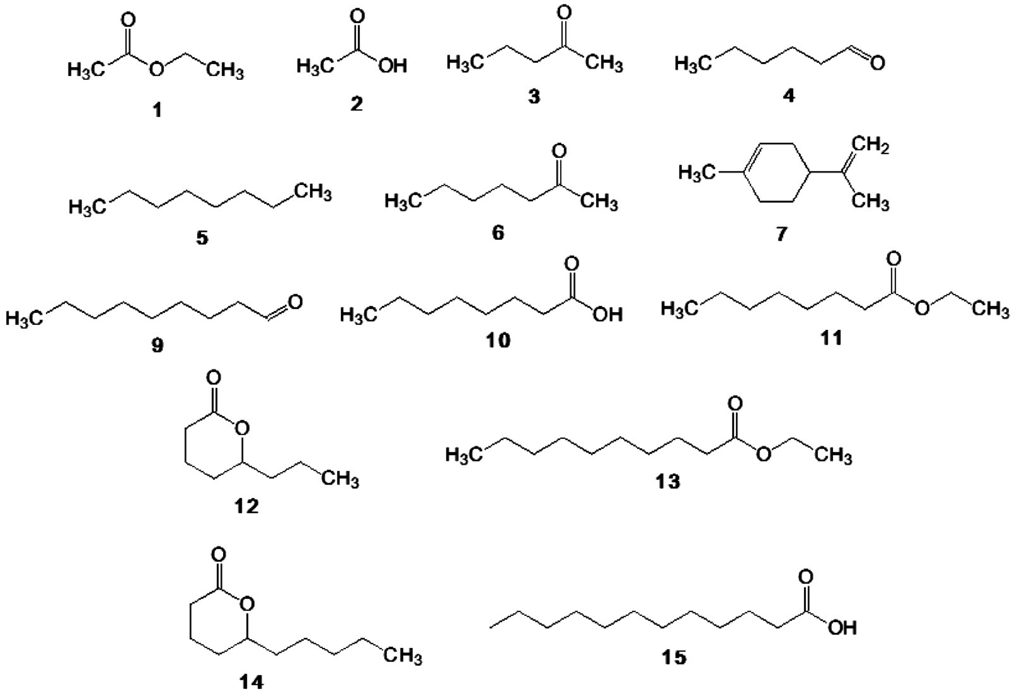

Figure 2. Structures of compounds which were identified in the VCO headspace: (1) Ethyl acetate; (2) acetic acid; (3) 2-pentanone; (4) hexanal; (5) n-octane; (6) 2-heptanone; (7) limonene; (9) nonanal; (10) octanoic acid; (11) ethyl octanoate; (12) δ-octalactone; (13) ethyl decanoate; (14) δ-decalactone; (15) dodecanoic acid. Numbers correspond to peaks in Figure 1 and Table 1. Compound 8 is unidentified.

Table 2. SPME-GCMS analysis of Volatile Organic Compounds in commercial Virgin Coconut Oil samples produced by the centrifuge method (n=7). "<LOD": below limit of detection; "<LOQ": detected but below limit of quantification. The values in parenthesis are the relative standard deviations. VCO samples with asterisk (*) were subjected to sensory evaluation.

Compound	Concentration in sample (ppm)						
	Cen1*	Cen2*	Cen3*	Cen4*	Cen5	Cen6	Cen7
Ethyl acetate (1)	ND	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOD
Acetic acid (2)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
2-Pentanone (3)	<LOQ	<LOQ	<LOD	<LOD	<LOQ	<LOQ	<LOQ
Hexanal (4)	<LOD	<LOQ	<LOQ	<LOD	<LOQ	1.06 (3.68%)	0.29 (7.35%)
n-Octane (5)	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD
2-Heptanone (6)	<LOQ	2.39 (2.34%)	<LOQ	<LOD	0.85 (3.95%)	3.02 (0.95%)	0.91 (8.49%)
Limonene (7)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Unknown (8)	1.90 (2.53%)	1.59 (1.72%)	0.98 (10.19%)	0.97 (3.98%)	1.04 (13.78%)	1.09 (5.91%)	0.83 (19.69%)
Nonanal (9)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Octanoic acid (10)	<LOQ	89.30 (4.19%)	<LOD	<LOD	<LOD	277.99 (13.47%)	<LOD
Ethyl octanoate (11)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
δ-Octalactone (12)	123.60 (2.55%)	112.31 (2.61%)	77.42 (14.52%)	69.75 (12.32%)	122.15 (10.38%)	127.71 (1.28%)	81.39 (4.09%)
Ethyl decanoate (13)	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD
δ-Decalactone (14)	<LOQ	<LOQ	<LOD	<LOQ	<LOD	<LOD	<LOD
Dodecanoic acid (15)	<LOD	<LOD	<LOD	211.58 (71.06%)	<LOD	<LOD	<LOD

Acetic acid has a significant loading (0.792) along PC2. Samples produced by centrifuge and expeller methods are generally found on the left side and are characterized by the high levels of δ-octalactone. Samples produced by fermentation are found on the right side and are characterized by their high levels of octanoic acid. The levels of acetic acid also contribute to the grouping of FWH5 and FNH5 with its high loading along PC2 (Figure

Table 3. SPME-GCMS analysis of Volatile Organic Compounds in commercial Virgin Coconut Oil samples produced by the expeller method (n=6). "<LOD": below limit of detection; "<LOQ": detected but below limit of quantification. The values in parenthesis are the relative standard deviations. VCO samples with asterisk (*) were subjected to sensory evaluation.

Compound	Concentration in sample, ppm (rsd)					
	Exp1*	**Exp2***	**Exp3**	**Exp4**	**Exp5**	**Exp6**
Ethyl acetate (**1**)	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOQ
Acetic acid (**2**)	<LOQ	<LOQ	<LOQ	14.96 (17.20%)	<LOD	<LOD
2-Pentanone (**3**)	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOQ
Hexanal (**4**)	<LOQ	<LOQ	0.26 (1.45%)	<LOD	<LOD	<LOD
n-Octane (**5**)	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD
2-Heptanone (**6**)	<LOQ	<LOQ	<LOQ	<LOQ	0.89 (129.71%)	1.79 (25.61%)
Limonene (**7**)	<LOD	11.44 (1.59%)	<LOD	<LOD	<LOD	<LOD
Unknown (**8**)	0.94 (1.69%)	0.74 (1.47%)	1.38 (3.88%)	1.19 (32.21%)	0.95 (22.40%)	0.43 (6.35%)
Nonanal (**9**)	2.27 (7.12%)	<LOQ	<LOD	<LOD	<LOD	<LOD
Octanoic acid (**10**)	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD
Ethyl octanoate (**11**)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD
δ-Octalactone (**12**)	82.19 (5.93%)	69.14 (3.52%)	112.09 (0.95%)	163.51 (25.48%)	159.10 (15.01%)	60.83 (22.93%)
Ethyl decanoate (**13**)	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD
δ-Decalactone (**14**)	<LOQ	<LOQ	<LOD	<LOD	257.31 (42.15%)	<LOD
Dodecanoic acid (**15**)	<LOD	<LOD	<LOD	883.35 (81.28%)	8613.88 (86.11%)	<LOD

Table 4. SPME-GCMS analysis of Volatile Organic Compounds in commercial Virgin Coconut Oil samples produced by fermentation without heat (n= 6). "<LOD": below limit of detection; "<LOQ": detected but below limit of quantification. The values in parenthesis are the relative standard deviations. VCO samples with asterisk (*) were subjected to sensory evaluation.

Compound	Concentration in sample, ppm (rsd)					
	FNH1*	**FNH2***	**FNH3***	**FNH4***	**FNH5**	**FNH6**
Ethyl acetate (**1**)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.87 (27.11%)
Acetic acid (**2**)	<LOD	<LOD	<LOQ	<LOQ	74.56 (6.26%)	<LOQ
2-Pentanone (**3**)	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOQ
Hexanal (**4**)	0.29 (4.81%)	0.68 (0.34%)	<LOQ	0.51 (30.88%)	0.41 (16.31%)	<LOQ
n-Octane (**5**)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
2-Heptanone (**6**)	5.23 (1.09%)	0.91 (2.17%)	<LOQ	<LOQ	<LOQ	0.83 (26.83%)
Limonene (**7**)	<LOQ	<LOD	<LOQ	<LOD	<LOQ	1.21 (35.77%)
Unknown (**8**)	1.17 (10.88%)	0.83 (3.19%)	0.31 (2.75%)	1.14 (64.49%)	1.10 (3.39%)	1.58 (25.88%)
Nonanal (**9**)	<LOQ	0.93 (1.77%)	<LOD	3.37 (38.55%)	<LOD	<LOD
Octanoic acid (**10**)	58.88 (17.42%)	160.11 (8.32%)	79.67 (9.28%)	800.79 (79.42%)	56.87 (26.22%)	<LOD
Ethyl octanoate (**11**)	<LOQ	<LOQ	<LOQ	19.75 (126.58%)	<LOQ	12.45 (20.59%)
δ-Octalactone (**12**)	96.06 (10.95%)	94.50 (2.83%)	<LOQ	188.23 (61.76%)	99.14 (3.63%)	196.84 (27.14%)
Ethyl decanoate (**13**)	<LOQ	<LOQ	<LOD	<LOQ	<LOD	<LOD
δ-Decalactone (**14**)	<LOQ	<LOQ	<LOD	261.01 (70.31%)	<LOD	<LOD
Dodecanoic acid (**15**)	<LOD	<LOD	377.02 (20.63%)	22079.89 (130.70%)	<LOD	<LOD

3B). Although the method of VCO production has a general effect on the composition of the VOCs, the quality of production can also affect the profile of the VOCs. For example, two centrifuge samples (Cen2 and Cen6) were grouped together with the fermentation samples, while one fermentation sample (FNH6) was grouped with the physical processing samples.

Table 5. SPME-GCMS analysis of Volatile Organic Compounds in commercial Virgin Coconut Oil samples produced by fermentation with heat (n= 5). "<LOD": below limit of detection; "<LOQ": detected but below limit of quantification. The values in parenthesis are the relative standard deviations. VCO samples with asterisk (*) were subjected to sensory evaluation.

Compound	Concentration in sample, ppm (rsd)									
	FWH1*		**FWH2***		**FWH3***		**FWH4***		**FWH5**	
Ethyl acetate (**1**)	<LOQ		<LOQ		<LOQ		<LOD		<LOQ	
Acetic acid (**2**)	22.45	(47.70%)	37.63	(7.67%)	16.77	(22.45%)	<LOQ		34.61	(0.76%)
2-Pentanone (**3**)	<LOQ		<LOQ	(9.19%)	<LOD		<LOD		<LOD	
Hexanal (**4**)	2.29	(30.00%)	1.54	(7.03%)	2.15	(6.07%)	0.47	(3.59%)	0.82	(62.76%)
n-Octane (**5**)	<LOQ		<LOQ		<LOQ		<LOQ		<LOQ	
2-Heptanone (**6**)	<LOQ		<LOQ		<LOD		<LOQ		<LOQ	
Limonene (**7**)	<LOD		<LOD		<LOD		<LOD		<LOD	
Unknown (**8**)	0.97	(24.53%)	1.77	(11.75%)	0.42	(20.85%)	0.43	(5.25%)	0.65	(3.08%)
Nonanal (**9**)	2.27	(21.43%)	<LOQ		<LOD		<LOD		<LOD	
Octanoic acid (**10**)	167.51	(25.64%)	199.82	(17.01%)	<LOD		309.61	(7.01%)	<LOD	
Ethyl octanoate (**11**)	<LOQ		<LOQ		<LOQ		<LOD		<LOQ	
δ-Octalactone (**12**)	116.58	(22.80%)	154.12	(13.22%)	<LOQ		<LOQ		68.65	(4.65%)
Ethyl decanoate (**13**)	<LOQ		<LOQ		<LOD		<LOD		<LOD	
δ-Decalactone (**14**)	<LOQ		<LOQ		<LOD		<LOQ		<LOD	
Dodecanoic acid (**15**)	<LOQ		<LOD		671.88	(118.25%)	2627.51	(17.46%)	<LOD	

Aroma Profile

Five descriptors were used for the aroma attributes of VCO: acid, cocojam, *latik*, nutty, and rancid aromas (Villarino et al. 2007). Data from eight panelists were considered after eliminating those who could not discriminate according to one-way ANOVA ($p<0.5$) (Stone et al. 1974). The mean descriptive ratings of aroma attributes of commercial VCO samples indicate that samples significantly differed in many of the attributes (Table 6).

Overall, centrifuge and expeller VCO tended to give lower acid and rancid aromas. However beyond this pattern, the various VCO samples, even those which were prepared using the same method, gave variable descriptive ratings. For example, Cen2, FNH1, FNH2, FWH1, and FWH2 had significantly ($p<0.05$) higher acid aroma intensity compared to the rest of the samples. On the other hand, Exp1, Exp2, and Cen1 had significantly ($p<0.05$) higher cocojam aroma.

PCR of VOCs and the sensory attributes of VCO

Determining the compounds which give rise to distinct sensory attributes is important since sensory quality plays a significant role in the overall quality of VCO. PCR can be used as a descriptive tool to study the relationship between the VOCs found in the headspace and the sensory attributes. A 5-member panel was also asked to describe the odor of the different VOCs found in the headspace of VCO using five aroma characteristics (i.e. acid, cocojam, *latik*, nutty, and rancid). Known amounts of the compounds were spiked in a VCO matrix and presented to the panelists to relate each compound to a particular VCO aroma characteristic. Lactones impart cocojam and *latik* aroma to VCO samples consistent with the description given by the olfactory panel; 2-heptanone and ethyl acetate at levels found in VCO impart a nutty aroma, while octanoic acid contributes the most to rancid aroma (Figure 4). These VOCs are well-described by the model since they lie near the 100% explained difference circle (Figure 4B). Samples produced by physical means (centrifuge and expeller) found on the upper right of the scores plot have higher levels of δ-octalactone, while samples produced by fermentation found on the lower left have higher levels of octanoic acid (Figure 4A).

Univariate ANOVA of VOCs and sensory attributes of VCO

Performing ANOVA on the levels of VOCs found in VCO, it was found that the levels of acetic acid, hexanal and *n*-octane distinguish the different processes from each other. The high levels of acetic acid, hexanal and *n*-octane found in VCO produced by FWH and FNH differentiate them from VCO produced by Exp and Cen methods.

Sensory attributes of VOCs in VCO

The volatile organic compounds found in the headspace of VCO samples can be classified into the following groups: acetic acid and ethyl acetate, free fatty acids (FFAs) and fatty acid ethyl esters, aldehydes, methyl ketones, δ-lactones, and hydrocarbons.

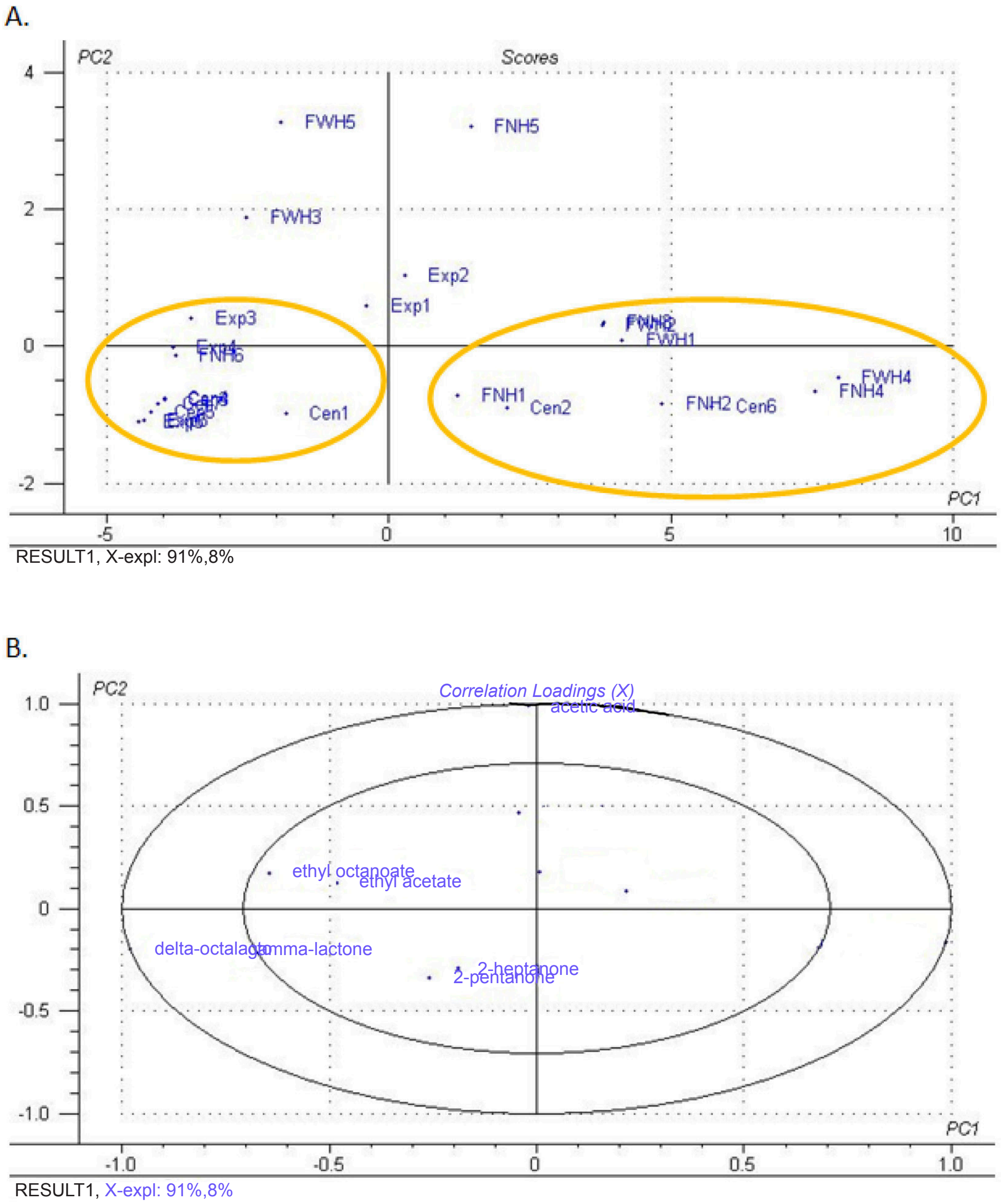

Figure 3. PCA analysis of VOCs which were identified in the 24 VCO samples by headspace SPME-GCMS analysis (See Tables 2-5). A. Scores plot. B. Correlation Loadings plot. The outer and inner circles in the correlation loadings plot represent 100% and 50% explained variance, respectively.

Acetic acid is known to be produced during fermentation by endogenous microflora in coconut (Lisdiyanti et al. 2003). It is known for its pungent smell and imparts an undesirable odor to VCO described as hydrolytic rancidity. The aromatic sensory threshold for acetic acid was estimated to be 3 ppm, whereas the SPME-GCMS LOD was 4.17 ppm. Acetic acid was not detected by SPME-GCMS analysis of any centrifuge-produced samples (Table 2); however, samples Cen 1 to Cen 4 gave acid aroma mean descriptive ratings ranging from 5.12 to 15.43 (Table 6). This suggests that acetic acid may be present at around the sensory aroma threshold and chemical LOD (3 - 4 ppm).

Octanoic acid was found to be more significant both in terms of amount present in the headspace (Tables 2-5), as well as its contribution to rancidity (Figure 4B). Samples produced by fermentation (Tables 4 and 5) had higher

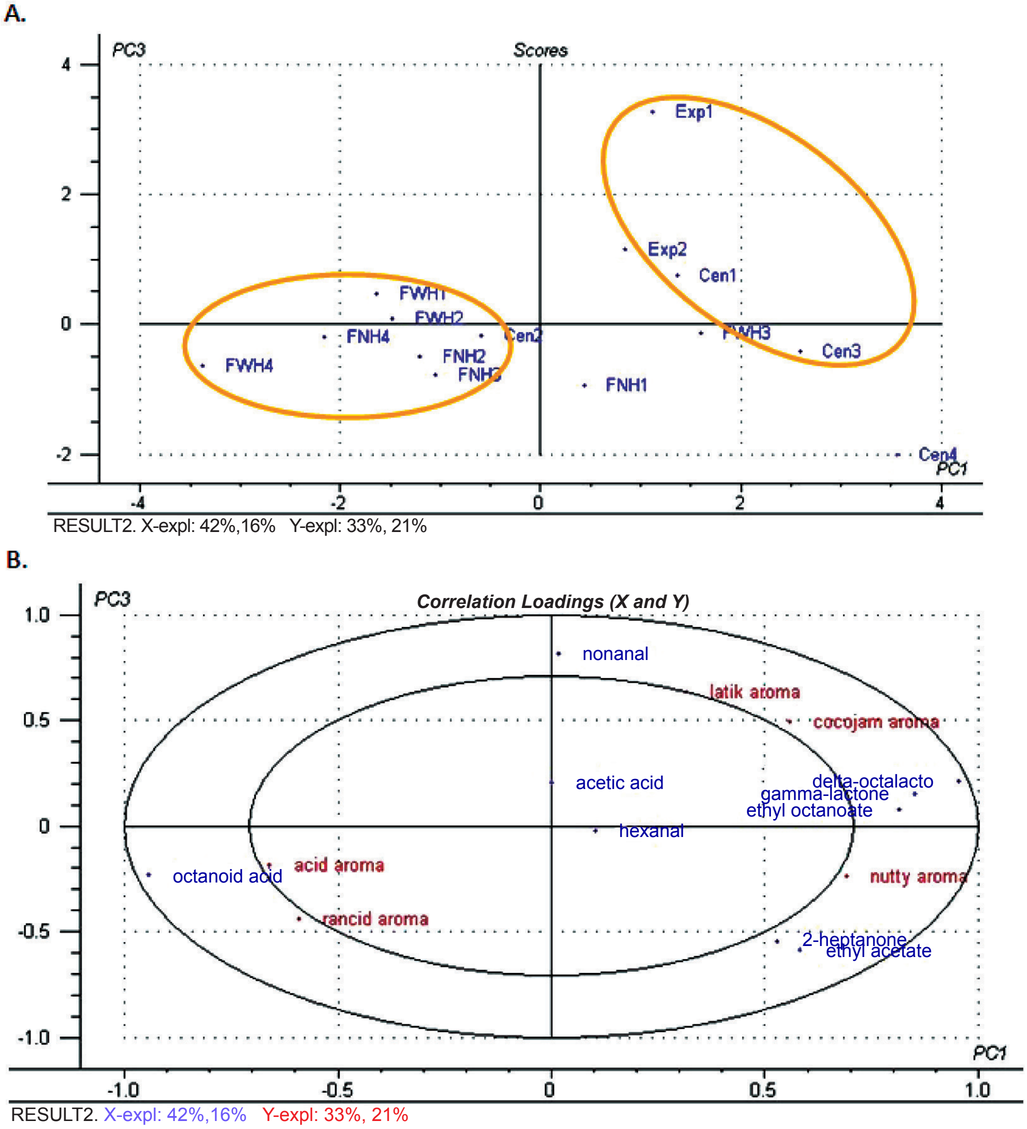

Figure 4. PCR analysis of VOCs and the aroma attributes of 14 VCO samples (See Tables 2-5, VCO samples marked with asterisk *). A. Scores plot. B. Correlation Loadings plot. The outer and inner circles in the correlation loadings plot represent 100% and 50% explained variance, respectively.

concentrations of octanoic acid (up to 800.8 ppm) in their headspace compared to the VCO produced by the centrifuge and expeller methods (Tables 2 and 3).

VCO is unique among vegetable oils because of its high level of dodecanoic acid (45 - 55% of all fatty acids). Consistent with this, high levels of dodecanoic acid were detected in the headspace of various VCO samples produced by both physical and fermentation methods. However, dodecanoic acid has a higher odor threshold compared to the shorter chain fatty acids and does not exert a significant olfactory effect on VCO.

Aldehydes, in particular hexanal and nonanal, are produced from the oxidation of linoleic acid and oleic acid respectively and are associated with undesirable smell described as oxidative rancidity. The low levels of hexanal (<LOD: ~2.29 ppm) and nonanal (<LOD: ~3.37 ppm) in the VCO headspace suggest that oxidation is not a major cause of rancidity (Figure 4B). Lactones arise from the cyclization and dehydration of γ- and δ-hydroxy acids (Kinsella et al. 1967). δ-Octalactone is responsible for the characteristic coconut aroma (Padolina et al. 1987, Maarse 1991). The majority of the VCO samples gave detectable amounts of δ-octalactone ranging from 68 to 196 ppm.

Methyl ketones are formed in coconut meat and coconut oil by the action of fungi and bacteria (Fernandez 1988). Methyl ketones are generally associated with

Table 6. Mean descriptive ratings* of aroma attributes of Vigin Coconut Oil samples.

VCO sample	Acid Aroma	Cocojam aroma	Latik Aroma	Nutty aroma	Rancid aroma
Cen1	9.93 ± 1.62^{cde}	35.38 ± 1.81^{a}	35.15 ± 1.62^{ab}	27.09 ± 1.39^{a}	9.18 ± 1.34^{f}
Cen2	15.43 ± 1.49^{a}	24.61 ± 2.73^{cde}	19.77 ± 2.19^{e}	21.88 ± 1.20^{c}	28.62 ± 2.79^{bc}
Cen3	8.25 ± 1.22^{efd}	28.53 ± 1.53^{bc}	27.26 ± 1.54^{cd}	27.28 ± 1.40^{a}	9.45 ± 1.55^{f}
Cen4	5.12 ± 0.80^{f}	14.82 ± 1.07^{gh}	18.45 ± 1.09^{e}	22.54 ± 1.50^{bc}	6.73 ± 1.14^{f}
Exp1	11.32 ± 2.05^{bcd}	31.88 ± 1.96^{ab}	44.33 ± 2.12^{a}	26.82 ± 1.19^{ab}	8.52 ± 2.12^{f}
Exp2	5.83 ± 1.06^{ef}	29.94 ± 2.17^{ab}	31.77 ± 1.40^{bc}	19.11 ± 1.20^{cd}	5.63 ± 1.18^{f}
FNH1	16.25 ± 1.39^{a}	20.78 ± 2.33^{def}	22.52 ± 1.74^{de}	20.69 ± 1.30^{cd}	35.18 ± 2.98^{a}
FNH2	15.00 ± 0.96^{ab}	17.78 ± 1.72^{fgh}	19.68 ± 1.60^{e}	16.57 ± 1.04^{de}	23.70 ± 2.10^{cd}
FNH3	9.20 ± 1.25^{cde}	25.71 ± 1.38^{cd}	30.06 ± 1.18^{bc}	19.02 ± 1.00^{cd}	26.81 ± 1.64^{bc}
FNH4	8.20 ± 1.06^{fde}	13.57 ± 1.02^{h}	20.14 ± 1.23^{e}	14.34 ± 0.86^{de}	17.02 ± 2.43^{e}
FWH1	15.92 ± 1.3^{a}	28.00 ± 2.27^{bc}	30.96 ± 2.41^{bc}	22.58 ± 1.30^{b}	32.58 ± 2.71^{ab}
FWH2	13.72 ± 0.98^{abc}	19.69 ± 1.31^{efg}	23.39 ± 1.74^{de}	16.88 ± 1.11^{de}	20.00 ± 1.85^{de}
FWH3	8.84 ± 1.19^{def}	25.31 ± 1.40^{cde}	27.90 ± 1.36^{cd}	19.87 ± 0.95^{cd}	27.61 ± 1.43^{bc}
FWH4	10.36 ± 1.32^{cd}	14.11 ± 1.03^{gh}	19.55 ± 1.30^{e}	16.16 ± 1.06^{de}	16.38 ± 1.88^{e}

the unpleasant odor described as ketonic rancidity (Kinderlerer & Kellard 1984). Variable amounts of these compounds were found in the headspace of all of the VCO samples analyzed. At >7.5 ppm, 2-heptanone gives a rancid aroma to VCO. However, none of the VCO samples contained 2-heptanone beyond 5.23 ppm.

SUMMARY AND CONCLUSIONS

The volatiles in the headspace of VCO samples produced by four VCO methods (expeller, centrifuge, fermentation with heat and fermentation without heat) were analyzed using headspace SPME-GCMS while their aroma characteristics were determined by a sensory panel. Fourteen compounds were identified in headspace of VCO samples using SPME-GCMS and confirmed by comparison with pure standards. Fermentation-produced samples were found to have higher levels of acetic acid and FFA in the headspace compared to VCO produced using the centrifuge and expeller methods.

Some of the VOCs may form by microbial conversion of FFA. For example, Kindelerer & Kellard (1984) proposed that 2-heptanone arises from decarboxylation of octanoic acid. δ-Octalactone may also arise from octanoic acid via δ-hydroxylation and lactonization (Lin & Wilkins 1970).

Applying PCA to the chemical and sensory data reveals that VCO produced through the centrifuge and expeller methods can be distinguished from samples produced by fermentation. Octanoic acid was found to exert the strongest influence in terms of acid and rancid aroma. However, the variability observed in both the chemical and aroma profiles in the VCO samples produced by both physical and fermentation processes suggests variability in the quality of VCO products.

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