PRODUCTION OF VIRGIN COCONUT OIL BY DIFFERENT WET METHODS AND DETERMINATION OF QUALITY PARAMETERS

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Abstract
Virgin Coconut oil (VCO) is a value added edible grade product of coconut. In general, different wet and dry methods were employed for the production of Virgin coconut Oil . Commercially VCO is producing by both dry and wet methods. In present study, VCO was produced in wet process by applying low temperature, freezing and centrifugation, ultra centrifugation, natural fermentation and induced fermentation (un-controlled) methods. Due to the auspicious demand in market for the VCO, organizations like Asian Pacific Coconut Community (APCC), Codex Alimentarius has designed quality control parameters in physical, chemical and microbiological aspects. Different physico-chemical and microbiological quality parameters were determined for all the VCO samples produced in all wet methods. All physical parameters were within the standards except the moisture content in VCO samples from fermentation method. In chemical characteristics, all the parameters of VCO produced from all methods were accordance with the Asian Pacific Coconut Standards. The fatty acid content of the VCOs produced in all the methods were showed the lauric acid in the major proportions. In the case of microbiological parameters, the microbial number is more in samples from fermentation methods it may be due to the presence of micro-organisms which may present in the fermentation process, but remaining all the samples were well within microbial quality. Highest amount of (6.23/100g) tochopherol is determined in the samples produced by the induced fermentation (uncontrolled conditions).

Keywords: Centrifugation method; Coconut Milk; Induced fermentation; Natural fermentation; Virgin Coconut Oil; Wet methods.

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1.INTRODUCTION

VCO differs from the commercial coconut oil in the way of its processing. VCO is a value added product (VAP) from coconut with numerous applications for mankind. Asian Pacific Coconut Community (APCC), The Philippine National Standards (PNS), Bureau of Product Standards (BPS) defined VCO as it is naturally processed either from ball copra (within the nut) or fresh coconut meat derivatives (coconut milk), free from additives, which has not undergone any chemical, high temperature processing changes after extraction in order to preserve its natural qualities (Fabian et al. 2007; Blance et al. 2001).

VCO has different food applications, Choo et al. (2010) succeeded in substitution of milk fat with VCO to produce nutritious ice cream with pleasant coconut flavor and aroma. Submersed chicken in VCO was showed the high storage stability at room temperatures (Aritonang et al. 2009). Mike foale (2003) reported that, VCO is acting as a major ingredient in the high quality cooking lotions, drinks due to its special flavor and aroma properties.

Administration of VCO has showed significant antithrombotic effect when compared to coconut oil. These properties of VCO may be attributed to the presence of biologically active unsaponifiable components like vitamin E, pro-vitamin A, polyphenols and phytosterols (Nevin *et al*. 2006). An open-label pilot study was reported by Kai ming liau et al. (2011) on the positive effect of VCO efficacy in weight reduction. Nevin et al. (2010) evaluated healing property of VCO, treated wounds with VCO healed much faster, as indicated by a decreased

time of complete epithelization and higher levels of various skin components. A randomized double-blind controlled clinical trial was reported by Agero et al. (2004) on the effect of VCO on mild to moderate xerosis, concluded that VCO positively showed the curing of Xerosis. Zakaria et al. (2011) determined the hepatoprotective nature of VCO. Olufunke et al. (2012) reported the protective effects of VCO on alcohol-induced oxidative stress. Mahadevappa et al. (2011) pointed that VCO has possess insulinotropic effects, shown in isolated perfused mouse islet with hypolipidemic effects.

It was reported that a blend of different essential oils (lemon oil, eucalyptus oil and lavender oil) and VCO was used to prepare massage oils (Sarunyoo songkro et al. 2010). Inhibition property of VCO against *Candida sp*. obtained from clinical specimens was reported by Ogbolu d et al. (2009). Yuniwart et al. (2012) reported the fatty acid in virgin coconut oil was potential as immunostimulant, which therefore could increase chicken immunity through the increase of lymphocyte T and Th-CD4.

Reza zamiri et al. (2011) reported that a laser ablation of a silver plate immersed in VCO was used for the fabrication of silver nanoparticles. VCO has recently become a more popular new raw material in the cosmetic industries (Sarmad et al. 2009) since VCO-in-water, a nano-emulsion in the form of cream stabilized by an emulsifier, was prepared by using the Emulsion Inversion Point method.

Present, there are many documented applications and health benefits of VCO. This functional, Value Added Product may be adulterated with cheap sources. To avoid the adulteration, determination of quality control parameters are very important. Major objectives of the present study are production of VCO by different wet methods, determination of different quality control parameters for produced VCO and comparison with the existing standers.

2. MATERIALS AND METHODS:

Production of VCO on Wet Basis- Collection and Processing of the Sample (Coconut Milk Extraction): Known weight of the depared coconut was taken in a kitchen grinder jar and ground for 10 min by adding water in 1:2 ratios. Ground mass was then transferred to the cheese cloth, squeezed manually to extract coconut milk; the same process was repeated twice and the coconut milk obtained was pooled up. After extraction of milk, the leftover residual coconut powder, a value added product (VAP) was dried in tray drier and preserved for further studies.

Coconut milk obtained was destabilized to separate VCO by different wet methods as follows.

1. Low Temperature Method (LTM) 2. Chilling and Centrifugation Method (C&C) 3. Centrifugation Method (CM) 4. Natural Fermentation Method (NFM) 5. Induced Fermentation Method (IFM) in Un controlled Condition (UC).

Production of VCO by LTM: The coconut milk is oil in water emulsion by composition consisting of different proteins (Seow and Gwee 1997). Proteins are the bimolecules which can coagulate at higher temperatures.

Coconut milk extracted from the known amount of depared coconut was taken in to a separating funnel and placed for 2 hrs without any agitation to separate water portion from the coconut milk. Slowly the cream portion and water portion gets separated, with cream on the top and water at the bottom. Later, the water was drained off and the top creamy portion (coconut milk emulsion with small amount of water) was collected into a borosilicate glass beaker. The beaker was then placed in thermostatic water bath at 50^0C and constantly maintained for 15-18 hrs. Proper care has been taken not to raise the temperature >50^0C.

Due to the maintenance of constant temperature for a long time, oil gets separated from the emulsion on the top of the beaker. The proteins were coagulated by heating and bounded fat particles were released and the oil droplets were combined together. Water was

present at the bottom of the beaker, coagulated protein portion was present in the middle and topmost layer was the VCO, it was filtered through whatman filter paper.

Production of VCO by C&C Method: Extracted coconut milk was taken in to a beaker and subjected to chilling in refrigerator at 4^0C for over knight. At this chilling temperature except the water, remaining portion was solidified on the top of the water layer (it is a portion of fat and proteinaceous matter). Bottom water portion was removed; total solidified portion was then separated. Allow the solid form to convert in to liquid at the room temperature.

Coconut milk was allowed for centrifugation process, which was done by taking the liquid coconut milk in to a 100 mL capacity centrifuge tube with lid in temperature controlled centrifuge or cooling centrifuge at 12000 rpm for 10 min, and the temperature was maintained at 26 ± 2^0C during the overall process. After centrifugation for 10 min the oil was separated on the top layer, it was separated by the suction in to another container. Pooled up oil from all batches was centrifuged again at same conditions to get clear VCO. This process was repeated for several times to determine the efficiency of process, oil from different batches were preserved in the glass/plastic bottles, weighed and yields were calculated.

Production of VCO by CM: The water and cream layers were separated from the milk in a separate funnel. Coconut cream was directly subjected to the centrifugation in the cooling centrifuge in 100 mL capacity contained plastic centrifuge tube with cap at 20,000 rpm for 20 min. The overall centrifugation process should be maintained at 26 ± 2^0C temperature with the cooling system.

After successful completion of centrifugation process, the total sample was divided into four portions solid mass, water, white suspended proteinous material and VCO from bottom to top of centrifugation tube. VCO was separated by suction in to another container. Pooled up VCO from all batches were centrifuged again maintaining the same conditions to get clear VCO. VCO produced was analyzed for its physico-chemical and microbiological quality parameters.

Production of VCO by NFM: The produced coconut milk was allowed for the natural fermentation in a separating funnel or drain contained vessel. The coconut milk was poured in a clean container and kept aside for 24-48 hrs at room temperature. After fermentation the coconut milk was destabilized in to coconut oil and protein portion. It consists of three parts; top of the vessel consists of VCO, middle non destabilized cream and in the bottom remains water. The water was drained off through the draining pump and the separated oil was collected in a beaker, and the oil was filtered through what man filter paper.

Production of VCO by IFM: Produced coconut milk was taken in autoclaved conical flask, allowed for sterilization under the U.V light in laminar airflow unit for 20 min. After sterilization, 2% of broth containing seed culture (probiotic strains of *Lactobacillus plantarum*) was added to the coconut milk under aseptical conditions in laminar air flow unit and the conical flask was made air tight by closing its mouth with cotton plug and placed in the shaking incubator for 48 hrs at 37^0C temperature. Due to fermentation the coconut milk was destabilized into VCO and proteinaceous portion.

After successful completion of fermentation, the fermented milk was centrifuged in a temperature controlled centrifuge at 27^0C and 6000 rpm for 10 min. Separated VCO was collected; pooled VCO from all batches were finally centrifuged to obtain clear VCO by maintaining the same conditions mentioned earlier. The same process was repeated for several times to study the production yield. VCO produced was analyzed for its physico-chemical and microbiological quality parameters.

Calculation of yield:

These processes were repeated for several times and process yields were calculated by using the following formula.

Process yield of VCO %=

$$\frac{\text{weight of the VCO was produced in the process}}{\text{Weight of the coconut taken for milk extraction}} \times 100$$

Determination of physico-chemical parameters: Quality control parameters, physical properties like specific gravity, Refractive index, Moisture content, Insoluble impurities and chemical properties like Saponification value, Iodine Value, Non-saponifiable matter, Polenske Value, Free fatty acid content and peroxide values were determined as according to AOCS methods.
Fatty acid profile (FAME) is determined for all the samples were determined by Gas chromatography IUPAC method. Total Plated Count was determined by aerobic plate count method. Total tochopherol composition is determined by HPLC.
***Statistical Analysis*:** All parameters were carried out in triplicate. Statistical mean of three values were presented in the study. Significant differences between means were determined by Duncan's multiple range tests and were considered to be significant when P≤0.05, based on SAS software (procedure followed is PROC ANOVA).

3. RESULTS AND DISCUSSION:

Production Yields of VCO in Different Methods:

Among the five wet methods in VCO production, highest yields were obtained in the centrifugation process followed by C&C, IFM (uncontrolled), NFM and LTM. In the former three cases, the VCO was separated by the centrifugation at different rotations and durations. In the later two methods (NFM and LTM) two distinct layers were formed hence, VCO was just decanted or used low centrifugation force; there is no need to use of centrifugation to separate VCO, yields in different methods were presented table 1.
In an earlier study (Brian 1975) it was reported around 30% of the yield in the LTM (50-60^{0}C) after centrifugation process where as in the present study (<50^{0}C) the yield is around 25% without centrifugation. This may be due to low temperature. In the IFM (UC), the yield of VCO was more in 28.47% both the fermentation methods.

Table 1: VCO yields (%) obtained in different dry and wet methods

S.NO	Equipment used to produce VCO	Yield percentage
1	LTM	25.20±0.735^a
2	C&C	28.62±1.011^c
3	CM	30.65±0.594^a
4	NFM	25.68±0.963^{ca}
5	IFM (U.C)	28.47±1.070^d

Note: Values followed by different letters (a, b, c, d) in column differs significantly from each other at P ≤ 0.05, based on SAS software.

Characterization of VCO-Physical Parameters:

Various physico-chemical and microbial parameters for VCOs produced in different methods were studied, reported in table 2 & 3 and compared with APCC standards.
Physical characteristics: In present study, VCOs produced in all the methods were shown Specific gravity in the range of 0.915-0.920. According to the APCC standards Specific gravity is 0.915-0.920. All the VCO samples produced from all methods were within the standard range of APCC. Refractive Index value of 1.4482 - 1.4491was observed for the VCO produced from different wet methods respectively. They are well within the APCC standard limits.
VCOs produced from wet methods were showed higher moisture content levels (0.40 - 0.56%) when compared with standards and they are within the range except NFM and IFM (U.C) were shown high in the moisture content. This may be due to the variations in processing conditions. Insoluble Impurities of 0.24 - 0.34% was present in VCOs produced from wet methods and they are within the standard limits. Physical characteristics (Specific gravity, Refractive index, Moisture content, Insoluble impurities) of the VCO samples were produced in this study are as accordance with the study of Raghavendra and Raghavarao (2011).

Table 2: Physical parameters (specific gravity, refractive index, moisture content, insoluble impurities) of VCO produced by different dry and wet methods

S.No	Method	Physical properties (Mean ± SD)			
		Sp. Gr	R.I	M.C	In.Im
1	LTM	0.920± 0.0055^{c}	1.4487± 0.0054^{db}	0.40±0.03^{cd}	0.031±0.06^{bc}
2	C&C	0.915± 0.0021^{b}	1.4482± 0.0017^{a}	0.42±0.01^{a}	0.024±0.21^{e}
3	CM	0.919± 0.0010^{bc}	1.4491± 0.0047^{d}	0.44±0.06^{ab}	0.026±0.011^{c}
4	NFM	0.918± 0.0028^{a}	1.4490± 0.0036^{ab}	0.52±0.08^{c}	0.034±0.06^{d}
5	IFM (U.C)	0.919± 0.0055^{dc}	1.4483± 0.0016^{b}	0.56±0.01^{d}	0.029±0.13^{c}
6	APCC	0.915 - 0.920	1.4480 - 1.4492	0.1 - 0.5	0.05

Note: Values followed by different letters (a, b, c, d, e) in column differs significantly from each other at $P \leq 0.05$, based on SAS software.

Sp.Gr=Specific gravity at 30^{o}C / 30^{o} C; R.I = Refractive index at 40^{o}C; M.C= Moisture content % by Wt; In. Im= Insoluble impurities % by mass

Chemical Characterization of VCO: Saponification values were observed between 252.4 - 254.3 for VCO samples produced by different methods. Saponification values are well within the APPC standard limits and also coincide with the values reported in earlier studies (Marina et. al. 2009: Yaakob et. al. 1992). Iodine value range of 4.2-9.5 was showed by VCOs produced by wet methods. All the VCOs produced in wet methods were within the standards of APPC and are correlated with reported study (Marina et. al. 2009).

Non saponifiable matter percentage of 0.36 to 0.40 was showed by VCO samples produced by different wet methods. All the VCOs produced in all methods showed the Non saponifiable matter values were within the standard APCC range and are correlated with earlier reported study of Marina et al. (2009).

Polenske value range of 13.1- 13.9 was determined for VCO produced from wet methods. All the VCOs produced in wet methods showed the Polenske values were Within the standard range and are correlated with earlier studies (Raghavendra and Raghavarao 2011; Mehlenbacher 1960).

Free Fatty Acid range of 0.29-0.49% was observed for VCO samples produced from wet methods. The free fatty acid values of all VCOs produced in wet methods are shown within the standard APCC range and they are correlated with Raghavendra and Raghavarao (2011); Laurelese and Resurreccion (1979); Sarunyoo (2010) and differed with Thieme (2006) reported studies.

Peroxide value range of 0.83 to 0.88 meq /kg was observed for VCOs produced from dry and wet methods respectively. All VCOs produced in wet methods were showed the peroxide values were well within the standard APCC range and correlated (Raghavendra and Raghavarao 2011) and differed (Rini 2009; Thieme 2006) with the reported values. All the chemical quality parameters data is depicted in the table 3.

Table 3: Chemical parameters (Saponification Value, Iodine Value, Unsaponifiable matter, Acid Value, Peroxide value, Polenske value, free fatty acid value) of the VCO produced in different dry and wet methods

S.No	Method	Chemical properties (Mean±SD)					
		S. V	I.V	N.S.M	P.V	Pol. V	FFA
1	LTM	253.0± 1.14^{d}	8.5± 0.93^{b}	0.36± 0.012^{ba}	0.84± 0.23^{e}	13.1± 0.1^{db}	0.29± 0.01^{cd}
2	C&C	254.3± 1.37^{bc}	4.2± 0.17^{ad}	0.40± 0.041^{cb}	0.83± 0.17^{bd}	13.4± 0.2^{a}	0.33± 0.05^{a}
3	CM	252.5± 1.02^{a}	7.2± 0.75^{a}	0.39± 0.026^{d}	0.88± 0.11^{a}	13.7± 0.4^{bc}	0.35± 0.02^{d}
4	NFM	252.4± 0.71^{ad}	9.5± 0.31^{ab}	0.38± 0.035^{cb}	0.86± 0.02^{dc}	13.9± 0.60^{dc}	0.46± 0.19^{e}
5	IFM (U.C)	252.5± 0.94^{c}	6.1± 0.99^{c}	0.40± 0.031^{a}	0.85± 0.01^{c}	13.9± 0.30^{e}	0.49± 0.15^{ac}
6.	APCC	250 - 260 min	4.1 - 11.00	0.2 - 0.5	≤3	13	≤ 0.5%

Note: Values followed by different letters (a, b, c, d, e) in column differs significantly from each other at $P \leq 0.05$, based on SAS software.
S.V= Saponification Value; I.V= Iodine Value; N.S.M = Unsaponifiable matter % by mass. max.; P.V= Peroxide value (meq/kg oil); Pol. V= Polenske value, FFA= Free fatty acid value %

Fatty Acid Profile: Fatty acid profile of all the VCO samples were analyzed and presented in table 4. In the study, $C_{8:0}$ concentrations between 8.8-9.7% were observed in VCO samples produced by wet methods. All the VCOs produced in wet methods were with standard range.$C_{10:0}$ amounts are present in the range of 5.8 to 6.4% in the VCO produced by wet methods. All the VCOs produced all methods in the present study $C_{10:0}$ is within the APCC range (4.5-8.0%). $C_{12:0}$ amounts are present 47.2 to 49.2% present in the VCO produced by different wet methods respectively. The $C_{12:0}$ in all the VCOs produced in wet methods in the present study were well within the APCC standards.

$C_{14:0}$ amounts are present in range of 19.5 to 20.9% in VCO produced by different wet methods respectively. All the VCO samples produced in wet methods in the present study were well within the APCC range (16.0-21.0%).

$C_{16:0}$ amounts are observed in the range of 7.0 to 8.4% in VCOs produced by different wet methods. The $C_{16:0}$ in all the VCOs produced in wet methods in the present study were well within the APCC (7.5-10%)

$C_{18:0}$ concentrations are present in the range of 2.3 to 3.0% in VCO samples produced by different wet methods. All the VCOs produced in wet methods in the present study are well within the APCC standards. $C_{18:1}$ fatty acid is present in range of 4.5 to 5.0% in VCO samples produced by different wet methods. The $C_{18:1}$ in all the VCOs produced in wet methods in the present study is well within the APCC range (5.0 to 10%) except the VCO from LTM (4.5%). It may be due to the geographical and varietal differences of the coconuts used in the present study.

Table 4: FAME of VCO samples produced by different dry and wet methods

S.No	Method	Fatty acid composition(%) of VCO produced in different methods							
		C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2
1	LTM	9.2	5.9	47.5	20.8	8.2	2.9	4.5	1.0
2	C&C	8.8	5.8	47.2	20.9	8.4	2.9	4.9	1.1
3	CM	9.3	6.2	47.5	20.1	9.7	3.0	4.9	1.1
4	NFM	9.4	6.2	48.1	19.8	7.5	2.7	5.0	1.2
5	IFM(U.C)	9.7	6.4	49.2	19.5	7.0	2.3	4.7	1.1
6	APCC	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

Table 5: Number of micro organisms (CFU) present in VCO samples produced by the different dry and wet methods

S.No	Method used to produce VCO	Total plate count (CFU/0.1mg of VCO)
1	LTM	4.2 ± 1.7^{c}
2	C&C	7.8 ± 1.9^{b}
3	CM	8.0 ± 1.4^{d}
4	NFM	31 ± 3.1^{c}
5	IFM (U.C)	49 ± 2.8^{a}
6	APCC	< 10

Note: Note: Values followed by different letters (a, b, c, d) in column differ significantly from each other at $P \leq 0.05$, based on SAS software.

$C_{18:2}$ concentrations are observed in range of 1.0 to1.2% in VCO samples produced by different wet methods. All the VCOs produced in dry and wet methods in the present study were well within the APCC standards.

The results of present study is correlates with the Dia (2009); Raghavendra and Raghavarao (2011); Fabian et al. (2007) Yaakob et al. (1992); Fabian *et al*. (2007).

Total Plate Count: Total plate counts of 4.2 to 49 CFU/ 0.1 mg were detected in VCO samples from different wet methods. All the VCOs produced in wet methods (except fermentation methods) were showed the total plate count of microbes and are within the standards and coincides with the reported studies (Sarunyoo et al.2010; Ian ken et al. 2006). But, the total plate count in VCOs from the NFM and IFM (UC) 31 and 49 CFU/0.1mg is observed respectively. In fermentative production of VCO, microbes played a major role. Some microbes may enter naturally and some may deliberately added in fermentation, may remains in VCO samples after separation. This may be one of the reasons for high amount of the microbes present in VCO samples, results related to microbial counts were depicted in table5.

Metallic Contaminants: Iron, Copper, Arsenic and Lead concentrations in VCO samples produced by different wet methods were depicted in table 6.

Table 6: Metals in (Iron, Copper, Arsenic, Lead) VCO samples from different dry and wet methods

S.No	Method	Metals as contaminants in the produced VCO mg/Kg of VCO			
		Fe	Cu	As	Pb
1	LTM	1.29	0.27	0.09	0.02
2	C&C	1.50	0.29	ND*	0.01
3	CM	1.21	0.32	ND*	0.02
4	NFM	1.08	0.33	0.02	ND*
5	IFM(U.C)	1.13	0.22	0.11	ND*
6	APCC	5	0.4	0.1	0.1

ND* = Not Detected.

Fe concentrations ranges of 1.08 to 1.50 mg/kg were present in the VCO samples produced by wet methods respectively. According to the USDA data base, concentration of Fe in the raw coconut is 2.23 mg/100gms; this may be one of the reasons for certain concentration of Fe in VCO. All the VCOs produced by wet methods in the present study are well within the APCC range i.e., Fe < 5 mg/kg of VCO. In the case of Cu, 0.22 to 0.33 mg/kg of concentrations were present in the VCO samples produced by wet methods respectively. According to the USDA reports 0.435 mg of Cu consists in 100 gms of coconut. The Cu in all VCOs produced wet methods in the present study are within the APCC range i.e., < 0.4 mg/kg.

Pb concentration 0.01to 0.02 mg/kg of were present in the VCO samples produced by wet methods respectively. However, Pb was not detected in NFM and IFM. All the VCOs produced in wet methods in the present study are within the APCC range i.e., of Pb < 0.1 mg/kg. As concentration 0.01to 0.02 mg/kg of were present in the VCO samples produced by wet methods. Whereas As concentration was not detected in VCO samples from CM and C&C methods. The As concentrations in all the VCOs produced in wet methods are within the APCC range i.e., < 0.1 mg/kg.

Metallic contamination values of present study correlated with Ian Ken et al. (2006) report.

Tocopherol Concentration: Tocopherol concentration of 5.71 to 6.12 mg/100 gms were present in the VCO samples produced by wet methods. Tocopherols in VCO samples produced from wet methods coincide with Raghavendra and Raghavarao (2011) and differed with study of Marina et al. (2009). Tocopherol concentration of VCO is presented in table7.

Table 7. Concentration of Tocopherols present in the VCO produced by different wet method

S.NO	Method used to produce VCO	Tocopherol in mg/100mg of VCO
1	LTM	5.71
2	C&C	6.09
3	CM	6.02
4	NFM	6.12
5	IFM (U.C)	6.23

4. CONCLUSIONS:

All the processes were suitable for the production of VCO in wet methods, but yields were varied. Where as in wet methods, highest

yield was obtained in CM so, this is recommended for the wet processing of VCO. Quality control parameters for VCO produced from all the methods were studied and compared well with standards. VCO samples from fermentation processes were slightly exceeded in moisture contents. This may be due to the wet processing. Metal impurities were also present in acceptable limits in all VCO samples. Fatty acid composition of all VCO samples produced from different wet methods was showed slight variation. $C_{8:0}$, $C_{10:0}$ and $C_{12:0}$ acid concentrations of all VCO samples were within the standards. In microbial quality control, samples from both induced and natural fermentation had more number of microbes than standards but, in induced fermentation, detected microbes are may be probiotic organisms, whereas the microbes involved in natural fermentation were not known exactly. However, VCO samples produced from other wet methods had microbes within the standards.

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