



# Engineering intervention for production of virgin coconut oil by hot process and multivariate analysis of quality attributes of virgin coconut oil extracted by various methods

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## Abstract

ICAR-Central Plantation Crops Research Institute, India, has designed and developed a virgin coconut oil (VCO) cooker for the extraction of oil by the hot process. However, a number of VCO production processes being followed in India and elsewhere cause variations in the physicochemical properties, which in turn potentially affect the nutritional and medicinal properties of VCO. The physical and biochemical properties of VCO from the hot process (VCO-Hot), fermentation (VCO-Fer), expelled from dried gratings (VCO-EDG), centrifugation (VCO-Cen), and conventionally prepared copra coconut oil (CCO) were investigated in light of the design concept of the VCO cooker. The nutritionally important total phenolic content (mg GAE/100 g) and antioxidant capacity of all the VCOs were found to be in the range of  $0.446 \pm 0.041$  (VCO-Cen) to  $2.867 \pm 0.152$  (VCO-Hot) and 3.87 mM Trolox equivalent (TE) (VCO-Cen) to 11.31 mM TE (VCO-Hot), respectively. Multivariate analysis revealed that quality attributes viz., total phenol, total flavonoid, and cupric ion reducing antioxidant capacity of VCO-Hot defined by principal component 1. Hierarchical clustering showed that the VCO-Hot belonged to the group with high total phenolic and flavonoids content and strong antioxidant capacity. Comparative biochemical properties along with multivariate analysis differentiated the various VCO samples.

## Practical Applications

Production of virgin coconut oil (VCO) by the hot process has been standardized by ICAR-CPCRI and the technology has been successfully adopted by several entrepreneurs. VCO has a tremendous export potential and hence has a greater demand in the international market. The quantum of VCO export from India has been 818 MT to various destinations such as the United States, Japan, Australia, United Kingdom, and Middle East (<https://www.coconutboard.in>). The export earnings of VCO have reached over Rs. 260 million in 2015–2016. The consumers are not aware of the different VCO production methods and the resultant properties of VCO (Manikantan

et al., Virgin Coconut Oil: Hot and Fermentation Process, Technical Bulletin No. 108, Centenary Publication 43, ICAR-CPCRI & AICRP on PHET, Kasaragod). Considering the commerce potential and nutraceutical importance, the quality profile of VCO produced in different methods has to be compared among and with the coconut oil produced from copra. Additionally, it is crucial to discriminate the various VCO samples based on their quality profile.

## 1 | INTRODUCTION

Virgin coconut oil (VCO) is extracted from the fresh and mature kernel of coconut (*Cocos nucifera* L.) through the application of physical methods such as cold pressing, expeller-pressed, centrifugal force, or by natural means utilizing microbes with or without the use of heat. Nevertheless, VCO production does not entail refining, bleaching, or deodorizing (RBD) processes that a conventional copra coconut oil undergoes. VCO has attained greater relevance owing to its nutritional properties, medicinal benefits and has been a component of a functional food (Marina, Man, & Amin, 2009; Villarino, Dy, & Lizada, 2007). VCO intake promotes cardiovascular health by improving serum lipid profiles. Marked reduction of bad cholesterol such as low-density lipoprotein and very low-density lipoprotein, triglycerides and concomitant improvement in high-density lipoprotein were documented with VCO uptake (Nevin & Rajamohan, 2004). Diet supplemented with VCO has provided improved anti-oxidation status in animal studies (Nevin & Rajamohan, 2006).

In general, the biochemical and physical characteristics of VCO and copra coconut oil were investigated to identify the bioactive components responsible for the observed health benefits. Spectroscopy and spectrometry techniques (NMR spectroscopy and HS-SPME coupled with GC-MS) could not differentiate the VCO and commercially produced RBD coconut oil (RCO), however, VCO recorded low diglyceride (1.55 w/w%) compared to RCO (4.1 w/w%) (Dayrit et al., 2007). Furthermore, Marina, Che Man, Nazimah, and Amin (2009) reported the superiority of VCO over RCO in terms of phenolics content.

Traditionally VCO is being produced from various processes including the use of a shallow pan to heat coconut milk and to extract oil. Continuous stirring of heated coconut milk is crucial for VCO production because a momentary lapse in the stirring causes charring of the milk that eventually sticks to the heating pan. Traditionally, VCO produced at the homestead farms has several drawbacks including the charring effect of coconut oil due to the uncontrolled heat and presence of inadvertent and undesirable microbial population, and so forth. To avoid these undesirable effects during the production of VCO, ICAR-CPCRI has devised a double-jacketed cooker to extract VCO utilizing heat (VCO-hot). This method of extraction involves the application of controlled heat to the coconut milk to extract VCO. The oil recovery from the hot process is 20–22% of the fresh weight of coconut endosperm (Manikantan et al., 2016). Cold processes involve extraction of VCO from microbes enabled fermentation (VCO-Fer)

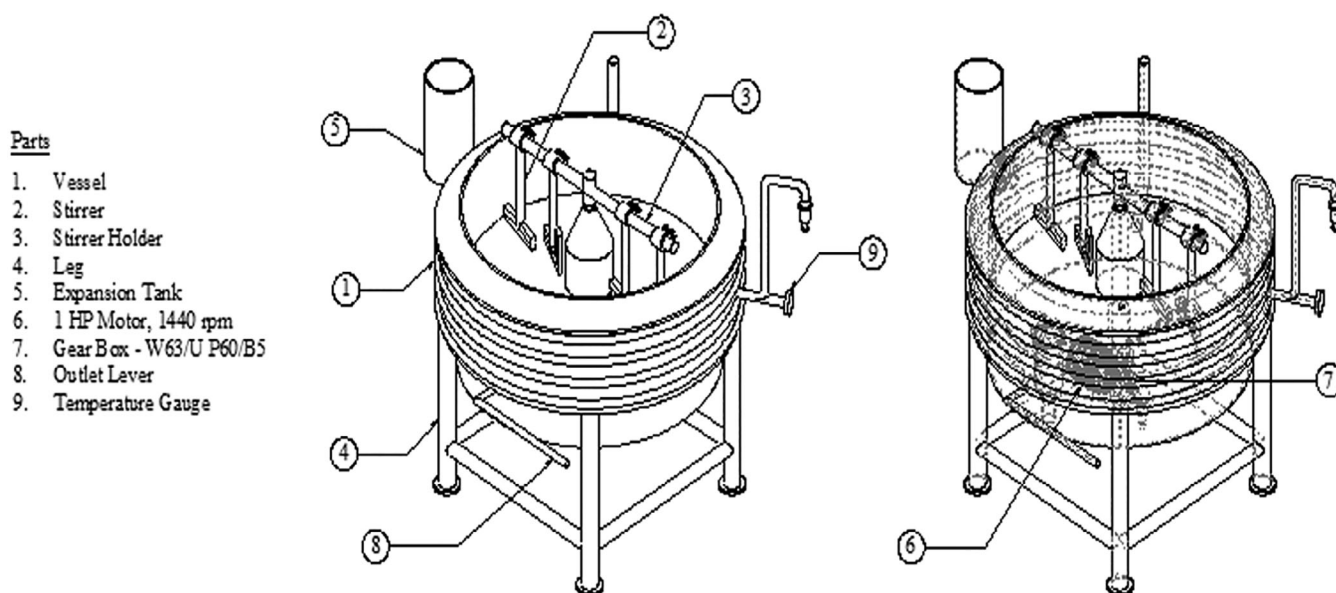
with an oil recovery of 16–18%, VCO extraction by applying centrifugal force (VCO-Cen) characterized with an oil recovery of 18–20%, and VCO obtained from the dried endosperm gratings in a process called extraction from dried gratings (VCO-EDG) with an oil recovery of 22–27% (Manikantan et al., 2016). Fatty acid and triacylglycerol components of various VCOs varied to some extent nevertheless, tocopherols content of oils did show little differences (Mansor, Man, Shuhaimi, Afiq, & Nurul, 2012). Investigations on the bioactive components revealed the high anti-oxidation capacity of the VCO from the hot process compared to the VCO from cold processes and copra oil (Srivastava, Semwal, & Majumdar, 2016).

The quality attributes and medicinal benefits of VCO have been compiled and reviewed comprehensively (Amri, 2011; Krishna, Gaurav, Singh, Kumar, & Preeti, 2010). Nonetheless, the quality profiles of different kinds of VCOs produced in India have not been compared and analyzed yet. Furthermore, VCO produced from India has a tremendous export potential and greater demand in the international market. The export earnings of VCO were at Rs. 50 million in 2013–2014 that has increased significantly to over Rs. 260 million in 2015–2016. The quantum of VCO export has been 818 MT to various destinations such as the United States, Japan, Australia, United Kingdom, and Middle East (<https://www.coconutboard.in>). Hence, it is imperative to generate the quality profile of VCO produced in India and compare it with that of coconut oil produced from copra. It is also essential to discriminate the samples based on the quality profile. In this work, the physicochemical properties of various VCOs namely, VCO-Hot (ICAR-CPCRI process), VCO-Fer, VCO-Cen, VCO-EDG, and copra coconut oil (CCO) are compared. Correlation and multivariate analysis were performed to define the quality parameters of various VCO from India.

## 2 | MATERIALS AND METHODS

### 2.1 | The design concept of VCO cooker

VCO production in the hot process involves the unit operation of heating the coconut milk. Traditionally VCO has been prepared by heating the coconut milk in a shallow depth pan at low flame. Continuous stirring is a laborious and high-energy consumption process. Hence, ICAR-CPCRI, Kasaragod, India, had designed and developed the VCO cooker (Figure 1) to extract the oil by the hot process. This article does not contain any studies with human or animal subjects.



**FIGURE 1** Engineering diagram of virgin coconut oil cooker

VCO cooker designed and developed by ICAR-CPCRI is a batch type cooker where the continuous stirring of coconut milk is accomplished by a mechanical setup using a reduction gearbox and an electric motor. VCO cooker was made up of stainless steel and contains a double-jacketed container. Thermic fluid (a mixture of synthetic hydrocarbons) was filled in the double-jacketed container, which facilitates the indirect process of heating. A mechanical stirrer of four arms was provided in the heating chamber of the cooker. Contact portions of the four arms were laminated with Teflon (Mathew, Madhavan, & Arumuganathan, 2014). The Teflon coated arms and/or stirrers were connected to an electric motor. The power requirement of the stirrer was calculated as suggested by Khurmi and Gupta (2006).

$$\text{Horse power required} = \frac{2\pi \times \text{RPM of the mixing arm} \times \text{torque, kg m}}{4,500}$$

The following assumptions were obtained from Mathew et al. (2014):

1. Self-weight of the mixing arm = 6 kg.
2. Coconut cream/milk to be mixed = 125 kg per batch.
3. Speed of the mixing arm (assumed) = 25 rpm.
4. Thus, coconut cream to be mixed per revolution = 40 kg.
5. Total weight = self-weight + material weight = 6 + 40 = 46 kg.
6. Contact distance in the container where the mixing is done = 0.60 m.
7. Torque required = load  $\times$  distance = 46  $\times$  0.60 = 27.6 kg m

$$\text{Therefore, the horse power required} = \frac{2 \times 3.14 \times 25 \times 27.6}{4,500} = 0.963 \approx 1 \text{ hp.}$$

Hence, 1 hp motor (1,440 rpm) was attached with a stirrer through a reduction gear (W63/U P60/B5).

## 2.2 | Chemicals, reagents, and VCO samples

All chemicals and solvents used in this analysis were of analytical grade. Ethanol, Folin-Ciocalteu's reagent, gallic acid, neocuproine and ammonium acetate copper chloride, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and potassium persulfate, n-Hexane, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), sodium nitrate, sodium hydroxide, acetic acid, chloroform, potassium iodide, potassium hydroxide, nitric acid, and hydrochloric acid were obtained from Sigma-Aldrich (Darmstadt, Germany).

VCO by hot process (VCO-Hot), fermentation process (VCO-Fer), expelled from dried gratings (VCO-EDG) were obtained from the endosperm of 11–12 months old, fully matured nuts of healthy coconut following standard procedures (Manikantan et al., 2016). Briefly, 11–12 months of matured coconuts were used for VCO extraction. In the hot process, the extracted coconut milk was allowed to separate into the coconut cream and coconut skim milk under refrigerated conditions. Coconut cream is processed in an in-house developed double-walled VCO cooker to coagulate the protein and release the oil (Mathew et al., 2014). In the fermentation process, the extracted milk is allowed to stand 20–24 hr in a food-grade plastic or stainless container under favorable conditions of temperature (35–40°C) and relative humidity (75%), leading to separation of the VCO layer (Manikantan et al., 2016). Similarly, fresh coconut endosperm gratings are dehydrated and the gratings are subjected to mechanical pressure to extract VCO (VCO-EDG). VCO from the centrifugation process (VCO-Cen) and conventionally

prepared copra coconut oil (CCO) were commercially available samples obtained from the market.

### 2.3 | Physicochemical properties

Around 3 g of VCO was used for the determination of physicochemical properties. Moisture content and a specific gravity of VCO samples were estimated following the method suggested by AOCS (Firestone, 2009) using moisture analyzer A&D MX-50 (Oxfordshire, UK). Free fatty acids (FFAs) content was determined according to the AOAC method (2005) (Horwitz, 2000). Standard IUPAC procedure was used for the determination of peroxide value (PV) and the saponification value of the VCO samples (Rigaudy & Klesney, 1992). PV was expressed as milli-equivalents (m-eq) of peroxide  $O_2$ /kg of VCO. The iodine value of the VCO samples was measured following the Wijs method (Firestone, 2009).

### 2.4 | Total phenolics and flavonoid content

The polyphenolic fraction in VCO samples was extracted using 10 ml of 80% ethanol in three successive extractions. The aliquot was mixed with 1:1 Folin-Cicolteau's reagent followed by the addition of 1.0 ml of 35%  $Na_2CO_3$  solution after 3 min and allowed to stand for 45 min in room temperature at dark condition. The total phenolic content (TPC) was spectrophotometrically estimated (absorbance at 760 nm, Shimadzu, Japan) and expressed as mg GAE per 100 g of VCO (Nevin & Rajamohan, 2006). Similarly, total flavonoid content (TFC) was measured and expressed as milligram of quercetin equivalent (QE)/100 g of oil (Zhishen, Mengcheng, & Jianming, 1999).

### 2.5 | Measurement of antioxidant potential

Cupric ion reducing antioxidant capacity (CUPRAC) of the oil extract was determined by following the protocol of Szydłowska-Czerniak and Tułodziecka (2014) with minor modifications. Sample extracts were made up to 1 ml with distilled water and 1 ml each of copper chloride ( $CuCl_2$ ), neocuproine, and ammonium acetate were added and mixed properly and incubated in dark for 30 min. Spectrophotometrical absorbance (Shimadzu, Japan) at 450 nm was recorded and the results were expressed as mM Trolox equivalent (TE)/kg of oil. ABTS + (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) free radical-scavenging assay was performed (Valantina & Neelamegam, 2015) and antioxidant potential of the oils are expressed as the ability of the extract to scavenge 50% of free radical  $ABTS \bullet + (IC_{50})$ .

### 2.6 | Estimation of trace elements

Trace elements, namely, Cu, Fe, and Zn present in VCO samples were estimated following the acid digestion method (Ang & Lee, 2005).

Quantitative measurement of trace elements present in VCO was performed using the atomic absorption spectrometer (Thermo Scientific ICE 3000 series, India).

### 2.7 | Statistical analysis

All the statistical computations were performed using the R software (Version: 3.5) (R Core Team, 2018). Principle component analysis (PCA) was performed through "prcomp" function of the R base library. The hierarchical clustering distance matrix was generated with the Euclidean distance method and the clusters were developed using the Ward D method using the "hclust" function of R base library. Furthermore, PCA-biplot analysis was done using the "facto-extra" package (Kassambara & Mundt, 2016) and "Performance Analytics" package (Peterson et al., 2018) of R, respectively.

## 3 | RESULTS AND DISCUSSION

### 3.1 | Physical properties of VCOs

The moisture content of VCOs was in the range of 0.22–0.29%, within the limits of Asian and Pacific Coconut Community (APCC) prescribed guidelines (Table 1). Slightly high moisture content in the samples of VCO-Fer and VCO-EDG compared to those of VCO-Hot and VCO-Cen could be ascribed to the absence of heat treatment or mechanical pressure. The relative density of oils [0.883 (VCO-Fer) to 0.986 (VCO-EDG)] suggests that the VCO-EDG has high density owing to the microscopic kernel particles. Saponification values (SV) showed slight but insignificant differences among the oil samples [ $251 \pm 1.01$  (CCO) to  $255 \pm 1.06$  (VCO-Hot) mg KOH/g]. However, the values comply with APCC standards for VCO. Also, the slight differences in the SV could be due to the different methods of VCO extraction. The iodine value of VCOs was low [ $5.23 \pm 0.321$  (VCO-Cen) to  $6.3 \pm 0.2$  (VCO-Fer)] compared to CCO [ $8.466 \pm 0.378$ ] however, the values are within the range delimited by APCC. Since iodine value is a measure of unsaturated fatty acids in the glycerol backbone, VCO and CCO have permissible levels of unsaturated fatty acids. The low iodine values of the VCOs signify that these oils comprise saturated fatty acids and are oxidatively stable. PV (milli. eq. peroxide/kg) of VCOs [ $0.24 \pm 0.017$  in VCO-Hot to  $1.233 \pm 0.208$  in VCO-EDG] comply with the APCC standards (<3 milli. eq.  $O_2$ /kg). PV is an indicator of the level of oxidative rancidity hence higher PVs of VCO-EDG and CCO indicate that these oils are prone to oxidative rancidity whereas PVs of VCO-Hot and VCO-Cen suggest their oxidative stability. Thus, among the various VCO extraction processes, hot and centrifugal methodologies yield stable oils compared to the traditionally prepared copra coconut oil. The FFA (%) of VCO samples is in the range of  $0.127 \pm 0.025$  (VCO-Hot) to  $0.293 \pm 0.025$  (VCO-Fer) whereas the CCO sample exhibited higher value  $0.323 \pm 0.025$  which could be due to the unhygienic postharvest management of copra in CCO method. Nonetheless, the values are less than the APCC

**TABLE 1** Physical properties of virgin coconut oils (VCOs) obtained from various processes (VCO-Hot: VCO from Hot process; VCO-Fer: VCO obtained from natural fermentation process; VCO-EDG: Oil expelled from dried gratings through mechanical means; VCO-Cen: VCO extracted by application of centrifugal force) and CCO: Copra coconut oil are presented along with APCC\* and Codex standards\*\* for VCO and coconut oil, respectively

Physical parameters	VCO-Hot	VCO-Fer	VCO-EDG	VCO-Cen	CCO	APCC standard*	Codex standard**
Moisture (%)	0.237 ± 0.083	0.296 ± 0.040	0.286 ± 0.070	0.220 ± 0.060	0.263 ± 0.045	0.1–0.5	No standard
Relative density	0.977 ± 0.005	0.883 ± 0.005	0.986 ± 0.005	0.896 ± 0.005	0.956 ± 0.005	0.915–0.92	–
Saponification value (mg KOH/g)	255 ± 1.06	252 ± 1.03	253 ± 2.13	253 ± 1.54	251 ± 1.01	250–260	–
Iodine value (g I <sub>2</sub> /100 g)	5.733 ± 0.057	6.300 ± 0.200	6.100 ± 0.173	5.230 ± 0.321	8.466 ± 0.378	4.1–11.0	6.3–10.6
Peroxide value (milli. eq. O <sub>2</sub> /kg)	0.240 ± 0.017	0.540 ± 0.040	1.233 ± 0.208	0.270 ± 0.020	1.066 ± 0.115	<3	<15
Free fatty acid (%)	0.127 ± 0.025	0.293 ± 0.025	0.143 ± 0.015	0.186 ± 0.005	0.323 ± 0.025	0.4–0.6	ND-0.7
Acid value (mg KOH)	0.307 ± 0.020	0.786 ± 0.030	0.416 ± 0.056	0.523 ± 0.020	0.900 ± 0.040	–	–

**TABLE 2** Antioxidant potential of virgin coconut oils (VCOs) and Copra coconut oil (CCO)

Biochemical parameters	VCO-Hot	VCO-Fer	VCO-EDG	VCO-Cen	CCO
Total phenol (mg GAE/100 g)	2.867 ± 0.152	0.566 ± 0.020	0.63 ± 0.121	0.446 ± 0.041	1.946 ± 0.041
Total flavonoid (mg QE/100 g)	6.6 ± 0.435	3.466 ± 0.288	5.16 ± 0.132	3.33 ± 0.305	5.353 ± 0.484
CUPRAC (mM TE/kg)	11.31 ± 0.495	4.67 ± 0.064	4.69 ± 0.468	3.87 ± 0.085	9.01 ± 0.446
ABTS IC <sub>50</sub> (mg/ml)	17.42	57.36	44.37	64.78	28.64

Total polyphenols and its constituent flavonoids were estimated followed by measurement of antioxidant capacity based on the reducing power of oils in cupric ion reducing antioxidant capacity (CUPRAC) and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays.

recommended standards (0.4–0.6%). Lower the FFA (%), greater is the taste and aroma of VCO, consequently VCO-Hot and VCO-Cen have desirable taste and aroma among the VCO samples. Corroborating the FFAs (%) of VCO, relatively high acid values (AVs) of 0.9 and 0.786 mg of KOH were recorded for CCO and VCO-Fer, respectively. Overall, this study reveals that the hot and centrifugal processes are suited for preserving the aroma, taste, and oxidative stability of the VCOs. Furthermore, the longer time involved in the drying of the coconut endosperm prior to VCO extraction in VCO-EDG and CCO processes could account for the relative increase in the quantum of FFAs causing rancid flavor.

### 3.2 | Antioxidant potential of VCOs

The TPC of VCO (expressed as mg GAE/100 g) ranged from 0.446 ± 0.041 (VCO-Cen) to 2.867 ± 0.152 (VCO-Hot) (Table 2). It indicates that VCO-Hot has high polyphenols compared to other VCOs. Similarly, high polyphenolic content in VCO obtained from the hot process was reported by Srivastava et al. (2016). The phenolic content of the hot extracted VCO (HEVCO), cold extracted VCO (CEVCO), and copra oil were found to be 650.35 ± 25.11, 401.23 ± 20.11, and 182.82 ± 15.24 µg/g, respectively (Srivastava et al., 2016). Furthermore, the chromatogram of phenolic components divulged that the

concentrations of gallic acid, ferulic, epicatechin, catechin, and p-coumaric derivatives among others were high in HEVCO compared to CEVCO (Srivastava et al., 2016). Authors reasoned that high temperatures during the hot process of VCO production could improve the incorporation of high phenolic substances (Marina, Man, & Amin, 2009; Molaveisi, Beigbabaei, Akbari, Noghabi, & Mohamadi, 2019; Seneviratne & Sudarshana Dissanayake, 2008; Srivastava et al., 2016). Contrarily, Ghani et al. (2018) reported that TPC of VCO from wet process such as fermentation (12.54 ± 0.96 mg GAE/g) was high compared to the dry processing during hot process evaporates excess moisture in coconut milk emulsion (VCO-Hot) improving the incorporation of phenolics (Mulyadi, Schreiner, & Dewi, 2018). Also, this discrepancy in the TPC of VCOs could be attributed to the differences in the standard phenolics used for its estimation, genotype or variety, age of the crop, and maturity stage during VCO extraction. The TFC of VCO also followed the pattern of TPC since flavonoids are constituents of total phenolics (Table 2). TFC varied from 3.33 ± 0.305 (mg QE/100 g) in VCO-Cen to as high as 6.6 ± 0.435 (mg QE/100 g) in VCO-Hot. The antioxidant capacity of oils was measured in terms of CUPRAC and that varied from 3.87 mM TE in VCO-Cen to 11.31 mM TE found in VCO-Hot. It is proved once again that total phenolics and consequently the antioxidant potential of oils are dependent on the method of VCO processing. VCO-Hot exhibited relatively high antioxidant activity attributed to its phenolic content.



Trace elements	VCO-Hot	VCO-Fer	VCO-EDG	VCO-Cen	CCO
Fe (ppm)	4.64 ± 0.091	3.3 ± 0.150	6.34 ± 0.168	4.50 ± 0.215	5.06 ± 0.061
Mn (ppm)	0.25 ± 0.096	1.55 ± 0.130	0.64 ± 0.113	0.36 ± .080	0.77 ± 0.146
Zn (ppm)	1.97 ± 0.0755	2.91 ± 0.117	3.35 ± 0.442	ND	ND

**TABLE 3** Trace elements content of various virgin coconut oil (VCO) preparations

The permissible concentration of iron in VCO according to Asian and Pacific Coconut Community (APCC) standard is <5 (µg/g); ND, not detectable.

**TABLE 4** Correlation analysis of various physicochemical parameters of virgin coconut oil (VCO) prepared by five different methods

	TPC	CUPRAC	TFC	Moisture	IV	FFA	AV	PV	ABTS.RSA	[Fe]	[Mn]	[Zn]
TPC	1	0.993 <sup>a</sup>	0.86 <sup>a</sup>	-0.169	0.287	-0.142	-0.201	-0.184	-0.941 <sup>a</sup>	0.048	-0.425	-0.182
CUPRAC		1	0.855 <sup>a</sup>	-0.113	0.347	-0.078	-0.141	-0.138	-0.947 <sup>a</sup>	0.04	-0.38	-0.173
TFC			1	-0.176	0.231	-0.389	-0.407	0.145	-0.937 <sup>a</sup>	0.45	-0.498	0.106
Moisture				1	0.171	0.283	0.211	0.26	0.033	-0.009	0.38	0.333
IV					1	0.733 <sup>a</sup>	0.75 <sup>a</sup>	0.619 <sup>a</sup>	-0.411	0.131	0.312	-0.293
FFA						1	0.988 <sup>a</sup>	0.271	0.15	-0.43	0.694 <sup>a</sup>	-0.316
AV							1	0.337	0.193	-0.364	0.681 <sup>a</sup>	-0.336
PV								1	-0.124	0.672	0.25	0.233
ABTS • RSA									1	-0.294	0.368	0.006
[Fe]										1	-0.487	0.11
[Mn]											1	0.392
[Zn]												1

Abbreviations: ABTS, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid); AV, acid value; CUPRAC, cupric ion reducing antioxidant capacity; FFA, free fatty acid; PV, peroxide value; TFC, total flavonoid content; TPC, total phenolic content.

<sup>a</sup>Significance at 1% level.

Similarly, IC<sub>50</sub> values of VCO-Hot are also low as found in ABTS radical scavenging assay suggesting the superiority of VCO obtained from the hot process in its antioxidant capacity.

### 3.3 | Trace elements in VCOs

Among the trace elements, Fe was in the range of 3.3 ± 0.150 ppm (VCO-Fer) to 6.34 ± 0.168 ppm (VCO-EDG) which was well within the permissible limit as per APCC standard (Table 3). On the other hand, Mn [range of 0.25 ± 0.096 ppm (VCO-Hot) to 1.55 ± 0.130 ppm (VCO-Fer)] and Zn [range of 1.97 ± 0.0755 (VCO-Hot) to 3.35 ± 0.442 ppm (VCO-EDG)] were relatively low. Trace elements such as Fe, Mn, and Zn are inadvertently introduced into the oils during the process of handling or extraction steps and increase the rate of oxidation of oils. Interestingly, the [Fe] [VCO-Fer: 3,300 ng/g] and [Zn] [VCO-Hot: 1,970 ng/g] found in VCO is way higher than in many other vegetable oils and the range of [Mn] in VCO ranged from 250 ng/g to 1,550 ng/g is within the range that is generally found in other vegetable oils (Manjusha, Shekhar, & Kumar, 2019). The [Fe], an important prooxidant, is within the range recommended by APCC hence, the quality and safety standards of VCO are assured for its oxidation potential.

### 3.4 | Correlation analysis

The highest significant positive correlations were observed between TPC and antioxidant potential measured in assay CUPRAC ( $r = .99, p < .01$ ) followed by correlation between FFA and AV ( $r = .98, p < .01$ ) (Table 4). The antioxidant potential of oils [CUPRAC] was directly related to the total amount of phenols. Significant correlation between total flavonoid ( $r = .85, p = .01$ ) and antioxidant activity provides strong evidence that the predominant source of antioxidant activity is derived from phenols and its sub-fraction flavonoids in VCO (Szydłowska-Czerniak & Tułodziecka, 2014; Srivastava et al., 2016; Ghani et al., 2018). Since AV is a measure of FFAs present in the oil, a positive correlation between both the parameters was observed. The heavy metal [Fe] was positively correlated with PV ( $r = .67, p < .01$ ) whereas [Mn] showed correlation with FFA ( $r = .69, p < .01$ ) and AV ( $r = .68, p < .01$ ), indicating that increase in Fe could result in enhanced PV. Similarly, the increase in Mn could lead to an increase in FFA and AV. Even though the moisture content of the oil did not show a significant correlation with other attributes, it is an important factor that predisposes VCO to spoilage during storage. The moisture content was positively correlated to FFA ( $r = .283$ ), PV ( $r = .26$ ), and AV ( $r = .21$ ).

**TABLE 5** Variance (%) and cumulative (%) proportion of principal components representing 13 virgin coconut oil (VCO) quality attributes obtained by five different methods

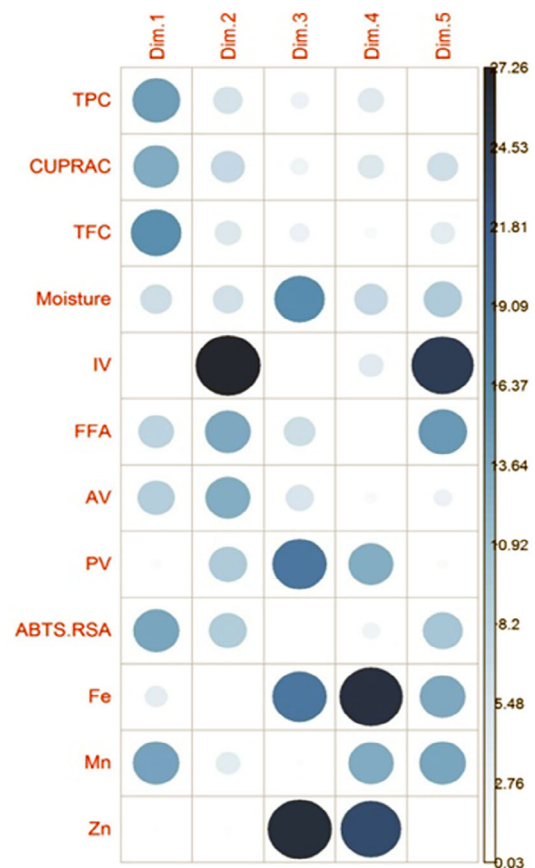
Statistical indices	PC1	PC2	PC3	PC4
Variance %	39.64	28.30	19.71	12.35
Cumulative%	39.64	67.94	87.65	100
SD	2.1809	1.8429	1.5379	1.2176
Eigen value	4.7565	3.3961	2.365	1.4824

### 3.5 | Principal component analysis

The principal component analysis (PCA) was used to study the relationship between the quality characteristics of various VCO products. PCA revealed that the initial four principal components defined 100% of the total variance of the VCO quality characteristics (Table 5). The first three of the principal components accounted for 87.65% of the variance observed. Ansari et al. (2009) extracted the first three principal components accounting for 88.65% of the total variation in heavy metals concentration (Zn, Cd, and Pb) present in 16 cultivars of sunflower seed oils. It was stated that the variability of 88.65% has to be considered as sufficient for explaining the heavy metal concentration in 16 different sunflower seed oil.

The principal component loadings for VCO quality characteristics are shown in Figure 2. The first principal component represented 39.64% of the total variability; total flavonoids, total phenols, and antioxidant activity (CUPRAC), ABTS radical scavenging activity and mineral [Mn] are the dominant variables. The second principal component accounted for 28.30% of total variability comprising characteristics such as iodine value, free fatty acids, and AV, while the third principal component represented 19.71% of total variability is dominant for [Zn], [Fe], PV, and moisture content.

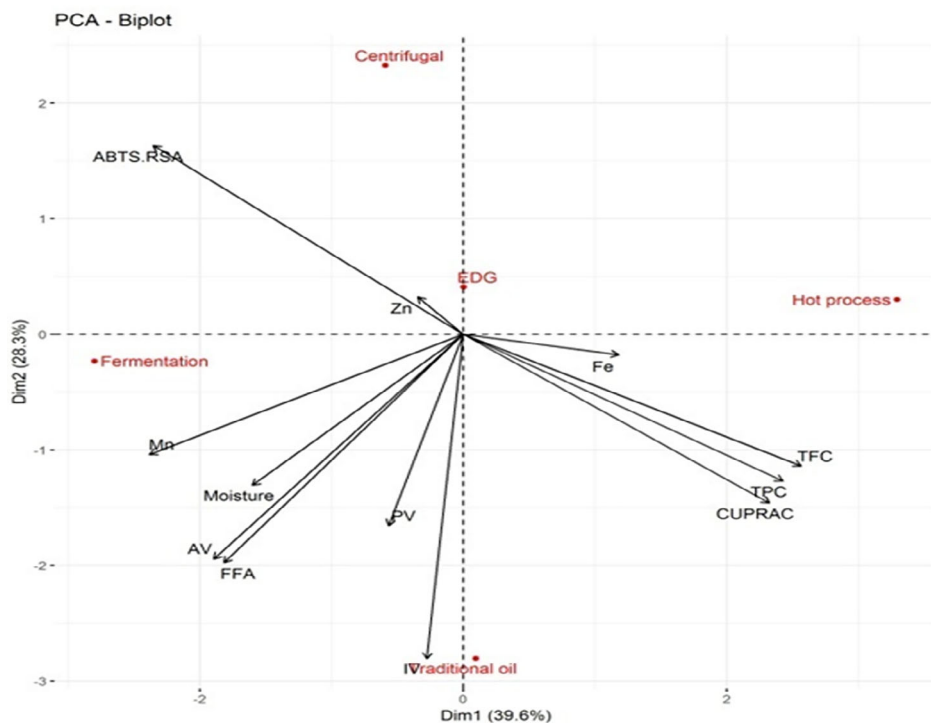
Figure 3 shows the correlation biplot (score plot) of the VCO quality characteristics on the first two principal components. The correlation score plots could distinguish different VCO production methods. Figure 3 depicts that the antioxidant potential as measured by CUPRAC assay, TFC and TPC are placed away from the principal component (PC1) suggesting the contribution of these biochemical parameters in delineating this PC. Their positive loading shows that their increase would result in an increase in the first PC. Similarly, mineral [Mn], AV, and FFA are far from PC1 with a negative loading indicating that their increase would result in a decrease in PC1. Besides, antioxidant potential, TFC and TPC are located close to one another indicating that they are positively correlated with each other but negatively correlated to a moisture content, which is placed on the opposite quadrant. FFA content and AV were located close to moisture content, showing that these two are very important attributes that depend heavily on the moisture content of VCO. VCO samples that were characterized by high moisture yielded high AV and FFA value. Iodine value, free fatty acid, and AV were highly negatively correlated and ABTS. RSA was positively correlated with the second PC.



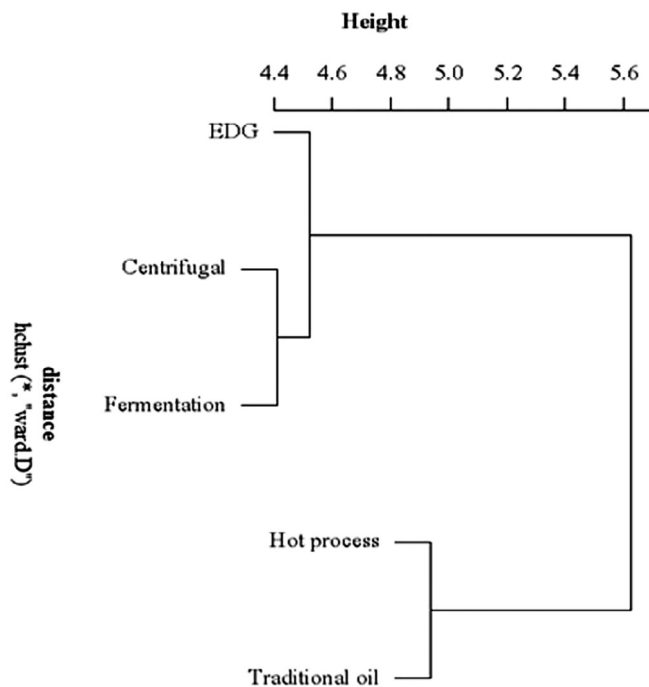
**FIGURE 2** Principal component loadings of quality attributes of virgin coconut oil

### 3.6 | Hierarchical clustering of VCO processes

The dendrogram resulting from hierarchical clustering had (Figure 4) two main clusters. The first cluster comprises VCOs prepared by traditional and hot processes. VCOs produced by these methods contain high total phenols, antioxidant capacity (CUPRAC, ABTS), and flavonoids compared to others. In the hot process, coconut milk is heated at 90–120°C for 3–4 hr (Mathew et al., 2014). The antioxidant activity and phenolics increase with treatment temperature and time (FSSAI, 2017). Therefore, the VCO obtained from the hot process is characterized by strong antioxidant activity. The increase in phenolic contents could be attributed to the denaturation of protein and degradation of intrinsic antioxidants agents (Molaveisi et al., 2019; Mulyadi et al., 2018). The second cluster comprises VCO-Cen, VCO-Fer, and VCO-EDG, which showed high FFA and AV compared to the VCO from the hot process, while the traditional method of oil production showed a much higher level of FFA and AV. Because of the unhygienic practices (improper drying and packaging of coconut, mishandling of oil extraction equipment, unhygienic collection, and bottling of extracted oil) followed in the traditional method of oil production leads to a higher amount of FFA and AV are observed. The permissible limit of moisture and AV in VCO according to the Food Safety and Standards Authority of India (FSSAI) standard are not



**FIGURE 3** Biplot for principal component-1 (PC1) and principal component-1 (PC2) based on the principal component analysis of different quality parameters of virgin coconut oils (VCOs) produced by five different processing methods



**FIGURE 4** Dendrogram based on hierarchical clustering of five different virgin coconut oil (VCO) processes based on the physicochemical properties

by using solar or mechanical drying to avoid fungal growth in copra, hygienic cleaning, and maintenance of extraction equipment, proper filtration of extracted oil, and bottling at the fresh hermetic container.

#### 4 | CONCLUSIONS

Overall, this investigation has provided insights from comparative physicochemical properties of various VCOs. The quality standards of various VCOs have been defined in respect of the physicochemical parameters enumerated by APCC and Codex Alimentarius. Furthermore, the correlation analysis showed that the total polyphenolic content and its constituent flavonoids are the important components that considerably contribute to the total antioxidant profile of VCO. The highest significant ( $p < .01$ ) positive correlation of  $r = .99$  was recorded between TPC and CUPRAC (mg Trolox/100 g) followed by the correlation ( $r = .98$ ) between FFA and AV. Multivariate analysis (PCA and cluster) could differentiate the VCO samples based on the quality profile. VCO-Hot clustered separately from the other VCOs but characterized with strong antioxidant potential owing to the TPC. Oils derived from the centrifugal and fermentation methods were clustered in the second group, which is characterized, by relatively weak antioxidant capacity and low phenolic and flavonoids content.

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>0.5% by weight and not >4, respectively (FSSAI, 2017). However, the FFA and AV can be reduced in the traditional method of oil production by maintaining proper conditions such as drying of coconut



## CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

## AUTHOR CONTRIBUTIONS

S.V.R., R.P., and K.B.H. conceived and designed the experiments; S.V.R. and T.R. performed biochemical analysis; R.P., M.R.M., and S.B. sample preparation and analysis; A.C.M. VCO hot process optimization; S. N. analysis of trace elements; S.S. statistical analysis; S.V.R., R.P., and K.B.H. drafted the manuscript; K.B.H. overall supervision of the work; all authors read and approved the final version of the manuscript.

## DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are included in this published article.

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