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RESEARCH

Detection of Oil Adulteration in Virgin Coconut Oil (VCO) Utilizing Chemometrics and Principal Component Analysis

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Abstract

Authentication of virgin coconut oil (VCO) is imperative to protect the interests of the consumers. An investigation was carried out to distinguish VCO from coconut oil (CO), palm oil (PO) and liquid paraffin utilizing biochemical quality parameters, including fatty acid composition, and principal component analysis (PCA). Various oil blends of VCO: PO, VCO: CO (both in 10% increments), VCO:CO:PO and VCO: liquid paraffin and CO: liquid paraffin were prepared. The oil blends were analyzed for quality features, fatty acid composition and the data was analyzed statistically. Biochemical attributes such as total phenolic content (TPC), total flavonoid content (TFC), iodine value (IV) and saponification value (SV) and fatty acids like lauric acid, myristic acid, palmitic acid and oleic acid were influential parameters to distinguish the oil samples at various levels of adulteration. Samples could be classified even with the adulteration level of as low as 10%. Principal component analysis produced two components distinguishing various adulterated oil samples. Multiple regression analysis provided predictive equation models with high coefficient of determination (R^2) and could help in adulteration quantitation. Hence, this study demonstrated the efficacy of chemometrics approach in distinguishing VCO from possible adulterants like PO, CO and liquid paraffin.

Keywords Adulteration detection, chemometrics · Food quality, principal component analysis · Virgin coconut oil

Introduction

Coconut (*Cocos nucifera* L.), is one of the important plantation crops, which belongs to the family Arecaceae, cultivated throughout the tropical and subtropical regions of the world. The crop is grown in 13 Mha spread over 90 countries, including the Philippines, Indonesia, Brazil, and Sri Lanka, with a production of 69,836.36 million nuts. In India, coconut is grown in an area of 2173 thousand hectares with a production of 20,309 million nuts (https://coconutboard.gov.in/Statistics.aspx). Coconut oil has a wide range of uses in both industrial and culinary purposes. Coconut oil is rich in medium-chain fatty acids (MCFAs) with profound health benefits (Man and Manaf 2006; Ramesh et al. 2019, 2021). However, olive oil, which is a popular edible oil, comprises higher monounsaturated fatty acid (MUFA) content (oleic acid- 72.34%) which helps reduce low-density lipoproteins (LDP) (Lechhab et al. 2022a).The volatile compounds like ethyl acetate (nutty aroma), acetic acid, 2-pentanone, hexanal, n-octane, 2-heptanone (nutty aroma), limonene, nonanal (citrus-like aroma), octanoic acid, ethyl octanoate, δ- octalactone, ethyl decanoate, δ- decalactone and dodecanoic acid contributes to the characteristic aroma of VCO among which ethyl acetate, limonene and nonanal could also be found in virgin olive oils (Santos et al. 2011; Lechhab et al. 2022b).

Among many plant-based edible oils, coconut oil is unique because it consists of more than 90 per cent of saturated fatty acids with traces of mono and poly unsaturated fatty acids (MUFA and PUFA), contains no cholesterol

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(Bharti et al. 2017), and could extensively increase the metabolism, immunity, and digestibility (Agarwal and Bosco 2017). Variety of coconut oils are available for edible purposes, mainly coconut oil from dried copra (unrefined grade), virgin coconut oil (VCO) from fresh kernel meat (unrefined grade), and refined, bleached, and deodorized (RBD) coconut oil. VCO is extracted from the fresh and mature kernel of coconut through physical methods such as cold pressing, expeller-pressed, centrifugal force, or by natural means utilizing microbes with or without using heat (Manikantan et al. 2016). VCO has more health benefits than the oil extracted from dried copra (Krishna et al. 2010; Ramesh et al. 2020). It has a multitude of uses in food, medicine, and industry.

The quantum of VCO export from India has been estimated to be 818 MT to destinations like the United Kingdom, Japan, Australia, the United States, and the Middle East (Anonymous 2020). The market value of VCO worldwide was 1.15 billion U.S. dollars in 2021 and is expected to be 1.28 billion U.S. dollars by 2022 (https://www.statista.com/statistics/875977/organic-virgin-coconut). Due to its nutritional value and market potential, VCO could be adulterated with conventional coconut oil or other oils of vegetables or animal sources of less value.

Adulteration in VCO due to various oils such as CO and palm kernel oil (PKO) was detected utilizing sophisticated instrumentation such as FTIR Fourier transform infrared spectroscopy (FTIR), high performance liquid chromatography (HPLC) (Manaf et al. 2007; Pandurangan et al. 2017; Jayatunga et al. 2020), differential scanning colorimetry (Marina et al. 2009; Marikkar 2019; Libish et al. 2011; Rohman et al. 2019) and application of principal component analysis (Marikkar and Yanty 2018). However, a baseline of information pertaining to the changes in the biochemical features of VCO owing to adulteration has not been performed. There is a necessity to develop a simple and cost-effective technique to verify the adulteration in coconut oil and other oil-based food products. Hence, this study is designed to investigate the alterations in the biochemical profile of VCO due to adulterants like vegetable oils (palm oil or coconut oil) or paraffin oil so that this quality profile could be used to devise cost-effective adulteration measurement techniques.

Material and Methods

Oil Samples

VCO extracted by the hot extraction process (Manikantan et al. 2016) was obtained from the Post-Harvest Technology Section of ICAR-CPCRI, Kasaragod, Kerala, India. Freshly extracted copra coconut oil (CO) was obtained from a commercial coconut oil mill at Kasaragod, Kerala, India. Commercially available palm olein (PO) and paraffin oil were purchased from an open market.

Chemicals and Reagents

All chemicals and solvents used in this analysis were of analytical grade. Ethanol, Folin–Ciocalteau's reagent, gallic acid, sodium carbonate, sodium nitrite, aluminium chloride, sodium hydroxide, quercetin, iodine, chloroform, potassium iodide, sodium thiosulphate, potassium hydroxide, hydrochloric acid, methanol, acetyl chloride and n-Hexane were obtained from Sigma Aldrich (Darmstadt, Germany).

Oil Blend Preparation

Treatments	VCO (%)	CO (%)	PO (%)	Paraffin (%)
T_1	100	0	0	-
T_2	80	20	0	-
T_3	70	30	0	-
T_4	60	40	0	-
T_5	40	60	0	-
T_6	20	80	0	-
T_7	80	0	20	-
T_8	70	0	30	-
T_9	60	0	40	-
T_{10}	40	0	60	-
T_{11}	20	0	80	-
T_{12}	0	80	20	-
T_{13}	0	70	30	-
T_{14}	0	60	40	-
T_{15}	0	40	60	-
T_{16}	0	20	80	-
T_{17}	60	20	20	-
T_{18}	50	25	25	-
T_{19}	40	30	30	-
T_{20}	0	100	0	-
T_{21}	0	0	100	-
T_{22}	90	0	0	10
T_{23}	0	90	0	10

Different oil blends comprising VCO, CO, PO and liquid paraffin, i.e., VCO + CO, VCO + PO, CO + PO, VCO + liquid paraffin and CO + liquid paraffin were prepared. Altogether, there were 23 treatments (including adulterated and unadulterated forms) of oil blends. Each blend was prepared in three replications, capped tight in amber glass bottles and stored at room temperature (25 ± 2 °C) for further analysis of biochemical parameters. Prior to storing, the oil blends were manually shaken to ensure uniform mixing of the

components. The oil blends were analyzed in crude form without any pre-treatment or dilution using solvents.

Analysis of Biochemical Parameters

The total polyphenol content (TPC) was estimated following Folin–Ciocalteau's method (Nevin and Rajmohan 2006) with slight modifications (Ramesh et al. 2020). The total flavonoid content (TFC) of the oil blends was estimated following the procedure of Zhishen et al. (1999). Briefly, total polyphenol and flavonoid fractions of the oil blends were extracted using organic solvents (Seneviratne et al. 2009). TPC was estimated by treating ethanolic oil extract (1 mL) with 1:1 FCR reagent (0.1 mL), followed by 20 per cent Na_2CO_3 (0.5 mL). The resultant reaction mixture, after 45 min of incubation, was measured in UV–vis spectrophotometer (Shimadzu UV-160 A) at 745 nm wavelength. The TFC was measured spectrophotometrically wherein the reaction mixture comprised ethanolic oil extract (1 mL), 5% sodium nitrite (0.3 mL) followed by 10% aluminium chloride (0.3 mL) and 1 M sodium hydroxide (2.5 mL). The absorbance of the reaction mixture was then measured at 510 nm.

The iodine value of different oil blends was estimated by the Hannus method (Virginia et al. 2013), with a few modifications. Oil sample dissolved in chloroform was treated with 20 mL of Hannus iodine solution (100 mL glacial acetic acid + 2.72 g iodine, simultaneously headed, cooled + 0.3 mL bromine), incubated in dark for 30 min and then15% potassium iodide solution (15 mL) was added to decompose excess iodine monobromide into iodine. Titration was carried out against 0.1N sodium thiosulphate solution where starch was used as an indicator.

The saponification value of the blends was determined according to the method enumerated by Zannat et al. (2019). One gram of oil blend was treated with 4% alcoholic KOH (25 mL) and refluxed for 30 min using a water condenser. It was later titrated against 0.5 N HCl in the presence of phenolphthalein indicator.

Gas Chromatography-based Profiling of Fatty Acids

Oil fractions were derivatized prior to analysis of fatty acid methyl esters (FAMEs). One drop of extracted oil was introduced into a 10 mL screw cap tube. One mL of hexane was added, followed by 0.5 mL of 0.05 mol L^{-1} sodium methanolate. The cap was tightened, and the solution was vortexed before heating in a water bath at 40 °C for 15 min. After heating, 2 mL of hexane and 3 mL of saturated sodium chloride solution were added. The solution was vortexed again and left at rest until the two phases were separated. The organic phase containing FAMEs was collected (supernatant) and filtered through a glass pipette equipped with 1 cm of anhydrous sodium sulfate. The FAMEs were collected in a clean 10 mL screw cap tube. The aqueous phase was washed twice with an additional 2 mL of hexane. The supernatant was collected after phase separation, filtered in a glass pipette as above, and added to the previous 10 mL tube. Another 5 mL of hexane was added toa final hexane volume of 10 mL, and a FAME concentration of about 1 mg mL^{-1}. Methyl-esterified oil samples were diluted in high-performance liquid chromatography (HPLC) grade n-hexane (40 µL FAME sample + 960 µL n-hexane) in the sample vial. The methyl-esterified samples (1 mL) were injected into the gas chromatograph (GC-2010, Shimadzu) using an auto injector (AOC-20i, Shimadzu) and capillary column (BPX 70, SGE Analytical Science, Austin, TX). The elutants were detected on a flame ionization detector (Shimadzu) under the conditions set for the analysis (Kumar et al. 2007). The injection mode was split (split ratio 1:50); injector temperature was 225 °C; nitrogen and air were carrier gases; pressure was set to 114.9 kPa; total flow was maintained at 68.9 mL min^{-1}; and column initial temperature was 100 °C with a temperature increase rate of 5 °C min^{-1}. A quantitative method was followed using an external standard of mixture of fatty acids (C6-C24). Fatty acid methyl ester standards (C6-C24; Sigma-Aldrich, Supelco, Bellefonte, PA, USA) were run earlier under similar conditions of analysis. The concentrations and area of each peak were computed using a data analysis method developed using different concentrations of standard FAMEs. The data thus acquired were analyzed using the GC Post-run analysis software (Shimadzu) (Ramesh et al. 2022).

Statistical Analysis

The data were subjected to analysis of variance using SAS software version 9.3 (SAS Institute Inc. 2011). The experiment was conducted in a completely randomised design (CRD) followed by the application of Duncan's Multiple range Test (DMRT). Further, principal component analysis (PCA) and multiple linear regression analysis of the data were carried out using the statistical software IBM SPSS statistics version 26 (Landau and Everitt 2003).

Results and Discussion

The quality parameters of VCO are quite distinct from other variants of coconut oils and palm oil (Marikkar and Yanty 2018; Ramesh et al. 2020). Hence, it was hypothesized that adulteration of VCO with other vegetable oils would cause significant variations in their biochemical features and fatty acid profiles. Accordingly, all the 23 combinations of oil blends were analyzed for biochemical attributes and fatty acid composition.

Total Polyphenol Content (TPC)

With an increase in the adulterant levels of PO in VCO and CO, TPC increased significantly, whereas among the VCO + CO blends (i.e., T_2, T_3, T_4, T_5, and T_6) TPC showed a significant decrease with an increase in CO content where the adulteration levels exceeded the threshold of 20 per cent (Fig. 1).The highest TPC among the pure oils was found to be in T_{21} (PO), followed by T_1 (VCO) and T_{20} (CO), showing 5.72, 3.08, and 1.69 mg gallic acid equivalent (GAE)/100 g, respectively. However, among the oil blends T_{17}, T_{18}, and T_{19}, no significant difference in TPC was observed (Table 1). At an adulteration level of 10 per cent in VCO and CO with paraffin oil, a significant decrease in the TPC of T_{22} (90VCO + 10liquid paraffin) and T_{23} (90CO + 10liquid paraffin) was observed compared to T_1 (VCO) and T_{20} (CO), respectively (Table 1). The increase in TPC of CO + PO and VCO + PO blends with an increase in PO levels is due to higher TPC of PO. A similar trend was observed in VCO + CO blends, where TPC decreased with an increase in CO concentration. As polyphenol content of paraffin oil is nil, CO and VCO samples with 10 per cent paraffin showed lower TPC.

The TPC of T_1 (VCO) was higher than T_{20} (CO) in this study, which was also reported by Dia et al. (2005) and Ramesh et al. (2020). This difference in the TPC content is due to the difference in the extraction process of VCO and CO, where a comparatively high temperature is employed during the extraction of VCO, which enables the effective incorporation of phenolics into the oil (Srivastava et al. 2016). Moreover, the process of refining, bleaching and deodorization (RBD), which a normal coconut oil undergoes, effectively decreases TPC (Ramesh et al. 2020).

Total Flavonoid Content (TFC)

TFC varied significantly among T_1 (VCO), T_{20} (CO) and T_{21} (PO) with 3.06, 2.12 and 6.82 mg QE/100 g, respectively. However, no significant difference was found between the blends of these oils. Increased levels of PO in VCO and CO resulted in an increasing trend of the TFC, whereas VCO blended with CO showed a decrease in the TFC with an increase in CO levels (Table 1; Fig. 2). The treatments T_{22} (90VCO + 10liquid paraffin) and T_{23} (90CO + 10liquid paraffin) showed low TFC than T_1 (VCO) and T_{20} (CO), respectively.

The TFC in T_1 (VCO) was higher than T_{20} (CO), as reported earlier (Ramesh et al. 2020). It was observed that T_{21} (PO) exhibited the highest TFC content among the oil samples. This trait followed a similar trend as that of TPC as flavonoids are a family of polyphenolic compounds. The increase in TFC of CO + PO blends and also in VCO + PO blends with an increase in PO levels are due to higher TFC of PO. Contrarily, in VCO + CO blends, TFC decreased with an increase in CO levels. As no TFC was nil in paraffin oil, CO and VCO samples with 10 per cent paraffin showed lower TFC.

Iodine Value (IV)

The lowest iodine value was observed in T_{20} (CO) (7.74 g I_2/100 g). As the IV of T_1 (VCO) (9.68 g I_2/100 g) was higher than that of T_{20} (CO) and lower than T_{21} (PO) (64.30 g I_2/100 g), it was observed that the IV among the VCO + CO blends decreased with an increase in the concentration of CO (Fig. 3). A significant difference in the IV was found between the treatments having at least 20 per cent difference in the concentration of CO. In the case of VCO + PO and CO + PO blends, there was a significant increase in the IV between the treatments where the adulteration of PO increased the threshold levels of ten per cent (Table 1). The IV of T_{22} (90VCO:10P) and T_{23} (90CO + 10liquid paraffin) was low compared to their respective pure oil samples. T_1 (VCO) and T_{22} (90VCO + 10liquid paraffin) differed significantly (Table 1).

The IV of T_{21} (PO) was found to be the highest because of the high degree of unsaturated fatty acids. Earlier, Haryati et al. (1998) reported that the IV of palm oil ranged from 29

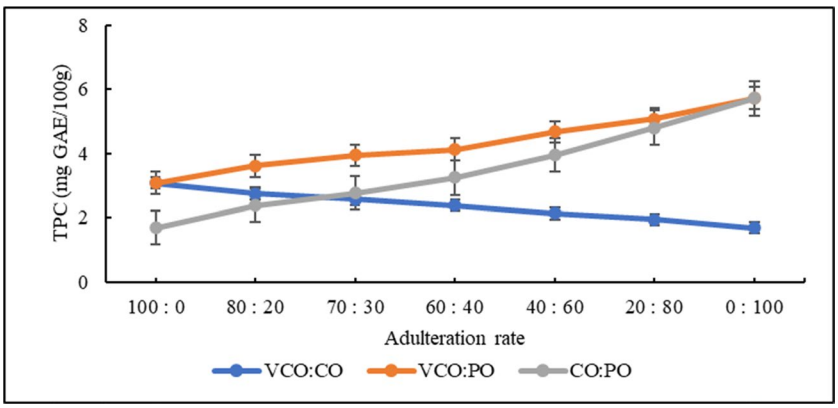

Fig. 1 Changes in total polyphenol content among the different VCO, CO, and PO blends

Table 1 TPC, TFC, IV and SV of VCO, CO, PO and P blends at different ratios

Treatment	TPC (mg GAE/100 g)	TFC (mg QE/100 g)	IV (g I_2/100 g)	SV (mg KOH/g)
T_1	$3.08^{g} \pm 0.05$	$3.06^{f} \pm 0.21$	$9.68^{i} \pm 0.14$	$269.20^{a} \pm 0.10$
T_2	$2.75^{hi} \pm 0.14$	$2.94^{fg} \pm 0.21$	$9.35^{ij} \pm 0.10$	$268.72^{a} \pm 0.59$
T_3	$2.58^{i} \pm 0.02$	$2.58^{gh} \pm 0.20$	$8.74^{ijk} \pm 0.14$	$267.30^{ab} \pm 0.20$
T_4	$2.39^{j} \pm 0.08$	$2.58^{gh} \pm 0.06$	$8.57^{ijk} \pm 0.17$	$266.86^{ab} \pm 0.12$
T_5	$2.13^{k} \pm 0.06$	$2.35^{hi} \pm 0.09$	$8.16^{ijk} \pm 0.20$	$265.73^{ab} \pm 0.06$
T_6	$1.94^{l} \pm 0.08$	$2.12^{i} \pm 0.06$	$7.80^{ijk} \pm 0.13$	$264.50^{b} \pm 0.31$
T_7	$3.61^{f} \pm 0.06$	$4.24^{d} \pm 0.16$	$18.17^{d} \pm 0.10$	$265.00^{b} \pm 1.58$
T_8	$3.94^{e} \pm 0.10$	$4.94^{c} \pm 0.16$	$22.63^{f} \pm 0.60$	$245.57^{e} \pm 1.62$
T_9	$4.12^{d} \pm 0.04$	$5.53^{b} \pm 0.13$	$28.00^{d} \pm 0.30$	$238.20^{f} \pm 2.07$
T_{10}	$4.67^{c} \pm 0.06$	$5.76^{b} \pm 0.21$	$37.31^{c} \pm 1.11$	$225.30^{h} \pm 2.07$
T_{11}	$5.07^{b} \pm 0.15$	$5.88^{b} \pm 0.21$	$46.19^{b} \pm 0.68$	$208.10^{j} \pm 2.12$
T_{12}	$2.39^{j} \pm 0.05$	$2.24^{hi} \pm 0.13$	$16.09^{h} \pm 0.54$	$258.00^{cd} \pm 1.16$
T_{13}	$2.77^{h} \pm 0.06$	$3.29^{ef} \pm 0.04$	$22.21^{f} \pm 0.26$	$245.57^{e} \pm 2.56$
T_{14}	$3.25^{g} \pm 0.06$	$3.53^{e} \pm 0.06$	$25.55^{e} \pm 2.95$	$234.13^{g} \pm 2.64$
T_{15}	$3.95^{d} \pm 0.08$	$3.65^{e} \pm 0.17$	$36.21^{c} \pm 1.03$	$221.20^{i} \pm 0.27$
T_{16}	$4.80^{c} \pm 0.14$	$4.47^{d} \pm 0.41$	$45.01^{b} \pm 0.90$	$207.03^{j} \pm 1.51$
T_{17}	$3.32^{g} \pm 0.06$	$2.23^{hi} \pm 0.04$	$19.79^{g} \pm 0.47$	$255.40^{d} \pm 2.99$
T_{18}	$3.33^{g} \pm 0.02$	$2.58^{gh} \pm 0.19$	$22.24^{f} \pm 0.15$	$247.00^{e} \pm 1.42$
T_{19}	$3.38^{g} \pm 0.07$	$2.47^{hi} \pm 0.10$	$22.88^{f} \pm 0.20$	$244.10^{e} \pm 2.39$
T_{20}	$1.69^{m} \pm 0.11$	$2.12^{i} \pm 00$	$7.74^{ijk} \pm 0.17$	$263.60^{b} \pm 0.80$
T_{21}	$5.72^{a} \pm 0.08$	$6.82^{a} \pm 0.21$	$64.30^{a} \pm 0.39$	$193.50^{k} \pm 0.41$
T_{22}	$2.88^{h} \pm 0.06$	$2.35^{hi} \pm 0.06$	$7.59^{ijk} \pm 0.14$	$264.46^{b} \pm 0.72$
T_{23}	$1.31^{n} \pm 0.15$	$0.82^{j} \pm 0.08$	$7.37^{k} \pm 0.31$	$260.1^{c} \pm 0.30$
CD at 1%	0.177	0.348	1.691	3.386

Treatments with significant differences are denoted with dissimilar notations, whereas those with no significant difference are denoted with the same notations as per Duncan's multiple range test (DMRT) at a 99 per cent level of confidence

T_1- 100 VCO: 0 CO: 0 PO, T_2- 80 VCO: 20 CO, T_3- 70 VCO: 30 CO, T_4- 60 VCO: 40 CO, T_5- 40 VCO: 60 CO, T_6- 20 VCO: 80 CO, T_7- 80 VCO: 20 PO, T_8- 70 VCO: 30 PO, T_9- 60 VCO: 40 PO, T_{10}- 40 VCO: 60 PO, T_{11}- 20 VCO: 80 PO, T_{12}- 80 CO: 20 PO, T_{13}- 70 CO: 30 PO, T_{14}- 60 CO: 40 PO, T_{15}- 40 CO: 60 PO, T_{16}- 20 CO: 80 PO, T_{17}-60 VCO: 20 CO: 20 PO, T_{18}-50 VCO: 25 CO: 25POT$_{19}$-40 VCO: 30 CO: 30 PO, T_{20}-0 VCO: 100 CO: 0 PO, T_{21}- 0 VCO: 100 PO, T_{22}- 90 VCO: 10 P, T_{23}- 90 CO: 10 P, VCO- virgin coconut oil, CO- Coconut oil, PO-Palm oil, P- Liquid paraffin

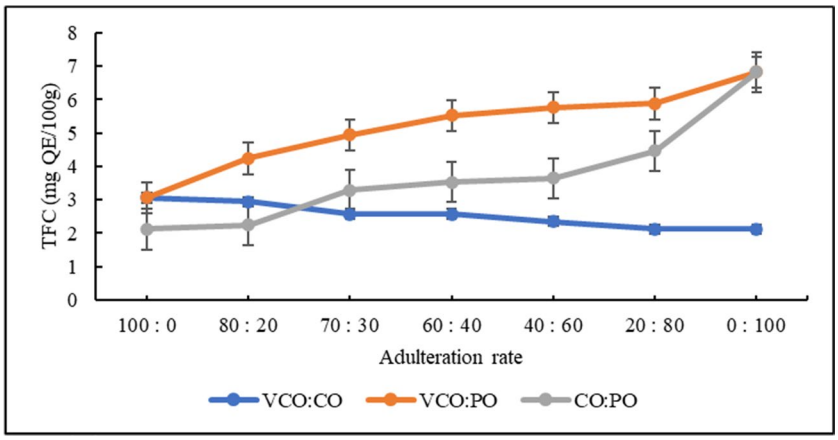

Fig. 2 Change in total flavonoid content among the different VCO, CO, and PO blends

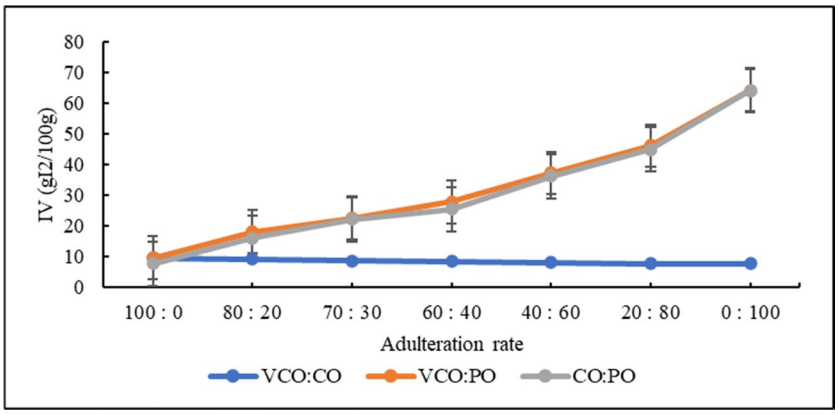

Fig. 3 Changes in iodine value among the different VCO, CO, and PO blends

to 60 g I_2/100 g. The IV of T_1 (VCO) and T_{20} (CO) were in accordance with the findings of Koh and Long (2012) and Srivastava et al. (2016). The increase in the IV of CO + PO blends and VCO + PO blends with an increase in PO levels is due to a higher IV of PO. A similar trend was observed in VCO + CO blends, where IV decreased with an increase in CO concentration. CO and VCO samples with 10 per cent paraffin oil showed lower IV since paraffin oil is chemically quite distinct from coconut oils.

Saponification Value (SV)

The saponification value of T_1(VCO) was the highest (269.20 mg KOH/g) among the treatments, whereas the lowest (193.50 mg KOH/g) was observed in T_{21} (PO). T_{20} (CO) showed a SV of 263.60 mg KOH/g. T_1 (VCO) and T_2 (CO) had SV in the range of APCC (Anonymous 2009) and codex standards (Anonymous, 1999), respectively. The treatments involving VCO + PO and CO + PO blends showed a significant reduction in SV with an increase in the concentration of PO (Table 1; Fig. 4). T_{22} (90VCO + 10 liquid paraffin) and T_{23} (90CO + 10liquid paraffin) also showed significantly low levels of SV compared to T_1 (VCO) and T_{20} (CO), respectively (Table 1).

SV of T_{21} (PO) was the lowest and was then followed by T_{20} (CO) and T_1 (VCO) with higher saponification values. The SVs of CO and VCO observed were similar to the findings of Ramesh et al. (2020) and Dia et al. (2005), respectively. The decline in SV of CO + PO blends and VCO + PO with an increase in PO levels is due to lower SV of PO. A similar trend was observed in VCO + CO blends, where SV decreased with an increase in CO levels. As no saponifiable matter could be found in paraffin oil, CO and VCO samples with 10 per cent paraffin showed lower SV.

Fatty Acid Profiles of Oil Blends

The total percentage of saturated fatty acids (SFA) found in T_1(VCO), T_{20} (CO) and T_{21} (PO) were 92.01, 92.23 and 46.83, respectively, and the total percentage of monounsaturated fatty acid (MUFA) was 6.55, 5.88, and 41.51, respectively. The oils [T_1(VCO), T_{20} (CO) and T_{21} (PO)] are characterised with a small percentage of polyunsaturated fatty acids (PUFA), 1.4 per cent, 1.54 per cent and 11 per cent, respectively. Total MCFAs observed in T_1(VCO), T_{20} (CO) and T_{21} (PO) were 58.39 per cent, 60.99 per cent, and 0.8 per cent, respectively. The pure oil blends of VCO, and CO, i.e., T_1 (VCO) and T_{20} (CO), showed a higher percentage of C12:0 (lauric acid) (47.42% and 49.96%) followed by C14:0

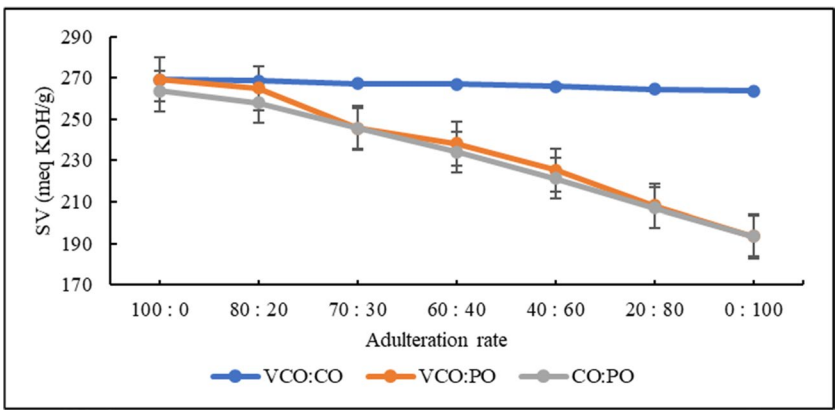

Fig. 4 Change in saponification value among the different concentration of VCO, CO, and PO blends

(myristic acid) (20.93% and 20.01%) and C16:0 (palmitic acid) (9.59% and 7.80%) whereas the PO, i.e., T_{21} (PO), showed a higher amount of C18:1 (oleic acid) (41.35%) followed by C16:0 (40.72%).

The fatty acid profiles of T_1 (VCO) and T_{20} (CO) were in accordance with the findings of Srivastava et al. (2016), Ajogun et al. (2020), and Koh and Long (2012). The fatty acid profile of T_{21} (PO) was similar to the findings of Koushik et al. (2015). As the fatty acid C12:0 was higher in T_{20} (CO) than in T_1 (VCO), it was observed that among the treatments involving the blends of VCO + CO, the concentration of C12:0 increased with an increase in the concentration of CO in VCO. As PO contained a high percentage of C16:0 and C18:1 and a lower percentage of C12:0 and C14:0 compared to CO and VCO, it was observed that, with the increase in the concentration of PO, among the treatments having VCO & PO, and CO & PO blends, C12:0 and C14:0 showed a significant decrease in its concentration and the concentration of C16:0 and C18:1 increased significantly. The concentration of all the fatty acids in T_{22} (90VCO + 10 liquid paraffin) and T_{23} (90CO + 10liquid paraffin) was observed to be significantly lower than T_1 (VCO) and T_{20} (CO), respectively. Variations in the concentration of these fatty acids due to adulteration are presented (Figs. 5, 6 and 7).

In order to understand the significance of variation in the biochemical properties of different blends at different adulteration levels, the DMRT was employed for the obtained data. Although each biochemical property solely could help detect the adulteration levels down to 20 percent, the combination of all four (i.e., TPC, TFC, IV and SV) enabled us to detect adulteration levels down to 10 per cent.

Principal Component Analysis (PCA)

The data obtained from the biochemical analysis and fatty acid profiling of different oil blends were subjected to principal component analysis as independent variables to

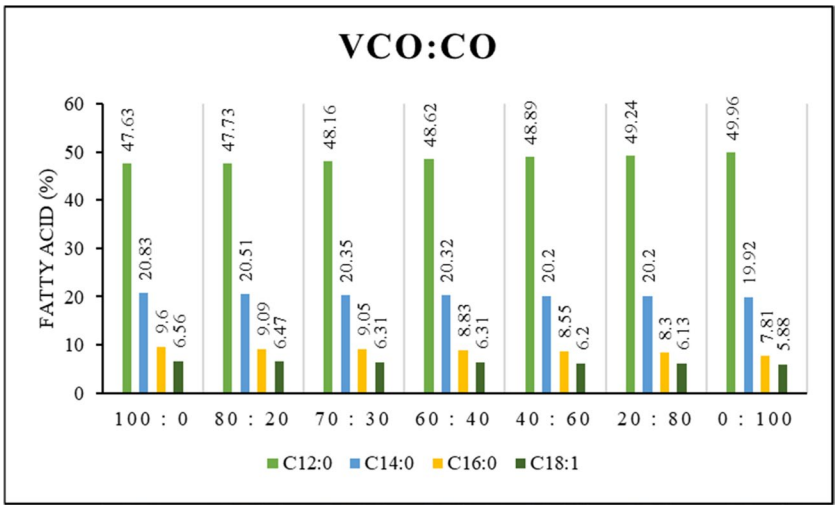

Fig. 5 Variation in the fatty acid profile of VCO and CO blends at different proportions. (C12:0, C14:0, C16:0 and C18:1)

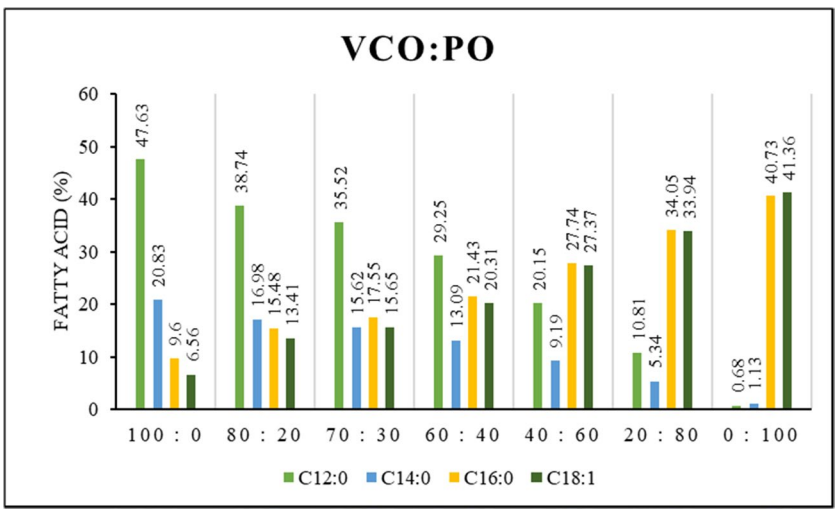

Fig. 6 Variation in the fatty acid profile of VCO and PO blends at different proportions. (C12:0, C14:0, C16:0 and C18:1)

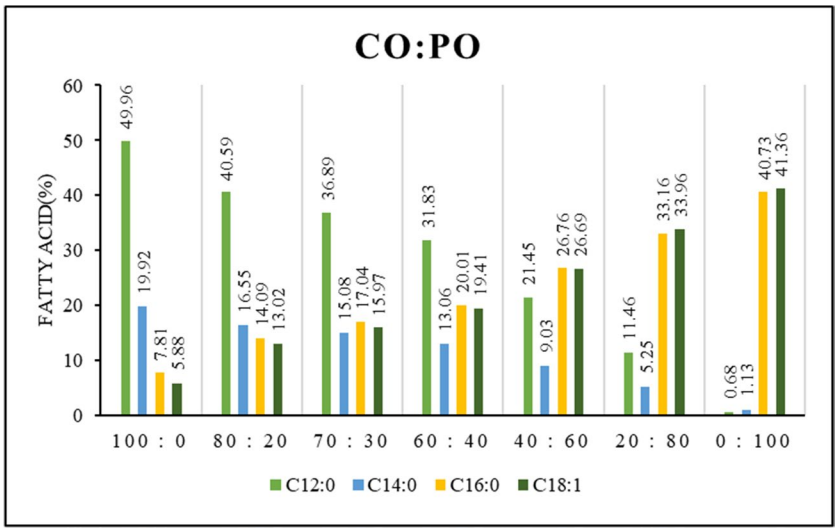

Fig. 7 Variation in the fatty acid profile of CO and PO blends at different proportions. (C12:0, C14:0, C16:0 and C18:1)

Table 2 Component matrix of the variables subjected to principal component analysis

Component Matrix		
Variables	Component	
	1	2
T_1	0.995	-0.095
T_2	0.995	-0.096
T_3	0.995	-0.099
T_4	0.995	-0.101
T_5	0.995	-0.103
T_6	0.994	-0.105
T_7	0.999	-0.034
T_8	1.000	-0.010
T_9	0.999	0.038
T_{10}	0.993	0.122
T_{11}	0.976	0.218
T_{12}	0.999	-0.045
T_{13}	1.000	-0.012
T_{14}	1.000	0.023
T_{15}	0.993	0.116
T_{16}	0.977	0.212
T_{17}	1.000	-0.030
T_{18}	1.000	-0.014
T_{19}	1.000	-0.001
T_{20}	0.994	-0.108
T_{21}	0.935	0.355
T_{22}	0.995	-0.094
T_{23}	0.994	-0.108
Eigenvalue	22.653	0.345
Variance (%)	98.491	1.501

T_1- 100 VCO: 0 CO: 0 PO, T_2- 80 VCO: 20 CO, T_3- 70 VCO: 30 CO, T_4- 60 VCO: 40 CO, T_5- 40 VCO: 60 CO, T_6- 20 VCO: 80 CO, T_7- 80 VCO: 20 PO, T_8- 70 VCO: 30 PO, T_9- 60 VCO: 40 PO, T_{10}- 40 VCO: 60 PO, T_{11}- 20 VCO: 80 PO, T_{12}- 80 CO: 20 PO, T_{13}- 70 CO: 30 PO, T_{14}- 60 CO: 40 PO, T_{15}- 40 CO: 60 PO, T_{16}- 20 CO: 80 PO, T_{17}-60 VCO: 20 CO: 20 PO, T_{18}-50 VCO: 25 CO: 25POT$_{19}$-40 VCO: 30 CO: 30 PO, T_{20}-0 VCO: 100 CO: 0 PO, T_{21}- 0 VCO: 100 PO, T_{22}- 90 VCO: 10 P, T_{23}- 90 CO: 10 P, VCO- virgin coconut oil, CO- Coconut oil, PO-Palm oil, P- Liquid paraffin

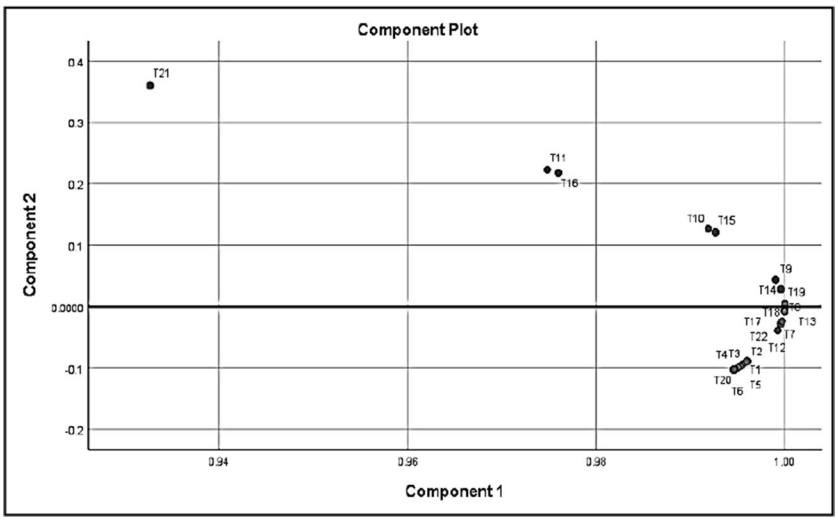

Fig. 8 Component score plot of principal component analysis

distinguish pure oils from those of their different blends at different levels of adulterants. By considering Eigenvalues, two principal components with eigen values of 22.653 and 0.345 were produced. These two PCs could explain 98.491% (PC1) and 1.501% (PC2) of variability. Table 2 represents the component matrix of the variables. Component score plot was plotted which revealed that the treatments with lower adulteration and pure VCO and CO samples appeared in PC1 block, whereas the pure palm oil sample and the treatments with higher levels of palm oil appeared in PC2 block (Fig. 8).

Multiple Linear Regression Analysis

A multiple linear regression analysis of various biochemical parameters was performed. TPC, TFC, IV and SV gave a fit regression model (model-3) for different blends of VCO + CO and VCO + PO each with an R^2 value of 1 and 0.998 respectively. For the blend of VCO + liquid paraffin, TPC alone gave a fit regression model with an R^2 value of 1.These regression models could be considered the best suitable fit to detect the levels of purity of the oil utilizing the equations thus obtained (Table 3).

Conclusion

This study was conducted to explore the feasibility of developing a simple, cost-effective method to detect adulterants in virgin coconut oil (VCO). It is concluded that a spectrophotometric quantitation of a combination of total polyphenolic content, total flavonoid content, iodine value, and saponification value could be an effective approach to detect the level of adulteration down to 10 per cent in VCO and coconut oil with palm oil and paraffin oil. Also, it is anticipated that differences in the quantitative and qualitative profiles of polyphenolic fractions among the oil blends coupled with spectroscopy could be a practical future line of research for adulteration detection.

Table 3 Linear regression models for biochemical properties of various blends

Model no	Regression model (% purity)	R-square value	Dubrin-Watson value
VCO + CO			
1	$Y = -119.116 + X_1\ 72.432$	0.989	3.448
2	$Y = -2560.905 + X_1 62.118 - X_2\ 21.326 + X_3\ 9.938$	0.998	
3	$Y = -323.318 + X_1\ 94.626 - X_2\ 59.419 + X_3\ 1.714 + X_4\ 80.456$	1.000	
VCO + PO			
1	$Y = 220.047 - X_1 38.663$	0.997	3.520
2	$Y = 159.198 - X_1 33.654 + X_2\ 0.005 + X_3\ 0.166$	0.997	
3	$Y = 183.381 - X_1\ 43.256 + X_2 0.253 + X_3\ 0.142 + X_4\ 2.904$	0.998	
VCO + liquid paraffin			
1	$Y = -54.00 + X_1 50.00$	1.000	-

X_1: TPC, X_2: IV, X_3: SV, X_4: TFC

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Author Contribution Study design and conceptualization: KBH, V, AKS & SVR; investigation: CMB, SVR; data analysis CMB, GSC, VJ, drafting of various sections of the manuscript: All authors and; Read and approved the final version of the manuscript: All authors.

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Data Availability All the data described in this research work is available within this manuscript.

Declarations

Conflict of Interest M.B. Cariappa declares that he has no conflict of interest. S.V. Ramesh declares that he has no conflict of interest. G.S. Chikkanna declares that he has no conflict of interest. J. Venkatesh declares that he has no conflict of interest. Vishnuvardhana declares that he has no conflict of interest. K.B. Hebbar declares that he has no conflict of interest. A.K. Singh declares that he has no conflict of interest.

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