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Physicochemical characterization and fatty acid profiles of testa oils from various coconut (*Cocos nucifera* L.) genotypes

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Abstract

BACKGROUND: ***Cocos nucifera*** **(L.) is an important plantation crop with immense but untapped nutraceutical potential. Despite its bioactive potential, the biochemical features of testa oils of various coconut genotypes are poorly understood. Hence, in this study, the physicochemical characteristics of testa oils extracted from six coconut genotypes – namely West Coast Tall (WCT), Federated Malay States Tall (FMST), Chowghat Orange Dwarf (COD), Malayan Yellow Dwarf (MYD), and two Dwarf × Dwarf (D × D hybrids) viz., Cameroon Red Dwarf (CRD) × Ganga Bondam Green Dwarf (GBGD) and MYD × Chowghat Green Dwarf (CGD) – were analyzed.**

RESULTS: The proportion of testa in the nuts (fruits) (1.29–3.42%), the proportion of oil in the testa (40.97–50.56%), and biochemical components in testa oils – namely proxidant elements Fe (34.17–62.48 ppm) and Cu (1.63–2.77 ppm), and the total phenolic content (6.84–8.67 mg GAE/100 g), and phytosterol content (54.66–137.73 mg CE/100 g) varied depending on the coconut genotypes. The saturated fatty acid content of testa oils (67.75 to 78.78%) was lower in comparison with that of coconut kernel oils. Similarly, the lauric acid (26.66–32.04%), myristic (18.31–19.60%), and palmitic acid (13.43–15.71%,) content of testa oils varied significantly in comparison with the coconut kernel oils (32–51%, 17–21% and 6.9–14%, respectively). Liquid chromatography–mass spectrometry (LC–MS) analysis revealed the presence of 18 phenolic acids in coconut testa oil. Multivariate analysis revealed the biochemical attributes that defined the principal components loadings. Hierarchical clustering analysis of the genotypes showed two distinct clusters.

CONCLUSION: This study reveals the genotypic variations in the nutritionally important biochemical components of coconut testa oils. The relatively high concentration of polyunsaturated fatty acids (PUFA) and polyphenol content in testa oils warrant further investigation to explore their nutraceutical potential.

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Supporting information may be found in the online version of this article.

Keywords: antioxidants; coconut testa; coconut cultivars; multi-variate analysis; polyphenol

INTRODUCTION

Coconut (*Cocos nucifera* L.), is an evergreen, tropical, monocot palm belonging to the family Arecaceae. It is an economically important plantation crop, grown throughout the humid and sub-humid coastal regions of the world. The crop is grown in an area of 13 million ha covering more than 90 countries including the Philippines, Indonesia, India, Brazil, and Sri Lanka with an estimated production of 69 836.36 million nuts. In India, coconut is grown in an area of 2.088 M ha with a production of 22 167.45 million nuts (source: Horticulture Division, Department of

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Agriculture and Cooperation, Ministry of Agriculture, Government of India). In the context of its economic importance and its remarkable role in sustaining the livelihood of tropical growers, the palm is appropriately called the 'tree of life'.[1]

Coconut oil, obtained from the coconut endosperm or kernel, is one of the most important oils in the tropical regions, with extensive applications not only as valuable edible oil but also in industrial processes. Coconut oil is rich in medium chain fatty acids (MCFAs), and health benefits have been attributed to it.[2] The MFCAs in coconut oils have attracted greater interest from the field of medicine since it became known that MCFAs have functional and nutritional attributes.[3,4] It has also been reported that phenolic components of the coconut oil are responsible for its high antioxidant capacity.[5-8] Coconut oil and its variants, such as virgin coconut oil (VCO), are generally obtained from the kernel or endosperm of coconut fruit and are processed in various ways.[7,8] Coconut oil has recently attracted significant attention with regard to its role in improving cardiovascular and liver functions related to obesity-induced health issues,[9] and for its antioxidant and neuroprotective effects.[10-13]

Coconut testa is a brown coat that covers the endosperm or kernel and is a known source of plant phenolics and antioxidants.[14-16] Coconut testa is obtained as a by-product of the coconut processing industry. It is utilized either as an animal feed, or as a starting material for biofuel production. At times it is discarded.[17,18] Virgin coconut oil obtained from coconut endosperm with testa has been shown to have relatively high polyphenolic content.[6,14] The quantitative chemical composition of the coconut endosperm, including its testa region, exhibited a marked variation across the endosperm from the inner region (cavity) towards outer region (testa) suggesting greater variability in the biochemical components of the endosperm.[19]

These earlier studies compared the physico-chemical properties of coconut testa oils with those of coconut oil without exploring the genotypic differences in the coconut cultivars. The biochemical characteristics of testa flour of select Sri Lankan cultivars revealed inter-varietal differences in the fat, protein, and carbohydrate content.[20] The current study therefore presents and discusses biochemical features of testa oils derived from the endosperms of select coconut genotypes in the context of our improved understanding of the nutraceutical potential of coconut and its products.

MATERIALS AND METHODS

Chemicals

All phenolic acid and flavonoid standards, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tris-2,4,6-tripyridyl-2-triazine (TPTZ), potassium persulfate, and neocuproine (2,9-dimethyl-1,10-phenanthroline) were purchased from Sigma-Aldrich Co., St Louis, MO, USA. Analytical-grade ethanol, methanol, acetone, acetic acid (glacial), sodium acetate, hydrochloric acid (conc.), ferric chloride, ammonium acetate, copper (II) chloride, Folin–Ciocalteu phenol reagent, and sodium carbonate were purchased from Merck KGaA, Darmstadt, Germany.

Coconut genotypes and testa removal

Mature nuts of coconut cultivars, namely West Coast Tall (WCT), Federated Malay States Tall (FMST), Chowghat Orange Dwarf (COD), Malayan Yellow Dwarf (MYD), and Dwarf × Dwarf (D×D hybrids) viz., Cameroon Red Dwarf (CRD) × Ganga Bondam Green Dwarf (GBGD) and MYD × Chowghat Green Dwarf (CGD), were obtained from the Division of Crop Improvement, ICAR-Central Plantation Crops Research Institute , Kasaragod, Kerala, India. The mature fruits of coconuts were dehusked, and the kernel, along with the intact testa, was removed from coconut shell using an in-house developed coconut de-sheller. The testa component of the kernel was carefully removed using the testa remover and was dried in a mechanical tray dryer.

Defatting of testa

Ten grams of coconut testa of each coconut genotype was placed in a cellulose cartridge (Fisher Scientific catalog number 12–101-100) and the fat components were extracted for 5 h using petroleum ether (boiling point 60–80 °C) in a Soxhlet apparatus.[16]

Measurement of physicochemical properties

Three grams of coconut testa oil was used to determine the physicochemical properties. The moisture content and specific gravity of testa oil samples were estimated using moisture analyzer A&D MX-50 (Abingdon, Oxfordshire, UK) and following a standard AOCS method (925.10).[21] Free fatty acid (FFAs) content was determined according to an AOAC method (Ca-5a-40).[22] Standard International Union of Applied and Pure Chemistry (IUPAC) procedure was followed to determine the peroxide value (PV) and the saponification value of the testa oil samples.[23] The PV is expressed as milli-equivalents (m-eq) of peroxide O_2 kg-$^{-1}$ of testa oil. The iodine value of the testa oil samples was measured following the Wijs method.[21] Briefly, a known quantity of testa oil was mixed in a conical flask containing a mixture of carbon tetrachloride (15 mL), Wij's reagent (25 mL), and potassium iodide solution (5%). The solution was kept in darkness for 30 min and the liberated iodine was titrated against 0.1 mol L^{-1} standard sodium thiosulfate solution using starch as indicator.

Estimation of total phenolics and flavonoid content

The polyphenolic fraction in testa oil samples was extracted using 10 mL of 80% ethanol in three successive extractions, and was pooled. The aliquot was mixed with 1:1 Folin–Cicolteau reagent followed by the addition of 1.0 mL of 20% Na_2CO_3 solution after 3 min, and allowed to stand for 45 min in room temperature in dark conditions. The total phenolic content (TPC) was spectrophotometrically estimated (absorbance at 760 nm, Shimadzu Corporation, Kyoto, Japan) and expressed as mg GAE per 100 g of testa oil.[5-7] Similarly, total flavonoid content (TFC) was measured and expressed as milligram of quercetin equivalent (QE)/100 g of oil.[7-24]

Determination of *in vitro* anti-oxidant potential

The radical scavenging activity of testa oils was determined by recording the absorbance diminution of DPPH when it interacts with testa oil.[25] The scavenging concentration (SC_{50}) – which refers to the concentration of samples required to scavenge 50% of the DPPH● – was calculated as Trolox served as a positive control and the results were expressed in μ mol TE/g. The ferric reducing antioxidant power (FRAP) and cupric ion reducing antioxidant capacity (CUPRAC) assays were performed following standard protocols.[26,27] Briefly, for CUPRAC assay, the oil 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 darkness for 30 min. Spectrophotometric absorbance (Shimadzu) at 450 nm was recorded. Similarly for the FRAP assay, a freshly prepared FRAP

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reagent consisted of 2.5 mL of a 10 m mol L^{-1} TPTZ solution in 40 m mol L^{-1} HCl, 2.5 mL of 20 m mol L^{-1} $FeCl_3$, and 25 mL of 0.1 mol L^{-1} acetate buffer (pH 3.6). About 0.250 mL of methanolic extracts of testa oil samples and 2 mL of FRAP reagent were transferred into a 10 mL volumetric flask and the volume was made up with distilled water. The mixture was kept at room temperature for 10 min and centrifuged at 13200 x*g* RCF for 10 min. The absorbance was measured at 593 nm against a reagent blank. Trolox was used as a reference and the results are expressed as μmol TE/g of oil sample.

Determination of chlorophyll and β-carotene content

Oil samples were homogenized and filtered immediately using a medium-size filter paper before the measurement of spectrophotometric absorbance. The oil samples were measured at 630 nm, 670 nm, and 710 nm using 10 mm spectrophotometer cell. The chlorophyll content was measured following the protocol suggested by IUPAC and the results are expressed as mg pheophytin per kg of oil.[28] Similarly, spectrophotometric measurement of β–carotene content in oils was performed by dissolving the homogenized samples in *n*-hexane and measuring the absorbance at 446 nm.[29]

Estimation of phytosterols content

The total phytosterol content of the oils was measured following the protocol described by Sabir *et al*.[30] Briefly, 1 g of oil sample was dissolved and diluted in 10 mL of chloroform. Three mL of diluted oil samples was mixed with 2 mL of Liberman–Burchard reagent and the volume was made up to 10 mL with chloroform. The reagent mixture was kept in darkness for 15 min followed by measurement of absorbance in UV–visible spectrophotometer (model UV-1601, Shimadzu). A blank mixture was also prepared following the same protocol without the addition of sample. To estimate the phytosterol content, a standard curve was developed using cholesterol as standard and results are presented as mg cholesterol equivalent (CE)/100 g of oil.

Characterization of fatty acid methyl esters (FAMEs) by gas chromatography

Oil fractions were derivatized prior to analysis of 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 NaCl 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 for a final hexane volume of 10 mL, and a FAME concentration of about 1 mg mL^{-1}.

Methyl-esterified testa oil samples were diluted in high-performance liquid chromatography (HPLC) grade *n*-hexane (40 mL FAME sample + 960 mL *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.[31] The injection mode was split (split ratio 1:50); terminal 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}. The amplified signals were transferred and recorded in a computer with GC-Solutions software (Shimadzu). 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).

Liquid chromatography–mass spectrometry (LC–MS) profiling of phenolic acids

The profiling of phenolic acids in coconut testa oil was performed following solvent extraction procedures described by Weidner *et al*.,[32] and Chen *et al*.,[33] with slight modifications. Briefly, the solvent extraction process utilized 80% methanol and the solvent was removed completely using a vacuum evaporator at 45° C. The dry residue, following solvent extraction, was hydrolyzed in 2 mol L^{-1} NaOH followed by acid hydrolysis in 2 mol L^{-1} HCL. The free phenolic acids were extracted using ethyl acetate and evaporated to dryness in vacuum at 45 °C. The resultant residue was dissolved in 1 mL of mass spectrometry (MS)-grade methanol filtered through 0.22 μm nylon filter prior to injecting in a liquid chromatography–tandem mass spectrometer (LC–MS/MS) (Waters UPLC H class system fitted with TQD MS/MS system) for the analysis of phenolic acids. The mobile phase used in the analysis comprised an aqueous phase of 0.1% formic acid in water (A) and an organic phase of 0.2% formic acid in methanol (B).[16] The phenolic acids were resolved on the analytical column BEH-C18 (2.1 × 50 mm, 1.7 μm) (Waters, Milford, MA, US) protected by a Vanguard BEH C-18 (Waters, Milford, MA,US) with the gradient flow of organic and aqueous phase in the flow rate of 0.1 mL min^{-1}. The column temperature was maintained at 25 °C during analysis and the sample injection volume was 5 μL. The eluted phenolic acids were monitored by a PDA detector and the eluted metabolites were pumped directly without any split into the TQD-MS/MS (Waters, Milford, MA, US) system optimized for the phenolic acids analysis.[16]

Estimation of trace elements

Trace elements, namely, Cu, Fe, and Zn, present in testa oil samples, were estimated following the acid digestion method.[34] Quantitative measurement of trace elements present in testa oil was performed using the iCE 3000 atomic absorption spectrometer (Thermo Scientific, Mumbai, India).

Statistical analysis

All the experiments were conducted in triplicate. Values are expressed as means ± standard deviations. Analysis of variance was computed using R software version 4.0.3[35] (R Foundation for Statistical Computing, Vienna, Austria). The significance of differences between the variety means (Fisher-LSD test) was determined using the Agricolae package.[36] The summary statistics and correlations among parameters were calculated using the corrplot package.[37] Multivariate analysis was performed with principal component analysis (PCA). The PCA results were visualized

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using Biplot constructed using factoextra package.[38] Cluster analysis was performed using Ward's method and squared Euclidean distance using the 'hclust' function of the R base library.

RESULTS

Physicochemical properties of testa oils

The physicochemical properties of testa oils of six coconut genotypes (WCT, FMST, COD, MYD, CRD × GBGD and MYD × CGD) are presented in Table 1 (Fig. 1). The proportion of testa as a percentage of nuts varied from 1.29 ± 0.30 in COD to 3.42 ± 0.54% in MYD. Similarly the proportion of oil in testa also varied among the genotypes. Testa obtained from CRD × GBGD yielded 50.56 ± 0.66% of oil compared to 40.97 ± 0.81% of oil in MYD-derived testa. Among the various physico-chemical properties of the testa oil, the refractive index (25 °C) and saponification value (mg KOH/g) showed insignificant differences among the coconut genotypes. The parameters to measure the rancidity due to free fatty acids (acid value mg KOH/g) and oxidative rancidity potential (peroxide value m eq O_2/kg) revealed that COD and MYD × CGD have relatively high acid values of 0.93 ± 0.02, 0.93 ± 0.05, respectively and peroxide values of 2.60 ± 0.04 and 2.32 ± 0.0, respectively, suggesting the testa oils from these genotypes are relatively prone to the oxidative rancidity. This is further corroborated by the iodine values (g I_2/100 g), which measure the degree of unsaturation of constituent fatty acids. MYD × CGD and COD showed values of 13.85 ± 0.11 and 13.58 ± 0.37, respectively. Analysis of trace elements in coconut testa oils revealed that the proxidants Fe (CRD × GBGD: 34.17 ± 0.70 to FMST: 62.48 ± 0.92 ppm) and Cu (WCT:1.63 ± 0.09 to MYD × CGD:2.77 ± 0.14 ppm) are present in considerable quantities, indicating the susceptibility of the oils to oxidative damage. The testa oils of COD and MYD × CGD showed relatively high Cu content accounting for the relatively high oxidation of these oils (Table 1).

Biochemical features and antioxidant potential of testa oils

The total phenolic content (TPC) of the testa oils of the coconut genotypes varied from 6.84 ± 0.09 to 8.67 ± 0.22 (mg GAE/100 g of oil). The testa oils of the genotypes FMST and CRD ×

Table 1. Comparative physico-chemical features of coconut testa oils from six *Cocos nucifera* L. genotypes

Component	WCT	FMST	COD	MYD	CRD × GBGD	MYD × CGD
Weight fractions of fruit						
Whole nut (g)	1375.88 ± 13.09^a	1205.5 ± 38.5^b	650 ± 55.86^d	536.17 ± 82.93^e	615.17 ± 20.82^d	763.33 ± 28.88^c
Testa(g)	23.33 ± 5.13^b	40.37 ± 0.12^a	8.31 ± 1.52^d	18.15 ± 2.67^c	14.22 ± 2.71^c	18.85 ± 2.00^{bc}
Proportion of testa in nut (%)	1.78 ± 0.61^{bc}	3.35 ± 0.12^a	1.29 ± 0.30^c	3.42 ± 0.54 ^{a}	2.31 ± 0.41^b	2.47 ± 0.17^b
Proportion of oil in testa (%)	43.73 ± 0.68^{ab}	45.02 ± 0.53^b	42.77 ± 1.18^{ab}	40.97 ± 0.81^{ab}	50.56 ± 0.66^a	49.40 ± 0.39^a
Properties of testa oils						
Refractive index (25 °C)	1.473 ± 0.005^b	1.464 ± 0.002^c	1.483 ± 0.003^a	1.473 ± 0.002^b	1.464 ± 0.002^c	1.482 ± 0.002^a
Acid value (mg KOH/g)	0.83 ± 0.02^b	0.82 ± 0.01^{bc}	0.93 ± 0.02 ^{a}	0.82 ± 0.02^{bc}	0.81 ± 0.01 ^{c}	0.93 ± 0.05^a
Iodine value (g I_2/100 g)	12.46 ± 0.21^b	11.26 ± 0.21^c	13.58 ± 0.37^a	12.54 ± 0.28 ^{b}	11.73 ± 0.39 ^{c}	13.85 ± 0.11^a
Peroxide value (meq O_2/kg)	2.14 ± 0.16^c	1.76 ± 0.03^d	2.60 ± 0.04^a	2.20 ± 0.05^c	1.87 ± 0.02 ^{d}	2.32 ± 0.03^b
Saponification value (mg KOH/g)	255 ± 1.01^b	258 ± 1.00^a	255 ± 2.51^{ab}	256 ± 1.73^{ab}	256 ± 0.57^{ab}	257 ± 2.30^{ab}
Trace elements in testa oils						
Fe (ppm)	54.8 ± 0.95^b	62.48 ± 0.92^a	51.16 ± 0.55^c	38.43 ± 1.26^d	34.17 ± 0.70^e	51.71 ± 0.57^c
Mn (ppm)	4.26 ± 0.08^d	6.67 ± 0.27^b	7.54 ± 0.09 ^{a}	5.61 ± 0.14^c	3.46 ± 0.24^e	5.51 ± 0.23^c
Zn (ppm)	4.69 ± 0.15^c	4.55 ± 0.13^c	5.66 ± 0.32^b	6.03 ± 0.21^a	4.62 ± 0.33^c	6.38 ± 0.14^a
Cu (ppm)	1.63 ± 0.09^d	2.18 ± 0.19^b	2.20 ± 0.13^b	1.92 ± 0.07^c	1.81 ± 0.07^{cd}	2.77 ± 0.14^a

Data are obtained from 5 ($n = 5$) independent samples of coconuts and presented as mean ± SD. Values with different superscript letters in each row indicate significant differences (one-way ANOVA test followed by Fisher's LSD test, $P < 0.05$) in physical characteristics among the varieties.

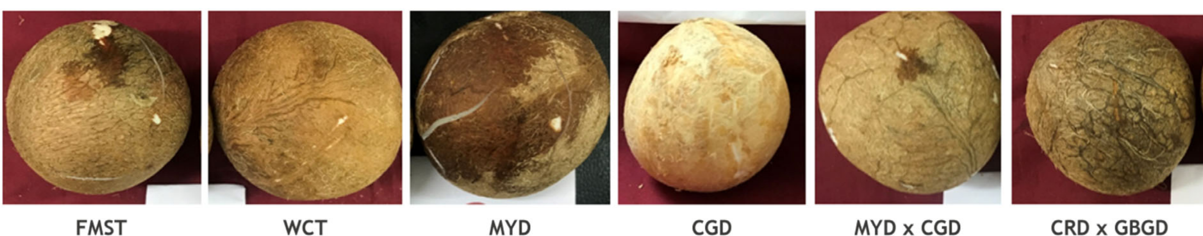

Figure 1. Deshelled coconut showing endosperm or kernels of various genotypes [Federated Malay States Tall (FMST), West Coast Tall (WCT), Malayan Yellow Dwarf (MYD), Chowghat Orange Dwarf (COD), and breeding lines MYD x CGD and CRD x GBGD] and their characteristic brown outer cover, the testa.

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Table 2. Biochemical features and antioxidant potential of testa oils from six *Cocos nucifera* L. genotypes

Component	WCT	FMST	COD	MYD	CRD × GBGD	MYD × CGD
Total polyphenols (mg GAE/100 g)	7.80 ± 0.24^{b}	8.67 ± 0.22^{a}	7.57 ± 0.40^{b}	7.56 ± 0.39^{b}	8.47 ± 0.41^{a}	6.84 ± 0.09^{c}
Total flavonoids (mg QE/100 g)	17.45 ± 0.60^{d}	20.58 ± 0.42^{a}	17.67 ± 0.22^{cd}	19.29 ± 0.32^{b}	19.51 ± 0.26^{b}	18.28 ± 0.63^{c}
Antioxidant capacity						
CUPRAC (μmol TE/g)	16.03 ± 0.86^{c}	20.34 ± 0.29^{b}	14.09 ± 0.41^{d}	16.89 ± 1.56^{c}	23.61 ± 0.157^{a}	10.37 ± 0.22^{e}
FRAP (μmol TE/g)	14.93 ± 0.65^{c}	21.94 ± 1.10^{b}	12.71 ± 1.06^{e}	17.97 ± 1.31^{c}	26.93 ± 0.42^{a}	12.82 ± 0.39^{e}
DPPH (μmol TE/g)	21.45 ± 2.08^{c}	31.95 ± 2.43^{a}	19.49 ± 1.03^{cd}	18.31 ± 1.06^{de}	28.92 ± 1.49^{b}	15.89 ± 0.46^{e}
Phytosterols (mg CE/ 100 g)	137.73 ± 16.88^{a}	122.39 ± 5.73^{b}	67.20 ± 7.73^{d}	54.66 ± 14.40^{f}	63.23 ± 8.04^{e}	77.52 ± 11.99^{c}
Chlorophyll (mg of pheophytin a kg^{-1})	24.74 ± 1.08^{b}	43.46 ± 3.48^{a}	8.79 ± 0.77^{e}	18.10 ± 1.18^{c}	14.70 ± 1.13^{d}	18.18 ± 1.81^{c}
β-carotene (ppm)	1.66 ± 0.11^{d}	3.84 ± 0.40^{a}	1.33 ± 0.11^{d}	3.27 ± 0.23^{b}	2.58 ± 0.24^{c}	2.77 ± 0.31^{c}

Data are obtained from 5 ($n = 5$) independent samples of coconuts and presented as mean ± SD. Values with different superscript letters in each row indicate significant differences (one-way ANOVA test followed by Fisher's LSD test, $P < 0.05$) in biochemical characteristic features including antioxidant potential of the testa oils of coconut genotypes.

Table 3. Comparative fatty acid composition of testa oils from six *Cocos nucifera* L. genotypes

Fatty acid	WCT	FMST	COD	MYD	CRD × GBGD	MYD × CGD
Caprylic acid	4.48 ± 0.27^{b}	3.66 ± 0.15^{c}	2.73 ± 0.20^{d}	4.18 ± 0.12^{b}	6.16 ± 0.06^{a}	2.78 ± 0.13^{d}
Capric acid	4.45 ± 0.07^{c}	4.74 ± 0.14^{b}	2.70 ± 0.12^{e}	4.35 ± 0.03^{c}	5.85 ± 0.05^{a}	3.25 ± 0.18^{d}
Lauric acid	28.64 ± 1.17^{b}	31.78 ± 0.38^{a}	26.66 ± 0.21^{c}	31.25 ± 1.01^{a}	32.04 ± 0.33^{a}	28.58 ± 0.66^{b}
Myristic acid	19.60 ± 0.24^{a}	19.17 ± 0.50^{abc}	18.31 ± 0.67^{d}	18.74 ± 0.30^{bcd}	19.30 ± 0.32^{ab}	18.41 ± 0.32^{cd}
Palmitic acid	14.62 ± 0.23^{b}	13.49 ± 0.50^{c}	15.75 ± 0.23^{a}	14.90 ± 0.28^{b}	13.43 ± 0.27^{c}	15.71 ± 0.35^{a}
Stearic acid	1.90 ± 0.05^{ab}	1.39 ± 0.49^{c}	1.58 ± 0.06^{bc}	1.87 ± 0.08^{ab}	2.18 ± 0.14^{a}	1.94 ± 0.07^{a}
Oleic acid	14.75 ± 0.52^{bc}	16.90 ± 0.11^{ab}	15.42 ± 0.54^{b}	13.95 ± 0.72^{c}	11.94 ± 0.09^{d}	17.63 ± 0.72^{a}
Linoleic acid	8.77 ± 0.47^{d}	9.57 ± 0.26^{c}	12.78 ± 0.26^{a}	9.62 ± 0.14^{c}	7.55 ± 0.31^{e}	10.83 ± 0.72^{b}
SFA	73.70 ± 1.29^{b}	74.24 ± 1.11^{b}	67.75 ± 0.85^{d}	75.29 ± 1.29^{b}	78.78 ± 0.21^{a}	70.6 ± 0.82^{c}
MUFA	14.75 ± 0.52^{bc}	16.90 ± 0.1^{e}	15.42 ± 0.54^{b}	13.95 ± 0.72^{c}	11.94 ± 0.09^{d}	17.63 ± 0.72^{a}
PUFA	8.77 ± 0.47^{d}	9.57 ± 0.26^{c}	12.78 ± 0.27^{a}	9.62 ± 0.14^{c}	7.55 ± 0.31^{e}	10.83 ± 0.73^{b}
MCFA	37.57 ± 0.99^{c}	40.17 ± 0.38^{b}	32.11 ± 0.53^{e}	39.77 ± 0.92^{b}	43.87 ± 0.12^{a}	34.54 ± 0.69^{d}

Data were obtained from the oil extracted from five independent samples ($n = 5$) of each variety, and all measurements were performed in duplicate. Values are expressed as relative percentages of total fatty acids, given as means ± SDs. Superscript letters in each row indicate statistically significant difference (one-way ANOVA test followed by Fisher's LSD test, $P < 0.05$). SFA: total saturated fatty acids; MUFA: total monounsaturated fatty acids; PUFA: total polyunsaturated fatty acid; MCFA: total medium chain fatty acids. The minor fatty acids found in testa oils are presented in representative chromatograms (supporting information, S3A through S3F).

GBGD exhibited highest TPC of 8.67 ± 0.22 and 8.47 ± 0.41 mg GAE/100 g, respectively whereas the least TPC content was found in genotype MYD × CGD (6.84 ± 0.09 mg GAE/100 g) (Table 2). No significant differences in the TPC of testa oils of dwarfs and the tall WCT. The total flavonoid content (TFC) of the testa oils ranged from 17.45 ± 0.60 (WCT) to 20.58 ± 0.42 (FMST) mg QE/100 g. Analysis of reducing power of the testa oils as measured by FRAP revealed that CRD × GBGD showed highest ferric reducing ability of 26.93 ± 0.42 (μmol TE/g) followed by FMST showing 21.94 ± 1.10 (μmol TE/g). The least ferric reducing ability was observed in oils obtained from COD and MYD × CGD. Similarly the measure of antioxidant potential of testa oils by CUPRAC revealed that CRD × GBGD showed highest CUPRAC activity of 23.61 ± 0.157 (μmol TE/g) followed by FMST 20.34 ± 0.29 (μmol TE/g) and lowest CUPRAC activity was exhibited by testa oils of MYD × CGD. The radical scavenging activity of the testa oil as quantified in DPPH assay varied from 15.89 ± 0.46 (MYD × CGD) to 31.95 ± 2.43 (FMST). The phytosterol content of the testa oil divulged that WCT showed the highest content of 137.73 ± 16.88 followed by 122.39 ± 5.73 mg CE/100 g (FMST) and the lowest phytosterol (54.66 ± 14.40) was found in the testa oil of the dwarf cultivar MYD.

Fatty acid composition of coconut testa oils

Table 3 presents the fatty acid composition of testa oils obtained from six genotypes of coconut. Eight major fatty acids were identified in testa oils, of which lauric acid ($C_{12:0}$) is predominant, contributing to 26.66–32.04% of the total followed by myristic acid ($C_{14:0}$) at 18.31–19.60%, palmitic acid ($C_{14:0}$) at 13.43–15.71%, oleic acid ($C_{18:1\ cis\text{-}9}$) at 11.94–17.63%, and linoleic acid ($C_{18:2}$) at 7.55–12.78%. The stearic acid ($C_{18:0}$) content varied from 1.39–2.18% among the coconut cultivars studied. The relative percentages of these fatty acids in the testa of the six coconut genotypes varied slightly. The highest lauric acid content of 32.04 ± 0.33% was found in the hybrid CRD × GBGD whereas the lowest of 26.66 ± 0.21 was found in the cultivar COD. The testa oils are characterized by low lauric acid content but have high PUFA, monounsaturated fatty acid (MUFA), and palmitic acid ($C_{14:0}$) contents

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Table 4. Phenolic acids in coconut testa oils (μg g^{-1} of oil). The LC–MS analysis of coconut testa oils revealed that the oils comprise 18 phenolic acids

Sl.No	Phenolic acids	μg g^{-1} of testa oil
1	Benzoic acid	0.0155 ± 0.006
2	*p*-Hydroxy benzoic acid	1.8277 ± 0.121
3	Salicylic acid	0.5558 ± 0.387
4	3-Hydroxy benzoic acid	0.2014 ± 0.016
5	*trans*-Cinnamic acid	3.7483 ± 0.826
6	2,4-Dihydroxybenzoic acid	0.3863 ± 0.144
7	Gentisic acid	0.9950 ± 0.142
8	Protocatechuic acid	0.4084 ± 0.375
9	*p*-Coumaric acid	67.4024 ± 4.796
10	*o*-Coumaric acid	2.2646 ± 0.334
11	Vanillic acid	5.3212 ± 0.090
12	Gallic acid	3.1029 ± 0.591
13	Caffeic acid	15.1428 ± 0.244
14	Ferulic acid	5.0450 ± 2.105
15	Syringic acid	0.0210 ± 0.001
16	Sinapic acid	0.6549 ± 0.379
17	Ellagic acid	0.4363 ± 0.010
18	Chlorogenic acid	0.0004 ± 0.000

compared with coconut kernel oils. Accordingly, the saturated fatty acids (SFAs) of testa oils ranged from 67.75 to 78.78% compared with 92% for SFAs found in coconut kernel oils. Similarly, lauric, myristic, and palmitic acid, which are predominantly found in coconut kernel oils, have percentages of 32–51%, 17–21%, and 6.9–14%, respectively (Deen *et al.*[39]) (Fig. 1).

Phenolic acid composition of testa oils

Liquid chromatography–mass spectrometry revealed the presence of 18 phenolic acids such as *p*-coumaric acid (67.40 ± 4.8 μg g^{-1}), caffeic acid (15.14 ± 0.24 μg g^{-1}), vanillic acid (5.32 ± 0.09 μg g^{-1}), ferulic acid (5.05 ± 2.10 μg g^{-1}), *trans*-cinnamic acid (3.75 ± 0.83 μg g^{-1}), and gallic acid (3.10 ± 0.59 μg g^{-1}) among others (Table 4). Phenolic acids such as benzoic acid, syringic acid protocatechuic acid and chorogenic acid, salicylic acid, 3-hydroxy benzoic acid, 2,4-dihydroxybenzoic acid, sinapic acid, and ellagic acid were found in minimal quantities (<1 μg g^{-1}) (Table 4).

Correlation analysis of biochemical features

The results of correlation analysis of various physico-chemical parameters of the testa oils are presented in Table S1 in the supporting information. Significant positive correlations were observed among the parameters viz., the iodine values, acid values, and peroxide values of the oils (Table S1 in the supporting information). These chemical parameters represent the degree of unsaturation of fatty acids of the oils and hence have a positive relationship with these parameters, which measure the keeping quality of the oils. The correlation analysis also indicates a significant positive relationship between TPC of oils with the measures of antioxidant potential (CUPRAC r = 0.88; DPPH r = 0.86; FRAP r = 0.77) (Table S2 in the supporting information). Thus, the antioxidant potential of the testa oils could be attributed to their relatively high polyphenol content. Similarly the total flavonoids in the testa oils exhibited high positive correlations with those of the FRAP assay based anti-oxidant measurement (FRAP r = 0.73) followed by DPPH (r = 0.67) and CUPRAC (r = 0.63), suggesting that the substantial portion of antioxidants in testa oils is derived from phenol and its subconstituents, flavonoids (Table S2 in the supporting information).

Principal component analysis

The principal component loadings for the biochemical characteristic features of coconut testa oils are presented in Fig. 2(A). The first principal component represented 62.8% of total variability and with parameters such as total phenolic content (TPC), total flavonoid content (TFC), antioxidant potential measured in the CUPRAC, FRAP, and DPPH assays. The second principal component represents 19.8% of total variability, comprising biochemical parameters such as phytosterol and chlorophyll b content. The third principal component represents the rest of the variability of 17.4% predominated by β-carotene content. Figure 2(B) shows the PCA biplot of the biochemical characteristics of testa oil on the first two principal components and this PCA biplot could differentiate the oils derived from all the six genotypes investigated here. The parameters TPC, TFC, FRAP, DPPH, and CUPRAC, which determine the antioxidant potential of the oil, have relatively high project values on PC 1 and hence are likely to influence PC1. Their negative loadings reveal that an increase in their values would cause a significant decrease in PC1. The vectors delineating the phytosterol content and chlorophyll content of the oils have high project values in PC2 and hence define that component. Similarly their negative loadings suggest that their values would inversely influence PC2. The phytosterol content and chlorophyll content of the testa oils exhibit no correlation with the antioxidant potential of the oils measured by FRAP and CUPRAC assays.

Similarly, the principal component loadings for the fatty acid composition of coconut testa oils are presented in Fig. 3(A). PC1 represents 73.2% of total variability being defined by the dominant fatty acids: caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, linoleic acid. PC2 represents 20.1% of total variability in the fatty acid composition, which is mostly defined by stearic acid and oleic acid.

Fig. 3(B) shows the PCA biplot of the fatty acid composition of testa oils depicting the first two principal components. This PCA biplot could differentiate the coconut genotypes. The fatty acids caprylic acid, capric acid, and the groups SFA and MCFA have relatively high project values on the PC1 hence contribute heavily in defining PC1. The positive loadings of these parameters also imply that they directly influence PC1. The fatty acids, stearic acid and oleic acid, and MUFAs are placed with high project values in PC2 and hence could contribute significantly to the definition of this component. The biochemically important MCFAs of coconut testa oils diverge from the MUFAs and hence are likely to show no correlation. The lauric acid and palmitic acid content of the testa oils show a negative correlation.

Further, the principal component loadings for the physicochemical characteristics of testa oils are shown in Fig. S1(A) in the supporting information. The first principal component represents 47.5% of the total variability; refractive index, acid value, iodine value, peroxide value are the dominant variables. The second principal component accounts for 24.4% of total variability comprising characteristics such as mineral content of the testa oil (Fe, Mn, Cu) and the proportion of oil in testa and testa content in the coconut fruit. Figure S1(B) in the supporting information depicts the PCA biplot of physicochemical characteristics of testa oil on the first two principal components and differentiates the testa oils obtained from all the six coconut genotypes.

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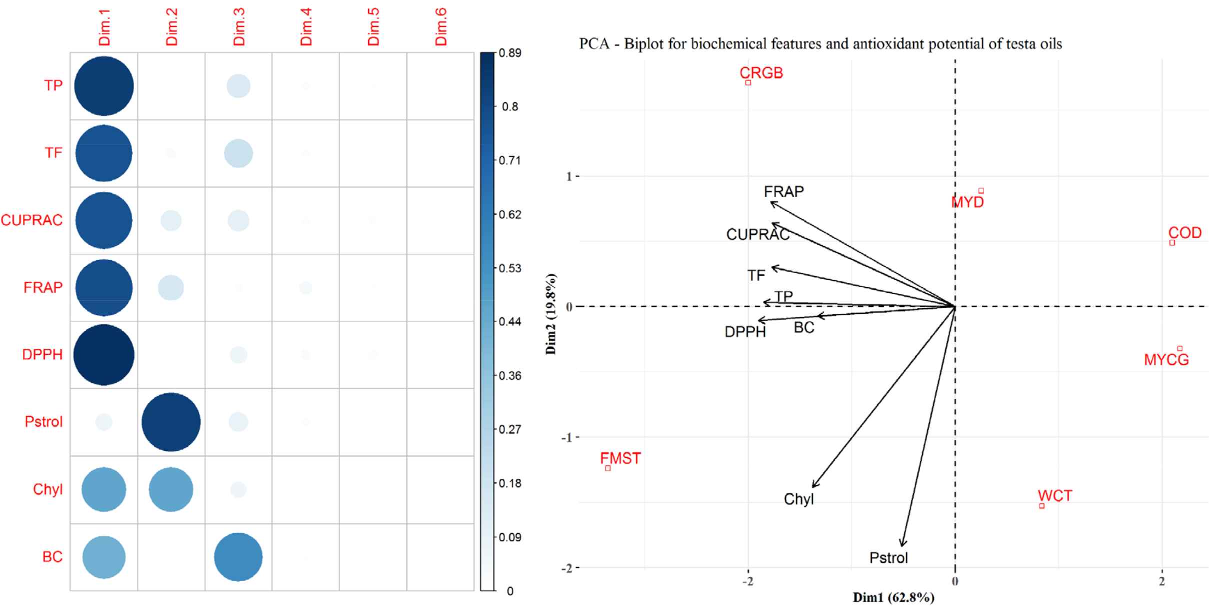

Figure 2. (A) Principal component loadings of biochemical attributes of coconut testa oils and (B). Biplot for principal component-1 (PC1) and principal component-1 (PC2) based on the principal component analysis of different biochemical parameters of testa oils obtained from six different coconut genotypes.

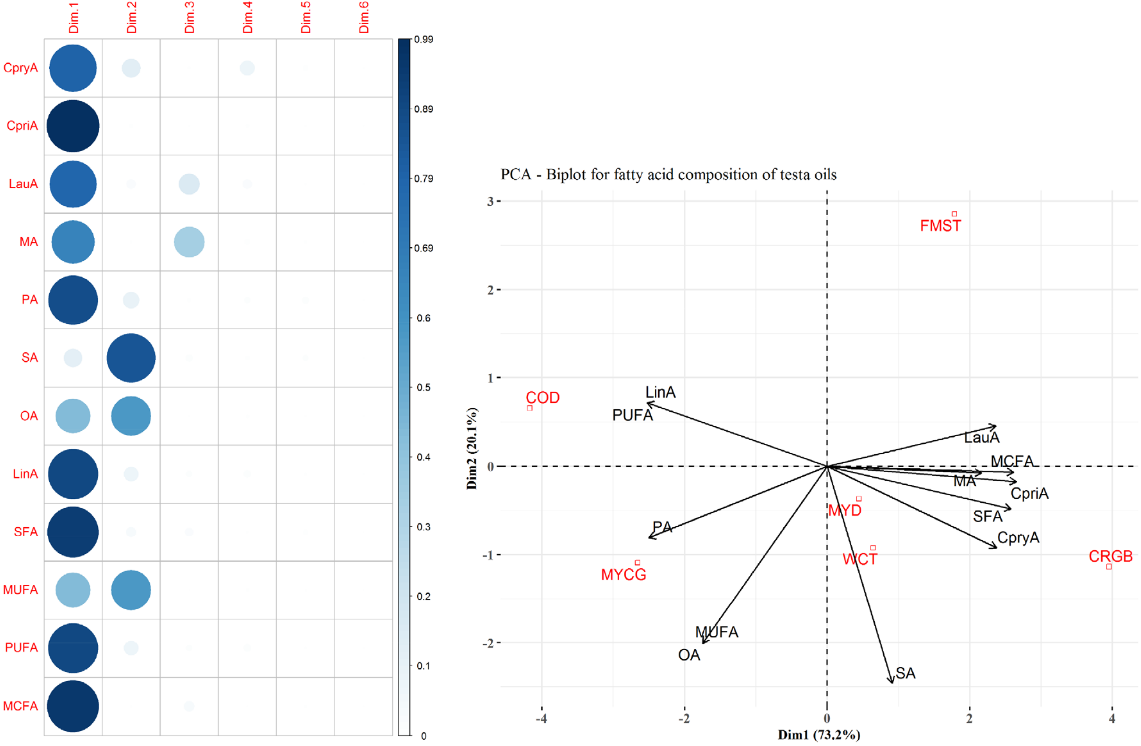

Figure 3. (A) Principal component loadings of fatty acids composition of coconut testa oils and (B). Biplot for principal component-1 (PC1) and principal component-1 (PC2) based on the principal component analysis of fatty acids composition of testa oils obtained from six different coconut genotypes.

The characteristic features such as refractive index, iodine value, acid value and peroxide value and trace element (Zn) have high project values at PC1 and hence could influence this principal component. However other trace elements such as iron, zinc, and copper have the potential to influence the PC2.

Hierarchical clustering of testa oils from coconut genotypes

The dendrograms resulting from hierarchical clustering based on individual analysis of physico-chemical, biochemical and fatty acid composition features display two distinct clusters (Fig. S2 in

the supporting information). The first cluster consists of the testa oils, which are derived from the tall genotypes (FMST, WCT), and the second cluster contains the testa oils of COD, MYD, and the D×D hybrids CRD × GBGD and MYD × CGD.

DISCUSSION

There has been a recent trend in food composition analysis and nutrition science advocating diets rich in phenolics and antioxidants. Coconut has attracted immense interest because of its nut oil, which is rich in MCFAs, with potential therapeutic cardioprotective effects. Virgin coconut oil is loaded with phenolics,[7,13] and defatted kernel is a rich source of dietary fiber.[5,6,40] The brown-colored outer coat of coconut kernel, which serves as a protective layer, called testa, imparts an undesirable dark color to coconut products such as desiccated coconut powder, coconut cream, and milk powder; hence it is removed from the kernel. Nevertheless, investigations have proven the nutritional importance of coconut testa, which is rich in phenolics, phytosterols, and tocopherols or tocotrienols.[14,16,41] The antioxidant richness of coconut testa and its potential anti-diabetic effects have also been proven.[42]

Despite these potential health benefits, investigations exploring the genotypic variations in the physico-chemical properties or biochemical potential of coconut testa oils are lacking. It is remarkable to observe that the biochemical characteristics of testa flour of select Sri Lankan coconut cultivars showed marked differences in their proximate composition.[20] The present study revealed that testa oils of coconut genotypes vary in oil percentage (40.97% to 50.56%) whereas prior analysis by Appaiah *et al.*[14] revealed that the percentage of fat in wet coconut testa was 34.7% and that of copra testa was found to be 59.8%. Thus the oil content of coconut testa shows considerable variation, which could be attributed to the different coconut genotypes being analyzed. There was similar variation in the trace elements: Fe content of copra testa and wet coconut testa was found to be 6.2 mg/100 g and 1.9 mg/100 g whereas the genotypes studied showed relatively high Fe content ranging from 3.4 mg/100 g (in CRD × GBGD) to 6.2 mg/100 g (FMST) in their respective testa oils.[14] Nevertheless, the relative content of Zn in the testa oils of coconut genotypes showed low values in the range of 0.45 to 0.63 mg/100 g as against the reported valued of Zn in coconut testa (1.6 to 3.0 mg/100 g).[14] Similarly, multiple VCO samples screened from the Malaysian market showed a relatively low Fe content of 0.45–14.53 ppm[43] compared to the values reported here (34 to 62 ppm) in coconut testa oils. However, VCOs are processed products compared to testa oils; hence there is a substantial variation in Fe content. The huge variations found in the trace elements imply that it is pertinent to investigate the effect of these metal proxidants in the keeping quality of these oils.

Coconut oil contains minor components such as phenolics and phytosterols, which contribute to its antioxidant potential. The total phenolic content (TPC) of testa oils (6.84 ± 0.09 mg GAE/100 g to 8.67 ± 0.22 mg GAE/100 g) was found to be high compared to the values of VCO obtained from different processing methodologies,[7,44] copra testa oil, and wet testa oils.[14] Nevertheless, the relative TPC of testa oils obtained from wet testa or copra testa is high than that in the oils from whole copra, the white portion of copra, the or endosperm,[14] suggesting the innate antioxidant potential of testa oils. Chromatography-based profiling of coconut testa oil divulged the presence of 18 phenolic acids, namely *p*-coumaric acid (67.40 ± 4.8 μg g^{-1}), caffeic acid (15.14 ± 0.24 μg g^{-1}), vanillic acid (5.32 ± 0.09 μg g^{-1}), ferulic acid (5.05 ± 2.10 μg g^{-1}), *trans*-cinnamic acid (3.75 ± 0.83 μg g^{-1}), gallic acid (3.10 ± 0.59 μg g^{-1}) and others. A recent study delineating the polyphenolic profile of oils in extracted from fresh and dried coconut testa also revealed the presence of protocatechuic acid, 4-hydroxy benzoic acid, and ferulic acid in both the oils. The flavonoids catechin and quercetin were also reported.[45] The phytosterol content of coconut testa oils was reported to be in the range of 42.52 to 50.97 mg/100 g[14] and had a mean value of 87 mg/100 g in whole coconut oil.[46] The phytosterol content of coconut testa oils reported from the dwarf genotypes or dwarf hybrids are in a similar range of 54.66 to 77.52 mg/100 g whereas the testa oils of tall cultivars show a significant increase 122.39 and 137.73 mg/100 g, indicating the further need for exploration of genotypic variation in the phytosterol content of the oils.

Most strikingly, our study has shown that testa oils are characterized by their low lauric acid content (26.66 ± 0.21 to 32.04 ± 0.33) but are rich in PUFA (7.55 ± 0.31 to 12.78 ± 0.27) and MUFA (11.94 ± 0.09 to 17.63 ± 0.72) when compared with coconut oil derived exclusively from the endosperm or kernel. Lauric acid, PUFA and MUFA contents of coconut kernel oil are 51.5 ± 0.5, 1.1, and 4.3%, respectively.[39] Hence, there is a decline in the SFA content of testa oil in the range of 67.75% to 78.78% in comparison with 92% found in coconut kernel oils.[39] Further, fatty acid composition of testa oils have a distribution pattern of lauric > myristic > palmitic acids[20,47] or lauric > myristic > oleic acids.[14] Among the coconut cultivars or genotypes reported here, the former pattern of relative predominance of palmitic acid over oleic acid content was found in dwarf cultivars (COD, MYD and CRD × GBGD) whereas the tall cultivars WCT and FMST and the hybrid MYD × CGD exhibit relatively high oleic acids (Table 3). Moreover variations in the fatty acid composition of coconut oils based on the cultivars are common.[48] An earlier report suggests differences in concentration in the glycerides across the transverse region of coconut.[49] Balachandran *et al.*[19] did not reveal any significant qualitative changes or concentration gradient in the biochemical components across the transverse section but similar changes in the fatty acid composition were observed across the inner to outer region of endosperm, substantiating the findings from this study.

CONCLUSIONS

To the best of our knowledge very little information has been available about the physical and biochemical features and fatty acid profiles of testa oils obtained from various cultivars of coconut. The chemical and biochemical features of coconut testa-derived oils varied to different extents depending on the cultivar. Biochemical components of the testa oils, namely proxidant elements (Fe 34.17–62.48 ppm; Cu 1.63–2.77 ppm), total phenolic content (6.84–8.67 mg GAE/100 g), and phytosterol content (54.66–137.73 mg CE/100 g) varied widely depending on the coconut genotypes. The testa oils of coconut have relatively high PUFA (7.55–12.78) and MUFA (11.94–17.63) content in comparison with coconut kernel oils. However, the high total phenolic content (6.84–8.67 mg GAE/100 g) of testa oils could give the oils appreciable antioxidant capacity. The various phenolic acids characterized in coconut testa oils emphasize the untapped nutritional potential of the crop and illustrate the genotypic differences in the biochemical properties of coconut testa oils. The large amount of coconut testa that remains unutilized or

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underutilized could be explored for the development of antioxidant-rich nutrient products.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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