

Mid-IR Spectral Characterization and Chemometric Evaluation of Different Solvent Extracts of Coconut Testa Flour

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Abstract

Fourier transform infrared (FTIR) spectroscopy is a fast analytical technique being successfully used in depicting the chemical characteristics of foods. The objective of this research was to use FTIR to characterize the fractionation behavior of coconut testa flour (CTF) under sequential extraction using three different solvents. In this study, CTF obtained from five Sri Lankan coconut cultivars namely; Gon Thembili, Ran Thembili, San Raman Tall, Tall x Tall and commercial hybrid were subjected to sequential extraction using hexane, ethyl acetate (EtOAc) and methanol (MeOH). FTIR spectral data were acquired within the range of 4,000–500 cm⁻¹ adopting the KBr pallet method. The contours of the FTIR spectra obtained for both hexane and EtOAc extracts were roughly similar, except for few regions. The presence of lipid molecules in these two extracts was confirmed by a dominant absorption peak due to ester linkage of triacylglycerols. Spectra of EtOAc extracts were nevertheless found to differ from those of hexane extracts by the presence of phenolic constituents since most of them contained additional peaks at ~3460 cm⁻¹ and ~1635 cm⁻¹. The spectral pattern of MeOH extracts differed significantly from those of the previous two sets of spectra due to drastic differences happened in the chemical composition. The occurrence of stretching vibrations of hydroxyl groups indicated the strong presence of carbohydrates and phenolic constituents in MeOH extracts. Existence of small amounts of proteins and lipids in this extract tended to give low-intense signal in the spectra. The distinctive differences of spectra of MeOH extracts from those of the other two extracts has been confirmed by principle component analysis (PCA), which exhibited 97% and 2% variance along principle component 1 and principal component 2, making up 98% of total variance. In conclusion, FTIR evaluation of various solvent extracts were helpful in identifying the changes in bimolecular distribution during sequential extraction of CTF. In addition, PCA has proven to be quite successful in discriminating the differences of both solvent extracts and cultivars.

Keywords

Coconut testa flour, Crude extracts, FTIR, Functional groups, Principle component analysis

Abbreviations

CTF: Coconut testa flour; **COM:** Commercial hybrid; **GT:** Gon Thembili; **RT:** Ran Thembili; **SR:** San Raman Tall; **TT:** Tall x Tall; **FTIR:** Fourier transform infrared spectroscopy; **PCA:** Principle component analysis; **PC1:** Principle component 1; **PC2:** Principle component 2

Introduction

Coconut testa (CT) is the brown color thin layer occurring on top of the white kernel of the coconut fruit. It has been shown that testa is composed of approximately 18% (w/w, wet basis) of the total weight of the coconut kernel [1]. Currently, it is utilized as a raw material for coconut testa oil production while the remaining partially defatted CT is reckoned as a byproduct of the industry. In an attempt to use it as a potential food source, the remnant was ground into fine particles to make coconut testa flour (CTF), which has 15% oil content. Previous studies reported from countries like India and Malaysia have confirmed the presence of various bioactive constituents in CT. As it was found to be a rich source of bioactive constituents like phenols, flavonoids, tannins and anthocyanin, it has been reckoned as a potential antioxidant and anti-hyperglycemic agent [1-4]. Pertaining to these findings, utilization of CTF as a source of edible flour is expected to provide several health benefits and would have beneficial economic and environmental impacts in the long run. However, chemical and nutritional studies focusing on CT obtained from Sri Lankan coconut cultivars are limited to few [5-6]. Hence, the characterization of biomolecular constituents of CTF of local coconut cultivars by FTIR spectroscopy is essential to advance further research into several other aspects.

FTIR spectroscopy is a rapid, time-saving analytical tool that is successfully used for chemical mapping of various agricultural produces. Application of FTIR to identify organic functional groups of biomolecules present in samples has led to detect biomass components in qualitative and quantitative ways [7]. Thus, this analytical method would be useful in the collective effort for detection of a range of different biomolecules present in a sample without relying on various sophisticated techniques. It is the main reason that FTIR has received much attention from researchers engaged in food analysis and authentication. The focus of this study was to establish the FTIR chemical mapping of different solvent extracts of CTF obtained from various Sri Lankan coconut cultivars. The chemical fractionation behavior of CTF in different organic solvents in a sequential manner has not been studied previously, but in fact it would facilitate the separation of its macro- and micro-bio molecular constituents based on differences in solvent polarities. Hence, the outcome of this study would be helpful in the identification of the presence of macro-biomolecules and minor-chemical constituents in different fractions of CTF based on polarity and inter-varietal differences among the local coconut cultivars.

Materials and Methods

Materials

Sampling

Five local cultivars of coconut Gon Thembili (GT), Ran Thembili (RT), San Raman (SR), Tall x Tall (TT) and Commercial (COM) hybrid were used for this analysis. For this study, twelve-month old mature coconuts, cultivated in the varietal blocks Coconut Research Institute, Lunuwila, Sri Lanka were collected. Nuts were processed in accordance with the method previously described by Marasinghe et

al. [5]. Briefly, disintegrated, and dried (at 70 °C) CT were subjected to cold press oil extraction using a micro-oil expeller (Komet DD85 machine, Germany). After collecting the oil, the remaining partially defatted CT (15% oil content) was collected and ground into fine particles to prepare CTF.

Crude extracts preparation

A 250 g portion of CTF of individual cultivar was sequentially extracted for 30 min with 1000 mL of hexane, EtOAc and MeOH in a sonicating machine (Rocker ultrasonic cleaner, model-Sonar 206) operating at 50 KHz. In each case, the extraction was repeated three times consecutively for equal time intervals. The solvent extracts of individual cultivar were concentrated under reduced-pressure using a rotary evaporator (Heidolph, Laborota 4000). The semi-solid extracts were freeze-dried using a bench top pro-freeze dryer (ESCO, model-FDL-2S8, Singapore). The crude extracts of individual cultivar were kept at -18 °C until further analysis.

FTIR measurements

FTIR measurements of the crude extracts was carried out as described by Mittal et al. [8] and Gunarathe et al. [9] with slight modifications. Around 1.5 mg of each crude extract was mixed with 90 mg of KBr (FT-IR grade, ≥99% trace metals basis, Sigma Aldrich) and turned into a pallet using a hydraulic press. The spectra were recorded using FTIR Nicolet iS50 spectrometer (Thermo Nicolet, Madison, WI) equipped with deuterated triglycine sulphate (DTGS) detector and KBr beam splitter. The data were collected in the mid-infrared region of 4000–500 cm⁻¹ by co-adding at 64 scans, resolution of 8 cm⁻¹. All spectra were ratioed against the blank background spectrum of pure KBr pallet and recorded as absorbance values at each data point in four replicates.

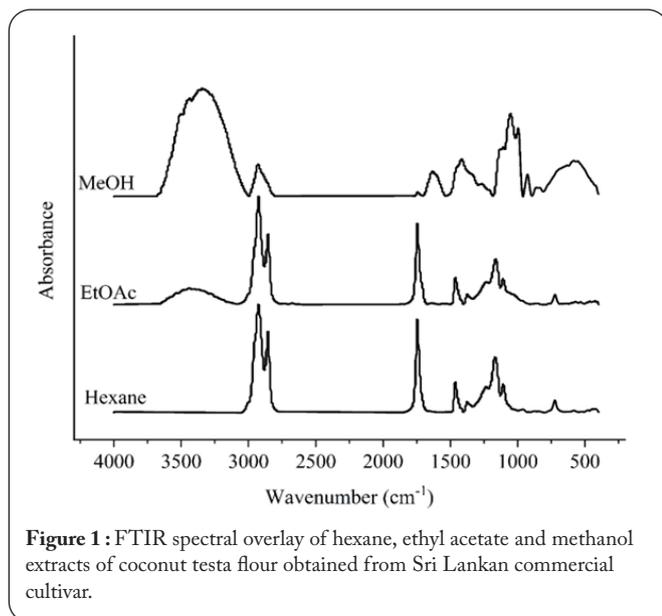
Spectral analysis

The manufacturer's software (OMNIC operating system, version 7.0 Thermo Nicolet) program was used for spectral pre-processing and qualitative analysis. The raw spectra of each cultivar were subjected to baseline correction, scale normalization respectively and the mean spectrum of the replicates was used for qualitative purposes. PCA was performed for spectral data of each crude extract using the Unscrambler 9.7 (Camo, USA) software. To conduct PCA, the spectral data of four replicates from all five cultivars were used.

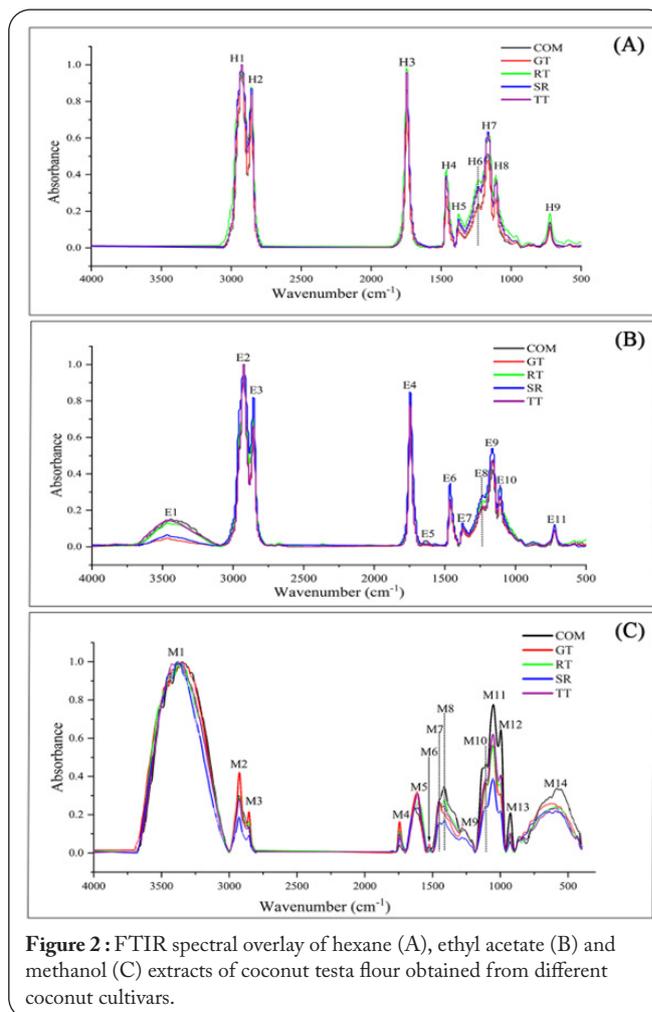
Results and Discussion

Characterization of hexane extracts

The FTIR spectrum corresponding to the hexane extract of COM cultivar is shown in figure 1 (hexane). Being a non-polar solvent, hexane is ideal for extraction of both non-polar and low-polar molecules in a food sample. Information provided in table 1 elaborates the vibrational frequencies of different organic functional groups associated with the biomolecules present in the crude extracts of hexane. According to figure 2A, FTIR spectral pattern of all cultivars exhibited roughly similar peak characteristics. Nonetheless, the peak intensities of different spectral bands of hexane extracts were found to vary, especially within the fingerprint region (1500–950



cm^{-1}) while the exact wavenumber of the peaks varied only within a narrow range (Table 1). As shown in figure 2A, the spectral characteristics of hexane extracts looks apparently similar to those of plant oils including coconut oil reported previously [10]. This observation is in accordance with the findings reported for other pure fats and oils as well [11–13]. According to a previous study, CTF contained considerable amounts of crude oil (~15 %) [5], which is more soluble in hexane due to its non-polar nature. Owing to this reason, the FTIR characteristic peaks of hexane extracts would be reflective of triacylglycerol molecules [10]. In figure 2A, the prominent peaks of H1 and H2 appearing at $\sim 2925 \text{ cm}^{-1}$ and $\sim 2855 \text{ cm}^{-1}$ were attributed to asymmetrical and symmetrical C–H stretching of methylene groups, respectively (Table 1). This was mainly due to the heavy presence of aliphatic chains attached to triacylglycerols. A distinct sharp peak at $\sim 1745 \text{ cm}^{-1}$ (H3) represented the C=O stretching vibration of saturated aliphatic ester moieties associated with lipid bio-



molecules (Table 1). This peak was usually found to appear distinctively in majority of the fats and oils as they are made up of 98% of triacylglycerol molecules [10]. When considering the spectra in exclusion of the fingerprint region ($1500 \text{ cm}^{-1} - 950 \text{ cm}^{-1}$), only H1, H2 and H3 peaks were mostly dominant to be within the range. This fact was further affirmed by the

Table 1: Characterization of FTIR spectra of hexane and ethyl acetate extracts of coconut testa flour.

Peak No	Wavenumber range* (cm^{-1})	Mode of vibration	Functional group	Reference
E1	3442-3470	O-H stretching	Alcohols, Phenols	[11-13]
E2, H1	2924-2925	C–H asymmetric stretching (CH_2)	Alkanes	[14, 15]
E3, H2	2855-2856	C–H symmetrical stretching (CH_2)	Alkanes	[14, 15]
E4, H3	1744-1745	C=O carbonyl ester stretching	Ester group	[14-17]
E5	1628-1642	C=O stretching	Aromatic-C(O)-OH.	[8]
E6, H4	1462-1463	C-H bending (CH_2, CH_3)	Alkanes	[14-16]
E7, H5	1374-1375	C-H bending (CH_3)	Alkanes	[14-16]
E8, H6	1230-1235	C-O stretching	Ester group	[14, 15]
E9, H7	1161-1169	C-O stretching	Ester group	[14-16]
E10, H8	1108-1111	C-O stretching	Ester group	[14]
E11, H9	722-723	$(\text{CH}_2)_n$ bending	Hydrocarbon	[14]

*Range of variation in wavenumbers at a particular peak among the different coconut cultivars namely, Gon Thembili, Ran Themili, San Raman, Tall x Tall, Commercial hybrid.

peaks occurring in the fingerprint region as they are mainly indicative of the presence of hydrocarbons and esters in the extracts. For instance, the peaks H4 and H5 appearing at $\sim 1462\text{ cm}^{-1}$, $\sim 1374\text{ cm}^{-1}$ were attributed to the C–H bending vibration of hydrocarbons, while peaks H6, H7 and H8 showing up at $\sim 1233\text{ cm}^{-1}$, $\sim 1165\text{ cm}^{-1}$ and $\sim 1110\text{ cm}^{-1}$ were stood for C–O stretching vibrations of ester linkages connected to triacylglycerols. Further, the peak H9 appearing at $\sim 722\text{ cm}^{-1}$ was due to the $(\text{CH}_2)_n$ bending vibration of hydrocarbon chains [15]. Interestingly, in the spectral overlay shown in figure 1 (hexane), a broad-blunt band within the ranger $3600\text{--}3400\text{ cm}^{-1}$ was totally absent. This could suggest that the stretching vibrations of O–H group associated with carbohydrates did not occur in the spectra confirming the absence of carbohydrates in hexane extracts of CTF.

Characterization of ethyl acetate extracts

The FTIR spectrum of EtOAc extract of COM cultivar is shown in figure 1 (EtOAc). When compared to hexane, EtOAc is generally said to be middle-polar by nature and therefore solubility would be different from that of hexane. According to figure 1 (EtOAc), the overall spectral features and exact wavenumbers of the peaks in the spectrum of EtOAc extracts were roughly similar to those of the spectral pattern seen before in hexane extracts, but with some exceptions. The spectral information provided in table 1 gives the details of vibrational frequencies of different organic functional groups associated with the biomolecules present in EtOAc extracts. As shown in figure 2B, the distinguishing sharp peak at $\sim 1745\text{ cm}^{-1}$ (E4) represented the C=O stretching vibration of saturated aliphatic ester moieties in triacylglycerols [10]. This affirms the presence of some amount of fatty molecules in the EtOAc fraction. When further scrutinizing the spectral patterns, it became evident that the EtOAc extract could contain some amount of mid-polar compounds containing hydroxyl groups. The occurrence of the broad blunt peak (E1) at $\sim 3450\text{ cm}^{-1}$, along with the weak peak at $\sim 1635\text{ cm}^{-1}$ (E5) was a distinguishable characteristic of the spectra of EtOAc extracts when compared to those of hexane extracts. The peak E1 is usually assigned to O–H stretching vibration of phenols, alcohols and simple sugars [9]. Meanwhile, the peak E5 is generally assigned to the stretching vibration of aromatic-C(O)–OH, confirming the presence of phenolic compounds in EtOAc extracts [8, 14, 15]. When comparing the different extracts of coconut cultivars, both SR and GT did not show E5 peak at $\sim 1635\text{ cm}^{-1}$ and also their peak intensities at $\sim 3450\text{ cm}^{-1}$ (E1) were relatively low. The noticeable variation in the intensities of the two peaks, namely E1 and E5 would be useful in interpreting the inter-varietal difference among CTF.

Characterization of methanol extracts

The FTIR spectrum of MeOH extract of COM cultivar is shown in figure 1 (MeOH). Among the three different solvents used in this study, MeOH is said to be the most polar by nature. Being a polar solvent, MeOH would facilitate the separation of molecules with more polar functional groups such as hydroxyl, amide, amines etc. The overlay of spectra shown in figure 2C compares the spectral patterns corresponding to MeOH extracts of different coconut cultivars. As shown in figure 2C, spectral features of MeOH extracts displayed considerable

differences from those of both hexane and EtOAc extracts. The information in table 2 gives the details of vibrational frequencies of different organic functional groups associated with various biomolecules present in the MeOH extracts. Although the overall contour of the spectra of MeOH extracts was mostly alike, slight changes in certain spectral regions were due to varietal differences in composition. As shown in figure 2C, MeOH extracts showed a prominent broad blunt peak at $\sim 3350\text{ cm}^{-1}$ (M1), which is usually assigned to O–H stretching vibration of polar molecules in a food sample. Some previous reports stated that the presence of O–H stretching vibrational groups was due to carbohydrates, phenolics or aliphatic alcohols present in MeOH extracts [11, 12]. In the FTIR spectrum of *A. calamus* extract, Mohani et al. [13] assigned the wide-band appearing at $\sim 3400\text{ cm}^{-1}$ to O–H stretching vibration of either glycosides or their derivatives. According to a previous study, CTF of locally available cultivars are rich source of phenolics and flavonoids that include caffeic acid, chlorogenic acid, ellagic acid, p-coumaric acid, vanillic acid, epigallocatechin gallate (EGCG), quercetin and rutin [6]. These compounds were also detected in the previous studies conducted among the coconut varieties grown in India and Malaysia [1–4]. Hence, the hydroxyl groups attached to these compounds would contribute to the above mentioned broad blunt peak at $\sim 3350\text{ cm}^{-1}$ (M1).

The peaks appearing at $\sim 2927\text{ cm}^{-1}$ (M2) and $\sim 2855\text{ cm}^{-1}$ (M3) are customarily assigned to C–H stretching vibrations of aliphatic carbon chain attachments in biomolecules [13]. However, the intensities of the C–H stretching vibrations of these alkyl group in MeOH extracts were much lower than those of both hexane and EtOAc extracts as seen before. This could be probably due to the fact that low amounts of fatty molecules were left in MeOH extracts after being sequentially extracted with hexane and EtOAc. As said before, the minor sharp peak appearing at $\sim 1744\text{ cm}^{-1}$ (M4) is generally attributed to the C=O stretching vibration of ester linkages [10, 18]. The low intensity of this band is a further confirmation to the fact that small amounts of fatty molecules were left in MeOH extracts after being sequentially extracted with both hexane and EtOAc. The presence of polar lipids was further supported by the appearance of peak (M9) at $\sim 1250\text{ cm}^{-1}$ in the fingerprint region which could be partially attributed to the C–O stretching of ester linkages [15]. The intensity variation of these peaks among different cultivars could be an indication of the existence of the varied amount of polar lipid moieties in them.

The presence of phenolic compounds in MeOH extracts was further affirmed by the appearance of peaks around $1615\text{--}1580\text{ cm}^{-1}$ (M5), $\sim 1510\text{ cm}^{-1}$ (M6), and $\sim 1450\text{ cm}^{-1}$ (M7) which are generally ascribed to C=C–C stretching vibrations of aromatic rings. According to Mohani et al. [13], the peaks appearing at $\sim 1500\text{ cm}^{-1}$ (M6) and $\sim 1450\text{ cm}^{-1}$ (M7) were due to existence of aromatic rings in the MeOH extract of *A. calamus*. Coates [19] stated that the peaks appearing at $\sim 1411\text{ cm}^{-1}$ (M8) and $\sim 1200\text{ cm}^{-1}$ (M9) were represented by the O–H bending and C–O stretching vibrations of phenolic compounds in the sample. Among the different cultivars, a noticeable variation was seen in the intensities of peaks M5 and M6, despite minimal variations in the intensity of peak M1.

Table 2: Characterization of FTIR spectra of methanol extracts of coconut testa flour.

Peak No	Wavenumber range ^a (cm ⁻¹)	Mode of vibration	Functional group	Reference
M1	3345-3376	O-H stretching, H-bonded, Normal polymeric OH stretching	Alcohols, Phenols	[11-13]
M2	2925-2927	C-H (CH ₂) asymmetric stretching	Alkanes	[19]
M3	2855-2857	C-H (CH ₂) symmetrical stretching	Alkanes	[19]
M4	1742-1744	C=O carbonyl ester stretching	Ester	[10, 18]
M5	1604-1611	C=C-C stretching	Aromatic ring	[19]
		C=O stretching (amide I)	Amide	[11]
M6	1523-1525	C=C-C stretching	Aromatic ring	[19]
		Amide II	Nitrogen compounds	[11]
M7	1448-1451	C=C stretching	Aromatic ring	[13]
M8	1411	O-H bending	Phenol or tertiary alcohol	[19]
M9	1267-1284	C-O stretching	Phenol	[19]
			Ester linkage	[15]
M10	1106-1107	C-O stretching	Secondary alcohol	[19]
M11	1052-1055	C-O stretching	Alcohol	[19]
M12	996-999	=C-H bending	Alkenes	[13]
M13	926-927	C-C vibration	Alkane	[17]
M14	593-627	O-H out-of-plane bending	Alcohol	[19]

^aRange of variation in wavenumbers at a particular peak among the different coconut cultivars namely, Gon Thembili, Ran Thembili, San Raman, Tall x Tall, Commercial hybrid.

Both GT and TT showed the highest intensity with respect to peak M5, which appeared within 1604-1611 cm⁻¹. The intensity of this peak (M5) was gradually decreased in the order of GT>TT>RT>SR>COM.

Peak M6 appeared as a minor in both GT and TT, but it happened to appear as a sharp peak with highest intensity value for GT. For cultivars SR and RT, this peak (M6) gradually became blunt while it was totally non-existence in the case of COM. For M7 appearing at ~1450 cm⁻¹, both GT and TT showed a mild sharp peak. For SR and RT, this peak was appeared to be blunt while it did not show up at all in the case of COM. The variations seen in the intensities of peaks M5, M6 and M7 were actually useful in describing the inter-varietal differences of phenolics occurring in CTF. According to a previous study reported by Gunarathne et al. [6], MeOH extracts of CTF is rich in phenolics; the phenolic content of the MeOH extract of CTF was relatively higher than those of EtOAc and hexane extracts [6]. These findings are in conformity with the results reported from other studies carried out elsewhere in the world [1-3]. According to these studies, likelihood of phenolics to be extracted into polar solvents like MeOH is higher when compared to those obtained with hexane and EtOAc.

When going through the spectra, some of the major peaks (peak M8, M10, M11 and M14) appearing in the fingerprint region could be ascribed to molecular vibrations of alcohols

[19]. The peak intensity variation of different cultivars was a distinctive feature within this region. Cultivar COM showed the highest while cultivar SR showed the lowest and rest of the cultivars (GT, RT, TT) were in between these two extremes. The complex region with overlapped bands appearing around 960-1130 cm⁻¹ was ascribed to the characteristic fingerprint of carbohydrates [20]. As mentioned earlier, carbohydrates containing hydroxyl groups are more likely to be dissolved in polar solvents like MeOH. Hence, it can be assumed that carbohydrates and glycosides were present in polar solvent fractions of CT as reported previously [2-3]. There is a possibility for polar groups such as amines and amides to exert some influence in the fingerprint region of mid-IR spectroscopy. Amir et al. [11] previously mentioned that the peaks showing up approximately at 1660 cm⁻¹ and 1550 cm⁻¹ are supposed to represent amide-I and amide II bands, respectively. Further to this, the peak at ~1610 cm⁻¹ also might be partially ascribed to the amide-I band which exerted its signal due to the stretching vibrations of C=O in amides. The peak at ~1530 cm⁻¹ might represent the amide-II band, which occurred due to the N-H bending and C-N stretching vibrations [11, 21]. As reported by Marasinghe et al. [5], CTF is a rich source of proteins, and hence these peaks in the spectra could be ascribed to moieties of protein molecules of CTF. Earlier findings of Appaiah et al. [3], which confirmed the presence of amino acids in polar solvent extracts of CT, could provide sufficient ground for assignment of amide I and II bands in the spectra.

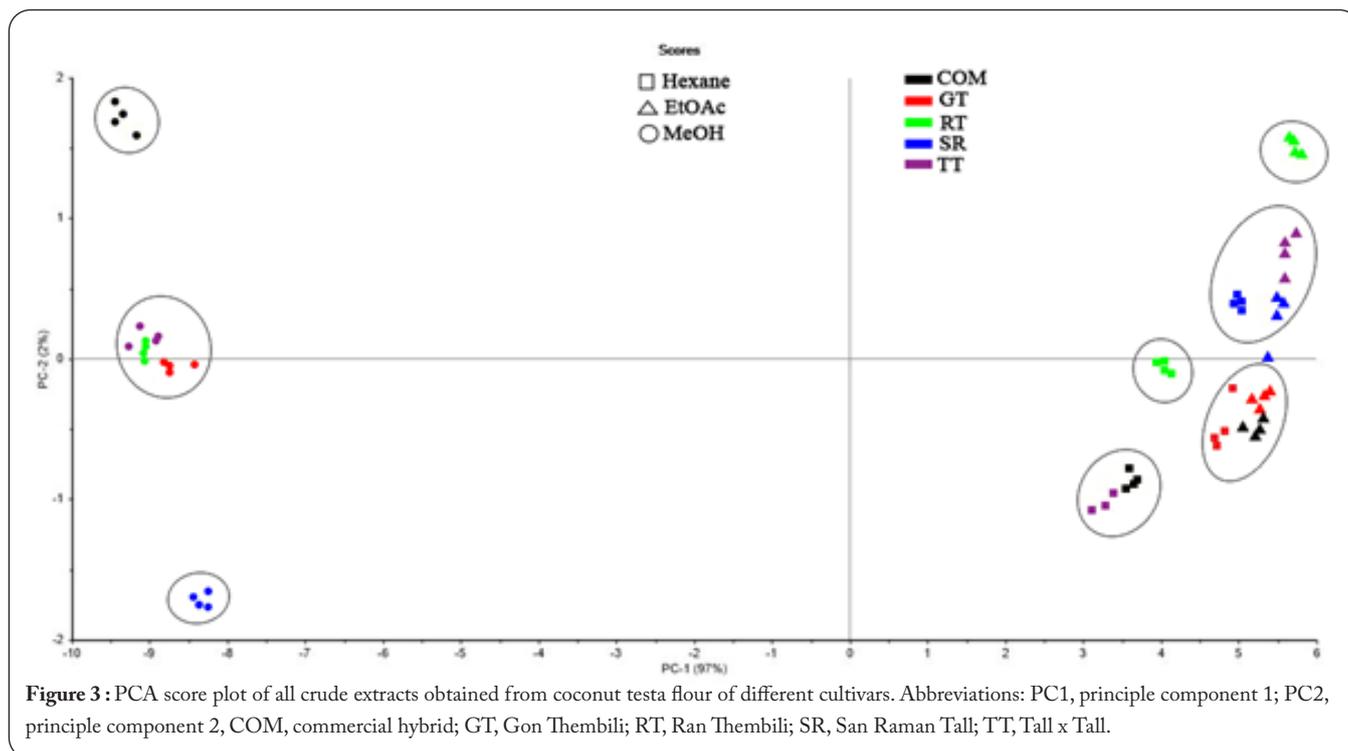


Figure 3: PCA score plot of all crude extracts obtained from coconut testa flour of different cultivars. Abbreviations: PC1, principle component 1; PC2, principle component 2, COM, commercial hybrid; GT, Gon Thembili; RT, Ran Thembili; SR, San Raman Tall; TT, Tall x Tall.

PCA analysis

PCA was applied to FTIR spectral data of all crude solvent extracts of CTF of different cultivars and presented as shown in figure 3. Previously, PCA was shown to be successful in discriminant analysis of rapeseed oils [14], legumes extracts [21] and animal fats [22]. These studies explained that the score plots represent the projection of samples defined by principal component 1 (PC1) and principal component 2 (PC2). PC1 is the linear combination of variables that explain the highest variation among samples, while PC2 is orthogonal to PC1 and exhibited the second largest variation [9]. Based on PCA of spectral data from all crude extracts, eight distinct groups were identified from the score plot of PC1 and PC2, which together accounted for 99 % variability. Out of the eight groups, MeOH extracts were clustered into three sub-groups in the left hand side while hexane and EtOAc extracts were clustered into five groups in the right hand side. This could be probably because the FTIR spectral patterns of hexane and EtOAc were mostly alike except for few regions, while the spectral pattern of MeOH extracts were distinctly different. Among MeOH extracts of GT, TT and RT remain as a single group, while the MeOH extracts of COM and SR were distinctly classified as separate groups on the upper left quadrant and lower left quadrant, respectively. As discussed earlier, both SR and GT did not show E5 peak at $\sim 1635\text{ cm}^{-1}$ and also their peak intensities at $\sim 3450\text{ cm}^{-1}$ (E1) were relatively low. The noticeable variation in the intensities of the two peaks, namely E1 and E5 would be useful in interpreting the inter-varietal difference among CTF. A closer look at the figure 3 will show that the hexane and EtOAc extracts of SR differently classified under two sub-clusters. This could be due to minor solubility differences in the two extracts.

Conclusion

This study demonstrated the use of FTIR spectroscopy as a rapid tool for chemical mapping of CTF through crude solvent extracts obtained with hexane, EtOAc and MeOH. While the FTIR spectral patterns of hexane and EtOAc were mostly alike except for certain regions, the spectral pattern of MeOH extracts were distinctly different. This was mainly due to changes happening in the major-biomolecule and minor-molecular distribution during the sequential extraction process with solvents of different polarities. The FTIR chemical mapping was successful in predicting the major biomolecules present in each solvent extract through the interpretation of organic functional groups. Fatty biomolecules were predominant in both hexane and EtOAc extracts while polar functional group having biomolecules such as carbohydrates, alcohols and polyphenols were dominant in MeOH extracts. The FTIR data elaborated not only the variance in the distribution of chemical constituents among different solvent extracts but also inter-varietal differences. The PCA model developed for spectral analysis gave a statistical explanation for differences arising out of different solvents and coconut cultivars. The PCA was able to discriminate them along with the variance of PC1 and PC2. This study affirms the effectiveness of FTIR spectroscopy as a rapid tool for chemical mapping of crude extracts of CTF in order to identify chemical compositional changes caused by dissolution of different solvents. Thus, the outcome of this study would provide a wide perspective for further research engagement for CTF.

Conflict of Interest

The authors declare no conflicts of interest.

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