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Physiochemical Analysis and Extraction of Anacardic Acid in Cashew Apple Vengurla 4, vengurla 7 Varieties: Chemical Profiling

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ABSTRACT

The physiochemical parameters significantly influence cashew apples and while rheological characteristics are pivotal in operational standardization from pulping to product development. This study elucidates the physiochemical dynamics in two Indian cashew apple varieties (‘*Vengurla 4’, ‘Vengurla 7’)*. Analysis encompassed moisture content, total soluble solids, titratable acidity, total sugar, reducing sugar, non-reducing sugar, protein content, fibre content, ash content, and ascorbic acid content. Results indicated similar physicochemical parameters in cashew apple varieties. Concurrently, moisture and ash content exhibited a lower trend in ‘*Vengurla 4’* variety compared to *‘Vengurla 7’* variety, while fibre content, protein content, sugar content levels lower compared to ‘*Vengurla 4’* variety. Moreover, extraction of Anacardic Acid (AnAc) in cashew apple by chemical, enzymatic, and ultra-technique method. Employing High Performance Liquid Chromatography (HPLC) for chemical profiling of AnAc samples were investigated. HPLC analysis delineated alterations in cardol, cardanol and AnAc levels C15:3, C15:2 and C15:1, emphasizing varietal differences. Understanding this importance of AnAc is imperative for development of new products and value addition in the food quality. Intriguingly, fully ripe mango pulp demonstrated varied sugar concentrations, with glucose, fructose, and sucrose exhibiting notable variability across ripened mangoes.

Key Words: Cashew Apple, Extraction, Food Product, Physiochemical, Postharvest

1. INTRODUCTION

A cashew (*Anacardium occidentale L.)* is a tropical fruit that produces a nut and an accessory fruit called the cashew apple [1]. India produces over 0.8 million tonnes of cashews annually, across more than 0.7 million hectares of land [2]. Besides the vast scale of cashew production also known for pioneering cashew processing and exporting cashew kernels across the globe. Over the years India has emerged as the global processing hub for the cashew industry [3]. Cashewnut undergo processing to new products, a versatile ingredient utilized in a myriad of products such as ice cream, beverages, and many more.

Cashewnut cherished globally in both fresh forms, owe much of their desirability to their unique nutritional profile. However, after harvesting the Cashewnut the cashew apples byproduct is produced. The pseudo-fruit, known as the cashew apple, is the part of the tree that connects it to the cashew nut, the real fruit, a well-known product worldwide [4]. The cashew apple is soft and juicy, and oval or pear-shaped structure, a hypocarpium that develops from the pedicel and the receptacle of the cashew flower, called the cashew apple [3]. It ripens into a yellow or red structure and about 5–11 cm long. It is edible and has a strong a sweet taste and used for development of delicious beverages such as fermented liquor kaju, feni, and other popular beverages.

However, the cashew apple contains valuable bioactive compounds, including anarcardic acid (AnAc), carotenoids (α-carotene, β-carotene and β-cryptoxanthin, vitamin C, phenols, and tannin [5]. Cashew apples contain high vitamin C [6], which is five times richer than other citrus fruit and possess anti-bacterial properties [7], have been proven to be effective in treating stomach ulcers and gastritis, which is usually caused by pH. AnAc from cashew nut shell liquid, a natural substance, have antioxidant, antimicrobial, and anti-inflammatory and modulate immune responses and angiogenesis [8]. Additionally, AnAc inhibiting breast and other cancer cell growth and total radical-trapping antioxidant potential (TRAP) assay showed lowered oxidative

damage-induced mutagenesis by co- and post-treatments with the cashew apple juices. Therefore, cashew nut shells have an internal part called the pericarp, containing a dark brown viscous liquid known as cashew nut shell liquid (CNSL) [9]. In CNSL alkyl phenol content, which is composed of anacardic acids , cardol and cardanols.

However, every ton of cashew nut, 8-10 tons of cashew apples are produced and only 9-10 % cashew apples are processed in the form of fenny [10]. The major issue in cashew apple utilization is its seasonal availability and short shelf life. The seasonal production of cashew apple is one of the largest bottlenecks for the processing industry. Also due to lack of awareness among people about the nutritional status of cashew apple and suitable technology, thousands tone of this fruit is spoiled every year. The cashew apples are highly perishable and cannot be stored for longer period [11].

Further, numerous researchers have reviewed and reported on cashew apples compositional, properties during processing. The bioactive properties of cashew apples and their fruit undergo modifications as they ripen, and these bioactive compounds are crucial for the standardization and development of product [6,12,13]. With these objectives in view, a study was conducted to ascertain develop effective techniques for extracting anarcardic acid (AnAc) from cashew apple powder to increase its utilization for product development. The extraction of anacardic acid (AnAc) from cashew apple powder is a challenging task due to its complex chemical structure and low solubility in water. Several extraction techniques have been reported in the literature, but there is no clear consensus on the best method for the extraction of anacardic acid (AnAc) from cashew shell. Therefore, the current study's goals are to examine proximate analysis of varieties (*Vengurla 4, vengurla 7*) of cashew apples and propose a relationship between physiochemical parameters, all of which are focused on two different varieties.

In this study, we investigate the physiochemical parameters of cashew apples and extraction of AnAc from cashew apple powder by chemical, enzymatic, ultra-techniques for further product development. Through a comprehensive analysis of physiochemical parameters and chemical profiling using HPLC (High Performance Liquid Chromatography) during extraction of AnAc, we aim to elucidate the dynamics of cashew apples and provide valuable insights for improving product developments, post-harvest handling practices and enhancing fruit quality.

2. MATERIALS AND METHODS

2.1Chemicals and raw materials

Ripe cashew apple fruits of *Vengurla- 4 & 7* variety were procured from the Instructional Farm of Department of Soil and Water Conservation Engineering, College of Agricultural Engineering and Technology, Dr. BSKKV, Dapoli, India. For titrable acidity studies, sodium hydroxide, buffer solutions, and phenolphthalein were sourced from Himedia Limited, Mumbai, India.

2.2 Material and methods

2.2.1 Selection of cashew apples

Ripe cashew apples were purchased from the Instructional Farm of the Department of Soil and Water Conservation Engineering, College of Agricultural Engineering and Technology, Dr. BSKKV, Dapoli, India, to start the cashew apple sample selection procedure. The cashew apples were brought to the lab within a day of being purchased, ensuring maximum freshness and reducing any possible post-harvest impacts. Each cashew apple was carefully hand-sorted upon arrival to ensure consistency in size, colour, and integrity. Cashew apples that showed symptoms of disease, infection, deterioration, or damage were quickly identified and removed away. Using a muslin cloth, the fresh cashew apple fruits were cleaned with tap water to get rid of any dirt or surface water. Cashew apples that were firm and ripe were examined to ascertain their physico-chemical characteristics.

2.2.2 Storage of cashew apples

Cashew apples were sorted and then immediately stored in regulated circumstances in the experimental trials, with a temperature range of 25–30°C and 65 ± 5% relative humidity. At the same temperature and humidity (25-30°C, 65 ± 5% RH), physicochemical and chemical profile analyses were then performed on the cashew application. Each evaluation was subjected to triplicate analyses to assure the reliability and consistency of the results. This systematic technique made it possible and HPLC analysis during the study, offering valuable the bioactive elements in cashew apples and the changes in the product.

2.3 Physiochemical analysis

2.3.1 Moisture content

Using the hot air oven method, a standardised process based on the methodology described by AOAC, 2010 was used to ascertain the moisture content of cashew apples. Samples were first taken from each variety of cashew apple. Using cleaned scissors, these samples were chopped into thin pieces, and precisely measured pieces (10–20 g) were placed onto dry, pre-weighed petri dishes. After that, the petri dishes with the cashew apple pieces were put in an oven that was set to 105 ± 2°C for around three hours. The samples of cashew apples were ensured to have their moisture removed while retaining their structural integrity attributable to this carefully regulated

drying procedure. To avoid moisture absorption, the petri dishes were taken out of the oven and placed in a desiccator after the drying time [14].

The following formula was used to determine the cashew apple moisture content:

$$\text{Cashew apple moisture content (db \%)} = (W_m - W_d) \times 100$$

Here, Wm represents the dish's initial weight (in g) with the cashew apple pieces before they have been dried, and *Wd* represents the dish's weight (in g) after the cashew apple pieces are dried.

2.3.2 Total soluble solid (TSS)

Using a mortar and pestle, 10 g of cashew apples were crushed for the purpose to determine the total soluble solids (TSS). After cheesecloth filtration of the obtained mixture and 2-3 mL of the filtrate were placed on a refractometer prism for measurement. A digital refractometer (ATAGO, Model: RX-5000) was used to measure the TSS content; results were represented in degrees Brix (°Brix), which indicates the amount of soluble solids present [15].

2.3.3 pH value measurement

The Hover labs pH meter, model No. HV-10-PH, which is renowned for its high precision up to 0.001%, was used in the study to measure the cashew apple's pH. Three grammes of cashew apple samples were mashed and diluted in ten millilitres of distilled water in order to perform the analysis. All samples were guaranteed to be consistent and uniform according to this preparation procedure. For every treatment, three measurements were made to assure the accuracy of the results of the study [16].

2.3.4 Titratable acidity (TA)

The procedure for calculating titratable acidity (TA) is described by Ranganna (2009). 20 g of cashew apples were dissolved in 100 10 mL of distilled water, and the mixture was then filtered. The presence of 1% phenolphthalein provided a slight pink shade, suggesting that the filtrate had reached the end point, which was titrated with 0.1 N NaOH. The percentage (%) represents the TA results [15].

2.3.5 Total sugar content, reducing and non-reducing sugar content

Using a 100 mL volumetric flask, add 50 mL of aliquot cleared and deleaded solution, 10 mL of HCl (1+ 1), and leave for 24 hours at room temperature. (The sample containing HCl can be heated to 700°C for 1 hour to achieve instant inversion.) Using a phenolphthalein indicator, precisely neutralise with a concentrated NaOH solution, then dilute to 100 mL [17]. Determine the total sugar as invert sugar by titrating against a mixture of Fehling A and B solutions (25 mL of Fehling's Solution may be used for this purpose). The following formula was used to determine the cashew apple ash content:

Reducing sugar (%) = mg. of invert sugar x vol. made up x 100/ TR x Wt. of sample x 1000

Total sugar (%) = (Total reducing sugar – Reducing sugar) x 0.95 + Reducing sugar

2.3.6 Protein content

The kjeldahl method was used for the determination of the protein content in cashew apple [17]. To digest the food sample for analysis, it is first weighed into a digestion flask and heated in the presence of sulphuric acid, anhydrous sodium sulphate, and a catalyst, such copper. Following the completion of the digestion, a tube connects the digestion flask to a receiving flask. The ammonium sulphate is subsequently changed into ammonia gas by adding sodium hydroxide, which makes the solution in the digesting flask alkaline. Titration of the ammonium borate produced with standard sulphuric or hydrochloric acid is then used to assess the nitrogen concentration, and the reaction's end-point is identified using an appropriate indicator. After followed the conversion factor used for analysis of protein content in cashew apple.

2.3.7 Fibre content

Samples of cashew apple waste analyzed for neutral detergent fibre (NDF),acid detergent fibre (ADF) and acid detergent lignin (ADL) as per the methods of Van Soestet al. (1991). SDF is precipitated with EtOH, and residue is then filtered, dried, and weighed. Total dietary fiber

(TDF) value is corrected for protein and ash content.

2.3.8 Ash content

The weigh a 5–10 g of cashew apple in a silica dish that is 7-8 cm in diameter [17]. Dry it on a water bath, light it on a burner and then burn it in a muffle furnace at 525–550°C for 4–6 hours, or until white ash forms. Weigh the dish once it has cooled. After an hour in the muffle furnace, let it cool and weigh it. determined the entire amount of ash by calculating the lowest weight.

The following formula was used to determine the cashew apple ash content:

$$\text{Cashew apple ash content (db \%)} = (W_3 - W_1) \times 100/(W_2 - W_1)$$

Here, W_1 represents the weight of empty dish (in g), W_2 weight of dish containing cashew apple sample, and W_3 represents the weight of dish containing ash.

2.4 Extraction of AnAc from cashew apple powder

The chemical, enzymatic, were utilized for extraction of AnAc from cashew apple powder, and ultra-techniques were utilized for analysis. The cashew apple powder was prepared by after the process of picking, sorting, washing, steam bleaching for 15 minutes or not, cutting into rounds, drying at 45-50°C in the oven or in the sun, grinding was carried out.

2.4.1 Chemical method

2.4.1.1 Sample Collection

The raw unripe cashew nut was collected from the Dr. BSKKV, Dapoli. The collected fruit were washed with sterile water and the peel was removed carefully without any flesh and dried for a week in shade at normal room temperature. The completely dried peel was then blended into fine powder. The fine powder was then packed and transferred to the laboratory for the analysis.

2.4.1.2 Preparation of extract

The extract was prepared using various solvents such as petroleum ether, hexane, and methanol. 25 g of powdered cashew apple was weighed and mixed with 250 ml of solvent and stirred under rotary shaker for overnight. After overnight stirring the extract was filtered with filter paper and the filtrate was then stored at room temperature for extraction process.

2.4.1.3 Reduction of calcium hydroxide to calcium anacardate

Filtrate 30 ml was taken in a round bottom flask and 60 ml of acetone was added to it and mixed well. To the above mixture calcium hydroxide (6 g) were added to stir continuously for 3½ hours using rotary shaker. After the incubation the white coloured powder starts to precipitate at the bottom was said to be calcium anacardate. The precipitate was filtered, washed with 85 mL of acetone, and dried for 2 hours under hot air oven at 50°C.

2.4.1.4 Calcium anacardate to anacardic acid

The dried calcium anacardate was then treated with 200 mL of sterile distilled water and 11M HCL and stirred for half an hour. The solution was then treated with 200 mL of petroleum ether or n-hexane and the obtained solution was dried over anhydrous sodium sulphate. After complete drying the pure white crystalline anacardic acid was obtained, characterized under UV-VIS spectrophotometer and fourier transform infrared spectrometer (FT-IR).

2.4.2 Enzymatic method

4 g of ground cashew nut was dispersed with distilled water in a material-to-water ratio (1:9) (w/v) to make a slurry and shaken in falcon (50 mL), then Viscozyme Cassava C enzyme was added (1% v/w E/S) into the slurry, and pH adjusted to 5.5 - 6.0. The samples were shaken for 3h at 50°C. At the end of treatment, the enzyme was deactivated by heating at 90°C for 5 minutes. The oil layer was taken out after centrifugation at 13,000 x g for 30 minutes and then evaporated at 60°C for 4h to remove residual moisture and then measured anacardic acid by HPLC method.

2.4.3 Analysis of AnAc from cashew apple powder by ultra-techniques methods

The AnAc were analysed by the ultraviolet-visible spectrophotometer (UV-DRS) and fourier transform infrared spectrometer (FT-IR) for chemical binding.

2.4.3.1 Ultraviolet-visible spectrophotometer (UV-VIS)

The UV- visible absorption spectra of extracted anacardic acid were recorded using Ultraviolet-visible spectrophotometer Hitachi U-2400PC, with the peak ranging from 200 to 800 nm using ultra-violet spectrum with an absorbance speed of 5 nm per minutes.

2.4.3.2 Ultra performance liquid chromatography (UP-LC)

The chemical binding of extracted anacardic acid were analyzed using UPLC system (Waters, Milford, MA, USA) coupled to a quadrupole/time-of-flight (QToF) mass spectrometer (Waters, Milford, MA, USA). The compounds were separated on an Acquity BEH C18 (150 × 2.1 mm^2, 1.7 µm; Waters, Milford, MA, USA) column operated at 40°C. The eluent system employed was a combination of A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) at a flow rate of 0.4 mL min^{-1}. The gradient varied linearly from 5 to 95% B (v/v) over 20 min. The sample injection volume was 5 µL. Mass spectra were obtained in the negative-ion mode over a mass range between 50 and 1180 Da. The spectrometer was operated with MSE centroid programming using a cone voltage of 40 V. The drying gas pressure was 35 psi at 370°C, while the nebulizer gas pressure was 40 psi. A capillary voltage of 3500 V and a 600 V spray shield voltage were used.

2.5 Statistical analysis

IBM Corp.'s SPSS version 20.0 (Armonk, NY, USA) was used to perform an analysis of variance (ANOVA) on

the physicochemical data gathered from all cashew apple sample observations. The cashew apple samples' physicochemical quality variations were evaluated using a Post Hoc Test (Duncan Test) with a 95% confidence interval and a significance level of $p < 0.05$. The mean ± standard deviation is used to display the data.

3.0 Results and discussion

3.1 Physiochemical analysis

3.1.1 Moisture content

When evaluating the quality of cashew apples, it is crucial to maintain and check on their moisture content. In this study, the moisture content of two cashew apple varieties was assessed. The variations in moisture content between the cashew apple samples are shown in Figure 1A. A significant moisture difference was noted across the two varieties of cashew apples. Notably the moisture content for cashew apple of varieties '*Vengurla-4'* and '*Vengurla-7'* was 84.40 ± 1.40 % and 86.60 ± 1.39 % respectively. '*Vengurla-4'* exhibited a lower moisture content behaviour, compared to the '*Vengurla-7'* variety of cashew apple. Statistical analysis revealed significant differences ($p< 0.05$) in moisture content among two varieties of samples. Previous studies have also reported a similar results of cashew apple varieties Attri, (2009) and Morton, (1987).

3.1.2 Total soluble solid (TSS)

A significant indicator of cashew apple quality, the TSS content has a direct impact on the apples' moisture content, flavour, and freshness. Significant water loss is indicated by a higher TSS score, which is frequently linked to the start of discolouration. Thus, vigilant TSS monitoring is necessary to preserve cashew apple quality. The variations in TSS levels between two cashew apple various kinds are illustrated in Figure 1B. Total soluble solids content did not differ significantly ($p < 0.05$), with "Vengurla-7" having the lowest TSS (13.21 ± 0.03 °Brix) and "Vengurla-4" having the greatest TSS (13.31 ± 0.03 °Brix).

3.1.3 pH values

A crucial factor affecting cashew apples' quality is their pH level, which represents enzyme activity and biological reactions. Two cashew apple varieties were used in this study, and their pH values were examined (Figure 1C). Cashew apples often have an acidic pH between 4 and 5, which helps with their microbiological stability and enzymatic profile. The pH values of the cashew apples for the *Vengurla-4'* and '*Vengurla-7'* varieties were recorded as 4.21 ± 0.01, and 4.31 ± 0.02, respectively, with the high pH found in *Vengurla-7'* variety. The results obtained are consistent with previous studies by Rosnah et al.,(2012), Sharma et al.,(2010) and Kardile et al.,(2014). The significant differences in were not shown pH values were observed among two varieties of cashew apples.

3.1.4 Titratable acidity (TA)

Monitoring TA is crucial for assessing cashew apple quality. The variations in TA across two varieties of cashew apples are shown in Figure 1D. The mean titratable acidity 0.75 ± 0.02 and 0.59 ± 0.02 for *'Vengurla-4'* and *'Vengurla-7'* varieties respectively. The differences in the acidity of *'Vengurla-4'* and *'Vengurla-7'* variety of cashew apple were not significant ($P \leq 0.05$). Similar type of trend was also observed and reported in literature for cashew apple, apples, mango, and pumpkin by Attri, 2009, Jan & Rab, 2012, Islam et al., 2013, Rahman et al.,2013 respectively.

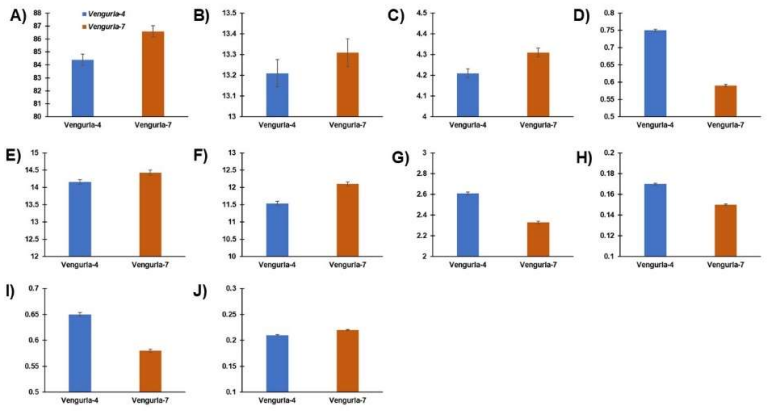

Figure 1: Physicochemical analysis of *'Vengurla-4'* and *'Vengurla-7'* variety of cashew apple; A) Moisture content; B) Total soluble solid; C) pH values; D) Titratable acidity; E) Total sugar content; F) Total reducing sugar; G) Non-reducing sugar content; H) Protein content; I) Fiber content; and J) Ash content.

3.1.5 Total sugar content

The sugar content of cashew apples can vary depending on the variety and location of the fruit, but generally ranges from 12 to 14%. Cashew apple contains good amounts of sugars including fructose and sucrose. The total suage content in across two varieties of cashew apples are shown in Figure 1E.The total sugar of cashew apple varieties *'Vengurla-4'* and *'Vengurla-7'* was determined. The total sugar content of the cashew apple of varieties *'Vengurla-4'* and *'Vengurla-7'*was 14.16 ± 0.47% and 14.43 ± 0.47%, respectively. Cashew apples are high in sugar and moisture, which makes them prone to rapid microbial spoilage and effect on final product. Similar finding was also reported for cashew apple by Attri, 2009 and Marc et al., 2012.

3.1.6 Total reducing and non-reducing sugar content

The reducing sugar content of cashew apples can vary, and depending on the variety. Two cashew apple varieties were used in this study, and their total reducing sugar content were examined and showed in Figure 1F and non-reducing sugar showed in in Figure 1G. Reducing the sugar content of cashew apples can be a complex process, as the sugar content is naturally present and varies depending on the stage of ripeness, environmental conditions, and variety of the fruit.

The reducing sugar of cashew apple of varieties *'Vengurla-4'* and *'Vengurla-7'* was determined. The reducing sugar for cashew apple of varieties *'Vengurla-4'* and *'Vengurla-7'* was 11.54 ± 0.31% and 12.10 ± 0.34%, respectively. Similar finding was also reported for cashew apple by Attri, 2009 and Marc et al., 2012. However, there are a few approaches that can be used to reduce sugar levels or manage sugar content for different purposes, such as processing or consumption. Reducing the sugar content in cashew apples might result in changes in taste, texture, and nutritional content. Since sugar is a significant factor in the flavor and appeal of the fruit, managing sugar reduction must be balanced with maintaining its palatability and health benefits.

The cashew apples, the predominant sugars are reducing sugars, primarily glucose and fructose. However, sucrose, a non-reducing sugar, is also present in varying amounts depending on the stage of ripeness. The non-reducing sugar for cashew apple of varieties *Vengurla-4'* and *'Vengurla-7'*was found to be 2.61 ± 0.68% and 2.33 ± 0.42%, respectively. The non-reducing sugar such as sucrose content in cashew apples would likely require intervention through selective harvesting, enzymatic treatment, or fermentation. However, these approaches are typically more relevant for processed products rather than fresh fruit.

3.1.7 Protein content

Cashew are primarily valued for their high vitamin C content, and while contain some protein, their protein content is relatively low compared to other sources like legumes, nuts, or seeds. The protein content of cashew apples is approximately 0.1–0.2 g per 100 g of fresh fruit. The protein content can vary depending on the ripeness of the fruit, the growing conditions, and the specific variety of cashew apple. The protein content of cashew apple of varieties *'Vengurla-4'* and *'Vengurla-7'* was determined and illustrated in Figure 1H. The protein content for cashew apple of varieties *'Vengurla-4'* and *'Vengurla-7'* was 0.17 ± 0.01% and 0.15 ± 0.01%, respectively. Similar finding was also reported by Morton, 1987.

3.1.8 Fibre content

Cashew apples are a good source of dietary fiber, and amount can vary based on factors like ripeness, variety, and growing conditions. On average, the fiber content of a cashew apple is approximately 0.5–3.0 grams of fiber per 100 g of fresh fruit. The variations in fibre levels between two cashew apple various kinds are illustrated in Figure 1I. The fibre content of cashew apple of varieties *'Vengurla-4'* and *'Vengurla-7'* was determined. The fibre content for cashew apple of varieties *'Vengurla-4'* and *'Vengurla-7'* was 0.65 ± 0.06% and 0.58 ± 0.04%, respectively. Similar type of trend was also observed and reported in literature for cashew apple by Nam et al.,2014.

3.1.9 Ash content

The ash content of a fruit refers to the mineral content left after the fruit is completely burned, which represents the inorganic compounds. This range can vary slightly depending on factors like the fruit's ripeness and the specific variety. The ash content between two cashew apple various kinds is illustrated in Figure 1J. The ash content of cashew apple of varieties *'Vengurla-4'* and *'Vengurla-7'* was determined. The ash content for cashew apple of varieties *'Vengurla-4'* and *'Vengurla-7'* was 0.21 ± 0.05% and 0.22 ± 0.05%, respectively. *Vengurla-4'* exhibited a lower ash content behaviour, compared to the '*Vengurla-7'* variety of cashew apple.

3.2 Extraction of AnAc by chemical method

The solubility data for natural CNSL in $SCCO_2$ under a range of operating conditions of pressure (100, 200, and 300 bar), temperature (40 and 50°C), and CO_2 flow rate (5, 10, and 15 g min^{-1} were established. The best $SCCO_2$ working conditions were found to be 50 °C and 300 bar at a flow rate of 5 g^{-1} min CO_2. Using 3 g of sample (CNSL/solid adsorbent = 1/2) under these $SCCO_2$ conditions, it was possible to quantitatively isolate high purity anacardic acid from crude natural CNSL (82% of total anacardic acid) within 150 min. Table 1 shows analysis of extraction of AnAc from cashew apple by chemical method.

Table.1 Analysis of extraction of AnAc from cashew apple by chemical method

Variety	M (g/mol)	Purity (%)	Mass (mg)	Final Volume (mL)	Stock solution (g/L)
Vengurla-4	340.40	90	7	5	4.75
Vengurla-7	348.52	95	5	5	4.75

3.3 Extraction of AnAc by enzymatic method

Anacardic acids, the main constituents of natural cashew nut shell liquid, are formed by a mixture of monoene, diene, and triene constituents. The long alkyl chains of anacardic acids come from the condensation of saturated or unsaturated fatty acids and phenolic compounds generated through acetate-malonate-derived pathways. Thus, palmitoleoyl-CoA can act as start group for extension by three malonyl-CoA units, with a reduction step during chain extension and aldol cyclization that yields anacardic acid. Anacardic acids are the most abundant (62.90%), and the triene component presents a higher yield, followed by diene and monoene with the same percentage. In the high-performance liquid chromatography (HPLC) analysis, a reverse phase chromatography column was used. Figure 2 and Table 2 illustrates the HPLC profile of the anacardic acid obtained from the cashew liquid shell liquid (CNSL).

Table 2. High performance liquid chromatography (HPLC) analysis of natural cashew nut shell liquid in *Vengurla 4 and Vengurla 7*.

Peak Number	Constituent	Retention Time (min)	Yield (%)
1	Anacardic acid triene	7.48	28.00
2	Anacardic acid diene	10.35	17.77
3	Anacardic acid monoene	15.93	17.13

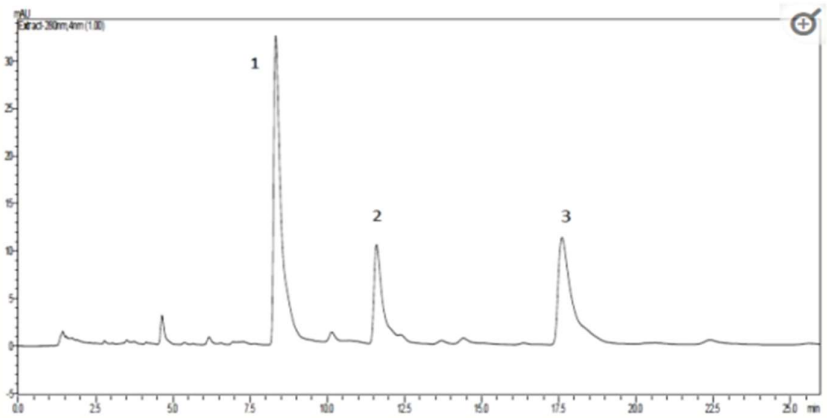

Figure 2. Illustrate the HPLC profile of the anacardic acid obtained from the cashew liquid shell liquid (CNSL).

3.4 Chemical profiling of AnAc from cashew apple powder by ultra-techniques methods

The AnAc were analysed by the ultraviolet-visible spectrophotometer (UV-DRS) and fourier transform infrared spectrometer (FT-IR) for chemical binding.

3.4.1 Ultraviolet-visible spectrophotometer (UV-VIS)

The anacardic acid powder was successfully extracted from the Cashewnut shell extract

and the characterization was further analyzed. The extracted anacardic acid was confirmed as anacardic acid using ultraviolet visible spectrophotometer and showed in Figure 3. The anacardic acid showed an absorbance range between 280nm to 300nm in '*Vengurla 4*' and '*Vengurla 7*' varieties.

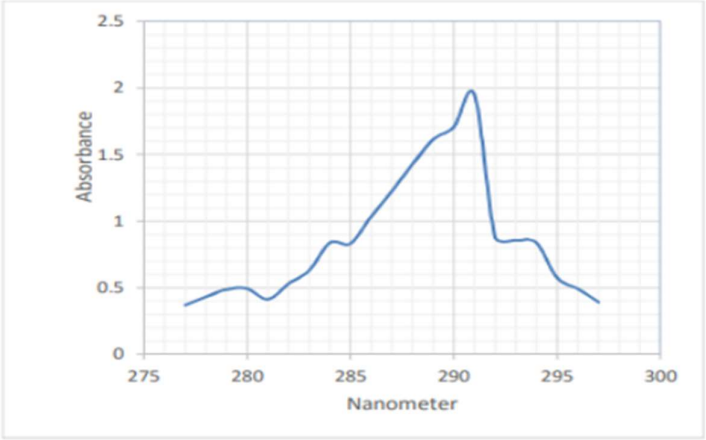

Figure 3. Illustrate the extracted anacardic acid was confirmed as anacardic acid using ultraviolet visible spectrophotometer.

3.4.2 Ultra performance liquid chromatography (UP-LC)

To confirm the structure of the AnAc, the samples were analyzed on an Acquity UPLC system (Waters, Milford, MA, USA) coupled to a quadrupole/time-of-flight (QToF) mass spectrometer (Waters, Milford, MA, USA). The compounds were separated on an Acquity BEH C18 (150 × 2.1 mm^2 , 1.7 µm; Waters, Milford, MA, USA) column operated at 40°C. The eluent system employed was a combination of A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) at a flow rate of 0.4 mL min^{-1}. The gradient varied linearly from 5 to 95% B (v/v) over 20 min. The sample injection volume was 5 µL. Mass spectra were obtained in the negative-ion mode over a mass range between 50 and 1180 Da.

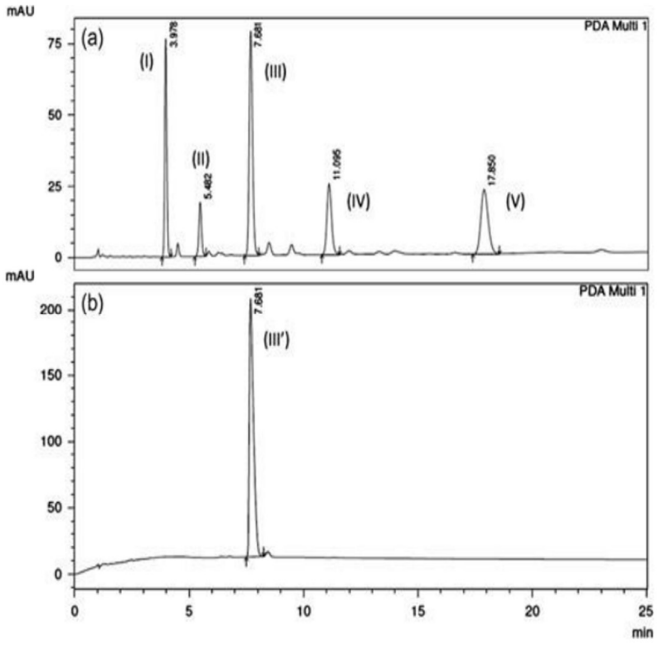

Figure 4: A) Chromatographic profile of CNSL (1 mg mL^{-1}) monitored at 280 nm for cardol (I); cardanol (II); anacardic acid triene (III), diene (IV) and monoene (V). B) Chromatographic profile for the external standard anacardic acid triene (1 mg mL^{-1}) monitored at 280 nm (III).

The spectrometer was operated with MSE centroid programming using a cone voltage of 40 V. The drying gas pressure was 35 psi at 370°C, while the nebulizer gas pressure was 40 psi. A capillary voltage of 3500 V and a

600 V spray shield voltage were used. The chromatographic separation conditions employed were based on the separation conditions previously established by Paramashivappa et al. to evaluate the alkyl phenols presents in CNSL. Exploratory experiments were performed to achieve the best separation results.

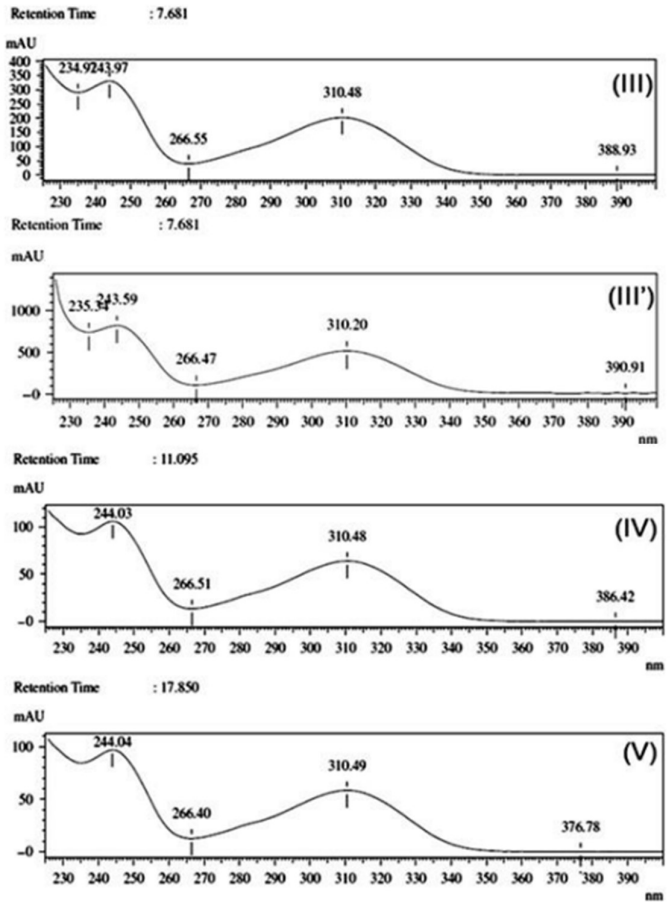

Figure 5: UV spectrum for anacardic acid (III) triene, (IV) diene and (V) monoene and the triene external standard (III).

The best results were achieved with a C18 column, a mobile phase with 80% of acetonitrile with acetic, and a flow rate of 1.5 mL min^{-1}. The peaks were identified using their retention times and UV spectra. Figure 4A shows the chromatographic profiles of CSNL, where peaks I–V were assigned to cardol, cardanol and the AnAc C15:3, C15:2 and C15:1, respectively. Figure 4B illustrated the chromatographic profile of the external standard of AnAc triene (15:3). Selectivity was evaluated by analysing the UV spectrum of the external standards used. The purities, obtained as a mean of three values, of the AnAc were 99.98, 99.98 and 99.97% for the (15:3), (15:2) and (15:1) species, respectively. The identity of the peaks was confirmed by their characteristic UV spectra (Figure 5). Throughout the chromatogram, no interference was detected.

4 CONCLUSION

In conclusion, this study examined the physiochemical analysis of two types of cashew apples. Several indicators, including moisture content, pH, TSS, TA, and sugar content interpretations, were carefully assessed to fully assess the influence of product development. the ratio of the physicochemical properties. The rheological behaviour was influenced by physiological fluctuations of several types and components other than soluble solids.

UV-VIS and UPLC offer comprehensive insights into these metabolic alterations, which are essential for comprehending and improving post-harvest procedures to improve nutritional value and product development. Anacardic acid levels during analysis are usually verified using ultra-methods. To modify flavour and produce new products, active ingredients like anacardic acid diene and anacardic acid monoene are essential.

Future studies should investigate the potential of AnAc for standardisation and improvement in the creation of several food products. Creating various goods based on AnAC may yield insightful information on new value-

added products. The study's overall findings emphasise the importance of cashew apples' physiochemical characteristics and active ingredients. The cashew apple has the potential to be widely used for the development of food products in research and development activities in this area continue.

CRediT authorship contribution statement

Sanjay H. Talware: Visualization, Formal Analysis, Investigation, Data Curation, Writing- Original Draft.

Gurunath V, Mote: Conceptualization, Methodology, Investigation, Supervision, Validation, Writing- Review & Editing.

Prathapan K. Pillai: Supervision, Writing- Review & Editing

Saubai B. Wakshe: Formal Analysis, Supervision, Validation, Writing- Review & Editing.

Declaration of competing interest

This article's authors declare no competing financial interests or personal relationships that may have appeared to affect the work reported.

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