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Research article

The feasible screening of genuine fresh palmyrah toddy and sugar or rice toddy using near-infrared spectroscopy

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ABSTRACT

The use of combination of Near-Infrared (NIR) absorption spectroscopic and k-mean multivariant statical cluster analysis as a screening tools for genuine fresh palmyrah toddy(G.F) and sugar (ATS) or rice toddy(ATS) is discussed. The quick and simple screening methods to ensure the authenticity of G.F is prime important to keep up its commercial value. Here we performed NIR spectroscopic analysis, k-mean multivariant analysis, and hierarchical cluster analysis to screen the G.F from ATR and ATS. For comparison, we performed chemical analysis and distinguished G. F from ATR and ATS. However, based on the NIR spectroscopic analysis together with the multivariant analysis G.F quickly screened from ATR and ATS. The plot of k-means cluster analysis and hierarchical cluster analysis shows three distinct clusters and it could be a useful tool to quickly screen the genuine toddy from artificial toddy.

1. Introduction

The Palmyrah palm (Borassus flabellifer) is widely distributed across Africa, South, and Southeast Asia, belonging to the Arecaceae family [1]. Its pristine sap is exceptionally sweet and nutrient-rich, known as "sweet toddy" or "neera," with a sugar content of 10–16.5 % w/v, primarily sucrose [2]. Fermentation of this sap yields alcoholic beverages like palmyrah toddy, with a typical 5 % alcohol content [3–7]. Yeast strains such as Saccharomyces chevalieri, *Saccharomyces cerevisiae*, Kloeckera apiculata, and *Schizosaccharomyces pombe* are crucial in the fermentation process, producing alcohol from the sap's sugars. Bacteria also contribute to the toddy's distinctive flavor [6,8,9].

Food, essential for sustenance, comprises carbohydrates, water, fats, and proteins. Adulteration, a persistent issue, involves lowering food quality for profit, posing health risks [10,11]. Frederick Accum's 1820 study highlighted harmful adulterants in food and drink, a concern since antiquity [12]. Economic adulteration, notably in the form of synthetic chemicals in milk, jeopardizes consumer health. Addressing this issue is critical for ensuring food safety and consumer trust [11,13,14].

Palmyrah toddy has become a significant resource in Sri Lanka's Northeast, providing substantial income for families [15]. It offers health benefits, being rich in nutrients like vitamin B complex and lauric acid, with potential to alleviate cold and flu symptoms [15, 16]. Concerns about adulterated toddy, artificially produced from sugar solution, necessitate stringent quality control measures.

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Recent studies focus on detecting adulterants in toddy using chemical tests, with sulphated ash concentration being a key indicator of authenticity. Ash values provide insights into a food's mineral content, crucial for determining its quality, authenticity, and purity. Addressing adulteration in toddy production is essential to safeguard consumer health and uphold product integrity [17].

The adulterants often have the same chemical compositions. The detection of adulteration is challenging because the chemical compositions are comparable [18]. However, there are a number of ways to identify and classify adulterations. Samples that are suspected could be selectively screened and assessed using more exacting techniques like carbon isotope ratio analysis [19]. The authentication procedure makes use of highly advanced analytical techniques like GC-MS, HPLC, GC, IR-MS, NMR, and DNA-based approaches [20]. While these methods based on GC-MS, HPLC, GC, IR-MS, NMR spectroscopy, and carbon isotope methodology have been published, those based on spectroscopic techniques have a lot to offer because of how simple, quick, and inexpensive they are to employ [21]. Modern instrumental analysis's high spectrum signal-to-noise ratio makes it possible to detect constituents at incredibly low concentrations as well as minor compositional variations between and within specimens with multiple elements [22].

Near-infrared spectroscopy (NIRS) is a technique used in analytical chemistry and various other fields to analyze the molecular composition of substances. It operates by measuring the absorption of near-infrared light by molecules. Near-infrared light has wavelengths longer than visible light but shorter than mid-infrared radiation. In NIRS, a sample is exposed to near-infrared light, and the light that passes through or is reflected off the sample is analyzed. Different molecules absorb light at different wavelengths, so by measuring the absorption of light at various wavelengths, scientists can identify and quantify the different components in a sample. This technique is widely used in pharmaceuticals, food and beverage industries, agriculture, environmental monitoring, and many other fields for tasks such as quality control, process monitoring, and research.

NIRS is a high-throughput, inexpensive, solvent-free, and non-destructive analytical instrument. The transition from the visible spectral range to the mid-infrared area is covered by near-infrared spectroscopy in vibrational spectroscopy. The NIR spectral area spans from 750 to 2500 nm (12,500-4000 cm^{-1}), with absorptions primarily linked to functional groups with the –CH, –OH, –NH, and –SH suffixes. The structure and composition of carbohydrates found in a variety of species are affected by chemical, physical, technical, or physiological processes, and these processes are frequently monitored using NIR spectroscopy [23]. This method works because the material is quickly and non-destructively analyzed without the use of chemicals. There are three regions that make up the NIR region. Region I, which covers the wavelength range of 800–1200 nm (12,500-8500 cm^{-1}) and is also referred to as "the Herschel region, the NIR region, and the short-wave NIR region, represents bands resulting from electronic transitions, overtones, and combinations modes. Stretching vibrations, various combination modes, and the first overtones of XH (X = C, O, and N) are all covered by Region II, which spans the wavelength range of 1200–1800 nm (8500-5500 cm^{-1}). Region III (1800–2500 nm or 5500-4000 cm^{-1}) is a combination mode region, and it's the last one. Regions II and III are used by many initiatives [24]. In order to improve the relevant information and lessen the impact of side information in the spectra, NIR spectra are pre-processed with mathematical treatments such as baseline correction, normalizations, derivatives, and smoothing [25]. A technique called near-infrared spectroscopy (NIRS) enables the analysis of carbohydrates in a wide range of samples. In the modern era, NIRS-chemometrics have established their efficacy in both qualitative and quantitative carbohydrate analysis.

The study of chemometrics uses statistical and mathematical techniques to interpret near-infrared spectra; it has been demonstrated that when these techniques are combined, their efficiency greatly enhances in-depth carbohydrate characterization. Principal component analysis (PCA) and partial least squares regression (PLSR) are two of the most well-known statistical techniques that can be applied to create NIR-chemometric models [25].

The method used to check the adulterate in toddy samples, should be a rapid and reliable method for screening the adulterate in toddy samples is important to avoid unwanted health issues due to consumption of alcoholic beverages. This study aims to explore the method to perform rapid screening of adulterate in toddy samples by using the NIR technique together with k-mean and hierarchical multivariant cluster analysis. Further, for comparison some chemical tests are performed to differentiate genuine toddy samples from the adulterate toddy via chemical methods.

2. Materials and methods

Location of Research work: This Research work was carried out in the analytical Laboratory at Palmyrah research Institute and Research Laboratory at Department of Chemistry, University of Jaffna.

Sample collection and preparation: Genuine fresh toddy was collected from different Palm Development Co-operative societies

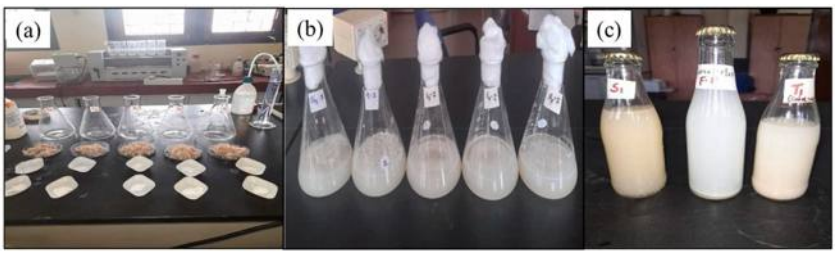

Fig. 1. Artificial toddy sample preparation by using different percentages of (a) cooked rice, (b) sugar. (c) Artificial toddy samples prepared by using sugar solution, genuine fresh toddy and Artificial toddy samples prepared by using cook rice.

in the Northern Province, Sri Lanka. Artificial toddy bottled samples were prepared at the Analytical laboratory of Palmyrah Research Institute. Samples of toddy were divided into various classes.

G.F: Genuine Fresh toddy, received from Palm Development Co-operative societies.

ATS: Artificial toddy samples prepared by using sugar solution.

ATR: Artificial toddy samples prepared by using cooked rice.

Artificial toddy preparation method by using cooked rice*: 0.5 g* NPK (Ammonium carbonate, Potassium di-hydrogen phosphate) nutrients, 1.0 g of commercial yeast, and 0.5 g of antibacterial agent (Sodium meta bi-sulphite) were added to the 500 mL of water in the conical flask (Fig. 1(a)). Cooked rice was added in different percentages such as 12 %,14 %,16 %,18 %, and 20 % as shown in Table 1 and Fig. 1. The flask was monitored daily to reach the maximum ethanol content and on the 7th day, maximum ethanol content was observed, then chemical tests were done. High adulterant cooked rice percentage of substance was taken for further analysis.

Artificial toddy preparation method by using sugar solution*: 0.5 g* NPK nutrients (Ammonium carbonate, Potassium di-hydrogen phosphate), 1.0 g commercial yeast, and 0.5 g of antibacterial agent (Sodium meta bi-sulphite) were added to the 16 % sugar solution in a conical flask. The flask was left for fermentation and when maximum ethanol content was obtained (9th Day), then chemical tests were conducted.

Then artificial toddy was prepared by using different amounts of sugar for chemical analysis (shown in Fig. 1(b)–(c)). The percentage of sugar used in the preparation of artificial toddy is shown in Table 1.

2.1. NIR spectra analysis

The Jasco V-570 UV/VIS/NIR spectrometer was used to measure the NIR absorbance spectra between 900 and 1300 nm at ambient temperature. The spectra were repeated three times, and the average of each NIR absorbance spectrum was then recorded. Using the Spectra Manager version 1.53, (Jasco Inc.), the average NIR absorbance spectra were exported as ASCII files.

2.2. Chemical analysis tests

Quantification of Phosphorous: A test tube containing 10 ml of ash filtrate was filled with 10 ml of 6 N HNO_3, 10 ml of 0.25 % ammonium monovanadate, and 10 ml of 5 % ammonium molybdate. After that, deionized water was used to bring the total volume to 100 ml. The solution's absorbance was then determined at 400 nm using a spectrophotometer. Other samples were treated in the same way. Using the phosphorus standard curve, the samples' phosphorous content was calculated [26].

Quantification of Na and K Content: Standard solutions of sodium and potassium for the calibration of flame photometry were prepared by serial dilution of chloride salt solutions of the respective metals. Each sample weighed 10.0 g, and they were burned to ash for 3 h at 600 °C in a muffle furnace. The ash was then dissolved in 10 ml of a solution of 2 M HNO_3, the solution was filtered, and the filtrate was then put into a volumetric flask with a capacity of 50 ml. The investigation of Na and K by flame spectroscopy was then performed using this solution.

Determination of Ethanol Content: Alcohol content was determined directly using Ebulliometer Dujardin-Salleron for each toddy sample at ambient temperature.

Determination of Total Ash: The clean crucibles were heated at 105 °C for 30 min in the oven. Then transferred into a desiccator to cool them. They were labelled and weighed. 5 g of each sample was weighed in the crucibles. The crucibles containing samples were heated over Bunsen flames until no more fumes were evolved. Then they were heated in a muffle furnace for 5 h at 550 °C. The crucibles were then transferred to the desiccator, cooled, and weighed [27].

Determination of Sulphated Ash: The sulphated ash concentration was determined by pipetting 10 ml of toddy sample into a silica crucible and igniting it in an electric furnace until the sample became charred. Then, 1.00 mL of H_2SO_4 was added, and the mixture was gradually heated until white fumes were no longer emitted and ignited at 500^{o}C. After a few drops of H_2SO_4 were added to the sample upon heating, the crucible was placed in the muffle furnace after cooling. The sample was ignited, and then allowed to cool before

Table 1
Artificial toddy samples prepared by using different percentage sugar and cooked rice. The samples, labelled as S1–S5, were prepared using varying amounts of sugar adulterant, while the samples labelled T1-T5 were prepared using different quantities of cooked rice.

Sample ID	Adulterant	Amount of adulterant (g/200 mL)	Percentage (w/v) %
G.F	None	0.0	0
S1	Sugar	16.0	8
S2	Sugar	20.0	10
S3	Sugar	24.0	12
S4	Sugar	28.0	14
S5	Sugar	32.0	16
T5	Cooked rice	24.0	12
T4	Cooked rice	28.0	14
T3	Cooked rice	32.0	16
T2	Cooked rice	36.0	18
T1	Cooked rice	40.0	20

being weighed. This process was continued as mentioned above until the sample's weight became constant [17].

Determination of the Total Acidity as Acetic Acid: An accurately weighed sample of sodium phthalate is used for the NaOH standardization by titration. The acidity of the toddy sample was assessed by titration with a standardized solution of 0.1 M sodium hydroxide using phenolphthalein as an indicator, and the results were expressed as acetic acid (%, w/v) content (AOAC, 2010a). Calculations were done to determine the amount of acetic acid in total acids.

Determination of Moisture Content: Five (05) g of the sample that was weighed to the closest milligram was taken in a moisture dish that had been previously dried and weighed. The dish with the lid was left unattended for 3 h while the side dried. As soon as the dish cooled, it was covered, moved to desiccators, and rapidly weighed (Official method of Analysis of AOAC International (2019) 21st edition.) Then the moisture content was calculated as follows;

$$\text{Moisture}\ (\%) = (M_1 - M_2)\ \text{x}100\ /\ (M_1 - M_0)$$

(Mo = weight of empty dish; M_1 = weight of fresh sample + dish; M_2 = weight of dried sample + dish)

Determination of Total Soluble Solids (TSS): A Refractometer was used to measure the sample's total soluble solids directly, and its results were stated in Brix [28].

Determination of conductivity: An Orion 4 Stars conductometer was used to assess conductivity. Tequila was poured over the sensor, and the conductivity was measured three times. All experiments were carried out at ambient temperature [29].

Determination of pH*:* After carefully pouring 10 mL of each sample into a clean beaker, the pH of the Palmyrah toddy samples was measured with a pH meter (Hanna Instruments, Switzerland) [30].

Determination of Brix value*:* Dipped hole was washed with distilled water and calibrated with distilled water. Then it was set to zero. Then a sample was put into the sample holding hole readings were got from the Brix meter desktop screen.

3. Results and discussion

Chemical analysis can provide valuable insights into differentiating genuine toddy from artificial toddy. Chemical analysis of genuine toddy would reveal the presence of natural compounds found in palm sap, such as sugars (glucose, fructose), organic acids (acetic acid, lactic acid), amino acids, vitamins, minerals, and ethanol resulting from fermentation. These compounds would be present in concentrations consistent with natural fermentation processes. Chemical analysis of artificial toddy may reveal the presence of synthetic additives such as artificial sweeteners, flavourings, stabilizers, and preservatives. These compounds would not be found in genuine toddy or would be present in concentrations inconsistent with natural fermentation processes. pH, acidity, total ash, total sulphated ash, sodium, potassium, phosphorous, ethanol content and conductivity of G.F, ATS, and ATR were analyzed and classified by using k-means multivariate statistical cluster analysis.

The fermented sap known as palm toddy is whitish has a pH of around 3.6, and contains 3.3–4.0 % alcohol in the pH and alcohol contents depending on several factors [31]. The pH level of palmyrah toddy depends on the environmental conditions in which it is collected, the stage of fermentation, and adulterants, etc [32]. The acidity of palmyrah toddy primarily arises via a natural fermentation process in which the sugar content in sap converts into alcohol and carbon dioxide in the presence of yeast and produces organic acids as byproducts. These organic acids, such as acetic acid, lactic acid, and others, contribute to the overall acidity of the toddy. The pH level of palmyra toddy can affect its taste and shelf life. A mildly acidic pH is typical for fresh toddy and contributes to its unique flavour profile. However, the continuation of fermentation of toddy may lead to higher acidity and affect the taste and aroma of toddy.

Fig. 2 shows that both genuine toddy samples and artificial toddy samples are mildly acidic nature and have a pH of about 3.75 to 3.5. The acidity measures the total number of hydrogen ions and the pH measures the concentration of free hydrogen ions in the solution. One would be tempted to believe there is a connection between the pH and the acidity of acetic acid in alcoholic beverages based on these definitions. However, there is no clear or consistent link between pH and acidity, and different juices with varied pH

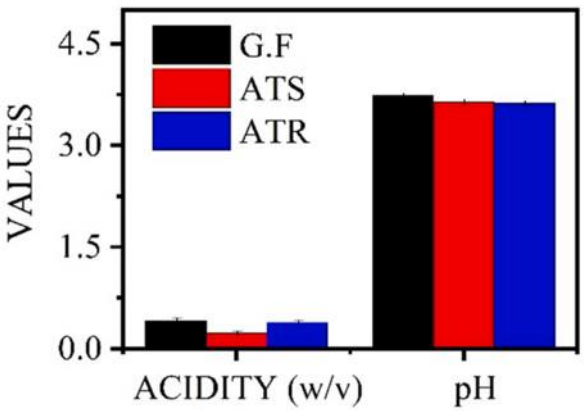

Fig. 2. Acidity and pH of genuine Fresh toddy, received from Palm Development Co-operative societies (G.F), artificial toddy samples prepared by using sugar solution (ATS), artificial toddy samples prepared by using cooked rice (ATR).

values can appear to have the same amount of acidity. The pH is affected by an acid's capacity to dissociate rather than its concentration, which is unrelated to it. The fermentation process results in the production of organic acid as a result of hydrolysis, biochemical reactions, and microbial metabolism. Although the presence of organic acids inhibits or reduces the growth of powerful foodborne pathogens during fermentation, it has an impact on the final product's color, aroma, and stability [33]. Fig. 2, illustrates that real fresh toddy has greater pH and acidity values than artificial toddy, which is adulterated with sugar and cooked rice. The total acid content of a genuine palmyrah toddy beverage sample ranged from 0.40 to 0.50 g acetic acid/L, while that of an artificial toddy ranged from 0.23 to 0.38 g acetic acid/L. The variations in the acid content and pH of the beverages mainly depend on the usage of different ingredients and substrates, and the differences in the conditions of fermentation of beverages. However, there is no significant difference in acidity and pH parameters between the artificial toddy from the genuine toddy.

The amount of inorganic components or minerals in a palmyrah toddy is indicated by the ash content which indicates the presence of inorganic matters as impurity. The inorganic matter is typically only present in trace amounts, is not harmful when present in small amounts, and is challenging to remove during the purifying process. High ash content demonstrates its unsuitability. Fig. 3 shows that genuine fresh toddy has ash contents of 0.200. When compared with artificial toddy samples, they show a relatively higher value than genuine toddy samples because of the addition of external mineral containing chemicals that function as nutrients. The primary purpose of the sulphated ash test is to quantify the amount of inorganic materials present in organic compounds. The larger association between sulphated ash content and the lower coefficient of variation (% Cv) is explained by the greater amount of sulphated ash [34]. Pure palmyrah jaggery samples which are made from sweet sap had a sulphated ash concentration in the range of 3.65 to 3.28 %, likewise, toddy is collected after fermentation of palmyrah sweet sap.

In contrast, the artificial toddy has lower sulphated ash content compared to fresh toddy. This means that there are fewer sulphate minerals present in the artificial version. These differences may be due to the composition of the ingredients used in making the artificial toddy, with the sugar likely contributing to the higher total ash content, while the fresh toddy might naturally contain more sulphates.

Fig. 4 illustrates the data on mineral contents (Na, K, P) in the genuine toddy samples and artificial toddy samples. Artificial toddy samples show more mineral content compared to genuine toddy samples. It may be due to the addition of chemicals from external sources as nutrients, and it is further supported by measurements of ash content.

The fermentation of the rice, an average of 10.5–13.5 % of ethanol was produced. Saccharomyces from Nigerian palm wine produced ethanol with a similar concentration of 12.2 %, which was observed for Sake-type fermentation [35]. These ethanol concentrations, however, were less than those made in Japan using industrial Sake yeast strains, where the ethanol percentage was estimated to be between 17 and 19 % [36]. The variations in the ability of yeast strains to ferment could be the cause of the ethanol levels. Furthermore, the maximum amount of ethanol in a fermentation medium may be determined by the varying alcohol tolerance levels of various strains, as shown by the absence of viable yeasts in the fermented liquid [37]. The higher conductivity observed in artificial toddy compared to fresh toddy suggests that artificial toddy is more conductive (shown in Fig. 5(a)). Conductivity is a measure of a substance's ability to conduct an electric current, and it is influenced by the presence of ions or charged particles in the solution. The higher conductivity could be attributed to a higher concentration of ions (charged particles) in the artificial toddy. This might be due to the ingredients used in making the artificial toddy, such as sugar or other additives, which can contribute to increased ionic content.

It is evident from Fig. 5(b) that sugar was used to make artificial toddy samples. Similar evolution patterns were seen for ethanol concentration throughout the fermentation process; more specifically, all concentrations steadily climbed from the start of the fermentation to a specified day, when they reached their maximum value. This rise in ethanol concentration is a result of primarily yeast-mediated alcoholic fermentation. The fermentation progressively transitioned into the stage that produces acid after reaching its maximum ethanol concentration. When they have reached their peak, the ethanol content will gradually decrease. This significant

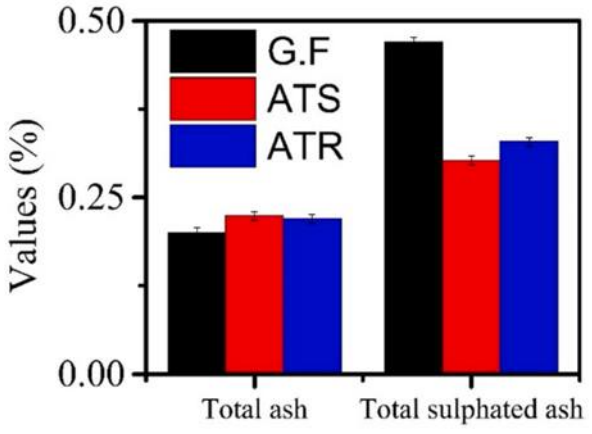

Fig. 3. Total ash and Total sulphated ash of genuine Fresh toddy, received from Palm Development Co-operative societies (G.F), artificial toddy samples prepared by using sugar solution (ATS), artificial toddy samples prepared by using cooked rice (ATR).

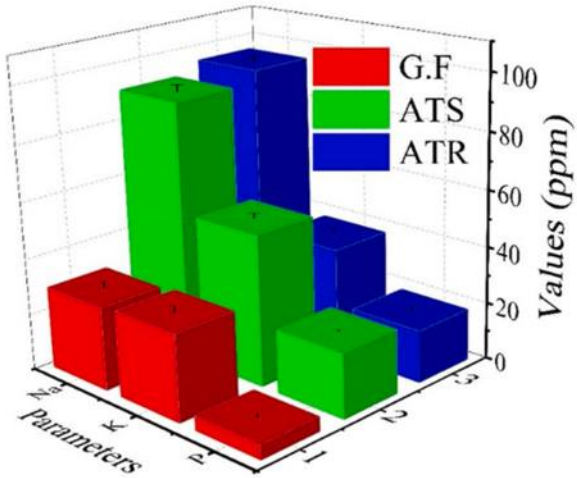

Fig. 4. Mineral contents (Na, K, P) of genuine Fresh toddy, received from Palm Development Co-operative societies (G.F), artificial toddy samples prepared by using sugar solution (ATS), artificial toddy samples prepared by using cooked rice (ATR).

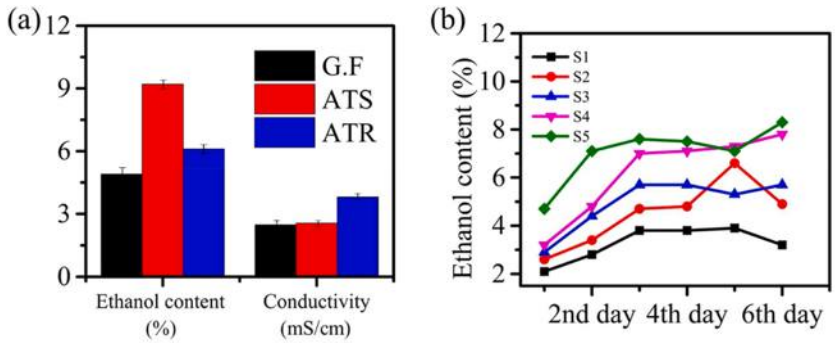

Fig. 5. (a) Conductivity and ethanol content of genuine Fresh toddy, received from Palm Development Co-operative societies (G.F), artificial toddy samples prepared by using sugar solution (ATS), artificial toddy samples prepared by using cooked rice (ATR). (b) Ethanol content of artificial toddy samples prepared by using sugar solution (ATS) prepared with different percentage sugar.

decline may be caused by esterification events taking place, and/or by some microorganisms using fatty acids in the process of producing ethanol [38].

The samples' Brix values were examined to establish the samples' sugar content. Brix content and acidity gradually rise with

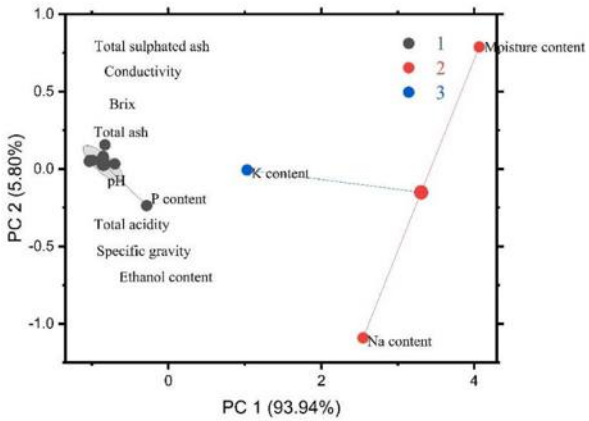

Fig. 6. k-means multivariate statistical cluster analysis of G.F, ATR and ATS based on measured chemical parameters.

increasing sugar content. Even though the acidity is rising, there are no discernible changes because it ranges between 0.0 and 0.5. Brix content is increased from S1 to S5 due to the increment in the addition of sugar to the artificial samples.

3.1. Cluster analysis based on chemical parameters of G.F, ATS and ATR

In a k-means multivariate statistical cluster analysis of chemical parameters (shown in Fig. 6) of fresh toddy and artificial toddy samples which was prepared by using sugar and rice with Principal Component (PC1) explaining 93.94 % of the variance and PC2 explaining 5.80 %, it indicates that most of the variance in the data is captured by PC1. This suggests that the primary differences between the samples are related to the patterns along PC1. PC2, although contributing some information, has less influence on the clustering. In the figure, there are three group sets of chemical parameters and it reflects that sodium, potassium, phosphorous, and moisture contents are much higher discrimination factors and the G.F toddy samples could be identified or separated based on such above parameters. Further the ethanol content much higher in ATS and ATR toddy samples than G.F toddy sample. Based on the chemical analysis of G.F toddy samples and ATS and ATR, and the k-means multivariate statistical cluster analysis the adulterated toddy samples could be identified. Although rather than performing all the chemical analysis, k-means multivariate statistical cluster analysis (shown in Fig. 7(c)) and hierarchical cluster plot (shown in Fig. 7(b)) of NIR data (shown in Fig. 7(a)) provide useful information to distinguish the G.F toddy samples and ATS and ATR toddy samples.

Further, screening of G.F from ATS and ATR was simply performed by using NIRS analysis together with k-means multivariate statistical cluster analysis. Characteristic peaks corresponding to different structures and different composition of sugars and metabolites.

NIR records the information of multiple frequencies and co-frequency of fundamental frequency vibration of a molecular single chemical bond, which is always dominated by an overlap of the multiple frequencies and co-frequency of groups containing hydrogen (such as C–H, O–H, N–H, S–H, and P–H). NIRS is primarily due to the anharmonicity of the molecular vibrations from the ground state to a higher energy level transition.

In Fig. 7, two wide absorption peaks can be seen in this NIR spectrum, one centered between 950 and 1000 nm and the other between 1150 and 1250 nm. The spectrum observed between 950 nm and 1000 nm due to presence of sucrose and starch in the toddy sample. The samples that were interfered with cooked rice and sugar exhibit noticeably higher absorption in the first region than the genuine toddy. Theanjumpol et al. [39] also noted that sucrose and starch absorb NIR radiation between 900 and 1000 nm. Ahmad Fairuz Omar et al. says, that absorption in-between 900 nm and 1000 nm, peak around 960 nm is due to the second overtone of the O–H stretching band [40]. Due to the various quantities of O–H groups that are present in sugars, the NIR absorption intensity of peaks varied. The small peak shift may be due to the availability of different structures of sugar such as glucose, sucrose, and other structures. Maria Lucia F. Simeone says NIR spectra demonstrated a correlation between the vibration bands for the O–H and C–H groups and the sugar constituents [41]. Although sugars have similar structures and share similar NIR absorption peaks. They can be distinguished by their absorption magnitude because they have different numbers of O–H groups and the intermolecular hydrogen bonds have a slight effect on the positions of the O–H and C–H absorption bands [42]. A slight variation of NIR absorption peaks may be attributed to several other factors including harvest time of toddy, geographical origin, and environmental condition as well [18]. Therefore, the comparing the absorption peaks and overtones available in NIR spectroscopic data at different wavelength may be used to distinguish the adulterations from the pure samples. Further, the genuine fresh toddy and adulterated with rice and sugar may consisting different compositions and metabolites [18]. k-means multivariate statistical cluster analysis and Hierarchical cluster multivariant analysis of NIR spectroscopic data were performed to the authenticity of different toddy samples obtained from different sources of adulteration and genuine toddy. Clustering is applied for a full set of NIR spectroscopic data to find out possible clusters and groups based on the peaks in NIR spectroscopy to quickly assess the authenticity of available toddy samples.

The hierarchical cluster plot shown in Fig. 7(b) and k-Means cluster analysis (Fig. 7(c) of NIR spectroscopic data shows three differentiate groups such as genuine and artificial toddy samples. $K = 3$ applied in k-mean cluster analysis and PC1, PC2 and PC3 shows 91.82 %, 6.60 % and 1.58 %. Owing to PC1, and PC2 covering almost 98.42 % of the composition in the sample, the k-means multivariate statistical cluster analysis performed only with PC1, and PC2. Three groups of clusters were identified and cluster plot with confidence eclipsed was drawn to separate the cluster. Separation of three clusters in k-mean cluster analysis gave the authenticity for genuine and adulterated toddy with different substrates.

Both k-means multivariate statistical cluster analysis of measured chemical parameters and K-means clustering of NIR spectroscopic data of G.F, ATR, and ATS are useful methods to distinguish the genuine fresh toddy (G.F) and artificial toddy (ATR and ATS). However, performing the screening of G.F, ATR, and ATS based on NIR spectroscopic analysis is a more rapid process than performing all the chemical analyses and is used to rapidly screen the genuine fresh toddy (G.F) in commercial applications.

4. Conclusion

This research illustrates the quick reliable screening method to distinguish the G.F, ATR, and ATS. Based on the chemical analysis sodium, potassium, and phosphorous contents are much higher discrimination factors, and the G.F, ATR, and ATS could be separated based on the above parameters. Further, G.F, ATR, and ATS are screened based on the NIR spectroscopic data together with Hierarchical cluster and k-means multivariate statistical cluster analysis. The plot of k-means cluster analysis and hierarchical cluster analysis of NIRS data shows three distinct clusters. Compared to chemical analysis, NIR spectroscopic analysis together with statistical analysis is a quick and reliable method and no sample preparation is required as follows in conventional chemical methods. A swift and precise screening of adulterants in toddy samples helps prevent potential health risks associated with consuming authentic toddy.

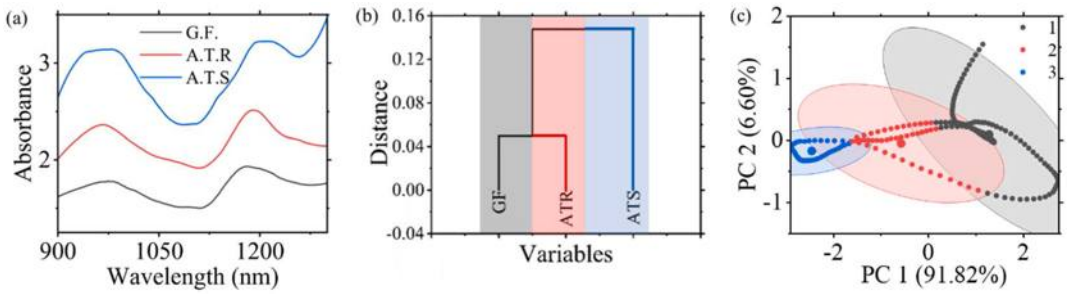

Fig. 7. **(a)** Smoothened NIR absorption spectra of genuine toddy (Black line), artificial toddy sample adulterated with cooked rice (ATR) (Red line) and artificial toddy sample adulterated with sugar solution (ATS) (Blue line) (b) hierarchical cluster plot of G.F, ATR and ATS. (c) k-means multivariate statistical cluster analysis of NIR spectroscopic data of G.F, ATR and ATS.

However, further work needs to analyze the toddy samples obtained from different sources, different locations, and together with a huge data set to find out the limitations of this method.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Sivatharshan Sivakumar: Formal analysis, Data curation. **Srivijeindran Srithayalan:** Resources, Project administration, Funding acquisition. **Kirushanthi Arunraj:** Writing – review & editing, Data curation. **Manjceevan Arumukham:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Data curation, Conceptualization. **Sashikesh Ganeshalingam:** Writing – review & editing, Supervision, Conceptualization. **Velauthamurthy Kugamoorthy:** Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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