

# Collection methods to preserve nutritive and physicochemical properties of unfermented coconut (*Cocos nucifera*) sap

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## Abstract

Quality evaluation of unfermented coconut (*Cocos nucifera*) sap was done using two sap collection methods involving application of Hal bark (*Vateria acuminata*) to the clay pots of 4L (TM) and novel sap collection method (NSCM), during 12 hours (from 6 pm to 6 am) collection period. The novel sap collecting device is a recent innovation by the Coconut Research Institute (CRI) – Sri Lanka. Twelve tapped coconut palms (CRIC 65, 45 years old) were used and filtered sap was stored (-18°C). Volume, pH, total soluble solids, alcohol content, total acidity, color, sugar profile, total phenols, EC50, ascorbic acid content and mineral in two types of unfermented coconut sap samples were determined. Yield of coconut sap (1569 ml) and alcohol content (zero) of TM and NSCM did not change significantly with the collection system. The results revealed that coconut sap collected from NSCM has a significantly higher pH (5.99), sucrose (13.71%), total sugar (19.99%) compared to the collected sap from TM. In contrast, the sap of TM method was significantly rich with total phenolic (65.90 mg GAE/100ml), EC50 (143.03 mg/ml), AEAC (0.2568 mg Vit C in 1g sample), browning index (6.76) and yellowing index (15.92). Moreover, Ca (39.3 mg/L), Fe (3.08 mg/L), Mn (0.96 mg/L), Sr (0.14 mg/L), Ba (0.33 mg/L) were significantly high in TM. Hence, the novel sap collection method can be concluded as the best approach for collecting quality unfermented coconut sap with its natural quality.

Keywords: coconut sap, sap collection, sugar profile, *Vateria acuminata*

## Introduction

The coconut tree, scientifically known as *Cocos nucifera* is a significant crop grown on plantations and has many different uses within tropical areas. Out of diverse utility of coconut tree, coconut sap which is obtained from coconut inflorescence is regarded as one of the major outcomes. Coconut sap-based sugar is a natural healthy sweetener that is produced from the unfermented coconut sap obtained from coconut inflorescence. The nectar which runs through phloem vessel is taken outside through special art of tapping and collected to clay pots during 6 hr - 12 hr time. The freshly oozed unfermented coconut sap (*meera* or *sweet toddy* - Sri Lanka/ *Neera* – India) is sweet oyster white (Xia et al., 2011) liquid and it is a rich source of sucrose (12%-18%) (Nathanael et al., 1960). It is fat free (0.02%) nectar with 0.23% of protein and it contains mineral such as Na and K (Barh and Mazumdar 2008). Total phenolic

content and total flavonoids content of unfermented coconut sap was identified as 21.7±0.48 mg GAE and 0.817±0.19 mg CE respectively (Hebbar et al., 2020). High nutrition composition of unfermented coconut sap increases the demand for value addition to sap based products such as coconut sugar, coconut jaggery, coconut treacle. Several microbes in atmosphere ferment sugars in *sweet toddy* into alcohol. The fermented coconut sap ("*Raa*") is a popular alcoholic beverage among rural South Asian people.

To protect the quality attributes of coconut sap, several preventive measures are used from ancient time such as application of lime (Ca (OH)<sub>2</sub>), bark of Hal tree (*Vateria acuminata*), peel of Mangosteen (*Garcinia mangostana* L.), bark of Kahata (*Careya arborea* roxb.) into the burned clay pots (Ranganathan et al., 2015). Moreover, synthetic preservatives such as sodium metabisulfites are also added to the clay pot to prevent the fermentation process. Addition of

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anti-fermenting agents preserves the sap from fermentation while reducing natural quality and purity of unfermented coconut sap (Samarajeeva et al., 1985). The collected sap using these means has negative impact of odour, taste and color. In addition, the traditionally collected sap is generally contaminated by insects, ants, dust particles and pollen due to the gap between clay pot and coconut inflorescence. Therefore, collection of unfermented coconut sap with its pure form is a challenging task. As a solution, a novel sap collecting device was introduced by Coconut Research Institute - Sri Lanka to reduce the fermentation by creating sealed environment with an ice box (Figure 1). Evaluation of the quality of coconut sap collected from new method is very essential to popularize it among coconut tappers.

In the field of traditional ayurvedic medicine, jaggery has been used as a natural calming agent since ancient times. Jaggery, which is a good source of iron, can help in reducing anemia (Sahu and Saxena, 1994). It has ability of cleaning the blood, lungs, stomach, intestine, respiratory track and curing ability against asthma, cough and congestion. Jaggery reduces the lungs damages created by coal, silica and dusts (Sahu and Saxena, 1994).

The aim of this research is to assess and contrast the physical, chemical, and nutritional characteristics of coconut sap obtained from a new sap collection device and the traditional way of sap collection.

## MATERIALS AND METHODS

The study was conducted at the Coconut Research Institute, Lunuwila, Sri Lanka. Unfermented coconut sap was collected from twelve different coconut palms (CRIC 65, 45 years old) at Bandirippuwa Estate. Clay pots and barks of *Vateria acuminata* ("hal"-Sinhala) were purchased from local stores. The novel sap collection device was fabricated at Coconut Research Institute, Lunuwila. It (Figure 1) is comprised of fixing unit (01), pipe connector (02), flexible hose for translocation of sap (03) and cooling compartment with collection can (04).

### Sample collection

Coconut sap was collected from April 2020 to July 2020. The selected 12 palm spadix were prepared for tapping by an experienced tapper for 21 days prior to collection of the coconut sap. To start the sap collection, 1-2 mm of slice at the top end of coconut inflorescence was removed using a special knife and the sap was collected using two treatments; Treatment 1: Traditional method (TM) - application of hal bark to the previously pre-sterilized clay pots of 4L and Treatment 2: Novel sap collection method (NSCM) (Figure 2). Unfermented coconut sap was collected during 12 hours intervals (from 6 pm to 6 am).

### Data Collection

The collected sap was filtered through a cotton cloth and volume of coconut sap and pH was measured at 25°C

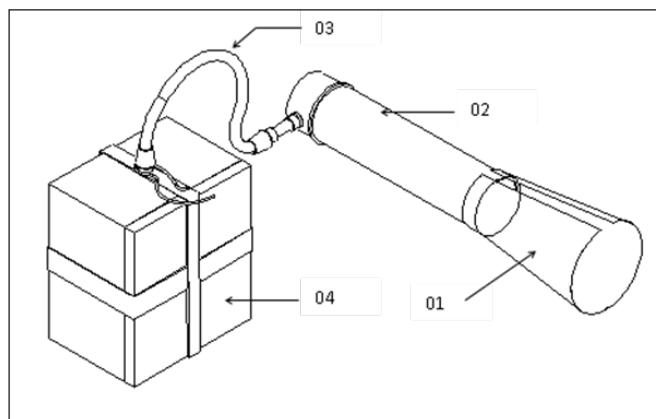


Figure 1. Diagram of novel sap collection device



Figure 2. Sap collection using TM and NSCM

temperature using a pH meter (Hach HQ40d). Total soluble solids (TSS) were measured using a manual refractometer (ATAGO-IE, JAPAN) with 0 % - 35 % measuring range. The alcohol content was measured using the ebulliometer (Dujardin-Salleron Model no: 360) and samples were stored at freezer (-18°C).

### Determination of total acidity

A volume of 10.0 mL from each sample was titrated with 0.01 M NaOH in the presence of phenolphthalein as an

indicator. Acidity was expressed as gram lactic acid per 100 ml of the sample (Li et al., 2011).

#### Color

Colors of samples were assessed visually. The color of the coconut sap sample was analyzed using Chroma Meter (B8205231, Japan) and the data were expressed based on the CIELAB scale in terms of L\* (black to white), a\* (green to red) and b\* (blue to yellow) values. Chroma (C\*) is measured by the colorfulness of the sample using  $(a^{*2} + b^{*2})^{1/2}$  equation (Granato and Masson, 2010). Browning Index (BI) and Yellowing Index (YI) of samples were determined according to the following equations (Subhashree et al., 2017).

$$BI = \frac{100(x - 0.31)}{0.17} \quad \text{Where } x \text{ is } x = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*}$$

$$YI = \frac{142.86 b^*}{L^*}$$

#### Sugar Profile

Coconut sap samples were diluted 100 times with Milli-Q water and filtered through a 0.45  $\mu\text{m}$  nylon syringe filter and 20  $\mu\text{l}$  was injected manually. The calibration curves for sucrose, glucose and fructose were constructed using the standard sugar solution from 0.05% to 2%. Sugar profile was analyzed using High Performance Liquid Chromatography (HPLC-Agilent1100) using a Waters™ Sugar-Pak™ column at 90°C, the sample was eluted with Milli-Q water at 0.5 ml/min flow rate and detected through RID detector (Agilent 1100). The concentration of each sugar type was determined using calibrated curves. Total sugar was measured by sums of sugar % in the sample.

#### Total phenolic content

Total phenolic content of the coconut sap samples was determined using Folin Ciocalteu's Regent (FCR) method with reference to Gallic acid standard. Ten times diluted samples (1 ml) were mixed with 5 ml of 10 % Folin Ciocalteu's reagent and kept in dark for 3 minutes. Then 4 ml of 7.5%  $\text{Na}_2\text{CO}_3$  solution was added and kept in dark in 30 min. One milliliter of distilled water was used as blank sample. The absorbance was measured by UV/VIS spectrophotometer (UV 1800 SHIMADZU) at 765 nm wave length against the Gallic acid standard curve (Odunola et al., 2015).

#### Antioxidant Activity by DPPH method

The DPPH scavenging activity of the coconut sap sample was determined using the method Phisut and Jirapron (2013) with some modifications. One milliliter of sample was mixed with 3.9 ml of 0.2 mM (DPPH) and mixed gently for 1min and kept in dark for 60 min. Milli-Q water (1 ml) was used as a control sample. Absorbance of each sample were measured at 517 nm using a UV spectrophotometer (UV 1800 SHIMADZU) with the blank sample of Milli-Q water and scavenging activity of each sample calculated by following equation. The Ascorbic acid concentration (0-50 ppm) series were prepared with Milli-Q water as a positive standard for antioxidant activity.

$$\text{Scavenging Activity} = \frac{\text{Abs (Control)} - \text{Abs (Sample)}}{\text{Abs (Control)}} \times 100$$

Where, Abs (Control) = Absorbance of solvent with Milli-Q water

Abs (Sample) = Absorbance of solvent with sample or standard

EC 50% value of graph was obtained by plotting the percentage of scavenging activity (Y) against the sample concentration (ppm) in (X). The regression line was built using  $(Y = MX+C, y = mx + C)$ . Calculation of mg of Ascorbic Equivalent Antioxidant capacity (AEAC) in g of sample is obtained by following equation (Odunola et al., 2015).

#### Ascorbic Acid content

Ascorbic acid content of two types of unfermented coconut sap samples were analyzed using 2,6-Dichlorophenol-indophenol visual titration method. Coconut sap sample (1 ml) was mixed with 10 ml of 3%  $\text{HPO}_3$  solution for titration. The dye factor and ascorbic acid content of sample was measured using following equations (Ranganna, 1986).

$$\text{DyeFactor} = \frac{\text{Abs (Control)} - \text{Abs (Sample)}}{\text{Abs (Control)}}$$

$$\text{Ascorbic acid content} \left( \frac{\text{mg}}{100 \text{ ml}} \right) = \frac{\text{titration Volume (ml)} \times \text{Volume Made up} \times 100}{\text{Sample volume for titration} \times \text{Sample volume for estimation}}$$

#### Macro elements and Micro elements

Macro elements (Na, K, Ca, Mg,) content of the samples was measured using Atomic Absorption Spectrophotometer (AAS) after acid digestion. Micro elements (Cd, Cr, Co, As, Ag, Be, Bi, Ga, In, Mo, Pb and V Fe, Zn, Mn, Cu, Sr, Ba, Ni) analysis was done by Inductive Coupled Plasma Mass Spectrophotometry (ICPMS) after microwave digestion.

#### Data Analysis

The significant variations of two samples with eight replicates were analyzed through t-test using Mini Tab 16 software after testing normality. Antioxidant activity was analyzed by ANOVA with the positive standard of Ascorbic acid. The mean separation of this test was done by Tukey's test.

## RESULTS AND DISCUSSION

#### Yield of coconut sap (Volume)

The yield of coconut sap depends on several factors such as environmental factors, physiological features of coconut palm and tapping skills. Yield of coconut sap of TM ( $1571 \pm 337$  ml) and NSCM ( $1568 \pm 308$  ml) did not change significantly with the collection system). A similar result was reported by Jananadevan in 2013 as 1.5 L of coconut sap has been reported per day in Sri Lanka. Therefore, the NSCM did not affect to



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the sap oozing volume although it creates a sealed condition between coconut inflorescence and NSCM.

### pH value of coconut sap

Significantly ( $p < 0.05$ ) higher pH value ( $5.99 \pm 0.71$ ) was observed in sap collected from novel collection method with its pure form. Ramalakshmi et al., (2018) reported that the pH value of unfermented coconut sap changed from 7.3 to 3.6 during 12 hr of natural fermentation process. That means the pH of sap can be decreased to 3.6 in the absence of preservatives. However, *Vateria acuminata* bark has elicited an ability of preserving the pH at  $5.34 \pm 0.13$  when it used for the unfermented coconut sap collection through traditional method. The antimicrobial activity of *Vateria acuminata* bark is the main reason for coconut sap preservation (Hewa Pathirana et al., 2018). According to the results, that NSCM produces sap having higher pH is more suitable for products like table sugar.

### Total soluble solids (TSS)

The total soluble solid content of coconut sap obtained from TM ( $16.55 \pm 1.30$  brix) and NSCM ( $16.15 \pm 0.44$ ) were not significantly ( $p > 0.05$ ) different. Total soluble solids of sap collected from new device developed by Central Plantation Crops Research Institute (CPCRI) in India reported as 15.5 to 18 brix while the traditional concepts reported 13 to 14 brix (Augustine and Hebbar et al., 2014). The brix of sap collected from the two methods of the present study were within the range of TSS of sap collected from new device of CPCRI.

### Alcohol content and Total acidity

The sap produced from both methods showed zero concentration of alcohol. Fermentation of sap may be within an initial stage of lactic acid fermentation. The acid compounds such as lactic, acetic, tartaric, and malic and citric acid are accumulated during the fermentation process (Samarajeeva et al., 1985). As a result of zero fermentation, acidity of NSCM ( $0.12 \pm 0.04$  g/100 ml) and TM ( $0.10 \pm 0.04$  g/100 ml) were very low and do not show a significant difference. Therefore, the sap collected from both methods can be used to collect unfermented coconut sap.

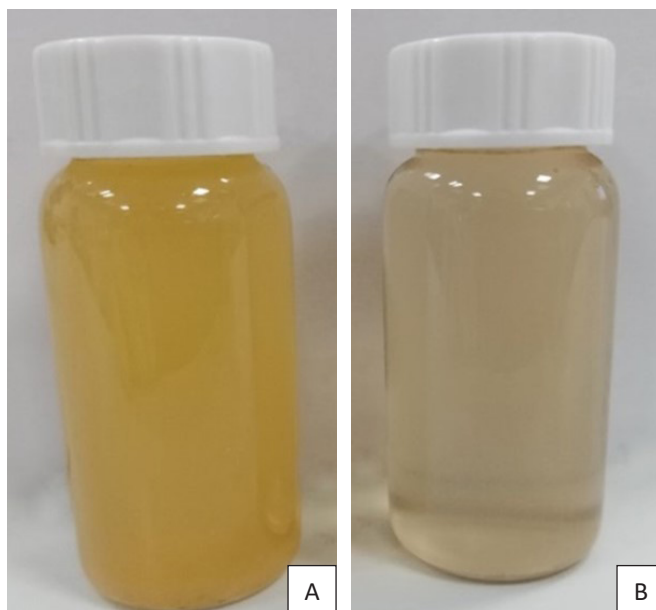


Figure 3. Sap with TM and NSCM

### Color

Visual observations of sap collected from the two methods are shown in Figure 03. The unfermented coconut sap collected from traditional method was brownish yellow while fresh coconut sap collected from novel sap collection method was oyster white in color. The difference might be due to the addition of *Vateria acuminata* bark to the collection pot in TM.

Visual observation is further confirmed by the results given in Table 1. The sap collected from NSCM is significantly ( $p < 0.05$ ) lighter compared to the TM due to it having phenolic compounds of hal bark. A previous study has reported that, 5.2 pH unfermented coconut sap had  $38.16 \pm 0.63$  lightness (Asgar et al., 2019) using a handheld portable colorimeter which is higher than the results of the current study. During the fermentation process the white color of sample has increased. Therefore, sample collected from NSCM can be concluded as less fermented than the one from TM.

The significant feature of coconut sap collected from TM was higher BI and YI (Table 1). Accumulation of *Vateria acuminata* bark constituents to the coconut sap has changed the natural color of the coconut sap and it gives a negative effect especially in beverage processing.

Table 1. Color parameters for coconut sap in two methods

Treatment	L*	a*	b*	C*	H*	YI	BI
TM	$21.55 \pm 1.94^b$	$-0.34 \pm 0.10^b$	$2.35 \pm 0.38^a$	$2.38 \pm 0.37^a$	$-1.42 \pm 0.06^a$	$15.92 \pm 4.09^a$	$6.76 \pm 1.71^a$
NSCM	$24.25 \pm 1.49^a$	$-0.01 \pm 0.11^a$	$0.74 \pm 0.47^b$	$0.76 \pm 0.45^b$	$-0.39 \pm 1.44^a$	$4.51 \pm 3.15^b$	$2.26 \pm 1.52^b$

Each value in table represents the mean of eight replicates. Means with different superscripts are significantly ( $p < 0.05$ ) different from each other's along each column. TM- coconut sap collected from traditional method; NSCM- Coconut sap collected from Novel sap collection device.

Table 2. Sugar profile of two types of unfermented coconut sap

Treatment	Sucrose (%)	Glucose (%)	Fructose (%)	Total sugar (%)
TM	9.47 ± 1.03 <sup>b</sup>	4.96 ± 0.36 <sup>a</sup>	4.37 ± 0.39 <sup>a</sup>	18.78 ± 0.45 <sup>a</sup>
NSCM	13.71 ± 0.76 <sup>a</sup>	2.76 ± 0.42 <sup>b</sup>	3.53 ± 0.15 <sup>b</sup>	20.00 ± 0.70 <sup>b</sup>

Each value in table represents the mean of eight replicates. Means with different superscripts are significantly ( $p < 0.05$ ) different from each other's along each column. TM- coconut sap collected from traditional method; NSCM- Coconut sap collected from Novel sap collection device.

Table 3. Macro and micro elements in coconut sap in two collection method

Mineral	Ca (mg/L)	Mg (mg/L)	Na (mg/L)	K (mg/L)	Fe (mg/L)	Zn (mg/L)	Mn (mg/L)	Cu (mg/L)	Sr (mg/L)	Ba (mg/L)	Ni (mg/L)
TM	39.3 ± 25.1 <sup>a</sup>	54.4 ± 23.3 <sup>a</sup>	440.2 ± 99.2 <sup>a</sup>	1278 ± 177 <sup>a</sup>	3.08 ± 2.00 <sup>a</sup>	1.02 ± 0.51 <sup>a</sup>	0.96 ± 0.07 <sup>a</sup>	0.29 ± 0.029 <sup>a</sup>	0.14 ± 0.12 <sup>a</sup>	0.33 ± 0.04 <sup>a</sup>	0.03 ± 0.00 <sup>a</sup>
NSCM	5.44 ± 3.02 <sup>b</sup>	59.9 ± 12.0 <sup>a</sup>	421 ± 20.5 <sup>a</sup>	1423 ± 165 <sup>a</sup>	1.00 ± 0.22 <sup>b</sup>	0.95 ± 0.41 <sup>a</sup>	0.08 ± 0.02 <sup>b</sup>	0.28 ± 0.04 <sup>a</sup>	0.01 ± 0.00 <sup>b</sup>	0.01 ± 0.00 <sup>b</sup>	0.03 ± 0.00 <sup>a</sup>

Each value in table represents the mean values. Means with different superscripts are significantly ( $p < 0.05$ ) different from each other's along each column. TM- coconut sap collected from traditional method; NSCM- Coconut sap collected from Novel sap collection device

### Sugar Profile and Total sugar

Sucrose, glucose and fructose are major sugar compounds in unfermented coconut sap. During the fermentation process, sucrose is broken down into glucose and fructose (reducing sugar) (Phaichamnan et al., 2010) by distinct groups of microbes. The sugar components of unfermented coconut sap collected from two methods were significantly different ( $p < 0.05$ ) (Table 2).

Sap collected from NSCM contained a higher concentration of total sugar and sucrose compared to the sap collected from TM. Further, sap collected from TM contained a higher percentage of reducing sugars (Table 2). Moreover, if the harvesting time of sap is high it also has a significant effect on sugar component of fresh coconut sap. Somawiharja et al., 2018 reported that 1.76% sucrose, 5.76% fructose and 4.46% glucose were observed in collected sap without preservatives. The results of this study showed that NSCM and TM have higher preservation ability against fermentation and NSCM is the better method for collecting unfermented coconut sap with high sucrose content compared to TM for product development.

### Total phenolic and DPPH Scavenging activity

The sap collected from TM with hal bark showed significantly ( $p < 0.05$ ) higher phenolic compounds (65.86 ± 12.91 GAE mg/100ml) than the sap collected from NSCM (22.80 ± 1.47 GAE mg/100ml). Similar concentration of total phenolic was identified by Asghar et al., 2019. Xia et al., (2011) identified that unfermented coconut sap has a higher concentration of Gallic acid (350 ± 1.36 µg/L) while the fermented coconut sap has high Caffeic acid (730 ± 1.29 µg/L) concentration. Therefore, most of the phenolics in unfermented coconut sap in this study can be in the form of gallic acid.

Higher free radical scavenging ability was observed in sap collected from TM (143.03 ± 27.10 mg/ml) than the sap from NSCM (294.00 ± 95.8 mg/ml). The positive control of ascorbic acid has 0.036 ± 0.00 mg/ml of antioxidant activity. This reveals that the pure coconut sap or unfermented coconut sap has very low antioxidant activity and phenolic content naturally. Moreover, the presentation of data as ascorbic acid equivalent antioxidant capacity (AEAC) directly compares the known potent of antioxidant ability with respective to the positive control of ascorbic acid. Significantly ( $p < 0.05$ ) lower values of AEAC were shown by the two types of coconut saps compared to the ascorbic acid with the figure of 0.26 ± 0.05 mg Vit C in a gram and 0.13 ± 0.03 mg Vit C in a gram for TM and NSCM respectively. The sap collected from TM has highest phenolic and free radical scavenging action than the sap collected from NSCM.

### Ascorbic acid content of unfermented coconut sap

Bremus et al., 2006 has identified that the production of ascorbic acid during the fermentation process is due to yeast. Similar results were shown by Ragu and Biju (2020) and they have found 112.3 mg/L ascorbic acid in unfermented coconut sap. The ascorbic acid content of sap collected from NSCM and TM was 87.45 ± 6.03 mg/L and 82.10 ± 10.1 mg/L respectively and were not significantly different. The ascorbic concentration in this study is less than previous finding; might have been due to the unfermented condition of coconut.

### Mineral content of unfermented coconut sap

Composition of macro and micro element of unfermented coconut sap are shown in Table 3.

Potassium is the major mineral in coconut sap and it did not show a significant difference ( $p > 0.05$ ) with the

collection method. Previous studies revealed that K content of unfermented coconut sap as  $960.87 \pm 12.50$  mg/L (Asghar et al., 2019) and  $1461 \pm 0.00$  mg/L (Hebbar et al., 2015) which are approximately closer to the values obtained from the practiced two methods. Sodium is the second most prominent macro element in coconut sap which is higher than the previous identification of  $183.21 \pm 1.42$  mg/L (Asghar et al., 2019). Only Calcium content of the two methods has changed significantly. Application of *Vateria acuminata* bark can be the major reason for additional Calcium contamination to the unfermented coconut sap.

Micro elements have enormous influence on human health. Sufficient dietary intakes of micro-elements are essential to maintain the proper functions in central nervous system, reproductive system, enzyme activities and energy metabolism and thus lead to serious illnesses. The micro elements of Cd, Cr, Co, As, Ag, Be, Bi, Ga, In, Mo, Pb and V were not detected in the two types of unfermented coconut sap samples. Sap collected from TM had significantly higher concentration of Fe, Mn, Sr and Ba (Table 3).

Most of the Sr in the human body is concentrated into the bones. Maximum intake of Sr should be 1.5 mg per day and excess amount can cause bone development deformities and skin rashes (Anupama et al., 2016). Results revealed that, the content of Sr in TM is not affected negatively in the human body.

Dietary Barium intake for adults has been found to be 0.75 mg/day including food and fluids (ICRP, 1975). Higher concentration of Ba in human body have been associated with elevated blood pressure, kidney and liver failure, stimulation of smooth, striated and cardiac muscles (Dietz et al., 1992). Therefore, high level of Ba contained in sap collected from TM might not become toxic.

## Conclusions

Two types of sap collection methods that have the capacity to prevent fermentation. Nevertheless, significant differences were observed between the two methods in terms of pH, ash, sugar components, color parameters, phenolic constituents, antioxidant properties, and certain minerals such as calcium, iron, manganese, strontium, and barium. The sap collected through the novel sap collection method (NSCM) had a higher sugar content, making it more suitable for producing products such as beverages, jaggery, and table sugar.

## Conflict of Interest

We do not have conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome. As Corresponding Author, I confirm that the manuscript has been read and approved for submission by all the named authors.

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