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Quality attributes of lime tapped and fresh palmyra sap: A comparative study

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Abstract
Palmyra palm (*Borassus flabellifer*) produces a non-alcoholic liquid exudate sap that is tapped from spathe of the tree. Traditional tappers use slaked lime in the collecting pot in order to avoid the rapid auto fermentation of the sap. Therefore, our study aims to carry out a comparative study between fresh sap (control) and lime tapped. The sap samples were subjected for sugar analysis (HPLC), mineral profile (ICP-MS), amino acid profile (UHPLC) and functional group (FTIR). Decrease in sugar content in control sap was observed due to the auto-fermentation. Potassium (115mg/100mL) was found to be the highest in the fresh sample followed by magnesium (34.46 mg/100mL) while lime tapped also showed similar pattern with potassium (119mg/ 100mL) to be the highest followed by magnesium (47.73 mg/100mL). Valine is an essential amino acid which was found to be in the highest concentration (11.739 mg/mL) in the sap. FTIR analysis confirmed that there was no significant chemical due to the presence of lime in the sap.

Keywords: palmyra, lime, mineral profile, amino acid profile, potassium, valine

Introduction

The demand for natural health drinks was increasing in last few years with increase in awareness in the consumers. Though there are many fruit-based beverages in the market the price of the natural packaged fruit juices was higher and was not affordable by all classes of people [1]. The Palmyra (*Borassus fabellifer*) sap is one such beverage which can be best natural health drink at affordable price. Palmyra palm is dioecious tall single stem tree that can grow up to 30 m height [2]. All the parts of tree are used in various application and it is tree of high economical value. The palm sap is one such product of great importance and economical value. The consumption of palm sap is most common in Asian countries due its enormous nutritive value and health benefits [3]. Palm trees are widely distributed along the coastal as well as inlands of India, Sri Lanka, Bangladesh, Myanmar, Indonesia and Africa [4]. In India, they are mostly cultivated in the states of Tamil Nadu, Kerala, Karnataka, Andhra Pradesh, Orissa, Maharashtra, West Bengal and also, in Andaman & Nicobar and Lakshadweep Islands [5].

The palm sap is nutritious fluid obtained from phloem of the tree rich in sugars, amino acids, minerals and vitamins with low glycaemic Index (GI 35) [6]. It is generally tapped from the month of January in the male trees while female trees produce this sap from the last week of March or first week of April. In India it is recorded that, a palm tree can produce maximum of 18 litres of sap per spadix per month. Palms yield up to 300 litres of sap for a period of six months of tapping with an average yield of about 50 litres of sap per spadix. The average yield of sap per palm per day is about 1.5 litres [7]. The sap and its value-added products are produced and marketed globally on a commercial scale.

Palms such as *Cocos nucifera* (coconut palm), *Arenga pinnata* (sugar palm), *Hyphaene spp.*, *Talipot buri*, *Elaeis guineensis* (African oil palm), *Phoenix sylvestris* (sugar date palm), *Corypha umbraculifera*, *Raphia spp.*, *Caryta urens*, *Borassus flabellifer* (palmyrah palm), *Phoenix dactylifera* (Date palm), *Areca catechu* (Betel nut palm) and *Bactris gasipaes* (Pejibaye- Peach palm) are of great importance Since the ages, the young inflorescences of these palms are tapped for many by products such as Fresh juices, Toddy, Wine, Syrup, Sugar and Jaggery [8]. The tip of the matured unopened inflorescence is cut for tapping when swelling develops at the base of the inflorescence. The spathe is tied with a coir or rope to avoid opening of inflorescence. Traditionally, sap is collected by inserting the inflorescence to an earthen pot. Sap yield varies with genetic potential of palm trees. The thin inflorescence sheath with long internodes is usually selected for tapping achieve high yield.

Despite of its enormous health benefits and nutritive value, natural fermentation of sap that occurs immediately after the tapping is the major problem with palm sap. The sap turns into white colour with a foul smell making it unfit for consumption. The fermented sap is known as ‘toddy’, which has a strong odour that makes it unpalatable despite being nutritious [9]. Thus, the auto-fermentation nature of palm sap to toddy is the major constraint of the sap industry. Eventually, unfermented sap and toddy are both chemically and nutritionally different from each other.

It is highly recommended to implement preventive measures during harvesting, processing and storage as the fresh sap is highly prone to fermentation therefore, collection of unfermented sap is challenging task. Palm sap is classified under low Glycaemic Index foods with GI of 35 and may be considered as diabetic friendly [10]. It is rich in inulin which can be used as a sugar replacer and is categorized as a prebiotic. Palm sap contains glutamic acid which is necessary for protein synthesis and it aids in digestive health [10]. The most appealing feature of this sap is the flavour and nutritional components.

The sap owed to its high sugar content it starts fermenting immediately after tapping because of natural macro flora. The natural fermentation of sap converts the natural sugars in sap into alcohol [6]. The initial fermentation of sap begins with *Lactobacillus* spp. and *Leuconostoc* spp. where the sap is acidified initially followed by fermentation by yeast that starts producing alcohol and finally Acetobacter and Gluconobacter species increases and produces acetic acid [6]. The spontaneous fermentation starts from the time it is tapped and comes in contact with air and is reported that it takes place predominantly by yeast *Saccharomyces cerevisiae* [11]. The traditional tappers generally use lime to inhibit the natural fermentation which is added into the pots before tapping [12]. Thus, this study aims to investigate and discuss the compositional changes in the sap tapped with and without the addition of lime.

Materials and Methods

Materials

The food grade lime ($Ca(OH)_2$) was purchased from the local market of Thanjavur (Tamil Nadu, India). Aqueous extract of the bark was coated in the inner lining of the collecting pots. All the required chemicals used for conducting this study were of analytical-reagent grade (Merck, Sigma, Chennai, Tamil Nadu, India) and utilised without further purification.

Tapping and collection of sap

Healthy palmyra palms were selected with mature without blossomed spike inflorescence for tapping sap. A sterile pot was attached to the spathe on different trees and tapping of sap was performed at Thulikkampatti village (10°43'57.1"N 79°06'51.0"E, Thanjavur district, Tamil Nadu, India) before the sunrise. The freshly tapped sap was filtered through Whatman filter papers having size of 0.1 mm and filtered sap was centrifuged at 5000 rpm for further use.

Quantitative Analysis of Selected Stored Sap Samples

Sugar profile analysis

The sugar profile (sucrose, glucose, fructose and maltose) analysis of fresh and selected hurdle treated sap samples was analyzed with high performance liquid chromatography (Shimadzu HPLC RID 10A, Japan) according to AOAC 982.14.

Mineral profile analysis

The mineral profile of fresh and selected hurdle treated sap samples were measured using AOAC 2011.14. The samples were digested with nitric acid in microwave digestion system and digested samples were filtered. Afterwards, filtrate is diluted with distilled water and injected into the ICP-MS system (Perkin Elmer Optima 8300 ICP-MS, Switzerland) and mineral content was measured against the standards.

Amino acid profile analysis

Amino acids present in the fresh and selected hurdle treated sap samples were determined using ultra-high performance liquid chromatography (UHPLC) according to AOAC 994.12. The sap samples (10 mL) were hydrolyzed with HCl (6M) in an ampoule containing 10 mg phenol (for protection of tyrosine) at 110 °C for 24 h. After acid hydrolysis, 30 mL of citrate buffer (pH 2.2) was added, and the pH was adjusted between 0.5 and 1 with 7.5 M NaOH and pH 2.2 with a 1 M NaOH. The sample obtained was diluted to 100 mL with citrate buffer after adding 1 mL of a nor leucine solution 50 μM (as an internal standard). The sample was filtered through a 0.2 μM nylon filter before being analyzed by HPLC. Sulphur-containing amino acids, cysteine and methionine were determined after pre hydrolysis oxidation with performic acids. The contents of the recovered amino acids were expressed in % (protein basis). The HPLC system (Berlin, Germany) was equipped with a UV-vis detector with two wavelengths, 440 nm and 570 nm, respectively for the proline and the other amino acids, and a cation exchange Waters C18 column (4.6 mm × 200 mm) (Dublin, Ireland). Resolution of amino acid derivatives was achieved using a four-buffer gradient system. The buffers used were: (A) 0.2 M Na citrate (pH 3.2), (B) 0.2 M Na citrate (pH 4.25), (C) 1.2 M Na citrate (pH 6.45) and (D) 0.4 M NaOH.

FTIR spectroscopy analysis

The functional groups changes made by hurdle treatment on Palmyra sap were analysed using Fourier transform infrared spectroscopy (FTIR). The analysis was carried out using Spectrum two FTIR, Spectrophotometer (PerkinElmer, USA). The spectra of the sap samples were recorded at a resolution of 4 cm^{-1} from 400 to 4000 cm^{-1} with 12 scans.

Statistical analysis

All the experimental data were conducted in triplicates and analyzed statistically. The means and standard deviation (SD) values were calculated and linear equation was developed using Microsoft Excel 2020 (Microsoft Corporation, Redmond, WA, USA).

Results and Discussion

Sugar profile of selected stored sap samples

The sugar profile of control and lime tapped sap sample were analyzed immediately after tapping. The control sap sample was observed to be slightly lower in sugar content compared to lime tapped sap sample and results are illustrated in Table 1. The decrease in sugar content in control sap was owing to the immediate auto-fermentation caused by the various microorganisms present in sap like *Saccharomyces* spp, *Lactobacillus* spp, *Leuconostoc* spp, *Streptococcus* spp and *Acetobacter* spp [13]. According to Dioha *et al*., (2010) [14] the invertase enzymes are produced during storage of sap at ambient condition was owing to the breakdown of sucrose into glucose and fructose. The addition of $Ca(OH)_2$ resulted in delayed the fermentation process which resulted higher sugar content in lime tapped sap sample [15].

Table 1: Sugar profile of control and lime tapped palmyra sap (g/100 ml)

Components	Control	Lime tapped
Glucose	1.41±0.03	1.70±0.03
Fructose	0.50±0.03	0.70±0.02
Sucrose	14.22±0.04	15.50±0.04
Maltose	0.30±0.02	0.50±0.02

Values represent means ±standard deviations.
Control: Sap tapped without lime
Lime tapped: Sap tapped with lime

Mineral profile of selected stored sap samples

Inductively coupled plasma mass spectroscopy (ICP-MS) is based on the principle of detecting samples by ionization. It is widely used to detect metals and non-metals in trace and ultra-trace level of concentration. Palm sap is a mineral rich drink comprise 4% of body weight and provide various metabolic functions [13]. The mineral content of control and lime tapped sap were analyzed using ICP-OES and there was no difference observed between each other's. The mineral content present in 100 ml of control and lime tapped sap were sodium 16.23±0.03 mg, potassium 115.02±0.03 mg, iron 0.38±0.02 mg, zinc 0.50±0.02 mg, magnesium 0.20±0.02 mg and manganese 0.29±0.02 mg (Table 2). The addition $Ca(OH)_2$ in lime tapped sap showed higher calcium content (3.30±0.04 mg) compared to control sap (1.30±0.03 mg). The slight increase in the mineral content of control sap was observed due to the initiation of natural fermentation process [16].

Table 2: Mineral profile of control and lime tapped palmyra sap (mg/100 ml)

Components	Control	Lime tapped
Sodium	16.23±0.03	16.23±0.03
Potassium	115.02±0.03	115.02±0.03
Iron	0.38±0.02	0.38±0.02
Zinc	0.50±0.02	0.50±0.02
Magnesium	0.20±0.02	0.20±0.02
Calcium	1.30±0.03	3.30±0.04
Manganese	0.29±0.02	0.29±0.02

Values represent means ± standard deviations.
Control: Sap tapped without lime
Lime tapped: Sap tapped with lime

Amino acid profiles of selected stored sap samples

The control and lime tapped sap samples were analysed with UHPLC method and 17 different amino acids were detected in both the sap samples which is depicted in Table 3. The lime tapped sap exhibited higher amino acid content compared to control sap. In both the sap samples of 100 ml the valine was observed to be higher. In control sap, the valine content was 11.74±0.05 mg/ml followed by methionine 7.29±0.04 mg/ml and lysine 2.43±0.03 mg/ml. The valine is an essential amino acid and an adult human requires about 4 mg/kg body weight [13]. Likewise, the valine content in lime tapped sap was higher 44.27±0.04 mg/ml followed by methionine 4.82±0.03 mg/ml, lysine 3.46±0.03 mg/ml and alanine 1.76±0.03 mg/ml. Methionine prevent from liver damage and viral infections and alanine plays a vital role in glucose-alanine cycle between tissue and liver. It is also good for re feeding syndrome. Methionine helps prevent liver damage and viral infections. Proteinogenic amino acid Pro line is the only amino acid with a secondary amine has beneficial effect in plant tissue development. Alanine plays a vital role in glucose-alanine cycle between tissue and liver [17].

Table 3: Amino acid profile of control and lime tapped palmyra sap (mg/100 ml)

Components	Control	Lime tapped
L-Aspartic acid	0.13±0.01	0.11±0.01
L-Glutamic acid	0.30±0.02	0.17±0.02
L-Serine	0.12±0.01	0.00±0.00
L-Histidine	0.15±0.02	0.19±0.02
Glycine	0.12±0.02	0.12±0.01
Threonine	0.00±0.00	0.08±0.01
Arginine	0.08±0.01	0.09±0.01
Alanine	0.56±0.04	1.76±0.03
Tyrosine	0.19±0.02	0.00±0.00
Cystine	0.00±0.00	0.00±0.00
Valine	11.74±0.05	44.27±0.04
Methionine	7.29±0.04	4.82±0.03
Phenylalanine	0.30±0.03	0.37±0.02
Isoleucine	0.00±0.00	0.22±0.02
Leucine	0.00±0.00	0.19±0.02
Lysine	2.43±0.03	3.46±0.03
Proline	0.00±0.00	0.00±0.00

Values represent means ± standard deviations.
Control: Sap tapped without lime
Lime tapped: Sap tapped with lime

FTIR spectroscopy of selected stored sap samples

The FTIR spectroscopic studies revealed different characteristic peak values (Fig.1) with various functional compounds present in control and lime tapped sap sample. FTIR spectra for lime tapped sap sample showed weak absorption on wavenumber of 3670 cm^{-1} was owing to presence of $Ca(OH)_2$. The stretching of OH bonded (water) was observed on wavenumber of 3271 cm^{-1} for control and lime tapped sap samples. The weak absorption of alkene (C=C) group was observed for control and lime tapped sap samples on wavenumber of 1636 cm^{-1} and 1652 cm^{-1}, respectively. The aromatic compound showed stretching of C=C on peak between 1435 cm^{-1} and 1417 cm^{-1} for control and lime tapped sap samples. The stretching of C-O (anhydride group) bonded was observed on absorption peak between 1041 cm^{-1} to 1108 cm^{-1} for both the sap samples. The bending of alkene (=C-H) group showed broad peak between 830 cm^{-1} to 990 cm^{-1} and stretching of C-Br (alkyl halide group) was observed on peak between 549 cm^{-1} to 601 cm^{-1} in both the sap samples. The extra peak was observed on wavenumber of 664.4 cm^{-1} and 667.4 cm^{-1} for control and lime tapped sap samples which corresponds to stretching of alkyl halide (C-Cl) group. No more additional peaks were observed. The FT-IR analysis confirmed that the lime tapped sap samples were not changed chemically due to the presence of $Ca(OH)_2$.

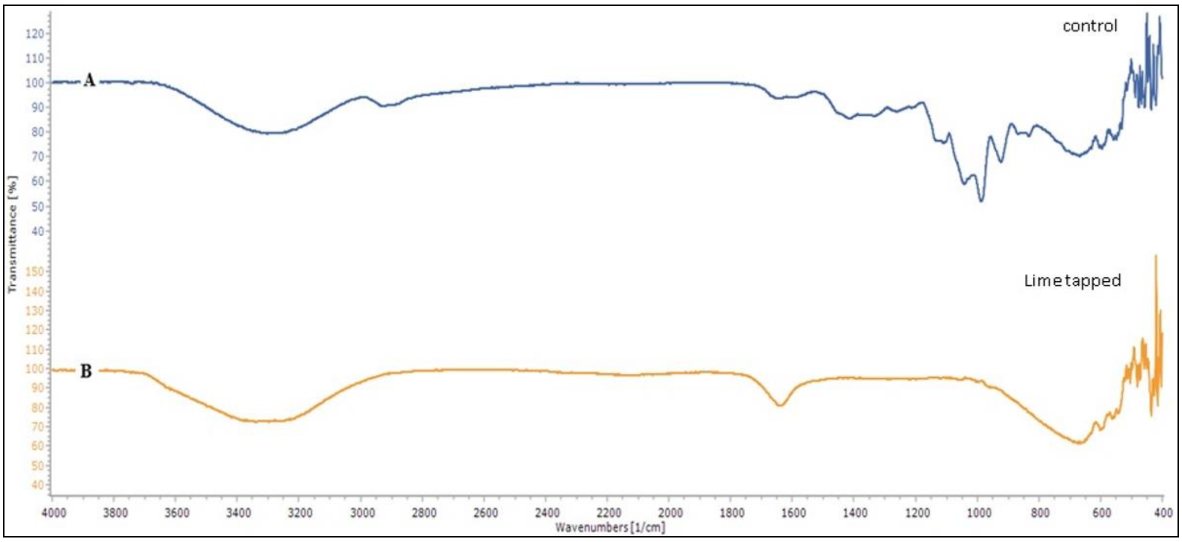

Fig 1: FTIR spectrum for control and lime tapped Palmyra sap samples

Conclusion

Traditional way of preventing fermentation using slaked lime Ca $(OH)_2$ is effective for the extending shelf life of palmyra sap. Fresh sap starts fermenting after 2-3hrs of tapping which is controlled by adding lime. The oxidation on addition of lime may be a reason of increase in the mineral content of the sap. Sap, a nutritious non- alcoholic sap produced from the Palm trees is also regarded as 'Tree of Hope' as it has numerous health benefits. The sap is highly energy dense and rich in minerals. But fermentation starts within 2-3 hours of tapping and it gets converted to an alcoholic drink known as Toddy. The addition of slaked lime was done to in hibited the fermentation process. There was no significant change observed in the chemical structure of the control and lime tapped sap from FTIR analysis. Potassium was found to be the highest in the fresh sample followed by magnesium in the fresh Sap sample while lime tapped sap, potassium was found having to be highest followed by magnesium. Valine is an essential amino acid which was found to be highest which has various health promoting properties. Challenges in preserving this sap include tapping, transportation and storage. Inhibition of fermentation at the time of collection is important in order to extend shelf life but least attempt has been made at the time of harvest to preserve this sap. Temperature of storage is also important to avoid fermentation; exposure to sunlight for long time will speed up the fermentation process. The low temperature transportation and storage facilities are to be established. Thus, it can conclude from our study that palmyra sap tapped with and without addition of lime is nutritious and has the potential to be regarded as a organic drink.

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