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ORIGINAL PAPER

Nutritional profiling of coconut (*Cocos nucifera* L.) inflorescence sap collected using novel coco-sap chiller method and its value added products

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Abstract
Coconut inflorescence sap is a phloem sap collected from the unopened coconut spadix. A new 'coco-sap chiller method' (CSCM) is developed to collect fresh and unfermented sap devoid of extraneous matter. A study was conducted to identify and compare the nutrients present in the sap collected by CSCM and the sap collected by traditional method (TM) using lime coated earthen pot, and also in the sap concentrate and coconut sugar prepared from sap collected by CSCM. Profiling of amino acids, phenolic acids, flavonoids and vitamins was also done using Ultra-Performance Liquid Chromatography/Tandem Mass Spectrometry (UPLC coupled with TQD-MS/MS). Distinct variation in physical and biochemical properties were recorded between the sap collection methods. The sap collected by CSCM was slightly alkaline (pH 7–8), golden brown in color, sweet and delicious and rich in nutrients and zero alcoholic. While the sap collected by TM was acidic (pH below 6), oyster white in color and gave astringent smell and had about 2.32% alcohol. Sap collected by CSCM was rich in essential amino acids like lysine, threonine and histidine. A total number of thirteen phenolic acids and seven flavonoids were identified from coconut sap, sap concentrate and sap sugar. Vanilic acid, syringic acid, *trans*-cinnamic acid and *p*-hydroxy benzoic acid are the major phenolic acids and catechin, hesperitin, and myricetin are the major flavonoids identified. Vitamin C, niacin, and tocopherol are the major vitamin identified. Except few vitamins, all the nutrients present in the sap were preserved in the sap concentrate and coconut sugar. In summary, coconut sap collected by CSCM was fresh and contained numerous health promoting biochemical constituents such as phenolics, flavonoids and vitamins. Hence it can be consumed as ready to serve non-alcoholic health drink. Similarly, nutrient rich sap concentrate and coconut sugar can be prepared from fresh sap collected by CSCM without the addition of any chemicals.

Keywords Coconut inflorescence sap · Phenolics · Vitamins · Antioxidant potential · UPLC MS/MS · Non-alcoholic healthy drink

Introduction

Coconut (*Cocos nucifera* L. Family: Arecaceae (Palmae)) is grown throughout the tropical and subtropical regions, and well recognized for its versatile uses. Globally it is cultivated in 12.3 million hectare across 93 countries, producing ~ 11.0 million tonnes (copra equivalent). India, Indonesia, Philippines, Thailand, and Sri Lanka are the major cultivators contributing for 78% of world area and production. India ranks third under cultivation, however first in productivity. Despite its versatile uses, the coconut farmers are in a precarious situation due to multiple issues associated with its cultivation such as price instability, pest and diseases, upsurge of input cost, fragmented land holdings etc. All these issues lead to utter negligence of coconut plantations, and thus, coconut farming becomes a non-profitable enterprise. Development and promotion of diverse coconut based value added products is a sustainable approach for regaining the lost glory of coconut cultivation [1]. Coconut inflorescence sap is one of the important value added products, and holds the

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potential role to revive the prospects of coconut farmers. It is the phloem sap collected by tapping of the unopened coconut inflorescence, rich in various nutrient and reported to be good digestive agent [2]. It is traditionally tapped and consumed by the people in the rural areas of the countries like India, Sri Lanka, Philippines, Indonesia, etc. nutrient dense products like coconut sugar, *jaggery,* sap concentrate and vinegar can also be prepared from the sap. The most significant characteristic of these products, especially coconut sugar prepared from sap is its low Glycemic Index (GI = 35) [3], and it can fulfill the global demand for low GI sugar. Tapping the trees for the sap collection and marketing of the sap as a health drink or the value added products derived from sap were found to be six to eight fold more profitable than the sale of nuts alone [2].

Due to richness of nutrients and long duration required for collection, sap gets fermented by the microbes, and thus, the collection of hygienic and unfermented sap is a challenge. In the traditional method (TM) of sap collection, an earthen pot or bamboo sac coated with lime is used to collect the sap. The sap collected by this method is partially fermented oyster-white and translucent liquid locally known as *neera*. Since, the sap collected by this method is in open vessel, it is contaminated with insects, pollen grains, etc. and emanates a fetid odor and hence it cannot be marketed as fresh juice, but can be used for the preparation of sugar, alcoholic drink called *toddy* and vinegar with the addition of chemicals [2, 4]. To overcome the issue, and to collect fresh unfermented sap, ICAR- Central Plantation Crops Research Institute (ICAR-CPCRI), Kasaragod, Kerala developed a simple ice-box or coco-sap chiller method (CSCM), a patented method [5], in which the sap is collected in a closed container which is maintained at low temperature (2–4 °C). Coco-sap chiller is a portable double jacketed device with PVC (polyvinyl chloride) pipe and have collection container of 2–3 L capacity [2, 6] which is placed in ice cubes. Half a kg ice is sufficient to keep the inside temperature cool for 10–12 h. Unlike the traditional method of sap collection, the sap collected by coco-sap chiller is free from insects, dust, ants etc., as it is a closed system. The sap collected using CSCM can be stored unfermented for any length of time under refrigerated condition (− 1 to − 3 °C). In order to avoid the ambiguity with the sap collected by traditional method (TM), the fresh, hygienic and unfermented sap collected by CSCM is registered as *Kalparasa* under 'Trademarks Registry of Intellectual Property India' in Mumbai. It is registered in the name of the Indian Council of Agricultural Research, New Delhi, with Trade Mark No. 2813919.

As the sap collected by CSCM is unfermented and rich in nutrients, it is ideal product for selling as a health drink. Further, the value added products like sap concentrate and coconut sugar can be prepared without the addition of chemicals [2]. Previously the nutrient content and volatiles was measured in the partially and fully fermented coconut sap [7, 8]. Since the nutrient profiling of sap collected by CSCM is not available, it is foremost important to identify the nutrients and other constituents like phenolics and antioxidants present in the freshly collected sap by CSCM and its value added products for their better utilization and promote them as a functional food. Thus, the aim of the study is to compare the biochemical and nutrient composition of coconut inflorescence sap collected both by TM and CSCM method, and also measure the biochemical constituents in value added products viz. sap concentrate and coconut sugar.

Materials and methods

Chemicals

All the chemicals used in the study are of analytical grades. All the standards including phenolic acids, flavonoids, amino acids and water and fat soluble vitamins, and TPTZ (2,4,6-tris-2,4,6-tripyridyl-2-triazine), were purchased from Sigma-Aldrich Co St. Louis, MO, United States of America. L(+)-ascorbic acid, 2, 6-dichlorophenol-indophenol, ethanol, methanol, acetic acid (glacial), sodium acetate, hydrochloric acid (conc.), sulphuric acid, ferric chloride, ammonium acetate, Folin–Ciocalteu's phenol reagent, sodium hydroxide, sodium bicarbonate, potassium sodium tartrate, sodium sulphate, ammonium molybdate, disodium hydrogen arsenate, ninhydrin, D-Glucose, etc. were purchased from Merck KGaA, Darmstadt, Germany. Water purified in the Milli-Q (Millipore) system was used to prepare the mobile phases. All mobile phases used for UPLC and MS/MS were filtered through membranes with a pore size of 0.2 µm. All the medias used in the microbial analyses were purchased from Himedia Chemicals, India.

Coconut inflorescence sap collection and preparation of sap concentrate and sugar

Selection of palm and initial preparation

Sap was collected from unopened inflorescences of the coconut palm (*Cocos nucifera* L; West Coast tall cultivar) at the farm of ICAR-CPCRI, Kasaragod, Kerala, India, during the summer season in the month of March and April. Healthy coconut trees (3 palms each) which had attained yield stability were selected for tapping. The unopened inflorescence (few days before its opening) was selected and tied around either with a strong coir or plastic rope, to prevent it from bursting, and was conditioned twice a day for a week using a mallet and hand massaged. The conditioning was done to make the spikelets within the inflorescence free as it is packed compactly inside the spadix, which in turn helps in easy flow of sap. After conditioning, tip of the inflorescence was sliced off and in a day or two sap starts oozing out from the cut surface of the spadix. The initial preparation was same for the collection of sap either by traditional method or using the coco-sap chiller method.

Collection of sap by traditional method (TM)

The sap trickling from the cut surface of the spadix is collected in an earthen pot connected to the spadix (Fig. 1a). In order to prevent the fermentation, lime is coated on the inner surface of the pot. The overnight sap was collected in the morning at 7 am and the day time sap was collected in the evening at 6 pm. The collected sap (Fig. 1b) was immediately filtered and pH and total soluble solids were measured using pH/Conductivity meter (Sartorious AG, Goettingen, Germany), and refractometer (Atago Co., Ltd, Japan) at 25 ºC, respectively. Simultaneously, sensory properties like appearance/color, taste/sweetness, were flavor/aroma measured using a 5-point hedonic scale judged by panelists consisting of 50 members (30 males and 20 females, aged between 18 and 75 years). The color and presence of extraneous matter was also noted purely based on visual observations. Then the samples were stored at – 20 ºC for further analysis.

Collection of fresh sap by coco-sap chiller method (CSCM)

The detailed method of preparation of coco-sap chiller and connection to the spadix for the collection of sap is described by Hebbar et al. [2] (Fig. 1c, d). Once the sap starts oozing, a coco-sap chiller is connected to the spadix and sap is collected in the food grade collection container which is housed inside the chiller with ice or gel packs. Ice cubes (half to 3/4th of a kg depending on climate and amount of sap) or 3 to 4 gel ice packets are sufficient to keep the container at 4 °C for 8–10 h. To prevent the pollen particles to fall in the sap, a stainless steel filter is placed above the collection container. The sap that trickles from the cut surface falls on the stainless steel filter and collects in the container. The pH and total soluble solids of the fresh sap collected at morning at 7 am and evening 6 pm was measured immediately. Simultaneously, sensory properties also measured as described in the previous section. Then the sap was stored at – 20 ºC for further analysis.

Microbial analysis

Fresh sap collected was immediately used for microbial analysis including bacteria, fungi and actinomycetes. The microbial analysis of sap samples was done using isolation-dependent microbiological protocol following the standard serial dilution and spread plating method. For enumerating bacteria, Nutrient Agar [9]; for fungi, Martin Rose Bengal [10]; for yeasts and moulds, Sabouraud Dextrose Agar and for actinomycetes, KenKnight & Munaiers agar medium [9], were used. Different dilutions of the sap samples were plated in triplicates on the respective medium and kept for incubation in BOD incubator at 28 ± 2 °C. Bacterial and yeast colonies were scored as colony forming units (cfu)/mL sap after 24 h of incubation, fungi and molds between 48 and 72 h and actinomycetes by a week time.

Preparation of coconut sap concentrate and sugar

Coconut sap concentrate and coconut sugar were prepared by evaporating the moisture from the fresh sap collected using CSCM at 115 °C using specially designed double jacketed cooker. Thermic fluid is filled inside the jacket to ensure efficient and uniform heat transfer (Fig. 2a). During the continuous heating process sap undergoes various changes in consistency, and at certain point fairly thick syrup (Brix 60°–70°) formed, which was collected and cooled to get coconut sap concentrate (Fig. 2b). Further

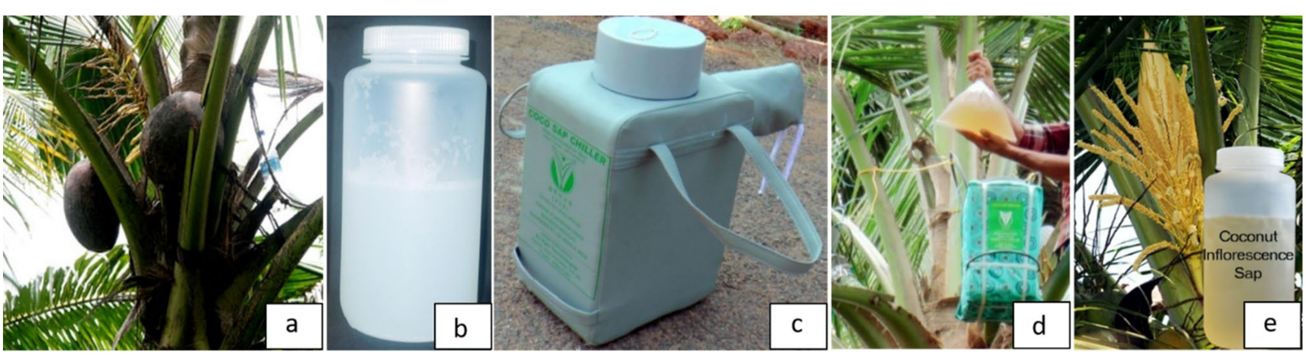

Fig. 1 Collection of coconut inflorescence sap by traditional method (TM) and coco-sap chiller method (CSCM). **a** Earthen pot connected to the unopened inflorescence to collect the sap; **b** Coconut sap collected by traditional method. **c** Coco-sap chiller; **d** Coco-sap chiller connected to the unopened inflorescence; **e** Coconut sap collected by coco-sap chiller method

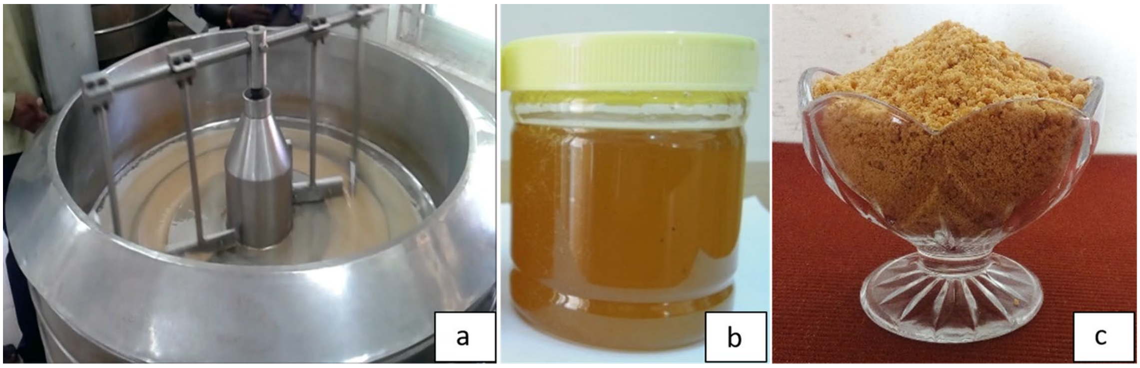

Fig. 2 Preparation of coconut sap concentrate and coconut sugar. Coconut sap concentrate and coconut sugar are prepared by evaporating the moisture from the fresh sap. **a** Specially designed double jacketed cooker; **b** Coconut sap concentrate; **c** Coconut sugar

heating led to conversion of viscous syrup into a crystal form and at this stage it was immediately cooled with continuous stirring to avoid lumps formation. The sugar crystals thus obtained were sieved to get uniform particle size (Fig. 2c) and used for the biochemical analysis.

Biochemical analysis

Coconut sap samples (collected by both TM and CSCM) stored in the − 20 °C was used for biochemical measurements. Total and reducing sugar content in the samples were measured by phenol–sulphuric acid method [11] and Nelson-Somogyi's method [12] using arsenomolybdate reagent, respectively. Vitamin C content was determined by 2,6-dichlorophenol-indophenol (DCPIP) method [13]. Free amino acids were estimated using ninhydrin method [14]. Alcohol content was measured by redox titration using the method described by Zoecklein et al. [15]. Total phenolic content (TPC) and total flavonoid content (TFC) were estimated by the Folin-Ciocalteu's method [16] and aluminium chloride method [17], respectively. Gallic acid and catechin were used as standard for TPC and TFC, and the results were expressed as mg Gallic acid equivalent (mg GAE) and mg catechin equivalent (mg CE), respectively. Antioxidant capacity was measured as FRAP (Ferric reducing antioxidant power) according to Benzie and Strain [18] using the stock solutions included 300 mM acetate buffer (3.1 g $CH_3COONa.3H_2O$ and 16 mL CH_3COOH, pH adjusted to 3.6), 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3•6H_2O$ solution. Ascorbic acid served as a positive control and results were expressed as milligrams of ascorbic acid equivalents antioxidant capacity (mg AEAC) per 100 mL for sap and per 100 g for sap concentrate and sugar.

Profiling of total amino acids, phenolic acids, flavonoids, fat and water soluble vitamins

The Ultra Pressure Liquid Chromatography- Mass spectroscopy (UPLC-MS) consists of an Acquity UPLC-H class coupled with Tandem (Triple) Quadrupole Detector (TQD) -MS/MS system (from Waters Inc., USA) with Electrospray ionization (ESI) source was used for profiling. Waters UPLC BEH-C18 column (2.1 × 50 mm) with 1.7 µm particles, protected by a Vanguard BEH C-18 with 1.7 µm guard column (Waters) was used and the column temperature was maintained at 25 ± 1 °C. Amino acids profiling was done after hydrolyzing the samples as described by Moore et al. [19] and Khalique et al. [20]. The samples were hydrolyzed using 6 M HCl under vacuum and the hydrolysates were dried under vacuum after neutralization, and dissolved in a known volume of mobile phase (0.1% formic acid in water), filtered using 0.2 µm nylon filter paper and injected to LC–MS/MS for analysis. The individual amino acids were eluted using the mobile phase which consisted of an aqueous phase of 0.1% formic acid in water (A) and organic phase of methanol: water (1:1) with 0.1% formic acid (B) and gradient flow was followed up to 20 min with the flow rate of 0.1 mL/min. Individual phenolic acids and flavonoids were extracted using 80% aqueous methanol (v/v) as described by Chen et al. [21]. The extracted sample was filtered through 0.2 µm nylon filter and injected to LC–MS/MS, and eluted using mobile phase consisted of an aqueous phase of 0.1% formic acid in water (A) and organic phase of 0.2% formic acid in methanol (B) and gradient flow was followed up to 18 min with the flow rate of 0.3 mL/min. Water soluble and fat soluble vitamins were extracted following a procedure as described by Santos et al. [22]. The sample was first extracted with 10 mM ammonium acetate:methanol 50:50 (v/v) containing 0.1% BHT followed by centrifugation and the supernatant was filtered through 0.2 µm nylon filter and injected into the LC–MS/MS system to determine the water soluble vitamin content. The solid residue from the first extraction was re-extracted twice with ethyl acetate containing 0.1% BHT for 15 min, centrifuged and filtered through 0.2 µm nylon filter prior to inject in a LC–MS/MS system to determine the fat soluble vitamin content. For water soluble vitamins, the mobile phase consisted of an aqueous phase of 0.1% formic acid in water (A) and organic phase of acetonitrile (B) was used. For fat soluble vitamins, the mobile phase consisted of acetonitrile (A) and 0.2% formic acid in methanol (B) was used. Linear gradient flow was followed upto15 min with the flow rate of 0.1 mL/min. The eluted compounds were subsequently identified and estimated by means of MS with ESI^{+ve}/ESI^{-ve} ionization followed by the Multiple Reaction Monitoring (MRM) method for amino acids, phenolic acids, flavonoids, fat and water soluble vitamins [23–26].

Statistical analysis

Data were statistically analyzed using SAS 9.3 [27]software Package and reported as mean ± standard deviation (SD). All experiments were replicated at least three times. Difference in the biochemical constituents between the sap collection methods, sap concentrate and sap sugar was evaluated by analysis of variance (ANOVA) and significant difference was considered at 5% level of significance ($p < 0.05$).

Results and discussion

Quality attributes of coconut sap collected by TM and CSCM

Distinct differences were noticed in terms of appearance and physical characteristics between the sap collected by TM and CSCM. Coconut sap collected by TM was oyster white (Fig. 1b), below neutral pH (6 or below) and gave an astringent smell as reported earlier [8], whereas, the sap collected by CSCM was sweet and delicious, golden brown or honey color with marginally alkaline pH (7–8), (Fig. 1e). The change in color from golden brown or honey color to oyster white could be due to addition of lime during the collection of sap and also due to the partial fermentation. Sensory evaluation indicated that sensory attributes were significantly high for sap collected by CSCM (4.7, based on 5-point hedonic scale) compared to sap collected by TM (1.2). The pH is a good indicator of the freshness of the sap, fermentation leads to change in pH from alkaline to acidic due to the production of organic acids [28], and from the study it is clear that the sap collected by CSCM is fresh and unfermented, whereas the sap collected by TM is partially fermented.

Total soluble solid content measured in terms of °Brix was high in the sap collected by CSCM (15.5–18) compared to TM (13–14), and this could be due to utilization of sugars present in TM collected sap collected by the microbes for their metabolism and further conversion of alcohol. Similarly, differences were observed for flavor. The sap collected by CSCM had sweet and delicious flavor whereas sap collected by TM had fetid smell. In CSCM, the sap was collected in closed system, and thus, the extraneous materials such as pollen, insects, bees, etc. were absent. In case of TM, bees and insects were attracted towards the collection pots due to the emission of volatile molecules from the collected sap, and thus the sap collected was contaminated with insects and other foreign matters. Alcohol content was high in sap collected by TM (2.32%), which was absent in sap collected by CSCM indicating the sap had not undergone any fermentation. This meets the Codex Alimentarius (International Food Standards WHO/FAO) definition of juice as “unfermented but fermentable juice, intended for direct consumption, obtained by the mechanical process from extractable fluid contents of cells or tissues, preserved exclusively by physical means”. Thus, the sap collected by CSCM is pure, non-alcoholic, devoid of added chemicals, and ready to serve health drink [2].

Microbial studies

Microbial studies were carried out in sap collected by both TM and CSCM, and the results indicated that the fresh coconut sap collected using CSCM had the least overall microbial population. Bacteria were the predominant community and *Bacillus* spp. being the preponderant genus. Fungi and yeasts numbers were in few hundred only. Contrary to these, the sap collected from traditional method had very high bacterial and yeast populations. The number of yeasts was in the range of n x 10^8 cfu/mL indicating brisk fermentation of the sugar to ethanol, thereby rapid conversion of sap to toddy. In all the sap samples analyzed, actinomycetes growth was not observed. The microbiological studies thus clearly confirmed that the fresh sap collected by CSCM method is having lesser microbiota than the sap collected using traditional method.

Biochemical constituents of sap, sap concentrate and sap sugar

Biochemical constituents including total and reducing sugar, free amino acids, phenolics, flavonoids and antioxidant potential were measured in coconut sap collected by CSCM and TM, sap concentrate and sap sugar and the results are presented in Table 1. The total sugar content of sap collected by CSCM was high (16.2 g/100 mL) compared to TM (9.20 g/100 mL) while reducing sugar content of sap

Table 1 Biochemical constituents of coconut sap collected by coco-sap chiller method (CSCM) and traditional method (TM), sap concentrate and coconut sugar

Constituents	Coconut sap (100 mL)		Sap concentrate (100 g)	Sap sugar (100 g)	CD
	TM	CSCM			
Total Sugars (g)	$9.20^{d} \pm 0.97$	$16.2^{c} \pm 0.33$	$72.6^{b} \pm 1.37$	$91.8^{a} \pm 1.01$	1.87
Non-Reducing sugars (g)	$7.95^{d} \pm 0.10$	$15.5^{c} \pm 0.34$	$69.6^{b} \pm 2.38$	$87.3^{a} \pm 5.91$	6.01
Reducing sugars (g)	$1.24^{c} \pm 0.87$	$0.68^{c} \pm 0.01$	$3.0^{b} \pm 1.01$	$4.69^{a} \pm 4.60$	1.38
Free Amino acids (g)	$0.413^{c} \pm 0.09$	$1.03^{c} \pm 0.10$	$2.77^{a} \pm 0.29$	$3.05^{a} \pm 0.07$	0.31
Total Phenolics* (mg GAE)	$14.8^{c} \pm 1.03$	$21.7^{c} \pm 0.48$	$92.3^{a} \pm 6.82$	$47.2^{b} \pm 20.7$	20.1
Total Flavonoids (mg CE)	$0.177^{c} \pm 0.02$	$0.817^{c} \pm 0.19$	$2.58^{b} \pm 0.09$	$4.76^{a} \pm 1.21$	1.16
FRAP (mg AEAC)	$8.34^{c} \pm 0.83$	$14.8^{b} \pm 0.21$	$26.7^{a} \pm 2.30$	$22.9^{a} \pm 4.12$	4.51

Values are given as mean ± Standard deviation of three independent samples (n = 3)

Different letters in each row after the mean value are significantly different at 5% level of significance according to Duncan's multiple range test (DMRT)

GAE Gallic acid equivalent, *CE* catechin equivalent, *FRAP* Ferric Reducing Antioxidant Power, *AEAC* ascorbic acid equivalents antioxidant capacity, *CD* Critical Difference at 5% level of significance

*Total phenolics as measured by Folin's Ciocalteu Method

collected using TM was high (1.24 g/100 mL) compared to CSCM (0.68 g/100 mL) (Table 1). Reducing sugar content is the indirect measure of the extent of freshness of the sap. If the sap is fresh and unfermented, the reducing sugar content should be lower than1.0 g/100 mL [28]. From the results obtained, it is clear that the sap collected by TM was partially fermented. The results obtained in the present study are in accordance with the sap collected from other palm families. The sap collected from *Phoenix dactylifera* consist mainly of sucrose (95.3%), followed by 2.51% glucose and 1.61% fructose (dry matter basis) [29]. The sugar concentrate and sugar prepared out of fresh sap had total sugar of about 72.6 and 91.8 g/100 g, respectively. As with fresh sap, coconut sugar is also found to contain 87% non-reducing sugar, mostly of sucrose [7]. Compared to starch foods or glucose, sucrose rich sugars are good for heath, as they are less fattening than starch or glucose, i.e., more calories can be consumed without gaining body weight. Further, they have low glycemic index (GI) and the coconut sap is reported to be low GI value [3]. Free amino acids, total phenolic content (TPC), flavonoid content (TFC) and antioxidant activity were found significantly higher in sap collected by CSCM compared to TM (Table 1). Sap collected by CSCM had 2.5, 1.5, 4.6 and 1.8 times higher amino acids, TPC, TFC and antioxidants, respectively, than the sap collected by TM. Continuous heating of sap caused significant reduction in phenolic content which was reduced from 92.3 mg in concentrate to 47.2 mg in coconut sugar. Since, phenolic compounds with antioxidant potential play a vital role in reducing oxidative stress, they are of great importance predominantly in human diets**.** Present study showed that the sap possess high antioxidant capacity as compared to other commonly consumed fruits like grapes, pear, watermelon, avacado, banana, lemon and peach [30].

Amino acid profile

Amino acid profiling of coconut inflorescence sap collected by CSCM, TM, sap concentrate and coconut sugar are given in the Table 2. Acidic amino acids viz. glutamic acid and aspartic acid are the predominant amino acids found both in coconut sap and its value added products. Sap collected by CSCM was rich in essential amino acids like lysine, threonine and histidine while sap collected by TM had higher content of leucine, methionine and valine. Both sap concentrate and sugar had high amount of essential amino acids. Along with amino acids, citrulline, a non-proteinogenic amino acid, also identified in all the samples analyzed. 3,4- dihydroxyphenylalanine otherwise called DOPA was detected in sap concentrate and coconut sugar which was not detectable in fresh sap collected by both methods. Glutamic acid was found maximum in all the samples studied. Heating caused significant reduction in serine, asparagine and citrulline content. Earlier studies suggested that serine was involved in the Strecker degradation reaction while heating [31]. Similarly, asparagine reacts with reducing sugars while heating [32], which leads to reduction in asparagine content. Aguayo et al. [33] reported that the heating also causes significant reduction in citrulline content. Most of the amino acids quantified in the sap were comparatively higher than the other fruit juices reported by Mostafa et al. [34]. Amino acids are one among the vital nutrients required for the human as they take part in various physiological processes, and from the present study it is clear that coconut sap

Table 2 Amino acid, phenolic acids and flavonoids composition of coconut sap collected by coco-sap chiller method (CSCM) and traditional method (TM), sap concentrate and coconut sugar

Compounds	Coconut sap (mg/100 mL)		Sap concentrate (mg/100 g)	Sap sugar (mg/100 g)	CD
	TM	CSCM			
Amino acids					
Alanine	$16.2^{c} \pm 0.3$	$15.1^{c} \pm 0.2$	$28.3^{b} \pm 0.6$	$84.5^{a} \pm 3.1$	3.99
Serine	$60.5^{b} \pm 1.1$	$58.1^{c} \pm 0.2$	$50.0^{d} \pm 1.2$	$78.0^{a} \pm 0.1$	2.03
Proline	$13.1^{c} \pm 0.9$	$14.6^{c} \pm 0.2$	$26.4^{b} \pm 0.9$	$112^{a} \pm 3.4$	4.5
Valine	$6.48^{c} \pm 0.4$	$6.10^{c} \pm 0.3$	$22.2^{b} \pm 1.0$	$50.9^{a} \pm 1.0$	1.92
Threonine	$13.7^{bc} \pm 0.3$	$12.2^{c} \pm 0.4$	$17.4^{b} \pm 0.1$	$59.1^{a} \pm 2.9$	3.67
Leucine	$0.64^{c} \pm 0.0$	$0.56^{c} \pm 0.0$	$4.27^{b} \pm 0.1$	$21.8^{a} \pm 1.4$	1.78
Asparagine	$0.86^{d} \pm 0.1$	$2.41^{b} \pm 0.2$	$1.36^{c} \pm 0.1$	$4.25^{a} \pm 0.0$	0.30
Aspartic acid	$83.7^{b} \pm 9.9$	$118^{a} \pm 5.5$	$96.8^{b} \pm 4.2$	$131^{a} \pm 4.6$	16.1
Lysine	$7.81^{c} \pm 0.2$	$5.93^{d} \pm 0.3$	$11.3^{b} \pm 0.1$	$64.5^{a} \pm 0.8$	1.06
Methionine	$5.90^{c} \pm 0.5$	$6.28^{c} \pm 0.3$	$17.6^{b} \pm 0.2$	$19.6^{a} \pm 0.6$	1.07
Histidine	$0.42^{c} \pm 0.0$	$0.65^{bc} \pm 0.0$	$1.14^{b} \pm 0.0$	$5.83^{a} \pm 0.4$	0.50
Phenylalanine	$2.14^{d} \pm 0.1$	$2.56^{c} \pm 0.0$	$28.7^{b} \pm 0.0$	$50.7^{a} \pm 0.1$	0.21
Arginine	$11.9^{d} \pm 0.3$	$17.3^{c} \pm 1.0$	$25.8^{b} \pm 0.5$	$53.7^{a} \pm 0.8$	1.73
Tyrosine	$0.29^{c} \pm 0.0$	$0.16^{c} \pm 0.0$	$1.30^{b} \pm 0.0$	$26.0^{a} \pm 0.5$	0.65
Tryptophan	$0.01^{c} \pm 0.0$	$0.01^{c} \pm 0.0$	$0.98^{a} \pm 0.0$	$0.18^{b} \pm 0.0$	0.03
Glutamic acid	$359^{b} \pm 28.9$	$626^{a} \pm 24.6$	$679^{a} \pm 20.1$	$394^{b} \pm 2.7$	53
Citrulline*	$6.38^{b} \pm 0.6$	$6.07^{b} \pm 0.2$	$8.04^{a} \pm 0.2$	$5.79^{b} \pm 0.2$	0.83
3,4-Dihydroxy-phenylalanine*	ND	ND	$3.33^{a} \pm 0.1$	$0.76^{b} \pm 0.0$	0.27
Phenolic acids					
Vanillic acid	$2.92^{c} \pm 0.13$	$3.54^{c} \pm 0.25$	$7.16^{b} \pm 0.38$	$12.8^{a} \pm 1.00$	1.37
Syringic acid	$1.80^{a} \pm 0.23$	$0.707^{b} \pm 0.08$	$1.14^{b} \pm 0.08$	$1.96^{a} \pm 0.31$	0.50
Ferullic acid	$0.302^{b} \pm 0.01$	$0.246^{b} \pm 0.02$	$0.756^{a} \pm 0.16$	$0.908^{a} \pm 0.21$	0.33
Caffeic acid	$0.042^{b} \pm 0.01$	$0.103^{b} \pm 0.03$	$0.144^{b} \pm 0.02$	$0.109^{b} \pm 0.00$	0.05
Gallic acid	$0.073^{b} \pm 0.00$	$0.044^{b} \pm 0.02$	$0.189^{a} \pm 0.02$	$0.203^{a} \pm 0.04$	0.06
p-Coumaric acid	$0.008^{c} \pm 0.00$	$0.030^{c} \pm 0.01$	$0.745^{b} \pm 0.01$	$1.27^{a} \pm 0.22$	0.27
o-Coumaric acid	$0.064^{b} \pm 0.00$	$0.062^{b} \pm 0.00$	$0.550^{a} \pm 0.20$	$0.706^{a} \pm 0.12$	0.29
2,4-Dihydroxy benzoic acid	$0.126^{a} \pm 0.02$	$0.015^{c} \pm 0.00$	$0.052^{b} \pm 0.01$	$0.037^{bc} \pm 0.01$	0.03
Gentisic acid	$0.104^{a} \pm 0.00$	$0.026^{b} \pm 0.01$	$0.089^{a} \pm 0.01$	$0.111^{a} \pm 0.01$	0.02
Protocatechuic acid	$0.182^{c} \pm 0.01$	$0.065^{d} \pm 0.00$	$0.271^{a} \pm 0.03$	$0.224^{b} \pm 0.00$	0.03
Trans-cinnamic acid	$0.636^{c} \pm 0.11$	$2.40^{b} \pm 0.15$	$1.75^{b} \pm 0.50$	$4.25^{a} \pm 0.31$	0.77
p-Hydroxy benzoic acid	$0.308^{b} \pm 0.01$	$0.963^{b} \pm 0.07$	$9.49^{a} \pm 1.76$	$9.41^{a} \pm 0.82$	2.41
Salicylic acid	$0.040^{b} \pm 0.00$	$0.477^{a} \pm 0.03$	$0.617^{a} \pm 0.16$	$0.653^{a} \pm 0.13$	0.26
Flavonoids					
Catechin	ND	$0.157^{c} \pm 0$	$0.868^{b} \pm 0.11$	$2.37^{a} \pm 0.22$	0.41
Rutin	$0.043^{b} \pm 0.01$	$0.078^{b} \pm 0.01$	$0.201^{a} \pm 0.01$	$0.192^{a} \pm 0.02$	0.04
Hesperetin	ND	$0.116^{b} \pm 0.00$	$0.140^{b} \pm 0$	$0.327^{a} \pm 0.07$	0.12
Myricetin	ND	$0.105^{b} \pm 0.02$	$0.135^{b} \pm 0.02$	$0.390^{a} \pm 0.04$	0.09
Quercetin	ND	$0.156^{c} \pm 0$	$0.235^{b} \pm 0$	$0.313^{a} \pm 0$	0.00
Apigenin	ND	ND	0.115 ± 0.01	0.230 ± 0.03	Ns
Coumarins					
Umbelliferone	$0.030^{c} \pm 0.01$	$0.056^{b} \pm 0.01$	$0.082^{a} \pm 0.01$	$0.078^{ab} \pm 0.01$	0.02

Values are given as mean ± Standard deviation of three independent samples (n = 3); ND- not detectable

Different letters in each row after the mean value are significantly different at 5% level of significance according to Duncan's multiple range test (DMRT)

*Non-protenogenic amino acids; CD- Critical Difference at 5% level of significance; ns – not significant

Table 3 Vitamin composition of coconut sap collected by coco-sap chiller method (CSCM) and traditional method (TM), sap concentrate and coconut sugar

Vitamins	Coconut sap (100 mL)		Sap concentrate (100 g)	Sap sugar (100 g)	
	TM	CSCM			CD
Water soluble vitamins					
Vitamin C (mg)	$16.3^{a} \pm 0.76$	$19.6^{a} \pm 0.95$	$6.72^{b} \pm 3.71$	$3.98^{b} \pm 1.12$	3.82
Thiamine (µg)	$0.021^{b} \pm 0.00$	$0.068^{b} \pm 0.02$	$1.35^{b} \pm 0.06$	$14.3^{a} \pm 1.16$	1.44
Niacin (µg)	$11.4^{b} \pm 0.7$	$14.9^{b} \pm 2.8$	$32.5^{a} \pm 3.6$	$34.7^{a} \pm 2.1$	6.31
Pyridoxine (µg)	$1.32^{c} \pm 0.12$	$2.35^{c} \pm 0.01$	$15.8^{b} \pm 5.4$	$101^{a} \pm 0.3$	6.79
Pantothenic acid (µg)	$1.64^{b} \pm 0.11$	$3.99^{b} \pm 0.08$	$13.4^{a} \pm 3.6$	$2.53^{b} \pm 0.2$	4.45
Biotin (µg)	$0.095^{b} \pm 0.01$	$0.073^{b} \pm 0.01$	$0.744^{b} \pm 0.06$	$2.51^{a} \pm 0.69$	0.86
Folic acid (µg)	$0.031^{b} \pm 0.00$	$0.036^{b} \pm 0.01$	$0.312^{a} \pm 0.07$	$0.260^{a} \pm 0.07$	0.13
Riboflavin (µg)	ND	ND	0.155 ± 0.04	0.248 ± 0.02	Ns
Fat soluble vitamins					
Ergocalciferol(µg)	$0.028^{d} \pm 0.00$	$0.074^{c} \pm 0.01$	$0.132^{b} \pm 0.01$	$0.171^{a} \pm 0.02$	0.03
Cholecalciferol (µg)	$0.062^{b} \pm 0.00$	$0.056^{b} \pm 0.00$	$0.256^{a} \pm 0.02$	$0.256^{a} \pm 0.02$	0.03
Tocopherol (µg)	$2.94^{d} \pm 0.46$	$7.20^{c} \pm 0.93$	$13.1^{b} \pm 2.32$	$19.6^{a} \pm 3.5$	3.15
Vitamin K1 (µg)	$0.601^{c} \pm 0.09$	$1.73^{bc} \pm 0.19$	$2.67^{b} \pm 0$	$7.35^{a} \pm 0.945$	1.20
Vitamin K2 (µg)	$0.428^{c} \pm 0.12$	$0.771^{c} \pm 0.12$	$3.42^{b} \pm 0.61$	$5.57^{a} \pm 0.61$	1.09

Values are given as mean ± standard deviation of three independent samples (n = 3)

ND not detectable, *CD* Critical Difference at 5% level of significance, *Ns* not significant

[a]Different letters in each row after the mean value are significantly different at 5% level of significance according to Duncan's multiple range test (DMRT)

and coconut sugar are rich in various amino acids and can be used substantially in daily diet.

Phenolic acids and flavonoids

A total number of 13 phenolic acids and seven flavonoids were identified from coconut sap, sap concentrate and sap sugar, and confirmed with commercial standards (Table 2). Out of 13 phenolic acids identified, eight phenolic acids belonged to hydroxybenzoic acid derivatives and four belongs to hydroxycinnamic acid derivatives and *trans*-cinnamic acid. Significant differences were observed for few phenolics acids viz. vanillic acid, ferulic acid, *p*-hydroxybenzoic acid and *trans*-cinnamic acid in sap collected by both CSCM and TM. Vanillic acid was found almost two-fold higher in sap collected by CSCM. This was followed by *p*-hydroxy benzoic acid and *trans*-cinnamic acid. Earlier, Xia et al. [7] also reported that phenolic acids such as caffeic acid, *p*-coumaric acid, protocatechuic acid and gallic acid were present in coconut sap. Significant differences were observed for flavonoid content in sap collected by both CSCM and TM and the content was very low in sap collected by TM. About five flavonoids namely catechin, rutin, hesperetin, myricetin, and quercetin were identified in sap collected by CSCM while only rutin was identified in sap collected by TM. Umbelliferone, a coumarine derivative, was also identified in sap collected by both methods. Sap concentrate and sugar prepared from sap collected by CSCM also contained higher levels of catechin, myricetin, hesperetin and quercetin. Apigenin was detected only in sap concentrate and sugar not in fresh sap. Presence of numerous health promoting phenolic acids and flavonoids in both fresh sap and its value added products make them functional food.

Vitamins

Vitamin content present in the sap collected by CSCM was significantly higher than the sap collected by TM (Table 3). Among the water soluble vitamins studied, vitamin C was at higher level in sap collected by CSCM (19.6 mg/100 mL) compared to sap collected by TM (16.3 mg/100 mL). Similarly, sap collected by CSCM contained considerably high amount of niacin, tocopherol, pantothenic acid and pyridoxine. Vitamin C content in the coconut sugar was significantly low in sap concentrate and sap sugar compared to fresh sap indicating its degradation under excess heating. Similarly, pantothenic acid and folic acid had also undergone degradation. Riboflavin could not be detected in sap collected by both methods, while it was detected in sap concentrate and sap sugar. Earlier studies also reported the presence of vitamins such as vitamin C, vitamin B complex, especially nicotinic acid in coconut sap [7, 35]. Among the fat soluble vitamins, tocopherol was found maximum followed by vitamin K1 (phylloquinone) and Vitamin K2 (menaquinone). Most of the fat soluble vitamins were present high in sap collected by CSCM compared to sap collected by TM. Heating caused substantial reduction in tocopherol content in sap concentrate and sap sugar, while other fat soluble vitamins are preserved in both the products.

Conclusions

Coconut inflorescence sap collected using novel coco-sap chiller method is fresh, unfermented and devoid of extraneous materials compared to sap collected by traditional method. Nutrient richness makes coconut sap superior than other fruit juices. As sugar and other nutrients are preserved in sap collected by CSCM, it could be easily processed into various value added products like sap concentrate and coconut sugar without the addition of chemicals. These products are rich in vitamins, phenols, flavonoids and antioxidants. These natural products have huge domestic as well as international market. To summarize, coconut sap collection using CSCM and its sale as health drink or processing into value added products ensures availability of quality products with various bioactive constituents to the consumer.

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