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Biochemical changes of coconut inflorescence sap on storage in different varieties

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Abstract
Aims: To study biochemical changes of coconut inflorescence sap on storage for one week in different varieties.
Methods and Results: The experiment was conducted at College of Agriculture, Padannakkad and Nileswaram farm, RARS Pilicode under irrigated condition during 2014-2016. Four varieties were selected for evaluation. Palms with similar age and morphological characters were selected as experiment units. The varieties tried were Malayan Yellow Dwarf, Kerasree, West Coast Tall and Keraganga**.** Fully emerged unopened bunches were selected for tapping and samples were collected. The sap collected from each variety was stored at refrigerated condition for one week and biochemical properties *viz*., pH, electrolyte concentration, reducing sugars, non-reducing sugars, total sugars, alcohol, phenol and vitamin C were assessed on 1st (D1), 3rd (D2) and 7th (D3) days of storage. The results are revealed that without any differentiation between the varieties there was a significant reduction in the pH, total sugar, reducing sugars and non reducing sugar while there was an increase in alcohol, vitamin C and phenol content.
Conclusions: The changes in CIS during storage for 1 week indicated that without any differentiation between varieties there was a decrease in pH, total sugar, reducing sugar and non-reducing sugar while there was an increase in alcohol and vitamin C content.
Significance and Impact of the study: To know the changes in biochemical properties of coconut inflorescence sap of different varieties on storage whereas there is no variation among the varieties with respect to biochemical properties of coconut inflorescence sap on storage.

Keywords: Coconut inflorescence sap, Varieties, Biochemical and nutritional changes on storage

Introduction
Coconut inflorescence sap (CIS) is extracted by a method called tapping which involves selective bleeding of unopened coconut inflorescence which is a traditional practice in all coconut growing countries. The exuding sap is a sweet translucent juice, oyster white in colour with high nutritive value. It is a rich source of reducing and non reducing sugars with plenty of minerals and vitamins. It is also a good source of iron, phosphorous and ascorbic acid. The most significant characteristic of coconut inflorescence sap is its low glycemic index an indication of the extent of sugar absorbed into the blood which makes it suitable even for consumption for diabetic patients (Manohar *et al*., 2007) [8]. In recent times there is a huge global demand for low GI sugars while its availability is limited. CIS which is a natural source of low GI sugars can fill up this gap. CIS is susceptible to natural fermentation to toddy within a few hours of extraction. Changes occur in nutritional and biochemical properties such as pH, total electrolyte concentration, total sugars, reducing sugars, non reducing sugars, vitamin C, alcohol, mineral elements and phenols. The utilization of coconut inflorescence sap as a beverage depends on its preservation in non alcoholic form under ambient condition. properties such as pH, total electrolyte concentration, total sugars, reducing sugars, non reducing sugars, vitamin C, alcohol, mineral elements and phenols. The utilization of coconut inflorescence sap as a beverage depends on its preservation in non alcoholic form under ambient condition. Fermentation of coconut inflorescence sap occurs in three stages which start with lactic acid fermentation followed by alcoholic fermentation and finally acetic fermentation. The microbial activity at each stage helps the activity of the micro-organisms in the subsequent stage.

Materials and Methods
The experiment was conducted at College of Agriculture, Padannakkad and Nileswaram farm, RARS Pilicode under irrigated condition. Four varieties were selected for evaluation. Palms with similar age and morphological characters were selected as experiment units.

The experiment was laid out in RBD replicated 5 times with four varieties as treatments *viz*.

T_1 – Malayan Yellow Dwarf
T_2 – Keraganga
T_3 – West Coast Tall
T_4 – Kerasree

Fully emerged unopened bunches were selected for tapping. The bunch was tied at many places to prevent opening of inflorescence and to facilitate sap flow. The first cut was made after four days. After the first cut, every day the cut surface was opened twice by slicing the cut edge again and the inflorescence was delicately beaten or tapped twice a day to stimulate flow of sap. The sap flow started 8 - 12 days after the first cut and the flow continues for 40 - 60 days. Slicing the cut edge and tapping were repeated every day. The sap was collected in a plastic container tied to the bunch. The sap collected from each variety was stored at refrigerated condition for one week and biochemical properties *viz*., pH, electrolyte concentration, reducing sugars, non-reducing sugars, total sugars, alcohol, phenol and vitamin C were assessed on 1st (D1), 3rd (D2) and 7th (D3) days of storage by following procedures.

The pH of sap was measured using electronic pH meter (Saini *et al*., 2001) [14]. The total electrolyte concentration of sap was measured by using Conductivity Bridge (Jackson, 1958) [5]. The total sugar content of sap was estimated as per the procedure outlined by (McCready *et al*., 1950) [10]**.** The estimation of reducing sugars in sap was done by dinitro salicylic acid (DNS) method (Somogyi, 1952). The observation under total sugars and reducing sugars were used for calculating non reducing sugars based on the procedure suggested by Ranganna (1977) [12] and expressed as percent on fresh weight basis. The vitamin-c content of sap was estimated by the volumetric method (Sadasivam and Manickam, 2008) [13]. Alcohol content of sap was estimated by titration method using potassium dichromate and sodium thiosulphate (William and Darwin, 1950) [19] Phenols content was estimated by Folin-Ciocalteau method (Mayr *et al.,* 1995) [9]. The data obtained were analyzed statistically and significance was calculated at ($p< 0.05$) levels of probability.

Results

The results on influence of storage on pH of coconut inflorescence sap from different coconut varieties are presented in Table 1.

1. **pH:** There was a significant reduction in pH of coconut inflorescence sap on storage. All the varieties behaved in the similar manner. The lowest pH of 3.72 was obtained in variety Kerasree one week after storage.
2. **Electrolyte concentration:** The electrolyte concentration of coconut inflorescence sap was significantly increased on storage. It increased from 0.19 dS m-1 on day 1 to 2.93 dS m-1 on day 3 and finally reached 3.92 dS m-1 on day 7. The highest electrolyte concentration of 4.38 dS m-1 was recorded in the variety Kerasree (T4) one week after storage, which was significantly higher than other varieties.
3. **Reducing sugars:** There was a significant reduction in reducing sugar content from day 1 (0.47 g 100ml-1) to day 3 (0.38 g 100ml-1). Beyond day 3 there was a sharp decline and it reached zero on day 7. All the varieties behaved in a similar manner.
4. **Non reducing sugars:** Non reducing sugars content decreased significantly from day 1 (10.06 g 100ml-1) to day 3 (8.67 g 100ml-1) and finally reached 6.31 g 100ml-1 on day 7.
5. **Total sugars:** There was a significant reduction in total sugars of coconut inflorescence sap on storage. All the varieties behaved in the similar manner.
6. **Alcohol:** The alcohol content of coconut inflorescence sap was significantly increased on storage. It increased from 0.07% on day1 to 1.93% on day 3 and finally reached 2.74% on day 7. The variety WCT recorded the highest alcohol content (3.38%) on day 7 which was significantly higher than all other varieties.
7. **Phenol:** The phenol content of coconut inflorescence sap was significantly influenced by storage. It increased from 0.49 mg 100ml-1 on day 1 to 0.94 mg 100ml-1 on day 3 and finally reached 1.43 mg 100ml-1 on day 7. All the varieties showed similar trend of results.
8. **Vitamin C:** There was a significant influence of storage on vitamin C content of CIS. It increased sharply from day 1 (1.64 mg 100ml-1) to day 3 (15.93 mg 100ml-1) and further increased to 16.78 mg 100ml-1 on day 7. The highest value was associated with T2 (17.18 mg 100ml-1) on day 7, which was on par with T3 (17.08 mg 100ml-1) on the same day.

Discussion

The present study showed that during storage of CIS, changes were observed in biochemical properties and nutritional qualities. These changes would have probably been induced by the fermentation of sugars. Natural fermentation of CIS consists of three stages *viz*., initial lactic acid fermentation, a middle alcoholic fermentation and a final acetic fermentation. At each stage, the microbial activity helps the activity of the micro-organisms in the next stage (Atputharajah *et al*., 1986).

1. **pH:** The storage of CIS sample resulted in a significant decrease in pH. The lowest pH was obtained in variety Kerasree one week after storage. This may be due to an acidification from lactic acid bacteria as reported by Xia *et al*., (2011) [20]. Similar results have been reported by Singaravadivel *et al*. (2012) [15]. Further Hebbar *et al*. (2015) [4] confirmed that fresh sap has slightly alkaline pH (7.5 - 8) and on fermentation a reduction in pH occurs and the pH attains values of 5.5 to 4.21 on 24 hours storage. Similar results were also reported by Stringini *et al*. (2009) [17] and Hebbar *et al*. (2015) [4].
2. **Electrolyte concentration:** The electrolyte concentration of coconut inflorescence sap increased significantly on storage. The highest electrolyte concentration was observed in the variety Kerasree. This might be due to the production of higher quantities of alcohols, phenols and vitamins during the fermentation of sugars.
3. **Reducing sugars:** Irrespective of the variety, the reducing sugar content of sap showed a significant reduction on storage. Sap of each variety showed a significant reduction in reducing sugar content and finally it reached zero on day 7. These findings are on line with the reports of Atputharajah *et al*. (1986), who reported that initially sucrose will be converted into glucose and fructose and the amount of these reducing sugars will get reduced with time as the microbes that involve in fermentation will use the reducing sugars for their energy needs. Konan *et al*. (2014) [6] also stated that the initial decrease of the reducing sugars could be as a result of the direct use of the residual glucose by microorganisms.
4. **Non reducing sugars:** The non-reducing sugar content was also reduced significantly on storage in the case of

all the varieties. This may be due to the conversion of sucrose to glucose and fructose during initial fermentation. Similar results were reported by Xia *et al.* (2011) [20]. However in the present study it could be observed that unlike reducing sugar, non reducing sugar was not completely exhausted indicating that the fermentation process doesnot end with 7 days of storage of coconut inflorescence sap.

5. **Total sugars:** Falling in line with the trend of results obtained for reducing and non reducing sugars, the total sugar content was also significantly reduced during storage. All the varieties behaved in the similar manner. Similar results were also reported by Eze and Ogan (1988) [3]. This fluctuation in sugar content in coconut inflorescence sap might be due to the utilization of sugar for energy and the conversion of sugars into other substances by microbial activity. Similar findings were also observed by Opara *et al*. (2014) [11].
6. **Alcohol:** As expected the alcohol content of coconut inflorescence sap was significantly increased on storage. The variety WCT gave the highest alcohol content of 3.38% on day 7. This was in harmony with the findings of Singaravadivel *et al*. (2012) [15] who found that alcohol content of coconut inflorescence sap increases on storage. It increased to 4.5% after 24 hours against the initial value of 0.2%.
7. **Phenol:** Irrespective of the variety studied, the total phenol content of sap increased on storage. Highest value was obtained on day 7 for all the treatments. The increase in the phenol content may be attributed to the degradation of glucoside bonds by the acids produced during natural fermentation which would have yielded polyphenolic compounds. The metabolism of certain microorganisms could have also contributed to the addition of polyphenols to the system. These results are in accordance with those reported by Landbo and Meyer (2004) [7] and Xia *et al*. (2011) [20].
8. **Vitamin C:** The perusal of results on changes in vitamin C content of CIS on storage revealed that the vitamin C content increased significantly on storage in the case of all the varieties wherein there was a sharp increase in vitamin C content from day 1 to day 3 and thereafter it appeared start stabilizing on day 7. The increased vitamin C content may be attributed to the increased activity of yeast which would have synthesized vitamin C during fermentation. Similar results were also reported by Bremus *et al*., (2006) [2], Singavardiel *et al*. (2012) and Syamala Devi *et al*. (2015) [18].

Table 1: Biochemical Changes in coconut inflorescence sap one week after storage.

Treatment combinations	pH	Electrolyte concentration (dS m^{-1})	Reducing sugars (g/100ml)	Non reducing sugars (g/100ml)	Total sugars (g/100ml)	Alcohol (%)	Phenol (mg/100ml)	Vitamin C (mg/100ml)
T1	5.56	2.17	0.24	8.95	9.19	1.28	0.92	11.23
T2	5.51	2.55	0.28	8.58	8.86	1.58	0.97	11.58
T3	5.54	2.01	0.29	7.74	8.04	2.00	0.95	11.78
T4	4.94	2.65	0.30	8.14	8.44	1.45	0.97	11.16
D1	6.7	0.19	0.47	10.70	10.55	0.07	0.49	1.64
D2	5.17	2.93	0.38	8.68	9.05	1.93	0.94	15.93
D3	4.29	3.92	0.00	6.31	6.31	2.74	1.43	16.75
T1D1	6.88	0.18	0.40	9.51	9.87	0.08	0.39	1.64
T1D2	5.42	2.60	0.33	8.81	9.12	1.48	0.94	15.54
T1D3	4.38	3.74	0.00	7.63	7.75	2.28	1.43	16.52
T2D1	6.6	0.19	0.47	9.32	9.70	0.04	0.54	1.36
T2D2	5.44	3.34	0.38	8.63	8.95	1.96	0.94	16.22
T2D3	4.5	4.14	0.00	7.44	7.58	2.76	1.43	17.18
T3D1	6.6	0.21	0.49	8.90	9.29	0.06	0.48	1.84
T3D2	5.44	2.40	0.40	8.21	8.54	2.56	0.94	16.44
T3D3	4.58	3.42	0.00	7.02	7.17	3.38	1.43	17.08
T4D1	6.72	0.19	0.51	9.10	9.49	0.08	0.55	1.72
T4D2	4.4	3.38	0.40	8.41	8.74	1.74	0.94	15.54
T4D3	3.72	4.38	0.00	7.22	7.37	2.54	1.43	16.22
CD – T (0.05)	0.09	0.07	0.016	0.17	0.15	0.05	0.04	0.17
CD – D (0.05)	0.07	0.06	0.014	0.14	0.13	0.05	0.04	0.14
CD - T D (0.05)	0.15	0.13	0.03	0.30	0.26	0.10	0.08	0.30

Author Contribution statement

All authors discussed the results and contributed to the final manuscript

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Conflict of interest

The authors declares that there is no conflict of interests regarding the publication of this paper

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