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Effect of Thermal Treatment on Keeping Quality of Palmyrah Sweet Sap

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Abstract: The palmyrah palm (*Borassus flabellifer*) is grows extensively in Northern part of Sri Lanka. Sweet sap is the most important product obtained from palm, could be extracted from both male and female inflorescences (dioecious) by tapping process. Harvested sap should be immediately processed due to the highly perishability as it under goes spontaneous fermentation via air born yeast microflora. The main objective is the study was to identify the optimum temperature and time for preservation of sweet sap and detected the suitable shelf life for bottled sweet sap via the physical, chemical, microbiological and sensory quality of preserved sweet sap. Traditionally quick lime is added to prevent the fermentation; phosphoric acid was selected at pH 8 based on the sensory analysis for the removal of lime as calcium phosphate. Delimed sweet sap was used for the study of thermal treatment in order to increase the keeping quality of sweet sap. Three experiments with different thermal treatments were conducted to preserve the sweet sap. Experiment 1 (preservatives such as citric acid and sodium metabisulphite) and as a result of gas formation due to the fermentation, Experiment 2 (thermal treatments of 60, 70 80 and 90 ^{o}C) were rejected. In the 3rd experiment the bottled sweet sap was heated at 105, 110 and 115 ^{o}C for different time intervals (15 and 30 min) and stored at room temperature (30±2 ^{o}C). There were no significant differences ($p<0.05$) in physicochemical (TSS, total and reducing sugar) and microbial (TPC and yeast and mould) evaluation of selected treatments at 60 days of storage. Based on sensory evaluation, thermal processing at 105 ^{o}C for 15 min was selected as the best treatment and it could be stored for 60 days without changing its native characteristics.

Keywords: Palmyrah, Preservation, Sweet sap, Temperature and Time

1. Introduction

The palmyrah palm (*Borassus flabellifer* L.) is present in tropical part of Sri Lanka, East Asia and Africa. The most important product of palmyrah palm is the sap. It could be extracted from the male or female

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inflorescences during January to August by the process known as "tapping (Theivendirarajah, 2008). Palmyrah inflorescence unfermented sap called as sweet toddy (neera or pathaneer), contained medicinal properties (Vengaiah *et al.*, 2013) and yielding a healthy nutritious drink. It is the one of the most important products, because it is rich in sugars, minerals (Kapilan *et al.*, 2015), if it is left exposed to the atmosphere; it undergoes both enzymatic and microbial fermentation within a couple of hours and become alcoholic beverage called as toddy (Davis and Johnson, 1987). Spontaneous fermentation of sap is caused by accumulation and growth of yeast from the air (Jeyaratnam *et al.*, 1984), at present arbitrary quantities of lime (calcium hydroxide) is used to arrest fermentation (Mary *et al.*, 2014). This is unsuitable for ready to serve drinks or for the preparation of natural treacle, sugar candy and jaggery as value added products. Consequently it needs further de-limeing steps for the production of value added products. However, the sap is seasonal and can be obtained only in the first and second quarter of a year, therefore the production of sap based products cannot be done throughout the year and also no approved preservation methods are available to preserve palmyrah sweet sap. This study was carried out with the aim to conserve the fresh sweet sap with increased the shelf life using suitable thermal preservation method. Therefore the main objective is this study was to identify the optimum temperature and time for the preservation of sweet sap and to detect the suitable shelf life period for storage of bottled sweet sap.

2. Materials and Methods

2.1 Sample Collection

Pooled palmyrah sweet sap was obtained from the Chavakachcheri palm development society and used for the preservation study.

2.2 Analysis of Fresh Sweet Sap

Collected sweet sap was filtered with muslin cloth and the initial parameters (pH and alcohol content) were measured to ensure the quality of sap.

2.3 Selection of De-limeing Agent

Food grade acids such as citric acid, maleic acid, phosphoric acid and tartaric acid were used to determine the suitable agent for neutralization of sap (de-limeing) and increase settling of sediments, A 5 points Hedonic scale test in terms of colour, flavour, appearance, mouth feel and overall acceptability was carried out for selection of best de liming agent of palmyrah sweet sap.

2.4 Selection of Suitable pH for De-limeing

Diluted (1:4) phosphoric acid (food grade) was added until the pH was brought for 7, 8, 9, 10 and 11. Then direct heat was applied to 60 ^{o}C, for the above pH adjusted sap separately and allowed to settle for 1 hour. Most suitable pH was selected through the sensory panel. A 5 points Hedonic scale test was carried out to the selection of optimum pH.

2.5 Preservation Techniques

Three experimental trials with different treatments were conducted during this study.

Experiment trial I: The pH of the de-limed sap was adjusted at 4.5 with citric acid then used for the treatments I and II (contained SMS - 50 ppm). Treatment III (pH = 8) was not contained no additives and used as a control. All the treatments were bottled then heated at 90 ^{o}C for 20 min and allowed to cool at room temperature. Then heat treated bottled sweet sap were used for sensory evaluation immediately.

Experiment trial II: The de-limed bottled sweet sap without any additives was heated at 60, 70, 80 and 90 ^{o}C for different time intervals (10, 20 and 30 min) and allowed to cool at room temperature. Then heat treated bottled sweet saps were stored at room temperature and were used for sensory evaluation at 24 h of storage.

Experiment trial III: The de-limed bottled sweet sap without preservative was heated at 105, 110 and 115 ^{o}C for different time intervals (15 and 30min) then allowed to cool at room temperature. Then heat treated bottled sweet saps were stored at room temperature and were analyzed at 30 day intervals to determine the shelf life of bottled sweet sap.

2.6 Sensory Analysis

The sensory evaluation (5 point hedonic scale test) was conducted by 30 untrained sensory panelists were selected from palmyrah research staff. The best treatment was selected in terms of colour, flavour, appearance, mouth feel and overall acceptability through statistical analysis.

2.7. Physiochemical Analysis

pH (Sension+ pH 31-Spain pH meter), total sugar (Miller, 1959), reducing sugar (Miller, 1959), alcohol (ebulliometerDujardin-Salleron), acidity (Sri Lanka Standard Institute, 1985) and brix value were evaluated in 30 days intervals.

2.8 Microbial Analysis

TPC (Sri Lanka Standard Institute, 1985) and yeast and mould count Sri Lanka (Standard Institute, 1992) were determined in monthly intervals.

2.9 Statistical Analysis

The sensory data with duplicate were analyzed using non-parametric procedure, according to the Friedman test using Minitab 16 software package.

3. Results and Discussions

3.1 Analysis of Fresh Sweet Sap

Fresh sweet sap was oyster white colour sap due to the addition of lime to arrest the fermentation. pH of the collected sap was 11.0, this results was agreed with Velauthamurty *et al.,* 2015 and there were no alcohol content.

3.2 De-limeing of Sweet Sap

Selection of de-limeing agent: Lime is

added to sap to prevent the fermentation (Mohanadas, 1974) due to the application of lime the pH of the sweet sap was more than 10. Therefore de-liming was carried out with different acids such as citric acid, malic acid, phosphoric acid, tartaric acid to reduce the pH of the sweet sap and form insoluble lime salts. Colloidal matters such as pectins, hemicelluloses, proteins and colored compounds are absorbed by the precipitated ions (Nair *et al.*, 2009). During the de-liming process sweet sap was heated at 60 °C to increase the precipitation of calcium phosphate and some colloids flocculation. Based on the median values of the sensory attributes, phosphoric acid (Figure 1) was selected as best acid among the selected acids. During this process, a wide range of chemical and physical reactions takes place in the sap. The main chemical reactions include precipitation of calcium phosphate, denaturation of proteins (and other organics, such as gums, pectins and waxes), inversion of sucrose due to the combined action of pH and temperature, degradation of reducing sugars to organic acids due to high pH and temperature, precipitation of organic and inorganic acid salts and formation of colour groups due to the polymerisation (either enzymatically or thermally) of phenolic compounds.

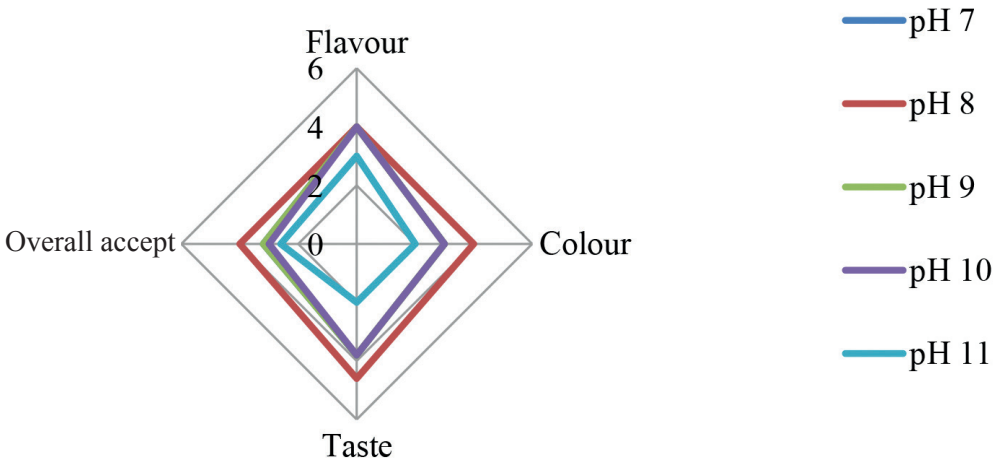

Figure 1: Web diagram for selection of de-limeing agent

Selection of pH for de-limeing: The initial pH of the sweet sap was more than 10 therefore it was de-limed to the appropriate pH such 7, 8, 9 and 10 and the best pH was selected based on the sensory attributes. There were no significance differences in median values of the flavour between different pH while colour, taste and overall acceptability were showed highest median values for pH 8. Calcium phosphate is more soluble at low temperatures, when the sap is heated there are competing reactions between the unreacted

calcium and phosphate, the formed calcium phosphate and sap constituents. Formation of calcium phosphate is spontaneous and complete at pH 7.8 with the heating process. Therefore, pH 8 was selected as the best de-limeing pH. Further, it had shown the highest value for quality attributes according to the web diagram (Figure 2). Australian cane sugar industry is best described as simple defecation of unwanted substance, is based on the addition of lime as lime saccharate to intermediate juice (72–76 °C) at pH of 7.8 (King, 1930).

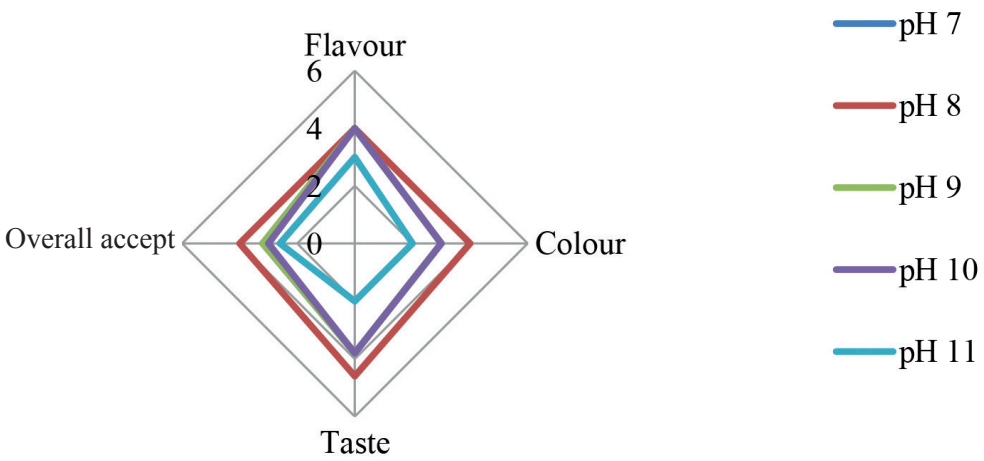

Figure 2: Web diagram for selection of pH

Heat treatments for bottled sweet sap: Sweet sap is highly susceptible to natural fermentation at ambient temperature within a few hours of collection from the palm. Once fermented, it transforms into toddy with 4 % alcohol. Using several technologies developed by various research institutes, sweet sap is processed and preserved in its natural form to retain the vitamins, sugar, and other nutrients beneficial for health. Heat preservation methods such as pasteurization and sterilization are necessary to preserve and extend the shelf life of the product.

Experiment I: Mohanadas (1974) preserved coconut sap at acidic pH, therefore this experiment was designed at acidic pH 4.5 (Treatment I), with preservative (Treatment II) and without preservative at pH 8 (Treatment III). Based on the sensory evaluation treatment I and II were rejected. While treatment III was selected as the best treatment at the 24 hours of storage.

Experiment II: Thermal treatments 60, 70, 80 and 90 °C were applied for 10, 20 and 30 min and stored at room temperature. All the above treatments were spoiled at 48 h of storage and gas formation was observed. Mohanadas (1974) reported that coconut sap could be preserved by the heat treatments: 80 °C for 25 min and 90 °C for 20 min using Lanka Glass Co. bottles. However, it was not recommended storage temperature.

According to the results of our experiments, it was found that sweet sap by heat treating 80°C for 30 min, could be stored for six months at 4°C.

Experiment III: Nair *et al*. (2013) reported that thermal processing of coconut neera at a temperature of more than 95 °C and reduction of thermal stress by addition of bio-preservative 'nisin' at a concentration of 10 ppm was found to enhance shelf life of coconut neera. Therefore, in this experiment different temperature, such as 95, 100, 105, 110, and 115 °C for different time intervals (15 min, 30 min) without preservatives were selected (T1−T10). Microbial analyses of thermal treated sweet saps were done at initial period of storage. Treatment (T1) (95 °C, 15min) , T2 (95 °C, 30 min), T3 (100 °C,15 min) and T4 (100 °C, 30 min) contained TPC and yeast and mould count which were more than microbiological tolerance limit (Sri Lanka Standard Institute, 1985). Therefore treatment 1, 2, 3 and 4 were rejected and the rest of the treatments (T5−T10) were analyzed at 30 days' time intervals (Table 1). Median value of colour (higher color intensity) and taste was less for treatments T8 and T10 due to the increasing rate of melanoidin formation through the Maillard reaction specifically monosaccharides present in the sap react with amino acids under the alkaline condition.

Table 1: Estimated median value obtained from sensory evaluation of selected treatments at initial time of storage

Quality attribute	T5 105°C, 15 min	T6 105°C, 30 min	T7 110°C, 15 min	T8 110°C, 30 min	T9 115°C, 15 min	T10 115°C, 30 min	P value
Flavour	4.000	4.000	4.000	4.000	4.000	3.000	0.00
Colour	5.000	4.833	4.083	3.917	4.000	3.167	0.00
Taste	4.896	3.979	4.062	3.895	4.062	3.479	0.00
Overall acceptability	4.938	4.271	4.021	3.937	4.021	3.021	0.00

3.3 Sensory Evaluation of Selected treatments

According Table 1 treatment 5 (Temperature 105 °C, Time 15 min) showed the highest estimated median value for quality attributes such as colour, taste, flavour and overall acceptability at initial time of storage. While there were no significant different in median value of quality attributes between the storage periods (Table 2). Therefore, treatment 5 was selected as the best treatment for bottling of sweet sap.

Table 2: Estimated median value obtained from sensory evaluation of best treatment (T5) at different storage period

Quality attributes	0 time	30 days	60 days
Flavour	4.000	4.750	4.750
Colour	5.000	4.917	5.000
Taste	4.896	4.708	5.000
Overall acceptability	4.938	4.958	5.000

3.4 Physicochemical Analysis

Reducing sugar: The reducing sugar content (Table 3) of bottled sweet sap treated with different treatments under different temperature and time period showed significant different between storage period. In the case of reducing sugar analysis 0.16 % was taken as a hypothetical mean (Theivendirarajah, 2008). According to the (t-test) result the p-value is 0.00, it is less than the tested value at 5 % significance level. So this statistical result strongly proved that whole value is greater than hypothetical mean value

Table 3: Amount of reducing sugar for selected treatments (g/100mL)

Temperature (^oC)	Time (min.)	Treatments	0 time	30 days	60 days
105	15	5	0.165±(0.012)	0.183±(0.014)	0.173±(0.011)
	30	6	0.159±(0.014)	0.185±(0.012)	0.186±(0.013)
110	15	7	0.164±(0.013)	0.190±(0.015)	0.178±(0.017)
	30	8	0.164±(0.011)	0.186±(0.015)	0.184±(0.014)
115	15	9	0.172±(0.012)	0.193±(0.013)	0.179±(0.012)
	30	10	0.157±(0.015)	0.186±(0.014)	0.216±(0.016)

Total sugar: According the Table 4 at 0 times of storage all treatments having more than 11 % of total sugar content. The fresh sap total sugar limit was 10-16 %. At 30 and 60 days of storage amount of total sugar was decreased with increasing the temperature and also decreased with increasing the storage period while the rate of decreasing amount of total sugar was less in 105 ^oC for 15 min than other treatments. Therefore 105 ^oC for 15 min is more suitable for the sweet toddy preservation.

Brix value: Brix is the measurement in percentage by weight of total soluble solids in sweet sap. According to the Table 5 the t-test result showed that the p-value as 0.00 and it seems that this value is lower than tested ($p<0.05$) significance level. Samples having the Brix value 11 was taken as a hypothetical mean (Jeyaratnam, 1986). These statistical results strongly proved that whole values were greater than the hypothetical mean value therefore all values are suitable for sweet toddy.

Table 4: Amount of total sugar for selected treatments (g/100 mL)

Temperature (°C)	Time (min.)	Treatments	0 time	30 days	60 days
105	15	5	12.664±(0.012)	9.639±(0.011)	8.427±(0.013)
	30	6	11.234±(0.015)	9.264±(0.014)	8.375±(0.016)
110	15	7	11.644±(0.018)	9.264±(0.015)	7.965±(0.016)
	30	8	11.600±(0.015)	8.942±(0.014)	7.364±(0.018)
115	15	9	11.165±(0.015)	7.460±(0.015)	7.434±(0.018)
	30	10	11.016±(0.014)	7.268±(0.011)	7.381±(0.013)

Table 5: Brix value (TSS) for selected treatments

Temperature (°C)	Time (min.)	Treatments	0 time	30 days	60 days
105	15	5	11.12	11.19	11.24
	30	6	11.11	11.12	11.23
110	15	7	11.05	11.08	11.13
	30	8	11.04	11.06	11.12
115	15	9	11.02	11.12	11.14
	30	10	11.00	11.06	11.12

TPC and Yeast count for the treated sweet toddy: The number of colony count (cfu/ml) in NA and PDA plates at 0 to 60 days of storage was less than that of limit (less than 50) present in the Sri Lanka Standard (Sri Lanka Standard Institute, 1985)

Alcohol and acidity content: Sugars present in sweet toddy undergo first alcoholic fermentation and then acidic fermentation consequently on the action of microorganism (Theivedirarajah, 1986) . There were no alcohol and acid formation in the all treatments. The total sugar content was decreased and reducing sugar was increased with storage however conversion of the sugars to alcohol via glucose metabolism was not observed. Therefore it was due to conversion of total sugar to reducing sugar and physic chemical changes in the sap with storage.

4. Conclusions

During the sweet sap collection spontaneous fermentation is take palace, which is prevented by the application of quick lime on the colleting pots. Phosphoric acid was found to be the best de-limeing agent based on the sensory, microbial and physic chemical characteristics. Preservation of sweet sap by heating at 105°C for 15 min was selected as

the best treatment and this heat treated sap was stored for 60 days at room temperature without changing its native characteristics.

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