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RESEARCH ARTICLE

Physicochemical characteristics of Palmyrah bottled toddy and their effect on storage

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ABSTRACT

In the northern and eastern parts of Sri Lanka, the cloudy whitish toddy is obtained by tapping young or matured inflorescence of both female and male palmyrah palm (Borassus flabellifer), preserved them by using pasteurization techniques and then sold. In the present study, the effect of heat and storage period on the physicochemical characteristics as well as nutritional and mineral content of fresh and preserved toddy were determined. Preserved toddy showed a significantly ($P<0.05$) higher alcohol (%) 3.80(±0.07), acidity (%) 0.60(±0.07), turbidity (NTU) 2,694.50 (±0.07), relative density 1.01 (±0.07) and total solid (g/L) 58.22 (±0.07) while the total soluble solids (^{o}brix) 5.93(±0.07) and pH 3.43 (±0.07) showed significantly lesser values compared to the fresh toddy. However, there was no significant ($P>0.05$) difference in protein 1.42(±0.01) g, fat 0.02(±0.00) g, iron 11.9(±0.3) mg, phosphorus 0.64(±0.04) mg, calcium 13.5(±0.5) mg and magnesium 5.3(±0.5) mg/100 mL. During pasteurization, total sugar and ascorbic acid content were significantly ($P<0.05$) decreased from 3.04(±0.06) to 2.80(±0.01) g/100 mL and 3.0(±0.01) to 2.1(±0.01), respectively. When pasteurized toddy was stored at room temperature, there were no significant differences between the storage period of twelve months in alcohol 2.9(±0.60), acidity 0.5(±0.10), TSS 7.9(±0.6), pH 3.6(±0.01) and TS 69.4(±9.3). In conclusion, the physicochemical characteristics of palmyrah bottled toddy were not influenced by the pasteurization process and period of storage of twelve months.

***Keywords**: Palmyrah, toddy, pasteurization, alcohol percentage and storage*

INTRODUCTION

Throughout the world, the group of most alcoholic beverages is produced by the natural alcoholic fermentation of sap, which is obtained locally by tapping method from many tropical palms of the Palmae family called toddy or palm wine (Okafor, 1978). There are common species of palms used for such a purpose; Palmyrah (*Borassus flabellifer*) (Theivendirarajah, and Chrystopher 1987), coconut (*Cocos nucifera*) (Singaravadivel *et al.*, 2012), wild date (*Phoenix sylvestris*) (Manel *et al.*, 2011), Kithul (*Nipa fruticans*) (Seneviratne and Dissanayake, 2016) and oil palm (*Elaeis guineensis*) (Samuel *et al.*, 2016).

Among the above, palmyrah toddy, which is highly produced in the Northern and Eastern parts of Sri Lanka, is a sugary, weighty, milky white, strongly effervescent, non-distilled product and rich nutrient source containing alcohol 0.5-6.0 % (v/v), total acidity 0.3-0.5 %(v/v), total solids, total sugar < 1.0% (w/v), protein, total ash 0.25 % (w/v) and vitamin C 39.58 mg L^{-1} (Theivendirarajah and Kumuthini, 1986). In addition, nutrient content, it also contains a dense population of wild yeast and bacteria. Thus, during the collection of toddy in earthen pots, it undergoes spontaneous fermentation by yeast converting sugars into alcohol. Subsequently, acetic acid fermentation carries out and then forms acids. Therefore, to prevent further fermentation, and also prolong the shelf life of the toddy, it is preserved by the bottling which includes the following steps such as filtration of fresh toddy, bottling, capping, and pasteurization (Mahilrajan *et al.*, 2014).

Palmyrah toddy bottling is still carried out as a small scales enterprise involving Palm Development Cooperative Societies situated in the Northern and Eastern parts of Sri Lanka. Generally, among lower-income people, it is used as a popular drink, and since the ancient period, it is believed to be suitable for health and eyesight and it also has helped as a sedative (Tuley, 1965). This low- expenditure product, in its all phases, is usually produced using only simple equipment. Due to the cheapness, low-income people commonly drink them. Further, good hygiene practices in their handling and consumption seem increasing their earnings.

With increasing urbanization in the county, the public has pursued knowledge about the availability of nutrients in the foods and also about the potential of interaction effect of food and nutrients. Therefore, this study was conducted to compare the physiochemical and nutritious features of bottled and fresh palmyrah toddy and their changes over storage time.

MATERIALS AND METHODS

Collection of Palmyrah toddy

Fresh and preserved (pasteurized) samples of three of 625 mL in a glass bottle were collected for the comparison of the physicochemical and nutritional analysis of traditional fermented toddy. For the storage study, two different batches of pasteurized toddy samples were collected from Atchuvelly Palm Development Cooperative Society, Jaffna, Sri Lanka, in July 2019. Samples were selected randomly and analyzing a single sample with triplicate was performed for each sample to reduce the variance and source depletion.

Effect of fermentation time on the alcohol production

Fresh toddy was taken immediately after collection and then its percentage of alcohol, pH and titratable acidity was measured at 4 h time intervals.

Analysis of the physicochemical parameter of the toddy

The fresh and preserved (stored for 24 h) toddy samples were used for the analysis of pH, total soluble solids, turbidity, specific gravity, total solids, titratable acidity, alcohol content, sugar content, total ash, crude protein, total fat, ascorbic acid content and mineral content.

Effect of storage period on characteristics of toddy

The effect of storage on physicochemical characteristics was studied by using preserved bottled toddy and stored at room temperature (28±2 ºC) then analysis (alcohol, total acidity, pH, TSS, and total solids) was conducted at 30 d interval for 12 months.

Physicochemical parameters

pH

A homogenous toddy sample (25 mL) was taken in a 25 mL clean glass beaker and its pH was measured by using a digital pH meter (Sension PH 31-Spain) at room temperature (28±2 °C). The standard buffers of pH 4, 7, and 10 pH were used to calibrate of pH meter. The electrode was rinsed and immersed in the sample and the pH was measured.

Estimation of total soluble solids

Using a hand refractometer, total soluble solid (TSS) was measured. One or two drops of the juice sample were kept on the prism and the glass plate was closed over the sample. Then, its reading was noted by looking through the piece of the eye. The sample was placed in between the refractometer's measurement prism and the cover layer. The shadow line created between the dark part and the illuminated part is the net result.

Estimation of turbidity

The turbidity of the toddy sample was determined by using a turbidity meter (HACH).

Determination of specific gravity

Clean and thoroughly dried specific gravity bottle weight was taken. Freshly boiled cooled distilled water was maintained at 28±2 ºC and filled up to the mark and the weight was taken. Water was removed and the bottle was dried again. The sample was filled and maintained at the same temperature and the weight was taken.

$$\text{Specific gravity at } 28\pm2\ ^{\circ}\text{C} = \frac{C-A}{B-A}$$

where;
C = weight of sample with specific gravity bottle
A = weight of empty specific gravity bottle

B = weight of specific bottle with water

Total solids

Total solids (TS) were measured according to the method set by (AOAC, 2000). Dry empty dish dried at 103±2 ºC for 30 min. The dish was taken out of the oven and kept in a dissector until it was cooled to room temperature. The weight of the empty dish was measured and 20 mL of samples were added. The sample was placed in an oven at 103±2 ºC for 3 h. The sample was taken out from the oven and kept in a dissector until it was cooled to room temperature. The weight of the dried sample was measured. Total solids in toddy were calculated by using the following equation:

$$\text{Total solids (w/v)} = \frac{\text{W sample} - \text{W dish}}{\text{W dry sample} - \text{W dish}} * 100$$

Determination of alcohol content

Toddy sample of 100 mL and sodium chloride were taken into a separating funnel to saturate the toddy. Then, petroleum ether (50 mL) was added, mixed well and allowed to stand for 15 min. The water layer was removed and poured into the distillation flask. The layer of petroleum ether was washed with 20 mL of saturated sodium chloride solution two times and added to the washing solutions into the distillation flask. The distillation was carried out and the distillate was collected and the alcohol content was measured at room temperature (28±2ºC) from each sample by using the ebulliometer Dujardin-Salleron and quantified in terms of percentage (v/v).

Determination of total acids content

The acidity of the toddy sample was estimated by titrating with a standardized solution of sodium hydroxide (0.1 M) using phenolphthalein as an indicator and the results were stated as acetic acid (%, w/v) content (AOAC, 2000). Accurately weighed sample of sodium phthalate was used for the NaOH standardization. The total acids contents in terms of acetic acid were determined.

Determination of nutritional content

Nutritional characteristics such as total ash content AOAC (2000), total fat AOAC (2000), crude protein (Pearson, 1976), total sugars (Lane and Enon method SLS 521: 1981), and ascorbic acid (2, 6- Dichlorophenolindophenol method) were determined.

Determination of mineral content

Sodium, potassium (flame photometry method), phosphorous (AOAC, 2000), iron (1, 10-phenanthroline method), calcium, magnesium (E.D.T.A. titration method, Vogel, 1989) were determined.

Statistical analysis

The results obtained from each trial with three replicates were taken to analysis of variance (ANOVA) by complete randomized design (CRD). Among the treatment, Least Significant Difference (LSD) was tested at 5% level using Minitab 19 software.

RESULTS AND DISCUSSION

During the collection of toddy, soon after inflorescence sap reaches the earthen pot alcohol undergoes fermentation with wild yeasts. Due to that physicochemical properties of the palmyrah toddy are changed. Fermentation depends on the collection time as diurnal (fermented toddy/evening collection) or nocturnal (fermented toddy/morning collection). This toddy is moderately sweet in taste and its alcohol content (v/v) (Theivendirarajah and Chrystopher, 1986) increases >5% due to the prolonged natural alcohol fermentation where yeasts grow by using sugar to produce pyruvic acid via glycolysis. Then, acetaldehyde is formed from ethyl alcohol due to reduction caused by NAD (Piskur *et al.* 2006). After that, the toddy becomes acidic due to acid fermentation of ethanol carried out by *Acetobacter aceti* to release acetic acid. Ethanol is produced by yeasts via metabolic activities (4-6%) and also other metabolic by-products such as esters, ketones, and higher alcohols are formed from the fermentation, which, if present in high concentrations, can impact the final aroma and flavour profile of the toddy (Hansen, 1999). These compounds are resulted from precursors of yeast metabolic pathways and some of them are important for the growth of the yeast (Brown and Hammond, 2003). These metabolic compounds and other constituents could be affected by heat and period of storage; therefore, analysis of physical-chemical and nutritional analysis of fresh and preserved toddy practicably may be used to detect the influence of these factors.

Effect of fermentation time on the alcohol production

The highest alcohol percentage (5.2%) was achieved at 14 – 20 h of prolonged fermentation (Figure 1) while the acidity was above 0.55% even though the maximum acidity of the bottled toddy was 0.60% (IS 8538 2004). Hence, fresh toddy has to be analyzed before 14 h of prolonged fermentation where it should contain alcohol within the range between 4.5 and 5.5%. The toddy is an excellent medium for microbial growth. It is consequently needed; proper collection trials under hygienic conditions must be followed to prevent bacterial contamination to avoid its fermentation competing with the yeast to produce acid as a substitute for alcohol.

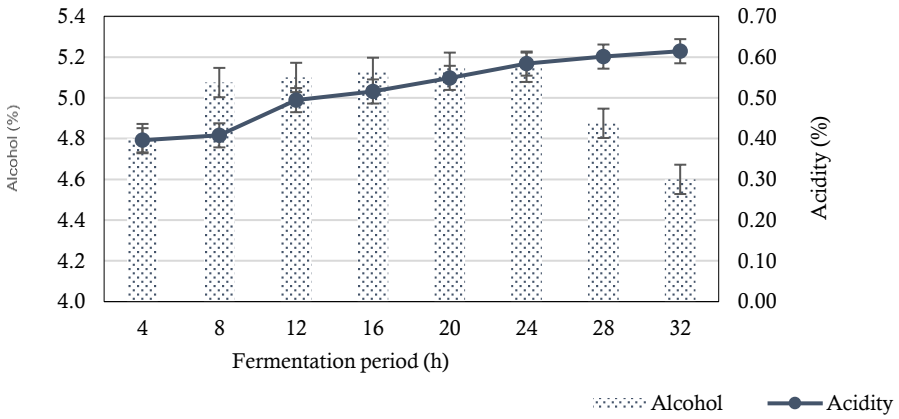

Figure 1: Effect of fermentation time on alcohol content and acidity of the fresh toddy.

Analysis of fresh and preserved bottled toddy

Bottled toddy is a pasteurized product, due to heat treatment that yeast fermentation is arrested and also metabolic pathway has not occurred. Therefore, above mention chemical characteristics did not show a change with the storage period. To improve the quality of the palmyrah bottled toddy, physicochemical characteristics are performed, and vital information on the pasteurization process, have to be recorded to retain the appreciable strength of alcohol and to extend the shelf life of the toddy. In this study, physicochemical, nutritional, and mineral analyses of fresh and preserved bottled toddy were compared to determine the effect of the preservation technique on the quality of the toddy.

Physicochemical analysis

There was no significant (P<0.05) change between fresh and bottled toddy in acidity and relative density among the physicochemical characteristics. Bottled toddy showed significantly (P<0.05) the highest values in alcohol, turbidity, and total solids and the lowest values in TSS and pH when compared with fresh toddy (Table 1).

The results from the physicochemical investigation exhibited that the values for the main parameters of palmyrah toddy tested such as alcohol, acidity, and turbidity exhibited within the reported values of a previous study (Mahilrajan *et al.*, 2014). Alcohol content was increased and pH was decreased due to the fermentation throughout the pre-pasteurization time during which multiplication of yeast cells increased values in turbidity and total solids of the toddy. TSS was decreased because of the utilization of soluble substances in the toddy via yeast cells. Sugars such as sucrose, fructose and glucose are mainly these soluble solids. It is expressed in "Brix" and is percentage-equivalent.

Table 1: Physicochemical parameters of fresh and bottled toddy. Each value in the table is represented as mean ± SD (n = 3). Values in the same rows followed by different letters are significantly different (P< 0.05).

	Parameters	Fresh toddy	Bottled toddy
1	Alcohol (w/w)	3.55 (±0.07)^{b}	3.80(±0.07)^{a}
2	Acidity (g/100 mL)	0.60(±0.01)^{a}	0.60(±0.07)^{a}
3	TSS (Brix)	6.75(±0.01)^{a}	5.93(±0.07)^{b}
4	pH	3.68(±0.01)^{a}	3.43(±0.07)^{b}
5	Turbidity (NTU)	2504.50(±0.07)^{b}	2694.50(±0.07)^{a}
6	Relative density	0.99(±0.01)^{a}	1.01(±0.07)^{a}
7	Total solids (g/L)	48.09(±0.01)^{b}	58.22(±0.07)^{a}
8	Specific gravity	0.99(±0.01)^{a}	1.01(±0.07)^{a}

Nutritional analysis

Among the nutritional factors, there were no significant (P>0.05) differences between fresh and bottled toddy in reducing sugar, protein, and fat content. While showing a significant decrease in total sugar and ascorbic acid, it also showed an increase in ash content (Table 2).

Table 2: Nutritional parameters of fresh and bottled toddy. Each value in the table is represented as mean ± SD (n = 3). Values in the same rows followed by different letters are significantly different (P<0.05).

	Nutritional parameters	Fresh toddy	Bottled toddy
1	Reducing sugar (g/100 mL)	2.69(±0.01)^{a}	2.70(±0.01)^{a}
2	Total sugar (g/100 mL)	3.04(±0.06)^{a}	2.80(±0.01)^{b}
3	Fat (g/100 mL)	0.02(±0.00)^{a}	0.02(±0.00)^{a}
4	Protein (g/100 mL)	1.41(±0.01)^{a}	1.42(±0.01)^{a}
5	Ash (g/100 mL)	0.18(±0.00)^{b}	0.19(±0.00)^{a}
6	Ascorbic acid (mg/100 mL)	3.0(±0.01)^{a}	2.1(±0.01)^{b}

Theivendrarajan (2008) reported that the ascorbic acid content of the toddy was 39.58 mg/l. Results of this study agree with that. However, there was a significant (P<0.05) decrease in bottled toddy due to the process of heating which leads to the reduction of vitamins. Asogwa and Onweluzo (2010) reported that vitamins present in legumes are reduced by the heating process. Fruits are missing vitamins because of their great sensitivity to oxidation and discharge into water-soluble substances during the processing (Davy *et al.*, 2000).

Analysis of minerals

There was no significant (P>0.05) difference between the mineral content of fresh and bottled toddy i.e. phosphorus, calcium, magnesium, and iron except for sodium and potassium (Table 3). Intracellular amounts of alkali metal cations mainly K^+ and Na^+ have to be maintained for survival of the yeast cells. While during the pasteurization of the toddy sample, enzyme activity of living cells is diminished. Hence, the balance of alkali metal cations may be disturbed. Therefore, the amount of Na^+ and K^+ may vary in bottled and fresh toddy samples. Not only the enzyme activity, but ions are determined by cell capacity, intracellular pH, and potential across the plasma membrane (Arino *et al.*, 2010).

Table 3: Mineral analysis of fresh and bottled toddy. Each value in the table is represented as mean ± SD (n = 3). Values in the same rows followed by different letters are significantly different (P<0.05).

	Mineral content (mg/100 mL)	Fresh toddy	Bottled toddy
1	Sodium (Na^+)	10.85(±0.05)^a	8.85(±0.05)^b
2	Potassium (K^+)	24.9(±0.4)^b	29.90(±0.3)^a
3	Phosphorous (P_2O_5)	0.48(±0.06)^a	0.64(±0.04)^a
4	Calcium (Ca^{2+})	10.5(±0.5)^a	13.5(±0.5)^a
5	Magnesium (Mg^{2+})	6.5(±0.5)^a	5.3(±0.5)^a
6	Iron (Fe^{2+})	10.8(±0. 1)^a	11.9(±0.3)^a

Effect of storage period on characteristics of toddy

The minimum and maximum results of physicochemical characteristics of the bottled toddy of two different batches during storage at room temperature (28±2 ^{o}C) are presented in Table 4.

Table 4: Range of physicochemical characteristics during the storage period.

Parameter	Batch I	Batch II
Alcohol % (v/v)	3.2-4.3	1.9-2.8
Acidity (%)	0.51-0.7	0.46-0.69
pH	3.55-3.68	3.57-3.76
TSS ^{o}brix	6.92-7.83	8.19-8.58
TS (g/L)	50.4-66.6	71.3-82.1

Palm Development Cooperative Societies are collecting the palmyrah toddy from different areas, pooled together and then used for the pasteurization process. Therefore, physicochemical characteristics of the toddy samples which are from different batches could differ because of climatic and seasonal changes of different areas. The average values of both batches for each chemical parameter are shown in Table 5. There were no significant (P>0.05) changes in all the parameters between the storage periods while all chemical characteristics showed a significant (P<0.05) difference between the batches.

Table 5: Physicochemical characteristics of bottled toddy during the storage period.

Months/ Parameters	Alcohol (%)	Acidity (%)	pH	TSS (°Brix)	TS (g/L)
1	2.7(±0.80)	0.46(±0.06)	3.59(±0.03)	7.96(±0.62)	73.0(±6.40)
2	3.2(±1.15)	0.47(±0.08)	3.63(±0.08)	7.71(±0.77)	69.5(±11.1)
3	3.1(±0.55)	0.48(±0.03)	3.69(±0.06)	8.01(±0.57)	65.0(±13.1)
4	3.1(±0.30)	0.55(±0.05)	3.72(±0.03)	7.92(±0.58)	70.4(±6.65)
5	2.8(±0.40)	0.54(±0.06)	3.72(±0.04)	7.86(±0.62)	67.5(±3.8)
6	2.9(±0.50)	0.65(±0.05)	3.68(±0.05)	7.97(±0.54)	62.8(±12.4)
7	2.9(±0.45)	0.65(±0.05)	3.68(±0.04)	7.79(±0.73)	67.6(±10.3)
8	3.1(±0.55)	0.69(±0.01)	3.65(±0.10)	7.74(±0.82)	68.6(±10.5)
9	3.0(±0.50)	0.65(±0.00)	3.65(±0.10)	7.76(±0.75)	70.6(±10.8)
10	2.9(±0.50)	0.50(±0.10)	3.61(±0.04)	8.09(±0.50)	73.7(±8.45)
11	3.1(±0.55)	0.46(±0.06)	3.57(±0.02)	8.03(±0.50)	72.2(±8.55)
12	2.6(±0.65)	0.48(±0.07)	3.58(±0.01)	8.01(±0.18)	72.5(±9.20)
Average/ month	2.9(±0.60)	0.5(±0.10)	3.6(±0.01)	7.9(±0.6)	69.4(±9.3)

The alcohol content and acidity of bottled toddy did not show any significant (P>0.05) difference during the storage period of a year (Figure 2). With improved awareness of alcoholic fermentation, greater stress is being placed on the role of yeasts in the formation of esters and higher alcohols resulting from precursors of yeast metabolic pathways. Some of them are important for the expansion of the yeast (Brown and Hammond, 2003), which give to the improvement of appreciable aroma and flavour of the toddy. Though these components are formed by most of the yeasts, depending on the strain of yeast, considerable distinction was observed in the relative amount of these complexes (Venkataramu *et al.,* 1977) during the storage of toddy.

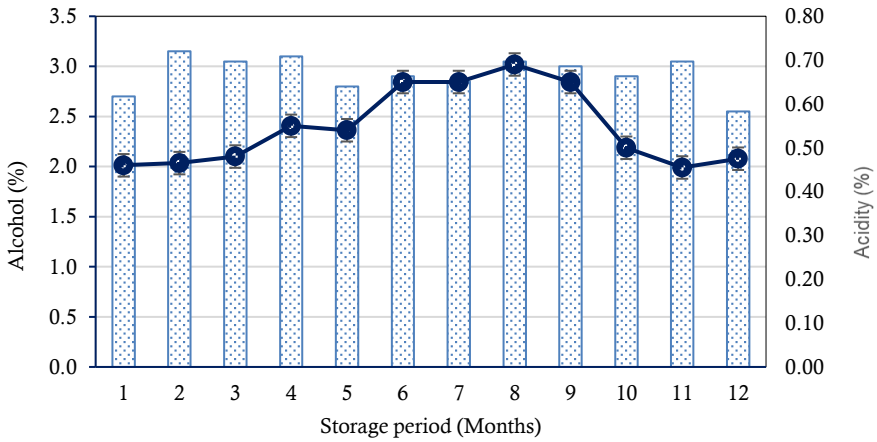

Figure 2: Alcohol content and acidity of bottled toddy during the storage period.

CONCLUSIONS

Physicochemical characteristics such as alcohol, acidity, turbidity, total solids, and relative density showed significantly higher values for preserved toddy when compared with fresh toddy with no significant difference in reducing sugar, fat, protein, and ash content and also mineral contents such as phosphorous, iron, calcium and magnesium. Also, there were no significant differences in alcohol, acidity, TS, TSS, and pH of preserved bottled toddy between the twelve months of storage periods. Therefore, palmyrah bottled toddy could be preserved for a period of twelve months by using the pasteurization process.

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