FORMULATION OF READY TO SERVE DRINK FROM PALMYRAH (BORRASUS FLABELLIFER L) FRUIT AND MODIFICATION OF ITS PROCESS TO IMPROVE SOME OF ITS SELECTED PROPERTIES

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Abstract
Although palmyrah fruits are seasonal they have excellent chemical and physical properties for the development of food and beverages. Though there is a demand for palmyrah ready to serve the beverage, existing commercial products are failed due to their poor quality. Hence this study was conducted to improve the quality of palmyrah ready to serve beverage by reformulation and modification of process. Pectin and citric acid were selected as stabilizer and acidulant through ranking test with 11 semi-trained panelists. Using general full factorial design, 18 treatments were carried out to optimize the levels of fruit (5 -12%), sugar (10-15 %) and pH (3.5-4.0) in the final formula. The final formulation was evaluated through 31 sensory panelists using 9 points hedonic scale. The formulated beverage contained 12 % fruit pulp, 12.5 % sugar and pH of 4.0. The level of pectin was adjusted to 0.66 % and the fruit pulp was subjected to homogenization (30000 rpm for 5 min). Chemical and nutrient analysis of the reformulated product revealed that the product was significantly better than the existing product in nutrients. It contained 0.14 % crude protein, 0.78 % crude fat, 0.41 % crude fibre, 0.17 % ash and 11.97 % total sugar. Total antioxidant capacity, total phenolic content, and inhibition of DPPH radical scavenging activity of prepared product were 4044.00 Ascorbic acid equivalent (AAE) mg/L, 137.57 gallic acid equivalent (GAE) mg/L and 12.43 % respectively. The developed product did not show any growth of yeast and mold and bacterial count during 10 weeks time. Hence the developed palmyrah fruit ready to serve drink showed better quality in the analyzed properties compared with commercially available products.

Keywords: palmyrah, antioxidant, homogenization, preserved pulp, ready to serve drink

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1. INTRODUCTION

Ready to Serve (RTS) drink is one of the types of fruit beverages. This study attempted to develop an RTS drink using palmyrah fruit. Palmyrah (*Borrasus flabellifer*) is wildly distributed throughout the tropics, mainly in the arid zones of the country. Palmyrah fruit pulp has been used by the people in Asian countries for decades. A number of beneficial characteristics of palmyrah fruit have been proved through researches. Flabelliferins of palmyrah fruit reduces the glucose absorption and decrease the blood cholesterol level. Further, palmyrah fruit is rich in antioxidants. They are beneficial in cardiovascular diseases and cancer (Sangheetha *et al*., 2015). Palmyrah Development Board, Jaffna, the sole producer of palmyrah fruit-based beverages, produces palmyrah fruit ready to serve drink and it contained palmyrah fruit pulp, sugar, sodium benzoate, citric acid, colour, and flavor. But the beverage does not meet the requirements specified in Sri Lanka Standards (SLS 729:1985). According to SLS, the ready to serve drink should contain a minimum of 5 % fruit juice. But, the amount of pulp used in the palmyrah fruit ready to serve drink is less than 5 %. Therefore the entire content of fruit pulp is not added and only the extract with water-soluble nutrients is added into the beverage. Moreover, the available drink cannot meet the expected quality. As a result, the market for the existing product is going down. Hence an urgent need has risen to reformulate the palmyrah ready to serve drink and to modify the process. Therefore the study was designed to find a new formulation to improve the quality of palmyrah fruit ready to serve the drink in the point of nutrient content, quality and shelf life through the reformulation, optimization and process modification.

2. MATERIALS AND METHODS

2.1 Materials

Preserved palmyrah fruit pulp was obtained from Palmyrah Development Board, Sri Lanka. Black skin variety palmyrah fruits was collected from Kaithady in Jaffna, Sri Lanka. All the chemicals used for the analysis were of analytical grade.

2.2. Determination of levels of parameters or ingredients for the experiment

According to the Sri Lankan Standard SLS 729:1985 for ready to serve drink published by Sri Lankan Standards Institute in 1985, the ready to serve drink should be constituted with minimum 5% of fruit pulp and sugar separately and acidity less than 1%. Three fruit pulp levels (10, 15, and 20%), three sugar levels (10, 15 and 20%), two pH levels (3.5 and 4.0) and three acidulants (tartaric acid, citric acid and malic acid) were evaluated using semi-trained sensory panelists at Palmyrah Research Institute, Sri Lanka.

2.3. Experimental design for a reformulation of palmyrah fruit pulp RTS drink

The major ingredients of the drink were palmyrah fruit pulp, sugar, water, citric acid, and pectin. The design of the experiments used for the optimization of major ingredients was full factorial design.

2.4. Preparation of palmyrah fruit pulp incorporated RTS drink

Fresh palmyrah fruits were sorted, cleaned and washed with running water following by distilled water and the tepals were removed. The fruits were kept at 200 °C till the formation of soap like foam ceased. Then the fruit was washed and peeled off and the pulp was extracted with water at the ratio of 1:1. Then the pulp was blended and homogenized to prepare the drink. The drink prepared with fresh fruit pulp showed relatively better taste and flavor than the drink prepared with preserved fruit pulp. In order to optimize the ingredients in the formulas, among the eighteen treatments (Table 1), treatment no 15 (pulp 12%, sugar 12.5% and pH 4.0) was selected through the preliminary sensory evaluation. The palmyrah pulp, pectin, and sugar were mixed and pasteurized at 90 °C for 1 minute. Then citric acid was added into the mixture and then the mixture was filled in sterilized glass bottles and pasteurized at 96°C for 20 minutes. The prepared RTS beverage bottles were kept at room temperature. In order to find the levels of ingredients to incorporate into the beverage, fruit pulp and sugar levels in the range of 10-20% evaluated while maintaining other ingredients were kept at the same in all products. Citric acid was selected as an acidulant and the pectin was selected as a stabilizer for RTS drink by using a preliminary sensory evaluation (data not shown).

Table 1 Design of experiments for the optimization of ingredients in palmyrah fruit RTS drink

Treatments	Pulp (%)	Sugar (%)	pH	Overall acceptability*
T1	5.0	15.0	4.0	142
T2	5.0	15.0	3.5	140
T3	5.0	12.5	4.0	62
T4	5.0	12.5	3.5	60
T5	5.0	10.0	4.0	58
T6	5.0	10.0	3.5	56
T7	8.5	15.0	4.0	140
T8	8.5	15.0	3.5	142
T9	8.5	12.5	4.0	63
T10	8.5	12.5	3.5	60
T11	8.5	10.0	4.0	57
T12	8.5	10.0	3.5	65
T13	12.0	15.0	4.0	82
T14	12.0	15.0	3.5	135
T15	12.0	12.5	4.0	**160**
T16	12.0	12.5	3.5	55
T17	12.0	10.0	4.0	57
T18	12.0	10.0	3.5	50

* Overall acceptability-based on the sensory evaluation

2.4.1. Selection of most preferable treatment

All 18 treatments were divided in to 3 groups according to the pulp percentage and each group was subjected to the sensory evaluation separately. Best two treatments were selected for each group. All 6 treatments were again tested by the untrained panelists and the best treatment was selected for the final formulation of the palmyrah fruit pulp RTS drink.

2.4.2. Minimization of the sedimentation

The pectin was optimized within the range of 0.60-0.69%. The best combination of pectin was selected based on the observation of viscosity and the rate of layer separation. A suitable processing method also selected from blending, centrifugation and homogenization to reduce the sedimentation.

Determination of physicochemical properties

Proximate composition of the beverages samples was analyzed using AOAC (2000). Total soluble solid content, pH and titratable acidity of the palmyrah beverages and the pulp was measured according to the methods described by Gunathilake (2012) with some modifications. Estimation of reducing sugar was analyzed according to the Miller (1959).

Determination of total phenolic content

The total polyphenol content of the palmyrah beverages and the pulp was estimated by the Folin–Ciocalteu method described by Gunathilake (2012) with some modifications. The concentration of total phenols was expressed as mmol gallic acid equivalents (GAE) per g dry weight (FW) of cooked leaves.

Determination of total antioxidant capacity

The total antioxidant capacity of the palmyrah beverages and the pulp was analyzed according to Gunathilake et al. (2018). The antioxidant capacity was expressed as ascorbic acid equivalents (AAE)/g cooked leaves.

Determination of DPPH radical scavenging ability

The DPPH radical scavenging ability of the beverages and the pulp was analyzed according to the method described in Gunathilake and Ranaweera (2016).

2.5. Shelf life study of RTS drink

The bottled ready to serve drink were stored at ambient temperature. Then the total plate count, yeast mold count, and organoleptic properties were analyzed in 3 weeks interval up to 12 weeks to estimate the shelf life. Further, Changes in pH, titratable acidity, and total soluble solid contents were also monitored within the storage period.

2.6. Statistical analysis

All data are presented as the mean ± standard deviation. All the samples were analyzed in triplicate and one-way analysis of variance (ANOVA) was performed using MINITAB 15 software.

3. RESULTS AND DISCUSSION

For the formulation of palmyrah RTS beverage, the levels of TSS, pH, pulp %, and pectin % as a stabilizer and citric acid % as the acidulant were determined. Based on a preliminary sensory evaluation (data not shown), 0.66% of pectin was accepted by the semi-trained panelists as a stabilizer. Homogenization process at 30000 rpm for 5 minutes was used as the best processing method to reduce the sedimentation. In the ranking test, the developed RTS drink was preferred than the drink available in the market. The preference level of palmyrah RTS drinks varied with different percentage of fruit pulp. Physicochemical properties of the formulated RTS beverage are shown in Table 2.

Table 2: Chemical properties of RTS drinks

Parameters	Commercial drink	Developed drink	P value
pH	4.63 ± 0.00	3.89 ±0.00	0.000*
Total soluble solid (Brix)	14.17 ± 0.00	14.75 ± 0.00	0.000 *
Titratable acidity (%)	0.05 ± 0.00	0.11 ± 0.01	0.004 *
Benzoic acid (ppm)	33.04 ± 4.40	86.41 ± 8.8	0.001 *

The drink prepared with 12% of pulp had the highest preference level as it contained the optimum taste of palmyrah fruit drink (Table 1). Nilugin and Mahendran, (2010) also reported that the RTS drink prepared using 12% of palmyrah pulp was selected as the most preferred treatment based on the physiochemical and organoleptic point of view. The drink prepared with 9% pulp had appreciably low sweetness. However, a bitter taste was observed in the drink prepared with 15% pulp (Jansz *et al.*, 1994). The low preference levels obtained for them may be due to characteristic bitterness. The palmyrah fruit pulp content in the RTS drink ranged between 5 to 12%. Based on the high preference level of the RTS drink (Table 1), 12% of pulp was selected as the upper level. However, the Brix value of RTS drink should be in the range of 10 to 15 % (Weerasekara *et al*., 2012; Nilugin and Mahendran, 2010). Generally, the RTS drink should contain a pH less than 4.3 according to Weerasekara *et al*.(2012).

Generally, xanthan gum, pectin, carboxymethyl cellulose are used as stabilizers for a fruit drink (Kahawa *et al.*, 1999). In this study, 0.5% of pectin and 0.5% of CMC were used as stabilizers. Pectin builds viscosity and mouthfeel, aids in pulp suspension and provides a preferable texture (Kahawa *et al.*, 1999). Therefore, the drink that contained pectin was the most preferred one while the drink that contained CMC was the least preferred drink based on a preliminary evaluation. Among the acidulants (citric acid, tartaric acid, and malic acid) evaluated for the formulations, at the preliminary trials, the drink that contained citric acid had the highest preference level since it keeps the taste of palmyrah fruit in the drink. Other acids may change the taste of palmyrah fruit in a drink (Theivendirarajah, 1991). Most of the commercially available drinks contain citric acid as acidulant. When considering the mean scores for the treatments with the pulp concentration of 5%, T1 and T2 have shown the highest mean scores for the attribute taste Table 1. Similarly, in the case of the mean scores for the treatments with the pulp concentration of 8.5%, T7 and T8 have shown the highest mean scores for the attribute taste. Since the amount of pulp was constant in all six treatments conducted with 5% and 8.5% of pulp, the preference level of taste was primarily determined by the amount of sugar used. The highest amount of sugar in T1and T2 and also in T7 and T8 was mainly responsible for the highest mean scores. High acidity drinks had a noticeable sour taste. Sugar in a drink can compensate the acidity and push the taste into a sweet range. Other attributes include color and odor is mainly depending on the amount of pulp. These attributes had nearly the same mean scores for all the six treatments which were conducted with 5% and 8.5% pulp because the same amounts of pulp were used.

In the case of the mean scores for the treatments with the pulp concentration of 12%, the pulp amount is high. It also influences in the sweetness of a drink. Therefore, the combination of sugar content and acidity determine the preference level for the taste of a drink. The best combination was observed in T14 and T15 as they had the highest mean scores. High acidity drinks had a noticeable sour taste. Other attributes include color and odor is mainly dependent on the amount of pulp. These attributes had nearly the same mean scores for all the treatments because the same amounts of pulp were used. Overall, T15 and T14 had the high mean scores for the preference level of all attributes. Among the six selected treatments, both T15 and T14 had the higher amount of pulp than others. Therefore, the pulp is a dominant constituent which can determine the preference level of RTS drink.

Although 0.5% of pectin was used in this study, 0.66% of pectin was accepted by the semi-trained panelists because sedimentation has occurred in 0.5% of pectin. 0.66% of pectin is an optimum level for preventing sedimentation and providing an optimum level of viscosity. Balaswamy *et al*, 2014 reported that 0.5% of pectin was used in RTS pineapple drink. It might be due to the addition of high pulp. Among the processes blending, centrifugation and homogenization, homogenization has been identified as the best method in increasing the uniformity and stability of RTS drink than other methods. 30000 rpm was used in the method of homogenization. Due to the high rpm, the particle sizes were reduced to a level that enough to maintain a better homogenization. Homogenization can decrease the viscosity. Beside pulp suspension was obtained in high rpm by preventing sedimentation (Kahawa *et al.*, 1999).The preference for the developed RTS drink was high compared to the other drinks available in the market. The color, odor, and taste of the developed RTS drink were almost similar to the palmyrah fruit as it contained 12 percent of pulp. Virtually, the amounts of pulp in commercially available drinks were very low because the pulp is strained while preparing the drink. Furthermore, essence and coloring are added to commercially available drinks.

Table 2 shows the physicochemical properties of the commercial and developed drinks and their values. There is a significant difference between the pH, total soluble solid, titratable acidity and benzoic acid content of the commercial and developed drinks ($p<0.05$).

The pH of fresh palmyrah fruit pulp ranged between 5.5-6 (Vijayakumari, 2014). The reduction in pH was mainly due to the addition of benzoic acid. The total soluble solids of fresh palmyrah fruit pulp were 16.5 (Vijayakumari, 2014). The pH and titratable acidity of commercial drink were 4.63 and 0.05 respectively while the pH and titratable acidity of developed drink was 3.89 and 0.11 respectively (Table 2). According to the Sri Lankan Standard for RTS drinks, maximum 1% of acidity was allowed (SLS 729: 1985). A higher amount of citric acid was added in developed drink than commercial drink and the developed drink contained a higher amount of pulp (12%) than the commercial drink. Therefore, the developed drink contained higher acidity and lower pH value than the commercial drink. The pH of an RTS drink should be lower than 4.3 (Weerasekara et al., 2012). The growth of Clostridium botulinum may be possible in the commercial drink as it contained the pH greater than 4.6. The higher amount of benzoic acid was added in developed drink than in commercial drink. According to the Sri Lankan Standard for RTS drinks, it should contain a maximum 120 ppm of benzoic acid (SLS 729: 1985). The added pulp was mainly responsible for the increased total soluble solid in the developed drink.

Proximate analysis of both developed drink and commercially available drink

Moisture of fresh Palmyrah Fruit Pulp was 75-80% (Jansz *et al.*, 2002). The protein content of fresh palmyrah fruit pulp was 0.7 % (Jansz *et al.*, 2002). Ash content of the fresh palmyrah fruit pulp was 1.2% (Vijayakumari *et al.*, 2014).The ash content is the organic residue remaining after the organic matter has been burnt away. It is not necessarily of exactly the same composition as the mineral matter present in the fresh palmyrah fruit pulp as there may be losses due to volatilization or some interactions between constituents. In the developed drink, all proximate contents include protein, fat, ash, reducing sugar, crude fiber, pectin and vitamin C were high in amounts compared to the commercial drink except total sugar. The high amount of sugar added in the commercial drink was the reason for the highest total sugar content. The higher amount of other proximate contents in the developed drink was due to the addition of fruit pulp whereas the pulp content was very low in the commercial drink because of the straining of the pulp. However, the percentages of proximate contents in the developed drink were lower than the preserved fruit pulp. It may due to the loss of nutrients because of the long procedures.

Table 3: Proximate content of RTS drinks

Constituents	Commercial drink	Developed drink	P value
Protein (%)	0.00±0.00	0.14 ±0,01	0.000
Fat (%)	0.00 ± 0.00	0.78 ± 0.06	0.000
Ash (%)	0.02 ± 0.01	0.17 ± 0.00	0.010
Acid insoluble ash (%)	0.00 ± 0.00	0 .00 ± 0.00	-
Total sugar (%)	12.75 ± 0.45	11.97 ± 0.90	0.256
Reducing sugar (%)	0.13 ± 0.02	0.57 ±0.08	0.001
Crude fiber (%)	0.00 ± 0.00	0.41 ± 0.01	0.000

Values are means of triplicates ± standard error.
*significantly different (p<0.05)

Table 3 shows the amount of the proximate content of commercial drink and developed drinks. There is a significant difference between the protein, fat, ash, reducing sugar, crude fiber, of both commercial drink and developed drinks (p<0.05).

Antioxidant analysis of developed drink and commercially available drink

Table 4 shows the antioxidant properties of the RTS beverage. The palmyrah pulp contained total antioxidants varying from 2.4-21.8 Trolox equivalent.g^{-1} determined by ABTS (Pathberiya and Jansz, 2005). In this study, total antioxidant activity, total phenolic content and DPPH radical scavenging capacity of preserved palmyrah fruit pulp were obtained 6553.33 AAE mg/L, 990 GAE mg/L and 1433.33% respectively. These all values were higher in developed drink than in commercial drink due to the addition of pulp (12%) in the developed drink.

The formulated drinks had significantly higher (p<0.05) total phenolic content and antioxidant activity. There is a significant difference between the total antioxidant activity, total phenolic content and radical scavenging capacity of the commercial and developed drinks (p<0.05).

Table 4: Antioxidant properties of palmyrah pulp and RTS drink

Parameters	Palmyrah pulp	Commercial drink	Developed drink	P value
Total antioxidant activity AAE (mg/L)	6553.33 ± 832	2895 ±111	4044 ± 34	0.000
Total phenolic content GAE(mg/L)	990 ± 121.24	17.09 ± 0.32	137.57 ± 10	0.000
Radical scavenging capacity (as a %)	1433.33 ±57.73	- 3.07 ±0.20	12.43 ±0.98	0.000

Values are means of triplicates ± standard error
*significantly different (p<0.05)

Changes in TSS, pH and titratable acidity with storage time in ambient temperature

Most significant changes in fruit products are due to biological factors especially microorganisms. The results of the microbial test revealed that the developed palmyrah RTS beverage was not deteriorated with microbial contamination. No bacterial growth was observed in the RTS beverages. Added sodium benzoate in preserved pulp inhibits yeasts, moulds, and bacteria. According to the Sri Lankan Standardfor RTS drink, the maximum total plate count is 50 CFU/Ml (SLS 729: 1985). The microbial growth was prevented by pasteurization, sterilization, hot filling and using benzoic acid. Pasteurization was done before and after bottling (90°C/ 1 min and 96°C/20 min). Glass bottles were sterilized at 99°C for 30 min. Crown cork lids were immersed in hot water at 99°C for 10 min. The acid medium may have prevented the development of some microbes. There was no microbial growth observed in developed RTS drink until 9 weeks of storage. Figure 1 shows the changes in total soluble solids content, titratable acidity, and the pH during storage. The total soluble solids, titratable acidity increased with the gradual passage of storage time (Fig 1), which might be due to hydrolysis of polysaccharides into monosaccharide and oligosaccharides (Hirdyani, 2015). The total soluble solids increased from 14.15 to 14.94% in 9 weeks. There was a slight increase in titratable acidity content during storage; this might be due to the formation of benzoic acid from benzoate. The titrable acidity increased from 0.11 to 0.135%.There was a significant decrease in pH during storage. The pH decreased from 3.89 to 3.7 for 9 weeks. This might be due to an increase in titratable acidity, as acidity and pH are inversely proportional to each other (Madurangi and Gunathilake, 2016). According to the Figure 1, developed RTS drink in an ambient temperature has the increasing trends of a total soluble solid with the storage time. The total soluble solids increased from 14.15 to 14.94% over 9 weeks.

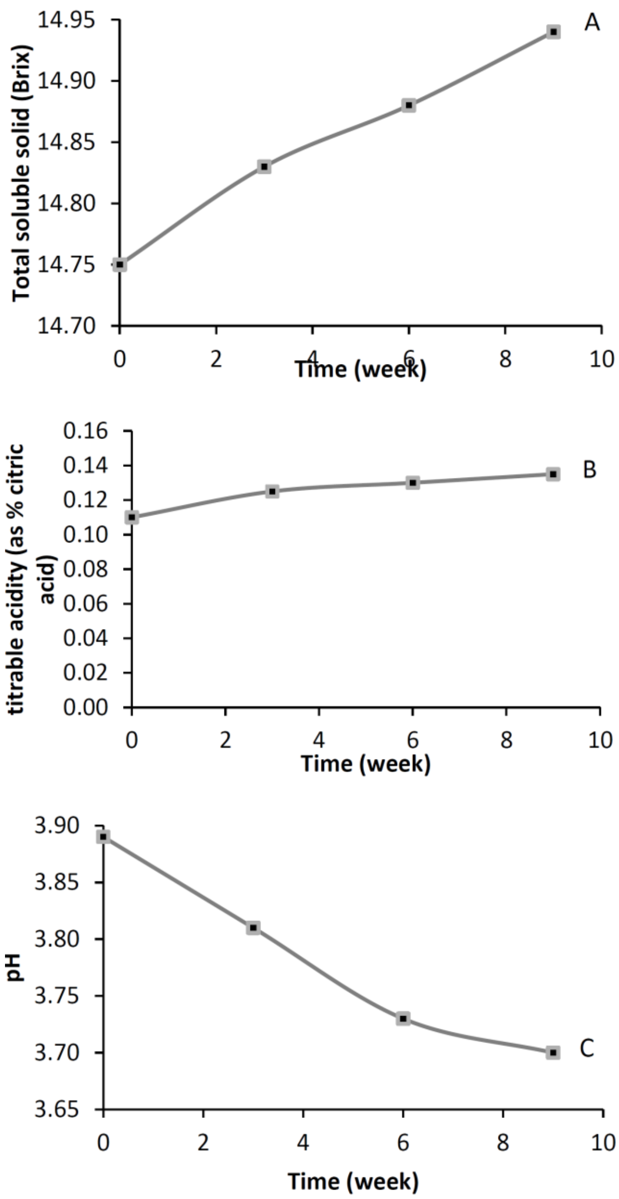

Figure 1: Changes in total soluble solid content (A), titratable acidity (B) and pH (C) of the formulated RTS beverage during the storage period

4. CONCLUSION

The research was conducted to enhance the utilization of locally available underutilized palmyrah fruit through the development of ready to serve fruit drink. The result showed that the most preferred reformulated RTS drink contained12% of palmyrah fruit pulp, 12.5% of sugar and 0.66% of pectin. The pH for this drink was 3.89. Significantly higher nutritional compositions and antioxidant activity were available in developed drink against commercially available drink. Citric acid was identified as the most effective acidulant among citric acid, tartaric acid, and malic acid. Similarly, among the stabilizers, pectin was more effective than CMC. Since there were no yeast, mold and bacterial colonies were observed until 9 weeks, the developed PRTS drink has the minimum period of 60 days of shelf life in an ambient temperature. Homogenization of pulp at 30000 rpm for 5 min can improve the quality of the final product due to the minimization of sedimentation. The sensory evaluation proved that the developed PRTS drink is mostly accepted than commercial PRTS drink.

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