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Microbial, Physicochemical and Sensory Evaluation of Preserved Palmyrah Fruit Pulp

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Abstract: Fruits of Palmyrah palm (*Borassus flabellifer*) are seasonal; therefore their fibrous (mesocarp) Palmyrah fruit pulp (PFP) extracted with water and should be preserved with lengthened shelf life to ensure its availability in local and international market throughout the year. Therefore a study on preservation of PFP was carried out with or without various concentrations of preservatives, Sodium benzoate (SB), Sodium metabisulphite (SMS) and combinations of the both at different ratio. pH of the PFP was adjusted to 3.8 with citric acid, heated in a water bath at 90°C for 20 Sec, preservatives were added, mixed well then bottled pulp was heated at 80°C for 30 min in thermostatic water bath and kept at room temperature (30°C) for 180 days. Initial pH with stabilization has come to about 4.2. Aliquots of them were withdrawn periodically (at 30 days intervals) and were analyzed for microbial, physicochemical and sensory characteristics. PFP alone (without preservatives) was spoiled with increasing pH by showing adverse characteristics (unacceptable odour) before 24 hours of storage. All the treatment showed significant ($p < 0.001$) increase in total soluble solid (10.82-13.10 °Brix) and declined in pH (4.42 - 4.14) was observed with a proportional increase in the acidity (0.71- 0.91%) for treatments of T1 - T5 (containing SB), T6 - T10 (containing SMS) and T11 - T15 (containing both SMS & SB) up to 180 days. But no colony (Total Plate Count) was observed in the pulp treated with SMS and with combination of SMS & SB at various concentrations up to 120 days of storage. Among the all treatments the pulp treated with SB were found to be inferior in both colour and flavour characteristics. Even though it was found that PFP treated with SMS, T6 - T10 could be stored for extended period of 180 days without any major changes in chemical, microbiological and sensory characteristics, whereas T7 (with SMS, 0.4g/l) was selected as the best treatment based on the overall acceptability.

Keywords: Palmyrah Fruit Pulp, preservatives, sensory evaluation

I. Introduction

Palmyrah (*B. flabellifer*) fruit is the oldest and most important tropical fruit. It is indigenous or naturalized throughout tropical and subtropical South and Southeast Asia. Palmyrah fruit is mostly used as fresh fruit for pinattu (Dried pulp) and oil cakes, but due to its perishable nature it cannot be stored for long period of time. Pulp is yellow in colour due to the presence of carotenoids (Provitamin A). It is a good source of vitamin C and contains appreciable amount of pectin [1]. Jeyaratnam (1986) [2] said that pulp contains appreciable amount of saponin and also believed that pulp provides dermatitis relief.

During peak of harvest season (Aug- Oct) large quantity of fruits are wasted due to limited shelf life in storage. In order to make the PFP available during the off season it has to be preserved with lengthened shelf life. Sales centres of Palmyrah Development Board, Katpakams sell bottled PFP to prepare fruit base edible products. But during storage period colour of the bottled pulp turns to yellowish brown. Despite the fact it has to be developed with favourable chemical treatment for the preservation of PFP. Because of its high fermentable nature under the influence of microbes, it is dried as Pinattu for short term preservation. But it is also preserved by making panampanam (diluted drink), cordial, crush and jam with moderate shelf life.

Sodium benzoate (SB) and potassium metabisulphite (PMS) are commonly used as preservatives for long term storage of fruit pulp because of their better antimicrobial activity [3]. The maximum level for the use of these chemicals in fruit preservation including pulp and purees as described in the Codex Standards adopted in 2001 and 2006 are 1000 mg/kg SB as benzoic acid and 500 mg/kg PMS as residual SO_2 [4]. Keeping in view these facts, this study was undertaken to find out the inhibitory effect of SB, SMS and both in different ratio with varying concentrations for microbial, chemical, physical and sensory quality of PFP stored at room temperature.

The aim of this study was to extend the shelf-life of the PFP by determining the best proportions of food additives like sodium benzoate and sodium metabisulphite (SB, SMS and both in combination) to be applied for preservation of PFP at room temperature (30°C). If storage of pulp can be improved for a long period, both PFP and its based food products will increase earnings in the Sri Lanka domestic and foreign markets.

II. Material And Methods

This research was approved by Research and Development division of Palmyrah Development Board.

2.1 Determination of Microbial Count

The method of Sri Lankan Standard: 516 Part 1: 1991 [5] was used.

Preparation of Nutrient Agar Plates

Plate Count Agar (PCA) HIMEDIA Laboratory Pvt. Ltd medium (2.35g) in a 250ml conical flask was dissolved in 40ml of distilled water by heating in a water bath, made up the volume to 100ml with the same, plugged with cotton wool well, sterilized at 121°C and 15lb in⁻² pressure for 15 min and then allowed to cool to 45°C.

Serial dilution

Sample (10g) was transferred into a labeled sterile dilution bottle, made up the volume to 100ml with peptone (HIMEDIA) water (peptone 1g, NaCl (HIMEDIA) 8.5g made up to 1000ml with water) under the sterile condition. Aliquots of it were taken after the thorough mixing by using vortex mixer (VELP SCIENTIFICA ZX3) and repeated the same process to obtain required dilution.

Microbial Count

Diluted sample (1ml) was transferred into each sterile plate. 20ml portion of the medium was poured to each plate in the laminar flow chamber (BIOBASE), mixed gently and allowed it to cool at room temperature. Plates of different dilutions were incubated at 37°C for 48hrs and the appeared colonies in the plates were counted and the total colonies were calculated. This experiment was repeated twice and the mean values (CFU/g) of these were calculated.

2.2 Physicochemical analysis

Total Soluble Solids (TSS)

Total soluble solids (TSS) of each sample were determined directly by using Refractometer (HSR500, Japan) at room temperature and expressed in terms of °Brix value.

Acidity

The method of SLS: 729:1985 [6] was used. The acidity of the given sample was determined as citric acid (%w/w) by titrating 10ml of sample against 0.1 N NaOH (SIGMA) using phenolphthalein (SIGMA) as an indicator.

pH

Homogenized sample (25ml) was taken in a clean beaker (25ml) and its pH was measured by using a digital pH meter (Sension PH 31-Spain) at room temperature.

2.3 Sensory evaluation

The method described by Larmond (1977) [7] was used. Selected sample was evaluated by a panel of judges from Palmyrah Research Institute staff with Oral Consent Scripts for sensory characteristics like colour, flavour, texture, mouth feel and over all acceptability. The judges were provided with prescribed questionnaires to record their observation. The information contained on the performance was 5 = Like very much; 4 = Like slightly; 3 = Neither like nor dislike; 2 = Dislike slightly; 1 = Dislike very much. The panelists expectorated the sample and rinsed mouth using water between samples.

2.4 Preparation of PFP

Well ripened Palmyrah fruits available in plenty in their season at Kaithady, Northern Region of Sri Lanka, were washed twice with potable water and their tepals (tops) were removed then again washed with potable water and dipped in hot water for few seconds. Then ectocarp (skin) was peeled manually, the remainder (nutlets) was macerated with warm water (nutlet: water in ml = 1:100). Diluted pulp (PFP) was extracted manually using sieve after the removal of seeds and insoluble fibres.

Adjustment of pH

Initial pH of the PFP was measured with pH meter (Sension+ PH 31-Spain) and then the pH of the pulp was adjusted to proper pH 3.8 with concentrated solution of food grade commercially available citric acid and mixed well.

Blending

Acidified pulp was blended by using electric blender at low speed for 5 min.

2.5. Effect of heating at 80°C for 30min Pasteurization in the preservation of PFP

PFP (pH3.8) poured into the capped clear glass bottle (100ml, without the addition of preservatives) was heated in a thermostatic water bath at 80°C for 30 minutes and then allowed to cool to room temperature [8,9] and stored for a period of 150 days. Aliquots of them were taken for the analysis.

2.6 Effect of preservatives in the preservation of PFP

Common preservative Sodium Benzoate and Sodium metabisulphate (Food Grade) available in local market were used. The PFP was heated in a thermostatic water bath GEMMYCO at 90°C for 20 sec., preservatives were added according to the TABLE1, mixed well and they were transferred into clear sterile glass bottles separately and capped well. The bottled pulp was heated in a thermostatic water bath at 80°C for 30 min and then allowed to cool to room temperature [8,9]. They were stored at room temperature for a period of 180 days. Aliquots of them were taken in 30 days interval for the analysis.

Statistical analysis

Results obtained from chemical analysis (pH, brix and acidity) with three replicate were subjected to three way ANOVA. The significant difference among the treatments was tested in Least Significant Difference (LSD) at 5 % level of significance using SAS (version 9) System software.

Friedman non-parametric statistical method was used to analyze the sensory evaluation data based on 5-point hedonic scales. In this data analysis 95% confidence interval was considered, and analysis was done using Minitab 13 software.

Table 1: Concentrations of different preservatives used in preservation of PFP, alone or their combination at varying ratio

Treatments	SB%, (w/v)	SMS%, (w/v)
T1	0.04	-
T2	0.08	-
T3	0.12	-
T4	0.16	-
T5	0.2	-
T6	-	0.03
T7	-	0.04
T8	-	0.05
T9	-	0.06
T10	-	0.07
T11	0.02**	0.015**
T12	0.04	0.02
T13	0.06	0.025
T14	0.08	0.03
T15	0.1	0.035

**Half of the concentration of each preservative used alone before was used together here.

III. Results And Discussion

Palmyrah fruit and their products have gained considerable importance by contributing significantly to the economy of Sri Lanka. On the other hand freshly extracted pulp is highly attractive in appearance and possesses good taste and aroma, but it deteriorates rapidly in 24h. This is mainly due to fermentation caused by moulds, yeasts and bacteria. The enzymes secreted by them may affect the colour and flavour adversely. Chemicals present in the pulp may react with one another and spoil its taste and aroma. Air coming in contact with the product may react with the glucosidal substances present in it. This deterioration must be avoided by application of the food preservation principle which first involves the prevention or delay of the microbial spoilage.

The present study was carried out to identify a suitable chemical preservative/s such as sodium benzoate, sodium metabisulphate either alone or in combination for satisfactory storage of PFP at room temperature. Efficiency of preservation and storage behavior of fruit pulp is depended on physicochemical characteristics such as acidity, pH and Total Soluble Solids (TSS) and biological parameters. Period of storage had shown a pronounced effect on physicochemical attributes of chemically preserved PFP.

3.1 Microbiological evaluation

Benzoic acid inhibits the growth of mold, yeast [10] and bacteria. It is either added directly or created from reactions with its sodium, potassium or calcium salt. The mechanism starts with the absorption of benzoic

acid into the cell. If the intracellular pH changes to 5 or lower, the anaerobic fermentation of glucose through phosphofructokinase is decreased by 95%. The efficiency of benzoic acid or benzoate is thus depended on the pH of the food [11]. Sodium metabisulphite releases SO_2 gas when added to water, SO_2 kills yeasts, fungi and some bacteria and also it acts as an antioxidant.

Microbial analysis of fresh PFP showed that total plate count (TPC) at the initial time was 2×10^6 cfu/g and also heat treated PFP at 80°C (without the addition of chemical preservatives) was spoiled before 15 days whereas the pulp containing preservatives (T1-T15) exhibited no microbial growth up to 120 days period of storage. At 150 days of storage, the mean TPC was significantly increased from 0 to 9 cfu/g for T5 to T1. A maximum mean value was recorded in T2 while minimum value was observed in T4. Treatments T6-T15 showed growth of microorganism at 180 days of storage but counts (cfu/g) were in the acceptable range given in SLS 730: 2010. Hence chemical preservatives decreased the microbial load significantly in PFP. These results are in accordance with the findings reported by Hussain *et al.*, (2003) [12] and Hashmiet *et al.*, (2007) [13] for mango pulp.

3.2. Physicochemical evaluation

3.2.1. Acidity and pH

There were interaction between preservatives, concentrations and storage period for acidity values while except preservatives for pH. Significantly higher mean pH was observed for PFP treated with SB (4.32) when compared with SMS (4.24) and both SB, SMS (4.21) and there were significant different ($p < 0.05$) between mean pH of the pulp with the storage period while which was decreased with period of storage. PFP treated with SMS (T6-T10) and both SB, SMS (T11-T15) showed less increase in pH compared with PFP treated with SB (T1-T5). This may be due to either utilization or neutralization of acidic compounds present in the pulp otherwise compound/s secreted by organism. This condition may facilitate organism/s to prolong their growth and thereby leads to deteriorate the pulp. Abbassiet *et al.*, (2009) [14] attributed the increase in pH and the decrease in titrable acidity with increased storage period of the mangoes.

The results relating to the increase in acidity and decrease in pH (Figure 1) during the storage of PFP are in complete agreement with other researchers [15]. pH plays dual role in the fruit juices by acting as a flavour promotion and preservation. Decrease in pH of the fruit pulp samples proportional to increase in acidity has been confirmed by several researchers and may be attributed to the presence of SB in the pulp samples [16, 17].

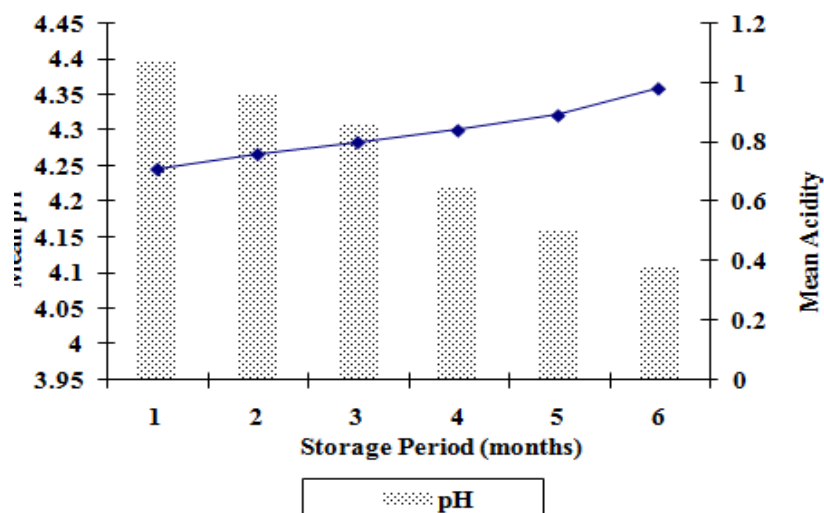


Fig 1: Change in pH and acidity of PFP incorporated with preservatives during the period of storage

Significantly higher mean acidity was observed for PFP treated with SB (0.88%) while no significant difference between pulp with SMS (0.79%) and both SB, SMS (0.80%). Change in acidity of PFP with the period of storage has been shown in Figure 1 while there were no significant different ($p < 0.05$) between 60, 90 and 120 days of the storage. While that acidity was significantly increased from 30-180 days. PFP treated with SMS (T6-T10) and both SB, SMS (T11-T15) showed less increase in percentage of acidity compared with PFP treated with SB (T1-T5). The increase in acidity may be ascribed to rise in the concentration of weakly ionized acid and their salts during storage and also due to formation of acid by degradation of polysaccharides and oxidation of reducing sugars or by breakdown of pectin substances and uronic acid [19, 17]

3.2.2 Total Soluble Solids (TSS)

There were interaction between all factors such as preservatives, concentrations and storage period. Amin *et al.*, (2008) reported the effect of time of fruit harvest affects the fruit quality. The variability in TSS in the PFP might be attributed to the alteration occurring in cell wall structure during ripening process. Moreover, various hydrolytic enzymes also affect complex carbohydrates changing them into smaller compounds. Significantly higher mean °brix was observed for PFP treated with both SB, SMS (13.00) when compared with SMS (11.02) and SB (12.15). TSS was significantly increased gradually up to a storage period of 180 days (TABLE 2). While there were no significant difference between 60 and 60 also 120 and 150 days of storage. PFP treated with both SB, SMS (T11-T15) and SB (T1-T5) showed more increase in °brix compared with PFP treated with SMS (T6-T10) while there was significance different between treatments.

Table 2: Effect of storage on TSS of the PFP (°Brix)

Treatments	Storage (days)						Mean	±SD
	30	60	90	120	150	180		
T1	11.83	11.84	11.89	11.95	11.90	12.00	11.9	0.06
T2	12.2	11.96	12.01	11.98	11.90	12.00	12.01	0.09
T3	12.17	12.03	12.11	12.48	12.25	12.50	12.26	0.18
T4	12.26	12.13	12.15	12.5	12.35	12.60	12.33	0.17
T5	12.43	12.17	12.19	12.38	12.25	12.30	12.29	0.09
T6	10.79	10.93	10.97	10.95	10.98	11.00	10.94	0.07
T7	10.86	10.90	10.93	10.98	10.95	11.00	10.94	0.05
T8	10.77	10.83	10.9	10.89	10.78	10.80	10.83	0.05
T9	10.86	10.92	11.26	11.45	11.40	11.50	11.23	0.25
T10	10.77	10.83	11.09	11.45	11.40	11.50	11.17	0.29
T11	12.65	12.9	12.91	12.95	12.90	13.20	12.92	0.16
T12	12.84	12.85	12.87	12.90	12.85	13.20	12.92	0.13
T13	12.86	13.03	12.29	13.20	12.95	13.60	12.99	0.39
T14	12.77	12.85	13.00	13.40	12.99	13.60	13.10	0.3
T15	12.71	12.78	13.01	13.30	13.20	13.60	13.10	0.31
Mean	11.92 ^d	11.93 ^d	11.97 ^c	12.18 ^b	12.07 ^b	12.29 ^a		
±SD	0.86	0.85	0.78	0.88	0.82	1.00		

Each value in the table is represented as mean ± SD (n = 3). Values in the mean row followed by a different letters (a-d) are significantly different (p < 0.05).

About half of the soluble sugars of PFP are mainly composed of fructose (3.4%), with about 6.6% sucrose and 3.5% glucose. The high sugar content of pulps from ripe fruits might be attributed to the transformation of starch into soluble sugars under the action of phosphorylase enzyme during ripening [19, 20] and water soluble pectin from insoluble proto pectin in lime squash and fruit bases, respectively [21, 22].

3.3 Sensory evaluation

Every fruit is selected by its visual appearance because colour of fruit is main attribute for judging the eatable quality of fruit and the same process is applied for the colour of PFP in this research. The values for colour of all the treated samples decreased during storage at ambient temperature. The PFP from various varieties collected from different production sites were not exactly at the similar ripening stage thus they may vary in colour and other sensory characteristics. Aina&Oladunjoye (1993) [23] reported that the colour change in mangoes is primarily associated with several biochemical changes, both degradation and synthesis of various classes of molecules including carotenoids in fruit.

A number of biochemical reactions or metabolic activities are involved in the ripening process of mango fruit such as increased respiration, ethylene production, change in structural polysaccharides causing softening, degradation of chlorophyll and synthesis of carotenoids, changes in carbohydrates or starch conversion into sugars, organic acids, lipids, phenolic compounds and a number of volatile compounds. All these changes lead to ripening of fruit with softening of texture to acceptable quality. These factors predominantly contribute towards developing a total sensory profile of the mango fruit [24].

The colour of T2 and T3 was spoiled and turned yellowish brown during 90 days of storage interval. Median values of colour score for T7 and T8 is high (44.5) when compared with other treatments while T3 showed very less score of median and also this median value decreased with increase concentration of preservatives (TABLE 3). Flavour is comprised of aroma and taste. The score for flavour decreased for PFP during storage at room temperature. Flavour score of T7 and T8 were higher than that of T12 and T13 during 180 days of storage while the scores noted for T2 and T3 were very less. Score of overall acceptability for T2 and T3 was less than other treatments when compared with others and median overall acceptability score at initial time of storage was highest for T7 (Figure 2).

Table 3: Effect of selected treatments on median value of sensory analysis at 180 days of Storage

	Flavour	Colour	Mouth feel	Texture	Overall acceptability
T2	14.5	14.5	17	19.5	15
T3	15.5	13	17	19.5	13
T7	42	44.5	37.5	31.5	37.5
T8	42	44.5	40.5	36.5	30
T12	37.5	34	39	41	33.5
T13	37.5	38.5	38	41	32.45

Organic acid and sugars ratio primarily creates a sense of taste which is perceived by specialized taste buds of the tongue. Thus, sweetness due to sugar and sourness from organic acids are dominant components in the mouth feel of many fruits [25]. But in PFP mouth feel is due to bitter compounds called flabelliferins which vary with many factors such as place at which palmyrah tree is grown, type of fruit and stage of ripening at which that fruit is tested. These factors play a major role in the assessment of its sensory qualities and acceptability [26]. In this study, T7 had highest overall acceptability at initial and 180 days of storage therefore based on the sensory characteristics the T7 was recognized as relatively better than the selected treatments (Figure 2).

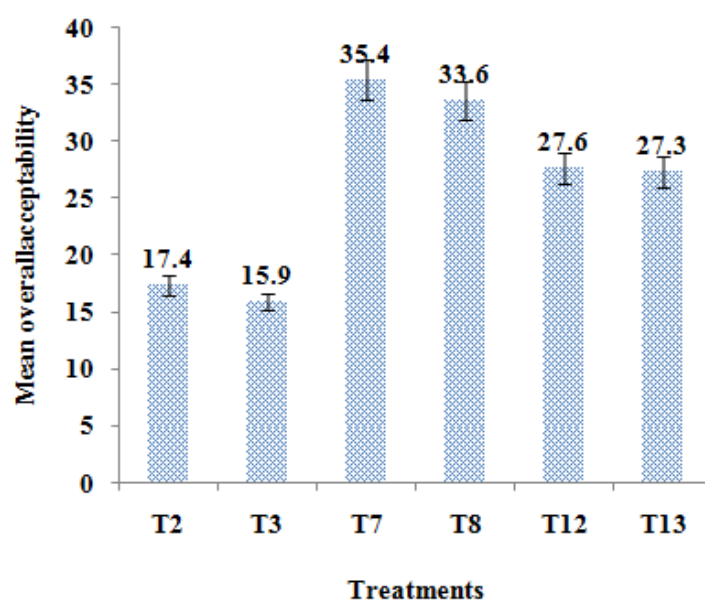


Fig 2: Effect of selected treatments on overall acceptability

IV. Conclusion

From this research, it is evident that storage of PFP incorporated with preservatives showed an increase in acidity and brix values besides the decreased level of microflora with time. However, according to the organoleptic evaluation done up to 180 days of period of storage PFP containing SB was rejected by panelists, whereas among the PFP containing SMS alone and combination of SB & SMS, PFP with SMS (0.4 g/l) was selected as better with respect to overall acceptability. Hence it is proved that pasteurization of PFP incorporated with SMS (0.4 g/l) at pH3.8 and 80°C for 30min is needed to store PFP for 6months without any loss of acceptable characteristics.

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

PN- made consultancy and revising the manuscript; RK carried out the research activities and revising the manuscript; SM carried out the research activities, statistical analysis and drafted the manuscript; SSV-coordinated & management of research activities. All authors read and approved the final manuscript.

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