Journal of Food Technology 10 (4): 117-123, 2012
ISSN: 1684-8462

Storage Stability of Cashew Apple Juice-Use of Chemical Preservatives

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Abstract: Cashew apples are being wasted across various parts of the cashew growing countries due to high perishability and short shelf life. The present study aims to preserve and improve shelf life of cashew apple juice using different combinations and concentrations of chemical preservatives. The efficiency of chemical preservatives was tested by analyzing sensory, physicochemical and microbiological qualities of the juice periodically. The results reveal that combination of sodium benzoate and sodium metabisulphite at 0.1 g L^{-1} each, sodium benzoate and citric acid at 0.1 g L^{-1} each and sodium metabisulphite and potassium metabisulphite at 0.05 g L^{-1} each, prolonged shelf life of cashew apple juice upto 20 days. Vitamin C and total sugars of the preserved samples were found to be almost stable. Sensory attributes also revealed good overall acceptability of the juice. Thus, cashew apple juice could be preserved using optimized chemical preservatives at household level.

Key words: Cashew apple juice, chemical preservatives, shelf life, physico-chemical characteristics, microbial analysis

INTRODUCTION

The cashew tree (*Anacardium occidentale* L.) is cultivated in 32 countries around the world with Brazil, India, Vietnam and Nigeria as the main cultivation centres (Honorato and Rodrigues, 2010). The tree bears two kinds of fruits, one the cashew nut (the true fruit) and the other, the cashew apple (pseudo fruit). Cashew nuts are commercially exploited in India and other parts of the world but cashew apples are rotten and wasted in the soil due to lack of proper preservation techniques. Cashew apple is fibrous, weighs about 75-80 g and is 6-10 cm in length (Maciel *et al*., 1986; Guilherme *et al*., 2007). It is a non-climacteric fruit found in three colours: yellow, orange and red with the pale yellow pulp (Sondhi and Pruthi, 1980; Rocha *et al*., 2006). For every ton of cashew nut, about 10-15 tonns of cashew apples are produced (Attri, 2009).

Cashew apple is rich source of carbohydrates, minerals, amino acids, carotenoids, phenolics (quercetin, anacardic acid, tannin), organic acids and anti-oxidants (Trevisan *et al*., 2006; De Carvalho *et al*., 2007; Honorato and Rodrigues, 2010). Cashew apple also contains vitamins such as thiamine, riboflavin, niacin hence, considered as first class source of energy (Azam-Ali and Judge, 2001). Cashew apples are taken as a cure for stomach disorders and are used to treat vomiting, children's diarrhoea, worms and syphilis. Cashew apple juice can be utilized as substrates for the production of dextransucrase, ethanol, biosurfactant, lactic acid, mannitol and many other value added products (Rocha *et al*., 2006; Chagas *et al*., 2007; Pinheiro *et al*., 2008; Fontes *et al*., 2009; Silveira *et al*., 2012).

Besides possessing nutritional, therapeutic properties and industrial applications, these are deterred due to various reasons short shelf-life, astringency and high perishability. Unlike other fruits, even during the fruiting season, these cannot be consumed at once in more quantity due to its astringent taste. Therefore, a proper method for preservation of cashew apple juice needs to be explored. In the present research, chemical preservatives namely, sodium benzoate, sodium metabisulphite, potassium metabisulphite, citric acid and benzoic acid were used for preservation and the juice was stored under refrigeration. Preservation of cashew apple juice not only minimises wastage of nutritious fruits but is also expected to derive economic benefits to rural farmers.

The juice extracted from cashew apples has pleasant flavour and if not consumed fresh, fermentation takes place and sour odour gets initiated resulting in spoilage. There are many factors responsible for spoilage of cashew apple juice which include physical, chemical, enzymatic and microbiological changes. The presence of oxygen

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also contributes to deterioration of fruit juices (Soares and Hotchkiss, 1999). Some of the adverse effects of dissolved oxygen on fruit juice quality include ascorbic acid degradation, increase in browning and growth of aerobic bacteria and molds (Meydev *et al*., 1977; Kennedy *et al*., 1992; Solomon *et al*., 1995; Soares and Hotchkiss, 1999). Microbiological spoilage is preventable to a large degree by a variety of chemical preservatives which prevent or inhibit the microbial growth (Gould, 1996). No single preservative is completely effective against all microorganisms. Sodium benzoate and benzoic acid, inhibit yeasts and molds (Banwart, 1989). Sodium benzoate has been Generally Recognized As Safe (GRAS) as a direct food additive and flavouring agent. Sodium and potassium metabisulphites reduce browning of cashew apple juice (Costa *et al*., 2003). Citric acid is known to decrease polyphenol oxidase activity in cashew apple juice (Queiroz *et al*., 2011).

The present study is focused to determine the concentration and combination of different preservatives to prevent the spoilage and to preserve the nutritional quality of the juice.

MATERIALS AND METHODS

Survey and processing of cashew apples: Different regions of Andhra Pradesh were surveyed for cashew plantations and cashew apples were procured from five selected regions namely Rajam, Parawada, Rajolu, Srikakulam and Yendada during the fruiting season. The collected cashew apples were first weighed, washed thoroughly in water to remove foreign particles. Juice was extracted using juice extractor with a yield of 780 mL kg^{-1} and filtered through sterilized muslin cloth. The detailed methodology is shown in Fig. 1.

Storage and analysis of fresh and preserved juices: The extracted juice was aseptically transferred to sterilized glass bottles that were numbered as 1-20 (Table 1). Ten different combinations and twenty different concentrations of chemical preservatives namely Sodium Benzoate (SB), Sodium Metabisulphite (SMS), Potassium

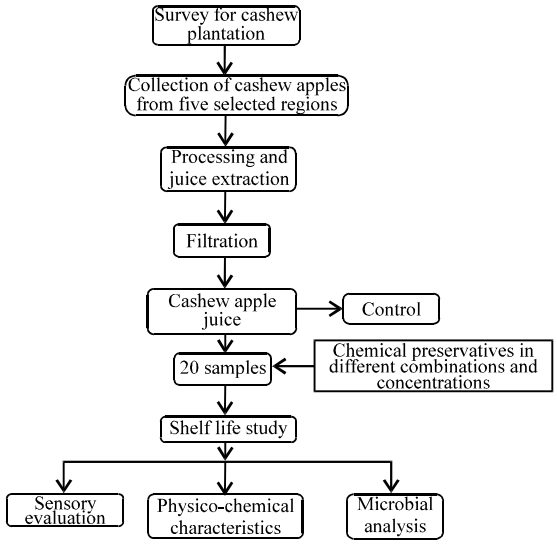

Fig. 1: Flow chart of cashew apple juice preservation

Table 1: Details of preserved samples and microbiological quality of the preserved cashew apple juice samples for a period of 30 days

Sample No.	Samples description	Concentration of the preservatives	Total count (log CFU mL^{-1})/No. of days: Bacteria 10	Bacteria 20	Bacteria 30	Yeasts 10	Yeasts 20	Yeasts 30	Molds 10	Molds 20	Molds 30
1	SB+SMS	0.05+0.05	6.30	6.47	6.60	6.60	6.84	7.00	6.60	6.90	7.00
2		0.1+0.1	-	-	-	-	-	6.30	-	-	6.90
3	SB+KMS	0.05+0.05	6.30	6.30	6.47	6.00	6.60	6.90	6.00	6.77	7.00
4		0.1+0.1	6.00	6.30	6.60	6.60	6.77	6.84	6.60	6.84	6.90
5	SB+CA	0.05+0.05	6.69	6.69	6.69	6.84	6.84	6.90	6.84	6.90	7.00
6		0.1+0.1	-	-	6.69	-	-	6.77	-	-	6.84
7	SB+BA	0.05+0.05	-	-	-	6.30	-	6.47	6.60	6.84	7.00
8		0.1+0.1	-	6.00	6.47	-	-	6.77	-	-	6.60
9	SMS+KMS	0.05+0.05	-	-	6.30	-	-	6.47	-	-	6.47
10		0.1+0.1	-	6.00	6.47	6.00	6.47	6.60	6.30	6.30	6.60
11	SMS+CA	0.05+0.05	6.30	6.30	6.69	6.47	6.47	6.77	6.30	6.30	6.47
12		0.1+0.1	6.00	6.00	6.00	6.00	6.30	6.47	6.30	6.30	6.60
13	SMS+BA	0.05+0.05	-	-	6.30	-	-	6.30	-	-	6.47
14		0.1+0.1	-	6.30	6.60	-	-	6.47	-	-	6.60
15	KMS+CA	0.05+0.05	6.00	6.30	6.47	6.30	6.30	6.60	6.30	6.30	6.30
16		0.1+0.1	6.47	6.47	6.60	6.30	6.60	6.60	6.47	6.47	6.69
17	KMS+BA	0.05+0.05	-	-	6.30	-	-	6.69	-	-	6.60
18		0.1+0.1	-	6.00	6.60	-	-	6.47	-	-	6.30
19	CA+BA	0.05+0.05	-	6.47	6.60	6.00	6.69	6.77	6.47	6.90	6.90
20		0.1+0.1	-	6.30	6.69	-	-	6.84	-	-	7.00

SB: Sodium Benzoate; SMS: Sodium Metabisulphite; KMS: Potassium Metabisulphite; CA: Citric Acid; BA: Benzoic Acid

Metabisulphite (KMS), Citric Acid (CA) and Benzoic Acid (BA) were added by constant stirring and samples were stored at 4°C. Replicates for each sample were also stored. Sensory evaluation, physico-chemical and microbiological analysis were performed for fresh (control) and preserved juice samples. The preserved samples were analysed at an interval of 10 days for 6 months.

Sensory evaluation: Sensory attributes were determined according to Stone and Sidel (1992). An informal panel of 10 untrained testers carried out the acceptance tests. The tests were performed by the hedonic rating option where panelists evaluated the degree of acceptability of samples based on colour, flavour, taste, sedimentation and overall acceptability. Sensory tests were performed in individual booths, in the morning shift (9:30-11:30 am) under white light. Samples, one per tester were served in transparent glass cups.

Physico-chemical analysis: All the samples were analysed for physico-chemical characteristics. Colour was determined by measuring absorbance at 420 nm (Abs_{420}) using dual beam UV-VIS spectrophotometer (ELICO BL-198). pH and Total Soluble Solids (TSS) were determined using digital pH meter (ELICO L1 614 pH Analyser) and digital ATAGO refractometer (ATAGO, PAL-Maple Pocket type), respectively. Vitamin C content and total sugars were determined according to Sadasivam and Manickam (1996). Total Titratable Acidity (TTA), sulphur dioxide content (SO_2), tannins and Polyphenol Oxidase (PPO) activity were measured according to methods recommended by Ranganna (1986).

Microbial analysis: Nutrient Agar media was used for the enumeration of bacteria and Rose Bengal Agar media for the enumeration of yeasts and molds. The juice containing media plates were incubated at 35±2°C for 24 h for Nutrient Agar and Rose Bengal Agar plates at 28±2°C for 7 days according to the methods of APHA (1992). The total number of bacteria, yeasts and molds per mL of the preserved juice samples were obtained by multiplying the number of Colony Forming Units (CFU) on the plate with 10^6 dilution factor and then was converted into logarithmic form. The experiment was repeated twice and data was represented as mean values (log CFU mL^{-1}).

Statistical analysis: Analysis of Variance (ANOVA) was performed with a significance level of $p \leq 0.05$ using the statistical package STATISTICA 6.0 (Stat-Ease Inc., Tulsa, OK, USA).

RESULTS

Sensory analysis

Colour: Colour of the preserved juice samples was found to be light yellow on the day of preservation and the mean values of the colour were observed to be between 7 (like moderately) and 8 (like very much). No significant differences ($p \leq 0.05$) were found among the preserved juices during 30 days of storage. However, the colour of the juices turned brown gradually after 30 days, accompanied with slight turbidity and sedimentation.

Flavour: No significant difference was found for the flavour of all the samples up to 30 days ($p \leq 0.05$) scoring a rating of 8 (like very much). The mean values of the stored samples were between 7 (like moderately) and 8 (like very much) which can be regarded as a satisfactory result. After 30 days, the comments of hedonic scale rating by panelists indicated unpleasant flavour which might be due to fermentation due to the action of microorganisms. The change in flavour of cashew apple juice is due to the presence of isobutyric and isovaleric acids in the juice (Maciel *et al*., 1986).

Taste: Significant difference ($p \leq 0.05$) in taste was observed in Samples S-2, 6, 9, 13, 15, 17 and 20 up to 30 days, securing score 8 and ranked as like very much. This result indicates the effectiveness of the respective preservatives in decreasing the tannin content (causing astringency) of the juice. Samples S-8, 14 and 18 were acceptable in terms of taste up to 20 days. Tastes of all other samples were ranked 6.0, i.e., like slightly up to 10 days.

Sedimentation: The added chemical preservatives were fully dissolved on the day of preservation of all the samples and no sedimentation was observed up to 10 days of storage under refrigeration. After 15 days of physical observation, though clarity of the preserved samples was maintained, sedimentation was observed in all the samples and these samples were ranked 7 (like moderately).

Overall acceptability: The mean values of overall acceptability of Samples S-2, 6, 8, 9, 13, 14, 15, 17, 18 and 20 were found to be between 6 (like slightly) and 7 (like moderately) with an average value of 6.5 indicating an acceptable score. In all the other samples, significant difference ($p \leq 0.05$) was observed at 95% confidence level throughout the storage period with a rating of 2.0 (dislike very much) indicating that these samples were not acceptable.

Table 2: Variation of pH, TTA, TSS, Tannins and PPO activity of samples showing good microbial quality (Samples S-2, 6, 9, 13 and 17)

Parameters / Samples/Storage time (days)	pH			TTA (malic acid) (%)			TSS (Brix) (%)			Tannins (%)			PPO (U mg^{-1})		
	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
S-2	3.68	3.45	3.20	0.203	0.213	0.245	13.5	13.2	13.0	0.84	0.83	0.82	2.34	2.36	2.40
S-6	3.72	3.50	3.48	0.280	0.302	0.309	14.5	14.5	14.3	0.48	0.43	0.39	2.58	2.59	3.30
S-9	2.59	2.53	2.44	0.290	0.340	0.360	12.6	12.4	12.6	0.87	0.85	0.81	3.75	3.78	3.99
S-13	4.70	4.30	3.70	0.250	0.260	0.270	11.9	11.7	11.5	0.53	0.50	0.48	3.29	3.35	3.40
S-17	4.53	4.36	4.29	0.319	0.325	0.341	12.7	12.1	11.3	0.64	0.61	0.57	2.67	2.71	2.76

TTA: Total Titratable Acidity; TSS: Total Soluble Solids; PPO: Polyphenol Oxidase

Physico-chemical analysis

pH: pH of the samples S-8, 10 and 19 was stable whereas decrease in pH was observed in all the other samples (3.6±0.6). However, the decrease was significant ($p \leq 0.05$) in Samples S-1, 2, 4, 5, 6, 9, 13 and 17 throughout the study period (Table 2). The decrease in pH with increase in shelf life could be probably attributed to the type of preservative used which was effective in completely removing the microorganisms. Further, at low pH, most of the bacteria will not grow and hence, the quality of the juice can be maintained (Ranganna, 1986).

Absorbance: Decrease in absorbance was observed in all the samples from 0.51±0.4 to 0.28±0.2 except in Samples S-14 and 17 for which absorbance was stable (0.48±0.4) towards the end period. Significant decrease in absorbance was observed in the Samples S-1, 2, 6, 9 and 12 at 95% confidence level ($p \leq 0.05$) throughout the storage period indicating clear and colourless juice. Stable absorbance indicates the efficiency of preservatives in retaining the colour of the juice.

Total Soluble Solids (TSS): The range of total soluble solids was found to be 11.6-14.6% Brix. Significant decrease ($p \leq 0.05$) in TSS of all the samples was observed throughout the storage period which might be due to the degradation of sugars with storage time. However, in Samples S-2, 6, 7, 9, 10, 13 and 17 decrease in TSS was not significant ($p \leq 0.05$) up to 30 days (Table 2).

Vitamin C (Ascorbic acid): The range of Vitamin C in fresh juice was found to be 123.9-184.46 mg/100 mL. Decrease in Vitamin C content was observed throughout the storage period in all the samples. However, the decrease was least (<5.5%) in Samples S-2, 5, 6, 9 and 14 (Fig. 2). The decrease in Vitamin C content is in conformity with studies in cashew apple juice preserved using 300 mg L^{-1} of sulphur dioxide (Costa *et al*., 2003). The decrease might be due to the presence of oxygen in the juice. The loss of ascorbic acid content in fruits might be minimized by treating with sulphur dioxide during processing or storage (Gregory, 1996).

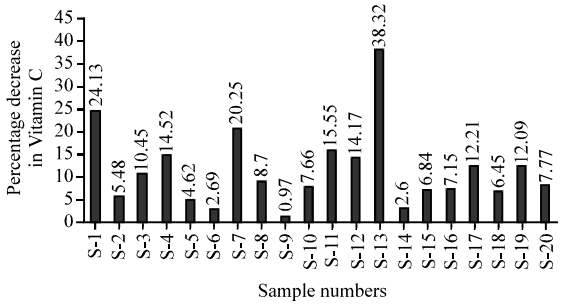

Fig. 2: Percentage decrease in Vitamin C content of different samples up to 30 days

Total Titratable Acidity (TTA): The range of TTA of fresh cashew apple juice was found to be 0.306-0.456% as malic acid. TTA was stable in Samples S-5, 11 and 18 whereas increase in TTA was observed in Samples S-2, 6, 9, 10, 14 and 16. The increase in acidity may be ascribed to rise in the concentration of weakly ionized acid and their salts during storage, i.e., change in pH (Table 2). The increase in titratable acidity might also be due to formation of acid by degradation of polysaccharides and oxidation of reducing sugars or by breakdown of pectic substances during processing (Hummel and Okey, 1950; Iqbal *et al*., 2001; Hussain *et al*., 2008). Decrease in TTA was observed with storage time in all the other samples however the decrease was significant in Samples S-1, 13, and 19 ($p \leq 0.05$). The decrease in TTA could be attributed to hydrolysis, oxidation or fermentation.

Sulphur dioxide (SO_2) content: Significant decrease ($p \leq 0.05$) in SO_2 content was observed with storage time in Samples S-1, 2, 14 and 16. In all the other samples preserved using metabisulphites (SMS and KMS), gradual decrease in SO_2 content was observed which was not significant ($p \leq 0.05$). The SO_2 content released by the metabisulphites is an efficient antimicrobial agent as well as ascorbic acid stabiliser which in turn depends on the pH of the juice. However, Mathooko and Kiniiya (2002) reported that high levels of sulphur dioxide (>400 ppm) impart characteristic pungent smell to the fruit juices.

Total sugars: The range of total sugars in fresh juice was found to be 8.4-14.8% (w/v). Figure 3 shows the

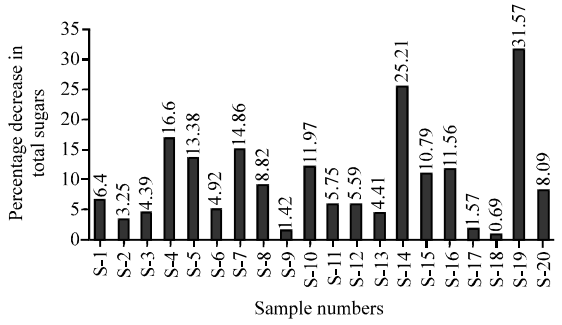

Fig. 3: Percentage decrease in total sugars of different samples up to 30 days

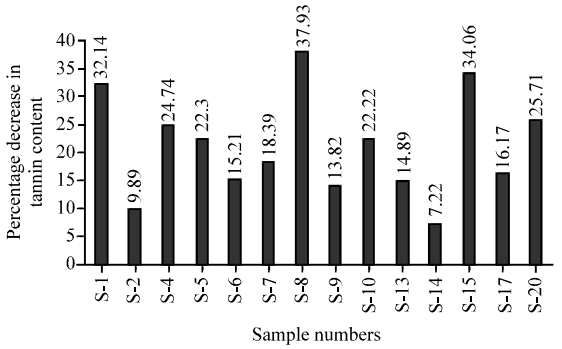

Fig. 4: Percentage decrease in tanin contant of different samples up to 30 days

percentage decrease in total sugars of all the preserved juice samples. The sugar content of all the preserved juice samples decreased at the end of the storage period but the decrease was not significant (<5%) in Samples S-2, 3, 6, 9, 13, 17 and 18. The decrease in sugars with time could be attributed to non-enzymatic browning reactions including condensation between reducing sugars and amino acids, caramelization and pigment destruction thus affecting the quality of the juices.

Tannins: Tannin content was found to be affected with storage time in different treatments. The effect of preservatives on tannin content of the juice samples is shown in Fig. 4. Increase in tannin content was observed in Samples S-3, 1 1, 12, 16, 18 and 19 whereas in all the other samples, tannin content decreased (up to <20%). Slight decrease in tannin content was observed in Samples 2, 6, 9, 13 and 17 (Table 2). This might be due to the efficiency of the respective combination of preservatives in precipitating tannins in the juice or due to the secretion of tannase by microorganisms which degrade tannins present in the juice (Ming-Shu *et al*., 2006).

Polyphenoloxidase activity (PPO): Slight increase in PPO activity was observed in Samples S-2, 6, 9, 13 and 17 which might be due to the degradation of tannins by the action of preservatives (Table 2). Decrease in PPO activity from 3.75±1.1 to 1.98±0.57 U mg^{-1} was observed in all the other samples throughout the storage period indicating the ineffectiveness of the preservatives in precipitating tannins. Citric acid and sodium metabisulphite are known to decrease PPO activity in cashew apple juice (Queiroz *et al*., 2011). This was clearly observed in sample containing sodium metabisulphite and citric acid at a concentration of 0.1 g L^{-1} each (S-12).

Microbial analysis: The total microbial count (bacteria, yeasts and moulds) present in preserved juice samples was not uniform. For up to 20 days, no microbial count was observed in preserved juice samples containing a combination of 0.1 g L^{-1} concentration of each of sodium benzoate and sodium metabisulphite (Sample-2), sodium benzoate and citric acid (Sample-6) and combination of 0.05 g L^{-1} concentration each of sodium metabisulphite and potassium metabisulphite (Sample-9), sodium metabisulphite and benzoic acid (Sample-13) and potassium metabisulphite and benzoic acid (Sample-17). Similarly, no microbial contamination was observed up to 10 days storage in juice samples preserved using combination of 0.1 g L^{-1} each concentrations of sodium benzoate and benzoic acid, sodium metabisulphite and benzoic acid, potassium metabisulphite and benzoic acid, citric acid and benzoic acid. After this period, growth of bacteria and moulds were observed in these samples. In all the other samples, microbial count was observed since the day of preservation. This indicates that the preservatives were inefficient in decreasing microbes.

DISCUSSION

The present study was conducted to optimize suitable concentration and combination of chemical preservatives to enhance shelf life of cashew apple juice. The shelf life of the juice preserved using different chemical preservatives (concentration and combination) showed variation in shelf life enhancement. The difference in enhancement of shelf life of the juices could be attributed to the difference in antimicrobial action (type, concentration and combination) of preservatives used.

Periodical analysis of the juice samples for the microbial count showed a progressive increase in the microbial growth with storage time though the rate of growth varied with different treatment combinations and concentrations. From the microbial data, it is clear that the total microbial count increased with the increase of storage period which could be due to variability in the chemical changes, specifically alteration in pH of the juice

which would take place resulting from the presence of the chemical preservatives in the juice samples. In Samples S-8, 14, 18 and 20, bacterial contamination was observed after 10 days of storage which might be due to improper handling, improper capping or combination of these. Sterile filtration of the juice prior to addition of preservatives might have reduced the bacterial count.

Sodium benzoate at 0.1 g L^{-1} concentration, in combination with any preservative is found to be effective in preserving the juice at least up to 10 days. But the same preservative at low concentration (S-1, 3, 5, 7) and in combination with KMS at high concentration (S-4) was not effective in decreasing microorganisms. The decrease in the microorganisms may be due to their presence in an unfavorable environment created by sodium benzoate. Sodium benzoate prevents the germination of bacterial spores. Sodium benzoate may have created hurdles which the organisms could not overcome. This may have led to physiological and metabolic distortion subsequently leading to death and decrease in population was observed. Hence, higher concentrations of sodium benzoate showed greater growth inhibition of microorganisms. This shows that the effect is concentration dependent.

On the other hand, Samples S-10, 11, 12, 15 and 16 were not acceptable in terms of reduction in microbes or retention of Vitamin C and sugars which might be attributed to high concentration of dissociated sulphurous acid which was unable to penetrate into the cell and inhibit cell division. Citric acid and benzoic acid at low concentration (0.05 g L^{-1} each) (S-19) was not efficient in preserving the juice, might be due to inefficiency of citric acid in maintaining low pH of the juice. However, citric acid in combination with sodium benzoate (S-5 and 6) and benzoic acid (S-20) in the specified concentrations was efficient rather than with metabisulphites. Though benzoic acid in combination with citric acid at 0.1 g L^{-1} (S-20) was efficient in decreasing microorganisms, it was not effective in retaining sugars and Vitamin C. The action of benzoic acid can be related to lowering the pH of the juice which does not favor growth of microorganisms. pH plays dual role in the fruit juices by acting as a flavour promotion and as preservative factor. And at low pH, the undissociated benzoic acid accumulates on the cell membranes or on various structures and surfaces of the bacterial and fungal cells, effectively inhibiting their cellular activity (16-19).

From Table 2, it can be observed that in Samples S-2, 6, 9, 13 and 17, pH and titratable acidity and tannins and polyphenol oxidase activity were inversely related. Whereas total soluble solids content and total sugars were directly proportional. In Samples S-13 and 17 though no microbial contamination was observed, negligible change in Vitamin C was observed at the end of 30 days storage period. Combination of sodium benzoate and citric acid (S-6) at 0.1 g L^{-1} concentration each was found to be stable up to 20 days. This result is in conformity with the research of Kurian and Peter (2007).

CONCLUSION

Considering all the parameters, the shelf life of cashew apple juice Samples S-2, 6 and 9 was prolonged up to 20 days in terms of retention of Vitamin C, sugars and decreasing microbial quality. Though these samples were slightly astringent, the samples were acceptable in terms of sensory and microbial quality during 20 days storage. This research revealed a simple and cost-effective method of preserving cashew apple juice at household level. Also, the research has the scope for utilizing cashew apples which are otherwise discarded in the field. Use of clarifying agents prior to addition of chemical preservatives would reduce the astringency and sterile filtration might reduce the microbial contamination. More detailed study is required for further enhancement of shelf life of cashew apple juice such as deaeration and heat treatment before bottling of the juice. Further, hydrolysable and condensed tannins present in the cashew apple juice needs to be analysed separately.

ACKNOWLEDGEMENTS

The researchers acknowledge funding received under the scheme Women Scientist Scholarship Scheme for Societal Programmes (WOS-B), Department of Science and Technology, Government of India for carrying out this research (Ref. No.: SSD/SS/033/2007) and to Department of Biotechnology, GITAM Institute of Technology for extending support during implementation of the research.

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