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Preservation of cashew apple juice using *Aloe vera* and hydroxychavicol

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ABSTRACT

Cashew apples are very nutritious and could be used the health, medicine and industry sectors if the high perishability and short shelf-life of the fruit and juice could be improved. Thus, the preservation of cashew apple juice for use in various applications is an important issue for food technologists. In this study, *Aloe vera* juice, hydroxychavicol and/or sterile filtration were employed to prolong the shelf-life of cashew apple juice. Juice quality (sensory, physicochemical and microbiological) was analysed after 15 days. The results showed that juice which underwent sterile filtration and was then preserved with hydroxychavicol was stable with preserved nutrient quality and medicinal properties for 45 days.

Keywords

Cashew apple juice
Aloe vera juice
Hydroxychavicol
Sterile filtration
Preservation

Introduction

Cashew apples are a rich source of carbohydrates, minerals, amino acids, carotenoids, phenolics, organic acids and vitamins, especially vitamin C (219 mg/100 ml) [1–3]. It is estimated that about 30 million metric tons of cashew apples are grown worldwide, with 64.14 tons produced in India and 9.95 tons in Andhra Pradesh [4]. Andhra Pradesh has the most land under cashew cultivation in India, at 165,560 hectares. About 10–15 tons of cashew apples are produced for every ton of cashew nut [5]. Despite their nutritional value, neither the fresh fruit nor the juice is consumed due to the presence of tannins and phenols, which impart an astringent taste. The juice can be utilized as a substrate for the production of value-added products but cannot be stored for prolonged periods due to its high perishability and short shelf-life. The juice possesses anti-bacterial, anti-oxidant and anti-mutagenic activity [6–8]. Cashew apples have medicinal properties and have been used in traditional medicine. In Brazil, fresh cashew apple juice is prescribed for stomach disorders and vomiting and as a gargle for sore throats. Fresh or distilled juice is a strong diuretic and is used to treat patients with uterine complaints and oedema. It also has sudorific and anti-syphilitic properties [9].

Although cashew apples have medicinal and therapeutic properties, they generally are not consumed like other fruits. The juice is subject to spoilage by microbial flora which results in an off-flavour. Therefore, to prevent microbial spoilage and increase the storage stability of the juice, methods such as hot fill, microfiltration and aseptic, high pressure and chemical treatments have been investigated [10–13]. However, no single method of preservation was effective in stabilizing nutrient quality and reducing astringency. In the hot fill, aseptic and high pressure methods, large amounts of energy are transferred to the juice, which triggers adverse reactions, leading to undesirable organoleptic and nutritional effects.

However, juice can be preserved using edible and medicinal herbs that inactivate microorganisms without altering juice quality or appearance. Antimicrobial compounds present in herbs can extend the shelf-life of unprocessed or processed juices by reducing microbial growth rate and/or viability. The inner gel of *Aloe vera* contains at least 75 active ingredients, including vitamins, enzymes, minerals, lignin, saponins, amino acids, sterols, sugars, salicylic acid and anthraquinones. However, no reports on their antimicrobial activity in preserving fruit juice have yet been published. Anthraquinones and saponins isolated from *Aloe vera* juice act against

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bacteria (both Gram-positive and Gram-negative), viruses, fungi and yeasts [14, 15]. Hydroxychavicol, a major phenolic component, obtained by aqueous extraction of *Piper betle* L. leaves, has been reported to possess antimicrobial activity. Time-kill studies found hydroxychavicol inhibited *Aspergillus* and yeast species with minimum inhibitory concentrations (MIC) of 125–500 µg/ml and 15.62–500 µg/ml, respectively [16, 17].

In this study, we sought to exploit the astringent principle for its medicinal value and to preserve the nutrient quality of cashew apple juice using *Aloe vera* juice, hydroxychavicol and/or physical methods such as sterile filtration.

Materials and methods

Materials

Commercial *Aloe vera* juice was procured from Lotus Enterprises, Visakhapatnam and hydroxychavicol was extracted from fresh *P. betle* leaves in the Plant Tissue Culture Laboratory of Gitam University according to the method of Peh and Wong [18]. Whatman glass microfibre filters (Whatman International, Maidstone, UK) and Millipore cellulose acetate filters (Millipore India, Bangalore, India) of pore size 0.45 and 0.22 µm were used for filtering cashew apple juice. Potassium metabisulphite (Koradia Exports, Ahmedabad, India) was supplied by Lotus Enterprises.

Extraction of hydroxychavicol

Fresh *P. betle* leaves (500 g) were cleaned under running tap water and left aside to drain. The leaves were then cut into small pieces, spread on plastic trays and dried in an oven-drier at 40°C for 3 days. After 72 hours of drying, the leaves were powdered using a blender and the powder stored in a sealed plastic bag in a refrigerator. Next, 10 g of dried powder was mixed with 150 ml of distilled water (1:15) and extraction was carried for 24 hours at 150°C using a Soxhlet extraction unit. The obtained extract was dried in an oven for 3 days at 40°C until the solvent had evaporated. The dried extract was weighed and stored.

Procurement and processing of cashew apples

Good quality cashew apples were procured from a farm in Yendada village, Visakhapatnam, Andhra Pradesh. The nuts of the cashew fruits were removed and the fruits weighed and washed thoroughly in distilled water to remove foreign particles. The fruits were then soaked in potassium metabisulphite solution (1 ppm) until juice was extracted using a screw press juice extractor (Process Masters Equipment, Pune, Maharashtra, India) with a yield of 780 ml/kg. The juice was filtered through a sterile muslin cloth to remove pulp particles.

Cashew apple juice preservation

Filtered samples (1 litre) of cashew apple juice were treated with *Aloe vera* juice at concentrations of 1 ml/l (S-1) and 3 ml/l (S-2). The same procedure was repeated with 50 µg/ml (S-3), 100 µg/ml (S-4), 500 µg/ml (S-5) and 1000 µg/ml (S-6) hydroxychavicol. Another juice sample (S-7) was passed through 0.45 and 0.22 µm filters for the complete removal of microbes (sterile filtration). In order to improve the shelf-life of the juice further, juice samples were also preserved using sterile filtration+hydroxychavicol 50 µg/ml (S-8) and sterile filtration+hydroxychavicol 100 µg/ml (S-9).

All samples were stored in duplicate in sterilized glass bottles under refrigeration at 4°C and analyzed for juice quality after 15 days.

Shelf-life evaluation of cashew apple juice

Sensory analysis

The preserved juice samples were analyzed organoleptically by 10 untrained panellists for colour, flavour, taste, sedimentation and overall acceptability. Sensory tests were performed in individual booths under white light. Samples, one per tester, were served in transparent glass cups. The samples were scored based on an organoleptic evaluation method [19] using a nine-point hedonic scale, ranging from 'dislike extremely' (score 1) to 'like extremely' (score 9).

Physico-chemical analysis

Colour was determined by measuring absorbance at 420 nm (Abs_{420}) using a dual beam UV-VIS spectrophotometer (ELICO BL-198). pH and total soluble solids (TSS) were determined using a digital pH meter (L1 614 pH analyser; Elico, Hyderabad, India) and a digital refractometer (PAL-Maple Pocket; Atago, Tokyo, Japan), respectively. Vitamin C content and total sugars were determined using the dichlorophenolindophenol (DCPIP) and phenol-sulphuric acid methods, respectively [20]. Polyphenol oxidase activity (PPO) was measured according to the Sigma quality control test procedure and expressed in units/mg enzyme. Total titratable acidity (TTA) was determined by titration with 0.1 N sodium hydroxide (Merck, Mumbai, India) and phenolphthalein as indicator (Qualigens Fine Chemicals, Mumbai, India), and tannins by the Folin-Denis method [21]. Phenols were determined using the Folin-Ciocalteu colorimetric assay and expressed as mg gallic acid equivalents/100 ml (GAE/100 ml) [22].

Microbial analysis

Nutrient agar medium was used for estimating the number of bacteria and Rose Bengal agar medium for estimating the number of yeasts and moulds. The juice-containing media plates were incubated at 35±2°C for 24 hours for nutrient agar and at 28±2°C for 7 days for Rose Bengal agar [23]. The total number of bacteria, yeasts and moulds per ml of the preserved juice samples were calculated by multiplying the number of colony forming units (CFU) on the plate with a 10^6 dilution factor and then converting the result into logarithmic form. The experiment was repeated twice and data were represented as mean values (log CFU/ml).

Results

Fresh cashew apple juice analysis

Fresh cashew apple juice was light yellow in colour with a pleasant flavour and astringent taste. The colour of the juice was due to the presence of carotenoids, mainly β-cryptoxanthin. Table 1 shows the physico-chemical characteristics of fresh cashew apple juice. Total bacteria, yeasts and moulds in fresh juice were in the range 7.14–7.51 log CFU/ml (Table 2).

Parameter	Value
pH	4.1±0.1
Absorbance at 420 nm	0.2±0.1
Total soluble solids (%Brix)	12.4±0.4
Total titratable acidity (% malic acid)	0.36±0.4
Viscosity (cP)	1.37±0.05
Vitamin C (mg/100 ml)	171.6
Total sugars (%)	11.4
Tannins (%)	0.88
Phenols (mg GAE/100 ml)	325±25
Polyphenol oxidase activity (U/mg)	3.40±0.02

Table 1 - Physicochemical characteristics of fresh cashew apple juice

Aloe vera juice as a preservative for cashew apple juice

Juice preserved using different concentrations of *Aloe vera* juice maintained its light yellow colour and astringent taste for up to 2 months, possibly because *Aloe vera* juice is colourless and clear and contributes phenols, respectively. However, a change in aroma was noticed after 15 days. Regarding physico-chemical analysis, a decrease in pH from 4.21 to 3.3±0.2 was seen towards the end of 2 months. The decrease in pH indicates an increase in juice acidity as a result of fermentation by yeasts, namely *Mucor, Aspergillus niger* and *Rhizopus*, which produce alcohol and acidic metabolites [24]. This was also observed in cashew apple juice preserved using cold and hot extracts of another natural preservative, *Aframomum danielli* [25]. Increases in TTA, tannins and phenols were observed up to 15 days but later stabilized up to 2 months. The TTA of these juices increased from 0.402 to 0.44±0.1. A similar decrease in pH with an increase in TTA was observed during the production of lacto-juice from sweet potato by fermentation using lactic acid bacteria [26] and in wine produced from sapota [27]. Tannins and phenols increased from 0.88 to 0.94±0.3% (Table 3) and 325±10.0 mg GAE/100 ml, respectively. A decrease in PPO activity from 3.39 to 2.29±0.3 U/mg was observed up to 15 days but later stabilized up to 2 months. Similarly, an increase in TSS from 12.1 to 13.7±0.3, and an increase in vitamin C and total sugars was also observed with an increase in *Aloe vera* concentration up to 15 and 30 days, respectively (Table 3), which might be due to the contribution of sugars, particularly mannose and vitamins, by the preservative.

Bacterial counts were reduced from 7.41 to 6.6 log CFU/ml up to 15 days but later increased (≤7.46 log CFU/ml) up to 2 months (Table 2). Growth of yeast in the juice resulted in an off-flavour, turbidity, alcohol and gas, while the growth of moulds produced unacceptable filaments in the juice.

Sample no./storage time (days)	No. of colonies on nutrient agar/Rose Bengal (log CFU/ml)														
	Bacteria					Yeasts					Moulds				
	0	15	30	45	60	0	15	30	45	60	0	15	30	45	60
1	7.41	6.60	7.25	7.39	7.46	7.25	6.69	6.90	7.11	7.25	7.20	6.77	6.95	7.11	7.27
2	7.41	6.84	7.20	7.36	7.49	7.25	6.77	7.07	7.23	7.36	7.20	6.69	7.07	7.25	7.38
3	7.32	6.47	6.84	7.0	7.44	7.25	–	–	6.30	6.69	7.17	–	–	6.0	6.60
4	7.32	7.0	7.15	7.30	7.41	7.25	–	–	6.0	6.47	7.17	–	–	6.47	6.47
5	7.32	7.04	7.11	7.25	7.38	7.25	–	–	6.47	6.60	7.17	–	–	6.30	6.47
6	7.32	7.11	7.17	7.27	7.44	7.25	–	–	6.30	6.69	7.17	–	–	6.30	6.69
7	7.51	–	–	–	6.30	7.14	–	–	6.30	6.77	7.23	–	–	6.0	6.60
8	7.32	–	–	–	6.47	7.25	–	–	–	6.30	7.17	–	–	–	6.30
9	7.32	–	–	–	6.60	7.25	–	–	–	6.47	7.17	–	–	–	6.60

Table 2 - Microbiological quality of the preserved juice samples

Sample no.	Vitamin C (mg/100 ml)						Total sugars (%)					Tannins (% tannic acid)				
	Storage time (days)					% Decrease after 60 days	Storage time (days)									
	0	15	30	45	60		0	15	30	45	60	0	15	30	45	60
1	171.6	179.5	173.4	157.2	142.8	16.78	11.4	11.7	11.7	11.8	11.8	0.88	0.91	0.91	0.91	0.91
2	171.6	186.3	170.8	158.4	135.1	21.27	11.4	11.9	12.0	12.1	12.1	0.88	0.96	0.96	0.95	0.96
3	171.6	171.3	170.7	166.4	153.2	1.39	11.4	11.3	11.3	11.3	11.3	0.88	0.91	0.91	0.91	0.91
4	171.6	169.9	169.1	161.7	152.6	3.14	11.4	11.3	11.1	11.0	11.0	0.88	0.94	0.94	0.94	0.94
5	171.6	168.2	164.7	154.2	147.5	10.19	11.4	11.2	11.1	10.6	10.4	0.88	0.96	0.96	0.96	0.96
6	171.6	161.1	156.3	146.6	134.6	21.56	11.4	11.0	10.8	10.3	9.8	0.88	0.99	0.99	0.99	0.99
7	171.6	171.4	154.6	133.6	125.5	2.97	11.4	11.3	11.2	10.9	9.7	0.88	0.87	0.87	0.87	0.87
8	171.6	171.4	170.8	170.4	169.9	0.99	11.4	11.4	11.4	11.4	11.4	0.88	0.89	0.89	0.89	0.89
9	171.6	170.2	169.9	169.2	168.4	1.86	11.4	11.3	11.3	11.3	11.3	0.88	0.9	0.9	0.9	0.9

Table 3 - Variation in vitamin C, sugar and tannin content in preserved juice samples

Hydroxychavicol as a preservative for cashew apple juice

Sensory evaluation showed that the juice preserved using different concentrations of hydroxychavicol changed from a light yellow to a brown colour. The intensity of the colour of the juice increased with extract concentration (Table 4). It was further observed that the astringency of the juice also increased with extract concentration. The changes in colour and taste of these samples might be due to the contribution of phenolic compounds by hydroxychavicol. However, no changes in flavour or sedimentation were observed during the 2 months.

The results showed that pH decreased from 4.3 to 3.6±0.3 in all samples preserved using the different concentrations of hydroxychavicol up to 15 days¬. Such a low pH does not favour bacterial growth and hence the juice can be maintained in good quality during storage [21]. TTA was found to be as stable in these samples as in fresh juice during the 2 months. The stability of TTA indicates that concentrations of organic acids, mainly malic acid, remained unaltered. With increased preservative concentration and storage time, a decrease in TSS (12.7 to 12.3±0.3), sugars and vitamin C (Table 3) was observed towards the end of 2 months. However, the decrease was not significant (1.39–3.14%) in samples preserved using low concentrations of hydroxychavicol (50 and 100 µg/ml). The decrease in vitamin C is likely due to oxidation, which occurs in fruit juices during storage, and is highly dependent on the presence of oxygen in the head space or dissolved in the juice. This result also corroborated previous findings by other authors. Reports reveal 25.65% and 26.74% decreases in vitamin C for cashew apple juice preserved using the hot fill and aseptic methods, respectively, over a shelf-life of 350 days [10]. Similarly, 29–59% of vitamin C in cashew apple juice and its blends was lost at the end of 240-day storage at room temperature [28]. It has been suggested that sulphur dioxide in the form of metabisulphites minimizes the loss of vitamin C during the processing or storage of fruits [29].

Tannins and phenol levels increased from 0.88 to 0.95±0.4% and from 325 to 340–395 mg GAE/100 ml, respectively. However, these increases were only observed up to 15 days and later stabilised, which might be due to inhibition of polyphenol oxidase after 15 days. PPO activity decreased from 3.4 to 2.93–3.2 U/mg up to 15 days and later stabilised. It was also observed that PPO activity was inversely proportional to tannin and phenol concentrations.

Thus, hydroxychavicol was efficient in removing yeasts and moulds in cashew apple juice up to 30 days, while the

Sample details	Colour (Abs_{420})				Visual clarity (%T660)			
Control	0.3				90.1			
Storage time (days)	15	30	45	60	15	30	45	60
Hydroxychavicol								
50 µg/ml	0.62	0.62	0.62	0.62	79.6	79.6	79.6	79.6
100 µg/ml	0.75	0.75	0.75	0.75	73.2	73.2	73.2	73.2
500 µg/ml	0.84	0.84	0.84	0.84	66.7	66.7	66.7	66.7
1000 µg/ml	0.97	0.97	0.97	0.97	54.6	54.6	54.6	54.6

Table 4 - Variation in colour and visual clarity with hydroxychavicol concentration

bacterial count only decreased from 7.32 to 6.84–7.17 log CFU/ml during this period (Table 2). The results indicate that hydroxychavicol extract exhibits better antifungal than antibacterial activity.

Efficiency of sterile filtration in cashew apple juice preservation

Juice subjected to sterile filtration was clear and colourless with a pleasant flavour and astringent taste similar to that of fresh juice. This was evidenced by the absorbance (0.16) and percent transmission (94.5%) of the juice, indicating that filtration was efficient in removing the pigments and substances that cause haze. Physicochemical analysis of the juice showed a decrease in pH from 4.0 to 3.5 towards the end of 2 months, whereas TTA and TSS were stable throughout the 2 months. Decreases in phenols from 325 to 320 mg GAE/100 ml, and in tannins, vitamin C and total sugars were observed (Table 3) when compared with the fresh juice, whereas PPO activity was stable throughout the 2 months. Further, sterile filtration inhibited the growth of bacteria up to 45 days, and of yeasts and moulds up to 30 days which later increased (6.0–6.30 log CFU/ml) as shown in Table 2.

Efficacy of sterile filtration combined with hydroxychavicol in extending the shelf-life of cashew apple juice

The addition of hydroxychavicol to the filtered juice (colourless) resulted in a brown colour which was stable up to 2 months. An increase in juice astringency was observed, which might have been due to the contribution of phenolic compounds by the hydroxychavicol extract. The juice was clear and transparent during the 2 months.

A combination of sterile filtration and hydroxychavicol (S-8 and S-9) preserved the juice best up to 45 days. The pH of these samples decreased from 4.35 to 3.3±0.1 towards the end of 2 months and TTA was stable. Such an environment (low pH and stable TTA) is not favourable for the growth of microorganisms so the nutrient quality of the juice (i.e., TSS and sugars) was stable up to 2 months. The stable TSS and TTA indicated that malic acid and sugars present in the juice samples remained unaltered. An increase in absorbance (0.2 to 0.3±0.1) was observed in these samples with increased concentrations of hydroxychavicol up to 15 days but was later stable. A slight decrease in vitamin C (0.99–1.86%) was observed in these samples towards the end of 2 months with increase in preservatives concentration (Table 3). Similarly, tannins, phenols and PPO activity were found to be almost stable. However, all these parameters were more stable in juice preserved using a low concentration of hydroxychavicol (S-8) than in sample S-9.

The microbial quality of these samples is shown in Table 2. The combination of sterile filtration and hydroxychavicol resulted in the inactivation of bacteria, yeasts and moulds in cashew apple juice for up to 45 days, which could be due to the efficiency of sterile filtration in removing bacteria together with the antifungal activity of hydroxychavicol.

Discussion

The ability of the natural food preservatives *Aloe vera* juice and hydroxychavicol to preserve cashew apple juice has been evaluated. In samples preserved using *Aloe vera* juice, an initial increase in vitamin C was observed up to 15 days, while an increase in tannins and total sugars was observed up to 45 days (Table 3). After this time point, vitamin C decreased while tannins and sugars were stable. One possible explanation for the initial increase in vitamin C and sugars is the contribution of active components of *Aloe vera* juice. The decrease in vitamin C in juice preserved using 50 and 100 µg/ml hydroxychavicol was comparatively less (1–3%) than in the sample preserved using *Aloe vera* juice (16–21%). It was also observed that the tannin content of the juices preserved using either of these preservatives rose with increased extract concentration (Fig. 1).

In juice samples preserved using hydroxychavicol, an increase in extract concentration was accompanied by a decrease in vitamin C content and total sugars, as illustrated in Figs. 2 and 3. A similar situation (i.e., a decrease in total sugars and TSS as must became wine) was observed in wine produced from sapota fruits, which indicates consumption of sugar by the yeast during ethanol production [27].

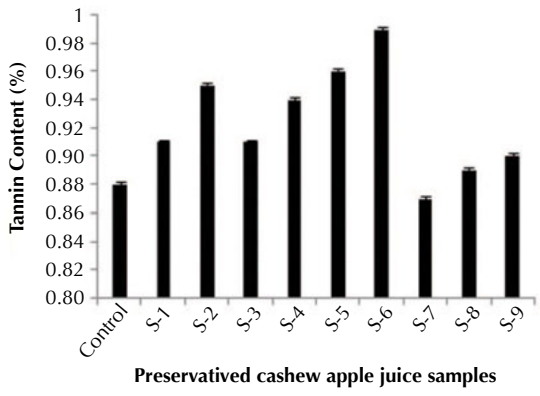

Figure 1 - Variation in tannin content of different samples after 45-days storage

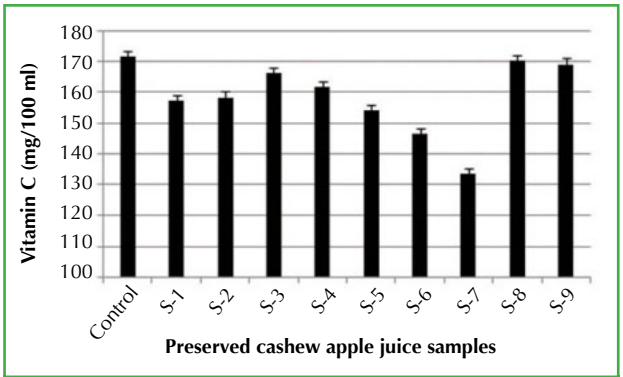

Figure 2 - Variation in vitamin C content of different samples after 45-days storage

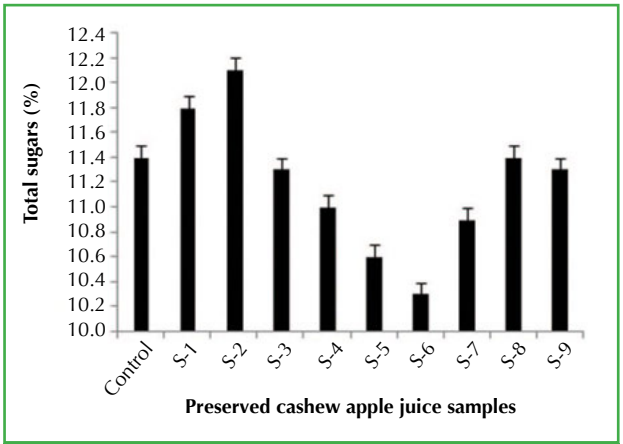

Figure 3 - Variation in total sugars of different samples after 45-days storage

A gradual fall in sugars and vitamin C was also observed during fermentation as the juice of cashew apple and of other fruits like jamun and bael fruits was made into wine [30–32]. A reduction in the vitamin C content of the must from 2.86 to 1.78 mg/100 ml in wine produced from sapota fruits was also seen [27]. The antimicrobial activity of juice preserved with *Aloe vera* juice was very poor. This might be due to the unstable nature of the active principle responsible for the inhibitory activity of *Aloe vera* juice against bacteria [33], yeasts and moulds. Nutrient quality was also changed in juice samples preserved with *Aloe vera* juice.

In the juice preserved using hydroxychavicol, though yeasts and moulds were not found up to 30 days, bacteria (6.84–7.17 log CFU/ml) were observed, which might be due to the direct addition of crude extract of hydroxychavicol to the juice. The addition of autoclaved extract might have eliminated this problem. Low concentrations of hydroxychavicol (50 and 100 µg/ml) were also more efficient in maintaining nutrient quality than high concentrations (500 and 1000 µg/ml). However, in order to remove bacteria, sterile filtration was employed using filters with very small pores (0.22 µm) which decreases the number of bacteria up to 45 days and of yeasts and moulds up to 30 days. The juice samples subjected to sterile filtration and preserved with low concentrations of hydroxychavicol have a prolonged shelf-life. However, juice preserved using higher concentrations of hydroxychavicol (500 and 1000 µg/ml) was not acceptable in terms of taste or aroma. The difference in the antimicrobial activity of *Aloe vera* juice and hydroxychavicol may be due to variation in the action of plant compounds present in the preservatives, that is, saponins, anthraquinones and dihydroxyanthraquinones in *Aloe vera* juice and polyphenolic compounds in hydroxychavicol. Cashew apple juice preserved using a combination of hydroxychavicol and sterile filtration could be marketed as a herb-enriched cashew apple juice as a remedy for common ailments such as the common cold, chronic diarrhoea, etc. The juice was stable regarding all biochemical and anti-oxidative properties except for a decrease in pH. The stable quantity of tannins, phenols and polyphenol oxidase reflects the health benefits of the juice, revealing its anti-oxidant potential.

Conclusion

This study investigated the preservation of cashew apple juice using *Aloe vera* and hydroxychavicol extracts. The results show that juice stored at 4°C and treated with a combination of hydroxychavicol at a concentration of 50 µg/ml and sterile filtration (0.22 µm Millipore membrane filters) preserves its nutrient quality (vitamin C and sugars) and medicinal properties (tannin content) and is microbiologically safe for up to 45 days. Although the organoleptic quality of the juice was not acceptable, it could be recommended to people with a sore throat or chronic diarrhoea. Treatment with a combination of sago (2 g/l), sterile filtration and citric acid and benzoic acid (0.1 g/l each) would improve the organoleptic and nutrient qualities of cashew apple juice [34]. Juice treated as described above could compete on the commercial market with other tropical beverages.

Conflict of interest

The authors declare they have no conflict of interest.

Human and Animal Rights

This article does not contain any studies with human or animal subjects performed by any of the authors.

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