

Original article

## Storage stability of a tropical fruit (cashew apple, acerola, papaya, guava and passion fruit) mixed nectar added caffeine

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**Summary** Nectars are formulated beverages with a fruit juice or pulp as its main component. A blend of fruits can also be used, with the advantage of combining sensory and nutritional properties of different fruits. The beverages may be added with some energetic component, as caffeine, known by its stimulating effect on central nervous system. The objective of this study was to develop mixed nectar of cashew apple, papaya, guava, acerola fruit and passion fruit added caffeine. The beverage was prepared with 35% juice blend and 10% sugar, added with 100 mg of caffeine L<sup>-1</sup> and 60 mg of sodium metabisulfite (SO<sub>2</sub>) L<sup>-1</sup> and 500 mg of sodium benzoate L<sup>-1</sup> as preservatives, packed in cleaned pasteurised bottles and heat processed at 90 °C for 30 s. Physicochemical, microbiological and sensory analyses of the beverage were performed initially (time zero) and during 6 months of storage at room temperature (about 25 °C). The products were microbiologically stable during storage. The vitamin C content decreased significantly throughout storage time, although it has remained relatively high. The product presented good sensory acceptance, which suggests its potential for market.

**Keywords** Caffeine, energetic beverages, nectar, shelf life, tropical fruits.

### Introduction

Beverages based on fruits are currently receiving considerable attention because their market potential is growing. Besides being delicious, these beverages are highly nutritious. They may be particularly useful in places where there is inadequate nutrition, which could lead to nutritional deficiency diseases (Zulueta *et al.*, 2007).

One of the most promising segments in Brazil is ready to drink fruit juice. This market share is being disputed by several industries, which look for possibilities of developing novel foods (Monteiro, 2006; Laboissière *et al.*, 2007). Nectars are ready-to-drink beverages usually formulated with a fruit juice or pulp, water, sugar and organic acid. Mixing two or more kinds of fruits can result in a new product with more vitamins and minerals, which presents some advantages, as the combination of sensory and nutritional properties of different fruits (Akinwale, 2000; Jain & Khurdiya, 2000).

Although blended fruit nectars have been frequently formulated, few involve tropical fruits.

Tropical fruits are widely accepted by consumers and are important sources of antioxidant compounds. Guava (*Psidium guajava* L.) is a cultivated species of the family Myrtaceae. The fruit is a very rich source of vitamin C, phenolic compounds, carotenoids and dietary fibres (Jawaheer *et al.*, 2003; Vasco *et al.*, 2008). Acerola fruit (*Malpighia emarginata* D.C.) has low sensory appeal and short fruit shelf life but high nutritional value, mainly vitamin C, carotenoids and anthocyanins, antioxidant pigments whose combination is responsible for the fruit red colour (Lima *et al.*, 2005). Cashew apple (*Anacardium occidentale* L.) is one of the fruits with the most expressive production in Brazil; however, <20% of its production is used by the fruit juice industry. This is often caused by the concentration of the harvesting season in 3 months, showing the importance of the agro-industry to add value to the final products. Cashew apple is a rich source of vitamin C, carotenoids and phenolic compounds (Assunção & Mercadante, 2003; Kubo *et al.*, 2006). The main form of utilisation of cashew apples is to process them into

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juice, but its high astringency tends to impair its acceptance. The formulation of mixed fruits and nectars may be an efficient way of reducing the negative impact caused by the cashew apple astringency (Sousa *et al.*, 2007). Papaya (*Carica papaya* L.) contains considerable levels of vitamin C, phenolic compounds and carotenoids (Luximon-Ramma *et al.*, 2003; Charoensiri *et al.*, 2009). Yellow passion fruit (*Passiflora edulis* Sims f. *flavicarpa* Degener) is a fruit much appreciated for its unique exotic flavour and yellow to reddish-orange colour because of the presence of carotenoids (Deliza *et al.*, 2004). Yellow passion fruit juice has good acceptability, pure or in combination with other juices and is considered an important source of vitamins, minerals, soluble and insoluble fibres (Righetto *et al.*, 1999).

Besides the mixture of fruits in beverages, the addition of stimulant compounds has been also studied. Caffeine, a powerful stimulating agent used in several beverage formulations, is a purine derived from xanthines. The stimulating effects of caffeine are attributed to its action on the human central neural system (Finnegan, 2003). Carvalho *et al.* (2007) added caffeine to a beverage formulated from cashew apple juice and coconut water, showing that this kind of product is a promising strategy to new products development.

The main goal of this work was to evaluate the storage stability as well as the consumers' acceptance during the storage of a stimulating blended beverage composed of cashew apple, passion fruit, papaya, guava and acerola fruit juices with addition of caffeine. This composition was previously selected as the most acceptable after a sensory study carried out with optimisation formulations (Sousa *et al.*, 2007). For a period of 180 days, the physicochemical, microbiological and sensory characteristics of this beverage, stored at room temperature ( $28 \pm 2$  °C), were evaluated every 30 days.

## Materials and methods

### Processing

Five pasteurised juices were blended in the following proportions: 40.00% cashew apple (*A. occidentale*, L.), 16.25% acerola (*M. emarginata* D.C.), 16.25% guava (*P. guajava* L.), 16.25% papaya (*C. papaya* L.) and 11.25% passion fruit (*Passiflora eludis* f. *flavicarpa*). The proportions of the juice were determined from the results of preliminary tests (Sousa *et al.*, 2007). The mixed nectar was formulated with 35% of the blended juice [14.0% cashew apple (*A. occidentale*, L.), 5.7% acerola (*M. emarginata* D.C.), 5.7% guava (*P. guajava* L.), 5.7% papaya (*C. papaya* L.) and 3.9% passion fruit (*P. eludis* f. *flavicarpa*)] and 10% sucrose in water, added with 100 mg of caffeine L<sup>-1</sup>. This amount is allowed by FAO/WHO (1997) and Brasil (2005), with

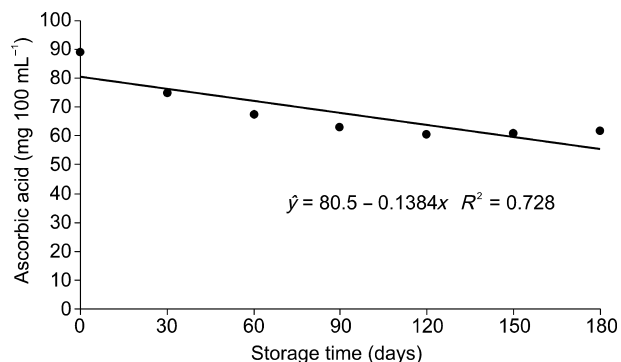
the maximum permissible level of caffeine being 350 mg L<sup>-1</sup> in energy drinks.

Sodium metabisulfite (60–40 mg of SO<sub>2</sub> kg<sup>-1</sup>) and sodium benzoate (500–200 mg kg<sup>-1</sup>) were added as preservatives. The beverage was heat processed (90 °C, 30 s), hot filled in 200 mL bottles and immediately closed with thread closures. The product was then cooled and stored at  $25 \pm 2$  °C. Three replicates of this nectar were prepared, and each of them was submitted to physicochemical analyses, which were done in triplicate. Sensory analyses were conducted with a homogenised mixture obtained from mixing the three replicates. Analyses were performed every 30 days during 6 months.

### Determinations

The following analyses were carried at the beginning of the storage time and every 30 days during 6 months of storage, to evaluate the product storage stability:

- Physicochemical analysis: total soluble solids was measured by a PR-101 digital refractometer (Atago, Norfolk, VA, USA); pH by a HI 9321 pHmeter (Hanna Instruments, Villafranca, Padovana, Italy) (potentiometer HANNA INSTRUMENTS, model HI 9321); titratable acidity was determined by titrimetry using NaOH solution (0.1 M) (AOAC, 1995); reducing and nonreducing sugars were determined by the method of Eynon and Lane according AOAC (1995), ascorbic acid was determined by means of a titration method, based on the reduction of the indicator, 2,6-dichlorophenol (Pearson, 1976) and caffeine was quantified by high performance liquid chromatography (HPLC) in a Varian ProStar (Palo Alto, CA, USA) chromatographic system composed of two high-pressure pumps model 201, a column oven Timberline model 101, a double channel UV-Vis detector ProStar model 342 and a Rheodyne injector with a 20-  $\mu$ L loop. Separation was achieved on a Microsorb C18 column (Varian, Palo Alto, CA, USA) (250 mm  $\times$  4.0 mm end capped with 5-  $\mu$ m spherical particles) thermostatted at 40 °C. Samples and standards were previously filtered with 0.45-  $\mu$ m nylon membrane (MFS, CA, USA). External standard method was used to build the calibration curve. The eluent was a mixture of water/methanol 70:30 (v/v), and the flow rate was 1.0 mL min<sup>-1</sup>. Detection was done at 285 nm, and caffeine peak was identified comparing retention time with the standard. The peak's identity was also confirmed spiking a sample with caffeine (Supelco Sigma-Aldrich, São Paulo, Brazil) (Carvalho *et al.*, 2007).
- Microbiological evaluations: counts of aerobic mesophiles, total and thermotolerant coliforms, molds and yeasts and *Salmonella* sp. (Downes & Ito, 2001);



**Figure 1** Vitamin C of the blended nectar added with caffeine as a function of the storage time.

- Sensory evaluation, carried out with forty untrained panellists were enlisted as suggest by Meilgaard *et al.* (1999) in laboratory test, consisting of: (i) acceptance tests for overall appearance, colour, flavour and overall acceptance, using nine-point structured hedonic scales (1: 'disliked extremely' to 9: 'liked extremely'); (ii) a purchase intent test, using a five-point structured scale (1: 'definitely would not buy' to 5: 'definitely would buy'). The tests were conducted in individual booths, under white light ('daylight'). The samples was refrigerated at  $6 \pm 2$  °C during 4 h before the evaluation and served at  $9 \pm 1$  °C. The panellists received 30 mL of each sample.

### Statistical analysis

The treatments were the seven storage periods 5 (0, 30, 60, 90, 120, 150 and 180 days) each replicated three times. For each physicochemical or sensory attribute, a model was fitted from the analytical results. The

models were analysed in terms of their significance (by *F*-test) and determination coefficient ( $R^2$ ). Additionally, a *t*-test was carried out to compare the overall acceptance of the product with that for a control treatment, not added with caffeine. Every statistical analyses were done using SAS program, software version 9.1 (SAS Institute, 2006), with significance established at  $P \leq 0.05$  level.

## Results and discussion

### Chemical and physicochemical determinations

Table 1 presents the characteristics of the blended nectar added with caffeine during 180 days of storage. Little changes were observed in the attributes monitored throughout storage time. Statistical analysis of the vitamin C, total soluble solids, ratio and colour values showed significant differences at 95% confidence level ( $P \leq 0.05$ ); however, only vitamin C adjusted to a regression model (Figure 1). The other evaluated parameters (titratable acidity, pH, reducing sugars, total sugars and caffeine) remained stable ( $P > 0.05$ ) during the storage time.

Soluble solids content was constant during the storage time in the range of  $15.0 \pm 0.0$ – $14.5 \pm 0.1$ ° Brix. Titratable acidity did change during the storage period, of 0.57–0.32, however, did not present significant difference ( $P > 0.05$ ). It was noted a fall in the acidity after the processing becoming stable after 30 days of storage. It can be because of the stabilisation of the additives (Kilcast, 2000). Some behaviour was noted for the ratio.

The caffeine content was very stable, around  $97.7 \pm 0.3$  and  $96.4 \pm 0.3$  mg L<sup>-1</sup>. Carvalho *et al.* (2007), in a beverage formulated with coconut water and cashew apple juice, found caffeine levels ranged from 93.6 to 101.1 mg L<sup>-1</sup> during 180 days of storage at

**Table 1** Physicochemical characterisation of the blended nectar added with caffeine as functions of the storage time [Value are reported in mean  $\pm$  SD of triplicate analysis of nine samples ( $n = 9$ )]

Determinations	Storage time						
	0	30	60	90	120	150	180
Ascorbic acid (mg per 100 mL <sup>-1</sup> )	88.9 $\pm$ 0.3	74.7 $\pm$ 0.7	67.2 $\pm$ 1.2	62.6 $\pm$ 0.4	60.2 $\pm$ 3.7	60.6 $\pm$ 1.7	61.8 $\pm$ 2.8
TSS (°Brix)	15.0 $\pm$ 0.0	15.6 $\pm$ 0.3	15.0 $\pm$ 0.0	14.6 $\pm$ 0.3	14.6 $\pm$ 0.3	14.5 $\pm$ 0.0	14.5 $\pm$ 0.1
TTA (g citric acid per 100 mL <sup>-1</sup> )	0.57 $\pm$ 0.00	0.34 $\pm$ 0.01	0.33 $\pm$ 0.01	0.33 $\pm$ 0.01	0.33 $\pm$ 0.01	0.33 $\pm$ 0.01	0.32 $\pm$ 0.01
Ratio	26.3 $\pm$ 0.0	46.6 $\pm$ 1.7	45.9 $\pm$ 0.8	44.0 $\pm$ 1.3	44.9 $\pm$ 0.7	44.4 $\pm$ 1.5	45.9 $\pm$ 0.8
pH	3.72 $\pm$ 0.01	3.72 $\pm$ 0.01	3.69 $\pm$ 0.01	3.64 $\pm$ 0.01	3.64 $\pm$ 0.01	3.67 $\pm$ 0.01	3.70 $\pm$ 0.01
Colour ( <i>L*</i> value)	39.5 $\pm$ 0.1	39.2 $\pm$ 0.1	38.8 $\pm$ 0.0	39.1 $\pm$ 0.1	39.0 $\pm$ 0.1	38.5 $\pm$ 0.8	38.8 $\pm$ 0.1
RS (g per 100 mL <sup>-1</sup> )	12.8 $\pm$ 0.2	17.9 $\pm$ 0.8	16.5 $\pm$ 0.9	19.8 $\pm$ 1.9	20.0 $\pm$ 0.4	17.1 $\pm$ 0.2	16.6 $\pm$ 1.2
NRS (g per 100 mL <sup>-1</sup> )	6.2 $\pm$ 0.5	0.2 $\pm$ 0.4	0.6 $\pm$ 1.1	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Caffeine content (mg L <sup>-1</sup> )	97.7 $\pm$ 0.3	96.4 $\pm$ 0.4	96.2 $\pm$ 0.4	96.6 $\pm$ 0.2	97.1 $\pm$ 0.3	97.2 $\pm$ 0.4	96.4 $\pm$ 0.3

TSS, total soluble solids; TTA, total titratable acidity; RS, reducing sugars; Ratio, relation: TSS/TTA; NRS, nonreducing sugars.

room temperature. Lima *et al.* (2009) also found stability in pH, soluble solids, titratable acid, total sugars and caffeine during 180 days of storage at room temperature ( $27 \pm 2$  °C) in glass-bottled pasteurised mixed of green coconut water and acerola fruit juice added with caffeine.

The linear model for ascorbic acid degradation determined in this study is in agreement with other studies (Burdulu *et al.*, 2006; Lima *et al.*, 2009). A loss of 30.5% ascorbic acid was noted after 6-month storage period. The decrease in the ascorbic acid content might be because of various factors that affect the stability of ascorbic acid. These factors include temperature, presence of oxygen in the headspace and light (Jawaheer *et al.*, 2003). Because the storage temperature was at room temperature (27 °C), the rate of degradation of ascorbic acid was relatively high. Assuming that glass containers are impermeable to oxygen, the principal causes of L-ascorbic acid destruction are oxidation by residual oxygen in the headspace followed by anaerobic decomposition and destructive influence of light (Maeda & Mussa, 1986). Being a photosensitive vitamin, vitamin C degradation during the storage period might be accelerated by the presence of light (Jawaheer *et al.*, 2003). Despite the vitamin C losses, its content at the end of the storage was 61.9 mg per 100 mL, i.e. only 100 mL of the beverage contained sufficient vitamin C to provide the recommended daily allowance (RDA) for adults, which is 45 mg (FAO/WHO, 2001). Because of the high vitamin C content of acerola, cashew apple and guava fruits, which were present in the nectar, even after this high loss, the beverage can still be considered a good source of vitamin C.

Statistical analysis of colour, expressed as  $L^*$  value, did not show significant difference ( $P \leq 0.05$ ) during the storage period. The average values ranged from  $39.5 \pm 0.1$  to  $38.8 \pm 0.1$ . According to Campos *et al.* (2002), Maillard reaction is a quick reaction and it is the major quality problem during storage at room temperature. It is largely affected by pH and temperature changes. Besides the darkening, which is a sensory parameter, it reduces the protein digestibility and inhibits some digestible enzyme reactions.

Statistical analyses of reducing and nonreducing sugars level did not present significant difference during the storage time. Reducing and nonreducing sugars content ranged of  $12.8 \pm 0.2$ – $16.6 \pm 1.2$  and  $6.2 \pm 0.5$ – $0.0 \pm 0.0$  g per 100 mL<sup>-1</sup>, respectively. This change is attributed to a slow acid hydrolysis of the nonreducing sugar (sucrose) added to standardise the beverage soluble solids, because the beverage was stabilised at acid pH values and weak acids easily hydrolyse sucrose (Bobbio & Bobbio, 1992). After 90 days of storage, the nonreducing sugar was almost completely hydrolysed to reducing sugar.

### Microbiological analyses

During the storage time, the counts of aerobic mesophiles of the beverage remained lower than 10 UFC mL<sup>-1</sup>, and the counts of yeasts and molds, lower than 100 UFC mL<sup>-1</sup>; the determination of total and thermotolerant coliforms presented values lower than 3 NMP mL<sup>-1</sup>; the presence of *Salmonella* sp. was not detected in 25 mL of the product. Therefore, the beverage remained safe from microbial contamination during storage at room temperature. It may be attributed to the combination of two preservation factors: the pH, which was below 4.0, the lower limit for bacterial growth (Alzamora, 1994), and the preservatives added.

### Sensory evaluation

The overall acceptance was not significantly affected ( $P > 0.05$ ) by the addition of caffeine, as indicated by the *t*-test result (Table 2). The mean hedonic ratings for both mixed nectars were not significantly different.

Table 3 presents the detailed sensory results for the product added with caffeine. At the beginning of the storage time, the product was well accepted by the panellists, with all the average hedonic scores near 7.0 ('liked moderately') and a positive average purchase intent (i.e. higher than 3.0 – 'might or might not buy').

The acceptance in terms of overall appearance and flavour did not significantly change with storage time. However, the overall acceptance decreased with time, which may be attributed to colour changes, because the colour acceptance also diminished. The purchase intent, however, remained stable.

**Table 2** Mean comparison between control and caffeine treatments for overall acceptance, by means of *t*-test

Treatment	(1)	(2)
Mean	7.48	7.07
Valid <i>N</i>	31	40
<i>t</i> -value	1.46	
<i>P</i>	0.15	

*N*, number of replications. (1) control treatment (without caffeine); (2) caffeine treatment.

**Table 3** Average scores of the acceptance tests of the blended nectar added with caffeine as functions of the storage time

Attribute	Storage time (days)						
	0	30	60	90	120	150	180
Appearance	7.2	7.1	6.6	6.2	6.2	6.4	6.3
Colour	7.2	7.3	6.1	6.1	5.9	5.9	5.9
Flavour	6.9	6.4	6.5	5.8	5.7	5.9	5.7
Overall acceptance	7.1	6.6	6.4	5.5	5.9	5.8	5.9
Purchase intent	3.7	3.5	3.5	3.0	3.1	3.0	3.2

## Conclusions

The mixed juice added caffeine presented some chemical changes during the 6 months of storage. The most affected component was vitamin C.

Good microbial stability was found for the product during the storage period, indicating that the thermal treatment was effective and good sanitary processing conditions were applied in the beverage preparation. It may be considered safe for consumption for at least 6 months.

The blended nectar was well accepted by the consumers during the storage period for all evaluated attributes (colour, flavour, overall quality and purchasing intention). The caffeine addition and physicochemical changes did not affect the sensory characteristics and acceptance. The beverage formulated is a tendency (trend) of world market, where there is a quest for beverages of mixed fruits, especially tropical fruits, gathering sensory and nutritional quality and, where possible, functional properties.

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