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Effect of food additives on the antioxidant properties and microbiological quality of red guava juice[1]

Efeito dos conservantes alimentares sobre as propriedades antioxidantes e qualidade microbiológica de suco de goiaba vermelha

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ABSTRACT - Guava is a tropical fruit that has a high potential for agroindustrial use, also known antioxidant property mainly attributed to vitamin C and carotenoids content. The objective of this study was to evaluate the influence of food preservatives, individually or combined, on the antioxidant properties of red guava nectar during storage. Nectars added of different additives were stored in the dark for 180 days and evaluated at the processing day and every 90 days for physico-chemical parameters, color, bioactive compounds content, total antioxidant activity (TAA), the microbiologica and sensory quality. Was not observed changes in quality parameters (P>0.05), since the guava nectars were presented in accordance with Standards of Identity and Quality required by Brazilian law. According to data for antioxidante compounds and total antioxidant activity (TAA), can conclude that nectars added sodium metabisulfite and sodium metabisulfite + sodium benzoate showed, after 90 days of storage, a lower reduction in total extractable polyphenols (TEP) and vitamin C content, being the TEP reduction directly correlated with TAA.

Key words: *Psidium guajava*. Chemical additives. Bioactive compounds. Stability.

RESUMO - A goiaba é um fruto tropical que apresenta elevado potencial de aproveitamento agroindustrial, além de conhecida propriedade antioxidante atribuída, principalmente, ao seu conteúdo de vitamina C e carotenóides. O objetivo desse trabalho foi avaliar a influência dos conservantes alimentares, individualmente ou associados, sobre as propriedades antioxidantes do suco de goiaba vermelha durante o armazenamento. Os sucos adicionados de diferentes conservantes foram armazenados na ausência de luz durante 180 dias e avaliados, no dia do processamento e a cada 90 dias, quanto aos parâmetros físico-químicos, a cor, ao conteúdo dos compostos bioativos, a atividade antioxidante total (AAT), a qualidade microbiológica e sensorial. Durante o armazenamento, não foi observado mudanças (P>0.05) nas características de qualidade dos sucos de goiaba, uma vez que se apresentaram de acordo com os Padrões de Identidade e Qualidade exigidos pela Legislação Brasileira. De acordo com os dados obtidos para os compostos antioxidantes e a atividade antioxidante total (AAT) pode-se concluir que os sucos de goiaba adicionados dos conservantes metabissulfito de sódio e metabissulfito de sódio + benzoato de sódio apresentaram, aos 90 dias de armazenamento, menor percentual de redução no conteúdo de polifenóis totais e de vitamina C, sendo os polifenóis o principal bioativo de influência na AAT.

Palavras-chave: *Psidium guajava*. Aditivos químicos. Compostos bioativos. Estabilidade.

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INTRODUCTION

In 2011, 38,948 million liters of juice were consumed worldwide. However, that is still a low per capita consumption, which favors the growth of the fruit juice market, making the fruit juice industry one of the largest agribusinesses in the world (FALGUERA; IBARZ, 2014).

Since the ripe guava is highly perishable when stored at room temperature, it is processed in various commercial products, including pulp, paste, canned slices in syrup and juice. Among these products, guava juice has become economically important in the market. The consumption of tropical fruit juice like guava is currently growing because it is natural, rich in nutrients and used as an alternative to other beverages such as soft drinks, tea and coffee (AKESOWAN; CHOONHAHIRUN, 2013).

The great national production, the high rate of perishability and lack of appropriate technology are some of the factors responsible for the high amount of waste in the fruit processing sector. The addition of chemical food additives to these products in order to improve the color, aroma, texture and flavor become necessary to benefit the food industry (QUEIROZ *et al*., 2006).

Chemical preservatives, a class of food additives, can be applied to maintain the quality and stability of fruit juices, resulting in satisfactory effects throughout the marketing period, given the market conditions and offering safety to the consumer. According to Damodaran, Parkin and Fennema (2010), these compounds show great technological importance during food processing, because they act as inhibitors of non-enzymatic browning and microbial growth, such as antioxidants and reducing agents, depending on the class used.

Among the most common preservatives used in food, according to the legislation for non-alcoholic beverages, are benzoic acid and its sodium, calcium and potassium (maximum permissible concentration 0.05g/100 mL), sorbic acid and its sodium, potassium and calcium (maximum permissible concentration of 0.08g/100 mL) and sulfur dioxide (maximum permissible concentration of 0.004 g/100 mL) (BRASIL, 2013).

The trends in global fruit juice point to the tropical fruit markets that emerge as an attractive potential because of the diversity of flavors, in addition to the nutritional and therapeutic value due to its high content of natural antioxidant such as carotenoids, polyphenols and ascorbic acid, in addition to flavonoids. Among the tropical fruits that have potential for agro-industrial use and thus participate in the composition of functional beverages, there is the guava (KAUR *et al*., 2011).

Studies have shown the influence of processing on the antioxidant properties of fruits (FERNANDES *et al*., 2006; MAIA; SOUSA; LIMA, 2007). However, there is little information related to the behavior of the chemical preservatives as the antioxidant components of processed fruit, making research necessary to quantify their influence and the action of the bioactive. Thus, this study aimed to evaluate the influence of different chemical food preservatives, isolated and / or associates, on antioxidant activity and microbiological and sensory attributes of guava juice sweetened during storage.

MATERIAL AND METHODS

Red guavas, of the Paluma variety, selected for their integrity and uniformity in the ripe maturity stage, obtained from the Ceara Supply Center - S / A (CEASA, Fortaleza - CE), were transported using coolers from the Fruits and Vegetables Laboratory (Department of Food Technology - UFC), where they were sanitized (100 mg of active chlorine / 1000 mL of water), pulped and processed.

The juices were composed of 40% puree, 50% potable water and 10% sucrose. Then preservatives were added as formulations shown in Table 1, considering as the control sample, juice without the addition of preservatives.

Subsequently, the mixture was homogenized and standardized in reference to the soluble solids content, according to the Identity and Quality Standard (IQS) for guava juice (11 °Brix) (BRASIL, 2003). After standardization, the juices were heated (90 °C, 60 s) (SILVA *et al*., 2010), and filled while hot in 200 mL glass containers, closed with a plastic screw cap and cooled under running water. The bottles were placed in cardboard boxes and stored at room temperature for 180 days. The processing of the juice was assessed every 90 days for physical and physicochemical parameters, antioxidant activity, microbiological quality and sensory attributes.

The pH parameters, titratable acidity (TA), in % of citric acid, soluble solids (SS) and in °Brix, were evaluated according to IAL (2008); and the color evaluation was performed using the CIE LAB system with the CR-400 colorimeter (Konica Minolta Sensing, Inc.) in reflectance mode (L*, a*, b*) according to the methodology described by AOAC (2005).

The vitamin C content was obtained through titration, using DFI (2,6-dichloro-phenol-indophenol) solution 0.2% until it had a permanent pink coloration according to AOAC (2005) and the results were expressed in mg of ascorbic acid / per 100g of fresh weight.

Table 1 - Formulations and amount of preservatives used in the preparation of the guava juice

Formulation	Preservative	Amount (g/100 mL)
Juice (Control)	-	-
Juice + Sodium metabisulphite (SM)	Sodium metabisulphite (expressed as sulfur dioxide)	0.004
Juice + Potassium sorbate (PS)	Potassium sorbate (expressed as sorbic acid)	0.08
Juice + Sodium benzoate (SB)	Sodium benzoate (expressed as benzoic acid)	0.05
Juice + SM+SB	Sodium metabisulfite*/Sodium benzoate **	0.002/0.025
Juice + SM+PS	Sodium metabisulphite*/Potassium sorbate**	0.002/0.04

*Expressed as sulfur dioxide; **Expressed as benzoic acid; Source: Brasil (2013)

The determination of total carotenoids, used the extraction solution of isopropyl alcohol: hexane (3:1) (HIGBY, 1962). The absorbance was measured at 450 nm using a UV-vis spectrophotometer (Shimadzu, model UV-1800) and the results were expressed as μg per 100 g of fresh weight (CARVALHO *et al*., 2014), according to the following calculation: $C\ (\mu g/100g) = (A \times volume\ (mL) \times 10^4/A^{1\%}_{1cm} \times weight\ (g) \times 100)$, where A = absorbance; *volume* = total volume of extract (50 mL); $A^{1\%}_{1cm}$ = ratio of β-carotene absorption in petroleum ether(2592); *weight* = weight of sample (g).

Total extractable polyphenols (TEPs) were determined according to the Folin-Ciocalteu method (HUANG; OU; PRIOR, 2005). The absorbance was measured at 700 nm using a UV-vis spectrophotometer, 30 minutes after the addition of the reagents. The results were expressed as mg of gallic acid equivalents (mg of GAE) per 100g of fresh weight.

Total antioxidant activity (TAA) was determined through testing with the 2,2’-azino-di-(3-ethylbenzthiazoline-6-sulphonic acid (ABTS) free radical, obtained through the reaction of 5 ml of 7 mM ABTS in 88 uL of 2.45 mM potassium persulphate. TAA values were obtained from the straight line equation: $y = ax + b$, substituting the y value with the absorbance equivalent of 1000 mM of Trolox, the results being expressed as TEAC (Antioxidant Activity Equivalent Trolox) in µM of Trolox per g of fresh weight (RUFINO *et al*., 2007).

The microbiological quality of juice analysis was performed using the commercial sterility test (APHA, 2013), as required by national law.

The formulations of the sweetened guava juice sensory were analyzed, by a group of 60 untrained tasters. The attributes analyzed were the flavor, color, appearance, body, aroma and overall acceptability, using the hedonic scale testing of nine points, and the purchase intent using the structured five-point scale (MEILGAARD; CIVILLE; CARR, 1991).

The experiment was conducted in a split plot design; with the juice formulation parcels and the storage time of the subplots. The results were presented as mean ± standard deviation. To compare the guava juice formulations, the Tukey test was performed. All analysis was performed with three replicates for each formulation. The significance level for all statistical analysis was 5% significance using the SAS statistical software version 8.1 (2006).

RESULTS AND DISCUSSION

The titratable acidity (TA), pH, and soluble solids (SS) of the guava juice did not differ statistically between the formulations and the storage time (P>0.05).

The treatment means for the TA during storage was 0.39 g citric acid / 100 g, a value different than what Fernandes *et al.* (2006) cited. This author studied the identity and quality standards of the unsweetened guava juice and they observed values between 0.82 and 0.87 g for the citric acid / 100 g (Table 2). The acidity values found in this study are in agreement with the identity and quality standard (IQS) established for the sweetened guava juice. The value established was a minimum of 0.12 g of citric acid / 100 g juice.

The mean pH of the treatments during storage was 4.0. According to Maia, Souza and Lima (2007), the pH is an intrinsic factor to the product and in food preservation. It is of great importance as a selective and microbial presence and in the occurrence of chemical interactions. It defines the accuracy of the industrial process to be applied, directly influencing the preservation of the product, in addition to the acidity of the basic components of food flavor.

The TA and pH values show that the treatments with or without food preservatives retained their values during storage, so the preservatives did not

Table 2 - Titratable acidity (TA), pH, soluble solids (SS) of the guava juice sweetened with and without the addition of preservatives during 180 storage days

Formulation	Titratable Acidity (TA) (g citric acid / 100 g)			pH			Soluble solids (SS) (°Brix)		
	Time (days)			Time (days)			Time (days)		
	0	90	180	0	90	180	0	90	180
Juice (Control)	0.39 ± 0.00	0.35 ± 0.00	0.38 ± 0.00	3.9 ± 0.1	3.8 ± 0.1	4.0 ± 0.1	11.9 ± 1.5	11.6 ± 0.5	11.8 ± 0.6
Juice+SM	0.39 ± 0.00	0.35 ± 0.00	0.39 ± 0.10	4.1 ± 0.4	3.8 ± 0.1	4.0 ± 0.1	10.4 ± 1.8	10.9 ± 0.7	11.1 ± 0.5
Juice+PS	0.39 ± 0.00	0.42 ± 0.10	0.39 ± 0.00	4.1 ± 0.1	4.0 ± 0.1	4.2 ± 0.1	10.9 ± 0.6	11.3 ± 0.3	11.3 ± 0.2
Juice+SB	0.38 ± 0.00	0.44 ± 0.10	0.40 ± 0.00	4.0 ± 0.1	3.9 ± 0.1	4.1 ± 0.0	11.1 ± 0.2	11.4 ± 0.1	11.5 ± 0.0
Juice+SM+SB	0.38 ± 0.00	0.36 ± 0.00	0.39 ± 0.00	4.1 ± 0.1	3.9 ± 0.1	4.2 ± 0.1	11.1 ± 0.1	11.4 ± 0.1	11.5 ± 0.0
Juice+SM+PS	0.39 ± 0.00	0.36 ± 0.00	0.42 ± 0.10	4.0 ± 0.0	3.8 ± 0.0	4.0 ± 0.0	11.3 ± 0.2	11.4 ± 0.1	11.5 ± 0.1
Mean	0.39 ± 0.01	0.38 ± 0.04	0.40 ± 0.01	4.0 ± 0.1	3.8 ± 0.1	4.18 ± 0.1	11.1 ± 0.5	11.3 ± 0.2	11.5 ± 0.2

SM - sodium metabisulphite; PS - potassium sorbate; SB - sodium benzoate

affect the percentage of the hydrogen ion or organic acid concentration in guava juice.

The soluble solids (SS) showed greater variation in formulations with sodium metabisulphite (SMS) during storage in the 10.4º-11.1º Brix. The juice as a control (C), showed the lowest oscillation in the storage function, the 11.9º-11.8º Brix (Table 2). The SS values remained on average at 11º Brix, this value is established by law as an IQS default for sweetened guava juice.

Ordóñez-Santos and Vázquez-Riascos (2010) investigated the effect of processing and storage in guava products. They obtained 8.84 and 24° Brix for pulp and nectar values respectively, with no significant variations during 240 storage days. Fernandes *et al.* (2006) studied unsweetened guava juice and found that it had a mean value of 8.70° Brix.

The mean lightness (L*) for all formulations ranged from 44.2 to 44.48 during storage (Table 3), remaining next to the central region of the scale, ranging from 0 to 100 black-white. This demonstrates that possible oxidative degradation reactions of heat-sensitive pigments, and non-enzymatic browning, such as oxidation of vitamin C and to a lesser extent, the Maillard reaction, which might occur in the product, did not influence this parameter.

For coordinate a*, in the green to red color variation, very similar values were observed regardless of juice formulations, and a significant reduction during storage was found, ranging from 6.49 to 6.00 for the processing day and end of the storage time (days zero and 180), respectively (Table 3). Decreased red color of guava juice, associated with the luminance values near the darker region may be due to the heat treatment. The high temperature can damage the structure of carotenoids, the pigments responsible for the color of the juice.

Table 3 - Color parameters (L*, a* e b*) of the guava juice sweetened with and without the addition of preservatives during 180 storage days

Formulation	L*			a*			b*		
	Time (days)			Time (days)			Time (days)		
	0	90	180	0	90	180	0	90	180
Juice (Control)	44.1 ± 1.1	42.8 ± 1.0	45.2 ± 2.4	6.4 ± 0.2	5.9 ± 0.2	6.3 ± 0.3	5.5 ± 0.4	6.2 ± 0.4	9.1 ± 0.7
Juice+SM	44.4 ± 1.4	45.3 ± 2.6	44.8 ± 2.1	6.8 ± 0.7	6.7 ± 0.9	6.3 ± 0.8	5.9 ± 0.8	6.4 ± 0.8	7.4 ± 0.4
Juice+OS	42.8 ± 1.2	45.2 ± 0.9	44.5 ± 1.9	6.1 ± 0.1	6.3 ± 0.5	5.9 ± 0.0	5.5 ± 0.4	7.4 ± 0.5	8.4 ± 1.1
Juice+SB	45.2 ± 1.3	44.8 ± 1.5	44.1 ± 0.7	6.6 ± 0.4	6.0 ± 0.2	5.8 ± 0.4	6.3 ± 0.5	6.8 ± 0.3	8.5 ± 0.6
Juice+SM+SB	44.9 ± 1.3	43.8 ± 1.4	44.8 ± 1.1	6.5 ± 0.4	5.9 ± 0.2	5.6 ± 0.2	6.1 ± 0.7	6.5 ± 0.6	8.1 ± 0.4
Juice +SM+PS	43.8 ± 2.6	45.0 ± 1.8	45.1 ± 2.3	6.6 ± 0.2	6.6 ± 0.9	6.1 ± 0.8	5.7 ± 1.1	6.8 ± 0.9	8.3 ± 1.1
Mean	44.2 ± 1.5	44.5 ± 1.6	44.8 ± 1.6	6.5 ± 0.4	6.2 ± 0.6	6.0 ± 0.5	5.9 ± 0.6	6.7 ± 0.6	8.3 ± 0.8

SM - sodium metabisulphite; PS - potassium sorbate; SB - sodium benzoate

In this study, the observation was made that independent of the applied formulation, all the samples showed coloration near the yellow tint, since all the coordinate b* values were positive and showed an increase over the storage period of 5.87 to 8.29 (Table 3).

There was no significant interaction between the guava juice formulations and the storage time in regards to vitamin C (P>0.05). Also, there was no statistical difference between the formulations and the average value of vitamin C formulations of 27.97 mg of ascorbic acid (AA) / 100g juice. There was a small decrease in these vitamin formulations in the average over the 180 days of storage (29.2 to 26.8 AA of mg / 100g of juice) (Table 4).

In regards to the variations in the vitamin C content in periods of 90 and 180 days, the study found that the formulation with sodium metabisulphite and sodium benzoate (SM + SB) showed a lower percentage of variation in the two periods, maintaining better vitamin C, while the formulation with potassium sorbate (PS) showed the greatest variation in the same period, with 8.2% to 90 days, and 12.4% for 180 days. However, by the end of the 180 storage days, the vitamin C content found in all samples supply the recommended dose for daily intake for an adult, 45 mg (BRASIL, 2005), the equivalent of 200 mL of juice.

In a study of unpasteurized sweetened guava juice, Silva *et al*. (2010) found that the vitamin C content analysis began with 33.9 mg of ascorbic acid and, after 200 days of storage, the content decreased to 21.6 mg ascorbic acid / 100 mL, with values lower than those presented in current research.

Studies have shown losses of vitamin C content due to processing and storage, heat treatment, storage temperature, and exposure to atmospheric oxygen and light, being in that the light incidence is one of the causes of the oxidation of this vitamin. This is due to the fact that it accelerates the reaction of ascorbic acid with amino groups, producing dark pigments through polymerization and therefore causing color loss and alterations to other sensory properties (FERNANDES *et al*., 2011).

The addition of chemical preservatives to guava juice did not affect the content of total extractable polyphenols (TEPs). A sharp decline during storage was observed: a reduction of 94.70% for the control treatment and variations of 96.4 and 95, 4% for all PS and SB + SM formulations, respectively (Table 4). However, the MS formulation showed a higher content of TEP (11.4 mg GAE / 100 g of juice) at the end of storage. Higher values were reported by Melo *et al*. (2008), 33.117mg GAE / 100g of juice. It is known that the concentration of TEP is influenced by many external factors, such as high temperature, which can destroy these compounds in juices on the market.

The carotenoid content statistically did not differ between formulations and during storage, with the variation 0.12 0.16 mg / 100g, with storage time for all formulations, obtaining an overall average of 0.13 mg / 100g (Table 4). The presence of carotenoids in this study is reflected in the results (a* and b* parameters) for juice color, since the average values of 6.24 and 6.95, respectively, correspond to the yellowish red color, characteristic of guava and of its products. However, β-carotene values higher than those found in this study were reported by Ordóñez-Santos and Vázquez-Riascos (2010) of 3.55 mg / 100 g in guava pulp.

The total antioxidant activity (TAA) through the ABTS method showed a significant interaction between the formulation and time of storage (P≤0.05). The treatments were analyzed on each day of storage separately. On the day of processing (time 0), the ABTS assay, control juice showed higher AAT, 11.88 mM of Trolox / g juice as the juice with potassium sorbate (PS) had the lowest value,

Table 4 - Vitamin C, total extractable polyphenols (TEP) and carotenoids of the guava juice sweetened with and without the addition of preservatives during 180 storage days

Formulation	Vitamin C (mg/100g)			Carotenoids (mg/100g)			TEP (mg/100g)		
	Time (days)			Time (days)			Time (days)		
	0	90	180	0	90	180	0	90	180
Juice (Control)	27.3 ± 3.5	26.6 ± 2.4	25.2 ± 3.3	177.6 ± 10.4	75.6 ± 7.7	9.4 ± 0.1	0.13 ± 0.0	0.15 ± 0.0	0.12 ± 0.0
Juice+SM	29.1 ± 1.0	28.2 ± 1.8	26.7 ± 2.2	191.7 ± 2.4	99.7 ± 15.2	11.4 ± 1.1	0.14 ± 0.0	0.16 ± 0.0	0.12 ± 0.0
Juice+OS	29.1 ± 1.8	26.7 ± 3.0	25.5 ± 1.6	179.5 ± 19.4	70.5 ± 6.8	6.5 ± 2.1	0.14 ± 0.0	0.16 ± 0.0	0.14 ± 0.0
Juice+SB	30.6 ± 0.9	28.9 ± 3.3	28.0 ± 1.3	177.6 ± 16.8	76.7 ± 17.8	10.1 ± 0.3	0.13 ± 0.0	0.16 ± 0.0	0.13 ± 0.0
Juice+SM+SB	30.7 ± 0.9	28.3 ± 1.2	28.3 ± 8.0	196.6 ± 45.7	79.6 ± 11.3	10.1 ± 0.7	0.13 ± 0.0	0.16 ± 0.0	0.12 ± 0.0
Juice +SM+PS	29.0 ± 4.3	29.0 ± 4.3	26.9 ± 6.5	187.5 ± 19.4	89.5 ± 8.9	8.6 ± 3.4	0.14 ± 0.0	0.16 ± 0.0	0.13 ± 0.0
Mean	29.2 ± 2.6	27.9 ± 2.5	26.8 ± 4.1	184.78 ± 21.28	81.92 ± 14.21	9.35 ± 2.12	0.13 ± 0.01	0.15 ± 0.01	0.12 ± 0.01

SM - sodium metabisulphite; PS - potassium sorbate; SB - sodium benzoate

8.6 mM of Trolox / g of juice. However, at 180 storage days, there was a significant reduction in the amount of AAT for all formulations (Table 5).

Higher values were cited by Thaipong *et al*. (2006), which ranged from 22.3 uM 34.4 the Trolox / g for red guava fruit clones.

Fruit has several compounds with antioxidant activity, which include ascorbic acid, carotenoids and polyphenols (ALMEIDA *et al.*, 2011; RUFINO *et al.*, 2010). The results here recorded show, through correlation analysis, that the decrease in the content of PET during storage (Table 4) significantly contributed to a reduction in TAA (ABTS method), and is evidenced by a positive correlation ($P<0.05$) ($R = 0.79$) between these variables (Table 6).

Several studies have reported the relationship between phenolic content and total antioxidant activity. Some authors found a high correlation between the phenolic compounds content and antioxidant activity (ALMEIDA *et al*., 2011; MAHATTANATAWEE *et al*., 2006; REDDY; SREERAMULU; RAGHUNATH, 2010; SILVA *et al*., 2007).

Although vitamin C is considered by some authors as the largest contributor to antioxidant activity, Almeida *et al.* (2011) demonstrated, through the ABTS method, that the correlation of vitamin C with the antioxidant activity of eleven tropical fruits, from northeastern Brazil, was low. Their study stated that the largest contribution to the total fruit antioxidant activity was related to composition of phenolic compounds.

Other compounds, such as carotenoids may be present in extracts and contribute to the antioxidant activity of the samples (MAHATTANATAWEE et al., 2006). However, in this study too, there was no verified correlation between AAT and the content of carotenoids. Despite these results, the antioxidant activity of fruit cannot be attributed solely to their phenolic content, but also to the actions of different antioxidant compounds present in fruit and possible synergistic effects and still unknown antagonists.

The results of the microbiological analysis show that the juices with the addition of chemical preservatives, individually or in combination, were in compliance with commercial sterility standards established by the current Brazilian law, RDC No. 12, even though the presence of molds and yeasts were observed in the control juice (Table 7). Therefore, it was found that the presence of food preservatives in sweetened guava juice was efficient enough to preserve the product during the studied storage period.

Table 5 - Total antioxidant activity (TAA) by ABTS method (µM de Trolox/ g) of the guava juice sweetened with and without the addition of preservatives during 180 storage days

Formulation	Tempo (dias)			
	0 days	90 days	180 days	Mean
Juice (Control)	11.88 ± 0.03 a	9.57 ± 2.55 a	6.0 ± 0.2 a	9.2 ± 3.0
Juice+SM	10.88 ± 0.42 b	6.74 ± 0.50 b	6.0 ± 0.3 a	7.9 ± 2.6
Juice+PS	8.6 ± 2.55 b	6.71 ± 0.23 b	5.8 ± 0.5 a	7.0 ± 1.4
Juice+SB	10.17 ± 0.13 b	5.91 ± 1.19 b	6.3 ± 0.9 a	7.5 ± 2.4
Juice+SM+SB	9.31 ± 1.43 b	6.54 ± 0.31 b	5.9 ± 0.1 a	7.3 ± 1.8
Juice +SM+PS	10.63 ± 0.45 b	6.45 ± 0.43 b	5.1 ± 1.9 a	7.4 ± 2.9

M - sodium metabisulphite; PS - potassium sorbate; SB - sodium benzoate; Means followed by the same letter in the column did not differ by Tukey test ($P<0.05$)

Table 6 - Pearson's correlation coefficient of vitamin C, total extractable polyphenols (TEP) and carotenoids of the guava juice sweetened with and without the addition of preservatives and total antioxidant activity (TAA) by ABTS method

Formulation	Pearson's correlation coefficient
	Total antioxidant activity (TAA) by ABTS method
Total extractable polyphenols (TEP)	0,79156*
Vitamin C	$0,20485^{NS}$
Carotenoids	$-0,02190^{NS}$

* = Significant at $P<0.05$; ^{NS} - Not significant at $P>0.05$

Table 7 - Results from microbiological analysis of the guava juice sweetened with and without the addition of preservatives

Formulation	*Clostridium butyricum/Facultative anaerobic*	*B. coagulans*	Mold and Yest	Lactic acid bacteria
Juice (Control)	Absence	Absence	Presence	Absence
Juice+SM	Absence	Absence	Absence	Absence
Juice+PS	Absence	Absence	Absence	Absence
Juice+SB	Absence	Absence	Absence	Absence
Juice+SM+SB	Absence	Absence	Absence	Absence
Juice +SM+PS	Absence	Absence	Absence	Absence

SM - sodium metabisulphite; PS - potassium sorbate; SB - sodium benzoate

Due to high concentrations of acids and low pH of fruit juices, these products have self-defense against microbial spoilage. Therefore, only organisms able to grow under such conditions will be capable of damaging these beverages. To ensure the quality and stability of fruit juice, these products are usually pasteurized at relatively high temperatures (90-98 °C) (FREITAS *et al*., 2006; GOUWS *et al*., 2005; HO *et al*., 2010). However, many types of yeast grow under anaerobic conditions and low pH, making it likely to grow in fruit juices.

In the sensory evaluation of the sweetened guava juice with different food preservatives, there was no significant difference ($P<0.05$) between the treatments tested for overall impression sensory attributes like flavor, appearance and purchase intent. There was stabilization of all attributes during storage.

For the overall impression the values of the results for all the juice formulations remained close at the end of the hedonic scale “like slightly”. However, the sodium metabisulphite formulation with potassium sorbate (SM + PS), presented the highest value, 6.7; only statistically different from the sodium metabisulphite with sodium benzoate (SM + SB) and potassium sorbate (PS) formulations (Table 8).

The taste parameter of the evaluated juices presented sensorial values represented by the hedonic terms “like slightly” to “like moderately”. The juice with SM + PS presented the highest value of the formulations, 6.6, not differing significantly from the sodium benzoate formulations (SB) and sodium metabisulphite (SM) formulations.

The appearance of juice showed mean values ranging from 6.4 to 6.7, which in hedonic terms is “like moderately” (Table 8). However, despite the juices containing SM and SB additives presenting the highest values, these were not statistically different from the other formulations.

The evaluation of purchase intent juices presented results between the terms “may buy, maybe not buy,” and “possibly buy, “highlighting the SM + PS formulation by the highest value differing significantly from the others (Table 8).

Table 8 - Results from sensory evaluation of the guava juice sweetened with and without the addition of preservatives

Formulation	Sensory attributes			
	Overall impression	Flavor	Appearance	purchase intent
Juice + Sodium metabisulphite (SM)	6.5 ab ± 1.5	6.3 a ± 1.7	6.7 ab ± 1.4	3.4 b ± 1.0
Juice + Potassium sorbate (PS)	6.2 b ± 1.7	5.8 c ± 2.0	6.5 b ± 1.6	3.1 b ± 1.2
Juice + Sodium benzoate (SB)	6.5 ab ± 1.6	6.2 ab ± 1.6	6.7 ab ± 1.2	3.4 b ± 1.1
Juice + SM+SB	6.3 b ± 1.5	5.9 bc ± 1.7	6.4 b ± 1.6	3.2 b ± 1.1
Juice + SM+PS	6.7 a ± 1.7	6.6 a ± 1.8	6.4 b ± 1.6	3.7 a ± 1.1

Means followed by the same letter in the column did not differ by Tukey test ($P<0.05$)

CONCLUSION

Vitamin C and carotenoids content remained constant during storage at room temperature, regardless of the presence or type of preservative used. The presence of the preservatives did not prevent the reduction of the total polyphenol content for the treatment and control with the addition of preservatives, which contributed to the significant decline in antioxidant activity of juice. The study found that the polyphenols showed high correlation with antioxidant activity. The preservatives used were effective in inhibiting microorganisms and verifying the presence of molds and yeasts in the control treatment (without the addition of preservatives). Formulations with the isolated metabisulphite and associated with potassium sorbate showed the highest sensory acceptance.

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