Quality and stability of concentrated guava puree added with *Hibiscus sabdariffa* extract

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Summary

The quality and stability of concentrated guava purees prepared under vacuum (345.23 kPa at 45 °C, 50 °C and 55 °C, 30 min), added with *Hibiscus sabdariffa* extract and stevia or glucose, were evaluated during refrigerated storage (6 months at 10 °C). Physico-chemical properties, chemical composition, microbiological and sensory properties, contents of bioactive compounds and antioxidant capacity were measured. A significant effect ($p < 0.05$) of vacuum concentration was observed. Control purees had low sensory quality compared with purees concentrated under reduced vacuum pressure. In these purees, addition of *Hibiscus sabdariffa* extract provided an attractive red colour containing 1.15–1.31 g·kg^{-1} of anthocyanins. Purees were rich in total dietary fibre, had contents of vitamin C from 1.39 g·kg^{-1} to 1.63 g·kg^{-1}, and contained total soluble polyphenols from 4.10 g·kg^{-1} to 7.02 g·kg^{-1} independently of concentration temperature, thus antioxidant capacity by 2-diphenyl-1-picryhydrazyl method was 151.45·183.01 μmol·kg^{-1} (expressed as Trolox equivalents). Storage caused slight changes in physico-chemical and chemical composition parameters in all purees. The purees were microbiologically stable, sensory acceptable and high antioxidant capacity was registered during storage. The combination of guava pulp and *Hibiscus sabdariffa* extract to prepare purees concentrated under reduced vacuum pressure has very promising and interesting phytochemical properties, and are source of dietary fibre and polyphenols.

Keywords

concentrated guava puree; *Hibiscus sabdariffa*; quality; stability; vacuum

Guava (*Psidium guajava* L.) is a fruit widely known and appreciated because of its sensory and nutritional qualities. It can be readily processed. Guava is consumed either as fresh fruit or as a variety of processed foods such as syrup pack, jam, jelly, nectar or puree. The market has a growing demand for these products together with fresh fruit, and such demand has become increasingly relevant especially in markets of Europe and the United States [1].

The combination of fruits, innovative additives and packaging can potentiate the functional characteristics of a food, providing that they can increase the content of macro- and micronutrients, dietary fibre and a wide range of phytochemicals, which individually or in conjunction may have important biological activities that promote health benefits [2]. It is known that guava is a rich source of vitamin C (four times more than orange) [3], dietary fibre [4, 5], carotenoids [6] and polyphenols [7]. On the other hand, dry calyces of *Hibiscus sabdariffa*, commonly known as Roselle or Jamaica, are used to produce a colourful drink consumed as a cold or hot beverage [8]. It was reported that the extract or beverage from *H. sabdariffa* contains soluble dietary fibre and polyphenols, which bestow therapeutic properties, especially in the treatment of hypertension and hyperlipidemia [9]. Therefore, the combination of guava pulp and *H. sabdariffa* extract can be used to

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prepare a colourful puree rich in dietary fibre and polyphenols.

Vacuum evaporation is a unit operation, in which water is evaporated at sub-atmospheric pressure. Under these conditions, concentration can be attained at lower temperatures [10]. In this manner, several objectives can be reached: a reduced rate of heat input to attain boiling temperature of water, rate of cool input to condense water, and the effects of temperature in foods [10]. The main use of vacuum technology is impregnation for incorporating additives in fruit and vegetable tissues, such as anti-browning agents, microbial preservatives or cryoprotectants [11]. Additionally, extraction of bioactive compounds was described in concentrated grape juice (1.50–10 kPa at 60 °C, 75 °C and 90 °C), increasing their availability [12] or stabilization of anthocyanins by impregnation with saccharose under vacuum pressure (4 kPa) prior to jam preparation [13], or addition of blueberry juice into the structure of fresh apple slices without a negative effect on bioactive compounds [14].

In this work, guava purees added with an extract of *H. sabdariffa* and concentrated under vacuum at moderate temperatures were evaluated measuring physico-chemical, chemical composition, microbiological and sensory attributes, as well as for the content of bioactive compounds and their antioxidant capacity during refrigerated storage.

MATERIALS AND METHODS

Raw material

Guava pulp of 'Media China' variety and *H. sabdariffa* calyces variety 'China', both donated by Purés y Derivados de Nayarit Company (PDN, Nayarit, Mexico), were utilized. Their physicochemical characteristics are shown in Tab. 1.

H. sabdariffa extract

Amount of 100 g of fresh calyces was dried at 60 °C in a convection oven for 12 h. Dried calyces (approx. 20 g) were added to 100 ml of purified water and heated at 60 °C for 30 min. The extract was filtered through Whatman No. 4 filter paper (Whatman International, Maidstone, United Kingdom) and the filtrate was used for further analysis [15].

Guava puree formulations

For each 100 g of Guava pulp, 15 ml of *H. sabdariffa* extract were added. The mixture was concentrated in a vacuum evaporation equipment (Model CV-6; Tecnodac, Monterrey, Mexico), with a capacity of 24 l that operates at 345.23 kPa and 45 °C, 50 °C or 55 °C for 30 min. Subsequently, a sweetener was added: glucose 250 $g \cdot kg^{-1}$ or stevia 20 $g \cdot kg^{-1}$, (97% of purity of rebaudioside A; Metco, Mexico City, Mexico). Control purees were prepared by the same procedure but were concentrated at 80 °C under atmospheric pressure as routinely elaborated by the producer. The puree formulations were packaged under vacuum in bags of high-density polyethylene (0.940–0.970 $g \cdot cm^{-3}$; Fast Sincere International Industrial, Hong Kong, China), pasteurized (pasteurizer Model KVV, Tecnodac) at 60 °C for 30 min, and then stored at 10 °C for 6 months.

Chemical composition

Moisture (Method 934.06), protein (Method 978.04), fat (Method 950.54) and ash (Method 940.26) contents were determined following the official AOAC methods [16]. Soluble carbohydrates were quantified by the phenol-sulphuric method [17]. Soluble dietary fibre, insoluble dietary fibre and total dietary fibre was analysed by the AOAC enzymatic-gravimetric method (Method 991.42) modified by Mañas and Saura-Calixto [18]. All other data were reported as grams per kilogram of fresh weight (FW).

Physicochemical analyses

Titratable acidity (Method 942.15), pH values (Method 981.12) and total soluble solids (Method 932.12) were determined according to the AOAC methods [16]. Colour changes were measured with a Minolta CR300 colorimeter (Konica Minolta, Osaka, Japan) by the L^* a^* b^* system and expressed as Hue angle (h).

Ascorbic acid content

Fresh samples (10 g) were homogenized with 25 ml of sulphuric acid (1.04 $mol \cdot l^{-1}$), 25 ml of distilled water, and 3 ml of starch solution (5% w/w). The mixture was titrated with potassium iodide-iodine solution (0.12 $mol \cdot l^{-1}$ and 0.02 $mol \cdot l^{-1}$, respectively), using an automatic titrator (Scott D-55122; Scott Instruments, Mainz, Germany), and the results were reported in grams per kilogram FW [19].

Total anthocyanins

Fresh sample (1 g) was mixed with 15 ml of acidified methanol (hydrochloric acid in ratio 1 : 10, v/v); the mixture was stirred for 10 min and centrifuged at 9380 $\times g$ at 4 °C. The supernatant was used to quantify total anthocyanins, employing the method of differential pH described by

Tab. 1. Physico-chemical parameters of guava pulp, extract of *Hibiscus sabdariffa* and the mixture guava-*H. sabdariffa*, before processing.

Parameter	Guava pulp	Extract of *H. sabdariffa*	Mixture of guava and *H. sabdariffa*
Titratable acidity [%]	0.6 ± 0.03	3.1 ± 0.02	1.0 ± 0.01
pH	4.00 ± 0.10	2.13 ± 0.10	3.20 ± 0.10
Total soluble solids [°Brix]	8.00 ± 0.20	12.60 ± 0.30	8.50 ± 0.10
Ascorbic acid [g·kg^{-1}]	3.01 ± 1.15	0.28 ± 0.12	3.05 ± 1.05
Moisture [g·kg^{-1}]	853.31 ± 11.31	905.80 ± 12.12	873.52 ± 12.40
Total anthocyanins [g·kg^{-1}]	ND	3.06 ± 0.21	1.84 ± 0.30
Total soluble polyphenols [g·kg^{-1}]	4.10 ± 1.31	2.75 ± 0.12 *	6.09 ± 1.05
Soluble dietary fibre [g·kg^{-1}]	3.15 ± 0.87	7.22 ± 0.39 *	4.54 ± 1.03
Insoluble dietary fibre [g·kg^{-1}]	61.28 ± 1.34	ND	61.24 ± 1.34
Total dietary fibre [g·kg^{-1}]	64.43 ± 1.56	ND	65.78 ± 1.82

Values are the mean ± standard deviation ($n \geq 3$, $p < 0.05$). Titratable acidity is expressed as percentage of citric acid. Content of ascorbic acid, moisture, total anthocyanins, total soluble polyphenols, soluble, insoluble and total dietary fibre is expressed in gram per kilogram of fresh weight.
ND – not determined. * – values are expressed as gram per litre.

GIUSTI and WROLSTAD [20]. Data were reported as grams per kilogram FW.

Total soluble polyphenols and antioxidant capacity

Total soluble polyphenols were extracted of 0.5 fresh sample with 20 ml of acidified methanol (0.8% of HCl 72.8 g·l^{-1}). It was stirred for 1 h, then centrifuged (Hermle Z306, Wehingen, Germany) for 30 min at 9380 ×*g* at 4 °C. A volume of 20 ml of acetone-water solution (80:20, v/v) was added and the extraction was repeated. The supernatants were combined and taken to 50 ml in a volumetric flask. Total soluble polyphenols were quantified in the extract using the Folin-Ciocalteu reagent following the method of MONTREAU [21]. Gallic acid was used as standard and results were expressed as grams of gallic acid equivalents per kilogram FW. The method of ferric-reducing antioxidant power (FRAP) assay described by BENZIE and STRAIN [22] and the 2-diphenyl-1-picryhydrazyl (DPPH) radical-scavenging assay described by PRIOR et al. [23] were used. A standard curve of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was employed to estimate the antioxidant capacity as micromoles of Trolox equivalent per kilogram FW.

Microbiological analysis

Microbiological evaluation in terms of total mesophilic aerobic bacteria, yeasts and moulds, psychrophilic and coliform bacteria counts were determined according to the methods described by DOWNES and ITO [24].

Sensory evaluation

Two tests were performed. The first one was a preference-ranking test, with 41 untrained judges to assess the preference in taste, colour and aroma of all concentrated purees. The second test utilized was the acceptation level test (taste, colour and aroma) with 25 untrained judges of concentrated purees (containing stevia or glucose) stored for 6 months at 10 °C. The scale was 1–4: 1 = dislike, 2 = indifferent, 4 = like [25].

Statistical analysis

All analyses were done in triplicate. Data were analysed by ANOVA using the software Statistica v.8 (StatSoft, Tulsa, Oklahoma, USA) with $p < 0.05$. Means comparison was made by the least significant difference (*LSD*, $\alpha = 0.05$). The first sensory evaluation was analysed by the non-parametric Friedman test by ranking ($\alpha = 0.05$). The second evaluation was analysed by Student's t-test ($\alpha = 0.05$).

RESULTS AND DISCUSSIONS

Chemical composition

The moisture content was different between treatments ($p < 0.05$) (Tab. 2). Control puree with glucose or stevia had lower moisture (679.11 g·kg^{-1} and 697.14 g·kg^{-1}, respectively) than purees concentrated under reduced vacuum pressure with stevia (832.75–832.91 g·kg^{-1}) and with glucose (726.18–727.90 g·kg^{-1}). This can be attributed to

Tab. 2. Chemical composition of purees concentrated under reduced vacuum pressure added with extract of *H. sabdariffa* and sweetened with stevia or glucose.

	Puree with glucose				Puree with stevia			
	Control	45 °C	50 °C	55 °C	Control	45 °C	50 °C	55 °C
Moisture [g·kg^{-1}]	679.11 ± 0.14^{d}	727.90 ± 0.60^{b}	726.18 ± 0.93^{b}	727.26 ± 0.27^{b}	697.14 ± 0.14^{c}	832.91 ± 1.45^{a}	833.75 ± 0.78^{a}	832.84 ± 0.57^{a}
Protein [g·kg^{-1}]	6.92 ± 2.30^{a}	7.26 ± 2.17^{a}	7.09 ± 3.15^{a}	6.90 ± 1.14^{a}	6.95 ± 1.42^{a}	6.82 ± 2.19^{a}	6.80 ± 1.32^{a}	6.91 ± 1.14^{ab}
Fat [g·kg^{-1}]	4.25 ± 1.62^{a}	4.23 ± 1.23^{a}	4.31 ± 1.12^{a}	4.28 ± 1.30^{a}	4.30 ± 1.51^{a}	4.29 ± 1.04^{a}	4.30 ± 0.93^{a}	4.18 ± 1.25^{ab}
Ash [g·kg^{-1}]	5.22 ± 0.54^{a}	5.20 ± 0.14^{a}	5.31 ± 0.92^{a}	5.33 ± 0.27^{a}	5.21 ± 0.54^{a}	5.24 ± 0.17^{a}	5.20 ± 0.61^{a}	5.21 ± 0.15^{a}
Soluble carbohydrates [g·kg^{-1}]	286.50 ± 1.36^{a}	254.36 ± 5.40^{b}	253.08 ± 2.61^{b}	253.21 ± 1.52^{b}	186.50 ± 1.32^{b}	149.72 ± 2.91^{c}	146.65 ± 3.51^{c}	148.47 ± 5.37^{c}
Soluble dietary fibre [g·kg^{-1}]	7.11 ± 1.32^{a}	4.29 ± 3.71^{b}	3.82 ± 2.06^{b}	3.78 ± 3.31^{b}	7.32 ± 2.16^{a}	5.31 ± 2.90^{b}	4.39 ± 1.73^{b}	4.91 ± 1.52^{b}
Insoluble dietary fibre [g·kg^{-1}]	112.71 ± 1.05^{a}	109.69 ± 2.82^{b}	106.82 ± 1.33^{b}	104.91 ± 3.72^{b}	112.32 ± 2.31^{a}	70.51 ± 1.62^{c}	67.88 ± 2.90^{cd}	73.83 ± 3.61^{c}
Total dietary fibre [g·kg^{-1}]	119.82	113.98	110.64	108.69	119.64	75.82	72.27	78.74

Values are expressed per kilogram of fresh weight as mean ± standard deviation ($n \geq 3$). Means with different superscripts in same row indicate significant difference using *LSD* test ($p < 0.05$).

Tab. 3. Physico-chemical parameters of purees concentrated under reduced vacuum pressure added with extract of *H. sabdariffa* and sweetened with stevia or glucose during storage for 6 months at 10 °C.

	Months of storage	Puree with glucose				Puree with stevia			
		Control	45 °C	50 °C	55 °C	Control	45 °C	50 °C	55 °C
Titratable acidity [%]	0	1.21 ± 0.09^{Ac}	1.34 ± 0.04^{Ab}	1.33 ± 0.10^{Ab}	1.33 ± 0.40^{Bb}	1.42 ± 0.6^{Aa}	1.43 ± 0.05^{Aa}	1.42 ± 0.04^{Aa}	1.44 ± 0.01^{Aa}
	3	1.12 ± 0.07^{Bb}	1.15 ± 0.05^{Bb}	1.14 ± 0.04^{Ab}	1.14 ± 0.09^{Bb}	1.13 ± 0.09^{Bb}	1.24 ± 0.05^{Ba}	1.21 ± 0.14^{Bab}	1.23 ± 0.07^{Ba}
	6	1.04 ± 0.07^{Cb}	1.09 ± 0.27^{Cab}	1.04 ± 0.27^{Bb}	1.04 ± 0.10^{Ab}	1.06 ± 0.07^{Bb}	1.13 ± 0.03^{Ca}	1.12 ± 0.06^{Ca}	1.12 ± 0.03^{Ca}
pH	0	3.17 ± 0.05^{Ba}	3.18 ± 0.11^{Ba}	3.15 ± 0.05^{Ba}	3.16 ± 0.10^{Ba}	3.09 ± 0.05^{Bb}	3.10 ± 0.02^{Bb}	3.10 ± 0.02^{Bb}	3.11 ± 0.01^{Bb}
	3	3.30 ± 0.02^{Ba}	3.30 ± 0.02^{Ba}	3.31 ± 0.01^{Ba}	3.30 ± 0.02^{Ba}	3.30 ± 0.02^{Ba}	3.32 ± 0.03^{Ba}	3.32 ± 0.02^{Ba}	3.31 ± 0.01^{Ba}
	6	3.48 ± 0.03^{Ab}	3.50 ± 0.03^{Aa}	3.52 ± 0.01^{Aa}	3.51 ± 0.08^{Aa}	3.58 ± 0.04^{Aa}	3.55 ± 0.03^{Aa}	3.53 ± 0.02^{Aa}	3.47 ± 0.06^{Aab}
Total soluble solids [°Brix]	0	30.57 ± 0.10^{Aa}	28.46 ± 0.25^{Aa}	30.30 ± 0.20^{Aa}	28.30 ± 0.10^{Aa}	18.57 ± 0.10^{Ab}	16.16 ± 0.15^{Ab}	16.56 ± 0.35^{Ab}	16.46 ± 0.32^{Ab}
	3	29.54 ± 0.02^{Aa}	28.83 ± 0.30^{Aa}	29.43 ± 020^{Aa}	29.93 ± 0.30^{Aa}	17.54 ± 0.02^{Ab}	16.86 ± 1.02^{Ab}	16.56 ± 0.32^{Ab}	16.63 ± 1.10^{Ab}
	6	28.34 ± 0.13^{Aa}	28.72 ± 0.04^{Aa}	28.26 ± 0.70^{Aa}	28.80 ± 0.10^{Aa}	16.65 ± 0.045^{Ab}	15.36 ± 0.46^{Ab}	15.23 ± 0.32^{Ab}	15.83 ± 0.45^{Ab}
Hue [°]	0	15.88 ± 0.16^{Ca}	10.83 ± 0.10^{Cb}	9.69 ± 0.04^{Cb}	10.33 ± 0.03^{Cbc}	15.88 ± 0.16^{Ca}	10.93 ± 0.19^{Cb}	10.40 ± 0.12^{Cb}	10.93 ± 1.08^{Cb}
	3	22.84 ± 0.63^{Ba}	13.63 ± 0.23^{Bb}	12.78 ± 0.06^{Bb}	12.15 ± 0.02^{Bb}	24.75 ± 0.63^{Ba}	14.99 ± 0.24^{Bb}	13.42 ± 0.17^{Bb}	13.62 ± 0.10^{Bb}
	6	34.16 ± 0.23^{Aa}	16.49 ± 0.78^{Ac}	16.20 ± 0.42^{Ac}	16.87 ± 0.01^{Ac}	43.84 ± 0.63^{Aa}	21.49 ± 0.78^{Ab}	21.47 ± 0.14^{Ab}	21.81 ± 0.37^{Ab}

Values are mean ± standard deviation ($n \geq 3$). Means with different uppercase letters in superscripts in the same column indicate significant difference ($\alpha = 0.05$). Means with different lowercase letters in superscripts in the same row indicate significant difference ($\alpha = 0.05$). 0 as month of storage means first day of processing.

the concentration temperatures and the type of added sweetener provided that the higher temperature (80 °C), which was employed in the control puree, increased the rate of water evaporation. Also, glucose has a direct influence because its hydroxyl groups can interact with water molecules through hydrogen bonds thus decreasing the free water [26] in purees with glucose.

It is important to mention that chemical composition data were dependent on moisture. As expected, all purees showed low protein (6.80–7.26 $g \cdot kg^{-1}$ FW), ash (5.20–5.33 $g \cdot kg^{-1}$ FW) and fat (4.18–4.31 $g \cdot kg^{-1}$ FW) contents and there was no significant effect ($p > 0.05$) of concentration, temperature and sweeteners. Data on protein and ash were similar to values reported previously for guava pulp (7.51 $g \cdot kg^{-1}$ and 5.62 $g \cdot kg^{-1}$ FW) but not for fat (1.72 $g \cdot kg^{-1}$ FW) [7]. This may be because differences could exist between guava varieties and because their chemical composition depends on genetic, climatic and ecological factors [7]. Purees concentrated under vacuum with stevia had 141.65–149.72 $g \cdot kg^{-1}$ FW of soluble carbohydrates and those with glucose contained 253.08–254.36 $g \cdot kg^{-1}$ FW because glucose was added on a weight level 12.5-times higher than stevia. Control purees had higher values of soluble carbohydrates (Tab. 2), which was caused by greater losses of water at 80 °C. In purees concentrated under reduced vacuum pressure with stevia, soluble carbohydrates content was similar to that of guava pulp without processing [7].

No significant effect ($p > 0.05$) of concentration-sweeteners and concentration-temperature was observed on dietary fibre. Soluble dietary fibre, insoluble dietary fibre and total dietary fibre variation in purees was highly dependent on moisture content. Therefore, control purees had higher dietary fibre (Tab. 2) because moisture was lower, which means that dietary fibre was more concentrated. It was reported that heat could increase soluble dietary fibre values by hydrolysis of insoluble dietary fibre, but total dietary fibre is not increased [2, 27]. The soluble dietary fibre content reported in guava pulp was 2.3–4.6 $g \cdot kg^{-1}$ FW as reported by JIMENEZ-ESCRIG et al. [5], which is lower than soluble dietary fibre values (3.78–7.32 $g \cdot kg^{-1}$ FW) of concentrated guava puree in this work, which was apparently caused by the concentration and addition of *H. sabdariffa* extract (Tab. 1). It was reported that some components of soluble dietary fibre contained in calyces could be transferred to the aqueous extract, such as arabinans and arabinogalactans of low relative molecular mass, that form soluble dietary fibre in *H. sabdariffa* [28]. These purees had total dietary fibre values higher than those reported in some fruits such as banana (18 $g \cdot kg^{-1}$ FW), grape (12 $g \cdot kg^{-1}$ FW), peach (16 $g \cdot kg^{-1}$ FW) and orange (11 $g \cdot kg^{-1}$ FW) [29]. In this sense, concentrated purees added with *H. sabdariffa* can be classified as having high dietary fibre content, according to the European Food Safety Authority [30] that takes a food product as rich in dietary fibre when its content is 25–30 $g \cdot kg^{-1}$ FW.

Physico-chemical analyses

At the day of preparation (month 0), the purees with glucose had the lowest titratable acidity and the highest pH values in comparison with purees prepared with stevia, irrespective of the concentration temperature (Tab. 3). This was because the high glucose content exerted protective effect on organic acids most likely through hydrogen bonds. Therefore, organic acids were not ionized and the stability of the total soluble solids/titratable acidity ratio was maintained [26]. During refrigerated storage for 6 months of all purees, pH values increased and titratable acidity decreased slightly, which could be attributed to oxidation of organic acids during this period [31]. As expected, total soluble solids were higher in purees added with glucose. At the start of processing (day one or month 0), h values were 9.69–10.93 (red colour) for all samples concentrated under reduced vacuum pressure, which were independent from the concentration temperature. Control purees had h values of 15.85 (brown-red colour) caused by the concentration temperature (80 °C) that probably affected the colour by structural damage to the anthocyanins [32]. After 3 months of storage, the samples concentrated under reduced vacuum pressure retained their red colour with the presence of a low level of brown pigments (12.15–13.62). Colour of control purees was completely brown (22.84–24.75). The purees were not protected from light during storage and this probably caused photodegradation of pigments [33] as can be observed in Fig. 1.

Ascorbic acid content

Ascorbic acid content increased before processing by addition of *H. sadbariffa* extract (Tab. 1) but, after vacuum concentration and storage, ascorbic acid content decreased to 2.99–3.02 $g \cdot kg^{-1}$ in purees with stevia and 2.30–2.44 $g \cdot kg^{-1}$ in purees with glucose, without statistical difference ($p > 0.05$) in reaction to concentration temperature (Fig. 1). In control purees, the loss was 90.9–97.4%. Guava pulp subjected to 80–95 °C suffered losses of up to 90% of ascorbic acid. Therefore, it is suggested that guava

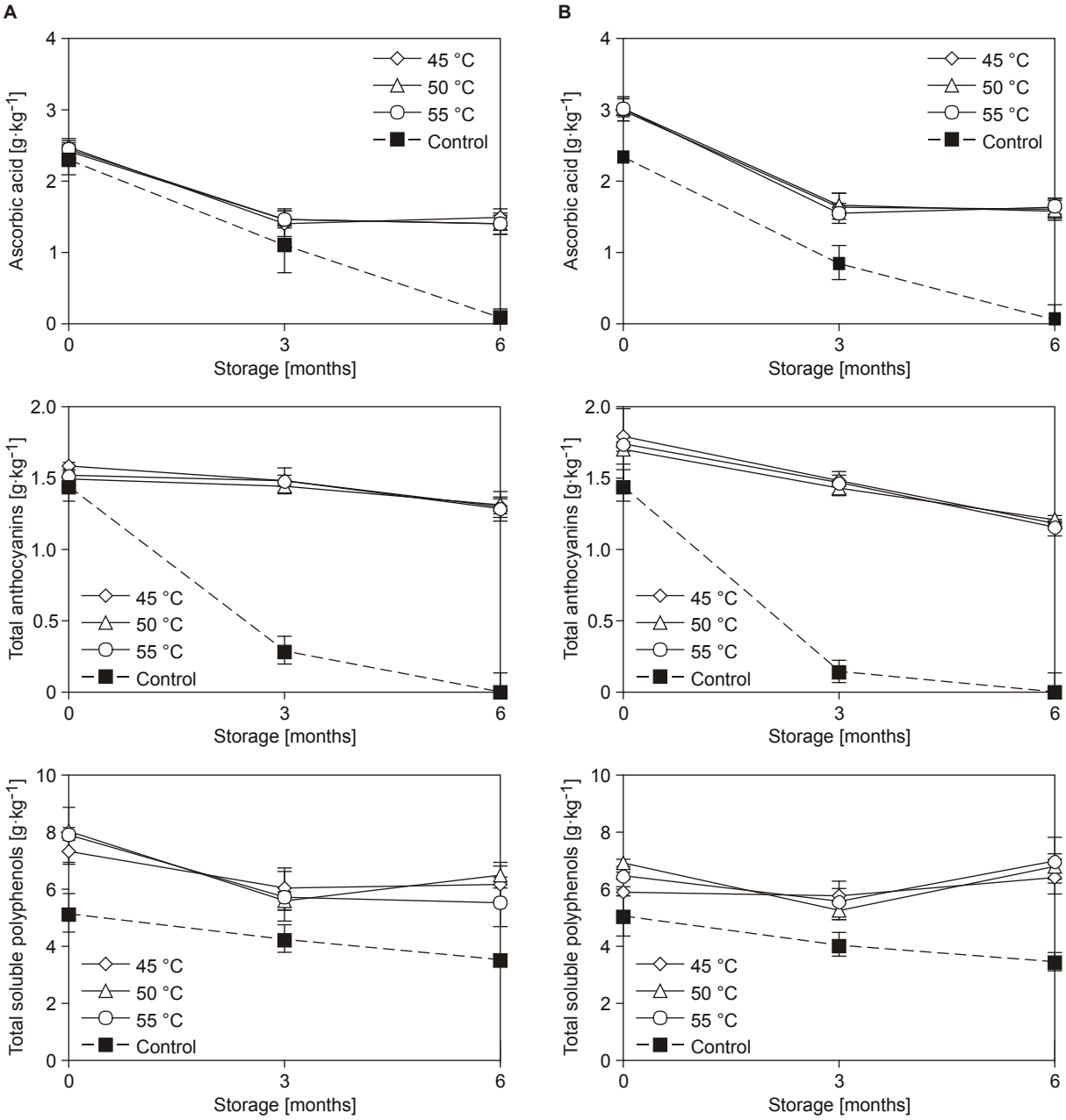

Fig. 1. Content of bioactive compounds in purees concentrated under reduced vacuum pressure, during storage for 6 months at 10 °C.

A – Puree with glucose, B – Puree with stevia.

pulp should be processed at temperatures below 75 °C, to avoid significant losses of ascorbic acid [34, 35]. Cavalcanti et al. [36] suggested that carbohydrates could form a complex with anthocyanins, proteins, tannins or antioxidants, which can increase their stability. It is possible that this occurred in purees with glucose because the ascorbic acid loss was smaller ($p < 0.05$). Although ascorbic acid content decreased (on average from 2.43 g·kg^{-1} to 1.62 g·kg^{-1}) after 6 months of storage in all purees concentrated by vacuum, ascorbic acid contents were higher than in the control samples as well as than those reported for orange (0.52 g·kg^{-1} FW), kiwi (0.91 g·kg^{-1} FW) and strawberry (0.60 g·kg^{-1} FW) [37]. This indicates that the combination of guava pulp with *H. sabdariffa* extract, and their concentration under vacuum, retained considerable amounts of vitamin C.

Total anthocyanins

An effect of concentration temperature, sweeteners and time of storage was observed on

total anthocyanins content ($p < 0.05$). A loss of 15.4–17.3% and 2.4–6.3% of total anthocyanins was noticed at the third month of storage for samples concentrated under reduced vacuum pressure with stevia or glucose, respectively, while 28.9–34.4% and 12.5–17.7% was found after 6 months of storage. In control purees, the loss was 99.9% of total anthocyanins (Fig. 1). The greatest conservation of total anthocyanins in purees with glucose concentrated under reduced vacuum pressure may be explained by the protective effect of glucose, as it may form glycosidic bonds to hydroxyl groups of anthocyanins and thus protect against oxidation [36, 38, 39].

Total soluble polyphenols and antioxidant capacity

The total soluble polyphenols content in the concentrated under reduced vacuum pressure samples with both sweeteners was greater than in control purees, but the contents of total soluble polyphenols decreased during storage in all purees (Fig. 1). Although total soluble polyphenols decreased, the concentrated under reduced vacuum pressure samples showed values of 5.54–7.02 g·kg^{-1} after 6 months of storage, which were higher than for guava pulp (4.10 g·kg^{-1}) and control purees (3.45–3.51 g·kg^{-1}). This result suggests that contents of bioactive compounds increased after the addition of *H. sabdariffa* extract, which made the product potentially functional. *H. sabdariffa* extract contained 2.87 g·kg^{-1} of total soluble polyphenols, which was lower than in guava pulp, due to the fact that a fraction of total soluble polyphenols are maintained in a by-product of the decoction process of *H. sabdariffa* calyces [8].

Antioxidant capacity values determined by DPPH and FRAP assays in total soluble polyphenols were lower in control purees ($p > 0.05$) (Fig. 2). High correlation between DPPH, total soluble polyphenols and total anthocyanins contents ($R^2 = 0.81$ and $R^2 = 0.97$) and between FRAP assay and content of ascorbic acid ($R^2 = 0.95$ and $R^2 = 0.99$) was determined. It was demonstrated previously that polyphenols

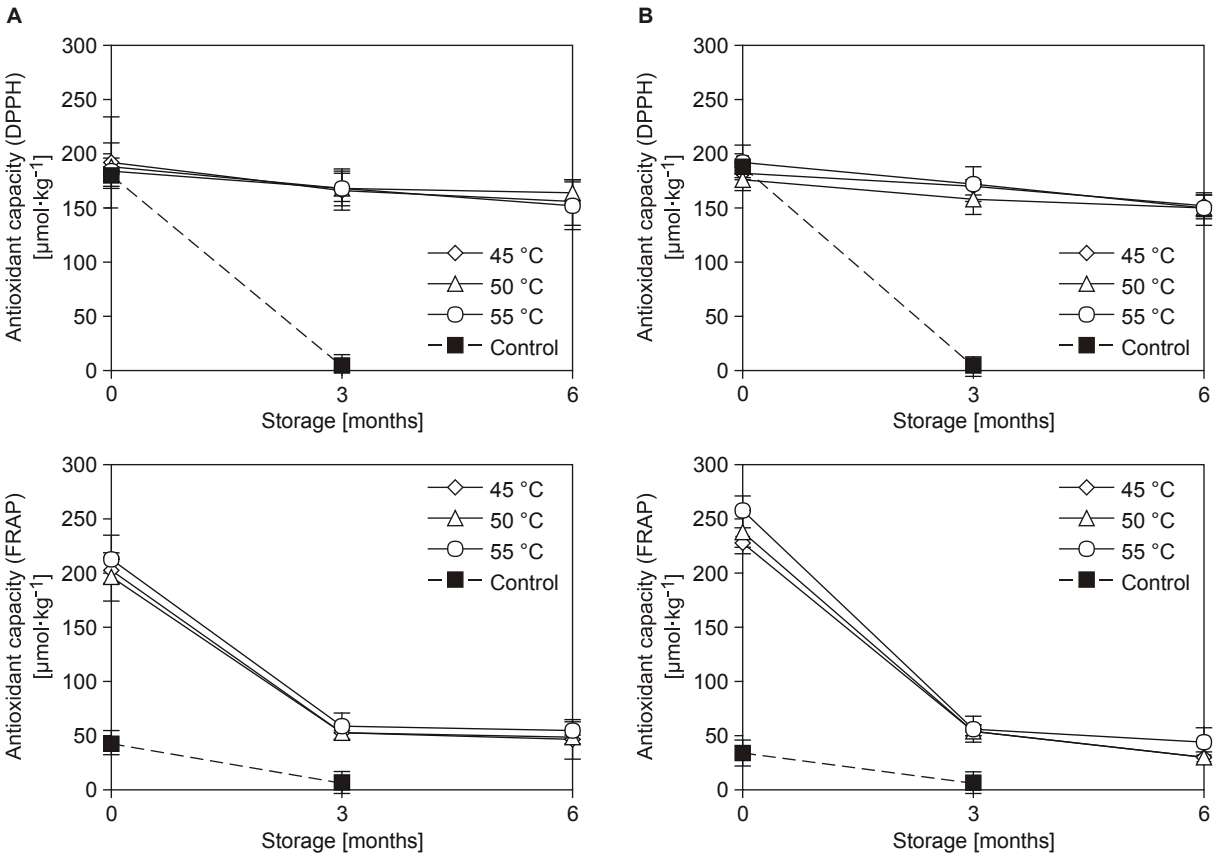

Fig. 2. Antioxidant capacity of purees concentrated under reduced vacuum pressure, during storage for 6 months at 10 °C.

A – Puree with glucose, B – Puree with stevia.
Antioxidant capacity determined by DPPH or FRAP assay is expressed as micromoles of Trolox equivalents per kilogram on fresh weight basis.

Tab. 4. Total score sum of sensory ranking evaluation of purees concentrated under reduced vacuum pressure, added with *H. sabdariffa* extract and sweetened with stevia or glucose at the day of processing.

	Puree with glucose				Puree with stevia			
	Control	45 °C	50 °C	55 °C	Control	45 °C	50 °C	55 °C
Taste	70^{b}	88^{a}	91^{a}	85^{a}	60^{b}	91^{a}	92^{a}	73^{a}
Colour	32^{b}	93^{a}	90^{a}	91^{a}	30^{b}	90^{a}	90^{a}	92^{a}
Smell	60^{b}	91^{a}	90^{a}	89^{a}	58^{b}	90^{a}	89^{a}	74^{a}

Means with different superscripts in same columns indicate significant difference ($p < 0.01$). Highest total sum of ranks indicated most preferred sample, while lowest total sum of ranks indicated least preferred sample.

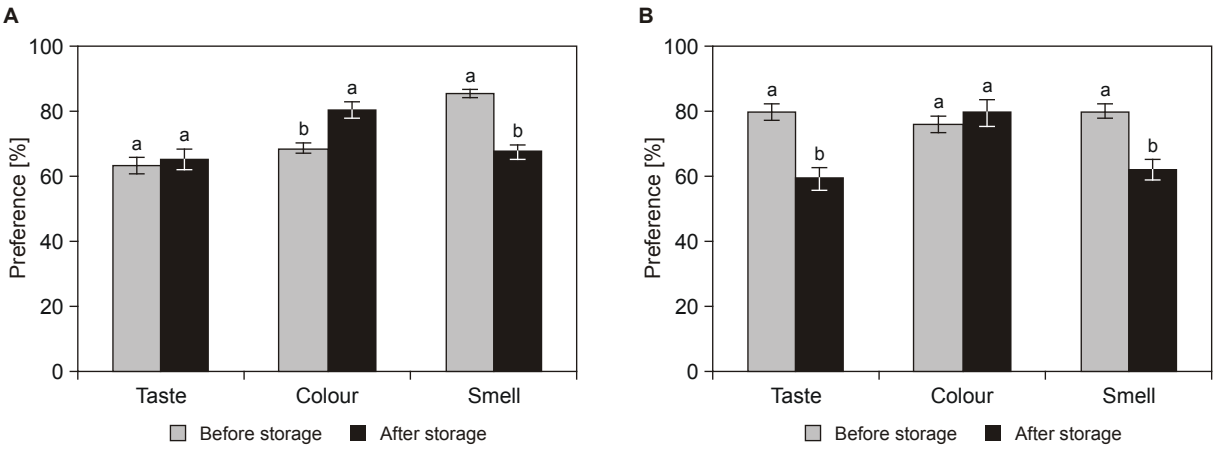

Fig. 3. Sensory evaluation of purees concentrated under reduced vacuum pressure at 50 °C and stored for 6 months.

A – Puree with glucose, B – Puree with stevia.

may operate via multiple mechanisms of radical-scavenging activity by metal scavengers, transferring electrons or donating hydrogen ions [5]. Therefore, it is clear that the addition of *H. sabdariffa* extract increases total soluble polyphenols content and antioxidant capacity.

Microbiological analyses

After 6 months of storage at 10 °C, microbial growth with values of 10 CFU·g^{-1} and 20 CFU·g^{-1} was observed only in samples concentrated under reduced vacuum pressure at 40 °C and 50 °C. There was no evidence of growth of coliforms and psychrophilic bacteria after 6 months of storage. All purees had pH values ranging from 3.09 to 3.58 (Tab. 3), which had an important influence on product stability [40]. The purees did not exceed the limits allowed by Codex Alimentarius [41], and therefore were considered microbiologically stable during the storage time.

Sensory analysis

In the results of the sensory preference-ranking test, there was no difference in taste, colour and aroma between samples concentrated under reduced vacuum pressure at different temperatures, but differences with the control purees were found (Tab. 4). The judges mentioned that control purees had a cooked flavour and a slight guava aroma. Purees that were concentrated at 50 °C obtained the highest sensory score and hence this treatment was used for the second sensory evaluation. Fig. 3 shows that judges preferred the taste and aroma of fresh concentrated puree in comparison with concentrated stored puree with stevia, because the latter had a slightly bitter aftertaste, although having 60% of preference. There was no difference in taste and colour in stored concentrated puree with glucose with respect to fresh concentrated puree. However, it had a score in aroma, probably because volatile compounds may have been lost during processing.

CONCLUSIONS

The highest amounts of soluble polyphenols, vitamin C and anthocyanins were conserved in purees concentrated under vacuum at 45 °C, 50 °C and 55 °C in comparison with control purees. The

purees were microbiologically, nutritionally and sensory acceptable after 3 months of storage at 10 °C. Addition of *H. sabdariffa* extract provided an attractive colour, dietary fibre and polyphenols to guava puree, resulting in a potentially functional product.

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