Guava purees with addition of agave fructans and natural sweeteners as potential functional products

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Summary

Concentrated guava purees formulated with native agave fructans (NAF), stevia, agave syrup or glucose, and pasteurized at 90 °C for 10 min, were evaluated. The purees were stored for 6 months at 10 °C. The physical-chemical parameters, nutritional composition, bioactive compounds, in vitro prebiotic activity, microbiological and sensory properties were measured. The added NAF increased the soluble dietary fibre content (from 7.43 $g \cdot kg^{-1}$ to 17.19 $g \cdot kg^{-1}$) and the contents of fructo-oligosaccharides in the guava purees. Also, the added sweeteners retained 83–88 % of soluble polyphenols (4.28–4.66 $g \cdot kg^{-1}$) and 58–64 % of ascorbic acid (2.51–2.59 $g \cdot kg^{-1}$) in the guava purees. The purees were microbiologically stable and sensorically acceptable. Furthermore, they showed prebiotic activity for a probiotic lactic bacterial culture (*Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus, Lb. acidophilus* and *Bifidobacterium lactis*). The combination of NAF and natural sweeteners at elaboration of guava purees improved the nutritional composition, prebiotic activity and conserved bioactive compounds, which are the properties of a potential functional product.

Keywords

guava puree; natural additives; functional product

Guava (*Psidium guajava* L.) is rich in vitamin C (ascorbic acid), vitamin E, niacin, dietary fibre (DF), and in bioactive compounds such as carotenoids and polyphenols. These features make this fruit a functional food [1].

The combination of guava pulp and natural additives for the elaboration of jams, jellies, juices, slices and concentrated purees can improve the nutritional properties and functional characteristics that might promote health benefits [2]. The substitution of saccharose by different types of natural sweeteners such as steviols (or stevia) and agave syrup, have gained importance in recent years mainly because of a tendency to decrease the sugar intake in products as jams, cakes or desserts [3]. The incorporation of stevia increased the stability of the colour and some polyphenols such as quercetin, gallic acid and rosmarinic acid during the storage of a roselle beverage [4]. The agave syrup is greatly demanded as a sugar substitute due to its low glycemic index [5]. On the other hand, the addition of trehalose (10 %) or glucose (10 %) to blackberry juices significantly reduced degradation of anthocyanins during the storage at 4 °C, whereas the opposite effect was observed when 10 % of fructose or saccharose was incorporated [6].

The fructans are also interesting additives considered as non-digestible carbohydrates, with prebiotic properties, being some of the most important ingredients used in the formulation of functional foods [7]. Mellado-Mojica

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and López-Pérez [8] mentioned that the simple sugars and a complex mixture of fructo-oligosaccharides (FOS) form the native agave fructans (NAF). They have a degree of polymerization (*DP*) ≥ 12–15, while poly-carbohydrates have *DP* from 15 to 30. *DP* depends upon the agave variety and internal (neoseries fructans) and external (graminans fructans) glucose units. The prebiotic properties of NAF were evaluated for their health benefits, particularly in people suffering from obesity and diabetes [9, 10]. Rendón-Huerta et al. [11] evaluated the prebiotic effect of fructans from *Agave angustifolia* measuring the microbial growth and reported an important increase in the growth of *Lactobacillus acidophilus, Lb. casei* and *Bifidobacterium lactis* when the fructan amounts were increased. Studies in vivo established that the addition of fructans, such as inulin, to the diet provides benefits on the lipid metabolism [12], appetite regulation [10], blood cholesterol decrease [13], body weight decrease and adiposity reduction [14].

This study evaluated the physical-chemical properties, nutritional composition, microbiological and sensory parameters, as well as the content of bioactive compounds and prebiotic activity of concentrated guava purees, to which NAF and natural sweeteners were added, during their storage at 10 °C.

MATERIALS AND METHODS

Chemicals and raw materials

Stevia (containing 97% of rebaudioside A) was purchased from Metco (Mexico City, Mexico); agave syrup from Bioagaves de la Costa (Nayarit, Mexico); glucose from Ingredion (Guadalajara, Mexico); NAF from Agaviotica (Monterrey, Mexico); citric acid from Weifang Ensign Industry (Shandong, China); ascorbic acid from Shandong Luwei Pharmaceutical (Shandong, China). Lactic culture was purchased from Danisco Company (Paris, France). De Man – Rogosa – Sharpe (MRS) medium was purchased from BD Mexico (Mexico City, Mexico).

'Portugal' guava (epicarp, and mesocarp without seeds) was used in this study and was do-

Tab. 1. Physical-chemical parameters and nutritional composition of fresh guava pulp, native agave fructans and agave syrup.

	Guava pulp	NAF	Agave syrup
Physical-chemical parameters			
Titratable acidity [%]	0.7 ± 0.1	ND	0.2 ± 0.0
pH	4.01 ± 0.01	6.64 ± 0.14	4.35 ± 0.01
Total soluble solids [°Brix]	10.73 ± 0.05	11.03 ± 0.05	75.01 ± 0.05
Water activity a_w	0.95 ± 0.04	0.41 ± 0.02	0.60 ± 0.01
Hue angle *h* [°]	94.96 ± 0.25	97.03 ± 0.24	45.34 ± 2.34
Nutritional composition [g·kg^{-1}]			
Moisture	853.7 ± 1.5	46.40 ± 2.4	252.6 ± 1.2
Total protein	6.45 ± 0.47	ND	0.17 ± 0.67
Fat	4.36 ± 0.54	ND	ND
Ash	3.72 ± 0.55	1.51 ± 0.05	1.69 ± 0.16
Glucose	35.04 ± 0.09	9.06 ± 0.97	123.4 ± 2.1
Fructose	27.20 ± 0.15	290.7 ± 1.2	530.1 ± 1.1
Saccharose	15.76 ± 2.73	6.72 ± 112	ND
Soluble dietary fibre	7.43 ± 1.25	236.0 ± 2.1	18.37 ± 1.53
Insoluble dietary fibre	84.32 ± 3.67	26.13 ± 0.08	ND
Total dietary fibre	91.74 ± 4.86	262.1 ± 2.2	ND
Ascorbic acid	2.21 ± 0.35	ND	ND
Total carotenoids	0.12 ± 0.05	ND	ND
Total soluble polyphenols	4.10 ± 0.01	ND	ND

Values are the mean ± standard deviation ($n \geq 3$, $p < 0.05$).
Titratable acidity was expressed as percentage of citric acid. Content of moisture, protein, fat, ash, glucose, fructose, saccharose, ascorbic acid, total carotenoids and total soluble polyphenols, soluble, insoluble and total dietary fibre are expressed as gram per kilogram of fresh weight of pulp or agave syrup.
NAF – native agave fructans (values are expressed in gram per kilogram of dry matter), ND – not detected.

nated by Purees and Derivatives of Nayarit (Tepic, Mexico). The physical-chemical parameters and nutritional composition of guava fresh pulp, NAF and agave syrup are shown in Tab. 1.

Preparation of guava puree formulations

The guava puree elaboration process is a trademark of company Purees and Derivatives of Nayarit. The guava purees were concentrated using a vacuum evaporation equipment (Model CV-6; Tecnodac, Queretero, Mexico), with 110 l capacity, operated under a reduced pressure (345.23 kPa), at (55 ± 3) °C for 30 min, until the concentrate reached 15–16 °Brix.

Three different batches of concentrated guava purees were prepared by adding 20 g·kg^{-1} stevia, 160 ml·kg^{-1} of agave syrup or 200 g·kg^{-1} of glucose. Into each batch, 30 g·kg^{-1} of NAF, 2.5 g·kg^{-1} of citric acid and 0.65 g·kg^{-1} of ascorbic acid were added. The control puree was a concentrated guava puree without a sweetener but with NAF, citric acid and ascorbic acid.

All puree formulations were packaged under vacuum into high-density polyethylene bags (0.940–0.970 g·cm^{-3}, 445 cm^3 of oxygen permeability; Fast Sincere International Industrial, Hong Kong, China), pasteurized using a Model KVV pasteurizer (Tecnodac, Monterrey, Mexico) at 90 °C for 10 min, and stored at 10 °C for 6 months.

Physical-chemical analysis

Titratable acidity (*TA*, method 942.15), pH (method 981.12) and total soluble solids (*TSS*, method 932.12) were determined according to AOAC official methods [15]. Colour changes were measured directly in the purees with a Minolta CR300 colorimeter (Konica Minolta, Osaka, Japan) in the L^* a^* b^* system, expressed as hue angle (h) and colour difference (ΔE) was calculated.

Nutritional composition

Protein (method 978.04), ash (method 940.26), fat (method 950.54) and moisture (method 934.06) contents were determined according to AOAC official methods [15]. Soluble dietary fibre (*SDF*), insoluble dietary fibre (*IDF*) and total dietary fibre (*TDF*) were analysed using the AOAC enzymatic-gravimetric method (method 991.42) [15] modified by Mańas and Saura-Calixto [16]. All data were reported in grams per kilogram of fresh weight (FW).

Simple sugars, total soluble carbohydrates and degree of polymerization of fructans

In all the samples, the degree of polymerization (*DP*) and simple sugars (glucose, fructose, and saccharose) were determined using high performance anion-exchange chromatography, coupled with a pulsed amperometric detector (HPAEC-PAD, Thermo Scientific Dionex-ICS 5000 system, Thermo Scientific, Waltham, Massachusetts, USA). The analyses were performed according to the method of Ortiz-Basurto et al. [17] with slight modifications.

The extractions were done using 0.5 g of sample and 25 ml of an ethanol solution (850 ml·l^{-1}). The mixture was stirred for 2 h at 80 °C and then centrifuged (9380 ×g, 40 min at 4 °C). The supernatant was dried and re-suspended in 1 ml of Milli-Q water (Merck Millipore, Billerica, Massachusetts, USA) for analysis. The extracts were filtered through a nylon membrane (0.45 μm pore size) and injected into HPAEC-PAD. A Dionex PA-200 column (0.4 cm × 5 cm, Thermo Scientific) at 35 °C was used. The separation was performed using a sodium acetate gradient (0–600 mmol·l^{-1}) in 100 mmol·l^{-1} NaOH with a flow rate of 0.4 ml·min^{-1}.

DP was estimated using a chicory inulin standard and concentrations of simple sugars were calculated with a calibration curve of glucose, fructose and saccharose standards. Total soluble carbohydrates were measured by the phenol-sulphuric method [18]. All data were expressed as grams per kilogram of FW.

Ascorbic acid, total carotenoids and total soluble polyphenols

The ascorbic acid content (*AA*) was determined according to the method of Suntornsuk et al. [19]. The samples (10 g) were homogenized with 25 ml of sulphuric acid (1.04 mol·l^{-1}), 25 ml of distilled water and 3 ml of starch solution (50 g·l^{-1}) as an indicator. The mixture was titrated with potassium iodide-diiodo solution (0.12 mol·l^{-1} and 0.02 mol·l^{-1}, respectively) and the results were expressed in grams per kilogram of FW.

The total carotenoid content (*TC*) was calculated with the method proposed by Cano and De Ancos [20]. The samples (2 g) were homogenized with 10 ml of ether-acetone mixture (80 ml and 20 ml, respectively for 100 ml) and with 0.5 g of $MgCO_3$. The mixture was stirred for 1 min and centrifuged at 11000 ×g for 30 min at 4 °C. The supernatant was recovered and homogenized with 15 ml of 200 g·l^{-1} NaCl solution. Ethereal extract was dried with 5 g of anhydrous sodium sulphate and the absorbance was measured at 448 nm (Model 6705; Jenway, Felsted, United Kingdom).

Quantification was performed using a calibration curve of β-carotene standard. The results

were expressed as grams of β-carotene per kilogram of FW.

A sequential organic aqueous extraction was used to evaluate the total soluble polyphenols (*TSP*) in 0.5 g of sample mixed with 20 ml of an acidified methanol solution (0.8 % of 72.8 $g \cdot l^{-1}$ hydrochloric acid) and 20 ml of acetone-water solution (80 : 20, v/v) according to the procedure described by Pérez-Jiménez et al. [21]. The mixture was stirred for 1 h, then centrifuged (Model Z306; Hermle, Wehingen, Germany) at 9 380 ×*g* for 30 min at 4 °C. A volume of 20 ml of acetone-water solution (80 : 20, v/v) was added to the residue and the extraction was repeated. The supernatants from each extraction step were combined and *TSP* were measured using Folin-Ciocalteu's reagent [22], with some modifications according to Alvarez-Parrilla et al. [23]. Aliquots (250 μl) of the supernatants were mixed with 1 000 μl of a 75 $g \cdot l^{-1}$ Na_2CO_3 solution. After 3 min, the Folin–Ciocalteu's reagent (1 250 μl) was added and the mixture was heated in a water bath for 15 min. A multimode microplate reader Synergy HT (Bio-Tek, Winooski, Vermont, USA) was used at 750 nm. The results were expressed as grams of gallic acid equivalents per kilogram of FW.

In vitro prebiotic assay

The prebiotic assay was carried out according to Farinha et al. [24] with some modifications. A lyophilized lactic culture YO-MIX205 LYO250 DCU (Danisco), which contained *Streptococcus thermophilus* (anaerobic), *Lactobacillus delbrueckii* subsp. *bulgaricus* (anaerobic), *Lb. acidophilus* (aerobic) and *Bifidobacterium lactis* (anaerobic), was used in the assay. The lactic culture was reactivated according to attached technical instructions. In an Erlenmeyer flask, 0.05 g the lactic culture was dissolved in 40 ml of MRS at pH 6.60 and incubated in a shaker (1.16 Hz) at 42 °C until optical density (*OD*) of 0.80–0.90 at 600 nm was reached. The prebiotic activity was measured with 50 ml of sterile MRS medium and 1 ml of sample, which contained 0.2 g of guava pulp, control puree, puree formulations or NAF. The mixture was inoculated with 5 ml of the re-activated lactic culture and then incubated in a shaker (1.16 Hz) at 42 °C. *OD* was measured at 600 nm each hour for 10 h. The results were expressed as *OD* values.

Microbiological analysis

The microbiological evaluation regarding yeasts and fungi, as well as the total mesophilic, psychrophilic and coliform bacteria counts were determined using the methods of Downes and Ito [25].

Sensory analysis

The preference level test (taste, colour and aroma) of the purees after storage for 6 months at 10 °C, was used with 100 untrained judges. The scale 1–4 was utilized: 1 – dislike, 2 – indifferent, 3 – like a little, 4 – like very much [26]. For taste test, the eyes of the judges were covered with cellophane lenses. Preference percentages were calculated.

Statistical analysis

All analysis were done in triplicate and the data were analysed by ANOVA, using the statistical software Statistica v.10 (Statsoft, Tulsa, Oklahoma, USA), at $p = 0.05$. The means comparison was made using the least significant difference (LSD, $\alpha = 0.05$). The sensory evaluation was analysed by Student's *t*-test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Physical-chemical analysis

Significant differences ($p < 0.05$) were observed in *TA* between the control puree and the puree with stevia (Tab. 2) on the day of preparation (month 0). The values of *TA* in these purees were 0.9 % with a pH of 3.69–3.70, while the puree with agave syrup and glucose maintained lower *TA* values. The lowest *TA* was attributed to the high content of simple sugars in the purees with agave syrup or glucose, which could exert a protective effect on the organic acids through hydrogen bonds [27]. The results coincided with the report of Chávez-Tapia et al. [28]. However, *TA* values slightly decreased and pH increased ($p < 0.05$) in all the purees during the time of storage, probably due to oxidation of organic acids, such as ascorbic acid [29].

The final pH was 3.73–3.83 indicating microbiological stability of the purees.

The puree with glucose had the highest *TSS* (29.43 °Brix) followed by the puree with agave syrup (25.33 °Brix), the puree with stevia (18.53 °Brix) and finally the control puree (18.52 °Brix). These results were dependent of the amount of sweetener, although there were no significant ($p < 0.05$) changes during the time of storage.

Glucose and fructose have the ability to link, through hydrogen bonds, with the free water of the foods [27]. Therefore, a_w was lower in the puree with glucose (0.90) and in the puree with agave syrup (0.92) compared to the puree with stevia and the control puree (both 0.95). During the time of storage, a_w was stable in the concentrated purees,

Tab. 2. Physical-chemical parameters of concentrated guava purees added with native agave fructans and natural sweeteners at the end of storage (6 months at 10 °C).

Parameters	Months of storage	Control puree	Puree with stevia	Puree with agave syrup	Puree with glucose
Titratable acidity [%]	0	0.9 ± 0.2^{aA}	0.9 ± 0.1^{aA}	0.8 ± 0.2^{bA}	0.8 ± 0.1^{bA}
	3	0.9 ± 0.1^{aA}	0.8 ± 0.1^{aB}	0.7 ± 0.1^{bB}	0.7 ± 0.2^{cB}
	6	0.8 ± 0.1^{aB}	0.8 ± 0.1^{aB}	0.7 ± 0.1^{bC}	0.6 ± 0.1^{bC}
pH	0	3.69 ± 0.05^{bC}	3.70 ± 0.05^{bC}	3.74 ± 0.05^{aC}	3.75 ± 0.01^{aB}
	3	3.73 ± 0.02^{bA}	3.73 ± 0.05^{bB}	3.75 ± 0.05^{aB}	3.77 ± 0.01^{aA}
	6	3.73 ± 0.03^{bA}	3.75 ± 0.01^{aA}	3.82 ± 0.01^{aA}	3.83 ± 0.05^{aA}
Total soluble solids [°Brix]	0	18.52 ± 0.32^{cA}	18.53 ± 0.15^{cA}	25.33 ± 0.20^{bA}	29.43 ± 0.05^{aA}
	3	18.43 ± 1.10^{cA}	19.70 ± 0.10^{cA}	24.97 ± 0.15^{bA}	29.63 ± 0.15^{aA}
	6	18.83 ± 0.45^{cA}	18.57 ± 0.15^{cA}	24.07 ± 0.20^{bA}	28.97 ± 0.15^{aA}
Water activity a_w	0	0.95 ± 0.01^{aA}	0.95 ± 0.01^{aA}	0.92 ± 0.01^{bA}	0.90 ± 0.01^{cA}
	3	0.95 ± 0.01^{aA}	0.95 ± 0.01^{aA}	0.92 ± 0.02^{bA}	0.90 ± 0.01^{cA}
	6	0.95 ± 0.01^{aA}	0.95 ± 0.01^{aA}	0.92 ± 0.01^{bA}	0.90 ± 0.01^{cA}
Hue angle *h* [°]	0	94.88 ± 0.16^{aC}	93.73 ± 0.40^{bA}	96.16 ± 0.40^{aA}	94.86 ± 1.09^{aA}
	3	85.84 ± 0.63^{aB}	89.29 ± 0.44^{aB}	88.77 ± 0.36^{aB}	86.83 ± 0.14^{bB}
	6	80.16 ± 0.23^{aA}	87.80 ± 0.29^{aC}	85.85 ± 0.76^{bC}	84.55 ± 0.55^{bC}
Colour difference (ΔE)	3	15.01	10.99	10.45	14.99
	6	20.14	12.10	11.43	19.15

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

which is a normal behaviour of the foods with high a_w [27].

The values of pH and *TA* indicated that the purees were physically, chemically and microbiologically stable during the storage [27].

The guava pulp had a *h* value of 94.96 (Tab. 1), which corresponds to the yellow colour. At the beginning of the processing (0 month), the *h* values were 93.73–96.16. The addition of sweeteners caused significant changes ($p < 0.05$) in the colour. The puree with agave syrup had the highest *h* value due to that the agave syrup, which is yellowish in colour. After 3 and 6 months of storage, the *h* values decreased (to 80.16–87.80).

The colour change from yellow to brown-yellow was evident during the storage and this coincided with the ΔE values. The greatest browning was observed in the control puree, which could be due to the high oxidation of ascorbic acid and/or due to photodegradation of others pigments. This coincided with the lack of sweeteners [29]. The sweeteners helped to maintain the colour of the purees.

Nutritional composition

The nutritional composition is shown in Tab. 3. The moisture content in the control puree was 824.11 g·kg^{-1}, in the puree with stevia was 823.45 g·kg^{-1} and in the puree with agave syrup was 800.13 g·kg^{-1}, while in the puree with glucose was 740.35 g·kg^{-1} (after 6 months of storage) with significant differences ($p < 0.05$) between them. The differences can be attributed to the quantity and type of sweetener added. Fructose and glucose have a direct influence on the moisture because they are hygroscopic, with hydroxyl groups that can establish hydrogen bonds with the water, which contributes to a reduction in the moisture and a_w [27].

All the purees had low protein (6.47–6.83 g·kg^{-1}), fat (4.27–4.48 g·kg^{-1}) and ash (3.52–3.85 g·kg^{-1}) contents (Tab. 3). After 6 months of storage at 10 °C, there was no significant change ($p > 0.05$) in the different purees. The data were similar to those of fresh guava pulp (Tab. 1). This indicates that the nutritional values were maintained during the storage.

The sweeteners had no significant effect ($p < 0.05$) on the dietary fibre, because they are not a part of it. *SDF* values (Tab. 3) and consequently *TDF* values were slightly higher ($p > 0.05$) in all the purees (17.13–26.74 g·kg^{-1} and 101.51–111.53 g·kg^{-1}, respectively) after 6 months of storage in comparison to fresh guava pulp (7.43 g·kg^{-1} and 91.74 g·kg^{-1}, respectively). *IDF* values were between 84.37 g·kg^{-1} and 84.82 g·kg^{-1} and did not show significant differences among the different purees ($p > 0.05$). The increase of *SDF*,

Tab. 3. Nutritional composition of concentrated guava purees added with native agave fructans and natural sweeteners at the end of storage (6 months at 10 °C).

Parameters [g·kg^{-1}]	Control puree	Puree with stevia	Puree with agave syrup	Puree with glucose
Moisture	824.11 ± 0.14^{a}	823.45 ± 3.18^{a}	800.13 ± 1.75^{b}	740.35 ± 3.76^{c}
Total protein	6.56 ± 1.50^{a}	6.47 ± 0.28^{a}	6.78 ± 0.86^{a}	6.83 ± 0.24^{a}
Fat	4.27 ± 0.92^{a}	4.32 ± 1.94^{a}	4.37 ± 1.87^{a}	4.48 ± 0.63^{a}
Ash	3.22 ± 0.54^{a}	3.72 ± 3.00^{a}	3.68 ± 1.00^{a}	3.85 ± 1.40^{a}
Soluble dietary fibre	17.16 ± 1.64^{a}	17.19 ± 1.43^{a}	26.74 ± 0.26^{b}	17.13 ± 0.15^{a}
Insoluble dietary fibre	84.37 ± 2.13^{a}	84.40 ± 3.47^{a}	84.82 ± 1.42^{a}	84.61 ± 2.38^{a}
Total dietary fibre	101.53 ± 1.12^{b}	101.51 ± 4.21^{b}	111.53 ± 1.32^{a}	101.74 ± 1.54^{b}

Values are the mean ± standard deviation ($n \geq 3$). Means with different small letters in superscripts in the same row indicate significant difference using LSD test ($\alpha = 0.05$).

Tab. 4. Simple sugars and total soluble carbohydrates in concentrated guava purees added with native agave fructans and natural sweeteners at the end of storage (6 months at 10 °C).

Carbohydrates [g·kg^{-1}]	Control puree	Puree with stevia	Puree with agave syrup	Puree with glucose
Glucose	42.84 ± 0.93^{c}	43.27 ± 0.18^{c}	50.87 ± 0.98^{b}	166.25 ± 0.70^{a}
Fructose	51.28 ± 1.15^{b}	51.39 ± 1.22^{b}	98.95 ± 0.71^{a}	50.96 ± 1.01^{b}
Saccharose	21.86 ± 1.63^{b}	21.96 ± 0.91^{b}	29.01 ± 0.66^{a}	21.70 ± 0.29^{b}
Total soluble carbohydrates	115.98 ± 1.22^{c}	116.63 ± 1.52^{c}	178.83 ± 3.58^{b}	238.92 ±7.64^{a}

Values are the mean ± standard deviation ($n \geq 3$), expressed in gram per kilogram of fresh weight. Means with different small letters in superscripts in the same row indicate significant difference using LSD test ($\alpha = 0.05$).

and consecutively *TDF*, in the control puree, puree with stevia and puree with glucose, was due to the concentration process and the addition of NAF. However, the highest value of *SDF* (26.74 g·kg^{-1}) in the puree with agave syrup was attributed to NAF and agave syrup because *SDF* was found in these additives (Tab. 1) [30, 31]. In general, *SDF* has nutritional importance due to its rheological properties, ability to affect the gastrointestinal content, increasing the viscosity of the medium triggering satiety signals and slowing down the gastric emptying. Moreover, *SDF* may serve as a substrate for the fermentative microbiota producing short-chain fatty acids, and it also stimulates the colonic blood flow and the electrolyte uptake [32]. Jiménez-Escrig et al. [33] and Chávez-Tapia et al. [28] considered the guava fruit rich in *TDF* (83.4–118.5 g·kg^{-1}). Thus, a portion of concentrated purees (100 g) can be classified as a source of DF and, in consequence, the purees are potential prebiotic products.

Simple sugars, total soluble carbohydrates and degree of polymerization of fructans

Significant differences ($p < 0.05$) were observed in the simple sugar and total soluble carbohydrate contents (Tab. 4) of the purees. This was due to the fact that the simple sugar content depended on NAF and the quantity of natural sweeteners added. The control puree and the puree with stevia had lower glucose (42.84 g·kg^{-1} and 43.27 g·kg^{-1}), fructose (51.28 g·kg^{-1} and 51.39 g·kg^{-1}) and saccharose (21.86 g·kg^{-1} and 21.96 g·kg^{-1}) contents, without significant differences between them. Stevia did not increase the carbohydrates content although the values were higher than those of guava pulp (Tab. 1). This is attributed to the concentration process and NAF addition, because NAF contains simple sugars (Tab. 1). The puree with agave syrup had the highest fructose content (98.95 g·kg^{-1}) by the addition of NAF and agave syrup. NAF are rich in fructose (530.1 g·kg^{-1}) and also contain glucose (123.4 g·kg^{-1}) (Tab. 1). In the puree with glucose, the glucose content (166.25 g·kg^{-1}) was the highest. The total soluble carbohydrates were 115.98 g·kg^{-1}, 116.63 g·kg^{-1} and 178.83 g·kg^{-1} for the control puree, the puree with stevia and the puree with agave syrup, respectively. Meanwhile, the puree with glucose contained 238.92 g·kg^{-1} total soluble carbohydrates, since the weight of the added glucose was 10 times more than at stevia and 1.5 times higher than at the puree with agave syrup.

DP values of the chicory inulin standard and

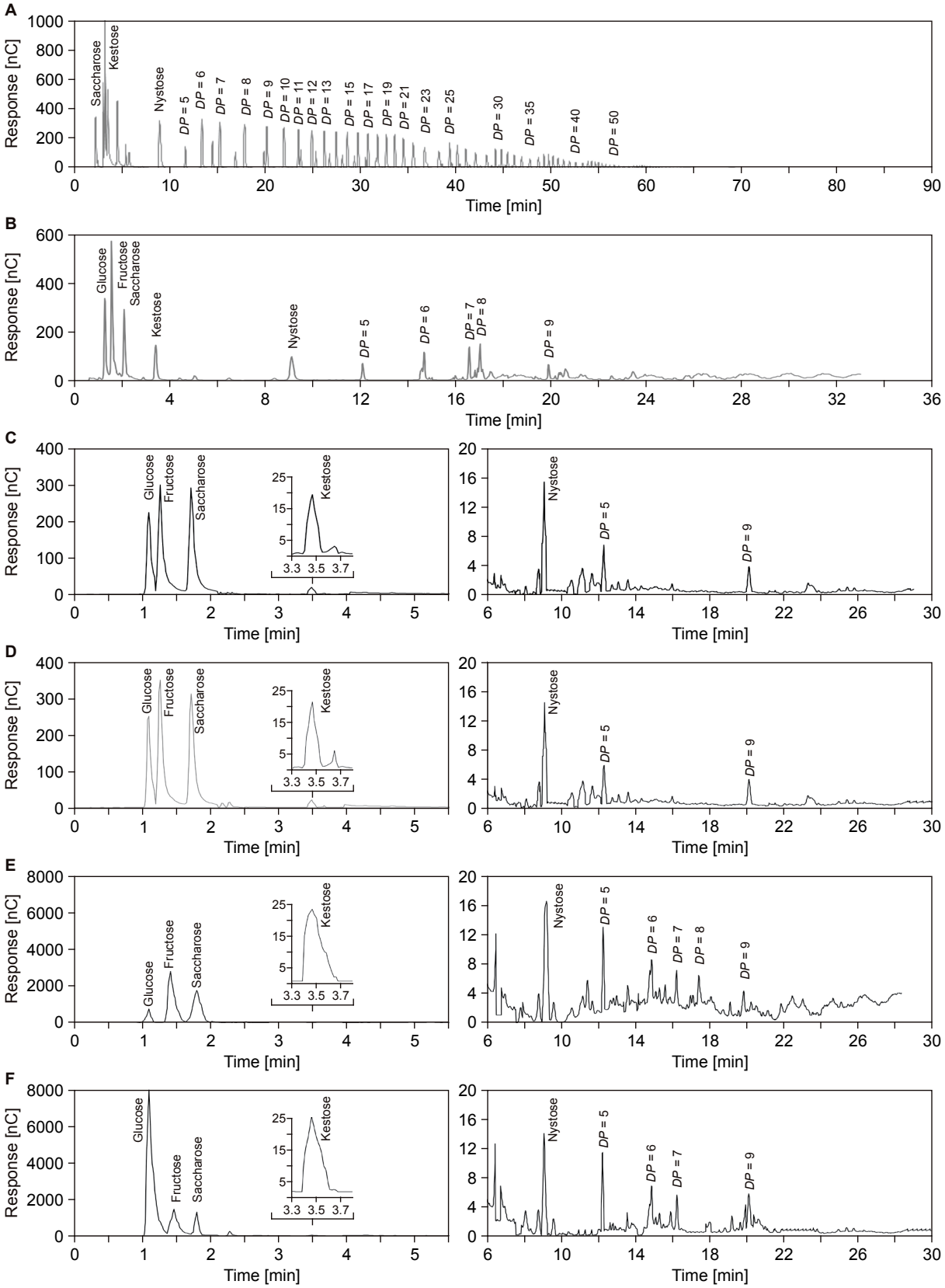

Fig. 1. HPAEC-PAD chromatogram profiles of fructan standards, control puree and concentrated guava purees with native agave fructans and natural sweeteners at the end of storage (6 months at 10 °C).

A - chicory inulin standard, B – native agave fructans, C – control puree, D – puree with stevia, E – puree with agave syrup, F – puree with glucose; *DP* – degree of polymerization.

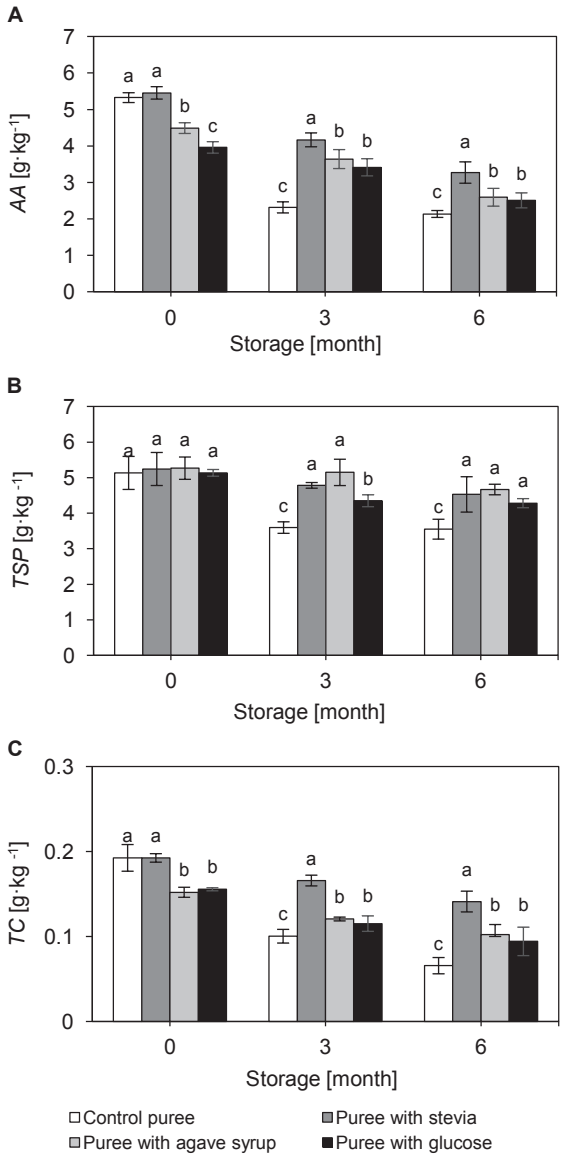

Fig. 2. Ascorbic acid, total soluble polyphenols and total carotenoids in concentrated guava purees added with native agave fructans and natural sweeteners during the storage at 10 °C.

A – ascorbic acid content (*AA*), B – total soluble polyphenols (*TSP*), C – total carotenoids (*TC*).

NAF sample are shown in Fig. 1A and Fig. 1B. The presence of FOS, namely, kestose (*DP* = 3), nystose (*DP* = 4), *DP* = 5 and *DP* = 9 was detected in all the purees (Fig. 1C, 1D, 1E and 1F). However, an increase in FOS was registered in the puree with agave syrup (Fig. 1E) in comparison to the control purees and the other puree formulations. This was due to the contents of FOS in agave syrup and in NAF. Mellado-Mojica and López-Pérez [8] reported that the agave syrup contained little glucose, abundant fructose, and abundant FOS (kestose, neokestose and nystose) as the outcome of hydrolysis of fructans. In the years later, the same authors [31] reported fructans of other *DP* in the agave syrup, such as inulotriose (*DP* = 3), kestopentaose (*DP* = 5) and *DP* = 6 to *DP* = 9. FOS with *DP* values 6, 7 and 8 in the puree with agave syrup (Fig. 1E) and in the puree with glucose (Fig. 1F) were identified.

The control puree and the puree with stevia only contained FOS with *DP* = 5 and *DP* = 9. It was demonstrated that pH lower than 4.0 decreased the fructan content due to its hydrolysis [34]. The control puree and the puree with stevia had pH of 3.73 and 3.75, respectively, after 6 months of storage at 10 °C. Therefore, it is possible that pH affected the content of FOS.

In all purees, low *DP* was observed. In comparison to the chicory inulin standard, this was because NAF are formed by a mixture of simple sugars, FOS and poly-carbohydrates with lower *DP* (3 to 9) in comparison with the chicory inulin (3 to 50) (Fig. 1A). Besides that, the heating process and a low pH possibly hydrolysed NAF during the storage. Glibowski and Bukowska [34] studied the effect of pH, temperature and heating time on fructans. They found that inulin degradation occurred when the temperature reached 80 °C at pH of 4.0. Despite this, it is clear that the addition of NAF as well as of agave syrup in the purees was important because they increased *SDF* content.

Ascorbic acid, total carotenoids and total soluble polyphenols

It is clear that the *AA* was higher in a puree with stevia (5.45 g·kg^{-1}) than in the control puree (5.35 g·kg^{-1}), puree with agave syrup (4.48 g·kg^{-1}) and puree with glucose (3.95 g·kg^{-1}) at the day of processing (Fig. 2A). Also, the *AA* values were higher in the concentrated purees than in the guava pulp (2.21 g·kg^{-1}) due to the addition of ascorbic acid in the formulations. It is important to point out that, in the control puree and the puree with stevia, the *AA* was higher than in those with of agave syrup or glucose, even though these sweeteners were added in greater quantity than was stevia. It was probably caused by the diluting effect when the agave syrup and glucose were added.

The *AA* decreased during the period of storage down to 52%, 40%, 42% and 36% in the control puree, puree with stevia, puree with agave syrup and puree with glucose, respectively, after 6 months of storage at 10 °C. The guava pulp treated by heat at 80–95 °C can present losses of

up to 90 % of *AA*, due to the lability of this vitamin [28]. It was suggested that the guava pulp should be processed at temperatures below 75 °C, to avoid these significant losses [35]. According to CHÁVEZ-TAPIA et al. [28], the concentrated guava purees lost at 45–55 °C up to 40–46% of *AA*. The foregoing indicates that the purees with natural sweeteners retained 58–64 % of *AA* after 6 months at 10 °C, in comparison to the control puree, thus a portion of 100 g can still cover the recommended daily intake.

All puree formulations showed a decrease of *TSP* during the storage (Fig. 2B). The control puree presented 69 % retention of *TSP*, while all the other formulations retained approximately 83–88 % of *TSP*. In comparison with the guava pulp (4.10 $g \cdot kg^{-1}$) and the control puree (3.55 $g \cdot kg^{-1}$), the concentrated purees with natural sweeteners had higher values of *TSP* (4.28–4.66 $g \cdot kg^{-1}$) after 6 months of storage. The data indicate that the sweeteners played an important role in the conservation of these compounds [36, 37]. PÉREZ-RAMÍREZ et al. [4] reported that incorporation of stevia increased the stability of anthocyanins and some polyphenols during the storage of a roselle beverage, although the authors mentioned that this effect was not previously reported. CORREA et al. [1] reported that the guava jam added with grape juice had an increased polyphenol content compared to the control formulation, and also that, during the storage, a reduction (36 %) of phenolic compounds took place. The presence of polyphenols in processed foods is important from a nutritional point of view because they have protective characteristics such as neuroprotection, anti-inflammatory and antioxidant [38]. Therefore, the increase and conservation of *TSP* in the concentrated purees gives them an additioned value.

The *TC* (Fig. 2C) was significantly different among the control puree and the purees with sweeteners during the time of storage ($p < 0.05$). On the day of processing, the *TC* was greater in the control puree and the puree with stevia (0.19 $g \cdot kg^{-1}$). In the puree with agave syrup and the puree with glucose, the lower values of the *TC* were determined, probably for the reasons discussed above. At the end of storage, a decrease ($p < 0.05$) in *TC* in all the purees was noted, the control puree having the lowest value (0.06 $g \cdot kg^{-1}$), probably due to the highest oxidation of carotenoids, considering that sweeteners had a protective effect in the other purees [37]. NORA et al. [39] reported that stability of carotenoids depended on factors such as dissolved oxygen residue in the sample, light, storage temperature and the food matrix.

In vitro prebiotic activity

The growth capacity of probiotic microorganisms varied in the different types of samples evaluated. The purees with sweeteners showed a higher growth (Fig. 3) of microorganisms than the control puree, while the fresh guava pulp and NAF exhibited the lowest growth capacity. Analysing the effect of each sample, it was observed that the puree with agave syrup promoted the higher growth of probiotic microorganims. This could be possible due to the puree with agave syrup having the highest *SDF* content, which included the highest FOS content (see Fig. 1E). FARHINA et al. [24] and VELÁZQUEZ-MARTÍNEZ et al. [40]

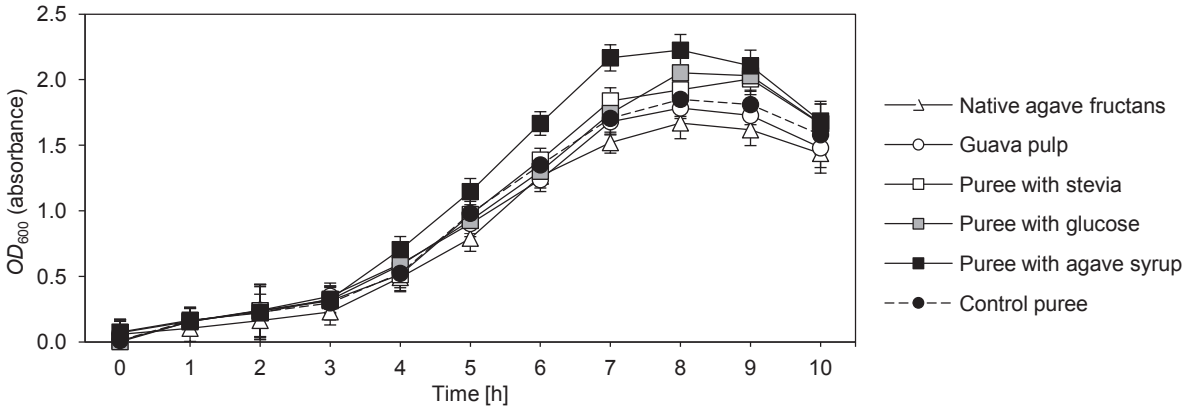

Fig. 3. In vitro prebiotic activity native agave fructans, guava pulp and concentrated guava purees added with agave fructans and natural sweeteners at the end of storage (6 months at 10 °C).

OD_{600} – optical density at 600 nm.

reported that fructans with a low *DP* offer better prebiotic activity. However, other authors reported that the prebiotic effect of the fructans is independent of *DP*, since some bacterial consortia showed growth depending on the size of the fructan chain. Also, the effectiveness depended on whether the microorganisms were aerobic or anaerobic, which in turn was directly related to the enzymatic capacity as well as probiotic metabolism, in particular with the secretion of fructanhydrolases [41–43]. On the other hand, the prebiotic activity was lower in NAF than in all the purees, although without significant differences ($p > 0.05$) with fresh guava pulp. It is possible that the sum of *SDF* from guava pulp and the added NAF in the purees caused the difference in the prebiotic activity with respect to NAF. The fruits rich in *DF* with the addition of fructans can be utilized in symbiotic formulations in the functional food industry thanks to the fact that the combination of different sources of *SDF* could increase the prebiotic properties [43, 44].

Microbiological analysis

At 6 months of storage at 10 °C, there was no growth of microorganisms in the purees. The pH is a strong factor in suppression of the microbial growth, because microorganisms can proliferate in a pH range of 4–10 and grow faster in a pH range of 5–7 [34]. The pH values of all the purees ranged from 3.5 to 3.8 (Tab. 2), which had an important positive influence on the stability of the product. Purees were stored at 10 °C, a temperature that restricts microbial growth and, therefore, they were microbiologically stable during the time of storage.

Sensory analysis

The sensory analysis demonstrated that the judges did not like the taste of the control puree, because of it was overly acidic. In the puree with stevia, the judges perceived a bitter remnant, but 75 % of them preferred it after 6 months of storage. The result of the sensory test was similar at the puree with agave syrup and the puree with glucose. The judges preferred the colour and aroma of the freshly prepared purees to those that were stored. This was because the stored purees presented browning and probably some of the volatile compounds were lost during processing, although the acceptance for them was 70–73 %. It is important to mention that colour of the control purees was the least preferred (55 %). These results are useful for continuing the study on the shelf life and the testing of different types of packaging.

CONCLUSIONS

All the formulations of guava purees added with NAF and natural sweeteners were physically, nutritionally and microbiologically stable during 3 months of cold storage (at 10 °C), although the colour changes were noted probably due to oxygen permeability in the bags as well as dissolved oxygen and gas headspace of the bags. The addition of NAF and natural sweeteners improved the quality of the purees increasing the *SDF* content as well as the retention of bioactive compounds and in vitro prebiotic activity. Therefore, these puree formulations are an alternative for obtaining potentially functional products.

Acknowledgements

The authors acknowledge the National Council for Science and Technology CONACYT (Mexico); Mr. John Philip Tolman (Purees and Derivatives of Nayarit, Tepic, Mexico) for the financial support (Grant No. 198552); and the valuable contribution of PhD Rosa Isela Ortiz-Basurto by her assistance in chromatographic analysis.

REFERENCES

1. Corrêa, R. C. – Haminiuk, C. W. – Sora, G. T. – Bergamasco, R. – Vieira, A.: Antioxidant and rheological properties of guava jam with added concentrated grape juice. Journal of the Science of Food and Agriculture, *94,* 2013, pp. 146–152. DOI: 10.1002/jsfa.6233.
2. Ajila, C. – Prasada Rao, U.: Mango peel dietary fibre: Composition and associated bound phenolics. Journal of Functional Foods, *5*, 2013, pp. 444–450. DOI: 10.1016/j.jff.2012.11.017.
3. Souza, V. R. – Pereira, P. A. – Pinheiro, A. C. M. – Bolini, H. – Borges, S. V. – Queiroz, F.: Analysis of various sweeteners in low-sugar mixed fruit jam: equivalent sweetness, time-intensity analysis and acceptance test. International Journal of Food Science and Technology, *48,* 2013, pp. 1541–1548. DOI: 10.1111/ijfs.12123.
4. Pérez-Ramírez, I. F. – Castaño-Tostado, E. – Ramírez-De León, J. A. – Rocha-Guzmán, N. E. – Reynoso-Camacho, R.: Effect of stevia and citric acid on the stability of phenolic compounds and *in vitro* antioxidant and antidiabetic capacity of a roselle (*Hibiscus sabdariffa* L.) beverage. Food Chemistry, *172,* 2015, pp. 885–892. DOI: 10.1016/j.foodchem.2014.09.126.
5. Foster-Powell, K. – Holt, S. H. – Brand-Miller, J. C.: International table of glycemic index and glycemic load values: 2002. The American Journal of Clinical Nutrition, *76*, 2002, pp. 5–56. ISSN: 1938-3207. <http://ajcn.nutrition.org/content/76/1/5.full.pdf+html>
6. Kopjar, M. – Jakšic, K. – Piližota, V.: Influence of sugars and chlorogenic acid addition on anthocyanin content, antioxidant activity and colour of

blackberry juice during storage. Journal of Food Processing and Preservation, *36,* 2012, pp. 545–552. DOI: 10.1111/j.1745-4549.2011.00631.x.
7. Corradini, C. – Lantano, C. – Cavazza, A.: Innovative analytical tools to characterize prebiotic carbohydrates of functional food interest. Analytical Bioanalytical Chemistry, *405*, 2013, pp. 4591–4605. DOI: 10.1007/s00216-013-6731-6.
8. Mellado-Mojica, E. – López-Pérez, M. G.: Análisis comparativo entre jarabe de agave azul (*Agave tequilana Weber var. azul*) y otros jarabes naturales. (Comparative analysis between blue agave syrup (*Agave tequilana Weber var. azul*) and other natural syrups.) Agrociencia*, 47,* 2013, pp. 233–244. ISSN: 1405-3195. In Spanish.
9. Urías-Silvas, J. E. – Cani, P. D. – Delmée, E. – Neyrinck, A. – López, M. G. – Delzenne, N. M.: Physiological effects of dietary fructans extracted from *Agave tequilana Gto* and *Dasylirion spp*. British Journal of Nutrition, *99*, 2008, pp. 254–261. DOI: 10.1017/S0007114507795338.
10. Santiago-García, P. A. – López, M. G.: Agavins from *Agave angustifolia* and *Agave potatorum* affect food intake, body weight gain and satiety-related hormones (GLP-1 and ghrelin) in mice. Food and Function, *5,* 2014, pp. 3311–3319. DOI: 10.1039/c4fo00561a.
11. Rendón-Huerta, J. A. – Juárez-Flores, B. I. – Pinos-Rodríguez, J. M. – Aguirre-Rivera, J. R. – Delgado-Portales, R. E.: Effects of different kind of fructans on in vitro growth of *Lactobacillus acidophilus, Lactobacillus casei and Bifidobacterium lactis.* African Journal of Microbiology Research, *5*, 2011, pp. 2706–2710. DOI: 10.5897/AJMR11.455.
12. Iñiguez-Covarrubias, G. – Lange, S. E. – Rowell, R. M.: Utilization of by products from the tequila industry: part 1: Agave bagasse as a raw material for animal feeding and fiberboard production. Bioresource Technology, *77*, 2001, pp. 25–32. DOI: 10.1016/S0960-8524(00)00137-1.
13. Rendón-Huerta, J. A. – Juárez-Flores, B. – Pinos-Rodríguez, J. M. – Aguirre-Rivera, J. R. – Delgado-Portales, R. E.: Effects of different sources of fructans on body weight, blood metabolites and faecal bacteria in normal and obese non-diabetic and diabetic rats. Plant Food for Human Nutrition, *67*, 2012, pp. 64–70. DOI: 10.1007/s11130-011-0266-9.
14. Moyano, G. – Sáyago-Ayerdi, S. G. – Largo, C. – Caz, V. – Santamaria, M. – Tabernero, M.: Potential use of dietary fibre from *Hibiscus sabdariffa* and *Agave tequilana* in obesity management. Journal of Functional Foods, *21*, 2016, pp. 1–9. DOI: 10.1016/j.jff.2015.11.011.
15. Horwitz, W. – Latimer, G. (Ed.): Official methods of analysis of AOAC International. 18th edition. Gaithersburg : AOAC International, 2005. ISBN: 0-935584-77-3.
16. Mañas, E. – Saura-Calixto, F.: Dietary fibre analysis: methodological error sources. European Journal of Clinical Nutrition, *49*, 1995, Supplement 3, pp. S158–S162. ISSN: 0954-3007.
17. Ortiz-Basurto, R.I. – Pourcelly, G. – Doco, T. – Williams, P. – Dornier, M. – Belleville, M.P.: Analysis of the main components of the aguamiel produced by the maguey-pulquero (*Agave mapisaga*) throughout the harvest period. Journal of Agricultural and Food Chemistry, *56*, 2008, pp. 3682–3687. DOI: 10.1021/jf072767h.
18. DuBois, M. – Gilles, K. A. – Hamilton, J. K. – Rebers, P. – Smith, F.: Colorimetric method for determination of sugars and related substances. Analytical Chemistry, *28*, 1956, pp. 350–356. DOI: 10.1021/ac60111a017.
19. Suntornsuk, L. – Gritsanapun, W. – Nilkamhank, S. – Paochom, A.: Quantitation of vitamin C content in herbal juice using direct titration. Journal of Pharmaceutical and Biomedical Analysis, *28*, 2002, pp. 849–855. DOI: 10.1016/S0731-7085(01)00661-6.
20. Cano, M. P. – De Ancos, B.: Carotenoid and carotenoid ester composition in mango fruit as influenced by processing method. Journal of Agricultural and Food Chemistry, *42*, 1994, pp. 2737–2742. DOI: 10.1021/jf00048a017.
21. Pérez-Jiménez, J. – Arranz, S. – Tabernero, M. – Díaz-Rubio, M. E. – Serrano, J. – Goñi, I. – Saura-Calixto, F.: Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. Food Research International, *4*, 2008, pp. 274–285. DOI: 10.1016/j.foodres.2007.12.004.
22. Montreau, F.: Sur le dosage des composés phénoliques totaux dans les vins par la methode Folin-Ciocalteau. (On the determination of total phenolic compounds in wines by the Folin-Ciocalteau method.) Connaissance de la vigne et du vin, *24*, 1972, pp. 397–404. ISSN 0010-597X. In French.
23. Alvarez-Parrilla, E. – De La Rosa, L. A. – Armarowics, R. – Shahidi, F.: Antioxidant activity of fresh and processed Jalapeño and Serrano peppers. Journal of Agricultural and Food Chemistry, *59*, 2011, pp. 163–173. DOI: 10.1021/jf103434u.
24. Farinha, L. L. – Sabo, S. S. – Porto, M. C. – Souza, E. C. – Oliveira, M. N. – Oliveira, R. P. S.: Influence of prebiotic ingredients on the growth kinetics and bacteriocin production of *Lactococcus lactis*. Chemical Engineering Transactions, *43,* 2015, pp. 313–318. DOI: 10.3303/CET1543053.
25. Downes, F. P. – Ito, K. (Eds.): Compendium of methods for the microbiological examination of foods. 4th ed. Washington, D. C. : American Public Health Association, 2001. ISBN-13: 978-0875531755.
26. O'Mahony, M. Sensory evaluation of food – Statistical methods and procedures. New York : Marcel Dekker, 1986. ISBN: 0-8247-7337-3.
27. Fennema, O. R. (Ed.): Food chemistry. 3rd ed. New York : Marcel Dekker, 1996. ISBN: 0824793463.
28. Chávez-Tapia, A. M. – Sáyago-Ayerdi, S. G. – García-Galindo, H. S. – García-Magaña, M. L. – González-Aguilar, G. A. – Montalvo-González, E.: Quality and stability of concentrated guava puree added with *Hibiscus sabdariffa* extract. Journal of Food and Nutrition Research, *55*, 2016, pp. 131–140. ISSN: 1336-8672 (print), 1338-4260 (online). <http://www.

vup.sk/download.php?bulID=1882>
29. Ioannou, I. – Hafsa, I. – Hamdi, S. – Charbonnel, C. – Ghoul, M.: Review of the effects of food processing and formulation on flavonol and anthocyanin behaviour. Journal of Food Engineering, *111*, 2012, pp. 208–217. DOI: 10.1016/j.jfoodeng.2012.02.006.
30. Moreno-Vilet, L. – Camacho-Ruiz, R. M. – Portales-Perez, D. P.: Chapter 11 – Prebiotic agave fructans and immune aspects. In: Ross-Waston, R. – Preddy, V. (Eds.): Probiotics, prebiotics, and symbiotics – Bioactive foods in health promotion. London : Academic Press, 2016, pp. 165–179. ISBN: 9780128021897. DOI: 10.1016/B978-0-12-802189-7.00011-3.
31. Mellado-Mojica, E. – López, M.G.: Identification, classification, and discrimination of agave syrups from natural sweeteners by infrared spectroscopy and HPAEC-PAD. Food Chemistry, *167,* 2015, pp. 349–357. DOI: 10.1016/j.foodchem.2014.06.111.
32. Lunn, J. – Buttriss, J. L.: Carbohydrates and dietary fibre. Nutrition Bulletin, *32*, 2007, pp. 21–64. DOI: 10.1111/j.1467-3010.2007.00616.x.
33. Jiménez-Escrig, A. – Rincón, M. – Pulido, R. – Saura-Calixto, F.: Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. Journal of Agricultural and Food Chemistry, *49*, 2001, pp. 5489–5493. DOI: 10.1021/jf010147p.
34. Glibowski, P. – Bukowska, A.: The effect of pH, temperature and heating time on inulin chemical stability. Acta Scientiarum Pollonorum Technologia Alimentaria, *10,* 2011, pp. 189–196. ISSN: 1644-0730 (print), 1889-9594 (online). <http://www.food.actapol.net/pub/5_2_2011.pdf>
35. Louarme, L. – Billaud, C.: Evaluation of ascorbic acid and sugar degradation products during fruit dessert processing under conventional or ohmic heating treatment. LWT – Food Science and Technology, *49*, 2012, pp. 184–187. DOI: 10.1016/j.lwt.2011.12.035.
36. Carbonell-Capella, J. M. – Barba, F. J. – Esteve, M. J. – Frígola, A.: High pressure processing of fruit juice mixture sweetened with *Stevia rebaudiana* Bertoni: Optimal retention of physical and nutritional quality. Innovative Food Science and Emerging Technologies, *18*, 2013, pp. 48–56. DOI: 10.1016/j.ifset.2013.01.011.
37. Cavalcanti, R. N. – Santos, D. T. – Meireles, M. A. A.: Non-thermal stabilization mechanisms of anthocyanins in model and food systems. An overview. Food Research International, *44*, 2011, pp. 499–509. DOI: 10.1016/j.foodres.2010.12.007.
38. Molino, S. – Dossena, M. – Buonocore, D. – Ferrari, F. –Venturini, L. –Ricevuti, G. – Verri, M.: Polyphenols in dementia: From molecular basis to clinical trials. Life Sciences, *161*, 2016, pp. 69–77. DOI: 10.1016/j.lfs.2016.07.021.
39. Nora, C. D. – Müller, C. D. R. – De Bona, G. S. – Rios, A. D. O. – Hertz, P. F. – Jablonski, A. – De Jong, E. V. – Flóres, S. H.: Effect of processing on the stability of bioactive compounds from red guava (*Psidium cattleyanum* Sabine) and guabiju (*Myrcianthes pungens*). Journal of Food Composition and Analysis, *34,* 2014, pp. 18–25. DOI: 10.1016/j.jfca.2014.01.006.
40. Velázquez-Martínez, J. R. – González-Cervantes, R. M. – Hernández-Gallegos, M. A. – Mendiola, R. C. – Aparicio, A. R. J. – Ocampo, M. L. A.: Prebiotic potential of *Agave angustifolia* haw fructans with different degrees of polymerization. Molecules, *19,* 2014, pp. 12660–12675. DOI: 10.3390/molecules190812660.
41. Biedrzycka, E. – Bielecka, M.: Prebiotic effectiveness of fructans of different degrees of polymerization. Trends in Food Science and Technology, *15*, 2004, pp. 170–175, DOI: 10.1016/j.tifs.2003.09.014.
42. Pompei, A. – Cordisco, L. – Raimondi, S. – Amaretti, A. – Pagnoni, U.M. – Matteuzzi, D. – Rossi, M.: *In vitro* comparison of the prebiotic effects of two inulin-type fructans. Anaerobe, *14*, 2008, pp. 280–286. DOI: 10.1016/j.anaerobe.2008.07.002.
43. Arrizon, J. – Hernández-Moedano, A. – Toksoy Oner, E. – González-Avila, M.: *In vitro* prebiotic activity of fructans with different fructosyl linkage for symbiotics elaboration. International Journal of Probiotics and Prebiotics, *9*, 2014, pp. 69–76, ISSN: 1555-1431.
44. Saad, N. – Delattre, C. – Urdaci, M. – Schmitter, J. M. – Bressollier, P.: An overview of the last advances in probiotic and prebiotic field. LWT – Food Science and Technology, *50*, 2013, pp. 1–16. DOI: 10.1016/j.lwt.2012.05.014.

Received 6 October 2016; 1st revised 22 February 2017; 2nd revised 4 June 2017; accepted 13 June 2017; published online 9 September 2017.