



Enhancement of health-promoting compounds of pineapple (*Ananas comosus* L.) by impregnation with LAB-fermented beetroot (*Beta vulgaris* L.) powder

Margarita García-Gómez¹ · Paola Hernández-Carranza¹ · Carolina Ramírez-López² · Karen H. Estévez-Sánchez³ · Irving I. Ruiz-López⁴ · Carlos E. Ochoa-Velasco¹

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Abstract

This study aimed to develop pineapple wedges added with health-promoting compounds (antioxidants, prebiotics, and probiotics) and evaluate their stability during storage. Fresh pineapple wedges were first predried (6 h at 60 °C), impregnated (1 min at 20 °C) with fermented solutions (*Lactobacillus acidophilus*) formulated with whey, inulin, and/or beetroot powder, and then thoroughly dried (40 °C for 1 h) to be stored at 20 ± 2 °C for 30 days. The physicochemical, total betalains (TB), total phenolic compounds (TPC), antioxidant capacity (AC), probiotic survival, and sensory acceptance of pineapple were evaluated at the beginning and end of the storage. Overall, storage time did not affect the physicochemical characteristics of pineapples (except titratable acidity). On the other hand, TB were incorporated into pineapples impregnated with fermented solutions containing beetroot, showing values of 31.69 to 31.86 mg/100 g. Adding inulin significantly enhanced the TPC in pineapples (12.7–26.2%, $p < 0.05$) compared with control pineapple. Therefore, pineapples with beetroot powder and inulin showed the highest AC (30.87–31.69 mg Trolox/g) after storage. By contrast, the probiotic survival remained up to 10⁶ colony-forming units (CFU)/g for 15 days of storage. However, after 30 days, the microbial load decreased to < 10² CFU/g. Sensory evaluation indicated that dried pineapples were not affected by the storage time and impregnation systems displaying values of 5–6 (I like it - I like it moderately). In conclusion, pineapple wedges impregnated with fermented solution based on whey, inulin, and beetroot showed the highest AC and an appropriate probiotic count to exert its beneficial effects for 15 days.

Keywords Antioxidant capacity · Betalains · Inulin · Drying process · Probiotics

Introduction

The pineapple (*Ananas comosus* L.), the king of fruits, is a tropical fruit from the Bromeliaceae family. It is appreciated for its sensory properties (juiciness and pleasant flavor and taste), being the third most consumed fruit after bananas and citrus fruits [1]. Besides sensory acceptance, pineapple is a rich source of phenolic compounds, vitamins, carotenoids, fiber, glycan, and minerals such as potassium, copper, and calcium [2]. These compounds give pineapple health-promoting properties such as anti-inflammatory, antioxidant, antimicrobial, anti-thrombotic, anti-edematous, and fibrinolytic activities [2, 3]. Pineapple is consumed not only in a fresh way but also canned, dried, jam, yogurt, juices, and candies, as well as fermented and non-fermented beverages, which

✉ Carlos E. Ochoa-Velasco
carlos.ochoav@correo.buap.mx

¹ Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla, Av. San Claudio y 18 Sur. Ciudad Universitaria. C.P., Puebla 72570, Puebla, México

² Instituto Politécnico Nacional, Centro de Investigación en Biotecnología Aplicada, Santa Ines Tecuexcomac-Tepetitla, km 1.5, Tepetitla de Lardizabal C.P., Tlaxcala 90700, México

³ Facultad de Ciencias de la Electrónica, Benemérita Universidad Autónoma de Puebla, Av. San Claudio y 18 Sur. Ciudad Universitaria. C.P., Puebla 72570, Puebla, México

⁴ Facultad de Ingeniería Química, Benemérita Universidad Autónoma de Puebla, Av. San Claudio y 18 Sur. Ciudad Universitaria. C.P., Puebla 72570, Puebla, México

increases its production but also its postharvest losses caused by its intrinsic properties (moisture and nutrients) and the lack of postharvest technologies [1, 4]. Drying is one of the most ancient methods for food preservation; nevertheless, it is still widely used for preserving agricultural products because it presents an interesting way to develop new products for commercial, economic, and sensory diversification [5].

Beetroot (*Beta vulgaris* L.) is a plant of the Chenopodiaceae family. It is highly appreciated by its different bioactive compounds such as betalains (red-purple betacyanins and yellow-orange betaxanthins), phenolic compounds (phenolic acids and flavonoids), carotenoids, vitamins, nitrates, and minerals, which confer several bioactivities such as antioxidants, anti-inflammatory, antihyperglycemic, antiviral, antimicrobial activities, among others [6, 7]. Interestingly, beetroot can act as a prebiotic due to its content of soluble and insoluble fibers and its total phenolic compounds. In this aspect, de Oliveira [8] pointed out that freeze-dried red beetroot increases the amount of *Lactobacillus* spp., *Enterococcus* spp., and *Bifidobacterium* spp. in the human bowel, showing a high positive prebiotic index (> 3.61) during colonic fermentation. In the same way, del Amo-Mateos [9] indicated that *L. rhamnosus* and *Bifidobacteria longum* grew on pectologosaccharides and pectin obtained from discarded red beetroot.

Probiotics are living microorganisms that have been demonstrated to improve human health by enhancing the gut microbiota [10]. Regular intake of adequate amounts of probiotics ($> 10^9$ CFU/mL or g) has been associated with reducing some gastrointestinal disorders and diseases, non-alcoholic fatty liver disease, obesity, type 2 diabetes, some types of cancer, and maintaining adequate skin, oral, and brain health [11, 12]. The action of probiotics is related to improving the host defense system, acting as antimicrobials, and producing metabolites with important functions [13]. Furthermore, when probiotic consumption is combined with prebiotics (substrates selectively utilized by host microorganisms), the health benefits obtained by the host increase since both act in a synergistic way, a beneficial interaction called synbiotics [14]. Additionally, although phenolic compounds have been known as antioxidant compounds, current research has indicated that they can act as prebiotics, modulating the composition of the intestinal microbiota in the colon [15].

Historically, probiotics have been found in dairy products; however, their incorporation into other food matrices such as fruits, fruit juices, desserts, peanut butter, mayonnaise, bakery products, and meat products is increasing [16, 17]. In this aspect, although probiotic inclusion in

fruits and the evaluation of their stability during storage at low temperatures has been studied in apples, strawberries, bananas, apricots, blueberries, and melons [18–20], their incorporation into pineapple alone or accompanying with prebiotics and antioxidants from beetroot have not been studied. Therefore, this study aimed to develop dried pineapple wedges impregnated with fermented solution (*L. acidophilus*) formulated with prebiotics (inulin) and antioxidant compounds (beetroot) and evaluate their stability during storage at room temperature.

Materials and methods

Vegetal material

Pineapples (*Ananas comosus*) were acquired from a local supermarket in Puebla, Mexico. The fruits were selected free from physical and microbiological apparent damage, with a full golden color (6-color scale according to the USDA color chart). They were then washed and disinfected with sodium hypochlorite solution (150 ppm), manually peeled using a stainless-steel knife, and cut into wedges (Size = $1 \times 4 \times 2$ cm; Weight = 3.59 ± 0.39 g) for further studies. Pure beetroot powder and organic inulin (blue agave) were obtained from Healthy Super Foods (Puebla, Puebla, Mexico) and Hacienda ORO de Agave (Guadalajara, Jalisco, Mexico), respectively. Whey powder (10% protein, 75% carbohydrates, 1.5% fat, and 1.15% sodium) was acquired from Food Technologies Trading (Ciudad de Mexico, Mexico).

Microorganisms and the fermentation process

Lactic acid bacteria (LAB) used in this study (*Lactobacillus rhamnosus* NRRL B-442, *Lactobacillus casei* NRRL B-1922, and *Lactobacillus acidophilus* NRRL B-4495) were obtained from the microbiological collection of the Facultad de Ciencias Químicas from the Benemérita Universidad Autónoma de Puebla were grown in de Man, Rogosa and Sharpe (MRS) broth (Merck, Darmstadt, Germany) for 24 h at 37 ± 2 °C. Then, 1 mL of each culture was individually inoculated in a sterilized (120 ± 2 °C for 15 min) solution of whey, inulin, and/or beetroot powder (Table 1) and grown for 24 h at 37 ± 2 °C. LAB-fermented solutions were used to impregnate pineapple wedges.

Impregnation and drying processes

The impregnation and drying processes of the pineapple wedges were conducted in three steps. Pineapple wedges

Table 1 Formulation of LAB-fermented solutions used for pineapple impregnation

Formulation	Whey (%)	Inulin (%)	Beetroot (%)	Water (%)
1	--	--	--	100
2	2	1	1	96
3	2	1	--	97
4	2	--	1	97
5	2	--	--	98

were first dried at 60 ± 3 °C for 6 h using a food dehydrator (Excalibur, model 3926 TB, California, USA). Trays were rotated 180° every 30 min along their longitudinal axis to ensure uniformity in the drying process. Afterward, the fruits were removed from the dehydrator and impregnated for 1 min (a time chosen in preliminary assays) with a selected LAB-fermented solution. A 1:15 solid-to-solvent ratio was used to avoid dilution of the impregnation medium [21]. Finally dried again at 40 ± 3 °C for 1 h. Dried fruits were stored in low-density polyethylene bags at room temperature (20 ± 4 °C) in a dark environment for analysis at the beginning and after 30 days of storage (Fig. 1).

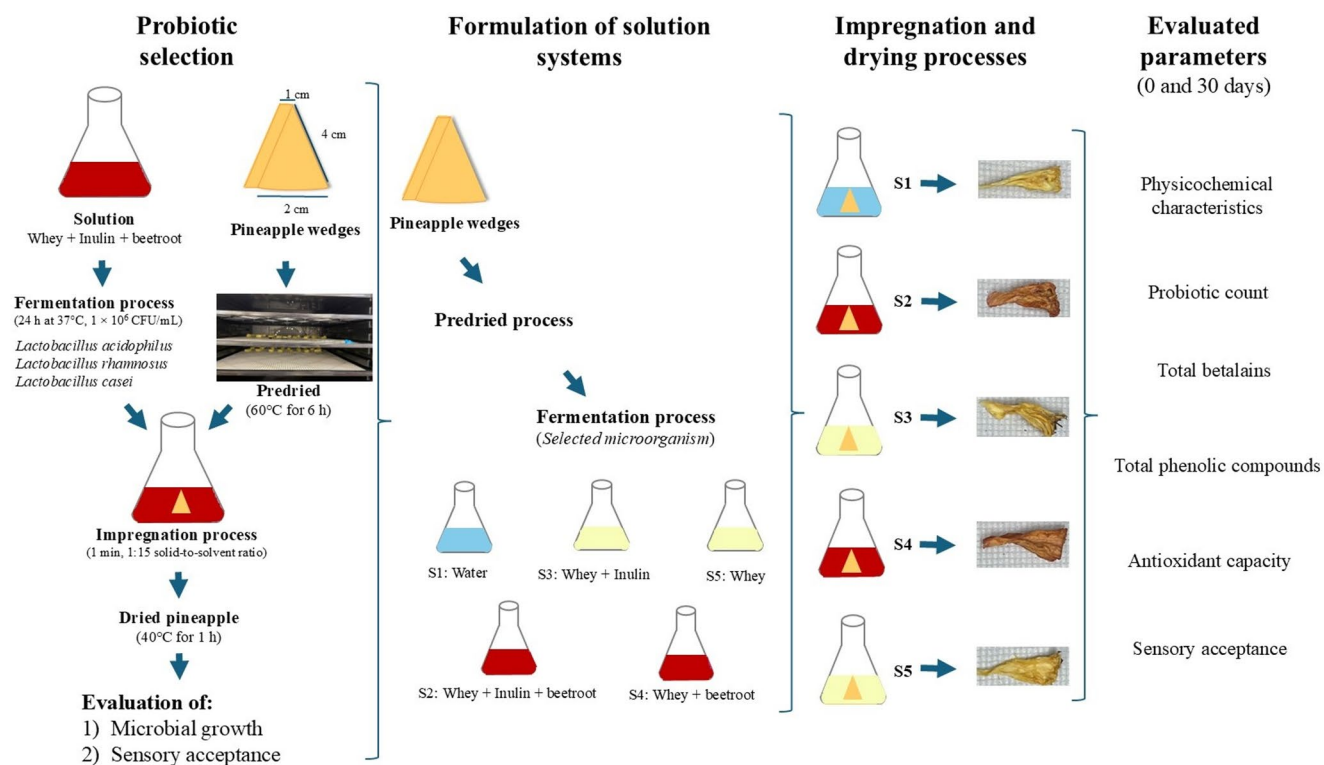
Selection of probiotic microorganisms

The selection among the three probiotic microorganisms to study the storage life of pineapple wedges with health-promoting compounds was carried out as follows:

fruits were predried (60 ± 3 °C for 6 h), impregnated with LAB-fermented solution composed of whey (2%), inulin (1%), and beetroot powder (1%), and thoroughly dried at 40 ± 3 °C for 1 h. Dried pineapple wedges were analyzed through probiotic survival and sensory acceptance (7-point hedonic scale), following the methodology proposed below.

Chemical-proximal analysis of beetroot powder and fresh pineapple wedges

The chemical composition of beetroot powder was analyzed using the Association of Official Analytical Chemists (AOAC) methods [22]. The moisture content was determined by oven-drying at 105 ± 2 °C (FE-292 model, FELISA, Zapopan, Jalisco, Mexico) until a constant weight was achieved (approximately 4 h). Ash content was determined by incineration at 525 ± 10 °C for 5 h. The fat content was determined using Soxhlet equipment (DEK-1, Prendo, Puebla, Puebla, Mexico) with hexane as solvent. Protein content was determined using the Kjeldahl method. Carbohydrates content was calculated by difference. Pineapple was only evaluated for moisture content as information about its chemical composition is found elsewhere.

**Fig. 1** Schematic representation of the methodology conducted in this study

Color of beetroot powder and pineapple wedges

The color parameters (L^* , a^* , and b^*) of beetroot powder and fresh and dried pineapple wedges were measured using a colorimeter Reader (TCR 200 model, TIME High Technology, Beijing, China). The color measurement was taken on the surface of pineapple wedges or a Petri dish containing beetroot powder. *Hue*, *Chroma*, and total color change (ΔE) were calculated as follows:

$$Hue = \tan^{-1} \left(\frac{b^*}{a^*} \right) \quad (1)$$

$$Chroma = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (3)$$

where L_0^* , a_0^* , and b_0^* and L^* , a^* , and b^* are the color parameters at the beginning and at the end of storage, respectively.

Physicochemical characteristics of dried pineapple wedges

Pineapple wedges (5 g) were homogenized with 50 mL of distilled water for their extraction procedure. The extract was cotton-filtered and used to evaluate the physicochemical characteristics of dried pineapple wedges. The pH was measured with a pH meter (pH 10 model, Conductronic, Puebla, Puebla, Mexico), following the 981.12 methodology [22]. The total soluble solids (TSS, °Brix) were evaluated with a digital refractometer (95200-08/01 model, Alla France, Maine and Loira, France), following the 932.12 methodology [22]. The titratable acidity (TA) was measured by titration with NaOH solution (0.1 N) and reported as a percentage of citric acid.

Extraction procedure for antioxidant quantification of beetroot powder and fresh and dried pineapple wedges

Beetroot extract was obtained by mixing one g of beetroot powder with 50 mL of distilled water. The extraction was conducted for 1 h (20 ± 2 °C) using a magnetic stirrer hot plate (SP-131325 model, Cimarec, USA) at 360 rpm. After this time, the extract was cotton-filtered and used for antioxidant quantification. On the other hand, pineapple extract, either fresh or dried, was obtained as previously described.

Total phenolic compounds (TPC)

The TPC were evaluated according to the methodology proposed by Aparicio-Fernández et al. [23] with some modifications. In brief, 1 mL of extract was combined with 1 mL of 0.1 N Folin-Ciocalteu reagent (Sigma Aldrich, CAS No. 12111-13-6, Toluca, Estado de México, Mexico), followed by adding 1 mL of Na_2CO_3 (Sigma Aldrich, CAS No. 497-19-8, Toluca, Estado de México, Mexico) solution (0.5% w/v) after 3 min. The mixture was allowed to stand for 30 min at room temperature, and the absorbance was read at 765 nm using a UV-Vis spectrophotometer (6405 model, Jenway, Essex, UK). Gallic acid (Sigma Aldrich, CAS No. 5995-86-8, Toluca, Estado de Mexico, Mexico) was used as standard, and the TPC were reported as mg gallic acid equivalents (GAE) per g of sample dry basis.

Total betalains (TB)

TB were reported as the sum of betacyanins and betaxanthins following the methodology proposed by Aparicio-Fernández et al. [23]. In brief, betacyanins and betaxanthins were determined at 535 and 483 nm using McIlvaine buffer as solvent. For betacyanins, a molecular weight of 550 g/mol and a molar extinction coefficient of 60,000 L/mol cm were used, while for betaxanthins, a molecular weight and a molar extinction coefficient of 308 g/mol and 48,000 L/mol cm were respectively used. The quantification of betalains was conducted using Eq. 4:

$$B = \frac{A \times DF \times MW \times 1000}{\epsilon \times l} \quad (4)$$

where B is the content of total betalains (mg/g), A represents the absorbance, DF is the factor of dilution, MW is molecular weight (g/mol), and ϵ and l represent the molar extinction coefficient and the cell path (1 cm), respectively.

Antioxidant capacity (AC)

The AC of beetroot and pineapple extracts was determined by the DPPH (2,2-difenil-1-picrilhidrazilo, Sigma Aldrich, CAS No. 1898-66-4, Toluca, Estado de Mexico, Mexico) radical scavenging activity, according to the methodology proposed by Hernández-Carranza et al. [24]. One mL of extract combined with 1 mL of DPPH radical (0.004%) was allowed to stand for 30 min in a dark environment at room temperature. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer, and

the AC was quantified using a standard curve of Trolox (Sigma Aldrich, CAS No. 53188-07-1, Toluca, Estado de Mexico, Mexico).

Probiotic survival

Probiotic survival was evaluated in the initial fermented solutions and the dried pineapple wedges at the beginning and for 30 days of storage at 20 ± 3 °C. One g of fermented solution or dried pineapple wedges was placed with 9 mL of peptone water and processed using stomacher equipment (model 400 C, Seward, England) for 5 min. Then, 1 mL of the sample was serially diluted in peptone water until 30–300 CFU were counted. The sample was plated in MRS agar and stored in an incubator (INO-650 M model, Prendo, Puebla, Puebla, Mexico) at 37 ± 2 °C for 24 h under anaerobic conditions.

Sensory acceptance

Dried pineapple wedges were sensory evaluated by 50 untrained judges (students of Benemerita Universidad Autonoma de Puebla, aged 20–24 years old, with 32 women and 18 men) who frequently consumed pineapples and beetroot (1–2 times a week). The judges were informed about the sensory test to ensure they fully understood the proposal of the study. They were informed about the ingredients in the samples, and it was made clear that their personal information (name, gender, and age) would be kept confidential and used solely for this assessment. The judges have the freedom to accept or decline participation in the sensory evaluation. The sensory test was conducted using a 7-point hedonic scale, where 1 represents “I dislike it very much,” 2 means “I dislike it moderately,” 3 indicates “I dislike it,” 4 stands for “Neither like it nor dislike it,” 5 signifies “I like it,” 6 means “I like it moderately,” and 7 represents “I like it very much.” Each judge received one wedge of treated pineapple to evaluate its odor, color, flavor, and overall acceptance. They were asked to give a rating between 1 and 7 on the hedonic scale. The sensory evaluation was carried out to select the probiotic microorganisms for further assessment of impregnation systems, and to evaluate the acceptance of dried pineapple wedges at the beginning and after 30 days of storage at room temperature.

Statistical analysis

All the experiments were conducted two times, and each analysis was done in triplicate. The results were analyzed using a comparison of means ($\alpha=0.05$) through analysis

of variance (ANOVA) using the Tukey's test in Minitab 15 software (Minitab Inc., State College, USA).

Results and discussion

Selection of the probiotic used for improving the functional quality of dried pineapples

The process of selecting the probiotics to be impregnated in pineapple was based on criteria of higher microbial growth and sensory acceptance (see Fig. 2). After the impregnation, all probiotics presented a high count ($6\text{--}8 \times 10^9$ CFU/100 g of dried pineapple wedges). Therefore, impregnated dried pineapple wedges can be considered a food with a high source of probiotic microorganisms according to the following scale: low $< 10^4$ CFUs/g, medium $10^4\text{--}10^7$ CFUs/g, and high $> 10^7$ CFUs/g [10]. However, among the microorganisms evaluated, *L. acidophilus* showed the highest microbial load ($p < 0.05$). In this respect, studies conducted by Zhang et al. [25] and Meng et al. [26] using *L. acidophilus* and *L. casei* have pointed out that *L. acidophilus* maintained a higher microbial load (> 8.5 log cycles) even at low pH (4.1–4.2); while the cell viability of *L. casei* was significantly reduced from 9 to 8 logs when pH was decreased to 4.2. On the other hand, in a study conducted by Chamberlain et al. [27] where *L. acidophilus* and *L. rhamnosus* were grown in glucose (1%) media with and without pomegranate extract. After 24-h culture, *L. acidophilus* showed higher growth in those media with pomegranate extract, while *L. rhamnosus* showed no significant effect by the media used in its culture. Therefore, it is possible to indicate that *L. acidophilus* can grow in various media at low pH and using different nutrient sources, such as those in pineapple wedges.

On the other hand, the sensory acceptance of dried pineapple wedges added with probiotics indicated that consumers perceptions were not affected by the microorganisms inoculated ($p > 0.05$), showing values in the range of 4.9–5.6 (I like it-I like it much) on a 7-point hedonic scale for all sensory parameters evaluated. Similar results (6.3–7.8 on 9-point hedonic scale) were obtained by Muyonga et al. [28] with pineapple slices dried using a refractance window drier and solar dried. Therefore, according to the results obtained, *L. acidophilus* was used to evaluate the effect of fermented solution and storage time on pineapple wedges.

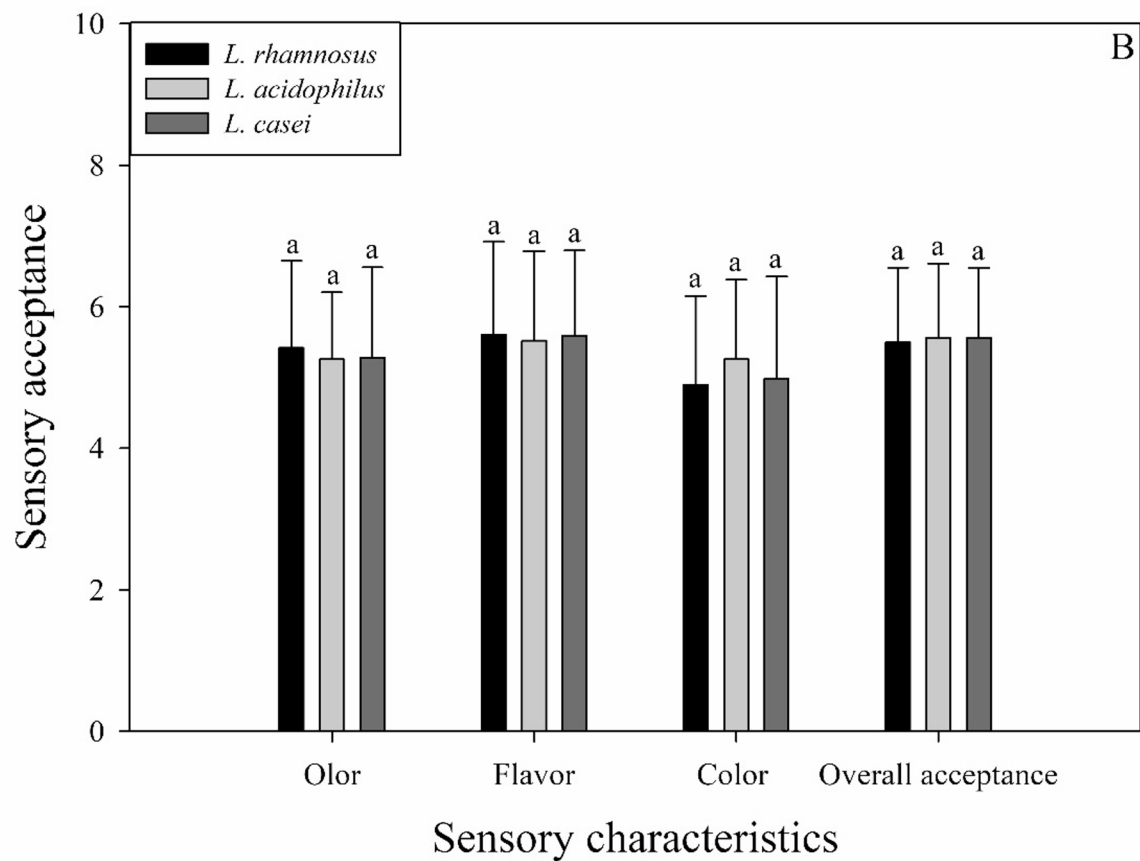
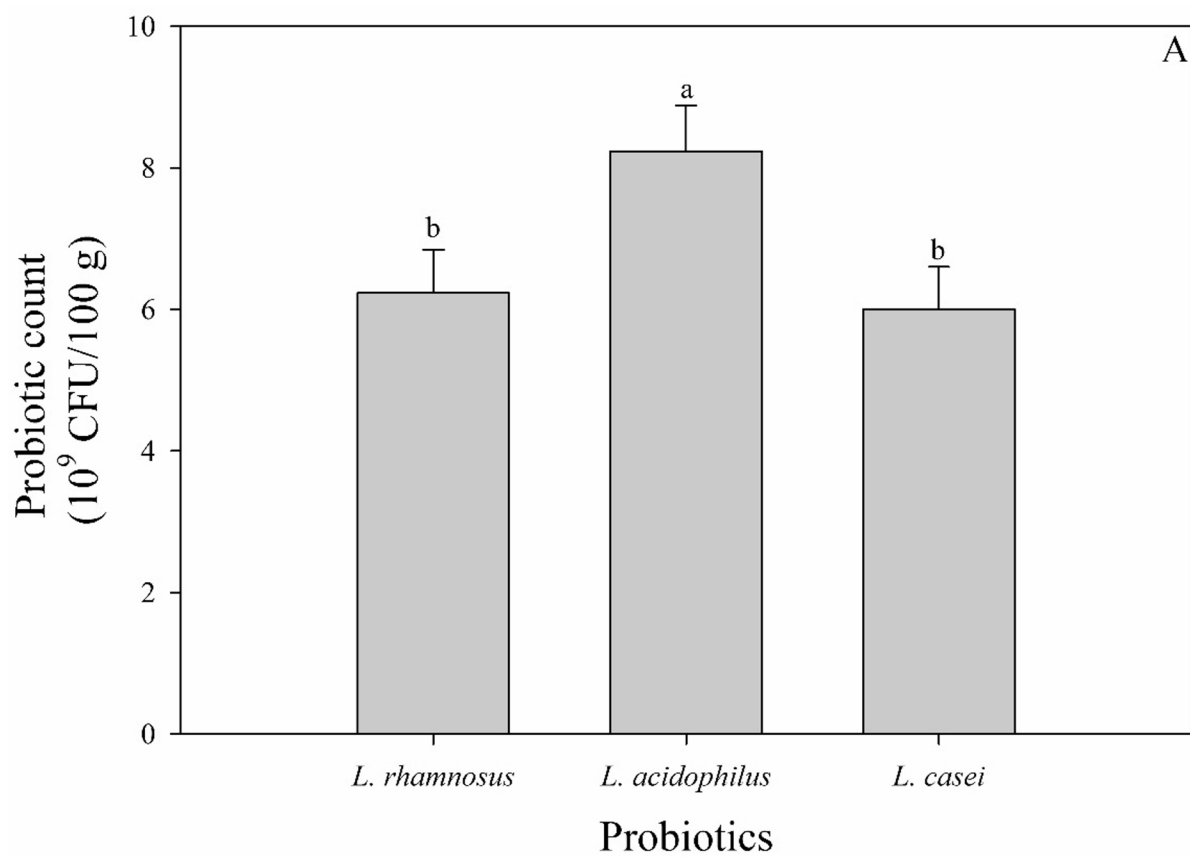


Fig. 2 Probiotic count (A) and sensory evaluation (B) of impregnated dried pineapple added with *L. rhamnosus*, *L. acidophilus*, and *L. casei*. Different letters a and b indicate significant differences ($p < 0.05$). Tin black bars indicate the standard deviation

Chemical-proximal, physicochemical, and antioxidant compounds of beetroot powder and fresh pineapple

Table 2 shows the proximal analysis and antioxidant compounds of beetroot powder and fresh pineapple. As observed, the moisture content of beetroot powder was below 5%, which is necessary in powder products to maintain adequate stability. On the other hand, pineapple presented a high moisture content ($89.63 \pm 0.20\%$) due to its fresh nature. The proximate analysis of beetroot powder was like that obtained by Mitrevski et al. [29], indicating moisture content, ash, protein, and total carbohydrates with values of 6.80 ± 0.18 , 3.80 ± 0.18 , 11.40 ± 0.20 , and $77.41 \pm 0.60\%$, respectively, for dried beetroot. The beetroot's nature determines the color parameters of the vegetal materials. In this sense, the color parameters of beetroot powder were 47.02 ± 1.83 , 24.10 ± 3.41 , and -3.04 ± 3.04 , represented by a shade of pink (#946075 of the hexadecimal color code), which is corroborated by the *Hue* angle (352.21) and *Chroma* (24.45) values. In the same way, pineapple color parameters ($L^* = 68.82 \pm 3.24$, $a^* = -3.78 \pm 1.14$, and $b^* = 45.02 \pm 0.90$) indicated a shade of yellow color (#bba854 of the hexadecimal color code).

The TPC and AC of beetroot were significantly higher (131.94 ± 0.13 mg GAE/g and 144.22 ± 5.00 mg Trolox/g, respectively) than those obtained in pineapple (26.53 ± 1.60 mg GAE/g and 27.57 ± 0.88 mg Trolox/g, respectively), which is of utmost importance since antioxidant compounds from beetroot were impregnated into the pineapple wedges. The content of TPC in beetroot was higher than those reported by Mitrevski et al. [29], who informed values of 45 mg GAE/g, while Ozaki et al. [30] indicated values of TPC of 2.92 mg GAE/g of beetroot powder; however, they indicated values of AC significantly higher (7.14 g Trolox/g) than those obtained in this study. On the other hand, the TPC and AC obtained in this study for pineapple were higher than those reported by Izli et al. [31]. They informed values of 4.67 mg GAE/g and 3.60 mg Trolox/g of pineapple. However, Huang et al. [32] indicated that TPC ranged from 11 to 15.5 mg GAE/g and EC_{50} (DPPH assay) from 3.4 to higher than 20 mg/mL for different varieties of pineapples. Moreover, beetroot powder used in this study presented adequate amounts of TB (58.22 ± 0.09 mg/g) distributed in 43.27 ± 0.09 mg/g and 14.96 ± 0.01 mg/g for betacyanins and betaxanthins, respectively. The values obtained in this study are slightly lower (154.2–306.5 mg

betanin/g and 67.52–159.4 mg vulgaxanthin/g) than those reported by Kerr and Varner [33]; who indicated that the drying method significantly affects the betalains beetroot content.

Effect of fermented solutions on quality parameters of dried pineapple during storage

The physicochemical characteristics of dried pineapple impregnated with fermented and non-fermented solutions during storage are presented in Table 3. As is observed, dried pineapple's moisture content was 22.16–26.72% without changes ($p > 0.05$) during the storage period. A low moisture content in dried fruits is required to avoid microbial growth and extend the shelf life of fruits and vegetables [34]. As part of the drying process, °Brix (%), pH, and TA also change from the initial values in fresh pineapple of 9.17 ± 0.64 (%), 4.10 ± 0.01 , and 2.35 ± 0.37 (% citric acid) to 42.52–50.97 (%), 3.80–4.03, and 2.86–3.63 (% citric acid), respectively, after drying process. In this aspect, the drying process concentrates the dry matter content, including organic acids that reduce the pH and increase the TA. After 30 days of storage, the TSS and TA increased due to the remaining enzymatic activity, such as pectin methyl esterase, polygalacturonase, amylase, and peroxidase, among others, which can still be active in dehydrated products [35].

The color parameters of dried pineapples are shown in Table 2. The drying process and the treatment significantly affected the pineapple color. Control pineapple (S1) and systems S3 and S5 displayed L^* , a^* , and b^* color parameters in the range of 56.91–67.47, 6.57–9.52,

Table 2 Chemical-proximal and antioxidant characteristics of beetroot powder and fresh pineapple.^a

Characteristics	Beetroot powder	Fresh pineapple
Moisture (%)	$4.37 \pm 0.39_b$	$89.63 \pm 0.20_a$
Ash (%)	7.79 ± 0.12	ND
Protein (%)	8.94 ± 0.30	ND
Fat (%)	0.29 ± 0.00	ND
Carbohydrates (%)	78.61	ND
L^*	$47.02 \pm 1.83_b$	$68.82 \pm 3.24_a$
a^*	$24.10 \pm 3.41_a$	$-3.78 \pm 1.14_b$
b^*	$-3.04 \pm 3.04_b$	$45.02 \pm 0.90_a$
<i>Hue</i>	$352.21 \pm 8.20_a$	$94.80 \pm 1.44_b$
<i>Chroma</i>	$24.45 \pm 2.99_b$	$45.18 \pm 0.91_a$
TPC (mg GAE/g)	$131.94 \pm 0.13_a$	$26.53 \pm 1.60_b$
AC (mg Trolox/g)	$144.22 \pm 5.00_a$	$27.57 \pm 0.88_b$
Betanin (mg/100 g)	43.27 ± 0.09	ND
Indicaxanthin (mg/100 g)	14.96 ± 0.01	ND
TB (mg/g)	58.22 ± 0.09	ND

^a Average $\pm$ standard deviation. Different letters a or b on the same line indicate statistical differences ($p < 0.05$). TPC: total phenolic compounds. AC: antioxidant capacity. TB: total betalains

Table 3 Physicochemical characteristics of impregnated pineapple wedges with LAB-fermented at the beginning and 30 days of storage.^a

Characteristics	S1		S2		S3		S4		S5	
	0	30 (d) ^b	0	30 (d)	0	30 (d)	0	30 (d)	0	30 (d)
Moisture (%)	23.36±1.57 _a	23.06±0.84 _a	22.16±0.39 _b	24.66±1.15 _a	22.79±0.72 _a	24.92±1.60 _a	26.72±2.70 _a	23.81±1.20 _a	23.20±1.90 _a	20.63±1.03 _a
TSS ^b	50.97±0.64 _b	54.98±1.55 _a	47.99±0.64 _b	57.89±0.64 _a	47.28±1.10 _b	54.80±0.84 _a	42.52±0.64 _b	45.45±0.64 _a	48.37±1.10 _b	58.23±0.00 _a
pH	3.92±0.01 _a	3.93±0.00 _a	3.80±0.10 _a	3.93±0.02 _a	3.86±0.06 _a	3.89±0.04 _a	4.03±0.01 _a	4.01±0.01 _a	3.98±0.02 _b	4.05±0.01 _a
TA ^c	2.86±0.07 _b	4.10±0.13 _a	3.46±0.26 _b	4.14±0.20 _a	3.63±0.07 _b	4.27±0.15 _a	2.86±0.07 _b	3.63±0.15 _a	3.03±0.07 _a	3.20±0.01 _a
L*	67.09±4.01 _a	59.73±4.13 _a	55.43±0.71 _a	50.00±6.33 _a	67.47±7.88 _a	57.90±3.70 _a	62.71±2.93 _a	54.89±5.21 _a	56.91±6.07 _a	53.50±1.85 _a
a*	7.50±1.97 _a	10.36±1.62 _a	19.90±4.68 _a	19.30±1.30 _a	6.57±5.71 _a	10.26±2.44 _a	15.37±1.11 _a	16.28±3.37 _a	9.52±1.29 _a	13.38±1.63 _a
b*	53.26±2.07 _a	44.62±4.63 _b	20.37±7.20 _a	28.20±5.87 _a	40.61±4.80 _a	27.81±1.20 _b	35.06±5.78 _a	32.64±4.39 _a	39.02±3.86 _a	39.87±3.90 _a
Hue	81.98±2.19 _a	76.73±3.40 _a	45.17±6.78 _a	55.01±7.77 _a	81.29±6.69 _a	69.77±4.76 _a	66.11±2.34 _a	63.18±7.62 _a	76.31±0.75 _a	71.26±3.92 _a
Chroma	53.81±2.01 _a	45.86±4.12 _b	28.61±7.91 _a	34.36±4.02 _a	41.34±5.59 _a	29.72±1.09 _b	38.30±5.70 _a	36.69±2.60 _a	40.16±4.04 _a	42.11±3.15 _a
ΔE	--	11.72±6.38 _{AB}	--	11.89±0.73 _{AB}	--	16.65±2.93 _A	--	9.94±3.38 _{AB}	--	6.19±2.16 _B

^aAverage ± standard deviation; ^bTotal soluble solids (°Brix); ^cTitrateable acidity (% citric acid); ^ddays. Different letters (a and b) within the same line for the same system are statistically different ($p < 0.05$). Different letters (A and B) for the same line are statistically different ($p < 0.05$).

and 39.02–53.26, respectively, with a shade of brown color, according to the Hex color code. Systems S2 and S4, which contain beetroot in their formulation, showed higher values of a^* (15.37–19.90) and lower values of b^* (20.37–35.06), giving them a shade of red-orange color, produced by the red color of beetroot (Table 2) and its capacity to act as a natural colorant [36]. These color characteristics are reflected in the Hue angle and Chroma, which are both lower in S2 and S4 systems, indicating a tendency to red color and less purity in their color, especially in system S2. In general, the color of pineapple is stable during storage without significant change ($p > 0.05$) in all parameters (except the b^* color parameter of S3). However, the total color change (ΔE) was calculated to evaluate the effect of storage on the color characteristics. The results showed that the pineapple wedges treated with a fermenting solution containing only whey had the lowest ($p < 0.05$) ΔE value (6.19 ± 2.16). On the other hand, the control pineapple (S1), system 2, and system S3 had the highest ΔE values. In this sense, inulin, which contains reduced sugar, makes the pineapple more susceptible to the Maillard reaction during storage [37].

Health-promoting compounds of dried pineapple wedges during storage

Figure 3 shows the pineapple wedges' TB, TPC, and AC after the impregnation process and 30 days of storage. It is observed that systems S2 and S4, which are impregnated with fermented solutions containing beetroot powder in their formulation, have TB (betacyanins 18.19–18.51 mg/100 g and betaxanthins 13.18–13.64 mg/100 g) in the range of 31.69–31.86 mg/100 g. Aguirre-García et al. [21] found values of approximately 20 mg/100 g and 12 mg/100 g of betacyanins and betaxanthins (TB = 32 mg/100 g) during the first minutes of apple wedges impregnated with beetroot juice. Betalains impregnation in pineapple wedges is paramount since they are an important source of antioxidant compounds with cardiovascular, anticancer, diabetes, neuroprotective, and anti-inflammatory, among other beneficial effects [38]. After 30 days of storage, TB were reduced ($p < 0.05$) in both S2 and S4. This reduction may be attributed to the light exposition during storage since pineapple wedges were stored using polyethylene bags. Interestingly, S2 presented the lowest TB reduction, which can be attributed to the presence of inulin, which improves the stability of betalains during storage [39]. In addition, oligosaccharides may serve as biostimulants to enhance plant resistance to various stress factors through several pathways, including the biosynthesis of betalains [39, 40].

Figure 3B shows the TPC of impregnated pineapple wedges. At the beginning of the storage system, S2 displayed the highest TPC (16.20 ± 0.27 mg GAE/g), whereas S1 and S5, corresponding to control pineapple and impregnated with fermented whey, showed lower TPC. This result indicated that both inulin and beetroot enhanced the TPC in pineapples. In addition, S2 also displayed the highest AC (28.40 ± 0.25 mg Trolox/g) of pineapple wedges ($p < 0.05$). In contrast, all other systems showed no significant differences ($p > 0.05$), demonstrating an average value of 3.28 mg Trolox/g. A strong correlation ($R^2 = 0.84$) between TPC and AC is observed, corroborating the impact of TPC on the AC of fruits and vegetables. As is seen in Table 2, beetroot contains a high amount of TPC and AC, which are impregnated in pineapple wedges. On the other hand, inulin can be metabolized by *L. acidophilus* during the fermentation process, producing metabolites derived from carbon metabolism (lactic, acetic, propionic, and butyric acids and exopolysaccharides) and derived from nitrogen metabolism (proteins, peptides, and amino acids), which can act as both reducing (affecting the TPC quantification) and antioxidant agents [41, 42].

After 30 days of storage, TPC and AC were significantly higher in pineapples S2 and S3, which contained both inulin in their formulation. During the storage of pineapple wedges, *L. acidophilus* is exposed to stress conditions (low moisture content, room temperature, and oxygen), having to induce adaptive responses and improve its resistance toward higher levels of oxidative stress, generating antioxidant compounds as a response [43]. Moreover, under drought conditions, some phytohormones trigger the production of antioxidant enzymes and osmoprotective agents, which may improve probiotic resistance [44]. Several studies have indicated that inulin can act as a nutrient source for lactic acid bacteria, protecting them during processing and storage [45, 46]. The survival of probiotics during storage is presented in Fig. 4. As observed, all formulated systems are adequate cultures for growing *L. acidophilus* because the microbial load was higher than 10^8 CFU/g. After the drying process, S2 and S3 demonstrated the highest microbial survival, which can be attributed to the protective characteristics of the inulin mentioned above [45]. After 15 days of storage, probiotic survival was significantly reduced; however, its amount ($> 10^7$ CFU) was up to those recommended to exert its benefit to human health when consuming 10 g of dried pineapple wedges [10]. The probiotic load in pineapple wedges was significantly reduced (1.0–1.4 logs) after 30 days of storage. It is worth noting that although the probiotic count is lower than the recommended dosage, beneficial effects can still be

obtained due to metabolites such as those quantified in antioxidant assays (postbiotics) and those obtained from dead probiotics cells, namely paraprobiotics, which have garnered attention due to their various effects, including intestinal and immunomodulatory, anticancer, and antimicrobial effects [47].

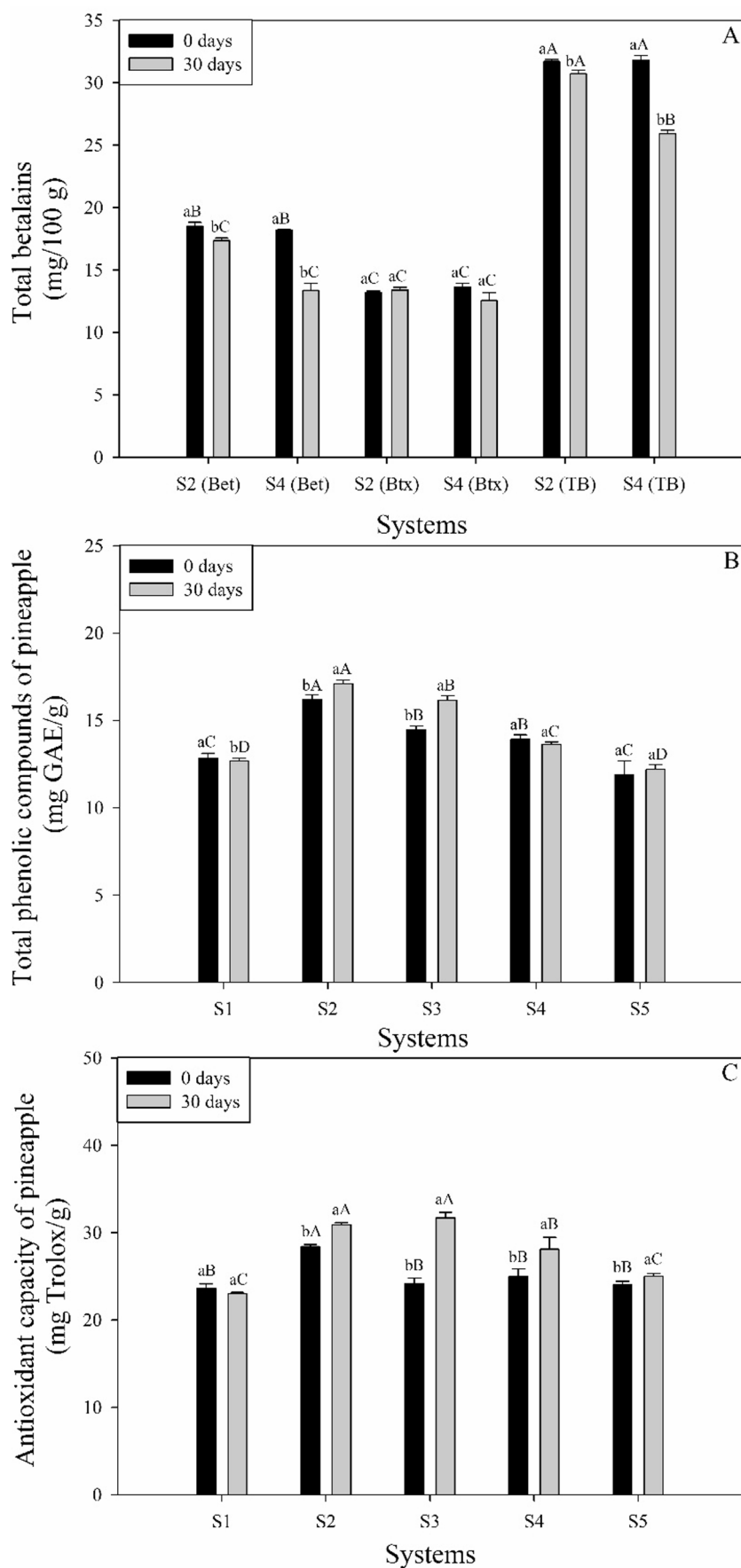
Sensory acceptance of pineapple wedges after 30 days of storage

Table 4 shows the sensory acceptance of pineapple wedges at the beginning and after 30 days of storage. As is observed, the values of acceptance in a 7-point hedonic scale ranged from 5.20 to 5.68 (I like it–I like it moderately), 4.80–5.56 (Neither like it nor dislike–I like it moderately), 4.33–5.80 (Neither like it nor dislike–I like it moderately), 5.24–5.88 (I like it–I like it moderately) for flavor, odor, color, and overall acceptance, respectively, without affectation ($p > 0.05$) of pineapple systems and storage time. Similar results were obtained by Chaudhary et al. [48], who evaluated the sensory acceptance (color, flavor, taste, texture, and overall acceptance) of pineapple dried using different methodologies and sugar impregnation concentrations. They gave values ranging from 6 to 8 (I like slightly–I like very much) in a 9-hedonic scale for the evaluated sensory parameters. In addition, they also pointed out that increasing the sugar concentration of the impregnation solutions (50 and 60 °Brix) improves the sensory acceptance of dried pineapple due to osmotic dehydration, which maintains the color, flavor, aroma, and nutritional components of fruit and vegetables [49].

Conclusions

This study aimed to develop pineapple wedges containing health-promoting compounds (antioxidants, prebiotics, and probiotics) and evaluate their stability during storage. The findings indicated that using fermented solution (whey, inulin, and beetroot) with *L. acidophilus* is a viable alternative to incorporating health-promoting compounds into pineapple wedges. In this aspect, pineapple wedges impregnated with fermented solution based on whey, inulin, and beetroot showed the highest TPC and AC at the beginning and end of the storage (30 days). However, the probiotic population remained at adequate concentrations to exert its health benefits for 15 days of storage. Therefore, despite maintaining the physicochemical and antioxidant characteristics and sensory acceptance of pineapple wedges for 30 days of storage, the probiotic survival limited their benefits to 15 days. Further studies must be conducted to evaluate other

Fig. 3 Total betalains (A), total phenolic compounds (B), and antioxidant capacity (C) of pineapple wedges at the beginning (black bars) and after 30 days (gray bars) of storage. Tin black bars indicate the standard deviation. Different letters a and b for the same sample at different storage days indicate significant differences ($p < 0.05$). Different letters A, B, C, and D for the same storage time are significantly different ($p < 0.05$)



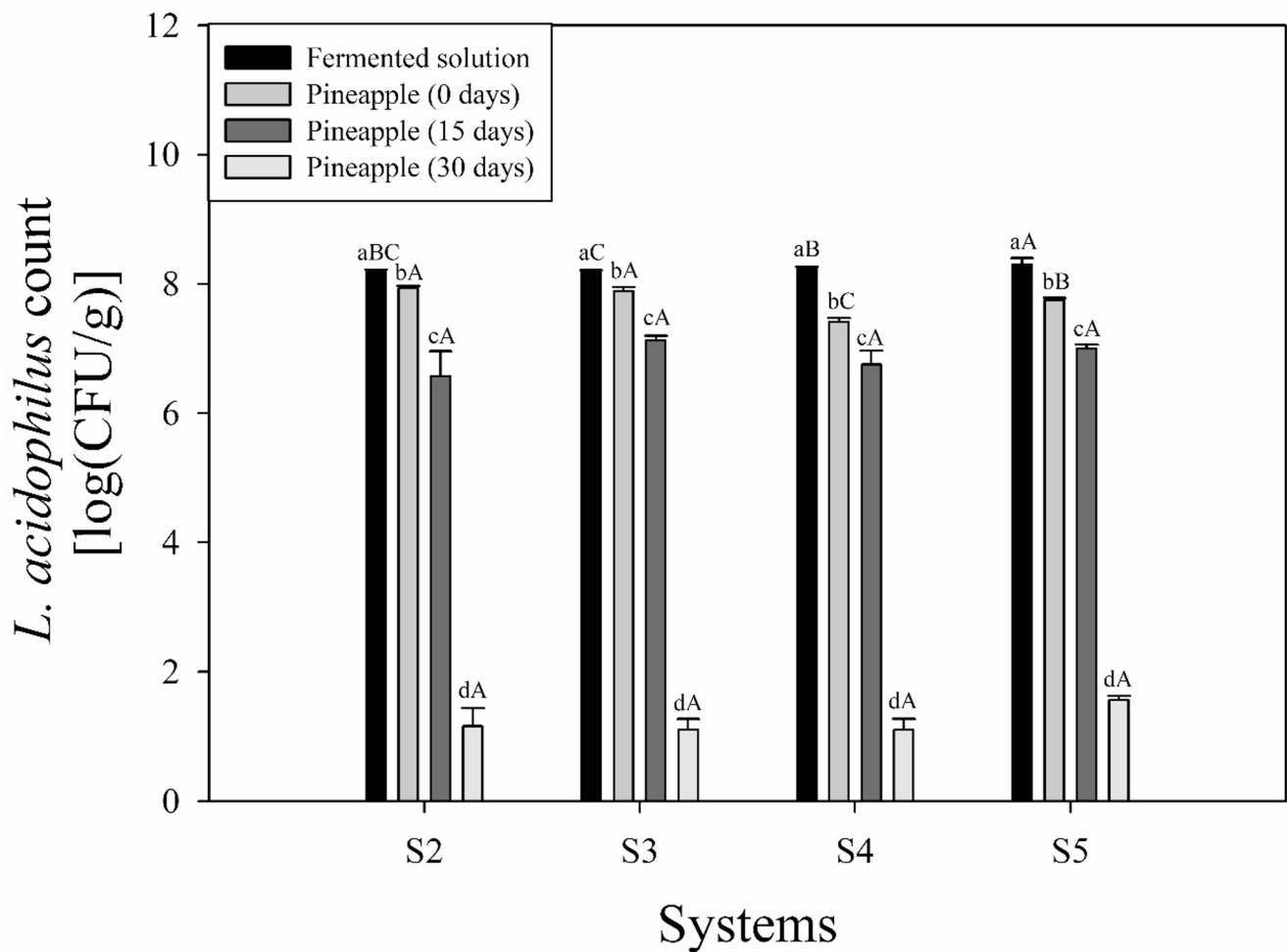


Fig. 4 Probiotic count of pineapple wedges at the beginning and after 30 days of storage. Tin black bars indicate the standard deviation. Different letters a, b, c, and d for the same sample at different storage days

indicate significant differences ($p < 0.05$). Different letters A, B, C, and D for the same storage time are significantly different ($p < 0.05$)

Table 4 Sensory acceptance of impregnated pineapple wedges with LAB-fermented after 30 days of storage at room temperature.^a

Sensory parameters	Storage time (days)	Systems				
		S1	S2	S3	S4	S5
Flavor	0	5.68 ± 0.88 _{aA}	5.24 ± 1.38 _{aA}	5.36 ± 1.28 _{aA}	5.40 ± 1.46 _{aA}	5.20 ± 1.54 _{aA}
	30	5.54 ± 0.95 _{aA}	5.54 ± 1.38 _{aA}	5.54 ± 1.53 _{aA}	5.38 ± 1.01 _{aA}	5.29 ± 1.63 _{aA}
Odor	0	5.56 ± 0.93 _{aA}	4.80 ± 1.10 _{aA}	4.80 ± 1.17 _{aA}	4.96 ± 1.45 _{aA}	4.80 ± 1.38 _{aA}
	30	5.04 ± 0.95 _{aA}	4.83 ± 1.04 _{aA}	4.92 ± 1.32 _{aA}	5.00 ± 1.25 _{aA}	4.96 ± 1.27 _{aA}
Color	0	5.80 ± 1.20 _{aA}	4.52 ± 1.69 _{aA}	5.24 ± 1.03 _{aA}	4.60 ± 1.44 _{aA}	5.48 ± 1.10 _{aA}
	30	4.83 ± 1.24 _{aA}	4.33 ± 1.20 _{aA}	4.08 ± 1.06 _{aA}	4.46 ± 1.18 _{aA}	4.63 ± 1.41 _{aA}
Overall acceptance	0	5.88 ± 0.87 _{aA}	5.24 ± 1.28 _{aA}	5.32 ± 1.17 _{aA}	5.28 ± 1.59 _{aA}	5.40 ± 1.13 _{aA}
	30	5.38 ± 1.01 _{aA}	5.54 ± 1.18 _{aA}	5.58 ± 1.02 _{aA}	5.46 ± 1.06 _{aA}	5.33 ± 1.49 _{aA}

^aAverage ± standard deviation. Different letters (a and b) within the same line for the same system are statistically different ($p < 0.05$). Different letters (A and B) for the same line are statistically different ($p < 0.05$)

methods for incorporating probiotics and maintaining their bioavailability in dried pineapples.

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Author contributions Margarita García-Gómez: Data Curation. Paola Hernández-Carranza: Investigation, Methodology. Carolina Ramírez-López: Data Curation, Supervision. Karen H. Estévez-Sánchez:

Methodology, Investigation. Irving I. Ruiz-López: Conceptualization, Formal Analysis, Validation. Carlos E. Ochoa-Velasco: Conceptualization, Formal Analysis, Funding Acquisition, Writing Original Draft.

Data availability The datasets obtained during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval and consent to participate Not applicable. There is no human and animal studies were carried out in these experiments.

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