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Use of green (*Opuntia megacantha*) and red (*Opuntia ficus-indica* L.) cactus pear peels for developing a supplement rich in antioxidants, fiber, and *Lactobacillus rhamnosus*

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Abstract

Cactus pear is an underused exotic fruit rich in health-promoting compounds with a high amount of peel in its composition. Thus, it is necessary to look for its application in the food industry. The aim of this study is to assess the proximal analysis, health-promoting compounds, sensory acceptance, and probiotic survival (*Lactobacillus rhamnosus*) of supplements based on red (RCPP) or green (GCPP) cactus pear peel. Both supplements presented a low moisture content (< 8%) and a high amount of dietary fiber (66-76%), with an adequate quantity of soluble dietary fiber (16.53 ± 1.84 and 19.78 ± 0.83%). Total phenolic compounds, betalains, and antioxidant capacity of the supplements were in the range of 392 to 543 mg GAE/100 g, 23 to 95 mg betalain/100 g, and 17 to 97 mmol Trolox /100 g, respectively, being always higher in RCPP, which was also better accepted by the consumers. After 5-h of gastrointestinal simulation, *L. rhamnosus* survival was about 5.79 x 10^6 CFU/mL for GCPP supplement, while 3.22 x 10^6 CFU/mL, were counted in RCPP supplement. Cactus pear by-products may be used for developing food supplements rich in fiber and probiotic bacteria widely demanded by the consumer.

Keywords: supplement; probiotic; viability; health-promoting compounds; dietary fiber.

Practical Application: Cactus pear peel is an alternative for elaborate supplements rich in health-promoting compounds.

1 Introduction

Fruit and vegetables are food commodities rich in fiber, vitamins, minerals, and phytochemicals widely demanded by consumers due to the health benefits that they give against heart diseases such as cardiovascular disease, type II diabetes, cancer, high blood pressure, overweight, obesity, among others (Patel, 2017; World Health Organization, 2020). During the processing of fruit and vegetables, different by-products (core, seed, peel, etc.) are obtained, which in many cases are discarded; however, they contain even higher amounts of health-promoting compounds than the fruit itself (Ayala-Zavala et al., 2011; Elhassaneen et al., 2016; Dimou et al., 2019).

The cactus pear is a sweet, juicy, exotic fruit appreciated by international consumers (Cota-Sánchez, 2016). Although Mexico is the largest producer of cactus pear worldwide, with more than 14,000,000 tons harvested per year (Secretaría de Agricultura y Desarrollo Rural, 2020), many of them are lost due to inadequate storage practices (Hernández-Carranza et al., 2019). Moreover, approximately 40% of the whole cactus pear fruit is peel, which is underused, despite the fact it contains several health-promoting compounds, such as phenolic compounds, betalains, and fiber (Hernández-Carranza et al., 2019). To present, different products have been developed using cactus pear by-products to improve their health-promoting composition, e.g., baked goods (Mahfouz & Abd-Elnoor, 2020; Parafati et al., 2020), yogurt (Hernández-Carranza et al., 2019), snacks (Namir et al., 2017), and margarine (Chougui et al., 2015). Therefore, the use of cactus pear peels for formulating value-added food commodities is currently on the rise.

On the other hand, it is well-known that a regular intake of viable probiotics (10^6 CFU per gram of food) displays several benefits to human health, such as improving lactose metabolism, reducing serum cholesterol and gastrointestinal infections, and increasing food digestibility (Liang et al., 2021; He et al., 2021). However, probiotics are widely found in dairy products, which in many cases are unacceptable for people intolerant to lactose, vegetarian, dairy allergies, or those people who are interested in low cholesterol foods (Ranadheera et al., 2017). Therefore, it is necessary to develop a new food matrix with added value, which at the same time contains probiotics and prebiotics (fiber), capable of improving their activity or growth in the gastrointestinal system (He et al., 2021). The aim of this study is to assess the proximal and antioxidant composition, sensory acceptance, and *Lactobacillus rhamnosus* viability after the gastric simulation process of supplements based on red or green cactus pear peel.

2 Material and methods

2.1 Cactus pear peels

Red (*Opuntia ficus-indica* L.) and green (*Opuntia megacantha*) cactus pears were acquired from San Sebastian Villanueva in

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the state of Puebla, Mexico. Fruits were selected in a fully ripe condition according to their external color and free from physical and microbiological appearance damage, washed with tap water, disinfected using a sodium hypochlorite solution (100 mg/L), and gently dried with paper towels. Peel was manually obtained using a stainless-steel knife, then cut into squares of 1 cm^2, and dehydrated in a Food Dehydrator (Excalibur, USA) at 60 °C until a constant weight was attained (24 h approximately). Dried peels were ground, sieved (300 μm), and stored in glass containers for further use.

2.2 *Lactobacillus rhamnosus*

Lactobacillus rhamnosus strain NRRL B-442 was obtained from the Benemerita Universidad Autonoma de Puebla culture collection (Puebla, Mexico). It was grown in Man, Rogosa, and Sharpe (MRS) broth at 37 °C for 24 h. Then, the broth was centrifuged (Premiere XC-2450, USA) at 4000 rpm for 20 min to obtain the microbial biomass. After that, 1 mL of biomass was mixed with 1 mL of glycerol (20%) aqueous solution and freeze-dried (Labconco, Benchtop, USA) at -40 °C, 0.060 mm of Hg, for 72 h. Finally, the microorganism was stored until use.

2.3 Elaboration of red or green cactus pear peel supplements

Supplements were made by mixing 9.0 g of red cactus pear peel (RCPP) or green cactus pear peel (GCPP), 1 g of *L. rhamnosus* (1×10^8 CFU/g), and 0.5 g of sucralose as sweetener. The supplements were stored in glass containers at dark and room temperature (22 ± 2 °C) conditions for further studies.

2.4 Characterization of the supplements

Proximal analysis

Supplements were characterized in their chemical composition using AOAC methods (Association of Official Analytical Chemists, 2019). Moisture was determined by over-drying at 105 °C until a constant weight was attained (4 h approximately). Ash was gravimetrically quantified after incinerating the samples at 525 °C for 5 h. Fat content was quantified gravimetrically after hexane extraction using a Soxhlet apparatus. Protein content was determined using Kjeldahl equipment; the sample was digested, neutralized, distilled, and estimated by multiplying the nitrogen content by 6.25 as a factor. Carbohydrates were calculated by difference. On the other hand, total dietary fiber (TDF) and insoluble dietary fiber (IDF) were determined by the gravimetric-enzymatic methodology. Supplements were treated with thermostable α-amylase, protease, and amyloglucosidase enzymes. After enzymatic hydrolysis, the residues were washed with alcohol 95% (v/v), alcohol 68% (v/v), and acetone or hot distilled water, ethanol 95% (v/v), and acetone to quantified TDF or IDF, respectively. Finally, the residues were dried and weighed. TDF and IDF were calculated using Equation 1. The soluble dietary fiber (SDF) was quantified as the difference between TDF and IDF:

$$TDF\ or\ IDF\,(\%) = \left[\frac{R - P - A}{S}\right] * 100 \quad (1)$$

where *R*, *P*, *A*, and S are the residues, protein, ash, and sample contents, respectively.

The L^* (Luminosity), a^* (red + to green -), and b^* (yellow + to blue -) color parameters were measured using a TCR 200 colorimeter (Beijing, China). Color was measured on the top of a Petri dish filled with the supplement. *Hue* and *Chroma* values were calculated using the followings equations (Equations 2-3):

$$Hue = tan^{-1}\left(\frac{b}{a}\right) \quad (2)$$

$$Chroma = \sqrt{a^2 + b^2} \quad (3)$$

Betalains

Total betalains (TB) were reported as the sum of both betacyanins and betaxanthins following the methodology proposed by Stintzing et al. (2005). One g of RCPP or GCPP supplement was mixed with 10 mL of McIlvaine buffer (pH 6.5) and stirred for 2 h at 300 rpm (room temperature) using a magnetic stirrer hot plate (Thermo Scientific Cimarec model SP131015Q, Waltham, MA). The extract was cotton-filtered and diluted with McIlvaine buffer until an absorbance in the range of 0.9-1 was obtained. A UV-Vis spectrophotometer (Jenway, model 6405, Staffordshire, UK) was used. The value of betacyanins and betaxanthins was calculated using the following equation (Equation 4):

$$TB\left(\frac{mg}{100\,g}\right) = \frac{A * DF * MW * 1000}{\varepsilon * l} \quad (4)$$

where *A* is the absorbance, *DF* is the dilution factor, *MW* is the molecular weight (308 and 550 g/mol for betaxanthins and betacyanins, respectively), *l* is the cell path (1 cm), and ε is the coefficient of molar extinction (48,000 and 60,000 L/mol for betaxanthins and betacyanins, respectively). Results were expressed as mg betalain/100 g dry weight (dw).

Phenolic compounds

To evaluate the total phenolic compounds and antioxidant capacity of supplements, an extraction process was conducted. Briefly, 1 g of the supplement was mixed with 50 mL of distilled water, stirred for 2 h at 300 rpm (room temperature) using a magnetic stirrer hot plate. The extract was cotton-filtered and immediately used for phenolic compounds and antioxidant capacity determinations. Total phenolic compounds (TPC) were assessed according to the methodology proposed by Hernández-Carranza et al. (2016) with modifications. One mL of extract was mixed with 1 mL of Folin-Ciocalteau reagent (0.1 M), after three min, 1 mL of Na_2CO_3 (0.05% w/v) was added and stored for 30 min in a dark environment at room temperature. The solution was read using a UV-Vis spectrophotometer at 765 nm. The TPC was quantified as mg of gallic acid equivalent (GAE)/100 g (dw) using a standard curve of gallic acid (slope: 0.004, intercept: -0.0392, and R^2: 0.996).

Antioxidant capacity

Antioxidant capacity (AC) was assessed by the inhibition of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. The determination was performed following the methodology proposed by Hernández-Carranza et al. (2016). One mL of extract was mixed with 1 mL of DPPH radical at 0.004% and stored for 30 min in a dark environment at room temperature. The solution was read using a UV-Vis spectrophotometer at 517 nm. A standard curve of Trolox was used for quantifying the antioxidant capacity, and the results were quantified as mmol of Trolox equivalent/100 g dw using a standard curve of Trolox (slope: 6.446, intercept: 2.007, and R^2: 0.998).

Gastrointestinal simulation process

The viability of *L. rhamnosus* under gastrointestinal simulation process was conducted following the methodology proposed by He et al. (2021). A gastric solution was formulated by solubilizing 3.2 g of pepsin (Sigma, P-700), 2 g of NaCl in 1 L of sterile distilled water (pH 2.0, adjusted with HCl). The intestinal solution was formulated by solubilizing 10 g of pancreatin (Sigma, P-1750) and 6.8 g of K_2HPO_4 in 1 L of sterile distilled water (pH 7.0, adjusted with NaOH). One g of RCPP or GCPP supplement was diluted with 25 mL of sterile distilled water and mixed for 2 h with 25 mL of gastric fluid at 37 °C and 110 rpm. Then, 25 mL of the gastric simulation was mixed with 25 mL of intestinal solution (3 h, 37 °C, and 110 rpm). Finally, 1 mL of each fluid was diluted with peptone water until the appropriate dilution for counting 30-300 CFU/mL in Petri dishes was obtained. *L. rhamnosus* was grown in MRS agar under anaerobic conditions at 35 ± 2 °C and counted after 24 h. *L. rhamnosus* (without supplement) was used as control.

Sensory acceptance

Consumer acceptance was assessed using a 9-point hedonic scale (Wichchukit & O'Mahony, 2015), where 1-indicates dislike very much and 9-indicates like very much. Ten mL of ready-to-drink supplement (10 g of supplement mixed with 200 mL of purified water) were provided to one hundred untrained judges who frequently consume products with fiber. The test was conducted with students (18-25 years old) of the Benemerita Universidad Autonoma de Puebla (Puebla, Mexico), who evaluated the flavor, color, and overall acceptance.

Statistical analysis

All determinations were made in duplicate, and each experiment was performed twice. Results were analyzed by analysis of variance using Minitab v.16 software (Lead Technologies Inc., USA). Tukey´s comparison test (α = 0.05) was used for deciding statistical differences among results.

3 Results and discussion

3.1 Supplements characterization

The proximate composition of GCPP and RCPP supplements is shown in Table 1. The main constituent of supplements was dietary fiber (66.26-75.65%), indicating that both supplements may be considered a good source of fiber. As in many other food products rich in fiber, in both supplements formulated, IDF represents the highest content of total fiber with a ratio of SDF: IDF of 1 : 2.4 and 1 : 3.6 for GCPP and RCPP, respectively; values like those reported in different cactus pear peel varieties. Interestingly, this relation was always higher in red color cactus pear (1 : 3.3 to 1 : 3.4) varieties compared to green color cactus pear (1 : 2.4 to 1 : 3.1) (Jiménez-Aguilar et al., 2015; Amaya-Cruz et al., 2019). Moreover, the SDF obtained in supplements is in the range of those values reported by Jiménez-Aguilar et al. (2015), who informed values of 11.3-17.2 g/100 g and 9.8-10.6 g/100 g for GCPP and RCPP of the same varieties reported in this study. The content of SDF is of paramount importance because it is the main responsible for the prebiotic potential of fiber (Garcia-Amezquita et al., 2018). In this aspect, the American Dietetic Association recommends a fiber intake of 25-30 g per day or 10-13 g/1000 kcal for adults (Slavin, 2003), so supplements formulated may be helpful to cover this recommendation. Commercial fiber supplements like *Plantago psyllium* (53.0%) have lower fiber values than these developed supplements. On the other hand, as powder supplements, a low moisture (< 8%) is desired for long shelf life (Monter-Arciniega et al., 2019). Overall carbohydrate (50.28-73.53), water (5.44-7.93%), lipids (1.52-2.87), and protein (0.92-1.98) values were like those reported by Monter-Arciniega et al. (2019) for purple cactus pear waste and commercial fibers of *Opuntia ficus.*

Table 1. Supplements made from cactus pear peel and *L. rhamnosus* proximate composition and color parameters[a].

Parameter	GCPP[b]	RCPP[c]
Moisture[d]	7.63 ± 0.15a	7.76 ± 0.51a
Ash[d]	1.74 ± 0.25a	1.28 ± 0.38a
Fat[d]	5.05 ± 0.04a	4.76 ± 0.12a
Protein[d]	0.25 ± 0.05b	0.33 ± 0.10a
Carbohydrates[e]	19.07 ± 0.12a	10.22 ± 0.28b
Total dietary fiber[d]	66.26 ± 1.40b	75.65 ± 2.20a
Insoluble dietary fiber[d]	46.48 ± 1.34b	59.12 ± 2.26a
Soluble dietary fiber[d]	19.78 ± 0.83a	16.53 ± 1.84b
L^*	48.38 ± 0.50a	29.28 ± 0.46b
a^*	-1.02 ± 0.10b	13.57 ± 0.29a
b^*	17.81 ± 0.56a	1.06 ± 0.19b
Hue	93.30 ± 0.33a	4.50 ± 0.91b
Chroma	17.84 ± 0.56a	13.61 ± 0.28b

[a] Average ± standard deviation. [b] Green cactus pear peel. [c] Red cactus pear peel. [d] Percentage in dry weight (dw)/100 g. [e] Carbohydrates were calculated by difference. Different letters in row are significantly different ($p < 0.05$).

Supplements showed a color according to the cactus pear variety from which they were made (Figure 1). Therefore, RCPP supplement has a higher a^* color and lower b^* color parameter, which placed it in the first quadrant (hue color) of the color chart (red to yellow color); while according to the hue color parameter of GCPP supplement is situated in the second quadrant of the color chart (yellow to green). According to the chroma parameter, both supplements present an opaque color, which is characteristic of this kind of vegetable fibers (oat, potato, pea, apple, and wheat) (Huber et al., 2016).

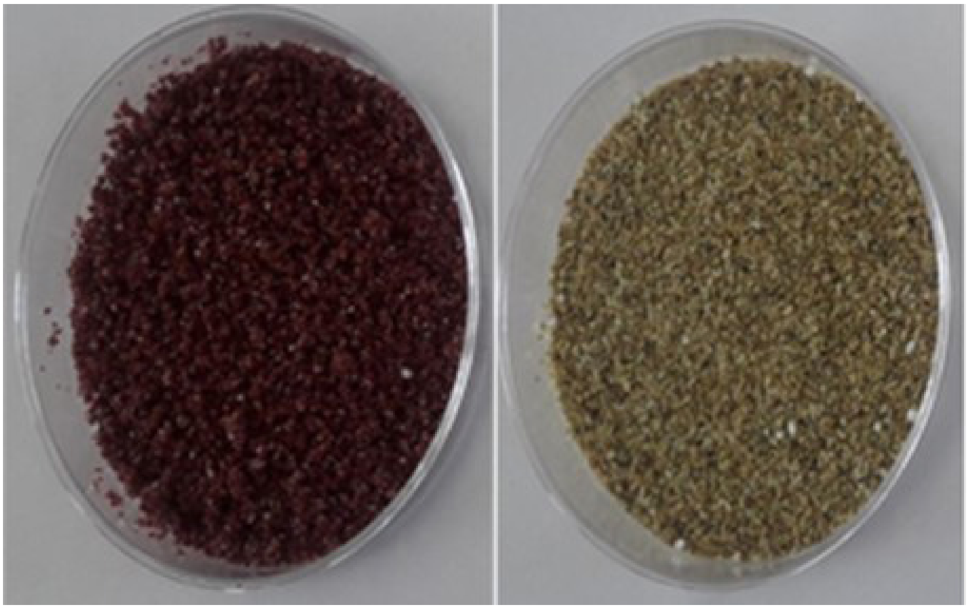

Figure 1. Supplements formulated with cactus pear peel and *L. rhamnosus*. Left: RCPP supplement. Right: GCPP supplement.

3.2 Bioactive compounds and antioxidant capacity of supplements

Bioactive compounds and antioxidant capacity of supplements made with cactus pear peel and *L. rhamnosus* are presented in Table 2. Cactus pear peel is a suitable source of phenolic compounds, betalain pigments, and in consequence, it contains a higher antioxidant capacity (García-Cayuela et al., 2019). Total phenolic compounds, total betalains, and antioxidant capacity of supplements were consistently higher in RCPP supplement. Wit et al. (2020), reported values of total phenolic compounds (78.52-100.96 mg GAE/kg), total betacyanins (0.56-7.77 mg/kg), and antioxidant capacity (93.33-95.63%, DPPH radical inhibition) always higher in RCPP than GCPP of the same variety (*Opuntia ficus-indica*). This may be attributed to the fact that red color cactus pear peel contains higher individual flavonoids like isorhamnetin, rutin, kaempferol, and their derivatives, and betalains (betanin, isobetanin betanidin, and Gomphrenin I) (García-Cayuela et al., 2019). It is of utmost importance to point out that the variation of bioactive compounds and antioxidant capacity depend on several factors such as the variety, ripening stage, climate, storage, methodology used, etc. (Aruwa et al., 2019).

Table 2. Bioactive compounds and antioxidant capacity in supplements made from cactus pear peel and *L. rhamnosus*[a].

Compound	GCPP[b]	RCPP[c]
Total phenolic compounds[d]	392.13 ± 4.29b	543.50 ± 4.34a
Total betalains[e]	23.00 ± 0.58b	95.43 ± 0.02a
Antioxidant capacity (DPPH)[f]	17.39 ± 2.64b	97.26 ± 3.26a

[a]Average ± standard deviation. [b]Green cactus pear peel. [c]Red cactus pear peel. [d]mg gallic acid equivalent (GAE)/100 g dw. [e]mg betalain/100 g dw. [f]mmol Trolox equivalents/100 g dw. Different letters in the row are significantly different ($p < 0.05$).

3.3 Lactobacillus rhamnosus survival during gastrointestinal simulation

As reported in several studies, the main probiotic challenge is through the gastrointestinal tract (stomach acid conditions and duodenal bile salts) and to reach the colon where they exert their function (He et al., 2021; Tripathi & Giri, 2014). Then, several approaches have been done to assess food matrices to carry out these beneficial microorganisms. However, the use of GCPP or RCPP has not yet been studied. In this regard, the initial microbial load of *L. rhamnosus* in formulated supplements was 8.10 ± 0.8 x 10^8 CFU/mL. During the gastric simulation a microbial reduction of 1.2 and 1.3-log cycles was observed for GCPP and RCPP supplement, respectively. This reduction is due to the low pH of the gastric fluid (Figure 2), which is the main factor against probiotics (Ranadheera et al., 2017). It is important to highlight that the intestinal process did not reduce ($p > 0.05$) the *L. rhamnosus* count in formulated supplements. Nevertheless, the lower microbial reduction was obtained in GCPP supplements probably due to its higher amount of SDF, which can be easily accessed by the *L. rhamnosus*, improving its gastrointestinal resistance (Guan et al., 2021). After, 5-h of the gastrointestinal simulation, the *L. rhamnosus* load was 5.8 x 10^6 and 3.2 x 10^6 for GCPP and RCPP supplements, respectively. While *L. rhamnosus* evaluated without supplement showed a microbial load of 2.2 x 10^3 CFU/mL after 5-h of process. According to the results obtained, it is possible to infer that cactus pear peel can protect the *L. rhamnosus* during the gastrointestinal simulation process because dietary fiber can entrap the microorganism and protect it from gastrointestinal fluids (Blaiotta et al., 2013).

3.4 Sensory evaluation

Figure 3 shows the color (Figure 3A), flavor (Figure 3B), and overall acceptance (Figure 3C) of RCPP and GCPP supplements. As is observed, both color and flavor of RCPP supplement were

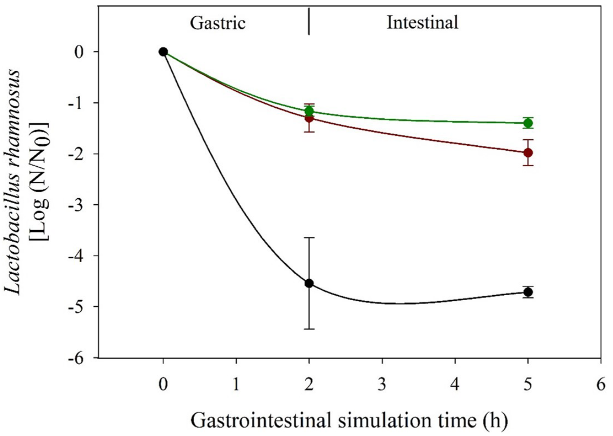

Figure 2. *Lactobacillus rhamnosus* survival curves in supplements of GCPP (green line), RCPP (red line), and without supplement (black line), during the gastrointestinal simulation.

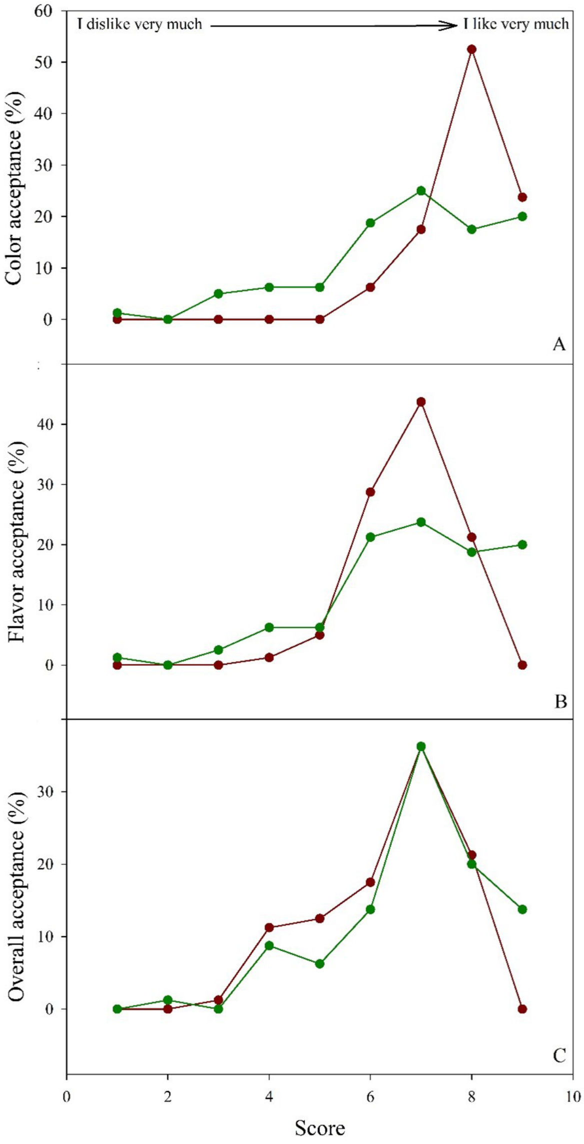

Figure 3. Color (A), flavor (B), and overall (C) acceptance of GCPP (green lines) and RCPP (red lines) supplements.

better accepted by the consumer, with 52.5% ("I like much") and 43.8% ("I like") of occurrence, respectively, while, for the same properties, 20% ("I like very much") and 23.8% ("I like") of occurrence, respectively, was observed for GCPP supplement. Both supplements were equally (36.3%, I like) scored in overall acceptance. Therefore, it is possible to infer that in general RCPP supplement was better accepted by the consumers, probably due to the pleasant red color of it provided by betalains and flavonoids (Amaya-Cruz et al., 2019). To our knowledge cactus pear peel drink-based added with probiotic has not been explored; however, this study indicated that supplements developed might be used for preparing a drink beverage added with probiotic and prebiotic widely demanded by consumers.

4 Conclusion

Results obtained in this study indicated that cactus pear peel is a good source of health-promoting compounds like dietary fiber (especially SDF), phenolic compounds, and betalains. Both green cactus pear peel and red cactus pear peel formulated supplements protected *L. rhamnosus* because a low microbial reduction was attained after gastrointestinal simulation. Moreover, formulated supplements were well accepted by the consumers, especially supplements made from the red cactus pear peel. This is a useful study because a value-added food commodity can be obtained from a by-product of the cactus pear industry.

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