Antioxidant fortification of yogurt with red cactus pear peel and its mucilage

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ABSTRACT

The addition of red cactus pear peel and its mucilage on color, bioactive compounds and antioxidant capacity (AC) of yogurt was investigated. Peel and its mucilage were dried and used for the formulation of different yogurt blends following a constrained mixture design. Color, total phenolic compounds, total flavonoids, total betalains and AC were evaluated in formulated yogurts. An optimization process was conducted thereafter, and the bioactive compounds and AC of this yogurt were evaluated before and after gastrointestinal simulation. Results indicated that cactus pear peel and its mucilage give a nice magenta color to the yogurts, and also increase the bioactive compounds and AC of them. Optimization procedure indicates that yogurt formulated with 5.5% of cactus pear peel and 7.5% of mucilage displays the highest bioactive compounds and AC; moreover, they were significantly increased ($p < 0.05$) by the simulated gastrointestinal process.

Fruit and vegetables are a well-known source of bioactive compounds; however, during their processing several by-products such as peel, seed, core, and pomace are obtained, reaching between 25% and 30% of wastes of a whole commodity group (Ayala-Zavala et al., 2011; Sagar, Pareek, Sharma, Yahia, & Lobo, 2018). These by-products possess in many cases different and/or higher content of bioactive compounds that the traditionally exploited portion of the vegetable; therefore, they may be used for the fortification and/or formulation of food products (Berto, Braga, Evelázio, Fernandes, & Campos, 2015; Elleuch et al., 2011; Helkar, Sahoo, & Patil, 2016).

Cactus pear is the fruit of *Opuntia ficus-indica* L., is a well-accepted fruit in Mexico due to its juicy and sweet flavor (Ochoa-Velasco & Guerrero-Beltrán, 2012). Different studies have indicated that cactus pear contains bioactive compounds such as polyphenols and betalains, which provide it high antioxidant capacity and attractive color (Ochoa-Velasco & Guerrero-Beltrán, 2012; Otálora, Carriazo, Iturriaga, Nazareno, & Osoiro, 2015). In this sense, betalains are natural plant pigments, which are commonly found in a wide variety of plants belonging to the Opuntia genus. The most common betalains present in cactus pear are the betacyanins (blue-purple color) and betaxanthins (red color) (Elleuch et al., 2011). These pigments are known for their health benefits, including their ability to reduce the risk of chronic degenerative diseases such as cancer, diabetes, obesity, chronic heart disease, among others (Tapsell, Neale, Satija, & Hu, 2016). On the other hand, the increase of chronic degenerative diseases such as cancer, diabetes, obesity, chronic heart disease, among others has driven consumers to demand foods with high content of bioactive compounds such as fiber, vitamins, pigments, minerals, phenolic compounds (Sharma et al., 2016; Tresserra-Rimbau et al., 2014). Thus, a frequent consumption of natural and formulated plant-based foods is necessary for an adequate intake of these compounds (Luna-Guevara, Luna-Guevara, Hernández-Carranza, Ruiz-Espinosa, & Ochoa-Velasco, 2019; Stagos et al., 2012).

1. Introduction

Tendencies and demands of consumers have been focusing on food products with high sensory and nutritional quality, as fresh as possible and free of synthetic additives or chemical preservatives (Asioli et al., 2017). However, the knowledge in nutrition and its relation on human health is constantly increasing and nowadays has focused on studying the effect of non-nutrient compounds and their relation to maintain good health and reduce the appearance of different diseases (Tapsell, Neale, Satija, & Hu, 2016). On the other hand, the increase of chronic degenerative diseases such as cancer, diabetes, obesity, chronic heart disease, among others has driven consumers to demand foods with high content of bioactive compounds such as fiber, vitamins, pigments, minerals, phenolic compounds (Sharma et al., 2016; Tresserra-Rimbau et al., 2014). Thus, a frequent consumption of natural and formulated plant-based foods is necessary for an adequate intake of these compounds (Luna-Guevara, Luna-Guevara, Hernández-Carranza, Ruiz-Espinosa, & Ochoa-Velasco, 2019; Stagos et al., 2012).
pigments which can be divided into red-violet betacyanins or yellow betaxanthins; these pigments are gaining popularity for using as a natural colorant in the food industry (Gengatharan, Dykes, & Choo, 2015). However, one of the main problems of cactus pear is its high amount of peel, representing about 40–45% of the whole fruit (Ochoa-Velasco & Guerrero-Beltrán, 2014, 2016). As many other fruits, the peel of cactus pear has a considerable amount of fiber and bioactive compounds, which are generally discarded. However, cactus pear peel is a good source of mucilage, which is a heteropolysaccharide constituted of L-arabinose, D-galactose, D-xyllose, L-rhamnose, D-galacturonic acid, etc. It has been associated with different health benefits (Otálora et al., 2015) and with a great capacity to absorb water and act as a hydrocolloid (Hernández-Carranza et al., 2019). However, there is evidence which indicates that these complex carbohydrates directly interact with the bioactive compounds of food, affecting their bioaccessibility and bioavailability in the gastrointestinal process (Palafox-Carlos, Ayala-Zavala, & González-Aguilar, 2011).

Yogurt is a dairy product obtained by the fermentation process carried out by Streptococcus thermophilus and Lactobacillus delbrueckii spp. bulgaricus (German, 2014). It is an excellent source of proteins, medium-chain fatty acids, vitamins, and minerals, and its continuous consumption causes a slight reduction in stomach pH reducing the risk of illnesses caused by pathogenic microorganisms (Hashemi, Hadi, Mesbahi, & Amin, 2015). Moreover, probiotic microflora of yogurt has shown to induce measurable health benefits, as it improves lactose digestion, reduces the acute diarrheal disorders, enhances the immune response in immunocompromised population, induces anti-tumorigenic activities and reduces risk of colon cancers (Fazilah, Ariff, Khayat, Rios-Solis, & Halim, 2018; Guarner et al., 2005). Although yogurt is one of the most complete foods, it is not considered a good source of bioactive compounds such as fiber, phenolic compounds, and pigments; therefore, its supplementing with plant-based products is necessary to avoid unstable coefficient estimates due to collinearity arising in highly constrained regions such as that given in Figure 1 (Cornell, 2002).

2.2. Yogurt and plant material

Fresh natural yogurt was purchased from a local producer of Puebla, Puebla, Mexico. Red cactus pear (Opuntia ficus-indica L.) was obtained from San Sebastian Villanueva, Puebla, Mexico. Fruits were selected according to their homogeneous red color, free from physical and microbiological appearance damage. Cactus pears were washed and disinfected using a sodium hypochlorite solution (100 mg L⁻¹) and dried with absorbent paper. The peel was manually obtained using a stainless-steel knife, then cut in squares (1 cm²) and dried at 60°C until constant weight (24 h approximately). Dried peel was grinded and sieved to obtain a fine powder (180 µm). To obtain the mucilage, peel powder and water were mixed in a 1:20 mass ratio (Hernández-Carranza et al., 2019). The mixture was stirred at 40°C, 300 rpm for 10 min. Then, it was centrifuged (Premiere XC-2450, USA) at 4000 rpm for 20 min, the supernatant was recovered and dried for 36 h at 60°C. Dried sample was grinded and sieved to obtain a fine powder (75 µm). Both peel powder and mucilage were stored at room temperature in a hermetic bottle covered from the light.

2.3. Formulation of natural yogurt added with cactus pear peel powder and its mucilage

The effect of the addition of red cactus pear peel ($i = 1$) and its mucilage ($i = 2$) to enhance the bioactive compounds and antioxidant capacity of natural yogurt ($i = 3$) was evaluated using a constrained mixture design with 9 formulations (Figure 1, Table 1), including the vertices ($A, B, C,$ and $D$), edges ($E, F, G,$ and $H$) and centroid ($I$) of the rhombus-shaped factor space. Cactus pear peel and mucilage were explored at the surface of each one using a precise colorimeter and lower bounds of the factor space. Cactus pear peel and mucilage were explored at the surface of each one using a precise colorimeter

$$
y = b_1 z_1 + b_2 z_2 + b_3 z_3 + b_{12} z_1 z_2 + b_{13} z_1 z_3 + b_{23} z_2 z_3
$$

where $y$ is the fitted response; $b_1, \ldots, b_{23}$ are the model parameters and $z_i$ ($i = 1, 2, 3$) represents the pseudocomponents calculated according to:

$$
z_k = \frac{x_k - L_k}{x_k - L_k}
$$

and

$$
z_i = \frac{x_i - x_i}{x_k - L_k}, \quad i \neq k
$$

In above equations $k$ is the component with the largest range ($R_i = U_i - L_i$, $i = 1, 2, 3$), where $U$ and $L$ are the upper and lower bounds of $x$ ($k = 3$ in this study). Transformation of regular compositions ($x$) to pseudocomponents ($z$) is necessary to avoid unstable coefficient estimates due to collinearity arising in highly constrained regions such as that given in Figure 1 (Cornell, 2002).

2.4. Color

The color parameters of fresh and formulated yogurt were evaluated at the surface of each one using a precise colorimeter.
The *L*, *a*, and *b* color parameter of the CIELAB color space were used to calculate the Hue and Chroma values following the next equations, respectively.

\[
\text{Hue} = \tan^{-1}\left(\frac{b^*}{a^*}\right) \tag{4}
\]

\[
\text{Chroma} = \sqrt{a^{*2} + b^{*2}} \tag{5}
\]

2.5. Bioactive compounds and antioxidant capacity determination

For determining the bioactive compounds and antioxidant capacity from red cactus pear peel powder, mucilage, fresh yogurt, and blends; extracts were obtained (Hernández-Carranza et al., 2019). The extract was obtained by placing 1 g of sample with 19 mL of distilled water, the mixture was stirred for 1 min using an electric stirrer (IKA Vortex 3, USA) and then centrifuged at 4000 rpm for 10 min. The supernatant was taken immediately to analyze the bioactive compounds and antioxidant capacity.

2.6. Total phenolic compounds

Total phenolic compounds (TPC) were evaluated according to the methodology proposed by Hernández-Carranza et al. (2016). One milliliter of an adequate dilution of extract was mixed with 1 mL of Folin-Ciocalteau reagent (0.1 M) and 1 mL of Na₂CO₃ (0.5% w/v) solution (3 min later). The mixture was incubated for 30 min in a dark environment at room temperature. Total phenolic compounds were read at 765 nm using a Jenway UV-Vis spectrophotometer (model 6405, Staffordshire, UK). To quantify the total phenolic compounds a standard curve of gallic acid was done. The result was expressed as mg of gallic acid equivalents (GAE) per 100 g of sample.

2.7. Total flavonoids

Total flavonoids (TF) were analyzed following the methodology proposed by Hernández-Carranza et al. (2016). 0.5 mL of an adequate dilution of extract was mixed with 0.5 mL of NaNO₂ (1.5% w/v) in an amber glass tube, the mixture was vortexed and let stand for 5 min. Later, 1 mL of AlCl₃ (3% w/v) was added and mixed for 1 min; then, 1 mL of NaOH (1 N) was added. The mixture was let to stand for 1 min and the absorbance was read at 490 nm using an UV-Vis spectrophotometer. To quantify the total flavonoids a standard curve of quercetin was done. The result was expressed as mg of quercetin per 100 g of sample.


2.8. Total betalains

Total betalains (TB) were reported as the sum of the betacyanins and betaxanthins following the methodology proposed by Stintzing, Herbach, & Mossierhammer (2005). Briefly, 1 mL of extract was diluted with McIlvaine buffer (pH 6.5, citrate-phosphate) to obtain absorption values between 0.9 and 1.0. The concentration of betacyanins and betaxanthins were determined spectrophotometrically at 535 and 483 nm, respectively. For betacyanins a molecular weight of 550 g/mol, and molar extinction coefficient of 60,000 L/mol cm were used; while for indicaxanthin a molecular weight 308 g/mol, and molar extinction coefficient 48,000 L/mol cm were employed.

\[ B = \frac{A \cdot DF \cdot MW \cdot 1000}{\varepsilon \cdot I} \]  

(6)

where \( B \) is the betacyanins or betaxanthins content (mg/100 g), \( A \) is the absorbance, \( DF \) is the dilution factor, \( MW \) is molecular weight (g/mol), \( I \) is the cell path (1 cm), \( \varepsilon \) is the extinction molar coefficient.

2.9. Antioxidant capacity

The antioxidant capacity (AC) was evaluated using both the iron (III) to iron (II) reduction (FRAP) assay and the inhibition of the DPPH (2,2-diphenyl-1-picyrylhydrazyl) radical. For the first one, the methodology proposed by Dorman, Kolar, Kahlos, Holm, & Hiltunen (2003) was used. One milliliter of extract was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 7.0) and 2.5 mL of potassium hexacyanoferrate solution (1% w/v) and let to stand for 30 min at 50°C. Then, 2.5 mL of trichloroacetic acid solution (10% w/v) was added and the mixture was centrifuged for 10 min (1000 rpm). At least, 2.5 mL of the supernatant was mixed with 2.5 mL of water and 0.5 mL of FeCl₃ solution (0.1% w/v) and the absorbance was read at 700 nm using an UV-Vis spectrophotometer. To quantify the reducing capacity a standard curve of ascorbic acid was done. The reducing capacity was expressed as mg of ascorbic acid (AA) per 100 g of sample. The inhibition of DPPH radical was conducted following the methodology proposed by Hernández-Carranza et al. (2016). One milliliter of an adequate dilution of extract was added in an amber glass tube and mixed with 1 mL of DPPH (0.004% w/v) solution. The mixture was stirred and let stand in dark environment at room temperature for 30 min. Absorbance was recorded at 517 nm using an UV-Vis spectrophotometer. A standard curve of Trolox was used for quantifying the antioxidant capacity. Results were expressed as mg of Trolox equivalent per 100 g of sample.

2.10. Effect of cactus pear peel and mucilage on bioactive compounds and antioxidant capacity of yogurt formulation

The change in a given response resulting from a change in the proportions of component \( i \) \((i = 1,2,3)\) while keeping constant the relative proportions of the remaining components is the effect of component \( i \) and was investigated using the Cox’s direction, that is, the line or ray projected from a reference mixture (the centroid) to the vertex \( x_i = 1 \) \((i = 1,2,3; \) Cornell, 2002). If the coordinates of reference mixture are denoted by \( \{r_1, r_2, r_3\} \) and the proportion of component \( i \) is changed in \( \Delta_i \), the formulations along Cox’s directions are obtained as:

\[ x_i = r_i + \Delta_i \]  

(7)

\[ x_j = r_j - \frac{\Delta_i r_j}{1 - r_i}, \quad j \neq i \]  

(8)

Coordinates defined in Equations (7) and (8) are further used in Equation (1) to obtain the responses (TPC, TF, TB, AC FRAP, AC DPPH) along the \( x_i \) ray.

2.11. Product optimization

The use of peel powder and mucilage added to natural yogurt was optimized to obtain a formulation with the highest level of bioactive compounds and antioxidant capacity. Yogurt blend was optimized with the desirability function approach (Cornell, 2002), involving the transformation of estimated responses \( y_i \) \((i = 1, 2, ..., q)\) to a desirability value \( d_i \) where \( 0 \leq d_i \leq 1 \). The desirability values are combined in the index:

\[ D = (d_1 \times d_2 \times \ldots \times d_q)^{1/q} \]  

(9)

The closer the value of \( D \) to 1, the higher overall desirability of the resulting blend. If \( L_i \) is the minimum acceptable limit of \( y_i \) and \( U_i \) is the limit above which a perfect desirability is achieved, then

\[ d_i = \begin{cases} 0 & y_i \leq L_i \\ \left( \frac{y_i - L_i}{U_i - L_i} \right)^t & L_i < y_i < U_i \\ 1 & y_i \geq U_i \end{cases} \]  

(10)

On the other hand, if response is unacceptable when \( y_i < L_i \) or \( y_i > U_i \) and desirability of \( y_i \) increases for \( L_i \leq y_i < M_i \) and decreases for \( M_i < y_i \leq U_i \), then \( d_i \) is given by

\[ d_i = \begin{cases} 0 & y_i \leq L_i \\ \left( \frac{y_i - L_i}{M_i - L_i} \right)^t & L_i < y_i < U_i \\ 1 & y_i \geq U_i \end{cases} \]  

(11)

In Equations (7) and (8), both \( s \) and \( t \) define the shape of the interpolation function, a straight-line in this study \((s = 1, t = 1)\). Desirability of TC \((y_1)\), TF \((y_2)\), TB \((y_3)\), AC FRAP \((y_4)\) and AC DPPH \((y_5)\) was evaluated with Equation (7), where \( L_i \) and \( U_i \) were set as the minimum and maximum responses in the experimental region predicted with Equation (1). The amount of peel powder and mucilage added to formulation was also controlled to achieve the highest level of bioactive compounds and antioxidant capacity without affecting other characteristics of product. This information was added to optimization problem by setting \( y_6 = x_1 \) and \( y_7 = x_2 \) and mapping these values into desirability functions with Equation (8). In this case, \( L_{6,7} = 0.1 \), \( M_{6,7} = 0.5 \) and \( U_{6,7} = 0.9 \). Finally, all responses \((y_6, i = 1, ..., q, q = 7)\) were combined in overall desirability index defined in Equation (6) and formulation was optimized to maximize its value.

2.12. Gastrointestinal simulation

Gastrointestinal simulation was conducted following the methodology proposed by Saura-Calixto, García-Alonso, Goñi, & Bravo (2000) with some modifications. Gastrointestinal simulation was divided into two steps, gastric and intestinal phase.
For the phase one, 1 g of optimum yogurt blend was placed with 19 mL of HCl-KCl (0.5 M, pH = 1.5) buffer and 0.2 mL of pepsin (Merck, 7190) solution (30% w/v). Sample was incubated at 40°C for 1 h under constant agitation (INO650 M, Mexico). For intestinal simulation, 1 mL of the gastric digestion was taken and added with 19 mL of phosphates buffer (0.1 M, pH = 7.5) and 1 mL of pancreatin (Sigma P-1750) solution (0.05% w/v). Sample was then incubated at 37°C for 6 h under constant agitation. At the end of the gastrointestinal process, samples were analyzed for their antioxidant capacity and bioactive compounds.

2.13. Statistical analysis

Linear regression and optimization procedures were performed with the Matlab R2010a program (MathWorks Inc., Natick, MA, USA). Mean comparisons (Tukey’s test) and correlation analysis were conducted using with the Minitab 15 software (Minitab Inc., PA, USA, 2008).

3. Results and discussion

3.1. Bioactive compounds and antioxidant capacity of yogurt, cactus pear peel powder, and its mucilage

Table 2 displays the bioactive compounds and antioxidant capacity of yogurt, peel and mucilage powders. As expected, yogurt showed a low amount of bioactive compounds and consequently a low antioxidant capacity; however, it had a higher content of total phenolic compounds and antioxidant capacity than those reported by Karasaşlan, Ozden, Vardin, & Turkgol (2011) and Najgebauer-Lejko, Sady, Grega, & Walczycza (2011), respectively; which may be attributed to the differences in the raw materials used in its preparing. On the other hand, mucilage powder presented both a higher amount of bioactive compounds and higher antioxidant capacity than the peel from which it was obtained. In this aspect, as mucilage is an aqueous extract obtained from peel powder, it could be that polarity of water increases the extraction of polar compounds with antioxidant capacity such as betalains, flavonoids and phenolic acids (Wybraniec et al., 2009; Yeddes, Chérif, Guyot, Sotin, & Ayadi, 2013). To date, no studies about the bioactive compounds and antioxidant capacity of mucilage obtained from cactus pear peel are reported, but cactus pear peel has been studied and the values of total betalains and antioxidant capacity obtained in this study are slightly higher than those reported by Aparicio-Fernández et al. (2018), who reported values of 112.6 mg betanin/100 g, 420.9 mg GAE/100 g and 879.1 mg AA/100 g for total betalains, inhibition capacity and reducing power, respectively.

3.2. Bioactive compounds, antioxidant capacity, and color of formulated yogurts

Table 3 shows the results of bioactive compounds, antioxidant capacity, and color obtained from the nine yogurts formulations. At least, an increase of 81.2%, 100.1%, 285.0%, 263.0%, and 635.0% for total phenolic compounds, total flavonoids, total betalains, inhibition capacity, and reducing power, respectively was observed by the addition of cactus pear and/or mucilage to the formulation A (yogurt alone). The highest value of bioactive compounds and antioxidant capacity was obtained with the higher concentration of peel and mucilage powder (Formulation D). Until now, no information has been found in the literature about the use of cactus pear or its by-product added to yogurt; however, the results obtained are higher than those reported by the addition of extract or waste from other plant-based foods. In this regard, Marchiani et al. (2016) informed an increase in 37.3–68.7% and 15–52% in the total phenolic compounds and antioxidant capacity, respectively, in yogurt added with grape pomace obtained from different cultivars. Similarly, Bertolino et al. (2015) reported an increase in 74.9–90.8% and 161–202% for the same responses in yogurt added with 6% of hazelnut skin. On the other hand, Chouchoui et al. (2013) evaluated the same antioxidant compounds that those evaluated in their study in yogurt added with grape seed extracts. They informed similar values of reducing power (223–311 mg AA/100 g); although total phenolic compounds and radical scavenging capacity were lower and higher, respectively, than the obtained in this study. Color parameters of formulated yogurts are presented in Table 3. It can be observed that the addition of cactus pear peel and/or mucilage significantly decreased (p < 0.05) the luminosity and increased the red color (a* color parameter) of the formulated yogurts, while b* color parameter did not show any tendency by the addition of the by-products. As the color of cactus pear peel and mucilage powder presented a deep red color, yogurts added with them had a pleasant magenta color with different intensities, thus hue and chroma values were increased by the addition of cactus pear peel and/or mucilage, while yogurt without powders shows a whitish color (Figure 2).

Pearson’s correlation coefficients between bioactive compounds and antioxidant capacity are given in Table 4. The antioxidant capacity of yogurts evaluated by the two methodologies showed a higher correlation with total phenolic compounds (0.984–0.988) and total flavonoids (0.748–0.808); therefore, the antioxidant capacity of yogurts could associate to those compounds (p < 0.05), and less to total betalains, that shows a lower correlation, thus the antioxidant capacity may be associated to another group of phenolic compounds such as phenolic acids, tannins or coumarins (Luna-Guevara et al., 2019). On the other hand, a negative and positive high correlation (p < 0.05) were observed for total betalains and L* (–0.840) and a* (0.862) color parameters, respectively. This behavior may be attributed to the intense red color presented by the red cactus pear peel and mucilage, which increases the red color, but reduces the luminosity of the yogurts.
### Table 3. Bioactive compounds, antioxidant capacity and color parameters of yogurt added with cactus pear peel powder and its mucilage.

| Blend | $x_1$ | $x_2$ | $x_3$ | TPC$^b$ | TF$^c$ | TB$^d$ | AC$^e$ (DPPH)$^f$ | AC$^e$ (FRAP)$^g$ | $L^*$ | $a^*$ | $b^*$ |
|-------|-------|-------|-------|---------|--------|--------|-----------------|-----------------|------|------|------|
| A     | 0     | 0     | 1     | 19.7 ± 0.9 | 21.1 ± 1.0$^D$ | 0.6 ± 0.0$^E$ | 33.6 ± 0.7$^I$ | 11.9 ± 0.6$^a$ | 78.9 ± 0.8$^a$ | −1.4 ± 1.6$^a$ | −4.4 ± 0.5$^D$ |
| B     | 0.1   | 0.0   | 0.9   | 49.1 ± 0.3$^F$ | 53.9 ± 4.1$^BC$ | 12.3 ± 0.1$^a$ | 189.0 ± 1.5$^a$ | 156.5 ± 0.6$^a$ | 49.3 ± 0.8$^a$ | 31.8 ± 1.3$^a$ | −1.9 ± 0.4$^B$ |
| C     | 0.1   | 0.9   | 0.0   | 61.3 ± 0.3$^D$ | 67.8 ± 5.2$^AB$ | 12.5 ± 0.0$^a$ | 272.6 ± 4.4$^a$ | 215.6 ± 0.0$^a$ | 49.5 ± 0.3$^a$ | 30.8 ± 1.7$^B$ | −3.8 ± 1.1$^D$ |
| D     | 0.1   | 0.1   | 0.8   | 83.8 ± 0.1$^A$ | 83.2 ± 2.1$^A$ | 11.7 ± 0.0$^a$ | 423.9 ± 25.8$^A$ | 349.7 ± 10.4$^a$ | 38.7 ± 1.0$^a$ | 33.6 ± 0.8$^a$ | −2.7 ± 0.5$^CD$ |
| E     | 0     | 0.05  | 0.95  | 42.2 ± 0.5$^G$ | 44.4 ± 3.1$^C$ | 5.6 ± 0.5$^D$ | 147.7 ± 0.1$^a$ | 115.3 ± 1.2$^a$ | 62.4 ± 0.0$^a$ | 27.6 ± 0.4$^a$ | 2.0 ± 0.6$^G$ |
| F     | 0.1   | 0.05  | 0.85  | 68.6 ± 1.2$^C$ | 44.4 ± 9.3$^C$ | 10.8 ± 0.2$^a$ | 330.0 ± 5.1$^a$ | 248.1 ± 2.5$^a$ | 41.8 ± 0.6$^a$ | 31.1 ± 3.3$^a$ | −1.3 ± 0.8$^G$ |
| G     | 0.05  | 0.95  | 0.05  | 35.7 ± 0.3$^I$ | 45.9 ± 5.2$^C$ | 6.2 ± 0.0$^a$ | 121.8 ± 7.3$^a$ | 87.7 ± 5.2$^a$ | 57.4 ± 0.5$^a$ | 29.3 ± 1.4$^a$ | −2.9 ± 1.1$^G$ |
| H     | 0.05  | 0.1   | 0.85  | 74.5 ± 1.4$^B$ | 78.1 ± 9.3$^A$ | 10.3 ± 0.0$^a$ | 337.7 ± 5.1$^a$ | 215.6 ± 0.0$^a$ | 39.5 ± 0.3$^a$ | 37.7 ± 0.4$^a$ | 1.4 ± 0.4$^a$ |
| I     | 0.05  | 0.05  | 0.9   | 56.7 ± 0.9$^B$ | 46.0 ± 4.5$^C$ | 12.6 ± 0.5$^a$ | 247.0 ± 11.1$^C$ | 179.3 ± 11.4$^a$ | 45.2 ± 0.1$^a$ | 37.6 ± 0.3$^a$ | 2.5 ± 0.3$^A$ |

$^a$Mean ± standard deviation, values followed by different capital letter within the same column are significantly different ($p > 0.05$);

$^b$Total phenolic compounds (mg GAE/100 g);

$^c$Total flavonoids (mg quercetin/100 g);

$^d$Total betalains (mg/100 g);

$^e$Antioxidant capacity;

$^f$mg Trolox/100 g;

$^g$mg of ascorbic acid/100 g

*Figure 2. Hue angle and Chroma color parameters of yogurt added with red cactus pear peel and mucilage.*

*Figura 2. Parámetros de color de ángulo de tono y croma de yogurt adicionado con cáscara y mucílago de tuna roja.*

### Table 4. Pearson’s correlation coefficient for bioactive compounds, antioxidant capacity and color parameters.

|       | TPC$^a$ | TF$^b$ | TB$^c$ | AC$^e$ (FRAP)$^d$ | AC$^e$ (DPPH)$^f$ | $L^*$ | $a^*$ | $b^*$ |
|-------|---------|--------|--------|-----------------|-----------------|------|------|------|
| TPC$^a$ | 0.787   | 0.556  | 0.984  | 0.988           | −0.706          | 0.682| −0.009|
| TF    | 1       | 0.321  | 0.748  | 0.808           | −0.444          | 0.386| −0.393|
| TB    | −1      | 0.535  | 0.532  | −0.840          | 0.862           | 0.383|
| AC (FRAP) | −1   | −1     | 1      | 0.977           | −0.699          | 0.662| 0.016|
| AC (DPPH) | −1   | −1     | −1     | 1               | −0.703          | 0.634| −0.078|
| $L^*$ | −1      | −1     | −1     | 1               | −0.848          | −0.271| 1    |
| $a^*$ | −1      | −1     | −1     | −1              | 1               | 0.574|
| $b^*$ | −1      | −1     | −1     | −1              | 1               | 1    |

$^a$Total phenolic compounds (mg GAE/100 g);

$^b$Total flavonoids (mg quercetin/100 g);

$^c$Total betalains (mg/100 g);

$^d$Antioxidant capacity;

$^e$mg ascorbic acid/100 g;

$^f$mg of Trolox/100 g. Bold numbers present a significant correlation ($p < 0.05$)
3.3. Optimization process using constrained mixture design and its validation under experimental condition

Table 5 shows the regression parameters and Pearson’s correlation coefficients of the second-order L-pseudocomponent model used in this study. As observed, proposed models achieved a satisfactory reproduction of experimental data ($R^2 > 0.76$, Figure 3); therefore, they can be used for the optimization of yogurt formulation. The statistical analysis of model parameters revealed that all blend components contributed to the observed response ($p < 0.05$). Moreover, a significant binary synergism was observed between peel-mucilage, peel-yogurt and mucilage-yogurt for TPC and between mucilage-yogurt for TB ($p < 0.05$), that is, the observed response in those mixtures is higher than the expected from averaging contribution from single components. On the other hand, components antagonism was observed for peel-mucilage and mucilage-yogurt in the case of TF ($p < 0.05$). A visual representation of response behavior can be observed in Figure 4(a-e). In addition, Cox’s plots can be used to examine the effect of components on studied responses (Figure 5). Cox’s plots show the effect of both adding and removing a certain component to a reference mixture (the centroid in this case, blend $I$), while the others are kept unaffected. It can be observed that TPC and AC evaluated by FRAP and DPPH methods have a similar response behavior (Figure 4(a,d,e)), a fact previously confirmed by its high correlation (>0.90), reaching a maximum response when peel and mucilage are in their highest levels. Moreover, Cox’s plots for these responses exhibit the same behavior, where TPC and AC (DPPH and FRAP methods) of the reference blend are increased with higher levels of peel and mucilage substitution and decrease when yogurt proportion is increased (Figure 5(a,d,e)). According to Figure 5(b), the TF increases by adding mucilage to the mixture, a fact also observed in Cox’s plot (Figure 4(b)). Here, the addition of peel powder to reference blend also causes an increase of TF, but it is much lower than the caused by adding mucilage to the blend. Finally, the analysis of TB in Cox’s plot (Figure 4(c)) revealed a complex behavior. Adding peel powder to reference formulation increases TB; however, addition of mucilage powder causes the increase of TB only up to a certain limit, beyond which response begins to decrease.

### Table 5. Regression analysis of constrained mixture design for the formulation of yogurt added with peel and mucilage powder of red cactus pear.

| Response | $b_1$ | $b_2$ | $b_3$ | $b_{12}$ | $b_{13}$ | $b_{23}$ | $R^2$ | L$^h$ | U$^h$ |
|----------|-------|-------|-------|----------|----------|----------|-------|-------|-------|
| TPC$^b$  | 77.1  | 88.1  | 56.7  | 5.88     | 5.82     | 6.98     | 0.998 | 19.5  | 84.1  |
| TF$^c$   | 48.7  | 138.9 | 46.6  | −59.9    | 13.9     | −56.2    | 0.851 | 26.1  | 79.6  |
| TB$^d$   | 18.2  | −0.12 | 11.5  | −0.19    | −3.29    | 15.6     | 0.762 | 1.57  | 12.6  |
| AC$^e$ (FRAP)$^f$ | 396.3 | 419.9 | 246.1 | 65.8     | 13.8     | 56.1     | 0.976 | 30.6  | 425   |
| AC$^e$ (DPPH)$^g$ | 339.1 | 359.9 | 178.3 | −26.1    | −23.7    | 8.01     | 0.989 | 16.1  | 343   |

$^a$Values in boldface are significant parameter estimates ($p < 0.05$); $^b$total phenolic compounds (mg GAE/100 g); $^c$total flavonoids (mg quercetin/100 g); $^d$total betalains (mg/100 g); $^e$antioxidant capacity; $^f$mg ascorbic acid/100 g; $^g$mg of Trolox/100 g; $^h$minimum ($L$) and maximum ($U$) predicted response in constrained experimental region (refer to Figure 1)

Figure 3. Fitness quality of second-order mixture models.

Figura 3. Calidad de ajuste de los modelos de mezcla de segundo orden.

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The desirability function approach was applied to optimize the formulation. The lower and upper limits for desirability function of TPC, TF, TB, and AC (DPPH and FRAP methods), as evaluated with fitted models within the constrained factor space, are shown in Table 5. The mapping of studied response into an overall desirability produced the behavior shown in Figure 4f. The maximum desirability was obtained for a yogurt formulated with 5.5% of cactus pear peel and 7.5% of mucilage ($D = 0.663$) was the best combination to get the desired responses. This formulation was experimentally evaluated and the results are shown in Table 6. As observed, a good agreement was found between predicted and experimental responses in optimized formulation. It is well known that bioactive compounds and antioxidant capacity are always evaluated...
Table 6. Predicted, experimental and gastrointestinal simulation responses of bioactive compounds and antioxidant capacity of optimum yogurt blend.

| Compounds | Predicted Experimental | Gastrointestinal Increase |
|-----------|------------------------|---------------------------|
| TPC^e     | 67.1 ± 0.31 g/100 g     | 807.8 ± 23.8g             | 1223.4 |
| TC^e      | 60.0 ± 0.31 g/100 g     | 245.5 ± 21.0g             | 246.3 |
| TB^d      | 11.5 ± 0.64 g/100 g     | 109.8 ± 13.5g             | 641.9 |
| AC^e (FRAP)^f | 308.2 ± 0.84 g/100 g  | 1070.0 ± 52.2g            | 429.4 |
| AC^e (DPPH)^g | 232.0 ± 5.4 g/100 g   | 577.7 ± 42.9g             | 131.4 |

^Formula: x1 = 0.055, x2 = 0.075 and x3 = 0.87. Mean ± standard deviation, values followed by different capital letter within the same row are significantly different (p < 0.05); 1) Total phenolic compounds (mg GAE/100 g); 2) total flavonoids (mg quercetin/100 g); 3) total betalains (mg/100 g); antioxidant capacity: 4) mg of ascorbic acid/100 g; 5) mg Trolox/100 g

^Formula: x1 = 0.055, x2 = 0.075 and x3 = 0.87. Mean ± deviation standard, valores seguidos por letras mayúsculas diferentes dentro de la misma fila son significativamente diferentes (p > 0.05); 1) Compuestos fenólicos totales (mg GAE/100 g); 2) flavonoides totales (mg de quercetina/100 g); 3) betalainas totales (mg/100 g); 4) capacidad antioxidante: 5) mg de ácido ascórbico/100 g; 5) mg de Trolox/100 g

4. Conclusions

Cactus pear peel and its mucilage were successfully incorporated into natural yogurt to give it a high amount of bioactive compound, antioxidant capacity and an attractive range of magenta color. Desirability function approach was successfully applied to formulate a yogurt with the highest bioactive compounds and antioxidant capacity, but with a reduced peel and mucilage substitution. Yogurt submitted to in vitro gastrointestinal process showed higher values of the bioactive compounds and antioxidant capacity than non-digested yogurt. Although further studies should be conducted, the results obtained in this study may be of great use for the fortification of yogurt using cactus pear by-products.

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Conflict of interest

The authors declare that they have no conflict of interest in the publication.

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