

# Enhancing sheep milk yogurt with prickly pear (*Opuntia ficus-indica* L.) flours (peel and pulp): nutritional, techno-functional, and sensory evaluation

Nuria Muñoz-Tebar,<sup>a\*</sup> Carmen Botella-Martínez,<sup>a</sup> Raquel Lucas-González,<sup>a</sup> José M. Lorenzo,<sup>b,c</sup> José Ángel Pérez-Álvarez,<sup>a</sup> Juana Fernández-López<sup>a</sup>  and Manuel Viuda-Martos<sup>a\*</sup> 



## Abstract

**BACKGROUND:** The aim of the present work was to evaluate the chemical composition and characterize the physicochemical, techno-functional and antioxidant properties of flours obtained from the peel and pulp of prickly pear and to assess the effect of their incorporation on the composition, physicochemical parameters, microbiology, antioxidant capacity and sensory properties of sheep milk yogurts.

**RESULTS:** The prickly pear flours stood out for their protein and fiber content, as well as for their high content of minerals. The antioxidant capacity was noticeable in both flours, although the peel was the one with the highest total betalain content and polyphenolic compounds. Likewise, firmness and consistency increased in yogurts with prickly pear without negative effects on the growth and development of lactic acid bacteria starter strains. Sensory evaluation results showed that only color was affected, and the yogurts enriched with prickly pear flours achieved good acceptance by the consumers.

**CONCLUSIONS:** Prickly pear flours could be a viable alternative in the development of dairy products such as yogurt with functional properties.

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**Keywords:** cactus pear; co-products; dairy foods; sheep yogurt; functional foods

## INTRODUCTION

The food market is continuously changing, and consumers are increasingly aware that food ingredients can exert a positive impact on their health, making it evident that there is a need for developing novel products with improved nutritional properties and health benefits. In order to stand out in a globalized and growingly competitive market as well as meet the consumer demands, food industries are constantly looking for new natural sources of functional ingredients since they have benefits on the human body beyond their nutritional value by reducing the risk of disease or improving consumers' health in addition to their nutritional value.<sup>1,2</sup>

Nowadays, yogurt is among the most preferred and widely used foods to incorporate functional ingredients as their consumption has increased worldwide mainly due to their benefits associated with human health such as the reduction of risk of colorectal cancer as demonstrated in a clinical study conducted by Michels

*et al.*,<sup>3</sup> as well as risk reduction of type 2 diabetes mellitus recently approved by the US Food and Drug Administration (FDA).<sup>4</sup> For yogurt manufacturing process, milk is fermented using a starter

\* Correspondence to: Manuel Viuda-Martos or Nuria Muñoz-Tebar, IPOA Research Group, Institute for Agri-Food and Agri-Environmental Research and Innovation (CIAGRO-UMH), Miguel Hernández University, 03312, Orihuela, Alicante, Spain, E-mail: [mviuda@umh.es](mailto:mviuda@umh.es) (Viuda-Martos) or [nmunoz@umh.es](mailto:nmunoz@umh.es) (Muñoz-Tebar)

a IPOA Research Group, Institute on Agri-Food and Agri-Environmental Research and Innovation (CIAGRO-UMH), Miguel Hernández University, Orihuela, Spain

b Centro Tecnológico de La Carne de Galicia, Rúa Galicia N° 4, Parque Tecnológico de Galicia, Ourense, Spain

c Área de Tecnología de los Alimentos, Facultad de Ciencias de Ourense, Universidad de Vigo, Ourense, Spain

culture only composed of two types of lactic acid bacteria (LAB): *Streptococcus thermophilus* and *Lactobacillus bulgaricus*,<sup>5</sup> which improve the digestion and therefore the human health and well-being.<sup>6</sup> Due to its remarkable nutritional profile, sheep milk outshines cow milk by offering higher concentrations of total solids (18.6% versus 12.1%), protein (7.0% versus 3.5%), lipid (6.6% versus 3.9%), and ash (0.9% versus 0.7%)<sup>5</sup> as well as greater calcium and, phosphorus content.<sup>7</sup> These outstanding nutritional aspects not only results in a firmer yogurt but also imparts a delightful creamy sour flavor and enhanced texture and viscosity.<sup>5,8</sup> Additionally, the presence of a proline-rich peptide called colostrinin in sheep milk highlights its potential therapeutic benefits, such as delaying the progression of Alzheimer's disease in humans.<sup>7</sup>

Fruits have been widely incorporated into yogurts to enhance not only their nutritional profile and potential health benefits but also to serve as natural stabilizing, coloring, and texturizing agents, improving their microstructure, color, and texture.<sup>9</sup> Several fruits such as strawberry, date, cactus pear, pomegranate, mango, black mulberry, and apple have been successfully used in the formulation of functional yogurts.<sup>9–14</sup> In addition, their co-products are valuable sources for extracting bioactive compounds that can be used for enriching foods. This not only promotes sustainable utilization but also aligns with the growing trend of health-conscious consumers seeking the potential benefits of functional foods for improved physical and mental well-being.<sup>11,13</sup> In this sense, *Opuntia ficus-indica* L. could be an interesting option to be incorporated in foods considering its nutritional and functional properties. *Opuntia ficus-indica* belongs to the *Cactaceae* family and it is characterized by thorny fruits that are oval in shape, abundant in seeds, and acidic taste.<sup>15</sup> This fruit is commonly known as prickly pear/cactus pear, tuna (Mexico), or higo chumbo (Spain) and is native to the arid and semi-arid regions of Mexico. Prickly pear is normally consumed as fresh or in artisanal products like jams and candies, which generates a substantial amount of waste in the form of peel (33–55% of total weight) and seeds during processing.<sup>16</sup> The main component of this fruit is water ranging from 820.1 to 945.9 g kg<sup>-1</sup> with a fat and protein content varying between 0.39 and 0.43 g kg<sup>-1</sup> and 8.7 and 9.0 g kg<sup>-1</sup> of edible portion, respectively.<sup>17,18</sup> Moreover, prickly pear is recognized as a rich source of minerals, including potassium, magnesium, calcium, and sodium.<sup>16,17</sup> The peels, particularly, are a valuable source of vitamin E ( $\alpha$ -tocopherol) and vitamin C, with a content of 17.60 g kg<sup>-1</sup> of total lipids, and 0.96 g kg<sup>-1</sup> of ascorbic acid equivalent, respectively.<sup>17,18</sup> Likewise, prickly pear peels are a source of bioactive compounds and pigments like betalains and (poly)phenolic compounds mainly flavonoids that according to the study conducted by Cano *et al.*<sup>19</sup> were of greater concentration than those found in the pulp. These characteristics make them ideal for the food industry, not only as colorants but also as functional ingredients with added health benefits. By utilizing these low-cost co-products, it is possible to promote sustainable practices and unlock the hidden potential within prickly pear fruit.

So, given the increasing consumer demand for foods with enhanced nutritional profiles and health benefits, coupled with the potential of fruit co-products as sources of functional ingredients, and the suitability of yogurt as a vehicle for them, this work addresses the need to explore the valorization of prickly pear co-products (peel and pulp) into flours, characterizing their physicochemical, technological, functional properties, and chemical composition and assess their impact on the technological

properties and quality of a widely consumed product like sheep milk yogurt.

## MATERIALS AND METHODS

### Pulp and peel powders

The fruits of the prickly pear (*O. ficus-indica*) cultivar 'nopal tradicional' were collected at the experimental field station of Miguel Hernandez University in Alicante, Spain at the commercial ripening stage with a weight ranged between 90 and 120 g. The harvest of the fruits was done during the summer of 2024. The fruits were transported to the IPOA research group's laboratory after harvesting. To remove the spines, the fruits were brushed under running tap water for 5 min and the peel was then manually separated from the pulp and each part was blended for 40 s using a vertical cutter. Both the peel and pulp were dried at 55 °C for 30 h in an air tunnel drier and a grinder mill and sieves were used to process the dried material into a powder (particle size < 417 µm), resulting in two distinct products: prickly pear peel flour and prickly pear pulp flour (Supporting Information, Fig. S1).

### Characterization of prickly pear flours

#### Physicochemical properties

The pH was determined using a pH-meter GLP21 (Crison, Barcelona, Spain), and color (CIE  $L^*a^*b^*$  coordinates) was measured in a CM-700 Minolta spectrophotometer (Minolta, Osaka, Japan), equipped with an illuminant  $D_{65}$  and a 10° observer angle. Water activity ( $a_w$ ) was determined in a Novasina TH-500 water activity analyzer (Novasina, Axair Ltd, Pfaffikon, Switzerland) at room temperature. All measurements were performed in triplicate.

#### Proximate composition

Total moisture content (determined by AOAC 925.45), protein (analyzed using AOAC 981.10), fat (AOAC 991.36), ash (measured according to AOAC 923.03), and dietary fiber (determined by AOAC 985.29) were assessed in triplicate following AOAC methods.<sup>20</sup> Mineral composition was analyzed in triplicate following the conditions described by Muñoz-Tebar *et al.*<sup>14</sup> Briefly, samples were digested in a microwave and quantification was performed using inductively coupled plasma mass spectrometry (ICP-MS, Shimadzu MS-2030; Shimadzu, Kyoto, Japan). The operating conditions were: carrier gas flow rate 0.70 L min<sup>-1</sup>, plasma gas flow rate 9.0 L min<sup>-1</sup>, auxiliary gas flow rate 1.10 L min<sup>-1</sup>, radio frequency set at 1.2 kW, and energy filter 7.0.

The extraction and quantification by high-performance liquid chromatography (HPLC) analysis (Hewlett-Packard 1100 series model; Hewlett-Packard, Woldbronn, Germany) of sugars and organic acids were carried out following the method described by Muñoz-Bas *et al.*<sup>21</sup> Samples were analyzed in triplicate and peaks were identified by comparing retention times with standards (organic acids, monosaccharides, and oligosaccharides from Supelco, Sigma-Aldrich, St Louis, MO, USA) and quantified using regression formulas derived from the standards.

#### Techno-functional properties

The water-holding capacity (WHC), oil-holding capacity (OHC), and swelling capacity (SWC) were assessed in triplicate according to Muñoz-Bas *et al.*<sup>21</sup> Results were reported as weight of water held (WHC) or oil held (OHC) per gram of prickly pear flour, and as milliliters per gram of prickly pear flour for SWC.

### (Poly)phenol content

(Poly)phenolic compounds were extracted following the same method described in a previous study.<sup>22</sup> The quantification was performed in triplicate using an Agilent HPLC 1200 series chromatograph (Agilent Technologies, Santa Clara, CA, USA) under the same conditions as described by Genskowsky *et al.*<sup>23</sup>

### Total betalain content

Total betalain content of prickly pear powders (pulp and peel) was determined according to the method described by Ravichandran *et al.*<sup>24</sup> The content of betaxanthins and betacyanins in the extracts was determined spectrophotometrically at 538 and 480 nm, respectively. The betalain concentration (BC) for each sample was calculated with the following formula:  $BC (mg L^{-1}) = [(A \times DF \times MW \times 1000)/(e \times l)]$ , where *A* is the absorbance, *DF* is the dilution factor, and *l* is the pathlength (1 cm) of the cuvette. For quantification of betacyanins and betaxanthins, the molecular weight (MW) and molar extinction coefficient (*e*) applied were  $MW = 550 g mol^{-1}$ ,  $e = 60\,000 L mol^{-1} cm^{-1}$  in water for betacyanins;  $MW = 308 g mol^{-1}$ ,  $e = 48\,000 L mol^{-1} cm^{-1}$  in water for betaxanthins.

### Antioxidant properties

The antioxidant properties of pulp and peel flours were evaluated in triplicate using different antioxidant assays: DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), FRAP (ferric ion reducing antioxidant potential) and FIC (ferrous ion chelating). DPPH and ABTS assays were conducted according to the methods outlined by Brand-Williams *et al.*<sup>25</sup> and Gullon *et al.*,<sup>26</sup> respectively. FRAP analysis was carried out with the procedure described by Oyaizu<sup>27</sup> and FIC using the method described by Mahdavi *et al.*<sup>28</sup>

### Sheep milk yogurts

#### Yogurt production

Three formulations of sheep yogurts were prepared as follows: control, 10 g kg<sup>-1</sup> pulp and 10 g kg<sup>-1</sup> peel powders from prickly pear fruit. Pulp and peel powders were added to the milk at the specified concentrations and homogenized until reaching a temperature of  $42 \pm 2$  °C. Subsequently, the starter culture (YO-MIX® 495; Danisco, Copenhagen, Denmark) was added at the concentration recommended by the manufacturer (10–20 DCU 100 L<sup>-1</sup>) and homogenized for 2 min. The samples were then distributed into sterile 100 mL containers and incubated at  $42 \pm 2$  °C until the pH reached 4.65–4.60. Finally, the yogurts were stored at 4 °C until analysis.

#### Physical–chemical properties

The pH of the yogurts was measured using a Crison GLP 21 pH meter (Crison), while titratable acidity (°Dornic, °D) was determined by titration with 0.11 mol L<sup>-1</sup> 0.11N sodium hydroxide (NaOH) using phenolphthalein as an indicator. All measurements were conducted in triplicate. CIELAB color coordinates (lightness *L\**, red/green coordinate *a\**, and yellow/green coordinate *b\**) were measured as detailed in an earlier section. Nine measurements for each sample were conducted.

#### Proximate composition

The composition of the yogurts was analyzed in triplicate after 24 h of production. Fat, protein, and total solids content were measured using a MilkoScan FT120 (FOSS, Hillerød, Denmark) calibrated for cream, while moisture (AOAC 925.45), total dietary

fiber (TDF, AOAC 985.29) and ash (AOAC 923.03) determined following AOAC methods.<sup>20</sup>

The mineral composition of the sheep yogurts enriched with pulp and peel from cactus pear was determined using the same methodology described in an earlier section.

#### Texture

Sheep yogurts were prepared in 100 mL sterile polypropylene containers (diameter = 55 mm; height = 70 mm) and texture was evaluated by back extrusion test using a Texture Analyzer TA-XT2i (Stable Micro Systems Ltd, Godalming, UK). Measurements were performed in triplicate and the results were expressed as firmness (N), consistency (N s), cohesiveness (N), and viscosity index (N s).

#### Sugar and organic acids content

Sugars and organic acid contents of the sheep yogurts enriched with pulp and peel from cactus pear was determined using the same methodology explained in an earlier section.

#### Microbiological quality

*Lactobacillus* spp. and *Streptococcus* spp. counts were measured by homogenizing 10 g of sample with 90 mL of sterile peptone water 1 g kg<sup>-1</sup> (w/v) and subsequent decimal dilutions. Duplicate seeding was performed on agar MRS for *Lactobacillus* spp. and on agar M17 for *Streptococcus* spp. followed by incubation at 37 °C for 48 h and under anaerobic conditions using Anaerocult A (Merck, Darmstadt, Germany) for 48 h, respectively. Enterobacteria counts as well as mold and yeast, were determined alongside by seeding on Petrifilm plates (3M, Madrid, Spain) in duplicate with subsequent incubation at 37 °C for 24 h and at 25 °C for 120 h, respectively. Results were expressed as log CFU g<sup>-1</sup> of yogurt.

#### (Poly)phenol and total betalain content

(Poly)phenol profile and total betalain content of sheep yogurts enriched with pulp and peel from cactus pear was determined using the same methodology described in earlier sections.

#### Antioxidant activity

Antioxidant properties of sheep yogurts enriched with pulp and peel from prickly pear fruit was determined as described in an earlier section.

#### Sensory analysis

Seventy-six participants (61% male, 39% female, aged between 18 and 40 years) were recruited from the Orihuela Polytechnic School of Miguel Hernández University (UMH) to evaluate the sheep yogurts formulated with cactus pear. Prior to commencing the analyses, all participants were briefed on the distinct features of the product they would be tasting and the nature of the analysis itself. They also provided their written informed consent. This study received approval from the Responsible Research Office at UMH (OIR- Reg. 211128200759, Ref. PRL.DTA.JPA.05.21, UMH, Elche, Alicante, Spain).

Sensory evaluations took place in the standardized sensory laboratory of UMH, meeting international standards and participants were situated in individual booths. Yogurt samples (10 g) were served in transparent plastic cups codified with random three-digit codes and participants rated the samples for odor, taste, color, acidity, firmness and overall acceptability using a nine-point hedonic scale ranging from 'dislike extremely' (1) to 'like

extremely' (9).<sup>29</sup> All participant were informed and gave their consent to participate in the sensory evaluation of the product and in the further processing of the data.

### Statistical analysis

Statistical analysis of data was performed using SPSS (IBM SPSS Statistics version 26; SPSS, Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was calculated using a confidence level of 95% to determine any significant differences ( $P < 0.05$ ) and Tukey test was carried out to determine the differences between the yogurts enriched with pulp or peel from prickly pear fruit.

## RESULTS AND DISCUSSION

### Characterization of prickly pear's pulp and peel flours

#### Physicochemical properties

The physicochemical properties (pH,  $a_w$ , and color) of the flours obtained from the edible (pulp) and non-edible (peel) parts of the prickly pear fruit are presented in Table 1. The peel flour exhibited a slightly higher pH value than the pulp flour ( $P < 0.05$ ), however, it was observed that the  $a_w$  was lower in the peel flour (0.442 versus 0.481). Both pH and  $a_w$  are indicators of food spoilage, so the low  $a_w$  and pH values suggest that the spoilage levels of both flours are minimal.<sup>30</sup> Water activity and pH values obtained were higher than those reported by Borchani *et al.*<sup>31</sup> for prickly pear peel flours and lower than those obtained for prickly pear fruit by Apablaza *et al.*<sup>32</sup> Likewise, it was found that the values of these parameters were comparable to those obtained in pitahaya pulp and peel flours from a recently published study carried out by Reyes-García *et al.*<sup>33</sup>

Color is one of the most important parameters in evaluating new ingredients with potential for incorporation into food matrices since product color affects consumer acceptance and purchase intention. As shown in Table 1, the color coordinates ( $L^*a^*b^*$ ) of the flours displayed significant differences ( $P < 0.05$ ), with the pulp flour reduced lightness and being more reddish and yellowish than the peel flour and this difference is mainly due to the inherent color variations between the fruit's parts. In this sense, a similar trend in color differences has also been observed in flours obtained from pitahaya pulp and peel.<sup>33</sup> Compared to other studies on prickly pear fruit and peel color properties, the values obtained for the peel flour were higher than those

**Table 1.** Physicochemical and techno-functional properties of pulp and peel flours obtained from prickly pear fruit (mean  $\pm$  standard deviation)

Parameter	Pulp	Peel	P-Value
pH	5.33 $\pm$ 0.01	5.38 $\pm$ 0.01	0.001
$a_w$	0.481 $\pm$ 0.002	0.442 $\pm$ 0.000	0.002
$L^*$	60.56 $\pm$ 1.00	64.15 $\pm$ 0.50	0.005
$a^*$	12.28 $\pm$ 0.43	11.40 $\pm$ 0.03	0.024
$b^*$	38.01 $\pm$ 2.29	33.03 $\pm$ 0.36	0.020
WHC (g g <sup>-1</sup> )	3.40 $\pm$ 0.33	3.77 $\pm$ 0.09	NS
OHC (g g <sup>-1</sup> )	1.28 $\pm$ 0.09	1.22 $\pm$ 0.16	NS
SWC (mL g <sup>-1</sup> )	9.78 $\pm$ 1.26	6.60 $\pm$ 0.29	0.013

Note:  $a_w$ , water activity; water-holding capacity (WHC) expressed as g of water g<sup>-1</sup> of sample; oil-holding capacity (OHC) expressed as g of oil g<sup>-1</sup>; swelling capacity (SWC) expressed as mL g<sup>-1</sup> sample. NS: ( $P > 0.05$ ).

reported by other authors for prickly pear peel powders.<sup>31,34,35</sup> However, the lightness and yellowness coordinates of the pulp flour were similar to those reported by Apablaza *et al.*<sup>32</sup> for prickly pear fruit pulp.

#### Proximate composition

Table 2 shows the gross composition of flours obtained from prickly pear (*O. ficus-indica*) pulp and peel, revealing significant differences ( $P < 0.05$ ) in all parameters. In general, moisture, fat, dietary fiber and protein values were higher in pulp flour compared to the peel, while peel flour had a higher ash content (105.9 versus 71.7 g kg<sup>-1</sup>). This fact has been previously observed in flours obtained from pitahaya pulp and peel, although the difference was more pronounced in that study (36.3 versus 116.3 g kg<sup>-1</sup> in pulp and peel, respectively).<sup>33</sup>

For peel flour, moisture values were similar to those reported by Parafati *et al.*<sup>36</sup> (81.70 g kg<sup>-1</sup>) and Bouazizi *et al.*<sup>34</sup> (91.1 g kg<sup>-1</sup>) in flours obtained from peel of different cultivar of prickly pear. However, the protein content in the current study's peel flour was much higher than the values reported by these authors (45.1 versus 35.8 and 33.0 g kg<sup>-1</sup>, respectively). Ash and fat contents were lower than those found by Borchani *et al.*,<sup>31</sup> Bouazizi *et al.*<sup>34</sup> and Parafati *et al.*<sup>36</sup> in prickly pear peel flours. When compared to flours from other fruit peels, the protein and fat content of the peel flour were comparable to those determined by Cangussu *et al.*<sup>37</sup> for seriguella (*Spondias purpurea* L.) peel flour.

Dietary fibers are well-recognized for their crucial role in developing new functional foods due to their technological properties and health benefits including their positive effects on

**Table 2.** Chemical composition of pulp and peel flours obtained from prickly pear fruit (mean  $\pm$  standard deviation)

Parameter	Pulp	Peel	P-Value <sup>a</sup>
<i>Proximal composition (g kg<sup>-1</sup>)</i>			
Moisture	110.1 $\pm$ 1.30	86.6 $\pm$ 2.86	0.000
Ash	71.70 $\pm$ 4.80	105.9 $\pm$ 0.71	0.000
Protein	73.73 $\pm$ 0.90	45.1 $\pm$ 0.92	0.001
Fat	27.42 $\pm$ 2.86	12.6 $\pm$ 2.14	0.002
Total dietary fiber	281.15 $\pm$ 13.7	330.6 $\pm$ 1.30	0.037
<i>Mineral content (mg kg<sup>-1</sup>)</i>			
Calcium	17 289.5 $\pm$ 853.2	26 670.31 $\pm$ 500.92	0.000
Copper	4.8 $\pm$ 0.12	3.60 $\pm$ 0.52	0.012
Iron	22.1 $\pm$ 1.12	47.22 $\pm$ 4.82	0.001
Potassium	15 773.8 $\pm$ 695.95	18 631.51 $\pm$ 1041.11	0.017
Magnesium	3365.91 $\pm$ 134.25	4508.23 $\pm$ 96.64	0.000
Manganese	06.8 $\pm$ 0.33	10.22 $\pm$ 0.30	0.000
Sodium	302.4 $\pm$ 13.12	140.32 $\pm$ 10.83	0.000
Phosphorus	1667.2 $\pm$ 92.65	972.51 $\pm$ 85.52	0.001
Zinc	19.2 $\pm$ 2.12	13.10 $\pm$ 1.13	0.011
<i>Sugars and organic acids (mg g<sup>-1</sup>)</i>			
Glucose	342.09 $\pm$ 6.87	290.74 $\pm$ 3.40	0.006
Fructose	325.92 $\pm$ 1.40	317.49 $\pm$ 3.66	0.013
Citric acid	15.09 $\pm$ 1.28	19.45 $\pm$ 0.24	0.042
Malic acid	10.57 $\pm$ 1.67	12.58 $\pm$ 1.68	NS
Fumaric acid	0.76 $\pm$ 0.06	2.00 $\pm$ 0.22	0.017

<sup>a</sup> NS:  $P > 0.05$ .

cardiovascular health, enhancement of intestinal function and insulin response, influence on fat and cholesterol absorption, and a decreased risk of certain cancers.<sup>38</sup> As shown in Table 2, both the peel and pulp have a significant dietary fiber content, which would suggest that both flours could be used as ingredients in the formulation of fiber-enriched foods. Compared to other studies on cactus pear dietary fiber, the content in the pulp flour was greater than the values reported by Albergamo *et al.*<sup>39</sup> in Tunisian *O. ficus-indica* pulp (40.6 versus 281.1 mg kg<sup>-1</sup>). Meanwhile, the fiber content in the peel flour was comparable to the findings of Parafati *et al.*<sup>36</sup> on prickly pear peel flour (330 versus 330.6 mg kg<sup>-1</sup>).

Finally, it has been observed that gross composition of prickly pear pulp and peel flours can vary widely in the literature due to factors such as the use of different varieties, analysis conditions, and the ripening stage of the fruit.

The results of the mineral content analysis (Table 2) revealed that the major elements found in both *O. ficus-indica* pulp and peel flours were calcium, potassium, magnesium, phosphorus, and sodium follow by iron, zinc, manganese and copper with statistical differences ( $P < 0.05$ ) between flours. These differences in mineral content between the peel and pulp have been previously observed in pitahaya flours, with the peel showing higher levels of calcium and potassium,<sup>33</sup> although in lower concentrations than those found in the present study.

It was found that the concentration of copper, sodium, phosphorus, and zinc was greater in pulp flour ( $P < 0.05$ ), while the rest of the minerals (calcium, iron, potassium, magnesium, and manganese) were significantly higher ( $P < 0.05$ ) in the peel flour, in fact it was noteworthy that calcium content in peel flour was almost twice higher compared to the pulp (26.67 versus 17.28 g kg<sup>-1</sup>). Mineral content of the pulp flour was higher than the values found by Lamia *et al.*<sup>40</sup> when they analyzed the *O. ficus-indica* fruit. These differences could be due to the fact that in the present study, the pulp was dehydrated, and this process normally causes a concentration effect on the fruit's macronutrients and micronutrients.

In the recent study carried out by Albergamo *et al.*<sup>39</sup> on the mineral content of different parts of the prickly pear fruit (pulp, peel, and seeds), the concentrations of magnesium and potassium found in the peel were lower than those present in the peel flour obtained for study. Similarly, Czech *et al.*<sup>41</sup> conducted a study on the comparison of the mineral content of the pulp and peel of various fruits (orange, lemon, pomelo, key lime, and grapefruit), confirming that the peel showed higher amounts of minerals and trace elements than the pulp. The concentration of minerals and trace elements in fruits can be affected by several factors, including the mineral composition of the soil where it is grown on, the composition of the irrigation water, weather conditions, and agricultural practices applied. Additionally, the variety of the fruit plays a significant role in determining its mineral content.<sup>41</sup>

#### Sugar and organic acids content

The flours obtained from prickly pear (both peel and pulp) primarily consist of glucose and fructose as the main sugars, along with citric, malic, and fumaric acids as the major organic acids. The sugars content was found higher ( $P < 0.05$ ) in pulp flour, while organic acids were higher in the peel, although significant differences ( $P < 0.05$ ) were only observed in citric acid (15.09 versus 19.45 mg g<sup>-1</sup>) and fumaric acid (0.76 versus 2.00 mg g<sup>-1</sup>), with higher concentrations found in the peel ( $P < 0.05$ ) compared to the pulp (Table 2). In this sense, Andreu *et al.*<sup>42</sup> reported a

similar composition of organic acids and sugars in various cultivars of *O. ficus-indica*, also noticing greater concentrations of organic acids in the peel and sugars in the pulp of the fruit.

#### Techno-functional properties

The techno-functional properties of the flours derived from cactus pear (*O. ficus-indica*) pulp and peel co-products, were measured as WHC, OHC, and SWC since flours formulated from vegetable co-products normally displayed good hydration properties. The results of these properties are illustrated in Table 1, noticing a significant increase ( $P < 0.05$ ) in OHC and SWC of the flour obtained from the pulp, while the WHC was slightly higher in the flour obtained from the prickly pear peel (3.77 versus 3.40 g water g<sup>-1</sup> sample).

As far as we are concerned, no studies have been conducted on the techno-functional properties of prickly pear pulp flour so far. However, several studies have examined these properties in flour derived from the peel. In this regard, WHC, OHC, and SWC values were higher than those obtained by Borchani *et al.*<sup>31</sup> and Bouazizi *et al.*<sup>34</sup> with the SWC being nearly six times greater (6.6 versus 0.95 mL water g<sup>-1</sup> sample) and the OHC almost twice higher (1.22 versus 0.91 g oil g<sup>-1</sup> sample).

Compared to flours obtained from other fruits, the prickly pear peel flour demonstrated a better WHC than the flour obtained from seriguela peel (3.97 versus 2.02 g water g<sup>-1</sup> sample),<sup>33</sup> whilst flours derived from pitahaya peel and pulp<sup>33</sup> exhibited superior techno-functional properties compared to those in the present study.

#### (Poly)phenol and total betalain content

(Poly)phenolic compounds found in fruits and their co-products represent a significant source of bioactive compounds with various functional properties, including antioxidant activity. Table 3 shows the (poly)phenolic profile of flours derived from both the edible and non-edible parts of prickly pear (*O. ficus-indica*) fruit. The main phenolic compounds identified in both flours were isorhamnetin-pentosyl-rutinoside, followed by isorhamnetin-pentosyl-hexoside, quercetin 3- $\beta$ -D-glucoside, isorhamnetin-rhamnose-rutinoside, and quercetin-3-O-rutinoside. Notably, the flour formulated with the peel contained a higher concentration of phenolic compounds than the pulp flour. This difference in (poly)phenolic content between the peel and pulp had also been observed in flours derived from pitahaya skin and pulp as reported Reyes-García *et al.*<sup>33</sup> Likewise, the (poly)phenolic profile analyzed in this study aligns with the findings previously reported in the literature for *O. ficus-indica* fruit peel and pulp.<sup>43,44</sup>

Betalains are water-soluble red and yellow pigments that can be found in *O. ficus-indica* (L.) in the form of betacyanins in the red-purple variety and betaxanthins in yellow-orange varieties.<sup>45</sup> These pigments possess several biological effects, such as antioxidant and anti-cancer properties, anti-lipidemic effects, hepatoprotective effects, and neuroprotective effects, among others.<sup>46</sup> Therefore, total betalain content of prickly pear peel and pulp flours were determined (sum of betacyanins and betaxanthins content) and the results are shown in Table 3.

As expected, the peel flour exhibited a significantly higher content ( $P < 0.05$ ) of total betalains compared to the pulp flour (114.40 versus 83.78 mg L<sup>-1</sup>) and this observation is consistent with previous findings by Reyes-García *et al.*,<sup>33</sup> who also reported higher total betalain content in flours obtained from pitahaya peel compared to pulp.

The concentration of betacyanins was significantly higher ( $P < 0.05$ ) in the peel than in the pulp (92.6 versus 69.7 mg L<sup>-1</sup>), obtaining a higher value than those reported by other authors in prickly pear peel.<sup>47,48</sup> Likewise, a lower concentration of betaxanthin was observed in both peel and pulp compared to betacyanins, a trend that has been previously noted by Sumaya-Martínez *et al.*<sup>49</sup> in different prickly pear varieties (values ranging from 8 to 147 mg indicaxanthin L<sup>-1</sup>).

Overall, the concentration of total betalains in both flours is significant, suggesting they could be valuable as ingredients for developing functional products or as natural colorants. In fact, betanin is a commercial colorant (E-162) currently used in dairy products, sauces, and soups.<sup>48</sup>

#### Antioxidant properties

The extracts obtained from plants and fruits contain a complex mixture of bioactive compounds and thus multiple mechanisms of action can be involved in the antioxidant activity. Therefore, it is crucial to use different antioxidant methods to achieve a comprehensive evaluation of their antioxidant potential.<sup>46</sup> The results of the antioxidant capacity of prickly pear flours evaluated with FRAP, DPPH, FIC, and ABTS are illustrated in Table 3. The peel flour showed a higher ( $P < 0.05$ ) antioxidant capacity with all methods used to determine the antioxidant capacity, compared to the pulp, which may be linked to its greater content of total betalains

and (poly)phenolic compounds, compared to the flour obtained from the pulp.

#### Effect of prickly pear flour in sheep milk yogurts

##### Coagulation curve

During typical yogurt fermentation, LAB decrease in pH causing the destabilization and gelation of the casein micelles, which can aggregate with the whey proteins through hydrophobic and electrostatic bonds creating three-dimensional acid milk gels whose structure and strength will determine the texture and mouthfeel of the yogurt.<sup>50</sup> The incorporation of both peel and pulp flours obtained from prickly pear slightly lowered the pH of the yogurt at the beginning of fermentation, likely due to the low pH values of both flours (Table 1). The pH decreased more rapidly in the yogurts containing prickly pear flours compared to the control yogurt, reaching the required final pH (4.65–4.60) in 6.30 h for the yogurts with prickly pear flours, while the yogurts formulated with only sheep's milk took an hour longer to reach the same pH. This behavior in the coagulation process during yogurt's fermentation has been previously observed in a similar study that we currently carried out about the fortification of goat yogurts with date co-products<sup>14</sup> and by Sah *et al.*<sup>51</sup> in yogurt formulated with pineapple powder. Based on the results it could be said that adding prickly pear flours (both pulp and peel) to yogurts might promote early formation of the protein network, explaining the faster acidification observed (Fig. S2).

**Table 3.** (Poly)phenolic profile and bioactive properties of pulp and peel flours obtained from prickly pear fruit (mean  $\pm$  standard deviation)

Compound	Pulp	Peel	P-Value <sup>a</sup>
<i>(Poly)phenolic profile (<math>\mu\text{g g}^{-1}</math>)</i>			
Quercetin derivative	5.82 $\pm$ 0.37	17.34 $\pm$ 1.64	0.021
Quercetin derivative	7.43 $\pm$ 0.75	25.71 $\pm$ 3.79	0.042
Isorhamnetin derivative	7.32 $\pm$ 1.10	20.92 $\pm$ 0.51	0.008
Isorhamnetin-rhamnose-rutinoside	86.87 $\pm$ 3.00	256.64 $\pm$ 32.47	0.035
Isorhamnetin-pentosyl-rutinoside	176.72 $\pm$ 3.82	587.83 $\pm$ 73.28	0.030
Isorhamnetin derivative	13.55 $\pm$ 0.41	48.99 $\pm$ 7.00	0.037
Quercetin-3-O-rutinoside	17.01 $\pm$ 0.86	88.41 $\pm$ 13.37	0.033
Quercetin 3- $\beta$ -D-glucoside	77.83 $\pm$ 3.77	333.61 $\pm$ 48.28	0.034
Quercetin derivative	7.88 $\pm$ 0.72	25.24 $\pm$ 3.17	0.033
Isorhamnetin-pentosyl hexoside	119.7 $\pm$ 5.98	431.83 $\pm$ 63.88	0.040
Isorhamnetin-3-O-glucoside	ND	8.09 $\pm$ 1.77	0.044
Quercetin-3-rhamnoside	ND	12.38 $\pm$ 0.61	0.002
5-Caffeoylquinic acid	ND	22.34 $\pm$ 1.62	0.005
Synapic acid hexoside	14.68 $\pm$ 0.44	9.23 $\pm$ 0.98	0.037
Caffeic acid	21.52 $\pm$ 0.11	12.58 $\pm$ 1.54	0.029
Ferulic acid-derivative	ND	17.09 $\pm$ 1.71	0.005
<i>Betalain content (mg L<sup>-1</sup>)</i>			
Betaxanthins	14.12 $\pm$ 1.11	21.82 $\pm$ 2.22	0.001
Betacyanins	69.67 $\pm$ 1.59	92.58 $\pm$ 4.20	0.001
Total betalain	83.78 $\pm$ 1.41	114.40 $\pm$ 6.34	0.001
<i>Antioxidant activity</i>			
FRAP (mg Trolox g <sup>-1</sup> )	8.41 $\pm$ 1.16	14.62 $\pm$ 0.77	0.002
DPPH (mg Trolox g <sup>-1</sup> )	1.26 $\pm$ 0.00	1.29 $\pm$ 0.01	0.001
ABTS (mg Trolox g <sup>-1</sup> )	1.94 $\pm$ 0.19	2.28 $\pm$ 0.06	0.04
FIC (mg EDTA g <sup>-1</sup> )	0.42 $\pm$ 0.02	0.45 $\pm$ 0.01	0.051

Note: DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); FRAP, ferric ion reducing antioxidant potential; FIC, ferrous ion chelating; ND, not detected.

<sup>a</sup> NS: ( $P > 0.05$ ).

### Physical–chemical properties

The results of incorporating prickly pear peel and pulp flours into sheep yogurt and their effects on the physicochemical parameters are shown in Table 4. The  $a_w$  value was not affected by the incorporation of prickly pear flour, obtaining similar values in all formulations (0.964–0.967), as already observed when saffron floral co-products extracts were incorporated in cow yogurts.<sup>52</sup> However, a significant increase in acidity was observed in yogurts formulated with prickly pear compared to the control (96 *versus* 115–120 °D). Taheur *et al.*<sup>15</sup> also noted an increase in the titratable acidity of kefir-based probiotic enriched with red prickly pear (*Opuntia dillenii*) powder, attributing this to phytochemical compounds such as phenols and organic acids present in cactus pear. The highest acidity value was recorded in the yogurts formulated with the peel flour, which could be explained by the higher content of phenolic compounds and organic acids found in the peel compared to the pulp (Tables 2 and 3).

Color is a crucial factor in the creation and marketing of fermented dairy products because it impacts consumer acceptance and preference. It can differ based on several factors, such as the type of milk used, the fermentation process and the different flavorings or ingredients added. Therefore, color properties (coordinates  $L^*a^*b^*$ ) of the sheep yogurts formulated with cactus pear flours was assessed noticing a significant effect ( $P < 0.05$ ) compared to the control sample. The brightness ( $L^*$ ) of the yogurts decreased with the addition of prickly pear flour, dropping from 81.82 to 78.07. However, the type of flour did not influence this change, as the  $L^*$  values were similar for both types of yogurts (Table 4). However, coordinates  $a^*$  and  $b^*$  significantly increased ( $P < 0.05$ ) in yogurts containing prickly pear flour (both peel and pulp), with  $a^*$  values ranging from −2.47 to 0.91 and  $b^*$  values from 6.51 to 11.84. This indicates that yogurts with prickly pear flours exhibited more reddish and yellowish colors compared to the control yogurt formulated only with sheep's milk, primarily due to the natural color of the flours itself (Table 1). Similar findings were observed in kefir-based probiotic dairy product enriched with red prickly pear powder<sup>15</sup> and in yogurts fortified with betalains and (poly)phenols from prickly pear extract encapsulated in a multiple emulsion,<sup>6</sup> and in a drinkable yogurt incorporating with purple cactus pear powder from the fruit juice.<sup>1,13</sup>

### Proximate composition

The effect of incorporating prickly pear pulp and peel flour on the proximate composition of the yogurts is shown in Table 5. Moisture and ash content were not affected by the addition of prickly pear flours, with moisture values ranging from 880.8 to 891.1 g kg<sup>−1</sup>, and ash content varying between 6.9 and 10.1 g kg<sup>−1</sup>. This trend has been previously noticed in the recent work that we have carried out on the fortification of goat yogurt with date flour.<sup>14</sup> On the contrary, the addition of prickly pear peel and pulp flours resulted in a significant ( $P < 0.05$ ) increase in protein, fat, and total solids content of the yogurts compared to the control, with the yogurt formulated with the pulp flour showing the highest values (Table 5). The differences observed between the yogurt incorporated with pulp and peel were consistent with those observed in the proximal composition of the flours, where pulp flour had the highest values of fat and protein (Table 2). Similar results regarding the increase in protein content due to fruit incorporation in yogurts have been previously reported in yogurt added with 50 g kg<sup>−1</sup> of white sapote fruit pulp<sup>54</sup> and in cow yogurt enriched with prickly pear pulp.<sup>55</sup> With regards to the increase in fat content, Dinkçi *et al.*<sup>55</sup> observed that the fat content of yogurt formulated with 20 g kg<sup>−1</sup> of hazelnut skin increased compared to the control.

Based on the results, it could be said that the incorporation of prickly pear flours (both pulp and peel) had a positive effect on the composition of yogurts, which would confirm its suitability as a new ingredient in the development of dairy products.

### Mineral composition

Mineral composition of sheep yogurts as affected by the incorporation of flours obtained from prickly pear's pulp and peel are presented in Table 5. The addition of prickly pear flour did not cause any significant change in the calcium, copper, iron, manganese and sodium content of the yogurts, while the concentration of potassium, magnesium, phosphorus and zinc increased significantly ( $P > 0.05$ ) in the yogurts formulated with prickly pear flours (both peel and pulp) compared to the control yogurt. The results align with the mineral content observed in the flours (Table 2), which showed high levels of potassium, magnesium, phosphorus and zinc, with greater concentrations in the peel ( $P < 0.05$ ) than in the pulp. This would explain the differences in the mineral content between the yogurts formulated with peel flour and those containing pulp flour. The potassium content of yogurts increased

**Table 4.** Effect of prickly pear incorporation on the physicochemical properties of yogurts elaborated with sheep milk (mean ± standard deviation)

Parameter	Control	1% Peel	1% Pulp	P-Value
pH	5.06 ± 0.11	4.92 ± 0.03	4.98 ± 0.02	NS
Acidity (°D)	96.00 ± 4.24 <sup>b</sup>	120.00 ± 1.41 <sup>a</sup>	115.00 ± 2.83 <sup>a</sup>	0.090
$a_w$	0.965 ± 0.001	0.964 ± 0.001	0.967 ± 0.002	NS
$L^*$	81.82 ± 0.42 <sup>a</sup>	78.07 ± 0.48 <sup>b</sup>	78.83 ± 0.35 <sup>b</sup>	0.000
$a^*$	−2.47 ± 0.05 <sup>a</sup>	0.91 ± 0.36 <sup>b</sup>	0.57 ± 0.02 <sup>b</sup>	0.000
$b^*$	6.51 ± 0.27 <sup>b</sup>	10.99 ± 0.65 <sup>a</sup>	11.84 ± 0.29 <sup>a</sup>	0.000
Firmness (N)	0.07 ± 0.01 <sup>b</sup>	0.18 ± 0.01 <sup>a</sup>	0.18 ± 0.00 <sup>a</sup>	0.000
Consistency (N s)	0.88 ± 0.10 <sup>b</sup>	2.62 ± 0.33 <sup>a</sup>	2.38 ± 0.21 <sup>a</sup>	0.000
Cohesiveness (N)	−0.03 ± 0.01 <sup>a</sup>	−0.12 ± 0.00 <sup>b</sup>	−0.11 ± 0.00 <sup>b</sup>	0.000
Viscosity index (N s)	−0.01 ± 0.01 <sup>a</sup>	−0.12 ± 0.01 <sup>b</sup>	−0.11 ± 0.01 <sup>b</sup>	0.000

Note: NS:  $P > 0.05$ . Different superscript lowercase letter in the same row indicates significant differences ( $P < 0.05$ ) due to prickly pear addition.  $a_w$ , water activity.

**Table 5.** Effect of prickly pear incorporation on the chemical compositions of yogurts elaborated with sheep milk (mean  $\pm$  standard deviation)

Parameter	Control	1% Peel	1% Pulp	P-value
<i>Proximate composition (g kg<sup>-1</sup>)</i>				
Moisture	891.12 $\pm$ 11.13	881.3 $\pm$ 0.73	880.82 $\pm$ 1.53	NS
Ash	9.01 $\pm$ 1.52	6.9 $\pm$ 3.44	10.12 $\pm$ 1.24	NS
Protein	19.64 $\pm$ 0.2q <sup>c</sup>	20.2 $\pm$ 0.33 <sup>b</sup>	21.01 $\pm$ 0.44 <sup>a</sup>	0.000
Fat	34.55 $\pm$ 0.31 <sup>c</sup>	35.2 $\pm$ 0.32 <sup>b</sup>	36.40 $\pm$ 0.32 <sup>a</sup>	0.000
Total solids	36.71 $\pm$ 0.30 <sup>b</sup>	40.8 $\pm$ 2.72 <sup>a</sup>	43.55 $\pm$ 0.32 <sup>a</sup>	0.000
<i>Mineral content (mg 100 g<sup>-1</sup> wet basis)</i>				
Calcium	142.42 $\pm$ 1.82	144.23 $\pm$ 2.65	139.14 $\pm$ 2.96	NS
Copper	0.02 $\pm$ 0.00	0.03 $\pm$ 0.01	0.01 $\pm$ 0.00	NS
Iron	0.24 $\pm$ 0.03	0.19 $\pm$ 0.02	0.20 $\pm$ 0.01	NS
Potassium	82.26 $\pm$ 2.45 <sup>c</sup>	104.57 $\pm$ 1.85 <sup>a</sup>	90.85 $\pm$ 1.10 <sup>b</sup>	0.000
Magnesium	13.62 $\pm$ 1.26 <sup>c</sup>	16.81 $\pm$ 0.20 <sup>a</sup>	14.20 $\pm$ 0.43 <sup>b</sup>	0.005
Manganese	0.01 $\pm$ 0.01	0.03 $\pm$ 0.01	0.02 $\pm$ 0.00	NS
Sodium	71.38 $\pm$ 8.23	68.07 $\pm$ 1.73	60.56 $\pm$ 1.67	NS
Phosphorus	128.28 $\pm$ 2.32 <sup>b</sup>	140.68 $\pm$ 1.97 <sup>a</sup>	126.56 $\pm$ 1.09 <sup>b</sup>	0.000
Zinc	0.33 $\pm$ 0.00 <sup>b</sup>	0.41 $\pm$ 0.05 <sup>a</sup>	0.29 $\pm$ 0.02 <sup>b</sup>	0.006
<i>Sugars and organic acids (g L<sup>-1</sup>)</i>				
Glucose	ND	0.24 $\pm$ 0.01 <sup>b</sup>	0.28 $\pm$ 0.00 <sup>a</sup>	0.006
Galactose	0.39 $\pm$ 0.00 <sup>c</sup>	0.53 $\pm$ 0.00 <sup>b</sup>	0.58 $\pm$ 0.00 <sup>a</sup>	0.000
Lactose	1.53 $\pm$ 0.01	1.43 $\pm$ 0.02	1.55 $\pm$ 0.07	NS
Citric acid	0.13 $\pm$ 0.00	0.14 $\pm$ 0.00	0.15 $\pm$ 0.01	NS
Lactic acid	0.09 $\pm$ 0.00 <sup>b</sup>	0.17 $\pm$ 0.02 <sup>a</sup>	0.20 $\pm$ 0.01 <sup>a</sup>	0.007

NS:  $P > 0.05$ ; ND, not detected. Values followed by the same letter in the same row indicate no significant differences ( $P > 0.05$ ) due to prickly pear addition.

from 0.82 to 1.04 mg kg<sup>-1</sup> in yogurts with peel flour, while phosphorus increased from 1.26 to 1.41 g kg<sup>-1</sup>. Phosphorus is a crucial mineral for human health, as it participates along with calcium in the formation of strong bones and teeth. Based on the present results, it could be said that yogurts formulated with prickly pear peel flour would provide 32% of the adequate intake (AI) established by the European Food Safety Authority (EFSA) (550 mg d<sup>-1</sup>). However, the control yogurts would cover 28% of the AI, indicating a 4% reduction compared to the yogurts formulated with prickly pear peel flour. The study carried out by Khalil *et al.*<sup>53</sup> on the incorporation of white sapote fruit to cow yogurts also revealed an increase in phosphorus (from 626 to 650 mg kg<sup>-1</sup>) and zinc (from 1.22 to 1.56 mg kg<sup>-1</sup>). Similarly, our previous study on the incorporation of date flour in goat yogurts showed an increase in potassium and zinc content compared to control yogurts formulated only with goat milk.<sup>14</sup>

Overall, it could be said that both prickly pear's peel and pulp flours could be suitable ingredients to increase the mineral content of foods, helping to fulfill the requirements of these micronutrients essential for human health and wellbeing.

#### Sugar and organic acids profile

Organic acids and soluble sugars play a significant role in both sensory properties and preservation during yogurt production. Therefore, the results of the organic acid and sugars of the sheep yogurts formulated with cactus pear are shown in Table 5. Lactic and citric acids were the main compounds ( $P < 0.05$ ) produced, with the highest concentrations found in yogurts formulated with pulp flour, followed by those containing the peel and finally the control yogurts. In terms of sugars, galactose and lactose were present in all yogurts, while glucose was only detected in those

that containing prickly pear flour ( $P > 0.05$ ), suggesting that its presence was mainly due to the glucose content of the prickly pear flours (Table 2).

This composition of organic acids and sugars aligns with a similar study that we previously conducted on goat yogurts enriched with date flour in which it was also observed that the glucose present in the reformulated yogurts came from the added flour since it was not detected in the control yogurts.<sup>14</sup>

#### Texture

The texture is a key factor in determining yogurt quality and consumer acceptance, with ideal yogurt being thick, smooth, and without signs of syneresis. Therefore, the texture characteristics of yogurt are essential for evaluating its quality. Table 4 shows the results of texture parameters, including firmness, consistency, cohesiveness, and viscosity index of the yogurts influenced by the incorporation of cactus pear flours. The firmness and consistency of the yogurts significantly increased ( $P < 0.05$ ) when prickly pear flour was incorporated while the consistency and index of viscosity decreased regardless of the part of the fruit used in the formulation (peel or pulp). Firmness and consistency of yogurt have been previously linked to its total solid content, which contributes to a stable and cohesive structure.<sup>56</sup> This is in line with the composition of the yogurts since those formulated with prickly pear flours presented higher values of total solids than the control yogurts (Table 5). Additionally, other components or added ingredients, such as the phenolic compounds present in cactus pear might help to stabilize the yogurt gel.<sup>57</sup>

This evolution in firmness and consistency due to fruit addition has been previously reported in our previous work when we incorporated 3% date flour in goat yogurts and by Du *et al.*<sup>57</sup> in

yogurts formulated with mulberry pomace ( $5 \text{ g kg}^{-1}$ ). Regarding viscosity index, decreased values were noted in yogurts with 150 and  $300 \text{ g kg}^{-1}$  of strawberry pulp<sup>58</sup> and those with 10, 30 and  $50 \text{ g kg}^{-1}$  of grape pulp.<sup>59</sup>

#### Microbial stability

The counts of starter culture LAB (*Lactobacillus* spp. and *Streptococcus* spp.) in sheep yogurt influenced by the incorporation of flours obtained from cactus pear's peel and pulp are shown in Fig. 1. The addition of prickly pear flour (both peel and pulp) did not impair the survival of the starter strains, obtaining similar values to those of the control yogurt ( $P > 0.05$ ) and varying from 8.37 to  $8.42 \text{ log CFU g}^{-1}$  in the case of *Lactobacillus* spp. and from 7.44 to  $7.46 \text{ log CFU g}^{-1}$  for *Streptococcus* spp. Likewise, all yogurts had viable counts for both strains of bacterial counts higher than the minimum concentration required ( $10^6 \text{ CFU g}^{-1}$ ) established by the Codex Alimentarius for yogurts.<sup>60</sup> *Enterobacteriaceae*, molds, and yeasts were not detected in any of the yogurts, confirming the proper hygienic quality of both the prickly pear flours and the manufacturing process.

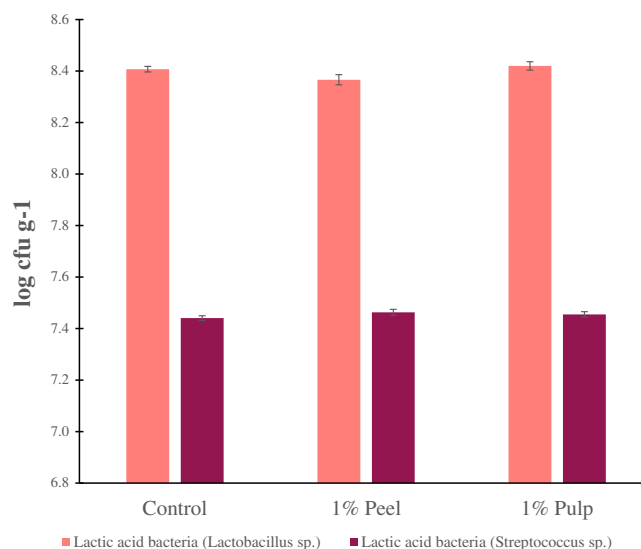
Kefir formulated with prickly pear (*O. dillenii*) powder elaborated by Taheur *et al.*<sup>15</sup> showed viable counts similar to those of the present work without negative effect on the growth of the starter LAB strains compared to the control. Similarly, Cenobio-Galindo *et al.*<sup>6</sup> reported that yogurt LAB counts were not affected when they incorporated a betalains and (poly)phenols extract from cactus pear encapsulated in a multiple emulsion (W1/O/W2) and Lugo-Zarate *et al.*<sup>13</sup> developed a drinkable yogurt added with juice powder from purple cactus pear (*O. ficus-indica*) powder reaching similar LAB counts than control yogurts formulated only with semi-skimmed milk.

The results indicate that prickly pear flours (either peel or pulp) are promising ingredients for yogurt and fermented milk production since their incorporation would not interfere with the growth of starter LAB strains maintaining the product's probiotic potential. Additionally, Carpena *et al.*<sup>61</sup> stated that prickly pear could be an appropriate and suitable carbon source with prebiotic properties for probiotic strains.

#### (Poly)phenol profile and total betalain content

(Poly)phenolic compounds have been shown to be safe and effective in treating numerous human diseases.<sup>62</sup> However, the levels of phenolics in different dairy products are highly limited or even absent. Prickly pear fruit is known to be a rich source of (poly)phenols, suggesting that yogurts enriched with prickly pear flours could have benefits on human health, although further research is needed to confirm this potential. As shown in Table 6, the yogurts enriched with prickly pear contained five phenolic compounds derived from the flour, with isorhamnetin-pentosyl-rutinoside being the major compound. This confirms that phenolic compounds were successfully transferred from the flour to the yogurt, even though the flour concentration was low. In terms of phenolic content, a pattern similar to the flours was observed, with higher concentrations found in yogurts formulated with peel flour compared to those with pulp ( $41.07 \text{ versus } 9.54 \mu\text{g g}^{-1}$ ). This increase in total phenolic content would explain the higher antioxidant capacity seen in yogurts enriched with prickly pear flours compared to traditional sheep yogurt.

The results for the total betalain content in yogurts supplemented with prickly pear flours are presented in Table 6. As expected, no betalains were detected in the control yogurts,



**Figure 1.** Effect of prickly pear incorporation on lactic acid bacteria (LAB) counts of sheep milk yogurts (mean  $\pm$  standard deviation).

while the formulations with prickly pear flours (both peel and pulp) showed the presence of betalains, with the highest levels found in the yogurts containing the peel. Yogurts with pulp showed values of  $3.24 \text{ mg L}^{-1}$ , while those incorporated with the peel reached a value of  $7.88 \text{ mg L}^{-1}$ . These results align with the betalain analysis of the flours (Table 3), where the peel exhibited higher total betalain content ( $114.4 \text{ versus } 83.8 \text{ mg L}^{-1}$ ).

In a similar study conducted by Lugo-Zarate *et al.*<sup>13</sup> an increase in total betalain content was also observed in drinkable yogurt when purple cactus pear powder was incorporated. Similarly, Cenobio-Galindo *et al.*<sup>6</sup> developed a multiple emulsion with an extract of betalains and prickly pear (poly)phenols that resulted in a significant increase in total betalain content compared to the control yogurts formulated only with cow milk ( $5.2 \text{ versus } 14.0 \text{ mg L}^{-1}$ ). The lower betalain content observed in the current study compared to those reported by other authors could be attributed to several factors such as the variety of prickly pear used, the processing methods of the fruit to obtain the flours, and the concentrations added to the yogurts. Based on these results, it can be concluded that betalains were successfully transferred from the flours (peel and pulp) to the yogurts formulated with them, indicating their potential as ingredients for developing yogurts or fermented milks enriched with bioactive compounds and functional properties. Although the total betalain amounts are not extremely high, this is mainly due to the low concentration of flours used. Therefore, further studies should be conducted with concentrations higher than 1% to evaluate their effect on technological properties of the yogurts as well as determine if greater betalain contents can be achieved in the formulated yogurt by using higher concentrations of prickly pear flours.

#### Antioxidant capacity

Table 6 illustrate the results of the antioxidant capacity of yogurts influenced by the addition of prickly pear flours. The antioxidant activity measured using four methods (FRAP, DPPH, FIC, and ABTS), showed a significant increase ( $P < 0.05$ ) in the yogurts formulated with prickly pear compared to the control. This increase

**Table 6.** Effect of prickly pear incorporation on polyphenolic profile and bioactive properties of yogurts elaborated with sheep milk (mean  $\pm$  standard deviation)

Compound	Control	1% Peel	1% Pulp	P-Value
(Poly)phenolic profile ( $\mu\text{g g}^{-1}$ )				
Isorhamnetin-rhamnose-rutinoside	ND	$6.04 \pm 0.30^a$	$1.33 \pm 0.04^b$	0.004
Isorhamnetin-pentosyl-rutinoside	ND	$15.43 \pm 0.59^a$	$3.55 \pm 0.09^b$	0.003
Quercetin 3- $\beta$ -D-glucoside	ND	$9.57 \pm 0.48^a$	$1.87 \pm 0.00^b$	0.004
Isorhamnetin-pentosyl hexoside	ND	$9.25 \pm 0.65^a$	$2.79 \pm 0.10^b$	0.010
5-Caffeoylquinic acid	ND	$0.78 \pm 0.04^a$	ND	0.003
Betain content ( $\text{mg L}^{-1}$ )				
Betaxanthins	ND	$2.99 \pm 0.74^a$	$1.28 \pm 0.00^b$	0.000
Betacyanins	ND	$4.89 \pm 1.06^a$	$1.96 \pm 0.21^b$	0.000
Total betain	ND	$7.88 \pm 0.32^a$	$3.24 \pm 0.21^b$	0.000
Antioxidant activity				
FRAP ( $\text{mg trolox g}^{-1}$ )	$7.16 \pm 0.33^a$	$14.06 \pm 0.27^a$	$12.76 \pm 0.21^b$	0.000
DPPH ( $\text{mg trolox g}^{-1}$ )	$0.06 \pm 0.00^b$	$0.10 \pm 0.00^a$	$0.09 \pm 0.01^a$	0.017
ABTS ( $\text{mg trolox g}^{-1}$ )	$0.15 \pm 0.03^b$	$0.27 \pm 0.01^a$	$0.25 \pm 0.00^a$	0.018
FIC ( $\text{mg EDTA g}^{-1}$ )	$0.44 \pm 0.08^b$	$0.58 \pm 0.01^a$	$0.47 \pm 0.02^b$	0.029

Note: DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); FRAP, ferric ion reducing antioxidant potential; FIC, ferrous ion chelating; ND, not detected. NS:  $P > 0.05$ . Values followed by a different letter in the same row mean significant no differences ( $P > 0.05$ ) due to prickly pear addition.

**Table 7.** Effect of prickly pear incorporation on the sensory characteristics of yogurts elaborated with sheep milk (mean  $\pm$  standard deviation)

Parameter	Control	1% Peel	1% Pulp	P-Value
Smell	$6.50 \pm 1.35$	$7.23 \pm 1.01$	$6.77 \pm 1.54$	NS
Color	$8.20 \pm 1.13^b$	$5.85 \pm 1.95^a$	$6.54 \pm 1.61^a$	0.006
Taste	$5.60 \pm 2.17$	$4.69 \pm 2.25$	$5.38 \pm 2.22$	NS
Acidity	$6.00 \pm 2.11$	$5.38 \pm 1.50$	$5.69 \pm 2.18$	NS
Firmness	$3.90 \pm 1.66$	$4.38 \pm 1.76$	$4.23 \pm 2.13$	NS
Overall acceptability	$5.80 \pm 1.13$	$4.92 \pm 2.10$	$5.23 \pm 2.01$	NS

Note: NS:  $P > 0.05$ . Different superscript lowercase letter in the same row indicates significant differences ( $P < 0.05$ ) due to prickly pear addition.

was more pronounced in yogurts added with peel flour, which correlates with their higher phenolic content and greater antioxidant capacity compared to the pulp (Table 3). The results indicate that all yogurts exhibited the strongest antioxidant activity with the FRAP method (capacity to reduce ferric ion ( $\text{Fe}^{3+}$ ) to ferrous ion ( $\text{Fe}^{2+}$ )), while the antioxidant activity measured by DPPH, FIC, and ABTS methods was less pronounced. FRAP values ranged from 7.16 to 14.06 mg Trolox  $\text{g}^{-1}$  sample, while DPPH and ABTS values varied from 0.06 to 0.27 mg Trolox  $\text{g}^{-1}$ . Finally, the FIC values oscillated from 0.44 to 0.58 mg EDTA  $\text{g}^{-1}$  sample. It is well known that yogurt possesses inherent antioxidant activity due to the amino acids and small molecular peptides produced during fermentation.<sup>63</sup> Therefore, the enhanced antioxidant capacity of yogurts formulated with prickly pear flours may be attributed to their (poly)phenol content, which can create a synergistic interaction between these compounds, peptides, and the metabolites generated by microbial activity during fermentation.<sup>64</sup> In this sense, improvements in the antioxidant capacity of dairy products due to the incorporation of prickly pear have been previously reported in yogurts fortified with red cactus pear peel powder<sup>35</sup> and in cow kefir enriched with red prickly pear fruit powder.<sup>15</sup>

### Sensory analysis

Food preferences and consumer acceptance are greatly influenced by sensory properties such as flavor, color, texture, and taste. Consequently, the popularity of yogurt depends on these sensory characteristics, and adding new ingredients to yogurt can expand consumer options and help the dairy industry to meet consumer demands and stand out in an increasingly competitive market. The color, smell, taste, acidity, firmness, and overall acceptability of the yogurts as affected by the addition of prickly pear flours are shown in Table 7, respectively. Supplementation with prickly pear flours significantly influenced the color of the yogurt, with the control yogurt receiving the highest score (8.20 over 9). For the rest of the attributes analyzed, all yogurt scores were acceptable (between 4 and 8), except for the firmness of the control yogurt, which scored 3.9. The overall acceptability scores indicated good acceptance of the yogurts formulated with cactus pear, with the yogurt containing pulp flour having the closest score to the control. Also, it is noteworthy that despite the increase in titratable acidity, there was no noticeable impact on the sensory perception of acidity by the consumers.

Consumer feedback revealed a preference for control yogurts and those with the pulp flour over those with peel. This preference could be due to the more pronounced color changes in yogurt formulated with peel flour and the potential increase in sweetness in yogurts with pulp flour, as the pulp is typically sweeter and less astringent than the peel. In relation to the effect of the incorporation of new ingredients on the sensory characteristics of yogurts, other authors have reported similar scores to control samples without negative effects on sensory characteristics in yogurts enriched with hazelnut skin and sapote fruit pulp.<sup>54,65</sup>

## CONCLUSIONS

The present work demonstrates that *O. ficus-indica* flours have remarkable properties to be potentially used as new ingredients in the dairy industry and that both the pulp and the peel could be used as ingredients to fortify sheep milk yogurts. Although prickly pear flours addition accelerates the acidification process, it does not prevent the gelation of milk proteins, resulting in a final product with the characteristic yogurt texture. Prickly pear flour-added yogurts exhibit higher levels of potassium, magnesium, phosphorus, and zinc, along with an increase in (poly)phenolic compounds and total betalain content compared to the control. Sheep yogurt's antioxidant capacity enhanced with the addition of *O. ficus-indica* flours without causing a negative effect on the growth of the LAB starter strains. Despite the differences in color detected by consumers, the yogurts formulated with cactus pear flour were well accepted with scores similar to the control yogurts. Dairy products (e.g., yogurt and fermented milks) formulated with the combination of probiotics and fruits can offer a wide variety of benefits due to their richness in natural bioactive molecules and compounds and the fact that together they can boost their health benefits. In addition, using prickly pear co-products, such as the peel, presents the advantage of utilizing an under-used source of ingredients, which could help mitigate the environmental impact of its processing.

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## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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