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Research Article

Physicochemical and functional properties of yogurt enriched with ultrafiltered juice of Red Prickly Pear (*Opuntia ficus-indica*) as a functional ingredient

Propiedades fisicoquímicas y funcionales de yogurt enriquecido con jugo ultrafiltrado de tuna roja (*Opuntia ficus-indica*) como ingrediente funcional

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Abstract

Yogurt has long been recognized as a health-promoting food for its bioavailability of protein, energy, probiotics and calcium, increasing digestibility, as well as preventing potential diseases beyond basic nutritional function. Recently, yogurt has become one of those foods whose functional properties are developed by adding functional substances with a potential positive effect on health. The objective was to evaluate the effect of ultrafiltered juice of red fruits of *O. ficus-indica* as a functional ingredient in yogurt. A completely randomized block design (CRBD) was used. The treatments were: yogurt with 0 % ultrafiltered red prickly pear juice (Y0) as a control; yogurt with 10 % ultrafiltered red prickly pear juice (Y1); yogurt with 20 % ultrafiltered red prickly pear (Y2); and yogurt with 30 % ultrafiltered red prickly pear juice (Y3). The variables to measure in each yogurt treatment were pH during lactic fermentation and the final product, syneresis, energy content, colorimetry, composition (total solids, protein, fat and ash), antioxidant activity (DPPH, ABTS and FRAP), phenol content and sensory evaluation. The incorporation of red prickly pear ultrafiltrates accelerated antioxidant activity and total phenolic contents. Finally, the sensory panel satisfactorily accepted the incorporation of red prickly pear ultrafiltered in yogurt.

***Keywords*: ultrafiltered, yogurt, antioxidant activity, phenolic content, sensory panel.**

Resumen

El yogurt ha sido reconocido desde hace mucho tiempo como un alimento que promueve la salud por su biodisponibilidad de proteínas, energía, probióticos y calcio, aumentando la digestibilidad, además de prevenir enfermedades potenciales más allá de la función nutricional básica. Recientemente, el yogurt se ha convertido en uno de los alimentos cuyas propiedades funcionales se desarrollan mediante la adición de sustancias funcionales con un efecto potencial positivo en la salud. El objetivo fue evaluar el efecto de jugo ultrafiltrado de frutos rojos de *O. ficus-indica* como ingrediente funcional en yogurt. Se utilizó un diseño de bloques completamente al azar (DBCA). Los tratamientos fueron: yogurt control 0 % jugo ultrafiltrado de tuna roja (Y0); yogurt con 10 % de jugo ultrafiltrado de tuna roja (Y1); yogurt con 20 % de jugo ultrafiltrado de tuna roja (Y2); y yogurt con 30 % de jugo ultrafiltrado de tuna roja (Y3). Las variables para medir en los tratamientos de yogurt fueron pH durante la fermentación láctica y en el producto final, sinéresis, contenido energético, colorimetría, composición (sólidos totales, proteína, grasa y ceniza), actividad antioxidante (DPPH, ABTS y FRAP), contenido de fenoles y evaluación sensorial. La incorporación de ultrafiltrados de tuna roja aceleró la fermentación del yogurt además se incrementó la sinéresis, el contenido de energía y pH. Los ultrafiltrados de tuna roja en yogurt aumentaron la actividad antioxidante y el contenido de fenoles totales. Finalmente, el panel sensorial acepto satisfactoriamente la incorporación de ultrafiltrados de tuna roja en yogurt.

***Palabras clave*: ultrafiltrado, yogurt, actividad antioxidante, contenido fenólico, panel sensorial.**

1. Introduction

Nowadays, the consumption of functional foods is in high demand because consumers consider that their health is linked with the foods they ingest (Dinkçi *et al.,* 2021). Foods or ingredients classified as functional foods resemble traditional ones but may have the potential to provide additional health benefits beyond the standard nutrient content, and should be incorporated as part of a normal diet for consumption in a local setting or specific culture (Shori *et al.,* 2022). Functional foods require new bioactive ingredients which can be used by the food industry (Faustino *et al.,* 2019). In the past years, studies about horticultural crops have revealed that these products have important functions in human health due to high levels of active biological compounds (Settar Unal *et al.,* 2022).

Cacti are a group species that live in arid areas; México hosts 518 species out of which 47.7 % are endemic (Martínez *et al.,* 2021). *Opuntia ficus-indica* (L.) Mill (Cactaceae) is a plant considered as functional food for its nutritional benefits on human health due to its high content of antioxidant compounds in its cladodes, flowers and fruits (Ayala *et al.,* 2021). The fruits of *O. ficus-indica* are berry-like and are known as "tunas" in Mexico, they contain flavonoids, phenolic acids, betalains, ascorbic acid, fatty acids, lignans and sterols (Martínez *et al.,* 2021). Betalains are derived from betalamic acid and are divided into two structural subgroups: betacianines and betaxantines. These compounds are extracted commercially from beetroot and are utilized commonly in the food industry as a color additive in many types of foods, such as dairy products, candies, soft drinks and some emulsified meat products (Bassama *et al.,* 2020). Many studies have investigated the properties

and interactions of the pigments of prickly pear (betalains), for example, characterizing and quantifying betalains and phenolic compounds (Gómez-Maqueo *et al.*, 2020); evaluating the antioxidant potential, antiinflamatory and anti hyperglucemic activity of the extracts of *O. ficus-indica* (Gómez-Maqueo *et al.*, 2019); and studying the potential of semi-processed juices from *O. ficus-indica* as a natural antimicrobial against Gram-negative and Gram-positive food pathogenic bacteria (Palmeri *et al*., 2020).

According to the lastest studies, the use of numerous additives, fruits or bioactive compounds of plant origin in the production of yogurt has a significant improvement in its quality and benefits such as antioxidant properties (Cenobio *et al.*, 2019; Tavakoli *et al.*, 2019; Won-Young *et al*., 2020). Yogurt is classified as a functional food and it is one of the most consumed dairy products (Jeong *et al.*, 2018). It is produced by the coagulation of proteins during fermentation with lactic acid bacteria such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus* under defined time and temperature (Kim *et al.*, 2019; Cho *et al*., 2020). These bacteria ferment lactose, producing lactic acid, carbon dioxide, acetic acid, dactyls, acetaldehyde and other components that give yogurt its characteristic, flavor and smell; and they must be viable and abundant in the final product (Shori *et al.*, 2022). Yogurt is a source of bioactive compounds that are formed during lactic fermentation and has a limited content of antioxidant activity. For this reason, the production of yogurt enriched with antioxidants from natural sources is of considerable interest and presents a novel approach for product development (Dabija *et al*., 2018). In summary, yogurt and red prickly pear are both functional foods that, when combined, may have physicochemical and functional properties that are beneficial to human health. Therefore, the objective is to evaluate the effect of ultrafiltered juice of red fruits of *O. ficus-indica* as a functional ingredient in yogurt.

2. Materials and methods

2.1 Materials

For the yogurt production, commercially available ultra-pasteurized milk, sugar, bacterial cultures, and ultrafiltered juice from red prickly pear (*O. ficus-indica*) were used, as shown in Table 1.

Table 1. Yogurt treatments with ultrafiltered red prickly pear juice (*O. ficus-indica*) as a functional ingredient.
Tabla 1. Tratamientos del yogurt con jugo ultrafiltrado de tuna roja (*O. ficus-indica*) como ingrediente funcional.

Ingredient (%) †	Treatment ‡			
	Y0	Y1	Y2	Y3
Ultrapasteurized milk	90.90	90.49	90.08	89.68
Saccharose	9.09	9.05	9.01	8.97
Bacteria cultures	0.01	0.01	0.01	0.01
PUJ *O. ficus-indica*	0.00	0.45	0.90	1.35

†PUJ: Pasteurized ultrafiltered juice. ‡ Y0: control yogurt; Y1: yogurt with 10 % ultrafiltered red prickly pear juice; Y2: yogurt with 20 % ultrafiltered red prickly pear juice; Y3: yogurt with 30 % ultrafiltered red prickly pear juice.

2.2 Methods

2.2.1 Yogurt processing

The elaborate process of yogurt formulation (Table 1) was done following the Karnopp method *et al.,* (2017) with some modifications. Saccharose was added to the ultra pasteurized milk and the mixture was heated to 42 °C. At this temperature, the freeze-dried bacterial cultures and pasteurized ultrafiltrate of red prickly pear juice were added to each treatment. The mixture was then incubated at 42 °C for 6 h. Subsequently, the products were cooled to 7 °C and kept at that temperature for 12 h for the analysis of the variables. The commercial ultra-pasteurized milk used in the experiment was composed of 33.1 % total solids, 3.1 % protein, 2.9 % fat and 0.6 % ash.

2.2.2 Physicochemical analysis

The pH of the yogurt samples was measured with a potentiometer (EcoSense pH1000A, YSI, Ohio, USA) during fermentation and in final products.

Yogurt syneresis was performed according to the method of Jeong *et al*. (2018). Samples of 10 g of yogurt were analyzed, which were centrifuged at 600 × g for 6 min at 4 °C. The supernatant (serum) obtained was weighed, and the syneresis was expressed as a percentage weight of the serum separated from the initial sample (Kim *et.al.,* 2020*)* using the following Eq. (1):

$$Syneresis\ (\%) = \left[\frac{separated\ serum\ (g)}{initial\ sample\ weight\ (g)}\right] \times 100 \qquad \textbf{Eq. (1)}$$

Gross energy was determined using an adiabatic bomb calorimeter (Bomb Calorimeter, Parr Instrument, Illinois, USA), using petroleum ether as the combustion reagent.

2.2.3 Colorimetry

The color was determined by the values of CIE L* (0 = black; 100 = white), CIE a* (- green; + red), CIE b* (- blue; + yellow), Chroma (C* = intensity of the color) and Hue angle (hue), with a colorimeter (CR 410, Konica Minolta, Japan). Furthermore, the color difference (ΔE) between the color of the samples (L2) compared to the control (L1), was calculated according to Dinkçi *et al*. (2021) with the following Eq. (2):

$$\Delta E = \sqrt{(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2} \qquad \textbf{Eq. (2)}$$

2.2.4 Proximal composition

The total solids content was determined according to the method 930.15 (AOAC, 2005) by gravimetric determination of the moisture loss of the sample dried for 3 h in an oven (Yamato Scientific America Inc. Constant Temperature Oven, Japan) at 103 °C. The percentage of ash according to the method 942.05 (AOAC, 2005) was considered as the residue after incineration of the samples at 550 °C in a muffle (Thermo Scientific, Waltham, MA) for 4 h. The percentage of protein was determined by the Kjeldahl method described by Wijesekara *et al*., (2022). In a digestion block, the samples were digested at 400 °C with sulfuric acid and catalyst (3 % $CuSO_4$, 97 % K_2SO_4). Subsequently, the samples were subjected to a Kjeldahl distiller with NaOH to distill the nitrogen from the combustion, which once obtained was titrated with hydrochloric acid (0.1 N). The results obtained were the quantification of total nitrogen in the samples, and through a conversion factor.

Fat quantification by method 920.39 (AOAC, 2005) consisted of quantifying the ether extract obtained by extracting triacylglycerides from the samples, using ether in an adiabatic calorimetric fat extractor (Parr Instrument, USA).

2.2.5 Preparation of yogurt-water extracts

The preparation of the yogurt-water extracts was done according to the method of Shori *et al.* (2022). A total of 40 g of each yogurt sample was homogenized with 10 mL of dH_2O. The pH of the samples had to be 4.0, then the samples were incubated in a water bath at 45 °C for 10 min. The samples were centrifuged at 5000 rpm at 4 °C for 10 min, and the supernatant was adjusted to pH 7.0 using NaOH (0.1 M). A second centrifugation was carried out (5000 rpm, 4 °C, 10 min) and the clear supernatant was used for subsequent analyses: DPPH, $ABTS^+$, FRAP and phenolic content.

2.2.6 Antioxidant activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was based on what was proposed by Jeong *et al*., (2018) with some modifications. The DPPH solution was dissolved in 80 % methanol, then the reagent was diluted 1:10 to an absorbance of 0.700 at 515 nm. The previously prepared yogurt-water extract (25 µL) was mixed with the DPPH solution (975 µL), and incubated in the dark at room temperature for 30 min. A mixture of 25 µL of ethanol and 975 µL of DPPH solution was used as a control. The antioxidant activity was calculated in accordance with Jeong *et. al.* (2018) as Eq. (3):

$$AA\ for\ DPPH \bullet\ (\%) = \left[1 - \left(\frac{Abs_{sample}}{Abs_{control}}\right)\right] \times 100\% \qquad \textbf{Eq. (3)}$$

The ABTS (2,2'–azino–bis–(3–ethylbenzothiazolin–6–sulfonic acid) assay was performed based on Jeong *et al*., (2018) with modifications. The ABTS reagent (14.8 mM) was mixed with 5 mM potassium sulfate, and the sample was incubated in the dark at room temperature for 16 hours. The working solution (ABTS) was diluted with dH_2O to an absorbance of 0.700 ± 0.05 at 734 before use. The yogurt-

water extract samples (10 μL) were mixed with 1 mL of ABTS solution and incubated in the dark at room temperature for 15 min. The percentage of antioxidant activity was calculated according to Jeong *et al*. (2018) in the following Eq. (4):

$$AA\ por\ ABTS\ (\%) = \left[1 - \left(\frac{Abs_{sample}}{Abs_{control}}\right)\right] \times 100\% \quad \textbf{Eq. (4)}$$

The methodology for FRAP was performed according to Schneider *et al.,* (2022) with some modifications. The solution was prepared by mixing acetate buffer (300 mM, pH 3.6) with 10 mM TPTZ solution (2,4,6-tripyridyl-s-triazine) in 40 mM HCl and 20 mM FeCl3, in a ratio of 10: 1:1 (v/v/v). The mixture of solutions was heated to 37 °C and 25 μL of diluted sample (1/5) or Trolox standard solution was added to 1 mL of FRAP solution. The absorbance was measured at 593 nm, after 30 min. The equation curve was based on Trolox concentrations between 0 and 1200 μM, and the results were expressed in μmol TE/L.

The Folin-Ciocalteu method (Wijesekara *et al.,* 2022) with some modifications was used to quantify the total phenolic content in the yogurt-water extracts. The analysis was based on a nine-point calibration curve from 0 to 0.032 mg/mL with intervals of 0.004 mg/mL gallic acid. The absorbance was measured at 760 nm before use. The results were expressed in gallic acid equivalents (mg AG/L).

2.2.7 Sensory evaluation

The sensory evaluation was carried out based on Shori (2020) with some modifications. The yogurt samples were evaluated on day 1 of storage. Sample preparation consisted of placing 20 g of each treatment in plastic containers, assigning them a random three-digit number. The panel consisted of 40 people (were students and professors, all yogurt consumers) and was divided into two sections. In the first section, 20 panelists evaluated the four treatments of replica one, while the other 20 assessed the same treatments in replica two. This way, the evaluation was conducted in replicates, ensuring that no different treatments were used, which allowed for more consistent and comparable results. The evaluation was carried out with a 5-point hedonic scale (5= I like it a lot, 4= I like it, 3= I neither like it nor dislike it, 2= I dislike it, 1= I dislike it very much), for the five attributes: color (presence of pink), milky odor, flavor, viscosity and overall acceptability.

2.2.8 Statistical analysis

The variables analyzed in the filtered, retained and pasteurized samples were considered as a treatment effect (T_i) to test the hypothesis H_0 (equality of treatments - no effect on the variables); this analysis was based on the general linear model "$y_{ij}=\mu+\tau_i+e_{ij}$" where "y_{ij}" refers to the study variable, "μ" refers to the global mean, "T_i" refers to the effect of the i treatment and "e_{ij}" is the experimental error.

In the yogurt experiment, a factorial design was first carried out to test the hypothesis (H_0) about the difference in pH between the treatments (Ti) in relation to the time of lactic fermentation: $yij=\mu+\tau i+tj+$

$(\tau i)_{ij(k)}+\beta+eij$; *yij*= study variable (pH); μ= overall mean; *Ti*=effect of the ith treatment; *tj*= effect of the jth fermentation time; $(Ti)_{ij(k)}$= effect of the treatment-fermentation time interaction; *B*= block effect; *eij* = experimental error.

In the treatments (Ti) of the final yogurt product, the hypothesis H_0 was tested based on: $yij=\mu+\tau i+\beta j+eij$; *yij*= study variable; μ= overall mean; *Ti*=effect of the ith treatment; *Bj*= effect of the ith block; *eij* = experimental error.

A value less than 0.05 (P-Value) was the criterion to reject H_0. The rejected variables were evaluated by a comparison of means (Tukey). Minitab® 17.1.0 (2013) software was used for data analysis.

Measurements of the colorimetry, pH and soluble solids variables were carried out seven times. Meanwhile, measurements of pH variables during lactic fermentation, antioxidant activity, betalains, phenolic content, total sugars, reducing sugars, proximal composition and water retention capacity were carried out in triplicate.

3. Results and discussion

3.1 Lactic fermentation

During lactic fermentation, the interaction of treatments and time showed no effect (P>0.05) for pH. Thus, the behavior of pH over hours is shown in Table 2. The pH was different (P<0.05) between the treatments at 0, 2 and 6 h. The yogurt with the highest content (Y3) of ultrafiltrates showed lower pH at hours 0 and 2, given that the pH of the pasteurized ultrafiltrate was 4.57.

Table 2. pH behavior during yogurt fermentation
Tabla 2. Comportamiento del pH durante la fermentación del yogurt

Treatment†	Time (h)			
	0	2	4	6
Y0	6.568^{a}	6.013^{a}	4.637	4.117^{a}
Y1	6.490^{a}	5.913^{b}	4.487	4.062^{b}
Y2	6.382^{b}	5.868^{b}	4.502	4.053^{b}
Y3	6.295^{b}	5.783^{c}	4.583	4.092^{ab}
SEM	0.034	0.102	0.056	0.028
P-Value	0.000	0.000	0.059	0.044

† Y0: control yogurt; Y1: yogurt with 10 % ultrafiltered red prickly pear juice; Y2: yogurt with 20 % ultrafiltered red prickly pear juice; Y3: yogurt with 30 % ultrafiltered red prickly pear juice. SEM: standard error of the mean. a-c Means in columns with different letters differ statistically (P<0.05).

Similar results were reported by Jeong *et al.* (2018), who used green tea powder in the yogurt formulation, where the pH was lower. Those authors indicated that the addition of phenolic compounds and organic acids can improve the metabolic activity of lactic acid bacteria in yogurt, leading to faster acidity of milk and, therefore, a shortening of fermentation times.

3.2 Physicochemical properties

Table 3 reports the physicochemical properties of the yogurt, such as pH, syneresis and energy. These properties showed a difference (P>0.05) between yogurt samples. Y0 presented the lowest syneresis value, and Y3 the highest energy value.

Table 3. Physicochemical properties of yogurt with ultrafiltered red prickly pear juice.
Tabla 3. Propiedades fisicoquímicas del yogurt con jugo ultrafiltrado de tuna roja.

Variables	Treatment†				SEM‡	P-Value
	Y0	Y1	Y2	Y3		
pH	4.01^{a}	3.99^{ab}	3.97^{b}	4.01^{a}	0.01	0.047
Syneresis %	60.73^{c}	65.50^{b}	68.56^{ab}	71.36^{a}	1.09	0.000
Energy (Kcal/kg)	4285.50^{b}	4164.50^{bc}	4061.00^{c}	4567.50^{a}	19.10	0.001

† Y0: control yogurt; Y1: yogurt with 10 % ultrafiltered red prickly pear juice; Y2: yogurt with 20 % ultrafiltered red prickly pear juice; Y3: yogurt with 30 % ultrafiltered red prickly pear juice. ‡ SEM: standard error of the mean. $^{a-c}$ Means in rows with different letters differ statistically (P<0.05).

The pigmentation of betalains is most stable in a pH range of 3 to 7, with betacyanins being more resistant to acidic conditions and betaxanthins being more stable at neutral pH (Schneider *et al.*, 2022). The addition of red prickly pear ultrafiltrates differed in pH value, Y0 and Y3 presented the highest value. The results obtained present more acidic values compared to previous research in which leaf extracts were added; For example, the addition of aqueous extracts of herbs in yogurt presented pH of 4.62 to 4.36 on day 1 of storage (Dabija *et al.*, 2018), and the addition of lotus leaf powder showed values of 4.39 and control of 4.49 (Da-Hee *et al.*, 2019).

Syneresis values differed significantly between treatments (P<0.05). Y0 presented the lowest percentage in terms of serum release compared to treatments added with red prickly pear ultrafiltrates. Similar results were obtained by Wijesekara *et al*., (2022), who added aqueous natural colorants to yogurt after fermentation, compared to this study in which the ultrafiltrates were added before fermentation, so adding the aqueous extracts before or after fermentation generates the same effect in the release of serum. T

The variation in the energy content of yogurt is due to the interpretation of the carbohydrate, protein and fat contents in the formulations (Ahmed *et al.*, 2023). Y3 presented the highest energy content, followed by Y0, Y1 and Y2. However, it would have been expected that the higher the concentration

of red tuna ultrafiltrate, the higher the energy content, but this was not the case for Y2, perhaps due to variations in the formulation.

3.3 Colorimetry

The color parameters evaluated in yogurt showed significant differences (Table 4). L*, b* and hue (Hue angle) decreased as the amount of ultrafiltrates added increased, while a* and saturation (Chroma) increased.

Table 4. Colorimetry in yogurt with ultrafiltered red prickly pear juice.
Tabla 4. Colorimetría en yogurt con jugos ultrafiltrados de tuna roja.

Colorimetry†	Treatment‡				SEM§	P-Value
	Y0	Y1	Y2	Y3		
L*	93.81[a]	79.63[b]	73.51[c]	69.66[d]	0.91	0.000
a*	-2.72[c]	18.68[b]	25.59[a]	29.46[a]	1.24	0.000
b*	9.41[a]	3.89[b]	2.98[b]	2.87[b]	0.44	0.000
Chroma	9.79[c]	19.22[b]	25.86[a]	29.7[a]	1.09	0.000
Hue Angle	106.20[a]	13.53[b]	7.86[c]	6.66[c]	0.99	0.000
ΔE	Ref	19.55[c]	27.45[b]	32.19[a]	0.11	0.000

† a*: red coordinates; b*: yellow coordinates; L*: luminosity; ΔE: color difference, Y0 as reference. ‡ Y0: control yogurt; Y1: yogurt with 10 % ultrafiltered red tuna juice; Y2: yogurt with 20 % ultrafiltered red tuna juice; Y3: yogurt with 30 % ultrafiltered red tuna juice. § SEM: standard error of the mean. [a-d] Means in rows with different letters differ statistically ($P<0.05$).

Cenobio *et al.* (2019) reported that L* increases in proportion to the concentration of the purple *O. ficus-indica* betalain emulsion. Also, the value of a* increases, since the emulsions tend to color red because of the presence of betalains. Parameter b* increases during the shelf life of the yogurt. In the results obtained, a* increased when adding 20 and 30 % of red tuna ultrafiltrates.

Various types of acids and their quantities cause different effects on the color of betalains (Guneser, 2021); an example is lactic acid produced during milk fermentation. Guneser (2021) described a relationship: as the shelf life of yogurt enriched with beet extract progressed, the lactic acid content, pH, and the value of L* and b* increased, while the value of a* decreased. In this case, the pH value decreased as the percentage of ultrafiltered red tuna juice increased and the a* value increased, so the use of betalains from red tuna is more stable at acidic pH, by obtaining greater pigmentation in red coordinates.

In addition, Guneser (2021) reported a significant positive correlation between Chroma values and betalain content. A similar effect occurred in the treatments, Chroma and a* increased as the

percentage of ultrafiltrates increased. The color difference between the samples results when 3.5 < ΔE < 5.0 (Schneider *et al.*, 2022), in treatments Y1, Y2 and Y3 the value was greater than 5.0.

3.4 Proximal composition

The results of the proximal composition such as total solids, fat and ash did not present significant differences (P>0.05), but the levels of proteins differed significantly (P<0.05) between treatments, with Y2 and Y3 having lower content (Table 5).

Table 5. Proximal composition of yogurt with ultrafiltrates of red prickly pear juice.
Tabla 5. Composición proximal en yogurt con ultrafiltrados de tuna roja.

Composition (%)	Treatment†				SEM‡	P-Value
	Y0	Y1	Y2	Y3		
Total solids	19.15	18.25	17.50	16.85	0.541	0.173
Raw protein	2.15^{ab}	2.50^{a}	1.75^{b}	1.65^{b}	0.075	0.011
Raw fat	0.90	1.10	1.10	0.65	0.125	0.201
Ash	0.65	0.60	0.60	0.55	0.029	0.292

† Y0: control yogurt; Y1: yogurt with 10 % ultrafiltered red tuna juice; Y2: yogurt with 20 % ultrafiltered red tuna juice; Y3: yogurt with 30 % ultrafiltered red tuna juice. ‡SEM: standard error of the mean. a-b Means in rows with different letters differ statistically (P<0.05).

The chemical composition of yogurt depends on the composition of the milk, the presence of additives and processes (Guneser, 2021). The fat and protein values of the four yogurt treatments are within the range established by NOM-181-SCFI/SAGARPA-2018, since the minimum percentage of protein must be at least 1.60 and the maximum percentage of butterfat must be 7.0. Although the percentage of protein decreased in the yogurt treatments as the amount of ultrafiltrates increased.

Guneser (2021) reported 13.13 g/100 g of dry matter, 3.01 g/100 g of fat, 2.50 g/100 g of protein and 0.85 g/100 g of ash, in yogurt with cow's milk. In the results obtained from Y0, Y1, Y2 and Y3, the fat, protein and ash contents were lower.

3.5 Antioxidant activity and total phenolic content

The antioxidant activity and total phenolic content are shown in Table 6. The ultrafiltrates increased the results obtained from DPPH, ABTS, FRAP and total phenolic content in Y1, Y2 and Y3, with Y3 presenting the highest values.

Table 6. Antioxidant activity and total phenolic content in yogurt with ultrafiltrates of *O. ficus-indica* red variety.
Table 6. Actividad antioxidante y contenido de fenólicos totales en yogurt con ultrafiltrados de *O. ficus-indica* variedad roja.

Variable†	Treatment‡				SEM§	P-Value
	Y0	Y1	Y2	Y3		
DPPH• %	2.69^{c}	15.86^{b}	27.02^{a}	33.21^{a}	1.97	0.000
ABTS•+ %	15.50^{d}	19.25^{c}	22.91^{b}	28.34^{a}	0.75	0.000
FRAP (μmol TE/L)	0.167^{c}	0.239^{b}	0.310^{ab}	0.356^{a}	0.018	0.000
CFT (mg AG/L)	22.74^{c}	34.69^{b}	39.68^{ab}	44.61^{a}	2.15	0.000

† TE: Trolox equivalents; TPC: Total phenolic content; FA: gallic acid. ‡Y0: control yogurt; Y1: yogurt with 10% ultrafiltered red tuna juice; Y2: yogurt with 20% ultrafiltered red tuna juice; Y3: yogurt with 30% ultrafiltered red tuna juice. § SEM: standard error of the mean. a-d Means in rows with different letters differ statistically (P<0.05).

The antioxidant activity reported by different methodologies such as DPPH, ABTS and FRAP, as well as the total phenolic content showed a similar behavior between the treatments, given that the antioxidant activity is correlated with the phenolic content (Dabija *et al.*, 2018); because as the percentage of red tuna ultrafiltrates increased in the treatments, the percentage of radical inhibition by DPPH, ABTS, and the content of μmol TE/L in FRAP increased, and in turn, the content of total phenols increased, with Y3 reporting the highest results.

In yogurt enriched with microencapsulated fruits of purple *O. ficus-indica*, similar results were obtained to those reported in the DPPH and ABTS assay (Cenobio-Galindo *et al*., 2019).

Schneider *et al.* (2022) mentioned that the betacyanin-rich fraction has a higher antioxidant activity and phenolic content than the betaxanthin-rich fraction. The ultrafiltered red tuna juice added to the different yogurt treatments presented a higher content of betacyanins (93.74 mg/L) than betaxanthins (39.18 mg/L), resulting in the increase in antioxidant activity and CFT in Y1, Y2 and Y3.

3.6 Sensorial evaluation

Table 7 shows the results obtained from the sensory evaluation of yogurt. The evaluation was conducted in replicates, using the same treatments, which allowed for data grouping. The pink color and flavor showed significant differences (P<0.05) between the treatments, as well as the milky odor, viscosity and overall acceptability (AG).

Table 7. Sensorial evaluation of yogurt with ultrafiltrates of *O. ficus-indica* juice red variety.
Tabla 7. Evaluación sensorial en yogurt con ultrafiltrados de *O. ficus-indica* variedad roja.

Treatment†	Pink color	Milky odor	Flavor	Viscosity	OA
Y0	1.750^{c}	4.000^{a}	4.250^{a}	4.000^{a}	4.000^{a}
Y1	4.000^{ab}	4.000^{a}	3.750^{b}	4.000^{a}	4.000^{a}

Y2	4.250[a]	4.000[a]	4.000[ab]	4.000[a]	4.000[a]
Y3	4.000[ab]	4.000[a]	4.000[ab]	4.000[a]	4.000[a]
P-Value	0.000	0.084	0.009	0.430	0.399

[†] Y0: control yogurt; Y1: yogurt with 10 % ultrafiltered red tuna juice; Y2: yogurt with 20 % ultrafiltered red tuna juice; Y3: yogurt with 30 % ultrafiltered red tuna juice. OA: Overall acceptability. [a-b] Means in columns with different letters differ statistically ($P<0.05$).

The yogurt treatments enriched with ultrafiltered red tuna juice (Y1, Y2, and Y3) received sensory scores similar to those of the control yogurt (Y0), without any negative effects. Wijesekara *et al.* (2022) reported that the use of natural pigments in yogurt production does not negatively impact sensory perception. However, in this study, the inclusion of red tuna ultrafiltrates resulted in a noticeable pink color and a distinct flavor profile. While Y0 did not exhibit pink pigmentation and was the panelists' accepted treatment in terms of flavor, Y2 and Y3 were also well-received, though their flavor scores were slightly lower than Y0.

4. Conclusions

The incorporation of red tuna ultrafiltrates into yogurt resulted in several significant effects on its properties. First, there was an acceleration in the yogurt fermentation process. Additionally, the inclusion of these ultrafiltrates increased syneresis, energy content, and pH, particularly in the Y3 sample. However, a decrease in L*, b*, and Hue angle values was also observed, indicating a noticeable color difference compared to yogurt without additives (Y0). Furthermore, the addition of ultrafiltrates reduced protein content in the Y2 and Y3 yogurt variants. From a health perspective, the presence of red tuna ultrafiltrates significantly boosted antioxidant activity (measured through the DPPH, ABTS, and FRAP methods) and increased total phenolic content. A sensory panel evaluated the yogurt with the ultrafiltrates positively, suggesting favorable consumer acceptance. This sensory evaluation was conducted on a pilot scale, so future research should include a larger group of consumers to obtain more reproducible and reliable results. The treatments containing 20% and 30% ultrafiltered tuna juice were the best-rated percentages by the panelists. Ultrafiltered red tuna appears to be a beneficial addition to yogurt, enhancing certain nutritional and functional aspects, though it also brings changes to the yogurt's physical and protein properties that warrant further consideration. Further large-scale studies involving a more substantial number of sensory panelists are recommended to gain more robust and representative data on consumer acceptance. Additionally, investigating the product's stability during storage—evaluating whether the antioxidant benefits and changes in physical properties are maintained over time—along with determining the product's shelf life under commercial conditions, would provide valuable insights.

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Author's contribution

Conceptualization, S.R.S.G. and L.S.F.; Methodology, A.V.P. and N.C.V.A.; Software, G.M.Z.; Validation, S.R.S.G., G.M.Z., and L.S.F.; Formal analysis, A.V.P. and G.M.Z.; Research, A.V.P., G.M.Z., and L.S.F.; Resources, C.A.H.M.; Writing-revising and editing, G.M.Z. and L.S.F.; Visualization, C.A.H.M.; Supervision, S.R.S.G.; Project management, S.R.S.G.; Fundraising, S.R.S.G., A.V.P., L.S.F., and G.M.Z.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Nomenclature

ABTS•+	2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)
DPPH•	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric Reducing Ability of Plasma

Greek symbols

yij	Study variable
μ	Overall mean
Ti	Effect of the ith treatment
tj	Effect of the jth fermentation time
$(Ti)_{ij(k)}$	Effect of the treatment-fermentation time interaction
B	Block effect
Bj	Effect of the ith block
eij	Experimental error

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