

Effects of microfiltration and ultrafiltration on the physicochemical and antioxidant related properties of juice from red prickly pear (*Opuntia ficus-indica*)

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ABSTRACT

The fruits of *Opuntia ficus-indica* have antioxidant, anticancer, and anti-inflammatory components. The aim of this study was to evaluate the effects of microfiltration and ultrafiltration on the physicochemical and antioxidant properties of red prickly pear juice (*Opuntia ficus-indica*). A completely randomized design with five treatments was used to examine the properties of clarified juice, microfiltered juice, juice retained following microfiltration, ultrafiltered juice, and juice retained following ultrafiltration. Physicochemical variables pH and dissolved solids, colorimetry, total and reducing sugar content, antioxidant activity, phenolic and betalain content were measured. Microfiltration and ultrafiltration improved ($P < 0.05$) color and betaxanthin content, while decreasing ($P < 0.05$) the total and reducing sugars content, pH, dissolved solids. Total sugars, phenolic compounds, and betacyanins decreased ($P < 0.05$) for juice retained following microfiltration. Filtered juice exhibited better ($P < 0.05$) antioxidant activity than retained juice. Microfiltration, as compared to ultrafiltration, improved the physicochemical and antioxidant related properties of microfiltered and retained red prickly pear juice.

1. Introduction

The global consumption of functional foods has grown a lot in the last few years because more people want products that are good for their health. The market was worth around USD 329 billion in 2023, and it is expected to expand by about 8–9 % each year over the next few years (Grand View Research, 2024). This trend is in line with scientific research that show people are becoming more interested in functional foods and bioactive chemicals since they may have health benefits (Ali & Rahut, 2019). This rise is linked to their recognized role in disease prevention and quality of life improvement, which has stimulated research focused on the development of novel functional products, particularly from non-traditional plant sources with agro-industrial potential (Faustino et al., 2019). Color is a key attribute influencing

consumer perception and acceptance, serving as an important quality indicator in both fresh and processed foods (Chaitanya Lakshmi, 2014). Growing demand for natural colorants has encouraged the exploration of plant-based alternatives that offer cleaner and safer functional properties (González-Aguayo, 2014).

Opuntia ficus-indica is the most widely cultivated species of the *Opuntia* genus, commonly known as "nopal" in Mexico, the world's largest producer and exporter (Chavez et al., 2021). The red prickly pear harvest occurs between May and September, with major production concentrated in Zacatecas, Puebla, and Guanajuato. In 2022, national production reached 88,759.64 tons, with a reported value of 365 billion Mexican pesos (SIAP, 2023).

Prickly pears are rich in betalains, nitrogen-containing water-soluble pigments derived from tyrosine. These compounds are classified as

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betacyanins (red-purple) and betaxanthins (yellow-orange) and exhibit strong antioxidant activity due to the presence of betalamic acid (García-Cruz et al., 2017). Their biological activities include anti-inflammatory, anticarcinogenic, antimicrobial and antioxidant effects (Castro-Enríquez et al., 2020; Sabtain et al., 2021).

Interest in *Opuntia* spp. has expanded due to their potential role in managing metabolic syndrome and diabetes (Angulo-Bejarano et al., 2019). Their pulp and seeds contain diverse bioactive compounds such as phenolics, vitamins, fatty acids, sterols, and flavonoids, with functional properties varying by cultivar (Ramírez-Rodríguez et al., 2020; Palmeri et al., 2020). Red varieties are rich in betanin, while yellow varieties contain indicaxanthin, both associated with significant biological activities (A. Gómez-Maqueo et al., 2019).

The stability of betalain pigments is influenced by several factors, including temperature, pH, water activity, enzymes, metals and light (Vergara et al., 2015). For this reason, clarification processes carried out under non-thermal conditions are preferred in order to minimize damage to these compounds (Hadj Sadok, 2019). In this context, membrane-based clarification processes such as microfiltration and ultrafiltration represent an attractive alternative, since these processes operate in non-thermal conditions while removing suspended solids and turbidity from the juice (Cassano et al., 2010; Castro-Muñoz et al., 2014).

Previous studies have evaluated the chemical composition and antioxidant properties of different prickly pear varieties (Rose & Norman, 2016; Andreu et al., 2017). In addition, storage at 4 °C and a pH range between 4 and 6 has been recommended to preserve betalain stability and functionality (Khan et al., 2016).

Several investigations have demonstrated that microfiltration using a 0.1 µm pore size allows a high degree of clarification by reducing suspended solids and turbidity in fresh orange prickly pear juice (Arboleda Mejía & Yáñez-Fernández, 2021). Likewise, de-pectinized yellow cactus pear juice processed by microfiltration (0.20 µm) and ultrafiltration (200 kDa) produced permeates with low protein content, enriched in antioxidant compounds (polyphenols, vitamin C), sugars, amino acids, and minerals (Cassano et al., 2010).

Ultrafiltration of purple cactus pear juice (30 kDa) induced significant changes in color properties, characterized by increased redness, due to the removal of high-molecular-weight polysaccharides (Castro-Muñoz et al., 2014). Additionally, ultrafiltration with a 100 kDa cut-off has been applied to xoconostle (*Opuntia joconostle*) juice to improve its commercial potential (Castro-Muñoz et al., 2017).

Juice obtained from purple cactus pear (*Opuntia dillenii* Haw.) has been processed under different membrane operating conditions, including microfiltration (0.1 and 0.2 µm), ultrafiltration (4 kDa), and nanofiltration (0.2 and 1 kDa), to evaluate their effects on clarification and quality (Tamba et al., 2019). Similarly, Vergara et al. (2015) evaluated ceramic and polymeric microfiltration membranes (0.2 µm), obtaining clear permeates with high betalain content, retained polyphenols, and significant antioxidant activity.

Although previous studies have mainly focused on membrane performance and operational conditions, the present work differs by applying a broader set of antioxidant capacity assays to characterize permeates and explore their potential applications. Membrane processing has also been employed beyond clarification, including the development of microencapsulated betalain ingredients (Castro-Muñoz et al., 2014) and yogurt enriched with ultrafiltered red prickly pear juice, which showed improved antioxidant capacity and phenolic content (Valadez-Pineda et al., 2024).

The objective of this study was to evaluate the effect of microfiltration (0.2 µm) and ultrafiltration (10 kDa) on the physicochemical and antioxidant related properties of red prickly pear juice (*Opuntia ficus-indica*). Ultrafiltration is widely used for juice clarification and bioactive compound recovery due to its non-thermal and additive-free nature (Loizzo et al., 2019; Cai et al., 2020). The properties evaluated included color, pH, soluble solids, sugars, betalains, phenolic content,

and antioxidant capacity.

2. Materials and methods

2.1. Raw material and equipment

The red prickly pears were obtained from a local establishment in Monterrey, NL, México. The reactants used were Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (Sigma-Aldrich), sodium potassium tartrate (Sigma-Aldrich); 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich), sodium acetate (Sigma-Aldrich); 3, 5-dinitrosalicylic acid (DNS) (Sigma-Aldrich); 2,4,6-tripyridyl-s-triazine (TPTZ; used for the ferric reducing/antioxidants power analysis (FRAP)) (Sigma-Aldrich), phenol (Sigma-Aldrich), gallic acid (Sigma-Aldrich), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Sigma-Aldrich, Buchs, Switzerland), sulfuric acid 95.0–98.0 % (J.T. Baker, Phillipsburg, NJ), sodium hydroxide (CTR Scientific, Monterrey, NL, Mexico); D-glucose (Bio Basic Canada Inc, Toronto, Canada), acetic acid (Jalmek, San Nicolas de los Garza, NL, Mexico), chloric acid (Jalmek), and ferric chloride (Jalmek), potassium persulfate (J.T. Baker). A spectrophotometer (Bio-Rad SmartSpec 3000, Hercules, CA, USA) was used for the analysis of total sugar, reducing sugar, DPPH, ABTS, FRAP, total phenolic content, and betalains content.

2.2. Experimental design

The experimental design was a completely randomized design, which was replicated two times with five treatments each. The experimental groups were clarified juice (CJ), microfiltered juice (MF), liquid juice retained following microfiltration (RMF), ultrafiltered juice (UF), and liquid juice retained following ultrafiltration (RUF).

The red variety fruits of *O. ficus-indica* obtained from a local establishment were harvested from a field situated in Nopaltepec, Mexico State, Mexico. For the experiment, 70 kg of red prickly pears were used. The fruit consisted of 46.70 % peel, 36.44 % pulp, and 16.86 % seeds. Through the CJ procedure (see below), 28.62 % of juice per prickly pear was obtained, resulting in a total yield of 20.48 L of clarified prickly pear juice, with a density of 1.022 kg/L.

In the MF and UF treatments, two processing cycles (clarification followed by microfiltration and ultrafiltration) were conducted with a capacity of 10 L each. The two processing cycles yielded 19.20 L of MF juice, with 8 L being retained, and 18.50 L of UF juice, with only 0.70 L being retained. The variables were measured in triplicate per experimental replicate ($n = 6$).

2.3. Clarified juice

The collection of CJ from red prickly pear pulp was elaborated similar to the method of Martínez et al., 2021. Fruits were washed with running water at room temperature, manually peeled, and the pulp was macerated to remove seeds. The macerated pulp was thoroughly homogenized to obtain a uniform batch prior to centrifugation.

Centrifugation was performed at 4 °C and 7500 g for 15 min using 50 mL Falcon tubes (Z400K, Hermle, Germany; Centrifuge 5804 R, Eppendorf, Hamburg, Germany). The use of multiple Falcon tubes corresponded to aliquots of the same homogenized batch. The supernatants obtained from all tubes were pooled and mixed to ensure compositional uniformity. The clarified juice was stored in the dark at 4 °C and processed within the same experimental session to minimize physicochemical changes.

2.4. Microfiltration and ultrafiltration

The microfiltration and ultrafiltration processes were conducted using a membrane microfiltration and ultrafiltration unit developed by

Membranology® (Swansea, UK). Microfiltration involved passing the CJ through a microfiltration cartridge filter (CFP-2-E-35A, GE Healthcare, Chicago, IL, USA) with a pore size of 0.2 µm. The operational temperature was maintained at 22 ± 2 °C. Following microfiltration, the retained juice (RMF) was stored in the dark at -20 °C for a maximum period of 30 days prior to analysis. Ultrafiltration was performed by filtering the MF permeate through an ultrafiltration cartridge filter (UFP-10-C-5A, GE Healthcare) with a pore size of 10 kDa. The retained juice (RUF) was also stored under the same conditions (-20 °C, dark) for a maximum of 30 days before analysis. These storage conditions were selected to minimize degradation of bioactive compounds, as low temperatures and absence of light have been reported to preserve the stability of phenolic compounds and betalains during storage.

2.5. Color parameters

Color parameters were evaluated following the method of Ruiz-Gutiérrez et al. (2017). The CIE Lab* coordinates, L* (lightness), a* (green to red axis), and b* (blue to yellow axis), were recorded using a CR-410 colorimeter (Konica Minolta, Ramsey, NJ, USA). Chroma (C*) and hue angle (h°) were subsequently calculated from a* and b* values using standard colorimetric equations. The overall color difference (ΔE) was determined according to Eq. (1).

$$\Delta E = [(L_c^* - L^*) + (a_c^* - a^*) + (b_c^* - b^*)]^2 \quad (1)$$

Where L*c, a*c, and b*c values correspond to the control clarified juice.

2.6. Physicochemical analyses

The physicochemical analysis included pH and a measure of dissolved solids (degrees Brix or °Bx). pH was determined using a potentiometer (HI 84,532 Fruit Juice Titratable Acidity, HANNA, Woonsocket, RI, USA), while °Bx was assessed using a refractometer (N-1EBX, Atago, Tokyo, Japan, USA).

2.7. Total sugars

The total sugar content was assessed using the phenol-sulfuric acid method (Nielsen, 2017). A calibration curve was employed spanning eight points ranging from 0 to 70 µg/mL of D-glucose (Bio Basic Canada Inc, Toronto, Canada) dissolved in distilled water. The reagents for this assay comprised 5 % phenol (Sigma-Aldrich, St. Louis, MO, USA), and 95.0–98.0 % sulfuric acid (CTR Scientific). Measurements were conducted using a spectrophotometer (Bio-Rad SmartSpec 3000) at a wavelength of 490 nm, and the results were expressed in mg/mL. Samples of CJ, MF, UF, and RUF were diluted at a ratio of 1/2000, while the RMF sample was diluted at a ratio of 1/1000.

2.8. Reducing sugars

Reducing sugars were quantified using the 3,5-dinitrosalicylic acid (DNS; Sigma-Aldrich, St. Louis, MO, USA) method (Lam et al., 2021). Two preliminary solutions were prepared before obtaining the DNS solution: the first solution contained 2 M NaOH (CTR Scientific) and sodium potassium tartrate (CTR Scientific), while the second solution consisted of 96 mM DNS. These solutions were mixed with distilled water to produce the final DNS solution. A calibration curve was constructed with eight points using glucose concentrations ranging from 0 to 2 mg/mL. The treatments were diluted at a ratio of 1/100 and measurements were taken at a wavelength of 540 nm.

2.9. Antioxidant activity

The antioxidant capacity of the samples was determined using DPPH,

following the method of Brand et al. (1995) with certain modifications. The DPPH solution was prepared by dissolving it in 80 % methanol (CTR Scientific), and then the reagent was further diluted to achieve a 1/10 ratio with an absorbance of 0.70 at 515 nm. For the calibration curve, eight points were utilized, employing Trolox solution as the standard with concentrations ranging from 0 to 1200 µmol. The results were recorded as Trolox equivalents (µmol TE/mL) using the linear equation derived from the curve.

The ABTS antioxidant assay was conducted following the method described by Martínez et al., 2021 with some modifications. Initially, potassium persulfate (Sigma-Aldrich) at 2.45 mM and the ABTS reagent at 7 mM were prepared then mixed to form the working solution. Absolute ethanol (CTR Scientific) was added to this solution until the absorbance reached 0.7 ± 0.02 at 732 nm. For calibration purposes, a series of eight points were established using a 4 mM Trolox solution spanning concentrations from 0 to 1200 µmol. Absorbance readings for both samples and the calibration points were taken at 732 nm.

The antioxidant capacity of the samples was evaluated using the ferric reducing antioxidant power (FRAP) assay, following the method described by Büyüktuncel et al. (2014). A freshly prepared FRAP reagent was used, consisting of 10 mL of 33.30 mM sodium acetate buffer (pH 3.6), 1 mL of 10 mM TPTZ solution (2,4,6-Tris(2-pyridyl)-s-triazine) in 40 mM HCl (Jalmek, San Nicolas de los Garza, NL, Mexico), and 1 mL of 20 mM FeCl₃•6H₂O (Jalmek, San Nicolas de los Garza, NL, Mexico). The mixture was pre-warmed to 37 °C, after which 25 µL of the diluted sample (1:5) or of Trolox solution was added to 1 mL of FRAP reagent. After incubating for 60 min, absorbance was measured at 593 nm. The Trolox calibration curve was constructed with concentrations ranging from 0 to 1200 µM in aqueous solution.

2.10. Total phenolic content

Total phenolic content (TPC) analysis was conducted using the Folin-Ciocalteu method, as described by Ruiz-Gutiérrez et al. (2017), with some adjustments. A calibration curve was prepared with nine concentration points ranging from 0 to 0.032 mg/mL of gallic acid (Sigma-Aldrich), with intervals of 0.004 mg/mL. Absorbance readings were taken at 760 nm. The results were expressed in terms of gallic acid equivalents (mg GA/100 mL), and the samples were analyzed at a dilution of 1/20.

2.11. Betalains content

The betalain content was evaluated by measuring the levels of betacyanins (βC) and betaxanthins (βX) in the samples, following the method of Martínez et al., 2021. The extinction coefficients used were E1 % = 60,000 L mol⁻¹cm⁻¹ at λ = 540 nm for βC, and E1 % = 48,000 L mol⁻¹cm⁻¹ at λ = 480 nm for βX. Results were reported as the total concentration of βC and βX. CJ samples were diluted to a ratio of 1/20, while MF, UF, RMF, and RUF samples were diluted to a ratio of 1/40.

2.12. Statistical analysis

The antioxidant related variables observed in the various filtrations and retained components were analyzed as treatment effects (Ti) to test the H₀ hypothesis (equality of treatments - no effect on the variables) using the general linear model. A P-value <0.05 was considered the criterion to reject H₀. Upon rejection of H₀, a Tukey's mean comparison test was conducted to discern differences in the content of antioxidant related compounds among the filtrations. Data analysis was performed using Minitab® Statistical Software 17.1.0 (2017) (Minitab LLC, State College, PA, USA).

3. Results and discussion

3.1. Colorimetry of filtered red prickly pear juice

Table 1 displays the results of the color parameter analysis of red prickly pear juice. All parameters showed differences ($P < 0.05$) between treatments. The UF treatment increased ($P < 0.05$) L^* , a^* , and Chroma but decreased ($P < 0.05$) b^* and Hue angle. RMF decreased ($P < 0.05$) L^* , a^* and Chroma; hence, values for L^* and a^* with RMF and RUF were lower ($P < 0.05$) than those for MF and UF. CJ increased ($P < 0.05$) b^* and Hue angle. These results improved these traits in respect to those obtained in a study of three wild and a commercial *Opuntia* spp. (Valero-Galván et al., 2021).

Kallel et al. (2023) observed that raw prickly pear juices subjected to microfiltration exhibited increased L^* values, indicating higher lightness, but decreased a^* and b^* values, reflecting reduced redness and yellowness. In contrast, the current study found that microfiltration not only resulted in significantly higher ($P < 0.05$) L^* values compared to ultrafiltration but also led to an increase ($P < 0.05$) in a^* , indicating enhanced redness. These differences could be due to a number of factors, such as the stage of maturity, the production zone, and the environmental conditions, which are known to affect the concentration and composition of plant metabolites (Lado et al., 2018; Medda et al., 2022; Verma & Shukla, 2015). Ascorbic acid is one of these metabolites. It has been shown to help keep the color of prickly pear juices stable because it is an antioxidant and protects betalains (Attoe & Von Elbe, 1982; Strack et al., 2003). However, ascorbic acid was not directly quantified in the present study; therefore, its contribution should be considered as a possible explanatory factor supported by literature rather than experimental evidence from this work.

RMF and RUF treatments presented the lowest ($P < 0.05$) Chroma values. In a study involving different varieties of *O. ficus-indica*, it was demonstrated that if the betalain content increased, the Chroma value decreased (Palmeri et al., 2020). This phenomenon could occur depending on the proportions of betaxanthins and betacyanins, as well as the color saturation or intensity.

Working with juice from several *O. ficus-indica* varieties, Palmeri et al. (2020) reported that red fruits showed a low Hue angle value (22.94), while white fruits showed high values (100.99). This difference is because the Hue value reflects the characteristics of reddening and yellowness (Palmeri et al., 2020). In the current study, CJ, RUF, and RMF had high ($P < 0.05$) values for Hue angle, while MF and UF had

lower ($P > 0.05$) values. Further research is needed to clarify these results due to conflicting results for a^* (redness) and b^* (yellowness).

To compare ΔE for the treatments, a "very distinct difference" was considered if $\Delta E > 3$, a "distinct difference" if $1.5 < \Delta E < 3$, and "no perceptible difference" if $\Delta E < 1.5$ (Palmeri et al., 2020). Therefore, ΔE values for MF, UF, and RUF did not show color differences compared to the CJ ΔE value, while RMF ΔE exhibited a very distinct difference. Color parameters are important signs of product quality and consumer acceptance, in addition to statistical differences. Higher L^* values mean that the juice is clearer and brighter, which are signs of high-quality juice. Changes in a^* and b^* are directly related to the composition of betalains and the stability of pigments, which influence visual appeal and marketability.

3.2. Physicochemical properties

In Fig. 1, results are presented for the physicochemical properties from the different purification treatments of red prickly pear juice. In terms of pH, MF showed the highest ($P < 0.05$) value, while the RMF exhibited the lowest ($P < 0.05$) pH. The pH of the filtered and retained juices were lower than that found in the "Rojo Pelón" variety of *O. ficus-indica* with a value of 6.23 (Zenteno-Ramírez et al., 2015), and lower than that exhibited by Tunisian red prickly pear juice before and after microfiltration (6.02 and 6.06, respectively) (Kallel et al., 2023). Tahir et al. (2019) reported *O. ficus-indica* juice as characterized by a high pH of 5.8 and an acidity of around 0.06 % as compared to other fruit juices such as orange, lemon, and pineapple. Tahir et al. (2019) also noted that alterations in acidity and pH affect the color characteristics and components of the juice. Additionally, processing methods for prickly pear juice such as acidification, heat treatment, refrigeration, and freezing have no effect on juice pH (Kgatla et al., 2011). In the current study membrane filtration resulted in differences in pH, but the results for CJ, MF, UF, and RUF were within the reported pH range for prickly pear juices from other studies (Kallel et al., 2023; Mejía & Yáñez-Fernández, 2021) the exception being RMF.

The lowest ($P < 0.05$) percentages of reducing sugars and total sugar were found in RMF and RUF, while CJ exhibited the highest ($P < 0.05$) percentages of these sugars. As seen in Fig. 1, RMF had lower ($P < 0.05$) values than MF for reducing sugars and for total sugar, while UF gave lower ($P < 0.05$) values than RUF only for reducing sugars. Differences in results following filtration may be attributed to the separation of biomolecules in heterogeneous suspensions, which relies on specific physicochemical properties such as molecular weight, charge, monomer composition, chemical affinity, or dielectric properties of dissolved solids (Kallel et al., 2023). Kallel et al. (2023) showed that green prickly pear juice contained more total sugars (71.01 g Eq glucose/100 mL) than the red variety (39.72 g Eq glucose/100 mL). However, microfiltration significantly decreased the amount in both varieties, to 55.5 g Eq glucose/100 mL in green fruits and 32.60 g Eq glucose/100 mL in red fruits.

Membrane pressure and pore size have significant effects on the retention of high molecular weight compounds (Ghosh et al., 2017), resulting in the presence of a high content of dissolved solids ($^{\circ}\text{Bx}$). Consequently, these suspended solids can interfere with refractive index measurements (Cassano et al., 2010; Mejía & Yáñez-Fernández, 2021). In the current study, all filter-treated samples contained lower ($P < 0.05$) dissolved solids in respect to CJ.

Mejía and Yáñez-Fernández (2021), working with orange prickly pear juice and a 0.1 μm microfiltration membrane, and Cassano et al. (2010), using juice from a yellow cultivar with a 0.2 μm membrane, both evaluated $^{\circ}\text{Brix}$ in relation to membrane processing of prickly pear juice. These authors concurred that, as membrane filtration progresses, the retained fraction tends to maintain a higher content of high molecular weight compounds. However, Vergara et al. (2015), working with juice from purple cactus pears using a 0.2 μm membrane, reported no significant differences in $^{\circ}\text{Brix}$ or total sugars between the permeate and

Table 1

Color analysis of clarified, microfiltered, ultrafiltered, and filter-retained red prickly pear juice.

Variables ¹	Treatment ²					P-value
	CJ	MF	RMF	UF	RUF	
L^*	37.95 $\pm$ 0.28 ^b	43.27 $\pm$ 1.89 ^a	38.51 $\pm$ 0.55 ^b	43.92 $\pm$ 2.55 ^a	38.66 $\pm$ 0.07 ^b	<0.001
a^*	58.01 $\pm$ 0.36 ^b	64.05 $\pm$ 0.37 ^a	55.97 $\pm$ 0.73 ^c	64.48 $\pm$ 1.62 ^a	57.08 $\pm$ 0.10 ^{b,c}	<0.001
b^*	18.59 $\pm$ 0.35 ^a	8.46 $\pm$ 1.56 ^c	13.89 $\pm$ 0.90 ^b	6.59 $\pm$ 5.97 ^c	16.71 $\pm$ 0.38 ^{a,b}	<0.001
Chroma	60.92 $\pm$ 0.24 ^b	64.63 $\pm$ 0.19 ^a	57.68 $\pm$ 0.50 ^d	65.07 $\pm$ 0.91 ^a	59.48 $\pm$ 0.13 ^c	<0.001
Hue angle	17.77 $\pm$ 0.42 ^a	7.52 $\pm$ 1.41 ^b	13.95 $\pm$ 1.03 ^a	5.90 $\pm$ 5.40 ^b	16.32 $\pm$ 0.35 ^a	<0.001
ΔE	Reference	-0.62 $\pm$ 0.54 ^c	3.09 $\pm$ 0.19 ^a	-0.22 $\pm$ 0.92 ^c	1.05 $\pm$ 0.17 ^b	<0.001

¹ L^* : lightness; a^* : greenness-redness; b^* : blueness-yellowness; ΔE : color difference.

² CJ: clarified juice; MF: microfiltered juice; RMF: juice retained following microfiltration; UF: ultrafiltered juice; RUF: juice retained following ultrafiltration.

^{a-d} Means $\pm$ standard deviation ($n = 6$) in rows and with different superscripts are significantly ($P < 0.05$) different.

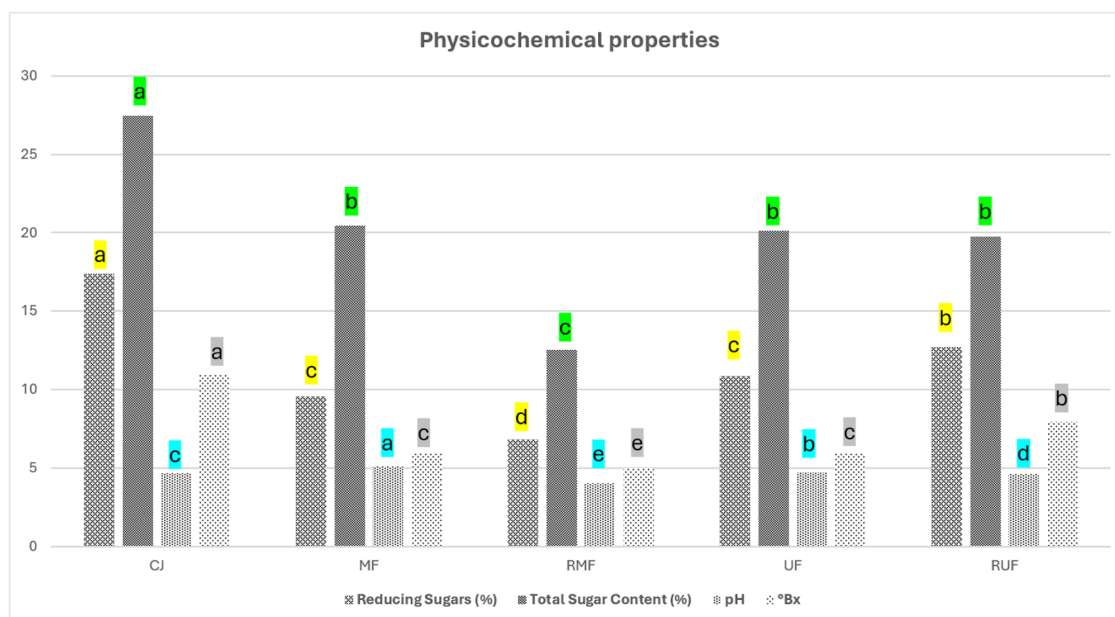


Fig. 1. Physicochemical properties of clarified, microfiltered, ultrafiltered, and filter-retained red prickly pear juice. ¹ CJ: clarified juice; MF: microfiltered juice; RMF: juice retained following microfiltration; UF: ultrafiltered juice; RUF: juice retained following ultrafiltration. °Bx: dissolved solids.

^{a-d} Means $\pm$ standard deviation ($n = 6$) in rows and with different superscripts are significantly ($P < 0.05$) different.

the retentate. The results of the presented study show that the RMF exhibited the lowest ($P < 0.05$) percentages of reducing sugars and total sugars, and °Brix. Therefore, it is possible that the prickly pear variety plays an important role in the retention of compounds in the retentate during the microfiltration process.

Jorge et al. (2025) reported that seasonal variation significantly influences the chemical composition of *Opuntia ficus indica* fruits, with winter harvested samples showing higher sugar, moisture, and α -tocopherol contents.

3.3. Antioxidant related properties

Total phenolic content, and betacyanins (β C) and betaxanthins (β X) content of the different treatments of red prickly pear juice are shown in Table 2. The clarification of red prickly pear through centrifugation (CJ) and RUF showed higher ($P < 0.05$) concentrations of total phenols than the MF and UF permeates as well as RMF. The lowest ($P < 0.05$) values for total phenols for all treatments were seen with RMF. Ghosh et al.

(2017) reported that membrane filtration with a pore size of 0.1 μ m acts as a barrier to macromolecules, such as polyphenols, in jamun juice. Those authors also reported that in membranes with pore sizes of 0.2 μ m and 0.45 μ m, polyphenols had easier flow through the filter. The result is a higher percentage of these molecules collecting in the permeates, with the 0.45 μ m pore filter reporting the highest concentration of polyphenols in the filtrate. The microfiltration was conducted using a membrane with a pore size of 0.2 μ m, and MF resulted in a higher ($P < 0.05$) quantity of phenols than RMF. This increased transfer of polyphenols through this membrane to the permeate reflects a behavior similar to that reported by Ghosh et al. (2017) and Vergara et al. (2015). In contrast, the ultrafiltration membrane pore size was 10 kDa, and the highest ($P < 0.05$) total phenols concentration was observed in RUF. The ultrafiltration results suggest that the membrane pore size should be equal to or greater than 0.2 μ m for enhanced red prickly pear juice phenol transfer through the membrane.

CJ showed the lowest ($P < 0.05$) concentration of betalains among the treatments, especially in terms of betacyanin (β C) content. Filtration generally resulted in higher ($P < 0.05$) values for betalain than those seen in the clarified juice. RUF had higher ($P < 0.05$) β C values, while RMF and RUF gave higher values ($P < 0.05$) for β X. Microfiltration resulted in similar ($P > 0.05$) levels of β C in MF and RMF, while β X was higher ($P < 0.05$) in RMF than in MF samples. Overall, RUF appears to render the best results for concentration of betalains found in red prickly pear juice.

However, due to the low molecular weights of betalains and their apparent relative ease of transport through the membrane pores (Cassano et al., 2010), both RMF and RUF appear to offer advantages for collection and concentration of betalains.

Kallel et al. (2023) presented different results from those presented in the current study, as microfiltration significantly decreased the content of both β C and β X in both red and green prickly pear juice. The difference in the current study was that, in this case, juice subjected to microfiltration first underwent centrifugation clarification, whereas Kallel et al. (2023) worked with juices without prior clarification treatment.

Sandate et al. (2020) report that after centrifugation of liquids derived from plant material, antioxidant compounds such as polyphenols and betalains increased concentration, as they are water-soluble

Table 2

Phenolic compounds, and betalain content of clarified, microfiltered, ultrafiltered, and filter-retained red prickly pear juice.

Variables ¹	Treatment ²					P-value
	CJ	MF	RMF	UF	RUF	
TPC (mg AG/100 mL)	39.79 $\pm$ 2.22 ^a	32.15 $\pm$ 0.31 ^b	21.41 $\pm$ 0.35 ^c	31.10 $\pm$ 0.24 ^b	39.79 $\pm$ 1.16 ^a	<0.001
β C (mg/L)	144.89 $\pm$ 3.94 ^d	209.61 $\pm$ 1.81 ^b	195.80 $\pm$ 9.03 ^{bc}	177.34 $\pm$ 4.04 ^c	267.80 $\pm$ 19.90 ^a	<0.001
β X (mg/L)	66.56 $\pm$ 1.09 ^b	77.60 $\pm$ 0.15 ^b	98.90 $\pm$ 8.84 ^a	65.62 $\pm$ 1.99 ^b	105.49 $\pm$ 8.27 ^a	<0.001

¹ TPC: Total phenolic content in gallic acid equivalents (AG); β C: betacyanins; β X: betaxanthins.

² CJ: clarified juice; MF: microfiltered juice; RMF: juice retained following microfiltration; UF: ultrafiltered juice; RUF: juice retained following ultrafiltration.

^{a-d} Means $\pm$ standard deviation ($n = 6$) in rows and with different superscripts are significantly ($P < 0.05$) different.

and are released from the pulp. In orange prickly pear juice that was previously centrifuged (Arboleda Mejía & Yáñez-Fernández et al., 2021) and processed using a 0.1 membrane, a higher concentration of betalains was observed in the permeate. In contrast, juice from a yellow cultivar subjected to a pectin pretreatment and processed through a 0.2 µm membrane showed a higher concentration of betalains in the retentate (Cassano et al., 2010). Similarly, purple prickly pear juice diluted 1:1 and processed using a 0.2 µm membrane resulted in betalain concentration in retentate (Vergara et al., 2015). These contrasting outcomes indicate that pretreatment conditions such as centrifugation, enzymatic pectin removal, and dilution play a critical role in determining the distribution of betalains during membrane processing. Previous studies have reported that both betacyanins and betaxanthins may be preferentially concentrated either in the permeate or in the retentate depending on processing conditions. In the present study, where centrifugation was applied as a pretreatment prior to microfiltration, distinct behaviours were observed for each betalain group: betaxanthins exhibited a higher concentration in the retentate, whereas betacyanins were more concentrated in the permeate. It is important to note that all these comparisons were conducted under microfiltration conditions, indicating that differences in betalains distribution cannot be attributed solely to membrane pore size, but rather to the combined effects of pretreatment, juice matrix composition, and interactions with macromolecular components during filtration. In this context, mucilage retention in the retentate has been reported when a membrane with a pore size of 0.1 µm was used following prior centrifugation and filtration through a 0.351 mm mesh, as described by Fernández-Martínez et al. (2024), supporting the role of matrix components in governing separation behaviour during microfiltration.

For the antioxidant activity (Fig. 2), all filtration treatments showed higher ($P < 0.05$) DPPH antioxidant activity than CJ, indicating that filtration concentrates a greater amount of antioxidant compounds. However, RMF showed higher ($P < 0.05$) DPPH antioxidant activity than all other treatments. ABTS antioxidant activity was decreased ($P < 0.05$) following filtration with the exception of RMF. FRAP antioxidant activity was highest ($P < 0.05$) in RMF followed by MF, while UF and RUF showed activity similar ($P > 0.05$) to that of CJ. In all cases, RMF had higher ($P < 0.05$) antioxidant activity than MF, UF, and RUF. Meanwhile, no differences ($P > 0.05$) were observed between UF and RUF for all analyses of antioxidant activity. Results suggest that these

functional compounds are not entirely permeable through microfiltration and ultrafiltration membranes.

Total phenolic content and antioxidant activity are two important signs of functional quality in fruit juices, in addition to statistical differences. Higher antioxidant activity means better oxidative stability, which could lead to a longer shelf life and better health benefits. The better antioxidant performance seen in RMF samples, and to a lesser extent in MF samples, shows that microfiltration is better than ultrafiltration for keeping bioactive compounds.

Mejía and Yáñez (2021) recommend the membrane microfiltration process because it is a gentle separation process that preserves bioactive compounds, reduces energy consumption, and operates with lower associated equipment costs. In terms of compounds with antioxidant characteristics, microfiltration of red prickly pear juice showed better characteristics (particularly in RMF) than centrifugation clarification and ultrafiltration.

On the other hand, the DPPH and FRAP methods exhibited similar trends for the treatments, despite the considerably different TE values, with RMF giving higher ($P < 0.05$) responses with both methods than the other treatments. However, the ABTS method showed different behavior and numerical results with CJ and RMF having values similar to each other ($P > 0.05$) and highest ($P < 0.05$) values compared to other treatments. The ABTS assay reflects a broad radical-scavenging capacity because it is applicable to both hydrophilic and lipophilic antioxidants (Prior et al., 2005; Re et al., 1999). In contrast, the DPPH and FRAP assays are particularly sensitive to compounds with strong hydrogen-donating and electron-transfer capacities (Benzie & Strain, 1996; Brand-Williams et al., 1995; Floegel et al., 2011), such as high-molecular-weight antioxidants that may be preferentially retained during microfiltration. Consequently, the ABTS response may be strongly influenced by a mixture of hydrophilic and lipophilic antioxidants that rapidly react with the $ABTS^{\bullet+}$ radical. As a result, similar ABTS values may be observed despite marked increases in DPPH and FRAP activities, reflecting inherent differences in assays chemistry, reaction pathways, and sensitivity.

4. Conclusions

Microfiltration and ultrafiltration improved the color and betaxanthin content of red prickly pear juice. Total sugars and reducing

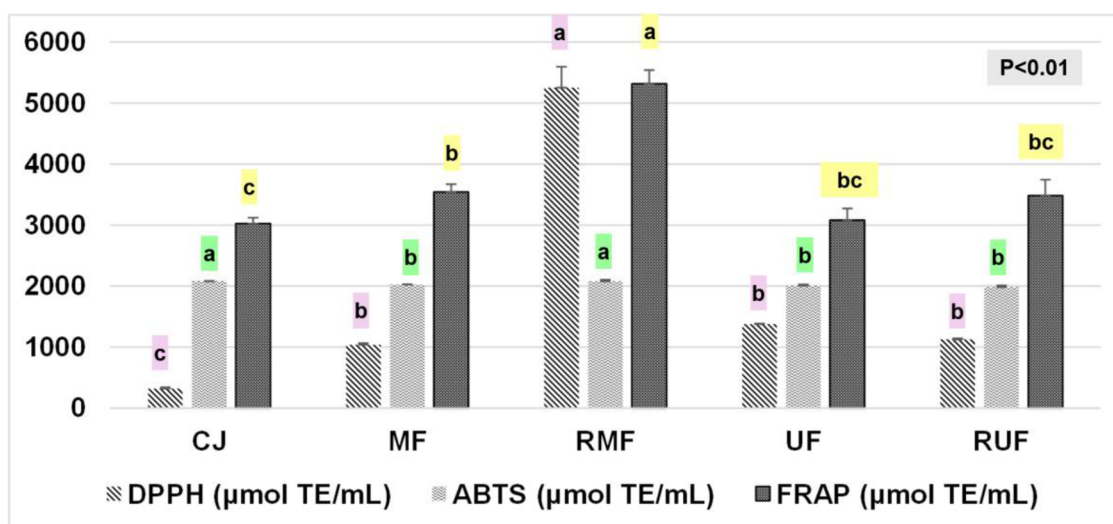


Fig. 2. Antioxidant activity, phenolic compounds, and betalain content of clarified, microfiltered, ultrafiltered, and filter-retained red prickly pear juice. DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid); FRAP: used for the ferric reducing/antioxidants power analysis; TE: Trolox equivalents; TPC: total phenolic content in gallic acid equivalents (AG); βC: betacyanins; βX: betaxanthins.

CJ: clarified juice; MF: microfiltered juice; RMF: juice retained following microfiltration; UF: ultrafiltered juice; RUF: juice retained following ultrafiltration.

^{a-d}Means ± standard deviation ($n = 6$) in rows and with different superscripts are significantly ($P < 0.05$) different.

sugars concentrations decreased following microfiltration and ultrafiltration. The physicochemical characteristics (pH, soluble solids, and total sugars), phenolic compounds, and betacyanins of the microfiltration retentates (RMF) decreased, but antioxidant activity (DPPH, ABTS, and FRAP) improved. The microfiltration process showed the best physicochemical and antioxidant related properties of red prickly pear juice, in both the permeate and in the retentate. Therefore, results suggested that microfiltration MF and RMF can be used for enriching different food products, and providing specific characteristics such as pigmentation, functional compounds (betalains), and antioxidants.

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Data availability

No data was used for the research described in the article.

CRediT authorship contribution statement

Luisaldo Sandate-Flores: Methodology, Conceptualization. **Alondra Valadez-Pineda:** Methodology, Formal analysis, Conceptualization. **Gerardo Méndez-Zamora:** Formal analysis, Data curation. **Nydia Corina Vásquez-Aguilar:** Methodology. **Eduardo García-Zambrano:** Investigation. **Roberto Parra-Saldívar:** Formal analysis. **Sugey R. Sinagawa-García:** Writing – original draft, Validation, Supervision, Conceptualization.

Declaration of competing interest

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

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Effects of microfiltration and ultrafiltration on the physicochemical and antioxidant related properties of juice from red prickly pear (*Opuntia ficus-indica*)

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