

Boosting betalain bioaccessibility from prickly pear peel through pulsed electric fields (PEF) and pressurized liquid extraction (PLE)

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ARTICLE INFO

Keywords:

Bioaccessibility
In vitro digestion
Pigments
Revalorization
Green technologies

ABSTRACT

Betalains are bioactive pigments with antioxidant and health-promoting potential, abundant in prickly pear (*Opuntia ficus-indica*) peel, an underutilized by-product. This study evaluated the efficiency of pulsed electric fields (PEF) and pressurized liquid extraction (PLE) for betalain recovery and their impact on compound bioaccessibility after *in vitro* digestion. Freeze-dried peels were treated with PEF (2.5 kV/cm, 50 kJ/kg) and PLE (50 °C, 1500 psi, pH 7), and compared to conventional aqueous maceration. Betalains were quantified by UV/Vis and characterized by LC-Q-TOF-MS/MS, while bioaccessibility was assessed using the INFOGEST protocol. PLE increased total betalain yield by 36.3%, whereas PEF enhanced bioaccessibility despite a lower initial concentration. Both techniques preserved 21–22 betalain derivatives, including indicaxanthin and betanin, showing high stability during digestion. These results highlight the potential of PEF and PLE as sustainable technologies for efficient extraction and stabilization of betalains, supporting circular bioeconomy strategies through the valorization of prickly pear peels.

1. Introduction

Betalains are natural water-soluble pigments found in various plant species and are known for their broad color spectrum, which ranges from red-violet (betacyanins) to yellow-orange (betaxanthins). These compounds are not only responsible for the vibrant coloration of many plants but also exhibit biological and functional properties of great interest, such as their antioxidant, anti-inflammatory and anti-carcinogenic potential (Gengatharan et al., 2015; Ho et al., 2022). Among the plant sources of betalains, prickly pear (*Opuntia ficus-indica*) has stood out in recent years for its high content of these pigments, making it a promising resource for applications in the food, pharmaceutical and cosmetic industries (Calvi et al., 2023; de Wit et al., 2019; Wang et al., 2023).

Importantly, the peel of the prickly pear, not just the flesh, deserves attention due to its high betalain content. The peel is considered an agricultural by-product, and its utilization represents a sustainable strategy for the extraction of betalains, contributing to the valorization of waste and the circular economy (Carreón-Hidalgo et al., 2023; de Wit

et al., 2019).

Prickly pear is a crop of great importance in arid and semi-arid regions with an estimated global production of more than 700,000 tons annually, with Mexico being the world's leading producer with 300,000–500,000 tons and 70,000 ha under cultivation, making it one of the country's most important crops (Arba et al., 2017; Naorem et al., 2024). Mexico is followed by countries such as Italy with more than 70,000 tons per year (Inglese et al., 2002; Liguori & Inglese, 2015). South Africa and Morocco are also characterized by their expansive production, with 150,000 ha being cultivated in Morocco (Oliveira & Tel-Zur, 2022). In this sense, when the fruit is processed into pulp, juices or jams, by-products such as the peel are produced, which represents approximately 30–40% of the total weight of the fruit (Melgar et al., 2017). This means that every year around 210,000 to 280,000 tons of prickly pear peel are generated worldwide as agro-industrial waste, a resource that in most cases is thrown away or used as animal feed without exploiting its potential as a source of bioactive compounds such as betalains (García-Cayuela et al., 2019).

In addition to the high content of betalains in prickly pear peel,

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<https://doi.org/10.1016/j.foodchem.2026.149325>

Received 27 June 2025; Received in revised form 12 April 2026; Accepted 17 April 2026

Available online 22 April 2026

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which varies according to growing conditions and variety (Melgar et al., 2017), it is also characterized by its content of bioactive compounds such as polyphenols and compounds with antioxidant capacity, making it a low-cost resource with high added value for the extraction of natural pigments and other functional ingredients (Castro et al., 2019a). To use this matrix as a source of compounds, the extraction process must be considered as a tool to achieve industrial sustainability, in order to promote the fulfillment of the Sustainable Development Goals and contribute to the circular economy and the transition towards sustainable food processing.

However, the efficient and sustainable extraction of betalains from prickly pear peels represents a significant challenge, as these compounds are sensitive to factors such as temperature, pH and light exposure (Castellar et al., 2003). So far, conventional extraction methods such as maceration with organic solvents have been used, which are effective but have their limitations in terms of efficiency, time and environmental impact (Cai et al., 2001). In this context, innovative technologies such as the application of high-intensity electrical pulses (PEF) and accelerated solvent extraction (ASE), also known as pressurized liquid extraction (PLE), have emerged as promising alternatives. These techniques allow for faster, more efficient and environmentally friendly extraction while preserving the integrity and functional properties of pigments and bioactive compounds (Barba et al., 2016; Calleja-Gómez, Pallarés, et al., 2024; Calleja-Gómez, Roig, et al., 2024).

In relation to these extraction processes, PEF technology has proven to be effective for the recovery of betalains from various sources such as beets, as it induces the permeabilization of cell membranes through the phenomenon of electroporation, which facilitates the release of pigments into the external environment without the need for high temperatures or environmentally harmful organic solvents and also reduces processing time and energy consumption (Bocker & Silva, 2022; Zhang et al., 2024). On the other hand, ASE technology has made it possible to achieve higher yields than with conventional methods by allowing the establishment of fixed conditions for temperature, treatment cycles and pressure (Calleja-Gómez, Pallarés, et al., 2024). However, it is not only the improvement in extraction that needs to be studied, but also the possible improvement in the bioaccessibility of these betalains extracted.

Against this background, this study aims to evaluate the potential of green technologies, such as PEF and PLE, to improve the extraction and bioaccessibility of betalains from prickly pears. It will also examine their potential applications and recent advances in extraction methods, with a particular focus on using agricultural by-products, such as fruit peels. Finally, the discussion will turn to future perspectives for optimizing these processes and integrating them into sustainable production chains.

2. Materials and methods

2.1. Sample preparation

The selected agri-food co-product from the pink prickly pear (*Opuntia ficus-indica*) was the peel, which was obtained from a local supermarket in València, Comunitat Valenciana, Spain. The prickly pear peel was subjected to a freeze-drying process (Model 10N, Gedilab S.L., Spain) to avoid the degradation of heat-sensitive compounds, such as betalains, during the drying process itself and during subsequent storage, thus increasing stability, which is particularly relevant considering its potential use as a standardized material for the formulation of functional ingredients. The use of dried material also allowed normalization of results on a dry weight basis and ensured reproducibility between extraction treatments. Moreover, PLE requires previously dried material to guarantee proper solvent penetration and homogeneous extraction under pressure, particularly when mixed with an inert dispersing agent (diatomaceous earth) to facilitate particle separation and optimal cell filling. Subsequently, the peel was crushed and homogenized by sieving until a fine powder was obtained. The dried peels

obtained were stored under vacuum and kept at -80°C until they were treated with sustainable technologies for the extraction of betalains.

2.2. Extraction conditions

The amount of dried peel used in each extraction system was determined by the operational constraints and maximum capacity of the respective equipment. For PEF, conductivity limitations and minimum working volume requirements (200 mL) restricted the solid-to-liquid ratio, whereas PLE required complete filling of the 35 mL extraction cell to ensure proper pressurized extraction. To allow comparison between technologies, all results were expressed on a dry weight basis (mg/kg DW).

2.2.1. Pulsed electric fields (PEF)

For the recovery of betalains from prickly pear peel, 1 g of the pulverized dry sample was taken and mixed with 200 mL of distilled water in a 900 mL capacity treatment cell from the PEF-Cellcrack III (German Institute of Food Technologies (DIL)) equipment (ELEA, Quakenbrück, Germany) located at the ALISOST research group lab, at the Faculty of Pharmacy and Food Sciences of the Universitat de València (Burjassot, Valencia, Spain) with two rectangular electrodes at a distance of 10 cm. Pulse frequency and duration were 2.00 Hz and 100 ms, respectively.

To determine the maximum amount of dried prickly pear peel that could be treated in the cuvette, the conductivity ($\mu\text{S}/\text{cm}$) was measured with a ProfiLine Cond 3310 conductometer (WTW, Xylem Analytics, Weilheim in Oberbayern, Germany) at a maximum of $800\ \mu\text{S}/\text{cm}$. In addition, the temperature fluctuation ($^{\circ}\text{C}$) was measured before and after treatment. Subsequently, the cuvette was inserted into the device and the treatment conditions from the previous optimization of the technology for the recovery of phenolic compounds were set, resulting in an electric field strength of $2.5\ \text{kV}/\text{cm}$, a specific energy of $50\ \text{kJ}/\text{kg}$ and a total of 33 pulses. The optimum additional shaking time for treatment was 0 min. After treatment, the extracts obtained were centrifuged at 4000 rpm for 15 min and filtered under vacuum to remove solid residues from the original matrix.

The extracts obtained had a final volume of 200 mL and were stored in the dark at -20°C until their use for the quantification and identification of the extracted betalains. The temperature before and after treatment did not vary ($20 \pm 0.5^{\circ}\text{C}$).

2.2.2. Pressurized liquid extraction (PLE)

For pressurized solvent extraction (PLE) of betalains, 4 g of the dry sample were taken and placed in a metal treatment cell with a capacity of 35 mL together with diatomaceous earth (size 6/60, RESTEK, Madrid, Spain) in a ratio of 1:2. A glass fiber filter was placed in the end part of the stainless-steel cell. The prepared cell was placed in the Dionex™ ASE™ 150 Heat Exchanger (ThermoFisher Scientific, Waltham, MA, USA) located at the ALISOST research group lab, at the Faculty of Pharmacy and Food Sciences of the Universitat de València (Burjassot, Valencia, Spain). In addition, 1 MPa of nitrogen was used to support the pneumatic system and purge the inserted cell. The standard operating conditions were: 5 min static time and heating period, 60% rinse volume, 60s purge time, 10 MPa extraction pressure and SST cell time.

In addition, the treatment conditions were established according to the previous optimization published by (Gómez-López et al., 2022) with some modifications: 1500 psi, 50°C extraction temperature, distilled water as solvent, pH 7 and 3 treatment cycles (15 min). In this sense, a treatment with sufficient pressure was sought, but without raising the temperature to a great extent in order to avoid thermal degradation. The temperature was set at 50°C and the solvent used was water instead of ethanol, as these pigments are easier to dissolve and extract. The extracts had a final volume of 35 mL and were stored in the dark at -20°C until they were used for the quantification and identification of the betalains.

2.2.3. Conventional extraction

To compare the extraction efficiency of both green technologies, the extracts obtained by PLE and PEF were compared with those obtained by conventional aqueous maceration. For the comparison with the extract obtained by PEF, 1 g of dried prickly pear peel was brought into contact with 200 mL of distilled water. The mixture was stirred without temperature at 400 rpm for the same time required for the entire treatment by PEF (15 min). Conductivity ($\mu\text{S}/\text{cm}$) and temperature ($^{\circ}\text{C}$) were measured again, the latter not deviating from 20°C during extraction.

For comparison with the extract obtained by PLE and to see the influence of the applied pressure, 4 g of dried prickly pear peels were taken and brought into contact with 35 mL of distilled water with a pH of 7. Stirring was carried out for 15 min at 400 rpm and 50°C , the total duration of the PLE treatment (3 cycles, 5 min/cycle). Finally, the extracts obtained were stored under the same conditions as the PEF and ASE extracts: in the dark, at -20°C until further use.

2.3. Analysis and characterization of betalain content

2.3.1. Betalain quantification

Betalain content was determined by spectrophotometry (UV/Vis) as described by (Nilsson, 1970). When needed, the sample was diluted with a phosphate buffer (pH 6.5) until an absorbance of 0.4–0.5 at 538 nm was achieved. Then, the absorbance of the samples was measured at 538 nm to quantify betacyanins and 476 nm to quantify betaxanthins. Absorbance at 600 nm was also measured to correct the interference of impurities. Betacyanin and betaxanthin concentrations in the extracts were expressed in mg/kg of dry weight. Total betalain content was calculated as the sum of betacyanins and betaxanthins.

2.3.2. Betalain identification

The identification of betalains was based on the procedure described by (Zoubiri et al., 2019) with some modifications. Separation of the compounds was performed on a polymer reversed phase column (PLRP-S; 250×4.6 mm, $5 \mu\text{m}$ particle size, $100 \text{ \AA}$ pore size; Agilent Technologies Inc., Palo Alto, CA, USA) using a linear gradient at a flow rate of $0.8 \text{ mL}/\text{min}$ (solvent A: 0.1% formic acid in water; solvent B: 0.1% formic acid in acetonitrile). The gradient profile was set under the following conditions: 0 min, 95% solvent A, 5% solvent B; at 60 min, 71% solvent A, 29% solvent B; at 62 min, 25% solvent A, 75% solvent B and at 69 min the conditions were restored to the initial setting (95% solvent A, 5% solvent B). The mass spectra of the compounds were obtained using an Agilent 1260 Infinity high performance-liquid chromatography system coupled to 6545 accurate-mass quadrupole Time-of-Flight (Q-TOF), with a Jet Stream Ionization source. Analysis was performed in positive electrospray ionization mode as follows: gas nitrogen flow rate $10 \text{ L}/\text{min}$ (400°C); drying gas nitrogen flow rate $8 \text{ L}/\text{min}$ (350°C); nebulizer pressure 50 psi, nozzle voltage 1 kV, capillary voltage 3.5 kV. Signals were recorded in the m/z 100–1200 range.

2.4. In vitro digestion process

A standardized INFOGEST *in vitro* digestion procedure described by (Brodkorb et al., 2019) was applied. For the gastric phase, 2 mL of a simulated gastric fluid (in mM: KCl 6.9, KH_2PO_4 0.9, NaHCO_3 25, NaCl 47.2, $\text{MgCl}_2(\text{H}_2\text{O})$ 0.1, $(\text{NH}_4)_2\text{CO}_3$ 0.5, pH 3.0), $1.25 \mu\text{L}$ of CaCl_2 (0.3 M) and 0.275 mL of porcine pepsin solution (25,000 U/mL) were mixed with 2.5 mL of extract. Then, pH was adjusted to 3.0 using HCl. Demineralized water was added to the gastric chyme to complete a volume of 5 mL. The headspace of the tubes was flushed with nitrogen before incubation at 37°C during 2 h in the dark, while end-over-end rotation (40 rpm). For the intestinal phase simulation, the gastric chyme was mixed with 2 mL of simulated intestinal fluid (in mM: KCl 6.8, KH_2PO_4 0.8, NaHCO_3 85, NaCl 38.4, $\text{MgCl}_2(\text{H}_2\text{O})$ 0.33, pH 7.0), 40 μL of CaCl_2 , 1.25 mL of pancreatin solution (800 U/mL based on trypsin activity) and 0.75 mL of bile solution (160 mM). NaOH (1 M) was used to adjust the

pH to 7 and water to complete the volume until 10 mL. Prior to incubation in the dark, at 37°C , 2 h, end-over-end rotation, the headspace of the tubes was flushed with nitrogen.

2.5. Bioaccessibility calculation

After digestion of the samples, a centrifugation (5500 rpm, 4°C , 10 min) was performed to separate soluble and non-soluble fractions. The supernatant containing the bioaccessible betalains was collected prior to the quantification by spectrophotometry and high-performance liquid chromatography (HPLC). Results are shown as the ratio of the bioaccessible compound concentration to the initial compound concentration in the sample, as indicated in Eq. (1) based on the study published by (Brodkorb et al., 2019).

$$\text{Bioaccessibility (\%)} = \frac{(\text{betalain (mg.kg}^{-1}) \text{ after digestion)}}{(\text{betalain (mg.kg}^{-1}) \text{ before digestion)}} \times 100 \quad (1)$$

2.6. Statistical analysis

Data are expressed as mean $\pm$ SD considering 3 replicates per extract ($n = 3$). For statistical analysis of the data, GraphPad Prism 8 software (GraphPad Software, San Diego, CA, USA) was used and a two-way analysis of variance (ANOVA) was performed to compare the data between the different extracts, considering a 95% confidence interval. The standard deviation is shown in the figures as error bars and the significant difference is expressed by lower case letters.

3. Results and discussion

3.1. Quantification of betalains from pulsed electric fields (PEF) extracts and effect on bioaccessibility

The determined content (mg/kg) of betacyanins, betaxanthins and total betalains in the extract obtained by pulsed electric fields (PEF) and its corresponding conventional extraction, as well as the quantified content after digestion, are shown in Table 1.

Regarding the extractive process, treatment by PEF showed a significantly lower content of total betalains, betacyanins and betaxanthins compared to conventional extraction ($p < 0.05$), with reductions of 17.7%, 17.9% and 15.5%, respectively, corresponding to a decrease of $43.8 \text{ mg}/\text{kg}$ in total betalain content. However, the ratio between the two types of betalains remains 1:1 (betacyanins: betaxanthins) in both methods, so the proportion of extracted betalains is not affected by electroporation of electric pulses.

On the other hand, the results observed after the digestive process were very remarkable. No significant difference ($p > 0.05$) was observed in the digested extracts between the extractive processes, showing a difference of only $6.9 \text{ mg}/\text{kg}$ of total betalains between the conventional and PEF-digested extracts. In addition to the quantitative differences, a slight variation in the betalain profile was observed after the digestion process, with an increase in betaxanthins compared to betacyanins (ratio 0.58 vs. 0.42). Thus, it was found that digestion affects not only the total content but also the profile of extracted betalains, regardless of the extractive methods studied.

In addition, Fig. 1 shows the bioaccessibility (%) of betacyanins, betaxanthins and total betalains after the digestive process in conventional and PEF extracts. As can be seen, PEF extraction greatly improved the bioaccessibility of betalains after the digestion process compared to aqueous maceration, so that despite the observation of lesser content of betalains in the original PEF extracts, it can be concluded that the treatment of prickly pear peel with electric pulses favors the bioaccessibility of these pigments, which is of particular importance for their assimilation in the body and the utilization of their functional properties.

In this sense, it is particularly important to highlight the role of PEF

Table 1

Content of betacyanins, betaxanthins and total betalains (mg/kg) in extracts from conventional and pulsed electric fields (PEF)-assisted extraction and their corresponding digests.

Method	Undigested			Digested		
	Betacyanins	Betaxanthins	Total betalains	Betacyanins	Betaxanthins	Total betalains
Conv	126.29 ± 11.20 ^a	117.50 ± 6.90 ^a	246.79 ± 18.07 ^a	87.69 ± 0.63 ^a	122.61 ± 4.13 ^a	210.30 ± 3.58 ^a
Ratio	0.52	0.48	1	0.42	0.58	1
PEF	103.71 ± 9.43 ^b	99.32 ± 13.54 ^b	203.03 ± 22.88 ^b	86.01 ± 19.58 ^a	117.41 ± 19.39 ^a	203.42 ± 38.97 ^a
Ratio	0.51	0.49	1	0.42	0.58	1
% increase	-17.88	-15.47	-17.73	-1.92	-4.24	-3.27

Different lower case letters indicate significant differences ($p < 0.05$) among PEF technology and its corresponding conventional.

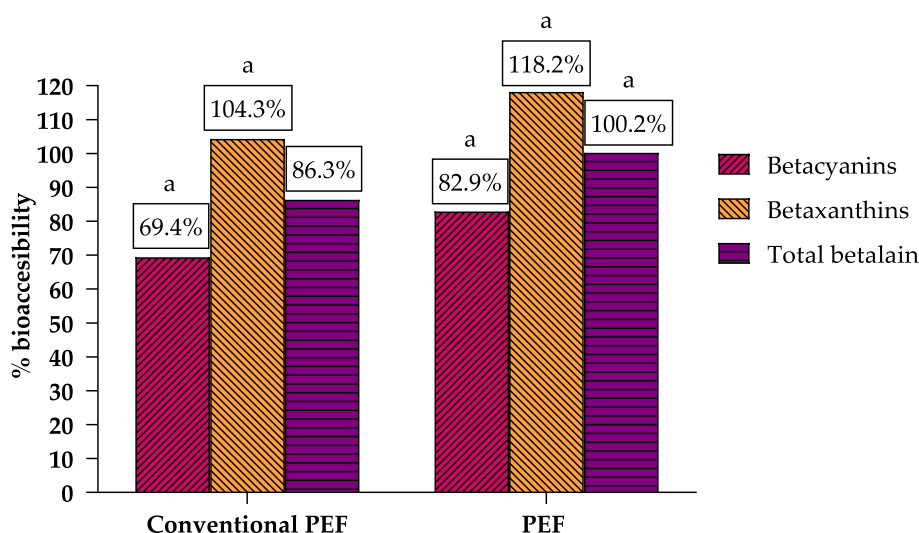


Fig. 1. Percentage bioaccessibility (%) of betacyanins, betaxanthins and total betalains according to the extracts obtained by pulsed electric fields (PEF) and conventional method after digestion. Different lowercase letters indicate statistically significant differences ($p < 0.05$) in betalain content (mg/kg) between extraction processes.

not only as an extractive process that allows higher recovery of bioactive compounds, but also as a sample treatment technology to increase bioaccessibility, whose combination with other efficient extractive processes would contribute to the optimal utilization of betalains as bioactive compounds.

Regarding the application of PEF for recovery and bioaccessibility studies on betalains, the published literature lacks detailed information on the effects of this technology, being beetroot one of the most studied matrices. In general, several authors have indicated that sustainable technologies, including PEF, play an important role. Surano et al. (2022) studied the extraction of pigments such as betalains from whole, unpeeled prickly pear fruits using a needle electroporation chamber, which allows the matrix to be treated without medium and the resulting juice to be analyzed. This approach resulted in increases ranging from 12.2% to 47.5% compared to the control. In addition, Koubaa et al. (2016) applied a more intensive treatment of 8–20 kV/cm together with a supplementary extraction of 1 h and observed that the process facilitated the diffusion of the pigments into the medium and increased the extraction in a voltage-dependent manner, with the highest amount observed after treatment with 20 kV/cm (50 mg betanin/100 g of fresh fruit).

Compared to the present study, it would be necessary to study whether more intense voltage treatments improve the recovery of betalains from ground and dried skin, and to seriously consider conductivity ($\mu\text{S}/\text{cm}$) as a major limiting factor in the extractive process. In addition, it would be beneficial to consider the possibility of combining sustainable extraction technologies such as ultrasound (USN), which has also shown a higher recovery of pigments thanks to the cavitation phenomenon (Gómez-López et al., 2021b; Koubaa et al., 2016; Silva

et al., 2020). However, most of the published studies focused on the general quantification of betalains rather than on in-depth research into the obtained ratios and bioaccessibility after each of the studied technologies. The influence of PEF on the bioaccessibility of other bioactive compounds has been studied by several authors. PEF treatment has been associated with increased bioaccessibility of other pigments such as carotenoids and phenolic compounds by altering the permeability of carrot cell membranes, an effect that is further enhanced when combined with heat treatment (López-Gómez, Elez-Martínez, Martín-Belloso, & Soliva-Fortuny, 2021a; López-Gómez, Elez-Martínez, Martín-Belloso, & Soliva-Fortuny, 2021b; López-Gómez, Elez-Martínez, Quiles-Chuliá, et al., 2021). Moreover, this increase has also been observed for antioxidant compounds in liquid matrices such as juices (Buniowska et al., 2017) and for the lipid fraction in microalgae such as *Chlorella vulgaris* (Canelli et al., 2022). Therefore, PEF is a promising technology to increase the bioaccessibility of betalains due to changes in microstructure (Ahmed & Kumar, 2023; Calleja-Gómez et al., 2022; Rybak et al., 2025). However, further studies are needed to optimize the applied conditions in relation to the extraction of betalains in prickly pear, although an increase in bioaccessibility was observed in the present study.

3.2. Quantification of betalains from pressurized liquid extraction (PLE) extracts and effect on bioaccessibility

The determined content (mg/kg) of betacyanins, betaxanthins and total betalains in the extract obtained by pressurized solvent extraction (PLE) and its corresponding conventional extraction as well as the quantified content after digestion are shown in Table 2.

Table 2

Quantification and profile of betacyanins, betaxanthins and total betalains content (mg/kg) in conventional and pressurized liquid extraction (PLE) extracts and their corresponding digests.

Method	Undigested			Digested		
	Betacyanins	Betaxanthins	Total betalains	Betacyanins	Betaxanthins	Total betalains
Conv	109.96 ± 6.24 ^a	105.44 ± 5.06 ^a	215.40 ± 11.28 ^a	76.95 ± 6.39 ^a	100.33 ± 5.20 ^a	177.29 ± 11.55 ^a
<i>Ratio</i>	0.51	0.49	1	0.43	0.57	1
PLE	141.56 ± 10.53 ^b	152.07 ± 4.28 ^b	293.64 ± 11.52 ^b	104.81 ± 1.61 ^b	167.01 ± 4.76 ^b	271.82 ± 6.12 ^b
<i>Ratio</i>	0.48	0.52	1	0.39	0.61	1
% increase	28.74	44.22	36.32	36.21	66.46	53.32

Different lower case letters indicate significant differences ($p < 0.05$) among PLE technology and its corresponding conventional.

In terms of extractive process, extraction by PLE significantly ($p < 0.05$) improved the content of betacyanins, betaxanthins and total betalains, with increases of 28.7%, 44.2% and 36.3%, respectively, with a difference of 78.2 mg/kg in total betalain content between PLE and conventional extracts. Furthermore, a very similar profile was observed between the conventional and PLE extracts in terms of the ratio of each betalain species, with a slight increase in the ratio of betaxanthins in the case of PLE compared to the conventional extraction (0.52 vs. 0.49, respectively).

After the digestive process, statistically significant differences ($p < 0.05$) were also found between the conventional and PLE extracts, with the PLE extract showing a strong increase in the content of betalains (53.3% of total betalains) and an even more significant increase in betaxanthins (66.5%). In terms of ratio, a remarkable increase in betaxanthins was observed in the PLE extract after digestion with respect to betacyanins (0.61 vs. 0.39), although this was also observed in the digested extract obtained by conventional processing.

In relation to the bioaccessible fraction, the bioaccessibility (%) of betacyanins, betaxanthins and total betalains after digestion of each of the PLE and conventional extracts is shown in Fig. 2. In this context, not only an increase in the betalains present in the PLE extracts was observed, but also an increase in the bioaccessibility of both types of betalains, which reached 92.6% of the total betalains in the PLE digested extracts, while it was 82.3% in the conventional digested extracts. Again, the increase in the bioaccessibility of betaxanthins stood out, which could indicate that both the digestive and extractive processes favor their extraction and bioaccessibility. In this sense, the PLE technology was very effective in the recovery of betalains from prickly pear peel.

Regarding the published literature, there is a great lack of

information on how accelerated solvent extraction (ASE) affects the recovery of pigments such as betalains, not only in prickly pear but also in other source matrices, with the most studied technologies being ultrasounds (USN) (Fernando et al., 2021; Gómez-López et al., 2021b; Ho et al., 2022; Koubaa et al., 2016; Silva et al., 2020) and microwave-assisted extraction (MAE) (Liu et al., 2017) to increase extraction and stability by avoiding thermal degradation. Regarding extractive processes based on the application of pressure, Xi and Luo (2016) suggested that pressure damages the cell walls and deforms the cells by increasing the solvent permeability and dissolution rate. In this sense, Castro et al. (2019b) observed that the application of high pressures increased the recovery of betalains between 6 and 17% from yellow prickly pear peel.

Regarding the bioaccessibility fraction, an increase in various compounds was observed following the use of ASE. However, there is a lack of information on how this technology affects the bioaccessibility of betalains from prickly pear cactus. Gómez-López et al. (2021a) studied the bioaccessibility and the amount of betalains in conventional methanol:water (1:1) extracts from *Opuntia stricta* peels after *in vitro* digestion and determined a bioaccessibility (%) of 42.58 ± 3.35 betalains after the intestinal phase. Therefore, the observed recovery of betalains is lower than that of ASE in the present study, which could be due to lower losses of betalains due to greater release from the original matrix and the influence of processing on bioaccessibility and stability (Medina et al., 2007). In addition, other authors reported increased bioaccessibility after ASE of other compounds such as polycyclic aromatic hydrocarbons (1500 psi, 100 °C) with up to 80% higher concentration than extraction with hydroxypropyl- β -cyclodextrin (HPCD) (Zhang et al., 2016). Therefore, the pressure and temperature exerted by ASE would contribute to a higher extraction from the original matrix and a solubilization of the compounds, making them more accessible to the action

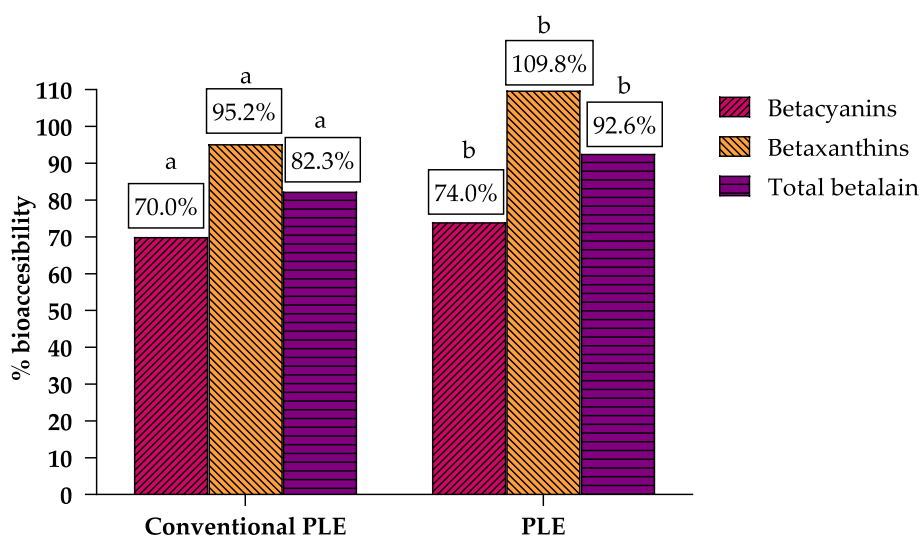


Fig. 2. Bioaccessibility (%) of betacyanins, betaxanthins and total betalains corresponding to extracts obtained by pressurized solvent extraction (PLE) and conventional methodology after digestion. Different lowercase letters indicate statistically significant differences ($p < 0.05$) in betalain content (mg/kg) between extraction processes.

of digestive enzymes.

3.3. Comparison between PEF and PLE

In order to establish an effective green technology for the recovery of betalains from prickly pear peel and other possible co-products, as well as to study the effects on their bioaccessibility, a comparison between pulsed electrical extraction (PEF) and pressurized solvent extraction (PLE) was carried out. It should be noted that the solid-to-liquid ratios differed between PEF and PLE due to inherent operational constraints of each technology. Therefore, the comparison reflects the extraction performance of each technology under technically feasible conditions rather than under identical processing parameters. To allow fair comparison, results were expressed on a dry weight basis (mg/kg DW). Fig. 3 shows the content (mg/kg) of each betalains in the extracts before and after the digestion process of the two technologies. As can be seen in Fig. 3, the PLE extractive technology significantly ($p < 0.05$) improved the recovery of betacyanins, betaxanthins and total betalains in both the original and digested extracts, with increases of 36.50%, 53.11% and 44.63%, respectively, with respect to PEF in original extracts. Compared to the corresponding conventional extracts, the increase in betalains was more pronounced: 152.07 ± 4.28 mg/kg total betaxanthins and 141.56 ± 10.53 total betacyanins in the undigested PLE extract.

However, the difference in the betalain content of PEF compared to PLE after digestion is less pronounced (Fig. 3b), with the increases of PLE over PEF smaller than those observed in the original extracts, with 21.86%, 42.25% and 33.63% for betacyanins, betaxanthins and total

betalains, respectively. Therefore, an 11% decrease in the difference in total betalain content between methodologies was observed after digestion.

In particular, a remarkable decrease in betacyanins content was observed after digestion, especially in the extracts obtained by PLE (26.0% reduction) compared to those obtained by PEF (17.1% reduction). This loss is consistent with the lability of betalains against high pH and oxidative conditions, which could favor its degradation during the intestinal phase of simulated digestion (Esatbeyoglu et al., 2015; Kanner et al., 2001). In contrast, the betaxanthin content increased after digestion in both methods, especially in the extracts obtained by PLE (9.8% increase). This phenomenon could be explained by the partial conversion of betalains into structurally related derivatives under gastrointestinal conditions, as well as by a possible release of conjugated forms, precursor compounds not initially detected, or protein aggregates formed during the digestion process (Montiel-Sánchez et al., 2021; Tesoriere et al., 2008).

The better performance of PLE extraction technology compared to PEF may be due to several factors. Among others, the temperature (50 °C) and pressure (1500 psi) applied during PLE would improve the solubilization and release of betalains from the plant matrix, resulting in higher recovery in the original extracts (Calleja-Gómez, Roig, et al., 2024; Dhua et al., 2022). Furthermore, although PLE presents higher extraction rate, a decrease in the relative advantage over PEF is observed after digestion, possibly due to the fact that PEF facilitates the extraction of betalains in more stable forms that are less susceptible to the digestion process.

However, although temperature and pressure may stress the extracted molecules, the selected conditions allow efficient recovery of betalains by combining pressure with a relatively low temperature to avoid thermal degradation. According to the observed results, PLE technology is more efficient than PEF technology in the recovery of betalains from prickly pear peels. However, it should be noted that both the PLE and PEF technologies allow an increase in the bioaccessibility of betalains, which is particularly pronounced in the case of the PEF technology, so that its application as a treatment is of great industrial interest.

As for the total betalain content, PLE showed a moderate decrease (7.4%) after digestion, while PEF remained completely preserved (non-significant change). These results suggest that although PLE is more efficient to obtain betalains in the native state, the extracts obtained by PEF exhibit greater stability to the simulated digestive environment, which could lead to a higher relative bioaccessibility.

3.4. Betalain profile identification

The identification of the betalains extracted by LC-Q-TOF-MS/MS in each of the pre- and post-digestion extracts obtained by PEF and PLE, as well as in their corresponding conventional extracts, is shown in Table 3. In this way, a characterization of the phytochemical profile of the extracts obtained with the applied technologies and their stability under simulated digestion conditions was performed based on the analysis in combination with the published literature on structural assignment.

In this context, 22 compounds were identified in the PLE extracts and 21 in the PEF extracts, with the exception of 2-decarboxy-neobetanin in PEF. In this sense, it was found that PLE extraction not only improved the quantitative recovery and bioaccessibility of betalains, but also increased the number of betalains identified by one compound compared to PEF. Furthermore, the profiles were similar, including both betacyanins and betaxanthins and reflecting a wide structural diversity. The most representative compounds identified included betanin, betanidin 5-O- β -sophoroside, muscaaurin I, γ -aminobutyric acid betaxanthin, and indicaxanthin, with a notable presence of amino acid-derived betaxanthins. Five betaxanthins derived from condensation with different amino acids were identified: γ -aminobutyric acid betaxanthin (GABA), proline betaxanthin (indicaxanthin), valine

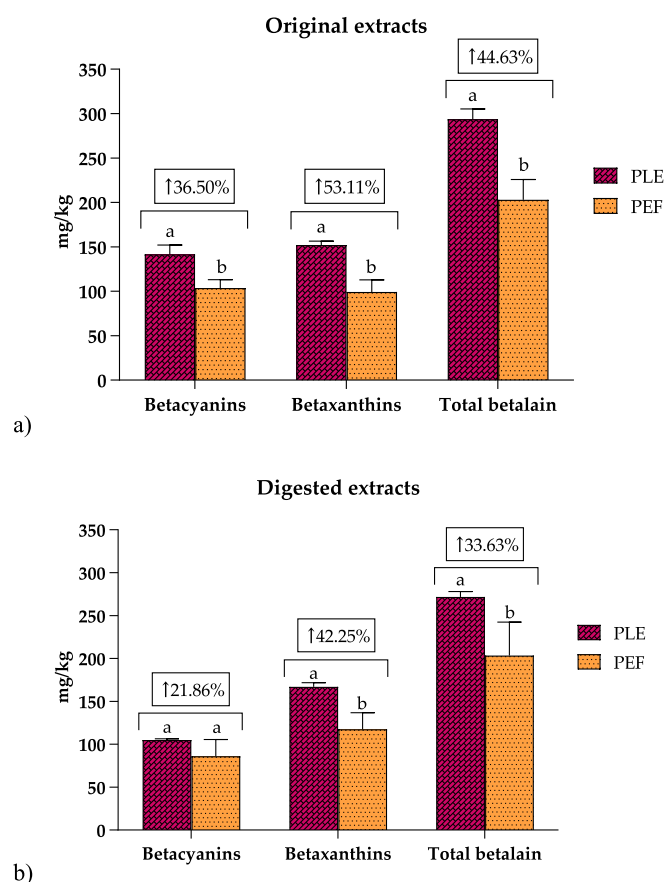


Fig. 3. Comparison of the quantified betalain content (mg/kg) in (a) original extracts and (b) digested extracts obtained by pressurized liquid extraction (PLE) and pulsed electric fields (PEF) from prickly pear peels. The extraction increases (%) of the PLE technology compared to PEF are indicated in the boxes. Different lower-case letters indicate statistically significant differences ($p < 0.05$) in betalain content (mg/kg) depending on the extractive process.

Table 3

Compounds identified by LC-Q-TOF-MS/MS in each of the extracts obtained by pulsed electric fields-assisted extraction (PEF), pressurized solvent extraction (PLE) and conventional aqueous maceration, as well as the compounds identified in their corresponding digests.

N°	tR (min)	Formula	Identification	Tof MS (m/z)	MS/MS (fragment ions (m/z)) (20 eV)	Undigested				Digested				References
						C. PEF	PEF	C. ASE	ASE	C. PEF	PEF	C. ASE	ASE	
1	3.52	C ₁₄ H ₁₃ N ₃ O ₈	Muscaaurin I	351.07	n.d	+	+	+	+	+	+	+	+	(Castellanos-Santiago & Yahia, 2008)
2	9.33	C ₁₃ H ₁₆ N ₂ O ₆	γ -aminobutyric-acid-betaxanthin	297.11	n.d	+	+	+	+	+	+	+	+	Barkociová et al. (2021); Wang, Liu, et al. (2020)
3	10.11	C ₃₀ H ₃₆ N ₂ O ₁₈	Betanidin 5-O- β -sophoroside	713.20	n.d	+	+	+	+	+	+	+	+	Barkociová et al. (2021); Wybraniec et al. (2007)
4	10.26	C ₂₄ H ₂₆ N ₂ O ₁₃	Betanin	551.15	389.10	+	+	+	+	+	+	+	+	Barkociová et al. (2021); Wang, Liu, et al. (2020)
5	11.64	C ₁₄ H ₁₆ N ₂ O ₆	Proline-betaxanthin (Indicaxanthin)	309.11	263.10/217.09/70.06	+	+	+	+	+	+	+	+	García-Cayuela et al. (2019); Wang, Liu, et al. (2020)
6	12.70	C ₂₂ H ₂₆ N ₂ O ₁₁	17-decarboxy-betanin	507.16	345.110	+	+	+	+	+	+	+	+	Sawicki and Wiczowski (2018); Wang, Liu, et al. (2020)
7	13.08	C ₃₀ H ₃₆ N ₂ O ₁₈	Isobetandin 5-O- β -sophoroside	713.20	n.d	+	+	+	+	+	+	+	+	Barkociová et al. (2021); Wybraniec et al. (2007)
8	13.25	C ₂₄ H ₂₆ N ₂ O ₁₃	Isobetatin	551.15	389.10	+	+	+	+	+	+	+	+	Barkociová et al. (2021); Wang, Liu, et al. (2020)
9	13.25	C ₂₂ H ₂₆ N ₂ O ₁₁	17-decarboxy-isobetatin	507.16	n.d	+	+	+	+	+	+	+	+	Sawicki et al. (2016); Wang, Liu, et al. (2020)
10	15.73	C ₁₄ H ₁₈ N ₂ O ₆	Valine-betaxanthin	311.12	n.d	+	+	+	+	–	–	–	–	Sawicki et al. (2016); Wang, Liu, et al. (2020)
11	15.77	C ₂₂ H ₂₆ N ₂ O ₁₁	2-decarboxy-isobetatin	507.16	345.11	+	+	+	+	+	+	+	+	Sawicki et al. (2016); Wang, Liu, et al. (2020)
12	15.78	C ₂₄ H ₂₆ N ₂ O ₁₃	Gomphrenin I	551.15	389.10	+	+	+	+	+	+	+	+	Barkociová et al. (2021); Wang, Liu, et al. (2020)
13	16.55	C ₂₂ H ₂₆ N ₂ O ₁₁	2-decarboxy-betanin	507.16	345.11	+	+	+	+	+	+	+	+	Sawicki and Wiczowski (2018); Wang, Liu, et al. (2020)
14	17.25	C ₂₇ H ₂₈ N ₂ O ₁₆	Phyllocactin	637.15	n.d	+	+	+	+	+	+	+	+	Barkociová et al. (2021)
15	17.98	C ₂₄ H ₂₆ N ₂ O ₁₃	Isogomphrenin I	551.15	389.10	+	+	+	+	+	+	+	+	Barkociová et al. (2021); Wang, Liu, et al. (2020)
16	18.72	C ₂₃ H ₂₄ N ₂ O ₁₁	17-decarboxy-neobetatin	505.15	n.d	+	+	+	+	+	+	+	+	Wang, Liu, et al. (2020)
17	19.84	C ₂₇ H ₂₈ N ₂ O ₁₆	Isophyllocactin	637.15	n.d	+	+	+	+	+	+	+	+	Barkociová et al. (2021); Wang, Liu, et al. (2020)
18	20.72	C ₂₃ H ₂₄ N ₂ O ₁₁	2-decarboxy-neobetatin	505.15	n.d	–	–	–	+	–	–	–	–	Wang, Liu, et al. (2020)
19	31.13	C ₁₈ H ₁₈ N ₂ O ₆	Phenylalanine-betaxanthin	359.12	315.13	+	+	+	+	+	+	+	+	Sawicki et al. (2016); Wang, Liu, et al. (2020)
20	36.54	C ₂₀ H ₁₉ N ₃ O ₆	Tryptophan-betaxanthin	398.13	354.14	+	+	+	+	+	+	+	+	Sawicki et al. (2016)
21	40.04	C ₃₄ H ₃₅ N ₂ O ₁₆ ⁺	6'-O-feruloyl-betanin	727.20	389.10	+	+	+	+	+	+	+	+	Sawicki and Wiczowski (2018); Wang, Liu, et al. (2020)
22	40.93	C ₃₄ H ₃₅ N ₂ O ₁₆ ⁺	6'-O-feruloyl-isobetatin	727.20	389.10	+	+	+	+	+	+	+	+	Sawicki and Wiczowski (2018); Wang, Liu, et al. (2020)

betaxanthin, phenylalanine betaxanthin and tryptophan betaxanthin. Also noteworthy is the identification of gomphrenin I, isogomphrenin I, phyllocactin and isophyllocactin, compounds characteristic of species such as *Opuntia* spp.

In terms of bioactive properties, several studies have shown that, in addition to pigmentation, betalains also have biological activities such as antioxidant, neuroprotective and anti-inflammatory effects (Barkociová et al., 2021; Gandía-Herrero et al., 2010; Wang, Zhang, et al., 2020). In particular, indicaxanthin, which is derived from the amino acid proline, has been extensively studied for its anti-inflammatory and anti-carcinogenic effects. In the study published by Allegra et al. (2018) on indicaxanthin contained in *Opuntia ficus-indica*, the ability of this pigment to inhibit the proliferation of metastatic melanoma cells through growth inhibition, apoptosis induction and reduced cell invasiveness was demonstrated, resulting in a decrease in tumor development when administered orally to mice. In addition, it has the ability to cross the blood-brain barrier and modulate cellular processes in neuronal models after oral administration, with an inhibition of neuronal excitability that could be beneficial in patients with neurodegenerative diseases (Gambino et al., 2018). Additionally, the functional properties of betanin, a betalain that is highly abundant in matrices such as beets and prickly pears, are also noteworthy for its antioxidant and antitumor properties, showing a selective cytotoxic effect on colon and glioma cancer cells (Saber et al., 2023; Salimi et al., 2021).

On the other hand, a high structural resistance to gastrointestinal conditions was observed regardless of the extraction process, which is fundamental for the development of their bioactive potential. In the case of PEF technology, all compounds were retained after digestion, with the exception of valine-betaxanthin, which was degraded by the digestive process. In the case of post-digestion PLE, valine-betaxanthin and 2-decarboxy-neobetanin were no longer detected.

Other authors have pointed out the structural resilience of betalains, as they are more stable under acidic conditions and towards digestive enzymes, which increases bioaccessibility and nutraceutical efficacy (Tesoriere et al., 2004; Wang, Liu, et al., 2020). For this reason, their use as functional ingredients in food or for the formulation of nutraceuticals would be of great interest to the industrial sector, in combination with the application of sustainable technologies that maximize the efficiency of extraction without undermining the commitment to sustainability.

The slight variability observed may be attributed to differences in molecular structure, polarity, and chemical stability. Structural features can influence solubility, susceptibility to hydrolysis, and oxidative degradation under processing and digestive conditions (Kumorkiewicz-Jamro et al., 2021). Furthermore, extraction technologies may differentially affect compound profiles depending on their sensitivity to temperature, pressure, or electric field-induced structural modifications. PEF electroporation and localized thermal effects, as well as pressurized solvent conditions in PLE, may alter selectively enhance or reduce the recovery of structurally distinct betalains. Similar structure and stability patterns during *in vitro* digestion have been reported in recent INFOGEST studies, highlighting that degradation are strongly compound specific (Trych et al., 2022).

Therefore, further studies systematically correlating betalain molecular structure with *in vitro* digestion behavior and modifications induced during processing are required. In particular, mechanistic research integrating advanced LC-MS/MS profiling with controlled processing variables would provide deeper insight into how emerging extraction technologies influence the digestive and functional performance of individual betalains.

3.5. Technology limitations and industrial scalability

Despite their effectiveness in recovering betalains from prickly pear peel, the limitations associated with PEF and PLE must be carefully considered when addressing industrial scale-up.

For PLE extraction, the sample must be previously dried and milled

to maximize solvent contact and prevent the formation of compact masses that hinder solvent percolation. Consequently, the use of a dispersing agent is typically required. In the case of prickly pear peel, drying enhances stability and enables sample standardization. However, it also increases processing costs and may become a critical factor during process scale-up. Moreover, freeze-drying is frequently selected to protect heat-sensitive pigments and is often used as a first option in laboratory scale studies, yet it is expensive and energy intensive relative to industrial alternatives. Recent studies report high energy demand for freeze-drying compared with other technologies and emphasize that the drying method can strongly influence overall process sustainability (Barp et al., 2023; Maharani Zudana et al., 2025). Therefore, for industrial translation of betalain ingredients, alternatives to lyophilization should be considered, such as spray-drying (with carriers or encapsulation), vacuum-based drying, or low temperature oven drying, which can produce stable powders at higher throughput and potentially lower energy consumption (Aburto et al., 2019; Grace et al., 2025; Kalambe & Guhe, 2026).

Nevertheless, PEF also presents industrial scale-up limitations. Increasing solids loading or enhanced ion release after electroporation can raise the electrical conductivity of the treatment medium, leading to higher Joule heating, narrowing the safe operating window, and potentially limiting the achievable electric field strength and treatment uniformity. This phenomenon has been documented in plant matrices rich in betalains, where PEF treatment has been shown to increase tissue conductivity because of membrane electroporation (Nowacka et al., 2019). As a result, PEF scale-up typically requires engineering solutions for temperature control, heat removal, and medium design, particularly when targeting thermolabile pigments. In the present study, only 1 g of sample could be introduced into the minimum required working volume of 200 mL, which substantially limited the solid-liquid (S/L) ratio.

In this context, an important methodological consideration is that PLE and PEF are different unit operations and therefore may impose different feasible S/L ratios. In PLE, the cell volume is fixed, and efficient operation requires complete and homogeneous filling. In contrast, PEF chambers may require a minimum working volume and are constrained by conductivity and thermal limits, making it difficult to match the high solids regime of PLE without redesigning the process line. For scale-up, these differences are highly relevant because industrial feasibility is driven by mass and energy balances. A useful way to connect lab comparability with industrial relevance is to report, alongside mg/kg DW, process metrics such as kg/mL of solvent per kg dry peel processed and kWh per kg extract (or per g betalains) recovered. Scale-up studies in PLE showed that S/L ratio become central design variables in industrial scenarios and it is a key factor in the transition from lab to pilot scale (da Rodrigues et al., 2025).

Given these green technology limitations, quantitative environmental assessment is essential. Wang et al. (2024), through a Life Cycle Assessment (LCA), concluded that the main environmental burdens associated with green technologies and solvents are primarily linked to solvent production and consumption, as well as thermal operations. Therefore, for the industrial upscaling of betalain valorization processes, a robust sustainability comparison between PEF, PLE, and conventional extraction requires process-specific LCA inputs, including pretreatments (drying and milling), extraction, solvent or treatment medium handling, and downstream concentration and stabilization steps such as spray- or vacuum-drying.

Finally, beyond extract yield and *in vitro* bioaccessibility, industrial scale-up requires demonstration of stability and functionality in real food matrices, sensory acceptance, and physiological relevance beyond static digestion. Prickly pear peel has already been explored as a functional ingredient in foods, showing an improved nutritional and functional profile when incorporated in bread while maintaining acceptable sensory scores at optimized inclusion levels (Parafati et al., 2020). Other applied studies have used prickly pear peel powder and extract in minimally processed fruit as coating systems and as a functional

ingredient in meat products, including sensory and quality assessment and showing feasibility but also dose dependent acceptability constraints (Lavelli et al., 2022; Parafati et al., 2021).

Therefore, future research should prioritize pilot-scale trials and stabilization strategies compatible with industrial processing, as well as *in vivo* studies or advanced absorption models coupled with product prototypes and sensory evaluation to establish a realistic performance.

4. Conclusions

This study demonstrates the potential of prickly pear peel as a sustainable and underutilized source of betalains, highlighting the relevance of applying green extraction technologies to agri-food by-products. The comparative evaluation of PEF and PLE showed different technological profiles, while PLE maximized initial betalain recovery, PEF enhanced relative bioaccessibility and compound stability under simulated gastrointestinal conditions. This suggested that although PLE maximizes initial extraction, PEF may provide additional benefits when physiological conditions after the digestion process are considered. From a food science perspective, the preservation of structural diversity observed through LC-Q-TOF-MS/MS analysis supports their potential incorporation into functional formulations. Moreover, the valorization of prickly pear peel through PEF and PLE aligns with circular economy strategies and contributes to the transition towards more sustainable food processing systems.

Nevertheless, several limitations must be acknowledged. The bioaccessibility results were obtained using the INFOGEST model, which does not account for intestinal absorption, metabolism, microbiota interactions, and systemic distribution. In addition, sensory properties and functional performance in real food matrices should be studied as the next step of the entire process, and economic or scalability analyses would be a key point in the industrial application of these technologies.

For these reasons, future research should include *in vivo* bioavailability studies, pilot-scale trials to evaluate industrial applicability, and integration of these extracts into food systems to assess stability, sensory acceptance, and functional performance. Furthermore, quantitative sustainability assessments, including LCA, would strengthen the environmental evaluation of these technologies. The exploration of higher electric field intensities and process combinations may contribute to the establishment of optimized and scalable extraction strategies. Finally, the application of these technologies to other betalain-rich matrices, such as different *Opuntia* spp., would allow the evaluation of their versatility and the establishment of more universal extraction parameters.

CRedit authorship contribution statement

Mara Calleja-Gómez: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Noelia Pallarés:** Writing – review & editing, Supervision, Conceptualization. **María Gaona-Ruiz:** Investigation, Formal analysis, Data curation. **Francisco J. Barba:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Celia Carrillo:** Writing – review & editing, Methodology, Formal analysis, Data curation, Conceptualization.

Funding

This research is part of the project PID2021-123628OB-C42 funded by MICIU/AEI/10.13039/501100011033/FEDER, UE. Mara Calleja-Gómez is a beneficiary of the predoctoral grant from the Conselleria d'Educació, Universitats i Ocupació of Generalitat Valenciana (CIACIF/2022/391).

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.

Acknowledgements

Authors would like to acknowledge Generalitat Valenciana (Spain) for the financial support (IDIFEDER/2018/046—Procesos innovadores de extracción y conservación: pulsos eléctricos y fluidos supercríticos) through the European Union ERDF funds (European Regional Development Fund).

Data availability

Data will be made available on request.

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