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Impact of sweet potato peels extracts obtained by pulsed electric fields on the growth of probiotic strains from *Lactobacillus* genus

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ABSTRACT

This work describes the first approach for the valorization of sweet potato (Sp) peels assisted by pulsed electric fields (PEF) to obtain extracts that enhance the growth of potential probiotic bacteria. Four different varieties were studied, namely white sweet potato (WSp, "O'Henry"), white with purple (WPSp or "Violeta Roja"), orange (OSp or "California") and purple (PSp, "Pepita"). Among them, the PEF extracts obtained from PSp had a noticeable content of carbohydrates, polyphenols and flavonoids (12.5 mg/mL, 4.34 mg GAE/g and 1.73 mg GAE/g, respectively), being in any case higher than those obtained by maceration extraction. The analysis by quantitative Nuclear Magnetic Resonance (qNMR) allowed us to identify sucrose, fructose and glucose as the main sugars extracted, with a total concentration close to 94 mg/g PSp for the PSp-PEF crude extracts, which entails an enhancement by up to 92% with respect to the conventional extraction. Furthermore, these extracts improved the growth kinetics of probiotic bacteria, *Lactobacillus* members and also, the production of short chain fatty acids (SCFA). This fact should be also noted since they are considered valuable platform chemicals to be transformed into high-added-value, thus proposing another way for valorization.

Industrial relevance: The relevance of this work lies in the necessity of searching new sustainable foods for their consumption, and the processing of these foods by means of innovative technologies. In light with this fact, the suitability of PEF-assisted extraction to be scaled-up at industrial level would pave the way for an efficient, notable conversion of wasted sweet potato (Sp) peels to potential prebiotics, boosting the recovery of these compounds by PEF technology with respect to traditional extraction methods and the health benefits.

1. Introduction

Roughly one-third of the global food supply is wasted along every step of the food distribution process before reaching consumers. This not only results in a substantial economic impact but also significant environmental consequences. As the global food production industry continues showing an exponential growth, these negative effects are scaling accordingly (FAO, 2022).

Most of agricultural side streams have few applications, being mainly used for composting or livestock feed. However, side streams' valorization represents an attractive alternative to reduce their quantity, transforming them into new products with an added value with potential applications in the cosmetic, pharmaceutical, or food industries (Thakur et al., 2024). In this sense, innovative non-thermal processing technologies such as ultrasound, high pressure processing, supercritical fluids, or pulsed electric fields (PEF) show a great potential for the side streams valorization (Chemat et al., 2020).

PEF is a non-thermal processing technology that has gained increasing attention in recent years for improving the extraction efficiency of nutrients and bioactive compounds from plant-based materials. This technology involves the application of high-intensity, short-duration electric pulses to a product, causing permeabilization of the cell membrane and thereby enhancing the release of intracellular compounds. In the food industry, PEF has been used for a wide range of applications, including juice extraction, oil recovery, and extraction of high-added-value from fruits and vegetables (Barba, Parniakov, &

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Wiktor, 2020).

Several studies have shown that PEF can significantly enhance the extraction efficiency of various nutrients and bioactive compounds, such as polyphenols, flavonoids, carotenoids, and vitamins from plant-based materials (El Kantar et al., 2018). The extraction efficiency of PEF differs according to several factors, including pulse frequency, duration, strength, and number of pulses. Additionally, the extraction efficiency can also be affected by the physicochemical properties of the plant-based materials, such as particle size, moisture content, and cell structure (Kim, Jeong, Ju, Jeong, & Lee, 2023).

In plant-rich diets, sweet potatoes (*Ipomoea batatas*) represent a nutritious and widely consumed root vegetable that has been studied extensively for its potential health benefits. It ranks among the world's most crucial, versatile, yet underutilized food crops, boasting an annual production exceeding 90 million tonnes (Alam, 2021). Sweet potato peels exhibit a spectrum of colors, ranging from white, cream, yellow, orange, pink, and red to purple. The flesh of sweet potatoes can also vary, appearing in shades of white, cream, yellow, orange, and even purple (Alam, Sams, Rana, Akhtaruzzaman, & Islam, 2020). Remarkably, sweet potatoes can be harvested multiple times throughout the year, yielding significantly a higher production compared to other leafy greens.

They are a good source of complex carbohydrates, fiber, carotenoids, vitamins, and minerals (Laveriano-Santos et al., 2022). Sweet potatoes are also known for their high content of antioxidants, such as β-carotene and polyphenols (Sun, Pan, Yang, Jia, & Guo, 2019). Furthermore, purple sweet potatoes have a high anthocyanin content, which is commonly known for its antioxidant properties (Gras, Nemetz, Carle, & Schweiggert, 2017). Not only tubers are a good source of bioactive compounds, but also peels contain bioactive compounds such as anthocyanins, carotenoids, and phenolic compounds (Amagloh et al., 2022). However, sweet potato peels are often discarded during food processing, resulting in a significant side streams (Zhu et al., 2019).

Previous researches have suggested that sweet potatoes may have numerous health benefits. For example, sweet potato extract was found to reduce inflammation in rats with colitis (Mu, Xu, Wang, Chen, & Chen, 2021) and potential anticancer properties (Asadi, Ferguson, Philpott, & Karunasinghe, 2017). Additionally, sweet potatoes may have potential benefits for lipid metabolism and beneficial effects on type 2 diabetes, as they have been shown to reduce total cholesterol levels and regulate blood glucose homeostasis (Kinoshita, Nagata, Furuya, Nishizawa, & Mukai, 2023). Interestingly, sweet potatoes may also have potential benefits as prebiotics promoting a balanced gut microbiome, as verified in *in vitro* and *in vivo* assays (Cao et al., 2022). Traditionally, prebiotics are defined as a selectively fermented ingredient that results in specific changes in the composition and/or the activity of the gastrointestinal microbiota, thus conferring benefits upon host health (Gibson et al., 2017). In this sense, the potential prebiotic effectwould be attributed to its high content of dietary fiber which are compounds resistant to digestion in the small intestine and are fermented by the microbiota in the colon, leading to the production of short-chain fatty acids (SCFAs) and other metabolites that promote the growth of beneficial gut bacteria and have anti-inflammatory effects (Liu et al., 2020). Among the human commensal bacteria, the genus *Lactobacillus*, also present in many fermented foods,play a crucial role in assisting the host's digestion of specific dietary components and protecting against harmful pathogens and also, playing a role in vaginal health (Matos & Leulier, 2014). Moreover, *Lactobacillus* members exhibit qualities that are highly desirable in commercial applications, serving as essential components in health supplements and tools within the food technology sector in the production of fermented dairy, meat, and vegetable products (Tang & Zhao, 2019). Due to their significant economic impact, Lactobacilli have been subject to extensive research, making them well-characterized in terms of genomics and their interactions with humans in both health and disease, especially when compared to other bacterial genera (Dempsey & Corr, 2022).

The aim of this study was the valorization of sweet potato peels through pulsed electric field-assisted extraction and the evaluation of the impact of the extracts on the growth of strains of the *Lactobacillus* genus.

2. Material and methods

2.1. Sweet potato varieties

Sweet potatoes (*Ipomea batatas*) var. "O'Henry" (WSp), "Violeta Roja" (WPSp), "Pepita" (PSp) and "California" (Osp) cultivated in the Valencian Community were purchased from the local market at Valencia (Spain) at January 2023. The samples were washed with tap water and manually peeled. Only the skin with the first layer of flesh was cut into small pieces of 2–3 mm^2 and then air-dried at 37 °C for 12 h. The samples stabilized were kept in airtight containers at 25 °C till the moment of analysis.

2.2. Maceration procedure

A maceration extraction (Conv) with water and magnetic stirring was applied as a reference procedure for the extraction of bioactive compounds from the skins of the sweet potato varieties studied: white (O'Henry), white with purple skin (Violeta Roja), purple (Pepita) and orange (California). The samples were mixed with sterile deionized water at a final concentration of 2% (*w*/w), meaning 4 g of peel were added to 196 mL of deionized water. The extraction was carried out in amber glass flasks and homogenized through agitation at 25 °C for 3 h using a thermostatic orbital shaker set at a rotational speed of 500 rpm. Afterward, the aqueous suspensions were filtered using Whatman No. 4 filter paper and divided into aliquots, which were stored at −20 °C until further analysis. Each suspension was prepared in triplicate to ensure accuracy and reproducibility.

2.3. Pulsed electric fields (PEF)-assisted extraction procedure

For the PEF-assisted extraction, the suspension described in Section 2.2 was prepared and treated using a laboratory-scale batch system PEF Cellcrack III (ELEA, Germany) located at the Faculty of Pharmacy of the Universitat de València, Spain. A chamber with a distance between two electrodes of 10 cm was used. Batches of samples weighing 200 g (196 g of water +4 g of sweet potato skin sample) were loaded into the chamber. An electric field intensity I of 3 kV/cm and a total specific energy input (WT) of 100 kJ/kg was applied. The treatment applied 45 pulses, each with a constant pulse duration (τp) of 100 μs and a pulse repetition frequency of 2 Hz (unipolar square wave pulse).

Following the PEF treatment, the suspensions were agitated for 3 h at 25 °C. The resulting extracts were filtered and stored at −20 °C until further analysis.

2.4. Sweet potato side streams characterization

2.4.1. Carbohydrate total content

Total-carbohydrate content was determined on the basis of previous works (Calleja-Gómez et al., 2022), according to the phenol-sulfuric method, with D-glucose solutions at 10–100 mg/L used as standard. Briefly, 500 μL of 5% phenol and 2.5 mL of sulfuric acid were added to a mixture composed of SP crude and standard solution (1/10, respectively). The crude was shaken and then incubated for 30 min at 25 °C. Afterwards, the value for absorbance measured at 490 nm provided us the total carbohydrates, further expressed in mg glucose/L solution (mg Glu/L).

2.4.2. Carbohydrate profile by NMR analysis

The carbohydrate profile of the PSp extracts obtained after PEF-assisted and conventional extraction was also analyzed by Nuclear

Magnetic Resonance (NMR). The dried crude extract was solubilized in D_2O (600 μL) and then passed through nylon syringe filters (13 mm diameter and 0.42 μm pore size) before being added to a 5 mm NMR tube. Afterwards, 50 μL of maleic acid 0.1 M was introduced in the tube as internal standard (IS) for further quantification, along with 20 μL of 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid sodium salt (TSP) solution acting as a reference. Chemical shifts were expressed in ppm.

The ^{1}H NMR spectrum was recorded on a Bruker Neo500 NMR spectrometer, operating at a frequency of 500.13 MHz for the ^{1}H nucleus. The following quantitative parameters were applied with a view to assure the complete relaxation of all nuclei: 16 scans, 90° pulse (8 μs), and relaxation delay (D1) 5 s, with a final acquisition time of 3.27 min. The temperature of the probe was adjusted to 25 °C. The amount of sugars was calculated by quantitative NMR (qNMR) according to the reported literature by using the relative method (Salgado-Ramos et al., 2022a; Salgado-Ramos et al., 2022b), on the basis of Eq. (1).

$$m(x) = \frac{I(x)}{I\,(IS)} \bullet \frac{N\,(IS)}{N\,(x)} \bullet n(IS) \bullet M(x) \quad (1)$$

I(x) and I (IS) refer to the area for a determined signal of the corresponding sugar (x) or internal standard, respectively; N (IS) and N (x) relate to the number of H nuclei involved in the above signals; n (IS) is the number of mmol of internal standard added to the sample and M (x) the molecular weight of each analyte.

The analysis of the PSp crude after fermentation with *Lactobacillus gasseri* ATCC 33323 was also characterized by using the above reported protocol.

2.4.3. Mineral content profile by ICP-MS

To analyze the content of minerals (Ca, Mg, Fe, Zn, P and Se), 1 mL of each extract was digested with 1 mL of 69% nitric acid (HNO_3) along with 250 μL of hydrogen peroxide (H_2O_2) at 180 °C in a microwave oven. The multi-elemental determination was performed by inductively coupled plasma mass spectrometry Agilent model 7990 ICP-MS (Agilent Technologies, CA, USA).

2.4.4. Polyphenols profile

The determination of total phenolic compounds (TPC) was carried out using the Folin-Ciocalteu colorimetric method, as described by (Singleton, Orthofer, & Lamuela-Raventos, 1999), with some modifications based on the work of (Martí-Quijal et al., 2021). Gallic acid was used as a standard for the calibration curve. The results of total phenolic content (TPC) were expressed as milligrams of gallic acid equivalent (GAE) per gram of dry weight (d.w.). Total flavonoid content was determined according to the method described by (Abou-Arab & Abu-Salem, 2010). 1 mL of the extract, 1 mL of 20% HCl (*v*/v), and 0.5 mL formaldehyde were added in a volumetric flask volume of 25 mL. The prepared samples were blown with nitrogen (N_2) and allowed to stand for 24 h at 25 °C, after which, the same Folin–Ciocalteu reaction as for the TPC was carried out. As a standard for TFC gallic acid was used and the results were expressed as milligrams of gallic acid equivalent (GAE) per gram of dry weight (d.w.)

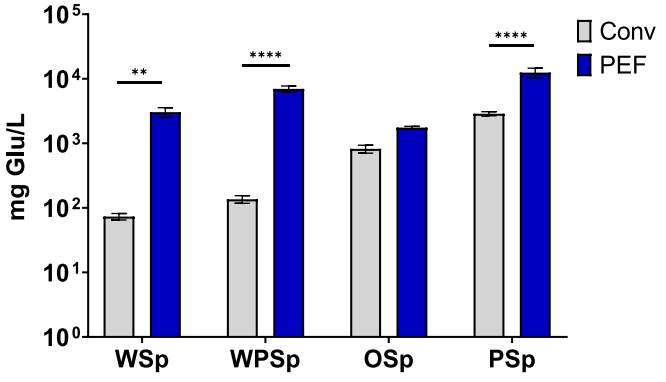

Fig. 1. Total carbohydrate content of extracts obtained by conventional and PEF technologies. Gray bars show results from conventional extracts and blue represents results obtained from PEF extracts. Each measurement was done in triplicate. *$p < 0.03$, **$p < 0.002$, ***$p < 0.0002$, ****$p < 0.0001$. WSp: white sweet potato (O'Henry), WPSp: white with purple skin (Violeta Roja), OSp: orange (California) and PSp: purple (Pepita). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.4.5. Impact on the potential probiotic bacterial growth kinetics

Potential *Lactobacillus* probiotic strains, *Lacticaseibacillus casei* BL23, *Lactobacillus gasseri* ATCC 33323, *Lactobacillus acidophilus* ATCC 4356 *and Lacticaseibacillus rhamnosus GG,* were cultivated in MRS (de Man, Rogosa, and Sharpe) media under aerobic and static conditions at 37 °C overnight.

Overnight cultures of each strain were collected through centrifugation and washed with PBS. Then, 96-well microtiter plates containing 200 μL of MRS minimum broth medium (without carbon source) was inoculated, achieving a final optical density of 0.05 at 600 nm. The growth behaviour was monitored in the presence of 10% of the conventional and PEF extracts obtained from the skin of different varieties of sweet potato. The plates were incubated at 37 °C and waved before each measure in a POLARStar plate reader (BMG). Changes in optical density at 600 nm were measured, and the growth data of the strains were modeled using the Gompertz equation (Zwietering, Jongenburger, Rombouts, & van' t Riet, 1990) with minor modifications as indicate (Chatterjee, Chatterjee, Majumdar, & Chakrabarti, 2015) to mathematically describe microbial growth (Eq. (2)). The equation is as follows:

$$y = N_0 + C exp\left(- exp\left[\left(\frac{\mu_{max} \cdot e}{C} (\lambda - t) + 1 \right) \right] \right) \quad (2)$$

The variable "y" represents the magnitude of growth at time "t" (in hours). "N_0" denotes the initial number of cells, while "C" signifies the change in cell count from the starting point to the stationary phase. "μ_{max}" stands for the maximum growth rate, representing the rate at which the cell count varies over time "λ" represents the duration of the lag phase in hours, and "e" represents the mathematical constant approximately equal to 2.7182.

2.4.6. Impact on microbial metabolite profile by short chain fatty acids (SCFAs) measurement

Short-chain fatty acids (SCFAs) concentration was determined using an Agilent GC 7890B-5977 GC–MS system equipped with a multipurpose sampler (Gerstel MPS). The GC column used was an Agilent DB-FATWAX with dimensions of 30 m × 0.25 mm × 0.25 μm, operated in split mode. The oven temperature program was set as follows: 100 °C for 3 min, then ramped to 100 °C at a rate of 5 °C per minute, followed by an increase to 150 °C for 1 min, further increased to 200 °C at a rate of 20 °C per minute, and finally held at 200 °C for 5 min. Helium was utilized as the carrier gas with a flow rate of 1 mL per minute.

To perform the GC–MS analyses the protocol described by (Eberhart, Wilson, O'Keefe, Ramaboli, & Nesengani, 2021) was followed. 3-methylvaleric acid is used as an internal standard at a concentration of 5 mM and standard curves for acetic, propionic, isobutyric, butyric, isovaleric, valeric and hexanoic acid were used for quantify the SCFAs in samples. The probiotic bacteria were grown overnight in minimal MRS supplemented with the extracts of interest. The cultures were then centrifugated at 13000 x g for 5 min at 4 °C and supernatants were filtered through 0,22 μm-pore-size nitrocellulose filters (Millipore, United States). Then, 200 μL of supernatant samples were mixed with 800 μL of internal standard solution, 1 mL of diethyl ether, and a spoonful of Na_2SO_4. The mixture was vortexed for 10 s and centrifuged 1500 x*g* for 2 min at 4 °C. Finally, 200 μL of the upper phase is transferred to a

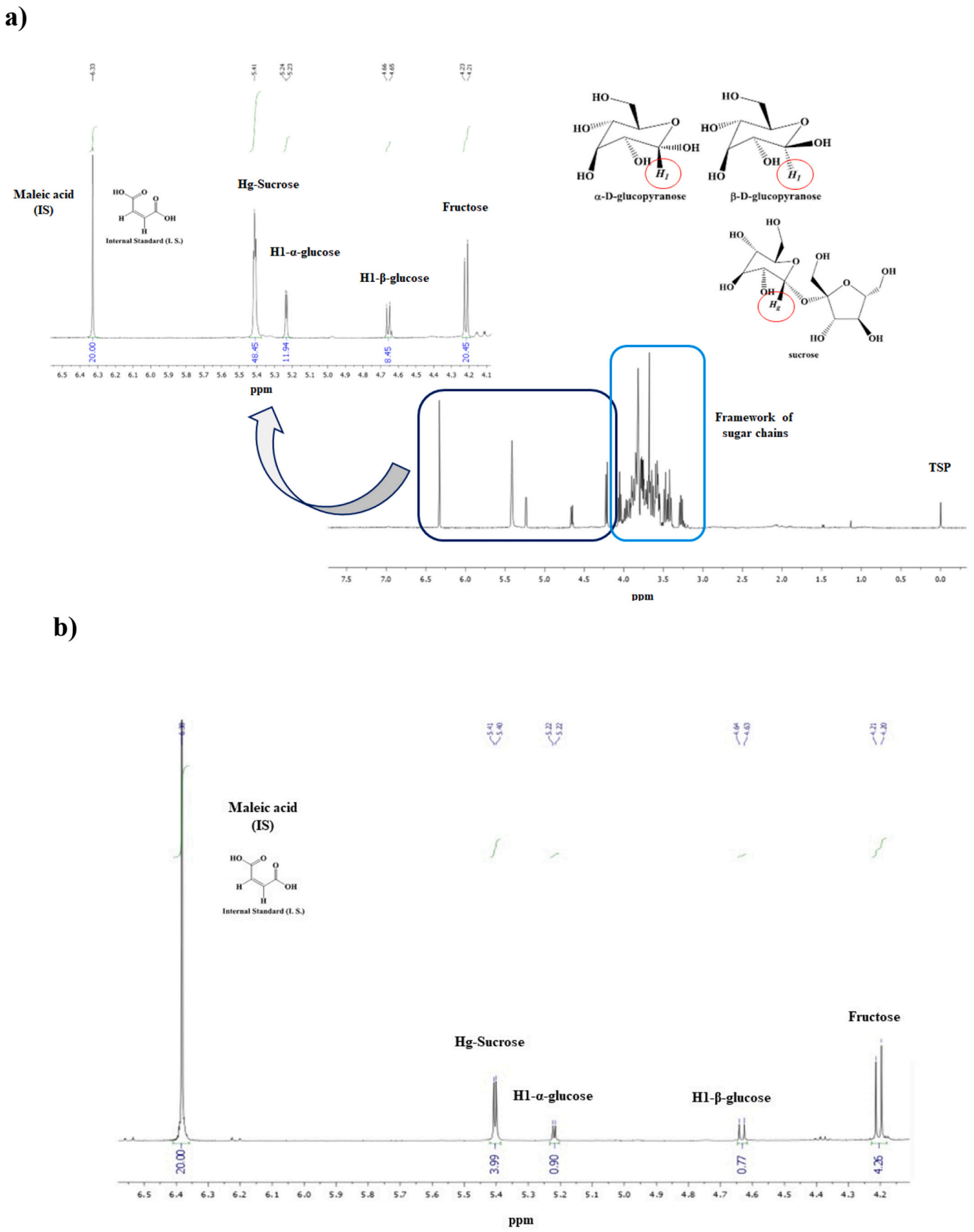

Fig. 2. ^{1}H NMR spectrum (D_2O, 500 MHz) of the water-soluble crude obtained by PEF-assisted (a) and conventional (b) extraction from purple sweet potato variety ("Pepita" (PSp)). The suppression of the signal solvent (4.8 ppm) was applied in any case. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Concentration (mg/g purple sweet potato (PSp)) of freely accessible carbohydrates extracted by PEF-assisted- and conventional extraction (Fig. 2) calculated by quantitative NMR (qNMR).

Analyte	Chemical shift (ppm)	I (x) (PEF)	PEF (mg/g PSp)	I (x) (conventional)	Conventional (mg/g PSp)
Maleic acid (IS)	6.38	20		20	
Sucrose	5.41	48.45	64.96	3.99	3.78
α-glucose	5.24	11.94	8.42	0.90	0.45
β-glucose	4.66	8.45	5.96	0.77	0.39
Total glucose			14.38		0.84
Fructose	4.23	20.45	14.43	4.26	2.13
Total (mg/g)			**93.77**		**6.75**

chromatography vial and analyzed by GC–MS.

2.5. Statistical analysis

Two-way ANOVA with Sidak multiple comparisons test was performed using GraphPad Prism 8 software. It was used to detect statistically significant differences between the extracts obtained and between the control and the sample-supplemented groups on the following parameters: mineral content, phenolic and flavonoid content, carbohydrate amount, bacterial growth parameters and SCFAs.

3. Results and discussion

3.1. Compositional profile

Total carbohydrate profile of the four varieties applying both a maceration extraction methodology (Conv) and the application of pulsed electric fields (PEF) are reported (Fig. 1).

As can be seen in Fig. 1, different carbohydrate contents were observed according to the varieties studied, independently of the applied treatment. Moreover, it was also observed a significant enhanced extraction (1752–12,495 mg Glu/L) of carbohydrates after PEF extraction compared to the conventional methodology (maceration) (73–2879 mg Glu/L).

Further, the soluble carbohydrate fraction was characterized by means of Nuclear Magnetic Resonance (NMR). In this regard, the application of quantitative NMR (qNMR) allowed us to determine the amount of each compound detected in the pertinent extract (Salgado-Ramos et al., 2022). In this work, mainly freely accessible carbohydrates (i.e., mono- and disaccharides) are expected, according to the TCC assay above reported.

The extracts recovered from "Pepita" variety (PSp) (purple sweet potato) were selected for the analysis, being also the most rich-carbohydrate crudes (Fig. 1). For the sake of example, the ^{1}H NMR analysis of the PEF crude was attached (Fig. 2a). As can be observed in the figure, the spectrum shows a typical profile of a rich-sugar, water-soluble extract (signals ranged from 5.5 to 3 ppm), which is in agreement with the reported literature for other matrices such as almonds (Salgado-Ramos, Martí-Quijal, et al., 2022a) or melon rind (Lucas-

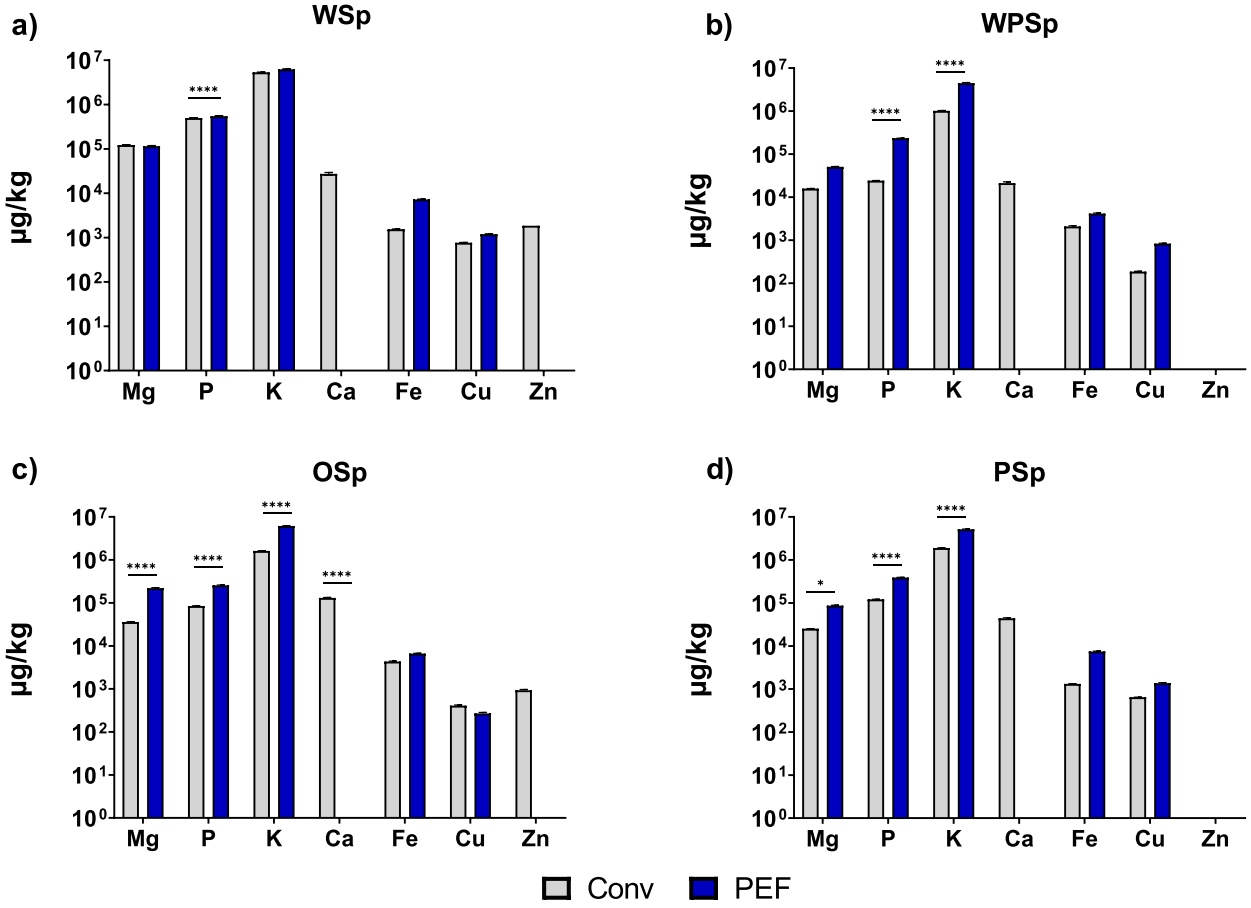

Fig. 3. Mineral content of sweet potato skin extracts. In gray the results corresponding to the extracts obtained by conventional methodology (Conv), in blue those obtained by pulsed electric fields (PEF). Each measurement was done in duplicate. a) WSp, b) WPSp, c) OSp, d) PSp. *p < 0.03, **p < 0.002, ***p < 0.0002, ****p < 0.0001. WSp: white sweet potato (O'Henry), WPSp: white with purple skin (Violeta Roja), OSp: orange (California) and PSp: purple (Pepita). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

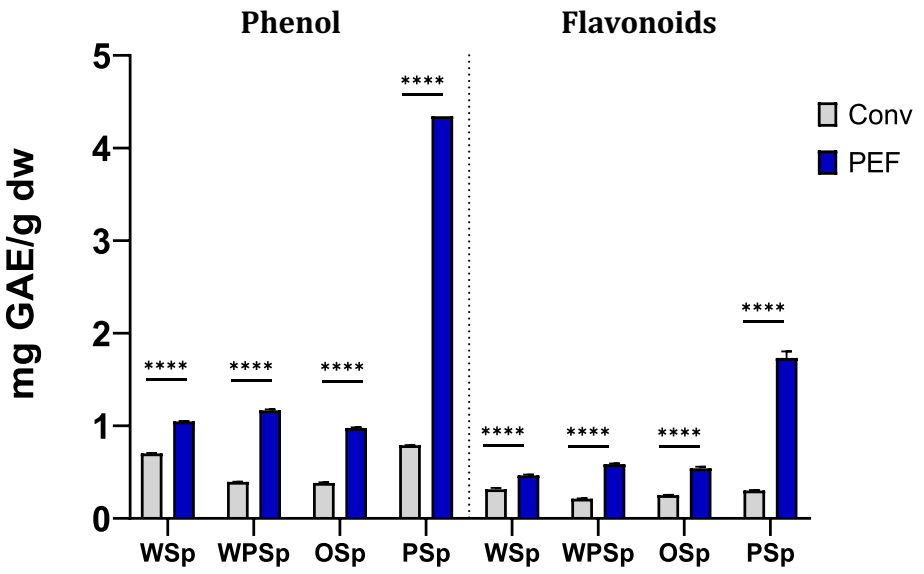

Fig. 4. Phenolic and flavonoid content of the extracts obtained from sweet potato skins expressed as milligrams of gallic acid equivalent (GAE) per gram of dry weight (dw). In gray the results correspond to the extracts obtained by maceration methodology (Conv) and in blue those obtained by pulsed electric fields (PEF). Each measurement was done in triplicate. ****$p < 0.0001$. WSp: white sweet potato (O'Henry), WPSp: white with purple skin (Violeta Roja), OSp: orange (California), and PSp: purple (Pepita). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Torres, Lorente, Cabañas, & Moreno, 2016). More specifically, sucrose, glucose and fructose were detected (Fig. 2a) and quantified (Table 1), on the basis of a previous research (Salgado-Ramos, Martí-Quijal, et al., 2022b). The conventional extracts also showed a similar profile, therefore only the targeted region (i.e., 5.4–4.2 ppm) was considered (Fig. 2b).

Briefly, and considering the experimental parameters applied (see *Section 2.4.4*), sucrose was the predominant metabolite for both extracts, with a concentration of 64.96 and 3.99 mg/g PSp for PEF and conventional crude, respectively. For the former, it entails a 4.5-fold increase with respect to total glucose (14.38 mg/g PSp) and fructose (14.43 mg/g PSp), both also present in the crude according to the ^{1}H NMR spectrum (Fig. 2). In this sense, anomeric protons, marked as H^{1} (Fig. 2), were suitable to identify glucose, whereas the proton attached to the carbon that forms the O-glycosidic bond (Hg) was considered for sucrose determination (Lucas-Torres et al., 2016). For fructose, the signal related to an intracycle hydrogen was evaluated, considering an NMR pattern of this compound. Overall, the results herein obtained were noticeable, since a noted sugar concentration of 93.77 mg/g PSp was detected in the crude after PEF application (Table 1). It supposes around 10% of the fresh SP skin weight, and entails a progress by up to 93% with respect to conventional extraction, which is worthy of note. At this regard, unfortunately, there is a scarceness in literature to compare these results, to the best of the authors' knowledge. Only Kurata et al. reported 1 g of carbohydrates / 100 g fresh weight from SP leaves, a 10-fold decrease compared to this work, which points out the role of PEF as an efficient approach for extraction (Kurata et al., 2017). However, when considering fresh SP, a remarkable carbohydrate concentration between 42 and 73 g/100 g SP was reported (Gou et al., 2023; H. Sun, Mu, Xi, Zhang, & Chen, 2014).

In summary, and in light the above results, an improvement in bacterial growth in the presence of SP peels after being processed by PEF is expected. Additionally, it should be noted out that monosaccharides serve as platform molecules for the development of valuable fine chemicals, such as levulinic acid or 5-hydroxymethylfurfural (Salgado-Ramos, Martí-Quijal, et al., 2022b), proposing alternative ways of upgrading for our matrix.

To continue characterizing the extracts that can be obtained from sweet potato peel varieties, the mineral profile was studied to see both the levels that can be obtained and whether there are differences between the extraction techniques used. Essential minerals are found in all varieties of sweet potatoes, although zinc could only be detected in the case of the white and orange varieties (Fig. 3).

The differences between both techniques (conventional vs PEF) are not very significant regarding the composition of the extracts, being Mg, P and K the minerals with the highest concentration and PEF the methodology with the best results obtained in terms of yields. However, in the case of Ca, extraction by PEF is very inefficient, drastically reducing the values compared to the results that can be obtained using a conventional methodology. This mineral composition, although in a lower concentration, is similar to that found in the tuber according to the Spanish food composition database (BEDCA) (https://www.bedca.net/bdpub/index.php), where potassium is the mineral with a significantly higher concentration than the rest.

Regarding the importance that the mineral content can have in improving the growth of beneficial bacterias such as *Lactobacillus*, the relationship between intestinal microbiota and the host's mineral status is symbiotic (Barone, D'Amico, Brigidi, & Turroni, 2022). Bacteria play a crucial role by producing enzymes that aid in the release of minerals from foods. For instance, phytase enzymes are instrumental in hydrolyzing plant phytic acid and releasing essential minerals like calcium, phosphate, and magnesium (Bohn, Josefsen, Meyer, & Rasmussen, 2007). Various minerals, including calcium, iron, zinc, magnesium, and phosphorus, have a significant impact on shaping the human gut microbiome (Bielik & Kolisek, 2021). These microbes utilize minerals for their growth and proper functioning, thereby preventing health issues linked to dysbiosis and pathobiont overgrowth (Hadadi, Berweiler, Wang, & Trajkovski, 2021). For example, studies have shown that phosphorus supplementation enhances microbial diversity and boosts the levels of short-chain fatty acids (SCFA), which are microbial metabolites produced during polysaccharide fermentation in the colon (Trautvetter, Camarinha-Silva, Jahreis, Lorkowski, & Glei, 2018). Magnesium also exhibits a positive influence on gut microbiome composition, as well as the metabolism of vitamins B1 and D, particularly in patients with metabolic syndrome, type 2 diabetes, and obesity (Piuri et al., 2021).

Total phenolic content was also evaluated showing the results as milligrams of gallic acid equivalent (GAE) (Fig. 4). In addition, among the phenols, the flavonoid content has been specifically evaluated as the most extensively studied phenolic compound concerning their impact on

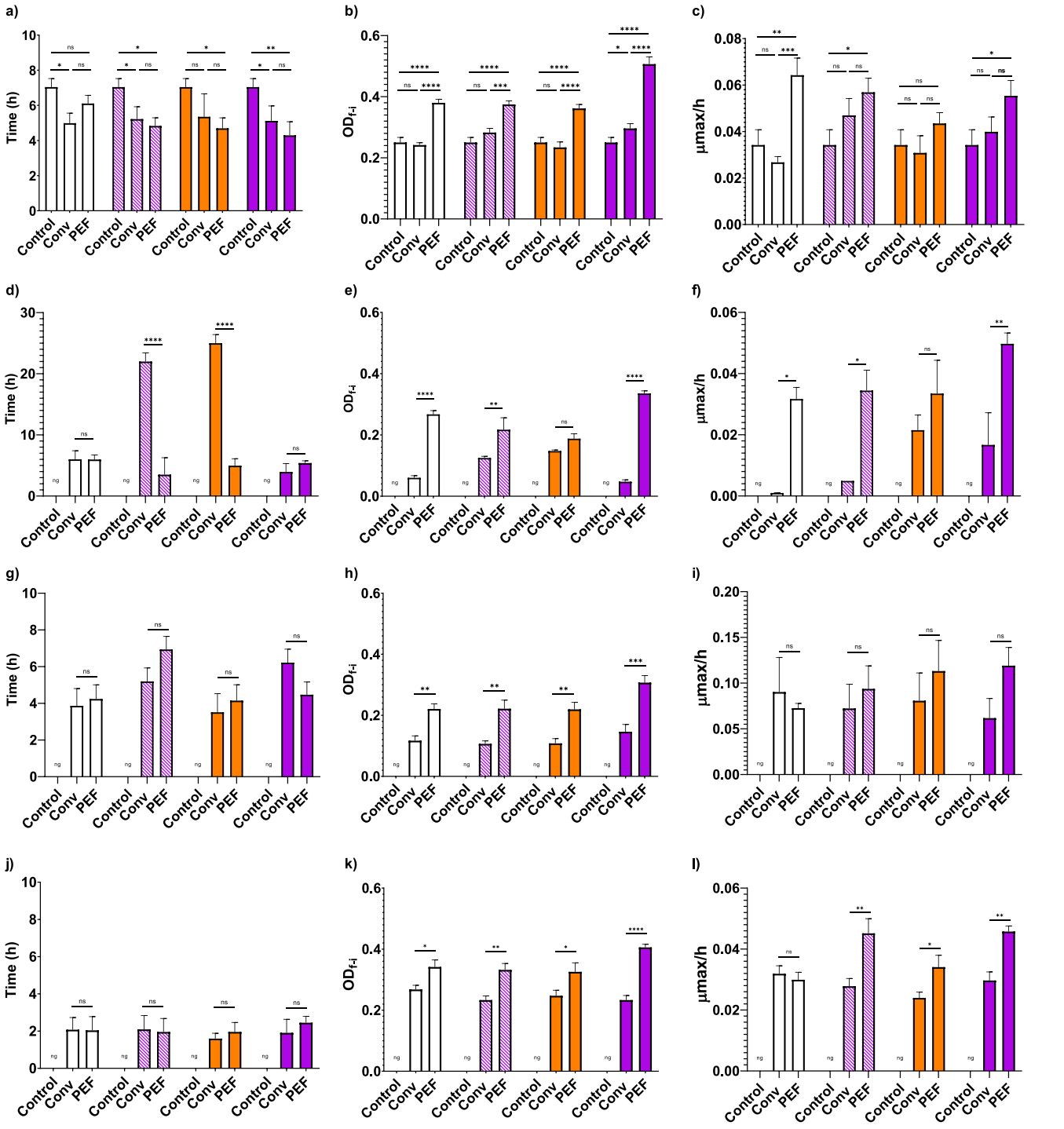

Fig. 5. *Lactobacillus* strains growth kinetics in presence of sweet potato extracts obtained by conventional and PEF methods. Delay before growth, the difference between initial and final cell numbers (OD_{f-i}) and maximum specific growth rate (µmax/h) of *Lactobacillus* spp. growth in mMRS (control) and supplemented with conventional extract (Conv) or pulsed electric fields extract (PEF). a), b) and c) *Lactobacillus casei.* d), e) and f) *Lactobacillus gasseri.* g), h) and i) *Lactobacillus acidophilus*. j), k) and l) *Lactobacillus rhamnossus*. The white bars represent the results of WSp (white sweet potato (O'Henry)), the white ones with purple lines those of WPSp (white with purple skin (Violeta Roja)), the orange ones those of OSp (orange (California)) and the purple ones those of PSp (purple (Pepita)). Each growth curve was performed in triplicate. ns: no significant, *p < 0.03, **p < 0.002, ***p < 0.0002, ****p < 0.0001, ng: no growth observed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the composition of the gut microbiota and the associated health benefits for the host (Wang, Qi, & Zheng, 2022).

The total phenolic content is higher in the sweet potato var. Pepita (PSp), is the same proportionally in the case of flavonoids. The phenolic content of the extracts obtained by conventional methodology (0.383–0.790 mg GAE/g dw) is higher than those obtained by (Šeregelj et al., 2020) (0.130–0.262 mg GAE/g dw), also in extracts of sweet potato peels, with the difference that in our case the extraction is carried out with water, and they carry it out with acetone and ethanol. Therefore, the sweet potatoes peels are a source of phenolic compounds, as shown by (Amagloh et al., 2022) when comparing the extractions of sweet potatoes with and without skin. However, regarding the

methodology used, the amount is significantly higher in the case of PEF extracts (0.977–4.343 mg GAE/g dw). In the case of flavonoids, the content also differs according to the variety studied. Previous studies have shown that there is a higher concentration in the purple variety (0.547 mg DAE/g dw) due mostly to its higher content of anthocyanins responsible for its coloration (Laveriano-Santos et al., 2022). The content that we have obtained in the peels (0.301 ± 0.005 mg GAE/g dw) is lower compared to those than can be obtained from the whole tuber in the case of the conventional methodology but applying PEF the results are significantly higher (1.733 ± 0.071 mg GAE/g dw).

According to the last definition of prebiotic, it is understood as "any substrate used selectively by a host microorganism conferring a beneficial effect on health" (Gibson et al., 2017). Compounds such as polyphenols and polyunsaturated fatty acids are also relevant due to their prebiotic potential, as has been verified in the case of flavonoids and carotenoids (Alves-Santos, Sugizaki, Lima, & Naves, 2020). This new perspective expands the possibilities to valorize side streams derived from the food industry. Clinical studies further confirmed the regulatory effect of polyphenols on human intestinal microorganisms. In line with both in vitro and in vivo animal studies, the supplementation of polyphenols, such as anthocyanins and flavonoids, has been found to elevate the levels of *Bifidobacterium* and *Lactobacillus* in the human gut (Tzounis et al., 2011). These two types of intestinal microorganisms are known for their protective properties, benefiting gut health. As an example, tests have been carried out with foods rich in polyphenols and flavonoids such as blueberries (Vendrame et al., 2011), almonds (Z. Liu et al., 2014), or red wine (Moreno-Indias et al., 2016). As a result, the number of *Bifidobacterium* and *Lactobacillus* increased, therefore, a diet rich in polyphenols can regulate the ratio of Firmicutes to Bacteroides in the human body. Taking these results into account, we proceeded to assess the effect of the extracts on the growth of *Lactobacillus* strains.

3.2. Impact on potential probiotic bacterial growth kinetics and metabolite profile after growth in sweet potato peel extracts

The primary probiotic species within the *Lactobacillus* genus encompass a range of strains, including L. *acidophilus*, *L. brevis*, *L. casei*, *L. delbrueckii* subsp. bulgaricus, *L. delbrueckii* subsp. lactis, *L. fermentum*, *L. gasseri*, *L. helveticus*, *L. johnsonii*, *L. paracasei* subsp. paracasei, *L. plantarum*, *L. reuteri*, and L. *rhamnosus*. Notably, among these, *L. rhamnosus* GG stands out as one of the most extensively researched, while L. *acidophilus* enjoys widespread use in commercial products (Dronkers, Ouwehand, & Rijkers, 2020). The realm of potential probiotics is vast, with numerous options, and the beneficial effects can be specific to the species or even the strain. In some cases, combining these probiotics with other probiotics or prebiotics may be necessary to maximize their effectiveness (Dempsey & Corr, 2022).

To check whether the extracts could be used as prebiotics, we first focused on the effect that extracts obtained from sweet potato peels had on the growth of four *Lactobacillus* species: *L. casei* BL23, *L. gasseri* ATCC 33323, *L. acidophilus* ATCC 4356 and *L. rhamnossus* GG. To be more restrictive, the bacteria were grown in an MRS medium without a carbon source (mMRS), trying in this way to see if there are differences in growth that can be directly related to the presence or absence of the extracts. Growth was modeled according to the Gompertz equation (see Section 2.8), representing the lag phase, the difference between the final and initial absorbance values and the specific growth rate of each strain that has been studied (Fig. 5).

In general, the *Lactobacillus* strains studied in this work showed a greater growth when supplementing the medium with the extracts. In the case of *L. gasseri*, *L. acidophilus* and *L. rhamnossus*, no growth was observed in the medium without supplementation, while a significant growth was found in the presence of the extracts.

Regarding the comparison between the conventional and PEF extracts, the difference between the final and initial growth and the growth rate are very significant, where the results are much better with the PEF extracts. According to the definition of prebiotic, when checking the effect of these extracts on the growth of bacteria it is expected that better results will be obtained in PEF extracts due to a higher content of carbon sources in addition to a higher content of polyphenols and minerals as we have verified. Above all, the purple sweet potato (var. Pepita) shows the best results and was selected to analyze the SCFA profile after growth in their presence. A 48-h fermentation of the *Lactobacillus* strains was carried out in mMRS medium supplemented with both the conventional extract and PEF-assisted methodology, using the mMRS medium without supplementation as a control. Before the identification and quantification of SCFA in the cultures of the 4 *Lactobacillus* strains, we performed a pH measurement, comparing the control medium with both media supplemented (Table 2).

Table 2
pH values measured after 48 h of growth in mMRS medium without supplementation and supplemented with the extracts obtained from the skins of purple sweet potatoes. Statistical significance was made by comparing the results against the control medium. Each measurement was done in duplicate. $^{*}p < 0.05$.

Microorganism	Control	Conventional	PEF
L. casei	6.505 ± 0.007	6.445 ± 0.007*	6.175 ± 0.007*
L. gasseri	6.525 ± 0.007	6.495 ± 0.007*	5.995 ± 0.007*
L. acidophilus	6.565 ± 0.007	6.585 ± 0.007*	6.195 ± 0.007*
L. rhamnossus	6.595 ± 0.007	6.525 ± 0.007*	6.495 ± 0.007*

Related to the results obtained previously where greater growth has been seen in the presence of the PEF extracts, the pH values are lower in the presence of this extract indicating the presence of a greater amount of acids in the medium characteristic of *Lactobacillus* growth. Therefore, we checked if this decrease in pH was due to the production of SCFA, that is, acetic, propionic, isobutyric, butyric, isovaleric, valeric and hexanoic acids. To do this, each compound and concentration was identified using GC–MS (Fig. 6).

Short-chain fatty acids (SCFAs) are synthesized by the gut microbiota through the metabolism of indigestible dietary components like fiber, oligosaccharides, and polysaccharides, utilizing distinct metabolic pathways (Fusco et al., 2023). Here, as expected, due to having obtained greater growth and its carbohydrate content, SCFA production is greater in the presence of each substrate compared to the control (without substrate). The amount of each SCFA studied is different depending on the *Lactobacillus* strain, which is related to its production capacity due to its metabolism (De Angelis & Gobbetti, 2016). Proof of this is the amount of acetic acid that is greater in the case of *L. acidophilus* while, in the rest of the acids studied, *L. gasseri* is the largest producer. In addition, the highest levels of SCFA have been obtained in the presence of the sweet potato skin extract obtained by PEF, indicating that the bioactive compounds present not only improved the growth of these strains but also increased the amount and diversity of SCFA by metabolizing them. These SCFAs play a vital role in benefiting the host in several ways including ensuring metabolic stability (Morrison & Preston, 2016), serving as an energy source for the cells lining the colon (Litvak, Byndloss, & Bäumler, 2018), influencing the activity of T regulatory cells (Park et al., 2015), and exhibiting anti-inflammatory properties (Vinolo et al., 2011).

In addition, and based on the above results, the PEF extract recovered after fermentation was also analyzed by ^{1}H NMR (Fig. 7). As *L. gasseri* was the largest producer of SCFA, as above mentioned, it was selected for the analysis.

Briefly, the main sugars detected in the original crude (i.e., sucrose, glucose, and fructose) were consumed after fermentation process, as expected, not being possible observe their signals (Fig. 7, chemical shift from 5.5 to 4.5 ppm). Additionally, the intensity of those related to the frameworks of sugar chains considerably decreases with respect to the initial analysis (see Fig. 2, 4.2–3 ppm). Instead of that, it is worth of noting those relate to the SCFA generated, from 2.5 to 0.5 ppm, in line

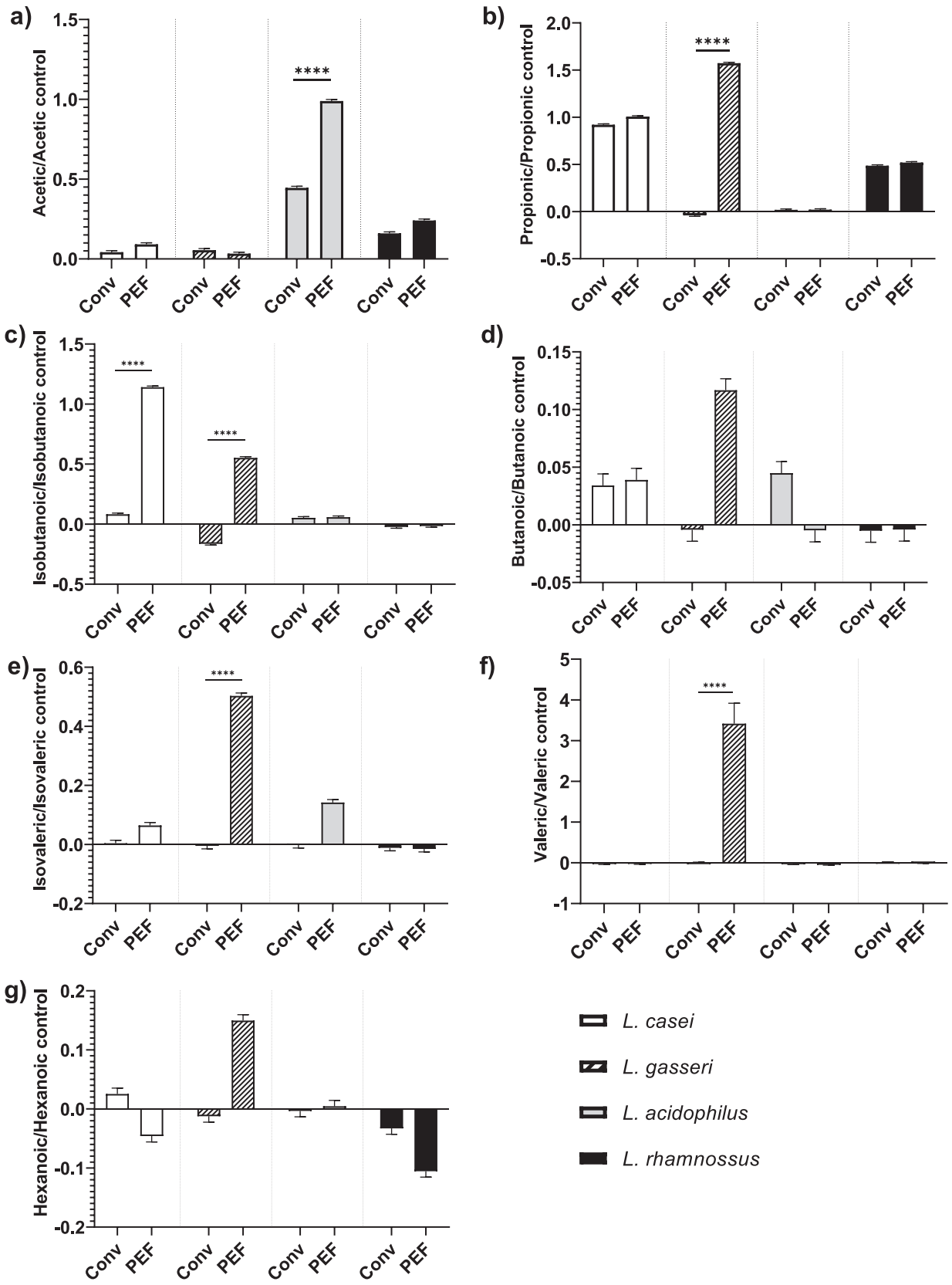

Fig. 6. Quantification of short chain fatty acids (SCFA) after growth for 48 h in the presence of extracts obtained from purple sweet potato peels. The baseline is set as the amount of each fatty acid in the control medium. a) Acetic acid, b) Propionic acid, c) Isobutanoic acid, d) Butanoic acid, e) Isovaleric acid, f) Valeric acid, g) Hexanoic acid. ****$p < 0.0001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

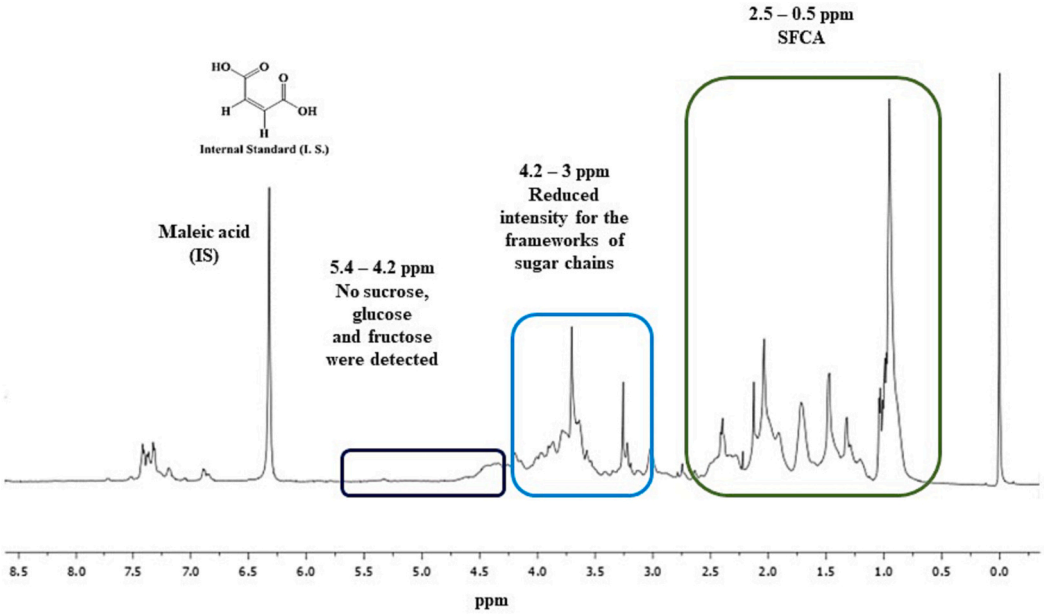

Fig. 7. ^{1}H NMR spectrum (D_2O, 500 MHz) of the water-soluble, PEF-treated crude derived from purple sweet potato variety ("Pepita" (PSp)) after fermentation with *L. gasseri.* The suppression of the signal solvent (4.8 ppm) was applied. Abbreviations: SCFA: short-chain fatty acids. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with the experiments carried out (Fig. 6). These signals fully agree with some previous studies in which the detection of different kind of fatty acids by NMR was reported, thus corroborating the results obtained (Salgado-Ramos et al., 2023).

4. Limitations

Our study has some limitations related to the characterization of the extracts and their use in a real situation. The methodology used only allows us to detect mono- and disaccharides, so it is possible that there are more complex soluble carbohydrates in the extract that we have not been able to observe. Furthermore, it would be necessary to study the bioactive compounds that reach the microbiota after digestion and improve the growth of beneficial bacteria, achieving a beneficial effect on the health of the host.

Despite these limitations, our study shows how side streams from the agricultural industry, such as sweet potato peels, can be revalued obtaining a wide variety of bioactive compounds of interest as well as a new sources of potential prebiotics. Our results confirm that PEF is a non-thermal processing technology with very promising results with plant matrices. Studies comparing conventional technologies with novel ones are necessary with the aim of selecting those that will provide us best results.

5. Conclusions

In this work, we verified that sweet potato peels, which are usually discarded during food processing, are a source of compounds that can improve the growth of specific strains of *Lactobacillus* genus, especially in the case of the purple variety (PSp). These results were especially promising in the extracts recovered by pulsed electric fields (PEF), an innovative technology which enables a boosted extraction of targeted analytes in a sustainable and efficient way. At this regard, a noted concentration of freely accessible carbohydrates (mainly sucrose, glucose and fructose) was detected by quantitative NMR with respect to the conventional extraction. Afterwards, the effect on the growth of *Lactobacillus* strain was studied, observing an increase in growth rate and total growth. Finally, a 48-h fermentation with *L. gasseri* showed a noticeable increase of short chain fatty acids (SCFA) in the PEF, PSp crudes, specially propanoic, isobutanoic and valeric acids, also serving as feedstock to produce biofuels as an alternative way of valorisation. This contribution supposes the first processing of sweet potato peels with PEF, paving the way to widen the state-of-art in this field, for instance, with new food and nutraceutical applications of the matrix, or methodologies which can intensify the protocol from a sustainable point of view. In this context, we should not only assess the by-products and waste that are discarded, but also the methodologies with which better results can be obtained.

CRediT authorship contribution statement

Manuel Bernabeu: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Manuel Salgado-Ramos:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Francisco J. Barba:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **M. Carmen Collado:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Juan M. Castagnini:** Writing – review & editing, Methodology, Formal analysis, Data curation.

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.

Data availability

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

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