Valorization of *Parmentiera aculeata* juice in growth of probiotics in submerged culture and their postbiotic production: a first approach to healthy foods

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**Abstract**

Nowadays, functional foods are greatly accepted by consumers because they improve health and are new sources for substrates to be explored. In this sense, *Parmentiera aculeata*, a plant distributed in Mexico with beneficial effects on health, has not been chemically explored. In this work, *P. aculeata* juice was used as carbon source to promote the growth of two probiotic *Lactobacillus* strains during submerged fermentation. Taguchi’s methodology with orthogonal array L9 was applied for culture conditions optimization. pH, agitation, and inoculum concentration variables, each with three levels, were evaluated and the best treatment was validated through a kinetic culture monitoring some postbiotics traits. We observed an increase in 1.76-times in cellular concentration of *L. plantarum* 14917, and the main produced postbiotics were short-chain fatty acids such as succinic, formic, acetic, propionic, and lactic acids, which are associated with the probiotic metabolism and are important for human health. In the best of our knowledge, this study is the first to describe the valorization of *P. aculeata* juice as substrate for growth of probiotic strains and future studies are required to gain further applications in functional food production.

**Keywords** Functional foods · *Parmentiera aculeata* · Posbiotics · Probiotics · Short-chain fatty acids

**Introduction**

Plant’s diversity from Mexico has potential applications in food biotechnology, however, multiple plant species remain still functionally uncharacterized, *Parmentiera aculeata* (Kunth) Seem (syn. *P. edulis* DC), which is a plant distributed in the Pacific area from Sinaloa to Chiapas, and in the Gulf region from Tamaulipas and San Luis Potosí to Yucatán (Andrade-Cetto and Heinrich 2005; Morales-Sánchez et al. 2015). *P. aculeata* tree can reach up to 15 m of height, with fruits from 15 to 20 cm long and 6.5 cm wide. It has longitudinal grooves and is yellow-green in color (Andrade-Cetto and Heinrich 2005). Their fruits, roots and bark are recognized in Mexican traditional medicine as treatment for diabetes and renal diseases, among others such as headaches, gallstones, deafness and diarrhea (Andrade-Cetto and Heinrich 2005; Morales-Sánchez et al. 2015). Some of these ailments have been confirmed in biological models, for example their hypoglycemic effects of lactucin-8-O-methylacrylate present in the chloroform extract from dried fruits. Also, the effect of lowering blood sugar levels was observed in a mice model with alloxan-induced diabetes (Pérez et al. 2000). In addition, the fruit extracts have cytotoxic activity and apoptosis induction in breast cancer cells (Estranislao-Gómez et al., 2016). Also, hypoglycemic (Pérez et al. 2000; Andrade-Cetto and Heinrich 2005) and antiurolithic effects have been reported (Morales-Sánchez et al. 2015).
et al. 2015). In this sense, the exploration of bioactive compounds present in *P. acuelata* could be applied for functional food formulation, where functional food and nutraceuticals have an increment demand because of their interesting health improvement effects. The first is defined as food products with appearance of traditional food that contains added ingredients to provide health-related benefits for humans. (Topolska et al. 2021). Whereas nutraceuticals are characterized by its presentation in pills, powders or other medicinal forms not generally associated with food (Penson and Banach, 2020). Probiotics and/or prebiotics are usually associated as the providers of those functionalities. Interestingly, probiotics have been associated to improve health in patient with any of the aforementioned diseases. Probiotics consists of lactic acid bacteria (LAB) of genus *Lactobacillus*, mainly (Kitazawa et al. 2020). *L. acidophilus, Lactobacillus casei, L. plantarum, L. rhamnosus* and *Bifidobacterium lactis* are the most commonly used strains during the formulation of new probiotic products from fruit- and vegetable-origin (Bernal Castro et al. 2017). During the growth of probiotics, soluble factors (biomolecules or metabolic by-products) are secreted by the bacteria (probiotic or non-probiotic) or released after bacterial lysis. These substances that will have a benefic effect in the host are known as postbiotics (Homayouni Rad et al. 2021; Kang et al. 2021). These compounds include enzymes, secreted proteins, short-chain fatty acids (SCFAs), hydrogen peroxide, ethanol, vitamins, secreted biosurfactants, amino acids, peptides, organic acids, endo- and exopolysaccharides, among others (Homayouni Rad et al. 2021; Lee et al. 2021).

On the other hand, to improve the growth of probiotic strains, culture and nutrimental condition’s optimization is mandatory. In this sense the submerged fermentation (SmF) in combination with Taguchi’s methodology can be used to improve the growth of different probiotic candidates. Taguchi’s methodology searches for the optimal conditions and is used in industries to improve the quality processes (Aguilar-Zarate et al. 2014). The aims of this work were 1) to find alternative sources of prebiotics, by demonstrate the ability of *P. aculeata* juice, used as carbon source, to promote the growth of two probiotic *Lactobacillus* strains during SmF, 2) to optimize the culture conditions of SmF to improve the cellular concentration of *Lactobacillus* strains and, 3) to characterize the postbiotics production during SmF by the probiotic *Lactobacillus* strains.

**Materials and methods**

**Culture medium and reagents**

The DeMan-Rogosa-Sharpe (MRS) broth were purchased from BD Bioxon (New Jersey, USA). All reagents used in this study were reactive grade and were purchased at Productos Químicos de Monterrey (Monterrey, Mex) and Jalmek Científica (San Nicolás de los Garza, México), standards were obtained from JT Baker (Phillipsburg, NJ, USA) or Sigma Aldrich Chemical Co. (St. Louis, MO).

**Vegetal material and strains**

Fruits from *P. acuelata* were collected in “El Gavilan” locality (22.5250397,-100.0429407), Ciudad Valles, San Luis Potosi, Mexico. The fruit was selected according to the color scale described by Angón-Galván (2006), focusing on fruits with a 3–4 color scale. Fruits were transported to Food Research Laboratory at FEPH-UASLP and conserved in plastic bags in refrigeration at 4–6 °C until further use.

Lactobacillus paracasei subsp. paracasei ATCC 25302 and *L. plantarum* subsp. *plantarum* ATCC 14917 were kindly provided by the Food Research Department at Autonomous University of Coahuila, Mexico.

**Preparation of juice from *P. acuelata***

The *P. aculeata* fruits were washed and disinfected with ionized 3% silver solution (Microdyn®). Then, fruits were cut, and the juice was obtained with Master Craft extractor. The collected juice was vacuumed, and the pH adjusted at the different values according to experimental design (Table 1) and centrifuged (Hermle, Z206A, Germany) at 5000 rpm for 5 min. Supernatant was transferred to 50 mL

| Serial no | pH | Agitation (rpm) | Inoculum concentration (%) |
|-----------|----|----------------|---------------------------|
| 1         | 1  | 1              | 1                         |
| 2         | 1  | 2              | 2                         |
| 3         | 3  | 2              | 1                         |
| 4         | 2  | 1              | 2                         |
| 5         | 3  | 3              | 2                         |
| 6         | 3  | 1              | 3                         |
| 7         | 2  | 2              | 3                         |
| 8         | 1  | 3              | 3                         |
| 9         | 2  | 3              | 1                         |
conical tubes and sterilized (Yamato, SM200, Japan) at 121 °C, 15 lb for 15 min. Before SmF cultivation, solids in the juice were eliminated by decantation.

Characterization of juice from \textit{P. aculeata}

Juice from \textit{P. aculeata} was characterized by standard technical analyses according to the Association of Official Analytical Chemist (AOAC, 2012) for humidity (Method 925.09) and ash (Method 923.03). For total soluble solids (TSS, °Brix), reducing sugars (RS) and titrable acidity (citric acid, % CA, and lactic acid, % LA) the Mexican Normativity were applied, as following: NMX-F-436-SCFI-2011, method Lane-Eynon from NMX-F-312-NORMEX-2016 and NMX-FF-011-1982. Additionally, pH was measured in an Oakton pH meter 700.

Taguchi methodology

Taguchi methodology was applied in four phases as previously described (Aguilar-Zarate et al. 2014): (1) design of experimental condition runs, (2) submerged fermentation, (3) experimental data analysis and prediction performance, and (4) validation. For phase 1, an L9 orthogonal array (3³) was used to include three important factors (pH, agitation, and inoculum concentration) at three levels (4.0, 5.0 and 6.0; 0, 75 and 150 rpm; 5, 10 and 15%, respectively), to define each SmF cultivation of the two \textit{Lactobacillus} probiotic strains (Table 1).

For submerged cultures

The \textit{Lactobacillus} strains were activated in assay tubes with 10 mL of MRS broth and incubated at 37 °C for 24 h. The inoculum for the fermentation was prepared from re-seeding the overnight culture in new broth and incubated for 16 h at 37 °C. Incubation of the treatments was carried out at 37 °C for 120 h in accordance with conditions described in Table 1. Growth was expressed as cells number per milliliter (cels/mL) from cells counts using a Neubauer’s chamber. The experimental was performed in duplicate.

Experimental data analysis and prediction performance

The results from the nine SmF cultivation runs were processed using the software Statistical version 7.1 (Statsoft, Tulsa, OK, USA).

Validation

The strain with the higher cellular concentration was validated as suggest the experimental data analysis using the optimal culture conditions. In addition, the SmF kinetic were cultured for 120 h and was monitored each 24 h to determine cellular concentration, pH changes, titrable acidity (citric acid as % CA, lactic acid as % LA), total soluble solids (TSS, °Brix), reducing sugars (RS, %) and postbiotic compounds. Cultures were realized in triplicates. All results were compared between statistical groups, using analysis of variance (ANOVA) and Tukey’s multiple comparisons.

Chemical profile from free-cell culture medium

Culture medium from each cultivation was centrifuged (Hermle, Z206A, Germany) at 5000 rpm for 5 min and free-cell culture medium (supernatants) was used for the following determinations: pH (Oakton, 700, Vernon Hills, USA), TA was determined according to procedure described in NMX-FF-011-1982 and was expressed as % CA, SS expressed in °Brix using the methodology described in NMX-F-436-SCFI-2011, ART using Lane-Eynon volumetric method described in NMX-F-312-1978. For postbiotics determination, supernatant samples were 0.4 μm syringe-filtered. Sugars, organic acids, and ethanol were measured by high-performance liquid chromatography (HPLC) in a Waters chromatograph (Milford, MA) equipped with a refractive index detector (2410, Waters). Compounds were separated using an Aminex HPX-87H (Bio-Rad, Hercules, CA) column operated at 50 °C with H₂SO₄ 0.25 mM as a mobile phase at 0.5 mL/min.

Table 2 Chemical profile of \textit{P. aculeata} juice used for submerged fermentations of \textit{Lactobacillus} strains

| Parameter                          | Content (%) |
|------------------------------------|-------------|
| Humidity                           | 88.70 ± 0.15 |
| Ash                                | 0.11 ± 0.00 |
| SST                                | 11.00 ± 0.00 |
| RS                                 | 9.04 ± 0.56 |
| Titrable acidity (% lactic acid)   | 0.25 ± 0.00 |
| Titrable acidity (% citric acid)   | 0.16 ± 0.00 |
| pH                                 | 4.93 ± 0.01 |

Results

The chemical profile of juice from \textit{P. aculeata} is presented in Table 2. The value of TSS was 11%, where RS corresponds to 9%. In addition, titrable acid was 0.16 and 0.25% expressed as citric and lactic acids, respectively. After SmF cultivations, experimental data analysis was analyzed for Taguchi methodology. In Fig. 1, the results of nine culture assays are presented, where the treatment 2 exhibited the major cellular concentration for both \textit{Lactobacillus} probiotic
strains. The conditions correspond to pH of 4.0, agitation of 75 rpm and inoculum concentration of 10%. The maximum cellular concentrations were $7.64 \times 10^8$ cells/mL for *L. plantarum* subsp. *plantarum* 14917 and $4.94 \times 10^8$ cel/mL for *L. paracasei* subsp. *paracasei* 25302. To validate the mathematical model, a cultivation of *L. plantarum* subsp. *plantarum* 14917 was carried out using the optimized conditions. A 1.76-fold increase in cell concentration ($1.36 \times 10^9$ cell/mL) was obtained compared to the expected value predicted by the Taguchi model (Fig. 2). First, the exponential growth of *L. plantarum* subsp. *plantarum* 14917 in SmF was observed in the first 24 h followed by a stationary phase in the next hours. A second growth increase was appreciated with the maximum growth at 120 h. In this sense, a decrease of pH to 3.28 (Fig. 2) and a titratable acidity of 0.69% citric acid was observed at 120 h. In addition, the consumption of 1°Brix (TSS) and 1.59% RS was evident at the end of the growth kinetics (Fig. 2).

The maximum substrate consumption was observed at 72 h with a consumption of 13.4 g/L and 9.5 g/L of glucose and xylose, respectively (Fig. 3). In addition, arabinose was not present during the kinetic growth. The main metabolites obtained at the end of the cultivation was lactic acid, with a production of 6.9 g/L in a typical culture from *L. plantarum* strains, but the maximum production was observed at 96 h with a value of 11.7 g/L. Additionally, the production of ethanol reached a maximum value of 0.2349 g/L at 72 h (Fig. 3). The production of SCFA was not statistically significant (Fig. 4). Initial and final concentrations of succinic acid were 0.3125 and 0.3198 g/L, respectively, indicating a 7.3 mg/L consumption. Formic acid initial and final values were 0.9520–0.6793, respectively, with a 0.2730 g/L consumption. Meanwhile acetic acid concentrations were 0.16636–0.73148 g/L, at the start and end of cultivation.
respectively, with a production of 0.5651 g/L, and 44 mg/L of propionic were produced at the end of the cultivation.

**Discussion**

The chemical profile of fruit juice from *P. aculeata* is related with the fruit maturity according to the color scale constructed, considering the fruit firmness, TSS and titrable acidity as reported in previous studies (Angón-Galván 2006). Our results were similar in humidity and TSS levels, but high in titrable acid expressed as citric acid and lower in ash content in comparison with previous reports (Angón-Galván 2006). These differences can be attributed to geographical conditions and the season of the year when the fruits were harvested, as referred by other authors (Angón-Galván 2006). The chemical profile of *P. aculeata* fruit juice contains sugars that can be used as substrate for the stimulation of probiotic growth, similar to previously reported fruit juices, such as apple, orange, pomegranate, among others (Jaiswal and Abu-Ghannam 2013; Londoño et al. 2015; Mousavi et al. 2011; Pérez-Leonard and Hernández-Monzón 2015; Perricone et al. 2014). However, few studies are focused in application of Taguchi’s methodology for probiotics growth optimization, but has been applied to describe the relationship with indole-3-acetic acid production in a symbiotic and non-symbiotic nitrogen-fixing bacteria (genera *Agrobacterium*, *Paenibacillus*, *Rhizobium*, *Klebsiella oxytoca*, and *Azotobacter*) to optimize the immobilization conditions for *Lactobacillus pentosus* cells (Shokri and Emtiazi 2010; Wang et al. 2020). In the best of our knowledge, our study is the first in describing the utilization of juice from *Parmentiera aculeata* as substrate for probiotic growth and its optimization using submerged cultures. Different sources of fruit and vegetables have been explored as substrates for growth of probiotics, for example, the juice from *Aloe vera* has been used for growth of *L. plantarum* and *L. casei* (González, Domínguez-Espinosa, and Alcocer, 2008; Pérez-Leonard and Hernández-Monzón 2015). While white cabbage (*Brassica oleracea* var. capitata) was used for the growth of other probiotics as well as *L. plantarum* ATCC 8014; *L. rhamnosus* ATCC 9595 and *Lactobacillus brevis* ATCC 8287 (Jaiswal and Abu-Ghannam 2013). Other authors have assayed pomegranate juice for growth of *L. plantarum* DSMZ 20174, *L. delbrueckii* DSMZ 20006, *L. paracasei* DSMZ 15996 and *L. acidophilus* DSMZ 20079 (Mousavi et al. 2011), and sweet lemon juice was fermented with *L. plantarum* LS5 (Hashemi et al. 2017).

Particularly, most of the studies reported an efficiency for probiotic effect (expressed as colony-forming units, CFU) of $1 \times 10^6$–$1 \times 10^{12}$/dosage (Guarner et al. 2017; Jurado-Gámez et al. 2013). In this study, we explored the efficiency by means of cellular growth expressed as cel/mL during the utilization of *P. aculeata* juice, since this plant is used as livestock feed and for traditional Mexican medicine (Andrade-Cetto and Heinrich 2005; Morales-Sánchez et al. 2015; Pérez et al. 2000). Results describing the growth of probiotic strains using MRS and Mueller-Hinton media have been reported previously, where cellular concentrations at 24–48 h were $2.5–4.5 \times 10^9$ cel/mL for microorganisms from gut microbiota such as *L. brevis*, *L. casei* and *Lactobacillus delbrueckii/Streptococcus thermophiles* (Niño Herrera et al. 2020).

Concerning to the growth conditions, particularly pH, it changed during the SmF fermentation at similar values as those previously reported. After 48 h of fermentation using *Aloe vera* juice with *L. plantarum* NCIMB 11718 and *L. casei* NRRL-1445 a final pH of 4.6 and 5.6, respectively, were observed (González et al. 2008). In another report, after 72 h of incubation at 37 °C, an increase of pH from 3.2 to 3.4–3.6 was observed in pure and mixed cultures of three *L. plantarum* strains (*L. plantarum* PTCC 1896, *L. plantarum* AF1 and *L. plantarum* LP3) using fermented bergamont juice (Hashemi and Jafarpour 2020). These results are similar with our results, where a pH of 3.35 and 3.28 at 72 and 120 h were obtained, respectively. Fermentation of apple juice with *L. plantarum* subsp. *plantarum* ATCC 14917 revealed a change in the initial pH 6.2 to a final pH of 3.68 in 72 h (Li et al. 2019). The same strains have been assayed in pomegranate fermentation for 24 h and pH of 3.5 (Mantzourani et al. 2019). Additionally, a probiotic beverage of pineapple juice was fermented with *L. plantarum* 299 V during 24 h with an observed pH value of 3.8 (Nguyen et al. 2019). The increase in titrable acidity was due to the carbohydrate metabolism of sugars present in the juice, causing a decrease of pH, this behavior was reported for other fruit juices (Vivek et al. 2019). The titrable acid values obtained for *L. plantarum* subsp. *plantarum* ATCC 14917 are lower in comparison to previous reports, values of 1.6 to 1.9% citric acid after 6 h in fermented sweet lemon juice with *L. plantarum* LS5 (Hashemi et al. 2017) were obtained. Similar values were reported for *L. acidophilus* DSMZ 20079, *L. plantarum* DSMZ 20174, *L. delbrueckii* DSMZ 2006, *L. paracasei* DSMZ 15996 in pomegranate juice fermentation (Mousavi et al. 2011).

The previously reported sugar consumption by the probiotic strains is similar to the values obtained in our study. *L. plantarum* MCC 2974 consumed $\sim 1^\circ$ Brix in 72 h during sohiong juice fermentation (Vivek et al. 2019). Additionally, *L. plantarum* consumed <1 Brix during tomato juice fermentation. Particularly, glucose consumption was variable during juice fermentation by probiotic strains. While *L. plantarum* LS5 consumed $\sim 2$ g/L of glucose during sweet lemon fermentation (Hashemi et al., 2017), three *L. plantarum* strains (*L. plantarum* subsp.
P. aculeata juice ethanol was observed. The growth of molds and yeasts (Lucumi-Banguero et al. 2021). Propionic acid, for a strong inhibitory effect controlling the reduction of intestinal pain symptoms (Moradi et al. 2021) and presents a similar inhibitory effect than acetic and lactic acid (Park et al. 2020). The production of these acids have associated with anti-obesity properties in experiments with probiotic strains (Ucar et al. 2020; Zhao et al. 2015). Xylose, which is other important source carbon for growth of probiotic strains (Ucar et al. 2020; Zhao et al. 2015). Xylose consumption in a range of 2.26–7.75 g/L have been reported for different Lactobacillus strains, such as L. pentosus, L. brevis and L. buchneri when were growth in cucumber juice supplemented with trehalose, xylose and L-citronelle. We observed a xylose consumption higher for L. plantarum in P. aculeata juice, a value of 8.6–9.5 g/L at 120 and 72 h, respectively.

In fermentation with LAB, the main product was lactic acid, with others organic acids such as formic and propionic (Hashemi and Jafarpour 2020). Analyzing the results of postbiotics production during the fermentation of sweet lemon juice with L. plantarum EM ~ 7.5 g/L of lactic acid was obtained at 48 h and a similar value was reported for fermented bergamot juice at 72 h with L. plantarum strains (Hashemi and Jafarpour 2020; Hashemi et al. 2017). Values of 5.71 g/L lactic acid were reported for fermented papaya juice with L. plantarum GIM1.140 at 48 h (Chen et al. 2018). In our study the higher lactic acid concentration was reached at 24 h, producing 1.5-times more acid (11.3 g/L) than those previously reported. The value of formic acid obtained in our study was similar that the reported for fermented bergamot juice with L. plantarum PTCC 1896, which exhibited a production of ~0.8 g/L formic acid (Hashemi and Jafarpour 2020), which was higher that the value obtained in fermented papaya juice with L. plantarum GIM1.140 at 48 h (0.1842 g/L formic acid) (Chen et al. 2018). The production of propionic acid during fermentation, has been associated with anti-obesity properties in experiments with animals treated with fermented juices, including also acetic acid (Park et al. 2020). The production of these acids have been reported (0.2315 g/L) in mixed fermentation with L. rhamnosus GG and L. plantarum A6 using whole teff at 15 h (Alemneh et al. 2021). Succinic acid is associated with the reduction of intestinal pain symptoms (Moradi et al. 2021) and presents a similar inhibitory effect than acetic and propionic acid, for a strong inhibitory effect controlling the growth of molds and yeasts (Lucumi-Banguero et al. 2021).

In addition, during the metabolite profile monitoring of fermented P. aculeata juice ethanol was observed. The obtained value in this study at 120 h was 0.235 g/L, which is higher than the value reported for mixed fermentation with L. rhamnosus GG and L. plantarum A6 during the fermentation of whole teff at 9–12 h with values of 0.044–0.037 g/L, respectively (Alemneh et al. 2021), but are lower in comparison with the produced values during bhaati jaanr production (a rice-based fermentation beverage) with L. plantarum L7, 3.8 g/L at 120 h (Giri et al. 2018).

Several studies have been focused in demonstrate the beneficial bioactivities of fermented juices, for example cabbage-apple juice and citrus juice fermented with Lactobacillus strains were evaluated for their positive effect on obesity and allergic rhinitis, among others (Harima-Mizusawa et al. 2016; Park et al. 2020). The results reported in the present work constitute a first approach to a future utilization for functional foods production, but numerous studies are required to confirm the potential of the fermented P. aculeata juice with probiotics as an interesting option for functional beverages.

**Author contributions** All authors contributed to the study conception and design. Methodology and writing were performed by TJLC. Supervision and technical suggestions were realized by MLCI and VEBH. Data and formal analysis were development by PAZ. Visualization, supervision, writing, and funding acquisition were realized by FV. All authors read and approved the final manuscript.

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**Declarations**

**Conflict of interest** The authors have no relevant financial or non-financial interests to disclose.

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