Valorization of Parmentiera aculeata juice in growth of probiotics in Submerged Culture and their postbiotic production: a first approach to healthy foods

Teresa de Jesús Lara-Cervantes
Universidad Autónoma de San Luis Potosí: Universidad Autonoma de San Luis Potosi
María Luisa Carrillo-Inungaray
Universidad Autónoma de San Luis Potosí: Universidad Autonoma de San Luis Potosi
Victor Emmanuel Balderas-Hernández
IPICYT: Instituto Potosino de Investigacion Cientifica y Tecnologica AC
Pedro Aguilar-Zárate
Tecnologico Nacional de Mexico
Fabiola Veana (f_veana@hotmail.com)
Tecnologico Nacional de Mexico https://orcid.org/0000-0003-4012-6161

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Abstract

Actually, functional foods are good accepted for consumers for to improves health and new sources of substrates are explored. However, *Parmentiera aculeate*, which is a plant distributed in Mexico, has not explored. The *P. aculeata* juice was used as unique carbon source of promote the growth of two probiotic strains in Submerged Fermentation. Taguchi methodology with orthogonal array L9 was applied for its optimization. pH, agitation and inoculum concentration with three levels were evaluated and the best treatment was validated through a kinetic culture with monitoring some postbiotics. We observed an increase of 1.76 times in cellular concentration for *Lactobacillus plantarum* 14917 culture and the main produced postbiotic were short-chain fatty acids such as succinic, formic, acetic, propionic and lactic acids, which are associated the metabolisms probiotic and are important in human health. This study is the first about valorization of *Parmentiera aculeata* juice as substrate for growth probiotic strain and future studies are necessaries for its application in functional food.

Introduction

Biology diversity in Mexico had potential application in Food Biotechnology. However, any species had not explored, as such as *Parmentiera acuelata* (Kunth) Seem (syn. *Parmentiera edulis* DC), which is a plant distributed in Pacific from Sinaloa to Chiapas, and in Gulf from Tamaulipas and San Luis Potosí to Yucatán (Andrade-Cetto and Heinrich 2005; Morales-Sánchez et al. 2015). The tree can measure up to 15 m and its fruit 15-20 cm long by 6.5 cm wide. It has longitudinal grooves and is yellow-green in color (Andrade-Cetto and Heinrich 2005). The fruit, root and bark are recognized in traditional Mexican medicine as treatment to diabetes, renal diseases, among other as headache, gallstones, deafness and diarrhea (Andrade-Cetto and Heinrich 2005; Morales-Sánchez et al. 2015). Some of these ailments have been confirmed in biological models as the hypoglycemic effects of lactucin-8-O-methylacrylate present in chloroform extract from dried fruit. Lowers blood sugar levels was presented when the mice model was induced to diabetes with alloxan (Perez et al. 2000). In addition, the fruit have cytotoxic activity e induction of apoptosis in mama cancer cell (Estanislao-Gómez et al. 2016), hypoglucemic (Pérez et al. 2000; Andrade-Cetto and Heinrich 2005) and antiruolitic effects (Morales-Sánchez et al. 2015). In these sense, the bioactive compounds can be used for functional food formulation, where functional food and nutraceuticals have increment demand for their interesting improves in health. The first is defined as food products with appearance of traditional food that contains added ingredients for provide health-related benefits for people. (Topolska et al. 2021). Whereas that, nutraceuticals are characterized by to be sold in pills, powders and other medicinal forms not generally associated with food (Penson and Banach 2020). Probiotics and/or prebiotics are usually associated for provided this functionality. Interesting, the probiotics have been associated to improve health in patient with any disease mentioned before. The probiotics are lactic acid bacteria (LAB) of genus *Lactobaccillus* mainly (Kitazawa et al. 2020). In addition, *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus rhamnosus* and *Bifidobacterium lactis* are the strains more used in formulation of new probiotic products of fruit and vegetable origin (Bernal Castro et al. 2017). During growth probiotics, soluble factors

(products or metabolic by-products) are secreted for bacteria (probiotic or non-probiotic), or released after bacterial lysis. These substances benefits to the host and are known as postbiotics (Homayouni Rad et al. 2021; Kang et al. 2021). These compounds can be 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; Nataraj et al. 2020; Wegh et al. 2019). Some important benefits of postbiotics are anti-inflamatory, anti-oxidant, anti-proliferative, anti-hypertensive, hypocholesterolemic, immumodulation, among others (Homayouni Rad et al. 2021; Lee et al. 2021). On the other hand, the Submerged Fermentations (SmF) are used in growth of probiotic strains and Taguchi methodology is less explored in this sense. This methodology searches the best condition (optimal conditions) applied in industries improves for quality process (Aguilar-Zarate et l. 2014) and can be applied in SmF for optimization of probiotic's growth. Therefore, the objectives of this work were 1) found alternative sources of prebiotics, mainly demonstrated the capability of juice from *Parmentiera aculeata* as unique carbone source of promote the growth of two probiotic strains in SmF, 2) optimize the SmF for see the major response in concentration cellular and, 3) characterizer the produced postbiotics during SmF.

Material And Methods

Culture medium and reagents

The DeMan-Rogosa-Sharpe 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), and standards were obtained from JT Baker (Phillipsburg, NJ, USA) or Simga Aldrich Chemical Co. (St. Louis, MO).

Vegetal material and strains

Fruits from *Parmentiera acuelata* were collected in "El Gavilan" locality (22.5250397, -100.0429407) from Valles City, San Luis Potosi, Mexico. The fruit selection was based according color scale described by Angon-Galvan (2006) with 3-4 from color scale. The fruits were translated to Food Research Laboratory from FEPH-UASLP and conserved in refrigeration until use.

Lactobacillus paracasei subsp. *paracasei* ATCC 25302 and *Lactobacillus plantarum* subsp. *plantarum* ATCC 14917 were provided by Food Reseach Department of Autonomous University of Coahuila, México.

Preparation of juice from P. acuelata

The fruits were washed and disinfected with ionidez silver 3% solution (Microdyn®). Then, fruits were cutted and the juice was obtained with Master Craft extractor. After that, the juice was vaccum and the pH was adjusted at the different values according to experimental model and was centrifuged (Hermle, Z206A, Germany) at 5000 rpm for 5 min. Supernatant was transferred at conic tubes of 50 mL and were sterilized (Yamato, SM200, Japan) at 121°C, 15 lb for 15 min. Previous to SmF, the juice was decanted for solids elimination.

Taguchi methodology

Taguchi methodology was applied in four phases as mentioned (Aguilar-Zarate et al. 2014): 1) design of experimental, 2) Submerged fermentation, 3) experimental data analysis and prediction performance, and 4) validation. For phase 1, a L9 orthogonal array (3^3) considered three important factors (pH, agitation and concentration inoculum) with three levels (4.0, 5.0 and 6.0; 0, 75 and 150 rpm; 5, 10 and 15%, respectively) for the cellular concentration during SmF of probiotic strains.

For submerge cultures

The strains were activated in assay tubes with 10 mL of Man-Rogosa-Sharpe Broth, which were incubated at 37°C for 24 h and the inoculum for the fermentation was prepared for re-seeding and culture of 16 h. Incubation of the treatments was carried out at 37°C for 120 hours as Table 1 suggested. Growth was expressed as cells per milliliter (cel/mL). for count in Neubauer´s chamber The experimental was performance in duplicated.

Table 1
Experimental matrix for orthogonal L9 array in Taguchi´s methodology

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

Experimental data analysis and prediction performance

The results of nine trials for SmF was processed using the software Statistical version 7.1 (Statsoft, Tulsa, OK, USA).

Validation

The strain with the higher cellular concentration was validated as suggest experimental data analysis with optimal culture conditions. In addition, the SmF was realized as kinetic culture at 120 h with a monitored each 24 horas of cellular concentration, pH changes, titrable acidity (TA, % citric acid (%CA)),

total soluble solids (TSS, °Brix), total reducers sugars (TRS, %) and postbiotic compounds. The culture was realized in triplicates. All results were compared between statistical groups, using analysis of variance (ANOVA) and Tukey's multiple comparisons, with a significance cutoff P value of <0.05.

Chemical profile from free cell culture medium

Finally, the culture medium was centrifuged (Hermle, Z206A, Germany) at 5000 rpm for 5 min and free-cell culture medium (supernatants) was used for the follow determinations: pH (Oakton, 700, Vernon Hills, USA), TA was determinated according to procedure described in NMX-FF-011-1982 and was expressed as % CA, SS expressed °Brix using the methodology described in NMX-F-436-SCFI-2011, ART using Lane-Eynon volumetric method described in NMX-F-312-1978 and. For postbiotic determination, supernatant samples were filtered using a 0.4 µm syringe filter. 10 µL of each sample were used for analysis. Sugars, organic acids and ethanol were measured by high-performance liquid chromatography (HPLC) in an Agilent chromatograph equipped with a refractive index detector, operated at 50°C (Agilent Technologies 1220 Infinity LC). Compounds were separated using a Rezex ROA Phenomenex-organic acid column operated at 60°C with 0.5 mL/min^{-1} of H_2SO_4 0.25 mM as a mobile phase and running time of 17 min.

Results

After that SmF, experimental data analysis for Taguchi methodology was realized. In Fig. 1 is presented the results of nine trials assays, where the treatment 2 exhibit the major cellular concentration for both probiotic strains. The conditions correspond to pH of 4.0, agitation of 75 rpm and inoculum concentration of 10%. The maximum cellular concentration were 7.64 x 10^8 cells/mL for *L. plantarum* subsp. *plantarum* 14917 and 4.94 x 10^8 cel/mL for *L. paracasei* subsp. *paracasei* 25302. In validation of the experiment with *L. plantarum* subsp. *plantarum* 14917, there was a 1.76-fold increase in cell concentration value (1.36 x 10^9 cell/mL) compared to the expected value predicted by the Taguchi model (Fig. 2). First, the behavior of *L. plantarum* subsp. *plantarum* 14917 in SmF is similar at the strains in the first 24 h and with stationary phase in the next hours. However, other increase is appreciated with the maximum growth at 120 h. In these sense, a decrease of pH 3.28 (Fig. 2) and titratable acidity of 0.69% citric acid was observed at 120 h. In addition, the consumption of 1 °Brix (SS) and 1.59% ART 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 not present during kinetic growth. The main metabolite is lactic acid, which had a production of 6.9 g/L in a typical culture from *L. plantarum* strains, but the maximum production is observed at 96 h with a value of 11.7 g/L. Additionally, the ethanol production was presented with the maximum value of 0.2349 g/L at 72 h (Fig. 3). Not significant statistically the production of SCFA were presented (Fig. 4). Succinic acid is 0.3125 and 0.3198 g/L initial and final, respectively, with production during fermentation of 7.3 mg/L. Formic acid values of 0.9520-0.6793 initial and final, respectively were observed, with a consumption of 0.2730 g/L. Acetic acid

0.16636-0.73148 g/L, respectively, with a production of 0.5651 g/L, and propionic acid 0.4964-0.5404 initial and final, respectively with a 44 mg/L.

Discussion

Diverse alternatives of substrates for probiotic growths have been explored as 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 and these are relationship with indole-3-acetic acid production in symbiotic and non-symbiotic nitrogen-fixing bacteria (genera *Agrobacterium, Paenibacillus, Rhizobium, Klebsiella oxytoca*, and *Azotobacter*) or immobilization conditions for *Lactobacillus penntosus* cells (Shokri and Emtiazi 2010; Wang et al. 2020). Our study is the first in describe the usage of juice from *Parmentiera aculeata* as substrate for probiotic growth and its optimization in Submerged Culture. Therefore, sources of fruit and vegetables has been explored as substrates for growth of probiotics, for example, the juice from *Aloe vera* has been used for growth of *Lactobaillus plantarum* and *Lactobacillus casei* (González et al. 2008; Pérez-Leonard and Hernández-Monzón 2015). While white cabbage (*Brassica oleracea* var. capitata) has been used for growth other probiotics as well as *Lactobacillus plantarum* ATCC 8014; *Lactobacillus rhamnosus* ATCC 9595 and *Lactobacillus brevis* ATCC 8287 (Jaiswal and Abu-Ghannam 2013). Other authors have assayed pomegranate juice for growth of *Lactobacillus plantarum* DSMZ 20174, *L. delbruekii* DSMZ 20006, *L. paracasei* DSMZ 15996 and *L. acidophilus* DSMZ 20079 (Mousavi et al., 2011), and sweet lemon juice has been fermented with *Lactobacillus plantarum* LS5 (Hashemi et al. 2017).

Particularly, most of the studies report that an efficiency for probiotic effect expressed as colony-forming units (CFU) is 1 x 10^6 – 1x 10^{12}/dosage (Guarner et al. 2017; Jurado-Gámez et al. 2013). However, in this study, we explorer only the behavior as cellular growth in cel/mL for utilization of juice from *P. aculeata*, since this plant is used as livestock feed and traditional mexican medicine (Andrade-Cetto and Heinrich 2005; Morales-Sánchez et al. 2015; Perez et al. 2000). In these sense, results for growth of probiotic strains using MRS and MH media have been reported previously, where cellular concentrations at 24-48 h are 2.5-4.5 x10^9 cel/mL for microorganisms from gut microbiota such as *Lactobacillus brevis, Lactobacillus casei* and L*actobacillus delbrueckii/Streptococcus thermophiles* (Niño Herrera et al. 2020). These results are superiors at those obtained in our study.

About growth conditions, particularly pH changes during fermentation, differences in values during fermentations have been reported. After 48 h of fermentation of *Aloe vera* juice with *L. plantarum* NCIMB 11718 and *L. casei* NRRL-1445 a final pH of 4.6 and 5.6, respectively, have been observed (González et al. 2008). However, similitudes with our results are observed by other authors. Therefore, after 72 h at 37°C the increase of pH at 3.2 from 3.4-3.6 was presented to pure and mixed cultures of three *L. plantarum* strains (*L. plantarum* subsp. *plantarum* PTCC 1896, *L. plantarum* AF1 and *L. plantarum* LP3) in fermented bergamont juice when are fermented in bergamot juice (Hashemi and Jafarpour 2020). These results are similar at our results with values of 3.35 and 3.28 at 72 and 120 h, respectively. Fermentation

of apple juice with *L. plantarum* subsp. *plantarum* ATCC 14917 reveals that pH 6.2 to final pH of 3.68 in 72 h (Li et al. 2019). The same strains have been assayed in promeganate fermentation for 24 h and pH of 3.5(Mantzourani et al. 2019). Additionally, a probiotic beverage of pineapple juice with *L. plantarum* 299V was produced and after 24 h of fermentation was observed a pH value of 3.8 (Nguyen et al. 2019). The increase in titrable acidity is due to carbohydrate metabolism for sugar presents in the juice and decrease of pH is observed, is behavior has been reported for fruit juices (Vivek et al. 2019). The titrable acid obtained for *L. plantarum* subsp. *plantarum* ATCC 14917 are minor 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). Same values has been reported for *Lactobacillus acidophilus* DSMZ 20079, *L. plantarum* DSMZ 20174, *L. delbrueckii* DSMZ 20006, *L. paracasei* DSMZ 15996 in pomegranate juice fermentation(Mousavi et al. 2011).

The reported sugar consumption for probiotic strains previously is similar to the value obtained in our study. *L. plantarum* MCC 2974 consumed at ~1° Brix in 72 h during sohiong juice fermentation (Vivek et al. 2019). Additionally, *L. plantarum* consumed < 1°Brix during tomato juice. Particularly, glucose consumption is variable during juice fermentation of probiotic strains. While *L. plantarum* LS5 consumed ~2 g/L of glucose during sweet lemon fermentation (Hashemi et al. 2017), three *L. plantarum strains (L. plantarum* subsp. *plantarum* PTCC 1896, *L. plantarum* AF1 and *L. plantarum* LP3) consumed ~4 g/L of glucose from fermentation of bergamot juice with(Hashemi and Jafarpour 2020). These values are minors that glucose consumption for *L. plantarum* ATCC 14917 in *P. aculeata* juice.

Other sugar monitoring in fermented *P. aculeata* was xylose, which is other important source carbon for growth probiotic strains (Ucar et al. 2015). Xylose consumption of 2.26-7.75 g/L have been reported for *Lactobacillus strains*, such as *L. pentosus*, *L. brevis* and *L. buchneri* when are fermented in cucumber juice supplemented with threalose, xylose and L-citrunille at time final of 60 h. 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 (equivalent to 1.1-5.9 times higher).

In fermentation with LAB the main product are lactic acid, and others organic acid such as formic and propionic acids (Hashemi and Jafarpour 2020). Analyzing the results of posbiotic production, during fermentation of sweet lemon juice with *L. plantarum* EM, ~7.5 g/L lactic acid is obtained to 48 h and similar value is 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 are reported for fermented juice papaya with *L. plantarum* GIM1.140 at 48 h (Chen et al. 2018). In this study this value is reached at 24 h and 1.5 times more is obtained at 48-120 h (11.3 g/L). The value of formic acid obtaining in our study is similar that 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), but is 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). Propionic acid has been monitoring during fermentation, which is associated to in anti-obesity properties in experiments with animals, including acetic acid. (Park et al. 2020).This last compounds have been reported as a value lower (0.2315 g/L) in mixed fermentation with *Lactobacillus rhamnosus*

GG/ *Lactobacillus plantarum* A6 when is fermented with whole teff at 15 h (Alemneh et al. 2021). The succinic acid is associated to reduction of symptoms intestinal and (Moradi et al. 2021) and presents a similar inhibitory effect sucs as acetic and propionic acids, since these present a strong inhibitory effect for control of molds and yeas growths (Lucumi-Banguero et al. 2021).

In addition, ther substance monitoring in fermented *Parmentiera aculeata* juice was ethanol. The value obtained in this study at 120 h (0.235 g/L) is higher that the value reported for mixed fermentation with *Lactobacillus rhamnosus* GG/ *Lactobacillus plantarum* A6 when is fermented with whole teff at 9-12 h with values of 0.044-0.037 g/L, respectively (Alemneh et al. 2021), but are minor compared with the produced ethanol during bhaati jaanr production based-raice fermentation beverage) with *L. plantarum* L7 3.8 g/L at 120 h (Giri et al. 2018).

In other sense, diverse studies have been focused in demonstrated the bioactivities of fermented juices, where fermented cabbage-apple juice and citrus juice using *Lactobacillus* strains have been evidenced in conditions such as obesity and allergic rhinitis, among others (Harima-Mizusawa et al. 2016; Park et al. 2020). Finally, the results suggest that fermented P. *acuelata* juice could be an interesting option in functional food where bioactivities will be evidenced. However, this study is a first approach to functional foods

Declarations

Author contributions

All authors contributed to the study conception and design. Methodology and writing were performed by TJ Lara-Cervantes and VE Balderas-Hernández. Supervision and technical suggestions were realized by ML Carrillo-Inungaray. Data and formal analysis were development by P. Aguilar-Zárate. Visualization, supervision, writing funding acquisition were realized by F. Veana. All authors read and approved the final manuscript.

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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Figures

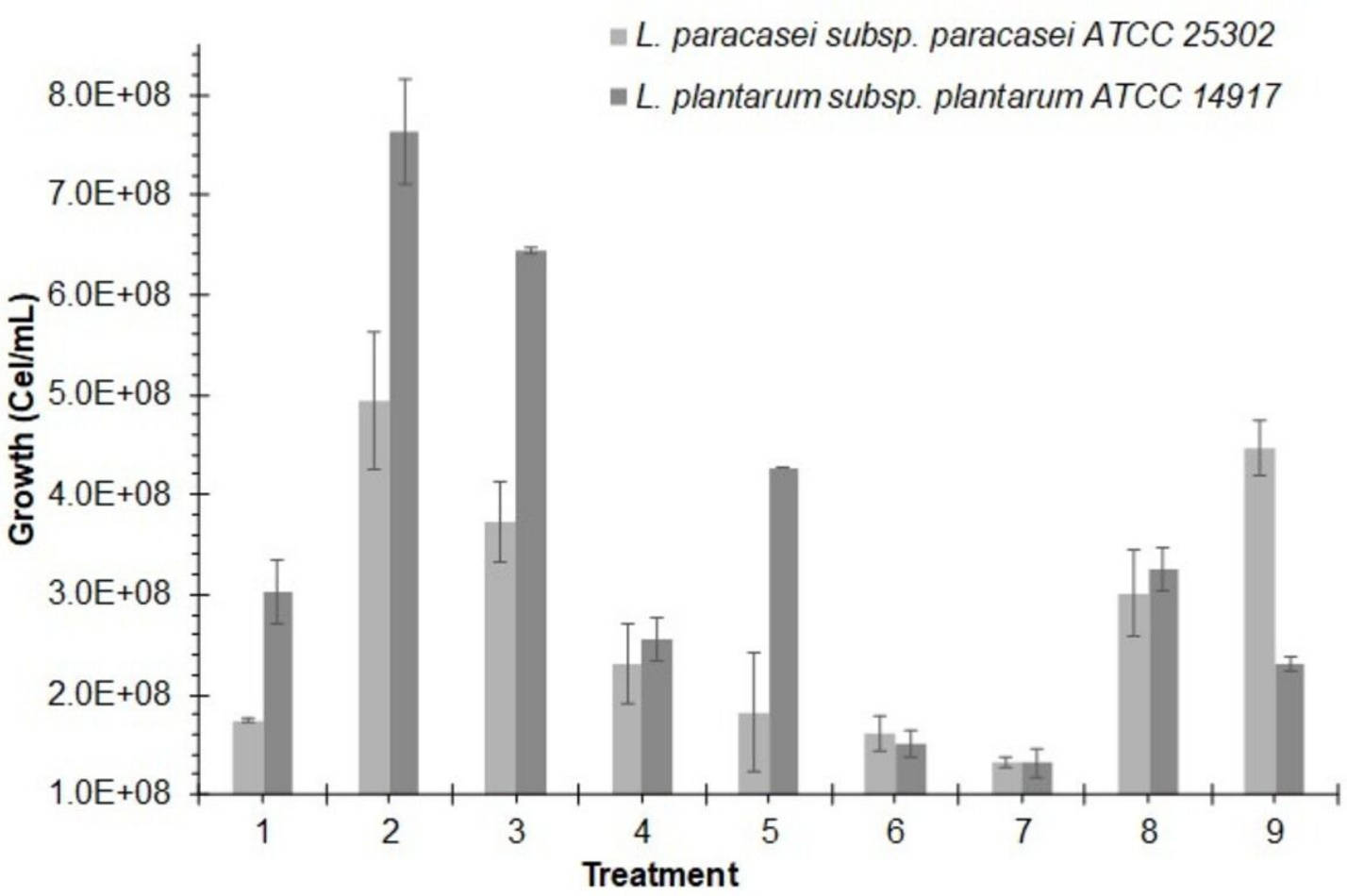

Figure 1

Results of experimental design using Taguchi's methodology for Submerged Fermentations

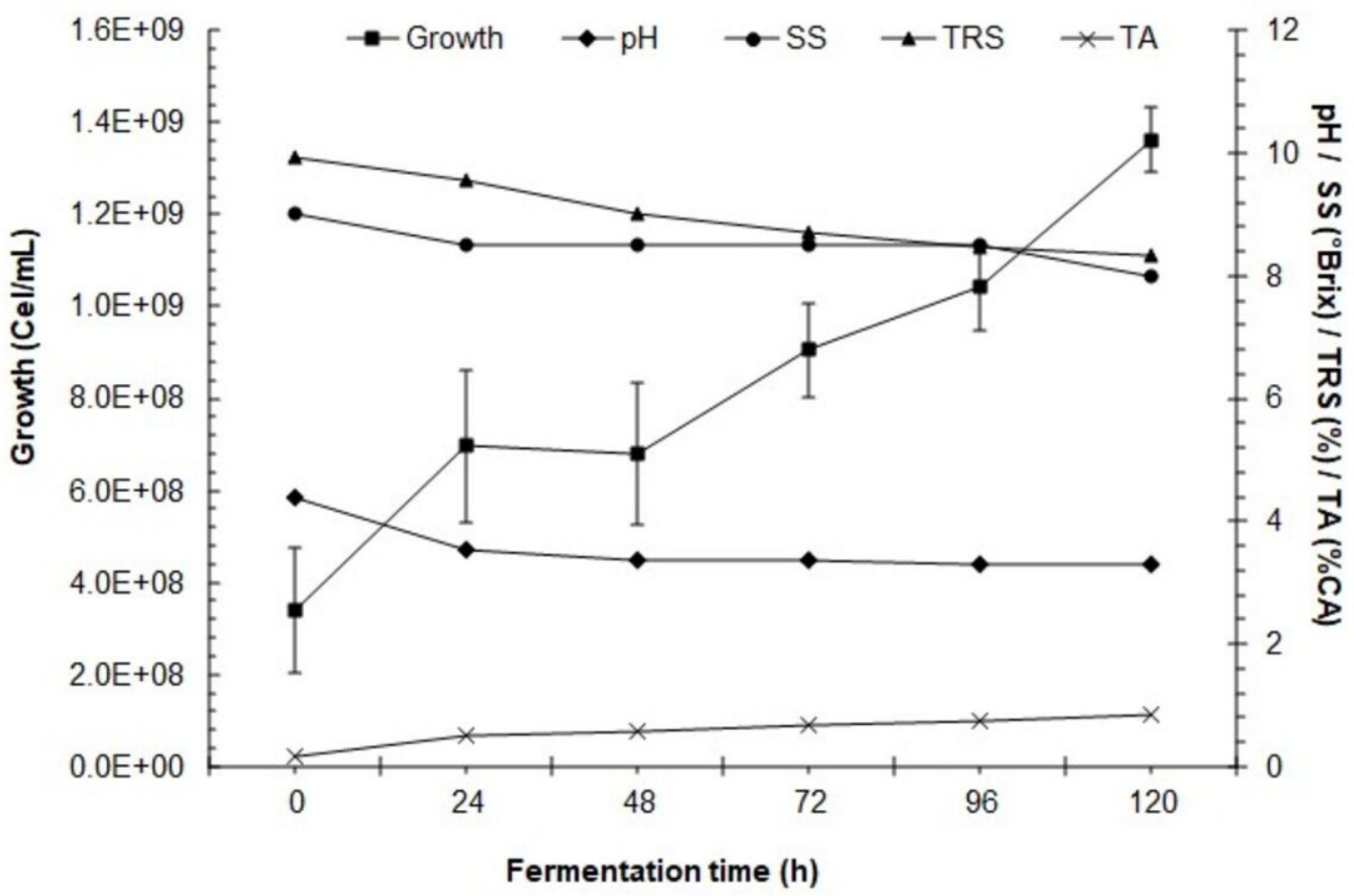

Figure 2

Growth, pH changes and substrate consumption from *L. plantarum* subsp. *plantarum* 14917 during kinetic SmF.

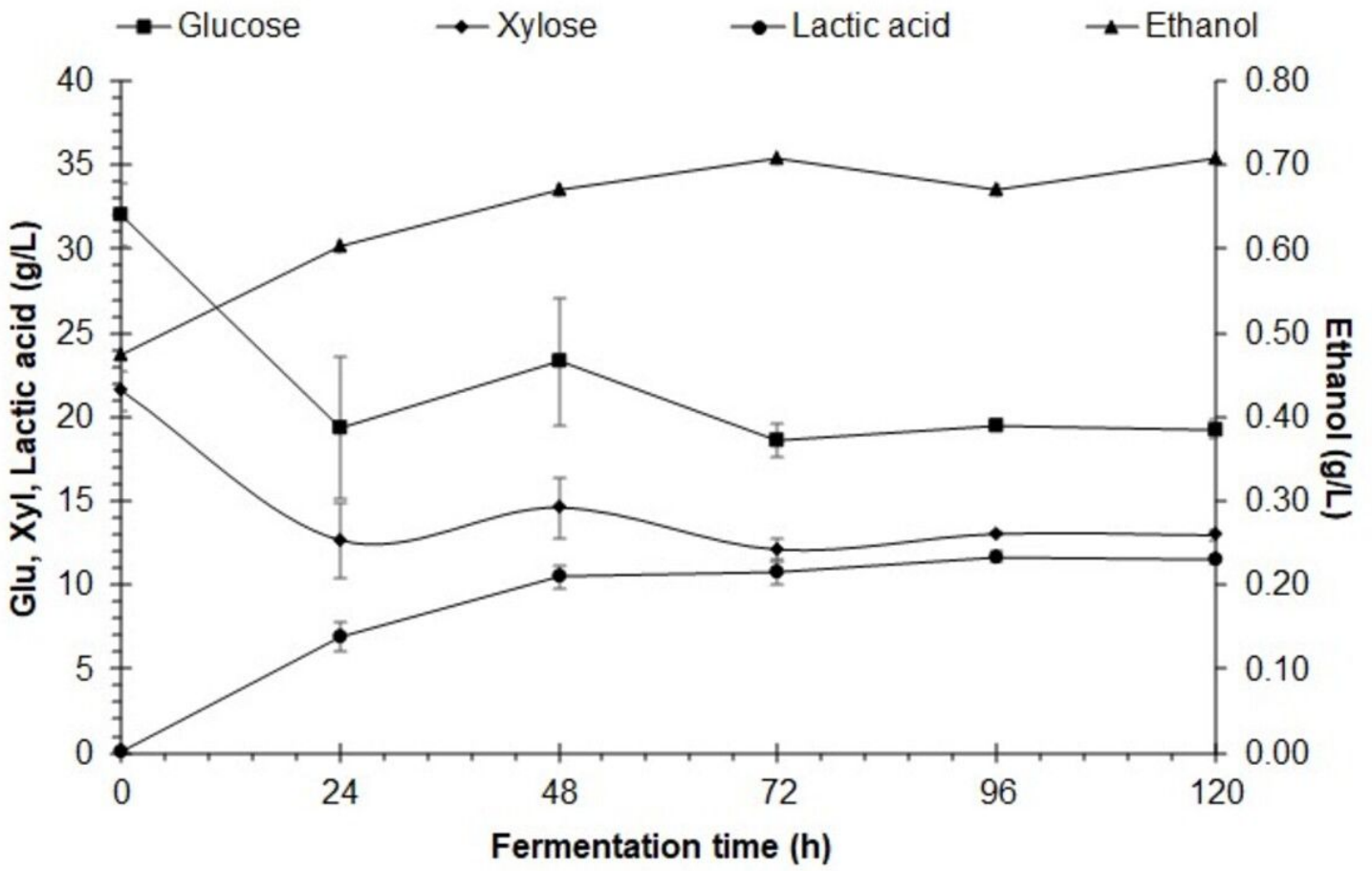

Figure 3

Susbtrates consumption and postbiotic production during SmF with juice from *P. aculeata* and *L. plantarum* subsp. *plantarum* ATCC 14917

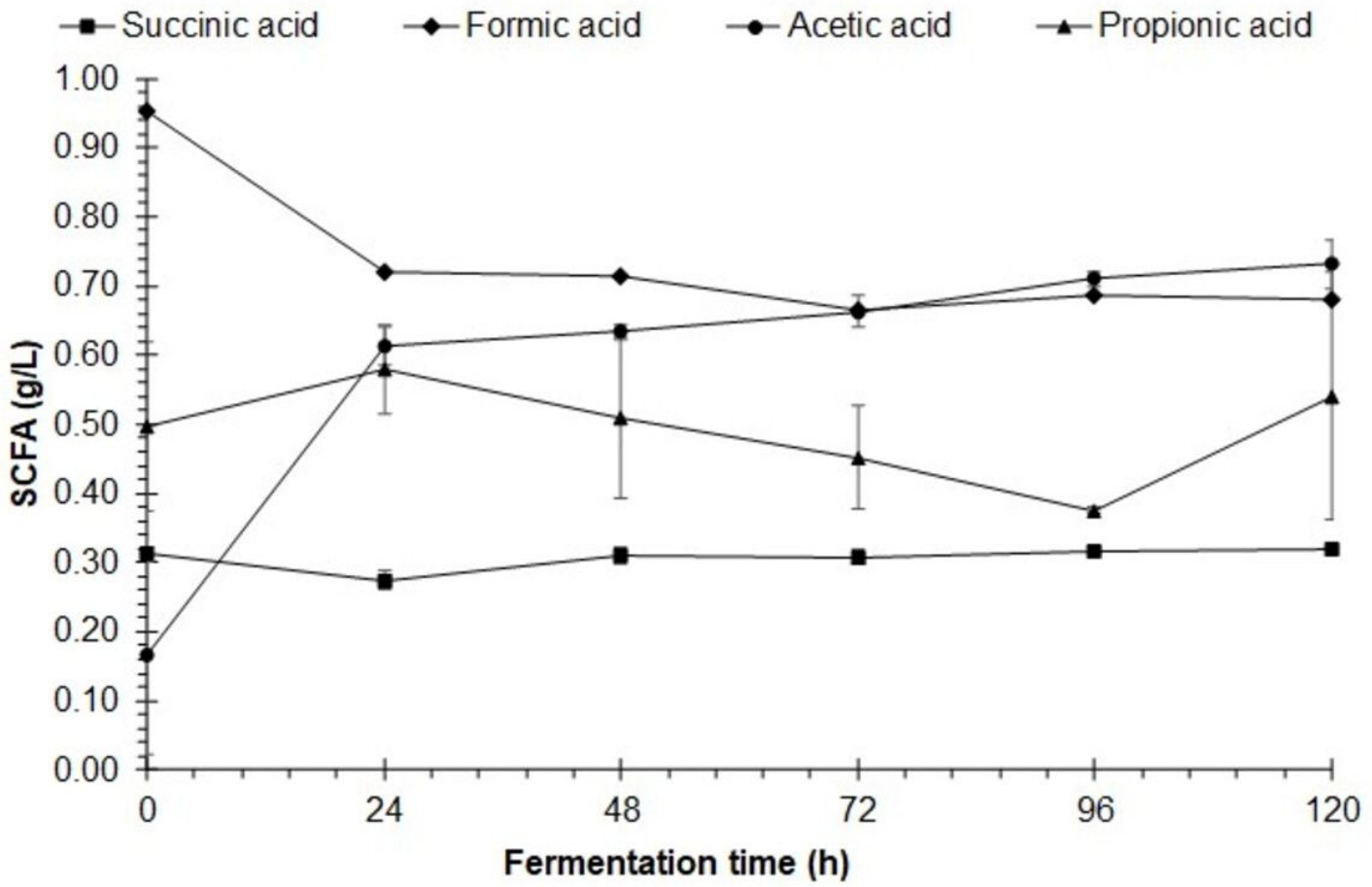

Figure 4

Postbiotic production during SmF with juice from *P. aculeata* and *L. plantarum* subsp. *plantarum* ATCC 14917