Sustainable Food Technology

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
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Cite this: *Sustainable Food Technol.*, 2025, 3, 1927

Biosurfactant from *Lactiplantibacillus plantarum* Tw226 produced using yacon juice and its functionality as emulsifier of essential oils in water emulsions

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The search for alternative culture media from agro-industrial sources to produce biosurfactants (BS) from lactic acid bacteria is of interest because it reduces production costs and allows circular production systems. This study aimed to evaluate the ability of *Lactiplantibacillus plantarum* Tw226 to produce BS in a nutrient medium based on yacon juice (BSY), a neglected and underutilized Andean tube, and to compare its functionality as an emulsifier for cinnamon bark and lemongrass essential oil-in-water emulsions. *L. plantarum* Tw226 reach a dry biomass yield of 1.10 ± 0.06 g L^{-1} using yacon juice supplemented with 25% MRS broth (MRSJ) and was similar to the one obtain with MRS. The BSY yield was 0.11 ± 0.03 g L^{-1} and has a surface tension of 43.48 ± 0.68 mN m^{-1}. The FTIR spectra shown that the functional groups of BSY, were similar to those presents in the BS produced in MRS broth (BSMRS). The emulsions of lemongrass and cinnamon bark oil, formulated BSY, had an initial droplet size of 425.9 ± 35.7 nm and 348.2 ± 19.9 nm, respectively. These sizes remained unchanged for 4 weeks, being smaller or similar to those stabilized with the BSMRS. The emulsion of cinnamon bark oil stabilized with BSY did not show creaming compared to the analogous stabilized with the BSMRS. Lemongrass emulsions presented creaming regardless of the BS used. In conclusion, *L. plantarum* Tw226 could produce a BS using yacon juice with 25% MRS broth which is useful as emulsifier.

Received 21st June 2025
Accepted 11th August 2025

DOI: 10.1039/d5fb00288e

rsc.li/susfoodtech

Sustainability spotlight

The search for alternative culture media from agro-industrial sources to produce biosurfactants from lactic acid bacteria is of interest because it reduces production costs and allows for circular production systems. This study explored the use of an alternative culture medium based on yacon, an underutilized Andean tuber, for the growth of *Lactiplantibacillus plantarum* Tw226 and the production of its cell-bound biosurfactant. Additionally, the biosurfactant functionality as an emulsifier for cinnamon bark and lemongrass essential oil-in-water emulsions was evaluated. The results obtained were encouraging since *L. plantarum* Tw226 could produce a biosurfactant using yacon juice supplemented with 25% MRS broth. This study also highlights the possibility of expanding the use of yacon

Introduction

The production of biosurfactants from lactic acid bacteria (LAB) is carried out through fermentations in which bacteria consume a substrate to produce the biomolecule of interest. In these biotechnological processes, the type of substrate on which the bacteria grow is fundamental in defining the properties of the product obtained, as well as its cost.

From the early studies of the production of biosurfactants from LAB at laboratory scale is commonly performed using Man Rogosa Sharpe (MRS) broth as the culture medium, which is a complex medium typically used for the detection and enrichment of lactobacilli, and whose main components are peptone, beef extract, yeast extract and glucose.[1] In recent years, there has been a surge in exploring new uses for food industry by-products, including their use as culture media to produce biomolecules such as biosurfactant. Certain strains of LAB have demonstrated the ability to produce biosurfactants from agro-industrial waste. This practice allows the reuse of by-products, prevents their disposal, reducing production costs, and opens

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up possibilities for waste exploitation and the development of circular production systems. Some of the by-products studied for biosurfactants production from LAB included cheese whey, supplemented with additional nutrients, that was useful for *Lactococcus lactis* 53, *Limosilactobacillus fermentum* ACA-DC 0185 growth.[2,3] Additionally, *Lactobacillus pentosus* was capable to produce a biosurfactant using sugars from agricultural residues of distilled grape pomace, hazelnut shells and walnut shells and *Lactobacillus plantarum* MGL-8 from mango by-products.[4,5]

Previous studies have identified *Lactiplantibacillus plantarum* Tw226 as a producer of a glycolipopeptidic cell-bound biosurfactant, which contains proteins, carbohydrates, and lipids in percentages of 64.0, 15.0, and 21.0% respectively. This biosurfactant is capable of emulsifying corn oil in water emulsions in the presence of humectant agents such as glucose (14%) or NaCl (5%) and its tensioactivity remains stable at pH between 5.00–7.00.[6,7] In addition, a recent study showed that it is as a possible emulsifier of natural multifunctional emulsions based on essential oil.[8]

The production of BS from *L. plantarum* Tw226 has primarily been studied using MRS medium, and in the search for alternative culture media, yacon juice has been proposed. Yacon (*Smallanthus sonchifolius*) is an Andean plant whose tuberous root is rich in prebiotic compounds, particularly fructooligosaccharides and inulin, which are known to enhance metabolic functions and the immune system. Nutritionally, on a dry basis, yacon root contains 94.15% carbohydrates, including simple sugars such as glucose and fructose, as well as fructans.[9] The yacon juice obtained by cold press showed 34.3% of carbohydrates and 58.9% of fructooligosaccharides on a dry basis.[10] Additionally, certain strains of *L. plantarum* are recognized for their ability to ferment fruit juices.[11] These properties suggest that yacon root juice could serve as a suitable medium for biosurfactant production.

The present study aimed to evaluate the capability of *L. plantarum* Tw226 to produce biosurfactant in a nutrient medium based on yacon juice and to compare its functionality as an emulsifier for cinnamon bark and lemon grass essential oils.

Experimental

Yacon juice production

Tubers of pesticide-free yacon from the Los Chorillos community in Jujuy, Argentina were used. Upon arrival at the laboratory, they were sanitized by washing with tap water, brushing to remove excess soil, and immersion in 200 ppm chlorinated water for 5 minutes. Then, they were peeled, cut into 1 cm^3 cubes and immersed in a 2.5% w/v citric acid solution for 5 minutes to reduce enzymatic browning. Finally, the cubes were blanched with steam for 5 minutes, cooled, and the juice was extracted using a commercial centrifugal juicer (Peabody, Argentina). To clarify the juice, two consecutive centrifugations were performed at 5590 × g for 15 minutes at 4 °C in a high-speed centrifuge (Eppendorf 5804 R, Germany).

Evaluation of the growth of *L. plantarum* Tw226 in culture media

The capability of *L. plantarum* Tw226 to grow using different concentrations of MRS broth or yacon juice was evaluated by the broth microdilution method using 96 well microplates. For this purpose, at a first stage,180 μL of nutrient medium of (i) MRS100: formulated according to the instruction provided by the supplier (Biokar, France); (ii) MRS75: dilution of MRS100 to 75%; (iii) MRS50: dilution of MRS100 to 50%; (iv) MRS25: dilution of MRS100 to 25%; (v) MRS12.5: dilution of MRS100 to 12.5%; (vi) J100: yacon juice at extraction concentration; (vii) J75: dilution of J100 to 75%; (viii) J50: dilution of J100 to 50%; (ix) J25: dilution of J100 to 25%; (x) J12.5: dilution of J100 to 12.5%. All dilutions were made with distillate water. An aliquot of 20 μL of an inoculum containing approximately 1×10^6 CFU mL^{-1} was added to each well. Absorbance was measured at 590 nm every 30 minutes for 48 h at 35.0 °C using a BioTek ELx808 microplate reader (BioTek Instruments, USA). Each system was tested in six replicates, with uninoculated broth included as a negative control and inoculated MRS broth as a positive control.

At a second stage, the growth of *L. plantarum* Tw226 was assessed in mixtures of yacon juice and MRS broth. Briefly, the following mixtures were formulated: (xi) MRS25J, comprising 75% J100 and 25% MRS100, and (xii) MRS12.5J, comprising 87.5% J100 and 12.5% MRS100. A volume of 75 mL of those culture media was placed in 250 mL Erlenmeyers flask and inoculated with 750 μL of *L. plantarum* Tw226 suspension containing approximately 1×10^6 CFU mL^{-1}. Those systems were incubated at 35 °C for 48 h, and aliquots were taken to measure the optical density using a BioTek ELx808 microplate reader. Each system was tested in three replicates.

In all cases, the inoculum was obtained from an overnight culture adjusted to 0.5 McF and diluted 1/10, the pH of the juice was adjusted with 1.5 M NaOH to reach the MRS broth pH (6.40), and it was autoclaved at 108° for 15 min. The yacon juice and the MRS broth were individually autoclaved and then mixed to avoid Maillard reaction.

Biosurfactant production

The biosurfactant production was carried out following the procedure proposed by Gudiña *et al.*, with certain modifications.[12] An aliquot of 75 mL of the medium was placed into an Erlenmeyer flask of 250 mL and *L. plantarum* Tw226 inoculum was added to achieve a concentration of 1×10^6 CFU mL^{-1} and incubated for 48 h at 37 °C and 120 rpm. Bacterial cells were washed twice with distilled water and resuspended in phosphate-buffer saline (PBS: 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 2.7 mM KCl and 150 mM NaCl with pH adjusted to 7.0) at a ratio of 1 : 6 relative to the volume of the culture medium. The suspension was shaken for 2 h at 150 rpm and a constant temperature of 25 °C to release the biosurfactant. It was then centrifuged to obtain the cell-free supernatant (CFS) containing the biosurfactant. Finally, to eliminate the lower molecular weight molecules, the CFS was dialyzed against deionized water using a 6–8 kDa pore diameter membrane (Merck Millipore,

Germany) and then lyophilized using a lyophilizer 1–2 LSCbasics (Christ, Germany).

Biosurfactant and dry biomass quantification

For the quantification of bacterial production, the dry biomass of the bacterial pellet was measured once the production was finished. For this purpose, the washed pellet was dried in a convection oven at 105 °C to a constant weight, and the grams of dry biomass per liter of nutrient medium were calculated. Also, was measured the weight of the lyophilized biosurfactant at the end of the production and calculated the yield per liter of nutrient medium.

Fourier-transform infrared spectroscopy analyses

To observe differences in the chemical composition of the lyophilized biosurfactant, Fourier transform infrared spectroscopy (FTIR) was performed. An aliquot of the sample was mixed with potassium bromide and pressed to produce a pellet. The infrared absorption spectra were recorded using a Thermo Electron OMNIC version 7.3, Nicolet 8700 FTIR system (Thermo Electron Corporation, Germany) with a spectral resolution of 4 cm^{-1} and a wavenumber range between 400 and 4.000 cm^{-1}. All measurements consisted of 32 scans, with the potassium bromide pellet used as a background reference.

Surface tension measurement

The surface tension of biosurfactant solutions produced using alternative culture media or MRS broth at a concentration of 5 g L^{-1} was measured using a pendant drop tensiometer (PAT-1, Sinterface Technologies, Germany). Droplets of the solutions were formed at the tip of a stainless-steel capillary immersed in a glass cuvette kept in a thermostat at 25.0 ± 0.1 °C. The drop profile was automatically fitted to the Young–Laplace equation to determine the surface tension.[13] Determinations were made in triplicate.

Emulsion preparation

The capability of the biosurfactant to emulsify cinnamon bark or lemon grass essential oils in water emulsions was evaluated. For this purpose, essential oil emulsions containing an equal amount (5000 ppm) of essential oil and the lyophilized biosurfactant were prepared in distillated water. The biosurfactants produced using MRS (BSMRS) and the alternative medium selected (BSY) were evaluated. The homogenization was performed using a high-speed disperser Ultraturrax IKA S10 (IKA, Germany) for 30 s at 15 000 rpm and 90 s at 30 000 rpm.

Emulsion droplet diameter

The emulsions were characterized using a Dynamic Light Scattering (DLS) equipment Zetasizer Pro (Malvern Panalytical, United Kingdom). Measurements with DLS were taken at a fixed angle of 173°, the refraction indexes were 1.60 and 1.483 for cinnamon bark and lemongrass oil, respectively. This technique determines the mean droplet diameter (Z-average) as a function of time. The emulsions were stored at 25.0 ± 0.1 °C, and measurements were taken every 7 days over a period of 28 days.

Emulsion physical stability

The physical stability of the emulsions was evaluated for 28 days at 25 °C by studying the variation in the backscattering (ΔBS%) profile as a function of the length of the tubes containing the sample and time. The analysis was performed using the Turbiscan CLASSIC 2 (Formulaction, France), using the multiple lightscattering technique. This method is useful for determining the mechanisms of emulsion. In addition, using the Software TurbiSoft Classic 2, it was possible to calculate the layer thickness and hydrodynamic parameters such as the migration velocity.

Data analysis

From the absorbance values, the bacterial growth curves were constructed; these were modeled by applying the Logistic Model[14] present in eqn (1):

$$\mathrm{Abs}_t = \mathrm{Abs}_{\min} + \frac{\mathrm{Abs}_{\max} - \mathrm{Abs}_{\min}}{1 + \exp^{[-\mu_{\max}(t - t_i)]}} \quad (1)$$

where $\mathrm{Abs}_{\min}$ corresponds to the minimum asymptotic absorbance, $\mathrm{Abs}_{\max}$ to the maximum asymptotic absorbance, t_i to the time at which the inflection time of the logistic curve occurred (h) and $\mu_{\max}$ to the maximum specific growth rate (h^{-1}). Abs and t correspond to the absorbance and time variables, respectively.

The averages of the biosurfactant and biomass yield, the surface tension measurement and the parameters of the growth models were calculated and compared using an ANOVA, with significant differences being determined using the Tukey test. In all cases, statistical significance was assessed at the 5% level ($p \leq 0.05$). The modeling and statistical analysis of the averages were carried out with the Statgraphiscs program (Centurion XV, Version 15.2.05).

Results and discussion

Growth of *L. plantarum* Tw226 in MRS and yacon juice

A preliminary test was conducted to evaluate the growth of *L. plantarum* Tw226 in MRS broth or yacon juice at different concentrations. The growth curves are shown in Fig. 1, the direct relation between microbial growth and absorbance value in the stationary phase was used as a criterion to analyze bacterial growth under different conditions.[14] After 48 h, the absorbance values were 1.83 ± 0.03 and 1.79 ± 0.02 for growth in MRS100 and MRS75, respectively, with no significant differences observed. However, greater decreases in MRS concentration resulted in a progressive decline in absorbance after 48 h of growth (Fig. 1). This phenomenon is likely due to the limitation in nutrient availability caused by dilution.[15]

Regarding *L. plantarum* Tw226 growth in yacon juice, the maximum absorbance was equal to 0.63 ± 0.03 in the undiluted yacon juice. The 75% v/v and 50% v/v dilutions showed a maximum absorbance equivalent to that of undiluted yacon

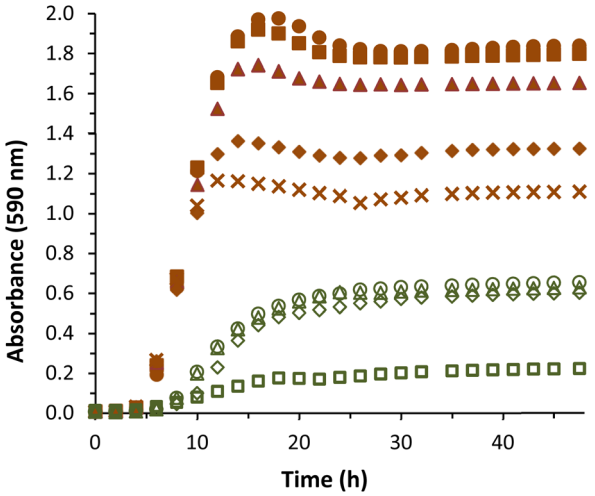

Fig. 1 Growth curves of *L. plantarum* Tw226 at 37 °C with agitation at 120 rpm. The strain was cultured in: full-strength MRS broth (●), MRS broth at 75% of its regular concentration (■), MRS broth at 50% of its regular concentration (▲), MRS broth at 25% of its regular concentration (◆), MRS broth at 12.5% of its regular concentration (×), yacon juice 100% (v/v) (△), yacon juice 75% (v/v) (○), yacon juice 50% (v/v) (◇) and yacon juice 25% (v/v) (□). All systems were adjusted at pH of 6.40.

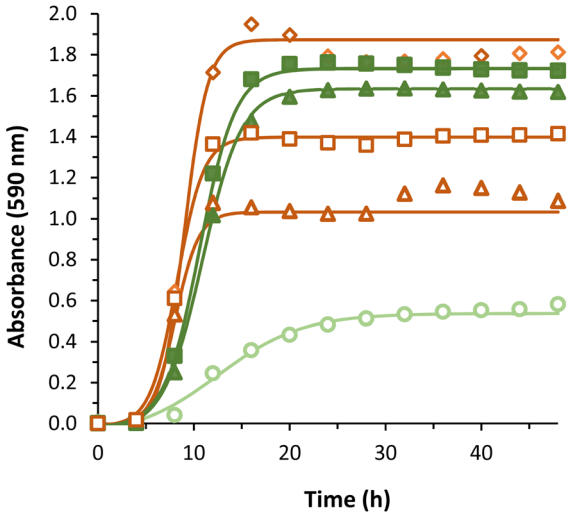

Fig. 2 Growth curves of *L. plantarum* Tw226 at 37 °C with agitation at 120 rpm. The strain was cultured in: full-strength MRS broth (◇), MRS broth at 25% of its regular concentration (□), MRS broth at 12.5% of its regular concentration (△), 75% (v/v) yacon juice (○), 75% (v/v) yacon juice supplemented with MRS broth 25% of its regular concentration (■), 75% (v/v) yacon juice supplemented with MRS broth 12.5% of its regular concentration (▲).

juice. A marked reduction in the absorbance was observed in the 25% v/v dilution of the juice. If the growth achieved using yacon juice is compared to that obtained with the MRS broth, it is observed that the absorbance at 48 h was significantly lower in all cases. Therefore, it is concluded that the nutrients provided by yacon juice are not sufficient to produce a growth like the one obtained with the traditional nutrient medium. To address nutrient deficits, systems were developed using yacon juice mixed with varying concentrations of MRS broth.

Growth in mixture of yacon juice and MRS broth

Fig. 2 shows the experimental values of the evolution of the growth of *L. plantarum* Tw226 over time and the curves adjusted to the Logistic model using as culture media MRS100, MRS25, MRS12.5, J75, MRS25J and MRS12.5J. The growth curves were effectively modeled using the Logistic model, as validated through ANOVA and correlation coefficient (R^2) analysis, which returned values ranging from 99.90% to 89.28% across all systems (Table 1). Although the model includes the Abs_{min} parameter, it was excluded from this analysis since it was near to zero.

The curve that presented the highest growth was the one in which the substrate was MRS100, followed by the curve containing yacon, MRS25J and MRS12.5J. The presence of nutrients provided by yacon juice, impacted the growth of *L. plantarum* Tw226, considering that the mix of the juice with MRS at 25%, and 12.5% resulted in statistically significant increases in Abs_{max} compared to the analogous system without yacon juice. In all systems with yacon juice, an increase in "t_i" and a decrease in "μ_{max}" was observed, this indicates an extended latency period and a reduced exponential growth rate. The increase in the latency period may be attributed to the adaptation period required by bacteria when transitioning from the inoculum, prepared in MRS broth, to the broth composed of yacon juice supplemented with MRS.[16]

Table 1 Parameters associated with modeling the growth curves of *L. plantarum* Tw226 in different nutrient media using the Logistic model[a]

	Abs_{max}	μ_{max} (h^{-1})	t_i (h)	R^2 (%)
MRS100	1.87 ± 0.03^{a}	0.82 ± 0.03^{a}	$8.99 \pm 0.15^{a,b}$	99.20
MRS25	1.40 ± 0.04^{b}	0.72 ± 0.06^{b}	$8.23 \pm 0.36^{a,c}$	99.28
MRS12.5	1.09 ± 0.10^{c}	0.83 ± 0.04^{a}	8.07 ± 0.43^{c}	98.57
J75	0.54 ± 0.06^{d}	0.23 ± 0.01^{c}	12.58 ± 0.40^{d}	99.08
MRS25J	1.73 ± 0.02^{e}	0.54 ± 0.02^{d}	10.62 ± 0.13^{e}	99.90
MRS12.5J	1.63 ± 0.05^{f}	0.50 ± 0.02^{d}	10.91 ± 0.05^{e}	99.90

[a] The means of each column with the same superscript letter do not differ significantly ($p < 0.05$). Abs_{max}: asymptotic maximum absorbance; μ_{max}: value associated with the growth rate; t_i: inflection time of the lag phase.

LAB, such as *L. plantarum*, are known for their extensive nutritional requirements, including amino acids, vitamins, purines, and pyrimidines.[17] It has been found that *L. plantarum* requires six key amino acids (isoleucine, leucine, valine, tyrosine, methionine, and phenylalanine) and nucleotides precursors for optimal growth in milk. While mineral salts are not essential, they do stimulate growth. In contrast yacon roots concentrate predominantly contains L-arginine, L-glutamic acid, L-proline, L-aspartic acid, and asparagine.[18,19] Additionally, the protein concentration in yacon juice is lower compared to MRS broth. Yacon juice contains only plant proteins, whereas MRS broth includes beef extract, yeast extract, and proteose peptone, which are rich sources of proteins and vitamins. In a previous

study, Palavecino Pripch *et al.* informs a protein concentration in yacon juice of 1.09% w/w in dry bases and if this value is express in wet basis reach just 0.63% w/w.[10] Therefore, the combination with MRS broth, even at a concentration of 12.5% v/v, would supplement the amino acids, vitamins and nucleotides precursors lacking in yacon juice, promoting the grow of *L. plantarum* Tw226.

Formerly, it was found that biosurfactant production by *L. plantarum* Tw226 was not achieved under optimal biomass growth conditions.[7] However, enough bacterial biomass is necessary for significant BS production. Therefore, based on the show results, it was decided to continue the studies with the "MRS25J" system. This decision was primarily due to its Abs_{max} values being closest to those obtained with MRS broth despite not having the optimal kinetic parameters (t_i and μ_{max}).

Biomass and biosurfactant production in mixture culture media

The dry biomass production reached a yield of 1.11 ± 0.11 g L^{-1} and 1.10 ± 0.06 g L^{-1} using MRS100 and MRS25J, respectively (Fig. 3, panel A). Although there were differences in Abs_{max} between MRS100 and MRS25J (Table 1), the dry biomass yield per liter of nutrient medium showed no significant differences, confirming that MRS25J is suitable for the growth of *L. plantarum* Tw226. Aditionally,the pH values at the end of the fermentation was 3.65 for MRS100, 3.29 for MRS25J, 4.25 for MRS25 and 4.30 for J. The pH values during acid lactic fermentation are closely related to growth, confirming that in all nutrients media *L. plantarum* Tw226 was capable of fermenting all nutrients media and there was a higher production of organic acids in MRS100 and MRS25J, which is evident based on the lower pH values.

The production of biosurfactant per liter of culture medium was 0.18 ± 0.02 and 0.11 ± 0.03 g L^{-1} for MRS100 and MRS25J, respectively, presenting significant differences. These yields were higher than those obtain with MRS25 and J, which reached a concentration of 0.03 ± 0.01 g L^{-1} of medium.

It was reported that the production of biosurfactant from *L. plantarum* increases in presence of higher concentrations of nitrogen and carbon sources.[20] As well, in an early study was found that for *L. paracasei* peptone is a very important nutrient source for biosurfactant production.[12] Based in this, the lack of nutrients on J and MRS25 could explain the lower production yield.

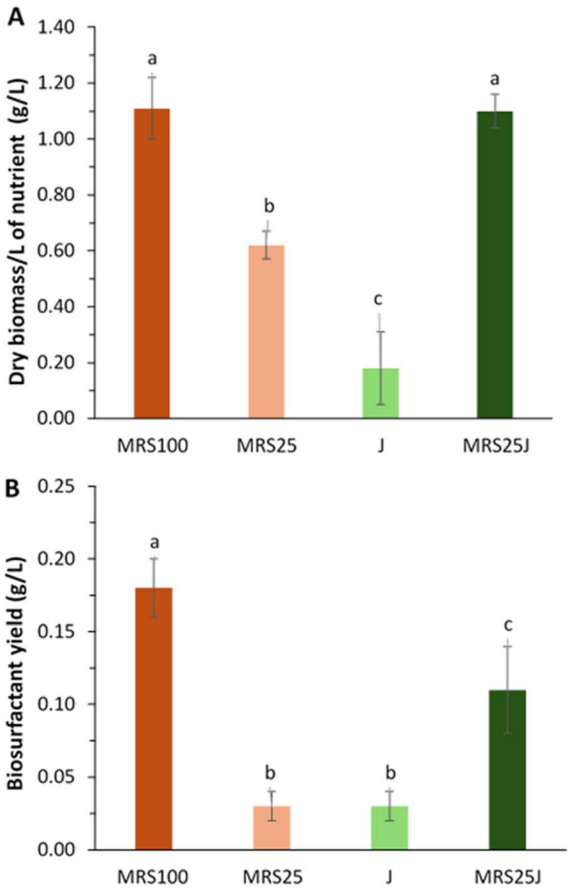

Fig. 3 Dry biomass of *L. plantarum* Tw226 per liter of culture medium (panel A) and biosurfactant yield per liter of culture medium (panel B).

Chemical characterization: fourier-transform infrared spectroscopy

Fig. 4 presents the FTIR spectra of the biosurfactant obtained from all the systems. The spectra of MRS25J and MRS100, reveal characteristic peaks of amide bond stretches and vibrations, typical of peptide bonds: amide I at 1653.3–153.6 nm, amide II at 1540–1542 cm^{-1}, and amide III at 1456.4–1455.7 cm^{-1}, 1399.2–1398.2 nm, and 1239.2–1239.9 cm^{-1}.[21] The amide I group is associated with C=O bond vibrations and, to a lesser extent, N–H bond vibrations. The amide II group combines N–H bond stretches and C–N stretch vibrations, with minor contributions from C–O, C–C, and N–C bonds. The amide III group involves a phase combination of N–H bending and C–N stretch vibrations, with small contributions from C–O bond flexion and

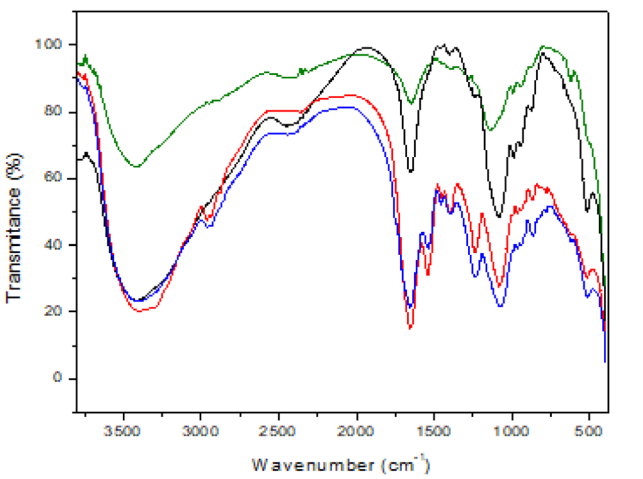

Fig. 4 FTIR spectrum of biosurfactants produced with MRS broth (—), yacon juice supplemented MRS broth at 25% of the regular concentration (—), yacon juice 75% v/v (—), MRS broth at 25% of the regular concentration (—).

C–C bond tension vibrations.[22] These findings indicate the presence of protein compounds in both biosurfactant samples. In contrast, the analyses of biosurfactant produced by the J and MRS25 systems, shown in Fig. 4 do not exhibit protein-related stretches.

Fig. 4 shows, for MRS25J and MRS100, peaks at 3413.2 and 3396.8 cm^{-1}, corresponding to the O–H bond stretch, and weak peaks at 2962 and 2957.9 cm^{-1}, corresponding to C–H bonds. Additionally, the characteristic peaks around 863.8 and 866.6 cm^{-1} indicate the presence of β-D-glycosidic bonds, while the peaks at 1083.3 and 1083.7 cm^{-1} correspond to the typical vibrations of the C–O–C group, commonly referred to as the PII band.[23,24] These peaks are characteristic of polysaccharides, suggesting that these two biosurfactant samples contain both protein and polysaccharide fractions. Similar peaks are observed in Fig. 4 (panels C and D), indicating that the compounds obtained from MRS25 and J systems exhibit similar polysaccharide functional groups.

In conclusion, the results indicate that the functional groups for the biosurfactant obtained with MRS100 and with MRS25J are similar. Also, both biosurfactants, presents a typical profile like others obtained from LAB in different conditions.[25,26] However, the MRS25 and J systems showed no presence of protein functional groups. Additionally, the absence of protein peaks in the BS produced by MRS25 and J is likely related to the lack of amino acids in the culture medium. It is possible that the few proteins available were needed to produce metabolites related to growth rather than to contribute to the chemical composition of the BS.

Surface tension

The surfactant activity of the products obtained was analyzed by measurement of the equilibrium surface tension of 5 g L^{-1} solutions made with biosurfactants produced with MRS100 (BSMRS) and MRS25J (BSY). The equilibrium surface tension refers to the tensioactive capacity of the biosurfactant; the lower it is, the more effective the surfactant activity. In Fig. 5, the evolution of the surface tension over time is shown. It can be seen three sections, an initial phase, where a sharp drop in surface tension is recorded, corresponding to a period in which the BS molecules move and position themselves at the interface. Then, the region where there is a gradual decrease in surface tension, in which the biosurfactant molecules settle at the interface, and finally, a region where the surface tension becomes constant, reaching the equilibrium.

The surface tension of water was 71.01 mN m^{-1}, and the equilibrium surface tension for the product obtained through the fermentation of MRS broth was 42.99 ± 1.29 mN m^{-1} and 43.48 ± 0.68 mN m^{-1} for the one obtained through the fermentation of yacon juice additionated with 25% of MRS, without significant differences between them. This assay confirms that the compounds produced can be considered a biosurfactant since they produce a reduction in surface tension higher than 8 mN m^{-1}.[12] The results obtained are typical for biosurfactants produced by LAB. A biosurfactant from *Lactobacillus plantarum* subsp. *plantarum* PTCC 1896

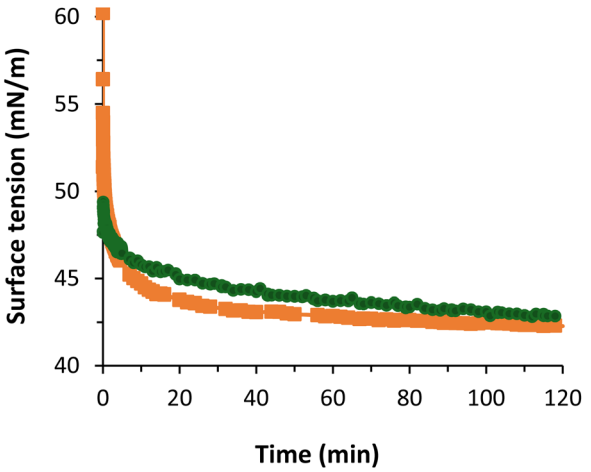

Fig. 5 Surface tension measurement of solutions of biosurfactant produced by *L. plantarum* Tw226 through fermentation of MRS broth (■) and yacon juice supplemented with 25% of MRS broth (●) in a concentration 5 g L^{-1}.

presented an equilibrium surface tension between 44.77 and 47.31 mN m^{-1} depending on the incubation time and the nutrient medium used, and the surface tension of the biosurfactant from *Lactiplantibacillus plantarum* OL5 was 37.2 mN m^{-1}.[27,28]

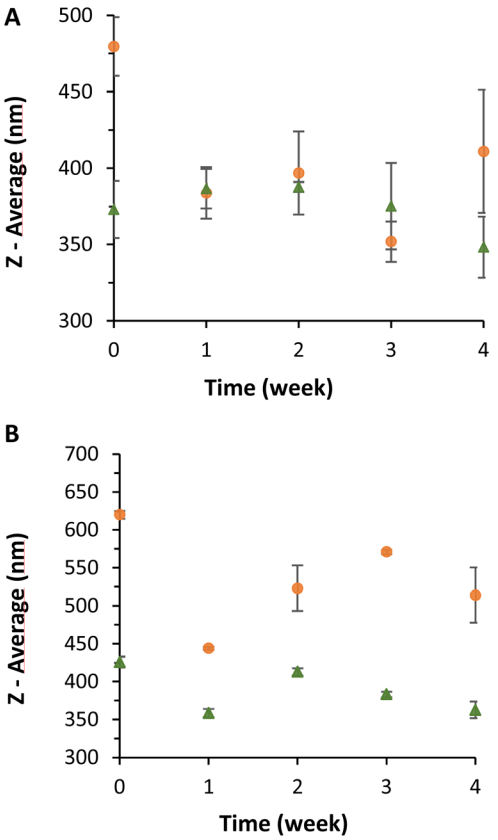

Fig. 6 Evolution of the Z-Average in center of the emulsions as a function of time at 25 °C. Emulsion with cinnamon bark essential oil (panel A). Emulsion with lemongrass essential oil (panel B). Emulsion emulsified with BSY (▲) or BSMRS (●).

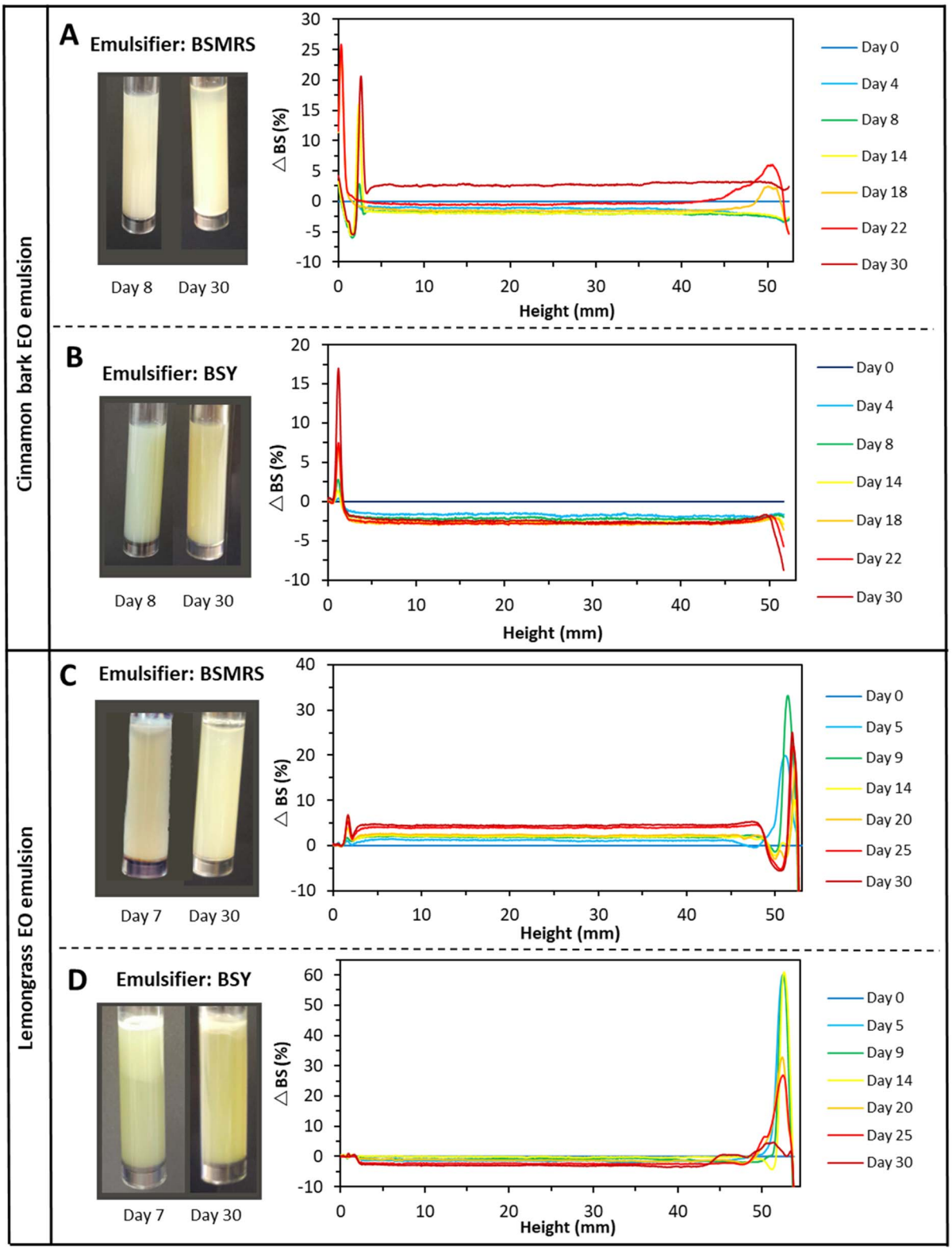

Fig. 7 Evolution of the increase in the backscatter of emulsions stored at 25 °C throughout the system for 30 days and photos of the emulsions stored for 7/8 and 30 days. Emulsion with cinnamon bark essential emulsified with biosurfactant produced with MRS broth (panel A) and biosurfactant produced with yacon juice 75% (v/v) supplemented with MRS broth at a 25% of its regular concentration (panel B). Emulsions with lemongrass essential oil emulsified with biosurfactant produced with MRS broth (panel C) and biosurfactant produced with yacon juice 75% (v/v) supplemented with MRS broth at a 25% of its regular concentration (panel D).

Essential oil emulsions characterization

BSMRS and BSY were used as emulsifiers for oil-in-water emulsions with both cinnamon bark essential oil (CBEO) and lemongrass essential oil (LGEO). Fig. 6 illustrates the evolution of the Z-average, which is related to the size of oil droplets in the emulsions over one month of storage at 25 °C. All the emulsions formulated can be classified as mini-emulsions as their average droplet diameter is between 100–1000 nm.[29] This characteristic is also reflected in the opaque appearance of the emulsions (Fig. 6), a phenomenon that happens in emulsion with a droplet size higher than 100 nm.[30]

Particularly, the CBEO emulsions emulsified with BSMRS and BSY exhibited initial Z-average of 479.6 ± 19.6 nm and 372.8 ± 18.7 nm, respectively, reaching 410.9 ± 40.0 nm and 348.2 ± 19.9 nm over one month. Despite fluctuations in Z-average during storage, these changes were not statistically significant ($P > 0.05$), and significant differences were observed only in the initial droplet size (week 0) which were smaller for the emulsified with BSY.

The LGEO emulsions emulsified with BSMRS and BSY presented initial droplet sizes of 620.3 ± 6.5 nm and 425.9 ± 35.7 nm, respectively, reaching 514.0 ± 36.6 nm and 362.6 ± 11.0 nm at the end of storage. The emulsion with LGEO emulsified with BSY consistently maintained a significantly smaller droplet size through the storage period compared to its counterpart emulsified with BSMRS.

Essential oil emulsions stability

The study of destabilization mechanisms through multiple light scattering is illustrated in the Fig. 7, in wich the high = 0 mm represents the bottom of the tube. Initially, all samples were homogeneous, as indicated by the linear variation of backscattering at zero time.

Emulsions containing CBEO exhibited an increase in ΔBS% at the bottom, indicative of a precipitate formation, which is visible to the naked eye. This precipitation took place on day 12 of storage in both cases and reach a maximum peak thickness of 0.66 mm and 0.33 mm for the emulsified with BSMRS and BSY respectively. Additionally, emulsions with BSMRS showed an increase in ΔBS% at the surface from day 17 of storage, suggesting destabilization due to creaming, this mechanism was not present in the emulsion with BSY. Considering the glycolipopeptic nature of the biosurfactants studied, the precipitation could be related to the formation of an insoluble complex because of the reaction between the surfactant and certain components of the essential oil. In a previous study it was found that CBEO contained terpenes, phenolic, esters and aromatic aldehydes,[31] being this compound able to bind through covalent crosslinking with protein amines.[32]

Furthermore, the emulsion with BSY presented a decrease of the ΔBS%, around −2.8%, along the center of the emulsion, phenomenon that used to be related with coalescence. However, as can be seen previously (Fig. 7), the Z-average in the center of the emulsion did not exhibit significant changes. It is worth noting that Turbiscan technology can detect coalescence 50 times earlier than the naked eye.[33] Also, it can be determined that the emulsions showed an acceptable stability according to Muñoz *et al.*, who state that a ΔBS% value below 5% is indicative of stability in a sample.[34] Therefore, even coalescence is not a reversible destabilization mechanism, it did not affect the droplet size significantly in the period studied.

Regarding emulsions with LGEO, emulsified with either BSY or BSMRS, demonstrated an increase in ΔBS% associated with creaming starting from the second day of storage, reaching the maximum point at the seventh day in both cases. The peak thickness on the top of the emulsion for the emulsion with BSY was of 1.61 mm, while the emulsified with BSMRS reached a value of 0.55 mm. Also, it is remarkable that the destabilization process was faster in the first three days of storage, as the migration velocity calculated through a linear regression on this section ($R^2 = 1.00$), is of 0.023 mm h^{-1} for the emulsion emulsified with BSY *versus* 0.010 mm h^{-1} for the emulsified with BSMRS. So, it looks like the creaming process of the emulsion with BSY happened faster than the one emulsified with BSMRS.

To summarize, according to the proposed analysis, the BSY seems to be a better stabilizer for CBEO emulsions than the BSMRS, considering that with the first there is no creaming and the precipitation is lower. But, in the case of the LGEO emulsion emulsified with BSY the creaming is faster than using BSMRS. For this reason, it could be concluded that the emulsifier capability depends on the essential oil used. Also, even both BS present the same functional groups, they do not show the same properties. Campolo *et al.* found that, for emulsions with different synthetic surfactants and different essential oils, the interaction between those ingredients plays an essential role and this bionomy is crucial for the drop size, Z-potential and polydispersity index determining the stability over the time.[35]

Essential oils emulsions can be used as flavored or preservative in foods. Minimal inhibitory concentrations (MIC) lower than 2500 ppm have been reported as preservatives against pathogenic or opportunistic microorganisms. For *Listeria monocytogenes*, *Escherichia coli* O157 : H7 and *Salmonella enteritidis* were informed concentrations of 600 ppm as MIC of cinnamon bark oil and was lower when it was incorporated in an emulsion.[36] Moreover, lemongrass essential oil emulsions presented a MIC of 469 ppm against *Zygosaccharomyces bailii* and 400 ppm against *Staphylococcus aureus.*[31,37] Even though the systems showed destabilization processes at the studied concentration of 2500 ppm, it should be noted that this emulsion can be used in a diluted form. Also, the formulations and homogenization process could be optimized to reach a higher stability.[30,35,37]

Conclusion

It was possible to obtain BSY, a biosurfactant produced through fermentation of a culture medium based on yacon juice (75% yacon juice/25% MRS broth) using *L. plantarum* Tw266. It presented similar functional groups and similar tensioactivity tothan BSMRS, the biosurfactant produced with MRS broth in the regular concentration. Even the BSY yield per liter of medium wasis lower than the one for BSMRS medium, the

reduction of the percentage of MRS and the use of an underexploited root as yacon gives both, economic and environmental benefits. The application of BSY as emulsifier of essential oils depends on the oil selected. The BSY seems to be a better stabilizer for cinnamon bark oil emulsions than the BSMRS, but in the case of the lemongrass emulsion stabilized with BSY the creaming is faster than using BSMRS. This study expands knowledge on the production of biosurfactants using alternative media, as well as the interaction between the essential oils/biosurfactant binomial produced by LAB. Therefore, this expands the uses of the yacon tuber by placing it within a biomolecule production cycle, thereby increasing its recognition and offering potential economic benefits to regional neglected agro-productions. In future studies would be interesting to analyze the antimicrobial, antioxidant and antibiofilm capability of BSY and the emulsions produced with it, to amplify it applications as a natural food additive.

Conflicts of interest

There are no conflicts to declare.

Data availability

Data will be made available on request.

Acknowledgements

This study received funding from the University of Buenos Aires. (grant number 20020220200033BA and 20020220400240BA), the National Research Council of Argentina, CONICET (grant number: 11220200100 114CO) and the National Agency of Scientific and Technical Research (grant number: PICT-2020-00437).

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