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Adding value to secondary streams of corn wet milling industry

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ABSTRACT

Corn steep liquor (CSL) is spontaneously fermented, mainly by thermophilic *Bacillus* strains, producing lipopeptide biosurfactants. These biosurfactants can be extracted with ethyl acetate at the same time that an aqueous phase is generated (named CSL-plus). The results showed that the biosurfactant extraction produced a liquor with a reduced content of fats and minor changes in the micronutrients concentration. The decrease in the fatty acid content is due to the extraction of biosurfactants and free fatty acids contained in the biosurfactant extract, composed mainly of C16 and C18 fatty acids. Therefore, it can be concluded that the extraction of biosurfactants gives a nutritional supplement (CSL-plus) with lower content of fats, improving the nutritional properties of CSL and consequently added value is given to the water stream generated. Moreover, it was demonstrated that the biosurfactant extract from CSL could be used to improve the solubilization of chicken broth cubes.

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KEYWORDS
Corn stream; nutritional supplement; biosurfactants; circular economy

AÑADIENDO VALOR A LAS CORRIENTES SECUNDARIAS DE LA INDUSTRIA DE FRACCIONAMIENTO DEL MAÍZ POR VÍA HÚMEDA

RESUMEN

El licor de lavado de maíz (CSL) es fermentado espontáneamente, principalmente por cepas termófilas de *Bacillus*, produciendo biosurfactantes lipopéptidos. Estos biosurfactantes se pueden extraer con acetato de etilo al mismo tiempo que se genera una fase acuosa (denominada CSL-plus). Los resultados mostraron que la extracción del biosurfactante produjo un licor con un contenido reducido de grasas y cambios menores en la concentración de micronutrientes. La disminución del contenido de ácidos grasos se debe a la extracción de biosurfactantes y ácidos grasos libres contenidos en el extracto biosurfactante, compuesto principalmente por ácidos grasos C16 y C18. Por tanto, se puede concluir que la extracción de biosurfactantes produce un suplemento nutricional (CSL-plus) con menor contenido de grasas, mejorando las propiedades nutricionales del CSL, y en consecuencia se le da un valor añadido a la corriente acuosa generada. Además, se demostró que el extracto biosurfactante de CSL podría usarse para mejorar la solubilización de cubos de caldo de pollo.

PALABRAS CLAVE
corriente de maíz; suplemento nutricional; biosurfactantes; economía circular

1. Introduction

In the food industry are produced an important number of secondary streams that treated properly can be converted in products of industrial interest, promoting a circular economy and the use of secondary raw materials (Miranda et al., 2021). An example of this is the use of corn steep liquor (CSL) to obtain biosurfactant extracts (Vecino et al., 2014, 2015), which have been demonstrated possess an enormous use in different sectors, including cosmetic, pharmaceutical and food industry (Rincón-Fontán et al., 2018, 2019, 2020). Hence, it has been demonstrated that biosurfactant extract obtained from CSL promote the growth of probiotic bacteria in liquid yogurts (López-Prieto et al., 2019) and it could be a good preservative for food industry against bacteria (López-Prieto et al., 2019a). Regarding other biosurfactants, Dilarri et al. (Dilarri et al., 2016) observed that the rhamnolipids produced by *Pseudomonas aeruginosa* LBI reduced the microbial contamination of different fruits when these were submerged in a solution of 1 g/L of rhamnolipids for five minutes, in comparison with submerging the same fruits in tap water or electrolyzed water following the same washing procedure. The addition of this biosurfactant increased the shelf-life of fruits in two days. In addition, Pirog et al. (Pirog et al., 2019) have used biosurfactants produced by *Nocardia vaccinii* IMV B- 7405 to increase the shelf-life of different vegetables including in the study red and green tomatoes, cucumbers and squashes. It was observed that the treatment of vegetables with the biosurfactant solution at, concentrations of 0.25 and 0.5 g/L, was more effective than washing them with tap water.

Regarding the biosurfactant extract obtained from CSL, this was defined by Rodriguez-López et al. (Rodríguez-López et al., 2016) as a multifunctional extract that possesses surfactant and antioxidant properties with a EC_{50} of 4.02–13.92 g/L, depending on the extraction process, being 2.54 g/L the EC_{50} of BHT (a synthetic compound with antioxidant properties commonly used in food industry) (Barbosa-Pereira et al., 2013). This biosurfactant extract content phenolic compounds and lipopeptides with surfactant properties, which were characterized in previous works (Rodríguez-López et al., 2020, 2016). The phenolic compounds include protocatechuic acid, vanillic acid, coumaric

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acid, ferulic acid, sinapic acid and quercetin. Protocatechuic acid being the major phenolic compound, among them, comprising 32% (Rodríguez-López et al., 2016); whereas the lipopeptide biosurfactant is composed of C16 and C18 fatty acids and amino acids compromising glutamic acid/glutamine, aspartic acid/asparagine, glycine, alanine, arginine, proline and leucine/isoleucine (Rodríguez-López et al., 2020).

However, after the extraction of biosurfactants, it is important to evaluate the nutritional variations produced in CSL as one of the main applications of this secondary stream could be its use as nutritional supplement for animal feeds. The extraction with ethyl acetate should promote the separation of biosurfactants from CSL, whereas the water-soluble components, contained in the aqueous phase of the raw CSL, should remain in the aqueous phase. The aim of this work was to evaluate if the extraction of biosurfactants from CSL modify its nutritional properties and evaluate the capacity of the biosurfactant extract under evaluation as dissolving agent, of chicken broth cubes, for its application as stabilizing agent in food industry, by promoting a circular economy framework and the valorization of secondary streams from food industry.

2. Materials and methods

2.1. Extraction of biosurfactants from corn steep liquor

Corn steep liquor (CSL) was provided by FeedStimulants company. CSL was diluted up to 50 g/L, in order to avoid emulsion formation during the extraction process, and then was subjected to liquid-liquid extraction with ethyl acetate using an aqueous stream-organic solvent relationship of 1:3 (*v:v*). The extraction was carried out at room temperature (22 ± 1°C) during 45 min under agitation at 150 rpm following the protocol published in previous works (Rodríguez-López et al., 2016). After reaching the equilibrium, the liquid and organic phase were separated in a decanter funnel overnight, and the organic phase was vacuum distilled to remove the ethyl acetate, obtaining a biosurfactant extract and an aqueous phase which was lyophilized, with a lyophilizer Telstar LyoQuest (Spain), in order to carried out different nutritional analysis. The ethyl acetate was recovered and used in the following extraction process.

2.2. Characterization of biosurfactant extract

After liquid-liquid extraction, the biosurfactant extract obtained from CSL was subjected to a validation analysis, measuring its surfactant capacity. This validation analysis is important as CSL is a residual stream where biosurfactants are produced in non-controlled conditions. Therefore, the biosurfactant extract was diluted in water up to concentration of 1 g/L and the surface tension and critical micellar concentration (CMC) of the extract was determined using a Krüss K20 EasyDyne tensiometer with a 1.9 cm platinum Wilhelmy plate at room temperature, following the procedure established in previous works (Vecino et al., 2014).

2.3. Analysis of corn steep liquor before and after biosurfactant extraction

After the extraction of biosurfactants, the aqueous phase of CSL was characterized based on elemental analysis, fatty acid analysis, micronutrients and Fourier-transform infrared spectroscopy (FTIR). Additionally, raw CSL was also characterized for comparative purposes. Raw corn steep liquor was designated as CSL and the corn steep liquor free of biosurfactants was named CSL-plus. Figure 1a shows a flow diagram of the extraction process of biosurfactants from raw CSL and Figure 1b shows real images of the aqueous phase (CSL-plus) and the organic phase containing the biosurfactants during the separation of phases. Moreover, Figure 1b includes a zoom of the interphase which is removed.

2.3.1. Elemental analysis

Lyophilized CSL and CSL-plus was subjected to elemental analysis (C, N, H, S) using an elemental analyzer (Fisons Carlo Erba EA-1108 CHNS-O, LabX, Midland, ON, Canada). Acetanilide and sulfanilamide standards were interspersed between samples in order to check the proper functioning of the equipment. Also, the biosurfactant extract obtained from

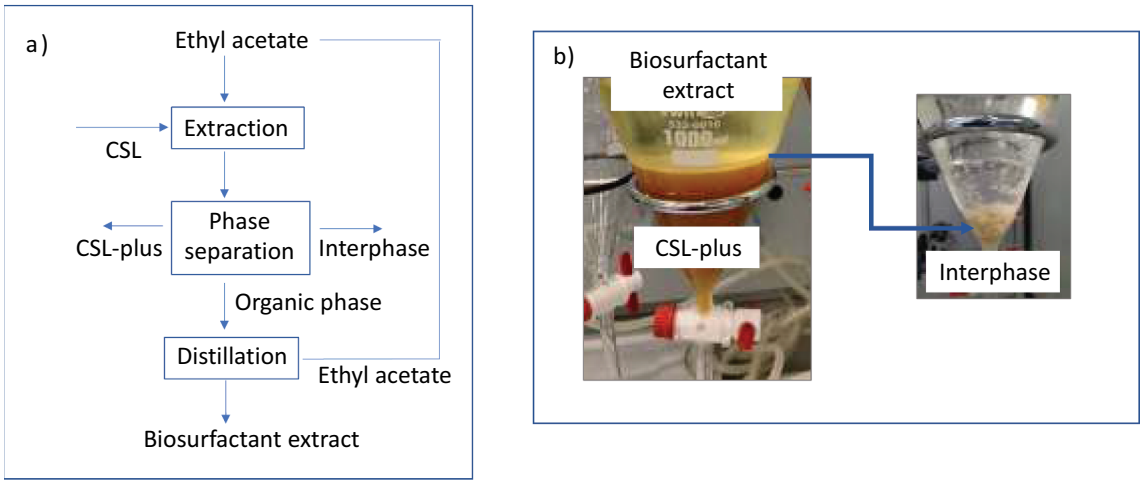

Figure 1. Flow diagram carried out for the extraction process of biosurfactant extract with ethyl acetate (a) and images of the different fractions generated during the separation of phases (b).

Figura 1. Diagrama de flujo para llevar a cabo el proceso para la extracción del extracto de biosurfactante con acetato de etilo (a) e imágenes de las distintas fracciones generadas durante la separación de fases (b).

raw CSL was subjected to elemental analysis following the same procedure.

2.3.2. Fatty acid analysis

Fatty acid content of biosurfactant extracts as well as lyophilized CSL and CSL-plus was determined by gas chromatography coupled to a mass spectrometer (CG-MS, Bruker Scion 451-GC), following the ISO-12,966-3:2009 law, based on the formation of methyl esters using trimethyl sulfonium hydroxide (TMSH), which is a method used to determine animals and vegetables fats and oils. Therefore, samples (10 mg) of CSL or CSL-plus were diluted in 500 µL of tert-butyl methyl ether (TBME) and 250 µL of TMSH, and then 1 µL of CSL and CSL-plus samples were injected in split less mode in a GS-Mass spectrometer equipped with a DB-WAX column (30 m x0.25 mm i.d. × 0.25 µm film thickness) at 250°C using helium as carrier, with a flow rate of 1 mL/min at 4°C. The electron impact ionization was carried out over a m/z range of 50–400 and 70 eV. Supelco 37 Component FAME mix from Sigma-Aldrich was used as control.

2.3.3. Minerals and micronutrients

Micronutrients including Ca, Cu, Fe, K, Mg Mn, Na, P and Zn were analyzed in diluted CSL and CSL-plus with an Optical Emission Spectrometer (OES Optima 4300DV) from PerKin Elmer. Samples were previously filtered (0.2 µm) and acidified (2% HNO_3).

2.3.4. Lactic acid

Lactic acid was determined in lyophilized raw CSL and CSL-plus by a chromatography system (Dionex ICS-3000) supplied by Thermo-Fisher Scientific. Moreover, an IonPac AS11-HC column (250 x 4 mm) and a conductivity detector were used for the determination and quantification of lactic acid (Dionex). The mobile phase was NaOH 50 mM at a flow rate of 1 mL/min. Before ion chromatography analyses, the samples were filtered using a 0.22 µm filter.

2.3.5. Fourier-transform infrared spectroscopy (FTIR)

CSL and CSL-plus were subjected to FTIR analysis with a Nicolet 6700 FTIR spectrometer (Thermo Scientific). For the analysis, 1 mg of lyophilized CSL and CSL-plus was ground with 10 mg of potassium bromide and pressed (7500 kg for 30 s) to produce translucent pellets, which were measured in a range of 400–4000 cm^{-1}, with a resolution of 4 cm^{-1} and an average range of 32 data scanning.

2.4. Application of biosurfactant extract from corn steep liquor as solubilizing agent

Moreover, in order to evaluate the solubilization properties of biosurfactant extract obtained from CSL, a cube of chicken broth (Avecrem-Gallina Blanca), composed by fats, carbohydrates, dietary fiber and proteins, was dissolved in 100 mL of biosurfactant extract solution (1 g/L) in Milli-Q water under agitation at 150 rpm during 5 and 10 min at room temperature (22 ± 1°C) and compared with a control, Milli-Q in absence of biosurfactant extract. In order to know the capability of the biosurfactant to increase the solubilization of chicken broth cubes, samples were centrifuged at 5000 rpm, 30 min at 4°C, and the dry weight of the precipitated was obtained after drying samples in oven at 105°C during 48 hours. Experiments were carried out by triplicate.

3. Results and discussion

3.1. Biosurfactant extract characterization

The biosurfactant extract obtained from CSL was composed by 1.25 ± 0.3% N, 40.1 ± 2.5% C, 6.8 ± 0.6% H, being the content of protein in the biosurfactant extract of 7.8% (Mariotti et al., 2008). This composition was in consonance with the composition of the biosurfactant extract obtained from different lots and from different brands of CSL (Rodríguez-López et al., 2020, 2016), demonstrating a high reproducibility despite that CSL is a secondary stream spontaneously fermented by different microorganisms, without controlling the operational conditions. The biosurfactant extract obtained from CSL, after different steeping process, possesses a quite homogeneous composition. Regarding the capacity to reduce the surface tension, the biosurfactant extract obtained from CSL, in the current work, was able to reduce the surface tension of water up to 39.3 ± 0.5 mN/m with a CMC of 320.2 ± 12.9 mg/L which was also in consonance with the results obtained in previous works (López-Prieto et al., 2020, 2019b).

3.2. Analysis of corn steep liquor before and after biosurfactant extraction

Table 1 shows the elemental composition of lyophilized raw CSL before (CSL) and after biosurfactant extraction (CSL-plus) , observing that both possess a similar elemental composition regarding C, N, H, and S. Hence, the extraction of the biosurfactant almost did not vary the protein concentration of both CSL, as the concentration of proteins in the biosurfactant extract was 7.8%, which was low in comparison with the concentration of proteins in CSL and CSL-plus (around 47–48%). However, the biosurfactant extract possess a slightly higher concentration of C than CSL and CSL-plus due to that CSL posseses an important amount of minerals and inorganic salts as well as lactic acid, that were not extracted during the separation process of the biosurfactant. The concentration of lactic acid in CSL was around 186 g/kg, which remained invariable after the extraction of biosurfactant. In addition, it was observed that during the extraction process, after the separation of phases, a small interphase is formed, between the aqueous and the organic phase, which is discharged (Figure 1b).

Table 1 also includes the composition in micronutrients (Ca, Cu, Fe, K, Mg Mn, Na, P and Zn) in CSL and CSL-plus, observing negligible changes in their composition except for the Ca content that decreased about 37.9% in CSL-plus in comparison with CSL, although the concentration of Ca in both nutritional supplements was low, 7.0 mg/kg and 4.3 mg/Kg for CSL and CSL-plus, respectively. The percentual differences regarding other micronutrients between CSL and CSL-plus was lower than 10%. These values were measured in the diluted CSL and CSL-plus, before and after extraction of biosurfactant.

Concerning the fatty acid content, it was observed important differences between CSL and CSL-plus. Figure 2 shows the relative abundance of fatty acid in CSL (Figure 2a) and CSL-plus (Figure 2b). For comparative purposes, in Figure 2 was also included the spectrum of fatty acid for the biosurfactant extract corroborating the presence of C16 and C18 fatty acids (Figure 2c). The fatty acids of CSL was composed by 22.7% of methyl

Table 1. Composition of the raw CSL and CSL-plus used in this study*.

Tabla 1. Composición del CSL inicial y CSL-plus usado en este estudio*.

Analysis	Units	CSL	CSL-plus
C	%	37.54 ± 0.15	37.45 ± 0.12
N	%	7.60 ± 0.20	7.67 ± 0.10
H	%	5.36 ± 0.99	5.93 ± 0.17
S	%	0.85 ± 0.10	0.77 ± 0.10
Protein	%	47.48 ± 0.28	47.93 ± 0.25
Ca	mg/kg	7.00 ± 0.20	4.35 ± 0.10
Cu	mg/kg	0.18 ±0.05	0.17 ± 0.01
Fe	mg/kg	2.16 ± 0.10	2.33 ± 0.20
K	mg/kg	849.56 ± 5.30	933.65 ± 4.70
Mg	mg/kg	227.92 ± 1.20	245.71 ± 1.15
Mn	mg/kg	1.09 ± 0.10	1.19 ± 0.10
Na	mg/kg	126.00 ± 0.70	125.88 ± 0.28
P	mg/kg	404.40 ± 1.28	430.11 ± 1.10
Zn	mg/kg	4.03 ± 0.11	4.34 ± 0.22
Lactic acid	g/kg	185.88 ± 0.99	185.55 ± 1.10

*Data of elemental analysis, protein and lactic acid composition are referred to the lyophilized CSL and CS-plus, whereas minerals or micronutrients are referred to the diluted CSL and CSL-plus (20 times).

*Los datos del análisis elemental, de las proteínas y de la composición del ácido láctico se refieren a los CSL y CS-plus liofilizados, mientras que los minerales o micronutrientes se refieren a los CSL y CSL-plus diluidos (20 veces).

palmitate, 2.6% methyl stearate, 20.8% of oleic acid methyl ester, 51.8% of 9,12-Octadecadienoic acid methyl ester, and 2.0% of linolenic acid methyl ester; whereas CSL-plus reduced the concentration of fatty acids about 96.7% detecting only methyl palmitate. Additionally, Rodríguez-López et al. (Rodríguez-López et al., 2020) demonstrated that the biosurfactant extract from CSL was composed of 49.1% free fatty acids; 25% lipopeptides, and 21% of phospholipids, although these percentages could vary a little bit depending on the precise separation processes between interface and organic phase and the grade of fermentation of CSL.

On the other hand, Figure 3 shows the FTIR analysis for CSL and CSL-plus (Figure 3a), the interphase observed during the extraction process (Figure 3b), and the biosurfactant extract (Figure 3c).

In Figure 3a is included a comparative of CSL and CSL-plus, observing that both possess a similar composition with a percentage of similarity of 87.7%. Moreover, Figure 3b shows the interphase obtained after biosurfactant extraction which is discharged. This interphase possesses an intermediate profile, regarding functional groups, between the CSL and the biosurfactant extract (Figure 3c) with a percentage of similarity of 65.3% with CSL. Regarding the FTIR analysis of the biosurfactant extract obtained from CSL this possess only a similarity of 18.5% with raw CSL, demonstrating that the extraction with ethyl acetate was highly selective for the biosurfactant extract. Pure fatty acids have strong absorption bands between 3000 and 2800 cm^{-1}, 1680 and 1775 cm^{-1}, and also in the fingerprint region of 1500 and 700 cm^{-1} (Koca et al., 2007). Some authors (Karoui et al., 2005; Koca et al., 2007) have reported that the increases in carbon chains and molecular weights of fatty acids resulting higher relative intensities of C-H stretching groups, which are most obvious in the symmetric C-H stretching region; whereas the band in the 3300 to 2500 cm^{-1} regions, correspond to the hydrogen-bonded O-H of most carboxylic acids, which are present not only in fatty acids but also in peptides and proteins. On the other hand, the spectrum of biosurfactant extract showed higher intensity between 3500 and 3100 cm^{-1} as the result of N-H and O-H stretching indicative of amine and hydroxyl groups related with the peptide fraction of the biosurfactant extract. In the FTIR spectra was signalized with a red circle the signal corresponding to the stronger absorption band corresponding to fatty acids, observing that this was smaller in the biosurfactant extract and higher in the interphase. This fact corroborated that an important fraction of the fatty acids contained in raw CSL were lost in the interphase because the biosurfactant extract, contained in CSL, produced a small emulsion with the fatty acids between the aqueous and the organic phase. This was also in consonance with the fatty acid observed in Figure 2, for CSL, CSL-plus and biosurfactant extract.

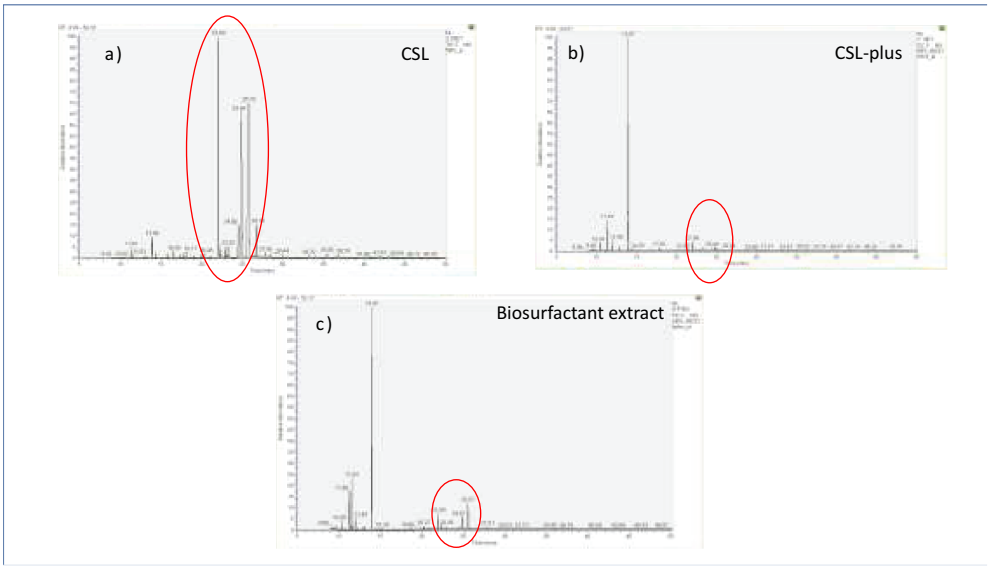

Figure 2. Mass spectrum of fatty acids for CSL (a), CSL-plus (b), and biosurfactant extract (c).

Figura 2. Espectro de masas de los ácidos grasos de CSL (a), CSL-plus (b), y extracto de biosurfactante (c).

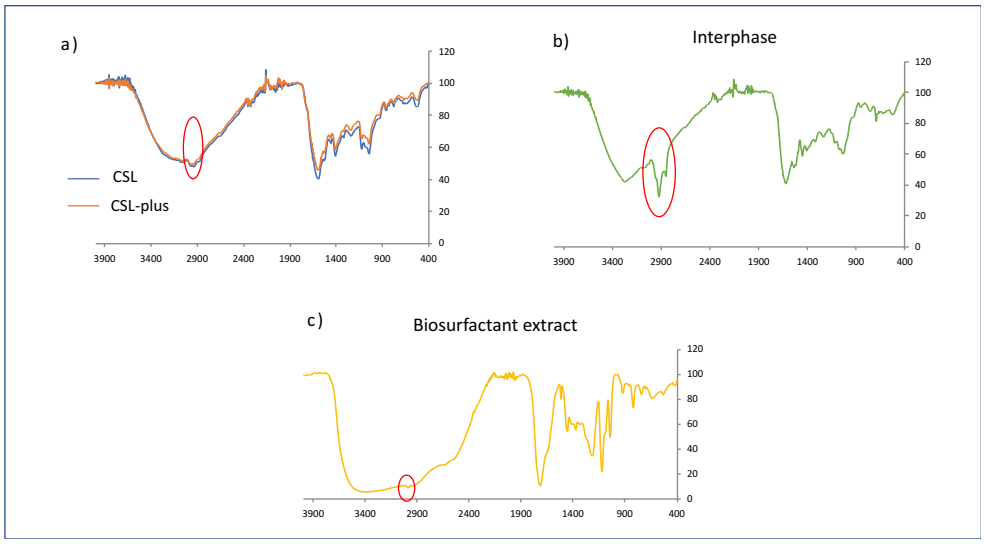

Figure 3. FTIR spectra of CSL and CSL-plus (a), the interphase (b), and the biosurfactant extract (c).

Figura 3. FTIR espectros de CSL y CSL-plus (a), la interfase (b), y el extracto de biosurfactante (c).

a)

b)

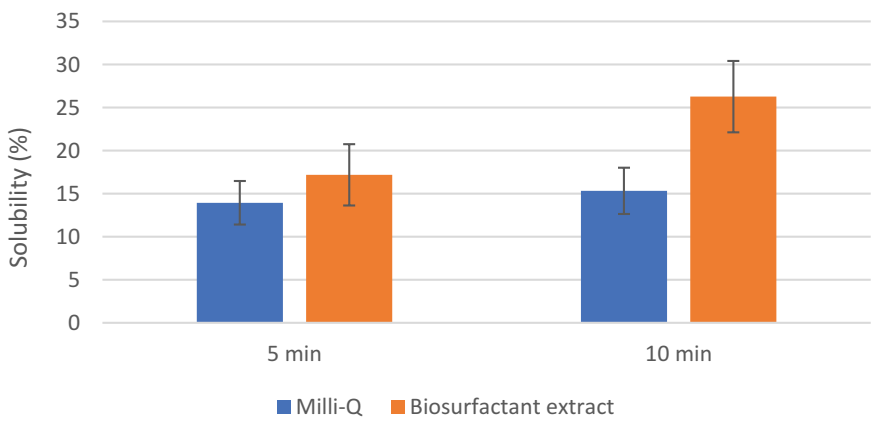

Figure 4. Macro view of the chicken broth cubes solubilization with Milli-Q water and biosurfactant solution, respectively (a); and the solubilization percentage obtained (b).

Figura 4. Visión macroscópica de la solubilización de los cubos de caldo de pollo en agua Milli-Q y disolución de extracto de biosurfactante, respectivamente (a); y el porcentaje de solubilización obtenido (b).

CSL is commonly used as a nutritional supplement for animal feed (Azizi-Shotorkhoft et al., 2016; Ullah et al., 2018) but in this work it has been demonstrated that it is possible to increase the added value of CSL by the extraction of biosurfactants, at the same time that it is obtained a CSL with a lower content of fat, improving its nutritional value.

3.3. Biosurfactant extract as solubilizing agent in food industry

In order to corroborate the solubilizing properties of the biosurfactant extract under evaluation, a cube of chicken broth was dissolved in water in presence of biosurfactant extract an compared with the solubilization of a cube of broth in water without biosurfactant extract. In Figure 4 it is observed that in presence of biosurfactant extract in the cube of chicken broth was dissolved in higher extent (26.3 ± 4.1%) that in absence of biosurfactant extract (17.2 ± 3.6%), being the solubilization of the cube chicken broth improved around 11%, based on dry weight, after 10 min of contact time. These results prove the potential of this biosurfactant extract as emulsifier and solubilizing agent in the food industry, hence it should be necessary to carry out deeper studies in further works.

4. Conclusions

The extraction of biosurfactants from CSL, a secondary stream of wet milling industry, increases its nutritional properties, reducing the fatty acid content and maintaining the other micronutrients in the same range of concentration. Therefore, the extraction of biosurfactants, from CSL, increases the possibilities of utilization of CSL as secondary raw material giving an improved nutritional supplement, at the same time that a valuable biosurfactant extract, with multiples applications in different industrial sectors, is obtained, promoting a circular economy and a synergy industrial. Moreover, it was proven the capacity of the biosurfactant extract under evaluation to increase the solubilization of concentrated chicken broth cubes, commonly used in the preparation of several foods, which usually are hardly dissolved in water.

Disclosure statement

No potential conflict of interest was reported by the authors.

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