Novel cosmetic formulations containing a biosurfactant from *Lactobacillus paracasei*

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ABSTRACT

Cosmetic and personal care products including toothpaste, shampoo, creams, makeup, among others, are usually formulated with petroleum-based surfactants, although in the last years the consume trend for “green” products is inducing the replacement of surface-active agents in these formulations by natural surfactants, so-called biosurfactants. In addition to their surfactant capacity, many biosurfactants can act as good emulsifiers, which is an extra advantage in the preparation of green cosmetic products. In this work, a biosurfactant obtained from *Lactobacillus paracasei* was used as a stabilizing agent in oil-in-water emulsions containing essential oils and natural antioxidant extract. In the presence of biosurfactant, maximum percentages of emulsion volumes (EV = 100%) were observed, with droplets sizes about 199 nm. These results were comparable with the ones obtained using sodium dodecyl sulfate (SDS), a synthetic well known surfactant with high emulsify capacity. Moreover, the biosurfactant and emulsions cytotoxicity was evaluated using a mouse fibroblast cell line. Solutions containing 5 g/L of biosurfactant presented cell proliferation values of 97%, whereas 0.5 g/L of SDS showed a strong inhibitory effect. Overall, the results herein gathered are very promising towards the development of new green cosmetic formulations.

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1. Introduction

Surfactants play important roles in cosmetic formulations due to their diverse properties such as wetting, solubilizing, emulsifying and foaming abilities, as well as detergency, among others. According to the European Commission regulation 2006/257/CE [1], a surfactant “lowers the surface tension of cosmetics, as well as it aids the uniform distribution of the product when used”. Two types of surfactants can be found in the market, those that are produced by chemical synthesis and those that are obtained from microorganisms by biotechnological processes. Currently, the market for beauty and personal care products is seeking for natural ingredients as alternatives to the commonly used chemicals [2]. In this sense, microbial surfactants (also known as biosurfactants) could be among those alternatives given that they are more biodegradable

and less cytotoxic than their chemical homologs with the advantage that they can be produced using renewable substrates [3,4].

Many applications have been proposed for biosurfactants including in the bioremediation of contaminated sites [5–8] or in the food industry [9,10] due to their ability to solubilize hydrophobic substances in oil–water interfaces. However, the costs involved in biosurfactants biotechnological production and recovery can hamper their application in those areas. On the contrary, despite these costs, biosurfactants can be used in cosmetics as this industry presents very high profits that can still overcome the costs involved. As previously mentioned, currently the cosmetic industry is interested in using natural compounds that can be labeled as “natural ingredients” [2].

Several cosmetic creams are formulated with essential oils from plants due to their occlusive, emollient and moisturizing properties on the skin [11]. Most of these oil-based substances require the presence of a stabilizing agent as emulsifiers and/or surfactants in order to obtain good emulsions. For instance, Vecino and collaborators [12] showed that a glycolipopeptide extracted from *Lactobacillus pentosus*, was a good stabilizing agent of oil-in-water (O/W) emulsions formulated with rosemary oil. Also,

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Bai and McClements [13] obtained O/W nanoemulsions with relatively small droplet diameters (<150 nm) at low surfactant/oil ratios (<1:10) using a rhamnolipid.

In addition, many cosmetic formulations contain antioxidants with a double function. They inhibit the oxidation of active principles in creams acting as preservatives, and also prevent the skin from free radicals scavenging, improving its condition [14].

Balboa and collaborators [15] formulated different cosmetic products, such as avocado cream, sun cream, massage oil and shower oil, incorporating natural antioxidants obtained from underutilized and residual vegetables and macro-algal biomass which showed a good biocompatibility for skin cells even when the concentration of antioxidant tested was higher than the values commonly used for synthetic antioxidants.

The aim of this work is to evaluate the potential use of a biosurfactant (produced by *Lactobacillus paracasei*) combined with several essential oils and a natural antioxidant extract (obtained from grape seeds) in the development of novel cosmetic formulations. Different emulsions will be formulated in presence of the biosurfactant that acts as a stabilizing agent and their cytotoxicity will be evaluated in comparison with other emulsions formulated with the chemical surfactant, SDS.

2. Materials and methods

2.1. Development of green cosmetic formulations

2.1.1. Biosurfactant production by *L. paracasei*

L. paracasei (isolated in a Portuguese dairy industry) was used for the production of biosurfactant [16]. The strain was grown in Petri dishes containing complete medium MRS agar (VWR, Belgium) at 37 °C for 24 h. The inoculum was prepared by solubilizing the cells from the plates with 5 mL of culture media (MRS broth de Man, Rogosa and Sharpe) and it was added into 250 mL Erlenmeyer flasks containing the rest of culture media (95 mL), afterwards it was incubated at 150 rpm and 37 °C.

For the production of biosurfactant, the fermentation medium was formulated with 33 g/L of glucose (Scharlau, Spain), 10 g/L of corn steep liquor (Sigma–Aldrich, China) and 10 g/L of yeast extract (Oxoid, UK). The medium was sterilized at 121 °C during 15 min and the chemostat fermentation was carried out in a 1.5 L Bioengineering AG® fermenter (Switzerland) at 200 rpm with a working volume of 1 L at 37 °C. The pH was adjusted to 5.85 with 4 M NaOH (Fisher, UK) for 24 h.

Afterwards, the biomass was separated from the fermentation medium by centrifugation at 9000 rpm during 20 min, it was washed two times with distilled water and re-suspended in about 167 mL of phosphate-buffered saline (PBS) (10 mM KH₂PO₄/K₂HPO₄ (Panreac, Spain) with 150 mM NaCl (Merck, Germany), with pH adjusted to 7.0). The ratio fermentation medium (containing the biomass)/PBS used for the extraction of the biosurfactant was 6:1. The extraction process was carried a room temperature (25 °C) during 2 h at 150 rpm [17]. Subsequently, the cells were removed by centrifugation (9000 rpm, 20 min) and the remaining supernatant liquid was filtered through a 0.2 µm pore-size filter (Whatman, GE Healthcare, UK). The solution containing the cell-bound biosurfactant was dialyzed against demineralized water at 4 °C in a Cellu-Sep® membrane (molecular weight cut-off 6000–8000 Da; Membrane Filtration Products, Inc., USA) for 48 h, and finally the biosurfactant was lyophilized using a lyophilizer CHRIST® Alpha 1–4 LD plus (Germany).

The surface activity of the biosurfactant was determined by measuring its surface tension using a KRÜSS K6 Tensiometer (KRÜSS GmbH, Germany) equipped with a 1.9 cm Du Noüy plat-

inum ring at room temperature. All determinations were carried out in triplicate.

2.1.2. Biosurfactant characterization

The elemental analysis of the *L. paracasei* biosurfactant was carried out by a chromatography analysis with thermal conductivity detection (TCD). C, N, H and S were determined using a Fisons Carlo Erba EA-1108 CHNS-O elemental analyzer. Hence, the amount of N was correlated with the protein content by multiplying it by a factor of 6.25 [18]. The carbohydrate and lipid content of the biosurfactant were determined by the phenol–sulfuric acid [19] and Folch [20] methods using D-glucose and cholesterol as standards, respectively.

2.1.3. Essential oils

The essential oils used in this work were provided by Gran Velada (Spain). These were extracted from wheat germ, almond, jojoba and rosemary.

2.1.4. Natural antioxidant extract from grape seeds

The antioxidant extract (AO) used in this work was obtained from grape seeds, which were kindly provided by the Oxvit Company (Barcelona, Spain). For comparison purposes, a synthetic antioxidant, (3)-tert-butyl-4-hydroxyanisole (BHA) (Merck, Germany) was used.

Previous to the preparation of the emulsions, the antioxidant capacity of this natural extract was evaluated according to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma–Aldrich, USA) radical scavenging method described elsewhere with slight modifications [21]. An aliquot of the natural extract (5 µL), dissolved in methanol, was added to 200 µL of DPPH solution (3.6×10^{-5} M), shaken vigorously on a vortex shaker and left to stand in the dark during 16 min at room temperature. Afterwards, the absorbance was measured at 515 nm in a Cytation 3 Imaging reader (BioTek, USA) spectrophotometer. All determinations were performed in triplicate.

The decrease in absorbance was converted to inhibition percentage of the DPPH (IP), according to Eq. (1):

$$IP = \frac{A_0 - A_{16}}{A_0} \times 100 \quad (1)$$

where A_0 and A_{16} are the absorbances of the sample at initial time and after 16 min of reaction, respectively.

The antioxidant extract concentration required to achieve 50% inhibition of the radical DPPH (equivalent concentration = EC₅₀) was determined from the linear regression curve obtained by plotting the different concentrations of antioxidant extract used against the IP of the DPPH.

2.2. Preparation of cosmetic formulations

The emulsions were formulated according to the procedure reported by Das and collaborators [22] with slight modifications. Different ratios between the hydrophobic phase (O), based on essential oil, and the hydrophilic phase (W), based on an aqueous solution containing the biosurfactant (BS), the sodium dodecyl sulfate (SDS) and/or the antioxidant extract (AO), were assayed. SDS is a synthetic surfactant widely used in the cosmetic industry, which was included in this study for comparison purposes.

The essential oils were mixed with the aqueous phase containing the BS, using an IKA T25 digital Ultra-turrax® (IKA Laboratory Equipment, Germany) at 18,000 rotations/min during 2 min at room temperature.

Different formulations were prepared, varying the concentration of BS, SDS and/or AO in the aqueous phase at different O/W ratios (1:3, 2:1, 3:1) (v/v). Table S1 (see supporting information)

shows the different emulsions performed specifying all the components and concentrations used.

2.3. Evaluation of the emulsions

2.3.1. Determination of the continuous and disperse phases

All emulsions systems are comprised by a continuous phase that suspends the droplets of the other element that is called the dispersed phase. In an O/W emulsion, the continuous phase is the water and the dispersed phase is the oil, while in water-in-oil (W/O) emulsions the oil is the continuous phase. To distinguish both emulsions systems, droplets of formulated emulsions were added to oil or water solutions determining in which phase droplets were solubilized.

2.3.2. Relative emulsion volume and emulsion stability

The relative emulsion volume (EV) and emulsion stability (ES) were quantified 1 h after the preparation of the emulsions (considering that 1 h corresponds to the initial time) and during 1–30 days at different time points, according to the methodology proposed by Das et al. [22]. These parameters were calculated following Eqs. (2) and (3), respectively.

$$EV(\%) = \frac{\text{Emulsion height (mm)} \times \text{Cross section area (mm}^2\text{)}}{\text{Total liquid volume (mm}^3\text{)}} \quad (2)$$

$$ES(\%) = \frac{EV(\text{at time } h)}{EV(\text{at } 1 \text{ h})} \times 100 \quad (3)$$

2.3.3. Droplet size characterization

The pictures of emulsion droplets were captured using a Leica DMI 3000B inverted microscope (Leica Microsystems, Germany) equipped with a high-sensitivity camera Leica DFC450C. Pictures were taken under a 20× objective at $20 \pm 2^\circ\text{C}$ and using the LAS 4.7 software.

Additionally, the droplets size was measured at room temperature ($\sim 25^\circ\text{C}$) using a Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK). For that purpose, 5 mL of the emulsion was disposed in plastic cuvettes and the results were processed with the Zetasizer software.

2.4. Cytotoxicity: sulforhodamine B (SRB) assay

In order to evaluate the cytotoxicity of the biosurfactant extracted from *L. paracasei*, as well as the emulsion systems, the mouse fibroblast cell line 3T3 was used. This cell line was grown in Dulbecco's Modified Eagle's Medium (DMEM) (Merck, Germany) supplemented with 10% fetal bovine serum (FBS) (Merck, Germany) and 1% ZellShield (Minerva Biolabs, Germany), at 37°C and 5% CO_2 .

Generally, the cells were propagated twice a week by washing the monolayer with PBS 1X pH 7.4 [137 mM NaCl, 2.7 mM KCl (Chem-Lab, Belgium), 10 mM Na_2HPO_4 (Scharlau, Spain) and 2 mM KH_2PO_4] solution, detaching them with trypsin-EDTA solution 0.05%/0.2% (w/v) (Merck, Germany) and dividing them at a 1:3 ratio.

3T3 cellular suspensions were seeded into 6-well plates at a concentration of 1×10^5 cells per well and were left to adhere for 24 h. Then, the culture media were replaced with 1 mL of fresh medium plus 1 mL of the different emulsions formulated with *L. paracasei* biosurfactant or with SDS, observing their effect on cell proliferation for 24 h. For comparison purposes, the cytotoxicity of each emulsion component (biosurfactant, SDS, antioxidant extract and oil) at different concentrations was also evaluated.

After discarding the medium by inversion, the wells were washed with PBS 1×. Then, 2 mL of 1% (v/v) acetic acid (VWR, Portugal) prepared in methanol (Fisher Scientific, Portugal) was added to each well and the plate was sealed with parafilm and

placed, overnight, at -20°C . Afterwards, the plates were inverted to discard the acetic acid/methanol solution and were incubated at 37°C until completely dry. A 500 μL volume of 0.5% (w/v) of Sulforhodamine B (SRB) (Sigma-Aldrich, Portugal) in 1% (v/v) acetic acid was added to each well. The plate was covered with foil and incubated 1.5 h at 37°C . The excess of non-ligated SRB was discarded and the wells were washed with 1% (v/v) acetic acid in distilled water. The plates were placed at 37°C to dry and 1 mL of 10 mM non-buffered Tris-base solution (Fisher Scientific, Portugal) was added to the wells. In the end, the volume was transferred to 96-well plate wrapped in foil and were shaken at room temperature. The absorbance was read at 540 nm using a microplate reader (Synergy HT, BioTek, USA).

The results were expressed as the percentage of cell proliferation as compared to the control (i.e. cells non-exposed to any substance) and they represent an average of ten independent wells per substance tested.

3. Results and discussion

In this work, a biosurfactant produced by *L. paracasei* (BS) was evaluated as a stabilizing agent of emulsions formulated with natural components based on essential oils and/or natural antioxidant extract. The BS reduced the surface tension of water by 25 mN/m and it possesses a critical micellar concentration (CMC) of about 1.35 g/L. This biosurfactant is composed by circa 14% C, 2% H, 3.4% N and S < 0.3%, being the percentage in proteins, carbohydrates and lipids, 21%, 6% and 25%, respectively. Thus, the *L. paracasei* is a glycolipopeptide biosurfactant.

In a preliminary screening, our emulsion systems based on different essential oils and biosurfactant were monitored during 1 month to select the most adequate essential oil for further assays with multiple components and using various oil/water ratios.

The essential oils studied were obtained from different natural sources such as wheat germ, almond, jojoba and rosemary. During the formulation of these emulsions using an O/W ratio of 2:2 (v/v), the essential oils were mixed with an aqueous solution containing the biosurfactant at its CMC. The emulsions formulated with these essential oils, in the presence of BS, were characterized as O/W emulsions.

Figs. 1 and 2 show the EV and ES after 1 month for each essential oil assayed. According to Willumsen and Karlson [23], a good emulsion-stabilizing agent should have the capacity to maintain at least 50% of the original EV after 24 h of emulsion formation. Emulsion systems based on wheat germ and rosemary oils exhibited the same behavior during the 3 days following emulsion formation with EV values of about 62.5%, while after 7 days and 4 days the emulsions formulated with wheat-germ oil or rosemary oil, respectively, decreased their EV values to 50%, and between 27.5% and 20% after 21 days of emulsion formation (see Figs. 1a and b). These EV values were higher than those obtained with jojoba oil that gave very unstable emulsion systems with EV values about 27.5% after 2 days of emulsion formation (Fig. 2b).

Contrarily, the emulsion formulated with almond oil and stabilized with BS showed the best results, with EV values of about 70% after 3 days and kept EV values above 50% after 15 days of emulsion formation (Fig. 2a). Moreover, almond oil showed 100% stability (ES) during the first 3 days, and afterwards it decreased to 80% after 7 days and to around 60% after 1 month (Fig. 2a).

The results herein achieved are comparable with those obtained by Chen and collaborators [24], which showed that O/W emulsions containing soybean oil and the biosurfactant produced by *Alcaligenes piechaudii* CC-ESB2 as stabilizing agent, exhibited an emulsification index about 80% after 24 h of emulsion aging using O/W ratio of 2:3 (v/v).

Fig. 1. EV and ES kinetics for the emulsions formulated with different essential oils: (a) wheat-germ oil and (b) rosemary oil.

Fig. 2. EV and ES kinetics for the emulsions formulated with different essential oils: (a) almond oil and (b) jojoba oil.

Furthermore, Velioglu and Urek [25] found that the biosurfactant produced by *Pleurotus djamor* in solid-state fermentation led to acceptable EV values working with sunflower oil at 24 h of emulsion formation, although after 48 h the EV values decreased below 50%.

The droplet size distribution of almond O/W emulsions was found to vary along time and these emulsions comprised mostly droplet sizes below 145 nm. Hence, after 1 day the droplet size distribution was homogenous consisting in a monodisperse emulsion with droplets sizes of about 100 nm; whereas after 7 days of emulsion formation it was observed a heterogeneous distribution of droplets consisting in a polydisperse emulsion with two droplet families at sizes between 10 and 100 nm. Vecino et al. [12] also reported a polydisperse emulsion when a biosurfactant produced by *L. pentosus* was used to stabilize an O/W emulsion based on rosemary oil with a droplet size < 100 μm .

3.1. Optimization of almond O/W emulsions

Based on the EV and ES values described above, almond oil was selected to obtain optima O/W emulsion systems with potential application in the cosmetic industry. In the mentioned preliminary screening, among the emulsion systems assayed, almond oil was found to be the most favorable oil for these emulsion systems, keeping EV values around 50% after 15 days of emulsion formation. Consequently, almond oil was selected as the oil phase, and therefore 15 days was the limit established for testing these emulsion systems.

The effect of surface-active agent (BS or SDS) concentration and O/W ratio was studied. Hence, different concentrations of BS or SDS (0.01, 0.1, 1 and 10 g/L) were dissolved in the aqueous phase and then mixed with almond oil following the same protocol aforementioned. The EV and ES were evaluated during 1 week after emulsion formation (see Fig. 3). It was found that to achieve EV values above 50%, comparable to those obtained with SDS, the BS concentration in the aqueous phase has to be higher than 1 g/L.

Fig. 3. EV and ES of almond oil emulsion systems stabilized with different concentrations of BS (a) or SDS (b) using O/W ratio of 2:2 (v/v).

Fig. 4. EV and ES values at different antioxidant concentrations in the almond O/W emulsion, formulated with (a) 1 g/L of BS using 2:1 O/W ratio (v/v) and (b) 10 g/L of BS using 3:1 O/W ratio (v/v).

Moreover, different O/W ratios (1:3, 2:1, and 3:1) (v/v) were used to evaluate the capacity of SDS or BS as stabilizing agents in emulsions containing almond oil. Previously to the emulsion formation, BS and SDS were added in the aqueous phase at concentrations of 1 and 10 g/L, respectively.

Tables 1 and 2 show EV, ES and size distribution of emulsion droplets after 7 days of emulsion formation for all the conditions assayed. It was observed that emulsions formulated at O/W ratio 2:1 (v/v) and O/W ratio 3:1 (v/v), with 10 g/L of BS in the aqueous phase, led to EV values around 50–57% with droplet sizes between 16.5–33.6 nm; whereas when the concentration of BS in the aqueous phase was reduced at 1 g/L, EV values around 70–82% were obtained. These emulsion systems were characterized by a droplet size distribution between 128 and 140 nm after 7 days of aging. In this case, lower concentrations of BS in the aqueous phase gave higher EV values, although the size distribution of droplets was more homogenous in the presence of higher concentrations of BS. Hence, 10 g/L of BS in the aqueous phase using O/W ratios of 2:1 (v/v) consisted in a monodisperse emulsion system.

On the other hand, using an O/W ratio of 1:3 (v/v), the size of droplets was even smaller, 10.1 nm or 19.2 nm, for emulsions containing 1 g/L or 10 g/L of BS respectively; however the EV values were below 50%.

The emulsions stabilized with SDS at concentrations of 1 g/L or 10 g/L using a O/W ratio of 1:3 (v/v) showed similar results to those obtained for emulsions stabilized with the BS, with EV values around 23–34% and droplet sizes between 5.5 and 7.5 nm. At higher O/W ratios, SDS showed higher EV values and higher size of droplets in the emulsion, following a similar behavior to that observed in the previous emulsions stabilized with BS.

Fig. 5. EV and ES values at different antioxidant concentrations in the almond O/W emulsion, formulated with (a) 1 g/L SDS and (b) 10 g/L SDS using O/W ratio of 2:1 (v/v).

Amania and Kariminezhad [26] also reported that emulsions stabilized with the biosurfactant produced by *Acinetobacter calcoaceticus* PTCC1318, showed more favorable EV values when using higher O/W ratio of 2:1 (v/v) as compared to lower ones (1:1, 1:2 and 1:3) (v/v).

Furthermore, Portilla-Rivera et al. [8] studied the emulsifying capacity of the biosurfactant from *L. pentosus* grown on sugars from different agricultural residues, reporting EV values around 50% similar to those achieved with the *L. paracasei* biosurfactant. However, in this case the assays were carried out with hydrophobic phases based on petrochemical hydrocarbons.

3.2. Incorporation of antioxidant extract in the almond O/W emulsions

An extract, obtained from grape seeds, was used as natural antioxidant extract. This antioxidant extract is soluble in water and has a similar antioxidant activity to the synthetic antioxidant BHA, showing EC₅₀ values of 0.28 ± 0.01 g/L and 0.24 ± 0.01 g/L, respectively.

Different concentrations of antioxidant extract (5, 10, 25, 50 and 100 g/L) were used in the presence of 1 g/L or 10 g/L of BS, at O/W ratios of 3:1 (v/v) and 2:1 (v/v), respectively. Additionally, emulsions containing 1 g/L or 10 g/L of SDS, in the aqueous phase, at O/W ratios of 2:1 (v/v) were used (see supporting information, Table S1).

Fig. 4 shows the EV and ES values obtained for the emulsions prepared with antioxidant extract at the concentrations above-mentioned, in emulsion systems containing 1 g/L (Fig. 4a) or 10 g/L (Fig. 4b) of BS. In general, the addition of the natural antioxidant extract allowed the formation of stable O/W emulsions with EV values above 50%; although it was observed that emulsion systems containing lower BS concentration (1 g/L) and lower antioxidant

Table 1

Droplet size distribution of different emulsion systems, using as variables the O/W ratio and the concentration of BS after 7 days of emulsion formation. Pictures were taken under a 20 × objective (----- 200 μm).

Emulsifier type: BS [Emulsifier] in W (g/L): 1 O/W ratio (v/v): 1:3			O/W ratio (v/v): 2:1	O/W ratio (v/v): 3:1
<p>EV (%) after 7 days: 28.3 ± 2.9 Droplets distribution: Polydisperse Droplet size (nm): 10.1 ± 1.9</p>			<p>EV (%) after 7 days: 68.9 ± 3.8 Droplets distribution: Polydisperse Droplet size (nm): 128.5 ± 23.3</p>	<p>EV (%) after 7 days: 81.7 ± 5.8 Droplets distribution: Polydisperse Droplet size (nm): 140.0 ± 3.3</p>
Emulsifier type: BS [Emulsifier] in W (g/L): 10 O/W ratio (v/v): 1:3			O/W ratio (v/v): 2:1	O/W ratio (v/v): 3:1
<p>EV (%) after 7 days: 35.8 ± 2.9 Droplets distribution: Monodisperse Droplet size (nm): 19.2 ± 4.0</p>			<p>EV (%) after 7 days: 56.7 ± 6.7 Droplets distribution: Monodisperse Droplet size (nm): 16.5 ± 1.4</p>	<p>EV (%) after 7 days: 50.0 ± 5.0 Droplets distribution: Polydisperse Droplet size (nm): 33.6 ± 7.4</p>

Table 2

Droplet size distribution of different emulsion systems, using as variables the O/W ratio and the concentration of SDS after 7 days of emulsion formation. Pictures were taken under a 20 × objective (----- 200 μm).

Emulsifier type: SDS [Emulsifier] in W (g/L): 1 O/W ratio (v/v): 1:3			O/W ratio (v/v): 2:1	O/W ratio (v/v): 3:1
<p>EV (%) after 7 days: 23.3 ± 7.6 Droplets distribution: Monodisperse Droplet size (nm): 5.5 ± 0.2</p>			<p>EV (%) after 7 days: 85.6 ± 1.9 Droplets distribution: Monodisperse Droplet size (nm): 64.5 ± 13.7</p>	<p>EV (%) after 7 days: 82.5 ± 17.3 Droplets distribution: Monodisperse Droplet size (nm): 64.9 ± 8.4</p>
Emulsifier type: SDS [Emulsifier] in W (g/L): 10 O/W ratio (v/v): 1:3			O/W ratio (v/v): 2:1	O/W ratio (v/v): 3:1
<p>EV (%) after 7 days: 34.2 ± 3.8 Droplets distribution: Monodisperse Droplet size (nm): 7.5 ± 0.5</p>			<p>EV (%) after 7 days: 86.7 ± 0.0 Droplets distribution: Monodisperse Droplet size (nm): 83.8 ± 4.2</p>	<p>EV (%) after 7 days: 70.8 ± 19.1 Droplets distribution: Monodisperse Droplet size (nm): 88.7 ± 12.7</p>

Fig. 6. Proliferation of 3T3 mouse cells exposed to the components and emulsion systems formulated with (a) BS or (b) SDS.

extract concentrations (5 to 25 g/L) showed EV values below 50%. However, O/W emulsions containing 10 g/L of biosurfactant and 5 g/L of antioxidant extract showed EV values about 100% after 7 days of emulsion formation.

On the other hand, Fig. 5 shows the EV and ES values for the emulsions prepared with antioxidant extract in the presence of SDS at 1 g/L (Fig. 5a) and 10 g/L (Fig. 5b). In this case, the differences observed between the emulsions obtained with 1 or 10 g/L of SDS and antioxidant extract concentration between 5 and 25 g/L were negligible. However, at the highest antioxidant extract concentrations evaluated (50 and 100 g/L), more favorable EV values were obtained at 1 g/L of SDS. These results were comparable with those obtained in emulsions stabilized with BS (10 g/L) using 5 g/L of antioxidant extract.

Regarding the size distribution of emulsions, when the antioxidant ingredient was incorporated to the almond O/W emulsions, the emulsions stabilized with BS showed a polydisperse emulsion with droplet size distributions between 177 and 235 nm, whereas in the emulsion formulated with SDS it was observed a homogeneous distribution of droplets comprising a monodisperse emulsion with droplets of about 178 nm.

Balboa and collaborators [15] also proposed the addition of an antioxidant extract, obtained from *Sargassum muticum* to different cosmetic models like avocado cream (O/W emulsion), massage oil and shower oil. The antioxidant assayed was not soluble in water and it was found that the extract could prevent the cosmetic lipid oxidation about 94%, 59% and 14% for avocado cream, shower oil and massage oil, respectively, after 34 days of storage.

3.3. Cytotoxicity of the almond O/W emulsions

Fig. 6 illustrates the cell proliferation of fibroblast cells after 24 h of exposure to the BS, SDS and the emulsions formulated with these components. Solutions containing 5 g/L of *L. paracasei* biosurfactant showed cell proliferation values of 97%. On the other hand, at the highest biosurfactant concentration assayed (10 g/L) the cell proliferation was over 64%, whereas 0.5 g/L of SDS showed a strong inhibitory effect.

However, the emulsions formulated with SDS and antioxidant extract showed low cell cytotoxicity, as 83% of cell viability was observed (Fig. 6). This fact suggests that the antioxidant compound had a positive effect on cells protecting them from the SDS.

Kim et al., [27] examined the toxicity of mannosylerythritol lipid (MEL) SY16 biosurfactant in mouse fibroblast L929 cells after 48 h of exposure. The results showed that the midpoint toxicity value of MEL was higher (5 g/L) in comparison with synthetic surfactants as LAS (linear alkylbenzene sulphonate) and SDS whose values were 0.01 g/L and 0.05 g/L, respectively. The data clearly suggest that MEL-SY16 is not harmful to human skin and eyes as compared to synthetic surfactants.

Furthermore, Burgos-Díaz and collaborators [28] studied the cytotoxicity and the anti-proliferative effects of a biosurfactant produced by *Sphingobacterium detergens* in 3T3 fibroblast and HaCaT keratinocyte cell lines after 24 h of exposure. The authors found that the purified fraction of the biosurfactant exhibited lower cytotoxicity than those obtained using SDS, hence indicating low skin irritability.

Balboa et al. [15] evaluated the skin irritability of different natural antioxidant extracts using the Episkin test (reconstructed human skin tissue), showing that these bioactive compounds almost did not affect the cell viability, obtaining values of cell proliferation of about 78–92%.

4. Conclusions

The results gathered in this work suggest that the BS produced by *L. paracasei* could be used as a natural ingredient in cosmetic formulations playing an important role as emulsifier agent in O/W emulsion systems, in combination with essential oils and natural antioxidant extract. The cell proliferation in the presence of the BS or in the presence of O/W emulsions containing the natural antioxidant extract and the BS was over 97%, with more favorable results than those obtained with SDS. These findings open new opportunities for the use of biosurfactants in cosmetic applications for example in creams as long as they ensure safety for consumers following the Regulation (EC) No 1223/2009 on cosmetic products.

Conflict of interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.colsurfb.2017.04.026>.

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