Water Practice & Technology

IWA PUBLISHING

Water Practice & Technology Vol 18 No 5, 1125 doi: 10.2166/wpt.2023.052

Basis for the formulation of *Bacillus subtilis* SPB1 biosurfactant to achieve low interfacial tension and high emulsification activities: development of a bio-based formula with potential use for personal care products

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ABSTRACT

Thanks to their multifunctional properties, such as foaming, emulsifying and skin hydrating activities biosurfactants are potentially used in the cosmetic industry. In the present work, firstly, skin cream formulations using chemical surfactant agents and lipopeptide biosurfactants isolated from *Bacillus subtilis* SPB1 were investigated. The efficiency of the selected formula, containing 15% biosurfactant, was tested and its physicochemical properties were satisfying. In fact, it shows a spreading ability of 23 mm, a water activity of 0.72 and a pH value of 5. In addition, its biological activities were interesting showing a zone of inhibition against Gram-positive bacteria ranging from 8 to 15 mm. Moreover, it presents an antiradical effect against diphenyl-1-picrylhydrazyl (DPPH) with an IC50 of the order of 68 mg/mL. Second, seeking a highly stable SPB1 biosurfactant formulation for an easy commercial trend, a combination of biosurfactant, Gum Arabic (GA) and glycerol was optimized using the Box–Behnken (BB) design. As analysed, the model predicts an optimal value of the surface tension (ST) equal to 40.18 $\pm$ 0.39 mN/m when the respective values of the percentages of SPB1 BioS, glycerol and GA are equal to 72.62, 7.5 and 1.5%. Under these conditions, the emulsification index (EI)-24% is around 81.76 $\pm$ 0.14%.

Key words: antimicrobial, antioxidant, emulsification activity, biosurfactant, Box–Behnken design, cream formulation

HIGHLIGHTS

- BioS-based formula has interesting biological and physicochemical properties.
- A Box–Behnken design permits to optimize a BioS-based formula composed of 72.62% SPB1 BioS, 7.5% glycerol and 1.5% Gum Arabic.
- The optimized formula has an EI-24% of 81.19 and reduces the ST of water to 41.4 mN/m.
- The BioS-based formula can be incorporated into cosmetic products, preventing water pollution and ecological toxicity.

ABBREVIATIONS

BB	Box–Behnken design
BioS	Biosurfactants
ST	Surface tension

INTRODUCTION

At present, many manufacturers producing personal skincare products use chemical surfactants as ingredients in their formulations, achieving in some cases concentrations of up to 50% (Adu *et al.* 2020; Moldes *et al.* 2021). Thanks to their amphiphilic property, surfactants play important roles in cosmetic formulations as they serve as wetting, solubilizers, dispersants, foaming and emulsion-forming agents (Ferreira *et al.* 2017; Bezerra *et al.* 2018). However, since about 50% of the chemical surfactants on the market are of petrochemical origin and are generated from non-sustainable resources, their increasing use by the cosmetic and pharmaceutical industries could be harmful to the skin and its microbiome (Adu *et al.* 2020; Moldes *et al.* 2021). On the other hand, as they attach to lipids and proteins on the epidermal layer of the skin, some of these surfactants are said to alter the skin

flora (Adu *et al.* 2020), causing allergic reactions (Bezerra *et al.* 2018; Moldes *et al.* 2021) and skin irritations (Bezerra *et al.* 2018; Adu *et al.* 2020). Another challenging issue associated with the employment of chemical tensio-active substances is the negative impact on the environment due to soil and groundwater contamination (Zulaikha *et al.* 2015; Moldes *et al.* 2021). In fact, after personal use, cosmetic products are discharged and can pass into the wastewater treatment plants, causing a huge negative effect on their performance (Juliano & Magrini 2017; Mousavi & Khodadoost 2019; Gkika *et al.* 2022). Also, the use of sunscreen cream allows for the discarding of huge quantities of chemical products into the seawater causing great damage to living organisms and disturbing the ecological equilibrium (Juliano & Magrini 2017). Thus, the use of chemical surfactants in the cosmetic formula is avoided.

Microbial biosurfactants (BioS) may be the ideal alternative to synthetic surfactants in commercial goods (Bezerra *et al.* 2018; Ceresa *et al.* 2021; Moldes *et al.* 2021). They are characterized by their stability under extreme conditions (pH, temperature and salinity), their biodegradability and their lower toxicity (Adu *et al.* 2020; Ceresa *et al.* 2021; Moldes *et al.* 2021). Moreover, they have great compatibility with human skin, protection and surface moisturizing effects, all of which are important aspects of an effective skincare routine (Adu *et al.* 2020).

Various factors based on the intended purpose of the product are involved in the selection of a BioS for a specific cosmetic product. Generally, the Critical Micelle Concentration (CMC), hydrophilic–lipophilic balance (HLB) and ionic performance are the key characteristics of BioS that determine their type and usage in cosmetic formulations. Besides their structural and chemical properties, BioS have biological activities that make these natural compounds multifunctional ingredients. Many interesting biological activities like the antibacterial, anti-adhesive, antiviral and antioxidant properties explain the growing interest of BioS in the pharmaceutical and biomedical industries (Mnif & Ghribi 2015a; Ceresa *et al.* 2021). It is thanks to their amphiphilic characteristics that BioS enter the bilipid membrane through hydrophobic interactions, resulting in physical changes to the membrane structure or disturbances in membrane protein conformations (Mnif & Ghribi 2015a, 2015b). This can alter its important functions such as energy transport and production and increase membrane permeability leading to metabolic leakage and causing its deterioration (Mnif & Ghribi 2015a, 2015b). This property results in different biological activities of BioS, including antibacterial and antifungal activities (Sharma *et al.* 2021), as well as antibiofilm formation (Adu *et al.* 2020).

As it is known, *Bacillus subtilis* SPB1 was able to co-produce lipopeptide compounds consisting of different isoforms of Surfactin, Iturin and Fengycin (Mnif *et al.* 2016a). The SPB1 BioS exhibits a broad spectrum of action, including antimicrobial activity against microorganisms with multidrug-resistant profiles (Ghribi *et al.* 2012a), insecticidal activity (Ghribi *et al.* 2012b), antifungal activity towards phytopathogenic fungi (Mnif *et al.* 2016b), anti-diabetic and anti-lipidemic properties in alloxan-induced diabetic rats (Zouari *et al.* 2015) with reduced toxicity towards living cells and antioxidant activity (Zouari *et al.* 2016). Moreover, the lipopeptide was able to reduce the surface tension (ST) of water along with solubilizing, mobilizing and emulsifying activities (Mnif *et al.* 2013a, 2013b). Otherwise, as developed by Bouassida *et al.* (2017, 2018), SPB1 lipopeptide was incorporated in toothpaste and detergent formulas. The toothpaste formula proved its cleaning ability on eggs with potentially important antimicrobial activity, which was very effective against *Klebsiella pneumonia* (Bouassida *et al.* 2017).

Owing to its excellent emulsification property, *B. subtilis* SPB1 BioS was used in the present work in a cosmetic cream formulation. Also, a Box–Behnken (BB) design was applied to optimize the quantities of SPB1 BioS, glycerol and gum Arabic (GA) as potential stable formulas containing BioS for commercial use. The formula was characterized by measuring the ST and the emulsification index (EI).

MATERIALS AND METHODS

Microorganism strain and biosurfactant production

B. subtilis SPB1 (HQ392822) was isolated from Tunisian hydrocarbon-contaminated soil and identified by morphological and 16S (rDNA) sequence analysis (Ghribi *et al.* 2012a, ERTY5). It was selected based on the high emulsification activity of its BioS, which belongs to the class of lipopeptides (Mnif *et al.* 2016a). It serves for BioS production by suspension of one loop of cells in 50 mL of fresh LB medium and incubated on a shaker until an OD_{600} of almost three is reached. This culture broth was used to inoculate the production medium, composed of glucose, yeast extract and other salts (KH_2PO_4, K_2HPO_4 and $MgSO_4$), to start with an initial optical

density of 0.15. After its incubation for 48 h at 37 °C and 150 rpm, the culture was centrifuged at 10,000 rpm and 4 °C for 20 min to remove cells and the supernatant served to extract BioS (Mnif *et al.* 2021a, 2021b, 2021c).

Preparation of the crude lipopeptide powder

The crude lipopeptide powder was prepared as described by Mnif *et al.* (2021a, 2021b, 2021c) and Bouassida *et al.* (2018). The supernatant-free cell was precipitated, by adding HCl solution (6 N) to achieve a final pH of 2.0, for 18 h at 4 °C. The white pellet formed was recuperated by centrifugation at 10,000 rpm and 4 °C for 20 min, dissolved in alkaline water. The pH was adjusted to 8 by NaOH 1N and the whole was centrifuged another time to eliminate the residual bacterial cells. The supernatant collected was followed by a second acid precipitation (HCl 6 N) and then centrifuged. The final pellet formed was washed three times with acid water (pH = 2) by repetitive centrifugation. The washed pellet was suspended in alkaline water (pH = 8) and then lyophilized. The obtained extract corresponds to the crude lipopeptide preparation used in the rest of this study.

Formulations of BioS-based skin cream

The crude BioS was used for the preparation of the skin cream formulation. As described by Kumar *et al.* (2012), with slight modifications, the formulation was composed of liquid paraffin (46%), white beeswax (23%) and purified water (31%).

Appropriate standard methods of fusion were adopted, where the solid fats were melted and mixed in hot water and known amounts of BioS or a chemical surfactant called sodium lauryl sulphate (SLS) were added at increasing concentrations of 5, 8, 10 and 15%. The different concentrations used define four formulations, as indicated in Table 1. The ingredients are stirred until a homogeneous cream is obtained. All preparations were packed in wide-mouthed plastic jars with screw-capped lids (Dally & Coulibaly 2007).

Table 1 | Physicochemical properties of formulated skin cream

Formulation	Témoin négatif T	Formulation 1		Formulation 2		Formulation 3		Formulation 4	
		F1-BioS	F1-SLS	F2-BioS	F2-SLS	F3-BioS	F3-SLS	F4-BioS	F4-SLS
Surfactant (%)	0	5		8		10		15	
pH	4.21	5.18	6.94	5.15	7.27	5.2	7.21	5.01	7.46
Water activity	0.506	0.811	0.970	0.844	0.970	0.842	0.964	0.720	0.974
Spreadability (mm)	30	26	37	22	38	24	37	23	50

Determination of pH

It was determined at room temperature (25 °C), using a previously calibrated pH meter (Metrohm-744 pH meter-Switzerland).

Determination of water activity (aw)

It was measured using a Novasina Aw Sprint TH-500 (Switzerland) at room temperature.

Determination of spreading ability

0.5 g of the formulation was placed at the center of a glass slide and covered with another glass slide. 1 kg of weight was carefully placed on a covered glass plate. After 10 min, the weight was removed and the diameter of the paste was measured in millimeters (Bouassida *et al.* 2017).

Determination of antioxidant activity

The formulation was tested for its antioxidant properties by using the free stable radical 2,2- diphenyl-1-picrylhydrazyl (DPPH) as described by Saadaoui *et al.* (2019).

Antimicrobial assay

The *in vitro* antibacterial activity of the tested formulation was evaluated against seven strains of microorganisms using the well diffusion method, as described by Bouassida *et al.* (2017): *Salmonella typhinirium* (ATCC 19430), *Enterobacter* sp., *Listeria monocytogenes* (ATCC 43251), *Klebsiella pneumoniae* (ATCC 13883), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922) and *Salmonella enterica* (ATCC 43972).

Elaboration of a liquid formula based on SPB1 BioS: optimization by a BB design

Design of the experiments

The experimental methodology was designed to formulate a liquid preparation composed of SPB1 BioS, glycerol and GA for further incorporation in different cosmetic preparations. Experimental designs were modelled to optimize the emulsification activity of the formula and the ST. SPB1 BioS (X_1) was used as an active ingredient and glycerol (X_2) and GA (X_3) were used as additives.

To determine the optimum levels of these variables, to predict the possible interactions between the selected factors and to elaborate on the best formulation with better functional properties, a BB design was adopted. The design was generated using Nemrod-W Version 2007 software (LPRAI, Marseille, France). Each variable was assessed at three coded levels (-1, 0, and $+1$). A total of 16 experiments were conducted, including 2^3 full factorial design experiments (runs N°1– 8), 4 axial points (runs N°9–12) and 4 replicates in the domain center (runs N°13 to 16) to estimate the variability of the experimental results (Table 2). The response values (Y) used in each trial were the average of the duplicates (Table 2). The formula's efficiency was evaluated by EI-24% and ST.

Table 2 | BB design and observed and predicted results for EI-24 and ST

				Y_1: EI-24		Y_2: ST (mN/m)	
N°Exp	X_1: SPB1 BioS (%)	X_2: Glycerol (%)	X_3: GA (%)	Observed	Predicted	Observed	Predicted
1	20	5	1.5	66.67	65.56	45.5	44.56
2	80	5	1.5	82.6	79.66	38.2	38.96
3	20	10	1.5	61.90	64.83	41.83	41.06
4	80	10	1.5	81.82	82.92	39.2	40.13
5	20	7.5	0.5	66.67	66.80	41.75	42.55
6	80	7.5	0.5	80.97	82.93	40.02	39.12
7	20	7.5	2.5	66.67	64.70	41.9	42.79
8	80	7.5	2.5	80.90	80.76	40.5	39.69
9	50	5	0.5	76.19	77.16	42.3	42.43
10	50	10	0.5	80.95	77.88	40.4	40.36
11	50	5	2.5	71.43	74.49	41.9	41.93
12	50	10	2.5	77.27	76.29	41.8	41.67
13	50	7.5	1.5	76.19	76.26	41.8	42.22
14	50	7.5	1.5	76.19	76.26	42.4	42.22
15	50	7.5	1.5	76.40	76.26	42.3	42.22
16	50	7.5	1.5	76.25	76.26	42.2	42.22
17	50	7.5	1.5	76.30	76.26	42.4	42.22

Statistical analysis and modeling

The data obtained from the response surface methodology with regard to the formula functional properties were subjected to an analysis of variance (ANOVA). ANOVA permits us to estimate the significance of each parameter and check for errors. EI-24% and ST were taken as responses (Y_1 and Y_2, respectively). An empirical model that could relate the response measured to the independent variables was obtained by the multiple regression analysis. The following quadratic equation describes the behavior of the system:

$$Y = b_0 + b_1{*}X_1 + b_2{*}X_2 + b_3X_3 + b_{1-1}{*}X_1^2 + b_{2-2}{*}X_2^2 + b_{3-3}{*}X_3^2 + b_{1-2}{*}X_1{*}X_2 + b_{1-3}{*}X_1{*}X_3 + b_{2-3}{*}X_2{*}X_3$$

where X_1, X_2 and X_3 were the coded factors studied (Table 2), b_0 the intercept, b_1, b_2 and b_3 the linear coefficients, $b_{1\text{-}1}$, $b_{2\text{-}2}$ and $b_{3\text{-}3}$ the squared coefficients and $b_{1\text{-}2}$, $b_{1\text{-}3}$ and $b_{2\text{-}3}$ the interaction coefficients.

The multi-linear regression analysis permits to estimate the model coefficients using the statistical software package (Nemrod-W by LPRAI Marseilles, France). The *F*-test with unequal variance (p 0.05) was used to assess the statistical significance of the model using multiple regression analysis. On the experimental data, regression analysis was performed and response surface graphs were generated. They represented the behaviour of the system. To describe the individual and cumulative effects of the variables, the two-dimensional graphical representation called the iso-response contour was plotted. They also allow for the prediction of potential correlations between them (Mnif *et al.* 2021c).

Characterization of the formula

Determination of the ST

The ST was measured according to the Du Noüy Ring method by a model Tensiometer Sigma 700 at room temperature. Distilled water was used as a negative control (Mnif *et al.* 2021a, 2021b).

Determination of the EI (24%)

As described by Ben Ayed *et al.* (2015), 2 mL sample was deposited in a tube containing 2 mL of kerosene. After rigorous agitation by the vortex for 2 min, we left the mixture at rest for 24 h at room temperature. The emulsification activity was determined by measuring the EI-24%, which was calculated as follows in the equation:

$$\text{EI} - 24\ (\%) = \frac{\text{Height of the emulsion}}{\text{Total height of the mixture}} \times 100$$

RESULTS AND DISCUSSION

Application of BioS in skin cream formulation

Cosmetics and personal care products are present in the lives of individuals on a daily basis in the form of toothpaste, shampoo, creams and makeup, among others (Ferreira *et al.* 2017; Bezerra *et al.* 2018; Sharma *et al.* 2021). The current trend among consumers is the use of natural ingredients in cosmetic products, as many of these items offer the same or even additional benefits as chemical-based products (Marinoa *et al.* 2020). Large companies in the cosmetic sector have an average of around 10,000 different cosmetic products and reformulate 25–30% of these products each year. About 10% of these reformulations depend on new active ingredients for the market or the industry. Such companies introduce up to 80 new ingredients into their product portfolios each year (Sarubbo *et al.* 2022). In this context, biosurfactants constitute an option for meeting the demand for new ingredients. For example, these biomolecules are used by Relipidium™ (body and face moisturizer, produced by BASF, Monheim, Germany), Sopholiance™S (deodorant, face cleaner and shower gel, produced by Givaudan Active Beauty, Paris, France) and Kanebo skincare (moisturizer, cleansing and UV filter, produced by Kanebo Cosmetics, Tokyo, Japan) (Adu *et al.* 2020). Moreover, some cosmetic firms in the USA have developed and marketed lipopeptide-based products that show a direct effect on the stimulation of collagen and elastin production (Mandal *et al.* 2013). In addition, the German chemical company Evonik, which managed to develop biotechnological methods to produce microbial biosurfactants on an industrial scale in 2010, is currently developing technologies for the production of rhamnolipids for application as foam promoters in cosmetic products, confirming the applicability and the interest of the cosmetic industry in the use of biosurfactants as new active ingredients in formulations (Sarubbo *et al.* 2022).

In this regard, we studied the possibility of incorporating the BioS obtained from *B. subtilis* as a stabilizing agent of the oil-in-water cosmetic cream formulation.

Evaluation of physicochemical properties

The majority of cosmetic creams are viscous liquids with opaque appearances or semi-solid preparations (Okafo *et al.* 2023) with an exact proportion of oily and watery phases that require a stabilizing agent such as emulsifiers and/or surfactants to produce good emulsions (Dao *et al.* 2018). Their rheological character and consistency depend on the emulsion type, oil-in-water (o/w) or water-in-oil (w/o) (Okafo *et al.* 2023). Surfactants are amphiphilic compounds containing a water-soluble head with hydrophilic groups and a tail that is insoluble in water, presenting hydrophobic groups. Thanks to this structure, these substances have the ability to diffuse in water and to place themselves between air/water or oil/water interfaces, solubilizing hydrophobic compounds in water and

giving stable emulsions (Moldes *et al.* 2021). The use of either SPB1 BioS or SLS as emulsion agents implies the homogeneity of the tested formulas.

Moreover, the results showed that the creams were soft, easy to spread, and applied easily to the skin.

The results of the physicochemical characteristics of the different formulations are presented in Table 1. It is clear that formulations based on chemical surfactants had a pH close to neutrality, while formulations based on BioS remained acidic, being suitable for skin application, since the pH of skin products should be in the range of 4.5–6.5 (Couto *et al.* 2020). Therefore, there would be a very minute risk of skin irritation (Okafo *et al.* 2023). It has been stated that the pH of the skin is slightly acidic, functioning as a natural defense against the growth of pathogenic bacteria or fungus, although this may be altered depending on body cells, age and the presence of natural skin components, such as fatty acids, which control human skin (Ferreira *et al.* 2017).

The spreadability of cream refers to its ability to spread evenly on the surface of the skin. This characteristic plays a vital role in administering the standard dose to different parts of the skin and determining its efficacy as a topical treatment (Okafo *et al.*, 2023). Spreadability is defined as the force required to apply the product on the skin until it is fully absorbed, and it is directly related to the cream's viscosity. (Böger *et al.* 2023). The spreading ability test showed that the BioS-formulated creams presented different values ranging from 22 to 26 mm. However, SLS-formulated creams presented values higher than 37 mm. Respecting the water activity (aw), all BioS-formulations presented comparable values between 0.81 and 0.84 with the exception of Formula 4, which had an aw of 0.72. Values close to those of BioS-formulation ranging between 0.96 and 0.975 were observed in the case of SLS-formulation. Das *et al.* (2013) reported that the 'aw' and spreading ability might reflect the consistency of the product, as the large spread area showed its better consistency. The analysis of these results allows us to conclude that the SPB1 BioS can replace SLS in the formulation of creams.

Antibacterial activities

Most cosmetic products are considered to be ideal growth media for microorganisms due to their composition. However, when a cosmetic product is contaminated with microorganisms, it can be harmful to consumers and may cause skin infections. Moreover, the proliferation of microorganisms can affect the stability, pH, colour or odour of products. Generally, the abolishment of an effective conservation system significantly increases the growth of several bacteria, mold or yeast (Berthele *et al.* 2014). It was reported that multifunctional antimicrobial compounds, pH adjustments in formulations, reducing the water activity in products and adequate packaging of finished products are all alternatives methods for limiting microbial growth (Dao *et al.* 2018).

The optimal pH for most bacteria's development has been reported to be around neutrality. Microbial development can be totally inhibited by pH extremes, whether acidic or basic. The pH range in which microorganisms can grow is determined by their species (Berthele *et al.* 2014).

In another hand, a minimum of 0.61 'aw' is required to support any microbial growth. Therefore, it was reported that the proliferation of microorganisms within a drug product can be controlled by reducing the water activity of the product (Dao *et al.* 2018).

In order to guarantee the conservation of the formulated cream, since the formulated skin cream has similar values of pH, we choose Formula 4 as it had the lowest 'aw' value for the rest of the experiments. As described in our previous work, the SPB1 BioS was effective against bacteria with multidrug-resistant profiles (Ghribi *et al.* 2012a). Therefore, we propose to investigate the antimicrobial capacity of the formulated BioS-added cream. As shown in Table 3, the formulated cream presented a clear inhibition zone against all of the tested bacteria, which indicated the level of antimicrobial activity present in the product. As remarked, the greatest inhibition

Table 3 | Antimicrobial activity of Formula 4 containing the SPB1 BioS against microorganisms (mm)

Bacteria	*Salmonella typhimurium* (ATCC 19430)	*Enterobacter* sp	*Listeria monocytogenes* (ATCC 43251)	*Klebsiella Enterococcus faecalis* (ATCC 13883)	*Enterococcus faecalis* (ATCC 29212)	*Escherichia coli* (ATCC 25922)	*Salmonella enterica* (ATCC 43972)
F4 (200 mg/mL)	13	10	10	15	10	11	8
Ampicillin (200 mg/mL)	15	22	12	28	23	38	24

diameter was observed against *K. pneumoniae* (15 mm). A larger zone of inhibition usually implies a higher antimicrobial agent efficiency (Bouassida *et al.* 2017).

Antioxidant activity

Environmental insult to human skin by ultraviolet (UV) radiation, as well as by cigarette smoke and air pollution, generates reactive oxygen intermediates that contribute to both acute and chronic skin damage. As largely recognized, the incorporation of antioxidants in skin care products protected effectively skin against free radical-mediated oxidative damage. Also, due to the rise in the photo damage in an aging population, the topical application of cosmetic products that contain antioxidants has become an important area of research in skin care products (Hoang *et al.* 2021).

Generally, BioS are well known by their antioxidant activity. In this study, a skin cosmetic formulation containing 15% BioS of SPB1 had an IC50 of the order of 68 mg/mL (Figure 1). The IC50 is the inhibitory concentration in mg/mL of product able to reduce the absorbance of DPPH by 50%. The lower the IC_{50} the higher the antioxidant activity (Theansungnoen *et al.* 2022). In our previous research, the scavenging effect of *B. subtilis* SPB1 BioS on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical at 1 mg/mL was 70.4% (IC50 = 0.55 mg/mL) (Zouari *et al.* 2016). Similarly, diverse glycolipid biosurfactants (Takahashi *et al.* 2012; Ribeiro *et al.* 2020; Silva *et al.* 2020) and lipopeptide biosurfactants (Yalcin & Çavuşoğlu 2010; Kiran *et al.* 2017; Jemil *et al.* 2017; Basit *et al.* 2018) have been shown to exhibit antioxidant properties. For lipopeptides, as reported by Jemil *et al.* (2017), the scavenging effect can be assigned to the presence of the hydrocarbon fatty acid chain and some active residues in the peptide ring. They can act as effective hydrogen atom or electron donors and are capable of scavenging free radicals like DPPH.

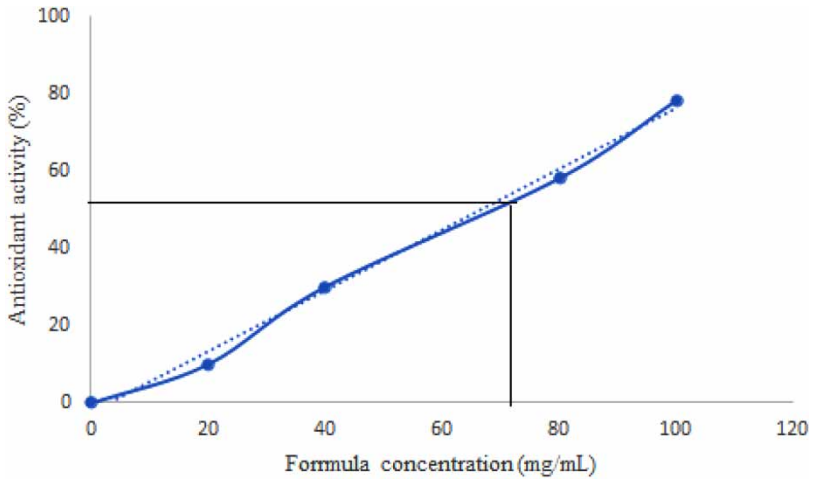

Figure 1 | The antioxidant activity of Formula 4.

Formula optimization by a BB design

A formulated product is the result of a combination or mixture of various materials of synthetic or natural origin, which are generally classified into two groups: active components and additives (Mnif *et al.* 2023). In this study, we tried to highlight a stable SPB1 BioS-based formulation that can be used in cosmetic products. Therefore, we used SPB1 BioS (X_1) as an active ingredient and glycerol (X_2) and GA (X_3) as additives.

Being non-toxic, edible and biodegradable, glycerol will provide important environmental benefits to the newly formulated products. It is a major ingredient in cosmetics and may be used as a lubricant to improve smoothness and as well as a humectant and moistener (Mellou *et al.* 2019) to improve skin hydration and cutaneous elasticity (Becker *et al.* 2019). Moreover, it possesses a keratolytic effect and anti-irritant properties (Becker *et al.* 2019). It, also, plasticizes the skin, reduces light scattering and accelerates the wound healing process. Additionally, it has been described as having diverse influences on the skin, including an antimicrobial effect.

GA, also called *Acacia gum*, is an amphiphilic branched polysaccharide. Thanks to their good emulsifying and encapsulating properties, high solubility and low viscosity in solution, it is widely used in the food and cosmetic

industries (Ashour *et al.* 2022). Being able to impart spreading properties, increase viscosity and provide a covering coating and a smooth feel, GA functions as a stabilizer in lotions and protective creams in cosmetics. It can be used, also, as an adhesive agent in a blusher and as a foam stabilizer in liquid soaps (Ashour *et al.* 2022).

We adopted a BB plan to determine the relative proportions of the different components for better emulsifying and surface properties of the prepared formulation. The operating conditions of the 17 experiments of the three-variable BB design are described by the lines of the experimental design shown in Table 2. The results presented in Table 2 are the average of three independent tests. It can be seen that the ST varies from 45.5 to 38.2 mN/m and those of the EI −24(%) from 61.9 to 82.6%, suggesting a wide variability of the results. This confirms the correct choice of the various factors and their assigned levels.

The mathematical models of the two responses studied for controlling the liquid formulations are written as in the following equations:

$$Y1 = 76.26 + 8.047{*}X_1 + 0.631{*}X_2 - 1.064{*}X_3 - 2.838{*}X_1^2 - 0.181{*}X_2^2 + 0,374X_3^2 + 0.998{*}X_1{*}X_2 - 0,017{*}X_1{*}X_3 + 0.27{*}X_2{*}X_3$$

$$Y2 = 42.22 - 1.63{*}X_1 - 0.58{*}X_2 + 0,2{*}X_3 - 0.798{*}X_1^2 - 0,24{*}X_2^2 - 0.380{*}X_3^2 + 1.168{*}X_1{*}X_2 + 0,082{*}X_1{*}X_3 + 0.450{*}X_2{*}X_3$$

With Y_1 and Y_2 referring, respectively, to the two recorded responses of EI-24% and ST and X_1, X_2 and X_3 are, respectively, the coded values of SPB1 BioS, glycerol and GA. The values of the coefficients are calculated by the multiple linear regression method.

The estimated values of the coefficients and their standard deviations and meanings are shown in Table 4. The overall quality of the regression is considered very well with respect to the values of the multi-linear correlation factors R^2 of 0.922 for EI-24% and 0.85 for ST. These values are close to 1 suggesting a strong correlation between experimental and theoretical values (Ghribi *et al.* 2012a). The analyses presented in Table 4 show that the variation of the two responses (EI-24% and ST) is strongly dependent on the three variables. Indeed, we note that most factors (simple interactions; double interactions and mutual interactions between the different factors) significantly affect the EI-24% and the ST.

Table 4 | Estimated effect, regression coefficient and corresponding *t* and *p* values for EI-24 and ST

	Coefficient		*F.* Inflation		Ecart-type		*t.* exp		Significance (%)	
Noun	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2
b_0	76.26	42.22			0.039	0.111	1,940	379.15	<0.01***	<0.01***
b_1	8.047	−1.63	1	1	0.031	0.088	258.8	−18.45	<0.01***	<0.01***
b_2	0.631	−0.58	1	1	0.031	0.088	20.31	−6.63	<0.01***	0.268**
b_3	− 1.064	0.204	1	1	0.031	0.088	−34.2	2.31	<0.01***	8.2
$b_{1\text{-}1}$	− 2.838	−0.798	1	1	0.043	0.121	−66.2	−6.57	<0.01***	0.277**
$b_{2\text{-}2}$	− 0.181	−0.240	1	1	0.043	0.121	−4.21	−1.98	1.36*	11.9
$b_{3\text{-}3}$	0.374	−0.380	1	1	0.043	0.121	8.74	−3.13	0.0944	3.51*
$b_{1\text{-}2}$	0.998	1.168	1	1	0.044	0.124	22.6	9.83	<0.01***	0.072***
$b_{1\text{-}3}$	− 0.017	0.082	1	1	0.044	0.124	−0.40	0.66	1.71	54.4
$b_{2\text{-}3}$	0.27	0.450	1	1	0.044	0.124	6.14	3.61	0.356**	2.25*

NS, nonsignificant.
***Significant at the level of 99.99%.
**Significant at the level of 99.9%.
*Significant at the level of 99%.

The validity of the two models described above is verified by the analysis of the variance presented in Table 5, which shows that the regression is significant for the two responses studied and confirms that the models adopted are adequate to predict the choice of variables to be used. This analysis shows that the total sum of the squares of the average deviations estimated with 12 degrees of freedom is divided into two sums of squares. The first,

Table 5 | ANOVA analysis for EI-24 and ST

Source de variance	Sum of squares		Degree of freedom	Mean square		*F*-value		Significance (Pr > *F*)	
	Y_1	Y_2	Y_1	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2
Regression	569.16	34.49	9	63.24	3.832	8,181.17	61.81	<0.01***	<0.01***
Residual	48.088	6.101	7	6.86	0.871				
Lack of fit	48.057	5.853	3	16.01	1.951	2,072.34	31.46	<0.01***	0.306**
Pure Error	0.030	0.248	4	0.007	0.062				
Total	617.15	40.59	16						

***Significant at the level of 99.99%.

estimated with 3 degrees of freedom, is due to regression and the second, estimated with 9 degrees of freedom, is due to residual variation. On the other hand, the analysis of variances shows that the regression was significant for the two responses studied and that the lack of validity is insignificant.

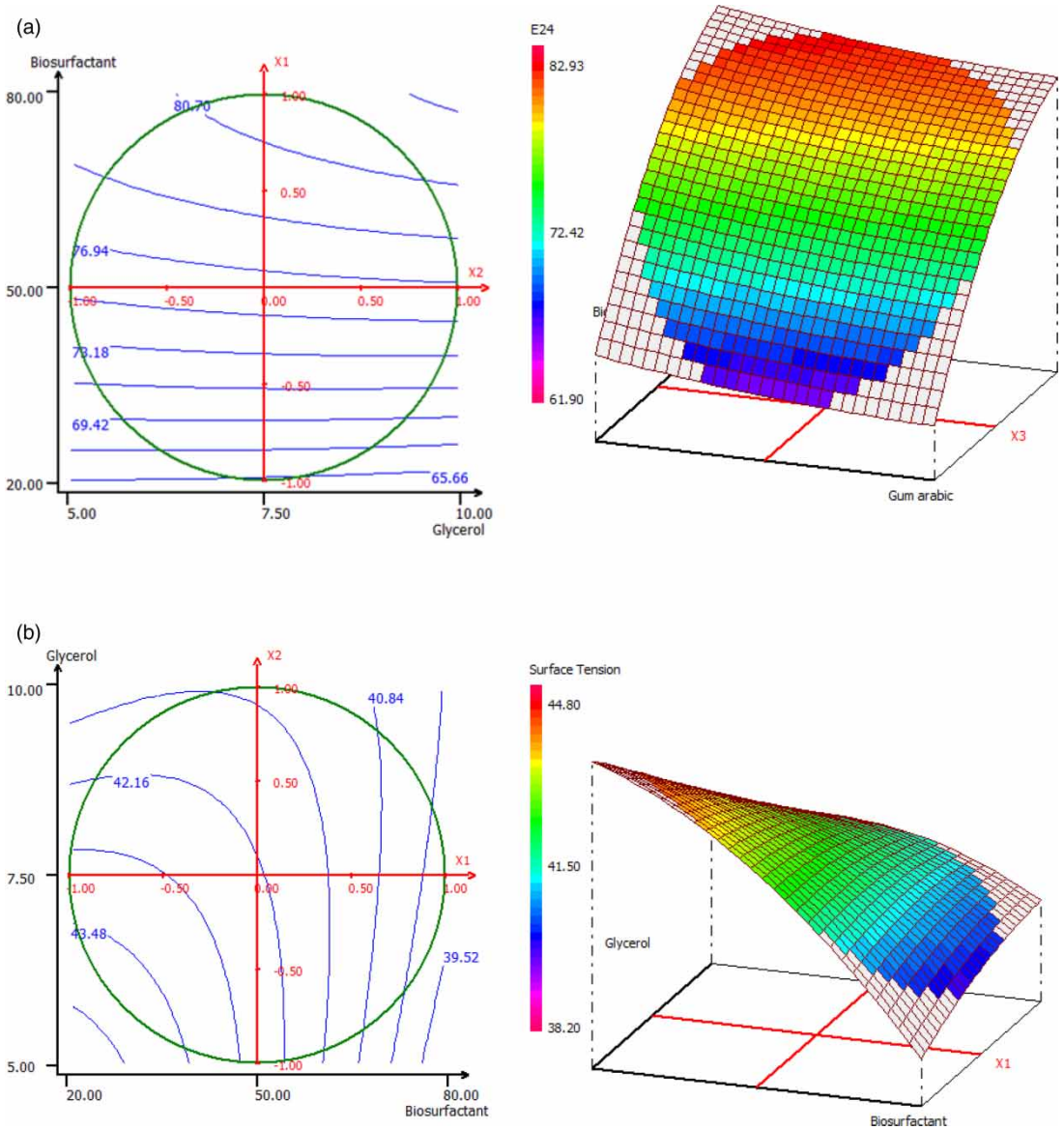

Figure 2 | Effect of the different ingredient's quantities on the functional characteristics of the formula (EI-24 (a) and ST (b)): Response surface plot (left) and its contour plot (right) of interaction between SPB1 BioS (X_1) and glycerol (X_2).

Based on the two theoretical models of the responses studied, the iso-response curves are presented by describing the interactions between two variables while setting the third at its central level (Mnif *et al.* 2021c). Indeed, for the EI-24% study, we set, first, the amount of GA at its zero level ($X_3 = 1.5\%$) (Figure 2(a)). The values of the SPB1 BioS and glycerol percentages, equal to 72.62% and 7.5%, respectively, resulted in an optimal EI-24% value of 80.72% (± 0.12). The iso-responses are almost parallel to the X_2 axis. This suggests that neither increasing nor decreasing the amount of glycerol can significantly affect the percentage of EI-24%, while any increase in the amount of SPB1 BioS improves the percentage of EI-24%.

As analysed in this study, the model predicts a maximum value of EI-24% equal to 80.72% when the respective values of the percentages of SPB1 BioS, glycerol and GA are equal to 72.62%, 7.5% and 1.5%. Similarly, when setting factor X_3 to its zero level ($X_3 = 1.5\%$), we notice (Figure 2(b)) that the ST value depends on the amount of SPB1 BioS (X_1) and glycerol (X_2) above its central level. In addition, we note the improvement in ST based on the amount of SPB1 BioS. Values of X_1, X_2 and X_3, respectively, equal to 77.62%, 8.33% and 1.5% allowed for an optimal value of the ST equal to 40.18 ± 0.39 mN/m. Under these conditions, the EI-24% is of the order of $81.76 \pm 0.14\%$. The optimum conditions obtained were confirmed by three separate experiments, the means of which correspond to an EI-24% of $81.19 \pm 0.35\%$ and a significant decrease of the ST to 41.4 ± 0.85 mN/m. Thanks to their surface activities, the elaborated formulation can be used in the cosmetic industry.

CONCLUSION

In the present work, skin cream formulations using a lipopeptide biosurfactant isolated from *B. subtilis* SPB1 were investigated. Their physicochemical properties and biological activities were satisfactory. The obtained results indicated that the SPB1 biosurfactant was as efficient as the chemical one, confirming its potential utilization in a cream formulation. From a perspective, other studies deem necessary, such as sensory analysis, stability and market studies in order to improve the commercialization of the product. Moreover, we were able to prepare a formulation based on a biosurfactant having an important emulsifying property favouring its exploitation in the cosmetic field.

CONSENT FOR PUBLICATION

All authors read the final manuscript and approved its submission to Water Practice and Technology.

FUNDING

Funding for this research work was granted by the Ministry of Higher Education and Research of Tunisia.

AUTHORS CONTRIBUTIONS

All authors directly participated in the planning, execution, or analysis of this study. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

This work has been supported by grants from the Tunisian Ministry of Higher Education, Scientific Research and Technology. It is a part of a research project on Biosurfactant Production, Characterization and Application.

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PUBLISHER'S NOTE

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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First received 20 November 2022; accepted in revised form 27 March 2023. Available online 10 April 2023