



Implementation of sustainable development goals in the cosmetics industry based on the example of cleansing cosmetics containing a surfactin-rich digestate extract

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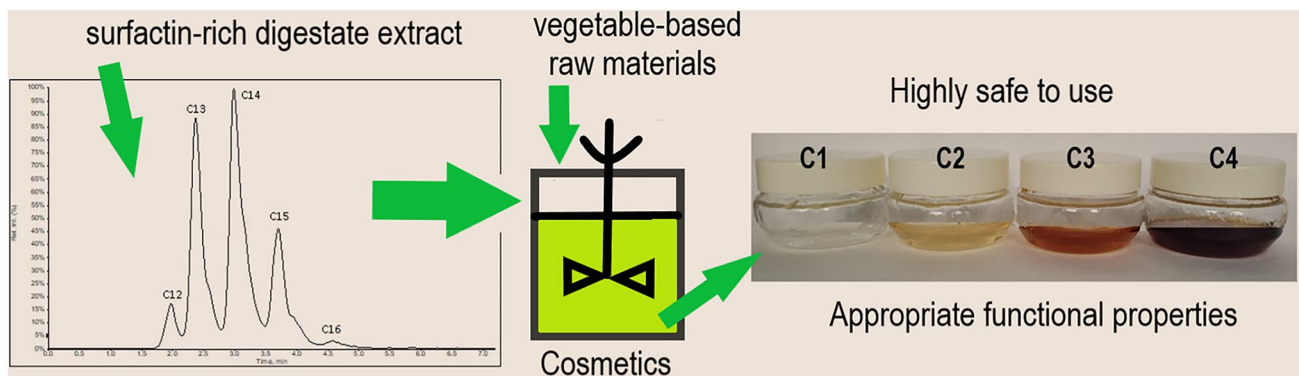
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Abstract

The implementation of sustainable development goals in the cosmetics industry is focused primarily on the phase of product design and the formulation of fully functional cosmetics based on raw materials of natural origin. The aim of the present study was to evaluate the feasibility of using a surfactin-rich digestate extract—a raw material of biotechnological origin—as an ingredient in cleansing cosmetics. A composition was designed, and shower gels based on the digestate extract were formulated. In the next stage of the study, the prototypical products were subjected to a quality assessment with an emphasis on the properties determining their functionality and safety of use by consumers. The shower gels formulated with raw materials of natural origin with the addition of the surfactin-rich extract show acceptable functional properties including viscosity, foaming ability and fat emulsification properties. Importantly, the use of the digestate extract was found to significantly improve the safety in use of the evaluated cosmetic prototypes. This finding is corroborated by a significant decrease in the zein value, which is a measure of the irritant effect of cosmetic formulations. A slight decrease in the ability to emulsify fats found for the tested prototypes confirmed their appropriate functionality and mild effect on the skin. Through the use of the surfactin-rich digestate extract, the cosmetics acquired a natural color derived from the extract. Colorimetric analysis shows that the color changes in the samples should be noticeable even by inexperienced observers.

Graphical Abstract



Keywords Sustainable products · Cosmetics · Biosurfactants · Surfactin · Safety · Quality

Introduction

The last decade has seen a major impact of the concept of sustainability throughout the cosmetics industry (Bom et al. 2019; Kolling et al. 2022). Consumers, and hence also cosmetics manufacturers, are increasingly interested in cosmetic products based on raw materials of natural origin which, by their definition, incorporate the principles of sustainable development at all stages of their life cycle (Amberg and Fogarassy 2019; Kapoor et al. 2019). In particular, widespread concerns about the safety of cosmetics and their impact on the environment have contributed to an increasing scientific interest in this area of study. Of all the stages in the life cycle of a cosmetic product, the development of product formulation and technology is the most crucial one. Designing formulations based on alternative ingredients recognized as more sustainable can be a significant challenge because of their instability, potential lack of the desirable functional parameters, issues related to the safety in use (i.e., effect of cosmetics on skin) (Seweryn 2018; Antignac et al. 2011; Nohynek et al. 2010) and esthetic considerations that are deemed very important by consumers. It is also important to note that the assessment of cosmetics formulated in line with the goal of sustainable development is a multifaceted process that takes into account environmental, social and economic factors as well as the quality and performance of the final product (Bom et al. 2019).

The growing demand for natural cosmetics has led to a recent major increase in the number of scientific studies investigating dedicated raw materials as well as formulations and manufacturing technologies of natural cosmetics (Savic et al. 2021; Goyal and Jerold 2021; Dini and Laneri 2021; Singh et al. 2022; Majchrzak et al. 2022; Fonseca-Santos et al. 2015). However, only some of the available studies evaluate complete cosmetic formulations based on raw materials of natural origin. Information related to this topic is still scattered and incomplete. Most reports address the application of plant extracts (Savic et al. 2021; Nizioł-Łukaszewska et al. 2017; Sikora et al. 2015), surfactants of natural origin (Klimaszewska et al. 2022; Seweryn and Bujak 2018; Nizioł-Łukaszewska and Bujak 2018) and raw materials of biotechnological origin (Rincón-Fontán et al. 2018; Bezerra et al. 2018; Ferreira et al. 2017; Vecino et al. 2017), including surfactin.

High hopes are also pinned on the application of raw materials of biotechnological origin in cosmetics. Biotechnological bioprocesses offer an opportunity to obtain a range of compounds with interesting properties for the cosmetics industry, such as vitamins (Mani 2020) and biopolymers (Velamakanni et al. 2022; Furtado et al. 2022; Luft et al. 2020; Domżał-Kędzia et al. 2019). Also,

there seems to be a great potential in the application of microorganisms in the production of surfactants. In fact, biosurfactants represent a viable alternative to the surfactants of plant or petrochemical origin that are widely used in cosmetics. They are fully natural and have a range of properties that make them potentially desirable cosmetic ingredients (Ferreira et al. 2017; Rincón-Fontán et al. 2018; Bezerra et al. 2018; Vecino et al. 2017; Jahan et al. 2020; Akbari et al. 2018; Lee et al. 2018; Lourith et al. 2009). The properties of surfactin seem to be particularly beneficial in this application. The compound is highly effective in reducing interfacial tension and has a low critical micelle concentration (Jahan et al. 2020; Ganesan and Rangarajan 2021; Bjerk et al. 2021). Research findings show that surfactin has a limited potential for skin irritation and toxicity compared to synthetic surfactants (Fei et al. 2020). Surfactin shows a number of characteristics that are suitable for use in cosmetics. Nonetheless, the range of applications of this compound is still fairly narrow because of high costs of production. Consequently, biosurfactants including surfactin are used in industrial practice only to a limited extent (Singh et al. 2022).

The use of ingredients derived from fermentation processes is known to bring a number of functional benefits in such formulations. Raw materials of this type have been scientifically validated to improve the bioavailability of ingredients that play a major role in skin care applications. Furthermore, the fermentation process itself, through an appropriate selection of processing conditions, offers an opportunity to obtain a specific range of compounds with desired functional properties. It has been shown that such raw materials may contain amino acids and peptides, vitamins, enzymes, fatty acids, minerals, antioxidants, etc. (Majchrzak et al. 2022; Mathur et al. 2020; Domżał-Kędzia et al. 2019; Sirilun et al. 2016). In conventional cosmetics, these compounds must be incorporated into the formulation at the production stage as separate raw materials. In contrast, digestate extracts are mixtures of components that may contribute to a major extent to the process of formulating cosmetic products. The compositions of digestate extracts are largely determined by the selection of microorganisms. Some raw materials obtained by fermentation may have a preservative effect on cosmetic systems (Majchrzak et al. 2022; Lee et al. 2020; Herman et al. 2019), while others display antioxidant or anti-aging effects (Majchrzak et al. 2022; Ziemlewska et al. 2021; Sivamaruthi et al. 2018). The use of digestate extracts as a completely natural source of ingredients that are known to nourish the skin and prevent skin irritation, as well as surfactants (biosurfactants), offering the advantage of not being obtained through chemical synthesis, may be a perfect solution for meeting sustainability goals in the cosmetics industry. It is important to note that the extract in question is a mixture of various ingredients in

water. Consequently, the production of the extract contributes to a significant reduction in the use of water, which is a waste product in the process of obtaining the raw material. Producing a digestate rich in biosurfactants (surfactin) is associated with considerably less water waste compared to the conventional process of obtaining dry surfactin as a commercially available cosmetic raw material. In the current approach to the production process of this biosurfactant, all the water required for manufacturing purposes is waste. In the prototypes of cleansing cosmetics developed for the purpose of the present study, the digestate extract replaces water in the formulation, which also contributes to reducing the final water consumption.

The study was carried out to explore the possibilities for using a surfactin-rich digestate extract in cleansing cosmetics. It was decided that in compliance with the sustainability policy the cosmetic prototypes would be formulated with raw materials of natural origin. The first stage involved a biotechnological process that was aimed to produce a digestate extract with a high content of surfactin with the use of bacteria of the species *Bacillus subtilis*. Tests were carried out on the raw material to confirm the presence of surfactin. The extract was then added to the shower gel prototypes that were formulated entirely with ingredients of natural origin. The composition of the formulations was chosen in such a manner that preservatives conventionally used in cosmetics would not be necessary. The analysis included formulations containing different concentrations of the digestate extract (4, 40; 80%). The raw material replaced water in the product composition. The reference sample used in the study was a cosmetic without the digestate extract. The cosmetic suitability of the surfactin-rich digestate extract was evaluated by analyzing the results of basic physicochemical and functional tests of the formulated product prototypes. The formulations were assessed against a range of criteria including viscosity, color, fat emulsification ability, skin irritation potential and skin drying effect. The aim was to determine whether the surfactin-rich digestate extract may serve as a valuable ingredient of cleansing cosmetics based on natural ingredients and to evaluate the potential benefits of the digestate extract in terms of the application properties of the finished product and its safety of use by consumers.

Materials and methods

Materials

Raw materials for cosmetics

The prototypes shower gels were made using certified, vegetable-based raw materials which are approved for the production of natural products according to COSMOS

standards: disodium cocoyl glutamate (trade name: Plantapon ACG 50; supplier: BASF, Ludwigshafen, Germany), xanthan gum (trade name: Cosphaderm X34; supplier: Cosphatec GmbH, Hamburg, Germany), propanediol (trade name: Cosphaderm Propanediol natural, supplier: Cosphatec GmbH, Hamburg, Germany), lactic acid (trade name: lactic acid 88%, supplier: WarChem, Warsaw, Poland), *Bacillus subtilis* ferment extract, propanediol, surfactin (supplier: InventionBio, Bydgoszcz, Poland) and distilled water.

Fermentation process for surfactin production with *Bacillus subtilis natto* KB1

Bacillus Subtilis fermentation extract was obtained according to (Lewińska 2021) with some modifications. The pre-culture was carried out at 37 °C with shaking the medium at 180 rpm. The initial optical density was set to OD₆₀₀ nm = 0.1. The cultures were grown at 37 °C with continuous shaking at 200 rpm for 72 h. The cultures were then centrifuged (14,000 × g) at 4 °C for 30 min (Sigma 6K15, 12,500 rotor, DJB Labcare Ltd., Newport Pagnell, UK) to remove the bacteria. The precipitation step was omitted and the liquid broth was subjected to ultrafiltration and then reverse osmosis (RO) in order to concentrate the surfactin (Mini UF-NF-RO bench-scale crossflow skid, Convergence). Finally obtained enriched surfactin sample was mixed with propanediol.

Methods

Bacillus fermented extract analysis: confirmation of the surfactin

The mass spectrometry (MS) detection of surfactin sample was performed using a triple quadrupole mass spectrometer (4500 QTRAP, AB Sciex, Concord, ON, Canada), equipped with an electrospray ionization source (ESI) working in positive and negative-scan modes. The surfactin sample was diluted with methanol and directly injected by insert pump with a 10 μL min⁻¹ flow rate. MS spectrum was obtained using full scan mode of the total ions with a mass scan of m/z 50–2000 Da. The identification of selected compounds was conducted by molecular mass and fragment of anion/cation entries of each compound and confirmed by MS² fragmentation. Chromatographic separation of identified surfactin homologues was performed by ultrahigh-performance liquid chromatography, UHPLC (Sciex Exion, AB Sciex, Concord, ON, Canada) coupled to mass spectrometer, working in the multiple reaction monitoring (MRM) scan mode. 100 × 4.6 mm chromatographic column Kinetex 3.5 μm XB-C18 100 Å with iso-butyl side chains and with TMS endcapping stationary phase with similar composition guard column (Phenomenex, Torrance, CA, USA) was used and

maintained at 30 °C. A binary solvent system comprising 0.1% (v/v) aqueous formic acid as solvent A and methanol as solvent B was used under gradient mode during 7.2 min of the run time. The elution conditions applied were as follows: 0.0–0.2 min 80% B, 0.2–6.2 min 85% B, 6.2–6.7 min 85% B and 6.7–7.2 min 80% B. The flow rate of the mobile phase was 0.6 mL/min and injection volume 10 µL. The eluent was monitored by electrospray ionization mass spectrometer (ESI–MS) under positive ion mode. The MS parameters were set as follows: capillary temperature of 650 °C, curtain gas at 35 psi, nebulizer gas at 50 psi and drying gas at 50 psi. 4500 V voltage was applied for positive ionization mode source. Nitrogen was used as curtain and collision gas. Data analysis was processed with Analyst 1.7.2 software.

Bacillus fermented extract analysis: surfactin's quantitative analysis

Surfactin's quantitative analysis was performed with the use of UHPLC-UV system produced by Waters (AcquityArc). Liquid samples after centrifugation were diluted to proper level with methanol, filtered through 0.22 µm nylon syringe filter and injected (10 µL). Detection was done with UV lamp set to 210 nm, and the column used for separation was Cortex C18 (4.6 × 50 mm, 2.7 µm). Mobile phases were water (A) and acetonitrile (B) (both with 0.1% addition of formic acid). The method was 10 min long with increasing B gradient from initial conditions (50% B). Quantitative analysis was done with external standard which was obtained from InventioBio SA biorefinery.

Determination of irritant potential: zein value

Irritant potential of the prototypes cosmetics was measured using zein test. The study was carried out using the automatic mineralization system Digester 8AR and the automatic nitrogen analyzer Kjeltac 8400 (Producer FOSS, Denmark). In the zein test procedure, 2 g of protein was solubilized in 40 g solution of shower gel (10% wt.). The amount of solubilized protein was determined by Kjeldahl analysis, and the result of the zein value procedure was expressed as mg of solubilized protein (calculated as nitrogen) in 100 mL of sample. The final result was the arithmetic mean of three independent measurements. The testing methodology was described by Seweryn and Bujak (2018) and Wasilewski et al. (2016).

Evaluation of ability to emulsify fatty soils

The ability to emulsify fatty soils was evaluated in tests conforming to the PN-C-77003 standard. The test consisted in determining the maximum weight of rapeseed oil which can be emulsified by 1 L of a washing bath containing 1

wt % of the evaluated formulation. The final result (mean value of three independent measurements) obtained in the test determining the ability of the evaluated formulation to emulsify fatty soils was expressed in grams of oil per liter of the evaluated formulation at the concentration of 1 wt %. The test methodology was described by Seweryn and Bujak(2018).

Evaluation of the foaming properties

Foaming properties were tested according to PN-74 C-04801 standard. Foam height was measured using cylinder of the Ross–Miles apparatus. 10% aqueous solutions of tested preparations were investigated. An aliquot of 50 mL of the solution was poured into the cylinder and 200 mL of the solution into a dropper. The dropper was placed 1 m above the liquid level in the cylinder and the dropper tap was turned on. The height of the foam formed in the cylinder was measured after 1 and 10 min. The measurement over time was used to calculate the foam stability (Wasilewski et al. 2022b).

Viscosity measurements

The viscosity of the model shower gel formulations was measured at 20 °C in triplicate, using a Brookfield rheometer DV2TRV with a small sample adapter and cylindrical spindle SC4 (Brookfield, WI, USA). An aliquot of 8 mL of the sample was used in each test. Different shear rates and shear stresses were applied to the sample, and the resulting rheogram was constructed to determine the rheological behavior (Wasilewski et al. 2022b).

Determination of the color parameters

The cosmetics shower gel prototypes, with and without surfactins extracts, were subjected to reflectance color measurements using a Konica Minolta CM-3600 (Konica Minolta, Sensing Singapore Pte Ltd., Tokyo, Japan) with color data software CM-S100w, SpectraMagic NX, version 1.07 (Konica Minolta Sensing Europe B.V., Nieuwegein, the Netherlands) and light source D65, which represented a daylight phase. The CIE LAB system (defined by the International Commission on Illumination in 1978) organized the perception of the three-dimensional color space represented in rectangular coordinates: L*, a* and b*. The L* axis is associated with the lightness of the color: A value of 100 corresponds to white and a value of 0 to black. The axes a* and b* are associated with the changes of red–green and yellow–blue, respectively (+ a* correlates with red, – a* with green, + b* corresponds to yellow, – b* to blue). The chromaticity coordinates (a*, b*) are represented in polar coordinates by the chroma (C*), the Euclidian distance from the lightness axis and

the hue angle (h^*), expressed in degrees, starting from the positive a^* axis (red) and turning in an anticlockwise direction.

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (1)$$

$$h^{\circ} = \arctan \frac{b^*}{a^*} \quad (2)$$

The differences (ΔE) between the color of the cosmetics with fermented extract and base cosmetic (without extracts) were calculated by the formula:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (3)$$

where ΔL^* , Δa^* and Δb^* are the mathematical differences between the cosmetics with extracts L^* , a^* and b^* and the base cosmetic L^* , a^* and b^* values.

Stability study

Stability was assessed under laboratory storage conditions. Samples were stored for 1 month under constant air temperature of 23 °C (air-conditioned room) and conditions of variable sunlight. In addition, the stability of the cosmetics was assessed with a mechanical loading test. After 24 h of detergent preparation, the samples were subjected to centrifugal force using a Rotofix 32 A (producer Zentrifugen) centrifuge. Samples were centrifuged at 2500 rpm for 15 min at room temperature. Microbiological pretesting was carried out. Microbiological stability of the shower gels was evaluated by tests using microcount® duo microbiological testers (Schülke & Mayr GmbH, Norderstedt, Germany). The test plates were stored at 28 °C, for 3 days for bacterial colony and fungi assays, and for 5 days for yeast and mold testing. After this time, the plates were visually evaluated and the number of microorganisms was determined, based on the test manufacturer's template. They were tested for fungal and bacterial colonies and molds or yeasts.

Statistical analysis

For zein value, ability to emulsify fatty soils, foaming ability, foaming stability factor and viscosity data were reported as a mean \pm standard deviation (SD), with 3 replicates for each sample ($n=3$). For comparing the statistical significance of values, ANOVA and a Tukey HSD post hoc test were applied. Differences were considered significant with the p -value was <0.05 . The Statistica version 10 software (StatSoft, Tulsa, OK, USA) was used for calculations.

Results and discussion

Production of surfactin-rich digestate extract

Surfactin is a cyclic lipopeptide described as a powerful biosurfactant and is natively produced by *Bacillus subtilis*. It can be easily detected with UV lamp due to the presence of carbonyl chromophore (210 nm) in peptide ring. It can be successfully separated from other sample components with the use of C18 resin containing columns. Additionally the ease of injection preparation makes HPLC–UV the method of choice in surfactin qualitative and quantitative measurements in liquid samples. Surfactin is a group of structural analogues so for the quantitative analysis their peak's areas were integrated and expressed as overall surfactin in sample (presented in Supplementary Material in Figure S1). During shake-flask fermentation process, *Bacillus subtilis* natto KB1 (which is wild, non-GMO strain) produces surfactin and secretes it to the external environment. Modified Landy medium and used parameters turned out to be most effective method to obtain good surfactin's yield based on the previous research (Lewińska 2021). After 72 h of fermentation, obtained liquid broth was centrifuged to remove bacteria cells and HPLC–UV analysis showed the surfactin yield equal 2.21 g/L. Such solution was subjected to reversed osmosis (RO), but before that step ultrafiltration was necessary to remove all residual cells and solid impurities. Then reversed osmosis was performed with stable crossflow set to 0.5 L/min. After 30 min, the concentrated solution was isolated in which surfactin's concentration achieved 14.9 g/L. It means that RO allowed sevenfold concentration. Then the solution was diluted with propanediol to produce final extract with 10 g/L of surfactin which was used for cosmetic formulations.

Confirmation of the presence of surfactin in the extract

Confirmation of surfactin was carried out through direct injection into an ESI–MS/MS in both negative and positive ionization modes. The fragmentation pattern by ESI–MS/MS analysis of recognized peaks was used to confirm the molecular identity. Analysis in positive mode revealed two set of specific m/z peaks. The first set of peaks belonged to the surfactin family, with strong signals at m/z 1016.6, 1030.5, 1044.5, 1058.5, 1072.6 and 1086.5 Da corresponding to sodiated surfactin homologues pseudomolecular ions $[M+Na]^+$. Second set of peaks corresponded to protonated pseudomolecular ions $[M+H]^+$ of surfactin at m/z 994.5, 1008.6, 1022.5, 1036.5 and 1050.6 Da.

Negative analysis mode confirmed set of peaks belonged to the surfactin family, with strong signals of $[M-H]^-$ at m/z 992.6, 1006.6, 1020.7, 1034.7 and 1048.6 Da. The set of sodiated peaks $[M+Na-H]^-$ with signals at m/z 1014.6, 1028.6, 1042.6, 1056.6 and 1072.6 Da was also detected. The presence of sodiated pseudomolecular ions in negative mode indicated very strong cation chelation ability of surfactin.

Full scan in positive and negative mode revealed surfactin isomers peaks with a mass difference of 14 Da, corresponding to CH_2 group. Moreover, protonated and sodiated surfactin's homologs were fragmented by the collision-induced dissociation (CID)—MS/MS. Their detected amino acid sequences were as follows: glutamic acid–leucine–leucine–valine–aspartic acid–leucine–leucine, but differed in the fatty acid moiety, which consisted β -hydroxy fatty acids of different lengths: C12, C13, C14, C15 and C16. This could be verified by the base fragment ion, m/z 685 Da, common to each precursor ion, as it was a cleavage product of glutamic acid–leucine and fatty acids–leucine, with the net charge retained in the hexapeptide residue (leucine–leucine–valine–aspartic acid–leucine–leucine). Another abundant fragment, common to every species analyzed, was observed at m/z 441 Da. It was the cleavage product of the leucine–leucine–valine–aspartic acid tetrapeptide residue.

The fragment ions of fatty acid in various detected precursor ions differed by 14 mass units (Da). For example, the fragment m/z 356.3 Da and its dehydrated form (m/z 338.3 Da) from the precursor ion at m/z 1022.5 Da were 14 Da smaller than their equivalents (m/z 370.26 and 352.26 Da) from the precursor ion with m/z 1036.5 Da.

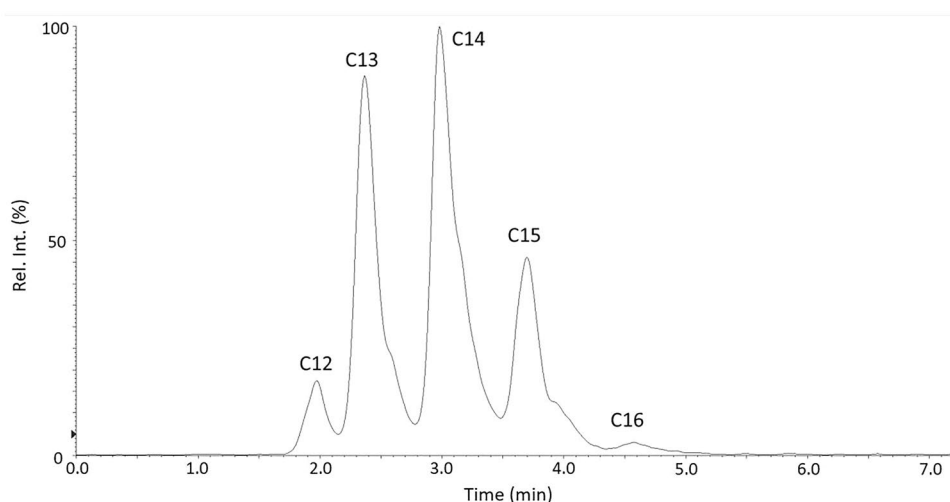
The MS/MS data from the series of precursor ions at m/z 992.6, 1006.6, 1020.6 and 1034.7 Da in negative mode revealed the loss of Leu-Leu-Asp residues (m/z 339.2) from the C terminus generating a complementary lipopeptide fragment with values of m/z 650.4, 664.4, 678.5 and 692.4 Da, respectively. The loss of the β -hydroxy fatty acid from the resulting lipopeptide chain was evidenced by the fragment ion m/z 452.3 (Glu-Leu-Leu-Val) and a further loss of glutamate from the N terminus by the fragment ion m/z 323.2 Da (Leu-Leu-Val).

The corresponding sodiated pseudomolecular ions $[M+Na^+H^+]^-$ at m/z 672.5, 686.4, 700.4 and 714 Da was also indicated with difference in product ion with a m/z of 23 Da. The set of mass peaks, with an interval of 14 Da, proved the presence of surfactin as a mixture of lipopeptide homologues with different length acyl chain corresponding to the number of carbon atoms: C12, C13, C14, C15 and C16. (Table 1). Chromatogram of separated surfactin homologues is presented in Fig. 1. The mass spectra obtained in

Table 1 Identified isomers of surfactin by ESI-MS/MS

Surfactin actual mass	Surfactin homologies	Retention time, min	Detected peaks m/z , Da			
			$[M+H]^+$	$[M+Na]^+$	$[M-H]^-$	$[M+Na-H]^-$
993	C12	2.0	994.5	1016.5	992.6	1014.6
1007	C13	2.4	1008.5	1030.5	1006.6	1028.5
1021	C14	3.0	1022.5	1044.5	1020.7	1042.6
1035	C15	3.7	1036.5	1058.5	1034.7	1056.7
1049	C16	4.6	1050.5	1072.6	1048.7	1072.6

Fig. 1 UPLC-MS/MS chromatogram of surfactin sample



positive and negative mode are presented in Supplementary Material in Figure S2 and S3, respectively.

Formulation and manufacturing technology of cosmetic prototypes

For the purpose of the study, shower gel formulations were developed based on ingredients listed in Table 2. The variable analyzed in the product prototypes was the concentration of the digestate extract.

The base (C1) for the shower gel production with digestate extract consists of (water, disodium cocoyl glutamate—6% w/w, xanthan gum—1% w/w and propanediol) in the proportion described in Table 2. Our new product was composed on the base and addition of 4% w/w (C2), 40% w/w (C3) and 80%w/w (C4) (Table 2). In addition to C1 (bases), water and propanediol was added to maintain the appropriate proportions of compounds used to produce gels.

The cosmetic prototypes were obtained using an MZUTL 5 homogenizing mixer (Urlinski, Warsaw, Poland). A total of 5 L of cosmetic formulation was obtained per single batch. The digestate extract and water were introduced into the mixer. Agitation (50 rpm) and heating (1 °C/min) were started. The contents were heated to 40 °C. Other ingredients were added in a sequence specified in the formula. The components were mixed until a homogeneous mixture was obtained. To maintain a constant concentration of propanediol in each sample (due to its presence in the digestate extract), it was added to the prototypes, as necessary, to achieve a constant concentration of 20%. The variable analyzed in the study was the concentration of surfactin in the digestate extract. A polymeric thickener (xanthan gum) was incorporated into the formulation by dispensing it onto the surface of the liquid during agitation. The ingredients were mixed until a clear homogeneous solution was obtained. In the final step, the pH was adjusted with lactic acid to a value of ~5.5.

Table 2 Model formulations of shower gel

Name according to INCI	Concentration [% w/w]			
	C1	C2	C3	C4
Aqua	to 100	to 100	to 100	to 100
Disodium Cocoyl Glutamate	6.0	6.0	6.0	6.0
Xanthan Gum	1.0	1.0	1.0	1.0
Propanediol	20.0	19.2	12.0	4.0
<i>Bacillus Subtilis</i> ferment extract, Propanediol, Surfactin	0.0	4.0	40.0	80.0
Lactic Acid	to pH 5.5			

INCI International nomenclature of cosmetic ingredients

The samples were left to stand for 24 h and stored at room temperature until the system was completely deaerated. Their stability was then tested. The formulated shower gel prototypes exhibited full physicochemical and microbiological stability during the period of sample storage under laboratory conditions: mechanical loading test and preliminary microbiological tests. Preliminary tests found that the products did not require any conventional (commonly used) preservative systems owing to the presence of propanediol in the formulations. Consequently, the prototypical shower gels were not stabilized with any preservatives. The main task of the preservative is to keep the product in the same microbiological purity in which it was produced. Unfortunately preservatives are known to cause physical instability in dispersed systems. Additionally, as usually they are toxic, irritating and allergenic, modern and ecological cosmetics are looking for components with antimicrobial functions (Nowak et al. 2021; Herman 2019; Dréno et al. 2019; Halla et al. 2018). Ferment from biotechnological processes are rich in natural substances with these properties (Majchrzak et al. 2022; Lee et al. 2020; Herman 2019). The surfactin-rich ferment used in our research was enriched with propanediol, which is an emerging class of multifunctional—ingredients that besides antimicrobial properties (Herman 2019; Kerdudo et al. 2015), possess skin-caring properties (Tse et al. 2021). In addition, the surfactin found in the extract also has antimicrobial properties. Previous studies have described its antimicrobial activities against various pathogenic bacteria, such as *Enterococcus faecalis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Chen et al. 2022; Fernandes et al. 2007). However, recent studies have questioned the general claim of antimicrobial properties of surfactin (Lilge et al. 2022).

Safety assessment of prototype cosmetics

Eco-friendly approach to the development of cosmetics requires the pursuit of sustainability goals which involve primarily the use of naturally derived ingredients in the process of cosmetic product formulation. Efforts to develop cosmetics that are consistent as closely as possible with the concept of sustainability should also take into account the aspect of safety in use and, more specifically, the potential impact of the product on the consumer (Dini et al. 2021; Boom et al. 2019; Barbulova et al. 2015). Cosmetics based on raw materials of natural origin are considered to be fully safe (Fonseca-Santos et al. 2015) and within the territory of the European Union, all marketed cosmetics are subject to a rigorous safety assessment in accordance with the legislation in force (Pauwels and Rogiers 2010). Even though commercially available products are certified as safe, there are occasional reports of adverse reactions experienced by consumers after using them. These include skin irritation

(Mijaljica et al. 2022; Seweryn 2018; Ananthapadmanabhan et al. 2004), contact allergies (Alani et al. 2013), sensation of dry skin or other intolerances associated with the contact of a given cosmetic with the skin (Mijaljica et al. 2022; Seweryn 2018; Ananthapadmanabhan et al. 2004).

As part of the study, the formulated prototypes of cleansing cosmetics were analyzed with regard to the risk of skin irritation following their application. The skin irritation effect of the cosmetic prototypes was evaluated with a method based on the zein value (ZV) which, according to state-of-the-art knowledge, is a measure of the irritant effect induced by cleansing cosmetics on the skin (Cohen et al. 2016; Wardet al. 1998; Pezron et al. 1996). The results are shown in Fig. 2.

The analyzed prototypes of cleansing cosmetics are characterized by relatively low ZV values, ranging from 15 to 80 mg N/100 mL. Based on the literature, these values indicate that the prototypical cosmetics have no skin irritation properties (Pezron et al. 1996). It is important to note that, compared to the reference formulation C1, an increase in the concentration of the surfactin-rich digestate extract in the cleansing cosmetic prototypes led to a significant decrease in the ZV value. The lowest ZV, i.e., 15 mg N/100 mL, was obtained for the sample C4 containing the studied extract at the highest concentration. The decrease in the value of the zein number, and hence the probability of skin irritation developing after the application of the cosmetic prototypes on the skin, is linked to the use of the surfactin-rich digestate extract in the formulation. The irritant effect of cleansing cosmetics is associated predominantly with potential electrostatic interactions between the surfactants present in the form of monomers and the proteins building the stratum corneum. In addition, surfactants may potentially interfere with the liquid crystalline structure of the intercellular cement in the stratum

corneum, which may damage the stratum corneum and impair the epidermal barrier. This type of action is mainly attributed to ionic (including anionic) surfactants, which are the primary ingredients in cleansing cosmetics. The established methods for reducing the irritant effect of cosmetics formulated with anionic surfactants mostly involve efforts to stabilize the micellar structure of surfactants in the bulk phase, increasing the size of aggregates forming in the solution or binding surfactant monomers in the surfactant–polymer system (Morris et al. 2022, 2019; Seweryn et al. 2018). In practice, it is possible to reduce the skin irritation effect of anionic surfactants by adding to the formulation anionic surfactants with proven low irritant potential (Seweryn and Bujak 2018; Klimaszewska et al. 2017), another type of surfactant (nonionic or amphoteric) (Klimaszewska et al. 2022), macromolecular compounds (including polymers or protein hydrolysates) (Bujak et al. 2015), hydrophobic substances (Wasilewski et al. 2016; Mukherjee et al. 2015) or plant-based extracts (Nizioł-Lukaszewska 2017).

The solution proposed by the authors was aimed at achieving a reduction in the irritant effect of the formulation through the use of a surfactin-rich digestate extract. In addition to containing a significant proportion of a biosurfactant, this raw material is a source of many cosmetologically valuable ingredients, including proteins, sugars, mineral salts and small amounts of hydrophobic components. Both surfactin, which has been shown in the literature to have a low skin irritation potential (Fei et al. 2020; Vecino et al. 2017), and the remaining ingredients may contribute to a significant decrease in the irritant effect of the entire cosmetic formulation by various mechanisms (increase in micelle size and stability, formation of complexes between macromolecular substances and surfactants leading to the binding of free monomers in the bulk phase). This effect was observed in the studies performed for the formulated cosmetic prototypes. Despite the addition of surfactants (in the form of surfactin in the digestate extract) into the formulation, a decrease in the irritant effect was noted, even though an increase in the concentration of conventional surfactants in aqueous solutions normally amplifies the irritant effect (Seweryn 2018). The observed decrease in the zein number, corresponding to a reduction in the skin irritation effect of cosmetics, is consistent with the findings of another study conducted by the Authors (Wasilewski et al. 2022a) to determine the possible applications of a levan-rich digestate extract. The study found that a decrease in the skin irritation potential of cosmetics is most likely attributable to the levan–surfactant interactions, the formation of levan–surfactant complexes and the presence of other ingredients with important cosmetic properties.

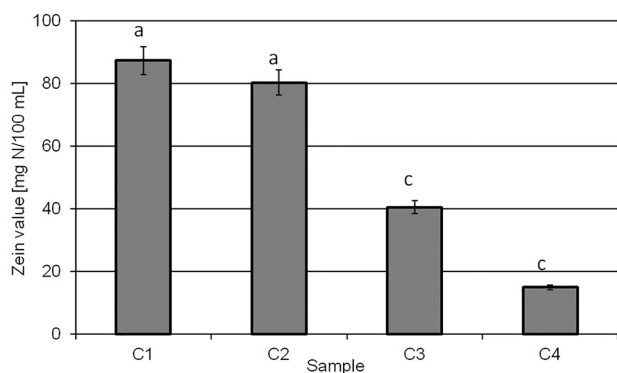


Fig. 2 Zein value for shower gels formulated with the digestate extract. The superscripts a, b and c next to points indicate significant ($p < 0.05$) differences between sample C1 and samples C2, C3 and C4 for zein value

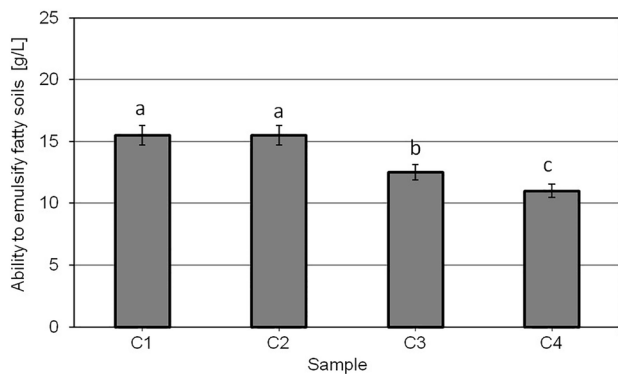


Fig. 3 Ability to emulsify fatty soils for prototypes shower gels. The superscripts a, b and c next to points indicate significant ($p < 0.05$) differences between sample C1 and samples C2, C3 and C4 for the value of the parameter evaluated

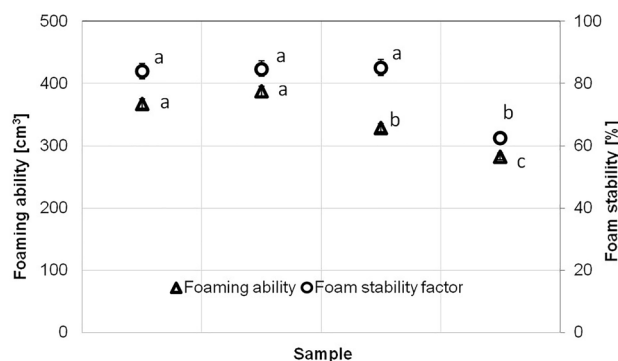


Fig. 4 Foaming properties of the investigated prototypes of shower gels. The superscripts a, b and c next to points indicate significant ($p < 0.05$) differences between sample C1 and samples C2, C3 and C4 for foaming ability and foam stability

Evaluation of functional properties of prototype cosmetics

In addition to aspects related to safety in use, and specifically the potential to cause skin irritation and drying, comprehensive evaluation of the quality of cleansing cosmetics by consumers also includes product properties associated with its intended function. Cleansing cosmetics are expected to deliver appropriate washing performance and good foaming properties (Seweryn and Bujak 2018; Seweryn et al. 2018). The formulated prototypes of cleansing cosmetics were evaluated to determine their ability to emulsify fatty soils and foaming capacity. The results are shown in Figs. 3 and 4.

Cosmetics are subject to rigorous quality assessment by consumers. With respect to body wash cosmetics, consumers expect not only appropriate performance related specifically to their intended purpose, but also specific features that prevent excessive skin drying after the process of skin

washing. The skin drying effect which may be observed after the application of cleansing cosmetics is attributed to excessive removal of fatty components from the skin's surface. This effect is closely linked to the skin washing process and the ability of the washing bath to emulsify and solubilize hydrophobic components from the skin. The greatest risks are associated with the extraction of components of the intercellular cement of the stratum corneum (Morris et al. 2022; Ananthapadmanabhan et al. 2013; Corazza et al. 2010), changes in the pH of the skin due to disturbances of the bacterial flora and disruptions affecting the hydro-lipidic film and the components of the natural moisturizing factor (Mijaljica et al. 2022; Ananthapadmanabhan et al. 2013; Ananthapadmanabhan et al. 2004). These processes may damage the integrity of the epidermal barrier, leading to excessive water loss from the epidermis and causing epidermal dryness (Green et al. 2022; Lukić et al. 2021; Ananthapadmanabhan et al. 2013; Corazza et al. 2010). The ability of cleansing cosmetics to emulsify fatty soils can be evaluated both as a parameter of product functionality and as a measure of safety, related to the potential negative impact of such cosmetics on the skin. Emulsification is one of the sub-steps of the body washing process that allows effective removal of soiling from the surface of the skin. By determining the value of this parameter, it is possible to evaluate the washing performance of cleansing cosmetics (Seweryn and Bujak 2018). On the other hand, excessively powerful emulsification of hydrophobic soiling from the surface of the skin may lead to the leaching of valuable lipid components from the epidermis. The values describing the ability of the prototypical cleansing cosmetics to emulsify fatty soils were found to be in the range of 15–11 g/L. The parameter reached the highest value in the reference formulation C1, while the lowest value was detected in the prototype C4 formulated with the highest evaluated concentration of the digestate extract. As the results show, incorporating the digestate extract into the cosmetic composition leads to a decrease in the evaluated parameter, which may have an impact on the performance of the cosmetic in terms of its intended use (washing effect). He developed cleansing formulations contain mild anionic surfactant disodium cocoyl glutamate which is known to be gentle for skin. The favorable safety profile (mildness/non-irritancy) of surfactin-rich shower gels could be probably attributed to their decreased ability to emulsify hydrophobic substances leading to the reduced skin drying upon cleansing process (which also stands for reference sample), but even more importantly to the mechanisms/substances responsible for the decrease in skin irritation potential (zein value).

The ability of body wash cosmetics to generate foam is an important factor contributing to consumers' assessment of product quality. In the opinion of consumers, the washing bath should preferably generate copious amounts of foam

exhibiting desirable sensory properties (thick and velvety) and stability. Crucially, foaming ability is a cosmetic parameter that does not translate in any way into the performance of cosmetic products. Its sole function is to enhance the consumer's experience during the washing process. The results of the foaming ability test performed for the prototypical cleansing cosmetics containing the surfactin-rich extract are shown in Fig. 4.

Foam volumes generated by the studied prototypical cosmetics ranged between 280 and 360 cm³. Compared to the reference formulation C1, a decrease in the evaluated parameter is noted as the concentration of the extract added to the formulation increases. The lowest foaming ability was determined for the cosmetic prototype C4 containing the extract at a concentration of 80%. Evaluation of foam stability showed a similar relationship, with a decrease in foam stability (up to 62%) observed solely in the formulation C4. For the remaining prototypes evaluated in the study, similar stability values as in the reference sample C1 were determined (85%).

In cleansing cosmetics, viscosity is another relatively important parameter contributing to perceived product quality. In industrial practice, the adjustment of this parameter in cosmetics of this type, which are surfactant-based systems, is typically accomplished through the addition of sodium chloride or polymeric rheology modifier. Obtaining the desired viscosity, especially in shower gels, is a prerequisite for positive consumer experience in terms of product dispensation from the package, spreading it on the skin and combining with water during the washing process. The rheological properties of cosmetics also have a bearing on their environmental safety. If cosmetic products are difficult to dispense, considerable quantities may stick to the walls of the package, reducing the efficiency of product use. What follows is that such cosmetics should be susceptible to shearing forces (package squeezing and shaking) that temporarily decrease the viscosity of products, thus facilitating their skin application (Wasilewski et al. 2022a; Gallegos and Franco 1999; Wasilewski and Seweryn 2016). The rheological behavior of model shower gels is shown in Fig. 5.

Viscosity and shear stress showed a nonlinear trend with increasing shear rate, indicating non-Newtonian behavior of all the model shower gels tested. It was observed that the apparent viscosity of the tested cosmetics prototypes decreased gradually with increasing shear rate value, which characterizes the shear-thinning pseudoplastic materials. The apparent viscosity increased with increasing surfactin digest content. However, the change in apparent viscosity at the same shear rate (in the range of 0.1 to 50 s⁻¹) between samples was not that significant ($p > 0.05$). The shear stress increases continuously with shear rate for all the samples. The shear-thinning behavior of investigated shower gel prototypes was fitted to the power-law model (Eq. 4).

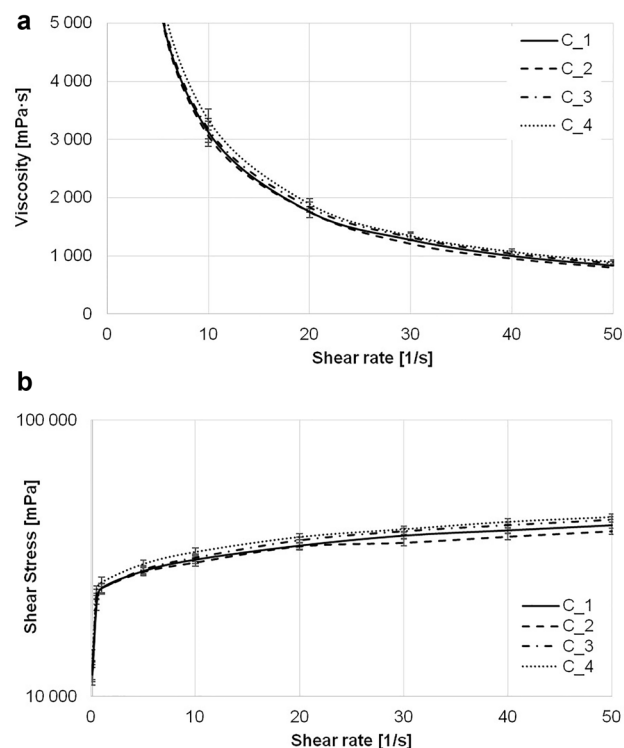


Fig. 5 Rheogram of shower gel prototypes. **a** Viscosity versus shear rate. **b** Shear stress versus shear rate

Table 3 Fit parameters to the power law of investigated shower gel prototypes

Sample	n	K	R^2
C1	0.16 ± 0.01	21,384 ± 1778	0.9973
C2	0.17 ± 0.01	21,795 ± 1778	0.9965
C3	0.18 ± 0.01	21,997 ± 1778	0.9976
C4	0.17 ± 0.01	23,478 ± 1778	0.9979

$$\eta = K\dot{\gamma}^{n-1} \quad (4)$$

where η is the apparent viscosity (mPa), $\dot{\gamma}$ is the shear rate (s⁻¹), n is the flow behavior index (dimensionless) and K is the consistency index (Pa·s ^{n}). For pseudoplastic fluids, $n < 1$ (Fan et al. 2022, García-Ochoa et al. 2000, Rottava et al. 2009). Table 3 shows the parameters of the power-law model for investigated shower gel prototypes.

When it comes to cleansing cosmetics, the purchasing decisions of consumers are determined to a major extent by their visual perceptions (Amberg and Fogarassy 2019). This is why most conventional cosmetics of this type are formulated with the addition of specially selected dyes that add color to the product to make it appear more visually appealing to consumers. Additives of this type are usually synthetic raw materials of organic origin, whose safety is

increasingly being called into question (Guerra et al. 2018). The trend not to use dyes is particularly evident in cosmetics that are marketed with the claims of being natural. This is motivated by the growing number of reports concerning adverse impacts of these ingredients on human health and symptoms of intolerance experienced by consumers. Cases involving various skin effects such as contact allergy have been reported (Borowska and Brzóška 2015). Consequently, following the trend toward naturalness, most natural cosmetics are free of such additives, and their color is derived from the raw materials used in the manufacturing process. The prototypical shower gels analyzed in the present study are dye-free and their color, as perceived by consumers, depends on the raw materials used. For the Authors, it was particularly important to establish the effects of the surfactin-rich digestate extract on the visual qualities of the formulated prototypes. To evaluate this aspect, an appropriate study was carried out, with the findings reported below. To determine the color difference of the tested model cosmetics, colorimetric assays were performed, and the results obtained are presented in Table 4.

The addition of surfactin-rich digest extract changed the appearance of the base cosmetic from colorless to colored products. In addition, the comparison of the L^* , a^* and b^* values indicated that the concentration of extract differentiated the color of cosmetics prototypes.

A cosmetic without extract was characterized by the highest L^* value and the lowest a^* and b^* values. As concentration of digest extracts increased, the L^* values decreased, but a^* and b^* increased indicating that the products became more red and more yellow with increasing concentration. Moreover, the chroma (C^*) values increased with extract concentration, demonstrating a significant change in the color saturation of the model cosmetics with the addition of the fermented extract to the base sample (without adding of

the extracts) and with the increase in its concentration in the products prototypes. The changes in H^0 values between the cosmetics prototypes indicated a change the color tonality in relation to the extract concentration. The calculated color difference, ΔE , compared to the C1 base cosmetic, showed good coloring power for C2, C3 and C4 products, with the highest ΔE value obtained for C4 cosmetics with the highest concentration of surfactin-rich digest extract. As indicated by literature data (Wan et al. 2019; Mokrzycki and Tatol 2011) if ΔE values are higher than 5, the color is perceived by the naked eye and perceived as a color effect.

The color differences between the cosmetics prototypes were clearly visible, with ΔE values of 6,18; 26.97 and 37.91 for C2, C3 and C4, respectively. Figure 6 shows the appearance of the shower gel prototypes.

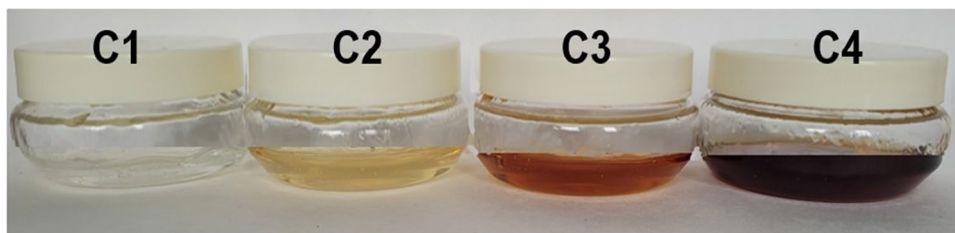
Conclusions

In line with the concept of sustainable development and new trends in the cosmetics industry, the underlying assumption of the study was to formulate and produce fully functional shower gels based on ingredients of natural origin. Special attention was paid to the possibility of using a surfactin-rich digestate extract as an all-natural raw material obtained through a biotechnological process. To this end, prototypical shower gels were designed and formulated, and subsequently studied to assess their quality. The study results showed that the formulated cosmetic prototypes exhibited satisfactory quality characteristics. The digestate extract incorporated into the formulations was not found to impair any cosmetic quality parameters related to functionality. All the parameters evaluated in the formulated shower gels (viscosity, foaming properties and ability to emulsify fats) reached similar values as in the reference formulation containing no

Table 4 Spectrophotometric data of the cosmetics prototypes

Sample	L^*	a^*	b^*	C^*	H^0	ΔE
						Cosmetic with Digestate Extract/Base Cosmetic
C1	37.55	-1.04	-1.55	1.87	56,17	-
C2	33.63	-1.00	3.23	3.38	-72,79	6,18
C3	25.44	0.76	22.48	22.49	88,06	26.97
C4	17.10	10.90	28.00	30.07	68,77	37.91

Fig. 6 Appearance of prototypes of shower gels



extract. The analysis of parameters related to the safety in use or, more specifically, the potential for skin irritation, none of the shower gels showed any irritant effect. The addition of the extract to the product formulation causes a significant (ca. 70%) decrease in the zein value, which is a measure of the severity of skin irritation effects. Colorimetric analysis of the formulated shower gels demonstrates that the addition of the surfactin-rich digestate extract changes the color of the samples toward the natural hue derived from the raw material used.—Consequently, there is no need to incorporate dyes into cosmetic formulations. The studies showed that it was feasible to formulate cleansing cosmetics consistent with the concept of sustainability based on ingredients of natural origin, including raw materials obtained through biotechnological processes, offering appropriate functional properties and highly safe to use in terms of their effects on users' skin.

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Author contributions AS and TW contributed to the study conception and design. Material preparation, data collection and analysis were performed by AS, TW, ZHB, MB, DP, MÅ and AL. Resourcing, investigation and data curation were done by AS, ZHB, MB and AL. The first draft of the manuscript was written by AS, TW, ZHB and AL, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

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

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