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Biosurfactant produced by *Candida utilis* UFPEDA1009 with potential application in cookie formulation

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ABSTRACT

Background: Biosurfactants are biomolecules that have the potential to be applied in food formulations due to their low toxicity and ability to improve sensory parameters. Considering the ability of yeasts to produce biosurfactants with food-friendly properties, the aim of the present study was to apply a biosurfactant produced by *Candida utilis* in the formulation of cookies.
Results: The biosurfactant was obtained with a yield of 24.22 ± 0.23 g/L. The characterization analysis revealed that the structure of a metabolized fatty acid with high oleic acid content (68.63 ± 0.61%), and the thermogravimetric analysis demonstrated good stability at temperatures lower than 200°C, potential for food applications. The biosurfactant also exhibited satisfactory antioxidant activity at concentrations evaluated, without cytotoxic potential for cell strains, L929 and RAW 264.7, according to the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The incorporation of the surfactant into the dough of a standard cookie formulation to replace animal fat was carried out, achieving a softer, spongier product without significantly altering the physical and physicochemical properties or energy value.
Conclusion: The thermal stability and antioxidant activity of the biosurfactant produced by *C. utilis* were verified, besides the positive contribution in the texture analysis of the cookies. Therefore, this biomolecule presents itself as a potential ingredient in flour-based sweet food formulations.
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1. Introduction

Some additives are used in the food industry to improve properties of foods as they have a large number of complex microstructures [1]. In this context, new products have been developed to enhance their application, taking into consideration different types of food processing. Among these compounds, biosurfactants are promising as constituents of these formulations because they have advantageous properties [2,3].

Biosurfactants have a hydrophilic portion, which may be a carbohydrate, amino acid, cyclic peptide, phosphate group, carboxyl acid or alcohol, and a hydrophobic portion, which may be a long-chain fatty acid, hydroxyl fatty acid, or α-alkyl-β-hydroxy fatty acid. The majority of biosurfactants are produced by a wide array of microorganisms, such as bacteria and yeasts, leading to different types, identified according to their chemical structure: lipopolysaccharides, lipoproteins, and complex biopolymers (high molecular weight); phospholipids, lipopeptides, and glycolipids (low molecular weight) [4,5,6,7].

Biosurfactants are promising compounds due to their greater resistance to adverse conditions found in food processing as well as their antioxidant activity and low toxicity. These natural compounds are capable of reducing the surface energy between phases and form electrostatic barriers, thereby preventing particle coalescence [8,9,10].

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Depending on molecular weight, biosurfactants may be more efficient both in reducing surface and interfacial tensions and in stabilizing oil emulsions in water [11,12]. In general, biosurfactants have a considerable advantage, as they can be produced from agro-industrial waste and are compatible with the environment [13].

Despite few reports on the use of these natural compounds in foods, studies published in the last ten years describe improvements in the texture, volume, and conservation of baked goods with the addition of rhamnolipid surfactants. Researchers reported improvements in the viscosity of food products when using a bioemulsifier isolated from *Enterobacter cloacae*, efficient emulsification of fat from meat products [4], the enhanced solubilization of aromas, and greater stability of salad dressings, in this case using a biosurfactant produced by the yeast *Candida utilis* [8,14]. The application of a biosurfactant characterized as a lipopeptide, produced by the actinobacteria *Nesterenkonia*, in muffin dough formulation, in substitution of baking powder and egg, also improved muffin smoothness and overall quality.

Therefore, the aim of the present study was to characterize and apply a biosurfactant, produced by *C. utilis* UFPEDA1009 as an ingredient to replace animal fat in a cookie formulation, and evaluating its effect on the physical and physicochemical properties as well as the texture of the end product.

2. Materials and methods

2.1. Materials and microorganism

All chemical reagents were of analytical grade. The frying oil used was obtained from local commerce in the city of Recife (Brazil) and was used without any further processing. The inputs for cookie formulations were obtained from supermarkets in the city of Recife, Pernambuco, Brazil. The yeast *C. utilis* UFPEDA1009, maintained in a yeast mold agar (YMA) medium was acquired from the culture collection of the Federal University of Pernambuco (Brazil). The inoculum was prepared by transferring the YMA culture medium to flasks containing 50 mL of yeast mold broth, followed by incubation (28°C/24 h/200 rpm).

2.2. Production and isolation of biosurfactant

The inoculum (2,0% *v*/v, 10^8 cells/mL) was added to a medium containing (*w*/*v*) 0.20% NH_4NO_3, 0.01% KH_2PO_4, 0.50% $MgSO_4.7H_2O$, 0.1% $FeCl_3$, 0.01% sodium chloride (NaCl), and 0.30% yeast extract, with the addition of 6.00% canola waste frying oil and glucose (pH 5.7), and incubated at 28°C and 150 rpm for 88 h [14]. The biosurfactant was extracted twice with ethyl acetate at a 1:4 (*v*/v) proportion with the noncentrifuged medium. The organic phase was submitted to centrifugation (2600 × g for 20 min), filtration, separation, and the addition of a saturated NaCl and Anhydrous magnesium sulfate ($MgSO_4$) for the removal of the aqueous phase, the filtrate isolated and dried (50°C).

2.3. Structural characterization of the biosurfactant

Samples of the isolated biosurfactant were submitted to nuclear magnetic resonance (NMR) analysis (Agilent 300), Fourier transform infrared (FT-IR) spectroscopic analysis (Perkin Elmer Spectrum 400), and gas chromatography coupled to a flame ionization detector (GC/FID) (Agilent Technologies 7890A) for the characterization of the structure. For NMR and FT-IR analyses, a previous biosurfactant purification procedure was performed, as follows: NaOH was added to the extracted biosurfactant for the formation of the carboxylic acid sodium salt, then washed with acetone and subjected to sintered glass filter filtration. After filtration, the precipitate was diluted with distilled water and acidified with HCl to recover the acid biosurfactant, which was extracted again with ethyl acetate, according to the extraction methodology. For GC/FID analysis, the extracted biosurfactant was submitted to the esterification process to obtain the fatty acid methyl esters. In this procedure, 25 mg of the biosurfactant was reacted with 0.5 mL of 0.5 mol/L potassium hydroxide solution under stirring on a vortex tube shaker for 2 min. Then hexane was added to separate esters from polar molecules, and the mixture was stirred again and centrifuged (4500 rpm for 6 min). Finally, the organic phase was filtered onto 0.22 μm porosity PTFE membrane (polytetrafluoroethylene).

2.4. Differential exploratory calorimetric and thermogravimetric analyses of the biosurfactant

The thermal analysis was performed based on Han et al. [15], in a sample of 50 mg of the isolated biosurfactant using a simultaneous thermal analyzer (STA 449 F3, NETZSCH). Successive heating, cooling, and heating steps were performed, with a heating and cooling rate of 10°C/min in a nitrogen atmosphere with an outflow of 50.0 mL/min in the range of 40°C to 400°C.

2.5. Antioxidant activity of biosurfactant

2.5.1. Total antioxidant capacity (TAC)

Total antioxidant capacity (TAC) was determined based on Prieto et al. [16], combining an aliquot of 0.1 mL at different concentrations of the isolated biosurfactant with 1.0 mL of a solution composed of sulfuric acid (600 mM), sodium phosphate (28 mM), and ammonium molybdate (4 mM) in tubes, capped and submitted to ebullition in a water bath at 90°C for 90 min, followed by the measurement of absorbance at 695 nm. The TAC was expressed in relation to a solution of ascorbic acid at a concentration of 1000 μg/mL, assumed to be 100%.

2.5.2. Sequestration of radical 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Following the method described by Brand-Williams et al. [17], 250 μL of a control methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (200 μM) was added to 40 μL of different concentrations of the isolated biosurfactant and absorbance was read at 517 nm after 30 min at rest sheltered from light. The inhibition activity (%I) was determined based on the percentage of DPPH eliminated, using the equation: $\%I = [(Abs_0 - Abs_1) / Abs_0] \times 100$, in which Abs_0 is the absorbance of the synthetic antioxidant controls (Trolox, 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid or BHT, 2,6-bis(1,1-dimethylethyl)-4-methylphenol) and Abs_1 is absorbance in the presence of the biosurfactant sample.

2.5.3. Sequestration of superoxide ion (SOD)

The isolated biosurfactant (50 μL) was diluted at different concentrations in phosphate buffer (150 mmol/L), 200 μL of methionine solution (65 mmol/L), 200 μL of EDTA solution (0.5 mmol/L), 200 μL of nitrotetrazolium blue chloride (NBT) solution (0.375 mmol/L), and 200 μL of riboflavin solution (0.5 mmol/L) were placed in a 50 μL tube [18]. The control was the same mixture without the biosurfactant. Tubes were exposed to fluorescent light for 15 min with dissipation, followed by the reading of absorbance at 560 nm. The %I of the photochemical reduction of NBT was calculated using the equation: $\%I = [(Abs_0 - Abs_1) / Abs_0 - AbsBLANK] \times 100$, in which AbsBLANK corresponds to the same composition as the control without exposure to fluorescent light.

2.6. Evaluation of the cytotoxic potential of the biosurfactant (MTT assay)

The MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was developed with mice fibroblast cells (L929) and

mouse macrophage cells (RAW 264.7) were kept in culture flasks at 37°C and 5% CO_2 [19]. Cells were detached with trypsin solution (0.5%) and added at a concentration of 10^5/mL to Dulbecco's Modified Eagle Medium (DMEM) in a 96-well microplate, followed by incubation at 37°C in a 5% CO_2 atmosphere for 24 h. Next, 10 μL of the isolated biosurfactant solutions at concentration of 200 μg/mL were added, followed by incubation under the same atmospheric conditions for 72 h. The DMEM medium was considered the negative control while phosphate buffer (150 mmol/L, pH 7.4) was used as the positive one. After 72 h, 25 μL of MTT (5 mg/mL) stain was added and incubated for three hours. After incubation, the culture medium with MTT was aspirated and 100 μL of dimethyl sulfoxide was added for the spectrophotometric reading at 560 nm. The percentage inhibition was calculated using GraphPad Prism 7.0 demo software).

2.7. Application of biosurfactant in cookie formulation

Cookie ingredients are listed in Table 1 and were based on an adapted standard formulation [10]. Pasteurized egg yolk was partially (50%) and totally (100%) replaced with the isolated biosurfactant in the dough of formulations A and B, totaling three different types of dough.

The ingredients were mixed in a planetary blender (Arno Ciranda) for 7 min. The dough was then rolled flat and cut into pieces with a diameter of 50 mm. The pieces were baked at 150°C for 5 min and 180°C for another 15 min, then cooled, weighed, packaged, and stored at 25°C -28°C for 24 h.

2.8. Physical properties of cookies

After baking, the weight, diameter, thickness, and spread factor of cookies were evaluated using the procedure described by literature [20,21]. Diameter was measured by randomly selecting four samples and placing them side by side for the measurement of the total diameter; cookies were then turned at 90° and the diameter was measured again. The final diameter was expressed as the mean of the two measurements divided by four. Thickness was measured by stacking four cookies one on top of another four times. The spread factor was determined by dividing the diameter by height.

2.9. Physicochemical analysis and determination of energy value of cookies

The moisture, ash, protein, carbohydrate, and lipid contents of cookies were determined based on the AOAC [22] and Bligh-Dyer [23]. The energy value was determined by summing the carbohydrate, lipid, and protein values multiplied by 4, 9, and 4, respectively.

2.10. Analysis of cookie texture with and without biosurfactant

The texture profile analysis (TPA = firmness, cohesiveness, adhesiveness, and elasticity) of the cookie dough before baking was determined using a texture analyzer (Brookfield CT3). After baking, only firmness was evaluated using the compression test at 50% of the original height at a constant velocity of 1 $mm.s^{-1}$). For the TPA, a second compression was performed after a 5 s interval; firmness was defined as the force at 50% of the height of the sample during the first compression. Cohesion was determined as the ratio between the compression work during the second compression and that during the first compression. Elasticity was calculated using the relative height of the remaining sample when the initial force was recorded during the second compression [10].

Table 1
Composition of cookie dough formulations.

Ingredients	Standard (%)	Formulation A (%)	Formulation B (%)
White flour	47.0	47.0	47.0
Margarine	20.0	20.0	20.0
Sugar	15.0	15.0	15.0
Vanilla extract	3.0	3.0	3.0
Baking powder	1.0	1.0	1.0
Pasteurized egg white	10.0	10.0	10.0
Pasteurized egg yolk	4.0	2.0	0.0
Biosurfactant	0.0	2.0	4.0
Total	100.0	100.0	100.0

2.11. Statistical analysis

Data were analyzed statistically using the one-way procedure in Statistica® (version 7.0), followed by a linear one-way analysis of variance model. Results were expressed as mean ± standard deviation determined from triplicate experiments. Differences were examined using Tukey's post hoc test and LSD multiple range test with a 95% significance level.

3. Results

3.1. Structural analysis of biosurfactant: FT-IR, NMR, and GC/FID

The biosurfactant isolated from *C. utilis* grown in a mineral medium supplemented with 6% canola waste frying oil and glucose was obtained with a yield of 24.22 ± 0.23 g/L.

Fig. 1 displays the infrared and NMR spectra of the isolated biosurfactant. As noted, the molecule exhibited regions of stretching between 1500 and 2000 cm^{-1} as well as between 2700 and 3000 cm^{-1}, indicating the possible presence of carbonyl groups (C═O) and simple bonds between carbon atoms (C—C), respectively. The stretching at approximately 1460 cm^{-1} corresponds to the double bond between carbon atoms.

The ^{1}H-NMR spectrum indicated the presence of hydrogen bonded to the carboxyl acid group between 10 and 11 ppm. The presence of hydrogen bonded to unsaturated carbon between 5 and 5.5 ppm was also observed. Very close signals were also found between 0.7 and 2.4 ppm, indicating the presence of methyl groups (from 0.7 to 0.9 ppm). The apolar region of the molecule (from 1.1 to 1.7 ppm), the presence of hydrogen bonded to unsaturated carbon (from 1.8 to 2.1 ppm), and hydrogen bonded to the carbon neighbor and to the unsaturated carbon (from 2.1 to 2.4 ppm) were also visualized. These results are confirmed by the ^{13}C-NMR spectrum, which displays a characteristic signal of the presence of carboxyl acid (180 ppm), double bonds between 120 and 140 ppm, and aliphatic carbon atoms in the region from 10 to 40 ppm.

The fatty acid percentage composition was calculated based on peak area normalization using an external standard (FAME Supelco™ C4-C24 mix, Bellefonte, PA, USA) (Table 2). Lauric, myristic, and nervous acids were not detected. Oleic acid was the predominant fatty acid (68.63% ± 0.61%). Based on the findings, one can affirm that the biosurfactant studied is composed of different proportions of fatty acids and has potential applications in food formulations due to its high nutritional value attributed to the greater percentage of fatty acids with 18 carbon atoms (oleic, linoleic, and linolenic acids).

3.2. Thermal analysis of biosurfactant

Regarding the thermogravimetry (TG) analysis (Fig. 2), the biosurfactant lost 0.77% of its mass at a temperature of 180.28°C. Beginning at 300°C, a significant reduction in mass occurred through to the final temperature (400°C), with a variation in the loss of mass in the range of 30.78%. Thermal degradation occurs when the loss of mass is approximately 5% [24]. The biosurfactant studied herein undergoes this type of degradation only at the beginning at 320°C.

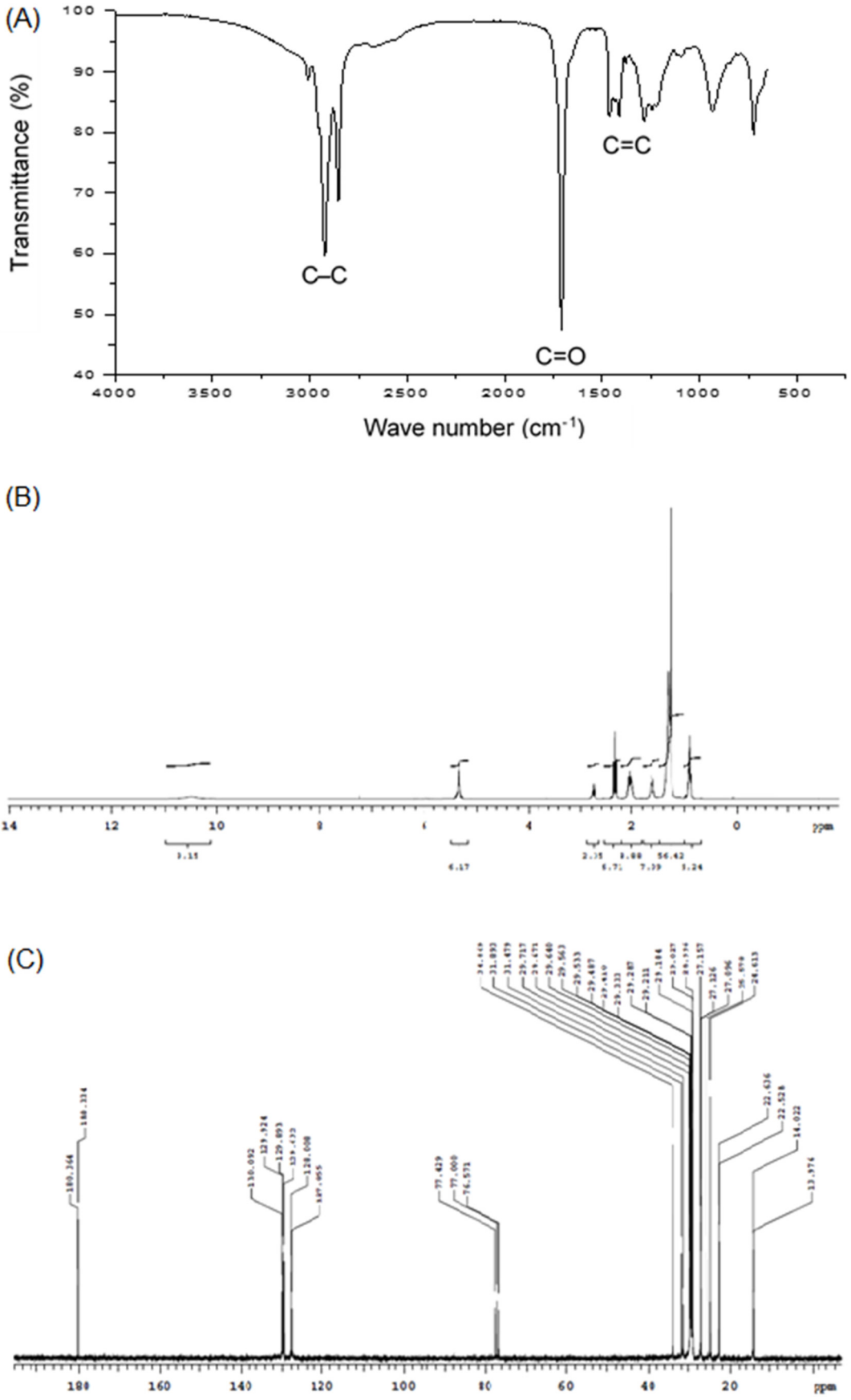

Fig. 1. (A) Infrared spectrum of biosurfactant produced by *C. utilis* in mineral medium supplemented with 6% waste canola fry oil and 6% glucose. (B) ^{1}H-NMR and (C) ^{13}C-NMR registered in deuterated chloroform of biosurfactant produced by *C. utilis* in mineral medium supplemented with 6% waste canola fry oil and 6% glucose.

Table 2
Fatty acid profile of biosurfactant produced by *C. utilis* in mineral medium supplemented with 6% waste canola fry oil and 6% glucose.

Fatty acid	Composition (%)
Lauric acid (C12:0)	ND
Myristic acid (C14:0)	ND
Palmitic acid (C16:0)	6.51 ± 0.02%
Linoleic acid (C18:2)	14.6 ± 0.67%
Oleic acid (C18:1)	68.63 ± 0.61%
Linolenic acid (C18:3)	3.69 ± 0.03%
Stearic acid (C18:0)	3.41 ± 0.04%
Eicosenoic acid (C20:1)	1.54 ± 0.09%
Arachidic acid (C20:0)	1.07 ± 0.02%
Behenic acid (C22:0)	0.55 ± 0.00%
Nervonic acid (C24:0)	ND

Already the differential scanning calorimetry (DSC), the thermogram demonstrated an exothermal peak with a crystallization temperature of 49.58°C (initial temperature: 42.08°C). Fusion peaks were observed at 169.58°C (initial temperature: 72.08°C) and 354.58°C (initial temperature: 317.08°C).

3.3. Antioxidant activity of biosurfactant

Table 3 displays the TAC (phosphomolybdenum complex) and inhibition (DPPH radical and SOD) of the biosurfactant solutions in percentage values.

Based on the results, the biosurfactant from *C. utilis* can be considered to be an antioxidant mainly in relation to the reduction of the phosphomolybdenum complex. Comparing the TAC percentages of the biosurfactant with the reference concentration of ascorbic acid (1000 μg/mL), the biosurfactant exhibited only 25% activity at a concentration of 1250 μg/mL, but more than 200% at a concentration of 20,000 μg/mL, demonstrating a linear relationship with the increase in the concentration. Regarding the DPPH assay, the antioxidant activity of the biosurfactant exhibited inhibition percentages with a different profile, which may be explained by differences in the

Table 3
Percentage of DPPH radical sequestration (%I) and total antioxidant capacity (% CAT) of different concentrations of biosurfactant from *C. utilis*.

Biosurfactant concentration (μg/mL)	%I (DPPH)	% TAC (ascorbic acid)	%I (superoxide ion)
20,000	16.52 ± 1.38	210.12 ± 0.21	21.64 ± 0.89
10,000	9.23 ± 1.61	114.45 ± 1.64	5.04 ± 1.89
5000	8.58 ± 0.06	73.27 ± 0.77	4.06 ± 0.61
2500	4.61 ± 1.12	43.08 ± 1.73	1.29 ± 0.31
1250	4.18 ± 0.59	25.35 ± 0.65	–

principle of the method and mechanisms of antioxidant action. This assay was used to evaluate the capacity of the biosurfactant and two standards (Trolox and BHT) to prevent the oxidation of the DPPH radical, reducing it to hydrazine and causing a change in color from purple to yellow, with a consequent reduction in absorbance [25]. Based on findings, the biosurfactant exhibited less antioxidant activity than Trolox and BHT, requiring a greater quantity to reduce the initial concentration of DPPH by 50% and cause the change in color. Thus, the biosurfactant does not have antioxidant activity at concentrations lower than 20 mg/mL when considering the DPPH reduction method and even has a low inhibition rate at this concentration (16.52% ± 1.38%) in comparison to the standards at a concentration of 1 mg/mL, which showed rates of 88.84% ± 0.25% and 83.37% ± 0.59% for trolox and BHT, respectively.

The SOD sequestration assay revealed that the biosurfactant has good capacity when analyzed by the riboflavin-light-NBT system, evidenced by the color change from blue to yellow, at concentrations above 20 mg/mL.

3.4. Cytotoxicity of biosurfactant

According to the data obtained, it was observed that the biosurfactant at a concentration of 200 μg/mL showed inhibition percentages of 15.83% against mice fibroblast cells (L929) and 0.00% against mouse macrophage cells (RAW 264.7), while the phosphate

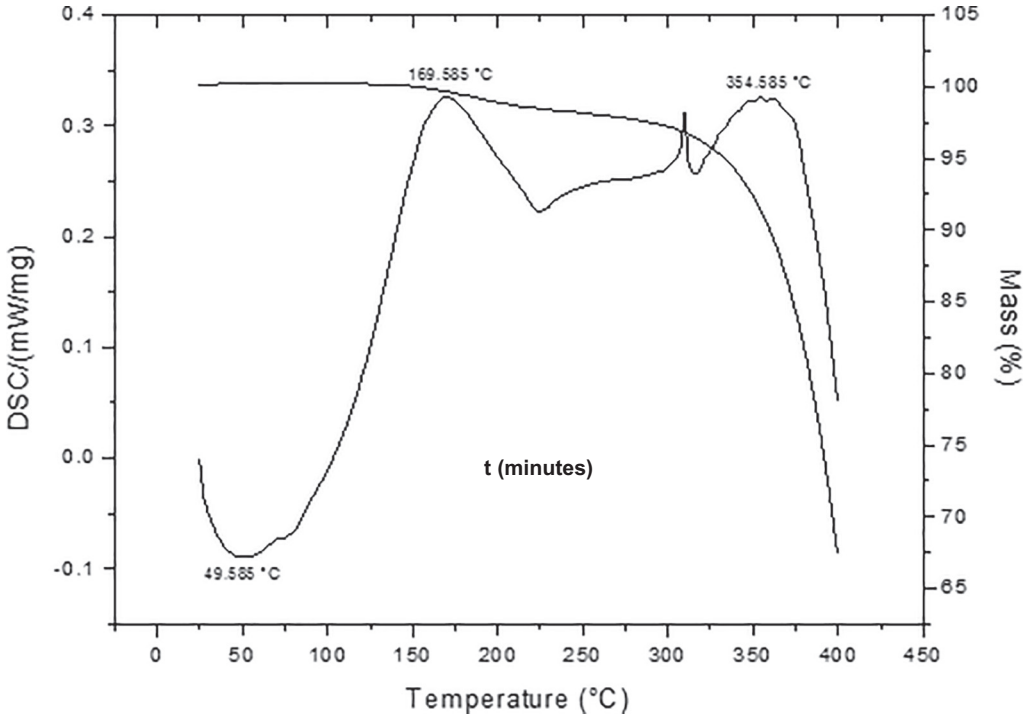

Fig. 2. TGA and DSC of biosurfactant produced by *C. utilis*.

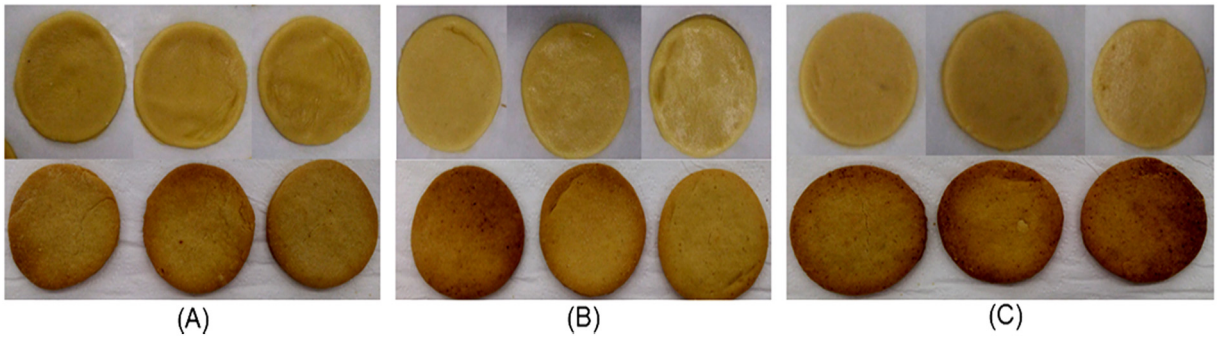

Fig. 3. Cookies before and after baking. (A): standard formulation, (B): Formulation A, and (C): Formulation B.

Table 4
Physical properties of cookies after baking.

Formulation	Weight (g)	Diameter (mm)	Thickness (mm)	Spread factor
Standard	6.92 ± 0.71^{a}	46.82 ± 0.50^{a}	7.34 ± 0.24^{a}	6.38 ± 0.05^{a}
A	6.38 ± 0.63^{a}	47.42 ± 0.20^{a}	7.65 ± 0.31^{a}	6.20 ± 0.17^{a}
B	6.54 ± 0.61^{a}	48.19 ± 0.96^{a}	7.64 ± 0.30^{a}	6.32 ± 0.37^{a}

Different letters in the same column denote significant differences ($p \leq 0.05$, Tukey test).

Table 5
Physicochemical composition and energy value of cookies with and without addition of biosurfactant.

Variable	Standard formulation	Formulation A	Formulation B
Moisture (%)	0.07 ± 0.01^{a}	0.02 ± 0.00^{b}	0.02 ± 0.00^{cb}
Ash (%)	1.53 ± 0.11^{a}	1.72 ± 0.25^{a}	1.70 ± 0.01^{a}
Lipids (%)	21.96 ± 0.79^{a}	24.58 ± 1.36^{ba}	27.96 ± 1.86^{c}
Proteins (%)	8.40 ± 0.04^{a}	7.95 ± 0.18^{b}	7.74 ± 0.03^{cb}
Carbohydrates (%)	68.05 ± 0.93^{a}	65.83 ± 1.81^{ba}	62.59 ± 1.88^{c}
Energy value (cal)	503.27 ± 5.05^{a}	515.96 ± 5.79^{ba}	532.94 ± 9.35^{c}

Different letters in the same line denote significant differences ($p \leq 0.05$, LSD test).

buffer (pH 7.4) showed values of 12.95% and 20.30% for L929 and RAW 264.7, respectively.

3.5. Application of biosurfactant in food formulation

The physical properties of cookies were determined after baking (Fig. 3). Table 4 displays the mean weight, diameter, thickness, and spread factor.

No significant differences were found among different formulations regarding the variables analyzed. Thus, the partial or complete replacement of pasteurized egg yolk with the biosurfactant does not lead to changes in the physical properties of cookies beyond a slight increase in the diameter.

Regarding the content of ash, lipids, carbohydrates, and energy value (Table 5), no significant differences were found between the standard formulation and the partially substituted formulation (Formulation A). The moisture content was low, ranging from 0.02% to 0.07%, with lower values when the yolk was replaced. Regarding the protein content, both alternative formulations showed lower values than the standard formulation, which is probably due to the absence of proteins in the biosurfactant and the presence of proteins in the yolk. Because of the increase in the content of lipids in Formulation B, the energy value of this formulation was consequently higher.

In the firmness, cohesion, and elasticity test of the three formulations before and after cooking (Table 6), it was observed that the substitution of the yolk by the biosurfactant led to insignificant differences in relation to all variables measured before cooking, in comparison with the standard formulation. The partial and total substitutions showed no significant differences, except for elasticity. After baking, a significant reduction in firmness occurred with the replacement of the yolk with the biosurfactant, with a greater reduction found when the replacement was complete (from 445.59 ± 15.52 to 354.93 ± 14.84 N). This may be due to the high lipid content of the biosurfactant, leading to a softer, spongier product.

4. Discussion

Considering FT-IR and NMR spectra similar to those found in this work and described in the literature, it can be stated that the biosurfactant produced by *C. utilis* is a type of carboxylic acid. Santos et al. [9], using *C. lipolytica* yeast in medium with 5% animal fat and 2.5% corn steep liquor, obtained a similar spectrum for the surfactant biomolecule, considering it with carboxylic acid structure.

In studies involving surfactant glycolipids, Silva et al. [26] and Vecino et al. [27] characterized the structure of biosurfactants produced by the bacteria *Pseudomonas cepacia* and *Lactobacillus pentosus*, respectively, and obtained a higher percentage (approximately 80%) of stearic acid reported as the main ones in structural characterization analysis of biosurfactants. According to Lopes et al. [28], the nutritional value of a molecule is high when the percentage of unsaturated fatty acids with 18 atom carbons is higher, because essential fatty acids are not

Table 6
Texture profile analysis of dough before and after baking.

Formulation	Before baking				Firmness (after baking) (N)
	Firmness (N)	Cohesiveness	Elasticity (mm)	Adhesiveness (mJ)	
Standard	63.57 ± 2.84^{a}	0.70 ± 0.02^{a}	0.77 ± 0.12^{a}	1.67 ± 0.29^{a}	445.59 ± 15.52^{a}
A	54.94 ± 3.49^{b}	0.44 ± 0.02^{b}	2.87 ± 0.32^{b}	2.00 ± 0.50^{b}	368.19 ± 7.63^{b}
B	45.65 ± 2.27^{cb}	0.46 ± 0.02^{cb}	0.83 ± 0.06^{ca}	2.25 ± 0.29^{cb}	354.93 ± 14.84^{cb}

Different letters in the same column denote significant differences ($p \leq 0.05$, Tukey test).
Elasticity and adhesiveness are classified in descending order.

synthesized by the human organism, which favors the use of such molecules in food systems.

In terms of industrial application, it is also necessary to evaluate the stability of the physical and chemical properties of the biosurfactant when exposed to temperature changes. Thereby, TG and DSC can be used as they allow the measurement of mass variation, thermal stability, free water, bonded water, purity, melting point, boiling point, phase diagrams, glass transitions, etc., or reaction products as a function of temperature when a substance is subjected to a controlled temperature program [29].

In the literature, there is no report on this type of analysis for biosurfactants produced by the yeast studied. However, Kourmentza et al. [24] found a similar fusion temperature (166.40°C) for a rhamnolipid produced by *Burkholderia thailandensis*, which is in agreement with the range reported in the literature (from 162°C to 181°C). According to Han et al. [15], biosurfactants with a higher fusion peak have greater thermal stability. Moreover, compounds with relatively high degradation temperatures are considered advantageous, as they have a broader range of applications at extreme temperatures. Therefore, as the biosurfactant will be submitted to a cookie baking process at a temperature of 180°C, it will not undergo a significant loss of mass and will remain stable and adequate for this application.

Another important parameter for food is the antioxidant activity, which can be evaluated by different methods, including capture or sequestration of organic radicals and ions or reduction of complexes, being visualized by the change in color of solutions proportional to the antioxidant capacity. From the phosphomolybdenum complex reduction method, biosurfactant can be considered a potential antioxidant agent in concentrations above 5000 μg/mL and can be applied in food formulations, as ascorbic acid is a recognized and highly used reducing agent.

By testing with DPPH, it was possible to evaluate the ability of biosurfactant and two standards (Trolox and BHT) to prevent the oxidation of the DPPH radical [24]. Significant differences in %I were found when comparing present findings with results obtained with *Lactobacillus* biosurfactants published by Merghni et al. [30], which demonstrated DPPH free radical scavenging activity of 74.6% and 77.3% at a concentration of 5 mg/mL, which is approximately tenfold higher than the rate achieved with the biosurfactant from *C. utilis* at the same concentration. Therefore, the biosurfactant cannot be considered antioxidant at concentrations studied when considering this method.

In the SOD sequestration assay, results were satisfactory and can be considered important for the application of the biosurfactant studied. This is due to the fact that, according to Vadivel and Biesalski [31], it is considered important to evaluate antioxidant capacity using sequestration methods, as SODs occur naturally in the human body, because they are produced during cellular respiration and they can destroy cells, damage the cell membrane and DNA, inactivate enzymes, and give rise to other free radicals. Therefore, biosurfactant has potential for application in foods that may be poor in antioxidants.

Evaluating cytotoxicity, Marques et al. [32] found similar results using this method and exposing 3 T6 mouse fibroblasts to a biosurfactant produced by *Rhodococcus* sp. 51T7, reporting lower toxicity in comparison to synthetic surfactants. Thus, with pertinent results found, *C. utilis* biosurfactant has even more potential for application in the food and even cosmetics industries.

Regarding the application of biosurfactant in the cookie formulation, one of the important characteristics in determining its quality is the dispersion factor, which is related to the diameter. In addition, this parameter is highly correlated with mass viscosity (lower viscosity translates into higher propagation factor), which may be influenced by lipid concentration. Thus, as there was an increase in lipid content resulting from the replacement of egg yolk by biosurfactant, an increase in diameter was observed as a consequence of the increase in mass spreading factor [10].

Still regarding the quality parameters of cookies, texture is considered as one of the main evaluated, as it affects the intensity and perception of sensory properties of foods, such as taste, being directly related to consumer acceptance. According to Pereira et al. [33], texture is significantly influenced by the fat present in the food, such as egg yolk, which is composed of a lipoprotein mixture, whose protein content is 16% and the lipid content is 35%. The lipid fraction of the yolk is composed of 66% triglycerides, 28% phospholipids, 5% cholesterol and small amounts of other lipids, which shows atherosclerotic power [34]. By replacing the yolk by the biosurfactant, which is, in turn, rich in omega 9, a monounsaturated fat with beneficial effects, associated with the prevention of cardiovascular disease [35], the biosurfactant formulation is a promising alternative for consumers seeking healthier foods with unique properties.

Comparing the texture findings obtained with the literature, Zouari et al. [10] reported similar results, with a significant reduction in firmness ($p \leq 0.05$) when adding a bioemulsifier produced by *Bacillus subtilis* SPB1 at concentrations above 0.5%. On the other hand, authors found greater cohesion and less elasticity with the addition of a bioemulsifier.

In general, it can be inferred that the biosurfactant exhibited functional properties similar to those of egg yolk, so it can be replaced by the biosurfactant analyzed without compromising characteristics of the final product.

From an economic point of view, the biosurfactant concentrations used in this work may be higher for food. However, the main objective was to completely replace the egg yolk with biosurfactant at the same concentrations to analyze the possibility of continuing biosurfactant application studies in this type of formulation. Studies are going on to test other concentrations based on results obtained in this study.

5. Conclusion

The yeast, *C. utilis,* is capable of producing a biosurfactant with satisfactory extraction yield using an agro-industrial residue (canola waste frying oil) in the cultivation medium that can be applied in systems with relatively high temperatures due to its proven thermal stability. Moreover, it has the potential for application in food formulations due to its studied antioxidant capacity and the absence of cytotoxicity. Therefore, the microbial surfactant studied herein has biotechnological potential for application in the food industry.

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Conflict of interest

The authors declare no competing interests.

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