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Candida utilis Biosurfactant from Licuri Oil: Influence of Culture Medium and Emulsion Stability in Food Applications

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Abstract

Biosurfactants (BSs) are natural, biodegradable compounds crucial for replacing synthetic emulsifiers in the food industry, provided their production costs can be reduced through the use of sustainable and low-cost substrates. This study evaluated the viability of licuri oil as a carbon source for BS production by *Candida utilis* and assessed the product's functional stability in food formulations. Production kinetics confirmed the yeast's efficiency, reducing the water surface tension to a minimum of 31.55 $mN \cdot m^{-1}$ at 120 h. Factorial screening identified a high carbon-to-nitrogen ratio as the key factor influencing ST reduction. The isolated BS demonstrated high surface activity, with a Critical Micelle Concentration of 0.9 $g \cdot L^{-1}$. Furthermore, the cell-free broth maintained excellent emulsifying activity (E_{24} > 70%) against canola and motor oils across extreme pH, temperature, and salinity conditions. Twelve mayonnaise-type dressings were formulated, utilizing licuri oil, and tested for long-term physical stability. Six formulations, featuring the BS in combination with lecithin and/or egg yolk, remained stable without phase segregation after 240 days of refrigeration, maintaining a stable pH and suitable microbiological conditions for human consumption. The findings confirm that the valorization of licuri oil provides a route to produce a highly efficient and robust BS, positioning it as a promising co-stabilizer for enhancing the shelf-life and natural appeal of complex food emulsions.

Keywords: emulsifier; food application; microbial surfactant; yeast

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1. Introduction

The increasing consumer demand for clean-label and sustainable foods has encouraged the food industry to replace synthetic additives with natural and eco-friendly ingredients. Surfactants are among the most widely used additives, as they improve texture, appearance, and stability in various food systems [1]. However, most commercial surfactants are

petrochemical-based, presenting issues such as toxicity, low biodegradability, and environmental persistence. This has created a clear need for safe, functional, and biodegradable alternatives [2].

Biosurfactants are promising natural substitutes for synthetic surfactants. These amphiphilic compounds, produced by microorganisms, can effectively reduce surface and interfacial tension. They offer several advantages, including biodegradability, low toxicity, and stability under extreme conditions of pH, salinity, and temperature. In food applications, biosurfactants act as emulsifiers and stabilizers, improving the consistency and shelf life of emulsified products such as sauces, dressings, and baked goods [3].

Despite these benefits, large-scale production of biosurfactants remains limited by high costs, mainly associated with substrate and downstream processing. The use of low-cost renewable substrates, particularly agro-industrial by-products and regionally available feedstocks, represents a sustainable strategy to reduce production expenses while simultaneously adding value to local resources [4,5].

Licuri oil (*Syagrus coronata*), a native palm oil from the semi-arid Caatinga region in northeastern Brazil, represents a valuable substrate for microbial biosurfactant production [6]. It is rich in medium-chain fatty acids and lipids, with physicochemical characteristics similar to coconut oil. Beyond its nutritional value, the use of licuri oil in bioprocesses supports regional bioeconomy and promotes the valorization of native plant resources [7,8].

The yeast *Candida utilis* is known for its ability to produce biosurfactants in lipid-rich media, due to its metabolic adaptability and secretion of surface-active biomolecules [9,10]. Combining *C. utilis* with licuri oil as the main carbon source may lead to the production of a stable and functional biosurfactant suitable for food applications.

This study aimed to (i) evaluate the feasibility of licuri oil as a substrate for biosurfactant production by *C. utilis* and determine the influence of culture medium components through a 2^4 factorial design with central replicates and (ii) assess the emulsifying and stabilizing performance of the biosurfactant in a mayonnaise-type salad dressing, focusing on its potential as a natural stabilizing agent for long-term emulsion stability.

2. Materials and Methods

The crude licuri oil (*S. coronata*) used as the main carbon source was obtained via mechanical pressing of the nuts and supplied by the Cooperativa de Produção da Região do Piemonte da Diamantina (COOPES), located in Capim Grosso, Bahia, Brazil (11°22′51″ S, 40°00′46″ W). All chemical reagents and culture media were of analytical grade.

2.1. Microorganism and Culture Medium

The yeast *C. utilis* (UFPEDA 1009) was obtained from the Culture Collection of the Department of Mycology at the Federal University of Pernambuco, Brazil. The strain was maintained at 5 °C on Yeast Mold Agar (YMA) containing (*w*/*v*): 0.3% yeast extract, 0.3% malt extract, 0.5% tryptone, 1% D-glucose, and 2% agar. For inoculum preparation, cells were cultivated in Yeast Mold Broth (YMB) with the same composition as YMA (without agar) under agitation (200 rpm, 28 °C, 24 h) using a C25KC incubator shaker (New Brunswick Scientific, Edison, NJ, USA). Cell concentration was adjusted to 10^6 cells mL^{-1} using a Neubauer chamber [11].

2.2. Production Kinetics

Preliminary batch fermentation was performed in 250 mL Erlenmeyer flasks containing 100 mL of production medium (licuri oil, glucose, and mineral salts) to determine the optimal production time. Cultures were incubated at 28 °C and 200 rpm for up to

168 h. Samples were collected at regular intervals (24–168 h) for biomass and surface tension determinations, performed in triplicate.

2.3. Evaluation of Culture Conditions

After determining the optimal fermentation time, a 2^4 full factorial design was applied using the Protimiza Experimental Design software (https://experimental-design.protimiza.com.br/, 1 March 2024) [12] to evaluate the effects of four variables: licuri oil (20–40 $g{\cdot}L^{-1}$), glucose (20–100 $g{\cdot}L^{-1}$), ammonium nitrate (1–3 $g{\cdot}L^{-1}$), and yeast extract (2–8 $g{\cdot}L^{-1}$). The experimental design included three replicates at the central point to estimate pure error and assess model reproducibility. The evaluated responses were surface tension reduction and emulsification indices using motor and canola oils. ANOVA was applied to verify the significance of the models.

2.4. Biosurfactant Isolation

Following the fermentation period, the biosurfactant was isolated from the culture broth without prior centrifugation. Isolation was performed using ethyl acetate in a 1:4 (v/v) ratio relative to the fermented medium. The organic phase was separated, and the procedure was repeated twice to maximize recovery. The collected organic phase was then centrifuged (4500 rpm for 15 min) and vacuum filtered. Saturated sodium chloride (NaCl) was added to the filtrate to facilitate the removal of residual aqueous phase, followed by the addition of anhydrous magnesium sulfate ($MgSO_4$) to eliminate residual moisture. The resulting material was filtered once more and dried in an oven at 50 ± 1 °C. This isolated product was utilized for Critical Micelle Concentration (CMC) determination [11].

2.5. Physicochemical Analyses

2.5.1. Surface Tension

Surface tension of cell-free supernatants (centrifuged at 4500 rpm, 20 min, 4 °C) was measured by the Du Noüy ring method using a Sigma 70 tensiometer (KSV Instruments Ltd., Helsinki, Finland) at 25 ± 1 °C.

2.5.2. Emulsification Index

The emulsification index (E_{24}) was determined according to Cooper and Goldenberg [13]. Equal volumes (1 mL) of the cell-free broth and oil were vortexed for 1 min, and the height of the emulsion layer was measured after 24 h. Only two oils—used motor oil and canola oil—were selected for testing, as they represent typical hydrophobic substrates of industrial and food relevance. E_{24} was determined according to Equation (1):

$$E_{24}\ (\%) = (he/H) \times 100 \quad (1)$$

where *he* represents the height of the emulsified layer and *H* the total mixture height.

2.5.3. Stability of Biosurfactant for Different Ranges of Parameters

The extracted crude biosurfactant was then tested for its stability across a wide range of different parameters (temperature, pH, and salinity). The ranges used for the different parameters were 30, 50, and 60 °C—temperature; 3, 5, and 7—pH; and 1, 5, and 10%—salinity concentrations, for 24 h. For these stability tests, the cell-free broth obtained from the culture run that resulted in the highest overall biosurfactant activity (lowest ST and high E_{24}) in the factorial screening was utilized.

2.5.4. Critical Micelle Concentration

The critical micelle concentration (CMC) was determined by plotting surface tension values against increasing concentrations of the isolated biosurfactant in distilled water. The CMC was defined as the concentration at which the surface tension reached a constant minimum value, indicating micelle formation [14].

2.6. Application in Salad Dressing Formulations

Salad dressing formulations were developed to evaluate the biosurfactant as a natural emulsifier, following the formulation proposed by Ribes et al. (2021) [15], with minor modifications. The base formulation consisted of (*w*/*w*) distilled water (50%), licuri oil (30%), vinegar (10%), starch (5%), sugar (1%), NaCl (0.5%), and citric acid (0.5%) (*w*/*w*). The emulsifiers—biosurfactant, pasteurized egg yolk, and lecithin—were incorporated individually or in combination at concentrations ranging from 0 to 3%, as detailed in Table 1. The control formulations were also prepared using Tween® 80 (Sigma-Aldrich, Darmstadt, Germany) instead of the biosurfactant at the same (*w*/*w*) concentration.

Table 1. Composition of salad dressing formulations containing different emulsifiers and concentrations.

Code	Biosurfactant (%) or Tween 80	Egg Yolk (%)	Lecithin (%)
BA/TA	3.0	0.0	0.0
BB/TB	2.0	0.0	0.0
BC/TC	1.0	0.0	0.0
BD/TD	0.5	0.0	0.0
BE/TE	0.5	2.5	0.0
BF/TF	0.5	0.0	2.5
BG/TG	0.5	1.5	1.0
BH/TH	0.5	1.0	1.5
BI/TI	0.0	3.0	0.0
BJ/TJ	0.0	2.0	1.0
BK/TK	0.0	1.0	2.0
BL/TL	0.0	0.0	3.0

The prefix "B" in the formulation code indicates the addition of the biosurfactant, while the prefix "T" indicates the addition of Tween 80, used as a comparative control at the respective concentrations listed in the second column.

A total of twelve formulations were prepared in sterile glass containers and stored under refrigeration at 8 °C. The emulsions containing Tween 80 were evaluated for their initial pH and for stability after 10 days of storage. Emulsions containing biosurfactants were monitored for stability after 60 and 240 days of storage, and those that remained stable up to 240 days were further analyzed for final pH and viscosity. The viscosity of each formulation was measured at 27 °C using a digital viscometer (VISDJ, Satra, London, UK) equipped with spindle 2 at 6 rpm.

2.7. Microbiological Analyses

The six biosurfactant-containing samples that remained stable after 240 days of refrigerated storage were evaluated for microbiological parameters. The analyses were conducted in the Laboratory of Animal Source Foods at the Federal University of Pernambuco, following the methods recommended by APHA (2015) [16]. The investigated parameters complied with current Brazilian regulations and included the determination of the most probable number (MPN) of total and thermotolerant coliforms, the enumeration of *Staphylococcus aureus*, and the detection of *Salmonella* sp. [17]. Although IN nº 161/2022 lists only these microorganisms, additional analyses were performed for mold and yeast

counts, total aerobic mesophiles, and *Pseudomonas aeruginosa* to validate quality control and determine the commercial shelf life.

2.8. Statistical Analysis

All experiments were carried out in triplicate, and the values are reported by calculating the average ± standard deviation. Analysis of variance (ANOVA) was performed to determine the statistical significance of the data.

3. Results

3.1. Growth Profile and Surface Tension Reduction

The growth curve of *C. utilis* cultivated in mineral medium supplemented with licuri oil is shown in Figure 1. Biomass concentration increased progressively during the 168 h fermentation, accompanied by moderate pH fluctuations. The surface tension of the culture medium decreased sharply from 71.01 to 31.55 mN·m^{-1} after 120 h, reaching a stable value thereafter.

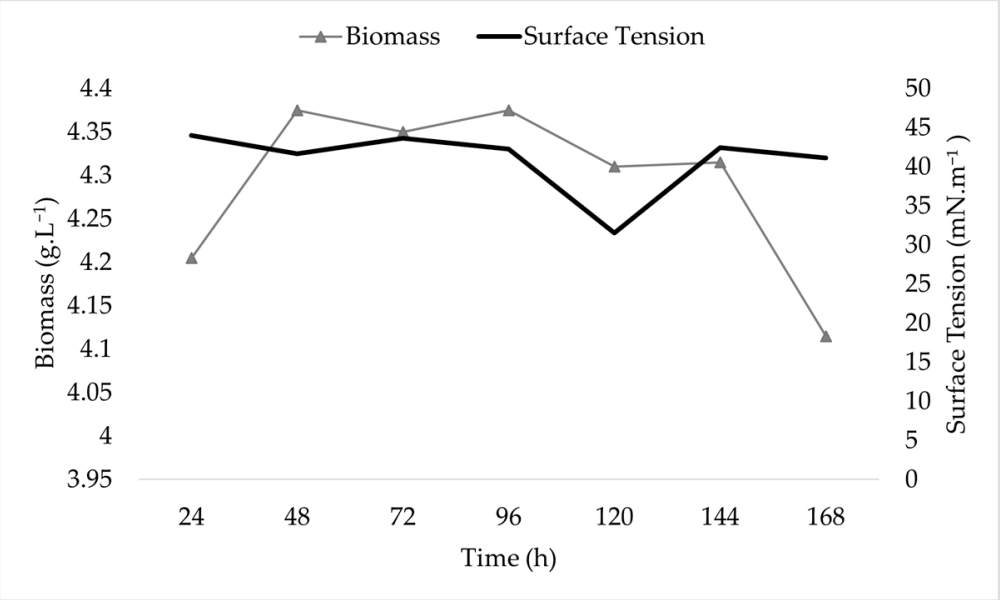


Figure 1. Biosurfactant production kinetics by *C. utilis* in mineral medium: Temporal variation of biomass (g·L^{-1}) and surface tension (mN·m^{-1}) over 168 h of fermentation.

This reduction in surface tension reflects the active synthesis and secretion of surface-active molecules by *C. utilis* during the exponential phase of growth. The profile suggests that the strain efficiently utilized licuri oil as a carbon source, producing compounds capable of lowering surface tension below 35 mN·m^{-1}, the benchmark value for efficient biosurfactants.

3.2. Evaluation of Culture Medium Parameters by Factorial Design

A 2^4 full factorial design, including three replicates at the central point, was employed to evaluate the effects of licuri oil (X_1), glucose (X_2), ammonium nitrate (X_3), and yeast extract (X_4) on three responses: surface tension (Y_1), emulsification index with motor oil (Y_2), and emulsification index with canola oil (Y_3). The experimental matrix and responses are presented in Table 2.

Table 2. Experimental matrix and observed responses for the Factorial Design (4 factors with central point), showing the coded levels of the independent variables (X_1 to X_4) and the measured responses: surface tension (Y_1 in $mN \cdot m^{-1}$), emulsification index with motor oil (Y_2 in %), and emulsification index with canola oil (Y_3 in %).

Run	X_1	X_2	X_3	X_4	Y_1 ($mN \cdot m^{-1}$)	Y_2 (%)	Y_3 (%)
1	−1	−1	−1	−1	42.83	75.00	6.67
2	1	−1	−1	−1	41.26	66.67	53.33
3	−1	1	−1	−1	45.93	66.67	5.00
4	1	1	−1	−1	32.17	66.67	33.33
5	−1	−1	1	−1	48.75	66.67	33.33
6	1	−1	1	−1	32.50	66.67	6.67
7	−1	1	1	−1	43.76	93.33	13.33
8	1	1	1	−1	34.56	90.00	75.00
9	−1	−1	−1	1	41.93	50.00	80.00
10	1	−1	−1	1	41.73	50.00	60.00
11	−1	1	−1	1	38.18	66.67	13.33
12	1	1	−1	1	44.73	66.67	0.00
13	−1	−1	1	1	43.62	66.67	0.00
14	1	−1	1	1	44.32	62.50	6.67
15	−1	1	1	1	46.79	66.67	25.00
16	1	1	1	1	42.65	94.44	25.00
17	0	0	0	0	39.47	25.00	20.00
18	0	0	0	0	40.45	66.67	13.33
19	0	0	0	0	45.42	66.67	4.55

X_1: licuri oil concentration; X_2: glucose concentration; X_3: ammonium nitrate concentration; X_4: yeast extract concentration. Coded Levels: (−1): low level; (0): central point; (+1): high level.

The surface tension values ranged from 33.7 to 47.8 mN m^{-1}. The lowest values—indicating higher biosurfactant production—were obtained in assays 4, 6, and 8.

Among them, assay 8 showed the most favorable overall performance, combining strong surface tension reduction (34.56 $mN \cdot m^{-1}$) with the highest emulsification indices for both oils (90.00% for motor oil and 75.00% for canola oil).

The ANOVA results for Y_1 are summarized in Table 3 and indicate that the regression was globally significant (p = 0.00164), confirming that at least one independent variable had a real influence on the response. The lack-of-fit test was not significant (p = 0.59024), suggesting that the model form was adequate. The coefficient of determination (R^2 = 55.12%) revealed that the model explained slightly more than half of the total variability in the data.

Table 3. ANOVA of the reparameterized linear model for surface tension (Y_1) reduction, identifying the statistically significant factors influencing the biosurfactant production.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Fcalc	*p*-Value
Regression	208.9	2	104.5	9.8	0.00164
Residual	170.1	16	10.6	—	—
Lack of Fit	149.7	14	10.7	1.1	0.59024
Pure Error	20.4	2	10.2	—	—
Total	379.0	18	—	—	—

After reparameterization, only the significant effects were retained, as represented in Equation (2):

$$Y_1 = 41.63 - 2.37X_1 + 2.73X_1X_4. \quad (2)$$

This model highlights licuri oil (X_1) as the main factor influencing surface tension, along with its positive interaction with ammonium nitrate (X_1X_4). The analysis of the response surface (Figure 2) shows that the maximum ST reduction (green region, approximately 38 $mN \cdot m^{-1}$) is achieved in the area combining the highest level of licuri oil (X1: 36 to 40 $g \cdot L^{-1}$) and the lowest level of ammonium nitrate (X4: 1 to 1.75 $g \cdot L^{-1}$). This pattern is a strong indication that biosurfactant synthesis by *C. utilis* is favored under high carbon-to-nitrogen (C/N) ratio conditions, a known strategy for inducing secondary metabolite production in yeasts.

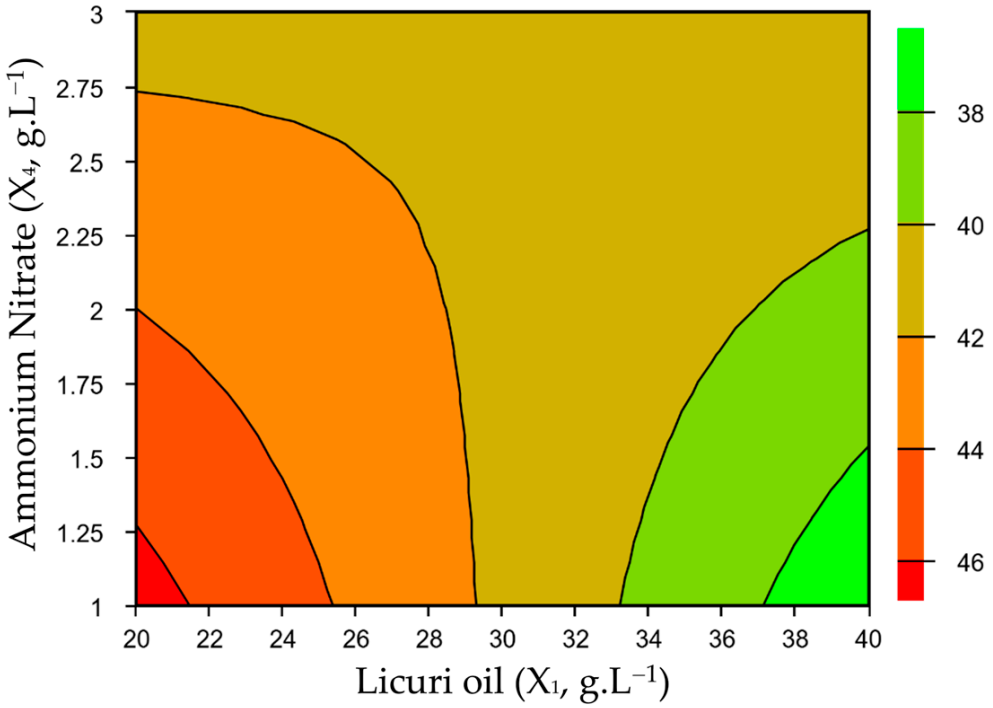


Figure 2. Response surface plot for surface tension (TS) showing the significant interaction between licuri oil concentration (X_1) and ammonium nitrate concentration (X_4) in the culture medium by *C. utilis*.

However, for the emulsification index responses (Y_2 and Y_3—motor oil and canola oil), the generated models were not found to be statistically significant or predictive. For the Y_2 response, the ANOVA *p*-value for regression was 0.69, indicating no statistically valid relationship between the four variables and the emulsifying activity. Although some individual runs showed high E_{24} results, a robust model could not be established using the linear factorial approach. Therefore, the discussion regarding emulsifying activity focuses on the raw performance data and the subsequent stability studies.

3.3. Emulsification Stability Under Environmental Stress and Critical Micelle Concentration

The biosurfactant exhibited remarkable stability under diverse environmental conditions (Table 4), maintaining emulsification indices above 50% even after exposure to variations in temperature (30–70 °C), NaCl concentration (1–10%), and pH (3–7). Emulsions prepared with canola oil remained particularly stable, with E_{24} values consistently exceeding 70% under all tested conditions. In contrast, motor oil emulsions displayed a slight decrease in stability at higher salinity levels, yet their E_{24} values remained above 60% at neutral pH, confirming the compound's overall structural resilience and versatility.

Table 4. Emulsification index (E24) stability of the biosurfactant produced by *C. utilis* under varying conditions of temperature, NaCl concentration, and pH against motor oil and canola oil (values are presented as mean ± SD, where SD stands for standard deviation).

Parameter	Condition	Motor Oil (% ± SD)	Canola Oil (% ± SD)
Temperature (°C)	30	60.00 ± 2.1 ^{b}	75.00 ± 1.8 ^{a}
	50	68.00 ± 2.5 ^{a}	72.00 ± 2.0 ^{a}
	60	64.00 ± 1.9 ^{ab}	70.00 ± 2.3 ^{a}
NaCl (%)	1	66.67 ± 1.7 ^{a}	70.00 ± 1.6 ^{a}
	5	62.50 ± 2.4 ^{b}	72.00 ± 1.8 ^{a}
	10	60.00 ± 1.5 ^{b}	64.00 ± 2.2 ^{b}
pH	3	72.00 ± 2.1 ^{a}	74.07 ± 1.9 ^{a}
	5	71.43 ± 2.0 ^{a}	71.43 ± 1.7 ^{a}
	7	70.37 ± 2.4 ^{a}	73.08 ± 2.1 ^{a}

Different lowercase letters indicate statistically significant differences among conditions for each oil according to Tukey's test ($p < 0.05$). Values sharing at least one letter do not differ significantly.

The relationship between biosurfactant concentration and surface tension is shown in Figure 3. The minimum surface tension (25.17 ± 0.03 mN·m^{-1}) was reached at a concentration of 0.9 g L^{-1}, corresponding to the critical micelle concentration (CMC).

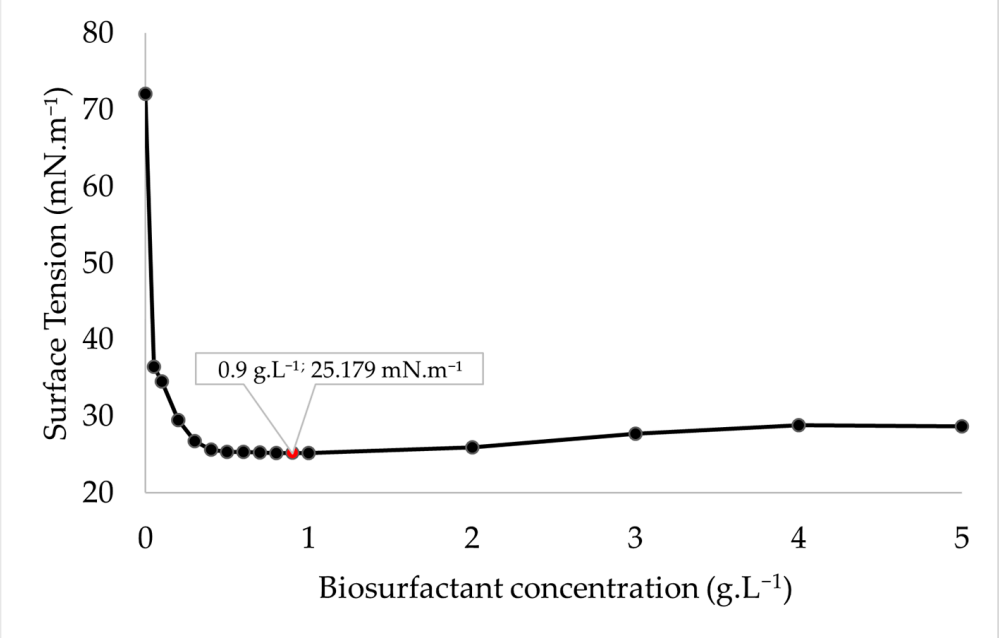


Figure 3. Critical micelle concentration (CMC) curve of the isolated biosurfactant produced by *C. utilis*.

3.4. Novel Application in Dressings

Visual observations of the formulations containing the biosurfactant (codes BA–BL) after 60 days, and the formulations containing Tween 80 (codes TA–TL) after 10 days, are presented in Figure 4.

After 60 days of storage, formulations BE (0.5% BS + 3% egg yolk), BF (0.5% BS + 3% lecithin), and BH (0.5% BS + 2% egg yolk + 1% lecithin) remained completely stable, showing no phase separation. Formulations BJ, BK, and BL, which contained only egg yolk and/or lecithin, also remained stable, confirming the synergistic stabilizing effect of phospholipids and proteins. Conversely, formulations lacking emulsifiers (BA–BD) displayed clear phase separation, demonstrating that the biosurfactant and natural emulsifiers are essential for

stability. Notably, formulation BG (0.5% BS + 1% egg yolk + 2% lecithin) showed instability despite having the same ingredients as BH, highlighting the importance of the proportion between egg yolk and lecithin.

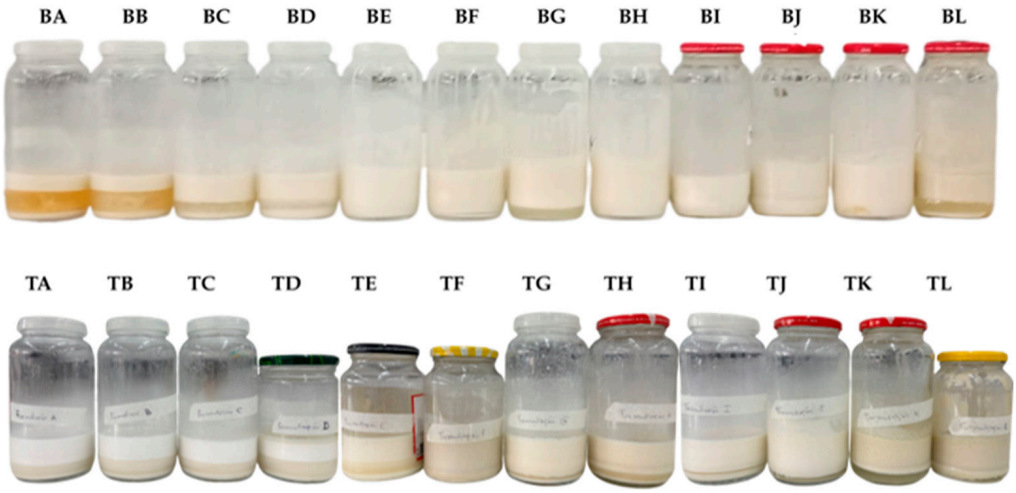


Figure 4. Physical stability of mayonnaise-type salad dressing formulations stored at 8 °C. Biosurfactant-based formulations (codes BA to BL) after 60 days of storage; Tween® 80-based formulations (codes TA to TL) after 10 days of storage. Formulations BE, BF, BH, BI, BJ, and BK remained stable without phase segregation.

The successful formulations (BE, BF, BH, BI, BJ, and BK) were then maintained under refrigeration for an additional 180 days, totaling 240 days of storage. All these formulations successfully maintained their physical integrity and stability, demonstrating consistent performance over long periods, as can be seen in Figure 5.

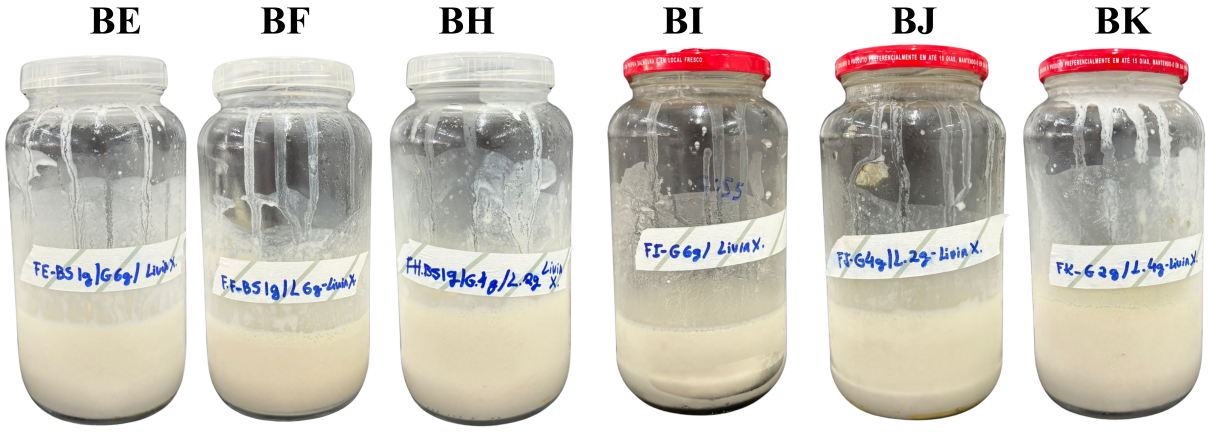


Figure 5. Stable mayonnaise-type dressing formulations (BE, BF, BH, BI, BJ, and BK) after the final storage period. These formulations included the biosurfactant produced by *C. utilis* (BE, BF, and BH) and/or natural emulsifiers (BI, BJ, and BK), demonstrating successful long-term physical integrity without phase segregation.

The pH and viscosity of the six most stable formulations during this period are presented in Table 5. An acidic pH was observed across all samples, with Formulation BI standing out for exhibiting the lowest pH and viscosity values. For the emulsions prepared with Tween® 80, the initial pH remained within the range of 2.11 to 2.74.

Table 5. Physicochemical parameters of the six stable emulsions after eight months of storage.

Sample	pH	Viscosity (mPa·s)
BE	3.78 ± 0.12^{a}	157 ± 2^{f}
BF	3.31 ± 0.08^{b}	300 ± 2^{e}
BH	2.95 ± 0.05^{c}	349 ± 3^{d}
BI	2.26 ± 0.03^{d}	21 ± 2^{c}
BJ	2.69 ± 0.10^{e}	285 ± 2^{b}
BK	2.33 ± 0.01^{d}	553 ± 1^{a}

Values are presented as mean ± standard deviation. Different lowercase letters within the same column indicate statistically significant differences according to Tukey's test ($p < 0.05$).

3.5. Microbiological Safety

In terms of microbiological safety, no growth of pathogenic or spoilage microorganisms was detected in the emulsions after 240 days of refrigerated storage (Table 6). The analysis includes the enumeration of total aerobic mesophiles, molds, and yeasts, and specific pathogens (*Salmonella* sp., *Staphylococcus* sp., and *Pseudomonas aeruginosa*) across three serial dilutions, demonstrating the sanitary quality of the products.

Table 6. Microbiological assessment of the six physically stable emulsion formulations (BE–BK) after 240 days of refrigerated storage (8°).

Sample	Dilution	Microorganisms Analyzed					
		Salmonella sp./25 g	Total Aerobic Mesophiles (CFU/g)	Thermotolerant Coliforms (MPN·g^{-1} at 45 °C)	Coagulase-Positive *Staphylococci* (CFU/g)	Molds and Yeasts (CFU/g)	*Pseudomonas aeruginosa* (CFU/g)
BE	10^{-1}	Absence	25	0	0	0	0
	10^{-2}	Absence	0	0	0	0	0
	10^{-3}	Absence	0	0	0	0	0
BF	10^{-1}	Absence	0	0	0	0	0
	10^{-2}	Absence	0	0	0	0	0
	10^{-3}	Absence	0	0	0	0	0
BH	10^{-1}	Absence	0	0	0	0	0
	10^{-2}	Absence	0	0	0	0	0
	10^{-3}	Absence	0	0	0	0	0
BI	10^{-1}	Absence	0	0	0	0	0
	10^{-2}	Absence	0	10^{1}	0	0	0
	10^{-3}	Absence	0	0	0	0	0
BJ	10^{-1}	Absence	0	1	0	0	0
	10^{-2}	Absence	0	0	0	0	0
	10^{-3}	Absence	0	0	0	0	0
BK	10^{-1}	Absence	0	0	0	0	0
	10^{-2}	Absence	0	0	0	0	0
	10^{-3}	Absence	0	0	0	0	0

A value of 0 indicates the absence of microbial cell growth.

4. Discussion

The integration of kinetic evaluation and functional testing demonstrated that *C. utilis* effectively produces a potent surface-active compound using licuri oil as the main carbon source. The rapid initial surface tension (ST) reduction suggests that biosurfactant synthesis commenced early, possibly during the exponential phase. However, the minimum ST of 31.55 mN·m^{-1} was definitively achieved at 120 h, coinciding with the transition to the stationary phase. This delayed production pattern is characteristic of secondary metabolism [18] and aligns well with the behavior observed for the related yeast *Candida mogii*, which, when cultivated on the same licuri oil substrate, achieved maximum BS production during the stationary/decline phase [11].

This mechanism is strongly supported by factorial analysis, which identified the high carbon-to-nitrogen (C/N) ratio as the key factor influencing ST reduction. This limitation-driven strategy is essential to stress the cells and redirect metabolic flux toward biosurfactant synthesis [19].

The functional parameters of the product confirm its strong competitive advantage. The lowest surface tension (ST) achieved was 25.17 ± 0.03 $mN \cdot m^{-1}$ at a critical micelle concentration (CMC) of 0.9 $g \cdot L^{-1}$. This CMC is highly competitive, positioning the product among the most efficient microbial surfactants for industrial applications. The CMC value obtained is significantly more efficient than those reported for *C. bombicola* (2.7 $g \cdot L^{-1}$) [20] and *Pichia pseudolambica* (16.0 $g \cdot L^{-1}$) [21]. Moreover, the minimal ST of 31.55 $mN \cdot m^{-1}$ achieved on licuri oil is highly competitive with, and cross-validated by, the performance of *C. utilis* grown on waste canola frying oil (CMC of 0.6 $g \cdot L^{-1}$ with ST of approximately 35 $mN \cdot m^{-1}$ [9] and *Pseudomonas* sp. (ST of 32 $mN \cdot m^{-1}$ at 1.0 $g \cdot L^{-1}$) [22].

The lower surface tension observed for the isolated product (25.17 $mN \cdot m^{-1}$) compared to the crude culture broth, 31.55 $mN \cdot m^{-1,}$ is attributed to the extraction process. The removal of interfering media components (such as salts and residual nutrients) facilitates tighter molecular packing at the air–water interface, enhancing the surface activity of the isolated compound [23].

This performance is further reinforced by recent studies utilizing the related yeast *C. mogii* on the same substrate, which achieved a highly comparable CMC of 0.8 $g \cdot L^{-1}$ and a minimum ST of 28.66 $mN \cdot m^{-1}$ [11]. This consistency reinforces the value of licuri oil as a strategic, regional feedstock. Moreover, the intrinsic safety of the strain (*C. utilis* holds GRAS status) and the proven innocuousness of its biosurfactant in previous toxicological and food application tests [9,10] provide a robust foundation for the safety and functional potential of the product derived from licuri oil.

The factorial screening successfully identified the variables critical for biosurfactant production, fulfilling its role as a process mapping tool. The linear model for surface tension (ST) showed a clear dependence on the licuri oil (X_1) and ammonium nitrate (X_4) interaction. This interaction confirms that a strategic nitrogen limitation is essential to stress the *C. utilis* cells and maximize the output of the secondary metabolite. Such behavior is consistent with reports that many yeast-derived biosurfactants (e.g., sophorolipids) are upregulated under low-nitrogen regimes, where excess carbon is redirected to surface-active compound biosynthesis [24,25]. Moreover, studies coupling hydrophobic carbon sources and variable nitrogen levels demonstrate that high carbon availability combined with moderate nitrogen favors product formation, while high nitrogen enhances growth at the expense of biosurfactant yield [26,27]. This finding is crucial for guiding future scale-up efforts toward a cost-effective medium design.

Conversely, the inability to establish robust predictive models for the Emulsification Index (Y_2 and Y_3) suggests that E_{24} activity is governed by complex, higher-order, or nonlinear interactions that could not be fully captured by the linear factorial approach. This outcome aligns with previous reports where emulsification responses exhibited significant variability and poor linear predictability. For instance, in a 2^4 factorial design applied to *Yarrowia lipolytica* [28], observed that while linear models adequately described surface tension reduction, the E_{24} response fluctuated considerably across different combinations of carbon and nitrogen sources, implying the presence of hidden nonlinear dependencies. Similarly, an RSM-based optimization study using *Pseudomonas aeruginosa* cultivated with rubber tree seed oil required the inclusion of quadratic and interaction terms to accurately predict E_{24} activity [29]. Comparable results were also reported for *Brevibacillus borstelensis*, in which a second-order model was necessary to describe the combined effects of nutrient components on emulsification performance [30]. Therefore, while the present factorial

design effectively guided the identification of nutrient ratios for maximal surface tension reduction, the interpretation of emulsification efficiency relies primarily on the direct experimental E_{24} outcomes rather than on predictive modeling. Future studies employing central composite or Box–Behnken designs could therefore capture these higher-order effects, improving predictive accuracy for emulsification-related responses.

The biosurfactant demonstrated strong functional activity, with E_{24} values exceeding 74% for the selected oils (canola and motor oil), validating its ability to effectively stabilize biphasic systems. These values are superior to those previously reported for *C. utilis* on other substrates. For example, *C. utilis* cultivated with waste frying canola oil and ammonium nitrate reached an E_{24} of 73% (using canola oil) under conditions involving nearly 6% oil, glucose, and low nitrogen (0.2% NH_4NO_3) [31].

Essentially, the compound maintained high emulsifying activity (>50%) across adverse conditions of pH (3 to 7), temperature (30 °C to 70 °C), and salinity (1% to 10%) in comparable studies. For example, *Bacillus aryabhattai* ZDY2 preserved E_{24} above 80% between 30–90 °C and still 75% at 10% NaCl (pH 5–10) [32]. *Pseudomonas mendocina* exhibited >70% emulsification with motor oil across most pH levels and stable performance under high salt conditions [33]. This structural robustness is non-negotiable for industrial applications. The stability profile confirms that the biosurfactant will retain its functionality during food processing and storage, where pH changes and thermal steps are routine, positioning it as a reliable ingredient against environmental fluctuations [34].

The most significant finding lies in the unprecedented application and validation of the product in food systems. The use of licuri oil not only as the fermentation substrate but also as the base oil in the mayonnaise-type dressings aligns perfectly with the current demand for integrated biorefinery and circular economy models.

The 240-day stability observed in six formulations provides strong proof of concept for the long-term functionality of the licuri-derived biosurfactant in complex food matrices. The clear failure of formulations containing only the biosurfactant (Formulations BA–BD), contrasted with the stability of blended systems (e.g., BE, BF, and BH), indicates that the *C. utilis* biosurfactant functions most effectively as a co-emulsifier or secondary stabilizer, rather than as a sole structural emulsifier. This result mirrors previous food-application studies where microbial biosurfactants alone were insufficient to guarantee prolonged physical stability: Campos et al. reported that mayonnaise containing only a *C. utilis* bioemulsifier lost stability within nearly 30 days unless combined with hydrocolloids (e.g., guar gum, CMC) [10]. Likewise, bakery and pastry applications (cookies, muffins, cupcakes) using biosurfactants required complementary formulation strategies (hydrocolloids, proteins, humectants) to maintain desirable texture and shelf life [35,36]. These convergent findings suggest that the biosurfactant's primary role in food formulations is to reinforce the interfacial film formed by primary emulsifiers (lecithin, egg yolk) and, together with rheology modifiers, to minimize droplet coalescence and creaming over extended storage. Therefore, the present results demonstrate the practical value of integrating the licuri-derived biosurfactant into multi-component formulations to achieve industrially relevant, long-term emulsion stability.

After evaluating important physicochemical parameters such as final pH and viscosity of the emulsions, it was observed that formulation BI presented the lowest values, being the one (among the three added emulsifying agents) that contains only egg yolk in its composition. Furthermore, it is worth noting that the concentration of biosurfactant used in this formulation is 0.5%, a divergent value compared with emulsions containing biosurfactant from Candida bombicola in association with guar gum and carboxymethylcellulose [35].

Although IN 161/2022 [37] lists only three microorganisms as quality control parameters, the food industry commonly monitors the presence of molds and yeasts in this type

of product due to their high tolerance to acidic environments. These microorganisms are the main agents responsible for spoilage in acidified sauces, and this analysis is important for quality control and shelf-life determination. Pinto et al. [38] evaluated the effect of biosurfactant in emulsions formulated with different gums and did not observe microbial growth of pathogens after 180 days of storage, corroborating the present study regarding the maintenance of safe emulsions after a long storage period without the use of prior thermal treatment.

5. Conclusions

This study demonstrated that the *C. utilis* biosurfactant produced using licuri oil as a carbon source exhibits promising physicochemical and functional characteristics for industrial and food applications. The factorial design effectively identified licuri oil and ammonium nitrate as the main variables influencing biosurfactant synthesis, confirming that mild nitrogen limitation enhances metabolite production. When applied in salad dressing formulations, the biosurfactant successfully acted as a co-emulsifier, particularly when combined with lecithin or egg yolk. The blended systems maintained physical stability for up to 240 days, confirming the compound's long-term compatibility and reinforcing its role as a secondary stabilizer in multiphase systems, as well as maintaining the appropriate pH and microbiological conditions for human consumption. These findings provide strong evidence of the potential of the licuri-derived biosurfactant as a sustainable alternative to synthetic surfactants for the food sector, adding functional and ecological value.

Despite the promising results, the predictive capacity of the linear factorial model for emulsification responses remained limited, indicating that biosurfactant activity may depend on complex, nonlinear interactions not captured in the present design. Future research should therefore employ response surface methodologies (RSM) or machine learning-based predictive modeling to elucidate these relationships more accurately. Additionally, scale-up studies in bioreactor systems are essential to assess process reproducibility and economic feasibility under controlled fermentation conditions.

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