# Production and Optimization of the Extraction Conditions of the Biosurfactant from *Candida Utilis* UFPEDA1009 with Potential Application in the Food Industry

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Biosurfactants are amphipathic molecules with capacity to reduce the surface and interfacial tension of aqueous solutions, arousing great industrial interest due to the diversity of structures and production from renewable sources, allowing the obtention of products with unique characteristics. In the food industry, for example, the use of biosurfactants produced by some yeasts is very promising, since they do not present any risk of toxicity and pathogenicity, and can be applied in emulsification, for improvement of mass texture, as antimicrobial agents and in the replacement of fats. Thus, the aim of this work was to produce a biosurfactant by the yeast *Candida utilis* UFPEDA1009 with potential application as an emulsifier in foods. The yeast was grown in mineral medium containing 6% canola frying oil and 6% glucose, under 150 rpm agitation for 88 h at 28 °C. After the production process, the surface and interfacial tension, yield and Critical Micelle Concentration (CMC), as well as the particle size distribution of emulsions were determined. The surface and interfacial tensions obtained were, respectively, 35.33 ± 0.19 and 2.53 ± 0.02 mN/m, with biosurfactant yield varying from 13.10 ± 0.04 to 48. 05 ± 0.21 g/L. The biosurfactant CMC was 0.6 g/L. The emulsions showed small particle sizes, with uniform shape and more separated particles, appearing resistance to coalescence. In general, it was observed that the increase in the concentration of the biosurfactant resulted in the decrease of the particle size, that is, the higher the concentration of a given biosurfactant, the greater the stability of the emulsion. The microbial surfactant was incorporated in the mass of a biscuit formulation in substitution of the animal fat, which showed improvements in the texture profile of its mass. Therefore, the biosurfactant produced is promising for application in food formulations as an emulsifier.

## 1. Introduction

Biosurfactants are tensioactive substances of natural origin that have the property of preferably being located between phases with different degrees of polarity, having hydrophilic (ionic, nonionic or amphoteric) and hydrophobic (hydrocarbon chain) moieties in their structure. These characteristics favor their ability to reduce surface and interfacial tensions, as well as to form microemulsions, allowing them to be used in several industrial sectors involving emulsification, detergency, lubrication, wetting, foaming, dispersions or solubilization of different phases (Bezerra et al., 2018).

Biosurfactants have several advantages over their chemical counterparts, as they can be produced from agroindustrial substrates and exhibit low toxicity, high biodegradability and compatibility with the environment, as well as greater selectivity, foamability and stability under extreme conditions of pH, temperature and salinity (Luna et al., 2018). However, its more extensive use presents as an obstacle the economic disadvantage of its production, since the market cost is still high when compared to the production of chemical surfactants (Radzuan; Banat; Winterburn, 2017). In this context, new perspectives of production with lower cost have been raised in the biotechnological processes aiming at the formulation of alternative means, composed of agroindustrial residues (Pinto et al., 2018).

In terms of application, biosurfactants are commercially important, since there is a tendency of the market to increase its production for different purposes, being classified according to their uses: 54 % as detergents, 13 % in the textile, leather and paper industries , 10 % in chemical processes, 10 % in the pharmaceutical and cosmetics industries, 3 % in the food industry, 2 % in agriculture and the remaining 2 % in other applications (Almeida et al., 2017). In the food sector, they are promising due to their antiadhesive activity and wide capacity to act as an emulsifying agent, indispensable in most foods containing oils and fats, being possible to produce them from microorganisms that fall under GRAS status (Generally Recognized as Safe), as some yeasts of the genus Candida (Mnif; Ellouz‑Chaabouni; Ghribi, 2017). Thus, the objective of this work was to produce a biosurfactant by yeast *Candida utilis* UFPEDA1009, as well as to optimize the extraction conditions to obtain a product with potential food application as emulsifier.

## 2. Material and methods

### 2.1 Microrganisms and maintenance

The yeast *Candida utilis* UFPEDA 1009 was used as the biosurfactant producer. The microorganism was maintained in the YMA (Yeast Mold Agar) medium composed by: yeast extract (0.3 %), malt extract (0.3 %), tryptone (0.5 %), D-glucose (1.0 %) and agar (5.0 %). To maintain cell viability, monthly samplings were conducted.

### 2.2 Medium of inoculum growth and biosurfactant production

The growth of the inoculum was carried out in YMB (Yeast Mold Broth) medium, which has the same composition as the YMA medium, excluding agar. The biosurfactant production process was carried out in a medium formulated with distilled water containing 6.00 % (v / v) canola frying oil, 6.00 % (v / v) D-glucose, 0.20 % $NH_4NO_3$, 0.01 % of $KH_2PO_4$, 0.50 % $MgSO_4.7H_2O$, 0.01 % $FeCl_3$, 0.01 % NaCl and 0.30 % yeast extract.

### 2.3 Preparation of inoculum and production of biosurfactant

The inoculum was standardized by transferring the yeast of YMA medium to flasks containing 50 mL of YMB medium and incubated (28 °C, 200 rpm) for 24 hours. After this time, the dilutions were performed until the desired final concentration of cells ($10^8$ cells/mL) was achieved. For the production of the biosurfactant, 2.0 % (v/v) of the inoculum was added in production medium at a concentration of 108 cells/mL. After addition, the media were incubated (150 rpm and 28 °C) for 88 h. After the incubation period, the media were subjected to centrifugation under stirring 3500 rpm for 20 minutes to obtain a metabolic fluid (free from biomass).

### 2.4 Determination of surface and interfacial tension

The surface tension of the biosurfactants was measured in the metabolic liquid using a KSV Sigma 700 (Finland) automatic tensiometer using the NOUY ring techinque. The interfacial tension was measured against n-hexadecane in the metabolic liquid (after filtration with a filter membrane of 0.45 μm in diameter).

### 2.5 Optimization the Isolation of biosurfactant

Five different extraction methods were tested for biosurfactant isolation, described by Johny (2013), Bhatia and Saharan (2015), Shah et al. (2017), Derguine-Mecheri et al. (2018) and Cameron et al. (1988). For this purpose, 100 mL of culture medium was used in each method studied.

To optimize the extraction conditions, a liquid-liquid extraction method was developed using ethyl acetate twice in the ratio 1:4 (v/v) with 100 mL of whole medium (not centrifuged). Then, the organic phase was subjected to centrifugation (2600 g for 20 minutes) and subsequent filtration. The filtrate was transferred back to the separatory funnel and saturated sodium chloride (NaCl) was added to separate the remaining aqueous phase. The organic phase was transferred to an erlenmeyer flask and anhydrous magnesium sulfate ($MgSO_4$) was added until granules were formed, filtered on qualitative filter paper and dried at 50 °C.

### 2.6 Determination of critical micelle concentration (CMC)

To obtain the CMC, 0.1 g of the biosurfactant isolated by the best extraction method previously selected was diluted to an initial concentration of 5 g/L, and successive dilutions were carried out with distilled water and the surface tensions of the respective dilutions were quantified with the aid of the NOUY ring technique.

### 2.7 Ligth microscopy of emulsions

The emulsions were prepared as described by Prasanna, Bell and Grandison (2012) using the biosurfactant solutions (½ CMC, CMC and 2XCMC) with motor oil and sunflower oil. For visualization under a light microscope (Nikon Eclipse E-100), a volume of 60 µL emulsion (after 24 hours storage at 27-28 °C) was added to a cavity slide, resting for 5 minutes for stabilization. Then, the particle size distribution was observed by means of an objective lens with magnification of 10 times.

### 2.8 Application of the biosurfactant in the cookie formulation

To prepare the cookies, a standard formulation adapted from Zouari et al. (2016) with the partial (A) and total (B) replacement of the egg yolk pasteurized by the isolated biosurfactant was used, being subjected to cooking (150 °C for 5 minutes and 180 °C for a further 15 minutes). Before cooking, the texture profile analysis (TPA) (firmness, cohesiveness, adhesiveness and elasticity) in the mass was performed using a Texture Analyzer (Brookfield CT3), as described by Zouari et al. (2016). After cooking, only the firmness was evaluated by the test of compression at 50 % of its original height (constant speed of 1 mm/s).

## 3. Results and Discussion

### 3.1 Surface and interfacial tension

One of the most important properties of a surfactant is its ability to reduce surface and interfacial tension through the formation of micelles. The higher the concentration of a surfactant in the medium, the greater the formation of micelles and the lower the surface and interfacial tensions. By evaluating the tension results found for the biosurfactant produced by *C. utilis*, it was observed that the biomolecule showed a good capacity to reduce the surface tension of the production medium from 57.75 ± 0.20 mN/m to 35.33 ± 0.19 mN/m, and interfacial tension from 40 mN/m to 2.53 ± 0.02 mN/m. According to Luna et al. (2013), surfactants that are capable of reducing water surface tension from 72 to 35 mN/m and interfacial tension against n-hexadecane from 40 to 1 mN/m are considered to be good surfactants, is considered low for values below 7 mN/m (Santos et al., 2016). Comparing this result with the results described by Campos, Stamford and Sarubbo (2014), who obtained an average value of 3.71 ± 0.15 mN/m using the same yeast, both are similar, being considered sufficiently effective the action of the biosurfactant. In relation to the values of surface tension, Almeida et al. (2017) obtained very close values using *C. tropicalis* UCP0996 in a medium containing 2.5% sugarcane molasses, 2.5 % frying oil and 2.5 % corn liquor: 34.12 ± 0, 07 mN/m (2 L bioreactor) and 35.60 ± 0.05 mN/m (50 L bioreactor). Approximate results were also found by Campos, Stamford and Sarubbo (2014) using *C. utilis* UFPEDA 1009 in different formulations of culture medium, obtaining values of 37.18 ± 1.01 to 48.06 ± 0.30 mN/m, the closest value being the canola oil residue as the carbon source.

### 3.2 Extraction yields

Table 1 shows the yield values obtained for each extraction methodology studied, considering the aspect of the extract obtained.

*Table 1: Yields and aspects of the isolated biosurfactant. Experiments were performed in triplicate and the results represent the mean ± standard deviation of the three independent experiments.*

| Solvent | Yield obtained (g/L) | Aspect | Reference |
|---|---|---|---|
| Ethanol + acetic acid | 48.05 ± 0.21 | Grainy | Cameron et al., 1988 |
| Ethyl acetate PA | 36.74 ± 0.38 | Oily | Bhatia; Saharan, 2015 |
| Acetone PA | 20.93 ± 0.18 | Oily and grainy | Johny, 2013 |
| Ethyl acetate PA | 38.93 ± 0.25 | Oily | Shah et al., 2017 |
| Ethyl acetate PA | 13.10 ± 0.04 | Oily | Derguine-mecheri et al., 2018 |
| Ethyl acetate PA | 24.22 ± 0.23 | Oily | Developed in laboratory |

According to Table 1, the highest yield was obtained from the method of Cameron et al. (1988), using the solvents ethanol and acetic acid in the ratio 3:1 (v/v) with the centrifuged medium; in addition to being superior to that found by the same authors using the same yeast (17.8 g/g). However, the appearance of the product

does not make it suitable for the intended application. Using ethyl acetate as extraction solvent, it was possible to obtain the biosurfactant in the oily form, with yields higher than those found by the authors: 19 g/L (Shah et al., 2017), 12 g/L (Derguine-Mecheri et al., 2018). In relation to economic viability, the laboratory developed method was the best choice, since it requires a lower volume of solvent (50 mL to 100 mL of medium) to the medium without the initial centrifugation and filtration steps, obtaining a clear oil extract and clear (not obtained with other methods), which is considered to be the most suitable for subsequent application in food formulations, replacing animal fat, since it is more homogeneous in terms of texture.

### 3.3 Critical Micelle Concentration (CMC)

The graphic describing the surface tension as a function of the biosurfactant concentration is presented in Figure 1. Since a good surfactant must have high efficiency and efficacy, the biosurfactant produced from *C. utilis* presented this capacity in concentrations lower than 1 g/L (0.6 g/L). As described by Santos et al. (2016), the CMC is reached at the point where a further increase in the biosurfactant concentration does not lead to an additional reduction in surface tension. While surface and interfacial tensions influence the efficacy of a surfactant, CMC reflects on the efficiency of the surfactant, ranging from 0.001 to 2 g/L for the biosurfactant concentration. Thus, the lower the concentration of the biosurfactant to reach this end, the greater its efficiency.
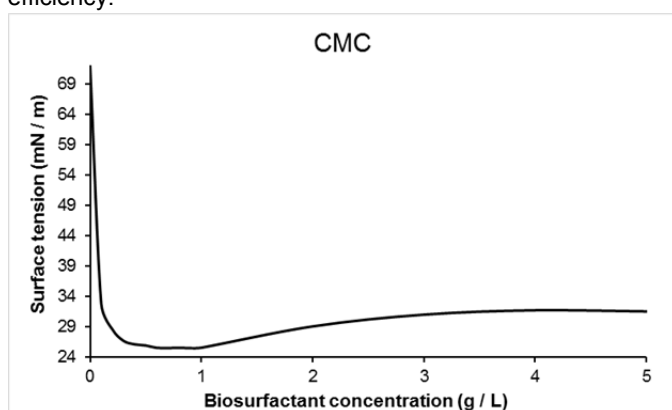


*Figure 1: Critical micelle concentration of the biosurfactant produced by C. utilis in medium supplemented with 6.00 % canola frying oil and 6.00 % D-glucose.*

This analysis was also performed for the biosurfactant produced by *Candida lipolytica* and showed a CMC value of 0.8 g L using a mineral medium supplemented with 6% soy frying oil and 1% glutamic acid (Rufino et al., 2014). Santos et al. (2013) using this same yeast in a medium composed of 5% animal fat and 2.5% corn liquor obtained a CMC equal to 0.3 g/L. A more recent study using a bacterium of the genus *Pseudomonas* for the production of biosurfactant, grown in mineral medium composed of 2% canola frying oil and 3% corn steep liquor (Soares da Silva et al., 2017) described a CMC value equal to that found in our work, of 0.6 g/L.

### 3.4 Particle Size Distribution

The evaluation of the particle size distribution of emulsions is important for the study of the physical properties of a biosurfactant, since it is possible to observe the droplet size of the emulsion, which may be related to several industrial processes such as flocculation (Han et al., 2015; Luo et al., 2017). The photomicrographs of emulsions of the biosurfactant solutions produced (½ CMC, CMC and 2XCMC) with the sunflower and motor oils are shown in Figure 2. According to Rocha e Silva et al. (2017) the size, shape and distribution of droplet size depends on various factors, the stability of the emulsion being inversely proportional to the size of the droplet. Observing Figure 2, the emulsions presented different particle sizes in relation to the two evaluated oils, besides the differences in the format and distance between the particles. The smaller the size and the more distant the particles from each other, the greater the resistance to coalescence. In general, it was observed that the increase in the concentration of the biosurfactant resulted in the decrease of the particle size, that is, the higher the concentration of a given biosurfactant, the greater the stability of the emulsion. To date, no records have been found in the literature of microscopic analysis of the emulsions using biosurfactant produced by yeasts, however, Han et al. (2015) performed this analysis with sunflower oil emulsions with exopolysaccharides produced by *Bacillus* species and with commercial guar gum emulsifier, obtaining photomicrographs similar to the emulsions of the biosurfactants with motor oil. Therefore, the results show that the biosurfactant produced by *C. utilis* has great potential to be used as an emulsifier.
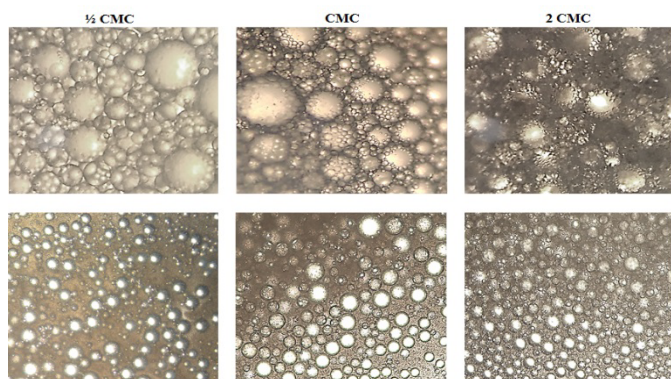
*Figure 2: Emulsions prepared with solutions (½ CMC, CMC and 2XCMC) of C. utilis biosurfactant in medium with 6.00% canola frying oil and 6.00% D-glucose. Sunflower oil (above) and motor oil (below).*

## 3.5 Analysis of the texture profile of cookies

In the analysis of the quality of a food, one of the main characteristics to be considered in the acceptance by the consumer is the result of the texture profile (Pereira et al., 2004), the fat being the ingredient that significantly affects this result, since it influences in the intensity and perception of sensory properties, such as taste. According to Table 2, it was observed that there were significant differences with the substitution of the animal fat (egg yolk) for the biosurfactant alone in relation to all texture parameters in the pasta before cooking. After the cooking process, significant decreases in firmness values were observed, mainly with total substitution (B) (from 445.59 ± 15.52 to 354.93 ± 14.84 N). Similar results of firmness were also found by Zouari et al. (2016), which showed a significant decrease (p ≤ 0.05) with the addition of a bioemulsifier produced by *Bacillus subtilis* SPB1 at concentrations above 0.5%. However, they obtained higher values of cohesiveness and lower values of elasticity. In general, the substitution of fat by the biosurfactant of *Candida utilis* presented improvements in the texture profile of the mass, being possible to replace the fat of animal origin by the evaluated biosurfactant, without prejudice to the characteristics of the final product.

*Table 2: Texture profile analysis of dough before and after baking. Different letters in columns denote significant differences between formulations (p ≤ 0.05, Tukey's test).*

| Formulation | Before baking | | | | After baking |
|---|---|---|---|---|---|
| | Firmness (N) | Cohesiveness | Elasticity (mm) | Adhesiveness (mJ) | Firmness (N) |
| 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[a] | 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[ba] | 354.93 ± 14.84[cb] |

## 4. Conclusions

The medium supplemented with agroindustrial residue and the optimization isolation of the biosurfactant with less volume of solvent favour its study and use by reducing the costs associated with the production process of the biomolecule. The results obtained for the production of the biosurfactant by *Candida utilis* demonstrate that this secondary metabolite presents promising properties in relation to surface tension and as emulsifying agent for both environmental and food purposes with potential industrial application.

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