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Production of Biossurfactant by *Candida guilliermondii* and Application in a Mayonnaise Emulsion

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In the era of globalization, many classic industries in the quest for innovation have increasing turned to biotechnology, which has enabled diverse research opportunities without exerting a negative effect on productivity. Considering the growing interest in alternative products that minimize environmental impacts natural additives produced by microorganisms, known as bioemulsifiers (surfactants with excellent emulsifying properties) have attracted the attention of researchers. Thus, the objective of the present study was to evaluate the production of biosurfactant by *Candida guilliermondii* (UCP0992) grown in low-cost medium containing 5% molasses, 5% corn steep liquor and 5% residual frying oil, for 120 hours at 200rpm. Tests were carried out to evaluate the properties of the biosurfactant and then were analysed seven formulations of mayonnaise, evaluating the stability with the addition of guar gum and the biosurfactant isolated in the formulation of mayonnaise. After 30 days of refrigeration, the samples were evaluated for phase separation and the growth of pathogens. According to the results obtained, it was observed that the biosurfactant obtained by *C. guilliermondii*, had the capacity to reduce the surface tension of water from 71 mN/m to 28 mN/m and a yield of 21 g/L with a Critical Micellar Concentration of 0.7 g/L. All the mayonnaise analysed using the biosurfactant remained stable, with no pathogenic microorganisms. With that, it can be concluded that the biosurfactant has potential for application in the food industry.

1. Introduction

In the era of globalization, many classic industries in the quest for innovation have increasing turned to biotechnology, which has enabled diverse research opportunities without exerting a negative effect on productivity (Campos et al., 2014). Considering the growing interest in alternative products that minimize environmental impacts natural additives produced by microorganisms, known as bioemulsifiers (surfactants with excellent emulsifying properties) have attracted the attention of researchers (Marcelino et al., 2020; Singh et al., 2018). Bioemulsifiers are used in the food industry is due to their emulsifying, foaming, humectant and solubilizing properties. Emulsification is particularly useful in the food industry and bioemulsifiers have the capacity to form stable emulsions, thereby improving the texture and creaminess of many products. These products can be used as emulsifiers in the processing of raw material, the control of the clustering of fat globules, the stabilization of aerated systems and the improvement of the consistency of fatty products (Campos et al., 2015). Yeasts of the genus *Candida* stand out among species of bioemulsifier-producing microorganisms due to their usefulness in food products (Bourdichon et al., 2012). However, some yeasts can produce biosurfactants in the presence of different types of substrates, such as carbohydrates. The use of different carbon sources changes the structure and properties of the biosurfactant produced, which can be useful in particular applications.

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A number of studies on biosurfactant-producing microbes, especially *Bacillus* (Durval et al., 2021), *Pseudomonas* (Bezerra et a., 2018), and *Candida* (Santos et al., 2021; Ribeiro et al.,2020a) e, have described the production process, type of biosurfactant produced, and industrial applications of these natural compounds. However, the pathogenic nature of many microorganisms restricts their range of uses, especially in the food industry. In this context, a growing number of studies have described biosurfactant production by yeasts, especially those of the genera *Candida*, *Pseudozyma*, and *Yarrowia.* The great advantage of using yeasts is the “Generally Regarded as Safe” (GRAS) status that most of these species have, which allows the application of byproducts from these microorganisms in the food and pharmaceutical industries (Silva et al., 2020).
Thus, the aim of the present study was to produce a non-toxic biosurfactant grown in low-cost medium with emulsifying properties for application as an additive in food systems.

2. Materials and methods

2.1 Microorganism

C. guilliermondii (UCP 0992) were tested as biosurfactant producers. Both strains were acquired from the culture bank of the Environmental Science Research Center of the Catholic University of Pernambuco.

2.2 Preparation of inoculum

The inoculum was transferred to a tube containing a YMA (Yeast Mold Agar*)* medium to obtain a young culture. Next, the sample was transferred to flasks containing 50 ml of YMB (Yeast Mold Broth*)* medium, followed by incubation with constant stirring at 200 rpm and 28 °C for 24 h. After this period, cell counts were performed in a Neubauer chamber until obtaining the desired final concentration of cells (10^8 CFU/ml).

2.3 Production of biosurfactant

Fermentations for the production of biosurfactant were performed in 1000 ml Erlenmeyer flasks containing 500 ml of production medium and incubated with the suspension of 10^6 CFU/ml. The inoculum was added and the media were kept under orbital stirring at 200 rpm for 144 h at a temperature of 28 °C. After the incubation period, the media were submitted to centrifugation with stirring at 4500 rpm for 20 minutes for the obtainment of the cell-free broth.

2.4 Determination of surface tension

The surface tension of the biosurfactant was measured in the cell-free broth with the aid of an automatic tensiometer (KSV Sigma 70, Finland), using the du Noüy ring method.
1987)

2.5 Determination of emulsification activity

For the determination of emulsification activity, the samples were centrifuged at 4500 rpm for 15 minutes and analyzed using the method proposed by Cooper and Goldenberg (1987).

2.6 Isolation of biosurfactant

The isolation of the biosurfactant produced by *C. guilliermondii*, with the method developed in the laboratory involved ethyl acetate and the cell-free broth with the non-centrifuged medium at a proportion of 1:4 (repeating twice). Next, the organic phase was submitted to centrifugation (4500 rpm for 20 minutes) followed by filtration. The yield of the isolated product was expressed as g/l.

2.7 Determination of critical micelle concentration (CMC)

To determine the CMC, 0.1 g of isolated biosurfactant was diluted to an initial concentration of 5 g/l, followed by successive dilutions with distilled water. The surface tension of the respective dilutions was quantified using the du Noüy ring method.

2.8 Application of biosurfactant as a food additive

The emulsifying property of the biosurfactant was tested in the formulation of seven different types of mayonnaise sauce obtained from commercial samples of the following ingredients (w / v): 40% sunflower oil, 40.3% water, 10% vinegar, 4% powdered egg (Naturovos LTDA, Brazil), 2% sugar, 2% salt, 1% mustard flour and 0.2% guar gum and 0.5% instant starch (Unilever LTDA, Brazil). The concentration of the isolated biosurfactant ranged from 0.2 to 0.8% (w/v). The formulas were then stored at 4 ºC for one month for future

analyses of their appearance, pH and viscosity (Shepherd et al., 1995). The tests were carried out in duplicate.
The best formulation out of the seven was chosen to carry on with the experiments. New emulsions were formulated with the same ingredients but in different proportions, as described in: Formula 1 - Starch; Formula 2 - Starch + Isolated *Candida* biosurfactant; Formula 3 - Starch + Guar gum; Formula 4 - Guar gum; Formula 5 – Isolated *Candida* biosurfactant + Guar gum and Formula 7-Isolated biosurfactant.
The sauces were then mixed for one minute at room temperature and then stored at 8 °C for one month in order to be analysed regarding appearance, pH and viscosity again. The emulsion stability of the sauces was analysed at each week.

2.9 Determination of viscosity of mayonnaise

The viscosity of the sauce formulas was determined at 22 °C in a Lamy Rheology viscometer (Shepherd et al., 1995).

2.10 Microbiological analyses

The mayonnaise formulations were analyzed at the end of the storage period for the microorganisms cited in the Resolution 12/2001 of the Brazilian Sanitary Surveillance Agency (Brasil, 2001).

3. Results and Discussion

3.1 Emulsification index

The emulsion stabilization capacity of a tensioactive compound is evaluated based on its capacity to maintain at least 50% of the original volume of the emulsion for 24 h after its formation. Considering this criterion, the biosurfactant produced by C. guilliermondii was able to form stable emulsions with corn oil (50%), sunflower oil (54%), soybean oil (48%) and motor oil (71.4%), which indicates a direction for further studies and possible environmental applications. Several authors consider quantities higher than 50% for emulsification to be significant.
A greater percentage of emulsification indicates the better emulsifying activity of a biosurfactant with a hydrophobic compound. Analyzing a biosurfactant produced by C. utilis grown in a medium supplemented with 5% canola frying oil and 6% glucose (Campos et al., 2014), obtained emulsification indexes with the biosurfactant produced by C. utilis, using mineral medium supplemented with 5% canola frying oil and 6% glucose, 43%, 73%, 73%, 33% and 30% for soybean, sunflower, corn, rice and motor oils, respectively (Luna et al., 2015) found emulsification indices of 78%, 21% and 24% for motor, corn and soybean oil, respectively.
According to Campos et al., 2015, the stability of an emulsion is evaluated as an indicator of surface activity, but the capacity to remain stable is not always associated with the reduction in surface tension. Although the physiological function of biosurfactants has not yet been fully clarified, several authors report the capacity to solubilize and emulsify different hydrophobic compounds.

3.2 Biosurfactant yield

The tested biosurfactant extraction method, with the best yield, was obtained with ethyl acetate (21 g / l).
In relation to viability, the method developed in the laboratory with ethyl acetate was a good better choice, as it requires a lower volume of solvent without the need for initial centrifugation and filtration steps, obtaining a clear, oily extract, considered more suitable. for later applications in food formulations. The yield of the biosurfactant produced by *C. bombicola* obtained by Silva et al. (2020) was 25.00 ± 1.02 g / L also using ethyl acetate

3.3 Determination of critical micelle concentration (CMC)

The CMC is the minimum concentration of a biosurfactant necessary for the maximum reduction in the surface tension of water and the onset of the formation of micelles. This concentration is used as a measure of the efficiency of a biosurfactant. A good biosurfactant should exhibit high efficiency and efficacy at concentrations less than 1.0 g / l. In the present study, the surface tension of water was gradually reduced from 72 to 30 mN/m with the increase in the concentration of the isolated biosurfactant. Beyond 0.7 g / l, no additional reduction in surface tension was found with the further increase in the concentration of the biosurfactant, indicating that the CMC had been reached (Figure 1).

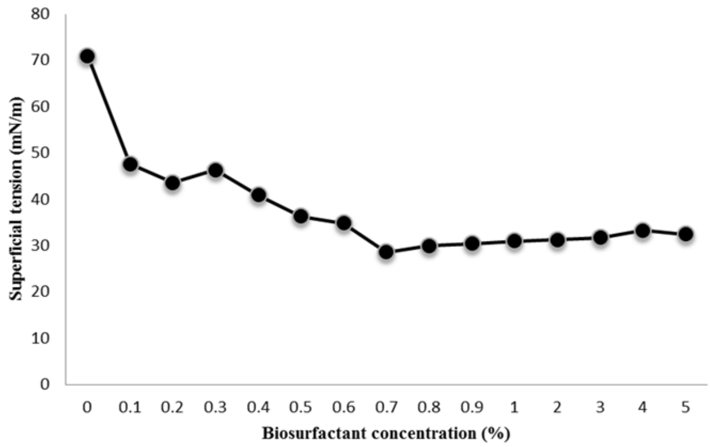

Figure 1: Critical micelle concentration of biosurfactant from C. guilliermondii UCP0996 grown in medium containing 5% molasses, 5% corn steep liquor and 5% waste frying oil

3.4 Application of biosurfactant as a food additive and mayonnaise sauces formulation

By definition, an emulsion is a heterogeneous system that consists of at least one immiscible liquid dispersed in another in the form of droplets. Emulsification plays an important role in the consistency, texture, phase dispersion and solubilization of aromas in the majority of products produced by the food industry. The function of an emulsifier is to stabilize the emulsion by controlling the clustering of globules and stabilizing aerated systems (Ghribi, 2016). The use of biosurfactants as emulsifiers occurs during the processing of raw materials to control the clustering of globules, stabilize aerated systems and improve the consistency of fat-based products (Santos et al., 2016). According to Campos et al. (2015), soybean oil and sunflower oil are among the most commonly used vegetable oils in the production of mayonnaise. The mustard powder has functional properties, serving as an emulsifier, stabilizer, agglutinating agent, conservative and antioxidant, besides conferring aroma to the product. The addition of vinegar reduces the pH, thereby inhibiting the growth of pathogenic microorganisms. Starch is often used as a texturizer in processed foods and to stabilize emulsions. Egg, especially the yolk, is a natural oil-in-water emulsion with proteins, lecithin and other phospholipids, which affects the viscosity and strength of the final mayonnaise emulsion. Guar gum is used as a thickener. All the formulas presented to be stable during the storage period (refrigeration) and no phase separation could be observed (Table 1). All the samples also presented a slight increase in pH, as opposed to the results presented (Kishk and Elsheshetawy, 2013), where the pH decreased from 4.3 to 3.5 after four weeks of storage.

Table 1: Mayonnaise formulation with addition of biosurfactant produced by C. guilliermondii in medium supplemented with 5% molasses, 5% corn steep liquor and 5% waste frying oil, after 30 days of cooling.

Concentration of biosurfactant (p/v) and formulations Mayonnaise	Initial pH	Final pH	Separation of phases *	Viscosity (mPa.S)
0.2	4.2	4.5	(-)	1415
0.3	4.2	4.5	(-)	1715
0.4	4.2	4.5	(-)	1699
0.5	4.2	4.6	(-)	1626
0.6	4.2	4.5	(-)	1645
0.7	4.2	4.5	(-)	1862
0.8	4.2	4.6	(-)	1625

*absence (-) or presence (+) of phase separation

Viscosity also remained constant, with small variations among the formulations. Mayonnaise is a non-Newtonian, pseudoplastic, thixotropic fluid with high consistency and rich in oils. Its viscosity diminishes with the increase in force applied, which leads to the alignment of molecules and a reduction in viscous friction. In other words, viscosity diminishes with the increase in the deformation rate (RPM) at a constant temperature (Bourne, 2002).

After one month of cold storage, the seven formulations with different concentrations of biosurfactant (0.2% to 0.8%) and guar gum were evaluated with regards to consistency. All formulations exhibited good emulsion stability and firmness due to the stabilizing capacity of the gum and the emulsifying action of the biosurfactant. Therefore, all seven formulations were submitted to microbiological analysis.

The mayonnaise formulations and their behavior during the four-weeks assessment. The formula containing the biosurfactant from *C. guilliermondii* at a concentration of 0.5% with the addition of 0.5% guar gum was selected to prepare the formulation of six different mayonnaise sauces. In the first week, formulas 1 and 2 exhibited phase separations (Figure 2), with visible aqueous phase at the bottom of the container. In the second week, formula 6 also exhibited phase separation. It is important to highlight that the use of isolated biosurfactant in sample 6 was not able to maintain the emulsion for 30 days. This could be explained by the difficulty in stabilizing the formula due to a large number of microstructures made up of proteins, carbohydrates and lipids combinations found in food (McClements et al., 2017). Formula 5 present to be the most creamy and stable, with higher resistance to shear and firm consistency and texture. The other formulas exhibited modifications in consistency and texture.

Figure 1: Mayonnaise sauce formulations, in the application of the biosurfactant produced by C. guilliermondii with medium supplemented with 5% molasses, 5% corn steep liquor and 5% residual frying oil, as a food additive, after 30 days in refrigeration.

As well, other authors also report the use of biosurfactants in emulsions and mayonnaise. However, recent studies with others microorganisms have been related using biosurfactants in food industry (Zouari et al., 2016; Kiran et al., 2017; Ribeiro et al., 2020a, 2020b; Silva et al., 2020).

3.5 Microbiological analysis

The microbiological analysis of the mayonnaise formulations was performed in accordance with Resolution 12/2001 of the Brazilian Sanitary Surveillance Agency (Brasil, 2001), which determines the type of laboratory analysis and limits for microorganism counts in microbiological analyses of foods destined for human consumption. None of the samples containing the biosurfactant at different concentrations (0.2 to 0.8%) exhibited microbiological contamination. There was an absence of *Salmonella* sp./25 g, levels of *Staphylococcus aureus* lower than 10 colony-forming units/ml and less than 3.0 MPN/ml of total coliforms at 45°C/g, with confidence intervals higher than 95%. All production steps were monitored following the good practices for the production of a microbiologically safe product (Campos et al., 2019) report similar results, in which samples containing a biosurfactant at a concentration of 0.7% (p/v) and guar gum exhibited no contamination by the same pathogens.

4. Conclusions

The novel biosurfactant produced by *C. guilliermondii* in a low-cost medium containing waste frying oil, molasses and corn steep liquor exhibited promising properties, such as the reduction of surface tension to 28.6 mN/m. The use of the biosurfactant added with gum demonstrated excellent performance in mayonnaise sauce formulations arising as a promising alternative for application in the food industry. In addition, biosurfactant demonstrated excellent performance in mayonnaise sauce formulations, presenting, potential application in foods and emulsions, constituting a promising new ingredient for use in the food industry.

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References

Brasil, Agência Nacional de Vigilância Sanitária (ANVISA). (2001). In Resolution-RDC n° 12, of January 2, 2001. Technical Regulation on Microbiological Standards for Food. Official diary. Brasília, DF.

Bezerra, K. G. O., Rufino, R. D., Luna, J. M., Sarubbo, L.A., 2018. Saponins and microbial biosurfactants: potential raw materials for the formulation of cosmetics, Biotechnology progress, 34, 1482–1493.

Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J.C., Gerds, M.L., Hammes, W.P., Harnett, J., Huys, G., Laulund, S., Ouwehand, A., Powell, I.B., Prajapati, J.B., Seto, Y., Ter Schure, E., Van Boven, A., Vankerckhoven, V., Zgoda, A., Tuijtelaars, S., Hansen, E.B., 2012. Food fermentations: Microorganisms with technological beneficial use. Int. J. Food Microbiol. 154, 87–97.

Bourne, M.,2002. Textura e viscosidade dos alimentos: conceito e medição . Elsevier.

Campos, J.M., Stamford, T.L.M., Sarubbo, L.A., 2014. Production of a bioemulsifier with potential application in the food industry. Appl. Biochem. Biotechnol. 172, 3234–3252.

Campos, J.M., Stamford, T.L.M., Rufino, R.D., Luna, J.M., Stamford, T.C.M., Sarubbo, L.A., 2015. Formulation of mayonnaise with the addition of a bioemulsifier isolated from *Candida utilis*. Toxicol. Reports 2, 1164–1170.

Campos, J.M., Stamford, T.L.M., Sarubbo, L.A., 2019. Characterization and application of a biosurfactant isolated from *Candida utilis* in salad dressings. Biodegradation, 8.

Durval I.J.B., Mendonça A.H.R., Rocha I.V., Luna J.M., Rufino R.D., Convertti A., Sarubbo L.A., 2020, Production, characterization, evaluation and toxicity assessment of a *Bacillus cereus* UCP 1615 biosurfactant for marine oil spills bioremediation. Marine Pollution Bulletin 157, 111357

Ghribi, D., 2016. Glycolipid biosurfactants : main properties and potential applications in agriculture Inès Mnif and Dhouha Ghribi a , b.

Kishk, Y.F.M., Elsheshetawy, H.E., 2013. Effect of ginger powder on the mayonnaise oxidative stability, rheological measurements, and sensory characteristics. Ann. Agric. Sci. 58, 213–220.

Kiran, G.S., Priyadharsini, S., Sajayan, A., Priyadharsini, G.B., Poulose, N., Selvin, J., 2017. Production of lipopeptide biosurfactant by a marine *Nesterenkonia sp*. and its application in food industry. Front. Microbiol. 8, 1–11.

Marcelino, P.R.F., Gonçalves, F., Jimenez, I.M., Carneiro, B.C., Santos, B.B., Silva, S.S., 2020. Sustainable Production of Biosurfactants and Their Applications. *Lignocellul. Biorefining* Technol. 159–183.

McClements, D.J., Bai, L., Chung, C., 2017. Recent Advances in the Utilization of Natural Emulsifiers to Form and Stabilize Emulsions. Annu. Rev. Food Sci. Technol. 8, 205–236.

Ribeiro, Beatriz Galdino, de Veras, B.O., dos Santos Aguiar, J., Guerra, J.M.C., Sarubbo, L.A., 2020a. Biosurfactant produced by *Candida utilis* UFPEDA1009 with potential application in cookie formulation. Electron. J. Biotechnol.

Ribeiro, Beatriz G., Dos Santos, M.M., Da Silva, I.A., Meira, H.M., De Oliveira, A.M., Guerra, J.M.C., Sarubbo, L.A., 2020b. Study of the biosurfactant production by *Saccharomyces cerevisiae URM 6670* using agroindustrial waste. Chem. Eng. Trans. 79, 61–66.

Santos, D.K.F., Rufino, R.D., Luna, J.M., Santos, V.A., Sarubbo, L.A., 2016. Biosurfactants: Multifunctional biomolecules of the 21st century. Int. J. Mol. Sci. 17, 1–31.

Santos E.M.S., Lira I.R.A.S., Meira H.M., Aguiar J.S., Rufino R.D., Almeida D.G., Casazza A.A., Converti A., Sarubbo L.A., Luna J.M., 2021, Energies, 14, 467.

Shepherd, R., Rockey, J., Sutherland, I.W., Roller, S., 1995. Novel bioemulsifiers from microorganisms for use in foods. J. Biotechnol. 40, 207–217.

Silva, I.A., Veras, B.O., Ribeiro, B.G., Aguiar, J.S., Campos Guerra, J.M., Luna, J.M., Sarubbo, L.A., 2020. Production of cupcake-like dessert containing microbial biosurfactant as an emulsifier. PeerJ 8, e9064.

Singh, R., Glick, B.R., Rathore, D., 2018. Biosurfactants as a Biological Tool to Increase Micronutrient Availability in Soil: A Review. Pedosphere 28, 170–189.

Zouari, R., Besbes, S., Ellouze-Chaabouni, S., Ghribi-Aydi, D., 2016. Cookies from composite wheat-sesame peels flours: Dough quality and effect of *Bacillus subtilis SPB1* biosurfactant addition. Food Chem. 194, 758–769.