PRODUCTION OF A BIOSSURFACTANT BY *Candida utilis* WITH THE USE OF LICURI OIL (*Syagrus Coronata*) AS A SUBSTRATE

Lívia X. de Araújo[1]*, Renata R. da Silva[2], Marcia V. da Silva[3] & Jenyffer M. G. Guerra[1]

[1] *Department of Chemical Engineering, Federal University of Pernambuco, Recife, PE, Brazil.*
[2] *Northeast Biotechnology Network (Renorbio), Federal Rural University of Pernambuco, Recife, PE, Brazil.*
[3] *Department of Biochemistry, Federal University of Pernambuco, Recife, Brazil.*
**Corresponding author's email adress: livia.araujo@ufpe.br*

ABSTRACT

Synthetic emulsifiers are widely used in the food industry. Although they make a very efficient contribution, these additives have been restricted by consumers due to demands to reduce the use of "artificial" or chemically synthesized additives in food. In this context, biosurfactants have emerged, which are biodegradable compounds produced by microorganisms, presenting a set of functions related to the stabilization of emulsions, improving the rheological aspects of food, as well as being less toxic than synthetic ones. This research concluded that the yeast Candida utilis is capable of producing a biosurfactant using licuri oil (Syagrus coronata) with the addition of glucose as a carbon source. The results obtained from the indirect determination of the biosurfactant, through its supernatant metabolic liquid, show that it reduced the water tension from 71.07 to 31.55 mN.m-¹ after a 120-hour fermentation with a yield of 20.6 g.L-1. This biosurfactant also showed stability in the emulsification of most vegetable oils, as well as motor oil, diesel oil and kerosene for the fermentation time mentioned. However, other issues need to be analyzed in order to optimize the overall process.

Keywords: Fermentation. Yest. Surface tension.

1 INTRODUCTION

Over the years, progress in biotechnology studies has made it possible to produce surfactants by microbiological means, known as biosurfactants. These have been used due to their advantages over those of chemical origin, such as biodegradability, specificity of action, resistance to adverse conditions (temperature, pH and salinity), low toxicity, specificity of action and digestibility.[1] Most of the studies in the literature show that the presence of biosurfactants in food reveals an arrangement of functions related to the stabilization of emulsions, improvement of rheological aspects in cookies, cakes, ice cream and sauces, acting on consistency, solubilization, texture and dispersion of phases.[2] Other studies have also revealed the antimicrobial potential of these compounds, which can inhibit the growth of deteriorating microorganisms, depending on the specificity.[3] Despite the various advantages of their use, these natural additives are unable to compete with synthetic additives in economic terms, due to the large financial investment required for production. [4]

In this context, it is becoming increasingly necessary to use low-cost waste materials as substrates, reducing manufacturing costs and the choice of microorganism. This research study looks at the use of Licuri oil (*Syagrus coronata*) as a substrate, as well as being very promising in terms of innovation. Licuri is widespread in the Northeast of Brazil, specifically in the Caatinga region, and is intensively exploited between the state of Bahia and the south of Pernambuco.[5] Known for its similarity to coconut oil and high stability, its high lipid content makes it a potential candidate for use in bioprocesses.[6] Its antimicrobial action is also explored, with the power to inhibit Staphylococcus aureus, and it can also be used in food preparations.[7]

The choice of microorganism is based on various studies by the scientific community over the last few decades, which have identified different species of Candida as important producers of biological surfactants. Its use is very efficient in media containing vegetable oils, due to the high content of free fatty acids, glycerols, proteins, glycolipids and unsaturated fatty acids, making it an excellent substrate for yeast growth, as shown by the studies carried out on Candida antarctica KCTC 7804 using soybean oil and *Candida lipolytica* from regional oily substrates (babassu, coconut and palm oil).[8,9]

2 MATERIAL & METHODS

The licuri kernels were used to extract the oil through mechanical extraction in a cold electric press and the crude oil extracted in this stage was the carbon source substrate for the production of the biosurfactant in the next stage. The yeast chosen for the production of the biosurfactant was *Candida utilis* (UFPEDA 1009), from the culture collection of the Biotechnology Department of the Catholic University of Pernambuco. The cultures were kept at 5°C in Yeast Mold Agar (YMA), with the following composition (w/v): yeast extract (0.3%), malt extract (0.3%), tryptone (0.5%), D-glucose (1%) and agar (5%). Repiques were carried out monthly to maintain cell viability.

The mineral medium for producing the biosurfactant was made up of 6% licuri oil, 6% glucose, 0.2% NH4NO3, 0.01% KH2PO4, 0.5% MgSO4.7H2O, 0.01% FeCl3, 0.01% NaCl and 0.3% yeast extract. The ingredients were dissolved and the medium sterilized in an autoclave at 120ºC for 20 minutes, with a final pH of 5.7. The sample was then transferred to flasks containing 50mL of another YMA medium and incubated under agitation at 200rpm at 28°C for 24 hours. After this period, dilutions were made until the desired final cell concentration (106 cells/mL) was obtained in a Neubauer chamber.

The fermentation to produce the biosurfactant was carried out in two 1000 mL Erlenmeyer flasks each containing 500 mL of the production medium. The flasks were incubated with the cell suspension and kept at 200 rpm in a rotary shaker at 28ºC for 120 hours. Surface tension was measured in an automatic KSV Sigma 70 tensiometer (Finland) using the NUOY ring technique. To determine the emulsification activity, the lowest surface tension obtained was analyzed according to the methodology proposed by Garcia and Cameron.[10,11] In this, 1 mL of hydrocarbons (motor oil, diesel oil, kerosene and petroleum) and 1 mL of vegetable oils (canola, cottonseed, soybean and corn) were added separately to 1 mL of cell-free metabolic liquid, obtained after centrifugation, in test tubes and shaken in a vortex for one minute. The stability of the emulsion was determined after 24 hours, and the emulsification index (E24) was calculated as the ratio between the height of the emulsion (he) and the total height (ht), the value being multiplied by 100.

The metabolic liquid from the 120 hours fermentation was first filtered to remove the cells and then subjected to the metabolic liquid extraction process using the method of Garcia and Cameron, which essentially consists of liquid-liquid extraction with ethyl acetate, twice, in a ratio of 1:4 with the non-centrifuged medium.[10,11] The organic phase was then subjected to centrifugation (200 rpm for 10 minutes) and subsequent filtration. The filtrate was transferred back to the separating funnel and a saturated solution of sodium chloride (NaCl) was added to separate the remaining aqueous phase. It was filtered again and transferred to a beaker, which was previously weighed and finally placed in an oven for 42 hours at 50°C.

3. RESULTS & DISCUSSION

Figure 1 shows the result for the emulsification index.

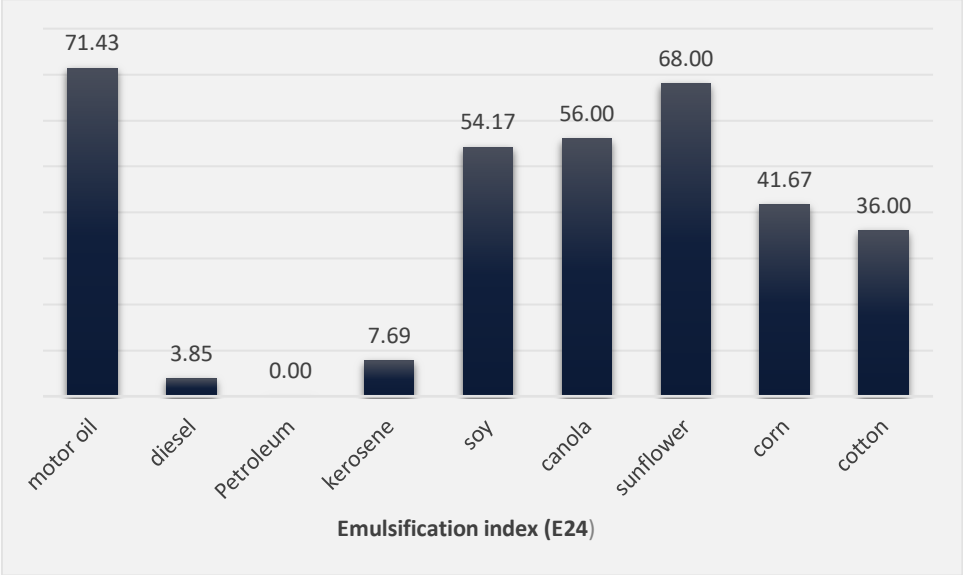

Figure 1. Emulsification index (E24) for 120h of fermentation

At this stage, the stability of the emulsification index formed was evaluated. The determination of E24 is directly related to the presence of biosurfactants in the cell-free fermented medium, where high emulsification rates are obtained, which will indicate a greater amount of biosurfactants.[12] A good biosurfactant has the ability to emulsify and stabilize the emulsification at around 50% of the original emulsion volume 24 hours after its formation.[13] This index was obtained for the result of 120 hours of fermentation, which decreased the surface tension of the water from 71.01 to 31.552 mN/m.

The bar graph presented shows a good result for the emulsification index for the fermentation carried out, with the highest percentage being obtained for motor oil. This shows that the biosurfactant formed by *Candida utilis* also has potential for applications in the remediation of degraded areas.[13] After 120 hours of fermentation, high percentages were obtained for soy, canola, sunflower, corn and cotton. An emulsification index of 3.85 was also obtained for diesel oil and 7.69 for kerosene. It can therefore be concluded that there was good biosurfactant production during this period. Since there is a relationship between the concentration of biosurfactant produced and the value of the emulsification index.[14] However, other effects that may influence the higher production of these biosurfactants should be analyzed, such as the influence of carbon and nitrogen concentrations, for example.

The biosurfactant was extracted using a process developed in the laboratory. The method has advantages over other conventional methods used, as it benefits from a smaller volume of solvent (ethyl acetate) in the main isolation phase. The yield obtained was 20.6 g/L. A recent study used the same extraction methodology to isolate the biosurfactant produced by C. utilis UFPEDA 1009 and obtained 24.22 ± 0.23 g/L .[15] If this same method were to be carried out in reactors with a larger capacity, the continuation of this research would indicate that on a larger scale the yield could be higher, since in this study the production of the biosurfactant was carried out in a shaker.

4. CONCLUSION

From this study, the results conclude that the yeast C. utilis produces a biosurfactant from licuri oil (*Syagrus Coronata*) as a substrate and with adequate surfactant and emulsifying activity. The experiment showed that 120 hours of fermentation decreased the surface tension of water from 71.01 to 31.552 mN/m with a yield of 20.6 g/L. This yield is promising for increasing the scale of production at an industrial level.

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