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

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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 with a range of functions related to stabilizing emulsions in 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 71.01 to 31.55 $mN.m^{-1}$ for a first fermentation of 120 hours. And in a second fermentation carried out after 144 hours, from 71.07 to 31.81 $mN.m^{-1}$. According to the surface tension and emulsification index, the ideal time for production was 120 hours. However, other issues should be analyzed to optimize the overall process.

Keywords: Yeast. Fermentation. 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% NH_4NO_3, 0.01% KH_2PO_4, 0.5% $MgSO_4.7H_2O$, 0.01% $FeCl_3$, 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.

Two fermentations to produce the biosurfactants were carried out in 250 ml Erlenmeyer flasks containing 100 mL of the production medium. The first fermentation lasted 216 hours and the second 144 hours. The flasks were incubated with the cell suspension and kept at 200 rpm on a rotary shaker at a temperature of 28ºC. To determine the biomass by dry weight, 90 ml of the culture was first centrifuged at 5000 rpm for 20 minutes and the supernatant was discarded. The cell pellet was then washed twice with distilled water to remove residues of the culture medium, centrifuged again, then mixed with 10 ml of ethyl acetate to remove oily substances and centrifuged again. After centrifugation, the biomass was dried in an oven at 105°C for 24 hours and weighed. This method followed the methodology proposed by Garcia and Cameron.[10,11] The surface tension was measured in an automatic tensiometer KSV Sigma 70 (Finland) using the NUOY ring technique.

To determine the emulsification activity, the samples chosen with the lowest surface tension obtained were analyzed using 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 the 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.

3 RESULTS & DISCUSSION

Figures 1 and 2 shows the results found for the first and second fermentations.

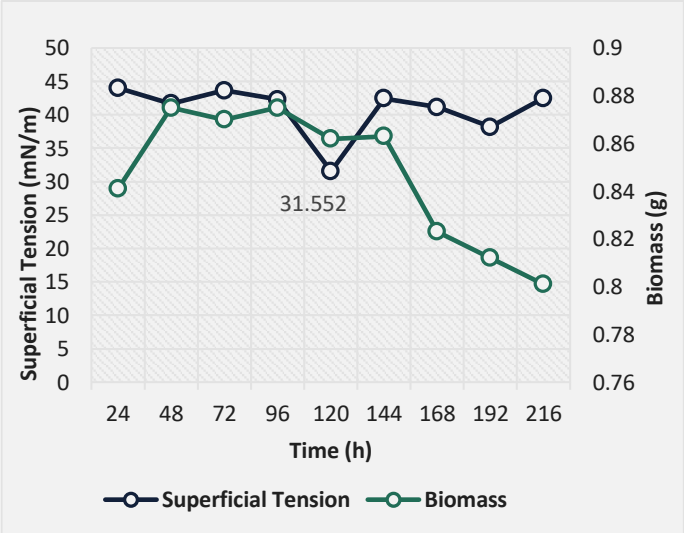

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Figure 1. Biomass and surface tension curve for 216 hours

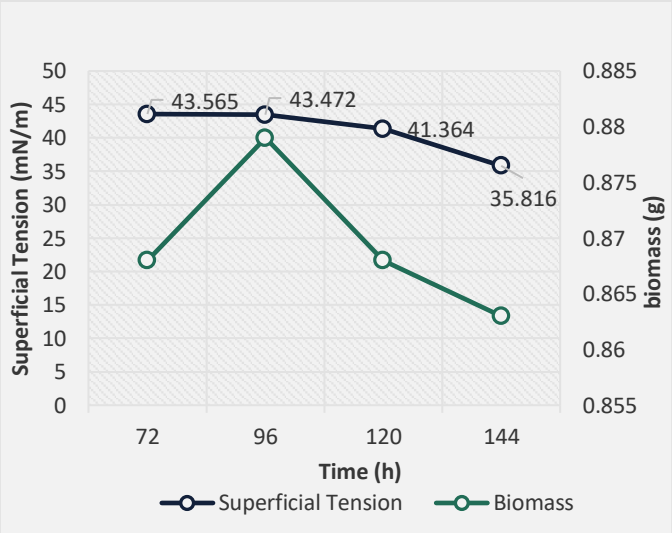

Figure 2. Biomass and surface tension curve for 144 hours

The result for the first fermentation from 24 to 216 hours shows that the lowest surface tension occurred at 120 hours, while the second fermentation from 72 to 144 hours shows the lowest tension at 144 hours. The biomass curves in the two figures follow the microbial growth process, which mostly has distinct phases, with an initial phase where the growth rate gradually increases over a certain period of time to a maximum value, and decreases at the same rate until it approaches zero.[12]

It is correct to say that the surface tension and biomass curves for the two fermentations do not have a linear correlation with time, and it is necessary to find an ideal fermentation time for the lowest surface tension point. An efficient biosurfactant is capable of lowering the surface tension of water, reducing it from approximately 72 to 35 mN.m-1, according to the literature.[1] The table shows that C. utilis, using licuri oil as a substrate, reduced the surface tension of water from 71.01 to 31.552 mN.m^{-1} for the first fermentation in 120 hours, and for a second fermentation carried out after 144 hours, from 71.07 to 35.816 mN.m^{-1}, making it viable to study for the production of biosurfactants. Other studies have used the yeast *Candida utilis* in their research, where in a medium containing residual canola frying oil, the biosurfactant showed great emulsification potential for application in the food industry.[13]

The results found for the emulsification indices are shown in Figure 3. At this stage, the stability of the emulsification index formed was assessed. The determination of E24 is directly related to the presence of biosurfactants in the cell-free fermented medium, where high emulsification indices are obtained, indicating a greater quantity of biosurfactants. A good biosurfactant has the capacity to emulsify and stabilize the emulsification at around 50% of the volume of the original emulsion 24 hours after its formation.[14] The graph presented shows a good result for the emulsification index for the two fermentations 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.[15]

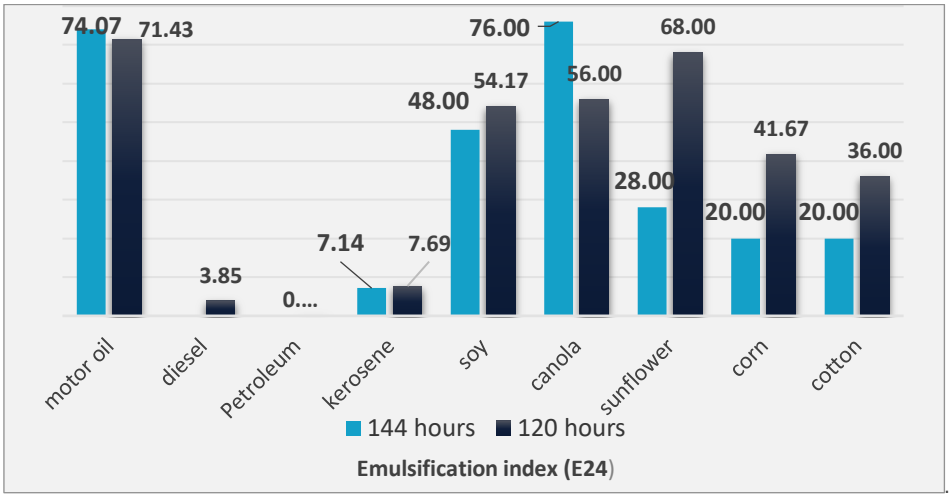

Figure 3. Emulsification index (E24) for 144h and 120h of fermentation

In the 120 hours fermentation, higher rates were obtained than in the 144-hour fermentation for soybean, canola, sunflower, corn and cottonseed oils, respectively. Also for 120 hours, an emulsifying index of 7.69 was obtained for kerosene and 3.85 for diesel oil, where the latter did not occur for the 144-hour fermentation. It can be concluded from this that there was a greater production of biosurfactant at 120 hours. Since there is a relationship between the concentration of biosurfactant produced and the emulsification index value.[11] However, other effects should be analyzed that could influence the greater production of these biosurfactants, such as the influence of carbon and nitrogen concentrations, for example.

4 CONCLUSION

From this study, the results conclude that the yeast C. utilis produces a biosurfactant from licuri oil (Syagrus Coronata) together with glucose as a substrate with surfactant and emulsifying activity suitable for application as a food additive. The best time to produce this biosurfactant, given its emulsification index, is 120 hours compared to a 144 hours fermentation. However, other effects that could enhance its emulsification should be analyzed.

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