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Emulsifying Capacity of Biosurfactants from *Chenopodium Quinoa* and *Pseudomonas Aeruginosa* UCP 0992 with Focus of Application in the Cosmetic Industry

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Biosurfactants from microorganisms and vegetables have a high potential to forming and stabilizing dispersed systems, providing stability in oil-in-water emulsions. The present study evaluated biosurfactants from *Chenopodium quinoa* and *Pseudomonas aeruginosa* as emulsifying agents, to incorporate vegetable oils with conditioning and fragrance properties. For both surfactants, the surface tension tests, emulsification index (E_{24}) and stability to different temperature and pH conditions were performed. The biosurfactant of *P. aeruginosa* decreased the water surface tension from 72 to 27 mN / m, whereas that of *C. quinoa* decreased to 31 mN / m. The emulsification index of the vegetable oils was higher when the biosurfactant of *P. aeruginosa* was used, reaching 71 % for the oil of rosemary. The extract of *C. quinoa* obtained the best result for coconut oil (51 %). When exposed to different temperatures, the two surfactants showed stability up to 100°C. Regarding the pH, in the range between 4 and 8 the extract of *C. quinoa* remained stable, on the other hand, for the biosurfactant of *P. aeruginosa*, the range between 6 and 10 were better for stability. In view of the results, it is concluded that the biosurfactants tested are capable of emulsifying oils widely used by the cosmetic industry in their formulations, presenting a good performance and stability, and therefore, an alternative for incorporation into cosmetic emulsion formulations, compatible with the human being and environment, providing new biotech products with high added value.

1. Introduction

An emulsion is a fluid state consisting of two immiscible liquid phases which mix and stabilize by the presence of an emulsifying agent. The best known examples are oil-in-water and water-in-oil emulsions, which are the basis of commercially important products used in the food, pharmaceutical and cosmetic industries (Weitz et al., 2019).

Surfactants are amphipathic molecules that facilitate the formation of emulsions by adsorption at the oil-water interface, reducing interfacial tension and improving the dispersion of oil droplets. In addition, the adsorbed surfactant forms a protective coating around the droplets, which inhibits their aggregation, improving long-term emulsion stability (Veverka et al., 2017).

Currently, there is a great interest in natural surfactants, produced by microorganisms and plants, as emulsifying agents, as they are efficient, biodegradable, stable and have reduced toxicity when compared to synthetic ones (Sarubbo et al., 2015).

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A very promising biosurfactant class is saponins. Widely distributed in the plant kingdom, saponins have a high potential for forming and stabilizing dispersed systems in food, cosmetics and pharmaceuticals (Bezerra et al., 2018). Saponin-containing emulsions performed very well and remained stable even when exposed to temperature, pH and salinity variations and could easily replace chemical surfactants in commercial products (Zhu et al., 2019).

Biosurfactants from microorganisms also provide stability in oil-in-water emulsions, which gives them potential for use in formulations, intended to incorporate vegetable and / or essential oils, such as creams, gels and conditioners (Ferreira et al. 2017).

In cosmetics, emulsions are the most commonly used physical form for integrating fat soluble, water soluble and even insoluble ingredients into stable systems. Emulsifying agents widely used in this area are polysorbate 80 (Tween 80) and sodium dodecyl sulfate (Pereira et al., 2018). However, lately there is a greater demand from consumers for products that use natural and renewable raw material in their composition, encouraging the search society for new natural assets for application in hygiene and beauty articles (Lukic et al., 2016).

Given the above, the present study evaluated the potential of two biosurfactants, derived from a vegetable (*Chenopodium quinoa*) and a microorganism (*Pseudomonas aeruginosa*), as emulsifying agents, to incorporate vegetable oils with conditioning and fragrance properties, focusing of application in the cosmetic industry.

2. Materials and Methods

2.1 Microorganism and vegetal material

Pseudomonas aeruginosa UCP 0992 was obtained from the culture collection of the Catholic University of Pernambuco, Brazil. The microorganism was maintained at 5ºC on agar nutrient broth containing beef extract (0.5 %), pepton (1 %), NaCl (0.5 %) and agar (5 %). Transfers were made to fresh agar slants each month to maintain viability.

Plant material was purchased from the public market in Recife. The seeds of *C. quinoa* were ground in a blender to a fine powder, so that they can be used to prepare the extract.

2.2 Production and isolation of biosurfactant from *P. aeruginosa*

The cultures of *P. aeruginosa* UCP 0992 were maintained in nutrient agar slants at 4 °C. For pre-culture, the strain from a 24 h culture on nutrient agar was transferred into 50 ml nutrient broth to prepare the seed culture. The cultivation condition for the seed culture was 28 °C, 150 rpm, and 10–14 h of incubation time. For liquid fermentation, a 2 % cell suspension of 0.7 OD (optical density) at 600 nm, corresponding to an inoculum of 10^7 CFU/ml, was inoculated into 500-ml flask containing 100 ml medium in distilled water based medium with 4% of vegetable oil refinery sludge residue and 0.5 % of corn steep liquor. The culture temperature and agitation rate was 28 ◦C and 150 rpm for 96h.

The biosurfactant was isolated from culture broth free of cells, obtained by centrifuging at 5000 g for 20 min. The supernatant was acidified with 6M HCl to pH 2.0 and an equal volume of $CHCl_3/CH_3OH$ (2:1) was added. The mixture was vigorously shaken for 15 min and allowed to set until phase separation. The organic phase was removed and the operation was repeated twice again. The product was concentrated and dissolved in water.

2.3 Preparation of *Chenopodium quinoa* extract

The extraction of biosurfactant from *Chenopodium quinoa* was done based on the methodology of Ribeiro et al. (2013), through a hydroalcoholic solution containing 50 % ethanol added to the seeds in a 1: 3 (w/v) ratio. The final solution was lyophilized to obtain crude extract. The surface tension test was performed to confirm the presence of biosurfactant in the extract. The processes were performed in duplicate.

2.4 Surface tension and CMC determination

The measurement of the surface tension was carried out on the cell-free broth, and on the *Chenopodium quinoa* extract, both at room temperature, by the ring method using a Sigma 70 Tensiometer (KSV Instruments Ltd., Finland). The surface tension of the water (70 mN / m) was used as a parameter.

The critical micelle concentration (CMC) was determined by measuring the surface tensions of dilutions of isolated biosurfactants in distilled water up to a constant value of surface tension. The value of CMC was obtained from the plot of surface tension against surfactant concentration.

2.5 Stability studies

Stability studies were done using isolated biosurfactant from *P. aeruginosa* and extract from *Chenopodium quinoa*. The effects of different temperatures (40, 60, 80 and 100 °C), and pH values (2.0, 4.0, 6.0, 8.0 and 10.0) in the biosurfactants were evaluated by determining surface tension.

2.6 Emulsifying activity with different hydrophobic compounds

Emulsification index was measured using the method described by Cooper and Goldenberg (1987), whereby 2 ml of hydrophobic compound was added to 2 ml of raw biosurfactant solution from *P. aeruginosa* or *C. quinoa* extract solution, in CMC and 2x CMC concentrations, in a graduated screwcap test tube, and vortexed at high speed for 2 min. The emulsion stability was determined after 24 h and the emulsification index was calculated by dividing the measured height of the emulsion layer by the mixture's total height and multiplying by 100. The oils used were coconut, sunflower, grape, lavender, cedar and rosemary.

3. Results and discussion

3.1 Surface tension and critical micelle concentration (CMC) of the biosurfactants

The critical micellar concentration of saponins, biosurfactants present in plants, varies according to the plant species from which it is extracted. This value can usually vary in the range of 0.05 % and 0.07 % when they are in their pure state; however, when present in raw plant extracts, the CMC presents higher values (Jiang et al., 2019). Studies by Jarzebski et al. (2018) and Samal et al. (2017) in which the CMC of *Verbascum nigrum* L. (mullein) and *Sapindus mukorossi* crude plant extracts was investigated, the CMC values of 1 % and 0.6 %, respectively, were found. In this study the CMC of *C. quinoa* crude extract was 0.33%, with a surface tension of 31 mN / m.

For *P. aeruginosa* 0992 the CMC was 0.9 g/L, and surface tension was 27mN / m. These values were also found by Silva et al. (2010), who used the same microorganism for the production of biosurfactant, corroborating with the results obtained in this work (Figure 1.)

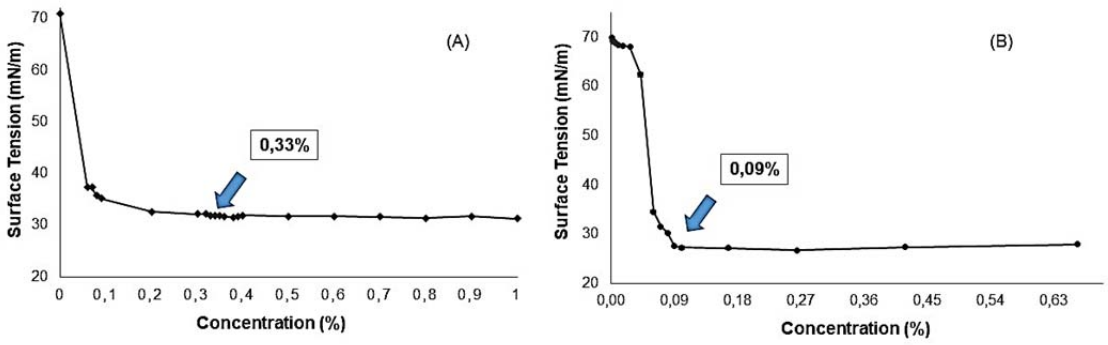

Figure 1. Surface tension versus concentration of extract of Chenopodium quinoa (A) and isolated biosurfactant produced by P. aeruginosa (B)

3.2 Stability studies

In stability tests under different temperature conditions, *C. quinoa* extract and crude *P. aeruginosa* biosurfactant showed stability at all temperatures tested (40, 60, 80 and 100 °C), maintaining the surface tension values at 31 and 27 mN / m, respectively (Figure 2).

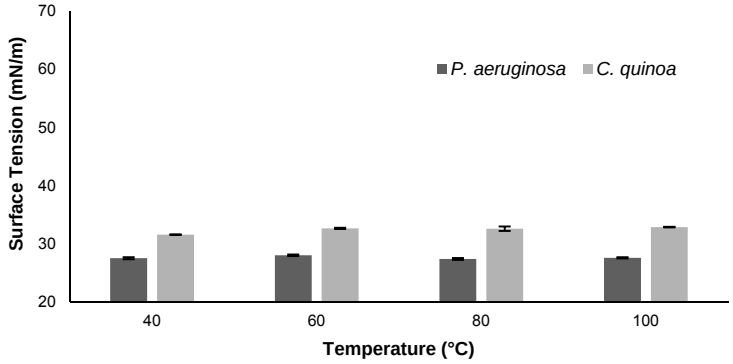

Figure 2. Influence of temperature on surface tension of biosurfactant from P. aeruginosa and extract from C. quinoa, at pH values 7 and 5, respectively.

In the range of pH values, stability was maintained for *P. aeruginosa* UCP 0992 biosurfactant in the range of 6 to 10. In the case of *C. quinoa* extract stability was maintained at pH values of 4 to 8 (Figure 3). Measurements were made at room temperature.

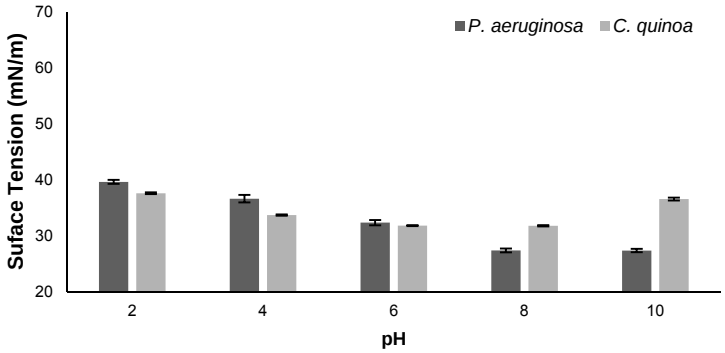

Figure 3. Influence of pH on surface tension of biosurfactant from P. aeruginosa and extract from C. quinoa

In the literature it is possible to find similar results, corroborating with those found in the present study. The stability of a biosurfactant produced by *P. aeruginosa* at different temperatures and pH values was studied by Chen et al. (2016). The biosurfactant was stable up to 100°C and between pH 6 and 8. Instability under acidic conditions is possibly due to insolubility and precipitation tendency of the biosurfactant at acidic pH. With respect to plant extracts, stability in the pH range between 5 and 9 was reported by Ralla et al. (2017).

3.3 Emulsifying activity with different hydrophobic compounds

When *C. quinoa* extract was used as emulsifying agent, the oils with the highest percentage of emulsification were coconut, sunflower and grape, with values between 41 % and 51 %, in the two concentrations tested 0.33 % and 0.66 % (CMC and 2x CMC). Among the essential oils, cedar was the one that most emulsified, with an index of 34 % at a concentration of 2x CMC (Figure 4). Ozturk et al. (2016), highlight the efficiency of saponins in the formation of stable emulsions in a wide range of conditions, thus being interesting molecules for use in various commercial fields, such as the food, cosmetics and pharmaceuticals industry.

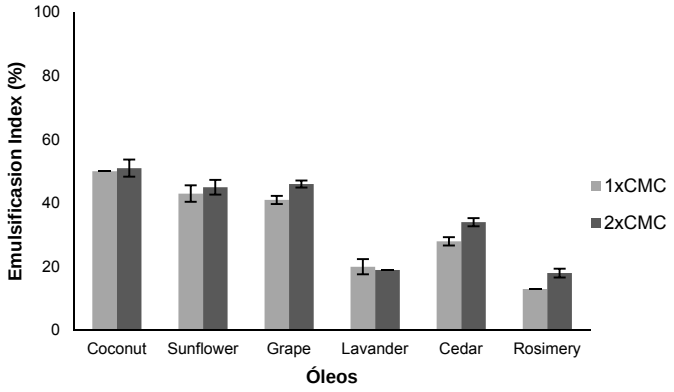

Figure 4. Emulsification index of extract from Chenopodium quinoa

When *P. aeruginosa* 0992 biosurfactant was used, all oils obtained emulsification rates above 53 %, reaching 71 % for rosemary oil at 2x CMC concentration, demonstrating the potential of this biosurfactant as emulsifier for these oils (Figure 5).

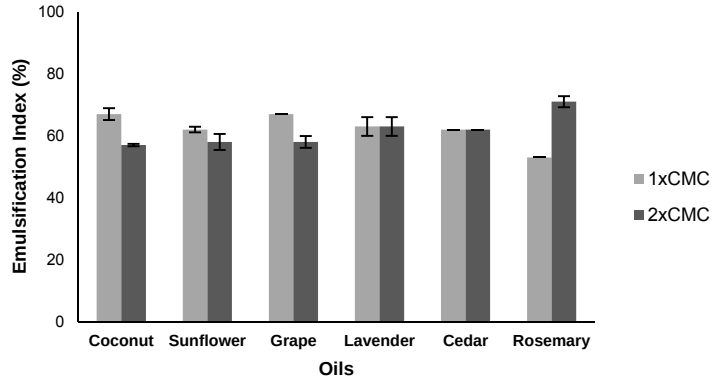

Figure 5. Emulsification index of biosurfactant from P. aeruginosa

The concentrations tested in the present study, based on the onset of micelle formation for each biosurfactant (CMC) and its double (2x CMC), show that with increasing concentration there is an increase in emulsifying potential.

Importantly, for a biosurfactant to be considered as a good emulsifying agent the criterion to be analyzed is the ability to form stable emulsions at least above 50 % for 24 h or more (Kreischer and Silva, 2017). Thus, P. aeruginosa biosurfactant was effective for all oils tested, and C. quinoa for coconut oil.

Another point to note is that in the cosmetic industry today, the concentration of emulsifying actives added in cosmetic formulations, such as creams and conditioners, varies between 1 and 6 % (Fujii et al., 2016; Pereira et al., 2018), concentrations much higher than those tested in the present paper. Therefore, it is possible that C. quinoa extract will achieve better emulsification rates with increasing concentration, especially for sunflower and grape oils, since at 2xCMC the 45% and 46% respectively have been reached.

4. Conclusion

This study demonstrated the properties as emulsion stabilizing agents of C. quinoa extract, especially for coconut oil, and crude P. aeruginosa UCP 0992 biosurfactant for all oils tested using small concentrations.

These actives showed stability over a wide temperature range, and at more acidic pH values like 4 and 6, range used in various cosmetic emulsions for skin and hair compatibility. These surfactants presented great biotechnological potential, especially with regard to their emulsification potential, demonstrating the feasibility of application as an additive in products such as cosmetics.

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