

Evaluation of the Emulsifying and Antioxidant Capacity of the Biosurfactant Produced by *Candida bombicola* URM 3718

Iverson Amaro da Silva^{a,c}, Káren Gercyane Oliveira Bezerra^{c,d}, Italo José Batista Durval^{c,d}, Charles Bronzo Barbosa Farias^{c,d}, Cláudio José Galdino da Silva Junior^{b,c}, Emilia Mendes da Silva Santos^{b,c}, Juliana Moura de Luna^{b,c}, Leonie A. Sarubbo^{a,b,c,d}

^a Department of Antibiotics, Federal University of Pernambuco, Avenida Arthur de Sá, S/N, Cidade Universitária, CEP: 50740-520, Recife-Pernambuco, Brazil

^b Center of Science and Technology, Catholic University of Pernambuco, Rua do príncipe, n 526, Boa Vista, CEP: 50050-900, Recife-Pernambuco, Brazil

^c Advanced Institute of Technology and Innovation (IATI), Rua Joaquim de Brito, n 216, Boa Vista, CEP: 50070-280, Recife-Pernambuco, Brazil

^d Northeast Biotechnology Network, Federal Rural University of Pernambuco, Rua Dom Manoel de Medeiros, S/N, Dois Irmãos, CEP: 52171-900, Recife-Pernambuco, Brazil
iversonsilva6@gmail.com

Biosurfactants are amphipathic molecules with hydrophobic and hydrophilic moieties, which can be obtained by bacteria, yeasts and filamentous fungi. In recent years, the scientific production with a focus on obtaining these bioproducts from industrial waste has increased. This alternative stems from the need to reduce production costs, since biosurfactants are not yet economically competitive against synthetic surfactants, which are petroleum-based, toxic and not biodegradable. Among the properties of biosurfactants we can cite the surface tension reduction, low toxicity, biodegradability, diversity, specificity, dispersing ability, emulsifying, demulsifying, antimicrobial and antioxidant activities. Such properties make biosurfactants attractive for application in several areas. In this sense, the present study evaluated the emulsifying and antioxidant capacity of the biosurfactant produced from low cost substrates by *Candida bombicola* URM 3718. The emulsifying capacity of the biosurfactant against vegetable oils was compared with guar gum, a vegetable origin emulsifier of common use in the food industry. The total antioxidant capacity (TAC) of the biomolecule was evaluated by the phosphomolybdenum method, seeking the reduction of molybdenum VI in molybdenum V by the antioxidant substance. According to the results, it was possible to verify that the biosurfactant presented excellent emulsification rates when compared to guar gum. The variation of the biosurfactant concentration had little effect on the results. On the other hand, in the results obtained for the antioxidant activity, it was verified that the increase of the concentration of the biosurfactant contributes to the increase of the percentage of TAC. Thus, it can be concluded that the biosurfactant obtained from low-cost substrates has potential for application in food systems, where emulsification and the introduction of antioxidants are extremely important for the useful life of food.

1. Introduction

Biosurfactants are natural molecules of an amphiphilic nature. Like surfactants, their molecules consist of a polar portion and an apolar portion. They can be produced by several microorganisms, including bacteria, filamentous fungi, and yeast, with different molecular structures and surface activities (Rocha e Silva et al., 2018). Its reduced toxicity is a feature that makes it highly competitive in industries that demand products with such property (Dolman; Wang; Winterburn, 2019).

Biosurfactants have been considered a safe and efficient alternative for the removal of hydrophobic contaminants and heavy metals. In addition to environmental decontamination, these compounds have the potential to be applied in the food industry, in the pharmaceutical and cosmetics industry and in the medical

field, as well as in the formulation of detergents, demonstrating their multifunctional characteristics. Thus, investing in strategies to improve the processing of these biomolecules is the path to the production of biosurfactants on an industrial scale (Santos et al., 2016).

In the food industry, chemically synthesized surfactants have been used in many formulations. Glycerol monostearate and carboxymethylcellulose are synthetic emulsifiers widely used in the food industries. Although they are extremely efficient, these additives have been restricted, especially by consumers, due to the demands to reduce the use of “artificial” or chemically synthesized additives in foods. Consequently, the increasing awareness of consumers has led to increased demand for more natural additives and ingredients (Campos et al., 2013).

Biosurfactants are not far behind, they have also been used, as an example we have lecithin and some proteins being used in salad dressings and cream and icing. However, since chemically synthesized surfactants have high toxicity, biosurfactants have gained prominence for their biodegradable and non-toxic nature. They are increasingly recommended as new functional additives for the food industry (Sharma, 2016). Furthermore, this growing awareness of replacing synthetic products with natural products reaches consumers who demand the replacement of more harmful products with natural ones and meet the same requirements. In this context, biosurfactants act very importantly as emulsifiers, and the particular combination of this characteristic with the antioxidant activity presented by the biocompound suggests its application as a food additive in food formulation (Ranasalva; Sunil; Poovarasan, 2014).

Emulsification has a role in the formation of consistency and texture, as well as in the phase dispersion and in the solubilization of aromas (Rahman; Gakpe, 2008). In general, the role of emulsifiers in foods is to promote the stability of the emulsion, controlling the agglomeration of fat globules and stabilizing aerated systems (Patino et al, 2008). By definition, an emulsion is a heterogeneous system, consisting of at least one immiscible liquid dispersed in another in the form of droplets (Sarubbo et al., 2001). Such systems have minimal stability, which can be increased by surfactant additives, finely divided solid that work by reducing the interfacial tension, decreasing the surface energy between the two phases and preventing the coalescence of the particles through the formation of steric and electrostatic barriers (Kosaric, 2001).

There are reports of the action of biosurfactants as emulsifiers for the processing of raw materials, with application in bakery products, influencing the rheological characteristics of flour, and meat products in the emulsification of fat. Emulsification plays an important role in consistency and texture formation, as well as phase dispersion and flavor solubilization (Radhakrishnan et al., 2011; Patino et al., 2008).

Regarding antioxidant activity in the food sector, the potential of natural compounds is of considerable attention. Biosurfactants have proved sufficient to replace existing synthetic antioxidants because they have significant antioxidant activity, since the generation of toxic compounds, development of rancidity and undesirable flavors are negative balances of lipid self-oxidation in addition to the fall in food security (Nitschke; Silva, 2017; Sharma, 2016).

In this sense, the objective of this work is to evaluate the emulsifying and antioxidant capacity of biosurfactant produced by *Candida bombicola* URM 3718.

2. Materials and Methods

2.1 Microorganisms

Candida bombicola yeast URM 3718, deposited in the Culture Collection of the Department of Mycology of the Federal University of Pernambuco, was used in the production of the biosurfactant. The microorganism was refrigerated at 5° C with YMA (Yeast Mold Agar) with the following composition yeast extract (0.3%), malt extract (0.3%), tryptone (0.5%), dextrose (1.0%), agar (3.0%)

2.2 Inoculum preparation

The inoculum of *C. bombicola* was prepared by transferring cells grown on a slant to 50mL of YMB (Yeast Mold Broth) consists of the same ingredients as YMA without agar. The seed culture was incubated for 24 hours at 28°C and agitated at 150 rpm. The inoculum (1%, v/v) was added to the medium at the rate of 10⁴ cells/mL.

2.3 Biosurfactant production

The fermentations for biosurfactant production by *Candida bombicola* URM 3718 were performed in medium formulated with distilled water containing 5% sugarcane molasses, 5% residual frying oil, and 3% corn steep liquor, with pH adjusted to 6.0 with a solution of HCl. Fermentations were performed in 1000 mL Erlenmeyer flasks containing 500 mL of the production medium and incubated with 5% of the pre-inoculum. The flasks were kept under orbital shaking at 180 rpm for 120 hours at 28 °C.

2.4 Determination of surface tension

The surface tension of the produced biosurfactant was measured in the cell-free metabolic liquid in KSV Sigma 700 tensiometer (Finland) using the NUOY ring.

2.5 Biosurfactant isolation

Isolation of the biosurfactant was performed from cell-free metabolic broth obtained after centrifugation (4400 rpm, 15 minutes, 4°C). Thereafter, the same volume of ethyl acetate (1:1 v/v) was added, the mixture being vigorously stirred for 15 minutes and allowed to stand to separate the layers. The samples were extracted twice. The organic phase was evaporated at 40 °C to solvent remove. The obtained residue was washed twice with hexane to remove the remaining oil and any hydrophobic substance, such as fatty acids and alcohols, which may have been formed during fermentation and the yield was obtained by gravimetry.

2.6 Emulsifying capacity

The emulsification activity of emulsifiers was compared with guar gum (plant origin) using the method of Prasanna, Bell, and Grandinson (2012). The oil (2 mL) was added to 2 mL of an emulsifier solution or gum solution (1%, w / v) in a screw-capped glass tube (100 mm x 13 mm) and the contents vortexed for 2 min at 50 Hz. After 24 h, the emulsification index (E24) was determined according to Equation 1:

$$E24 = (he/ht) \times 100 \quad \text{(Equation 1)}$$

Where: he is the height of the emulsion layer, and ht is the total height of the mixture in mm. Different vegetable oils were used as substrates (corn oil, soybean oil, sunflower oil, canola oil, and peanut oil). All samples were stored at 27 °C (Han et al., 2015).

2.7 Antioxidant capacity

The total antioxidant capacity (TAC) of the compounds was evaluated by the phosphomolybdenum method, which consisted of the ability of the composition to reduce molybdenum and form the phosphate-molybdate complex (Pietro et al., 1999). The formation of this complex results in the color change from yellow to bluish purple. Different concentrations of the composition (100 µL) were mixed in 1 mL with phosphate-molybdate complex (40 mM ammonium molybdate / 60 mM sulfuric acid, 280 mM sodium phosphate), then incubated in a water bath at 90 °C for 90 minutes. Absorbances were read on a spectrophotometer at 695 nm. For calculation purposes, ascorbic acid was considered as 100% antioxidant activity; the activity was calculated according to equation 2:

$$\% \text{ Antioxidant Capacity} = [(Abs1 - Abs0) / Abs0 - Abs AA] \times 100 \quad \text{(Equation 2)}$$

Where: Abs0 is the absorbance of the control, Abs1 is the absorbance of the compound, and Abs AA absorbance of ascorbic acid.

3. Results and Discussion

3.1 Determination of surface tension

The surface tension result for the biosurfactant produced by *Candida bombicola* URM 3718 was 30.790 ± 0.04 mN.m⁻¹. According to Akbari et al. (2018), biosurfactants with the ability to reduce water surface tension from 72 to 35 mN.m⁻¹ are active and are therefore considered suitable surfactants.

Similar results were found by Jadhav, Pratap, and Kale (2019) using *Starmerella bombicola* MTCC 1910 in medium containing 10% sunflower oil refinery residue as carbon source and obtaining surface tension value of

$35.5 \pm 0.52 \text{ mN.m}^{-1}$. Shah et al. (2017) also used *Starmerella bombicola* ATCC 22214 grown in medium with 10% palm oil, and the obtained surface tension was 35.35 mN.m^{-1} .

3.2 Emulsifying capacity

The emulsification index (E₂₄) is a qualitative and rapid method for evaluating the emulsifying properties of surfactants. According to Campos et al. (2015), the stability of an emulsion is indicative of biosurfactant surface activity, although the emulsification capacity of the bioproduct is unrelated to its ability to reduce surface tension. The results obtained for the emulsifying capacity of Guar Gum and the biosurfactant produced by *Candida bombicola* URM3718 are shown in Tables 1 and 2, respectively.

Table 1: Guar gum emulsification index against vegetable oils

Guar Gum (1%)	E ₂₄ (%)				
	Soy Oil	Corn Oil	Canola Oil	Sunflower Oil	Peanut Oil
	48.11±1.33	66.77±0.15	52.59±1.05	30.49±0.85	42.36±1.38

Table 2: Biosurfactant emulsification index against vegetable oils

Biosurfactant	E ₂₄ (%)				
	Soy Oil	Corn Oil	Canola Oil	Sunflower Oil	Peanut Oil
0.3%	41.16±0.32	39.29±0.00	44.64±0.27	49.78±0.31	62.70±0.89
0.6%	45.81±3.50	48.28±0.00	45.44±1.40	54.61±0.79	68.97±0.00
1.2%	51.78±0.08	56.33±0.48	50.86±1.22	56.72±2.19	69.48±0.73

Guar gum, used as a comparative as it is documented by the Food and Drug Administration (FDA) for food purposes, is a fiber obtained from the endosperm of *Cyamopsis tetragonolobus*, the scientific name of the Guar plant.

By analyzing the results, it is possible to state that both emulsifiers were able to satisfactorily emulsify the oils studied, especially the biosurfactant that obtained high levels for all oils, being the increase of the biosurfactant concentration a determining factor for the increase emulsification indexes. Comparing the results obtained with values found in the literature for biosurfactant emulsification indices of *Candida* species against vegetable oils, Pinto et al. (2018) used *Candida bombicola* to produce biosurfactant from industrial waste in medium containing 5% cane molasses, 5% corn steep liquor, and 5% residual soybean oil. It obtained 28% emulsification rates for canola oil, 26 % for corn oil, and 30% for soybean oil. Campos, Stamford, and Sarubbo (2014) used biosurfactant produced by *Candida utilis* UFPEDA 1009 in medium containing residual canola oil and obtained 73, 73, 43, and 33% for sunflower, corn, soybean and rice oils, respectively. Corroborating, thus, the affirmation of the application of biosurfactant produced in alimentary systems.

3.3 Antioxidant capacity

Lipid oxidation and enzymatic activities are significant problems in the food industry, which can result in changes in the chemical composition of the food, thus reducing its quality and shelf life (Gahruie; Niakousari, 2017). Antioxidants play a central role in neutralizing these processes, and their action can be verified through several mechanisms: inhibiting free radicals, sequestering oxygen, or chelating metal ions that catalyze oxidative reactions (Cörmet; Gökmen, 2018). The antioxidant capacity of the produced biosurfactant was verified, and the results are expressed in table 3.

According to Table 3, the biosurfactant did not present satisfactory results about the reduction technique of the phosphomolybdenum complex. According to Pietro et al. (1999), this analysis is based on the reduction of molybdenum VI to molybdenum V by the test substance, resulting in the medium color change from light yellow to green. Comparing the percentages of the total antioxidant capacity (TAC) of the biosurfactants with the reference concentration of ascorbic acid ($2000 \mu\text{g.mL}^{-1}$), at the concentration of $2000 \mu\text{g.mL}^{-1}$ the biosurfactant showed 25.47% of activity. The results presented a linear relationship, indicating that the

increase of biosurfactant concentration favors the increase of its activity. Thus, in higher concentrations, the biosurfactant produced has the potential for application in foods lacking antioxidants.

Table 3: Percentages of total antioxidant capacity (% TAC) of biosurfactant

Concentration of Biosurfactant ($\mu\text{g.mL}^{-1}$)	% TAC
2000.00	25.47 \pm 3.18
1000.00	11.16 \pm 0.37
500.00	7.65 \pm 4.53
250.00	7.01 \pm 0.46
125.00	3.47 \pm 0.07
62.50	2.80 \pm 0.21

4. Conclusions

In the present study, *Candida bombicola* URM 3718 yeast produced biosurfactant from agro-industrial residues with excellent surfactant and emulsifier activity, being the most efficient biosurfactant that Guar Gum in emulsifying vegetable oils used in food. The biosurfactant did not show good antioxidant activity. However, the results indicated the feasibility of applying biosurfactant in food systems.

Acknowledgments

This study was funded by the Foundation for the Support of Science and Technology of the State of Pernambuco (FACEPE), the National Council for Scientific and Technological Development (CNPq), and the Coordination for the Improvement of Higher Level Education Personnel (CAPES). The authors are grateful to the Centre of Sciences and Technology of the Universidade Católica de Pernambuco and to the Advanced Institute of Technology and Innovation (IATI), Brazil.

References

- Akbari, S.; Abdurahman, N. H.; Yunus, R. M.; Fayaz, F.; Alara, O. R., 2018, Biosurfactants - A New Frontier for Social and Environmental Safety: A Mini Review, *Biotechnology Research and Innovation*, 2, 81-90, DOI: 10.1016/j.biori.2018.09.001.
- Campos, J. M.; Stamford, T. L. M.; Rufino, R. D.; Luna, J. M.; Banat, I.; Sarubbo, L. A., 2013, Microbial biosurfactants as additives for food industries, *Biotechnol. Progress*, 29, 1097–1108. DOI: 10.1002/btpr.1796.
- 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. Rep.*, 2, 1164-1170, DOI: 10.1016/j.toxrep.2015.08.009.
- 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, DOI: 10.1007/s12010-014-0761-1.
- Cömert, E. D.; Gökmen, V., 2018, Evolution of food antioxidants as a core topic of food science for a century. *Food Res. Int.*, 105, 76-93, DOI: 10.1016/j.foodres.2017.10.056.
- Dolman, B. M.; Kaisermann, C.; Martin, P. J.; Winterburn, J. B., 2016, Integrated sophorolipid production and gravity separation. *Process Biochemistry*, 54, 162-171, DOI: 10.1016/j.procbio.2016.12.021.
- Gahruie, H. H.; Niakousari, M., 2017, Antioxidant, antimicrobial, cell viability and enzymatic inhibitory of antioxidant polymers as biological macromolecules. *Int. J. Biol. Macromol.*, 104, 606-617, DOI: 10.1016/j.ijbiomac.2017.06.021.
- Han, Y.; Liu, E.; Liu, L.; Zhang, B.; Wang, Y.; Gui, M.; Wu, R.; Li, P., 2015, Rheological, emulsifying and thermostability properties of two exopolysaccharides produced by *Bacillus amyloliquefaciens* LPL061. *Carbohydr Polym.*, 115, 230–237, DOI: 10.1016/j.carbpol.2014.08.044.
- Jadhav, J. V.; Pratap, A. P.; Kale, S. B., 2019, Evaluation of sunflower oil refinery waste as feedstock for production of sophorolipid. *Process Biochem*, 78, 15-24, DOI: 10.1016/j.procbio.2019.01.015.

- Kosaric, N., 2001, Biosurfactants and their application for soil bioremediation. *Food Technol. Biotechnol.*, 39, 295-304.
- Nitschke, M.; Silva, S. S., 2017, Recent food applications of microbial surfactants. *Critical Reviews in Food Science and Nutrition*, 58, 631-638. DOI: 10.1080/10408398.2016.1208635.
- Patino, J. M. R.; Sánchez, C. C.; Niño, M. M. R., 2008, Implications of interfacial characteristics of food foaming agents in foam formulations. *Advances in Colloid and Interface Science*, 140, 95-113, DOI: 10.1016/j.cis.2007.12.007.
- Pietro, P.; Pineda, M.; Aguilar, M., 1999, Spectrophotometric quantification of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal. Biochem.*, 269, 337-341, DOI: 10.1006/abio.1999.4019.
- Pinto, M. I. S.; Ribeiro, B. G.; Guerra, J. M. C.; Rufino, R. D.; Sarubbo, L. A.; Santos, V. A.; Luna, J. M., 2018, Production in bioreactor, toxicity and stability of a low-cost biosurfactant. *Chem. Eng. Trans.*, 64, 595-600, DOI: 10.3303/CET1864100.
- Prasanna, P. H. P.; Bell, A.; Grandison, A. S., 2012, Emulsifying, rheological and physicochemical properties of exopolysaccharide produced by *Bifidobacterium longum* subs. *Infantis* and CCUG 52486 and *Bifidobacterium infantis* NCIMB 702205. *Carbohydr. Polym.*, 90, 533-540, DOI: 10.1016/j.carbpol.2012.05.075.
- Radhakrishnan, N.; Kavitha, V.; Madhavacharyulu, E.; Gnanamani, A.; Mandal, A. B. 2011, Isolation, production and characterization of bioemulsifiers of marine bacteria of coastal Tamil Nadu. *Indian Journal of Geo-Marine Sciences*, 40, 76-82.
- Rahman, P. K. S. M.; Gakpe, E., 2008. Production, characterization and applications of biosurfactants – review. *Biotechnology*, 7, 360-370.
- Ranasalva, N.; Sunil, R.; Poovarasam, G., 2014, Importance of Biosurfactant in Food Industry. In: *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)*, 6-9.
- Rocha e Silva, N. M. P.; Meira, H. M.; Almeida, F. C. G.; Silva, R. C. F. S.; Almeida, D. G.; Luna, J. M.; Rufino, R. D.; Santos, V. A.; Sarubbo, L. A., 2018, Natural surfactants and their applications for heavy oil removal in industry. *Separation & Purification Reviews*, 47, 1-15, DOI: 10.1080/15422119.2018.1474477.
- Sarubbo L. A.; Porto, A. L. F.; Campos-Takaki, G. M., 2001. Bioemulsifier production in batch culture using glucose as carbon source by *Candida lipolytica*, *Appl. Biochem. Biotechnol.*, 95, 59-67.
- Shah, M. U. H.; Sivapragasam, M.; Moniruzzaman, M.; Talukder, M. M. R.; Yusup, S. B.; Goto, M., 2017, Production of sophorolipids by *Starmerella bombicola* yeast using new hydrophobic substrates. *Biochem. Eng. J.*, 127, 60-67. DOI: 10.1016/j.bej.2017.08.005.
- Sharma D., 2016, Applications of Biosurfactants in Food. In: *Biosurfactants in Food, Springer Briefs in Food, Health, and Nutrition*, Sharma, D. (ed.), 43-80. DOI: 10.1007/978-3-319-39415-2_3.
- Santos, D. K. F.; Luna, J. M.; Rufino, R. D.; Santos, V. A.; Sarubbo, L. A., 2016, Biosurfactants: multifunctional materials of the XXI century. *International Journal of Molecular Sciences*, 17, 401, 2016. DOI: 10.3390/ijms17030401