

**DEVELOPMENT OF A
MULTIFUNCTIONAL
LIPSTICK WITH
SOPHOROLIPIDS
PRODUCED BY
*Starmmerella bombicola***

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Abstract: The demand for sustainable formulations with natural bioactive ingredients has been intense, in order to meet the current market demands, which reveal that this sector is on the rise. Among the most used makeup products is lipstick, which for greater acceptance by consumers requires a formulation with multifunctional properties. Sophorolipids are microbial biosurfactants that exhibit biological properties such as moisturizing effect, antimicrobial and antioxidant activity, which are desirable characteristics for application in cosmetic formulations. Essential oils are also widely used in cosmetic formulations due to their antimicrobial and antioxidant activities. Thus, this study aimed to produce and characterize a lipstick with the addition of sophorolipids produced by *Starmerella bombicola* in association with palmarosa essential oil. The lipsticks were developed using 1g of sophorolipids and 0.2g of palmarosa essential oil. Sophorolipids showed an antioxidant activity of 59.40%. The lipstick formulation containing sophorolipids and palmarosa oil showed normal organoleptic parameters (appearance, color and odor), melting point (63 °C), breaking point (89 g), maximum occlusiveness (85.60), spreadability (201.5 mm²) and moisture retention greater than 90%. The use of sophorolipids combined with essential oils for the development of lipstick formulations resulted in a product with multifunctional properties that are very attractive for the cosmetic and pharmaceutical industries.

Keywords: *Starmerella bombicola*; Soforolipídios; Cosmetics; Lipstick.

INTRODUCTION

Lipstick is a worldwide popular cosmetic product that attracts consumers for its characteristics, such as type of pigment, spreadability, texture, pleasant smell, taste, and dermatological safety (Esposito; Kirilov, 2021). The addition, of components in the formulation that act as bioactive agents can provide multifunctional properties to the lipstick, such as ultraviolet protection, antioxidant, antimicrobial, moisturizing and emollient activities. These properties add value to the product and promote greater acceptance in the consumer market (Kamairudin et al., 2014).

Sophorolipids are biosurfactants composed of a disaccharide of glucose (sophorose) linked to a long fatty acid chain. They are mainly produced by non-pathogenic yeasts such as *Starmerella bombicola*. These molecules have good skin compatibility, moisturizing properties and biological activities and can be used in acne, dandruff and body odor treatments (Lourith and Kanlayavattanukul, 2009). Our research group has already reported the antimicrobial activity of sophorolipids against pathogenic microorganisms: *S. aureus*, *Escherichia coli*, *Salmonella*, *Proteus mirabilis*, *S. aureus*, *Enterococcus faecium* (Fontoura et al., 2020). The combination of sophorolipids and lactic acid resulted in an additive interaction, reducing the concentration of active compounds necessary for efficacy against *S. aureus* and *Listeria monocytogenes*, by 50% and 75%, respectively (Silveira et al., 2021). Recently, it was shown that the association of sophorolipids and palmarosa essential oil resulted in a self-preserving cosmetic formulation, with great stability and effective antibacterial activity against acne-causing microorganisms (FILIFE et al., 2022).

Thus, the objective of this work was to develop and characterize a lipstick containing sophorolipids and palmarosa essential

oil. The results obtained can contribute to the development of a multifunctional and sustainable formulation of great interest to the cosmetic industry.

MATERIAL AND METHODS

PRODUCTION OF SOPHOROLIPIDS BY *Starmerella bombicola*

Sophorolipids were produced by fermentation with *Starmerella bombicola* (ATCC® 22.214™) in 250 mL Erlenmeyers flasks with 50 mL of culture medium composed of (g/L): glucose 100, avocado oil 100, yeast extract 2.5, monobasic potassium phosphate 0.5 and magnesium sulfate 0.5. The flasks were incubated in an orbital shaker at 30 °C, 150 rpm, for 240 h. Cultivation was stopped by centrifugation (9956 x g, 15 min, 4°C). The recovery of sophorolipids was performed by solvent extraction, according to the protocol described by Fontoura et al. (2020). Antioxidant activity was performed by the DPPH method (2,2-diphenyl-1-picrylhydrazyl) according to Srikanth et al., 2015.

LIPSTICK DEVELOPMENT

Two lipstick formulations were developed, a control base formulation (FB) and a formulation with sophorolipids and essential oil of palmarosa (FS), as described in Table 1. For the preparation of FB, all ingredients from phase 1 were pre-mixed and heated until reaching a temperature of 80 °C, the same procedure was carried out with the phase 2 ingredients. The phase 3 ingredients were mixed and heated until the solution became homogeneous. Afterwards, phase 2 was poured onto phase 1 by hand stirring. Then the ingredients from phases 3, 4, 5 and 6 were added while still under manual stirring. The FS has all the ingredients of the FB plus the incorporation of phase 7 into phase 4. After the incorporation of all the ingredients, the

formulations were poured into the lipstick molds, allowing to cool for 20 minutes. Then, the lipsticks were removed from the moulds and stored.

CHARACTERIZATION OF FORMULATIONS

The preliminary evaluation of the formulations was carried out for fifteen days, where the samples were subjected to stress conditions in order to accelerate the appearance of possible signs of instability. The samples were exposed to cycles of 24 hours of heating in an oven at 45 ± 2 °C and cooling in refrigerators at 4 ± 2 °C. (Brazil, 2004). The formulations were evaluated in terms of characteristics: appearance, color and odor. The formulation was classified as normal, with no change; slightly modified; modified and heavily modified (Brasil, 2010). Melting and breaking points were determined according to Giovanini et al., 2019. The occlusion factor was determined according to Wissing and Muller, 2002. Density was evaluated using a glass pycnometer. The spreadability test was performed according to Borghetti; Knorst, 2006. Moisture holding capacity was evaluated according to Zhao et al. 2012. The pH was determined using a potentiometer.

RESULTS AND DISCUSSION

PRODUCTION AND ANTIOXIDANT ACTIVITY OF SOPHOROLIPIDS

The production of sophorolipids using glucose (100 g/L) and avocado oil (100 g/L) was 71.83 g/L, with a yield of 51.97% (11 days, 240 h) and the biomass production was 5.98 g/L. Sophorolipids were evaluated for antioxidant activity and the results showed that concentrations of 5, 10, 20, 30, 40 and 50 mg/mL had, respectively, an inhibitory capacity equal to 34.06, 59.40, 69.21, 92.10, 93.19 and 99.46%. The minimum inhibitory capacity (IC₅₀) of sophorolipids was found at

a concentration of 10 mg/mL, the same used in the formulation of lipstick with sophorolipids, performing 59.40% of inhibition.

CHARACTERIZATION OF COSMETIC FORMULATIONS

The results obtained from the preliminary stability test of FB and FS are showed on Table 2. The parameters analyzed do not show any type of significant change. According to ANVISA (National Health Surveillance Agency) the study of the stability of cosmetic products consists of a series of tests to obtain information on the stability of products in terms of previously specified limits, with the objective of defining shelf life and conditions of ideal storage (Brasil, 2006).

The analysis of organoleptic parameters (aspect, color and odor) showed that there was no significant change in the formulations. The samples maintained a normal appearance, without macroscopic changes such as phase separation, precipitation or turbidity. The color remained slightly yellowish white, characteristic of the ingredients ingredients used in the formulation. The odor identified is characteristic of palmarosa essential oil.

The melting point obtained was 59 °C for FB and 63 °C for FS, similar to the value of 66 °C obtained by Giovanini et al. (2019) in lipsticks with photoprotective properties. Kamairudin et al. (2014), obtained melting points of 41 to 51 °C in natural lipsticks based on pitaya seed oil, while Kasparaviciene et al. (2016), reported melting points of 51 to 69 °C in lipsticks with beeswax. Considering that this product is intended for the lips and that the ability to melt during application is expected, but without melting at higher temperatures, the value obtained in FS is desirable and is in the acceptable range for the consumer, which goes from 60.6 to 64 °C (Rajin; Bono; Mun, 2007).

The increase in the FS melting temperature

Table 1 Ingredients used to lipsticks production.

Step	Ingredients	FB	FS
		(%; p/p)	(%; p/p)
1	Refined Castor Oil	QSP.100 g	QSP.100 g
1	Almond oil	12,0 g	12,0 g
1	BHT	0,2 g	0,2 g
2	Cetyl alcohol	5,2 g	5,2 g
2	Beeswax	14,0 g	14,0 g
2	Carnauba wax	12,0 g	12,0 g
3	Decyl oleate	24,0 g	24,0 g
3	Menthol	0,4 g	0,4 g
4	Propylene glycol	6,0 g	6,0 g
5	Palmarosa essential oil	0,2 g	0,2 g
6	Neolone	0,4 g	0,4 g
7	Sophorolipid	-	1,0 g

Table 2 - Evaluation of the preliminary stability of lipsticks

Tests	FB day 0	FS day 0	FB day 15	FS day 15
Aspect	Normal	Normal	Normal	Normal
Color	Normal	Normal	Normal	Normal
Smell	Normal	Normal	Normal	Normal
Fusion point (°C)	59	63	59	63
Breaking point (g)	85	89	83	89
occlusive factor (6 h)	80,47	85,60	80,11	84,31
pH	6,0	6,0	6,0	6,0
Density (g/cm ³)	0,9981	0,9856	0,9889	0,9806
spreadability (mm ²)	179,10	201,5	187,27	203,64
moisture retention (%)	90,83	91,57	89,02	92,33

FB: base formulation (control), FS: formulation containing sophorolipids and palmarosa essential oil.

compared to the value obtained in the FB can be explained by the presence of the bioactive compound, due to the hydrating and humectant properties of the sophorolipids, which guarantees a higher concentration of water in the lipstick providing greater rigidity and elevation of the temperature of fusion (Adu et al., 2020).

The breaking point of FB lipsticks was found to be 85 g, while the breaking point for FS was 89 g. Giovanni et al. (2019) developed multifunctional lipstick that broke under the mass of 91 g. According to Hayati and Chabib (2016), the ideal is for the lipstick to support 77 to 106 g, since hardness is an important physical property for the lipstick to remain stable during application on the lips and during product transport (Bono; Mun; Rajin, 2006).

The occlusion factor of FB and FS can be seen in Figure 1. The analysis of the data obtained shows that the occlusion factor of the formulations decreased over time (6, 24 and 48 h) and that the formulation containing sophorolipids showed a higher value the base formulation at all times, with maximum occlusion equal to 85.60 (6 h). According to Ziefenmeyer (1992), active compounds with occlusive potential directly influence skin hydration, preventing the evaporation of water from the skin to the environment, and this property may be associated with the crystallinity of the lipid structure of sophorolipids (Wissing; Muller, 2002).

The pH is an important factor for the stability of the lipstick, which influences the acceptance of the product in the market (Oliveira et al., 2003). FB and FS had a pH equal to 6.0. These values are desirable for lip cosmetics, since pH values between 6.0 and 7.0 are ideal for compatibility with the pH of human saliva and lips (Ribeiro, 2010). Furthermore, this pH value facilitates the solubility and permeation of sophorolipids in

the skin of the lips, since the solubility of this bioactive compounds varies according to the pH of the medium, and it is fully soluble at pH 6.0 (Van Bogaert et al. 2011; Varvaresou and Iakovou, 2015). The density of the control base formulation (FB) was 0.9981 g/cm³. The formulation containing sophorolipids and essential oil of palmarosa (FS) presented a density of 0.9856 g/cm³, lower than the density of water and FB. This value is justified by the presence of oily compounds in the formulation, including the active one, which is a biosurfactant belonging to the glycolipid class (Ashby; Solaiman, 2010).

The spreadability profile of the FB and FS are showed on Figure 2. The maximum spreadability obtained for the FB was 179.10 mm². The FS, under the same conditions, showed a spreadability of 201.5 mm². Data analysis showed that the formulations presented an increase in the spreadability profile when the weight of 27.8182 g was used, before that it remained constant. The spreadability of FS exceeds that of FB, demonstrating that the incorporated active was responsible for this increase, which can promote greater acceptability of the product in the market by consumers, since spreadability is essential for an easy application of lipstick on the lips (Rafferty et al., 2018; Montenegro et al, 2015).

The moisture retention capacity of FB and FS after 96 h were, respectively, 90.83% and 91.57%. The results showed that at 43% (RU), lipstick with sophorolipids retains a large amount of water and has a high hydration capacity. The moisture retention capacity is of great importance for cosmetic formulations (Jiménez-Pérez et al., 2018), and it is suggested that the high moisturizing capacity of the developed formulations occurs due to the hydration potential of sophorolipids (Adu et al.; 2020), and other ingredients such as almond oil (Patzelt et al., 2012) and beeswax

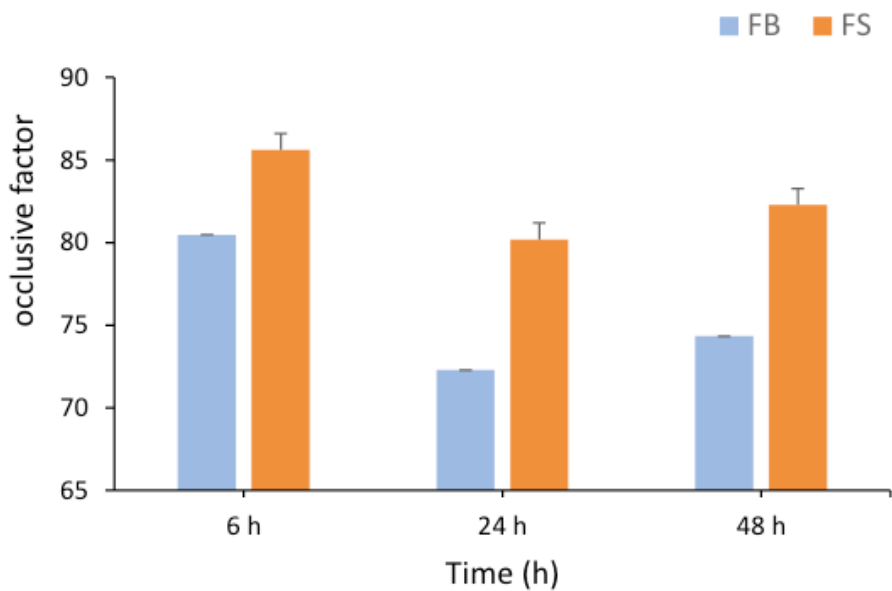


Figure 1 – Occlusive factor of the control base formulation (FB) and formulation with sophorolipids (FS)

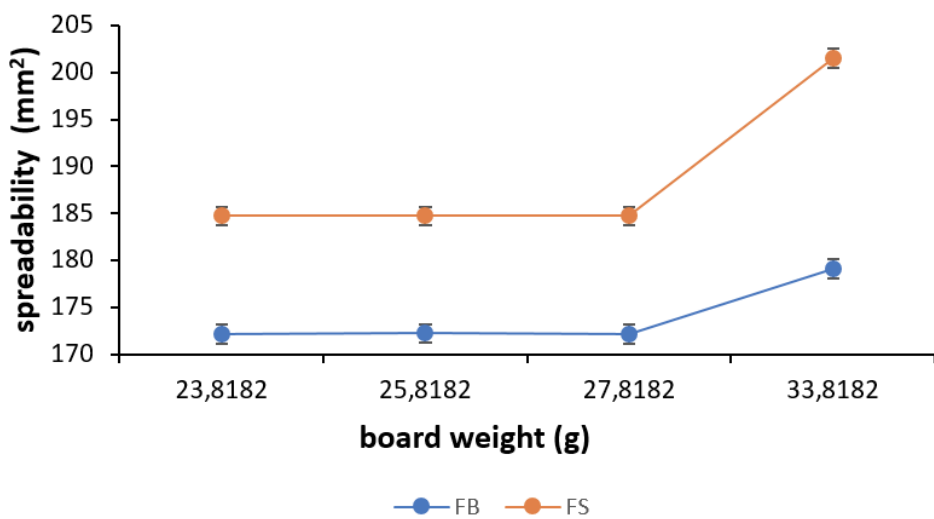


Figure 2. Spreadability of control base formulations (FB) and formulation with sophorolipids (FS)

(Kasparaviciene et al., 2016).

bioactive ingredient for cosmetics.

CONCLUSIONS

The lipstick formulation containing sophorolipids and palmarosa oil showed excellent organoleptic parameters, ideal pH and density and good spreadability. Thus, the present study demonstrates that microbial sophorolipids in association with essential oils have innovative application potential as a

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REFERENCES

- ABIHPEC. Associação Brasileira da Indústria de Higiene Pessoal, Perfumaria e Cosméticos. **Caderno de tendências 2019-2020**. Brasília, 2018. Disponível em: <https://abihpec.org.br/publicacao/caderno-de-tendencias-2019-2020/>. Acesso em: 13 de maio de 2021.
- Adu, S. A.; Naughton, P. J.; Marchant, R.; Banat, I. B. Microbial Biosurfactants in Cosmetic and Personal Skincare Pharmaceutical Formulations. **Pharmaceutics**, v. 12, n. 11, p. 1099-2020, nov. 2020.
- Ashby, R. D., Zerkowski, J. A., Solaiman, D. K. Y.; Liu, L. S. Biopolymer scaffolds for use in delivering antimicrobial sophorolipids to the acne-causing bacterium *Propionibacterium acnes*. **New Biotechnology**, v.28, n.1, p.24–30, 2011.
- Bono, A.; Mun, C. H.; Rajin, M. Effect of various formulation on viscosity and melting point of natural ingredient based lipstick. *Studies in Surface Science and Catalysis*, v. 159, p.
- Borghetti, G. S. Knorst, M. T. Desenvolvimento e avaliação da estabilidade física de loções O/A contendo filtros solares. **Revista Brasileira de Ciências Farmacêuticas**, v. 42, p. 531–537, dez. 2006.
- BRASIL. Agência Nacional de Vigilância Sanitária (ANVISA). **Guia de estabilidade de produtos cosméticos**. 1.ed. Brasília: ANVISA, 2004, 52p.
- BRASIL. Agência Nacional de Vigilância Sanitária (ANVISA). **Guia de controle de qualidade de produtos cosméticos**. 1.ed. Brasília: ANVISA, 2010.
- Ceresa, C.; Fracchia, L.; Fedeli, E.; Porta, C.; Banat, I. M. Recent Advances in Biomedical, Therapeutic and Pharmaceutical Applications of Microbial Surfactants. **Pharmaceutics**, v. 13, n. 4, p. 466, mar. 2021.
- Eposito, C. L.; Kirilov, P. Preparation, Characterization and Evaluation of Organogel-Based Lipstick Formulations: Application in Cosmetics. **Gels**, v. 7, n. 3, jul, 2021.
- Filipe, G. A.; Bigotto, B. G.; Baldo, C.; Gonçalves, M. C.; Kobayashi, R. K. T.; Lonni, A. A. S. G.; Celligoi, M. A. P. C. Development of a multifuncional and self-preserving cosmetic formulation using sophorolipids and palmarosa essential oil against acne-causing bacteria. **Journal of Applied Microbiology**. Nova Jersey, p.1-9, 2022.
- Fontoura, I, C. C., Saikawa, G. I. A.; Silveira, V. A. I.; Pan, N. C.; Amador, I.R.; Baldo, C., Racha, S. P. D., Celligoi, M. A. P. C., Antibacterial Activity of Sophorolipids from *Candida bombicola* against Human Pathogens. *Brazilian Archives of Biology and Technology*, v.63, p.1, 2020.
- Giovanini, I. R. T.; Alves, P. E.; De Siqueira, L. B. O.; Martins, L. L. B.; dos santos, E. P. Desenvolvimento de maquiagem multifuncional: batom com propriedade fotoprotetora, emoliente e hidratante. **Iniciação Científica Cesumar**, v. 21, p. 71.82, jun. 2019.
- Hayati, F.; Chabib, F. Formulation and evaluation of herbal lipsticks from carrot (*Daucus carota* L) extract. **International Journal of Pharmacy and Pharmaceutical Sciences**, v. 8, n. 3, p. 403-405, 2016.

- Jiménez-Pérez, Z. E., Singh, P., Kim, Y.-J., Mathiyalagan, R., Kim, D.-H., Lee, M. H., Yang, D. C. Applications of Panax ginseng leaves-mediated gold nanoparticles in cosmetics relation to antioxidant, moisture retention, and whitening effect on B16BL6 cells. **Journal of Ginseng Research**, v.42, n.3, p.327–333, 2018.
- Kamairudin, N.; Gani, S.S.; Masoumi, H. R.; Hashim, P. Optimization of natural lipstick formulation based on pitaya (*Hylocereus polyrhizus*) seed oil using D-optimal mixture experimental design. **Molecules**, v. 19, n. 10, p. 16672-16683, oct. 2014.
- Kasparaviciene, G.; Savickas, A.; Kalveniene, Z.; Velziene, S.; Kubiliene, L.; Bernatoniene, J. Evaluation of Beeswax Influence on Physical Properties of Lipstick Using Instrumental and Sensory Methods. **Evid Based Complement Alternat Med**, 2016.
- Lourith N, kanlayavattanakul M. Natural surfactants used in cosmetics: glicolipids. *International Journal of Cosmetic*, v.31, p.255–261, 2009
- Lydon, H. L; Baccile, N.; Callaghan, B.; Marchant, R.; Mitchell, C. A; Banat, I. M. Adjuvant Antibiotic Activity of Acidic Sophorolipids with Potential for Facilitating Wound Healing. **Antimicrob Agents Chemother**, v. 61, n. 5, may. 2017.
- Maeng, Y.; Kim, K.T.; Zhou, X.; Jin, L.; Kim, S. K.; Kim, H. Y.; Lee, S.; Park, H. J.; Chen, X.; Kong, M.; Cai, L.; Li, X. A novel microbial technique for producing high-quality sophorolipids from horse oil suitable for cosmetic applications. **Microb Biotechnol**, v. 11, n. 5, p. 917-929, jul. 2018.
- Moldes, A. B.; Rodríguez-López, L.; Rincón-Fontán, M.; López-Prieto, A.; Vecino, X.; Cruz, J. M. Synthetic and Bio-Derived Surfactants Versus Microbial Biosurfactants in the Cosmetic Industry: An Overview. **Int J Mol Sci**, v. 22, n. 5, feb. 2021.
- Montenegro, L.; Rapisarda, L.; Ministeri, C.; Puglisi, G. Effects of Lipids and Emulsifiers on the Physicochemical and Sensory Properties of Cosmetic Emulsions Containing Vitamin E. **Cosmetics**, v. 2, p. 35–47, mar. 2015.
- Oliveira, F. F. Contribuição da análise no desenvolvimento de formulações de batons. **Dissertação de mestrado submetido ao Instituto de Química – Universidade de São Paulo, São Paulo, 2003.**
- Patzelt, A.; Lademann, J.; Richter, H.; Darvin, M. E.; Schanzer, S.; Thiede, G.; Sterry, W.; Vergou, T.; Hauser, M. In vivo investigations on the penetration of various oils and their influence on the skin barrier. **Skin Res Technol**, v. 18, n. 3, p. 364-369, ago. 2012.
- Paulino, B. N.; Pessoa, M. G.; Mano, M. C. R.; Molina, G.; Neri-Numa, I. A.; Pastore, G. M. Current status in biotechnological production and applications of glycolipid biosurfactants. **Applied Microbiology and Biotechnology**, v. 100, n. 24, p. 10265–10293, 2016.
- Rafferty, D. W.; Dupin, L.; Zellia, J.; Giovannitti-Jensen, A. Predicting lipstick sensory properties with laboratory tests. **Int J Cosmet Sci**, v. 40, n. 5, p. 451 -460, set. 2018.
- Rajin, M.; Bono, A.; Mun, C. H. Optimisation of natural ingrediente based lipstick formulation by using mixture design. **Journal of Applied Sciences**, v. 7, n. 15, p. 2099-2103, 2007.
- Ribeiro, D. C. C.; Produção e análise sensorial de batom. **Trabalho de Conclusão de curso apresentado ao Curso de Graduação em Química – Instituto Municipal de Ensino Superior de Assis, Assis, 2010.**
- Silveira VAI, Kobayashi RKT, Oliveira AGJ, Mantovani MS, Nakazato G, Celligoi MAPC (2021). Antimicrobial effects of sophorolipid in combination with lactic acid against poultry-relevant isolates. *Brazilian Journal of Microbiology*, v. 1, p. 143-153.
- Shu, Q.; Lou, H.; Wei, T.; Liu, X.; Chen, Q. Contributions of Glycolipid Biosurfactants and Glycolipid-Modified Materials to Antimicrobial Strategy: A Review. **Pharmaceutics**, v. 13, n. 2, p. 227, fev. 2021.
- Srikanth, R.; Siddartha, G.; Sundhar, R.C.H.; Harosh, B.S.; Janaki, R.M.; Uppuluri, K.B. Antioxidant and anti-inflammatory levan produced from *Acetobacter xylinum* NCIM2526 and its statistical optimization. **Carbohydrate Polymers**, v. 123, p. 8–16, 5 jun. 2015.
- Varvaresou, A.; Iakovou, K. Biosurfactants in cosmetics and biopharmaceuticals. **Letters in Applied Microbiology**, v.61, p. 214--223, 2015.
- Van Bogaert, I.N. A; Zhang, J; Soetaert, W.B. Microbial synthesis of sophorolipids. **Process Biochemistry**, v.46, p.821–833, 2011.

Westfall, A.; Sigurdson, G. T.; Giusti, M. M. Antioxidant, UV protection, and antiphotaging properties of anthocyanin-pigmented lipstick formulations. **Journal of Cosmetic Science**, v. 70, n. 2, p. 63-76, apr. 2019.

Wissing, S. A.; Muller, R. H. The influence of the crystallinity of lipid nanoparticles on their occlusive properties. **International Journal of Pharmaceutics**, v. 242, n. 1-2, p. 377-379, 2002.