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BIOTECHNOLOGY

Development of a facial biocosmetic containing levan, almond and cinnamon oils with antioxidant and moisturizing properties

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ABSTRACT. The addition of natural molecules such as microbial exopolysaccharides in cosmetics is a trend in the current market, adding properties and improving the product quality. Therefore, the aim of this study was to develop a facial biocosmetic formulation containing microbial levan, almond and cinnamon oils. The centroid-simplex design was used to evaluate the spreadability, antioxidant activity, moisture retention capacity and viscosity of formulations. Since it is a facial cosmetic, the formulation was optimized using the intermediate viscosity. The optimized formulation with intermediate viscosity was 75% (0.75 g) levan and 25% (2 mL) almond oil, without the addition of cinnamon oil. This formulation was submitted to 90 days under different exposure conditions, and the results showed a spreadability of 805 mm^2, pH and density ideal for the facial area, with an antioxidant activity of 72%, hydration capacity of 100.3%, viscosity with no-Newtonian behavior, and normal organoleptic properties when stored at room and low temperature. The formulation with levan associated with almond oil showed potential for application in the facial area, with high antioxidant properties, moisturizing intermediate viscosity and stability for 90 days. The utilization of centroid-simplex design allowed the development of a biocosmetic with desired characteristics just by adjusting the concentrations of the bioactive.

Keywords: exopolysaccharides; cosmetic; antioxidant activity; moisturizer; personal care products.

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Introduction

The skin undergoes progressive morphological and physiological changes with the increase of age, which can be caused by genetic (endogenous aging) and extrinsic factors (exogenous aging), such as chronic exposure to light, pollution, ionizing radiation, chemicals, or toxins (Zouboulis et al., 2019). Several mechanisms trigger the natural aging process, including the oxidative stress theory of free radicals (Wickens, 2001). Due to its chemical instability, free radicals can capture oxygen from other biologically active molecules, leading to tissue damage and, consequently, contributing to the skin aging process (Jaradat et al., 2018). Therefore, the antioxidant activity of cosmetic ingredients is crucial in delaying the aging process (Jiménez-Pérez et al., 2018).

Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are widely used in cosmetic formulations. However, some studies suggest that high consumption of synthetic antioxidants results in potential health risks. Sato et al. (1987) found that the topical application of BHA and BHT to the dorsal skin of mice induced skin tumors in 15 and 35%, respectively. Additionally, these compounds are potentially disturbing for the environment because they can bioaccumulate and possess high aquatic toxicity (National Industrial Chemicals Notification and Assessment Scheme [NICNAS], 2017).

The development of new cosmetic formulations with natural ingredients is gaining attention, due to the current beauty and personal care market that seeks for natural and eco-friendly alternatives to the conventional synthetic substances (Ferreira et al., 2017). In this sense, levan is an exopolysaccharide with high antioxidant action (Liu, Luo, Ye, & Zeng, 2012; Srikanth et al., 2015; Taylan, Yilmaz, & Dertli, 2019;

Bouallegue et al., 2020). It consists of fructose units linked by β-glycosidic linkages (2 → 6) and is produced from sucrose by microorganisms, such as *Bacillus subtilis* and *Zymomonas mobilis* (Oliveira, Silva, Buzato, & Celligoi, 2007; Bersaneti, Pan, Baldo, & Celligoi, 2017).

This polysaccharide possesses many attractive features for cosmetic application, such as hydrating and moisturizing properties comparable to hyaluronic acid, stimulation of fibroblasts and keratinocyte proliferation (Domżał-Kędzia et al., 2019), skin irritation-alleviating (Kim, Kim, Ryo, Lee, & Kim, 2003), anti-inflammatory action and skin whitening effect (Srikanth et al.,2015). Moreover, according to the Cosmetic Ingredient Review (CIR) Expert Panels, levan meets all safety criteria to be incorporated in cosmetic formulations, showing no cytotoxicity or allergic reactions to skin or eye (Öner, Hernández, & Combie, 2016).

Although levan has excellent cosmetic properties, the combination of active ingredients in the same delivery system represents innovation in cosmetology, as it increases the pre-existing bioactive action, and may also add new properties to the product (Lacatusu et al., 2018). Almond oil is commonly used in skin creams, and anti-aging products due to its properties as an emollient, moisturizer, antioxidant, and photoprotective effect (Sultana, Kohli, Athar, Khar, & Aqil, 2007; Ahmad, 2010). Cinnamon essential oil presents antioxidant and antimicrobial activities against a wide range of pathogens (Herman, Herman, Domagalska, & Młynarczyk, 2012; Zhang, Liu, Wang, Jiang, & Quek, 2016; Chuesianga et al., 2019) and can be added in cosmetic formulations, enhancing the antioxidant action, and adding antimicrobial properties to the product (Silva et al., 2020).

The objective of this study was to develop a sustainable and innovative biocosmetic with microbial levan, which has antioxidant and moisturizing properties, combined with compounds of vegetable origin, almond oil and cinnamon oil, to enhance the properties of the cosmetic.

Material and methods

Production of levan by *Bacillus subtilis* natto

Levan was produced by *Bacillus subtilis* natto in the fermentation medium (g L^{-1}): sucrose, 400; yeast extract, 2; KH_2PO_4, 1; $(NH_4)_2SO_4$, 3; $MgSO_4$ ($7H_2O$), 0.6; $MnSO_4$, 0.2; and ammonium citrate, 0.25 (Bersaneti et al., 2017). Fermentation was conducted in 2 L Erlenmeyer flasks with 500 mL of medium, pH 7.0, 150 rpm, 37°C, for 24 hours and 0.2 g L^{-1} of inoculum (Bersaneti, Mantovan, Magri, Mali, & Celligoi, 2016). Fermentation was stopped by centrifugation at 9000 rpm for 15 min. at 4°C. Levan was precipitated from the supernatant with absolute ethanol 1:1.5 v v^{-1} (medium: ethanol) for 12 hours at 4°C and centrifuged at 9000 rpm for 20 min. (Viikari & Gisler, 1986). High mass levan (4146 kDa) was dialyzed against distilled water for 48 hours and lyophilized.

Cosmetic formulations

The oil/water (o w^{-1}) emulsion was studied using a simplex-centroid design with nine formulations varying the components X_1 (levan), X_2 (cinnamon oil) and X_3 (almond oil). The maximum concentrations of the components were 1 g, 0.5 and 8 mL for variables X_1, X_2 and X_3, respectively (Table 1). The response variables were (Y1) spreadability, (Y2) antioxidant activity, (Y3) moisture retention and (Y4) viscosity.

The formulations were prepared using the phase inversion emulsification method. Phase 1 was prepared with 72.3 mL of distilled water and 3.0 g of glycerin. Phase 2 was prepared with 4.0 g of cetyl alcohol, 3.8 g of stearyl alcohol, 3.0 g of ethoxylated cetostearyl alcohol 20 OE, 2.0 g of glycerin monostearate, 5.0 g of 1000 olive oil and 5.0 g of decyl oleate. Phases 1 and 2 were heated to 75°C, and phase 2 was poured over phase 1 under agitation. After that, at temperatures below 40°C, 0.4 mL of preservative, 1.5 mL of cyclomethicone, levan, almonds and cinnamon oil were added to the formulations (Table 1). The formulations were adjusted to pH 4.5 with citric acid solution.

Formulation characterization

The formulation selected by the simplex-centroid design was exposed to different conditions: high temperature (37 ± 2°C), low temperature (5 ± 2°C), room temperature (28 ± 2°C), and indirect or direct exposure to light radiation (Agência Nacional de Vigilância Sanitária [ANVISA], 2004). The parameters stability and spreadability, physical and chemical properties, antioxidant activity, moisture retention capacity (hydration) and organoleptic properties were analyzed at 2, 30, 60 and 90 days.

Stability and spreadability

Stability was verified by phase separation. Five grams of the formulation was centrifuged at 3000 rpm for 30 min at room temperature and allowed to rest for 24 hours. After, the macroscopic visualization it was interpreted as normal (without phase separation), phase separation, or coalescence (ANVISA, 2004). Spreadability was evaluated with 1 g of sample using glass plates on graph paper. The formed perpendicular diameters were measured to determine the comprehensive surface at a temperature of 25 ± 2°C (Borghetti & Knorst, 2006). Weights of 2.0, 4.0, and 10 g were used in one-minute intervals. The tests were performed in triplicate. The spreadability was calculated using the Equation 1:

$$Ei = \frac{(d^2 . \pi)}{4} \quad (1)$$

Where Ei is the spreadability of the sample for a given weight in square millimeters (mm^2); d: average diameter in millimeters (mm).

Physicochemical analysis

The pH was measured in the samples diluted in distilled water (10%) (Garbossa & Maia Campos, 2016). Density (d) was evaluated using a glass pycnometer with a volume of 10 mL at 20°C. Water density was used as a standard. The empty pycnometer was weighed (M0), followed by the addition of water until it overflowed. The pycnometer was dried outside, and weight was obtained (M1). Subsequently, the formulation was also weighed (M2) (Agência Nacional de Vigilância Sanitária [ANVISA], 2007). Equation 2 was used for the calculation:

$$d = \frac{M2 - M0}{M1 - M0} \quad (2)$$

Viscosity was determined by a digital rotational viscometer (Marte MDV-8). Approximately 200 g of the formulation and the number 4 spindle with 0.6 speeds were used: 1.5, 3.0, 6.0 and 12.0 rpm (shear rates) at 25°C (ANVISA, 2007). The results were plotted on a graph correlating the apparent viscosity given in cP (centiPoise) and the shear rate.

Antioxidant activity

The antioxidant activity was evaluated by the DPPH method (2,2-diphenyl-1-picryl-hydrazil). For the assay, 1 mL of the sample (formulation at 2.5 mg mL^{-1}) and 0.3 mL of the DPPH solution were incubated in the dark for 30 min. at room temperature followed by reading at λ = 517 nm. The blank solution was composed of 1 mL of the formulation and 0.3 mL of ethanol. The control sample was composed of 1 mL of ethanol and 0.3 mL of the DPPH solution (Srikanth et al., 2015). The inhibition rate (%) of the free radical was calculated according to Equation 3:

$$\% \text{ inhibition} = \frac{(\text{control Abs} - \text{sample Abs})}{\text{control Abs}} \text{ x } 100 \quad (3)$$

Moisture holding capacity

The moisture holding was evaluated by gravimetry according to Zhang, Wang, Han, Zhao, and Yin (2012) and Zhao, Fan, Wang, and Jiang (2013). In previously tared crucibles, 1 g of the formulation and 1 ml of water were mixed. The crucibles were stored for 96 hours (room temperature) in a moisture desiccator sealed with saturated K_2CO_3 solution (43% relative humidity - RH). The moisture retention capacity (Ru) was evaluated by the percentage of residual water in the samples, calculated by Equation 4:

$$Ru\ (\%) = \frac{Pt}{P0} \text{ x } 100 \quad (4)$$

where P0 is the weight of distilled water added to the samples and Pt is the weight of water after the tested times.

Organoleptic properties

The color and odor of the formulation were evaluated in the initial and final time of storage (2 and 90 days). The color was verified through visual analysis in white light and the odor using the smell. The results were interpreted as normal (no change), slightly modified, modified, and intensely modified (ANVISA, 2004).

Statistical analysis

The results of the simplex-centroid design were submitted to analysis of variance (ANOVA) and regression analysis using Statistica version 7.0 software. The model was validated using the t test, where the value of the best response of the experiments was compared with the responses presented by the optimized cosmetic formulation (estimated by the model).

Results and discussion

Analysis of cosmetic formulations

The results of the cosmetic formulations were evaluated by the simplex-centroid design and are shown in Table 1. The formulation containing 100% almond oil (test 3) showed greater spreadability (1197.6 mm^2), antioxidant activity (66.5%) and viscosity (42079 cP). The formulation with 50% levan and 50% cinnamon oil (test 4) showed the highest moisture retention rate (94.32%).

Table 1. Simplex-centroid design for the development of a facial cosmetic formulation with *Bacillus subtilis* levan, cinnamon oil and almond oil. The responses were (Y1) Spreadability (mm^2), (Y2) Antioxidant activity (%), (Y3) Moisture retention (%) and (Y4) Viscosity (cP).

Runs	Factor levels (%)			Variable responses			
	X_1	X_2	X_3	Y_1 (mm2)	Y_2 (%)	Y_3 (%)	Y_4 cP
1	100	0	0	1160.0	36.5	93.20	33733
2	0	100	0	1041.2	33.6	93.91	26645
3	0	0	100	1197.6	66.5	93.02	42079
4	50	50	0	821.4	30.4	94.32	41413
5	50	0	50	827.1	46.3	92.92	35394
6	0	50	50	982.3	50.7	93.45	38575
7	33.3	33.3	33.3	944.1	41.0	88.70	32435
8	33.3	33.3	33.3	1017.4	47.9	89.10	31939
9	33.3	33.3	33.3	963.2	38.4	89.01	31473

Symbols	Factors	Coded levels		
		100 %	50 %	33,3 %
(X_1)	Levan (g)	1	0.5	0.333
(X_2)	Cinnamon oil (mL)	0.5	0.25	0.17
(X_3)	Almond oil (mL)	8	4	2.67

Viscosity is a crucial parameter in the cosmetic formulation, and the appropriate consistency and/or fluidity may be selected according to the body region of application (ANVISA, 2004). As the aim of this study was to formulate a facial moisturizer, an intermediate viscosity was selected for the new formulation. The optimization of the formulation considering the responses of spreadability, antioxidant activity, moisture retention and intermediate viscosity is shown in Figure 1.

According to the results, the formulation should contain 75% levan and 25% almond oil (0.75 g of levan and 2 mL of almond oil) without the addition of cinnamon essential oil. The validation was performed in triplicate, and the average result of spreadability (1056.1 mm^2), antioxidant activity (43.33%), moisture retention (95.17%) and viscosity (32925 cP) did not show a significant difference ($p < 0.05$) between the predicted and the experimental model. Therefore, the optimized formulation was characterized for a period of 90 days under different exposure conditions to verify its stability.

Formulation characterization

Stability and spreadability:

The formulation did not show phase separation or visible changes and was classified as normal at the end of 90 days. The spreadability indicates the area that topical formulation spreads on the skin in mm^2. At 90 days, all conditions had a similar average spreadability of 805 mm^2. Samples exposed to radiation showed lower spreadability. The spreadability plays a fundamental role in determining the effectiveness and acceptance of the product by the consumer (Montenegro, Rapisarda, Ministeri, & Puglisi, 2015). Some authors have reported a relationship between viscosity and spreadability for topical formulations, suggesting that the lower the viscosity and surface tension, the greater the spreadability on the skin (Lardy, Vennat, Pouget, & Pourrat, 2000; Deuschle, Deuschle, Bortoluzzi, & Athayde, 2015).

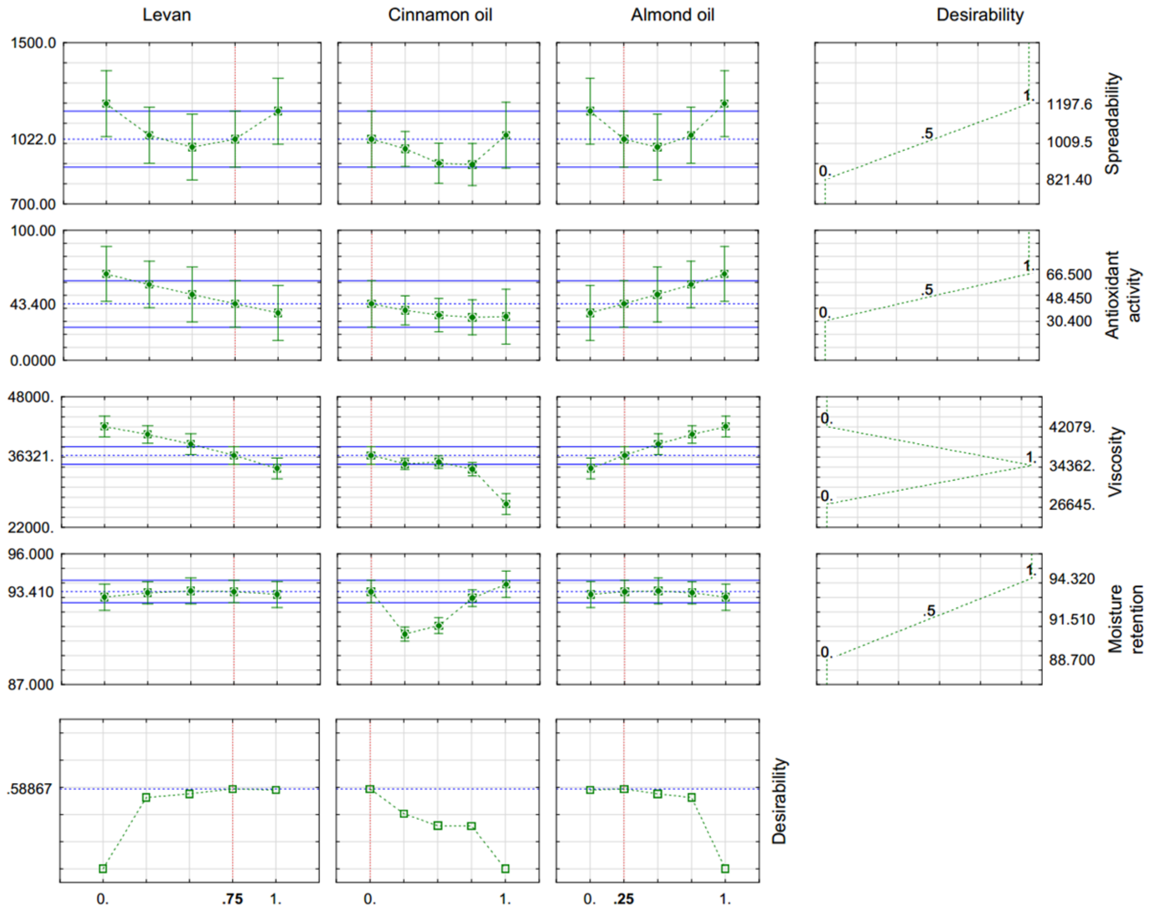

Figure 1. Optimization of spreadability responses with variation of levan and cinnamon and almond oils.

Physicochemical analysis:

The formulation did not show changes in density at all studied times, showing normal results. After 90 days of exposure to light radiation and high temperature, the formulation showed a drop in pH, ranging from 4.5 to 4.2 and 4.3, respectively, staying outer of the pH range of the facial skin (4.5 to 5.5). When maintained at low temperature, room temperature and without exposure to light radiation, the formulation showed an increase in pH, ranging from 4.5 to 4.9, 4.8 and 4.9, respectively, presenting the ideal pH range of the skin. The decrease in the pH of cosmetic emulsions stored at high temperatures was also verified by Kim and Lee (2018), who reported a gradual decrease in pH on the studied samples.

At the end of 90 days, the apparent viscosity (cP) of the cosmetic formulation remained close to the initial viscosity under the different exposure conditions. Viscosity showed typical non-Newtonian or pseudoplastic behavior. This behavior is considered desirable for topical preparations, as it improves the spread of the product in the skin. This behavior is the most common in cosmetic formulations and is characterized by a gradual decrease in apparent viscosity with an increase in the shear rate (Garbossa & Maia Campos, 2016). Thus, viscosity cannot be expressed by single values (Correa, Camargo Junior, Ignacio, & Leonardi, 2005).

Antioxidant activity:

At the initial study time (2 days), the formulation showed an antioxidant activity of 43%. At 90 days, this activity increased in all conditions of exposure. The formulation showed better results when kept at room temperature and without exposure to light radiation, obtaining 72% and 65% antioxidant activity, respectively. When stored at low and high temperatures (under light radiation), the antioxidant action was 56.55 and 51%, respectively.

Domżał-Kędzia et al. (2019) also evaluated the antioxidant activity using the DPPH method in cosmetics type o/w emulsion with levan by *B. subtilis* natto KB1, obtaining 57.3% for the sample tested. In the present study, it was possible to obtain a higher value (72%) of antioxidant activity with the formulation containing levan by *B. subtilis* natto.

The action of antioxidants is to neutralize free radicals formed in the skin; thus, cosmetic products with the addition of these ingredients have been increasing because they act in anti-aging and maintain skin health.

Moisture retention capacity:

The moisture retention capacity of the cosmetic formulation exposed to different exposure conditions is shown in Table 2. At 90 days, the formulation showed a decrease in moisture retention capacity under the following conditions: low temperature, no light radiation, room temperature, light radiation, and high temperature. The results showed that when stored at low temperature, the formulation reaches 100% moisture retention capacity.

The results suggest that the hydrating capacity of the formulation is mainly due to the high moisture retention potential of levan (Bouallegue et al., 2020) and other components of the formulation, such as glycerin (Shi et al., 2010) and almond oil, which prevents water evaporation from the skin (Costa & Santos, 2017).

Table 2. Moisture retention capacity of the cosmetic formulation at 2, 30, 60 and 90 days, under different exposure conditions.

Exposure	Moisture retention in 43% UR (%)			
	2 days	30 days	60 days	90 days
Luminous radiation	94.2	95.6	85.1	78.5
No light radiation	94.2	94.8	99.3	97.8
High temperature	94.2	95.2	83.9	74.2
Low temperature	94.2	95.3	98.6	100.3
Room temperature	94.2	96.1	87.7	82.1

Organoleptic properties:

In the initial time (2 days), the formulation presented a white color and pleasant odor, considered normal and standard. Changes in color were observed in the formulation exposed to elevated temperature and room temperature for 60 days, showing yellow (modified) and pale yellow (slightly modified) colors, respectively. At the end of 90 days, this color remained stable, and no darkening was observed. The formulation kept without radiation exposure was also classified as slightly modified, with a change from white to pale yellow. Kim and Lee (2018) also observed that when stored at high temperature, emulsions show greater changes in color, changing from pale yellow to dark brown after 25 days. When the odor was analyzed, the only condition that presented a change in the formulation was exposure to light radiation, changing from modified at 30 days to intensely modified (total absence of the initial fragrance) in 90 days.

Conclusion

For the development of a facial cosmetic with intermediate viscosity, the ingredients concentrations were optimized in 75% levan and 25% almond oil. This formulation showed good spreadability, pH and density compatible for the facial area, normal organoleptic properties, antioxidant activity, moisturizing capacity and high stability at 90 days when stored at room and low temperature, without exposure to light radiation. Therefore, the microbial levan associated with almond oil adds value to biocosmetics due to its antioxidant and moisturizing properties in the same product.

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