

Hidratante labial usando oleoresina e óleo essencial de canela: avaliação da segurança microbiológica e estabilidade estendida e prolongada

Lip balm using cinnamon oleoresin and essential oil: microbiological safety assessment with accelerated and extended stability

Bálsamo labial con oleorresina y aceite esencial de canela: evaluación de seguridad microbiológica y estabilidad acelerada y prolongada

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Resumo

Devido ao crescimento da indústria cosmética, são necessários desenvolvimentos e melhorias para o setor produtivo e seus produtos, para que atendam à demanda de consumo e também aos padrões de qualidade impostos pelos órgãos de inspeção. Em vista disso, um ramo que se desenvolveu para atender a essas necessidades é o dos fitocosméticos, que usam nos constituintes de suas formulações de origem vegetal com atividades biológicas. O objetivo deste trabalho foi comparar as formulações de hidratantes labiais incorporando-se oleorresina e óleo essencial de canela (*Cinnamomun verum*), além de testar a segurança microbiológica e a estabilidade acelerada e estendida dos produtos formulados. Os testes revelaram que as formulações propostas são estáveis e seguras microbiologicamente, o que se deve à atividade antimicrobiana da oleorresina e óleo essencial de canela utilizados nas formulações.

Palavras-chave: Hidratante labial; Canela; Oleorresina; Óleo essencial.

Abstract

Due to the growth of the cosmetics industry, developments and improvements are needed for the productive sector and its products to meet consumer demand and the quality standards imposed by inspection bodies. As a result, an area has developed to meet these needs that is known as phytocosmetics. This area involves the inclusion of constituents with of plant origin in the cosmetic product formulation. The objective of this work was to compare the formulations of lip moisturizers incorporating oleoresins and essential oils of cinnamon (*Cinnamomun verum*), in addition to testing the microbiological safety and the Accelerated and Extended Stability of the formulated products. The tests revealed that the proposed formulations are microbiologically stable and safe, which is due to the antimicrobial activity of the oleoresin and cinnamon essential oil used in the formulations.

Keywords: Lip balm; Cinnamon; Oleoresin; Essential oil.

Resumen

Debido al crecimiento de la industria cosmética, se necesitan desarrollos y mejoras para el sector productivo y sus productos, a fin de satisfacer la demanda del consumidor y también

los estándares de calidad impuestos por los organismos de inspección. En vista de esto, una rama que se ha desarrollado para satisfacer estas necesidades es la de los fitocosméticos, que utilizan en los componentes de sus formulaciones de origen vegetal con actividades microbiológicas. El objetivo de este trabajo fue comparar las formulaciones de humectantes labiales que incorporan oleorresinas y aceites esenciales de canela (*Cinnamomun verum*), además de probar la seguridad microbiológica y la estabilidad acelerada y extendida de los productos formulados. Las pruebas revelaron que las formulaciones propuestas son microbiológicamente estables y seguras, lo que se debe a la actividad antimicrobiana de la oleorresina y el aceite esencial de canela utilizados en las formulaciones.

Palabras clave: Bálsamo labial; Canela; Oleorresina; Aceite esencial.

1. Introduction

A cosmetic is defined as any substance or preparation intended to be placed in contact with the various external parts of the human body (epidermis, capillary system, nails, lips and external genital organs) or with the teeth and mucous membranes of the oral cavity, with the purpose of modifying their appearance, correcting body odour and/or protecting them by keeping them in good condition. Cosmeceuticals are intended to perform their functions, such as protection, whitening, tanning, anti-wrinkle, deodorants, anti-aging and nails and hair care as a cosmetic product, but also claim to have biologically active ingredients with medicinal benefits (Aranaz et al., 2018).

Products with topical action should be formulated to ensure that the active compounds efficiently cross the different layers of tissue to maximize their effectiveness. Although the skin constitutes a multi-layered organ, it is the outermost layer, which exerts the greatest barrier action to the permeation of drugs (Lane, 2013). The lips are an extremely sensitive region and have skin three times thinner than the other regions of the body. The lips do not have follicles, do not produce sebaceous secretions and are not covered, unlike other regions of the body, by the protective lipid film. Therefore, lips are very prone to dehydration and cracking. In addition, as saliva is constantly secreted from within the mouth and has saline properties, cosmetic products for this region require the pH must be compatible with the pH of human saliva (Galembeck & Csordas, 2009).

Lip balm is a cosmetic product similar to lipstick and is employed to avoid lip dryness and provide protection against adverse environmental factors. The product in question should possess the following characteristics: pleasant taste, resistance to temperature variations,

softness in the application, easy intentional removal and adherence (Fernandes et al., 2013). In addition to the use of waxes, oils and butters, which are responsible for providing consistency, antioxidants, preservatives, fragrances, dyes and pigments can be used in lip balm formulations (Denavarre, 1975; Barel, Paye & Maibach, 2009).

Since ancient times, natural resources are well known as a source of biologically active substance to be used as cosmetics or cosmeceutical products (Aranaz et al, 2018). Among them are essential oils and oleoresins, which are natural products of the secondary metabolism of plants. Essential oils and oleoresins are known to present a broad spectrum of action, including antioxidant activity and antibacterial activity. Even though the antimicrobial mechanism of action of essential oils and oleoresins is not fully understood, it is believed that the activity occurs due to their hydrophobicity (Martinelli et al., 2017; Rosa et al, 2018).

Essential oils have a recognized aesthetic and therapeutic potential and are mainly used for their bactericidal, virucide, antioxidant and anti-inflammatory activities, but also for their fragrant character, which can cause a sense of well-being. Several ways to use essential oils are possible: inhalation, ingestion, or application to the skin. Pure essential oils are almost never applied directly to the skin, as they can be irritating, but are diluted in other vegetable oils (olive oil, sunflower seed oil, etc.). The application to the skin is performed in the context of massages, local treatments (infections) or when using perfumes (main constituents) (Marrot & Soeur, 2015).

According to Eiserle & Rogers (1972), essential oils and oleoresins are especially important flavour constituents for hot processed foods. However, according to the authors, essential oils tend to volatilize during processing due to the heat and humidity present, losing some of the flavouring components. However, as they contain essential oils and fixatives compounds, oleoresins tend to depress volatilization and are preferred as flavouring materials. This is because solvent extraction removes not only essential oil from the material, but also other non-volatile constituents and these are retained in the product in the heating process and tend to fix or stabilize the most volatile essential oil (Eiserle & Rogers, 1972).

In order to obtain an ideal formula, the cosmetic must be stable, and for this to happen the evaluation of the stability of the products must be one of the most important factors in the execution of the processes involved (Zanin, Miguel, Chimell, & Dalmaz, 2001). Therefore, the objective of this study was to obtain cinnamon oleoresin, as well as to evaluate the antimicrobial properties of the oleoresin and the essential oil and employ them as additives in the preparation of organic lip balms enriched with vitamin E. In addition, the accelerated and extended stability analysis, as well as the microbiological safety assessment of the products.

Therefore, the present study aimed to use essential oil and cinnamon oleoresin in the production of lip balms, evaluating whether these additives differ in the microbiological stability and safety of the cosmetic product as suggested by Eiserle & Rogers (1972) for food.

2. Methodology

Obtaining the oleoresins: The cinnamon oleoresin obtention were carried out using 30 grams of the material and 350 mL of absolute anhydrous alcohol (99.3%) in a Soxhlet apparatus for two hours at 80°C. After extraction, the solvent was concentrated using a rotary evaporator.

Antimicrobial activity of the oleoresins and essential oils: The cinnamon essential oil was purchased from Ferquima Industria e Comércio Ltda. Different microorganisms were used, as Gram-negative bacteria *E. coli* and as Gram-positive bacteria *S. aureus* and *B. cereus*. The bacterial cultures were diluted to a final concentration of 10⁶ cells/mL and 500µL of these solutions were spread on Petri dishes, separately, containing Muller Hinton Agar. Over the broth were deposited the oleoresin and essential oil under study. The Petri dishes were incubated at 37°C for 24 h and after that, the size of inhibition zones was measured in millimetres. These analyses were performed in triplicate.

Gas Chromatography coupled to Mass Spectroscopy (GC-MS) of Cinnamon essential oil and oleoresin: according to Eiserle & Rogers (1972) oleoresin have in their constitution essential oils and other compounds, of a non-volatile nature, that act as fixatives and tend to depress the volatilization of essential oils during thermal processing. Thus, an evaluation by Gas Chromatography coupled with Mass Spectrometry of the essential oil and oleoresin used in this work was carried out to investigate their chemical composition. For this, 1 g of the samples was dissolved in 10 ml of HPLC grade acetone. The solution was vortexed and kept in an ultrasonic bath for 10 minutes. Then the samples were filtered through a 0.45 µm RC filter before analysis. The analyses were performed in a High-Resolution Gas Chromatograph coupled to a Mass Spectrometer Detector - Shimadzu, model GC / MS-QP2010. GCMS, using an Agilent DB-5MS column (30 m x 0.25 mm - 0.25 µm). The equipment conditions were: Injector temperature at 220°C, Splitless Injection Mode with 2-minute sampling time, Linear speed flow control mode, 15.7 psi pressure, total flow of 19.4 mL. min⁻¹, Flow in the column of 1.49 mL.min⁻¹, Linear speed of 45.0 cm.sec⁻¹, Purge flow of 3.0 mL.min⁻¹, Split ratio of 10, Column temperature in Thermogradient mode (80°C for 2 minutes, 140°C and 280°C). The Parameters of the Mass Spectrometry Detector were: Ion

source temperature at 200°C, Interface temperature at 280°C, Solvent cut-off time of 3 minutes, Detector voltage in relation to the result of the Tuning, Initial detection in 3.5 minutes, Final detection time in 17.0 minutes, SCAN acquisition mode, 0.25 second acquisition time, SCAN mass/load ratio (m/z) from 40 to 600 and volume of injection of 1 µL.

Preparation of lip balms: 45 g of coconut oil, 24 g of Candelilla wax and 45 g of cupuaçu butter were weighed, taken to the water bath at 100°C to form a homogeneous mixture which was given the named base. As the objective was the analysis of the influence of cinnamon oleoresin/essential oil and vitamin E to the base, 6 different types of lip balms were prepared varying the compositions. In all variations 18 g of base were used. For the balms containing vitamin E, four capsules of the vitamin were added in each sample. To the balms containing the oleoresin or essential oil, 5 drops were added in each variation. All the samples prepared were stored in an acrylic container, properly labelled according to the following nomenclature and composition:

- Control 1: base;
- Control 2: base + vitamin E;
- Control 3a: base + Cinnamon oleoresin;
- Control 3b: base + Cinnamon essential oil;
- Sample 1: base + vitamin E + Cinnamon oleoresin;
- Sample 2: base + vitamin E + Cinnamon essential oil.

Balm Stability Tests: Two stability tests were carried out on the prepared balms, the Accelerated Stability Test (AST) and the Extended Stability Test (EST). The AST lasts 15 days and the EST lasts 90 days. For this, the test formulations were subjected to stress conditions in order to accelerate the appearance of possible signs of instability according (ANVISA, 2012). The samples in this study were kept in the following environments and temperatures:

- Oven: T = 40°C;
- Refrigerator: T = 5°C;
- Freezer: T = -5°C;
- Cycles: 24 h at 45°C and 24 h, at -5°C;
- At 25°C under UV light;
- At 25°C protected from UV light.

The entire stability assessment was carried out in triplicate for the environment and

temperature under study. The analyses were carried out every 3 days over a period of fifteen days (AST) and every 15 days for 90 days (EST), investigating the organoleptic characteristics, which are those perceptible by the human senses. The appearance, colour and odour of the prepared balms were analysed, which can be classified as normal or unchanged (N); slightly modified (SM); modified (M) and intensely modified (IM).

Evaluation of Microbiological Safety of the products: for the evaluation of the microbiological safety of the balms, the research of *Escherichia coli* was carried out, as well as, of *Staphylococcus* and *Pseudomonas*, according to ANVISA (2012). For *E. coli* research, 1 g of sample was aseptically transferred to 9 mL of saline. Then, 1 ml of the solution was transferred to the Petri dish containing Eosin Methyl Blue agar (EMB) and the dish was incubated at $36 \pm 1^\circ\text{C}$ for 24 hours. After this period, it was observed whether there was a growth of colonies, as well as their characteristics (if present, they are black metallic colonies). When there is growth of suspicious colonies, the biochemical series for the identification of *E. coli* must be followed. For the research of *Staphylococcus* and *Pseudomonas*, 1 g of sample was aseptically transferred to 9 mL of soy-casein broth and this broth was incubated at $36 \pm 1^\circ\text{C}$ for 24 to 48 hours. Then, it was sown in Petri dishes containing Vogel Johnson agar for *Staphylococcus* research, and in cetrimide agar plates for *Pseudomonas* research. The plates were incubated at $36 \pm 1^\circ\text{C}$ for 24 hours. After this period, it was observed whether there was growth and the characteristics of the colonies. For *Staphylococcus*, the presence of yellowish colonies or the appearance of specific characteristics must be confirmed with the catalase and coagulase tests. For *Pseudomonas*, on the other hand, the presence of blue-green colonies must be confirmed by the microorganism by the biochemical series.

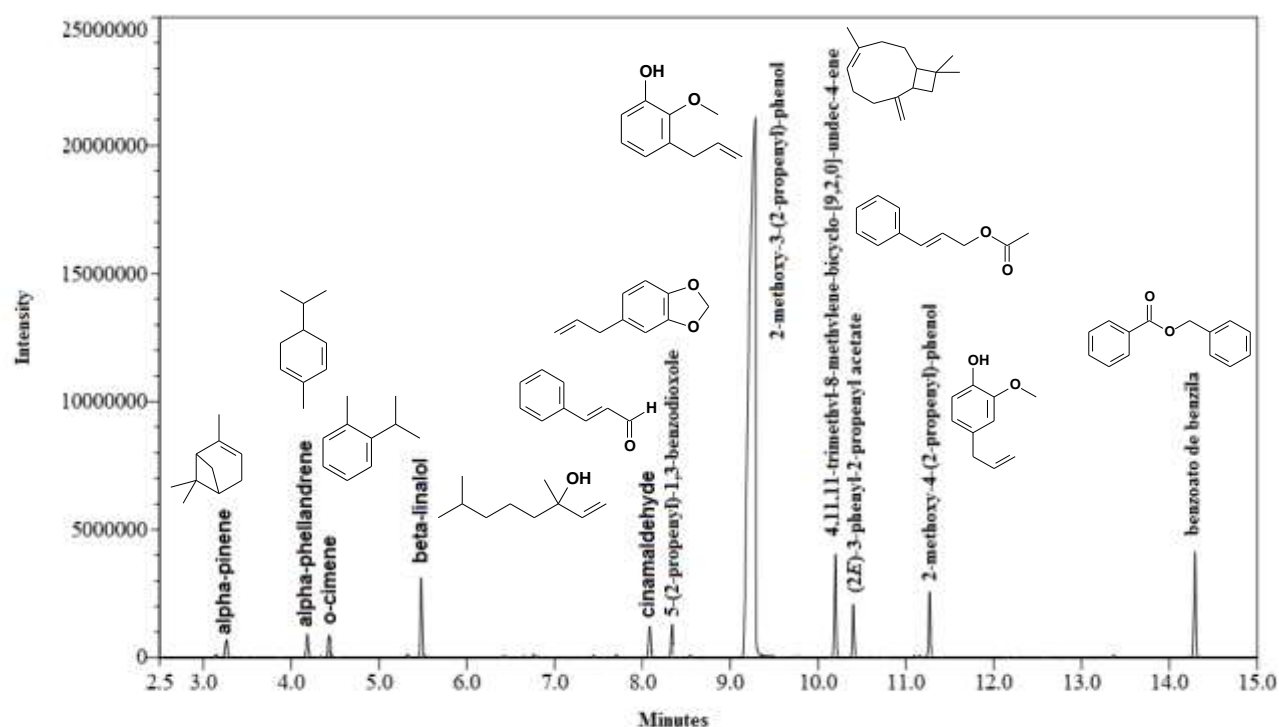
3. Results and Discussion

Antimicrobial activity of the cinnamon oleoresin and essential oil: Cinnamon oleoresin and essential oil inhibited entirely the growth of the microorganism tested. These results are important to verify if it is necessary a high antimicrobial activity to be used in a cosmetic product to inhibit the growth of pathogenic microorganisms.

GC-MS of Cinnamon essential oil and oleoresin: According to the GC-MS analysis, the cinnamon essential oil and oleoresin studied are completely different both in the amount of compounds present and in the nature of the composition itself. GC-MS of cinnamon essential oil showed 11 peaks between 3.2 and 14.3 minutes (Figure 1), while cinnamon

oleoresin showed 5 peaks between 1.2 and 13.4 minutes (Figure 2). The compounds present in essential oil and cinnamon oleoresin and their chemical structures are listed in each chromatogram.

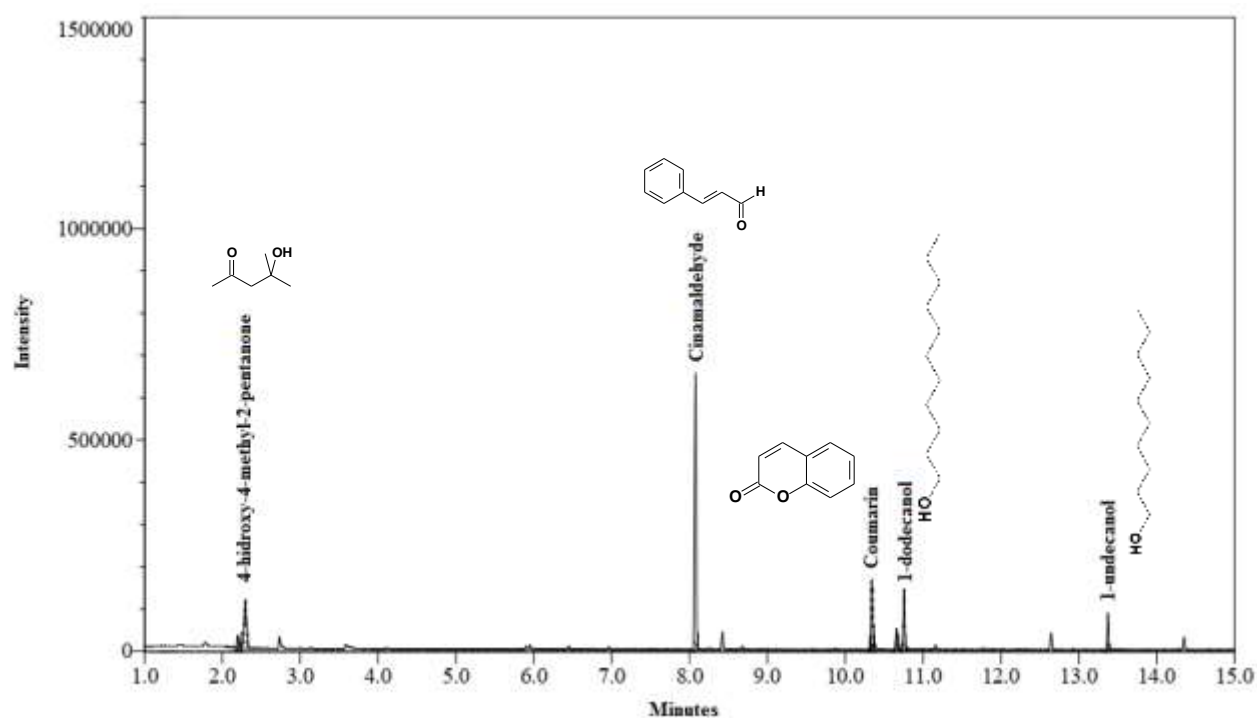
Figure 1: Chromatogram and compounds present in cinnamon essential oil studied.



Source: Authors.

Da Silva et al. (2012) studied the biological activity of pinenes and demonstrated that both enantiomers, α - and β -pinene have antimicrobial activity. However, only (+) enantiomers show such activity. According to Dorman & Deans (2000), alcohols have bactericidal activity against vegetative cells. Still according to the authors, aldehydes have high antimicrobial activity, possibly due to the conjugation of the aldehyde group to a C=C double bond in a highly electronegative arrangement. The authors also suggest that an increase in electronegativity improves antimicrobial activity, since electronegative compounds can interfere with biological processes by electron transfer and react with nitrogenous components, such as proteins and nucleic acids, inhibiting the growth of microorganisms. According to Hyldgaard et al. (2012), although the mode of action of cinnamaldehyde is inconclusive, aldehydes covalently bind to the amino groups of DNA and proteins, reducing their normal function. Cinnamaldehyde still interacts with the cell membrane.

Figure 2: Chromatogram and compounds present in cinnamon oleoresin studied.



Source: Authors.

For Goodarzi, Hadjiakhoondi, Yassa, Khanavi & Tofighi (2016) heterocyclic compounds containing the methylenedioxy functional group, such as 5-(2-propenyl)-1,3-benzodioxol also known as safrole, have a wide range of biological activities, such as antitumor, antibacterial, antifungal, antiparasitic, antimalarial, antioxidant, pesticide and herbicide. For Shah & Shelar (2018), both 2-methoxy-3-(2-propenyl)-phenol and 2-methoxy-4-(2-propenyl)-phenol also have antimicrobial activity. De Campos (2017) reports the biological activity of benzyl benzoate, present in extracts of green propolis. In the review on coumarin antimicrobial activity written by Smyth et al. (2009), the authors showed that these compounds are selective for Gram-positive microorganisms.

Taking into account cinnamon oleoresin, cinnamaldehyde, alcohols and coumarin are reported as antimicrobial compounds. But since cinnamaldehyde is the major compound, it probably is the responsible for the antimicrobial activity. Considering cinnamon essential oil, α -pinene, o-cymene, β -linalool, cinnamaldehyde, 5-(2-propenyl)-1,3-benzodioxol, 2-methoxy-3-(2-propenyl)-phenol, 2-methoxy-4-(2-propenyl)-phenol and benzyl benzoate, have antimicrobial activity reported in the literature. Although, as 2-methoxy-3-(2-propenyl)-phenol it is the major compound in the sample, it is probably the responsible for the

antimicrobial activity. Thus, this study goes against the one proposed by Eiserle & Rogers (1972), as it is possible to verify by the results obtained that the compounds present in cinnamon essential oil are not found in cinnamon oleoresin. Only cinnamaldehyde coexists in both essential oil and oleoresin. It is noteworthy that, there was no analysis of the non-volatile compounds present in the studied oleoresin to prove the existence of such compounds and to know their composition.

Stability Tests with the balms under study: When performing the AST, variations in the organoleptic properties were observed in the evaluation of aspects, colour and odour in the studied samples, performing each test in triplicate for the two comparisons, with oleoresin and essential oil. The results of the tests are shown in Table 1. As can be observed from Table 1, both samples with essential oil and with oleoresin at 25°C exposed to UV light were more unstable. The characteristics observed in the product with essential oil were slightly altered in the sixth and fifteenth day of evaluation, with the presence of water droplets in the three samples. For the oleoresin samples, instability also started to appear on the sixth day of evaluation, with the presence of water droplets in the samples (presence of condensation), showing a slight increase in this aspect over the following days. Another slightly moderate instability observed in the aspect analysis was the presence only on the fifteenth, day of water droplets in the samples with oleoresin at 25°C without UV light. The explanation for this effect in the samples, is because the air present between the lids and the surface of the products was saturated with water vapor that ended up condensing.

It should be noted that there were no cracks in the samples analysed and it is believed that this occurred due to the presence of vitamin E in the formulations. This is because, according to Rowe, Sheskey and Quinn (2009) and Barel, Paye and Maibach (2009), vitamin E acts attracting the water molecules, acting as a humectant. Therefore, as there was vitamin E in these formulations, the water molecules present in the products did not dissipate to the external environment, which in turn did not cause the appearance of cracks.

Table 1. Accelerated Stability Test data.

		Control 1					Control 2					Control 3A					Control 3B					Sample 1					Sample 2																													
Storage time (days)		0	3	6	9	1/2	15	0	3	6	9	12	15	0	3	6	9	12	15	0	3	6	9	12	15	0	3	6	9	1/2	1/5	0	3	6	9	1/2	1/5																			
Freezer -5°C	Aspect	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N														
	Colour	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N												
	Odour	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N											
Refrigerator 5°C	Aspect	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	S	S	S	L	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N													
	Colour	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N											
	Odour	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N									
Cycle	Aspect	N	N	N	S	S	M	N	N	N	N	N	N	N	N	N	M	S	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N											
	Colour	N	N	N	S	S	S	N	N	N	S	S	S	N	N	N	S	S	S	N	N	N	S	S	S	N	N	N	S	S	S	N	N	N	S	S	S	N	N	N	S	S	S	N	N	N	S	S	S	N						
	Odour	N	N	S	N	N	N	N	N	S	N	N	N	N	N	M	N	N	M	N	N	S	N	N	S	N	N	S	N	N	N	N	N	N	N	N	N	N	S	N	N	N	N	N	N	N	N	S	N	N	N					
25°C without UV light	Aspect	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N										
	Colour	N	N	N	N	N	S	N	N	N	N	N	S	N	N	N	N	N	S	N	N	N	S	N	S	N	N	N	N	N	N	S	N	N	N	N	N	N	N	N	N	S	N	N	N	N	N	S	N	N	N					
	Odour	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N				
25°C with UV light	Aspect	N	N	N	N	S	N	N	N	N	N	S	N	N	N	N	N	N	N	N	N	S	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N						
	Colour	N	N	M	M	S	M	N	N	M	M	S	M	N	N	M	M	S	M	N	N	M	M	S	M	M	N	N	M	M	S	M	M	N	N	M	M	S	M	N	N	M	M	S	M	N	N	N	N	S	M	N	N			
	Odour	N	N	S	N	N	N	N	N	S	N	N	N	N	N	M	N	N	S	N	N	S	N	N	S	N	N	S	N	N	N	N	N	N	N	N	N	N	N	N	S	N	N	N	N	N	N	N	N	S	N	N	N			
Oven	Aspect	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N				
	Colour	N	N	N	S	S	S	N	N	N	S	S	S	N	N	N	S	S	S	N	N	N	S	S	S	N	N	N	S	S	S	N	N	N	S	S	S	N	N	N	S	S	S	N	N	N	S	S	S	N	N	N	S	S	S	N
	Odour	N	N	S	S	N	N	N	N	S	S	N	N	N	N	S	S	N	M	N	N	S	S	N	N	N	N	S	S	N	N	N	N	N	N	N	N	N	N	S	S	N	N	N	N	N	N	N	N	N	N	S	S	N	N	

Legend: N - Normal, SM - Slightly Modified, M - Modified, IM - Intensely Modified. Source: Authors.

For the colour analysis, no variations were observed for samples kept at low temperatures, such as in the freezer (-5°C) and refrigerator (5°C) for the two comparisons. In the remaining conditions, samples and controls with oleoresin showed instability at 25°C with UV light, demonstrating a slightly moderate change to a lighter colour on the sixth and ninth test days and remaining stable from the fifteenth day. Another observation is a small modification on the fifteenth day for the cycle control, which showed a darker colour. For the samples with essential oil, changes were observed in the controls stored under UV light, with instability on the sixth and ninth day and in the controls at 25°C without UV light, only on the ninth day, with a slight lightening of the sample as an alteration. For the control 1 maintained in the cycle condition, a darkening was observed on the sixth day, which remained constant in the next days of analysis for the three samples.

Some ingredients, especially colouring solutions and pigments can change their colour due to oxidation reactions that occur when they are exposed for a long time to oxygen. In addition, products that contain oily-based materials in their formulation and are exposed to air or extreme temperatures can degrade by oxidation (Galembeck & Csordas, 2009). In such a way, it can be concluded that the samples and controls that were present in the conditions of cycles and at 25°C under UV light underwent oxidation of the compounds present in the candelilla wax. Another factor also responsible for the oxidation reaction is the action of UV light (Galembeck & Csordas, 2009). From this it is possible to affirm the samples and controls that were exposed to solar radiation suffered oxidation, therefore, causing their colour variation. It shows the exposure condition was a factor that caused a slow rate of degradation of the compounds present in the product formulations, mainly those present in candelilla wax. This slow rate of degradation is due to the presence of vitamin E in the formulations, an antioxidant.

The last organoleptic characteristic analysed was the odour of the studied samples. Analysing the two sets, instabilities were observed when the samples were exposed to higher temperatures. Evaluating the first set, the changes appeared from the twelfth day of storage at 25°C with UV light and on the fifteenth day for samples in the oven (40°C). For the second set, the instabilities arose from the ninth day for tests at 25°C with UV light and on the fifteenth day for samples stored in an oven (40°C). All the mentioned samples had a slightly moderate instability change, with attenuation of the characteristic odour of cinnamon and started to have a slight odour of candelilla wax. When an oil-based product is exposed to air or extreme temperatures, it can degrade by oxidation, generating the unpleasant rancid odour.

Taking this aspect into account, vitamin E was added to the formulation as an antioxidant (Galembeck & Csordas, 2009). Thus, no rancid odour was observed in any of the controls or samples analysed and as only the attenuation of the characteristic odour of cinnamon was observed to accentuate the characteristic odour of candelilla wax, it is possible to conclude that vitamin E acted in reducing the effects caused by the oxidation reaction, one of which is the rancid odour.

However, as can be seen from the results obtained in the stability tests performed, there was no difference in the stability of the samples in the two sets analysed. Therefore, it is possible to affirm that, although oleoresin does not contain the same compounds present in the essential oil of cinnamon used in this work, the compounds present in oleoresin were sufficient to maintain the same stability observed for the samples with essential oil. Thus, it is possible to affirm that although they do not have the same chemical composition, both cinnamon oleoresin and essential oil are additives that can be used in the manufacture of cosmetic products, with antimicrobial and preservative action.

To complement the AST, the EST was performed following the same procedures as the AST test, removing the samples kept in a condition of temperature cycles. The results obtained from such an evaluation are shown in Table 2. For the analysis related to the aspect, a small modification was noted for the samples with essential oil on the ninetieth day of the test for low temperatures such as freezer (-5°C) and refrigerator (5°C) and for the test in the oven (40°C). All of the aforementioned had a slightly drier appearance than the reference, stressing that there were no cracks, probably due to the presence of vitamin E in the samples, proving that the release of water molecules present in the products happened very slowly and gradually. For the sample at 25°C with UV light, instability was observed from the seventy-fifth day with the disappearance of water droplets but forming a slightly moderate to oleaginous aspect. Finally, the samples with oleoresin showed the same mildly dry appearance on the ninetieth day for freezer storage, but without cracks, and for the samples at 25°C without UV light the formation of small water droplets was noticed on the last evaluation day. For the sample exposed to UV light, the presence of water droplets and a slightly more oleaginous aspect was also noticed from the seventy-fifth day, which proves that the air present between the caps and the product surfaces was saturated with steam water and its condensation happened gradually.

For the colour analysis of the samples, it was observed a gradual variation in the analysed products. Control A showed a slight colour change on the ninetieth day of storage in an oven (40°C) and at 25°C without UV light, showing a slightly darker colour than the reference. For the sample at 25°C with UV light, a progressive loss of colour was observed from the thirtieth day, showing a slightly white colour at the end of the test. The control 2 that found a more significant colour change were for samples exposed to sunlight gradually losing their colour at high temperature. Therefore, observing the results, the oxidation occurred slowly and gradually and can be justified due to the addition of vitamin E in the formulations. Finally, the odour of the samples showed mostly mild changes in the last days of evaluation of the controls, thus losing the natural aroma of their essences (cinnamon). However, in the last organoleptic analysis, the odours did not coincide mostly with the odour of the samples at the beginning of the test, proving that oxidation, a probable cause of the colour change, is not the only responsible for the odour change, but also the volatility of the components present in the formulation of the products.

Evaluation of Microbiological Safety of the products: According to the microbiological safety assessment of the products studied, there was no *E. coli*, just as there was no *Staphylococcus* and *Pseudomonas* growth. In this way, it is possible to conclude that both essential oil and cinnamon oleoresin are equally effective antimicrobial additives, as none of the products showed growth of pathogenic microorganisms in the researches carried out compared to control 1. The different chemical composition of the essential oil and cinnamon oleoresin studied did not compromise the antimicrobial action of these additives. Therefore, it can be concluded that the fact that oleoresin does not contain all the compounds present in the essential oil did not compromise the antimicrobial action of this additive. In other words, is possible to say that the synergistic action of the various compounds present in the essential oil does not increase its antimicrobial action and that, probably, cinnamaldehyde is the compound responsible for the antimicrobial activity of both the essential oil and oleoresin, or if this is in greater quantity, this may also be the possible explanation for the activity in both additives. According to Orth (1984) all cosmetic products are subject to contamination with microorganisms. However, the growth of fungi, bacteria and spores in cosmetic products depends on a series of physical and chemical factors, such as the availability of water, the composition of the product that can serve as nutrients for microorganisms, storage temperature, among others. For Carvalho & Pachione (1989), the lack of hygiene in manufacturing and the low stability of the constituents of the formulation are the main factors that contribute to the contamination of non-sterile products. However,

according to McCarthy (1984), microbial contamination can cause visible changes, such as changes in colour, odour, viscosity, in addition to being able to cause toxic reactions to the user.

4. Conclusions

Due to the growing consumption of cosmetic products, the industries of the sector, in addition to increasing their production lines and also meeting the criteria imposed by the quality agencies, need to improve their products, so new research for the development and formulation is being performed. The products formulated in this work met the criteria related to stability tests, both accelerated and extended, even with variations found in the products, these did not interfere in the synergistic relationships of its components, leaving them stable. The microbiological evaluation of the formulated products showed that the formulated products are 100% microbiologically safe.

In future studies other essential oils and oleoresins will be used to test the same properties.

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