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| cetlogo ***CHEMICAL ENGINEERING TRANSACTIONS***  ***VOL. , 2024*** | A publication of  aidiclogo_grande |
| The Italian Association  of Chemical Engineering  Online at www.cetjournal.it |
| Guest Editors:  Copyright © 2023, AIDIC Servizi S.r.l. **ISBN** 979-12-81206-XX-X; **ISSN** 2283-9216 | |

Biotechnology of natural dyes: Obtaining pigment biomass from *Beta Vulgaris* and *Zea Mays Ceratina* and production of Cosmetic Products

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The cosmetic industry manufactures products that contain substances that produce harmful effects (toxic or carcinogenic) to the consumer's health, softening or moisturizing substances such as MOSH (Mineral Oil Saturated Hydrocarbons) and MOAH (Mineral Oil Aromatic Hydrocarbons) or heavy metals such as lead when ingested through an application on the lips generate allergic reactions or intoxication. To solve this problem, we propose the extraction of natural pigments using Beta vulgaris (beet) and Zea mays ceratina (purple corn) by dehydration and sedimentation methods. Doses of 0.5 g, 1.0, g, and 1.5 g of beet pigment were applied, with 3 g of purple corn pigment in all doses. The results showed a pasty consistency lipstick with a density of 0.85 g/cm³, softening temperature at 71.8 °C, and melting point at 98.5 °C, also identified an area reduction of 28.2% in the mechanical test. It is concluded that natural colorants can be used in beauty and personal care products with the consequent reduction of environmental impact and health effects instead of using colorants based on synthetic chemical products.

* 1. Introduction

Multiple colorants are used in the cosmetics industry to enhance presentation and attract consumers' attention (Messaraa et al., 2020). The colorants used in this industry are generally manufactured with chemicals containing heavy metals such as lead, chromium, arsenic, and other synthetic substances, which can negatively affect health and generate impacts on the environment (Safavi et al., 2019). Several studies on the content of heavy metals in lipsticks have confirmed their use for several years (Al-Saleh et al., 2019). Recently, a Mexican study determined the presence of lead at an average concentration of 1,457 ppm in lip products (Rodríguez Rosalío et al., 2023). Although this concentration is within the limits allowed by the FDA (Food and Drug Administration), a risk persists, so the precautionary principle should prevail, considering that continuous use on the lips could affect consumers' health.

Despite the dangers posed by these chemicals (Öztaş et al., 2024), they continue to be used or replaced by others to improve their quality in the color palette, increase their shelf life, or any other desired properties. Indirect ingestion of heavy metals causes health problems such as allergies and hypersensitivity (Sharma et al., 2021), and it should also be considered that by bioaccumulation, they could even have carcinogenic effects (Mohammed et al., 2023).

There are European regulations on banned substances (lead) in cosmetic products (EC REGULATION No 1223., 2009), as well as concentration limits; however, it should be considered that many have been extensively studied, being necessary to establish permanent control policies (Arshad et al., 2020). Therefore, replacing these chemicals with natural products such as plant pigments is the safest approach. In this sense, the objective of the research was to obtain biomass of natural pigments from Beta vulgaris (sugar beet) and Zea mays var. ceratina (purple corn) to test the production of lipsticks then and characterize them.

The results will offer a viable alternative to mitigate the environmental and health impacts of the toxicological risk of products made with hazardous chemical components. Replacing these substances with natural ingredients will maintain their protective properties against ultraviolet (UV) rays, while natural pigments, oils, and aromas will enhance lip beauty. Thanks to them, herbal lipsticks containing safe and beneficial nutrients have been developed (Vigneshwaran et al., 2023).

* 1. Methodology

The research consisted of preparation and treatment processes of pigment biomass *from Beta vulgaris and Zea mays var. ceratina*, production of lipsticks, and characterization of the prototypes. The following stages were followed:

* + 1. Obtaining raw materials

*Beta vulgaris and Zea mays were purchased at a food market. These products were chosen according to their organoleptic characteristics with due sanitary care (use of mask, cap, and gloves).*

* + 1. Preparation of the pigment biomass of *Beta vulgaris and Zea mays var. ceratina*

Samples were washed and dried at room temperature to extract Beta vulgaris pigment. After cutting, the material was dehydrated at 73°C for 72 hours. The dehydrated sheets were crushed to obtain biomass with a suitable composition for lipstick production and sieved according to ASTM 422 standard (-200 mesh), yielding the pigment in powder form. A solution was prepared by dissolving 250 g of biomass in 250 mL of water (Fig. 1). Physicochemical parameters were measured using multiparameter equipment (Gondo EZODO). Anthocyanin concentration was determined through serial dilution and spectrophotometry at 440 nm wavelength, calculating concentration from absorbance according to Beer's law.

For Zea mays var. ceratina pigment, 3 kg of grains were weighed, washed, and dried for 48 hours at room temperature. The grains were crushed and sieved to 100 mesh (0.150 mm), then washed three times to obtain a solution that underwent sedimentation. The resulting pigmented biomass was dried for 5 days at room temperature after complete water removal. Twenty grams of this biomass were dissolved in 60 mL of distilled water to characterize the solution's physicochemical properties (see Figure 2).

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| Figure 1: Beta vulgaris pigment biomass | Figure 2: Zea mays var. ceratina pigment biomass |

* + 1. Production of cosmetology products: lipsticks

Lipsticks were prepared with doses of 0.5, 1.0, and 1.5 g of pigmented biomass of Beta vulgaris and 3 g of pigmented biomass of Zea mays var. ceratina, to which complementary components of almond oil, cocoa butter, beeswax, eucalyptus oil, and glycerine were added. This formulation was tested in triplicate in three groups. Table 1 shows the doses of the components used.

Table 1: Components for making lipsticks

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Prototype | *Beta vulgaris* pigment biomass (g) | *Zea mays* var. ceratina pigment biomass (g) | Beeswax (g) | Cocoa butter (g) | Glycerin (mL) | Almond oil (mL) | *Eucalyptus* essential oil (mL) |
| 1 | 0.5 | 3.0 | 3.0 | 2.7 | 1.0 | 0.5 | 0.05 |
| 2 | 1.0 | 3.0 | 3.0 | 2.7 | 1.0 | 0.5 | 0.05 |
| 3 | 1.5 | 3.0 | 3.0 | 2.7 | 1.0 | 0.5 | 0.05 |

The generated prototypes were made based on the researcher's experience and the literature consulted, making lipstick molds that were subsequently dried and refrigerated.



Figure 3: Made lipstick

* + 1. Characterization of Lipstick Prototypes

The comprehensive characterization of lipsticks is fundamental for ensuring high-quality, safe, and effective products that comply with current cosmetic industry regulations. In this study, we evaluated multiple parameters across several categories. Physicochemical properties (Romanowsky and Shueller, 2006) included consistency, density, melting point, softening point, and hydrogen potential (pH). The microbiological assessment followed the Andean Technical Regulation on Microbiological Technical Specifications for Cosmetic Products (2019), determining the presence of potential pathogens including Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, as well as aerobic mesophiles. Additionally, we analyzed organoleptic parameters (Varas-Arribasplata et al., 2021) such as color, odor, and flavor. We also measured the surface shrinkage of the lipstick samples (Gładysz et al., 2021) this allows for increased mechanical strength and minimal deformation due to the application force, as illustrated in Figure 4.



Figure 4: Lipstick area reduction test

* 1. .Results
     1. Physicochemical parameters of the pigment biomass of Beta vulgaris and Zea may

The conductivity of Beta vulgaris was low compared to Zea mays (Table 2). Conductivity is an indicator of the presence of minerals in beet. There are potassium, sodium, calcium, magnesium, and iron; sugars such as sucrose (between 15-20 %), fructose, and glucose; and other components such as vitamins A, B1, and B2; proteins, pigments (betaine), choline, glutamine, saponosides and phytoestrogens (Barel et al., 2009). The pH is close to neutral, making it ideal as a raw material for producing cosmetic products, including lipsticks. The redox potential indicates the chemical energy of exchange between solutes and the ions present, influencing bacterial development (Bouslimani et al., 2019). Zea mays var. ceratina scientific studies indicate that it contains starch around 80%, sugars 10 %, proteins 11 %, minerals and vitamins (B complex and ascorbic acid) approximately 2%, but the most important thing is the content of the pigments (between 1.5 % and 6.0 %), called anthocyanins (cyanin-3-glucose C3G, which is its main colorant) from the group of flavonoids and phenolic compounds with natural antioxidant and anticancer properties (Guillén-Sánchez et al., 2014).

Table 2: Physicochemical analysis of the pigment biomass of Beta vulgaris and Zea mays var. ceratina

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Pigment biomass solution | Conductivity electrical (uS/cm) | Potential hydrogen (pH) | Potential redox (mV | Temperature  (°𝑪) |
| *Beta Vulgaris* | 9.69 | 6.90 | 208.3 | 20.11 |
| *Zea mays* var. ceratina | 497.00 | 5.39 | -092.00 | 20.10 |

* + 1. Characteristics of lipsticks

Lipsticks are one of the most used products in cosmetology, so they must have unique characteristics that make them attractive (such as color) along with properties that make them beneficial without risk to people's health. The pencils made in this research based on pigment biomass of *Beta vulgaris* (beet) and *Zea mays* var. ceratina (purple corn) presented a pasty consistency in the three prototypes, with density, softening temperature, and pH presented in Table 3. These characteristics, at their optimal level, allow lipsticks to be easy to apply, form a soft and uniform covering film on the surface, and increase the temperature of the lips due to thixotropic properties. They must also have a shiny appearance, be stable to light and heat, and have physicochemical stability (Benaiges, 2004). The lip prototypes had a high melting point between 90-98 °C, which indicates the presence of beeswax, along with the characteristic of pasty consistency and adequate shine (Ordis, 2013).

Beeswax also gives the pigment biomass characteristics of plasticity and flexibility, thus avoiding possible breakage during its use (Benaiges, 2004).

Table 3: Physicochemical parameters of lipsticks

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Prototype | Consistency | Density  (g/m3) | Melting point (°C) | Softening temperature (°C) | pH |
| 1 | doughy | 0.79 | 92 | 67.1 | 5.49 |
| 2 | doughy | 0.80 | 96 | 68.4 | 5.77 |
| 3 | doughy | 0.84 | 98 | 71.6 | 6.51 |

The lipstick prototypes presented zero presence of microbiological agents, as shown in Table 4; that is, they presented good dermatological tolerance due to the natural ingredients in their composition with the presence of antioxidants such as anthocyanins in the lipstick. *Zea mays ceratina*. All this gives it the property of microbiological stability (Barrientos, 2005)

Table 4: Microbiological parameters of lipsticks

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Prototype | *Staphylococcus*  *Aureus* (UFC/g) | *Pseudomonas*  *Aeruginosa* (UFC/g) | *Escherichia*  *Coli* (UFC/g) | *Mesófilos*  *Aerobios* (UFC/g) |
| 1 | Absence of colonies | Absence of colonies | Absence of colonies | Absence of colonies |
| 2 | Absence of colonies | Absence of colonies | Absence of colonies | Absence of colonies |
| 3 | Absence of colonies | Absence of colonies | Absence of colonies | Absence of colonies |

Regarding the organoleptic parameters (see Table 5), the color was beautiful in the formulation of prototype 3, and the smell was aromatic of almond, with an acceptable flavor. This good smell is also favored by the presence of antioxidants from the biomass of *Zea mays* var. ceratina that prevent the oxidative degradation of the oils (Benaiges, 2004)

Table 5: Organoleptic analysis of lipsticks

|  |  |  |  |
| --- | --- | --- | --- |
| Prototype | Color | Olor | Flavor |
| 1 | colorless | Aromatic | Acceptable |
| 2 | faint | Aromatic | Acceptable |
| 3 | accentuated | Aromatic | Acceptable |

Area reduction in lipsticks is the ability to cover a large area with a small amount of product. In the investigation, it was found that in the three prototypes, with the same initial area and applied force, the effort was equal. Still, prototype 3 had a minor area reduction (see Table 6) compared to the others (Disser, 2006). This is an essential feature in the lipstick industry.

Table 6: Rheological characteristics of lipstick prototypes

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Prototype | Initial area (mm2) | Applied force (N) | Effort (N/mm2) | End area (mm2) | Area Reduction |
| 1 | 1252.18 | 7.0 | 5.590 x 10-3 | 701.77 | 78.4 |
| 2 | 1252.18 | 7.0 | 5.590 x 10-3 | 885.24 | 41.5 |
| 3 | 1252.18 | 7.0 | 5.590 x 10-3 | 976.97 | 28.2 |

3.3 Toxicology of natural dyes

The toxicity of natural pigments obtained from *Beta vulgaris* and *Zea mays* var. ceratina is significantly lower compared to traditional synthetic dyes; according to information from scientific journals, these natural pigments, being extracted from plant sources such as corn and beets, have beneficial properties for the skin and are less likely to cause adverse effects compared to artificial dyes. Therefore, their use in products such as lipsticks can be a safer and healthier alternative with positive impacts on the skin (Salinas et al., 2005). Therefore, plant-based cosmetics are safe for human health, unlike synthetic cosmetics, regular use of artificial colors in lipsticks can cause serious side effects such as skin discoloration, skin irritation, acne, and cancer (Anilkumar and Dhanaraju, 2021).

Natural pigments, according to scientific literature, do not have adverse effects on health; on the contrary, it has been demonstrated that the colorant of *Beta vulgaris* behaves as a cytotoxic compound (Kapadia et al., 2011), i.e., for cancer problems, it has also been determined the action of the colorant betaine against the aggregation of β-amyloid (A β ), which causes Alzheimer's disease (Inamura et al., 2022); as for the pigment of *Zea mays* L., its anthocyanin content has potential therapeutic action against oxidative stress (Magaña et al., 2020); also, the pulp of Beta vulgaris, helps to reduce synthetic contaminants, for example, helps in the removal of the turquoise blue-G reagent Gemazol from an aqueous solution, in an investigation the removal of 234.8 mg g-1 was obtained at 25 °C and an initial pH value of 2.0 (Zümriye A. and I. Alper I., 2006), these azo dyes are mainly harmful to health (Correa-Mahecha et al., 2023).

* 1. Conclusion

Three prototypes of lipsticks were made using the pigment biomass of *Beta vulgaris* and *Zea mays* var. ceratina as the primary raw material, with perfect consistency and color. Also, they presented good physicochemical characteristics, microbiological characteristics, organoleptic and rheological properties such as area reduction. Prototype three exhibited the best properties, the same one made with 1.5 g of *Beta vulgaris*, 3 g of *Zea mays ceratina,* and other natural complementary additives. In this way, it is established that using biomass products containing natural pigments is a sustainable alternative with environmental advantages in the cosmetic industry.

Acknowledgments

The authors and the GITA and GIGATRE groups thank César Vallejo University for promoting scientific research.

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