UNIVERSIDADE DE SÃO PAULO
FACULDADE DE CIÊNCIAS FARMACÊUTICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM FÁRMACO E MEDICAMENTOS
ÁREA DE PRODUÇÃO E CONTROLE FARMACÊUTICOS

Cosmetic attributes (oiliness reduction and firmness) from face masks composed of red, green and black clays

Atributos cosméticos (redução de oleosidade e melhora de firmeza) de máscaras faciais compostas por argilas vermelha, verde e preta

Fernanda Daud Sarruf

Tese para obtenção do grau de
DOUTOR

Orientador: Prof. Dr. André Rolim Baby

São Paulo

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Tese para obtenção do grau de Doutor

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ABSTRACT

SARRUF, F.D. Cosmetic attributes (oiliness reduction and firmness) from face masks composed of red, green and black clays. **2024. Thesis (PhD) – Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, 2024.**

The appearance of skin strongly influences people's self-esteem and well-being. Among the characteristics that affect the most, we can cite acne and wrinkles. Therefore, minimizing those characteristics by using cosmetics has important value for the consumer. Nowadays, there is a demand for natural ingredients, mainly concerning cosmetic products. The use of clays in beauty care is old, with therapeutic uses since Prehistory. There is an essential advantage in using clays for cosmetic purposes, as it is a low-cost, environmentally friendly, natural, and abundant ingredient, which is chemically inert with a low level of toxicity (when in adequate conditions), easy to apply and remove, dries, and hardens fast. In this research work, we aimed at developing and investigating the effect of formulations containing red, green, and black clays as active components in oiliness reduction, and firmness and elasticity improvement *in vivo*, as well as characterizing clays mineralogically. All clays presented different compositions. Black clay was the one with the simplest mineralogic content, lowest density, and smallest particle size. It was the richest in Si and Al. Green clay presented expandable smectite and the highest density. Red clay presented the largest particle average size and was the richest in iron content. By thermal analysis, clays presented two characteristic events: the water loss (dehydration) and the dihydroxylation of kaolinite, turning into meta kaolinite. In Sebumetry, clays showed a significant reduction of skin oiliness on the forehead in both concentrations after 2h of contact compared to control, baseline, and placebo. However, after 4h (2h after removal), no significant difference was observed with the control. In the Cutometry, clays did not present significant efficacy in skin firmness and elasticity improvement when compared to control and placebo sites, thus, further studies should be performed comparing the efficacy of different vehicles with dispersed clays. Finally, clay mask formulations were developed with black, red, and green clay dispersed in an oil-free gel vehicle, which proved to be adequate for oily skin.

Keywords: Clay. Mineralogic composition. Efficacy assessment. Face mask.

RESUMO

SARRUF, F.D. Atributos cosméticos (redução de oleosidade e melhora de firmeza) de máscaras faciais compostas por argilas vermelha, verde e preta. **2024. Tese (Doutorado) - Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, 2024.**

A aparência da pele influencia fortemente autoestima e o bem-estar das pessoas. Dentre as características que mais afetam podemos citar acne e rugas. Portanto, minimizar estas características com o uso de cosméticos tem um valor importante para o consumidor. Atualmente existe uma procura por ingredientes naturais, principalmente no que diz respeito a produtos cosméticos. O uso das argilas nos cuidados de beleza é antigo, com usos terapêuticos desde a Pré-História. Há uma vantagem essencial na utilização de argilas para fins cosméticos, pois é um ingrediente de baixo custo, ecologicamente correto, natural, e abundante, que é quimicamente inerte e tem baixo nível de toxicidade (quando usado em condições adequadas), fácil de aplicar e remover, seca, e endurece rapidamente. Neste trabalho de pesquisa, objetivamos desenvolver e investigar o efeito de formulações contendo argilas vermelha, verde e preta como componentes ativos na redução da oleosidade e na melhoria da firmeza e elasticidade *in vivo*, bem como caracterizar mineralogicamente as argilas. Todas as argilas apresentaram diferentes composições. A argila preta foi a de conteúdo mineralógico mais simples, mais baixa densidade, e menor tamanho de partícula. Foi a mais rica em Si e Al. A argila verde apresentou esmectita expansível e mais alta densidade. A argila vermelha apresentou o maior tamanho médio de partícula e foi a mais rica em ferro. Segundo a análise térmica, as argilas apresentaram dois eventos característicos: a perda de água (desidratação) e a desidroxilação da caulinita, transformando-se em meta-caulinita. Na Sebumetria, as argilas demonstraram significativa redução na oleosidade da pele na testa em ambas as concentrações após 2h de contato comparada ao controle, basal e placebo. Porém, após 4h (2h após remoção), nenhuma diferença significativa foi observada com o controle. Na Cutometria, as argilas não apresentaram Eficácia significativa na melhora da firmeza e elasticidade quando comparadas aos sítios controle e placebo, portanto, mais estudos devem ser realizados comparando a eficácia de diferentes veículos com argilas dispersas. Finalmente, as formulações de máscaras de argilas foram desenvolvidas com argilas preta, vermelha e verde dispersas em um veículo em gel livre de oleosidade, o qual provou ser adequado para pele oleosa.

Palavras-chave: Argila. Composição mineralógica. Avaliação de eficácia. Máscara facial.

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LIST OF ABBREVIATIONS

Min = minutes

°C = Celsius degrees

mL = milliliters

µL = microliters

µg/cm^2 = micrograms per square centimeter

mg/cm^2 = milligrams per square centimeter

RDC = resolution of the collegiate board

MPA = Multi Probe Adapter

t0 = baseline measurement

t2h = measurement 2 hours after product application

t4h = measurement 4 hours after product application

INCI = International Nomenclature of Cosmetic Ingredients

Rpm = Rotations per minute

SEM = scanning electron microscopy

EDS = Energy Dispersive Spectroscopy

SUMMARY

1. Introduction

Skin appearance directly influences people's self-esteem and well-being. Among the conditions that affect most, we can cite acne and wrinkles (GUPTA; GILCHREST, 2005; GUPTA; GUPTA, 1998). Therefore, minimizing characteristics that occur in these conditions by using cosmetics has great value for the consumer.

Clays correspond to the inorganic fraction of several types of soil and are composed by inorganic minerals (denominated clay-minerals). They may also contain organic matter, impurities in the form of salt, and residual and amorphous minerals. Clays used in cosmetics contain metals such as aluminum, iron, magnesium, and titanium, which contribute to the functions of their use (BALDUINO, 2016; ZAGUE, 2007). Clays may be used in cosmetics as active components, for example, for oiliness reduction, skin whitening, firmness improvement, hydration, etc. (SARRUF et al., 2024). Therefore, they are an interesting active to be applied on acneic and mature skin to improve their characteristics.

Clays can be incorporated in several types of cosmetic formulations, both as active ingredient and as formulation component, due to their properties such as cation exchange capacity, rheological properties, color, particle size, and functional properties like the adsorption of secretions, microorganisms, oiliness, sweat and dirt. As examples we can mention exfoliants, sunscreens, soaps, shampoos, toothpastes, deodorants, makeup products, and face masks (SARRUF et al., 2024). As stated by Velasco and co-workers (2016), "clays are mostly used in face masks due to their high absorbency levels on skin surface, such as greases, toxins and even bacteria and viruses". Hence, this was the cosmetic form of clays application selected for this research work.

Face masks are cosmetic formulations to be applied on the face with a thick layer and further removed after a certain time (normally of 10-30 minutes according to the composition and aim), with a frequency of 1-2 times a week. They can have many effects according to their components, like cleaning, lifting effect, rejuvenation *etc*. Face masks composed of clay-material dispersed in a vehicle are called "Clay Face Masks" (ZAGUE, 2007).

Clinical trials in humans are of great importance to understand the real effectiveness of active cosmetic components and cosmetic preparations. These evaluations must be carried out in accordance with the ethical principles established by legislation, to guarantee the protection of research participants (AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA (ANVISA), 2012).

By the above, this research involved the in vivo instrumental assessment of cosmetic attributes related to oiliness, firmness and elasticity of the association of red, green, and black clays incorporated into cosmetic face masks.

2. Literature review (https://doi.org/10.3390/cosmetics11010007)

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Review

The Scenario of Clays and Clay Minerals Use in Cosmetics/Dermocosmetics

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Abstract: The use of clays in beauty care comes from ancient times, with therapeutic use since prehistory, and it is considerably relevant in the current cosmetic industry worldwide. In our review, we described types of clay and clay minerals used in cosmetics and dermocosmetics, compositions, usages as active compounds and cosmetic ingredients/starting materials, and observations about formulation techniques. From this review, we observed that although much scientific and specialized literature has reported the characterization of clays, only some involved efficacy tests when incorporated into cosmetic products, mainly concerning haircare applications. Our review could be considered and encouraged in the coming years to provide scientific and technical information for the cosmetic industry regarding the multifunctional use of clays and clay minerals.

Keywords: clays; physiochemical properties; efficacy; delivery; safety; formulation

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1. Introduction

The cosmetic industry has been using natural components in products since the beginning of history. The use of clays in beauty care is old, with therapeutic uses since prehistory [1–3]. Reports of treatments with "medicinal earth", mainly constituted by clay minerals, are present in ancient civilizations' scriptures, such as ancient China, Egypt, and Greece [2,4]. Nowadays, there is a demand for natural ingredients by consumers, mainly for cosmetic products [5].

Minerals used for cosmetic purposes are mainly natural clay minerals due to the high cost and difficulty in industrial production. Concerning topical use, they are applied either as cosmetics or dermatological protectors [2,3], acting as starting materials, adjuvants, or active ingredients, and as vehicles.

A cosmetic product is a preparation made for external topic application (skin, hair, lips, nails) or to be applied in the oral cavity (teeth and mucous membranes) aiming to clean, beautify, perfume, change its appearance, correct body odors, protect, and/or keep in good condition [6–9]. According to our point of view, cosmetics and dermocosmetics can act further ahead as previously described, also contributing to the health maintenance of the consumers/patients. A broad range of cosmetic products can be formulated with clay minerals to clean, moisturize, and treat/improve skin conditions, like gynoid lipodystrophy, dandruff, seborrheic dermatitis, and acne [8]. By improving such conditions, they act indirectly on consumers' health, life quality, and self-esteem.

There is an important advantage in using clays for cosmetic purposes as they are a low-cost, environmentally friendly, natural, and abundant component, which are easy to apply and remove, dry and harden fast, and present low toxicity risk when used in adequate conditions, respecting the specifications of official compendiums [8,10–14]. They also are used due to their high surface area, rheological properties, and excellent ion exchange capability [11,14,15].

Cosmetics with clays can be formulated in several forms, such as ointments, gels, creams (emulsions), and pastes. Those products must be formulated considering their adequate rheology profile (must be appropriate to maintain contact with application region) and sensory aspects (must be cosmetically acceptable), which are influenced by the clays' physicochemical properties (e.g., particle size) [16]. Due to their composition, uses, and properties, clays are relevant in cosmetology. In such products, clays can act either as active or as cosmetic ingredients, influencing stability, rheology, color, etc. As actives, they are widely used for skin cleansing, oil reduction/control (skin and scalp), substance adsorption, antiaging, ultraviolet (UV) radiation protection (sunscreens), and ion exchange with skin, in the function of color, qualitative and quantitative mineralogical composition, particle size, and shape, structure, and ion exchange capacity. They can also be used in makeup products [2,16–19].

Despite the importance of clays and clay minerals in the cosmetic industry, specialized literature about this theme is scarce. Therefore, in our review, we provided updated information concerning clays that are used in cosmetics/dermocosmetics, both as active and as starting materials, and gathered investigation findings to better understand future perspectives and deficiencies in this proficuous field.

2. Clays x Clay Minerals

The term "clay" does not have a consensus in the specialized/scientific literature. As such, it is difficult to define due to the variety of materials known as "clays" and for being used in several areas, such as chemistry, mineralogy, geology, etc. [20]. Therefore, clays are classified as natural inorganic rock or soil materials, composed of finely divided particles (inferior to 2 μm) with some plasticity while mixed with water and which hardens after drying. They are formed by clay minerals, organic matter, salt impurities, feldspars, and other minerals, like quartz, dolomite, calcite, iron, and/or aluminum oxides [5,6,13,15,18,20–22]. It is applied to all small-sized particles found in soils or sediments including phyllosilicates, quartz, feldspars, carbonates, sulphates, iron and aluminum oxides, humus, and other mineral and/or organic components [6,13]. Clays can be formed by one clay mineral, or (more commonly) a mixture of clay minerals with the prevalence of one. Those clay minerals give clays several properties that allow their use in many industries [13].

2.1. Types of Clay

Clays for cosmetic use are produced in many colors and the color difference is caused by the proportion of the minerals in it. As a raw material for cosmetic purposes, they are commercialized based on their color [11,15,16,18,20,21]. The color, which is a reference for the purchaser, is mentioned during the marketing and is often used aligned to the clay's properties [1].

The presence and proportion of minerals in clays interfere with their color and formulation stability [21]. Their colors also result from the crystalline structure in a certain state, being affected by composition, oxidation of structural cations, ionic charge, and ion position. The amount of water can also affect the color aspect. Color can come from matter associated with clay [1]. According to Gubitosa and co-workers (2019), different clays can be found in nature depending on the presence of iron and its chemical state. For example, clays with bivalent iron present a green color, those with trivalent iron are red, and those that do not contain iron are white [5].

Rautureau and co-workers (2017) correlated color of clay minerals to the structural ions they contain. For example, white clays present Al^{3+} and Mg^{2+}; yellow clays present Fe^{3+}; red clays present Mn^{3+}, Fe^{3+}, Co^{3+}, and Ti^{4+}; and green clays present Fe^{2+}, Fe^{3+}, Cr^{3+}, and Ni^{2+} [1].

The clays of each color present different cosmetic attributes due to their composition, as shown in Table 1 [18,20,21]. However, literature correlating color to cosmetic use and concerning chemical/mineralogical compositions of clays are infrequent [11,15]. Matike and co-workers (2011) correlated clay colors with sunscreen ability and mentioned that most clay soils used as UV filters corresponded to hematite's and goethite's colors, which are iron oxides that are responsible for reddish and yellowish colors [16].

Table 1. Clay classification according to their color.

Color	Present Elements/ Composition [18,20]	Cosmetic Use
Yellow	Rich in silicon dioxide	Rejuvenation, skin purification, and hydration [18,20] Prevention of bacterial infection on skin, cleansing, sunscreen, and body beautification [16] Antiaging, remineralizing, illuminating, hydration, nourishing, cleansing, tonifying, eliminates residues [4] Tensor effect, activates microcirculation, and contributes to ionic balance [23]
Beige	Rich in silicon, aluminum, titanium; low in Fe and hydrated aluminum silicate content	Astringent, purifying (adsorbs oil), and moisturizing [20] Tissue protection, purification, astringent, hydrating, remineralizing, skin whitening, and oiliness absorption [18] Body beautification [16]
White	Hydrated aluminum silicate, aluminum, sulfur, iron, boron, potassium, and calcium [18]	Skin whitening, moisturizing, and helps in oil removal [20] Whitening, oiliness absorption, and hydration [18] Anti-acne, whitening, use in skincare preparations [15] Cleansing, sunscreen, and body beautification [16] Wrinkle smoothing, whitening [4]
Grey	Rich in silica	Antiedematous, anti-aging, measure reduction [20] Antiedematous [18] Sunscreen and body beautification [16]
Brown	Rich in silicon, aluminum, titanium; low iron content	Purifying, astringent, and moisturizing [18,20] Antiacne, antiaging, and anticellulite cosmetic products [18] Soothing and cleansing [15] Body beautification [16]
Black	Rich in aluminum and silicon; low iron content [18,20] Also contains titanium, aluminum and magnesium silicate, calcium and magnesium carbonate, silicon oxide, zinc, and sulfur [18]	Skin rejuvenation, whitening, and oil absorption [18,20] Cellulite and stretch marks improvement [18]
Pink	Rich in Fe_2O_3 and CuO [18,20] Hydrated aluminum silicate [18] Pink clay is a mixture of red and white clays in which composition can include quartz, smectite, illite, and kaolinite. Its color is normally related to the presence of iron as hematite—Fe_2O_3 [24]	Sensitive, delicate, dehydrated, tired skin, with soothing action [18,20] Rosacea, localized fat, cellulite, and tissue flaccidity [18] Skin nourishing, depurative, cleansing, decongestant, slightly tensor, revitalizing, exfoliating, toning effect, elasticity increase, skin shine, and smoothness improvement, relaxing, and antioxidant [4] Pink clay is normally softer and less adsorbent than green clay [24] Antioxidant and soothing effect on skin; commonly used on sensitive and dry face skin [24]
Green	Fe_2O_3 associated with calcium, magnesium, potassium, manganese, phosphorus, zinc, copper, aluminum, silicon, selenium, cobalt, and molybdenum	Astringent, invigorating, stimulating, drying, and bactericidal actions [20] Skin oiliness reduction, cleansing, body beautification [16] Oily skin improvement [21] Oily, acneic skin, and oily hair improvement [18,23] Absorbent, adsorbent, purifying, pores tightening, calming, softening, repair skin cells [15] Blood circulation improvement, toxin removal, draining, used for massage, exfoliating, emollient, oiliness control, acneic skin [4]
Red	Rich in Fe_2O_3 and CuO	Skin rejuvenation and measure reduction [18,20] Cleansing skin, sunscreen [23] Dry skin improvement [21] Blood circulation improvement, blood flow increase [4]

Wargala and coworkers (2021) reported that the application of clays in cosmetics is directly related to their composition. For example, clays rich in Si present hydration properties, mitigate skin inflammatory processes, and can be used to contribute to skin regeneration/protection; and clays rich in Al provide hydration, pigment dispersion, and adsorption of melanin. For clays containing Si, Al, Ca, Ti, Fe, and K, the authors mentioned antiseptic, antibacterial, and regenerative activities, as well as cell renewal action, circulation activation, and adsorption of impurities [25].

2.2. Structure and Composition

Clays are composed by solid, liquid, and gas substances—solid particles form a skeleton and the spaces between these particles are filled by gas and/or liquid [18]. Mineralogic composition, particle shape (laminar or fibrous), and particle granulometric distribution are the main factors that determine clay's physicochemical properties and properties of the final product obtained [18,21]. Therefore, it is important to know these characteristics when developing a cosmetic to choose formulation components and preparation technique, culminating in a stable formulation [21]. Other characteristics that differentiate clay types are ion exchange capability, the nature of the exchanged cations, specific area, dispersion viscosity, plasticity, among others [18].

Clays may be found in different types of soils due to their structure, colors, and metals that compose them, which contribute to their function. Clays used in cosmetics are formed by metal such as aluminum, iron, magnesium, and titanium, which contribute to their functions [20,21].

Clay minerals are the mineral constituents of clays, which are normally crystalline and formed by hydrated aluminum silicate [20]. They contain in their composition silicon, aluminum, water, and frequently iron, alkali metals, and alkaline earth metals [18]. According to Daneluz and co-workers (2020), in general, clay minerals contain Si, Al, Fe, Ti, Mg, Ca, K, Na, phyllosilicates, oxides, carbonates, kaolinite, chlorides, etc. According to the authors, these elements are important for cosmetology due to their effect on skin.Some examples include hematite (Fe_2O_3) which acts as pigment, opacifier, antiseptic, and stimulates cell renewal; rutile (TiO_2) which provides photoprotection; kaolinite which provides renewal of the skin, hydration, and soothing effect; and ZnO and MgO which are invigorating [19].

According to Balduíno (2016), the complexity and amount of different clay minerals in clays makes it difficult to classify them and the author considers that there are no equal clays—each one will differ in at least one property. This variability is due to varied geology formation conditions [20]. Crystalline clay minerals can be divided in two classes, which are composed by families, groups, and subgroups, as we can see in Table 2 [20,26].

Table 2. Classification of clay minerals [6,13,18,20,21,26].

Class	Family	Group	Subgroup
Silicates with lamellar structure (phyllosilicates)	1:1-type layers	Kaolinite-serpentine	Kaolinite, halloysite, nacrite, dickite, chrysotile, antigorite, lizardite
	2:1-type layers	Smectites	Dioctahedral: beidellite, montmorillonite, nontronite
		Vermiculites	----
		Talc	Illite, celadomite, fengita, fussite, muscovite
Silicates with fibrous structure	2:1-type layers	Palygorskite-sepiolite	Palygorskite (known as attapulgite), sepiolite

Note: The word 'bentonite' is employed for a plastic, colloidal, swelling clay consisting of a smectite mineral regardless from its origin [11].

According to Table 2, clay minerals can be divided into (a) 1:1-type layers or 1:1 structure, and (b) 2:1-type layers or 2:1 structure.

(a) 1:1-type layers or 1:1 structure is when one tetrahedral layer is bond to one octahedral layer [1,8,13,18,20].
(b) 2:1-type layers or 2:1 structure is when one octahedral layer is between two tetrahedral layers, forming a kind of "sandwich" [8,13,18,20]. These layers compose the unitary crystalline structure of the clay mineral [18].

There is also a specific third type of layer with intercalation of 2:1-type layer and an additional octahedral layer ("hydroxide layer"). This occurs in the chlorite family.

These structures are based on a perfect model. However, natural clay minerals present defects on their ions concerning nature and position, which influences their properties. Disturbed zones favor ion and molecule trapping from the external medium, thus influencing their properties [1].

The proportion of tetrahedral and octahedral layers can vary between clay minerals. The bond between those crystallin layers determines the different clay mineral structures and families they belong to (Table 2) [20].

Chemical bonds between atoms inside the layer are covalent and, therefore, strong. Adjacent layers are connected parallelly one above the other by Van der Waals bonds and, therefore, are considered weak. The space between layers is called interlayer space. This allows layers separation when submitted to excess of water or under mechanical force [20,21].

These two structure types behave differently when dispersed in polar solvents [6,9,13,27]:

- 1:1-type minerals do not swell in contact with polar solvents [6,13].
- 2:1-type minerals swell in contact with polar solvents, creating structured systems with interesting rheological properties [6]. They lead typically to gels with pseudoplastic behavior. After hydration, a tridimensional net is built leading to sharply higher viscosity. When tension is applied, most of the structure breaks as shear occurs [13].

According to Moraes and co-workers (2017), 1:1 and some 2:1 clay minerals (like talc, pyrophyllite, illite, palygorskite, and sepiolite) do not swell in polar solvents; chlorites swell occasionally; and smectites and vermiculite do swell. Smectites can easily swell, thus forming a clay-gel with pseudoplastic behavior [27].

Tetrahedral layers are formed by an atom of silicon in the center and four atoms of oxygen in the vertices (SiO_4). Silicon atoms (Si^{+4}) may be replaced by aluminum (Al^{+3}) and occasionally by iron (Fe^{+3}), causing negative charges on the faces [18,20,21].

Octahedral layers are formed by six hydroxyl groups or oxygen atoms in the vertices of an octahedron, and an atom of either aluminum (Al^{+3}), magnesium (Mg^{+2}), or iron (Fe^{+2}) in the center [20,21]. The substitution of aluminum by magnesium or iron also causes charge deficiency and the particle's surface becomes negatively charged. This is compensated by the adsorption of interlamellar cations like Na^+ in the layers' faces [21].

Therefore, most clay minerals are negatively charged on the faces (as mentioned above) and have a pH-dependent charge on the edges (positive in acid or neutral solutions and may become negative with pH increase) [8,21]. The fact that clay minerals are charged is one of the main reasons for their cation exchange capacity, which is one of the aspects responsible for their use in cosmetics [8]. Based on charge, clay minerals can also be classified as cationic (most abundant) and anionic clay minerals (uncommon) [8].

High repellent potential in the layers' surfaces contributes to increasing the space among them, causing water penetration in the interlayer space. Therefore, some clay minerals (mainly smectites) have an expansive structure where all layer surfaces are open for hydration [21].

Several characterization techniques may be used to identify clay components, behavior, and structure. As examples, we can mention X-ray diffraction, X-ray fluorescence, scanning electronic microscopy, thermal analysis, and Fourier transform infrared spectroscopy. These techniques allow verification that most clay minerals are hydrated aluminum silicates, which present a defined crystalline structure and may contain non-clay materials, organic and inorganic substances, adsorbed cations, organic matters, and soluble salts. These components interfere with the mineralogical composition and properties of each clay [18].

3. Demands for Cosmetic Use

Among 4500 minerals known today, and only around 30 are used in the pharmaceutical and cosmetic industries (including kaolin, talc, smectites, and fibrous clays) due to safety requirements they must fulfill. According to the Cosmetic Ingredient Review (CIR) (2023), kaolin has the most reported uses in cosmetics, followed by bentonite [28]. Clays must be submitted to a series of purification treatments and characterization tests to meet strict pharmacopeial specifications before cosmetic use [29]. Clays, as actives and as starting materials, must [4,6,8,12–15,18,20,27,30]:

- Fulfill chemical requirements—stability, purity, and chemical inertia.
- Fulfill physical requirements—texture, water content, particle size (must present fine granulometry), and be pH compatible with the region of application.
- Fulfill toxicological requirements—zero or extremely low toxicity, safe, and microbiological purity. The high absorption capacity of clays may cause them to accumulate potentially toxic trace elements, which must be verified. Clays must be submitted to the decontamination process assuring microbiological safety before incorporating into cosmetics/dermocosmetics.

Concerning chemical and physical properties, they should have:

(a) High surface area (which contributes to adequate rheology).
(b) High absorption and adsorption capacity.
(c) High cationic exchange capability.
(d) Favorable colloidal dimension.
(e) High refraction index and heat retention.
(f) Low hardness (must be soft to apply on skin).
(g) Astringency.
(h) Low toxicity.
(i) Chemical inertia.
(j) Pleasant or neutral colors.

Therefore, the most used clay minerals in this segment are smectites (montmorillonite, saponite, and hectorite), fibrous clay minerals (palygorskite and sepiolite), kaolinites, and talc [4,5,12,20]. Clay minerals' high adsorption capacity allows them to adsorb toxins, impurities, oiliness, secretion, bacteria, and viruses. The high cationic exchange capability may offer vital chemical elements to the organism that are present in minerals, such as sulfur, phosphorus, sodium, potassium, magnesium, copper, iron, zinc, and manganese [4] The clay's cation exchange capacity, together with other formulation characteristics, may interfere with the percutaneous depth that ions may reach in the cutaneous tissue and, even, the absorption. This has a direct impact on clay's safety and efficacy [14].

Natural deposits of those clay minerals are rarely pure and may present chemical composition variations. They are composed of two or more clay minerals mixed with variable amounts of non-clay materials (for example quartz, feldspars, carbonates, oxides, amorphous materials, and organic matter). Thus, before use, clay raw materials are treated to increase and achieve quality patterns. The physical and chemical treatments to which clays are submitted may include desiccation, pulverization, bleaching, magnetic separation, size fractionation, chemical modification, and drying, among others. In some cases, it is necessary to remove specific associated substances that exceed pharmacopoeia requirements or modify appropriate properties (e.g., quartz, heavy metals, dolomite) [8,9,12,27]. To exemplify clay purity requirements, "food-grade bentonite should contain no more than 5 mg/kg arsenic, no more than 15 mg/kg lead, and no more than 1000 colony-forming units (CFU)/g aerobic microbes. Bentonite should be negative for Escherichia coli in 25 g"; and "food-grade kaolin should contain no more than 3 mg/kg arsenic and no more than 10 mg/kg lead" [28]. International regulations must be considered before selecting clays to be used in cosmetics aiming to fulfill safety requirements. Therefore, selecting suppliers is of utmost importance. According to Bastos and Rocha (2022), current studies evidenced the need to establish quality criteria and certification for clay-based products for topical/cosmetic use and to adopt methodologies for clay decontamination before incorporating them into formulations, to achieve the required limits assuring safety [14].

According to Silva (2011), the most important clay properties for their usage choice are mineralogical composition, particle shape and granulometric distribution, plasticity, mechanical resistance, linear drying retraction, compaction, thixotropy, reactive surface (absorption, ionic exchange, swelling), low toxicity, as well as therapeutic and viscosity dispersion [18]. Also, they must be easy to apply and remove, and dry quickly in contact with skin [18]. In addition to the several positive properties of clays to be considered for the cosmetic industry, it is not trivial to obtain a clay ingredient, regarding the relevant characteristics. Commercially available clays for cosmetic use must include the following information: substance identification (mineralogical and chemical composition), hazard identification, handling and storage, stability, reactivity, toxicology, and physicochemical properties [15].

Despite the abundance of clay minerals in nature, some have been synthetized to allow obtaining purer raw materials with a homogeneous structure and composition, as well as lower contaminations, thus meeting industrial requirements. Also, they can be enriched with mineral elements, such as Zn, Co, and Ni [8]. In addition, clay minerals can be modified to improve performance and expand their applications. Modifications can be chemical alterations to promote surface reactivity (homoionic clays), interactions with organic substances to improve hydrophobicity (organoclays) and incorporation into polymers to create clay–polymer composites [8,9].

Natural deposits of clays used for cosmetics are rarely pure and may vary in chemical composition. Therefore, clays used as raw materials for cosmetics benefit from improvement techniques to eliminate accessory minerals, enhance physicochemical properties, and increase quality by physical and chemical treatments such as magnetic separation, flotation, drying, calcination, bleaching, size fractionation, among others [12].

Toxicological and Safety Aspects of Clays and Clay Minerals

Clays must present a zero to low toxicity profile for further use in cosmetics/dermocosmetics, as well as being submitted to purification processes. Due to their high adsorbing capacity, they may accumulate toxic substances, heavy metals (such as Sb, As, Cd, Pb, Ni, and Tl), and micro-organisms, which must be well-verified before use. Also, the presence of organic matter is commonly associated with pathogenic microorganisms in clays; therefore, as a rule, before using clays in cosmetic products, they undergo fine processing to reduce potentially toxic elements and pathogens—for example: refining, beneficiation, and sterilization/decontamination [25,31].

Clays' and clay minerals' toxicology can be assessed by in vitro and in vivo assays. However, in vitro assays are mainly for screening purposes and are useful to obtain mechanism-derived information [32]. Toxicologic assays' results may vary according to the type of clay, route of administration, dose, experimental times, etc.

Maisanaba and co-workers (2015) studied/reviewed toxicological aspects of clay minerals and derived nanocomposites frequently used in food packaging (mainly kaolinite, montmorillonite, and sepiolite), and concluded that toxicological evaluation is needed when taking into account that clays have distinct toxicological profiles and their modification can alter it. Toxicity results in the literature vary thoroughly, with most in vitro toxicity showing cell death and toxic effects (oxidative stress, etc.), although in vivo studies in humans and animals demonstrated lower toxicity [32].

The dose is an important issue regarding the safety of cosmetic ingredients. Amongst clays intended for cosmetic use, kaolin presents the highest maximum concentration usage in leave-in formulations (up to 53.2% for manicuring preparations, 16% in leave-on dermal formulations), followed by bentonite (8% for face and neck formulations) [28]. Also, some clay ingredients have been reported to safely be used in formulations that may be incidentally ingested, like kaolin in lipsticks (up to 14.5%); and formulations that may get in contact with the eyes, like kaolin in eye shadows (up to 8.5%). As far as cosmetic regulations in the European Union regarding clays is concerned, there are no restrictions on attapulgite, clay, fuller's Earth, hectorite, illite, or montmorillonite. Bentonite and kaolin are listed in Annex IV (allowed colorants) as they contain calcium, magnesium, or iron carbonate, ferric hydroxide, quartz-sand, and mica, among others as impurities [28]. Clays have been submitted to bioavailability toxicokinetic studies using human skin as a model membrane and diffusion cells to assess (trans)cutaneous permeation of heavy metals (such as lead, arsenic, chromium, and aluminum) after application of three clay pastes (white montmorillonite, kaolin, and clay). Diffusion cells were incubated for 24 h, and diffusion and storage liquids were analyzed for metal content. No detectable quantity of heavy metals was found, leading to the conclusion that traces of heavy metal in the clay pastes did not penetrate the skin [28]. An acute inhalation assessment was performed following OECD TG 403, in which rats were exposed to 3.856 mg/L air of clay composed of illite (75%), kaolin (19%), and montmorillonite (6%) versus a control group (air). Both groups were exposed for 4 h and observed for 14 days. As a result, neither mortality nor clinical signs of toxicity were observed. Also, dermal sensitization was not reported in human repeated insult patch tests (HRIPTs) with a foot mask containing 3.5% bentonite, a clay mask containing 3.8% bentonite (108 subjects), a face cream containing 7.5% bentonite, a lip product containing 14.5% kaolin, and a clay mask containing 40% kaolin. However, one subject in a study of a clay mask containing 14.5% kaolin involving 103 subjects had moderate erythema and edema with papules through the induction and challenge phase. From this, it is considered that kaolin, attapulgite, bentonite, clay, fuller's Earth, hectorite, illite, and montmorillonite are safe to be used in cosmetics in the mentioned concentrations [28].

According to Gomes and co-workers (2021), topically applied clays may cause exposure to toxicity due to persistent skin adsorption of potentially toxic elements and compounds. Although rare, clays may harm human health when the particles are persistently inhaled (respiratory diseases, lung cancer, mesothelioma, or pneumoconiosis); persistently ingested, for example, in geophagy (which should not be the case for cosmetics); or dermally adsorbed (podoconiosis). The conditions' severity depends on the dose and the exposure duration. This justifies the importance of the sanitary safety of clays selected to be used in topical samples [25,31].

Heavy metals, such as Sb, As, Cd, Pb, Ni, and Tl, have been banned by the European Commission and restricted by the FDA with strict limits for cosmetics. Metals in cosmetics can accumulate on the skin and cause allergic reactions and internal organ damage (Hg and Pb)—topical and systemic side effects [25].

Another toxicological aspect that should be considered is related to the talc. Carcinogenic fibrous materials (asbestos) could be detectable in cosmetic-grade talc, which is used as a protective, abrasive, absorbent, anticaking, filler, opacifier, lubricating, and refreshing ingredient. Also, talc should not be applied on the skin when its barrier is not intact. Cosmetic talc's purity is required to be at least 90% [25,31]. According to Wargala and co-workers (2021), the toxicity of the minerals is mostly related to the existence of asbestos or quartz from mining procedures [25].

4. Important Considerations When Formulating Clay-Containing Cosmetics

When formulating cosmetics with clays, parameters such as particle size and shape/morphology, temperature, pH, agitation time, and speed, as well as other ingredients influence product stability and clays' dispersion in the medium [9,21]. The formulation's pH is also important, concerning the effect and product safety on the skin—ideally, it should be compatible with the skin's value (slightly acid) in cleansing and beautification cosmetics, for example [16].

When preparing a face mask, for example, clay hydration (dispersion in water) is a critical step. It involves incorporation, powder humectation, and fragmentation of agglomerates. This step is affected by temperature, pH value, and agitation parameters (speed and time), as well as product composition. The stability of this dispersion may be affected by interactions between clay particles and between those particles with the liquid. The use of humectants (propylene glycol and glycerin, for instance), for example, helps retain water in the formulation and avoids its dehydration, thus enhancing product stability and improving product use [21].

When laminar clays are dispersed in polar solvents, a rigid network is formed by face–face and face–edge interactions. Also, laminar silicate gels are sensitive to electrolytes, which may influence the formed structure. As for fibrous clays, they form a 3D structure in water composed of interconnecting fibers, and they retain their stability in the presence of electrolytes in high concentrations [6,27].

Another important aspect is their swelling property. As previously mentioned, high repellent potential in layers' surfaces contributes to increasing the space between them, causing water penetration in the interlayer space. Therefore, when they are dispersed in the aqueous medium, the solvent is trapped between those layers by solvation. Swelling involves the separation of those layers until reaching balance [21]. However, some clay gels may contract upon standing, expelling interstitial liquid (syneresis) [9].

Swelling is a required property to achieve high-viscosity systems. Smectite gels can swell by absorbing liquids and increasing volume, and the resulting material presents thixotropic behavior. Bentonite can swell to approximately 12-times its volume except in the presence of organic solvents [9,27]. Clay's swelling degree can be influenced by several factors, such as present clay mineral types (expansive or non-expansive), the addition of electrolytes (may increase interaction between particles), and the presence of other hydrophilic substances that will compete with clay for water [21].

The formation of gel clays is influenced by the type of mineral and preparation technique. For example, the simple addition of water to bentonite does not lead to gel formation. To jellify it, bentonite must be sprinkled on hot water and the dispersion must rest for 24 h with occasional stirring after the clay has become wetted. Bentonite may also be dispersed in water after being triturated with glycerin or being previously mixed with a powder, like ZnO. To achieve dispersion in cold water, high-speed mixing equipment is required to enable swelling [9]. Another relevant factor to consider is that formulated products should have adequate consistency to be suitable for cosmetic use. Viscosity must allow product application, and it must allow the product to remain in contact with the application area at least until achieving the desired effect. As far as concentration is concerned, the amount of clay applied to the formulation may vary from a small proportion until almost the total final mass [9].

5. Properties in Cosmetic Products for Skincare and Haircare

5.1. Minerals

There are several minerals used as active ingredients in cosmetic products (Table 3). Their activity depends on their physical and physicochemical properties, as well as their chemical composition [30]. Minerals with a high refraction index and good light scattering properties, like oxides, can be used in photoprotective formulations. Those with high sorption capacity and large surface areas may be used in powders and emulsions. The ones with proper hardness can be used as abrasives in toothpastes [8,30]. Those with antiseptic properties (for example, borax, zincite, and goslarite, among others) are highly astringent, and astringency is controlled by their concentration. They may be incorporated in liquid (lotions) and solid formulations (powder) and can be used in deodorants/antiperspirants. They are toxic in high concentrations, so one should avoid continuous application on extensive skin areas or application on damaged skin [30].

Table 3. Minerals used as actives in cosmetic formulations.

Group	Mineral	Cosmetic Use	Other Relevant Characteristics
Oxides	Rutile (TiO_2)	Physical UV filter, protection against visible light, dermatological protector	High refraction index
	Zincite (ZnO)	Physical solar filter, UV filter, protection against visible light, dermatological protector, antiseptic	High refraction index
Carbonates	Calcite ($CaCO_3$)	Abrasive and polishing agent in toothpastes	Non-toxic, proper hardness to be used in toothpastes
	Hydrozincite ($Zn_5(CO_3)_2(OH)_6$) and smithsonite ($ZnCO_3$)	Dermatological protector	High sorption capacity
Sulphates	Epsomite ($MgSO_4.7H_2O$) and mirabilite ($Na_2SO_4.10H_2O$)	Bathroom salt	High water-solubility
	Chalcanthite ($CuSO_4.5H_2O$), zincosite ($ZnSO_4$), and goslarite ($ZnSO_4.7H_2O$)	Antiseptic	High astringent capacity
	Alum ($KAl(SO_4)_2{\cdot}12H_2O$)	Antiseptic and deodorant	High astringent capacity
Chlorides	Halite (NaCl) and sylvite (KCl)	Bathroom salt	High water-solubility
Phyllosilicates	Smectites (montmorillonite, saponite, hectorite), and talc	Dermatological protector, cosmetic creams, powders, and emulsions, makeup products	Opacity and high sorption capacity
	Kaolinite	Dermatological protector, cosmetic creams, powders, and emulsions, face masks, makeup products, anti-inflammatories	Opacity and high sorption capacity, heat retention capacity
	Palygorskite, sepiolite, and mica (muscovite)	Cosmetic creams, powders, and emulsions	Opacity and high sorption capacity
Others	Sulphur (S)	Antiseptic, keratolytic reducer	High astringent capacity
	Greenockite (CdS)	Keratolytic reducer	Reacts with cysteine in keratinocytes
	Borax ($Na_2B_4O_7.10H_2O$)	Antiseptic	High astringent capacity
	Niter (KNO_3)	Desensitizing agent in toothpastes	Non-toxic, high water-solubility

Adapted from Carretero and Pozo (2010) [30]; Moraes et al. (2017) [27].

Minerals can be used as antibacterial agents depending on high sorption properties, large surface area, mineral content (release minerals that are toxic to bacteria), pH and oxidation state, and structure. The structure must ease the sorption of nutrients and/or disrupt bacterial envelope and/or impair bacterial metabolites' efflux. In general, the antibacterial effects from minerals come from the exchange of their soluble constituents that are toxic to bacteria [8].

Although kaolinite has a low cation exchange capacity and relatively small surface area compared to other groups, it can still absorb small substances such as proteins, bacteria, and viruses, justifying its use in cosmetics [27].

Minerals with high sorption capacity (kaolinite, talc, smectites) can be applied in dermatological protectors, which are solid or semisolid compositions that protect skin against external agents, exudations, and liquid excretions. Those minerals adhere to the skin, forming a film that provides mechanical protection against external agents, as well as taking up skin exudations. They also produce a water-poor medium unfavorable to bacterial growth and sorb bacteria, viruses, grease, and toxins, thus presenting some antiseptic activity [2,3,13,30]. Also, minerals used in deodorant formulations can eliminate gases responsible for bad odor [6]. The mentioned properties could also allow those minerals to be used in antipollution cosmetics, which is an increasingly used and desired claim in the market in skin and haircare products. In that case, minerals could be applied on skin to protect it against pollutants from the environment, which favor the skin aging process. This is a field that should be explored in future research.

Minerals with high refraction index and that also scatter light (rutile and zincite) are suitable as UV filters in photoprotective formulations. Their effectiveness as filters also depends on their stability against degradation by UV radiation. Natural rutile is not used; rutile's synthetic analogous (synthetic TiO_2) is used instead. Synthetic TiO_2 is a white powder with a high refraction index that reflects UV radiation and presents good photostability. Its light-scattering property depends on particle size—bigger particles (around 230 nm) scatter visible light, while smaller particles (around 60 nm) scatter UV rays and reflect visible light [8,30]. As synthetic TiO_2 may give a white appearance on the skin due to bigger particle distribution, currently it has been used in very small particle sizes to avoid this undesirable effect. A size of 50 nm is considered an optimum particle size to provide good photoprotection without being white on the skin [30].

Iron oxides are commonly used in cosmetics depending on their color. Among them we can mention Fe_2O_3, which is the most used iron oxide in cosmetics. According to Wawrzynczak and co-workers (2016), iron pigments are considered stable and safe, mainly when synthesized. The authors mentioned that synthetic production allows the elimination of impurities normally found in natural minerals [33].

Regarding toothpastes, minerals can be used as desensitizing agents for sensitive teeth (niter), or as abrasive/polishing agents (calcite). Niter releases K^+ ions when dissolved in contact with saliva, which act on nerve endings in the dentine to inhibit pain sensation [30]. Also, hydroxyapatite mineral particles in micro- or nanocrystalline forms can be added to kinds of toothpaste, where, through in vitro and in situ studies, they were deposited on and restored demineralized enamel surfaces. Hydroxyapatite toothpastes can remineralize enamel lesions, reduce/prevent demineralization, and present a caries reduction/prevention effect, without the risk of fluorosis [34,35].

Clay minerals with opacity and high sorption capacity (for example, palygorskite, sepiolite, kaolinite, smectites, and talc) are applied to cosmetic compositions (solid and semisolid) as opacifiers, mattifiers, and for imperfection coverage. They also form a protective film on the skin, adsorb excessive oiliness and toxins, and increase adherence to the preparation [6,13,30]. Talc is widely used in a diversity of applications in cosmetic products. It is odorless and can be micronized to an ideal particle size, becoming a white powder. As it absorbs grease, it can be incorporated into makeup face products as a mattifier and/or oil control component [27]. Talc is also widely used in children's cosmetics for its sorption and fluidity properties to absorb humidity and sweat in the diaper's zone. It cleans, deodorizes, lubricates skin surfaces, and acts as an antiseptic. It also keeps skin folds lubricated, avoiding friction [6,13]. Micas are used in makeup cosmetics, like lipsticks and eyeshadows due to their high reflectance and iridescence. They are also applied to moisturizers to provide a luminous effect on skin [13,27,30].

5.2. *Clays*

Clay use depends on the type of clay mineral (mineralogical composition), type of layer (clay structure), and chemical composition. Also, differences in texture may affect rheological properties and adsorption capacity, even among identically structured clays [6,15]. Application temperature also affects their use. When using clay minerals as facial masks, they can be applied directly on the skin at room temperature. However, when used to treat acne, it is advisable to apply at higher temperatures, as the heat increases perspiration and opens pilosebaceous orifices, thus favoring efficacy [11]. On inflamed areas, the application temperature should be lower than body temperatures, so that the mixture of water and clays will cool the inflamed treated area, acting as an anti-inflammatory agent [2].

Clays' properties related to cosmetic applications, in general, are related to surface (surface area, charges, cation exchange capacity, etc.), rheological (thixotropy, viscosity, etc.), physical (color, particle size and shape, opacity, reflectance), mechanical [8,16,36], and functional properties. Clays used in cosmetics have functional properties, such as the adsorption of skin secretions, dirt, oiliness, sweat, toxins, bacteria, and viruses (which comes from their high cation exchange capacity); rejuvenating; skin cleansing; slight physical exfoliation; carrying of active substances; antiseptic; and regenerative; astringent; lifting effect; whitening; moisturizing; and can contribute to the improvement of inflammatory processes of boils, and acnes [2–4,8,10,16,18,20,21,37].

As examples of cosmetic products that may contain clays, we can mention exfoliants, masks, sunscreens, soaps, shampoos, toothpastes, deodorants, makeups (foundations, eye shadows, lipsticks, etc.), and facial skincare products, among others [4,5,36]. According to Velasco and co-workers (2016), "clays are mostly used in face masks due to their high absorbency levels on skin surface, such as greases, toxins and even bacteria and viruses". They are also used for cleansing and lifting effects. Still, there are few studies concerning the impact of clay masks on skin biomechanical properties [11]. Face masks can contain more than 25% of solid phase dispersed in liquid and are applied on the skin during 10 to 25 min in a layer of 1 to 2 mm thick. After water evaporation, the mask hardens and contracts, causing mechanical tension, slight physical exfoliation, and astringency [13,21].

Another important characteristic that allows clays to be used in cosmetics is their detergent property. Some clays behave like detergents when wet with water, as well as remove impurities, what makes them an excellent choice for products like soaps and shampoos [5]. This property also enables their use as emulgents or emulsifiers [36].

Topical formulations using clays as active components, like facial masks, applied to skin during a certain period, trigger a flow that transports metabolic products, cell particles, and bacterial toxins out of the skin to adhere to the clay. In addition, clay particles absorb excess of sebum, impurities and skin exudates, cleanse pores, and improve blood flow, thus enhancing oxygen and nutrients' supply to skin [2,6,38].

The absorption capacity of cutaneous exudates may be related to particles' porosity—porous particles from minerals with a large surface area can adhere to the skin, forming a film with mechanical protection and oil retention properties [22]. Meier and co-workers (2012) studied the efficacy of facial masks with clay and jojoba oil against mild acne. The results showed that the proposed treatment reduced acne lesions, such as papules, pustules, and comedones. For the study, participants used the product 2–3 times a week for 6 weeks with 15–20 min contact per application and further removal. Lesions were counted before and after treatment for comparison [38].

As clays have high absorption power, they can adhere to the skin and form a pellicle that protects against external chemical and physical agents [5,10]. This property is important for the retention of skin oiliness, contributing to the skin regenerating potential [22]. Due to their capacity to eliminate excessive oiliness and toxins from the skin, they are considered effective against several dermatological conditions, such as acne [2,37].

As they are rich in sulphidric acid, they present bactericide, and fungicide properties [4]. Lately, clays have received special attention concerning their potent antimicrobial properties. Studies demonstrated the in vitro broad-spectrum antibacterial activity of an iron-rich clay that was applied to treat Buruli ulcers. However, studies revealed that only a few deposits showed antibacterial properties [39]. In 2020, Gomes and co-workers published an overview of antibacterial clays. The authors reported that clays did not present bactericidal characteristics when in a dry state, i.e., bactericidal activity only occurs in hydrated clays. Also, not every clay presents such activity, and only some have this function and against certain types of bacteria. Clays containing illite and smectite, that bear ferrous iron in the structures, and clays bearing one or more ferrous iron-rich-associated phases (pyrite, marcasite, magnetite, pyrrhotite, and goethite) present antibacterial activity, since they can inhibit microorganisms' growth or disrupt cell membranes. In addition to iron, other metals/ions contribute to antibacterial function of clays, such as Ag, Cu, Zn, and Au [40].

Clays with high amounts of silicon mean they can be used for skin hydration and to contribute to skin renewal/regeneration [37]. Clays with high sorption capacity may be used in cosmetics as an opacifier, mattifier, and for skin imperfection coverage [5]. Massage practices with clays suspended in liquid vehicles explore their slight abrasive effect for physical exfoliation of the skin. This property is also used in shampoos and soaps. For toothpastes, clays can also be used as abrasives, as well as for their properties of impurity absorption [1].

They may also be used as physical filters in photoprotective formulations [10,16,17]. To be used as filters, they must have a high refraction index and optimal light dispersion properties [36]. This property is also affected by particle size. Small particle sizes allow better skin coverage, reducing the intensity of UV radiation reaching the skin [16].

In addition, they are capable of invigorating tissues, activating microcirculation, presenting lifting effect, softening skin, and reducing oiliness due to absorption properties [18].

As clays are rich in iron, silicon, magnesium, titanium, and potassium, they present antibacterial, antiseptic, and regenerative efficacy; contribute to cell renewal; absorb impurities; and activate microcirculation, therefore, are suitable to be used as active compounds for numerous cosmetic products. The importance of those minerals in cosmetology stems from their assumed effects on skin, e.g., iron is an antiseptic and catalyzes cell renewal; silicon helps to renew/regenerate and hydrate the skin; zinc and magnesium are invigorating; potassium acts on circulation and tissue invigoration; titanium is used as a UV filter [18,22]. Therefore, they have been successfully used in haircare and hair therapy through application protocols on the scalp in patients with seborrheic dermatitis, psoriasis, dandruff, and seborrhea [5,41]. In those cases, clays may be associated with essential oils for synergic effect to clean, nourish, and revitalize the scalp [23].

The application of clays on the scalp (for example, as hair treatment masks) allows the removal of dead cells (slight physical exfoliation); stimulates local cutaneous microcirculation (thus nourishing the scalp); eliminates impurities, dirt, excessive oiliness, and toxins by adsorption; and also acts as a seborregulator [23,41]. Damazio and Makino (2017) [23] published several hair therapy protocols with clays associated with essential oils to treat different scalp conditions, for example:

- Treatment for scalps affected with dandruff and seborrhea—after cleaning the scalp with a neutral shampoo, apply a hair mask on the scalp composed of 10 mL of the same shampoo, 3 drops of peppermint (*Mentha piperita*), 3 of lemon (*Citrus limon*), and 3 of petitgrain (*Citrus aurantium*) essential oils and 5 g of yellow clay thoroughly mixed; then, cover with plastic film and leave for 20 min. Rinse completely after that and apply hair conditioning [23].
- Protocol for chemically treated hair (bleached or straightened, for instance)—after cleaning the scalp with a neutral shampoo, apply a hair mask on the scalp composed of 10 mL of the same shampoo, 3 drops of lavender (*Lavandula angustifolia*), 3 of chamomile (*Chamomilla recutita*), and 3 of copaiba (*Copaifera langsdorffii*) essential oils and 5 g of white clay thoroughly mixed; then, cover with plastic film and leave for 20 min. Rinse completely after that and apply hair conditioning [23].

Clays incorporated in cosmetics protect the skin against external damaging agents, like UV radiation, acting as a physical barrier and increasing sun protection factor (SPF) [6]. This is improved by its high surface area, which allows effective skin coverage. Therefore, when applied to sunscreens, this property offers a great advantage. Still, the magnitude of UV protection depends on its mineralogical composition [10,37,42], as well as the type of the vehicle.

Studies showed that smectite and kaolinite clays incorporated into sunscreens were effective in reflecting/scattering/absorbing UV radiation between the wavelengths of 250 to 400 nm. This is probably related to their composition, as clays' UV protection capability was shown to depend on iron oxides' concentration among their components; the higher the amount of iron oxides (Fe_2O_3) in the minerals, the better the protection against UV rays [10,17].

Clays were also found to contain other physical protectors, such as titanium dioxide (TiO_2), zinc oxide (ZnO), and silicon oxide (SiO). The amount of these protectors in clay's composition may also present a positive effect on clay's photoprotective efficacy [10,17]. Also, clays' particle size was shown to influence their photoprotective efficacy [17]. On the other hand, clays containing higher iron concentrations present stronger colors, affecting the final product aspect [10].

Dusenkova and co-workers (2015) researched the use of Latvian illite clays in sunscreens. They proved them to be effective in improving protection due to high iron content, mentioning the advantage of clay's brown color which allows its use as a pigment in facial sunscreens [42]. Hoang-Minh and co-workers (2010) assessed the UV protection of several types of clays (some types of kaolins, bentonites, among others), discussing the influence of mineralogical parameters on photoprotective efficacy. According to the authors, clays have UV protection potential due to absorbing or reflecting radiation, which could be influenced by particle size and chemical composition. They measured UV transmission of cream samples containing clays using an Analytik Jena AG Specord 50 photometer. They found that samples had different levels of UV transmission, which varied across UVA and UVB spectral ranges. They concluded that the hematite (Fe_2O_3) content of clay minerals significantly affected the samples' UV protection behavior. They observed different patterns of relations between Fe_2O_3 content and photoprotection efficacy, comparing expandable and non-expandable clay minerals, which could be explained by their arrangement in the cream samples. Therefore, UV protection of clay minerals was found to be dependent on their hematite content and expandability [43].

Not only are clays used in cosmetics as actives but also as starting materials. In this case, they are added to formulations to improve stability and rheology, as thickening or suspending agents, and to carry active substances, allowing the development of formulations with active controlled liberation [1,13,18,21]. Their functionality depends highly on particle morphology and surface electric charge [1].

6. Clays Used as Formulation Starting Materials

Cosmetic starting materials are components incorporated into formulations to improve the physicochemical characteristics of the active substances and improve/allow the formulation process and application [8]. Clays in cosmetics can be used as raw materials for solid, liquid, and semisolid samples based on their properties. Among those properties, we can mention high adsorption and swelling, high cation exchange capacity, large surface area, water miscibility and hydration ability, dispersity, thixotropy, opacity, and color [8,19,44]. They can be used as lubricants, grease absorption agents, carriers, inert bases, protectors, heat release controllers, and so on [5].

Clay minerals dispersed in polar solvents tend to form gels with characteristics between solid and liquid, whose rheological behavior differs depending on the type of clay mineral used, concentration, and presence of other molecules/ions in the composition [9,13]. It can be dilatant (less frequent), pseudoplastic, or thixotropic. As they form a tridimensional structure that is easily deformed and rearranged, they are usually incorporated into semisolid cosmetic preparations, like dental gels or mascara. This is because those formulations need to be easily deformed as a liquid during application, and then restore to their initial shape (more solid) at rest [13,27].

They are often used to stabilize emulsions or suspensions, increase their viscosity, modify systems' rheological behavior, carry active substances, and are used as adsorbents or absorbents [5,19,36,44]. To improve the stability of suspensions, clays may be used as agents to delay sedimentation. The same is applied to emulsions [44], to avoid phase separation in a short period of time. Stabilization of those formulations occurs because of the gel-forming capacity of clay minerals and due to their presence on interface boundaries, which occurs because of their colloidal size, surface charges, and high surface areas [9].

In semisolid cosmetics, clay minerals may be used for two main reasons: to stabilize dispersed systems and to modify rheology. This is related to the presence of charge on their surface, their colloidal dimension, and their capacity to form different structures when dispersed in polar media [9,44]. Also, clay minerals are adsorbed and act as a physical barrier in the interface, thus preventing stability issues, like flocculation and coalescence, as well as acting as an emulsifier [13,21].

Clay minerals may also be used to formulate Pickering emulsions. In Pickering emulsions, colloidal solid particles (colloidal surfactants) act as stabilizing agents in the interface between two liquid phases. It is possible to formulate stable emulsions using only solid particles as emulsifiers, and some clay minerals may be used with this aim. The characteristics of the obtained Pickering emulsions will depend on the properties of the chosen solid particles [45–47].

Ashby and Binks (2000) studied emulsions stabilized by laponite (synthetic smectite clay with uniform particle size) [45]. Lu and co-workers (2014) prepared Pickering emulsions with fibrous palygorskite clay particles, which formed a three-dimensional network to stabilize the formulation [48]. Kpogbemabou and co-workers (2014) formulated oil-in-water Pickering emulsions stabilized using three different phyllosilicates—kaolin, halloysite, and palygorskite [49].

Clay minerals' fine texture and plasticity ease the application of makeup products and increase their durability over the skin. Their oil control property also improves makeup's water resistance without making the skin dry. As they provide excellent coverage, sorption, and adhesion, they have been used in facial treatments to hide imperfections and fine lines [8].

Phyllosilicates may have several functions in cosmetic formulations, such as: thickening or suspending agents, binders, anti-caking agent, emulgent, adherent, diluent, lubricant, and stabilizers in emulsions. They can also be used to facilitate the incorporation of hydrophobic actives in formulations, as they enable their dispersion [13]. In Table 4, we list different uses of clays as raw materials in cosmetics/dermocosmetics.

Table 4. Uses of clay as starting material/raw material in cosmetic formulations [3,6,9,13,19,26,44].

Clay Type	Use
Kaolinite	Emulsifying agent (creams and pastes), suspending and anticaking agent (in liquid formulations), thickening agent
Talc	Emulsifying agent (creams and pastes), suspending and anticaking agent (in liquid formulations)
	Secondary emulsifying agent in makeup products (as it remains in the interface between water and oil phases)
	Diluent and lubricant in powder formulations; can ease cosmetic powder compaction (e.g., face powders); diluent for pigments in makeup formulations
	Filler, absorbent, protection agent in formulations like creams and pastes
Bentonite * and purified bentonite	Emulsifying agent (creams, ointments, and gels), suspending and anticaking agent (in gels, emulsions, pastes, and suspensions), improve formula stabilization (due to surface electronic charges that promote repulsion between particles and avoid formation of aggregates)
	Rheological additive in toothpastes
	Emulsion stability additive
	Thickener in topical suspensions
	Thickener, suspending and thixotropy agent in liquid makeup products

Table 4. *Cont.*

Clay Type	Use
Magnesium aluminum silicate	Emulsifying agent (creams, ointments, and gels), suspending and anticaking agent (in gels, emulsions, pastes, and suspensions), improve formula stabilization (due to surface electronic charges that promote repulsion between particles and avoid formation of aggregates)
	Rheological additive, gelling agent. Can be applied to pigment suspensions
Magnesium trisilicate	Suspending and anticaking agent
	Gelling in non-polar organic solvents in antiperspirants, lotions, suntan products, nail lacquers, lip products
Smectites	Emulsifying agent, thickening agent, suspending, and anticaking agent
	Some smectites (e.g., mixture of montmorillonite and saponite) are used as thickening or gelling agents in cosmetic gels **
	Smectites can be mixed with pigments to dilute them—this mixture can be used in makeup products (10–25% pigments) or incorporated in emulsions (3–10% pigments)
Palygorskite	Emulsifying agent, thickening agent, suspending and anticaking agent in topical suspensions, pastes, creams, etc.
Vermiculite	Diluent and binder, emulsifying agent, thickening agent, anticaking agent, flavor corrector, carrier of active compounds
Hectorite	Thickener, suspending and thixotropy agent in lotions, shampoos, and liquid makeup products
Synthetic hectorite	Viscosity agent in toothpastes and shampoos
	Thixotropy in toothpastes, emulsions, and shampoos
	Suspending agent in liquid makeup products

* Used at 0.5–5.0% (*w*/*w*) as suspending agent. Its gelling properties are reduced by acids and increased by bases [44].
** Used at 1.0–2.0% (*w*/*v*) to slightly increase viscosity and 10.0% (*w*/*v*) for accentuated increase [13].

7. Clays Used as Delivery Systems

Components, including clay minerals, can be used in formulations to target active release [50]. Currently, clays have been explored as active ingredients/drug delivery systems. They can be used as vectors to transport substances to their targets in an organism, thus benefiting pharmaceutical and cosmetic industries [1,26]. They can interact with formulation components and affect bioavailability by influencing on actives' liberation and stability [3,6,13].

Clay minerals can be used as auxiliary components to maintain the active dose in the treated area due to viscosity increase, better skin adhesivity, and active concentration on the treated site [6]. Clay minerals can interact with organic molecules by different mechanisms, such as hydrophobic interactions (kaolinites, smectites, and others with neutral sites), hydrogen bonding (clays with oxygen surfaces), cation exchange (smectite, vermiculite, illite), etc. Based on these interactions and on their swelling properties, clay minerals are effective in delaying and targeting drug release, as well as in improving solubility. So, they can be used to increase active stability and alter drug delivery patterns, creating extended and/or site-specific delivery systems [50].

One example of clays used as a delivery system in cosmetics is in sunscreens. In those products, clays improve the stability of organic UV filters, as well as allowing the slowing of the filters' release, avoiding close contact with the skin, therefore, preventing cutaneous reactions and allergies, and improving water resistance [3,5]. Sepiolite and smectites can form complexes with organic UV absorbers, enabling their use in sunscreens. This allows the use of lower concentrations of active components [2], which contributes to improving formulation efficacy and safety.

Another example is the combination of vitamins (e.g., B and C) and antioxidants with clay minerals to deliver these actives to the skin for cosmetic purposes [8].

Halloysites have been used in nanotechnology due to their tubular morphology, which allows their use as drug carriers in the dermatological field. Their nanotubular structure can accommodate active molecules inside, enabling the controlled delivery of substances to the target area [27].

Clay nanotubes have also been used for haircare cosmetics, as shown by Panchal and co-workers (2018). The authors developed halloysite clay nanotubes formed by rolled sheets of aluminosilicate kaolin, a natural abundant biocompatible clay with low cost, which were loaded with dyes for hair coloring, allowing an efficient coloring procedure. The halloysite clay nanotubes were used as a coating on hair via physical adsorption and self-assembly not involving any additional chemical treatment. The same mechanism can be used to transport other actives in haircare formulations [51].

Clay nanoparticles are a promising alternative to nanomaterials for cosmetic use. Clay minerals, like kaolinite and halloysite, have been used for the encapsulation of essential oils, obtaining nanohybrids for several applications. An example for cosmetic use is a bio-based antimicrobial mosquito repellent composed of *Curcuma aromatica* and *Zanthoxylum limonella* essential oils onto montmorillonite clays dispersed in a methyl ester of the castor oil. Also, the mixture of clay and essential oils can be used as a flavor and fragrance nanodelivery system [52].

8. Clays and Clay Minerals Used in Spas and Aesthetic Medicine

Clay minerals, mainly smectites and kaolinite, are widely used in spas and aesthetic medicine in geotherapy, pelotherapy, and paramuds, even before the industry started incorporating them in cosmetic products [2,3,27]. Polymineralic clays are also used [3].

Geotherapy corresponds to clay minerals mixed with water and applied directly on the skin, as a layer. It is used mainly as a facial treatment against boils, acne, ulcers, seborrhea, blackheads, and spots, among others. In spas, when the treated area is extensive, it can be applied as mud baths, where the whole area is immersed in the mixture [2,3].

Pelotherapy consists of clay minerals mixed and matured with sea or salt-lake water, or minero-medicinal water resulting in a peloid [2]. Carretero (2020) recently wrote a review about clays in pelotherapy (should be read for further information). The article mentions a new definition of a peloid: "a peloid is a maturated mud or muddy suspension (more precisely, a muddy dispersion) with . . . cosmetic properties composed of a complex mixture of fine-grained materials (mineral and/or organic), with mineral water, seawater, salt-lake water, and commonly organic compounds from biological metabolic activity" [53,54]. The maturing process alters some clay minerals' properties, thus improving their efficacy; it cools more slowly, decreases grain size, increases plasticity, and improves absorption capacity. It is normally applied on the skin for 20–30 min in layers covered with an impermeable material to preserve the heat. This causes vasodilatation and perspiration, favoring its effect [2]. However, depending on the purpose, it can also be applied cold [3].

Paramuds consist of a mixture of paraffin with an inorganic material which is usually clay. They are applied hot for 20–30 min in layers covered with an impermeable material to preserve temperature. This therapy moisturizes skin and enhances penetration of other active substances, as pores are dilated, and superficial circulation is stimulated. All these actions are due to the provided heat [2,3,53]. The presence of paraffin allows paramuds not to adhere to the skin, so they can be conveniently removed after treatment. However, it has reduced efficacy since, unlike peloids, there is no exchange between paramuds and skin apart from heat exchange [53].

These approaches are used in spas for their properties of (1) high sorption capacity—to eliminate the excess of grease and toxins from the skin; (2) high cation exchange capacity—to enable the exchange of nutrients; (3) rheology—must be adequate to enable the formation of a consistent paste with good plastic properties for easy application and skin adhesion; (4) grain size—ideally must be small and soft for application to be pleasant; (5) cooling index/heat retention capacity—in some therapies they are applied hot to treat dermatological conditions; and (6) adequate pH—must be compatible with the skin to avoid irritation [2,3].

Among those properties, we can highlight high cation exchange capacity, which is presented mainly by peloids containing clays with smectites (minerals sensitive to ion exchange). This property enables ion exchange with minero-medicinal water, modifying the composition of the liquid part of the peloid, thus enhancing bioavailability of ions to the treated skin [53].

Geotherapy and pelotherapy can be applied as face masks (mainly in beauty treatment), cataplasms (applied on small areas), or mud baths (on extensive areas), depending on the treatment area. They can be applied hot (40–45 °C) or cold, depending on the aims. Paramuds are always applied hot (40–45 °C) and as cataplasms [3].

In beauty treatment, geotherapy, pelotherapy, and paramuds should be applied hot for the following reasons: (1) it allows moisturizing of the skin since perspiration cannot evaporate during therapy application due to the impermeable material, thus remaining retained on the skin; (2) it increases cutaneous absorption of active ingredients; (3) it stimulates local circulation and acts as an anti-inflammatory; and (4) it improves cutaneous cleaning and treatment of dermatological conditions (as acne, ulcers, and seborrhea) since it increases perspiration and sebaceous secretions, as well as opens pilosebaceous orifices, thus improving clay mineral's sorption properties and, therefore, efficacy [2,3,11].

9. Conclusions

A review of clays and clay minerals used in cosmetology was carried out, evidencing and discussing the numerous attributes of such substances, from ingredient processing and characterization to safety and efficacy establishment. They can be used in several types of cosmetics/dermocosmetics, both as active ingredients and/or as starting materials, due to their properties, like sorption, cation exchange capacity, physical exfoliation, and swelling, among others. In addition, they can be formulated in distinct types of cosmetic/pharmaceutical forms. However, most specialized literature justifies their efficacy based on theories, and only a few report proven efficacy using specific assays to suggest or demonstrate cosmetic attributes. Most papers that showed efficacy tests concerning clays used in photoprotection performed comparative in vitro spectrophotometric methods, which are not accepted for product registration, for instance. As far as haircare is concerned, scarce literature was found. Therefore, we believe our review, as organized with the discussed issues, could be considered in the coming years in the cosmetic field to provide information for the related industry.

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3. Aims

3.1. General aim

Considering the selected active compounds (1) red clay – selected for its antiaging properties; (2) green clay – selected by its astringent, stimulating, drying, bactericidal, and healing properties; and (3) black clay – selected by its rejuvenating, healing, and oil absorption properties, we aimed at developing clay face masks and challenging their *in vivo* efficacy regarding skin oiliness reduction and firmness.

3.2. Specific aims

The specific aims of this research were:

a) Characterization of the clays.
b) Preparation of samples containing the association of red, green, and black clays.
c) Determination of cosmetic attributes of the samples by *in vivo* methods in humans, involving objective methodologies (oiliness, firmness, and elasticity).

4. Material and Methods

4.1. Raw materials

The raw materials used for this research are listed below by INCI name, batch, and manufacturer / distributor.

a) INCI: Sodium benzoate – Batch: AUTO275546 (Mapric®)
b) INCI: kaolin – Green clay – Tersil® G – Batch: 00048/19 (Terramater®)
c) INCI: kaolin – Black clay – Tersil® CB – Batch: 1997 (Terramater®)
d) INCI: kaolin – Red clay – Tersil® CDR – Batch: 2097 (Terramater®)
e) Aristoflex AVC – INCI: Ammonium Acryloyldimethyltaurate/VP Copolymer – Batch: AUTO279778 (Mapric®)
f) INCI: Glycerin – Batch: PS-012153/F01 (PharmaSpecial®)
g) INCI: Propylene glycol – Batch: PS-012094/F01 (PharmaSpecial®)
h) INCI: Disodium EDTA – Batch: AUTO279693 (Mapric®)
i) FocusGuard PE MIT – INCI: Phenoxyethanol (and) Methylisothiazolinone – Batch: 220630 (Alianza Magistral®)
j) Belsil® OW 2100 – INCI: PEG-12 Dimethicone – Batch: UC03329 (Alianza Magistral®)
k) DUB B1215 – INCI: C12-15 Alkylbenzoate – Batch: 18100546 (Alianza Magistral®)
l) INCI: Citric acid – Batch: 36884 (Purifarma®)

4.2. Packing material

a) Clear Glass Wide Mouth Jars with 60 mL capacity and metal caps

4.3. Equipment, Instruments and Accessories

a) Analytical balance – Model: AUX220 – Shimadzu®
b) Semi-analytical balance – Model: ARD110 – Ohaus®
c) Suntest CPS+ Solar Light®

d) pHmeter Digimed DM-22 with glass probe
e) Heating plate IKA® C-MAG HS 7
f) Reverse osmosis – Model: Osmose 10 LX - Gehaka®
g) Scanning Electron Microscope (SEM) – JSM-6460LV with Energy Dispersive Spectroscopy system (EDS) – Jeol®
h) Sputtering – Denton Vacuum Desk II
i) Multi Probe Adapter (MPA) 580 – Courage & Khazaka® – with Corneometer, Sebumeter and Cutometer probes
j) X-ray diffractometer Bruker D8 Da Vinci
k) X-ray fluorescence (FRX) analyzer Epsilon 4
l) Rheometer model Haake Mars II with Rheoscope module (Haake, Germany)
m) Thermo-hygrometer Instrutherm® model HT-750
n) Analytical balance Marte AUW220D (Shimadzu Corporation®, Japan)
o) Particle size analyzer model CILAS 1090 (Quantachrome Instruments®, United States)
p) Ultrapycnometer 1000 (Quantachrome Instruments®, United States)
q) Differential Scanning Calorimeter DSC 7020 (Seiko Instruments, Tokyo, Japan)
r) Thermogravimetry/Differential Thermal Analyzer Exstar TG/DTA 7200 (Seiko Instruments, Tokyo, Japan)

5. Methods

5.1. Characterization of the clays

5.1.1. Scanning Electron Microscopy (SEM)

The clays were submitted to Scanning Electron Microscopy analysis using JSM-6460LV Scanning Electron Microscope with Energy Dispersive Spectroscopy system (Jeol®), to characterize their surfaces.

Therefore, the samples were transferred to a carbon double sided tape placed on the SEM sample holder (**Figure 1**). The samples were coded as follows[1]: AP = black clay; AVT = green clay; AV = red clay.

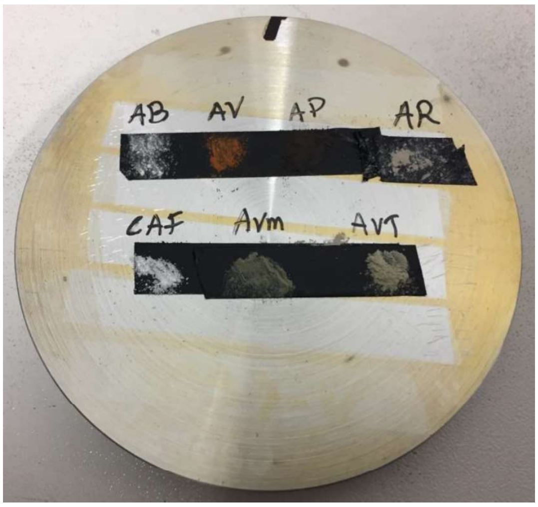


Figure 1. SEM sample holder with clays

The sample holder containing the samples was then submitted to the sputtering procedure to cover samples with gold to improve electron conductivity.

Afterwards, the sample holder was inserted on the SEM equipment for surface morphology analysis.

5.1.2. pH measurement

The pH (hydrogen potential) values of each clay were obtained in replicates of three using a calibrated pHmeter equipment. Therefore, clays were previously dispersed in distilled water in a proportion of 1:9 immediately before measurements in accordance with current Brazilian Pharmacopoeia (AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA (ANVISA), 2019).

[1] Codes AB, CAF, AR, and AVM are irrelevant to this research work and were used elsewhere.

5.1.3. Thermal Analysis

The thermal properties of clay samples were determined by Differential Scanning Calorimetry (DSC) and Thermogravimetry (TG) assessments at DEINFAR Laboratory ("Laboratório de Desenvolvimento e Inovação Farmacotécnica").

5.1.3.1. Differential Scanning Calorimetry (DSC)

The DSC analysis was performed with the equipment Differential Scanning Calorimeter DSC 7020 (Seiko Instruments, Tokyo, Japan), using a heating ramp of 30 to 450°C, and a heating rate of 10°C/min. Therefore, clay samples were placed in hermetic aluminum pans, using masses between 3 and 6 mg. As inert atmosphere, nitrogen was employed at a 50 mL/min flow. The equipment was previously calibrated using metallic indium standard (ΔHfusion = 28.57 J/g and Tonset melting point = 156.6°C) (ZAGUE, 2007).

5.1.3.2. Thermogravimetry (TG)

The thermogravimetry (TG) was performed with the equipment Thermogravimetry/Differential Thermal Analyzer Exstar TG/DTA 7200 (Seiko Instruments, Tokyo, Japan), using a heating ramp of 30 to 600°C, and a heating rate of 10°C/min. Sample mass for each analysis lied between 3 and 6.5 mg (according to the material's characteristic), conditioned in hermetic alumina pan. As inert atmosphere, nitrogen was employed at a 100 mL/min flow. The equipment was previously calibrated and verified concerning mass loss using calcium oxalate standard.

5.1.4. Real density assessment

For the real density assessment, clay samples were transferred to the equipment's sample holder with nominal volume of 10.8 cm^3 until three quarters of it was fulfilled. The holder containing each sample was weighted in analytical balance (Marte AUW220D - Shimadzu Corporation, Japan) and inserted in the equipment

Ultrapycnometer 1000 (Quantachrome Instruments®, United States) for volume and density reading, as described in United States Pharmacopeia (UNITED STATES PHARMACOPEIAL CONVENTION, 2019). Five volume measurements were performed using helium gas (He) as purging gas, in replicates of three, to determine average density value per sample.

5.1.5. Particle size distribution by Laser Diffractometry

Clay samples' particle size distribution was determined by laser diffraction using Particle size analyzer model CILAS 1090 (Quantachrome Instruments®, United States). For green clay we used wet dispersing module, and for the other clays dry dispersing module.

5.1.5.1. Wet dispersion analysis (green clay)

Samples were dispersed in purified water (dispersing agent) and submitted to reading without using ultrasound. Only stirring activation and measurement time of 60 seconds were employed. The amount of sample was enough to maintain obscuration between 15 and 20%. The Franhoufer model was used to calculate the diameters and the graphs were obtained using the Size Experts program.

5.1.5.2. Dry dispersion analysis (other clays)

Samples were dispersed in a compressed air jet at a pressure of 200 mbar, and measurement time of 30 seconds, with obscuration between 1 and 5%. The Franhoufer model was used to calculate the diameters and the graphs were obtained using the Size Experts program.

5.1.6. X-ray diffraction

The mineralogical composition analysis was performed using X-ray Diffraction Laboratory's method in Geoanalytical Multiuser Center – Institute of Geosciences of the University of São Paulo (IG-USP).

Clay samples were transferred to the sample holder, and the diffractograms were obtained using a Bruker D8 Da Vinci X-ray diffractometer, with copper K-alpha radiation (λ = 1.5418 Angstrom), operating at 40 KV and 40 mA, and angle sweep of 2 to 70° 2-teta. Data were analyzed with Match! Software from the COD database (Crystallographic Open Database).

5.1.7. X-Ray Fluorescence

Clay samples were submitted to chemical assessment (elements' determination) by X-ray fluorescence diffraction technique, in Alex Stewart International do Brasil Ltda. Laboratory. Therefore, samples were prepared and pressed into a briquette before inserting in the equipment X-ray fluorescence analyzer Epsilon 4. This chemical semiquantitative analysis aimed to characterize clays concerning oxides (CORRÊA, 2006).

5.2. Development of the face masks

The development of the cosmetic formulations involved elaborating facial clay masks containing red, green, and black clays as active components.

5.2.1. Formulation composition and preparation

Red clay was selected for its antiaging properties. Green clay was selected due to its astringent and healing properties. As to black clay, it was selected due to its rejuvenating, healing, antioxidant and oil absorption properties (BALDUINO, 2016).

Formulations containing the association of red, green, and black clays were prepared in three different concentrations: F01 - placebo or control formulation (where no actives were added), F02 - minimum concentration formulation (with 3% of each active component), and F03 - maximum concentration formulation (with the addition of 7% of each active component). The concentration of each substance was established in accordance with Brazilian legislation. Compositions are described in **Table 1**.

Table 1. Composition of the cosmetic samples

Components			Concentration (%w/w)		
	Component name	**INCI name**	**F01**	**F02**	**F03**
Base formulation	Aristoflex® AVC	*Ammonium Acryloyldimethyltaurate/VP Copolymer*	2.0	2.0	2.0
	Glycerin	*Glycerin*	3.0	3.0	3.0
	Propylene glycol	*Propylene glycol*	3.0	3.0	3.0
	Disodium EDTA	*Disodium EDTA*	0.1	0.1	0.1
	FocusGuard PE MIT	*Phenoxyethanol (and) Methylisothiazolinone*	0.5	0.5	0.5
	Belsil® OW 2100	*PEG-12 Dimethicone*	4.0	4.0	4.0
	DUB B1215	*C12-15 Alkylbenzoate*	4.0	4.0	4.0
	Distilled water	*Aqua*	q.s.	q.s.	q.s.
	Citric acid (50% w/w solution)	*Citric acid*	**	**	**
Active compounds	Red clay - Tersil® CDR	*Kaolin*	0.0	3.0	7.0
	Green clay - Tersil® G	*Kaolin*	0.0	3.0	7.0
	Black clay -Tersil® CB	*Kaolin*	0.0	3.0	7.0

Legend: INCI = International Nomenclature of Cosmetic Ingredients; q.s. = *quantum sufficit* (enough amount to complete weight); w/w = weight/weight.

** Enough amount added to achieve final formulation pH value of 5.5-6.5.

Clays were individually sieved in 60 mesh sieve and transferred to a previously identified open mouth jar. This process enabled better incorporation of all clays in the prepared formulation. Then, sieved clays were weighted and transferred to a porcelain mortar to be homogenized and grinded.

C12-15 Alkylbenzoate, PEG-12 Dimethicone and *Glycerin* were weighted and transferred to the same beaker and stirred with glass stick. This mixture was then transferred to the porcelain mortar with the clays and stirred with the pistil until homogenization and formation of a paste. Part of the formulation's water was then weighted and added to this mixture under stirring (10% of total formulation for F01 and F02, and 20% of total formulation for F03).

In a stainless-steel mug, the EDTA was solubilized in the remaining amount of water. Then, the preservative and *Propylene glycol* were weighted and added. This mixture was homogenized before the addition of the polymer (*Ammonium Acryloyldimethyltaurate/VP Copolymer*). The polymer was incorporated slowly under stirring until the formation of a gel and stirred until no lump was observed.

Finally, the content of the porcelain mortar was added to the stainless-steel mug to be incorporated in the gel under agitation with a mixer until complete homogenization.

The final formulations were transferred to the proper packing material.

5.3. Efficacy assessment of the developed face masks

5.3.1. Ethical Assessment of the Study Protocols

The research was conducted in accordance with current Brazilian legislation and following the ethical principles described in RDC 466/2012. The study protocol and product information were previously submitted to the approval of the Research Ethics Committee of Faculty of Pharmaceutical Sciences, University of São Paulo, to protect all study participants.

5.3.2. Participants' Consent

Before each assay, the aim and methodology of all assessments were clarified to the participants, and they signed an informed consent term previously approved by the Ethics Committee (Annex C). The informed consent term was read aloud to the participants before they signed it.

The selected participants were all capable of accomplishing each research protocol and of understanding all stages of the process.

5.3.3. Inclusion Criteria

The general inclusion criteria determined in the protocols are listed below.

General inclusion criteria:

- Gender: male and female
- Healthy participants
- Intact skin on the experimental region
- Phototypes I to IV
- Absence of irritation or allergy history to the material used in the studies.
- Having signed the informed consent term
- Participants that want to be part of the study without financial profit. They were compensated for the transportation and feeding expenses.
- Participants apt to accomplish the study protocol demands.

5.3.4. Exclusion Criteria

The general exclusion criteria determined in the protocols are listed below.

- Allergy to the test product category
- Pregnant or lactating women
- Immunodeficiency
- Active atopic dermatitis
- Kidney, heart and/or liver transplant
- Solar erythema on the test region caused by intense sun exposure of 1 month before the study.
- Forecast of intense sun or ultraviolet lamps exposure during the study
- Use of the following medications: corticoids, antihistamines, immunosuppressants, retinoids, anti-inflammatory

- Any conditions that may interfere with the assessments according to the principal investigator.
- History of non-adherence to the study protocol
- People directly involved with the protocol and their family.
- Participants who refuse to participate in the study.

The participants were also asked not to alter their diet, cosmetic and hygiene habits, exercise routine and contraceptive methods during the research. They were also asked not to use products from the same category of the investigational sample on the test region along the study.

5.3.5. Sebumetry

The assessment of skin oiliness (sebumetry) of the participants was performed using the probe Sebumeter® (Courage & Khazaka) coupled to the equipment Multi Probe Adapter - MPA (Courage & Khazaka).

It consists of a non-invasive, fast, safe, and effective method for the assessment of product efficacy in skin oiliness reduction. It is based on the skin oiliness adsorption on the probe's indicator tape, converting it from opaque to transparent (RIZER, 1999). During measurement, the probe's tape is pressured against the skin for 30 seconds to collect the produced sebum, culminating in the increase of the tape transparency proportional to the amount of sebum produced. The transparency change is measured by a sensor inside the equipment MPA, leading to obtaining a value between 50 and 300 $\mu g/cm^2$ to indicate the amount of sebum per skin area (COURAGE & KHAZAKA, 2020).

In this assay, 20 participants with oily skin on the face T-zone, aged between 18 and 45 years, were selected. They were submitted to an acclimatization period of 30 min under standardized environmental conditions (20 ± 2°C temperature and 50 ± 5% relative humidity) before all measurements. Then, a site was marked on the experimental region (forehead) of the participants using a template, inside which the baseline oiliness measurements were taken in replicates of three (t0).

After the baseline measurement, the investigational products were applied standardizedly on the marked sites using a 1 mL disposable syringe and were spread with finger covered with a disposable latex finger cot at a ratio of 2.0 mg/cm^2.

Afterwards, new oiliness measurements were taken on the following experimental times: 2 hours after application (t2h); and 4 hours after application (t4h).

5.3.6. Cutometry

The cutometry assessment was performed using the Cutometer® (Courage & Khazaka) probe coupled to the equipment Multi Probe Adapter - MPA (Courage & Khazaka) for firmness and elasticity determination.

The principle of the technique is based on the suction method. The system creates negative pressure, and the skin is pulled inside the probe aperture. Then the pressure stops, and the skin is released to return. Inside the probe, sensors determine the skin penetration depth. The cutaneous resistance to the negative pressure applied corresponds to firmness (the lower the penetration inside the probe, the more the skin is firm). The skin's ability to return to the original position corresponds to elasticity (the closer to the original position the skin returns, the more this skin is elastic) (COURAGE & KHAZAKA, 2021).

In this assay, 20 participants aged between 30 and 60 years were selected to participate. They were submitted to an acclimatization period of 30 min under standardized environmental conditions (20 ± 2°C temperature and 50 ± 5% relative humidity) before all measurements. Then, a site was marked on the experimental region (malar region on the cheeks) of the participants using a template, inside which the baseline firmness and elasticity measurements were taken with three suction-relaxation cycles (t0).

After the baseline measurement, the investigational products were applied standardizedly on the marked sites using a 1 mL disposable syringe and were spread with finger covered with a disposable latex finger cot at a ratio of 2.0 mg/cm^2.

Afterwards, new firmness and elasticity measurements were taken on the following experimental times: 2 hours after application (t2h); and 4 hours after application (t4h).

5.4. Statistical treatment of the obtained data

All data was statistically assessed (where applicable) to determine whether clays and their concentrations influenced on product efficacy. Statistical analyses were performed using Minitab software.

6. Results and Discussion

The SEM allows obtaining a magnified, three-dimensional image of unmodified clay surface to observe features, like surface, morphology, texture (particle arrangements), and growth mechanics of crystals and crystallites (BOHOR; HUGHES, 1971). Green, red, and black clays were submitted to SEM coupled with an Energy Dispersive Spectroscopy (EDS) to allow a semi-quantitative assessment of the elements present in their composition. To confirm their mineralogical composition, an X-ray diffraction analysis was additionally performed. **Figures 2** to **4** illustrate the results for each clay for the EDS analysis. **Table 2** presents the percentual weight of each element per sample (reminding that the analysis is semi-quantitative).

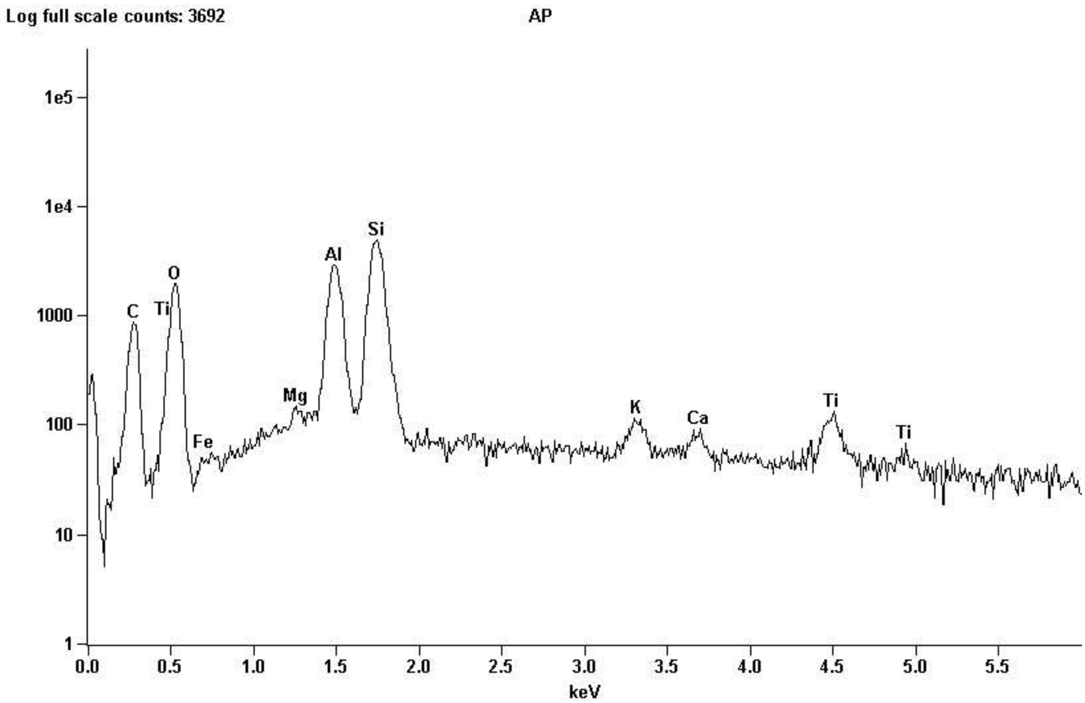


Figure 2. EDS analysis of Black Clay.

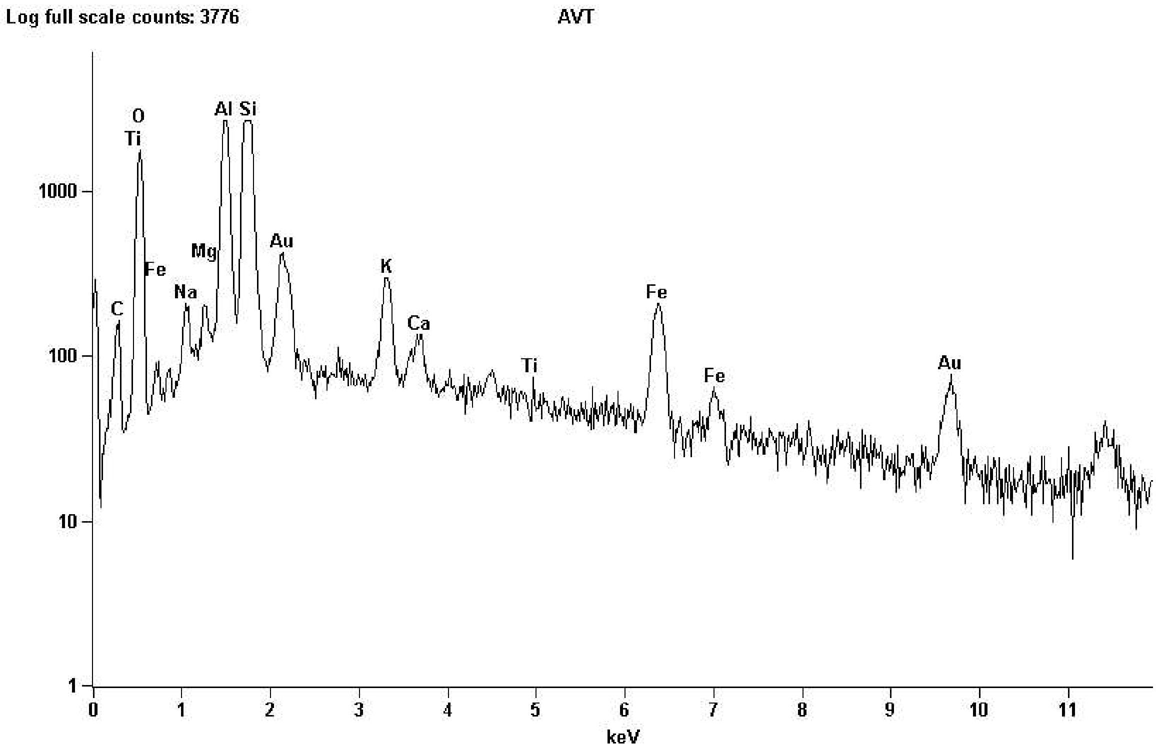


Figure 3. EDS analysis of Green Clay

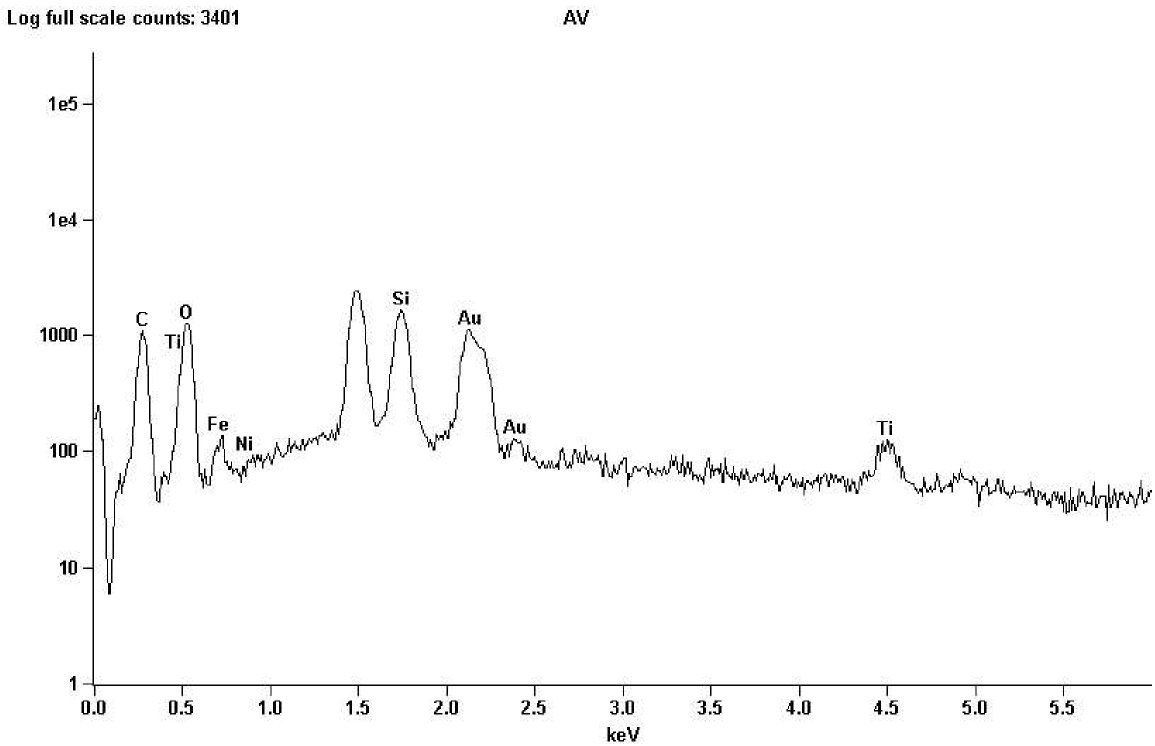


Figure 4. EDS analysis of Red Clay

Table 2. Weight percentage of each element on clay samples by SEM coupled with EDS.

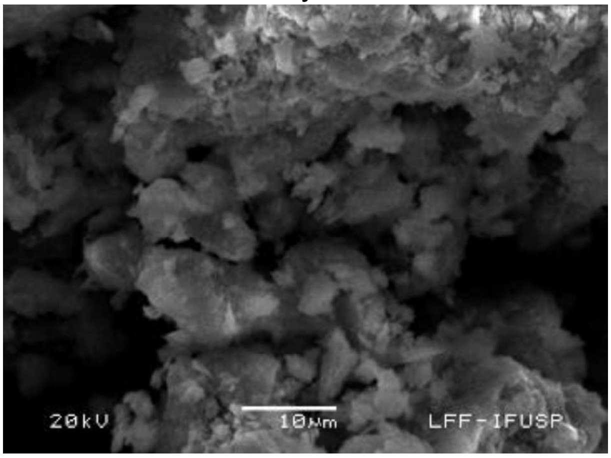
Element	Black Clay		Green Clay		Red Clay	
	% Weight	% Weight Error	% Weight	% Weight Error	% Weight	% Weight Error
C	43.15	+/- 1.01	17.45	+/- 0.90	41.51	+/- 0.88
O	35.33	+/- 0.49	37.46	+/- 0.45	28.76	+/- 0.67
Mg	0.02	+/- 0.02	0.23	+/- 0.04	---	---
Al	6.81	+/- 0.08	9.76	+/- 0.12	---	---
Si	12.64	+/- 0.09	21.16	+/- 0.15	3.19	+/- 0.08
K	0.28	+/- 0.02	1.75	+/- 0.04	---	---
Ca	0.17	+/- 0.02	0.49	+/- 0.04	---	---
Ti	0.62	+/- 0.06	0.16	+/- 0.05	0.75	+/- 0.08
Fe	0.97	+/- 0.06	3.19	+/- 0.18	4.87	+/- 0.18
Na	---	---	0.59	+/- 0.09	---	---
Ni	---	---	---	---	0.17	+/- 0.08
Total	100.00	---	100.00	---	100.00	---

The size of the peak is proportional to the amount of that element in the sample. Gold (Au) is always detected in the referred graphs, as the samples are covered in gold during preparation to improve electron conductivity (sputtering) (ERIK LUYK, 2019). As we can observe from the graphs:

- Elements found in black clay:
  - Higher amounts: carbon (C), oxygen (O), aluminum (Al), and silicon (Si).
  - Lower amounts: iron (Fe), titanium (Ti), potassium (K), and calcium (Ca).
- Elements found in green clay:
  - Higher amounts: carbon (C), oxygen (O), aluminum (Al), silicon (Si) and Iron (Fe).
  - Lower amounts: titanium (Ti), sodium (Na), magnesium (Mg), potassium (K), and calcium (Ca).
- Elements found in red clay:
  - Higher amounts: carbon (C), oxygen (O), silicon (Si) and Iron (Fe).
  - Lower amounts: titanium (Ti), and nickel (Ni).

The SEM micrographs from each clay, with 2000x and 5000x magnifications, are illustrated in **Figures 5** to **7** and each type of sample presented a different morphology.

Black clay – 2000x



Black clay – 5000x

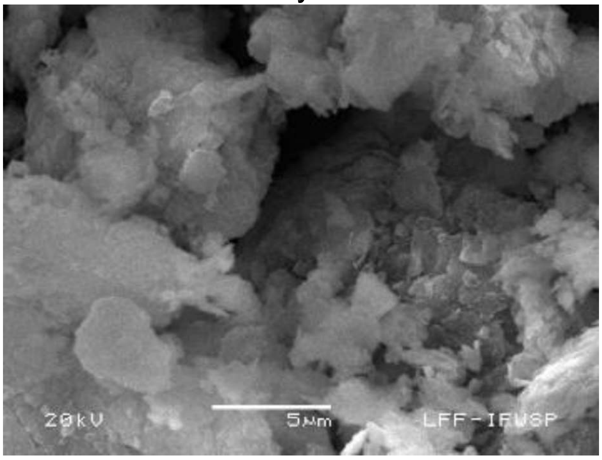
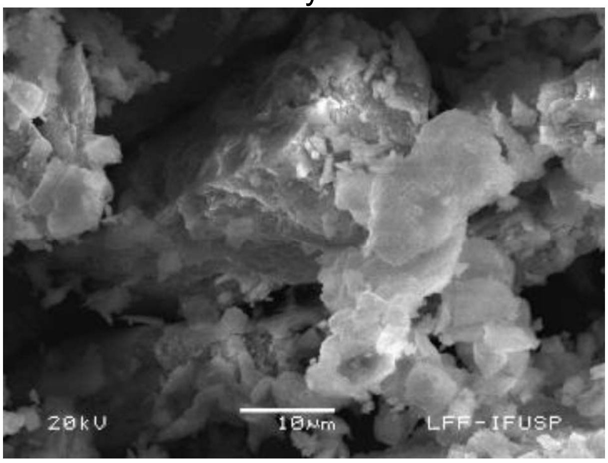


Figure 5. Micrographs of Black clay for morphology assessment by SEM (2000x and 5000x).

Green clay – 2000x



Green clay – 5000x

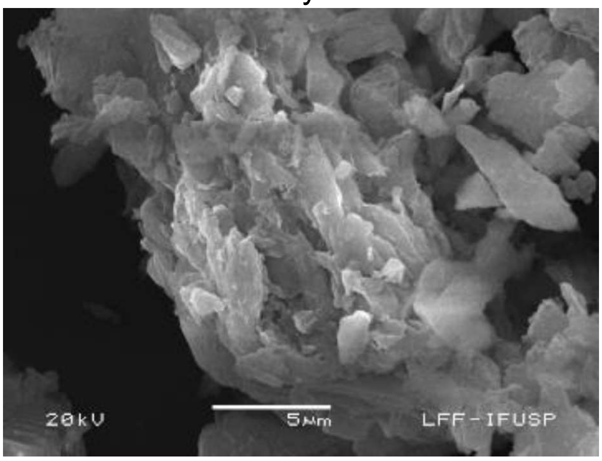
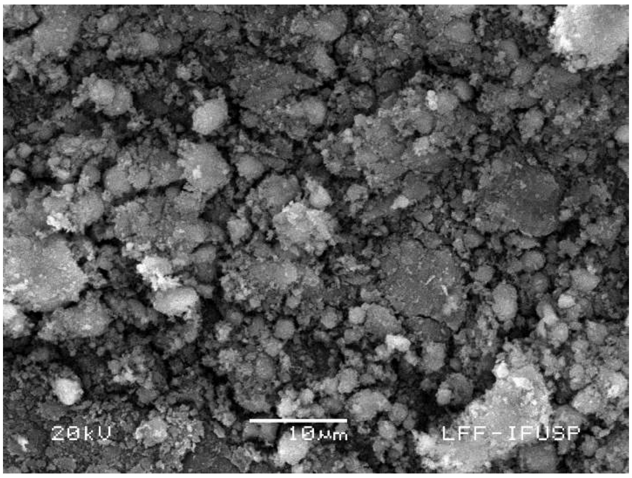


Figure 6. Micrographs of Green clay for morphology assessment by (2000x and 5000x).

Red clay – 2000x

Red clay – 5000x



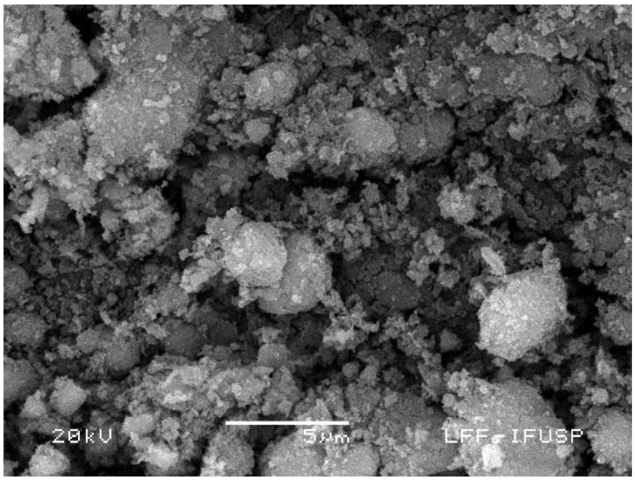


Figure 7. Micrographs of Red clay for morphology assessment by (2000x and 5000x).

When comparing the morphology of the three types of clays assessed in this research, we observed that they presented different structures. Red clay has smaller particles with a more rounded shape and does not form a layer pattern. Among all three, green clay is the one with the most layer-like appearance, with different layers forming a cohesive structure. As to black clay, it appears to have several layered structure blocks, and we can see the layers better when increasing the micrograph magnification to 5000x.

SEM technique allows morphology visualization, to better understand and visualize differences between clay types. According to Bohor and Hughes (1971), SEM technique allows the analysis of morphology/configuration, texture (particle arrangements) and growth mechanics of crystalline units. According to the authors, SEM offers a magnified, 3D-view of unmodified clay surface with dept of focus (BOHOR; HUGHES, 1971).

Keller and co-workers (1986) mention that crystal morphologies of clay-mineral families, as observed in SEM, are characteristically unique, and that the morphology and texture assessment by SEM can be used to identify individual species (KELLER; REYNOLDS; INOUE, 1986).

Corrêa (2006) also assessed clay samples' morphology by SEM and compared the results with his findings from X-ray diffraction analysis. The author observed aspects related to morphology, particle dimensions and mineralogic composition. His findings showed that the SEM analysis corroborated with the X-ray diffraction analysis, where kaolinite hexagonal darker plates were found beside gibbsite lighter agglomerates. The author described the gibbsite as agglomerates of whiteish particles sometimes with tubular forms. Kaolinite was described as normally smaller than 1µm, appearing as small plates occasionally with hexagonal profiles with lighter color (CORRÊA, 2006). These lighter gibbsite agglomerates could be also observed for red clay, as it has a different pattern and morphology compared to the other two clays assessed. X-ray diffraction detected the presence of this clay-mineral in red clay, and not in black and green ones (data shown further in results and discussion). Kaolinite was detected in all clays assessed, but the plates' shape was clearer to be observed in green and black clays.

When we consider clays' INCI names, all the assessed clays are considered as "kaolin", which means they present the same INCI name and are considered as the same clay-mineral. Therefore, when looking at a cosmetic product composition only, we cannot differentiate these types of clays. However, when assessing the morphology by SEM technique, we can observe that they have different characteristics, which justify their different behavior and efficacy when in contact with hair and the skin. For cosmetics, clays are mainly identified by their color. Based on that, further research should be performed exploring clays commercialized for cosmetic use with the comparison between clays of the same color (sold as the same raw material) from different suppliers.

The pH values obtained for each clay are described in **Table 3**. All assessed clays presented pH values tending to acid, with green clay being the only one near neutrality. This pH result was different from the one observed by Zague, 2007, concerning green clay (ZAGUE, 2007).The author also assessed the pH value of green clay used for cosmetic formulations and detected an alkaline behavior, with average pH of 8.7 ± 0,01, to which was attributed to the presence of calcium carbonate. This difference proved that even though commercialized with the same name and INCI, green clays from different suppliers may have different compositions and therefore they behave differently.

Table 3. pH values of each clay.

Clays	Average pH value (n=3)
Green	6.39 ± 0,01
Red	4.49 ± 0,02
Black	3.96 ± 0,06

Keller and Matlack (1990) assessed the pH values of several clay suspensions collected from soils at different locations (KELLER; MATLACK, 1990). The authors found that pH behaved differently according to soil composition:

- The pH of clear supernatant water over halloysite is around 4 (acid).
- Like its polymorph halloysite, kaolinite suspensions presented pH values between 3.9 and 4.6.
- Dickite also generated acid pH (3.7).
- Illite presented pH of 5.5 – 6.0.
- Smectite presented pH of 4.5 – 5.0.

The authors concluded that "the pH of the supernatant fractions of clay soils in natural field occurrences and in laboratory suspensions tends to be on the acid side, pH 4-5, for the kaolin family of minerals". Illite and smectite, due to Na, NH_4 and K content in mobile positions, tend to present higher pH values, around 5.5 – 8.0 (KELLER; MATLACK, 1990).

When analyzing X-ray diffraction results, we found that green clay was the only one composed by illite and smectite, together with kaolinite (results shown further in **Table 8**). This is probably the reason why it presented the highest pH value among them, corroborating with Keller and Matlack, 1990.

Thermal analysis methodologies have been used in cosmetic analysis for quality control and product development, as they consist of techniques involving measurement of physical properties of a substance and/or its reaction subproducts in function of temperature and/or time, while submitted to a controlled temperature program under controlled environment (BRETZKE, 2015; LIMA, 2016).

The DSC thermo-analytical technique measures the difference between the energy supplied to a thermally stable reference material and the test sample in function of temperature while the materials are submitted to a controlled temperature program. Both sample and reference were placed in identical capsules located on the thermoelectrical disc and were heated by a single heat source to obtain the DSC curves (LIMA, 2016). This allows the assessment of thermal transitions (which are transformations where the substance can gain or lose energy in heat form), such as exothermic (thermal transitions where the material releases heat – example: crystallization temperature, oxidation temperature), and endothermic processes (thermal transitions where the material absorbs heat – example: fusion temperature, evaporation temperature, glass transition temperature). Each type of transformation demands a certain amount of energy to occur (BRETZKE, 2015). In the DSC curves, the ascending peaks correspond to exothermal events, while descending peaks correspond to endothermal events (LIMA, 2016). The DSC curves for each clay (black, green, and red) are presented in **Figures 8** to **10** and the DSC results are in **Table 4**.

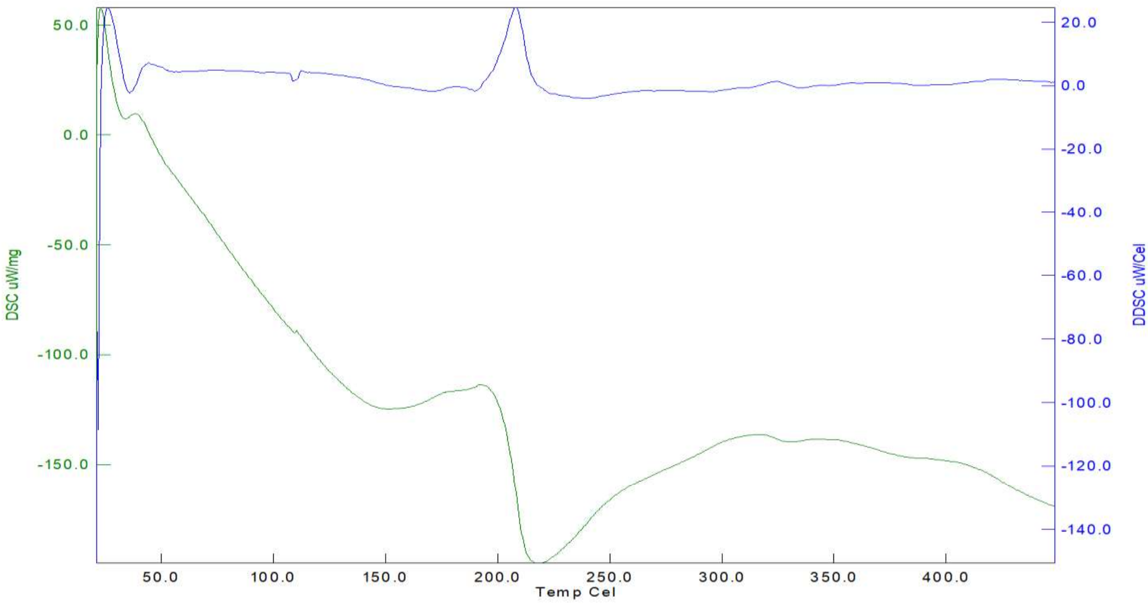


Figure 8. DSC curve obtained for black clay.

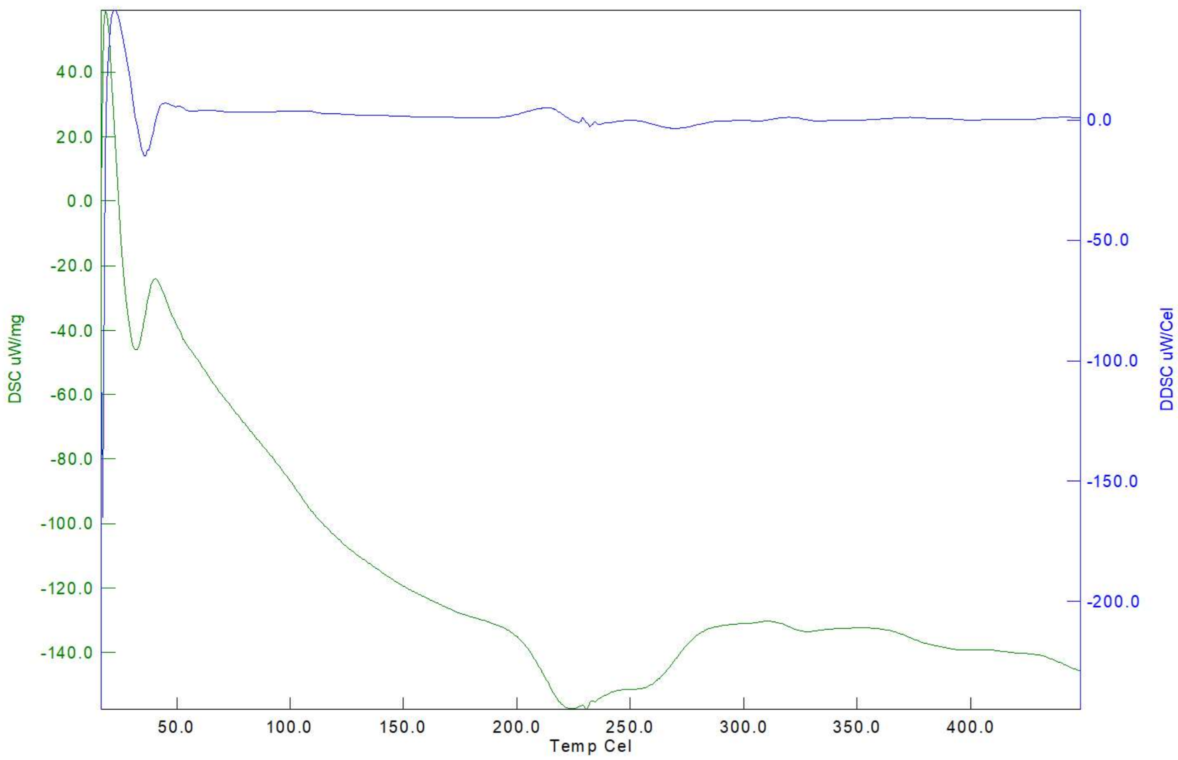


Figure 9. DSC curve obtained for green clay.

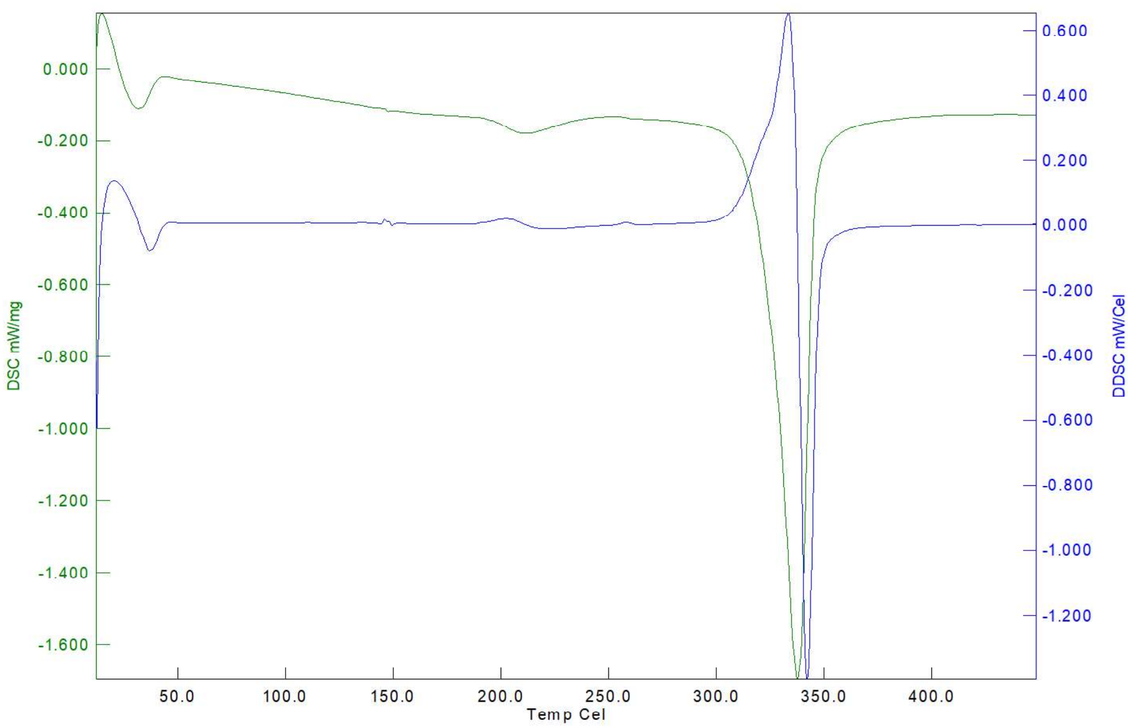


Figure 10. DSC curve obtained for red clay.

Table 4. DSC obtained results for each clay type.

Clay sample	On set (°C)	End set (°C)	Exothermic peak (°C)	Area (mJ/mg)	Area (uV.s/mg)
Black clay	201.7	263.0	217.7	21.2	-
Red clay	322.5	345.6	337.7	161.7	152
Green clay	203.4	245.9	230.6	8.74	-

The TG analysis supplies information about mass variations in function of time and/or temperature under standardized atmospheric conditions. Therefore, the equipment used has a precise balance, to weight the sample and register mass along the assay, as temperature increases. In the end, a thermogram is obtained to verify sample's composition and thermal stability, as well as the stability of its intermediate products and of formed residue. The DTG is the first derived from the TG curve, and their "steps" correspond to mass variations. The DTG representation is easier to visualize (BRETZKE, 2015; LIMA, 2016). A substance may gain or lose mass according to the occurred phenomenon. For example, it may gain mass after oxidation reactions where non-volatile oxides are formed; it may lose mass after the evaporation of components such as water or other volatiles. The obtained TG curves for each clay (black, green, and red) are presented in **Figures 11** to **13**.

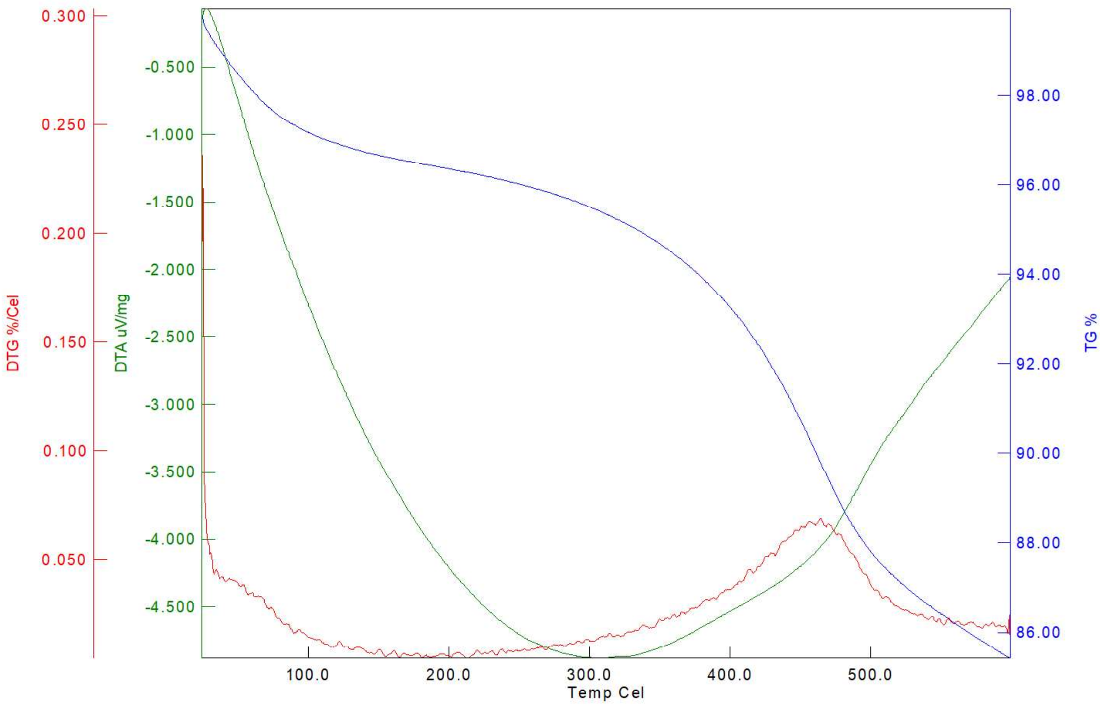


Figure 11. TG/DTG curve obtained for black clay.

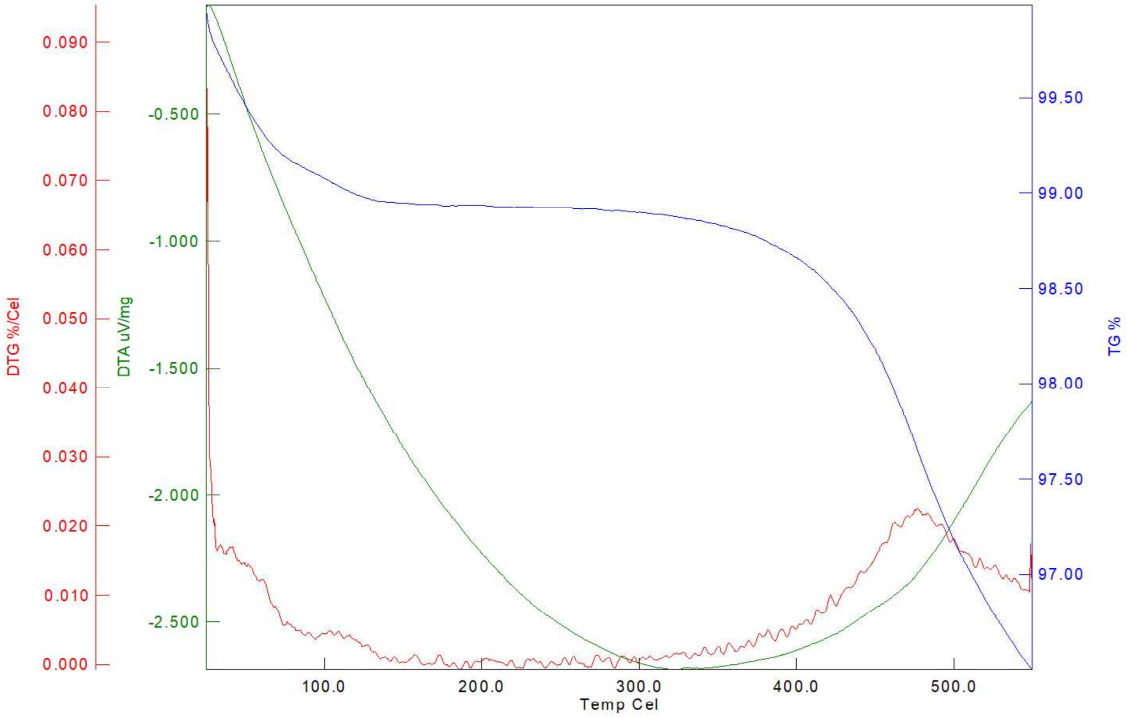


Figure 12. TG/DTG curve obtained for green clay.

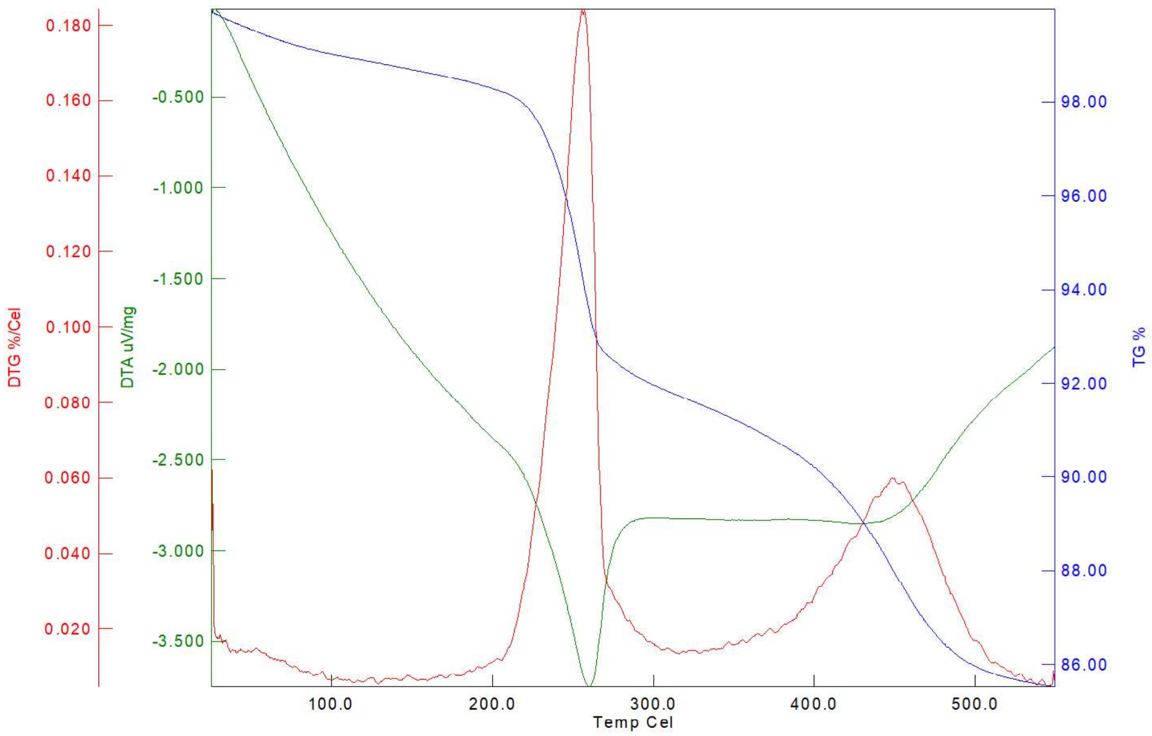


Figure 13. TG/DTG curve obtained for red clay.

The obtained TG results for each clay type, with mass loss percentages per temperature rate, are summarized in **Table 5**.

Table 5. TG obtained results for each clay type.

Clay sample	Mass loss between 25 and 125°C	Mass loss between 425 and 550°C	Residue at 540°C
Black clay	2.87%	6.10%	86.64%
Red clay	1.08%	3.80%	85.57%
Green clay	0.98%	1.95%	96.61%

When verifying thermal analysis of clays, they normally present two types of events: (1) at lower temperatures, normally between 50 and 200°C, they present dehydration events, with the evaporation of water from internal and external surfaces; and (2) at higher temperatures, normally above 400°C, they lose mass probably due to dihydroxylation of kaolinite (BRETZKE, 2015; FAVERO et al., 2016; ZAGUE, 2007). The dihydroxylation of kaolinite causes its transformation into meta kaolinite, as high temperatures provoke calcination of this clay-mineral, with loss of the reticulate water. The formed meta kaolinite is an amorphous aluminum silicate with a disordered structure (BRETZKE, 2015).

By the results on **Table 5**, we noticed a first thermal event (dehydration) at lower temperatures, between 25 and 125°C, with 2.87% mass loss for black clay, 1.08% for red clay, and 0.98% for green clay. This meant that dehydration was more significant for black clay compared to the others. Concerning the second thermal event (dihydroxylation of kaolinite) that occurred at 425 – 550°C, there was an amount of 6.10% mass loss for black clay, 3.80% for red clay, and 1.95% for green clay. Considering these results, black clay was more sensitive to mass loss when submitted to temperature compared to other clays for both thermal events.

When observing granulometry and composition assays (described in the following topics), black clay was the one with smallest average diameter (smallest particle size) and simplest composition amongst all. This might have affected its capacity to dehydrate and suffer reactions when submitted to temperature compared to the others, as smaller particles are more exposed. Small particles present a bigger exposed contact surface area, thus being more susceptible to reactions (SCHWARZE et al., 2007). However, further research should be conducted to prove this hypothesis.

Green clay, on the other hand, presented the lowest mass loss in TG assay, and is the only clay where expandable clay mineral (smectite) was found in X-ray diffraction assessment.

As to the DSC values, shown in **Table 4** all clays presented exothermic peaks probably due to kaolinite decomposition. Still, the amplitude and intensity of this event was different for each clay, what corroborated with the findings from Bretzke (2015). According to literature, the extent of kaolinite dihydroxylation may vary depending on chemical, physical and mineralogical characteristics of clays, thus leading to a variation in exothermic peaks' temperatures (BRETZKE, 2015). In our research, exothermic peaks occurred at 217.7°C for black clay (event from 201.7 to 263.0°C), at 337.7°C for red clay (event from 322.5 to 345.6°C), and at 230.6°C for green clay (event from 203.4 to 245.9°C).

Zague (2007) studied thermal behavior of green clay and found different results. The author observed an endothermic peak at 96.93°C which was attributed to clay's dehydration (water evaporation from the internal and external surfaces of the particles). No event was observed for green clay around 200°C. Still, another endothermal event was observed for pink clay and aluminum and magnesium silicate (around 270°C), which was attributed to the beginning of dihydroxylation of the layers of this reticle (ZAGUE, 2007).

Beside thermal analysis, the real density of clays was determined. The weighted mass (g), volume (cm^2), and real density (g/cm^2) values obtained for each clay are described in **Table 6**.

Table 6. Mass, volume, and density values of each clay (n=3).

Results / Sample		Green clay	Black clay	Red clay
Weighted mass (g)		4.260	3.839	3.576
Volume (cm^2)	M1	2.5721	2.4339	2.2150
	M2	2.5497	2.4198	2.1817
	M3	2.5326	2.4129	2.1808
	A	**2.5514**	**2.4222**	**2.1925**
Density (g/cm^2)	M1	1.6563	1.5773	1.6145
	M2	1.6708	1.5865	1.6391
	M3	1.6821	1.5910	1.6398
	A	**1.6696**	**1.5849**	**1.6310**

Legend: M1 = measurement 1; M2 = measurement 2; M3 = measurement 3; A = average value

Clay samples' particle size distribution (granulometric assessment) was determined by laser diffraction and the results are represented in **Figures 14** to **16** and detailed in **ANNEX E**. The results are also summarized in **Table 7**.

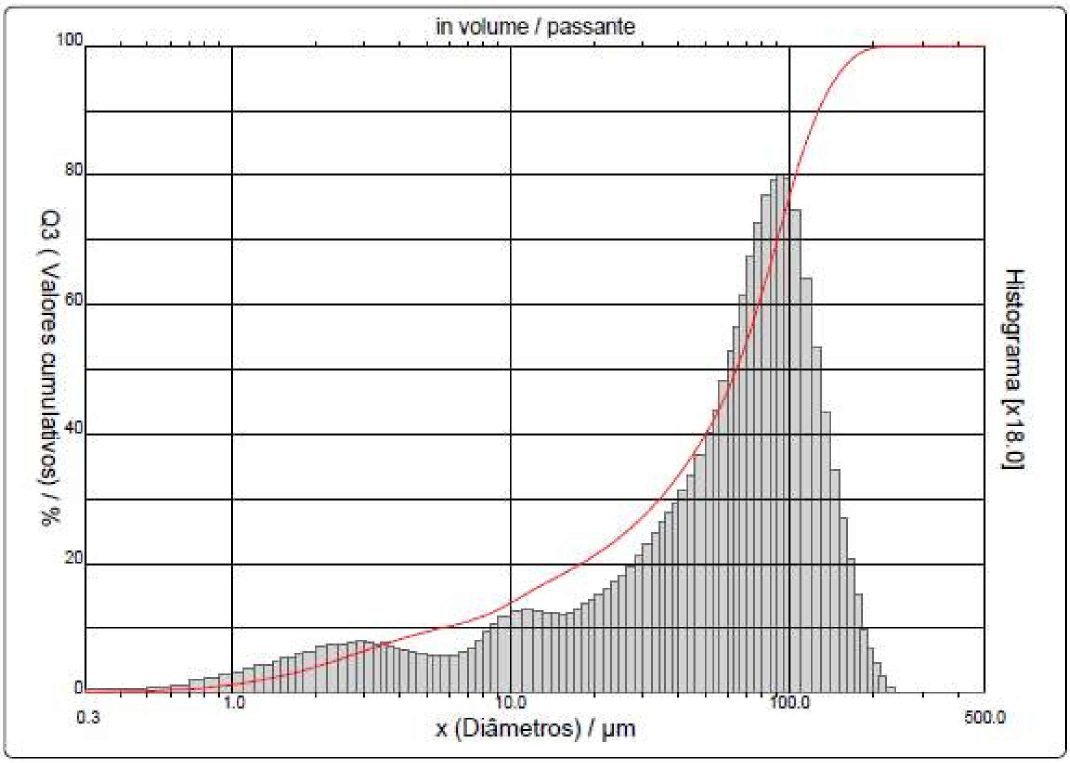


Figure 14. Particle size distribution volume (bars) and cumulative distribution volume (dashed line) of clays by laser diffraction – Black clay

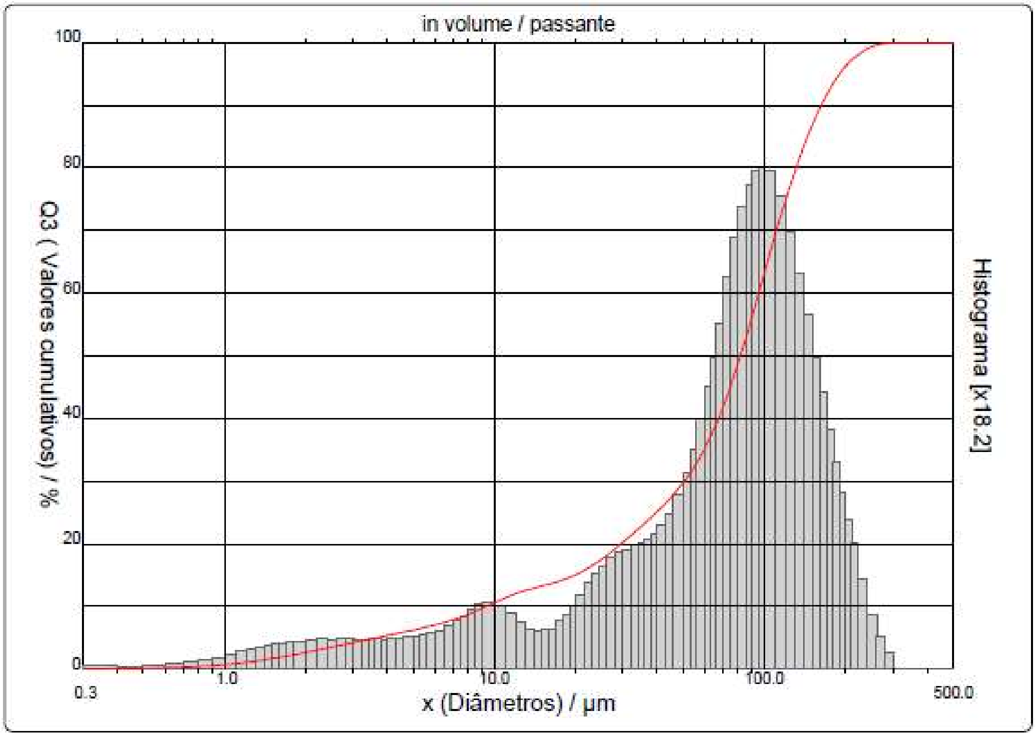


Figure 15. Particle size distribution volume (bars) and cumulative distribution volume (dashed line) of clays by laser diffraction – Red clay

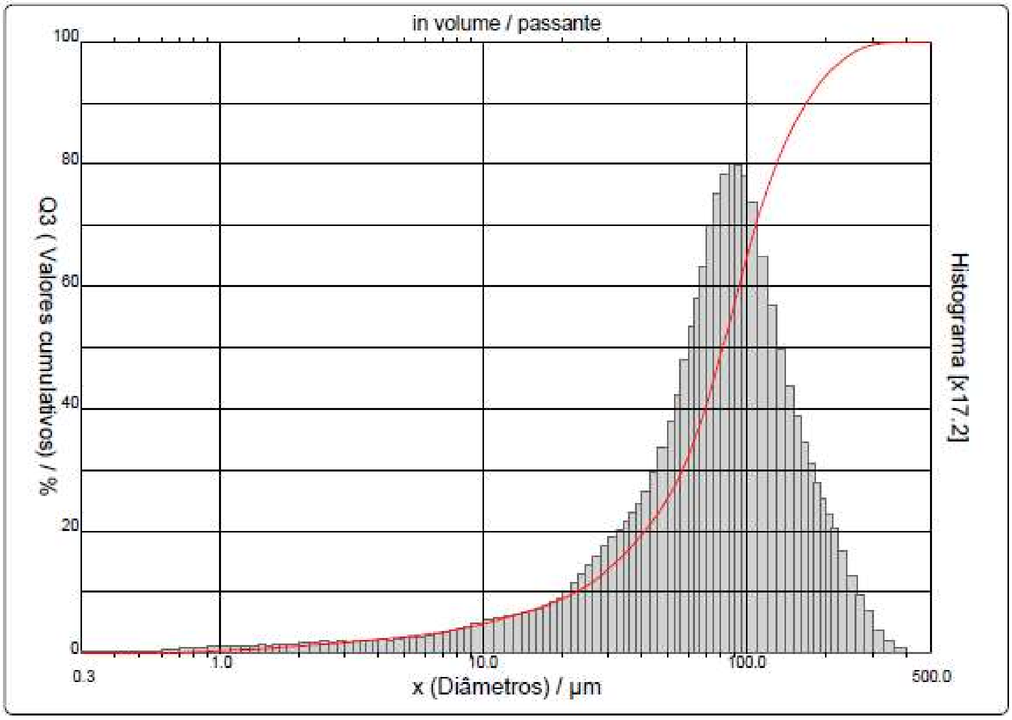


Figure 16. Particle size distribution volume (bars) and cumulative distribution volume (dashed line) of clays by laser diffraction – Green clay

Table 7. Particles size distribution profile (average) for each clay type assessed (CILAS readings).

Clay sample	10% Diameter (µm)	50% Diameter (µm)	90% Diameter (µm)	Average Diameter (µm)
Black clay	4.10	59.62	121.86	61.70
Red clay	22.65	91.33	189.12	101.64
Green clay	7.44	81.47	157.78	82.67

Black clay had the smallest particle sizes (average diameter 61.70 µm) and presented the lowest real density average (1.5849 g/cm^2). Red clay presented the highest diameter average and therefore biggest particles (101.64 µm) although its density was similar to green clay's (1.6310 and 1.6696 g/cm^2 respectively).

Bretzke (2015) assessed granulometry of several types of clays, including green, black, and red, and observed different results. According to the author, clays presented an average size between 2 and 16 µm and kaolin was the material with the smallest size. In the author's findings, green clay presented the biggest granulometry (BRETZKE, 2015).

Clays contain a mixture of substances with several particle sizes, from smallest to biggest (such as quartz). They are composed of two or more clay minerals mixed with variable amounts of non-clay materials (for example quartz, feldspars, carbonates, oxides, amorphous materials, and organic matter) (BRETZKE, 2015; GHADIRI; CHRZANOWSKI; ROHANIZADEH, 2015; MORAES et al., 2017; SILVA-VALENZUELA et al., 2018; VISERAS et al., 2007). Higher amounts of quarts in clays, which normally occur in lighter-colored ones, tend to present bigger size particles, and, therefore, a rougher feel on the skin (BRETZKE, 2015).

Considering our findings, among the three clays, black one should be the smoother to be applied on skin, and red clay should be the roughest. However, all three assessed clays are commercialized for cosmetic use in clay masks. Among the commercially available clays from this supplier, they are the ones with bigger granulometry due to their application. To apply on non-rinsible products such as sunscreens or makeup powders, this is not the clay of choice, as granulometry for this category should be smaller.

Silva-Valenzuela and co-workers (2018) state that "softness and small particle size are important for skin therapeutic and cosmetic purposes, such as face masks and clay body creams." (SILVA-VALENZUELA et al., 2018).

According to literature, inorganic fractions from the soil can be classified according to their particle size as: (1) sand (particles larger than 50 μm); (2) silt (particles between 2 and 50 μm); and (3) clay (particles smaller than 2 μm) (BESQ et al., 2003; ZAGUE, 2007). All clays presented an average diameter above 50 μm, thus indicating that part of them is not classified as clay and there is the presence of other minerals, such as quartz. The presence of quartz was proved in all three clays by X-ray diffraction analysis.

Mineralogical analyses of the clays were carried out by X-ray diffraction and the result allowed us to verify that the clays were different from one another in composition (**Table 8**). Data were analyzed using Match! Software from Crystallographic Open Database.

- Black clay was mainly composed of kaolinite and quartz. It is mineralogically simpler than the other two, and its color is probably due to the presence of organic matter, as no manganese minerals were detected.
- Red clay was composed of kaolinite, quartz, gibbsite (aluminum hydroxide), and hematite. Hematite is the component responsible for its reddish color. The presence of gibbsite indicates that it consists of a material that is lixiviated by weathering.
- Green clay was composed of kaolinite, quartz, illite, and smectite. Smectite is an expandable clay-mineral. The identification of the expandable clay-mineral in the sample was carried out after sample saturation with ethylene glycol, where a peak expansion from 14 to 17 Angstrom was observed.

Table 8. X-ray diffraction results obtained for black, red, and green clays.

Clay	Assay condition	Kaolinite	Illite	Smectite*	Quartz	Hematite	Gibbsite
Black	A	X			X		
	B	X			X		
Red	A	X			X	X	X
	B	X			X		X
	C	X			X		X
Green	A		X	X	X		
	C	X	X	X			

Legend: A = total sample pressed on a glass slide; B = clay fraction sample decanted on glass slide; C = clay fraction sample saturated with ethylene glycol. * Mineral from smectite group, possibly montmorillonite.

The samples' diffractograms are presented in **Figures 17** to **23**. The presence of kaolinite in all clays and of smectite in green clay indicated application capacity in dermatological care products to protect skin against external agents (for example, antipollution effect), exudations, and liquid excretions. Those minerals adhere to skin, forming a film that provides mechanical protection against external agents and take up skin exudations. They also produce a water-poor medium unfavorable to bacterial growth and sorb bacteria, viruses, grease, and toxins, thus presenting some antiseptic activity (CARRETERO, 2002; CARRETERO; GOMES; TATEO, 2006; CARRETERO; POZO, 2010; DARÉ et al., 2015). Kaolinite and smectite also have high sorption capacity, form protective film, cover imperfections, and adsorb excessive oiliness and toxins. Hence its indication for oily and acneic skin (CARRETERO; POZO, 2010; DARÉ et al., 2015; LÓPEZ-GALINDO; VISERAS, 2004).

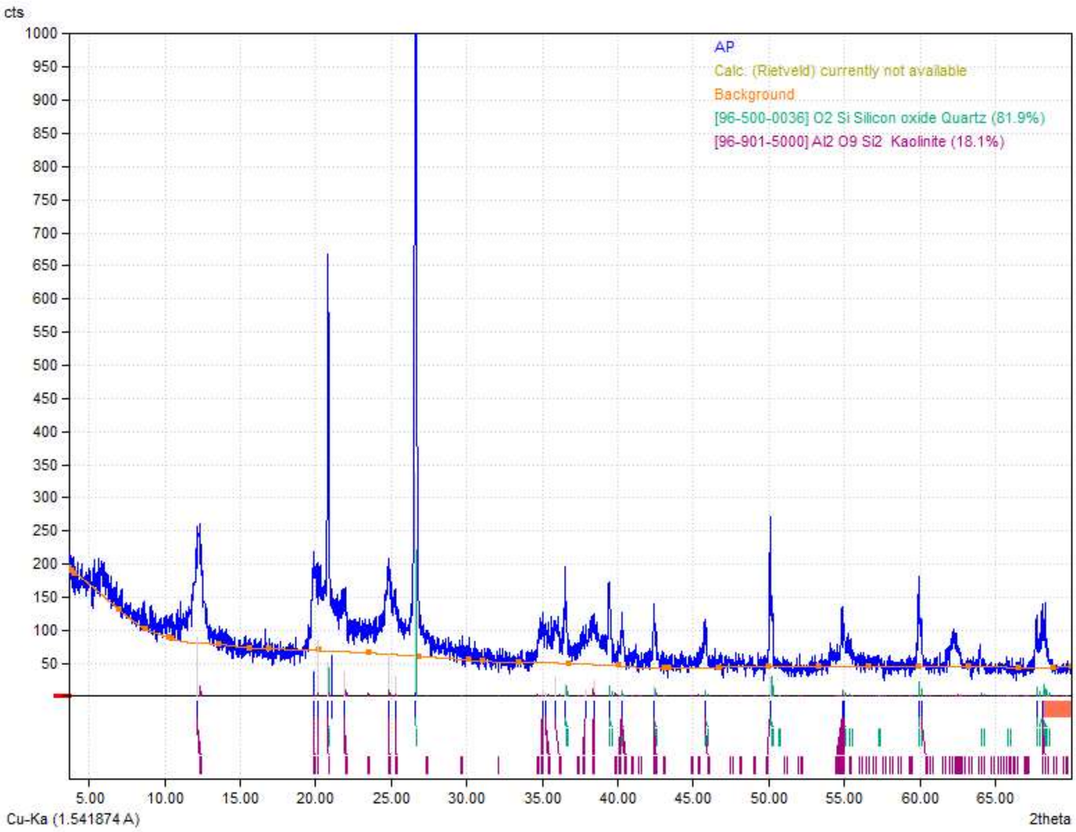


Figure 17. Diffractogram obtained from X-ray diffraction analysis of natural clay samples – Black clay (total sample pressed on a glass slide).

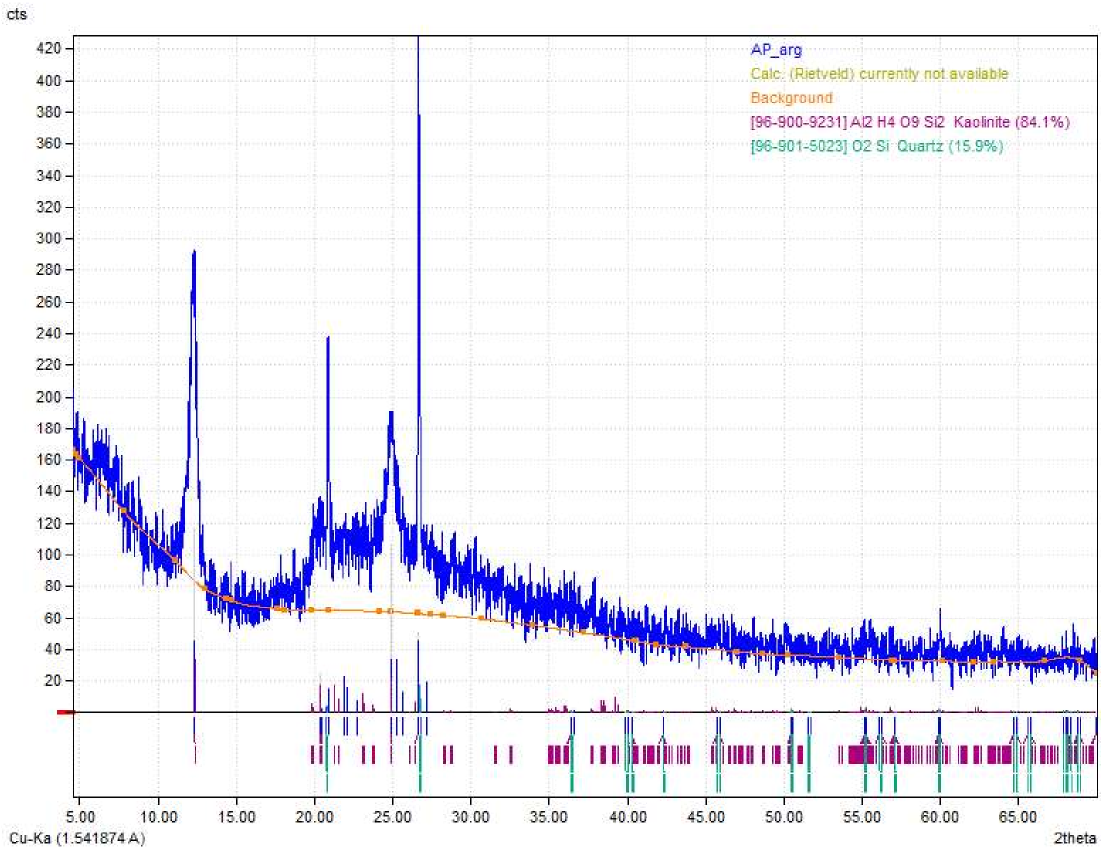


Figure 18. Diffractogram obtained from X-ray diffraction analysis of natural clay samples – Black clay (clay fraction sample decanted on glass slide).

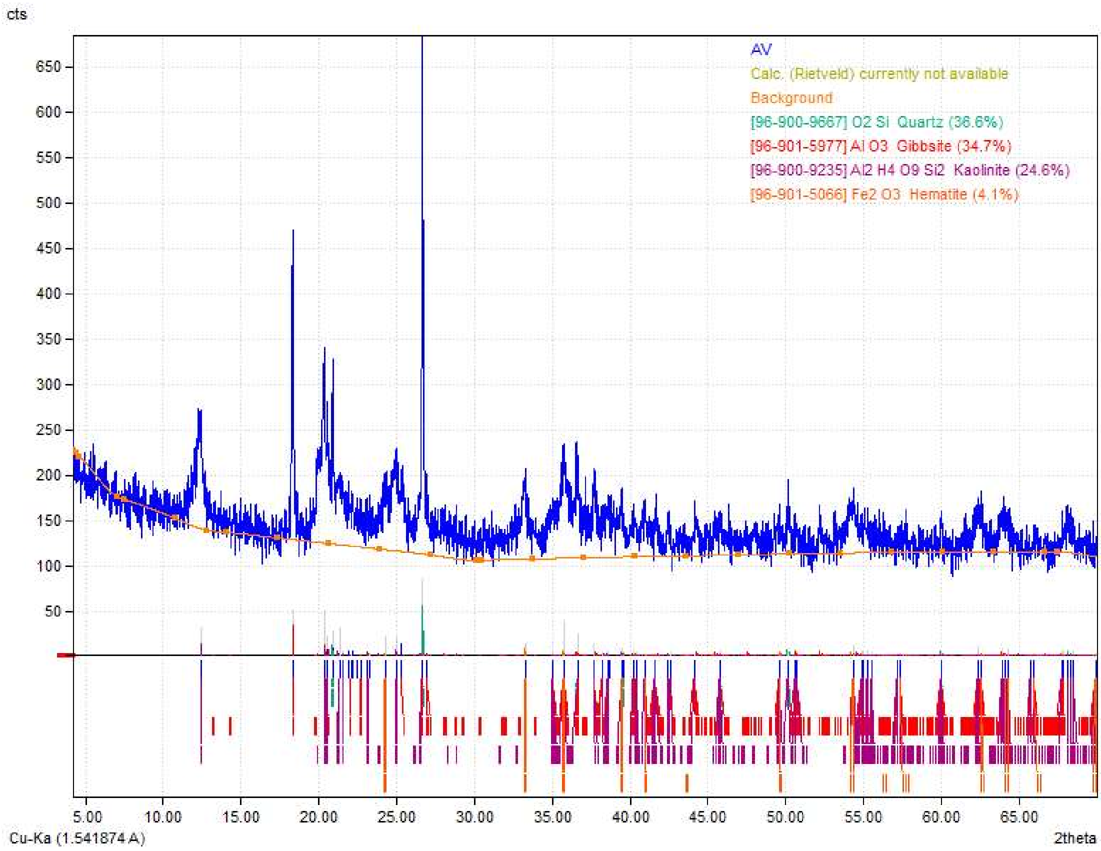


Figure 19. Diffractogram obtained from X-ray diffraction analysis of natural clay samples – Red clay (total sample pressed on a glass slide).

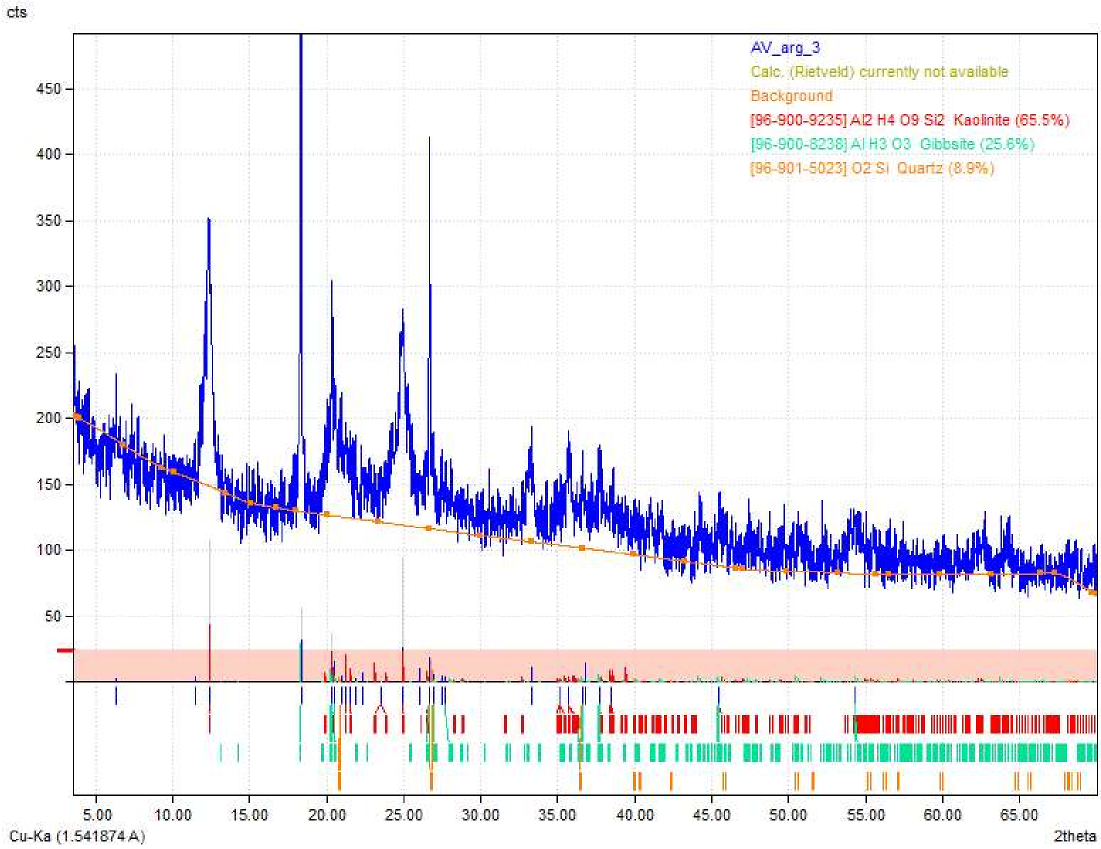


Figure 20. Diffractogram obtained from X-ray diffraction analysis of natural clay samples – Red clay (clay fraction sample decanted on glass slide).

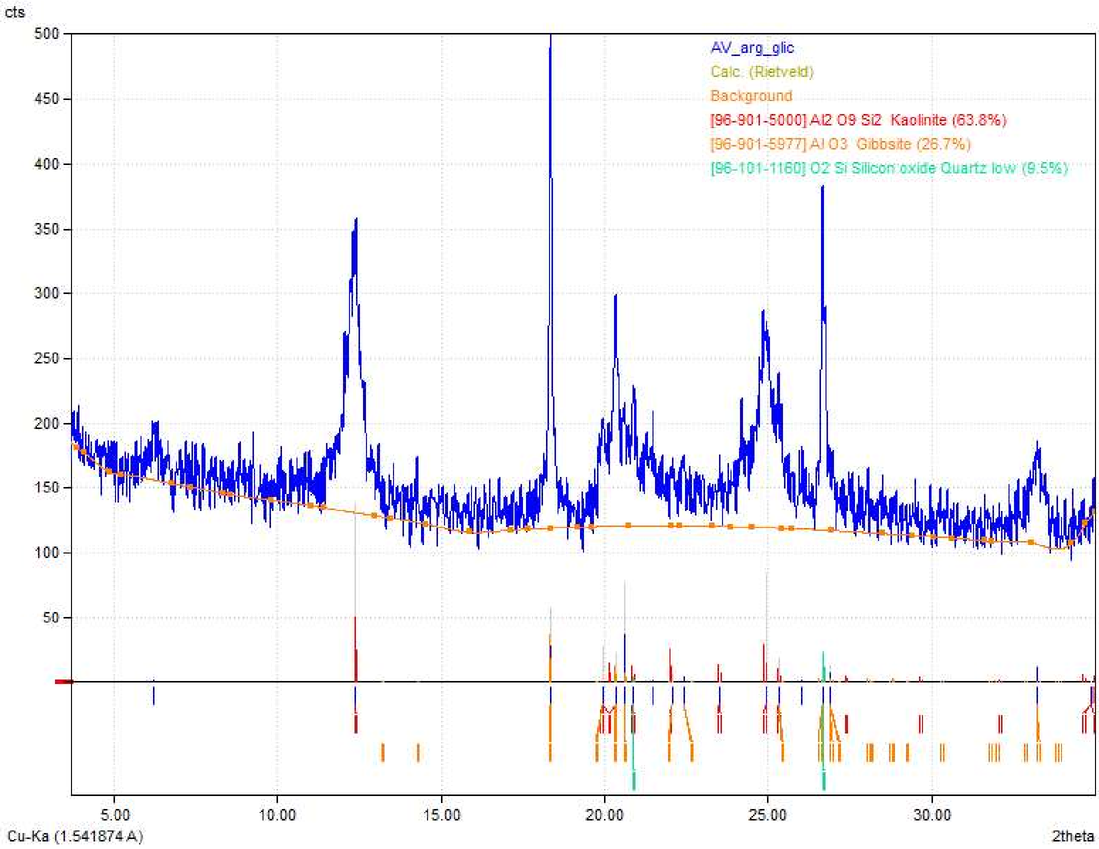


Figure 21. Diffractogram obtained from X-ray diffraction analysis of natural clay samples – Red clay (clay fraction sample saturated with ethylene glycol).

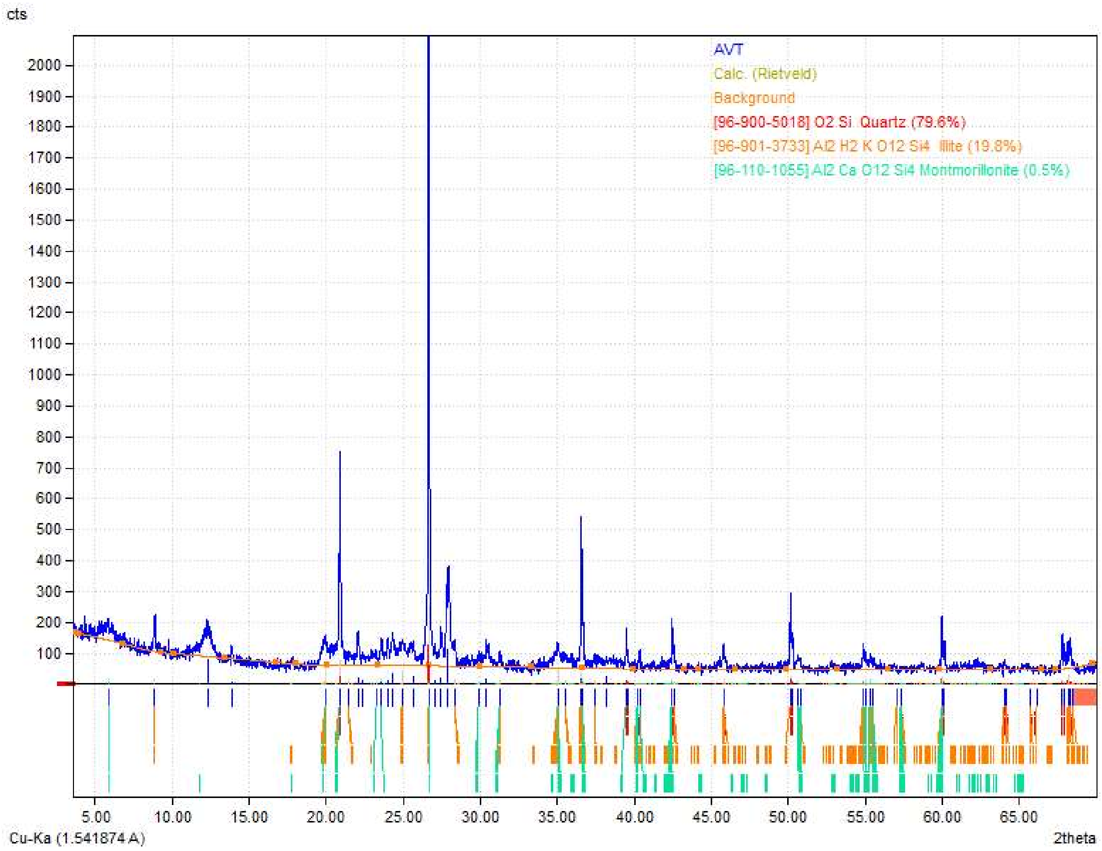


Figure 22. Diffractogram obtained from X-ray diffraction analysis of natural clay samples – Green clay (total sample pressed on a glass slide).

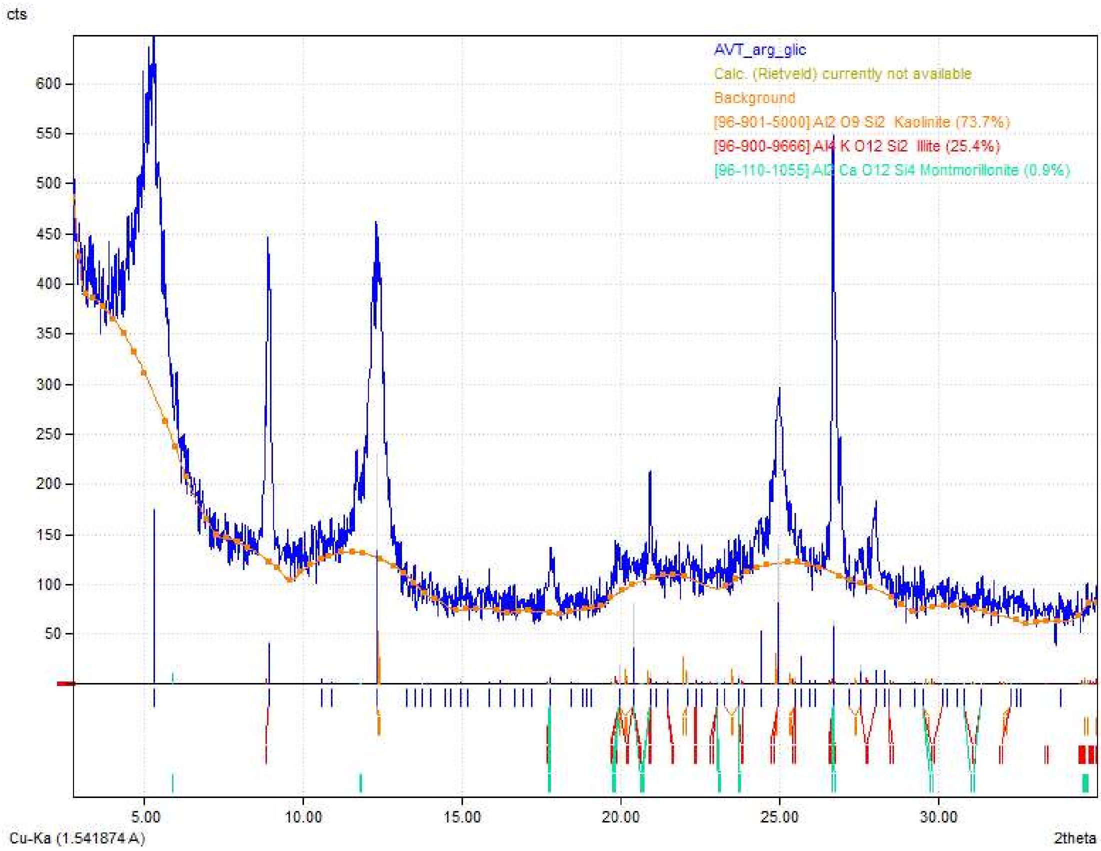


Figure 23. Diffractogram obtained from X-ray diffraction analysis of natural clay samples – Green clay (clay fraction sample saturated with ethylene glycol).

Clays were submitted to semi-quantitative determination of elements in oxide state by X-ray Fluorescence using the equipment Epsilon 4, in accordance with the method developed by Alex Stewart International Laboratory. The results are described in **Table 9**.

Table 9. X-ray Fluorescence results of each clay.

Element	Multielement Calibration / Screening (%)		
	Black Clay	Green Clay	Red Clay
Na_2O	0.0 ppm	1.148	0.0 ppm
MgO	0.0 ppm	0.643	0.0 ppm
Al_2O_3	24.658	19.749	37.259
SiO_2	56.417	58.182	33.948
P_2O_5	0.028	0.018	0.025
SO_3	0.212	0.151	0.120
Cl	0.003	0.002	0.0 ppm
K_2O	0.416	1.840	0.264
CaO	0.235	0.721	0.027
TiO_2	1.157	0.303	2.151
V_2O_5	0.022	0.006	0.047
Cr_2O_3	0.011	0.014	0.030
MnO	0.017	0.013	0.015
Fe_2O_3	1.539	2.330	13.295
NiO	0.002	0.001	0.005
CuO	0.002	0.001	0.004
ZnO	0.003	0.006	0.004
Ga_2O_3	0.004	0.002	0.007
As_2O_3	4.2 ppm	1.1 ppm	0.006
SeO_2	0.9 ppm	0.0 ppm	/
Rb_2O	0.002	0.005	0.001
SrO	0.014	0.032	0.002
Y_2O_3	0.004	0.001	0.004
ZrO_2	0.053	0.016	0.136
Nb_2O_5	0.010	0.011	0.022
MoO_3	3.1 ppm	2.2 ppm	7.5 ppm
PdO	2.4 ppm	2.6 ppm	0.2 ppm
Ag_2O	0.077	0.077	0.112
CdO	0.0 ppm	0.4 ppm	1.2 ppm
SnO_2	0.008	0.007	0.011
Sb_2O_3	0.002	0.002	0.003
BaO	0.032	0.051	0.013
CeO_2	0.022	0.007	0.012
Eu_2O_3	0.008	0.010	0.038
HfO_2	7.1 ppm	4.1 ppm	0.004
Ta_2O_5	0.006	0.010	0.022
WO_3	8.9 ppm	0.003	0.0 ppm
IrO_2	0.0 ppm	0.0 ppm	0.0 ppm
PtO_2	0.0 ppm	0.0 ppm	0.0 ppm
HgO	5.0 ppm	0.0 ppm	1.7 ppm
Tl_2O_3	0.0 ppm	0.0 ppm	0.0 ppm
PbO	0.005	0.001	0.002
Bi_2O_3	0.0 ppm	0.0 ppm	0.0 ppm
ThO_2	0.002	2.5 ppm	0.005
P.F	0.0 ppm	0.0 ppm	0.0 ppm
F	15.026	14.628	12.397

Element	Multielement Calibration / Screening (%)		
	Black Clay	Green Clay	Red Clay
Rh	0.0 ppm	0.0 ppm	0.9 ppm
Re	0.0 ppm	0.0 ppm	0.0 ppm
Au	0.1 ppm	0.0 ppm	0.0 ppm
U	4.0 ppm	1.3 ppm	7.2 ppm
Yb_2O_3	8.8 ppm	0.003	0.006
Nd_2O_3	0.0 ppm	0.0 ppm	/
TeO_2	/	0.006	/
GeO_2	/	0.4 ppm	/
Br	/	/	3.0 ppm

All clays were rich in Al_2O_3 and SiO_2. This profile corroborates with the findings from Favero and co-workers (2016), as they mentioned that Si, Al, Fe and K were the elements constituting the major amount of the studied samples after fluorescence diffraction analysis (FAVERO et al., 2016).

Clays with high amount of Si indicates its efficacy in skin tissue reconstitution, skin hydration, and mitigation of cutaneous inflammatory processes (SARRUF et al., 2024). Clays with high amount of Al is relevant for cosmetic preparations, due to their healing activity, hydration, and melanin absorption (CARRETERO; POZO, 2010; FAVERO et al., 2016). Clays having Si, Al, Fe, Ca, Ti and K can be employed as antibacterial, regenerative, antiseptic, and contribute to cell renewal, impurity adsorption, and circulation activation (CARRETERO; POZO, 2010; FAVERO et al., 2016).

Red clay was the richest in the amount of Fe_2O_3. This could be justified as it is the only clay where hematite was detected by X-ray diffraction (FAVERO et al., 2016). Clays rich in iron oxides are likely to improve sun protection of cosmetic products (MATIKE; EKOSSE; NGOLE, 2011). The same result was obtained by Bretzke (2015), who tested several clays and found that red clay also presented the highest amount of iron content (BRETZKE, 2015).

There is an advantage to using clays for cosmetic purposes, as it is a low-cost, natural, and abundant component, which is chemically inert and presents low toxicity when used in adequate conditions (COSTA, 2015). Cosmetics with clays can be formulated in several cosmetic forms, such as ointments, gels, creams, and pastes. Those products must be formulated considering their viscosity (must be appropriate to maintain contact with application region) and sensory aspects (must be cosmetically acceptable), which are influenced by the clay physicochemical properties (MATIKE; EKOSSE; NGOLE, 2011). According to Velasco and co-workers (2016), the most used form concerning clays in cosmetics is face masks. This is due to their high absorption of substances on skin surface like oiliness, grease, toxins, and microorganisms. Hence, this was the cosmetic form of clays application selected for this research work.

In cosmetics, clays can act either as active or as starting material, influencing the stability and rheology (SARRUF et al., 2024). As actives, they are widely used for skin cleansing, substances adsorption, detoxification, UV filter in sunscreens, and ion exchange with skin, due to their color, composition, particle size, structure, and ion exchange capacity (CARRETERO, 2002; MATIKE; EKOSSE; NGOLE, 2011; NG'ETICH et al., 2014).

According to the manufacturer literature, black clay is the most noble among clays, and has rejuvenating properties besides favoring cell renewal (MAPRIC, 2021). According to Balduino (2016), black clay properties include rejuvenation, whitening, healing, and oil absorption (BALDUINO, 2016).

Several previous formulation prototypes were developed until achieving the one elected to this research. As far as actives' concentrations are concerned, we adopted for minimum concentrations 3% of each clay (sum = 9%), and for maximum concentrations 7% of each clay (sum = 21%). Polymer concentration adopted was 2% for best viscosity. **Table 10** below summarizes the first obtained formulations and preparation techniques.

Table 10. First obtained formulations and preparation techniques (F-a and F-b)

	Components		**Concentration (%w/w)**	
	Component name	**INCI name**	**F-a**	**F-b**
1	Aristoflex® AVC	*Ammonium Acryloyldimethyltaurate/VP Copolymer*	2.0	2.0
2	Glycerin	*Glycerin*	3.0	3.0
3	Propylene glycol	*Propylene glycol*	3.0	3.0
4	Disodium EDTA	*Disodium EDTA*	0.1	0.1
5	FocusGuard PE MIT	*Phenoxyethanol (and) Methylisothiazolinone*	0.5	0.5
6	Belsil® OW 2100	*PEG-12 Dimethicone*	4.0	4.0
7	DUB B1215	*C12-15 Alkylbenzoate*	4.0	4.0
8	Distilled water	*Aqua*	q.s.	q.s.
9	Citric acid	*Citric acid*	*	*
10	Green clay (Terramater)	*Kaolin*	3.0	7.0
11	Red clay (Terramater)	*Kaolin*	3.0	7.0
12	Black clay (Terramater)	*Kaolin*	3.0	7.0

Preparation technique:
Clays were weighted and transferred to a porcelain mortar to be grinded and homogenized. Then, 6 and 7 were added to the mixture to wet the clays and form a paste. In a stainless-steel mug, 4 was solubilized in water. Then, 2, 3 and 5 were added and homogenized. After, 1 was added slowly and stirred with glass stick until complete polymer hydration. At last, the content of the porcelain mortar was added to the stainless-steel mug under agitation with a mixer until complete homogenization.

Final aspect observed and conclusion:
Formulations' consistencies were adequate. However, both presented black dots spread in the formulation which did not disappear even after thorough agitation with the mixer (images not taken). Therefore, preparation technique was modified maintaining the same formulations.

Legend: INCI = International Nomenclature of Cosmetic Ingredients; q.s. = *quantum sufficit* (enough amount to complete weight). *Enough amount to obtain adequate pH for preparation technique.

For formulation prototypes "F-c" and "F-d" all clays were sieved before incorporating to avoid the formation of black dots. Also, glycerin was tested together with part of the water to wet clays before incorporating them into the gel, to ease the dispersion. Both formulations were approved for the following steps. Final formulations and preparation technique are described in **Table 11**, together with components' functions in the investigational product.

Table 11. Composition and preparation technique of the formulations' final (approved) versions.

	Final Formulations' Components		Concentration (%w/w)	
	Component name (commercial and INCI)	Function	F-c	F-d
Base formulation	Aristoflex® AVC - *Ammonium Acryloyldimethyltaurate/VP Copolymer*	Gelling agent for aqueous system. Forms anionic gels with cold process and increases formulation's viscosity	2.0	2.0
	Glycerin	Humectant, retains water in the formulation to avoid mask drying	3.0	3.0
	Propylene glycol	Humectant, retains water in the formulation to avoid mask drying	3.0	3.0
	Disodium EDTA	Chelating agent	0.1	0.1

	FocusGuard PE MIT - *Phenoxyethanol (and) Methylisothiazolinone*	Broad spectrum preservative agent with low allergenic potential	0.5	0.5
	Belsil® OW 2100 - *PEG-12 Dimethicone*	Improves formulation sensorial and stability besides acting as co-emulsifier. Also improves spreadability and reduces tacky sensation. Avoids complete mask dehydration while drying	4.0	4.0
	DUB B1215 - *C12-15 Alkylbenzoate*	Emollient without oily sensorial. Avoids complete mask dehydration while drying	4.0	4.0
	Distilled water - *Aqua*	Formulation vehicle and solvent	q.s.	q.s.
	Citric acid (50% w/w solution)	pH corrector	**	**
Active compounds	Red clay - Tersil® CDR (Terramater) - *kaolin*	Active component for skin rejuvenation	3.0	7.0
	Green clay - Tersil® G (Terramater) - *kaolin*	Active component for oiliness reduction, astringency, healing, and antibacterial effect	3.0	7.0
	Black clay -Tersil® CB (Terramater) - *kaolin*	Active component for skin rejuvenation, whitening, healing, and oil absorption	3.0	7.0

Preparation technique:

Clays were individually sieved in 60 mesh sieve and transferred to a previously identified open mouth jar. Then, sieved clays were weighted and transferred to a porcelain mortar to be homogenized and grinded.

C12-15 Alkylbenzoate, PEG-12 Dimethicone and *Glycerin* were weighted and transferred to the same beaker and stirred with glass stick. This mixture was then transferred to the porcelain mortar with the clays and stirred with the pistil until homogenization and formation of a paste. Part of the formulation's water was then weighted and added to this mixture under stirring (10% of total formulation for F-c and 20% for F-d).

In a stainless-steel mug, the EDTA was solubilized in the remaining amount of water. Then, the preservative and *Propylene glycol* were weighted and added. This mixture was homogenized before the addition of the polymer (*Ammonium Acryloyldimethyltaurate/VP Copolymer*). The polymer was incorporated slowly under stirring until the formation of a gel and stirred until no lump was observed.

Finally, the content of the porcelain mortar was added to the stainless-steel mug to be incorporated in the gel under agitation with a mixer until complete homogenization.

Formulation images:

F-c:	
F-d:	

Legend: INCI = International Nomenclature of Cosmetic Ingredients; q.s. = *quantum sufficit* (enough amount to complete weight); w/w = weight/weight.

** Enough amount added to achieve final formulation pH value of 5.5-6.5.

Based on formulation prototypes F-c and F-d, formulations F01, F02 and F03 were developed and corresponded to placebo, minimum concentration version (3% of each clay) and maximum concentration version (7% of each clay), respectively.

Efficacy assessment was performed for formulations F01, F02 and F03 compared to a control site (untreated) using MPA equipment (Courage & Khazaka) after 2 and 4 hours of application, compared to t0 (baseline). Raw data is presented in **ANNEX A**. Data was statistically compared to verify the influence of time and treatment on the variables "sebumetry value", "R0 cutometry value", (regarding skin firmness) and "R7 cutometry value" (skin elasticity). The detailed statistical assessment is in **ANNEX B**.

Oiliness assessment was performed using the Sebumeter® equipment (Sebumetry technique). The equipment's probe contains a mat of synthetic tape, whose part gets exposed at the top to get in contact with the participant's skin for 30 seconds. During this period, the skin's sebum is adsorbed by the tape, which becomes partly transparent (the transparency is proportional to the amount of sebum collected). After sebum collection, the probe is inserted in the equipment's aperture, where a photocell is located and measures the tape's transparency. The light transmission represents sebum content, given as µg sebum / skin cm^2. The oilier the skin, the more transparent the tape (COURAGE & KHAZAKA, 2020).

Sebumetry assessment was performed on the participants' forehead to analyze the influence of clays on skin oiliness. This area was selected due to the higher sebum production tendency in the T-Zone (forehead, nose, and chin) as well as the space available to mark all 4 required sites (untreated control, F01, F02 and F03). Average sebumetry values per experimental time and treatment are presented in **Figure 24**.

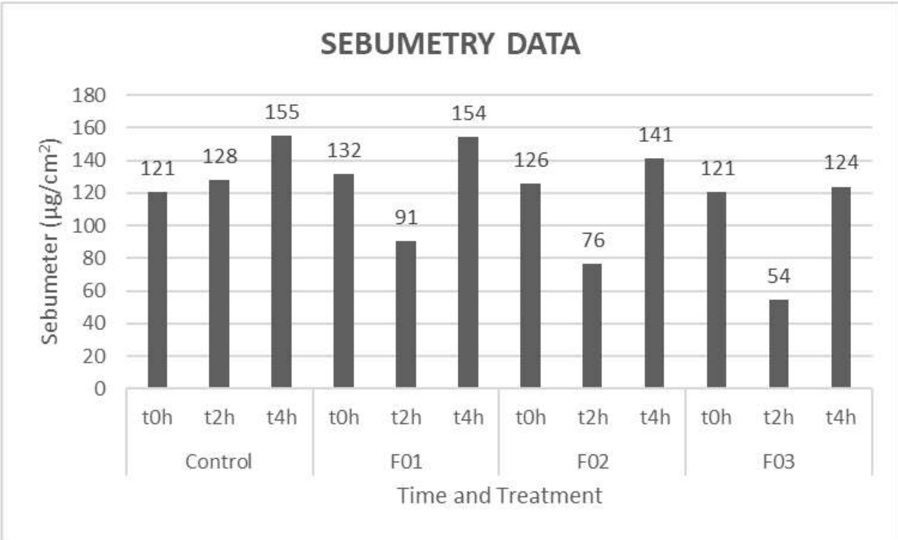


Figure 24. Average sebumetry values per time and treatment

For the sebumetry analysis, each formulation was applied on the marked test site after baseline measurement, in accordance with a randomization table. This procedure allows to avoid bias concerning site location, thus making the study more robust and reliable (JUNIOR, 2006).

After 2 hours of contact with the product (t2h), each formulation was removed with a cotton pad slightly moistened with water, which was passed 3 times in each site with standardized pressure. In each passage, the cotton pad area was renewed. The same procedure was applied to the control site, where no product was applied. This standardized procedure assured complete product removal for all sites without wetting the skin, which avoided interference in the measurements. This lack of interference could be confirmed with the control site's t2h readings.

When comparing t2h with baseline sebumetry measurements, we observed that clays were able to reduce skin oiliness significantly for both concentrations (maximum and minimum), with more significant reduction in maximum amounts (7% of each clay). This proves both efficacy of the active components as well as concentration relevance in the conditions applied. These findings corroborated with literature, as clays are known to adsorb skin oiliness and impurities (CARRETERO, 2002; CARRETERO; GOMES; TATEO, 2006). They have high absorption capacity of skin grease and exudates, and great astringency efficacy, due to their porosity and cation exchange capacity (FAVERO et al., 2016; VELASCO et al., 2016). Still, further research could be performed to compare each clay separately and verify which is more effective concerning sebum reduction. Literature leads us to believe that green clay would perform better than the others.

We also observed that the placebo (F01) caused significant decrease in oiliness in t2h compared with baseline value, which was unexpected, as no component of the formulation is known to adsorb sebum. We can discard methodology inadequacy, as this behavior was not observed in the control site. However, the sebumetry decrease observed for the placebo was significantly smaller compared with the sites treated with clay formulations, therefore not invalidating their efficacy. Still, placebo's efficacy indicates that it would be a good vehicle for oil control formulations.

For t4h reading, measurement was performed 2h after formulations' removal procedure to verify clays' sebum regulation capacity and/or possible "rebound effect". The average values at t4h indicated that the skin reestablished its baseline conditions concerning sebum amount, thus becoming comparable with the control in all sites. This proved that neither did clays present a "rebound effect" to worsen skin conditions due to excessive sebum removal, nor did they regulate sebum production along time. Therefore, clays were effective to remove skin sebum by adsorption during their contact with skin surface, corroborating with literature (SARRUF et al., 2024). When developing an anti-acne formulation, sebum regulation is a relevant factor; so, as clays did not present this effect, it should be associated with other anti-acne sebum-regulator active components for better product efficacy against acne.

It is noteworthy to mention that sebum values in control (untreated) site increased along time, even though participants remained in an acclimatized room at 20 ± 2°C during the whole study. All participants initiated the research earlier in the morning (between 8-10h am, with most of them around 8-9h am) and finished around midday (12-14h). We suggest that this behavior was caused by the cutaneous increase in sebum production along time. However, to establish a correlation between time of the day and sebum production, further research needs to be performed.

Verschoore and co-workers (1993) studied the influence and correlation of the circadian rhythm hormonal factors and sebum excretion on the chest and forehead, assessed with Sebutape. They showed that increase in sebum excretion was related to the increase in the number of secreting follicles. The authors found that the number of secreting follicles on the forehead presented significant differences along the circadian cycle, where in the chest it was almost constant. On the forehead, the number of active follicles increased considerably between 8 and 14h (VERSCHOORE et al., 1993). These findings corroborate with our results in the control site measurements.

Firooz and co-workers (2016) assessed the daytime changes of skin biophysical characteristics, such as skin hydration, sebum amount, and color index, among others. Concerning sebum assessment, it was performed using sebumetry technique on the forearm at 8, 12, and 16h under standardized environmental conditions. When comparing skin oiliness results over time, the authors did not find statistically significant differences, unlike our findings (FIROOZ et al., 2016). Still, the authors used a different measurement site (forearm). This leads us to believe that skin sebum secretions behave differently according to body region.

Cutometry assessment was performed to analyze the influence of clays on skin firmness and elasticity. The Cutometer is a non-invasive device that consists of a probe with a small circular opening connected to a device that generates negative pressure (vacuum bomb). Therefore, when placed on the skin, a defined period of suction is applied, followed by release, causing the skin to enter the probe opening and return when suction is released. The sensor located inside the probe detects how deep skin went inside the probe and how far from the original point it returned after suction (BONAPARTE; ELLIS; CHUNG, 2013; CAMARGO JUNIOR, 2010; COURAGE & KHAZAKA, 2021).

Each reading was set to be performed using 3 cycles consisting of suction (2 seconds) and relaxation (2 seconds). For each cycle, a graph was obtained with "Deformation" as the y axis, and "Time" as x axis. The graph model (skin deformation curve) is presented in **Figure 25** below. The parameters given by the skin deformation curve are described in **Table 12**. Firmness and elasticity results were calculated from the parameters generated in the graph.

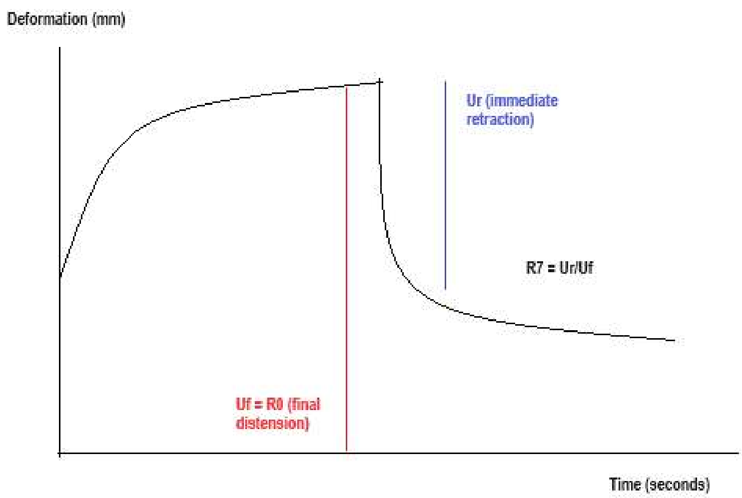


Figure 25. Skin deformation curve obtained by Cutometer® (Courage-Khazaka) – suction and relaxation cycle.

Source: Adapted from (CAMARGO JUNIOR, 2010; COURAGE & KHAZAKA, 2021)

Table 12. Parameters obtained from the skin deformation curve.

Calculated Parameter	Meaning
R0 = Uf	Maximum amplitude – **corresponds to skin firmness**
R1 = R	Minimum amplitude
R2 = Ua/Uf	Situated between maximum amplitude and skin recovery capacity (rough elasticity). The closer to 1 the value (corresponding to 100%), the more elastic the curve.
R3	Last maximum amplitude
R4	Last minimum amplitude
R5 = Ur/Ue	Liquid elasticity. The closer to 1 the value (corresponding to 100%), the more the elasticity.
R6 = Uv/Ue	Ratio between skin viscoelasticity and immediate distension.

R7 = Ur/Uf	Biologic elasticity. Ability of the skin to return to its initial position after deformation. **Corresponds to skin elasticity**
R8	Area above the curve, given by Uf and by suction time. The lower the value, the more elastic the curve.

Source: Adapted from (CAMARGO JUNIOR, 2010; COURAGE & KHAZAKA, 2021)

For the current research, skin firmness (R0 values) and skin elasticity (R7 values) were compared between test sites for each experimental time. Average cutometry values per experimental time and treatment are presented in **Figures 26** and **27**.

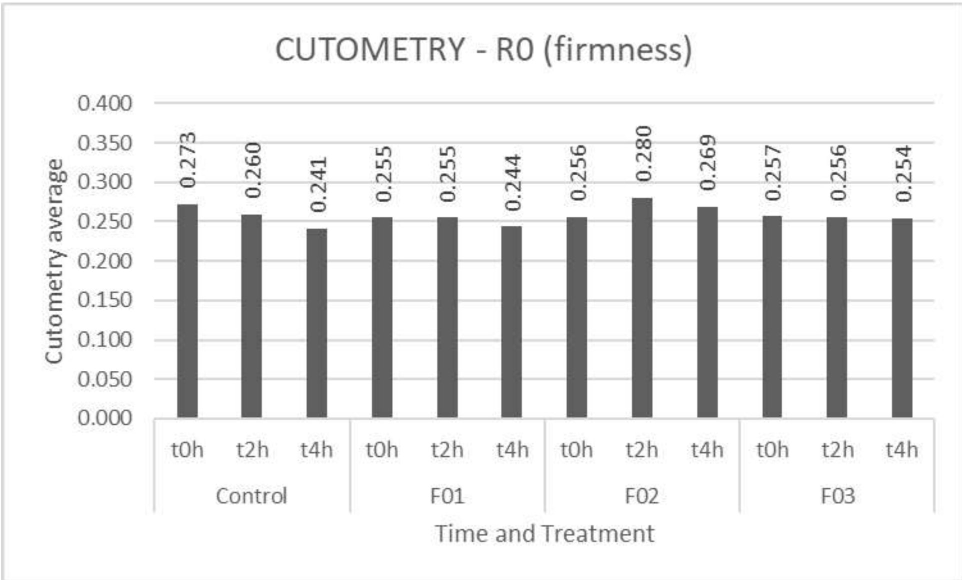


Figure 26. Average cutometry values per time and treatment – R0 (skin firmness)

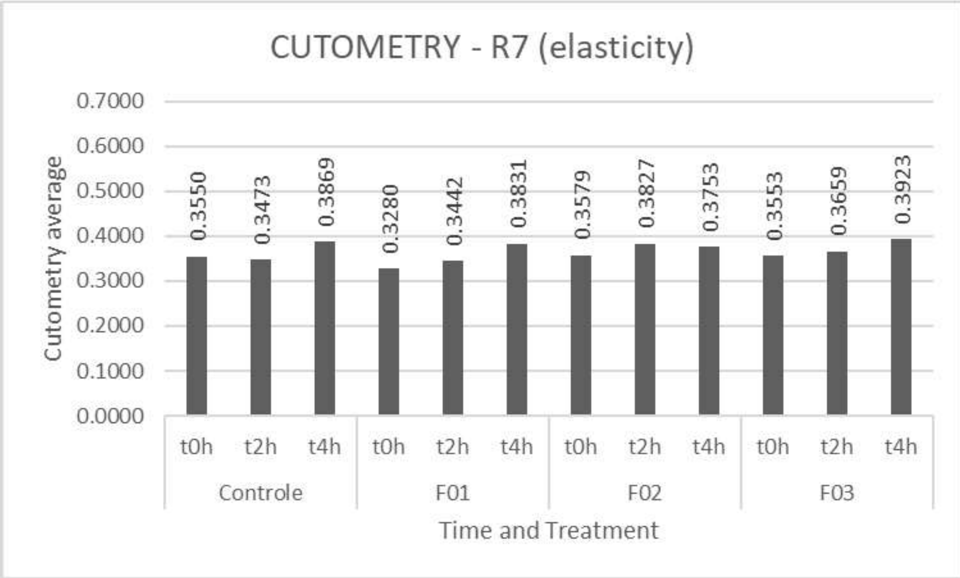


Figure 27. Average cutometry values per time and treatment – R7 (skin elasticity)

For the cutometry analysis, each formulation was applied on the marked test site after baseline measurement, in accordance with a randomization table. After 2 hours of contact with the product (t2h), each formulation was removed with the same procedure described for Sebumetry. This standardized procedure assured complete product removal for all sites without wetting the skin, which avoided interference in the measurements. This lack of interference could be confirmed with the control site's t2h readings.

When statistically comparing R0 values (regarding skin firmness), no significant difference was observed along time for any test site. However, when looking at average firmness values, F02 (minimum clay concentration), there was a tendency to increase firmness at t2h (after removal from 2h of contact) and a return to baseline at t4h, which could be indicative of a lifting effect (JUNIOR, 2006; VISERAS et al., 2021). However, this behavior was not observed for F03 (maximum clay concentration), for which average firmness values remained constant. Our findings concerning firmness values were unexpected when compared to the literature, as, in theory, clays used in these formulations are known for their lifting effect (VISERAS et al., 2021). According to Balduino (2016) and Silva (2011), both red and black clays are used for skin rejuvenation and have lifting effect, as well as other relevant characteristics which could justify their use in antiaging cosmetics (BALDUINO, 2016; SILVA, 2011). Still, although literature was found mentioning clays' ability to improve skin firmness in theory, no literature was found showing statistically significant cutometry improvement concerning the use of clays against control sites. Velasco and co-workers (2016) also assessed firmness and elasticity of clay face masks applied on the skin by cutometry and found that there were no statistically significant differences in cutometry values between times and between formulations. They therefore concluded that "the composition of the different clays did not affect the skin viscoelasticity behavior in the short-term clinical study".

After water evaporation, clay masks tend to harden and contract, causing mechanical tension (DARÉ et al., 2015; ZAGUE, 2007). Our formulation tended to take longer to harden and did not dry completely as does a mask composed by the simple mixture of water and clay, as observed by experience. This might have been the reason why the lifting effect was neither observed nor mentioned to have been felt by the study participants. Still, further research comparing the same clays incorporated in different formulation vehicles should be conducted to test this hypothesis.

As far as elasticity results are concerned (R7 values), no significant difference was observed between treatments, with a slight tendency in all test sites to increase with time, including in control and placebo sites. Our elasticity results also corroborated with findings from Velasco and co-workers (2016), who assessed skin elasticity after application of clay mineral face masks in a short-term study (experimental times: baseline, t20min, t1h, and t2h after mask removal). The authors too found no significant differences between the experimental times per treatment.

Concerning the obtained results for cutometry assessments and the sensations informed to have been felt by study participants, further research should be conducted to assess the firmness and elasticity of clays incorporated into different vehicles compared to clay applied after its simple mixture with water to verify the influence of the dispersion medium in the short-term results. Also, long-term assessments should be analyzed, with measurements at least after 30 days of treatment.

7. Conclusions

- All clays were characterized and presented different compositions. Black clay was the one with the simplest mineralogic composition, lowest density, and smallest particle size. It was rich in Si and Al. Green clay presented expandable clay mineral (smectite) and the highest density. Red clay presented the largest particle average size and was the richest in iron content.
- All clays were characterized by thermal analysis and presented two characteristic events: water loss (dehydration) and dihydroxylation of kaolinite, turning into meta kaolinite.
- In Sebumetry assessment, clays showed significant reduction of skin oiliness on the forehead in both concentrations adopted after 2h of contact compared to control, to baseline, and to placebo formulation. However, after 4h (2h after removal), no significant difference was observed compared with the control.
- In Cutometry assessment, clays did not present significant efficacy in skin firmness and elasticity improvement when compared to both control and placebo test sites. Further studies should be performed comparing the efficacy of different vehicles with dispersed clays.
- Clay mask formulations were developed with black, red, and green clay dispersed in an oil-free gel vehicle, which proved to be adequate for oily skin.

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ANNEX A – RAW DATA (SEBUMETRY AND CUTOMETRY)

SEBUMETRY DATA																
Part. Nb.	Participants Characteristics				Control			F01			F02			F03		
	Initials	Gender	Age	Phototype	t0h	t2h	t4h	t0h	t2h	t4h	t0h	t2h	t4h	t0h	t2h	t4h
1	CSS	F	35	III	166	133	157	162	51	185	157	94	145	164	49	169
2	VHSM	M	29	III	194	211	242	192	119	220	128	154	213	187	157	216
4	ABB	F	29	IV	121	124	220	171	135	218	178	96	195	161	42	204
5	LPPS	M	42	III	121	103	202	156	99	211	86	78	114	99	58	150
6	JPV	F	23	II	155	148	170	157	119	159	172	116	176	129	125	132
9	AAH	F	45	III	128	182	190	166	94	180	152	86	201	185	92	190
10	FDS	F	36	III	107	162	211	85	128	128	75	69	131	88	26	123
12	NSN	F	26	III	197	188	185	163	129	110	184	121	149	86	11	79
13	GRS	F	26	IV	151	147	153	145	167	193	157	110	177	167	113	159
15	JRP	F	23	III	126	162	168	156	31	74	133	19	73	80	14	52
16	MBA	F	33	III	73	86	59	90	77	82	103	16	56	90	13	56
17	CFH	F	24	III	78	157	183	91	33	143	93	24	171	85	19	90
19	LND	F	33	III	66	55	78	67	46	98	54	14	69	62	24	123
23	CIVR	F	41	III	93	94	105	74	75	135	97	76	142	102	56	131
26	BBS	F	27	IV	78	74	161	75	26	145	71	18	126	92	17	72
29	DLF	F	23	IV	73	68	85	133	84	89	121	91	124	98	57	90
30	SPC	M	31	III	96	58	94	99	53	232	131	78	173	92	26	99
31	ARP	F	30	III	197	204	202	228	152	213	192	157	204	194	110	192
32	GBL	F	25	III	69	60	60	93	96	152	105	60	107	124	48	78
33	ARM	F	18	IV	122	145	176	128	99	119	129	52	79	128	29	71

CUTOMETRY DATA																
Part. Nb.	Participants Characteristics				R0 Parameter (skin firmness)											
					Control			F01			F02			F03		
	Initials	Gender	Age	Phototype	t0h	t2h	t4h	t0h	t2h	t4h	t0h	t2h	t4h	t0h	t2h	t4h
1	CSS	F	35	III	0.432	0.439	0.346	0.428	0.479	0.284	0.433	0.523	0.448	0.291	0.337	0.301
5	LPPS	M	42	III	0.384	0.276	0.270	0.446	0.324	0.225	0.346	0.358	0.345	0.294	0.300	0.314
7	ASS	M	49	IV	0.292	0.298	0.298	0.262	0.212	0.315	0.286	0.354	0.282	0.319	0.324	0.269
8	NR	F	56	IV	0.275	0.297	0.272	0.168	0.169	0.114	0.305	0.301	0.221	0.165	0.159	0.208
9	AAH	F	45	III	0.189	0.217	0.134	0.240	0.192	0.161	0.118	0.156	0.134	0.192	0.179	0.142
10	FDS	F	36	III	0.516	0.342	0.322	0.549	0.438	0.440	0.505	0.454	0.465	0.456	0.384	0.431
11	MA	F	42	III	0.160	0.191	0.169	0.152	0.223	0.169	0.210	0.267	0.272	0.299	0.271	0.312
14	RMN	F	48	IV	0.482	0.414	0.388	0.326	0.341	0.383	0.250	0.293	0.294	0.312	0.311	0.274
16	MBA	F	33	III	0.285	0.282	0.227	0.270	0.319	0.301	0.406	0.411	0.442	0.432	0.382	0.364
18	CASOP	F	52	IV	0.294	0.371	0.336	0.306	0.323	0.331	0.283	0.336	0.346	0.271	0.306	0.347
19	LND	F	33	III	0.307	0.305	0.233	0.311	0.329	0.298	0.251	0.219	0.227	0.287	0.265	0.181
21	JMN	F	34	IV	0.257	0.236	0.217	0.321	0.397	0.337	0.247	0.325	0.276	0.285	0.279	0.249
22	DDP	F	37	II	0.240	0.192	0.223	0.208	0.183	0.174	0.297	0.273	0.211	0.301	0.267	0.300
23	CIVR	F	41	III	0.156	0.174	0.203	0.222	0.194	0.182	0.192	0.246	0.230	0.154	0.177	0.188
24	AKM	M	48	III	0.148	0.168	0.154	0.110	0.176	0.173	0.220	0.230	0.244	0.195	0.217	0.247
25	KJCM	F	35	IV	0.324	0.300	0.293	0.136	0.119	0.139	0.175	0.201	0.228	0.210	0.244	0.205
27	MMGBA	F	41	IV	0.205	0.203	0.233	0.198	0.184	0.258	0.178	0.247	0.242	0.247	0.250	0.279
28	EDS	F	60	III	0.150	0.161	0.151	0.147	0.234	0.233	0.104	0.121	0.110	0.136	0.099	0.105
30	SPC	M	31	III	0.217	0.191	0.225	0.167	0.096	0.177	0.139	0.151	0.115	0.144	0.162	0.210
31	ARP	F	30	III	0.141	0.133	0.132	0.136	0.173	0.185	0.174	0.143	0.239	0.144	0.200	0.152

CUTOMETRY DATA																
Part. Nb.	Participants Characteristics				R7 Parameter (skin elasticity)											
					Control			F01			F02			F03		
	Initials	Gender	Age	Phototype	t0h	t0h	t2h	t4h	t0h	t0h	t2h	t4h	t0h	t2h	t4h	t0h
1	CSS	F	35	III	0.3773	0.3622	0.4075	0.3061	0.2401	0.3063	0.3072	0.3040	0.3817	0.2955	0.3175	0.2857
5	LPPS	M	42	III	0.2109	0.3949	0.2963	0.2511	0.3704	0.5867	0.3902	0.5391	0.5188	0.3537	0.4433	0.3885
7	ASS	M	49	IV	0.3116	0.2752	0.4631	0.3244	0.3868	0.5651	0.3217	0.4548	0.3759	0.4138	0.4074	0.3903
8	NR	F	56	IV	0.4364	0.5017	0.5404	0.2857	0.3550	0.6053	0.5180	0.4817	0.6471	0.3212	0.4214	0.4423
9	AAH	F	45	III	0.3598	0.3456	0.3209	0.4750	0.3802	0.2981	0.3559	0.3846	0.3284	0.4219	0.4469	0.4577
10	FDS	F	36	III	0.2829	0.3655	0.4099	0.3151	0.3379	0.3114	0.3901	0.3921	0.4172	0.3618	0.3411	0.3109
11	MA	F	42	III	0.3937	0.1361	0.2604	0.2697	0.2422	0.2426	0.2714	0.3633	0.3493	0.3478	0.3506	0.3301
14	RMN	F	48	IV	0.2075	0.2150	0.2655	0.3190	0.2199	0.2663	0.1880	0.2969	0.2857	0.2468	0.1576	0.2372
16	MBA	F	33	III	0.3123	0.2766	0.3348	0.2963	0.3197	0.3189	0.3276	0.3309	0.3529	0.3218	0.3455	0.3764
18	CASOP	F	52	IV	0.3844	0.2075	0.2143	0.3856	0.2848	0.3142	0.3145	0.2321	0.2197	0.3838	0.3595	0.3112
19	LND	F	33	III	0.3583	0.3902	0.3734	0.3505	0.3799	0.4698	0.4024	0.4155	0.4537	0.3937	0.4075	0.3867
21	JMN	F	34	IV	0.3424	0.4322	0.4055	0.3427	0.3728	0.4036	0.4170	0.4985	0.4058	0.2702	0.3584	0.3936
22	DDP	F	37	II	0.3042	0.3438	0.3632	0.3798	0.4098	0.3621	0.3232	0.3919	0.3033	0.2658	0.3333	0.3500
23	CIVR	F	41	III	0.3910	0.3448	0.3596	0.3468	0.3299	0.3571	0.3646	0.3293	0.4000	0.3247	0.2938	0.4043
24	AKM	M	48	III	0.3243	0.3750	0.3961	0.2636	0.2955	0.2197	0.2000	0.2783	0.2377	0.3231	0.3548	0.2996
25	KJCM	F	35	IV	0.4969	0.4900	0.5051	0.3015	0.4034	0.3669	0.4914	0.3333	0.3728	0.3905	0.4303	0.4683
27	MMGBA	F	41	IV	0.3268	0.3498	0.3648	0.2323	0.3859	0.2752	0.4101	0.3887	0.3719	0.3927	0.3720	0.4373
28	EDS	F	60	III	0.3733	0.3975	0.3907	0.2177	0.2308	0.3047	0.1923	0.2893	0.1727	0.2500	0.3737	0.4476
30	SPC	M	31	III	0.4654	0.3141	0.5289	0.4192	0.3958	0.5311	0.4101	0.4040	0.4000	0.5972	0.3086	0.5286
31	ARP	F	30	III	0.4397	0.4286	0.5379	0.4779	0.5434	0.5568	0.5632	0.5455	0.5105	0.4306	0.4950	0.5987

ANNEX B – STATISTICAL ANALYSIS (SEBUMETRY AND CUTOMETRY)

Statistical analysis of Sebumetry – Skin Oiliness (Minitab)

Modelo Linear Generalizado: Sebumetria versus Tempo; Tratamento

Método

Codificação de fator	(-1; 0; +1)

Informações dos Fatores

Fator	Tipo	Níveis	Valores
Tempo	Fixo	3	0; 2; 4
Tratamento	Fixo	4	0; 1; 2; 3

Análise de Variância

Fonte	GL	SQ (Aj.)	QM (Aj.)	Valor F	Valor-P
Tempo	2	131002	65501	29,41	0,000
Tratamento	3	40810	13603	6,11	0,001
Erro	234	521193	2227		
Falta de ajuste	6	31262	5210	2,42	0,027
Erro puro	228	489931	2149	*	*
Total	239	693006			

Sumário do Modelo

S	R2	R2(aj)	R2(pred)
47,1945	24,79%	23,19%	20,89%

Coeficientes

Termo	Coef	EP de Coef	Valor-T	Valor-P	VIF
Constante	118,54	3,05	38,91	0,000	
Tempo					
0	6,12	4,31	1,42	0,157	1,33
2	-31,18	4,31	-7,24	0,000	1,33
Tratamento					
0	16,01	5,28	3,03	0,003	1,50
1	6,96	5,28	1,32	0,189	1,50
2	-4,01	5,28	-0,76	0,448	1,50

Equação de Regressão

Sebumetria = 118,54 + 6,12 Tempo_0 - 31,18 Tempo_2 + 25,06 Tempo_4
+ 16,01 Tratamento_0
+ 6,96 Tratamento_1 - 4,01 Tratamento_2 - 18,96 Tratamento_3

Ajustados e Diagnósticos para Observações Atípicas

Obs.	Sebumetria	Ajuste	Resíd	Resíd Pad	
22	211,00	103,37	107,63	2,31	R
38	204,00	103,37	100,63	2,16	R
51	59,00	159,61	-100,61	-2,16	R
59	60,00	159,61	-99,61	-2,14	R
78	228,00	131,62	96,38	2,07	R

R Resíduo grande

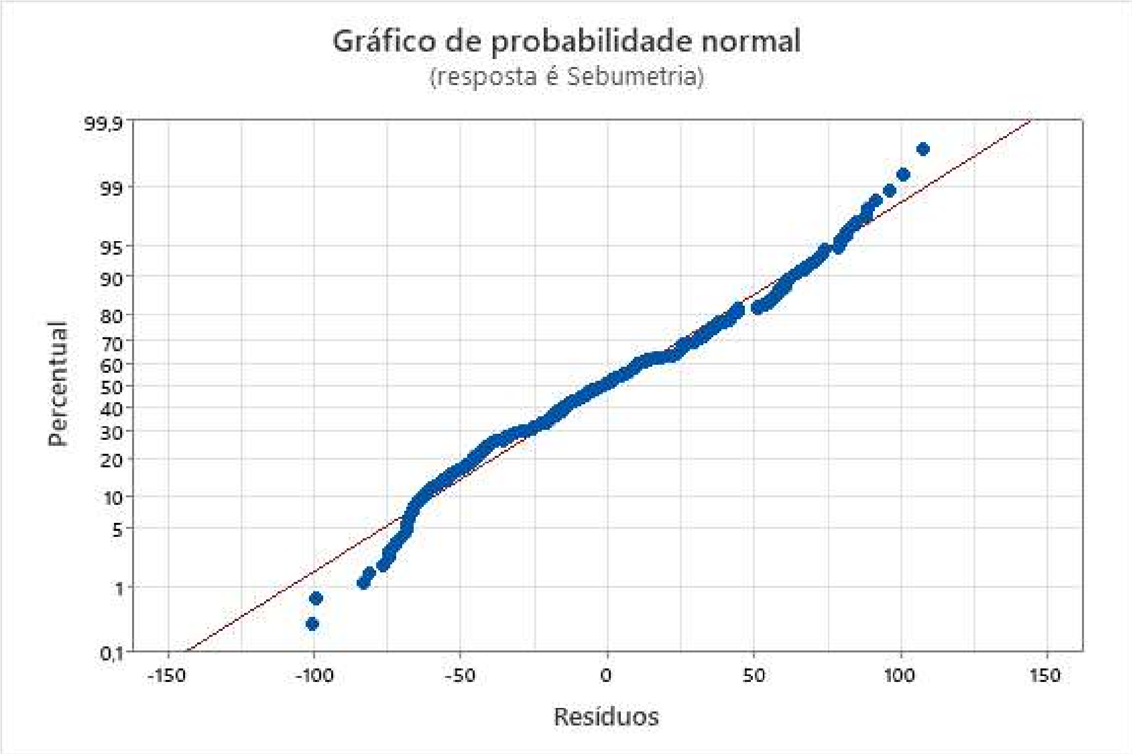
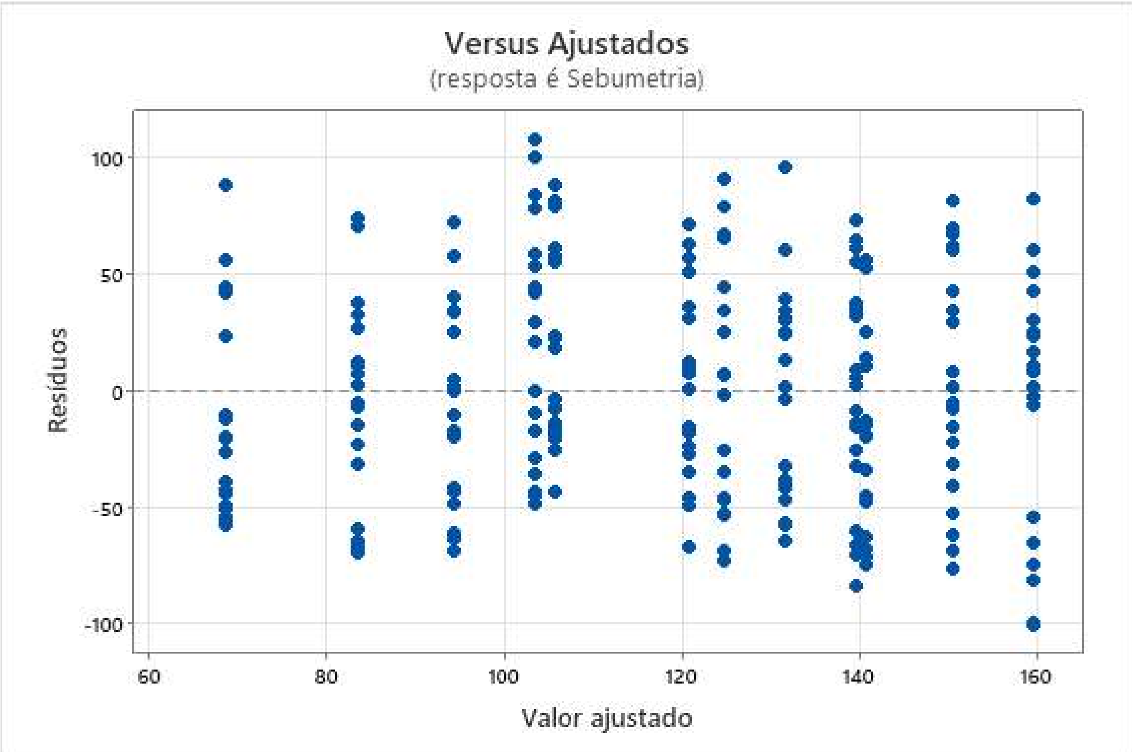
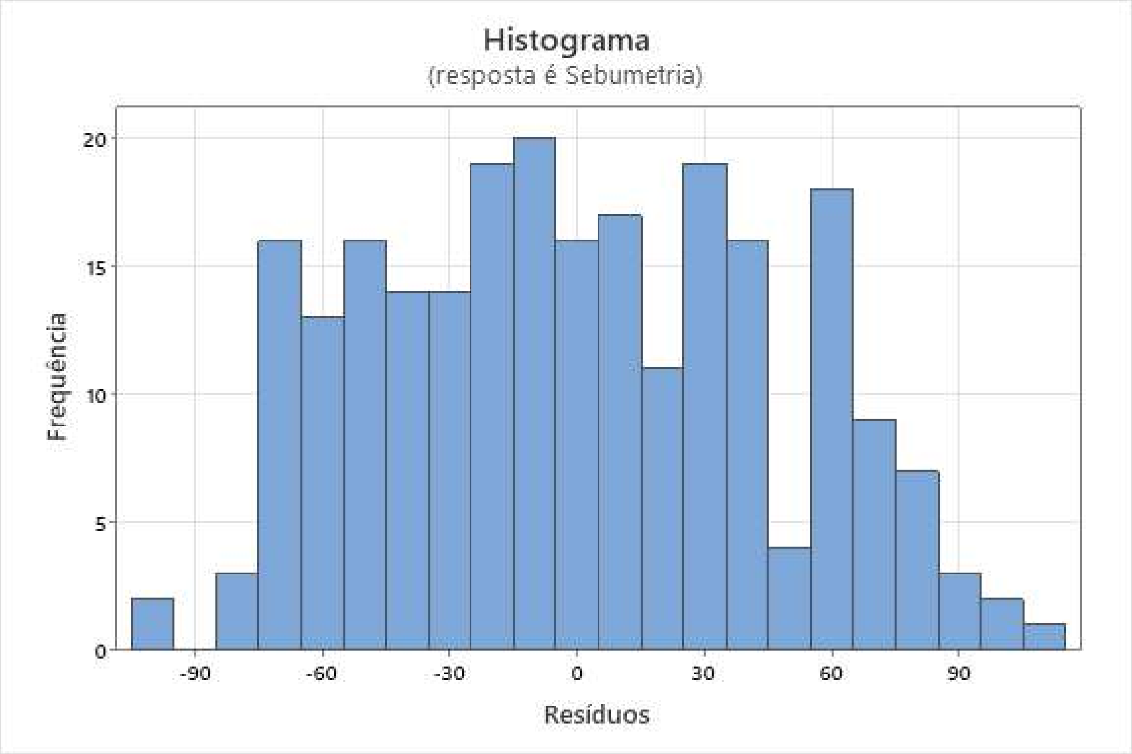
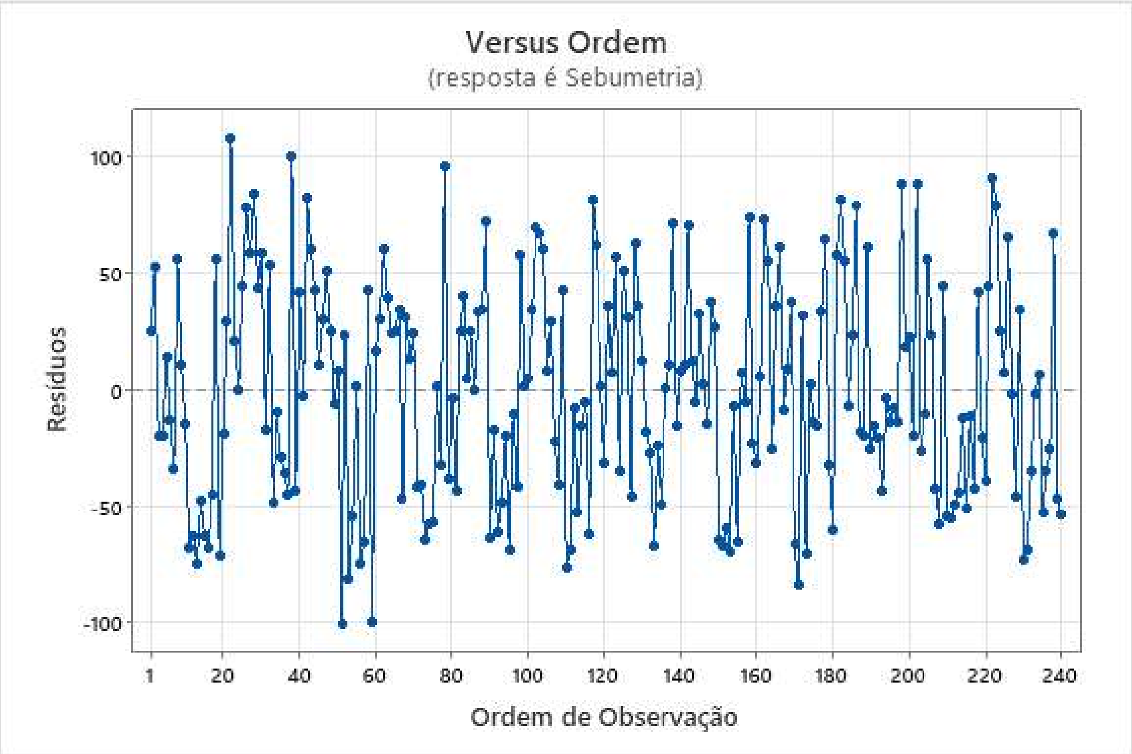


Gráfico de probabilidade normal
(resposta é Sebumetria)
Percentual
99,9
99
95
90
80
70
60
50
40
30
20
10
5
1
0,1
-150
-100
-50
0
50
100
150
Resíduos


Versus Ajustados
(resposta é Sebumetria)
Resíduos
100
50
0
-50
-100
60
80
100
120
140
160
Valor ajustado


Histograma
(resposta é Sebumetria)
Frequência
Resíduos
20
15
10
5
0
-90
-60
-30
0
30
60
90


Versus Ordem
(resposta é Sebumetria)
Resíduos
Ordem de Observação
100
50
0
-50
-100
1
20
40
60
80
100
120
140
160
180
200
220
240

Statistical analysis of Cutometry – Firmess R0 (Minitab)

Modelo Linear Generalizado: Cutometria R0 (firmeza) versus Tempo; Tratamento

Método

Codificação de fator	(-1; 0; +1)

Informações dos Fatores

Fator	Tipo	Níveis	Valores
Tempo	Fixo	3	0; 2; 4
Tratamento	Fixo	4	0; 1; 2; 3

Análise de Variância

Fonte	GL	SQ (Aj.)	QM (Aj.)	Valor F	Valor-P
Tempo	2	0,00507	0,002537	0,28	0,757
Tratamento	3	0,00935	0,003116	0,34	0,795
Erro	234	2,13520	0,009125		
Falta de ajuste	6	0,01264	0,002107	0,23	0,968
Erro puro	228	2,12256	0,009309	*	*
Total	239	2,14962			

Sumário do Modelo

S	R2	R2(aj)	R2(pred)
0,0955237	0,67%	0,00%	0,00%

Coeficientes

Termo	Coef	EP de Coef	Valor-T	Valor-P	VIF
Constante	0,25825	0,00617	41,88	0,000	
Tempo					
0	0,00187	0,00872	0,21	0,830	1,33
2	0,00446	0,00872	0,51	0,610	1,33
Tratamento					
0	-0,0004	0,0107	-0,04	0,969	1,50
1	-0,0068	0,0107	-0,64	0,525	1,50
2	0,0101	0,0107	0,94	0,347	1,50

Equação de Regressão

Cutometria R0 (firmeza) = 0,25825 + 0,00187 Tempo_0 + 0,00446 Tempo_2
- 0,00633 Tempo_4
- 0,0004 Tratamento_0 - 0,0068 Tratamento_1
+ 0,0101 Tratamento_2
- 0,0028 Tratamento_3

Ajustados e Diagnósticos para Observações Atípicas

Obs.	Cutometria R0 (firmeza)	Ajuste	Resíd	Resíd Pad
6	0,5160	0,2597	0,2563	2,72 R
8	0,4820	0,2597	0,2223	2,36 R
62	0,4460	0,2533	0,1927	2,04 R
66	0,5490	0,2533	0,2957	3,13 R
81	0,4790	0,2559	0,2231	2,37 R
106	0,4400	0,2451	0,1949	2,07 R
126	0,5050	0,2702	0,2348	2,49 R
141	0,5230	0,2728	0,2502	2,65 R
166	0,4650	0,2620	0,2030	2,15 R
186	0,4560	0,2573	0,1987	2,11 R

R Resíduo grande

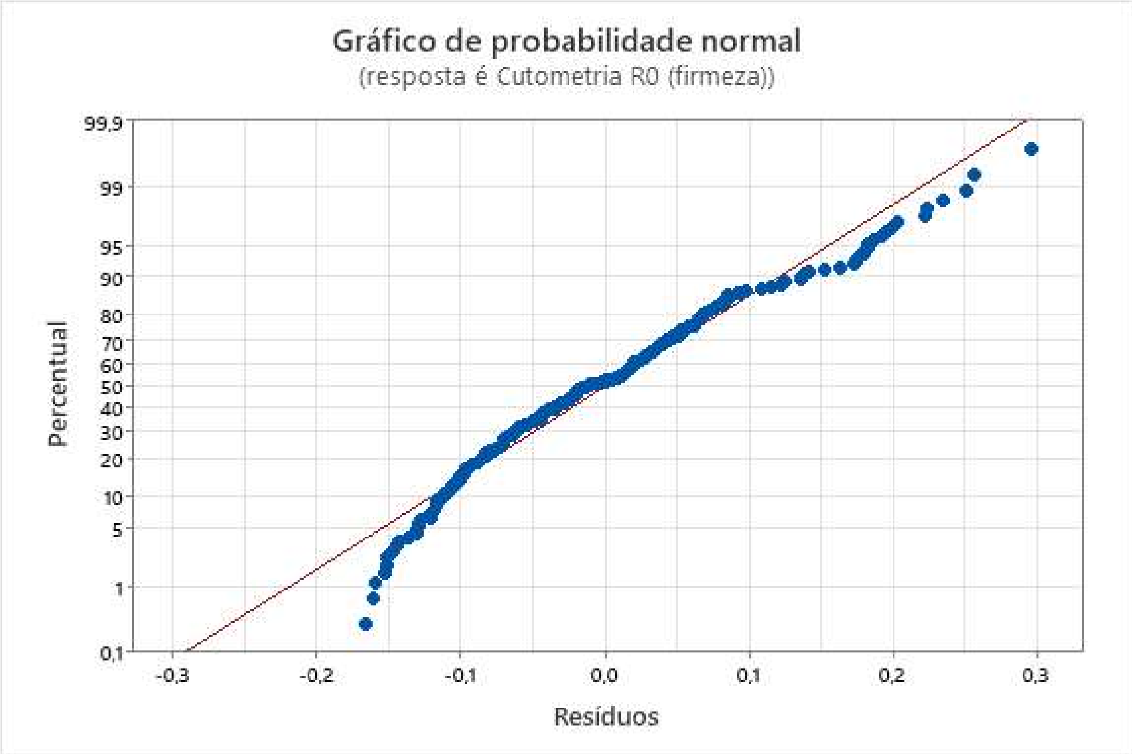
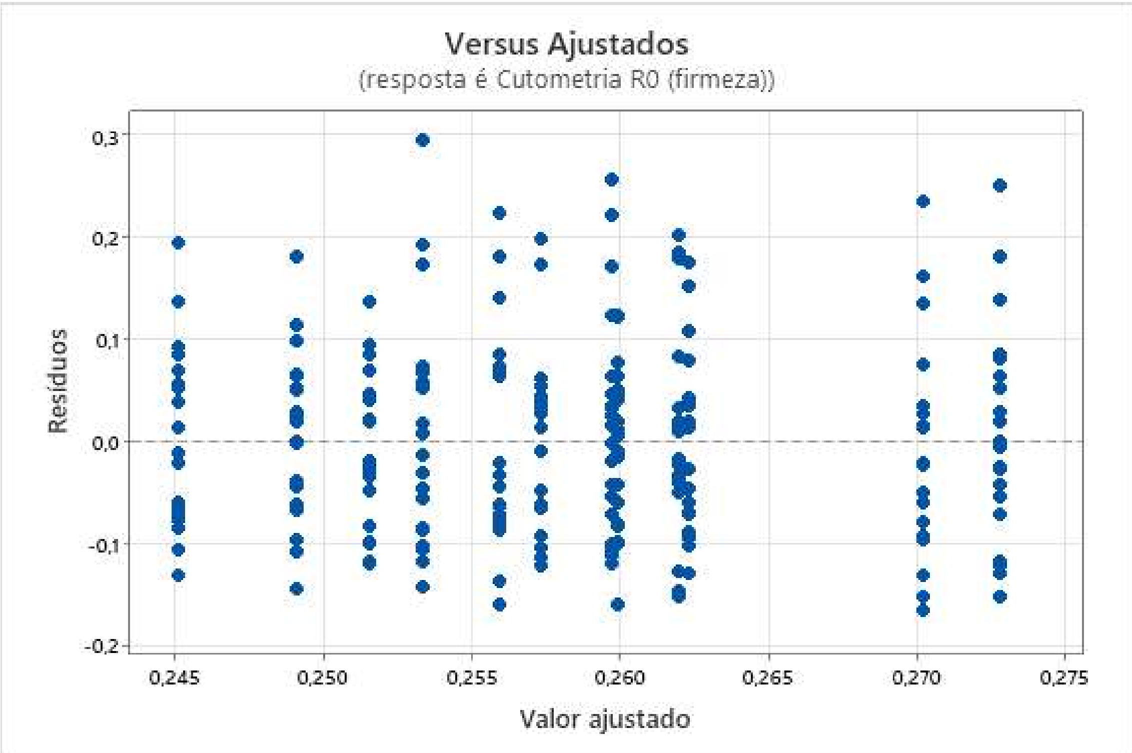
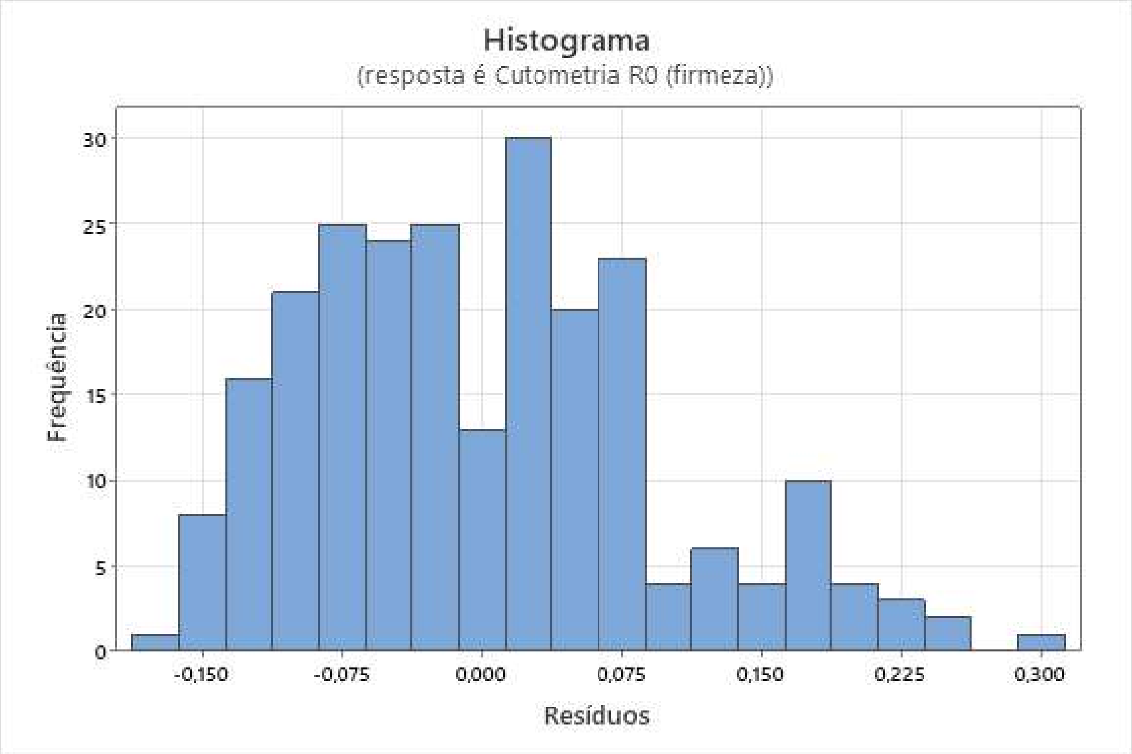
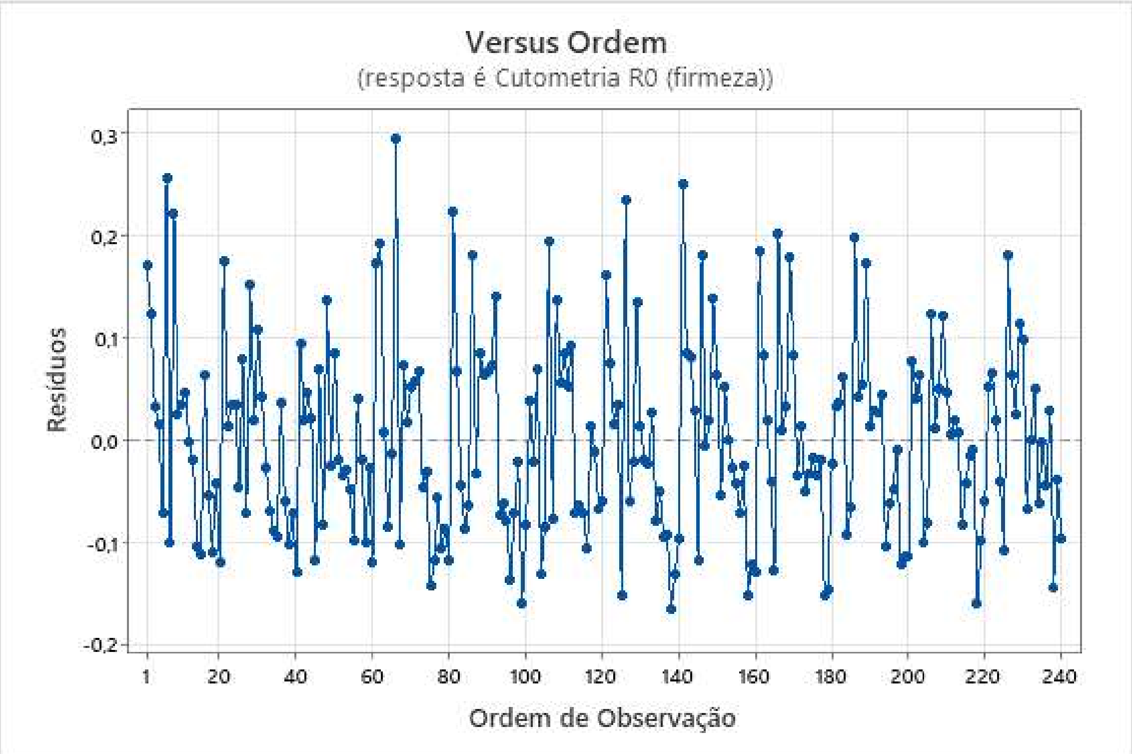


Gráfico de probabilidade normal
(resposta é Cutometria R0 (firmeza))
Percentual
99,9
99
95
90
80
70
60
50
40
30
20
10
5
1
0,1
-0,3
-0,2
-0,1
0,0
0,1
0,2
0,3
Resíduos


Versus Ajustados
(resposta é Cutometria R0 (firmeza))
Resíduos
0,3
0,2
0,1
0,0
-0,1
-0,2
0,245
0,250
0,255
0,260
0,265
0,270
0,275
Valor ajustado


Histograma
(resposta é Cutometria R0 (firmeza))
Frequência
Resíduos
30
25
20
15
10
5
0
-0,150
-0,075
0,000
0,075
0,150
0,225
0,300


Versus Ordem
(resposta é Cutometria R0 (firmeza))
Resíduos
Ordem de Observação
0,3
0,2
0,1
0,0
-0,1
-0,2
1
20
40
60
80
100
120
140
160
180
200
220
240

Statistical analysis of Cutometry – Elasticity R7 (Minitab)

Modelo Linear Generalizado: Cutometria R7 (elasticidade) versus Tempo; Tratamento

Método

Codificação de fator	(-1; 0; +1)

Informações dos Fatores

Fator	Tipo	Níveis	Valores
Tempo	Fixo	3	0; 2; 4
Tratamento	Fixo	4	0; 1; 2; 3

Análise de Variância

Fonte	GL	SQ (Aj.)	QM (Aj.)	Valor F	Valor-P
Tempo	2	0,05229	0,026144	3,26	0,040
Tratamento	3	0,01586	0,005285	0,66	0,578
Erro	234	1,87848	0,008028		
Falta de ajuste	6	0,01834	0,003057	0,37	0,895
Erro puro	228	1,86014	0,008159	*	*
Total	239	1,94663			

Sumário do Modelo

S	R2	R2(aj)	R2(pred)
0,0895975	3,50%	1,44%	0,00%

Coeficientes

Termo	Coef	EP de Coef	Valor-T	Valor-P	VIF
Constante	0,36449	0,00578	63,02	0,000	
Tempo					
0	-0,01543	0,00818	-1,89	0,060	1,33
2	-0,00446	0,00818	-0,55	0,586	1,33
Tratamento					
0	-0,0014	0,0100	-0,14	0,887	1,50
1	-0,0127	0,0100	-1,27	0,205	1,50
2	0,0075	0,0100	0,75	0,456	1,50

Equação de Regressão

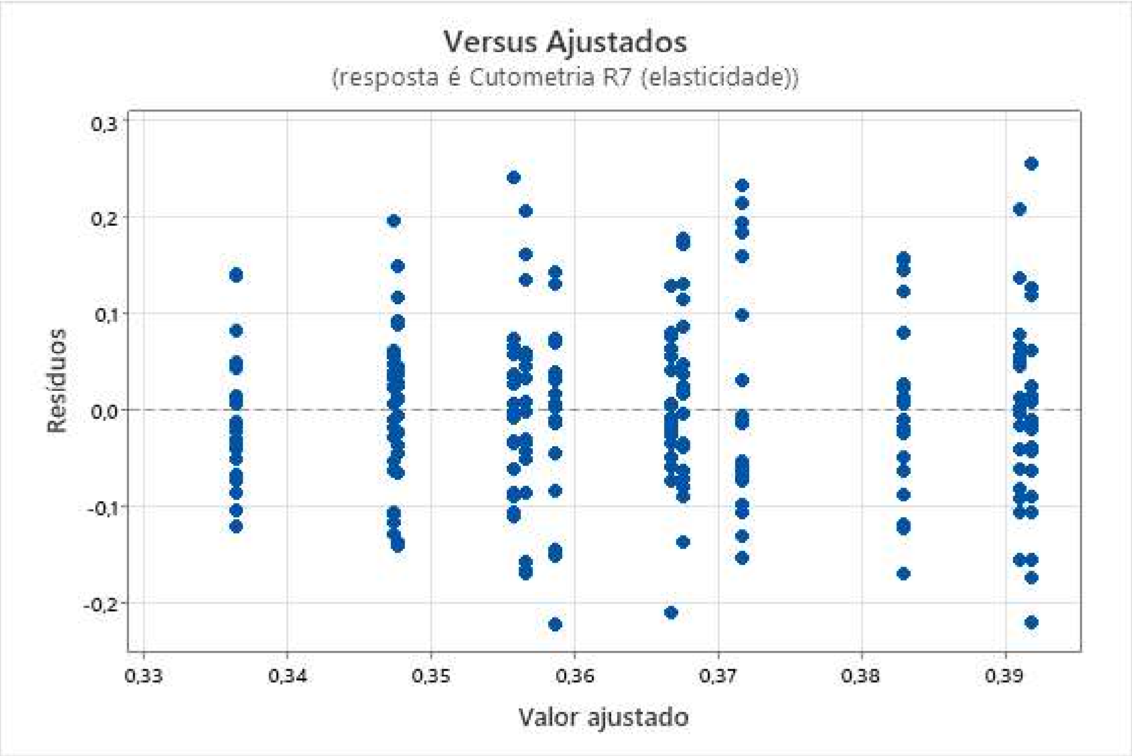
Cutometria R7 (elasticidade) = 0,36449 - 0,01543 Tempo_0 - 0,00446 Tempo_2 + 0,01989 Tempo_4 - 0,0014 Tratamento_0 - 0,0127 Tratamento_1 + 0,0075 Tratamento_2 + 0,0067 Tratamento_3

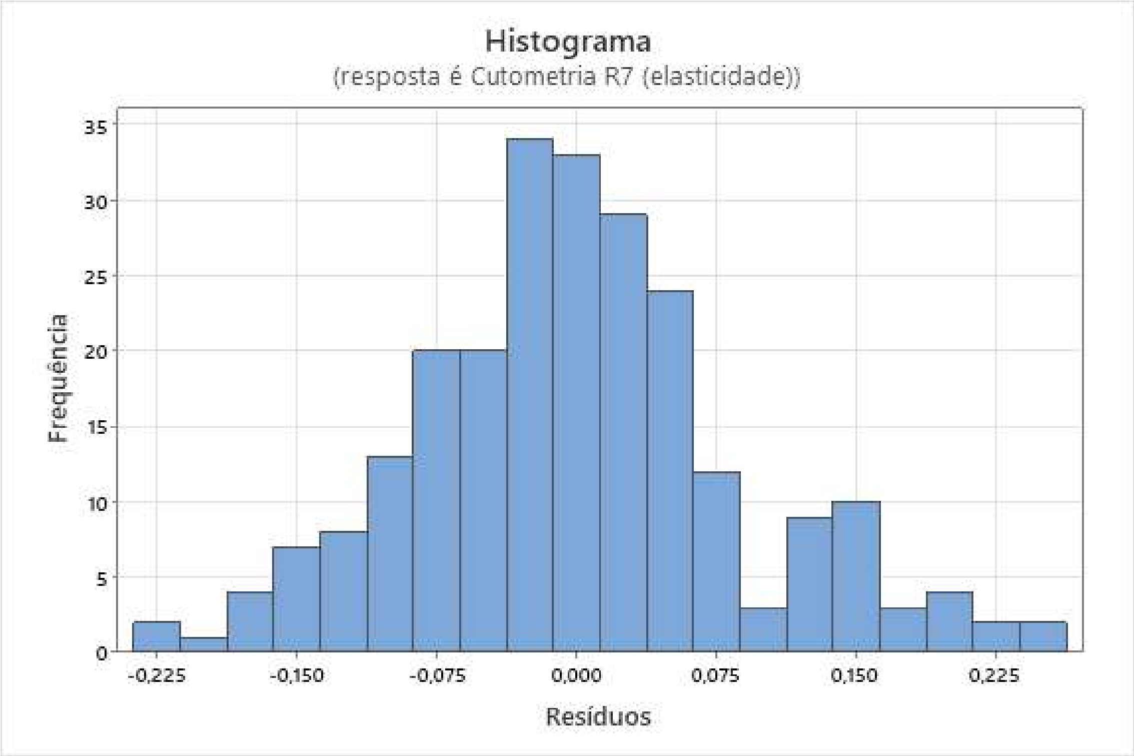
Ajustados e Diagnósticos para Observações Atípicas

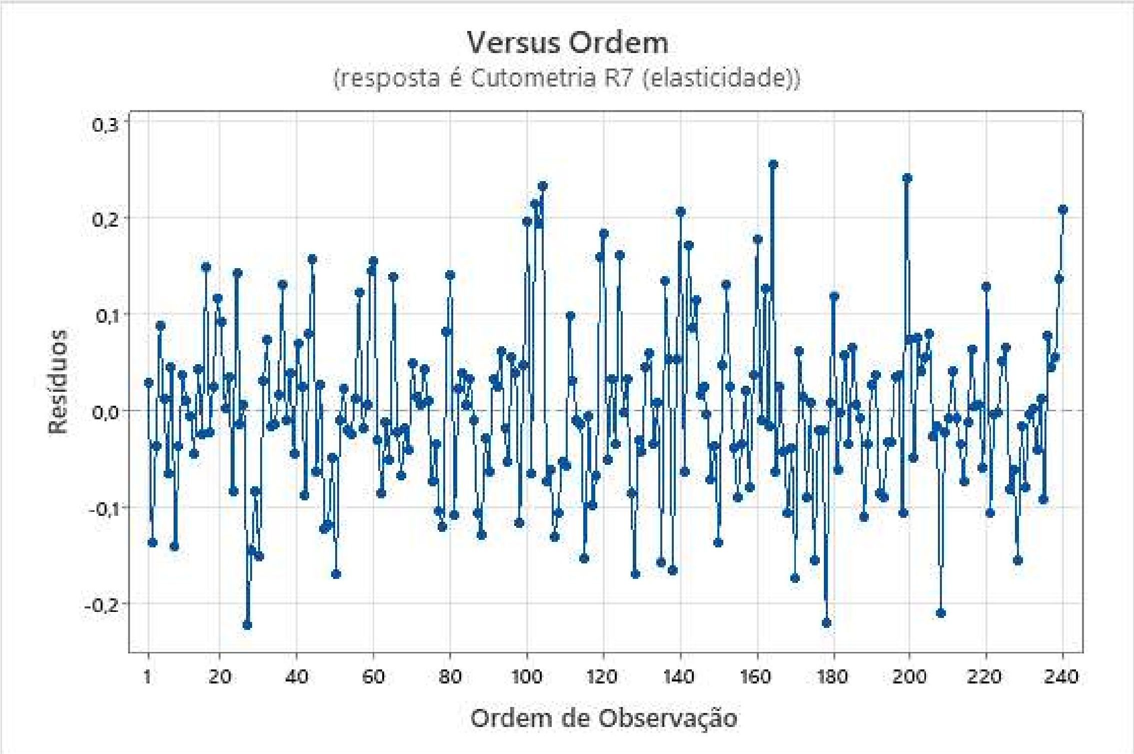
Obs.	Cutometria R7 (elasticidade)	Ajuste	Resíd	Resíd Pad	
27	0,1361	0,3586	-0,2225	-2,52	R
100	0,5434	0,3473	0,1961	2,22	R
102	0,5867	0,3717	0,2150	2,43	R
103	0,5651	0,3717	0,1934	2,19	R
104	0,6053	0,3717	0,2336	2,64	R
120	0,5568	0,3717	0,1851	2,09	R
140	0,5632	0,3565	0,2067	2,34	R
160	0,5455	0,3675	0,1780	2,01	R
164	0,6471	0,3919	0,2552	2,89	R
178	0,1727	0,3919	-0,2192	-2,48	R
199	0,5972	0,3557	0,2415	2,73	R

208	0,1576	0,3667	-0,2091	-2,36 R
240	0,5987	0,3911	0,2076	2,35 R

R Resíduo grande




Histograma
(resposta é Cutometria R7 (elasticidade))
Frequência
35
30
25
20
15
10
5
0
-0,225
-0,150
-0,075
0,000
0,075
0,150
0,225
Resíduos


Versus Ordem
(resposta é Cutometria R7 (elasticidade))
Resíduos
0,3
0,2
0,1
0,0
-0,1
-0,2
1
20
40
60
80
100
120
140
160
180
200
220
240
Ordem de Observação

ANNEX C – INFORMED CONSENT TERMS

Universidade de São Paulo
Faculdade de Ciências Farmacêuticas

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO – TCLE

AVALIAÇÃO INSTRUMENTAL DA EFICÁCIA DE PRODUTO COSMÉTICO - CINÉTICA

1. Informações do Participante da Pesquisa (para preenchimento)

Nome: ____

Documento de Identidade (número): __________________________ Sexo: ( ) M ( ) F

Local de Nascimento: ______________________ Data de Nascimento: ___ / ___ / ______

Endereço: ____

Complemento: ________________ Bairro: _________________ CEP: ________________

Cidade: ____ Estado: ______________

Telefone (residencial): ___________________ Telefone (celular): ____________________

2. Dados sobre a Pesquisa

Título da Pesquisa: Atributos cosméticos provenientes da associação entre argilas: ensaios *ex vivo* e *in vivo*

Pesquisador Responsável: Dr. André Rolim Baby / fone: (11) 3091-2358 e (11) 94225-0033
- Professor Doutor da Universidade de São Paulo (Faculdade de Ciências Farmacêuticas)

Pesquisador Associado: Fernanda Daud Sarruf / fone: (11) 97152-3795
- Pesquisadora da Universidade de São Paulo (Faculdade de Ciências Farmacêuticas)

Tempo de duração do ensaio: 1 dia por participante

Tempo de permanência do participante na Instituição: 5 horas

Risco da Pesquisa: Mínimo

Faixa etária dos participantes da pesquisa: 18 a 60 anos

Você está sendo convidado a participar de uma pesquisa que tem como objetivo avaliar a eficácia na melhora da firmeza e da elasticidade, e na redução de oleosidade da pele de produtos cosméticos. Pedimos que leia detalhadamente este Termo de Consentimento e, apenas se concordar com os itens mencionados, assine-o em duas vias (uma delas será entregue a você). Todas as dúvidas que surgirem durante ou após o estudo serão prontamente esclarecidas.

Para a realização deste estudo, você comparecerá ao local da pesquisa (Laboratório IPclin - Instituto de Pesquisa Clínica Integrada Ltda, situado à Rua Doutor Leonardo Cavalcante, 314 – Centro – Jundiaí/SP) em apenas um dia, permanecendo no local por um período máximo de cinco horas.

Nesse dia, serão marcados locais no seu rosto com caneta cirúrgica para delimitar as regiões da pele onde os produtos serão aplicados. Então, serão realizadas medidas iniciais com os equipamentos que avaliam a oleosidade da pele (Sebumeter®) e a firmeza / elasticidade (Cutometer®). Essas medidas não são invasivas e não causam dor nem desconforto. Na medida, usamos sondas que são colocadas em cima do seu rosto, e lemos o valor obtido no visor do computador do equipamento.

Então, os produtos serão aplicados nas regiões demarcadas usando seringa descartável sem agulha. Uma das áreas será mantida virgem (sem aplicação de produto). Essa área virgem será mantida como controle.

Depois de duas e de quatro horas da aplicação dos produtos, serão realizadas novas medidas com os dois equipamentos. Durante esse período, você permanecerá em uma sala, com ar condicionado ligado, e não poderá remover os produtos do seu rosto.

Solicitamos que durante o período de estudo (incluindo três dias antes do início do ensaio) você não altere seus hábitos de higiene, dieta, medicamentos, cosméticos e exercícios; e não se exponha excessivamente ao sol. Solicitamos que você não aplique qualquer produto no local de realização do teste desde três (03) dias antes até a finalização da pesquisa.

Todas as substâncias que compõem o produto são de uso habitual e são consideradas seguras para a finalidade que se destinam e na forma de aplicação proposta. No entanto, ainda que raras, ressaltamos que reações inesperadas, decorrentes da aplicação tópica do(s) produto(s) em teste, podem ocorrer, incluindo dermatites de contato e/ou alérgica com intensidades variáveis, podendo ser graves.

Garantimos que os cuidados e acompanhamento necessários serão fornecidos pelos pesquisadores e médico dermatologista durante todas as etapas do estudo. Caso você necessite de assistência médica, decorrente da pesquisa, ou esclarecimentos, favor entrar em contato com os pesquisadores responsáveis pela pesquisa: Dr. André Rolim Baby, nos telefones (11) 3091-2358 e (11) 94225-0033 e/ou Fernanda Daud Sarruf, no telefone (11) 97152-3795, ou pessoalmente no Laboratório de Cosmetologia da Faculdade de Ciências Farmacêuticas da Universidade de São Paulo, localizada na Avenida Professor Lineu Prestes, n°580, Bloco 15; ou ainda no Laboratório IPclin - Instituto de Pesquisa Clínica Integrada Ltda, situado à Rua Doutor Leonardo Cavalcante, 314 – Centro – Jundiaí/SP onde será realizada a pesquisa. Os pesquisadores estarão disponíveis 24 horas por dia para atendê-lo, inclusive em feriados e finais de semana.

Em caso de reações adversas que necessitem de acompanhamento ambulatorial e/ou internação, os responsáveis pela pesquisa lhe acompanharão até o **Hospital Universitário da Universidade de São Paulo** (Av. Professor Lineu Prestes, 2565, Cidade Universitária, São Paulo, SP, 05508-000, telefone: (11) 3091-9200) ou no SUS, que possui infraestrutura adequada para qualquer tratamento que se faça necessário. Garantimos que todas as despesas decorrentes do tratamento serão arcadas, de forma integral, pelos responsáveis por esta pesquisa.

Você não obterá nenhum benefício financeiro e à sua saúde, pois este é um produto cosmético. No entanto, você estará ajudando, como participante desta pesquisa, a ampliar o conhecimento sobre a segurança e eficácia de produtos cosméticos. Garantimos que as suas despesas, decorrentes exclusivamente da participação nesta pesquisa, serão completamente ressarcidas.

Nota: em decorrência do tempo de permanência dos participantes (até 5 horas), será fornecido um lanche a estes entre as medidas de duas e de quatro horas com os equipamentos.

Todas as informações obtidas e declaradas por você serão tratadas de maneira confidencial, sendo que apenas as pessoas diretamente ligadas a este estudo e autoridades legais poderão ter acesso, sem com isso, violar a confidencialidade. Se os resultados do estudo forem publicados, sua identidade continuará sendo preservada.

Você pode retirar sua participação a qualquer momento do estudo, comunicando sua desistência ao pesquisador responsável pelo seu acompanhamento, sem que isso acarrete qualquer consequência negativa a você.

Consentimento Pós-Esclarecido

Declaro que, após ter sido convenientemente esclarecido pelo pesquisador e ter entendido o que me foi explicado, consinto em participar do presente Protocolo de Pesquisa. Declaro ter recebido uma via (não uma cópia) original deste Termo com todas as páginas rubricadas e com a assinatura do pesquisador responsável.

São Paulo, ______ de _________________ de ________.

_________________________________ _

Assinatura do Participante de Pesquisa

_________________________________ _

Assinatura do Pesquisador Responsável

Para qualquer questão, dúvida, esclarecimento ou reclamação sobre aspectos éticos relativos a este protocolo de pesquisa, favor entrar em contato com o **Comitê de Ética em Pesquisa** da **Faculdade de Ciências Farmacêuticas** da **Universidade de São Paulo**: Av. Prof. Lineu Prestes, 580, Bloco 13 A, Butantã, São Paulo, CEP: 05508-000, telefones (11) 3091-3622 e (11) 3091-3677, e-mail: **cepfcf@usp.br**.

ANNEX D – ETHICS COMMITTEE APPROVAL LETTER

FIRST APPROVAL (INITIAL PROJECT)

USP - FACULDADE DE CIÊNCIAS FARMACÊUTICAS DA UNIVERSIDADE DE SÃO PAULO - FCF/USP

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: ATRIBUTOS COSMÉTICOS PROVENIENTES DA ASSOCIAÇÃO ENTRE CAFEÍNA E ARGILAS: ENSAIOS EX VIVO E IN VIVO

Pesquisador: André Rolim Baby

Área Temática:

Versão: 3

CAAE: 15193119.6.0000.0067

Instituição Proponente: Faculdade de Ciências Farmacêuticas da Universidade de São Paulo

Patrocinador Principal: CNPQ

DADOS DO PARECER

Número do Parecer: 3.998.887

Apresentação do Projeto:

A boa aparência da pele impacta diretamente na autoestima. Por este motivo, há interesse no desenvolvimento de produtos que sejam comprovadamente eficazes quanto à melhora de atributos cosméticos. Argilas são amplamente utilizadas no mercado cosmético/estético no auxílio ao tratamento destas condições. A cafeína é uma metilxantina com propriedades antioxidante e anticelulite difundida no meio cosmético; porém, não há estudos em literatura avaliando o desempenho da combinação dessas classes de compostos ativos em preparações faciais frente a atributos cosméticos desejáveis para essa região experimental. Esta pesquisa tem como objetivo o preparo de amostras contendo associações de cafeína a argilas verde e rosa com posterior determinação de sua eficácia frente a atributos cosméticos diversos por métodos in vivo e ex vivo. As amostras serão submetidas a avaliações subjetivas e objetivas de eficácia in vivo, empregando-se as técnicas de sebumetria, cutometria, análise de imagem e avaliação dermatológica por meio de escalas extraídas de literatura. Ademais, será determinada a eficácia antioxidante das mesmas. Ao final, o desempenho dos produtos investigados, para cada ensaio realizado, será estatisticamente comparado para verificar a influência de cada substância, bem como de eventuais efeitos de sinergismo.

Objetivo da Pesquisa:

Avaliação da eficácia frente a atributos cosméticos de amostras contendo a associação de cafeína e

Endereço: Av. Prof. Lineu Prestes, 580, Bloco 13A, sala 112
Bairro: Butantã **CEP:** 05.508-000
UF: SP **Município:** SAO PAULO
Telefone: (11)3091-3622 **Fax:** (11)3031-8986 **E-mail:** cepfcf@usp.br

argilas verde e rosa, por estudos em humanos.

Avaliação dos Riscos e Benefícios:

Riscos:

O grau dos riscos associados ao estudo pode variar de pessoa para pessoa, levando em consideração as diferentes características fisiológicas e pessoais dos participantes. Todas as matérias primas utilizadas no produto são aprovadas para uso tópico e não são tóxicas. Existem desconfortos e riscos mínimos para o participante do estudo, entretanto, como qualquer produto, poderá causar reações inesperadas como "vermelhidão", "inchaço", "coceira" e "ardor" nos locais de aplicação deste.

Benefícios:

Possibilidade de comprovação da eficácia do produto investigacional frente à acne, rugas, aumento da firmeza e elasticidade da pele, redução da oleosidade, garantindo à comunidade um produto eficaz.

Comentários e Considerações sobre a Pesquisa:

Neste estudo serão inicialmente preparadas diferentes formulações de máscara facial contendo cafeína associada a argilas verde e rosa (conforme descrito na Tabela 1 do Projeto). As argilas utilizadas para preparo das amostras serão previamente caracterizadas. As formulações desenvolvidas serão previamente submetidas a análises preliminares de estabilidade, e serão armazenadas de modo a garantir a integridade dos produtos ao longo da pesquisa.

As formulações serão avaliadas por meio de ensaios in vivo e ex vivo. Os ensaios in vivo serão realizados no IPclin Instituto de Pesquisa Clínica Integrada Ltda. Os ensaios in vivo envolvem: Avaliação instrumental da redução da oleosidade da pele por Sebumetria; Avaliação instrumental da firmeza e elasticidade da pele por Cutometria; Avaliação instrumental fotográfica do desempenho do produto investigacional por captura de imagem da face antes e após uso, com equipamento Reveal Imager - Canfield®, e avaliação instrumental de eficácia - Sebumetria e Cutometria; Avaliação subjetiva dermatológica do produto investigacional quanto a: não comedogenicidade, não acnegenicidade, eficácia redutora de acne, eficácia redutora de rugas. Os ensaios ex vivo serão realizadas no Laboratório de Cosmetologia da FCF-USP (determinação da atividade antioxidante ex vivo por tape stripping com posterior determinação da peroxidação lipídica).

Considerações sobre os Termos de apresentação obrigatória:

Trata-se de uma reavaliação, onde o autor responde as questões levantadas pelo CEP, anexa as declarações solicitadas e atualiza o cronograma. A folha de rosto, declarações estão de acordo com as normas e os TCLEs foram corrigidos adequando a linguagem e esclarecendo melhor o participante. O autor envia uma carta resposta com as solicitações indicadas conforme solicitado. Considero que foram realizadas as correções e inadequações, tornando o projeto adequado e condizente com as normas vigentes.

Recomendações:

Sem recomendações.

Conclusões ou Pendências e Lista de Inadequações:

Aprovado, sem pendências ou inadequações.

Considerações Finais a critério do CEP:

APROVADO

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1369459.pdf	29/01/2020 20:04:22		Aceito
Outros	Declaracao_equipe_de_pesquisa.pdf	29/01/2020 20:03:34	André Rolim Baby	Aceito
Outros	Carta_Resposta_CEPUSP_argila_ARB_jan2020.pdf	29/01/2020 20:02:51	André Rolim Baby	Aceito
Declaração de Instituição e Infraestrutura	Declaracao_IPclin.pdf	29/01/2020 20:02:17	André Rolim Baby	Aceito
Projeto Detalhado / Brochura Investigador	PROJETO_CEP_doutorado_PLBR_27JAN2020_GRIFADO.pdf	29/01/2020 20:01:53	André Rolim Baby	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	27JAN2020TCLE_tape_stripping_GRIFADO.pdf	29/01/2020 20:01:34	André Rolim Baby	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	27JAN2020TCLE_instrumentais_com_teste_em_uso_GRIFADO.pdf	29/01/2020 20:01:19	André Rolim Baby	Aceito
TCLE / Termos de	27JAN2020TCLE_instrumentais_ante	29/01/2020	André Rolim Baby	Aceito

USP - FACULDADE DE CIÊNCIAS FARMACÊUTICAS DA UNIVERSIDADE DE SÃO PAULO - FCF/USP

Continuação do Parecer: 3.996.887

Assentimento / Justificativa de Ausência	braco_GRIFADO.pdf	20:01:08	André Rolim Baby	Aceito
Folha de Rosto	Folha_rosto_CEP.pdf	27/01/2020 14:53:52	André Rolim Baby	Aceito
Declaração de Pesquisadores	Declaracao_part_fds.pdf	06/06/2019 11:26:31	André Rolim Baby	Aceito
Declaração de Pesquisadores	Declaracao_part_ARB.pdf	06/06/2019 11:26:18	André Rolim Baby	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

SAO PAULO, 29 de Abril de 2020

Assinado por:
Neuza Mariko Aymoto Hassimotto
(Coordenador(a))

SECOND APPROVAL (AMENDMENT)

USP - FACULDADE DE CIÊNCIAS FARMACÊUTICAS DA UNIVERSIDADE DE SÃO PAULO - FCF/USP

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: ATRIBUTOS COSMÉTICOS PROVENIENTES DA ASSOCIAÇÃO ENTRE ARGILAS: ENSAIOS EX VIVO E IN VIVO

Pesquisador: André Rolim Baby

Área Temática:

Versão: 4

CAAE: 15193119.6.0000.0067

Instituição Proponente: Faculdade de Ciências Farmacêuticas da Universidade de São Paulo

Patrocinador Principal: CNPQ

DADOS DO PARECER

Número do Parecer: 5.532.318

Apresentação do Projeto:

A boa aparência da pele impacta diretamente na autoestima. Por este motivo, há interesse no desenvolvimento de produtos que sejam comprovadamente eficazes quanto à melhora de atributos cosméticos. Argilas são amplamente utilizadas no mercado cosmético/estético no auxílio ao tratamento destas condições. Esta pesquisa tem como objetivo o preparo de amostras contendo associações de argilas com posterior determinação de sua eficácia frente a atributos cosméticos diversos por métodos in vivo e ex vivo. As amostras serão submetidas a avaliações objetivas de eficácia in vivo, empregando-se as técnicas de sebumetria e cutometria. Ademais, será determinada a eficácia antioxidante das mesmas. Ao final, o desempenho dos produtos investigacionais para cada ensaio realizado será estatisticamente comparado para verificar a influência de cada substância, bem como eventuais efeitos de sinergismo.

Objetivo da Pesquisa:

Avaliar a eficácia frente a atributos cosméticos de amostras contendo a associação de argilas, por estudos em humanos.

Avaliação dos Riscos e Benefícios:

Riscos:

Riscos: O grau dos riscos associados ao estudo pode variar de pessoa para pessoa, levando em consideração as diferentes características fisiológicas e pessoais dos participantes. Todas as matérias primas utilizadas no produto são aprovadas para uso tópico e não são tóxicas. Existem desconfortos e riscos mínimos para o participante do estudo, entretanto, como qualquer produto, poderá causar reações inesperadas como "vermelhidão", "inchaço", "coceira" e "ardor" nos locais de aplicação deste.

Benefícios:
Comprovação da eficácia do produto investigacional frente ao aumento da firmeza e elasticidade da pele, redução da oleosidade, garantindo à comunidade um produto eficaz.

Comentários e Considerações sobre a Pesquisa:
Trata-se de um projeto de Doutorado e a partir do estudo, espera-se comprovar e comparar o aumento da firmeza e elasticidade da pele e redução da oleosidade com o uso de formulação cosmética com argila. O projeto foi anteriormente aprovado pelo CEP; entretanto, devido a pandemia de Covid-19, os pesquisadores notificam as alterações no projeto de pesquisa.

Considerações sobre os Termos de apresentação obrigatória:
Os seguintes termos foram apresentados e estão em conformidade (folha de rosto, declarações dos componentes da equipe e TCLE).

Recomendações:
As alterações efetuadas no projeto inicial não impactam os princípios éticos envolvidos
na condução da pesquisa. Recomenda-se a aprovação da emenda.

Conclusões ou Pendências e Lista de Inadequações:
Sem pendências.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1962431_E1.pdf	20/06/2022 12:16:07		Aceito
Folha de Rosto	folhaDeRosto_JUN2022_ASSIN.pdf	20/06/2022 12:15:18	André Rolim Baby	Aceito
Projeto Detalhado	PROJETO_CEP_doutorado_PLBR_JUN	07/06/2022	André Rolim Baby	Aceito

/ Brochura Investigador	022_GRIFADO.pdf	17:11:25	André Rolim Baby	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	JUN2022TCLE_tape_stripping_GRIFADO.pdf	07/06/2022 17:10:37	André Rolim Baby	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	JUN2022TCLE_instrumentais_cinetica_GRIFADO.pdf	07/06/2022 17:09:57	André Rolim Baby	Aceito
Outros	EMENDA_JUN2022.pdf	07/06/2022 17:09:21	André Rolim Baby	Aceito
Outros	Declaracao_equipe_de_pesquisa_GRIFADO.pdf	07/06/2022 17:07:29	André Rolim Baby	Aceito
Declaração de Instituição e Infraestrutura	Declaracao_IPclin.pdf	29/01/2020 20:02:17	André Rolim Baby	Aceito
Declaração de Pesquisadores	Declaracao_part_fds.pdf	06/06/2019 11:26:31	André Rolim Baby	Aceito
Declaração de Pesquisadores	Declaracao_part_ARB.pdf	06/06/2019 11:26:18	André Rolim Baby	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

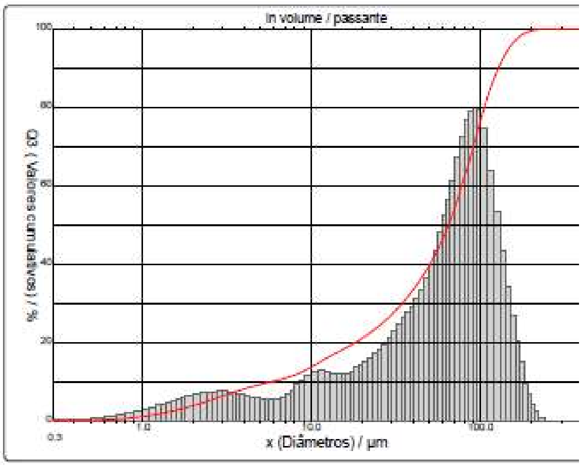
SAO PAULO, 18 de Julho de 2022

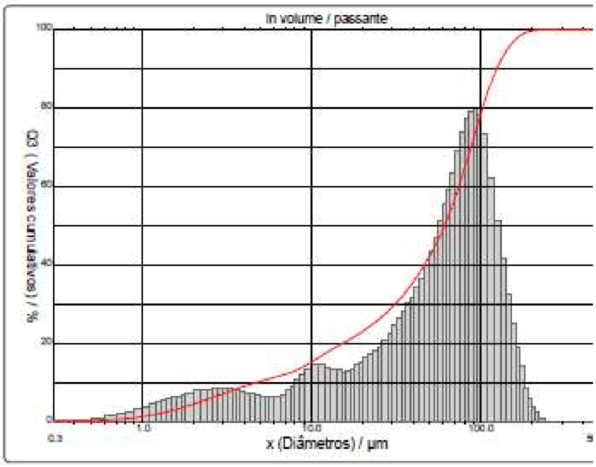
Assinado por:
Mauricio Yonamine
(Coordenador(a))

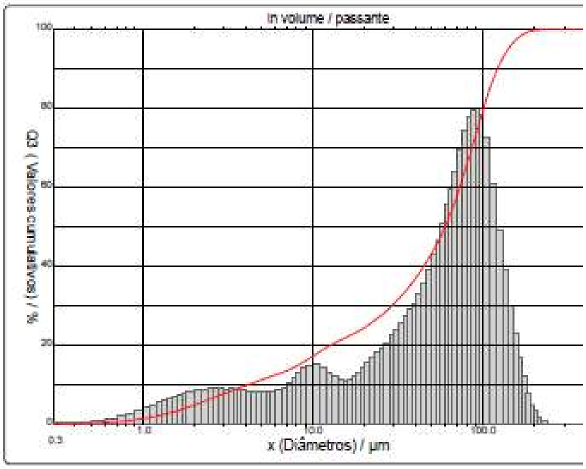
Endereço: Av. Prof. Lineu Prestes, 580, Bloco 13A, sala 112
Bairro: Butantã **CEP:** 05.508-000
UF: SP **Município:** SAO PAULO
Telefone: (11)3091-3622 **Fax:** (11)3031-8986 **E-mail:** cepfcf@usp.br

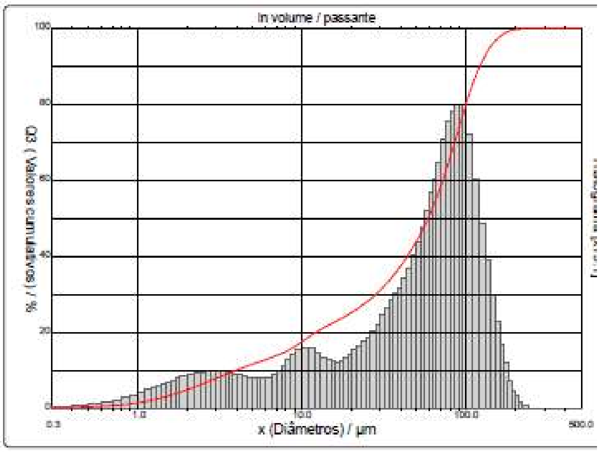
ANNEX E – PARTICLE SIZE DISTRIBUTION GRAPHS / RESULTS

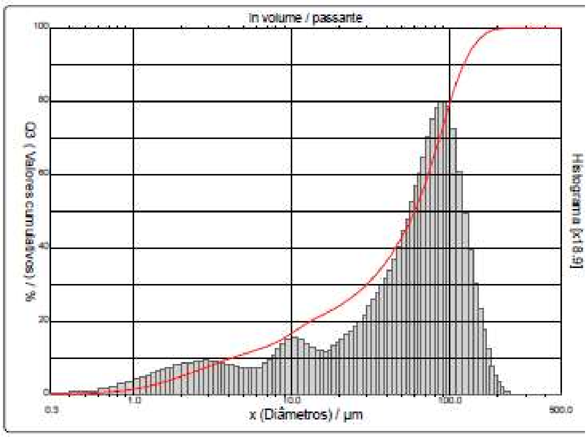
Particle size distribution of Black clay – graphs per sample and overlapped curves:

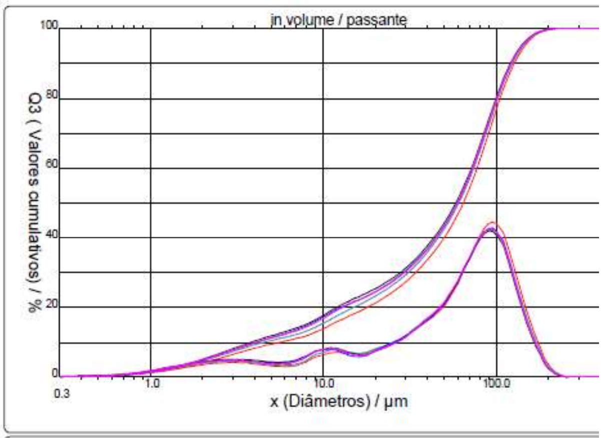




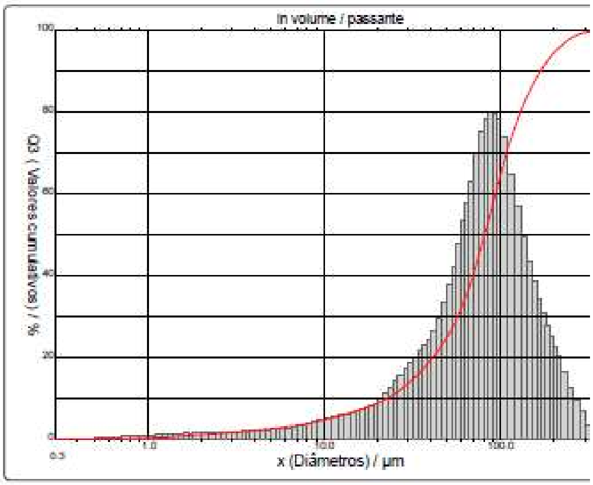


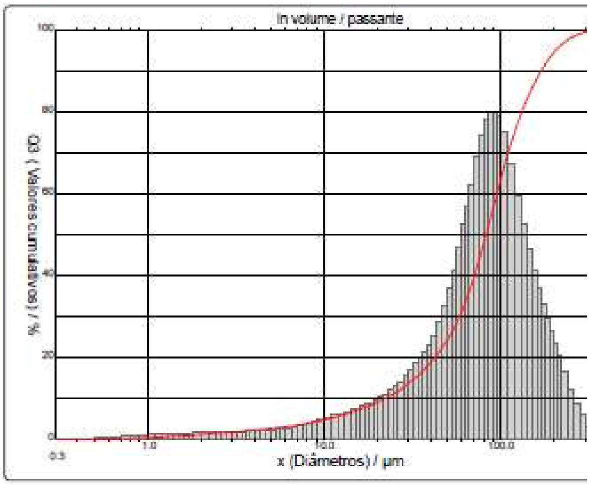


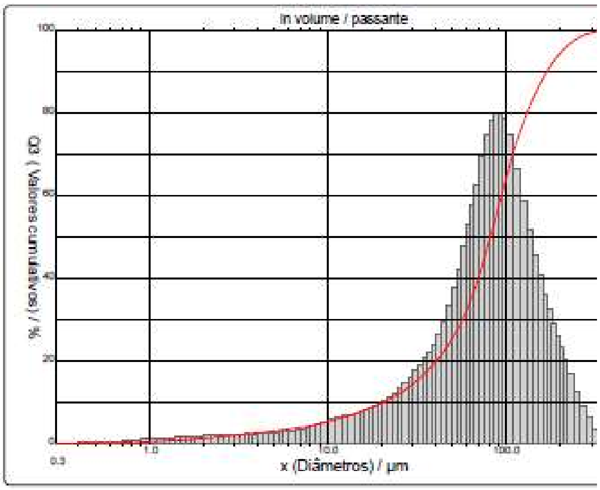


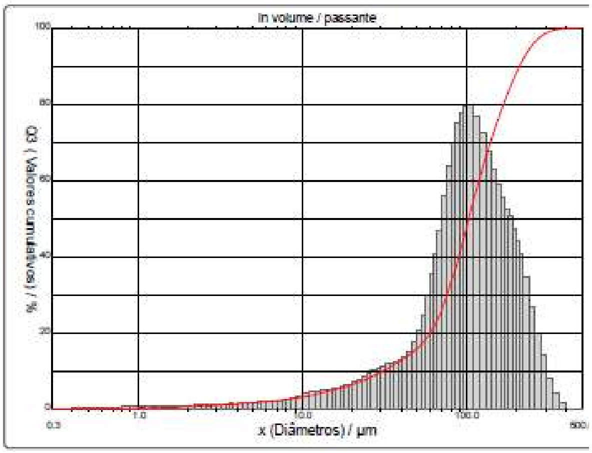


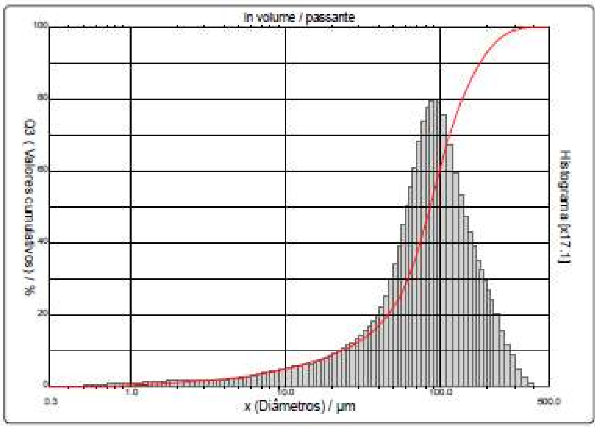
Particle size distribution of Red clay – graphs per sample and overlapped curves:

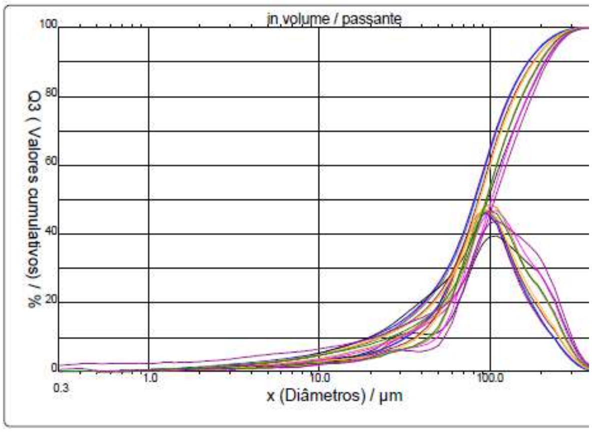




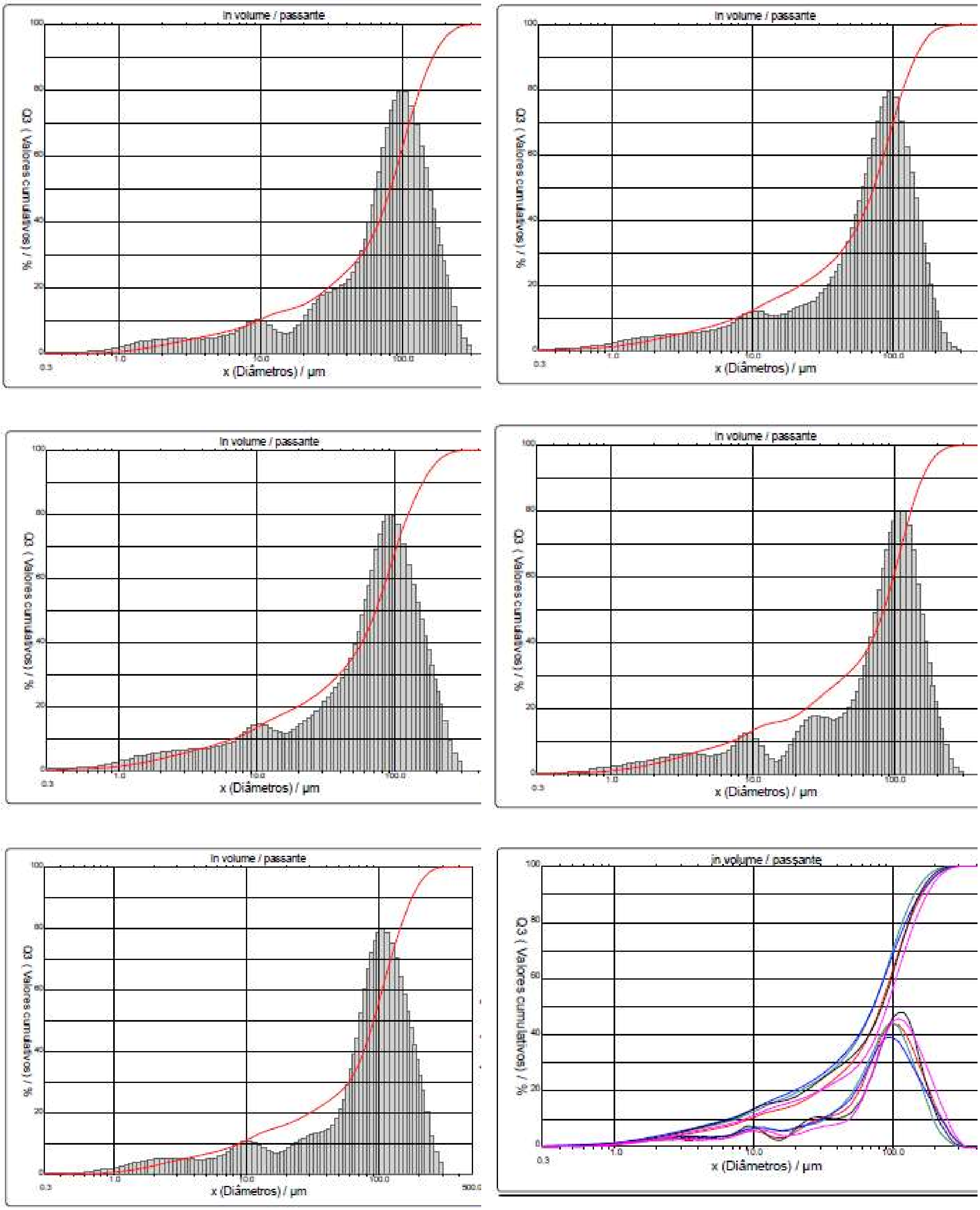








Particle size distribution of Green clay – graphs per sample and overlapped curves:



APPENDIX A – CERTIFICATE OF ANALYSIS OF CLAYS (SENT BY THE MANUFACTURER)

Terramater
INGREDIENTES ATIVOS MINERAIS

Argilas do Brasil Com. de Insumos Ltda., Rua Donato Pereira, 93 Fazenda – CEP 88306-220
Itajaí - SC Fone (47) 3344-0029

Produto: Tersil G Lote: 00048/19	Fab: 20 de agosto de 2019 Val: 4 anos	
	Data: 28/8/2019	Pág: 1 de 1

CERTIFICADO DE ANÁLISE

ANÁLISE	ESPECIFICAÇÃO	RESULTADO
Características organolépticas		
Aspecto	Pó fino	De acordo
Cor	Creme a Amarelo esverdeado	De acordo
Odor	Característico	De acordo
Características físicas		
Distribuição Granulométrica em Suspensão (diâmetro médio)	Max. 30,00 µm	14,45 µm
Perda por dessecação	Máx. 3,00%	1,13%
Material orgânico máximo	Máx. 2,00%	0,20%
Pico Exotérmico (DSC)	600 – 601,2 °C	600,6 °C
Área Superficial	35 - 40 m^2/g	38,18 m^2/g
Capacidade de troca catiônica	67-73 eq/g	70 eq/g
Análise química		
SiO_2	57,00 – 73,00%	64,84%
Fe_2O_3	2,50 – 6,50%	4,43%
Al_2O_3	13,00 – 23,00%	18,40%
Metais pesados totais	< 50 ppm	De acordo
Metais pesados		
Arsênio	Máx. 4 ppm	2 ppm
Antimônio	Máx. 0,20 ppm	< 0,05 ppm
Cádmio	Máx. 0,50 ppm	0,15 ppm
Chumbo	Máx. 20 ppm	10,7 ppm
Cobalto	Máx. 25 ppm	14,2 ppm
Cromo	Máx. 50 ppm	45 ppm
Selênio	Máx. 1 ppm	< 1 ppm
Lantânio	Máx. 80 ppm	55,8 ppm
Níquel	Máx. 25 ppm	22,6 ppm
Mercúrio	Máx. 1 ppm	0,02 ppm
Análise Microbiológica		
Contagem de bactérias	<100 UFC/g	De acordo
Contagem de bolores e leveduras	<10 UFC/g	De acordo
Coliformes totais e fecais	Ausência	De acordo
Clostridium sp.	Ausência	De acordo
Pseudomonas aeruginosa	Ausência	De acordo
Staphylococcus aureus	Ausência	De acordo

Condições de armazenagem: armazenar em local fresco, seco, arejado, ao abrigo da luz e a temperatura ambiente.
Por ser uma matéria prima de origem natural, pode haver variações de cor e composição entre os lotes.

Metodologia: Farmacopéia Brasileira 4ª ed., parte 1, 1998.
ACME Labs – Vancouver – Canadá
Métodos internos Terramater
Metais Pesados: Digestão multiácida e leitura em ICP/MS - SGS Laboratórios – Brasil

Técnico Responsável: Pedro E. Bretzke CRF-SC 4055

Terramater — INGREDIENTES ATIVOS MINERAIS		
Argilas do Brasil Com. de Insumos Ltda. Rua Donato Pereira, 93 Fazenda – CEP 88306-220 Itajaí - SC Fone (47) 3344-0029		
Produto: Tersil CB Lote: 1997	Fab: 12 de janeiro de 2019 Val: 4 anos	
	Data laudo: 14/1/2019	Pág: 1 de 1

CERTIFICADO DE ANÁLISE

ANÁLISE	ESPECIFICAÇÃO	RESULTADO
Características organolépticas		
Aspecto	Pó fino	De acordo
Cor	Preto	De acordo
Odor	Característico	De acordo
Características físicas		
Distribuição Granulométrica em suspensão (diâmetro médio)	Máx. 15,00 µm	14,52 µm
Perda por dessecação	Máx. 6,5%	1,32%
Material Orgânico Máximo	Máx. 7,0%	5,8%
Pico Exotérmico (DSC)	602 – 604,0 °C	603,5 °C
Area Superficial	38 - 42 m^2/g	40,57 m^2/g
Capacidade de troca catiônica	58 - 64 eq/g	60 eq/g
Análise química		
SiO_2	52,00 – 68,00%	63,62%
Fe_2O_3	0,50 – 2,00%	0,96%
Al_2O_3	22,00 – 40,00%	31,85%
TiO_2	1,50 – 3,25%	2,05%
Metais pesados		
Arsênio	Máx. 03 ppm	2 ppm
Cádmio	< 0,10 ppm	< 0,10 ppm
Chumbo	Máx. 20 ppm	18 ppm
Cobalto	Max. 10 ppm	5 ppm
Cromo	Máx. 20 ppm	18 ppm
Selênio	Máx. 0,5 ppm	0,1 ppm
Níquel	Máx. 10 ppm	5 ppm
Mercúrio	< 0,20 ppm	< 0,10 ppm
Neodímio	< 0,20 ppm	< 0,10 ppm
Análise Microbiológica		
Contagem de bactérias	< 100 UFC/g	De acordo
Contagem de bolores e leveduras	< 10 UFC/g	De acordo
Coliformes totais e fecais	Ausência	De acordo
Clostridium sp.	Ausência	De acordo
Pseudomonas aeruginosa	Ausência	De acordo
Staphylococcus aureus	Ausência	De acordo

Condições de armazenagem: armazenar em local fresco, seco, arejado, ao abrigo da luz e a temperatura ambiente.

Por ser uma matéria prima de origem natural, pode haver variações de cor e composição entre os lotes.

Metodologia: Farmacopéia Brasileira 4ª ed., parte 1, 1998.
ACME Labs – Vancouver – Canadá
Métodos internos Terramater
SGS labs - Brasil

Técnico Responsável: Pedro E. Bretzke CRF-SC 4055

Argilas do Brasil Com. de Insumos Ltda. Rua Donato Pereira, 93 Fazenda – CEP 88306-220
Itajaí - SC Fone (47) 3344-0029

Produto: Tersil CDR Lote: 2097	Fab: 18 de julho de 2019 Val: 4 anos	
	Data: 23/07/2019	Pág: 1 de 1

CERTIFICADO DE ANÁLISE

ANÁLISE	ESPECIFICAÇÃO	RESULTADO
	Características organolépticas	
Aspecto	Pó fino	De acordo
Cor	Vermelho Escuro	De acordo
Odor	Característico	De acordo
	Características físicas	
Distribuição Granulométrica em suspensão (diâmetro médio)	Máx. 15,00 µm	10,88 µm
Perda por dessecação	Máx. 5,00%	1,04%
Pico Exotérmico (DSC)	600,00 – 602,00 ºC	600,8 ºC
Área Superficial	20 - 22 m^2/g	20,00 m^2/g
Capacidade de troca catiônica	58 - 62 eq/g	60 eq/g
	Análise química	
SiO_2	35,00 – 45,00%	40,28%
Fe_2O_3	7,00 – 15,00%	11,02%
Al_2O_3	30,00 – 40,00%	34,65%
	Metais pesados	
Arsênico	Máx. 03 ppm	2,00 ppm
Cádmio	<0,10 ppm	<0,10 ppm
Chumbo	Máx. 20 ppm	19 ppm
Cobalto	Max. 10 ppm	5 ppm
Cromo	Máx. 20 ppm	16 ppm
Selênio	Máx. 0,5 ppm	0,2 ppm
Níquel	Máx. 10 ppm	2 ppm
Mercúrio	<0,20 ppm	<0,10 ppm
Neodímio	<0,20 ppm	<0,10 ppm
	Análise Microbiológica	
Contagem de bactérias	<100 UFC/g	De acordo
Contagem de bolores e leveduras	<10 UFC/g	De acordo
Coliformes totais e fecais	Ausência	De acordo
Clostridium sp.	Ausência	De acordo
Pseudomonas aeruginosa	Ausência	De acordo
Staphylococcus aureus	Ausência	De acordo

Condições de armazenagem: armazenar em local fresco, seco, arejado, ao abrigo da luz e a temperatura ambiente.
Por ser uma matéria prima de origem natural, pode haver variações de cor e composição entre os lotes.

Metodologia: Farmacopéia Brasileira 4ª ed., parte 1, 1998.
SGS Laboratórios
Métodos internos Terramater

Técnico Responsável: Pedro E. Bretzke CRF-SC 4055