Development of a Solid Organic Shampoo Formulation

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Thesis to obtain the Master of Science Degree in

Biological Engineering

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November 2018

The work presented in this thesis was performed at the company UniBio Lda (Pêro Pinheiro, Portugal), during the period February-July 2018, under the supervision of Eng. Ricardo Spencer. The thesis was co-supervised at Instituto Superior Técnico by Prof. Helena Pinheiro.

Abstract

The present work is focused on the development of a solid organic shampoo in order to respond to the current demands of consumers, offering an innovative product within the organic cosmetics market. In this context, three lines of shampoo were developed: a line for oily hair, another for normal hair and a third one for dry and damaged hair. The experimental conditions and ingredients required to develop a product able to wash and moisturize, and at the same time remain solid before and after washes, were tested. Secondly, the developed laboratory-scale process was scaled up to commercial scale, highlighting the differences between the two. Among the product quality tests, microbiological analysis and dry weigh determinations were performed to calculate the content of water and volatiles present in the shampoo and whether microbial contamination was present. A preservative was not added to the shampoo, since the contents of water and volatiles were low. The microbiological tests confirmed that it was not necessary to add a preservative, since the shampoo medium was not propitious for bacterial and fungal growth. Under these conditions, the product was legally approved according to the EU regulation 1223/2009. A sustainable, sulphate-free, silicone-free, plastic-free solid shampoo was obtained with abundant and creamy foam, allowing a quick and improved washing. Finally, packaging design was also part of the project: a method for hanging the bar shampoo using cork materials was created and recycled paper was used for packaging.

Keywords: Solid bar shampoo, organic cosmetics, sulphate-free shampoo, silicone-free shampoo, plastic-free shampoo, vegan shampoo, sustainable shampoo.

Resumo

A presente dissertação teve como objetivo a produção de um champô biológico sólido, de forma a responder às necessidades atuais dos consumidores, oferecendo um produto inovador no mercado dos cosméticos biológicos. Neste contexto, produziram-se três linhas de champô, nomeadamente, para cabelos oleosos, normais e secos e estragados. Testaram-se as condições e ingredientes necessários para desenvolver um produto que seja capaz de lavar e hidratar, mas que se mantenha sólido antes e depois das lavagens. De seguida, o processo desenvolvido à escala laboratorial foi adaptado à escala comercial, realçando-se as diferenças entre as duas. Para testar a qualidade do produto, realizaram-se análises microbiológicas e determinações em balança de medida de humidade para calcular o teor água e voláteis e avaliar a presença de contaminação microbiana. Concluiu-se que o teor em água não era significativo, pelo que não foi necessário adicionar conservante ao champô. Isto foi confirmado pela análise microbiológica, visto que o meio do champô se revelou como não propício ao crescimento de fungos e bactérias. Nestas condições, o produto foi aprovado legalmente segundo o regulamento EU 1223/2009. Obteve-se um champô sólido vegano, sustentável, sem sulfatos, silicones ou plástico, com espuma abundante e cremosa, permitindo uma rápida lavagem. Finalmente, o design da embalagem também fez parte do projeto e criou-se um sistema para pendurar o champô com material de cortiça e uma embalagem em papel reciclado.

Palavras-chave: Champô sólido, cosmetica biológica, champô sem sulfatos, champô sem plásticos, champô sem silicones, cosmética vegana, cosmética sustentável.

Acknowledgements

First of all, I would like to thank my parents. To my mom, for being my support, adviser and confidant. To my dad, for always encouraging me and for all the caring and comprehension.

To Engineer Ricardo Spencer, who allowed me to realize this dream in the development of a cosmetic product, for transmitting me all his passion and knowledge for cosmetics and for the help and guidance provided during the internship.

To Professor Helena Pinheiro, for accepting to be my supervisor, for the excellent guidance during the internship and for being always available with all the attention and patience.

To Professor Ana Cristina Viegas for all the help in the lab with the microbiological analysis.

To Solange Bolas for being an amazing colleague and friend in UniBio's lab.

To Maria João Reis, for the packaging design of the shampoos and for recording the promotional video.

To Claúdia Batista and all the UniBio's team for the patience and help.

To my aunt Jajã and cousin Ana for the help given when I was writing my thesis.

To my family in Lisbon (Paula, Xana, Paulo, Cat) for all the support during this 5 years.

To my grandmother Laurinda for all the kindness and dedication. To my grandmother Dinorah for being my lucky star.

To all my family for the support and kindness.

To my boyfriend, Joaquim Braga, for all the kindness and help when I was writing my thesis, for always trying to cheer me up and for being my role model in academic life in the past year.

To the *flowers* (Inês Ferreira, Mariana Ávila, Catarina Costa, Mafalda Santos) for all the company and friendship during these 5 years.

To Eunice Cordeiro and Daniela Coelho, for always reminding me of home, and for being there for me besides the distance.

To Miguel Brito and Gabriela Montenegro for being my partners.

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Acronyms

CoA	Coenzyme A
CMC	Critical Micelle Concentration
DP	Dermal Papilla
DS	Dermal Sheath
DMAPA	Dimethylaminopropylamine
GMO	Genetically Modified Organism
HS	Hair Shaft
INCI	International Nomenclature of Cosmetic Ingredient
IRS	Inner Root Sheath
IF	Intermediate Filament
SCI	Sodium Cocoyl Isethionate
SDS	Sodium Dodecyl Sulphate
SI	Sodium Isethionate
SC	*Stratum Corneum*
TEWL	Transepidermal Water Loss
TWL	Total Weight Loss
VCO	Virgin Coconut Oil

1. Framework and Goals

In a society where the environmental awareness of the damages caused by the daily use of chemicals in skin and hair care influences the consumer's attitude towards buying organic products, it is important to provide consumers with a wider range of these products. This would lead to a significant impact on human health and would promote a more sustainable economy.

In Europe, a cosmetic is defined as a "perfume or product used to clean, change appearance, correct odours, protect and maintain skin and hair in good condition". The European Union requires that cosmetic products placed on the EU market to be "safe", that is, they must not cause damage to human health when applied under normal or reasonably foreseeable conditions of use (Trueb, 2001). The manufacturers are responsible for the safety of their products and must ensure that they undergo an expert scientific safety assessment before they are sold. However, many cosmetic brands sell their products with chemicals on the legal thresholds, and in this case, safety becomes questionable.

Consumers buy all sorts of products that may dry out, damage or lead to a continued degraded state of the hair. These products often contain harmful ingredients for humans and environment, for example petroleum-based polymers, silicones, plastics and synthetic and harsh chemicals, such as sulphates, which can be aggressive to the scalp and may strip hair of its natural oils. Therefore, organic hair products reduce the level of daily exposure to damaging chemicals, have sustainably sourced organic and biodegradable ingredients, protect biodiversity and some are packed with recycled content. They also contain herbal infusions, mild surfactants, butters, oils and pure essential oils that provide a clean, hydrated, and, above all, healthy hair.

In Portugal there are only a few companies that produce and sell organic cosmetics and some of them are expanding in the market and gradually marking their position. UniBio Lda is a good example of a company that produces and sells organic cosmetics, such as soaps, massage oils, elixirs and toothpastes. The company wanted to start producing a haircare line, which together with other products would constitute its new brand: *Unii- Organic Skin Food.*

Having this in mind, the present project was implemented, namely developing and formulating an innovative product, a solid shampoo bar.

Shampooing is by far the most frequent form of cosmetic hair treatment. In the beginning, shampoos were made from soap or mixtures of soaps but soon this was no longer accepted or used for several reasons. Therefore, new shampoo products were formulated, more precisely, in the form of clear or opaque liquids, lotions, pastes, gels, foam aerosols or dry absorbent powder products (Tibbetts, 1990), however, bar shampoos are still not as well known in the market as the other shampoos mentioned above, highlighting this product as quite innovative.

The aim of this work was to develop, in the UniBio Lda lab, three types of solid shampoos, for oily, normal and dry/damaged hair, that lasted longer than a traditional liquid shampoo, were eco-friendly and did not contain any sulphates, plastics, silicones or other additives that would extensively strip the natural moisture of the hair and scalp.

Not only different experimental conditions and procedures had to be tested, but also diverse raw materials and combinations of ingredients had to be used to obtain the expected solid texture and incorporation of specific active ingredients for each type of hair. The shampoo performance should be tested, namely washing the scalp with adequate foam formation and creaminess, in order to evaluate the conditioning, texture, detangling and cleanliness obtained. The final product – solid shampoo - is supposed to remove surface grease, dirt, skin debris and natural skin secretions from the hair shaft or scalp without leaving residues to provide hair conditioning natural agents to enhance the body and shine of the hair to repair damage and finally moisturize it.

2. State of the Art

2.1. Hair

Hair is one of the most important characteristics of mammals, since it is involved in a wide range of functions such as thermoregulation, physical protection, dispersion of sweat and sebum, sensory and tactile functions, and social interactions, protecting the body against heat, cold, sunlight, pollution, injuries and impacts (Schneider, 2009).

2.1.1. Hair types

The human hair can be divided into three types: terminal hairs, *vellus* hairs and intermediate hair. The first are macroscopically long (>2 cm), thick (>0.03 mm), pigmented and are present in scalp, eyebrows, eyelashes, beards and pubic areas. The *vellus* hairs, in contrast, are non-pigmented, thinner than 30 μm, silky, do not grow longer than 2 cm and are scattered all over the body and, for example, are found diffusely over hairless areas of face and bald scalp. Intermediate hair has an intermediate length and shaft size and is found on the arms and legs of adults. The only parts of the human body that have no hair growing are the mucous membranes, the palms of the hands, the soles of the feet and the lips (Toll, 2004) (Paus & Cotsarelis, 1999).

2.1.2. Hair Follicle

Hair follicles are located at the epidermis extending down through the dermis and project into the subcutaneous adipose layer and generate hair (Figure 1), a flexible tube of fully keratinized epithelial cells. At the base, the growing hair follicle extends into the hair bulb surrounding the dermal papilla (Khidhir, 2010).

Several layers (the inner root sheath, the outer root sheath, the vitreous membrane and the connective tissue sheath) compose the hair follicle which hold and frame the hair (Figure 2). Focusing on the inner root sheath, it surrounds the hair fiber from the bulb up to the level of the sebaceous gland and has three distinct cell layers: the outer Henle's layer, the Huxley layer and the inner cuticle (Figure 2) (Khidhir, 2010).

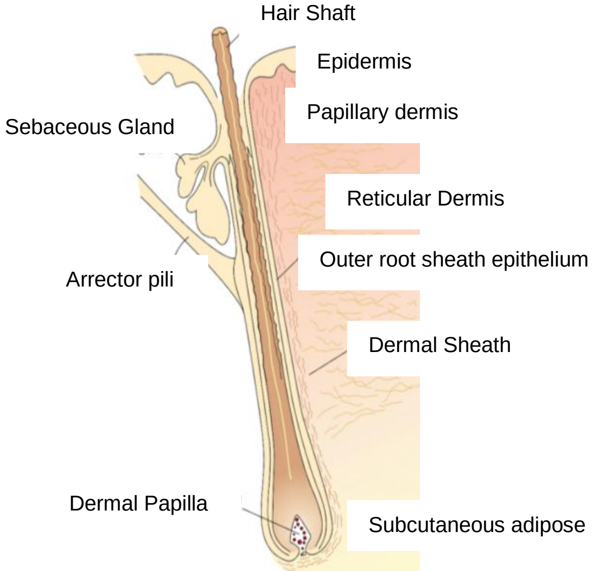


Figure 1: Diagram of a human hair follicle (Jahoda & Reynolds, 2001).

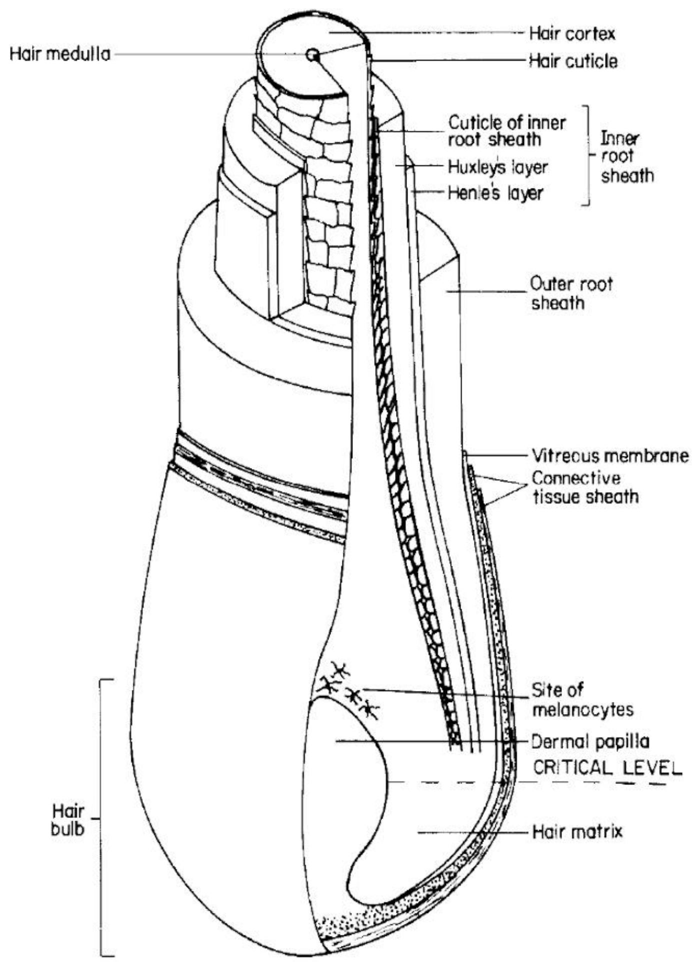


Figure 2: Lower part of a human hair follicle. (Khidhir, 2010).

2.1.3. Hair fiber structure

Hair is a filamentous biomaterial consisting mainly of proteins, in particular, keratin (approximately 80% w/w), which is an α-helical protein with high cysteine content (Wagner & Kunihiko, 2007). Hair fibers have a hierarchical structure similar to other α-keratin materials, such as wool, nails, claws, and horns present in mammals (Yu & Yang, 2017).

Morphologically, a human hair is divided into different units: cortex, cuticle, and medulla (which is not always present) (Wagner & Kunihiko, 2007).

The typical diameter of a hair fiber is 50–100 μm and the fiber is covered by an outermost layer, a hydrophobic lipid cuticle (Figure 3). This layer consists of dead skin cells, which overlap in layers, each having an average length of 60 μm and a thickness of about 0.5 μm. Moreover, 5–10 scales overlap creating a total thickness of approximately 5 μm (Yu & Yang, 2017).

The morphology of the cuticle edges is affected by weathering, combing, and brushing, with more severe damage seen on long hair fibers. The normal cuticle has a smooth appearance, allowing light reflection and restraining the friction between the hair shafts. It is responsible for the luster and texture of the hair (Sinclair, 2007).

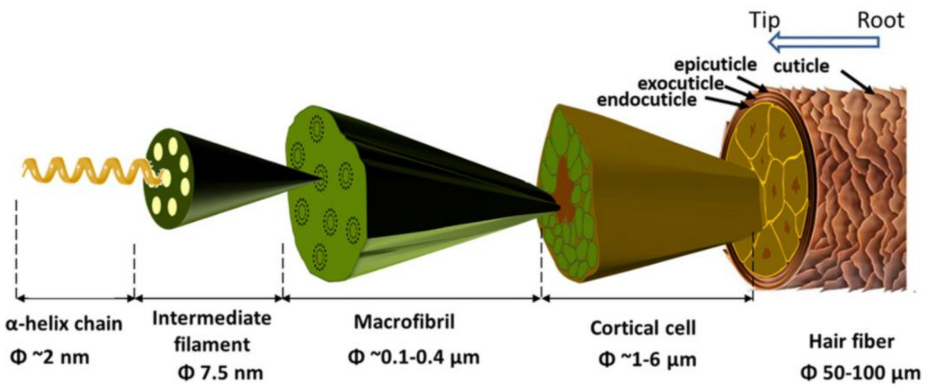


Figure 3: Representation of hierarchical structure in human hair starting at α-helix chains. (Yu & Yang, 2017)

The central core of hair is the cortex that forms around 80% of the hair strand and gives the hair its colour. The core is composed of cortical cells (100 µm long and 1–6 µm thick) which are spindle-shaped with various sizes, and are aligned along the main axis of the fiber (Figure 3). The main axis is composed of macrofibrils (diameter of 0.1– 0.4 µm) which in turn are constituted by intermediate filaments (IF) embedded in a matrix with high-sulphide content.

One IF has a diameter of approximately 7.5 nm and is formed of eight protofilaments (Figure 3). Each protofilament is composed in turn of four right-handed α-helix chains and as a result, a total of thirty-two chains form an IF (Wagner & Kunihiko, 2007) (Figure 3).

The α-keratin chains have a large number of sulphur-containing cysteine residues. Cysteine residues in adjacent keratin filaments form covalent disulphide bonds, establishing a strong crosslink between adjacent keratin chains. These bonds are responsible for the shape, stability, and texture of the hair. *Van der Waals* interactions and hydrogen bonds also link the keratin chains together. However, these weaker bonds can be overcome with the action of water (Sinclair, 2007).

The medulla is a slightly packed, disordered region in the middle of the fiber which is surrounded by the cortex (Yang, 2014). The function of the medulla is not well known but may provide thermo-regulatory properties to hair taking into account the airspaces within the medulla and contribute to a higher volume/body to the hair. Normally, coarse fibers contain a medulla, instead of very fine fibers that usually do not. (Syed, 2018)

2.1.4. The chemical composition of hair

Hair fibers have 65–95 wt% of proteins and up to 32 wt% of water. The rest of the components are lipid pigments and others. Additionally, glycine, threonine, aspartic and glutamic acid, lysine, cysteine, and tyrosine are the aminoacids that compose the major part of hair (Rigon, 2013). Therefore, chemically the properties of human hair are dominated by the α-keratin (Yu & Yang, 2017).

2.1.5. Hair cycle

2.1.5.1. Morphogenesis

Initially, in the embryonic stage, the skin is composed of a single layer of epidermal stem cells. Afterward, as mesenchymal cells aggregate below the epidermis to form the dermis, the development of the hair follicle begins (Figure 4). The aggregates of mesenchymal cells spot the location of the new hair follicle and stimulate the epithelial stem cells to grow and produce a hair follicle. As the follicle grows, the inner layers differentiate into concentric cylinders to create the central hair shaft (HS) and the inner root sheath (IRS), the channel that surrounds the central hair shaft (Alonso & Fuchs, 2006). The dermal portion of the hair follicle can be divided into two compartments, the dermal papilla (DP) and dermal sheath (DS). The DP is located at the base of the hair follicle and travels with the epithelial down growth and becomes enveloped by the hair bulb. The follicle becomes fully mature as its bulb nears the bottom of the dermis. The proliferative cells go on with the division, producing progeny cells which differentiate to form the growing hair exiting the skin surface (Alonso & Fuchs, 2006).

During the production of a hair fiber, the follicle assumes dynamic changes from a growing phase (anagen), to a remodeling phase (catagen), and to finish to a quiescent phase (telogen), only to start growing again (M.Lavker, Sun, & Oshima, 2003).

2.1.5.2. Anagen

The anagen phase is the hair shaft growth phase, due to an increased metabolic activity of the matrix cells at the bottom of the follicle which is pushed into to dermis by the cell division. The cell cycle length of the matrix cells is approximately 18 hours and the daughter cells move upwards, adopting one lineage of the IRS and HS (Figure 4). The duration of anagen determines the length of the hair and is dependent upon continued proliferation and differentiation of matrix cells at the follicle base (Alonso & Fuchs, 2006).

The most part of the hair follicles are in the anagen phase (85% - 90%), and new cells are added to the bottom of the hair, elongating the strand bit by bit (Organic Cosmetic Science School, 2018).

2.1.5.3. Catagen

The catagen phase corresponds to the dynamic transition between anagen and telogen (Figure 4). Melanin is the pigment that gives human skin, hair, and eyes their colour, and is produced by cells called melanocytes. After the anagen phase, the hair follicle stops producing melanin through a signal sent by the body and the melanocytes undergo apoptosis. The cell death of follicular keratinocytes that produce keratin also takes place in this phase (Paus & Cotsarelis, 1999).

At the end of the catagen stage, the dermal papilla condenses and moves upward and stays below the hair-follicle (Paus & Cotsarelis, 1999). If this fails, the cycle stops and the hair is lost (Khidhir, 2010). Cell division stops and the base of the hair shaft becomes fully keratinized and forms the dry, white node characteristic of a ‘club’ hair, that has been cut from the blood supply and remain in the place until is shed. The bulb begins to degenerate and the follicle shrinks rapidly and pushes the hair, reducing the firmness on the hair strand, facilitating its removal (Stewart, 1992).

It is considered to be the phase that corresponds to the renewal of the hair. The catagen phase lasts approximately 2-3 weeks (Organic Cosmetic Science School, 2018).

2.1.5.4. Telogen

During the telogen stage, the hair shaft matures and is eventually shed from the follicle. This means that is the resting phase of the hair cycle. The hair follicle does not undergo cell division at this stage, there are no melanocytes in the follicle and the hair does not have any nutrition or blood supply. If the hair is still in the follicle, it is totally keratinized.

This phase usually lasts two or three months before the scalp follicles go again to the anagen phase, repeating the cycle. However, the time that follicles stay in this quiescent period depends on two factors: the body area and the age of the person, for example, for scalp hair this period of time is about ten weeks and for the general body surface is about two a six weeks (Stewart, 1992).

It is not clear if the shedding is an active, regulated or passive process, that is, if occurs at the beginning of the anagen stage when the new hair grows and weakens the anchor that keeps the club hair in the scalp, leading to a fall out of the hair (Paus & Cotsarelis, 1999) (Organic Cosmetic Science School, 2018).

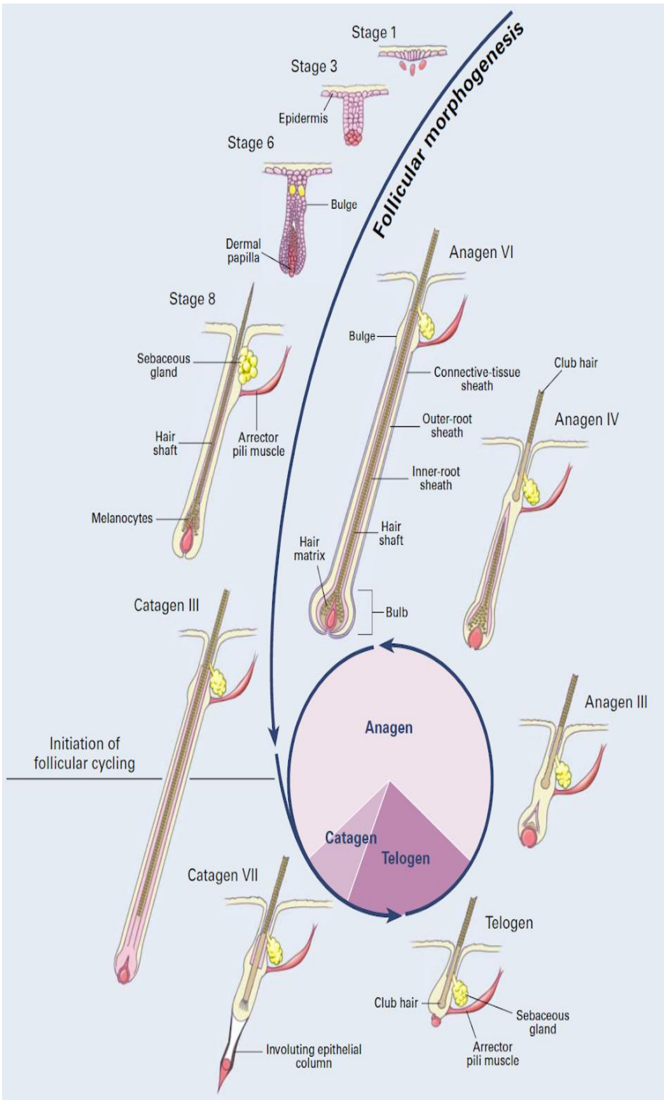


Figure 4: Hair follicle cycle. (Paus & Cotsarelis, 1999)

2.2. Shampoos

2.2.1. General Aspects

Shampoo is a hair care product used to remove the sebum, skin particles, dandruff, environmental pollutants and other contaminant elements. The shampoo ingredients and characteristics can vary according to the type and condition of the hair (limp, bleached, permed, dyed, dry, greasy, short or long); according to the season, age, lifestyle, frequency of shampoo use and the use of conditioning products such as conditioners, moisturizing masks, styling products and others; and finally, according to the specific problems related to the physiologic condition of the scalp, for example: dandruff or seborrhoea. Moreover, individual preferences for aroma, texture and foaming characteristics, ease of rinsing and wet combing affect the choice of the consumer of a certain type of shampoo over another (Bouillon, 1988). For this reason, a diverse range of shampoos must be available and it is necessary to develop new and innovative products to satisfy the needs of the consumers.

Hair is composed by a hydrophobic protein and retains dirt due to the presence of sebum, which is an excretory product of the sebaceous glands. The bulk of sebum is a mixture of squalene, esters of glycerol, cholesterol, wax esters, fatty acids and free cholesterol, which are synthesized by the sebaceous gland (Picardo, Ottaviani, Camera, & Mastrofrancesco, 2009). The most important products of sebaceous secretion are squalene and wax esters that provide the skin and hair surface a hydrophobic coating. The disadvantage of this lubricating oil is that it entraps pollutants such as environmental dust, smoke, greases, also entraps debris from the scalp, hair sprays, and styling products. Also, microbial enzymatic activity affects the composition of the sebum, modifying it over time (Bouillon, 1988).

Therefore, the main intention is to remove the dirt that is entrapped in the hair through the following steps: weak the physicochemical forces that link the dirt to the hair; carry it into the aqueous vehicle and finally disperse it and at the same time avoid redeposition on the fiber. This involves numerous interactions between water and air, lipids and water, solids (for example hair) and water and is accomplished by surfactants (Bouillon, 1988).

2.2.2. Surfactants used in Shampoos

A surfactant is a blend of surface active agents. They are organic compounds that are both lipophilic and hydrophilic (amphiphilic) and have the ability to orient themselves according to the polarities of two opposing phases. The hydrocarbon fatty chain constitutes the non-polar part of the surfactant and gets in contact with the lipophilic part of the interface (fatty material). The polar group (hydrophilic) which is located at the end of the fatty acid chain strongly interacts with water molecules by dipole or ion-dipole interactions (water-soluble) and removes the dirt that is entrapped (Bouillon, 1988).

2.2.2.1. Surfactants Classification

Surfactants are classified into four different categories according to the type of the polar end: Anionics, Cationics, Amphoterics and Nonionics (Figure 5) (Rieger & Rhein, 2017).

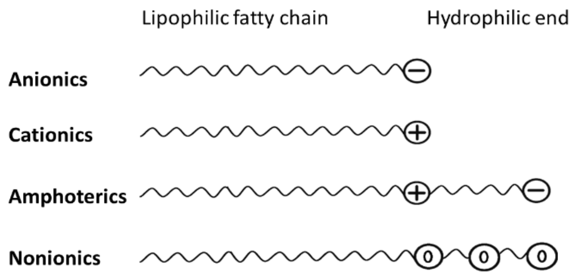


Figure 5: Different types of surfactants according to the type of the polar end.

Anionics

The surfactants that have a hydrophilic head carrying a negative charge are defined as anionics. Those types of surfactants have excellent emulsifying, detergent and foaming properties which are the main reason for their use in shampoos. Also, they are divided into five major chemical classes:

- Acylated Amino Acids
- Carboxylic Acids
- Sulfonic Acid Derivatives
- Sulfuric Acid Derivatives
- Phosphoric Acid Derivatives

(Rieger & Rhein, 2017)

Cationics

These surfactants carry a positive charge on their polar group and are also divided in different categories, as follows:

- Quaternarie amines
- Alkyl Amines
- Alkyl Imidazolines

(Rieger & Rhein, 2017)

Since cationic surfactants show affinity for the hair fibres and provide softness and mildness, the lather is poor and the redeposition of the dirt onto the hair may occur. These surfactants do not clean, being the conditioning of the hair their main function. Also, the incompatibility with anionic surfactants limits their use together in shampoos formulations.

Amphoterics

If the hydrophilic head behaves as a function of pH, that is, carry a positive charge at low pH and a negative charge at high pH, the surfactants are classified as amphoterics. There are two categories of amphoterics:

- Alkylamido Alkyl Amines

- Alkyl Substituted Aminoacids.

(Rieger & Rhein, 2017)

The amphoterics surfactants are usually used in a combination with anionic surfactants, to give the desired properties of the formulation (Bouillon, 1988).

Non ionics

In this case, the surfactants do not have an ionic end. Instead, have a repeated oxyethylenic pattern as hydrophilic element. The most used type of non ionic surfactants are:

- Polyoxyethylene derivatives

$$\mathbf{R}-X(\underbrace{CH_2\text{-}CH_2\text{-}O}_{\text{ethylene oxy}})_nH \qquad (X = O \text{ or } COO \text{ or } CONH)$$

- Polyglycerol derivatives

$$\mathbf{R}-X(CH_2\text{-}CHOH\text{-}CH_2O)_nH$$

$$\mathbf{R}-X(CH_2\text{-}\underset{\displaystyle CH_2OH}{\underset{|}{CH}}\text{-}O)_nH$$

- Fatty ethanolamide

$$\mathbf{R}-CO\text{-}NH\text{-}CH_2\text{-}CH_2OH$$

(Bouillon, 1988)

Besides the poor foaming potential, they have good emulsifying, dispersing and detergent properties and are usually considered as the mildest surfactants.

2.2.2.2. Surfactants Adsorption

The balance between hydrophilic and hydrophobic parts of the molecule provides unique properties to the surfactant system in what regards accumulation at different interfaces and association in solution (to form micelles) (Kirk-Othmer, 2013).

The interfacial free energy per unit area is the minimum amount of work required to create or expand the interface and is measured when the interfacial tension between two phases is determined. A surfactant is a substance that at low concentrations adsorbs at interfaces in the system and changes the amount of work required to expand those interfaces. Therefore, adsorption of surfactant molecules at interfaces lowers the interfacial tension (Kirk-Othmer, 2013).

2.2.2.3. Micellization

The micelle formation is the process in which surfactants aggregate in solution forming micelles. Micellization is an important process since phenomena such as detergency and solubilization depends on the existence of micelles and also because it reduces the surface or interfacial tension caused by the reduction of contact between the hydrocarbon chain and water, which in turn lower the free energy of the system. The micelles are structured with the polar groups (heads) oriented toward the aqueous solvent and hydrophobic chains directed to the interior of the aggregate (Kirk-Othmer, 2013).

Critical Micelle Concentration (CMC)

The critical micelle concentration represents the concentration of surfactants above which surface-active molecules in solution aggregate to form larger units called micelles (Figure 6). From this concentration up the surface tension remains constant (Figure 6) and each surfactant has a singular CMC value at a certain temperature and electrolyte concentration. This concentration can be measured by surface tension, light scattering or fluorescence spectroscopy (Kirk-Othmer, 2013).

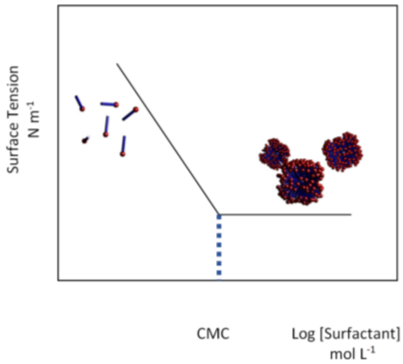


Figure 6: Variation in surface tension as a function of the logarithm of total concentration of surfactant.

2.2.3. Formulating an Organic Shampoo Bar

To formulate a mixture able to clean the scalp and hair and at the same time moisturize it without causing dryness is a complex task. In addition, most of the examples shown above are from surfactants used in conventional shampoos, which in the case of an organic shampoo are not used due to their aggressiveness towards the hair. Therefore, the aim is to formulate a mixture of mild surfactants (solid and liquid) to clean the hair, butters and oils to nourish it, hardeners and powders to give the desired texture and toughness and finally the aroma.

The formulation includes several classes of ingredients with specific quantities necessary to create a shampoo (Table 1).

Table 1: Example of a formulation with the quantities of each type of ingredient (Spencer, 2018).

Ingredient	Quantity (%)
Surfactants	50-65
Hardeners	0-15
Conditioning Agents	0-12
Active Ingredients	0-8
Powdered Active Ingredients	0-10
Essential Oils	0-1
Preservative	If necessary

2.2.3.1. Surfactants

A mixture of surfactants must be devised that provides the desired properties of detergency and foaming, cleaning the dirt and the debris imprisoned on the scalp. The most commonly used are fatty alcohol sulphates, but in the context of organic shampoo, the surfactants to be used are mild and accepted in organic cosmetics.

Therefore, the base of the formulation is often constituted by an anionic surfactant that would offer a lot of foam and detergency, as told before.

Surfactants form micelles when added in suitable concentrations, as was previously said. However, in some cases some surfactants can stay apart of the micelles as monomers and interact with the proteins of the scalp, causing irritation (Secchi, 2008). To increase the mildness and reduce the potential irritation caused by anionic surfactants (though mild) a mixture of amphoteric and non ionics is usually added forming larger and stable micelles, reducing the number of monomers and lowering the CMC of the system and consequently the irritation (Secchi, 2008).

2.2.3.2. Bar Hardeners

To achieve the toughness and the desired consistency of the solid shampoo bar, hardeners are particularly needed. Thus, fatty acids and fatty alcohols should be used to harden the bar. In addition, vegetable origin waxes can also be used as hardeners since they are complex mixtures of alcohols, fatty acids and esters, are very resistant to moisture, oxidation and microbial degradation. They also provide stability, enhancing the viscosity and consistency of the mixture.

2.2.3.3. Conditioning Agents

The conditioning agents are used to provide a greater softness and gloss to the hair and to improve disentangling, essential for dry and damaged hair. In this case of organic solid shampoo these are essentially oils and butters. These can help balance the effect of surfactants of removing oiliness from the hair, preventing hair from drying out. However, they have to be added in certain amounts to ensure hair nutrition but, at the same time, guarantying the hardness of the shampoo bar.

2.2.3.4. Active Ingredients

Depending on the kind of shampoo, different types of ingredients are added corresponding to the specific requirements of the shampoo. These can vary depending on whether the shampoo is for oily hair, dry hair, dandruff hair, sensitive hair or normal hair. Humectants, emollients, proteins, clays or other ingredients can be used for this purpose.

2.2.3.5. Preservatives

In the case of solid shampoo, there may be a need to add preservative or not, depending on the amount of water used in the formulation and on the conditions under which it is made. To prevent microbiological contamination, the use of preservative might be necessary.

2.2.3.6. Aroma

Regarding the aroma, essential oils provide the desired fragrance to the shampoo. Aromatic plants and their natural oils are used for different purposes such as alleviate tension and fatigue, produce a sense of relaxation, anxiety-relieving and anti-inflammatory effects and provide antimicrobial, hepatoprotective, antiviral and anticarcinogenic activities. Essential oils give a long-lasting refreshing feeling and obviously impart a pleasing aroma, shine and conditioning (Szumnya & Figiel, 2010).

2.2.4. Tested Ingredients

2.2.4.1. Surfactants

Sodium Cocoyl Isethionate

Isethionates are anionic surfactants, more precisely, sulfonic acid derivatives. Isethionates are esters formed from isethionic acid ($HOCH_2$ CH_2SO_3H) and long-chain alkanoic acids. They are used in cosmetics mainly as salts, and are also strong acids, not undergoing self-hydrolysis in aqueous system (Rieger & Rhein, 2017).

Sodium Cocoyl Isethionate (SCI) is the sodium salt of the coconut fatty acid ester of isethionic acid (Figure 7):

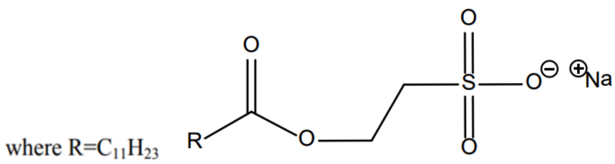


Figure 7: Sodium Cocoyl Isethionate structure (Burnett, Heldreth, & Bergfeld, 2013).

SCI is formed by reacting sodium isethionate (SI) with either the fatty acid mixture from coconut oil or the corresponding chlorides. The principal methods of producing SCI are (1) Fatty Acid Chloride Route - Liquid-Solid Reaction (Figure 8) and (2) Direct Condensation - Esterification (Figure 9).

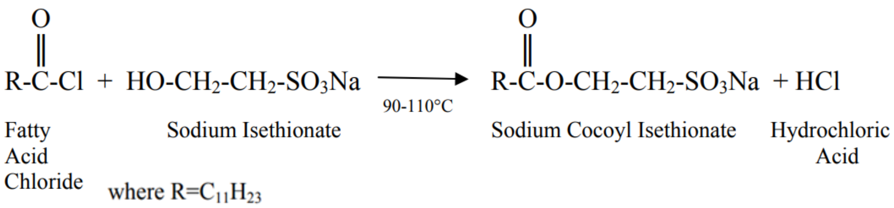


Figure 8: Fatty Acid Chloride Route - Liquid-Solid Reaction for producing Sodium Cocoyl Isethionate (Keller & Heckman, 2006).

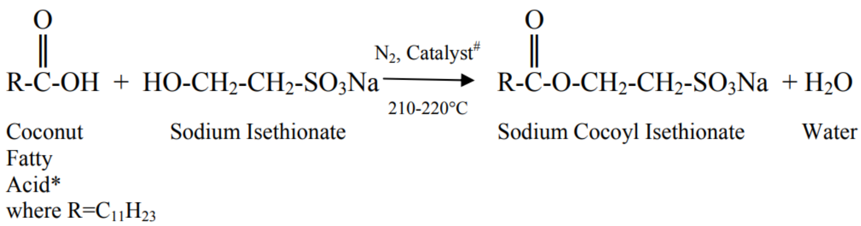


Figure 9: Direct Condensation – Esterification for producing Sodium Cocoyl Isethionate (Keller & Heckman, 2006).

The catalyst in Figure 9 could be H_2PO_4, H_2SO_4, PtO_2, Boric Acid, ZnO, $ZnSO_4$ or others.

Due to the outstanding mildness, skin compatibility and emollient properties of sodium cocoyl isethionate, it has been recently, frequently used in shampoos and other cosmetics. The fatty acids contained in SCI are derived from the coconut oil, and they are responsible for the mildness that the SCI bring to the scalp.

Several studies have proven that SCI is less damaging to the skin barrier than other surfactants such as sodium dodecyl sulfate (SDS). This is due to the fact that SDS forms small micelles in aqueous solutions capable of penetrating the aqueous pores in the *stratum corneum* (SC), the outermost layer of the epidermis, inducing skin barrier perturbation (Ghosh & Blankschtein, 2008).

In vitro studies demonstrate that the radii of SCI micelles (33.5$\pm$1 Å) determined by dynamic light scattering measurements are larger than the aqueous pores of the SC (29$\pm$5 Å), which results in inhibition of penetration. Since the micelles cannot penetrate the skin barrier, skin barrier perturbation does not occur. In consequence, this inability of the micelles to provoke skin barrier perturbation is the reason for the mildness of the SCI (Ghosh & Blankschtein, 2008).

Cocamidopropyl Betaine

To improve scalp and hair compability and to optimize the performance of primary surfactants, they are combined with secondary surfactants. One of the most used class of secondary surfactants are the amidopropyl betaines, outstandingly, cocamidopropyl betaine, which is an amphoteric zwitterion surfactant (alkylamido alkyl amine) derived from coconut fatty acid. As a result of its outstanding mildness, antiseptic properties and other favourable attributes, cocamidopropyl betaine has been increasingly used in shampoos, shower gels and other cosmetic products (Herrwerth & Leidreiter, 2008).

The first step of the production of cocamidopropyl betaine is the reaction of coconut fatty acids with 3-dimethylaminopropylamine (DMAPA) (Figure 10), yielding cocamidopropyl dimethylamine (an amidoamine). DMAPA can be added in slight excess to ensure the conversion of the entire fatty acid source, and afterwards is removed by distillation (Herrwerth & Leidreiter, 2008).

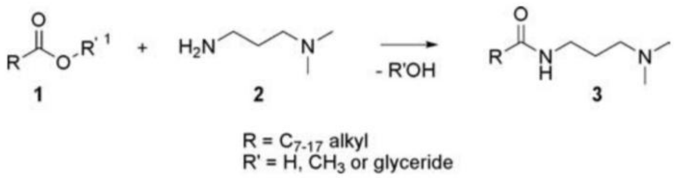


Figure 10: First step of the production of cocamidopropyl betaine (Herrwerth & Leidreiter, 2008).

In the second process step (Figure 11), conversion of cocamidopropyl dimethylamine into cocamidopropyl betaine is established through the reaction with chloroacetic acid and sodium hydroxide or directly with sodium monochloroacetate. (Herrwerth & Leidreiter, 2008).

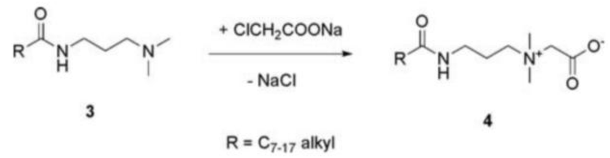


Figure 11: Second step of the production of cocamidopropyl betaine (Herrwerth & Leidreiter, 2008).

Coco Glucoside

Coco glucoside is a non-ionic surfactant included in the alkyl poly glucoside group, and it is obtained from renewable raw materials such as a mixture of fatty alcohols from coconut oil which react with glucose from corn, potato or wheat (Aguirre, 2014). It is completely biodegradable and is used to increase the foaming capacity of the surfactant mixture and for cleansing and conditioning.

2.2.4.2. Bar Hardeners

Stearic Acid

Stearic acid is a long chain and saturated fatty acid with 18 carbon atoms. Stearic acid can be found in oils, butters and even in the human body muscles. Due to its thickening properties, it is frequently used in cosmetic products intense hardening is desired. Despite not being an emulsifier, it has the ability to stabilize emulsions which is considerably favourable for the formulation (Nichols, 2018).

Cetearyl Alcohol

Cetearyl alcohol is an oil soluble blend of cetyl and stearyl alcohol that has emollient properties and for this reason it moisturizes the hair and scalp preventing it from drying out. Also, it is used as a thickener providing the toughness that the solid shampoo requires (Nichols, 2018).

Carnauba Wax

Carnauba wax is obtained from the leaves of *Copernicia prunifera*, a species of palm tree native to north-eastern Brazil. Since it is a plant-based source, carnauba wax is a smart alternative for beeswax. It is the hardest natural wax available and is composed mainly of wax esters, fatty acids, fatty alcohols and resins. It is soluble in ethanol but not in water, and also its melting point is one of the highest compared to other natural waxes (appendix B) (Happy Happy Vegan, 2018).

2.2.4.3. Conditioning Agents

Coconut Oil

Virgin coconut oil (VCO) is processed natural oil extracted directly from fresh and mature coconut kernel by natural and mechanical means with or without heating and without chemical treatment and refining procedure. The solvent extraction method and dry and wet methods can be used for the extraction of coconut oil. Solvent extraction method has several negative aspects such as high safety hazard, low quality oil and environmental risk. In wet method, oil is extracted through coconut milk by heating and non-heating processes. In the case of heating process, oil is obtained by direct heating of coconut milk. Considering the non-heating process the oil is extracted through aqueous enzymatic extraction process, supercritical fluid extraction process and fermentation process (Agarwal & Bosco, 2017).

VCO has great skin and hair benefits such as emollient properties and also reduces the transepidermal water loss (TEWL). Also, coconut oil provides hydration, retaining water molecules and penetrating the *stratum corneum*.

The main chemical constituents of coconut oil are presented in Table 2:

Table 2: Chemical composition of coconut oil (Varma, 2018).

Fatty acid	%
Caprylic acid C8:0	8.42
Capric acid C10:0	6.01
Lauric acid C12:0	50.97
Myristic acid C14:0	19.18
Palmitic acid C16:0	7.12
Stearic acid C18:0	2.71
Oleic acid C18:1	4.73
Linoleic acid C18:2	0.83

Lauric acid is a medium-chain triglyceride and is rapidly assimilated into the hair, exhibiting smoothing and texturizing properties. Capric and caprylic acids are also medium-chain triglycerides, which have antimicrobial properties. Linoleic acid moisturizes hair, promoting moisture retention and also exhibit anti-inflammatory properties, ideal for sensitive hair. Oleic acid stimulates the growth of thicker and stronger hair, maintaining the softness of the fibers and finally eliminates dandruff (New Directions Aromatics, 2018).

Shea Butter

Shea butter is a vegetable fat from West Africa, extracted from kernels of the shea tree, *Vitellaria paradoxa* or *Butyrospermum parkii*. Fat extraction is mainly performed by traditional methods such as roasting and pressing the kernels, churning the obtained liquid with water and afterwards, boiling, sieving and cooling. The fruit, kernels and butter constitute the valuable products of the tree. The pulp of the fruit contains sugars, proteins, calcium, ascorbic acid and iron.

The principal use for shea nuts is in chocolate production, as a cocoa butter replacer. On the other hand, with the progress trend toward organic and natural ingredients in hair products, shea butter is highly demanded by cosmetic industries (Maranz & Wiesma, 2004).

This butter is basically composed by triglycerides with oleic, stearic, linoleic and palmitic fatty acids and unsaponifiables such as triterpenes, tocopherol, phenols and sterols. The high percentage of unsaponifiables provides anti-inflammatory, antioxidant and moisturizing properties. Shea butter can be used as hair softener since it seals the moisture by coating each strand of hair in a thin layer of fat.

Cocoa Butter

Cocoa butter is the predominant commercial product obtained from seeds of *Theobroma cacao* and is an important ingredient in food, pharmaceutical, cosmetic and other industries. About 50% of the weight of cocoa seeds is cocoa butter, which is extracted through different approaches, such as ultrasound-assisted extraction, pressurized liquid extraction, microwave extraction and supercritical fluid extraction (Roiaini, 2016).

Cocoa butter is composed of approximately 98% of esterified fatty acids (Table 3). The principal fatty acids in cocoa butter are palmitic, stearic, oleic and linoleic acid (Roiaini, 2016).

Table 3: Fatty acid composition of cocoa butter (Roiaini, 2016).

Fatty Acid	Percentage (%)
Stearic Acid (C18:0)	33.7–40.2
Oleic Acid (C18:1)	26.3–35
Palmitic acid (C16:0)	25–33.7
Linoleic Acid (C18:2)	1.7–3

Since it contains a high percentage of fatty acids, cocoa butter is a great emollient and moisturizer, and as it was told above regarding the fatty acids, has anti-inflammatory properties. Therefore, it neutralizes the action of free radicals in the body, caused by external agents that trigger cellular aging, contributes to the regeneration of damaged and dry hair and readily absorbs into the top layer of skin and hair (Organic Cosmetic Science School, 2018).

2.2.4.4. Active Ingredients

Emollients

Emollients are responsible for softening and smoothing the skin and hair, *i.e.*, they are moisturizers that spread themselves across the hair fiber, forming a layer of protection and avoiding any moisture loss.

Olive Oil

Since old times people have used the fruit and oil of *Olea europaea*. Nowadays, the oil not only has been used in food industry but also in cosmetics as skin moisturizer, lip balm, hand lotion, shampoo, soap, massage oil, etc. Fatty acids, triglycerides, tocopherols, carotenoids, squalene, sterols and polyphenols are the main constituents of olive oil and contribute to its unique properties for the hair and skin. The unsaponifiables and polar compounds are responsible for the anti-inflammatory effect and, on the other hand, the presence of polyphenols provides the free radical-scavenging effect of virgin olive oil, avoiding lipid oxidation (Aburjai & Natsheh, 2003).

Açai Fruit Oil

Euterpe oleraceae is a large palm from South America that provides berries, juice, fruit powder and oil which are used in food, beverage, pharmaceutical and cosmetic industries. The açai oil is extracted by cold pressing from açai berries and has a high content in oleic acid (50-55%), palmitic acid (25-30%), phytosterols and phenolic acids. Therefore, the outstanding properties of this oil are the thermal and oxidation stability, conditioning properties and unique sensory characteristics (Organic Cosmetic Science School, 2018).

Castor Oil

Castor oil is a viscous fluid obtained from the seeds of *Ricinus communis*. The major component of castor oil is ricinoleic acid which has smoothing and moisturizing qualities for the hair, acting as barrier agent for the fibers. Hydrogenated castor oil and its esters are emollients for hair and skin formulations.

Glyceryl Oleate

Glyceryl oleate is the monoester of oleic acid and glycerin, and thus is produced from oils that have high contents of oleic acid, such as peanut oil, pecan oil or olive oil. Glyceryl oleate is part of the human hair lipids and has outstanding re-fatting properties, reduces roughness and the combing force of wet hair and enhances hair gloss (Making Cosmetics, 2018)

Humectants

Humectants are substances able to bond with water molecules, that is, they attract water from the environment, increasing the moisture content of the hair. The humectants are present in different processes involving the *stratum corneum* and water retention such as those described in the following paragraphs.

Scalp Skin and Hair Moisturization

One of the principal functions of the *stratum corneum* is to avoid evaporation of water from the epidermis to the surrounding environment when the human organism is exposed to dry conditions. If the water is not retained by the SC, dry skin will start appearing, meaning that when the content of water in the SC is reduced, the skin dries. A percentage of 15% to 25% of water at the skin surface and 40% of water at the SC border correspond to a healthy SC. When the content of water decreases below 10%, scaling on the skin becomes evident. Water permeability of the epidermis, the water retaining-properties of the SC and the rate of evaporative water loss constitute the principal elements that influence SC water content (Fluhr, 2008).

Hydration of the SC is fundamental for a normal and healthy skin and hair physiology and depends on the phenotype of the corneocytes (differentiated keratinocytes that compose the SC), on the composition of the physical packing of extracellular lipids and finally on whether or not the corneocytes are surrounded by highly hygroscopic compounds (Fluhr, 2008).

Since corneodesmolysis is regulated by numerous enzymes (serine proteases and glycosidases), the hydration of the SC is essential to avoid the retention of corneocytes on the skin surface and consequently the formation of scales (Fluhr, 2008).

The main reason for the use of humectants is due to their tendency to attract water for the skin by absorption under certain conditions. Since humectants are soluble in water, they have water-absorbing ability and represent highly hygroscopic substances. Therefore, they interact with the SC, creating a reservoir in the depth of the SC with lipid bilayers changing their water-binding or hydrophilic properties, but without disruption of its liquid crystallinity structure. The humectant causes the expansion of the corneocytes and intercellular expansion between them, enhancing skin barrier properties and improving the water-holding ability of the SC, which results in a better moisturization (Fluhr, 2008).

<u>Skin Barrier Function - *Stratum Corneum* Lipids Phase Transition</u>

The organization and interactions between corneocytes and intercellular bilamellar lipids ensure the entity of the skin barrier. The balance between solid and liquid crystalline phases is determined by the quantity of water and the degree of fatty acid unsaturation and is essential for preventing water loss, since a pure liquid crystal system, produced by an all-unsaturated fatty acid mixture, causes a fast water loss through the bilayers with a moderate barrier action and a solid system formed with an all-saturated fatty acid mixture causes an extreme water loss due to cracks in the solid phase. To prevent water loss, the balance between the two phases is required (Fluhr, 2008).

Skin dryness results from an elevated level of skin lipids in the solid state. Thus, a good moisturizer will sustain a higher proportion of lipids in the liquid state, and, therefore the humectants prevent the phase transtition of the *stratum corneum* from liquid to solid crystalline structure, preventing *water* loss and improving skin barrier properties (Fluhr, 2008).

<u>Desquamation: Regulated Degradation of the Desmosomes</u>

Desmosomes are structures through which keratinocytes are attached. The balance between synthesis and degradation of desmosomes is regulated by internal and external factors (such as surface pH, humidity, calcium ion gradient) and are required to maintain a healthy skin. The desmosomes of the SC (corneodesmosomes) and the *van der Waal's* forces holding the intercellular lipid lamellae ensure the SC cohesion. When the cohesive forces are weakened, the corneocyte begin to shed (desquamation). This process is regulated by two enzymes, namely, kallikren 5 and 7, and the hydratation of the SC is essential for the normal action of these enzymes. Thus, the activity of these enzymes is influenced by the increased hydration of the SC, caused by the presence of a humectant and a highly humid environment (Fluhr, 2008).

<u>*Vegetable Glycerin*</u>

Glycerin or glycerol is a highly hygroscopic, odorless, viscous, and sweet-tasting fluid with low toxicity. It is a trihydroxy alcohol generally obtained by saponification, and it is a multifaceted ingredient used in the food and cosmetic industry (as a solvent, humectant and dispersant of natural gums and thickeners) (Fluhr, 2008).

Glycerol hygroscopicity and water solubility are due to the three hydrophilic hydroxyl groups in its chemical structure (Figure 12).

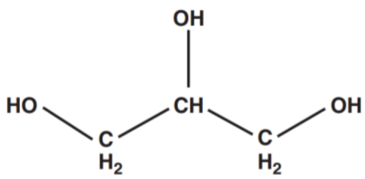


Figure 12: Glycerol chemical structure (propane-1,2,3-triol; trihydroxypropane; $C_3H_8O_3$).

Beyond the hydrating properties, glycerol has other biological and biophysical effects on skin and hair such as anti-irritant properties and helps in the process of wound healing.

Panthenol

Panthenol, dexpanthenol or d-panthenol is the correspondent alcohol of pantothenic acid, a water-soluble vitamin called vitamin $B_{5,}$ a major constituent of the hair. D-panthenol is water and alcohol soluble and is rapidly converted to D-pantothenic acid in the body, and has a very low toxicity. Panthotenic acid is necessary for the synthesis of coenzyme A (CoA) which is involved in enzyme-catalyzed reactions that are important in the metabolism of fatty acids, proteins, carbohydrates, sterols, gluconeogenesis, steroid hormones and porphyrins. Panthenol is widely used in pharmaceutical and cosmetics industries for its outstanding properties, such as hygroscopic properties, which allow an improvement of SC hydration and a reduction of transepidermal water loss. It works by attracting water from the surrounding environment which results in an increase of elasticity and softness of the skin, avoiding cracking or scaling, as explained above. Although panthenol is added to various types of cosmetic products, its anti-inflammatory activity is valuable for baby care preparations (Ebner, Heller, & Rippke, 2002).

Also, fibroblast proliferation is determinant for wound healing, and *in vivo* experiments with dexpanthenol have demonstrated increased proliferation of human fibroblasts (Ebner, Heller, & Rippke, 2002).

Finally, it was also confirmed that TEWL is notably affected by dexpanthenol (Ebner, Heller, & Rippke, 2002).

Proteins

Hydrolized Wheat Protein

Since proteins have poor water solubility, for use in cosmetics they are previous hydrolyzed, increasing their solubility (Secchi, 2008). Due to the low molecular weight range of the hydrolyzed proteins, they can penetrate easily into the hair shaft and form a thin protective film on hair. They can also reduce skin irritation caused by anionic surfactants, fighting skin dryness and providing a moisturizing effect (Secchi, 2008).

Hydrolyzed proteins have a brownish colour and a distinctive odour and this is due to the Maillard reaction at hight temperatures between the proteins and the accompanying carbohydrates (oligosaccharides) (Sun & Zhuang, 2012).

Powdered Active Ingredients

Activated Charcoal

Activated charcoal or carbon is a black powder and a form of carbon that is activated by subjecting it to very high temperatures that modify its internal structure, resulting in smaller pores and increasing its surface area. The activation of charcoal modifies its particles into very porous surfaces able to adsorb chemicals present in a liquid or a gas. Also, the activated carbon has a negative charge which attracts positively charged molecules such as some toxins and dirt (Naturally Curly, 2018).

Thus, it is an excellent additive to include in shampoo formulations, since it removes the dirt and the oiliness of the hair by adsorption.

<u>Grey Clay</u>

Clays are mineral-rich elements derived from a range of sources including volcanic ash, rocks, soil, or sediments. They are combinations of minerals such as calcium, magnesium, potassium and silica, and are a porous material which has the ability to adsorb oil, bacteria and impurities present on the hair strands and scalp. Thus, it eliminates oil, dead cells and flakiness without stripping the natural oils and stimulates hair growth (New Directions Aromatics, 2018).

2.2.4.5. Essential Oils

Essential oils are extracted from flowers, grasses, herbs, fruits, peels of citrus fruits, seeds, leaves and roots.

They are produced by cold-pressure squeezing of vegetable material or by distillation, which are processes that are laborious and expensive.

Essential oils, when incorporated in a hair care product, provide shine and conditioning effects and provide good hair texture and a longer lasting aroma (Aburjai & Natsheh, 2003)

Rosemary Essential Oil

Rosemary or *Rosmarinus officinalis* of the family *Labiatae*, is an aromatic plant with an intense pleasing smell suggestive of pine wood (Szumnya & Figiel, 2010). Rosemary is cultivated essentially in Mediterranean countries, and is used to not only help condition, but also to encourage healthy hair to grow (Aburjai & Natsheh, 2003).

Rosemary essential oil has antibacterial and circulation boosting properties in addition to its antioxidant properties. It also promotes blood circulation and vasodilatation in the scalp and slows down hair loss (Jalali-Heravi, Moazeni, & Sereshti, 2011).

Sweet Orange Essential Oil

Sweet orange (*Citrus sinensis*) oil is mainly composed by terpene hydrocarbons providing flavour and fragrance. It has more than 90% of limonene which is found in most of the citrus fruit peel oils and is used in different cleaning applications (Aburjai & Natsheh, 2003).

The most outstanding benefit is its high content of vitamin C which dilates the blood capillaries when applied to the hair, improving blood circulation and promoting hair growth.

Geranium Essential Oil

Pelargonium graveolens oil is extracted through steam or water plus steam distillation and is extensively used in cosmetic industry and aromatherapy.

Geranium oil is a cleansing, toning and sharpening oil and it has a strong rose-like perfume. It balances the oil content in the scalp and hair. However it should be used with care since there is a possibility of it originating contact dermatitis in hypersensitive individuals (Aburjai & Natsheh, 2003).

Lemongrass Essential Oil

Lemongrass (*Cymbopogon citratus*) is a plant that contains 1 to 2% of essential oils on a dry basis with wide variation of their chemical composition depending on genetic diversity, habitat and agronomic treatment. Lemongrass essential oil is defined by a high content of citral, which is used as a raw material for the production of ionone (aroma compounds in essential oils), vitamin A and betacarotene (red pigment in plants and fruits) (Tzortzakis, 2007). Lemongrass has antimicrobial activities, fortifies hair follicles and calms an itchy and irritated scalp. Additionally, it is a natural bug repellant, treats headaches and relieves stress.

3. Materials and Methods

3.1. Raw Materials

In this subchapter, the ingredients which were used during the experiments are presented, not only with the common name but also with the designation under the INCI (International Nomenclature Cosmetic Ingredient) nomenclature (Table 4). INCI is an international coding system for the classification of cosmetic ingredients, recognized and adopted worldwide, created to standardize the ingredients in the labelling of cosmetic products. The ranges of values defined for each ingredient used, as well as their supplier, are provided in appendix A.

Table 4: List of ingredients used in the present work.

	Common Name	**(INCI) Name**	**Physical State**
Surfactants	Sodium Cocoyl Isethionate	Sodium Cocoyl Isethionate	Powder/Flakes/ Needles
	Cocamidopropyl Betaine	Cocamidopropyl Betaine	Liquid
	Coco Glucoside	Coco Glucoside	Liquid
Bar Hardeners	Stearic Acid	Stearic Acid	Powder
	Cetearyl Alcohol	Cetearyl Alcohol	Flakes
	Organic Carnauba wax	*Copernicia cerifera* Wax	Pellets
Conditioning Agents	Organic Coconut Oil	*Cocos nucifera* Oil	Solid
	Organic Shea Butter	*Butyrospermum Parkii* Butter	Solid
	Organic Cocoa Butter	*Theobroma cacao* Seed Butter	Solid
Active Ingredients	Olive Oil	*Olea europaea* Fruit Oil	Liquid
	Organic Açai Fruit Oil	*Euterpe oleracea* Fruit Oil	Liquid
	Organic Argan Oil	*Argania spinosa* Kernel Oil	Liquid
	Organic Tamanu Oil	*Calophyllum tacamahaca* Oil	Liquid
	Organic Castor Oil	*Ricinus communis* Seed Oil	Liquid
	Vegetable Glycerin	Vegetable Glycerin	Liquid
	Panthenol	Panthenol	Liquid/Powder
	Glyceryl Oleate	Glyceryl Oleate	Liquid/Solid
	Hydrolyzed Wheat Protein	Hydrolyzed Wheat Protein	Liquid
	Grey Clay	Illite	Powder
	Activated Charcoal	Charcoal Powder	Powder
Essential Oils	Organic Rosemary Essential Oil	*Rosmarinus officinalis* Oil	Liquid
	Organic Sweet Orange Essential Oil	*Citrus sinensis* Oil	Liquid
	Organic Geranium Essential Oil	*Pelargonium graveolens* Oil	Liquid
	Organic Lemongrass Essential Oil	*Cymbopogon flexuosus* Leaf Oil	Liquid
Others	Water	*Aqua*	Liquid

3.2. Equipment

In this subchapter, the equipment and material which were used during the experiments are presented in the following Tables (Tables 5 and 6). Also, part of the material and equipment is differentiated as having been used for the lab and scale-up process (Table 6).

Table 5: Equipment used in the present work.

Equipment	Brand and model
Balance	RADWAG PS 4500/C/2
Heating Plate	Tristar
Thermostatic Bath	ARBO Lab WB12
Laser Thermometer	INFRARED DT8380
Stirrer	Heidolph- Hei-Torque 400
Mikrocount dipslides® duo	Schülke
Laminar Flow Chamber	BioAir TopSafe 1.2
Dry Weight Measurement Balance	Metler Toledo HB43-S
Incubator 1	ARALAB CL II
Incubator 2 (in a room)	Memmert
pH electrode	METTLER TOLEDO Five Easy TM F20
External Thermometer	Rotronic Hygrolog HL-20D

Table 6: Different equipment and materials used in lab scale and scale-up process.

<table>
<tr><th>Equipment and Material</th><th>Lab Scale</th><th>Scale-Up</th></tr>
<tr><td rowspan="2">Heatproof Glass Container</td><td rowspan="2">0,450 Liters
Diameter- 7 cm
Height- 11 cm</td><td>2 Liters
Diameter- 12 cm
Height- 18 cm</td></tr>
<tr><td>5 Liters
Diameter- 19 cm
Height- 27,5 cm</td></tr>
<tr><td>Heatproof Plastic Container</td><td>Not used</td><td>5 Liters
Diameter- 18 cm
Height- 23 cm</td></tr>
<tr><td>Stirring Shafts with Rotors</td><td> **1**
Diameter-4 cm
Height- 40 cm
Submergence -20-30 cm
 **2**
Diameter-6 cm
Height- 40 cm
Submergence -20-30 cm</td><td>
3
Diameter- 8 cm
Height- 50 cm
Submergence- 30-40 cm</td></tr>
<tr><td>Molds</td><td>
25- 30 grams

45-50 grams</td><td>
75-85 grams</td></tr>
</table>

3.3. Operational Procedures for Shampoo Production

The experiments took place between February 18th and July 27th of 2018 in UniBio's laboratory located in Sintra, where the air temperature oscillated throughout the length of the experiments, according to the seasons.

Regarding the experimental procedure for each shampoo, it should be noted that the process for oily hair shampoo has the addition of one more phase compared to the other two shampoos. Thereby, to illustrate the developed manufacturing process, two flowcharts were created: one representing the procedure for oily hair shampoo (Figure 13) and other representing the procedure for normal and dry/damaged hair shampoos (Figure 14):

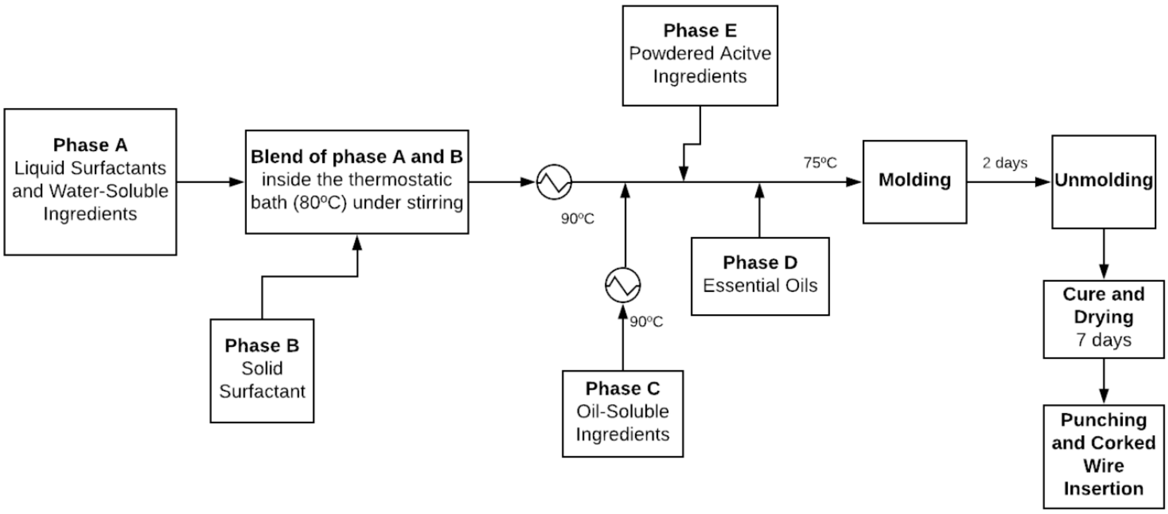
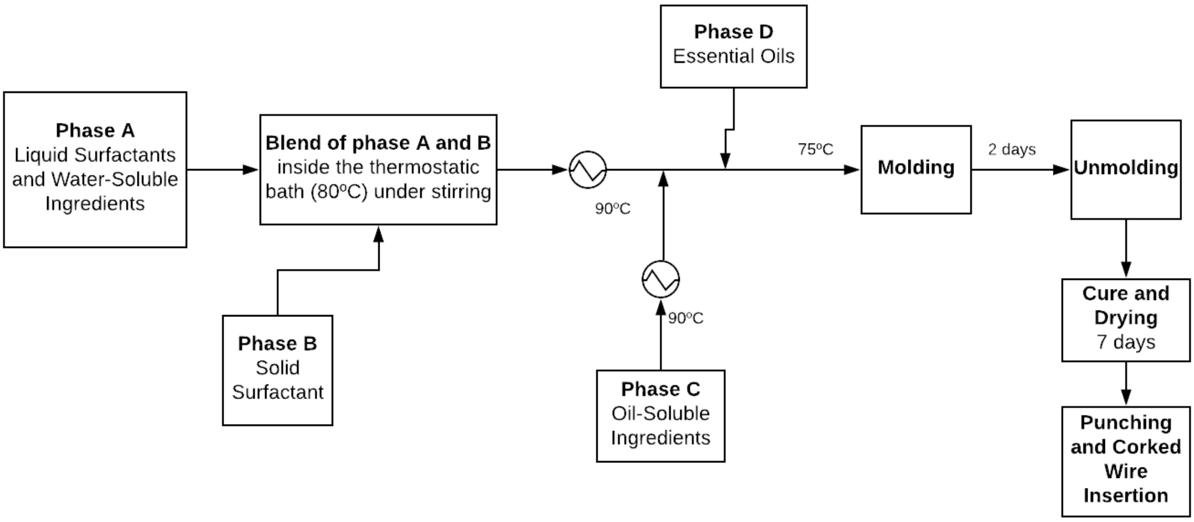


Figure 13: Oily hair shampoo flowchart.



Also, the operational procedures were described not only for the lab scale process, but also the for the scale-up process.

3.3.1. Laboratory-Scale

3.3.1.1. Surfactants and Liquid Phase

The liquid surfactants (cocamidopropyl betaine and coco glucoside) and the water-soluble ingredients with the exception of hydrolyzed wheat protein (phase A) were weighted on the scale and placed into a glass heatproof container. The heatproof container with phase A was placed into a hot water bath at 80ºC. Afterwards, this phase was used to melt and incorporate the solid surfactant phase (phase B). Phase B, which was previously weighted into a glass heatproof beaker, was slowly added to phase A which was placed in the glass heatproof container inside hot water (80ºC), and stirred (with impeller 1 or 2) until it was completely melted and a thick mixture was formed (Figure 15).

Figure 15: Blend of phases A and B.

The heating step was performed by two different approaches. The first was a more traditional method, using a double boiler and a heating plate (Figure 16), and the second (more accurate method) in a thermostatic bath (Figure 17). In both methods, the water that was placed either in the bath or in the double boiler had to be at the same level as the mixture inside the glass heatproof container, to ensure that it remained at the desired temperature. The glass heatproof container was always kept inside of the bath until the end of the protocol.

Figure 16: Phase B dissolution and incorporation using a double boiler and a heating plate.

Figure 17: Phase B dissolution and incorporation using a thermal bath.

To monitor both the water bath and mixture temperatures, a laser thermometer was used. At this stage, the water bath was kept at 80ºC and the mixture reached around 60ºC. In early procedures, other bath temperatures were used, up to 95-100ºC.

Phase B was incorporated under stirring and that procedure was also carried out in two different ways, more specifically: (1) manually (Figure 16) or (2) by using a stirrer (between approximately 50 and 250 rpm) (Figure 17) with different types of impellers (1 and 2) according to the size of the sample and the corresponding stirring requirements.

3.3.1.2. Oil Phase

The oils, oil-soluble ingredients and hydrolyzed wheat protein (phase C) were previously weighted and placed together in a glass heatproof beaker. Thereafter, the beaker was placed inside a water bath at 90ºC, until all components had been dissolved (Figure 18). For this step, there was no need of special stirring, since they mixed easily only with the help of a stirring rod. In this case, the water was also at the same level as the oil phase inside the container, providing a faster melting.

Figure 18: Phase C melting inside of a double boiler.

3.3.1.3. Incorporation of Phase C with Phases A and B

When melted, phase C was added to the mixture of phase A and B (glass heatproof container in hot water bath at 90ºC) under stirring. Before the phase C was added, the hot water bath temperature was increased to 90ºC. The stirring speed was dependent on the size of the sample and on the type of impeller. The mixture was stirred until a low viscosity and homogeneous mixture were obtained.

3.3.1.4. Powdered Active Ingredients Phase

The steps mentioned above are common to all three formulations. However, in the case of the oily hair shampoo, there is an additional step which corresponds to the powdered active ingredients phase (phase E). After phase C has been incorporated, the powder phase (phase E), which was previously weighted into a glass beaker, was added to the glass heatproof container, at the same bath water temperature (90ºC), with the contents reaching around 75ºC. Also, this phase was added all at once, under low stirring speed (approximately 50 rpm), preventing powder from escaping and cause losses (Figure 19).

Figure 19: Mixture with powder active ingredients added.

3.3.1.5. Essential Oils

The next part of the protocol was the addition of the essential oils (phase D) which provided the desired perfume and aromas. After the essential oils have been weighted into a glass beaker, these were immediately covered, avoiding their evaporation and loss. Right before the mixture was poured into the molds, essential oils were added to the glass heatproof container under stirring.

3.3.1.6. Molding and Curing

With the mixture in the glass heatproof container at a temperature of at least 75ºC, it was spooned into the molds (Figure 20), with the mixture under stirring. The shampoos were kept inside the molds during 48 hours inside the warehouse at a temperature and humidity corresponding to the atmospheric conditions. These parameters were monitored with an external thermometer. After 48 hours inside the molds the shampoos were removed from the molds and placed in trays inside the warehouse at room temperature (Figure 21).

Figure 20: Scoop used to transfer the mixture and the mixture placed in the mould.

Figure 21: Stored Shampoos for the drying and curing step.

3.3.2. Scale-Up

In the scale-up process, the procedure was similar with the exception of the materials and equipment (Table 6).

Regarding the heatproof container, three different types of vessels were used: (1) a 2-L glass heatproof container suitable for intermediate size samples, (2) a 5-L plastic heatproof container for large size samples and (3) a 5-L glass heatproof container also for large size samples (Table 6).

Considering the heating step of phase A and B, it was only performed in the thermostatic bath, excluding the heating plate and double boiler method. In relation to the agitation, it was only accomplished by using impeller 3 (between approximately 150 and 500 rpm), throughout the protocol (Table 6).

Concerning the materials used at both scales, the last difference corresponds to the molds. For the laboratory scale, two types of molds were used: one containing 25-30 grams of shampoo and the other 45-50 grams. For the scale-up process, the molds were larger, had a cylindrical shape and contained between 75 and 85 grams of product.

A final step was added in the scale-up process. Seven days after removing the shampoos from the molds, they were ready to be punched with a 1 mm needle, in order to insert a corked wire. Two corked wires (35mmx1mm) were used to provide a line to hang the shampoo, with the aid of a recycled cork piece previously cut and used as a stopper (Figure 22 and 23).

Figure 22: Pieces of cork used as stoppers and two inserted corked wires.

Figure 23: Hung shampoo with corked wire.

3.3.3. Water Content Determination

Dry weigth tests were performed on some samples, to determine their content in water and volatiles. The drying was made to 3 replicates of different formulations. First, the sample was cut into strips, and each strip was weighted in an analytical balance before drying. A dry weight measurement balance was used and the sample dried at a temperature of approximately 106°C until constant weight. The drying was done inside a fume hood to prevent the release of toxic compounds, such as sodium cocoyl isethionate. After drying, the strip sample was cooled in a desiccator for about 20 minutes in order to prevent water absorption and also allowing the sample cooling. Afterwards, the sample strip was reweighed in the analytical balance, and the content of volatiles that had been evaporated was determined by weight difference.

The sample mass was obtained by the difference between the total and the plate mass (equation 1):

$$Sample\ Mass = Total\ Mass - Plate\ Mass \quad (1)$$

The total weight loss (TWL) was calculated by dividing the volatiles mass by the sample initial mass (equation 2):

$$\text{Total Weight Loss (TWL)} = \frac{\text{Lost volatiles mass}}{\text{Sample mass before drying}} \quad (2)$$

3.3.4. Microbiological Analysis

In order to execute the microbiological control of the product, quick microbiological test kits were used. The mikrocount® duo dipslides (Schülke) are plastic nutrient media slides coated on both sides with a agar media, namely one for detection of general microbial growth (universal medium) and another for detection of fungal growth (antibiotic media). These were used for the combined determination of the total plate count and for the detection of yeasts and moulds (Figure 24).

Figure 24: Mikrocount® duo dipslides coated with a special agar (Schülke & GmbH, 2018)

The application of the mikrocount® dipslides procedure was performed in a laminar flow chamber to avoid any contamination. The chosen method was the contact inoculation, which is suitable for surfaces and solid samples. First, the shampoo samples were cut along the smallest centre axis with a disinfected x-acto knife and then both sides of the nutrient media carrier were presses manually onto the sample surfaces and held for about 10 seconds (instead of 5 seconds, according to the supplier's instructions in Appendix D). Thereafter, the mikrocount® dipslides were incubated during 14 days in incubator 1 or 2 at 30 ºC and regularly inspected for the appearance of colonies.

3.4. Analytical Procedures

3.4.1. pH Measurement

In order to monitor the pH of the solid shampoos, a 1:3 (by weight) dilution was performed in hot water to obtain a solution with the dissolved shampoo. Thus, the pH of the achieved hot solution was measured using a pH meter.

3.4.2. Quantitative Evaluation of the Shampoos

Between each formulation, almost all shampoos after the cure were quantitatively evaluated in different parameters such as visual characteristics, texture, application to wet hair and effects on dry hair. On a scale of 1 to 5, several features were evaluated within these parameters such as:

Visual Characteristics

Colour (Compare the colour of the obtained shampoo with a white and a completely black colour): 0- White; 5- Black.

Granules (Evaluate the presence of granules on the surface and on the inner surface after cut the shampoo along the smallest center axis): 0- Has several granules; 5- Does not have granules.

Cracks (Visually evaluate the presence of cracks): 0- Has several cracks; 5- Does not have cracks.

Brightness (Compare visually the brightness of the obtained shampoo to a high gloss and an opaque colour): 0- Lusterless; 5- Bright.

Unmolding (Compare the shape of the obtained shampoo with the shape of the used mold): 0- The shape does not correspond to the mold; 5- The shape corresponds perfectly to the mold.

Texture

Toughness (Press the shampoo with the finger, evaluating its ability to resist or deform): 0- Soft; 5-Tough.

Roughness (Swipe with the finger on the surface of the shampoo, assessing its irregularities): 0- Smooth; 5- Rough.

Release Colour (Put the finger on the surface of the shampoo and evaluate if it has the same colour of the shampoo or if it has a greasy feeling): 0- Release 5- Does not release colour.

Cohesion (Evaluate the shampoo's consistency not only by the appearance but also by slightly squeezing it): 0-Non-cohesive; 5-Cohesive.

Application to Wet Hair

Foaming duration (Measure the time between applying the shampoo and the foaming to appear): 0- After a considerable time; 5- Instantly.

Foaming creaminess (Apply the foam in the hand and evaluate how well it spreads): 0- Not creamy (Does not spread well); 5- Creamy (Spreads very well).

Foaming (Measure the dispersion of the foam): 0- No foam; 5- Considerable foam quantity.

Bubble size (Measure the size of bubbles): 0- Approximately 1 millimetres; 5- Approximately 0.1 millimetres.

Wet combing (Pass the comb through the wet hair and evaluate its ability to comb from the root to the ends without any stop): 0- Difficult combing; 5- Easy combing.

Effects on Dry Hair

Dry combing (Pass the comb through the dried hair and evaluate its ability to comb from the root to the ends without any stop): 0- Difficult combing; 5- Easy combing.

Coating (Touch the washed hair and feel and see if there is any residue of dirt): 0- Have residues; 5- Does not have any residue.

Volume (Compare the size of wet and the dry hair): 0- Without volume; 5- With volume.

Gloss (Compare visually the gloss obtained after drying with the gloss of the wet hair): 0- Lusterless; 5- Bright.

Static (Evaluate the dispersion of loose hair strands in relation to a piece of hair): 0- Have static electricity (Has many scattered hairs); 5- There is no static electricity (Does not have scattered hairs).

Dryness (Evaluate the friction between the hair strands and the visual aspect of the hair): 0- Dry hair; 5- Moistened hair.

Washing of the Hair

Note that the washing of the laboratory hair was performed by initially soiling the hair with 0.5 g of olive oil and then passing 4 times the solid shampoo over the hair. After applying the shampoo, the hair was rubbed with the hands in order to evaluate the parameters of the foam. Therefore, the hair was rinsed with water in order to evaluate the above referred parameters.

Panel Choice

The evaluations were carried out by a group of 7-10 people who had previously been trained with a control shampoo and gained experience with other shampoo samples. The evaluations were carried out through questionnaires that were separately answered by each person taking into account all the formulations that were evaluated.

4. Results and Discussion

In this work, the oily hair shampoo was the first to be produced and its formulation constituted the basis for the other two shampoos (normal and dry/damaged hair shampoos).

4.1. Oily Hair Shampoo

Between February 27th and May 17st of 2018, 40 tests were performed to formulate the oily hair shampoo and to achieve the desired properties such as texture, colour, toughness, cohesion, foaming duration, foaming creaminess, and conditioning. Multiple ingredients and experimental conditions were tested in order to achieve improvements between each formulation.

The initial goal was to formulate a shampoo with the characteristics and quantities of each type of ingredient according to Table 1. In all formulations, the essential oils were randomly chosen, *i.e.*, the definitive aroma was only chosen for the final formulation.

It should be noted that in all the Tables presented in the results the quantities of used ingredients result from a normalization of their base ranges given in appendix A. Also, the formulations are identified according to the type of shampoo: O for oily hair, D for dry and damaged hair and N for normal hair.

4.1.1. Incorporation of Sodium Cocoyl Isethionate

4.1.1.1. Formulation O1

Table 7: Formulation O1 of oily hair shampoo.

Phases	Component	Quantity (% of the base range)
A	Cocamidopropyl Betaine	96.40
B	Sodium Cocoyl Isethionate	32.03
C	Cetearyl Alcohol	44.29
	Organic Coconut Oil	96.33
	Hydrolyzed Wheat Protein	39.25
E	Activated Charcoal	99.50
	Grey Clay	42.57
D	Peppermint Essential Oil	62.10

Since SCI is the powdered surfactant and the goal was to obtain a solid shampoo, this ingredient was used in a higher quantity in comparison with the liquid surfactant. The initial plan was to dissolve the SCI (phase B) first and then mix it with the liquid surfactant (phase A). Thereafter, the intent was to add the active ingredients with a lower melting point than the SCI (phase C) in a heat resistant glass container and melt them all together. Later, the goal was to blend them with the surfactants obtaining a liquid mixture. The last step before inserting the essential oil (phase D) was to incorporate the previously obtained mixture with the powder phase (E), resulting in a fluid mixture to place into the mold.

The activated charcoal and the grey clay were especially chosen for the oily hair shampoo due to their adsorbent properties, grabbing the oil particles present in dirty oily hair.

First, a simple formulation was performed and an attempt was made to dissolve the powder SCI directly in the double boiler at 210ºC, since its melting point is around 190ºC (appendix B). However, it burned immediately, concluding that it is not possible to dissolve this powder surfactant directly without mixing with any liquid.

As the first approach was not successful, it was attempted to incorporate the SCI otherwise, that is, by adding it to the powder phase (phase E) without dissolving, as done with the other powder ingredients. On the other hand, the quantity of liquid ingredients was not sufficient to incorporate the powder phase and to create a manageable mixture.

As a conclusion, SCI can only be inserted with the previous dissolution with other liquids, in this case, liquid surfactants such as cocamidopropyl betaine and coco glucoside.

4.1.1.2. Formulation O9

Table 8: Formulation O9 of oily hair shampoo.

Phases	Components	Quantity (% of the base range)
A	Cocamidopropyl Betaine	29.80
	Coco Glucoside	0.180
B	Sodium Cocoyl Isethionate (Needles)	59.60
C	Cetearyl Alcohol	38.21
	Stearic Acid	57.21
	Organic Coconut Oil	21.47
	Organic Shea Butter	4.690
E	Grey Clay	77.43
	Activated Charcoal	0
D	Lemongrass Essential Oil	22.10

In this formulation, SCI was previously mixed with coco glucoside and cocamidopropyl betaine, as told above, and an extremely viscous mixture was obtained (Figure 25).

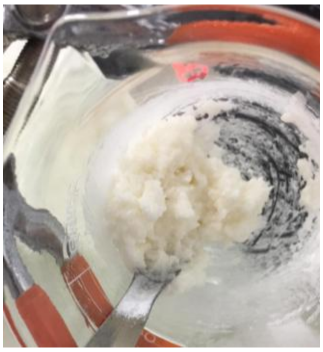


Figure 25: Phase A and B mixed in formulation O9.

To continue the dissolution of SCI needles, and to achieve a less viscous mixture, the previously melted phase C was added (Figure 26).

Figure 26: Phase A, B, and C mixed in formulation O9.

After phase E and D were added, the mixture was poured into the mold. However, several cracks were detected in the final shampoo due to the presence of air in the mixture. Several granules were also visible, demonstrating that the SCI was not perfectly incorporated (Figure 27). This may be due to the fact that the agitation step was performed manually.

Figure 27: Formulation O9 of oily hair shampoo with visible imperfections.

4.1.2. Addition of Water in the Formulation

In line with an ingredient supplier's suggestion, water was added to the formulation, since it is a suitable solvent for sodium cocoyl isethionate.

4.1.2.1. Addition of Water in the Formulation at Phase A

Formulation O16

Table 9: Formulation O16 of oily hair shampoo.

Phases	Components	Quantity (% of the base range)
A	Cocamidopropyl Betaine	30.00
	Coco Glucoside	36.36
	Water	10.53
B	Sodium Cocoyl Isethionate	40.00
C	Stearic Acid	57.14
	Cetearyl Alcohol	14.29
	Organic Coconut Oil	20.00

Table 8: (Continuation) Formulation O16 of oily hair shampoo.

Phases	Components	Quantity (% of the base range)
C	Organic Shea Butter	23.08
	Hydrolyzed Wheat Protein	25.00
E	Grey Clay	42.86
	Activated Charcoal	0
D	Lemongrass Essential Oil	4.000

Regarding this formulation, water was added in a considerable percentage (10.53% of the base range), which together with the increase in the percentage of coco glucoside (36.36% of the base range), would contribute to an improved dissolution of the SCI granules. Moreover, the quantity of SCI was reduced to 40% of the base range in order to facilitate the process described above.

Considering the possibility of the visible granules being cetearyl alcohol pellets, which were not well dissolved in phase C, the amount of this ingredient was likewise decreased to 14.29% of the base range.

To prevent crack occurrence in the shampoo, the amount of organic shea butter was increased to 23.08% of the base range and grey clay diminished to 42.86% of the base range, with the intent to provide a more fluid mixture which would prevent air from remaining in the shampoo. The fluid mixture would also contribute to a better molding, allowing an improved cohesion of the shampoo without cracks.

Nonetheless, when phases C and E were added, the mixture became extremely viscous, which was not expected (Figure 28). This could be caused by water evaporation during the procedure and/or due to some air incorporated into the mixture, given that water was added at the beginning of the experimental procedure at phase A with a stirring time of 54 minutes at temperatures close to 95/100ºC.

Figure 28: Shampoo mixture (formulation O16) at 54 minutes of agitation.

Formulation O22

Table 10: Formulation O22 of oily hair shampoo.

Phases	Components	Quantity (% of the base range)
A	Cocamidopropyl Betaine	9.200
	Coco Glucoside	24.73
	Water	78.95
B	Sodium Cocoyl Isethionate	17.60
C	Stearic Acid	34.86
	Cetearyl Alcohol	34.86
	Organic Coconut Oil	11.47
	Organic Cocoa Butter	15.69
	Glyceryl Oleate	7.560
	Panthenol	7.560
	Hydrolyzed Protein	6.500
E	Grey Clay	19.43
	Activated Charcoal	21.00
D	Lemongrass Essential Oil	16.84

Concerning this formulation, the intent was to largely increase the quantity of water (to 78.95% of the base of range) in order to evaluate its ability to enhance the fluidity of the mixture. Nevertheless, to ensure the toughness of the shampoo, the liquid surfactants had a decrease to 9.20% and 24.73% of the base range (cocamidopropyl betaine and coco glucoside respectively), as well as the coconut oil (to 11.47% of the base range), and the organic shea butter was replaced by a harder butter – cocoa butter. In addition, in previous formulations which were not mentioned, new active ingredients, namely, glyceryl oleate and panthenol, already had been added in order to intensify the conditioning and hydration of the hair.

In order to prevent water evaporation during the procedure, the temperatures were kept under 85ºC from the beginning to the end, and also, the stirring time was reduced (40 minutes).

At the beginning when phases A and B were merged, the mixture was liquid. When adding phase C, it turned heterogeneous and particularly thick. Thus, water ability to liquefy the mixture was not confirmed.

Considering that this formulation contained a significant quantity of water, it was chosen that the mixture would stay in the molds for 5 days, in order give the shampoo more time to harden. Nonetheless, after 5 days, the shampoo was unmolded and it was noticed that it was extremely soft, thus being concluded that no water was evaporated during the cure and that the water amount in the next attempted formulation should be lower.

4.1.2.2. Addition of Water in the Formulation after Phase E

Formulation O23

Table 11: Formulation O23 of oily hair shampoo.

Phases	Components	Quantity (% of the base range)
A	Cocamidopropyl Betaine	10.00
	Coco Glucoside	27.27
B	Sodium Cocoyl Isethionate	16.67
C	Stearic Acid	50.00
	Cetearyl Alcohol	21.43
	Organic Coconut Oil	13.33
	Organic Cocoa Butter	38.46
	Glyceryl Oleate	33.33
	Panthenol	0
	Hydrolyzed Wheat Protein	25.00
E	Grey Clay	28.57
	Activated Charcoal	0
F	Water	52.63
D	Lemongrass Essential Oil	21.05

As it was told before, the correspondent shampoo of formulation O22 remained soft, so the amount of water had to be reduced to 52.63% of the base range. Since that stearic acid and cocoa butter are strong hardeners that provide toughness, which was not sufficient in the prior formulation, these two ingredients were increased to 50% and 38.46% of the base range respectively.

The aim of this formulation was to test if there is any difference in the absorption of water in the mixture, through inserting it at the beginning of the protocol (phase A) or after phase E.

The initial temperature was 75ºC and after 14 minutes from the start, phase C was added at 80ºC. When phase E was added (20 minutes at 81.9ºC) the mixture had the desired consistency to be inserted into the mold.

Phase F and D were added almost simultaneously, and at that time, the mixture became heterogeneous and viscous.

After inserting the shampoos into the molds, they were stored at 14.4ºC and 67.4% of humidity. After two days, the shampoos were removed and it was possible to see the following the characteristics (Figure 29):

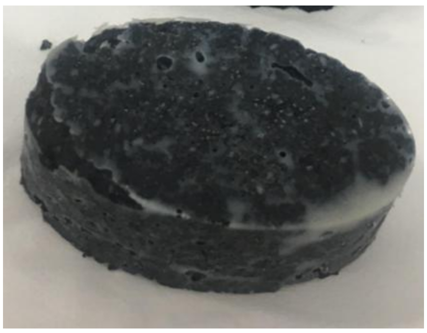


Figure 29: Formulation O23 of oily hair shampoo.

1. Several cracks were observed due to the mixture viscosity when poured into the molds.
2. The consistency of the shampoo did not fulfill the expected requisites (too soft), which means that the cocoa butter did not give the necessary hardness to the shampoo.
3. There were also two visible colours in both of the shampoos, indicating the heterogeneity of the mixture, which may have been caused by the briefer stirring time of this formulation (24 minutes).

In a nutshell, the conclusion is that the addition of water causes a phase separation, since most of the mixture is constituted by water-insoluble ingredients. This does not depend on the phase where it is added. Therefore, this contributes directly to an increase in viscosity and heterogeneity. As a result, water should be added in phase A to dissolve the SCI, in smaller or non-existent quantities in the following formulations of the shampoo, taking into account the above-justified fact.

4.1.3. Shampoo Hardness and Consistency

4.1.3.1. Formulation O26

Table 12: Formulation O26 of oily hair shampoo.

Phases	Components	Quantity (% of the base range)
A	Cocamidopropyl Betaine	18.30
	Coco Glucoside	35.18
	Water	21.95
B	Sodium Cocoyl Isethionate	27.87
C	Stearic Acid	63.57
	Cetearyl Alcohol	21.29
	Organic Coconut Oil	20.87
	Organic Shea Butter	14.92
	Glyceryl Oleate	39.44
	Panthenol	0.440
	Hydrolyzed Wheat Protein	25.75
E	Grey Clay	29.43
	Activated Charcoal	5.250
D	Organic Lemongrass Essential Oil	37.89

To overcome the weaknesses of formulation O23, several changes were carried out in this test.

As stated before, the quantity of water was once again diminished to 21.95% of the base range, in order to try to prevent phase separation. To melt the powder surfactant, water was added at phase A, and also, the quantity of liquid surfactants was slightly increased to 18.30% and 35.18% of the base range for cocamidopropyl betaine and coco glucoside, respectively (since the water had been reduced). This way, the obtained mixture would be less viscous, simplifying its entry into the mold.

Regarding the final hardness of the shampoo, the introduction of cocoa butter was not as efficient as it was expected, so it was withdrawn. Thus, shea butter was re-added and stearic acid was increased again to 63.57% of the base range, in order to balance the removal of the cocoa butter.

Considering the amount of activated charcoal in this formulation, it is confirmed to be higher in comparison with formulation O23 (5.25% of the base range). This was done to avoid the two-colour appearance of the shampoo, ensuring its uniform black colour. In addition, it was also important to increase the total stirring time to ensure the homogeneity of the blend (40 minutes).

Given that in the shampoos corresponding to the previous formulations, a lot of product was lost during the washes, it was necessary to reduce the quantity of butter (shea butter) to 14.92% of the base range, in order to build a less greasy and more cohesive shampoo.

Therefore, phases A and B were mixed at 75ºC under stirring during 11 minutes. At that time, phase B was apparently well dissolved (Figure 30).

Figure 30: Phase B dissolved in phase A after 11 minutes of stirring in O26.

After 36 minutes of stirring and after phase C and E were added, the mixture presented a good viscosity and consistency, liquid enough to be poured in the molds (Figure 31).

Figure 31: Phase A, B, C, and E mixed after 36 minutes in formulation O26.

At 40 minutes of stirring and after phase D was added, the mixture with the desired viscosity was easily poured into the mold (Figure 32).

Figure 32: Shampoo mixture of formulation O26 inserted into the mold.

The shampoo was stored at 18.0 ºC and 71.0 % of humidity during 1 day. The following day, it was removed and the shape was perfectly corresponding to the mold, with few apparent granules, and with a good consistency, hard and cohesive (Figure 33).

Figure 33: Shampoo (formulation O26) removed from the mold after 24 hours of storage at 18.0 ºC and 71.0 % of humidity.

However, when the shampoo was vertically cut, undissolved sodium cocoyl isethionate was found in the middle of the shampoo (Figure 34). In addition, after the first contact with water, the shampoo showed more SCI, which was previously unseen (Figure 35). That probably means that the sodium cocoyl isethionate was not sufficiently well dissolved and, perhaps, the stirring time of phase A and B together should be increased above 11 minutes.

Figure 34: Shampoo (formulation O26) cut vertically.

Figure 35: Half of the shampoo (formulation O26) after its first contact with water.

4.1.3.2. Formulation O32

Table 13: Formulation O32 of oily hair shampoo.

Phases	Components	Quantity (% of the base range)
A	Cocamidopropyl Betaine	27.70
	Coco Glucoside	43.82
	Water	5.310
B	Sodium Cocoyl Isethionate	33.20
C	Stearic Acid	49.00
	Cetearyl Alcohol	29.07
	Organic Coconut Oil	0.200
	Organic Shea Butter	1.620
	Olive Oil	15.00
	Glyceryl Oleate	33.55
	Hydrolyzed Wheat Protein	30.00
E	Grey Clay	96,71
	Activated Charcoal	3.500
D	Organic Rosemary Essential Oil	24.21

In formulation O26, the shampoo had a good consistency and cohesion. On the other hand, after its contact with water, and despite the granules appearance, a considerable amount of product was also lost during the wash. To counter this effect, a new approach was tested: in formulation O32, the quantity of grey clay was increased in order to create a dense shampoo with a larger portion of solid ingredients.

Simultaneously, a new ingredient was inserted into the formulation: the olive oil. In addition to all its favorable properties for the hair, this ingredient would have the function of homogenizing the shampoo, aggregating the clay particles that were present in the mixture, with the goal of binding all the ingredients, in order to accomplish a greater cohesion. In this case, as the olive oil is supposed to be an ingredient that alredy adds hydration properties to the shampoo, it was decided that shea butter and coconut oil would have a smaller percentage in this formulation (1.62% and 0.20% of the base range respectively).

Initially, before phase E was inserted, the mixture was extremely liquid. However, when this phase was inserted, the mixture became too viscous and solid, preventing its pouring in the molds by the usual method. The insertion into the mold required manual compression, which means that the percentage of solids in relation to the liquids was too high.

Since the mixture already had a solid consistency when it was placed in the mold, the time inside the mold was smaller (8 hours) at 21.4ºC and 60.3% of humidity.

Bearing in mind that the method used was manual compression and that the mixture was not liquid enough, after the cure, the shampoo presented several cracks due to the air that was trapped inside mixture (Figure 36).

Figure 36: Shampoo (formulation O32) removed from the mold after 8 hours of storage at 21.4ºC and 60.3% of humidity.

Another distinctive of this shampoo is the fact that its colour had turned gray, due to the larger percentage of clay (96.71% of the base range) for almost the same percentage of activated charcoal (3.50% of the base range).

4.1.3.3. Formulation O37

Table 14: Formulation O37 of oily hair shampoo.

Phases	Components	Quantity (% of the base range)
A	Cocamidopropyl Betaine	37.30
	Coco Glucoside	47.55
B	Sodium Cocoyl Isethionate	37.40
C	Stearic Acid	56.14
	Cetearyl Alcohol	21.50
	Organic Coconut Oil	16.73
	Organic Shea Butter	14.77
	Glyceryl Oleate	46.44
	Olive Oil	2.250
	Hydrolyzed Wheat Protein	4.000
E	Grey Clay	42.29
	Activated Charcoal	15.25
D	Organic Rosemary Essential Oil	26.32

Considering the results obtained previously, and the tests performed on the hair of the previous formulations, some adjustments were performed in the present formulation, such as:

- The water was withdrawn in this formulation in order to evaluate the ingredients affinity and their ability to render the mixture fluid.
- The quantity of sodium cocoyl isethionate was slightly (to 37.40% of the base range) increased to enhance the foam during the wash.
- Coconut oil and the shea butter were increased (to 16.73% and 14.77% of the base range respectively) to provide the necessary hydration to hair strands.
- The quantity of grey clay was considerably reduced to 42.29% of the base range, in order to obtain a mixture with a lower viscosity.
- The activated charcoal was increased to 15.25% of the base range for the black colour to prevail instead of gray.

At first when phases A and B were mixed, and since there was no water in phase A, a viscous mixture was formed, and the dissolution of SCI was a difficult task. Nevertheless, with the chosen portion of oils and butters which were melted and added in phase C, the mixture became liquid and easy to pour into the molds.

The shampoos were stored at 20.9 ºC and 71.5 % of humidity during 48 hours. After these two days, they were removed from the molds (Figure 37).

Figure 37: Shampoos (formulation O37) removed from the molds after 48 hours of storage at 20.9 ºC and 71.5% of humidity.

As can be seen in Figure 37, the results obtained correspond to the goals of this formulation: the consistency of the mixture, the colour, the toughness, the cracks and granules absence and also the shape were in line with the expected. On the other hand, when in contact with the skin before washing, the shampoo caused a greasy feeling, leaving it oily and with colour (Figure 38).

Figure 38: Oily skin after its contact with the shampoo (formulation O37).

The greasy feeling given by the shampoo is caused by the greater amount of coconut oil and shea butter and other ingredients contained in phase C in comparison with the other formulations (with water).

4.1.3.4. *Formulation O40*

Table 15: Formulation O40 of oily hair shampoo.

Phases	Components	Quantity (% of the base range)
A	Cocamidopropyl Betaine	40.80
	Coco Glucoside	45.27
B	Sodium Cocoyl Isethionate	36.10
C	Stearic Acid	57.00
	Cetearyl Alcohol	21.43
	Organic Coconut Oil	17.40
	Organic Shea Butter	15.31
	Glyceryl Oleate	11.11
	Olive Oil	0
	Hydrolyzed Wheat Protein	100.0
	Panthenol	11.11
	Carnauba Wax	19.80
E	Grey Clay	21.29
	Activated Charcoal	12.50
D	Organic Rosemary Essential Oil	22.10

The aim of the formulation O40 was to solve the situation described above, removing the greasy feeling of the shampoo and adding a bit more hardness. This formulation is very similar to formulation O37, with the exception of glyceryl oleate that was reduced to 11.11% of the base range in the same order of magnitude than the other similar active ingredients (hydrolyzed wheat protein, olive oil, and panthenol).

The new ingredient responsible for hardness and the removal of the greasy feeling was carnauba wax since it is one of the hardest waxes and it has the ability to absorb some of the oiliness from the remaining ingredients. It has a melting point of 82 °C to 86 °C (appendix B), which forces the melting temperature of phase C to be higher than before. Part of the percentage of grey clay from the previous formulation was removed to be possible to add carnauba wax.

The final mixture (all phases mixed) had a good viscosity and a manageable consistency, thus allowing an easy molding. They were stored at 25.6 ºC and 68.1% of humidity for 48 hours. After this time, they were unmolded and the curing process started (Figure 39).

Figure 39: Shampoos (formulation O40) removed from the molds after 48 hours of storage at 25.6 ºC and 68.1% of humidity.

The results obtained correspond to all the requirements that were planned for the oily hair shampoo, namely:

- A completely black shampoo that highlights the activated charcoal and clay, which are indicated for oily hair due to its adsorption properties.
- A shampoo with the absence of undissolved granules and without numerous cracks.
- A shampoo when unmolded had with the exact shape of the mold.
- A cohesive shampoo, which allows not losing much product during the washes.
- A shampoo with the necessary hardness preventing it from breaking during the washes.
- A shampoo without a greasy feeling, which does not allow the colour to be transmitted to the skin.
- A creamy foam that not only allows washing the hair from the root to the tips but also that quickly appears when in contact with water (Figure 40).

Figure 40: Formulation O40 hair foaming test.

- A shampoo that not only removes the oiliness of hair (main function), but at the same time does not strip extensively the natural oils from the hair allowing its hydration and softness after washing.

4.1.4. Quantitative Evaluation of the Shampoo

The shampoos that were referenced in the previous chapters were evaluated according to what was tested in each formulation and the need to test the product on hair (Table 16 and Figures 41 and 42).

Table 16: Evaluated shampoos referenced in the previous chapters.

		Formulation								
	Features	**O1**	**O9**	**O16**	**O22**	**O23**	**O26**	**O32**	**O37**	**O40**
Visual characteristics	Colour	5	4	3	5	1	5	3	4	5
	Granules	4	2	5	2	2	3	4	5	5
	Cracks	2	1	1	1	1	4	3	4	4
	Brightness	4	3	2	5	2	2	2	2	4
	Unmoulding	3	4	3	0	2	5	3	5	5
Texture	Toughness	3	5	5	0	2	5	4	5	5
	Roughness	4	4	4	0	2	5	5	5	5
	Cohesion	0	2	2	1	1	5	4	5	5
	Release colour	2	2	5	1	5	3	3	5	5
Application to wet hair	Foaming Duration			4			4		3	5
	Foaming			4			4		4	5
	Foaming Creaminess			4			4		4	5
	Bubble Size			5			4		4	4
	Wet Combing			3			5		5	5
Effects on dry hair	Cleanliness			4			5		5	5
	Coating			5			3		5	5
	Volume			3			4		3	4
	Dry Combing			5			5		5	5
	Gloss			4			4		5	5
	Static			5			5		5	5
	Dryness			3			2		5	5

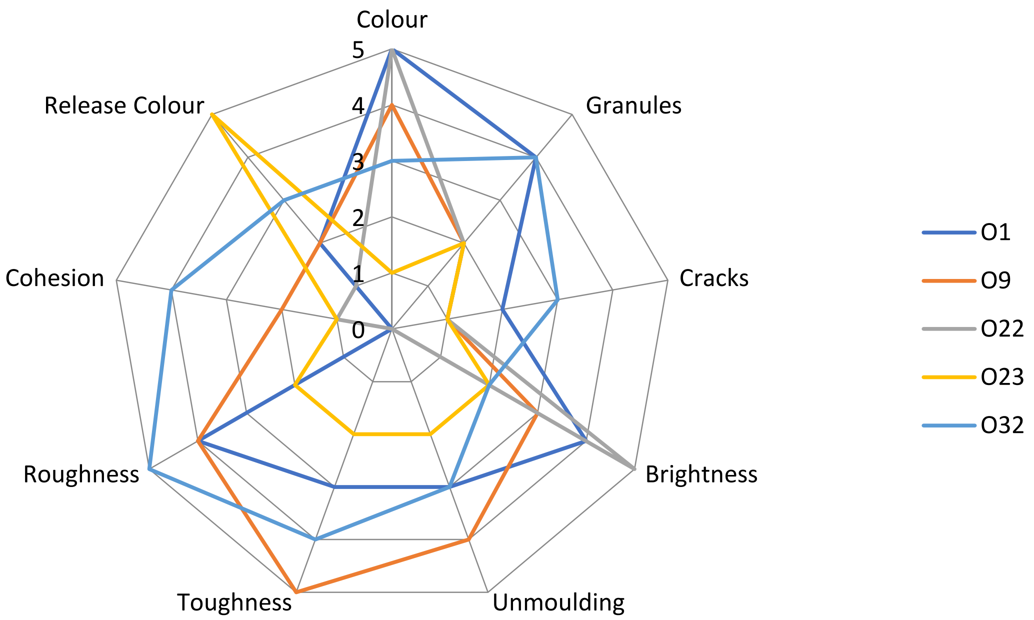


Figure 41: Quantitative evaluation (visual characteristics and texture) of oily hair shampoos corresponding to formulations O1, O9, O22, O23 and O32.

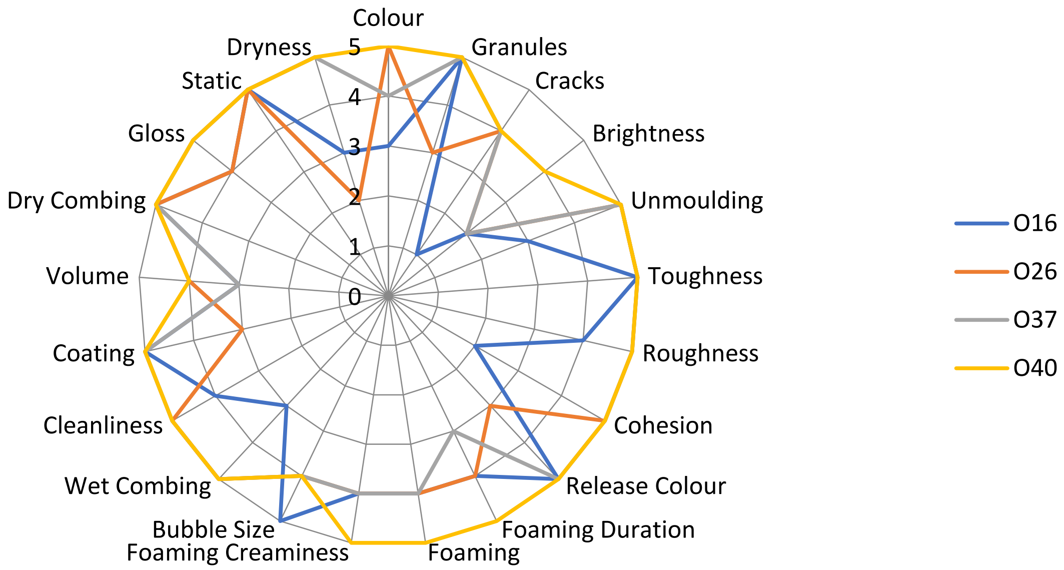


Figure 42: Quantitative evaluation (visual characteristics, texture and hair tests) of oily hair shampoos corresponding to formulations O16, O26, O37 and O40.

4.2. Dry and Damaged Hair Shampoo

Based on the shampoo formulation for oily hair (formulation O40), a shampoo for dry and damaged hair was created. Between May 22nd and June 22nd of 2018, 8 tests were performed to formulate the shampoo and to achieve the outlined goals for its features.

Considering the previous shampoo, the first change was to remove the grey clay and the activated charcoal, since these ingredients are specific for the adsorption of hair oil particles, and are not suitable in this case. It is therefore proposed to replace these two ingredients with others that meet the needs of dry hair, promoting its hydration and repairing.

It should be noted that in all the Tables presented in the results the quantities of used ingredients result from a normalization of their base ranges given in appendix A.

4.2.1. Effect of Glycerin

4.2.1.1. Formulation D1

Table 17: Formulation D1 of dry and damaged hair shampoo.

Phases	Components	Quantity (% of the base range)
A	Cocamidopropyl Betaine	38.50
	Coco Glucoside	80.80
	Vegetable Glycerin	10.66
B	Sodium Cocoyl Isethionate	59.30
C	Stearic Acid	81.00
	Cetearyl Alcohol	20.00
	Organic Coconut Oil	18.40
	Organic Shea Butter	18.57
	Glyceryl Oleate	32.00
	Olive Oil	26.00
	Hydrolyzed Wheat Protein	25.00
	Organic Jojoba Oil	25.75
D	Organic Lemongrass Essential Oil	70.00

In addition to removing activated charcoal and clay, two new ingredients were added to achieve the goal of hair strands hydration. One of them is vegetable glycerin which has highly emollient and humectant properties and is responsible for the transepidermal water loss reduction and for maintaining the adequate level of moisture in the *stratum corneum*, allowing hair flexibility.

The other new ingredient is jojoba oil which is a moisturizer, providing the protection against dryness, breakage, and split ends.

After the addition of phase C, it has been confirmed that the stirring (173 rpm) was not enough to homogenize the mixture (phase C was separated from the others). To counter this separation, the stirring speed was increased to 231 rpm and after 6 minutes of mixing with this stirring speed, the mixture became apparently homogeneous. Nevertheless, when the mixture was placed into the molds, the phases separated again, and the mixture became heterogeneous once more. This is probably due to the glycerin addition, as it is water soluble and has no affinity with the rest of the mixture, as it was seen above.

A few minutes after the mixture has been placed into the molds, it was found that it was still liquid, which was not the case in the oily hair shampoos. This is due to the fact that clay and activated charcoal were not present in this shampoo. Since the clay is composed of minerals, it has a high melting point, as well as the activated charcoal (appendix B), the mixture stayed liquid during more time due to the lower melting point of the mixture. When the shampoos were removed from the molds, the heterogeneity was less evident, that is, it vanished when the solidification occurred. Afterwards, the shampoos were stored at 19.9 ºC and 67.5% of humidity.

However, when the washing test occurred, the shampoo was quite soft and easy to break (Figure 43).

Figure 43: Shampoo (formulation D1) after the wash.

4.2.1.2. Formulation D4

Table 18: Formulation D4 of dry and damaged hair shampoo.

Phases	Components	Quantity (% of the base range)
A	Cocamidopropyl Betaine	48.90
	Coco Glucoside	78.40
	Panthenol	25.00
B	Sodium Cocoyl Isethionate	53.20
C	Stearic Acid	78.40
	Cetearyl Alcohol	59.60
	Organic Coconut Oil	19.60
	Carnauba Wax	43.88
	Glyceryl Oleate	17.75
	Olive Oil	0.750
	Hydrolyzed Wheat Protein	13.50
	Organic Argan Oil	26.00
D	Organic Rosemary Essential Oil	62.22

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In this formulation, the moisturizer and emollient properties of a different oil were tested. Regarding the new oil, it was argan oil, a moisturizer, conditioner, and softener for the hair strands, since it has vitamin E and fatty acids, which not only promote healthy growth but also promote shine and gloss, contributing to a more manageable hair, which is desirable for this shampoo. The argan oil was added in the same quantity as the jojoba oil in the previous formulation.

In order to provide the required hardness to the shampoo, shea butter was replaced by carnauba wax and cetearyl alcohol was increased to 59.60% of the base of range since it can be used as a hardener. In contrast, glycerin was replaced by panthenol in attempt to avoid the heterogeneity.

The insertion of the mixture into the molds was easily executed and the homogeneity was also confirmed. Considering that 20 minutes after the insertion the mixture was almost totally solidified (Figure 44), it is concluded that the carnauba wax increases the melting point of the mixture (appendix B) causing its fast solidification. The shampoo was stored at 21.1 ºC and 77.2% of humidity.

Figure 44: Solified shampoo (formulation D4) after 20 minutes of the insertion.

On the other hand, when the shampoo was tested on the hair, despite the excellent and immediate foam, it was found that replacing the butter with the wax and removing the glycerin led to a dry and dehydrated hair after washing. This leads to conclude that the shampoo needs the glycerin in order to increase the moisture content and consequently the hydration.

After washing, the shampoo kept its shape and it was confirmed that not much product was lost in the washes (Figure 45).

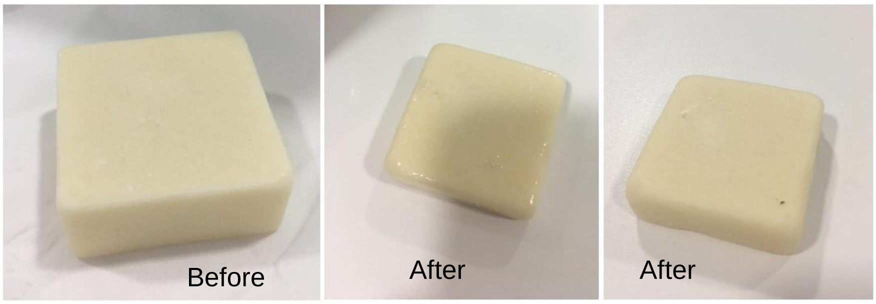

Figure 45: Shampoo (formulation D4) before and after washing.

4.2.2. Shampoo Hardness and Consistency

4.2.2.1. Formulation D7

Table 19: Formulation D7 of dry and damaged hair shampoo.

Phases	Components	Quantity (% of the base range)
A	Cocamidopropyl Betaine	49.20
	Coco Glucoside	79.00
	Panthenol	33.00
	Vegetable Glycerin	44.44
B	Sodium Cocoyl Isethionate	46.20
C	Stearic Acid	79.20
	Cetearyl Alcohol	40.00
	Organic Coconut Oil	29.40
	Carnauba Wax	16.33
	Glyceryl Oleate	0.500
	Olive Oil	0
	Hydrolyzed Wheat Protein	0.500
	Organic Argan Oil	27.75
D	Geranium Essential Oil	43.43

To maintain the same toughness obtained by carnauba wax and, at the same time, achieve a shampoo that moisturizes dry hair, glycerin was added as in formulation D1. However, in order to minimize the oil absorbing effect of carnauba wax, it was reduced to 16.33% instead of 43.88% of the base of range. In order to provide extra moisturization, the panthenol and coconut oil contents were increased 33% and 29.40% of the base of range, respectively.

In this formulation, even though the mixture presented heterogeneity caused by glycerin when it was inserted into the molds, when the shampoo solidified this went unobserved.

Regarding the mixture pouring into the mold, it was equally easy, which allowed the shampoo to have a shape without cracks and a softer texture due to the reduction of carnauba wax. Taking into account the test in the hair, the foam was very creamy and rapidly spread from root to the ends, making it easy to wash. This translates into the need to, not only, increase the quantity of carnauba wax, but also, keep the softness and moisture which this shampoo gave to the hair strands.

4.2.2.2. Formulation D8

Table 20: Formulation D8 of dry and damaged hair shampoo.

Phases	Components	Quantity (% of the base range)
A	Cocamidopropyl Betaine	50.00
	Coco Glucoside	80.00
	Panthenol	16.67
	Vegetable Glycerin	11.11
B	Sodium Cocoyl Isethionate	50.00
C	Stearic Acid	60.00
	Cetearyl Alcohol	20.00
	Organic Coconut oil	30.00
	Organic Shea Butter	14.29
	Glyceryl Oleate	0
	Olive Oil	0
	Hydrolyzed Wheat Protein	0
	Carnauba Wax	27.77
	Organic Açai Oil	12.50
D	Organic Geranium Essential Oil	44.44

Considering this formulation, in order to provide the required hardness, the goal was to add shea butter together with a higher percentage of carnauba wax (27.77% instead of 16.33% of the base of range), rather than replace it. Consequently, the glycerin and the panthenol occupied a smaller percentage in this formulation in comparison with formulation D7. Also, stearic acid and cetearyl alcohol were reduced to 60% and 20% of the base of range respectively, since the shea butter and the carnauba wax were already contributing to a tougher consistency of the shampoo. In order to give a touch of excellence to the hair fibers, since these are dry and damaged, the argan oil was replaced by the açai oil, restoring hair strands and giving them shine and softness. As a result, the obtained shampoo corresponded to all the requirements for dry and damaged hair shampoo, such as:

- A shampoo that when unmolded had the exact shape of the mold.
- A cohesive shampoo, which allows not losing much product during the washes.
- A shampoo with the necessary hardness preventing it from breaking during the washes.
- A creamy foam that not only allows washing the hair from the root to the tips but also that quickly appears when in contact with water.
- A shampoo that provides the necessary moisture and hydration to the hair strands which are damaged, restoring and giving them back the shine and softness.

4.2.3. Quantitative Evaluation of the Shampoo

Between each formulation, almost all shampoos were evaluated quantitatively in different parameters. The shampoos that were referenced in the previous chapters were evaluated according to what was tested in each formulation (Table 21 and Figure 46)

Table 21: Evaluated shampoos referenced in the previous chapters.

		Formulation			
	Features	**D1**	**D4**	**D7**	**D8**
Visual characteristics	Cracks	4	5	4	4
	Brightness	4	4	4	4
	Unmoulding	4	5	5	5
Texture	Toughness	2	5	4	5
	Roughness	2	5	4	5
	Cohesion	2	5	4	5
	Release colour	2	5	3	5
Application to wet hair	Foaming Duration	5	5	5	5
	Foaming	5	5	5	5
	Foaming Creaminess	4	5	5	5
	Bubble Size	4	4	4	5
	Wet Combing	4	2	3	5
Effects on dry hair	Cleanliness	5	5	5	5
	Coating	3	5	4	5
	Volume	5	5	4	4
	Dry Combing	4	1	2	5
	Gloss	5	3	3	5
	Static	5	5	5	5
	Dryness	4	2	3	5

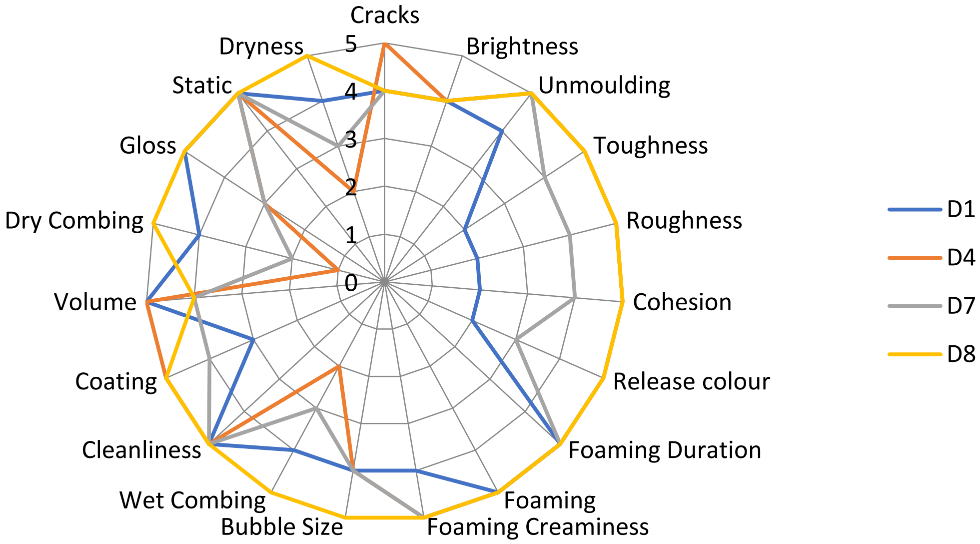

Figure 46: Quantitative evaluation (visual characteristics, texture and hair tests) of dry/damaged hair shampoos corresponding to formulations D1, D4, D7 and D8.

4.3. Normal hair shampoo

The last shampoo to be created was the normal hair shampoo and on June 27th, a test was performed in order to produce a shampoo that was the intermediate between the oily and dry/damaged hair shampoo.

It should be noted that in the Table 22, the quantities of used ingredients result from a normalization of their base ranges given in appendix A.

4.3.1. Formulation N1

Table 22: Formulation N1 of normal hair shampoo.

Phases	Components	Quantity (% of the base range)
A	Cocamidopropyl Betaine	50.00
	Coco Glucoside	90.00
	Vegetable Glycerin	11.11
	Panthenol	16.67
B	Sodium Cocoyl Isethionate	52.50
C	Stearic Acid	60.00
	Cetearyl Alcohol	20.00
	Organic Coconut oil	30.00
	Organic Shea Butter	14.29
	Carnauba Wax	27.77
	Glyceryl Oleate	0
	Olive Oil	12.50
	Hydrolyzed Wheat Protein	0
D	Organic Lemongrass Essential Oil	44.44

Considering the oily hair formulations, the first dissimilarity to notice was the removal of the grey clay and the activated charcoal, since these ingredients are specific for the adsorption of hair oil particles, which are not necessary for normal hair. Regarding the dry/damaged hair formulations, the açai oil was not present in the formulation for normal hair, since this oil is extremely repairing for hair strands, and is not essential for hair strands which are not damaged.

In contrast, the coco glucoside and sodium cocoyl isethionate were increased to 90% and 52.50% of the base of range respectively, in order to replace the extra hydration component which was given by the açai oil. The remaining part of the percentage corresponding to the açai oil in dry hair formulation D8 was transferred to olive oil in order to provide a hydration component, although lower than the required for dry hair.

In conclusion, a shampoo suitable for hair without special needs such as oil removal or extra moisturization was obtained, guaranteeing hair cleaning and hydration at the same time.

4.3.2. Quantitative evaluation of the shampoo

The normal and hair shampoo was also evaluated in different parameters and since the goals for normal hair shampoo were achieved in the first formulation, only one evaluation was performed (Table 23 and Figure 47):

Table 23: Evaluation of formulation N1 (normal hair shampoo).

	Features	Formulation N1
Visual characteristics	Cracks	4
	Brightness	4
	Unmoulding	5
Texture	Toughness	5
	Roughness	5
	Cohesion	5
	Release Colour	5
Application to wet hair	Foaming Duration	5
	Foaming	5
	Foaming Creaminess	4
	Bubble Size	4
	Wet Combing	4
Effects on dry hair	Cleanliness	5
	Coating	5
	Volume	4
	Dry Combing	4
	Gloss	5
	Static	5
	Dryness	4

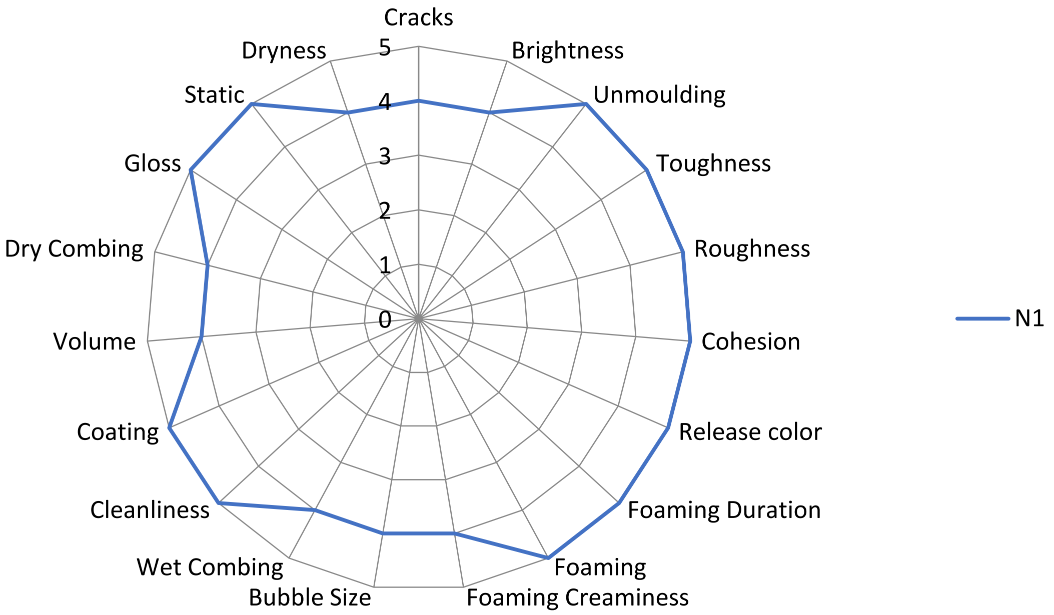

Figure 47: Quantitative evaluation (visual characteristics, texture and hair tests) of normal hair shampoo corresponding to formulation N1.

4.4. Mixture Pouring into the Molds

Several techniques of pouring the mixture into the molds were tested in order to choose the most efficient one for the manufacturing procedure.

Until formulation O36 of oily hair shampoo, the mixture was poured by removing the glass vessel that contained the mixture from the bath and poured directly into the molds. However, this method promotes the cooling of the mixture, and thus, making it difficult to insert the last shampoos (due to their already lower temperature). For instance, in formulation O14 of oily hair shampoo, the above-mentioned effect was confirmed, since shampoo 1 was the first to be placed and shampoo 3 the last one (Figure 48).

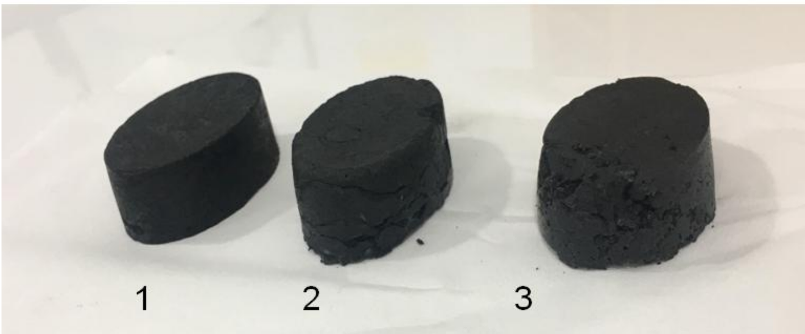

Figure 48: Shampoos of formulation O14 inserted at different times and temperatures.

Considering that the shampoo mixture 1 was inserted immediately after being removed from the bath at a temperature above 70°C, it had the exact shape of the mold, without cracks and a uniform consistency.

In the case of shampoo 2, the mixture was at a lower temperature, thus being harder to insert it. As a result, this shampoo owned several cracks and holes which were responsible for its irregular texture.

Taking into account that the shampoo 3 was inserted at the lowest temperature, it was not possible to insert the mixture directly, so manual compression was used to fill the mold with the mixture. As a consequence, the shampoo turned extremely irregular due to the air that was trapped inside the mixture during the manual compression process.

As a conclusion, this method is neither efficient nor reproducible, whereby the mixture must be poured around the same temperature in all samples to ensure equality between them.

Therefore, the solution for this situation lies on leaving the glass container in permanent heating inside the bath and take portions of the mixture into the molds with a suitable scoop for the size of the molds. This way, the shampoos are all produced under the same conditions (temperature and time outside the bath), guaranteeing the fluidity of the mixture and its easy insertion into the molds. Consequently, it was possible to make several shampoos in one formulation.

4.5. Drying and Curing time

After the shampoos removal from the molds, they undergo a drying process, which is responsible for the evaporation of some volatiles and the greasy feeling elimination that remains after their removal. In this case, it was determined how long it would take for these to evaporate all the volatile ingredients and to be ready for sale and consumer use. For that purpose, they were placed in trays in the warehouse at room temperature (in this period the measured temperatures and humidity values were between 20.1ºC and 74.6% and 25.9ºC and 72.9%).

After 2 days of drying in the temperature and humidity conditions referred above the shampoos were not ready to be packed and sent to stores due to its transpiration phenomenon, which causes the existence of droplets in the shampoo surface (Figure 49).

Figure 49: Droplets in shampoo surface (formulation O40) after 2 days of drying (20-30ºC).

After 3 days from the beginning of the process, some droplets on the surface were still visible in several shampoos. So, it was concluded that 3 days were still not enough time to complete the drying process (Figure 50).

Figure 50: Droplets in shampoo surface (formulation O40) after 3 days of drying (20-30ºC).

Finally, after 7 days the drying process can be interrupted since all the shampoos were perfectly dry with no drops and no greasy feeling (Figure 51).

Figure 51: Shampoos surface (formulation O40) after 7 days of drying (20-30ºC).

4.6. Corked Wire Insertion

To hang the shampoo in the bath and prevent it from being placed in a dish and getting wet, it was decided to use corked wire, not only because it is a Portuguese product but mainly because it is impermeable to liquids and gases.

First, a 3 mm wire was used and it was decided to place the wire during the mixture pouring into the mold, *i.e.*, half of the blend was added, then the wire and finally, the rest of the mixture was then inserted (Figure 52).

Figure 52: Shampoo with 3mm corked wire inserted during molding.

As the wire had a very high thickness, it prevented the adequate molding of the shampoo, destroying it and not being efficient in use. Therefore, this was the reason why this wire was discarded.

After that, a 1 mm wire was used and with the help of a flat piece of cork, a wire with a brake or stopper was created (Figure 53).

Figure 53: 1 mm wire with a break.

In order to use this wire, it was necessary to pierce the shampoo after curing. For that purpose, 3 needles were tested: 0.75 mm, 1 mm and 2 mm. The needle chosen was 1 mm, which created the hole with necessary diameter for the passage of the wire (Figure 54).

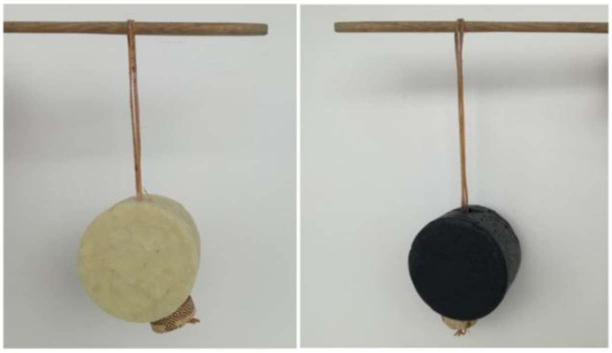

Figure 54: Hung shampoo by the corked wire with the cork stopper.

4.7. Water Content Determination

In order to decide whether it would be necessary to introduce a preservative in the formulation, the amount of water and volatiles in the shampoo was determined. For that purpose, two oily hair formulations were chosen, namely, formulation O33 (with water) and O37 (without water).

First, with the technical data sheet and the purity of each ingredient given by the suppliers, it was possible to estimate the maximum amount of water present in each formulation. Note that the mass of water was obtained by multiplying the water content in each ingredient by the mass of each ingredient in the formulation (Table 24 and 25).

Table 24: Estimated maximum water content in formulation O33 of oily hair shampoo.

	Water Content (%)	Ingredient Mass (g)	Water Mass (g)
Cocamidopropyl Betaine	70	8.42	5.89
Coco Glucoside	60	5.65	3.39
Water	100	2.17	2.17
Vegetable Glycerin	0.5	3.23	0.02
Sodium Cocoyl Isethionate	0	28.03	0.00
Stearic Acid	0	7.06	0.00
Cetearyl Alcohol	0	307	0.00
Organic Coconut Oil	0	2.96	0,00
Glyceryl Oleate	0	1.40	0.00
Hydrolyzed Wheat Protein	82	1.40	1.15
Grey Clay	0	5.63	0.00
Activated Charcoal	0	1.92	0.00
Organic Rosemary Essential Oil	0	0.39	0.00
Total		71.33	**12.62**

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Table 25: Estimated maximum water content in formulation O37 of oily hair shampoo.

	Water Content (%)	Ingredient Mass (g)	Water Mass (g)
Cocamidopropyl Betaine	70	9.8	6.86
Coco Glucoside	60	6.59	3.95
Sodium Cocoyl Isethionate	0	29.42	0
Stearic Acid	0	6.32	0
Cetearyl Alcohol	0	2.86	0
Organic Coconut Oil	0	5.36	0
Organic Shea Butter	0	2.8	0
Glyceryl Oleate	0	1.49	0
Olive Oil	0	0.78	0
Hydrolyzed Wheat Protein	82	0.83	0.68
Grey Clay	0	3.54	0
Activated Charcoal	0	1.15	0
Organic Rosemary Essential Oil	0	0.43	0
Total		71.37	**11.49**

Then, to obtain otherwise the content of water and volatiles present in the sample, drying tests were performed and the results are presented in the Tables 26-31.

Table 26: Results obtained from drying - sample 1 of formulation O33.

Plate Mass	2.8648 g
Total Mass (Sample+Plate) before Drying	6.3931 g
Sample Mass	3.5283 g
Drying Temperature	106ºC
Drying Time	16.04 min
Total Mass after Drying	6.2607 g
Lost Volatiles Mass	0.1324 g
TWL	0.0375 g/g

Table 27: Results obtained from drying - sample 2 of formulation O33.

Plate Mass	0.3763 g
Total Mass (Sample+Plate) before Drying	3.5734 g
Sample Mass	2.7536 g
Drying Temperature	105ºC
Drying Time	17.12 min
Total Mass after Drying	3.4172 g
Lost Volatiles Mass	0.1562 g
TWL	0.048 g/g

Table 28: Results obtained from drying - sample 3 of formulation O33.

Plate Mass	3.1573 g
Total Mass (Sample+Plate) before Drying	5.912 g
Sample Mass	2.7547 g
Drying Temperature	105ºC
Drying Time	12.21 min
Total Mass after Drying	5.8149 g
Lost Volatiles Mass	0.0971 g
TWL	0.0350 g/g

Table 29: Results obtained from drying - sample 1 of formulation O37.

Plate Mass	3.1612 g
Total Mass (Sample+Plate) before Drying	4.9266 g
Sample Mass	1.7654 g
Drying Temperature	104ºC
Drying Time	15.07 min
Total Mass after Drying	4.8647 g
Lost Volatiles Mass	0.0619 g
TWL	0.0350 g/g

Table 30: Results obtained from drying - sample 2 of formulation O37.

Plate Mass	3.2058 g
Total Mass (Sample+Plate) before Drying	6.3896 g
Sample Mass	3.1838 g
Drying Temperature	104ºC
Drying Time	54.22 min
Total Mass after Drying	6.0372g
Lost Volatiles Mass	0.3254 g
TWL	0.1106 g/g

Table 31: Results obtained from drying - sample 3 of formulation O37.

Plate Mass	3.1991 g
Total Mass (Sample+Plate) before Drying	5.7448 g
Sample Mass	2.5457 g
Drying Temperature	105ºC
Drying Time	11.58 min
Total Mass after Drying	5.6520 g
Lost Volatiles Mass	0.1248 g
TWL	0.049 g/g

Taking into account the results, it can be verified that the values that were obtained in the drying are very different from the values obtained by estimation, according to the data of the suppliers. An explanation for this difference may be related to some water evaporation during the shampoo manufacturing process since it is performed at high temperatures. Another possible explanation is related to the drying time of the sample, as it can be seen in sample 2 of formulation O37. In this sample, the drying time was much higher compared to all the other samples from the 2 formulations. This was due to a difference in the protocol: during the drying of this sample, the door of the hood was closed (which did not happen in the other drying procedures), and its ventilation caused instability in the dish of the balance, resembling a change of weight. Thus, the drying did not stop until the door of the hood was opened, stopping the ventilation which in turn stopped affecting the weight on the scale. Supposedly, if this had not happened, the drying time would have been between 10 to 20 minutes, and the TWL would be much smaller (between 0.03 and 0.05 g/g), as found in the other samples. It would also be expected that, regardless of how long the sample remained on the scale at 105 °C after the drying time has ended, the mass of lost volatiles remained the same. However, this did not happen and the mass of volatiles lost continued to increase, leading to the conclusion that if the door did not open and the sample continued to dry, the mass lost would increase and possibly reached the estimated mass of water calculated before. Presenting these values to Oxford Biosciences, an accredited company that offers cosmetic product safety reports, it was concluded that it would not be necessary to add preservative for the shampoos. The Oxford Biosciences also legally approved the shampoo according to regulation EU 1223/2009.

4.8. Microbiological Analysis

In order to evaluate the quality of the product at a microbiological level, and to confirm the adequacy of the non-addition of preservative to the shampoo, the microbiological analysis was performed.

4.8.1. Microbiological Analysis 1- July 20th

The first analysis was performed on July 20th, and the microbiological tests were executed on 6 samples of different productions of the 3 lines of shampoo. In addition, a replica of each of the samples was performed, leading to a total of 12 kits (Table 32).

Table 32: Microbiological analysis samples.

Shampoo type	Production
Oily Hair Shampoo (formulation O40)	July 18^{th}- production 1
	July 18^{th}- production 2
Normal Hair Shampoo (formulation N1)	July 2^{nd}
	July 18^{th}
Dry/Damaged Hair Shampoo (formulation D8)	July 9^{th}
	July 18^{th}

Note that the July 9^{th} result of the dry/damaged shampoo samples may contain a contamination from the July 18^{th} sample because when the former was cut, the x-acto knife still had traces of the latter shampoo even after being disinfected. Also note that the production of July 18^{th} of dry/damaged hair shampoo was performed after the previous disinfection of the molds and of all the material and equipment used during the process, which did not happen with the other samples.

Seven days after the start of the incubation of the kits at 30ºC (July 27^{th}), what looked like a fungus and a bacterium were visible on the July 18^{th} sample of normal hair shampoo (Figure 55) and an apparent bacterium colony on the July 9^{th} sample of dry/damaged hair shampoo, replica 2 (Figure 56). The contamination probably occurred due to the direct contact between the producer and the sample during the production, despite the use of gloves and mask.

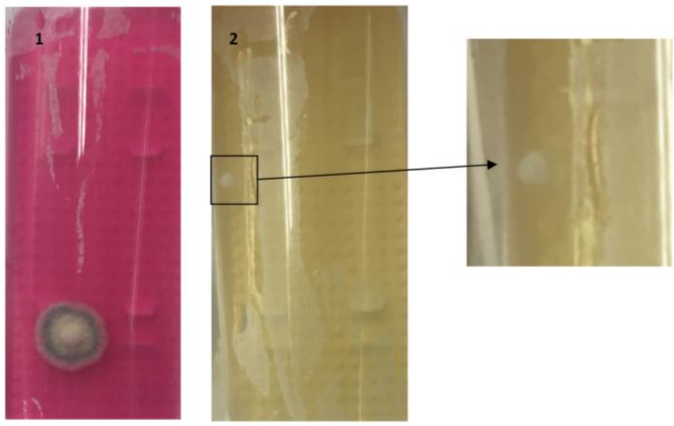

Figure 55: Presumable fungi (1) and bacteria (2) colonies on the culture kit from the July 18^{th} sample of normal hair shampoo.

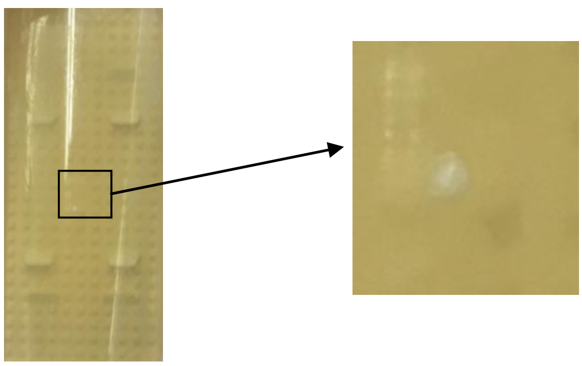

Figure 56: Presumable bacteria colony on the culture kit from the July 9^{th} sample of dry/damaged hair shampoo, replica 2.

4.8.2. Microbiological Analysis 2- September 20th

The samples used in the first analysis were kept in a confined space without light at room temperature. On September 20th the microbiological analysis was repeated to the same samples as before, in order to evaluate the consistency of the results and to verify how long the shampoo samples would be good for consumption from a microbiological point of view. Therefore, as the shampoos were already cut, the instructions given in the appendix D were immediately followed with the same alteration in the protocol as before referred in the subchapter 3.3.5 of the chapter 3 Materials and Methods.

After 13 days of incubation, there was no evidence of bacteria or fungi growth on the culture kits from all the samples, which leads to the conclusion that the shampoo medium is not propitious to the growth of fungi and bacteria. As a conclusion, the bacteria and fungi previously detected in the first analysis were there still in viable form due to the short time after the production (2 days and 11 days respectively). The unfavourable growth conditions of the shampoo (such as the low content of water in the formulations) lead to the fungus and bacteria death after the considerable storage time following the production (2 months).

4.9. Scale-Up Process

After all the targets were reached for a small scale production of shampoos, it was necessary to increase the scale for a larger production process. As a result, the scale was increased stepwise, and the necessary adjustments were performed, corresponding to the demands of each step of scale increase in the process.

4.9.1. Glass and Plastic Beakers

Initially, the total mass of the mixture was 60 grams and the scale increase started at this value. Gradually larger quantities were produced in each batch, up to 3 kilograms of shampoo (several batches of 3 kilograms). When productions were made for 2 kg of shampoo, the 2-L glass beaker was no longer large enough to be used as the heatproof container inside the thermostatic bath. As the 5-L glass beaker was not initially available, a 5-liter plastic beaker was used provisionally. However, a liquid mixture was not obtained and is not adequate to be poured into the molds, and since the process and the formulation were exactly the same, it was concluded that the problem came from the beaker material (Figure 57).

Figure 57: Viscous mixture inside the plastic beaker (formulation N1, 2550 grams).

This leads to the conclusion that even though the temperature of the bath was around 90/95 ºC °C, the 5-L plastic beaker did not provide adequate heat transfer from the bath water to the mixture. Effectively, the thermal conductivity of the plastic (Propylene 0.33 W/(m K)) (Engineering Tool Box, 2018) is lower than that of glass (0.8 W/(m K)) (Protolab, 2018), which results in a lower temperature in the mixture inside the beaker (51ºC instead of approximately 75ºC). Therefore, it was necessary to use a glass beaker to ensure good heat transfer between the water and the mixture (Figure 58).

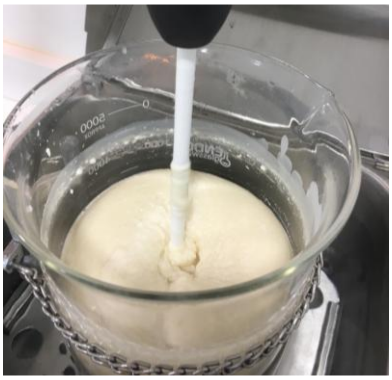

Figure 58: Liquid mixture inside the glass beaker (formulation O40, 3000 grams).

4.9.2. Filling Machine

With the increase in the production scale, it was found that the beaker and the thermal bath would not be suitable productions above 3 kilograms. This is due to the mixture inside the beaker reaching a height which would exceed the level of the water bath and thus not being properly heated.

Therefore, for larger scale productions it would be necessary to use a filling machine with heating and mixing devices suitable for viscous fluids (appendix C), instead of a beaker and water bath and the filling of the molds with scoops.

4.10. Other Process Parameters

4.10.1. Sodium Cocoyl Isethionate Dissolution

The time required for complete dissolution of SCI in the liquid surfactants of different productions of dry and damaged hair shampoo was analyzed as function of (1) the bath temperature at which each process occurred in samples with similar masses (Figure 59) and (2) mass and agitation level in samples with different masses (Figure 60).

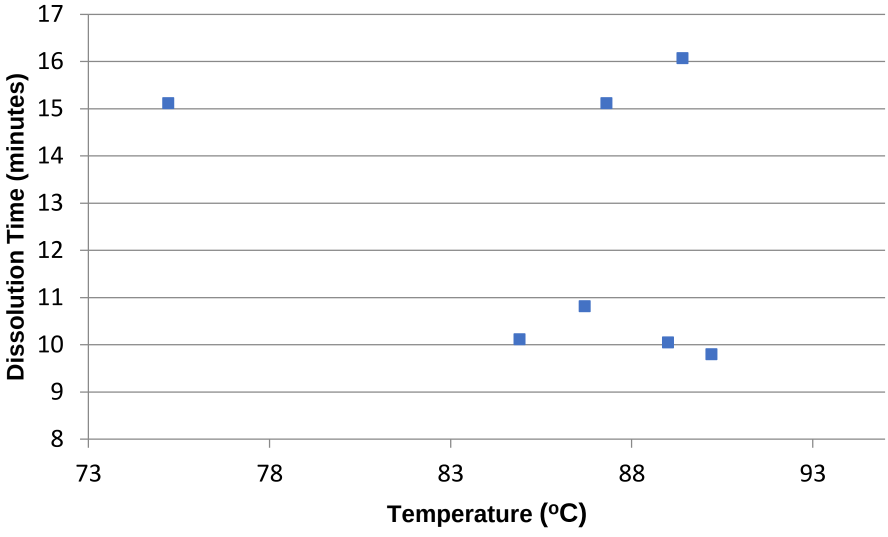

Figure 59: Dissolution time of SCI in liquid surfactants as a function of the bath temperature.

Through the analysis of Figure 59, it was concluded that the dissolution time does not have a tendency to decrease or increase with the increase in the bath temperature, as long as it is above 70-75 °C. That is, as long as the process is occurring at a high temperature, the SCI can dissolve easily in the liquid surfactants and the time of this process does not depend on the temperature from the above-mentioned value.

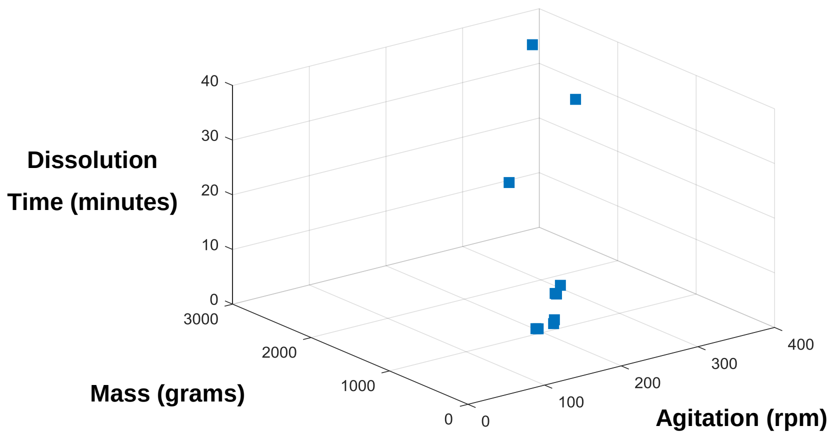

Figure 60: Dissolution time of SCI in liquid surfactants as a function of mass and agitation level.

Regarding the dissolution time as a function of the mass of the sample in Figure 60, it was confirmed that the time increases with the increase of the sample mass. This is due to the greater difficulty in transferring heat from the thermostatic bath water to the mass inside the container. That is, since the mass of SCI is larger, the contact area with the walls of the container, in relation to the working volume, becomes smaller and consequently, the heat transfer is slower, leading to an increase in the dissolution time.

In order to try to overcome the increase of the dissolution time with the increase in mass, it was attempted to increase the stirring power applied to the mixture favouring the contact with the walls of the container, and improving heat transfer. However, this strategy was not sufficient to overcome the increase in mass factor, that is, the dissolution time increased even as the stirring rate increased.

4.11. Final Formulations

In this chapter additional aspects related to the final formulations of the 3 lines of shampoo are presented.

4.11.1. Other Product Parameters

Final Shampoos Essential Oils

When the final formulations were obtained, the essential oils which would compose each of the shampoos were chosen. For the dry and damaged hair shampoo an exotic mixture of geranium, sweet orange, and rosemary essential oils was preferred, in order to continue the exotic effect of açai oil. For the normal hair shampoo, the mixture was the same as the dry hair with the exception of geranium essential oil. Finally, maintaining the base of the aroma of the 3 shampoos (rosemary essential oil), lemongrass essential oil was added for oily hair shampoo.

Final Shampoos pH

The pH of the 3 shampoos was also determined to confirm if they were suitable for the hair. The scalp pH is 5.5, and the hair shaft pH is 3.67 (Dias & Almeida, 2014) and hair care products with a pH in this range of values are beneficial for hair health.

For the dry and damaged hair shampoo, the pH was measured in 3 different samples of this shampoo and the results were 6.31, 6.32 and 6.30. In the case of oily and normal hair shampoos, only one sample of each was used to determine the pH and the obtained results were 6.86 and 6.49, respectively.

Comparing the obtained results with the values mentioned above, it is confirmed that the pH of the shampoos is slightly higher the recommended range. The formulations which have an alkaline pH may increase the negative electrical net charge of the hair fiber surface and consequently increase the friction between the fibers, causing frizz (Dias & Almeida, 2014).

On the other hand, comparing the obtained pH of the shampoos with the pH of a regular soap (which is used by some people to wash the hair), it is confirmed that is significantly lower since the pH of most of the soaps is approximately 10. Thus, the importance of washing the hair with the shampoo instead of soap is emphasized for several reasons:

- The pH of the shampoo is closer to the optimum pH for the hair. Since the hair has no ability to readjust the pH as the skin has, as a long-term effect the continuous use of soaps will damage the hair and the scalp.
- The possibility to add the active ingredients to the formulations without destroying them as would happen in the soap formulations. This is also due to the high pH of the soap.
- The shampoo foam characteristics are not dependent on the hardness of the water as it is with soap. Hard water has a high content of calcium and magnesium cations which react with the anions of the soap and precipitate as scum. This reduces

cleaning efficiency and also lays down an insoluble layer of these precipitates on the hair scalp, damaging it (Kirk-Othmer, 2013).

4.11.2. Comparison with a Control Shampoo

Taking into account the characteristics obtained in the final shampoos, it was decided to compare these features with those of another shampoo of the same type (organic solid shampoo). This shampoo is produced by a French company named Lamazuna, and it is natural, sustainable, vegan, not tested on animals and approved by PETA (an animal rights organization) (Figure 61). The Lamazuna shampoo was used as a control to evaluate the different parameters of the produced shampoo in this work.

Figure 61: Lamazuna shampoo for oily hair.

In this specific case, as the control shampoo used was for oily hair, the comparison was made with the produced shampoo formulation for oily hair. The characteristics present in the subchapter 3.3.6 were then evaluated in both shampoos, with the results shown in Figure 62.

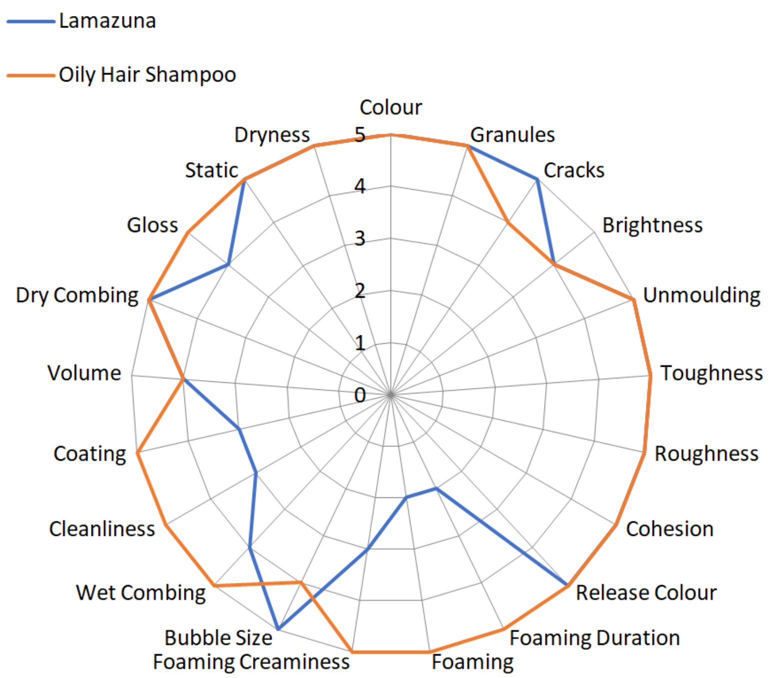

Figure 62: Comparison of the chacateristics of the Oily Hair Shampoo produced in the present work and those of the Oily Hair Shampoo from Lamazuna.

Analyzing the previous graph, it is confirmed the two shampoos have different characteristics, namely:

- The produced Oily Hair Shampoo was evaluated with a stronger ability to wash and clean the hair in comparison with the Lamazuna shampoo.
- The Lamazuna shampoo has less and smaller cracks than the produced shampoo.
- The foam of both shampoos is extremely different since the Lamazuna shampoo has poor foam with small bubbles, making difficult the washing of the hair from its root to the tips. On the other hand, the produced shampoo is characterized by rich and creamy foam which appears immediately after its contact with water, allowing an easy and quick wash.

Despite the fact that the evaluations were more perceptible when tested in real hair (mainly in relation to the cleaning and coating aspects), some of them (such as foaming quantity and creaminess) were also visible in the lab test hair (Figure 63).

Figure 63: Foaming test in lab test hair of the produced Oily Hair Shampoo and of the Lamazuna Oily Hair Shampoo.

4.11.3. Final Shampoo Packages and Labels

UniBio's design team created the packaging for the 3 shampoos (appendix E). For that purpose, it was relevant to sum up all the important information that should be on the package:

- The mass of the shampoo and its composition in INCI nomenclature.
- A brief description of the most relevant features of the shampoo.
- A stamp of a vegan product without sulfates, plastic and silicones.
- The equivalence between the solid shampoo and the ordinary liquid shampoo, in terms of quantity.
- The directions of use and the involved precautions for the shampoo.

In addition to the information described above, the two most relevant ingredients were selected and added to the name of the shampoo on the main division of the package (appendix E). The common chosen ingredient for the 3 shampoos was the olive oil since it is a Portuguese product and it is suitable for all types of hair as long as it is added in the right quantity. Thus, the idea that olive oil is of all the 3 shampoos was emphasized. Regarding the other ingredients, those chosen for labelling were activated charcoal for oily hair shampoo, açai oil for dry hair and rosemary for normal hair (appendix E).

4.11.4. Legal Approval and Certification

According to the European Union (EU) legislation, only agricultural products and foods which have been certified can be marketed as organic. However, such legal limitation does not exist for cosmetic products. The absence of specific regulations in Portugal and at EU level on organic cosmetics has led to the development of certifications, to ensure consumers compliance with a set of environmental values and safety levels in the use of these products.

Thus, there are a number of global certification bodies that carry out inspections and ensure that the cosmetic products are actually natural and organic, according to their defined criteria. There are many of these organizations worldwide and below is a brief summary of the criteria defined by the main European certification bodies (Table 33).

Table 33: Organizations certifying organic cosmetics in Europe and criteria required for the certification of each company. (Glow Organic, 2018) (Green Goji, 2018).

	ECOCERT Founded in France in 1951, it is an independent organization and one of the most trusted international certification for organic products. Its principles are: • The use of biodegradable and recyclable ingredients derived from renewable resources and produced using environmentally friendly processes. • The absence of genetically modified organisms (GMO), parabens, nanoparticles, silicon, PEG, synthetic perfumes and dyes, phenoxyethanol, animal-derived ingredients. • Containing a minimum amount of natural ingredients in the products obtained from organic farming. According to ECOCERT, the natural and organic ingredients are divided into 2 different labels: • **Natural and Organic Cosmetic Label:** A minimum of 95% of all plant-based ingredients and a minimum of 10% of all ingredients in the formula must come from organic farming. • **Natural Cosmetics Label:** A minimum of 50% of all plant-based ingredients and a minimum of 5% of all ingredients in the formula must come from organic farming.
	Soil Association Organic The UK largest organic certification body standards are based on principles that aim to maximize the proportion of organic ingredients, minimize synthetic ingredients, minimum processing of ingredients and clear labelling, so that consumers can make an informed choice about the product they are buying. There is also a criterion for organic products that should be considered as not being detrimental to human health and the environment in their production and use, and should not be tested on

	animals. According to Soil Association Organic, cosmetics are divided into 2 different categories: **Made with xx% organic ingredient**: Must contain at least 70% of organic ingredients. **Organic**: Should contain more than 95% of organic ingredients.
	COSMOBIO One of the most important associations in France that certify ecologically made products and organic cosmetics which are divided into 2 labels: **BIO Label:** Contains at least 95% natural ingredients, or created from natural sources. Contains at least 95% of organic plant ingredients. At least 10% of the product is made from organic farming. **ECO Label:** Contains at least 95% natural ingredients, or created from natural sources. Contains at least 50% of organic plant ingredients. At least 5% of the product is made from organic farming.
	COSMOS COSMOS Standard (COSMetic Organic Standard) was developed at European level by BDIH (Germany), Cosmebio & Ecocert (France), ICEA (Italy) and Soil Association (UK). Under the Cosmos standard, it was agreed that if an ingredient, technology or process may pose a risk to health or the environment, it will not be permitted. For this reason, nanomaterials, GMOs, irradiation and animal testing are not allowed. **Cosmetic products under organic certification**: • At least 95% of the agro-ingredients physically processed have to be of organic production. After a transitional period of 36 months after the entry into force of this standard (January 2010), the remaining agro-ingredients physically processed must be of organic production if they are available (in quantity and quality); • After a transition period of 60 months after the entry into force of this standard, at least 30% of the agrochemicals processed chemically must be of organic origin. • At least 20% of the total product should be organic; • Exceptionally, for rinsing products, non-emulsified aqueous products, and products with at least 80% of minerals or mineral ingredients, at least 10% of the total product should be organic. **Cosmetics under natural certification**: There is no requirement to use a minimum level of organic ingredients. This standard also defines a set of requirements in terms of storage, production and packaging and requires the existence of an Environmental Management Plan that covers the entire production process and resulting waste products and waste, effectively implemented. The use of cleaning materials whose ingredients comply with this standard is also required.

However, achieving a certification is a time consuming and expensive process. Since there are no auditors in Portugal, they would have to come from abroad to analyze the process, the ingredients, the conditions of production, and other aspects referred above. Therefore, this is a process that entails many costs which will be more appropriate as soon as UniBio has more products ready to be certified together and minimize costs.

On the other hand, the produced solid shampoo was legally approved by Oxford Biosciences according to regulation EU 1223/2009, which sets obligatory requirements for cosmetic products that have been made available on the market within the European Union. In order to protect the health and safety of EU citizens, EU Regulation 1223/2009 regulates several aspects related to the manufacturing and labelling of products such as safety, maintaining an accessible product information file, obligation to supply information and labelling requirements for cosmetic products.

4.11.5. Price

After the production of several shampoos these were put up for sale for 7,90€ in different physical stores such as Organii (Baixa-Chiado, Príncipe-Real, Lx Factory, Saldanha and Porto) and in Maria Granel (Alvalade). Organii was the first Portuguese company specialized in organic cosmetics that also sells all the produced products. On the other hand, Maria Granel was the first organic grocery store and was also interested in selling the produced solid shampoos. It is also possible to buy the shampoos on the websites of both companies (Figures 64 and 65).

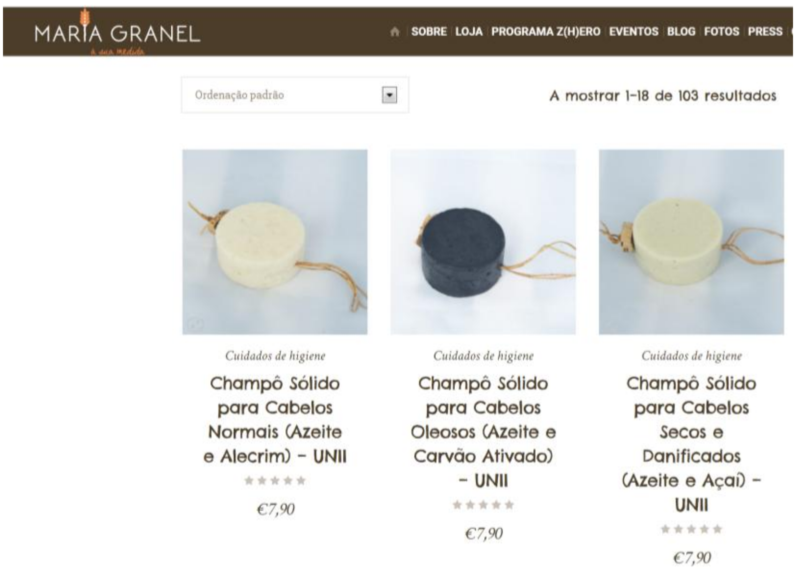

Figure 64: Produced Solid Shampoos for sale on the Maria Granel website.

Figure 65: Produced Solid Shampoos for sale on the Organii website.

In comparison with the control shampoo (Lamazuna- 12,50€, 55g), the price is lower and the mass of the shampoo is bigger (85g), which results in a very competitive price but at the same time assuring the profit of the product.

5. Conclusions and Future Improvements

Regarding the development and production of the three lines of shampoo, it was possible to conclude that after the oily hair shampoo production, the other two shampoos were easily reproducible, by adding to the formulation the specific ingredients for each type of hair. In fact, from the moment the manufacturing process was defined and the desired characteristics were reached, it was only necessary to manipulate the active ingredients according to the needs of the hair. In a nutshell, three products were obtained in accordance with the previously delineated expectations.

When determining the content of water and volatiles present in different formulations samples, it was concluded that they were low and for this reason, it was not required a preservative. This was confirmed through the microbiological tests of the final formulations since the shampoo medium was not propitious for bacterial and fungal growth.

The pH of different samples for the three shampoo lines was determined, and the results obtained (between 6.31 and 6.86) are slightly higher than the recommended range (between 3.7 and 5.5approximately).

When comparing the features of the obtained shampoo with the control shampoo (Lamazuna shampoo), the shampoo produced in this work proved to be better in the foam quantity and creaminess which allowed a quick and improved washing of the hair.

Finally, a hanging system with cork materials was created, as well as the shampoo packaging with recycled paper.

In order to improve the work done, the pH of the shampoo should be adjusted with citric acid in a way that this pH is closer to the hair pH. This will provide a less aggressive washing of the hair, and the increasing of the electrical charge of the hair cuticle will be avoided.

Other procedures could be tested in order to evaluate the different parameters of each shampoo, for example, the use of a colorimeter to evaluate the color of the shampoo.

Further stability tests could be performed such as long-term photostability and odour tests, to examine the product and its components feasibility, as well as the determination of the product shelf-life.

To achieve the absence of the microorganisms after the production, the molds and the material should be previously disinfected with an alcoholic solution. In addition, the microbiological analysis of shampoos after use could be performed in order to confirm the non-contamination of the product.

The water content determination methodology should be reviewed, *i.e.*, other process should be used instead of dry weight determinations to obtain better results. Other parameters could be tested in addition to humidity, namely, water activities of different samples with different formulations.

An automatic or semi-automatic filling machine with heating and mixing devices should be necessary if the scale of production increase. However, the heating fluid should not be water, in order to ensure the mixture fluidity.

In conclusion, the goals set at the beginning of this work were accomplished. However, the above-mentioned changes can be implemented to improve not only the quality of the shampoos, but also the manufacturing process.

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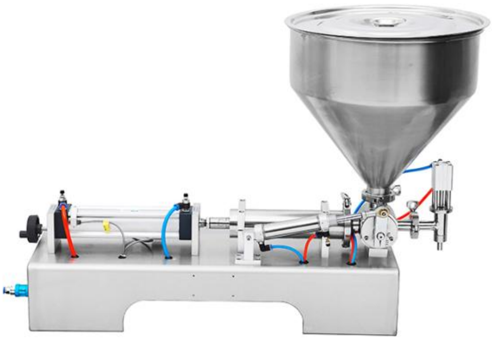
Appendix

Appendix B- Ingredients Melting Points and sources of values

Sodium Cocoyl Isethionate	190.6-191.6ºC	(Personal Care, Products Council, 2018)
Stearic Acid	68.8ºC	(PubChem)
Cetearyl Alcohol	52ºC	(PubChem)
Coconut Oil	24ºC	Krishna, A. G. (2010)
Shea Butter	52.1ºC	(Adomako, 1977)
Cocoa Butter	53.4ºC	(Adomako, 1977)
Carnauba Wax	82-86ºC	(Happy Happy Vegan, 2018)
Activated Charcoal	>3500ºC	(PubChem)

Appendix C – Example of a commercial Filling Machine (Beespacker, 2018).



Label	Bespacker
Suitable for	Honey, Oil, Cream, Hair shampoo and others bad liquidity products.
Power	500 W
Automatic Grade	Semi-Automatic
Air Pressure	0,4-0,6 MPa
Filling Speed	5-30 Bottles/min

Model	Voltage	Filling volume	Filling precision	Dimension	Weight
G1WGD	110V/220V	5-50ml	within 1%	90*30*35cm 45*45*45cm	20KG 4KG
		10-100ml	within 1%	97*39*37cm 45*45*45cm	25KG 4KG
		50-500ml	within 1%	97*39*37cm 45*45*45cm	30KG 4KG
		100-1000ml	within 1%	115*40*38cm 45*45*55cm	32KG 4KG
		300-2500ml	within 1%	125*40*38cm 45*45*55cm	38KG 5KG
		1000-5000ml	within 1%	127*39*43cm 43*43*46cm	41KG 5KG

Appendix D - Mikrocount® dipslides Instructions (Schülke & GmbH, 2018).

Hygiene testing with a system.

Easy, safe and reliable – mikrocount® dipslides.

Application of mikrocount® dipslides

Contact inoculation:

- For surfaces and solid samples.
- Hold both sides of the nutrient media carrier onto the surface for about 5 seconds without touching the agar with your hands.
- The pivot joint of the agar slide offers additional convenience.

Streak test:

- Use a sterile swab to collect samples from points that are difficult to access or from highly viscous liquids.
- The swab is then streaked out onto the agar surface of the slide in order to transfer the microorganisms to be cultivated.

Dip:

- The slide is dipped into the liquid which is to be tested for a few seconds.
- Allow any excess liquid to run from the nutrient media carrier to ensure safe transportation of the dipslides.

Incubation:

- Incubation in closed tubes
- mikrocount® TPC
  24 – 48 h at 25 °C – 30 °C
- mikrocount® TPC/E
  24 – 48 h at 25 °C – 30 °C
- mikrocount® duo
  24 – 48 h at 25 °C – 30 °C
  Detection of yeasts and moulds 3 – 5 days at 25 °C – 30 °C

Appendix E – Final Shampoo Packages

Oily Hair Shampoo

Normal Hair Shampoo

Dry and Damaged Hair Shampoo

Appendix References

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