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Diana Raquel Silva Monteiro

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Dissertação no âmbito do Mestrado em Biotecnologia Farmacêutica, orientada pela Doutora Andreia Alves e pelo Professor Doutor António Ribeiro e apresentada à Faculdade de Farmácia da Universidade de Coimbra

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Resumo

Vários produtos dermocosméticos têm uma capacidade antisséptica limitada e inexata. Neste contexto, foram apresentadas no mercado novas gamas de produtos que incluem óleos essenciais, mas cujo resultado não é eficaz.

O desenvolvimento de novas formulações de dermocosméticos, com melhores propriedades antissépticas e, de preferência, incorporando componentes de base natural, como os óleos essenciais, torna-se imperativo. Assim, neste trabalho, foram produzidas e caracterizadas emulsões à base de óleos essenciais constituídos por componentes com ação antimicrobiana verificada, como o timol, através da incorporação de surfactantes de base natural e estabilização com biopolímeros. Estes sistemas de emulsão foram avaliados quanto à sua capacidade antimicrobiana, através da determinação da sua concentração mínima inibitória (CMI), com vista à sua aplicação no desenvolvimento de produtos inovadores na área da cosmética.

Produtos com capacidade antisséptica sempre tiveram um papel preponderante na nossa sociedade, dada a capacidade de controlo da proliferação de microrganismos presentes na pele, reduzindo o risco de desenvolvimento de infeções.

Foram realizados estudos essenciais para a produção de emulsões estáveis, como a determinação da concentração micelar crítica (CMC) de diversos surfactantes de base natural em estudo, como o lauril glicosídeo, decil glicosídeo, glicosídeo de coco, cocamidopropril betaína, tween 20 e tween 80.

Depois foram produzidas emulsões com sistemas modelo, como o óleo vegetal e óleo de côco, tendo como objetivo a determinação dos seus índices de emulsificação (IE), de forma a se selecionar os surfactantes com maior capacidade de emulsificação e estabilização destes óleos. De forma a se obter emulsões mais estáveis ao longo do tempo, tendo em vista a aplicação futura que se pretende para estes sistemas, foi também avaliada a influência da presença de biopolímeros, goma xantana (GX) e carboximetil celulose 700.000 (CMC 700.000), nestas emulsões.

A atividade antimicrobiana de seis óleos essenciais (tomilho, orégãos, eucalipto, menta, limão e árvore de chá) foi avaliada em quatro estirpes bacterianas, *E. coli ATCC 8739, P. aeruginosa DM, S. aureus ATCC 6538, E. faecalis ATCC 29212*, através da determinação da CMI, utilizando o método de microdiluição em placa.

Numa última fase, após a seleção dos óleos de tomilho, orégãos e menta, como os que possuem maior capacidade antibacteriana, foram produzidas emulsões contendo estes óleos individualmente e na forma de mistura, de modo a avaliar o efeito sinergístico entre os mesmos, pela determinação das suas CMI´s, utilizando, também, o método de microdiluição em placa.

Com o presente estudo foi possível a produção e estudo de emulsões de base natural com óleos essenciais, muito promissoras para aplicação no desenvolvimento de produtos de dermocosmética inovadores e com propriedades diferenciadas.

Palavras-Chave: óleos essenciais, emulsões, produtos cosméticos, propriedades antimicrobianas.

Abstract

Several dermo cosmetic products have a limited and inaccurate antiseptic ability. In this context, have been presented at the market new product ranges that include essential oils, but whose result it is not effective.

The development of new dermocosmetic formulations, with better antiseptic properties and, preferably, incorporating natural-based components, such as essential oils, becomes imperative. Thus, in this work, we produced and characterized emulsions based on essential oils constituted by components with verified antimicrobial action, such as thymol, through the incorporation of natural-based surfactants and stabilization with biopolymers. These emulsion systems were evaluated for their antimicrobial capacity, by determining their minimum inhibitory concentration (MIC), with a view to their application in the development of innovative cosmetic products.

Products with antiseptic capacity have always played an important role in our society, given their ability to control the proliferation of microorganisms present on the skin, reducing the risk of developing infections.

Essential studies were conducted for the production of stable emulsions, such as determining the critical micellar concentration (CMC) of several natural-based surfactants under study, such as lauryl glycoside, decyl glycoside, coconut glycoside, cocamidopropril betaine, tween 20 and tween 80.

Then emulsions were produced with model systems, such as vegetable oil and coconut oil, aiming to determine their emulsification indexes (IE), to select the surfactants with the highest emulsification and stabilization capacity for these oils. In order to obtain more stable emulsions over time, considering the future application intended for these systems, the influence of the presence of biopolymers, xanthan gum (GX) and carboxymethyl cellulose 700,000 (CMC 700,000), on these emulsions was also evaluated.

The antimicrobial activity of six essential oils (thyme, oregano, eucalyptus, mint, lemon and tea tree) was evaluated on four bacterial strains, E. coli ATCC 8739, P. aeruginosa DM, S. aureus ATCC 6538, E. faecalis ATCC 29212, by determining MIC using the microdilution assay.

In a last phase, after the selection of thyme, oregano and mint oils as having the highest antibacterial capacity, emulsions containing these oils individually and as a mixture were

produced in order to evaluate the synergistic effect between them, by determining their MICs, also using the microplate dilution method.

With this study it was possible to produce and study natural-based emulsions with essential oils, very promising for application in the development of innovative dermocosmetic products with differentiated properties.

Keywords: essential oils, emulsions, cosmetic products, antimicrobial properties.

List of abbreviations

ATCC – American Type Culture Collection

ATP – Adenosine triphosphate

CAGR – Compound annual growth rate

CLSI – Clinical and Laboratory Standards Institute

CMC – Carboxymethyl cellulose

CMC – Critical micelle concentration

CMC 700.000 – Carboxymethyl cellulose 700.000

CPP – Critical packing parameter

DMSO – Dimethyl sulfoxide

EEO – Eucalyptus essential oil

EI – Emulsification index

EOs – Essential oils

FDA – Food and Drug Administration

GG – Guar gum

HLB – Hydrophilic-lypophilic balance

LB – Luria Bertani

LEO – Lemon essential oil

LPS – Lipopolysaccharides

MEO – Mint essential oil

MH – Muller Hinton

MIC – Minimum inhibitory concentration

MOAs – Mechanisms of action

O/W – Oil/Water

OEO – Oregano essential oil

P_c – Molecular packing parameter

PEGs – Polyoxyethylene esters

PMF – Protonmotive force

TEO – Thymus essential oil

T_K – Krafft temperature

TTO – Tea tree essential oil

UFA – Unsaturated fatty acids

W/O – Water/oil

XG – Xanthan gum

1 Introduction

1.1 Cosmetic Industry and the benefits of natural compounds in cosmetic products

As established by the EU regulations, a cosmetic is classified as "any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours" (Regulation (EC) n.° 1223/2009 of the European Parliament and of the Council on Cosmetic Products, 2009).

The cosmetic industry provides a wide range of important and daily care products, including hair care, personal care, cleaner, perfume, and makeup. Cosmetic products are always being improved and innovated. For this, cosmeceuticals products, items with one or more bioactive components designed to enhance health and beauty, have grown in popularity (Pitman, S., 2014).

The CAGR (Compound annual growth rate) for the worldwide cosmetics industry is predicted to be 4.3 percent from 2016 to 2022, and it is expected to achieve $429.8 billion this year (Market, V.F., 2016). There is a list composed of synthetic compounds used in the production of cosmetics, of this list, less than twenty percent of the twelve thousand chemical ingredients were found to be safe (Karr, S. *et al*., 2013). As a result, serious issues have been expressed in recent years about the rising use of cosmetic products, drugs, and several other personal care items such as antiseptics, repellents, and nutraceuticals. These concerns are related with the environmental effects of cosmetic items, i.e., their biocompatibility, bioaccumulation, and toxicity potential in waterways (Gao, P. *et al*., 2018).

Approximately 25,000 different components can be used to produce cosmetics, and the majority of them fall under the broad category of "lipids," which includes waxes, oils, and fats. Still, it seems that getting these substances from natural sources, like plants and animals, rather than manufactured versions is preferable (Kulkarni, C.V., 2016).

Several chemical compounds or cosmetic additives, including synthetic musks (Liu, N. *et al*., 2014), ultraviolet filters (Ramos, S. *et al*., 2016) and microplastics (Conley, K. *et al*., 2019), are not really and successfully removed by wastewater treatment facilities. Additionally, as a result of employing sludges as fertilizers, these concentrate in sewers throughout wastewater treatment and make their way into the aquatic environment (Díaz-Cruz, M.S. *et al*., 2009).

Cosmetics, as opposed to drugs, provide the most serious environmental dangers since they are used continuously over the course of a person's life. Due to their intended usage on exterior surfaces and lack of metabolic conversion, they are introduced in the environment in considerable amounts while being washed and taking a shower (Ternes, T.A. *et al*., 2004).

Due to these concerns, the investigation and development of new cosmetic products, using natural and safe components it is extremely important to overcome the main problems reported.

In the present work, the stability and behavior of essential oils will be evaluated, through the formation and study of emulsions for a future application in the production of new cosmetics. Once essential oils are natural compounds and has excellent recognized properties, like antimicrobial activity, this study can contribute to the substitution of compounds with undesirable effects in products of different industries such as cosmetics, food, or pharmaceutical.

1.2 Surfactants in cosmetic industry

Surfactants or surface-active agents are amphipathic or amphiphilic compounds that are composed of a polar (hydrophilic head) and a non-polar moiety (hydrophobic tail) (Lourith, N. and Kanlayavattanakul, M., 2009, Schramm, L.L. *et al*., 2003, Seweryn, A., 2018) and can be described as molecules capable of self-associate to create different molecular structures, called aggregates (Desai, J.D. and Banat, I.M., 1997, Schramm, L.L. *et al*., 2003). The hydrophilic component is attracted to polar solvents such as water and it includes electrolytically dissociable (basic and acidic) groups and nonionic groups (Hydroxide, sugar, and thiol). Typically, the acidic groups are sulphonic ($R-S(=O)_2-OH$), sulphate (SO_4^{2-}), phosphate (PO_4^{3-}), and carboxylic (R-COOH) groups. The basic groups are usually primary ($R-NH_2$), secondary (R_2NH), and tertiary amine (R_3N), and primary, secondary, and tertiary pyridine groups. The hydrophobic tail exhibits affinity for non-polar solvents, and it is made up of hydrocarbon or alkyl chains with 8-22 carbons (Sakamoto, K. *et al*., 2017), which can be dissociated into fatty acids and derivatives, ramified or non-ramified (Falbe, J., 2012, Seweryn, A., 2018).

One of main properties of surfactants is to decrease the interfacial tension of liquid-liquid systems, such as oil/water (O/W) or water/oil (W/O), and the surface tension of aqueous solutions, such as air/water solutions (De, S. *et al*., 2015). The interfacial tension is the force that draws molecules together at the interface between two fluids (Cong, Y. *et al*., 2020, Meybodi, M.K. *et al*., 2015). The presence of surfactants in aqueous medium leads to a decrease in surface tension, since its molecules take some of the position of water molecules

present in water´s surface. Thus, surfactant molecules and water molecules are attracted to one another with less force than water molecules are attracted to each other, which results in a decreasing of the contraction force that causes surface tension (Hu, D. *et al*., 2016, Mohapatra, S. *et al*., 2014). In O/W systems, the surfactant molecules will act at the O/W interface, replacing part of water and oil molecules, this event is known as surfactant adsorption. So, the surfactant molecules reorganize themselves in the interface in such a way, that, in on side of the interface, the hydrophilic portion is in contact with the water, and, on the other side of the interface, the hydrophobic portion is in contact with the oil. This results in a more stronger interaction among oil and water interface than the previous contact before adding the surfactant (Azodi, M. and Nazar, A.R.S., 2013, Xu, J. *et al*., 2013).

Nowadays, surfactants are used for more than simply cleaning, they also act as effective emulsifiers, dispersing, foaming and defoaming agents due to their surface activating property (Desai, J.D. and Banat, I.M., 1997, Sakamoto, K. *et al*., 2017). In the cosmetic industry, surfactants are essential compounds since it promotes the mixing of two immiscible liquids, such as oil and water, allowing a product to permeate skin and hair, and keep a product stable for long periods of time (Sakamoto, K. *et al*., 2017).

1.2.1 Classification of surfactants

The surfactants are categorized based on their hydrophilic portion. They can be classed into two groups: ionic and non-ionic. The ionic group can even be subclassified into anionic, cationic and zwitterionic (or amphoteric) (Carson, C.F. *et al*., 2002, Dave, N. and Joshi, T., 2017), and it can be shown in **Figure 1**.

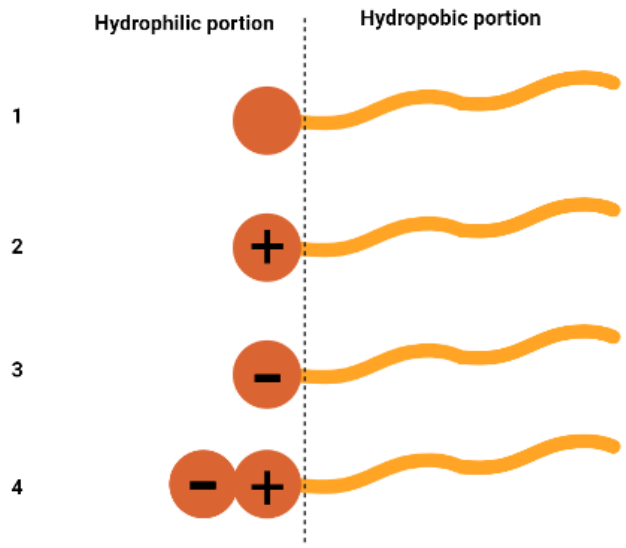


Figure 1: Surfactant classification based on the hydrophilic head. Non-ionic surfactant (**1**), Cationic surfactant (**2**), Anionic surfactant (**3**) and Zwitterionic surfactant (**4**).

1.2.1.1 Anionic surfactants

In this type of surfactants, the hydrophilic portion dissociates into anions in aqueous medium. Typically, the hydrophilic portions are: sulfonate ($-SO_3^-$), sulfate ($-OSO_3^-$), carboxylate ($-COO^-$), sulfobetaine ($-N(CH_3)_2C_3H_6SO_3^-$) and carboxybetaine ($-NR_2CH_2COO^-$) (Sakamoto, K. *et al*., 2017). The hydrophobic tail is composed for alkyl chains with a length of C_{12}-C_{18} of saturated/unsaturated aliphatic groups. They are the most commonly used class of surfactants due to its great cleaning capabilities in shampoos, dishwashing solutions and detergents. This type of emulsifiers is nontoxic (Dave, N. and Joshi, T., 2017).

1.2.1.2 Cationic surfactants

In this case the hydrophilic portion dissociates into cations in aqueous solutions. They function well as emulsifiers. Due to its effective and powerful antibacterial properties, this type of surfactants is used as topical antiseptics, hand and bathroom sanitizers. Besides these applications, the cationic surfactants are even used as textile conditioners due to attraction of cationic surfactants by negative charges, they can bond to each other and give the fabric a plush and cozy feel (Dave, N. and Joshi, T., 2017). One of the examples of cationic groups is quaternary ammonium ($-R_4N^+$) (Sakamoto, K. *et al*., 2017).

1.2.1.3 Zwitterionic surfactants

Zwitterionic or amphoteric surfactants present a hydrophilic portion made up of cationic and anionic groups. The anions are often, carboxylate, sulphate and sulphonate and the cations are, usually, ammonium. This type of surfactants is lesser found than anionic,

cationic and non-ionic surfactants. Due to their extreme mildness, effectiveness in treating skin conditions, and powerful foaming capabilities, zwitterionic surfactants are ideal for usage in personal care (shampoos and other cosmetics) and home cleaning items (dishwashing detergents) (Dave, N. and Joshi, T., 2017).

1.2.1.4 Non-ionic surfactants

In non-ionic surfactants, the hydrophilic head do not dissociate in ions in water solutions, as is the case with phenol (C_6H_6O), alcohol (CH_3OH), glycerol ($C_3H_8O_3$), sorbitol ($C_6H_{14}O_6$), ether (R–O–R′), ester (R–COO–R′) and amide (CO-NH) groups (Dave, N. and Joshi, T., 2017, Sakamoto, K. *et al.*, 2017). Owing to lack of a charge, these surfactants resist to water action. Among the several applications of these emulsifiers, stands out dishwashing detergents, home cleansers, laundry detergents, as good fat removers, and drug delivery (Dave, N. and Joshi, T., 2017).

1.2.2 Critical micelle concentration (CMC)

At a given concentration, the surfactant molecules organize, rearrange themselves and associate with each other creating an aggregate form designated as micelle, typically a spherical micelle (self-assembled molecules cluster) (Bhosle, M.R. *et al.*, 2020, Massarweh, O. and Abushaikha, A.S., 2020, Naseri, N. *et al.*, 2018, Sakamoto, K. *et al.*, 2017), such is shown in **Figure 2**.

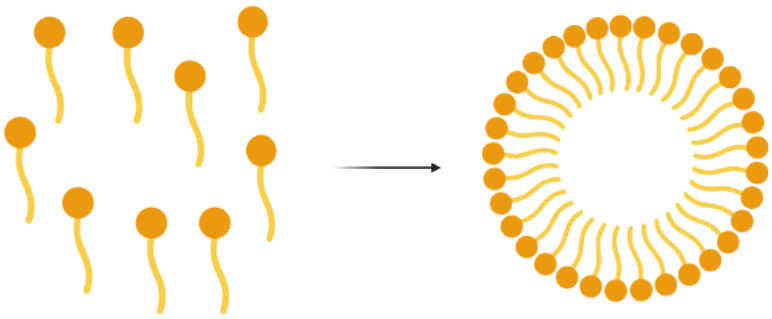


Figure 2: Surfactants molecules rearrange into micelles.

The concentration above which surfactants create micelles is known as the critical micelle concentration (CMC) (Massarweh, O. and Abushaikha, A.S., 2020, Sakamoto, K. *et al.*, 2017). Beyond the micellization, above CMC, the surfactant molecules can rearrange themselves into vesicles and bilayers (De, S. *et al.*, 2015). Due to this feature, surfactants improve the availability and solubility of non-polar compounds (Whang, L.-M. *et al.*, 2008). Usually, surfactant effectiveness is measured using the CMC parameter. Therefore, for more

efficient surfactants, the CMC value is lower because a lower amount of surfactant is required to reduce surface tension (Desai, J.D. and Banat, I.M., 1997), as is shown in **Figure 3**. A surfactant's CMC is influenced by its molecular structure (such as the length of its hydrophobic portion) (Glennie, A.R. *et al.*, 2006), the salinity and ionic constitution of the solution (Bratovcic, A. and Nazdrajic, S., 2020), pressure conditions, temperature, pH, and other variables (Harutyunyan, L.R. and Harutyunyan, R.S., 2019). In addition to surface tension, when the CMC is reached other physical and chemical parameters drastically change such as electrical and thermal conductivity, and viscosity (Sabahi, N. *et al.*, 2017).

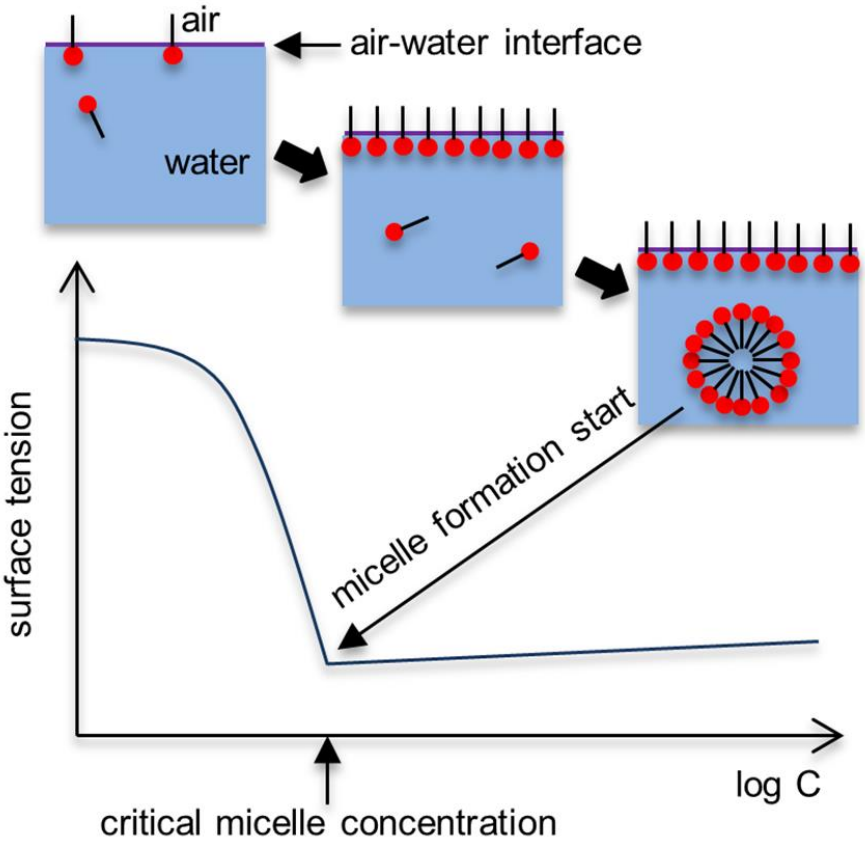


Figure 3: Schematic representation of the decrease in surface tension with increasing surfactant concentration and consequent CMC determination. (Adapted from (Ueno, M. *et al.*, 2016))

1.2.3 Critical packing parameter (CPP)

At the level of aggregates that can be formed, besides micelles there are others, depending on a factor, known as molecular packing parameter (P_C) or critical packing parameter (CPP), and it can be calculated through the formula in **Equation 1.**

$$CPP = \frac{v_0}{l_0 a} \qquad \textbf{Equation 1}$$

In the equation v_0 is the volume of the surfactant hydrophobic portion, l_0 is the length of the surfactant hydrophobic portion, a is the surface area of the hydrophilic portion at the surface of the aggregate (Nagarajan, R., 2002).

The CPP factor defines the connection among a surfactant molecule´s shape and the types of aggregation that can occur in water medium (Massarweh, O. and Abushaikha, A.S., 2020). Therefore, based on the value of CPP, it is possible to hypothesize the form and geometrical structure of molecular aggregates based on the molecular structure of surfactants. If $0 < CPP \leq 1/3$, merely spherical micelles are present in solution. When $1/3 < CPP \leq 1/2$, hexagonal or rod-shaped aggregations are most common. Whereas, $1/2 < CPP \leq 1$, an equilibrium among the head group and tail lengths result in planner aggregates with a bilayer shape that resembles a sheet (vesicles) (Holmberg, K. *et al*., 2002). The $CPP > 1$ form inverted micelles or inverted hexagonal phases (H_{II}) (Stuart, M.C. and Boekema, E.J., 2007). The **Figure 4** illustrates the influence of the CPP parameter on the morphology of the aggregates that are formed.

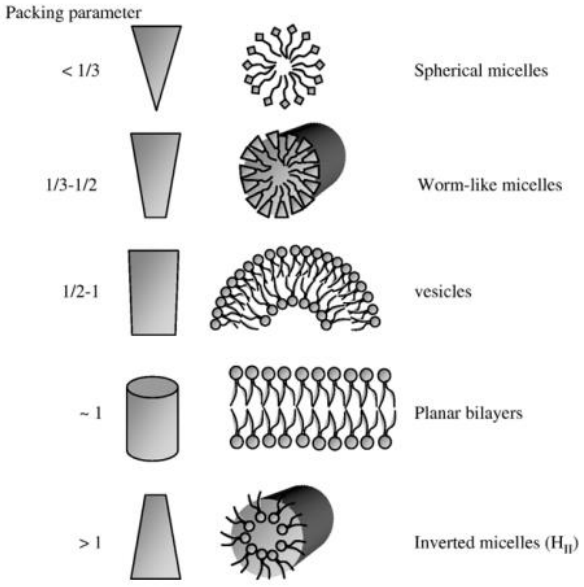


Figure 4: Aggregates structures that are formed based on CPP parameter. (adapted from (Stuart, M.C. and Boekema, E.J., 2007))

1.2.4 Krafft temperature, Krafft point and Cloud point

The Krafft temperature (T_K), commonly referred as the critical micelle temperature, is a significant factor associated with the CMC (Islam, N. *et al*., 2015, Roy, J.C. *et al*., 2014). The T_K is the temperature at which the surfactant solubility significantly increases, and a continuous temperature rise until reaches the CMC and hence leading to a micelle formation (Malik, N.A. and Ali, A., 2016). Under the Krafft temperature, the formation of surfactant micelles is not

possible (Dicharry, C. *et al*., 2016, Dölle, S. *et al*., 2012), and surfactant tended to be insoluble (Moon, T.L. *et al*., 2001, Srivastava, A. *et al*., 2019). Other important parameter is Krafft point that indicate the temperature at which the solubility of surfactants matches their CMC, since at this point de hydrophobic chains starts to melt and that helps the dissolution of surfactant molecules into micelles and monomers (Dave, N. and Joshi, T., 2017).

However, there is types of surfactants that do not have Krafft point, as non-ionic surfactants. When temperature rises, the non-ionic surfactants solubility drops, and they start to lose their surface-active features above a temperature shift known as the cloud point. This happens when a phase of inflated surfactant micelles splits above the cloud point (Schramm, L.L. *et al*., 2003). The transition is detectable due to a sharp rise in dispersion turbidity. Thus, the cloud point is used as a gauge of the solubility of this type of surfactants, since a surfactant with higher value of this parameter also has a greater hydrophilic feature (Sakamoto, K. *et al*., 2017).

1.3 Emulsification

Emulsification is the process by which a liquid is dispersed into another, resulting in droplets (dispersed phase) suspended in another liquid (the continuous phase) and, consequently, the blending of two immiscible liquids (De, S. *et al*., 2015, Jaiswal, M. *et al*., 2015, Travis, P.M., 1926), originating an emulsion. Emulsions are usually unstable so to help in this process it is necessary an additional component called emulsifier or surfactant, which allows the emulsification through surrounding the droplets and forming a thin layer. Usually, the droplets size is between 0.1 and 100 μm (Jaiswal, M. *et al*., 2015).

1.3.1 Types of emulsions

There are two types of emulsions: O/W and W/O emulsions. O/W emulsions have oil globules that are spread in the continuous phase, water. W/O emulsions consist of a continuous oily phase in which water is scattered as droplets (Barkat, A.K. *et al*., 2011, Travis, P.M., 1926). The **Figure 5** shows the two types of emulsion in question.

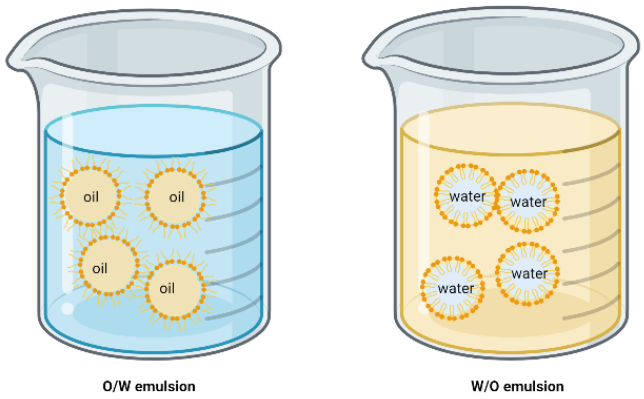


Figure 5: Types of emulsions.

1.3.2 Hydrophilic-lipophilic balance (HLB)

An extremely helpful approach for categorizing surfactants according to how well they dissolve in water is the hydrophilic-lipophilic balance (HLB). This numeric value parameter indicates the relative surfactant affinity for water and oil. Therefore, surfactants with HLB values of three to six, i.e., low HLB, are applied to W/O emulsions since the lower the HLB number, the more lipophilic the surfactant is, so they stabilize W/O emulsions. While surfactants with HLB values, i.e. high HLB, of seven to twenty are applied to O/W emulsions, since the higher the HLB number, the more hydrophilic the surfactant is, hence stabilizing O/W emulsions (Barkat, A.K. *et al*., 2011, Mohamed, A.I.A. *et al*., 2017, Schramm, L.L. *et al*., 2003). This parameter is based on a concept known as Bancroft's rule, which define that the continuous phase is that in which a surfactant dissolve more easily. Accordingly, surfactants that are soluble in oil, often produce water-in-oil emulsions whereas surfactants that are soluble in water typically produce oil-in-water emulsions (Mohamed, A.I.A. *et al*., 2017).

1.3.3 Types of instabilities that occur in emulsions

Emulsions are thermodynamic systems with a high associated instability because of the strong interfacial tension among the two phases (Becher, P., 1983). In an emulsion four types of instabilities can occur, such as: coalescence, flocculation, creaming and Ostwald ripening, which can be seen in **Figure 6**.

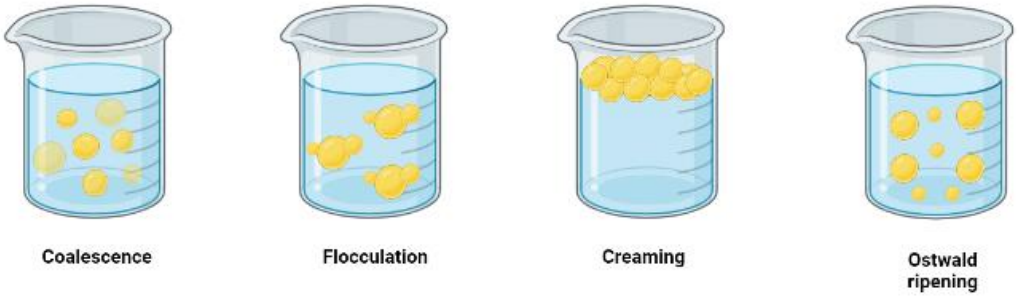


Figure 6: The four types of emulsions instabilities.

1.3.3.1 Coalescence

Coalescence is the kind of emulsion instability that happens when interfacial layer is disrupted and as the result, the droplets combine to produce bigger globules (Shao, P. *et al*., 2020). Emulsion stabilization against coalescence can be reached by adding high molecular weight or high boiling point compounds (Herbert, A. *et al*., 1996).

1.3.3.2 Flocculation

Flocculation is the formation of a big aggregation through the combination of tiny emulsion particles. This is a reversible instability e and it precede coalescence. Flocculation can be caused by an overabundance of surfactant in the continuous phase. That happens due to a depletion mechanism which is described as a system with large amounts of micelle-forming surfactants, the droplets begin to approach one another at a distance nearer than emulsifier micelles diameter and because of the decrease of entropy, micelles separate from the interparticle region. Because of this process, the osmotic pressure in the space among the globules decreases, creating an attraction force between them. As a result, particle flocculation takes place (Barkat, A.K. *et al*., 2011).

1.3.3.3 Creaming

Creaming occurs when the disperse phase splits out and creates a layer on the surface of the continuous phase (Shao, P. *et al*., 2020). It is noteworthy that the dispersed phase in creaming stays in droplet form such that shake might redisperse it. By increasing the continuous phase's viscosity, creaming can be reduced. Typically, in O/W emulsions, the creaming layer forms on top of the continuous phase, since the dispersed droplets are less dense than the other phase. Whereas in W/O emulsions the creaming layer forms below the continuous phase since the dispersed droplets are denser than the other phase (Barkat, A.K. *et al*., 2011).

1.3.3.4 Ostwald ripening

Ostwald ripening is the expansion of one disperse particle at the detriment of another that is smaller due to the different chemical potential of the content of the two globules (Shao, P. *et al*., 2020, Taylor, P., 1995).

1.4 Polymers in cosmetic industry

Polymers are made up of several repeated units designated monomers, often organized in the shape of a chain. Through the chemical reaction of monomers, polymers are created. When the right conditions are met, monomers can interact with molecules of the same kind or a different type to form polymer chains. Natural polymers were created because of this process in nature, whereas synthetic polymers were produced in laboratory (Namazi, H., 2017). Having said that, according to the source from which these compounds are obtained they can be categorized as natural, from biological material such as plants or animals, semi-synthetic, polymers from natural sources that have undergone chemical modification, or synthetic polymers, from artificial source, i.e., produced by mankind. Specifically in cosmetic products they are present as basic ingredients since they are fundamental in the creation of high-performance goods. Due to the variety in terms of polymers structure, they promote a diversity of roles such as thickening and emulsifying agents, conditioners, and foaming stabilizers and destabilizers (Dias-Ferreira, J. *et al*., 2020, Gawade, R.P. *et al*., 2020).

In fact, these macromolecules are included in a variety of formulations used in the beauty industry, including fragrance, cosmetics, and nail care. For example, they are used in hair formulations, such as shampoos, conditioners, tip repair, hair color, hydrating treatments, and fixing solutions, and in skin care items including hydrating lotions, sunscreen, body oils and liquid soaps (Dias-Ferreira, J. *et al*., 2020). All these goods have unique uses and applications, as well as various compositional, production, and physical and chemical properties that require a wide range of polymers (Alves, T. *et al*., 2020).

Besides the applicability of these components in cosmetics, they also could be part of physic sector, mechanical and chemical engineering, textile sector and pharmaceutical area. Thus, it is possible to employ both natural and synthetic polymers as composites, coverings, synthetic rubber, ceramic products, caulking, textile fibers, plastic. In fact, the science field, such as, biochemistry, biophysics, biomedicine and molecular biology are the main areas which polymers and polymeric chemistry have a key role in their innovative development (Namazi, H., 2017).

In cosmetics, the most often applied polymers are carboxymethyl cellulose (CMC), guar gum (GG), modified starch, xanthan gum (XG) (Alves, T. *et al*., 2020). Considering their applications in cosmetics, xanthan gum and carboxymethyl cellulose were chosen as polymers for this work.

1.4.1 Xanthan gum (XG)

Xanthan gum is a natural anionic extracellular polysaccharide with high molecular weight. This polymer is the result of fermentation process carried out by a Gram-negative bacterium designated *Xanthomonas campestris* (Bhattacharya, S.S. *et al*., 2013, Chaturvedi, S. *et al*., 2021). Since each monomeric unit of this polymer contains several functional groups, such as ester, hydroxyl, and carboxyl, which are uniformly distributed in polymeric chain, XG is totally soluble for both hot and cold water. This confers stability under several conditions such as temperature, acidity, and enzymes action, allowing the XG to maintain solution viscosity constant at low concentrations and throughout a broad variety of temperatures and pH values (He, J. *et al*., 2017, Nor Hayati, I. *et al*., 2016).

Beside these features, XG does not present toxicity associated and it is considered by US Food and Drug Administration (FDA) safe to use in food items without any quantitative restriction (Bhattacharya, S.S. *et al*., 2013). So, for that, it has been extensively used in O/W emulsions, as polymeric stabilizers, food preservatives and rheology modifiers in cosmetic, food and pharmaceutical sectors, respectively (Nor Hayati, I. *et al*., 2016, Xue, J. and Ngadi, M., 2009, Zhang, X. *et al*., 2013). The **Figure 7** represent the chemical structure of XG.

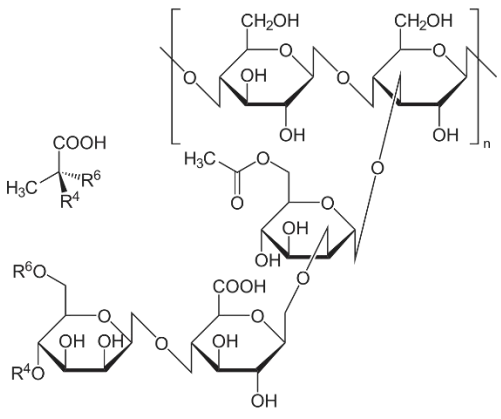
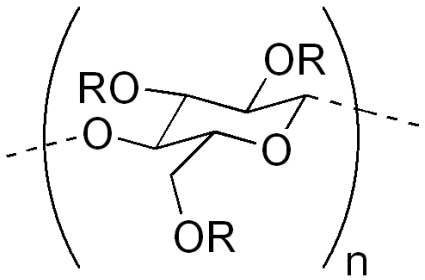


Figure 7: Chemical structure of XG.

1.4.2 Carboxymethyl cellulose (CMC)

Carboxymethyl cellulose is a semi-synthetic, anionic, linear, and water-soluble polysaccharide derivate of cellulose (Rahman, M.S. *et al*., 2021). The synthesis of CMC was once reliant on wood-based plants since celluloses were mostly extracted from, not only wood, but another plant precursors which inherently had a high proportion of cellulose fibers (Fengel, D., 1969, Revol, J.F. and Goring, D., 1981, Scott, D.S. *et al*., 1988). The main structural changes among cellulose and CMC are a few carboxymethyl units (-CH_2COOH) in the CMC architecture that take the place of few of the hydroxyl groups that are found in the natural cellulose backbone (Rahman, M.S. *et al*., 2021).

Concerning the marketplace, CMC has been manufactured at the commercial level, since the twenties. Particularly because of this polymer´s biodegradability and lack of associated toxicity, CMC is being used in wide range of sectors, such as cosmetic, culinary and pharmaceutical goods, to increase the viscosity of solutions, to stabilize emulsions, and as a way to enhance texture (Fengel, D., 1969). The **Figure 8** represent the chemical structure of CMC.



$R = H \text{ or } CH_2CO_2H$

Figure 8: Chemical structure of CMC.

2 Introduction of essential oils and their application in cosmetic industry

Essential oils (EOs) also called as volatile oils are concentrated hydrophobic liquids, which contain compounds responsible for their distinctive scents. (Burt, S., 2004) They are the product of the secondary metabolism of the plants (Fraenkel, G.S., 1959), so they are reaped from plant materials such as flowers, buds, leaves, bark, roots, seeds, fruits, and wood (Burt, S., 2004).

Typically, the EOs consists of a complex set of volatile compounds which can be classed regarding their chemical structure as terpenes, terpenoids (or oxygenated terpenes) and phenols (Hyldgaard, M. *et al*., 2012, Rao, J. *et al*., 2019), which will be discussed below.

Nevertheless, the composition of these oils varies according to certain parameters, such as harvest time, geographical origin, and extraction procedure. (Demuner, A.J. *et al*., 2011, Nannapaneni, R. *et al*., 2009, Paibon, W. *et al*., 2011) Even the fact that they are extracted from different parts of the same plant influences their composition (Delaquis, P.J. *et al*., 2002).

The EOs exhibit over 60 individual components, the major components of which constitute about 85% of the overall EOs, whereas minor constituents are usually found in trace amounts (Senatore, F., 1996).

The essential oils throughout history have had several applications including in the food industry, cosmetics, and even health care, mainly in aromatherapy. EOs were often employed in the cosmetic business in the past because of their well-known scent qualities. Yet, additional intriguing qualities of several essential oils have been investigated, proven, and used in cosmetics (Lubbe, A. and Verpoorte, R., 2011, Nohynek, G.J. *et al*., 2010). Numerous studies demonstrated that due to their antibacterial properties against a variety of bacterial strains, EOs may be employed as preservation ingredients in cosmetics (Bakkali, F. *et al*., 2008, Nakatsu, T. *et al*., 2000). Since at considerably low concentrations, EOs inhibit the growth of harmful pathogens in cosmetics (Yorgancioglu, A. and Bayramoglu, E.E., 2013). Due to natural origin of EOs, they can take place of synthetic compounds, that endanger the health of the human being, and contribute with their antimicrobial and preservative characteristics for the cosmetic products (Adwan, G. *et al*., 2012, Claffey, N., 2003, Yorgancioglu, A. and Bayramoglu, E.E., 2013).

In plants, essential oils also play a fundamental role in protecting against predators, in communication among plants, and even in attracting insects for pollination (Hanif, M.A. *et al*., 2019). Besides this, the organic compounds of low molecular weight that make up the EOs have an antimicrobial activity widely well described and recognized (Hyldgaard, M. *et al*., 2012). The verified antimicrobial activity of EOs is directly correlated to their major constituents and their synergistic action with minor components.

Among the components with high antimicrobial activity, phenols and oxygenated terpenoids stand out. Whereas terpenes and the other components, including ketones, such as β-myrcene, α-thujone, and geranyl acetate, exhibit lower antimicrobial activity when compared to phenols and terpenoids (Bassolé, I.H. and Juliani, H.R., 2012).

2.1 Chemical composition of essential oils

The chemical compounds present in essential oils are mainly hydrocarbons, thus presenting carbon and hydrogen as structural blocks. In essential oils, isoprene (C5H8) is the most common fundamental hydrocarbon unit (Hanif, M.A. *et al*., 2019).

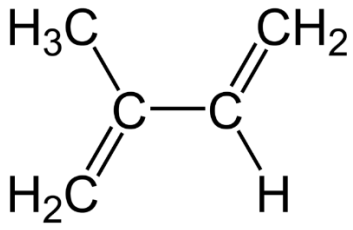


Figure 9: Chemical structure of isoprene unit.

The chemical composition of the constituents of EOs is quite important since its antimicrobial action is directly related to the way in which their components act (Bakkali, F. *et al*., 2008, Burt, S., 2004). Considering this, three groups of compounds can be highlighted: terpenes, terpenoids and phenols.

2.1.1 Terpenes

The most common elements of essential oils are terpenes which are produced in the cytoplasm of a wide range plant cells (Hyldgaard, M. *et al*., 2012). Terpenes are made up of several isoprene units joined together in a cyclic or non-cyclic chains (Sikkema, J. *et al*., 1995). Terpenes are classified as monoterpenes, diterpenes or sesquiterpenes based on the number of isoprene units in the molecule. Monoterpenes have the chemical formula C10H16 and are comprised of two isoprene units. This type of terpenes is found mostly in citrus, rosemary, sage, and lemongrass oils and include limonene, myrcene, α-pinene, and β-pinene. Sesquiterpenes have the chemical formula C15H24 and are made up of three isoprene units, such as β-caryophyllene (Rao, J. *et al*., 2019). Monoterpenes (C10) and sesquiterpenes (C15), which combine two and three isoprene units, respectively, are the two most common terpenes found in EOs (Bakkali, F. *et al*., 2008).

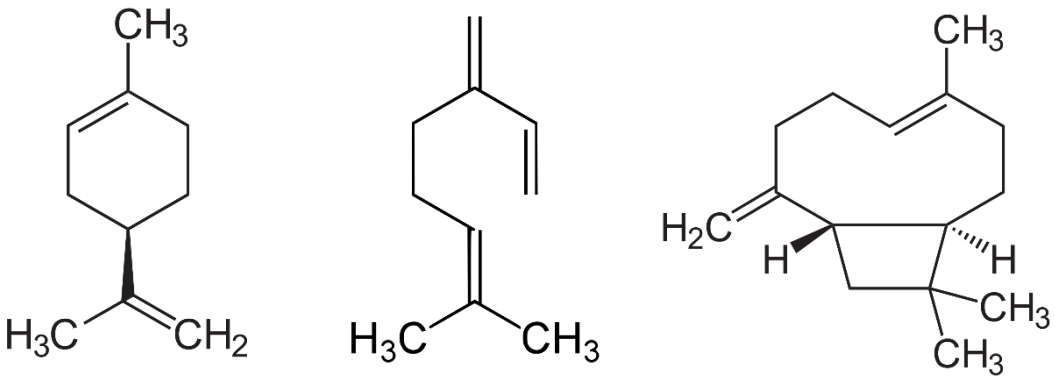


Figure 10: Chemical structure of limonene, myrcene and β-caryophyllene, respectively.

2.1.2 Terpenoids

Terpenoids, commonly referred as isoprenoids, are a broad and diversified family of naturally occurring isoprene-derived chemicals present in essential oils. In contrast to terpenes, isoprene units in terpenoids are joined and changed in several ways, including the addition or deletion of methyl groups and the introduction of oxygen atoms to the isoprene unit (Bakkali, F. *et al*., 2008). Based on the number of isoprene units present, terpenoids can be classed as monoterpenoids, sesquiterpenoids, or diterpenoids. Another way of categorizing terpenoids is by the number of cyclic structures they have (Rao, J. *et al*., 2019). Examples of some terpenoids are: 1,8-cineole, linalool and terpinen-4-ol.

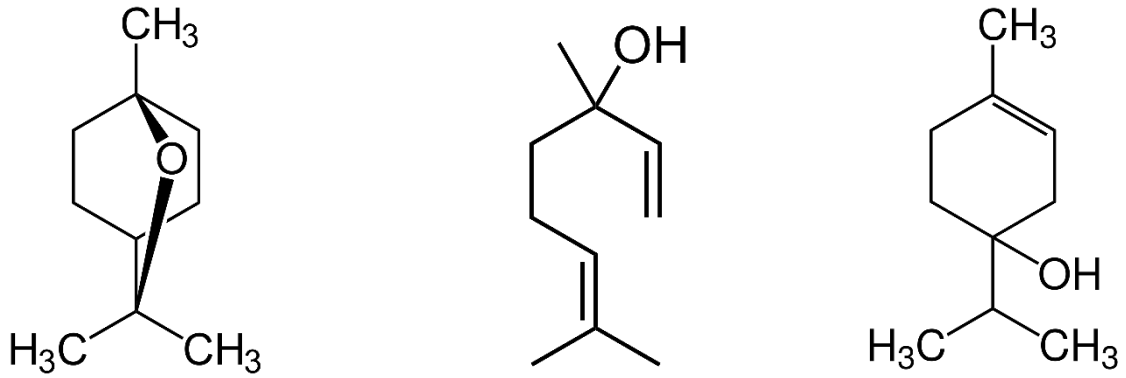


Figure 11: Chemical structure of 1,8-cineole, linalool and terpinene-4-ol, respectively.

2.1.3 Phenols

Phenols, commonly referred as terpenoid phenols, are a class of chemical components which include an aromatic ring with a hydroxyl group. Thymol, carvacrol and eugenol are examples of terpenoid phenols that are present in cinnamon, oregano, clove, and thyme EOs

(Rao, J. *et al*., 2019). In the phenolic compounds, the presence and the position of the hydroxyl group in the benzene ring seems to have influence in their action, mainly in antimicrobial action (Hyldgaard, M. *et al*., 2012).

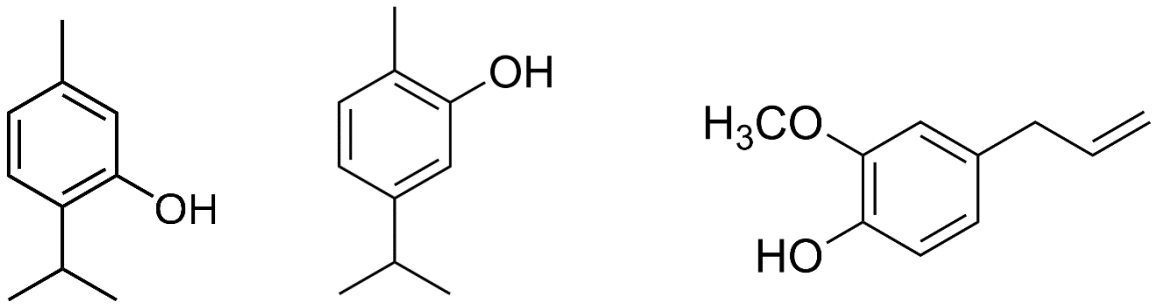


Figure 12: Chemical structure of thymol, carvacrol and eugenol, respectively.

2.2 Essential oils in study

2.2.1 Thymus Essential Oil

Thyme is a fragrant and therapeutic herb that it has gained more commercial appeal. It is part of the Lamiaceae or Labiatae family, and it typical species are *T. serpyllum* (wild thyme) and *T. vulgaris* (ordinary thyme) (Badi, H.N. *et al*., 2004, Nabavi, S.M. *et al*., 2015). Regarding to thyme essential oil (TEO) chemical composition, it comprises two phenolic terpenoids, thymol (37-55%) and carvacrol (0.5-5.5%), and monoterpenes such as, γ-terpinene (30.90%) (Borugă, O. *et al*., 2014), *p*-cymene (9.1–22.2%), linalool (2.2–4.8%), 1,8-cineole (0.2–14.2%) and α-pinene (0.9–6.6%) (Amiri, H., 2012, Burt, S., 2004, Nickavar, B. *et al*., 2005). Thyme is in the top ten most popular oils in the world due to its several applications, for example, it acts as a natural preservative, as a powerful antioxidant and antimicrobial (Ben-Jabeur, M. *et al*., 2015, Rasooli, I. *et al*., 2006), and adds flavor and smell to a broad range of foods and beverages and items of personal care, such as soaps, perfumes, oral solutions, and cosmetics (Cosentino, S. *et al*., 1999, Schulz, H. *et al*., 2003). Besides these applications, the TEO also exhibits antimicrobial activity, which is attributed to its main compounds, the phenolic monoterpenoids, thymol and carvacrol (Montes-Belmont, R. and Carvajal, M., 1998).

2.2.2 Oregano Essential Oil

Oregano is an herb that is part of the Lamiaceae family, which includes as primary representative species *Oregano vulgare* (Hercules, S., 2017). Oregano essential oil´s (OEO) main compounds are the phenolic isomers thymol and carvacrol, and, at a lower percentage, *p*-cymene and γ-terpinene, two of their precursors monoterpenes (Kokkini, S. *et al*., 1997). After researching several Greek oregano species, gathered from various geographic locations,

suggest that despite the variation in the concentrations of the primary compounds within different species, their combined levels can account for up to 90% of the overall oil composition (Kokkini, S. *et al*., 1997). Since ancient times, oregano has been used as a common spice and as essential oil is known for having antimicrobial and antispasmodic properties (Nostro, A. *et al*., 2004). In culinary and alcoholic beverage preparations, oregano are frequently employed as aromatics (Aligiannis, N. *et al*., 2001). Due to its high thymol and carvacrol content, oregano essential oil has the strongest antioxidant capacity and has notable benefits in inhibiting fat oxidation (McKay, D.L. and Blumberg, J.B., 2006). Though, owing to its potent fragrance, which adversely affects the food's organoleptic features, the use of such essential oil as a food preservative is somewhat restricted (Lambert, R. *et al*., 2001). In addition to these applications, several highly bioactive chemicals with acaricidal, insecticidal (Cetin, H. *et al*., 2009), and perhaps antibacterial properties have been found in oregano essential oils, against bacteria that cause food poisoning and food spoilage (Mith, H. *et al*., 2014, Nabavi, S.M. *et al*., 2015). This antimicrobial potential of OEO is related with the phenolic monoterpenoids compounds, thymol and carvacrol (Baratta, M.T. *et al*., 1998, Force, M. *et al*., 2000, Manohar, V. *et al*., 2001, Marino, M. *et al*., 2001, Mockute, D. *et al*., 2003, Stiles, J.C. *et al*., 1995).

2.2.3 Eucalypt Essential Oil

Eucalyptus is a plant that belongs to Myrtaceae family (Tyagi, A.K. and Malik, A., 2011). Regarding to Eucalyptus essential oil (EEO) composition, this includes monoterpenes, such as, *p*-cymene, α-pinene, β-pinene and D-limonene, monoterpenoids (oxygenated monoterpenes), as is the case 1,8-cineole (eucalyptol), linalool and sesquiterpenoids (oxygenated sesquiterpenes) (Broker, M. and Kleinig, D., 2006, Dhakad, A.K. *et al*., 2018). Among these compounds, 1,8-cineole stands out as the main compound, with a range of percentages 44-84 % (Limam, H. *et al*., 2020). EEOs are gaining popularity in a wide range of industries, including the alternative medicine, pharmaceutical, cosmetic, perfume, and culinary industries (Goldbeck, J.C. *et al*., 2014).The antimicrobial action associated to EEO is due to certain compounds such as, 1,8-cineole, α-pinene, β-pinene and limonene (Raju, G. and Maridas, M., 2011). Instead of the concentration of a unique compound, antimicrobial activity of this oils was shown to be related to the synergistic interactions of major and minor constituents (Posadzki, P. *et al*., 2012).

2.2.4 Mint Essential Oil

Mint, the genus *Mentha,* is an herb that belongs to Lamiaceae family. The mint essential oils (MEOs) are mainly made up of aromatic monoterpenoids, with menthol (20-60%) and mentone (5-35%) standing out as the major components, and followed by, depending on

species, isomenthone, limonene, β-caryophyllene and β-pinene (Kalemba, D. and Synowiec, A., 2019, Stringaro, A. *et al*., 2018). The two most significant mint species in terms of production and trade are peppermint (*M. x piperita*), and corn mint (*M. canadensis*, *M. arvensis* or Japanese mint) (Singh, P. and Pandey, A.K., 2018, Wongpornchai, S. *et al*., 2006). Corn mint is used for oils production (Naeem, M. *et al*., 2017), peppermint is used too for essential oils mainly due to its major compounds, menthol and menthone (Mimica-Dukić, N. *et al*., 2003). The terpenoids and the aromatic compounds, due to their many benefits, have been applied to several domains, in food, such as flavouring agents, pharmaceuticals, in medicine preparations, cosmetics and perfumery industry (Mahendran, G. and Rahman, L.U., 2020, Singh, P. and Pandey, A.K., 2018). In addition to these applications, this oil, as well as the previous sections, also has antimicrobial activity, which is owed to the presence of bioactive molecules, such as menthol and menthone, however, a higher content of menthol has been revealed to be more efficient and active than menthone (Anwar, F. *et al*., 2019, Kalemba, D. and Synowiec, A., 2019).

2.2.5 Lemon Essential Oil

Citrus limon, also referred as lemon, is a tree from Rutaceae family (Klimek-Szczykutowicz, M. *et al*., 2020). The barks of citrus fruits are de primary source of citrus EOs (Mahato, N. *et al*., 2019). The lemon essential oil (LEO) presents as major components, monoterpenoids, particularly, D-limonene (Abad-García, B. *et al*., 2012). Of these, the quantity-dominant compounds are, usually, limonene (69.9%), β-pinene (11.2%), γ-terpinene (8.21%), E-citral (geranial, 2.9%), neral (Z-citral, 1.5%) and linalool (1.41%) (González-Molina, E. *et al*., 2010, Kaskoos, R.A., 2019, Russo, M. *et al*., 2015). Due to its rich chemical constitution, this oil is present in some sectors, such as cosmetic and food industries (Abad-García, B. *et al*., 2012, García-Salas, P. *et al*., 2013, Russo, M. *et al*., 2015). Concerning antimicrobial activity of this EO, although limonene be the major compound in LEO, there is no correlation between the antibacterial action of these oil and the amount of limonene, so it is likely that a synergistic effect among major and minor components are responsible for the antimicrobial effect of this oil (Ambrosio, C.M.S. *et al*., 2019, Djabou, N. *et al*., 2013, Raspo, M.A. *et al*., 2020).

2.2.6 Tea Three Essential Oil

The tea three belongs to *Melaleuca* genus from the Myrtaceae family, and the brand name used to sell the essential oil obtained from this genus is Tea three oil (TTO). Besides this name, this EO is also called Australian tea three oil or Cajupat oil, since the plants that produce it are indigenous to that area (Yasin, M. *et al*., 2021). The most outstanding species at the level of TTO commercialization are *M. alternifolia, M. cajuputi, M. bracteata* and *M.*

quinquenervia (Hnawia, E. *et al*., 2012, Padalia, R.C. *et al*., 2015, Zhang, J. *et al*., 2021). This type of oil offers multiple benefits in the health and cosmetics sectors since it is a powerful bactericide, fungicide, and antiseptic. It is also highly safe and effective (Budhiraja, S.S. *et al*., 1999). The volatile oils of melaleuca species include a wide range of aromatic constituents (Trilles, B.L. *et al*., 2006), which includes monoterpenoids such as terpinene-4-ol, p-cymene, α-pinene and α-terpinene (Yasin, M. *et al*., 2021). Terpinene-4-ol (40%), γ-terpinene (23%) and α-terpinene (10%) make up the majority of this oil (Brophy, J.J. *et al*., 1989, Santos, R.C.V. *et al*., 2014). In addition to the compounds already mentioned, TTO also has limonene in its constitution (Brophy, J.J. *et al*., 1989). Regarding antimicrobial activity of TTO, this is associated to the major compound terpinene-4-ol which is the main antibacterial compound present in this oil (Carson, C.F. *et al*., 2006, Santos, R.C.V. *et al*., 2014). The antimicrobial characteristics of this oil are related to the impairment of respiration and the leakage of bacterial cell membrane components (Yasin, M. *et al*., 2021).

2.3 Antimicrobial properties of Essential oils against Gram-positive and Gram-negative bacteria

The antibacterial action of EOs is unlikely to be due to a single mechanism; multiple locations in a cell are likely to be targeted (Carson, C.F. and Riley, T.V., 1995). It is not easy to anticipate an organism sensitivity to a given EO, since it differs from strain to strain (Burt, S., 2004). Nevertheless, Gram-negative bacteria are known to be, typically, more resistant than Gram-positive bacteria (Trombetta, D. *et al*., 2005). This happens as a result of the presence Gram-negative bacteria's outer membrane which are constitute by lipopolysaccharides (LPS). The outer membrane functions as a barrier against macromolecules and hydrophobic substances. This increases Gram-negative bacteria's resistance to the predominantly hydrophobic antibacterial constituents of EOs (Nikaido, H., 2003).

The Gram-positive bacteria are surrounded by a strong peptidoglycan wall that is insufficiently dense to resist tiny antibacterial compounds, hence permitting access to the cell membrane (Hyldgaard, M. *et al*., 2012, Zinoviadou, K.G. *et al*., 2009). In addition, the lipophilic ends of lipoteichoic acid found in Gram-positive bacteria's cell membranes may facilitate the entry of hydrophobic EO compounds (Cox, S. *et al*., 2000). This hydrophobic nature enables EOs to disrupt the lipids of bacterial cell membranes in Gram-positive bacteria and, to a lesser degree, in Gram-negative bacteria (Knobloch, K. *et al*., 1986, Sikkema, J. *et al*., 1994) and enabling cellular material and ions to escape (Carson, C.F. *et al*., 2002, Helander, I.M. *et al*., 1998). It may not always imply apoptosis since certain cell permeability is tolerated, but severe leakage or loss of critical elements might result in death (Denyer, S., 1991).

2.3.1 Mechanism of action of essential oils

Multiple mechanisms of action (MOAs) have been hypothesized for essential oils' antimicrobial effect, which would include modifying the fatty acid profile of the cell membrane, harming the cytoplasmic membrane, and decreasing the protonmotive force (PMF) (**Figure 13**) (Di Pasqua, R. *et al.*, 2007, Oussalah, M. *et al.*, 2006). Several studies have linked the MOAs of essential oils to their capacity to permeate bacterial cell outer membranes and cytoplasmic membranes, hence destroying the cellular structures, making vulnerable to the essential oils in the vicinity (Burt, S., 2004, Calo, J.R. *et al.*, 2015, Hyldgaard, M. *et al.*, 2012, Swamy, M.K. *et al.*, 2016). Whenever bacteria are exposed to several essential oils, the antimicrobial MOAs of essential oils may include diverse activities at the cell outer membrane and inside the cytoplasm (Rao, J. *et al.*, 2019). The outer membrane is nevertheless able to retain its fluidity, under some conditions, due to self-defense mechanisms, which include modifying the degree of fatty acids saturation, the carbon chain size, the ramification position, the cis/trans isomerization, and the conversion of unsaturated fatty acids (UFAs) into cyclopropanes in the cellular membrane (Di Pasqua, R. *et al.*, 2006, Siroli, L. *et al.*, 2015).

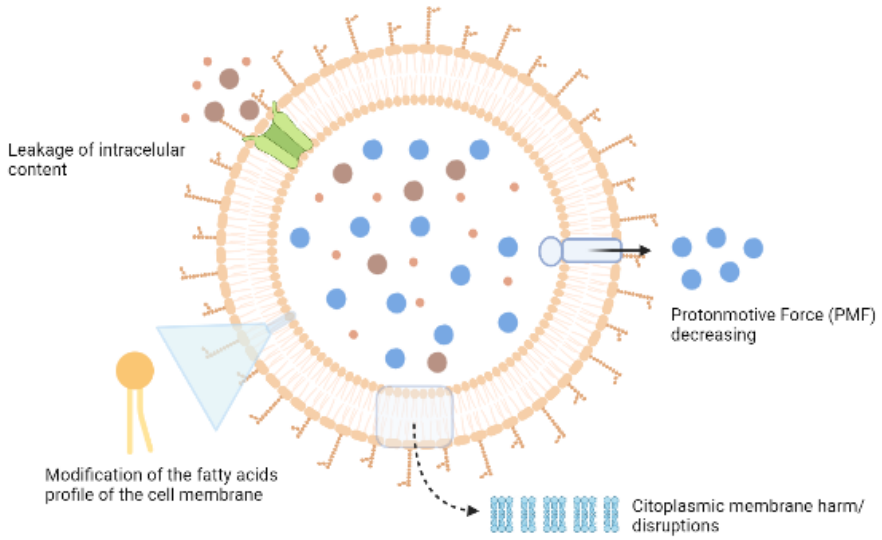


Figure 13: Mechanisms of action of essential oils in the bacterial cells.

The Antimicrobial action of terpenes has been related to their ability/skill to suppress respiration and other energy-dependent mechanisms at the level of the cell membrane (Griffin, S.G. *et al.*, 1999). Nevertheless, when employed as a single component, terpenes' antibacterial action is ineffective (Hyldgaard, M. *et al.*, 2012).

Regarding terpenoids, the MOA is largely attributable to a change in the fatty acid content of bacteria cellular membrane, leading to changes in the membrane permeability and leaking of intracellular components (Trombetta, D. *et al*., 2005).

Many compounds of EOs, especially phenols, seem to have specific MOAs, working as a proton exchanger. Thus, decreasing the pH gradient throughout the cell membranes, particularly when their penetration is facilitated by other components. Eventually, the collapse of PMF and the depletion of adenosine triphosphate (ATP) culminate in apoptosis (Ultee, A. *et al*., 2002). Subsequently, there is iron loss and intracellular content leakage (Helander, I.M. *et al*., 1998, Rhayour, K. *et al*., 2003). Phenols, similarly to terpenoids, are known for causing structural and functional harm to bacteria' cytoplasmic membranes (Lambert, R. *et al*., 2001, Oussalah, M. *et al*., 2006). Enzymes inhibition, including ATPase, histidine decarboxylase, amylase, and protease, is promoted by the hydroxyl group present in these compounds struture. Due to perturbed cellular respiration, the inhibition of ATPase may be crucial for cell death (Swamy, M.K. *et al*., 2016).

2.3.1.1 Terpenes

Large-scale experiments using limonene, α-pinene, β-pinene, and α-terpinene, which exhibit minimal or nonexistent antibacterial activity, suggest that terpenes do not have substantial antimicrobial activity (Dorman, H.J. and Deans, S.G., 2000). Another important monoterpene is *p*-cymene, one of the main elements present in some EOs, such as thyme, at high concentrations, has no antibacterial action when used alone (Bagamboula, C.F. *et al*., 2004), since in its structure the benzene ring does not have a hydroxyl group attached (Bagamboula, C.F. *et al*., 2004, Carson, C.F. and Riley, T.V., 1995), but it has the ability to improve/enhance/increase the effect of some components, such as carvacrol (Ultee, A. *et al*., 2002). *p*-cymene has a strong affinity for membranes and induces swelling, and this helps the entry of carvacrol into the cell, but it does not impact membrane permeability. It does lower the enthalpy and melting point of the membranes (Cristani, M. *et al*., 2007). Although *p*-cymene has a minimal impact on cell protein production, its action on membrane potential can influence cell motility (Burt, S.A. *et al*., 2007).

2.3.1.2 Terpenoids

Terpenoids are a large class of antimicrobial compounds that are effective against a wide range of microorganisms (Dorman, H.J. and Deans, S.G., 2000). Among the existing terpenoids, the following stand out for their antimicrobial capacity: 1,8-cineole, linalool, camphor, geranyl acetate, neryl acetate, geraniol, nerol, terpinen-4-ol, and cinnamaldehyde

(Rao, J. *et al*., 2019). The functional groups contained in terpenoids are connected to their antibacterial activities (Tahlan, V., 2014). It has been discovered that the presence of delocalized electrons and a hydroxyl group are required for antibacterial activity in phenolic terpenoids (Hyldgaard, M. [*et al*.], 2012).

Terpenoids and terpenes seem to have phospholipid bilayer as their central target due to their lipophilic characteristics. Inhibition of electron transport, protein translocation, and enzyme-dependent processes, such as phosphorylation, are therefore the main repercussions (Dorman, H.J. and Deans, S.G., 2000, Laciar, A. *et al*., 2009). As a result of Gram-negative bacteria's outer membrane, which is made up of hydrophilic lipopolysaccharides, this creates a barrier that allows them to tolerate hydrophobic antimicrobial agents more effectively. Therefore, Gram-positive bacteria, which lack this barrier, would be more vulnerable to EOs containing terpenoids (Dung, N.T. *et al*., 2008, Ennajar, M. *et al*., 2009, Laciar, A. *et al*., 2009, Mkaddem, M. *et al*., 2009).

2.3.1.3 Phenols

As it has been revealed in several studies, EOs with significant amounts of phenols show high antimicrobial activity (Bassolé, I.H. and Juliani, H.R., 2012, Cosentino, S. *et al*., 1999, Dorman, H.J. and Deans, S.G., 2000, Rota, M.C. *et al*., 2008).This antimicrobial activity of phenols seems to be related to their hydroxyl group, which is amplified by the benzene ring. Crucial to the antimicrobial action of EOs is the existence of a free hydroxyl group in phenols, as is the case with carvacrol, as well as an electron delocalization system (Ben Arfa, A. *et al*., 2006, Ultee, A. *et al*., 2002). The lack of a free hydroxyl group to interchange their proton limits their ability to change the stability of bacteria´s cell membrane (Rao, J. *et al*., 2019). Furthermore another factor that seems to contribute to the antimicrobial efficacy of these compounds is the relative position of the hydroxyl group in the phenolic ring (Rao, J. *et al*., 2019).

Carvacrol and thymol, phenolic terpenoids, can disaggregate the Gram-negative cells' outer membrane, thereby releasing lipopolysaccharides and boosting the cytoplasmic membrane's permeability to ATP. Carvacrol is thought to enhance membrane permeability by pushing the fatty acid chains in the phospholipids apart, forming channels across the membrane (Ultee, A., 2000).

3 Goals

3.1 General goal

As several products in the dermocosmetic area have limited antiseptic properties, new product ranges have appeared that take advantage of essential oils, but the results are still not effective. Thus, the need arises for new products that are more effective at the antiseptic level and at the same time of natural origin, safeguarding the environment.

Therefore, the general goal of this work was the production and stabilization of essential oils based emulsions, through natural components and the determination of its antibacterial capacity on Gram-positive and Gram-negative bacteria, a future application in the cosmetic area.

4 Materials and Methods

4.1 Essential oils in this study

In this study the antimicrobial activity of six essential oils, Thymus (*Thymus hyemalis*), Oregano (*Origanum vulgare*), Eucalyptus (*Eucalyptus globulus*), Mint (*Mentha arvensis* or *Mentha canadensis*), Lemon (Citrus lemon Peel oil), and Thea Tree (*Melaleuca alternifolia*), purchased in Gran Velada, was assessed.

4.2 Surfactants under study

Based on the most commonly used surfactants in the cosmetic field, the following surfactants were chosen for this work: tween 20 and tween 80 (Maccelli, A. *et al*., 2020), purchased in PanReac AppliChem, and, lauryl, decyl and coco glucosides (Fiume, M.M. *et al*., 2013), and coco betaine (cocamidopropyl betaine) (Burnett, C.L. *et al*., 2012), purchased in Gran Velada. In **Figure 14** is represented the chemical structure of each surfactant selected.

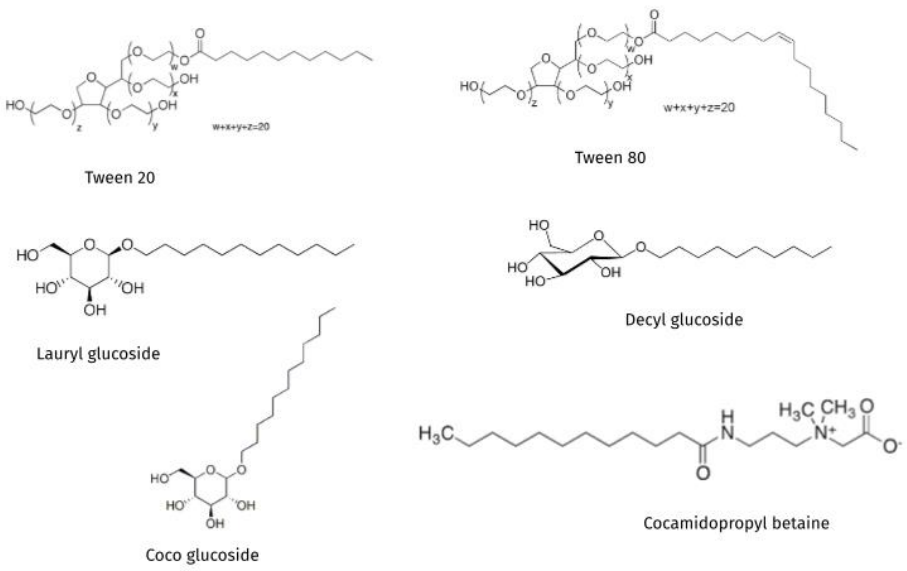


Figure 14: Chemical structure of surfactants used in this study.

4.3 Preparation of surfactant solutions

For each surfactant under study, five solutions were prepared with percent mass (% m/m) of 0.1%, 1%, 2.5%, 5%, and 10%.

Upon preparation of surfactant solutions, each of these was homogenized at 1500 rotations per minute (rpm) on the stir plate, until complete dissolution of the surfactants was achieved.

The lauryl glucoside was the only surfactant for which a 10% solution was not prepared, because this surfactant does not dissolve well at 10%, so the maximum concentration prepared was 5%.

4.4 Determination of surface tension

The determination of the surface tensions of the samples was performed in a force tensiometer of the Attension brand, model *Sigma 700/701*, using a Wilhelmy plate. As experimental conditions, a penetration height of 10 mm of the Wilhelmy plate in the sample was defined, and the tests were performed at a temperature of 25°C. All tests were performed in triplicate. The means and standard deviations for each test are presented.

4.5 Preparation of polymers solutions

As mentioned above, the polymers chosen for this work were xanthan gum and carboxymethyl cellulose 700.000 (CMC 700.000). These polymers solutions were prepared with concentrations of 3% and 5% for CMC 700.000 and XG, respectively, and then these were added in the preparation of the final emulsions.

4.6 Preparation of emulsions using three different agitation methods

The influence of the stirring method on the formation of emulsions was evaluated. The emulsions were prepared at room temperature, for 5 minutes and at a maximum speed of 1500 rpm, with different stirring methods: vortex, magnetic and mechanical stirring. Thus, the variables temperature time and maximum speed were kept constant, with a view to making the future large-scale production process as simple and inexpensive as possible.

To evaluate which surfactant is best among those described to obtain more stable emulsified layers, emulsions were produced using coconut oil and sunflower oil. Since coconut oil solidifies at lower temperatures it has a very similar hue to the emulsion and thus the emulsified layers were easily confused with the solidified oil, which led to erroneous results. With sunflower oil this difficulty is overcome as it remains liquid over a wide temperature range and thus colorless, allowing the emulsified layer to be visible after the emulsion production process, leading to reliable results.

Each emulsion, with each type of surfactant, was produced in triplicate and the length of the emulsified layer was measured at 0 hours and after 24 hours and 48 hours, then the average of the length of the emulsified layer was calculated and the emulsification index, a parameter described below, was determined.

The final emulsions were produced using EOs and EOs mixes. The total concentration of the essential oils is 50% of the emulsion. In mixtures of two EOs this proportion becomes 25% of each and in mixes of three EOs 16.7% of each.

4.6.1 Vortex

For the preparation of the emulsions 1.5 g of surfactant solution of each concentration mentioned above plus 1.5 g of oil (coconut or sunflower) was weighed into an Eppendorf tube. Then were placed in the vortex (Multi reax-heidolph) at a speed of 1500 rpm for 5 minutes. The **Figure 15** represents this procedure.

Figure 15: Procedure of emulsion preparation using vortex.

4.6.2 Magnetic stirring

The preparation of the emulsions for this stirring method was started by adding 5 g of surfactant solution of each concentration already mentioned, followed by 5 g of oil (coconut or sunflower) to 20 mL flasks. Magnetic stirrers were then added to each flask to facilitate the homogenization of the two phases, and then each flask was placed on the stirring plate (Velp® Scientifica) at a speed of 1500 rpm for 5 minutes. This is shown in **Figure 16**.

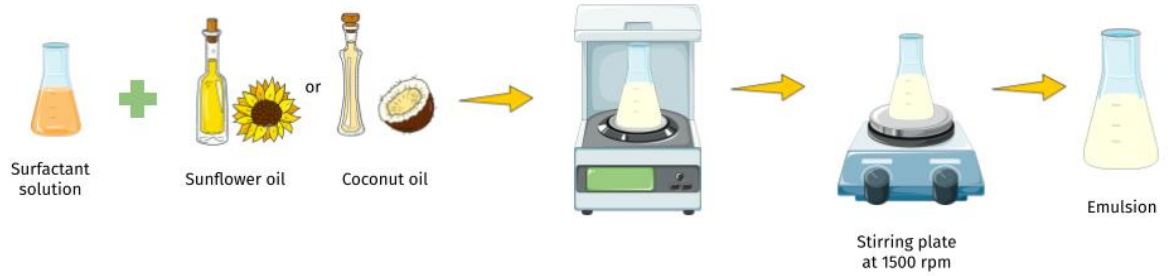


Figure 16: Schematic representation of emulsion production using stirring plate.

4.6.3 Mechanical stirring

For the preparation of the emulsions, 25 g of surfactant solution of each concentration already mentioned, plus 25 g of oil (coconut or sunflower) were added to a beaker. Then the two phases were homogenized using a mechanical stirrer (Velp® Scientifica) at a speed of 1500 rpm for 5 minutes, as can be seen in **Figure 17.**

Figure 17: Process of emulsion production using mechanical stirring.

4.7 Emulsion production using EOs

The final, with EOs, emulsions were prepared by adding 0.75 g of a 2.5% coco glucoside solution with 0.75 g of a 5% XG solution or a 3% CMC 700.000 solution, followed by the addition of 1.5 g of EO or EOs mixture. The emulsification process was carried out on the stir plate at 1500 rpm for 20 minutes. The stirring method, the surfactant solution and the polymer solutions chosen to prepare final emulsions, were the ones that allowed the formation of bigger and more stable emulsified layers over time. The **Figure 18** shows the procedure of the final emulsions prodution.

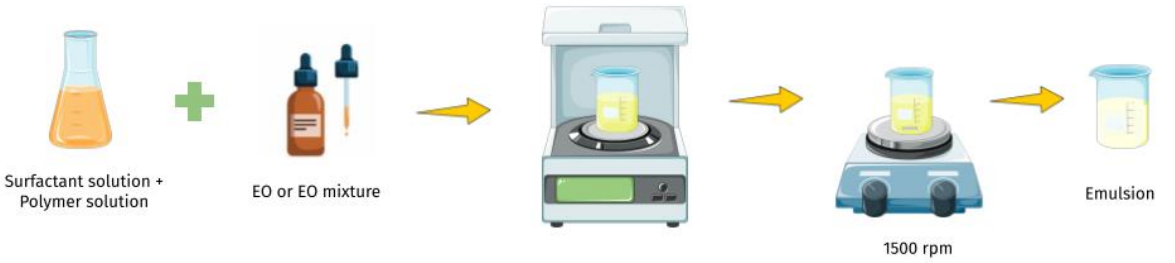


Figure 18: The final emulsion production using the essential oils and magnetic stirring.

4.8 Determination of Emulsification Index

The Emulsification Index (EI) is defined as the ratio among the length of emulsified layer (cm) and the entire length of the solution (cm) (Sumiardi, A. *et al*., 2018). The **Figure 19** shows a schematic representation of the parameters measured for the determination of the emulsification index.

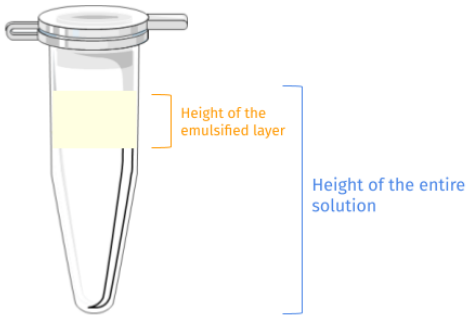


Figure 19: Scheme of the measured parameters that are used to determinate the emulsification index.

The **Equation 2** shows the formula to calculate the EI (%).

$$\mathrm{EI} = \frac{\text{Heigh of the emulsified layer (cm)}}{\text{Heigh of the entire solution (cm)}} x\ 100 \qquad \textbf{Equation 2}$$

4.9 Bacterial strains

For this work, two Gram-positive and two Gram-negative bacteria were used in order to evaluate the effectiveness of the antimicrobial activity of the compounds under study. The bacterial strains from the American Type Culture Collection (ATCC), including Gram-negative *Escherichia coli* ATCC 8739, and Gram-positive *Staphylococcus aureus* ATCC 6538, and *Enterococcus faecalis* ATCC 29212, were used. Besides these bacterial strains, the Gram-negative bacterial *Pseudomonas aeruginosa* DM from the Faculty of Pharmacy collection, was also studied. All these strains were preserved at -80°C in the Microbiology Laboratory of the Pharmacy Faculty of the University of Coimbra.

The choice of this model was based on the use of the EOs and emulsions under study in the cosmetic area. This was done by considering the microorganisms that are usually present where the cosmetic products act, in order to test the inhibition potential of the antimicrobial compounds present in the EOs. *Staphylococcus spp.* are part of the skin microbiota. *Enterococcus spp*, are found in the mouth. *E. coli* is not so common on the skin, but like the previous two Gram-positive, is used in the present study as model of Gram-negative bacteria that can be

encountered on the skin, including the moist parts of the human body. Although *P. aeruginosa* is not commonly present in the natural microbiota of the skin, it was tested because is a bacteria that shows often an extended antimicrobial resistance profile, quite different from *E. coli*. So, if *P. aeruginosa* is inhibited, other Gram-negative bacteria present in the skin flora, with weaker resistance profiles, will also be.

For the assays performed, the bacterial strains were thawed and then cultured into a petri dish containing Luria-Bertani (LB) (HiMedia Laboratories) agar (VWR Chemicals). The same Petri dishes were then incubated at 37°C for 18 to 24 hours in a *Binder FD240* incubator.

4.10 Antimicrobial activity assessment

4.10.1 Determination of Minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) is set as "the minimum concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation" (Andrews, J.M., 2001). The MIC was determined by performing the microdilution assay that will be described below.

4.10.1.1 Microdilution Assay for EOs

This bacterial susceptibility test was performed in 96-well microtiter plates according to CLSI standards (Humphries, R.M. *et al*., 2018).

Initially, for the microdilution assay, it was prepared a suspension of each bacterial strain under study, containing 5 mL of sterile Milli Q water and well-isolated bacterial colonies. The bacterial strains were incubated overnight at 37°C in LB agar plates. Then the turbidity standard for each suspension was achieved comparing to the 0.5 Mc Farland turbidity standard, which is equivalent to 5×10^5 colony forming units CFU/ml (Humphries, R.M. *et al*., 2018).

During the test, several concentrations of essential oils were assessed, starting with the concentration of 1% of each oil until it reached the concentration at which no growth was visible.

The assay was carried out by adding 100 µL of Muller-Hinton (MH) broth (HiMedia Laboratories) to each well of the plate. Then sterile Milli Q water, the essential oil under study and 2 microliters (1%) of dimethyl sulfoxide (DMSO) were added, in the first well of the row of the plate. The microdilution procedure is the same for all oils, with only the volume of water and oil added varying depending on the concentration of oil required in the first well.

Thereafter, the two-fold dilutions were performed consecutively by taking 100 µL from the first well, of a row, to the second well and so on. Finally, 10 µL of bacterial inoculum are added to each well. The assay was performed in a *Faster BH-EN2005* laminar flow camera.

The growth controls were as follows: Growth Control 1 with 100 µL of MH broth, water volume used in the assay and 10 microliters of bacterial inoculum. The Growth Control 2 with 100 µL of MH broth, water volume used in the assay, 2 µL of DMSO and 10 microliters of bacterial inoculum (suspension).

The sterility controls were as follows: Control 1: 100 µL of medium only, Control 2: 100 µL of medium and 2 µL of DMSO, Control 3: 100 µL of medium and Milli Q water, other controls: 100 µL of medium, each type of oil and 2 µL of DMSO.

Finally, the 96-well microtiter plates were covered up and incubated at 37°C for 18 to 24 hours. Initially, it was noticed that all wells, even the sterility controls, showed turbidity and precipitate, likely due to the action of essential oils with the proteins in the medium, which avoid the differentiation of wells that showed growth and those that did not. To understand whether the turbidity was due to chemical reaction (with no bacteria) or bacterial growth, a loop of each well was stroked on LB agar and incubate at 37°C overnight.

After this period the Petri dishes were observed, and the MIC was determined. The essential oil concentration that corresponded to bacterial growth inhibition of about 90%, was considered the MIC of the essential oil under discussion. For each EO the assay was performed in triplicate and reproduce in, at least, two independent assays.

The **Figure 20** shows a schematic overview of the procedure performed to determine the MICs of the EOs.

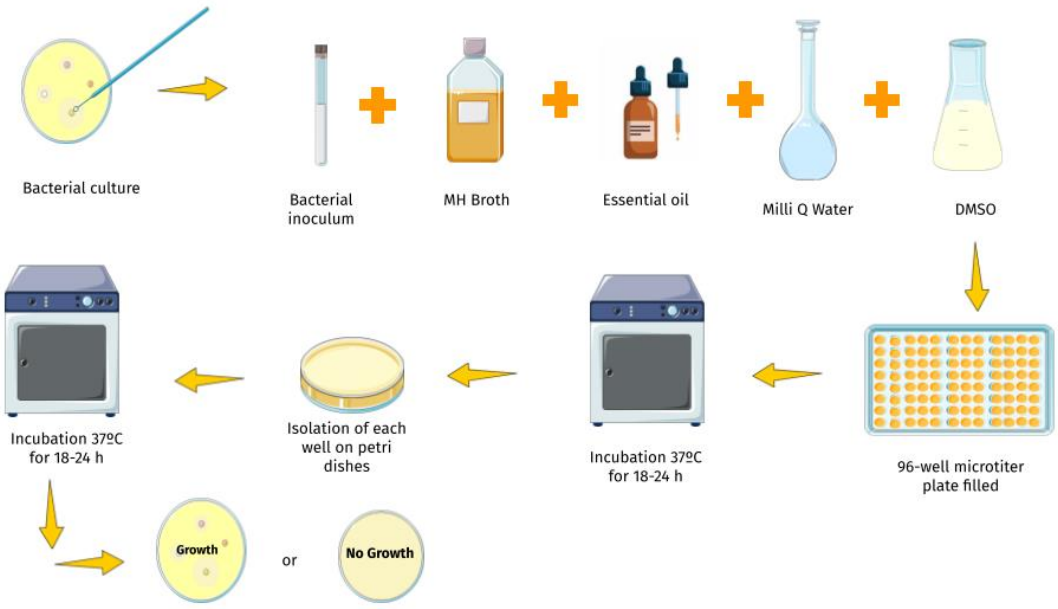


Figure 20: Schematic representation of the microdilution assay procedure for determining the MICs of EOs.

4.10.1.2 Microdilution assay for Emulsions

Bacterial suspensions were initially prepared under the conditions previously described. After that, 100 µL of MH broth were added to each well of the plate, followed by 100 µL of emulsion in the first well of each row. Then, after homogenization of the first well, two-fold dilutions were performed by taking 100 µL from the first well of a row to the second well and so on until the desired concentration was reached. After serial dilution, 100 µL of inoculum solution were added to each well.

Regarding the growth control only 100 µL of MH broth and 100 µL of bacterial inoculum solution were added.

Concerning sterility controls, one of the controls had only 100 µL of MH broth and the other controls: 100 µL of MH broth and 100 µL of each emulsion.

The assay was performed in the laminar flow camera, and after that the 96-well microtiter plate was incubated at 37°C for a period of 18 to 24 hours. The observation of the results was performed after inoculation of a loop of each well suspension and incubation as previously described (see section **Microdilution assay for EOs**).

Finally, after this period the Petri dishes were analyzed, and the MIC was determined. The emulsion concentration that corresponded to bacterial growth inhibition of about 90%,

was considered the MIC of the emulsion under study. For each emulsion the assay was performed in triplicate and reproduce in, at least, two independent assays.

Considering this, below is the **Figure 21** for the microdilution test procedure to determine the MICs of the emulsions.

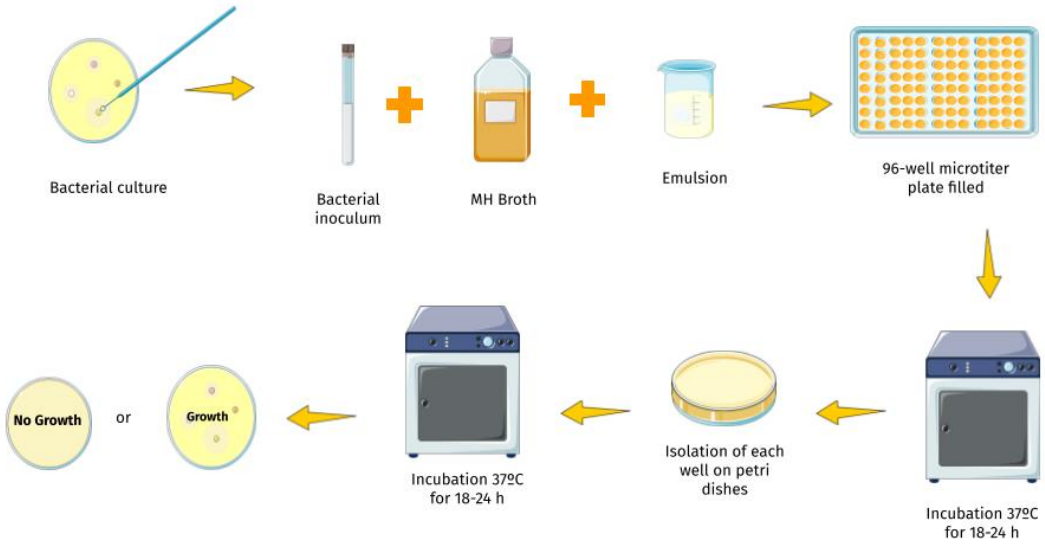


Figure 21: An overview of the microdilution assay procedure for determining the MICs of emulsions.

5 Results and Discussion

5.1 Determination of CMC and the lowest surface tension of surfactants

The CMC of the six surfactants under study, as well as the lowest surface tension value, were determined through tensiometry tests, where the decrease in surface tension (γ) values was evaluated with increasing surfactant concentration in water. The **Figure 22** shows the graphs of the CMC determination, and in **Table 1** all these CMC values are presented as well as the smallest γ, of the surfactants individually. The CMC was determined in triplicate for all surfactants, and this parameter was measured by fitting the straight lines in the two concentration regions of the graphs and by the intersection of these straight lines.

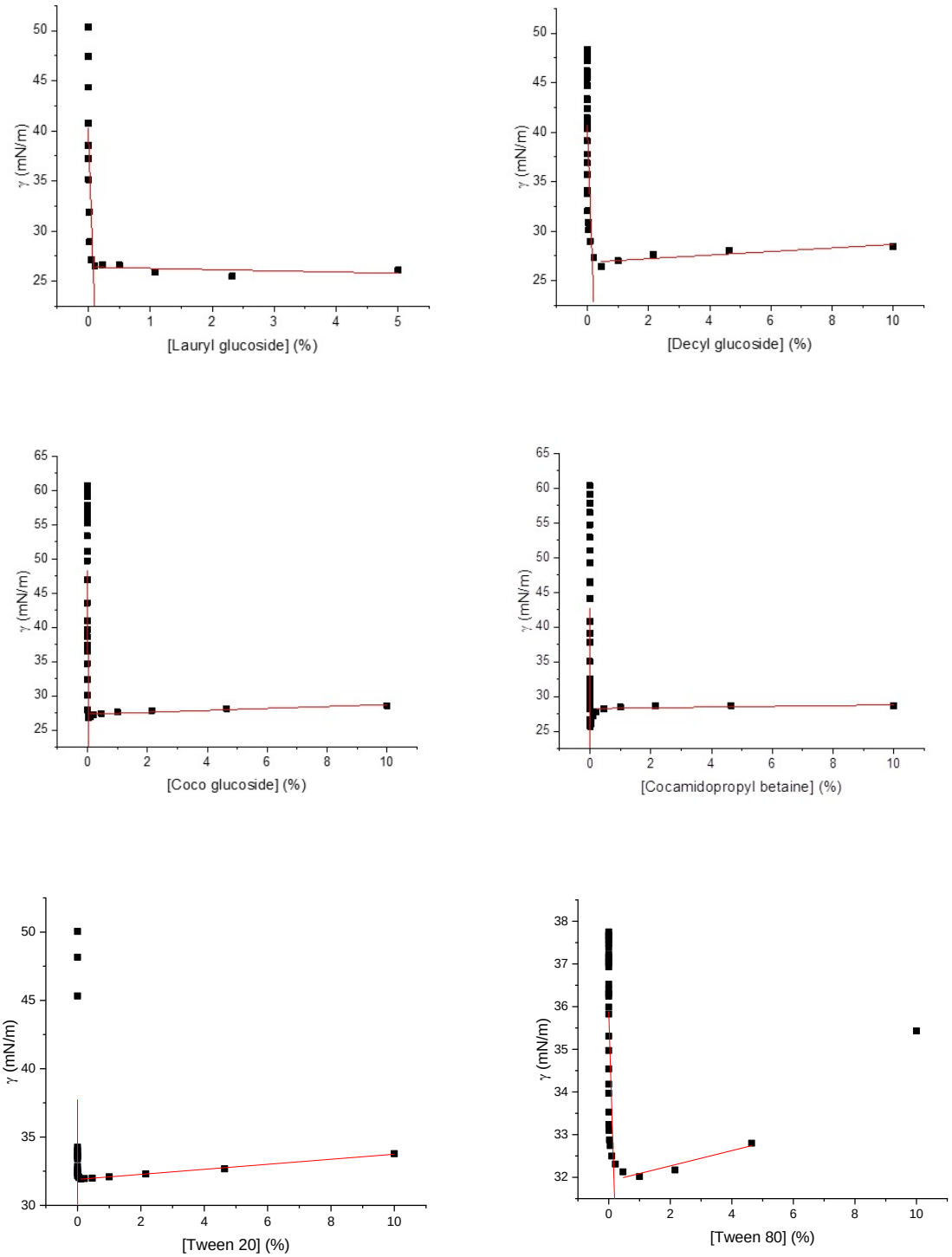


Figure 22: Graphic representation of the results obtained from the determination of CMC for de six surfactants under study.

Table 1: CMC values with respective standard deviations and the lowest γ for each surfactant under study.

Surfactant	CMC (%)	γCMC (mN/m)
Cocoamidopropyl betaine	0.0054 (±0.0010)	28.29 (±0.05)
Coco glucoside	0.0335 (±0.0028)	27.21 (±0.07)
Decyl glucoside	0.1678 (±0.0025)	26.93 (±0.08)
Lauryl glucoside	0.0081 (±0.0004)	26.53 (±0.21)
Tween 20	0.0047 (±0.0013)	31.51 (±0.61)
Tween 80	0.1758(±0.0055)	32.01 (±0.06)

The evaluation and determination of CMC and the lowest values is of utmost importance for the application of surfactants in cosmetic products. Surfactants with lower CMC values will always be preferred, since a smaller amount of them is needed to obtain microstructures, such as micelles, capable of emulsifying hydrophobic molecules, in this case oil.

Analyzing the CMC values determined, it is observed that cocamidopropyl betaine, lauryl glucoside, and tween 20 are the surfactants that present the lowest CMC values. In parallel, the surfactants that showed the best ability to lower the surface tension of water were the glucosides (decyl-, lauryl- and coco glucosides), since the lowest surface tension values were obtained with the 3 glucosides under study, although all tested surfactants are promising to the formation of emulsions.

Cocamidopropyl betaine, coco glucoside and lauryl glucoside are constituted by hydrophobic chains with a length of 12, 8-14 and 12-14 carbons (C), respectively. In the group of surfactants under study, these are the most hydrophobic, with the monomers of these surfactants having a higher capacity to adsorb at the water-air interface, filling it completely and leading the remaining monomers to self-aggregate, initiating the formation of micelles.

Tween 80 consists of C18 chains with an unsaturation between C9-C10 what makes this more hydrophobic than tween 20. That is, because it has such a large alkyl chain, when it is in contact with water, the monomers will move more quickly to the interface, to reduce the interaction of these chains with the water. At the interface, since they have larger chains, they will also occupy the surface much faster, leading to micelles starting to form in the water at lower concentrations.

A lower surface tension value will be important for the specific intended application of these surfactants: the formation of emulsions. Since the lower the water surface tension value, implemented by the presence of the surfactant, greater the adsorption capacity of the surfactant at the water-air interface, easier it is to promote the mixing of two immiscible components, (O/W, in this work), and thus to form a more stable emulsion.

Comparing the different CMC values of tween 20 and tween 80 in the literature, this parameter was in the range of 0.06% for tween 20 (Jan, a.K., 2017, Mahmood, M.E. and Al-Koofee, D.A., 2013), and for tween 80, this value was 0.02% (Jan, a.K., 2017, Mahmood, M.E. and Al-Koofee, D.A., 2013). Comparing the CMC values present in literature with those obtained in our measurements, it is possible to conclude that CMC of tween 80 was bigger (0.1758%) than what was measured for tween 20 (0.0047%). This variation may be due to differences in suppliers or possibly the definition of the intersection of the lines that allow obtaining the CMC value. Throughout this chapter, the discussion will be based on the CMC values of tween 20 and tween 80 present in the literature.

Regarding the obtained CMC values for each surfactant, as mentioned earlier these values are similar to those in the literature for some coco glucoside which is 0.05% (Chen, R.K.Z. and Wee, S.C., 2021). However, the CMC values obtained for lauryl,decyl and and for cocamidopropyl betaine are lower than the literature values of 0.09% (Gao, Y. *et al*., 2014), 0.6 - 0,7% (Lopez, O. *et al*., 2001, Shinoda, K. *et al*., 1961) and 0.03% (Basheva, E.S. *et al*., 2019), respectively, this may again be due to differences in the surfactant suppliers.

5.2 Evaluation of the emulsifying capacity of surfactants

As mentioned above, the magnetic stirring method was chosen for the preparation and production of the emulsions, since the vortex and the mechanical stirrer showed a relative discrepancy in the EIs at 24 and 48 hours and consequently also high standard deviations. This is associated with a lower stability of the emulsion over time, thus demonstrating that these two methods were not very effective in emulsions production.

The emulsions were then produced using sunflower oil to define, based on the EIs, which is the best surfactant and its concentration in order to obtain more stable emulsions. The EIs were determined by measuring the height of the emulsified layer after 24 hours and 48 hours, to evaluate the emulsifying properties of each surfactant. Moreover, it is important to note that each determined EI comes from the average calculation of the assays performed in triplicate.

The **Figure 23** shows the graphs of the EIs obtained for lauryl glucoside, decyl glucoside, coco glucoside, cocamidopropyl betaine, tween 20 and tween 80 at 0.1, 1, 2.5, 5 and 10%.

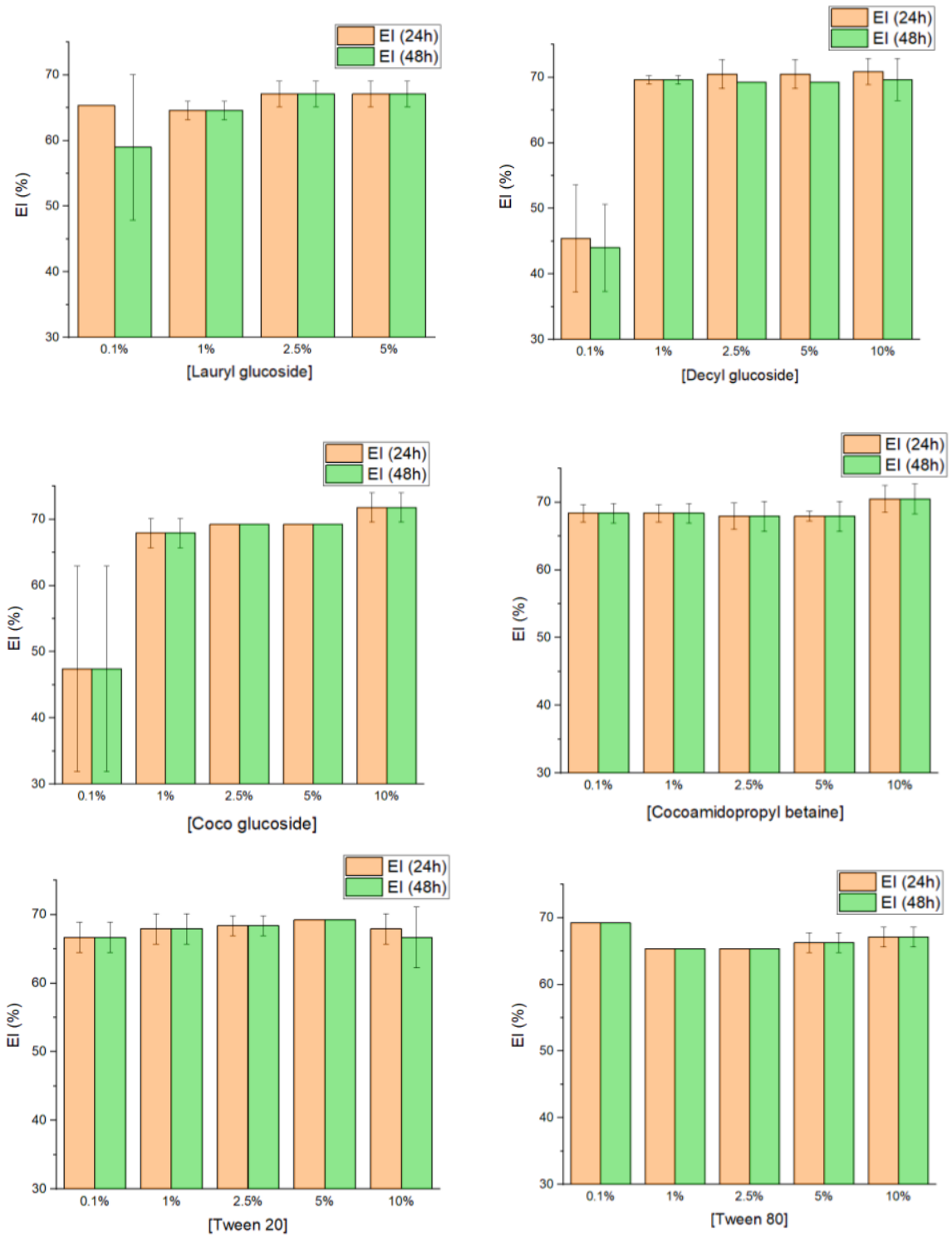


Figure 23: Graphical representation of the calculated EIs (%) at 24 and 48 hours of the emulsion produced with lauryl glucoside, decyl glucoside, coco glucoside, cocamidopropyl betaine, tween 20 and tween 80 (0.1, 1, 2.5, 5 and 10%) and sunflower oil by magnetic stirring method (1500 rpm).

Regarding the results present in the **Figure 23**, all of them have EI higher than 60% and relatively low standard deviations, with certain exceptions.

Comparing the results obtained for the different surfactants, at the 0.1% concentration, as mentioned above, the three glucosides form less unstable emulsions, while cocamidopropyl betaine and tweens at this concentration already show higher EIs and lower standard deviations, which is indicative of stable emulsions at this concentration.

It was found that the emulsion produced at this concentration of glucoside surfactants were unstable since the emulsified layer did not remain intact over time, which was corroborated with the visual analysis of these emulsions, particularly with the decyl and lauryl, verifying phase separation already at 24 hours and this separation increased after 48 hours.

According to table I, lauryl has the lowest CMC values and is the surfactant that contributed the most to the decrease in surface tension, which would be expected that at 0.1% this surfactant would already present a great ability for emulsification. However, despite all the conditions being met at this level to obtain stable emulsions, there are other equally important factors that seem to influence the stability of the emulsion that is formed, such as the constitution of sunflower oil itself, the interactions and number of interactions that are established between the oil molecules and the alkyl chain of surfactants, and problems at the level of homogenization.

Sunflower oil is rich in polyunsaturated lipids, being mainly composed of linoleic acid (C18:2) (Santos, A. *et al*., 2015). Therefore, as the alkyl chain of the constituents of oil is quite large, the number of possible interactions between them and the surfactants that can be established are also high. Sunflower oil has constituents with large chains, there will be more area available for the surfactant chains to adsorb and emulsify the oil.

Being 0.1% a low concentration of surfactant this may affect the total incorporation of the oil droplets by the surfactant molecules because fewer micelles will be formed and thus less capacity to compartmentalize the fat molecules inside. This may result, together with a possible inefficient homogenization, in oil molecules that are not sufficiently encompassed in the surfactant micelles and therefore not fully mixed with the aqueous phase. Thus, the oil being in contact with the aqueous phase, will lead to more unstable emulsions because it tends to escape from the hydrophilic environment, and being less dense than the aqueous phase, will migrate to the surface, contributing to phase separation.

As for the decyl and coco glucosides these are constituted by C8-10 and C12-14 chains, respectively and in addition they have CMC values, in the case of decyl greater than 0.1% and in the case of coco less than 0.1%. Comparing this information with the results obtained in **Figure 23**, it is possible to conclude again that the length of the alkyl chain is very important for the stabilization of the emulsion since coco glucoside despite containing a CMC below 0.1% as it has a small alkyl chain, similar to lauryl, this leads to unstable emulsions over time, once surfactants with small alkyl chains are less hydrophobic. The decyl besides the alkyl chain length being even less than the lauryl and coco glucosides, also has a CMC value higher than 0.1%, so the small alkyl chain combined with a CMC greater than 0.1%, makes this surfactant, at this concentration, is in an amount below the necessary to produce micelles and to stabilize oil molecules, thus leading to emulsions that are also unstable.

Like the results obtained in this work, also in the literature, in assays already performed, it was found that surfactant's hydrophobic portion determines the emulsifying capacity in general, as do its length and composition. The longer the alkyl chain length, the greater the stability of the emulsion created. With bigger alkyl chain length the surfactant solubility in the oil phase increase creating a very stable emulsion (Geetha, D. and Tyagi, R., 2012, Sukkary, M. *et al*., 2007).

Concerning the remaining surfactants, although, similarly to lauryl, cocamidopropyl betaine also presents only twelve carbons in the alkyl chain. This surfactant even at a concentration of 0.1% presents stable emulsions and with high EIs, which can probably be explained not only by the low value of CMC, around 0.005%, also associated with a great capacity to reduce surface tension, but also the fact that this is the only surfactant under study that is amphoteric or zwitterionic, which influences the interactions between this one and the oil due to its polar portion, because the polar groups being more ionic have the ability to break the ordered structure of the water molecules around the hydrocarbon chain of the surfactant monomers contributing to the free energy of micelle formation (Herrmann, K., 1966). So despite the alkyl chain being small compared to the length of the oil, and although the CMC is not much lower than that of lauryl, probably, for this particular surfactant, being amphoteric, this lowest value of CMC, a 0.1% surfactant concentration may be sufficient to form micelles that surround the oil droplets and allow an efficient incorporation of these droplets into the aqueous phase and consequently result in a more stable emulsion.

Finally, Tween 20 and Tween 80 also present stable emulsions with high IEs at all concentrations, including 0.1%. Comparing these two surfactants, they have very similar surface tensions, but the CMC values are different, with Tween 20 having a CMC value well

below that of Tween 80 and the CMC value of the last one being above 0.1%, which would be expected that with Tween 20 the emulsion formed would already be stable at 0.1% surfactant, but not with Tween 80. However, and although the CMC value is not the expected, evaluating the results of the graphics in the Figure 19, these results are in accordance with what is advocated in the literature in which the length of the alkyl chain is preponderant in stabilizing the emulsions that are formed and consequently in the parameters that depend on this, such as the CMC and surface tension. Thus tween 80 being more hydrophobic, C-18, than tween 20, C-12, presents a lower CMC value than the latter and therefore lower than 0.1% hence the values of EIs are higher for tween 80.

Thus, it is possible to conclude that the size of alkyl chains plays a major role in the ability to form and stabilize emulsions, since it affects essential parameters for understanding emulsion formation, such as CMC and surface tension values. Surfactants with low CMC values and a higher capacity to lower water surface tension will have a higher adsorption capacity at interfaces (water-air, water-oil), thus being more capable of forming the essential structures for emulsion formation.

As the goal is to use a surfactant in the lowest possible concentration and at the same time ensuring a fulfillment of the commitment of emulsions with high EIs levels but also stable over time, coco glucoside ended up being the surfactant chosen at 2.5%, since at this concentration, is ensured that there is enough surfactant to produce micelles to surround all oil droplets and consequently produce stable emulsions. Despite the minimal differences compared to cocamidopropyl betaine, coco glucoside has higher EIs than cocamidopropyl betaine and all the other surfactants at the 2.5% concentration, the minimum concentration chosen. Besides the EI of coco glucoside at 2.5% being high and equal for 24 and 48 hours, the standard deviation has a value of 0, which indicates that coco glucoside at 2.5% produces a stable emulsion over time.

With these emulsification studies, was possible the choice of the best surfactant and its concentration to be used in the production of the final emulsions, already containing essential oils.

5.3 Selected polymers for incorporation into the final emulsions containing EOs

Given the experience that the Science 351 has in developing emulsions for various areas, including cosmetics, it was decided with the research team to study the GX and CMC 700,000 because it was known that they were excellent polymers in terms of stabilization and

are also the most used for formulations in this area. Therefore, these two polymers were incorporated and studied in the final emulsions.

5.4 Determination and evaluation of EI of final emulsions with EOs

The final emulsions with all the ingredients (surfactant, polymers and essential oils) were produced and each of them containing 1.5 g of essential oils, 0.75g of 2.5% coco glucoside solution and 0.75g of polymer solution, in the case of the 3% CMC 700.000 solution and the 5% XG solution.

Three EOs were chosen to produce the final emulsions, based on the lowest MIC: oregano (O), thymol (T) and mint (M). In addition to producing emulsions with each of these oils, emulsions containing a mixture of two of these oils (T+O or M+O) and the three oils (T+M+O) were also produced, and the MIC of each of these emulsions be then determined. In **Figure 24** are presented the EI obtained for the emulsions produced with coco glucoside (2.5%), essential oils of oregano, thymus and mint, and CMC 700.00 and XG as polymers.

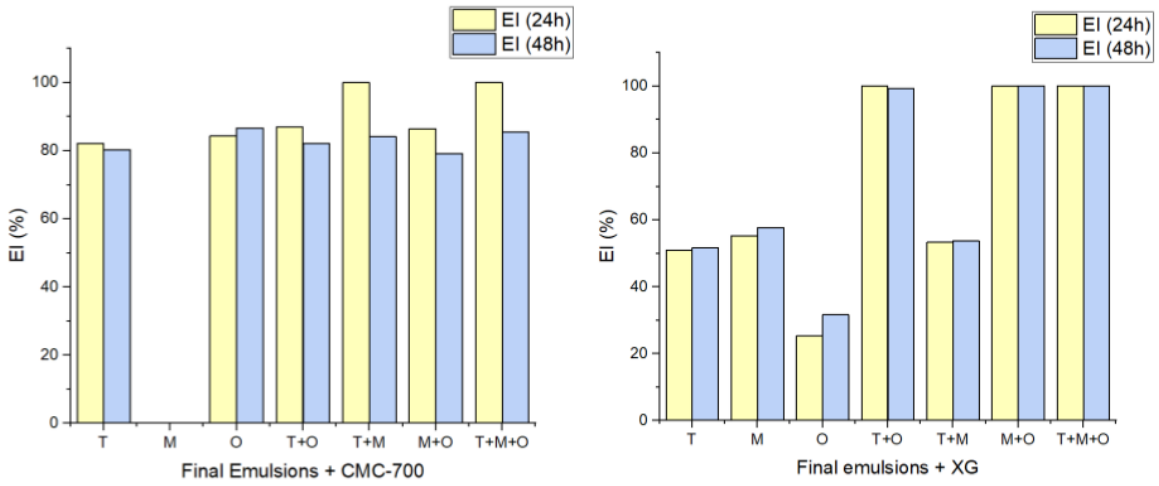


Figure 24: Graphical representation of the calculated EIs (%) at 24 and 48 hours of the final emulsion produced with essential oils of oregano (O), thyme (T) and mint (M), coco glucoside (2.5%), CMC 700.000 (3%) and XG (5%), by magnetic stirring method (1500 rpm), for 20 minutes, at 20°C.

Comparing the two graphs in the **Figure 24** regarding the action of the two polymers in the stabilization of emulsions, the highest EIs are achieved with CMC 700.000, except for the mint emulsion, which shows no emulsified layer. However, it is with xanthan gum that there is a greater stability of emulsions compared to CMC 700.000, since with XG the EIs determined after 24 and 48 hours remain practically unchanged.

In **Figure 24** it is also possible to see that in some cases, both with XG and CMC 700.000, the EIs increase from 24 to 48 hours, which may be due to the restructuring of the

structures formed between the oil and the surfactants or possibly to the difficulty in defining the emulsified layer, leading to some measurement errors.

Another factor with influence on the EIs obtained are the constituents of the EOs, since they can act as co-surfactants allowing better solubilization and consequently better stabilization of the emulsions. A study based on clove, thyme and oregano EOs showed that clove EO had a superior power to form stable emulsions compared to the other two oils, which was due to the abundance and co-surfactant behavior of its major component, eugenol (Edris, A. and Malone, C., 2012). Thus, based on this information, it can be supposed that the higher EIs values obtained for both polymers can be due to the co-surfactant behavior of the phenolic major components and the other components of the EOs and in the case of the mixture of the oils may be due to the synergistic effect of the majority components and the minor components of the oils, working as co-surfactants and, thus, helping in the emulsification process, and contribute to momentary stability of emulsions, which can explain EIs of 80% and 100%.

In the results present in **Figure 24** it is also possible to see that in the emulsions with mint oil, containing the polymer carboxymethyl cellulose, there is no formation of an emulsified layer, something that does not happen with the same emulsion but containing XG, and since this is a single result, not having been prepared in triplicate, maybe it happened because some problem in the homogenization process.

However, as the preponderant factor in stabilizing emulsions is the length of the alkyl chains of surfactants, this stabilization does not seem to be long lasting since essential oils, as compared to sunflower oil, have in their constitution, as mentioned in previous sections, diterpene phenolic components such as menthol, carvacrol and thymol, all C-10, which do not have long alkyl chains. This results in small oil droplets and consequently, a greater difficulty for the surfactant to encompass these molecules, thus these oil droplets eventually escape resulting in faster phase separation, such can be seen, for certain emulsions, in **Figure 25**, and in **Figure 24** by the differences of the EIs values from 24 to 48 hours in emulsions with CMC 700.000.

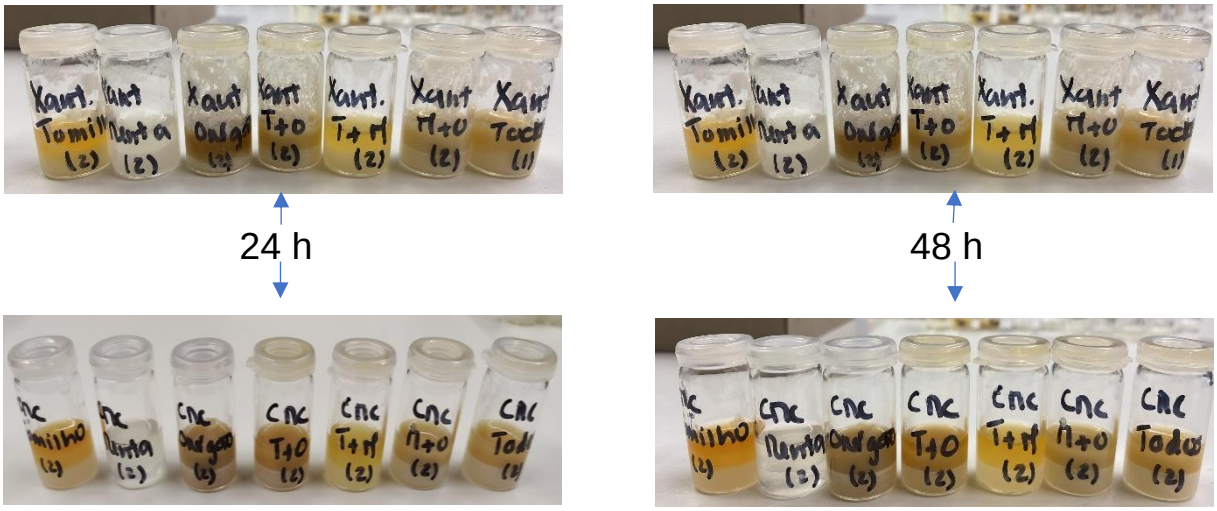


Figure 25: All the emulsions under study 24 and 48 hours after they were produced and with each of the surfactants under evaluation. At the top of the figure are the emulsions containing XG at 24 and 48 hours after they were produced and at the bottom of the image are the emulsions containing CMC and also at 24 and 48 hours after they were produced.

Given the above observation, this is where the importance of the polymer chosen for stabilization comes in. As mentioned in the introduction, polymers play a key role in long-term emulsion stabilization, however there are polymers with greater stabilization capacity than others due to several factors including their structure. This is the case with CMC 700.000 and XG.

XG plays an important role in stabilizing emulsions due to the formation of an adsorbed layer on the surface of the oil droplet and a scaffolding structure in the continuous phase (Ougiya, H. *et al*., 1997). XG's ability to stabilize emulsions also results from the capability of this polymer to increase viscosity (Fioramonti, S.A. *et al*., 2015, Taherian, A.R. *et al*., 2011) and due to its double helix structure that results in the formation of a secondary structure, network structure (Wang, A. *et al*., 2017), which very efficiently traps the emulsion droplets (Cai, X. *et al*., 2020), which will decrease the movement of oil droplets and consequently the number of collisions, thus preventing phase separation, i.e. coalescence, one of the main instabilities of emulsions (Krstonošić, V. *et al*., 2015). Another author considered that this and other biopolymers can prevent flocculation and coalescence by combined mechanisms. XG is a thickening agent so it will increase the viscosity of the emulsion and consequently the collisions of oil droplets in emulsions are compromised (Bouyer, E. *et al*., 2012). This polysaccharide polymer is then a texture modifier (Owens, C. *et al*., 2018) and because it has long polymeric chains it is easy to dissolve in cold water (Fang, F. *et al*., 2020).

For carboxymethyl cellulose, a water-soluble cellulose derivate, it has been demonstrated that it maintains high droplet size homogeneity in O/W emulsions (Hayati, I.N. *et al*., 2009). As for its structure, this polymer has carboxylic groups, especially propionic acid ($CH_2CO=O$) which is suspected to influence the formation of uniform and small oil droplets (Dickinson, E., 2003, Mann, B. and Malik, R., 1996). Like XG, carboxymethyl cellulose is also a thickening agent, thus increasing the viscosity of emulsions (Radi, M. and Amiri, S., 2013).

Studies comparing the stabilizing capacity of CMC compared to XG showed that with the first polymer there was a faster phase separation, which is characterized by a creamy top layer and a transparent bottom layer corresponding to the continuous phase, which indicated the occurrence of flocculation during storage, leading to phase separation (Xu, X. *et al*., 2017). While emulsions with CMC to which XG was added, there was no phase separation, which was attributed to the good dissolution characteristics of XG in water due to its long polymer chain and its great thickening capacity that increases the viscosity of the emulsion, which prevents the accumulation of oil droplets and consequently phase separation (Hayati, I.N. *et al*., 2016). An earlier study evaluated the stabilizing ability of four polymers, two of them being XG and CMC, and concluded that XG has a greater ability to stabilize emulsions than CMC (Cao, Y. *et al*., 1990).

Thus, considering the above, we can conclude by observing the graphs in **Figure 24** that the XG has a greater stabilizing capacity than the CMC 700.000, so we can see through the IEs that the emulsified layer remains almost constant between 24 and 48 hours after the production of the emulsion, the same does not happen with the CMC 700.000, with a decrease in this layer from 24 to 48 hours.

In summary, the stability of emulsions is the result of a number of parameters like pH, polymer concentration, ionic strength, emulsification process and the nature of the polymer, all of which influence the microstructures that form in emulsions and therefore their stability (Bouyer, E. *et al*., 2012, Schubert, H. and Engel, R., 2004).

5.5 Determination of MIC of essential oils

Initially, the MIC of thyme, oregano, eucalyptus, mint, lemon and tea tree oils were determined individually for the four strains under study, *E. coli ATCC 8739, P. aeruginosa DM, S. aureus ATCC 6538, E. faecalis ATCC 29212*.

The **Table 2** shows the MIC values obtained for each strain and oil in question, which resulted from the average of the trials performed in triplicate in at least two independent trials.

Table 2: MIC (%) of the thymus, oregano, eucalyptus, mint, lemon and tea tree EOs for *E. coli ATCC 8739, P. aeruginosa DM, S. aureus ATCC 6538, E. faecalis ATCC 29212.*

	MIC (%) of Essential Oils					
Bacterias	Thymus (Thymol 50% -55%)	Oregano (Thymol 3% - 5%)	Eucalyptus	Mint	Lemon	Thea Tree
E.coli ATCC 8739	0.5	0.25	4	1.75	10	1
P.aeruginosa DM	14	1.25	14	45	16	14
S.aureus ATCC 6538	0.375	0.125	6	0.5	8	2
E.faecalis ATCC 29212	1	0.188	8	3	14	7

By analyzing the data in **Table 2** it can be seen that overall, with certain exceptions, Gram-positive bacteria have lower MIC values than those obtained for Gram-negative bacteria. Other reports have also found that OEs are more effective generally against Gram-positive than Gram-negative bacteria (Hammer, K.A. *et al*., 1999, Prabuseenivasan, S. *et al*., 2006, Smith-Palmer, A. *et al*., 1998), which agrees with these results. However, in this present study it was found that in some cases *E. faecalis ATCC 29212* and *S. aureus ATCC 6538* had lower susceptibility than *E. coli ATCC 8739*. In one study *E. coli* was found to be more susceptible than other Gram-positive bacteria under study. This was justified by the fact that the antimicrobial activity of EOs is due to the specificity of the functional groups of their compounds for multiple or single targets. Some components of the oils have the ability to damage the outer membrane of Gram-negative bacteria, such as *E. coli*, which leads to the release of intracellular and membrane material, entering the cell and causing detrimental effects to the same (Helander, I.M. *et al*., 1998).

Regarding the bacteria under study, in Gram-positive bacteria there is a higher resistance to antimicrobials for *E. faecalis ATCC 29212* while in Gram-positive bacteria *P. aeruginosa DM* stands out with the highest MIC values, not only compared to *E. coli ATCC 8739* but also to the two Gram-positive bacteria. This resistance associated with *P. aeruginosa DM* and Gram-negative bacteria in general is due to the difference in cell wall structure since Gram-negative bacteria possess an outer membrane that Gram-positive bacteria do not. This membrane consists of lipopolysaccharides (Bezić, N. *et al*., 2003) porins, transmembrane proteins, and polar portions (O-polyssacharides), which allow the entry of hydrophilic

compounds, hindering the entry of hydrophobic compounds, such as EOs components and their consequent effects (Mayaud, L. *et al*., 2008, Nazzaro, F. *et al*., 2013). In addition, the resistance associated with this type of bacteria can also be synergized to the presence of efflux mechanisms, which allow toxic compounds, such as antibacterial compounds, to be removed from inside the cell (Pearson, J.P. *et al*., 1999).

Comparing the different MIC values for the six oils and the four bacteria in the study, TEO, OEO and MEO are the EOs with the lowest MIC values for the bacteria in question, not reaching 2% for most of them, except for *P. aeruginosa DM* with an MIC value of about 14% for TEO and 45% for MEO. For the remaining EOs the MIC values are significantly above 2%.

5.5.1 MIC of TEO

The MIC values obtained in this study for TEO for *E. coli ATCC 8739*, *P. aeruginosa DM*, *S. aureus ATCC 6538* and *E. faecalis ATCC 29212* were 0.5%, 14%, 0.375% and 1%, respectively. For other authors, the MIC determined was about 0.25% (Rusenova, N. and Parvanov, P., 2009) and 0.35% (Mayaud, L. *et al*., 2008) for *E. coli*, 2% (Rusenova, N. and Parvanov, P., 2009) and >10% (Mayaud, L. *et al*., 2008, Sikkema, J. *et al*., 1994) for *P.aeruginosa*, 0.29% (Mayaud, L. *et al*., 2008), 0.31% (Donaldson, J.R. *et al*., 2005) and 0.5% (Rusenova, N. and Parvanov, P., 2009) for *S. aureus,* and 1% (Rusenova, N. and Parvanov, P., 2009) 6.3% (Mayaud, L. *et al*., 2008) for *E. faecalis*. Considering the described information, it is verified that the MICs obtained in this work agree and are quite similar with the results of others, however, some of the values mentioned above, for example for *P. aeruginosa* and *E. faecalis*, are more discrepant. These values may be due to several factors such as different cultivation locations of the plants and harvesting times, a variety of plant types and species (Imelouane, B. *et al*., 2009), different bacterial strains, different OE suppliers or even different methodologies.

According to the obtained results, it can be concluded that TEO shows great antimicrobial capacity, which is justified by the presence of three very important phenolic compounds thymol, carvacrol and eugenol (Cosentino, S. *et al*., 1999, Naidu, A., 2000, Rota, M.C. *et al*., 2008, Skočibušić, M. *et al*., 2006). The mechanism associated with thymol, carvacrol and eugenol action may due to the proton donor of their hydroxyl group that interact with bacteria membranes, providing protons across the membrane, which lead to the waste of the energy that come from the proton flux leading to a reduction of intracellular ATP accompanied by an increase in the external side, loss of intracellular components, such as potassium ions, and structural changes in plasmatic membrane (Di Pasqua, R. *et al*., 2007, Donsì, F. and Ferrari, G., 2016). Besides thymol and carvacrol other components that stand out are γ-terpinene and p-cymene, to which the antimicrobial activity of this oil is also attributed. Although p-cymene,

the precursor of carvacrol, it does not show antibacterial activity on its own (Dorman, H.J. and Deans, S.G., 2000), p-cymene has a synergistic effect when acting together with carvacrol, since p-cymene has the ability to swell the bacterial cell membrane, which facilitates the entry of carvacrol into the cell (Rota, M.C. *et al*., 2008). In addition to the contribution of the synergistic effect between p-cymene and carvacrol to the antimicrobial activity of the oil, there is also such an effect between p-cymene, thymol and γ-terpinene, the three major components of this oil (Delgado, B. *et al*., 2004, Gallucci, M.N. *et al*., 2009), and even between the majority and minority components (Borugă, O. *et al*., 2014). Among the various mechanisms exerted by lipophilic compounds present in OEs is the damage of bacterial membranes, which leads to imbalances of inorganic ions and consequently unbalance of pH homeostasis (Cowan, M.M., 1999).

5.5.2 MIC of OEO

For OEO the MIC values were 0.25%, 1.25%, 0.125% and 0.188% for *E. coli ATCC 8739*, *P. aeruginosa DM*, *S. aureus ATCC 6538* and *E. faecalis ATCC 29212*, respectively. While in the literature the values were 0.1% (Man, A. *et al*., 2019) and 0.24% (Mayaud, L. *et al*., 2008), for *E. coli,* 2.0% (Rusenova, N. and Parvanov, P., 2009) and 2.19% (Mayaud, L. *et al*., 2008) for *P. aeruginosa DM,* 0.006% (Rusenova, N. and Parvanov, P., 2009), 0.24% (Mayaud, L. *et al*., 2008), 0.42% (Donaldson, J.R. *et al*., 2005) for *S. aureus,* and 0.125% (Rusenova, N. and Parvanov, P., 2009) and 0.8% (Man, A. *et al*., 2019) for *E. faecalis*. These results are in conformity with those obtained in other studies, with, in certain cases, some differences which may be due to the factors indicated in the TEO as well.

The proven antimicrobial properties for OEO are mainly associated with its major components aromatic terpenes such as carvacrol, thymol and p-cymene (Esen, G. *et al*., 2007, Soylu, S. *et al*., 2007). One of the recognized effects of thymol and carvacrol is the ability to disrupt the outer membrane of Gram-negative bacteria through the release of the lipopolysaccharide content, which alters the permeability of the cell (Guarda, A. *et al*., 2011). Beside the synergistic effect among p-cymene and carvacrol, previously mentioned, another possible effect associated with carvacrol is related to its acidity, i.e. hydrogen-bonding ability, which seems to influence the loss of the proton gradient that destabilizes the protonmotive force and thus the cytoplasmic membrane, leading to apoptosis (Ben Arfa, A. *et al*., 2006, Ultee, A. *et al*., 2002). Minority components such as γ-terpinene also seem to influence the antimicrobial activity found. Since carvacrol and thymol show a synergistic effect, and since γ-terpinene and p-cymene are precursors of the first two, it is possible that there is also a

synergistic effect between this component and p-cymene (Burt, S.A. and Reinders, R.D., 2003, Gilles, M. *et al*., 2010).

Despite all the aforementioned contributions of the different components to the antimicrobial activity of this oil, another study concluded that the synergistic effect between p-cymene and carvacrol, compared to the individual components, seems to be the pivotal effect for the greater membrane destabilization and dysregulation of proton exchange (Ultee, A. *et al*., 2002).

5.5.3 MIC of EEO

Regarding the MIC values obtained in this assay, they were 4%, 14%, 6%, 8% for *E. coli ATCC 8739*, *P. aeruginosa DM*, *S. aureus ATCC 6538* and *E. faecalis ATCC 29212*, respectively. In other studies, the values obtained were 2.25% (Mayaud, L. *et al*., 2008) and 8% (Aldoghaim, F.S. *et al*., 2018) for *E. coli*, 8% (Aldoghaim, F.S. *et al*., 2018) and >10% (Mayaud, L. *et al*., 2008) for *P. aeruginosa*, 4 % (Aldoghaim, F.S. *et al*., 2018) and 7.19% (Mayaud, L. *et al*., 2008) for *S. aureus*, 2% (Ait-Ouazzou, A. *et al*., 2011) and > 8% (Aldoghaim, F.S. *et al*., 2018) for *E. faecalis*. In certain cases, there is then a starker difference in MIC values, which may be due to the factors listed for TEO as well.

The antimicrobial ability of EEO may be mainly due to the major component 1,8-cineole, whose strong antimicrobial activity has already been proven for various bacteria (Rosato, A. *et al*., 2007, Sonboli, A. *et al*., 2006). However, other studies consider that the antimicrobial activity of this oil is due to components such as p-cymene, γ -terpinene, and limonene. Since essential oils are composed of a mixture of compounds it is difficult to state that the antimicrobial activity is due to a specific compound, because the higher concentration of a particular compound does not necessarily imply that it is responsible for greater antimicrobial activity (Rota, M.C. *et al*., 2008), and therefore it is to consider the contribution of synergistic or antagonistic effect of the constituents of oils for the antimicrobial activity of the same (Ait-Ouazzou, A. *et al*., 2011).

By analyzing the results obtained for this oil it is also possible to see a higher susceptibility to a Gram-negative bacterium, *E. coli ATCC 8739* compared to the Gram-positive bacteria in the study *E. faecalis ATCC 29212* and *S. aureus ATCC 6538*. These results were also obtained in another study, in which unexpectedly Gram-positive bacteria were less susceptible to the effect of EEO than Gram-negative bacteria. According to the authors, this may be due to the action of components such as 1,8 cineole (eucalyptol), p-cymene, cis-geraniol and terpinolene, which may be involved in the unloading of the lipopolysaccharide membranes of

Gram-negative bacteria, changing the conformation of the membrane and increasing its permeability (Marino, M. *et al*., 2001).

5.5.4 MIC of MEO

Concerning the MIC values determined in this study, they were 1.75%, 45%, 0.5%, 3% for *E. coli ATCC 8739*, *P. aeruginosa DM*, *S. aureus ATCC 6538* and *E. faecalis ATCC 29212*, respectively. The values documented are 1% (Muntean, D. *et al*., 2019, Shahbazi, Y., 2015) for *E. coli*, 1% (Rusenova, N. and Parvanov, P., 2009) and 2 % (Muntean, D. *et al*., 2019) for *P. aeruginosa,* 0,5% (Muntean, D. *et al*., 2019) and 0.25% (Rusenova, N. and Parvanov, P., 2009) for *S. aureus*, and 1% (Rusenova, N. and Parvanov, P., 2009) for *E. faecalis*.

Regarding the antimicrobial effect of this oils, one of the main actions of this oil is the destruction of protoplasm by lysis, however preserving the cell wall´s structure. Between all the compounds in the oil, menthone and menthol, are the major compounds that seem to have a key role in the antimicrobial effect, which is already highly described by several authors (Badea, M.L. *et al*., 2019, Cao, L. *et al*., 2009, Oke, F. *et al*., 2009). It has been documented that monoterpenes, like menthol, can harm both Gram-negative and Gram-positive bacteria´s membranes. In specific, menthol disrupts the lipid portion of the cell membranes, altering how permeable the membrane is and allowing the intracellular content to leak out (Russo, A. *et al*., 2016). Besides the combination of menthol and other compounds of the oils, such as, limonene and menthone with the other compounds of the oil, that are present in low percentages, may lead a synergistic effect (Antolak, H., 2018, Lopes-Lutz, D. *et al*., 2008, Singh, R. *et al*., 2015).

It should be noted that *P. aeruginosa* presents an MIC value much higher than documented in the literature, which may be due to an antagonistic effect of the different components of the oils specifically for this bacterium, since the greater or lesser antibacterial capacity of an oil is dependent on the bacterial strains used in each study and the part and composition of the plant used for the oil production (Antolak, H., 2018, Lopes-Lutz, D. *et al*., 2008, Singh, R. *et al*., 2015), or even because is a clinical strain.

5.5.5 MIC of LEO

As can be seen in **Table 2**, for LEO the MIC values were 10%, 16%, 8%, 14% for *E. coli ATCC 8739*, *P. aeruginosa DM*, *S. aureus ATCC 6538* and *E. faecalis ATCC 29212*, respectively. For LEO the values were 6.3% for *E. coli*, > 10 % and 12.5% for *P. aeruginosa* (Man, A. *et al*., 2019), 1.25% (Moosavy, M. *et al*., 2017) for *S. aureus*, and 12.5% for *E. faecalis* (Man, A. *et al*., 2019).

Although limonene is the majority component of LEO, antimicrobial activity of LEO is not due to this component alone, as studies conducted on this component alone have shown that it does not show antimicrobial activity when alone (Fancello, F. *et al*., 2016). Thus, the influence of minority components may be predominant for antimicrobial activity. In a study conducted with another oil, compounds such as citral and linalool, also present in this oil, were shown to be probably important for this effect when used against Gram-positive and Gram-negative bacteria (Yi, F. *et al*., 2018). As with the other oils, the mechanism of action of these oils is to alter the structure of plasma membranes, which results in an increase in membrane permeability and membrane damage that affects proton flux and hence energy production and transmembrane transport (Swamy, M.K. *et al*., 2016). In addition, the expression of virulence factors, the quorum sensing process and biofilm production are also affected (Nazzaro, F. *et al*., 2013).

5.5.6 MIC of TTO

And finally, for TTO the MIC values were 1%, 14%, 2% and 7% for *E. coli ATCC 8739*, *P. aeruginosa DM*, *S. aureus ATCC 6538* and *E. faecalis ATCC 29212*, respectively. The values obtained in other study were and 0.62% for *E. coli*, >10% for *P. aeruginosa*, 1.05% for S. aureus (Mayaud, L. *et al*., 2008) and 1% for *E. faecalis* (Rusenova, N. and Parvanov, P., 2009).

In this type of oil, the major component is terpinene-4-ol, which is also involved in the antibacterial activity of this oil (Carson, C.F. *et al*., 2006, Santos, R.C.V. *et al*., 2014). As far as the mechanisms of action of this oil are concerned, this are also associated with leakage of the compounds present in the membrane and hindering the antimicrobial respiration (Yasin, M. *et al*., 2021).

Thus, comparing the different oils it is possible to see that TEO, OEO and MEO were the ones with the lowest MIC values, which in the first two cases is most likely due to the carvacrol and thymol and the synergistic effects between these and other components such as p-cymene. The MEO, even without carvacrol and thymol in its composition, presents MIC values that are also quite low, which is due to other components in its constitution that seem to have antimicrobial capacity, which leads to conclude that this antibacterial activity of the oils is not only due to carvacrol and thymol, but also to other components present.

Still, knowing that thymol has a high antimicrobial capacity, the MIC of an 8% solution of this compound was also determined, which can be seen in **Table 3**.

Table 3: MIC (%) of the 8% thymol solution for *E. coli ATCC 8739, P. aeruginosa DM, S. aureus ATCC 6538, E. faecalis ATCC 29212.*

Bacteria	MIC (%)
E.coli ATCC 8739	1
P.aeruginosa DM	2
S.aureus ATCC 6538	0.5
E.faecalis ATCC 29212	0.5

Comparing the values on **Table 3** with those of the oils, which correspond to complex mixtures of components, the goal is to conclude if in fact the antimicrobial activity of the oils containing this component is due exclusively to it or, as other authors have suggested, if there may be other components or synergistic relationships, or other types, such as antagonistic, established between this and the other components and also between the remaining components (Rao, J. *et al*., 2019).

These work values are in agreement with those of another study, in which 0.5% for *S. aureus* and 0.3% for *E. coli* were obtained (Tippayatum, P. and Chonhenchob, V., 2007). Thus, when comparing the MICs of the thymol solution with those of the thymol-containing oils, such as thyme, which contains 50-55% thymol, and oregano, which contains between 3% and 4% thymol, the MICs of these oils are mostly lower than those of the solution containing only thymol, which may be due to other components present in the oil that also contribute to their antimicrobial activity. Therefore, the antimicrobial activity of the oils containing thymol is not only due to this compound, but also to other compounds that interact with thymol and even with each other, leading to a synergistic effect that results in a lower MIC in the oils. This observation is also supported by other studies in which the essential oils had a higher antimicrobial capacity than their majority components in combination, which may also suggest that the minority components also contribute to the antimicrobial activity through a synergistic relationship of the same (Jiang, Y. *et al*., 2011, Rota, M.C. *et al*., 2008, Sarrazin, S.L.F. *et al*., 2012).

5.6 Determination of MIC of emulsions

Based on the above information, thyme, oregano and mint oils were chosen for the formulation of the final emulsions due to their low MIC values indicating that lower concentrations of these oils are required to prevent visible bacterial growth.

In **Table 4 and 5** are represented the MIC values obtained for each emulsion and bacterial strains in question, which resulted from the average of the trials performed in triplicate in at least two independent trials.

Table 4: MIC (%) of the thymus, oregano and mint emulsions for *E. coli ATCC 8739, P. aeruginosa DM, S. aureus ATCC 6538, E. faecalis ATCC 29212* with CMC 700.000 polymer.

	MIC (%) of Emulsions						
Bacterias	Thymus + CMC	Oregano + CMC	Mint + CMC	Thymus + Oregano + CMC	Thymus + Mint + CMC	Oregano + Mint + CMC	Thymus + Oregano + Mint + CMC
E. coli ATCC 8739	0.049	0.012	0.195	0.006	0.049	0.012	0.008
P .aeruginosa DM	ND	ND	ND	ND	ND	ND	ND
S. aureus ATCC 6538	0.0976	0.024	0.195	$9{,}54 \times 10^{-5}$	0.024	0.006	0.0041
E .faecalis ATCC 29212	0.0976	0.012	0.195	7.6×10^{-4}	0.0976	0.024	0.0163

ND-Not Detected

Table 5: MIC (%) of the thymus, oregano and mint emulsions for *E. coli ATCC 8739, P. aeruginosa DM, S. aureus ATCC 6538, E. faecalis ATCC 29212*, with XG polymer.

	MIC (%) of Emulsions						
Bacterias	Thymus + XG	Oregano + XG	Mint +XG	Thymus + Oregano + XG	Thymus + Mint + XG	Oregano + Mint + XG	Thymus + Oregano + Mint + XG
E. coli ATCC 8739	0.049	0.024	0.049	0.006	0.049	0.012	0.008
P. aeruginosa DM	ND	ND	ND	ND	ND	ND	ND
S .aureus ATCC 6538	0.049	$9{,}54 \times 10^{-5}$	0.024	0.012	0.049	0.024	0.0081
E. faecalis ATCC 29212	0.0015	$9{,}54 \times 10^{-5}$	0.012	1.9×10^{-4}	0.012	0.003	0.00125

ND- Not Detected

By observing **Tables 4** and **5** we can see that all the MIC values are much lower than those for the essential oils alone. This can be explained by greater stabilization of the oil drops, and consequently of their compounds, increasing their activity (Hussein, A. *et al*., 2019, Nirmal, N.P. *et al*., 2018). The oil droplets get dimensions that allow a better dispersion of the oil drops in the aqueous phase, increasing the contact area between the oil drop itself and the bacterial cells (Shinoda, K. and Kunieda, H., 1973, Valizadeh, A. *et al*., 2018). This contributes to a more targeted antimicrobial action of the oil since its components more easily interact

with the bacteria membrane. The main actions associated with emulsions are membrane disruptions that lead to the release of intracellular contents such as potassium ions, proteins, carbohydrates and DNA (Moghimi, R. *et al*., 2016a)

Comparing even the two polymers and knowing that they do not present antimicrobial activity, based on the knowledge of Science 351 in working with these polymers, it can be seen that the MIC values are quite low for both polymers. However, for XG, although not significantly lower, these values are lower than for CMC 700.000, which may be due to the emulsions formed being even more stable with XG, which allows no fast phase separation, thus maintaining the oil droplets with the desirable dimensions for a beneficial contact area between them and the bacterial cell and leading to greater interaction between the components of these oils and the cells. This information concerning the polymers is in line with the earlier conclusion concerning the increased stability of emulsions with XG.

Studies that determined MIC values for TEO emulsion, obtained MIC values of about 0.04% (Moghimi, R. *et al*., 2016b) and 0.05% (He, Q. *et al*., 2022) which are very close to the 0.049%, obtained in this work for *E. coli*. Also for *S. aureus*, the MIC of the emulsion of this oil was determined, and the value obtained was 0.05% (He, Q. *et al*., 2022) , which is also close to the value obtained in this work of about 0.0976%, for the emulsion with CMC 700.000 and 0.049% for the emulsion with XG.

Comparing the values obtained in this work with the study of other authors, it can be seen, for example, for the OEO emulsion, for *S. aureus* and *E.coli* bacteria that the MIC values are about 0.0312% (Enayatifard, R. *et al*., 2021) so they are quite close to the values obtained in this work, about 0.049 % and 0.0976 %. for *S. aureus*, and 0.012% and 0.024% for *E. coli*, depending on the polymer used, which for the last bacterium is still below what is described in the literature, which is very beneficial at the industrial level because less emulsion is needed to inhibit this bacterium. In this work, not only for the OEO but also for the other oils and blends, in this study too, *P. aeruginosa* continued to grow normally, and there was no action from the emulsion (Enayatifard, R. *et al*., 2021).

In addition to evaluating the antimicrobial activity of the emulsions of each oil individually, MIC values were also determined for emulsions containing two of these oils and emulsions containing all three oils. This combination of the oils was intended to assess whether this mixture would be beneficial in contributing to an increase in the antimicrobial capacity of the emulsions containing them. The mixing of several oils or their compounds can result in four types of antimicrobial actions: indifferent, antagonistic, additive, or synergistic (Delaquis,

P.J. *et al*., 2002). The antagonistic and synergistic effect of the combination of the different oils is dependent on two factors: the type of EO and the bacterial strains under study. The synergistic or antagonistic effects arising from the mixture of oils is probably due to the interactions established between the various components of the different oils (Rao, J. *et al*., 2019).

When comparing the MIC values of the emulsions for the oil blend with those for the individual oils, the MIC values are mostly lower for the oil blends than for the individual oils, suggesting a synergistic effect due to interactions between the components of the different oils (Rao, J. *et al*., 2019). Several studies have evaluated the interaction of the phenolic compounds, carvacrol, thymol and eugenol, with other compounds, e.g. monoterpenes, and the contribution of their interactions to the antimicrobial activity and they found that there was indeed a synergistic effect due to the combination of these phenols with the monoterpenes, such as between thymol and p-cymene (Delgado, B. *et al*., 2004). The structure of thymol differs from that of p-cymene due to the existence of a hydroxyl group attached to the benzene ring in the former case, leading to an increase in the antimicrobial activity of p-cymene (Rusenova, N. and Parvanov, P., 2009).

Thus, it is possible to conclude that emulsions containing oil blends are more beneficial due to the interactions that form between the components of the different oils, increasing the antimicrobial activity and decreasing the MIC value.

6 Conclusion

Essential oils emerged as natural alternatives to synthetic compounds that are often toxic to humans and other living beings, and can be applied to the most diverse areas, such as agriculture, in the pharmaceutical sector, food industry and the cosmetics area. The fact that essential oils have several fundamental properties allow the formulation of new products with antimicrobial activity without the need to resort to synthetic components that have been increasingly proven to be toxic. Thus, the need to resort to natural compounds is increasingly in vogue, since they are a cheap source and great availability, without causing environmental concerns.

Regarding the present work it can be concluded that: of the surfactants tested, coconut glycoside was the one that presented the highest EIs values and a high stability at 24 and 48 hours after emulsion production, in most of the concentrations tested. As for the polymers, XG showed a higher stabilizing capacity of the emulsions after 24 and 48 hours.

From the oils studied it can be concluded that oregano, thyme and mint are the ones with the highest antimicrobial activity, and therefore lower MICs. As regards the emulsions with the essential oils alone and the emulsions with the mixture of oils, it was found that the latter show lower MICs than the oils individually, which may be due to the synergistic effect of the components of the different oils that will contribute to an increase in the antimicrobial activity of the emulsions containing the mixed oils.

When assessing the antimicrobial capacity not only of the oils individually, but also of the emulsions, mostly Gram-positive bacteria were more susceptible than Gram-negative bacteria, which may be due to the outer membrane of Gram-negatives. And since skin bacteria are mostly Gram-positive, these oils have several applications in cosmetics, allowing the formulation of products with the most diverse purposes.

In the future it will also be possible to test and compare the MICs of emulsions with essential oils, with and without the addition of thymol, since it has a high antimicrobial activity, and to evaluate whether the addition of thymol would be beneficial to further increase the antimicrobial capacity of the emulsions. It will also probably be possible to produce emulsions with an equal mixture of the surfactants with which better results were obtained, such as coco glucoside and cocamidopropryl betaine, in order to assess whether the action of both in the same emulsion could further increase the EIs and the stability of the emulsions, compared to their action alone, It may also be tested the action of the mixture of the two polymers in emulsions, in order to assess their joint action in stabilizing emulsions in the long term, which

opens the way to another test that involves the characterization of emulsions, by determining the stability, i.e. through stability studies over 48 hours.

Thus, this study proved the antimicrobial activity of the tested oils and their emulsions, which will pave the way for the development of innovative cosmetic products capable of inhibiting bacterial activity, without the need to add synthetic compounds, often associated with environmental pollution and toxicity.

7 Bibliography

ABAD-GARCÍA, Beatriz; GARMÓN-LOBATO, Sergio; BERRUETA, Luis A; GALLO, Blanca; VICENTE, Francisca - On line characterization of 58 phenolic compounds in Citrus fruit juices from Spanish cultivars by high-performance liquid chromatography with photodiode-array detection coupled to electrospray ionization triple quadrupole mass spectrometry. *Talanta*. Vol. 99. (2012). p. 213-224.

ADWAN, Ghaleb; SALAMEH, Yousef; ADWAN, Kamel; BARAKAT, Ali - Assessment of antifungal activity of herbal and conventional toothpastes against clinical isolates of *Candida albicans*. *Asian Pacific journal of tropical biomedicine*. Vol. 2. n.° 5 (2012). p. 375-379.

AIT-OUAZZOU, Abdenour; LORÁN, Susana; BAKKALI, Mohammed; LAGLAOUI, Amin; ROTA, Carmen; HERRERA, Antonio; PAGÁN, Rafael; CONCHELLO, Pilar - Chemical composition and antimicrobial activity of essential oils of *Thymus algeriensis*, *Eucalyptus globulus* and *Rosmarinus officinalis* from Morocco. *Journal of the Science of Food and Agriculture*. Vol. 91. n.° 14 (2011). p. 2643-2651.

ALDOGHAIM, Fahad S; FLEMATTI, Gavin R; HAMMER, Katherine A - Antimicrobial activity of several cineole-rich Western Australian Eucalyptus essential oils. *Microorganisms*. Vol. 6. n.° 4 (2018). p. 122.

ALIGIANNIS, Nektarios; KALPOUTZAKIS, E; MITAKU, Sofia; CHINOU, Ioanna B - Composition and antimicrobial activity of the essential oils of two Origanum species. *Journal of agricultural and food chemistry*. Vol. 49. n.° 9 (2001). p. 4168-4170.

ALVES, Thais; MORSINK, Margaretha; BATAIN, Fernando; CHAUD, Marco; ALMEIDA, Taline; FERNANDES, Dayane; SILVA, Classius; SOUTO, Eliana B.; SEVERINO, Patrícia - Applications of Natural, Semi-Synthetic, and Synthetic Polymers in Cosmetic Formulations. *Cosmetics*. Vol. 7. (2020). p. 75.

AMBROSIO, Carmen M. S.; IKEDA, Natália Y.; MIANO, Alberto C.; SALDAÑA, Erick; MORENO, Andrea M.; STASHENKO, Elena; CONTRERAS-CASTILLO, Carmen J.; DA GLORIA, Eduardo M. - Unraveling the selective antibacterial activity and chemical composition of citrus essential oils. *Scientific Reports*. Vol. 9. n.° 1 (2019). p. 17719.

AMIRI, Hamzeh - Essential oils composition and antioxidant properties of three thymus species. *Evidence-Based Complementary and Alternative Medicine*. Vol. 2012. (2012).

ANDREWS, J. M. - Determination of minimum inhibitory concentrations. *J Antimicrob Chemother*. Vol. 48 Suppl 1. (2001). p. 5-16.

ANTOLAK, H - Czy zowska. *Kregiel, D. Activity of Mentha piperita L. Ethanol Extract against Acetic Acid Bacteria Asaia spp. Foods*. Vol. 7. (2018).

ANWAR, Farooq; ABBAS, Ali; MEHMOOD, Tahir; GILANI, Anwarul-Hassan; REHMAN, Najeeb-ur - Mentha: A genus rich in vital nutra-pharmaceuticals—A review. *Phytotherapy Research*. Vol. 33. n.° 10 (2019). p. 2548-2570.

AZODI, Masood; NAZAR, Ali Reza Solaimany - Experimental design approach to investigate the effects of operating factors on the surface tension, viscosity, and stability of heavy crude oil-in-water emulsions. *Journal of dispersion science and technology*. Vol. 34. n.° 2 (2013). p. 273-282.

BADEA, Monica Luminita; ICONARU, Simona Liliana; GROZA, Andreea; CHIFIRIUC, Mariana Carmen; BEURAN, Mircea; PREDOI, Daniela - Peppermint essential oil-doped hydroxyapatite nanoparticles with antimicrobial properties. *Molecules*. Vol. 24. n.° 11 (2019). p. 2169.

BADI, Hassanali Naghdi; YAZDANI, Darab; ALI, Sajed Mohammad; NAZARI, Fatemeh - Effects of spacing and harvesting time on herbage yield and quality/quantity of oil in thyme, *Thymus vulgaris* L. *Industrial crops and products*. Vol. 19. n.° 3 (2004). p. 231-236.

BAGAMBOULA, C. F.; UYTTENDAELE, Mieke; DEBEVERE, Johan - Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. Flexneri*. *Food Microbiology*. Vol. 21. (2004). p. 33-42.

BAKKALI, F.; AVERBECK, S.; AVERBECK, D.; IDAOMAR, M. - Biological effects of essential oils--a review. *Food Chem Toxicol*. Vol. 46. n.° 2 (2008). p. 446-75.

BARATTA, M Tiziana; DORMAN, HJ Damien; DEANS, Stanley G; BIONDI, Daniela M; RUBERTO, Giuseppe - Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oils. *Journal of Essential Oil Research*. Vol. 10. n.° 6 (1998). p. 618-627.

BARKAT, Ali Khan; NAVEED, Akhtar; HAJI, Muhammad Shoaib Khan; KHALID, Waseem; TARIQ, Mahmood; AKHTAR, Rasul; MUHAMMAD, Iqbal; HAROON, Khan - Basics of pharmaceutical emulsions: A review. *African Journal of Pharmacy and Pharmacology*. Vol. 5. n.° 25 (2011). p. 2715-2725.

BASHEVA, Elka S; DANOV, Krassimir D; RADULOVA, Gergana M; KRALCHEVSKY, Peter A; XU, Hui; UNG, Yee Wei; PETKOV, Jordan T - Properties of the micelles of sulfonated methyl esters determined from the stepwise thinning of foam films and by rheological measurements. *Journal of colloid and interface science*. Vol. 538. (2019). p. 660-670.

BASSOLÉ, I. H.; JULIANI, H. R. - Essential oils in combination and their antimicrobial properties. *Molecules*. Vol. 17. n.º 4 (2012). p. 3989-4006.

BECHER, Paul - Encyclopedia of emulsion technology. *Basic theory*. Vol. 1. (1983). p. 58-125.

BEN-JABEUR, Maissa; GHABRI, Emna; MYRIAM, Machraoui; HAMADA, Walid - Thyme essential oil as a defense inducer of tomato against gray mold and Fusarium wilt. *Plant Physiology and Biochemistry*. Vol. 94. (2015). p. 35-40.

BEN ARFA, A; COMBES, S; PREZIOSI-BELLOY, L; GONTARD, N; CHALIER, P - Antimicrobial activity of carvacrol related to its chemical structure. *Letters in applied microbiology*. Vol. 43. n.º 2 (2006). p. 149-154.

BEZIĆ, Nada; SKOČIBUŠIĆ, Mirjana; DUNKIĆ, Valerija; RADONIĆ, Ani - Composition and antimicrobial activity of *Achillea clavennae* L. essential oil. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*. Vol. 17. n.º 9 (2003). p. 1037-1040.

BHATTACHARYA, Shiv Sankar; SHUKLA, Seema; BANERJEE, Subham; CHOWDHURY, Purojit; CHAKRABORTY, Prithviraj; GHOSH, Amitava - Tailored IPN hydrogel bead of sodium carboxymethyl cellulose and sodium carboxymethyl xanthan gum for controlled delivery of diclofenac sodium. *Polymer-Plastics Technology and Engineering*. Vol. 52. n.º 8 (2013). p. 795-805.

BHOSLE, Manisha R; JOSHI, Supriya A; BONDLE, Giribala M - An efficient contemporary multicomponent synthesis for the facile access to coumarin-fused new thiazolyl chromeno [4, 3-b] quinolones in aqueous micellar medium. *Journal of Heterocyclic Chemistry*. Vol. 57. n.º 1 (2020). p. 456-468.

BORUGĂ, O; JIANU, C; MIȘCĂ, C; GOLEȚ, I; GRUIA, AT; HORHAT, FG - *Thymus vulgaris* essential oil: chemical composition and antimicrobial activity. *Journal of medicine and life*. Vol. 7. n.º Spec Iss 3 (2014). p. 56.

BOUYER, Eléonore; MEKHLOUFI, Ghozlene; ROSILIO, Véronique; GROSSIORD, Jean-Louis; AGNELY, Florence - Proteins, polysaccharides, and their complexes used as stabilizers for emulsions: alternatives to synthetic surfactants in the pharmaceutical field? *International journal of pharmaceutics*. Vol. 436. n.º 1-2 (2012). p. 359-378.

BRATOVCIC, Amra; NAZDRAJIC, Sanela - Viscoelastic behavior of synthesized liquid soaps and surface activity properties of surfactants. *Journal of Surfactants and Detergents*. Vol. 23. n.º 6 (2020). p. 1135-1143.

BROKER, MIH; KLEINIG, DA - Field Guide to Eucalyptus vol. 1 South-eastern Australia. Bloomings. Melbourne, 2006.

BROPHY, Joseph J; DAVIES, Noel W; SOUTHWELL, Ian A; STIFF, Ian A; WILLIAMS, Lyall R - Gas chromatographic quality control for oil of Melaleuca terpinen-4-ol type (Australian tea tree). *Journal of Agricultural and Food Chemistry*. Vol. 37. n.º 5 (1989). p. 1330-1335.

BUDHIRAJA, Shalini S; CULLUM, Malford E; SIOUTIS, Susie S; EVANGELISTA, Lucio; HABANOVA, Susan T - Biological activity of *Melaleuca alternifolia* (tea tree) oil component, terpinen-4-ol, in human myelocytic cell line HL-60. *Journal of manipulative and physiological therapeutics*. Vol. 22. n.º 7 (1999). p. 447-453.

BURNETT, Christina L; BERGFELD, Wilma F; BELSITO, Donald V; HILL, Ronald A; KLAASSEN, Curtis D; LIEBLER, Daniel; MARKS JR, James G; SHANK, Ronald C; SLAGA, Thomas J; SNYDER, Paul W - Final report of the Cosmetic Ingredient Review Expert Panel on the safety assessment of cocamidopropyl betaine (CAPB). *International journal of toxicology*. Vol. 31. n.º 4_suppl (2012). p. 77S-111S.

BURT, S. - Essential oils: their antibacterial properties and potential applications in foods--a review. *Int J Food Microbiol*. Vol. 94. n.º 3 (2004). p. 223-53.

BURT, S. A.; VAN DER ZEE, R.; KOETS, A. P.; DE GRAAFF, A. M.; VAN KNAPEN, F.; GAASTRA, W.; HAAGSMAN, H. P.; VELDHUIZEN, E. J. - Carvacrol induces heat shock protein 60 and inhibits synthesis of flagellin in *Escherichia coli* O157:H7. *Appl Environ Microbiol*. Vol. 73. n.º 14 (2007). p. 4484-90.

BURT, Sara A; REINDERS, Robert D - Antibacterial activity of selected plant essential oils against *Escherichia coli* O157: H7. *Letters in applied microbiology*. Vol. 36. n.º 3 (2003). p. 162-167.

CAI, Xuran; WANG, Yan; DU, Xianfeng; XING, Xiaoyan; ZHU, Guilan - Stability of pH-responsive Pickering emulsion stabilized by carboxymethyl starch/xanthan gum combinations. *Food Hydrocolloids*. Vol. 109. (2020). p. 106093.

CALO, Juliany Rivera; CRANDALL, Philip G; O'BRYAN, Corliss A; RICKE, Steven C - Essential oils as antimicrobials in food systems–A review. *Food control*. Vol. 54. (2015). p. 111-119.

CAO, Li; SI, Jian Yong; LIU, Yan; SUN, Hong; JIN, Wen; LI, Zhan; ZHAO, Xiao Hong; LE PAN, Rui - Essential oil composition, antimicrobial and antioxidant properties of *Mosla chinensis* Maxim. *Food Chemistry*. Vol. 115. n.º 3 (2009). p. 801-805.

CAO, Yunhong; DICKINSON, Eric; WEDLOCK, David J - Creaming and flocculation in emulsions containing polysaccharide. *Food Hydrocolloids*. Vol. 4. n.º 3 (1990). p. 185-195.

CARSON, C. F.; RILEY, T. V. - Antimicrobial activity of the major components of the essential oil of *Melaleuca alternifolia*. *J Appl Bacteriol*. Vol. 78. n.º 3 (1995). p. 264-9.

CARSON, Christine F; HAMMER, Katherine A; RILEY, Thomas V - *Melaleuca alternifolia* (tea tree) oil: a review of antimicrobial and other medicinal properties. *Clinical microbiology reviews*. Vol. 19. n.º 1 (2006). p. 50-62.

CARSON, Christine F; MEE, Brian J; RILEY, Thomas V - Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. *Antimicrobial agents and chemotherapy*. Vol. 46. n.º 6 (2002). p. 1914-1920.

CETIN, Huseyin; CILEK, James E; AYDIN, Levent; YANIKOGLU, Atila - Acaricidal effects of the essential oil of *Origanum minutiflorum* (Lamiaceae) against *Rhipicephalus turanicus* (Acari: Ixodidae). *Veterinary parasitology*. Vol. 160. n.º 3-4 (2009). p. 359-361.

CHATURVEDI, Surabhi; KULSHRESTHA, Sanchita; BHARDWAJ, Khushboo; JANGIR, Rekha - Microbial Polymers: Applications and Ecological Perspectives. Singapore: Springer Singapore, 2021. Cap. - A Review on Properties and Applications of Xanthan Gum.

CHEN, Randy Kong Zheng; WEE, Sia Chee - PERFORMANCE EVALUATION OF ALPHA-OLEFIN SULFONATE (AOS), COCO GLUCOSIDE AND DECANE IN CREATING WINSOR TYPE-III MICROEMULSION. *Platform: A Journal of Engineering*. Vol. 5. n.º 3 (2021). p. 38-59.

CLAFFEY, N - Essential oil mouthwashes: a key component in oral health management. *Journal of clinical periodontology*. Vol. 30. (2003). p. 22-24.

CONG, Yanxia; ZHANG, Weinong; LIU, Changsheng; HUANG, Fenghong - Composition and oil-water interfacial tension studies in different vegetable oils. *Food Biophysics*. Vol. 15. n.º 2 (2020). p. 229-239.

CONLEY, Kenda; CLUM, Allan; DEEPE, Jestine; LANE, Haven; BECKINGHAM, Barbara - Wastewater treatment plants as a source of microplastics to an urban estuary: Removal efficiencies and loading per capita over one year. *Water Research X*. Vol. 3. (2019). p. 100030.

COSENTINO, S.; TUBEROSO, C. I.; PISANO, B.; SATTA, M.; MASCIA, V.; ARZEDI, E.; PALMAS, F. - In-vitro antimicrobial activity and chemical composition of Sardinian Thymus essential oils. *Lett Appl Microbiol*. Vol. 29. n.º 2 (1999). p. 130-5.

COWAN, Marjorie Murphy - Plant products as antimicrobial agents. *Clinical microbiology reviews*. Vol. 12. n.º 4 (1999). p. 564-582. ISSN: 1098-6618

COX, SD; MANN, CM; MARKHAM, JL; BELL, Hanssel C; GUSTAFSON, JE; WARMINGTON, JR; WYLLIE, S Grant - The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). *Journal of applied microbiology*. Vol. 88. n.º 1 (2000). p. 170-175.

CRISTANI, M.; D'ARRIGO, M.; MANDALARI, G.; CASTELLI, F.; SARPIETRO, M. G.; MICIELI, D.; VENUTI, V.; BISIGNANO, G.; SAIJA, A.; TROMBETTA, D. - Interaction of four monoterpenes contained in essential oils with model membranes: implications for their antibacterial activity. *J Agric Food Chem*. Vol. 55. n.º 15 (2007). p. 6300-8.

DAVE, N; JOSHI, T - A concise review on surfactants and its significance. *Int. J. Appl. Chem*. Vol. 13. n.º 3 (2017). p. 663-672.

DE, Sourav; MALIK, Susanta; GHOSH, Aniruddha; SAHA, Rumpa; SAHA, Bidyut - A review on natural surfactants. *RSC advances*. Vol. 5. n.º 81 (2015). p. 65757-65767.

DELAQUIS, P. J.; STANICH, K.; GIRARD, B.; MAZZA, G. - Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *Int J Food Microbiol*. Vol. 74. n.º 1-2 (2002). p. 101-9.

DELGADO, Begoña; FERNÁNDEZ, Pablo S; PALOP, Alfredo; PERIAGO, Paula M - Effect of thymol and cymene on *Bacillus cereus* vegetative cells evaluated through the use of frequency distributions. *Food Microbiology*. Vol. 21. n.º 3 (2004). p. 327-334.

DEMUNER, Antonio Jacinto; ALMEIDA BARBOSA, Luiz Claudio; GONÇALVES MAGALHAES, Cassia; DA SILVA, Cleber Jose; ALVARES MALTHA, Celia Regina; LELIS PINHEIRO, Antonio - Seasonal Variation in the Chemical Composition and Antimicrobial Activity of Volatile Oils of Three Species of Leptospermum (Myrtaceae) Grown in Brazil. *Molecules*. Vol. 16. n.º 2 (2011). p. 1181-1191.

DENYER, SP - Biocide-induced damage to the bacterial cytoplasmic membrane. *Mechanisms of action of chemical biocides*. (1991). p. 171-188.

DESAI, Jitendra D; BANAT, Ibrahim M - Microbial production of surfactants and their commercial potential. *Microbiology and Molecular biology reviews*. Vol. 61. n.º 1 (1997). p. 47-64. ISSN: 1092-2172

DHAKAD, Ashok K; PANDEY, Vijay V; BEG, Sobia; RAWAT, Janhvi M; SINGH, Avtar - Biological, medicinal and toxicological significance of Eucalyptus leaf essential oil: a review. *Journal of the Science of Food and Agriculture*. Vol. 98. n.º 3 (2018). p. 833-848.

DI PASQUA, Rosangela; BETTS, Gail; HOSKINS, Nikki; EDWARDS, Mike; ERCOLINI, Danilo; MAURIELLO, Gianluigi - Membrane toxicity of antimicrobial compounds from essential oils. *Journal of agricultural and food chemistry*. Vol. 55. n.º 12 (2007). p. 4863-4870.

DI PASQUA, Rosangela; HOSKINS, Nikki; BETTS, Gail; MAURIELLO, Gianluigi - Changes in membrane fatty acids composition of microbial cells induced by addiction of thymol, carvacrol, limonene, cinnamaldehyde, and eugenol in the growing media. *Journal of agricultural and food chemistry*. Vol. 54. n.º 7 (2006). p. 2745-2749.

DIAS-FERREIRA, João; FERNANDES, Ana R; SORIANO, José L; NAVEROS, Beatriz C; SEVERINO, Patricia; DA SILVA, Classius Ferreira; SOUTO, Eliana B - Biopolymer Membranes and Films. Elsevier, 2020. - Skin rejuvenation: Biopolymers applied to UV sunscreens and sheet masks.

DÍAZ-CRUZ, M. S.; GARCÍA-GALÁN, M. J.; GUERRA, P.; JELIC, A.; POSTIGO, C.; ELJARRAT, E.; FARRÉ, M.; LÓPEZ DE ALDA, M. J.; PETROVIC, M.; BARCELÓ, D.; PETROVIC, M.; BARCELÓ, D. - Analysis of selected emerging contaminants in sewage sludge. *TrAC Trends in Analytical Chemistry*. Vol. 28. n.º 11 (2009). p. 1263-1275.

DICHARRY, Christophe; DIAZ, Joseph; TORRÉ, Jean-Philippe; RICAURTE, Marvin - Influence of the carbon chain length of a sulfate-based surfactant on the formation of CO2, CH4 and CO2–CH4 gas hydrates. *Chemical Engineering Science*. Vol. 152. (2016). p. 736-745.

DICKINSON, Eric - Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food hydrocolloids*. Vol. 17. n.º 1 (2003). p. 25-39.

DJABOU, Nassim; LORENZI, Vanina; GUINOISEAU, Elodie; ANDREANI, Stéphane; GIULIANI, Marie-Cécile; DESJOBERT, Jean-Marie; BOLLA, Jean-Michel; COSTA, Jean; BERTI, Liliane; LUCIANI, Anne - Phytochemical composition of *Corsican Teucrium* essential oils and antibacterial activity against foodborne or toxi-infectious pathogens. *Food Control*. Vol. 30. n.º 1 (2013). p. 354-363.

DÖLLE, Sarah; LECHNER, Bob-Dan; PARK, Ji Hyun; SCHYMURA, Stefan; LAGERWALL, Jan PF; SCALIA, Giusy - Utilizing the Krafft Phenomenon to Generate Ideal Micelle-Free Surfactant-Stabilized Nanoparticle Suspensions. *Angewandte Chemie International Edition*. Vol. 51. n.º 13 (2012). p. 3254-3257.

DONALDSON, Jack R; WARNER, Steven L; CATES, Rex G; GARY YOUNG, D - Assessment of antimicrobial activity of fourteen essential oils when using dilution and diffusion methods. *Pharmaceutical biology*. Vol. 43. n.º 8 (2005). p. 687-695.

DONSÌ, Francesco; FERRARI, Giovanna - Essential oil nanoemulsions as antimicrobial agents in food. *Journal of biotechnology*. Vol. 233. (2016). p. 106-120.

DORMAN, H. J.; DEANS, S. G. - Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J Appl Microbiol*. Vol. 88. n.° 2 (2000). p. 308-16.

DUNG, N. T.; KIM, J. M.; KANG, S. C. - Chemical composition, antimicrobial and antioxidant activities of the essential oil and the ethanol extract of *Cleistocalyx operculatus* (Roxb.) Merr and Perry buds. *Food Chem Toxicol*. Vol. 46. n.° 12 (2008). p. 3632-9.

EDRIS, AE; MALONE, CFR - Preferential solubilization behaviours and stability of some phenolic-bearing essential oils formulated in different microemulsion systems. *International journal of cosmetic science*. Vol. 34. n.° 5 (2012). p. 441-450.

ENAYATIFARD, Reza; AKBARI, Jafar; BABAEI, Amirhossein; ROSTAMKALAEI, Seyyed Sohrab; HASHEMI, Seyyed Mohammad Hassan; HABIBI, Emran - Anti-microbial potential of nano-emulsion form of essential oil obtained from aerial parts of *Origanum Vulgare* L. as Food Additive. *Advanced Pharmaceutical Bulletin*. Vol. 11. n.° 2 (2021). p. 327.

ENNAJAR, M.; BOUAJILA, J.; LEBRIHI, A.; MATHIEU, F.; ABDERRABA, M.; RAIES, A.; ROMDHANE, M. - Chemical composition and antimicrobial and antioxidant activities of essential oils and various extracts of *Juniperus phoenicea* L. (Cupressacees). *J Food Sci*. Vol. 74. n.° 7 (2009). p. M364-71.

ESEN, Gülden; AZAZ, Ayse Dilek; KURKCUOGLU, Mine; BASER, Kemal Husnu Can; TINMAZ, Ahmet - Essential oil and antimicrobial activity of wild and cultivated *Origanum vulgare* L. subsp. *hirtum* (Link) letswaart from the Marmara region, Turkey. *Flavour and Fragrance Journal*. Vol. 22. n.° 5 (2007). p. 371-376.

FALBE, Jürgen - Surfactants in consumer products: Theory, Technology and Application. Springer Science & Business Media, 2012.

FANCELLO, Francesco; PETRETTO, Giacomo Luigi; ZARA, Severino; SANNA, Maria Lina; ADDIS, Roberta; MALDINI, Mariateresa; FODDAI, Marzia; ROURKE, Jonathan P; CHESSA, Mario; PINTORE, Giorgio - Chemical characterization, antioxidant capacity and antimicrobial activity against food related microorganisms of *Citrus limon* var. *pompia* leaf essential oil. *LWT-Food Science and Technology*. Vol. 69. (2016). p. 579-585.

FANG, Fang; LUO, Xuan; BEMILLER, James N; SCHAFFTER, Samuel; HAYES, Anna MR; WOODBURY, Travest J; HAMAKER, Bruce R; CAMPANELLA, Osvaldo H - Neutral

hydrocolloids promote shear-induced elasticity and gel strength of gelatinized waxy potato starch. *Food Hydrocolloids*. Vol. 107. (2020). p. 105923.

FENGEL, Dietrich - The ultrastructure of cellulose from wood. *Wood Science and Technology*. Vol. 3. n.º 3 (1969). p. 203-217.

FIORAMONTI, Silvana A; MARTINEZ, María J; PILOSOF, Ana MR; RUBIOLO, Amelia C; SANTIAGO, Liliana G - Multilayer emulsions as a strategy for linseed oil microencapsulation: Effect of pH and alginate concentration. *Food Hydrocolloids*. Vol. 43. (2015). p. 8-17.

FIUME, Monice M; HELDRETH, Bart; BERGFELD, Wilma F; BELSITO, Donald V; HILL, Ronald A; KLAASSEN, Curtis D; LIEBLER, Daniel; MARKS JR, James G; SHANK, Ronald C; SLAGA, Thomas J - Safety assessment of decyl glucoside and other alkyl glucosides as used in cosmetics. *International Journal of Toxicology*. Vol. 32. n.º 5_suppl (2013). p. 22S-48S.

FORCE, Mark; SPARKS, William S; RONZIO, Robert A - Inhibition of enteric parasites by emulsified oil of oregano in vivo. *Phytotherapy Research*. Vol. 14. n.º 3 (2000). p. 213-214.

FRAENKEL, G. S. - The raison d'ĕtre of secondary plant substances; these odd chemicals arose as a means of protecting plants from insects and now guide insects to food. *Science*. Vol. 129. n.º 3361 (1959). p. 1466-70.

GALLUCCI, Mauro Nicolas; OLIVA, M; CASERO, C; DAMBOLENA, J; LUNA, Agustin; ZYGADLO, J; DEMO, M - Antimicrobial combined action of terpenes against the food-borne microorganisms *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*. *Flavour and fragrance journal*. Vol. 24. n.º 6 (2009). p. 348-354.

GAO, Peng; LEI, Tingting; JIA, Liming; YURY, Badmatsybenov; ZHANG, Zhaohan; DU, Yingqiu; FENG, Yujie; XING, Baoshan - Bioaccessible trace metals in lip cosmetics and their health risks to female consumers. *Environmental Pollution*. Vol. 238. (2018). p. 554-561.

GAO, Yuyang; YANG, Xiuquan; BAI, Liang; ZHANG, Jun - Preparation and physiochemical properties of disodium lauryl glucoside sulfosuccinate. *Journal of Surfactants and Detergents*. Vol. 17. n.º 4 (2014). p. 603-608.

GARCÍA-SALAS, Patricia; GÓMEZ-CARAVACA, Ana María; ARRÁEZ-ROMÁN, David; SEGURA-CARRETERO, Antonio; GUERRA-HERNÁNDEZ, Eduardo; GARCÍA-VILLANOVA, Belén; FERNÁNDEZ-GUTIÉRREZ, Alberto - Influence of technological processes on phenolic compounds, organic acids, furanic derivatives, and antioxidant activity of whole-lemon powder. *Food chemistry*. Vol. 141. n.º 2 (2013). p. 869-878.

GAWADE, Rohini P.; CHINKE, Shamal L.; ALEGAONKAR, Prashant S. - Polymer Science and Innovative Applications. Elsevier, 2020. Cap. - Chapter 17 - Polymers in cosmetics.

GEETHA, Daz; TYAGI, Rashmi - Alkyl poly glucosides (APGs) surfactants and their properties: a review. *Tenside surfactants detergents*. Vol. 49. n.º 5 (2012). p. 417-427.

GILLES, Martin; ZHAO, Jian; AN, Min; AGBOOLA, Samson - Chemical composition and antimicrobial properties of essential oils of three Australian Eucalyptus species. *Food Chemistry*. Vol. 119. (2010). p. 731-737.

GLENNIE, Andrew R; MOHAREB, Michael M; PALEPU, Rama M - Thermodynamic and related properties of alkyl cationic surfactants based on dimethyl and diethyl ethanol amines. *Journal of dispersion science and technology*. Vol. 27. n.º 5 (2006). p. 731-738.

GOLDBECK, Júlia Coswig; DO NASCIMENTO, José Edmilson; JACOB, Raquel G; FIORENTINI, Ângela Maria; DA SILVA, Wladimir Padilha - Bioactivity of essential oils from *Eucalyptus globulus* and *Eucalyptus urograndis* against planktonic cells and biofilms of *Streptococcus mutans*. *Industrial Crops and Products*. Vol. 60. (2014). p. 304-309.

GONZÁLEZ-MOLINA, E; DOMÍNGUEZ-PERLES, R; MORENO, DA; GARCÍA-VIGUERA, C - Natural bioactive compounds of *Citrus limon* for food and health. *Journal of pharmaceutical and biomedical analysis*. Vol. 51. n.º 2 (2010). p. 327-345.

GRIFFIN, Shane G; WYLLIE, S Grant; MARKHAM, Julie L; LEACH, David N - The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. *Flavour and Fragrance Journal*. Vol. 14. n.º 5 (1999). p. 322-332.

GUARDA, Abel; RUBILAR, Javiera F; MILTZ, Joseph; GALOTTO, Maria Jose - The antimicrobial activity of microencapsulated thymol and carvacrol. *International journal of food microbiology*. Vol. 146. n.º 2 (2011). p. 144-150.

HAMMER, Katherine A; CARSON, Christine F; RILEY, Thomas V - Antimicrobial activity of essential oils and other plant extracts. *Journal of applied microbiology*. Vol. 86. n.º 6 (1999). p. 985-990.

HANIF, Muhammad Asif; NISAR, Shafaq; KHAN, Ghufrana Samin; MUSHTAQ, Zahid; ZUBAIR, Muhammad - Essential Oils. (2019). p. 3-17.

HARUTYUNYAN, Lusine R; HARUTYUNYAN, Romik S - Effect of amino acids on micellization and micellar parameters of anionic surfactant alpha olefin sulfonate C14–C16 in aqueous solutions: surface tension, conductometric, volumetric, and fluorescence studies. *Journal of Chemical & Engineering Data*. Vol. 64. n.º 2 (2019). p. 640-650.

HAYATI, Ibrahim Nor; CHING, Cheong Wai; ROZAINI, Mohd Zul Helmi - Flow properties of o/w emulsions as affected by xanthan gum, guar gum and carboxymethyl cellulose interactions studied by a mixture regression modelling. *Food Hydrocolloids*. Vol. 53. (2016). p. 199-208.

HAYATI, Ibrahim Nor; MAN, Yaakob Bin Che; TAN, Chin Ping; AINI, Idris Nor - Droplet characterization and stability of soybean oil/palm kernel olein O/W emulsions with the presence of selected polysaccharides. *Food hydrocolloids*. Vol. 23. n.º 2 (2009). p. 233-243.

HE, Jiarong; ZHONG, Haoxiang; WANG, Jinglun; ZHANG, Lingzhi - Investigation on xanthan gum as novel water soluble binder for LiFePO4 cathode in lithium-ion batteries. *Journal of Alloys and Compounds*. Vol. 714. (2017). p. 409-418.

HE, Qiao; ZHANG, Lianjiao; YANG, Zhehao; DING, Tian; YE, Xingqian; LIU, Donghong; GUO, Mingming - Antibacterial mechanisms of thyme essential oil nanoemulsions against *Escherichia coli* O157:H7 and *Staphylococcus aureus*: Alterations in membrane compositions and characteristics. *Innovative Food Science & Emerging Technologies*. Vol. 75. (2022). p. 102902.

HELANDER, Ilkka M; ALAKOMI, Hanna-Leena; LATVA-KALA, Kyösti; MATTILA-SANDHOLM, Tiina; POL, Irene; SMID, Eddy J; GORRIS, Leon GM; VON WRIGHT, Atte - Characterization of the action of selected essential oil components on Gram-negative bacteria. *Journal of agricultural and food chemistry*. Vol. 46. n.º 9 (1998). p. 3590-3595.

HERBERT, AL; MARTIN, MR; GILBERT, SB - Pharmaceutical Emulsions and Microemulsions, Pharmaceutical Dosage Forms Disperse Systems. Marcel Dekker, INC, New York and Basel, USA, 1996.

HERCULES, Sakkas - Antimicrobial Activity of Basil, Oregano, and Thyme Essential Oils. *Journal of Microbiology and Biotechnology*. Vol. 27. n.º 3 (2017). p. 429-438.

HERRMANN, KW - Micellar properties of some zwitterionic surfactants. *Journal of Colloid and Interface Science*. Vol. 22. n.º 4 (1966). p. 352-359.

HNAWIA, Edouard; BROPHY, Joseph J; CRAVEN, Lyn A; LEBOUVIER, Nicolas; CABALION, Pierre; NOUR, Mohammed - An examination of the leaf essential oils of the endemic Melaleuca (Myrtaceae) species of New Caledonia. *Journal of Essential Oil Research*. Vol. 24. n.º 3 (2012). p. 273-278.

HOLMBERG, Krister; SHAH, Dinesh Ochhavlal; SCHWUGER, Milan J - Handbook of applied surface and colloid chemistry. Wiley-Blackwell, 2002.

HU, Dan; MAFI, Amirhossein; CHOU, Keng C - Revisiting the thermodynamics of water surfaces and the effects of surfactant head group. *The Journal of Physical Chemistry B*. Vol. 120. n.° 9 (2016). p. 2257-2261.

HUMPHRIES, Romney M.; AMBLER, Jane; MITCHELL, Stephanie L.; CASTANHEIRA, Mariana; DINGLE, Tanis; HINDLER, Janet A.; KOETH, Laura; SEI, Katherine; HARDY, Dwight; ZIMMER, Barbara; BUTLER-WU, Susan; BARD, Jennifer Dien; BRASSO, Bill; SHAWAR, Ribhi; DINGLE, Tanis; HUMPHRIES, Romney; SEI, Katherine; KOETH, Laura; KRAFT, Colleen Suzanne - CLSI Methods Development and Standardization Working Group Best Practices for Evaluation of Antimicrobial Susceptibility Tests. *Journal of Clinical Microbiology*. Vol. 56. n.° 4 (2018). p. e01934-17.

HUSSEIN, AM; MAHMOUD, KF; HEGAZY, NA; KAMIL, MM; MOHAMMAD, AA; MEHAYA, FM - Efficiency of micro and nano encapsulated orange peel essential oils on quality of sponge cake. *J Environ Sci Tech*. Vol. 12. (2019). p. 26-37.

HYLDGAARD, M.; MYGIND, T.; MEYER, R. L. - Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. *Front Microbiol*. Vol. 3. (2012). p. 12.

IMELOUANE, B; AMHAMDI, H; WATHELET, Jean-Paul; ANKIT, M; KHEDID, K; EL BACHIRI, A - Chemical composition and antimicrobial activity of essential oil of thyme (*Thymus vulgaris*) from Eastern Morocco. *Int. J. Agric. Biol*. Vol. 11. n.° 2 (2009). p. 205-208.

ISLAM, Nazrul; SHARKER, Komol Kanta; SARKER, Khokan Chandra - Salt-induced modulation of the Krafft temperature and critical micelle concentration of benzyldimethylhexadecylammonium chloride. *Journal of Surfactants and Detergents*. Vol. 18. n.° 4 (2015). p. 651-659.

JAISWAL, Manjit; DUDHE, Rupesh; SHARMA, PK - Nanoemulsion: an advanced mode of drug delivery system. *3 Biotech*. Vol. 5. n.° 2 (2015). p. 123-127.

JAN, abdul Khaliq - Surface and thermodynamic study of micellization of non ionic surfactant/diblock copolymer system as revealed by surface tension and conductivity. *Journal of Materials and Environmental Science*. Vol. 8. (2017). p. 1161-67.

JIANG, Yang; WU, Nan; FU, Yu-Jie; WANG, Wei; LUO, Meng; ZHAO, Chun-Jian; ZU, Yuan-Gang; LIU, Xiao-Lei - Chemical composition and antimicrobial activity of the essential oil of Rosemary. *Environmental toxicology and pharmacology*. Vol. 32. n.° 1 (2011). p. 63-68.

KALEMBA, Danuta; SYNOWIEC, Agnieszka - Agrobiological interactions of essential oils of two menthol mints: *Mentha piperita* and *Mentha arvensis*. *Molecules*. Vol. 25. n.º 1 (2019). p. 59.

KARR, S; HOUTMAN, A; INTERLANDI, J - Toxic bottles? On the trail of chemicals in our everyday lives. *American Environmental Science for a Changing World; Kate Ahr, P., Ed*. (2013). p. 54.

KASKOOS, Raad A - Essential oil analysis by GC-MS and analgesic activity of *Lippia citriodora* and *Citrus limon*. *Journal of essential oil bearing plants*. Vol. 22. n.º 1 (2019). p. 273-281.

KLIMEK-SZCZYKUTOWICZ, Marta; SZOPA, Agnieszka; EKIERT, Halina - *Citrus limon* (Lemon) phenomenon—a review of the chemistry, pharmacological properties, applications in the modern pharmaceutical, food, and cosmetics industries, and biotechnological studies. *Plants*. Vol. 9. n.º 1 (2020). p. 119.

KNOBLOCH, K; WEIGAND, H; WEIS, N; SCHWARM, HM; VIGENSCHOW, H - Action of terpenoids on energy metabolism. Walter de Gruyter: Berlin, Germany, 1986.

KOKKINI, Stella; KAROUSOU, Regina; DARDIOTI, Antonia; KRIGAS, Nikos; LANARAS, Tom - Autumn essential oils of Greek oregano. *Phytochemistry*. Vol. 44. n.º 5 (1997). p. 883-886.

KRSTONOŠIĆ, Veljko; DOKIĆ, Ljubica; NIKOLIĆ, Ivana; MILANOVIĆ, Maja - Influence of xanthan gum on oil-in-water emulsion characteristics stabilized by OSA starch. *Food Hydrocolloids*. Vol. 45. (2015). p. 9-17.

KULKARNI, Chandrashekhar V - Lipid self-assemblies and nanostructured emulsions for cosmetic formulations. *Cosmetics*. Vol. 3. n.º 4 (2016). p. 37.

LACIAR, A; VACA RUIZ, ML; CARRIZO FLORES, R; SAAD, JR - Actividad antibacteriana y antioxidante del aceite esencial extraído de *Artemisia echegarayi* Hieron.(Asteraceae). *Revista argentina de microbiología*. Vol. 41. n.º 4 (2009). p. 226-231.

LAMBERT, RJW; SKANDAMIS, Proteus N; COOTE, Proteus J; NYCHAS, G-JE - A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of applied microbiology*. Vol. 91. n.º 3 (2001). p. 453-462.

LIMAM, Hajer; JEMAA, Mariem Ben; TAMMAR, Sonia; KSIBI, Nour; KHAMMASSI, Saber; JALLOULI, Selim; DEL RE, Giovanni; MSAADA, Kamel - Variation in chemical profile of leaves essential oils from thirteen Tunisian Eucalyptus species and evaluation of their antioxidant and antibacterial properties. *Industrial Crops and Products*. Vol. 158. (2020). p. 112964.

LIU, Nannan; SHI, Yali; LI, Wenhui; XU, Lin; CAI, Yaqi - Concentrations and distribution of synthetic musks and siloxanes in sewage sludge of wastewater treatment plants in China. *Science of The Total Environment*. Vol. 476-477. (2014). p. 65-72.

LOPES-LUTZ, Daíse; ALVIANO, Daniela S; ALVIANO, Celuta S; KOLODZIEJCZYK, Paul P - Screening of chemical composition, antimicrobial and antioxidant activities of Artemisia essential oils. *Phytochemistry*. Vol. 69. n.º 8 (2008). p. 1732-1738.

LOPEZ, O; COCERA, M; PARRA, JL; DE LA MAZA, A - Influence of the alkyl chain length of alkyl glucosides on their ability to solubilize phosphatidylcholine liposomes. *Colloids and surfaces A: physicochemical and engineering aspects*. Vol. 193. n.º 1-3 (2001). p. 221-229.

LOURITH, Nattaya; KANLAYAVATTANAKUL, Mayuree - Natural surfactants used in cosmetics: glycolipids. *International journal of cosmetic science*. Vol. 31. n.º 4 (2009). p. 255-261.

LUBBE, Andrea; VERPOORTE, Robert - Cultivation of medicinal and aromatic plants for specialty industrial materials. *Industrial crops and products*. Vol. 34. n.º 1 (2011). p. 785-801.

MACCELLI, Alessandro; VITANZA, Luca; IMBRIANO, Anna; FRASCHETTI, Caterina; FILIPPI, Antonello; GOLDONI, Paola; MAURIZI, Linda; AMMENDOLIA, Maria Grazia; CRESTONI, Maria Elisa; FORNARINI, Simonetta; MENGHINI, Luigi; CARAFA, Maria; MARIANECCI, Carlotta; LONGHI, Catia; RINALDI, Federica - *Satureja montana* L. Essential Oils: Chemical Profiles/Phytochemical Screening, Antimicrobial Activity and O/W NanoEmulsion Formulations. *Pharmaceutics*. Vol. 12. n.º 1 (2020). p. 7.

MAHATO, Neelima; SHARMA, Kavita; KOTESWARARAO, Rakoti; SINHA, Mukty; BARAL, EkRaj; CHO, Moo Hwan - Citrus essential oils: Extraction, authentication and application in food preservation. *Critical reviews in food science and nutrition*. Vol. 59. n.º 4 (2019). p. 611-625.

MAHENDRAN, Ganesan; RAHMAN, Laiq-Ur - Ethnomedicinal, phytochemical and pharmacological updates on Peppermint (*Mentha× piperita* L.)—A review. *Phytotherapy Research*. Vol. 34. n.º 9 (2020). p. 2088-2139.

MAHMOOD, May Essa; AL-KOOFEE, Dhafer AF - Effect of temperature changes on critical micelle concentration for tween series surfactant. *Global Journal of Science Frontier Research Chemistry*. Vol. 13. n.º 4 (2013). p. 1-7.

MALIK, Nisar Ahmad; ALI, Anwar - Krafft temperature and thermodynamic study of interaction of glycine, diglycine, and triglycine with hexadecylpyridinium chloride and hexadecylpyridinium bromide: A conductometric approach. *Journal of Molecular Liquids*. Vol. 213. (2016). p. 213-220.

MAN, Adrian; SANTACROCE, Luigi; IACOB, Romeo; MARE, Anca; MAN, Lidia - Antimicrobial activity of six essential oils against a group of human pathogens: A comparative study. *Pathogens*. Vol. 8. n.º 1 (2019). p. 15.

MANN, B; MALIK, RC - Studies on some functional characteristics of whey protein-polysaccharide complex. *Journal of food science and technology (Mysore)*. Vol. 33. n.º 3 (1996). p. 202-206. ISSN: 0022-1155

MANOHAR, Vijaya; INGRAM, Cass; GRAY, Judy; TALPUR, Nadeem A; ECHARD, Bobby W; BAGCHI, Debasis; PREUSS, Harry G - Antifungal activities of origanum oil against *Candida albicans*. *Molecular and cellular biochemistry*. Vol. 228. n.º 1 (2001). p. 111-117.

MARINO, Marilena; BERSANI, Carla; COMI, Giuseppe - Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. *International journal of food microbiology*. Vol. 67. n.º 3 (2001). p. 187-195.

MARKET, Vertical Farming - Global Opportunity Analysis and Industry Forecast, 2017-2023. *Allied Market Research: Philadelphia, PA, USA*. (2016).

MASSARWEH, Osama; ABUSHAIKHA, Ahmad S. - The use of surfactants in enhanced oil recovery: A review of recent advances. *Energy Reports*. Vol. 6. (2020). p. 3150-3178.

MAYAUD, L; CARRICAJO, A; ZHIRI, A; AUBERT, G - Comparison of bacteriostatic and bactericidal activity of 13 essential oils against strains with varying sensitivity to antibiotics. *Letters in applied microbiology*. Vol. 47. n.º 3 (2008). p. 167-173.

MCKAY, Diane L; BLUMBERG, Jeffrey B - A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.). *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*. Vol. 20. n.º 8 (2006). p. 619-633.

MEYBODI, Mahdi Kalantari; SHOKROLLAHI, Amin; SAFARI, Hossein; LEE, Moonyong; BAHADORI, Alireza - A computational intelligence scheme for prediction of interfacial tension between pure hydrocarbons and water. *Chemical Engineering Research and Design*. Vol. 95. (2015). p. 79-92.

MIMICA-DUKIĆ, N.; BOZIN, B.; SOKOVIĆ, M.; MIHAJLOVIĆ, B.; MATAVULJ, M. - Antimicrobial and antioxidant activities of three Mentha species essential oils. *Planta Med*. Vol. 69. n.º 5 (2003). p. 413-9.

MITH, Hasika; DURE, Rémi; DELCENSERIE, Véronique; ZHIRI, Abdesselam; DAUBE, Georges; CLINQUART, Antoine - Antimicrobial activities of commercial essential oils and

their components against food-borne pathogens and food spoilage bacteria. *Food science & nutrition*. Vol. 2. n.º 4 (2014). p. 403-416.

MKADDEM, M.; BOUAJILA, J.; ENNAJAR, M.; LEBRIHI, A.; MATHIEU, F.; ROMDHANE, M. - Chemical composition and antimicrobial and antioxidant activities of Mentha (*longifolia* L. and *viridis*) essential oils. *J Food Sci*. Vol. 74. n.º 7 (2009). p. M358-63.

MOCKUTE, Danute; BERNOTIENE, Genovaite; JUDZENTIENE, Asta - The β-ocimene chemotype of essential oils of the inflorescences and the leaves with stems from *Origanum vulgare* ssp. *vulgare* growing wild in Lithuania. *Biochemical systematics and ecology*. Vol. 31. n.º 3 (2003). p. 269-278.

MOGHIMI, Roya; ALIAHMADI, Atousa; MCCLEMENTS, David Julian; RAFATI, Hasan - Investigations of the effectiveness of nanoemulsions from sage oil as antibacterial agents on some food borne pathogens. *LWT-Food Science and Technology*. Vol. 71. (2016a). p. 69-76.

MOGHIMI, Roya; GHADERI, Lida; RAFATI, Hasan; ALIAHMADI, Atousa; MCCLEMENTS, David Julian - Superior antibacterial activity of nanoemulsion of *Thymus daenensis* essential oil against *E. coli*. *Food chemistry*. Vol. 194. (2016b). p. 410-415. ISSN: 0308-8146

MOHAMED, Abdelhalim I. A.; SULTAN, Abdullah S.; HUSSEIN, Ibnelwaleed A.; AL-MUNTASHERI, Ghaithan A. - Influence of Surfactant Structure on the Stability of Water-in-Oil Emulsions under High-Temperature High-Salinity Conditions. *Journal of Chemistry*. Vol. 2017. (2017). p. 5471376.

MOHAPATRA, SS; JHA, JM; SRINATH, K; PAL, SK; CHAKRABORTY, S - Enhancement of cooling rate for a hot steel plate using air-atomized spray with surfactant-added water. *Experimental Heat Transfer*. Vol. 27. n.º 1 (2014). p. 72-90.

MONTES-BELMONT, R.; CARVAJAL, M. - Control of *Aspergillus flavus* in maize with plant essential oils and their components. *J Food Prot*. Vol. 61. n.º 5 (1998). p. 616-9.

MOON, Theodore L; BLANZAT, Muriel; LABADIE, Laurent; PEREZ, Emile; RICO-LATTES, Isabelle - Synthesis and physicochemical study of new surfactants derived from carboxylic acid sugars. *Journal of dispersion science and technology*. Vol. 22. n.º 2-3 (2001). p. 167-176.

MOOSAVY, MH; HASSANZADEH, P; MOHAMMADZADEH, E; MAHMOUDI, R; KHATIBI, SA; MARDANI, K - Antioxidant and antimicrobial activities of essential oil of Lemon (*Citrus limon*) peel in vitro and in a food model. *Journal of food quality and hazards control*. Vol. 4. n.º 2 (2017). p. 42-48.

MUNTEAN, Delia; LICKER, Monica; ALEXA, Ersilia; POPESCU, Iuliana; JIANU, Calin; BUDA, Valentina; DEHELEAN, Cristina Adriana; GHIULAI, Roxana; HORHAT, Florin; HORHAT, Delia - Evaluation of essential oil obtained from *Mentha× piperita* L. against multidrug-resistant strains. *Infection and Drug Resistance*. Vol. 12. (2019). p. 2905.

NABAVI, Seyed Mohammad; MARCHESE, Anna; IZADI, Morteza; CURTI, Valeria; DAGLIA, Maria; NABAVI, Seyed Fazel - Plants belonging to the genus Thymus as antibacterial agents: From farm to pharmacy. *Food chemistry*. Vol. 173. (2015). p. 339-347.

NAEEM, M.; AFTAB, Tariq; IDREES, Mohd; SINGH, Minu; ALI, Akbar; KHAN, M. Masroor A.; UDDIN, Moin; VARSHNEY, Lalit - Modulation of physiological activities, active constituents and essential oil production of *Mentha arvensis* L. by concomitant application of depolymerised carrageenan, triacontanol and 28-homobrassinolide. *Journal of Essential Oil Research*. Vol. 29. n.º 2 (2017). p. 179-188.

NAGARAJAN, R. - Molecular Packing Parameter and Surfactant Self-Assembly: The Neglected Role of the Surfactant Tail. *Langmuir*. Vol. 18. n.º 1 (2002). p. 31-38.

NAIDU, AS - Natural food antimicrobial systems. CRC press, 2000.

NAKATSU, Tetsuo; LUPO JR, Andrew T; CHINN JR, John W; KANG, Raphael KL - Biological activity of essential oils and their constituents. *Studies in natural products chemistry*. Vol. 21. (2000). p. 571-631.

NAMAZI, Hassan - Polymers in our daily life. *BioImpacts: BI*. Vol. 7. n.º 2 (2017). p. 73.

NANNAPANENI, R.; CHALOVA, V. I.; CRANDALL, P. G.; RICKE, S. C.; JOHNSON, M. G.; O'BRYAN, C. A. - *Campylobacter* and *Arcobacter* species sensitivity to commercial orange oil fractions. *Int J Food Microbiol*. Vol. 129. n.º 1 (2009). p. 43-9.

NASERI, Neda; AJORLOU, Elham; ASGHARI, Fatemeh; PILEHVAR-SOLTANAHMADI, Younes - An update on nanoparticle-based contrast agents in medical imaging. *Artificial cells, nanomedicine, and biotechnology*. Vol. 46. n.º 6 (2018). p. 1111-1121.

NAZZARO, Filomena; FRATIANNI, Florinda; DE MARTINO, Laura; COPPOLA, Raffaele; DE FEO, Vincenzo - Effect of essential oils on pathogenic bacteria. *Pharmaceuticals*. Vol. 6. n.º 12 (2013). p. 1451-1474.

NICKAVAR, Bahman; MOJAB, Faraz; DOLAT-ABADI, Reza - Analysis of the essential oils of two Thymus species from Iran. *Food chemistry*. Vol. 90. n.º 4 (2005). p. 609-611.

NIKAIDO, H. - Molecular basis of bacterial outer membrane permeability revisited. *Microbiol Mol Biol Rev*. Vol. 67. n.º 4 (2003). p. 593-656.

NIRMAL, Nilesh Prakash; MEREDDY, Ram; LI, Li; SULTANBAWA, Yasmina - Formulation, characterisation and antibacterial activity of lemon myrtle and anise myrtle essential oil in water nanoemulsion. *Food chemistry*. Vol. 254. (2018). p. 1-7.

NOHYNEK, Gerhard J; ANTIGNAC, Eric; RE, Thomas; TOUTAIN, Herve - Safety assessment of personal care products/cosmetics and their ingredients. *Toxicology and applied pharmacology*. Vol. 243. n.° 2 (2010). p. 239-259.

NOR HAYATI, Ibrahim; WAI CHING, Cheong; ROZAINI, Mohd Zul Helmi - Flow properties of o/w emulsions as affected by xanthan gum, guar gum and carboxymethyl cellulose interactions studied by a mixture regression modelling. *Food Hydrocolloids*. Vol. 53. (2016). p. 199-208.

NOSTRO, Antonia; BLANCO, Anna R; CANNATELLI, Maria A; ENEA, Vincenzo; FLAMINI, Guido; MORELLI, Ivano; SUDANO ROCCARO, Andrea; ALONZO, Vittorio - Susceptibility of methicillin-resistant staphylococci to oregano essential oil, carvacrol and thymol. *FEMS microbiology letters*. Vol. 230. n.° 2 (2004). p. 191-195.

OKE, Feyza; ASLIM, Belma; OZTURK, Sahlan; ALTUNDAG, Senol - Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten. *Food Chemistry*. Vol. 112. n.° 4 (2009). p. 874-879.

OUGIYA, Hiroshi; WATANABE, Kunihiko; MORINAGA, Yasushi; YOSHINAGA, Fumihiro - Emulsion-stabilizing effect of bacterial cellulose. *Bioscience, biotechnology, and biochemistry*. Vol. 61. n.° 9 (1997). p. 1541-1545.

OUSSALAH, Mounia; CAILLET, Stephane; LACROIX, Monique - Mechanism of action of Spanish oregano, Chinese cinnamon, and savory essential oils against cell membranes and walls of Escherichia coli O157: H7 and Listeria monocytogenes. *Journal of food protection*. Vol. 69. n.° 5 (2006). p. 1046-1055.

OWENS, Cory; GRIFFIN, Kristen; KHOURYIEH, Hanna; WILLIAMS, Kevin - Creaming and oxidative stability of fish oil-in-water emulsions stabilized by whey protein-xanthan-locust bean complexes: Impact of pH. *Food Chemistry*. Vol. 239. (2018). p. 314-322. ISSN: 0308-8146

PADALIA, Rajendra C; VERMA, Ram S; CHAUHAN, Amit; GOSWAMI, Prakash; VERMA, Sajendra K; DAROKAR, Mahendra P - Chemical composition of *Melaleuca linarrifolia* Sm. from India: a potential source of 1, 8-cineole. *Industrial Crops and Products*. Vol. 63. (2015). p. 264-268.

PAIBON, W.; YIMNOI, C. A.; TEMBAB, N.; BOONLUE, W.; JAMPACHAISRI, K.; NUENGCHAMNONG, N.; WARANUCH, N.; INGKANINAN, K. - Comparison and evaluation of volatile oils from three different extraction methods for some Thai fragrant flowers. *Int J Cosmet Sci*. Vol. 33. n.º 2 (2011). p. 150-6.

PEARSON, James P; VAN DELDEN, Christian; IGLEWSKI, Barbara H - Active efflux and diffusion are involved in transport of *Pseudomonas aeruginosa* cell-to-cell signals. *Journal of bacteriology*. Vol. 181. n.º 4 (1999). p. 1203-1210.

PITMAN, S - Organic personal care market likely to post double-digit annual growth to 2020. *Cosmeticsdesign. com*. (2014).

POSADZKI, Paul; ALOTAIBI, Amani; ERNST, Edzard - Adverse effects of aromatherapy: a systematic review of case reports and case series. *International Journal of Risk & Safety in Medicine*. Vol. 24. n.º 3 (2012). p. 147-161.

PRABUSEENIVASAN, Seenivasan; JAYAKUMAR, Manickkam; IGNACIMUTHU, Savarimuthu - In vitro antibacterial activity of some plant essential oils. *BMC complementary and alternative medicine*. Vol. 6. n.º 1 (2006). p. 1-8.

RADI, Mohsen; AMIRI, Sedigheh - Comparison of the Rheological Behavior of Solutions and Formulated Oil-in-Water Emulsions Containing Carboxymethylcellulose (CMC). *Journal of Dispersion Science and Technology*. Vol. 34. n.º 4 (2013). p. 582-589.

RAHMAN, Md. Saifur; HASAN, Md. Saif; NITAI, Ashis Sutradhar; NAM, Sunghyun; KARMAKAR, Aneek Krishna; AHSAN, Md. Shameem; SHIDDIKY, Muhammad J. A.; AHMED, Mohammad Boshir - Recent Developments of Carboxymethyl Cellulose. *Polymers*. Vol. 13. n.º 8 (2021). p. 1345.

RAJU, G; MARIDAS, M - Composition, Antifungal and Cytotoxic activities of Essential oils of *Piper barberi* fruits. *Int J Bio Technol*. Vol. 2. n.º 2 (2011). p. 100-105.

RAMOS, Sara; HOMEM, Vera; ALVES, Arminda; SANTOS, Lúcia - A review of organic UV-filters in wastewater treatment plants. *Environment International*. Vol. 86. (2016). p. 24-44.

RAO, Jiajia; CHEN, Bingcan; MCCLEMENTS, David Julian - Improving the Efficacy of Essential Oils as Antimicrobials in Foods: Mechanisms of Action. *Annual Review of Food Science and Technology*. Vol. 10. n.º 1 (2019). p. 365-387.

RASOOLI, Iraj; REZAEI, Mohammad Bagher; ALLAMEH, Abdolamir - Ultrastructural studies on antimicrobial efficacy of thyme essential oils on *Listeria monocytogenes*. *International journal of infectious diseases*. Vol. 10. n.º 3 (2006). p. 236-241.

RASPO, Matías Alejandro; VIGNOLA, María Belén; ANDREATTA, Alfonsina Ester; JULIANI, Héctor Rodolfo - Antioxidant and antimicrobial activities of citrus essential oils from Argentina and the United States. *Food Bioscience*. Vol. 36. (2020). p. 100651.

Regulation (EC) N° 1223/2009 of the European parliament and of th Council on Cosmetic Products. (30 november 2009) [accessed: 15th june 2022]. Available in: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02009R1223-20201203&from=en.

REVOL, J-F; GORING, DAI - On the mechanism of the mercerization of cellulose in wood. *Journal of Applied Polymer Science*. Vol. 26. n.° 4 (1981). p. 1275-1282.

RHAYOUR, Khadija; BOUCHIKHI, Touria; TANTAOUI-ELARAKI, Abdelrhafour; SENDIDE, Khalid; REMMAL, Adnane - The mechanism of bactericidal action of oregano and clove essential oils and of their phenolic major components on *Escherichia coli* and *Bacillus subtilis*. *Journal of essential oil research*. Vol. 15. n.° 5 (2003). p. 356-362.

ROSATO, Antonio; VITALI, Cesare; DE LAURENTIS, Nicolino; ARMENISE, Domenico; MILILLO, Maria Antonietta - Antibacterial effect of some essential oils administered alone or in combination with Norfloxacin. *Phytomedicine*. Vol. 14. n.° 11 (2007). p. 727-732.

ROTA, María C; HERRERA, Antonio; MARTÍNEZ, Rosa M; SOTOMAYOR, Jose A; JORDÁN, María J - Antimicrobial activity and chemical composition of Thymus vulgaris, Thymus zygis and Thymus hyemalis essential oils. *Food control*. Vol. 19. n.° 7 (2008). p. 681-687. ISSN: 0956-7135

ROY, Jagadish Chandra; ISLAM, MD; AKTARUZZAMAN, Gazi - The effect of NaCl on the Krafft temperature and related behavior of cetyltrimethylammonium bromide in aqueous solution. *Journal of Surfactants and Detergents*. Vol. 17. n.° 2 (2014). p. 231-242.

RUSENOVA, N; PARVANOV, P - Antimicrobial activities of twelve essential oils against microorganisms of veterinary importance. *Trakia Journal of sciences*. Vol. 7. n.° 1 (2009).

RUSSO, Alessandra; FORMISANO, Carmen; RIGANO, Daniela; CARDILE, Venera; ARNOLD, Nelly Apostolides; SENATORE, Felice - Comparative phytochemical profile and antiproliferative activity on human melanoma cells of essential oils of three lebanese Salvia species. *Industrial Crops and Products*. Vol. 83. (2016). p. 492-499.

RUSSO, Marina; BONACCORSI, Ivana; COSTA, Rosaria; TROZZI, Alessandra; DUGO, Paola; MONDELLO, Luigi - Reduced time HPLC analyses for fast quality control of citrus essential oils. *Journal of Essential Oil Research*. Vol. 27. n.° 4 (2015). p. 307-315.

SABAHI, Nasim; RAZFAR, Mohammad Reza; HAJIAN, Mansour - Experimental investigation of surfactant-mixed electrolyte into electrochemical discharge machining (ECDM) process. *Journal of Materials Processing Technology*. Vol. 250. (2017). p. 190-202.

SAKAMOTO, Kazutami; LOCHHEAD, Howard; MAIBACH, Howard; YAMASHITA, Yuji - Cosmetic science and technology: theoretical principles and applications. Elsevier, 2017.

SANTOS, AC; PATTEKARI, P; VEIGA, F; LVOV, Y; RIBEIRO, AJ - SONICATION-ASSISTED LAYER-BY-LAYER NANOPAR-TICLES OF RESVERATROL. 2015.

SANTOS, Roberto Christ Vianna; LOPES, Leonardo Quintana Soares; DOS SANTOS ALVES, Camilla Filippi; FAUSTO, Viviane Pedroso; PIZZUTTI, Kauana; BARBOZA, Victor; DE SOUZA, Marcia Ebling; RAFFIN, Renata Platchek; GOMES, Patricia; TAKAMATSU, Daisuke - Antimicrobial activity of tea tree oil nanoparticles against American and European foulbrood diseases agents. *Journal of Asia-Pacific Entomology*. Vol. 17. n.º 3 (2014). p. 343-347.

SARRAZIN, Sandra Layse Ferreira; OLIVEIRA, Ricardo Bezerra; BARATA, Lauro Euclides Soares; MOURÃO, Rosa Helena Veras - Chemical composition and antimicrobial activity of the essential oil of *Lippia grandis* Schauer (Verbenaceae) from the western Amazon. *Food Chemistry*. Vol. 134. n.º 3 (2012). p. 1474-1478.

SCHRAMM, Laurier L; STASIUK, Elaine N; MARANGONI, D Gerrard - 2 Surfactants and their applications. *Annual Reports Section" C"(Physical Chemistry)*. Vol. 99. (2003). p. 3-48.

SCHUBERT, H; ENGEL, R - Product and formulation engineering of emulsions. *Chemical Engineering Research and Design*. Vol. 82. n.º 9 (2004). p. 1137-1143.

SCHULZ, Hartwig; QUILITZSCH, Rolf; KRÜGER, Hans - Rapid evaluation and quantitative analysis of thyme, origano and chamomile essential oils by ATR-IR and NIR spectroscopy. *Journal of Molecular Structure*. Vol. 661. (2003). p. 299-306.

SCOTT, Donald S.; PISKORZ, Jan; BERGOUGNOU, Maurice A.; GRAHAM, Robert; OVEREND, Ralph P. - The role of temperature in the fast pyrolysis of cellulose and wood. *Industrial & Engineering Chemistry Research*. Vol. 27. n.º 1 (1988). p. 8-15.

SENATORE, Felice - Influence of harvesting time on yield and composition of the essential oil of a thyme (*Thymus pulegioides* L.) growing wild in Campania (Southern Italy). *Journal of agricultural and food chemistry*. Vol. 44. n.º 5 (1996). p. 1327-1332.

SEWERYN, Artur - Interactions between surfactants and the skin – Theory and practice. *Advances in Colloid and Interface Science*. Vol. 256. (2018). p. 242-255.

SHAHBAZI, Yasser - Chemical composition and in vitro antibacterial activity of *Mentha spicata* essential oil against common food-borne pathogenic bacteria. *Journal of pathogens*. Vol. 2015. (2015).

SHAO, Ping; FENG, Jieru; SUN, Peilong; XIANG, Ning; LU, Baiyi; QIU, Dan - Recent advances in improving stability of food emulsion by plant polysaccharides. *Food Research International*. Vol. 137. (2020). p. 109376.

SHINODA, Kōzō; KUNIEDA, Hironobu - Conditions to produce so-called microemulsions: Factors to increase the mutual solubility of oil and water by solubilizer. *Journal of Colloid and Interface Science*. Vol. 42. n.º 2 (1973). p. 381-387.

SHINODA, Kozo; YAMAGUCHI, Tokio; HORI, Ryohei - The surface tension and the critical micelle concentration in aqueous solution of β-D-alkyl glucosides and their mixtures. *Bulletin of the Chemical Society of Japan*. Vol. 34. n.º 2 (1961). p. 237-241.

SIKKEMA, J.; DE BONT, J. A.; POOLMAN, B. - Mechanisms of membrane toxicity of hydrocarbons. *Microbiol Rev*. Vol. 59. n.º 2 (1995). p. 201-22.

SIKKEMA, Jan; DE BONT, Jan A; POOLMAN, Bert - Interactions of cyclic hydrocarbons with biological membranes. *Journal of biological Chemistry*. Vol. 269. n.º 11 (1994). p. 8022-8028. ISSN: 0021-9258

SINGH, Pooja; PANDEY, Abhay K - Prospective of essential oils of the genus Mentha as biopesticides: A review. *Frontiers in plant science*. Vol. 9. (2018). p. 1295.

SINGH, Rajinder; SHUSHNI, Muftah A. M.; BELKHEIR, Asma - Antibacterial and antioxidant activities of *Mentha piperita* L. *Arabian Journal of Chemistry*. Vol. 8. n.º 3 (2015). p. 322-328.

SIROLI, Lorenzo; PATRIGNANI, Francesca; GARDINI, Fausto; LANCIOTTI, Rosalba - Effects of sub-lethal concentrations of thyme and oregano essential oils, carvacrol, thymol, citral and trans-2-hexenal on membrane fatty acid composition and volatile molecule profile of *Listeria monocytogenes*, *Escherichia coli* and *Salmonella enteritidis*. *Food Chemistry*. Vol. 182. (2015). p. 185-192.

SKOČIBUŠIĆ, Mirjana; BEZIĆ, Nada; DUNKIĆ, Valerija - Phytochemical composition and antimicrobial activities of the essential oils from *Satureja subspicata* Vis. growing in Croatia. *Food chemistry*. Vol. 96. n.º 1 (2006). p. 20-28.

SMITH-PALMER, A; STEWART, J; FYFE, Lorna - Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Letters in applied microbiology*. Vol. 26. n.º 2 (1998). p. 118-122.

SONBOLI, Ali; BABAKHANI, Babak; MEHRABIAN, Ahmad Reza - Antimicrobial activity of six constituents of essential oil from Salvia. *Zeitschrift für Naturforschung C*. Vol. 61. n.º 3-4 (2006). p. 160-164.

SOYLU, Soner; YIGITBAS, H; SOYLU, EM; KURT, Ş - Antifungal effects of essential oils from oregano and fennel on *Sclerotinia sclerotiorum*. *Journal of applied microbiology*. Vol. 103. n.º 4 (2007). p. 1021-1030.

SRIVASTAVA, Alok; CHAHAR, Vikas; SHARMA, Vishal; SWAIN, Kollola K; HOYLER, Friedrich; MURTHY, Ganti S; SCHERER, Ulrich W; RUPP, Hildegard; KNOLLE, Friedhart; MAEKAWA, Miyuki - Study of toxic elements in river water and wetland using water hyacinth (*Eichhornia crassipes*) as pollution monitor. *Global Challenges*. Vol. 3. n.º 6 (2019). p. 1800087.

STILES, John C; SPARKS, William; RONZIO, Robert A - The inhibition of *Candida albicans* by oregano. *Journal of Applied Nutrition*. Vol. 47. (1995). p. 96-102.

STRINGARO, Annarita; COLONE, Marisa; ANGIOLELLA, Letizia - Antioxidant, Antifungal, Antibiofilm, and Cytotoxic Activities of Mentha spp. Essential Oils. *Medicines*. Vol. 5. n.º 4 (2018). p. 112.

STUART, Marc CA; BOEKEMA, Egbert J - Two distinct mechanisms of vesicle-to-micelle and micelle-to-vesicle transition are mediated by the packing parameter of phospholipid–detergent systems. *Biochimica et Biophysica Acta (BBA)-Biomembranes*. Vol. 1768. n.º 11 (2007). p. 2681-2689.

SUKKARY, MM; SYED, NA; AIAD, I; AZAB, WIME - Synthesis and characterization of some alkyl polyglycosides surfactans. *Journal of Dispersion and Technology*. Vol. 2. (2007). p. 129-137.

SUMIARDI, Ade; SOETARTO, Endang; SUSILANINGSIH, Dwi - Screening and characterization of biosurfactant produced by bacterial consortium in degrading polycyclic aromatic hydrocarbon compound. 2018.

SWAMY, Mallappa Kumara; AKHTAR, Mohd Sayeed; SINNIAH, Uma Rani - Antimicrobial properties of plant essential oils against human pathogens and their mode of action: an updated review. *Evidence-Based Complementary and alternative medicine*. Vol. 2016. (2016).

TAHERIAN, Ali R; BRITTEN, Michel; SABIK, Hassan; FUSTIER, Patrick - Ability of whey protein isolate and/or fish gelatin to inhibit physical separation and lipid oxidation in fish oil-in-water beverage emulsion. *Food Hydrocolloids*. Vol. 25. n.º 5 (2011). p. 868-878.

TAHLAN, Varun - Antimicrobial Activity Of Essential Oil Emulsions And Possible Synergistic Effect On Food Borne Pathogens. 2014.

TAYLOR, P. - Ostwald ripening in emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. Vol. 99. n.º 2 (1995). p. 175-185.

TERNES, Thomas A; JOSS, Adriano; SIEGRIST, Hansruedi - Peer reviewed: scrutinizing pharmaceuticals and personal care products in wastewater treatment. *Environmental science & technology*. Vol. 38. n.º 20 (2004). p. 392A-399A.

TIPPAYATUM, Panitee; CHONHENCHOB, Vanee - Antibacterial Activities of Thymol, Eugenol and Nisin Against Some Food Spoilage Bacteria. 2007.

TRAVIS, P. M. - Theory of emulsions and emulsification. *Journal of Oil & Fat Industries*. Vol. 3. n.º 1 (1926). p. 21-25.

TRILLES, Bénédicte L; BOMBARDA, Isabelle; BOURAÏMA-MADJEBI, Saliou; RAHARIVELOMANANA, Phila; BIANCHINI, Jean-Pierre; GAYDOU, Emile M - Occurrence of various chemotypes in niaouli [*Melaleuca quinquenervia* (Cav.) ST Blake] essential oil from New Caledonia. *Flavour and Fragrance Journal*. Vol. 21. n.º 4 (2006). p. 677-682. 1

TROMBETTA, Domenico; CASTELLI, Francesco; SARPIETRO, Maria Grazia; VENUTI, Vincenza; CRISTANI, Mariateresa; DANIELE, Claudia; SAIJA, Antonella; MAZZANTI, Gabriela; BISIGNANO, Giuseppe - Mechanisms of Antibacterial Action of Three Monoterpenes. *Antimicrobial Agents and Chemotherapy*. Vol. 49. n.º 6 (2005). p. 2474-2478.

TYAGI, Amit Kumar; MALIK, Anushree - Antimicrobial potential and chemical composition of *Eucalyptus globulus* oil in liquid and vapour phase against food spoilage microorganisms. *Food Chemistry*. Vol. 126. n.º 1 (2011). p. 228-235.

UENO, Momoko; ISOKAWA, Naho; FUEDA, Kazuki; NAKAHARA, Suzuka; TESHIMA, Hinako; YAMAMOTO, Nanami; YOKOYAMA, Haruka; NORITSUGU, Yukina; SHIBATA, Koushi; MIYAGAWA, Kourin; TANAKA, Seiko; HIRANO, Takashi; FUJITO, Ayako; TAKASHIMA, Ayaka; KANNO, Kenichi - Practical Chemistry of Long-Lasting Bubbles. *World Journal of Chemical Education*. Vol. 4. n.º 2 (2016). p. 32-44.

ULTEE, A.; BENNIK, M. H.; MOEZELAAR, R. - The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. *Appl Environ Microbiol*. Vol. 68. n.º 4 (2002). p. 1561-8.

ULTEE, Annemieke - Bactericidal action of carvacrol towards the food pathogen *Bacillus cereus*: a case study of a novel approach to mild food preservation. Wageningen University and Research, 2000.

VALIZADEH, Alireza; SHIRZAD, Mahdieh; ESMAEILI, Fariba; AMANI, Amir - Increased antibacterial activity of cinnamon oil microemulsionin comparison with cinnamon oil bulk and nanoemulsion. *Nanomedicine Research Journal*. Vol. 3. n.º 1 (2018). p. 37-43.

WANG, Aiqin; LI, Yiming; YANG, Xiaolong; BAO, Mutai; CHENG, Hua - The enhanced stability and biodegradation of dispersed crude oil droplets by Xanthan Gum as an additive of chemical dispersant. *Marine Pollution Bulletin*. Vol. 118. n.º 1-2 (2017). p. 275-280.

WHANG, Liang-Ming; LIU, Pao-Wen G; MA, Chih-Chung; CHENG, Sheng-Shung - Application of biosurfactants, rhamnolipid, and surfactin, for enhanced biodegradation of diesel-contaminated water and soil. *Journal of hazardous materials*. Vol. 151. n.º 1 (2008). p. 155-163.

WONGPORNCHAI, S; PANDAN, W; PETER, KV - Handbook of herbs and spices. *England: Publishing Limited and CRC Press LLC*. (2006). p. 453-459.

XU, Jiafang; ZHANG, Yang; CHEN, Haixiang; WANG, Pan; XIE, Zhenhua; YAO, Yongji; YAN, Youguo; ZHANG, Jun - Effect of surfactant headgroups on the oil/water interface: An interfacial tension measurement and simulation study. *Journal of Molecular Structure*. Vol. 1052. (2013). p. 50-56.

XU, Xingfeng; LUO, Liping; LIU, Chengmei; MCCLEMENTS, David Julian - Utilization of anionic polysaccharides to improve the stability of rice glutelin emulsions: Impact of polysaccharide type, pH, salt, and temperature. *Food Hydrocolloids*. Vol. 64. (2017). p. 112-122.

XUE, Jun; NGADI, Michael - Effects of methylcellulose, xanthan gum and carboxymethylcellulose on thermal properties of batter systems formulated with different flour combinations. *Food hydrocolloids*. Vol. 23. n.º 2 (2009). p. 286-295.

YASIN, Mursleen; YOUNIS, Adnan; JAVED, Talha; AKRAM, Ahsan; AHSAN, Muhammad; SHABBIR, Rubab; ALI, Muhammad Moaaz; TAHIR, Ayesha; EL-BALLAT, Enas M; SHETEIWY, Mohamed S - River Tea Tree Oil: Composition, Antimicrobial and Antioxidant Activities, and Potential Applications in Agriculture. *Plants*. Vol. 10. n.º 10 (2021). p. 2105.

YI, Fengping; JIN, Ruyue; SUN, Jing; MA, Baodi; BAO, Xiaoli - Evaluation of mechanical-pressed essential oil from Nanfeng mandarin (*Citrus reticulata* Blanco cv. Kinokuni) as a food preservative based on antimicrobial and antioxidant activities. *Lwt*. Vol. 95. (2018). p. 346-353.

YORGANCIOGLU, Ali; BAYRAMOGLU, Eser Eke - Production of cosmetic purpose collagen containing antimicrobial emulsion with certain essential oils. *Industrial crops and products*. Vol. 44. (2013). p. 378-382.

ZHANG, Jiawei; WANG, Yang; FENG, Yixi; DU, Shushan; JIA, Liming - Contact toxicity and repellent efficacy of essential oil from aerial parts of *Melaleuca bracteata* and its major compositions against three kinds of insects. *Journal of Essential Oil Bearing Plants*. Vol. 24. n.° 2 (2021). p. 349-359.

ZHANG, Xiaodan; MA, Ji; LIU, Ningan; LI, Guicun; CHEN, Kezheng - Impact of Calcining Active Material (α− Fe2O3) on Binder Selection in Lithium-Ion Batteries. *Journal of The Electrochemical Society*. Vol. 160. n.° 8 (2013). p. A1163.

ZINOVIADOU, Kyriaki G; KOUTSOUMANIS, Konstantinos P; BILIADERIS, Costas G - Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef. *Meat Science*. Vol. 82. n.° 3 (2009). p. 338-345.