Assessment of microfluidization for the preparation and characterization of submicron emulsions of essential oils

Evaluación de la homogeneización por microcanales para la preparación y caracterización de emulsiones submicrónicas de aceites esenciales

Ph.D. Thesis

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Abstract

This Ph.D. Thesis has been carried out in the frame of the research project "Evaluación de la homogeneización por microcanales para la preparación y caracterización de emulsiones submicrónicas de aceites esenciales" (CTQ2015-70700-P) funded by the Spanish Ministerio de Economía y Competitividad (MINECO) and by the European Commission (FEDER program).

Essential oils are gaining increasing interest due to the strong consumers' demand for more biodegradable, biocompatible and natural products. Essential oils (EOs) fulfil these requirements and exhibit antibacterial, antifungal, antiviral and antioxidant properties, which made them very attractive to be used in some food, pharmaceutical or cosmetic products. Nevertheless, their high hydrophobicity and volatility hinder their incorporation in many products. In addition, EOs are very sensitive to external factors, so they need further protective measures to enhance or preserve their properties for a longer time. Emulsification is one of the most efficient techniques for the incorporation and preservation of essential oils. Emulsions help to preserve the essential oil properties and dampen the strong taste and smell typical of these oils. Oil in water (O/W) emulsions can be used to either avoid or reduce the evaporation of volatile components and to incorporate essential oils into an aqueous phase, despite of their marked hydrophobic properties, which result in a very low solubility in water. In order to obtain stable emulsions it is required a third component, an emulsifier. Moreover, the evolution of droplet sizes and droplet size distribution (DSD) among other parameters must been controlled to check the shelf life of emulsions. A high proportion of fine droplets and narrow distributions enhance the physical stability of

emulsions. High-pressure homogenization is one of the mechanical methods used to achieve the desired droplet size distribution. Thus, this Ph.D. Thesis is focused on the evaluation of the microchannel homogenization, based on the microfluidization (Microfluidizer) technique, to prepare oil-in-water emulsions of essential oils. The essential oils selected for this research were thyme and rosemary oils. Alkyl polypentoside nonionic surfactant derived from wheat biomass, which possess ECCOCERT label, was used as emulsifier.

This Ph.D. Thesis is divided in seven chapters published in scientific journals. Firstly, a brief introduction of essential oils is given, including composition, properties, applications and drawbacks, focused on thyme and rosemary oils. Furthermore, the introduction includes the key concepts of emulsion science as well as of stabilizer and main emulsification methods, focused on microfluidization.

Chapter 1 is a preliminary study, which shows the physical stability and droplet size distribution of emulsions formulated with thyme EO using commercial non-ionic surfactants of different families (ethoxylated sorbitan esters, alkyl polypentosides and sucroesters) and different HLB values (from 9 to 16.5). The alkyl polypentoside surfactant, Appyclean 6548 formed the most stable emulsions. This surfactant is solid at room temperature and immiscible in water. Appyclean 6548 was used for the rest of studies included in this Ph.D. Thesis.

Chapter 2 illustrates the influence of heating temperature (from 55^{o}C to 75^{o}C) of a mixture of thyme essential oil and Appyclean 6548 in a ratio of 10:1, on its physical stability. In a second step, two emulsions were homogenised using the abovementioned mixture of thyme oil and Appyclean 6548 as the dispersed phase at 55^{o}C and 70^{o}C. Similar values of

droplet sizes, viscosity and physical stability were obtained, regardless of the heating temperature of the dispersed phase.

Chapter 3 deals with the influence of the pressure applied and the number of passes through the Microfluidizer high pressure homogenizer for 40wt% thyme oil and 4wt% Appyclean. Slightly differences were found for DSD but emulsions passed through the Microfluidizer at 5000 psi (34.5 MPa) and 1 pass exhibit the best physical stability as indicated by the minimum value of the so-called, Turbiscan stability index, TSI.

Chapter 4 shows the effect of welan gum concentration on the rheological properties, droplet size distribution and physical stability of thyme oil/W emulsions. This work demonstrated that it is possible to control the rheology with welan gum due since it is an excellent rheological modifier. However, this gum was not able to increase the physical stability of these emulsions.

Chapters 5, 6 and 7 focus on the assessment of rosemary essential oil emulsions. Chapter 5 investigated the processing conditions and formulation variables of rosemary oil emulsions formulated with Appyclean 6548. The optimal emulsion was achieved at 20 wt% rosemary oil and 4 wt% Appyclean 6548 processed at 2500 psi (17.2 MPa) after 3 passes. Stable nanoemulsions exhibiting a high stability and with a volumetric mean diameter of 149 nm were obtained. These formulation and processing conditions were fixed for the following studies.

The target of Chapter 6 is to investigate the addition of welan gum (WG) and advanced performance xanthan gum (APXG) at the optimal formulation and processing conditions of rosemary oil and Appyclean 6548 emulsions. For this purpose, small amplitude oscillatory shear (SAOS)

measurements, creep tests, and flow curves were carried out. Furthermore, laser diffraction and multiple light scattering techniques were used to determine the droplet size distribution and the physical stability for all emulsions studied. Slightly differences were observed between nanoemulsions formulated with APXG or WG.

Chapter 7 presents the influence of evaporation time on rheological properties, DSD, microstructure and physical stability of biodegradable emulsions formulated with rosemary oil and Appyclean 6548. Thanks to vacuum evaporation it is possible to control the rheological properties and obtain long-term physical stability and greater resistance against creaming for the nanoemulsions studied.

Finally, the main conclusions of this Ph.D. Thesis are presented.

Resumen

Esta tesis doctoral forma parte del proyecto de investigación “Evaluación de la homogeneización por microcanales para la preparación y caracterización de emulsiones submicrónicas de aceites esenciales” (CTQ2015-70700-P) financiado por el Ministerio de Economía y Competitividad (MINECO) y la Comisión Europea (Programa FEDER).

Los aceites esenciales suscitan gran interés debido a la fuerte demanda de los consumidores por productos más biodegradables, biocompatibles y naturales. Los aceites esenciales (AEs) cumplen estos requisitos y, además, presentan propiedades antibacterianas, antifúngicas, antivirales y antioxidantes, lo que los hace muy interesantes para ser utilizados en productos alimentarios, farmacéuticos o cosméticos. Sin embargo, su elevada hidrofobicidad y volatilidad dificultan su incorporación en muchos productos. Asimismo, los AEs son muy sensibles a factores externos, por lo que necesitan otras medidas de protección para mejorar o conservar sus propiedades durante más tiempo. La emulsificación es una de las técnicas más eficaces para la incorporación y conservación de los aceites esenciales. Las emulsiones ayudan a preservar las propiedades de los aceites esenciales y a suavizar el fuerte sabor y olor típicos de estos aceites. Las emulsiones de aceite en agua (O/W) pueden utilizarse para evitar o reducir la evaporación de los componentes volátiles e incorporar los aceites esenciales en una fase acuosa, a pesar de que presentan muy baja solubilidad en agua. Para obtener emulsiones estables se requiere un tercer componente, un emulsionante. Además, es necesario controlar la evolución del tamaño de la gota y la distribución de tamaño de gota (DTG), entre otros parámetros, para comprobar la vida útil de las emulsiones. Una alta proporción de gotas pequeñas y distribuciones estrechas mejoran la

estabilidad física de las emulsiones. La homogeneización a alta presión es uno de los métodos mecánicos utilizados para conseguir la distribución de tamaño de gota deseada. Así, esta tesis doctoral se centra en la evaluación de la homogeneización por microcanales, basada en la técnica de microfluidización (Microfluidizer), para preparar emulsiones de aceite-en-agua de aceites esenciales. Los aceites esenciales seleccionados para este proyecto fueron los de tomillo y romero. Como emulsionante se utilizó el tensioactivo no iónico alquil-polipentósido derivado de la biomasa de trigo, que posee certificado ECCOCERT.

Esta tesis doctoral se divide en siete capítulos publicados en revistas científicas. En primer lugar, se hace una breve introducción sobre los aceites esenciales, incluyendo su composición, propiedades, aplicaciones e inconvenientes, centrada en los aceites de tomillo y romero. Además, la introducción incluye los conceptos clave de la ciencia de las emulsiones, así como de los estabilizadores y los principales métodos de emulsificación, centrados en la microfluidización.

El capítulo 1 es un estudio preliminar, que muestra la estabilidad física y la distribución del tamaño de gota de emulsiones formuladas con aceite esencial de tomillo utilizando tensioactivos no iónicos comerciales de diferentes familias (ésteres de sorbitán etoxilados, alquil polipentósidos y sucroésteres) y diferentes valores de HLB (de 9 a 16,5). El tensioactivo del tipo alquil polipentósido, Appyclean 6548, formó las emulsiones más estables. Este tensioactivo es sólido a temperatura ambiente e inmiscible en agua. Appyclean 6548 se utilizó para el resto de los estudios incluidos en esta tesis doctoral.

El capítulo 2 muestra la influencia de la temperatura de calentamiento (de 55^{o}C a 75^{o}C) de una mezcla de aceite esencial de tomillo y Appyclean 6548

en una proporción de 10:1, sobre su estabilidad física. En un segundo paso, se prepararon dos emulsiones utilizando la mezcla de aceite de tomillo y Appyclean 6548 como fase dispersa a 55^{o}C y a 70^{o}C. Se obtuvieron valores similares de tamaño de gota, de viscosidad y de estabilidad física, independientemente de la temperatura de calentamiento de la fase dispersa.

El capítulo 3 investiga la influencia de la presión aplicada (de 5000 a 15000 psi) y del número de pasadas (1 y 2) a través del homogeneizador de alta presión Microfluidizer para un 40 m/m% de aceite de tomillo y un 4 m/m% de Appyclean. Se encontraron ligeras diferencias en las DTGs, pero las emulsiones pasadas por el Microfluidizer a 5000 psi (34,5 MPa) y 1 pasada presentaron la mejor estabilidad física, obteniéndose el valor mínimo del llamado índice de estabilidad Turbiscan, TSI.

El capítulo 4 muestra el efecto de la concentración de goma welan (de 0 a 0,2 m/m%) sobre las propiedades reológicas, la distribución del tamaño de gota y la estabilidad física de las emulsiones aceite de tomillo/agua. Este trabajo demostró que es posible controlar la reología de las emulsiones con goma welan debido a que es un excelente modificador reológico. Sin embargo, esta goma no fue capaz de aumentar la estabilidad física de las emulsiones estudiadas.

Los capítulos 5, 6 y 7 se centran en la evaluación de las emulsiones de aceite esencial de romero. El capítulo 5 investiga las variables de procesado (presión de 2500 a 15000 psi y número de ciclos de recirculación de 1 a 10) y de formulación (concentración de fase dispersa de 10 a 50 m/m%) de las emulsiones de aceite de romero formuladas con Appyclean 6548. La emulsión más estable se consiguió con un 20 m/m% de aceite de romero y un 4 m/m% de Appyclean 6548 procesado a 2500

psi (17,2 MPa) y 3 pasadas. Se obtuvieron nanoemulsiones estables que mostraban una gran estabilidad y con un diámetro medio volumétrico de 149 nm. Estas condiciones de formulación y procesado se fijaron para los siguientes estudios.

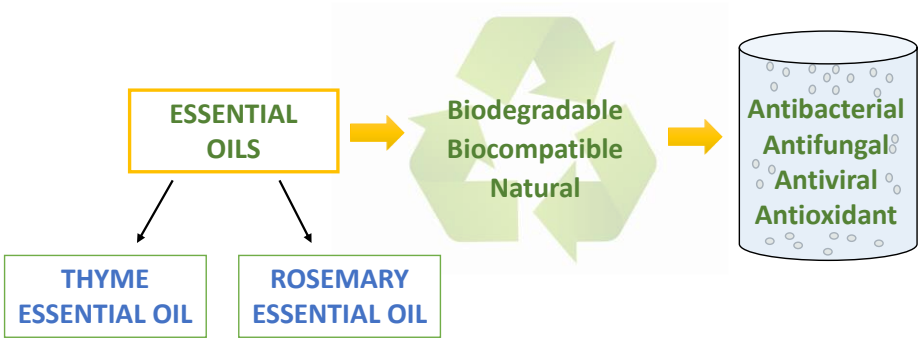
El objetivo del capítulo 6 es investigar la adición de goma welan (WG) y goma xantana "*advanced performance*" (APXG) (de 0 a 0,5 m/m%) en las condiciones óptimas de formulación y procesado de las emulsiones de aceite de romero y Appyclean 6548. Para ello, se llevaron a cabo mediciones de cizalla oscilatoria de pequeña amplitud (SAOS), pruebas de fluencia y curvas de flujo. Además, se utilizaron técnicas de difracción láser y de retrodispersión múltiple de luz para determinar la distribución del tamaño de gota y la estabilidad física de todas las emulsiones estudiadas. Se obtuvieron nanoemulsiones estables a una concentración de goma de 0,4 m/m%. Sin embargo, se encontraron ligeras diferencias entre las nanoemulsiones formuladas con APXG y WG.

El capítulo 7 presenta la influencia del tiempo de evaporación a vacío (de 10 a 60 min) en las propiedades reológicas, la DTG, la microestructura y la estabilidad física de las emulsiones formuladas con aceite de romero y Appyclean 6548. Gracias a la evaporación a vacío es posible controlar las propiedades reológicas y obtener una estabilidad física a largo plazo y una mayor resistencia al cremado para las nanoemulsiones estudiadas.

Finalmente, se presentan las principales conclusiones de esta tesis doctoral.

Introduction

State-of-the-art in essential oil emulsions



1. Introduction

1.1. Essential oils

In the last few decades, a growing social concern about environment is reflected in the strong demand for ecological ingredients. The development of sustainable products has risen because of the growing consumer interest in natural products. Scientifics are looking for new formulations, which fulfil environmental requirements and consumer needs. Essential oils (EOs) may satisfactorily contribute to this demand be a good choice due to the fact that they are considered natural components, environmentally friendly and easily degradable [1].

EOs are complex mixtures of volatile compounds derived from aromatics plants and secondary metabolites[2]. As defined by the International Organization for Standardization (ISO), the term essential oil is reserved for a "product obtained from vegetable raw material, either by distillation with water or steam, or from the epicarp of citrus fruits by a mechanical process, or by dry distillation" (ISO 9235, 1997); that is, by physical means only. The extraction methods of essential oils may be classified into two categories: classical and innovating methods. Hydro distillation, solvent extraction, and cold pressing are some examples of classical extraction techniques. However, these methods usually can undergo chemical alterations, which lead to loss quality of extracted EOs. New extraction techniques have demonstrated that reduce extraction times, energy consumption, solvent use, CO_2 emissions and exhibit high efficiency [3,4]. Supercritical fluid extraction, ultrasound and microwave assisted extractions are innovating techniques for EOs isolation which show the abovementioned advantages over traditional ones [5–7].

In general, EOs are volatile, immiscible in aqueous liquids and characterized by a strong odour. They contain bioactive compounds, mainly terpenoids, especially mono- and sesquiterpenoids and its derivatives in the form of aldehydes, alcohols, esters, phenolic ethers, and ketones [8,9]. Some of them also contain non-terpenoid products like phenylpropanoids, and, more rarely, nitrogen- and sulphur molecules [10]. Terpenoids are, by far, the most significant group of natural compounds as far as essential oils are concerned. Around 90% of bioactive essential oils are constituted of monoterpenes [11]. Some of the major compounds of EOs are monoterpene hydrocarbons (myrcene citronellene, p-cymene, limonene, α-pinene, and γ-terpinene), monoterpene alcohols and aldehydes (geraniol, linalool, nerol and citral, cuminal), sesquiterpene hydrocarbons (β-caryophyllene, germacrene D, and humulene), sesquiterpene alcohols and acids (spathulenol, caryophyl, patchoulol and geranic acid, benzoic acid) sesquiterpenes lactones (costunolide), phenol compounds (eugenol, thymol, carvacrol, and catechol), esters (bornyl acetate, ethyl acetate), and coumarins (fumarin, benzofuran) [2,12–16]. Figure 1 shows the structure of some of the main components of EOs.

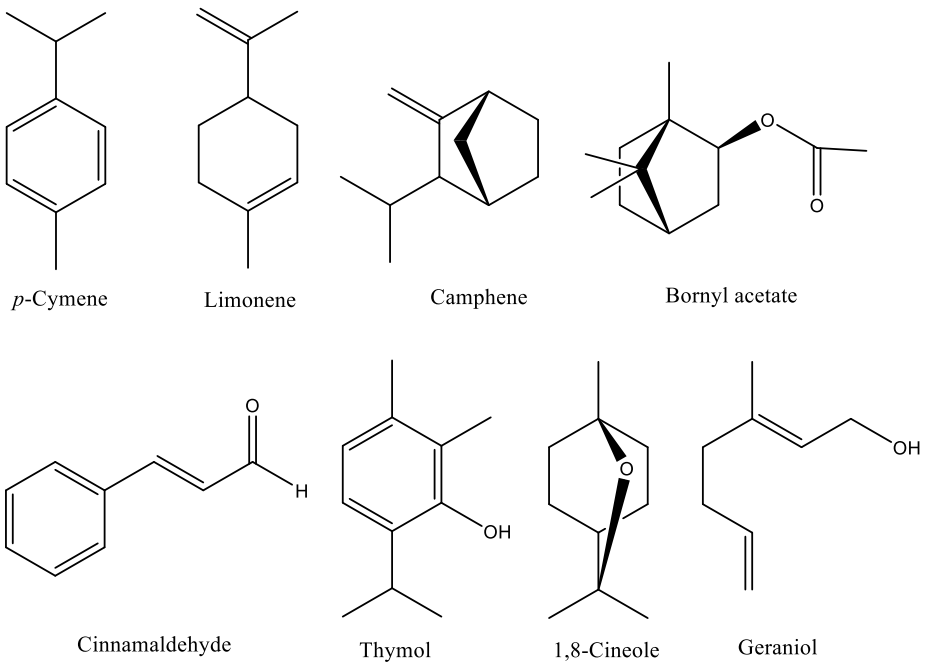


Figure 1. Chemical structures of the major compounds of EOs.

Internal and external factors vary the chemical composition of EOs. Type of plant part, which EOs are extracted from, species types, plant origin, stage of maturity and state of the whole plant have been included as internal factors. For example, a recent study has demonstrated that oregano essential oil showed variation in the chemical composition and biological properties at different growth stages [17]. External conditions include environmental factors, cultivation conditions or and extraction methods [18–21]. It is known that genetic and environmental factors play an important role in the biosynthesis, accumulation and distribution of secondary metabolites [22]. Thus, differences in chemical composition is directly related to differences in the functional properties of essential oils.

EOs may contain about 20-60 compounds at different concentrations. However, each essential oil is always characterized by two or three main

components (around the 20-70% of total mass) that determined the chemo type of the oil [11,23]. Thanks to their chemical composition, EOs exhibit functional properties like antioxidant, antimicrobial, antifungal, anti-inflammatory, anticancer, antiviral, sedative and analgesic properties [24–31]. In addition, many EOs are also used as flavouring and fragrance agents [32,33]. All these properties justify why EOs are widely used in the food and cosmetic industries, as well as in the pharmaceutical and medical fields, among others.

Within the food industry, essential oils can be used as natural antimicrobial, antioxidant, antibacterial and antifungal agents [34,35]. These properties extend food shelf life, reducing or eliminating the presence of food borne microorganisms. In addition, new food packaging are being used to protect food from external factors that can provoke their degradation [36,37]. Some essential oils have been tested as natural food preservatives in several investigations. For example, thyme and oregano EOs can be applied to protect goat cheese against eight spoilage and pathogenic bacteria [38,39]; Clove and thyme EOs can be used in functional packaging in deboned chicken meat film since they possess antimicrobial activity against *B. subtilis, S. aureus, and E. coli*. oil and *L. monocytogenes*, respectively [40,41],

Essential oils are widely used in cosmetic and personal care products. Skin care and maintenance, repairing, dentifrices and toothpastes, odour improvement, hair removal ,skin softeners, shower gels and body lotions, are some examples of products containing EOs [42]. As mentioned above, essential oils possess antioxidant and antimicrobial properties, which increase the shelf life of products. The antimicrobial activity protects them against a wide range of bacterial strain[43,44]. Thyme, oregano,

eucalyptus and mint plants have demonstrated antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Aspergillus fumigatus* and *Candida albicans* [45]. Moreover, mint and eucalyptus oils give cooling and refreshing sensations to the skin and mouth. On the other hand, the so-called cosmeceuticals are cosmetic products with pharmacological effects [46]. For instance, topical applications of chamomile, geranium and rosemary essential oils products have shown to be effective in the treatment of acne and eczema due to their antibacterial and anti-inflammatory properties [47]. Essential oils can be used as natural preservative ingredients because of their antimicrobial properties, replacing some synthetic chemicals in cosmetic products. They also play a relevant role in the perfume and cosmetic industries, mainly due to their well-known fragrance compounds [48]. Furthermore, they are widely used in a more specialized area (aromatherapy) [49]. Aromatherapy EOs inhalation has demonstrated that relieve the perception of stress, anxiety and depression and also, that improves the sleep quality [50,51]. Lavender oil is one of the most used EO in massage or inhalation aromatherapy [31,52,53].

Furthermore, EOs are used in many branches of medicine such as in pharmacy, balneology and massage [2]. In medicine, EOs are used against drug-resistant microbial pathogens, reducing the growth of bacteria or destroy the bacterial cells [16,54,55]. Additionally, several chemical ingredients of EOs have demonstrated to act on inflammatory and other processes associated with cardiovascular diseases [56]. In addition, recent research have shown the potential value of EOs as new sources of anticancer therapeutic strategies [29,30,57].

In the field of agriculture, the importance of EOs as eco-friendly natural biocides and insect repellents are gaining more interest due to their antimicrobial and repellent activities against insects and other kinds of arthropods [58]. They are natural resources that can replace synthetic pesticides and herbicides.

After an overview of essential oils, composition, extraction methods, properties and applications, the following parts have been focused on thyme and rosemary essential oils.

1.1. Thyme and rosemary essential oils

Thyme and rosemary EOs belong to the Lamiaceae family, which is characterised by a strong antioxidant power. Both are classified by the United States Food and Drug Administration (FDA) as generally recognized as safe (GRAS) [59].

Thyme essential oil (TEO) is extracted by steam distillation from leaves of the plant *Thymus vulgaris*. Thyme EO is characterised by a high antioxidant activity due to their main compounds: thymol, carvacrol, ρ-cymene, γ-terpinene, limonene, 1,8-cineole, and α-pinene [60]. TEO not only has shown important antioxidant activity but also antibacterial, antimicrobial, and antifungal. These properties make it suitable for use as natural food preservative in meat, cheese, milk or bakery products among others [38,60–62].

Rosemary essential oil (REO) is steam distilled from flowers or leaves of *Rosmarinus officinalis*. As thyme, rosemary EO is characterized by a strong antioxidant activity. Among their main components are 1,8-cineole, α-pinene, limonene and camphorer camphene, borneol, bornyl acetate and α-terpineo [63].

The European Food Safety Authority (EFSA) considered rosemary oil for its use as a natural food preservative (EFSA, 2008). It can be found in the EFSA list with the number EFSA-Q-2003-140 [64]. Thus, rosemary is the only spice commercially available for use as an antioxidant in the United States and Europe [65]. Numerous publications have shown the use of REO as food preservative agent because it prevents against oxidation and microbial contamination. In addition, recent studies have reported that REO extracts can be used in the treatment of different diseases, due to its hepatoprotective potential as well as therapeutic potential for Alzheimer's disease and stress-related psychiatric disorders [66,67]. Components of REO such as carnosol and carnosic acid have demonstrated chemo preventive, antitumoral and antimetastatic activities. This is an important advance in the treatment of angiogenesis diseases [68].

1.2. Drawbacks of essential oils

Despite the abovementioned beneficial properties of EOs, they present several disadvantages that must be noted.

Essential oils are formed by an array of many hydrophobic and highly volatile compounds, which are known to be susceptible to oxidation and polymerization processes. These degradation processes depend on external factors such as oxidation, light or temperature. In addition, the chemical composition and the presence of impurities may also cause destabilization of EOs [69].

The autoxidation processes are accelerated by the ultraviolet (UV) light and visible (Vis) light, causing the hydrogen abstraction that results in the formation of alkyl radicals. REO is very sensitive to daylight that change the chemical composition while TEO compounds are not usually altered to the same extent [70].

Controlling the storage temperature is also essential to minimise the risk of destabilization. High temperature accelerates chemical reactions that lead to a changing chemical composition. REO has demonstrated higher physical stability at lower temperature and oxidation reactions could be hindered during storing time at refrigerator temperature [71].

The oxidation reactions are one of key causes for spoilage of EOs. Half-filled containers showed more marked changes in composition and physicochemical properties of EOs than when only little or no headspace was present.

Metal impurities such as copper or ferrous ions can promote autoxidation. These impurities can come from distillation in primitive stills or during storage in metallic containers.

Furthermore, EOs have a strong odour and flavour. Many terpenes are bitter in taste and many, specially the terpenic hydrocarbons are poorly soluble in water and seem not to have strong contribution to the antifungal activity [72].

Moreover, EOs are under regulatory limitations [73]. The FDA establishes that fragrances and flavours may be listed under the word “fragrance” or “flavour” (FDA, Code of Federal Regulations, Title 21)[74]. In 1992, the SCCNFP (Scientific Committee on Cosmetic Products and Non-food products) identified 26 fragrance allergens (SCCNFP/0017/98), 18 of which can be found as single compounds of EOs. These 26 ingredients are declared as potentially allergenic substances (PASs) by the EU Regulation on Cosmetic Products and they are listed into the Annex II and Annex III of regulation (EC) 1223/2009 of the European parliament and of the council

of 30 November 2009 on cosmetic products (Regulation EC 1223/2009) [75,76]

In spite of the high volatility and the susceptibility against environmental effects, legal limitations and their high prices, EOs are getting more and more interest.

1.3. Emulsions

Due to the main drawbacks of EOs (highly volatility, hydrophobicity and very sensitive to external factors), they need further protective measures, not only to improve or preserve their properties for a longer time and to increase their uptake and bioavailability but also to mask the strong taste and the typical smell. For this purpose, oil-in-water (O/W) emulsions are one of the chosen formulations for EOs. Emulsions are colloidal thermodynamically unstable dispersions in which a liquid is dispersed in a continuous liquid phase in the form of droplet [77]. They are formed by two immiscible liquids (oil and water) and an emulsifier.

1.4. Stabilizers

Despite emulsions are thermodynamically unstable systems; the stability of emulsions can be enhanced by adding substances known as stabilizers: such as emulsifiers, texture modifiers or weighting agents.

1.4.1. Surfactants

Surfactants play an essential role to form emulsions. They enhance the formation of droplets during homogenization due to the ability to decrease the interfacial tension [78]. In addition, surfactants provide certain physical stability because of the viscoelastic and physicochemical properties of the interfacial film they form surrounding the droplet

interface. This avoids, hinders or delays flocculation and coalescence among droplets.

The HLB (hydrophilic–lipophilic balance) value is the main property to characterize surfactants. This concept was introduced by Griffin as an empirical scale from 0 to 20 for non-ionic surfactants. Generally, emulsifiers with HLB values in the range of 3-8 form W/O emulsions whereas emulsifiers which are acceptable for O/W emulsions have HLB values of about 8-18 [79].

In a preliminary study of this Thesis, different families of surfactants were used such as ethoxylated sorbitan esters and sucroesters. However, a sucroester surfactant called Applyclean showed the best results to achieve the objectives for this Ph.D. project. For this purpose, Appyclean surfactant was used for the rest of studies. Appyclean is the trade name of alkyl polypentoside (APP) surfactants. They are non-ionic new surfactants derived from wheat straw that possess the ECOCERT label. The APPs are synthesized by Fisher-type glycosylation of fatty alcohols and pentoses (xylose and arabinose) derived from cereal products [80]. The reactivity of the transglycosylation reaction depends on the nature of pentose's origin, being higher for wheat straw (Fig. 2). The use of dimethyl sulfoxide (DMSO) also improve the solubility and increase the yield of alkyl pentosides [81]. Alkyl polypentosides (APP) and alkyl polyglycosides (APG) are sugar-based surfactants derived from carbohydrate, which show several advantages in comparison with others surfactants such as pH neutrality, excellent stability, biocompatibility, biological degradability, low toxicity and are characteristically non-irritating [8]. Furthermore, these surfactants tend to present a low critical micellar concentration (CMC) [9]. However, APPs and APGs show different properties that can be observed in figure 3. In this

Thesis project, Appyclean 6548 (D- pentose, oligomeric C14/18 alkyl glycosides) was used as surfactant. It possesses an HLB value of 9.25. It is solid at room temperature since it contains C16-C18 hydrocarbons and does not dissolve in water, but in oil. It is 100% natural or from natural origin and 100% active matter.

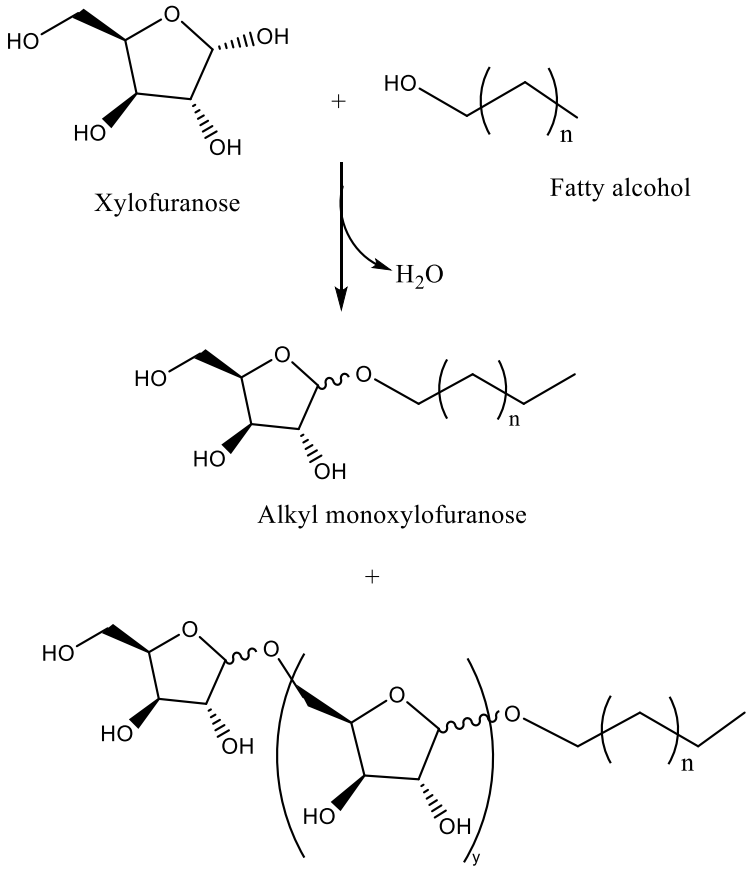


Figure 2. Example of xylofuranose glycosylation. (Martel et. al., 2010) [80]

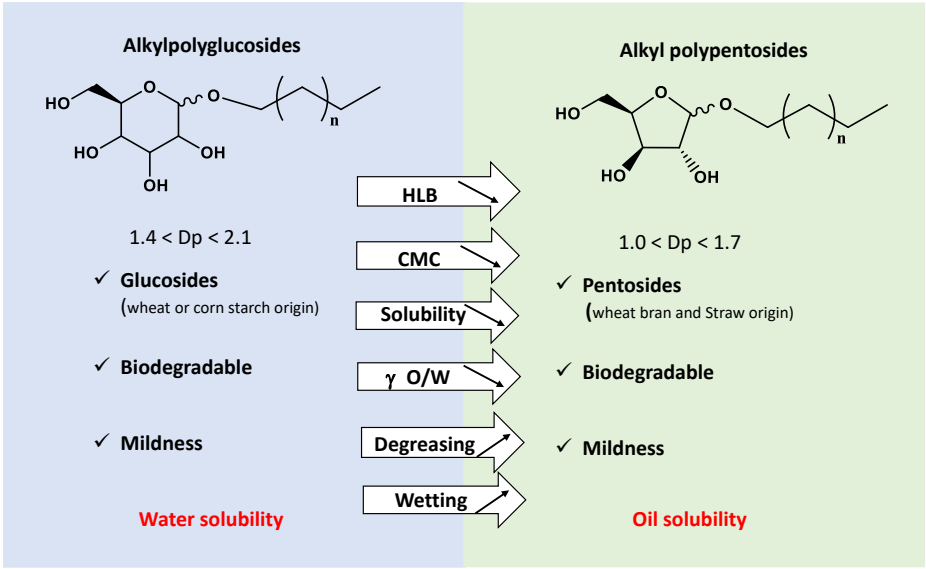


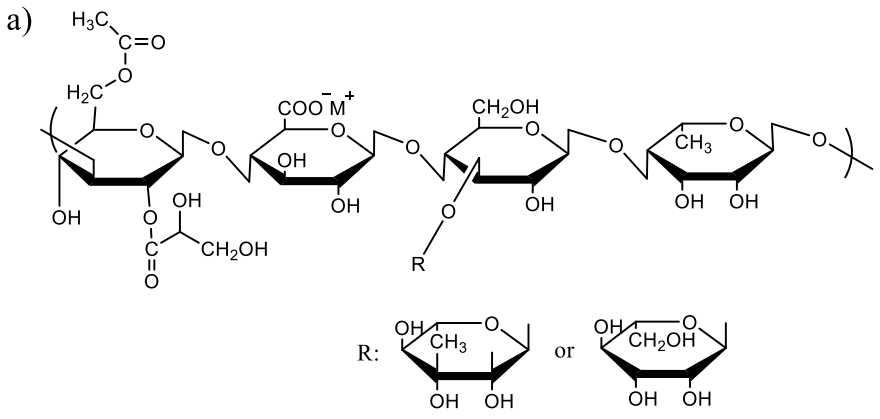
Figure 3. Main property differences between APG from glucose and xylose (Martel et al., 2010)[80].

1.4.2. Texture modifiers

Texture modifiers are substances that increase the viscosity (thickening agent) or gel (gelling agent) the aqueous phase. They may be used to provide desirable textural characteristics, or to stop gravitational separations. Biopolymers are the most used texture modifiers. They are incorporated to the aqueous phase in O/W emulsions. Thickening agents or gelling agents are usually an individual type of biopolymer or a mixture of different types of biopolymers. Sugars (e.g., sucrose, high fructose corn syrup (HFCS)), polyols (e.g., glycerol, sorbitol), polysaccharides (e.g., xanthan, pectin, carrageenan, alginate) and proteins (e.g., gelatin, whey protein isolate (WPI), serum protein isolate (SPI)) are some examples of the most commonly biopolymers used as thickening agents or gelling agents. In this Thesis project, different biopolymers have been used. Welan gum (WG), advanced performance xanthan gum (APXG), diutan

gum (DG) and rhamsan gum (RG). However, WG and APXG demonstrated the best results for the purpose of this research.

WG is an anionic extracellular polysaccharide produced by the microorganism *Sphingomonas sp.,* namely from *Alcaligenes sp.,* ATCC 31555. WG is mainly used for building materials and chemical industrial applications, although may be used in some countries as additive in food products, in which it can act as a thickening, suspending, binding, emulsifying, stabilizing and viscosifying agent [82]. On the other hand, XG is widely used as additive (European code: E415) for a great variety of food products and also for others markets, such as those of the cosmetic, pharmacy, agrochemistry or chemical industries. XG is a natural high molecular weight anionic polysaccharide produced by aerobic fermentation of sugars by the microorganism *Xanthomonas campestris*. Specifically, APXG is suitable for sauces, milk and cream products or beverages [83]. This APXG yields faster hydration, it is able to hydrate in higher levels of salts and acids and increase the viscosity of aqueous solutions in comparison with standard xanthan gum (private communication from the manufacturer).



Welan Gum

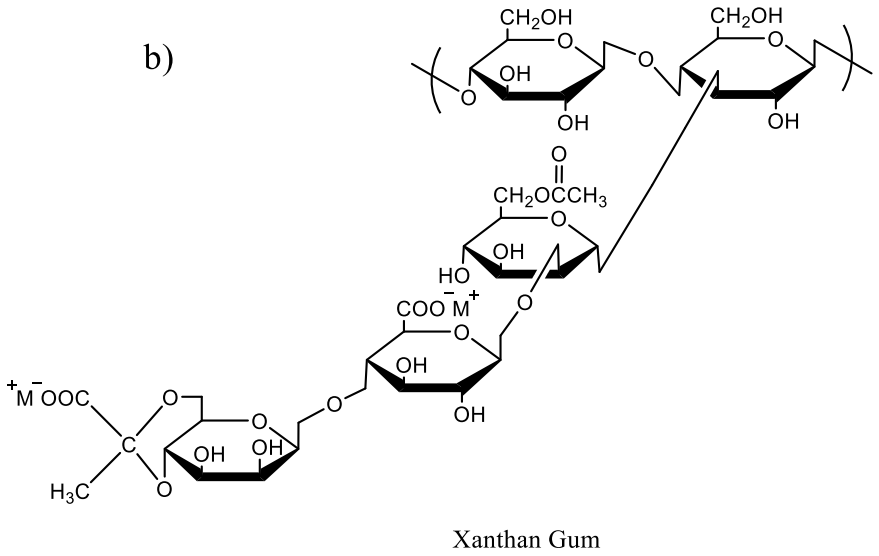


Figure 4. a) Chemical structure of welan gum and b) xanthan gum (Xu et al.,)[84].

1.5. Emulsion formation. High-energy methods

Emulsion formation requires energy to form droplets of the dispersed phase due to the fact that it is a non-spontaneous process. Mechanical energy is applied to the system to break up the oil droplets and to disperse them into the water phase. Different high-energy devices can be to prepare emulsions. Namely, colloid mills, rotor-stators, high-pressure homogenizers, ultrasonic homogenizers and membranes. Rotor stator devices such as Ultra-Turrax or Silverson provide enough energy to produce macroemulsions. On the other hand, the formation of submicron and nanoemulsions needs a greater amount of energy [77]. The most common emulsification devices to obtain fine droplets are high-pressure valve homogenizers (HPvH) and Microfluidizers. In this section, only is the microfluidization technique reviewed.

1.5.1. Microfluidization

In Microfluidizers, the coarse emulsion feeds the inlet reservoir. Due to a pneumatic pressure, the gross emulsion is forced to flow through microchannels (see figure 5). These microchannels are the so-called interaction chambers. While in some interaction chambers two streams of the feeding emulsions are forced to impinge over each other at very high velocities producing intense forces, in others the feeding stream is forced to hit several times the microchannels walls. Interaction chambers play an important role due to the fact that they are microchannels designed with specifical both geometrical designs and minimum diameter values [85]. There are two different types of interaction chambers: Z-type and Y-type (figure 6). In this project, the F12Y and H30Z chambers, which have a minimum diameter of 75 µm and 200 µm, respectively, have been used [86]. They not only have different geometries and minimum diameters but also can reach different maximum shear rates. Namely, these chambers can reach up to $8x10^6\ s^{-1}$ (F12Y) and $2x10^6\ s^{-1}$ (H30Z), respectively[87] .

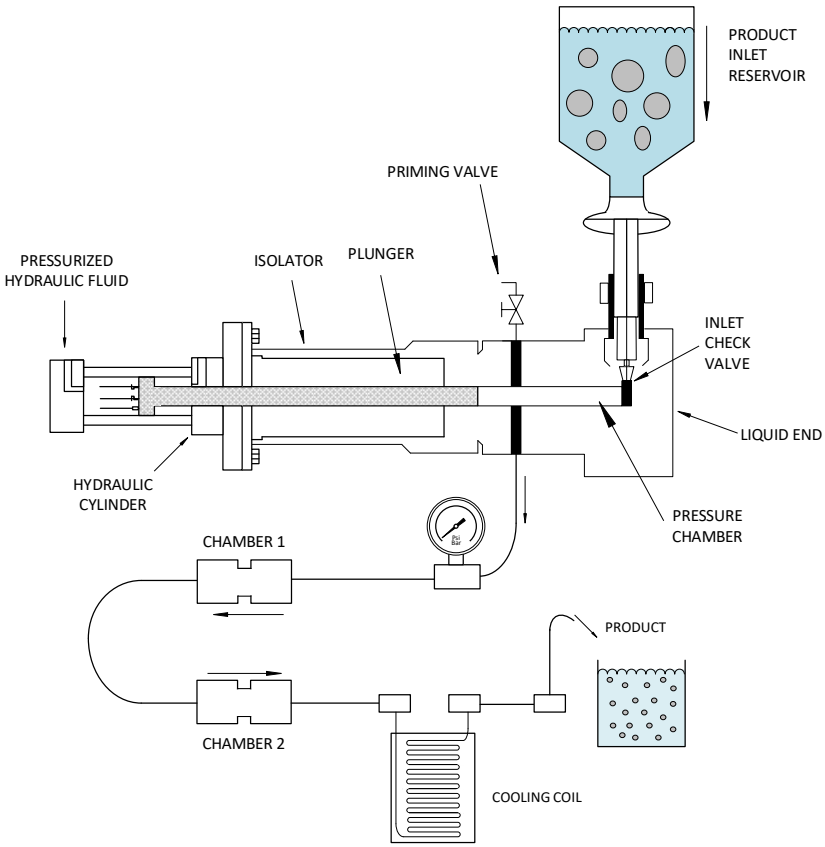


Figure 5. Scheme of the Microfluidizer

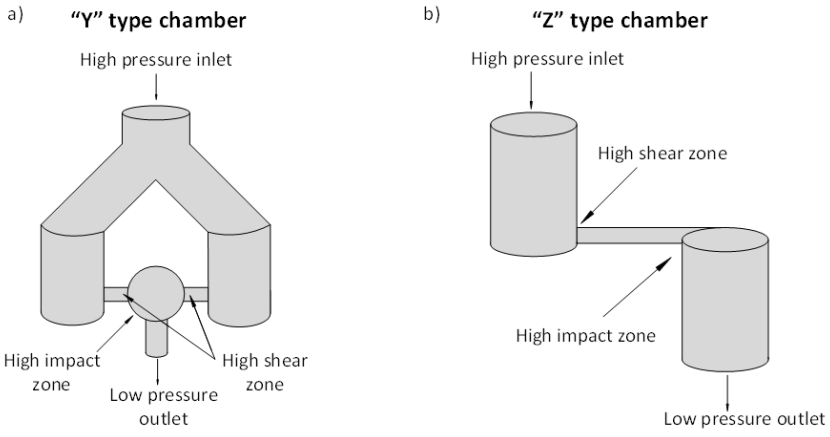


Figure 6. Scheme of a) Y-type and b) Z-type interaction chambers.

Thus, nanoemulsions can be achieved by the combination of processing variables such as premixing, number of cycles, homogenization pressure and type of interaction chambers [88]. The inlet and outlet temperatures are also production variables to be considered. Microfluidization can also be used as an encapsulation technique. Encapsulation not only increases the antimicrobial potency of essential oils by controlled release and facilitating close interaction with the microorganisms but also enhances emulsion appearance and stability [89,90]. In addition, the use of this technique is very important to obtain fine droplets between 50 and 500nm. It is known that droplet sizes under 500 nm lead to enhanced absorption and bioavailability of active ingredients [91]. The preparation of essential oil nanoemusions has been reported in several articles [92,93], although the use of biobased surfactants derived from wheat biomass or comprehensive studies on physical stability had not been considered before this Ph.D. Thesis

Thus, the overall objective of this Ph. D. Thesis is to contribute to gain a deeper insight into the formulation and processing conditions for essential oil emulsions using a novel bio-based surfactant. Thyme and rosemary essential oil emulsions are added value products, which can be useful for replacing or decreasing synthetic antioxidants used in many fields. More extensive information is provided in each chapter of this thesis.

References

[1] F. Reyes-Jurado, A.R. Navarro-Cruz, C.E. Ochoa-Velasco, E. Palou, A. López-Malo, R. Ávila-Sosa, Essential oils in vapor phase as alternative antimicrobials: A review, Crit. Rev. Food Sci. Nutr. 60 (2020) 1641–1650. doi:10.1080/10408398.2019.1586641.

[2] K.H.C. Baser, G. Buchbauer, Handbook of Essential Oils Science, Technology, and Applications, 2010. doi:doi:10.1201/9781420063165-c3.

[3] A. El Asbahani, K. Miladi, W. Badri, M. Sala, E.H.A. Addi, H. Casabianca, A. El Mousadik, D. Hartmann, A. Jilale, F.N.R. Renaud, A. Elaissari, Essential oils: From extraction to encapsulation, Int. J. Pharm. 483 (2015) 220–243. doi:10.1016/j.ijpharm.2014.12.069.

[4] M.D. Luque De Castro, M.M. Jiménez-Carmona, V. Fernández-Pérez, Towards more rational techniques for the isolation of valuable essential oils from plants, TrAC - Trends Anal. Chem. 18 (1999) 708–716. doi:10.1016/S0165-9936(99)00177-6.

[5] M. Yousefi, M. Rahimi-Nasrabadi, S.M. Pourmortazavi, M. Wysokowski, T. Jesionowski, H. Ehrlich, S. Mirsadeghi, Supercritical fluid extraction of essential oils, TrAC - Trends Anal. Chem. 118 (2019) 182–193. doi:10.1016/j.trac.2019.05.038.

[6] H. Benmoussa, A. Farhat, M. Romdhane, J. Bouajila, Enhanced solvent-free microwave extraction of Foeniculum vulgare Mill. essential oil seeds using double walled reactor, Arab. J. Chem. 12 (2019) 3863–3870. doi:10.1016/j.arabjc.2016.02.010.

[7] F. Chen, S. Liu, Z. Zhao, W. Gao, Y. Ma, X. Wang, S. Yan, D. Luo, Ultrasound pre-treatment combined with microwave-assisted hydrodistillation of essential oils from Perilla frutescens (L.) Britt. leaves and its chemical composition and biological activity, Ind. Crops Prod. 143 (2020) 111908. doi:10.1016/j.indcrop.2019.111908.

[8] K. Skalicka-Woźniak, I. Garrard, Counter-current chromatography for the separation of terpenoids: A comprehensive review with respect to the solvent systems employed, Phytochem. Rev. 13 (2014) 547–572. doi:10.1007/s11101-014-9348-2.

[9] S.M. Njoroge, N.T.L. Phi, M. Sawamura, Chemical composition of peel essential oils of sweet oranges (citrus sinensis) from uganda and rwanda, J. Essent. Oil-Bearing Plants. 12 (2009) 26–33. doi:10.1080/0972060X.2009.10643687.

[10] M. Zuzarte, L. Salgueiro, Essential oils chemistry, in: Bioact. Essent. Oils Cancer, Springer International Publishing, 2015: pp. 19–61.

doi:10.1007/978-3-319-19144-7_2.
[11] F. Bakkali, S. Averbeck, D. Averbeck, M. Idaomar, Biological effects of essential oils - A review, Food Chem. Toxicol. 46 (2008) 446–475. doi:10.1016/j.fct.2007.09.106.
[12] L. Caputo, F. Nazzaro, L. Souza, L. Aliberti, L. De Martino, F. Fratianni, R. Coppola, V. De Feo, Laurus nobilis: Composition of Essential Oil and Its Biological Activities, Molecules. 22 (2017) 930. doi:10.3390/molecules22060930.
[13] R. Ait Babahmad, A. Aghraz, A. Boutafda, E.G. Papazoglou, P.A. Tarantilis, C. Kanakis, M. Hafidi, Y. Ouhdouch, A. Outzourhit, A. Ouhammou, Chemical composition of essential oil of Jatropha curcas L. leaves and its antioxidant and antimicrobial activities, Ind. Crops Prod. 121 (2018) 405–410. doi:10.1016/j.indcrop.2018.05.030.
[14] C.S.B. Damasceno, N.T. Fabri Higaki, J.D.F.G. Dias, M.D. Miguel, O.G. Miguel, Chemical Composition and Biological Activities of Essential Oils in the Family Lauraceae: A Systematic Review of the Literature, Planta Med. 85 (2019) 1054–1072. doi:10.1055/a-0943-1908.
[15] A.K. Pandey, P. Singh, The Genus Artemisia: a 2012–2017 Literature Review on Chemical Composition, Antimicrobial, Insecticidal and Antioxidant Activities of Essential Oils, Medicines. 4 (2017) 68. doi:10.3390/medicines4030068.
[16] M.K. Swamy, M.S. Akhtar, U.R. Sinniah, Antimicrobial properties of plant essential oils against human pathogens and their mode of action: An updated review, Evidence-Based Complement. Altern. Med. 2016 (2016). doi:10.1155/2016/3012462.
[17] A. Bouyahya, N. Dakka, A. Talbaoui, A. Et-Touys, H. El-Boury, J. Abrini, Y. Bakri, Correlation between phenological changes, chemical composition and biological activities of the essential oil from Moroccan endemic Oregano (Origanum compactum Benth), Ind. Crops Prod. 108 (2017) 729–737. doi:10.1016/j.indcrop.2017.07.033.
[18] M. Moghaddam, L. Mehdizadeh, Chemistry of Essential Oils and Factors Influencing Their Constituents, in: Soft Chem. Food Ferment., Elsevier, 2017: pp. 379–419. doi:10.1016/b978-0-12-811412-4.00013-8.
[19] A.C. Figueiredo, J.G. Barroso, L.G. Pedro, J.J.C. Scheffer, Factors affecting secondary metabolite production in plants: Volatile components and essential oils, Flavour Fragr. J. 23 (2008) 213–226. doi:10.1002/ffj.1875.
[20] S. Melito, G.L. Petretto, J. Podani, M. Foddai, M. Maldini, M.

Chessa, G. Pintore, Altitude and climate influence Helichrysum italicum subsp. microphyllum essential oils composition, Ind. Crops Prod. 80 (2016) 242–250. doi:10.1016/j.indcrop.2015.11.014.

[21] R. Rehman, M.A. Hanif, Z. Mushtaq, A.M. Al-Sadi, Biosynthesis of essential oils in aromatic plants: A review, Food Rev. Int. 32 (2016) 117–160. doi:10.1080/87559129.2015.1057841.

[22] N. Verma, S. Shukla, Impact of various factors responsible for fluctuation in plant secondary metabolites, J. Appl. Res. Med. Aromat. Plants. 2 (2015) 105–113. doi:10.1016/j.jarmap.2015.09.002.

[23] C. Genova, A. Grottoli, E. Zoppis, C. Cencetti, P. Matricardi, G. Favero, An integrated approach to the recovery of travertine biodegradation by combining phyto-cleaning with genomic characterization, Microchem. J. 156 (2020) 104918. doi:10.1016/j.microc.2020.104918.

[24] R. Amorati, M.C. Foti, L. Valgimigli, Antioxidant activity of essential oils, J. Agric. Food Chem. 61 (2013) 10835–10847. doi:10.1021/jf403496k.

[25] J. Nie, R. Li, Z. Jiang, Y. Wang, J. Tan, S. Tang, Y. Zhang, Antioxidant activity screening and chemical constituents of the essential oil from rosemary by ultra-fast GC electronic nose coupled with chemical methodology, J. Sci. Food Agric. 100 (2020) 3481–3487. doi:10.1002/jsfa.10388.

[26] M. Ghavam, M.L. Manca, M. Manconi, G. Bacchetta, Chemical composition and antimicrobial activity of essential oils obtained from leaves and flowers of Salvia hydrangea DC. ex Benth., Sci. Rep. 10 (2020) 1–10. doi:10.1038/s41598-020-73193-y.

[27] M. Ribeiro, M. Simões, Antimicrobial Activity of Essential Oils, in: Heal. Consequences Microb. Interact. with Hydrocarb. Oils, Lipids, Springer International Publishing, 2020: pp. 335–356. doi:10.1007/978-3-030-15147-8_28.

[28] R. Donato, C. Sacco, G. Pini, A.R. Bilia, Antifungal activity of different essential oils against Malassezia pathogenic species, J. Ethnopharmacol. 249 (2020) 112376. doi:10.1016/j.jep.2019.112376.

[29] M. Basholli-Salihu, R. Schuster, A. Hajdari, D. Mulla, H. Viernstein, B. Mustafa, M. Mueller, Phytochemical composition, anti-inflammatory activity and cytotoxic effects of essential oils from three *Pinus* spp., Pharm. Biol. 55 (2017) 1553–1560. doi:10.1080/13880209.2017.1309555.

[30] E. Fitsiou, A. Pappa, Anticancer Activity of Essential Oils and Other Extracts from Aromatic Plants Grown in Greece, Antioxidants. 8

(2019) 290. doi:10.3390/antiox8080290.
[31] K. Tomi, M. Kitao, H. Murakami, Y. Matsumura, T. Hayashi, Classification of lavender essential oils: sedative effects of *Lavandula* oils, J. Essent. Oil Res. 30 (2018) 56–68. doi:10.1080/10412905.2017.1377122.
[32] A.A. Mariod, Effect of essential oils on organoleptic (smell, taste, and texture) properties of food, in: Essent. Oils Food Preserv. Flavor Saf., Elsevier Inc., 2016: pp. 131–137. doi:10.1016/B978-0-12-416641-7.00013-4.
[33] N. Singh, M. Yadav, S. Khanna, O. Sahu, Sustainable fragrance cum antimicrobial finishing on cotton: Indigenous essential oil, Sustain. Chem. Pharm. 5 (2017) 22–29. doi:10.1016/j.scp.2017.01.003.
[34] F. Donsì, G. Ferrari, Essential oil nanoemulsions as antimicrobial agents in food, J. Biotechnol. 233 (2016) 106–120. doi:10.1016/j.jbiotec.2016.07.005.
[35] A. Belasli, Y. Ben Miri, M. Aboudaou, L. Aït Ouahioune, L. Montañes, A. Ariño, D. Djenane, Antifungal, antitoxigenic, and antioxidant activities of the essential oil from laurel (*Laurus nobilis* L.): Potential use as wheat preservative, Food Sci. Nutr. 8 (2020) 4717–4729. doi:10.1002/fsn3.1650.
[36] R. Ribeiro-Santos, M. Andrade, N.R. de Melo, A. Sanches-Silva, Use of essential oils in active food packaging: Recent advances and future trends, Trends Food Sci. Technol. 61 (2017) 132–140. doi:10.1016/j.tifs.2016.11.021.
[37] A. Grant, S. Parveen, All natural and clean-label preservatives and antimicrobial agents used during poultry processing and packaging, J. Food Prot. 80 (2017) 440-444. doi:10.4315/0362-028X.JFP-16-146.
[38] S. Zantar, F. Yedri, R. Mrabet, A. Laglaoui, M. Bakkali, M.H. Zerrouk, Effect of *Thymus vulgaris* and *Origanum compactum* essential oils on the shelf life of fresh goat cheese, J. Essent. Oil Res. 26 (2014) 76–84. doi:10.1080/10412905.2013.871673.
[39] N. Khorshidian, M. Yousefi, E. Khanniri, A.M. Mortazavian, Potential application of essential oils as antimicrobial preservatives in cheese, Innov. Food Sci. Emerg. Technol. 45 (2018) 62–72. doi:10.1016/j.ifset.2017.09.020.
[40] F.T. Sarıcaoglu, S. Turhan, Physicochemical, antioxidant and antimicrobial properties of mechanically deboned chicken meat protein films enriched with various essential oils, Food Packag. Shelf Life. 25 (2020) 100527. doi:10.1016/j.fpsl.2020.100527.
[41] L. Hernández-Ochoa, Y.B. Aguirre-Prieto, G. V. Nevárez-Moorillón, N. Gutierrez-Mendez, E. Salas-Muñoz, Use of essential oils and

extracts from spices in meat protection, J. Food Sci. Technol. 51 (2014) 957–963. doi:10.1007/s13197-011-0598-3.

[42] I.T. Carvalho, B.N. Estevinho, L. Santos, Application of microencapsulated essential oils in cosmetic and personal healthcare products - A review, Int. J. Cosmet. Sci. 38 (2016) 109–119. doi:10.1111/ics.12232.

[43] A. Kunicka-Styczyńska, M. Sikora, D. Kalemba, Antimicrobial activity of lavender, tea tree and lemon oils in cosmetic preservative systems, J. Appl. Microbiol. 107 (2009) 1903–1911. doi:10.1111/j.1365-2672.2009.04372.x.

[44] N.Y.O. Muyima, G. Zulu, T. Bhengu, D. Popplewell, The potential application of some novel essential oils as natural cosmetic preservatives in an aqueous cream formulation, Flavour Fragr. J. 17 (2002) 258–266. doi:10.1002/ffj.1093.

[45] A. Yorgancioglu, E.E. Bayramoglu, Production of cosmetic purpose collagen containing antimicrobial emulsion with certain essential oils, Ind. Crops Prod. 44 (2013) 378–382. doi:10.1016/j.indcrop.2012.11.013.

[46] A. Orchard, S. Van Vuuren, Commercial Essential Oils as Potential Antimicrobials to Treat Skin Diseases, Evidence-Based Complement. Altern. Med. 2017 (2017). doi:10.1155/2017/4517971.

[47] A. Orchard, S.F. Van Vuuren, A.M. Viljoen, G, Kamatou, The in vitro antimicrobial evaluation of commercial essential oils and their combinations against acne, Int. J. Cosmet. Sci. 40 (2018) 226–243. doi:10.1111/ics.2018.40.issue-3.

[48] A. Sarkic, I. Stappen, Essential Oils and Their Single Compounds in Cosmetics—A Critical Review, Cosmetics. 5 (2018) 11. doi:10.3390/cosmetics5010011.

[49] B. Ali, N.A. Al-Wabel, S. Shams, A. Ahamad, S.A. Khan, F. Anwar, Essential oils used in aromatherapy: A systemic review, Asian Pac. J. Trop. Biomed. 5 (2015) 601–611. doi:10.1016/j.apjtb.2015.05.007.

[50] F. Fazlollahpour-Rokni, S.A. Shorofi, N. Mousavinasab, R. Ghafari, R. Esmaeili, The effect of inhalation aromatherapy with rose essential oil on the anxiety of patients undergoing coronary artery bypass graft surgery, Complement. Ther. Clin. Pract. 34 (2019) 201–207. doi:10.1016/j.ctcp.2018.11.014.

[51] M. kyoung Lee, S. Lim, J.A. Song, M.E. Kim, M.H. Hur, The effects of aromatherapy essential oil inhalation on stress, sleep quality and immunity in healthy adults: Randomized controlled trial, Eur. J. Integr. Med. 12 (2017) 79–86. doi:10.1016/j.eujim.2017.04.009.

[52] A. Nasiri, M.A. Mahmodi, Z. Nobakht, Effect of aromatherapy massage with lavender essential oil on pain in patients with osteoarthritis of the knee: A randomized controlled clinical trial, Complement. Ther. Clin. Pract. 25 (2016) 75–80. doi:10.1016/j.ctcp.2016.08.002.

[53] L. Franco, T.J.J. Blanck, K. Dugan, R. Kline, G. Shanmugam, A. Galotti, A. Von Bergen Granell, M. Wajda, Both lavender fleur oil and unscented oil aromatherapy reduce preoperative anxiety in breast surgery patients: A randomized trial, J. Clin. Anesth. 33 (2016) 243–249. doi:10.1016/j.jclinane.2016.02.032.

[54] S. Tariq, S. Wani, W. Rasool, K. Shafi, M.A. Bhat, A. Prabhakar, A.H. Shalla, M.A. Rather, A comprehensive review of the antibacterial, antifungal and antiviral potential of essential oils and their chemical constituents against drug-resistant microbial pathogens, Microb. Pathog. 134 (2019) 103580. doi:10.1016/j.micpath.2019.103580.

[55] A. Man, L. Santacroce, R. Iacob, A. Mare, L. Man, Antimicrobial Activity of Six Essential Oils Against a Group of Human Pathogens: A Comparative Study, Pathogens. 8 (2019) 15. doi:10.3390/pathogens8010015.

[56] T.U. De Andrade, G.A. Brasil, D.C. Endringer, F.R. Da Nóbrega, D.P. De Sousa, Cardiovascular activity of the chemical constituents of essential oils, Molecules. 22 (2017) 1539. doi:10.3390/molecules22091539.

[57] K. Blowman, M. Magalhães, M.F.L. Lemos, C. Cabral, I.M. Pires, Anticancer Properties of Essential Oils and Other Natural Products, Evidence-Based Complement. Altern. Med. 2018 (2018). doi:10.1155/2018/3149362.

[58] L.S. Nerio, J. Olivero-Verbel, E. Stashenko, Repellent activity of essential oils: A review, Bioresour. Technol. 101 (2010) 372–378. doi:10.1016/j.biortech.2009.07.048.

[59] G. Nieto, Biological Activities of Three Essential Oils of the Lamiaceae Family, Medicines. 4 (2017) 63. doi:10.3390/medicines4030063.

[60] M. Pateiro, F.J. Barba, R. Domínguez, A.S. Sant'Ana, A. Mousavi Khaneghah, M. Gavahian, B. Gómez, J.M. Lorenzo, Essential oils as natural additives to prevent oxidation reactions in meat and meat products: A review, Food Res. Int. 113 (2018) 156–166. doi:10.1016/j.foodres.2018.07.014.

[61] M. Ben Jemaa, H. Falleh, M.A. Neves, H. Isoda, M. Nakajima, R. Ksouri, Quality preservation of deliberately contaminated milk using thyme free and nanoemulsified essential oils, Food Chem.

217 (2017). doi:10.1016/j.foodchem.2016.09.030.
[62] N.D. Gonçalves, F. de L. Pena, A. Sartoratto, C. Derlamelina, M.C.T. Duarte, A.E.C. Antunes, A.S. Prata, Encapsulated thyme (Thymus vulgaris) essential oil used as a natural preservative in bakery product, Food Res. Int. 96 (2017) 154–160. doi:10.1016/j.foodres.2017.03.006.
[63] G.N. Mezza, A. V. Borgarello, N.R. Grosso, H. Fernandez, M.C. Pramparo, M.F. Gayol, Antioxidant activity of rosemary essential oil fractions obtained by molecular distillation and their effect on oxidative stability of sunflower oil, Food Chem. 242 (2018) 9–15. doi:10.1016/j.foodchem.2017.09.042.
[64] EFSA, Use of rosemary extracts as a food additive, ESFA J. 721 (2008) 1–29. http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/721.pdf.
[65] B. Bozin, N. Mimica-Dukic, I. Samojlik, E. Jovin, Antimicrobial and antioxidant properties of Rosemary and Sage (Rosmarinus officinalis L. and Salvia officinalis L., Lamiaceae) essential oils, J. Agric. Food Chem. 55 (2007) 7879–7885. doi:10.1021/jf0715323.
[66] S. Habtemariam, The Therapeutic Potential of Rosemary (Rosmarinus officinalis) Diterpenes for Alzheimer's Disease, Evidence-Based Complement. Altern. Med. 2016 (2016). doi:10.1155/2016/2680409.
[67] M.O. Villareal, A. Ikeya, K. Sasaki, A. Ben Arfa, M. Neffati, H. Isoda, Anti-stress and neuronal cell differentiation induction effects of Rosmarinus officinalis L. essential oil, BMC Complement. Altern. Med. 17 (2017) 1-10. doi:10.1186/s12906-017-2060-1.
[68] A. López-Jiménez, M. García-Caballero, M.Á. Medina, A.R. Quesada, Anti-angiogenic properties of carnosol and carnosic acid, two major dietary compounds from rosemary, Eur. J. Nutr. 52 (2013) 85–95. doi:10.1007/s00394-011-0289-x.
[69] C. Turek, F.C. Stintzing, Stability of Essential Oils: A Review, Compr. Rev. Food Sci. Food Saf. 12 (2013) 40–53. doi:10.1111/1541-4337.12006.
[70] C. Turek, F.C. Stintzing, Evaluation of Selected Quality Parameters to Monitor Essential Oil Alteration during Storage, J. Food Sci. 76 (2011) 1365–1375. doi:10.1111/j.1750-3841.2011.02416.x.
[71] C. Turek, F.C. Stintzing, Impact of different storage conditions on the quality of selected essential oils, Food Res. Int. 46 (2012) 341–353. doi:10.1016/j.foodres.2011.12.028.
[72] G. Ben Salha, R. Herrera Díaz, O. Lengliz, M. Abderrabba, M. Labidi, Effect of the Chemical Composition of Free-Terpene Hydrocarbons

Essential Oils on Antifungal Activity, Molecules. 24 (2019) 3532. doi:10.3390/molecules24193532.
[73] A. Chisvert, M. López-Nogueroles, P. Miralles, A. Salvador, Perfumes in Cosmetics: Regulatory Aspects and Analytical Methods, in: Anal. Cosmet. Prod. Second Ed., Elsevier, 2018: pp. 225–248. doi:10.1016/B978-0-444-63508-2.00010-2.
[74] CFR - Code of Federal Regulations Title 21, (n.d.). https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm (accessed November 5, 2020).
[75] Draft pre-opinion of the scientific committee on cosmetic products and non-food products intended for consumers concerning fragrance allergy in consumers. A review of the problem Analysis of the need for appropriate consumer information and identification of consumer allergens, 1999.
[76] Regulation (EC) 1223/2009 of the European Parliament and the Council of 30 November 2009 on Cosmetic Products., (n.d.). https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02009R1223-20160812&from=EN (accessed November 5, 2020).
[77] T.F. Tadros, Emulsion Science and Technology: a general introduction, Emul. scien. and tech. 1 (2009) 1-55. doi:10.1002/9783527626564.
[78] P. Walstra, Principles of Foam Formation and Stability, Chem. Eng. Scien. 48 (1993) 333-349. doi:10.1007/978-1-4471-3807-5_1.
[79] W.C. Griffin, Classification of surface-active agents by "HLB," J. Soc. Cosmet. Chem. 1 (1946) 311-326.
[80] F. Martel, B. Estrine, R. Plantier-Royon, N. Hoffmann, C. Portella, Development of agriculture left-overs: Fine organic chemicals from wheat hemicellulose-derived pentoses, Carbohydr. Sust. Devepññ. (2010) 79-115. doi:10.1007/128_2010_54.
[81] C. Ludot, B. Estrine, N. Hoffmann, J. Le Bras, S. Marinkovic, J. Muzart, Manufacture of decyl pentosides surfactants by wood hemicelluloses transglycosidation: A potential pretreatment process for wood biomass valorization, Ind. Crops Prod. 58 (2014) 335–339. doi:10.1016/j.indcrop.2014.04.039.
[82] V. Kaur, M.B. Bera, P.S. Panesar, H. Kumar, J.F. Kennedy, Welan gum: Microbial production, characterization, and applications, Int. J. Biol. Macromol. 65 (2014) 454–461. doi:10.1016/j.ijbiomac.2014.01.061.
[83] B. Katzbauer, Properties and applications of xanthan gum, Polym. Degrad. Stab. 59 (1998) 81–84. doi:10.1016/s0141-3910(97)00180-

8.
[84] L. Xu, G. Xu, T. Liu, Y. Chen, H. Gong, The comparison of rheological properties of aqueous welan gum and xanthan gum solutions, Carbohydr. Polym. 92 (2013) 516–522. doi:10.1016/j.carbpol.2012.09.082.
[85] J. Muñoz, M.C. Alfaro, L.A. Trujillo-Cayado, J. Santos, M.J. Martín-Piñero, Production of food bioactive-loaded nanostructures by microfluidization, in: Nanoencapsulation Food Ingredients by Spec. Equip., 2019. doi:10.1016/b978-0-12-815671-1.00007-x.
[86] Microfluidics Co. Chamber User Guide Innovation Through Microfluidizer ® Processor Technology, 2018.
[87] L.A. Trujillo-Cayado, J. Santos, P. Ramírez, M.C. Alfaro, J. Muñoz, Strategy for the development and characterization of environmental friendly emulsions by microfluidization technique, J. Clean. Prod. 178 (2018) 723–730. doi:10.1016/j.jclepro.2018.01.028.
[88] F. Villalobos-Castillejos, V.G. Granillo-Guerrero, D.E. Leyva-Daniel, L. Alamilla-Beltrán, G.F. Gutiérrez-López, A. Monroy-Villagrana, S.M. Jafari, Fabrication of Nanoemulsions by Microfluidization, Nanoemulsions Formul. Appl. Charact., Academic Press, 2018: pp. 207–232. doi:10.1016/B978-0-12-811838-2.00008-4.
[89] M. Valdivieso-Ugarte, C. Gomez-Llorente, J. Plaza-Díaz, Á. Gil, Antimicrobial, antioxidant, and immunomodulatory properties of essential oils: A systematic review, Nutrients. 11 (2019) 2786. doi:10.3390/nu11112786.
[90] L. Salvia-Trujillo, M.A. Rojas-Graü, R. Soliva-Fortuny, O. Martín-Belloso, Impact of microfluidization or ultrasound processing on the antimicrobial activity against Escherichia coli of lemongrass oil-loaded nanoemulsions, Food Control. 37 (2014) 292–297. doi:10.1016/j.foodcont.2013.09.015.
[91] E. Acosta, Bioavailability of nanoparticles in nutrient and nutraceutical delivery, Curr. Opin. Colloid Interface Sci. 14 (2009) 3–15. doi:10.1016/j.cocis.2008.01.002.
[92] J. Santos, N. Calero, L.A. Trujillo-Cayado, M.J. Martín-Piñero, J. Muñoz, Processing and formulation optimization of mandarin essential oil-loaded emulsions developed by microfluidization, Materials (Basel). 13 (2020) 3486. doi:10.3390/MA13163486.
[93] R. Llinares, P. Ramírez, J. Carmona, F. Carrillo, J. Munñoz, Formulation and optimization of emulsions based on bitter fennel essential oil and EO/BO block copolymer surfactant, Colloids Surfaces A Physicochem. Eng. Asp. 536 (2018) 142–147. doi:10.1016/j.colsurfa.2017.07.027.

Objectives

The aim of this Ph.D. Thesis is to master the formulation tools and the so-called microfluidization technique to prepare submicron oil in water emulsions of rosemary and thyme essentials oils with enhanced physical stability, using a novel bio-based surfactant.

In the first study, the objective was to select the best surfactant to be used in the following stages of this Ph.D. Thesis research project. For this purpose a sweep of emulsifiers, representing various HLBs, involving ethoxylated sorbitan esters (Tween 20, Tween 60 and Tween 80) and sucroesters (SP 70, Appyclean 6548 and Appyclean 6552), were tested in terms of droplet size, size distribution and physical stability. This study reached the following targets:

1. The required HLB for the ~~oil~~ surfactant used as emulsifier.
2. Emulsions of thyme essential oil showing a good physical stability could be prepared.

The second work package dealt with the influence of temperature at which the mixture thyme oil/Appyclean 6548 (App.6548) had to be heated before emulsification and its effects on the properties and stability of final emulsions. The following goals were achieved:

1. The physicochemical properties of thyme essential oil and Appyclean 6548 were analysed.
2. The effect of the heating temperature of the mixture TEO/App.6548 was determined
3. A relationship between the heating temperature of the TEO/App.6548 system and the properties of final emulsions.

4. The influence of energy applied during the primary emulsification on the properties of emulsion has been determined.

The third work package investigated the influence of pressure and number of passes through the high-pressure Microfluidizer homogenizer of emulsions formulated with thyme essential oil and Appyclean 6548. The milestone achieved was

1. To determine the optimum both pressure and number of passes through the Microfluidizer for thyme essential oil emulsions.

To apply the knowledge acquired from the previous investigations to obtain stable concentrated emulsions of thyme oil and Applyclean 6548, the fourth work package investigated the rheological properties, DSD and physical stability of thyme oil emulsions as a function of welan gum concentrations. The objective achieved was:

1. To study of the role of biopolymer on the properties of emulsions.

The fifth, sixth and seventh work packages focussed on the assessment of rosemary essential oil emulsions. The fifth work package investigated the processing conditions and formulation variables of rosemary oil emulsions formulated with Appyclean 6548. This study reached the targets described below:

1. Determination of the optimum oil/surfactant mass ratio
2. Pressure and number of passes through the Microfluidizer which yield the smallest droplet sizes and the best physical stability.

The sixth work package investigated the addition of two different biopolymers at the optimum formulation and processing conditions of

rosemary oil and Appyclean 6548 emulsions. The objective of this study was:

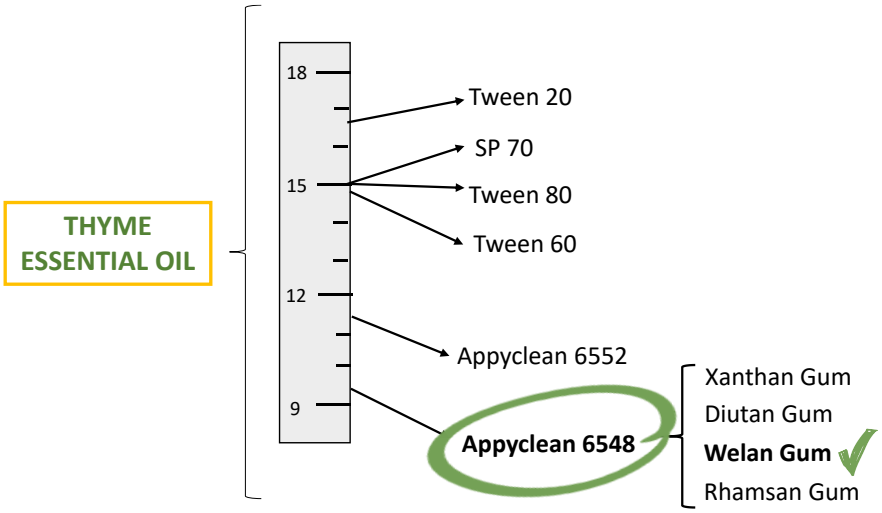
1. Comparison of xanthan and welan gums on the rheological properties, DSD and physical stability.

The seventh work package combine the microfluidization technique and vacuum evaporation in order to enhance the rheological properties of rosemary oil emulsions. The objective of this work was:

1. To stablish a relationship between the processing time under vacuum evaporation and the rheological properties and physical stability of rosemary emulsion.

Chapter 1

Effect of emulsifier HLB and stabilizer addition on the physical stability of thyme essential oil emulsions



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Effect of emulsifier HLB and stabilizer addition on the physical stability of thyme essential oil emulsions

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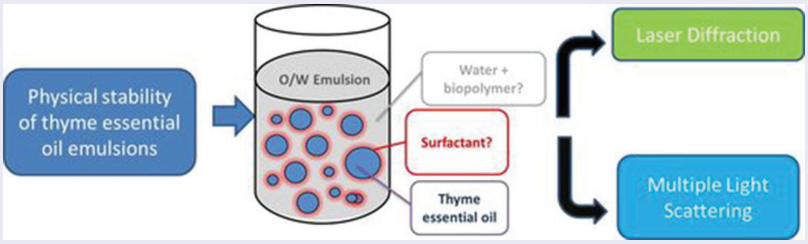
ABSTRACT

The objective of this work was to formulate and to further improve the stability of emulsions based on thyme essential oil. Several nonionic surfactants of different nature and with different hydrophilic–lipophilic balance (HLB) values were investigated. The surfactant with optimal HLB found for the thyme essential oil was Appyclean 6548 (HLB: 9-9.5). Afterwards, stabilizing biopolymers were added in order to improve emulsion stability. Properties of emulsions were evaluated in terms of droplet size and physical stability. Thyme essential oil/W emulsions formulated with a new biodegradable emulsifier (alkyl polypentoside) and welan gum as stabilizer were obtained with high shelf-life.

GRAPHICAL ABSTRACT



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KEYWORDS
Emulsifier; emulsion; multiple light scattering; stability; thyme essential oil

Introduction

Essential oils are natural hydrophobic compounds which contain a complex mixture of non-volatile and volatile compounds.[1] They are used in a wide variety of applications as functional ingredients in the food, pharmaceutical, agrochemical, sanitary and cosmetics industries[2,3] thanks to the fact that, apart from giving aroma, they also act as antibacterial, antifungical, antiviral and antioxidant agents,[2,4–7] and are classified by the United States Food and Drug Administration as generally recognized as safe (GRAS).[8] Nevertheless, several factors limit the incorporation of essential oil. These include a high hydrophobicity[1] and high volatility.[9] For many applications, oil-in-water (O/W) emulsions can be used to enhance solubilization and reduce the evaporation problem.[1]

In this work, emulsions containing thyme essential oil as oil phase were prepared. It is known that the main component in Thymus Vulgaris essential oil is thymol[10] (Nguefack et al., 2012), among other lipophilic terpenoids, phenylpropanoids and short-chain aliphatic hydrocarbon derivates.[11] Thyme essential oil has been shown to have inhibitory activities against various bacteria and yeasts.[12]

Emulsions are thermodynamically unstable colloidal dispersions in which a liquid is dispersed in a continuous liquid phase in the form of droplets. Smaller droplet size and narrow distributions confer advantages on emulsions, such as high physical stability or consistency. However, it is difficult to produce stable emulsions of thyme essential oil since these emulsions undergo one or several destabilization mechanisms, such as flocculation, coalescence, Ostwald ripening or gravitational separation. It should be noted that the use of emulsifiers whose hydrophilic lipophilic balance (HLB) value is close to the required HLB for oil can enable the formulation of stable emulsions. For this purpose, different surfactants, representing various HLBs, composed of ethoxylated sorbitan esters (Tween 20, Tween 60 and Tween 80) and sucroesters (SP 70, Appyclean 6548 and Appyclean 6552), were tested. The main novelty of this study was to use new surfactants derived from renewable raw materials such as wheat biomass (Appyclean series). This is a contribution to development of natural surfactants and to a rational and sustainable solution to agricultural wastes, like wheat straw. To the authors knowledge there are not available scientific articles dealing with this type of wheat-biomass

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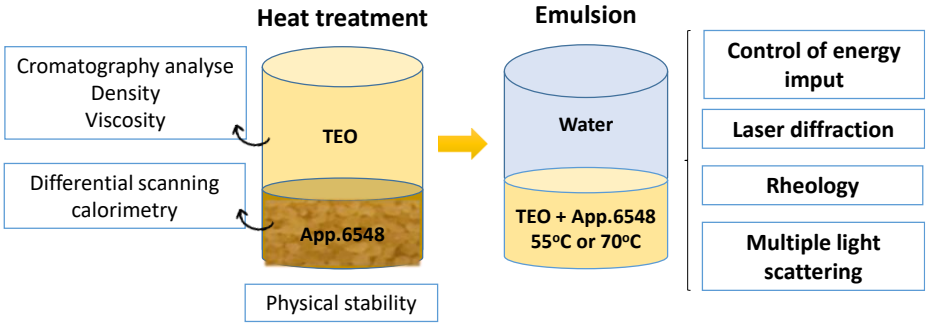
Chapter 2

Effect of heating temperature of a novel wheat-derived surfactant on a mixture of thyme essential oil/surfactant and on the final emulsions



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Effect of heating temperature of a novel wheat-derived surfactant on a mixture of thyme essential oil/surfactant and on the final emulsions

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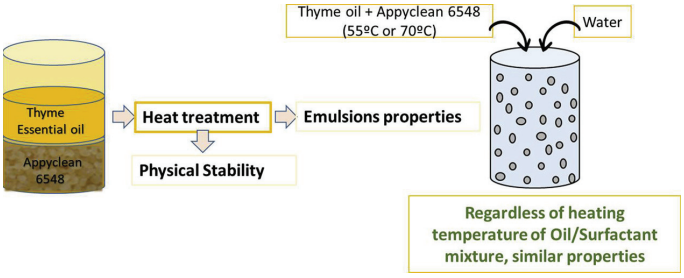
GRAPHICAL ABSTRACT



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ABSTRACT

Emulsions formulated with natural compounds are increasingly interesting in fields such as food, cosmetics or pharmaceutics. Surfactants play a significant role in the stability of emulsions. In this work, the influence of the temperature at which a mixture of thyme essential oil and surfactant is heated, on its physical stability and on properties and physical stability of emulsions formulated with this mixture as the dispersed phase, were investigated. A new bio-surfactant derived from wheat straw was employed as an emulsifier. It is an alkyl poly pentoside which is poorly soluble in water and solid at room temperature, thus, it is necessary to melt it prior to being used with the oil. As a previous step, both a physical characterization of thyme essential oil used in this work and a thermal study of surfactant were carried out. DSC technique applied to the surfactant showed that this was completely melted at 50 °C. From this information, several temperatures of heating (from 55 °C to 75 °C) for an oil and surfactant mixture in a ratio of 10:1, were applied in order to know the effect on the physical stability of the dispersion. Multiple light scattering technique was used for this purpose. It was found that slightly lower stability for the dispersion heated was at 55 °C and an optimum value at about 70 °C. For this reason, two thyme essential oil/water emulsions were prepared. One of them, from a dispersed phase previously heated at 55 °C and another one, from a dispersed phase previously heated at a higher temperature (70 °C). Then, their influence on the droplet size distribution, flow behaviour and physical stability was studied. No significant differences were observed between properties of both emulsions exhibiting similar values of mean droplet sizes, viscosity and stability.

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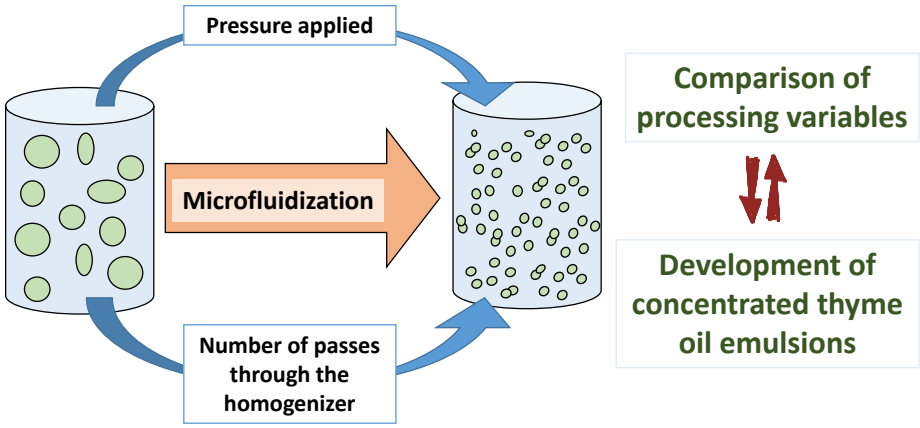
Chapter 3

Processing variables with high pressure homogenizer as Microfluidizer® influencing on droplet size distribution and physical stability of thyme essential oil/W emulsions



Variables de procesado en homogeneizador de alta presión, Microfluidizer®, que influyen en la distribución de tamaños de gota y estabilidad física de emulsiones aceite esencial de tomillo/agua

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Processing variables with high pressure homogenizer as Microfluidizer® influencing on droplet size distribution and physical stability of thyme essential oil/W emulsions

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SUMMARY

The aim of this work consisted of to investigate the influence of the pressure applied with high pressure homogenizer with Microfluidizer® technology (2500, 5000, 7500, 10000, 12500 y 15000 psi) on the droplet size distribution and physical stability of O/W emulsions formulated with thyme essential oil and an alkyl poly pentoside as an emulsifier. In addition, the number of passes (1 or 2 cycles) through the homogenizer is also studied. In order to study the quality of emulsions, techniques such as laser diffraction and multiple light scattering have been used together. It has been shown that, in general, for thyme essential oil emulsions stored at 4ºC, the lower the pressure applied and the number of passes through the homogenizer the better the physical stability being the main mechanisms of destabilization the creaming and the so-called oiling off. This work contributes to the development of ecological emulsions with the added value of that they have antimicrobial properties which is very interesting in industries such as food, cosmetics or agrochemical.

Keywords: Laser diffraction, Multiple light scattering, Green solvent, Emulsion, Surfactant.

RESUMEN

El objetivo de este trabajo ha consistido en investigar cómo influye la presión aplicada en homogeneizador de alta presión con tecnología Microfluidizer® (2500, 5000, 7500, 10000, 12500 y 15000 psi) sobre la distribución de tamaños de gota y estabilidad física de una emulsión O/W formulada con aceite esencial de tomillo y un alquilpolipentósido como emulsionante. Adicionalmente se estudia la influencia del número de pasadas (1 y 2) a través del homogeneizador. Para estudiar la calidad de las emulsiones se han utilizado conjuntamente técnicas como la difracción láser y el *multiple light scattering*. Se ha demostrado que, en general, para las emulsiones de aceite esencial de tomillo almacenadas en frío, a una temperatura de 4ºC, cuanto menor es la presión aplicada y el número de pasadas a través del homogeneizador mejor es la estabilidad física de las mismas siendo los principales mecanismos de desestabilización el cremado y el conocido como *oiling off*. Este trabajo contribuye al desarrollo de emulsiones ecológicas que tienen además el valor añadido de poseer propiedades antimicrobianas, propiedades de gran interés en industrias como la alimentaria, la cosmética o la agroquímica.

Palabras clave: Difracción láser, Retrodispersión múltiple de luz, Disolvente verde, Emulsión, Tensioactivo.

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1. INTRODUCTION

El desarrollo sostenible aplicado a la industria química justifica hoy día el uso de disolventes verdes y tensioactivos ecológicos en el desarrollo de nuevas formulaciones.

Los aceites esenciales son compuestos aromáticos derivados de productos naturales que poseen actividad biológica de amplio espectro[1]. Precisamente gracias a sus propiedades antioxidantes, antifúngicas y antivirales, tienen numerosas aplicaciones como ingredientes funcionales en alimentación, cosmética, farmacia o medicina[2-4]. Por ejemplo, se ha demostrado que el aceite esencial de tomillo es capaz de inhibir algunos tipos de bacterias y levaduras[5]. Estas propiedades se atribuyen a sus componentes fenólicos y la interacción que ejercen con las membranas de las células de los microorganismos. El aceite esencial de tomillo, como todos los aceites esenciales, es hidrófobo por lo que para mejorar su dispersabilidad se introduce en forma de emulsión. Ahora bien, para que una emulsión pueda formarse es necesaria la incorporación de agentes emulsionantes. En este trabajo se ha utilizado un alquil polipentósido obtenido a partir de una materia prima renovable (trigo) y con escasa o nula toxicidad, por lo que puede ser considerado un tensioactivo ecológico.

Numerosos son los factores que determinan las propiedades finales y estabilidad física de la emulsión. Entre ellos se encuentra el procesado. Este puede llevarse a cabo con equipos rotor-estator, membranas u homogeneizadores de alta presión (de válvulas o con tecnología Microfluidizer®).

El objetivo de este trabajo ha sido evaluar la influencia de la presión y del número de pasadas a través de un homogeneizador de alta presión con tecnología Microfluidizer® sobre la distribución de tamaños de gota, diámetros medios y estabilidad física de emulsiones O/W formuladas con aceite esencial de tomillo como fase dispersa y un tensioactivo ecológico derivado de trigo como emulsionante. Para conseguir este objetivo se han combinado técnicas como la difracción láser y *multiple light scattering.*

2. MATERIALES Y MÉTODOS

2.1 Materiales

La fase dispersa de las emulsiones constituida por el aceite esencial de tomillo (Thymus Vulgaris) (938g/L a 25ºC) fue suministrada por Bordas Chinchurreta S.A. Como emulsionante se ha utilizado un alcohol C14/C18-poliglucósido (HLB de 9-9,5) proporcionado por Wheatoleo y conocido comercialmente como Appyclean 6548. Como fase continua se ha utilizado agua ultrapura de calidad Milli-Q®.

2.2 Métodos

2.2.1. Preparación de las emulsiones

Emulsiones concentradas con un 40% en peso de aceite de tomillo y 4% en peso de tensioactivo, han sido preparadas en un homogeneizador de alta presión con tecnología Microfluidizer® (Microfluidizer® M110P) que utiliza una cámara de cizalla en forma de Y, modelo F12Y. Este homogeneizador es alimentado con emulsiones groseras o pre-emulsiones obtenidas haciendo uso de un equipo rotor estator Silverson L5M con una malla de homogeneización. Destacar que el tensioactivo Appyclean 6548 es soluble en la fase oleosa y se presenta en forma sólida por lo que, antes de ser usado se calienta en estufa junto con el disolvente a una temperatura de 60ºC durante 1 hora. A continuación se procede a preparar la pre-emulsión siguiendo dos etapas. Una primera etapa en semicontinuo, en la que la fase dispersa es añadida lentamente a la fase continua mientras se agita a 2000 rpm durante 180s. Una segunda etapa en discontinuo en la que se agita la mezcla a la misma velocidad hasta alcanzar los 210 s. Una vez preparada la emulsión grosera, se alimenta el homogeneizador M110P y se procesa utilizando como variables la presión: 2500 psi (17,2 MPa), 5000 psi (34,5 MPa), 7500 psi (51,7 MPa), 10000 psi (68,9 MPa), 12500 psi (82,7 MPa) y 15000 psi (103,5 MPa) y el número de pasadas (1 y 2 pasadas). Durante la preparación se utilizó un baño termostático para mantener la temperatura constante a 20ºC. Posteriormente se almacenaron en frío a 4ºC. Se prepararon dos lotes de cada una de las muestras.

2.2.2. Distribución de tamaños de gota

La distribución de tamaños de gota se determinó mediante difracción láser con un equipo Mastersizer X (Malvern). Las medidas se realizaron por duplicado a lo largo del tiempo de envejecimiento presentándose los resultados como la media de los mismos. A partir de la distribución de tamaños de gota (DTG) se han obtenido los diámetros medios (diámetro medio de Sauter, $D_{3,2}$ y diámetro medio volumétrico, $D_{4,3}$) así como el parámetro span el cual ha sido utilizado para evaluar la polidispersidad de los tamaños de gota. Estos parámetros se calculan mediante las ecuaciones que a continuación se indican:

$$D_{3,2} = \frac{\sum_{i=1}^{N} n_i d_i^3}{\sum_{i=1}^{N} n_i d_i^2} \qquad (1)$$

$$D_{4,3} = \frac{\sum_{i=1}^{N} n_i d_i^4}{\sum_{i=1}^{N} n_i d_i^3} \qquad (2)$$

donde d_i es el diámetro de la gota, N es el número total de gotas y n_i es el número de gotas que tienen el diámetro d_i.

$$span = \frac{D(v,0.9) - D(v,0.1)}{D(v,0.5)} \qquad (3)$$

donde D(v,0.9), D(v,0.5) y D(v,0.1) son los diámetros acumulativos al 90%, 50% y 10% respectivamente.

2.2.3. Estabilidad física

La estabilidad física de las muestras se ha monitorizado mediante el equipo Turbiscan Lab Expert (Formulaction) el cual utiliza la técnica de retrodispersión múltiple de luz (*multiple light scattering).* Los resultados se presentan como porcentaje de variación de

luz retrodispersada o modo referencia (ΔBS%), de tal forma que a cada barrido se le sustrae el resultado del primero. De esta forma se aprecian mejor los cambios que tienen lugar en los perfiles de luz retrodispersada. Para su análisis se ha utilizado el Índice de estabilidad de Turbiscan, aplicado a toda la altura de la muestra, de tal forma que tiene en cuenta todos los procesos de desestabilización que tienen lugar en ella[6]. Cuanto más elevado es su valor, menos estable es la muestra. Se obtiene mediante la ecuación:

$$TSI = \sum_j \left| scan_{ref}(h_j) - scan_i(h_j) \right| \qquad (4)$$

3. RESULTADOS Y DISCUSIÓN

En la figura 1 se muestra, a modo de ejemplo, la distribución de tamaños de gota de la emulsión preparada a 10000 psi en función del número de pasadas a través del homogeneizador M110P, para un tiempo de envejecimiento de 24 h. Como puede observarse, las distribuciones son bimodales caracterizándose por presentar un primer máximo de población de gotas por debajo de 1 micra y un segundo pico a valores de aproximadamente 2 ó 3 µm. La existencia de esta segunda población de gotas está causada por un fenómeno de re-coalescencia, inducido por un exceso de energía mecánica durante el proceso de emulsificación[7,8]. En general, se encuentra que, a este tiempo de envejecimiento, al aumentar el número de pasadas la distribución se desplaza hacia tamaños menores de gota pero, al mismo tiempo, se produce un aumento del número de gotas de mayor tamaño, es decir, aumenta la recoalescencia de gotas.

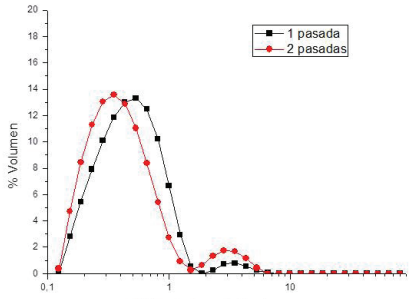


Figura 1. *Distribución de tamaños de gota de la emulsión aceite esencial de tomillo/W procesada en homogeneizador de alta presión M110P a 10000 psi como una función del número de pasadas. Tiempo de envejecimiento: 24 h*

El efecto de la presión sobre la distribución de tamaños de gota a las 24 horas de envejecimiento se ilustra en la figura 2, donde a modo de ejemplo se representan las distribuciones para 1 pasada a través del M110P. La presión influye de forma similar al número de pasadas, es decir, las distribuciones de tamaños de gota se desplazan ligeramente hacia la izquierda y al mismo tiempo el segundo máximo de población de gotas se incrementa.

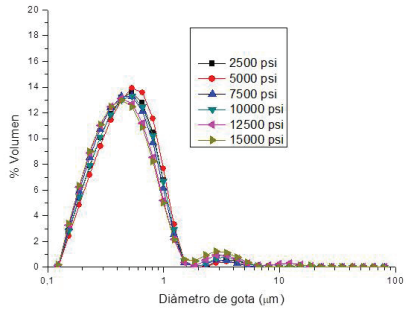


Figura 2. *Distribución de tamaños de gota de emulsiones aceite esencial de tomillo/W procesadas en homogeneizador de alta presión M110P, 1 pasada, como una función de la presión aplicada. Tiempo de envejecimiento: 24 h*

El tiempo de envejecimiento desplaza la distribución hacia mayores tamaños y provoca un aumento de la población de gotas correspondiente a tamaños del orden de 2-3 micras. Este comportamiento se ilustra en la figura 3 donde, a modo de ejemplo, se representa la DTG para emulsiones aceite esencial de tomillo/W procesadas en M110P, 1 pasada a 5000 psi.

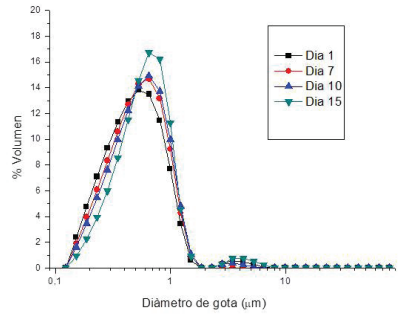


Figura 3. *Distribución de tamaños de gota de la emulsión aceite esencial de tomillo/W procesada en homogeneizador de alta presión M110P, 1 pasada a 5000 psi como una función del tiempo de envejecimiento.*

Los diámetros medios de Sauter y volumétricos así como el span en función de la presión ejercida en M110P y del número de pasadas están recogidos en la tabla 1. En primer lugar, destacar los bajos valores que presentan los diámetros medios; todas las emulsiones estudiadas presentan valores muy por debajo de la micra. Se puede hablar, pues, de emulsiones submicrónicas. Un análisis de dicha tabla revela que: a) en general D3,2 no varía significativamente ni con el número de pasadas ni con el tiempo de envejecimiento aunque sí se observa que a altas presiones o mayor número de ciclos tiende a ser menor y b) a diferencia de lo que le ocurre al diámetro medio de Sauter, el diámetro medio volumétrico, más sensible a la agregación de gotas, sí muestras variaciones significativas tanto con el tiempo de envejecimiento como con el número de pasadas. Respecto a la presión, D4,3 alcanza un valor máximo a 12500 psi. Con respecto al número de

pasadas, D4,3 se mantiene prácticamente invariable a presiones bajas, mientras que a partir de 10000 psi las diferencias de este parámetro entre una y dos pasadas son más significativas. El parámetro span, el cual determina la polidispersidad de la emulsión, también muestra, en general, un aumento de su valor tanto con el número de pasadas como con el tiempo de envejecimiento. Está claro que al aumentar la energía aplicada (mayor presión o mayor número de pasadas) el efecto de la recoalescencia de gotas se incrementa.

Tabla 1. *Diámetro medio de Sauter, diámetro medio volumétrico y span en función de la presión, número de ciclos y tiempo de envejecimiento de emulsiones aceite esencial de tomillo/W procesadas en homogeneizador de alta presión M110P*

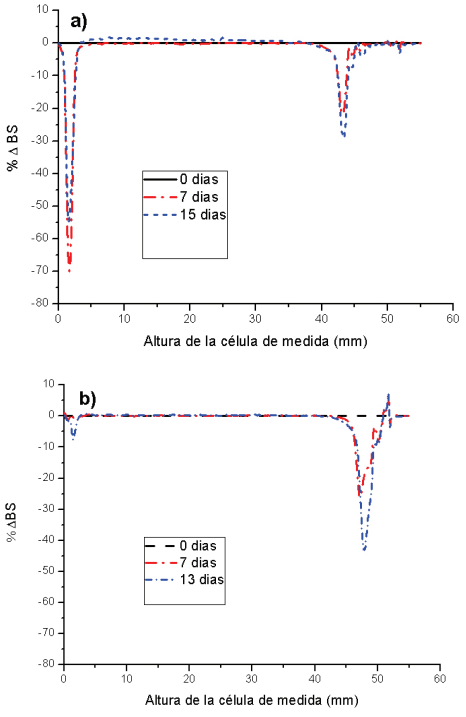
Presión (psi)	Pasadas	Tiempo Envejecimiento (días)	$D_{4,3}$ (µm)	$D_{3,2}$ (µm)	Span
2500	1	1	0,52±0.03	0,36±0.01	1,58±0.06
		7	0.63±0.03	0.39±0.02	1.5±0.2
	2	1	0,51±0.03	0,31±0.01	1,707±0.09
		7	0.75±0.02	0.35±0.01	2.2±0.03
5000	1	1	0,52±0.05	0,37±0.01	1,475±0.07
		7	0.68±0.06	0.37±0.03	2.42±0.06
	2	1	0,51±0.03	0,38±0.01	1,676±0.07
		7	0.61±0.02	0.38±0.03	1.75±0.05
7500	1	1	0,50±0.01	0,35±0.01	1,565±0.02
		7	0.71±0.03	0.38±0.02	2.12±0.04
	2	1	1,40±0.06	0,37±0.01	1,75±0.02
		7	1.61±0.01	0.38±0.05	1.76±0.09
10000	1	1	0,54±0.03	0,36±0.03	1,59±0.06
		7	0.62±0.03	0.38±0.04	1.79±0.03
	2	1	0,55±0.03	0,31±0.01	1,98±0.02
		7	0.74±0.04	0.35±0.03	4.86±0.09
12500	1	1	0,58±0.01	0,33±0.01	1,899±0.02
		7	0.75±0.04	0.38±0.05	3.76±0.08
	2	1	0,65±0.03	0,35±0.01	1,727±0.1
		7	1.15±0.03	0.37±0.06	6.65±0.07
15000	1	1	0,52±0.05	0,32±0.01	1,765±0.7
		7	0.75±0.01	0.36±0.02	3.76±0.08
	2	1	0,60±0.02	0,32±0.01	1,923±0.3
		7	0.71±0.04	0.35±0.02	4.0±0.1

En la figura 4 se muestran los perfiles de luz retrodispersada frente a la altura de la célula de medida que contiene la muestra como una función del tiempo de envejecimiento para emulsiones procesadas a 5000 psi y 15000 psi a 1 pasada (figuras 4a y 4b, respectivamente) y procesada a 5000 psi 2 pasadas (figura 4c). En la figura 4a se observa un descenso del *%delta-backscattering* en la zona baja y alta del vial mientras que en la zona central del mismo los perfiles permanecen prácticamente constantes. De acuerdo con la teoría de *multiple light scattering* este comportamiento se asocia a un proceso de cremado de gotas como consecuencia de la diferencia de densidades entre las fases continua y dispersa. Además, las gotas al migrar hacia la parte superior de la célula de medida, chocan unas con otras, se fusionan y, finalmente provoca coalescencia de gotas en la fase cremada de ahí el descenso observado en la parte superior del vial. Este último proceso se conoce como *oiling off*. Si comparamos los resultados con los mostrados por la emulsión sometida a mayor presión (figura 4b), se encuentra que el proceso de desestabilización por cremado ha disminuido considerablemente mientras que el proceso de *oiling off* aumenta. Lo mismo ocurre con la emulsión sometida a dos pasadas, figura 4c, donde se observa una menor disminución del *%delta-backscattering* en la zona baja de la célula de medida y una mayor disminución de éste en la zona alta de la célula de medida. Este resultado puede atribuirse al hecho de que a altas presiones o al aumentar el número de pasadas, como se ha comentado anteriormente, el tamaño medio de Sauter, aunque no de forma significativa, es ligeramente menor. Se sabe que cuando el tamaño es pequeño el proceso de cremado está más desfavorecido[9-10]. No obstante, sigue existiendo un ligero proceso de desestabilización por cremado. A este hecho se suma que bajo estas condiciones, más energía, el segundo pico de las distribuciones de tamaños de gota aumenta debido a un exceso de energía mecánica (recoalescencia) lo cual implica la existencia de gotas de mayor tamaño que, una vez en la fase cremada se fusionan provocando una mayor desestabilización por coalescencia.

Con el objetivo de cuantificar la cinética de desestabilización por expulsión de aceite, se determinó el *% oiling off (Oil_{off})* en función del tiempo de envejeciendo haciendo uso de la siguiente ecuación:

$$\%Oil_{off} = \frac{H_{free}}{H_B} x\ 100 \qquad (5)$$

donde es la altura del aceite libre y es la altura total de la emulsión.



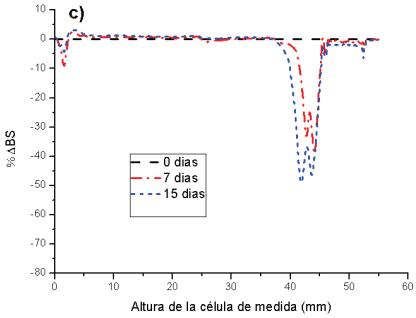


Figura 4. *Delta-backscattering frente a la altura de la célula de medida que contiene a la muestra como una función del tiempo de envejecimiento de la emulsión aceite esencial de tomillo/W procesada en a) homogeneizador de alta presión M110P, 1 pasada a 5000 psi, b) homogeneizador de alta presión M110P, 1 pasada a 15000 psi y c) homogeneizador de alta presión M110P, 2 pasadas a 5000 psi.*

En la figura 5 se representa el proceso de desestabilización que ocurre en la zona alta de la célula de medida, es decir, la expulsión de aceite debido a un proceso localizado de coalescencia[11]. Hay una primera etapa de retraso en la expulsión de aceite que da paso a la misma y a la separación visual de fases. Independientemente del número de pasadas se ha de destacar que la emulsión que más tarde comienza a expulsar aceite es la procesada a 5000 psi. Del resto de emulsiones destacar que aquella procesada a 10000 psi es la que expulsa aceite a una velocidad menor.

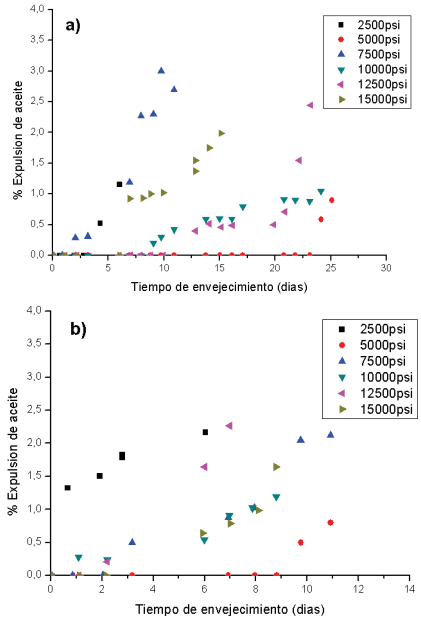


Figura 5. *Porcentaje de expulsión de aceite frente al tiempo de envejecimiento en función de la presión aplicada a emulsiones aceite esencial de tomillo/W procesadas en homogeneizador de alta presión M110P a) 1 pasada y b) 2 pasadas.*

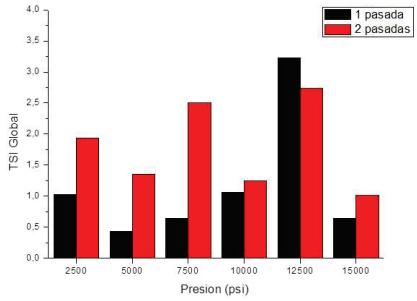


Figura 6. *TSI global frente a la presión aplicada en homogeneizador de alta presión M110P a los 7 días de envejecimiento de emulsiones aceite esencial de tomillo/W como una función del número de pasadas.*

Un análisis del parámetro TSI global a los 7 días de envejecimiento (figura 6) nos permite analizar la estabilidad de las diferentes emulsiones investigadas considerando todos los procesos de desestabilización que en ellas tienen lugar. Cuanto más elevado es el valor de este parámetro más inestable es la emulsión. De acuerdo con este criterio, se puede afirmar que, independientemente del número de pasadas, la emulsión menos estable es aquella procesada a 12500 psi. Este resultado es coherente con los obtenidos por difracción láser donde, debe recordarse, que eran estas emulsiones las que presentaban un diámetro medio volumétrico más elevado. Respecto al número de pasadas, en general 1 pasada produce emulsiones más estables que si el número de veces que la emulsión pasa a través del homogeneizador M110P se duplica. Analizando simultáneamente ambas variables se puede concluir que la emulsión más estable es aquella procesada a 5000 psi y 1 pasada.

CONCLUSIONES

En este estudio se han podido obtener emulsiones concentradas de aceite esencial de tomillo y agua empleando un homogeneizador de alta presión con tecnología Microfluidizer®. Estas emulsiones satisfacen la necesidad actual de desarrollar productos sostenibles ya que están formuladas con productos verdes. Todas las emulsiones procesadas se desestabilizan por cremado y *oiling off* si bien al aumentar la energía aplicada durante su procesado la desestabilización por cremado disminuye mientras que la coalescencia en la fase cremada aumenta. La consideración de todos los procesos de desestabilización permite concluir que la emulsión aceite esencial de tomillo/W con mayor estabilidad física es la procesada a 5000 psi y 1 sola pasada.

AGRADECIMIENTOS

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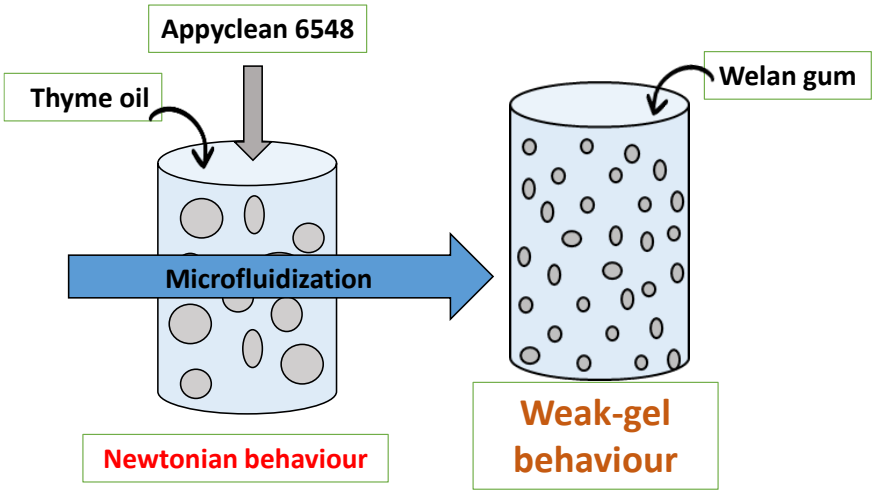
REFERENCIAS

1. Asbahani, A.; Miladi, K.; Badri, W.; Sala, M.; Addi, E.H.A.; Casabianca, H.; El Mousadik, A.; Hartmann, D.; Jilale, A.; Renaud, F.N.R.; Elaissari, A. Essential oils: from extraction to encapsulation. *Int. J. Pharm*. **2015**, *483*, 220-243.
2. Bakkali, F.; Averbeck, D.; Idaomar, M. Biological effects of essentials oil-a review. Food Chem. Toxicol., **2008**, *46*, 446-475
3. Dima, C.; Dima, S. (2015). Essential oils in foods: extraction, stabilization and toxicity. *Curr. Opin. Food Sci.*, **2015**, *5*, 29-35
4. Seow, Y.X.; Yeo, C.R.; Chung, HL.; Yuk, H.-G. Plant essential oils as active antimicrobial agents. *Crit. Rev. Food Sci.Nutr.*, **2014**, *54*, 625-644
5. Gaysinsky, S.; Davidson, P.M.; McClements, D.J.; Weiss, J. Formulation and characterization of phytophenol-carrying antimicrobial microemulsions. *Food Biophys*., **2008**, *3*, 54-65
6. Lesaint, C.; Glomm, W. R.; Lundgaard, L. E. and Sjöblom, J. Dehydration efficiency of AC electrical fields on water-in-model-oil emulsions. *Colloids Surf., A* . ***2009***. 352(1), 63-69.
7. Lobo, L. and Svereika, A. Coalescence during emulsification – 2. Role of small molecule surfactants, *J. Colloid Interface Sci.,* **2006,** *261*, 498–507.
8. Jafari, S.M..; Assadpoor, E.; Bhandari, B. Re-coalescence of emulsion droplets during high-energy emulsification, *Food Hydrocolloids*, **2008**, *22* , 1191–1202.
9. Chanamai, R.; McClements, D. J. Dependence of Creaming and Rheology of Monodisperse Oil-in-Water Emulsions on Droplet Size and Concentration. *Colloids Surf., A* . **2000**, 172 (1–3), 79–86.
10. Martin-Piñero, M.J.; Santos, J.; Trujillo-Cayado, L.A.; Garcia, M.C.; Afaro, M.C. Influencia Del Tensoactivo En La Formación y Estabilidad Física de Emulsiones Ecológicas Concentradas, *Afinidad*, **2017.** *75*(581).
11. Udomrati, S.; Khalid, N.; Gothani, S.; Nakajima, M.; Uemura, K.; Kobayashi, I. Formulation and characterization of esterified xylo-oligosaccharides-stabilized oil-in-water emulsions using microchannel emulsification. *Colloids Surf., B*, **2016** , *148*, 333-342.

Chapter 4

Influence of the welan gum biopolymer concentration on the rheological properties, droplet size distribution and physical stability of thyme oil/W emulsions



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Influence of the welan gum biopolymer concentration on the rheological properties, droplet size distribution and physical stability of thyme oil/W emulsions

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ABSTRACT

The objective of this work is to obtain a stable and natural antimicrobial delivery system. Thus, the effect of the addition of a natural polysaccharide such as welan gum on the linear viscoelastic properties, flow behaviour, droplet size distribution and physical stability of thyme oil/W emulsions formulated with a wheat-derived surfactant was studied. All emulsions obtained show submicron diameters regardless of the concentration of welan. Emulsion without gum shows Newtonian behaviour under steady shear. Meanwhile, emulsions containing welan gum show a weak gel-like behaviour with higher G′ and G″ values on increasing the content of gum in the emulsion. Their flow curves illustrate a shear thinning behaviour with much greater viscosity than that exhibited by emulsions without gum. This behaviour fits well to the Cross model. The main destabilization process of thyme oil/W emulsion without gum is creaming versus flocculation and coalescence in emulsions containing welan. Rheology, diffraction laser and multiple light scattering techniques have proved that welan gum is an important rheological modifier for thyme oil/W ecological emulsions, it being possible to control the rheological properties of these emulsions by adjusting the concentration of gum. However, welan gum does not improve the physical stability of these emulsions.

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1. Introduction

Nowadays, many industries are looking for new ecofriendly and sustainable products, which can be used in different applications. Ecological emulsions are one of these products. Finding the right formulation which fulfils environmental and non-toxicity requirements is essential for researchers within both the research and industrial fields.

As far as formulations are concerned, one of the most important ingredients in emulsions is the surfactant. Surfactants are amphiphilic molecules formed by a polar head and a lipophilic tail which can be grouped by the charge of their polar head groups: cationic, anionic, amphoteric (zwitterionic) or non-ionic. In addition, surfactants can also be classified by their hydrophilic lipophilic balance (HLB) value. The range of HLB values is from 0 to 20. HLB <9 refers to lipophilic surfactants and HLB >11 to hydrophilic. Therefore, generally for emulsions of type O/W the emulsifiers have an HLB between 8 and 18 and for W/O emulsions of 3–8 [1]. It should be noted that the use of emulsifiers whose hydrophilic lipophilic balance (HLB) value is close to the required HLB for oil could enable the formulation of stable emulsions. In this work, a non-ionic surfactant, specifically the alkyl poly pentoside commercially known as Appyclean 6548 (D-xylofuranose, oligomeric tetradecyl and octodecyl glycoside, C14, C18 alcohol) is used. This surfactant possesses an HLB value of 9.25 which has been shown to be the optimum HLB value necessary to obtain stable thyme essential oil emulsions [2]. Appyclean 6548 is a newly-developed surfactant derived from renewable raw materials such as wheat biomass. The alkyl poly pentoside surfactants are made of pentosides derived from hemicellulose [3], which is not digestible by human intestinal digestive enzymes. However, this kind of food fiber could be utilized by members of the intestinal microbial microbiota like a prebiotic product [4]. In addition, this surfactant fulfils the requirements to be used in ecolabel formulations.

In this work, thyme essential oil is used as oil phase. This essential oil is classified as a GRAS product by the United States Food and Drug Administration [5]. Thyme Vulgaris, has very high antioxidant activity due to its main components: thymol, carvacrol, γ-terpinene, myrcene, linalool, p- cymene, limonene, 1,8-cineole, and α-pinene. Among other essential oils, thyme oil has relatively strong antimicrobial activity which it makes it suitable for use in the food field [6,7], Thyme oil is found to be effective against *Salmonella typhimurium L. monocytogenes*, *A. hydrophila* and autochthonous flora spoilage in meat products [8]. Nevertheless, thyme essential oil emulsions might undergo one or several destabilization mechanisms, such as flocculation, coalescence, Ostwald ripening or creaming. The incorporation of biopolymers into the

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Chapter 5

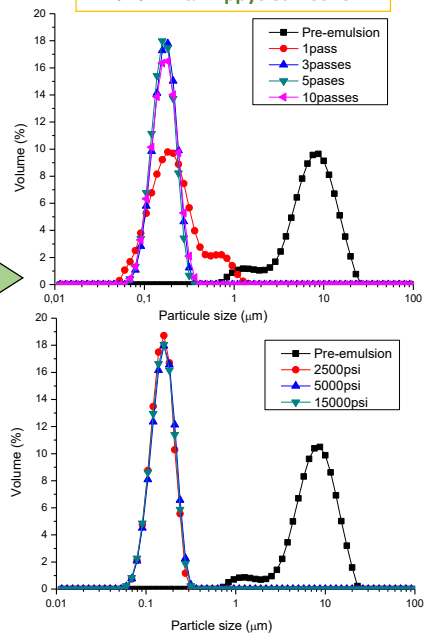
Development of rosemary essential oil nanoemulsions using a wheat biomass-derived surfactant

Influence of **rosemary essential oil** concentration (10-50wt%)

Wheat derived surfactant (**Appyclean 6548**)

Microfluidization

20 wt% rosemary essential oil and **4 wt% Appyclean 6548**



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Development of rosemary essential oil nanoemulsions using a wheat biomass-derived surfactant

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ABSTRACT

In the present work aqueous-based emulsions formulated with bio-based solvents and surfactants were studied. The droplet size distribution, rheology and physical stability of rosemary essential oil/water emulsions formulated with an emulsifier derived from wheat waste (alkyl polypentoside) were investigated as a function of the dispersed phase concentration (10–50 wt%) by means of laser diffraction, multiple light scattering and rheology measurements. Subsequently, processing variables, such as the pressure and the number of microfluidization passes, were studied to the best formulation (20 wt% rosemary oil). The laser diffraction technique revealed that monodispersed submicron emulsions were obtained for oil phase concentrations below 20 wt%. All emulsions showed Newtonian behavior, except for the emulsion containing 50 wt% oil, which exhibited shear-thinning properties. Moreover, the main destabilization mechanism of all the emulsions was creaming. The combination of techniques used demonstrated that the emulsion containing 20 wt% rosemary essential oil (REO) and prepared by microfluidization at 2500 psi (17.2 MPa) exhibited the longest physical stability and the smallest droplet size after 3passes. This research is a contribution to sustainable development not only by using chemicals derived from renewable raw materials but also by achieving stable emulsions with a low surfactant/oil mass ratio.

1. Introduction

In recent years, the utilization of renewable resources/raw materials is gaining increased significance. The development of sustainable products has increased due to the strong demand for ecological ingredients as a result of social concern about the environmental impact of industrial products in many fields [1]. Essential oils (EOs) are very interesting products derived from natural plants which contain complex mixtures of non-volatile and volatile compounds [2]. Among other qualities they exhibit various biochemical characteristics such as antibacterial, antifungal, antiviral and antioxidant properties [3–7]. Thanks to their biochemical compounds,essential oils are widely used in many fields, such as the food, pharmaceutical and cosmetics industries [6]. Rosemary essential oil (*Rosmarinus officinalis L.*) belongs to the Lamiaceae family and it mainly contains monoterpenes and monoterpene derivates, the major chemical constituents being α- pinene, 1,8-cineole, camphor, myrcene, camphene, borneol and verbenone [8–11]. Due to this composition, rosemary essential oil (REO) has been reported to exhibit antioxidant [10], antimicrobial [12], aromatherapeutical [13] and anticancerproperties [4,14]. REO is sensitive to light, temperature and oxidative reactions. These processes may result in a loss of quality and pharmacological properties [15]. Due to its high volatility [16] and its sensitivity to environmental effects [11], REO needs further protective measures to enhance or preserve its properties for a longer time.

Emulsification is one of the most efficient techniques for the preservation of essential oils. An emulsion is a thermodynamically unstable dispersion of two immiscible liquids of different polarity and of different hydrophobicity and hydrophilicity. One of the liquids forms small droplets, which are dispersed in the continuous phase formed by the other liquid. The liquid that forms the droplets is known as the dispersed phase (internal phase) and the liquid containing these droplets is the continuous phase (external phase) [17]. Emulsions not only help to preserve the essential oil properties, but also help to mask the strong taste and smell typical of these oils. Oil in water (O/W) emulsions can be used to prevent the evaporation of the volatile components and enhance their solubility, reducing the hydrophobic problem [2]. An emulsion must also contain an emulsifier. Bio-based surfactants have attracted increasing attention within the research and industrial sector due to their performance, cost and environmentally friendly characteristics. Alkyl poly pentosides (APPs) are non-ionic and renewable

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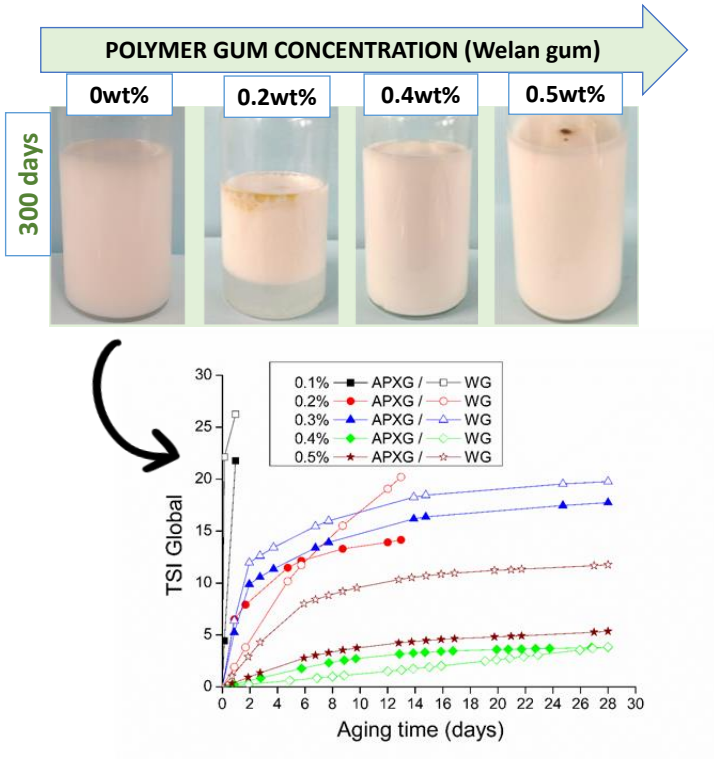
Chapter 6

Characterization of novel nanoemulsions, with improved properties, based on rosemary essential oil and biopolymers



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Characterization of novel nanoemulsions, with improved properties, based on rosemary essential oil and biopolymers

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Abstract

BACKGROUND: Nowadays, it is of great interest to develop stable and sustainable formulations that act as nanocarriers of active ingredients. In this work, the droplet size distribution, rheology and physical stability of nanoemulsions with improved properties containing rosemary essential oil and biopolymers as a function of the concentration of these polysaccharides were investigated.

RESULTS: Mean diameters below 150 nm were achieved, indicating nanostructures were obtained. Regardless of gum type, a gel-like structure and a shear thinning behaviour was achieved. In addition, an increase of G', G'' and viscosity and a decrease of J_0, J_1, J_2, λ_1 and λ_2 with increasing gum concentration were observed, due to the formation of a three-dimensional network in the aqueous phase. Slight differences between nanoemulsions containing welan or xanthan were found. Creaming, depletion flocculation and gel aggregation were the main destabilization processes at low, intermediate and high gum concentration, respectively. A 0.4 wt% gum nanoemulsion exhibited the best physical stability.

CONCLUSION: These stable and sustainable nanoemulsions with improved rheological properties contribute to the development of biodegradable and non-toxic food or agrochemical products.
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Keywords: nanoemulsion; rosemary essential oil; biopolymer; rheology; physical stability

INTRODUCTION

In recent years, numerous studies about nanoemulsions have been developed due to the interest of these formulations as delivery systems for lipophilic active compounds. However, nanoemulsions are Newtonian and lack the desired rheological properties for some applications. Thus, for example in the case of an emulsion-based agrochemical or food product, this product should possess a non-Newtonian behaviour with high viscosity at rest in order to prevent its destabilization during lifetime, but at the same time, with low viscosity under an applied stress in order to facilitate the handling. In addition, a gel-like structure is also interesting due to the fact that is correlated with a higher stability of emulsions against creaming. This rheological behaviour can be obtained with these structured nanoemulsions which can be used not only in agrochemical or food applications but also in pharmaceutic, medical or cosmetic applications. Currently, another aspect to consider when formulating a product is the growing interest of consumers in the use of natural products and sustainable raw materials.

Natural polymers, that are strongly demanded due not only to their thickening, stabilizing or emulsifying properties, but also their biocompatibility, biodegradability and non-toxicity, can be used to enhance the rheological properties and improve the shelf life of nanoemulsions. Specifically, xanthan gum (XG) advanced performance and welan gum (WG) were used in this study.

XG is a natural high molecular weight anionic polysaccharide produced by aerobic fermentation of sugars by the microorganism *Xanthomonas campestris*.[1] There are numerous products in different fields which use XG. Cleaners, coatings, polishes and products in the agricultural and food industry also use XG.[2] Specifically, advanced performance food grade XGis suitable for sauces, milk and cream products or beverages.

WG is an anionic extracellular polysaccharide produced by the micro-organism *Sphingomonas* sp., namely from *Alcaligenes* sp., ATCC 31555.[3] Although WG is rarely used in emulsions, in the last few years, there have been a number of studies concerning its use in this type of formulations. For example, WG may be used as an ingredient in food products in which it can act as a thickening, suspending, binding, emulsifying, stabilizing and viscosifying agent.[3]

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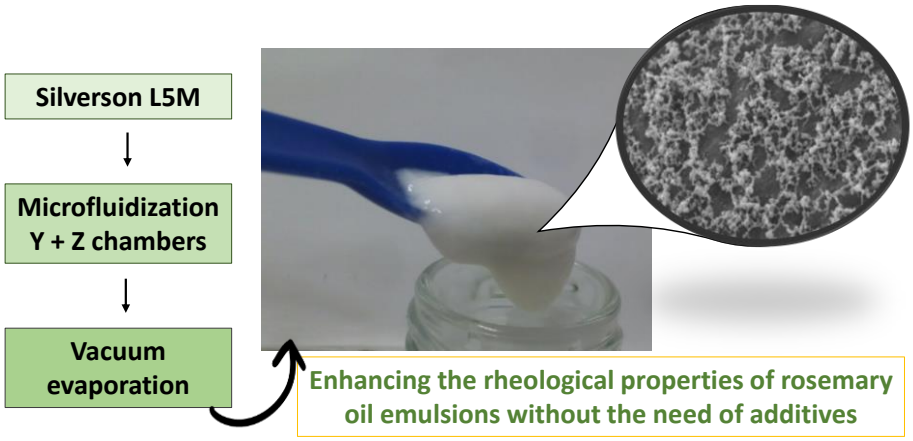
Chapter 7

Improvement of the rheological properties of rosemary oil nanoemulsions prepared by microfluidization and vacuum evaporation



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ABSTRACT

Commercial products with specific textures and rheological properties can be made by controlling the processing variables by evaporation. In this research, we investigate the effect of evaporation time on rheological properties, DSD, microstructure and physical stability of biodegradable emulsions formulated with rosemary oil and Appyclean 6548, an alkyl polypentoside surfactant derived from wheat straw. Results showed that the rheological properties of emulsions depend greatly on time of evaporation and aging time. This method makes it possible to improve the viscosity and viscoelasticity compared with emulsions without evaporation. The main destabilization process was flocculation. Emulsions undergoing higher evaporation time also exhibited coalescence. In conclusion, rosemary nanoemulsions obtained by vacuum evaporation showed high stability and great resistance against creaming, making these appropriate for use in functional foods or cosmetic products.
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Introduction

Droplet sizes play an important role in the stability of emulsions and the release capacity of bioactive compounds into the human body. A decrease in droplet size below 500 nm leads to an enhancement in the absorption and bioavailability of active ingredients [1].

To achieve nanoemulsions, the most common emulsification methods consist of applying mechanical energy to the system in order to break up the oil droplets and disperse these into the water phase. Typically, high-pressure valve homogenizers (HPvH) or Microfluidizers are effective in reducing the droplet size to the nanoscale. Small droplet sizes could be achieved by the combination of processing variables such as premixing, the number of cycles and homogenization pressure which affect the final nanoemulsion properties [2]. However, nanoemulsions are usually Newtonian and lack the desired rheological properties for some applications.

Evaporation could be a new method to develop nanoemulsions with specific rheological properties. The evaporation technique increases the temperature and breaks up the emulsion, which leads to the evaporation of water [3]. It has been shown that the droplet size distributions of evaporated emulsions are not affected [4,5]. The properties of emulsions prepared by the evaporation technique are influenced by variables such as oil concentration, type of surfactant and operating conditions [6]. Insoluble water surfactants and small oil droplet sizes decrease the evaporation rate and the quality of the condensate liquid [7]. Temperature and working pressure are important operating conditions in emulsion evaporation. These variables has been previously studied by different authors [3–6]. In addition, the time that emulsions undergo vacuum evaporation is also an important variable. Increasing evaporation time increases the mass of water which is vaporized. The ratio surfactant/oil (S/O) of nanoemulsion increases as a function of time during vacuum evaporation. The S/O ratio of the final emulsion affects the microstructure (level of flocculation), the physical stability (creaming and coalescence) [8] and the rheological properties (viscosity and viscoelasticity). A 3-D network of flocculated oil droplets is formed, which results in an improvement in the rheological properties and the stability of nanoemulsions due to the aggregation of the droplets and their interactions.

In this study, rosemary essential oil (REO) was used as dispersed phase. It belongs to the Lamiaceae family, which has been demonstrated to show high antioxidant activity [9]. In particular, REO exhibits high antioxidant and antibacterial activity against L. monocytogenes bacteria, *Escherichia coli*, *Salmonella indiana* and *Listeria innocua* [10,11] due to the presence of its major compounds such as α- pinene, 1,8-cineole, camphor, myrcene, camphene, borneol and verbenone. [12] These REO properties are potentially

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Conclusions

This Ph.D. Thesis is a contribution to gain a deeper insight into the formulation and processing conditions for essential oil emulsions, using a novel bio-based surfactant derived from wheat biomass. The next paragraphs summarize the most relevant results for thyme and rosemary oil emulsions:

1. Identification of the best HLB value for thyme essential oil.

Only the emulsifiers with HLB < 12 (Appyclean 6548 and Appyclean 6552) were able to form emulsions containing 40 wt% thyme oil. Laser diffraction and multiple light scattering techniques showed creaming for both emulsions and, additionally coalescence for emulsion with Appyclean 6552. The most stable emulsion was that formulated with **Appyclean 6548 (HLB = 9.25)**, the most hydrophobic emulsifier investigated. Emulsions containing 40 wt% thyme oil and 4 wt% Appyclean 6548 exhibited a Sauter mean diameter around 1 μm, using a Silverson L5M rotor-stator homogenizer equipped with an emulsor screen at 7000 rpm. Subsequently, several biopolymers were used as stabilisers, the most stable emulsions being formulated with welan gum.

2. Investigation of the influence of temperature at which the mixture thyme oil/Appyclean 6548 is heated and its effects on the properties and stability of final emulsions.

Differential scanning calorimetry (DSC) analysis showed that Appyclean 6548 was completely melted at 50^{o}C. Multiple light scattering was used to determine the stability of the mixture thyme oil/Appyclean 6548, in a ratio of 10:1 at temperatures from 55^{o}C to 75^{o}C. The highest TSI value was achieved at 55^{o}C. Subsequently, emulsions formulated with 40 wt% thyme

oil and 4 wt% Appyclean 6548 (heated at 55^{o}C and 70^{o}C) were prepared by Silverson L5M at 7000rpm. **Both emulsions showed similar properties:** monomodal droplet size distribution, Sauter mean droplet size around 1 µm, Newtonian behaviour and creaming followed by oiling off as destabilization mechanisms, **regardless of the heating temperature**. Nevertheless, dispersed phase prepared at 70^{o}C resulted in slightly better stability.

3. Determination of the optimal processing conditions of emulsions formulated with 40 wt% thyme essential oil and 4 wt% Appyclean 6548.

The combination of laser diffraction and multiple light scattering techniques demonstrated that the lower the pressure applied and the number of passes through the homogenizer the better the physical stability. The main destabilization mechanisms were creaming and oiling off. All emulsions showed bimodal distributions and submicron droplet Sauter mean diameter. Thus, the difference among droplet sizes were not significant although the multiple light scattering technique showed slightly **better stability for emulsions prepared at 5000 psi (34.47 MPa) after one pass through the high-pressure homogeniser.**

4. Study of the role of biopolymer on the properties of thyme oil emulsions.

O/W emulsions of submicron Sauter mean droplet size range were obtained with a Microfluidizer M100P at 2500 psi (17.23 MPa) one pass formulated with 6 wt% Appyclean 6548 and 40 wt% thyme essential oil. The addition of welan gum biopolymer enhanced the rheological properties of emulsions. Emulsion without gum showed Newtonian

behaviour and did not exhibit linear viscoelastic region whereas all emulsions with gum presented and a weak gel like behaviour that became stronger with welan concentration. Steady shear flow properties of emulsions containing gums fitted fairly well the Cross model equation, exhibiting shear thinning behaviour. Multiple light scattering demonstrated that creaming was the main destabilization mechanism for emulsions without gum, which decreased with increasing gum concentration. Conversely, flocculation was the most important destabilization process for emulsions with gum, the faster kinetics destabilization being observed at 0.2 wt% welan gum. **This gum does not make possible to increase the physical stability but improve the rheological properties.**

5. Investigation of the processing conditions and formulation variables of rosemary oil emulsions formulated with Appyclean 6548.

Regardless of the oil concentration used, the use of microfluidization and Appyclean 6548 surfactant enabled nanoemulsions to be formed. However, the oil concentration did influence the DSD, rheological properties and physical stability of all emulsions studied. Laser diffraction technique showed bimodal distribution after one pass. **Three passes at 2500 psi** (17.23 MPa) were required to obtain reproducible DSDs. **Monodispersed nanoemulsions** (span = 0.75 and $D_{4,3}$ = 149 nm) were obtained at **4 wt% Appyclean 6548 and 20 wt% rosemary oil.** The main destabilization process was creaming for all emulsions. However, 30-50 wt% rosemary oil emulsions showed some coalescence which is more significant at the highest concentration. All emulsions exhibited Newtonian behaviour, except for the 50 wt% of rosemary oil which

exhibited shear thinning behaviour. In addition, laser diffraction results demonstrated that either an increase in the number of passes or in pressure did not improve either the droplet size distribution or the mean droplet sizes.

6. Comparison of xanthan and welan gums on the rheological properties, DSD and physical stability.

The influence of welan gum (WG) and of an advanced performance xanthan gum (APXG) concentrations on the rheological properties, DSD, and physical stability of emulsions was investigated. Emulsions containing WG exhibited G´ and G´´ higher than those containing APXG but, at the same time, they were more dependent on the concentration. Regarding the droplet sizes, nanoemulsions were obtained and remained stable except for emulsions that underwent coalescence (0.3 wt% APXG and 0.3 wt% WG and 0.5 wt% WG). The main destabilization process depended on the concentration of biopolymer:

- At low gum concentration, emulsions showed creaming as a predominant mechanism.
- At intermediate gum concentration, emulsions exhibited depletion flocculation, which sometimes yielded coalescence, subsequently.
- **At 0.4 wt% WG or APXG, emulsions showed the greatest stability.**
- At 0.5 wt%, emulsions underwent coalescence according to the results obtained by laser diffraction. Coalescence became more pronounced in emulsions formulated with WG.

7. Stablishing a relationship between time under vacuum evaporation and rheological properties and physical stability of rosemary emulsion.

Vacuum evaporation is a very effective technique to produce concentrated nanoemulsions of rosemary oil and Appyclean 6548 with a long-term stability and high viscosity without the need of additives. It is possible to obtain nanoemulsions with desirable rheological properties controlling the vacuum evaporation time. Rheological measurements demonstrated that emulsions with an initial oil concentration of 20 wt% subjected to 10 min and 20 min showed G" over G´ and Newtonian behaviour. In contrast, emulsions subjected to 30 min and 40 min exhibited G´ values greater than G" and shear thinning behaviour, which shifted to very shear thinning after 50 min and 60 min. The increase of both moduli and viscosity are due to the aggregation of droplets, which form a 3D network. Rheology, laser diffraction and multiple light scattering were important tools to clarify the destabilization mechanisms, which were taking place simultaneously in these emulsions:

- Emulsions treated for 10 min and 20 min showed a main destabilization process by creaming, which is justified by the low values of viscosity of these systems. These emulsions showed an increase in droplet size possibly caused by depletion flocculation.
- Emulsions treated for 30 min and 40 min underwent a clear increase in droplet size by depletion flocculation, that subsequently led to coalescence
- Emulsions treated for 50 min and 60 min were the most concentrated emulsions (oil concentrations of 18.6 wt% and 29.3 wt% were reached, respectively). A network based on packed

droplets were formed yielding a weak gel-like structure which became stronger with the increase in the interactions among droplets. The main destabilization process consisted of an increase in droplet size by depletion flocculation, which subsequently led to coalescence.

Conclusiones

Esta tesis doctoral contribuye a la idoneidad de la formulación y el procesado de emulsiones de aceites esenciales, utilizando un nuevo tensioactivo derivado de biomasa de trigo. A continuación, se muestra un resumen de los resultados más relevantes para las emulsiones de aceite de tomillo y aceite de romero:

1. Identificación del valor óptimo de HLB requerido para emulsiones de aceite esencial de tomillo.

Solo los tensioactivos con HLB < 12 (Appyclean 6548 y Appyclean 6552) pudieron formar emulsiones con un 40 m/m% de aceite de tomillo. Las técnicas de difracción láser y retrodispersión múltiple de luz (*multiple light scattering*) mostraron desestabilización por cremado para ambas emulsiones y, además, coalescencia para la emulsión con Appyclean 6552. La emulsión más estable fue la formulada con **Appyclean 6548 (HLB = 9,25)**, el tensioactivo más hidrófobo investigado. Las emulsiones formuladas con 40 m/m% de aceite de tomillo y 4 m/m% de Appyclean 6548 mostraron un diámetro medio de Sauter alrededor de 1 µm, usando un homogeneizador rotor-estator Silverson L5M equipado con una malla de homogeneización a 7000 rpm. Posteriormente, se utilizaron varios biopolímeros como estabilizadores, formulándose las emulsiones más estables con goma welan.

2. Investigación de la influencia de la temperatura a la que se calienta la mezcla aceite de tomillo/Appyclean 6548 y sus efectos sobre las propiedades y estabilidad en las emulsiones finales.

El análisis de calorimetría diferencial de barrido (DSC) mostró que el Appyclean 6548 funde completamente a 50°C. Se utilizó la retrodispersión múltiple de luz para determinar la estabilidad física de la mezcla aceite de

tomillo/Appyclean 6548, en una proporción de 10:1 a temperaturas de 55°C a 75°C. El valor de TSI más alto se alcanzó a 55°C. Posteriormente, se prepararon emulsiones formuladas con 40 m/m% de aceite de tomillo y 4 m/m% de Appyclean 6548 (calentado a 55^{o}C y 70^{o}C) mediante Silverson L5M a 7000 rpm. **Ambas emulsiones mostraron propiedades similares:** distribución monomodal del tamaño de gota, diámetro medio de Sauter alrededor de 1 µm, comportamiento newtoniano y desestabilización por cremado seguido de expulsión de aceite (*oiling off*), **independientemente de la temperatura de calentamiento.** Sin embargo, la fase dispersa preparada a 70°C mostró una estabilidad física ligeramente mejor.

3. Determinación de las condiciones óptimas de procesado de emulsiones formuladas con 40 m/m% de aceite esencial de tomillo y 4 m/m% de Appyclean 6548.

La combinación de las técnicas de difracción láser y retrodispersión múltiple de luz demostraron que cuanto menor es la presión aplicada y el número de pasadas a través del homogeneizador, mejor es la estabilidad física de las emulsiones. Los principales mecanismos de desestabilización fueron el cremado y *oiling off*. Todas las emulsiones mostraron distribuciones bimodales y tamaño de gotas submicrónicas (diámetro medio de Sauter). Por tanto, la diferencia entre los tamaños de gota no fue significativa, aunque la técnica de retrodispersión múltiple de luz mostró una **estabilidad ligeramente mejor para las emulsiones preparadas a 5000 psi (34,47 MPa) una pasada a través del homogeneizador de alta presión.**

4. Estudio de la concentración de un biopolímero en las propiedades de las emulsiones de aceite de tomillo.

Se obtuvieron emulsiones O/W de diámetro medio de Sauter submicrónico con Microfluidizer M100P a 2500 psi (17,23 MPa) una pasada formulada con 6 m/m% de Appyclean 6548 y 40 m/m% de aceite esencial de tomillo. La adición de goma welan mejoró las propiedades reológicas de las emulsiones. La emulsión sin goma mostró un comportamiento newtoniano y no presentó propiedades viscoelásticas, mientras que todas las emulsiones con goma presentaron un comportamiento de gel débil que se hizo más fuerte con la concentración. Las propiedades de flujo de las emulsiones que contienen goma se ajustan bastante bien a la ecuación del modelo de Cross, mostrando un comportamiento pseudoplástico. La técnica de retrodispersión múltiple de luz demostró que el cremado fue el principal mecanismo de desestabilización de las emulsiones sin goma, que disminuía al aumentar la concentración de goma. A la inversa, la floculación fue el proceso de desestabilización más importante para las emulsiones con goma, observándose la desestabilización más rápida al 0,2 m/m% de goma welan. **Esta goma mejora las propiedades reológicas pero no permite incrementar la estabilidad física de estas emulsiones.**

5. Investigación de las condiciones de procesado y variables de formulación para emulsiones de aceite de romero formuladas con Appyclean 6548.

Independientemente de la concentración de aceite utilizada, el uso de la tecnología de microfluidización y el tensioactivo Appyclean 6548 permitió que se formaran nanoemulsiones. Sin embargo, la concentración de aceite influyó en la distribución de tamaños de gota (DTG), en las propiedades reológicas y en la estabilidad física de todas las emulsiones estudiadas. La

técnica de difracción láser mostró una distribución bimodal después de una pasada. Se requirieron **tres pasadas a 2500 psi (17,23 MPa)** para obtener DTG reproducibles. Se obtuvieron nanoemulsiones monodispersas (span = 0,75 y D4,3 = 149 nm) al **4 m/m% de Appyclean 6548 y al 20 m/m% de aceite de romero**. El cremado fue el principal proceso de desestabilización para todas las emulsiones. Sin embargo, las emulsiones de aceite de romero al 30-50 m/m% mostraron cierta coalescencia que fue más significativa a la concentración más alta. Todas las emulsiones exhibieron comportamiento newtoniano, excepto a 50 m/m% de aceite de romero que mostró un comportamiento pseudoplástico. Además, los resultados de difracción láser demostraron que un aumento en el número de pasadas o un aumento de la presión no mejoraba ni la distribución del tamaño de gota ni sus tamaños medios.

6. Comparación de goma xantana y goma welan sobre las propiedades reológicas, DTG y estabilidad física.

Se investigó la influencia de la goma welan (WG) y de goma xantana "*advanced performance*" (APXG) sobre las propiedades reológicas, DTG y estabilidad física de las emulsiones. Las emulsiones que contienen WG mostraron G´ y G´´ más altas que las que contienen APXG pero, al mismo tiempo, fueron más dependientes de la concentración. Con respecto al tamaño de gota, se obtuvieron nanoemulsiones y se mantuvieron estables excepto por las emulsiones que experimentaron coalescencia (0,3 m/m% de APXG, 0,3 m/m% de WG y 0,5 m/m% de WG). El principal proceso de desestabilización dependió de la concentración de biopolímero:

- A baja concentración de goma, las emulsiones mostraron cremado como mecanismo predominante de desestabilización.

- A una concentración intermedia de goma, las emulsiones mostraron floculación por agotamiento (*depletion flocculation*), que posteriormente, podría producir coalescencia.
- **Al 0,4 m/m% WG o APXG, las emulsiones mostraron la mayor estabilidad.**
- Al 0,5 m/m% las emulsiones mostraron coalescencia según la técnica de difracción láser. La coalescencia se hizo más pronunciada en emulsiones formuladas con WG.

7. Establecer una relación entre el tiempo de evaporación a vacío, las propiedades reológicas y la estabilidad física de la emulsión de romero.

La evaporación a vacío es una técnica muy eficaz para producir nanoemulsiones concentradas de aceite de romero y Appyclean 6548 con una estabilidad a largo plazo y alta viscosidad sin necesidad de aditivos. Es posible obtener nanoemulsiones con propiedades reológicas deseables controlando el tiempo de evaporación al vacío. Las mediciones reológicas demostraron que las emulsiones con una concentración inicial de aceite del 20 m/m% sometidas a 10 min y 20 min mostraron G" sobre G´ y comportamiento newtoniano. Por otro lado, las emulsiones sometidas a 30 min y 40 min mostraron valores de G´ mayores que G" y un comportamiento pseudoplástico que, pasa a ser muy pseudoplástico (*very shear thinning*) tras 50 y 60 min en el rotavapor. El aumento tanto de los módulos viscoelásticos como de la viscosidad se debe a la agregación de gotas, que forman una red 3D. La reología, difracción láser y retrodispersión múltiple de luz fueron herramientas importantes para aclarar los mecanismos de desestabilización, que tenían lugar simultáneamente:

- Las emulsiones sometidas a vacío durante 10 min y 20 min mostraron el cremado como proceso principal de desestabilización, que se justifica por los bajos valores de viscosidad de estos sistemas. Estas emulsiones mostraron un aumento del tamaño de las gotas, posiblemente causado por la floculación por agotamiento.
- Las emulsiones tratadas durante 30 min y 40 min experimentaron un claro aumento del tamaño de gota por floculación por agotamiento, que posteriormente condujo a coalescencia.
- Las emulsiones tratadas durante 50 min y 60 min fueron las más concentradas (se alcanzaron concentraciones de aceite de 18,6 y 29,3 m/m%, respectivamente). Se formó una red basada en gotas empaquetadas que dio lugar a una estructura débil de tipo gel que se hizo más fuerte con el aumento de las interacciones entre las gotas. El principal proceso de desestabilización consistió en un aumento del tamaño de las gotas por floculación de agotamiento, que posteriormente condujo a la coalescencia.

Articles related to this Ph.D. Thesis published in journals ranked in the Journal of Citation Reports (JCR).

Martin, M. J., Trujillo, L. A., Garcia, M. C., Alfaro, M. C., & Muñoz, J. (2018). Effect of emulsifier HLB and stabilizer addition on the physical stability of thyme essential oil emulsions. Journal of Dispersion Science and Technology, 39(11), 1627-1634.

Martin-Piñero, M. J., Carmona, J. A., Muñoz, J., & Alfaro-Rodriguez, M. C. (2019). Effect of heating temperature of a novel wheat-derived surfactant on a mixture of thyme essential oil/surfactant and on the final emulsions. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 579, 123649.

Martín-Piñero, M. J., Trujillo, L. A., García, M. C., Muñoz, J., & Alfaro, M. C. (2019). Variables de procesado en homogeneizador de alta presión, Microfluidizer®, que influyen en la distribución de tamaños de gota y estabilidad física de emulsiones aceite esencial de tomillo/agua. Afinidad, 76(585).

Martin-Piñero, M. J., García, M. C., Muñoz, J., & Alfaro-Rodriguez, M. C. (2019). Influence of the welan gum biopolymer concentration on the rheological properties, droplet size distribution and physical stability of thyme oil/W emulsions. International journal of biological macromolecules, 133, 270-277.

Martin-Piñero, M. J., Ramirez, P., Muñoz, J., & Alfaro, M. C. (2019). Development of rosemary essential oil nanoemulsions using a wheat biomass-derived surfactant. Colloids and Surfaces B: Biointerfaces, 173, 486-492.

Martin-Piñero, M. J., García, M. C., Santos, J., Alfaro-Rodriguez, M. C., & Muñoz, J. (2020). Characterization of novel nanoemulsions, with improved properties, based on rosemary essential oil and biopolymers. Journal of the Science of Food and Agriculture.

Martin-Piñero, M. J., Muñoz, J., & Alfaro-Rodriguez, M. C. (2020). Improvement of the rheological properties of rosemary oil nanoemulsions

prepared by microfluidization and vacuum evaporation. Journal of Industrial and Engineering Chemistry, 91, 340-346.

Martín Piñero, M. J., Santos García, J., Trujillo-Cayado, L. A., García González, M. D. C., & Alfaro Rodríguez, M. D. C. (2018). Influencia del tensoactivo en la formación y estabilidad física de emulsiones ecológicas concentradas. *Afinidad: Revista de Química Teórica y Aplicada, 75 (581), 9-15.*

Santos, J., Calero, N., Trujillo-Cayado, L. A., Martín-Piñero, M. J., & Muñoz, J. (2020). Processing and Formulation Optimization of Mandarin Essential Oil-Loaded Emulsions Developed by Microfluidization. Materials, 13(16), 3486.

Muñoz, J., Alfaro, M. C., Trujillo-Cayado, L. A., Santos, J., & Martín-Piñero, M. J. (2019). Production of food bioactive-loaded nanostructures by microfluidization. In Nanoencapsulation of Food Ingredients by Specialized Equipment (pp. 341-390). Academic Press.

Articles related to this Ph.D. Thesis in preparation.

Martin-Piñero, M. J., Muñoz, J., & Alfaro-Rodriguez, M. C., Vladisavljević G.T. Controlled production of eco-friendly emulsions formulated with essential oils in premix membrane emulsification.

Martin-Piñero, M. J., Mileti O., Baldino N., Alfaro-Rodriguez, M. C. Muñoz, J., Gabriele D. The effect of biodegradable surfactant at rosemary essential oil/water interface on the interfacial rheology.

Martin-Piñero, M. J., Muñoz, J., & Alfaro-Rodriguez, M. C. Rosemary emulsions produced by vacuum evaporation. A comparison between two plant-derived surfactants.

Contributions to Scientific Conferences

Martín Piñero, María José, Alfaro Rodriguez, Maria Carmen, Muñoz Garcia, Jose: Welan versus Xanthan gum as stabilisers of rosemary emulsions. A rheological and physical stability study. Ponencia en Congreso. 3er Congreso de Estudiantes de Doctorado en Química. Universidad de Sevilla, - Facultad de Química. 2019

Martín Piñero, María José: Emulgeles de Aceite Esencial de Romero. Ponencia en Jornada. VI JORNADA DE I+D+I y PROGRAMA DE DOCTORADO. Escuela Politecnica Superior. 2019

Martín Piñero, María José: Sinergias Universidad-Empresa en investigación aplicada. Sector petroquímico. Mesa redonda de Jornada. Ponencia. Facultad de química. 2019

Santos García, Jenifer, Martín Piñero, María José, Carmona Gallego, Jose Antonio, Calero Romero, Nuria, Muñoz García, Jose: Development of an emulgel type format based on rosemary essential oil and a fumed silica. Influence of a shear post-treatment on its final properties. Ponencia en Congreso. 7th Iberian Meeting on Rheology (IBEREO 2019). - Porto, Portugal. 2019

Muñoz García, Jose, García González, Maria Carmen, Carmona Gallego, Jose Antonio, Martín Piñero, María José, Alfaro Rodriguez, Maria Carmen: Shear flow of diutan gum aqueous dispersions. Poster en Congreso. 7th Iberian Meeting on Rheology (IBEREO 2019). - Porto, Portugal. 2019

Martín Piñero, María José, Santos García, Jenifer, Trujillo Cayado, Luis Alfonso, García González, Maria Carmen, Alfaro Rodríguez, Maria Carmen: Rheology and physical stability of rosemary essential oil emulsions. Poster en Congreso. 7th Iberian Meeting on Rheology (IBEREO 2019). - Porto, Portugal. 2019

Carmona Gallego, Jose Antonio, Ramírez del Amo, Pablo, Martín Piñero, María José, Llinares, Rubén, Muñoz García, Jose: Parallel superposition of welan gum solutions. Poster en Congreso. Annual European Rheology Conference 2018. Sorrento (Italy). 2018

Martín Piñero, María José, Ramírez del Amo, Pablo, Alfaro Rodríguez, Maria Carmen, Santos García, Jenifer, Muñoz García, Jose: Development of Thyme Essential Oil Emulsions and a Natural Biopolymer. Poster en Congreso. II Congreso Estudiantes Doctorado en Química. Sevilla. 2018

Martín Piñero, María José, Ramírez del Amo, Pablo, Alfaro Rodríguez, Maria Carmen, Santos García, Jenifer, Muñoz García, Jose: Applications of microfluidization to the fabrication of thyme essential oil in water emulsions containing welan gum. Poster en Congreso. Loughborough University Research Conference 2018. Ciudad: Loughborough País: Reino Unido. 2018

Martín Piñero, María José: Formulación y estabilidad física de emulsiones concentradas de aceite esencial de tomillo. Ponencia en Congreso. I Congreso de Estudiantes de Doctorado en Química. Universidad de Sevilla, - Facultad de Química. 2017

Martín Piñero, María José, Alfaro Rodríguez, Maria Carmen, Trujillo Cayado, Luis Alfonso, García González, Mari Carmen, Muñoz García, Jose: Desarrollo de emulsiones concentradas formuladas con aceite esencial de tomillo. Ponencia en Congreso. XIV Simposio de Investigadores Jóvenes de la Real Sociedad Española de Química. Badajoz. 2017

Martín Piñero, María José, García González, Mari Carmen, Ramírez del Amo, Pablo, Muñoz García, Jose, Alfaro Rodríguez, Maria Carmen: Estabilidad Física de Mezclas de Aceite Esencial de Tomillo y un Tensioactivo no Iónico. Ponencia en Congreso. IV Jornada de Investigación y Posgrado en la EPS. Escuela Politécnica Superior (Universidad de Sevilla). 2017

Ramírez del Amo, Pablo, Trujillo Cayado, Luis Alfonso, Carmona Gallego, Jose Antonio, Martín Piñero, María José, García González, Mari Carmen: Interfacial rheology and emulsifying properties of bio - based surfactants obtained from coconut oil. Poster en Congreso. Ibereo 2017. Valencia, España. 2017