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Treball Final de Grau

Improvement of ginger oil stability by encapsulation in alginate-carrageenan-chitosan blended beads.

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"Los obstáculos no son más que la salsa del triunfo"

Mark Twain

En primer lloc, voldria agrair aquest treball principalment a les meves tutores, la Dra. Alicia Maestro Garriga i la Dra. Carme González Azón, per tota la seva dedicació, disposició i ajuda durant tots aquests mesos. També, a la Sharmaine Atencio que em va facilitar l'inici al laboratori.

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CONTENTS

SUMMARY

The tendency to create innovative ingredients or new functional foods is growing. Encapsulation is a process aimed at the protection and controlled release of active principles. This study aims to provide better protection for ginger essential oil.

It has been based on a preliminary study in which the ginger oil was encapsulated with the Büchi model-390 and where the essential oil coating was a hydrogel consisting of a mixture of sodium alginate with Kappa-carrageenan and with a final chitosan coating, that is, the oil was wrapped in the hydrogel layer. The main conclusion of the previous study was that encapsulation protects ginger oil from oxidation but there is still room for improvement, and a deeper research is required, a part of which is carried out in the present work.

In this work, unlike the previous study, the emulsion of ginger oil was encapsulated with the aqueous phase, so that the oil is already trapped in the hydrogel. Three formulations were prepared for the encapsulation of ginger oil, the hydrogel discussed before, the hydrogel with natural reddish tint and the hydrogel with methylene blue, to try to offer a better protection against light. These three formulations were subjected to different storage conditions varying three parameters; temperature, contact with light and contact with air. Contact with the light by placing the samples in the case or, cabinet, temperature by locating them or not in the refrigerator and contact with air by covering or not them.

The encapsulated ginger oil gave lower values of oxidation compared to non-encapsulated control oil. The oil extracted from the capsules stored in a refrigerator and covered, showed lower values of oxidation products throughout the storage period. The red dye used in gel capsules provides the best results and improves the protection of ginger oil against unwanted oxidation.

Keywords: Encapsulation, alginate, Kappa-carrageenan, chitosan, ginger oil

RESUM

La tendència a crear ingredients innovadors o nous aliments funcionals està en creixement. L'encapsulació és un procés destinat a la protecció i alliberació controlada de principis actius. Aquest estudi pretén proporcionar una millor protecció a l'oli essencial de gingebre.

S'ha partit d'un estudi previ en el que s'encapsulava l'oli de gingebre amb el Büchi model-390 i on el recobriment de l'oli essencial era un hidrogel que consistia en una mescla d'alginat de sodi amb Kappa-carragenat i amb un recobriment final de quitosà, és a dir, l'oli quedava embolcallat per la capa d'hidrogel. La principal conclusió de l'estudi anterior va ser que l'encapsulament protegeix l'oli de gingebre de l'oxidació, però encara hi ha marge de millora i es requereix una investigació més profunda, una part de la qual es fa en el present treball.

En aquest treball, a diferència de l'estudi previ, es va encapsular l'emulsió d'oli de gingebre amb la fase aquosa, de manera que l'oli ja queda atrapat en l'hidrogel. Es van preparar tres formulacions per l'encapsulament de l'oli de gingebre; l'hidrogel anterior, l'hidrogel amb tint vermellós natural i l'hidrogel amb blau de metilè, per intentar oferir una millor protecció contra la llum. Aquestes tres formulacions van ser sotmeses a diferents condicions d'emmagatzematge on varien tres paràmetres; el contacte amb la llum, la temperatura i el contacte amb l'aire. El contacte amb la llum col·locant les mostres a la vitrina o a l'armari, la temperatura localitzant-les o no a la nevera i per últim el contacte amb l'aire cobrint-les o no.

L'oli de gingebre encapsulat va donar valors més inferiors d'oxidació en comparació amb l'oli de control no encapsulat. L'oli extret de les càpsules emmagatzemades a la nevera i cobertes, va mostrar valors inferiors dels productes d'oxidació durant tot el període d'emmagatzematge. El tint vermell utilitzat en càpsules de gel proporciona els millors resultats i millora la protecció de l'oli de gingebre conta l'oxidació no desitjada.

Paraules clau: Encapsulació, alginat, Kappa-carragenat, quitosà, oli de gingebre

1. INTRODUCTION

Nowadays, people's concern for their health and nutrition has increased. Consumers are more interested in food products that have a positive impact on their health. We are talking about functional foods that provide benefits that are not only nutrition [1]. Functional foods are always presented in the form of food for ordinary consumption in the conventional diet. Food with added compounds obtained from medicinal plants are an example [2].

Some extracts of plants seem to have relevant effect in the cure of diseases, especially with antioxidant, anti-inflammatory, antidiabetic and antitumor properties. Ginger, the rhizome of Zingiber officinale, is commonly consumed as a dietary condiment, and generally seems to be promising for the treatment of various diseases. It also shows to have a role in the prevention of cancer by inactivating and activating several molecular pathways [3].

The extract of Zingiber officinale is known as ginger essential oil or just ginger oil. Essential oils are the volatile lipophilic components extracted from plants. Some of them can be easily degraded and must be protected of environment. Therefore, commercial applications of these EOs require an adequate formulation consisting, for example, of biodegradable compounds that protect them from degradation through encapsulation [1]. In this study we want to encapsulate ginger oil to protect it from degradation oxidation.

Encapsulation of essential oils constitutes an interesting technology used in the food industry, preventing their volatilization and oxidation and extending the useful life of these biological components [4].

1.1. **Encapsulation**

Encapsulation is a useful tool to improve the controlled delivery of bioactive molecules (for example, antioxidants, minerals, vitamins, fatty acids ...) and living cells (for example, probiotics) in food [5]. Encapsulation favors the release of active principles at controlled rate for extended periods and under specific conditions.

In the food industry, the encapsulation process can be applied for several reasons. The most important ones are to provide improved stability in the final products and during processing, to increase their resistance to heat, humidity, air, light, oxygen ...; to decrease the evaporation and degradation of volatile active substances, such as aromas; to mask unpleasant flavors; to avoid reaction with other components in food products such as oxygen or water; to offer a controlled release; and even to immobilize cells or enzymes in food processing applications [5].

There are many techniques that can be used to encapsulate food ingredients. The selection of a method depends on economics, sensitivity of active principles, size of capsule desired, physical/chemical properties of both core and coating, applications for the food ingredients and the release mechanism.

Physical methods of encapsulation include spray drying, extrusion, air suspension, coating, spray cooling and chilling, co-crystallization and multi-orifice centrifugal extrusion. Encapsulation processes involving both physical and chemical techniques include coacervation, liposome entrapment and inclusion complexation. Interfacial polymerization is a chemical method for entrapping food materials [6].

Encapsulation is a process in which active agents are completely covered and protected by a physical barrier. There are two ways to encapsulate. One in which the active principle is inside or in the core and is covered by shell of the capsule, outer layer or matrix. The other in which the active principle is trapped and mixed with the matrix. In the present work, the second encapsulation method is used, not the core-shell method. Matrix must be food grade, biodegradable and capable of forming a barrier between the active principle and its environment. The particles usually have a diameter of some nm to a few mm [5].

1.1.1. Ionic gelation

In this work, ionic gelation method is used as encapsulation technique. The formation of the gel comes from an alginate salt solution and an external or internal calcium source, where the calcium ion diffuses to meet the polymer.

The degree of gelation depends on the hydration of the alginate, the concentration of the calcium ion and the content of the G-blocks [7]. It has also been observed that the gelation kinetics and gel properties may depend on the type of counter ion, the monovalent ion of the alginate salt (K or Na). There are two processes for ionic gelation: external gelation and internal gelation.

The external gelation process occurs when an alginate solution is introduced as droplets into another solution containing calcium ions. Starting at the interface, the calcium ion that is on the external solution will produce gelation and progress towards the interior of the gel as it becomes saturated, so that the sodium ion (alginate salt) will be displaced by the divalent cation solubilized in water, and gelation occurs. $CaCl_2$ is the salt most used for its high percentage of available calcium [8].

The internal gelation process consists of the controlled release of calcium ion from an internal source of calcium salt previously dispersed in the sodium alginate solution. The release of the calcium ion can occur in two ways:

- The calcium salt is insoluble at neutral pH but soluble at acidic pH. It is necessary to add an organic acid that when diffusing to the salt allows the acidification of the medium getting solubilize the calcium ions, promoting gelation. In this case, the most commonly used calcium salts are calcium carbonate, calcium citrate and tricalcium phosphate.

- The calcium salt is partially soluble. The internal gelation process consists of the addition to the alginate-calcium salt mixture, a sequestering agent such as phosphate, sulphate or sodium citrate. By adding a sequestrant, this binds to free calcium, thus delaying the gelling process.

Addition of and acid promotes release of calcium and subsequent alginate gelation.

The main difference between the external and internal gelation mechanism is the origin of the calcium source. If what is intended is the control of the sol-gel transition, in the external gelation process the factors to be manipulated are the concentration of calcium and composition of the polymer. While, for the internal gelation process, the solubility and concentration of the calcium salt, concentration of the sequestering agent and the organic acid used must be considered [9].

1.2. **Emulsions**

In the present work, an oil-in-water emulsion (O / W) where the aqueous continuous phase contains a mixture of alginate and k-carrageenan and the organic phase is ginger oil is dropped in a calcium chloride-chitosan bath to from gel beads. This emulsion must be stable long enough for encapsulation. Once gelation occurs, oil droplets become immobilized. The term emulsion designates a system composed of two immiscible liquids, one dispersed in the other, in a kinetically stable manner, although being thermodynamically unstable. Food emulsions are complex systems whose stability is determined by many parameters. An emulsion must keep the interfacial area constant to preserve its stability. Emulsifiers as surfactant are used to achieve kinetically stable emulsions.

1.2.1. Surfactants

The surfactants are amphiphilic substances, they are formed by two parts, one hydrophilic and the other hydrophobic. It is a molecule with superficial capacity, with capacity to be absorbed in the interfaces, so that the interfacial tension is reduced and can increase the stability of the emulsions [10].

Tween® 80 is a non-ionic surfactant, that type of surfactants is those that do not contain dissociable functional groups (ionizable) and, therefore, do not dissociate in water in ions [11].

All emulsifiers have a hydrophilic head which is generally composed of a water-soluble functional group (polar part) and a glue generally composed of a fatty acid or a fatty alcohol (a polar part) [11].

The HLB (hydrophilic-lipophilic balance) system was devised by Griffin in 1949. An emulsifying agent is assigned an HLB number, which is characteristic of its relative polarity. The hydrophilic-lipophilic equilibrium system takes advantage of the fact that a more hydrophilic interfacial barrier favors O / W emulsions, while a less polar barrier favors O / W emulsions to evaluate surfactants and emulsifying agents [11]. The HLB number of the surfactant gives an idea of its affinity in water or organic solvents, so that the high HLB number would be of a hydrophilic surfactant and would serve to stabilize emulsions or O / W, and vice versa.

1.3. **Hydrogel matrix for encapsulation**

Hydrogels are presently under investigation as matrices for the controlled release of bioactive molecules, pharmaceutical proteins, for the encapsulation of living cells and for protection of active principles. For these applications, it is often required that the gels degrade under physiological conditions. This means that structure must disintegrate to ensure a good biocompatibility of the hydrogel [12].

Among the polymers used as encapsulation material are alginate, carrageenan, chitosan, xanthan gum or pectin [13]. Among these polysaccharides, the most common is sodium alginate which can form a highly versatile, biocompatible and non-toxic matrix for the protection of active components, cells and mainly probiotic microorganisms sensitive to heat, pH, oxygen, and other factors in which food is exposed during processing and storage.

However, alginate matrix is too porous and does not offer enough protection in many cases. It has a low crosslinking density and does not provide the necessary barrier effect [14]. This is achieved by combining the alginate with other polysaccharides, in this study it is done with Kappa-carrageenan and chitosan [15].

1.3.1. Sodium alginate hydrogel

Alginate is a biodegradable polysaccharide of natural origin that is found in the structure of the cell wall in a brown seaweed belonging to the *Phaeophycean* family. Its main function is to give rigidity, elasticity, flexibility and water binding capacity.

After extracting it with sodium hydroxide and filtering it, sodium chloride is added to precipitate the alginate and obtain the salt of sodium alginate $(NaC_6H_70_6)_n$, whose molecular weight is in the range 32000 - 400000 g/mol. The alginates appear as a mixture of salts of the cations that are commonly found in seawater, mainly Ca^{2+}, Mg^{2+} and Na^{+} [16].

This hydrocolloid belongs to the family of linear polysaccharides and consists of two monomeric units linked by 1-4 glyosidic bonds; β-D mannuronic acid (M) and α-L guluronic acid (G). Alginates are grouped or distributed constituent homopolymers of type G blocks (-GGG-), M blocks (-MMM-) or heteropolymers in which the blocks M and G alternate (-MGMG-) (Figure 1.1.) [17].

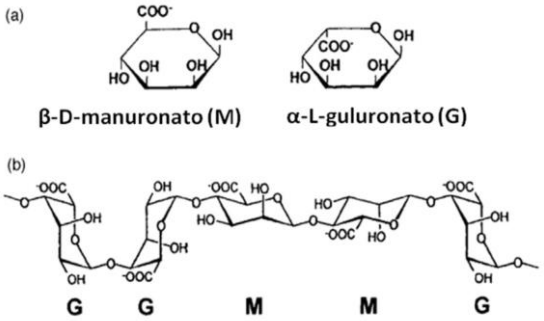

Figure 1.1. Structural characteristics of alginates: (a) alginate monomers, (b) chain conformation [19].

The distribution of these blocks depends on the nature of the alginate and its growth conditions and will determine properties such as swelling or viscoelasticity.

In this way, a greater amount of G blocks produces a harder and brittle gel, while a higher proportion of blocks M results in a softer and more elastic hydrogel [18], as the blocks that participate un gelation are only the G ones.

This polymer owes its polyanionic character to the carboxyl groups (-COOH) that appear in its chain and that are responsible for forming salts such as sodium alginate [18].

One of its most interesting properties is the ability to crosslink and form hydrogels by the ionic interaction between divalent cations such as calcium and carboxyl groups of neighbouring polymer chains of G type [18].

Calcium chloride, a solid salt at room temperature, is one of the most used reagents to ionically crosslink alginate due to its high percentage of available calcium and has been used in this work for its high solubility in water and ease of handling. The crosslinking process consists of the replacement of sodium by calcium and the union of two chains of alginate through the two positive charges of calcium [17].

These salts are formed by three blocks M, G and MG. When two chains of block G are aligned, coordination sites are formed. Due to the shape of the loops of these chains, there are cavities between them that are sized to accommodate the calcium ion and are also coated with carboxylic

groups and other electronegative oxygen atoms. It is when the gelation model of the alginate known as "egg box" (Figure 1.2.) is given [18].

In this way, the stability and resistance of the gel is related to the proportion and length of G blocks.

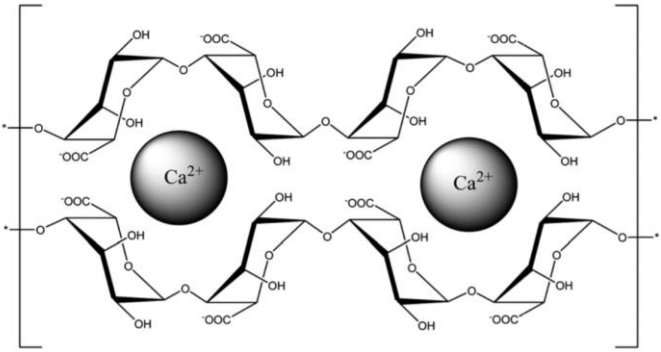

Figure 1.2. Egg box model that describes the structure of alginate gel [19].

1.3.2. Kappa- carrageenan

Carrageenan is a group of linear sulphated polysaccharides, present in the cell structure of rodophyceae algae. It is used much more widely than agar as emulsifier, gelling, thickening and stabilizing agent in pharmaceutical and industrial formulations.

There are three main commercial classes of carrageenan: Kappa (*k*) (Figure 1.3.), which has only one negative charge per disaccharide and produces strong, rigid gels; the Iota-type (*i*), which has intermediate sulphate content and produces soft gels that provide excellent freeze/thaw stability, and lambda-type (*λ*), which is highly sulphated, less likely to form a gel structure, but forming gels when mixed with proteins rather than water. K-carrageenan hydrogel provides a firm gel structure [20].

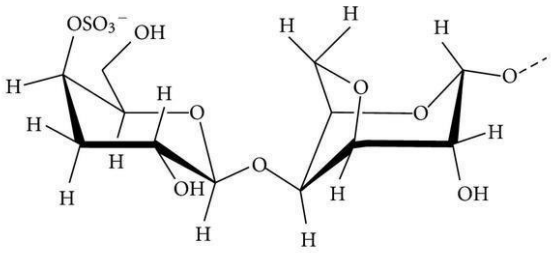

Figure 1.3. Kappa-carrageenan chemical structure [21].

1.3.3. Chitosan

Chitosan is a natural lineal polysaccharide obtained from the deacetylation of chitin, a component structurally like to cellulose. It is present in the cell wall of bacteria and in the exoskeleton of crustaceans and insects. Besides being a biocompatible material, non-toxic, stable, sterilizable and degradable by enzymes, it is very present in biomedicine and biotechnology for its properties mucoadhesive, antimicrobial and macrophage activation capacity [22].

Chitosan is a copolymer formed by 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose with β - (1-4), can react with many negatively charges surfaces/polymers and also to chelate metal ions (Figure 1.4.) [23].

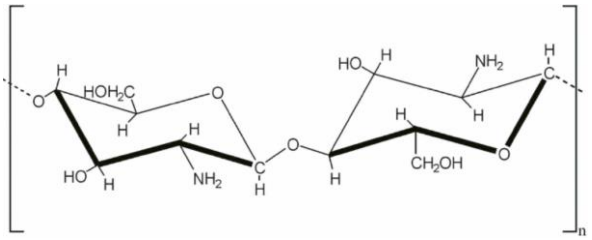

Figure 1.4. Chitosan chemical structure [24].

The degree of deacetylation is, together with the molecular weight, one of the decisive characteristics in the crystallinity, degradation and cellular response of chitosan.

Are properties that depend on the processing of the starting polymer and its nature, respectively. Obtaining levels of deacetylation greater than 90% requires times and high temperatures that can degrade the polymer. Chitin is insoluble in its native form, but chitosan is soluble in water [25].

Chitosan is an excellent encapsulation material thanks to properties such as facilitating the release of active ingredients in the intestine and giving the particles greater stability, a more effective form and a homogeneous size [26].

1.4. **Ginger oil**

Medicinal plants are a source of great economic value throughout the world. *Zinigiber officinale* is an important plant with several medicinal and nutritional values. Ginger is consumed worldwide as a spice and flavoring agent and is attributed to have many medicinal properties, such as cardio-protective, anti-inflammatory, anti-microbial, antioxidant, anticancer properties, etc [27-28].

The constituents of ginger oil are numerous and vary depending on the place of origin and whether the rhizomes are fresh or dry. We could classify the chemical compounds of this essential oil in volatile and non-volatile. The volatile components consist mainly of sesquiterpene hydrocarbons, predominantly zingiberene (35%), curcumene (18%) and farnesene (10%). Non-volatile pungent compounds include gingerols, shogaols, paradols and zingerone. In the fresh ginger rhizome, the gingerols were identified as the major active components, which are a homologous series of phenols. The most abundant is [6]-gingerol [29].

1.4.1. Oxidation and rancidity

There are two alterations of great importance for foods, as they can cause deterioration or loss of quality: oxidation and the action of microorganisms. The process of oxidation produces important consequences in food; collected in a process called rancidity. This oxidative rancidity is produced by autooxidation of the double bonds that have unsaturated fatty acids and other organic compounds and the consequent formation of potentially toxic compounds such as peroxides or hydroperoxides. Such compounds can polymerize and decompose giving rise to unwanted molecules [30].

After this oxidation, aldehydes, epoxides and other products such as ketones and acids are formed. These products suppose the apparition of undesirable flavors and odors, and in occasions also changes in the color of the foods and a loss of nutrients. Factors such as light, heat, humidity, presence of free fatty acids and certain inorganic catalysts such as iron and copper salts are responsible for the process of oxidation. To avoid this, easily oxidizing components should be stored in opaque containers that do not let light in, in cool, dark places and at a temperature around 18ºC. Also, the presence of antioxidants can partially avoid this process [31].

The peroxide value is the most widely used indicator of fast oxidation, as it measures the peroxides and hydroperoxides formed during the initial primary stages of oxidation. It is a good indicator of oil quality, a parameter that indicates the degree of freshness of stored oils.

In this work we talk about an essential oil, and these do not contain fatty acids like oils. However, and because studies have been found in which it is done ([32] and [33]); you can extrapolate the information given about these fatty acids to an essential oil such as our ginger oil. The method to quantify rancidity of fatty acids will therefore be used to quantify oxidation levels of ginger oils.

2. OBJECTIVES

The main objective of this work is to encapsulate the ginger essential oil into polysaccharide-gel beads to protect it against oxidation, and to measure the protection level. In order to reach this general objective, some specific objectives must to be reached:

- As ginger oil is not water soluble, it must to be emulsified in the polysaccharides water solution that will be further gelled. Therefore, it is required to formulate a suitable O/W emulsion stable enough to allow gelation process. It includes to decide if it is necessary to use a surfactant and, in this case, which of them should be used for our mixture.

- Encapsulation of ginger oil-polysaccharides emulsion with Buchi-390 through dropping it over a calcium-chitosan promoting gelation of the continuous phase, formed by alginate and kappa-carrageenan.

- Encapsulation of ginger oil-polysaccharides emulsion with Buchi-390 through dropping it over a calcium-chitosan promoting gelation of the continuous phase, formed by alginate and kappa-carrageenan, with blue dye and red dye

- Study of the degradation of ginger oil by oxidation over time in different storage conditions (refrigerator, cupboard, open). Check effect of air, light and temperature and compare oxidation of free oil, encapsulated in non-colored, red and blue beads.

- Check by microscope if the pearls have the expected diameter by the nozzle used.

- Section pearls and visualize the state of these inside

3. EXPERIMENTAL SECTION

3.1. **Materials**

3.1.1. Matrix-former materials for encapsulation

a. Ginger oil, natural FCC, Food Grade (Sigma Aldrich)
b. Sodium Alginate LS (Panreac)
c. Kappa-carrageenan (Sosa Ingredients)
d. Calcium Chloride Anhydrous ≥ 97% (Sigma Aldrich)
e. Chitosan (Sigma Aldrich)
f. Milli-Q water (Millipore)
g. Surfactant Tween® 80 (Sigma Aldrich)
h. Red natural dye. Glycerine, natural extract of black carrot, citric acid and water (Sosa Ingredients)
i. Blue dye. Methylene blue. (Sigma Aldrich)

3.1.2. Ginger Oil extraction

a. Methanol (≥ 99,9% purity, Sigma Aldrich)
b. n-Hexane (≥ 99,9% purity, Panreac)

3.1.3. Determination of Peroxide Value

a. Chloroform ≥99% (Sigma Aldrich)
b. Glacial Acetic Acid ≥ 99,5% (Panreac)
c. Potassium Iodide ≥99,5% (Panreac)
d. Sodium thiosulphate penta-hydrate ≥ 99,5% (Sigma Aldrich)
e. Starch (Sigma Aldrich)

3.2. **Equipment**

3.2.1. Büchi encapsulator model B-390 (BÜCHI Labortechnik AG, Switzerland)

The technology of the B-390 encapsulator (Figure 3.1.) works on the principle of laminar jet breaking of a liquid stream in equal size beads / capsules by the application of a controlled vibration frequency for the liquid. The production technique is called "Prilling pro vibration". Nozzles of different sizes are available, so that it is easy to adjust the production conditions, in order to obtain the desired, particle size. Extrusion of a liquid polymer (containing the material to be encapsulated) through a selected nozzle results in the formation of a laminar flow liquid jet. A vibratory frequency controlled in amplitude is imposed in this jet and causes it to break into uniform small droplets of equal size. For the BÜCHI encapsulator this force is applied by the vibration of liquid polymer in a chamber (bead production unit) before it is extruded through the nozzle. To prevent coalescence of the droplets during rupture jet and / or upon entering the gelation bath, an electric charge is induced on the surface of the droplets using an electrostatic voltage system. In this system an electric potential is applied between the nozzle and an electrode, placed directly below the nozzle. The droplets fall through the electrode, which is charged and deviated from its vertical position so that its impact occurs over a larger area in the hardening solution. The size of the droplets produced, and the production rate depend mainly on the size of the nozzle, the flow rate and the viscosity of the extrusion liquid, and the frequency of vibration applied. Viscosity will depend on the polymer and concentration chosen. The other parameters can all be controlled using the BÜCHI Encapsulator, allowing the operator to pre-determine the size and characteristics of the beads and capsules that are produced [34].

Figure 3.1. Encapsulator Büchi B-390 [34].

3.2.2. OPTIKA Vision Pro (OPTIKA Microscopes, Italy)

The microscope is an instrument that allows objects to be observed that are too small to be seen in a simple view. Optical microscopy is the most basic type of microscope, its operation is based on a set of visible lenses to increase the image of a sample. Through the lenses it is passed to visible light, and thus generate an enlarged image.

They are available for laboratory and industrial applications, they are easy to use, and you get good image quality.

When using the integrated camera with the USB, the connection to the PC will be simple and fast [35].

3.2.3. Turbiscan™ Classic MA 2000 Stability Analyzer from Formulaction SA

The propagation of light through a dispersion and its change over time can be used to characterise the system physic-chemical stability. Mie's theory for the scattering of light by small spherical particles indicates that there is a simple relationship between the turbidity and the interfacial area of the emulsion/dispersion.

This section mainly concerns the independent and anisotropic scattering of light from an emulsion or suspension in a cylindrical glass measurement cell, in relation with the optical

analyser TURBISCAN MA 2000. Indeed, photons undergo many scattering events in an optically thick dispersion before escaping the medium and entering a receiver aperture [36].

The heart of TURBISCAN MA 2000 (Figure 3.2.) is a detection head which moves up and down along a flat-bottomed glass cylindrical cell.

The detection head is composed of a pulsed near infrared light source (wavelength L=850 nm) and two synchronous detectors.

The transmission detector receives the light which goes through the sample (0° from the incident beam), while the backscattering detector receives the light scattered by the sample at 135° from the incident beam.

The detection head scans the total length of the sample, acquiring transmission and backscatter data during the time and intervals desired.

Figure 3.2. Turbiscan^{TM} Classic MA 2000 Stability Analyzer from Formulation SA [37].

The signal is first treated by a TURBISCAN MA 2000 current-to-voltage converter. The integrated microprocessor software handles data acquisition, analogue to digital conversion, data storage, motor control and computer dialogue [36].

3.2.4. Rotation Rheometer HAAKE MARS III, equipped with thermostatic bath G25

The HAAKETM MARS™ rheometer is designed to perform rheology, controlled frequency (CR), controlled stress (CS) and controlled deformation (CD) tests.

The HAAKE MARS III is suitable for all industries and has specific accessories for the polymer industry, petrochemicals, paints, inks and coatings, pharmaceuticals and cosmetics.

These products have a complex flow and deformation behavior; it is necessary to understand their viscoelastic properties and conditions of processing and design of final products for specific products [38].

The shear rate sweep test of this study was carried out using this device, equipped with a bath thermostatic F6 and a HAAKE C25 container for precise control of temperature (25°C). To process the data obtained, HAAKE RheoWin DataManager software version 3.12 (Thermo Fisher Scientific, Inc.) was used.

3.2.5. Ultra-Turrax® model T25 basic IKA WERKE

This is high performance dispersion instrument that produces high shear by the presence of a stator and a rotor with a small gap. It offers a wide range of speeds from 3,000 to 25,000 rpm that allows users to work at circumferential speeds even with small rotor diameters [39].

In the present work a speed of 11000 rpm was used to homogenize sodium alginate with water, to mix this with Kappa-carrageenan and for the preparation of $CaCl_2$. A speed of 15000 for the extraction of the ginger oil and a speed of 22000 rpm for 10 minutes to prepare the O / W emulsion.

3.2.6. Rotary evaporator IKA RV 10 digital with thermostatic bath IKA HB 10 basic

A rotary evaporator is a device used in chemical laboratories (organic laboratories, especially). Rotary evaporation is the process of reducing the volume of a solvent by distributing it as a thin film through the interior of a container at elevated temperature and reduced pressure. This promotes rapid removal of excess solvent from less volatile samples. The apparatus consists of a motor unit that rotates the evaporation flask, a vacuum system, a hot water bath and a condenser. In addition, a vacuum cleaner or vacuum pump should be placed, as well as a protection trap and a round bottom flask containing the sample to be concentrated. The universal heating bath allows quick heating times, the container can be metallic or a crystallization dish.

This prevents the solvent from freezing during the evaporation process. The solvent is removed under vacuum, trapped with a condenser and collected for easy reuse or disposal. The condenser unit has specially designed glass tubes that use an extremely efficient 1500 cm² surface. An important aspect of digital RV 10 are the sophisticated security features. The motorized lift has an automatic lift function in case of power cut to avoid overheating of the solvent. The safety temperature circuits can be configured individually. The dry run protection, the adjustable lower end stop, and the toilet block function complete the line of safety features. In addition, IKA offers a selection of coated glassware for greater security needs [40].

3.3. **Methodology**

3.3.1. Preparation of sodium alginate and *Kappa*-carrageenan solution

A solution of sodium alginate 1% w / v was prepared by the addition of weighted portions of sodium alginate in measured volumes of Milli-Q water. The solution was homogenized using an Ultra-Turrax homogenizer, at a speed of 11,000 rpm and room temperature.

Subsequently, a 1.5% w / v solution of *Kappa*-carrageenan in milli-Q water previously heated to 60 degrees to facilitate its dissolution was prepared. It was also mixed with the Ultra-Turrax®.

Then, the mixture of alginate and *Kappa*-carrageenan was prepared by combining the two hydrocolloid solutions at 80:20 v / v ratio of alginate to *kappa*-carrageenan solution.

After the solution is completely homogenized, is stored for 24 hours at room temperature to assure complete hydration and eliminate all the air bubbles inside. All polymeric solutions were prepared 24 hours prior to further gelling and analysis.

3.3.2. O/W emulsion preparation

It is necessary to emulsify the ginger oil in the polymeric watery solution which will become the continuous phase, then homogenize the emulsion at high speed to produce small droplets of ginger oil in water polysaccharides solution. The use of the Ultra-Turrax® mechanical device at 22000 rpm for 10 minutes serves to intensively break oil drops, resulting in a much smaller size, an O/W emulsion with a concentration 10% ginger oil was prepared [10].

3.3.2.1. Measurement of the stability of the emulsion

In this study it was necessary that the ginger oil/water polysaccharides emulsion was stable for at least 30 minutes, time necessary for the encapsulation. The emulsifier used in the production of ginger oil emulsion has been Tween 80. Two types of emulsion were prepared; the first with 10% ginger oil and the second with 20% of it. And with these it was tried to add in both 0.1% and later 0.25% of Tween 80 and to see the effects of the emulsifier in stability with the Turbiscan™.

3.3.3. Preparation of calcium chloride solution

A solution of 1% calcium chloride w / v was prepared by the addition of weighted portions of calcium chloride in measured volumes of Milli-Q water.

3.3.4. Preparation of chitosan solution

Solutions 0.01% w / v chitosan were prepared. In 500 mL of water, 0.05 g of chitosan and 10 mL of acetic acid were added and mixed with a magnetic stirrer for 30 minutes.

Then, the mixture of calcium chloride and chitosan was prepared by combining the two solutions in a ratio of 90:10. It was homogenized with Ultra-Turrax®.

Two methods for the preparation of beads are described in bibliography. The one-step method is the method of dispersing the oil in the polysaccharide solution and this emulsion is dropped into a solution containing both calcium chloride and chitosan. The two-step method is the method comprising as a first step the dispersion of the product in the polysaccharide solution and then dropping this dispersion in a solution containing calcium chloride and a second step where the beads are suspended in a solution of chitosan for hardening [41].

According to the method of preparation, these alginate particles coated with chitosan show different mucoadhesive. By means of this second step, a ticker and well packaged chitosan is processed around the alginate-based bead, while in the one-step method it retains its flexibility.

3.3.5. Encapsulation of ginger oil

The encapsulation of ginger oil was carried out with the BÜCHI B-390 encapsulator (Figure 3.3.). Three different materials were prepared for the matrix. The three consisted of solutions of 1% w/v of alginate and 1.5% w/v o kappa-carrageenan in a ratio of 80:20 v: v of each solution. The difference between these three materials was the addition of dye. The first had no dye, the second a natural red dye (120 drops) and finally one with methylene blue dye (75g). The obtained capsules were hardened in the solution of 1% w/v $CaCl_2$ and 0.1% w/v of chitosan for 10 minutes with stirring. The $CaCl_2$-chitosan water solution was also dyed in the same way as the three matrices discussed above.

The chosen nozzle was 750 µm in diameter, it was attached to the assembled pearl production unit of the encapsulator. Then, the unit was attached to the carrier plate. A beaker containing approximately 200 ml of 1% w / v calcium chloride solution and 0.1% w / v chitosan was placed under the nozzle and a magnetic stirrer was used. The grounded clip was placed on the edge of the beaker, so that one end was in contact with the liquid. The pressure bottle was filled with the alginate-k-carrageenan solution (one of the three) and screwed into the assembled lid. It was tightened by turning the valve clockwise to close the silicone tube. The supply of external pressurized air was opened, and the value was established at 450 mbar. The electrode, frequency and amplitude were adjusted to 380 V, 180 Hz and 6, respectively. The liquid flowed regulating valve was opened by turning counterclockwise until the liquid flows through the silicone tubing and the pearl production unit to the nozzle, where it forms a continuous liquid jet.

Three different batches of capsules loaded with ginger oil were produced, which vary according to the formulation of the matrix material.

Figure 3.3. Actual experimental set-up during the production of alginate beads.

3.3.5.1. Measurement of flow rate of alginate solution during encapsulation

The density of the preparation that would pass through the feeding tube of the BÜCHI encapsulator was found. A beaker was taken, and known volume of the preparation was introduced. The resulting weight divided by volume experimentally revealed the density.

After setting all the working parameters of the encapsulator (amplitude, frequency, electrode voltage, pressure and opening of the valve), the flow rate was calculated.

A petri dish was placed between the beaker and the nozzle of the encapsulator. All the parameters were activated, and the valve was opened so that the emulsion circulated through the feeding tube and it started to come out through the mouthpiece. Once the droplets were formed, the petri was removed, and the solution was dropped in the beaker. The flow of the beads was collected for exactly 30 seconds. Then, this glass, which had previously been tared, was weighted. Was calculated by Eq. 3.1. as follows:

$$flow\ rate\ (\frac{ml}{s}) = \frac{\frac{(\text{weight of alginate solution collected in the beaker in grams})}{\text{density of alginate solution in g/ml}}}{30\ \text{seconds}} \quad \textbf{Eq. 3.1}$$

3.3.5.2. Measurement of diameter of alginate beads and corresponding standard deviation

The alginate beads without dye were examined under the previously calibrated optical microscope. The size of the bead was expressed as diameter of the beads in micrometres. The mean and the standard deviation of the obtained measurements were calculated.

3.3.5.3. Ginger Oil extraction

The extraction method was based on SunWaterhouse et al. (2011) with modifications [42]. The dried capsules (5 g) were transferred to a 250 mL beaker. Then 150 mL of methanol was added. The resulting mixture was homogenized using an Ultra-Turrax T25 digital homogenizer (IKA, Germany) at 15,000 rpm for 1 min. This mixture was filtered with a Büchner funnel and to this same 150 mL of hexane was added. The resulting mixture was vigorously stirred to facilitate transfer of the oil containing methanol (aqueous phase) to the hexane layer (organic phase). This mixture was introduced in a separator funnel where methanol was discarded(bottom). The oil was recovered by evaporation of hexane using a rotary evaporator at 55 ° C and 250 mbar with the increase of the rotation speed from 20 to 250 rpm, until no condensation of hexane could be observed.

3.3.5.4. Primary oxidation measurement

The classical method for the quantification of hydroperoxides is the determination of the peroxide value (PV). The content of hydroperoxides, generally referred to as PV, is determined by an iodometric method [43].

This is based on the reduction of the hydroperoxide group (ROOH) with iodide ion (I^-). The amount of iodine (I_2) released is proportional to the concentration of peroxide present. The released I_2 is evaluated by a concentration against a standardized solution of sodium thiosulfate ($Na_2S_2O_3$) using a starch indicator. The chemical reactions involved in the determination of PV are given below (Figure 3.4.):

$$2\,\mathrm{KI} + 2H^+ + \mathrm{ROOH} \rightarrow \mathrm{ROH} + 2\mathrm{K}^+ + \mathrm{I}_2 + H_2O$$

$$2Na_2\mathrm{S}_2\mathrm{O}_3 + \mathrm{I}_2 \rightarrow Na_2\mathrm{S}_4\mathrm{O}_6 + 2\,\mathrm{NaI}$$

Figure 3.4. Chemical reactions in PV

In a 250 mL Erlenmeyer a sample of ginger oil was weighed, 10 mL of a chloroform solution and 15 mL of acetic acid were added and stirred until dissolved. Next, 1 mL of a saturated solution of potassium iodide (KI) was added, vigorously stirred and left to stand in the dark at room temperature for 5 minutes. After that time, 75 mL of milli-Q water was added. The released iodine was titrated with a previously standardized solution of sodium thiosulfate ($Na_2S_2O_3$, 0.01 mol / L), shaking vigorously and using 1 mL of the starch solution (10 g / L) as indicator, until the disappearance of the blue color. The peroxide value (PV), expressed in milliequivalents of active oxygen per kilogram of oil, was calculated by the following equation 3.2.

$$PV\left(\frac{meq}{Kg}\right) = \frac{100\,V\,T}{m} \qquad \textbf{Eq. 3.2}$$

Where:

V the number of mL of the standardized thiosulfate solution used for the determination, in milliliters;

T the exact molarity of the sodium thiosulfate solution used, in moles per liter;

m the weight of the test portion, in grams.

The method is applicable to all animal and vegetable fats and oils, fatty acids and their mixtures with peroxide values of 0 meq to 30 meq (milliequivalents) of active oxygen per kilogram [44]. In the present work, it is extended to use this method with essential oils since bibliography has been found where it is applied [45].

3.3.5.4.1. Standardization of sodium thiosulphate 0,1N

Sodium thiosulfate is very unstable due to its hygroscopic properties and therefore the prepared solution must be standardized, in order to carry out a good analysis. To carry out the standardization, 3 g of potassium iodide was weighed and transferred to an Erlenmeyer of 500 mL capacity. The potassium iodide is dissolved in 10 mL of deionized water and then 5 mL of concentrated hydrochloric acid is added. In parallel, a 0.1 N solution of potassium dichromate was prepared, and 25 mL of this solution was added to the Erlenmeyer. After the addition, the resulting solution was left for 5 minutes in a dark place and then 200 mL of deionized water was added.

The sodium thiosulfate solution to be standardized was placed in a 25 mL burette and the brown solution was titrated from iodine to a transparent yellow. At this time, 2 mL of starch indicator was added, and the solution was titrated until became a strong blue color. In all steps of the titration, the solution was stirred vigorously with a stirring magnet.

The concentration of the sodium thiosulphate solution was calculated as follows in the Eq. 3.3.

$$N\ Na_2S_2O_3 = \frac{N\ K_2Cr_2O_7\ x\ 25}{V} \qquad \textbf{Eq. 3.3}$$

Where:

N = Normality of the solution [equivalent/L],

V = total volume of $Na_2S_2O_3$ used during the titration

3.3.6. Rheology

Rheology is the study of the deformation and flow of matter. Study the properties that determine the behaviour of the matter, as well as the answers when stress and / or tension are applied [46].

In industry, quality controls can be applied using rheology, determination of product texture, viscoelastic behaviour, yield stress and other rheological properties. In the present work, the shear rate (gamma) was studied, which is the speed gradient established in the liquid as a result of the shear stress. This test was performed to measure the stationary viscosity of the gels at different shear rates in the range of 0.01 to 500 s^{-1}. Behaviour of the gel by the response of the viscosity at different shear rates can be seen from the test

Each unique material has its own behaviour when it is subject to flow, deformation or tension. Depending on its viscosity behaviour as a function of cutting speed, stress, history of deformation ..., fluids are characterized as Newtonian or non-Newtonian.

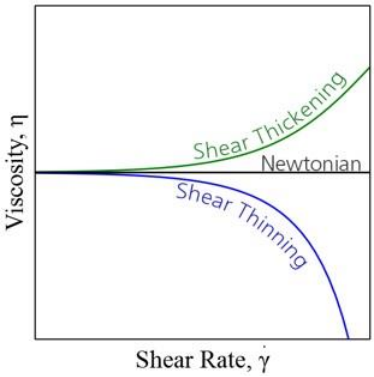

Figure 3.5. Viscosity of Newtonian, Shear Thinning and Shear Thickening fluids as a function shear rate. [47].

The viscosity of Newtonian fluids will remain constant (Figure 3.5.) no matter how fast they are forced to flow through a pipe or channel (the viscosity is independent of the cutting speed).

The viscosity of non-Newtonian fluids depends on the shear rate (thinning or shear thickening). A fluid is thickening by shearing if the viscosity of the fluid increases as the shear rate increases (Figure 3.5). The fluids are diluted by shearing if the viscosity decreases as the shear rate increases [47].

3.3.7. Electron Microscopy

The object of these techniques is the interaction of electrons with matter and the way to obtain information both structural and characterization of defects. It offers a solution to the problems presented by optical microscopes that cannot obtain atomic resolution since the wavelength of the incident radiation is too large. The electronic microscopes reach to solve objects of the angstrom order, and therefore, at least, atomic resolution can be obtained [48].

In the electron microscope, a beam of electrons impinges on a sample and the interaction of these electrons with the atoms of this, arise signals that are captured by some detector or, projected directly on a screen. Within the family of electronic microscopes are the transmission electron microscope (TEM) and the scanning electron microscope (SEM).

Each of them allows the study of different characteristics of a sample. The SEM provides information on morphology and surface characteristics, while with the TEM we can observe the internal structure and ultrastructural details.

In TEM the electrons that penetrate through the studied sample are used for the formation of the image, while in SEM, the electrons are used that are mainly reflected on the surface.

3.3.7.1. Field Emission Scanning Electron Microscopy (FESEM)

The exclusive high-power optics of JSM-7100F [49] is a device that allows the observation of the surface of materials, obtaining images of up to 1,000,000 magnification and a resolution of 1.2 nm resolution. A sample is introduced through the sample exchange air bag chamber. The sample chamber is always kept in high vacuum. The unique exchange mechanism of samples of an action allows you to insert and extract a sample with a simple operation. The operation is based on the incidence of an electronic beam on a conductive sample. This beam focuses on a point where a sweep of the surface is carried out. The result of the interaction is the emission of various signals: backscattered electrons, secondary electrons and X-rays, among others. These signals are picked up by detectors that process them, then they are amplified and transformed into electrical signals that are encoded as pixels in a monitor. The high increase for the study of nanostructures is easily obtained [50].

In the cry microscopy unit, at the Scientific and Technological Centre of the University of Barcelona explained they performed the Cryo-sectioning for TEM and the procedure was follows:

Each bead of sample was mounted onto sample pins with 5 µL 30 % dextran PBS and frozen in liquid nitrogen. Pins with frozen samples were transferred to a pre-cooled (-100 ºC) EM FC7 cryoultramicrotome (Leica Microsystems, Vienna, Austria) from the Cryo-microscopy Unit from the CCiTUB. Squared block faces with side measures ranging from 260 to 300 µm were trimmed using a 45º cryotrim diamond blade (Diatome, Biel, Switzerland) at -100 ºC. 60-nm cryosections were cut at -100 ºC with a 35º diamond knife (Diatome, Biel, Switzerland) with a clearance angle of 6° and at cutting speeds between 0.3 and 1 mm/s. Ribbons were picked-up with a 1:1 mixture of 2% methyl cellulose (25 centipoises, Sigma-Aldrich) and 2.3 M sucrose and next, thawed and stored on formvar coated 200 mesh nickel grids (EMS, Hatfield, USA) at 4°C. The grids were then stained with 2% uranyl oxalate [2% uranyl acetate and 0.15 M oxalic acid (Fluka); pH 7] and 0.4% UA in 2% methylcellulose.

4. RESULTS AND DISCUSSION

4.1. Parameters for the encapsulation process

In this section there are included the bases to define the values used for the constant parameters for the encapsulation. These parameters were defined in two previous works [51-52]. Here they are explained in a very summarized way.

First the alginate concentration (1% w / v) was fixed. In the study carried out by Lupo-Pasin (2015) [53] it was determined that lower values of this concentration produced a too weak gel matrix and higher a too high viscosity non-manageable for gelation. In this study, the appropriate concentration of calcium chloride (1% w / v) was also determined, since it guarantees complete gelation and prevent or minimize syneresis (expelling of water along time).

Afterwards, four parameter values had to be defined that must be kept constant in the BÜCHI Encapsulator, with the help of both works commented at the beginning of this section and the Encapsulator Guide [34] where information such as that of table 4.1 is extracted.

One of them is the size of the nozzle. In our case it will be of 750 µm, to obtain pearls of between 1150 to 1800 µm, an optimal size for its study and the use of alginate (1% w / v).

The next two parameters were the pressure at 450 mbar, the 380 V electrode and the vibration amplitude of 6, as indicated in table 4.1. The electrode is necessary for the dispersion of the newly created spheres and to avoid agglomerations. The amplitude or intensity of vibration does not influence the production of pearls too much, although if the value of the amplitude is too high it can cause an unstable liquid current.

Nozzle (µm)	Flow rate range (ml/min)* (Production)	Air pressure (bar)	Optimal frequency range (Hz)**	Amplitude	Size range of produced beads (µm)
80	1.1	0.5 - 0.7	1300 - 3000	1 - 4	120 - 200
120	1.5 - 1.8	0.5 - 0.7	1000 - 2500	1 - 4	200 - 300
150	2.3 - 2.8	0.4 - 0.6	800 - 1800	1 - 3	260 - 350
200	3.5 - 4.5	0.4 - 0.6	600 - 1200	1 - 3	350 - 450
300	6.0 - 8.0	0.3 - 0.5	400 - 800	1 - 3	550 - 700
450	11 - 15	0.3 - 0.5	200 - 500	1 - 4	700 - 1150
750	19 - 25	0.3 - 0.5	40 - 300	6-9	1150 - 1800
1000	30 - 40	0.3 - 0.6	40 - 220	6-9	1600 - 2400

Table 4.1. Parameter range to produce Ca-alginate beads (in the sizes ranges outlined) when using the single nozzle system on the Encapsulator B-390 and B-395 Pro [34].

4.1.1. Flow rate

When performing three weighing for a known volume it was found that the alginate together with the ginger oil had a density of 0.975 ± 0.014 g / ml. This was necessary to know the flow rate. When carrying out the three replicas of the experiment, a flow velocity of 0.735 ± 0.006 ml / s was obtained.

4.1.2. Rheology

The viscosity of the extruded mixture in the Büchi-390 encapsulator is one of the process parameters that influence the encapsulation.

The samples of different alginate and Kappa-carrageenan ratio have different properties and therefore it is necessary to determine the conditions in which the sample remains stable to achieve a good extrusion by the encapsulator. The shear thinning behavior allows the extrusion of a hydrogel by the application of shear stress during encapsulation.

In this work a hydrogel was prepared at volume ratio of 80:20 of 1% (w / v) alginate solution and 1.5% (w / v) kappa-carrageenan.

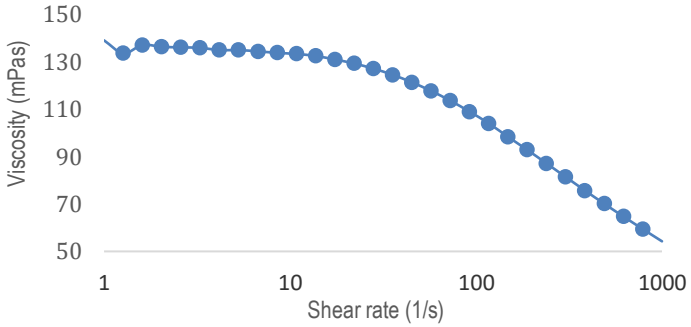

Figure 4.1. Shear thinning 1% Alginate + 1,5 % Kappa-carrageenan at 80:20 v/v ratio

As seen in Figure 4.1 the apparent viscosity of the alginate solution used in the experimental design decreases significantly with the increase in cutting speed. It is then shown thinning by cutting (non-Newtonian fluid), as demonstrated in the study Belščak-Cvitanović [15].

4.2. **Stability of O/W ginger oil/polysaccharide-water emulsions**

4.2.1. Surfactant for O/W emulsion

The use of emulsifiers can decrease the interface tension of the two immiscible liquid phases and prevent coalescence through electrostatic and/or steric barriers. The emulsifier works by forming a film around the dispersed granules thus preventing the coalescence and separation of the dispersed phase.

The hydrophilic-lipophilic balance of surfactants (HLB) provides information on the hydrophilic-lipophilic preference of the surfactant for its application in fields such as detergency or the stabilization of emulsions. Assigns to each surfactant a dimensionless number between 0 and 20 for the nonionic surfactants that provides information on the solubility in water and oil. This range is extender to higher values for ionic surfactants.

In this way it is known that HLB of surfactant/s used must be chosen depending on the emulsion being prepared. There are several formulas to calculate the HLB values of non-ionic surfactants. We can calculate the values for polysorbates (Tweens) and sorbitan esters (Spans) with the formula [54]:

HLB =% hydrophilic weight part in the surfactant molecule / 5 **Eq. 4.1**

The percentage by weight of each group in a molecule or mixture dictates the behaviour that a molecular structure will exhibit.

It is also possible to calculate the HLB values directly from the chemical formula using empirically determined group numbers. In that case, the formula looks like this [54]:

HLB = 7 + Σ(number of hydrophilic groups) - Σ (number of lipophilic groups) **Eq. 4.2**

The emulsifiers that have lower HLB value, are very useful in water-in-oil emulsions, due to their great solubility in oil phases, on the contrary, those with higher HLB value are soluble in water and suitable to prepare O/W emulsions [54].

In table 4.2. it can be verified that for an O / W emulsion (our case) the HLB number is between the value of 8 and 18.

Table 5. A Summary of HLB Ranges and Their Application

HLB range	Application
3 to 6	W/O emulsifier
7 to 9	Wetting agent
8 to 18	O/W emulsifier
13 to 15	Detergent
15 to 18	Solubilizer

Table 4.2. HLB ranges and their application [55].

In table 4.3. where are the HLB values corresponding to each emulsifier it is verified that Tween® 80 has a value of 15.

Nombre	Surfactante	HLB
Span 85	Trioleato de sorbitol (NI)	1,8
Span 65	Triestearato de sorbitol (NI)	2,1
Span 80	Monooleato de sorbitol (NI)	4,3
Span 60	Monoestearato de sorbitol (NI)	4,7
Span 40	Monopalmitato de sorbitol (NI)	6,7
Span 20	Monolaurato de sorbitol (NI)	8,6
Tween 81	Igual al Span 80 con poli-EO (NI)	10,0
Tween 65	Igual al Span 65 con poli-EO (NI)	10,5
Tween 21	Igual al Span 20 con poli-EO (NI)	13,3
Tween 60	Igual al Span 60 con poli-EO (NI)	14,9
Tween 80	Igual al Span 80 con poli-EO (NI)	15,0
Tween 40	Igual al Span 40 con poli-EO (NI)	15,6
Tween 20	Igual al Span 20 con poli-EO (NI)	16,7
Triton X-15	Octil-fenol-1,5 EO (NI)	3,6
Triton X-35	Octil-fenol-3,5 EO (NI)	7,8
Triton X-45	Octil-fenol-4,5 EO (NI)	10,4
Triton X-114	Octil-fenol-7,5 EO (NI)	12,4
Sipex SB	Dodecil sulfato de sodio (AI)	40,0
Sipon L-22	Dodecil sulfato de amonio (AI)	31,0
Sipon LT6	Dodecil sulfato de trietanolamina (AI)	34,0
Neodol 25-7	Alcohol primario (C12-15) poli-EO (NI)	12,0

Table 4.3. Hidrophilic- lipophilic balance [55].

4.2.2. Influence of concentration and surfactant on stability

It was required that the emulsion was stable enough to allow gelation process through BÜCHI, it means about half an hour. Once the pearls are formed that stability is not important as droplets of oil are already immobilized inside the beads as they are surrounded by the gel. It was checked if the use of tween 80 was required or if mixing well with the Ultra-Turrax was enough. Turbiscan™ was used to measure stability of emulsions.

The detection head of the device explores the entire length of the sample (approximately 100 mm), acquiring transmission and backscatter data every 5 minutes over half an hour.

The results will be represented in the following manner. The vertical axis represents the diffuse reflectance R (in%) normalized with respect to a standard non-absorbent reflector, representing the Back Scattering (%) and the horizontal axis represents the height of the sample in mm (z = 0 mm corresponds to the bottom of the measuring cell).

Two types of emulsions were prepared; the first one with 10% by weight of dispersed phase (ginger oil) and the second with 20% of it. 20 mL of fresh emulsion was introduced in a tube for the backscattering study along the tube and it was analysed with time, taking measurements every 5 minutes for half an hour.

The two emulsions behave differently, and graphically can be seen that in the first case there is no variation of the profile over this half an hour, indicate stable behaviour this time (Figure 4.2),

on the other hand, backscattering variation is observed in the profile with time, indicating that the 20% was not stable enough for encapsulation process (Figure 4.3).

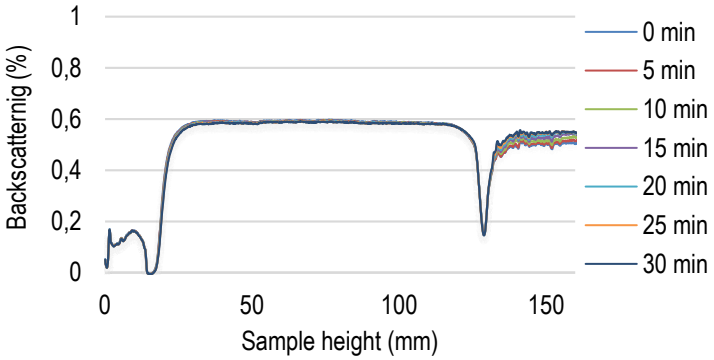

Figure 4.2. Backscattering 10% ginger oil, 0% Tween® 80.

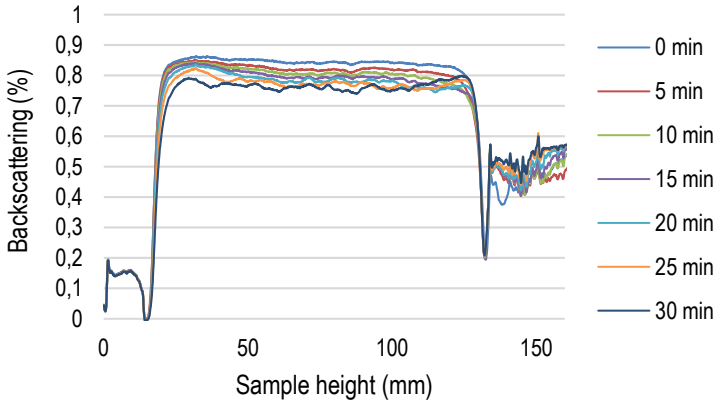

Figure 4.3. Backscattering 20% ginger oil, 0% Tween® 80.

Results indicate that emulsion with 10% of ginger oil was stable at least for 30 minutes, the required time for gelation process.

On the other hand, 20% emulsion was unstable. We add the surfactant Tween® 80 to stabilize the emulsion. It was tested with a surfactant concentration of 0-1% and 0-25%. It can be observed how the surfactant fulfils its function and less variation of backscattering is observed in the graphics (Figure 4.4 and 4.5), indicating a more stable emulsion.

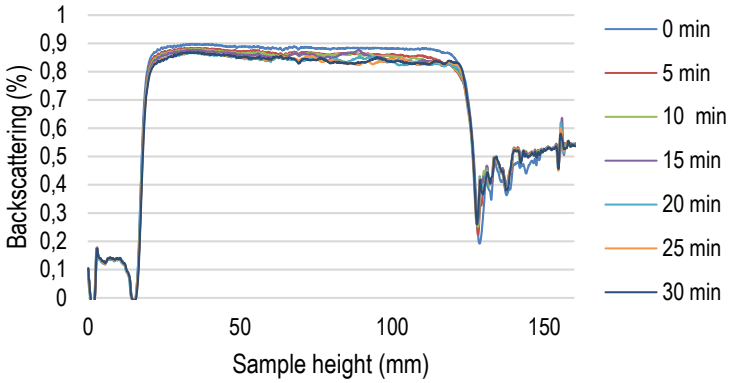

Figure 4.4. Backscattering 20% ginger oil, 0,1% Tween® 80.

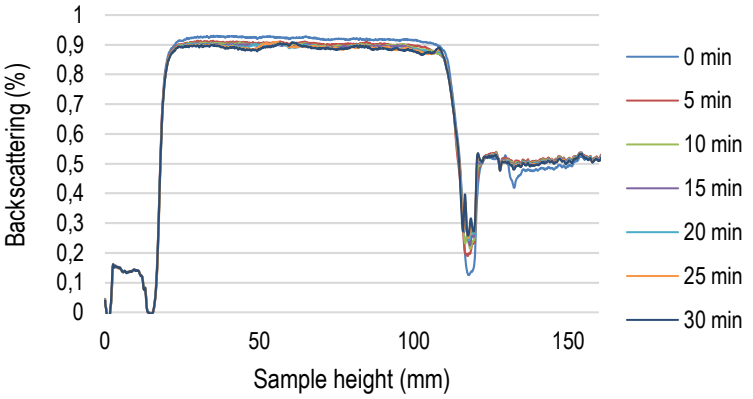

Figure 4.5. Backscattering 20% ginger oil, 0,25% Tween® 80.

Even so, it is seen graphically how the emulsion containing 10% ginger oil is still more stable than the one containing 20% with 0.25% Tween® 80. Moreover, the use of Tween 80 presented and added problem, that was the formation of foam.

Therefore, beads will be prepared using 10% dispersed phase in the absence of surfactant.

4.3. **Measure of diameter beads**

The diameter of the encapsulated beads was measured using OPTIKA Vision Pro. It was found that as indicated in the Büchi use guide, using a 750 µm nozzle, pearls from 1150 to 1800 µm are obtained. As can be seen in figure 4.6 in this study, an average diameter of 15996.43 ± 413.61µm was obtained.

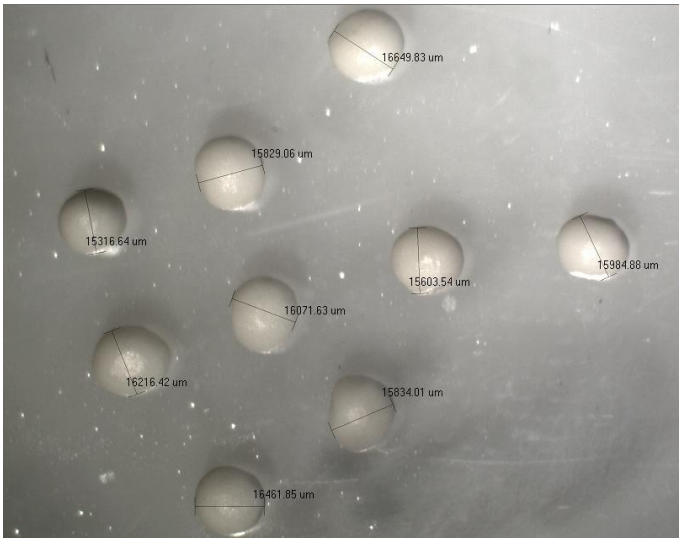

Figure 4.6. Measure of diameter real pearls

4.4. **Transverse view of the capsules by Scanning Electron Microscopy**

The samples were frozen with liquid nitrogen in the TEM unit. The team was responsible for cold mounting them on a metal thumbtack and observing them with their microscope, but they did not visualize themselves well. So, the samples that had previously been frozen, cut and already at room temperature were transferred to the Field Emission Scanning Electron Microscopy unit.

The beads, when hydrated, had no adhesion and were transferred to a sample holder containing an adhesive tape and adapted to the microscope. This sample was frozen again with liquid nitrogen to be able to introduce it inside the microscope that works under vacuum, otherwise it could be drained. Then for a few hours the sample was brought to room temperature and observed.

In the gelation process, the oil droplets were distributed throughout the cross-linked alginate-Kappa carrageenan gel. When cutting this frozen pearl and as seen in Figure 4.7, the ball is retracted and looks like it could be closed again.

It can be seen that the pearl is empty inside. It has been considered that it could be for two reasons. That, during the encapsulation process, there is a certain coalescence due to the high shear when passing through the nozzles. Or, that the film that remains crosslinked between the drops is very weak and due to frozen cutting-defrost cycle, it breaks / collapses and the oil escapes and hence it looks hollow.

It follows that the outer layer does not break and has no porosities due to the content of chitosan and more $CaCl_2$ due to external gelation, as can be seen in Figure 4.8.

The syneresis is shown in Figure 4.9. The outer surface is very wrinkled since the pearl has lost water.

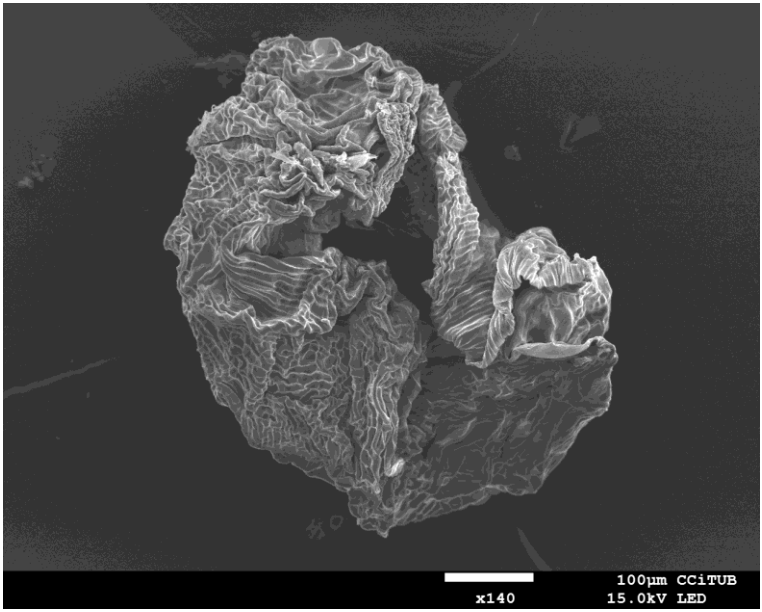

Figure 4.7. Image obtained in the SEM

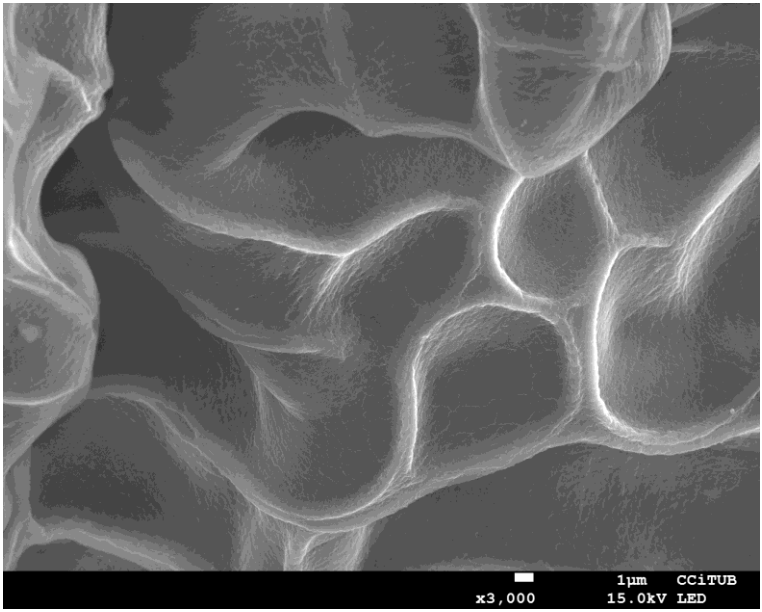

Figure 4.8. Image obtained in the SEM

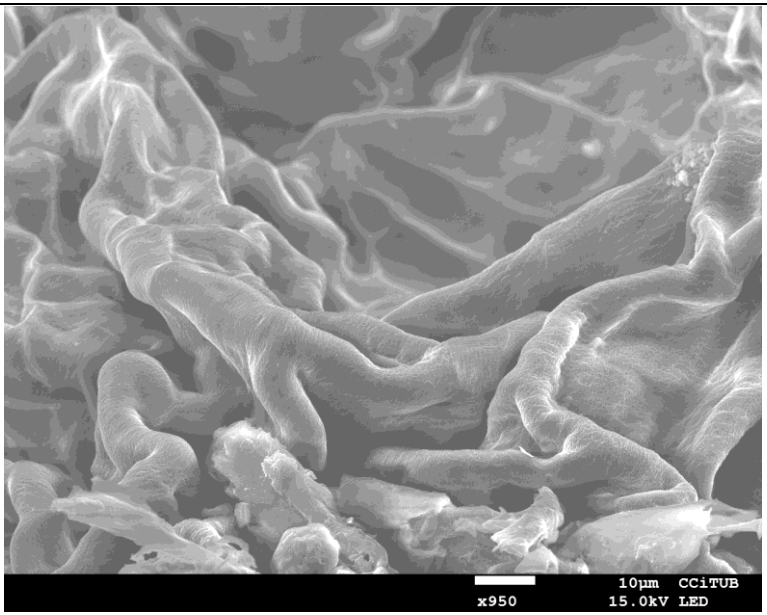

Figure 4.9. Image obtained in the SEM

4.5. **Stability against oxidation of encapsulated ginger oil**

As explained in section 3.3.5, three different solutions were prepared for the matrix. The first without dye, the second was dyed with a natural red dye and the third was dyed with methylene blue. The result was the one shown in Figure 4.10.

Figure 4.10. Result of the production of alginate pearls without dye, red dye and blue dye.

Once the three different pearl samples were produced, every four days and for a period of 30 days the same experiment was performed for each sample. The repetitive experiment consisted in extracting the oil from weighted amount of beads and performing the peroxide determination method (PV), both processes explained in the previous sections. The peroxide value from which we begin to consider the stale essential oil is 30 meq.

In all the graphs that we find below you can see how PV values start at 2 day's time. The start day, the PV has a value of 0 in all cases, so we ignore it when plotting. You can also see in all the graphs as the experimental values, the points, they are linked by lines and it is to facilitate the understood

The first experiment that was done was with the beads that did not contain dye, as can be seen in Figure 4.11.

We wanted to analyze what type of storage made the rancidity time of the ginger oil slow down. A sample quantity was in the showcase, another in the locker and another in the refrigerator (4ºC). In the first place, it was analyzed what effect the exposure of the sample had with the external light. Comparing results of the showcase and the locker. It was concluded that as the days passed, and the beads had less contact with the light, the oil was maintained better, and lower values of PV were obtained. Secondly, the effect of the contact of the sample with oxygen of the air was analyzed. The three samples discussed above were duplicated; one completely covered with a film and the other covered with it, but leaky (to avoid fouling). In table 4.4 and figure 4.7 as the days go by oil contained in the beads covered with film preserved in a better way as shown by the lower PV values obtained.

Third, the effect of the temperature difference between the locker and refrigerator samples was analyzed, since contact with light and oxygen are the same between these two storages. At colder, the oxidation rate decreases.

Fourth and last, the control experiment was carried out, that is, ginger oil mixed with the hydrogel but not encapsulated and compared with the results obtained from the encapsulated pearls. Where it was demonstrated that the encapsulation is effective and will slow down the oxidation time of the essential oil.

Days	Control	Showcase uncovered	Showcase covered	Locker uncovered	Locker covered	Refrigerator uncovered	Refrigerator covered
2	8.56 ±0.30	7.44 ±0.31	5.91 ±0.08	5.14 ±0.18	4.37 ±0.43	4,31 ±0.27	3.95 ±0.71
6	10.81 ±0.73	8.74 ±0.39	7.91 ±0.20	8.14 ±0.21	6.09 ±1.61	5,49 ±0.74	4.25 ±0.35
10	20.40 ±0.44	19.05 ±0.10	18.85 ±0.12	13.36 ±2.96	8.16 ±0.61	6,14 ±0.34	5.33 ±0.22
14	31.06 ±0.63	23.68 ±2.13	20.07 ±0.37	16.27 ±0.27	15.34 ±0.31	9,91 ±0.78	8.11 ±0.44
18	33.70 ±0.53	25 ±4.58	20.89 ±2.62	19.22 ±3.49	16.39 ±4.37	10,73 ±0.31	8.7 ±0.58
22	41.65 ±1.96	29.79 ±7.55	22.74 ±1.85	20.55 ±3.20	19.33 ±0.93	13,04 ±0.35	11.53 ±1.26
26		33.06 ±3.91	25.33 ±1.19	20.62 ±2.88	19.72 ±0.50	13,92 ±0.87	12.69 ±0.20
30		40.19 ±1.78	37.02 ±2.31	20.62 ±2.44	20.89 ±0.48	16,76 ±0.30	15.22 ±1.45

Table 4.4. Peroxide values white pearls with different exposure to oxygen and light.

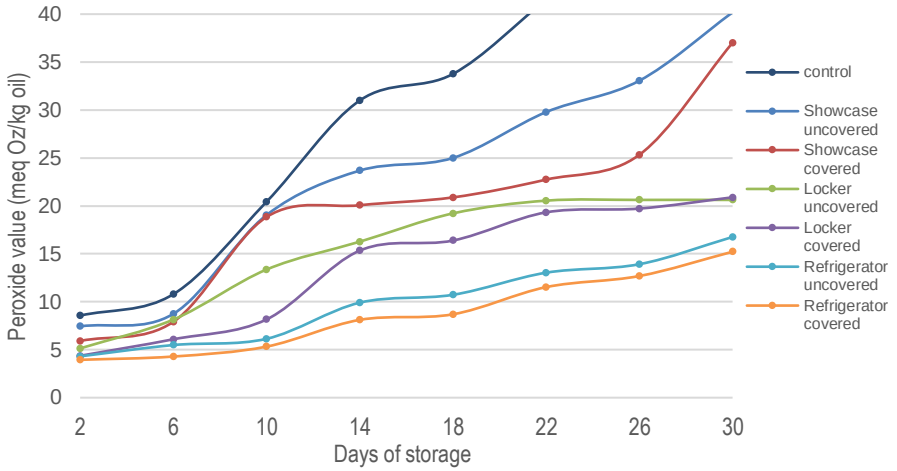

Figure 4.11. Peroxide values white pearls with different exposure to oxygen and light.

The second experiment was performed with the beads tinted with red dye, as can be seen in figure 4.10.

The first thing we could see was that the $CaCl_2$ also had to be tinted in the same way as the alginate, otherwise the beads would be tinted a lighter color as it is lost during encapsulation process du to diffusion of dye to the calcium bath. That is, if the two solutions were dyed, we avoided diffusion, and, in this way, we obtained beads with a more intense color (Figure 4.12).

Figure 4.12. Alginate and $CaCl_2$ dyed red in the same way

As happened with the beads without dye, it was demonstrated that in those that were protected from contact with air kept the oxidation of oil was retarded; what we wanted to test now was if the dye protected the sample from contact with light. So, a sample was placed in the showcase and another in the locker. And, a third in the refrigerator to see the effect of temperature.

In table 4.5 and in figure 4.13 you can check and draw the same conclusion as with the undyed beads, as the days passed, in the beads with less contact with the light, the oil was maintained better, and lower values of PV were obtained. It is also concluded that the dye is not opaque and lets light in. If this were not the case, it would not influence where the sample was located and whether it gave direct light or not. Looking at the curves in Figure 4.9 and comparing the three storages with the control one (without encapsulating), you can see how they are very close together and very far from the control one. It can be said that the dye allows the light to pass, but since there is not as much difference between the three samples as in the previous experiment, it is concluded that the dye provides some protection.

It was again proved that the refrigerated samples have a higher oxidation time.

Days	Control	Showcase	Locker	Refrigerator
2	8.56 ± 0.30	5.28 ± 0.89	4.61 ± 0.68	3.57 ± 0.58
6	10.81 ± 0.73	6.94 ± 0.93	6.29 ± 0.89	5.53 ± 1.36
10	20.40 ± 0.44	8.47 ± 1.32	6.64 ± 0.85	5.91 ± 1.34
14	31.06 ± 0.63	9.48 ± 1.29	8.07 ± 1.83	6.72 ± 2.03
18	33.70 ± 0.53	10.98 ± 1.03	10.31 ± 1.14	7.79 ± 0.70
22	41.65 ± 1.96	12.68 ± 0.24	11.36 ± 0.14	8.9 ± 0.66
26		15.1 ± 0.86	12.75 ± 0.77	10 ± 0.34
30		22.96 ± 0.32	14.72 ± 0.70	11.57 ± 0.44

Table 4.5. Peroxide values red pearls with different exposure to light uncovered

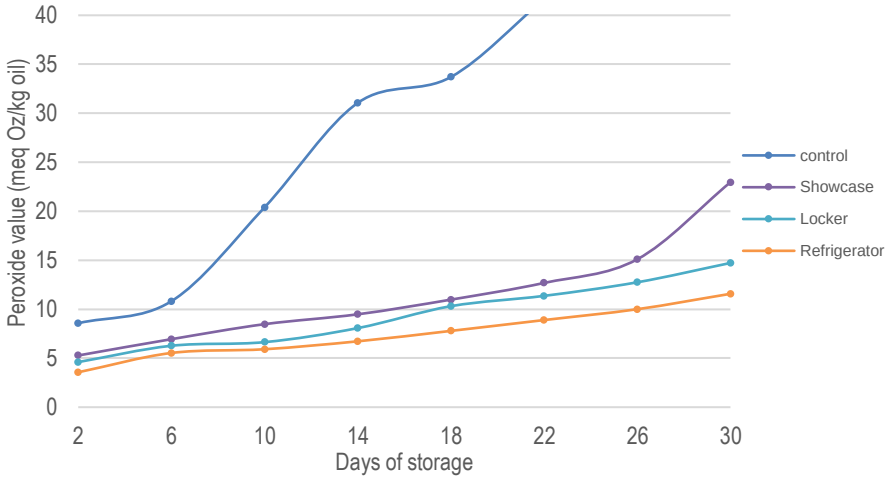

Figure 4.13. Peroxide values red pearls with different exposure to light uncovered

The third experiment was carried out with the pearls stained with methylene blue, as can be seen in figure 4.14.

Again, the $CaCl_2$ had to be tinted in the same way as the alginate, otherwise the pearls would be tinted a lighter color, and we wanted to avoid diffusion.

The sample was distributed in the same way as in the previous two experiments, in the showcase, in the locker and in the refrigerator.

In table 4.6 and in figure 4.15 you can check and draw the same conclusion as with the red tinted pearls, as the days went by and the pearls had less contact with the light, the oil was maintained better, and lower PV values were obtained. It is also concluded that the dye is not opaque and lets light in. If this were not the case, it would not influence where the sample was located and whether it gave direct light or not.

It was again proved that the refrigerated samples have a higher oxidation time In this experiment you can see how until the tenth day the values of the control sample are very close and even lower than the encapsulated pearls. This gives an index that the methylene blue hinders in some way the protection of the essential oil. It will be discussed in figures 4.16, 4.17 and 4.18.

Figure 4.14. Alginate and $CaCl_2$ dyed blue in the same way

Days	Control	Showcase	Locker	Refrigerator
2	8.56 ± 0,30	13.35 ± 1.08	11.6 ± 1.02	9.44 ± 0.81
6	10.81 ± 0.73	16 ± 0.66	15.2 ± 0.78	10.3 ± 0.85
10	20.40 ± 0.44	16.5 ± 0.99	15.82 ± 0.85	10.69 ± 0.59
14	31.06 ± 0.63	20.08 ± 2.04	16.75 ± 0.06	12.05 ± 0.95
18	33.70 ± 0.53	23.07 ± 0.99	19.77 ± 1.28	16.59 ± 0.52
22	41.65 ± 1.96	28.53 ± 0.31	27.3 ± 0.06	18.23 ±0.74
26		31.71 ± 0.11	29.39 ± 0.71	23.81 ± 0.41
30		40.19 ± 1.78	37.02 ± 2.31	24.65 ± 2.44

Table 4.6. Peroxide values blue pearls with different exposure to light uncovered

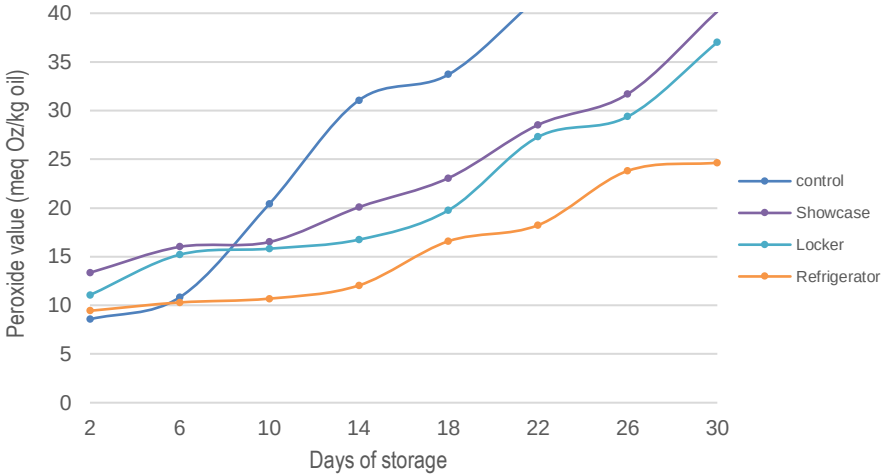

Figure 4.15. Peroxide values blue pearls with different exposure to light uncovered

Once the three experiments and the three replications were carried out, we could make a comparison between the non-tinted and tinted pearls with the two dyes and draw conclusions.

Figure 4.16. reflects the comparison of the three types of pearls in the showcase, figure 4.17 reflects the comparison of the three types of pearls in the cabinet and finally figure 4.18 reflects the comparison of the three types of pearls in the refrigerator.

In the following graph (figure 4.16) in the red pearls located in the showcase after being encapsulated for 30 days, the ginger oil gives a PV of 22.96 ± 0.32. That is, the oil has not yet been oxidized. In contrast, in the non-tinted beads with blue dye, the ginger oil is rancid about the 22th day of its encapsulation.

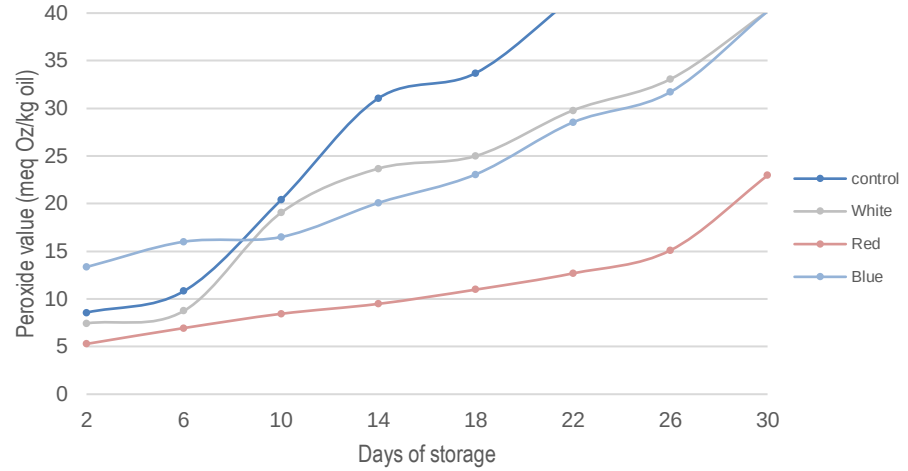

Figure 4.16. Peroxide values pearls in showcase with different exposure to light uncovered

In the next two graphs (figure 4.17 - 4.18) you can also see how the red pearls are the ones that best maintain the ginger oil and how those that are tinted blue are oxidized before those that do not contain dye.

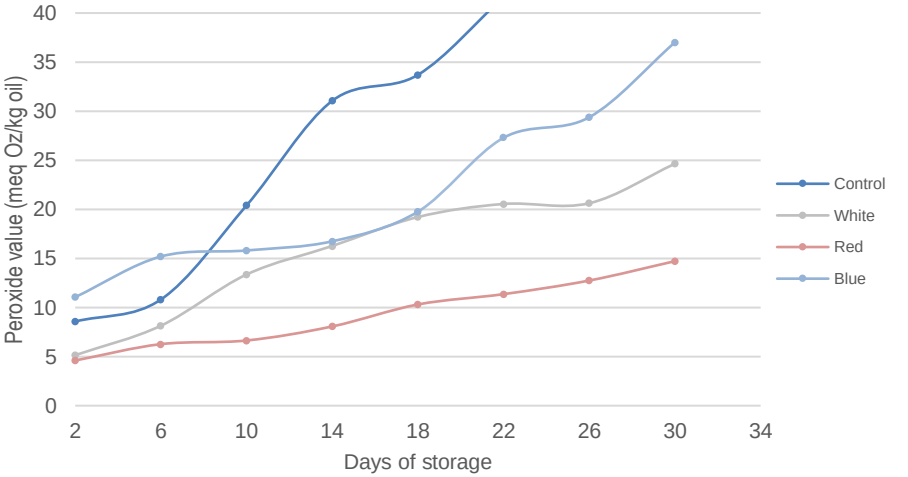

Figure 4.17. Peroxide values pearls in locker with different exposure to light uncovered

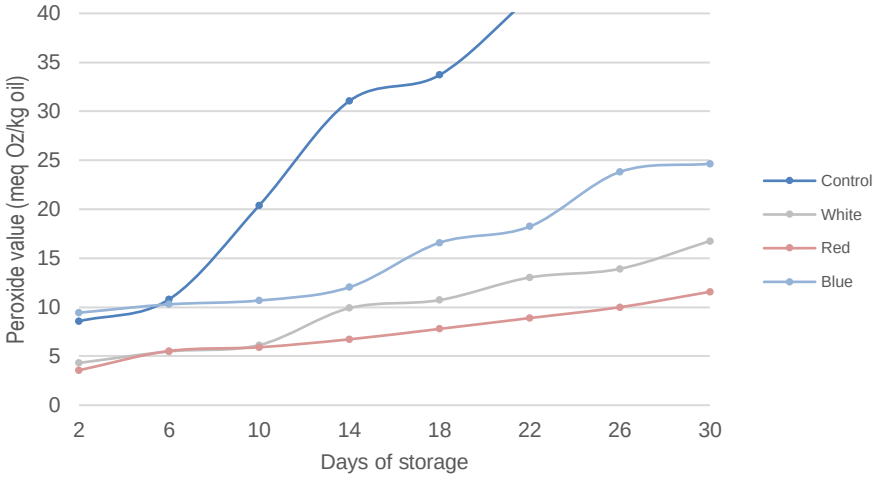

Figure 4.18. Peroxide values pearls in refrigerator with different exposure to light uncovered

It was not expected that, in the pearls stained with methylene blue, the ginger oil would oxidize faster than even those that did not contain dye. It seems that an oxidation-reduction reaction occurs between the oil and the methylene blue in which the methylene blue is reduced, and the oil is oxidized [56].

The first conclusion that can be drawn from them and globally is that the control experiment, that is, without encapsulating, gives higher PV values than any encapsulated one. So, it is concluded that the encapsulation lengthens the oxidation time of the essential oil of ginger.

If we plotted together these last three graphs, it could be visually checked as the best option of all and to lengthen the oxidation time would be the encapsulation with red natural dye and keep the beads in the fridge.

Finally, comment that the samples stored in the refrigerator, apart from keeping the ginger oil in better conditions, were the only samples that did not appear mustiness.

5. CONCLUSIONS

The conclusions that can be derived from this work are the following:

- The 10% O / W emulsion ginger oil/ alginate k-carrageenan aqueous solution is stable enough for the time required for its encapsulation and the use of a surfactant is not necessary. Tween 80 surfactant improves stability, but even with this surfactant, with 20% dispersed phase remains more unstable than with 10% oil. It is decided to work without surfactant and thus also avoid the formation of foam derived from use.

- Pearl diameters between 1150 and 1800 µm were obtained as indicated in the Büchi Guide if a 750 µm nozzle is used.

- The free ginger oil (control) is oxidized much faster than the encapsulated ginger oil, indicating that encapsulation offers some protection.

- Encapsulated ginger oil has a longer oxidation time with less contact with air. The oil that is in the pearls covered with plastic film is better maintained than those that are covered with the perforated film.

- Encapsulated ginger oil has a longer oxidation time with less contact with light. The oil found in the pearls stored in the locker is maintained better than those stored in the showcase.

- Encapsulated ginger oil has a longer oxidation time at low temperature (4ºC). The oil found in the pearls stored in the refrigerator stays better than those stored in the locker. In addition, the appearance of mold is avoided.

- Ginger oil encapsulated in red beads has a longer oxidation time than the one where beads are not dyed. Therefore, the red dye protects from light and decreases the rancidity of the essential oil.

- The use of methylene blue as a dye does not give the expected results; it seems to favor the oxidation of the oil through some reaction or some catalyst effect as ginger oil is oxidized more or less in the same extent as without the dye regardless the darkness of the blue color obtained.

Although the dye obstructs the passage of light into the pearls, favors oxidation by other paths, resulting in similar in similar results than without dye.

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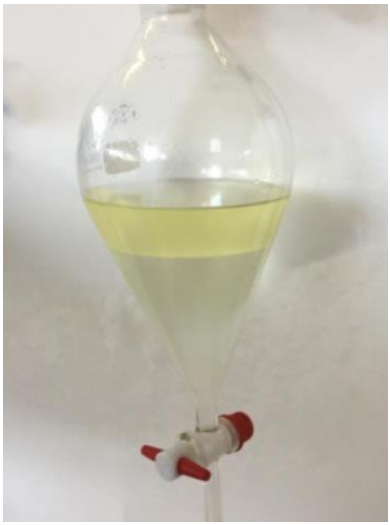
ACRONYMS

EO	Essential oil
O/W	Oil-in-water emulsion
% w/w	Solution concentration expressed in percentage weight/weight
%w/v	Solution concentration expressed in percentage weight/volume
%v/v	Solution concentration expressed in percentage volume/volume
meq O_2/Kg	Milliequivalents of active oxygen per Kilogram of oil
$CaCl_2$	Calcium chloride
PV	Peroxide Value
SEM	Scanning Electron Microscopy
FESEM	Field Emission Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
HLB	Hydrophilic-lipophilic balance of surfactants
mg	Milligrams
mL	Millilitres

ANNEXES

ANNEX 1. a) Filtered by Büchner, the lower part contains methanol and the upper part hexane with ginger oil. b) Recovery of the oil by evaporation of the hexane by means of a rotating equipment.



ANNEX 2. Two extra images obtained in the SEM.