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**Preparation and characterization of oil in water in water (O/W/W)
emulsions with food applications**

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Gràcies als meus avis per estar sempre amb mi, per ajudar-me a fer camí cap al meu futur i per estimar-me. Gràcies a la meva família, a la meva mare i al meu tiet. Gràcies a totes les amistats que heu estat al meu cantó, els companys d'universitat que s'han convertit en amics, els que han anat arribant i els de tota la vida, gràcies a la família que s'escull.

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SUMMARY

Food industry is increasingly aware of the importance of maintaining a healthy diet. For this reason, this industry tries to obtain the same products with less fat content. Elaboration of emulsions allows obtaining similar textures to traditional food products, such as sauces. Oil in water emulsions (O/W) are most common in this industry, however, by making multiple emulsions a wide variety of textures can be obtained. In the production process it is possible to choose oily phase proportion, controlling the amount of fats of the final product.

The objective of this work is to obtain an oil in water in water emulsion (O/W/W) with a low oil content. For this, firstly, oil in water emulsions have been prepared by emulsifying sunflower oil in casein with different types of agitation until obtaining the desired drop size and stability. A small proportion of oil has been chosen respect to the amount of casein. To ensure enough stability of this emulsion for a period of time, it has been stabilized by the addition of different percentages of surfactant until founding the most suitable concentration. The size distribution of oil droplets of the emulsions and its stability has been determined.

Once the O/W emulsion has been characterized, it has been dispersed in alginate by different types of agitation, obtaining a multiple emulsion of oil in water in water (O/W/W). This emulsion has been characterized, determining emulsion drop size and its stability.

Subsequently, this multiple emulsion has been gelled with different concentrations of a calcium chloride solution and its rheological behavior has been evaluated and comparing it with a commercial sauce, specifically, mayonnaise.

Oil contained amount in resulting emulsion is lower, so it is an interesting proposal for the food industry to achieve obtaining emulsions with industrial sauces texture and less content in oil.

Key words: food emulsions, external gelation, characterization of emulsions, sodium alginate, casein, calcium chloride, multiple emulsions.

RESUMEN

La industria alimentaria cada día está más concienciada sobre la importancia de mantener una alimentación saludable. Por este motivo, se intentan elaborar los mismos productos con menor cantidad de grasas. Mediante la elaboración de emulsiones se pueden obtener texturas parecidas a productos alimentarios tradicionales, como podrían ser salsas. Las emulsiones aceite en agua (*oil in water*) son las más comunes en esta industria; no obstante, mediante la elaboración de emulsiones múltiples se puede obtener una gran variedad de texturas. En el proceso de producción se puede elegir la proporción de fase oleosa, controlando la cantidad de grasas del producto final.

El objetivo de este trabajo es obtener una emulsión de aceite en agua en agua (*oil in water in water*) con un bajo contenido en aceite. Para ello, primeramente, se ha preparado la emulsión de aceite en agua (O/W) emulsionando aceite de girasol en caseína con diferentes tipos de agitación hasta obtener el tamaño de gota y estabilidad deseados. Se ha elegido una proporción de aceite pequeña respecto a la cantidad de caseína. Para asegurar la estabilidad de esta emulsión durante un periodo de tiempo suficiente, se ha estabilizado mediante la adición de diferentes porcentajes de tensioactivo hasta encontrar la concentración más adecuada. Se ha obtenido la distribución de tamaño de gotas de aceite de la emulsión y su estabilidad.

Una vez caracterizada la emulsión O/W, se ha dispersado ésta en alginato mediante distintos tipos de agitación, obteniendo una emulsión múltiple de aceite en agua en agua (O/W/W). Se ha caracterizado la emulsión, determinando el tamaño de gota de emulsión y su estabilidad.

Posteriormente, se ha gelificado esta emulsión múltiple con diferentes concentraciones de una solución de cloruro de calcio y se ha evaluado su comportamiento reológico comparándolo con el de una salsa comercial, concretamente, mayonesa.

La cantidad de aceite contenida en la emulsión resultante es pequeña, de manera que es una propuesta interesante para la industria alimentaria conseguir obtener emulsiones con una textura parecida a la de salsas industriales y con menor contenido en aceite.

Palabras clave: emulsiones alimentarias, gelificación externa, caracterización de emulsiones, alginato de sodio, caseína, cloruro de calcio, emulsiones múltiples.

1. INTRODUCTION

1.1. EMULSIONS

An emulsion is a dispersion of one of two immiscible phases within the other one in form of little droplets. The liquid droplets are dispersed in a continuous phase. There are different emulsion types, according to the size of droplets in the dispersed phase and the structure of the emulsion:

- Oil in water and water in oil macroemulsions: these have a size range of 0.1-5 μm .
- Nanoemulsions: these have a size range of 20-100 nm. They are kinetically stable.
- Micellar emulsions or microemulsions: these have a size range of 5-50 nm. They are thermodynamically stable.
- Double and multiple emulsions, like oil in water in water emulsion.
- Mixed emulsions: these emulsions consist of two different disperse droplets that do not mix in a continuous medium. (*Borwankar R. P. et al., 2003*)

Another way to classify emulsions is by the nature of the dispersed and the continuous phase, as simple and multiple emulsions:

- Water in water (W/W)
- Oil in oil (O/O)
- Oil in water (O/W)
- Water in oil (W/O)
- Oil in water in water (O/W/W)
- Water in oil in water (W/O/W)
- Oil in water in oil (O/W/O)
- Water in water in oil (W/W/O)

Emulsions are thermodynamically unstable, and their stability depends on:

- Droplet size: the emulsion is more stable with smaller droplet sizes.
- Viscosity of continuous phase: the emulsion is more stable with higher viscosity values.
- Specific density of the two phases: the emulsion is more stable with smaller difference of densities between the phases. (*F. Tadros, Tharwat, 2013*)

Emulsions also can be classified by the volume fraction of disperse phase:

- Dilute emulsions: $\Phi < 0.20$. The fluid has a Newtonian behavior, where the viscosity dependence with speed gradient is lineal. The interaction between the drops is lower. Some examples of food dilute emulsions are milk and ice-cream.
- Concentrated emulsions: $0.20 < \Phi < 0.74$. The fluid starts to have a no Newtonian behavior to become a pseudoplastic behavior. Some examples of food concentrated emulsions are butter and margarine.
- Highly concentrated emulsions: $\Phi > 0.74$. This is the maximum volume fraction of close-packed monodisperse spheres, where drops have a spherical structure. If the volume fraction of disperse phase overcomes this value, the drops are deformed as a polyhedral structure and are separated from each other by a thin film of continuous phase. Some examples of food highly concentrated emulsions are sauces and mayonnaise. (*Ayşe Kara et al., 2015*)

Variety of volume fraction of disperse phase results in a wide variety of rheological behaviors and textures usable in food industry. This viscoelastic behavior is characterized by viscoelastic modulus G' and G'' .

Storage modulus (G') characterizes the elastic response and it is associated with the storage energy on the material.

The loss modulus (G'') characterizes the viscous behavior and it is associated with the dissipated energy by the material.

When the modulus G' is greater than G'' , the behavior is more elastic than viscous. It means that the material recovers his initial structure when a speed gradient is not applied on it.

If the material is more viscous than elastic, it not returns to initial state.

When these modulus have the same value, the product changes his elastic behavior to viscos behavior. The storage energy is equal at the dissipated energy: the sample has a solid behavior, not liquid. On concentrated emulsions, G' and G'' not cross or they do it at lower frequencies. On diluted emulsions, this cross is at higher frequencies.

1.2. WATER IN WATER EMULSIONS

Water in water emulsions are a dispersion of an aqueous phase into another aqueous solution. Food products often contain these emulsions because they can improve organoleptic and health properties. These emulsions have low interfacial tension and they are biocompatible. However, their stability is not high, so it is quite common to gel the emulsion.

Polymers constituting the emulsion are incompatible and it produces the phase separation. In this case, casein and alginate are two polymers incompatible between them. Casein will be continuous phase of oil in water emulsion that will be emulsified on alginate, which will be continuous phase of oil in water in water emulsion.

Casein is a phosphoprotein found in mammalian milk. This protein is a natural emulsifier on oil in water emulsions which resists high temperatures. The casein micelles are constituted by a 92% of casein and an 8% of calcium phosphate colloidal salt and this protein represents the 80% of the total protein quantitate of the milk.

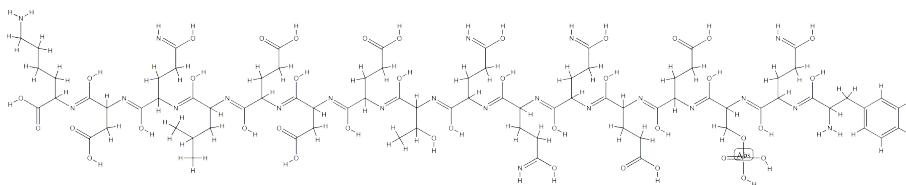


Figure 1. Casein molecule (www.chemicalbook.com, accessed June 7, 2019)

The casein micelle is a very complex polymer aggregate and interacts via hydrophobic and electrostatic interaction and calcium bridging. A typical casein micelle contains about 10000 casein molecules. Casein has stabilizer and emulsifier power because of this protein is amphipathic and it adheres on the surface of the emulsion substances. For this reason, casein reduces the interfacial tension stabilizing the emulsion. (Guo, Mingruo; Wang, Guorong, 2016)

Furthermore, alginate is one of the most used polymers because is biocompatible and low toxicity. This natural polysaccharide is typically obtained from brown seaweed. This polymer has the ability to be gelled constituting a structure is known as “egg-box”.

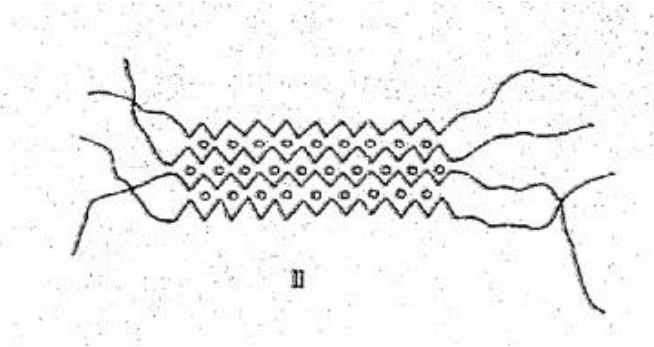


Figure 2. Egg-box structure of alginate. (A. Rees, David *et al.*, 1973)

This structure, showed on Figure 2, consists on the fact that calcium ions are situated like bridges between the groups with negative charge of the guluronic acid, which corresponds to alginate. The eggs represent the calcium ions characteristics of gelation (coming from, for example, calcium chloride) and the box represents the polysaccharide chains. Alginate has gelation properties in the presence of multivalent cations like calcium ion. (A. Rees, David *et al.*, 1973)

Emulsion formation and their gelation enable to achieve textures applicable to food industry.

1.3. FOOD EMULSIONS

Emulsions are very important in the food industry. Many natural and processed foods consist as emulsions, for example, cream and mayonnaise are water in oil emulsions, and margarine and butter are water in oil emulsions.

Usually, food emulsions consist on oil in water emulsion. However, it is possible to produce multiple emulsions, as oil in water in water emulsions, also carrying out their gelation to achieve a wide variety of textures applicable to food products.

The concentration of droplets in an emulsion is characterized by the dispersed-phase volume fraction. This property determines the texture of the food.

Nowadays, healthy alimentary habits are becoming an important factor to the people, consequently, to the food industries. The society is concerned about the consume of food rich in fats is because an excessive consume of these products can produce damages on the health of the people and several problems like obesity or cholesterol. For this reason, it is interesting to obtain adequate textures with less grease content.

The dispersed-phase volume fraction is easy to manipulate because the concentration of the ingredients could be controlled in the manufacturing process. It allows control of oily quantitate, in oil in water emulsions. It is important to improve food products and become they healthier. (*Borwankar R. P. et al., 2003*)

1.4. OIL IN WATER EMULSIONS

Oil in water emulsions are a dispersion of an oily phase into an aqueous solution. These emulsions are quite stable, but if it could be necessary, they can be stabilized by using surfactants. The studied oil in water emulsion is composed by sunflower oil as dispersed phase and casein as continuous phase.

Sunflower oil is one of the most important vegetable oils employed on food industry. The refined sunflower oil has a pale-yellow color and a mild flavor. It has a very low melting point, below 0 °C but it stays clear at refrigeration temperatures. This is an advantage for the formulation of sauces and emulsions that must be kept cold. (*Cuesta, C.; Sánchez-Muniz, F.J., 2003*)

The reduce amount of polyunsaturated fatty acids makes high oleic sunflower oil especially stable against oxidation. (*Cuesta, C.; Sánchez-Muniz, F.J., 2003*)

1.5. OIL IN WATER IN WATER EMULSIONS

Oil in water in water emulsions are a dispersion of an oil in water emulsion into an aqueous solution. The studied oil in water in water emulsion is composed by sunflower oil in casein emulsion as dispersed phase and alginate as continuous phase.

The advantage of oil in water in water emulsions (generally, of the water in water emulsions) respecting to oil in water emulsions is that the first do not emulsify oil components so they have lower interfacial tension.

1.6. STABILIZATION PROCESS

An emulsion is a thermodynamically unstable dispersion of several immiscible liquids.

Emulsions can be destabilized by different phenomena:

- Creaming and sedimentation: when external forces, like gravitational or centrifugal, exceed the thermal motion of the droplets produced by agitation, a concentration gradient is developed on the system. It produces the movement of the drops to the top (direct sedimentation) when the density of the drops is lower than the density of the medium, or to the bottom (inverse sedimentation or creaming) when the density of the drops is larger than the density of the medium. The droplets may form a close-packed array at the top or the bottom of the system.
- Flocculation: when there is not enough repulsion to keep the droplets apart to distances where the van der Waals attraction is weak, the aggregation of the drops is produced.
- Ostwald ripening: a mass transfer is produced from the smaller drops to bigger drops since the smallest droplets disappear.
- Coalescence: the fusion of two or more droplets into bigger drops is produced because of the thinning and disruption of the liquid film between these droplets. (Borwankar R. P. et al., 2003)

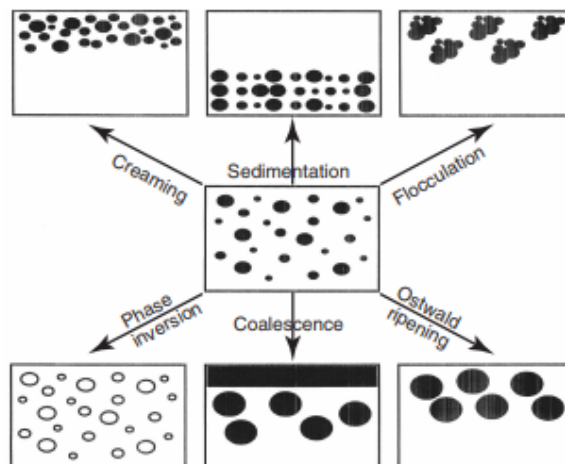


Figure 3. Destabilization phenomena. (Borwankar R. P. et al., 2003)

Because of the thermodynamically instability of the emulsions and to prevent the happening of these destabilization process, it is necessary to carry out a stabilization process.

Oil in water emulsion has higher values of interfacial tension so it is necessary to reduce it with a surfactant addition.

A surfactant is an amphiphilic molecule composed of a hydrophobic part and a hydrophilic part. They minimize the energy required for the emulsion formation by reducing oil-water interfacial tension. Polar part of surfactant is enticed by interphase polar part and the same succeeds with non-polar chain, which is enticed by interphase non-polar part. It is showed at Figure 4. Surfactant non-polar fraction forms hydrogen bonds with aqueous phase and hydrophobic fraction is aggregated with other surfactant molecules by Van der Waals forces. (F. Tadros, *Tharwat*, 2013)

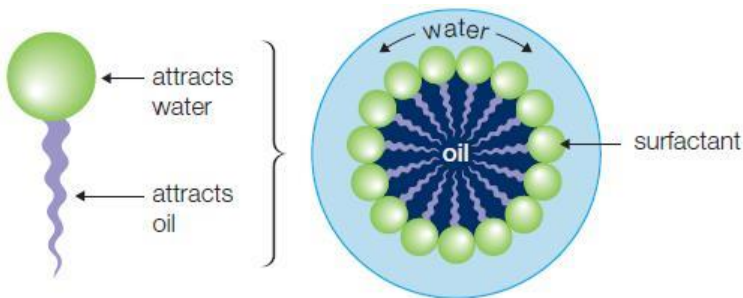


Figure 4. Surfactant representation on oil in water emulsion. (Grote, *matthias*, 2016)

To select the appropriated surfactant, it is necessary to know HLB number associated to sunflower oil. This number represents the hydrophilic proportion of the surfactant and for every material, there is a better value of HLB. A mixture of surfactants with similar structure as oil will stabilize better than only one surfactant. (F. Tadros, *Tharwat*, 2013)

The oil in water in water emulsion must be stabilized too. The interfacial tension in the interphase between the oil in water emulsion and the water phase must be overcome to be a stable emulsion. The agitation can reduce this interfacial tension for a short time but not definitely, so it is convenient to do the gelation of the oil in water in water emulsion. The external gelation process consists on the diffusion of the calcium ion which surrounds the alginate solution.

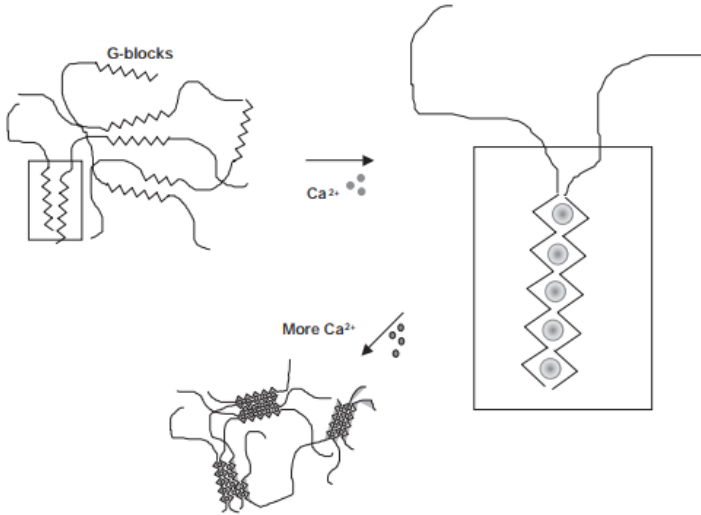


Figure 5. Alginate gelation with calcium ions: the “egg-box model”. (*Fjaereide et al., 2010*)

The formation of the gel starts at the interface because the surface is saturated with calcium ions and it produces that the sodium ion from the alginate is displaced by the calcium cation. Calcium cations usually provide of calcium chloride (CaCl_2), which is the salt with higher percentage of available calcium.

1.7. CHARACTERITZATION OF EMULSIONS

The oil in water in water emulsion is a liquid which becomes more solid because of the gelation process. It is described by viscoelastic behavior. Because of this, it is necessary to study:

- the stationary flow viscosity: the viscosity of the emulsion applying different speed gradients.
- the viscoelastic behavior: the viscoelastic modulus applying different rotation frequency.

Dynamic viscosity describes the tendency of a fluid to resist, by internal friction, the “mutually nonaccelerated laminar displacement of two adjacent layers”. For Newtonian flow, viscosity depends linearly on speed gradient. If not, it is a no Newtonian flow. (*George, Naziem et al., 2018*)

Viscoelastic behavior is characterized by viscoelastic modulus, as stated at 1.1.

A rheometer is the equipment used to measure the rheological properties of materials. This device is capable of measuring viscosity and elasticity of non-Newtonian materials under a wide range of conditions.

Measuring process consists on putting samples between the two parts of the according accessory, such as plate/cone or plate/plate. The top part exerts a rotational shear stress on the sample because of a torque is applied on it. The shear rate is measured. (*Fernandez-Nieves, Alberto; Manuel Puertas, Antonio, 2006*)

2. OBJECTIVES

The objective of this work is to obtain food sauces with an adequate texture for consumer acceptance and with less fat content. To achieve this objective, oil in water in water emulsions (O/W/W) will be studied as alternative to classical oil in water emulsions (O/W). The following tasks will be carried out:

1. Study of oil in water emulsions (O/W). Emulsions of a typical food oil, sunflower oil, in a casein solution will be studied. Different methods of preparation will be tested, and emulsions will be characterized regarding drop size and stability for selecting a good preparation method.
2. Study of oil water in water emulsions (O/W/W). Emulsions of sunflower oil in casein solutions will be emulsified by different methods and resultant emulsions will be characterized to select a good preparation method. Influence stirring and addition of disperse phase will be studied.
3. Study of O/W/W gelation. O/W/W emulsions will be gelled with calcium ion to stabilize the emulsions and achieve the desired rheological behavior. Different amounts of calcium ion will be used, and resultant gelled emulsions will be characterized regarding stationary flow viscosity and viscoelastic behavior.
4. Comparison of rheological behavior of prepared emulsions with commercial sauces. Commercial sauces will be characterized regarding stationary flow viscosity and viscoelastic behavior and will be compared with rheological behavior of prepared emulsions.

3. MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. Components

- Sodium alginate LS (Panreac). Sigma-Aldrich.
- Casein from bovine milk. Sigma-Aldrich.
- Sodium azide: NaN_3 , suitable for prevent the organisms development. Molecular weight: 65.01 g/mol. Sigma-Aldrich.
- Deionized water: H_2O , Milli-Q water (ultra-pure Millipore water system, Milli-Qplus 185 filter) supplied by Merck Millipore.
- Sunflower oil: 5% palmitic acid, 6% stearic acid, 30% oleic acid, 59% linoleic acid. La Masia.
- Span 80 Sorbitan monooleate: $\text{C}_{24}\text{H}_{44}\text{O}_6$, surfactant suitable for stabilization. Molecular weight: 428.60 g/mol. Sigma-Aldrich.
- Tween 85 Polyoxyethylenesorbitan Trioleate: $\text{C}_{60}\text{H}_{108}\text{O}_8 \cdot (\text{C}_2\text{H}_4\text{O})_n$, surfactant suitable for stabilization. Molecular weight: 16.04 g/mol. Sigma-Aldrich.
- Calcium chloride anhydrous: CaCl_2 , $\geq 97.0\%$, suitable for stabilization. Molecular weight: 110.98 g/mol. Sigma-Aldrich.
- Mayonnaise sauce: 64% soybean oil, water, 5% egg yolk (barn), 5% white wine vinegar (6% acid), sugar, salt, modified corn starch, lemon juice concentrate, antioxidant (calcium disodium EDTA (E385)), paprika extract, color carotenes (E165a). Hellmann's.
- Fluorescein 5(6)-isothiocyanate (FITC): $\text{C}_{21}\text{H}_{11}\text{NO}_5\text{S}$, suitable for fluorescence, mixture of 2 components. Molecular weight: 389.38 g/mol. Sigma-Aldrich.

- Dimethyl sulfoxide (DMSO): $(\text{CH}_3)_2\text{SO}$ ACS reagent. Molecular weight 78.13 g/mol. Merk-KGaA.
- Sudan III: $\text{C}_{22}\text{H}_{16}\text{N}_4\text{O}$, suitable for observing the color-change phenomenon on nonpolar substances, such as oils, fats and greases. Molecular weight: 352.39 g/mol. Sigma-Aldrich.
- Rhodamine B isothiocyanate: $\text{C}_{29}\text{H}_{30}\text{ClN}_3\text{O}_3\text{S}$, suitable for protein labelling. Molecular weight: 536.08 g/mol. Sigma-Aldrich.

3.1.2. Equipment

- Analytical balance Mettler Toledo AB204-S/FACT. Maximum: 220g
- Optical microscope with digital camera connected to computer which has Motic Images Plus 2.0 software.
- Turbiscan MA 2000. This dispositive allows to study the stability of concentrated dispersions through the process of Backscattering. The equipment measures the intensity of the light that the dispersion transmits and reflects, and it measures the changes produced over time.
- Basics magnetic stirrer hot plate Heidolph MR Hei-Standard with temperature control by means of Heidolph EKT3001 probe (maximum stirring capacity: 1500 rpm).
- pH-meter Mettler Toledo Seven Easy.
- Thermostatic bath
- Ultra Turrax T.25 basic IKA-WERKE. It is a high frequency homogenizer for volumes from 1 to 2000 mL with speed indicator. His range of speeds is from 3000 to 25000 rpm.
- Mastersizer 2000 Malvern Panalytical (UK) with a dispersion unit Hydro 2000G.
- Olympus BX51 optical and fluorescence microscope. Images taken by interferential contrast technology with Nomarski prism. The microscope is interfaced to an Olympus DP73 camera connected to computer which has Stream Essentials Olympus software.

- Thermo Scientific, Haake Mars III. Modular Advanced Rheometer System. The software used for this study is RheoWin DataManager and RheoWin JobManager and the cone/plate (4°/60 mm) sensor.

3.2. PREPARATION OF EMULSIONS

3.2.1. Preparation of polymer rich phases

Casein stock solution is prepared by weighing casein from bovine milk powder on an analytical balance according to the desired concentration, which is 10% by weight. The powder is transferred to a beaker and then the deionized water is added to prepare the solution. It is recommended to add a part of the water before adding the powder to facilitate the homogenization of the solution. It is important to not add all the water because afterwards it will be necessary to adjust the pH adding sodium hydroxide solution. Sodium azide is added to the solution by weighing on an analytical balance according to the desired concentration, which is 0.04% by weight, and adding it to the solution. The solution is mixed using a magnetic stirrer at a 700 rpm and at atmospheric temperature for 24 hours.

Alginate stock solution is prepared by weighing sodium alginate powder on an analytical balance according to the desired concentration, which is 2% by weight. The powder is transferred to a beaker and then the deionized water is added to prepare the solution. Sodium azide is added to the solution by weighing on an analytical balance according to the desired concentration, which is 0.04% by weight, and adding it to the solution. The solution is homogenized using an Ultra-Turrax stirrer at 15000 rpm and at atmospheric temperature for 5 minutes. Then, it is stored for 24 hours inside the refrigerator to allow it to hydrate and eliminate all the air bubbles formed because of the agitation.

The concentration of the solutions is determined by a tie line studied on intern research group documents.

Although sodium azide is added to both solutions, these are deteriorated after a week approximately. So, it would be necessary to prepare these solutions again.

To adjust the pH at value of 8.0, the needed amount of sodium hydroxide solution 1M is added to each of solutions.

Then, both solutions are added in respective mass proportions to centrifugate tubes, taking care that all the tubes must weigh the same. These tubes are submerged in water in a thermostatic bath fixed at 25 °C to produce the phase separation. The separation process to obtain casein rich phase and alginate rich phase is carried out with Pasteur pipette and every solution is stocked in beakers.

3.2.2. Preparation of oil in water emulsion

It is necessary to stabilize the emulsion adding a surfactant with an HLB of 10, mixing Tween 85 and Span 80.

The sunflower oil is weighed on an analytical balance, according to a 20% by weight. The oil in water emulsion is prepared by adding the oil with a Pasteur pipette, drop by drop, for 5 minutes, while it is on agitation. This emulsion is left to repose to de-aerate it for 24 hours.

3.2.3. Preparation of oil in water in water emulsion

The oil in water in water emulsion is prepared by weighing the according quantitate of alginate rich phase and, separately, by weighing the according quantitate of the oil in casein emulsion, which is 20% by weight. This emulsion is added on the alginate rich phase with a Pasteur pipette, drop by drop, for 5 minutes, while it is on agitation.

Total amount of oil in oil in water in water emulsion is 4% by weight.

3.3. DROP SIZE MEASUREMENT

The drop size characteristic of each emulsion is determined by the observation with microscope by differential interference contrast technic with Nomarski prism. In DIC technic, the illumination starts as a plane polarized beam of light. This is divided into two beams with different polarization angles separated horizontally by a distance roughly equal to the resolution of the objective lens. These two beams pass through the sample and changes in refractive index are produced due to the structure of the specimen. After passing through the sample and the objective lens, the horizontal separation between the beams is removed and the two beams have the same polarization angle. In the microscope's intermediate image plane the beams interfere to produce an amplitude contrast which reflects the optical path difference between the

two beams produced by the sample. The differential in DIC is the difference in optical path length of the two beams.

Also, the drop size distribution is determined with laser diffraction by using a *mastersizer*. A monochromatic beam strikes on a continuous flow of particles. The particles, which minimum size is like the wavelength, diffract the visible light. The light is dispersed to different angles and different intensities according to the particles size. Between the detector and the sample there is a lens which focalize the diffracted light on a detector point. The higher diffraction angle is, the smaller drop size is. The drop size distribution it is calculated by applying Mie and Fraunhofer theories, which considers that the particles are spherical also considers the dispersion and the absorption of part of the radiation incident on a particle. When the sample is an emulsion it is necessary to know the refraction index of both phases to applicate the Mie Theory. (*Research group documents proportioned by CSIC*)

Another method used to determine drop size is fluorescence microscopy. The sample is excited with light of the wavelength that is absorbed by the fluorophores and these emit light of longer wavelengths with a different color. A spectral emission filter separates the illumination light from the emitted fluorescence. In this case, the casein shines front to oily phase and alginate, which seems black.

Fluorescein isothiocyanate (FITC) is the original fluorescein molecule. It is orange and his absorption maximum value is at 495 nm. While excitation, it emits a yellow-green color with an emission maximum at 525 nm. (*Sigma-Aldrich*) It is necessary to save FITC and DMSO with refrigeration. At the time to prepare the sample, DMSO is at ambient temperature and it becomes liquid. With a micrometer, 500 μL of DMSO are added to the vial which contains FITC. 1 μL of FITC is added on casein solution for each solution milliliter. This sample is analyzed with a fluorescence microscopy.

3.4. STABILITY CHARACTERIZATION

The stability of the emulsion is characterized by using the Turbiscan, which analyses the stability by backscattering process. A part of the emitted light passes through the tube and the most part are scattered by the particles in the sample. The equipment detects the intensity of the both beams of light and it permits direct monitoring of local physical heterogeneities. By this

method, it is possible to study the destabilization process and the stability of the sample over time.

The measurement principle of the Turbiscan range is based on multiple light scattering: the backscattered light by the sample, which depends on the diameter of the particles, their volume fraction and the relative refractive index between the dispersed and continuous phases, and the transmitted light through the sample.

The analyzed emulsion is introduced on a Turbiscan tube with a Pasteur pipette, taking care that the sample cannot touch the wall tube. The tube must be introduced on the equipment at the same position every time.

3.5. STABILIZATION PROCESS

The sunflower oil in casein in alginate emulsion is stabilized by gelation.

Gelling solution is prepared by weighing calcium chloride powder on an analytical balance according to the desired concentration. The powder is transferred to a volumetric flask and then the deionized water is added to the mark. The flask is held and it is agitated manually since the calcium chloride has been dissolved.

To gel the emulsion, it is set on agitation using a magnetic stirrer at a 600 rpm and at atmospheric temperature and the according quantitate of gelling solution is added on the surface of the emulsion, drop by drop, with a Pasteur pipette. After 5 minutes, the magnetic stirrer is turned off.

3.6. RHEOLOGICAL CHARACTERIZATION

The rheological characterization is carried out with the HAAKE-MARS rheometer with a cone/plate sensor: the cone has an inclination of 4 degrees and the plate has 60 mm of diameter. The rheometer is connected to a thermostatic bath which maintains a constant temperature of 25°.

The characteristics parameters of the viscoelastic behavior are the modulus G' and G'' , as stated at 1.1.

The sample is put on the plate and the cone descends up to the sample. The software determines the position "zero" of measures.

At first, it is necessary to measure the shear stress threshold. When this value is exceeded, the fluid cannot return to his initial state. After this, the tests executed are:

- the stationary test: this analyze the stationary flow viscosity, so the rheometer measures the viscosity of the sample applying different speed gradients. The equipment is programmed to applicate a speed gradient of 0.1 s^{-1} for 3 minutes to distribute evenly the sample. Then, it is applicated a speed gradient of 0.01 to 500 s^{-1} and the values of viscosity are registered.
- the oscillatory test: this analyze the viscoelastic behavior, so the rheometer measures the viscoelastic modulus applying different rotation frequency. The equipment is programmed to produce a rotation with frequencies of 0.01 to 10 Hz and to register the values of viscoelastic modulus.

4. STUDY OF OIL IN WATER EMULSIONS (O/W)

4.1. PRELIMINARY STUDY OF THE PREPARATION OF OIL IN WATER EMULSIONS

4.1.1. Election of the composition of casein and alginate to prepare casein rich phase and alginate rich phases in equilibrium

Casein stock solution is prepared as stated at 3.2.1. A little quantitate of Rhodamine B isothiocyanate is added to be able to observe better the phase separation, because this colorant tint proteins (casein).

Alginate stock solution is prepared as stated at 3.2.1.

To adjust the pH at value of 8.0, an according quantitate of sodium hydroxide solution 1M is added to both solutions. To adjust casein solution, it is necessary to add approximately a 0.1% of the solution and to adjust alginate solution, it is necessary to add approximately a 0.04% of the solution.

Both solutions are added in equal mass proportions to 8 centrifugate tubes, taking care that all the tubes must weigh the same. The studied proportions are shown in table 1:

Table 1. Proportions of the solutions to study which is the adequate.

% mass alginate	20	30	40	50	60	70	80	90
% mass casein	80	70	60	50	40	30	20	10

These tubs are submerged in water in a thermostatic bath with a temperature of 25°C for 24 hours.

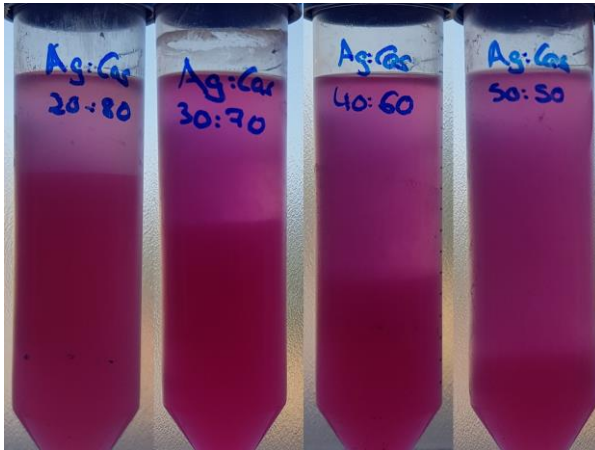


Figure 6. Phase separation of alginate solution and casein solution.

On Figure 6, phase separation of alginate solution and casein solution by different proportions is shown. The phase proportion chosen is 50:50 (or 1:1) because it allows to obtain more alginate than casein, but casein is obtained too. The separation process to obtain casein rich phase and alginate rich phase is carried out with Pasteur pipette and every solution is stocked in beakers.

4.1.2. Preparation of the emulsion by different methods

The sunflower oil is weighed on an analytical balance and it is tinted with a little quantitate of sudan III to facilitate the observation of oil drops. The necessary quantity of oil is a 20% on weigh of the total weigh emulsion. The oil in water emulsion is prepared by adding the oil with a Pasteur pipette, drop by drop, for 5 minutes. This addition is made on three different emulsion process:

- using a magnetic stirrer at a 700 rpm
- using Ultra Turrax at 11000 rpm
- using Ultra Turrax at 22000 rpm

The resultant emulsions are observed on optical microscope and the three samples are analyzed by the Mastersizer to obtain the drop size distribution. Also, the stability of these emulsions is analyzed by Turbiscan (backscattering process).

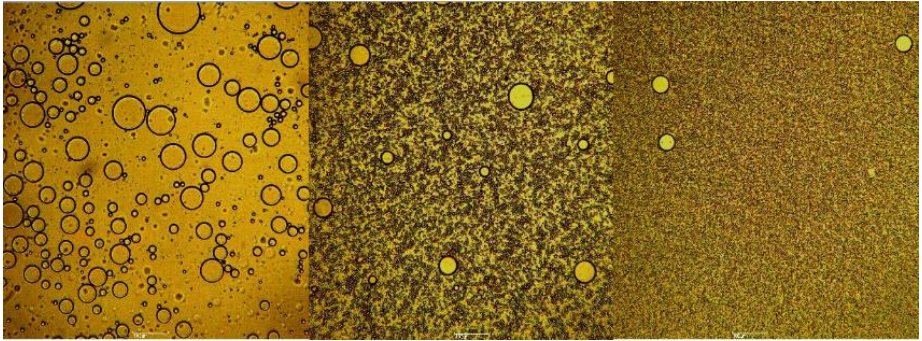


Figure 7. Microscope picture of the o/w emulsion homogenized by using (from left to right) magnetic stirrer 700 rpm 40x – Ultra Turrax 11000 rpm 40x – Ultra Turrax 22000 rpm 40x.

The scale line equivaless at 10 μm .

On Figure 7 it is possible to see air bubbles on all the emulsions and a wide variety of drop size.

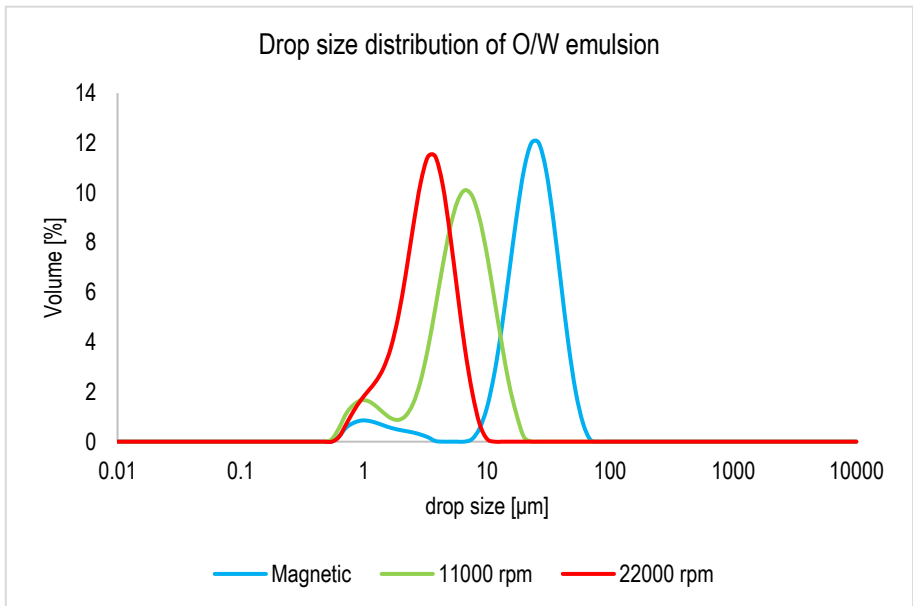


Figure 8. Drop size distribution obtained by using Mastersizer of the o/w emulsion homogenized by using magnetic stirrer 700 rpm – Ultra Turrax 11000 rpm – Ultra Turrax 22000 rpm

The best way to prepare sunflower oil in casein emulsion is by using the Ultra Turrax at a rotation speed of 22000 rpm because the two other methods produces a little spike with smallest drop size than the other spike. Selecting the method by using Ultra Turrax at 22000 rpm the distribution of the drop size is more homogeneous.

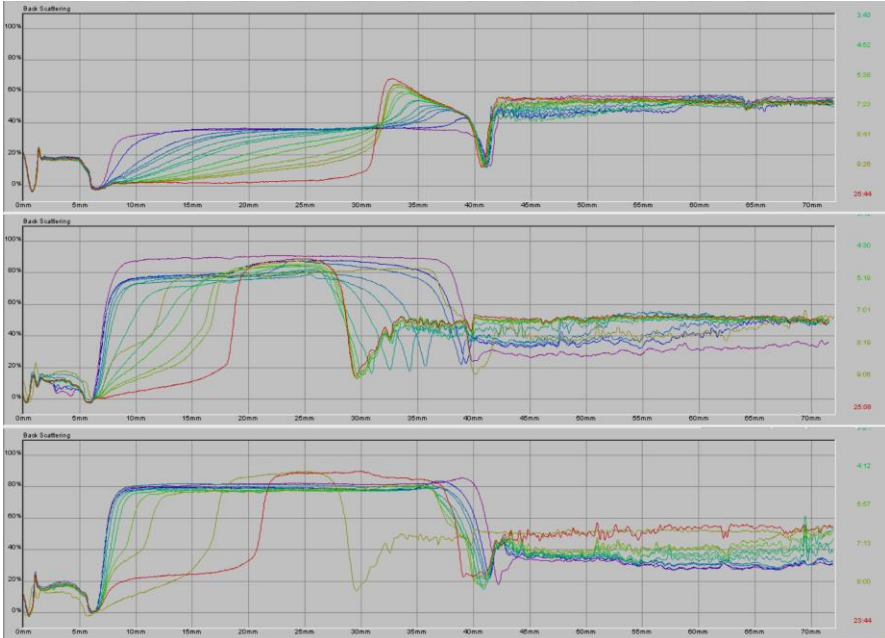


Figure 9. Backscattering pictures obtained by using Turbiscan of the o/w emulsion homogenized by using (from top to bottom) magnetic stirrer 700 rpm – Ultra Turrax 11000 rpm – Ultra Turrax 22000 rpm.

The results of the backscattering study represented on Figure 9 show that the oil in water emulsion prepared by agitation at 22000 rpm is the most stable emulsion until 7 hours. So, their stability study also concludes that the best way to prepare the sunflower oil in casein emulsion is by using Ultra Turrax at 22000 rpm.

The changes on the top of the tube (at 40 mm approximately) are because of the disappearance of emulsion foam. This foam is formed because there are air bubbles in the emulsion.

The changes on the bottom of the tub (at 7 mm approximately) are produced because of destabilization phenomena. The oily phase detaches from the casein phase displacing to the top by sedimentation. Also, the results of the backscattering study shows that air bubbles

ascend from bottom to top. For this reason, backscattering process shows a change from 7 to 40 mm approximately. It is not necessary to use Ultra Turrax to prepare the emulsion because the interfacial tension on these emulsions is lower. So, the best way to prepare the sunflower oil in casein in alginate emulsion is by using magnetic agitation at 700 rpm.

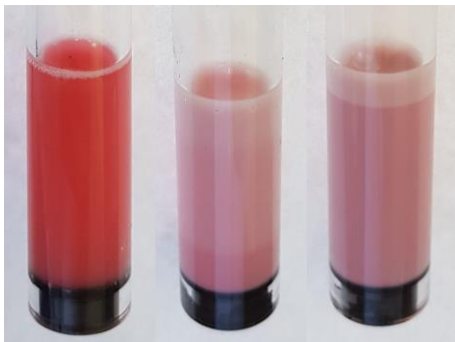


Figure 10. Oil in water emulsion at 0 hours prepared by using (from left to right) magnetic stirrer at 700 rpm – Ultra Turrax at 11000 rpm – Ultra Turrax at 22000 rpm.

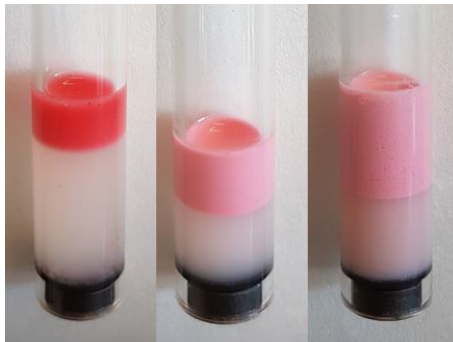


Figure 11. Oil in water emulsion at 24 hours prepared by using (from left to right) magnetic stirrer at 700 rpm – Ultra Turrax at 11000 rpm – Ultra Turrax at 22000 rpm.

The pictures show that the air bubbles disappear from the emulsion, because the occupied part of the tube is decreased from the initial state to 24 hours later.

Oily phase is rose-red because sunflower oil is colored with Sudan III to facilitate microscope observation.

On figure 10, foam formed by the additional air introduced by Ultra Turrax is evident.

Also, it is possible to observe the phase separation produced by sedimentation phenomena. Oily phase is on the top and casein phase is on the bottom.

To verify that the emulsions have air bubbles, the density of this emulsion has been determinate, and it is $0.80 \text{ g}\cdot\text{mL}^{-1}$. It shows that the emulsion has air bubbles because it is constituted largely from water, which density is $1.00 \text{ g}\cdot\text{mL}^{-1}$. To de-aerate it, the emulsion is left covered for 24 hours, when their density is $1.00 \text{ g}\cdot\text{mL}^{-1}$.

4.1.3. Stabilization of the emulsion by using surfactants

The stability emulsion at 24 hours is not the desired so it is necessary to add a surfactant with an according value of HLB to reduce the interfacial tension between phases. For this case, the HLB value is 10. (*Grubenmann, Arnold; Mollet, Hans, 2001*)

HLB value is obtained by mixing two surfactants: Span 80 and Tween 85. These surfactants have an HLB value of 4.3 and 11 respectively. (*Sigma-Aldrich*)

To prepare the surfactant mixture, an according quantity of Span 80 is weighted by analytical balance. For Tween 85, this process is the same. These surfactants are liquid at atmospheric conditions so, to weight them, they are put on a small beaker.

Resultant solution has 85% of Tween 85 and 15% of Span 80.

To conserve it, the mixture is saved in a small covered bucket.

Surfactant mixture is added on oil in water emulsion with a Pasteur pipette on different proportions to determine which is the most convenient to maintain the stability more than 24 hours.

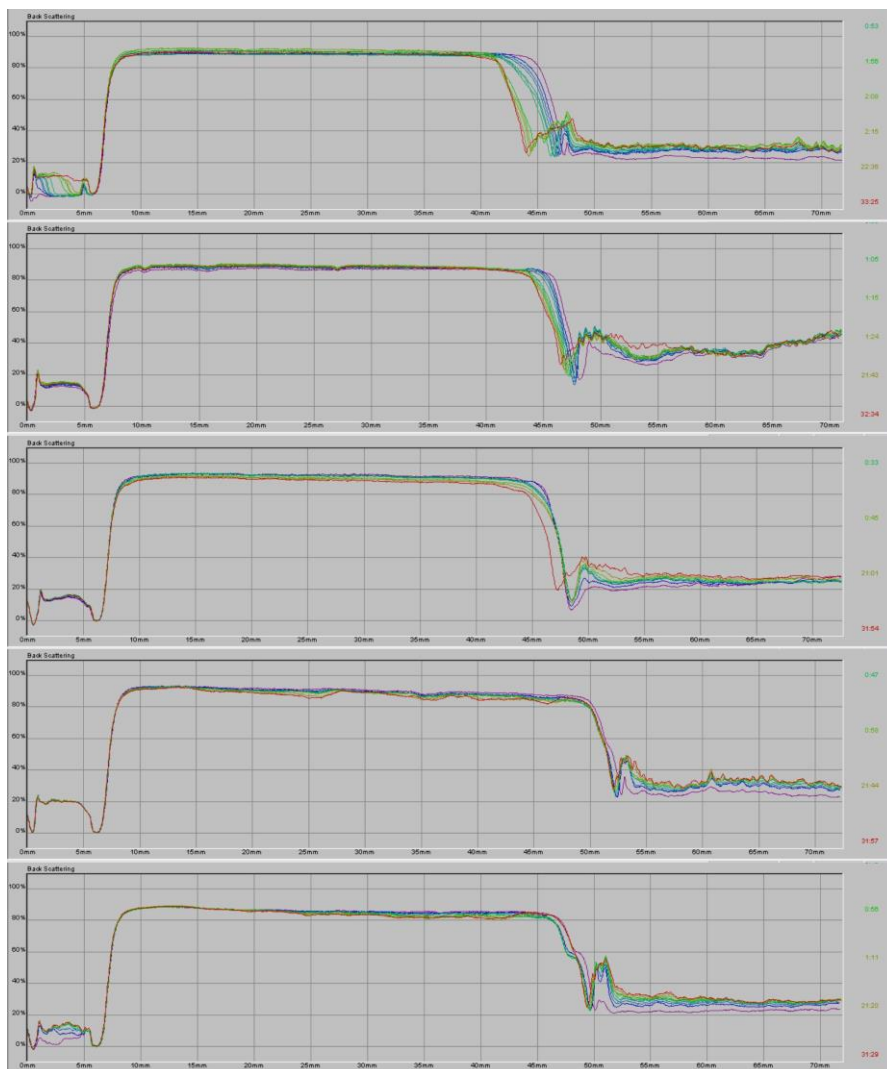


Figure 12. Backscattering pictures obtained by using Turbiscan adding surfactant to the oil in water emulsion on proportions (from top to bottom) 0.25% - 0.50% - 1.00% - 1.50% - 2.00%.

The backscattering study represented on Figure 12 shows that the emulsion with 0.50% of surfactant solution is enough to maintain the stability more than 33 hours.

De-aerating the emulsion and by adding surfactant solution, there is not any destabilization phenomena, for the studied time.

4.2. PREPARATION AND CHARACTERIZATION OF THE OIL IN WATER EMULSIONS

4.2.1. Drop size

To determine the drop size of the sunflower oil emulsified on casein, the sample has been observed by differential contrast microscopy and fluorescence microscopy.

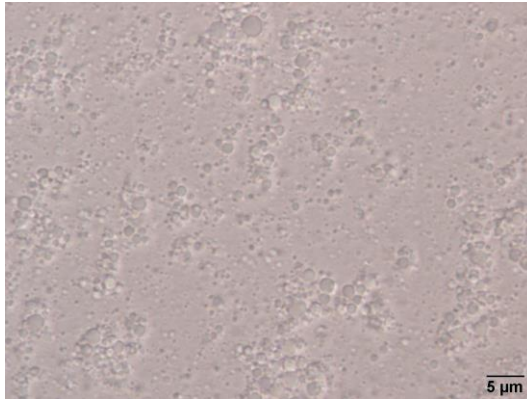


Figure 13. Differential contrast microscopy picture of the oil in water emulsion.

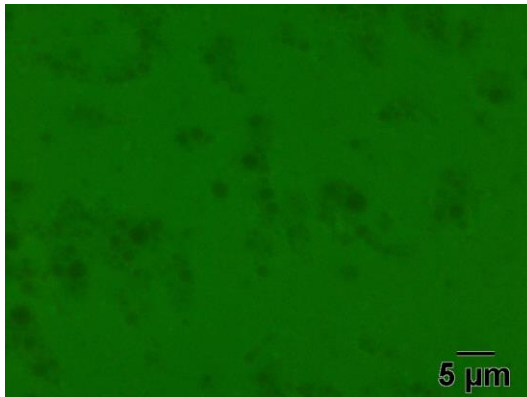


Figure 14. Fluorescence microscopy picture of the oil in water emulsion.

On figures 13 and 14, it is observed that there are not an only drop size of oil, but there is a drop size distribution which corresponds to the determined by Mastersizer (Figure 7).

Fluorescence picture is not very clear, but it is possible to watch different size drops in the sample. The dark drops are sunflower oil and the light background is casein.

The mean diameter of these drops is 3.224 μm . This drop size corresponds to a microemulsion.

4.2.2. Stability of the emulsion

To determine the stability of the emulsion, a sample of it is analyzed by using turbiscan, with backscattering method.

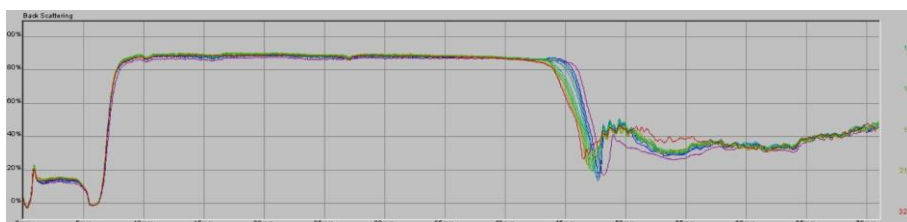


Figure 15. Backscattering picture obtained by using Turbiscan – sunflower oil in casein emulsion prepared with Ultra Turrax at 22000 rpm and by adding 0.50% of surfactant solution.

Figure 15 shows that the emulsion is stable until 33 hours.

5. STUDY OF OIL IN WATER IN WATER EMULSIONS (O/W/W)

5.1. PRELIMINARY STUDY OF THE PREPARATION OF OIL IN WATER IN WATER EMULSIONS

5.1.1. Preparation of the emulsion by different methods

Alginate stock solution is prepared as stated at 3.2.1.

To adjust the pH at value of 8.0, an according quantitate of sodium hydroxide solution 1M is added to alginate solutions. It is necessary to add a 0.04% of the solution approximated.

A little quantitate of Sudan III is added to be able to observe better the oil drops, because this colorant tint non-polar substances, like oils.

Sunflower oil in casein in alginate emulsion is prepared as stated at 3.2.3.

The addition of the oil in water emulsion on alginate is made on two different emulsion process:

- using a magnetic stirrer at a 700 rpm
- using Ultra Turrax at 22000 rpm

The resultant emulsions are observed on optical microscope and their stability is analyzed by Turbiscan (backscattering process). It is not possible to analyze the drop size distribution on Mastersizer because the sample is too opaque.

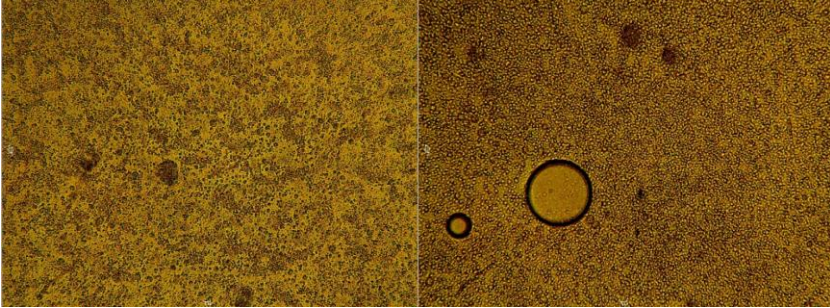


Figure 16. Microscope picture of the o/w/w emulsion homogenized by using (from left to right) magnetic stirrer 700 rpm 40x – Ultra Turrax 22000 rpm 40x. The scale line equivaless at 10 μm .

The drop size is very small on both samples and the emulsion prepared with Ultra Turrax has air bubbles, so it is preferable to use magnetic agitation at 700 rpm.

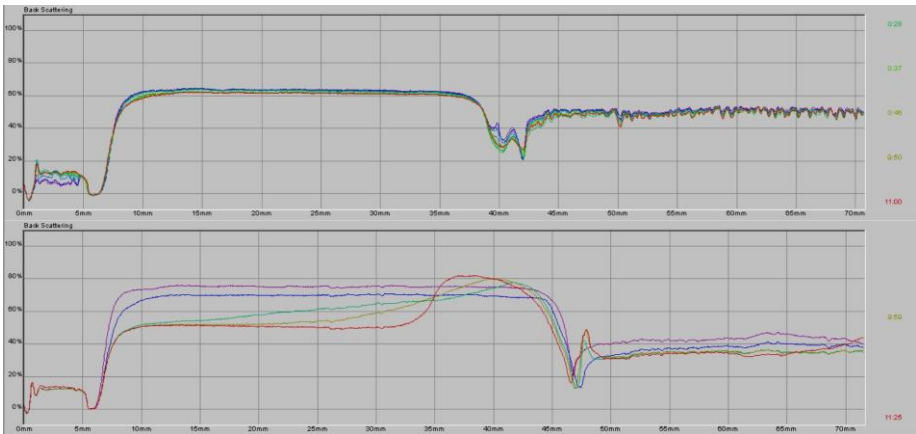


Figure 17. Backscattering pictures obtained by using Turbiscan of the o/w/w emulsion homogenized by using (from top to bottom) magnetic stirrer 700 rpm – Ultra Turrax 22000 rpm.

The results of the backscattering study shows that the emulsion prepared by using Ultra Turrax is not stable because this equipment introduces air. These air bubbles ascend from bottom to top. For this reason, backscattering process shows a change from 7 to 35 mm approximately. From 35 to 50 mm there also are air but shaped like foam. It is possible to observe on the backscattering study, from 50 mm to 30 mm, the sample is destabilized because the air leaves it. It is not necessary to use Ultra Turrax to prepare the emulsion because the

interfacial tension on these emulsions is lower. So, the best way to prepare the sunflower oil in casein in alginate emulsion is by using magnetic agitation at 700 rpm.

5.2. PREPARATION AND CHARACTERIZATION OF THE OIL IN WATER IN WATER EMULSIONS

5.2.1. Drop size

To determine the drop size of the sunflower oil in casein in alginate emulsion, the sample has been observed by differential contrast microscopy and fluorescence microscopy.

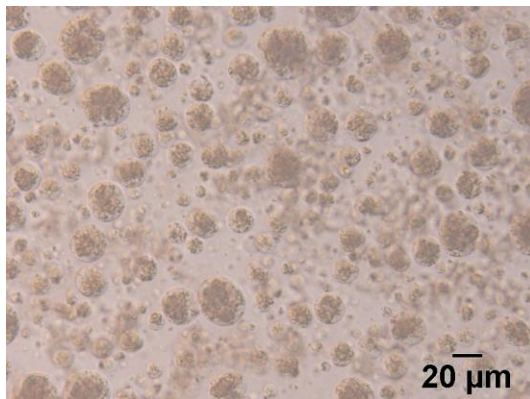


Figure 18. Differential contrast microscopy picture of the oil in water in water emulsion.

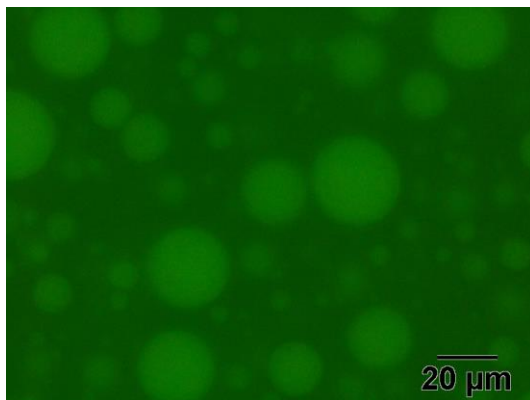


Figure 19. Fluorescence microscopy picture of the oil in water in water emulsion.

Is observed that there is not an only drop size, but it is more homogeneous than the drop size distribution of oil in water emulsion (Figure 13).

Fluorescence picture is not very clear, but it allows observe that there is not only drop size. The light drops are casein, which contains sunflower oil, but it is difficult to observe. And the dark background is alginate.

The oil in water emulsion drop size is between 15 to 25 μm .

5.2.2. Stability of the emulsion

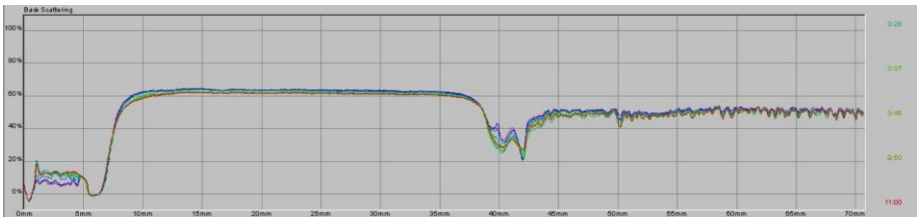


Figure 20. Backscattering picture obtained by using Turbiscan – sunflower oil in casein in alginate emulsion prepared with magnetic stirrer at 700 rpm.

Figure 20 shows that for 11 hours, the emulsion prepared by magnetic agitation at 700 rpm is sufficiently stable to conclude that it will be stable for a long time.

At the top of tube there is not instabilities, so the here is not air bubbles. Also, at the bottom of tube there is not instabilities, so the emulsion is stable.

6. STUDY OF OIL IN WATER IN WATER EMULSIONS GELATION

To stabilize the sunflower oil in casein in alginate a sodium chloride solution is added on the emulsion. The preparation process of gelation solution and her addition is carried out as stated at 3.5.

At first, preliminary tests serve to prove if the gelation is not only carried out on the surface. To prepare it, a sample of the oil in water in water emulsion is put on a turbiscan tube. On the surface of the emulsion, 2 mL of gelation solution with 1% of calcium chloride are added with a Pasteur pipette.

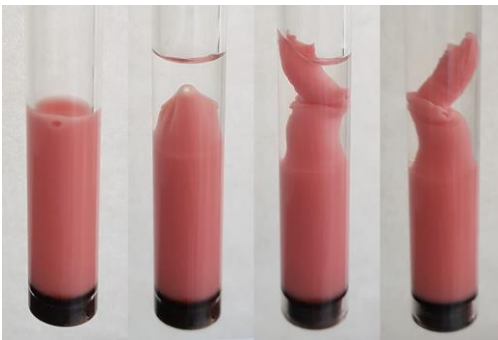


Figure 21. Gelation emulsion evolution – preliminary test.



Figure 22. Emulsion gelled - preliminary test.

The addition of the gelation solution produces that the emulsion starts to go up. When the gel arrives to the top, it stops to go up since a little quantity of calcium chloride solution is added on it. Then, emulsion starts to go up again.

24 hours later, the sample is put out of the tube and, as it is possible to see on Figure 22, the emulsion is completely gelled.

Another preliminary test with small samples consists on the addition of gelation solution of the emulsion with different proportions. The problem is that the quantity of water could not be maintained constant on different samples.



Figure 23. Gelation test with different gelation solutions with mass percentages, from left to right, of 0.25%, 0.50%, 0.75% and 1.00%.

All solutions are gelled, and they have the same behavior than the samples on Figure 21.

Emulsions are yellowish because of the addition of fluorescein to oil in water emulsion.

Calcium chloride solution is added on the emulsion on different proportions. Four solutions are prepared as stated on 3.5. in the way that 10 mL of gelation solution are added on 30 mL of oil in water in water emulsion. The quantity of water added on the samples is the same.

The proportions are prepared to obtain determined concentrations of calcium chloride on the emulsion (see table 2).

Table 2. Mass proportions and concentrations on the emulsion of calcium chloride for every emulsion.

Emulsion name	Emulsion A	Emulsion B	Emulsion C	Emulsion D
Concentration of CaCl_2 [mM]	6	7	8	9
Mass percentage of CaCl_2 (%)	0.0027	0.0031	0.0036	0.0040

The gelation solution is added with Pasteur pipette on the surface of the emulsion while it is on magnetic agitation at 600 rpm to homogenize it and gelify all the emulsion homogeneously.

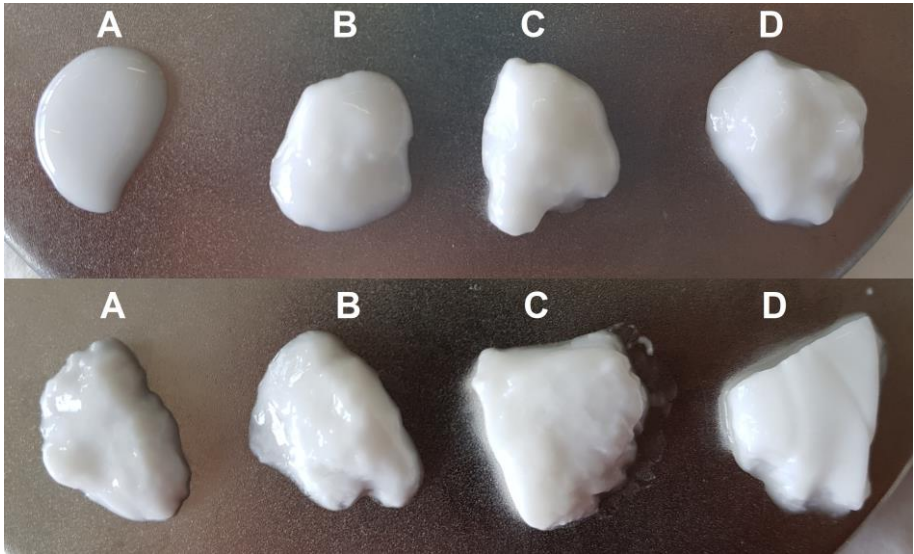


Figure 24. Gelled emulsion at 0 hours (on the top) and at 24 hours (on the bottom).

Figure 24 shows that at initial time, emulsion A is quite liquid, without lumps. It is not gelled. All these emulsions are opaque with a whiteness color.

Emulsion B has some lumps. At this concentration, the emulsion starts to be gelled.

Emulsions C and D seem similar. Both are gel, but emulsion D has some lumps, it is not homogeneous.

At 24 hours, emulsion A is gel and the others are more gelled than at 0 hours, but the change is not such significant.

7. COMPARISON OF RHEOLOGICAL BEHAVIOUR OF PREPARED EMULSIONS WITH COMMERCIAL SAUCES

7.1. STATIONARY TEST

To determine the shear stress applied on the stationary tests, it is necessary to carry out a shear stress sweep.

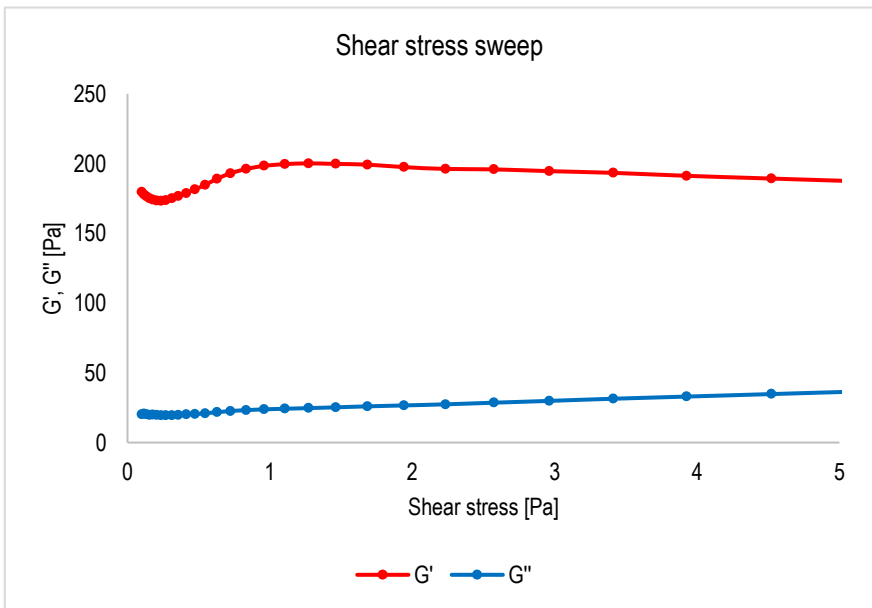


Figure 25. Shear stress sweep with emulsion D (with a calcium chloride concentration 9 mM).

Figure 25 shows shear stress sweep test carried out with an emulsion D sample. The shear stress selected is 1 Pa, but it is valid choosing another shear stress, while the moduli are quite constant.

The stationary test is carried out 5 minutes after the emulsion gelation, at 24 hours and at 96 hours.

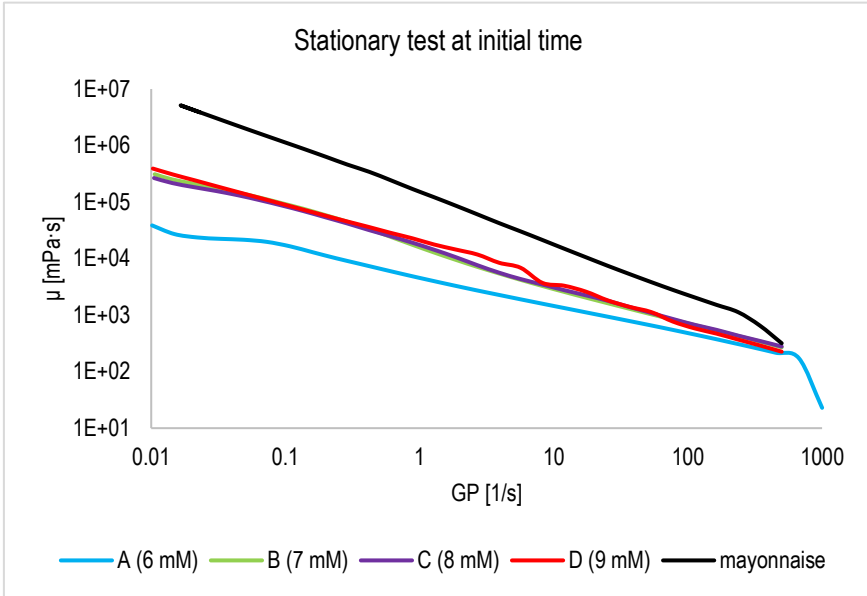


Figure 26. Stationary test of gelled emulsions at initial time (5 minutes).

Figure 26 shows that the emulsion leaves the equipment because of the high-speed gradient. Because of it, the next tests are executed with a speed gradient from 0.01 to 500 s^{-1} , not to 1000 s^{-1} .

All emulsions have the same behaviour, but emulsion A has viscosity values quite lower than the other, for low speed gradients. Viscosity values for emulsions B, C and D are practically overlapping, so have viscosity values very similar. Their viscosity is higher than emulsion A viscosity, but it remains lower than mayonnaise viscosity.

As speed gradients are becoming increasingly, viscosity decreases. At speed gradient of 500 s^{-1} , emulsions B and C and mayonnaise sauce have a similar value of viscosity, see table 3.

Table 3. Viscosity values for emulsions and mayonnaise at a speed gradient of 500 s^{-1} – at initial time.

	Emulsion A	Emulsion B	Emulsion C	Emulsion D	Mayonnaise
μ [mPa·s]	206	270	278	225	317

Emulsion A is not completely gelled, so has lower viscosity values; but D emulsion has strange behaviour. His viscosity should be higher. It may be due to a preparation error by a human fault because the sample is put on the plate 5 minutes of the agitation stop. These 5 minutes not include setting the sample on the plate and it would be a little different. Also, this sample has lumps, it is not homogeneous. This may be the source of the change on the viscosity tendency.

No emulsion achieves mayonnaise viscosity value.

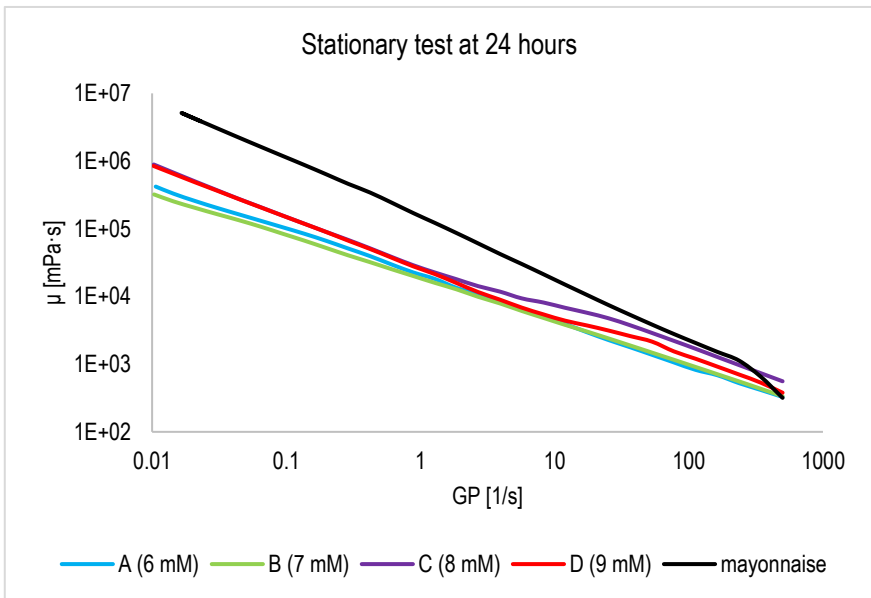


Figure 27. Stationary test of jellied emulsions at 24 hours.

Figure 27 shows that at 24 hours of gelation, viscosity values have increased but the tendency is the same. The irregularity of the D emulsion is also present at 24 hours. The viscosity value of emulsion C is much higher than other samples.

All the samples overcome mayonnaise viscosity value at a determined speed gradient.

Table 4. Viscosity values for every emulsion and mayonnaise at a speed gradient of 500 s^{-1} – at 24 hours.

	Emulsion A	Emulsion B	Emulsion C	Emulsion D	Mayonnaise
μ [mPa·s]	327	335	558	378	317

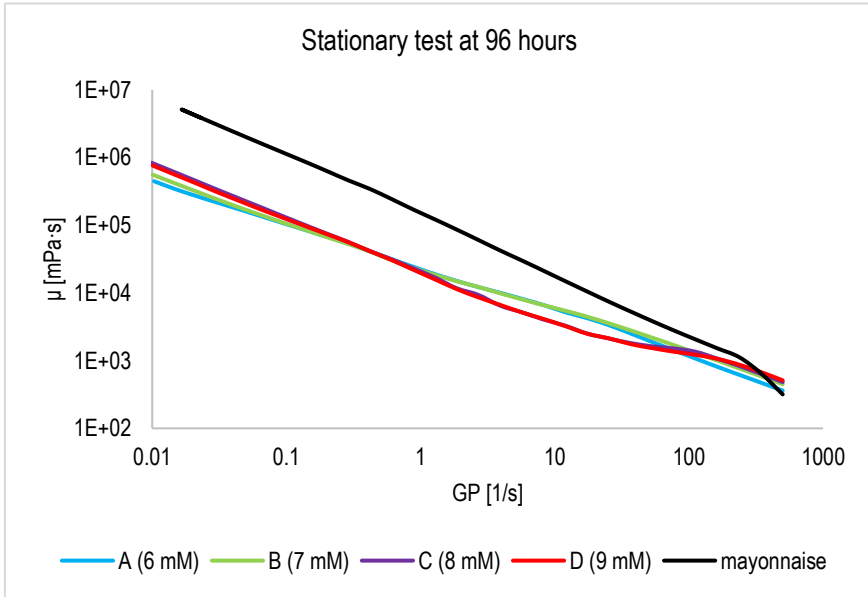


Figure 28. Stationary test of jellied emulsions at 96 hours.

Figure 28 shows that at 96 hours of gelation, viscosity values have increased but the tendency is the same. It seems that the viscosity tendency with the speed gradient it is the same. Letting the samples rest for 96 hours, the viscosity increases when the concentration of gelation solution increases. The irregularities of the emulsions C and D disappear. All the viscosity values overcome mayonnaise's, at speed gradient values lower than 500 s^{-1} .

Table 5. Viscosity values for every emulsion and mayonnaise at a speed gradient of 500 s^{-1} – at 96 hours.

	Emulsion A	Emulsion B	Emulsion C	Emulsion D	Mayonnaise
μ [mPa·s]	360	449	482	511	317

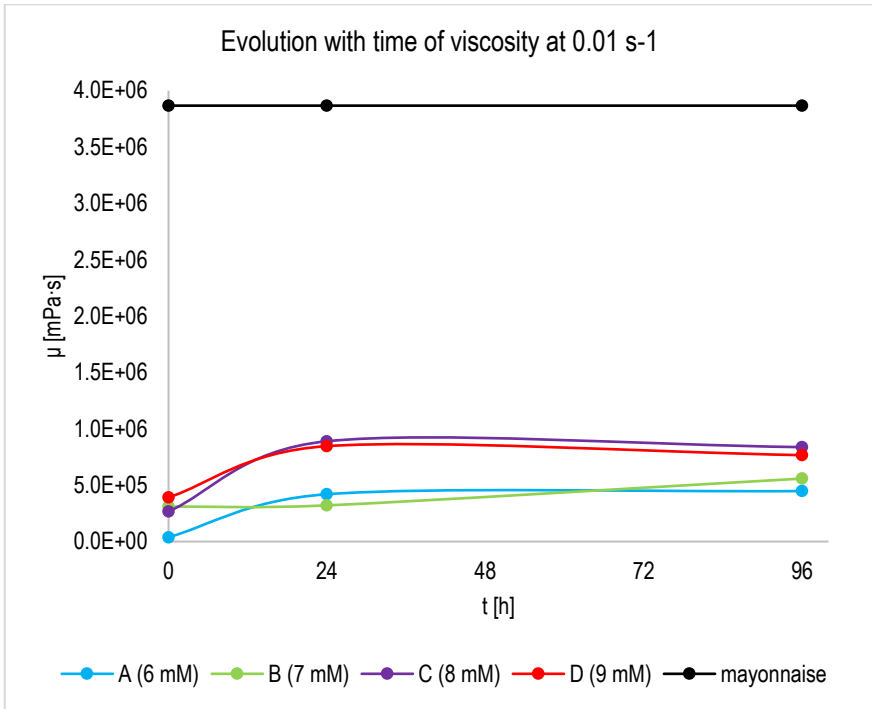


Figure 29. Stationary test of jellied emulsions with a speed gradient of 0.01 s^{-1} .

Figure 29 shows that at initial time, no emulsions achieve mayonnaise viscosity value. Emulsions A, C and D viscosity changes from initial time to 24 hours and then this value is quite constant. Emulsion B viscosity changes little from initial time to 24 hours and then it increases for 96 hours.

The viscosity behaviour of emulsions C and D are very similar, and emulsion D viscosity at the end of the test (96 hours) is higher to emulsion C viscosity.

Viscosity values of the emulsions are between $3 \cdot 10^4$ and $9 \cdot 10^5 \text{ mPa} \cdot \text{s}$ (see Figure 44 on Annex 2) and viscosity value of mayonnaise is above $3,9 \cdot 10^6 \text{ mPa} \cdot \text{s}$.

To increase viscosity values, the volume fraction of disperse phase could be increased. It would be adding sunflower oil in casein emulsion to alginate.

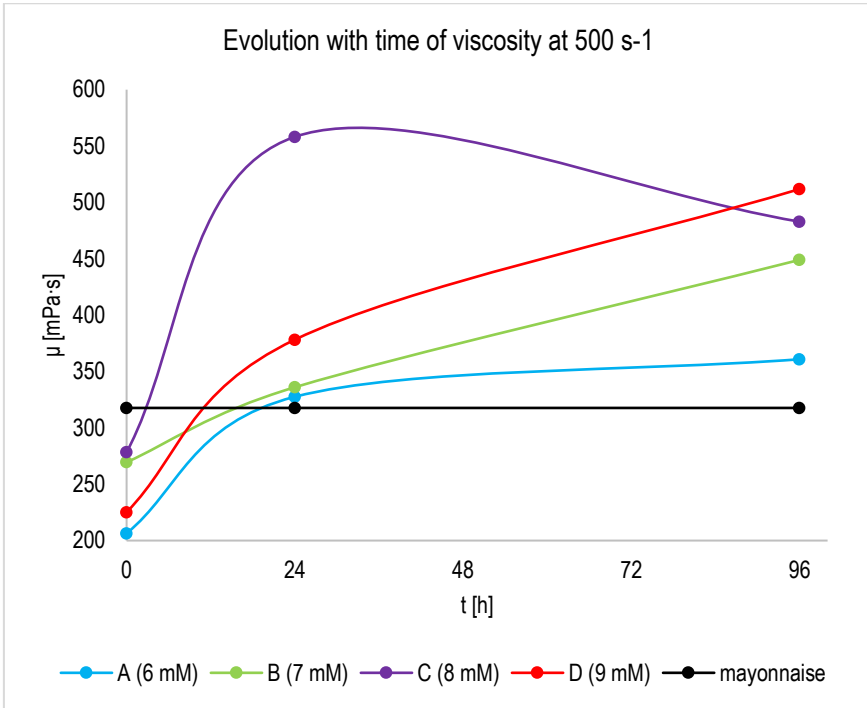


Figure 30. Stationary test of jellied emulsions with a speed gradient of 500 s^{-1} .

Figure 30 shows that at initial time, no emulsions achieve mayonnaise viscosity value but at 24 hours, all the emulsions do it.

Emulsion A viscosity increases from initial time to 24 hours and then this value continues increasing but less.

Emulsion B viscosity increase quite constant during this time. Emulsion D has a similar behaviour, but his change from initial time to 24 hours is greater.

Emulsion C has an irregular behaviour: from initial time to 24 hours his viscosity increases a lot with a very higher value (approximately $550 \text{ mPa}\cdot\text{s}$). From 24 to 96 hours, this value decreases, and his viscosity is lower than D emulsion viscosity, which is expected because emulsion C has a lower calcium chloride concentration than emulsion D. It is an irregularity that could be for a human fault.

Viscosity values of the emulsions are between 200 and $550 \text{ mPa}\cdot\text{s}$ and viscosity value of mayonnaise is above $320 \text{ mPa}\cdot\text{s}$.

7.2. OSCILLATORY TEST

The oscillatory test is carried out as stated at 3.6. 5 minutes after the emulsion gelation, 24 hours and at 96 hours.

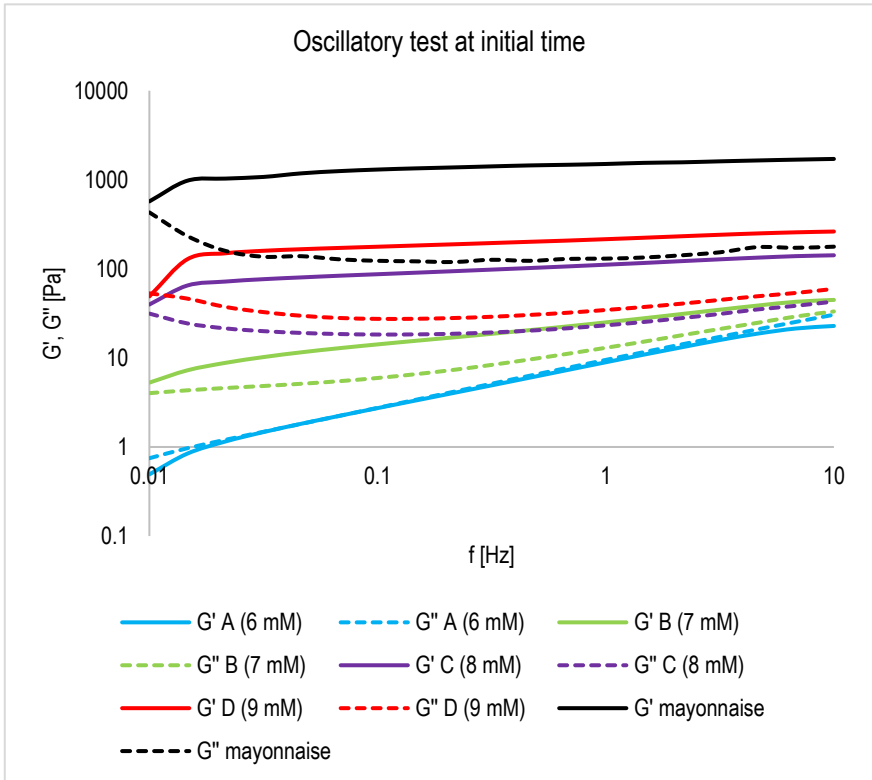


Figure 31. Oscillatory test of jellied emulsions at initial time (5 minutes).

Figure 31 shows that the viscoelastic modulus tendency is the same for emulsions C and D and mayonnaise, but the mayonnaise values are higher than the emulsions. At first, from 0.01 to 0.1 Hz, approximately, G' increases and G'' decreases. This increase is greater from 0.01 to 0.03, approximately. For 0.01 to 10 Hz, modulus values increase but lightly, these are quite constant.

Emulsion B tendency is like above emulsions but G' and G'' modulus difference is smaller. Modulus values increases more than the other emulsions (steeper line).

Emulsion A tendency is different respect the other samples. G' and G'' modulus are practically overlapped.

All emulsions have G' values higher than G'' values, it means that the samples have more elastic behavior than viscos. It means that the sample recovers his initial structure when the speed gradient is not applied.

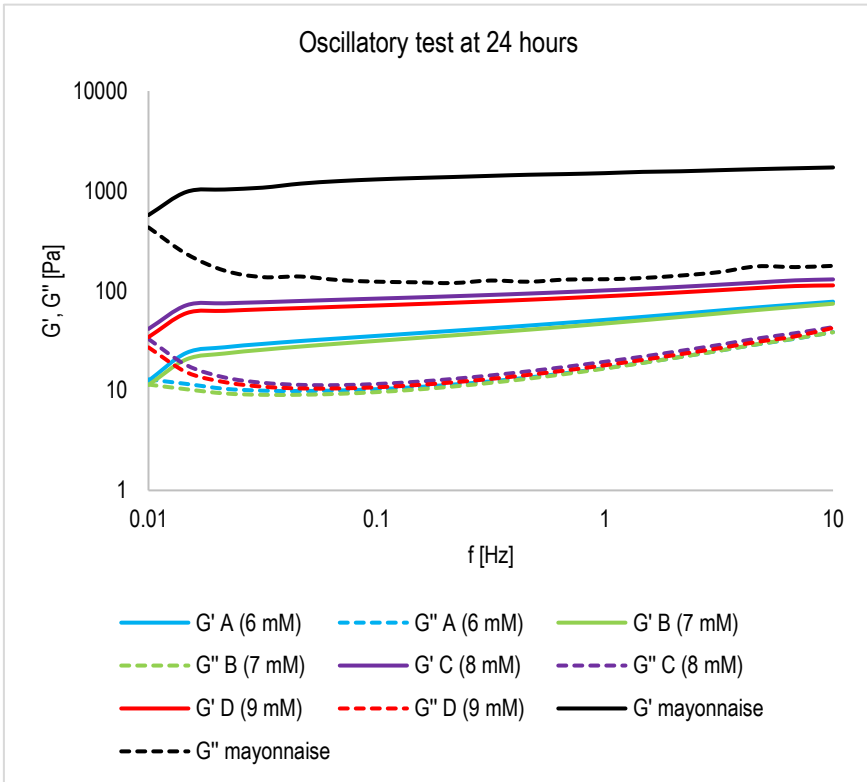


Figure 32. Oscillatory test of jellied emulsions at 24 hours.

Figure 32 shows that the viscoelastic modulus tendency is the same for all emulsions and mayonnaise, but the mayonnaise values are higher than the emulsions. This tendency is the same that at initial time.

A and B viscoelastic modulus tendency are practically overlapped, and C and D viscoelastic modulus tendency are also practically overlapped.

Respect to initial test, emulsions A and B values are increased, emulsion C values are quite constant and emulsion D values are decreased.

The behavior remains elastic: G' is upper to G'' .

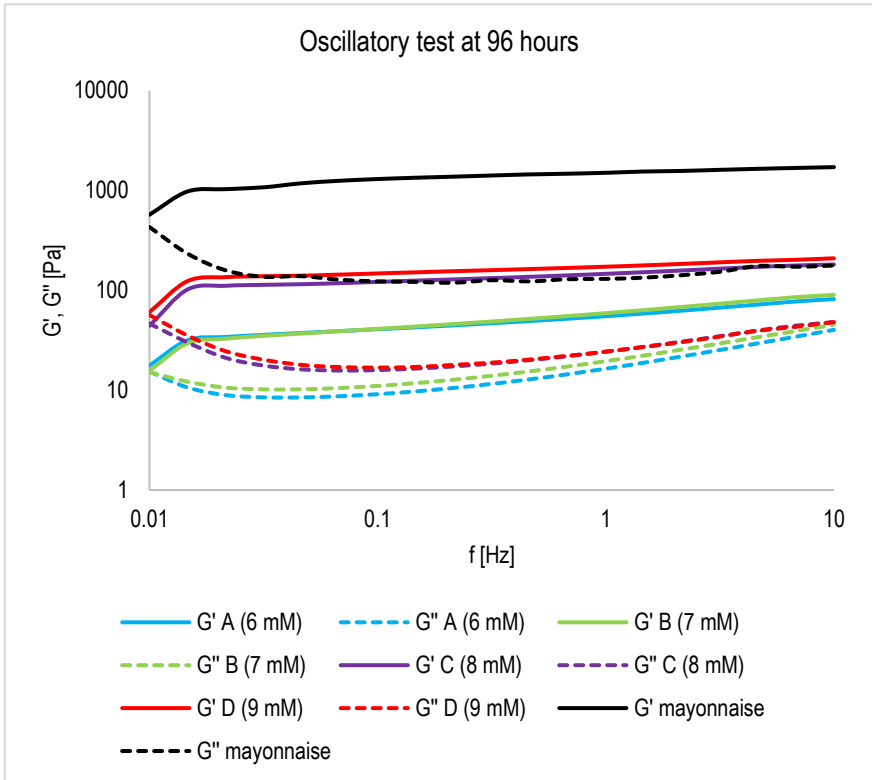


Figure 33. Oscillatory test of jellied emulsions at 96 hours.

Figure 33 shows that the viscoelastic modulus tendency is the same for all emulsions and mayonnaise, but the mayonnaise values are higher than the emulsions. This tendency is the same, but it seems that the increasing of viscoelastic modulus by increasing the frequency is more pronounced that at initial time and at 96 hours.

A and B viscoelastic modulus tendency are practically overlapped, and C and D viscoelastic modulus tendency are also practically overlapped, like at 24 hours.

In respect to the test at 24 hours, all emulsions values are slightly increased.

Loss modulus mayonnaise has lower values than storage modulus values of emulsions C and D as of 0.1 Hz approximately.

The behavior remains elastic: G' is upper to G'' .

As emulsions are more jellied, viscoelastic modulus increases. It might mean that adding more concentrated gelation solution to emulsion, it could achieve mayonnaise viscoelastic modulus values.

Also, it is important to consider the wide variety of mayonnaise textures, which could be more viscous or less.

8. CONCLUSIONS

Oil in water emulsion in casein rich phase is prepared by dispersing the oil with Ultra Turrax at high speed in the aqueous phase. A surfactant is needed for achieving the adequate stability, and de-aerating emulsion is necessary to remove air bubbles.

The oil in water emulsions prepared present drop sizes about 3 μm , which corresponds to a microemulsion by drop size classification, and are stables for more than 33 hours.

Oil in water in water emulsion in alginate rich phase is prepared by dispersing the oil in water emulsion with magnetic agitation in the aqueous phase.

The oil in water in water emulsions prepared present drop size distribution between 15 to 25 μm and are stables for more than 11 hours. This resultant emulsion has a 4% by weight of oil.

Gelation is necessary to achieve the desirable texture, seemly to mayonnaise texture. Oil in water in water emulsion is gelled with calcium chloride solution. Emulsion starts seeming a gel at a minimum concentration of gelation solution of 7 mM and at 8 mM emulsion is gelled. Over time, emulsion becomes more gelled.

A rise in concentration of calcium chloride increases slightly viscosity values. A rise in speed gradient decreases viscosity values.

The gelled emulsion has an elastic behavior (G' is higher than G''). Viscoelastic tendency is equal for all emulsions: a rise in concentration of calcium chloride increases viscoelastic modulus values.

Emulsions viscosity is less than mayonnaise at low speed gradients but as speed gradient increases, emulsions viscosity lightly overcomes sauce viscosity.

Emulsions and industrial sauce viscoelastic tendencies are equals, but industrial sauce values are larger. The higher concentration of calcium chloride studied is insufficient to obtain similar values of viscosity and viscoelastic modulus. It could be necessary to increase calcium chloride concentration to obtain similar values as industrial sauce.

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ACRONYMS

O/W	oil in water emulsion
W/W	water in water emulsion
O/W/W	oil in water in water emulsion
μ	viscosity
GP	speed gradient
F	frequency
G'	storage modulus
G''	lost modulus
Emulsion A	oil in water in water emulsion with 6 mM of calcium chloride
Emulsion B	oil in water in water emulsion with 7 mM of calcium chloride
Emulsion C	oil in water in water emulsion with 8 mM of calcium chloride
Emulsion D	oil in water in water emulsion with 9 mM of calcium chloride

APPENDICES

APPENDIX 1: EMULSION STUDY

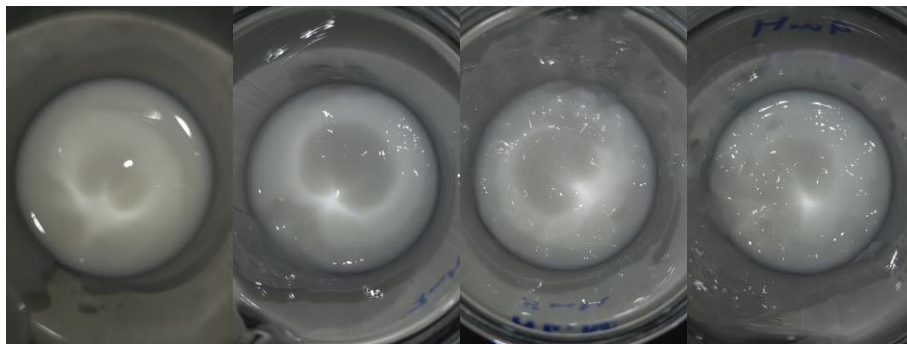


Figure 34. Oil in water in water gelled emulsions at initial time. From left to right: emulsions A, B, C and D.



Figure 35. Oil in water in water gelled emulsions at 24 hours. From left to right: emulsions A, B, C and D.

APPENDIX 2: GELATION STUDY

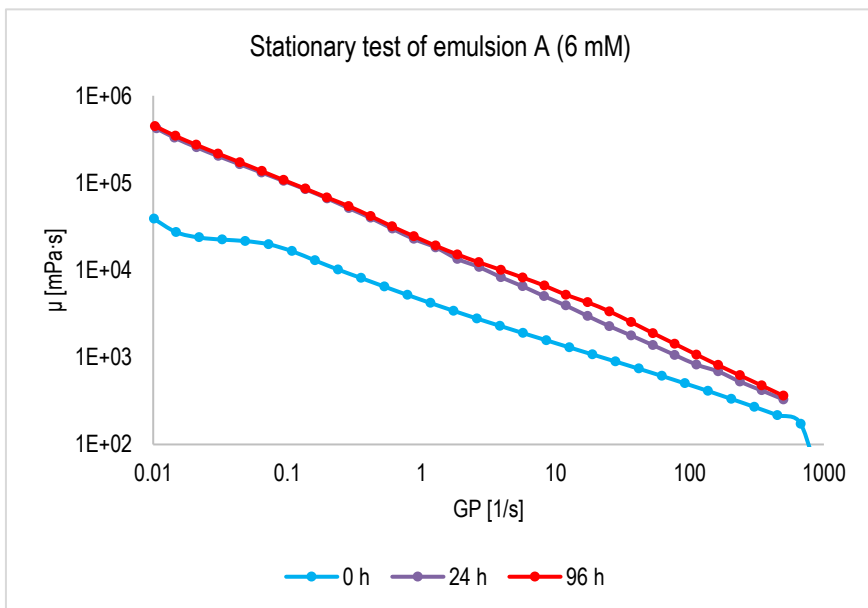


Figure 36. Stationary test of emulsion A (6 mM) over time.

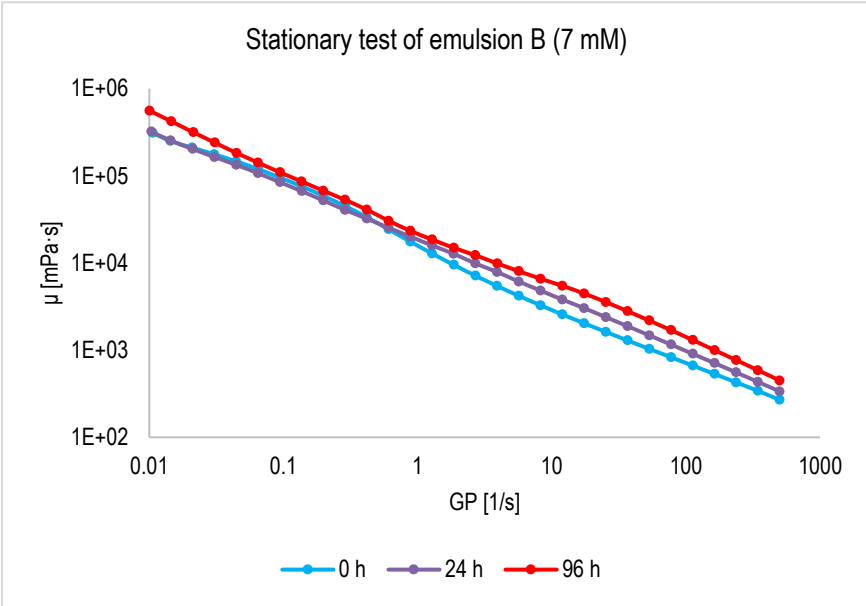


Figure 37. Stationary test of emulsion B (7 mM) over time.

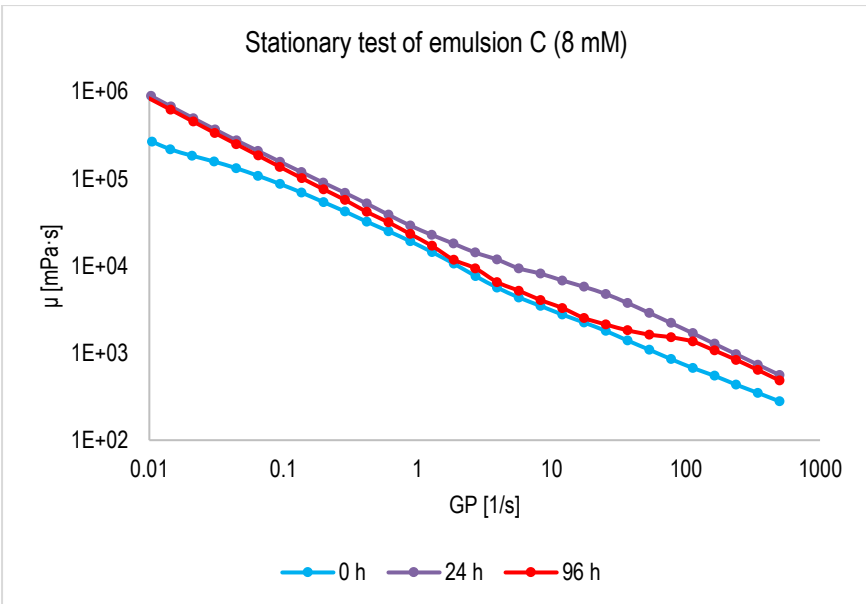


Figure 38. Stationary test of emulsion C (8 mM) over time.

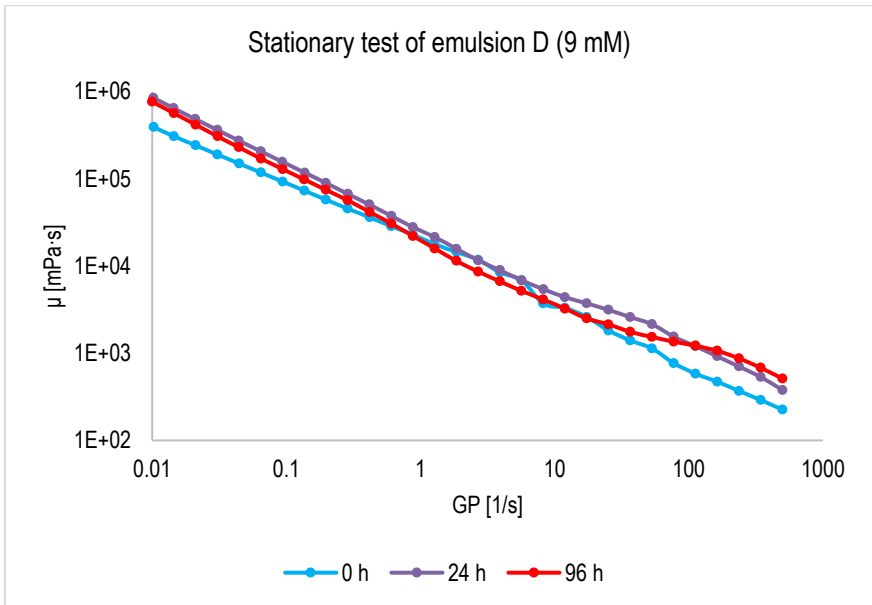


Figure 39. Stationary test of emulsion D (9 mM) over time.

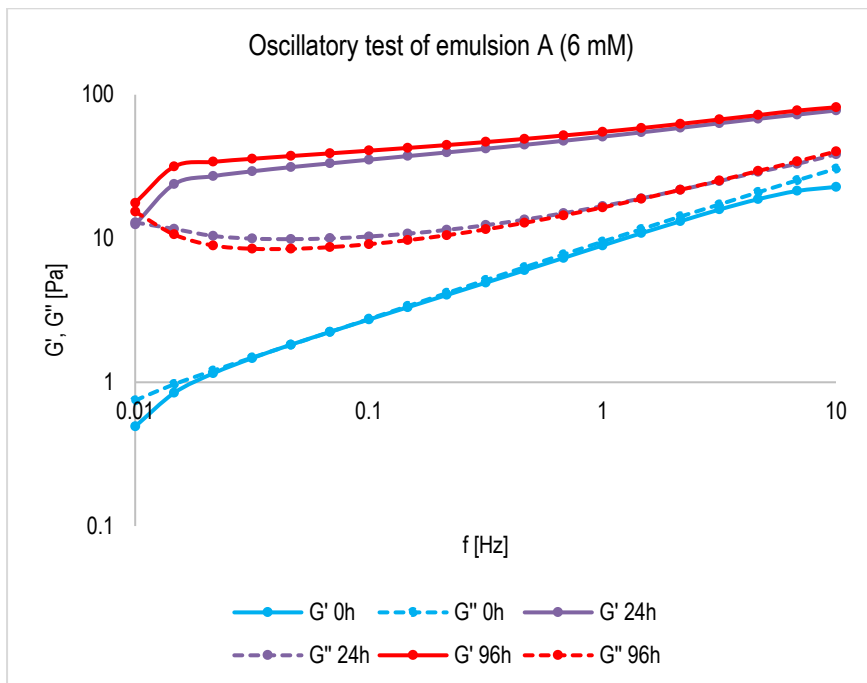


Figure 40. Oscillatory test of emulsion A (6 mM) over time.

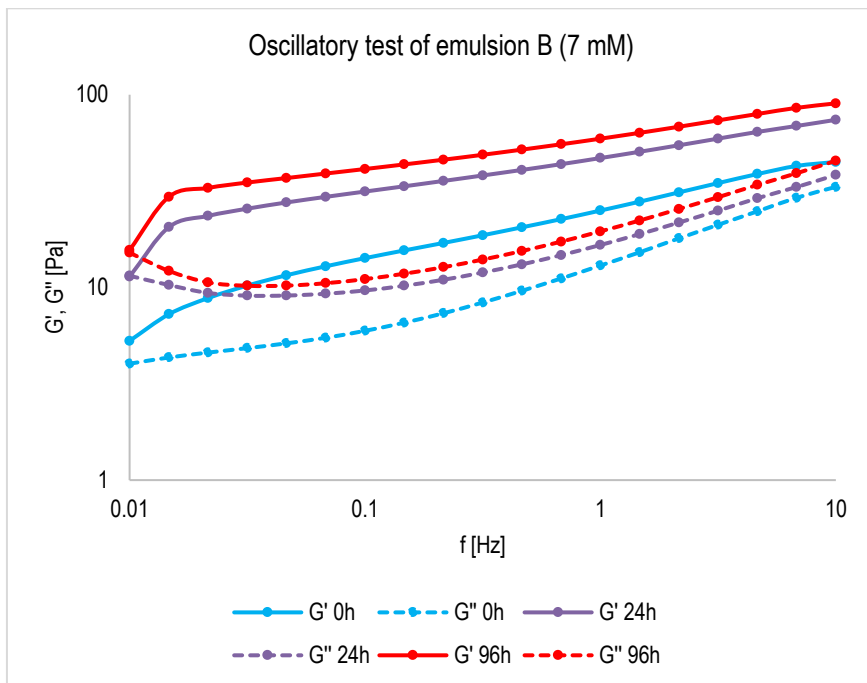


Figure 41. Oscillatory test of emulsion B (7 mM) over time.

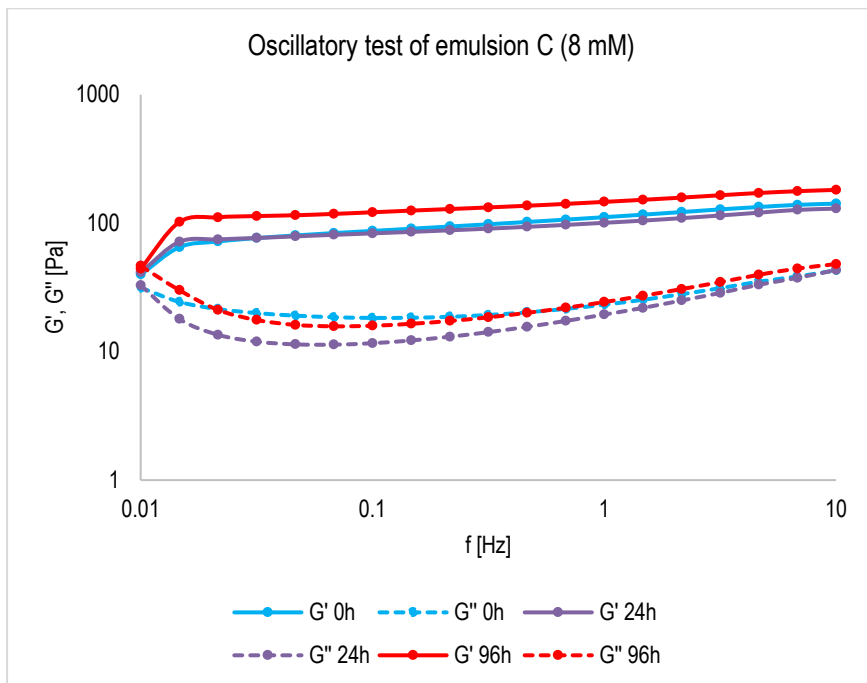


Figure 42. Oscillatory test of emulsion C (8 mM) over time.

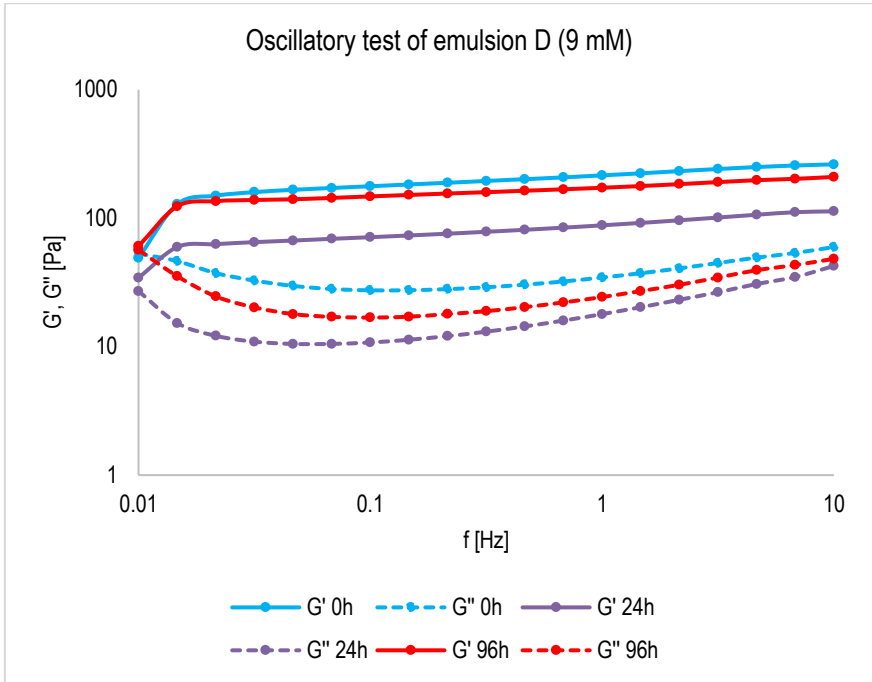


Figure 43. Oscillatory test of emulsion D (9 mM) over time.

Table 6. Oil in water in water emulsions viscosity values at 0.01 s^{-1} over time.

Time [h]	μ_A (6 mM) [mPa·s]	μ_B (7 mM) [mPa·s]	μ_C (8 mM) [mPa·s]	μ_D (9 mM) [mPa·s]	$\mu_{\text{mayonnaise}}$ [mPa·s]
0	38,643	310,870	266,253	391,386	3,867,233
24	419,883	321,081	889,619	846,424	3,867,233
96	447,838	558,727	837,681	766,502	3,867,233

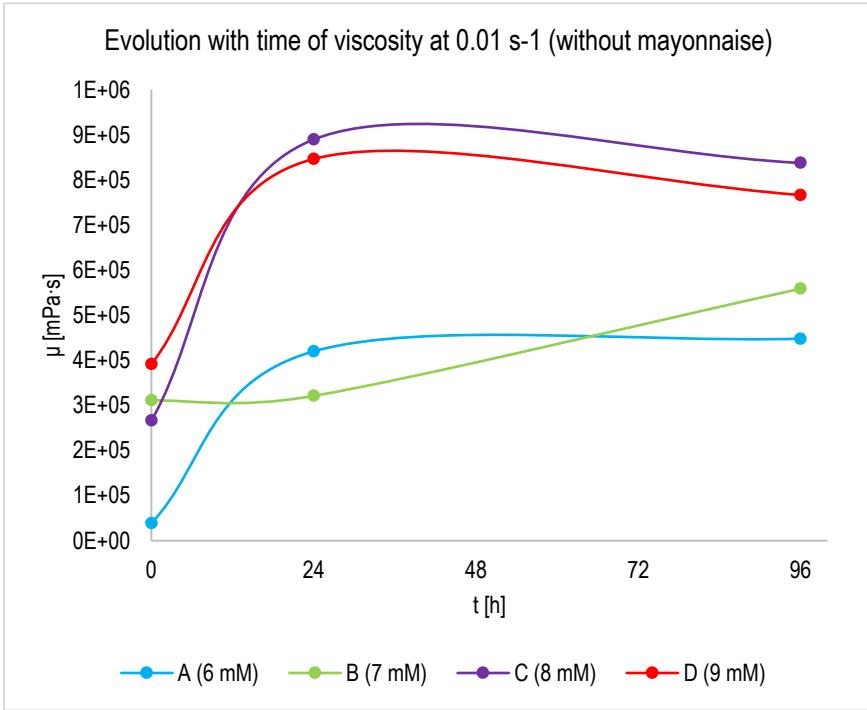


Figure 44. Evolution with time of viscosity at 0.01 s⁻¹ without mayonnaise.

Table 7. Oil in water in water emulsions viscosity values at 500 s⁻¹ over time.

Time [h]	μ _A (6 mM)	μ _B (7 mM)	μ _C (8 mM)	μ _D (9 mM)	μ _{mayonnaise}
	[mPa·s]	[mPa·s]	[mPa·s]	[mPa·s]	[mPa·s]
0	206	270	278	225	318
24	328	336	558	378	318
96	361	449	483	512	318

