# STABILITY IN PLANT-BASED CREAMS

Estabilidad en cremas con ingredientes de origen vegetal.

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## RESUMEN

Se ha llevado a cabo un estudio sobre el efecto de dos emulgentes y cuatro estabilizantes en el comportamiento de una emulsión de aceite en agua (O/W) formulada con aceite altamente insaturado. Para ello se prepararon 8 tipos de emulsiones O/W estabilizadas por homogeneización a alta presión (150 bar), con un contenido fijo de aceite vegetal (10,9% en peso). Como emulgentes se utilizaron emulsificador 1, por un lado, y emulsificador 2, por otro, a una concentración fija de 0,65%, en ambos casos, y diferentes estabilizantes (1, 2, 3 y 4). Las emulsiones fueron caracterizadas en cuanto a la distribución del tamaño de partícula, viscosidad, pH y estabilidad física. Los resultados mostraron que las emulsiones presentaron un pH comprendido entre 6 y 6,5, y fueron fácilmente dispersables en agua. La viscosidad dinámica de las cremas estuvo comprendida entre 100 y 226,5 cP, siendo la V8 la que presentó la mayor viscosidad. El análisis de la microestructura de la emulsión reveló que los diámetros medios de las partículas oscilaron entre 4,2 y 13 $\mu$m, siendo la V6 (estabilizante 3, 0,34%) la que presentó el mayor tamaño de los glóbulos de grasa, mientras que la V8 fue en donde se detectó

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el menor diámetro de las partículas (4,2 ± 1,51 $\mu$m). El análisis de la estabilidad física de las emulsiones por el método estático reveló que todas las emulsiones fueron estables a baja temperatura (6 ºC) durante los 14 días del ensayo. En los ensayos a temperatura ambiente (22 ºC) y a alta temperatura (40 ºC) la crema V8 fue la que mostró la mayor estabilidad durante todo el periodo ensayado. El análisis de estabilidad física de las emulsiones, utilizando el método dinámico (LUMiSizer®), mostró que la emulsión V8 fue la más estable de todas las formuladas, indicando que la adición del estabilizante 4 a una concentración 0,15% mejoró tanto la estabilidad física como el tamaño de las partículas de la crema obtenida.

**Palabras Claves**: aceite vegetal, emulsificador, estabilizante, LUMiSizer®.

## ABSTRACT

A study has been carried out on the effect of two emulsifiers and four stabilizers on the behavior of an oil-in-water (O/W) emulsion formulated with highly unsaturated oil. For this, 8 types of O/W emulsions stabilized by high pressure homogenization (150 bar) were prepared, with a fixed content of vegetable oil (10.9% by weight). On the one hand, emulsifiers 1 and 2, which were used at a fixed concentration of 0.65%, and different stabilizers (1, 2, 3 and 4) were tested. The emulsions were characterized in terms of particle size distribution, viscosity, pH and physical stability. The results showed that the emulsions had a pH between 6 and 6.5 and were easily dispersible in water. The dynamic viscosity of the creams was between 100 and 226.5 cP, with V8 being the one with the highest viscosity. The analysis of the microstructure of the emulsion revealed that the mean diameters of the particles ranged between 4.2 and 13 $\mu$m, with V6 (stabilizer 3, 0.34%) having the largest size of the fat globules, while V8 was where the smallest diameter of the particles (4.2 ± 1.51 $\mu$m) was detected. The analysis of the physical stability of the emulsions by the static method revealed that all the emulsions were stable at low temperature (6 ºC) during the 14 days of the test. In the tests at room temperature (22 ºC) and at high temperature (40 ºC), the V8 cream was the one that showed the greatest stability throughout the period tested. The physical stability analysis of the emulsions, using the dynamic method (LUMiSizer®), showed that the V8 emulsion was the most stable of all the formulated ones, indicating that the addition of stabilizer 4 at a concentration of 0.15% improved the stability physical as the size of the particles of the cream obtained.

**Keywords:** vegetable oil, emulsifier, stabilizer, LUMiSizer®.

# INTRODUCTION

One of the main factors contributing to the negative impact of climate change and unhealthy life style, is the consumption of high levels of animal based foods products such as meat and milk (Poore & Nemecek, 2018; Springmann, Clark, Mason-D'Croz, Wiebe, Bodirsky, Lassaletta, de Vries, Vermeulen, Herrero, Carlson, Jonell, Troell, DeClerck, Gordon, Zurayk, Scarborough, Rayner, Loken, Fanzo, Godfray, Tilman, Rockström & Willett, 2018), for therefore, there is great concern in looking for alternatives, in order to change into a plant based diet, with the objective of improving human health and zero waste (Willett, Rockström, Loken, Springmann, Lang, Vermeulen, Garnett, Tilman, DeClerck, Wood, Jonell, Clark, Gordon, Fanzo, Hawkes, Zurayk, Rivera, Vries, Sibanda & Murray, 2019).

Although there are plant-based creamers on the market today, including almond, coconut, soy, rice, and oat-based milk substitutes (Sethi, Tyagi & Anurag, 2016; Chalupa-Krebzdak, Long & Bohrer, 2018), there are many consumers who are reluctant to adopt these plant-based creamers because they don't like how it tastes dairy milk, or due to the fact that animal suffering in factory farming. For this reason, it is necessary to advance in the study of the physicochemical basis of the functional attributes of vegetable creams so that they can be developed as products that are more acceptable to a wider range of consumers. In this sense, research carried out in recent years

has been focused on specific aspects of the formation or properties of plant-based creams, as well as their preparation, composition or nutritional profile (Sethi et al., 2016), and more recently on designing plant-based creams that simulate sensory (appearance, texture, etc.) and gastrointestinal attributes of milk of animal origin (McClements, Newman & McClements, 2019). In this line, the food industry is working on the development of a range of affordable, convenient, desirable, nutritious and sustainable plant-based products as milk substitutes.

**Production of plant-based creams**

A cream is an emulsion made up of two immiscible liquids (usually oil and water), one of which is dispersed as small spherical droplets in the other. Emulsions can be conveniently classified according to the relative spatial distribution of oil and water phases. A system consisting of oil droplets dispersed in an aqueous phase is called an oil-in-water (O/W) emulsion, e.g., milk, cream, mayonnaise, etc. A system consisting of water droplets dispersed in an oil phase is called a water-in-oil (W/O) emulsion, e.g., margarine and butter. The substance that forms the droplets in an emulsion is called the dispersed, discontinuous, or internal phase, while the substance that forms the surrounding liquid is called the continuous, or external, phase. In most foods, droplet diameters are usually between 100 nm and 100 μm, but recently there is a growing interest in the use of emulsions with smaller diameters ($d < 100$ nm), due to their physicochemical properties (McClements et al., 2019).

The process of converting two separate immiscible liquids into an emulsion, or of reducing the size of droplets in a preexisting emulsion, is known as homogenization. In the food industry, this process is usually carried out using high-energy methods that use mechanical devices (homogenizers) to form droplets by subjecting the phases to disruptive forces, e.g., high-speed mixers, high-pressure valve homogenizers and colloid mills (Figure 1). However, under certain

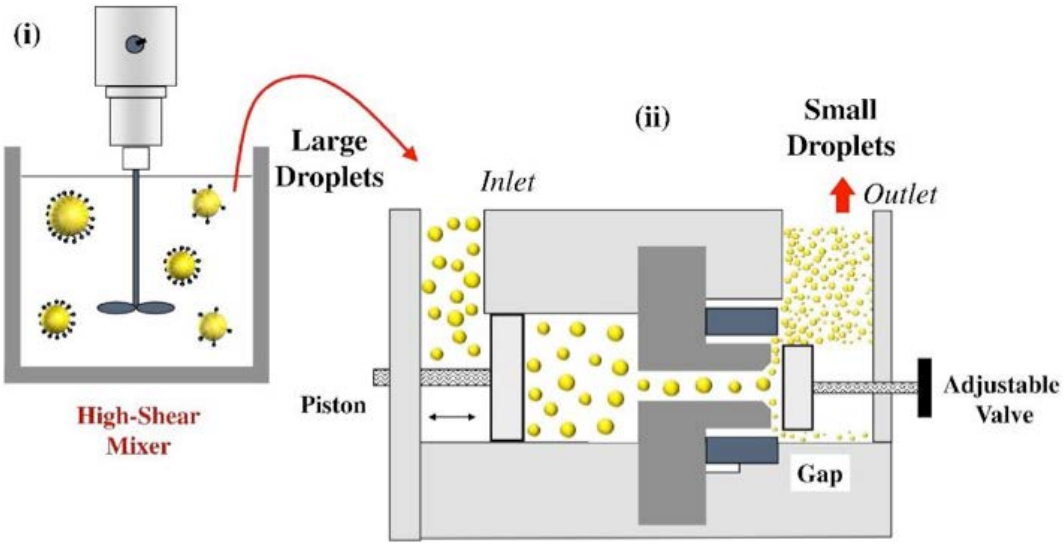


Figure 1.- Schematic diagram of the typical two-step procedure used to produce oil-in-water emulsions using a high energy method (i) a coarse emulsion is formed using a high shear mixer; (ii) a fine emulsion is formed by passing the coarse emulsion through a high-pressure valve homogenizer (taken from McClements & Jafari, 2018).

circumstances, it is also possible to form emulsions using low-energy methods that are based on the spontaneous formation of small droplets when two phases are mixed (Traynor, Burke, Frías, Gaston & Barry-Ryan, 2013).

An emulsion is generally achieved through the application of mechanical energy that allows the interface to be deformed to such an extent that it causes the formation of droplets, and subsequently their division into even smaller droplets. Droplet size is an important property because it determines the shelf life stability, consistency, and rheological properties of an emulsion (Aswathanarayan & Vittal, 2019). In many cases the goal of emulsification is to produce droplets as fine as possible ($d < 1\ \mu m$) (Anton & Vandamme, 2011). In the food industry, high pressure homogenizers are the most used (Schultz, Wagner, Urban, & Ulrich, 2004). High-pressure homogenization based on microchannel technology (microfluid) is a methodology used to obtain fluid emulsions with submicrometric mean diameters and narrow droplet size distributions, since they can reach extremely high shear rates (Vladisavljević, Kobayashi & Nakajima, 2012). Microfluidization processing involves passing a coarse emulsion through an interaction chamber using a high-pressure pumping device (Jafari, He & Bhandari, 2007). The interaction chamber consists of two flow channels that are designed to cause two coarse emulsion streams to collide with each other at high velocities, thus creating a very high shearing action that provides an exceptionally fine emulsion (Wang, Neves, Isoda & Nakajima, 2015).

It is possible to form an emulsion by homogenizing oil and water, both phases usually separate quickly in a system consisting of a layer of oil (lower density) over a layer of water (higher density). This is because droplets tend to merge with their neighbors when they collide, eventually leading to complete phase separation. The driving force for this process is the fact that the contact between oil and water molecules is thermodynamically unfavourable, thus emulsions are thermodynamically unstable systems. It is possible to form emulsions that are kinetically stable (metastable) for a reasonable period (a few days, weeks, months, or years), by including substances known as stabilizers. A stabilizer is any ingredient that can be used to improve the kinetic stability of an emulsion and can be classified as an emulsifier, texture modifier, or weighting agent, depending on its mode of action (Berton-Carabin, Sagis & Schroën, 2018).

An emulsifier is an amphipathic molecule, that is, they have polar and non-polar regions in the same molecule, of low or high molecular weight, which tend to migrate and adsorb quickly at the oil-water interface, favoring the formation of drops with less consumption of energy, and therefore the formation of the emulsion, by reducing the interfacial tension. An emulsifier is not the same as a stabilizer, as it does not exhibit significant interfacial activity. Examples of low molecular weight emulsifiers include surfactants, polar lipids, and glycolipids. Among the high molecular weight emulsifiers, we can mention proteins, lipoproteins, block copolymers (synthetic) and some exuded polysaccharides such as gum Arabic (Dickinson, 2003).

A stabilizer is a chemical compound normally of a macromolecular nature that, when hydrated in the aqueous phase, gives an O/W emulsion physical stability for a long time. The stabilization of the emulsion is achieved by restricting the mobility of the droplets of the dispersed phase, thanks to the increase in viscosity and, sometimes, in the viscoelasticity, of the continuous phase. Stabilizers contribute to emulsion stability by mainly favoring steric interactions between droplets, although electrostatic interactions can also be significant. These compounds not only increase the viscosity, but also modify the viscoelastic and therefore rheological properties of the emulsion. The polymers used to stabilize O/W food emulsions are mainly hydrocolloids of a polysaccharide nature (food gums). They can be classified into

natural, semi-synthetic and synthetic. Examples of the former can be carrageenan, locust bean gum, pectins, xanthan and gellan gums, and starches obtained from cereals. Among the semisynthetics, the modified starches and propylene glycol alginate can be highlighted, while the polyoxyethylated type polymers can be mentioned among the synthetic ones (Lopes da Silva, Gonçalves & Rao, 1993; Muñoz García, Alfaro Rodríguez & Zapata Guillén, 2007). To avoid destabilization problems, the concentrations of the biopolymers must be properly adjusted to avoid alterations in the emulsion caused by flocculation induced by bridges between adsorbed macromolecules in nearby droplets or by expulsion of macromolecules from the continuous medium (Dickinson, 2009).

Texture modifiers can be divided into two categories based on their mode of operation and the rheological characteristics of their solutions: thickening agents and gelling agents. Thickening agents are ingredients used to increase the viscosity of the continuous phase of emulsions, while gelling agents are ingredients used to form a gel in the continuous phase of emulsions. Therefore, texture modifiers improve emulsion stability by retarding droplet movement. In the food industry, the most widely used thickening and gelling agents are usually polysaccharides or proteins in O/W emulsions and fat in W/O emulsions.

A weighting agent is a substance that is added to the dispersed phase (droplets) to decrease the density contrast between the droplets and the surrounding liquid, thus delaying gravitational separation. Bulking agents are commonly used in the beverage industry to increase the density of flavor oil droplets, and this improve cream stability. When developing an emulsion-based product, it is extremely important to identify the most appropriate stabilizer or combination of stabilizers to use, based on the instability mechanisms in the system.

During the homogenization process, the emulsifying agents tend to adsorb on the surface of the drops, forming a protective barrier that prevents aggregation, facilitating a homogeneous distribution of the particles that make up the system and substantially reducing surface tension (Berton-Carabin et al., 2018). The repulsive interactions induced in the interface prevent future flocculation and coalescence phenomena, stabilizing the emulsion for long periods of time (Bot, Cossuta & O'Mahony, 2021).

With respect to the oil phase of the emulsion, a variety of different oils of vegetable origin are available that can be used to build this type of emulsion, including coconut, corn, flaxseed, olive, palm, soybean, and sunflower oils. Each of these oils varies in its molecular composition, physicochemical properties, sensory attributes, and nutritional profile. Coconut oils are composed almost entirely of medium chain saturated fatty acids. As a result, they are highly stable to lipid oxidation but may have some adverse health effects compared to unsaturated fats, for example, they may promote heart disease due to their high level of saturated fat (Ludwig, Willett, Volek & Neuhouser, 2018). In contrast, oils containing high levels of long-chain polyunsaturated (ω-3) fatty acids, although more susceptible to lipid oxidation, are more beneficial to health. Differences in the melting/crystallization behaviors, viscosities, and interfacial tensions of edible oils also affect the formation and stability of oil-in-water emulsions and must be taken into account when formulating them (McClements, 2015).

## STABILITY OF FOOD EMULSIONS

The term "emulsion stability" refers to the ability of said emulsion to resist changes in its properties over time: the more stable the emulsion, the slower its properties change. An emulsion can become unstable due to physical changes (alteration in the spatial distribution or structural organization of the molecules) or chemical changes (changes in the chemistry of its components). There is a variety of physico-

chemical mechanisms that may be responsible for alterations in emulsion properties, and it is usually necessary to establish which of these mechanisms are important in the system under consideration before effective strategies can be developed to improve emulsion properties stability. Figure 2 schematically shows a series of the most common physical mechanisms that are responsible for the instability of food emulsions. Creaming and sedimentation are forms of gravitational separation (McClements, 2015). Creaming describes the upward movement of droplets due to having a lower density than the surrounding liquid, while sedimentation describes the downward movement of droplets due to having a higher density than the surrounding liquid.

There are different potential strategies for overcoming gravitational separation, including:

**- Reduce particle size**: One of the most effective means of reducing gravitational separation in this type of product is to ensure that most of the particles are relatively small (D < 300 nm). For constructed fat droplets, the particle size can be reduced by ensuring that the homogenization process is efficient, i.e., the droplets are below a critical size and the particle size distribution is narrow. Droplet size can usually be reduced by increasing the energy intensity and duration of the homogenizing device used (McClements, 2015). For example, with a high-pressure valve homogenizer or microfluidizer, droplet size can be decreased by increasing the homogenizing pressure and the

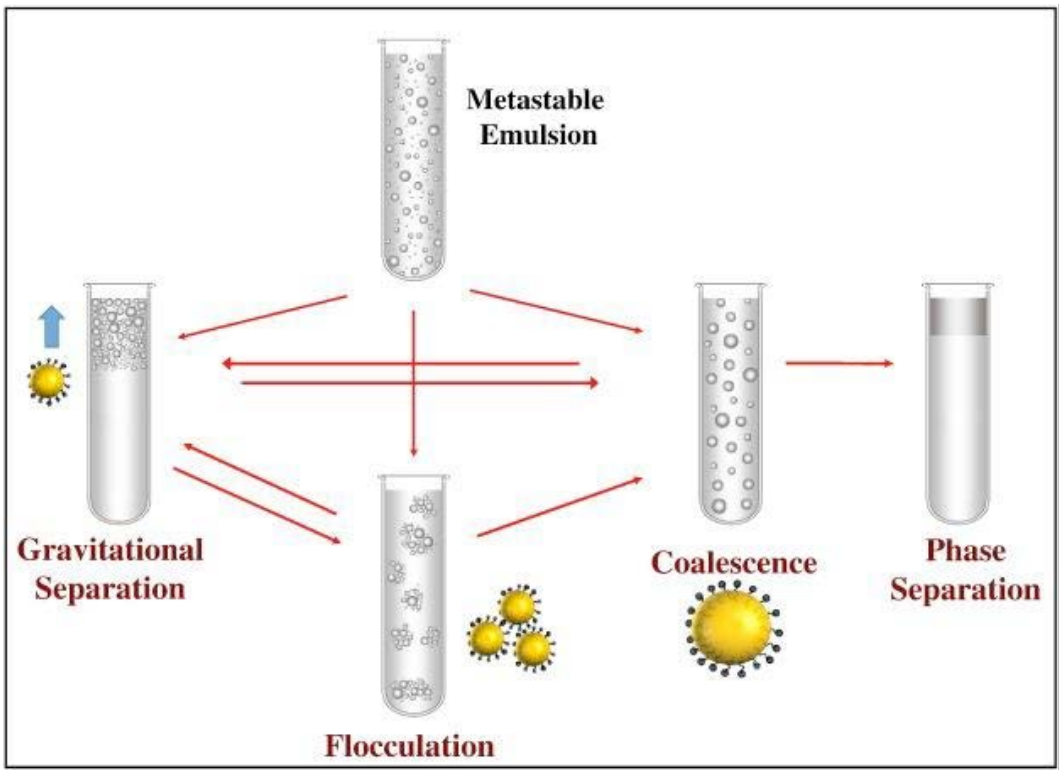


Figure 2. Food emulsions can become unstable through a variety of physical mechanisms, including creaming, sedimentation, flocculation, coalescence, and phase inversion (taken from McClements & Jafari, 2018).

number of passes through the device. Furthermore, it is important to inhibit aggregation of individual oil bodies or fat droplets once they have formed, because this will lead to an increase in effective particle size.

**- Increase viscosity**: The viscosity of the aqueous solution surrounding the fat droplets or oil bodies can be increased by adding thickening agents, which are typically biopolymers such as guar gum, locust bean gum or xanthan gum. However, care must be taken to control the level used so as not to promote instability by inducing depletion or bridging flocculation (McClements, 2015).

**- Decrease density difference:** In principle, gravitational separation can be inhibited by reducing the density difference between the colloidal particles and the surrounding liquid (McClements, 2015). In practice, this is often difficult to achieve because natural oils have a limited range of viscosities (generally between about 910 and 930 kg/m$^3$). However, for plant-based systems, this could be done by wrapping dense biopolymers around the fat droplets to increase their density. Generally, the density of fat globules increases as the solid fat content increases, which can reduce the difference in density between the oil and water phases. However, care must be taken that partial coalescence does not occur; otherwise, this will promote the aggregation of fat globules, which can alter the stability and texture of the product (Fredrick, Walstra & Dewettinck, 2010).

**- Particle aggregation.** The shelf life of plant-based products can be reduced due to the aggregation of fat bodies or fat droplets they contain (McClements, 2015). Particle aggregation can affect product quality in several ways: (i) it can lead to the formation of antistatic particles; (ii) can promote faster gravitational separation carrying a visible layer of cream or sediment; and (iii) may negatively alter the mouthfeel of the product. In general, the tendency for particle aggregation to occur within a colloidal dispersion is governed by the balance of attractive and repulsive interactions that operate between the particles (McClements, 2015). The main attractive forces are hydrophobic and van der Waals interactions, while the main repulsive ones are steric and electrostatic interactions. All colloidal particles attract each other through Van der Waals forces, so it is always important to generate some sort of repulsive force to prevent aggregation. In general, both the magnitude and range of the repulsive forces must be large enough to overcome the attractive forces.

There are two main types of particle aggregation in colloidal dispersions, such as plant-based creamers: flocculation and coalescence (Figure 2). In flocculation, the particles come together and form a group, but each individual particle retains its original integrity. This type of aggregation can occur when the long-range repulsive forces are insufficient to overcome the long-range attractive forces, but the interfacial layers are robust enough to resist disruption. In coalescence, the particles fuse together after coming into close contact. Coalescence often occurs in emulsions when the interfacial coating around the fat droplets is not robust enough. This instability mechanism can be overcome by choosing an emulsifier or other substance that forms a robust coating around fat droplets or oil bodies. For example, a layer of dietary fiber can be deposited on the surface of the particles through electrostatic deposition (Guzey & McClements, 2006). This layer increases electrostatic and steric repulsion between droplets, preventing them from getting too close together. In addition, it can form a solid coating that is difficult to break, inhibiting droplet fusion.

As described above, many of the most important properties of emulsion-based food products are determined by the size of the droplets. If all the droplets within an emulsion have the same dimensions, it is called a monodisperse emulsion, but if a variety of droplet sizes are present, it is called a polydisperse emulsion. Real food emulsions always contain a distribution of droplet sizes and are characterized

by the average droplet size. Information about the size of particles within an emulsion can be obtained using various analytical methods, including microscopy, light scattering, particle counting, and sedimentation methods (Mengual, Meunier, Cayre, Puech & Snabre, 1999; McClements, 2007).

**Quantification of emulsion stability**

A variety of analytical methods and protocols have been developed to monitor the stability of vegetable creams (McClements, 2015). The degree of creaming or sedimentation can be determined by measuring the thickness of the layers of cream, whey and/or sediment formed in a sample.

Gravitational separation (static method) is usually followed by simple visual observation of samples stored in transparent containers over time, using digital photography to record changes in their appearance during storage. The problem with this approach is that it is often difficult to clearly discern the boundary between the different layers in a sample (Choi, Won, Park & Chang, 2014). For this reason, more sophisticated methods have been developed to measure the change in particle concentration with sample height over time.

Gravitational separation can be determined using commercial devices that measure the reflection/transmittance of a laser beam that scans the entire sample during storage, e.g., the Turbiscan. Other commercial devices speed up this process by applying a centrifugal force to the sample, for example, the Lumifuge and the LUMiSizer® Multi-Sample Analytical Centrifuge that allows simultaneous measurement of transmitted light intensity as a function of time and position over the entire length of the sample (Figure 3). The data is displayed as a function of the distance from the center of rotation to the position within the sample (transmission profiles). Transmission profiles contain the kinetic information for the separation process and allow particle characterization (Sobisch, Lerche, Detloff, Beiser & Erk, 2006; Chiu, Chang, Chiang, Kuo & Wang, 2011).

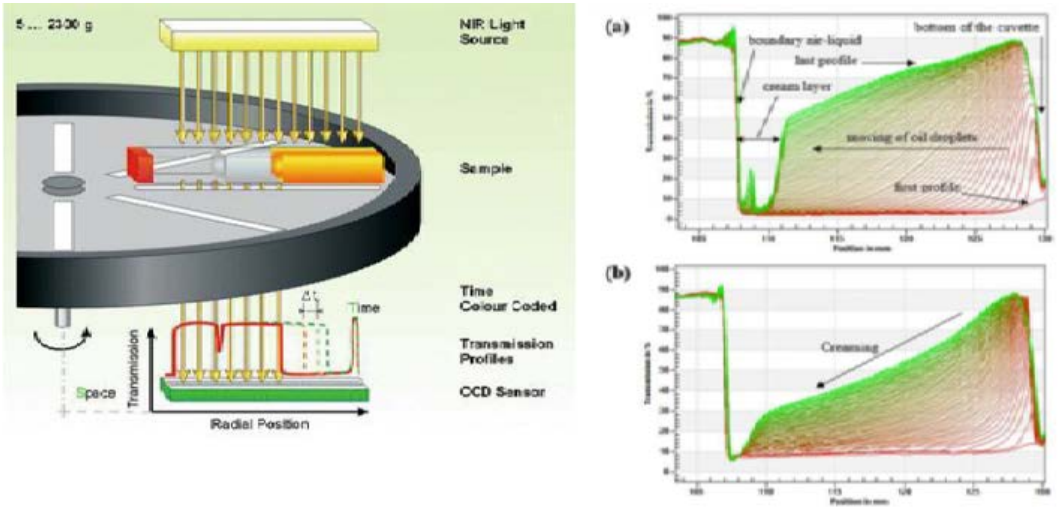


Figure 3. Schematic configuration and measurement principle of the LUMiSizer® analytical photocentrifuge (taken from Gross, Herrmann, Blech, Pinnapireddy, Garidel & Bakowsky, 2018), and transmission profiles of lecithin-stabilized emulsions (taken from Dammak & José do Amaral Sobral, 2018).

## OBJETIVES

For the development of this researching, the objective has been set out to deepen the knowledge of the effect of different ingredients on the stability in plant based creams, improving their sensory qualities, for a greater acceptance by consumers, in order to being able to reduce the number of animal based foods products, in their diet, for health, ethical and environmental reasons. To this end, different emulsions have been prepared in which the impact of different additives on parameters indicative of stability in said creams is analyzed, such as: particle size distribution, viscosity, pH and physical stability.

## MATERIALS AND METHODS

### Materials

For the elaboration of the vegetable creams object of the test, the following products were used: oat base, vegetable oil from two sources (1 and 2), emulsifier (1 and 2), stabilizers (1, 2, 3 and 4) all of them from different suppliers.

### Preparation of vegetable creams

400 g of 8 different types of O/W emulsions whose composition is shown in Table 1 were prepared.

The different components, once weighed, were mixed with the help of a magnetic stirrer at a temperature between 40 and 50 ºC, to facilitate the solubilization of the carbohydrates and the dispersion of the aqueous and lipid phases. To reduce the size of the fat globules and stabilize the emulsion, the different mixtures were introduced into a homogenizer and subjected to a pressure of 150 bar. Sample V1 was used as a control.

### Pasteurization of vegetable creams

Once the respective emulsions (V1-V8, see Table 1) were obtained, they were pasteurized at 100 ºC for 6 min. Next, the creams were cooled with water at room temperature, with gentle agitation, prior to storage at low temperature (refrigerator, 6 ºC).

Table 1. Composition of the different creams produced (V1, control; V2-V8, the different components are varied, with respect to the control).

| Ingredients | Concentration (g) / 100 g of cream | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **V1** | **V2** | **V3** | **V4** | **V5** | **V6** | **V7** | **V8** |
| **Oat base** | 47,56 | 47,56 | 47,56 | 47,56 | 47,56 | 47,56 | 47,56 | 47,56 |
| **Water** | 27,25 | 27,25 | 27,25 | 27,25 | 27,25 | 27,25 | 27,25 | 27,25 |
| **Vegetable oil 1** | 10,9 | | 10,9 | 10,9 | 10,9 | 10,9 | 10,9 | 10,9 |
| **Vegetable oil 2** | | 10,9 | | | | | | |
| **Emulsifier 1** | 0,65 | 0,65 | | 0,65 | 0,65 | 0,65 | 0,65 | 0,65 |
| **Emulsifier 2** | | | 0,65 | | | | | |
| **Stabilizer 1** | 0,13 | 0,13 | 0,13 | 0,13 | 0,13 | 0,13 | 0,13 | 0,13 |
| **Stabilizer 2** | 0,03 | 0,03 | 0,03 | 0,04 | | | | |
| **Stabilizer 3** | | | | | 0,17 | 0,34 | | |
| **Stabilizer 4** | | | | | | | 0,12 | 0,15 |
| **Sea salt** | 0,11 | 0,11 | 0,11 | 0,11 | 0,11 | 0,11 | 0,11 | 0,11 |

**Characterization of creams: pH and Viscosity**

For the determination of the pH, a laboratory pH meter was used. To measure the viscosity, an oscillating body rotational viscometer was used using the spindle R2 and a stirring speed of 100 rpm, all of them at room temperature. Units were expressed in centipoises (cP).

**Microscopic determination of floc sizes**

The size distribution of the flocs in the creams was determined by light microscopy, using a computer-connected Optika B-350 photomicroscope. Motic Images Plus version 2.0ML was used as image capture and processing software. To calibrate the size determination program, an integration graticule of 1 mm length with 100 divisions was used. Each division corresponds to 10 $\mu$m.

For the microscopic observation of the creams, a homogeneous sample was taken, and a drop was placed on a slide, then covered with a coverslip to be able to observe the preparation on a plane. Each preparation was photographed at 400x magnification in different fields, and the photos obtained were later analyzed with the image analysis software.

**Measurement of emulsion stability**

Two protocols were used for the stability studies of the creams: a static and a dynamic method.

*1. Static method*

10 mL of emulsion was transferred to a 15 mL polypropylene screw cap graduated plastic test tube. The tubes were stored for 14 days at different temperatures: 6°C (refrigerator), room temperature. (23 ºC) and in an oven at 40ºC. The stability (SR), at 7 and 14 days, was calculated as the percentage of the height of the aqueous phase with respect to the initial volume (Mirhosseini, Tan & Taherian, 2008).

*2. Dynamic method*

To determine the stability of the creams by the dynamic method, a LUMiSizer® multisample analytical centrifuge (L. U. M. GmbH, Berlin, Germany) was used, which allows characterizing the emulsions by simultaneously measuring the intensity of the transmitted light as a function of time and position along the entire length of the sample (Figure 3). It allows analyzing the velocity distribution of particles and drops to detect the change in particle size in coalescence and flocculation processes, and the separation of phases in sedimentation or creaming processes. According to the transmission and retro-scattering profiles of light, obtained along the vial, it is possible to know the dynamic behavior of the emulsions, which translates into the possibility of detecting the two main destabilization phenomenon that affect the homogeneity of dispersions such as particle migration (creaming and sedimentation) and particle size variation or aggregation (coalescence and flocculation) (Mengual et al., 1999).

The following parameters were used: 0.4 mL of dispersion, exposure time 15,200 s, speed 2867 rpm, wavelength 865 nm, LUM cell 2 mm temperature 25 °C. All measurements were repeated and tested at least twice. The analysis of results, using the SEPView software, allowed to determine the instability index and its evolution over time.

**RESULTS**

**Elaboration of the creams**

For the preparation of the creams, a base mixture (V1) composed of a suspension of oat in water (47.56%), water (27.25%), vegetable oil 1 (10.9%), emulsifier 1 (0.65%), stabilizer 1 (0.13%) and stabilizer 2 (0.03%) as thickeners, in addition to sea salt (0.11%). From this base cream, some of the components were modified to determine what effect they had on their sta-

bility. Their compositions are shown in Table 1 (see Materials and Methods), the changes introduced, are the next ones:

- In cream 2, the type of lipid phase was modified (vegetable oil 2). The same concentration was maintained, although the oil tested came from another supplier.
- In cream 3, the emulsifier was modified, the emulsifier 2 was used in this case.
- In cream 4, the concentration of the stabilizer 2 was modified.
- In cream 5, the stabilizer 2 was changed for stabilizer 3 at a concentration of 0.17 g/100 g of cream.
- In cream 6, the concentration of stabilizer 3 was increased to 0.34 g/100 g of cream.
- In cream 7, stabilizer 2 was replaced by stabilizer 4 at a concentration of 0.12 g/100 g of cream.
- And in cream 8, the concentration of stabilizer 4 was increased to 0.15 g/100 g of cream.

Once the different creams were prepared, they were pasteurized (section 3.3, Materials and Methods) to avoid the development of microorganisms that could affect their stability during the development of the investigation.

**Characterization of creams: Viscosity and pH**

Among the physical-chemical properties of emulsions are viscosity, pH, stability, droplet size and size distribution. Viscosity is basically defined as the resistance to deformation presented by a fluid subjected to a shear stress. The viscosity of emulsions depends on their characteristics: external phase viscosity, internal phase proportion, droplet size, internal phase viscosity, electro-viscous effects, formulation effects. The rheological behavior of fluids is subject to the way in which deformation occurs when a shear stress is applied. The variables that affect the rheological behavior of the emulsions obey the following order of importance: the internal phase content, the size and distribution of drop sizes, the viscosity and rheological behavior of the continuous phase, the temperature, the viscosity of the internal phase and, finally, if any, the non-hydrodynamic interactions between the droplets.

To measure the viscosity, an oscillating body rotational viscometer was used, using the R2 spindle and a stirring speed of 100 rpm for all mixtures and at room temperature. Units were expressed in centipoises (cP).

Table 2 shows the viscosity values determined at different times, the mean and the stan-

Table 2. Viscosity and pH of the different creams obtained. The viscosity was measured at different times (10, 20, 30 and 60 seconds).

| | Viscosity (cP) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Sample** | **10 ‘’** | **20”** | **30”** | **60”** | **Mean** | **Standar desviation** | **pH** |
| **V1** | 116,6 | 115,9 | 115,4 | 114,3 | 115,55 | 0,97 | 6,3 |
| **V2** | 158,7 | 155,1 | 157 | 147,6 | 154,6 | 4,89 | 6,44 |
| **V3** | 100,3 | 101,5 | 100,5 | 98,5 | 100,2 | 1,25 | 6,41 |
| **V4** | 150,9 | 147,4 | 146,1 | 142,6 | 146,75 | 3,43 | 6,43 |
| **V5** | 113,4 | 112,7 | 112 | 110,9 | 112,25 | 1,07 | 6,24 |
| **V6** | 151,2 | 160,8 | 159,9 | 147 | 154,725 | 6,73 | 6,05 |
| **V7** | 188,4 | 178,6 | 171,6 | 161,6 | 175,05 | 11,31 | 6,46 |
| **V8** | 232 | 228 | 225 | 221 | 226,5 | 4,65 | 6,38 |

dard deviation, as well as the pH value of each of the creams. The results obtained reveal that the average viscosity of the creams ranged from 100.2 to 226.5 cP. Regarding the pH, all the creams presented values between 6.3 and 6.45, a range that favours the stability of the emulsion (McClements et al., 2019).

**Microscopic determination of floc sizes**

Many of the most important properties of emulsion-based food products are determined by droplet size that determines shelf life, appearance, texture, flavor profile, and biological fate (McClements, 2015). Consequently, it is important to be able to control the size of droplets in emulsions.

To determine the size of the fat globules in the different emulsions, the procedure described in Materials and Methods (section 3.5) was followed. Figure 4 shows a photomicrograph of a microscopic preparation of the V8 sample obtained at 400x magnification. All creams were tested under the same conditions.

Using the image analysis software, the different diameters of the particles observed in different fields were determined, using an integration reticule of 1 mm length with 100 divisions, to calibrate the size determination program. Figure 5 shows the diameters of the particles of the different creams obtained in a point cloud graph.

The substitution of vegetable oil 1 for another from another origin (vegetable oil 2,

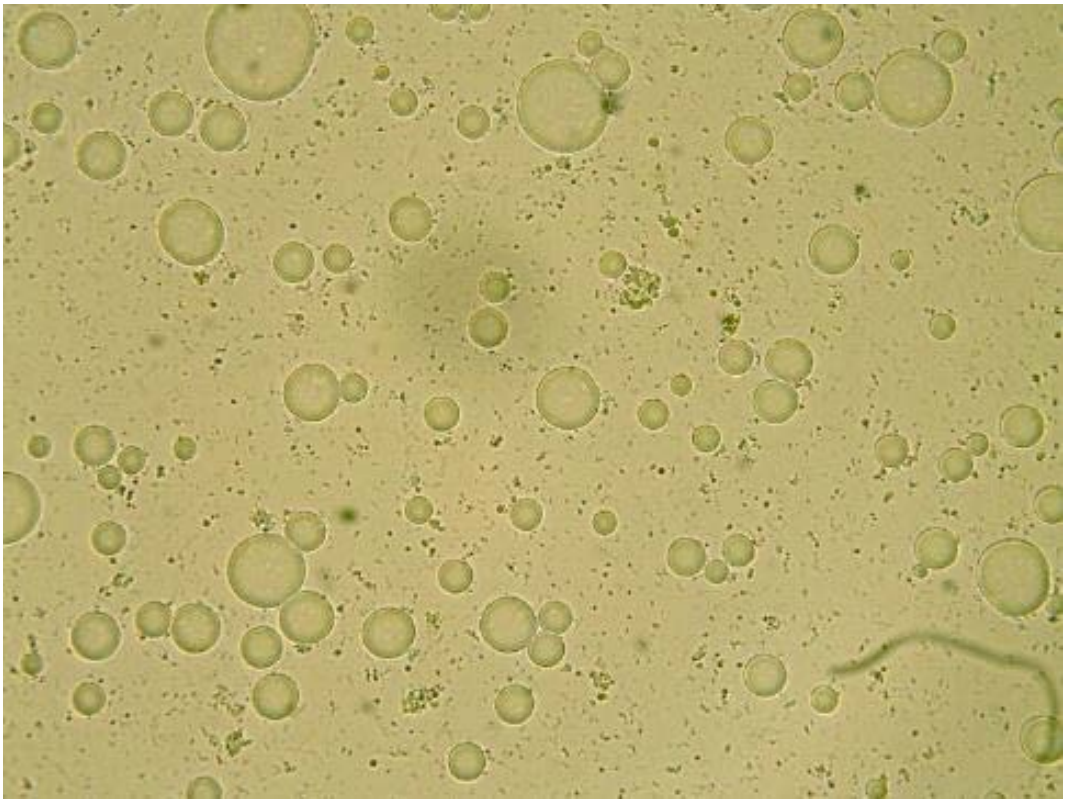

Figure 4.-. Photomicrograph of a preparation of V8 cream obtained at 400x magnification.

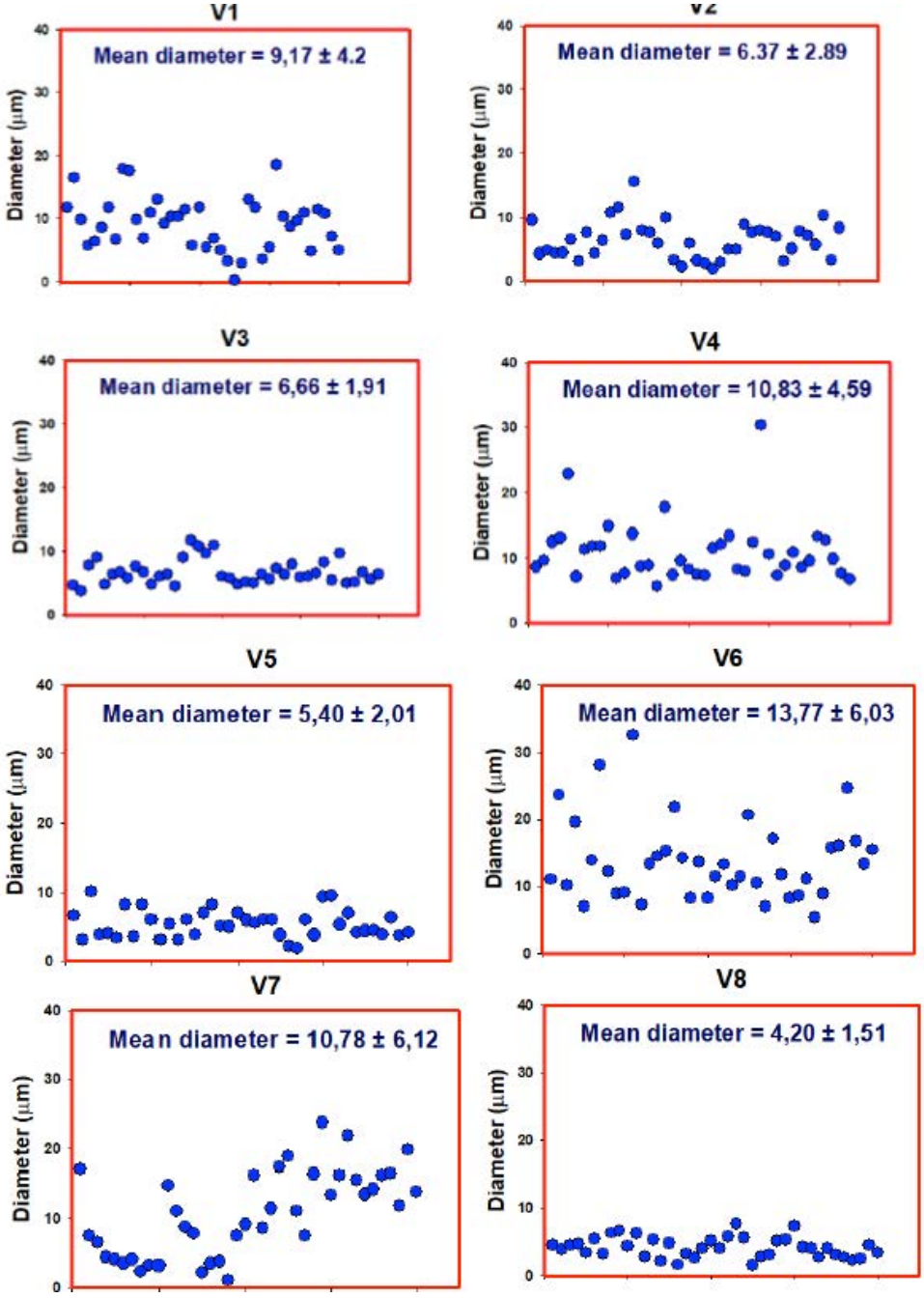


Figure 5. Representative distribution of the particle sizes ($\mu$m) in the different emulsions prepared. Numerical data represent the mean diameter of 40 fat globules quantified in different fields of the preparation. For each sample, the mean diameter and standard deviation are indicated.

V2), although it did modify the viscosity (Table 2), did not significantly alter the diameter of the particles. The substitution of emulsifier 1 for emulsifier 2 (V3) did not significantly modify the diameter either. The increase in the concentration of stabilizer 2 (V4) reduced the average diameter of the particles, although it was not significantly different from the control. The substitution of the thickening agent (stabilizer 2) for stabilizer 3 (V5) did not modify the diameter of the particles either, although a reduction in the average diameter was observed. However, increasing the concentration of the gelling agent (stabilizer 3) produced an increase in the average size of the particles, higher than the control, although the differences were not significantly different from the statistical point of view. The substitution of stabilizer 2 for stabilizer 4 (V8) did produce a significant reduction in the diameter of the fat globules, reaching the lowest mean value (4.2±1.51 $\mu$m).

If all the droplets within an emulsion have exactly the same dimensions, it is called a monodisperse emulsion, but if a variety of droplet sizes are present, it is called a polydisperse emulsion (McClements, 2015). The results reveal that all the prepared samples can be classified as polydisperse emulsions.

**Measurement of emulsion stability**

Physical stability refers to the ability of an emulsion to remain unchanged against the main alteration processes for a certain period of time. Oil-in-water emulsions tend to be thermodynamically unstable due to the large interfacial area between the oil phase and the water phase (McClements, 2004). The tendency to rearrange towards its initial state leads to local changes in size and concentration of particles or droplets. Therefore, the stability analysis is based on the measurement of these changes. Two protocols were used for the stability studies of the creams: a static and a dynamic method.

*Static method*

To analyze the gravitational separation of the emulsion, transparent containers are used, and the evolution of the samples stored in them is observed over time. The degree of creaming or sedimentation can be determined by measuring the thickness of the layers of cream or whey formed in a sample. For this, 10 mL of emulsion were transferred to a 15 mL graduated plastic test tube made of polypropylene with a screw cap (Figure 6). The tubes were stored for

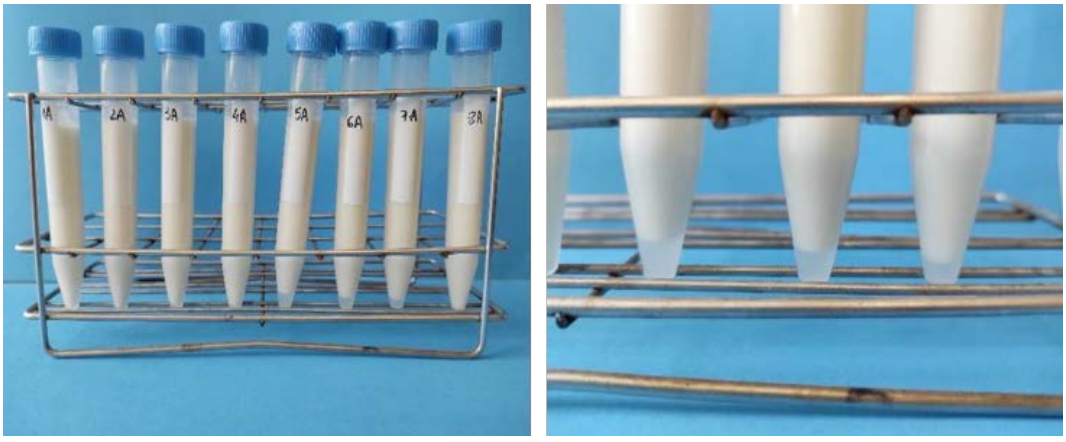


Figure 6. System used to determine the stability of emulsions by the static method. The phase separation can be seen in the lower part of the tubes, the cream, having less density, remains in the upper part.

14 days at different temperatures: 6 ºC (refrigerator), room temperature (23 ºC) and in an oven at 40 ºC. The stability (SR) was calculated as the percentage of the height of the aqueous layer with respect to the initial volume (Mirhosseini et al., 2008). Stability tests were performed on the prepared samples and a record of the phase behavior was made, observing a separation of the aqueous phase towards the bottom of the tubes and the emulsified phase remaining at the top of the tubes (Figure 6).

Table 3 shows the results obtained in the determination of stability by the static method. It should be noted that all the prepared creams were stable at low temperature (6 ºC) during the tests. At room temperature (22ºC) creams V3, V5, V6 and V7, at 7 days showed phase separation and at 14 days the two most stable creams were V4 and V8. The stability of the emulsions decreased considerably with increasing temperature, with phase separation being observed in all the creams. Of all of them, the most stable, in order, were V8, in which at 14 days only a phase separation of 1.2% was observed, and V4, in which the phase separation was 6%.

*Dynamic method*

To confirm the stability results, the stability of the creams was determined by the dynamic method using a LUMiSizer® multisample analytical centrifuge (L. U. M. GmbH, Berlin, Germany), which allows emulsions to be characterized by simultaneous measurement of light intensity transmitted as a function of time and position over the entire length of the sample. It allows analyzing the velocity distribution of particles and drops to detect the change in particle size in coalescence and flocculation processes, and the separation of phases in sedimentation or creaming processes. According to the transmission and retro-scattering profiles of light, obtained along the vial, it is possible to know the dynamic behavior of the emulsions, which translates into the possibility of detecting the two main destabilization phenomena that affect the homogeneity of dispersions such as particle migration (creaming and sedimentation) and particle size variation or aggregation (coalescence and flocculation) (Mengual et al., 1999).

Table 3.- Determination of the stability of the creams by the static method. The percentages of the aqueous phase formed by altering the emulsion at different incubation temperatures and at two test times: 7 and 14 days are shown.

| | **% Aqueous phase (after 7 days)** | | | **% Aqueous phase (after 14 days)** | | |
|---|---|---|---|---|---|---|
| **Sample** | **40ºC** | **Room T[a] (22ºC)** | **6ºC** | **40ºC** | **Room T[a] (22ºC)** | **6ºC** |
| **V1** | 0,5 | 0 | 0 | 7 | 3 | 0 |
| **V2** | 18 | 0 | 0 | 24 | 4 | 0 |
| **V3** | 45 | 0,25 | 0 | 50 | 8 | 0 |
| **V4** | 3 | 0 | 0 | 6 | 0 | 0 |
| **V5** | 4 | 2 | 0 | 9 | 7 | 0 |
| **V6** | 12 | 1,5 | 0 | 10 | 5 | 0 |
| **V7** | 7 | 1 | 0 | 4 | 2 | 0 |
| **V8** | 0,5 | 0 | 0 | 1,2 | 0 | 0 |

The following parameters were used: exposure time 15,200 s, speed 2867 rpm, wavelength 865 nm, LUM cell 2 mm temperature 25 °C. All measurements were repeated and tested at least twice. The analysis of results, using the SEPView software, allowed to determine the instability index.

Figure 7 shows the transmission profiles in space and their evolution over time of the different emulsions during analytical centrifugation.

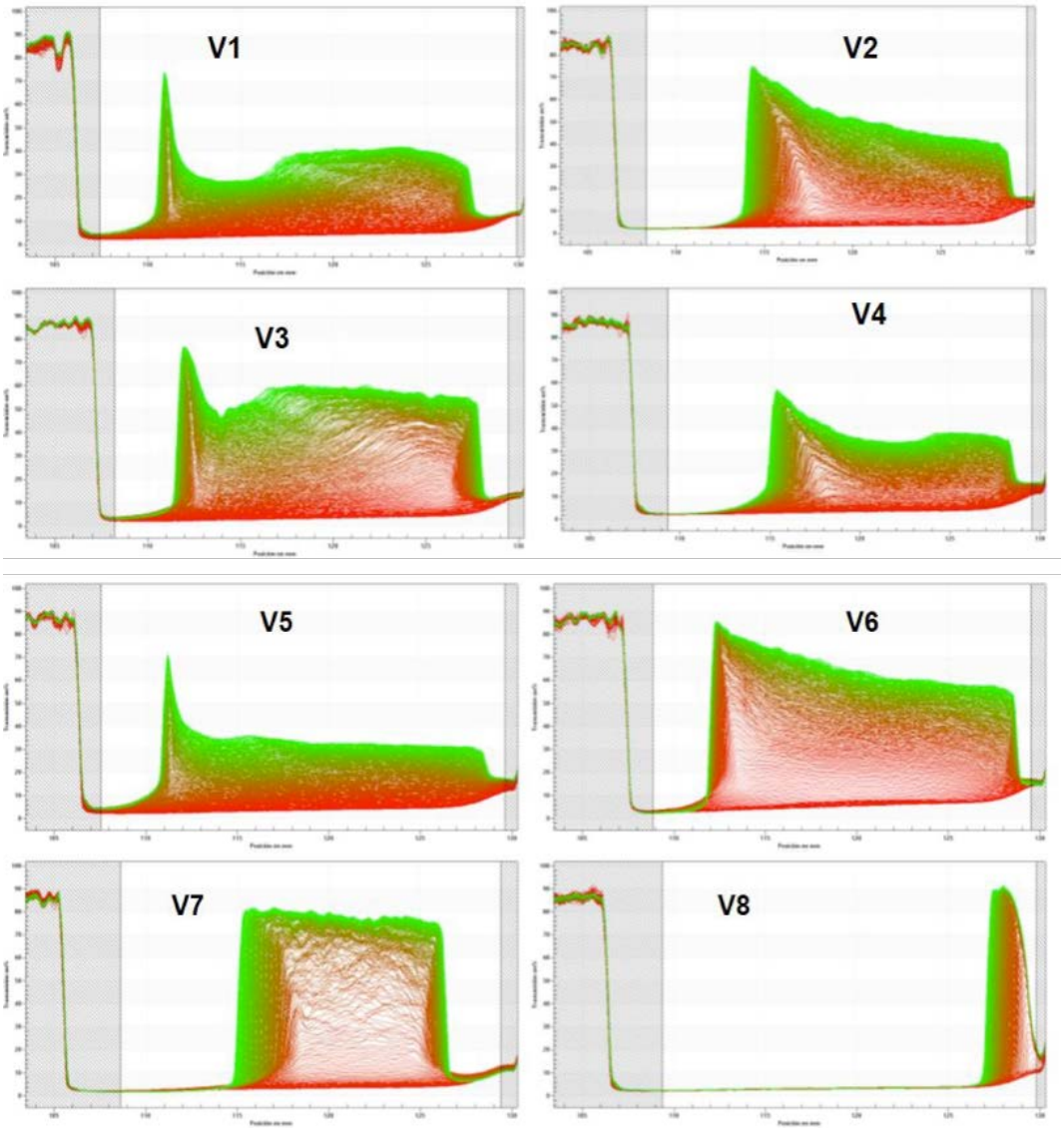


Figure 7. Transmission profiles obtained during the centrifugation of each of the creams obtained with the LUMiSizer®. Exposure time 15,200 s, speed 2867 rpm, wavelength 865 nm, LUM cell 2 mm temperature 25 °C.

The air-liquid phase boundary (concave meniscus) shows high transmission and is located to the left of 107 mm from the center of rotation, while the low transmission to the right of 107 mm represents the cream layer. The greater the change in transmission of the general profile with centrifugation, the less stable the emulsion will be (Li, Wang, Liu, Xu, Cao & Sun, 2018). The change of vegetable oil 1 in the control mixture (V1) increased the transmission profile (vegetable oil 2, V2) suggesting a reduction in cream stability. The substitution of the emulsifier 1 for emulsifier 2 also increased the transmittance profile (V3).

The increase in the concentration of the stabilizer 2 thickener practically did not modify the transmitted light profile, although the size of the cream was greater (V4). The substitution of stabilizer 2 for the gelling agent stabilizer 3 (0.17%) did not modify the transmission profile (V5) with respect to the control (V1), however, by doubling the concentration of stabilizer 3, transmission profiles (V6) were obtained. far superior to control. The substitution of stabilizer 2 for stabilizer 4 at a concentration of 0.12% did not improve the behavior of the cream during centrifugation, giving higher transmission profiles than the control, although it was observed that the size of the cream was greater. However, when increasing the concentration of this thickener (stabilizer 4) to a concentration of 0.15%, a significant reduction in the transmission profile (V8) was observed.

From the transmittance values, the instability index can be determined, whose values, at the end of the test, are shown in Figure 8. The lower the instability index, the more stable the emulsion obtained will be. Thus, the results obtained reveal that the V8 was the most stable cream of all the creams produced.

## DISCUSSION

The substitution of vegetable oil 1 for another from a different supplier (vegetable oil 2) increased the viscosity suggesting that the composition of the lipid phase, in relation to density, melting point, viscosity and surfa-

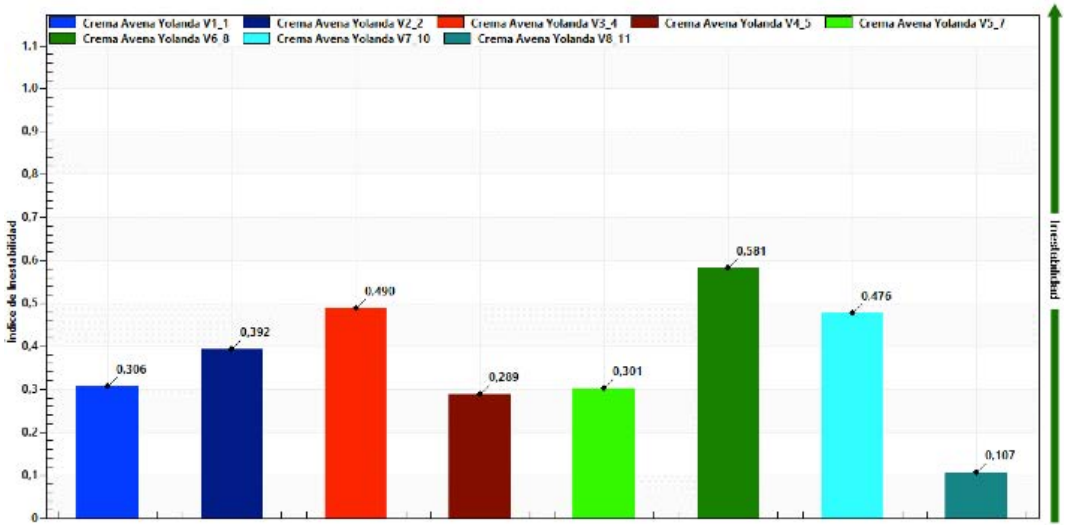


Figure 8. Instability index of the creams obtained after the analysis of the transmission profiles using the LUMiSizer®. Exposure time 15,200 s, speed 2867 rpm, wavelength 865 nm, LUM cell 2 mm temperature 25 °C.

ce tension, may have an important role in the elaboration of emulsions and consequently in their stability (McClements, 2015). Substituting emulsifier 1 for emulsifier 2 had a negative effect on viscosity, so it was decided to keep emulsifier 1 to make the rest of the creams. Lecithin is perhaps the most widely used natural emulsifier in the food, pharmaceutical, cosmetic and biotechnological industries due to its dispersive and emulsifying properties. It is a mixture of phospholipids, glycolipids and fatty acids. The polar structure of the lecithin molecules makes them excellent emulsifying agents by acting as a bridge between the lipids (apolar) and the aqueous phase (polar). Depending on the source of emulsifier, its composition varies, and in our case the differences between emulsifier 2 and emulsifier 1 lie in part in the composition of the different phospholipids, fatty acids and degree of unsaturation (Bot et al., 2021), which could explain the differences observed in the viscosity values.

Increasing the stabilizer 2 concentration from 0.03 to 0.04 improved the viscosity of the cream. The substitution of stabilizer 2 for stabilizer 3, at a concentration of 0.17 g/100 g of cream, did not modify the viscosity, although its value increased by doubling the concentration of this thickener. However, the substitution of the thickener for stabilizer 4 did significantly modify the viscosity (V7) with respect to the control (V1), and this effect was dependent on the concentration (V8).

Regarding the measurement of emulsion stability, although the static method is quite didactic and easy to carry out, it has some drawbacks related to the observation of the formation of the interface, due to the fact that during the separation the aqueous phase remains cloudy enough to indistinguishable from the emulsified phase. On the other hand, from the analysis of the transmission profiles, using the dynamic method, it is concluded that the change in the lipid phase, the emulsifier 2, high concentrations of stabilizer 3 and low concentration of stabilizer 4 generated more unstable emulsions than the control, while with greater concentration of stabilizer 4 tested, the most stable cream of all the creams produced was obtained (V8).

## CONCLUSIONS

The conclusions derived from this research work are the following:

1. The substitution of the oil phase (vegetable oil 1) for another of a different origin (vegetable oil 2) slightly increases the viscosity and reduces the average diameter of the particles, however, these changes do not have a direct effect on the stability of the emulsion, since the instability increases.
2. The substitution of the emulsifier 1 for emulsifier 2 slightly reduces the viscosity, increases the diameter of the fat globules and reduces the stability of the emulsion, possibly caused by differences in the composition of phospholipids and fatty acids.
3. The increase in the concentration of the stabilizer 2 has a direct effect on the viscosity, reduces the diameter of the particles and increases the stability of the emulsion.
4. The increase in the concentration of stabilizer 3, although it increases the viscosity, has a negative effect on the size of the particles and stability, suggesting that the concentration used is above the optimum, causing a destabilization of the oil/water interface, due to its high polarity.
5. The substitution of stabilizer 2 for stabilizer 4, at a concentration of 0.15%, increases the viscosity, reduces the size of the particles and increases the stability of the emulsion estimated with both methods, even being quite stable at high temperature.

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