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Escuela de Ciencias Biológicas e Ingeniería

TÍTULO: Study of the effect of microemulsions from Zingiber officinale extracts on broiler chicken production

Trabajo de integración curricular presentado como requisito para la obtención del título de Ingeniero Biomédico

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Dedication

I dedicate this thesis to my parents, who have always watched over me and my well-being, no matter the distance or the place from where they were taking care of me. To my brother who has been my greatest support and drive to continue. To my aunt, who made it possible for me to continue studying with the certainty that my family would be fine. Finally, to everyone who has been there for me in my best and worst moments and has always had good wishes for me.

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Resumen

Debido al trascendente uso de extractos fitoquímicos como aditivo en alimentos para diferentes animales, facilidad de acceso a ellos, costos módicos y diversos estudios que demuestran su funcionamiento como promotores de crecimiento, este estudio se centra en el uso de microemulsiones como sistema portador y de liberación de principios activos, que impulsará la efectividad del extracto de *Zingiber Officinale* en pollos broiler. Diferentes métodos de caracterización fueron herramientas clave para el desarrollo y estudio del producto resultante (microemulsión) como de sus componentes. Fourier transform infrared spectrometry, de la mano de la marcha fitoquímica, nos brinda una firma espectral respaldada con el estudio organoléptico del extracto. Dynamic Light Scattering junto con un estudio reológico nos proporcionan información del tamaño de partícula, comportamiento moléculas, viscosidad, plasticidad, elasticidad y derrame del producto. Para llegar a la formulación final de 18.16% tensoactivo, 34.91% fase orgánica and 46.93% fase acuosa, se realizaron varias pruebas de graduación de los componentes, así como del uso de varios tensioactivos: hexadecyltrimethylammonium brom, Sodium Lauryl Sulfat, y SPAN 60, Lecitina de yema y soya grado alimenticio. La administración del producto a la muestra de 200 pollos se realizó desde el día 8 al 42, en donde ya mostraron estar listos para el consumo. Se realizó un estudio estadístico para mostrar si la efectividad es significativa, llegando a la conclusión de que la administración adicional de este promotor de crecimiento sí favorece a la ganancia de peso de los pollos broiler.

Palabras Clave:

Microemulsiones, fitoquímicos, pollos de engorde, jengibre, *Zingiber officinale*.

Abstract

Due to the transcendent use of phytochemical extracts as feed additives for different animals, easy access to them, low costs and several studies that demonstrate their performance as growth promoters, this study focuses on the use of microemulsions as a carrier and release system of active ingredients, which will boost the effectiveness of *Zingiber Officinale* extract in broiler chickens. Different characterization methods were key tools for the development and study of the resulting product (microemulsion) and its components. Fourier transform infrared spectrometry, together with phytochemistry, provides us with a spectral signature supported by the organoleptic study of the extract. Dynamic Light Scattering together with a rheological study provides us with information on particle size, molecular behavior, viscosity, plasticity, elasticity and spillage of the product. To arrive at the final formulation of 18.16% surfactant, 34.91% organic phase and 46.93% aqueous extract phase, several component graduation tests were performed, as well as the use of several surfactants: hexadecyltrimethylammonium brom, Sodium Lauryl Sulfat, and SPAN 60, yolk lecithin and food grade soy lecithin. The product was administered to the sample of 200 chickens from day 8 to 42, when they were ready for consumption. A statistical study was carried out to show if the effectiveness is significant, reaching the conclusion that the additional administration of this growth promoter does favor broiler chick weight gain.

Keywords:
Microemulsions, phytochemicals, broilers, ginger, *Zingiber officinale*.

Contents

Chapter 1

Introduction

For years, various methods have been experimented with, to improve farm animal production, seeking to achieve a balance between animal welfare, environmental care, best quality products and preserving or increasing profits for the owner. These methods can be summarized as: conditioning of physical spaces that improve the quality of life of the farm animal, feed with quality certification and in defined portions according to days of birth and sex, constant and well-planned checks with control parameters that serve as a basis for monitoring and recording the development of these animals, and the use of growth promoters [1][2].

However, feeding is one of the most important parameters to consider in animal care and welfare, because it is reflected in key areas for the farmer such as: health, reproduction, weight gain, milk and meat production [3]. The implementation of nutritional supplements as growth promoters arises as an alternative in the optimization of animal production, to improve both performance and efficiency in the production of meat, milk or eggs [4].

Growth promoters are substances that are added in a controlled and planned way to feed the animals, allowing them to control their development, benefiting mainly in control parameters such as daily weight gain, meat quality, birth rate, mortality and milk or egg production [5]. There is no a single type of growth promoter, according to the place where they act or their results, they are classified as: androgens, estrogens, probiotics and antibiotics [6]. The latter are the ones that have been used the most, and the ones that have had the most negative consequences in several areas.

Motivation and problem statement

Many of the existing products on the market bring with them certain disadvantages or problems that cannot be ignored, such as: a) accumulation of antibiotic residues in meat and eggs: being responsible for the appearance of resistant bacteria in humans [4], b) economic losses: due to treatments against antibiotic-resistant bacteria or poor implementation of these supplements, c) digestion problems: due to the presence of components that cannot be digested by chickens, d) risk of disease: as another consequence of antibiotics, the natural balance of their intestine may be affected, e) contamination of water and/or soil: generated by chemical residues in these supplements [7][4][8]. This is how the idea of using plant extracts, natural additives, herbal products or mixtures of ingredients of natural origin as growth promoters to avoid to deal with all these problems arose.

Justification

Plant extracts have been used countless times as growth promoters because they produce no adverse effects on the animal. Additionally, the bioactive components of plants include: appetite stimulators, feed intakers, enhancemers of endogenous digestive enzyme secretion, stimulators of the immune response, antibacterial, antiviral and antioxidant actives. Not to mention that these types of extracts are cheaper and easier to access compared to other products on the market. This makes them a clear option when choosing a growth promoter [9].

Several studies have focused on demonstrating the effectiveness of the use of *Zingiber officinale*, commonly known as ginger, in different forms such as: dry root, powder, aqueous extract, combined, essential oil, among others. Althought, obtaining dissimilar or even contradictory results. According to Thomas et al., ginger in combination with turmeric, at different percentages, had significant influence on body weight, weight gain, feed intake, ready-to-cook performance, and net profit per kg live weight from 0 to 8 weeks in chicks [9]. Asghar et al. in their study "Exploration of *Zingiber officinale* effects on growth performance, immunity and gut morphology in broilers" showed that ginger had positive impact on cholesterol, triglycerides and gut microbiota [10]. In addition, ginger provided

improvement in growth and gut morphometry of broilers. Thus, it could be a better substitute for growth-promoting antibiotics [10]. More studies will be presented in the section state of the art. The effectiveness of the use of ginger microemulsions in the growth and development of broilers from Las Mercedes - Santo Domingo - Ecuador will be tested. The figure 1.1 is a photograph of the ginger rhizome, as it is ready for sale and/or consumption.

Figure 1.1: Rhizome of *Zingiber officinale*. Own authorship.

Contribution

Despite the amount of research in this area, there are just few studies that test nano or microemulsions as a delivery system. Micro and nano emulsions can be containers and carriers of active ingredients [11], which in animal feed means a better absorption of them. They also have the capacity to trap and protect substances, with possibly a controlled release from the interior of one phase to another. In addition, this type of emulsions improves feed preservation and protects unstable components [12]. This thesis provides a substantial contribution in the development of new methods for inclusion of phytochemical extracts in the animal diet of farm animals, specifically broilers.

1.1 Objectives

Under these considerations, the present work poses the following objectives.

1.1.1 General objective

To determine the effect of the use of *Zingiber Officinale* microemulsions as a growth promoter in broiler chickens.

1.1.2 Specific objectives

- To obtain a suitable microemulsion formulated with biocompatible raw material from natural origin. Using the mixed research method, integrating both qualitative and quantitative research.
- To compare the productivity of a private broiler farm using this emulsion as growth promoter through a statistically comparison using minimal significant difference assay.
- To test the effectiveness of emulsions as delivery system to improve the effect of *Zingiber Officinale* extract, as a growth promoter in broilers.
- To characterize the physical properties of the formulated emulsion by means of qualitative and quantitative characterization methods.

The theoretical basis for the development of the proposed objectives are as follow.

1.2 Growth promoters in farm animals

The nomadic human being, through agriculture and animal husbandry, managed to give way to sedentary life and advance by leaps and bounds to what we know today. Since then, animal husbandry became a means of survival [13].

Farm animals are essential species for mankind, representing the core of several activities they perform. For example, in feeding with direct products such as meat, eggs,

leather, wool and plumage, as well as with by-products such as milk, yogurt, cheese, among other important foods for the human diet. They are also used to perform tasks such as loading and transporting, planting and farming, and finally they offer protection and companionship to humans and to other animals. The types of farm animals are: bovine (cows, bulls and oxen), poultry (birds such as chickens, ducks, turkeys and geese), equine (mules, donkeys and horses), swine (pigs), sheep (lambs and sheep), goats (goats and similar animals), fish (edible domestic fish) and exotic (alpacas, camels, buffaloes, ostriches and other animals not typically found on farms).

The study and preparation for the care of these animals has gained ground over the years, supporting the improvement in feeding and breeding methods for farm animals. In terms of feeding, farm animals must have a feeding program. This program must be able to satisfy the nutritional requirements of each species, breed, sex and physiological state, supplying adequate amounts of feed, as well as quality feed. This feed must provide the necessary energy for the animal to perform well, as evidenced in health, herd welfare and productive and reproductive parameters such as birth weight, weaning weight, weight gain, milk and meat production and calving interval [3].

Likewise, different ways have been sought to obtain improvements in animal production, when we talk about improvements in animal production, we refer to everything necessary to keep livestock (all the different farm animals mentioned above) in a good state of health [14]. In order to reach adequate levels of production and meet all the requirements so that the animal protein provided is of the best quality and represents a good cost-benefit ratio for the producers [15]. Animal welfare is no longer an ethical proposition but an area of optimization that can improve the profitability of a business [16]. This is how the use of food complements or supplements, which do not harm animal welfare, came about.

The complements or supplements used additionally in the feeding of farm animals should be implemented according to the farm, the type of animal, the ages and physiological states, as well as being easy to handle and obtain [17]. Feed supplementation seeks to cover the requirements of the animals to increase productive responses. According to the

basic nutrients: energy, protein, minerals, vitamins and water, supplements are developed to help meet these needs [17]. Among these supplements, the so-called growth promoters stand out.

Supplementation is used as a strategy to increase production, because it decreases the amount of time animals spend grazing, without reducing the total amount of dry matter consumed, which has an important effect on the energy balance [18], to allow controlling the growth and development of farm animals, due to the increase in feed conversion efficiency, daily weight gain, meat quality and milk production [5].They have an action on protein anabolism, which results in a greater amount of muscle or meat in significant proportions, with a lower fat content.

Additionally, these food supplements reduce the production period, saving time and money in poultry production [6]. Among the types of growth promoters we have:

-**Androgens**: which act on muscle cells, producing muscular hypertrophy with a decrease in plasma amino acids and plasma urea with a positive nitrogen balance, with a decrease in urine excretion and an increase in STH somatotrophin [6].
-**Estrogens**: which act at the pituitary level, stimulating the production of STH, thyrotrophin (TSH) and adrenocorticotrophin (ACTH) [6].
-**Probiotics**: these are microbial inoculums that improve the intestinal microbial balance, however there is no research confirming their mode of action in the digestive tract [6].
-**Antibiotics**: their objective is to increase weight gain and feed conversion efficiency. They are added to minimize bacterial infections and control hepatic abscesses, common in feedlot fattening [6].

The agricultural area and its production is very broad, so we focus this study on a small branch, the production of broiler chickens. In Ecuador, broiler production has increased in recent years. CONAVE (National Corporation of Poultry Farmers of Ecuador) mentions that Ecuador produces all the chicken meat and table eggs consumed in the country. In 2020, 494 thousand tons of chicken meat were produced from the raising of 263 million

broiler chickens, concluding that on average an Ecuadorian consumes 28 kg of chicken per year per capita [19].

The effects of the use of growth promoters date back to the 1940s, when chickens were fed feed containing chlortetracycline residues to facilitate vitamin B12 absorption. There, chickens showed higher growth rates, high resistance to infection and faster feed conversion than chickens that were not fed feed containing antibiotics [20].

Since then, the use of synthetic growth promoters (SGP; such as antibiotics or beta-antagonists) or natural growth promoters (NGP; such as plant extracts, essential oils, fungal-derived enzymes, exogenous enzymes, direct-feeding microbes, prebiotics, phytobiotics, guanidinoacetic acid, spirulina, algal-derived polysaccharides and synbiotics) has expanded in their use for various animal species [21].

There are national and international entities, such as: the Food and Agriculture Organization of the United Nations (FAO), the World Organization for Animal Health (OIE), the United States Food and Drug Administration (FDA), the European Food Safety Authority (EFSA) and the Ministries of Agriculture and Livestock of each country. All these entities provide standards and guidelines for the regulation of food and feed supplements provided to farm animals, with the purpose of promoting animal health and welfare and veterinary public health worldwide [22], offering information on food-related risks, protecting consumers along the food chain [23], inspecting and providing the necessary preparation for farm management [24], as well as ensuring that additives and ingredients used in animal feed are safe and suitable for use [25].

The Ministry of Agriculture of the Government of Chile mentions that the control of animal feedstuffs is extremely necessary, since these feedstuffs can become carriers of disease agents or chemical, physical or biological contaminants, which can later be transmitted to humans [26]. In addition, they clarify that feed to be used in farms and other animal production establishments must come from establishments under official control and that the population must be adequately informed about this feed, for its proper commercializa-

tion and use [26]. The Ministry of the Presidency of Spain, issued an official state bulletin (BOE) in November 2019, where it provides information about infractions and sanctions related to animal safety and nutrition, regulations for feed producing or importing establishments, among other things, with the purpose of regulating the animal feed sector, as well as establishing the conditions that products intended for animal feed must comply with [27]. Compliance with and/or monitoring of these regulations makes it possible to guarantee food safety, avoid the ingestion of products contaminated with chemical, physical or biological agents and control the food chain from the grassroots level.

The use of phytochemicals as growth promoters has gained ground in the world of feed supplements for broilers and other animals. The present work focuses on the specific use of zingiber officinale to analyze its impact on the development of broiler chickens. Scientific articles published in the last 4 years, and one article from 2012, were used as the basis of information. We extracted from them, essentially the size of the group we worked with and the replicates used, the form in which they used ginger (either powder, dried root, chopped ginger, etc.), and the results they obtained. Google Scholar, Scopus and PubMed were the main sources to obtain both articles and additional information for this section. For a better and quicker understanding of these studies, they have been summarized in the table 1.1 below.

1.3 Broiler growth control parameters

Worldwide production of chicken meat has been increasing in recent years, making it more accessible and available in many places, becoming the main sources of low-cost protein [34]. Broilers are birds raised primarily for meat. Production parameters are intended to present an overview of the production performance of the flock, these parameters can be: related to body development, related to production or related to the final product, the measurement of these parameters is a process of gathering orderly, precise and reliable information collected during the production. To define the parameters to be measured, it is necessary to know the type of poultry to be produced, the equipment to be used, the

Table 1.1:

Summary of previous work and research together with the results obtained.

Reference	Group	Ginger shape	Result
[9]	240 chicks: 4 replicates of 10 each	Diet based on commercial desi chick starter. T1: C, T2: C + 0.50% T*, T3: C + 0.75% T*, T4: C + 0.50% ginger, T5: C + 0.75% ginger, T6: 0.50% T*+ 0.50% ginger + C.	Significant influence of ginger in combination with turmeric on parameters such as: body weight, feed consumption, among others.
[10]	1500 chicks: 5 treatment groups, each with 6 replicates.	5 experimental diets using basal diet. Antibiotics positive control, 3 g/kg (g of ginger/kg of diet, 6 g/kg, 9 g/kg and no antibiotics negative control.)	Supplementation with 0.6% ginger in the basal diet significantly improved growth performance and intestinal morphometry of broilers.
[28]	420 chickens: 4 groups and each group was further divided into 4 replicates, with 15 birds in each group.	Ginger powder at levels of 0%, 0.2%, 0.4% and 0.6%.	Substantial differences in body weight and weight gain between groups. Birds fed ginger had higher feed conversion and lower feed consumption than control birds.
[29]	128 chicks: 32 birds with four replicates.	Ginger root powder at levels of 0%, 0.5%, 1% and 1.5%.	The results showed that the inclusion of ginger root powder at 0.5% and 1% levels in the diet had a cholesterol-lowering effect, and the chick can tolerate up to 1.5% without adverse effects on growth and blood parameters.

[30]	120 chicks: 4 treatments with 5 replicates of 6 birds each.	Ginger powder at levels of 0, 2.5, 5.0 and 7.5 g/kg feed.	The inclusion of ginger powder had a positive impact on body weight, body weight gain, feed consumption, performance index, carcass characteristics and net profit per bird.
[31]	99 chicks: 3 treatments, with 3 replicates of 11 chicks.	T1 (control), T2 (1% garlic) and T3 (1% ginger)	Garlic and ginger at inclusion levels of 1% each improved growth and hematology.
[32]	200 broilers: 4 treatments with 20 replicates each.	Ginger powder at levels of 0, 0.25, 0.50 and 0.75 g of ginger in 1 kg of feed.	Ginger can be used as a growth promoter in broiler production.
[33]	132 chicks: 4 groups, with 3 replicates each.	Ginger flour at 0.0%, 0.5%, 1.0% and 1.5% levels.	Increased weight gain at reduced cost and up to a level of 1.5% to achieve reduced fat content in the meat.

Note: "T" it is the way in which the author designates the treatment he is using. The percentages presented are a representation of the amount of ginger in the chicken feed.

economic objectives of the farm and the geographical area. There are production parameters for broilers that facilitate the control and management of broiler production. This makes it easier to know if the management is adequate or not, and allows correct decisions to be made to correct the problem(s) that may affect production.

Commercial broilers have a high rate of rapid growth, high feed conversion rate, viability, yield and meat quality [35]. The goal in responsible broiler growing is to achieve the required flock performance in terms of bird welfare, live weight, feed conversion, uniformity and meat yield within economic constraints [36]. The most commonly used parameters in broiler farms are: birds starting to birds finishing day (n), viability, cumulative day mortality %, body weight in grams, feed consumption (g/a/d), feed conversion in kg/kg (kilograms of food / kilograms of weight gained), flock uniformity %, feed efficiency, daily weight gain (GDP), production index (PI), production efficiency factor (PEF), coefficient of variation (%CV), feed conversion factor (FCR), adjusted feed conversion factor (FCA), feed efficiency factor (FEP), feed efficiency (%CV) [36] [37]. A brief definition and calculation formulas for the aforementioned production parameters are detailed below:

Body weight:

Weight of standing bird (g).

Viability:

This is linked to the possibility of obtaining a uniform and non-negative rate of profit in production. [38].

$$\text{Viability} = \frac{\text{Current stock of birds (n x 100)}}{\text{Initial stock of birds (n)}} \tag{1.1}$$

Mortality (daily and cumulative %):

This presents the number of dead birds expressed as a percentage. It is recommended not to exceed 3 deaths per 10,000 birds [36] [37].

$$\text{Mortality} = \frac{\text{Number of deaths x 100}}{\text{Current stock of birds (n)}} \tag{1.2}$$

Feed consumption:

Feed consumption represents 60% to 70% of fixed production costs [36] [37].

$$\text{Feed consumption} = \frac{\text{Total feed offered (kg) x 1000}}{\text{Initial stock of birds (n)}} \tag{1.3}$$

Feed conversion (kg/kg):

Expresses the amount or units of feed that must be consumed per bird to produce one unit of product as meat. Conversion should be as low as possible to obtain the highest product yield [36] [37].

$$\text{Feed conversion} = \frac{\text{Total feed offered (kg) per flock}}{\text{Total kg of broiler - chick weight 1 day of age}} \tag{1.4}$$

Feed efficiency:

The number of kilograms of live chicken produced with one ton of feed (1000 kg) [36] [37].

$$\text{Feed efficiency} = \frac{\text{1000 kg feed}}{\text{Feed conversion}} \tag{1.5}$$

Daily weight gain (DWG):

It is the weight gained in relation to its days of age.

$$\text{DWG} = \frac{\text{Average final weight (kg) of standing bird}}{\text{Age (total days of breeding)}} \tag{1.6}$$

Production Index (PI):

The PI is measured in points or units that summarizes the above parameters and provides a figure that rates the flock, the higher the PI the higher the productivity of the flock [36] [37].

$$\text{PI} = \frac{\text{Average DWG (kg) x viability}}{\text{Average feed conversion per bird x 10}} \tag{1.7}$$

Production Efficiency Factor (PEF):

The higher this value, the better the technical performance. This calculation is strongly biased by daily gain. When comparisons are made between different environments, they should be made at similar slaughter ages. It is also known as the European Production Efficiency Factor (EPEF) [36] [37].

$$\text{FEP} = \frac{\text{Viability x live weight in kg}}{\text{Age in days x FCA}} \tag{1.8}$$

Percent Coefficient of Variation (%CV):

The higher the %CV, the more uniform and less variable the flock will be. The %CV is an important tool for estimating flock live weight [36] [37].

$$\%\text{CV} = \frac{\text{(Standard deviation) x 100}}{\text{Average body weight}} \tag{1.9}$$

Feed conversion factor (FCR):

The lower the FCR, the more efficient a bird (or sample of birds) will be at converting feed consumed into body live weight. It is especially important for broilers to have a good FCR, as they are usually processed to a target live weight and customers want to get as much marketable meat as possible [36] [37].

$$\text{FCR} = \frac{\text{Total feed consumed}}{\text{Total live weight}} \tag{1.10}$$

Adjusted Feed Conversion Factor (Adjusted FCA):

Adjusted FCA is a useful calculation when you want to measure how a flock is performing compared to the common target weight. It is also useful when making breed comparisons, as they can be analyzed against a specific target weight [36] [37].

$$\text{Adjusted FCA} = \frac{\text{Actual FCA + (target body weight - actual body weight)}}{\text{Factor}} \tag{1.11}$$

The factor in the above equation, depending on the units of measurement used, will

change. For mixed flocks, a factor of 10 lb, 4.5 kg or 4500 g should be used, depending on the unit of measure. This equation provides a good estimate of adjusted FCA for broiler performance comparison. However, it is important to note that adjusting the FCA to target weights beyond ± 0.5 lb / 0.227 kg / 227 g of the actual weight can distort the comparison[36] [37].

The chicks are ready for slaughter at an age of approximately 45 days. Considering that they have followed the four stages of the recommended feeding program, providing starter feeds during the incubation period, physiological transition and the first 14 days. On day 15 to 24 they are provided with grower feeds, from 25 days of age finisher feeds are usually provided and after 42 days of age they will require additional finisher feeds. Additionally, it should be considered that finisher feed represents the largest proportion of the total feed intake and feed cost of a broiler. To finally proceed to the feed withdrawal period in order to eliminate the risk of pharmaceutical residues in the meat and conclude the feeding plan effectively [36].

1.4 Digestion and metabolism in broiler chickens

Broilers, like animals of different species and breeds, have a unique metabolism and digestion. Knowledge of this metabolism is essential because it is important to their growth and health. Lipids, proteins and carbohydrates are the main sources of dietary energy in broilers, making up the largest proportion of their diet [39].

Water: Water makes up 55 to 75% of the body of the bird and about 65% of the egg. There is a strong correlation between feed and water intake. Water softens the feed in the crop and prepares it for grinding in the gizzard. In addition, it is necessary that the water contains the necessary minerals for the health and proper growth of the chicken [40].

Lipids: Involving fatty acids, there are a series of both metabolic and anabolic processes that create biologically important molecules such as triglycerides (TG), and phospholipids. The digestion of fat globules constituted by TG starts in the gizzard (muscular stomach)

and increases until it reaches the small intestine [41]. Lipid droplets decrease in size and mix with bile salts and pancreatic secretions, facilitating their contact with pancreatic lipase [42]. Enzymatic metabolism takes place in the small intestine, where pancreatic lipase produces the hydrolysis of TG bonds. The hydrolyzed lipids are returned to the gizzard (entero-gastric reflex) before being absorbed by the duodenum and the anterior part of the jejunum [41].

Protein: The body of a mature chicken is made up of more than 65% protein [40]. Broilers have diets rich in protein, because protein is a major component of biologically active compounds in the body [43]. The higher the crude protein level, the lower the crude fiber level and the better the performance of the chicks, especially in the first days of hatch [44]. The main source of vegetable protein is soybean meal, which is a good source of protein. This meal provides a very complete amino acid profile, and is a cost-effective energy source in formula [45].

Carbohydrates: They constitute the largest proportion in poultry feed, being the most important source of energy in chicken nutrition. This energy is used in vital functions such as the conservation of body temperature or movement [46]. The chicken does not have the enzyme system required to digest cellulose and other complex carbohydrates, so it is responsible for converting part of the component into crude fiber. The most important sources of carbohydrates for broilers are corn and wheat [40]. It should be taken into consideration that when the amount of carbohydrates is greater than the amount required by the animal, this will be stored in the form of glycogen and when there is an excess, it will be converted into fat in the animal's organism, constituting adipose tissue [46].

1.5 Emulsions

According to the Spanish Agency of Medicines and Health Products (AEMPS) "an emulsion is defined as a dispersed system, stabilized by the addition of a suitable emulsifier, of two immiscible phases, where both the internal and external phases are liquid. The particle size of the internal phase varies between 0.5 and 100 µm " [47]. Concepts such as internal

phase and external phase come to light. The internal phase, also known as dispersed or discontinuous, is that which is contained in the form of globules or micelles in the external phase. The external phase is known as dispersing or continuous [48]. An emulsion is a thermodynamically unstable dispersion of two or more immiscible or partially miscible liquids, which can become kinetically stable due to the presence of surfactants that have the ability to adsorb on the surfaces of the droplets [49].

1.5.1 Types of Emulsions

According to the substance that forms the external or internal phase of the emulsion or the droplet size they have, they can be classified as: two-phase emulsions, nanoemulsion, microemulsion and multiple emulsions [11]. Although the type of emulsion depends on the volume ratio of the phases, the viscosity of the phases and the preparation technology, it is generally the surfactant that determines the type of emulsion, using Bancroft's rule as a parameter. Bancroft's rule indicates that the external phase of an emulsion will always be the liquid in which the emulsifier dissolves or accumulates [50]. Figure 1.2 shows visually single and multiple emulsions, showing the organic part in yellow and the aqueous part in blue. In addition, a table 1.2 summarizing both the types of emulsions and their defining characteristics is presented.

1.5.2 Emulsifier

In addition, emulsifier or surfactant are chemical substances, which are used to reduce the surface tension between the two phases, allowing the formation and stabilization of emulsions. The difference between emulsifier and surfactant is that an emulsifier usually forms emulsions without foam and a surfactant generates emulsions that produce foam and are mainly used as detergents [48]. These agents are usually classified by the HLB (Hydrophilic-Lipophilic Balance) system, where each agent has a specific HLB number, which is used to predict the surfactant properties of a molecule. The HLB evaluates the relative strength of the hydrophilic and lipophilic ends of each emulsifier, so that the higher the value, the higher the hydrophilic character and the lower the lipophilic character, and vice versa. Unfortunately, this method is limited by the absence of HLB data for many

Table 1.2:

Summary of emulsion types and some of their defining characteristics.

Types of emulsions		**Characteristics**
Two-phase emulsions	Oleo-aqueous - Oil - water (O/W or O/A)	The external phase is aqueous and the internal phase is oily. By Bancroft's rule, generally these types of emulsions use emulsifiers that are more soluble in oil. They are known as hydrophobic emulsions [51][50][48].
	Aqueous-oil - Water - oil (W/O or A/O)	The external phase is oily and the internal phase is aqueous. By Bancroft's rule, generally these types of emulsions use emulsifiers that are more soluble in water. They are known as hydrophilic emulsions [51][50][48].
	Silicone (W/S)	The external and emulsifying phases are silicone derivatives and the internal phase is aqueous [51][50][48].
Microemulsions	They are dispersed systems containing droplets of dimensions smaller than 0.05 microns, but they are considered a bicontinuous structure in which there is no external or internal phase, being thermodynamically stable [51][50][48]	
Nanoemulsions	They have a droplet size of less than 100 nm and have an appearance similar to microemulsions. Although they may have high kinetic stability, they are not systems in thermodynamic equilibrium [51][50][48]	
Multiple emulsions	Water-acceptance-water (W/O/W)	A small aqueous phase is included in a larger oily phase, which in turn is dispersed in an aqueous phase [50][48].
	Accepted-water-accepted (O/W/O)	A small oily phase is included in a larger aqueous phase, which in turn is dispersed in an oily phase [51][50][48].

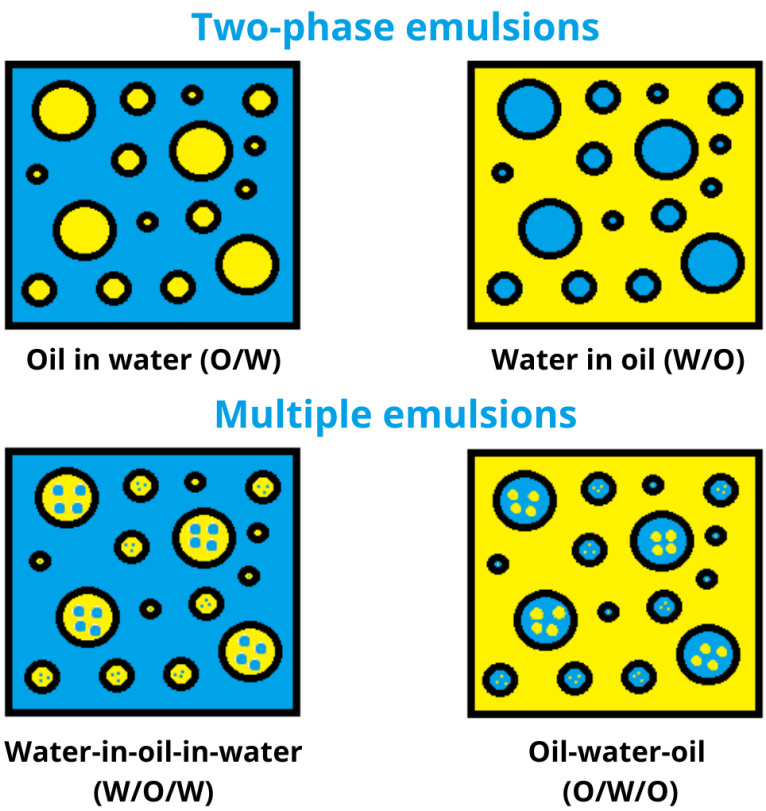

Figure 1.2: Simple and multiple emulsions. Own authorship.

chemical ingredients. [48]. The Figure 1.4 shows graphically the scale of HLB values and the type of agents it represents.

Surfactants can be classified according to: chemical constitution and application characteristics. In terms of chemical constitution they are subdivided into: primary or true emulsifiers, secondary or stabilizing emulsifiers and insoluble emulsifiers. Primary emulsifiers are all those that act directly on the surface tension, they are the only ones that simultaneously facilitate the obtaining of the emulsion and help in its stability, in this subgroup we have ionic and non-ionic surfactants. Secondary surfactants have almost no action on surface tension, and hardly help the stability of the product by mechanical means. Finally, insoluble surfactants form part of inorganic gels and it is not known whether stability is due to the accumulation of powder at the interface or to the increase of the viscosity of the external phase.

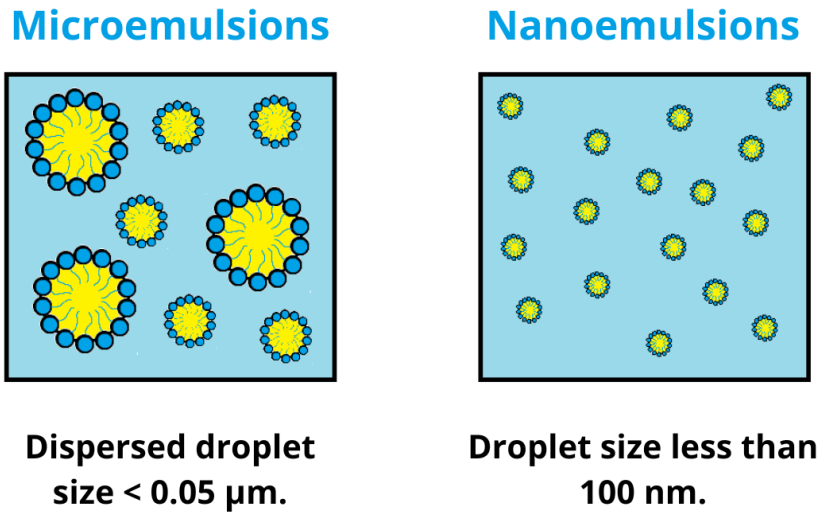

Figure 1.3: Nano and microemulsions. Own authorship.

Depending on their application, surfactants can be classified as emulsifiers, detergents, dispersants and solubilizers. Emulsifiers are emulsion promoters; for each type of emulsion it will be necessary to establish which emulsifier or system of emulsifiers is most suitable. Detergents favor or promote the process of residue removal from a surface by transforming from lipophilic to hydrophilic. A dispersant is used to keep solid particles homogeneously distributed in a liquid medium; surfactants do not by themselves create dispersion, but reduce the energy necessary for it to form. Finally, the solubilizer promotes the solubilization of poorly soluble substances [50]. See table 1.3.

1.5.3 Emulsion formation mechanisms

Different preparation equipment can be used for the formation of emulsions. The conventional ones are: the use of mechanical agitation, or high-pressure homogenization, colloid mills, ultrasonic homogenization among others. The choice of this equipment will depend on the emulsion components, the type of emulsion to be obtained and the scale of production.

High-speed mechanical agitation: This method is considered the simplest method for preparing emulsions. Emulsion is achieved by rotating an impeller connected to a motor inside a vessel. The shape of the impeller and the speed at which it rotates results in a cer-

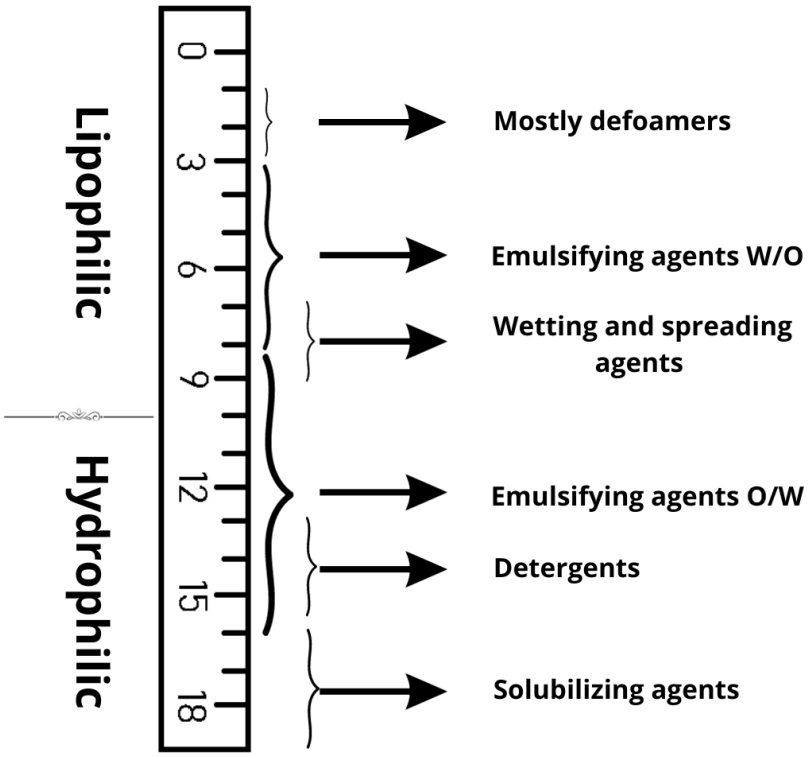

Figure 1.4: Scale of hydrophilic-lipophilic balance values for various surfactants. Own authorship.

tain flow pattern of the liquid inside the vessel, which will result in the desired emulsion [52].

High pressure homogenization: They are used to achieve high uniformity and higher stability, as well as to decrease the particle size in large droplet dispersions. This dispersion is compressed by passing through a reduced area section. The decrease in droplet size of the dispersed phase is due to the passage through the narrowing, causing an increase in flow velocity and high shear stress. It is used for the preparation of emulsions with not very high viscosity and for technical emulsions [52].

Colloid mill: its function is based on the movement of a rotor, which rotates at very high speed in the proximity of a conical stator. In order to create a high turbulence and favor mixing and particle size reduction, the conical stator has an irregular surface. The rotor-stator gap can be adjusted to control the final product size. This type of mill is used

Table 1.3:

Summary of the aforementioned classification of emulsifiers.

Classifier			
Chemical constitution	Primary or True	Ionic	Anionic
			Cationic
			Amphoterics
		Non-ionic	
	Secondary or Stabilizers		
Surfactant application	Insoluble		
	Emulsifier		
	Detergent		
	Dispersant		
	Solubilizer		

for the production of fine emulsions, stable colloidal solutions, suspensions and homogeneous mixtures [53].

Ultrasound: This equipment uses frequencies between 40 and 100 kHz. This equipment contacts a dispersion of coarse droplets with a piezoelectric material or magnetostriction. Like homogenizers, this equipment reduces the droplet size of coarse dispersions [52].

Methods for production of microemulsions and nanoemulsions

There are two types of methods for the production of micro and nanoemulsions: low energy methods and high energy methods.

High energy methods are commonly used methods to produce nanoemulsions. Devices such as microfluidization, high shear stirrers, high pressure homogenizers, jet disperser method and ultrasonic generators are used. The higher the energy input, the smaller the droplet size [54] [55].

High pressure homogenization: in addition to the above, it uses hydraulic shear, intense turbulence and cavitation as forces to produce nanoemulsions [55].

Microfluidization: A technique that uses a high-pressure displacement pump to produce fine nanoemulsions. Liquids from two opposing microchannels collide with each other in a common impact zone, developing high pressure that results in high shear. This method is not suitable for preparing large quantities of nanoemulsion [55].

Sonication: In addition to the information provided in previous sections, this method is widely used for small-scale production of nanoemulsion. To achieve a dispersed phase droplet size of 20 nm, it is necessary to optimize the design of the ultrasonic reaction chambers [55].

Jet disperser: In this method, two jets coming out of orifices (with a diameter between 0.3 and 0.5 mm) opposite each other, collide with each other. The jet diffuser can be used at high pressures up to 400 MPa, because both the disperser and the orifice plate contain no moving parts [55].

Low-energy methods, on the other hand, are systems that use the internal chemical energy of the system, and since they require simple agitation they are usually more energy efficient. Additionally, these methods can produce smaller droplet sizes than high-energy methods [54]. Among these low-energy methods are: stepwise addition of oil to a surfactant mixture with water, gradual addition of water to a solution of the surfactant in oil and mixing all components in the final composition, pre-equilibrating the samples prior to emulsification. Additionally, low energy methods include: spontaneous emulsification, phase inversion methods and solvent displacement method [55] [12].

Phase inversion method: It is also known as condensation method and it is based on the transition of a phase during the emulsification process. It can be achieved by: constant composition or constant temperature [55]. This method is limited by complexity, precision requirements and the use of synthetic surfactants [55]. There are two methods that can be used in phase inversion:

1. **Constant composition**: It is achieved by modifying the spontaneous curvature of nonionic surfactants with temperature (PIT phase inversion temperature method).
2. **Constant temperature**: It is achieved by varying the composition of the system using the emulsion inversion point (EIP) method.

Spontaneous emulsification: This can occur at room temperature without the need for special equipment. It is achieved by adding water to the oil and emulsifier solution step by step at constant temperature while gently stirring to produce O/W nanoemulsions. Emulsification depends on: surface tension, interfacial viscosity and phase transition, surfactant structure and concentration or proportions of each component [55].

Solvent displacement method: Similarly, it is achieved at room temperature by pouring the organic phase containing oil dissolved in a solvent (such as ethanol or acetone, which can be removed later by vacuum evaporation) into the aqueous phase containing surfactant [55].

Approaches to nanoparticle fabrication

The physical or chemical methods that can be used for the development of nanoparticles have two types of approaches: bottom-up and top-down.

Bottom-up: This approach refers to the construction of nanoparticles atom by atom [56]. It consists mainly of the condensation of atoms in the gas phase or in solution. The methods used in this approach are: supercritical fluid synthesis, colloidal method, photochemical and radiochemical reduction, microwave irradiation, use of dendrimers, solvothermal synthesis, sol-gel method, spinning and plasma spraying [56] [57]. Chemical methods are the most convenient for obtaining uniform and small nanoparticles [57].

Top-down: This approach refers to the reduction of material size, down to the nanometer scale. In other words, it consists of the division of solids into small portions, where methods such as: grinding, attrition, chemical methods, thermal evaporation, chemical

vapor deposition, preparation of gaseous clusters, ion implantation, external energy source or volatilization of a solid followed by condensation of its components can be used [56] [57]. In the case of the use of energy sources, we can highlight the use of ablation or laser irradiation of metals in a liquid medium. In turn, ultrasonic irritation also arises, where a liquid is irradiated with high intensity ultrasound. These energy sources can also be used in post-processing to modify the size, shape and composition of the nanostructure [58]. This approach is used to a lesser extent because, for the most part, complex instrumentation is required, which makes them expensive [57]. In this work a low energy methos with an approach type top-down was used, applying mechanical rotational shear stress.

1.5.4 Emulsions rupture mechanisms

It is important to mention that there are breakdown processes that lead to instability in emulsions. These processes should be studied both to prevent and to correct them. Emulsion breakdown involves four different mechanisms: creaming, flocculation, coalescence and Ostwald ripening [49][59]. The most important rupture mechanisms are presented visually in Figure 1.5 and summarized in table 1.4.

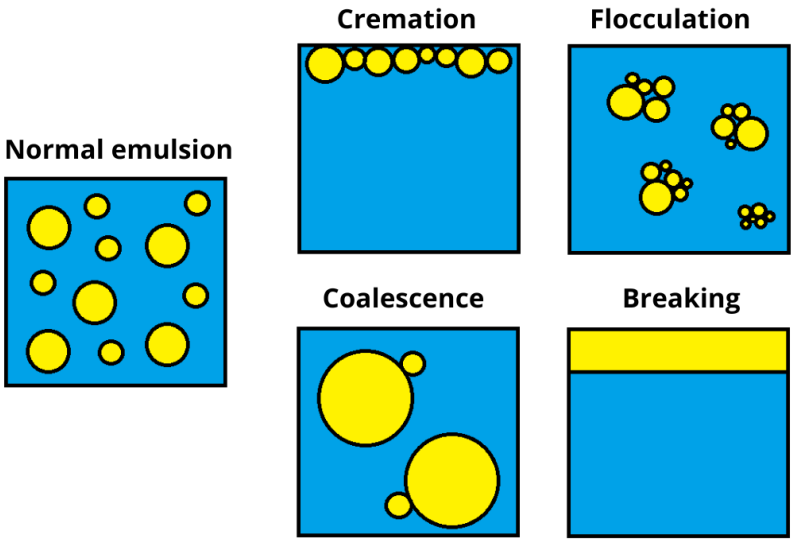

Figure 1.5: Emulsion rupture mechanisms. Own authorship.

Table 1.4:

Breakdown mechanisms involved in the stability of an emulsion summarized.

Mechanism	**Features**
Creaming/Sedimentation	It is a mechanism caused by gravity. Droplets concentrate on the surface without varying the droplet size distribution. Sedimentation is a similar process, but the droplets are concentrated at the bottom [49] [59].
Flocculation	The droplets adhere but do not coalesce. This process is controlled by an equilibrium between Van der Waals attractive forces and steric repulsive forces [49] [59].
Coalescence	The droplets coalesce into larger droplets. The larger the droplet size, the greater the tendency to coalesce [49] [59].
Ostwald ripening	It is due to the molecular diffusion of the component of the dispersed phase through the dispersing phase, from the small droplets to the large ones, until the small ones disappear [49] [59].

1.5.5 Characterization

Characterization is the identification of a material by studying its physical, chemical or structural properties [59]. In the case of emulsions, the characterization can be quantitative or qualitative.

Qualitative Characterization

In this case, it is the organoleptic characterization of the emulsion and its separate components, describing them also with techniques like phytochemical screening and qualitive FTIR.

Phytochemical march

Phytochemicals are naturally present in different plants, and are capable of having both positive and negative effects. Among the most important phytochemicals we have: alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, etc. [60]. With the use of various extraction techniques, it is possible to separate the phytochemicals from their plant material of origin.

These techniques are mainly based on maceration, infusion, percolation, infusion, digestion, extraction, among others [61]. The study and understanding of these organic chemicals is due to the fact that they facilitate the identification and extraction of the active principles, thus being able to isolate them in their pure form [62]. This identification makes it possible to predict to a certain extent the pharmacological action that a plant or substance containing the identified phytochemical could have; additionally, these pure forms make it possible to create "mold" for the development of synthetic drugs [63].

Phytochemical techniques are inexpensive and easy to perform, as it depends mainly on the type of solvent used. The phytochemical march is a totally quantitative method, providing only information on the presence or absence of a specific phytochemical or functional group, according to the reagent preparation used [61].

Fourier transform infrared spectrometry

Spectroscopic techniques are relatively simple, reproducible, non-destructive and have the advantage that they do not require a large amount of sample for analysis [64]. Infrared (IR) spectrometry has been a common method to investigate and delve into the structure, bonding and chemical functional groups of different materials from original or synthetic origin [65]. This type of analysis can be used to characterize samples in the form of liquids, solutions, pastes, powders, films, fibers and gases [66]. Fourier transform infrared spectrometry (FTIR) allows a qualitative and semi-quantitative analysis of the chemical composition of emulsions. With this method we can know the functional groups responsible for chemical or physical aging without destroying the internal equilibrium of the emulsion [67]. The spectral bands from FTIR are specific to each molecule due to vibrational bond-IR interactions.

The FTIR peaks are relatively narrow and, in many cases, can be associated with the vibration of a specific functional group, as well as with the vibration of a specific chemical bond in the molecule [64]. As for the procedure, the infrared radiation passes through an interferometer and comes into contact with the sample, the sample absorbs certain wavelengths and allows the passage of others that will be captured by a detector. The

fourier transform decodes the signal, which results in sprectrum, used to characterize and quantify the material. In other words, IR has atomic vibrations of a molecule, resulting in absorption and/or transmission of energy [66] [68]. For a better understanding, figure 1.6 provides an IR spectrum of palmitic acid, along with the peak values and formula of this compound.

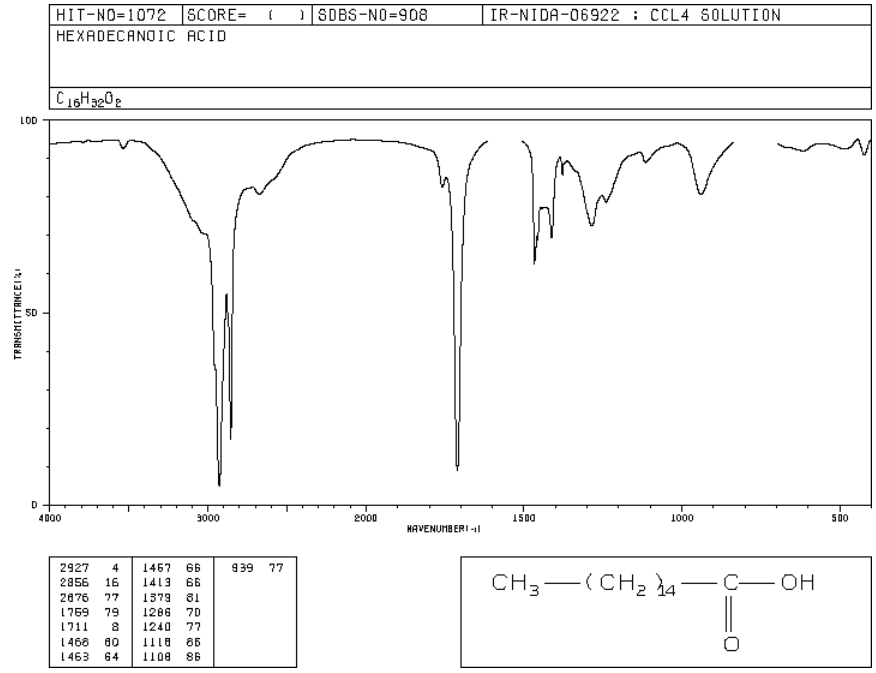

Figure 1.6: Spectrum of hexadecanoic acid known as palmitic acid.
Taken from: SDBSWeb : https://sdbs.db.aist.go.jp (National Institute of Advanced Industrial Science and Technology, date of access: 2023/08/03).

Quantitative characterization

Quantitative characterization is a process that establishes the relative amount of one or more species, or analytes, present in an emulsion. In other words, it allows us to determine the amount, in numbers, of a particular substance present in the sample [69]. Allowing a better understanding of the behavior of such emulsions in different environments and applications. In emulsions, the characteristics that are usually analyzed are: drop size, stability, viscosity, chemical composition, among others [70]. This thesis will use 3 methods for quantitative characterization of emulsions, which are: dynamic light scattering, infrared spectrometry and rheology, which will be detailed below.

Dynamic Light Scattering

Known as DLS or photon spectrometry or quasi-elastic light scattering, this technique is based on radiation-matter interaction [59]. It is a very powerful tool for studying the diffusion behavior of macromolecules in solution [71]. The DLS is a simple, easy and reproducible implement for particle size determination, executable in laboratory environments from biosafety level 1 upwards. The equipment has easy-to-use digital interfaces along with the possibility of full data analysis. This technique is non-invasive, does not require much sample preparation, nor does it require pre-calibration [72].

Its operation is based on a beam of monochromatic light (laser light) encountering a solution containing macromolecules. Depending on the size and shape of the macromolecules the light will be scattered in certain directions and then the intensity of the scattering is recorded by a detector. When the movement of the particles is monitored over time, information about the size of the molecules can be obtained, being it large when they scatter slowly and small when they scatter rapidly [71]. The operation is presented visually and simplified in Figure 1.7.

The movement of the molecules depends on the size, temperature and viscosity of the solvent [73]. Temperature is an important factor to consider when performing DLS measurements because it affects the viscosity of the solvent [74].

> "The digital autocorrelator then correlates intensity fluctuations of scattered light with respect to time (ns-μs) to determine how rapidly the intensity fluctuates, which is related to the diffusion behaviour of macromolecules." [71].

Rheology

A rheological study in emulsions is important because it provides information about the stability and microstructure of emulsions. Rheological properties allow us to evaluate emulsions and understand the best formulation, processing, handling, storage and trans-

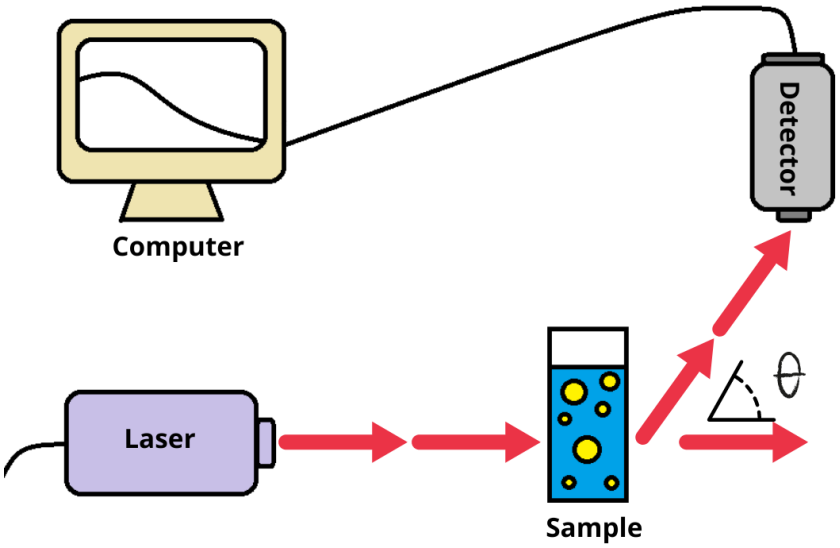

Figure 1.7: DLS operation. Own authorship.

portation needed to avoid problems of breakage or instability [75]. In the following, characteristics of the rheology of dilute systems will be presented in brief outlines.

Rheology of dilute systems

Regarding rheology of dilute systems, it is known that in these systems the droplets are so far apart that they do not interact with each other. They have the advantage that just considering the mechanics of a droplet is enough to develop the relationships that govern the macroscopic rheology of the system [75]. In dilute systems we have: single emulsions and multiple emulsions type A, B and C.

Simple emulsions: In simple emulsions, the hydrodynamic tension ($\eta_c\dot{\gamma}$) tends to stretch the droplet into a filamentary shape, while the interfacial tension ($\gamma\backslash$R) tends to contract the droplet into a sphere of radius R. The relationship between hydrodynamic tension and surface tension is represented by the capillary number. That is, as the capillary number increases, the droplet elongates and the angle between the major axis of the droplet and the direction of flow decreases [75].

Multiple emulsions: Regarding multiple emulsions we have type: A; where the glob-

ules can have a core-shell morphology, B and C; where the globules have a more complex morphology, being formed by several internal droplets [75].

Type A emulsions: In this type of multiple emulsions it must be taken into consideration that we have several layers that will form the globule, with different viscosities and radii. The inner core droplet will have radius "a" and the outer liquid layer will have thickness b-a. In terms of viscosities (η) we list: η_1; continuous phase (fluid 1), η_2; outer shell liquid (fluid 2), η_3; inner number liquid (fluid 3). The interfacial tension is measured between fluids 1 and 2 and between fluids 2 and 3 [75].

Type B and C emulsions: Due to the structural similarity between these two types of emulsions, they have been grouped together to present their most important characteristics. Two steps are followed for their rheological study: First; the viscosity of the individual globule of one of these multiple emulsions, which is itself the globule of a single emulsion, is obtained (hence the viscosity equation of a single emulsion is used). Second, the globules of these emulsions are treated as "homogeneous" droplets of viscosity obtained above [75]. In type A, B and C emulsions, it is necessary to take other considerations depending on the emulsion.

Rheology of moderately concentrated systems

In these systems the neighboring droplets are able to interact with each other hydrodynamically. It is important to mention that 3 approaches are used to model the rheological behavior in these emulsions: self-consistent effective medium approach; the emulsion is treated as an "effective medium", homogeneous and with equal rheological properties, cellular model approach; the emulsion droplets reside in well-defined unit cells in which the flow field is the same and differential effective medium approach; the emulsion has an initial continuous phase to which droplets are added until the final volume fraction of droplets is reached [75]. Single emulsion systems and multiple emulsions type A, B and C should also be considered, these are also analyzed using the above mentioned approaches.

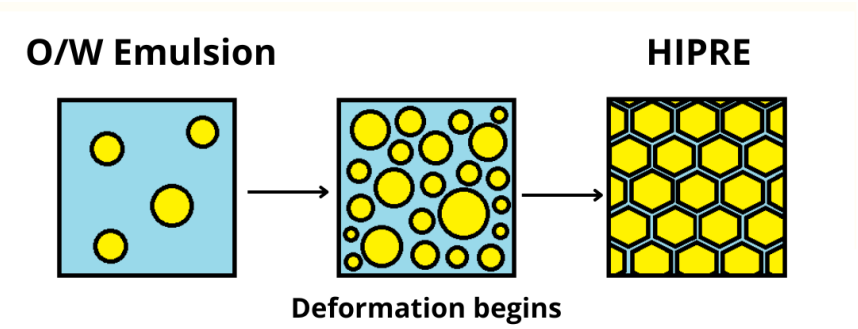

Figure 1.8: HIPREs emulsions. Own authorship.

Rheology of highly concentrated systems

In these systems the droplets touch each other without any significant deformation. They are high internal phase ratio emulsions (HIPREs) and are often referred to as gel emulsions. The droplets deform against their neighbors and take the shape of a polyhedron. In single emulsions the rheological properties are highly dependent on the average droplet size and polydispersity. In multiple emulsions the elasticity is mainly governed by the internal droplets when the volume fraction of internal droplets within the globule is large [75]. The figure 1.8 shows in a simplified form the droplet shape in a HIPRE emulsion.

Chapter 2

Materials and methods

2.1 Materials

2.1.1 Instruments

The glass and/or other materials used for the development of this research work are listed in this section.

- Polypropylene falcon tubes
- Beakers
- Parafilm
- Stirring rod
- Spatula
- Micropipettes
- Erlenmeyers
- Test tubes
- Magnetic stirrer
- Filter paper
- Scalpel
- Glass bottles with blue cap

2.1.2 Reagents

This section lists the reagents used for both the production of the growth promoter and its characterization used during the present research work.

- Sodium Lauryl Sulfate
- Hexadecyltrimethylammonium bromide (CTAB)
- Food grade soy lecithin
- SPAN 60
- Iodine
- Sodium hypochlorite
- Distilled water
- Copper acetate
- Glacial acetic acid
- Sulfuric acid
- Hydrochloric acid
- Sodium hydroxide
- Potassium hydroxide
- Acetic anhydride
- Ferric chloride
- Potassium iodide
- Copper sulfate
- Sodium potassium tartrate
- 95% ethanol

- Chloroform
- Gelatin

2.1.3 Equipment

This section lists the equipment used for both emulsion characterization and emulsion development. In addition, some specifications necessary for the understanding of its use are detailed.

- Stove
- Vortex agitator
- Heating plate
- Analytical balance
- pH potentiometer with calibration buffer
- Refractometer
- UV transilluminator
- **IKA T25 digital ULTRA-TURRAX ®:** Homogenizer for volumes from 1 to 2000 mL with speed indicator. Speed range: 3000 to 25000 rpm. Metallic and plastic dispersion elements; we use disposable plastic ones for their easy acquisition. Maximum supported viscosity of 5000 mPas. The principle of the rotor and stator is based on acceleration forces, shear stresses, intense thrust and high turbulence leading to optimal mixing [76].
- **LABCONCO FreeZone 2.5 Liter Benchtop Freeze Dryer:** Freeze dryer that removes a maximum of 2 liters of water in 24 hours. The lyophilization rate will be lower for samples that are not frozen. For optimum performance it should be at an ambient temperature of 21°C or colder [77].
- **Nanopartica Horiba SZ - 100 series:** Equipment produced in Kioto-Japan. Flexible analytical tool for characterizing the physical properties of small particles. It can

be used as a dynamic light analyzer (DLS) for particle size. The lower limit is influenced by the concentration, the strength with which the sample scatters light and the presence of unwanted large particles. The upper limit is influenced by the density of the sample. Measuring range: Particle diameter: 0.3 nm - 8.0 μm. Measuring accuracy: Particle size: Complies with ISO 13321/22412. NIST-traceable polystyrene latex particle standard: 100 nm measurement accuracy = +/- 2%. Measurement time: Approx. 2 minutes for particle sizes. Provides size measurements at 90° and 173°. Sampling cell: cuvette cell. Sampling volume: 12 μL - 4 mL (depends on cell capacity). Use 21 CFR part 11 software [78].

- **Agilent Cary 630 FTIR:** U.S. equipment. FTIR spectrometer featuring a 25 mm optical aperture and short internal optical path in the interferometer that provide performance levels associated with large laboratory systems. Unique Flexture system that drives the moving mirror of the interferometer providing reliability. Solid state laser provides long life, reliable operation and accuracy. Interchangeable sampling accessories that do not require user alignment. Uses MicroLab pharmaceutical FTIR software for simplified data processing, visualization and analysis [79].

- **Malvern/Bohlin Instruments Rheometer System CVO-100:** Equipment created by Malvern Panalytical, a UK company. Cone/plate rotational viscometer. Precision instruments designed to provide accurate rheological measurements on a wide variety of materials. Rheometer Software V6.32 Rheometer Software V6.21.2.0 2. Torque Range 0.5000 to 100 mN-m [80].

2.1.4 Software

This section mentions the software with which we have interacted for characterization, statistical analysis and text editor for the realization of this project.

- RStudio version 2023.06.1
- Overleaf version 2023
- Software Agilent MicroLab FTIR

- 21 CFR part 11
- Rheometer software V6.21.2.0 2

2.2 Methods

For the preparation of the growth promoter, an emulsion is formulated, which consists of an organic phase, an aqueous phase and the surfactant. To make both the aqueous phase (aqueous organic extract) and the emulsion, a general procedure was followed as detailed below. Likewise, different surfactants such as: CTAB, sodium lauryl sulfate, egg yolk lecithin, span 60 and food grade soy lecithin were tested in order to find the most viable option for our objectives.

2.2.1 General procedure

Aqueous ginger extract:

1. Wash the ginger (previously stored in a humidity and light free environment) until the skin is completely clean. In this way we avoid having to peel the ginger.
2. Immerse the ginger in a 0.5% sodium hypochlorite solution (5 mL of sodium hypochlorite in 1000 mL of water), for approximately 5 minutes.
3. Rinse the ginger with plenty of water until all traces of chlorine are removed. In this way we avoid contamination by chlorine and other external factors.
4. Cut the ginger with a mirepoix type of cut. See figure 2.1 as a reference. This size allows the filling of the jar as well as an easy extraction and obtaining of the ginger extract.
5. Introduce the ginger, previously chopped, into the glass jars with blue cap, fill until the ginger is between the neck and shoulder of the bottle.
6. Fill with distilled water until the bottle is full, making sure to cover the ginger completely.

7. Close the bottle and cover the lid with parafilm to avoid any contamination. Figure 2.1 shows the ginger ready to move to the next stage.

8. Place the extract at room temperature and away from any light.

9. Allow to macerate for 48 to 72 hours, for use in the emulsion.

Note: The use of gloves and mask is recommended during the whole process. It is also recommended to decontaminate all the instruments that will be used during the process.

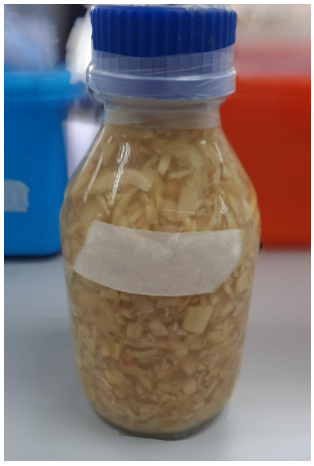

Figure 2.1: Aqueous extract. Own authorship.

General emulsion

1. Weigh in grams, with the aid of an analytical balance and beakers, each of the components: aqueous extract for the aqueous phase, vegetable oil for the organic phase and the surfactant. The order of these components will be specified according to the type of surfactant used.

2. Seal the beakers with the components using parafilm for their isolation from the environment, until we have to homogenize. Figure 2.2 shows the beakers filled with the 3 phases ready to homogenize.

3. Homogenize, first at low speed from 2000 rpm to 3000 rpm until the 3 elements of the emulsion are combined.

4. Homogenize at maximum speed of 25000 rpm until the desired emulsion of creamy consistency and homogeneous color (light beige) is obtained. To facilitate the emulsification, make rotating movements from bottom to top of the beaker.

5. Carefully remove from the homogenizer and store in jars with lids, sealed with parafilm.

Figure 2.2: Beakers after weighing and sealing procedure. Own authorship.

Note: The use of gloves and mask is recommended during the whole process. Likewise, it is recommended the previous decontamination of all the instruments that will be used during the process. Label each one of the bottles with the date of elaboration and the appropriate dosage. During homogenization, avoid rubbing the sides and base of the beakers.

Specifications according to the type of surfactant:

Small tips will be detailed to obtain better results and facilitate emulsification with different types of surfactants.

CTAB and sodium lauryl sulfate: In both cases the aqueous extract should be weighed, then the oil and finally the surfactant (powdery or granular). The high foam production should be taken into consideration, so it is preferable to use bottles with wide necks to avoid spills and loss of product.

SPAN 60: In this case it is necessary to consider first weighing the liquid materials, both the aqueous and the organic phase, finally the solid span is added. And then take it to the homogenizer. Keep it at low speed long enough to dissolve the span 60 lamellae, the time may be longer than with other types of surfactant.

Lecithin obtained from egg yolk: In order to use this type of surfactant, we perform the following procedure:

1. Clean the eggshell with 70% ethanol and disposable paper towels.
2. Break the shell and extract the egg yolk, discarding the white and the shell.
3. Remove the membrane covering the yolk, using a spatula.
4. Store the obtained yolks, free of membrane, in glass jars with lids. Avoid overfilling these jars for easy freeze-drying.
5. Seal the jars with parafilm and place in the freezer.
6. When the yolks are completely frozen, remove the lid and pierce the parafilm.
7. Place in the freeze dryer and leave for approximately 2-3 days, until the yolks are completely freeze-dried.
8. Additionally, to use in the emulsion, the freeze-dried yolk should be weighed first, then the vegetable oil and finally the aqueous extract.

Food grade soy lecithin: In this case the soy lecithin should be weighed first, then the aqueous extract and finally the vegetable oil. Before carrying out the first low-speed homogenization, stir manually with a stirring rod until most of the lecithin is dissolved; this will facilitate homogenization with the IKA T25.

Chapter 3

Results and discussion

3.1 Emulsion components characterization

Both the characterization methods and the results obtained during this work are shown below.

3.1.1 Phytochemical screening

A phytochemical run was performed with at least 3 replicates per assay, for each extract produced and used during this degree work. All these qualitative tests are based on complexation reactions, due to the presence of the organic group that is reacting with a transition metal, which in turn have enough free orbitals to accept electrons from external pi orbitals of those organic compounds, when the electrons between these two are shared, a colored compound is generated, giving as a positive result the presence of the functional group tested. For example, in the case of alkaloids, metals such as Bi, Fe, Cu can produce these colorings or even precipitates with characteristic colors. For the study of carbohydrates present or not in the sample, Barfoed's test uses copper acetate and glacial acetic acid, whose reddish coloration would indicate the presence of carbohydrates. In the case of other functional groups such as reducing sugars, these qualitative reactions are determined by "redox" mechanisms, where the sugar is the reducing agent and the transition metal used such as Cu can be reduced from Cu2+ to Cu1+ obtaining the characteristic red precipitate, while the sugar is oxidized to a simpler compound or even CO2, depending on the strength of the chemical reaction. Keller-Killani for the presence of cardiac glyco-

sides, would show a blue solution when interacting with the filtrate, due to its use of ferric chloride, glacial acetic acid and sulfuric acid. Biuret test for amino acids and proteins bases its characterization on potassium hydroxide and copper sulfate, giving rise to a pink coloration as an indicator of their presence. To better summarize the reagents used, the following table 3.1 is presented.

3.1.2 FTIR

Infrared analyses were performed on 4 different extracts, from different days, produced and used during the development of the present work. For the aqueous phase, both distilled water and bottled water were used. In this case, it was taken into consideration that the use of bottled water in the case of yolk generated a large amount of bacterial growth, leading to the instability of the emulsion. On the other hand, with distilled water and the use of food grade soy lecithin, this contamination and/or bacterial propagation was avoided. Additionally, the same analysis was also performed for both the surfactant and the oil used. The figures shown for all the spectra were provided by the equipment software. It is important to emphasize that [64] was taken as a guide for the analysis of the spectra obtained.

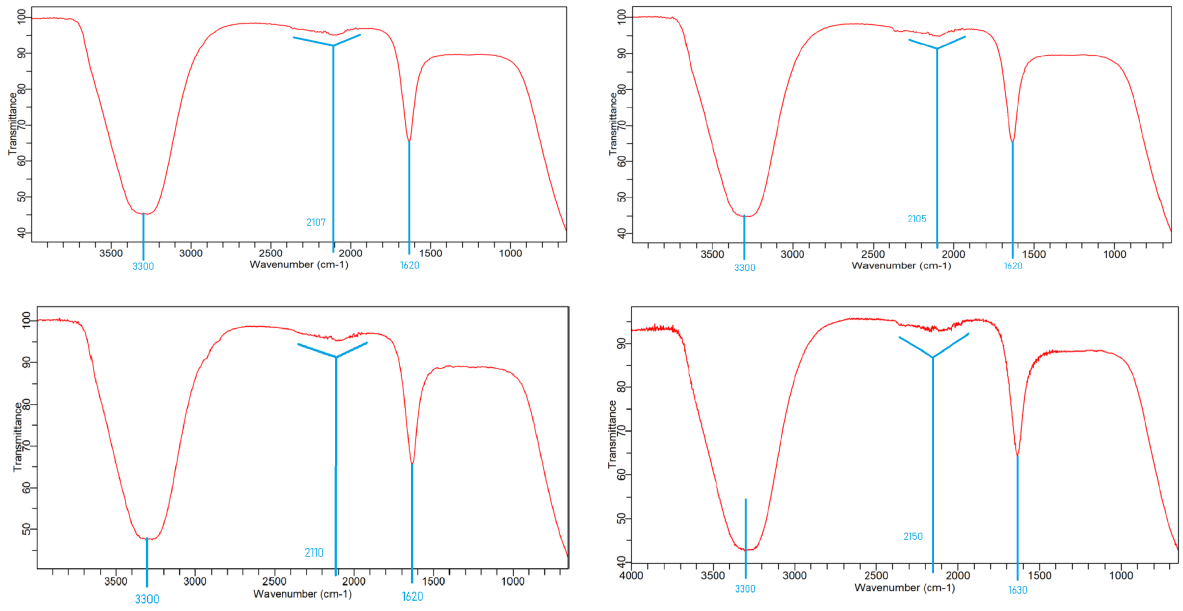

Figure 3.1: Spectra obtained from different samples of aqueous extract of *Zingiber Officinale*.

Table 3.1:
Phytochemical march results.

Compound	Test	Reagents	Result
Alkaloids	Wagner	Iodine + potassium iodide + distilled water	Positive
Carbohydrates	Barfoed	Copper acetate + glacial acetic acid	Negative
Reducing sugars	Fehling	Copper sulfate + distilled water + sodium potassium tatrate + NaOH	Positive
Cardiac Glycosides	Keller-Killani	Glacial acetic acid + ferric chloride + sulfuric acid	Positive
Proteins and amino acids	Biuret	Copper sulfate + ethanol + KOH	Positive
Flavonoids	Alkaline reagent	NaOH + hydrochloric acid	Positive
Phenolic compounds	Test for Cartenoids	Chloroform + sulfuric acid	Positive - low
Tannins	Gelatin	Gelatin solution + NaCl	Positive
Phlobatannins	Foam	Hydrochloric acid	Negative
Phytosterols	Salkowski	sufuric acid	Positive
Cholesterol	No name	chloroform + acetic anhydride + sulfuric acid	Positive
Terpinoids	No name	chloroform + sulfuric acid	Positive
Triterpinoids	Salkowski	sulfuric acid	Positive
Diterpenes	Copper acetate	distilled water + copper acetate	Positive
Lignins	Labat	gallic acid	Negative
Quinones	Alcoholic KOH	alcoholic potassium hydroxide	Negative
Anthraquinones	Borntrager	ammonia solution	Negative
Coumarins	NaOH	NaOH + Chloroform	Positivo
Gums and Mucilages	Alcohol	distilled water + absolute alcohol	Negative
Resins	Acetic anhydride	Acetic anhydride solution + sulfuric acid	Negative
Fixed Oils and Fat	Spot	Extract pressed on filter paper	Positive
Volatile Oils	Fluorescence	Extract filtered till saturation	Positive

Note: At least 3 replicates were performed for each extract, before being used to prepare the microemulsion. For more detailed information on how to perform each of the tests, please visit to reference [61].

According to the peaks obtained in the spectra of the figure 3.1, we have coincidence in the four spectra shown of a very pronounced peak at ~ 3300 cm^{-1} with a transmittance between 40 and 50, being the indicator of amide A bands (carbonyl group attached to an amine group) derived from N-H stretching modes in proteins and nucleic acids. Additionally we can notice in Table 3.1 that we also obtained positive in proteins and amino acids, thus having a double verification. As mentioned before, proteins constitute more than 65% of an adult chicken and constitute an important part of its diet, forming part of biologically active compounds within its organism. Subsequently, we have a set of peaks forming a curvature whose midpoint varies between ~ 2150 cm^{-1} to ~ 2105 cm^{-1} wavelength and with an approximate transmittance between 90 to 100, signifying the presence of a combination of hindered rotation and O-H bending, i.e. water. Being an aqueous extract these peaks were expected and corroborate the presence of water. Finally, we have a last very intense peak at approximately ~ 1620 cm-1, they could have variations of 10^{-1} and a transmittance between 60 and 70, this peak means the presence of nucleic acids due to the stretching of the base carbonyl and the annular respiration mode, in addition to an amide region, again this information is corroborated with the phytochemical march presented in table 3.1. The presence of amides, as well as amino acids, indicates that the organic extract of *Zingiber Officinale* presents proteins, and could have hormones and vitamins, important substances in the diet of farm animals.

In the spectrum obtained with a sample of commercial soy lecithin (Figure 3.2), we can observe peaks at ~ 3300 cm^{-1}, indicating the presence of amide bands, related to proteins and nucleic acids. To the right, it is followed by a group of 3 consecutive peaks at ~ 3007 cm^{-1}, indicating the presence of C-H rings, at ~ 2915 cm^{-1} signifying the presence of cholesterol, phospholipids and creatine, CH, CH2 and CH3 stretching vibrations, and the third peak at ~ 2850 cm^{-1} being CH, CH2 stretching bands, indicating lipids and fatty acids. We have a peak between ~ 2105 cm^{-1} and ~ 2090 cm^{-1}, this is due to the combination of hindered rotation and O-H bending (water), a peak at ~ 1990 cm^{-1} shows the presence of a second order band. A peak at ~ 1738 cm^{-1} is a sign of C=O stretching, presence of polysaccharides, lipids and hemicellulose, as well as vibrational stretching indicating phospholipids. Another at ~ 1650 cm^{-1} is due to amide absorption, C=O vi-

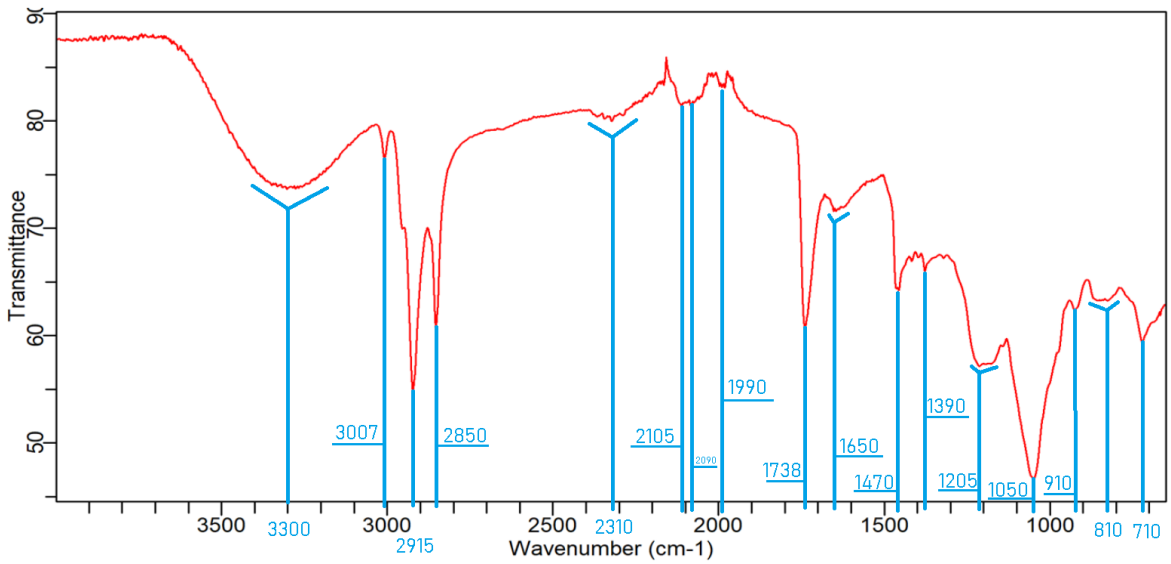

Figure 3.2: Spectrum obtained with a sample of commercial soy lecithin.

brational stretching of the amide, C=C stretching of uracil, NH2 guanine, C2=O cytosine and other nitrogenous bases. The peak at $\sim$ 1470 cm^{-1} is a CH2 bending of methylene chains in lipids, the peak at $\sim$ 1390cm^{-1} is due to carbon particles, at $\sim$ 1205 cm^{-1} we have a weak peak representing little presence of polysaccharide rings, and at $\sim$ 1050 cm^{-1} are phosphates and oligosaccharides, antisymmetric P-O-C stretching modes of phosphate ester and C-OH stretching of oligosaccharides. Finally, we have 3 peaks: $\sim$ 910 cm^{-1}, $\sim$ 810 cm^{-1} and $\sim$ 710 cm^{-1}, these being signals from the phosphodiester region, CH ring deformations and out-of-plane bending vibrations.

As can be seen in the figure 3.3 ,the yolk lecithin and commercial grade lecithin show similarities in their spectra, such as in the peaks $\sim$ 3300 cm^{-1} - $\sim$ 3290 cm^{-1}, $\sim$ 3000 cm^{-1}- $\sim$ 3005 cm^{-1}, $\sim$ 2910 cm^{-1}- 2915 cm^{-1}, $\sim$ 2850 cm^{-1}, $\sim$ 1738 cm^{-1}- $\sim$ 1745 cm^{-1}, $\sim$ 1620 cm^{-1}- $\sim$ 1650 cm^{-1}, $\sim$ 1470 cm^{-1}, $\sim$ 1390 cm^{-1}, $\sim$ 1205 cm^{-1}- $\sim$ 1215 cm^{-1}, $\sim$ 1050 cm^{-1}- $\sim$ 1080 cm^{-1}, $\sim$ 910 cm^{-1}, $\sim$ 710 cm^{-1}. In addition, they have notable differences, the group of 3 peaks $\sim$ 2105 cm^{-1}, $\sim$ 2090 cm^{-1} and $\sim$ 1990 cm^{-1} which are second order bands and a combination of hindered rotation and O-H bending (water). The yolk spectrum also has additional peaks at: $\sim$ 1180 cm^{-1}, $\sim$ 890 cm^{-1}, $\sim$ 700 cm^{-1}, $\sim$ 680 cm^{-1}, being respectively, an Amide III, C-O and C-C deoxyribose region band, and out-of-plane bending vibrations. These results show that both lectins could provide similar results at the time of emulsifi-

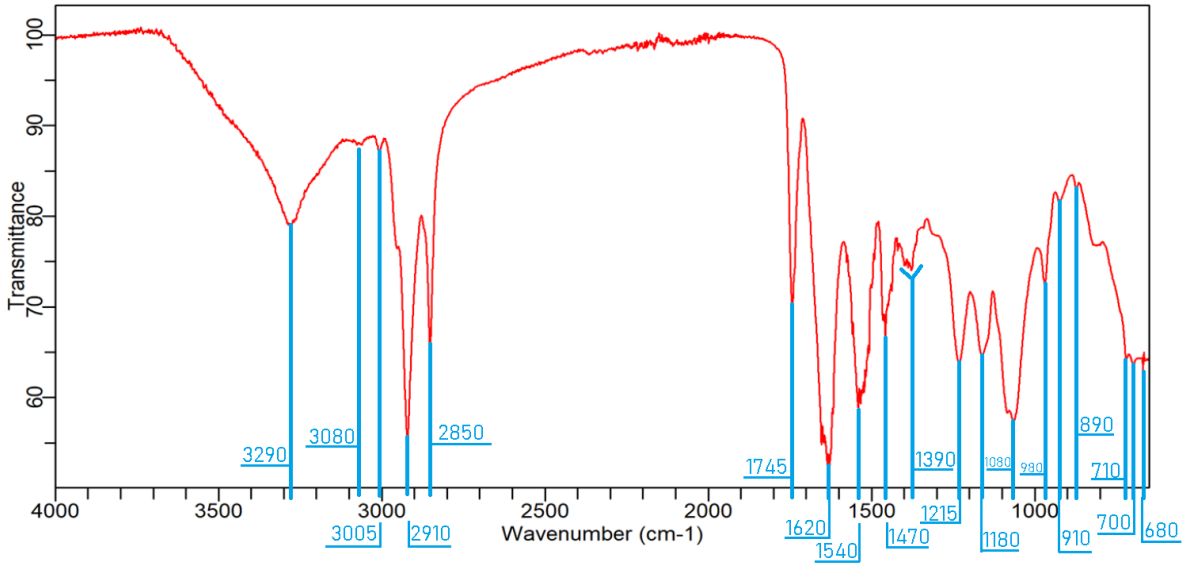

Figure 3.3: Spectrum obtained with a sample of lecithin obtained from egg yolk.

cation, however the small variations in peaks and the additional peaks presented by the yolk lecithin could be the cause of the instability in the emulsion, as well as favoring the production of microorganisms and bacterial development.

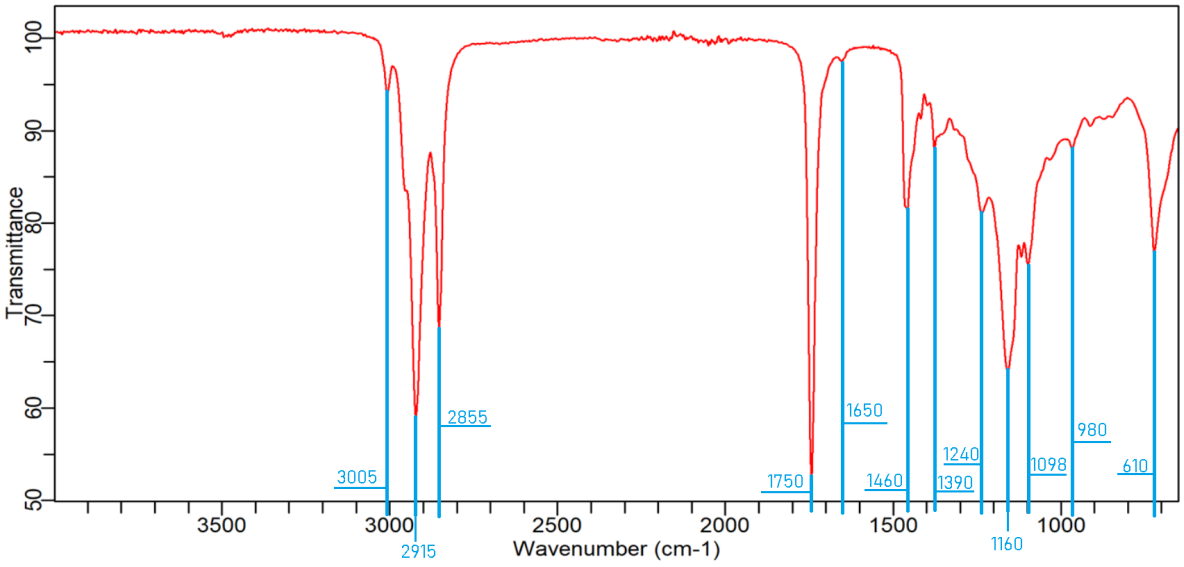

Figure 3.4: Spectrum obtained with a sample of soybean and palm oil.

The organic phase selected are fatty acids obtained from vegetable oils, due to their ease of obtaining and low prices. Specifically, we will use soybean and palm vegetable oil

(Alesol). The resulting spectrum of the soybean and palm oil sample gave us approximately 12 peaks noticeable to the naked eye in figure 3.4. The first three peaks on the left: $\sim$ 3005 cm^{-1} show the presence of O-H and C-H bonds, as well as possible C-H rings of lipids and fatty acids, the peak at $\sim$ 2915 cm^{-1} is the signal for the presence of cholesterol, phospholipids and creatine, resulting from CH, CH2 and CH3 stretching vibrations and a peak at $\sim$ 2855 cm^{-1} also shows the presence of lipids and stretching of methylene chains in membrane lipids. On the right we have the remaining peaks, $\sim$ 1750 cm^{-1} presents a double C=C chain of lipids and fatty acids, $\sim$ 1650 cm^{-1} indicates the predominance of C=O, C=C and NH2 stretching vibration, resulting in nitrogenous bases such as adenine, thymine, guanine and cytosine, $\sim$ 1460 cm^{-1} the presence of collagen and possible presence of kerosene, $\sim$ 1390 cm^{-1} is the result of the presence of carbon particles, $\sim$ 1240 cm^{-1} are phosphate stretching modes and originate from phosphodiester groups of nucleic acids, $\sim$ 1160 cm^{-1} is a CO stretch, 1098 cm^{-1} is a symmetric PO2 stretch, the last peaks at $\sim$ 980 cm^{-1} and $\sim$ 610 cm^{-1} represent polysaccharide-cellulose and a deformation of the phenyl ring. The similarities between the spectrum of the oil and the surfactant, as well as with the spectrum obtained from the extract, allow us to understand the easy emulsification and stability obtained at the end of this work.

3.2 Emulsion development

First, several formulation tests were carried out, varying surfactants and amounts of the phases, until the ideal formulation for our study was reached, with better stability over time and at room temperature, see table 3.1.

Table 3.1:
Different formulations and results obtained.

Surfactant (mg)	Aqueous (mg)	Organic (mg)	Result
		CTAB	
10	3.45	1.54	In less than 3 days, flocculation and cremation were present.
5	3.99	1	
5	4.75	0.25	
5	3.74	1.25	
5	3.245	1.75	
5	4.754	0.25	
5	4.495	0.5	
5	4.245	0.75	
1.5	70	28.5	In less than 3 days there is flocculation. The emulsion is very foamy.
2	70	28	
1	70	29	
0.1	35	14.9	It showed both cremation and flocculation in less than 4 days.
6	4.754	0.249	The phases were separated immediately.
6	4.245	0.749	

Surfactant (mg)	Aqueous(mg)	Organic(mg)	Result
Sodium Lauryl Sulfate			
1.5	70	28.5	Phase separation is achieved in less than 2 days.
2	70	28	
1	70	29	
5	3.5	1.4	Cremation and flocculation took less than 4 days.
7.5	3.5	1.35	
2.5	3.5	1.25	
100	22.5	1.25	In less than 3 days there is phase separation.
10	4.75	0.15	
Food or commercial grade soy lecithin			
1.2	14	4.8	Mostly liquid emulsions. 24 hours later, granulation and rupture could be noticed.
1.5	14	4.5	
1.8	14	4.2	
0.9	5.1	14	It is a creamy emulsion, in 24 hours it presented air bubbles. After 4 days, the emulsion lost stability.
1.2	4.8	14	Creamy emulsions, in 24 hours separated into 2 phases: oil and cream.
1.5	4.5	14	
1.8	4.2	4.2	
0.6	9.85	9.85	It presents separation from the fourth day on.
0.9	9.55	9.55	
1.2	9.4	9.4	It is a more stable formulation than the one with surfactant 0.9.

Surfactant(mg)	**Aqueous(mg)**	**Organic(mg)**	**Result**
1.5	9.25	9.25	Sores were observed. After 4 days the emulsion broke.
1.8	9.1	9.1	
4	10	6	It presents a very yellowish coloration, and loses stability after 2 days.
1.5	10	7	It lasts approximately 4 days before losing stability.
0.9	5.1	14	Very creamy emulsion, loses stability after 3 days.
1.2	9.4	9.4	At 24 hours there is presence of 1 cm of liquid at the bottom of the tube. At 7 days there is separation into cream, emulsion and liquid.
1.2	9	9	
1.5	9.4	9.4	
1.2	9	9.8	
1.2	9.8	9	Approximately 0.5 cm of liquid at the bottom of the tube. At 7 days there is separation into 3 phases (cream, emulsion and liquid).
1.5	8	9.5	At 24 hours, oil droplets were present on the surface and approximately 0.5 cm of liquid at the bottom of the tube. At 7 days it separated into 2 phases, increasing the liquid to more than 1 cm.
1.5	8	9.5	

Surfactant(mg)	**Aqueous(mg)**	**Organic(mg)**	**Result**
2	5	9	It showed many air bubbles and oil separation at 24 hours.
1.5	8	9	A yellow, liquid precipitate was witnessed at the bottom of the tube, approximately 1 mm.
0.96	2.5	1.86	It shows stability for at least 8 days.
		Egg yolk lecithin	
1.2	9.4	9.4	At 24 hours there was phase separation.
2.5	8	9	In one day there are many bubbles and separation.
1.5	9.5	8	
2	5	9	At 8 days they present cremation and sulfide smell.
2	3.5	9	Loses stability the same day.
2	8	9.5	At 24 hours there is separation of liquid at the bottom, at 6 days there is cremation and sulfide odor.
5	5	5	It has a sulfurous odor and a cream layer forms on the surface after approximately 2 days.

Surfactant(mg)	Aqueous(mg)	Organic(mg)	Result
		SPAN 60	
1.5	9	3	They are white, frothy, thick and have a good odor. In 7 days, the liquid separates and accumulates in the center of the emulsion, causing it to break.
2	9	5	
2	10	4	
0.8	10	4	
2.3	10	4	

After making all these formulations, using the same botanical extract of *Zingiber Officinale* and organic phase, reviewing their stability time and the way in which the breakdown occurs and additionally, taking into consideration the ease of obtaining and cost-benefit ratio, food or commercial grade soy lecithin was chosen as the best surfactant option. Expressed as a percentage, the emulsion is made up of 18.16% surfactant, 34.91% organic phase and 46.93% aqueous extract phase. In the figure A2 and A1 in appendicces shows the breakdown of some formulations with both commercial and egg soy lecithin, and the breakdown of emulsions with SPAN. Also, there is an image of the find bottled product sent to test (figure 3.5).

Figure 3.5: Microemulsion packaged and labeled with the necessary dosage for 8 days, prior to shipment. Own authorship.

The present formulation chosen, has been developed without the presence of preservatives, this could represent an advantage from the point of view of production costs, nutritional, it will avoid interactions with the microbiome of the animals, but it is possible that the shelf life of the product is diminished due to microbial degradation. For the present study, the product was administered to the animals no more than 7 days after its elaboration date, since the formulation showed physicochemical and microbiological stability of no less than 8 days, there were no organoleptic problems of the product during its administration and the animals showed no signs of gastrointestinal disease.

However, for storage of the product, it was recommended to refrigerate it and this did not affect the physicochemical and organoleptic characteristics of the product either. On the other hand, for prolonged storage, a greater study of shelf life determination should be carried out, although the infrastructure in equipment of the laboratories of the UITEY are limited for this type of studies.

3.2.1 DLS

A microemulsion with the following particle size characteristics was obtained. The test was performed with a dilution of the samples 1:10, under the conditions of 173° as the degree of dispersion angle, 25 °C as the ambient temperature, 0.895 mPas as the average viscosity of the dispersion, the dispersion form will be standard and monodisperse. In addition, the test was performed in triplicate with 3 replicates. The replicates (composition information) and the values obtained during the characterization are presented below; the nomenclature used should be taken into consideration: L: for food lecithin, A: for aqueous phase, O: organic phase, PI: for polydispersion index and RI: for refractive index.

Replicate 1:

L: 3.9055 g - A: 10.5678 g - O: 7.5209 g - RI: 1.3333

Repetition 1	Repetition 2	Repetition 3
PI: 1.052	PI: 0.905	PI: 0.785

Replicate 2:

L: 3.9878 g - A: 10.1182 g - O: 7.4991 g - RI: 1.3337

Repetition 1	Repetition 2	Repetition 3
PI: 0.543	PI: 1.547	PI: 1.201

Replicate 3:

L: 3.9640 g - A:9.9799 g - O:7.4349 g- RI: 1.3322

Repetition 1	Repetition 2	Repetition 3
PI: 4.448	PI: 1.238	PI: 0.181

The average polydispersity index (PI) is 1.325, this value close to 1 means that the samples had a low polydispersity. Therefore, there are no defined groups of different particle or droplet sizes, rather there is only one group in the size range of approximately 1800 to 8000 nm in diameter. Having mostly particles with an approximate diameter of 1800 to 3500 nm, these values can be seen in the figures 3.6, 3.7, and 3.8 (pages 60, 61 and 62) or also in the tables in the appendix.

3.2.2 Rheology

To carry out this study on the deformation suffered by the emulsion, viscosity and rupture, we performed two tests: controlled stress and controlled rate for each of the 3 undiluted samples. We used a plate-plate (pp) 20 mm contact and a 1000 μm gap. In addition to a round-trip analysis with thixotropic area (TA) results to improve their analysis. The figures presented were provided by the equipment software (see pages 63 and 64).

Stress controlled and Rate controlled.

Sample	Composition	Stress controlled TA	Rate controlled TA
1	L:3.9055 - A:10.5678 - O:7.5209	147.53	6.9567
2	L:3.9878 - A:10.1182 - O:7.4991	56.562	15.553
3	L:3.9640 - A:9.9799 - O:7.4349	365.63	8.4671

TA: Thixotropic area

The thixotropic area can be viewed as the area between the outward and return curves in both tension and controlled speed. The average thixotropic area obtained from the 3 replicates in controlled tension is 189.91, which is significantly higher than that obtained in controlled speed, which is 10.3256. of the replicates. The second replicate is the most stable in controlled tension, with a value of 56.562, but in controlled rate it is the least stable, this may be due to the presence of bubbles in the replicate . On the other hand, the first and third replicates are the most stable at controlled rate, with 6.9567 and 8.9567 and with 6.9567 and 8.4671 respectively, being the third replicate the most stable and with possible presence of air bubbles. In addition, most of the graphs start and end at the same or close points, indicating the absence of emulsion breakage. For more detailed values, please refer to the tables in the appendix section or the figures 3.9 and 3.10.

3.3 Formulation testing

In order to include the dose of the formulation in the daily diet, this one was use the following dosage table 3.2 that was given to the 200 chicks from day 8 of hatching until day 42. The amount of emulsion (18.16% lecithin, 34.91% organic phase and 46.93% aqueous phase) will be given in grams and will depend only on their age in days and the percentage of extract that the animals should ingest (0.5% daily). It is emphasized that the in vivo nutrition tests carried out with the test subjects (broiler chicken), as well as the approval protocols for the development of such tests, were carried out by another group of researchers from the Universidad de las Fuerzas Armadas (ESPE) de Santo Domingo (graduation project under elaboration by Peñaherrera Velez Kennia Geomara at Department of Life Sciences - Universidad de las Fuerzas Armadas ESPE). However, the results of female chicken will be analyzed in this study through a paired t-test to corroborate both the effectiveness and ineffectiveness of the promoter. Below is the data table 3.3 provided by the ESPE researchers that we will use for the T-test.

Table 3.2:

Dosage for broiler chickens.

Age	Food consumed	Extract 0.5%	Lecithin	Oily phase	Total emulsion
8	37	0.185	0.072	0.138	0.394
9	43	0.215	0.083	0.160	0.458
10	50	0.25	0.097	0.186	0.533
11	57	0.285	0.110	0.212	0.607
12	64	0.32	0.124	0.238	0.682
13	72	0.36	0.139	0.268	0.767
14	74	0.37	0.143	0.275	0.788
15	78	0.39	0.151	0.290	0.831
16	85	0.425	0.164	0.316	0.905
17	91	0.455	0.176	0.338	0.969
18	103	0.515	0.199	0.383	1.097
19	110	0.55	0.213	0.409	1.172
20	114	0.57	0.220	0.424	1.214
21	118	0.59	0.228	0.439	1.257
22	123	0.615	0.238	0.457	1.310
23	128	0.64	0.248	0.476	1.364
24	133	0.665	0.257	0.495	1.416
25	137	0.685	0.265	0.509	1.459
26	144	0.72	0.278	0.535	1.532
27	150	0.75	0.290	0.558	1.598
28	156	0.78	0.302	0.580	1.662
29	160	0.8	0.309	0.595	1.704
30	164	0.82	0.317	0.610	1.747
31	167	0.835	0.323	0.621	1.779
32	170	0.85	0.329	0.632	1.811
33	174	0.87	0.337	0.647	1.854
34	177	0.885	0.342	0.658	1.885
35	179	0.895	0.346	0.666	1.907
36	182	0.91	0.352	0.677	1.939
37	186	0.93	0.360	0.692	1.981
38	190	0.95	0.367	0.706	2.024
39	193	0.965	0.373	0.718	2.056
40	197	0.985	0.381	0.733	2.099
41	203	1.015	0.393	0.755	2.162
42	208	1.04	0.402	0.773	2.216

Note: Age measured in days. Other parameters were measured in grams.

Table 3.3:

Weekly weight of broilers.

HS	HC
	43.1
211.95	214.65
388.75	418.05
691.6	722.8
1216.0	1035.5
1547.5	1633.0
1920	2066.05
2547.9	2847.5

Note: HS: Female without emulsion.

HC: Female with emulsion.

3.3.1 Shapiro test

To perform the t-test, we first need to perform some normality tests. We performed Shapiro test (for our sample size) to probe it if female chicken weight values without emulsion (HS) and female chicken with emulsion (HC) follow a normal distribution.

Hypotheses for Shapiro test for HS:

H_0: The proportion of weekly growth in females without emulsion (weight in grams) is normally distributed.
H_1: The proportion of weekly growth in females without emulsion (weight in grams) is not normally distributed.

Hypotheses for Shapiro test for HC:

H_0: The proportion of weekly growth in females with emulsion (weight in grams) is normally distributed.
H_1: The proportion of weekly growth in females with emulsion (weight in grams) is not normally distributed.

Our confidence percentage is 95%, therefore the alpha value is 5%. For both HS and HC, we have p-values greater than alpha. It means, the weekly growth ratio for females with and without emulsion follows a normal distribution. These values can be seen in the

Table 3.4:

Shapiro test female.

HS		HC	
W	p-value	W	p-value
0.94903	0.7014	0.94043	0.6153
Alpha = 0.05			

Table 3.5:

Paired T-Student for females.

Paired t-test			
t	p-value	df	mean difference
-2.6585	0.0156	7	-90.8875
Alpha = 0.05			
T-critical = 2.3646			

table 3.4 with data obtained from RStudio Team (2020) [81].

3.3.2 T-Student

After confirming the normal distribution of group (female chicken), with and without emulsion, we proceeded to perform the paired t-student test. In order to demonstrate that the inclusion of the promoter in the feed consumed daily by the chicks, positively modifies the weight of the females, favoring the increase and/or gain of weekly weight. We proceeded to perform the corresponding t student tests.

Hypothesis for HS and HC are:

H_0: The mean in weight of females fed without emulsion = the mean in weight of females fed with emulsion.
H_1: The mean in weight of females fed without emulsion $\neq$ the mean in weight of females fed with emulsion.

With a p-value of 0.0156, lower than alpha of 0.05, we can reject H0. In other words, the inclusion of the promoter in the daily feed consumed by the chicks positively modifies the weight of the females, favoring weekly weight gain and/or increase. These values can be seen in the table 3.5 with data obtained from RStudio Team (2020) [81]. This result

agrees with those obtained by other authors, such as those shown in Table 1.1 Despite not having performed the same statistical tests due to the different sample sizes compared to other works.

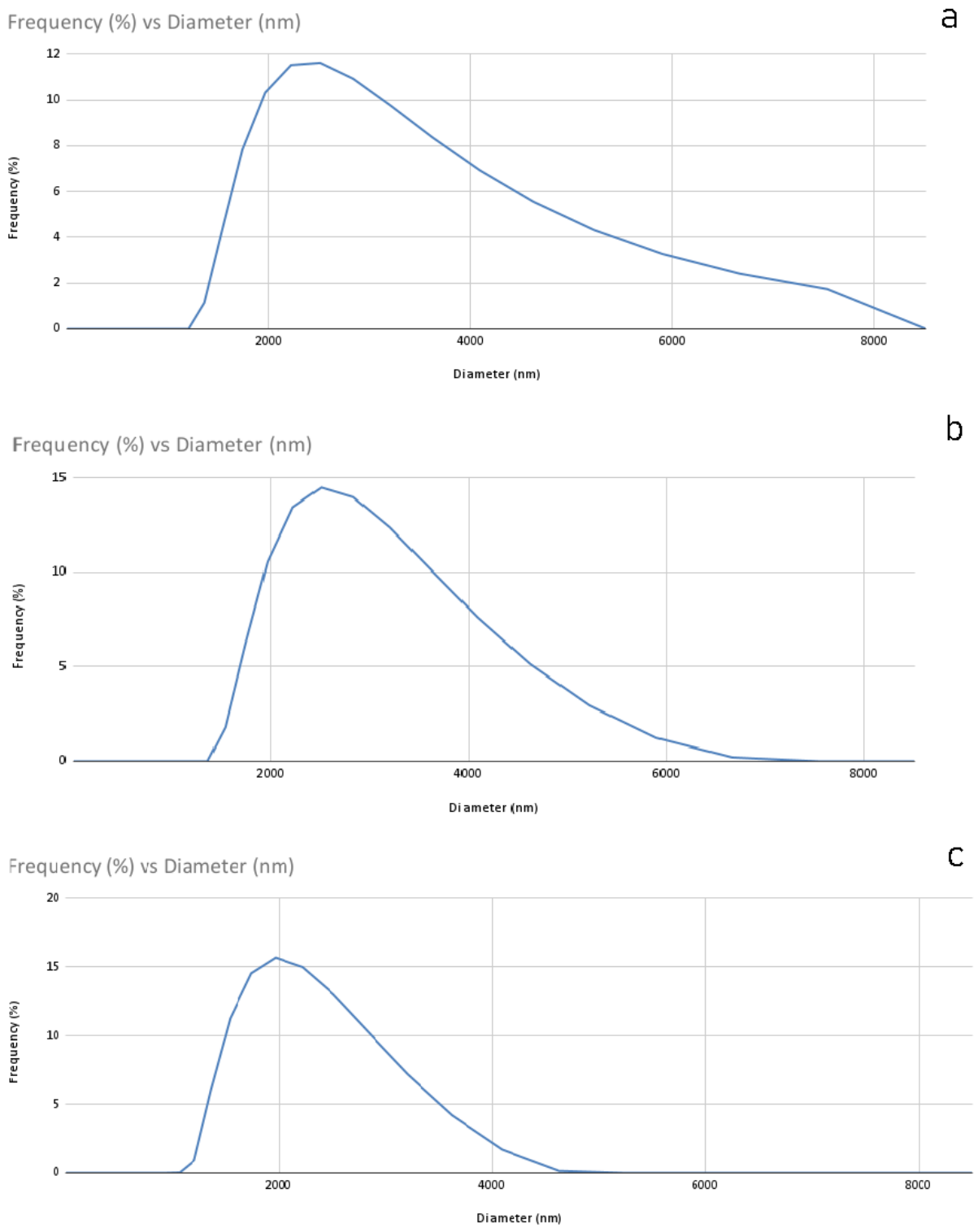

Figure 3.6: Frequency (%) vs diameter (nm) Sample 1: a. Repetition 1. b. Repetition 2. c. Repetition 3. Own authorship.

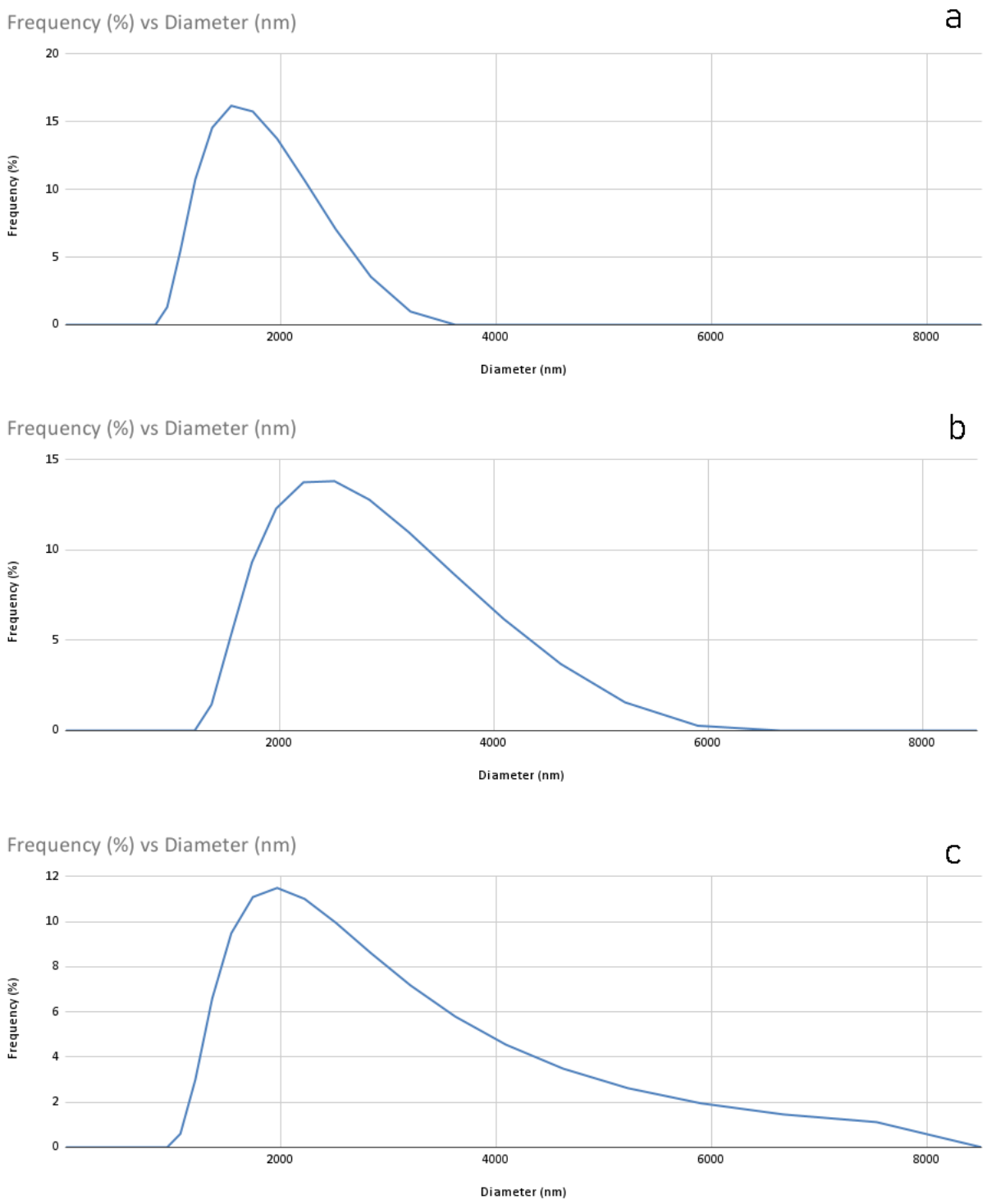

Figure 3.7: Frequency (%) vs diameter (nm) sample 2. a. Repetition 1. b. Repetition 2. c. Repetition 3. Own authorship.

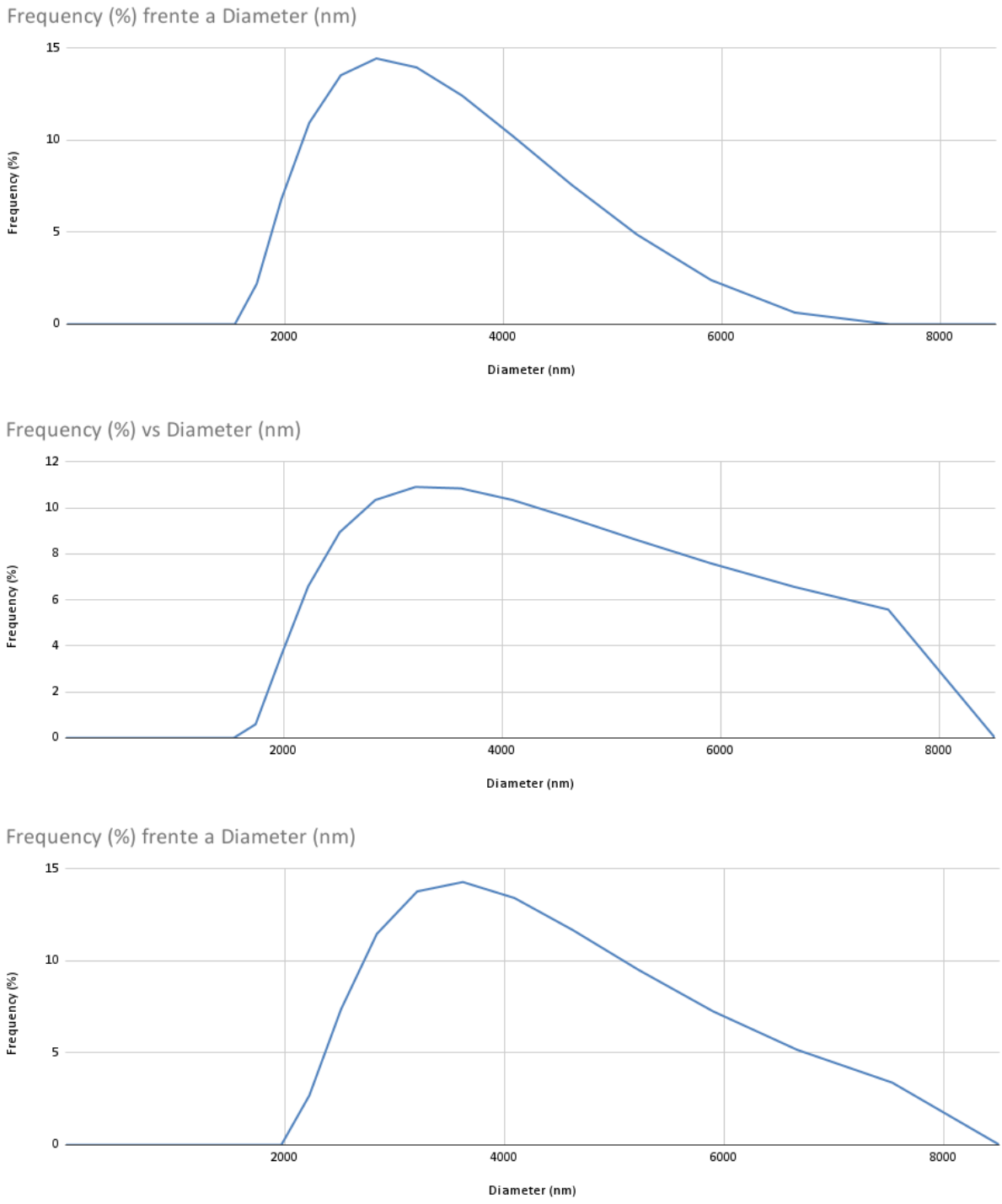

Figure 3.8: Frequency (%) vs diameter (nm) sample 3. a. Repetition 1. b. Repetition 2. c. Repetition 3. Own authorship.

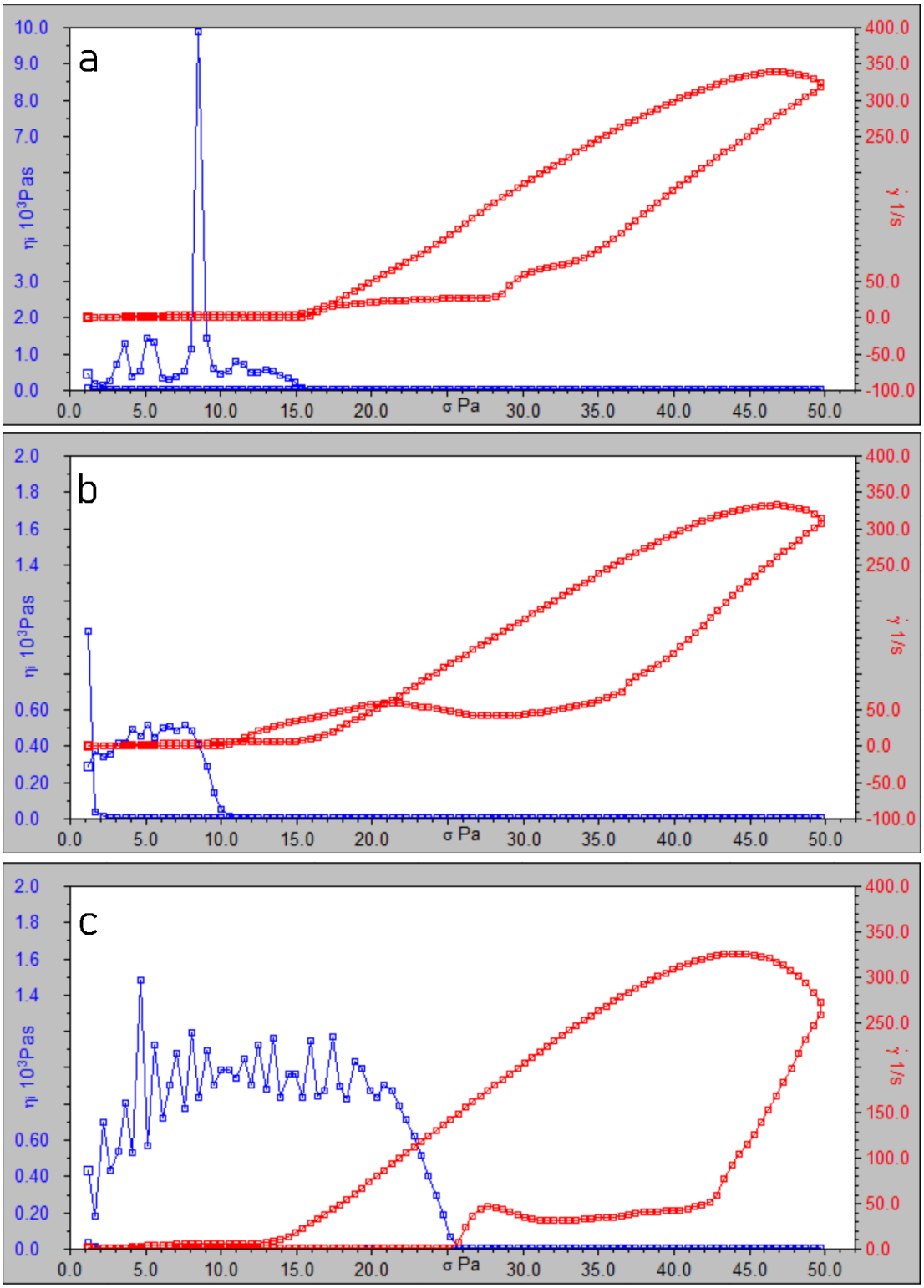

Figure 3.9: Stress controlled: in red we can observe the relation Shear rate (1/s) vs Shear stress (Pa) and in blue the graph shows the Instantaneous viscosity (Pas) vs Shear stress (Pa). a. Replicate 1. b. Replicate 2. c. Replicate 3.

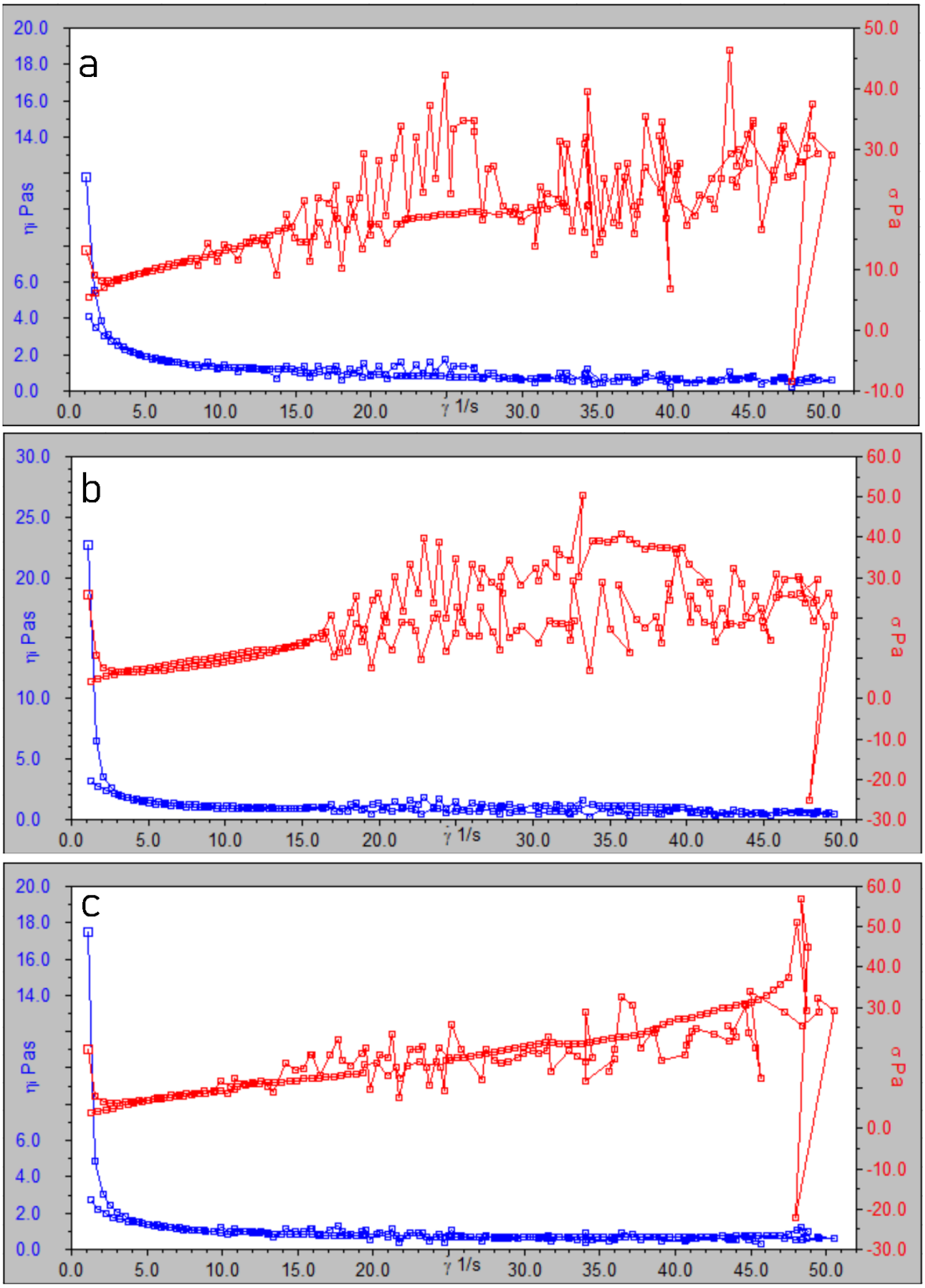

Figure 3.10: Rate controlled: in red we can observe the relation Shear stress (Pa) vs Shear rate (1/s) and in blue the graph shows the relation Instantaneous viscosity (Pas) vs Shear rate (1/s). a. Replicate 1. b. Replicate 2. c. Replicate 3.

Chapter 4

Conclusions

Having concluded this work, the following conclusions were reached.

- Through multiple analyses and tests of different materials, a suitable microemulsion formulated with biocompatible raw material of natural origin was obtained, which showed stability of no less than 8 days over time at room temperature, due to the nature of these raw materials, and the microemulsion provide, ideal complements for the animal diet. It is a nutritional and growth enhancer dietary complement.

- A comparison was made of the productivity of a private broiler farm using the emulsion as a growth promoter, the results were provided in the statistical test section, for a more detailed view of this comparison can be directed to the study conducted by researchers of the ESPE (graduation project under elaboration by Peñaherrera Velez Kennia Geomara at Department of Life Sciences - University of the Armed Forces ESPE). In addition, these researchers were in charge of the in vivo tests, as well as of maintaining the protocols necessary for these trials.

- The efficacy of the microemulsion as an administration system to improve the effect of Zingiber Officinale extract as a growth promoter in broiler chickens was proven, obtaining significant differences in the weights of females fed with microemulsion respect to group without microemulsion, evidencing the usefulness of this product.

- This work was also able to characterize the physicochemical properties of the formulated emulsions, showing both qualitative and quantitative results. It provided spectrum prints of each of the components used in the emulsion: extract, soybean

and palm oil, and soybean lecithin, obtaining a microemulsion of O/W type among other studies with their results.

- It is necessary to carry out tests to determine the effectiveness of the product in males, and due to the fact that phytochemicals are widely used in different farm animals, it is advisable to test the microemulsions in other species of these animals.
- On the other hand, the formulation could be perfectible, although it has been possible to obtain a homogeneous mixture, with good stability over time, and it has been possible to make it without the use of preservatives, which is an advantage, but not necessarily good from the microbiological point of view, which for the purposes of this study was not a variable to be considered since the product was administered shortly after it was formulated.

Future work:

Having checked with the present work the effectiveness of this emulsion as a growth promoter and being an excellent replacement for the pre-existing ones in the market, it is expected to open projects with the community, small and large farms. Provide training on the topic of growth promoters and to produce this product in a natural way and for the use of these farms, which are usually the ones that supply small towns. Being a profitable, healthy and easily accessible option, not only for those who produce for commercial purposes, but also for people who raise and consume their own farm animals.

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Appendices

.1 Appendix 1.

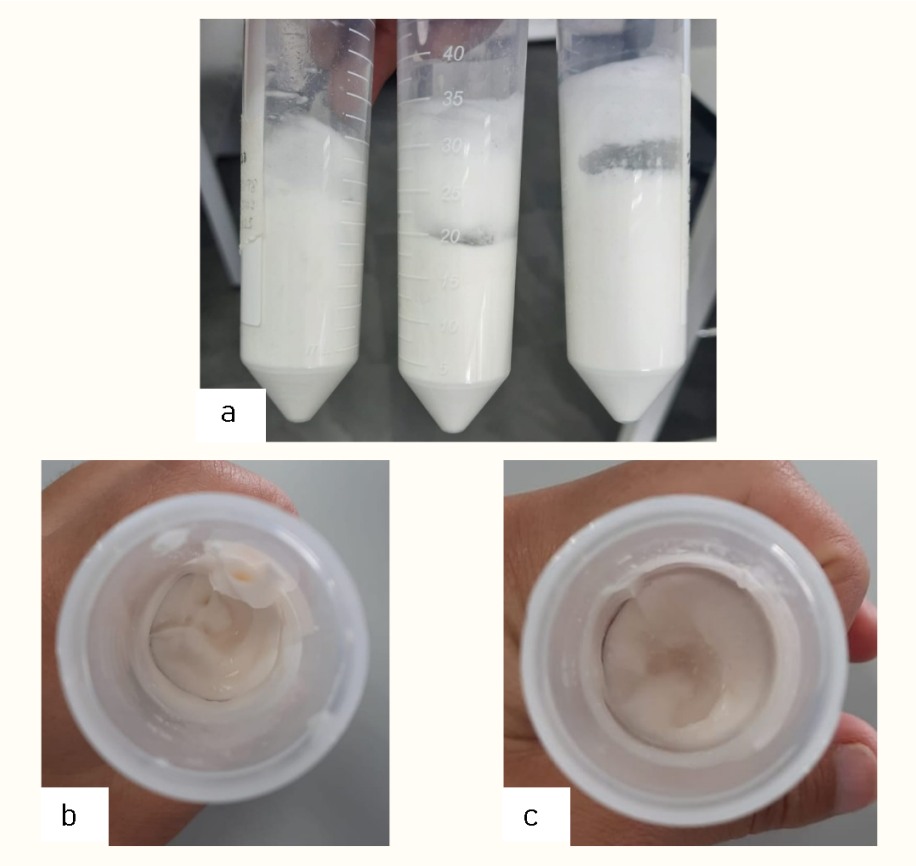

Figure A 1: Emulsions made with SPAN 60: a. From left to right the emulsions T: 1.5 A:9 O:3, T:2 A:9 O:5, T:2 A:10 O:4. b. and c. show destabilization, a faint pinkish liquid is evident in the center of the emulsion. Own authorship.

Table A 1: DLS data sample 1, repetition 1.

Diameter(nm)	Frequency(%)	Diameter(nm)	%)	Diameter(nm)	(%)
0.34	0	10.34	0	315.27	0
0.38	0	11.68	0	356.27	0
0.43	0	13.2	0	402.44	0
0.49	0	14.91	0	454.69	0
0.55	0	16.84	0	513.71	0
0.62	0	19.03	0	580.41	0
0.7	0	21.5	0	655.76	0
0.8	0	24.29	0	740.89	0
0.9	0	27.45	0	837.07	0
1.02	0	31.01	0	945.74	0
1.15	0	35.03	0	1068.52	0
1.3	0	39.58	0	1207.24	0
1.47	0	44.72	0	1363.97	1.134
1.66	0	50.53	0	1541.04	4.323
1.87	0	57.09	0	1741.1	7.832
2.11	0	64.5	0	1967.14	10.32
2.39	0	72.87	0	2222.51	11.516
2.7	0	82.33	0	2511.05	11.613
3.05	0	93.02	0	2837.04	10.927
3.45	0	105.1	0	3205.35	9.764
3.89	0	118.74	0	3621.48	8.37
4.4	0	134.16	0	4091.63	6.925
4.97	0	151.57	0	4622.91	5.55
5.61	0	171.25	0	5222.96	4.318
6.34	0	193.48	0	5901.02	3.267
7.17	0	218.6	0	6667.1	2.408
8.1	0	246.98	0	7532.65	1.733
9.15	0	279.04	0	8510.56	0

Table A 2: DLS data sample 1, repetition 2.

Diameter(nm)	Frequency(%)	Diameter(nm)	%)	Diameter(nm)	(%)
0.34	0	10.34	0	315.27	0
0.38	0	11.68	0	356.27	0
0.43	0	13.2	0	402.44	0
0.49	0	14.91	0	454.69	0
0.55	0	16.84	0	513.71	0
0.62	0	19.03	0	580.41	0
0.7	0	21.5	0	655.76	0
0.8	0	24.29	0	740.89	0
0.9	0	27.45	0	837.07	0
1.02	0	31.01	0	945.74	0
1.15	0	35.03	0	1068.52	0
1.3	0	39.58	0	1207.24	0
1.47	0	44.72	0	1363.97	0
1.66	0	50.53	0	1541.04	1.805
1.87	0	57.09	0	1741.1	6.113
2.11	0	64.5	0	1967.14	10.549
2.39	0	72.87	0	2222.51	13.442
2.7	0	82.33	0	2511.05	14.484
3.05	0	93.02	0	2837.04	13.972
3.45	0	105.1	0	3205.35	12.366
3.89	0	118.74	0	3621.48	10.119
4.4	0	134.16	0	4091.63	7.613
4.97	0	151.57	0	4622.91	5.147
5.61	0	171.25	0	5222.96	2.952
6.34	0	193.48	0	5901.02	1.232
7.17	0	218.6	0	6667.1	0.208
8.1	0	246.98	0	7532.65	0
9.15	0	279.04	0	8510.56	0

Table A 3: DLS data sample 1, repetition 3.

Diameter(nm)	Frequency(%)	Diameter(nm)	%)	Diameter(nm)	(%)
0.34	0	10.34	0	315.27	0
0.38	0	11.68	0	356.27	0
0.43	0	13.2	0	402.44	0
0.49	0	14.91	0	454.69	0
0.55	0	16.84	0	513.71	0
0.62	0	19.03	0	580.41	0
0.7	0	21.5	0	655.76	0
0.8	0	24.29	0	740.89	0
0.9	0	27.45	0	837.07	0
1.02	0	31.01	0	945.74	0
1.15	0	35.03	0	1068.52	0.028
1.3	0	39.58	0	1207.24	0.924
1.47	0	44.72	0	1363.97	6.09
1.66	0	50.53	0	1541.04	11.248
1.87	0	57.09	0	1741.1	14.536
2.11	0	64.5	0	1967.14	15.669
2.39	0	72.87	0	2222.51	14.989
2.7	0	82.33	0	2511.05	13.014
3.05	0	93.02	0	2837.04	10.262
3.45	0	105.1	0	3205.35	7.192
3.89	0	118.74	0	3621.48	4.206
4.4	0	134.16	0	4091.63	1.699
4.97	0	151.57	0	4622.91	0.145
5.61	0	171.25	0	5222.96	0
6.34	0	193.48	0	5901.02	0
7.17	0	218.6	0	6667.1	0
8.1	0	246.98	0	7532.65	0
9.15	0	279.04	0	8510.56	0

Table A 4: DLS data sample 2, repetition 1.

Diameter(nm)	Frequency(%)	Diameter(nm)	%)	Diameter(nm)	(%)
0.34	0	10.34	0	315.27	0
0.38	0	11.68	0	356.27	0
0.43	0	13.2	0	402.44	0
0.49	0	14.91	0	454.69	0
0.55	0	16.84	0	513.71	0
0.62	0	19.03	0	580.41	0
0.7	0	21.5	0	655.76	0
0.8	0	24.29	0	740.89	0
0.9	0	27.45	0	837.07	0
1.02	0	31.01	0	945.74	1.304
1.15	0	35.03	0	1068.52	5.474
1.3	0	39.58	0	1207.24	10.743
1.47	0	44.72	0	1363.97	14.559
1.66	0	50.53	0	1541.04	16.179
1.87	0	57.09	0	1741.1	15.756
2.11	0	64.5	0	1967.14	13.743
2.39	0	72.87	0	2222.51	10.663
2.7	0	82.33	0	2511.05	7.056
3.05	0	93.02	0	2837.04	3.547
3.45	0	105.1	0	3205.35	0.977
3.89	0	118.74	0	3621.48	0
4.4	0	134.16	0	4091.63	0
4.97	0	151.57	0	4622.91	0
5.61	0	171.25	0	5222.96	0
6.34	0	193.48	0	5901.02	0
7.17	0	218.6	0	6667.1	0
8.1	0	246.98	0	7532.65	0
9.15	0	279.04	0	8510.56	0

Table A 5: DLS data sample 2, repetition 2.

Diameter(nm)	Frequency(%)	Diameter(nm)	%)	Diameter(nm)	(%)
0.34	0	10.34	0	315.27	0
0.38	0	11.68	0	356.27	0
0.43	0	13.2	0	402.44	0
0.49	0	14.91	0	454.69	0
0.55	0	16.84	0	513.71	0
0.62	0	19.03	0	580.41	0
0.7	0	21.5	0	655.76	0
0.8	0	24.29	0	740.89	0
0.9	0	27.45	0	837.07	0
1.02	0	31.01	0	945.74	0
1.15	0	35.03	0	1068.52	0
1.3	0	39.58	0	1207.24	0
1.47	0	44.72	0	1363.97	1.439
1.66	0	50.53	0	1541.04	5.187
1.87	0	57.09	0	1741.1	9.319
2.11	0	64.5	0	1967.14	12.302
2.39	0	72.87	0	2222.51	13.757
2.7	0	82.33	0	2511.05	13.819
3.05	0	93.02	0	2837.04	12.79
3.45	0	105.1	0	3205.35	10.987
3.89	0	118.74	0	3621.48	8.697
4.4	0	134.16	0	4091.63	6.179
4.97	0	151.57	0	4622.91	3.391
5.61	0	171.25	0	5222.96	1.563
6.34	0	193.48	0	5901.02	0.269
7.17	0	218.6	0	6667.1	0
8.1	0	246.98	0	7532.65	0
9.15	0	279.04	0	8510.56	0

Table A 6: DLS data sample 2, repetition 3.

Diameter(nm)	Frequency(%)	Diameter(nm)	%)	Diameter(nm)	(%)
0.34	0	10.34	0	315.27	0
0.38	0	11.68	0	356.27	0
0.43	0	13.2	0	402.44	0
0.49	0	14.91	0	454.69	0
0.55	0	16.84	0	513.71	0
0.62	0	19.03	0	580.41	0
0.7	0	21.5	0	655.76	0
0.8	0	24.29	0	740.89	0
0.9	0	27.45	0	837.07	0
1.02	0	31.01	0	945.74	0
1.15	0	35.03	0	1068.52	0.599
1.3	0	39.58	0	1207.24	2.995
1.47	0	44.72	0	1363.97	6.594
1.66	0	50.53	0	1541.04	9.488
1.87	0	57.09	0	1741.1	11.091
2.11	0	64.5	0	1967.14	11.496
2.39	0	72.87	0	2222.51	11.009
2.7	0	82.33	0	2511.05	9.957
3.05	0	93.02	0	2837.04	8.611
3.45	0	105.1	0	3205.35	7.177
3.89	0	118.74	0	3621.48	5.795
4.4	0	134.16	0	4091.63	4.55
4.97	0	151.57	0	4622.91	3.487
5.61	0	171.25	0	5222.96	2.623
6.34	0	193.48	0	5901.02	1.952
7.17	0	218.6	0	6667.1	1.458
8.1	0	246.98	0	7532.65	1.118
9.15	0	279.04	0	8510.56	0

Table A 7: DLS data sample 3, repetition 1.

Diameter(nm)	Frequency(%)	Diameter(nm)	%)	Diameter(nm)	(%)
0.34	0	10.34	0	315.27	0
0.38	0	11.68	0	356.27	0
0.43	0	13.2	0	402.44	0
0.49	0	14.91	0	454.69	0
0.55	0	16.84	0	513.71	0
0.62	0	19.03	0	580.41	0
0.7	0	21.5	0	655.76	0
0.8	0	24.29	0	740.89	0
0.9	0	27.45	0	837.07	0
1.02	0	31.01	0	945.74	0
1.15	0	35.03	0	1068.52	0
1.3	0	39.58	0	1207.24	0
1.47	0	44.72	0	1363.97	0
1.66	0	50.53	0	1541.04	0
1.87	0	57.09	0	1741.1	2.195
2.11	0	64.5	0	1967.14	6.798
2.39	0	72.87	0	2222.51	10.95
2.7	0	82.33	0	2511.05	13.534
3.05	0	93.02	0	2837.04	14.447
3.45	0	105.1	0	3205.35	13.958
3.89	0	118.74	0	3621.48	12.427
4.4	0	134.16	0	4091.63	10.199
4.97	0	151.57	0	4622.91	7.584
5.61	0	171.25	0	5222.96	4.873
6.34	0	193.48	0	5901.02	2.401
7.17	0	218.6	0	6667.1	0.635
8.1	0	246.98	0	7532.65	0
9.15	0	279.04	0	8510.56	0

Table A 8: DLS data sample 3, repetition 2.

Diameter(nm)	Frequency(%)	Diameter(nm)	%)	Diameter(nm)	(%)
0.34	0	10.34	0	315.27	0
0.38	0	11.68	0	356.27	0
0.43	0	13.2	0	402.44	0
0.49	0	14.91	0	454.69	0
0.55	0	16.84	0	513.71	0
0.62	0	19.03	0	580.41	0
0.7	0	21.5	0	655.76	0
0.8	0	24.29	0	740.89	0
0.9	0	27.45	0	837.07	0
1.02	0	31.01	0	945.74	0
1.15	0	35.03	0	1068.52	0
1.3	0	39.58	0	1207.24	0
1.47	0	44.72	0	1363.97	0
1.66	0	50.53	0	1541.04	0
1.87	0	57.09	0	1741.1	0.595
2.11	0	64.5	0	1967.14	3.477
2.39	0	72.87	0	2222.51	6.591
2.7	0	82.33	0	2511.05	8.944
3.05	0	93.02	0	2837.04	10.345
3.45	0	105.1	0	3205.35	10.913
3.89	0	118.74	0	3621.48	10.85
4.4	0	134.16	0	4091.63	10.347
4.97	0	151.57	0	4622.91	9.562
5.61	0	171.25	0	5222.96	8.618
6.34	0	193.48	0	5901.02	7.602
7.17	0	218.6	0	6667.1	6.575
8.1	0	246.98	0	7532.65	5.581
9.15	0	279.04	0	8510.56	0

Table A 9: DLS data sample 3, repetition 3.

Diameter(nm)	Frequency(%)	Diameter(nm)	%)	Diameter(nm)	(%)
0.34	0	10.34	0	315.27	0
0.38	0	11.68	0	356.27	0
0.43	0	13.2	0	402.44	0
0.49	0	14.91	0	454.69	0
0.55	0	16.84	0	513.71	0
0.62	0	19.03	0	580.41	0
0.7	0	21.5	0	655.76	0
0.8	0	24.29	0	740.89	0
0.9	0	27.45	0	837.07	0
1.02	0	31.01	0	945.74	0
1.15	0	35.03	0	1068.52	0
1.3	0	39.58	0	1207.24	0
1.47	0	44.72	0	1363.97	0
1.66	0	50.53	0	1541.04	0
1.87	0	57.09	0	1741.1	0
2.11	0	64.5	0	1967.14	0
2.39	0	72.87	0	2222.51	2.687
2.7	0	82.33	0	2511.05	7.368
3.05	0	93.02	0	2837.04	11.465
3.45	0	105.1	0	3205.35	13.777
3.89	0	118.74	0	3621.48	14.286
4.4	0	134.16	0	4091.63	13.418
4.97	0	151.57	0	4622.91	11.677
5.61	0	171.25	0	5222.96	9.509
6.34	0	193.48	0	5901.02	7.259
7.17	0	218.6	0	6667.1	5.168
8.1	0	246.98	0	7532.65	3.386
9.15	0	279.04	0	8510.56	0

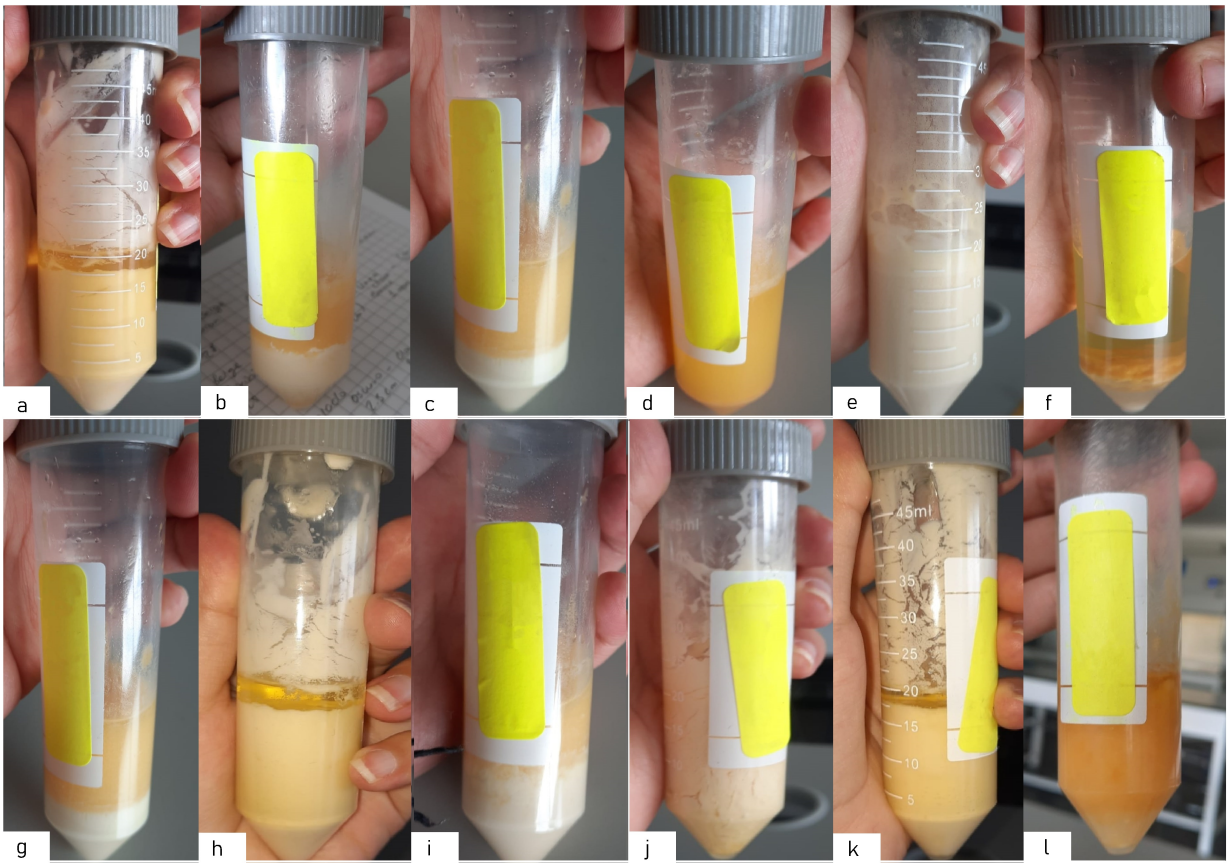

Figure A 2: Presentation of some examples of emulsion breakdown or loss of stability: a. LSC T: 1.2 A: 14 O:4.8 b. LSC T:0.9 A: 5.1 O:14 c. LSC T:1.5 A:10 O:7 d. LY T:2 A:3.5 O:9 e. LSC L:0.97 A:2.5 O: 1.86 f. LSC T: 1.8 A; 4.2 O:4.2 g. LSC T:1.5 A:4.5 O:14 h. LSC L:4 A:10 O:6 i. LY T: 1.2 A:9.4 O:9.4 j. LSC T:1.5 H:9.25 O:9.25 k. LSC T: 0.9 A:5.1 O:14 l. LY T:5 O:5 A:5, where LSC: commercial soy lecithin, LY: lecithin from yolk, T: surfactant, A: aqueous phase, O: organic phase. Own authorship.

Table A 10: Rheology: controlled stress, sample 1.

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
0,002876	1,25	434,5
0,01015	1,737	171,1
0,01662	2,225	133,9
0,01186	2,713	228,7
0,004513	3,201	709,2
-0,002925	3,689	1261
-0,01102	4,189	380,1
-0,008961	4,676	521,9
-0,003641	5,164	1418
0,004337	5,652	1303
0,01909	6,152	322,2
0,02256	6,64	294,4
0,0206	7,128	346
0,01462	7,616	520,8
0,007154	8,103	1133
0,0008703	8,603	9886
0,006415	9,091	1417
0,01587	9,579	603,5
0,02372	10,07	424,5
0,02087	10,55	505,7
0,01407	11,04	784,7
0,01613	11,54	715,4
0,02442	12,03	492,6
0,02579	12,52	485,4
0,0234	13,01	555,7
0,02654	13,49	508,3
0,03427	13,98	408
0,04663	14,48	310,5
0,07467	14,97	200,5
0,2042	15,46	75,69
1,9	15,94	8,393
7,169	16,43	2,292
11,85	16,93	1,429
14,93	17,42	1,167
16,72	17,91	1,071
17,81	18,4	1,033
18,69	18,88	1,01
19,65	19,37	0,9857
20,74	19,87	0,9583
21,44	20,36	0,9498

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
21,99	20,85	0,9479
22,58	21,34	0,9449
23,14	21,82	0,9429
23,45	22,31	0,9516
23,84	22,81	0,9567
24,47	23,3	0,952
25,1	23,79	0,9476
25,47	24,27	0,9529
25,6	24,76	0,9674
25,75	25,26	0,9811
25,96	25,75	0,9919
26,26	26,24	0,9993
26,52	26,73	1,008
26,72	27,21	1,018
27,06	27,7	1,023
27,51	28,2	1,025
32,54	28,69	0,8815
43,9	29,18	0,6646
52,71	29,66	0,5628
58,86	30,15	0,5123
62,97	30,64	0,4866
66,13	31,14	0,4709
68,86	31,63	0,4593
70,9	32,12	0,453
72,48	32,6	0,4498
74,63	33,09	0,4434
77,72	33,59	0,4322
81,8	34,08	0,4166
87,31	34,57	0,3959
93,75	35,05	0,3739
101	35,54	0,352
108,5	36,03	0,332
116,5	36,53	0,3135
124,7	37,02	0,2969
133,2	37,51	0,2816
141,7	37,99	0,2681
150,1	38,48	0,2564
158,4	38,97	0,2461
166,7	39,47	0,2368
174,6	39,96	0,2288
182,3	40,44	0,2218
190,2	40,94	0,2153

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
197,6	41,42	0,2097
205,2	41,92	0,2043
212,6	42,41	0,1995
219,9	42,9	0,1951
227,1	43,38	0,191
234,5	43,88	0,1871
241,7	44,37	0,1836
248,8	44,86	0,1803
255,8	45,35	0,1773
262,7	45,83	0,1745
269,6	46,32	0,1718
276,7	46,82	0,1692
283,5	47,31	0,1669
290,2	47,8	0,1647
296,9	48,29	0,1626
303,4	48,77	0,1607
310,2	49,27	0,1589
316,6	49,76	0,1572
323	49,75	0,154
328	49,26	0,1502
331,9	48,78	0,147
334,7	48,29	0,1443
336,5	47,79	0,142
337,5	47,3	0,1401
337,8	46,81	0,1386
337,4	46,32	0,1373
336,5	45,84	0,1362
335,1	45,35	0,1353
333	44,85	0,1347
330,7	44,36	0,1341
328	43,87	0,1338
324,9	43,37	0,1335
321,7	42,9	0,1333
318,2	42,4	0,1333
314,4	41,91	0,1333
310,4	41,42	0,1334
306,2	40,93	0,1337
301,7	40,43	0,134
297,3	39,96	0,1344
292,6	39,46	0,1349
287,7	38,97	0,1354
282,7	38,48	0,1361
277,6	38	0,1369

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
272,3	37,5	0,1377
267,1	37,02	0,1386
261,6	36,52	0,1396
256	36,03	0,1407
250,4	35,54	0,1419
244,8	35,06	0,1432
238,9	34,56	0,1446
233,1	34,07	0,1462
227,2	33,58	0,1478
221,2	33,09	0,1496
215,3	32,6	0,1515
209,2	32,12	0,1535
202,9	31,62	0,1558
196,7	31,13	0,1582
190,4	30,64	0,1609
184,1	30,15	0,1638
177,7	29,67	0,1669
171,3	29,18	0,1703
164,6	28,68	0,1742
157,8	28,19	0,1786
151	27,7	0,1834
144,2	27,21	0,1888
137,1	26,73	0,1949
129,8	26,24	0,2022
122	25,74	0,2109
114,4	25,25	0,2208
107	24,76	0,2315
100,1	24,28	0,2426
93,58	23,79	0,2542
87,53	23,3	0,2662
81,51	22,8	0,2797
75,66	22,31	0,2949
69,85	21,82	0,3124
64,08	21,34	0,333
58,37	20,85	0,3572
52,7	20,36	0,3864
46,87	19,86	0,4237
41,19	19,37	0,4704
35,49	18,89	0,5321
29,6	18,39	0,6212
24,12	17,91	0,7425
18,83	17,41	0,9245
14,23	16,92	1,189

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
10,37	16,43	1,585
7,452	15,95	2,14
5,488	15,45	2,815
4,516	14,96	3,312
4,043	14,47	3,579
3,973	13,98	3,519
4,047	13,5	3,335
4,117	13,01	3,159
4,2	12,51	2,978
4,299	12,02	2,796
4,419	11,53	2,609
4,462	11,04	2,475
4,352	10,56	2,426
4,294	10,06	2,342
4,178	9,568	2,29
4,003	9,08	2,268
3,836	8,593	2,24
3,616	8,105	2,241
3,322	7,617	2,293
3,079	7,117	2,311
2,853	6,629	2,323
2,492	6,141	2,465
2,12	5,654	2,667
1,838	5,166	2,81
1,504	4,678	3,111
1,162	4,178	3,596
0,9063	3,69	4,071
0,6734	3,202	4,755
0,4674	2,715	5,808
0,2875	2,227	7,746
0,1117	1,727	15,46
0,01666	1,239	74,37

Table A 11: Rheology: controlled stress, sample 2.

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
0,004368	1,25	286,1
0,004685	1,737	370,9
0,006579	2,237	340,1
0,007732	2,713	350,9
0,007684	3,213	418,1
0,008856	3,701	417,9
0,008545	4,189	490,2
0,01038	4,676	450,3
0,01011	5,176	511,9
0,0126	5,652	448,6
0,0123	6,152	500,3
0,01313	6,652	506,6
0,01473	7,128	483,9
0,01475	7,616	516,4
0,01693	8,116	479,3
0,02119	8,603	406
0,03198	9,091	284,3
0,06799	9,591	141,1
0,2009	10,07	50,11
1,221	10,55	8,642
5,532	11,05	1,998
10,87	11,54	1,062
15,81	12,03	0,7608
20,04	12,53	0,6251
23,35	13,01	0,5569
26,26	13,49	0,5139
28,89	13,99	0,4844
31,28	14,48	0,463
33,48	14,97	0,4471
35,75	15,47	0,4327
37,83	15,94	0,4215
39,99	16,43	0,4109
42,33	16,93	0,4
44,54	17,42	0,3911
46,88	17,91	0,382
49,37	18,41	0,3728
51,78	18,88	0,3647
54,19	19,37	0,3575
56,1	19,87	0,3542
57,36	20,36	0,3549
57,83	20,85	0,3605

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
58,05	21,35	0,3677
57,91	21,82	0,3769
56,92	22,31	0,3919
55,55	22,81	0,4106
53,98	23,3	0,4316
52,16	23,79	0,456
50,27	24,29	0,4831
48,67	24,76	0,5088
47,07	25,26	0,5366
45,1	25,75	0,5709
43,3	26,24	0,6059
42,52	26,73	0,6285
42,08	27,23	0,6471
41,7	27,7	0,6643
41,54	28,2	0,6789
41,42	28,7	0,693
41,37	29,18	0,7052
41,87	29,66	0,7084
43,08	30,16	0,7002
44,7	30,64	0,6855
46,31	31,14	0,6725
47,46	31,64	0,6667
48,83	32,12	0,6577
51,03	32,6	0,639
53,01	33,1	0,6244
54,74	33,59	0,6137
56,65	34,08	0,6016
59,04	34,58	0,5857
62,97	35,05	0,5566
66,98	35,54	0,5307
70,3	36,04	0,5127
74,77	36,53	0,4886
86,58	37,02	0,4275
95,61	37,52	0,3924
100,7	37,99	0,3772
106,7	38,48	0,3608
112,5	38,98	0,3466
119	39,47	0,3316
126,8	39,96	0,3151
135,9	40,46	0,2977
145,4	40,94	0,2815
155,4	41,43	0,2667
166,1	41,93	0,2524

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
176,4	42,41	0,2405
186,9	42,9	0,2295
197,5	43,4	0,2197
207,5	43,88	0,2115
216,8	44,37	0,2046
225,9	44,87	0,1986
234,4	45,35	0,1934
243,1	45,83	0,1886
251,7	46,33	0,1841
260	46,82	0,1801
268,1	47,31	0,1764
276,3	47,81	0,173
284	48,29	0,17
291,7	48,77	0,1672
299,4	49,27	0,1646
306,8	49,76	0,1622
313,9	49,75	0,1585
319,6	49,26	0,1541
324,1	48,78	0,1505
327,4	48,29	0,1475
329,6	47,79	0,145
331	47,3	0,1429
331,6	46,81	0,1412
331,5	46,32	0,1398
330,8	45,84	0,1386
329,5	45,35	0,1376
327,7	44,85	0,1368
325,5	44,36	0,1363
322,9	43,87	0,1359
319,9	43,39	0,1356
316,5	42,9	0,1355
312,9	42,4	0,1355
309,1	41,91	0,1356
305	41,42	0,1358
300,6	40,93	0,1362
296,1	40,45	0,1366
291,4	39,96	0,1371
286,4	39,46	0,1378
281,4	38,97	0,1385
276,1	38,48	0,1394
270,8	38	0,1403
265,4	37,51	0,1413

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
259,9	37,02	0,1424
254,1	36,52	0,1437
248,4	36,03	0,1451
242,6	35,54	0,1465
236,7	35,06	0,1481
230,8	34,57	0,1498
224,6	34,07	0,1517
218,6	33,58	0,1536
212,5	33,09	0,1557
206,5	32,6	0,1579
200,4	32,12	0,1603
194,2	31,63	0,1628
187,9	31,13	0,1656
181,8	30,64	0,1685
175,6	30,15	0,1717
169,5	29,67	0,175
163,3	29,18	0,1787
157,1	28,69	0,1826
150,7	28,19	0,187
144,6	27,7	0,1916
138,3	27,21	0,1967
132,1	26,73	0,2023
125,9	26,24	0,2083
119,7	25,74	0,2151
113,5	25,25	0,2224
107,4	24,76	0,2306
101,2	24,28	0,2399
94,87	23,79	0,2507
88,6	23,3	0,263
81,9	22,8	0,2784
75,56	22,31	0,2953
69,16	21,82	0,3156
62,97	21,34	0,3388
57	20,85	0,3658
51,25	20,36	0,3973
45,59	19,86	0,4357
40,23	19,37	0,4816
35,02	18,89	0,5392
29,87	18,4	0,6159
24,76	17,91	0,7232
19,66	17,41	0,8853
15,11	16,92	1,12

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
11,37	16,43	1,446
8,629	15,95	1,848
6,859	15,45	2,252
5,987	14,96	2,499
5,753	14,47	2,515
5,827	13,98	2,4
6,039	13,5	2,235
6,169	13,01	2,109
6,159	12,51	2,031
5,985	12,02	2,008
5,999	11,53	1,922
5,931	11,04	1,862
5,581	10,56	1,892
5,186	10,06	1,939
4,866	9,568	1,966
4,479	9,081	2,027
4,141	8,593	2,075
3,837	8,105	2,112
3,567	7,617	2,136
3,34	7,117	2,131
3,03	6,629	2,188
2,714	6,142	2,263
2,344	5,654	2,412
2,029	5,166	2,547
1,72	4,678	2,72
1,292	4,178	3,234
0,9041	3,69	4,082
0,5909	3,203	5,42
0,3595	2,715	7,551
0,1541	2,227	14,45
0,05115	1,727	33,76
-0,0012	1,239	1032

Table A 12: Rheology: controlled stress, sample 3.

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
0,002886	1,25	433
0,009685	1,737	179,4
0,003194	2,225	696,7
0,006287	2,713	431,5
0,005957	3,213	539,4
0,004614	3,701	802
0,007931	4,189	528,2
0,003154	4,676	1483
0,009072	5,164	569,3
0,005042	5,652	1121
0,008553	6,152	719,3
0,007338	6,64	904,9
0,006628	7,128	1075
0,009821	7,616	775,4
0,00679	8,103	1193
0,01038	8,603	829,1
0,008312	9,091	1094
0,01061	9,579	902,7
0,01019	10,07	987,8
0,01076	10,55	981,2
0,01176	11,04	939,2
0,01108	11,54	1042
0,01336	12,03	900,3
0,01116	12,52	1121
0,01482	13,01	877,8
0,01167	13,49	1156
0,01675	13,98	834,6
0,01501	14,48	964,7
0,01556	14,97	961,7
0,01848	15,46	836,3
0,01391	15,94	1146
0,01951	16,43	842,4
0,01939	16,93	873,2
0,01497	17,42	1164
0,02006	17,91	892,6
0,02228	18,4	825,6
0,01832	18,88	1031
0,0195	19,37	993,4
0,02276	19,87	873
0,0245	20,36	831,1
0,02322	20,85	897,9

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
0,02441	21,34	874
0,02769	21,82	788
0,03154	22,31	707,3
0,03675	22,81	620,8
0,04527	23,3	514,7
0,05935	23,79	400,8
0,08345	24,27	290,9
0,133	24,76	186,1
0,4104	25,26	61,56
7,469	25,75	3,448
24,13	26,24	1,087
36,17	26,73	0,7389
42,73	27,21	0,6368
45,68	27,7	0,6064
45,54	28,2	0,6192
43,33	28,69	0,6621
39,89	29,18	0,7314
36,97	29,66	0,8024
34,43	30,15	0,8756
32,18	30,64	0,9521
30,82	31,14	1,01
30,55	31,63	1,035
30,57	32,12	1,051
30,74	32,6	1,061
30,85	33,09	1,073
31,39	33,59	1,07
32,13	34,08	1,061
33,2	34,57	1,041
33,62	35,05	1,043
33,92	35,54	1,048
34,56	36,03	1,042
35,46	36,53	1,03
37,33	37,02	0,9917
39,1	37,51	0,9592
40,27	37,99	0,9436
40,8	38,49	0,9435
41,05	38,97	0,9493
41,45	39,47	0,9522
41,3	39,96	0,9675
41,52	40,44	0,974
43,14	40,94	0,9491
45,86	41,42	0,9032

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
48,36	41,92	0,8668
51,56	42,41	0,8225
58,85	42,9	0,7289
77,2	43,38	0,562
91,99	43,88	0,477
104,2	44,37	0,4259
115	44,86	0,3902
125,8	45,35	0,3606
138,8	45,83	0,3302
152,7	46,32	0,3034
167,3	46,82	0,2798
182,9	47,31	0,2586
198,8	47,8	0,2405
214,8	48,29	0,2247
230,3	48,77	0,2118
244,9	49,27	0,2012
258,5	49,76	0,1925
271,5	49,75	0,1833
282,5	49,26	0,1744
292,1	48,76	0,1669
299,8	48,29	0,1611
306,6	47,79	0,1559
312	47,3	0,1516
316,3	46,81	0,148
319,6	46,32	0,145
322,1	45,82	0,1423
323,8	45,35	0,1401
324,9	44,85	0,1381
325,2	44,36	0,1364
325	43,87	0,135
324,2	43,39	0,1338
322,9	42,89	0,1328
321,2	42,4	0,132
319,2	41,91	0,1313
316,8	41,42	0,1308
314	40,93	0,1304
311	40,45	0,13
307,6	39,95	0,1299
303,9	39,46	0,1298
300	38,97	0,1299
295,9	38,48	0,13
291,6	38	0,1303
287,2	37,51	0,1306

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
277,6	36,52	0,1315
272,6	36,03	0,1322
267,6	35,54	0,1328
262,4	35,06	0,1336
257	34,57	0,1345
251,4	34,07	0,1355
245,9	33,58	0,1365
240,3	33,09	0,1377
234,6	32,61	0,139
228,8	32,12	0,1404
223	31,63	0,1418
216,9	31,13	0,1435
210,9	30,64	0,1453
204,9	30,15	0,1472
198,9	29,67	0,1492
192,6	29,18	0,1515
186,5	28,69	0,1539
180,1	28,19	0,1565
173,9	27,7	0,1593
167,7	27,21	0,1623
161,3	26,71	0,1656
155,2	26,24	0,169
148,8	25,74	0,1729
142,6	25,25	0,1771
136,4	24,76	0,1815
130,2	24,28	0,1864
123,9	23,78	0,1919
117,9	23,3	0,1976
111,6	22,8	0,2042
105,5	22,31	0,2115
99,27	21,82	0,2198
92,94	21,34	0,2296
86,37	20,84	0,2412
79,97	20,36	0,2546
73,17	19,86	0,2714
66,66	19,37	0,2906
60,37	18,89	0,3128
54,36	18,4	0,3385
48,5	17,9	0,3691
43,04	17,41	0,4045
37,74	16,92	0,4483
32,56	16,43	0,5048

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
27,42	15,95	0,5815
22,26	15,45	0,6938
17,44	14,96	0,8579
13,23	14,47	1,094
9,925	13,98	1,409
7,606	13,5	1,774
6,246	13,01	2,082
5,716	12,51	2,188
5,553	12,02	2,165
5,562	11,53	2,073
5,562	11,04	1,986
5,636	10,56	1,873
5,704	10,06	1,763
5,778	9,569	1,656
5,751	9,081	1,579
5,701	8,593	1,507
5,536	8,105	1,464
5,199	7,617	1,465
4,698	7,117	1,515
4,259	6,629	1,557
3,935	6,142	1,561
3,568	5,654	1,585
3,188	5,166	1,62
2,671	4,678	1,752
2,025	4,178	2,064
1,496	3,69	2,466
1,097	3,203	2,919
0,7429	2,715	3,655
0,4192	2,227	5,312
0,167	1,727	10,34
0,03682	1,239	33,66

Table A 13: Rheology: controlled rate, sample 1.

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
1,107	13,05	11,78
1,654	9,121	5,515
2,142	8,162	3,811
2,627	8,035	3,058
3,113	8,315	2,671
3,599	8,499	2,362
4,097	8,783	2,144
4,582	9,208	2,009
5,068	9,471	1,869
5,553	9,651	1,738
6,052	10,02	1,656
6,536	10,33	1,58
7,022	10,68	1,521
7,508	11,05	1,472
7,993	11,35	1,42
8,492	11,73	1,381
8,977	12,08	1,346
9,462	12,45	1,316
9,947	12,7	1,276
10,43	13,06	1,252
10,92	13,49	1,236
11,42	13,92	1,219
11,9	14,37	1,207
12,39	14,8	1,195
12,87	15,22	1,182
13,36	15,68	1,174
13,84	16,24	1,173
14,34	16,7	1,165
14,83	17,14	1,156
15,65	21,31	1,362
15,98	11,26	0,7048
16,56	21,72	1,312
17,21	20,82	1,21
17,7	23,97	1,354
17,85	18,48	1,035
18,45	16,67	0,9036
19,27	21,87	1,135
19,56	29,15	1,49
19,98	15,67	0,7841
20,57	28,03	1,363
20,98	18,8	0,8961

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
21,53	28,45	1,322
22	33,65	1,53
22,44	18,45	0,8222
23,07	31,8	1,379
23,51	22,83	0,9712
23,97	37,1	1,548
24,34	25,08	1,03
24,92	42,1	1,689
25,31	22,56	0,8913
25,54	33,24	1,301
26,13	34,5	1,32
26,85	34,49	1,284
26,9	32,68	1,215
27,4	18,11	0,661
27,76	26,61	0,9589
28,11	27,01	0,9607
28,81	20,46	0,71
29,42	19,02	0,6465
29,94	17,95	0,5996
30,99	19,76	0,6377
31,24	23,57	0,7544
31,71	19,96	0,6293
32,74	20,81	0,6356
33,02	19,62	0,5943
32,84	20,49	0,624
32,97	30,65	0,9298
34,21	16,1	0,4707
34,4	20,37	0,5921
34,49	20,63	0,5982
34,36	39,34	1,145
35,43	15,97	0,4509
36,43	27,18	0,7461
36,52	17,36	0,4753
36,87	25,36	0,6878
37,53	15,96	0,4253
38,24	26,76	0,6997
39,2	22,77	0,5808
39,68	26,29	0,6626
39,16	32,11	0,8201
39,9	6,785	0,17
39,29	34,27	0,8723
40,31	21,55	0,5345

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
41,51	18,88	0,4548
42,61	24,99	0,5865
43,9	29,18	0,6647
44,47	29,82	0,6706
45,05	27,5	0,6105
44	24,78	0,5632
44,22	23,69	0,5356
45,4	34,24	0,7543
44,98	32,27	0,7173
46,71	24,78	0,5304
47,51	30,73	0,6468
47,29	30,04	0,6352
47,17	33,01	0,6998
47,61	25,17	0,5287
48,01	25,52	0,5317
49,28	37,27	0,7562
48,96	29,95	0,6118
47,95	-8,59	0,1792
50,58	28,89	0,5711
49,29	32,13	0,6518
49,62	29,14	0,5872
48,5	27,86	0,5746
48,56	27,69	0,5703
47,34	33,63	0,7103
46,77	26,4	0,5644
45,9	16,47	0,3589
45,4	34,55	0,761
44,3	27,17	0,6134
43,85	46,17	1,053
43,24	25,11	0,5808
42,83	19,9	0,4646
42,51	21,68	0,5101
41,8	22,26	0,5326
40,95	17,16	0,419
40,27	24,69	0,6131
40,28	26,98	0,6697
40,56	27,64	0,6815
40,35	25,65	0,6358
39,61	18,51	0,4674
38,27	35,26	0,9215
37,83	21,14	0,5588
37,7	19,11	0,507
37,49	20,52	0,5474

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
36,99	27,5	0,7434
36,12	17,83	0,4936
35,51	25,07	0,7061
35,19	14,57	0,4142
34,24	31,76	0,9275
34,85	12,57	0,3607
34,18	30,77	0,9002
33,4	16,31	0,4884
32,57	31,17	0,9569
32,44	21,54	0,6641
31,76	22,48	0,7076
31,38	20,7	0,6595
30,88	13,95	0,4516
30,98	19,82	0,6396
30,64	20,33	0,6637
29,87	19,13	0,6406
29,59	20,32	0,6869
29,25	19,63	0,671
28,56	19,09	0,6684
27,51	19,41	0,7058
27,15	19,58	0,7212
26,66	19,46	0,7301
26,18	19,29	0,7369
25,68	19,18	0,7469
25,19	19,11	0,7587
24,71	19,02	0,77
24,22	18,87	0,7789
23,72	18,69	0,788
23,25	18,57	0,7987
22,75	18,33	0,8057
22,27	18,15	0,8152
21,78	17,43	0,8004
22	17,51	0,796
21,1	14,37	0,6812
20,58	17,38	0,8445
20,04	17,58	0,8773
19,42	13,47	0,6939
18,9	18,65	0,9871
18,59	21,64	1,164
18,09	10,29	0,569
17,59	19,76	1,123
17,18	13,99	0,8141

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
16,65	17,71	1,064
16,24	15,51	0,9547
15,8	14,48	0,9165
15,36	14,56	0,9475
15	15,17	1,011
14,38	19,07	1,326
13,76	9,085	0,6605
13,11	14,64	1,116
12,93	14	1,082
12,21	15,3	1,253
11,8	14,42	1,222
11,24	11,67	1,039
10,61	13,66	1,288
10,27	14,1	1,373
9,808	11,29	1,151
9,22	14,37	1,558
8,586	10,6	1,235
8,14	11,74	1,442
7,654	11,41	1,491
7,157	11,12	1,554
6,671	10,81	1,621
6,185	10,47	1,693
5,7	10,15	1,781
5,214	9,689	1,858
4,728	9,341	1,976
4,23	8,994	2,126
3,745	8,501	2,27
3,257	8,038	2,468
2,777	7,569	2,726
2,299	6,926	3,013
1,802	6,179	3,43
1,316	5,388	4,095

Table A 14: Rheology: controlled rate, sample 2.

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
1,135	25,68	22,63
1,636	10,5	6,42
2,141	7,397	3,455
2,627	6,76	2,573
3,125	6,341	2,029
3,611	6,434	1,781
4,097	6,503	1,587
4,582	6,438	1,405
5,068	6,617	1,306
5,553	6,718	1,21
6,051	6,948	1,148
6,537	7,12	1,089
7,023	7,443	1,06
7,52	7,63	1,015
7,994	7,8	0,9757
8,492	8,102	0,9541
8,977	8,249	0,9189
9,463	8,625	0,9115
9,948	8,945	0,8992
10,45	9,186	0,8794
10,92	9,517	0,8715
11,42	9,914	0,8683
11,9	10,26	0,8617
12,39	10,71	0,8646
12,88	11,11	0,8627
13,37	11,51	0,861
13,85	12,11	0,8747
14,34	12,69	0,8845
14,83	13,51	0,9108
15,31	14,09	0,9201
15,8	14,98	0,9478
16,3	16,04	0,9844
16,96	20,55	1,212
17,5	11,39	0,6509
18,23	21,11	1,158
18,58	25,3	1,362
18,92	13,82	0,7303
19,59	24,12	1,231
20	25,92	1,296

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
20,49	18,72	0,9135
21,06	30,2	1,434
21,57	21,5	0,9966
22,06	33,26	1,508
22,58	25,85	1,145
22,96	39,63	1,726
23,54	23,39	0,9933
23,95	38,69	1,615
24,42	19,75	0,8088
25,02	34,34	1,372
25,42	18,72	0,7364
26,05	33,14	1,272
26,63	27,27	1,024
26,7	32,12	1,203
27,31	28,59	1,047
28,07	25,89	0,9226
27,99	29,94	1,07
28,46	34,2	1,202
29,25	28,08	0,96
30,19	32,07	1,062
30,36	29,1	0,9586
30,85	33,6	1,089
31,52	29,98	0,951
31,58	36,84	1,166
31,71	35,45	1,118
32,48	34,02	1,047
33,27	50,3	1,512
33,03	30,05	0,9095
33,88	38,82	1,146
34,37	38,81	1,129
34,85	38,62	1,108
35,35	39,21	1,109
35,82	40,56	1,132
36,32	39,44	1,086
36,81	38,39	1,043
37,29	36,97	0,9914
37,77	37,42	0,9907
38,27	37,22	0,9724
38,76	37,26	0,9613
39,24	37,06	0,9443
39,73	37,34	0,9399
40,21	33,13	0,824
41,46	28,69	0,692

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
40,94	28,71	0,7014
41,49	25,89	0,6241
41,9	13,82	0,3299
42,59	18,1	0,4251
43,05	31,97	0,7427
43,56	28,43	0,6528
43,86	20,27	0,4622
44,52	25,21	0,5662
44,98	18,97	0,4217
45,84	30,76	0,671
46	25,64	0,5574
46,38	29,21	0,6298
47,31	29,91	0,6322
48,46	24,38	0,5031
47,36	26,06	0,5502
48,56	29,4	0,6054
48,28	23,56	0,488
49,11	17,87	0,3638
47,99	-25,35	0,5282
49,62	20,49	0,413
49,29	25,99	0,5272
48,3	19,18	0,397
47,4	29,42	0,6207
47,71	23,54	0,4934
46,85	25,66	0,5476
45,81	24,99	0,5456
45	17,48	0,3885
45,52	14,18	0,3115
44,89	22,04	0,4911
44,22	19,77	0,4471
43,56	18,03	0,414
42,84	18,33	0,4277
42,32	22,26	0,5258
41,8	18,06	0,432
41,16	18,6	0,4518
40,7	22,02	0,541
40,24	25,14	0,6247
40,31	18,73	0,4648
39,37	35,73	0,9075
38,91	24,38	0,6265
38,88	28,31	0,7283
38,36	13,71	0,3573

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
38,22	17,33	0,4534
38,01	19,97	0,5254
37,27	17,4	0,4669
36,79	19,29	0,5243
35,63	28,02	0,7864
36,28	11,21	0,309
35,07	16,89	0,4817
34,48	28,77	0,8342
33,72	6,853	0,2033
32,68	29,08	0,89
32,39	17,57	0,5422
32,47	14,39	0,4431
32,76	19,24	0,5872
31,91	18,44	0,5778
31,47	18,41	0,5852
31,07	19,05	0,613
30,43	13,52	0,4442
29,35	17,79	0,6063
28,94	16,86	0,5826
28,51	14,96	0,5247
27,9	27,62	0,9899
27,9	11,84	0,4243
27,45	16,5	0,6011
26,59	22,56	0,8483
26,51	15,5	0,5849
25,95	15,34	0,5911
25,13	22,54	0,897
25,03	15,88	0,6346
24,39	11,72	0,4807
23,82	20,81	0,8738
23,54	19,88	0,8445
22,81	9,457	0,4147
22,42	16,56	0,7385
22,12	18,73	0,8468
21,55	18,61	0,8638
20,87	11,96	0,5729
20,25	15,42	0,7618
20,35	20,53	1,009
19,52	7,548	0,3868
19,12	17	0,8891
18,55	18,34	0,9885
18,04	11,55	0,6404

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
17,68	16,06	0,9085
17,1	10,3	0,6022
16,54	16,54	0,9995
16,4	14,76	0,9001
15,3	13,48	0,8806
14,96	13,07	0,8733
14,48	12,86	0,8881
13,99	12,55	0,8968
13,51	12,29	0,9102
13,02	12,02	0,9231
12,52	11,99	0,9577
12,04	11,79	0,9794
11,55	11,48	0,9935
11,07	11,22	1,014
10,58	10,92	1,032
10,08	10,6	1,052
9,595	10,32	1,076
9,111	10,03	1,101
8,625	9,601	1,113
8,128	9,37	1,153
7,654	9,162	1,197
7,156	8,835	1,235
6,671	8,571	1,285
6,185	8,263	1,336
5,699	7,959	1,397
5,136	7,62	1,484
4,74	7,286	1,537
4,242	7,074	1,668
3,756	6,677	1,778
3,27	6,313	1,931
2,785	5,908	2,122
2,287	5,343	2,336
1,793	4,803	2,678
1,327	4,211	3,174

Table A 15: Rheology: controlled rate, sample 3.

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
1,124	19,6	17,45
1,646	7,935	4,82
2,118	6,364	3,004
2,618	6,187	2,364
3,102	6,236	2,01
3,588	6,31	1,759
4,085	6,5	1,591
4,571	6,65	1,455
5,057	6,883	1,361
5,542	7,078	1,277
6,04	7,272	1,204
6,525	7,49	1,148
7,012	7,744	1,104
7,496	7,927	1,057
7,983	8,2	1,027
8,481	8,448	0,9962
8,965	8,651	0,9649
9,452	8,941	0,946
9,948	11,61	1,167
10,56	10,35	0,9795
11,03	10,21	0,9259
11,51	10,73	0,9326
12	11,11	0,9262
12,58	11,55	0,9181
13,01	10,09	0,7754
13,39	9,009	0,673
14,23	16,12	1,133
14,88	14,2	0,9542
15,45	14,65	0,9482
15,87	18,08	1,139
15,99	17,93	1,121
16,48	13,02	0,7898
17,18	18,24	1,062
17,7	21,86	1,235
18,03	16,62	0,9215
18,53	15,18	0,819
19,23	18,5	0,9622
19,52	19,95	1,022
19,84	9,626	0,4853
20,49	18,07	0,8818
21,02	17,38	0,8266

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
21,3	23,08	1,084
21,74	7,624	0,3507
22,44	19,28	0,8591
22,99	19,48	0,8473
23,25	20,11	0,8648
23,71	10,6	0,4471
24,43	19,89	0,8142
25,08	17,34	0,6914
25,09	16,8	0,6696
25,58	17,35	0,6785
26,06	17,47	0,6704
26,55	17,59	0,6624
27,03	17,96	0,6643
27,52	18,36	0,6671
28,02	18,85	0,6726
28,51	19,27	0,6761
28,99	19,53	0,6736
29,48	19,83	0,6726
29,96	20,12	0,6715
30,45	20,53	0,6744
30,94	20,92	0,6759
31,43	21,19	0,6741
31,92	21,13	0,6621
32,4	20,92	0,6456
32,89	20,83	0,6333
33,39	20,88	0,6254
33,87	20,96	0,6189
34,35	21,19	0,6168
34,84	21,49	0,6167
35,33	21,78	0,6164
35,81	22,2	0,62
36,31	22,48	0,6192
36,79	22,67	0,6161
37,28	22,84	0,6125
37,77	23,29	0,6168
38,25	23,96	0,6264
38,75	24,72	0,6379
39,24	25,51	0,6501
39,72	26,27	0,6613
40,21	26,84	0,6674
40,69	27,07	0,6652
41,19	27,49	0,6673

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
41,67	28,02	0,6724
42,16	28,46	0,675
42,64	28,89	0,6774
43,13	29,59	0,6861
43,63	29,85	0,6842
44,12	30,26	0,6859
44,6	30,78	0,6903
45,09	30,95	0,6864
45,57	31,63	0,6941
46,05	32,72	0,7105
46,56	34,19	0,7343
47,04	35,49	0,7546
47,52	37,16	0,7819
48,08	50,89	1,058
48,74	29,05	0,596
48,39	56,77	1,173
48,88	44,82	0,917
47,99	-22,17	0,462
50,58	29,05	0,5743
49,49	32,11	0,6489
49,59	28,73	0,5794
48,44	25,1	0,5182
47,29	28,53	0,6034
44,98	33,73	0,7501
45,73	12,3	0,2689
45,39	19,86	0,4375
44,91	23,45	0,5222
44,76	30,24	0,6755
43,89	23,91	0,5448
43,57	25,28	0,5803
44,11	22,63	0,5129
43,66	21,39	0,4899
42,57	23,27	0,5465
41,44	24,56	0,5927
41,12	23,94	0,5822
40,76	20,48	0,5025
40,97	22,19	0,5417
40,7	18,16	0,4462
39,11	16,86	0,431
38,67	23,7	0,6129
38,59	24,34	0,6308
37,76	19,65	0,5204

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
37,17	30,28	0,8145
36,47	32,33	0,8867
36,06	19,47	0,54
35,64	13,91	0,3904
36,05	16,91	0,469
34,12	11,43	0,3351
34,06	28,72	0,843
34,59	17,49	0,5057
34,08	16,49	0,4839
33,55	17,84	0,5316
33	19,09	0,5787
31,78	14,03	0,4416
31,62	22,51	0,7118
31,58	18,98	0,6011
31	18,56	0,5989
30,63	19,12	0,6244
29,99	18,51	0,6174
29,61	17,46	0,5896
28,95	16,53	0,571
28,54	16	0,5606
28,04	16,65	0,5939
27,54	19,32	0,7018
27,2	11,85	0,4358
25,87	19,6	0,7576
25,25	25,75	1,02
24,83	16,69	0,6724
24,81	9,226	0,3719
24,46	14,92	0,6099
24,18	16,46	0,6807
23,54	14,88	0,6323
23,11	16,38	0,7089
22,31	15,44	0,6919
21,87	12,18	0,557
21,58	15,13	0,7011
21,01	12,96	0,6168
20,25	16,1	0,795
19,47	15,59	0,8005
19,35	13,48	0,6965
18,87	13,28	0,7038
18,37	13,23	0,7202
17,88	12,94	0,7235
17,4	12,76	0,7335
16,91	12,58	0,7441

Shear Rate 1/s	Shear Stress Pa	Instantaneous Viscosity Pas
16,43	12,39	0,7542
15,94	12,35	0,7749
15,44	12,1	0,7835
14,96	11,68	0,781
14,47	11,56	0,7987
13,99	11,39	0,8144
13,5	11,16	0,8263
13,02	11,07	0,8505
12,52	10,87	0,8687
12,03	10,74	0,8925
11,55	10,58	0,9163
10,84	12,28	1,133
10,87	9,487	0,8726
10,35	8,346	0,8068
9,917	9,028	0,9104
9,485	9,211	0,9711
8,792	9,276	1,055
8,214	8,467	1,031
7,659	8,346	1,09
7,161	8,208	1,146
6,675	7,989	1,197
6,189	7,631	1,233
5,704	7,436	1,304
5,218	6,916	1,325
4,732	6,544	1,383
4,183	6,095	1,457
3,762	5,7	1,515
3,275	5,272	1,61
2,789	4,858	1,742
2,303	4,472	1,942
1,804	3,944	2,186
1,327	3,552	2,677