Master Thesis in Food Technology

Effect of High-Pressure Homogenization and Heat Treatment on the Emulsification Properties of Oat Protein Concentrate

by

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Preface

This report presents the results of master thesis project at the Department of Food and Nutrition at the Faculty of Engineering at Lund University. The project was carried out in collaboration with Lantmännen from January to June 2022.

This thesis report would not have been possible without the support of my supervisor Björn Bergenståhl from Lund University. Thank you for supporting my growth and widening my understanding of the world of proteins and emulsions during the thesis. A special thanks to Maria Elena Damas from Lantmännen for supplying me with PrOatein powder and giving me the opportunity to work with it. Thank you for insightful discussions and your contagious enthusiasm throughout the thesis process. A big thanks for giving me a tour at your facilities in Kimstad and introducing me to your team, who assisted me with solubility analysis. I would also like to thank Hans Bolinsson and Olexandr Fedkiv for training me to use different equipment in the laboratories at Kemicentrum. Lastly, thank you Lars Nilsson for your input as an examinator and Natalie Longmore together with Philip Bergsell as opponents.

Thank you!

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Abstract

This master thesis project was performed in collaboration with an agricultural cooperative Lantmännen Oats AB, who has developed a dry protein powder concentrate PrOatein™. The powder consists of oat proteins, dextrins, lipids and β-glucans. The aim of the thesis was to investigate the powder's functionality in a liquid emulsion and see if it can be improved by pre-treating it with thermal heating and high-pressure homogenization (HPH) under different pH conditions. The emulsification properties of the protein concentrate were first evaluated in the means of the protein's solubility and its efficacy of producing small oil droplets in different pre-treatment conditions. The effect of high-pressure treatment nor heating in slightly alkaline conditions (pH 7.5) was statistically proved. However, the solubility was highest, when the sample was heated to 85 ºC and high-pressured with 600 bars. In slightly acidic conditions (pH 4.5) the effect of high-pressure treatment was significantly proved. Under acidic conditions, the increased temperature during heat treatment and increased pressure during homogenisation decreased the solubility.

The light scattering analysis showed that the particle size in pH 7.5 was smaller at all pre-treatment conditions compared to pH 4.5 The smallest particle size was achieved by treating the sample with pH 7.5 with 85 ºC and 800 bars. The effect of HPH and heat treatment on particle size was also significantly proved.

The stability analysis showed that heat and high-pressure treatment can delay the sedimentation, which is the dominating instability mechanisms in emulsions consisting of 5 % w/v PrOatein™ powder and 3 % v/v oil. The emulsion sedimented, when the oil content was increased to 10 %, suggesting that the optimal ratio of oil and PrOatein™ in a liquid emulsion should be 1:1.

Popular Summary

Plant-based proteins have an important role to play in our diet. Protein deficiency can trigger loss of muscle mass, which increases the risk of injuries. Moreover, it can increase our appetite and consequently increase the calory intake. However, the food developers face challenges when using plant-based proteins in liquid food products due to their more complex nature compared to animal-origin proteins. But what are those challenges of plant-protein and how to overcome them?

To answer this question let's take a deeper look on oat proteins. Oats are fascinating grains that have an interesting nutritional profile. Why so? Maybe you have heard of oat β-glucans and their positive effect on health as they can lower the cholesterol level and decrease the risk of cardiovascular disease. That's not the only health benefit that oats can provide. They are also rich in oat oil, that has high concentration of polyunsaturated fatty acids. And most importantly, oats are source of protein, that could alleviate the health problems mentioned before. Oats are also one of the few plants that thrive here in the rather cold North, making them a good source of local plant-proteins.

Now as we know the importance of proteins and Nordic oats, we can talk about the challenges of oat proteins for the food industry. You probably have not noticed too many protein shakes or protein rich cooking creams with oat protein in the market. One reason for this is the low solubility of oat proteins. Low solubility of proteins in such products results in two unpleasant phenomena. One being the development of sediment layer in the bottom and the other one, the separation of oil in such products. Scientifically speaking, the products including oil and water are called 'emulsions'. The addition of fat in liquid emulsions (as cooking creams, protein shakes, milk alternatives are) is desired for improved mouthfeel. For example, the fat makes the milkshake nice and silky and less 'watery'.

The possibilities to increase the use of oat protein powder developed by Lantmännen Oat AB under a trademark PrOatein, that incorporates oat protein, oil, β-glucans and dextrins, is investigated in this thesis project. The thesis explores the potential of heating and high-pressure homogenization to overcome the limitations of using this nutritious and protein rich powder in liquid food systems such as emulsions.

Contents

1 Introduction

1.1 Background

Oats have one of the highest protein content among cereals, which gives them a good potential to play a significant role in modern plant-based diets. The lack of gluten in protein composition makes oats suitable for people with celiac disease. Moreover, globulins being the dominating protein fraction in oats, have more balanced amino acid profile compared to popular *Triticeae* cereals (barley, rye, wheat), rich in prolamin fraction. However, the major throwback of oat protein is its low solubility, especially around neutral or slightly acidic pH, which limits the application of oat protein as an emulsifier in liquid foods (Mäkinen et al. 2017).

Lantmännen is an agricultural cooperative owned by 19 000 Swedish farmers. They produce two oat powders branded as PrOatein™ and PromOat® for food product formulation. The first has the oat protein as the main ingredient, while in the latter it is the β-glucan. In addition, Lantmännen produces Avenacare with β-glucans for formulation of cosmetical products such as skin and hair care (Lantmännen Oats AB, n.d.). Lantmännen's different sectors are devoted to importing, marketing and selling agricultural machinery; producing bioenergy and developing and marketing food products (Lantmännen Oats AB, n.d.).

In 2019 Lantmännen purchased an oat ingredients processing factory in Kimstad to meet the growing demand for oat products. The company has also partnered with industrial research centre ScanOats and is the majority owner of biotech company CropTailor (Askew, 2019). The company invests 250-300 million SEK annually in various projects from plant breeding to consumption (Lantmännen, n.d.-a) and has oats in the centre of its strategic investments (Askew, 2019).

This project aims to investigate the use of oat bran protein powder PrOatein™ supplied by Lantmännen Oats AB in Kimstad in liquid emulsions and evaluate if different pre-treatment methods could help to overcome its functional limitations.

1.2 The Purpose of the Project

The aim of the project is to investigate the characteristics of emulsions prepared with PrOatein™ powder after they have been treated with thermal heating and high-pressure homogenisation under different pH conditions. Emulsions can be characterised by various attributes, which were limited to oat protein solubility, particle size, emulsion stability and emulsion capacity. The knowledge about the effect of those pre-treatments on the emulsion incorporating PrOatein™ powder is important for expanding its application in liquid emulsions. The effect of homogenisation, heat

and pH has been investigated on some plant-protein isolates, but not on a complex system, where oat proteins, lipids and polysaccharides concurrently interact as they do in PrOatein™.

The project aims to answer following questions:

A) Could the solubility of oat protein be improved by applying high pressure homogenisation and heat treatment on the PrOatein™ powder?
B) Which treatment conditions provide particle and droplet size needed for a stable emulsion?
C) What are the underlying instability mechanisms in emulsions incorporating PrOatein™ and what is the optimal amount of oil that could provide a stable emulsion?
D) How does the pH shift influence PrOatein™ emulsions?

1.3 The Scope of the Project

The PrOatein™ powder is a complex food matrix, where the ingredients could be affected by the mentioned treatments in a different way. Oat proteins consist of four fractions with individual pI, that influences the solubility and functionality of oat protein. Moreover, that ratio of those fractions depends on environmental factors during growing period and the genotype of the variety.

Due to the limit of resources available for this project, it is aimed to provide a general overview about the effect of the pre-treatments on the behaviour of the oat protein emulsions. Thus, the more detailed characterisation of oat protein is out of scope in this project. The project can rather be seen as a springboard for future investigation of the effect of the treatments on the isolated ingredients found in the powder.

2 Theoretical Background

2.1 Oats

Oats (*Avena sativa*) are one of the major cereal crops with an annual global production of 25 million tonnes (Price & Welch, 2013). In Europe, the share of oats from the total cereal production is 2.6 % (8.06 million tonnes). The largest oat producer in Europe is Poland with 1.5 million tonnes produced annually, while in Sweden the annual production is around 800 000 tonnes (Eurostat, 2017). Oat products have been traditionally consumed for breakfast in the form of porridge or cereals (Price & Welch, 2013).

Oats need temperate climate for growth and can be successfully grown in cold, wet conditions. They are less sensitive to acidic soils and require less agro-chemical and fertiliser input compared to other grains. The oat grain consists of 18-36 % hull, that is removed when milled. After milling, the groats are left, which can be processed into oatmeal, oat flakes or oat flour products by cutting, rolling or grinding (Price & Welch, 2013). The groats are composed of the bran, germ and endosperm as seen on Figure 2.1. The protein can be found in different parts of the groat, but in more concentrated form in the bran layer surrounding the groat and in the germ (Miller & Fulcher, 2011). In the central endosperm, the concentration of starch is the highest instead. Here, also the lipids can be found in more concentrated form (Miller & Fulcher, 2011). In total, the oat groats contain approximately 15-20 wt. % proteins (Peterson, 2011), 8 wt. % lipids and 55 wt. % starch (Sosulski & Sosulski, 1985).

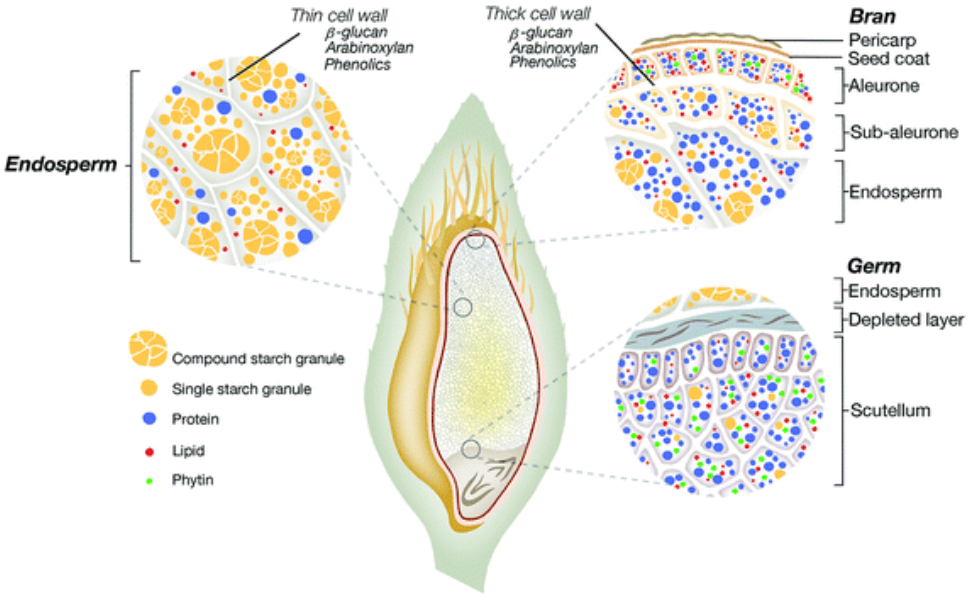

Figure 2.1 Structural representation of the oat grain (Grundy et al., 2018).

2.2 PrOatein™

The coarse oat protein powder PrOatein™ (from now on referred as "PrOatein" or "protein powder") used in the project was supplied by Lantmännen Oats AB and had 95.9 % dry matter containing 53 % protein (Lantmännen Oats AB, 2022). Additionally, it contains approximately 20-24 % of oat dextrin, 16-19 % of oat oil and 2-3 % of oat β-glucan (Lantmännen Oats AB, n.d.). Those ingredients provide functional and health benefits in a food product and are discussed further in coming chapters.

The oat protein is extracted from non-GMO oats from commercial varieties Kerstin and Galant (Damas, 2022b) grown in Nordic countries (Lantmännen Oats AB, n.d.). The process line of the protein powder begins with the dry milling of the oat kernels and separating the different components. The oat bran is then subjected to wet milling processes, where enzymes are used to separate the different fractions. After the centrifugation, the protein part is spray dried and β-glucans drum dried. Lastly, the protein powder is packaged in a high-hygiene area. The fibres and dextrins, that are separated during the process are used for ethanol, biofuel or feed production (Damas, 2022a). The process line applies no chemicals, which supports the use of PrOatein in clean label products (Lantmännen Oats AB, n.d.).

PrOatein stands out among other plant-based protein products by having low sodium content as no pH adjustment is done during the extraction of the protein (Damas, 2022a). The PrOatein is described to have mild and neutral taste. Some additional properties of the product are its light-brown colour, non-stickiness, and good wettability. The product is most commonly used in dry food applications such as in breads, cakes, biscuits, but also in powdered beverages. On the ingredients label, the powder can be listed simply "oat protein" (Lantmännen Oats AB, n.d.).

2.3 Proteins

Proteins in human diet are responsible for keeping the muscles in a good condition, as well as controlling the immune responses and functioning of the cells (Nasrabadi et al., 2021). On a chemical level, proteins are classified as linear heteropolyelectrolytes, with a backbone consisting of α–L amino acids linked with peptide bonds (Walstra, 2002c). The alpha (α) amino acid consists of α-carbon, which is bound to amine and carboxylic acid functional groups. The α-carbon is also linked with a side group, where 20 different structures exist. During a dehydration synthesis, two amino acids will be linked with peptide bond. The peptide bond forms between the carboxylic tail and amino head of two amino acids (Flatt, 2019). The degree of polymerization (a.k.a. the number of amino acids) can range from 50 to over 100. The side groups differ in polarity, charge, sulphur

containment, aromatic and aliphatic characteristics and thus define the chemical reactivity of the protein molecule (Walstra, 2002c).

The properties of the protein depend on its composition and structure. The primary structure describes the sequence of amino acid residues. The secondary structure describes the arrangement of those residues. Tertiary structure describes the three-dimensional structure of a protein, which can be globular, fibrous, or disordered. The tertiary structure is unique for each protein and has evolved to fulfil a certain function. Most of the proteins are globular, where hydrophilic amino acid side groups are exposed on the surface, while the hydrophobic ones are packed inside. However, in some cases, the primary structure does not allow packing hydrophobic residuals in the core, and they can instead appear on the surface. Still, in most globular proteins, many sulphur bridges between different regions of peptide chains support tight packing.

In order to limit the contact between water and apolar groups, the proteins can aggregate into larger units and develop a quaternary structure. Those aggregates can be broken down by pH or temperature alteration (Walstra, 2002c).

2.3.1 Oat Proteins

The dominating protein fraction (more than 55 wt. % counted on total protein) in oats is globulin, which make them different from wheat and other cereals, where prolamins are dominating (Miller & Fulcher, 2011). In oats, the prolamins (a.k.a. avenins) are present at 4-14 wt. %. In addition, albumins account for 9-20 wt. % and the remaining is covered by glutenin fraction. The amino acid composition and thus the ratio of different protein fractions in oats can vary significantly between the varieties. In some studies, it has been found that instead of globulin, the glutenin fraction is instead dominating (Peterson, 2011).

Oat globulins have been well studied. They have a compact globular structure and high denaturation temperature (around 112 ºC) (Marcone et al., 1998). Their solubility in water at neutral and slightly acidic pH is poor. This means, that those globulins have shown very poor functionalities in liquid food applications (Loponen et al., 2007). The four protein fractions are soluble in different conditions. Globulins are soluble in dilute salt solutions, glutenins in strong acid or alkaline solution, albumins in water at pH 6.6 and prolamins in ethanol (Bergenståhl, 2020a).

2.3.2 Solubility

Solubility is often prerequisite for good emulsification, gelling and foaming properties (Jiang et al. 2015). Solubility of proteins depends on the properties of the groups at the surface (Walstra, 2002c). In general, the proteins show lowest solubility at their isoelectric point (pI), which is individual for each oat protein fraction. For 12 S globulins the pI has found to be around 5, prolamins around 5-9, albumins 4-7.5. As apparent, the variations in the ratio between the protein fractions bring about the fluctuation of the average pI of oat protein (Mäkinen et al., 2017).

Solubility can be influenced by various chemical and physical changes, among what, the most relevant one to the present master thesis (i.e. heating, pH shifting and homogenisation) are explained in greater detail in following chapters. It is important to remember, that the presence of other compounds can also influence the solubility of protein. For example, it has been found that the presence of alcohol at moderate concentration decreases the surface tension and thus increases the solubility of hydrophobic proteins. Sugars have reported to reduce the solubility (Walstra, 2002c).

Solubility is often measured as a % of protein in a supernatant collected after centrifugation of the samples compared to the total amount of protein present. The results may depend on the stirring and centrifugation conditions (Walstra, 2002c).

2.3.3 Denaturation

Denaturation means the unfolding of the protein from its native conformation. It can be provoked by changes in the environmental conditions, such as changes in temperature, pH, high pressure or by contact with hydrophobic surfaces (as air or oil). Since, the hydrophobic parts of the protein unravel and expose themselves at the surface, then the solubility is decreased. Additionally, the denaturation causes decrease in surface activity, increased risk of proteolysis and reactivity to other compounds (Walstra, 2002c).

In some cases, refolding of the protein to the native state can be induced, however, the properties of the native protein are seldom recovered. While the protein molecule is in unfolded state, it can form intermolecular bonds between hydrophobic regions of different protein molecules giving rise to the formation of aggregates. The aggregates further suppress the refolding and cause imperfections at refolding, making it impossible to recover the functionality of native protein (Walstra, 2002c).

2.4 Oat β-glucans

Oats can contain about 3-7 % of β-glucans, varying between cultivars and environmental conditions. The β-glucans are found in the bran and endosperm cell walls in oats. The molecular structure of a β-glucan is shown on Figure 2.2.

β-glucans are linear non-starch polysaccharides, containing D-glucose units bound by β-(1→3) or β-(1→4) linkages. Those β-linkages make the molecule indigestible by human enzymes in the small intestine and thus they reach the large intestine (Ahmad & Kaleem, 2018). On the other hand, the β-(1→3) linkages give the structure solubility (Bergenståhl, 2020b), which makes the oat β-glucans available for gut microbiota. Gut microbes ferment the β-glucan polymers and produce short chain fatty acids, that have proven to have several health benefits, such as having anti-inflammatory effect (Spina, et al. 2007).

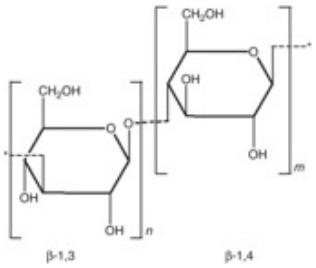

Figure 2.2 Chemical structure for cereal β-glucan (Figure from Ahmad & Kaleem, 2018).

β-glucans can also effectively bind water and increase the viscosity of intestinal contents. In addition, they can bind bile acids and increase their excretion. That could promote bile acid synthesis from hepatic cholesterol, thus decrease the absorption of intestinal cholesterol and its enterohepatic circulation (Marlett, et al., 1994). Those mechanisms are suggested to be the reason for decreased levels of low-density lipoprotein (LDL) cholesterol levels observed with increased β-glucan intake. High cholesterol level is a risk factor for cardiovascular disease (CVD), the world's number one cause of deaths (WHO, 2021).

In food product development, there is a great interest in use of β-glucans for those health effects. In addition, β -glucans have several functionalities in food matrices. In food products, they act as thickening, stabilizing and texturizing properties. They can provide alternative to traditional stabilizers and thickeners such as gum arabic, carrageenan, guar gum or modified starches (Ahmad & Kaleem, 2018).

2.5 Dextrins

Dextrins are hydrolysed from any starch if heated in the presence of water, acid or enzymes. Dextrins are more water-soluble and produce less viscous solutions than the starch, where they

originate from. Dextrins can be classified as white, yellow dextrins and British gums depending on their appearance (BeMiller, 2003).

Dextrins are characterized by their dextrose equivalency (DE). DE reflects the degree of hydrolysis of the breakdown product from starch. While the DE of starch is 0, then the DE value of D-glucose is 100. Maltodextrins have DE value between 5 to 19. Some properties as for example particle size, sweetness and solubility of maltodextrins increase as the DE increases. In food applications, maltodextrins have been widely used to improve the mouthfeel and texture of the food product. They also absorb flavour oils and other nonaqueous liquids, making them of use as encapsulating agents for essential oils and other flavours. Apart from that, they are used as carriers for spray-dried and extruded flavours, bulking agents, sweeteners and fat replacers. In hard confections they can aid maintaining the moisture levels by preventing sugar crystallization (BeMiller, 2003).

From oat flour and bran, low-DE (DE value less than 3) and more crystalline maltodextrins can be produced. More crystalline maltodextrins are less soluble. They give fatty mouthfeel and form soft creamy gels when hydrated. (BeMiller, 2003). Due date, the oat dextrins are not well studied and the DE of the dextrins in PrOatein is unknown (Damas, 2022a).

2.6 Oat Lipids

Oats contain more lipids than any other cereal grain and are good source of unsaturated fatty acids. Lipids could influence the adhesive properties of oat starch and thus influence their functionality. They also contribute to the flavour of oats. The unique flavour of oats originates from the lipid oxidation products and N-heterocyclic compounds developed during heat processing of the oat groats. High lipid content makes the oats susceptible to oxidative and hydrolytic rancidity, which causes bitter taste. The hydrolytic processes could be initiated by lipases when the cell has been damaged. It is thus necessary to inactivate the lipases during oat product production (Zhou et al., 1999).

Lipid removal from oat starch has shown to decrease the gelatinization temperature, peak viscosity and swelling factor of the starch at pH>4. The formation of amylose-lipid complexes, where saturated fatty acid chain is packed in the core of the amylose helix (Zhou et al., 1999), can thus influence the functionality of the PrOatein.

2.7 Emulsions

Emulsions are food dispersions, where both the continuous and dispersed phases, are in liquid form, such as oil and water. Oil-in-water emulsions, where the surfactant is soluble in water, are for example milk and mayonnaise. The other type of emulsions is water-in-oil, where the surfactant is soluble in oil. The last type includes margarine and butter. As apparent, in food industry, it is often needed to mix two immiscible liquids to supply the market with tasty food products. Those, liquids, need surface active compounds in order to mix in one another and form a stable system. The surfactant has to succeed in reducing the interfacial pressure and preventing the emulsion droplets to coalesce to provide a stable emulsion. Thus, an emulsion consists of oil, water and surfactant and needs an external energy to compensate the increased interfacial tension and area (Walstra, 2002a).

The drops in an emulsion for food applications are most often produced by agitation, including stirring, beating or homogenizing. During the agitation, not all of the mechanical energy is transferred for drop formation, but also for temperature increase (Walstra, 2002a). Generally, smaller drops increase the stability of the emulsion. However, as the Laplace pressure, that prevents the deformation of the particle, increases as the particle size decreases, it is often difficult to achieve very small emulsion drops (Walstra, 2002a).

For food manufacturers, it is often necessary to know the minimum amount of surfactant for a maximum amount of oil needed for a stable emulsion. This is described by the surfactant's emulsifying capacity. The maximum emulsifying capacity is just before the breaking point of the emulsion or its conversion into water-in-oil emulsion. There exists no standard for measuring the breaking point in practical setting and several methods, including optical, rheological, and electrical conductivity measurements have been implemented. Emulsification capacity depends on the size of the droplets, but also on surfactant concentration and temperature (McClements, 2016c).

As proteins are not soluble in oil, they can only be used in emulsions where the continuous phase of an emulsion is polar (Walstra, 2002a). As the proteins are amphiphilic (i.e. they have hydrophobic and hydrophilic parts) molecules, they can act as surfactants and be used in emulsions, such as in protein shakes and other plant-based beverages as well as in light cooking creams.

2.8 Destabilisation Mechanisms

At constant volume, the energy of the system always thrives to be minimal. At such level, the entropy of the system can be maximum. Entropy is defined as a measure of disorder and has

highest value when the molecules in the system do not have any attraction or repulsion forces between them. This would allow the particles to be positioned in the system randomly at any location possible. However, in emulsions, a great tension at the oil and water interfaces is generated. To minimize the interfacial free energy, the molecules of the less-dense liquid rise to the surface and coalesce (Walstra, 2002b). This makes the system thermodynamically unstable without constant input of energy that could alleviate the tension at the interfaces.

In order to maintain a somewhat stable emulsion, the surfactant in the emulsion has to prevent the coalescence through electrostatic repulsion. As previously mentioned, the protein side groups can be positively and negatively charged, which depends on the environmental factors such as pH, temperature and ionic strength. Thus, the droplets that are stabilized by the same emulsifier have same electrical charge, which creates repulsion between the particles.

The electrical charge on the surface can be characterised by surface charge density (σ, amount of electrical charge per unit surface area) and the electrical surface potential (Ψ_0, free energy needed to increase the surface charge density from zero to σ). Electrostatic repulsion has to overcome the van de Waals attraction force between the oil droplets to ensure the stability. The success to overcome it depends on the thickness and composition of the interfacial layer (McClements, 2016a).

Surface active compounds can alleviate the problem somewhat, but they are not suitable for the long-term stability of the system. Thus, in food industry, the food matrices for emulsion needs different compounds working in synergy to form and maintain a homogenous system for a desired period. The stability depends on the particle size, solubility, volume fraction of different compounds and the density of dispersed phase (Walstra, 2002a).

2.8.1 Sedimentation and Creaming

Sedimentation and creaming are examples of gravitational separation of the phases in the emulsion and are often the dominating instability mechanisms in a food system. Gravitational separation is provoked by the density differences between dispersed droplets and continuous phase. The droplets having higher density will move downwards and sediment, while the smaller density of the droplets causes their migration to the upper layers and creaming (McClements, 2016b). Sedimentation and creaming rate (v) are in a proportional relationship with the particle size as described by Stokes' law (Equation 2.1):

$$v = \frac{2(\rho_2 - \rho_1)g}{9\eta} r^2 \qquad \textit{Equation 2.1}$$

where ρ_1 is the density of the continuous phase

ρ_2 is the density of the dispersed particle

η is the viscosity of the continuous phase

g is acceleration due to gravity

r is the radius of the particle.

In addition to particle size and density, the creaming and sedimentation rate is also dependent on the viscosity of the continuous phase. In a more viscous solution, the velocity of particle movement is decreased and thus the instability mechanisms are delayed (McClements, 2016b).

Stokes' equation has some limitations in practical use. It assumes the particles to have no interaction and to be in ideal spherical shape. Thus, the actual sedimentation and creaming rate in more concentrated ($\varphi > 5\%$) emulsions differ from computations (Nilsson, 2021).

A cream layer (Figure 2.3, iii.) in an emulsion is eventually formed as the oil droplets migrate on the top. The thickness of the cream layer depends on the initial droplet concentration and the nature of the droplet packing. Tight packing is likely when droplets have small polydispersity. In loosely packed cream layer, there is an attractive interaction between the droplets, which does not support rearrangement after the droplet has "settled down". If the droplets in cream layer are not too strongly attached, then the emulsion can be redispersed by mild agitation. The polydispersity of the particles also affects the creaming rate. In a polydisperse emulsion, the small droplets can be trapped between the larger ones and when the droplets are attracted to each other, then a gel network can be formed and droplet movement restricted (McClements, 2016b).

Gravitational separation is often not appealing for the consumer as the cream layer has a very viscous and oily appearance coupled with unpleasant mouthfeel. Creaming over prolonged period can eventually cause flocculation or coalescence leading to the formation of pure oil layer on top (Figure 2.3, iv.). It is thus important to control the rate of gravitational separation (McClements, 2016b).

2.8.2 Flocculation

While in the case of coalescence (Figure 2.3, i.), the particles that have been collided, merge into one single large droplet, then flocculation (Figure 2.3, ii.) occurs as the droplets preserve their integrity after being associated with another particle. This kind of aggregation of droplets in food emulsions causes development of flocs. The development of flocs can have either retarding or enchasing effect on the creaming rate, depending on the nature of interaction between the flocs.

In an emulsion, where the flocs do not interact, the creaming rate can be increased due to increase in the effective size of flocs. In concentrated emulsions, strong attraction between flocs decreases the rate of creaming as three-dimensional network is formed. Loose packing of the droplets within the flocs also decreases the rate of creaming as the density contrast between particles and surrounding fluids is decreased (McClements, 2016b).

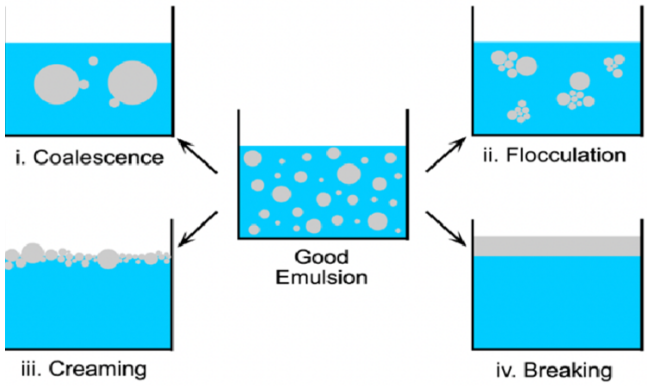

Figure 2.3 Instability mechanisms in emulsions (Khan et al., 2011).

2.9 Particle Size

The stability of the emulsions depends on the particle size as seen before from the Stokes' law (Equation 2.1, p. 16). Smaller emulsion droplets generally increase the stability of the system (Walstra, 2002a). Homogenization is often used in food applications to decrease the particle size, which increases the stability of the colloidal system. However, Liu and McGrath (2005) found that the effect on homogenization can differ. While the droplet size in low-oil content emulsion decreased after homogenizations, then the droplet size in high-oil content emulsion increased (Liu & McGrath, 2005).

Two most common ways for particle size distribution determination are microscopy and light diffraction analysis. The analysis under the microscope reveals more about the shape and size of the individual dispersed particles and makes it possible to identify aggregates. This method is rather slow for giving representative results as only extremely small samples can be investigated at once. On the other hand, methods based on light diffraction are faster and allow to make conclusion about size distribution in bigger sample volume (Nilsson, 2021).

2.10 Techniques for Protein Modifications

There is a growing interest on modifying the functionality of plant-based proteins, due to their poor functionality causing broad limitations for their applications. The modification methods can be classified as physical, chemical, and biological. The first has gained significant interest as it poses

no risk of possible chemical residuals from the process in the final product. It is important to select the modification method carefully as they can influence the functional, nutrition and organoleptic properties of proteins (Nasrabadi et al., 2021).

2.10.1 Heating

Heat can be generated and applied on the product in different ways. In addition to conventional thermal heating, methods as ohmic, microwave, radio frequency and infrared irradiation have been applied on plant-proteins (Nasrabadi et al., 2021). The response to temperature differs among proteins. Hydrophilic proteins may increase their solubility with temperature increase (Walstra, 2002c).

During conventional thermal heating, the plant-based proteins' functionality is changed through protein unfolding. As the protein unfolds from its native state, the hydrophobic side chains from the inside of the molecule will be exposed on the surface. However, extreme heating can have reverse effect on the functionality. In addition to improved functional properties, heat treatment on plant-proteins also improves the digestibility and nutritional properties through inactivation of anti-nutritional compounds in plant proteins (Nasrabadi et al., 2021).

Ohmic heating is an emerging alternative for thermal heating (Rodrigues et al., 2021). The heat is generated by an electric current passing the product. A comparison of thermal and ohmic heating effects on soybean proteins found that the first decreases the heating time and is more successful in improving emulsifying ability (Nasrabadi et al., 2021).

2.10.2 Homogenisation

As mentioned earlier, small droplets result in increased stability of an emulsion. Different machines are used to decrease the droplet size, for example stirrers, colloid mills, homogenizers. Very high-pressure treatment (over 1000 bars) causes denaturation of proteins (Walstra, 2002c). Interestingly, the packing density of proteins after being exposed to very high pressure, has been observed to be lower than after low pressure treatment. The covalent bonds in the primary structure are pressure insensitive (up to 10–15 kbar), which suggests that the changes in volume are due to hydration changes and non-covalent interactions between protein molecules (Mozhaev & Masson, 1996). There is a lack of thorough understanding of the underlying mechanisms on how the pressure modifies the protein structure, but in favourable conditions, moderate pressure could stabilize the protein, while very high pressure causes unfolding (Walstra, 2002c).

2.10.3 pH shifting

pH shifting is an example of chemical treatment of proteins. It is often used as a pre-treatment for other modification methods. The denaturation and unfolding are evoked at both, extremely high and low, pH values. Most food proteins show highest solubility and improved functionality at more alkaline pH (Nasrabadi et al., 2021). However, it has been found that heating at alkaline conditions, generates lysinoalanine (LAL). LAL is an amino acid derivate, that has been found to cause cytomegaly in the rat and mouse kidney. Due date, its toxicity to humans is not proved and thus there is no guidelines for LAL content in food products (Alavi et al., 2021).

The solubility strongly increases, when the pH is away from the isoelectric point of the protein. At the extremely high pH values, the proteins have a high charge, which makes them well-soluble, despite of the hydrophobic groups at the surface (Walstra, 2002c).

2.11 Experimental Planning

Emulsions that incorporate PrOatein powder are complex food systems with a great potential to provide oat β-glucans, lipids and proteins to the diet. However, since the oat protein has found to have poor solubility, its use in liquids has been limited. Moreover, the presence of other compounds could affect the powder's ability to interact in an emulsion. This makes it interesting and important to study the emulsification properties of oat protein, when other components are also present. Since proteins constitutes the largest part of the powder, it has been given a more attention in this project.

The food systems' properties can be enhanced when pre-treated, providing a solution for the limited application of PrOatein powder. In this thesis, the effect of industrially more feasible pre-treatment methods such as pH shifting, heating and high-pressure homogenisation (HPH) are studied.

Drawn from the theoretical framework, it is thus intended to investigate, which pre-treatment parameters (temperature, pH, pressure at homogenisation) result in the most optimal emulsifying properties of PrOatein, regarding the emulsion stability and capacity, that are dependent on the surfactant's solubility and particle size.

3 Methods

3.1 Preparation of Oil-in-Water Emulsion

The emulsions were prepared in glass jars by first mixing 5 % w/v of the PrOatein dry powder (Lantmännen Oats AB, batch number KM21L24900) with 3 % v/v (counted on the total mixture) rapeseed oil Zeta (Di Luca & Di Luca AB, Sweden), purchased from a local grocery store and then adding purified water. The samples were then shaken by hand to mix the ingredients. The pH was adjusted to 7.5 or 4.5 with either 1 M sodium carbonate or 1 M acetic acid solutions respectively. The mixture was pre-homogenised for 30 s at 20 000 rpm by a disperser (UltraTurrax). The emulsion was then heated in a water bath until the core temperature of the sample reached 35, 65 or 85 ºC and kept at this temperature for 5 min. The samples were immediately removed from the water and cooled in a cool water bath until room temperature (25 ºC) was reached. This was followed by single-stage homogenisation by using high pressure homogeniser PandaPlus 2000 (GEA Niro Soavi S.p.a, Italy). The samples were circulated once at either 200, 600 or 800 bars.

3.2 Determination of the Particle Size

3.2.1 Aim

The aim of the particle size study was to understand the effect of heat and high-pressure homogenisation treatment on the average particle size and volume distribution in emulsions with different pH.

3.2.2 Method

The information about particle size was collected by analysing the samples in Mastersizer 2000 (Malvern Panalytical Ltd., UK). The instrument applies dynamic laser light scattering technique to estimate the particle size and volume distribution. Mastersizer 2000 relies on Mie theory that estimates the particle size from the angular scattering of electromagnetic light from the dispersed particles by knowing the difference between refractive indices of the dispersant and the sample (Malvern Instruments, 2007). It is important to mention, that such instruments assume to have spherical and homogenous particles. (McClements, 2006a) Two important parameters that influence the results are obscuration rate (i.e. sample concentration) and the stirring speed of dispersing unit (Schalkwijk & Sotomayor, 2020). The results can also be sensitive to the occurrence of multiple scattering, which creates discrepancies from model fitting. The particle size can be

presented as surface- or volume-weighted mean diameter. The difference between these values increases with an increase in the index of polydispersity (McClements, 2006a).

5 w/v % PrOatein emulsions with pH 7.5 and 4.5 containing 3 v/v % of oil were treated with high temperature (35, 65 and 85 ºC) and high pressure (200, 600 and 800 bars). Particle size was analysed within 20 min after the high-pressure homogenisation. Analysis were conducted by dropping 2–3 drops from the sample in the water unit. Duplicates were withdrawn from the sample and analysed in triplicates. During the analysis, the obscuration rate of the laser beam, that reflects the concentration of the sample, was aimed to be between 5-20 %. The speed of the stirrer was aimed to always be around 2000 rpm to minimize the errors from the set up. The dispersant was water with a refractive index of 1.33 and the refractive index of the sample was set to 1.5. The measurement was considered reliable, when the results fitted the suggested optical model well (i.e. weighted residuals were less than 1 %). Result analysis report (an example can be found in Appendix A) was generated from where the volume-weighted mean diameter (D[4,3]) (Equation 3.1) was reported:

$$D[4,3] = \frac{\Sigma d^4}{\Sigma d^3} \qquad \textit{Equation 3.1}$$

where d is diameter of the particle.

3.3 Determination of the Oil Droplet Size and Emulsion Microstructure

3.3.1 Aim

Microscopy photos were analysis to observe the effect of heat and pressure on the structure of the emulsion droplet. In addition, the microscopy photos were analysed to detect the changes in droplet size in different layers formed during emulsification capacity study.

3.3.2 Method

The photos were taken of all samples right after the particle size analysis and 24 h after the beginning of the emulsification capacity study. The microstructure of the samples was studied using an optical light microscope (Olympus BX50, Japan). The sample was mounted on a microscope slide and covered with a thin glass. At least 3 photos with a magnification of 10x or 20x were taken of the samples and two were chosen for droplet size analysis. The representative droplet was considered as the 3rd largest particle (all photos can be found in Appendix B). Average droplet size has been reported with standard deviation.

3.4 Protein Solubility

3.4.1 Aim

The oat protein solubility was measured to investigate the effect of the combination of heat and high-pressure treatment, as well as the presence of other ingredients on the solubility of oat proteins present in 53 % protein powder.

3.4.2 Method

The solubility was determined by measuring the remaining protein in the supernatant collected after centrifugation. The measurement was performed by determining the protein content using Kjeldahl method. For the protein solubility analyses 18 % w/v PrOatein dispersions were prepared in pH conditions 4.5 and 7.5 in a similar manner as described in section 3.1 excluding the addition of oil. Dispersions were treated with heat and high pressure at 35 ºC or 85 ºC and 200 or 600 bars respectively. The samples were stored at 4 ºC overnight. The dispersions were taken to room temperature and then centrifuged for 15 min at 20 000 x *g* (11K rpm). 3 g of supernatant was collected and transferred to glass tubes. 2 tablets (each containing 3.5 g potassium sulphate, 0.105 g copper sulphate and 0.105 g titanium dioxide) and 15 ml of sulfuric acid (95 %) were added into the tubes. The samples were heated in the Digestor 2520 (FOSS, Denmark) at 420 ºC for 1 h. The samples were distilled in Kjeltec 8400 (FOSS, Denmark). The samples were then titrated with HCl until the blue colour was changed to purple. The volume and concentration of HCl was used to calculate the % of protein in the sample.

3.5 Study of Emulsion Stability

3.5.1 Aim

The only surface-active component having emulsifying properties was assumed to be oat protein, which has shown poor emulsification properties in previous studies. However, the powder includes other components, that could complement the poor emulsification properties of oat protein. The aim of the stability study was to understand the main destabilization mechanisms in PrOatein emulsions over 48 h and the effect of HPH and heat treatment on delaying those mechanisms.

3.5.2 Method

Alkaline (pH 7.5) and acid (pH 4.5) PrOatein emulsions containing 3 v/v % of oil were treated with heat treatment (35 ºC or 85 ºC) and high pressure (200 or 600 bars). The stability mechanisms for each sample were then studied in duplicates for 1 hour in Turbiscan, following measurements of the sediment height with a ruler after 24 h and 48 h.

The underlying working principle of Turbiscan is similar to the one of Mastersizer 2000 (see section 3.2.2). The machine utilises Mie theory of light scattering to produce unique backscattering (BS) profile for a sample (an example available in Appendix C), where the destabilisation mechanisms could be precisely and easily read. The instrument scans the 20 ml cylindrical glass cell and reports the change (%) in BS. The first scan is a reference scan, against what all the following scans are weighed. The produced BS profile describes the changes in particle diameter and volume fraction over time and space. Creaming can be identified from the BS report, when there has been increase in BS in the top layer of the sample as the particles will migrate from the bottom. On the contrary, the sedimentation is characterised by increase in BS in the bottom layer as the particles migrate from the top. In addition, the *Peak Thickness* function in the software displays the increase of the sediment layer over time (Formulaction SA, n.d).

Measurements were considered reliable, if the meniscus did not have any imperfections and if the meniscus did not move over time. Imperfections in meniscus can occur due to breakage of air bubbles or movement of the glass cell and will affect the interpretation of results (Formulaction SA, n.d.).

3.6 Creaming Phenomenon in PrOatein Emulsions

3.6.1 Aim

The stability analysis over 48 h detected no creaming. Thus, the volume of oil phase was incrementally increased to estimate the maximum volume of oil that could be incorporated in PrOatein emulsions.

3.6.2 Method

Samples were prepared as described in section 3.1. The oil content was increased to 5 %, 10 % and 15 %. The observations about cream layer were made at 24 h after storing at room temperature. The cream layer was observed visually and in microscopy to detect the increase in droplet size and to describe the layers.

3.7 Statistical Analysis

3.7.1 Aim

Two-way ANOVA analysis was performed to identify statistical differences of particle size and solubility between samples with different pre-treatments.

Two-way ANOVA analysis was used to investigate the effect of two variables (heating and HPH) on the dependent variable (particle size or solubility) and to determine if there is an interaction effect of those variables. ANOVA test computes the *F*-value by comparing the variance in mean of each group to the variance of the overall dependent variable. A higher *F*-value indicates smaller variance within the groups compared to higher variance between groups. If $F > F_{crit}$, then significant difference with a confidence interval of 95 % ($p = 0.05$) can be concluded and the null hypotheses rejected. Two-way ANOVA allows to test three null hypotheses at once:

- $H_{0,1}$ "There is no difference between the first independent variable group means"
- $H_{0,2}$ "There is no difference between the first independent variable group means"
- $H_{0,3}$ "The effects of two independent variables does not depend on each other (no interaction)."

4 Results and Discussion

4.1 Oat Protein Solubility

Oat protein solubility was analysed from the supernatants collected from the samples, that had undergone varying heat and high-pressure pre-treatment as well as pH shifting. The changes in the solubility are presented in Tables 4.1-4.3. Two-way ANOVA analysis with replication found no significant effect ($p>0.05$) of heat nor high pressure on the solubility in the samples with pH 7.5 treatment ($F_{heat} = 2.26$ and $F_{pressure} = 6.63$ both $< F_{crit} = 7.71$). Similarly, no significant effect of heat in samples at pH 4.5 ($F_{heat} = 0.32 < F_{crit} = 7.71$), but effect of pressure on the solubility was significant ($F_{pressure} = 8.64 > F_{crit} = 7.71$). In samples with native pH, there was also a significant effect of pressure ($F_{pressure} = 8.17 > F_{crit} = 7.71$), but no significant effect of heat ($F_{heat} = 0.71 < F_{crit} = 7.71$). The pH shift towards more alkaline environment increased the solubility at least two times at all pre-treatment conditions compared to samples with pH 4.5. At higher pH the net charge of protein is increased, which improves the solubility in water, as the molecule becomes more polar.

4.1.1 Effect of High-Pressure Homogenisation

Although, the effect of homogenization on solubility cannot be statistically proved at all pH settings, there seems to be an increase in solubility when applying high pressure homogenization. To prove the effect significantly, more replicates with different temperature and homogenization treatments should be analysed. Positive effect of high-pressure homogenization was found by earlier master thesis by Vikenborg and Stensson (2020), who found a statistically significant effect of 100-200 bars pressure on the solubility of oat protein with pH 6.42 and 3.15 while no significant effect was found between pH 4.8-5.24.

The oat protein solubility showed highest value after it had been exposed to heat treatment of 85 ºC and pressure of 600 bars. High-pressure homogenisation causes denaturation of proteins as suggested by Walstra (2002c). Thus, during the treatment the protein molecules are likely to be broken down and their hydrophobic core will be exposed to the surface. The hydrophobic parts of different protein molecules could then aggregate via non-covalent bonds into bigger units to decrease the contact of apolar groups and water. This was suggested by Yuan et al. (2011) who studied soy protein isolate. They found that high homogenization pressure (300 bars) treatment could promote more dense re-packing of hydrophobic residues towards the core of the protein molecule and thus increase the solubility. They found a correlation between the surface hydrophobicity and solubility. The effect of high-pressure homogenization on the solubility of other plant proteins has been different. Bader et al. (2011) found the positive effect of very high-pressure homogenization (1500 bars) on lupin bean isolate. Study on lentil proteins showed increase in solubility only until the pressure was increased to 1500 bars, but at higher pressure conditions, the solubility was decreased (Saricaoglu, 2019).

Table 4.1 Solubility (%) of samples with pH 4.5.

Pressure (bars) / Temp (°C)	200	600
35	0.23 ± 0.02	0.20 ± 0.02
85	0.22 ± 0.01	0.19 ± 0.00

Table 4.2 Solubility (%) of samples with pH 7.5.

Pressure (bars) / Temp (°C)	200	600
35	0.49 ± 0.04	0.62 ± 0.12
85	0.44 ± 0.04	1.19 ± 0.47

Table 4.3 Solubility (%) of samples with native pH.

Pressure (bars) / Temp (°C)	200	600
35	0.29 ± 0.00	0.36 ± 0.03
85	0.29 ± 0.02	0.33 ± 0.03

4.1.2 Effect of Heat

The effect of heat on the solubility of oat protein among samples was rather unnoticeable and not significantly proven at any pre-treatment conditions. The solubility showed slight decrease as the heating temperature increased. The effect of heat treatment on oat protein has been previously studied by Runyon et al. (2015). In their study, the oat goats underwent steaming at 102 ºC following drying at 110–120 ºC. The heat treatment decreased the solubility of oat proteins extracted from the groats by 50% compared to extraction from non-heated groats. The soluble protein content was found to be 4.1 wt % in heated samples. It is worth mentioning, that in their study the proteins were extracted at pH 9.5, which probably increased the solubility even more, compared to the results found in the present thesis project.

4.2 Effect of Heat Treatment and High-Pressure Homogenization on Particle Size

The PrOatein heated and high-pressure homogenized emulsions were analysed by using light scattering to estimate the average particle size in the sample. A two-way ANOVA with replicates found that both treatments have significant effect on the particle size (F_{heat} = 20.9 and $F_{pressure}$ = 19.5 both > F_{crit} = 4.3) in slightly alkaline (pH 7.5) samples. However, no interaction effect was found ($F_{interaction}$ = 2.1 < F_{crit} = 3.6). In acidic environment (pH 4.5) significant effect of both treatments on the particle size (F_{heat} = 42.1 and $F_{pressure}$ = 687 both > F_{crit} = 4.3) was found. In acidic environment, there was also an interaction effect of the two treatments ($F_{interaction}$ = 10.0 > F_{crit} = 3.6).

The heat and high-pressure homogenisation decreased the average particle or aggregate size at both pH levels studied (Figure 4.1). The smallest particle size (6.6 µm) was achieved by treating the emulsion with 85 ºC and 800 bars at pH 7.5. The minimizing effect of high pressure on particle size can be explained by the turbulence created in the homogenizer's chamber, which has been suggested to be the main reason for fragmentation of drops by Håkansson et al. (2011). Turbulence causes the particles to break down and destroys the aggregates of proteins.

In addition to physical disruption of aggregates during HPH, the effect of pH can be seen. Most samples had smaller particle size at pH 7.5 compared to pH 4.5. At higher pH, the oat

proteins might have obtained larger charge density, which could promote the repulsive interactions between polysaccharides in the sample (maltodextrins and β-glucans) and decrease the dominating particle size. Proteins are positively charged under acidic conditions, whereas the polysaccharides in the sample could have a negative charge (Yildiz et al., 2018). This could evoke attraction between protein and polysaccharides in acidic environment. The presence of larger aggregates at lower pH levels was confirmed by microscopy photos (Appendix B). In addition to protein-polysaccharide interactions, the intramolecular interactions of oat proteins in acid conditions could be favoured. Increase of particle size in soy protein dispersions in acid conditions was attributed by Yuan et al. (2012) to increased hydrophobicity of the protein molecule as their surface net charge is decreased.

Higher pressure and heat treatment formed emulsions with higher proportion of small particles. According to McClements (2016a), small and uniform particles increase the stability of the emulsion and are thus pre-requisites for (meta)stable emulsions. However, more research about oat dextrins and surface hydrophobicity of oat proteins is needed to motivate the possible protein-polysaccharide and intramolecular protein interactions in the sample explaining the decrease of the average particle size during HPH.

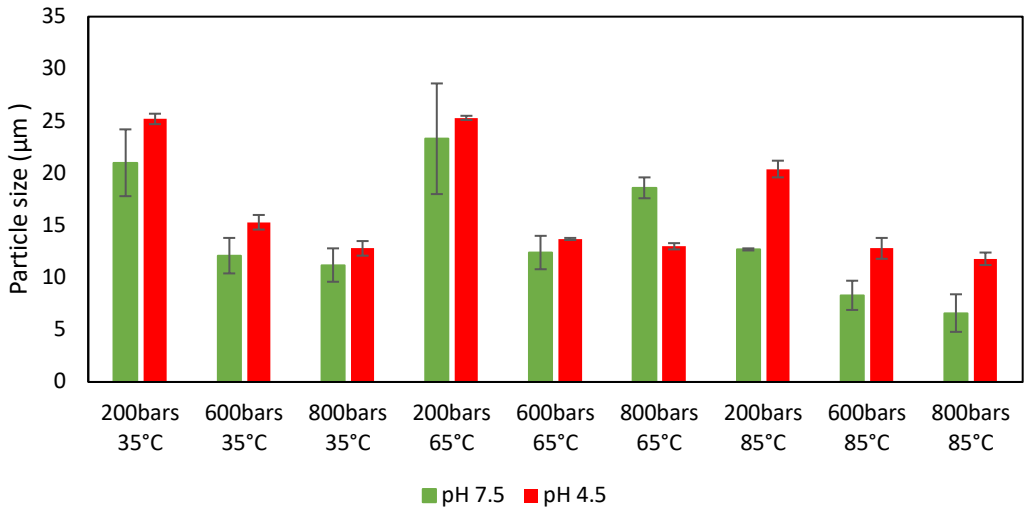

Figure 4.1 Average particle size (D [3,4]) of heated and high-pressure homogenized oat protein emulsions. Error bars depict standard deviation.

4.3 Oil Droplet Size and PrOatein Structure Under Microscopy

The light scattering gave an approximate of the average droplet size in the sample and allowed to analyse larger amount of sample. The samples were then analysed under the microscope to complement light scattering measures with better understanding of the size of the individual oil droplets and special arrangement of the droplets. The average size of oil droplets in samples is presented in Tables 4.4 and 4.5. A two-way ANOVA with replicates found only significant effect

of high-pressure homogenisation on droplet size in alkaline samples (pH 7.5) (F_{heat} = 0.07 and $F_{pressure}$ = 5.61, F_{crit} = 4.26). No effect of pre-treatments on the oil droplet size in acid environment was found (F_{heat} = 2.80 and $F_{pressure}$ = 0.25, both < F_{crit} = 4.26).

In alkaline samples, the size of oil droplets was decreased as the pressure was increased. At the same time, the intensification of heat treatment from 35 ° to 65 ºC increased the droplet size after being high pressured with 600 and 800 bars. Higher solubility of the oat protein increases its emulsification capacity and thus produces small oil droplets. The smallest droplet size was measured after heat treatment of 35 ºC and high-pressure treatment of 800 bars.

Although the oil droplet size is small at both pH levels, the microscopy photos showed higher aggregation of oil droplets in pH 4.5 than in 7.5. That suggests that oat protein is able to cover the surface of oil droplets at both pH levels, but the repulsive forces between protein molecules at pH 4.5 are not strong enough to prevent aggregation of the droplets (Figures 4.2 and 4.3).

Table 4.4 Emulsion droplet size (μm) in samples with pH 4.5.

Pressure (bars) / Temp (°C)	200	600	800
35	4.98 ± 0.21	3.20 ± 0.04	4.94 ± 0.54
65	4.18 ± 0.53	4.62 ± 0.32	3.34 ± 0.23
85	2.80 ± 0.75	3.41 ± 0.54	3.78 ± 1.06

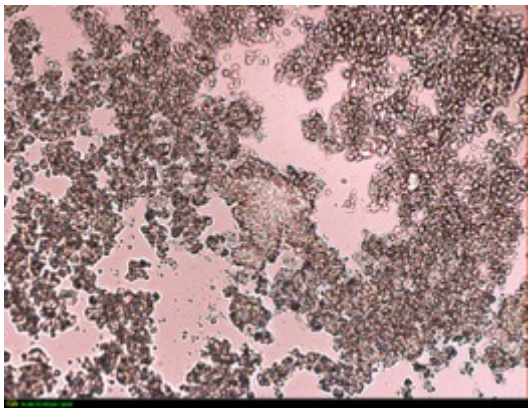

Figure 4.2 Microscopy photo of sample with pH 4.5, heat treatment of 35 ºC and HPH of 600 bars.

Table 4.5 Emulsion droplet size (μm) in samples with pH 7.5.

Pressure (bars) / Temp (°C)	200	600	800
35	4.11 ± 0.13	2.29 ± 0.32	1.53 ± 0.25
65	2.81 ± 0.54	2.75 ± 0.60	2.49 ± 0.67
85	2.77 ± 0.47	2.65 ± 0.21	2.22 ± 0.03

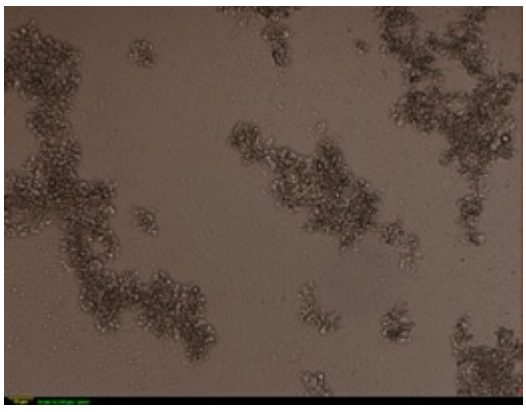

Figure 4.3 Microscopy photo of sample with pH 7.5, heat treatment of 35 ºC and HPH of 600 bars.

4.4 Effect of Heat Treatment and HPH on Emulsion Stability

4.4.1 Sedimentation Rate During the First Hour

The emulsions were scanned in Turbiscan to investigate the destabilization mechanisms. The results of backscattering profiles (an example in Appendix C) revealed that the main destabilization mechanism in emulsions containing 3 % v/v oil and 5 % w/v PrOatein powder is sedimentation. None of the samples developed a cream nor oil layer on top after the first hour.

The sedimentation rate was obtained from the slope of a linear regression line, where the height of the sediment layer was plotted against time as shown on Figure 4.4. The sedimentation rate in acid samples was at least 2 times higher in all pressure and temperature conditions (except at 35 ºC and 600 bars that can be treated as an outliner) than in alkaline samples. Stronger high-pressure treatment (600 bars) delays the beginning of the sedimentation by approximately 1000 s. in alkaline and 2000 s. in acid samples at both temperature treatments. As the particle size decreases with high pressure treatment, it takes more time to them to sediment (McClements, 2016b).

In addition to the effect of decreased average particle size confirmed by the particle size analysis, it could be assumed that the viscosity of the continuous phase increased with stronger heat and pressure treatment. Stokes' equation describes the inversible relation between sedimentation rate and the viscosity of the continuous phase. As the viscosity increases, the sedimentation rate decreases because of the immobility of the particles. In those samples, oat protein, dextrins and on a smaller extent β-glucans are likely to contribute to the viscosity increase. Brückner-Gühmann and colleagues (2021) studied the gelling of oat protein at pH 4.5 and 8 and heating temperatures at 90 ºC and 120 ºC. They found that pH 8 and 120 ºC induced a gel with strongest elastic properties. It has also been found, that the dextrins with low DE value increase the viscosity of the solution when heated (Sun et al., 2010). However, the effect of β-glucans on the decreased sedimentation rate when the homogenisation pressure is increased can assumed to only be minimal. The viscosity of β-glucan solution is dependent on its molecular weight, which decreases with increased pressure. Moreover, oat β-glucans have been found to provide more viscous solutions at 37 ºC rather than 85 ºC (Mäkelä et al., 2017).

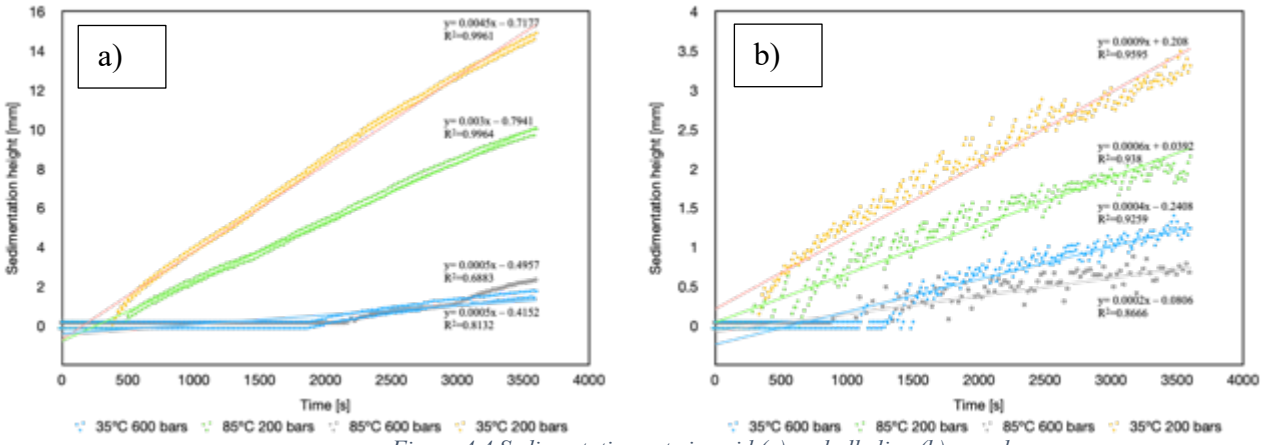

Figure 4.4 Sedimentation rate in acid (a) and alkaline (b) samples.

4.4.2 Development of sediment layer over 48 h

The samples were kept at room temperature to observe the development of the sediment layer over 48 h.

The sediment layer had highest proportion from the whole volume in the acid sample treated with 85 ºC and 600 bars (75 ± 16 %) (Figure 4.5), while the alkaline sample treated with 600 bars and 85 ºC developed lowest amount of sediments (2 %) (Figure 4.6).

The % of sediment layer from the volume of the whole sample is plotted on Figure 4.7. Photos of all samples are available in Appendix D. The increase in temperature from 35 to 85 ºC in alkaline samples resulted in little decrease in the sediment layer, while the homogenization with 600 bars significantly decreased the volume of sediments compared to 200 bars. In contrary, in acid samples, the higher pressure and temperature seem to increase the volume of sediments.

Figure 4.5 Appearance of the sample with pH 4.5, exposed to 85 ºC heat treatment and 600 bars.

Figure 4.6 Appearance of the sample with pH 7.5, exposed to 85 ºC heat treatment and 600 bars.

As the high-pressure treatment of both, alkaline and acid samples, reduced the particle size, then the changes in the development of sediment layer after 48 h indicate different rate in particle aggregation into bigger flocs, that eventually sediment. Alkaline samples produce more

homogenous emulsion, where repulsive forces between particles can be assumed, while in acid samples attractive forces occur between hydrophobic residuals of protein molecule. This could result in faster aggregation of protein particles in acid samples compared to alkaline ones and explain the differences between the development of sediment layer in alkaline and acid samples.

The sediment layers were also visually different. While in alkaline samples the sediment layer appeared to be densly packed and increase over time, then in acid samples, the sediment layer was more porous and looser, showing decrease over time. This suggests that the sediment layer in acid samples only became denser over time. One explanation to that could be, that acid environment produced larger flocs with varying size, that sedimented as a porous layer, while the aggregates in alkaline samples were more uniform. However, more detailed insight into large particles in the emulsion could increase the knowledge of underlying forces.

None of the samples developed oil layer on top, suggesting that oat protein could successfully emulsify the oil droplets at both pH levels. The low solubility of oat protein could be compensated by soluble maltodextrins in the sample. In a set of experiments investigating the effect of the presence of maltodextrins on the stability of faba bean proteins by Alavi et al. (2021), it was found that the presence of maltodextrins produces more stable emulsions. The authors suggested that while heating the protein in the presence of maltodextrins, they form conjugates with high solubility. Those conjugates adsorb onto the oil/water interface and emulsify the oil.

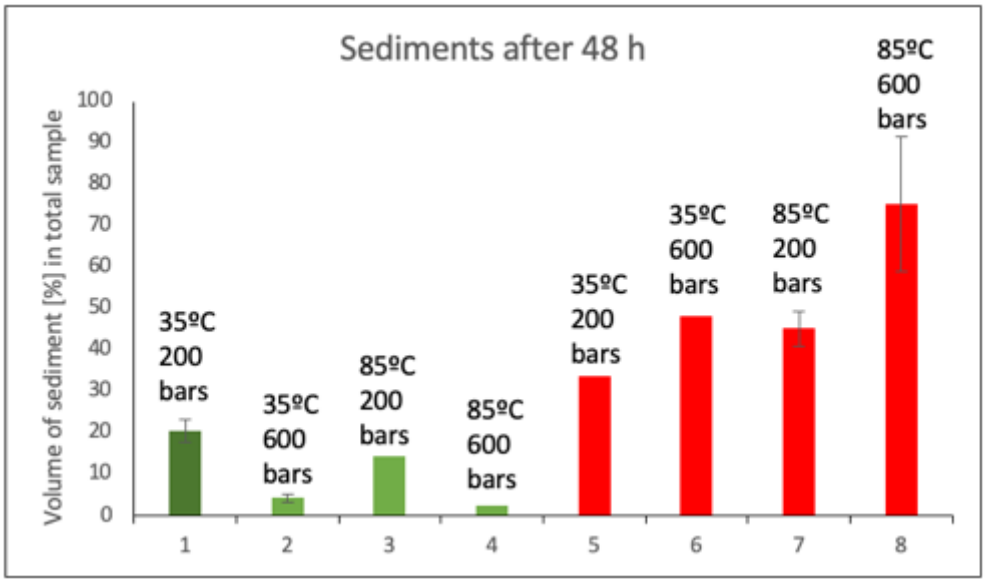

Figure 4.7 Volume of sedimentation after 48 h in relation to the total volume. Green bars represent samples with pH 7.5 and red bars pH 4.5. Error bars depict standard deviation of the mean.

4.5 Effect of Increased Oil Content on the Appearance of the System

Samples with native and pH 7.5 heated to 85 °C were investigated to evaluate the impact of high-pressure treatment on the emulsification capacity of the oat protein powder. The appearance of the system can be seen on Figure 4.8. Only alkaline samples were chosen for this experiment and compared to samples with no pH shift as in previous analysis about particle size and solubility, only alkaline samples exhibited potential for being used in homogenous emulsions.

In a simple emulsion system, consisting of oil, water and surfactant, most often upper creamed layer, middle emulsion and lower serum layer are developed. The droplet-depleted serum layer is usually identified below the emulsion layer (McClements, 2016b), but in those samples the phenomenon occurred in reversed matter. In addition to emulsion and serum layer, a development of sedimented emulsion layer was identified. The sedimented emulsion layer is a more dense layer than emulsion layer and consists of insoluble proteins that have aggregated with the oil. As the insoluble protein has higher density than water, then the layer has sedimented. In those samples, the emulsion started to sediment after the oil content was increased to 10 %.

At 5 % oil content, one of the samples – 200a – also developed a distinct thick dark sediment layer. This is assumed to consists of very big insoluble protein and carbohydrate particles, that have sedimented. As seen previously, the solubility of oat protein was increased with the increase of homogenisation, and thus in other samples the formation of thick sediment layer was prevented.

At 10 % oil content all but AL200b had separated into two layers, suggesting that lower pressure treatment and thus the presence of larger particles could be of advantage when producing products with increased oil content.

At any oil content, none of the samples developed an oil layer on top. When the oil content is increased to 10 or even 15 %, then the emulsion layer developed a more thick consistency, unappealing to consumers. An emulsion produced with 5 % w/v oat protein powder provided a rather stable system with liquid appearance when oil content was around 3-5 % v/v counted on the whole system. Those experiments thus suggest that the oat protein powder to oil ratio shall be around 1:1. However, more precise studies, including the characteristics of rheology and microstructure of each layer could help to give more accurate conclusions of the impact of pressure and heating on the formation of those layers.

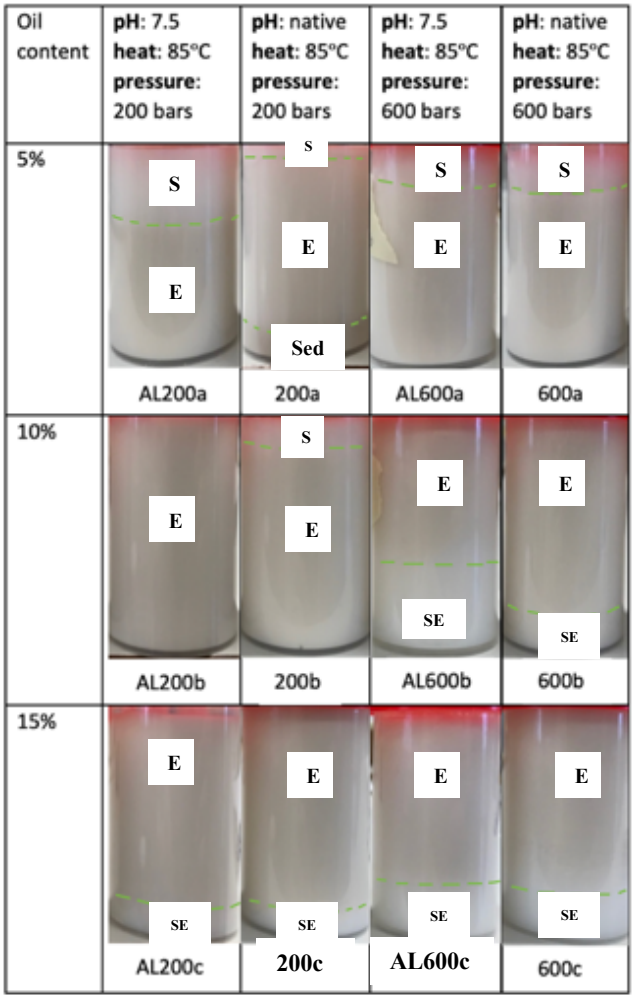

Figure 4.8 Observation of different layers under destabilization of the emulsions with varying oil content.

5 Conclusion

This project can be seen as a fundament for following development process of oat protein rich nutritious products. Oat bran protein powder containing oat proteins, dextrins, lipids and β-glucans as major ingredients was evaluated in terms of its application in liquid food products with a focus on oat protein's emulsification properties. It has been previously known that oat protein has poor solubility, which limits its usage in liquid foods. Thus, the aim of this project was to evaluate the potential of pre-treatment in the form of pH shifting, heating and high-pressure to produce more stable emulsion.

Oat protein solubility was analysed from the supernatants collected from the samples, that had been treated with heat and high-pressure. No significant effect ($p>0.05$) of heat nor high pressure on the solubility in the samples with alkaline pH treatment was found. Similarly, there was no significant effect of heat treatment on solubility in samples at pH 4.5. However, the effect

of pressure on the solubility was statistically proven. Although, the effect of homogenization on solubility was not statistically proved for all pH settings, there was an increase in solubility among alkaline samples. The highest of solubility (1 %) was measures in the supernatant collected from a sample heated to 85 ºC and treated with high pressure of 600 bars.

The droplet and particles size of the emulsion were investigated by laser scattering and microscopic methods. It was found that high pressure homogenisation and heating have significant effect on the particle size in alkaline (pH 7.5) samples. The effect was also found in acidic environment. The smallest particle size (6.6 μm) was achieved by treating the emulsion with 85 ºC and 800 bars at pH 7.5. Thus, homogenisation coupled with heat treatment can effectively reduce the particle size in an emulsion. Smaller particles are favoured in emulsions as they delay the gravitational separation mechanisms in emulsions.

The stability mechanisms of the emulsion were observed over 48 hours. The sedimentation rate in acid samples was at least 2 times higher in all pressure and temperature conditions than in alkaline samples. Stronger high-pressure treatment delays the beginning of the sedimentation both temperature treatments. Pressure and heat treatment had different effects on the development of the sediment layer in acid and alkaline samples. When applying higher pressure and stronger heat treatment to alkaline samples, then the final height of the sediment was decreased, while in acid samples lower temperature and pressure decreased the sediment height.

The findings suggest that emulsions produced with 5 % w/v oat protein powder, could be used to create an emulsion with maximum amount of oil around 3-5 % v/v counted on the whole system. Those experiments revealed that the oat protein to oil ratio shall be around 1:1. At higher oil content, the emulsioon will sediment within only one day.

In products with higher viscosity and oil content, lower pressure might me of advantages due to the ability of bigger particles to immobilize the oil droplets. In contrary, in products with low viscosity, higher pressure might be favourable due to significant decrease in particle size and delayed sedimentation rate.

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Appendix A An example of the result report from particle size analyzer. The parameters that were paid attention to are circled in red.

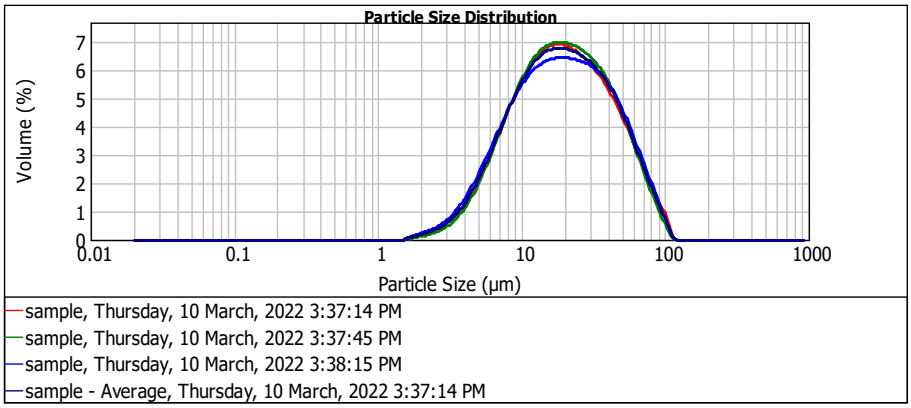
MALVERN INSTRUMENTS

MASTERSIZER 2000

Result Analysis Report

Sample Name: sample - Average	**SOP Name:**	**Measured:** Thursday, 10 March, 2022 3:37:14 PM	
Sample Source & type:	**Measured by:** Master	**Analysed:** Thursday, 10 March, 2022 3:37:15 PM	
Sample bulk lot ref:	**Result Source:** Averaged		
Particle Name: Oat drink	**Accessory Name:** Hydro 2000SM (A)	**Analysis model:** General purpose (spherical)	**Sensitivity:** Normal
Particle RI: 1.500	**Absorption:** 0.001	**Size range:** 0.020 to 1000.000 um	**Obscuration:** 4.08 %
Dispersant Name: Water	**Dispersant RI:** 1.330	**Weighted Residual:** 0.610 %	**Result Emulation:** Off
Concentration: 0.0073 %Vol	**Span :** 2.471	**Uniformity:** 0.76	**Result units:** Volume
Specific Surface Area: 0.44 m²/g	**Surface Weighted Mean D[3,2]:** 13.635 um	**Vol. Weighted Mean D[4,3]:** 25.424 um	

d(0.1): 6.548 um **d(0.5):** 19.188 um **d(0.9):** 53.969 um

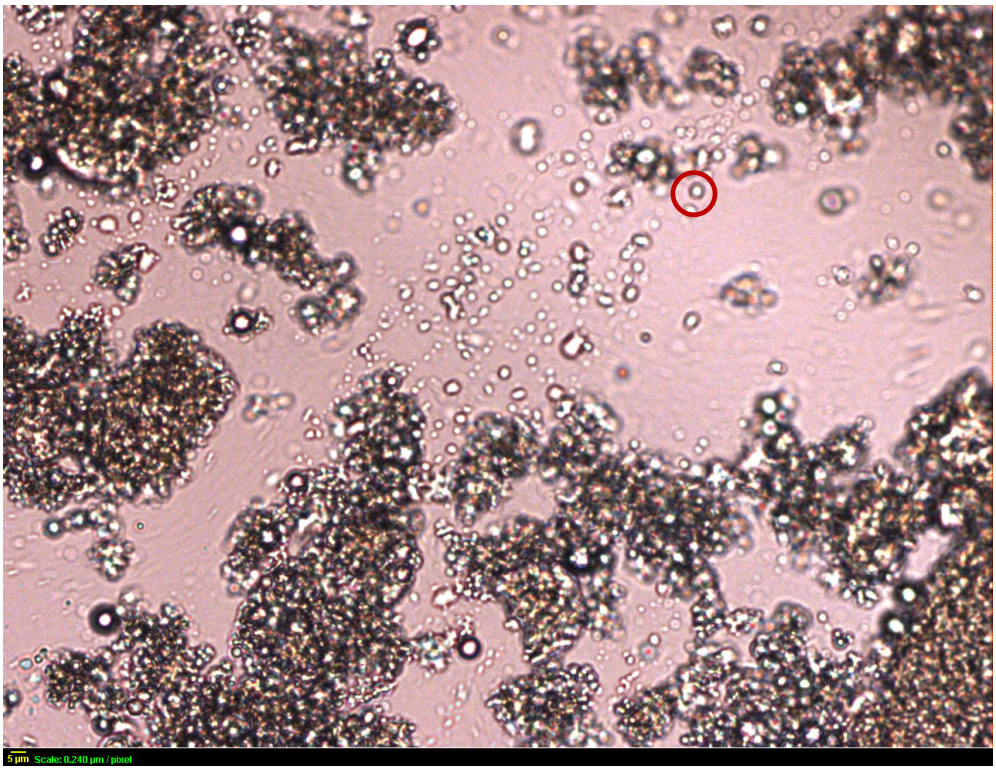


Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.020	0.00	0.096	0.00	0.458	0.00	2.193	0.17	10.496	3.58	50.238	2.57
0.022	0.00	0.105	0.00	0.502	0.00	2.405	0.21	11.509	3.75	55.085	2.27
0.024	0.00	0.115	0.00	0.551	0.00	2.637	0.27	12.619	3.89	60.399	1.95
0.026	0.00	0.126	0.00	0.604	0.00	2.891	0.35	13.837	3.99	66.226	1.62
0.029	0.00	0.138	0.00	0.662	0.00	3.170	0.45	15.172	4.05	72.616	1.31
0.032	0.00	0.152	0.00	0.726	0.00	3.476	0.59	16.635	4.08	79.621	1.01
0.035	0.00	0.166	0.00	0.796	0.00	3.811	0.75	18.240	4.08	87.303	0.72
0.038	0.00	0.182	0.00	0.873	0.00	4.179	0.94	20.000	4.06	95.726	0.45
0.042	0.00	0.200	0.00	0.957	0.00	4.582	1.16	21.930	4.01	104.961	0.13
0.046	0.00	0.219	0.00	1.050	0.00	5.024	1.41	24.045	3.95	115.088	0.00
0.050	0.00	0.240	0.00	1.151	0.00	5.508	1.68	26.365	3.88	126.191	0.00
0.055	0.00	0.264	0.00	1.262	0.00	6.040	1.97	28.909	3.78	138.366	0.00
0.060	0.00	0.289	0.00	1.384	0.00	6.623	2.26	31.698	3.66	151.716	0.00
0.066	0.00	0.317	0.00	1.517	0.01	7.262	2.56	34.756	3.51	166.353	0.00
0.073	0.00	0.348	0.00	1.664	0.07	7.962	2.85	38.109	3.32	182.402	0.00
0.080	0.00	0.381	0.00	1.824	0.09	8.730	3.12	41.786	3.11	200.000	
0.087	0.00	0.418	0.00	2.000	0.13	9.573	3.36	45.817	2.86		
0.096		0.458		2.193		10.496		50.238			

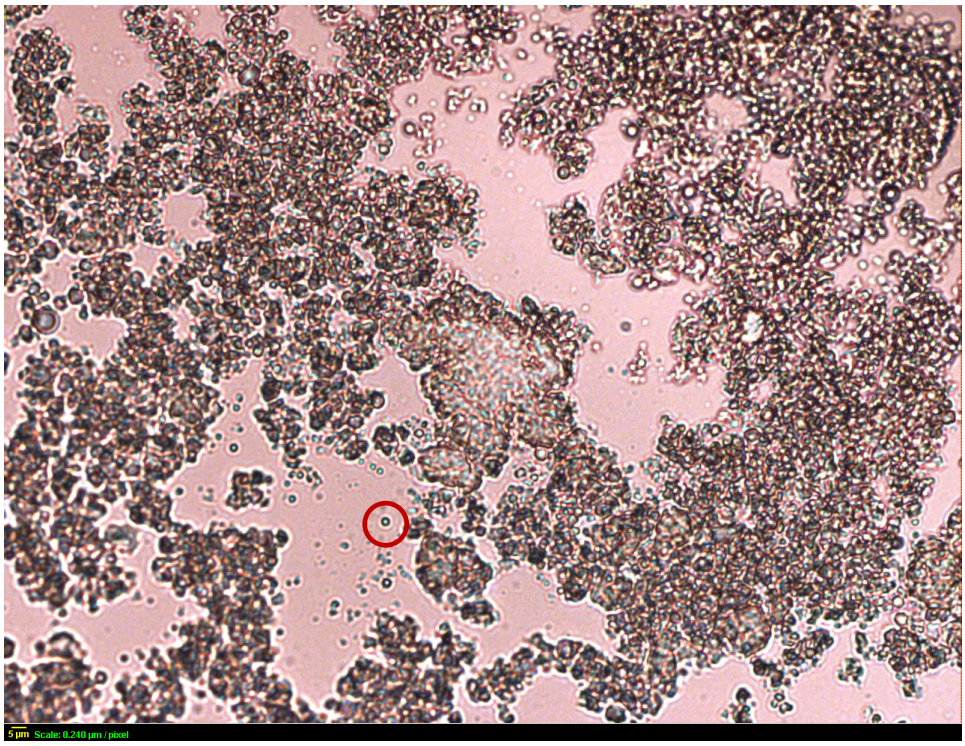
Operator notes:

Appendix B Microscopy photos for evaluation of droplet size and structural changes. Representative droplet circled red.

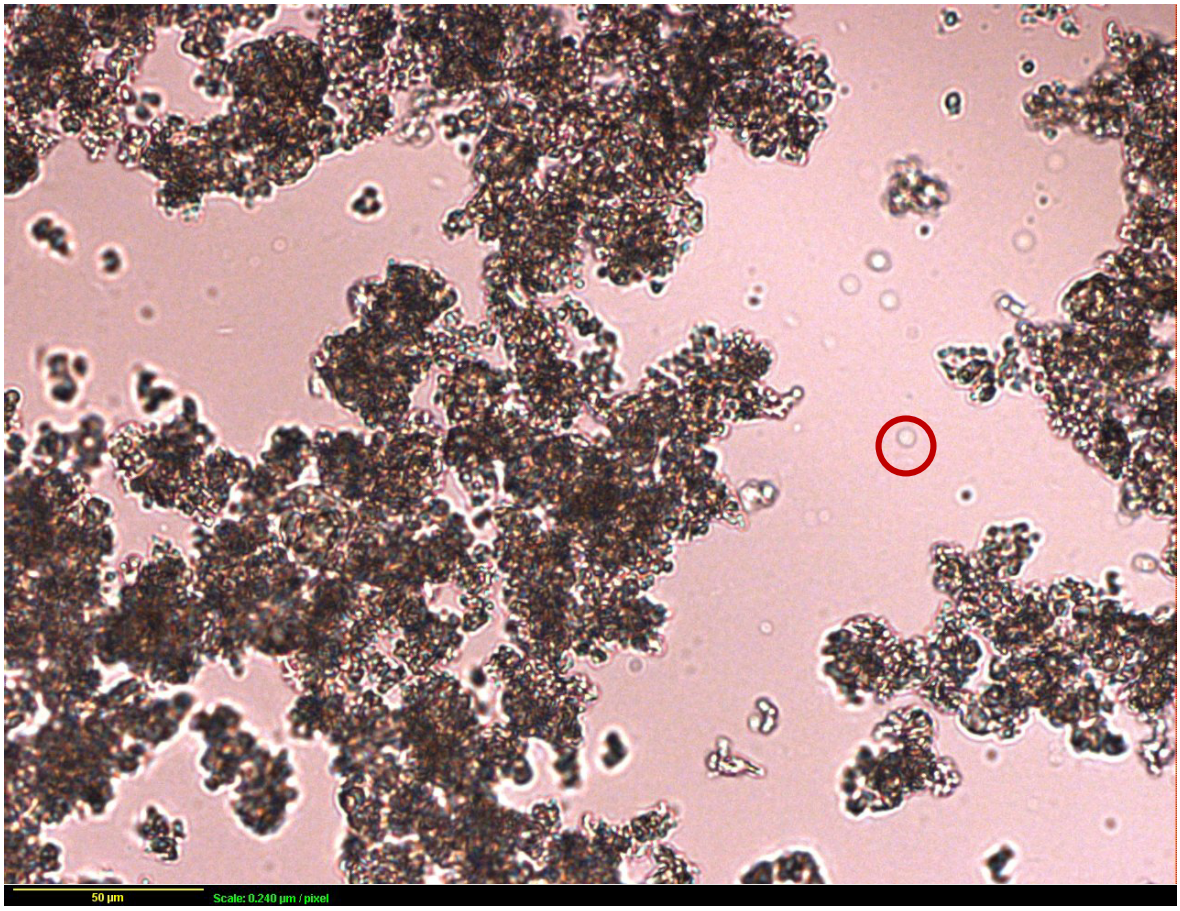
pH 4.5 35 d 200 bars



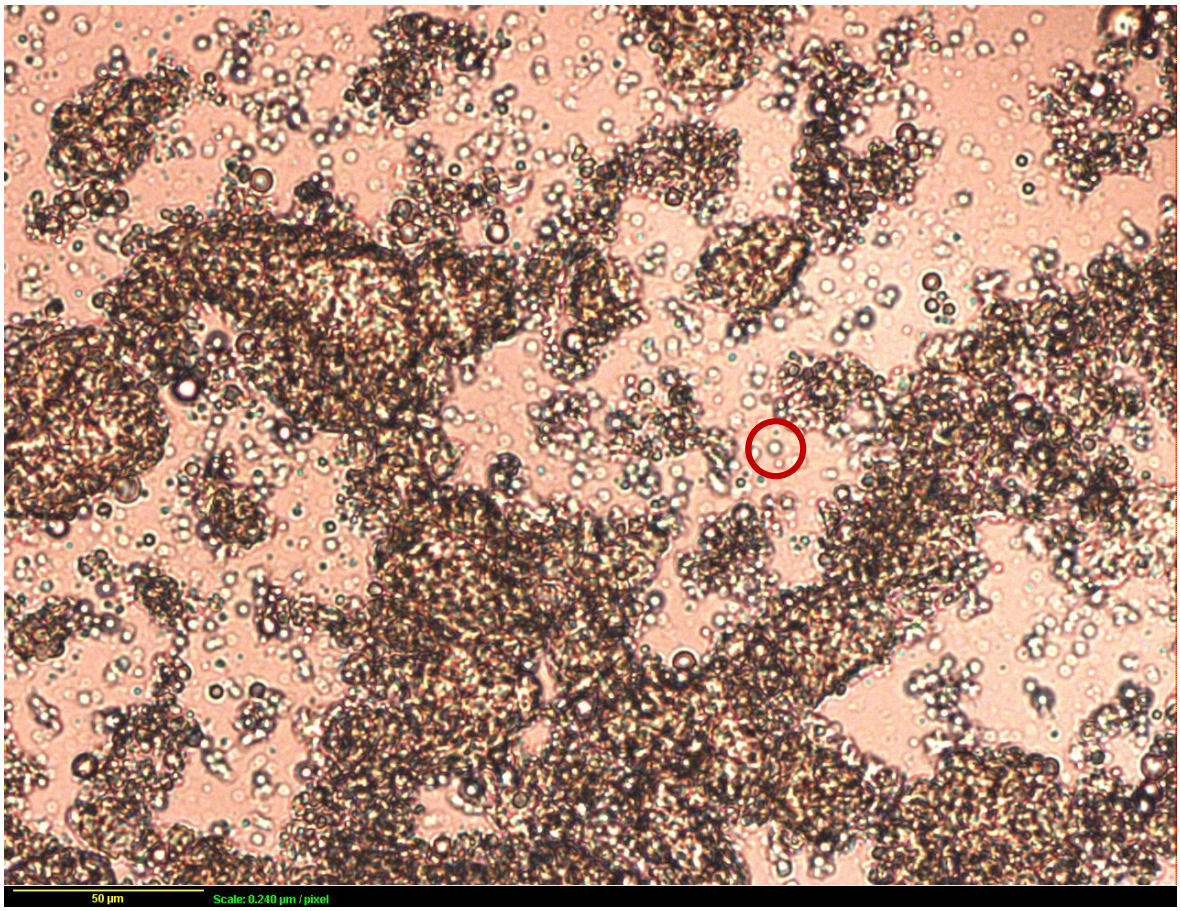
pH 4.5 35 d 600 bars



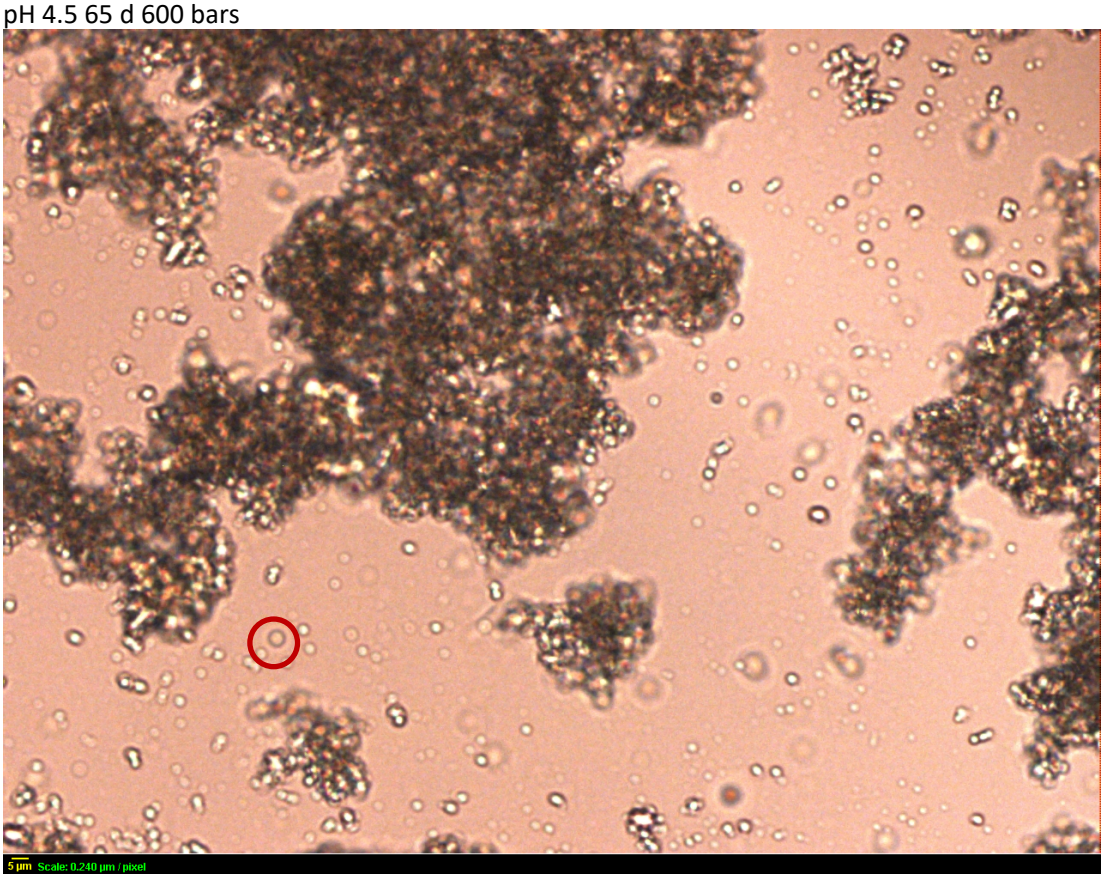
pH 4.5 35 d 800 bars



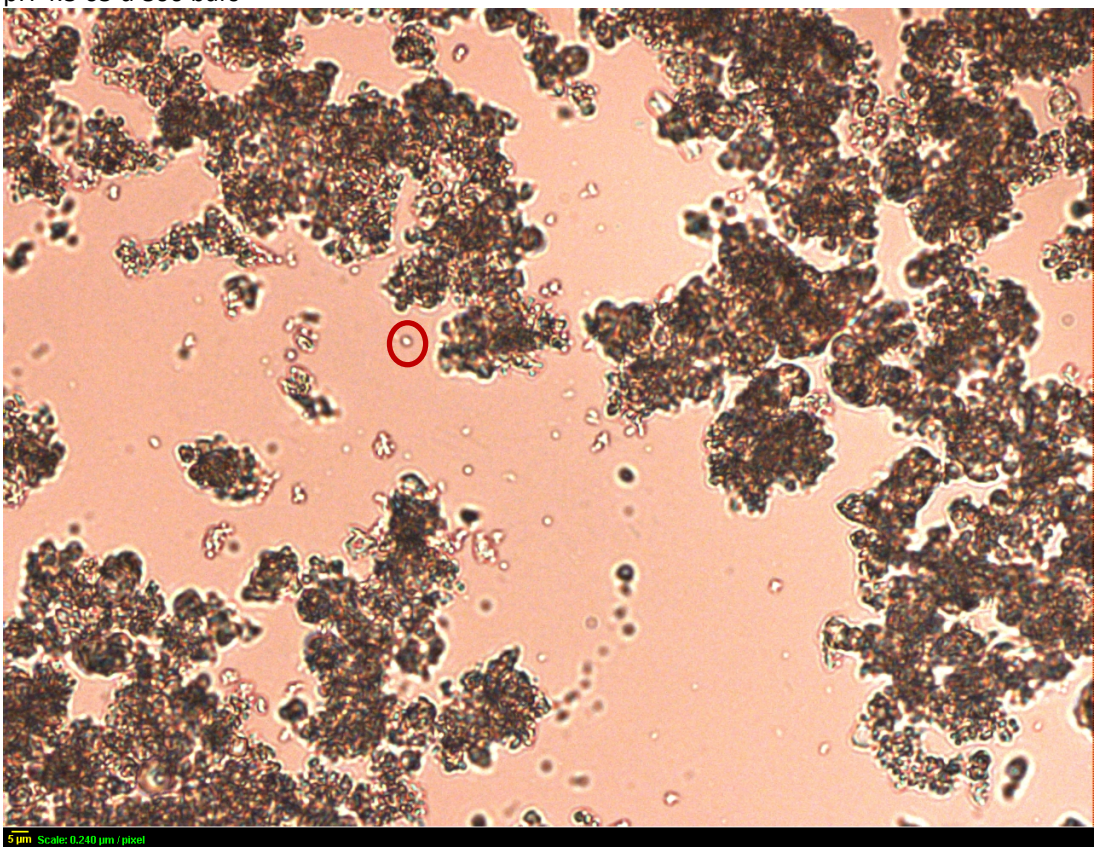
pH 4.5 65 d 200 bars



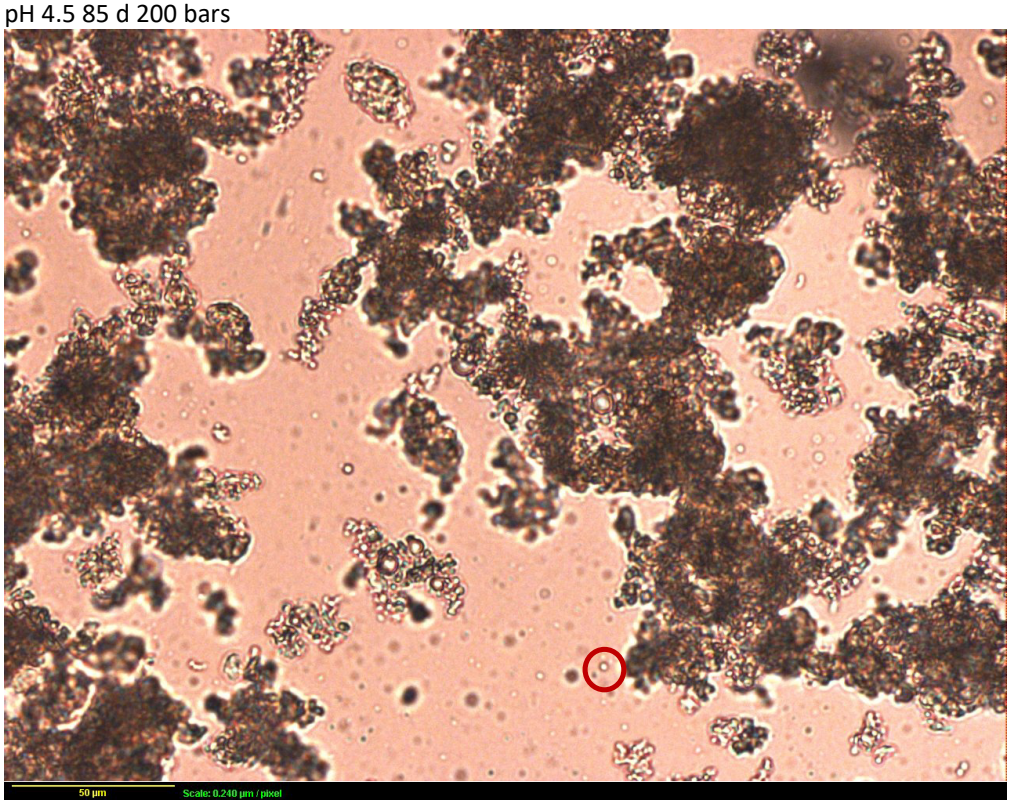
pH 4.5 65 d 600 bars



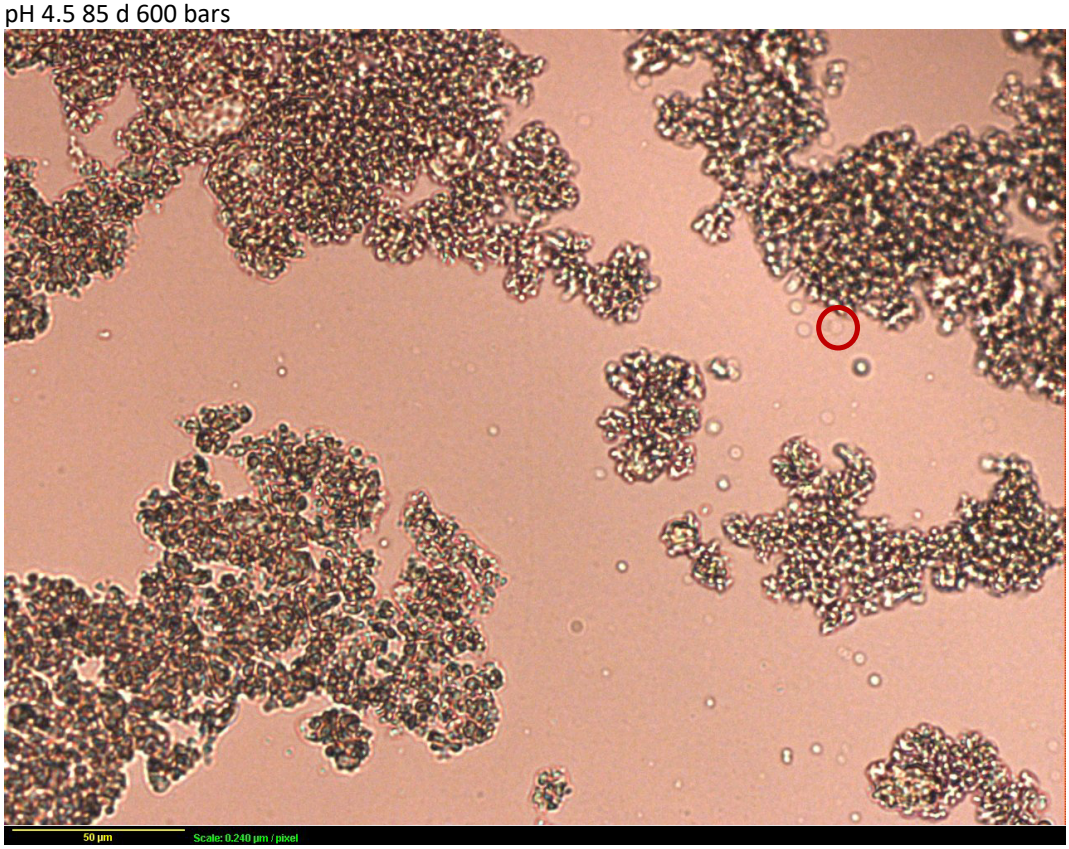
pH 4.5 65 d 800 bars



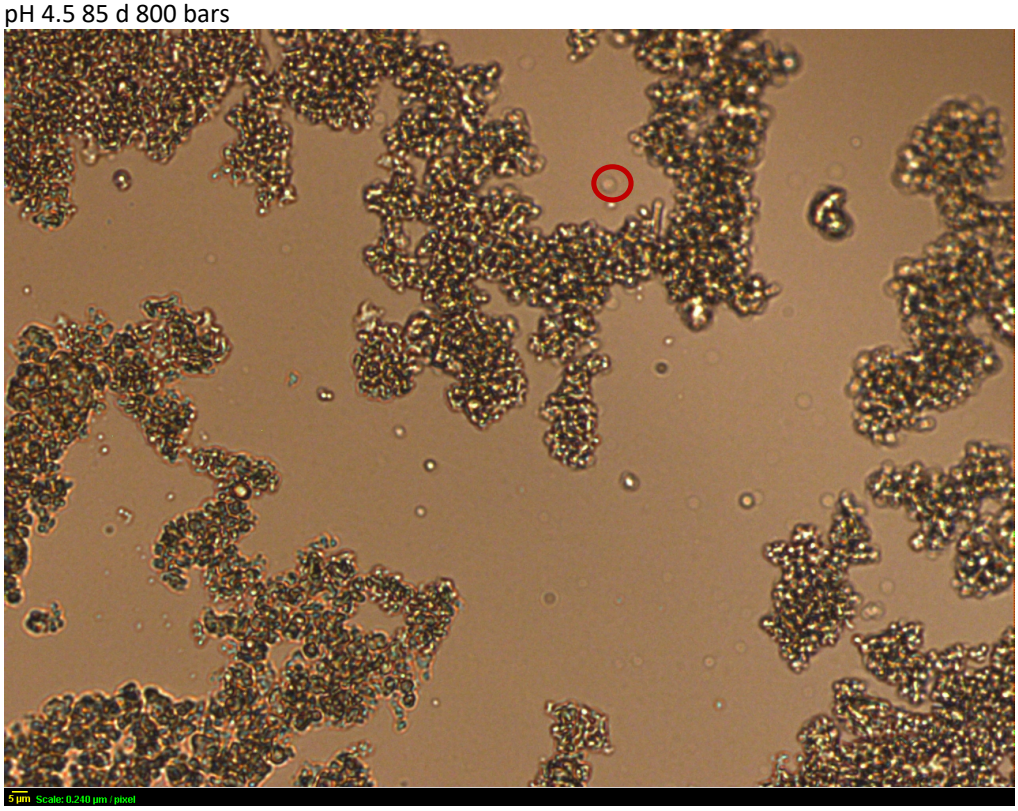
pH 4.5 85 d 200 bars



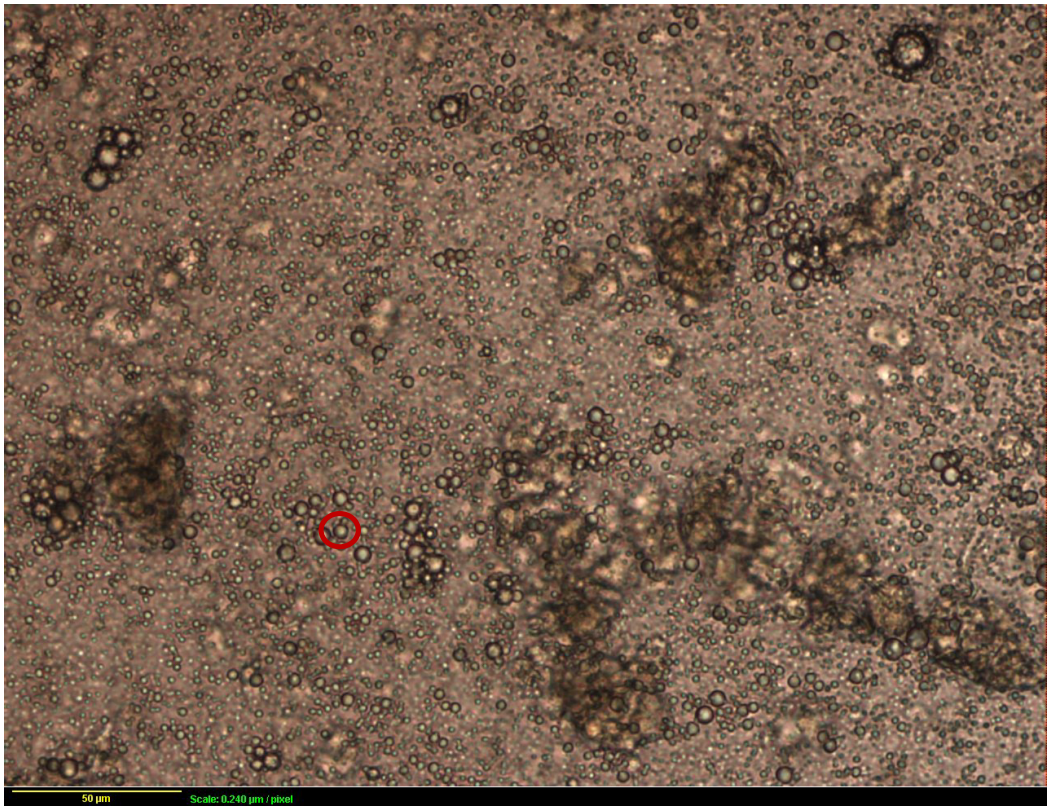
pH 4.5 85 d 600 bars



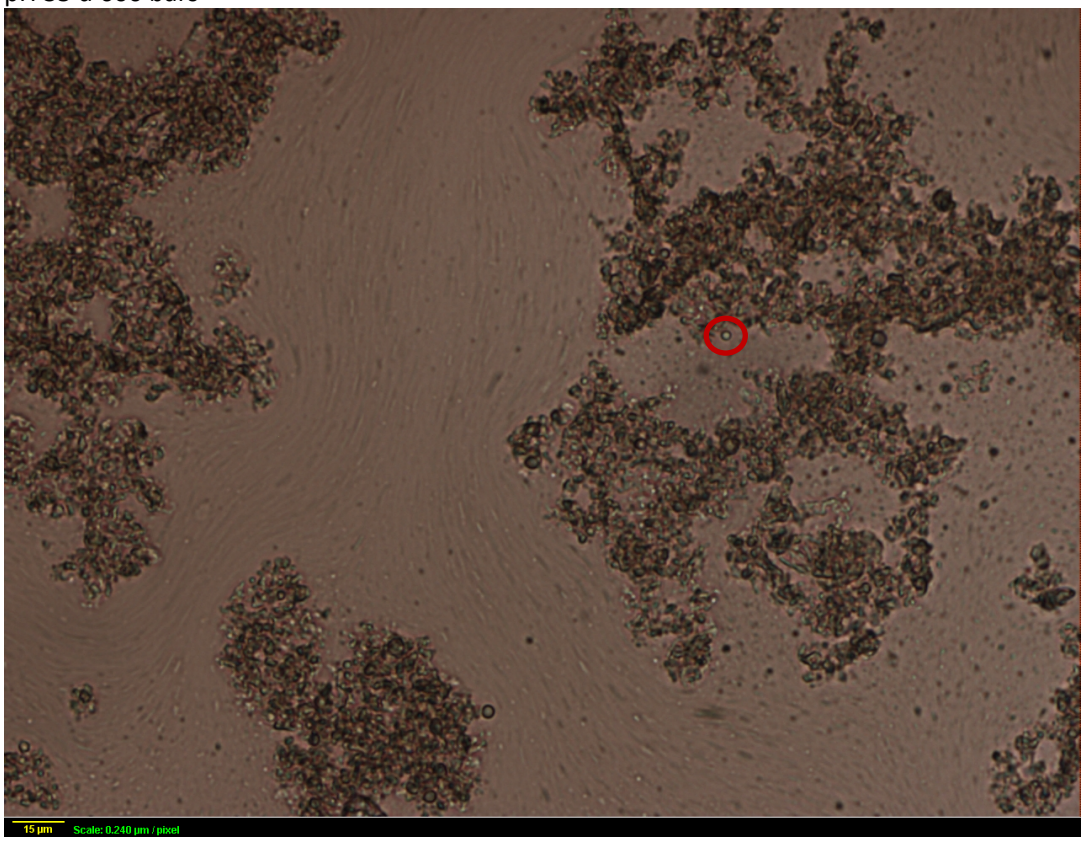
pH 4.5 85 d 800 bars



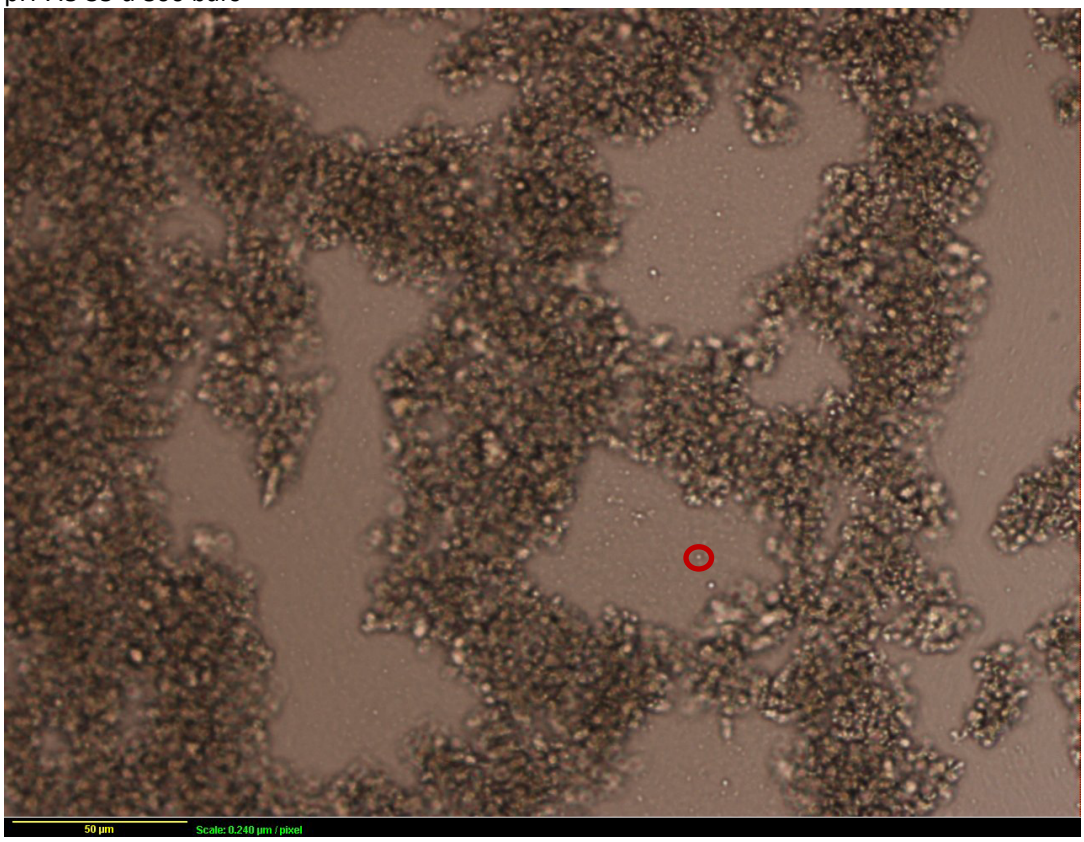
pH 7.5 35 d 200 bars



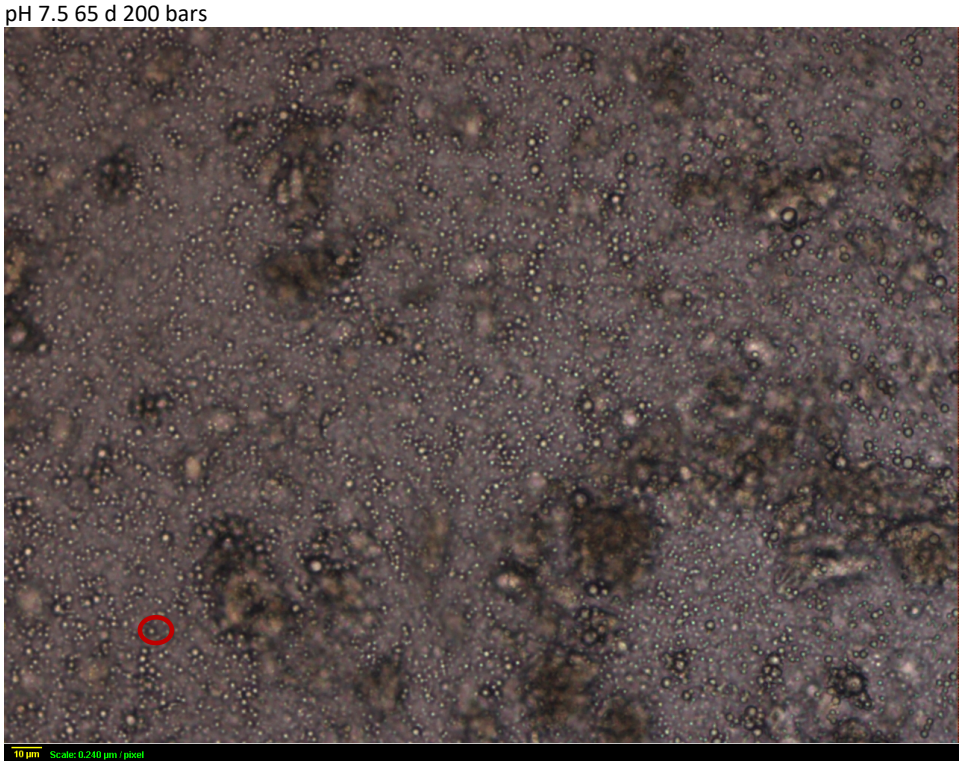
pH 35 d 600 bars



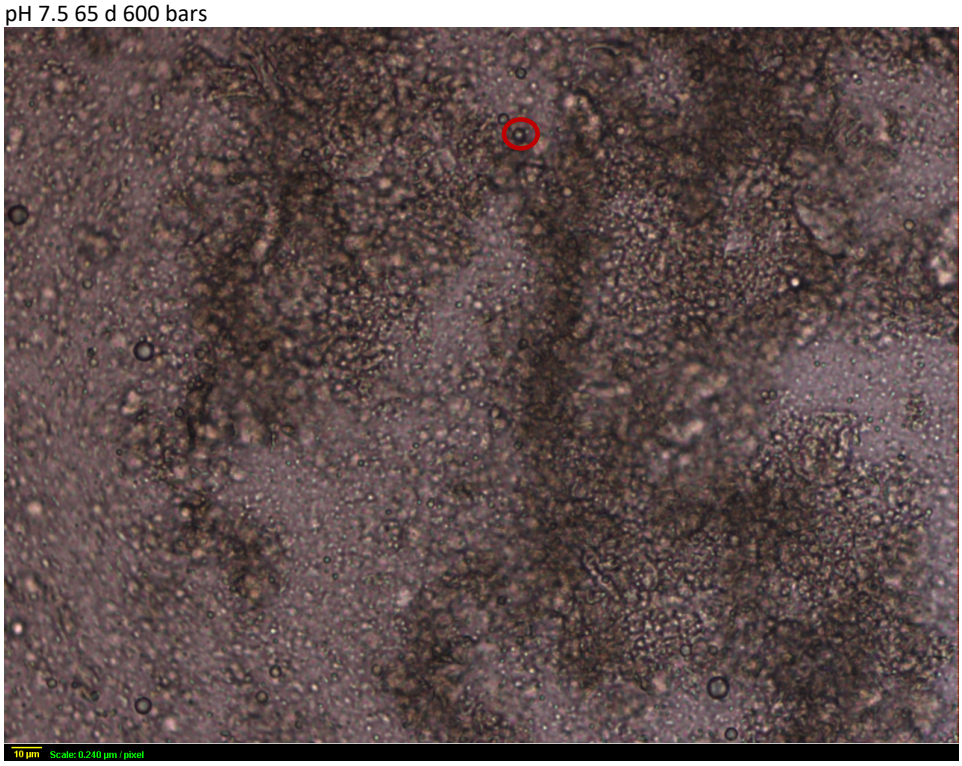
pH 7.5 35 d 800 bars



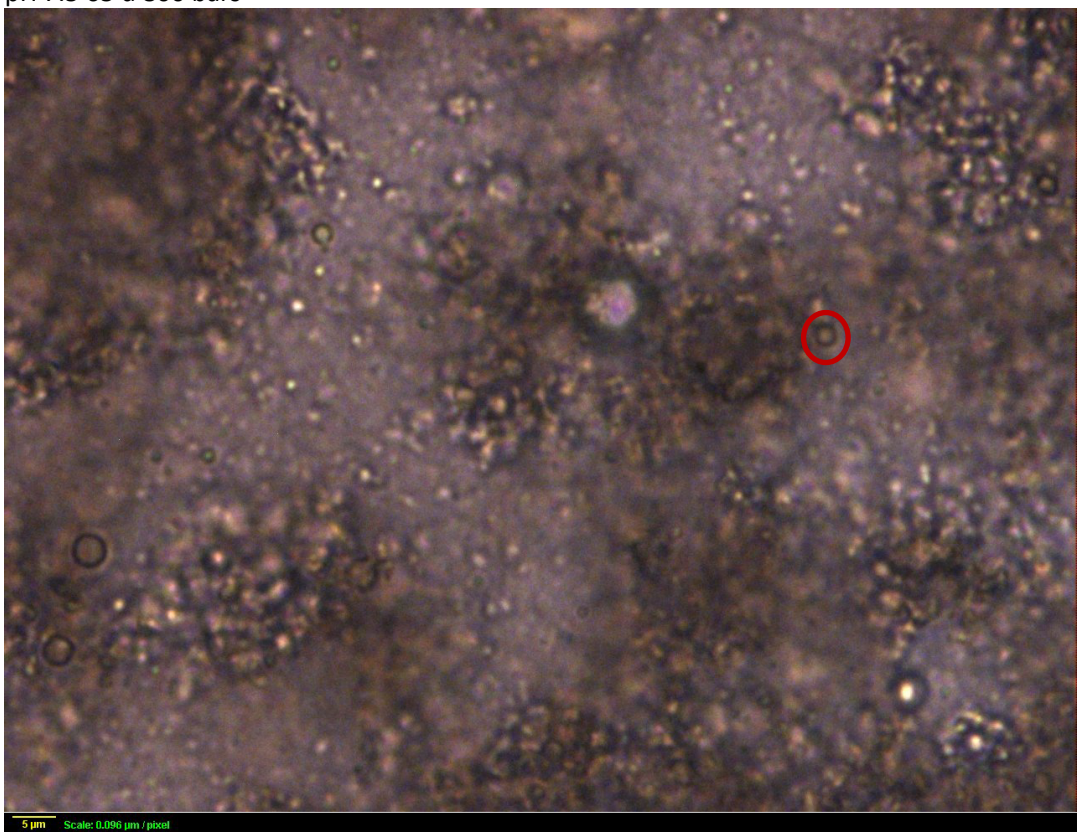
pH 7.5 65 d 200 bars



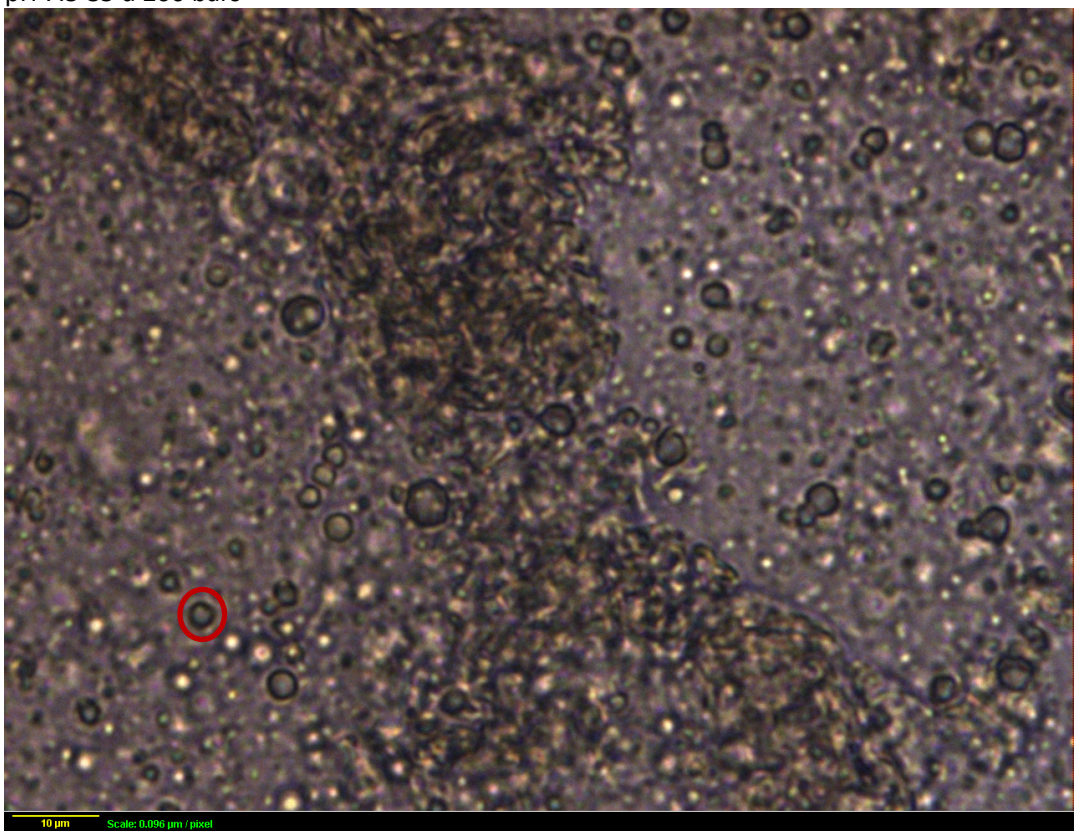
pH 7.5 65 d 600 bars



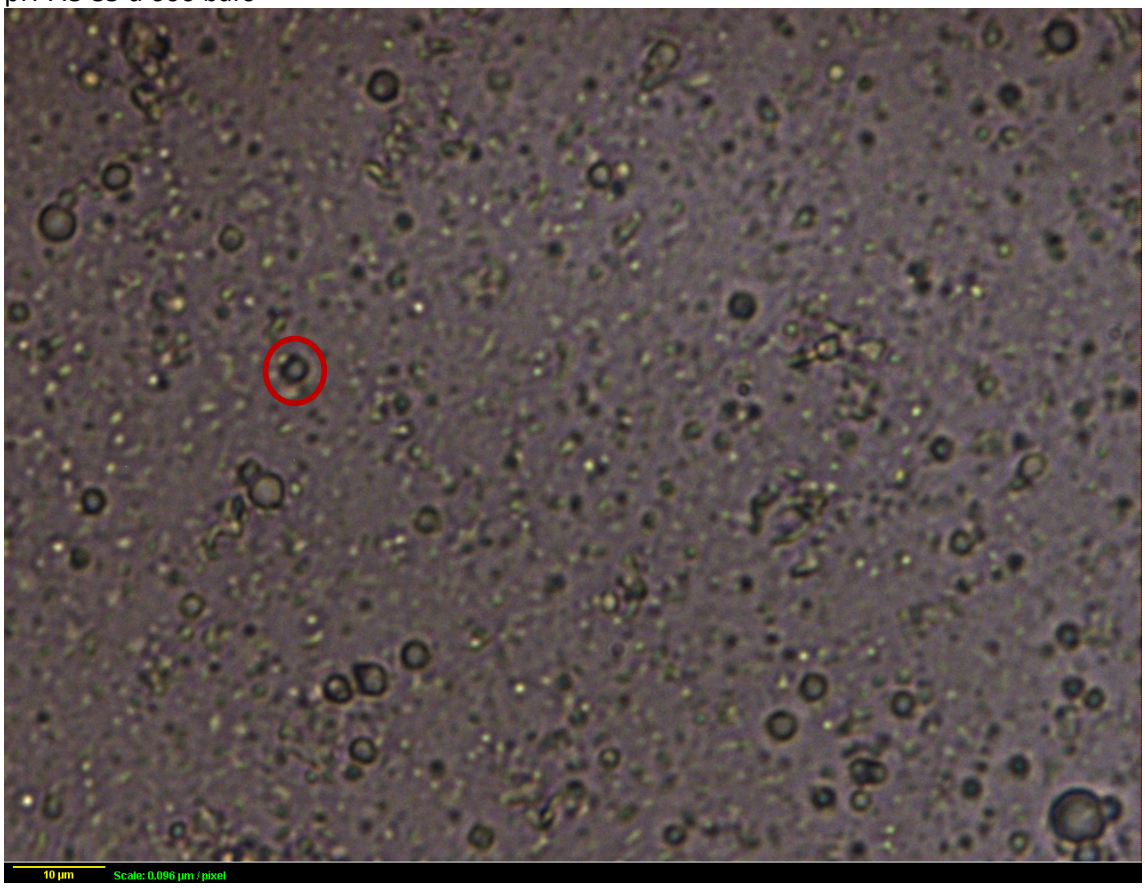
pH 7.5 65 d 800 bars



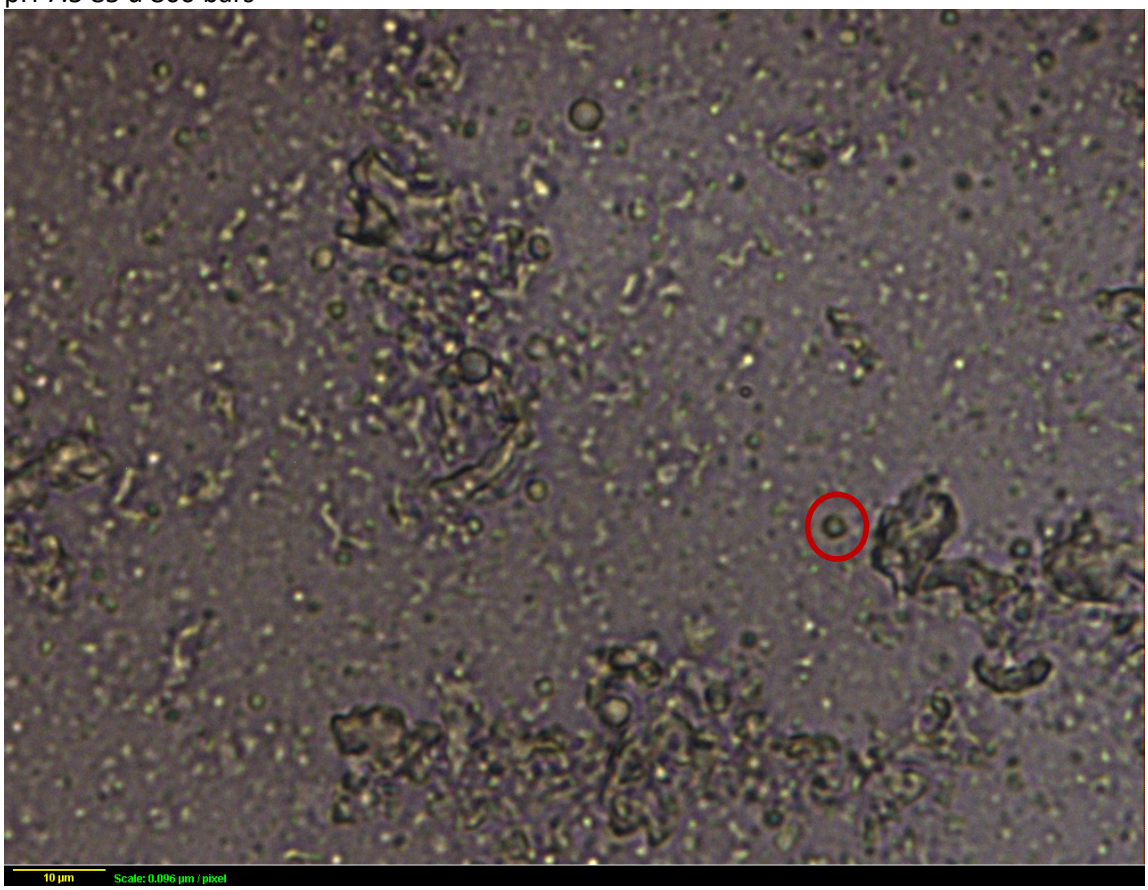
pH 7.5 85 d 200 bars



pH 7.5 85 d 600 bars

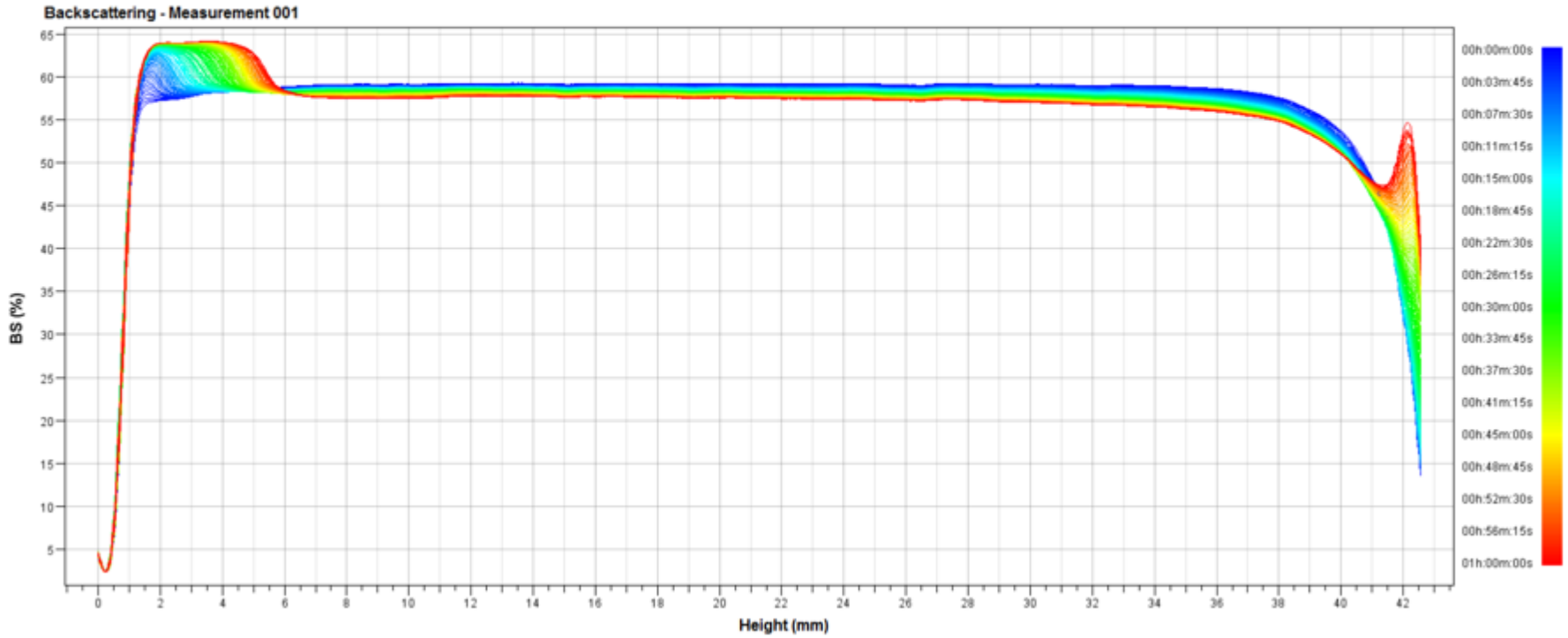


pH 7.5 85 d 800 bars



Appendix C Example of backscattering report from Turbiscan. The example represents a sample with pH 7.5 treated with 35 ºC and 200 bars.

Raw Data - BS



Appendix D Stability of samples over 48 h.