An evaluation of the role of PromOat® on the stability of oil-in-water emulsions

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Popular Science Abstract

In the past years, there has been a major shift towards the consumption of healthier, more sustainable, and more convenient food products. Consumers actively demand for natural ingredients, and for the absence of food additives or components which are hard to read or pronounce. The food industry has replied to these concerns by developing new products or reformulating existing products, replacing controversial ingredients which are regarded as unnatural from a consumer's perspective. Reformulating or developing a new product is no easy task- very often, food additives are incorporated to enhance the ability of a product to remain fresh or apt for consumption over longer periods of time. Therefore, food producers are bound to find natural and healthy substitutes which can satisfy consumer desires without compromising the functionality of the product.

Emulsions comprise the foundation of many of the food products consumers eat on a regular basis. Common examples include sauces, dressings, milk, and ice cream, among others. All of these products contain a fat-based ingredient, like oil or butter, and a water-soluble/polar component such as water or vinegar. It is common knowledge that oil and water do not mix together, hence a typical challenge when dealing with emulsions is stability. In this context, certain ingredients are added to ensure that these two components remain in a homogenous state throughout the whole shelf-life of the product.

β-glucans are dietary fibers found in the kernels of oats and other cereals. Regular consumption of β-glucans has been associated with the maintenance of normal blood LDL-cholesterol, and a reduction of post-prandial glycemic response (to avoid the so-called sugar spikes). On top of this, this ingredient has been widely used as a functional component in a variety of products due to its ability to modify the viscosity and texture of these products, thus aiding in extending their shelf-life. There is widespread acceptance among consumers when it comes to the inclusion of oat β-glucans in a food product, given their health benefits and their natural origin.

Studying the functionality of oat β-glucans as a stabilizing agent in food emulsions appears as an interesting alternative to tackle both the technological challenges associated with emulsions, but also the rising pressure that consumers are exerting on food producers. The present study seeks to contribute to existing knowledge within the field of food emulsions and oat β-glucans, aiming to clarify the mechanisms of action of this ingredient in order to achieve more successful product applications.

An evaluation of the role of *PromOat® on the stability of oil-in-water emulsions*

Department of Food Technology, Engineering and Nutrition, Lund University.
Bernardo Paul

Abstract

Background. In the past years, there has been a major shift towards the consumption of healthier, more sustainable, and more convenient food products. These trends have triggered a response from the food industry, instigating the reformulation of several products. The challenge is removing or replacing certain ingredients, without sacrificing certain elements such as taste, texture, or shelf-life. Swedish cooperative Lantmännen produces and sells *PromOat®*, a β-glucan product derived from oats. β-glucans have been used in product formulations as a means to improve quality and stability during storage, by modifying the texture and appearance of sauces, salad dressings, cakes, bread, and ice creams. Successful applications with oat β-glucans require an understanding of the rheological behavior of the hydrocolloid and an investigation of the underlying mechanisms that are generated when the ingredient is added into a food matrix. **Methods**. The study involved three main steps: (1) the determination of the critical overlap concentration of the hydrocolloid (2) an evaluation of the potential surface activity of the contaminant proteins present in the powder, and (3) an evaluation of the role of oat β-glucans on the stability towards creaming of a reference oil-in-water emulsion using droplet profiling. **Results.** The critical overlap concentration of the hydrocolloid is 0.0309 g/mL (3.09% m/m). There is a tenfold increase in the viscosity of the solution when the critical overlap concentration is reached due to enhanced entanglement and overlapping of the polymer chains. Oat β-glucan solutions exhibit a shear-thinning behavior and there is no evidence of gel formation, presumably due to the high molecular weight of the polysaccharide. The contaminant proteins in the powder have little-to-no surface activity mainly as a consequence of the poor solubility of oat proteins. The oat β-glucan powder does not provide long-term stability on its own and the addition of an emulsifier in the system is a necessary step to achieve kinetic stability. Emulsion systems containing oat β-glucans at a concentration of c* or higher have remained stable over a period of 30 days, achieving creaming rates in the order of 0.002 $mm.min^{-1}$. Although no visual evidence of instability was detected, the droplet profile of these samples exhibited mild flocculation. A mechanism of depletion is generated when the hydrocolloid is added into the food matrix, given the concentration gradient between the depletion zones in the vicinity of the oil droplets and the bulk polymer solution. At concentrations lower than c*, the depleting mechanism dominates over the stabilizing effect provided by the increase in viscosity from the addition of the hydrocolloid, and the emulsions are rendered unstable. On the other hand, the threshold concentration at which the repulsive effect from the rising viscosity starts to compensate for the attractive flocculating effect from depletion is in the order of 0.6 of the c*. Other possible food matrices including, for instance, a lower oil fraction have not been tested and could be investigated in future research. **Conclusion**. Oat β-glucans in *PromOat®* appear as an alternative to other more traditional hydrocolloids used in the food industry. The critical overlap concentration of the powder is 0.0309 g/mL (3.09% m/m) and the application of this hydrocolloid in food emulsions, in combination with an emulsifier, imparts kinetic stability for over 30 days, when the range of application is above or equal to the c*.

Keywords*: oat β-glucans, emulsion stability, critical overlap concentration, droplet profiling, depletion.*

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Background

In the past years, there has been a major shift towards the consumption of healthier, more sustainable, and more convenient food products. In this sense, consumers have raised their awareness about what components are included in the foods they eat on a daily basis, avoiding extensive ingredient lists and ingredients that seem unnatural like the *E-numbers*, which consumers associate with negative health effects. These trends have thus triggered a response from the food industry, instigating the reformulation of several products in their portfolios but also the launching of new, *clean label* products, as a way to satisfy consumers' demand *(Asioli et al., 2017).* The challenge for producers is to remove or replace certain ingredients, without sacrificing sensory elements such as taste and texture, or the ability of the product to stay fresh over a long period of time *(Morrison, 2020).*

Swedish cooperative Lantmännen produces and sells *PromOat®*, a β-glucan product derived from oats. Oat β-glucans are polysaccharides with a combination of β (1-3) and β (1-4) linkages. The European Food Safety Authority (*EFSA*) has authorized health claims arising from the consumption of β-glucans, namely the maintenance of normal blood LDL-cholesterol concentrations and a reduction of post-prandial glycemic responses *(EFSA, 2011)*. Apart from these nutritional qualities, β-glucans have also been used in product formulations as a means to improve quality and stability during storage, by modifying the texture and appearance of sauces, salad dressings, cakes, bread, and ice creams, among others *(Havrlentova et al., 2013).*

Hence, the aim of this project is to investigate the functionality of oat β-glucans in *PromOat®* as an additive in food emulsions, evaluating whether or not the inclusion of said ingredient can have a positive effect on the stability of food emulsions. This work aims to contribute by broadening the existing knowledge with respect to the role of *PromOat®* in food emulsions, aiding Lantmännen in its task to satisfy both present and potential future customers.

1. Introduction

1.1 Emulsions: general aspects

An emulsion is a system comprising two immiscible fluids, where one of the phases is dispersed in the form of droplets within the other. Depending on the composition of the phases and the potential presence of surface-active substances, two main types of emulsions are distinguished, namely oil-in-water (O/W) and water-in-oil (W/O) emulsions *(Weiss and Muschiolik, 2007).* Typical food emulsions, like mayonnaise and salad dressings, belong to the first category as the oil droplets are dispersed in the aqueous phase *(Yang and Lai, 2003).* Given the immiscible nature of the phases, mechanical energy must be applied for the molecules to come together. The energy expenditure compensates for the cost of increasing the total interface area between the two components *(Meller and Stavans, 1996).* Consequently, emulsions are unstable from a thermodynamics perspective, and the free energy present at the interface will eventually usher the separation of the phases *(Ritzoulis, 2013).*

1.2 Mechanisms of instability

1.2.1 Gravitational Separation

In an oil-in-water emulsion, the difference in density between the oil droplets and the aqueous continuous phase leads to a net gravitational force which acts upon the droplets. As the density of most edible oils (ca. 0.93 g/mL) is lower than the density of water (ca. 1.00 g/mL), there is a tendency for the droplets to move upwards, often referred to as creaming *(McClements, 2016)*. The effect of this is the separation of the emulsion into two layers; a more opaque droplet-rich layer and a less opaque droplet-depleted layer. Consumers view such a separation as an undesirable trait due to the fact that the oil-rich layer has a higher viscosity, so the mouthfeel and the taste of the product will be different than that of the more dilute fraction. Prolonged creaming may also lead to flocculation, coalescence, and oiling off given that the oil droplets are in close contact with one another *(McClements, 2016)*.

The physical basis for gravitational separation is described by *Stoke's Law* (***Equation 1*****).** The velocity at which an isolated rigid particle (oil droplet) creams is determined by the upward gravitational force that the particle experiences – due to the lower density of the oil droplet as compared to the surrounding medium – and the hydrodynamic frictional force which hinders the movement, while the particle is moving up through the medium *(McClements, 2016)*.

Equation 1

$$v_{\text{Stokes}} = -\frac{2gr^2(\rho_2-\rho_1)}{9\eta}$$

Where v_{Stokes} is the velocity of creaming (the sign determines the direction of the movement), r is the radius of the particle, g is the acceleration due to gravity, ρ_2 the density of the continuous phase, ρ_1 the density of the dispersed phase, and η the viscosity of the continuous phase.

1.2.2 Ostwald ripening

Ostwald ripening is a process by which large oil droplets grow in size as a result of the dissolution of smaller droplets through the continuous phase. The process is driven by the dependence of the solubility of the droplets with respect to size (***Equation 2)***, and by the fact that there is a net reduction in the number of particles with a consequent formation of larger oil droplets. This transformation is thermodynamically favorable due to the fact that the total interfacial energy decreases as the smaller droplets merge into a larger one *(Kabalnov, 2001).*

Equation 2

$$c(r) = c(\infty)\exp\left[\frac{2\sigma V_m}{rRT}\right] \approx c(\infty)\left(1 + \frac{2\sigma V_m}{rRT}\right)$$

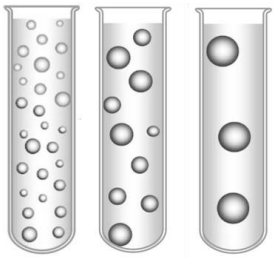

Figure 1. Process of Ostwald ripening. Adapted from *(McClements, 2016).*

Ostwald ripening is usually not significant in food emulsions since the mutual solubility between the oil phase (rapeseed, corn, and sunflower) and the aqueous phase is low, and a considerable solubility is a necessary condition for mass transport to occur *(McClements, 2016).*

1.2.3 Droplet aggregation

The oil droplets in a food emulsion move constantly as a result of thermal energy and gravitational or shear forces that act upon them. The movement can result in collisions and interactions between different droplets within the vicinity of one another. These collisions can lead either to the droplets moving away from each other or to a state of aggregation, depending whether attractive or repulsive interactions govern the collision. When the droplets do come in contact with one another, a thin film of aqueous phase is formed between them. For the droplets to effectively collide, the aqueous film must be forced out and this poses a hydrodynamic challenge arising from the frictional forces generated by the aqueous phase flowing out. On top of this, the net interaction of attractive and repulsive forces has a direct impact on the frequency of collisions. ***Figure 2*** below displays the different scenarios that can take place when it comes to droplet aggregation, depending on the nature of the interactions. If the energy barrier is very high, a situation of a secondary (shallow) minimum is achieved, and the droplets move away from one another. Weak flocculation takes place when the energy barrier is also high, but the secondary minimum is deep. The droplets will then come together, but a thick film of aqueous phase lies between them. Coagulation occurs when the energy barrier is low while a strong short-range repulsion exists. The droplets will thus strongly flocculate, but a layer of thin film will still separate them. Finally, coalescence will happen when the underlying short-range repulsion is not present when the primary minimum is achieved, as the absence of the repulsive force cannot hinder the droplets from coming in close contact with one another *(McClements, 2016).*

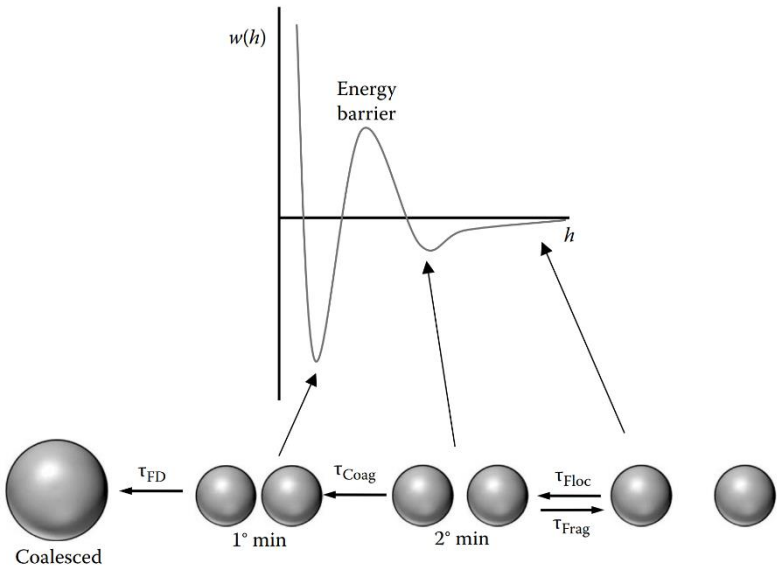

Figure 2. Droplet aggregation scenarios. Adapted from *(McClements, 2016).*

Droplet aggregation, in the form of flocculation or coalescence, has a negative impact on emulsion stability. When oil droplets flocculate, the individual integrity of each droplet is maintained but the effective droplet size within the emulsion increases. As per *Stoke's Law*, an increased particle size increases the rate of gravitational separation and the overall velocity of creaming. The same applies for coalescence, where the merging of smaller droplets into a single larger one will also increase the rate of creaming as a consequence of the size enlargement *(McClements, 2016).*

The addition of non-adsorbing polymers may sometimes induce flocculation as a result of depletion forces. This form of instability is associated with an osmotic pressure difference between the interdroplet gap (film of aqueous phase) and the mass of continuous phase in the vicinity of the droplets. The concentration gradient between the two regions will generate an attractive force between the droplets, and the thin film of aqueous phase will tend to be pressed out of the interdroplet region, thus causing flocculation *(Dickinson, 2009).*

1.2.4 Phase inversion

During phase inversion, the dispersed and continuous phases in an emulsion invert. This means that an oil-in-water emulsion will shift to a water-in-oil emulsion or vice versa. Said process is a necessary step in the production of several food products, namely butter and margarine *(McClements, 2016).* The onset of phase inversion is marked by alterations in the composition or the environment of the emulsion. These changes could involve a modification in the volume fraction of the dispersed phase, the type of emulsifier used, the concentration of the emulsifier, the temperature at which the emulsion is being stored, and whether the emulsion is undergoing agitation *(McClements, 2016).*

Phase inversion has been described and modeled as a complex and dynamic phenomenon, comprising instances of flocculation, coalescence, and droplet break-up. Temperature mediated phase inversion, for instance, is often linked to an induced modification in the structure of emulsifiers. A change in the

structure of the hydrophilic head of the emulsifier can lead to an increased affinity to the oil phase in the emulsion and a consequent transformation in how the emulsifier is arranged at the interface. (*Perazzo et al., 2015).*

Common applications for the process of phase inversion include nano emulsions, the production of cosmetics, and certain petroleum applications such as the transportation of heavy oil (*Perazzo et al., 2015).* The impact of phase inversion is disregarded in this project since the emulsions will not be subjected to any of the aforementioned inducing factors, but also ensuring that the dispersed phase volume fraction (ϕ) is below 0.6, preventing the appearance of catastrophic phase inversion *(McClements, 2016).*

1.2.5 Chemical and Biochemical stability

The mechanisms that have been previously outlined refer to the physical instability of food emulsions. However, there are a number of chemical and biochemical reactions that can have a negative impact on the quality of an emulsion. Lipid oxidation is a form of instability leading to the generation of off-flavors and other undesirable compounds. Furthermore, some of these compounds are surface active and this can directly impact the physical stability of the emulsion by interacting on an interfacial level. Another area of interest when it comes to biochemical stability is the use of enzymes. Enzyme hydrolysis, for example, has been used as a means to improve stability during storage, by cross-linking the interfacial layers that surround the lipid droplets. Colors and flavors are sometimes added to emulsions to enhance sensory attributes like taste and appearance. These compounds are also subject to degradation, as a consequence of a prolonged exposure to light or an unfavorable pH *(McClements, 2016)*. Even though the chemical/biochemical mechanisms herein described are relevant when it comes to evaluating the stability of food emulsions, these aspects are considered to be out of the scope of this project and only physical stability will be further discussed.

1.3 How to stabilize emulsions

So far it has been observed that the thermodynamic instability of food emulsions stems from the inherent nature of its components. Even though emulsions are unstable from a thermodynamic viewpoint, there are still ways by which an emulsion can remain in a kinetically stable (*metastable)* state for a prolonged period of time. A stable – *metastable* - emulsion is defined as one where no perceivable changes take place when it comes to the size distribution of the droplets, their state of aggregation, or the spatial arrangement, over a defined period of time (*Dickinson, 2003).* In general terms, emulsion stability requires that the inter particle repulsion (between the oil droplets) exceeds the joint attractive forces generated by gravity, convection, Brownian motion, and the underlying short-range Van der Waals forces *(Dickinson, 2009).* In practice, emulsions are kinetically stabilized by modifying the size of the oil droplets, by inclusion of surface-active emulsifiers, and by addition of stabilizing/thickening agents *(Lobo, Wasan and Ivanova, 1991).*

1.3.1 Emulsion formation: primary and secondary homogenization

Emulsion formation or emulsification is the process by which an emulsion is formed, or by which the droplet size within an existing emulsion is reduced *(Håkansson, 2019).* The creation of an emulsion from its individual components is denominated primary homogenization, while the reduction in size of the droplets in an emulsion is known as secondary homogenization. It is common practice to prepare food emulsions in a stepwise manner, that is, an initial formation of a coarser emulsion and a subsequent reduction in the droplet size (*McClements, 2016)*.

As previously noted, emulsion formation is thermodynamically unfavorable; an increase in the number of droplets and a reduction in their diameter results in a larger interfacial area, and a consequent increase in interfacial energy. On the other hand, there is a positive contribution to stability due to an increase in the entropy of mixing, which is higher for the emulsion than for the individual components. The entropic effect is, however, not very significant considering the droplet size of traditional food emulsions *(Håkansson, 2019).* A reduction in droplet size, though, will kinetically stabilize the emulsion as predicted by *Stoke's Law*, where the velocity of creaming is directly proportional to the square of the radius.

To compensate for the increase in interfacial energy, a considerable amount of mechanical energy must be imparted onto the emulsion to achieve emulsification. There are two main high-energy emulsification systems, namely high-pressure homogenizers (HPH) and rotor-stator mixers (RSM). The former is mainly used for products with low-to-medium viscosities (1-200 mPa.s) whereas the latter is used for products with higher viscosities *(Håkansson, 2019).* In this regard, only the HPH technology is relevant for the type of emulsions that are evaluated in this project. The mechanism of action of an HPH involves a piston which creates a pressure (up to 500 MPA) that accelerates the fluid through a narrow gap, exposing it to high shear stress and cavitation. The fluid is deformed and twisted as a consequence of the high mechanical stress *(Yong et al., 2017).* Droplet breakdown is also visualized in the outlet chamber of the HPH, where the jet meets the stagnant fluid, generating turbulence and dissipating heat *(Håkansson, 2019).*

1.3.2 Inclusion of a surface-active emulsifier: soybean lecithin

The formation of a stable emulsion requires not only the formation of smaller droplets, as previously mentioned, but also the inclusion of an emulsifier that can hinder the droplets from merging back together after homogenization. Emulsifiers are classified into two main categories: small-molecule surfactants such as monoglycerides, polysorbates, sucrose esters; or lecithin and macromolecular emulsifiers, which are usually proteins derived from milk and eggs (*Dickinson, 2003).* During homogenization, emulsifiers play a vital role by decreasing the interfacial tension between the oil droplets and the aqueous phase, allowing for an overall reduction in the free energy which is a necessary condition for the disruption of the droplets. Emulsifiers will also protect the oil droplets by coating them, thus preventing the latter to coalesce with each other. Said protection should persist even in those cases where the emulsion is subjected to varying environmental conditions, such as pH, ionic strength, temperatures, and other ingredient interactions *(McClements, 2016)*. There are a few parameters which are key for an emulsifier to provide appropriate emulsification. First, the ratio of emulsifier-to-oil should be enough to allow for all the surface of the droplets to be covered completely. Second, the way the emulsifier is adsorbed onto the surface is determined both by the adsorption time, and the probability that the emulsifier will actually adsorb onto

the surface when it comes in contact with the droplets (adsorption efficiency). In both cases, the higher the parameter, the better the emulsification process. Third, the emulsifier should reduce the interfacial tension in such a way that the interfacial rheology is altered, and the droplets can be reduced in size. Lastly, the effectiveness of the emulsifier also lies in the strength of the protective layer; a stronger protection leads to the formation of smaller droplets *(McClements, 2016)*.

Soybean lecithin is a mixture of phospholipids, namely phosphatidylcholine and phosphatidylethanolamine, obtained as a by-product during the production of soybean oil. The phospholipids are separated from the oil in a degumming step, by precipitation with water in the form of a liquid crystalline phase *(Rydhag and Wilton, 1981).* To obtain oil-free, granular lecithin (95-97%) an acetone treatment is needed *(List, 2015).* Due to its amphiphilic nature, lecithin can concentrate at the interface of emulsions, reducing interfacial tension and allowing for the disruption of the oil droplets *(van Nieuwenhuyzen and Szuhaj, 1998).* In food formulations, usage levels of soybean lecithin range from 1-5% for water-in-oil emulsions, and 5-10% for oil-in-water emulsions, based on the oil fraction *(List, 2015).* Phosphatidylcholine is key for the stabilization of emulsions and the main mechanism of action is the formation of a well-ordered lamellar structure, comprising both mono and bilayers. Phosphatidylethanolamine, on the other hand, forms reversed hexagonal structures which are not easily arranged at the interface due to steric hindrance *(van Nieuwenhuyzen and Szuhaj, 1998).* At a pH of around 6.2, soybean lecithin has been shown to confer greater stability to oil-in-water emulsions against creaming as reflected by a reduced droplet size, when compared to samples with a more acidic pH (*Comas, Wagner and Tomás, 2006).* Granular de-oiled soybean lecithin, *Epikuron 100G™* provided by Cargill was used in this project as the emulsifier.

1.3.3 Inclusion of a stabilizing/thickening agent: oat β-glucans (*PromOat®)*

Cereal β-glucans are cell wall polysaccharides present in the grains of certain cereals, namely oats, barley, rye, and wheat *(Nie, Cui and Xie, 2018).* From a molecular perspective, β-glucans are linear homopolysaccharides of d-glucopyranosyl residues which display a combination of β (1-3) and β (1-4) linkages by which residues consisting of consecutive β (1-4) linkages are separated by a single β (1-3) bond *(Lazaridou and Biliaderis, 2007).* In oats (*Avena sativa*), β-glucans are distributed uniformly throughout the endosperm and aleurone cells, though some enrichment in the subaleurone layer has been reported. The content of β-glucans in oats ranges from 2-6%, and the extractability depends on the severity of the extraction method. Mild extraction conditions, such as neutral pH and room temperature, lead to an extractability rate of 30-70%. An alkaline extraction, on the other hand, results in a higher extraction though some hydrolysis of the polysaccharide chains occurs, ultimately leading to the formation of lower molecular weight β-glucans *(Nie, Cui and Xie, 2018).*

As other polysaccharides, and given their affinity to water, β-glucans are classified as hydrocolloids. Hydrocolloids form viscous dispersions and/or gels when they are dispersed in water. By modifying the rheology of the food system, hydrocolloids have a direct impact on the viscosity (flow behavior) and the texture of a food product, which is vital when it comes to both sensory properties and long-term stability *(Saha and Bhattacharya, 2010)*. Hydrocolloids modify the viscosity of a solution as a result of physical entanglement of conformationally disordered random coils *(Saha and Bhattacharya, 2010)*, leading to an

increased resistance to flow when subjected to shearing forces. The concentration of a hydrocolloid in solution has a direct impact on viscosity; for low concentrations (dilute solutions), the increase in viscosity is linear and the behavior is that of a Newtonian fluid. As the concentration increases and reaches a point where the molecules start to overlap with each other, the viscosity increases sharply as a result of this enhanced molecular entanglement. This concentration is known as the critical overlap concentration (c*), and depends on the intrinsic viscosity of the hydrocolloid, or the molecular volume occupancy *(Goff and Guo, 2019).* Above c*, the dispersion exhibits shear thinning behavior *(Saha and Bhattacharya, 2010).* The determination of c* contributes to a deeper understanding of β-glucans in solution, leading to more successful product applications *(Burkus, 2003).* The viscosity of β-glucan solutions is also determined by certain factors, like temperature, pH, molecular weight, and the source of extraction. β-glucan solutions tend to remain stable over a pH range of 2-10 and are stable towards temperature, although temperatures higher than 75°C lead to a decrease in viscosity. When it comes to molecular weight, higher molecular weight β-glucans tend to form more viscous solutions *(Ahmad and Kaleem, 2018).*

The gelling properties of β-glucan solutions have also raised interest with regards to the role of β-glucans as a functional ingredient. β (1-3) linked cellotriosyl units form junction zones connected by hydrogen bonds and trapping water. The strands aggregate with one another, resulting in the formation of a gel network with cluster-cluster aggregation *(Brummer et al., 2014).* The ability of β-glucans to form gels is associated with the ratio of cellotriosyl to cellotetraosyl units present in the cereal structure. In this sense, wheat β-glucans are more prone to form gels than barley and oat, as the ratio of cellotriosyl to cellotetraosyl units is 4, 3, and 2 respectively *(Lazaridou, Biliaderis and Izydorczyk, 2003).* Low molecular weight β-glucans exhibit a faster gelation process whereas higher molecular weight β-glucans produce better organized gels, involving interchain associations over a longer chain segment. As a consequence, higher molecular weight gels have a higher melting point *(Lazaridou, Biliaderis and Izydorczyk, 2003).*

For this project, oat β-glucans were provided in the form of a powder (*PromOat®*) by Lantmännen Oats. The β-glucan content was 34.0% ± 2.0% on a dry basis, a protein content of 3.5%, a fat content of 6.5%, a carbohydrate content of 42.5% and a fiber content of 6.0%, excluding the β-glucans. The molecular weight was in the range of 950.000 g/mole *(Lantmännen, 2020)*.

1.4 Methods to monitor emulsion stability

There are several experimental approaches to monitor variations in the stability of food emulsions. As described in **Section 1.2.1**, the effects of gravitational separation can be predicted by means of mathematical models like *Stoke's Law* and other more complex correlations. Information about the densities of each phase, the droplet size, and the rheology of the continuous phase is necessary. In practice, mathematical predictions have not proven to be very effective due to the fact that they disregard inherent interactions within the food matrix, like the hindrance generated by a more concentrated oil fraction or the negative impact of flocculation which increases the droplet size and the velocity of creaming *(McClements, 2007)*. Emulsion stability has also been studied by visual observation. The samples are usually placed in transparent tubes which are stored for defined periods of time and under specific environmental (stress) conditions. Two distinct layers are normally observed; a lower serum layer, more dilute and less opaque, and an upper cream layer which is more opaque due a higher concentration of oil

droplets. The height of these two layers is measured over time and the creaming index can be calculated as a function of the total height of the emulsion and the height of the serum layer. The slope of a creaming index and time curve can be used as an estimate of the creaming velocity. Although simple and inexpensive, this method is particularly ineffective when the boundary between the two layers is not clearly observed with the naked eye *(McClements, 2007)*. Droplet profiling is a more recent technique, providing a measure of how the droplet concentration varies in relation to the emulsion height over time *(McClements, 2007).* Emulsions are placed in glass vials and a diode emits a light source through the sample. There are two optical sensors; one which receives the light that is transmitted through the sample (transmission detector) and one that receives the light backscattered by the sample (backscattering detector). The result is an accurate macroscopic fingerprint of the migration phenomena taking place in the emulsion, allowing for the detection of creaming, sedimentation, and flocculation, among others. The method is versatile, rapid, reproducible, and non-destructive *(Turbiscan Lab, 2020).* Droplet flocculation can also be monitored through optical microscopy and particle size analysis *(McClements, 2007).*

1.5 Droplet profiling – Turbiscan® LAB Analyzer

The **Turbiscan® LAB Analyzer** functions as a scanner. The optical reading head scans the sample lengthwise, acquiring data from transmitted and backscattered light every 40µm. The backscattering signal is used to analyze opaque and concentrated dispersions and is thus relevant in the field of food emulsions *(Turbiscan Lab, 2020).* The backscattering signal is a function of the mean photon transport free path, and the latter is a function of the concentration and diameter of the particles (see ***Equation 3*** **and** ***4***)

Equation 3

$$BS \approx \left[\frac{1}{l^*}\right]^{1/2}$$

Equation 4

$$l^* = \left[\frac{2d}{3\Phi(1-g)Q_s}\right]$$

Where *BS* is the backscattering signal, *l** is the photon transport mean free path, *d* is the diameter, Φ is the concentration, *g* is an asymmetry factor, and Q_s is a scattering efficiency factor.

A more concentrated (opaque) emulsion will thus have a higher backscattering signal than a dilute (less opaque/watery) emulsion. With this in mind, the stability of the emulsions can be analyzed by observing how the signal changes along the height of the glass cell. When the oil droplets start to migrate due to creaming, a droplet-rich layer will be formed at the top of the glass cell, and the backscattering signal will increase towards the top of the cell. The droplet-deprived layer at the bottom of the cell will consequently experience a reduction in the backscattering signal. Droplet flocculation or coalescence can be perceived as a diverging (up-and-down) signal in the middle of the droplet profile. A summary of the different scenarios is presented in ***Table 1*** below. Typical backscattering curves for the three different scenarios are presented in **Appendix 1.**

Table 1. Description of the different signals for each of the different instability phenomena. Bottom, Middle, and Top refer to the relative position within the glass cell. Adapted from *(Turbiscan Lab, 2020)*

Delta BS	Bottom	Middle	Top	Phenomenon
Case 1	Up	-	Down	Sedimentation
Case 2	-	Up/Down	-	Flocculation/Coalescence
Case 3	Down	-	Up	Creaming

1.6 Definition of the objectives of the project

The experimental design of this project is centered around three main notions which are essential to understand what role, if any, do oat β-glucans in *PromOat®* have upon the kinetic stabilization of oil-in-water emulsions. The first point is to determine the critical overlap concentration of the β-glucans in *PromOat®*. Knowledge of this parameter is key both to understand what the rheological behavior of this hydrocolloid is, but also to define the appropriate range of concentrations that should be utilized when including this ingredient in a product formulation. Details on how the c* is calculated are presented in **Section 2.1** and **Section 3.1.** The second part involves an evaluation of the potential surface activity of *PromOat®*. In order to understand the mechanism of action of this ingredient with respect to the stability of oil-in-water emulsions, a preliminary analysis of the surface activity of the contaminant proteins present in the powder is needed. The idea behind this analysis is to elucidate whether or not this ingredient can provide combined emulsifying and stabilizing effects. Details on how the evaluation is performed are presented in **Section 2.2** and **Section 3.2**. The third part of the experimental design deals with understanding the implications of adding varying concentrations of *PromOat®* on the stability of oil-in-water emulsions. The aim is to investigate what underlying mechanisms arise when different concentrations of the hydrocolloid are incorporated into the food emulsion matrix, and to clarify if these mechanisms have stabilizing or destabilizing effects. Details on how the investigation is conducted are presented in **Section 2.3** and **Section 3.3**.

2. Materials and Methods

2.1 Determination of the critical overlap concentration of PromOat®

2.1.1 Viscosity measurements

Viscosity measurements were determined at a fixed shear rate of 50 s^{-1} by means of a ***Kinexus Pro+ Rheometer***, using a smooth concentric cylinder bob (C25 SC0053SS: PC25 DIN C0052 AL) and a smooth cup (PC25 C0003SS). The rheometer was first calibrated with a 10% m/m sucrose solution of a known viscosity (1.337 mPa.s @ 20°C). Five samples with increasing *PromOat®* concentration were prepared, dissolving the powder in the sucrose solution solvent. The concentrations used were 0.02%, 0.04%, 0.06%, 0.08%, and 0.10%. The apparent viscosity of each sample was measured ten times at 50 s^{-1}, controlling the temperature at 20°C. A mean value for each sample was calculated.

2.1.2. Determination of the intrinsic viscosity and the critical overlap concentration.

The reduced viscosity was calculated as per ***Equation 5*** below for each of the samples above. The intrinsic viscosity was then calculated by extrapolating the reduced viscosity to concentration zero with a linear regression.

Equation 5

$$\boldsymbol{\eta_{red} = (\eta - \eta_0) / \eta_0\, c}$$

where:
η_{red}: reduced viscosity
η: viscosity of the sample
η_0: viscosity of the 10% m/m sucrose solution
c: concentration of the sample in g/mL

The critical overlap concentration c* was finally calculated as per ***Equation 6*** *(Burkus, 2003).*

Equation 6

$$\boldsymbol{c^* \approx 2.5/[\eta]}$$

where:
η: intrinsic viscosity

2.1.3. Measurement of the viscosity/shear profile of PromOat®

The viscosity of different aqueous solutions of *PromOat®* was determined by means of a ***Kinexus Pro+ Rheometer***, using a smooth concentric cylinder bob (C25 SC0053SS: PC25 DIN C0052 AL) and a smooth cup (PC25 C0003SS). The concentrations of *PromOat®* were 0.50%, 1.00%, 2.00%, 3.09%, 4.00%, and 5.00%. The apparent viscosity of each sample was measured ten times at a shear rate 100 s^{-1}, controlling the temperature at 20°C. A mean value for each sample was calculated.

2.2 Evaluation of the potential surface activity of *PromOat®*

2.2.1 Preparation of the samples

The potential surface activity of the proteins in *PromOat®* was assessed by preparing 25% oil-in-water emulsions (V,W,X,Y, and Z), consisting of rapeseed oil, an acetic acid/lactic acid and sodium hydroxide buffer (pH = 6.2), and varying concentrations of *PromOat®* (1.00%, 3.09%, 5.00%, 10.00% and 25.00% respectively) always counted with respect to the concentration of the oil phase. The samples were homogenized in a two-step homogenization process using a ***Panda Plus GEA Lab Homogenizer*** *(GEA Homogenizers, 2020)*, controlling the pressure at 300 bar, and allowing each one of the emulsions to recirculate three times through the equipment. The samples were finally transferred to individual 50mL, graduated polypropylene tubes with a screw cap.

Table 2. Oil-in-water emulsion samples with an oil fraction of $\phi = 0.25$ and increasing concentrations of *PromOat®.*

ID	Rapeseed oil (g)	PromOat (g)	Buffer pH 6.2 (g)
V	25.00	0.25	74.75
W	25.00	0.75	74.25
X	25.00	1.25	73.75
Y	25.00	2.50	72.50
Z	25.00	6.25	68.75

2.2.2 Measuring the particle size (droplet size)

The size of the oil droplets was determined immediately after homogenization by means of laser diffraction particle size analysis, using the ***Mastersizer 2000*** *(Malvern Panalytical | Mastersizer, 2020).* The refractive indices were 1.33 for water and 1.47 for the rapeseed oil, and the system was configured to use deionized water as the dispersant, adding between 3-5 drops of undiluted emulsion until the laser obturation was in the range of 2.0% ± 0.2% as per the instructions manual.

2.2.3 Evaluation of the stability of the emulsions

The stability of the emulsions was evaluated both by visual inspection and by optical light microscopy utilizing an ***Olympus BX50*** optical microscope. Samples were stored at room temperature (20°C) and monitored to detect any possible sign of instability. A microscopy analysis was performed after 1h of preparing the samples using a magnification of 10x.

2.3 Evaluation of the role of *PromOat®* as a stabilizing agent in oil-in-water emulsions

2.3.1 Preparation of the samples

In order to evaluate the functionality of *PromOat®* as a stabilizing agent in oil-in-water emulsions, two different sets of emulsions were prepared. Again, in this case, rapeseed oil and an acetic acid/lactic acid and sodium hydroxide buffer (pH = 6.2) were used in combination with soybean lecithin and varying concentrations of *PromOat®*. A master emulsion was first prepared for each set by combining the rapeseed oil, the emulsifier, and the buffer. The master emulsion was pre-homogenized using an ***Ultra-Turrax®*** disperser at 20.000 rpm. The mix was further homogenized in a two-step homogenization process using a ***Panda Plus GEA Lab Homogenizer*** *(GEA Homogenizers, 2020)*, controlling the pressure at 300 bar, and allowing the emulsion to recirculate three times through the equipment. Using a master emulsion as a starting point ensures that all the samples have the same initial oil droplet size.

Table 3. Master emulsion (ϕ = 0.50) used for the first set of emulsions

Ingredient	Quantity (g)
Rapeseed oil	250.0
Soybean lecithin	12.5
Buffer	237.5
Total	500.0

The master emulsion was subsequently mixed with solutions containing the buffer and *PromOat®*, as per ***Table 4*** below. It is worth noting that the desired final oil fraction of ϕ = 0.25 was obtained in each sample by diluting the original master emulsion (ϕ = 0.50) in half by adding the corresponding quantity of buffer solution and *PromOat®.* All of the samples were prepared in duplicates.

Table 4. Oil-in-water emulsion samples with an oil fraction of ϕ = 0.25 and increasing concentrations of *PromOat®.*

Fraction of c*	ID	Master emulsion (g)	PromOat (g)	Buffer pH 6.2 (g)
0.00	A	50.00	0.00	50.00
0.25	B	50.00	0.77	49.23
0.50	C	50.00	1.55	48.45
0.75	D	50.00	2.32	47.68
1.00	E	50.00	3.09	46.91
1.50	F	50.00	4.64	45.36
2.00	G	50.00	6.19	43.81

A second set of emulsions was prepared, following the steps that were previously described, to further investigate the behavior of the hydrocolloid for concentrations lower than the critical overlap concentration. All the samples were prepared in duplicates.

***Table 5*.** Oil-in-water emulsion samples with an oil fraction of ϕ = 0.25 and increasing concentrations of *PromOat®* in the range of 0.10 to 0.60 of the c*.

Fraction of c*	ID	Master emulsion (g)	PromOat (g)	Buffer pH 6.2 (g)
0.10	O	50.00	0.31	49.69
0.20	P	50.00	0.62	49.38
0.30	Q	50.00	0.93	49.07
0.40	R	50.00	1.24	48.76
0.60	S	50.00	1.55	48.45

2.3.2 Evaluation of the stability of the emulsions

2.3.2.1 Preparation of the samples

The stability of the emulsions was monitored through droplet profiling, employing the **Turbiscan LAB Analyzer**. 20 mL of the different emulsions were pipetted into the glass cells, ensuring the glass surface was completely clean to avoid distortions in the results. The pipette was inserted into the cell to prevent air bubble formation in the middle of the vial, and the potential menisci were flattened by carefully hitting the glass cell against the holder. A black cap, specifically designed to optimize the positioning of the cell in the instrument, was used in combination with a rubber seal to prevent leakage *(Turbiscan Lab, 2020).* The samples were then labeled sticking a label on the curved side of the black cap. The samples were stored at room temperature for 30 days, making sure that no agitation occurred after the first measurement, as this would otherwise produce divergences in the droplet profile.

2.3.2.2 Determination of the viscosity of the samples

Viscosity measurements were determined at a fixed shear rate of $100s^{-1}$ by means of a ***Kinexus Pro+ Rheometer***, using a smooth concentric cylinder bob (C25 SC0053SS: PC25 DIN C0052 AL) and a smooth cup (PC25 C0003SS). The apparent viscosity of each sample was measured ten times at a shear rate of 100 s^{-1} controlling the temperature at 20°C. A mean value for each sample was calculated.

2.3.2.2 Determination of the droplet profiles

The glass cells were scanned with the instrument directly after the emulsions were prepared, using the single scan setting, and setting the temperature at 20°C. First, the scans were carried out during 1h in 5min intervals. The frequency of the measurements was then modified to one measurement every 30min, for the first 8h, and finally to one measurement every 24h. The aim was to detect any possible instability that could arise both during the early and the late stages of the experiment.

2.3.2.3 Determination of the creaming rate

The progression of the cream layer for each sample was examined with the *Peak Thickness* function of the instrument, which allows for a representation of how the turbidity changes with height, as a result of the migration of the oil droplets to the surface of the glass cells. By keeping track of the position of the oil droplets and the thickness of the cream layer as a function of the time increments previously defined, a height (of the cream layer) against time curve was constructed. For each sample, a linear regression of the data points was performed, and the creaming rate was determined as the slope of the regression line. As each sample was prepared in duplicates, two scatter plots were obtained, and two creaming rates were calculated.

2.3.2.4 Statistical analyses

To establish whether there is a significant difference between the creaming rates of the samples, a one-way ANOVA was conducted with **IBM SPSS Statistics 26**. The significance level was set at $p < 0.05$ (2 tailed) and the ANOVA was followed by Tukey's post-hoc test.

3. Results and Discussion

3.1 Critical overlap concentration of PromOat®

Table 6. Apparent viscosity of the samples measured at a fixed shear rate of 50 s^{-1}. The value presented is a mean of ten measurements. The difference between the measurements was no more than 3%.

Concentration (g/ mL)	Apparent viscosity (mPa.s)
0.0000	1.34 ± 0.03
0.0002	1.38 ± 0.03
0.0004	1.43 ± 0.03
0.0006	1.52 ± 0.03
0.0008	1.65 ± 0.03
0.0010	1.83 ± 0.04

The reduced viscosity was then calculated as per ***Equation 5***, and the results were plotted against the concentration as depicted on ***Figure 3*** below.

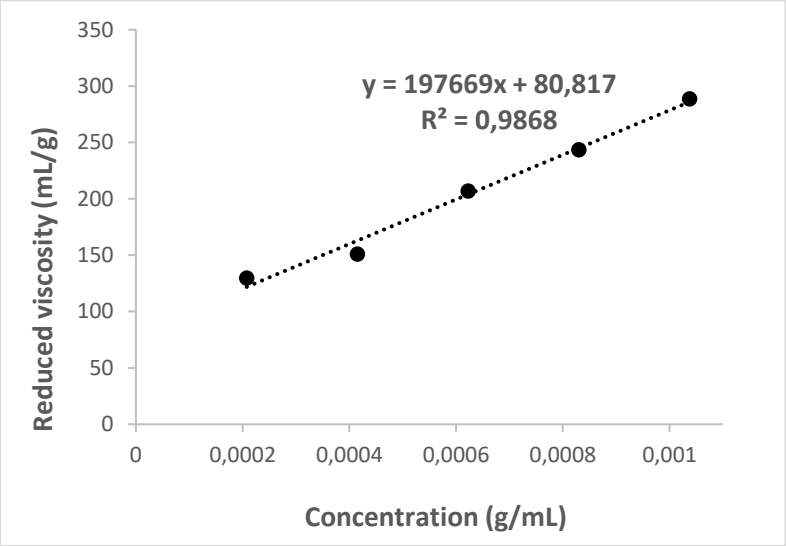

Figure 3. Reduced viscosity (mL/g) as a function of the concentration (g/mL) of the samples.

By linearly extrapolating the reduced viscosity to zero concentration, the intrinsic viscosity can be determined. The value is **80.8167 ± 9.1001 mL/g** and is a measure of the individual molecular occupancy of the molecules in the solvent. This parameter is associated with the molecular weight of the polysaccharide, as described by the *Mark-Houwink* relationship *(Burkus, 2003).*

Equation 7

$$[\eta] = K' M_r^{\alpha}$$

Where η is the intrinsic viscosity, M_r is the relative molecular weight, and K' and α are "stiffness" parameters that can be obtained graphically with a double logarithmic plot of η and M_r *(Burkus, 2003).*

In this context, the determination of the intrinsic viscosity may be used as an indirect approximation of the molecular weight of the polysaccharide, given that a standard curve can be constructed with samples of known molecular weights. Although the molecular weight of *PromOat®* has already been determined by Lantmännen, having knowledge of the intrinsic viscosity is relevant when comparing different hydrocolloids with each other. Molecules with a higher molecular size, for example, occupy a larger volume in the solution, and the entanglement of the individual molecules takes place at lower concentrations (i.e. the critical overlap concentration is lower) *(Salovaara, 2007).*

The critical overlap concentration was thus calculated from ***Equation 6.*** The value is **0.0309 ± 0.0035 g/mL** (3.09 %m/m) for *PromOat®* and **0.0105 ± 0.0018 g/mL**, with respect to the β-glucan fraction present in the powder (34%).

To further verify the accuracy of this result, the rheological behavior of different *PromOat®* solutions was investigated as per **Section 2.1.3**. The concentrations used were 0.50%, 1.00%, 2.00%, c* (3.09%), 4.00%, and 5.00%.

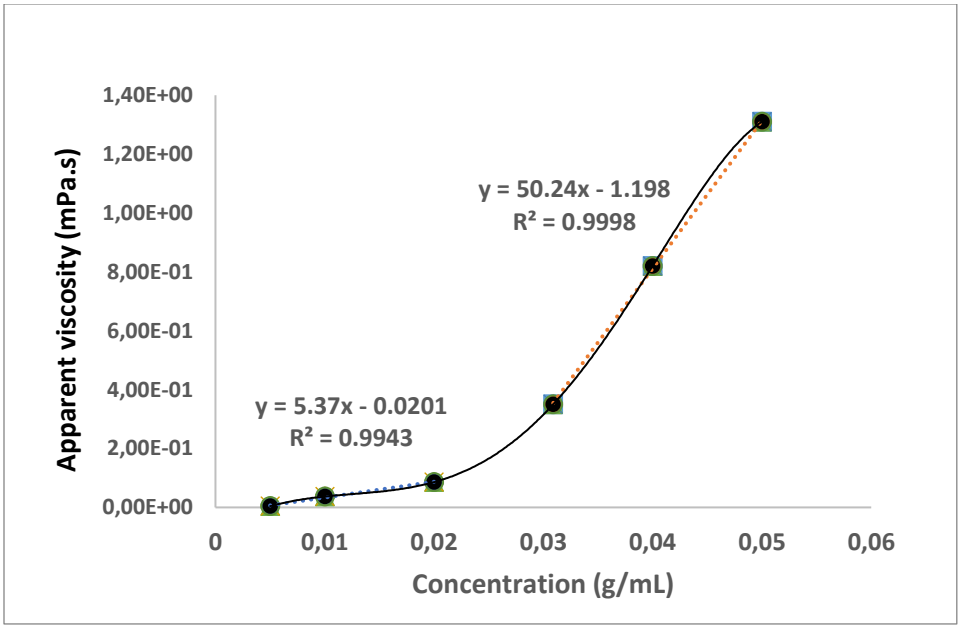

Figure 4. Apparent viscosity (mPa.s) for the different PromOat® solutions as a function of concentration (g/mL).

The behavior of *PromOat®* falls into two distinct linear regions, and this is typical for most disordered polysaccharide solutions. As previously described, the individual polymer molecules are separated from the neighboring molecules in a dilute solution, so viscosity increases linearly with concentration, though not steeply. When the critical overlap concentration is reached at 3.09%, the polymer becomes entangled and these chain-to-chain interactions result in a steep viscosity increase (as visualized by the almost ten-fold increase in the slope of the curve from 5.37 to 50.24). The calculated value is also in line with previous rheological investigations performed on oat β-glucans, where the c* parameter varied from 0.25 g/dL to 1.10 g/dL, depending on the molecular weight of the polymer *(Agbenorhevi et al., 2011).*

Solutions of random coil polysaccharides normally exhibit a shear thinning behavior as a result of the alignment of the polymer molecules in the direction of the flow deformation. The flow behavior of oat β-glucans can sometimes differ from this pattern, as the flow curves continue with an upward trend. As previously noted, oat β-glucans are gel formers and it is the formation of the gel in the form of a continuous network where the molecules are cross-linked through intramolecular associations or aggregations that is responsible for this deviation *(Salovaara, 2007).*

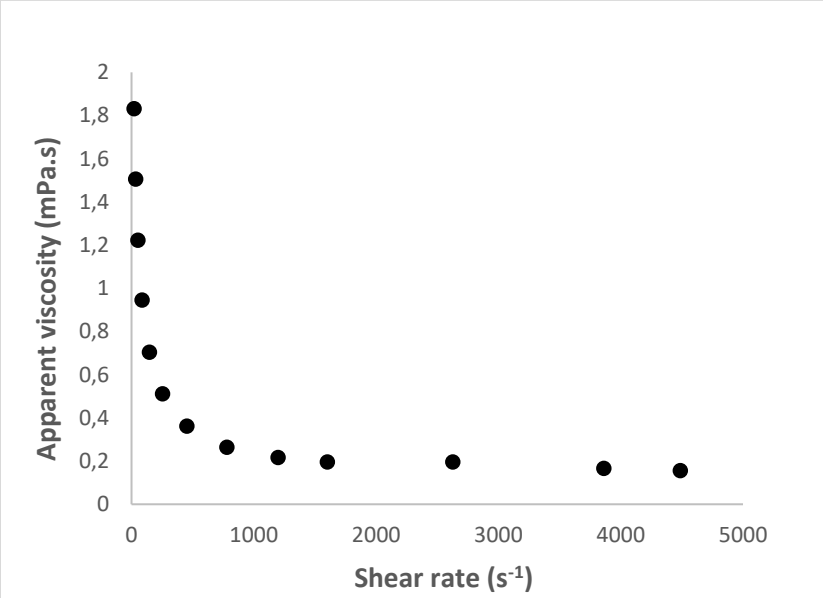

Figure 5. Apparent viscosity (mPa.s) for a solution containing 3.09% PromOat® as a function of the shear rate

Figure 5 shows the flow behavior for PromOat® as a function of the shear rate. At lower shear rates, the polymer chains get disentangled at the same rate as new entanglements are formed and the viscosity remains high. As the shear rate is increased, the number of disentanglements exceeds that of the entanglements (which are a result of Brownian motion), and the viscosity drops sharply. The shear-thinning behavior is evident at this point. A further increase in the shear rate leads to the molecules being practically fully disentangled, yielding a low and constant viscosity *(Rayner et al., 2015).* In this regard, it seems as if there is no apparent gel formation that could possibly alter how the solution behaves upon shear deformation. The formation of the gel is determined by the molecular weight of the β-glucan, by the ratio of cellotriosyl to cellotetraosyl units present in the cereal structure, and by the time that the sample is stored. Oat β-glucans are poor gel formers when compared to other cereals like wheat and barley, given that the ratio of cellotriosyl to cellotetraosyl units is lower for oats. Furthermore, the flow behavior of the samples was determined with freshly prepared solutions and this could have had an impact as the sample could have been measured before the onset of gel formation. However, only lower molecular weight (< 250.000 g/mol) oat β-glucan samples tend to exhibit gel formation at an appreciable rate enough to be observed within the time frame of such an experiment (minutes to hours) *(Salovaara, 2007).* Since the molecular weight of PromOat® is said to be in the range of 950.000 g/mol, gel formation is not apparent for these samples.

3.2 Evaluation of the potential surface activity of *PromOat®*

As the protein content of PromOat® is in the range of 3.5%, a potential migration of these contaminant proteins to the interface of the oil-in-water emulsion, and a subsequent adsorption of these proteins at the interface, could stabilize the emulsion droplets and deter creaming.

The average particle size (volume weighted mean) was determined for each of the emulsions V, W, X, Y, and Z to assess whether the inclusion of *PromOat®* affected the size of the droplets, potentially acting as a deterrent of creaming.

Table 7. Particle size expressed as volume weighted mean D (4,3) of the different emulsions with increasing PromOat® concentrations. The concentration of PromOat® is counted with respect to the oil fraction.

ID	PromOat (%)	Particle size (µm)
V	1.00	22.04
W	3.00	13.95
X	5.00	10.47
Y	10.00	8.41
Z	25.00	15.67

As seen on ***Table 7***, the droplet size decreased with increasing PromOat® concentrations (from V to Y), as would be expected in a scenario where the emulsifier content is raised, lowering the interfacial free energy, and allowing for a more significant disruption of the droplets during homogenization *(Pinnamaneni et al., 2003).* For the sample with the highest concentration of PromOat® (sample Z), the droplet size was higher than for sample Y. This could be attributed to the fact that the viscosity of sample Z was higher, triggering a viscous stabilization that supposes an additional resistance against droplet breakup in the process of homogenization *(Håkansson, 2019).* From these preliminary results, a lower creaming velocity would be expected for sample Y, given that the droplet size is the lowest among the group (disregarding the difference in viscosity between the samples).

However, all the samples were completely unstable only after 35 min of being homogenized. A cream layer of oil at the top of the polypropylene tube was rapidly observed (see ***Figure 6*** below), and there was evidence of flocculation. Previous research using oat proteins has indicated that these proteins show poor functionality as emulsifiers, mainly due to their poor solubility *(Zhang et al., 2015).* Solubility is key for an emulsifier to be effective; only soluble proteins can migrate to the oil-water interface and adsorb at the surface of the oil droplets. Due to their amphiphilic nature, soluble proteins rearrange at the interface and partially denature, binding to the oil phase at the hydrophobic end and exposing the hydrophilic head to the aqueous continuous phase *(Burger and Zhang, 2019).* The poor solubility of oat proteins could thus account for the lack of effectiveness as an emulsifier. Moreover, the fact that *PromOat®* only contains 3.5% of protein suggests that the concentration of these proteins, considering that the solubility is essentially very low, is not enough to provide an emulsifying effect.

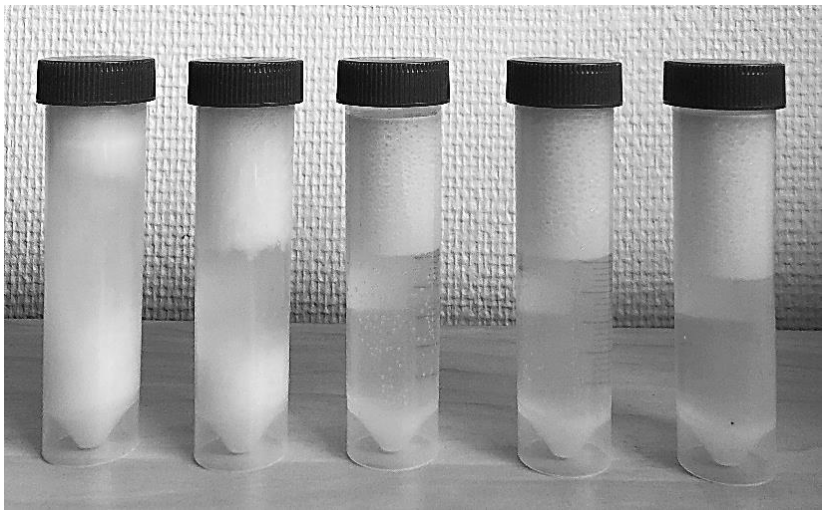

Figure 6. Visual appearance of the samples after 35min. From left to right (V, W, X, Y, and Z)

The nature of the instability mechanism was further investigated by means of optical microscopy as can be seen on ***Figure 7*****.** Microscopy images reveal that the oil droplets have aggregated and come together, although the individual integrity seems to be preserved (flocculation). As previously mentioned, the formation of flocs increases the overall particle size within the emulsion (reaching values in the range of 100μm), and it is this large particle size which is responsible for such a rapid destabilization of the samples (the velocity of creaming is proportional to the square of the radius of the droplets). Although all the samples were completely unstable after 35min, no clear distinction as to which sample destabilized first was established. This is evidently a consequence of the simplicity of the selected method to evaluate the stability of said samples, as the naked eye does not detect minor divergences between the different creaming rates. Nevertheless, the results provide strong evidence that the proteins in *PromOat®* have little-to-no surface activity, which is a consequence of both the poor solubility of the oat proteins and the low content of proteins in the powder. In this regard, the selection of the method is deemed appropriate due to the fact that using a more complex method would probably yield the same conclusion, at the expense of rendering the experiment cumbersome and more time-consuming.

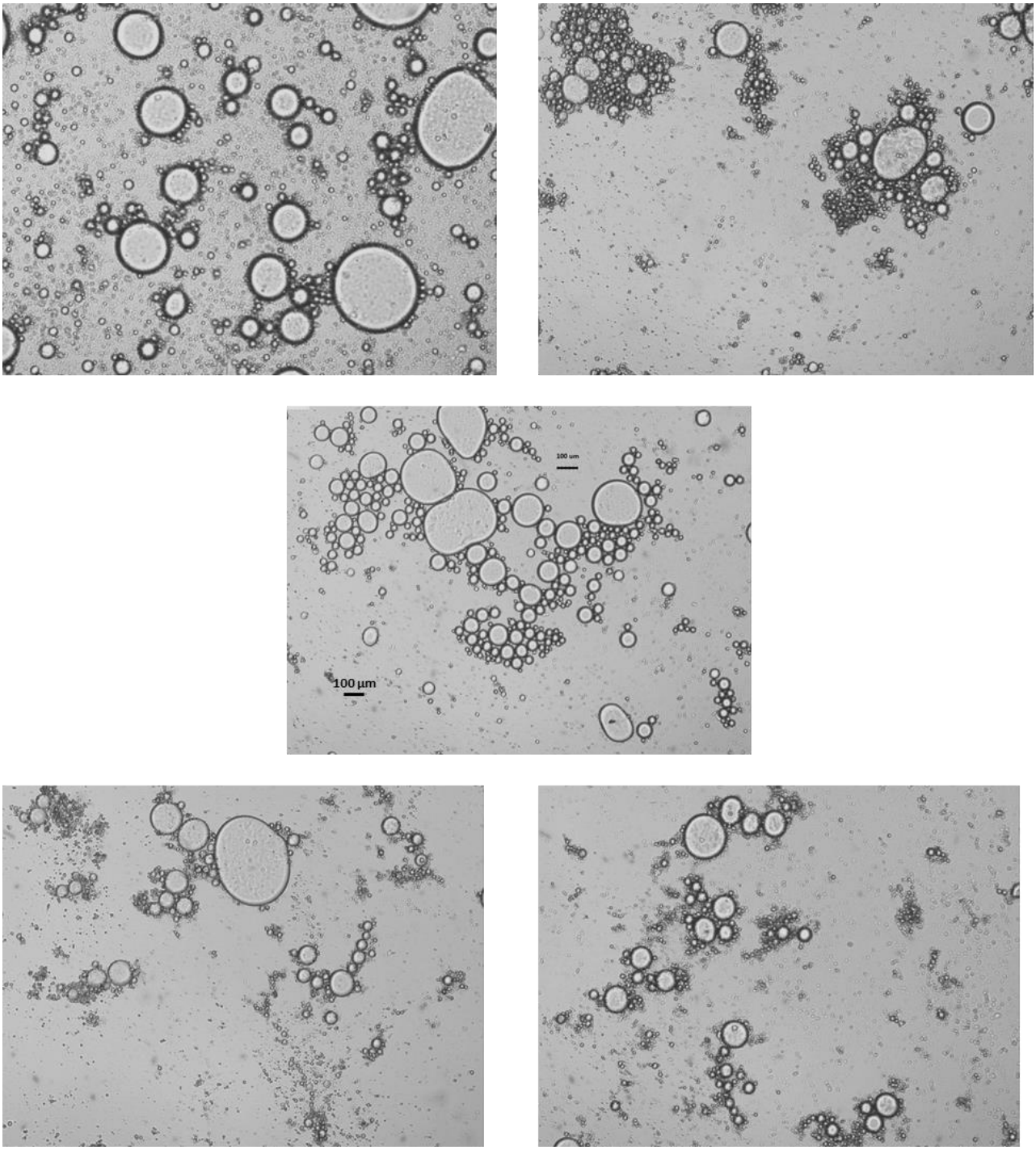

Figure 7. Microscopy analysis of the samples using a magnification of 10x. From left to right and top to bottom (V, W, X, Y, and Z)

3.3 Evaluation of the stability of the emulsions using droplet profiling

Once it was established that the proteins in *PromOat®* do not have surface activity, three different sets of emulsions were prepared as described in **Section 2.3.1.** Different concentrations of *PromOat®* were used in combination with soy lecithin, the latter providing the emulsion system with an emulsifying effect.

A macroscopic fingerprint of the migration phenomena was established for all the samples by means of the **Turbiscan® LAB Analyzer**. As a way to illustrate how the output of the instrument was analyzed, the reference sample (Sample A, 0% *PromOat®*) is used as an example.

Figure 8 shows how the backscattering signal decreases at the bottom of the glass cell (from the blue curve to the red curve) while, at the same time, there is an increase of the backscattering signal at the top of the cell. This is a typical scenario of creaming, as described in **Section 1.5**. As Sample A contains no hydrocolloid, there is no deterrent towards the gravitational force acting upon the emulsion, so the oil droplets eventually migrate to the top of the cell, thus producing a reduction in the turbidity at the bottom of the vial (and an increase at the top). Single-scan analyses were performed during a 90min period, already showing evidence of creaming at this point. The inclusion of *PromOat®* as a stabilizing agent appears as a necessary step to increase the viscosity of the samples and prevent the separation of the multicomponent system by dropping down the rate of creaming, as predicted by *Stoke's Law* (*Rayner et al., 2015).*

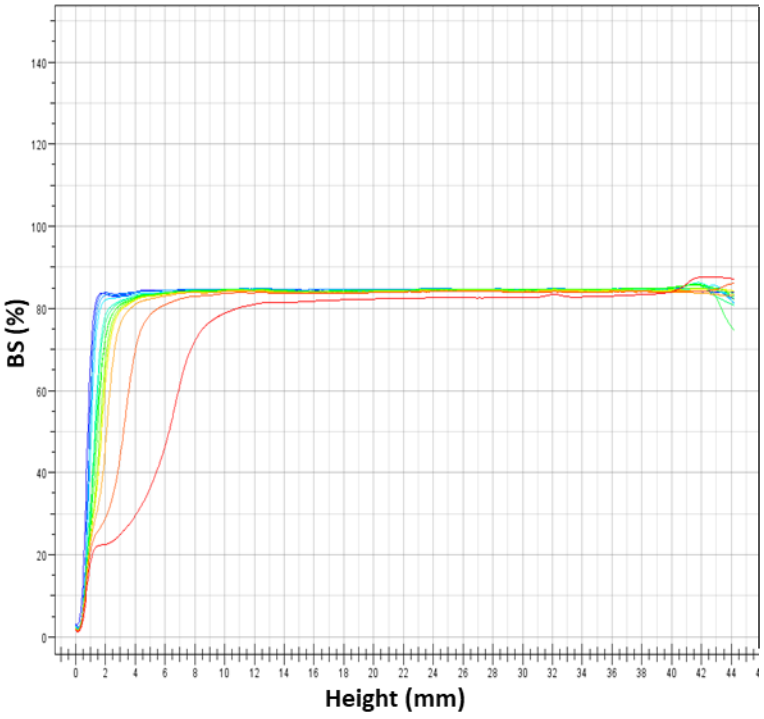

Figure 8. Single-scan analysis of Sample A as obtained from the **Turbiscan® LAB Analyzer**

By using the *Peak Thickness* function in the **Turbiscan® LAB Analyzer**, a scatter plot of the height of the cream layer as a function of time can be constructed. The slope of the regression line approximates the creaming rate for Sample A. The creaming rate was **0.833 mm/min**.

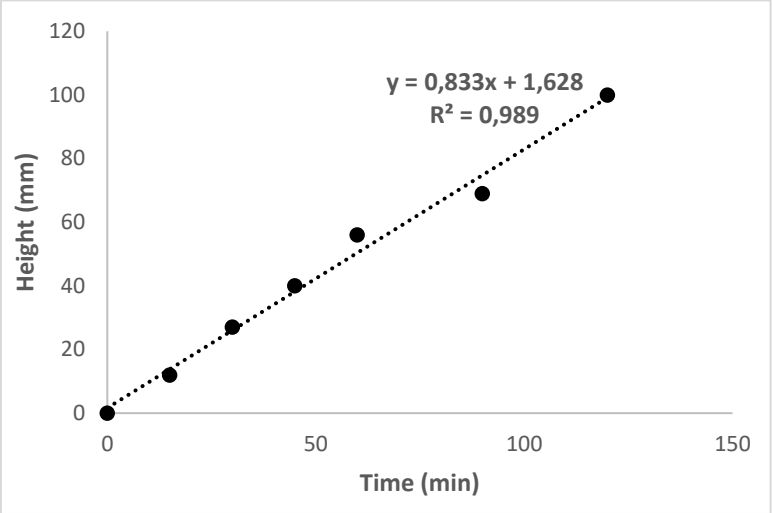

Figure 9. Height of the cream layer as a function of time for Sample A.

The viscosity of the samples was also determined as per Section **2.3.2.2** and the results are summarized in ***Table 8*** below.

Table 8. Viscosity of the emulsions with an oil fraction of ϕ = 0.25. The difference between the measurements was no more than 3%.

ID	Fraction of c*	Apparent viscosity (mPa.s)
A	0	1.30 ± 0.04
O	0.1	1.43 ± 0.04
P	0.2	2.86 ± 0.09
B	0.25	3.56 ± 0.11
Q	0.3	4.04 ± 0.12
R	0.4	5.37 ± 0.16
C	0.5	6.72 ± 0.20
S	0.6	8.06 ± 0.24
D	0.75	10.07 ± 0.30
E	1	55.60 ± 1.67
F	1.5	460.00 ± 13.80
G	2	923.00 ± 27.69

It is evident from ***Table 8*** that the higher the concentration of the hydrocolloid in the formulation, the higher the viscosity of the emulsion. Based on *Stoke's Law*, where the creaming rate is inversely proportional to the viscosity of the continuous phase, the expected trend for the creaming rate of the emulsions should follow the order A > O > P > B > Q > R > C > S > D > E > F > G.

The *Peak Thickness* function was thus applied to each sample and its duplicates as a way to experimentally determine the creaming rate for the set of emulsions. A mean creaming rate was established as the average between the slopes of the respective regression lines. The results are depicted on ***Figure 10*** below.

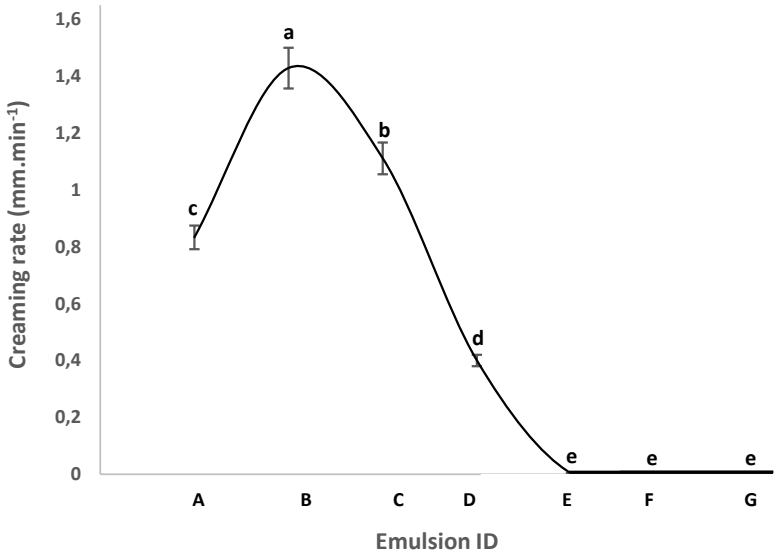

Figure 10. Creaming rate for the emulsions (A, B, C, D, E, F and G). The data is presented as mean values of duplicate measurements. The difference between the duplicates was no more than 5%. Different superscripts indicate significantly different values ($p < 0.05$; Tukey's test). For samples E, F, and G the error bars are not visible because the mean value of the creaming rate is approximately zero and the standard deviation is consequently very low.

The creaming rate follows the order B > C > A > D > E > F > G which is not in line with the prediction from *Stoke's Law*, even though the viscosity of both sample B and C is greater than that of sample A. The one-way ANOVA analysis shows that there is a significant difference between the creaming rates of sample A, B, C, and D, and that there is no significant difference between samples E, F, and G (at concentrations higher or equal than the c*). The fact that sample B and C have a higher creaming rate than A may be attributed to the fact that non-adsorbed polymers, like oat β-glucans, can promote droplet flocculation in oil-in-water emulsions through a depletion mechanism. In an oil-in-water emulsion containing a non-adsorbing polysaccharide, there is a region surrounding each oil droplet which is polymer depleted. In this context, a concentration gradient (osmotic pressure) is generated between these polymer depleted regions and the bulk polymer solution. To minimize the free energy and reduce the osmotic potential differences, the system will tend to lower the volume of depletion zones. The latter is achieved by oil droplets approaching each other resulting in an overlapping of the depletion zones. A direct consequence of this droplet-to-droplet association is flocculation and this process promotes gravitational separation by increasing the effective size of the oil droplets *(Grundy et al., 2018).*

The macroscopic fingerprint of Sample B is presented below. The backscattering signal rapidly lowers at the bottom of the glass cell, and there is evidence of flocculation in the middle part of the graph. The increase of the backscattering signal at the top of the glass cell displays the fact that the sample was completely creamed after 40min. The depleting effect of the oat β-glucans is thus manifest.

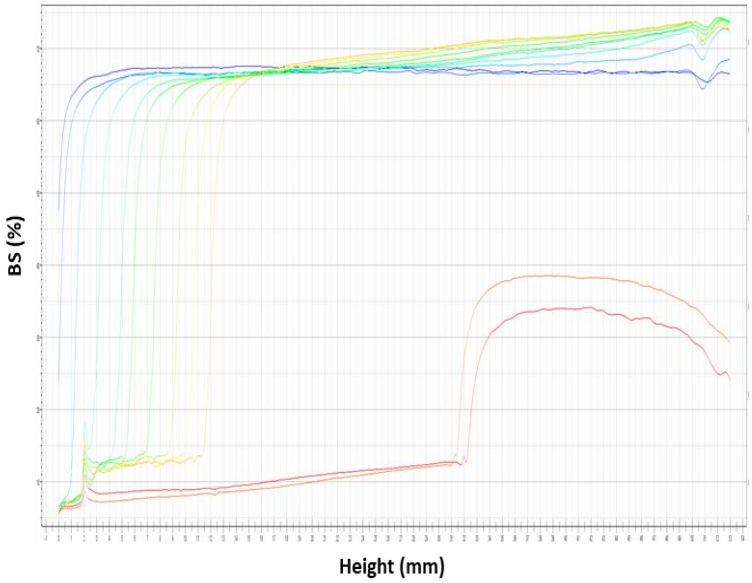

Figure 11. Single-scan analysis of Sample B as obtained from the **Turbiscan® LAB Analyzer**

At higher polymer concentrations, as for Sample D, E, F, and G, the creaming rate is lower than that of the reference sample (A). As it was previously noted, hydrocolloids impart stability by modifying – increasing - the viscosity of the continuous phase. From ***Figure 10***, it seems as if emulsions containing fractions of c* of 0.75 or higher are more stable towards creaming than both the reference sample and samples containing concentrations lower or equal than 50% of the c*. Although non-adsorbing polymers induce flocculation through a depletion mechanism, further increments in the concentration of the hydrocolloid in the formulation can re-stabilize the emulsion by conferring a very high apparent viscosity, thus preventing the attractive forces from dominating the stability of the emulsion *(McClements, 2016).*

All the emulsions with a concentration of c* or higher (E, F, and G) appear as stable throughout the duration of the experiment (30 days). The droplet profile from the **Turbiscan® LAB Analyzer (*Figure 12*)** displays mild flocculation though no alteration in the appearance can be perceived with the naked eye. Using concentrations above or equal to the critical overlap concentration allows for the polymer chains to entangle, leading to the formation of a viscoelastic network. It is this network which hinders the oil droplets from diffusing and approaching each other, thus preventing the depletion zones from overlapping *(Grundy et al., 2018).* This notion is illustrated by the results of the one-way ANOVA, due to the fact that the creaming rate decreases in a statistically significant manner when the concentration is higher or equal to c*, to the point where no instability is perceived (the creaming rate is ca. **0 mm.min^{-1}**).

Table 9 Creaming rate of samples E, F, and G. The data is presented as mean values of duplicate measurements
.

ID	Creaming rate ($x\ 10^{-3}$ mm.min^{-1})
E	2.86 ± 0.06
F	2.22 ± 0.04
G	2.08 ± 0.04

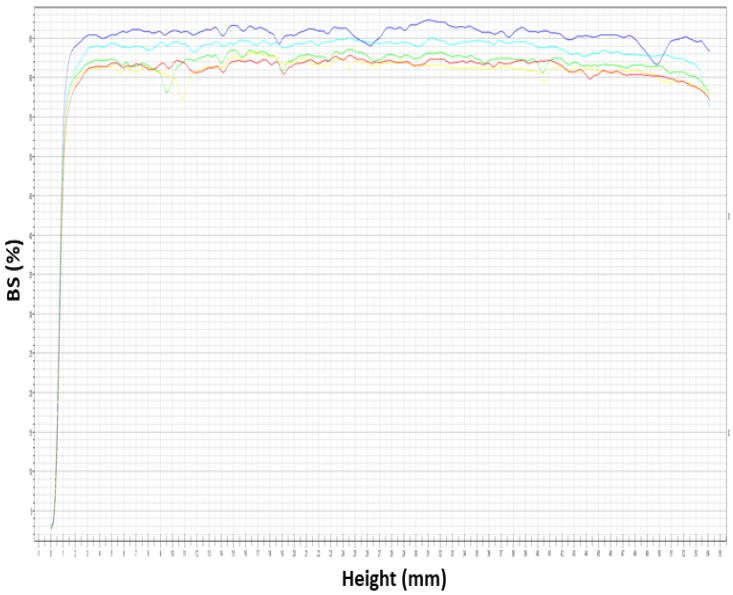

Figure 12. Single-scan analysis of Sample E as obtained from the **Turbiscan® LAB Analyzer**

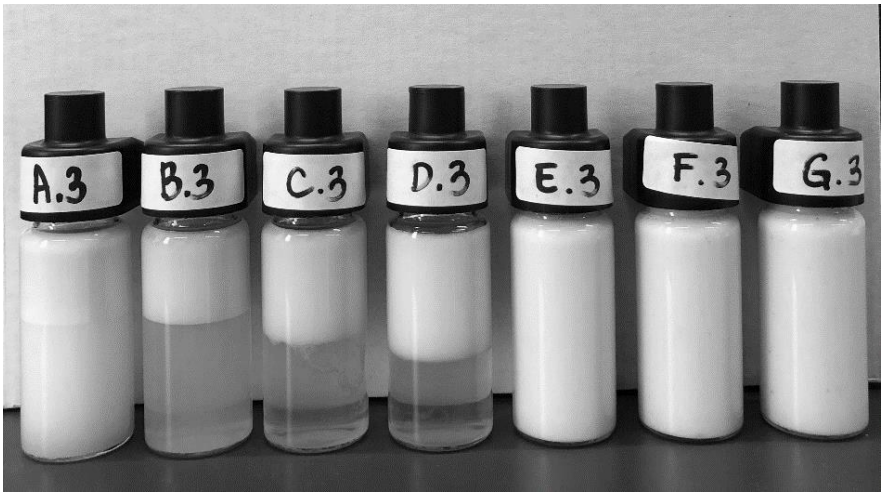

Figure 13. Appearance of the samples (A, B, C, D, E, F and G) after 30 days.

The effect of the depletion mechanism was further analyzed for samples O, P, Q, R, and S to clearly determine the threshold upon which the stabilizing effect of the hydrocolloid counterbalances the destabilizing effect. The creaming rate for these samples was calculated using the *Peak Thickness* function and the results were analyzed by means of a one-way ANOVA. As can be seen from ***Figure 14***, the creaming rate of sample A is statistically lower than the creaming rates for samples O, P, B, Q, and R (up to 40% of c*). In other words, the destabilizing effect as a consequence of depletion results in a net attractive force which leads to the interaction of the oil droplets and the consequent flocculation. For a concentration of 60% of c* (Sample S), the creaming rate is significantly lower than A. In this sense, the stabilizing effect of the hydrocolloid, due to the increased viscosity, seems to counteract the depleting forces, resulting in a more stable emulsion. Therefore, the threshold concentration for which the re-stabilizing effect of the hydrocolloid counterbalances the depletion mechanism is said to be around 0.6 of the c*.

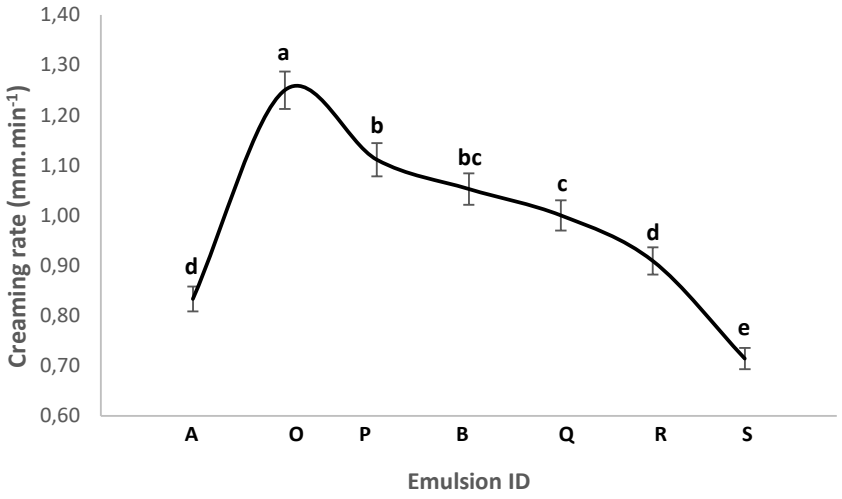

Figure 14. Creaming rate for the emulsions (A, O P, B, Q, R, and S). The data is presented as mean values of duplicate measurements. The difference between the duplicates was no more than 5%. Different superscripts indicate significantly different values ($p < 0.05$; Tukey's test).

In any case, only samples E, F, and G appear as stable from a kinetic perspective. This means that when using this hydrocolloid in a product formulation, the recommended range of application should be in the range of the critical overlap concentration (or higher), as lower concentrations fail to stabilize the emulsions over a long period of time. These results prove the importance of an accurate determination of the c*, contributing to a deeper understanding of the role of β-glucans in a formulation, leading to more successful product applications *(Burkus, 2003).*

4. Conclusion

Successful product formulation requires a deep understanding of the interaction between the different ingredients within a food matrix. Food emulsions are complex by nature, and the characteristics of their components render the emulsions unstable from a thermodynamic perspective. An appropriate selection of an emulsifier and a thickening/stabilizing agent that can provide the emulsion with long-term kinetic stability is thus essential.

In this regard, different food emulsions containing a rapeseed oil fraction of 25%, 5% of soybean lecithin as an emulsifier, and a pH 6.2 buffer solution containing acetic acid, lactic acid and sodium hydroxide have been analyzed. Varying concentrations of *PromOat®* have been tested in order to determine the most appropriate range of concentrations to be used in a food matrix with these characteristics. It is worth noting that the addition of an emulsifier is a necessary step since the contaminant proteins in *PromOat®* have little-to-no surface activity and poor solubility, these aspects being crucial parameters when determining the effectiveness of an emulsifier.

The adequate range of concentrations to apply *PromOat®* in food emulsions has been calculated based on understanding the rheological behavior of this hydrocolloid in solution. The critical overlap concentration, that is, the concentration at which the polymer chains being to overlap generating a significant increase in viscosity, was calculated as 0.0309 g/mL. In line with this, only those samples with a concentration of c* or higher had a negligible creaming rate, in the order of 0.002 $mm.min^{-1}$, appearing as stable throughout the duration of the experiment. For those samples with a *PromOat®* concentration below the c* (0.6 and lower), the destabilizing forces as a result of the depletion mechanism dominate over the stabilizing effect of an increased viscosity provided by the introduction of the hydrocolloid.

In conclusion, *PromOat®* appears as an alternative to other more traditional hydrocolloids used in the food industry. The critical overlap concentration of the powder is 0.0309 g/mL (3.09% m/m) and the application of this hydrocolloid in food emulsions containing rapeseed oil and soybean lecithin at a pH of 6.2 imparts kinetic stability for over 30 days, when the range of application is above or equal to the c*.

5. Further considerations

Food emulsions are intricate systems and the interaction of the different components defines the underlying stabilizing/destabilizing mechanisms which act upon them. The present study applies primarily to an emulsion of the specific characteristics herein described, so the results of the study should be interpreted in a prudent manner.

In this sense, a wider array of emulsions should be tested in order to assess whether the mechanisms and theories that have been discussed can also be of relevance in a broader field of products. For instance, different oil fractions could be examined as a way to control for the hydrodynamic effect that is generated when the presence of oil droplets hinders the movement of migrating droplets in their vicinity. Another pertinent aspect could be the evaluation of oat β-glucan powders with varying molecular weights. As previously described, the ability of β-glucans to form gels is linked to the molecular weight of the polysaccharide, so it would be interesting to understand whether the formation of a gel has an impact on the stability of an emulsion or not.

In any case, this study provides a systematic protocol which can be extrapolated to other food emulsions containing oat β-glucans, and this serves as the basis for future studies within the field, contributing to the development of more successful product applications with this ingredient.

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Appendix A

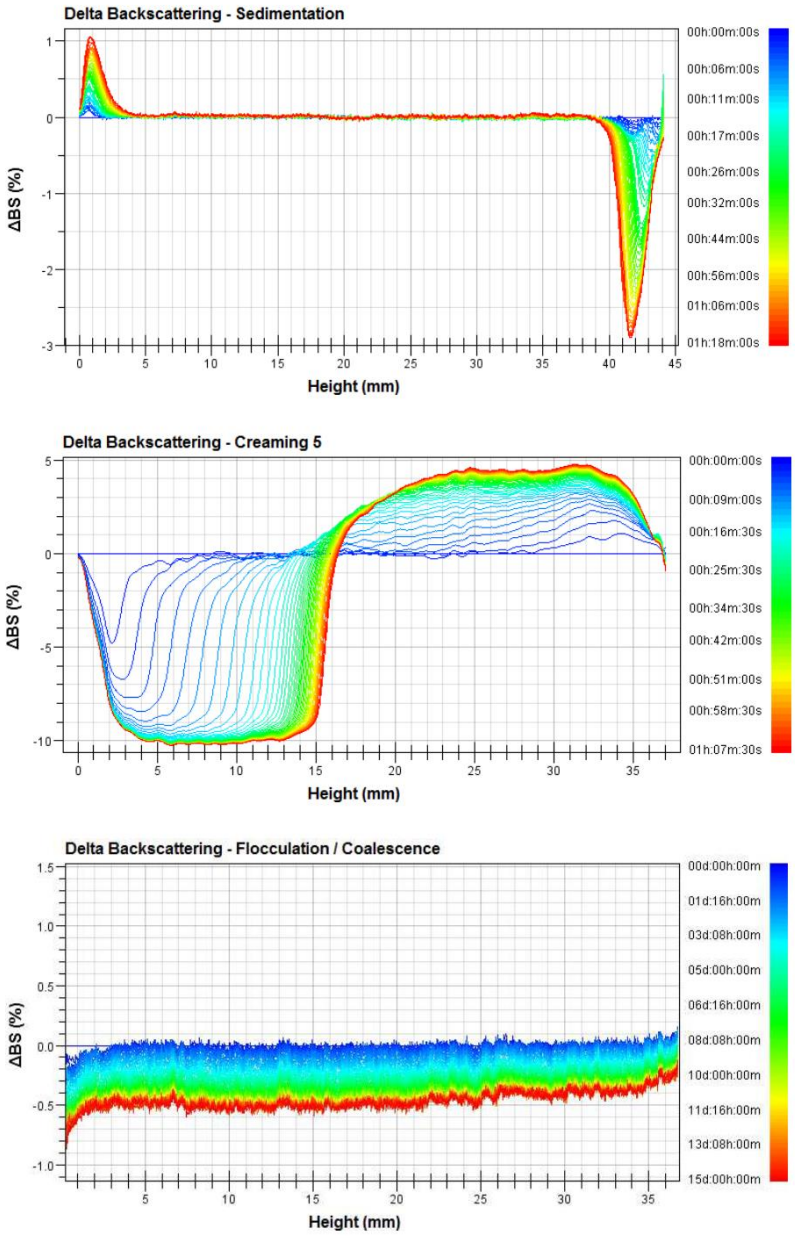

Figure 15. Sedimentation, Creaming and Flocculation/Coalescence phenomena as depicted in the **Turbiscan® LAB Analyzer**. Adapted from *(Turbiscan Lab, 2020).*

Appendix B

Table 10. One-way ANOVA results using Tukey's test as the post-hoc analysis for samples A to G.

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	B	-.6133*	.02143	.000	-.6982	-.5284
	C	-.2526*	.02143	.000	-.3375	-.1676
	D	.4520*	.02143	.000	.3671	.5369
	E	.8263*	.02143	.000	.7414	.9113
	F	.8266*	.02143	.000	.7417	.9116
	G	.8268*	.02143	.000	.7419	.9117
B	A	.6133*	.02143	.000	.5284	.6982
	C	.3607*	.02143	.000	.2758	.4457
	D	1.0653*	.02143	.000	.9804	1.1502
	E	1.4396*	.02143	.000	1.3547	1.5246
	F	1.4399*	.02143	.000	1.3550	1.5249
	G	1.4401*	.02143	.000	1.3552	1.5250
C	A	.2526*	.02143	.000	.1676	.3375
	B	-.3607*	.02143	.000	-.4457	-.2758
	D	.7046*	.02143	.000	.6196	.7895
	E	1.0789*	.02143	.000	.9940	1.1638
	F	1.0792*	.02143	.000	.9943	1.1641
	G	1.0794*	.02143	.000	.9944	1.1643
D	A	-.4520*	.02143	.000	-.5369	-.3671
	B	-1.0653*	.02143	.000	-1.1502	-.9804
	C	-.7046*	.02143	.000	-.7895	-.6196

	E	.3743*	.02143	.000	.2894	.4593
	F	.3746*	.02143	.000	.2897	.4596
	G	.3748*	.02143	.000	.2899	.4597
E	A	-.8263*	.02143	.000	-.9113	-.7414
	B	-1.4396*	.02143	.000	-1.5246	-1.3547
	C	-1.0789*	.02143	.000	-1.1638	-.9940
	D	-.3743*	.02143	.000	-.4593	-.2894
	F	.0003	.02143	1.000	-.0846	.0852
	G	.0005	.02143	1.000	-.0845	.0854
F	A	-.8266*	.02143	.000	-.9116	-.7417
	B	-1.4399*	.02143	.000	-1.5249	-1.3550
	C	-1.0792*	.02143	.000	-1.1641	-.9943
	D	-.3746*	.02143	.000	-.4596	-.2897
	E	-.0003	.02143	1.000	-.0852	.0846
	G	.0002	.02143	1.000	-.0848	.0851
G	A	-.8268*	.02143	.000	-.9117	-.7419
	B	-1.4401*	.02143	.000	-1.5250	-1.3552
	C	-1.0794*	.02143	.000	-1.1643	-.9944
	D	-.3748*	.02143	.000	-.4597	-.2899
	E	-.0005	.02143	1.000	-.0854	.0845
	F	-.0002	.02143	1.000	-.0851	.0848

		Subset				
	N	1	2	3	4	5
G	2	.0022				
F	2	.0024				
E	2	.0027				
D	2		.3770			
A	2			.8290		
C	2				1.0816	
B	2					1.4423
Sig.		1.000	1.000	1.000	1.000	1.000

Appendix C

Table 11. One-way ANOVA results using Tukey's test as the post-hoc analysis for samples A to S

		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	B	-.4148*	.07865	.002	-.7077	-.1220
	C	-.1451	.07865	.776	-.4379	.1478
	D	.4471*	.09632	.008	.0884	.8058
	E	.8214*	.09632	.000	.4627	1.1801
	F	.8217*	.09632	.000	.4630	1.1804
	G	.8219*	.09632	.000	.4632	1.1806
	O	-.4072*	.09632	.018	-.7659	-.0485
	P	-.2698	.09632	.257	-.6285	.0889
	Q	-.1609	.09632	.860	-.5196	.1978
	R	-.0718	.09632	1.000	-.4305	.2869
	S	.0660	.09632	1.000	-.2927	.4247
B	A	.4148*	.07865	.002	.1220	.7077
	C	.2698	.07865	.087	-.0231	.5626
	D	.8619*	.09632	.000	.5032	1.2206
	E	1.2363*	.09632	.000	.8776	1.5949
	F	1.2365*	.09632	.000	.8779	1.5952
	G	1.2367*	.09632	.000	.8780	1.5954
	O	.0077	.09632	1.000	-.3510	.3663
	P	.1450	.09632	.921	-.2137	.5037
	Q	.2539	.09632	.329	-.1048	.6126
	R	.3430	.09632	.068	-.0157	.7017

	S	.4808*	.09632	.004	.1221	.8395
C	A	.1451	.07865	.776	-.1478	.4379
	B	-.2698	.07865	.087	-.5626	.0231
	D	.5921*	.09632	.000	.2335	.9508
	E	.9665*	.09632	.000	.6078	1.3252
	F	.9668*	.09632	.000	.6081	1.3255
	G	.9670*	.09632	.000	.6083	1.3256
	O	-.2621	.09632	.290	-.6208	.0966
	P	-.1247	.09632	.969	-.4834	.2339
	Q	-.0159	.09632	1.000	-.3745	.3428
	R	.0732	.09632	1.000	-.2855	.4319
	S	.2111	.09632	.574	-.1476	.5698
D	A	-.4471*	.09632	.008	-.8058	-.0884
	B	-.8619*	.09632	.000	-1.2206	-.5032
	C	-.5921*	.09632	.000	-.9508	-.2335
	E	.3743	.11122	.098	-.0398	.7885
	F	.3746	.11122	.098	-.0395	.7888
	G	.3748	.11122	.097	-.0394	.7890
	O	-.8542*	.11122	.000	-1.2684	-.4401
	P	-.7169*	.11122	.000	-1.1311	-.3027
	Q	-.6080*	.11122	.001	-1.0222	-.1938
	R	-.5189*	.11122	.008	-.9331	-.1047
	S	-.3811	.11122	.088	-.7953	.0331
E	A	-.8214*	.09632	.000	-1.1801	-.4627
	B	-1.2363*	.09632	.000	-1.5949	-.8776

	C	-.9665*	.09632	.000	-1.3252	-.6078
	D	-.3743	.11122	.098	-.7885	.0398
	F	.0003	.11122	1.000	-.4139	.4145
	G	.0005	.11122	1.000	-.4137	.4146
	O	-1.2286*	.11122	.000	-1.6428	-.8144
	P	-1.0912*	.11122	.000	-1.5054	-.6771
	Q	-.9823*	.11122	.000	-1.3965	-.5682
	R	-.8933*	.11122	.000	-1.3074	-.4791
	S	-.7554*	.11122	.000	-1.1696	-.3412
F	A	-.8217*	.09632	.000	-1.1804	-.4630
	B	-1.2365*	.09632	.000	-1.5952	-.8779
	C	-.9668*	.09632	.000	-1.3255	-.6081
	D	-.3746	.11122	.098	-.7888	.0395
	E	-.0003	.11122	1.000	-.4145	.4139
	G	.0002	.11122	1.000	-.4140	.4143
	O	-1.2289*	.11122	.000	-1.6431	-.8147
	P	-1.0915*	.11122	.000	-1.5057	-.6773
	Q	-.9826*	.11122	.000	-1.3968	-.5685
	R	-.8935*	.11122	.000	-1.3077	-.4794
	S	-.7557*	.11122	.000	-1.1699	-.3415
G	A	-.8219*	.09632	.000	-1.1806	-.4632
	B	-1.2367*	.09632	.000	-1.5954	-.8780
	C	-.9670*	.09632	.000	-1.3256	-.6083
	D	-.3748	.11122	.097	-.7890	.0394
	E	-.0005	.11122	1.000	-.4146	.4137

	F	-.0002	.11122	1.000	-.4143	.4140
	O	-1.2291*	.11122	.000	-1.6432	-.8149
	P	-1.0917*	.11122	.000	-1.5059	-.6775
	Q	-.9828*	.11122	.000	-1.3970	-.5686
	R	-.8937*	.11122	.000	-1.3079	-.4795
	S	-.7559*	.11122	.000	-1.1701	-.3417
O	A	.4072*	.09632	.018	.0485	.7659
	B	-.0077	.09632	1.000	-.3663	.3510
	C	.2621	.09632	.290	-.0966	.6208
	D	.8543*	.11122	.000	.4401	1.2684
	E	1.2286*	.11122	.000	.8144	1.6428
	F	1.2289*	.11122	.000	.8147	1.6431
	G	1.2291*	.11122	.000	.8149	1.6432
	P	.1374	.11122	.978	-.2768	.5515
	Q	.2462	.11122	.560	-.1679	.6604
	R	.3353	.11122	.182	-.0788	.7495
	S	.4732*	.11122	.017	.0590	.8874
P	A	.2698	.09632	.257	-.0889	.6285
	B	-.1450	.09632	.921	-.5037	.2137
	C	.1247	.09632	.969	-.2339	.4834
	D	.7169*	.11122	.000	.3027	1.1311
	E	1.0912*	.11122	.000	.6771	1.5054
	F	1.0915*	.11122	.000	.6773	1.5057
	G	1.0917*	.11122	.000	.6775	1.5059
	O	-.1374	.11122	.978	-.5515	.2768

	Q	.1089	.11122	.996	-.3053	.5231
	R	.1980	.11122	.809	-.2162	.6122
	S	.3358	.11122	.180	-.0784	.7500
Q	A	.1609	.09632	.860	-.1978	.5196
	B	-.2539	.09632	.329	-.6126	.1048
	C	.0159	.09632	1.000	-.3428	.3745
	D	.6080*	.11122	.001	.1938	1.0222
	E	.9823*	.11122	.000	.5682	1.3965
	F	.9826*	.11122	.000	.5685	1.3968
	G	.9828*	.11122	.000	.5686	1.3970
	O	-.2462	.11122	.560	-.6604	.1679
	P	-.1089	.11122	.996	-.5231	.3053
	R	.0891	.11122	.999	-.3251	.5033
	S	.2269	.11122	.665	-.1873	.6411
R	A	.0718	.09632	1.000	-.2869	.4305
	B	-.3430	.09632	.068	-.7017	.0157
	C	-.0732	.09632	1.000	-.4319	.2855
	D	.5189*	.11122	.008	.1047	.9331
	E	.8933*	.11122	.000	.4791	1.3074
	F	.8935*	.11122	.000	.4794	1.3077
	G	.8937*	.11122	.000	.4795	1.3079
	O	-.3353	.11122	.182	-.7495	.0788
	P	-.1980	.11122	.809	-.6122	.2162
	Q	-.0891	.11122	.999	-.5033	.3251
	S	.1378	.11122	.977	-.2763	.5520

S	A	-.0660	.09632	1.000	-.4247	.2927
	B	-.4808*	.09632	.004	-.8395	-.1221
	C	-.2111	.09632	.574	-.5698	.1476
	D	.3811	.11122	.088	-.0331	.7953
	E	.7554*	.11122	.000	.3412	1.1696
	F	.7557*	.11122	.000	.3415	1.1699
	G	.7559*	.11122	.000	.3417	1.1701
	O	-.4732*	.11122	.017	-.8874	-.0590
	P	-.3358	.11122	.180	-.7500	.0784
	Q	-.2269	.11122	.665	-.6411	.1873
	R	-.1378	.11122	.977	-.5520	.2763

		Subset				
VAR00012	N	1	2	3	4	5
S	2	.7581				
A	2	.8192				
R	2		.8959			
Q	2			.9850		
B	2			1.0355	1.0355	
P	2				1.0939	
O	2					1.2313
Sig.		.174	.065	.324	.205	1.000