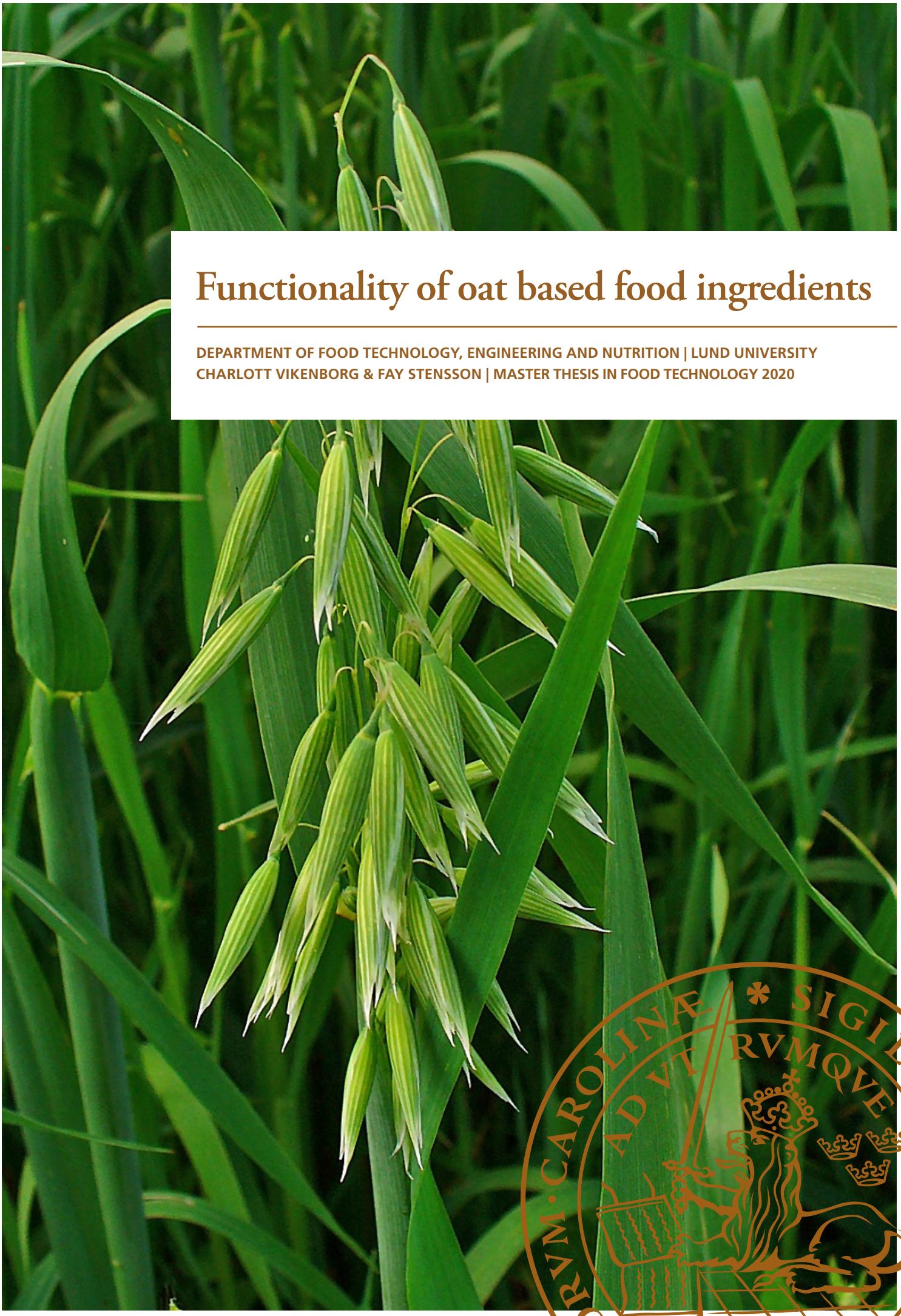

Functionality of oat based food ingredients

DEPARTMENT OF FOOD TECHNOLOGY, ENGINEERING AND NUTRITION | LUND UNIVERSITY
CHARLOTT VIKENBORG & FAY STENSSON | MASTER THESIS IN FOOD TECHNOLOGY 2020

Master Thesis in Food Technology

Functionality of oat based food ingredients

by

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Department of Food Technology, Engineering and Nutrition, in cooperation with Lantmännen

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Abstract

This master thesis work was performed in collaboration with the agricultural cooperative Lantmännen. With the ambition to take a world leading role in the development of unique products based on oats, Lantmännen has developed two oat based powders; one protein powder, PrOatein™, and one β-glucan powder, PromOat®. The aim of this master thesis project was to investigate the functional properties of the products and evaluate their potential use as ingredients in food formulations. Based on this, potential food product prototypes using PrOatein™ and PromOat® as ingredients were developed.

The analysis of PrOatein™ was aimed to investigate the protein solubility and how it was affected by changes in pH and ionic strength, and the combination of the two. It was found that solubility showed no or little change in a pH range of 3.14-6.42. Ionic strengths in the range of 7.63-1030 mmol/L had effect on solubility at pH 3.15, 4.80 and 5.24 but not at pH 6.42 and 4.32. The overall solubility is rather low, ≈5% of the total protein. High pressure homogenisation as a pretreatment was shown to somewhat increase the solubility, decrease particle size, increase sedimentation time and increase the total volume of the sediment.

The analysis of PromOat® was aimed to investigate the rheological properties and how it was affected by changes in pH and ionic strength, as well as at what concentration a yield stress was present. PromOat® showed Newtonian flow behavior at a concentration of 1% w/v and shear thinning behavior at concentrations of 3, 6 and 9% w/v. The flow behavior was unaffected in a pH range of 3.14-6.42 and at ionic strengths in the range of 6.83-1010 mmol/L. Yield stress was seen above a PromOat® concentration of 7.4% w/v. Overall, PromOat® serves well to be used as a viscosity modifier in food products.

The part of the project devoted to product development aimed to develop prototypes of high protein (≥20 energy%) and high fiber (≥3 g fiber/100 kcal) products. High concentration of PrOatein™ gave a tendency of astringency to the mouthfeel of the formulation while PromOat® gave a sensation of filmyness. However, a combination of the two seemed to counteract the astringency and filmyness that the ingredients contributed with separately. It was concluded that the combination of PrOatein™ and PromOat® make good ingredients for the formulation of a smoothie and a pudding with suitable viscosity and a pleasant mouthfeel. The developed food products contained sufficient amounts of PromOat® and PrOatein™ to be claimed as high protein and high fiber products.

Sammanfattning

Detta examensarbete utfördes i samarbete med lantbrukskooperativet Lantmännen. Lantmännen har ambitionen att ta en världsledande roll i utvecklingen av unika produkter baserade på havre och har till följd av det utvecklat två havrebaserade pulver; ett proteinpulver, PrOatein™, och ett β-glukanpulver, PromOat®. Syftet med detta examensarbete var att undersöka produkternas funktionella egenskaper och utvärdera deras potential till att användas som ingredienser i livsmedelsformuleringar. Baserat på detta, utvecklades även två prototyper av livsmedelsprodukter där PrOatein™ och PromOat® är de huvudsakliga ingredienserna.

Analysen av PrOatein™ innebar främst att undersöka proteinlösligheten och hur den påverkades av förändringar i pH och jonstyrka, samt kombinationen av de två. Resultaten visade att lösligheten i ett pH-intervall mellan 3.14-6.42 påverkades inget eller lite. Jonstyrkan varierades i intervallet 7.63-1030 mmol/L och påverkade proteinlösligheten vid pH 3.15, 4.80 och 5.24 men inte vid pH 6.42 och 4.32. Proteinlösligheten var överlag låg, ≈5% av den totala mängden protein. Högtryckshomogenisering som förbehandling visade sig att öka proteinlösligheten något men minskade även partikelstorleken, ökade sedimentationstiden och ökade sedimentets totala volym.

Analysen av PromOat® innebar främst att undersöka de reologiska egenskaperna hos pulvret och hur det påverkades av förändringar i pH och jonstyrka, liksom vid vilken koncentration systemet visar en skjuvspänning. PromOat® system vid 1% vikt/volym hade Newtonskt flödesbeteende och PromOat® system vid 3, 6 och 9% vikt/volym var skjuvförtunnande. Flödesbeteendet påverkades inte i ett pH-intervall mellan 3.14-6.42 eller vid en jonstyrka i området 6.83-1010 mmol/L. Skjuvspänning erhölls vid en PromOat®-koncentration av ≥7.4% vikt/volym. Generellt, anses PromOat® vara effektiv för att användas som viskositetsreglerare i livsmedelsprodukter.

I den del av projektet som ägnades åt produktutveckling låg fokus på att utveckla två prototypsprodukter av högt protein- (≥20 energi%) och högt fiberinnehåll (≥3 g fiber/100 kcal). Hög koncentration av PrOatein™ gav en tendens till en sandig munkänsla medan PromOat® gav en känsla av filmighet vid högre koncentrationer. En kombination av de två verkade motverka dessa oönskade upplevelserna som de två ingredienserna bidrog med separat. Slutsatsen var att kombinationen av PrOatein™ och PromOat® visade sig vara fördelaktiga för formulering av en smoothie och en pudding som erhöll en lämplig viskositet och en behaglig munkänsla. De utvecklade livsmedelsprodukterna innehöll tillräckliga mängder PromOat® och PrOatein™ för att kunna anses vara produkter med högt protein- och hög fiberinnehåll.

Popular Science summary - Potential of oat based food ingredients

Consumers seek more healthy and sustainable diets and more and more people are adapting to a more plant based diet. This has awakened a huge interest for food companies to produce more plant based products and the market is exploding with new, innovative products to meet this need. But, what is the actual potential of plant based ingredients and how can they be used in food applications to live up to the increasing demand of more sustainable food products?

The fact that people want to reduce their meat consumption is clear. 2018 the meat consumption in Sweden was reduced by 2 kg per person and year, for the second year in a row. However, changing to plant based raw materials comes with many challenges. Vegetable proteins do not have the same nutritional quality as proteins of animal origin, and therefore a person with a vegetarian diet needs to be more aware of food sources to attain adequate amounts of essential amino acids. Also, using plant based raw material in food applications comes with processing difficulties for the industry to live up to a desired texture, taste and nutritional value.

Lantmännen produces a range of oat ingredients out of Nordic oats. PromOat® beta-glucan is a soluble oat bran fibre which can provide important health and functional benefits as well as it can be used as a thickening agent to provide stability and a creamy mouthfeel. Proatein™ oat protein contains > 50% protein and has high levels of essential amino acids as well as having a neutral taste. Oats have been harvested in Sweden for over 2000 years and have great water tolerance and require lower summer heats compared to other cereals, making it perfectly suitable to grow in the Swedish climate. Oats are generally considered healthy and nutritious as they are full of fibers and other vitamins and minerals.

The functionality of these two ingredients from Lantmännen, PromOat® and PrOatein™, and their potential use as food ingredients have been investigated in this project. Despite a low protein solubility, PrOatein™ can enrich products with protein of high nutritional quality. It appears rather stable and changes in pH and ionic strange has little effect on functional properties, such as solubility and water holding capacity. By homogenising the powder in liquid, protein particles become six times smaller. This significantly decreases the sedimentation rate, thus increases the stability and has desired outcome to the sensory experience, giving a smooth mouthfeel. PromOat® has the ability to stabilise systems and can serve as a thickening agent due to the capability to increase the viscosity. Furthermore, it adds to the nutritional value due to the high fibre and β-glucan content. The two powders in combination, increases the gelling capacity and seem to counteract some less desired properties of the two powders individually, such as sandiness from PrOatein™ and filmyness from PromOat®. The knowledge of the functionality of the powders was used to develop two prototype products using the two powders. This resulted in one berry and cardamom smoothie and one mocha pudding. The products contain >20 energy % from protein and >3 g fibre per 100 kcal and can therefore be claimed as both high protein and high fibre products.

To conclude, PromOat® and PrOatein™ have high potential to be used in food applications and can help to meet the fast-growing demand of nutritious plant based food products.

Preface

This report is a result of a Master Thesis at the Institution of Food and Nutrition at the Faculty of Engineering at Lunds University, in collaboration with Lantmännen. The project covers 30 credits and took part from January-June 2020.

A special thank you to our supervisor Björn Bergenståhl at Lunds University for his involvement and commitment in supporting us through the project and guiding us to make the correct decisions. Also, thank you to our supervisors at Lantmännen, Mats Larsson and Emma Nordell, for supplying us with the powders and for encouraging us through the project. We would also like to express our gratitude to the CropTailor team of the department of Applied Biotechnology at Lunds University, especially Lars Sjögren, for support, knowledge and supply of equipment and chemicals for the BCA-analysis, as well as the enthusiasm for us to deliver good results. A thank you to Magdalena Bergh for being a further support from Lantmännen and to Marie Wahlgren for your role as the examinator for the project. Lastly, thank you Anna-Malin Hallberg and Ronja Wennerström for the useful comments and interesting questions during the opposition.

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1. Introduction

1.1 Background

Lantmännen is an agricultural cooperative owned by 20 000 Swedish farmers, with grain as the heart of their operations. Lantmännen is part of the Industrial Research Centre ScanOats which formed in 2017 and was given 100 million SEK from the Swedish Foundation for Strategic Research, SSF, to do research about oats. Their ambition is to take a world leading role in the development of unique products and applications based on oats. As a result of this, Lantmännen has developed two oat based powders; one protein powder, PrOatein™ and one β-glucan powder, PromOat®, produced at their site in Kimstad. Currently, Lantmännen only sells the powders to customers and are not using the powders as an ingredient in any of their products. This is of high interest as there is a large demand of new innovative plant based products.

1.2 Project task and purpose

The task of the project includes analysing the functionality of the PrOatein™ and PromOat® powders and their possible use as food ingredients with the purpose to provide Lantmännen with useful information of future applications of the powder.

The project expects to answer the following questions:

- What powder property parameters have the major effect on the functionality of the two powders?
- How important is the effect of their behavior in food applications?
- What are the possibilities for the powders to be used as food ingredients?

The following four phases summarises how the project was performed:

- Literature study of the major parameters that are of importance for proteins and polysaccharides in food applications.
- Screening of the key properties of the two powders and their behavior and functionality.
- Systematic experimental analysis of the two powders and data interpretation.
- Applying the knowledge of the powder functionalities to develop prototypes of food products.

1.3 The scope of the project

The functionality of food ingredients and influencing factors is wide and complex. Some of the functional properties that are considered to have the major importance for the powders and their use as food ingredients have been analysed in more detail. Due to time limitation, other properties have been analysed more briefly. The food product development part provides two prototypes of food products and does not include all steps to achieve a final product that is ready for the market. It shall instead be used as a base for future work and progression with the ingredients. The effect of the processing on the raw material in the powders is not thoroughly analysed, thus the starting point for the functional analysis are the powders themselves.

1.4 Disposition of the report

The report is divided into four major parts. The first part is the theoretical background describing general facts about oats, the major functional properties that are of importance for proteins and polysaccharides as well as how these aspects correlate with food manufacturing and properties of foods. The second part describes the experimental methods used for the analysis as well as courses of actions used for data interpretation. The third part presents the results of the analysis, discusses the findings and correlates the results to the functional properties as

food ingredients. It also includes the results of the procedure and progression of the food product development. The last part summarises all the findings in a conclusion. Beyond these four major parts, the report also includes an Appendix section which includes the raw data from the analysis and calculations.

2. Theoretical background

2.1 Oat products from Lantmännen

Lantmännen is responsible for the production of a range of oat ingredients from high quality non-GMO Nordic oats. In this project, the ingredients analysed are PrOatein™[1] and PromOat®[2] Betaglucan. The production of the powders takes place in Kimstad, Sweden, and is performed according to the flow chart in *Figure 2.1* (Larsson, 2020). The manufacturing process is performed without the need of chemicals and solvents. The powders do not inherently contain gluten but gluten can be present in the powders due to cross-contamination in the fields where crops containing gluten are cultivated, or due to cross-contamination during transportation. Other than gluten, the powders do not contain any allergens complying with regulations where the product is manufactured and sold. The powders shall be stored in a clean and dry environment, away from odorous material, at max 25°C. The shelf life of PrOatein is 365 days and PromOat 548 days (Lantmännen Oats AB, 2020g).

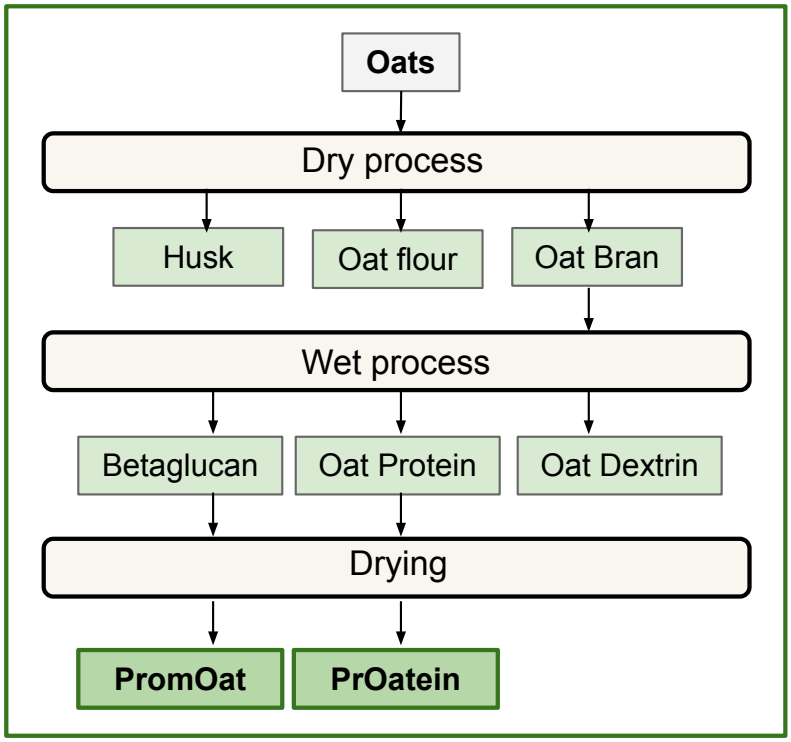


Figure 2.1. Processing scheme for the production of PrOatein and PromOat.

2.1.1 PrOatein™ Oat Protein

PrOatein is a natural protein concentrate powder prepared from oat bran. It is beige in colour and has a neutral oat aroma. The nutritional information can be seen in *Table 2.1* (Lantmännen Oats AB, 2020f). PrOatein has a minimum protein content of 50% (Lantmännen Oats AB, 2020d) and the batch used for the analysis in this project, had a protein content of 54.6% and a dry matter of 95.9% (Lantmännen Oats AB. 2020b).

PrOatein is vegan friendly and can serve as an alternative to other protein sources and increase the protein content in a wide range of products. There is a fast-growing demand for protein-enriched, nutritious food products and supplements and PrOatein can help manufacturers to meet these demands. Lantmännen states some functional benefits of PrOatein; the powder is fine and free-flowing, does not stick and has good wettability. It is also easy to use and handle (Lantmännen Oats AB, 2020d).

[1]PrOatein™ is a preceding trademark by Lantmännen and this applies to all times "PrOatein" is mentioned in the report.
[2]PromOat® is a trademark by Lantmännen and this applies to all times "PromOat" is mentioned in the report.

Table 2.1. Nutritional information PrOatein.

Nutritional information	Nutrients per 100 g
Energy	1770 kJ
Calories	423 kcal
Total fat Saturated fat Monounsaturated fat Polyunsaturated fat	13 g 2.6 g 4.2 g 5.6 g
Carbohydrate Sugars	21 g 0.7 g
Fiber	4.6 g
Protein	53 g
Salt	0.11 g
Iron	5.8 mg
Calcium	174 mg
Potassium	327 mg

2.1.2 PromOat® Betaglucan

PromOat is a natural soluble dietary fiber powder made from the oat grain, rich in (1-3, 1-4) β-glucans with an average molecular weight of 1 200 000 Dalton (Bergh, 2020). The powder appears creamy in colour, has a neutral oat aroma and is stated soluble in water. The nutritional information can be seen in *Table 2.2* (Lantmännen Oats AB, 2020g). The β-glucan content is 32% or greater (Lantmännen Oats AB, 2020e) and 34.6% in the batch used for the analysis in this project. The moisture content was 3.9% (Lantmännen Oats AB, 2020c).

Table 2.2. Nutritional information PromOat.

Nutritional information	Nutrients per 100 g
Energy	1351 kJ
Calories	323 kcal
Total fat Saturated fat	6.5 g 1.2 g
Carbohydrate Sugars	42.5 g 2.0 g
Fiber β-glucan	40 g 34 g
Protein	3.5 g
Salt	0.17 g
Calcium	9.5 mg
Potassium	765 mg

β-glucans are known for several health and functional benefits and by the addition of PromOat into food and beverages, it can be possible for manufacturers to claim specific health benefits of the product. Lantmännen states some functional benefits of PromOat, such as clean taste, neutral colour and no graininess, high solubility and its easiness to use and handle. Due to moisture management, it can improve the shelf life of products and it can also be used as a stabiliser and viscosity modifier or as a fat substitute due to the fat-mimicking properties (Lantmännen Oats AB, 2020e).

2.2 Oats

Oats, *Avena sativa*, is a commonly grown cereal in the temperate parts of the world. It is mainly cultivated for its use as animal feed but it is also a commonly used crop for human consumption. The plants, which can grow to 1.5 m in height, have good ability to grow under poor conditions, such as in low nutrient- or acidic soils (Oats | grain, 2020). Compared to other cereals, oats are high in lipids and proteins. It normally has a high lipase activity, which

can be a problem in combination with the high lipid content. If not denatured, the lipases can cause lipid oxidation in milled oat products leading to a limitation in shelf life (Rahim, 2013). The oat grain consists of the germ, endosperm and bran, covered by an outer hull layer with hair like outgrowths, as seen in *Figure 2.2*. The major part of the grain is the endosperm and the outer bran layer protects the endosperm and the germ (Beloshapka, Buff, Fahey and Swanson, 2016).

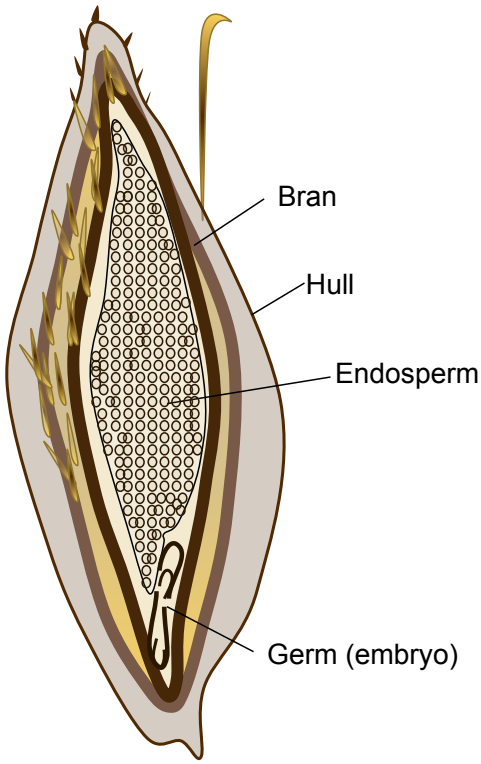


Figure 2.2. Cross section of the oat grain structure. Figure is modified from (Cereal processing - Nonwheat cereals, 2020)

2.2.1 Oat protein

Oats consist of 15-20 wt.% protein. The proteins can be divided into fractions that are classified according to their solubility; albumins (9-20 wt.%), globulins (70-80 wt.%), prolamins (4-14 wt.%) and the glutenins covering the remaining part (Runyon, Nilsson, Alftrén and Bergenståhl, 2013). The albumins are water soluble and their solubility have not shown to decrease in water solutions with low salt concentrations. They are however more affected by exposure to heat leading to coagulation of the proteins. The globulins are soluble in low concentrated salt solutions and insoluble in high concentrated salt solutions (described by the salting in and salting out phenomena) but not soluble in pure water solutions. The glutenins are soluble in solutions with low concentrations of an acid or a base, and the proteins in the prolamin fractions are soluble in water solutions with an ethanol concentration of 70% (Rahim, 2013). The oat prolamins are commonly called avenins. The avenins have shown lack of certain immunogenic epitopes that are present in the prolamins in other cereals like wheat, barley and rye. These epitopes can provoke celiac disease (CD) in people that are genetically susceptible to the disease. Thanks to this, the oat itself can be considered safe for people suffering from CD. However, there is still a risk that the oats can be cross-contaminated by other gluten containing cereals during cultivation, harvesting and later steps along the production chain (Gilissen, van der Meer and Smulders, 2016).

In most other cereals the most common storage proteins are the prolamins, but unlike other cereals the major storage protein in oats are the globulins which also take up the largest fraction of the total protein. This is an important contributor to the high nutritional quality of the oat proteins. Protein quality is defined both by the digestibility of the protein but also by the concentration of the essential amino acids that the body cannot synthesise itself (Hoffman and Falvo, 2004). The globulins have shown to have a balanced amino acid profile and a higher

concentration of the essential amino acid lysine compared to the prolamins, which makes it beneficial from a nutritional point of view. However, the lysine content is still below the FAO standards (4.2 g/100 g protein compared to the recommendation 4.5 g/100 g protein) (Arendt and Zannini, 2013). The amino acid profile for total oat, the oat bran and for PrOatein can be seen in *Table 2.3.*

*Table 2.3. Amino acid profile of total oat, oat bran and for PrOatein. * = essential amino acids.*

Amino acid	**Total oat [g/100 g]** (Oats, 2020)	**Oat bran [g/100 g]** (Oat bran raw, 2020)	**PrOatein [g/100 g powder]** (Lantmännen Oats AB. 2020a)	**PrOatein [g/100 g protein]** (Lantmännen Oats AB. 2020a)
Tryptophan*	0.234	0.335	0.7	1.3
Threonine*	0.575	0.502	1.4	2.6
Isoleucine*	0.694	0.668	1.9	3.5
Leucine*	1.28	1.37	3.7	6.7
Lysine*	0.701	0.760	1.6	2.9
Methionine*	0.312	0.335	0.9	1.6
Cysteine	0.408	0.576	1.0	1.8
Phenylalanine*	0.895	0.908	2.7	4.9
Tyrosine	0.573	0.668	1.9	3.5
Valine*	0.937	0.964	2.5	4.6
Arginine	1.19	1.28	3.4	6.2
Histidine*	0.405	0.410	1.1	2.0
Alanine	0.881	0.872	1.8	3.3
Aspartic acid	1.45	1.58	3.5	6.4
Glutamic acid	3.71	3.75	10.4	19
Glycine	0.841	0.947	1.7	3.1
Proline	0.934	0.982	2.4	4.4
Serine	0.750	0.890	1.8	3.3

2.2.2 β-glucans

β-glucans are non-starch polysaccharides and are a part of the total dietary fiber. The general β-glucan content in oats is 2.5-6.6%, mostly located in the bran. Monomers linked with a mix of β-(1→3) and β-(1→4) linkages build up the structure of the β-glucans, which is illustrated in *Figure 2.3* (Phillips and Williams, 2014, pp. 600-638).

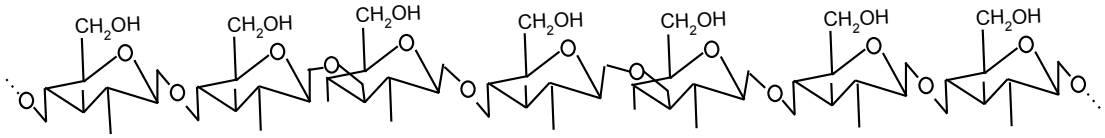


Figure 2.3. General structure of β-glucans, where the frequency of β-(1→3) and β-(1→4) linkages of the polymers vary. Figure is modified from (Phillips and Williams, 2014, pp. 600-638).

The molecular weight of oat β-glucans varies between 65 000-3 000 000 g/mol. The solubility of β-glucans is highly influenced by the molecular weight and it has been shown that β-glucans <200 000 g/mol are insoluble. An increase in the molecular weight seems to have a strong positive linear relationship with apparent viscosity and mechanical stability. In highly concentrated acid solutions at low pH, hydrolysis of β-glucans can occur. As the majority of food systems have a pH >2, the stability of β-glucans is usually not an issue in food processing (Phillips and Williams, 2014, pp.600-638).

β-glucans, together with other hydrocolloids like guar gum, alginates, inulin and pectin, have several mechanisms of action on the metabolic effect in the body. They have the capability to form gels and to increase the viscosity in solutions. In the body this can by others slow down gastric emptying, increase nutrient absorption and lower the postprandial glucose response, LDL cholesterol synthesis and fat absorption. These actions can have a positive effect in glycemic control of type 2 diabetes, cardiovascular disease prevention and weight management. It has

been shown that by consuming 6 g of β-glucans from oat/day for over six weeks, the total and LDL cholesterol will be lowered, having a hypercholesteolemic effect (Phillips and Williams, 2014, pp.600-638). Consumption of ≥3 g of oat β-glucans per day is supported by both the European Society of Cardiology and the US National Cholesterol Education Program to lower serum cholesterol and blood glucose levels (Oat beta glucans, 2020).

Cereal β-glucans are used in several foods and serve well for industrial colloid applications, for example as thickening and gelling agents in soups or as fat replacers in fat free products. β-glucans compare well with guar- and locust bean gum (Phillips and Williams, 2014, pp.600-638).

2.3 Protein solubility

Protein solubility usually ranges between 0-35% for globular proteins and can be seen as the concentration at which the solution has reached its saturation concentration. The structure of the protein, temperature and the properties of the surrounding solution are factors that have an impact on the solubility. For globular proteins, the electrostatic interactions present at the surface are of great concern for the solubility in water. The solubility increases with the amount of hydrophilic groups on the surface, and is poorer with a higher amount of hydrophobic groups (Walstra, 2001, Chapter 7 - Proteins).

2.3.1 Protein structure

The backbone of the amino acid sequence makes up the primary structure of a protein. There are 20 different amino acids and all have the same core structure of an amine- and a carboxylic acid group. The side group of the amino acid varies and defines the hydrophobicity, charge and chemical activity. The various molecular properties of the primary structure folds into α-helixes, β-strands, β-sheets or random coil, building up a secondary structure. The tertiary structure is the overall three dimensional structure of the polypeptide which the secondary structures form when they interact, primarily due to non-covalent interactions of the different side groups. The tertiary structure of proteins in aqueous solution will orient itself in a way that hydrophobic groups cluster inside the structure and the hydrophilic groups will be exposed to the surrounding water. Proteins with multiple polypeptide chains further conform into a quaternary structure, where individual polypeptide chains form a protein complex. (Wilde, 2014, pp.5-8) (Walstra, 2001, Chapter 7 - Proteins).

2.3.2 Denaturation of proteins

Denaturation arises when a protein loses its tertiary structure and the unique shape of the protein is disrupted. If the primary structure is still intact, the denatured protein can refold into its functional form, but may also stay denatured. Denaturation most likely decreases the solubility of the protein but also makes it non-functional in other ways, including loss of biological activity, increased hydrodynamic size and susceptibility for attack by proteolytic enzymes. Refolding is prevented by aggregation of unfolded molecules, conformation change from cis to trans, reshuffling of sulphur bridges, deamination or crosslinking (Walstra, 2001, Chapter 7 - Proteins).

The rate of heating and cooling is important to consider in food applications as this can cause denaturation of the proteins. Extreme pH, solvent quality, exposure to chemicals, high pressure and shear stress are also things that can cause denaturation (Walstra, 2001, Chapter 7 - Proteins).

2.3.3 Impact of ionic strength

The concentration of ions in a solution can be denoted as the ionic strength [mol/L] and can have an impact on the solubility of proteins if they are charged (Arnaut, Formosinho and Burrows, 2007, pp. 223-250). At dilute ion concentrations, an increase in ionic strength can result in a phenomena called salting in, which facilitates the solubility of the proteins. This occurs as a result of electrostatic interactions between proteins and is mainly applicable on globular proteins that can be seen as large ions. The ion activity at saturation, a, can be used as an expression of solubility and should be constant at any temperature. As can be seen in *Equation 2.1*, the ion activity is proportional to the solubility concentration, c, with a proportionality constant, γ, known as the activity constant.

$$a = c \cdot \gamma \qquad \text{Equation 2.1}$$

Since a is constant, a decrease in γwould lead to an increase in c, hence an increase in solubility. An expression for γcan be seen in *Equation 2.2*.

$$\gamma_{\pm} \approx exp[\frac{-0.37z^2\kappa}{1+\kappa R}] \qquad \text{Equation 2.2}$$

R denotes the radius for the globular protein [nm], z is the valence and is the Debye-parameter. The equation is valid for room tempered water, and not as applicable at high valances or ionic strengths. The Debye-parameter is dependent on the ionic strength, I, according to *Equation 2.3*:

$$\kappa \approx 3.2\sqrt{I} \qquad \text{Equation 2.3}$$

From this, it can be seen that by increasing the ionic strength, I, the activity coefficient, γ, will decrease leading to an increase in solubility. This is the phenomena known as salting in. However, the ionic strength (i.e. the concentration of ions) needs to be low enough to not disturb interactions between the water molecules and the hydrophilic groups on the protein (Walstra, 2001, Chapter 7 - Proteins).

At high ionic strengths, the opposite can occur, a phenomena called salting out, that will decrease the solubility of the proteins and may also lead to precipitation. The charges on the protein surface attract the counter ions from the solution, and the surface together with the counter ions form something called a diffuse double layer. This double layer shields the charges and decreases the repulsive forces between the proteins as the opposite charges cannot "sense" each other. At high salt concentrations this double layer gets more compact which allows the proteins to get closer without getting repelled from each other. If they get close enough, attractive hydrophobic interactions will take over and lead to aggregation and precipitation of the protein aggregates (Walstra, 2001, Chapter 6 - Polymers). Salting out is commonly used for extraction of protein from a solution (Ciborowski and Silberring, 2016, pp. 51-62).

2.3.4 Isoelectric point

Proteins contain many different amino acids, resulting in a wide range of pKa values, thus the charge of the protein can vary a lot over pH ranges in foods. At the isoelectric point (IP) of the protein, there will be as many positive as negative charges on the protein surface, resulting in a net charge of zero. This will lead to a decrease in the repulsive forces and the attractive forces between the molecules will take over. This attraction can result in aggregation of protein and cause a decreased solubility close to the IP. When the pH is altered, the charge distribution over the molecule and the electrostatic interactions between the proteins will change. Far away from the IP, the protein is either more negatively or positively charged leading to repulsive forces acting between the proteins. The relationship between IP and solubility of the proteins can also be explained by looking at *Equation 2.1-2.2* above, describing the relationship between solubility concentration, c, and the activity constant, γ. From *Equation 2.2* it can be seen that γdecreases for larger valances, z, which is what happens to proteins at pH far away from their IP. As mentioned above, a decrease in γleads to an increase in c, hence a higher solubility (Walstra, 2001, Chapter 6 - Polymers).

2.4 Water holding capacity of protein

The water content in food varies greatly and the ability of the protein matrix to absorb and retain bound, hydrodynamic, capillary and physically entrapped water against gravity is defined as the water holding capacity, WHC. The values of the WHC are used to describe the amount of embedded water in the polymer network, which will affect the swelling of the dry mass and proteins usually have a WHC between 2-7 g water/g protein. Polar groups and (to a lesser extent) dipoles can bind water molecules (Walstra, 2001, Chapter 8 - Water Relations),

implying that proteins with a lot of charged amino acids usually bind large amounts of water (Phillips and Williams, 2014, p. 404). The WHC is of importance for the quality and formulation of food products as it can have an impact on the texture, colour and moisture related sensory attributes, such as juiciness, of a product (Zayas, 1997, pp.76-77). Amino acid composition, molecular mass, protein conformation, ionic strength, composition and inhomogeneity of the dry matter, temperature and water content are parameters that will affect the WHC. The WHC and the protein matrix stability is also affected by non-protein compounds, such as fibers and phenolic compounds, as well as the genetic variety of the crop and the degree of protein denaturation (Phillips and Williams, 2014, p.406).

2.5 Rheological functionality of polysaccharides

2.5.1 Rheological behavior

Rheology describes the relation between force, deformation and time and is important for the sensory attributes of a food, how to design and dimension a production plant and to further investigate the potential in ingredient functionality by correlating the relation between molecular structure, consistency and shelf life. The deformation of matter depends on the tension, compression and shear applied. The shear stress, σ [Pa], is the force divided by the area in which the force is applied and the shear rate, γ [s^{-1}], is the difference in the distance of the deformation of the material in which the force has created, also described as the velocity gradient, illustrated in *Figure 2.4*. The viscosity, μ [Pa · s] is the shear stress divided by the shear rate: σ/γ, also called the proportionality constant. A high viscosity is the result of particles disturbing the applied flow field by bumping into each other, the viscosity therefore increases with increased volume fraction of the dispersed phase (Pashley and Karaman, 2004). Elasticity refers to the tendency of a system to recover its shape and dimensions when an applied stress is removed.

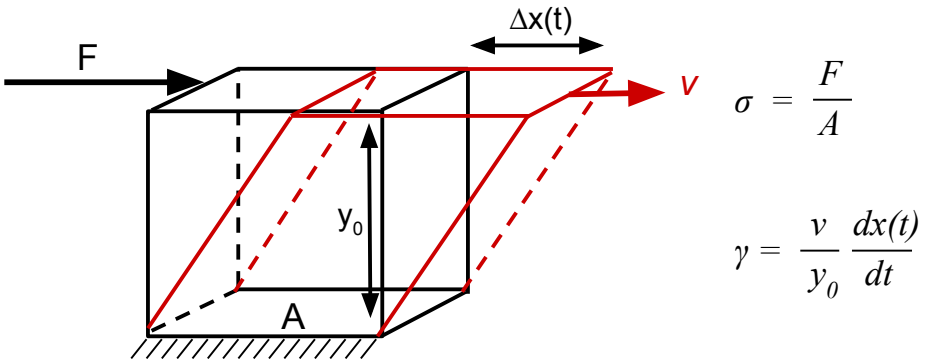


Figure 2.4. The deformation of matter when a force is applied. Figure is modified from (Innings, 2019).

A fluid can be Newtonian, shear thinning or shear thickening and its behavior can be fitted to the power law, see *Equation 2.4,* where n is a constant. A Newtonian liquid has a viscosity which is equal to the friction between the molecules and is independent of the shear rate. A non-Newtonian behavior arises from disturbances in the flow pattern caused by sample components and the fluid either increases in viscosity with increasing shear rate (shear thickening, $n>1$) or decreases in viscosity with increasing shear rate (shear thinning, $0<n<1$) (Innings, 2019). Some systems appear solid up to a certain shear stress before they begin to flow. This value is called the yield stress [Pa] and is a good measure of the firmness of a system (Walstra, 2001, Chapter 17 - Soft Solids). In *Figure 2.5,* the different flow behaviors are illustrated.

$$\sigma = \eta \cdot \gamma^n \qquad \textit{Equation 2.4}$$

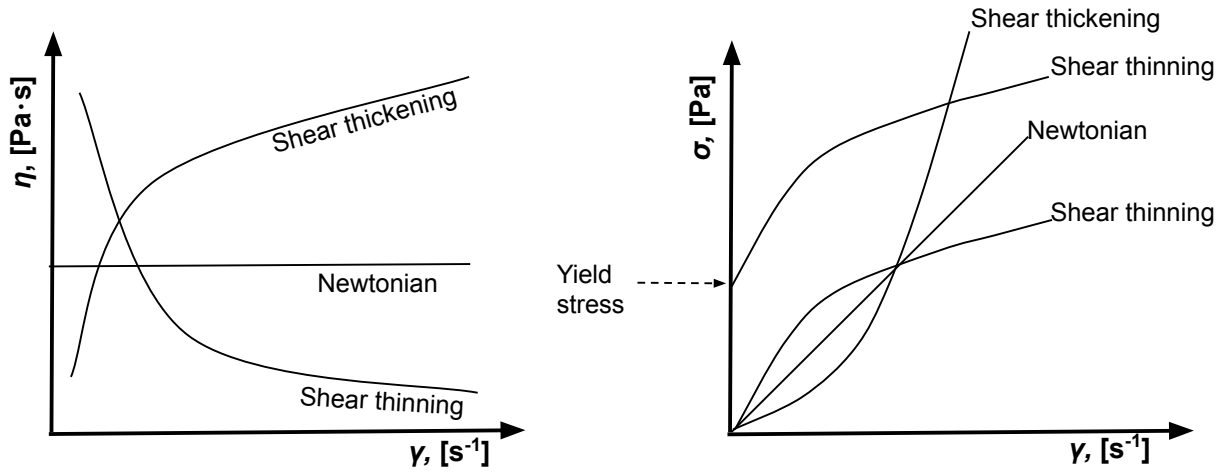


Figure 2.5. Flow behavior for Newtonian and non-Newtonian fluids.

2.5.2 Gels and gel formation

A gel consists mainly of a solvent that shows a solid character provided by a space filling network. A gel shall show no syneresis (leakage of liquid) or shall not fracture. For the motion of particles to be prevented, the gel must hold a significant yield stress and for the motion of the solvent to be prevented it is necessary that the viscosity of the continuous liquid is high. Linear polymers forming gels are crosslinked with various types of junctions; stacked double helixes, partly stacked triple helices or egg-box junctions. The particle material determines the strength of the junctions and they usually contain about 30% of the polymer material. There are no covalent bonds present between the molecules, it is van der Waals attraction, hydrophobic interactions and hydrogen bonding that give the gel formation. For charged polymers, ionic bonds can also be involved (Walstra, 2001, Chapter 17 - Soft Solids).

A gel can be strong or weak and the side chains of the polysaccharide is important for the gel formation. The volume occupied by a polymer in a solution and the extent of steric hindrance depends on chain stiffness, side group interactions and state of expansion as well as branching of the molecule, polydispersity and presence of heteropolymers. Bulky side groups enhance stiffness, which gives a large change in enthalpy when they entangle (Walstra, 2001, Chapter 6 - Polymers) (Walstra, 2001, Chapter 17 - Soft Solids).

The entanglement, or gel formation, depends on shear rate. When exposed to a shear rate, the entanglement stretches and deforms into a new conformation (Pashley and Karaman, 2004). The new orientational alignment depends on the magnitude of the shear rate as well as the rotary diffusion time. Firstly, the shear rate will cause the molecules and particles to rotate in the shear flow. At low shear rates, no alignment of the polysaccharide to the flow will occur and the viscosity will remain high. It can also be explained by the fact that the entanglements that are loosened, are as many as the ones that are formed. If a yield stress is present, new bonds form at the same rate as bonds are broken, and thus there will be no deformation and the system will not flow. An increase in shear rate causes the particles to align in the orientation of the flow to cause less flow disturbance, which makes the viscosity lower as the time allowed for entanglements to form is very short. When the polymers become fully entangled, the viscosity will become very low. The change from high to low viscosity occurs at lower shear rates for polysaccharides with larger molar mass, greater stiffness of the polymer molecule and at a better solvent quality (Walstra, 2001, Chapter 6 - Polymers). For food applications, it is desired for a gel to be subsequently deformed in the mouth to allow swallowing (Walstra, 2001, Chapter 17 - Soft Solids). As polysaccharides are not precisely spherical, the entanglement will stretch when exposed to a shear rate and deform into a new conformation, thus these systems usually show shear thinning behavior (Pashley and Karaman, 2004).

2.5.2.1 Chain overlap

In very dilute solutions, the polymer will not sense the presence of other polymers and is only affected by the solution. In concentrated polymer solutions, there are mutual interactions between polymers in the solution and at a certain concentration, *c**, the chains can overlap and entangle. At *c** the viscosity of a solution increases sharply, see *Figure 2.6,* and this depends on the volume occupied by the polymer in the solution. The larger the volume occupied, the lower *c** and the viscosity increase is stronger at higher concentrations as there is a larger amount of entanglements per unit volume (Goodwin, 2009). Galactomannans, e.g. guar gum, has a *c** concentration at 0.25 g/100 mL at an ionic strength of 0.1 mol/L, and shows high similarity with β-glucans (Walstra, 2001, Chapter 6 - Polymers).

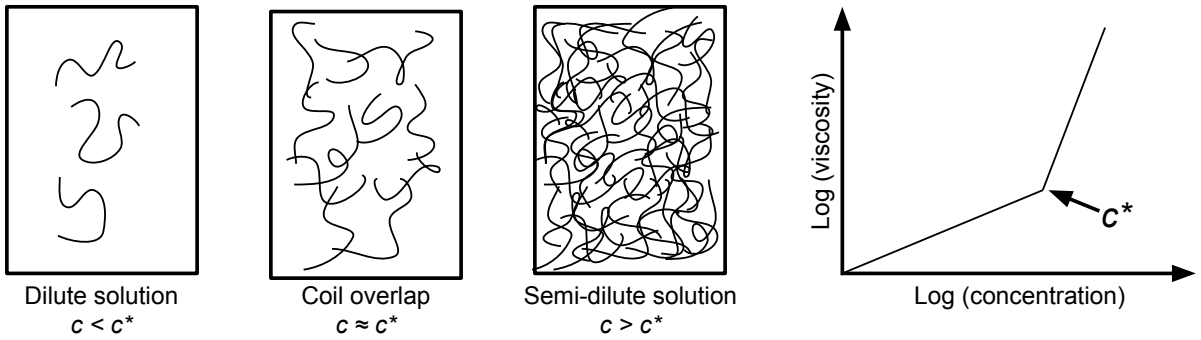


Figure 2.6. An illustrative picture of the entanglement at different concentration and the increase in viscosity at c. Modified from (Rayner, Östbring and Purhagen, 2015).*

2.5.3 Ionic strength and pH dependence

The conformation of a polysaccharide in an aqueous solution is affected by the electrical charges, depending on the amount of charged groups on the molecules, the pH and the ionic strength. Like charges repel, making the molecules more expanded in the solution than for a neutral polymer. This implies that the electrical charge of the molecule will affect the conformation and viscosity. With increasing ionic strength, the sense of repulsion between similar charges of the charged polysaccharides are screened and the expansion of the molecule in the solution will decrease, lowering the viscosity. At very high ionic strengths, the charged polymer is similar to a neutral one (Walstra, 2001, Chapter 6 - Polymers). High concentration of divalent ions however, might have the opposite effect as they can cause bridging between the polysaccharides and increase gelation (Walstra, 2001, Chapter 6 - Polymers).

2.6 Sedimentation

Stokes law, *Equation 2.6,* describes the sedimentation of particles in a gravitational field. v_{Stokes} is the sedimentation rate of the particles, d is the diameter of the particles, g is the gravitational force =9.81 [m/s2], $\Delta\rho$ is the density difference between the phases and η is the viscosity.

$$v_{Stokes} = d^2 g \Delta\rho / 18\eta \qquad \textit{Equation 2.6}$$

As the sedimentation rate is proportional to the square of the particle size, a decrease in particle size will have a large effect in slowing down sedimentation. Stokes law assumes spherical particles and that the settling of particles is not influenced by the presence of other particles. At particle concentrations above a few % however, the influence by other particles will slow down the sedimentation rate and $v_{Stokes} > v_{Sedimentation}$. The phenomenon is called 'hindered settling' and is of great importance in food applications. At a dispersed phase concentration at 40%, the settling rate is almost zero (Bergenståhl, 1995). The volume fraction of the dispersed phase has a huge influence on sedimentation (Nilsson, 2019).

2.7 Food application of polymers

2.7.1 Protein

Proteins are commonly used in food products for its nutritional properties but also to form and stabilise emulsions, foams, suspension and to form gels (Walstra, 2001, Chapter 7 - Proteins). The functionality will vary depending on what interactions that take place between the protein and the water and it is of major importance to have good knowledge of the preparation and processing of proteins to be able to use them in food applications (Phillips and Williams, 2014, pp. 600-638). To allow a product to have the claim that it is high protein, the protein in the product shall provide > 20% of the energy value (20 E%) (Nutrition claims - Food Safety - European Commission, 2020).

Proteins are generally not suitable to be used as gelling agents as a high protein concentration is needed to achieve a gel with a high viscosity. Despite this, they are commonly used for this purpose. Gel formation proceeds when the protein unfolds to an open conformation and functional groups are exposed to the environment where they can interact with each other. At low concentrations, the proteins may precipitate but at higher concentrations, network formation takes place in the system and a gel can be formed (Phillips and Williams, 2014, pp. 600-638). The denatured proteins can aggregate to form particles and then further aggregate into a space filling network. The two mechanisms usually happen simultaneously and can take minutes to hours. Considering protein mixtures, the gel formation is very complex as the different proteins will react in different ways (Walstra, 2001, Chapter 17 - Soft Solids).

2.7.2 Polysaccharides

The solubility, ability to form a gel and state of expansion varies widely for polysaccharides. To be able to increase the viscosity, a polysaccharide needs to be adequately soluble in the solution. The concentration of the polysaccharide will affect the apparent viscosity which affects the consistency (Walstra, 2001, Chapter 6 - Polymers). Non-digestible carbohydrates are capable of forming viscous solutions or gels and are commonly used as thickening or gelling agents or to stabilise dispersions by slowing down aggregation and sedimentation (Phillips and Williams, 2014, pp.600-638). As charged polysaccharides are more expanded in the solution, they are effective thickening agents (Walstra, 2001, Chapter 6 - Polymers).

Low molecular β-glucans have a shorter gelation time compared to high molecular β-glucans. Generally, β-glucans from oats have more junction zones which allows a faster gelation rate (Phillips and Williams, 2014, pp. 600-638).

2.7.3 Polymer mixtures

Systems of proteins and polysaccharides in combination can be used to obtain a desired texture or as fat replacers. In complex formation with polysaccharides, the protein solubility has been shown to be improved, especially close to the isoelectric point of the protein (Phillips and Williams, 2014, pp. 600-638) .If two polymers that by themselves lack the ability to form gels are mixed, it is still possible for weak gels to form due to weak interactions between the two. Closely packed systems can appear as fluid gels (e.g. yoghurt) containing concentrated gel particles, or pastes (e.g. peanut butter and quark) containing finely dispersed material that have a yield stress but can still deform (Walstra, 2001, Chapter 17 - Soft Solids).

2.7.3.1 Depletion flocculation

Depletion flocculation is an instability mechanism that can occur if a polymer is introduced into a dispersion, and induces flocculation of the dispersed particles. This mechanism can be explained by the behavior of a polymer close to, or at a particle surface, which depends on its affinity to the surface. This affinity can be quantified with the use of the surface interaction parameter, χ_S, that gives the net interaction between the solution, polymer and the surface. If this interaction parameter is below the critical value $\chi_{S,\,crit}$, the polymer will not adsorb to the present surface but move from the interface to the bulk solutions. This will create a so-called “depletion zone” that will have a lower concentration of the polymers compared to in the bulk solution. The depletion zones of different

particles in the colloidal dispersion can overlap and if the distance between these particles is smaller than the diameter of the polymer, it can induce aggregation of the particles (Jenkins and Snowden, 1996).

2.7.4 Sensory evaluation

The perceived texture when eating a food can be described by the mouthfeel. However, the experienced sensation is very complex and also depends on interactions of nerve impulses coming from what you see, smell and touch. It highly depends on the individual and the conditions when eating, as well as intensity and duration of the flavor and the temperature of the food in the mouth. For example, some foods are solid at room temperature, and then melt in the mouth. When evaluating a sensory perception, the conditions in the mouth should be attempted to be mimicked. The deformation in the mouth is dominated by elongation, especially for viscoelastic material. The strain rate in the mouth during biting and chewing is usually 2 s^{-1}. It should also be noticed that saliva gives a lubricating effect, as well as diluting the food. Inhomogeneity in the food, e.g. if a pudding contains fruit pieces, is also something that affects the perception (Walstra, 2001, Chapter 17 - Soft Solids).

The particle size does not only influence the sedimentation rate, but also sensory properties like sandyness. Particle sizes as small as 25 μm can be detected by the palate (Engelen and van der Bildt, 2008).

2.8 Evaluation of the theoretical background for the experimental plan

For the use of protein- and dietary fiber powders in food applications the functional properties of the ingredients needs to be taken into consideration.

A high protein solubility is crucial for the stability and mouthfeel of aqueous products and is dependent on the surface properties of the protein and its interactions with the surrounding water. This is affected by changes in ionic strength and pH and these two parameters might be altered within suitable ranges for food products to investigate if the protein solubility is higher at any setting. Homogenisation is a common process used in the food industry to create homogeneous and more stable systems. It has the potential to break protein aggregates and decrease the size of the protein particles. The effect of homogenisation is therefore interesting to study as it may have an effect on the solubility and the sedimentation of particles according to Stokes law. The relation between proteins and water can also be analysed by the WHC which can be of importance for texture, colour and moisture of a product.

β-glucans have proven to have several mechanisms of actions in the body that can be favorable for health. They have the capability to form gels and can therefore be used as stabilisers or fat replacers. Based on this, it is of interest to investigate the rheological properties of PromOat.

There is a demand for more plant based products on the market, both from sustainability and health aspects. However, using plant based raw material in food applications comes with processing difficulties for the industry to be able to live up to a desired texture, taste and nutritional value. PromOat and PrOatein have high nutritional quality and might therefore be used as food ingredients and result in competitive food products. A combination of the two might affect the gel formation of the system and result in a favorable texture.

These conclusions of the theoretical background are used as motivation for the choice of analysis performed on the powders in this master thesis project.

3. Methods

3.1 PrOatein

3.1.1 Preparation of PrOatein dispersions

3.1.1.1 Aim

PrOatein dispersions were prepared in order to obtain homogeneous dispersions of PrOatein powder for further analysis.

3.1.1.2 Method

PrOatein dispersions at different powder concentrations were prepared according to *Table 3.1.* Water was added to the dried powder and mixed with a spoon until the total volume was obtained. Depending on the total volume needed, the amounts were adjusted, keeping the same ratio of powder and water.

Table 3.1. Mixing ratios for different % w/v PrOatein.

PrOatein conc. [% w/v]	PrOatein powder [g]	Total volume [mL]
1	1	100
3	3	100
5	5	100
6	6	100
9	9	100
10	10	100

3.1.2 Homogenisation

3.1.2.1 Aim

In an attempt to increase protein solubility of the powder and stability of the dispersions, PrOatein dispersions were homogenised to break possible aggregates in the powder and create smaller particles.

3.1.2.2 Theoretical explanation

High pressure homogenisation is a mechanical process, in which a liquid product is forced through a narrow valve under intense energy release. The breakup of the particles is a result of turbulence that is formed when the liquid exits the narrow valve. As it enters, the pressure drops rapidly causing the formation of cavities. It is the combination of the forces from the collapse of the cavities, turbulence and shear that impacts the break up and decrease in particle sizes (Shechter, 2016).

3.1.2.3 Method

Samples were prepared by mixing PrOatein powder and water at the desired ratio with a hand mixer. The samples were homogenised using the high pressure homogeniser GEA Niro Soavi Panda Plus at a pressure of 100-200 bars and recirculated 7 times.

3.1.3 Buffering capacity PrOatein

3.1.3.1 Aim

Determine the buffering capacity of PrOatein, as well as preparing PrOatein dispersions at a set pH, a titration curve was produced.

3.1.3.2 Method

Non-homogenised PrOatein dispersions at 3 and 5% w/v as well as homogenised dispersions at 5% w/v (recirculated 2, 5 and 7 times) were prepared and titrated with 0.1 mol/L HCl. The dispersion was mixed using a magnetic stirrer and the pH was measured continuously. The pH change for each volume of added 0.1 mol/L HCl was noted. The amount of µmol HCl/g PrOatein to reach the desired pH was calculated.

3.1.4 BCA-method for protein solubility

3.1.4.1 Aim

The concentration of proteins in solution was measured with Bicinchoninic Acid Protein Assay (BCA) to determine the solubility at different settings of the system.

3.1.4.2 Theoretical explanation

Under alkaline conditions, proteins will form a complex with Cu^{2+}. The peptide bonds in cysteine, cysteine, tryptophan and tyrosine are able to further reduce Cu^{2+} to Cu^{1+} and the amount of protein that is present will determine the amount of reduction. Due to the purple-blue complex that Cu^{1+} forms with BCA in alkaline conditions, absorbance measurements in a plate reader at 562 nm can be performed to obtain the amount of protein. The concentration range that is applicable is 200-1000 µg/mL and it is necessary to perform an appropriate dilution to record absorbance measurements within the linear range. The working reagent consists of Bicinchoninic Acid Solution (containing bicinchoninic acid, sodium carbonate, sodium tartrate and sodium bicarbonate in 0.1 mol/L sodium hydroxide (NaOH), with a final pH 11.25) and copper reagent (Copper(II) Sulphate Pentahydrate 4% w/v). When protein is mixed with the working reagent colour development starts. In the standard procedure the reaction is performed at incubation at 37°C but can be accelerated if higher temperatures are used and the sensitivity can increase if the incubation is performed for a longer time. Contrary, lower temperatures can slow down the colour development. The protein standard is Bovine Serum Albumin (BSA) and together with blank (only working reagent and no protein) the final protein concentration of the unknown samples can be determined by comparison with the standard curve. A standard curve shall be generated for each new assay (Sigma Aldrich, 2020).

3.1.4.3 Preparation of samples

The BCA-analysis was performed on homogenised and non-homogenised PrOatein dispersions. The samples were analysed at different ionic strengths and at different pH within a suitable range for food applications, as well as on combinations of the two. The combinations were prepared in eppendorf tubes according to the volumes given in *Table 3.2*, all containing a final PrOatein concentration of 5% w/v and a total volume of 1 mL.

Table 3.2. Volumes for preparation of PrOatein samples at different pH and ionic strength for the BCA-analysis.

pH	Ionic strength [mmol/L]	PrOatein dispersions 10% w/v [µL]	0.5 mol/L NaCl [µL]	5 mol/L NaCl [µL]	0.1 mol/L HCl [µL]	H_2O [µL]
6.42	7.36	500	0.00	0.00	0.00	500
6.42	57.4	500	100	0.00	0.00	400
6.42	507	500	0.00	100	0.00	400
6.42	1010	500	0.00	200	0.00	300
5.24	12.1	500	0.00	0.00	47.0	453
5.24	62.1	500	100	0.00	47.0	353
5.24	512	500	0.00	100	47.0	353
5.24	1010	500	0.00	200	47.0	253
4.80	17.2	500	0.00	0.00	98.0	402
4.80	67.2	500	100	0.00	98.0	302
4.80	517	500	0.00	100	98.0	302
4.80	1020	500	0.00	200	98.0	202
4.32	21.2	500	0.00	0.00	138	362
4.32	71.2	500	100	0.00	138	262
4.32	521	500	0.00	100	138	262
4.32	1020	500	0.00	200	138	162
3.15	30.4	500	0.00	0.00	230	270
3.15	80.4	500	100	0.00	230	170
3.15	530	500	0.00	100	230	170
3.15	1030	500	0.00	200	230	70

3.1.4.3 Method

The samples used for the analysis were prepared according to *Section 3.1.4.3* above. Every sample was prepared and analysed in quadruplicates. After preparation, the samples were looped in an orbital mix for 2 hours to get the proteins fully solubilised in the liquid phase. After 2 hours, the samples were centrifuged at 14 500 rpm for 15 minutes in Eppendorf miniSpin Plus Centrifuge to separate insoluble particles from the solution. The supernatants were separated from the pellets into new eppendorf tubes and further diluted to fit the interval for the absorbance measurements. 25 µL of the sample was added to 200 µL BCA reagent in a 96-well microplate. The plate was incubated at 37°C for 30 minutes and was then let to cool for 15 minutes to reach room temperature. The absorbance at 562 nm was measured in BioTek Epoch2 microplate reader. The results were compared to a standard curve made with bovine serum albumin (BSA) solution. The BSA standard solutions were prepared according to *Table 3.3.*

Table 3.3. Preparation of BSA standard solutions.

Conc. BSA [µg/mL]	BSA [µL]	H_2O [µL]	Amount [µg]
0	0	300	0
200	60	240	5
400	120	180	10
600	180	120	15
800	240	60	20
1000	300	0	25

3.1.5 Water holding capacity

3.1.5.1 Aim

PrOatein was analysed to determine the water holding capacity, WHC, of the powder.

3.1.5.2 Method

9 eppendorf tubes were weighed and labelled. 6.00 mg and 9.00 mg of PrOatein was added to three tubes respectively. 1.00 mL of water was added to every tube and the tubes were looped for 1 hour to let the powder and water mix. The tubes were centrifuged at 14 500 rpm for 15 minutes in Eppendorf miniSpin Plus Centrifuge, followed by removal of as much supernatant as possible. This was repeated a second time. After centrifugation, the tubes were placed in an eppendorf heater, Grant QBD2). The samples were first heated at 70°C for 45 minutes, and the weight was recorded. The samples were further heated at 105°C for 2 hours, and the weight was recorded. The samples were left in an incubator at 37°C for 18 hours and the final weight was recorded.

3.1.6 Particle size determination

3.1.6.1 Aim

PrOatein samples were analysed in microscopy with the aim to investigate the size of the particles. Homogenised and non-homogenised PrOatein dispersions were compared to see if homogenisation affected the particle size.

3.1.6.2 Method

A droplet of homogenised and non-homogenised PrOatein dispersions at 5% w/v, was placed on a microscopy glass and was covered by a cover glass. The samples were analysed in Olympus BX50 Microscope at 20x objective and pictures were taken for further analyses of particle size.

The particle size was determined by printing large images of the microscopy pictures with an inserted scale and measuring the diameter, *d,* of the particles by hand with a ruler. The amount of particles, *n,* with approximately the same size were summarised and the total volume for each size was calculated. The volume weighted average diameter, *d*(4.3), was calculated according to *Equation 3.1*.

$$d(4.3) = \frac{\sum_i (\frac{\pi d_i^3}{6} \cdot n_i \cdot d_i)}{\sum_i (\frac{\pi d_i^3}{6} \cdot n_i)} \qquad \textit{Equation 3.1}$$

3.1.7 Sedimentation analysis

3.1.7.1 Aim

The sedimentation of PrOatein dispersions was analysed to gather knowledge about the stability of the systems.

3.1.7.2 Method

25.0 mL of homogenised and non-homogenised dispersions at 5% w/v and 10% w/v were poured into small tubes. The amount of sediment was determined by measuring the height of where the lower sediment layer was separated from the upper layer with a ruler, starting from the bottom of the tube. This was repeated over time until there was no change in the system.

3.2 PromOat

3.2.1 Preparation of PromOat dispersions

3.2.1.1 Aim

PromOat dispersions were prepared in order to obtain homogenous dispersion of PromOat powder for further analysis.

3.2.1.2 Method

PromOat dispersion at different powder concentrations at different ionic strengths and at set pH, was prepared according to *Table 3.4.* The amount of acid needed was obtained from the titration curves, see *Figure 4.1* in *Section 4.1*. The salt solutions were prepared in advance by mixing the amount of salt with water. The liquid/s was mixed with water to obtain a total volume of 100 mL and placed in the refrigerator. When refrigerator temperature was obtained, the dried powder was added in small amounts while stirring with a fork. The preparation was followed by mixing with a hand mixer for approximately 5 seconds.

Table 3.4. Mixing ratios for different % w/v PromOat at different ionic strengths and pHs.

PromOat concentration [% w/v]	pH	Ionic strength [mmol/L]	PromOat powder [g]	Mass NaCl [g]	0.1 mol/L HCl [mL]	Total volume [mL]
1	5.95	2.28	1.00	0.00	0.00	100
3	5.95	6.83	3.00	0.00	0.00	100
3	5.95	56.8	3.00	0.242	0.00	100
3	5.95	507	3.00	2.42	0.00	100
3	5.95	1010	3.00	4.84	0.00	100
3	4.95	7.89	3.00	0.00	1.05	100
3	4.03	9.22	3.00	0.00	2.40	100
3	3.01	12.5	3.00	0.00	5.70	100
6	5.95	13.7	6.00	0.00	0.00	100
9	5.95	20.4	9.00	0.00	0.00	100

3.2.2 Buffering capacity PromOat

3.2.2.1 Aim

To be able to determine the buffering capacity of PromOat, as well as preparing PromOat dispersions at a set pH, a titration curve was produced.

3.2.2.2 Method

PromOat dispersion, 2% w/v, was prepared and titrated 0.1 mol/L HCl. The dispersion was mixed using a magnetic stirrer and the pH was measured continuously. The pH change for each volume of added 0.1 mol/L HCl was noted. The amount of µmol HCl/g PromOat to reach the desired pH was calculated.

3.2.3 Rheological behavior

3.2.3.1 Aim

Flow curves for different settings of PromOat dispersions were analysed to investigate the rheological behavior of which the powder in aqueous solutions generates.

3.2.3.2 Method

PromOat dispersions were prepared and the shear rate and shear viscosity at a set shear stress interval was measured in a shear stress controlled rheometer, Malvern Kinexus Pro, at 20°C. The flow behavior was determined by rotational measurements where the shear rate and shear stress was altered to obtain the apparent viscosity. A bob and cup rotational cylinder, *Figure 3.1,* is the measuring geometry used and is appropriate if the samples are still somewhat liquid.

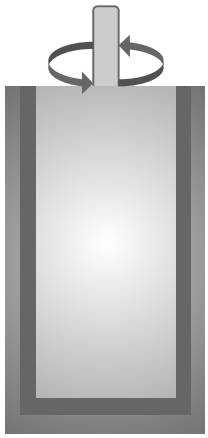


Figure 3.1. A rotational bob and cup viscometer.

The settings of the rheometer can be seen in *Table 3.5.* The shear stress interval [Pa] was set and adapted in order to obtain a shear rate interval within the range of 0-100 s^{-1} and the samples per decade was set and adapted to obtain full deformation of the matrix and therefore the most reliable results. Flow curves were obtained by plotting the shear rate against the shear viscosity.

Table 3.5. Settings for rheological measurements in Rotational Viscometer for different % w/v PromOat.

PromOat concentration [% w/v]	Shear stress start [Pa]	Shear stress end [Pa]	Samples /decade	Geometry
1	0.15 0.05	5 5	10 10	Smooth cup and smooth bob
3	1	100	10	Smooth cup and smooth bob
6	10 10	350 350	10 5	Smooth cup and smooth bob
9	5 100	500 500	5 5	Smooth cup and grooved bob

3.2.4 Yield stress determination

3.2.4.1 Aim

The yield stress was determined to see at what % w/v PromOat, the system needs a force to flow.

3.2.4.2 Method

A glass bead with a radius of 2.5 mm and a weight of 0.17 g was placed on PromOat dispersions at different % w/v to see at which concentration the glass bead remained on top of the gel. When the bead does not sink, the resulting force of the gravitational force of the glass bead and the lifting force from the gel can be converted into the yield stress at that specific concentration of PromOat.

3.3 Statistical analyses

3.3.1 Aim

Statistical analysis was performed in order to draw statistical conclusions from the results.

3.3.2 One-Way ANOVA

The one-way ANOVA was performed in Microsoft Excel (version 11929.20776) and compares the variances of the mean score within each group to the variance of the mean score between the groups. The null-hypothesis is that the mean scores in each group are all the same. The test calculates the ratio, F, between the variance of the mean score between groups and the variance of the mean score within groups. The ratio is being compared to a F_{crit}-value that depends on the degrees of freedom within or between the groups. If $F > F_{crit}$, there is a statistically significant difference between the groups mean scores at a set significant level, p, of 0.05 in this case. p is the probability that the null hypothesis is being rejected even though it is true.

There are some assumptions drawn when applying the one-way ANOVA test which can be affected if the size variations between the groups are too large. The assumptions are that the test results within the groups are normally distributed and that the groups have the same standard deviations. (McDonald, 2014)

3.3.3 Tukey-Kramer Post Hoc Test

The one-way ANOVA tells if there is a statistically significant difference between groups but not between which groups. If comparing more than two groups, a post hoc test therefore needs to be applied to get more detailed information from the test. For this, the Tukey-Kramer Post Hoc in Microsoft Excel (version 11929.20776) was used which pairwise compares the means to find between which groups there is a significant difference. From the test you calculate a q-value for each pair that is compared to a q_{crit}-value. If $q > q_{crit}$, there is a statistically significant difference between the groups mean score at a set significant level (in this case 0.05) (McDonald, 2014).

3.4 Curve fitting

3.4.1 Aim

To draw smooth curves through data points, methods for curve fittings are necessary. It is also useful when determining a mean curve from multiple results of the same trials and to find a value between a pair of data points.

3.4.2 Spline functions

Mathematically a spline function consists of small pieces of polynomial interpolation connecting the data points which are used when data interpolation and/or smoothing is necessary. x_i data points with corresponding y_i values of the function can be fitted to the splined function, S, where $S(x_i)=y(x_i)$ for i =0, 1, 2...n, where n is the amount of data points. $S(x)$ can be parameterised as seen in *Equation 3.2*, where $p(x)$ is a polynomial function of x. The polynomial used can be changed but the spline function is joined together with certain continuity and interpolatory conditions where $p(x)$ is fitted so that $S(x)$, $S'(x)$ and $S''(x)$ are continuous in the interval of x_0 - x_n (Einarsson, 1974). The curve fitting was performed in MatLab R2017a.

$$S(x) = \begin{cases} p_1(x) & x_0 < x_1 \\ \vdots & \\ p_n(x) & x_{n-1} < x < x_n \end{cases} \qquad \textit{Equation 3.2}$$

3.4.3 Creating mean curves with for loop

A for loop allows a repetition of a requested command to be executed a specific number of times. The first statement in the command is executed first, followed by the second statement and proceeds to the final number. By this method, the for loop can run over an interval of *x*-values, note and save the *y*-value for each curve at the current *x* and generate a mean value for each *x*-value of the interval.

3.5 Product development

3.5.1 Aim

To provide potential prototype food products using PromOat and PrOatein which can serve as a base for future product development.

3.5.2 Method

Homogenised PrOatein and PromOat were mixed at different ratios to investigate what structure of the system that was obtained. The methodology of the product development was thereafter continuously planned and modified based on the evaluation from the previous step to develop the most suitable and favorable product/s and this is documented as results. Sensory evaluations were performed by the authors of this report. Appropriate functional and theoretical analysis were performed on the final product/s in order to give a reasonable product description.

4. Results and discussion

4.1 Buffering capacity

Titration curves for non-homogenised PrOatein (3 and 5% w/v) as well as for 5% w/v homogenised samples (recirculated 2, 5 and 7 times) resulted in five titration curves. They all had a similar appearance and it can be concluded that homogenisation does not affect the buffering capacity. The average titration curve is seen as the black line in *Figure 4.1* (raw data can be seen in *Appendix A)*. The initial pH of PrOatein samples, without addition of HCl was pH 6.44. The pH dropped rather constantly until it reached a pH just above 2. When HCl is added, the amino- and acid groups on the protein have the ability to bind and release hydrogen ions and counteract the pH change, thus it takes some time for the pH to stabilise. When stable, the pH remains the same if nothing in the system is changed. The values of the curve were used to prepare samples of the different powders at a set pH by adding the amount of µmol HCl/g powder according to the curve. The titration curve was verified by measuring the pH of a PrOatein sample after addition of 150 µmol HCl/g powder. This should, according to the titration curve result in a pH 5.03 and the measured pH was 5.00, thus the determination of the pH according to the titration curve is considered accurate.

The titration curve from PromOat can be seen as the red line in *Figure 4.1* (raw data can be seen in *Appendix A).* The initial pH of PromOat samples, without addition of acid was pH 5.95. The pH dropped rather quickly until it reached a pH just above pH 2. The values of the curve were used to prepare samples of the different powders at a set pH by adding the amount of µmol HCl/g powder according to the curve. For the PromOat dispersions analysed, the pH was also measured externally for the exact value.

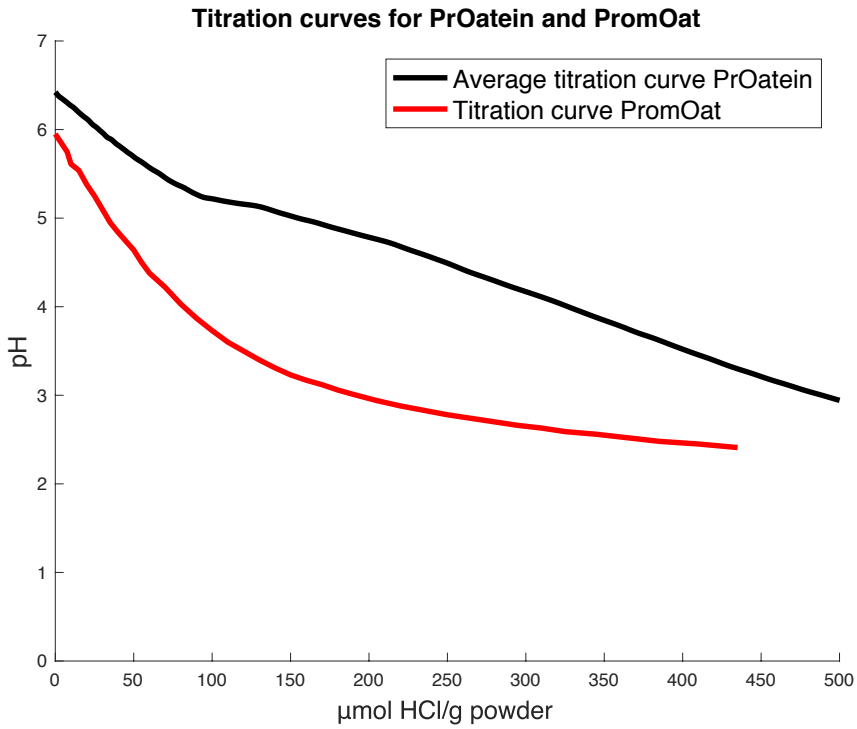


Figure 4.1. Titration curve PrOatein (black) and PromOat (red).

By comparing the titration curves of the different powders, it can be seen that to reach a pH ≈4, it was necessary to add 320 µmol HCl/g PrOatein and 90 µmol HCl/g PromOat, making the buffering capacity for PrOatein 3.5 times higher than for PromOat. Also, the slope from the initial pH to pH 3 is steeper for PromOat than PrOatein and it can be determined that the buffering capacity of PrOatein is larger than for PromOat. This is not surprising, as protein, with many charged groups, has large capacity to neutralize the added acid and PrOatein has a protein

content of 55%, compared to PromOat which only has a protein content of 3.5%, making it rather charge neutral. What is worth to notice is that there are also several other ions present that have buffering capacity.

Close to, or at the isoelectric point for a protein, the buffering capacity is assumed to be at its lowest due to the lower net charge leading to less capacity to get protonated and counteract the pH decrease from the hydrogen ions. This would result in a steeper slope of the curve around that pH. As the titration curve for PrOatein appears almost linear throughout the pH range, it is hard to detect a possible IP for the PrOatein. As PrOatein contains a wide range of different proteins which all have an individual IP, determination of the IP of the dispersion is complex as the total IP of the system would symbolise all these proteins combined.

4.2 Final ionic strength

The presence of ions in PrOatein and PromOat, and the effect of addition of NaCl and HCl, to the final ionic strength in the solutions was determined in order to evaluate the actual ionic strength of the samples analysed. The amount of ions in the PrOatein and PromOat powder was given by the product specification sheet, obtained from Lantmännen. The amounts of the cations were summarised to obtain the final ionic strength, µmol/g PrOatein and PromOat respectively, and the results can be seen in *Table 4.1*.

Table 4.1. Ionic strength determination of PrOatein and PromOat.

Ions	Molar mass ions [g/mol]	Mass cations [g/100 g PrOatein]	Mass cations [g/g PrOatein]	Ionic strength [µmol/g PrOatein]	Mass cations [g/100 g PromOat]	Mass cations [g/g PromOat]	Ionic strength [µmol/g PromOat]
K^+	39.1	0.327	0.00327	83.6	0.765	0.00765	196
Fe^{2+}	55.8	0.00580	0.0000580	1.04	0	0	0.00
Ca^{2+}	40.1	0.174	0.00174	43.4	0.00950	0.0000950	2.37
Na^+	23.0	0.0440	0.000440	19.1	0.0680	0.000680	29.6
			Final ionic strength [µmol/g PrOatein]	**147**		**Final ionic strength [µmol/g PromOat]**	**228**

The final ionic strength, calculated with the amount of cations, of the prepared samples of PrOatein 5% w/v and PromOat at different % w/v can be seen in *Table 4.2* and *Table 4.3* respectively. The choice of the final ionic strengths were determined by a reasonable salt concentration interval of food products and the same reasoning applies to the choice of the pH interval. For the analyses performed, HCl 0.1 mol/L was chosen to alter the pH according to the titration curve for PrOatein and PromOat in *Figure 4.1.* The amount of cations is approximated to represent the total ionic strength, as there is the same amount of counteracting anions to each cation respectively. This applies as long as the amount of divalent ions are not the majority. There are some divalent ions present in the powders but the ionic strength contribution from the powders is low compared to the amount of added NaCl, thus their contribution to an increased ionic strength is considered negligible.

According to *Table 4.2-4.3*, a lowering of the pH by addition of HCl, will increase the ionic strength. An increase of powder concentration will also increase the final ionic strength, thus the % w/v of the powders for the majority of the analysis was kept the same to avoid differences. The addition of HCl as well as the cation contribution of the powders to the final ionic strength can be seen as almost negligible for some settings (higher ionic strengths at lower pH), but contributes more for others. As the final ionic strength is close to, but not equal to the molarity obtained from just the added NaCl, the final ionic strength according to the tables above, are the ones used in the results. It shall be noted that no sample can be prepared without any ionic strength, due to the neutral contribution to the ionic strength from the powders.

Table 4.2. Final ionic strength of PrOatein dispersions 5% w/v at different NaCl-concentrations and pH.

pH	Conc. NaCl solution [mmol/L]	Cation contribution from HCl [mmol/L]	Cation contribution from PrOatein [mmol/L]	Final ionic strength in solution [mmol/L]
6.42	**0**	0	7.36	7.36
	50	0	7.36	57.4
	500	0	7.36	507
	1000	0	7.36	1010
5.24	**0**	4.70	7.36	12.1
	50	4.70	7.36	62.1
	500	4.70	7.36	512
	1000	4.70	7.36	1010
4.80	**0**	9.80	7.36	17.2
	50	9.80	7.36	67.2
	500	9.80	7.36	517
	1000	9.80	7.36	1020
4.32	**0**	13.8	7.36	21.2
	50	13.8	7.36	71.2
	500	13.8	7.36	521
	1000	13.8	7.36	1020
3.15	**0**	23.0	7.36	30.4
	50	23.0	7.36	80.4
	500	23.0	7.36	530
	1000	23.0	7.36	1030

Table 4.3. Final ionic strength of PromOat dispersions 1, 3, 6 and 9% w/v at different NaCl-concentrations and pH.

pH	Conc. NaCl solution [mmol/L]	Cation contribution from HCl [mmol/L]	PromOat conc. [% w/v]	Cation contribution from PromOat [mmol/L]	Final ionic strength in solution [mmol/L]
5.95	**0**	0	1	2.28	2.28
	0	0	3	6.83	6.83
	0	0	6	13.7	13.7
	0	0	9	20.5	20.5
	50	0	3	6.83	56.8
	500	0	3	6.83	507
	1000	0	3	6.83	1010
4.95	**0**	1.05	3	6.83	7.88
4.03	**0**	2.40	3	6.83	9.22
3.01	**0**	5.70	3	6.83	12.5

4.3 Particle size determination of PrOatein

Microscopy pictures of non-homogenised and homogenised PrOatein samples can be seen in *Figure 4.2*. By measuring the diameter of particles of different sizes and counting the amount of particles with the given size, the volume weighted diameter, $d(4.3)$, was calculated, the raw data is seen in *Appendix B*. The diameter of the particles is approximately **60 µm for non-homogenised samples** and **<10 µm for homogenised samples**. It can easily be noted that homogenisation significantly decreased the particle size, making the average size over 6 times smaller.

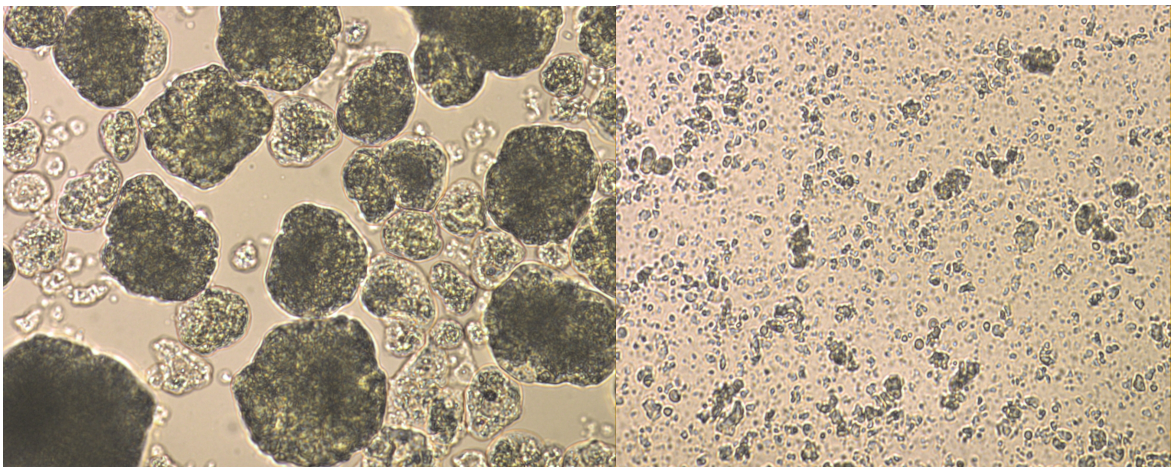


Figure 4.2. Microscopy pictures of non-homogenised (left) and homogenised (right) PrOatein dispersions at 20x magnification.

The results are assumed to be representative for the entire sample although the amount of particles analysed is only a small portion of the entire sample. An improvement would be to make calculations of the particle size from more pictures, as well as decreasing the magnification of the non-homogenised sample to see more particles in the same picture.

4.4 Sedimentation analysis of PrOatein dispersions

Sedimentation of PrOatein powder 5 and 10% w/v in water for homogenised and non-homogenised samples were analysed over time by measuring the height of the sediment at different times until the sediment layer remained constant, t =150 hours (raw data and pictures over time can be seen in *Appendix C*). The soluble proteins are approximately 4% and will remain in the upper water layer, thus the final sediment height assumes to have ≈96% of the total protein in the dispersion. The height of the sediment at different times were firstly converted to a % of the total sediment height and then to the % protein of the total protein in the sediment. These values were plotted against the time and can be seen in Figure 4.3. The final volume of the sediment in relation to the total volume of the sample can be seen in Figure 4.4.

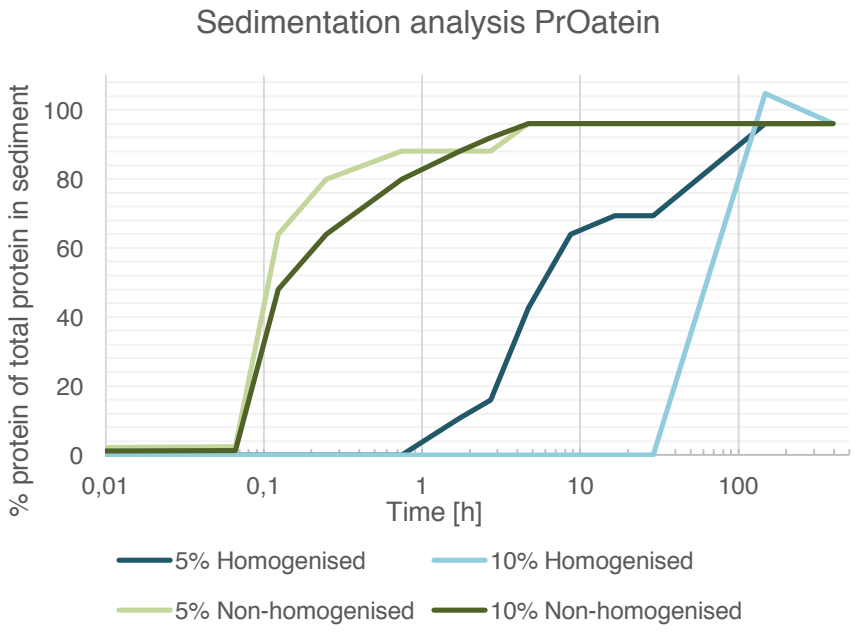


Figure 4.3. % protein of the total protein in the sediment for homogenised and non-homogenised PrOatein samples at 5 and 10% w/v.

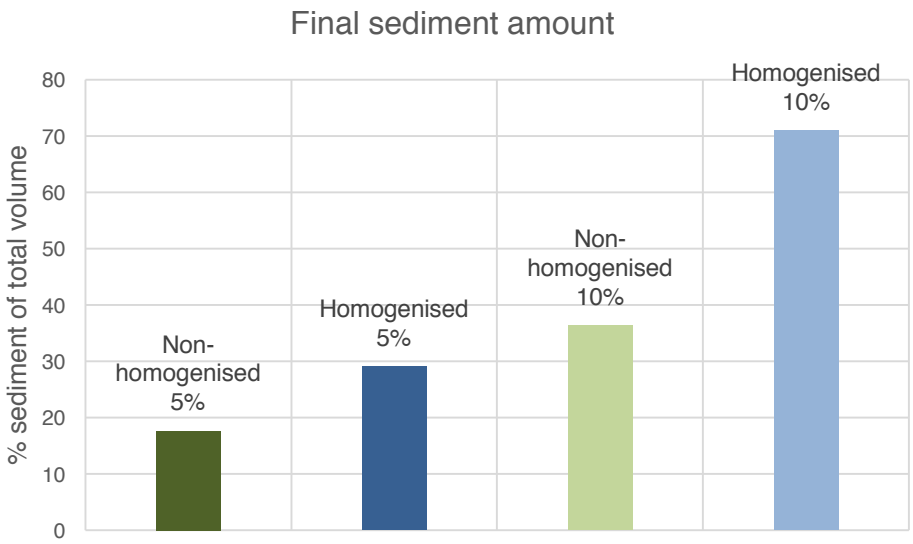


Figure 4.4. Final sediment volume in relation to the total volume of the sample for homogenised and non-homogenised PrOatein samples at 5 and 10% w/v.

It can be easily noticed that the non-homogenised samples reach the final sedimentation height after approximately 5 hours, compared to the homogenised samples which reached the final sedimentation height after more than 100 hours. This can be explained by the decrease in particle size of the homogenised samples slowing down sedimentation rate of the PrOatein dispersions, due to the phenomena described by Stokes Law. This kept the particles evenly distributed in the dispersion for a longer time, increasing the storage stability. It can be concluded that homogenisation is necessary to decrease the sedimentation rate and improve the stability of the PrOatein dispersions, due to the major decrease in particle size. The velocity of the sedimentation rate is hard to determine only using Stokes Law as particles at concentrations above a few % will influence the sedimentation rate of other particles and hinder their sedimentation, thus $v_{Stokes} > v_{Sedimentation}$. This could explain why 5% w/v reaches their full sedimentation height faster than 10% w/v.

The homogenisation did not only decrease the sedimentation rate, it also increased the % of sediment of the total volume in the dispersion. As the amount of powder is equal between the homogenised and the non-homogenised samples, the larger ratio of sediment is probably a result of a less dense sediment layer for the homogenised samples. The reduced particle size decreases sedimentation rate and results in an increased number of particles in the suspension. This will probably lead to an increased number of collisions between particles and the adhesive contacts between particles might have led to the formation of a more porous sediment with a larger volume. One interesting observation was that for the homogenised samples at 10% w/v, the sediment did not start at the bottom and increase with time. Instead, it was the upper water layer that increased and the bottom sediment layer that became more and more compact over time. It was only visible to see this upper water layer after >100 hours. Before this, the dispersion appeared homogeneous. An explanation to this could be that the particles in the powder have created a partially consolidated aggregated structure. The upper water layer could be a result of a time dependent compression of the aggregated structure leading to the phase separation.

To determine what concentration of the powder that is necessary for the aggregated structure to fill the space of the entire system, the g PrOatein/volume sediment was plotted against the % of PrOatein in the system for both homogenised and non-homogenised samples, as seen in *Figure 4.5*. The g PrOatein/volume sediment was calculated by dividing the % w/v PrOatein in the system with the % of sediment of the total volume. In this interpretation, it is assumed that this relationship is linear and a linear curve was fitted to the data points. At the point where these lines cross the line of g PrOatein/volume sediment = PrOatein in system (black line in *Figure 4.5)* gives an indication of what concentration % w/v of homogenised and non-homogenised dispersions that is necessary to fill up the entire network. The results show that this point is **25% w/v for non-homogenised** PrOatein

and **12.5% w/v for homogenised** PrOatein. At these concentrations there is no problem with stability as the particles of PrOatein in water will remain evenly distributed.

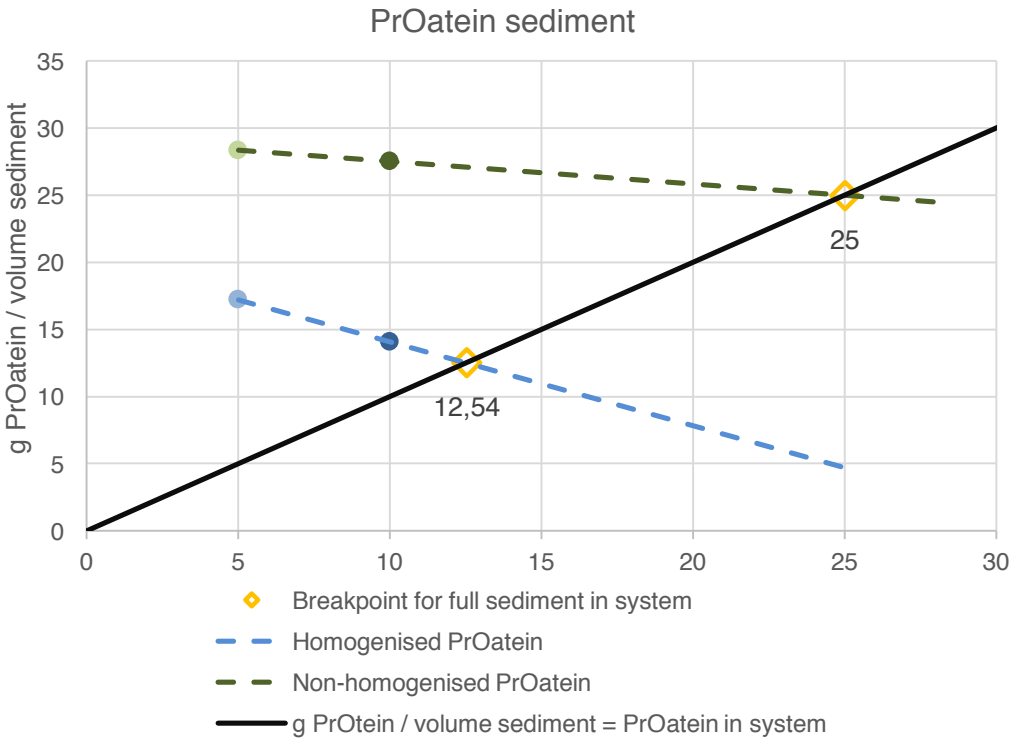


Figure 4.5. PrOatein need for a full aggregated structure in the system for homogenised and non-homogenised samples.

4.5 PrOatein solubility

The protein solubility and its effect by homogenisation and changes in pH and ionic strength have been investigated using the BCA-method. All measurement data from the different trials can be seen in *Appendix H* and all statistical data in *Appendix D*.

4.5.1 pH dependence

To investigate how the protein solubility depends on the pH of the solution, the concentration of soluble protein of PrOatein dispersions, 5% w/v, at different pH was measured with the BCA-method. The samples were prepared with 0.1 mol/L HCl to obtain pHs 6.42, 5.24, 4.80, 4.32 and 3.15, according to the titration curve in *Figure 4.1*. The concentration of soluble proteins for homogenised and non-homogenised samples can be seen in *Figure 4.6* and is given as a percentage of the original protein concentration in the dispersion. The error bars show the standard error of mean for the measurements.

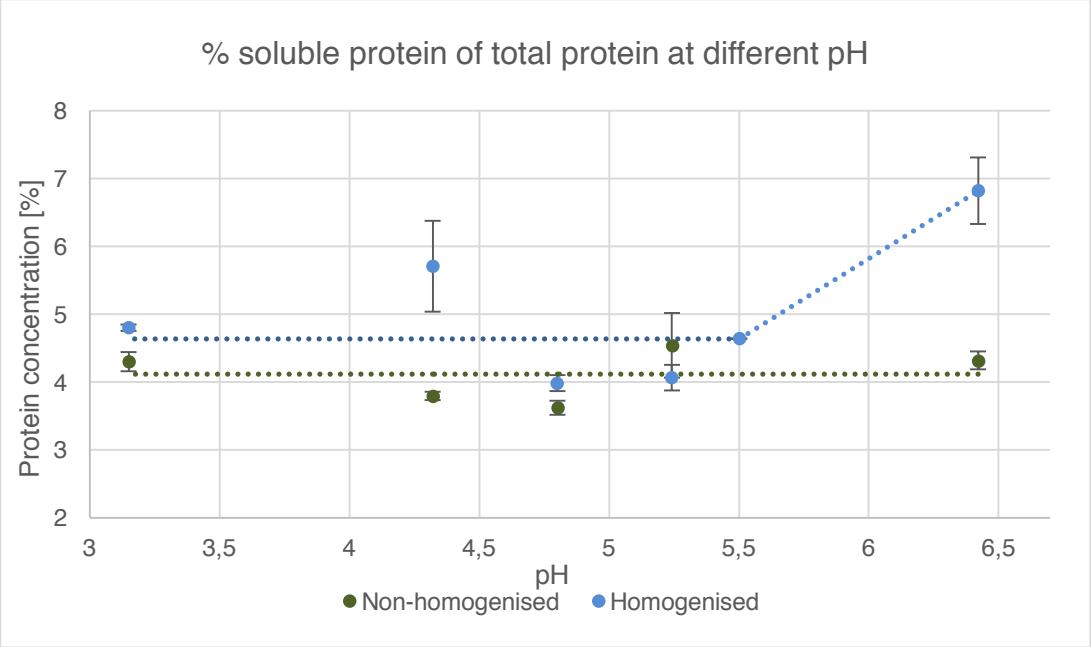


Figure 4.6. Data points and fitted curves showing solubility of homogenised and non-homogenised samples in HCl-solutions at different pHs. The error bars show the standard error of the mean.

The solubility for the samples ranges between 4-7% for the homogenised samples and 3.5-4.5% for the non-homogenised samples. There were statistically significant differences in solubility between the homogenised and non-homogenised samples at pH 6.42 and pH 3.15, as determined by one-way ANOVA (pH 6.42: $F(1,67)=42.8$, $p=9.87\cdot10^{-9}$, pH 3.15: $F(1,14)=9.84$, $p=7.27\cdot10^{-3}$) at the $p<0.05$ level. There were no statistically significant differences in solubility between the homogenised and non-homogenised samples at pH 5.24, 4.80 and 4.32 as determined with the same test (pH 5.24: $F(1,14)=0.769$, $p=0.395$, pH 4.80*:* $F(1,6)=3.81$, $p=9.85\cdot10^{-2}$, pH 4.32*:* $F(1,10)=3.42$, $p=9.43\cdot10^{-2}$).

When analysing the measurement data, the homogenised samples indicate to have the highest solubility around pH 6 and the lowest solubility around pH 5. The non-homogenised samples indicate to have lowest solubility around pH 4.5 and highest between pH 5-5.5 followed by a small decrease up to around pH 6.5. Statistical analysis on the pH-dependence were performed on the homogenised and non-homogenised samples separately. The one-way ANOVA showed statistically significant differences in solubility between the different pHs for the homogenised samples but not for the non-homogenised samples (homogenised: $F(4,43)=5.44$, $p=1.24\cdot10^{-3}$, non-homogenised: $F(4,72)=1.21$, $p=0.316$) at the $p<0.05$ level. To find out where in the pH range this statistically significant difference was noticeable, the Tukey-Kramer Post Hoc test was performed at the level of $p<0.05$. The test indicated that the solubility at pH 6.42 was statistically significantly different from pH 5.24 and 4.80. Due to the small influence on solubility from the change in pH, the pH range in which the analyses are performed are probably too narrow and more extreme pHs may be necessary to get more noticeable differences in solubility.

4.5.2 Ionic Strength Dependence

To investigate how the change in the ionic strength influenced protein solubility, the concentration of soluble protein in PrOatein samples, 5% w/v, at different ionic strength was measured with the BCA-method. The samples were prepared with 0, 0.05, 0.5 and 1 mol/L NaCl solutions. No acid was added to adjust the pH, hence the dispersions were at pH 6.42. The concentration of soluble proteins for homogenised and non-homogenised samples can be seen in *Figure 4.7* and is given as a percentage of the original protein concentration in dispersion. The error bars show the standard error of the mean for the measurements. Due to the salt contribution from the PrOatein powder itself, the initial ionic strength is 7.36 mmol/L.

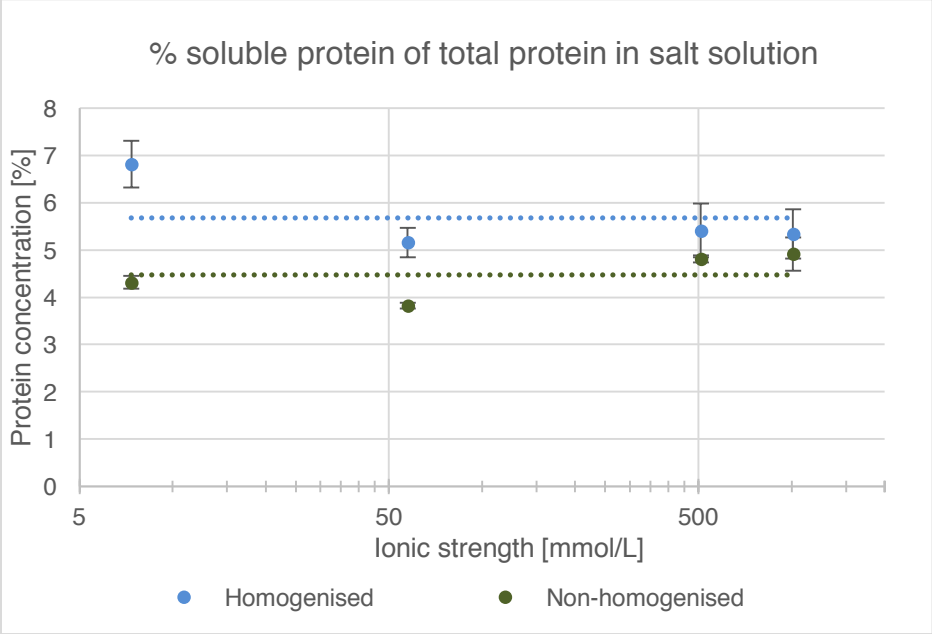


Figure 4.7. Data points and fitted curves showing solubility of homogenised and non-homogenised samples at different ionic strengths at pH 6.42. The error bars show the standard error of the mean.

The data points for both the homogenised and non-homogenised samples indicate a decrease in solubility between 7.36-57.4 mmol/L. The solubility for the homogenised samples seems somewhat unchanged up to 1010 mmol/L mmol/L, while for the non-homogenised samples a small increase is apparent. An initial interpretation of the results could be that the slightly higher solubility at low ionic strength for the homogenised samples could be a result of a salting in behavior, followed by a slight salting out of the protein when the ionic strength increased. For the non-homogenised samples, they seem to have a minima in solubility around 50 mmol/L.

Statistical analysis on the ionic strength dependence were performed on the homogenised and non-homogenised samples separately. The one-way ANOVA did not show any statistically significant differences in solubility between the different ionic strengths for any of the samples (homogenised: $F(3,37)=2.31$, $p=0.092$, non-homogenised: $F(3,65)=2.16$, $p=0.101$) at the $p<0.05$ level. Hence, no statistically significant ionic strength dependence can be seen within the analysed interval of the ionic strength, giving a horizontal line between all of the data points.

As there was no statistical support that there would be any ionic strength dependence within the groups, an analysis to see whether there is any difference between all homogenised vs. all non-homogenised samples were performed. This showed statistically significant differences in solubility between the homogenised and non-homogenised samples ($F(1,109)=36.7$, $p=1.97\cdot10^{-8}$).

4.5.3 Different pH and ionic strength

To investigate if the ionic strength influences solubility of the protein differently depending on the charge distribution of the protein molecule, the solubility was measured in solutions at the same ionic strengths as above but at different pHs. As the HCl contributes to the ionic strength, the final ionic strengths at which the measurements were made, differ a bit from the prepared dispersions. The results from the measurements at pH 3.15, 4.32, 4.8, and 5.24 can be seen in *Figure 4.8.* The error bars show the standard error of mean. The presented results are from analyses performed on the homogenised samples as these were considered to be of most interest since previous results indicated a slightly higher solubility for those. In the figure, the curves bind the data points together to visualise the solubility behavior. However, they do not necessarily indicate that the dependence is statistically different, which is further discussed below.

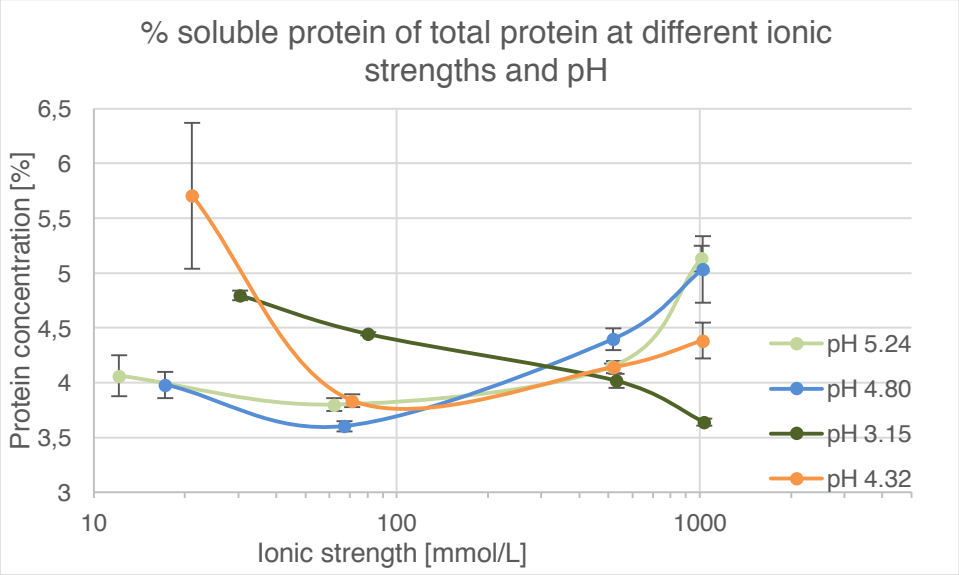


Figure 4.8. Data points and curves showing solubility at different ionic strengths and pH for homogenised PrOatein samples. The error bars show the standard error of mean.

There were statistically significant differences between the group means at every pH except at pH 4.32, as determined by one-way ANOVA (pH 3.15: $F(3,16)=105$, $p=9.69\cdot10^{-11}$, pH 4.32*:* $F(3,16)=2.33$, $p=0.113$, pH 4.80*:* $F(3,12)=9.59$, $p=1.65\cdot10^{-3}$, pH 5.24: $F(3,16)=8.85$, $p=1.09\cdot10^{-3}$) at the $p<0.05$ level.

To find out where in the range of different ionic strengths the statistically significant differences were noticeable, the Tukey-Kramer Post Hoc test was performed at the level of $p<0.05$. For pH 3.14, the test indicated that the mean values at the different ionic strengths were all statistically significantly different from each other. For pH 4.80, the test indicated that the mean values at 17.2 and 67.2 mmol/L were statistically significantly different from the mean value at 1020 mmol/L. For pH 5.24, the test indicated that the mean values at 12.1, 62.1 and 512 mmol/L were all statistically significantly different from the mean values at 1010 mmol/L.

The charge distribution of the protein differs at the different pH as they are closer or further away from their IP. The net charge of the molecule will be larger further away from the IP at which the net charge is zero. This is illustrated in *Figure 4.9.*

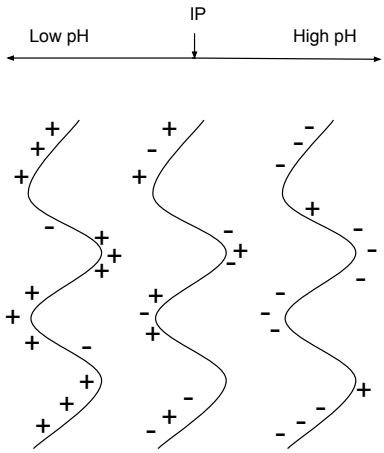


Figure 4.9. Illustration of how the charge distribution on a protein changes on opposite sides of the isoelectric point.

At pH 4.80 and 5.24, the increase in ionic strength leads to a higher solubility which goes in line with the theory for salting in, described with *Equation 2.1-2.3* in *Section 2.3.3.* However, the equation is not as reliable for too high valences which could be the case for the proteins at pH 3 and a reason to why the proteins show a salting out behavior instead. Due to the higher amounts of charges around pH 3, this reasoning would imply that the IP is

closer to 4.80 and 5.24. Since the sample contains many different proteins this would be an indication of a total IP for the dispersion. If comparing the different curves it can be noticed that the solubility at lower ionic strengths are lower at pH 4.80 and 5.24 compared to at pH 4.32 and 3.14. This could also be an indication that the net charge of the proteins are larger at the lower pH and therefore enables more hydrophilic interactions.

The salting out behavior for pH 3.14 could possibly also be explained by the fact that oats consists of 70-80 wt.% globulins, which are soluble in low concentrated salt solutions, and insoluble in high concentrated salt solutions. Far away from the IP, as explained above, when the hydrophobic interactions between the proteins is less, this could allow for all proteins to be exposed to the environment, also the globulins, and their low solubility at high ionic strengths may be more noticeable.

4.5.4 Overall solubility

The behavior of % soluble protein at pH 3.15-6.42 and ionic strengths of 7.36-1030 mmol/L, describing the experimental setup, can be seen in *Figure 4.10* for non-homogenised PrOatein samples and *Figure 4.11* for homogenised PrOatein samples. The lines connect the settings with the same solubility with the values given on the lines.

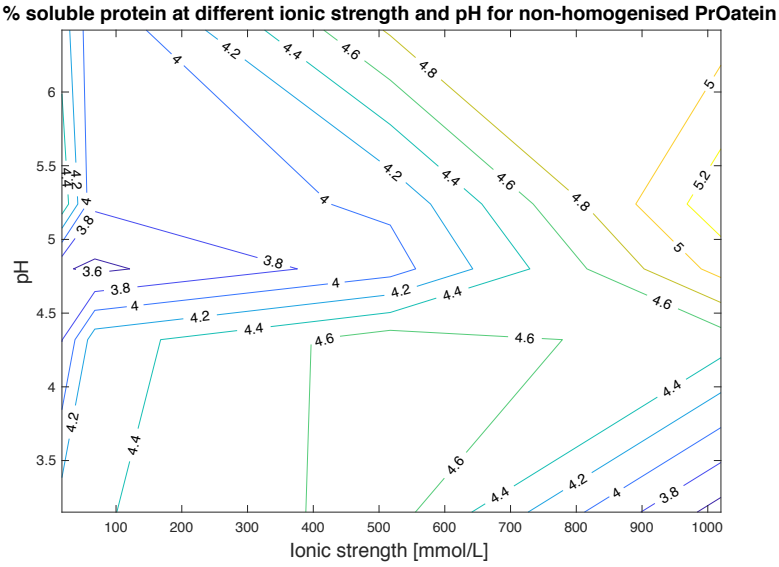


Figure 4.10. Contour plot of non-homogenised PrOatein samples showing % soluble protein at different pH and ionic strengths.

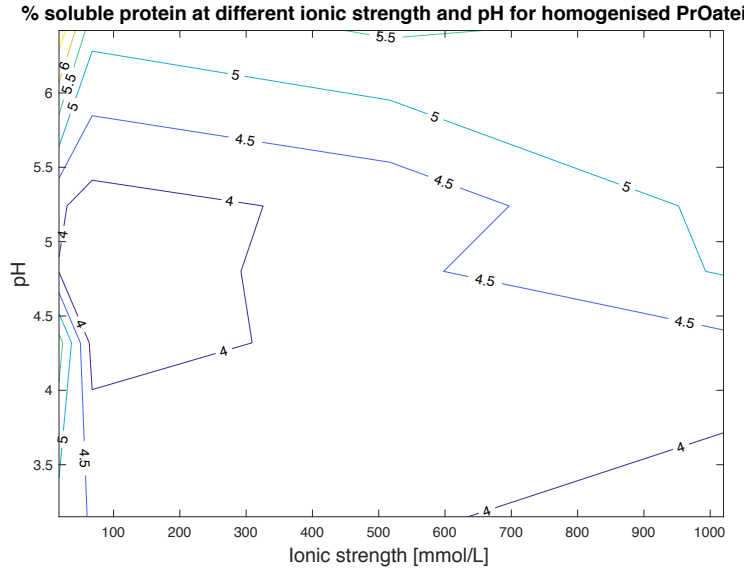


Figure 4.11. Contour plot of homogenised PrOatein samples showing % soluble protein at different pH and ionic strengths.

The solubility of the protein in PrOatein is generally low. A reason for the overall low solubility, ≈4-6%, could be that the PrOatein powder is sterilised during processing. During this tough heat treatment, the proteins most likely denature which is followed by hydrophobic parts of the protein getting exposed, leading to aggregation of protein particles and formation of bigger aggregates with other residues like starch molecules. Due to this, the refolding of proteins to their native form is prevented and the initial protein solubility will decrease.

Differences in pH and ionic strength that are reasonable to use for food applications have low or no significant effect on the protein solubility which suggests that the protein particles are rather stable and do not easily get affected by changes in the surrounding. Homogenisation however, seems to somewhat improve the protein solubility from approximately 4% for non-homogenised samples to approximately 6% for homogenised samples. An explanation to why homogenisation improves the solubility of the protein might be that aggregates are disrupted enabling refolding of the denatured proteins and some solubility might be regained.

The protein solubility is measured with the BCA-method, which relies on the proteins forming a complex and reducing Cu^{2+} to Cu^{1+} under alkaline conditions, which gives a colored complex that can be determined by absorbance measurements at 562 nm. The absorbance is proportional to the amount of protein present in the sample. It is only the peptide bonds of cysteine, cysteine, tryptophan and tyrosine that are able to further reduce Cu^{2+} to Cu^{1+} and contribute to the colour change. If these amino acids in PrOatein are lower than for the standard, the obtained colour change may be underrepresented, giving a lower solubility than what is correct.

4.6 Water holding capacity

The WHC of PrOatein was determined to be **4.52±0.12 g water/g protein** (see *Appendix E* for raw data and calculations). The amount of protein in the samples were calculated based on a protein content in PrOatein of 55% and an insoluble protein fraction of 96%. The value lies within the middle range of 2-7 g water/g protein that is common for proteins. As PrOatein consists of more than just oat protein, the WHC is probably also affected by other compounds in the powder such as fibers which also have the possibility to bind water.

A study performed by Boucheham *et al*, showed that maize powder had a WHC of 5.31±0.02 g water/g sample compared to rice (2.36±0.07 g/g), which can be explained by the high dietary fiber content in maize (Boucheham, Galet, Patry and Zidoune, 2019). The WHC based on the PrOatein powder and not the protein content, could also be of interest and was calculated to **2.07±0.05 g water/g PrOatein.** This value seems rather low and could have correlation with the overall low solubility of PrOatein. It is the hydrophilic polar groups and dipoles which have the ability to bind water, if these are low, the hydrophobic groups are probably higher which lowers both the solubility and the WHC. Larger numbers of charged amino acids normally leads to higher WHC, and as the distribution of charges normally changes with pH, it would have been interesting to also investigate if the pH would have an effect on the WHC. However, since the previous results from the pH dependence on protein solubility did not have any large effects, it could be assumed that it would not have a big impact on WHC either.

4.7 Rheological behavior of PromOat

4.7.1 Flow curves of different % w/v PromOat

The shear rate, $\dot{\gamma}$, [s^{-1}] was plotted against the shear viscosity, η, [Pa·s] to see the flow behavior of the PromOat mixtures. The mean flow curves for 1, 3, 6 and 9% w/v PromOat can be seen together in a logarithmic scale in *Figure 4.12.* In *Figure 4.13, 4.14, 4.15* and *4.16* the logarithm of the shear rate is plotted against the shear viscosity for 1, 3, 6 and 9% w/v respectively, for all trials of the same setting, accompanied with the mean curve. The figures are accompanied with a mean flow curve. All the samples for the different % w/v PromOat are prepared without addition of salt but the final ionic strength of the samples is not equal as PromOat contributes with an ionic strength of 228 µmol/g powder. This would imply an ionic strength of 2.28 mmol/L for 1% w/v and approximately 10 times larger for 9% w/v, 20.5 mmol/L. The results might therefore be seen as non-comparable. However, as

discussed below in *Section 4.7.2*, the ionic strength has no effect on the viscosity of the mixtures and the differences are therefore not considered to affect the results.

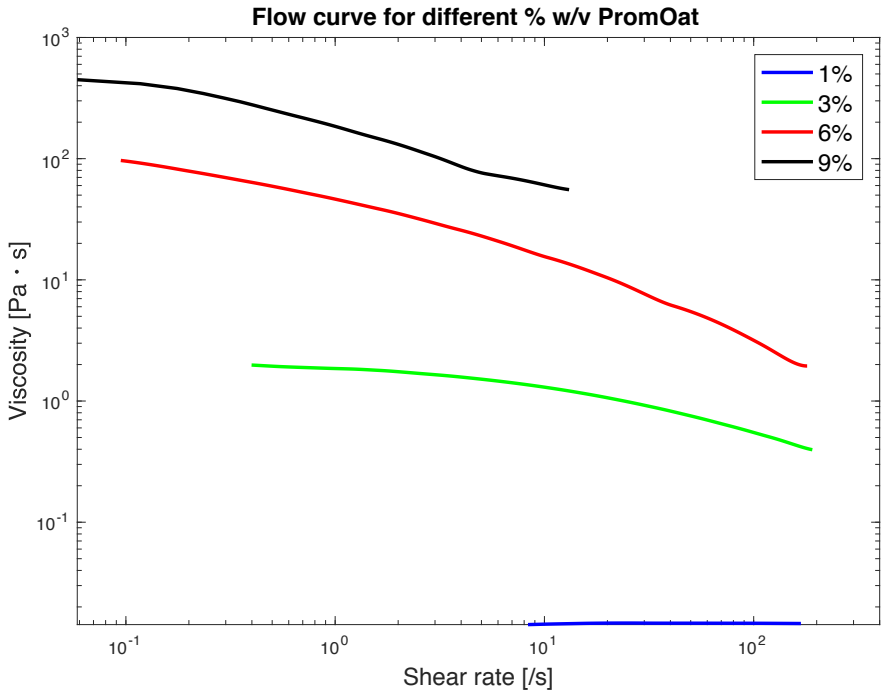


Figure 4.12. Flow curve for 1, 3, 6 and 9% w/v PromOat.

1% w/v PromOat shows a Newtonian flow behavior with a viscosity of approximately 14 mPa·s.

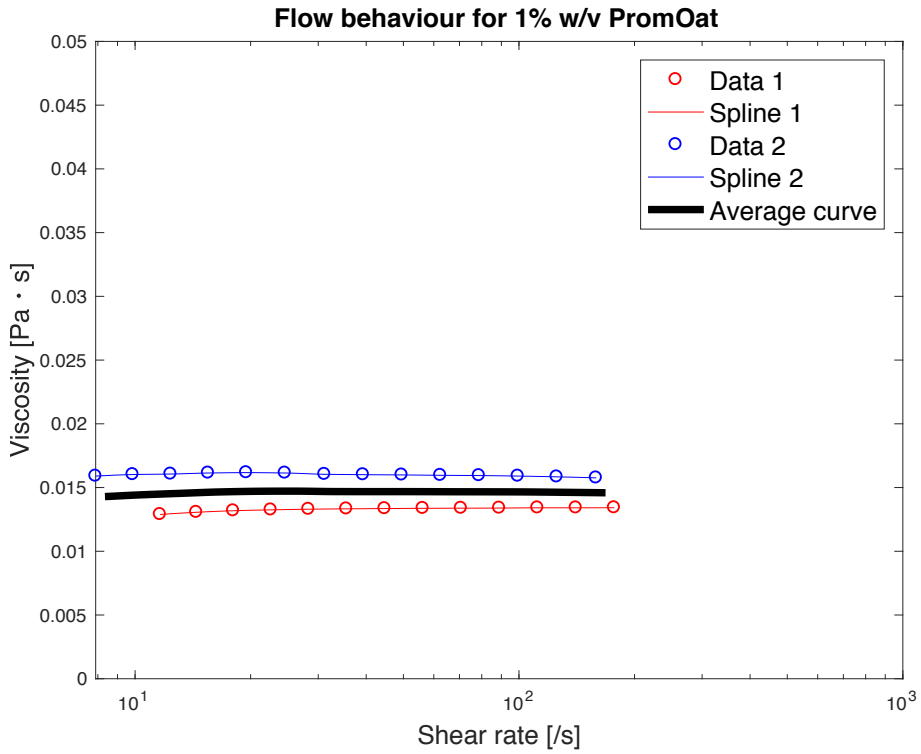


Figure 4.13. Flow curve for 1% w/v PromOat.

3% w/v PromOat shows a shear thinning behavior with a mean viscosity of 1.80 Pa·s at a shear rate of 1 s^{-1}. The variation is bigger at low shear rates and the curves coincide more at shear rates above 100 s^{-1} where they seem to flatten out to a viscosity of below 0.5 Pa·s.

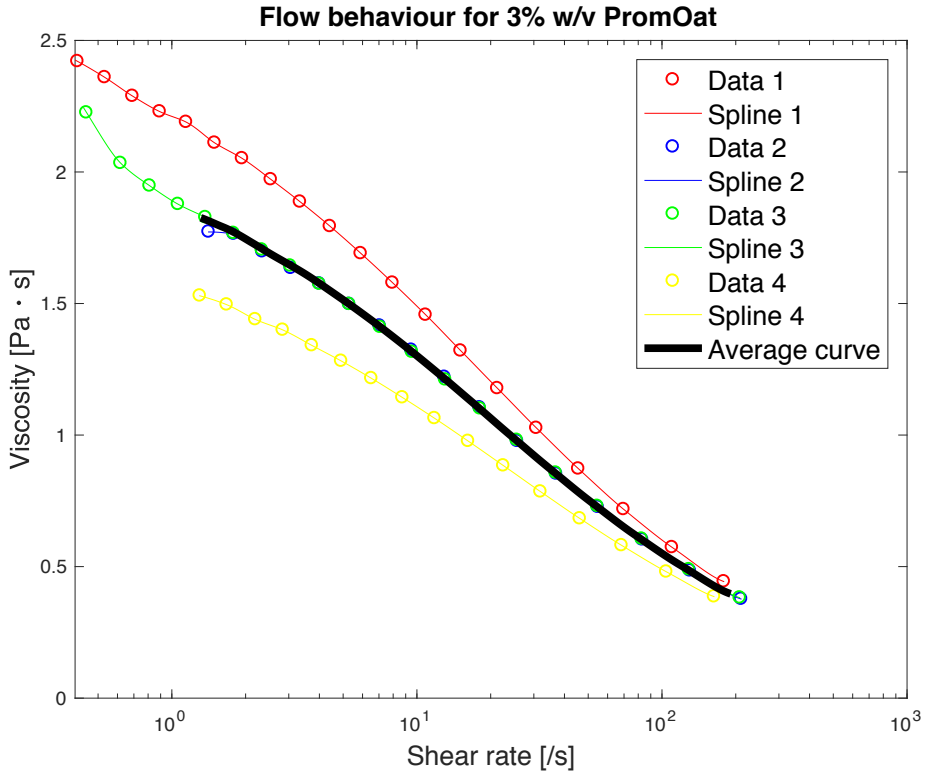


Figure 4.14. Flow curve for 3% w/v PromOat.

6% w/v PromOat shows a shear thinning behavior with a viscosity of approximately 45 Pa·s at a shear rate of 1 s^{-1}. The curve flattens out to approximately 2 Pa·s at shear rates above 150 s^{-1}.

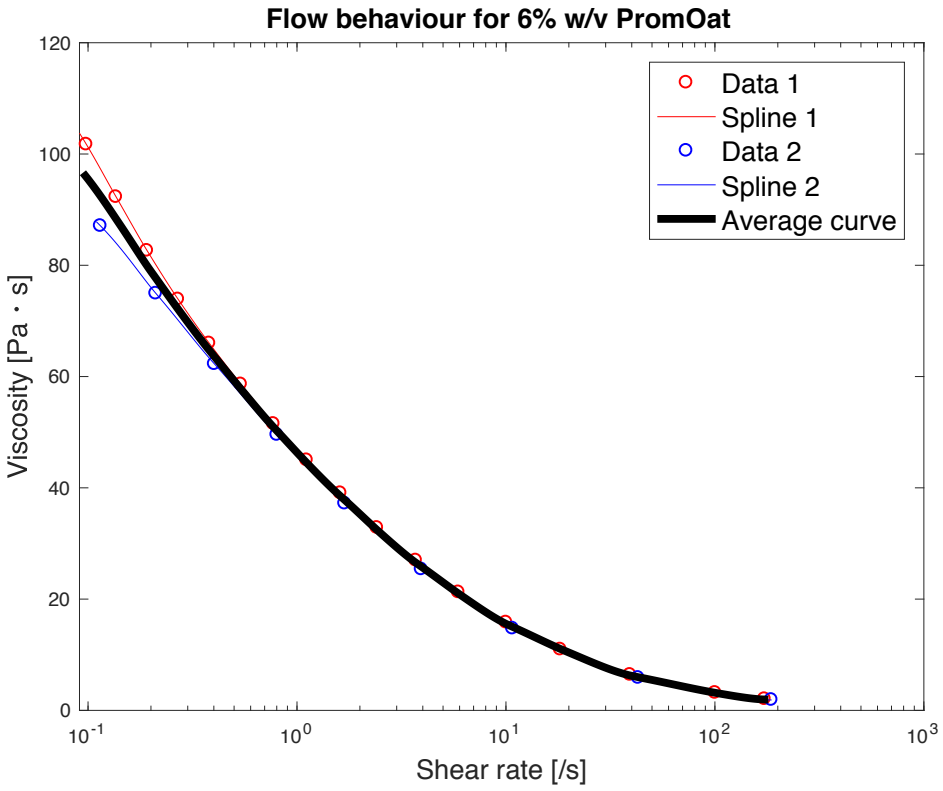


Figure 4.15. Flow curve for 6% w/v PromOat.

9% w/v PromOat shows a shear thinning behavior with a viscosity of approximately 180 Pa·s at a shear rate of 1 s^{-1}. The curve flattens out to slightly below 4 Pa·s at shear rates above 100 s^{-1}. The mean splined function was ended at 60 s^{-1} because at higher shear rates it gave a misleading flow behavior due to the limited amount of data points. Therefore, an approximate curve fitting was drawn from 60-100 s^{-1} which correlates well with the given data and represents the flow at the higher shear rates.

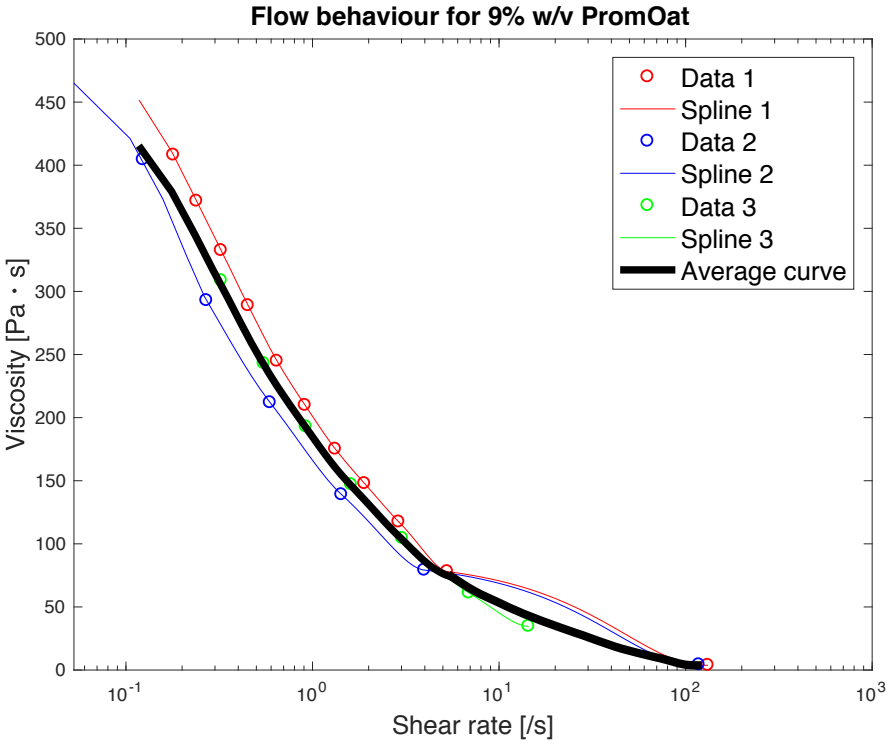


Figure 4.16. Flow curve for 9% w/v PromOat.

The viscosity increases with increased amount of powder and the difference is most noticeable at low shear rates. The high viscosity for higher concentrations is a result of more particles colliding with each other and disturbing the flow field. At high shear rates, all mixtures have a lower viscosity and the shear thinning behavior is explained by polymers disentangling and aligning in the direction of the flow. The number of disentanglements exceeds the number of entanglements that have the time to reform and the viscosity decreases as the disturbances in the flow field are less. At the highest shear rate region, the polymers are fully disentangled which results in the low and more constant viscosity.

The obtained values from the different trials for the same system, differentiate from one another. This is most likely due to lack of reproducibility of the sample preparation as the powder was somewhat difficult to dissolve in water, especially at high concentrations. This resulted in some mixtures containing small lumps of powder, reducing the amount of interactions with water.

4.7.2 Ionic strength dependence

The shear rate, $\dot{\gamma}$, [s^{-1}] was plotted against the shear viscosity, η, [Pa·s] to see the flow behaviour of the PromOat mixtures 3% w/v at final ionic strengths of 6.83, 56.8, 507 and 1010 mmol/L, as seen in *Figure 4.17*. The viscosity varies between 1.50-2.20 Pa·s at low shear rates but flattens out to a more uniform behavior at higher shear rates.

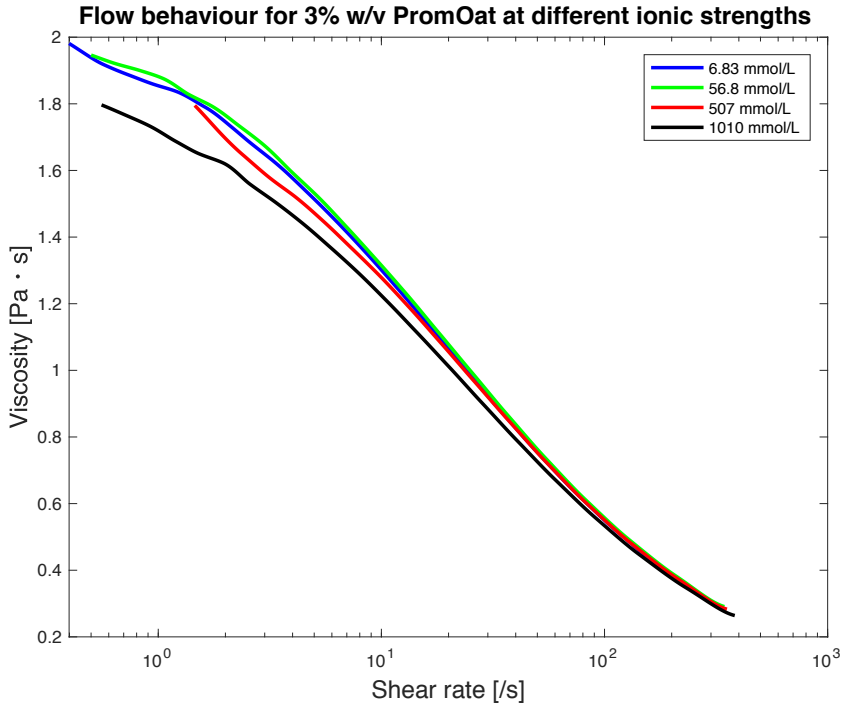


Figure 4.17. Flow curve for 3% w/v PromOat at a final ionic strength of 6.83, 56.8, 507 and 1010 mmol/L.

A comparison of the viscosity at shear rates 1, 50 and 100 s^{-1} for the different ionic strengths can be seen in *Figure 4.18,* and was done to investigate if there were any statistically significant differences between the different ionic strengths (raw data is presented in *Appendix I).*The error bars symbolise the standard error of mean. With the results obtained, it is not possible to determine any ionic strength dependence on the viscosity of the PromOat mixtures. As the molecules do not have a lot of charges which can interact with the added ions, this result is not surprising.

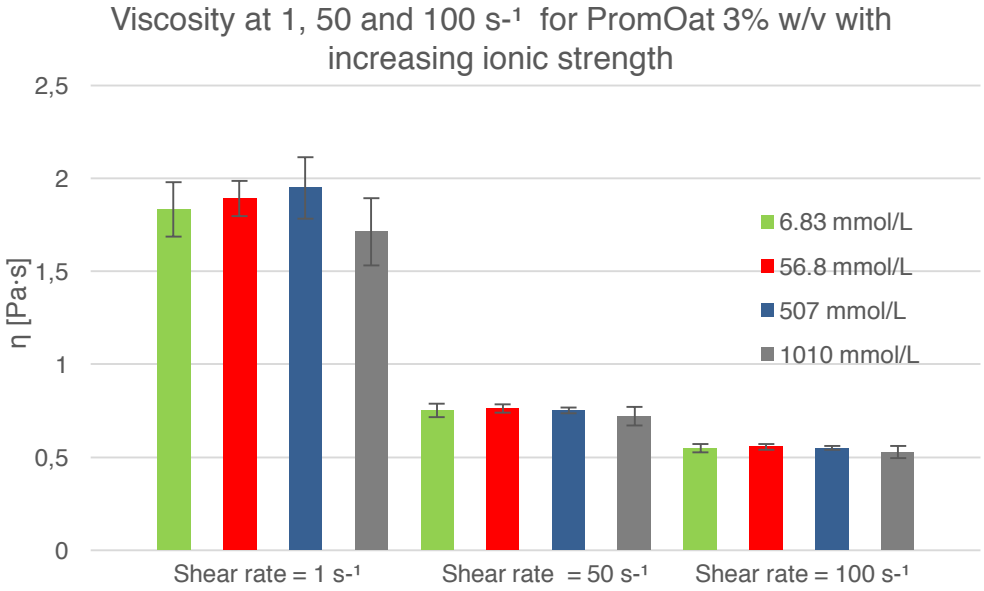


Figure 4.18. The mean viscosity of PromOat 3% w/v at increasing ionic strengths at shear rates 1, 50 and 100 s^{-1}. The error bars show the standard error of mean.

4.7.3 pH dependence

The shear rate, γ, [s^{-1}] was plotted against the shear viscosity, η, [Pa·s] to see the flow behaviour of the PromOat mixtures 3% w/v at pH 6.32, 5.35, 4.16 and 2.84. The pH was set by addition of HCl 0.1 mol/L according to the

titration curve, seen in *Figure 4.19.* The pH was also measured in the prepared mixtures and a mean value of the measured pH is the pH that is presented in the results. The viscosity varies between 1.50-2.20 Pa·s at low shear rates but flattens out to a more uniform behavior at higher shear rates.

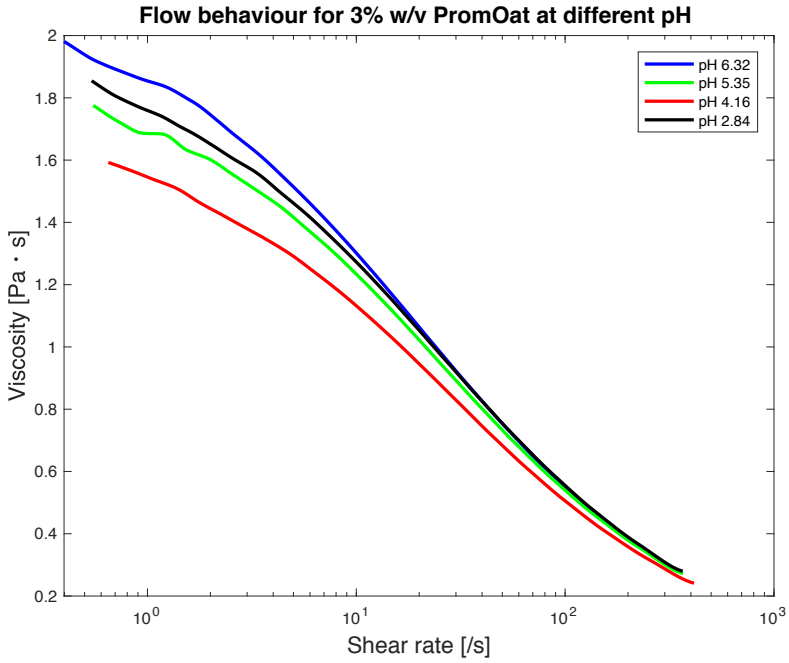


Figure 4.19. Flow curve for 3% w/v PromOat at pH 6.32, 5.35, 4.16 and 2.84.

A comparison of the viscosity at shear rates 1, 50 and 100 s^{-1} for the different pHs can be seen in *Figure 4.20,* and was done to investigate if there were any statistically significant differences between the different pHs (raw data is presented in *Appendix I).* The error bars symbolise the standard error of the mean. With the results obtained, it is not possible to determine any pH dependence on the viscosity of the PromOat mixtures. As for the ionic strength dependence, this result is not surprising as the molecules do not have a lot of charges which can interact with the added acid and will therefore not affect the interactions between PromOat and water, which would result in a change of the rheological properties.

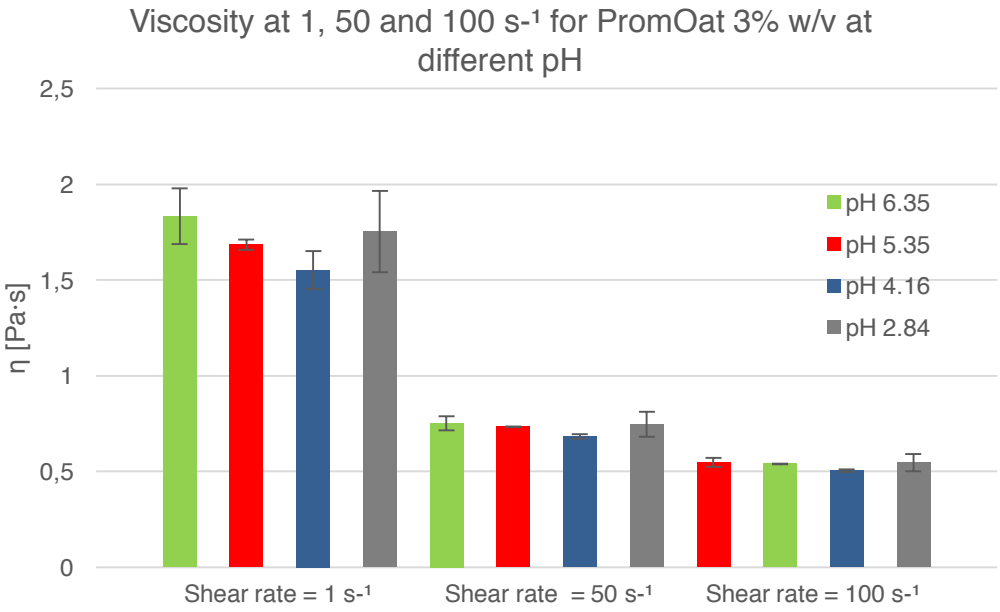


Figure 4.20. The mean viscosity of PromOat 3% w/v at decreasing pH at shear rates 1, 50 and 100 s^{-1}. The error bars show the standard error of mean.

All the samples for analysis of the pH dependence of PromOat are prepared without the addition of salt. However, the addition of HCl will affect the final ionic strength of the samples. The largest difference of the ionic strength is between the samples at pH 6.32 and pH 2.84. At pH 6.32, only the salt in the powder contributes to the ionic strength of 6.83 mmol/L, compared to the final ionic strength of the samples at pH 2.84, 12.5 mmol/L. As discussed above in *Section 4.7.2*, the ionic strength has no effect on the viscosity of the mixtures and the differences are therefore not considered to affect the results.

4.7.4 Yield stress determination

1% w/v PromOat shows a Newtonian behavior, thus there is no yield stress for that concentration. For 9, 6 and for 3% w/v PromOat, see *Figure 4.21*, the shear rate, $\dot{\gamma}$ [s^{-1}], was plotted against the shear stress, τ [Pa].

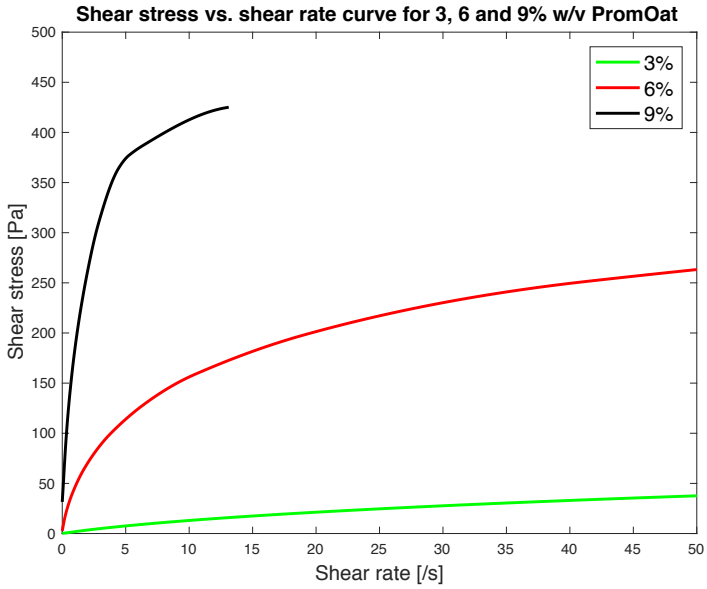


Figure 4.21. The shear stress [Pa] at different shear rates [s^{-1}], for 3, 6 and 9% w/v PromOat.

In order to have a yield stress, a certain shear stress is needed for the system to start flowing, and the shear stress needs to be >0 at very low shear rates. From the figure above, it is possible to interpret that there would be a yield stress for 9 and 6% w/v, but this appearance is not as noticeable for 3% w/v, thus the breakpoint for where the yield stress arises is somewhere between 3-6% w/v. The interpretations of precise values of the yield stress from the figure are hard, thus the conclusions of the yield stress are somewhat vague. For this reason, the yield stress analysis was complemented with a test which determined at what % w/v PromOat was necessary to keep a small glass bead from sinking in the gel. 10% of the glass bead remained on top of the surface at 10% w/v PromOat, 28% above the surface at 11% and 40% above the surface at 12% and yield stresses were calculated to 15.4, 21.4 and 27.3 Pa respectively (see raw data in *Appendix F)*. In *Figure 4.22*, the obtained yield stresses were plotted against the % w/v PromOat and a linear curve was fitted to the data to be able to approximate the yield stress at different % w/v PromOat. The breakpoint for where the yield stress arises is 7.4% w/v PromOat, which is equal to a β-glucan content of 2.6%. This seems like a reasonable result, also concerning the appearance of the flow curve.

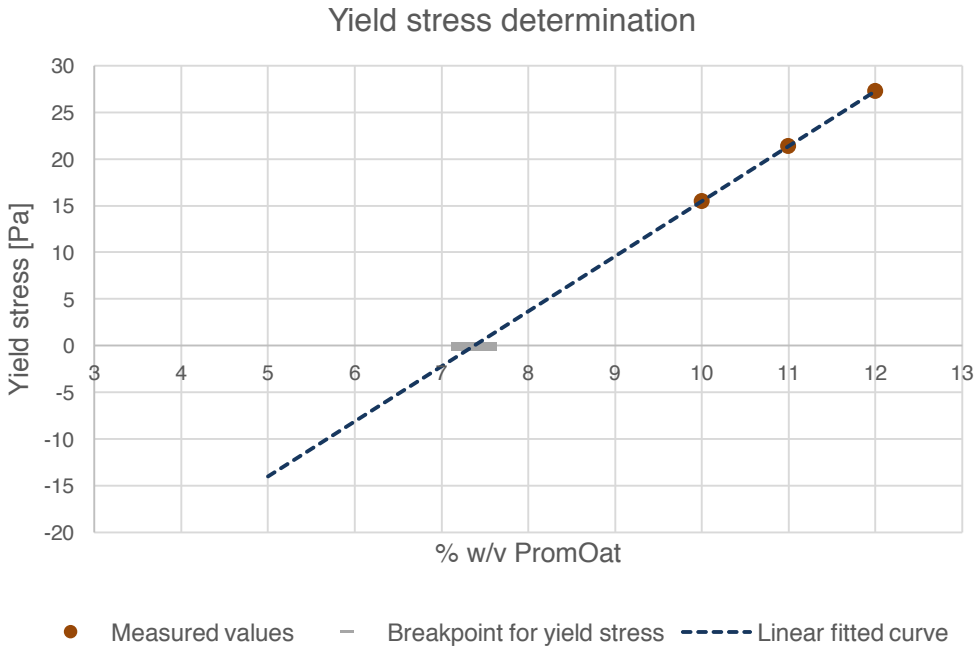


Figure 4.22. Yield stress at different % w/v PromOat.

An adequate yield stress can enable that particles in a PromOat dispersion will not sediment and is of great importance as the dispersion then would remain stable. The resulting force of homogenised and non-homogenised PrOatein particles was calculated in the same way as for the glass bead. Giving a pressure of 4.22 mPa for homogenised samples (particle diameter <10 µm) and 27.8 mPa (particle diameter ≈60 µm) for non-homogenised samples. These values are very low and conclusions can therefore be made that if the PromOat content is >7.4% w/v, particles in PrOatein will remain stable in the system.

4.8 Food application of the powders

4.8.1 PrOatein

The protein solubility is a limiting factor for the use of PrOatein in food applications, especially in products with high water content in which high solubility is of great importance. Examples of such applications could be to use the oat proteins as emulsifiers, which PrOatein can be assumed to not be suited for. However, the emulsifying properties of PrOatein have not been investigated which would be necessary to be able to draw more accurate conclusions regarding this. Homogenisation slightly improves the protein solubility, but the most significant improvement from homogenisation is the reduced particle size which leads to a slower sedimentation and a more stable dispersion. If higher values in solubility are wanted, other methods to improve the protein solubility are necessary for a wider application functionality of the powder. As the tough heat treatment of the powder is believed to be a reason for the low solubility, examples could be changes in processing conditions for the powder or addition of ex. α-amylase to break down starch that may surround protein aggregates and limit interactions with surrounding water. However, it is worth to notice that mitigating the heat treatment step could result in a high lipase activity and a reduced shelf life of the product.

The WHC also affects the applicability of the powder in food products and the retention of water in a product can have a big impact on the texture and sensory properties. PrOatein could possibly be used in applications in which enhancing the moisture in a product or modifying the texture into a more swelled or gelated structure is desired.

Homogenisation of PrOatein in water decreases the sensation of sandiness when consumed. Sandiness is described by the possibility to sense the particles, the threshold for where it is possible to sense particles in the palate is when the diameter is >25 µm. Homogenisation decreased the average particle size to <10 µm and explains why the homogenised samples had a better mouthfeel.

Plant based protein sources for food formulations are of large interest both considering greater sustainability, lower production costs and an increased trend in vegetarian and vegan diets. In 2018, the meat consumption in Sweden was reduced by 2 kg per person and year, for the second year in a row (Röös, 2012). However, generally plant based proteins have a lower content of essential amino acids and a less balanced composition, why it is recommended to combine different plant sources for a full nutritional profile. A comparison of the amount of essential amino acids in some protein isolates and PrOatein can be seen in *Figure 4.23* (Gorissen et al., 2018). As seen in the figure, whey protein has the highest total content of essential amino acids, followed by PrOatein. PrOatein is considered to be a very competitive protein powder compared with other plant sources but even compared to whey and will add to the nutritional quality of a product.

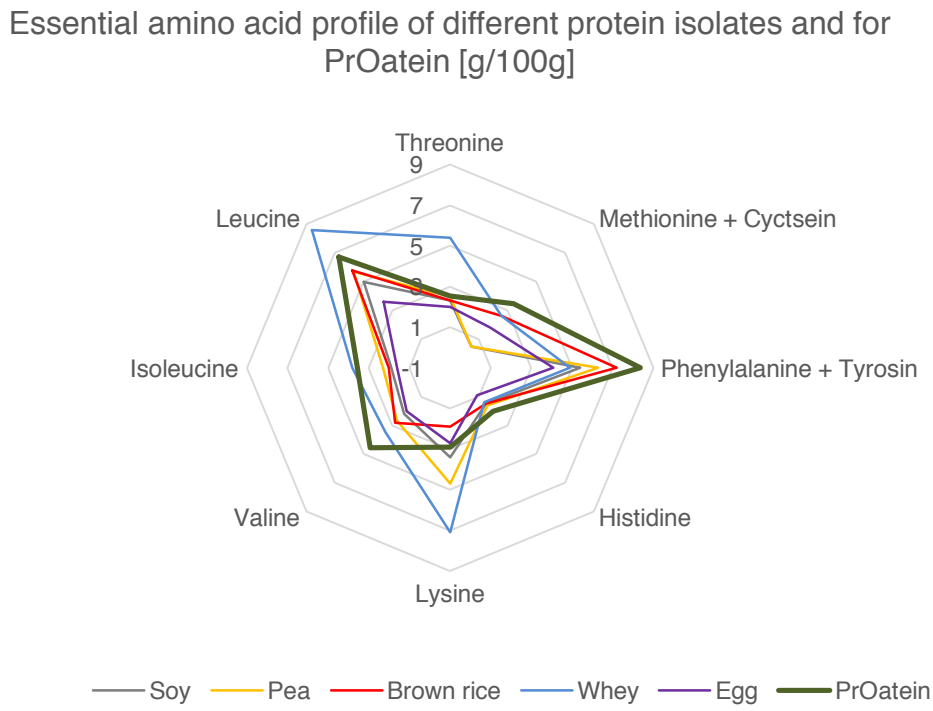


Figure 4.23. Essential amino acid profile of soy, pea, brown rice, whey and egg protein isolates and for PrOatein. A table of the values is listed in Appendix G.

4.8.2 PromOat

As discussed in *Section 4.7*, addition of PromOat powder in water affects the rheological properties of the dispersion by increasing the viscosity and PromOat is considered an effective thickening agent. This could make it a suitable ingredient in low fat products in which a similar sensation that a full fat product would have given, is desired. PromOat can also be suitable as a stabilising agent in products as the higher viscosity will stabilise the dispersion by slowing down sedimentation and aggregation. If a yield stress is present, the motion of particles can be fully prevented. The critical overlap concentration, c*, was determined to 3.1% w/v PromOat (1.05% in respect to the β-glucan content of 34%) (Paul, 2020). At this concentration the viscosity will steeply increase as the polymer can now entangle and overlap. At 3% w/v the viscosity of PromOat dispersions has a viscosity that is ≈100 times higher at 1 s-1. This is of importance if PromOat shall be used as a thickening agent as the polymer chains are able to entangle above ≈3% w/v and will therefore have a large impact on the viscosity at low shear rates. The value of c^* is interesting to compare with other hydrocolloids to evaluate their capacity of increasing the viscosity. β-glucans compare well with locust- and guar bean gum, which have a c^*=0.25% w/v and according

to the results, a higher content of β-glucans would be necessary to allow the chains to overlap. The downside with this, is the higher costs.

From a nutritional point of view however, a high β-glucan content is advantageous as the nutritional value of such products would increase which could make PromOat more favorable compared to other thickeners. It will contribute well to the fiber and the β-glucan content and open possibilities for the producer to set nutritional claims for the product.

β-glucans can hydrolyse at pH <2, so the pH of the food product where PromOat is put, should be above this pH. This will most likely not be a problem as hardly no food products are this acidic.

4.8.3 Product development

4.8.3.1 Development of structure

Based on the results obtained from the analysis on the functionalities of the powders, mixtures of PromOat and PrOatein powder in water were prepared at a variation of % w/v, as seen in *Table 4.4.* This was performed to investigate what ratios that could be suitable for food products, such as smoothies and puddings.

Table 4.4. Initial structure analysis of PrOatein and PromOat combinations in water.

% w/v PrOatein	% w/v PromOat	Note
5	1	Least viscous
	2	Not very viscous, drinkable
	3	Viscous, non-drinkable
10	1	Not very viscous, drinkable
	2	Moderately viscous, hardy drinkable
	3	Most viscous, non-drinkable

The results showed that a wide range of systems can be obtained by mixing different ratios of the powders. Some systems were well suited for smoothie like products (PrOatein 5-10% w/v combined with PromOat at 1-2% w/v), and some systems suited well for pudding like products (PrOatein 5-10% w/v combined with PromOat at 2-3.2% w/v). The properties of the gel were also affected by the combinations of the powders and the PrOatein seemed to increase the gelling capacity of PromOat. At a high PrOatein, >5% w/v, a astringent tendency is noted, but this is masked by an increasing PromOat content. The downside with an increasing PromOat content however, is a sensation of filmyness in the mouth. This sensation is described by a feeling of a thin layer maintaining in the mouth for an undesired long time. This filmyness is however masked by an increasing PrOatein content. By combining the two powders at different ratios, an optimal proportion can be used to mask these two undesired sensations.

The combinations of the powders in water without taste addition did not result in a tasty outcome. The taste from the oats was clear and an off-taste from the oat protein was noted. To give a more desired neutral product, PrOatein powder was homogenised in oat drink (Oatly 1.5% fat) instead of water at different proportions (2.5-5% w/v PrOatein and 1.5-3% w/v PromOat). The base of the oat drink resulted in a better mouthfeel and taste and was chosen as the preferred base for future product development.

The nutritional properties of the powders is high and the combinations of the two powders can be used to develop products with a high protein and high fiber content. To enable to state these claims for the product it requires a protein content contributing to >20% of the total energy and a fiber content having >3 g of fiber/100 kcal (Nutrition claims - Food Safety - European Commission, 2020). The previous structure analysis showed that the combination of the powders at different ratios can result in one consistency of something more fluid, like a smoothie, and one consistency of something less fluid and more gel like, like a pudding. These two products were chosen to be

developed further. The ratios of the powders were chosen to obtain the claim and also leave room to add ingredients that will add to the energy and nutrient value of the final product.

4.8.3.2 Development of smoothie

A smoothie base of 3.5% w/v homogenised PrOatein in oat drink combined with 1.5% w/v PromOat was used to develop a smoothie. The initial phase of the product development included investigation of what ingredients that should be added to give a desired final product, as seen in *Table 4.5.* The product needed some sweetness which was achieved by the addition of Stevia. Stevia was chosen to keep the calorie content low which facilitates for the product to maintain the high protein and high fiber claim. It was preferred over other sweeteners due to it having a more natural association and seems to be more accepted by consumers. Some other combinations were also tested, but with a more undesired outcome.

Table 4.5. Initial flavor development of the smoothie.

Flavor combination	Note
Carrot and ginger juice Cinnamon Cardamom Stevia	Nice flavor combination with potential for further development. It was however hard to balance the ingredients.
Blueberries Coconut Stevia	The blueberries and coconut suited well with the cereal base but the amount of coconut needed for the desired taste contribution made the product too high in energy.
Blueberries Cardamom Stevia	The sourness from the blueberries and the interesting taste from the cardamom showed high potential to mask the aftertaste of the cereals and seemed to be a good combination.

It can be noted that many taste combinations are possible to give a desired outcome but due to time limitations, only one was chosen for further development. To conclude, the blueberry cardamom smoothie showed the highest potential for this and the following steps in the product development can be seen in *Table 4.6.* The sweetness, sourness, mouthfeel, masking off-taste and flavor balance were evaluated from a scale from 1-5; 1: Dislike very much, 2: Dislike, 3: Moderate, 4: Like and 5: Like very much. The overall perception is the mean value of the scores of the different parameters. The modification and motivation for the modification of the product development steps is also noted in the table. The improvement from the initial product to the final product can be seen in *Figure 4.24.*

The nutritional value, ingredients list and a prototype packaging describing the final product is seen in *Figure 4.25* and data about the pH, viscosity and rheological behavior can be seen in *Table 4.7.* The viscosity at 2 s^{-1} is chosen as it is the shear rate in the mouth when biting and chewing.

Table 4.6. Smoothie product development steps. The different parameters were scored from 1-5 (1: Dislike very much, 2: Dislike, 3: Moderate, 4: Like and 5: Like very much). The overall perception is the mean value of the different parameters.

Product development step	Modification	Motivation for the modification	Sweetness	Sourness	Mouthfeel	Masking off-taste	Flavor balance	Overall perception
Initial	No added flavors. 3.5% w/v PrOatein, 1.5% w/v PromOat		1	1	3	1	1	**1.40**
1	Added blueberry	Mask off-taste from cereals, flavor contribution	1	3	3	2	2	**2.20**
2	Decreased to 3.2% w/v PromOat, addition of Stevia	Make it more drinkable, more sweetness necessary	4	3	5	2	2.5	**3.30**
3	Added cardamom	Mask after-taste from cereals, flavor contribution	4	3	5	4	3	**3.80**
4	Added vanilla powder	Make the sweetness more round	5	3	5	4	3.5	**4.10**
5	Added lingonberries	Flavor and sourness contribution	5	4	5	4	4	**4.40**
Final	Added citric acid	To achieve a desired sourness	5	5	5	4	4	**4.60**

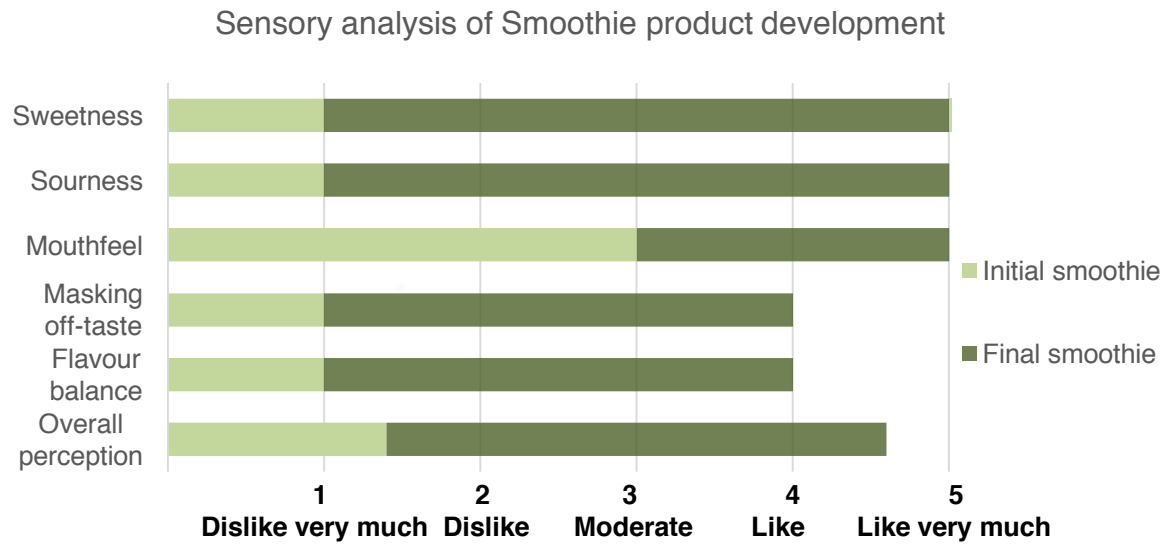


Figure 4.24. The improvement from the initial product to the final product of the smoothie.

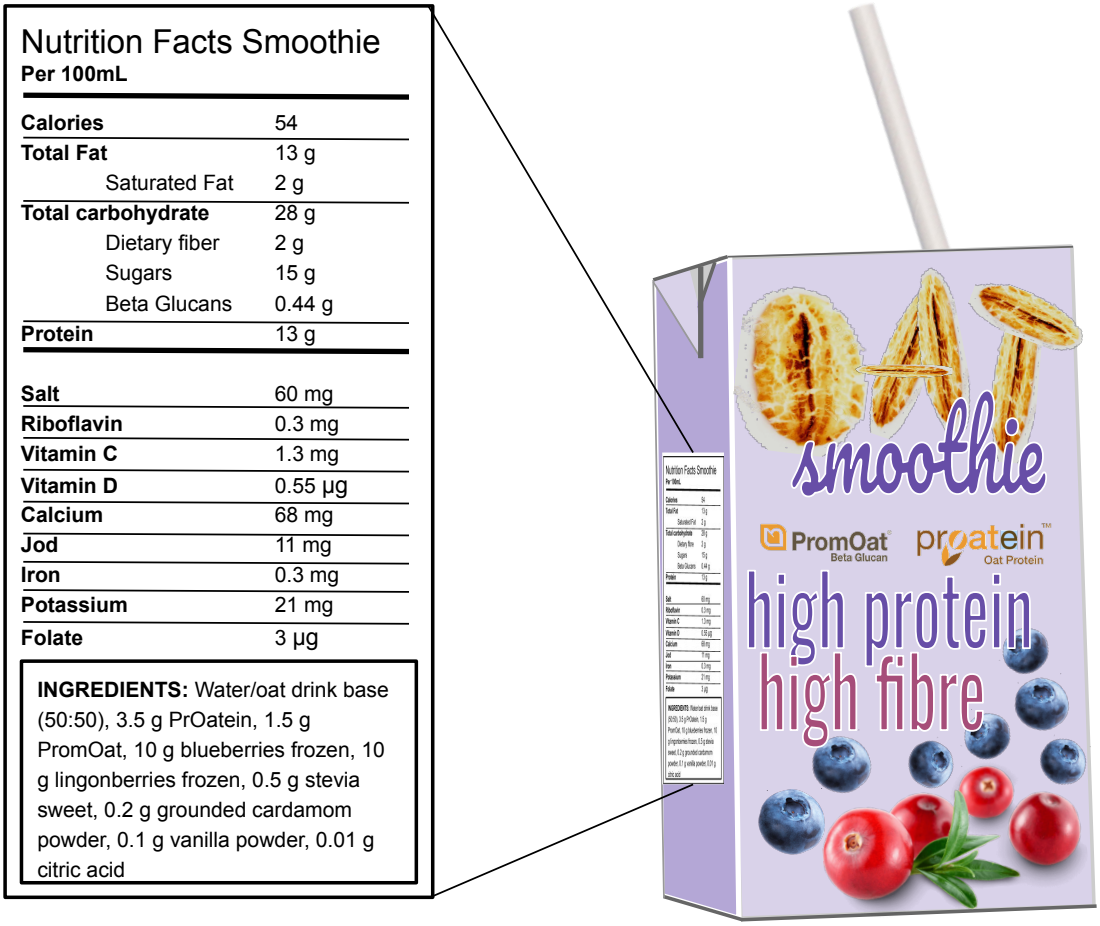
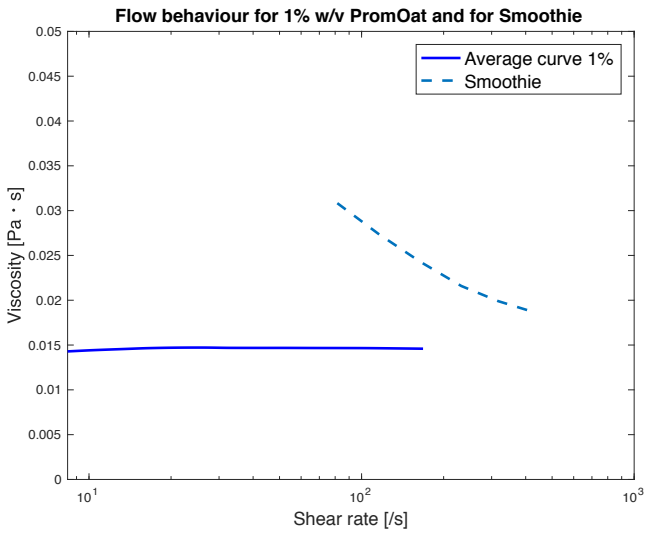


Figure 4.25. Nutritional value of the smoothie and final recipe together with a prototype packaging.

Table 4.7. Data about the developed smoothie.

pH	4.07
Density	898 kg/m^3
Viscosity at shear rate 2 s^{-1}	46.1 mPa · s
Rheological behavior	Shear thinning
Yield stress	No
Dilutable in water	Yes



The final product is high in fiber and protein and with a serving size of 300 mL it will give approximately half of the amount of β-glucans recommended/day to maintain normal cholesterol levels. The smoothie is easily drinkable and can be preferably consumed with a straw. When consumed, the taste from the lingonberries and blueberries will quickly satisfy the initial perception of the product, contributing with a lot of taste and a natural sourness. The sourness is balanced by the sweetness from stevia and sweet aroma from the vanilla powder. Slightly after

swallowing, the taste of cardamom will spread in the mouth, which effectively masks the aftertaste from the oat protein and stevia and the final sensory experience is accomplished. The masking of the off-taste is however something that could be adjusted further and may be something that consumers find undesired with the product. Due to the health benefits associated with the product, the smoothie is still considered competitive on the market and should be promoted as a high protein and high fiber product with Nordic ingredients and taste. The pungency, sourness, saltiness, bitterness and astringency counteract the sweetness of the product in different ways and are described as vectors in *Figure 4.26.*

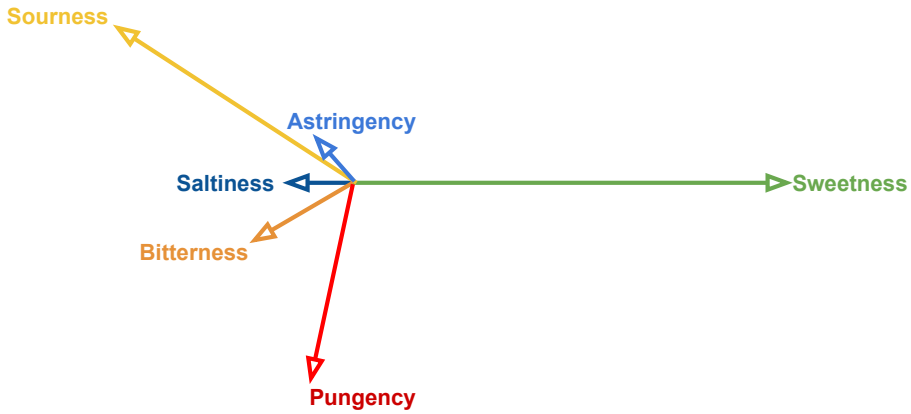


Figure 4.26. The taste parameters sweetness, pungency, sourness, saltiness, bitterness and astringency are described as vectors for the smoothie.

The appearance of the smoothie can be seen in *Figure 4.27.* The smoothie will after 1-2 days give rise to some phase separation, where a water layer will form beneath the major part of the fluid. By only flipping the container upside down the two layers are homogeneous again. Due to this, the product shall have a note on the package indicating that it should be shaken before consumption.

Figure 4.27. Visual appearance of the smoothie after storage (left), when quickly shaken (middle) and from above (right).

4.8.3.3 Development of pudding

A base of 4.9% w/v homogenised PrOatein in oat drink combined with 3.2% w/v PromOat was used to develop a pudding with coffee mocha (chocolate and coffee) taste. The pudding is suitable to be consumed as a snack or dessert, thus more round and sweet flavors were chosen before fruity and sour flavors. Coffee was added to aim for a more mature perception of the product.

The steps of the product development can be seen in *Table 4.8.* The sweetness, bitterness, masking off-taste and flavor balance were evaluated from a scale from 1-5; 1: Dislike very much, 2: Dislike, 3: Moderate, 4: Like and 5: Like very much. The overall perception is the mean value of the scores of the different parameters. The modification and the motivation for the modification of the product development steps is also noted in the table. The improvement from the initial product to the final product can be seen in *Figure 4.28.*

Table 4.8. Pudding product development steps. The different parameters were scored from 1-5 (1: Dislike very much, 2: Dislike, 3: Moderate, 4: Like and 5: Like very much). The overall perception is the mean value of the different parameters.

Product development step	Modification	Motivation to modification	Sweetness	Bitterness	Masking off-taste	Flavor balance	Overall perception
Initial	No added flavors. 4.9% w/v PrOatein, 3.2% w/v PromOat		1	4	1	1	**1.75**
1	Added coffee, cacao powder and stevia	Mask off-taste from cereals, flavor and sweetness contribution	4	4	3	2.5	**3.38**
2	Added more coffee and cacao powder	Need to mask off-taste from cereals more, the product needed more flavor	4	3	4	3	**3.50**
3	Less stevia and more agave to mask aftertaste	Attempt to mask aftertaste from sweetener	4	3	4	3	**3.50**
4	Less stevia and added vanilla powder	Attempt to mask aftertaste from sweetener as well as the bitterness from the coffee and cacao	4	4	5	3.5	**4.13**
Final	Kept initial amount of stevia and the vanilla powder	The vanilla contributed to a better flavor balance and there was a need to keep the initial sweetness	5	5	5	4	**4.75**

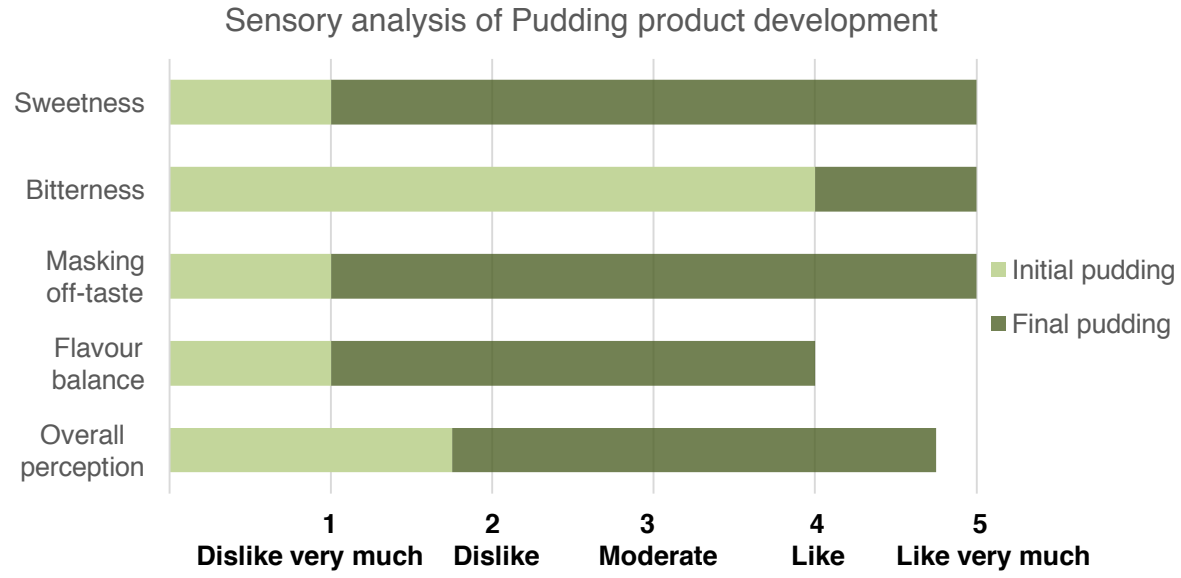


Figure 4.28. The improvement from the initial product to the final product of the pudding.

The nutritional value, the ingredients list and a prototype packaging describing the final product is seen in *Figure 4.29* and data about the pH, viscosity and rheological behavior can be seen in *Table 4.9.* The viscosity at 2 s^{-1} is chosen as it is the shear rate in the mouth when biting and chewing.

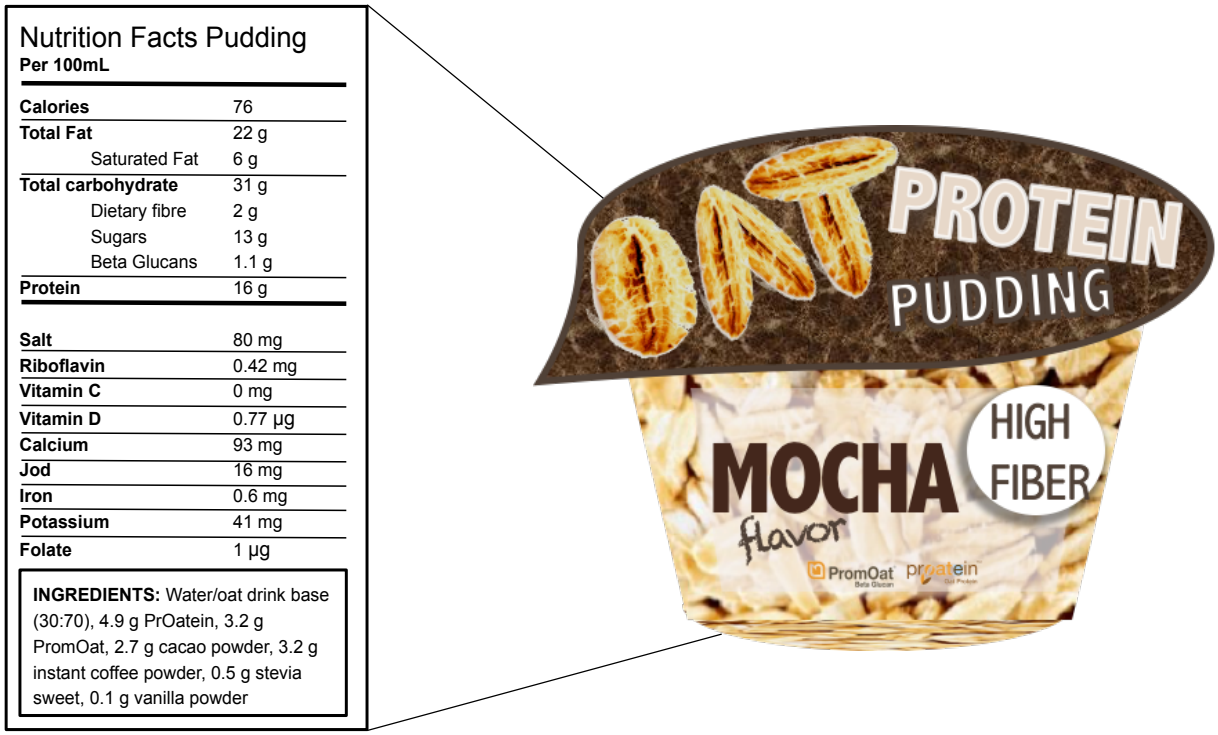
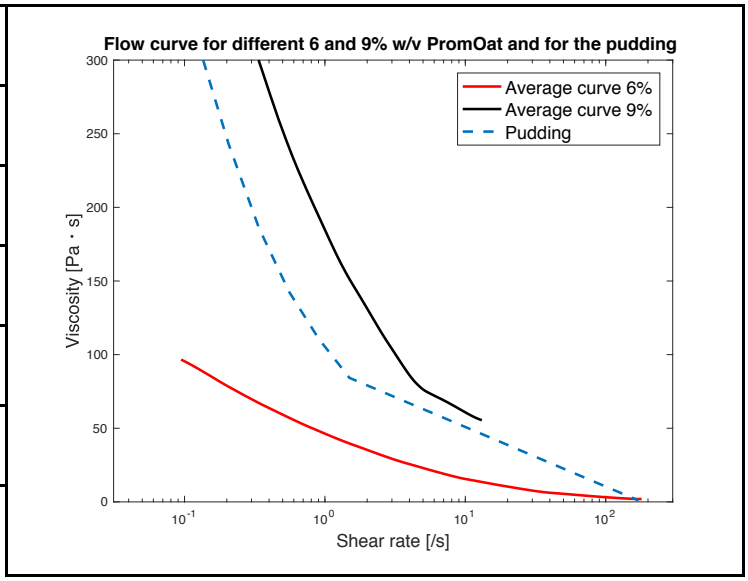


Figure 4.29. Nutritional value and final recipe of the pudding and a prototype packaging.

Table 4.9. Data about the developed pudding.

pH	5.02
Density	1110 kg/m^3
Viscosity at shear rate 2 s^{-1}	82.4 Pa · s
Rheological behavior	Shear thinning
Yield stress	Yes
Dilutable in water	Yes



The flow behavior of the pudding, seen in the figure above, is somewhere in between the flow curves of previously analysed 6% and 9% w/v PromOat. At a shear rate of 1 s^{-1}, the viscosity is approximately 50 times higher for the pudding than for PromOat at 3% w/v which clearly indicates that PromOat in combination with PromOat has an significant increase in the viscosity. The pudding also seems to have a yield stress. According to the results obtained from the yield stress experiments on PromOat, yield stress is assumed to arise at a concentration >7.4% w/v, which is above the concentration of 3.2% w/v used to prepare the pudding. This insinuates that the particles from PrOatein also contribute to the formation of the gel structure in the system. It can be believed that the PrOatein

particles have formed an aggregated structure in between the fibers from PromOat, enhancing the gel formation in the system. This could be the result of the phenomena depletion flocculation. If the polymers from PromOat do not have very high affinity to the particle surfaces of PrOatein, they will not adsorb to the surfaces but create depletion zones in the interface between the surface and the solution. If depletion zones of different particles overlap and if the distance between the particles is smaller than the diameter of the polymers, it can induce aggregation of the particles from PrOatein and increase the viscosity.

The final product is high in fiber and protein and with a serving size of 300 mL it will exceed the recommended/daily intake of β-glucans to maintain normal cholesterol levels. The pudding is preferably consumed with a spoon. The sensory experience is described by a rather strong and mature taste from the coffee and cacao which effectively masks the off-taste from the cereals. The bitterness is balanced by sweetness from stevia and the aroma from the vanilla powder. It is possible to dilute the pudding in water, which gives a smooth mouthfeel when diluted with the saliva in the mouth. There is still some sensation of filmyness of the pudding, but this sensation is not necessarily negative in a pudding application. The reason for the flavor balance not scoring a 5 (like very much) is due to the aftertaste from the stevia and is something that could be adjusted further. The product should be promoted as a healthy and delicious snack or dessert and should be a very competitive product on the market due to the high fiber and protein content. The pungency, sourness, saltiness, bitterness and astringency counteract the sweetness of the product in different ways and are described as vectors in *Figure 4.30.*

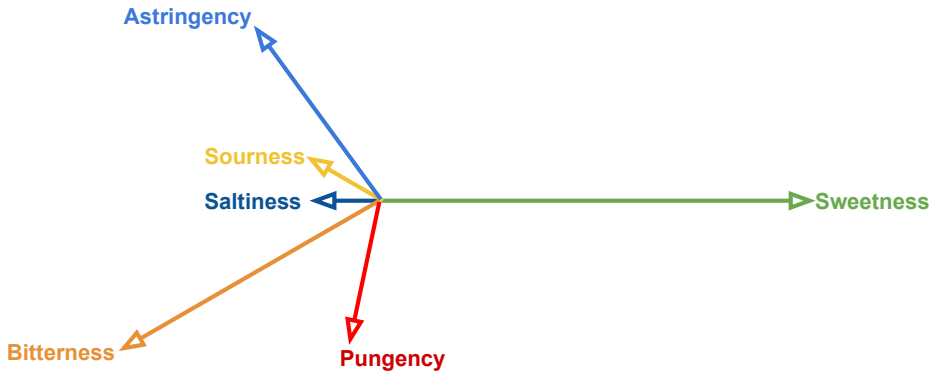


Figure 4.30. The taste parameters sweetness, pungency, sourness, saltiness, bitterness and astringency are described as vectors for the pudding.

The visual appearance of the pudding can be seen in *Figure 4.31.* The gel formed is very stable and when being centrifuged, the pudding remains intact, only small vanilla powder particles can be visualised at the bottom of the centrifuged tube. The pudding appears very smooth. When pressure is applied by for example the bottom side of a spoon, the pudding is rubbery and elastic and when being stuck by a knife there will be a visible cut in the gel which after some time disappears.

Figure 4.31. Visual appearance of the pudding after centrifugation (left), from above (middle left), from above when stuck by a knife (middle right) and from the side (left).

4.8.3.4 Final product comparison

In order to be able to see whether the two products that were developed by PromOat and PrOatein will be competitive products on the market, a comparison between existing products has been done, seen in *Figure 4.32*. The products chosen for the comparison are products that are comparable with the smoothie and the pudding with the major differences in the protein sources used (peas, soy, oats or milk). There are many other products in which the products could be comparable, but the following analysis has been made only to provide a brief overview of how the developed products can find their place on the market. Green dots (🟢) shows that the product satisfies the criteria to the left, yellow dots (🟡) shows that it somewhat lives up to the criteria and red dots (🔴) indicates that it does not live up to the criteria. The settings for the criteria can be seen in *Table 4.10.*

Table 4.10. Setting criteria for the market comparison.

	🟢	🟡	🔴	**Note**
High protein	>20 E% comes from protein	10-20 E% comes from protein	<10 E% comes from protein	For a product to be claimed high protein, the protein shall provide >20 E% (Nutrition claims - Food Safety - European Commission, 2020). The other ranges are set for comparison.
High fiber	The product has >3g fiber/100 kcal product	The product has 0-3g fiber/100 kcal product	The product contains no fiber	For a product to be claimed high fiber, it shall contain >3 g fiber/100 kcal (Nutrition claims - Food Safety - European Commission, 2020). The other ranges are set for comparison.
Low in calories	<20 kcal per 100 mL	20-50 kcal per 100 mL	>50 kcal per 100 mL	For a beverage to be claimed low in energy, it shall contain <20 kcal/100 mL (Nutrition claims - Food Safety - European Commission, 2020). The other ranges are set for comparison.
No added sugars	Contains no added sugars	-	Has added sugars	Sweeteners are not counted as sugars.
Plant based	Origin of ingredients are plant based	-	Origin of ingredients are not plant based	
Possibility to be locally produced	It is possible to produce all the majority of the ingredients in Sweden	Contains some ingredients that cannot be produced in Sweden	The majority of the ingredients cannot be produced in Sweden	Only considers the main ingredients and not flavor additives like agave syrup and cardamom.
Low carbon footprint	<0.6 kg CO_2 per kg protein source	0.6-1 kg CO_2 per kg protein source	1 kg CO_2 per kg protein source	Values are taken from Mat-klimat-listan (Röös, 2012). Oats are counted as grains, soy and peas as legumes. This is based on complex calculations and the values are only approximate.
Price	<20 SEK/L	20-30 SEK/L	>30 SEK/L	Based on prices from ICA (Handla mat online, 2020). The prices for the smoothie and pudding are speculative.

	Smoothie	Pudding	Alpro Protein Chocolate	Nije Propud Chocolate Pudding	Arla Protein Mjölkdryck Blåbär	Oatly Oatdrink	Sproud Chocolate Drink
							
High protein							
High fiber							
Low in calories							
No added sugars							
Plant based							
Possibility to be locally produced							
Low carbon footprint							
Price							

Figure 4.32. Market comparison of the developed smoothie and pudding, together with existing products on the market; Alpro Protein Chocolate (Soya High Protein Chocolate, 2020), Nije Propud Chocolate Pudding (Protein Pudding Chocolate 200 g — NJIE (EN), 2020), Arla Protein Mjölkdryck Blåbär (Arla® Protein Mjölkdryck Blåbär 5 dl, 2020), Oatly Oatdrink (Havredryck | Oatly | Sweden, 2020) and Sproud Chocolate Drink (SPROUD CHOCOLATE - Sproud, 2020).

Generally, what makes the smoothie and the pudding unique is their high content in both protein and fiber, which is not present in any other of the products in the comparison. Compared to other additional products, no plant based products that are considered both high protein and high fiber are present on the Swedish market today. Two of the products in the comparison (Nije Propud Chocolate Pudding and Alpro Protein Chocolate) contain carrageenan, cellulose gum and xanthan gum which are added to obtain a desired viscosity and to stabilise the system. Addition of these ingredients may be undesired from a consumers point of view due to an arising trend of more neutral products. The addition of PromOat satisfies the need of stability and altered viscosity, but will also contribute to the nutritional value of the product and will therefore not influence these possible concerns. The price is the potential drawback of the product and has not been analysed thoroughly. The smoothie will most likely have a high price with the current recipe as it contains a large amount of berries. This is something that can be modified for future development by adding flavorings that keep the good flavor but enable the berry content to be less. However, a high berry content will also add to the nutritional value of the product as it contains a lot of vitamins and minerals.

5. Conclusions

5.1 PrOatein

Analysis of the protein solubility of PrOatein was performed on both homogenised and non-homogenised at pH ranges of 3.2-6.4 and ionic strengths between 7-1000 mmol/L. The solubility showed no statistically significant differences of the pH dependence without addition of salt for the non-homogenised samples. For the homogenised samples, the solubility at pH 6.42 was statistically significantly higher than the solubility at pH 5.24 and 4.80. When increasing the ionic strength, while not regulating the pH (pH 6.42), there were no statistically significant differences in solubility for any of the samples. At different pHs, the homogenised samples had a statistically significant ionic strength dependence at pH 3.15, 4.80 and 5.24, but not at pH 4.32. Overall, the solubility of PrOatein is low, ≈5% soluble protein of total protein in the powder, and the pH and ionic strength have little or no significant effect on the protein solubility within the range that are reasonable to use in food applications.

Homogenisation gave a statistically significant increase in solubility from 4 to 6% soluble protein of total protein in the powder. It also decreased the particle size from approximately 60 µm to <10 µm. At a PrOatein concentration of 5 and 10% w/v, homogenisation increased the time to reach total sedimentation height from about 5 hours to >100 hours. It also decreased the amount of PrOatein needed to form a full aggregated structure and a stable gel.

The water holding capacity of PrOatein was 2.1 g water/g PrOatein and 4.5 g water/g protein in the powder. The latter being in the middle range of 2-7 g water/g protein that is common for proteins. Conclusions regarding the WHC of the oat proteins are somewhat hard to draw due to the presence of many other compounds in the powder.

In food products where high protein solubility is required, PrOatein is not considered a suitable ingredient. However, it is believed to have good potential as an ingredient in a food product in which the stability of the dispersion can be aided by stabilising agents or homogenisation. PrOatein also adds to the nutritional quality of a product as the protein content contains a high amount of essential amino acids.

5.2 PromOat

Rheological analysis on PromOat showed Newtonian flow behavior at a concentration of 1% w/v with a viscosity of approximately 14 mPa·s. Shear thinning behaviors appeared at concentrations of 3, 6 and 9% w/v, and viscosity increased with increasing concentration. Differences in pH and ionic strength had no effect on the flow behavior of the samples. After interpretation of the results, an approximate yield stress was present at a concentration of 7.4% w/v or higher. However, the deficiencies in the experimental set up makes it possible to question the accuracy of the result.

PromOat is an effective thickening agent, which makes it suitable as a stabilising agent in unstable dispersions or as a fat replacer in low fat products. It is also considered a suitable gelling agent in high viscosity products such as puddings. From a nutritional point of view, PromOat can contribute well to the fiber-, in particular the β-glucan, content and opens possibilities for the producer to set nutritional claims for the product.

5.3 Product Development

Based on the results from the functionality analysis of PromOat and PrOatein, a smoothie and a pudding were decided to be the best options for product development. It was concluded that at a high PrOatein concentration, >5% w/v, a astringent tendency was noted, but this could be masked by an increasing PromOat content. The downside with an increasing PromOat content however, was that it gave a sensation of filmyness in the mouth. The filmyness could however somewhat be masked by an increased amount of PrOatein. A suitable viscosity for each product, as well as a pleasant mouthfeel, was developed by combining the two powders. PromOat and

PrOatein were added in sufficient amounts for both of the products to be claimed as high protein and high fiber products.

The smoothie had a PrOatein concentration of 3.5% w/v and a PromOat concentration of 1.5% w/v. The best flavor combination was concluded to be blueberry and cardamom. The blueberry gave a nice freshness and acidity to the product while the spiciness from the cardamom worked well by masking the slight hint of cereal flavor from the powders. Vanilla powder was added to give more of a roundness to the taste. Stevia was used as sweetener to avoid the high calorie contribution from sugar. This was necessary to maintain the high protein and fiber claim of the product. The smoothie had a pH of 4.07, a viscosity of 46.1 mPa · s at a shear rate of 2 s-1 and showed no yield stress.

The pudding had a PrOatein concentration of 4.9% w/v and a PromOat concentration of 3.2% w/v. The best flavor combination for the pudding was concluded to be cacao and coffee to obtain a coffee mocha flavor. The pudding is intended to be used as a snack or dessert, thus more round and sweet flavors were aimed for and the coffee flavor was intended to give the product a more mature perception. Vanilla powder was added to balance the bitterness from the coffee and the cacao. As for the smoothie, stevia was used as sweetener to avoid the high calorie contribution from sugar. The pudding had a pH of 5.02, a viscosity of 82.4 Pa · s at a shear rate of 2 s^{-1} and showed a yield stress.

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Appendixes

Appendix A. Titration data for PrOatein and PromOat

Table A.1. Raw data for the pH at increasing amount of HCl for PrOatein and PromOat dispersions..

Homogenised (2 recirculations)		Homogenised (5 recirculations)		Homogenised (7 recirculations)		Non-homogenised (1)		Non-homogenised (2)		PromOat	
µmol HCl/g PrOatein	pH	µmol HCl/g PrOatein	pH	µmol HCl/g PrOatein	pH	µmol HCl/g PrOatein	pH	µmol HCl/g PrOatein	pH	µmol HCl/g PromOat	pH
0	6.5	0	6.43	0	6.37	0	6.44	0	6.37	0	5.95
1	6.49	4	6.38	4	6.31	3.33	6.35	4	6.31	7.5	5.75
2	6.46	8	6.31	6	6.29	10	6.24	6	6.29	10	5.61
4	6.44	12	6.23	8	6.26	13.33	6.2	8	6.26	15	5.54
6	6.41	16	6.17	10	6.23	16.67	6.18	10	6.23	20	5.38
8	6.38	20	6.09	12	6.21	20	6.1	12	6.21	25	5.25
10	6.35	24	6.04	14	6.18	23.33	6.04	14	6.18	30	5.1
12	6.33	28	5.97	16	6.15	26.67	6	16	6.15	35	4.95
14	6.3	32	5.91	18	6.12	30	5.96	18	6.12	40	4.84
16	6.27	36	5.85	20	6.08	33.33	5.9	20	6.08	50	4.64
18	6.24	40	5.79	22	6.07	36.67	5.82	22	6.07	55	4.5
20	6.21	44	5.73	24	6.03	40	5.77	24	6.03	60	4.38
22	6.19	48	5.67	26	6	43.33	5.72	26	6	70	4.22
24	6.16	54	5.59	28	5.97	46.67	5.64	28	5.97	80	4.03
26	6.13	60	5.49	30	5.95	50	5.63	30	5.95	90	3.87
28	6.11	70	5.34	32	5.91	53.33	5.59	32	5.91	100	3.73
30	6.08	80	5.3	34	5.88	56.67	5.55	34	5.88	110	3.6
32	6.05	90	5.3	36	5.86	60	5.5	36	5.86	120	3.5
34	6.02	100	5.3	38	5.83	63.33	5.46	38	5.83	130	3.4
36	6	110	5.3	40	5.8	66.67	5.44	40	5.8	140	3.31
38	5.98	120	5.22	44	5.73	71.67	5.4	44	5.73	150	3.23
40	5.95	130	5.15	48	5.66	73.33	5.39	48	5.66	160	3.17
42	5.92	140	5.08	52	5.6	76.67	5.4	52	5.6	170	3.12
44	5.9	150	5.02	56	5.56	83.33	5.35	56	5.56	180	3.06
46	5.87	160	4.98	60	5.49	90	5.3	60	5.49	190	3.01
48	5.85	170	4.98	64	5.44	96.67	5.26	64	5.44	205	2.94
50	5.81	180	4.94	68	5.4	103.33	5.24	68	5.4	220	2.88
52	5.79	190	4.88	72	5.34	110	5.22	72	5.34	235	2.83
56	5.73	200	4.81	76	5.3	116.67	5.23	76	5.3	250	2.78
60	5.69	210	4.74	80	5.25	126.67	5.16	80	5.25	265	2.74
64	5.64	220	4.69	84	5.25	136.67	5.12	84	5.25	280	2.7
68	5.59	230	4.63	92	5.25	146.67	5.06	92	5.25	295	2.66
72	5.54	240	4.56	100	5.25	156.67	5.02	100	5.25	310	2.63
76	5.49	250	4.5	110	5.23	166.67	4.98	110	5.23	325	2.59
80	5.45	260	4.44	120	5.17	180	4.92	120	5.17	345	2.56
84	5.41	270	4.38	130	5.13	193.33	4.88	130	5.13	365	2.52
88	5.37	280	4.31	140	5.09	206.67	4.83	140	5.09	385	2.48
92	5.32	290	4.24	150	5.05	220	4.75	150	5.05	410	2.45
96	5.27	300	4.17	160	5	233.33	4.67	160	5	435	2.41
100	5.24	310	4.09	170	4.95	246.67	4.58	170	4.95		
104	5.22	320	4.01	180	4.91	260	4.51	180	4.91		
112	5.15	330	3.92	190	4.84	273.33	4.41	190	4.84		
120	5.08	340	3.84	200	4.8	286.67	4.31	200	4.8		
128	5.02	350	3.75	210	4.75	300	4.22	210	4.75		
136	4.99	360	3.65	220	4.68	313.33	4.12	220	4.68		
144	4.95	370	3.57	230	4.64	326.67	4.01	230	4.64		
152	4.9	380	3.49	240	4.59	340	3.88	240	4.59		
160	4.86	390	3.41	250	4.54	353.33	3.78	250	4.54		
168	4.82	400	3.33	260	4.5	366.67	3.66	260	4.5		
176	4.82	410	3.26	270	4.44	380	3.55	270	4.44		
188	4.77	420	3.19	280	4.36	393.33	3.45	280	4.36		
200	4.72	430	3.13	290	4.29	406.67	3.35	290	4.29		
212	4.68	440	3.06	300	4.22	420	3.27	300	4.22		
224	4.63	460	2.96	310	4.14	433.33	3.19	310	4.14		
236	4.58	470	2.91	320	4.07	446.67	3.12	320	4.07		
248	4.53	480	2.86	330	3.99	460	3.05	330	3.99		
264	4.45	490	2.81	340	3.9	473.3	2.99	340	3.9		
280	4.34	500	2.77	350	3.82	486.67	2.93	350	3.82		
296	4.22			360	3.73	500	2.88	360	3.73		

312	4.12		370	3.65	513.33	2.83	370	3.65	
328	3.99		380	3.56	526.67	2.78	380	3.56	
344	3.85		390	3.48	540	2.74	390	3.48	
352	3.79		400	3.4	553.33	2.7	400	3.4	
360	3.73		410	3.33	566.67	2.66	410	3.33	
368	3.66		420	3.25	580	2.62	420	3.25	
376	3.59		430	3.19	593.33	2.59	430	3.19	
384	3.53		440	3.13	606.67	2.56	440	3.13	
392	3.47		450	3.07	620	2.53	450	3.07	
400	3.4		460	3.02	633.33	2.5	460	3.02	
408	3.35		470	2.96	646.67	2.47	470	2.96	
416	3.29		480	2.91	660	2.45	480	2.91	
424	3.23		490	2.87	673.33	2.42	490	2.87	
432	3.18		500	2.82	686.67	2.4	500	2.82	
440	3.13								
448	3.09								
460	3.02								
472	2.95								
480	2.92								
490	2.87								
500	2.83								
512	2.77								
522	2.72								
532	2.68								
542	2.64								
552	2.6								
562	2.56								
572	2.53								
582	2.49								
592	2.44								
602	2.42								
612	2.38								
640	2.25								

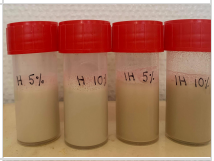
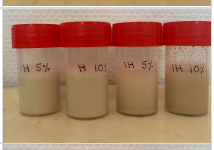
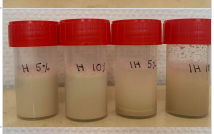
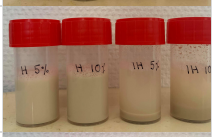
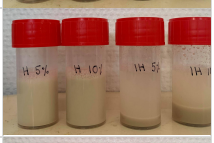
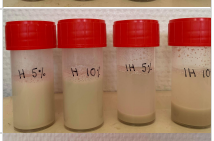
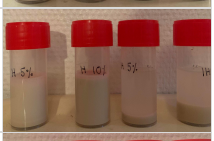
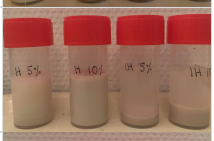
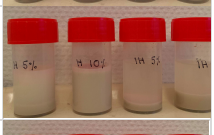
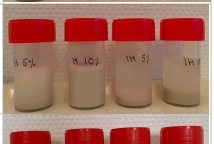
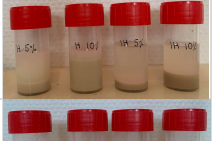
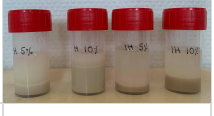
Appendix B. Particle size determination

Table B.1. Raw data for particle size determination..

Homogenised PrOatein					**Non-homogenised PrOatein**				
Measured diameter, *d* [mm]	**Amount of particles, *n***	**Sum of volume [mm^3]**	**d·sum of volume**		**Measured diameter, *d* [mm]**	**Amount of particles, *n***	**Sum of volume [mm^3]**	**d·sum of volume**	
0.0148	2	0.00000342	0.000000050739		0.0715	3	0.000576	0.0000412	
0.00742	2	0.000000427	0.00000000317		0.0557	7	0.000632	0.0000352	
0.00593	4	0.000000438	0.00000000260		0.0368	4	0.000102	0.00000375	
0.00445	78	0.00000360	0.0000000160		0.0318	4	0.0000674	0.00000214	
0.00297	30	0.000000410	0.00000000122		0.0286	8	0.00009823	0.00000281	
0.00223	38	0.000000219	0.000000000488		0.0223	6	0.0000347	0.000000772	
0.001484	107	0.000000183	0.000000000271		0.0159	7	0.0000147	0.0000002345	
0.000742	217	0.000000046	0.0000000000344		0.009542	7	0.00000318	0.0000000304	
Sum:	478	0.00000875	0.00000007455		0.00795	8	0.00000210603	0.0000000167	
			d(4.3) [nm]:	**8.52**	0.00636	8	0.0000010783	0.00000000686	
					0.00318	35	0.000000590	0.00000000188	
					Sum:	46	0.00153	0.0000862	
								d(4.3) [nm]:	**56.2**

Appendix C. Sedimentation Analysis

Table C.1. Raw data of sedimentation height over time for different PrOatein samples.

Time [h]	Sediment height [mm]				Picture
	Homogenised 5% w/v	Homogenised 10% w/v	Non-homogenised 5% w/v	Non-homogenised 10% w/v	
$1 \cdot 10^{-10}$	0	0	0	0	
0.667	0	0	0.15	0.15	
0.125	0	0	4	6	
0.25	0	0	5	8	
0.75	0	0	5.5	10	
1.75	1	0	5.5	11	
2.75	1.5	0	5.5	11.5	
4.75	4	0	6	12	
8.75	6	0	6	12	
16.75	6.5	0	6	12	
29.25	6.5	0	6	12	
149.25	9	24	6	12	
400	9	22	6	12	
Initial height of tube [mm]	31	31	34	33	

Appendix D. Statistics

D.1 pH dependence

D.1.1 One-Way Anova, Excel

Table D.1. Result from a One-Way Anova analysis in Excel: comparison between homogenised and non-homogenised samples at pH 6.42.

SAMMANFATTNING						
Grupper	*Antal*	*Summa*	*Medelvärde*	*Varians*		
Icke-Homogeniserat	20	136,3611	6,818055	5,049996		
Homogeniserat	49	211,5093	4,316516327	0,8989		
ANOVA						
Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Mellan grupper	88,87742	1	88,87741768	42,81029	9,87E-09	3,984049
Inom grupper	139,0971	67	2,076076164			
Totalt	227,9745	68				

Table D.2. Result from a One-Way Anova analysis in Excel: comparison between homogenised and non-homogenised samples at pH 5.24.

Anova: En faktor						
SAMMANFATTNING						
Grupper	*Antal*	*Summa*	*Medelvärde*	*Varians*		
Icke-Homogenisera	8	36,3545	4,544313	2,076367		
Homogeniserat	8	32,5114	4,063925	0,323164		
ANOVA						
Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Mellan grupper	0,923089	1	0,923089	0,769391	0,395209	4,60011
Inom grupper	16,79671	14	1,199765			
Totalt	17,7198	15				

Table D.3. Result from a One-Way Anova analysis in Excel: comparison between homogenised and non-homogenised samples at pH 4.80.

SAMMANFATTNING						
Grupper	*Antal*	*Summa*	*Medelvärde*	*Varians*		
Icke-Homogeniserat	4	14,4909	3,622725	0,058158		
Homogeniserat	4	15,9275	3,981875	0,077005		
ANOVA						
Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Mellan grupper	0,257977	1	0,257977445	3,817266	0,098541	5,987378
Inom grupper	0,40549	6	0,067581729			
Totalt	0,663468	7				

Table D.4. Result from a One-Way Anova analysis in Excel: comparison between homogenised and non-homogenised samples at pH 4.32.

Anova: En faktor						
SAMMANFATTNING						
Grupper	*Antal*	*Summa*	*Medelvärde*	*Varians*		
Icke-Homogenisera	4	15,1745	3,793625	0,021409		
Homogeniserat	8	45,6351	5,7043875	4,061659		
ANOVA						
Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Mellan grupper	9,736036	1	9,73603555	3,416652	0,094289	4,964603
Inom grupper	28,49584	10	2,849583808			
Totalt	38,23187	11				

Table D.5. Result from a One-Way Anova analysis in Excel: comparison between homogenised and non-homogenised samples at pH 3.15.

Anova: En faktor						
SAMMANFATTNING						
Grupper	*Antal*	*Summa*	*Medelvärde*	*Varians*		
Icke-Homogeniserat	8	34,4127	4,3015875	0,18387		
Homogeniserat	8	38,3929	4,7991125	0,017314		
ANOVA						
Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Mellan grupper	0,990125	1	0,990124503	9,843003	0,007272	4,60011
Inom grupper	1,408284	14	0,100591714			
Totalt	2,398409	15				

Table D.6. Result from a One-Way Anova analysis in Excel: pH dependence on non-homogenised samples.

SAMMANFATTNING						
Grupper	*Antal*	*Summa*	*Medelvärde*	*Varians*		
6,42	49	249,2814	5,08737551	4,229537		
5,24	8	32,5114	4,063925	0,323164		
4,8	4	15,9275	3,981875	0,077005		
4,32	8	45,6351	5,7043875	4,061659		
3,15	8	38,3929	4,7991125	0,017314		
ANOVA						
Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Mellan grupper	15,68511625	4	3,921279063	1,206219	0,315678	2,498919
Inom grupper	234,0637699	72	3,250885693			
Totalt	249,7488862	76				

Table D.7. Result from a One-Way Anova analysis in Excel: pH dependence on homogenised samples.

SAMMANFATTNING						
Grupper	*Antal*	*Summa*	*Medelvärde*	*Varians*		
6,42	20	136,3611	6,818055	5,049996		
5,24	8	32,5114	4,063925	0,323164		
4,8	4	15,9275	3,981875	0,077005		
4,32	8	45,6351	5,7043875	4,061659		
3,15	8	38,3929	4,7991125	0,017314		
ANOVA						
Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Mellan grupper	64,24121	4	16,06030223	5,437916	0,001238	2,588836
Inom grupper	126,9959	43	2,953392785			
Totalt	191,2371	47				

D.1.2 Tukey-Kramer Post Hoc

Table D.8. Result from the Tukey-Kramer Post Hoc test in Excel: pH dependence on homogenised samples.

Tukey-Kramer Post Hoc					α	0.05	
					q_{crit}	4.04	
Kombinationer		Difference	*n* (Group 1)	*n* (Group 2)	*SE*	*q*	Results
6,42	5,24	2,75413	20	8	0,508352	5,417761	> q_{crit}
6,42	4,8	2,83618	20	4	0,665589	4,261157	> q_{crit}
6,42	4,32	1,1136675	20	8	0,508352	2,19074	< q_{crit}
6,42	3,15	2,0189425	20	8	0,508352	3,971543	< q_{crit}
5,24	4,8	0,08205	8	4	0,744151	0,11026	< q_{crit}
5,24	4,32	1,6404625	8	8	0,607597	2,699919	< q_{crit}
5,24	3,15	0,7351875	8	8	0,607597	1,209992	< q_{crit}
4,8	4,32	0,48	4	8	0,744151	0,64503	< q_{crit}
4,8	3,15	1,65	4	8	0,744151	2,217291	< q_{crit}
4,32	3,15	1,17	8	8	0,607597	1,925619	< q_{crit}

D.2 Ionic strength dependence

D.2.1 One-Way Anova, Excel

Table D.9. Result from a One-Way Anova analysis in Excel: comparison between homogenised and non-homogenised samples at 7.36 mmol/L.

Anova: En faktor

SAMMANFATTNING

Grupper	*Antal*	*Summa*	*Medelvärde*	*Varians*
Homogeniserat	20	136,3611	6,818055	5,049996
Icke-Homo	49	211,5093	4,316516	0,8989

ANOVA

Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Mellan grupper	88,87742	1	88,87742	42,81029	9,87E-09	3,984049
Inom grupper	139,0971	67	2,076076			
Totalt	227,9745	68				

Table D.10. Result from a One-Way Anova analysis in Excel: comparison between homogenised and non-homogenised samples at 57.4 mmol/L.

Anova: En faktor						
SAMMANFATTNING						
Grupper	*Antal*	*Summa*	*Medelvärde*	*Varians*		
Homogeniserat	8	41,2828	5,16035	0,898912		
Icke-Homogeniserat	4	15,3049	3,826225	0,020547		
ANOVA						
Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Mellan grupper	4,746372	1	4,74637204	7,469864	0,021081	4,964603
Inom grupper	6,354027	10	0,63540271			
Totalt	11,1004	11				

Table D.11. Result from a One-Way Anova analysis in Excel: comparison between homogenised and non-homogenised samples at 507 mmol/L.

SAMMANFATTNING						
Grupper	*Antal*	*Summa*	*Medelvärde*	*Varians*		
Homogeniserat	8	52,2769	6,5346125	12,32959		
Icke-Homogeniserat	8	38,5105	4,8138125	0,056884		
ANOVA						
Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Mellan grupper	11,84461	1	11,8446106	1,912507	0,188346	4,60011
Inom grupper	86,70534	14	6,19323862			
Totalt	98,54995	15				

Table D.12. Result from a One-Way Anova analysis in Excel: comparison between homogenised and non-homogenised samples at 1007 mmol/L.

SAMMANFATTNING						
Grupper	*Antal*	*Summa*	*Medelvärde*	*Varians*		
Homogeniserat	8	57,8534	7,231675	14,17651		
Icke-Homogeniserat	8	39,3284	4,91605	1,140361		
ANOVA						
Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Mellan grupper	21,44848	1	21,44847656	2,800635	0,116414	4,60011
Inom grupper	107,2181	14	7,658433113			
Totalt	128,6665	15				

Table D.13. Result from a One-Way Anova analysis in Excel: comparison between homogenised and non-homogenised samples at all of the different ionic strengths. .

SAMMANFATTNING						
Grupper	*Antal*	*Summa*	*Medelvärde*	*Varians*		
Icke-Homogenisera	69	304,6531	4,415262319	0,834298		
Homogeniserat	42	259,1456	6,170133333	4,432978		
ANOVA						
Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Mellan grupper	80,40181	1	80,40180595	36,7479	1,97E-08	3,928195
Inom grupper	238,4843	109	2,187929491			
Totalt	318,8861	110				

Table D.14. Result from a One-Way Anova analysis in Excel: ionic strength dependence on non-homogenised samples.

SAMMANFATTNING						
Grupper	*Antal*	*Summa*	*Medelvärde*	*Varians*		
7,361	49	211,5093	4,31651633	0,8989		
57,36	4	15,3049	3,826225	0,020547		
507,4	8	38,5105	4,8138125	0,056884		
1007,4	8	39,3284	4,91605	1,140361		
ANOVA						
Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Mellan grupper	5,142692	3	1,71423067	2,159837	0,101235	2,745915
Inom grupper	51,58954	65	0,79368521			
Totalt	56,73223	68				

Table D.15. Result from a One-Way Anova analysis in Excel: ionic strength dependence on homogenised samples.

SAMMANFATTNING						
Grupper	*Antal*	*Summa*	*Medelvärde*	*Varians*		
7,361	20	136,3611	6,818055	5,049996		
57,36	8	41,2828	5,16035	0,898912		
507,4	7	37,8952	5,4136	2,655615		
1007,4	6	32,0512	5,34186667	1,980034		
ANOVA						
Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Mellan grupper	23,96858	3	7,98952801	2,3081	0,092426	2,858796
Inom grupper	128,0762	37	3,4615179			
Totalt	152,0447	40				

D.3 Different pH and Ionic Strength

D.3.1 One-Way Anova, Excel

Table D.16. Result from a One-Way Anova analysis in Excel: ionic strength dependence on homogenised samples at pH 5.24.

SAMMANFATTNING						
Grupper	*Antal*	*Summa*	*Medelvärde*	*Varians*		
12,06	8	32,5114	4,063925	0,323164		
62,06	4	15,2015	3,800375	0,017759		
512,06	4	16,5927	4,148175	0,004499		
1012,1	4	20,53	5,1325	0,075515		
ANOVA						
Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Mellan grupper	4,240989	3	1,413662921	8,851067	0,001088	3,238872
Inom grupper	2,555467	16	0,159716676			
Totalt	6,796456	19				

Table D.17. Result from a One-Way Anova analysis in Excel: ionic strength dependence on homogenised samples at pH 4.80.

SAMMANFATTNING						
Grupper	*Antal*	*Summa*	*Medelvärde*	*Varians*		
17,16	4	15,9275	3,981875	0,077005		
67,16	4	14,4125	3,603125	0,010926		
517,2	4	17,5905	4,397625	0,050451		
1017,2	4	20,1359	5,033975	0,488158		
ANOVA						
Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Mellan grupper	4,506717	3	1,50223911	9,590701	0,001648	3,490295
Inom grupper	1,87962	12	0,15663497			
Totalt	6,386337	15				

Table D.18. Result from a One-Way Anova analysis in Excel: ionic strength dependence on homogenised samples at pH 4.32.

SAMMANFATTNING						
Grupper	*Antal*	*Summa*	*Medelvärde*	*Varians*		
21,16	8	45,6351	5,7043875	4,061659		
71,16	4	15,3416	3,8354	0,018477		
521,2	4	16,567	4,14175	0,015607		
1021,2	4	17,5347	4,383675	0,141774		
ANOVA						
Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Mellan grupper	12,64916	3	4,21638586	2,32956	0,113089	3,238872
Inom grupper	28,95919	16	1,80994915			
Totalt	41,60834	19				

Table D.19. Result from a One-Way Anova analysis in Excel: ionic strength dependence on homogenised samples at pH 3.15.

SAMMANFATTNING						
Grupper	*Antal*	*Summa*	*Medelvärde*	*Varians*		
30,36	8	38,3929	4,7991125	0,017314		
80,36	4	17,7852	4,4463	0,001618		
530,4	4	16,0762	4,01905	0,021697		
1030,4	4	14,5706	3,64265	0,005466		
ANOVA						
Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Mellan grupper	4,088666	3	1,362888587	105,07	9,69E-11	3,238872
Inom grupper	0,20754	16	0,012971238			
Totalt	4,296206	19				

D.3.2 Tukey-Kramer Post Hoc, Excel

Table D.20. Result from the Tukey-Kramer Post Hoc test in Excel: ionic strength dependence on homogenised samples at pH 5.24.

Tukey-Kramer Post Hoc, homogeniserat								
					α	0.05		
					q crit	4.046		
Kombinationer		Difference	*n* (Group 1)	*n* (Group 2)	*SE*	*q*	Results	
12,06	62,06	0,26355	8	4	0,173052	1,522956	< *q crit*	
12,06	512,06	0,08425	8	4	0,173052	0,486849	< *q crit*	
12,06	1012,1	1,068575	8	4	0,173052	6,17489	> *q crit*	
62,06	512,06	0,3478	4	4	0,199823	1,740542	< *q crit*	
62,06	1012,1	1,332125	4	4	0,199823	6,66653	> *q crit*	
512,06	1012,1	0,984325	4	4	0,199823	4,925988	> *q crit*	

Table D.21. Result from the Tukey-Kramer Post Hoc test in Excel: ionic strength dependence on homogenised samples at pH 4.80.

Tukey-Kramer Post Hoc, Homogeniserat							
						α	0.05
						q_{crit}	4.199
Kombinationer		Difference	*n* (Group 1)	*n* (Group 2)	*SE*	*q*	Results
17,16	67,16	0,37875	4	4	0,197886	1,913984	< q_{crit}
17,16	517,2	0,41575	4	4	0,197886	2,10096	< q_{crit}
17,16	1017,2	1,0521	4	4	0,197886	5,316706	> q_{crit}
67,16	517,2	0,7945	4	4	0,197886	4,014944	< q_{crit}
67,16	1017,2	1,43085	4	4	0,197886	7,23069	> q_{crit}
517,2	1017,2	0,63635	4	4	0,197886	3,215746	< q_{crit}

Table D.22. Result from the Tukey-Kramer Post Hoc test in Excel: ionic strength dependence on homogenised samples at pH 3.15.

Tukey-Kramer Post Hoc, Homogeniserat							
						α	0.05
						q_{crit}	4.046
Kombinationer		Difference	*n* (Group 1)	*n* (Group 2)	*SE*	*q*	Results
30,36	80,36	0,3528125	8	4	0,049316	7,154061	> q_{crit}
30,36	530,4	0,7800625	8	4	0,049316	15,81751	> q_{crit}
30,36	1030,4	1,1564625	8	4	0,049316	23,44986	> q_{crit}
80,36	530,4	0,42725	4	4	0,056946	7,502765	> q_{crit}
80,36	1030,4	0,80365	4	4	0,056946	14,11257	> q_{crit}
530,4	1030,4	0,3764	4	4	0,056946	6,609809	> q_{crit}

Appendix E. Water holding capacity

Table E.1. Values for calculation of the water holding capacity, WHC, of PrOatein.

Sample	Mass tube [g]	Mass PrOatein [g]	Mass wet pellet [g][1]	Mass dry pellet [g][2]	Mass evaporated water [g][3]	WHC [g water/g insolubles][4]	WHC [g water/g protein][5]	Mass bound water [g][6]	WHC [g water/g PrOatein][7]
6%									
1	1.25	0.0619	0.184	0.0555	0.128	2.31	4.27	0.122	1.96
2	1.25	0.0604	0.182	0.0545	0.127	2.34	4.43	0.122	2.01
3	1.25	0.0603	0.199	0.0543	0.145	2.55	5.04	0.139	2.30
9%									
1	1.26	0.0894	0.278	0.0816	0.196	2.40	4.55	0.188	2.11
2	1.25	0.09	0.265	0.0822	0.183	2.23	4.22	0.175	1.95
3	1.23	0.0901	0.278	0.0832	0.195	2.34	4.43	0.188	2.09
						Mean value:	**4.52**	**Mean value:**	**2.07**
						Std:	0.116	**Std:**	0.0482

1. After centrifugation.
2. After centrifugation and heating to constant weight.
3. Mass of wet pellet subtracted by mass of dry pellet.
4. Mass evaporated water divided by mass of dry pellet.
5. Assuming that 96% of the protein in PrOatein is insoluble and a protein content of 55% in the powder.
6. Mass of wet pellet subtracted by the mass of added PrOatein.
7. Mass of bound water divided by mass of added PrOatein.

Appendix F. Yield stress determination with glass bead

Table F.1. Values used for calculations of the yield stress.

Radius, $r_{glass\ bead}$ [m]	0.0025
Volume, $V_{glass\ bead}$ [m^3]	0.0000000654
Area, $A_{glass\ bead}$ [m^2]	0.0000785
Mass, $m_{glass\ bead}$ [kg]	0.00017
Gravitational constant, g [m/s^2]	9.81
Density water, ρ_{water} [kg/m^3]	997

Table F.2. Equations used for calculations of the yield stress.

	Equations
$V_{under\ surface}$ [m^3]	$V_{glass\ bead} \cdot$ % of bead under surface
$F_{gravity,\ glass\ bead}$ [N]	$m_{glass\ bead} \cdot g$
$F_{lifting\ force\ water}$ [N]	$\rho_{water} \cdot V_{under\ surface} \cdot g$
$F_{resulting}$ [N]	$F_{gravity,\ glass\ bead} - F_{lifting\ force\ water}$
Yield stress [Pa]	$F_{resulting}/A_{under\ surface}$

Table F.3. Results for calculations of the yield stress for 10, 11 and 12% w/v PromOat.

% w/v PromOat	**% of bead under surface**	**Yield stress [Pa]**
10	90	15.5
11	72	21.4
12	60	27.3

Appendix G. Amino acid profile

Table G.1. Essential amino acid content [g/100g] of protein isolates of different origin (Gorissen et al., 2018) and for PrOatein (Lantmännen Oats AB, 2020a). Tryptophan was not measured in the study and therefore not presented in the comparison.

Essential amino acid	Soy	Pea	Brown rice	Whey	Egg	PrOatein
	g/100g raw material					
Threonine	2.3	2.5	2.3	5.4	2	2.55
Methionine + Cysteine	0.5	0.5	2.6	2.6	1.8	3.45
Phenylalanine + Tyrosine	5.4	5.4	7.2	4.9	4.1	8.36
Histidine	1.5	1.6	1.5	1.4	0.9	2
Lysine	3.4	4.7	1.9	7.1	2.7	2.91
Valine	2.2	2.7	2.8	3.5	2	4.54
Isoleucine	1.9	2.3	2	3.8	1.6	3.54
Leucine	5	5.7	5.8	8.6	3.6	6.73
Sum:	22.2	26.3	26.1	37.3	18.7	34.08

Appendix H. Measurement data from BCA-analysis of protein solubility

Table H.1. % soluble protein of total protein from all trials and replicates of the BCA-analysis, accompanied with the mean value, for analysis performed at different pH and ionic strengths for homogenised and non-homogenised PrOatein samples at 5% w/v.

		% soluble protein of total protein	
pH	**Ionic strength [mmol/L]**	**Homogenised samples**	**Non-homogenised samples**
6.42	**7.36**	6.5912	2.4378
		7.0828	3.9121
		7.3451	3.8139
		7.4696	4.4574
		5.9839	3.8256
		6.9201	4.011
		8.759	4.148
		7.2513	6.2447
		8.2571	6.6652
		10.5238	6.0366
		12.2952	5.8205
		9.8168	6.3531
		5.4674	6.2037
		4.8747	4.4809
		5.4645	4.4258
		4.7722	4.4114
		4.2879	4.315
		4.2505	5.83
		4.2894	5.5897
		4.6586	5.6066
			5.4667
			4.3801
			4.1395
			4.058
			3.9024
			3.2806
			3.4212
			3.1678
			3.463
			3.5171
			3.6542
			3.7178
			3.53
			3.2498
			4.2967
			4.0315
			3.9941
			4.5752
			3.8652
			4.2655
			4.1462
			4.0047
			3.7694
			3.862
			3.9262
			3.9897
			3.7295
			3.7418

			3.7744
	57.4	3.0066 5.2103 5.7033 5.0747 5.4212 5.3172 6.2755 5.274	3.8297 3.6366 3.8547 3.9839
	507	8.9033 4.2132 5.9385 4.6725 4.8432 4.7956	4.7763 5.0954 4.3722 4.9345 4.8738 4.6429 5.0772
	1010	7.6974 6.4476 4.4234 4.2161 4.5495 4.7172	5.8614 7.0673 4.9946 4.8601 4.2262 4.0917 4.1776 4.0495
5.24	**12.1**	4.4271 3.4982 3.3231 3.3641 4.263 4.5099 4.4308 4.6952	3.5074 7.619 3.1625 3.355 4.2409 4.7097 5.2905 4.4695
	62.1	3.6234 3.8037 3.9458 3.8286	3.7562 3.7524 3.9095 3.9074
	512	4.1165 4.0703 4.2176 4.1883	4.0126 4.2561 3.9124 3.9897
	1010	5.265 5.0447 4.7937 5.4266	5.2269 5.0222 6.3409 4.738
4.80	**17.2**	3.6711 3.959 4.3465 3.9509	3.3451 3.9326 3.6273 3.5859
	67.2	3.5531 3.496 3.7385	3.4283 3.6094 3.5789

		3.6249	3.6155
	517	4.5399 4.6381 4.2103 4.2022	3.9692 3.9344 3.8482 3.8919
	1020	4.6589 6.0811 4.6706 4.7253	5.0085 5.1023 4.9543 5.2112
4.32	**21.2**	9.581 7.8689 4.9875 3.567 5.0601 5.6571 4.6864 4.2271	3.9364 3.7453 3.6103 3.8825
	71.2	4.0386 3.7817 3.767 3.7543	4.7477 4.441 4.0308 4.0308
	521	4.0308 4.3145 4.148 4.0737	4.2637 3.914 5.8657 4.7761
	1020	4.9026 4.0396 4.1905 4.402	4.2549 4.3795 4.693 4.6862
3.15	**30.4**	4.6236 4.6901 4.7347 4.7393 4.8755 4.8029 4.8894 5.0374	4.6432 4.6385 4.6283 4.7364 3.8923 4.3575 3.759 3.7575
	80.4	4.4051 4.4256 4.4571 4.4974	4.3373 4.4114 4.4138 4.3443
	530	4.2344 3.9018 3.9788 3.9612	3.6492 3.6768 3.7433 3.6879
	1030	3.7516 3.5868 3.6161 3.6161	3.5168 3.537 3.4646 3.5443

Appendix I. Raw data for statistical analysis of flow behavior

Table I.1. The viscosity [Pa·s] at shear rates 1, 50 and 100 s^{-1} *for 3% w/v for the different trials prepared with increasing ionic strength of 6.83, 56.8, 507 and 1010 mmol/L, as well as the mean value and the standard error of mean.*

	Ionic strength [mmol/L]			
Shear rate [s-¹]	**6.83**	**56.8**	**507**	**1010**
1	2.211	1.987	1.784	1.893
	1.693	1.796	2.115	1.534
	1.892			
	1.536			
Mean value	1.83	1.89	1.95	1.71
Std:	0.146	0.0956	0.166	0.180
50	0.8375	0,7861	0.7371	0.7693
	0.7552	0.7389	0.7682	0.6716
	0.7571			
	0.6610			
Mean value	0.753	0.763	0.753	0.720
Std:	0.0361	0.0236	0.0156	0.0489
100	0.6004	0.5721	0.5391	0.5611
	0.5513	0.5401	0.5606	0.4968
	0.5528			
	0.4894			
Mean value	0.548	0.556	0.550	0.529
Std:	0.0228	0.0160	0.0108	0.0322

Table I.2. The viscosity [Pa·s] at shear rates 1, 50 and 100 s^{-1} *for 3% w/v for the different trials prepared at pH 6.35, 5.36, 4.16 and 2.84, as well as the mean value and the standard error of mean.*

	pH			
Shear rate [s-¹]	**6.35**	**5.36**	**4.16**	**2.84**
1	2.211	1.658	1.454	1.965
	1.693	1.7123	1.651	1.540
	1.892			
	1.536			
Mean value	1.83	1.67	1.95	1.75
Std:	0.146	0.0273	0.166	0.213
50	0.8375	0.7319	0.6702	0.8110
	0.7552	0.7344	0.6954	0.6801
	0.7571			
	0.6610			
Mean value	0.753	0.733	0.683	0.746
Std:	0.0361	0.00125	0.0126	0.0655
100	0.6004	0.5382	0.4978	0.5904
	0.5513	0.5403	0.5126	0.5036
	0.5528			
	0.4894			
Mean value	0.548	0.539	0.505	0.547
Std:	0.0228	0.00105	0.00740	0.0439