The effect of high pressure homogenization on emulsions containing oat β-glucan

A study of viscosity, stability and molecular weight

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Abstract

This master thesis project has been conducted in cooperation with Lantmännen AB who are producers of PromOat® - an oat β-glucan powder concentrate. The main objective of this thesis was to investigate the properties of emulsions containing PromOat® oat β-glucan, focusing on the viscosity, stability, and molecular weight before and after high pressure homogenization (HPH) at pressures of 100, 150 and 200 bars.

The viscosity of emulsions was measured on a shear rate range from 0.1 to 100/s and extrapolated to zero shear force. Results showed a significant decrease in viscosity with HPH and its increasing pressures in both research and control samples, indicating its disruptive character on β-glucan aggregates.

Stability analysis of oat β-glucan emulsions through backscattering measurements indicated no significant differences among the research samples, suggesting that HPH did not affect the overall stability of the emulsions. Obtained results in light of documented loss of viscosity with HPH suggest stabilizing effect of oil droplet size reduction. Control samples showed variations in backscattering behavior, indicating the influence of HPH on stability. Light microscopy images revealed smaller oil droplet sizes in emulsions subjected to HPH pressures, confirming its well documented disruptive effect on oil droplets.

Asymmetrical flow field-flow fractionation (AF4) analysis of oat β-glucan samples using multi-angle light scattering (MALS) and differential refractive index (dRI) detectors showed an increase in average molar mass and average root-mean-square radius with higher HPH pressures, suggesting that re-aggregation of oat β-glucan had occurred under different circumstances than in non-homogenized sample which manifests itself in increased average molar mass and root-mean-square radius.

In conclusion, HPH was found to significantly decrease the viscosity and disrupt the aggregate structure of oat β-glucan in emulsions. The overall stability of the emulsions was not significantly affected. The molecular properties of β-glucan, such as molar mass and root-mean-square radius, were influenced by HPH treatment. These findings may contribute to a better understanding of the HPH impact on oat β-glucan properties in emulsions.

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Chapter 1
Introduction

Emulsions are thermodynamically unstable systems made of two immiscible liquids. To improve their stability certain substances which delay phase separation are added. One group of such substances are stabilizers which enhance the viscosity of continuous phase and thus slow down the displacement of dispersed phase in time[1]. Common technique used in emulsion production which significantly reduces the oil droplet size is HPH[2].

Oat β-glucan, a partially soluble dietary fiber derived from oat endosperm cell walls, has gained considerable attention in last 20 years due to its beneficial health effects [3, 4]. Incorporating oat β-glucan into emulsions offers a promising approach to develop functional food products with improved nutritional value[4]. However, the impact of HPH on the properties of oat β-glucan has to be established when developing such products.

This thesis aims to investigate the influence of HPH on the viscosity and stability of oat β-glucan containing emulsions as well as on the molecular weight of oat β-glucan itself. Understanding these effects is crucial for optimizing the processing parameters and being able to produce emulsions with desired properties.

In the viscosity analysis, the viscosity of oat β-glucan emulsions was measured before and after HPH at pressures of 100, 150, and 200 bar. Analogical experiment was conducted using water dispersions of the polysaccharide to confirm the role of oat β-glucan in viscosity loss due to processing conditions. Stability is a critical aspect of emulsion performance. The stability of oat β-glucan emulsions was assessed through backscattering measurements, which provide insights into the changes in light scattering behavior. The emulsion stability was additionally examined using light microscopy, which revealed HPH induced size reduction of oil droplets. The molecular weight of oat β-glucan, an essential parameter for its functional properties, was determined using AF4 analysis with the use of MALS and dRI detectors.

By analyzing the viscosity, stability, and molecular weight of oat β-glucan in emulsions under different HPH conditions, this work contributes to a deeper understanding of the impact of HPH on oat β-glucan and thus contributes to advancement of food science and technology.

Chapter 2
Background

2.1 Oat β-glucan

Oats contain on average $4.5-5.5\%$ of β-glucan. It occurs mainly in endosperm cell walls with the highest abundance in the sub-aleurone layer [5]. Oat β-glucan is a linear, high molar mass (M) polysaccharide that in the food industry is used as a thickener or stabilizer [6]. Its functional properties in pair with its beneficial effects on health make it a valuable component in food formulations both for producers and consumers.

2.1.1 Physicochemical properties

Oat β-glucan is a linear polysaccharide composed of β-(1→3),(1→4) linked D-glucopyranosyl units [7]. Approximately 90% of oat β-glucan structure is comprised of sections containing three or four β-(1→4) subunits joined together by β-(1→3) linkage. Ratio of the three to four unit building blocks ranges from 1.4 to 2.3. The remaining 10% consists of oligosaccharides comprised of 5 to 16 subunits [3]. Weight-average molar mass ($\overline{M}$) of oat β-glucan has been reported to be between $6.5 \cdot 10^4$ and $3.1 \cdot 10^6$ g/mol but a more general approximation of M mentions a range of several hundreds kDa to be the most common [3]. Viscosity of oat β-glucan solutions depends on its concentration, M and the temperature [8].

2.1.2 Functionality

Oat β-glucan is a polysaccharide that is partially soluble in water. Due to its water-holding property and swelling in aqueous environment it has the ability to form viscous solutions [1]. Under appropriate conditions it can also produce gels namely when the oat β glucan molecules aggregate and form a three-dimensional network. Higher degree of gelling is associated with the aforementioned three subunit segments which increase the probability of aggregation ([9]). Increased gel formation was also appointed to increased number of low M oat β-glucan molecules which may enhance aggregation [10].

This viscosity-enhancing functionality is one of the main reasons for its extensive use in the food industry. It can be used simply as a thickener to achieve desired consistency of viscous products such as sauces and dressings. Water absorption ability of oat β-glucan can be employed to improve moisture retention in baked

goods such as muffins in order to prolong the shelf-life and enhance crumb softness [7]. It can be also incorporated into products with reduced fat content to mimic the viscosity, texture and mouthfeel of the full fat equivalent. Oat β-glucan aqueous solutions exhibit shear-thinning characteristics, where viscosity decreases with increasing shear rate. This property can be utilized to improve the spreadability of products such as low-fat cream cheese[6].

Increased viscosity can also have a stabilizing effect on emulsions. In a system where water is the continuous phase of an emulsion oat β-glucan can be utilized. Higher viscosity of the continuous phase slows down the gravitational displacement of dispersed components and in turn preserves the stability of an emulsion for longer time [1].

It is worth to mention that oat β-glucan is odorless [3]. When used in a food formulation it will not impact the flavor of the product. This characteristic makes it applicable for a wide range of food stuff.

By leveraging the functional properties of oat β-glucan, food producers can develop innovative food products with improved texture and stability.

2.1.3 Effects on health

Oat β-glucan has been extensively studied for its numerous health benefits, particularly its effects on cardiovascular health and glycemic control. One of the most well-established health benefits of oat β-glucan is its ability to reduce levels of total and low-density lipoprotein (LDL) cholesterol. Additionally, it has no significant effect on high-density lipoprotein (HDL) cholesterol levels [11]. de Morais Junior et al. (2023) meta-analysis results conclude that there is no significant difference between oat and β-glucan isolate effects on human health indicating that this polysaccharide is the main bioactive compound of the grain [11]. The magnitude of the beneficial health effects depend on factors such as the dose and duration of consumption. The cholesterol-lowering effect is attributed to the ability of oat β-glucan to form a viscous gel in the gut, which inhibits bile acids and cholesterol absorption and promotes their excretion [12, 13, 14].

In addition to its cholesterol-lowering properties, oat β-glucan has been found to have positive effects on glycemic control. Studies have shown that oat β-glucan slows down gastric emptying by hindering mixing of gastric juices with food. It can also attenuate the postprandial rise in blood glucose levels by slowing the absorption of glucose in the small intestine [15]. However, low M fractions did not exhibit such an effect suggesting that it is connected to solubility and aggregation [10, 16]. This can be particularly beneficial for individuals with diabetes or those at risk of developing the condition.

Other potential health benefits of oat β-glucan include its prebiotic effects on gut microbiota [13]. As a soluble fibre it can be fermented in the small intestine and be a substrate for short chain fatty acids production - especially the butyric acid. Butyric acid hinders growth of degenerated colon cells and evokes their apoptosis which in effect reduces the risk of colon cancer development [17].

For producers to currently declare an oat β-glucan related health claim in European Union there are certain conditions to fulfill. They have to inform the consumers that beneficial effect of oat β-glucan is acquired through an intake of

3 g per day. Another condition is that its amount in a quantified portion of a product has to be 1 g minimum. [18]

2.2 Emulsions

Emulsions are colloidal dispersions consisting of two immiscible liquids, where one liquid is dispersed in the other. Main types of emulsions are oil-in-water (O/W) and water-in-oil (W/O) where first part of the name describes the dispersed phase and the last one the continuous phase [1]. In order to obtain such a system providing external force is required which will disrupt one phase in the other. One example of such operation is HPH.

2.2.1 Instability mechanisms

Emulsions are inherently thermodynamically unstable systems and various factors can contribute to their destabilization. Several instability mechanisms can lead to emulsion breakdown which can occur individually or in combination, depending on the specific formulation and environmental conditions. Understanding the instability mechanisms is crucial for formulating stable emulsions. Main instability mechanisms are described below [1].

Sedimentation and creaming

Creaming or sedimentation occurs when the dispersed phase migrates towards the top or bottom of the system, respectively. This phenomenon is driven by the density difference between the phases, as well as the gravitational force acting on the droplets. Creaming is typically observed in oil-in-water emulsions, where the oil droplets rise to the top, while sedimentation occurs in water-in-oil emulsions, causing the water droplets to settle at the bottom. Emulsions with higher concentrations of dispersed phase are less sensitive to creaming [1].

Flocculation

Flocculation refers to the aggregation of emulsion droplets, leading to the formation of clusters or flocs. This can occur due to attractive interparticle forces, such as van der Waals forces or electrostatic interactions. Flocculation can propel creaming as flocs are bigger than individual droplets and gravitate to the top faster than individually [1].

Coalescence

Coalescence is the process by which two or more emulsion droplets fuse together and form larger droplets. This happens through droplets coming into close contact. This instability similarly as the previous one increases the creaming rate as larger droplets travel faster to the top. This mechanism is driven by diminution of interfacial energy and is influenced by factors such as droplet size, interfacial tension, and collisions between droplets [1].

2.2.2 Stability factors

Emulsions are bound to separate into phases they consist of. To delay this fate certain strategies can be implemented. Reduction of the dispersed phase droplet size improves the stability of emulsions significantly. The principle of droplet size reduction involves mechanical disruption of the system. This is often achieved through processes such as HPH. The smaller the size of dispersed droplets the longer its re-association time [1].

Another strategy is increasing the viscosity of the continuous phase. The resistance against sedimenting or creaming species increases making it more difficult for them to displace [1].

Surfactants or surface active polymers are also a mean for stability enhancement. They reduce the interfacial tension and can also provide repulsion between the droplets be it electrostatic or steric, preventing their association [1].

Formulation and environment parameters are also relevant for emulsion stability. These include temperature, emulsifier concentration and pH. Increased temperature can reduce the viscosity of the continuous phase and hinder its stabilizing effect. Insufficient concentration of emulsifier in the emulsion will result in partial coverage of oil droplets and less efficient protection against instability mechanisms. pH can affect surfactants by eliminating the charge which introduces repulsion between dispersed droplets. pH can also affect a type of surface active polymers which are proteins. At given isoelectric point certain proteins precipitate and stop stabilizing the emulsion [1, 19].

In conclusion, understanding the mechanisms of emulsion instability is essential for formulating stable emulsion systems. By considering the various instability mechanisms and employing appropriate strategies, it is possible to enhance emulsion stability and substantially extend the shelf life of an emulsion-based products.

2.3 High pressure homogenization

HPH is a widely used technique in the food industry for the production of stable emulsions. It plays a crucial role in achieving a finely dispersed phase by reducing the droplet size and improving the interfacial interactions between the phases.

This size reduction of dispersed phase is achieved through sending a pressurized liquid through a narrow gap (10-100 µm) due to which a significant pressure drop occurs and shearing against the geometry and itself [20]. After passing the gap the liquid enters a chamber where combination of shearing, turbulence and cavitation occur. Disruption of oil droplets is speculated to occur mostly through turbulent interactions [21].

By reducing droplet size, improving emulsifier functionality and enhancing stability, HPH contributes to the development of high-quality emulsion-based food products.

Chapter 3
Aim

To maximize the potential of oat β-glucan functional properties in food formulations the effect of relevant processing operations used in production has to be studied. One of those processing operations is HPH on which this project focuses on.

The main aim of this thesis is to investigate the effect of HPH on the viscosity and stability of oat β-glucan emulsions as well as molecular weight of the polysaccharide in emulsion matrix.

The specific objectives are to determine the influence of different homogenization pressures (100, 150, and 200 bar) on the viscosity of oat β-glucan emulsions, assess the stability of these emulsions through backscattering measurements and analyze the molecular weight, particle size and structure of oat β-glucan using AF4 coupled with MALS and dRI detectors.

This master thesis project findings will hopefully contribute to a better understanding of the relationship between the processing conditions and the properties of oat β-glucan which may have implications for its application in food and beverage products.

Through learning the effect of HPH on oat β-glucan the processing parameters can be properly selected to obtain desired properties of the polysaccharide when used in emulsions.

Chapter 4
Methodology

4.1 Materials

Samples were prepared using PromOat® oat β-glucan powder concentrate containing 34% of β-glucan - batch KM22H13522 (Läntmannen AB, Sweden), Nutralys® F85M pea protein isolate containing 80% of total protein (Roquette Frères, France), rapeseed oil (ICA AB, Sweden) and deionized water. Sucrose solutions were prepared using white fine crystal sugar (ICA AB, Sweden) and deionized water. AF4 carrier liquid consisted of NaN_3 (VWR Chemicals, Belgium), $NaNO_3$ (Scharlab, Spain) and Milli-Q Gradient A10 system purified water (Millipore Co., USA).

4.2 High pressure homogenization

Mixtures for emulsion preparation were prepared with 2 % (w/w) oat β-glucan, 0.5 % (w/w) pea protein, 5 % (w/w) oil and water. First, oat β-glucan was gradually added to whirling water and mixed for 5 minutes using T 25 Ultra-Turrax with S 25 N - 18 G dispersing tool (IKA-Werke GmbH & Co. KG, Germany) at 24 000 rpm. Afterwards it was left to hydrate overnight with magnetic stirring on 500 rpm. Next, oil and pea protein were added to the β-glucan dispersion and mixed using T 25 Ultra-Turrax at 24 000 rpm for 5 minutes.

The instrument used for HPH was GEA Lab Homogenizer PandaPLUS 2000 (GEA Niro Soavi S.p.A., Italy). The β-glucan mixture samples were homogenized using one stage at three different pressures (100, 150 and 200 bars).

4.3 Viscosity

The emulsions' viscosity was measured using StressTech HR High Resolution Oscillatory Rheometer and RheoExplorer version 5.0 software (REOLOGICA Instruments AB, Sweden). The measurements were performed using a cup and bob geometry (CC 25). 16 ml of each sample material was deployed into the instrument's cavity and flow curve was measured at 20℃ with a shear rate ranging from 0.1 to 100 $1/s$. The curves were then calibrated using a 40 % sucrose solution (6.2 $mPa \cdot s$ at 20℃) [22]. Control samples were prepared using only oat β- glucan by

mixing it with water and hydrating it overnight as described in Section 4.2. Samples' viscosity was measured promptly after finishing homogenization or mixing. Measurements were repeated nine times per sample.

4.4 Creaming rate

The emulsions' stability was investigated swiftly after they had been processed using U12 - E13 Turbiscan LAB Analyzer (Formulaction, France). Approximately 20 ml of sample material was poured into a glass cell which was then tightly secured with a cap and inserted into the equipment's chamber. The temperature during the measurements was 20℃. Backscattering measurements were taken every minute for a time span of an hour. Unit of backscattering is %. Changes in backscattering throughout sections of the sample can indicate occurrence of certain instabilities as presented in Table Table 4.1.

Table 4.1: Instability phenomena corresponding to changes in backscattering [23]

Δ BS	Bottom	Middle	Top	Instability
Case I	⇑	-	⇓	Sedimentation
Case II	⇓	-	⇑	Creaming
Case III	⇓	⇓	⇓	Flocculation or coalescence

Control samples were prepared by mixing rapeseed oil, pea protein and water using Ultra Turrax similarly as described for emulsion preparation in Section 4.2. Measurements were performed in triplicates.

4.5 Light microscopy

Oil droplet size of oat β-glucan emulsions was investigated using an Olympus BX50F4 light microscope (Olympus Optical Co. Ltd., Japan). Samples were diluted with water directly on glass slides and observed under 100x magnification. Images were retrieved using a DFK 41AF02 color camera through IC Capture 2.4 software (The Imaging Source, Germany).

Based on a representative light microscopy image per examined oat β-glucan emulsion, an average oil droplet diameters was established. Third largest oil droplets in the images were assumed to represent the average oil droplets size in the examined emulsions. The image scale and oil droplet diameter were measured using ImageJ software.

4.6 Molecular weight

Oat β-glucan molecular weight and particle size was examined through AF4 analysis. The equipment used was an Eclipse 3+ Separation System with Dawn

Heleos II MALS detector and Optilab T-rEX dRI detector (Wyatt Technology, Germany). The carrier liquid used for the analyses was a solution of 0.02 % (w/v) NaN_3 and 10 mM $NaNO_3$ in pure water. A long channel with a 190 µm thick and 26.3 cm long (tip to tip) spacer was selected for the equipment setup. Spacer thickness was then assessed through calibration with bovine serum albumin. The actual thickness obtained through that measurement was found to be 159 µm. A polyethersulfone (PES) membrane was chosen with a 5 kDa cut-off.

Samples for AF4 analysis were 0.4% (w/w) oat β-glucan dispersions in water hydrated overnight similarly as described in Section 4.2. Samples were then treated the same way as the emulsion samples. Afterwards, NaN_3 and $NaNO_3$ were added to the samples in concentrations corresponding to the ones in the carrier liquid and mixed using magnetic stirring for a minute. Samples were then filtered using a 25 mm syringe filter with 0.45 µm pore size surfactant-free cellulose acetate (SFCA) membrane (Thermo Fisher Scientific Inc., USA).

Analysed volume for each sample was 40 µl which was injected onto the channel at a flow rate of 0.2 ml/min for 10 minutes. Next, a focusing step was carried out for 5 minutes at a focusing flow rate of 1 ml/min. Elution began at 16 minutes. Detector flow rate was at 0.5 ml/min throughout the entire analysis. Cross flow during elution was decaying exponentially with a half time of 4.5 minutes from 1 ml/min to 0.1 ml/min. This flow rate was maintained for additional 20 minutes. The cycle concluded with 5 minutes of elution without cross flow and 5 minutes of elution with injection also without cross flow.

The molar mass (M) and root-mean-square radius (r_{rms}) were obtained using Astra software version 5.3.4.14 (Wyatt Technology, Germany). MALS data was taken from detectors 9-14. Fitting of the data was performed using Berry method as suggested in literature [24]. The specific refractive index increment (dn/dc) of β-glucan in aqueous solution was set to 0.146 ml/g [8].

The hydrodynamic radius (r_{hyd}) was calculated using FFFHydRad 2.0 - software made in Matlab by Andreas Håkansson utilising equations presented in a publication co-authored by him [25]. Graphic representation of data was performed using Matlab software version R2022a (MathWorks Inc., USA).

4.7 Statistical analysis

The analysis of data was performed using one-way ANOVA and Tukey's post-hoc test at confidence level of 95% ($p<0.05$). Both analyses were conducted in Windows Excel version 16.0 (Microsoft Corporation, USA).

Chapter 5
Results

5.1 Viscosity

Viscosity (η) of emulsions containing oat β-glucan without HPH (no HPH) and after at 100 (100 bar), 150 (150 bar) and 200 (200 bar) bar is presented in Table 5.1. These values were obtained by extrapolating viscosity to zero shear force on logarithmic scale plots.

Table 5.1: Viscosity of oat β-glucan containing emulsions without homogenization and after at given pressures

	no HPH	100 bar	150 bar	200 bar
$\eta\ [mPa \cdot s]$	$304^a \pm 16$	$212^b \pm 6$	$214^b \pm 5$	$219^b \pm 11$

± - standard deviation
mean values obtained from 9 measurements
a-b - different lower case letters represent statistically significant effect of homogenization pressure on examined oat β-glucan emulsions ($p<0.05$)

In order to investigate if the decrease in emulsions' viscosity is directly related to oat β-glucan the same experiment was performed for a water dispersion of said polysaccharide. Results of that experiment are presented in Table 5.2.

Table 5.2: Viscosity of oat β-glucan dispersions in water without homogenization and after at given pressures

	no HPH	100 bar	150 bar	200 bar
$\eta\ [mPa \cdot s]$	$164^a \pm 8$	$130^b \pm 3$	$134^b \pm 2$	$133^b \pm 5$

± - standard deviation
mean values obtained from 9 measurements
a-b - different lower case letters represent statistically significant effect of homogenization pressure on examined oat β-glucan dispersions ($p<0.05$)

A graphic display of viscosity depending on the processing for control and research samples is presented in Figure 5.1.

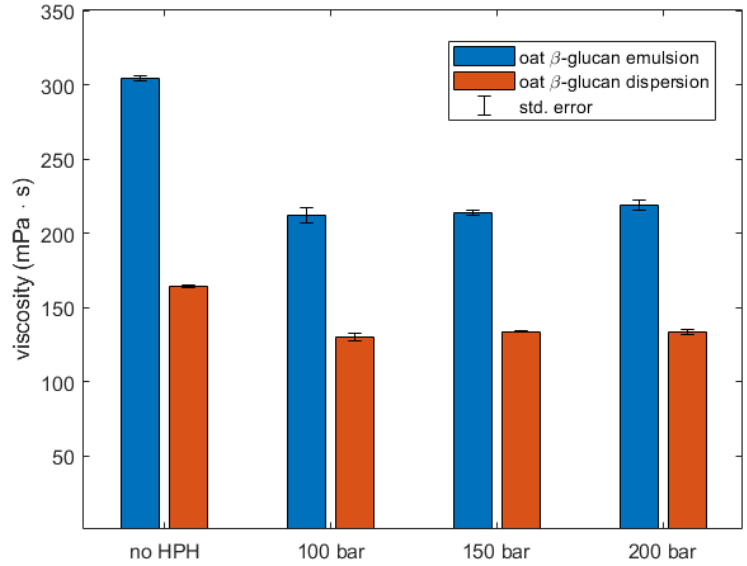

Figure 5.1: Summary of viscosity measurements for oat β-glucan emulsions and dispersions

5.2 Stability

Emulsions stability is presented in Table 5.3 as a general direction of backscattering in each of the three height sections of the samples (Table 4.1). Two exemplary graphs showing changes in backscattering for both research and control samples can be found in Appendix Figures A.8 and A.9. Table 5.4 shows stability as backscattering averages per sections of each sample. These averages were derived from the difference in backscattering between the final backscattering measurement and the first one at time 0. Negative and positive values correspond to backscattering decrease and increase respectively.

Table 5.3: Backscattering behaviour in oat β-glucan emulsions

	No HPH	100 bar	150 bar	200 bar
Top	⇓	⇓	⇓	⇓
Middle	⇓	⇓	⇓	⇓
Bottom	⇓	⇓	⇓	⇓

measurements initiated no longer than 3 minutes after finishing sample processing
backscattering behaviour based on 3 measurements

Table 5.4: Backscattering variance [%] in oat β-glucan emulsions

	No HPH	100 bar	150 bar	200 bar
Top	$-1.0^a \pm 0.3$	$-1.1^a \pm 0.1$	$-1.0^a \pm 0.3$	$-0.8^a \pm 0.2$
Middle	$-0.9^a \pm 0.4$	$-0.6^a \pm 0.2$	$-0.5^a \pm 0.2$	$-0.4^a \pm 0.1$
Bottom	$-0.6^a \pm 0.3$	$-0.6^a \pm 0.2$	$-0.4^a \pm 0.2$	$-0.5^a \pm 0.3$

± - standard deviation
mean values obtained from 3 measurements
a - the same lower case letters in each row represent statistically insignificant effect of homogenization pressure on stability of oat β-glucan emulsions ($p>0.05$)

Backscattering behaviour in control samples as well as its average values per sample section are shown in Table 5.5 and Table 5.6 respectively.

Table 5.5: Backscattering behaviour in emulsion control samples

	No HPH	100 bar	150 bar	200 bar
Top	⇑	⇑	⇑	⇑
Middle	⇓	-	⇓	-
Bottom	⇓	⇓	⇓	⇓

measurements initiated no longer than 3 minutes after finishing sample processing
backscattering behaviour based on 3 measurements

Table 5.6: Backscattering variance [%] in emulsion control samples

	No HPH	100 bar	150 bar	200 bar
Top	$2.8^a \pm 1.2$	$2.9^a \pm 0.1$	$2.1^a \pm 1.2$	$2.0^a \pm 0.3$
Middle	$-3.2^a \pm 0.5$	$-0.0^b \pm 0.1$	$-0.4^b \pm 0.6$	$-0.1^b \pm 0.0$
Bottom	$-10.8^a \pm 0.8$	$-1.7^b \pm 0.2$	$-2.2^b \pm 0.8$	$-1.6^b \pm 0.3$

± - standard deviation
mean values obtained from 3 measurements
a-b - different lower case letters in each row represent statistically significant effect of homogenization pressure on stability of control emulsions ($p<0.05$)

Emulsions stability investigated using light microscopy is shown in Figure 5.2.

Presented images are sections of the entire images for the sake of better visibility. Entire images can be found in Appendix Figures A.1 to A.4.

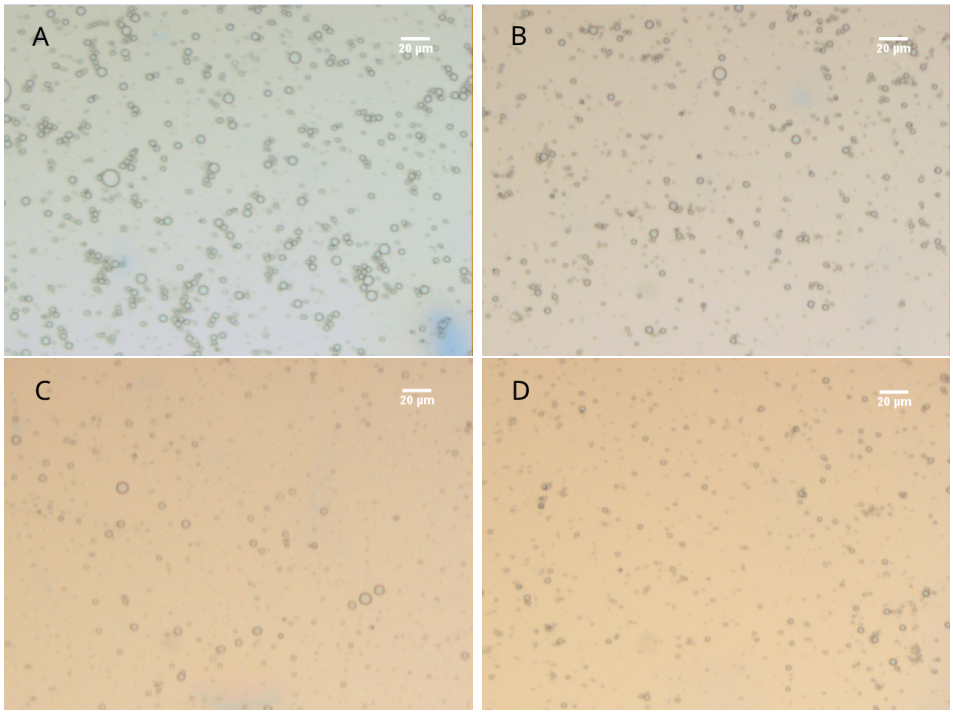

Figure 5.2: Light microscopy images of oat β-glucan emulsions after different processing treatments. ***A*** - no HPH, ***B*** - 100 bar, ***C*** - 150 bar and ***D*** - 200 bar HPH. White scale bar represents 20 µm

The results of oil droplet size assessment in oat β-glucan emulsions are gathered in Table 5.7. Threshold for the droplet detection was circularity from 0.7 to 1 which ought to exclude aggregates from selection which can elevate the calculated mean oil droplet diameter value.

Table 5.7: Volume mean average diameter of oil droplets in oat β-glucan emulsions based on >200 droplets - image analysis using ImageJ software

	No HPH	100 bar	150 bar	200 bar
d(4,3) [µm]	7.6	6.0	6.1	4.9

Based on the obtained droplet size data size categories were established and size distribution plots were made for oat β-glucan emulsion samples Figure 5.3.

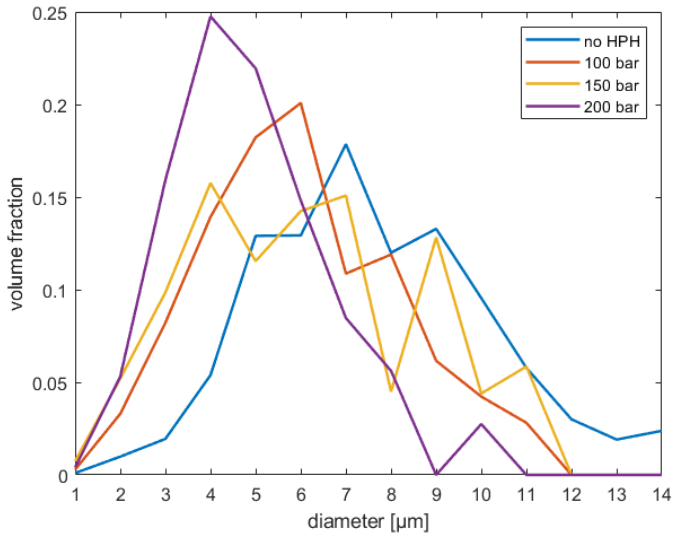

Figure 5.3: Size distribution of oil droplets as fractions of the total volume

Additional analysis of sedimentation rate (ν_{sed}) based on Stokes' equation was performed for oat β-glucan emulsion samples (Table 5.8). The equation can be found here [26]. The density of continuous phase has been estimated to be 1100 kg/m^3 and density of oil approximated to 920 kg/m^3. The exact input data and calculations are included in Figure A.7.

Table 5.8: Sedimentation rate approximation in oat β-glucan emulsions using Stokes' equation

	No HPH	100 bar	150 bar	200 bar
ν_{sed} [10^{-10} m/s]	9.3	4.7	4.7	3.1

5.3 Molecular weight

A brief summary of oat β-glucan physical quantities calculated with ASTRA software such as average molar mass ($\overline{M}$) and average root-mean-square radius ($\overline{r_{rms}}$) are included in Table 5.9. These quantities were calculated based on 100% of injected samples' mass fraction.

Table 5.9: Oat β-glucan samples AF4 analysis summary - retrieved from ASTRA results report

	No HPH	100 bar	150 bar	200 bar
$\overline{M}$ [10^6 g/mol]	5.9 ± 0.8	14.6 ± 3.1	16.1 ± 1.9	15.2 ± 2.0
$\overline{r_{rms}}$ [nm]	37.0 ± 15.9	43.6 ± 17.7	58.2 ± 8.8	71.3 ± 8.4
m_{rec} [%]	92	100	105*	102*

$\overline{M}$ - weight-average molar mass
$\overline{r_{rms}}$ - weight-average root-mean-square radius
m_{rec} - mass recovery, ratio of detected mass to mass injected onto AF4 channel
* - illogical values possibly due to carry-over of injected samples in between analyses

Based on raw data from AF4 analyses obtained through ASTRA a comparison of lowest and highest M of oat β-glucan samples was prepared Table 5.10.

Table 5.10: Lowest and highest M of oat β-glucan samples depending on processing - retrieved from ASTRA

	No HPH	100 bar	150 bar	200 bar
M_{min} [g/mol]	$1.48 \cdot 10^4$	$1.60 \cdot 10^4$	$2.25 \cdot 10^4$	$2.93 \cdot 10^4$
M_{max} [g/mol]	$1.44 \cdot 10^8$	$2.91 \cdot 10^8$	$8.32 \cdot 10^7$	$7.46 \cdot 10^7$

M_{min} - lowest M detected during AF4 analysis
M_{max} - highest M detected during AF4 analysis

Figures 5.4 and 5.5 show oat β-glucan samples' M and r_{rms} with RI fractograms in the background. Time 0 on the X axes corresponds to 16th minute of entire analysis which is when the elution begins. Fractogram from MALS can be found in Appendix A.5. Spectrum of oat β-glucan M depending on undergone treatment is presented in Figure 5.4.

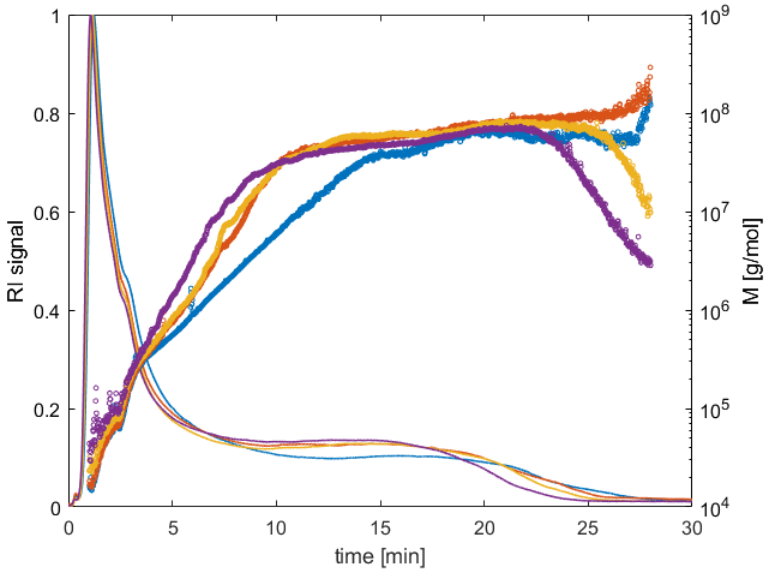

Figure 5.4: Distribution of M in oat β-glucan samples depending on processing

Similarly, a range of root-mean-square radii (r_{rms}) obtained from oat β-glucan samples is shown in Figure 5.5.

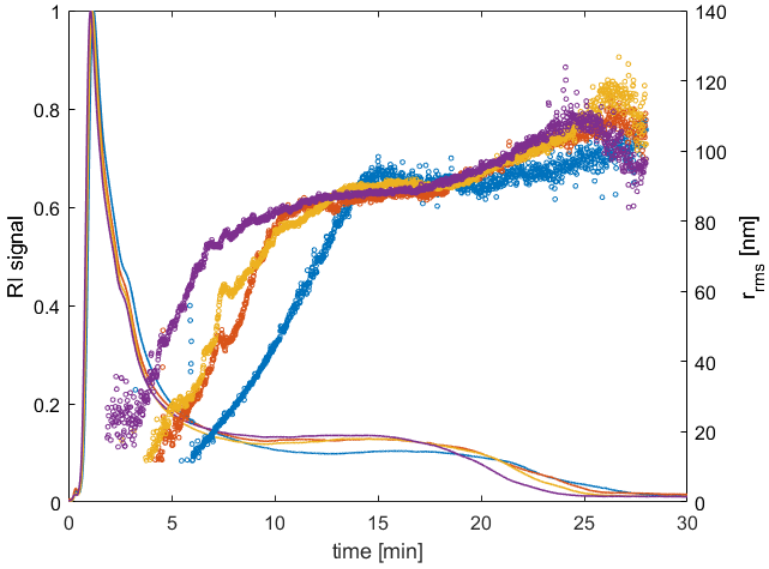

Figure 5.5: Distribution of r_{rms} in oat β-glucan samples depending on processing

A graph of apparent density against r_{hyd} is shown in Figure 5.6. Ratio of r_{rms} to r_{hyd} versus M is presented in Figure 5.7.

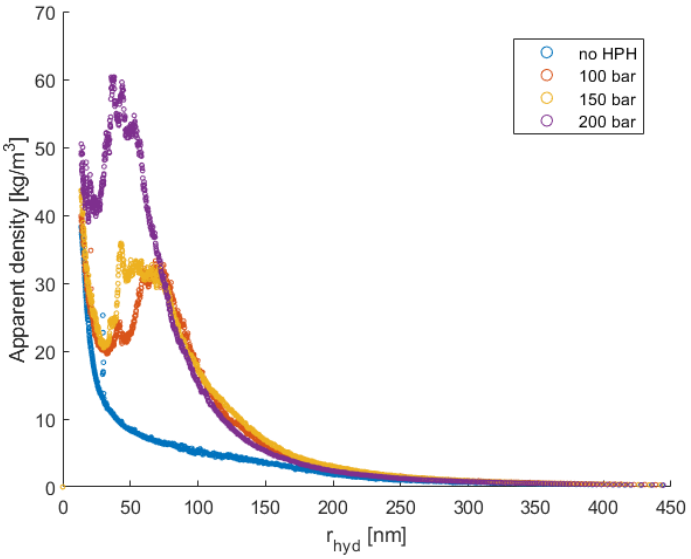

Figure 5.6: Apparent density against r_{hyd} in oat β-glucan samples depending on processing

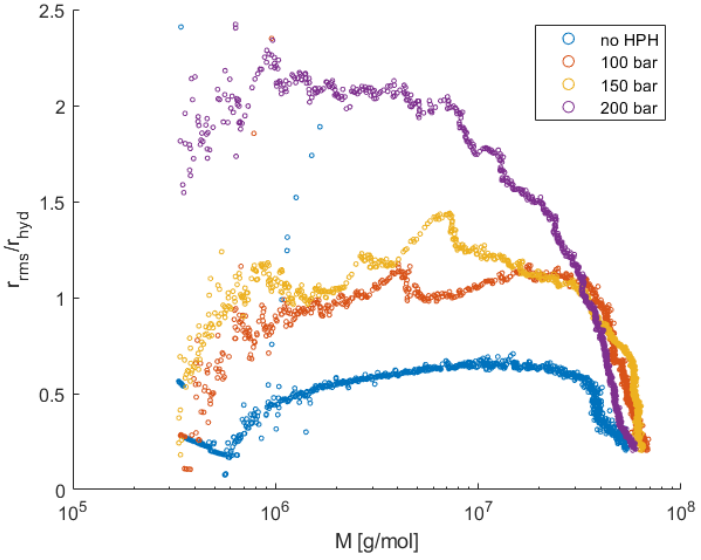

Figure 5.7: r_{rms} to r_{hyd} ratio versus M fraction of oat β-glucan samples depending on processing

The data in Figure 5.7 is shown for M starting from $3 \cdot 10^5$ g/mol because the limit of detection for MALS is approximately r_{hyd}<10 nm [27]. A plot with full range of M data can be found in Appendix A.6.

Chapter 6

Discussion

6.1 Viscosity

Based on viscosity measurements of oat β-glucan emulsions (Table 5.1) there is a statistically significant difference between non homogenised sample and the ones treated with HPH irrespectively of the pressure (100, 150 and 200 bars). Viscosity of homogenised samples did not differ significantly among the examined pressures.

Similar results were obtained for control samples (Table 5.2). Non homogenised sample's viscosity was significantly higher than the one of the homogenised samples. Viscosity of homogenised control samples did not show statistically significant differences from each other.

Viscosity of control samples compared to emulsion samples is approximately 50% lower for non homogenised samples and approximately 40% lower for the homogenised ones. This proves the viscosity-enhancing character of oat β-glucan in emulsions.

There is a statistically significant loss of viscosity caused by HPH to both types of samples. For emulsion samples it is 30% and for control samples it is 20% loss. These results suggest that HPH affects oat β-glucan viscosity-enhancing abilities but also that the decrease in viscosity is not exclusively oat β-glucan related. This can be most likely caused by the reduction of oil droplets size through HPH treatment.

A possible cause of oat β-glucan related viscosity loss suggested in the literature is the disruption of polysaccharide's aggregates [28]. Kivelä et. al [28] measured viscosity of oat β-glucan dispersion after HPH at 6, 30 and 60 MPa. Viscosity decreased substantially after the HPH compared to the control sample. It should be noted that the samples passed the homogenizer 4 times which most likely led to permanent changes in the polysaccharide's structure. Nonetheless, the disruptive effect of HPH can be observed in the aforementioned article. Rojas et al. [29] examined the effect of HPH at 6, 12 and 24 MPa on waxy barley starch. They established that the overlap concentration (c*) increased with homogenization and its consecutive pressures. This increase translates to a decrease in exact cause of this effect.

6.2 Stability

Oat β-glucan emulsions stability was not affected significantly by HPH in a statistical sense. All oat β-glucan samples backscattering data was relatively similar to each other (Table 5.4). Decrease in backscattering for a given section suggests clarification and its increase implies accumulation of particles. An instability mechanism observed in the polysaccharide-enriched emulsions through decrease of backscattering in all sample sections in time (Table 4.1) was flocculation or coalescence (Table 5.3). Lower number of dispersed particles scatter light less and thus the detection of backscatter decreased overtime in the oat β-glucan samples. Microscopy images revealed that both instabilities in fact occurred simultaneously in these samples.

Control samples stability was affected by HPH. The difference in backscattering between non-homogenised sample and the treated ones is statistically significant for middle and bottom sections of the glass cell (Table 5.6). The instability mechanism detected in the control samples was creaming (Table 5.5).

The differences between backscattering in control and oat β-glucan samples' are statistically significant for all samples sections with the exception of the middle section for 150 bar treated samples. Backscattering variance among both types of samples in top sections range from 52 to 66%. In the bottom sections - from 65 to 94%. This indicates that the oil droplets traveled faster to the top in control samples compared to the research ones. Both of these observations can be explained by the aforementioned higher viscosity of oat β-glucan emulsions which retards oil droplets displacement in the samples' volume. Because of this there is no creaming as in control samples but rather flocculation and coalescence.

Additional examination of oat β-glucan emulsions under the microscope showed that oil droplet size decreased after HPH (Figure 5.2). Image analysis using ImageJ showed that the differences in oil droplet size among HPH samples did not follow a declining trend despite the expectations. Samples' 100 and 150 bars average oil droplets size were similar rather than decrease with pressure. This can be explained by the software incapability to fully detect merged droplets as individual ones and their interpretation as bigger droplets. Sedimentation rate calculated for oat β-glucan emulsions depending on processing conditions reveals that the rate is the highest for sample with no HPH and the lowest for 200 bar sample proving that small oil droplet size travels to the top slower than large one which translates to increased emulsion stability. Oil droplets size distribution Figure 5.3 shows reduction of size range with HPH and increasing pressures in favor of the smaller size droplets. The droplet sizes are larger than expected with the use of HPH. Droplet size for HPH under 200 bars is expected to be around 1 µm - rather it is around 5 µm [30]. This could be explained by an insufficient amount of emulsifier in the formulation or its insufficient emulsifying properties. Plant protein gain more and more popularity as emulsifiers [1]. Evaluation of their performance compared to other types of emulsifiers is in order to assess whether they are technologically valuable components to use for emulsion production.

6.3 Molecular weight

Results shown in Table 5.9 suggest that M increases after HPH but does not differ substantially among applied pressures of homogenization. r_{rms} seems to gradually increase with homogenization and its pressure. The $\overline{M}$ of non-homogenized oat β-glucan sample is in agreement with results found in literature [31].

Graphs depicting ranges of M and r_{rms} (Figure 5.4, Figure 5.5) are comparable to the results presented in Table 5.9. Zielke et al. [32] examined oat β-glucan M and r_{rms} extracted from oat flour. The quantities were obtained through AF4 analysis with MALS and dRI detectors. Their results show M range from 10^5 to 10^8 g/mol which is similar to the one presented in Table 5.10.

In Figure 5.4 the slopes of HPH treated samples are relatively similar to each other until 25 minutes mark of analysis. For that same time range the slope of the non-homogenized sample is flatter indicating that the number of lower M species of oat β-glucan is higher in that sample. This translates into lower $\overline{M}$ value of non-homogenized sample which is what is visible in the AF4 summary table (Table 5.9.

In Figure 5.5 the slopes of analysed samples vary among each other more than in the previous graph representing molar mass distribution (Figure 5.4). Sample processed under 200 bars has relatively the smallest amount of particles with low r_{rms}. Samples processed under 150 and 100 bars are relatively similar to each other with an apparent difference at 7 minutes when 150 bar sample shows presence of bigger radius species than 100 bars. Non-homogenised sample has the highest amount of small particles. The amount of big species is relatively similar among HPH samples and higher than in non-homogenized sample. This analysis is also in agreement with the results presented in the AF4 summary table (Table 5.9).

Figure 5.6 shows that the density of small radii particles of oat β-glucan samples is relatively high and increases with homogenization and its increasing pressures. For no HPH sample the density drastically decreases from the very beginning ranging from more than 40 to less than 10 kg/m^3 for sizes 50 nm and lower. From that point the density slope is more gentle and keeps on declining towards the higher radii. Density for radii size $\leq$ 50 nm in 100 and 150 bar samples is very similar to each other and ranges from more than 40 to more than 20 kg/m^3. Around 50 nm there is a density increase for the 150 bar sample and density drop for 100 bar sample. At approximately 80 nm r_{hyd} the density of 100 and 150 bar samples drops. The samples' plots become similar again and maintain the similarity until the end of the x axis range. 200 bars sample has the highest maximal density which is around 60 kg/m^3 at approximately 50 nm. Afterwards the density drops in a similar fashion as for the other HPH samples following a similar curvature. These results indicate increase in density of oat β-glucan species smaller than 200 nm for HPH samples compared to the control sample (no HPH). For the species larger than 200 nm the density did not differ significantly depending on samples' processing.

r_{rms}/r_{hyd} ratio can indicate a conformation of a polymer in solution [33]. Figure 5.7 shows the differences of the radii ratio among analysed samples. Li et al. Figure 6.1 examined r_{rms} and r_{hyd} different M fractions of oat β-glucan. The individual samples were obtained through gradient ammonium sulphate precipitation

of oat β-glucan extract. The radii were calculated based on static and dynamic light scattering measurements of different M oat β-glucan samples. Based on their results radii ratios were calculated for each M sample and put in comparison to the results from own research. Their results align with the results obtained for a sample with no HPH both do not go above 0.7.

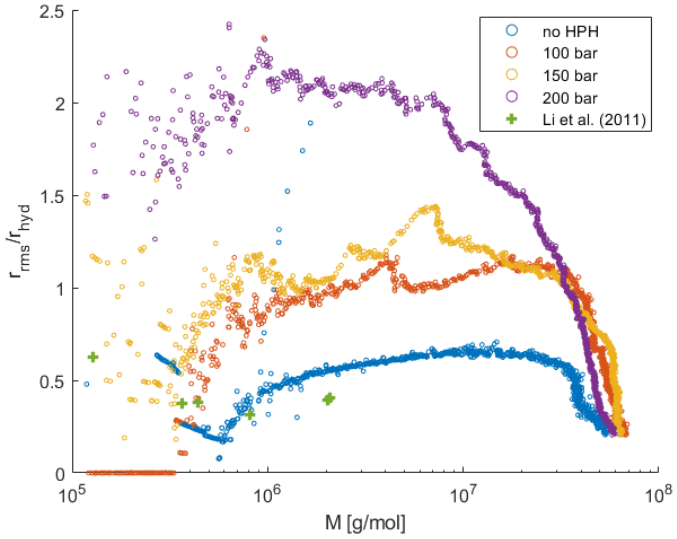

Figure 6.1: r_{rms}/r_{hyd} versus M fraction of oat β-glucan samples (own elaboration based on Li et. al [8] and own data)

r_{rms}/r_{hyd} plots for all oat β-glucan samples have more or less pronounced arc shapes. For samples after HPH there are more than one conformations at analyzed M spectrum. The ratio for sample with no HPH is lower than 0.7 for displayed M fraction. The ratio values equal to 0.7 and under correspond to micro gel conformation which is also described as a highly swollen structure [33]. Both 100 and 150 bar samples display similar r_{rms}/r_{hyd} range although 150 bar plot is slightly higher than the other one. Radii ratio for 100 bars begins below 0.7 and continues rising until slightly above 1. Values 1 - 1.5 correspond to branched molecule [31]. At M larger than $3 \cdot 10^7$ the ratio value begins to sharply decrease until below 0.5 where it goes back to micro gel structure as in the lower M section. 150 bar sample displays a similar pattern with a difference of a higher peak which does not go above 1.5 which points to a branched molecule as mentioned before. 200 bars sample scatter shape resembles a tilted arc to the right side. At the beginning the lowest value of radii ratio is slightly below 1.5 - branched molecule conformation. At M $10^6 - 10^7$ g/mol radii ratio displays values above 2 which in theory correspond to a random coil conformation. At M around 10^7 g/mol the ratio values decrease until below 0.5 corresponding to micro gel structure. The decrease is softer than in the other plots. 200 bar sample exhibits the biggest range of conformations throughout the M than the others.

The general radii ratio increase and thus broader spectrum of conformations is observed with HPH and increasing pressures on oat β-glucan samples. This phenomenon can be explained by a physical, reversible degradation and further re-aggregation of the polysaccharide particles. Pressures seem to have an effect on the degradation potentially causing higher degree of aggregation with higher pressures. This effect could be beneficial to examine more in depth and with higher pressures.

Zielke et al. [31] examine the r_{rms}/r_{hyd} of oat β-glucan extracted from oat flour using AF4 with MALS and dRI detectors. Their results compared to the ones for non HPH sample show an inverted fashion of ratio distribution for M range from 10^5 to 10^7 g/mol where highest values (>2) correspond to lower M fraction and lowest (<0.7) for higher M fraction. The difference may arise from the method of sample preparation which differs substantially and the processing performed on the sample material before analysis.

6.4 General

The stability of oat β-glucan emulsions was not affected by HPH although viscosity decreased significantly after HPH. Possible explanation of this phenomenon is that the stability measurement results depict the simultaneous effect of droplet size reduction and viscosity loss in homogenized samples. Smaller droplet size of dispersed phase provides higher stability of an emulsion. Continuous phase with lower viscosity has a weaker stabilizing effect on the emulsion. Both these occurrences overlapping can result in an insignificant difference between processed samples.

Molecular weight measurements were conducted days after sample preparation unlike the other analyses which were performed right after samples' processing. It was initially expected that the M of oat β-glucan would correlate with viscosity and decrease in the homogenized samples. Instead M and r_{rms} increased. This might be a result of β-glucan re-aggregation. Differences in M and r_{rms} between the analyzed samples may arise from aggregation into structures different than before HPH. Conducting measurements (viscosity, stability, molecular weight) at different time spans from the moment of sample preparation step may be responsible for contradicting results. Measuring overtime changes in oat β-glucan viscosity, M and r_{rms} post-HPH could be beneficial to better understand the relationship between the three physical quantities.

Kivelä et al. (2010) measured the viscosity of oat β-glucan dispersions before and after HPH at days 0, 2 and 8 [28]. Viscosity of HPH treated samples did not change overtime. Homogenization conducted as part of their research included four passes through the two-stage homogenizer rather than one pass through one-stage homogenizer. These processing conditions might have been more aggressive on oat β-glucan and potentially lead to permanent changes that negatively affected its re-aggregation abilities. Harsh HPH conditions have been shown to lead to depolymerization of oat β-glucan [28], [34]. Mäkelä et al. (2017) measured viscosity of oat β-glucan solutions at days 1, 4 and 7. They found that viscosity of a non-oxidized solution of oat β-glucan dissolved at 37°Cwas increasing significantly in

time. Processing conditions of examined samples were more gentle than in Kivelä et al. (2010) and so possibility of re-aggregation after HPH at 100, 150 and 200 bars can not be rejected.

In relation to the health aspects, the increase in M due to HPH induced re-aggregation may be therapeutically beneficial. Higher M species have been linked to the viscosity increase in the intestines which is linked to the aforementioned health effects [16, 15]. The degree of said increase has to be evaluated in comparison to the non-HPH oat β-glucan sample and relevant research in nutrition to examine if it has a significant effect on the beneficial effect on health. Analysis of the HPH effect on oat β-glucan and positive health effects associated with it could be of relevance.

Chapter 7

Conclusion and future prospects

The decrease of oat β-glucan emulsions viscosity with increasing homogenization pressures is statistically significant. The viscosity values were considerably lower after homogenization at 100, 150, and 200 bar compared to the non-homogenized emulsion. The decrease in viscosity indicates that homogenization pressure affects the rheological properties of oat β-glucan emulsions.

The backscattering variance in oat β-glucan emulsions showed no significant effect of homogenization at the investigated pressures on the emulsion's stability in contrast to control samples. Emulsions containing the polysaccharide in their formula exhibited coalescence/flocculation instability mechanism compared to control samples exhibiting creaming. The backscattering variance of oat β-glucan was significantly lower compared to control samples. Homogenization pressure did not have a significant impact on the stability of oat β-glucan emulsions.

Light microscopy images of oat β-glucan emulsions showed a decrease in oil droplet size with increasing homogenization pressure. The average oil droplet size decreased from 7.6 µm to 4.9 µm after homogenization at 200 bar. This indicates that higher homogenization pressure leads to smaller oil droplets in the emulsions.

The $\overline{M}$ of oat β-glucan samples increased with HPH treatment. The $\overline{r_{rms}}$ increased with HPH and its consecutively pressures. Statistical analysis of results to determine if the effect of HPH was significant was not possible. Calculations of average quantities were performed automatically by software. Obtained results suggest that higher homogenization pressure affects the molecular weight and size distribution of oat β-glucan.

Overall, the homogenization pressure has a significant effects on the viscosity of oat β-glucan emulsions. However, it does not significantly affect the stability of the emulsions. $\overline{M}$ and $\overline{r_{rms}}$ increased with HPH.

As a future prospect, an analysis of overtime changes in viscosity, M and r_{rms} of oat β-glucan post HPH could be beneficial for better understanding of the influence HPH has on the polysaccharide.

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Appendix A
Data

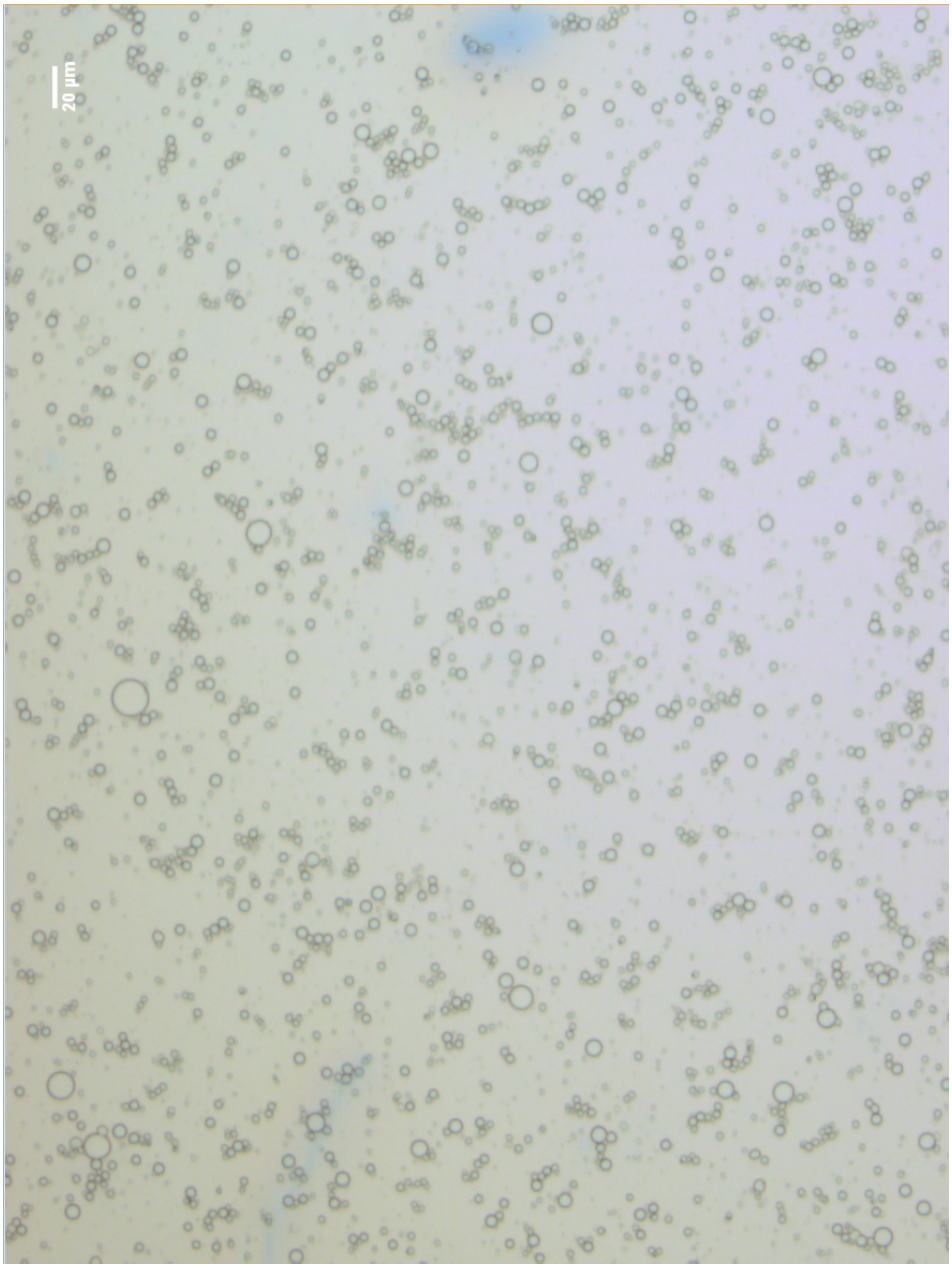

Figure A.1: Light microscopy image of oat β-glucan emulsion without HPH

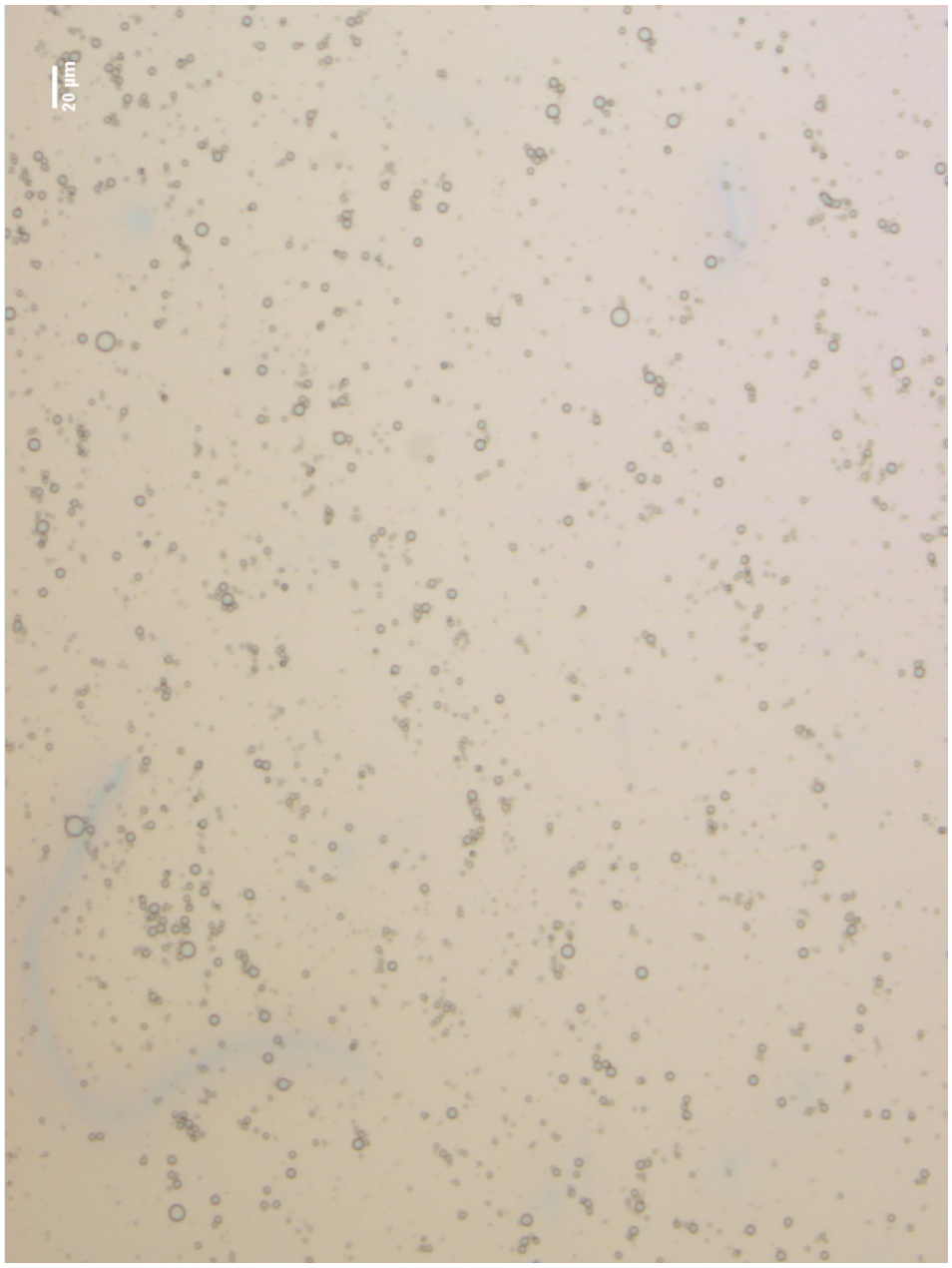

Figure A.2: Light microscopy image of oat β-glucan emulsion after 100 bar HPH

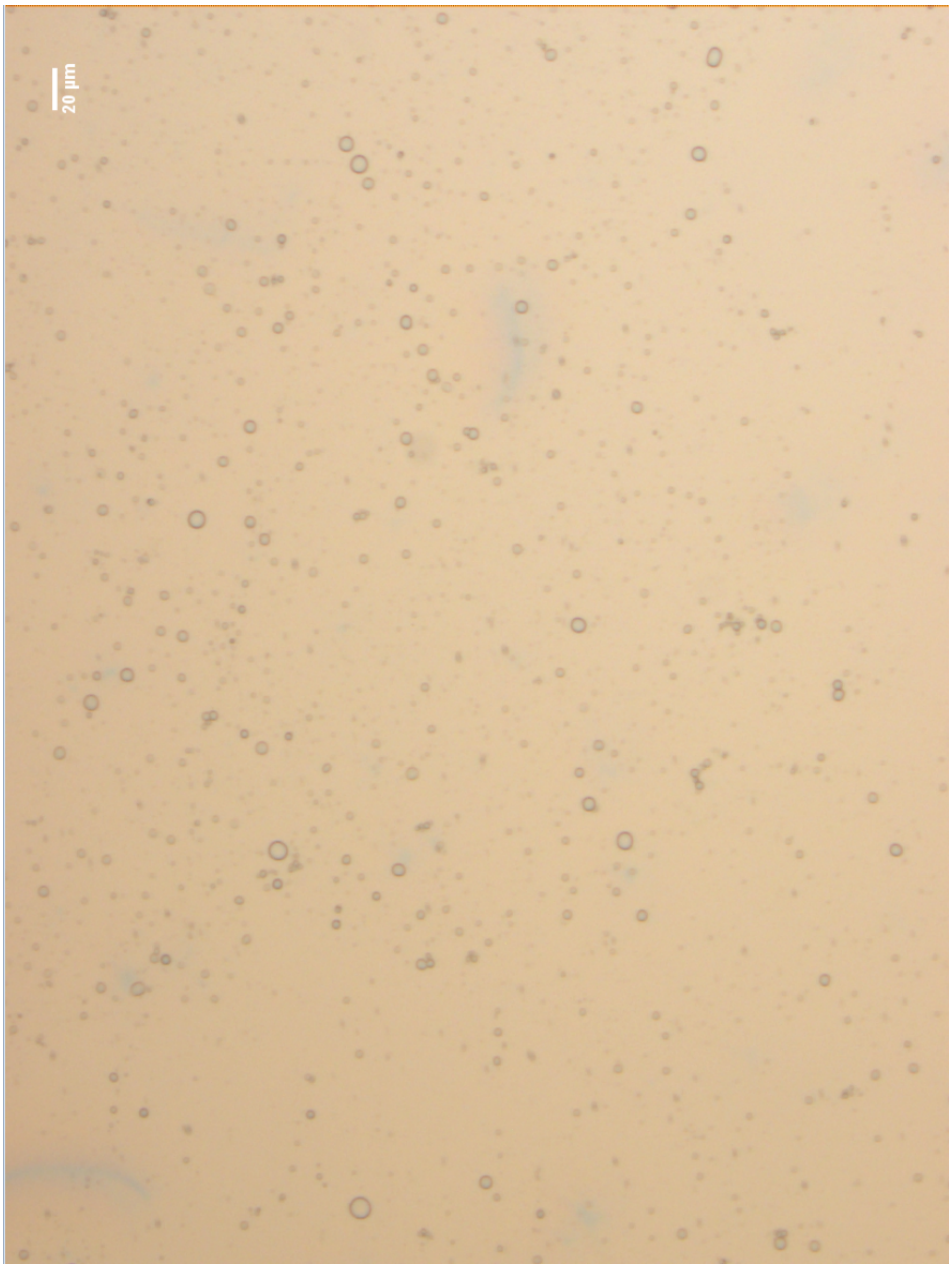

Figure A.3: Light microscopy image of oat β-glucan emulsion after 150 bar HPH

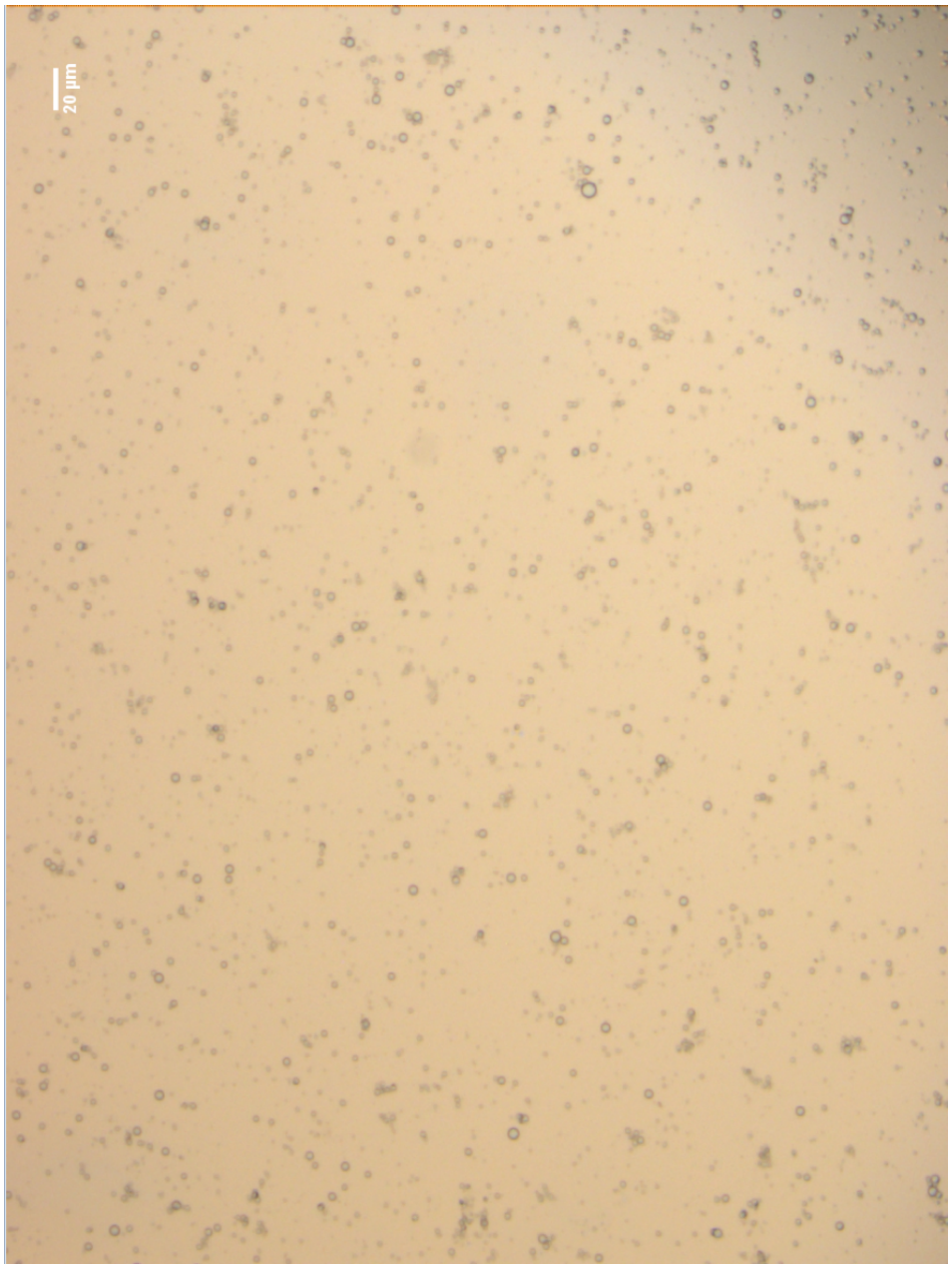

Figure A.4: Light microscopy image of oat β-glucan emulsion after 200 bar HPH

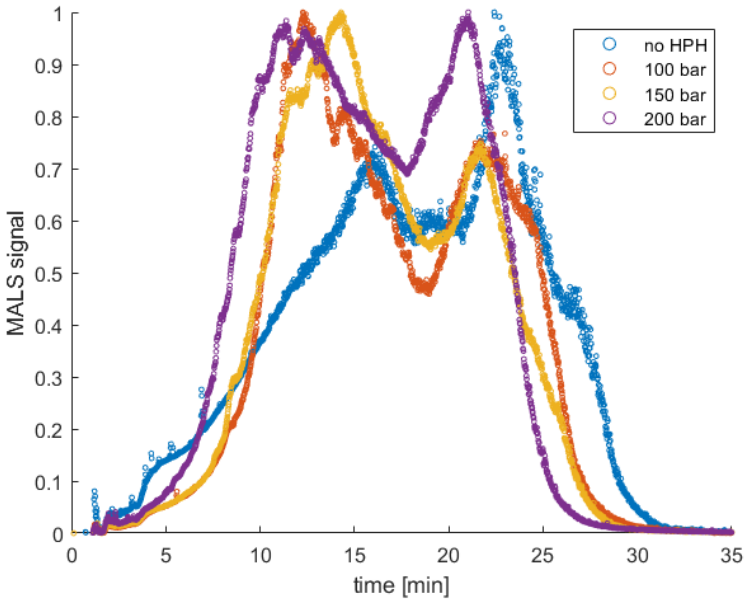

Figure A.5: MALS fractogram for oat β-glucan samples

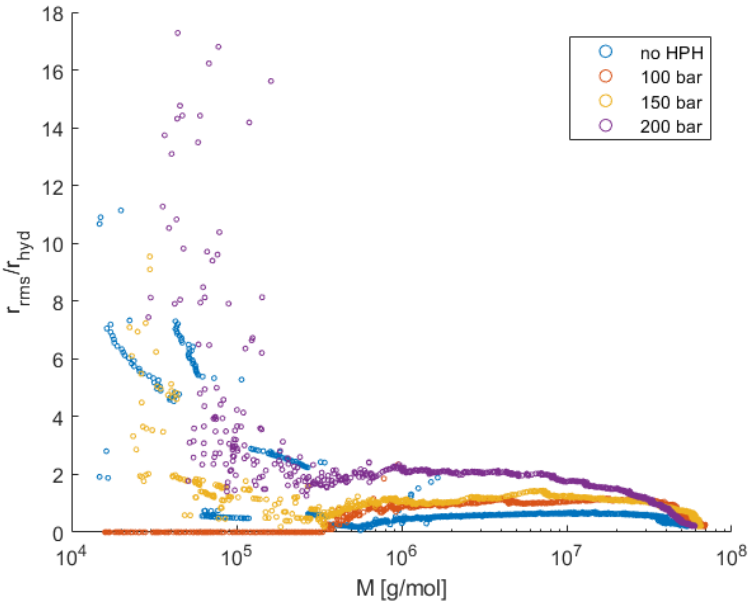

Figure A.6: r_{rms} to r_{hyd} ratio versus M in oat β-glucan samples

ρ_p	1100	kg/m^3	R^2_nohph	1,444E-11	m
ρ_s	920	kg/m^3	R^2_100bar	9,3025E-12	m
g	9,81	m/s^2	R^2_150bar	9E-12	m
			R^2_200bar	6,0025E-12	m
η_nohph	0,164	Pa s	v_sed	9,3E-10	m/s
η_100bar	0,13	Pa s	v_sed	4,7E-10	m/s
η_150bar	0,134	Pa s	v_sed	4,7E-10	m/s
η_200bar	0,133	Pa s	v_sed	3,1E-10	m/s

Figure A.7: Data and calculations for sedimentation rate based on Stokes' equation

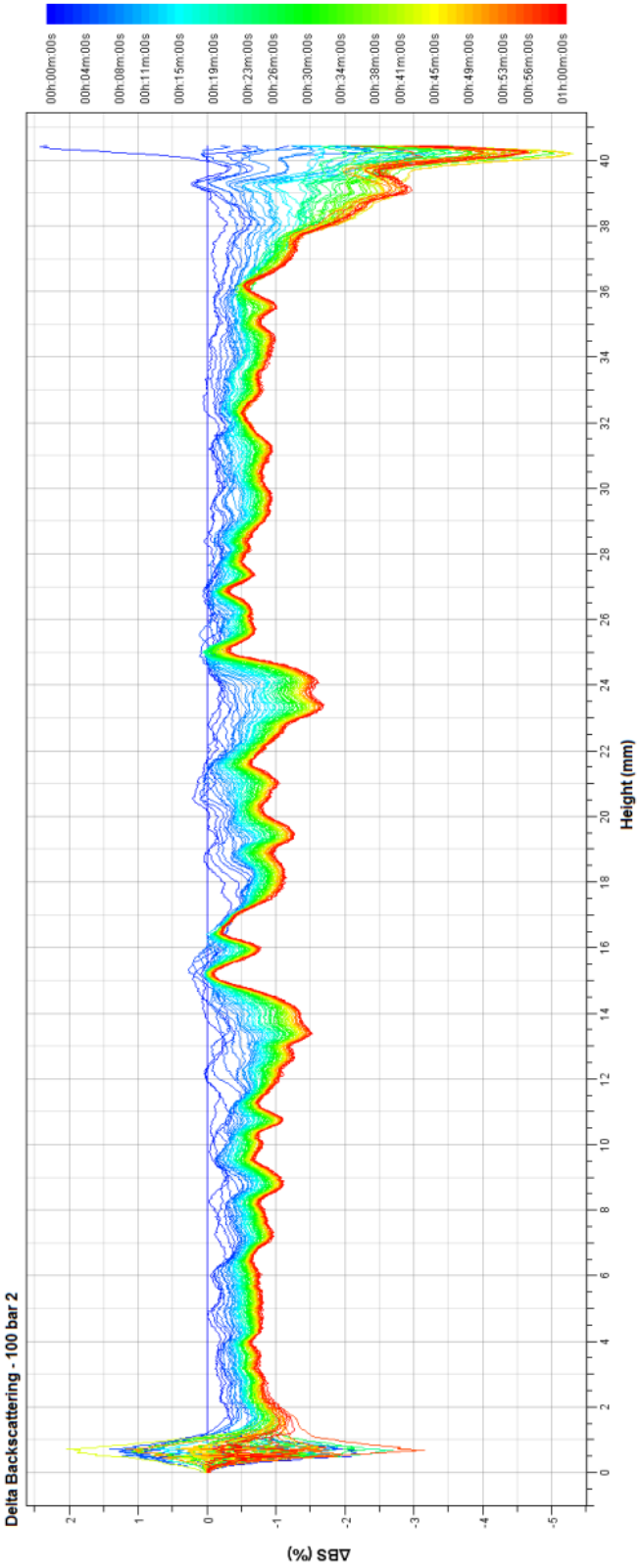

Figure A.8: Backscattering changes in 100 bar oat β-glucan emulsion - derived from Turbiscan

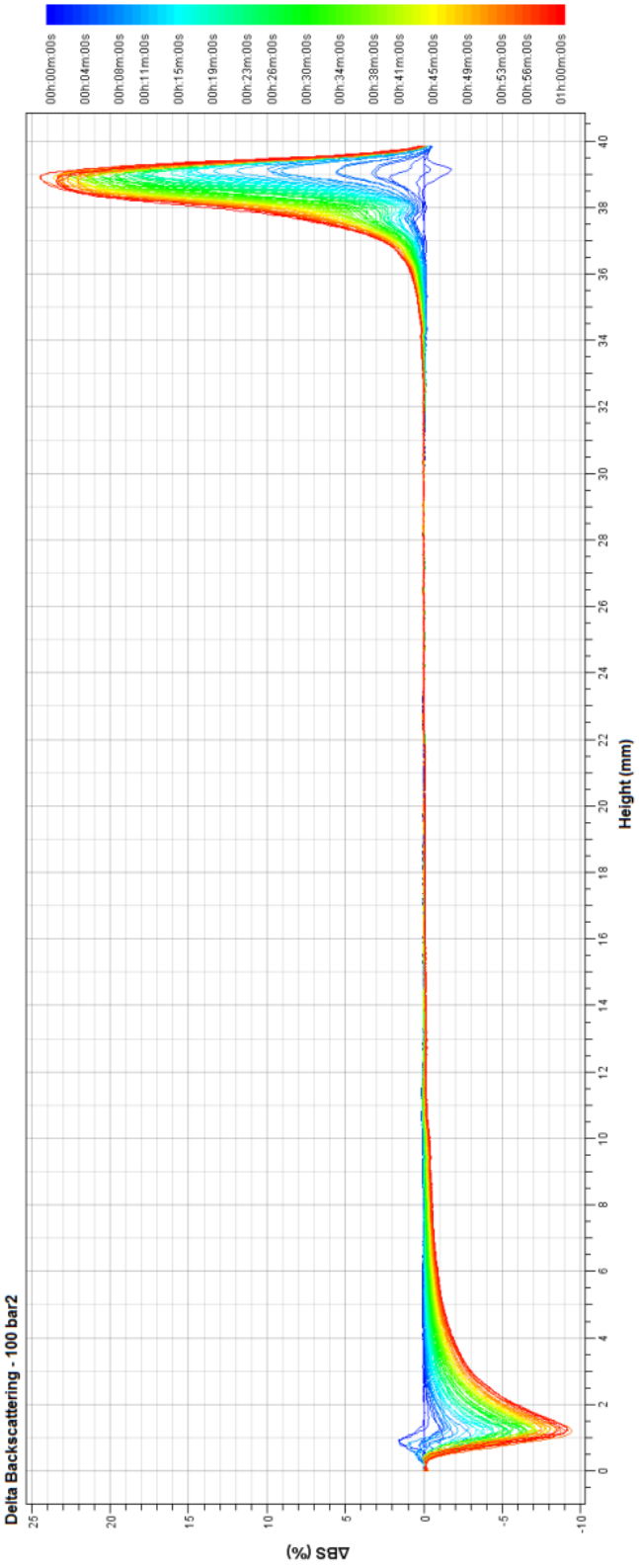

Figure A.9: Backscattering changes in 100 bar emulsion control sample - derived from Turbiscan