Investigating the role of the different molar mass fractions of a pectin rich extract from onion towards its emulsifying and emulsion stabilizing potential

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Abstract

A pectin rich nitric acid extract produced from onion flesh (NA) showed promising emulsion stabilizing potential. At both pH 2.5 and pH 6.0, emulsions produced with the NA sample as sole emulsifying and emulsion stabilizing compound displayed stable oil droplet size distributions. Moreover, the emulsions showed good stability against creaming. Interestingly, the pectin rich acid extract was characterized by a bimodal molar mass distribution which led to the research objective to investigate the possible role of both polymer fractions in the promising emulsion stabilizing potential. To investigate this, both fractions were separated using centrifugal ultrafiltration.

Structural characterization revealed the presence of mainly short chain galactans in the low molar mass fraction (NA-LMM) while the high molar mass fraction (NA-HMM) consisted mainly of pectin. Rheological analysis clearly demonstrated the viscosity increasing effect of NA-HMM in solution while the viscosity of a NA-LMM solution at equal concentration was comparable to pure water. Emulsions produced with NA-LMM destabilized quickly during refrigerated storage by extensive coalescence and creaming at pH 2.5. Despite presenting a somewhat better stability at pH 6, also in this case fast destabilization was observed. Contrarily, NA-HMM showed promising emulsion stabilizing potential. Oil droplet size distributions remained stable during storage at both pH 2.5 and pH 6.0. Creaming was observed for NA-HMM at pH 6.0 although to a lesser extent as for NA-LMM stabilized emulsions. Based on these results, it can be concluded that NA-LMM does not contribute to the emulsion stabilizing functionality of NA. Promising functionality is mainly attributed to the high molar mass polymers.

Keywords: Onion pectin; Molar mass; Fractionation; Emulsion stability; Viscosity

1 Introduction

Today, an extensive range of emulsion-based food products are available on the market. The majority of these products are industrially manufactured and consist of an aqueous and an oil phase in which several food ingredients and components can be dissolved. Due to the large contact area between oil and water, these systems are thermodynamically unfavorable. In order to produce stable food emulsions with a desired and extended shelf life, emulsifiers and emulsion stabilizing compounds are commonly added (McClements & Gumus, 2016; Walstra, 1993). Many of the compounds used for this purpose by industrial producers today are synthetically manufactured low molecular weight surfactants such as Tweens and Spans. Contrastingly, there has been a rising consumer demand for more natural, sustainable and environmentally friendly food products over the last decades (McClements & Gumus, 2016). Consequently, food producers are faced with the challenge of finding ways to shift from the use of synthetic emulsifiers towards the use of natural alternatives.

Over recent years, interest has grown in the potential use of pectin to stabilize oil-in-water emulsions (o/w emulsions). Originally, the emulsion stabilizing potential of pectin, together with other thickeners, was attributed to its viscosity increasing effect in the aqueous phase. Certainly this may have a positive effect on emulsion stability, but there is more to the promising potential of pectin besides this factor (Ngouémazong et al., 2015). Recent research showed pectin is able to adsorb at the oil-water interface and can be considered a surface active polymer (Funami et al., 2011; Schmidt, Schmidt, et al., 2015; Verkempinck et al., 2018). Additionally, adsorbed pectin can stabilize oil droplets against coalescence in two distinct ways. Parts of the pectin chains and/or branches of the pectin structure which protrude away from the interface into the continuous aqueous phase increase emulsion stability by steric hindrance. Secondly, non-methoxylated galacturonic acid residues of pectin chains can be negatively charged at higher pH values. Electrostatic repulsion between pectin chains adsorbed

on different oil droplets can contribute to long-term stability as well (Funami et al., 2007, 2011; Ngouémazong et al., 2015). These findings suggest pectin could play multiple roles in stabilizing o/w emulsions and therefore it is interesting to study the use of this natural polymer for this food technological purpose.

Pectin chains are complex macromolecules whose structure can be chemically and/or enzymatically modified (Voragen et al., 2009). Structural changes already take place during plant growth and development (e.g. fruit ripening) but targeted structural modifications of pectin can also be executed after pectin extraction from plant tissues (Ngouémazong et al., 2015). Although the structural complexity of pectic polymers is actually responsible for its multifunctional nature, it makes pectin research extra challenging. This is evident when looking at previous studies on the emulsifying and emulsion stabilizing capacity of pectin. Many different pectin properties have been suggested to attribute to its aforementioned functionality. Research papers mention pectin related proteinaceous moieties (Akhtar et al., 2002; Funami et al., 2007, 2011; Leroux et al., 2003; Williams et al., 2005), the degree of methyl esterification (DM) and acetylation (Leroux et al., 2003; Schmidt, Koch, et al., 2015; Verkempinck et al., 2018) and the presence of covalently bound ferulic acid in the pectin structure of sugar beet pectin (Chen et al., 2016). These structural characteristics all determine the amphiphilic character of the polymer chains. Furthermore, another structural feature of pectin polymers, not directly linked to its amphiphilicity, seems extremely important in this research: the molar mass of the pectic polymers. In fact, molar mass is actually often mentioned in literature to influence the functionality of pectin as well. However, the difference with other structural properties is that it influences multiple properties. The molar mass of pectin polymers determines its adsorption kinetics, influences the polymer conformation and consequently the accessibility of surface active groups, and is the main factor that determines the viscosity of a

pectin solution (Akhtar et al., 2002; Alba & Kontogiorgos, 2017; Leroux et al., 2003; Ngouémazong et al., 2015; Schmidt, Koch, et al., 2015).

Previous research showed good potential of a pectin rich onion extract to serve as an emulsifying and/or emulsion stabilizing agent (Neckebroeck et al., 2021). In the current study, the goal is to further investigate this pectin rich sample and to better understand the origin of the promising functionality. The first part of the results and discussion section will introduce the promising potential of the pectin rich nitric acid extract to the reader (section 3.1). As the pectin rich acid extract displayed a bimodal molar mass distribution, it was opted to particularly focus on the different molar mass fractions to gain in-depth insight into the emulsifying and emulsion stabilizing potential. It was hypothesized that both the low as well as the high molar mass polymer fraction present in the sample could contribute to emulsion stability. To investigate this hypothesis, it was aimed to separate both fractions and consequently study their individual potential to stabilize o/w emulsions (section 3.2). Samples were compared based on molecular structure, physicochemical properties and their ability to form and stabilize o/w emulsions during refrigerated storage. Understanding the structural and functional properties of the separated fractions may lead to further and better insight into the functionality of the original pectin rich acid extract.

2 Materials and Methods

2.1 Materials

Chopped onions (*Allium cepa* L.) and commercial sunflower oil were purchased from a local supermarket. Ultrapure water (organic free, 18.2MΩ cm resistance) was supplied by a Simplicity™ 150 water purification system (Millipore, Billerica, USA). Unless mentioned otherwise, all chemicals used were of analytical grade.

2.2 Pectin extraction and fractionation

2.2.1 Production of Alcohol Insoluble Residue

The procedure of McFeeters & Armstrong (1984) was used to isolate cell wall material from chopped onion pieces as an alcohol insoluble residue (AIR). 60 g of wet tissue was suspended in 192 ml 95% ethanol using a high speed mixer (3 times for 6 s) (Buchi mixer B-400, Flawil, Switzerland). After filtration (filter paper Machery-Nagel MN 615 Ø 90 mm), the residue was resuspended in 96 ml 95% ethanol, mixed and filtered again. Subsequently, the resulting residue after this step was resuspended once more in 96 ml technical acetone and finally filtered to obtain the AIR. The AIR was dried overnight at 40 °C, grounded with mortar and pestle and stored in a desiccator until use.

2.2.2 Nitric acid extraction

An acid extraction was executed on the AIR following the procedure described by Willemsen et al. (2017). Briefly, 60 g AIR was suspended in 4 L of demineralized water and incubated at 80 °C for 30 minutes while stirring to enhance suspension formation. Subsequently, the pH of the suspension was adjusted to 1.6 using 7 M HNO_3 and extraction proceeded for 60 minutes at 80 °C under mild stirring. After fast cooling in an ice bath, the suspension was centrifuged at room temperature for 10 min at 4000 g. The supernatant was separated from the pellet and the pH of the former was adjusted to 6.1. The resulting pectin rich solution was extensively

dialyzed against demineralized water for 48h (Spectra/Por® Dialysis Membrane, 3.5 kDa MWCO), lyophilized (Alpha 2-4 LSC, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany) and stored in a desiccator until use. This sample is further referred to as the nitric acid extract (NA). Due to some practical issues, the nitric acid extraction was performed in duplicate resulting in two batches of NA sample referred to as NA b1 and NA b2.

2.2.3 Acid extract fractionation

To fractionate the nitric acid extract of onion into fractions with different molar mass distributions, ultrafiltration was applied using centrifugal concentrators (Vivaspin® 20 ultrafiltration unit, Sartorius AG, Göttingen, Germany). The ultrafiltration units are equipped with vertical twin PES-membranes (1,000 kDa MWCO) through which smaller polymers can migrate while large polymers are retained on the membranes. Briefly, a 0.2% (w/v) solution of the acid extract was brought in the units on the membranes. After centrifugation for 60 minutes at 5500 g and 20 °C, the filtrate was collected as the fraction containing the nitric acid extracted polymers of low molar mass (NA-LMM). The concentrate retained on the membranes was redissolved overnight in ultrapure water and consequently centrifugation was repeated under identical conditions as described above. After dissolving, the retained concentrate of this second centrifugal ultrafiltration step was collected as the fraction containing the nitric acid extracted polymers of high molar mass (NA-HMM). Both collected fractions were dialyzed against demineralized water for 48h (Spectra/Por® Dialysis Membrane, 3.5 kDa MWCO), lyophilized (Alpha 2-4 LSC, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany) and stored in a desiccator until use. Some practical issues forced the repetition of the NA sample production. As a consequence, the NA-LMM sample was produced from NA b1 and NA-HMM was produced from NA b2 according to the procedure described in this section.

2.3 Structural characterization of the pectin samples

2.3.1 Degree of methyl esterification

The DM was measured using Fourier transform infra-red spectroscopy (FT-IR; IRAffinity-1, Shimadzu, Kyoto, Japan) following the procedure and calibration curve described by (Kyomugasho et al., 2015). FT-IR analysis was performed in quadruplicate.

2.3.2 Galacturonic acid content

The galacturonic acid (GalA) content of the samples was determined spectrophotometrically. Before analysis, samples were hydrolyzed with H_2SO_4 following the method described by Ahmed & Labavitch (1978). The hydrolysis was performed in duplicate and each hydrolysate was analyzed in triplicate (25 °C, wavelength 520 nm, GENESYS^{TM} 30 Visible Spectrophotometer, Thermo Scientific, Massachusetts, USA). The spectrophotometric method of analysis can be consulted in Blumenkrantz & Asboe-Hansen (1973).

2.3.3 Monosaccharide composition

The GalA content is related to the pectin content but it is equally important to determine the content of other monosaccharides as well. Monosaccharides in the samples can originate from pectin sidechains and from co-extracted non-pectin polysaccharides. The content of fucose, rhamnose, arabinose, galactose, xylose and mannose in the samples was determined using high performance anion exchange chromatography coupled to a pulsed amperometric detector (HPAEC-PAD). Before analysis, the samples were hydrolyzed in 4 M trifluoroacetic acid (TFA) at 110 °C for 1.5 h followed by immediate cooling in an ice bath. Neutralization of the remaining TFA was performed by first drying the samples under N_2 at 45 °C, washing with 1 M ammonium hydroxide and finally drying the samples a second time under N_2 at 45 °C (De Roeck et al., 2008; Stolle-Smits et al., 1997). The fully dried hydrolyzed samples were redissolved in ultrapure water and filtered (Chromafil® A-45/25, 0.45 μm pore size, Macherey-

Nagel, Düren, Germany) before being injected into the HPAEC-PAD system. The hydrolysis was performed in duplicate.

Identification and quantification were performed by use of calibration curves using a mixture of commercially available standards of all seven monosaccharides to be quantified. Further details on the preparation of these standards and on the Dionex ICS-6000 system (Dionex, Sunnyvale, CA, USA) used for the HPAEC-PAD analysis can be found in Neckebroeck et al. (2020). The analysis in the current study was performed exactly as described in the work mentioned.

2.3.4 Protein content

The calculation of the protein content of the samples was based on the total nitrogen content which was determined in triplicate for each sample using an elemental analyzer (EA 1108 CHNS–O elemental analyzer, CE- Instruments/Thermo Scientific, Waltham, MA, USA) using the Dumas method (Santiago et al., 2018). A conversion factor of 6.25 was applied to the total nitrogen content to calculate the protein content of each pectin sample.

2.3.5 Molar mass distribution

Lyophilized samples were dissolved overnight in ultrapure water at a concentration of 0.2% (w/v). After dissolving, samples were filtered before injection (Chromafil A-45/25, 0.45 mm pore size, Macherey-Nagel Gmbh, Duren, Germany). Consequently, 100 µl of each sample was injected and analyzed by means of high performance size exclusion chromatography coupled to a multiangle laser light scattering detector (HPSEC-MALLS). Elution was performed using a 0.1 M acetic acid buffer (pH 4.4) containing 0.1 M $NaNO_3$ at a flow rate of 0.5 ml/min over a series of size exclusion columns kept at 35 °C (Ultrahydrogel 250, 1000 and 2000 with exclusion limits of 8×10^4, 4×10^6 and 1×10^7 g/mol, respectively, Waters, Milford, MA, USA). The concentration of the eluted polymers was calculated based on the refractive

index (RI) signal and a dn/dc value of 0.146 ml/g (Shodex RI-101, Showa Denko K.K., Kawazaki, Japan). The molar mass was calculated from the MALLS signal (PN3621 detector, Postnova analytics, Germany) applying the Debye fitting method (up to second order) by the NovaMals software (version 1.2.0.0, Postnova analytics, Germany). Each sample was analyzed in triplicate. The analysis performed was previously described by Shpigelman, Kyomugasho, Christiaens, Van Loey, & Hendrickx (2014). The average molar mass of the two individual peaks was calculated as follows: for peak 1, the part of the molar mass distribution between minute 35 and minute 51 of elution was taken into account, while for peak 2, the part from minute 51 of elution until the end of elution set at 60.5 minutes was taken into account.

2.4 Physicochemical characterization of the pectin rich samples

2.4.1 ζ-potential

The ζ-potential of all samples was measured as a function of pH by capillary electrophoresis (Zetasizer NanoZS, Malvern Instruments, Worcestershire, United Kingdom). 1% (w/v) solutions were prepared overnight and subsequently diluted 1:100 (v/v) in ultrapure water set at specific pH values (pH 1.5 to 7). Analysis was performed applying an electrical voltage over two electrodes of capillary cells containing the samples. The resulting velocity of charged polymers was measured by a laser beam and used to obtain the electrophoretic mobility. The ζ-potential was calculated in the instrument's software from the electrophoretic mobility using the equation of Henry. Each sample at one particular pH value was inserted twice into the equipment and additionally analyzed in duplicate with a minimum of 12 runs per measurement. Reported values are averages of these four analytical values.

2.4.2 Dynamic interfacial tension

An important characteristic of emulsifiers is their capacity to lower the interfacial tension by adsorbing at the interface. This adsorption enhances droplet disruption and stabilizes emulsified droplets. Therefore, the dynamic interfacial tension of an oil droplet in aqueous

solutions of the produced samples was measured using a pendant drop tensiometer (CAM 200, KSV Instruments, Finland). First of all, commercial sunflower oil was purified exactly as described in Neckebroeck et al. (2020) using the inorganic adsorbent Florisil®. The purified sunflower oil was used to create an oil droplet in an 0.2% (w/v) solution of the produced samples set at pH 2.5 or 6.0. After droplet formation, optical droplet profile determination was started immediately and performed for 1 hour and in duplicate for every sample and pH condition.

2.4.3 Flow behavior

The flow behavior of the three samples was studied using a stress-controlled rheometer (MCR 302, Anton Paar, Graz, Austria) at 25 °C based on a method described by Bernaerts et al. (2018) with slight modifications. A double wall Couette geometry (DG26.7, internal radius 12.3 mm, external radius 13.3 mm and measuring height 40 mm) was used. All samples were dissolved overnight, brought to pH 2.5 or 6.0 and adjusted to a final concentration of 1% (w/v). First, the samples were presheared for 30 s at a shear rate of 10 s^{-1} followed by 300 s of rest before each measurement. Next, steady-shear measurements were performed by increasing the shear rate logarithmically from 1 to 100 s^{-1} and applying each shear rate until steady state was reached, with a maximal measuring time of 40 s per shear rate. Finally, a minimum torque of 0.1 µN m was set to ensure reliability of the data.

2.5 Emulsion preparation

Coarse 5% (w/v) oil-in-water (o/w) emulsions were prepared by mixing purified sunflower oil and a 1% (w/v) solution of the lyophilized samples using a high speed mixer at 9500 rpm for 10 min (Ultra-Turrax T25, IKA, Staufen, Germany). Emulsions of all samples were prepared at both pH 2.5 and pH 6.0 since at these conditions distinctly different charge densities exist on the polymer chains in the samples (see **Figure 2**). After mixing, coarse emulsions were

immediately further homogenized using high pressure homogenization at 100 MPa (STANSTED SPCH-10, Homogenising Systems, UK) for one cycle.

2.6 Evaluation of physical stability of emulsions

To investigate the physical stability of the final emulsions, they were stored at 4 °C for 14 days. On day 0 (i.e. day of emulsion production), 1, 4, 8 and 14 of storage, the particle size distribution and the microstructure of the emulsions were analyzed and macroscopic pictures were taken to visually evaluate noticeable creaming. Furthermore, the creaming rate was analyzed in an accelerated way as well by means of a direct stability analysis on the day of emulsion production using an analytical centrifuge at 20 °C.

2.6.1 Volume-weighted particle size distribution

Laser diffraction (Beckman Coulter Inc, LS 13 320, Miami, Florida, USA) was used to determine the particle size distributions and d_{43} of all emulsions on each analysis day. Emulsions were first shaken to resuspend possible creamed oil droplet fractions before inserting it into the stirring tank of the equipment. Scattered laser light was detected at multiple angles and transformed into a particle size distribution using the Mie theory (refractive index of purified sunflower oil was experimentally determined to be 1.467). Each emulsion was analyzed in duplicate and the equipment was set to perform two runs per repetition. Presented results are average values of those four measurements. Reporting the d_{43} as average particle size was preferred as this parameter is sensitive to the presence of large oil droplets, which makes it a good parameter to consider when evaluating emulsion stability.

2.6.2 Microstructure

The microstructure of the emulsions was visualized using light microscopy. Imaging was performed with an Olympus BX-51 light microscope (Olympus, Optical Co. Ltd., Tokyo, Japan) equipped with an Olympus XC-50 digital camera (Olympus, Optical Co. Ltd., Tokyo,

Japan) and coupled to an image-analysis software (cell^F, Soft Imaging Systems, Münster, Germany). Micrographs were taken using a 40x magnification and an exposure time set at 10 ms of two test tubes of each emulsion on each analysis day.

2.6.3 Accelerated physical stability test

Emulsions were centrifuged (1308 g at 20 °C) in an analytical centrifuge (LUMiFuge, LUM GmbH, Berlin, Germany) while simultaneously recording laser extinction profiles over the full length of the emulsion. An instability index was calculated based on the Space- and Time-resolved Extinction Profiles (STEP®) Technology® as described in detail in Detloff, Sobisch, & Lerche (2014). The instability index can range from 0 (theoretical perfect stability) to 1 (theoretical full destabilization of the emulsion). A total of 1000 extinction profiles were recorded for each emulsion during centrifugation at a rate of one profile each minute. Similar to the other stability evaluation techniques described above, this stability analysis was performed in duplicate and only on the day of emulsion production to avoid interference of possible destabilization phenomena occurring after (short) storage.

3 Results and discussion

3.1 Emulsifying and emulsion stabilizing potential of the pectin rich onion extract

From a functionality point of view, previous research showed that the pectin rich onion extract (NA) displayed a very good emulsifying and emulsion stabilizing potential both at pH 2.5 and pH 6.0 (Neckebroeck et al., 2021). Indeed, evaluating the oil droplet size distributions of o/w emulsions stabilized by 1% NA in **Figure 5A** and the corresponding d_{43} in **Figure 6A**, very little changes in oil droplet size were noticed during refrigerated storage at both pH values. Micrographs of NA stabilized emulsions at pH 2.5 and pH 6.0 displayed homogeneous emulsions with predominantly small oil droplets. Visually, the micrographs showed no differences in oil droplet size due to coalescence or flocculation during a 14 days storage period at 4 °C (**Figure 7**). Furthermore, after 14 days of storage, no creaming was observed in NA stabilized emulsions as can be seen in **Figure 8**. Complementary, an accelerated creaming stability test by centrifugation was performed (**Figure 9**). The curves of the NA stabilized o/w emulsions (dashed lines) demonstrate that after 16 hours of centrifugation still no plateau value for the instability index was reached. This indicates that even at accelerated conditions creaming was a relatively slow process which corresponds with the absence of a visual creamed layer in the emulsions stored at gravitational conditions.

Looking at the structural characteristics of the NA sample (**Table 1** and **Figure 1**), its distinct bimodal molar mass distribution was remarkable. As two polymer fractions with different average molar mass could be distinguished, it is to be unraveled whether both fractions contribute to the emulsifying and emulsion stabilizing potential of the NA pectin rich onion extract. To verify this hypothesis, it was aimed to separate both fractions and study their individual functionality (section 3.2). Additionally, structural and physicochemical properties of both fractions were characterized. In this way, it can be investigated whether both fractions

consist of different kinds of polymers which may vastly influence their functionality and furthermore impact their role in the observed emulsion stabilizing potential of the NA extract.

3.2 Structural and physicochemical characterization of NA-HMM and NA-LMM in light of their potential to stabilize emulsions

3.2.1 Structural characterization

All results of the determined structural properties are presented in **Table 1**. Besides, molar mass distributions of all samples are depicted in **Figure 1**. For practical reasons, two separate batches of the onion acid extract were prepared. The first batch (NA b1) was used as a starting point to prepare the NA-LMM sample while the second batch (NA b2) was used to prepare NA-HMM. Some minor differences between both batches need to be mentioned. NA b2 contained less GalA and slightly more galactose than NA b1 although the galactose content was considered high for both batches. The difference in glucose content was remarkable and consequently it seemed there were more glucose containing polymers co-extracted in NA b2 compared to NA b1. It is unclear what the origin of these glucose molecules exactly is as it is unlikely that residual cellulose is present in the sample since this polymer will not be solubilized during extraction and monomeric glucose or sucrose is expected to be removed during dialysis. High glucose levels are however in line with the results reported by Ng et al. (2000) for the fleshy parts of onions. Despite these minor differences and most importantly, both batches clearly displayed a bimodal molar mass distribution (**Figure 1**). As in this research, we aim and focus on the separation of the two molar mass fractions of the original onion NA sample, it was concluded that both batches were suitable to be further used for fractionation purposes. Results for the NA sample mentioned in sections 3.2.2 and 3.2.3 represent the NA b1 sample.

Considering the structural properties of NA-LMM, the results clearly showed a low GalA content indicating the presence of only a small amount of pectin. Given the very high galactose levels, it can be concluded that this sample mostly consists of short chain galactans. The presence of these short chain galactans most probably explains the high galactose content in the original NA sample as well. Golovchenko, Khramova, Ovodova, Shashkov, & Ovodov (2012) already suggested the presence of short chain galactans in isolated polysaccharide material of onion. The concentration profile of the molar mass distribution of NA-LMM in **Figure 1** indicated substantial enrichment of the desired fraction of low molar mass with only a minor presence of polymers with medium molar mass which eluted between minute 45 and minute 50 of the HPSEC-MALLS analysis. Additionally, the molar mass of NA-LMM had a lower average value compared to the polymers present in this fraction of the original NA sample.

The NA-HMM sample clearly showed an enrichment of the higher molar mass fraction in the molar mass distribution (**Figure 1**). The sample has a higher GalA and a lower galactose content compared to the original NA b2 sample used as starting material. This can be explained by the removal of some low molar mass polymers which were found to be mainly short chain galactans. The DM of the pectin present is highly comparable to the DM of the original NA sample.

Based on the molar mass distributions depicted in Figure 1, NA-LMM and NA-HMM are individually enriched with polymers of low and high molar mass respectively. The following sections will discuss the results obtained regarding the physicochemical and functional properties of the fractionated samples.

3.2.2 Physicochemical characterization

In **Figure 2**, the ζ-potential of the three samples is represented as function of pH of the aqueous solution. NA and NA-HMM showed nearly identical profiles which can be explained by the GalA content of these samples and the similar DM. It was contemplated that almost all GalA present in the NA sample ends up in the NA-HMM fraction after fractionation. Moreover, a similar DM was measured for NA and NA-HMM and consequently, similar charge densities could be expected for both samples. Oppositely, the charge density of NA-LMM was anticipated to be very low due to the low GalA content. The ζ-potential analysis showed that increasing the pH induced a clear negative charge for NA-LMM. The low amount of GalA and possibly the protein moieties present might be responsible for this. Analyzing charge density is relevant for compounds aimed to be used for emulsion stabilization as an increased charge density of molecules adsorbed at the oil-water interface normally results in electrostatic repulsive forces and consequently an increased emulsion stability.

Adsorption of amphiphilic compounds at the oil-water interface, decreases the interfacial tension. This ability is important for emulsifiers as it facilitates droplet break up during emulsification and increases the stability forming an interfacial layer surrounding the oil droplets. In **Figure 3**, the interfacial tension as a function of time is displayed for all samples. Immediately after starting the analysis, the polymers present in the surrounding, aqueous phase spontaneously adsorbed at the interface decreasing the interfacial tension over time. At both pH 2.5 and pH 6.0, a fast decrease of the interfacial tension was observed during the first minutes of analysis for the NA sample. A clear decrease was also observed for NA-LMM at pH 2.5 while this was not the case at pH 6.0. Furthermore, no clear decrease of the interfacial tension is observed for the NA-HMM sample at both pH values. Based on these results, it can be concluded that neither NA-LMM nor NA-HMM spontaneously adsorbed at the interface as fast as the original NA sample at both pH conditions.

When considering samples with different molar masses, it can be expected that these samples exhibit different viscosity increasing capacities. According to the Mark-Houwink relation, the intrinsic viscosity of polymers in solution mainly depends on the molar mass of the polymers (Picout & Ross-Murphy, 2007). The viscosity of 1% (w/v) solutions of all samples was measured using shear rate sweep rheological measurements. The experimental values at a shear rate of 10 s^{-1} are represented in **Figure 4**. Identical trends were observed at other shear rates (Supplementary Material S1). The molar mass of NA-LMM seems to be too low to have a significant effect on the solution viscosity. The viscosity of NA-LMM solutions at both pH values was only slightly higher than 1 mPa.s at higher shear rates and consequently they merely had a viscosity comparable to pure water. Comparing the results for NA and NA-HMM, two interesting observations can be made. Firstly, at both pH values NA-HMM created a higher solution viscosity compared to NA which can be attributed to the selective enrichment of high molar mass polymers in the NA-HMM samples. Secondly, at pH 6.0 both NA and NA-HMM displayed higher viscosities than at pH 2.5. As at pH 6.0, NA and NA-HMM bear more negative charges compared to pH 2.5 (**Figure 2**), more electrostatic interactions can take place between positively charged divalent ions in the samples and the negatively charged polymers (mainly pectin).

3.2.3 Emulsifying and emulsion stabilizing potential

Several physical phenomena can cause o/w emulsion instability. Flocculation and coalescence influence the oil droplet distribution and oil droplet size. Flocculation can be considered, to some extent, a reversible mechanism, while coalescence is an irreversible process and ultimately leads to emulsion breakage. Next to these two phenomena, creaming is another very important and common destabilization phenomenon. While creaming as such can be reversed by simple agitation of the emulsion, oil droplets in close vicinity to each other might upon extended storage cause further irreversible destabilization by coalescence. It is clear that

studying emulsion stability is very complex and maintaining emulsion stability during storage can be challenging.

After emulsion preparation using one of the three samples of interest at a time, emulsion stability was evaluated through various analysis techniques. Laser diffraction was used to evaluate the evolution of the oil droplet size over storage. The oil droplet size distributions for all emulsions formulated are presented in **Figure 5** for the day of emulsion production (day 0), day 8 and day 14 of refrigerated storage. **Figure 6** displays the according average oil droplet sizes (presented as d_{43} values) of all emulsions determined on each analysis day. Examining the emulsified oil droplets by means of microscopy is a very useful additional technique that can differentiate between flocculation and coalescence phenomena, since this is not always possible only by laser diffraction analysis. The microstructure of all emulsion under consideration is visualized in the images presented in **Figure 7**. The presented pictures are taken on the day of emulsion production (i.e. day 0) and after 14 days of refrigerated storage. Next to studying particle size to evaluate the possible influence of flocculation and coalescence, the extent of creaming in the samples was studied as well. Macroscopic pictures of the emulsions stored on the shelf were taken each day of analysis to visually observe the presence of a creamed layer in the emulsions under gravitational conditions (**Figure 8**). Furthermore, an accelerated stability test was performed using an analytical centrifuge. The change of the instability index during centrifugation is displayed in **Figure 9**.

In case of the oil droplet size distributions for the NA-LMM stabilized emulsion at pH 2.5, the distribution ranges from small oil droplets of around 1 µm until oil droplets of almost 100 µm already on the day of emulsion production. At the beginning of storage, the oil droplet population is dominated by small oil droplets, while after 8 and 14 days of storage the amount of small oil droplets in the population decreased and the particle size distribution shifts more towards larger droplets. This observation is supported by the micrographs: on day 0, some

larger oil droplets were visible in the emulsion as well as a lot of smaller ones while at day 14 some very large and medium sized oil droplets were found together with some remaining small ones. Clearly, coalescence occurred in this emulsion and possibly the extent of coalescence is increased due to rapid creaming (see also below). At pH 6.0, smaller oil droplets were created than at pH 2.5 in presence of NA-LMM. Even though some larger particles were observed under the microscope and by laser diffraction, the destabilization due to coalescence occurred to a lower extent compared to pH 2.5. The similar oil droplet size distribution at day 14 compared to day 0 is probably caused by inaccurate sampling as the micrograph of this final day clearly shows the presence of larger oil droplets and some flocculation. Therefore it is expected that the oil droplet size distribution of day 14 would be similar to that of day 8 showing the presence of larger oil droplets. Both emulsions stabilized by NA-LMM displayed visible creaming from day 1 of storage onwards (**Figure 8**). At pH 2.5 almost complete clarification of the bottom layer is observed after 14 days of storage. This is confirmed by the rapid increase of the instability index during the accelerated stability analysis (**Figure 9**). Clearly, the NA-LMM stabilized emulsions at pH 2.5 have poor stability, suffering from both strong coalescence and fast creaming. Both phenomena probably facilitating and accelerating each other. Although dynamic interfacial tension measurements reveal spontaneous adsorption of NA-LMM at pH 2.5 as observed in **Figure 3**, this spontaneous adsorption is clearly not enough for this sample to be a good emulsion stabilizing compound under these conditions. NA-LMM therefore shows limited capabilities in emulsion stabilization, due to poor interfacial stabilization and poor viscosifying properties.

For NA-HMM, the oil droplet size distributions showed little changes over a 14 day storage period. Consequently, the corresponding d_{43} values for the NA-HMM stabilized emulsions did not increase considerably over time. The values for NA-HMM at pH 2.5 remained constant and this emulsion was arguably even more stable than the NA stabilized emulsion at pH 2.5.

At pH 6.0 in case of NA-HMM, it seemed that during homogenization the oil is not as evenly fine dispersed compared to the NA stabilized emulsion (**Figure 5 and 6**). As explained before, NA-HMM seemed unable to decrease the interfacial tension at both pH values by spontaneous adsorption. Even though oil droplets and polymers experience high forces and shears during homogenization, it can be expected that also under these conditions adsorption at the interface will be less efficient for the NA-HMM sample compared to NA. This eventually affected the oil droplet size when the surrounding pH induced a high charge density on the polymers in the sample. The micrographs of both emulsions stabilized by NA-HMM showed many small oil droplets homogeneously distributed in the images. No visual signs of coalescence or flocculation are noticeable. However, the NA-HMM stabilized emulsion at pH 6.0 showed some visual creaming from day 4 of storage (**Figure 8**). The accelerated analysis confirmed that this emulsion creamed faster compared to the NA-HMM emulsion at pH 2.5 (**Figure 9**). Although a higher viscosity was observed at pH 6.0 for this sample, the larger oil droplet sizes seemed to have a dominating effect on the creaming rate of the emulsion.

The increase of the instability index during centrifugation is complementary to the observations under gravitational conditions. Both NA-LMM stabilized emulsions and the NA-HMM stabilized emulsion at pH 6.0 display fast creaming behavior compared to the other emulsion where no creamed layer was observed macroscopically. Overall it can be concluded that after the selective elimination of most polymers with low molar masses, NA-HMM still shows promising emulsion stabilizing potential. This is mainly attributed to the potential to increase solution viscosity and to the presence of chargeable GalA units, factors which are in turn linked to the high molar mass of this NA-HMM fraction.

4 Conclusion

A pectin rich extract from onion flesh, produced by nitric acid (NA) extraction, was found to adequately stabilize o/w emulsions. Interestingly, the polymers present in this sample displayed a bimodal molar mass distribution. Ultracentrifugation resulted in two distinct fractions, NA-LMM and NA-HMM, enriched in polymers of low and high molar mass respectively. Structural characterization of these separated fractions demonstrated that NA-LMM mainly exists of short chain galactans and contained only very little pectin. The presence of these galactan polymers explained the high galactose content of the original NA extract. The NA-HMM sample contained most of the pectin polymers after fractionation and presented a similar DM as the original NA sample. Overall, comparing the molar mass distributions of all three samples, the ultrafiltration technique applied allowed an adequate separation of both polymer fractions. In line with the expectations, the experimental work showed that NA-LMM had no viscosity increasing effect when solubilized in a 1% (w/v) concentration. Contrastingly, the NA and NA-HMM samples increased viscosity at pH 2.5 to about 9 mPa.s and 17 mPa.s and at pH 6.0 to about 15 mPa.s and 25 mPa.s respectively. An increased viscosity of the continuous phase could decrease oil droplet mobility in emulsions. Moreover, it was demonstrated that NA and NA-HMM displayed a higher viscosity at pH 6.0 compared to pH 2.5.

The NA-LMM emulsion at pH 2.5 displayed extensive coalescence and creaming and was consequently considered unstable already in an early stage of storage. At pH 6.0, NA-LMM performed slightly better in stabilizing an emulsion yet several destabilization phenomena affected the stability of this emulsion as well. Generally, NA-HMM presented good emulsion stabilizing potential. Only minor creaming from day 4 of storage onwards was visible for the NA-HMM stabilized emulsion at pH 6.0 but no significant oil droplet size increase was observed. Overall, it can be stated that the low molar mass polymer fraction present in the NA sample did not substantially contribute to the functionality of this sample to stabilize o/w

emulsions. The polymers with higher molar mass, consisting to a large extent of pectin chains, provided the functional potential since NA-HMM stabilized emulsions well when solely applied.

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Declaration of interests

Declarations of interest: none

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Tables

Table 1. Structural properties of both batches acid onion extract and both fractionated samples. All values are expressed as average values ± standard deviation. (GalA: galacturonic acid; DM: degree of methyl esterification; MM: molar mass)

	NA b1	NA-LMM	NA b2	NA-HMM
GalA content (mg/g)	359.26 ± 21.96	25.24 ± 13.31	284.45 ± 6.16	440.36 ± 14.93
Neutral sugars (mg/g)				
Rhamnose	12.23 ± 0.54	ND	12.65 ± 0.20	15.43 ± 0.78
Arabinose	12.79 ± 0.66	15.24 ± 0.42	10.98 ± 0.24	5.05 ± 0.80
Galactose	291.11 ± 8.57	627.32 ± 6.86	329.87 ± 37.63	259.65 ± 21.69
Fucose	ND	ND	ND	ND
Glucose	52.23 ± 1.71	75.80 ± 3.77	178.60 ± 12.78	146.66 ± 8.45
Xylose	6.37 ± 0.96	ND	ND	1.26 ± 0.41
Mannose	ND	ND	ND	1.70 ± 0.43
DM (%)	56.41 ± 0.21	ND	52.76 ± 1.33	55.66 ± 0.42
Protein content (% w/w)	6.28 ± 0.08	5.13 ± 0.04	5.34 ± 0.11	5.26 ± 0.04
MM peak 1 (kDa)	1150.00 ± 30.00	N/A	2220.00 ± 105.36	2213.33 ± 205.51
MM peak 2 (kDa)	59.90 ± 8.67	25.37 ± 1.15	130.33 ± 19.14	N/A

ND: not detected
N/A: not applicable

Figure captions

Figure 1. Molar mass distributions displaying the molar mass (thick lines on top of the graph) and concentration (thin lines at the bottom of the graph) profiles for NA b1 (full black), NA-LMM (light grey), NA b2 (dashed black) and NA-HMM (dark grey).

Figure 2. ζ-potential as function of pH for 1% w/v solutions of (▲) NA, (■) NA-LMM, (○) NA-HMM.

Figure 3. Dynamic interfacial tension of a droplet of purified sunflower oil in 0.2% solutions of NA (dashed lines), NA-LMM (full lines) and NA-HMM (dotted lines). Dash-dotted lines represent measurements in ultrapure water. Black lines represent measurements at pH 2.5, while grey lines represent measurements at pH 6.0.

Figure 4. Viscosity at a shear rate of 10 s^{-1} for 1% solutions of NA, NA-LMM and NA-HMM. Full black bars represent measurements at pH 2.5 while the shaded bars represent measurements at pH 6.0.

Figure 5. Oil droplet size distribution of 5% o/w emulsions stabilized by 1% (w/v) (A) NA, (B) NA-LMM and (C) NA-HMM for the day of emulsion production (full lines), after 8 days of storage (dotted lines) and after 14 days of storage (dashed lines). Black lines represent emulsions at pH 2.5, while grey lines represent emulsions at pH 6.0

Figure 6. Evolution of the average oil droplets size (expressed as d_{43}) during refrigerated storage of 5% o/w emulsions stabilized by 1% (w/v) (A) NA, (B) NA-LMM and (C) NA-HMM. Black symbols and lines represent emulsions at pH 2.5, while grey lines represent emulsions at pH 6.0.

Figure 7. Micrographs, obtained with light microscopy, of 5% o/w emulsions stabilized by 1% (w/v) NA, NA-LMM and NA-HMM at pH 2.5 and pH 6.0. Images displayed for the day of emulsion production (day 0) and day 14 of refrigerated storage. Scale bars represent a length of 100 µm.

Figure 8. Macroscopic pictures of 5% o/w emulsions stored at 4 °C stabilized by 1% (w/v) NA, NA-LMM and NA-HMM. For each set of pictures from left to right: day of emulsion production (day 0), day 1, day 4, day 8 and day 14 of refrigerated storage at 4°C. Black arrows indicate visual cream layers.

Figure 9. Instability index of 5% o/w emulsions stabilized by 1% NA (dashed lines), NA-LMM (full lines) and NA-HMM (dotted lines). Black lines represent measurements at pH 2.5 while grey lines represent measurements at pH 6.0.