Proceedings

Functionalisation of Pectin by Ultra High Pressure Homogenisation †

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† Presented at the 1st International Electronic Conference on Food Science and Functional Foods, 10–25 November 2020; Available online: https://foods_2020.sciforum.net/

Submitted: date; Accepted: date; Published: date

Abstract: Pectin is well-known plant-based hydrocolloid extensively used in food and beverage industry for formation and/or enhancement of product texture and stability. Versatile functionalities of different pectin types (e.g. gelling, thickening, colloidal stabilization) are predetermined by their structure. The aim of this work was to explore possibility to alter structure of pectin by pilot-scale ultra-high dynamic pressure treatment, as a physical modification approach, and potentially enhance its functionality.

High esterified unstandardized apple (AU) and citrus pectin (CU) solution (2.5% (m/m)) were treated by ultra-high pressure homogenizer (UHPH) at three different pressure levels and characterized with respect to their apparent viscosity, gelling behavior (gelation time, temperature and melting point), gel strength, molecular weight (MW) and on-line viscosity using size-exclusions chromatography coupled to refractive index, light scattering and online differential viscometer. The results indicated that UHPH impacted the MW in a source dependent manner. Treated CU and AU pectin exhibited a small decrease in the average MW and a more pronounced decrease in the intrinsic viscosity, likely due to more significant UHPH effect on the larger pectin molecules. In addition, the smaller (in volume) AU pectin presented a more compact conformation in solution. On the macroscopic level, those changes resulted in statistically significant decrease in apparent viscosity. UHPH CU pectin exerted decrease in gelation time and increase in gelation temperature, but not on statistically significant level. Even though treatment caused decrease in apparent viscosity of CU and AU pectin it had no effect on their respective gel strengths.

From the obtained results, it can be concluded that UHPH process has a potential to modify the structure and flowing behavior of pectins, but further research is needed in order to elucidate all the changes in pectin functionality and potential benefits of this ubiquitous and multifunctional hydrocolloid treated by UHPH.

Keywords: Ultra high pressure homogenization (UHPH), pectin; functionality; structure

1. Introduction

Pectin is well-known plant-based hydrocolloid that found numerous applications in food and other related industries (e.g. pharmaceutical). As a functional ingredient, pectin has been extensively used in food and beverage industry for various applications such as gelling, thickening or colloidal stabilization [1]. Pectin is usually commercially obtained from two major sources, apple pomace and citrus peel (juice industry side-streams). Thus, majority of commercially available pectin for different applications originates from these two side-streams [2].

Although being natural polysaccharide with possible and health-promoting effect its industrial application is still limited, partially due to its techno-functional properties, favouring utilization of animal-based hydrocolloids (gelatine) or stabilizers with possible adverse health effects (carrageen).

It is well known that versatile functionalities of pectin are predetermined by its structure and that by modifying pectin structure (e.g. enzymatically, chemically etc.) different functionalities are obtained [1]. Ultra-High Pressure Homogenization (UHPH) is a technique, known for its ability to modulate the structure and functionality of polysaccharides and different polymers by ultra-high dynamic pressure (up to 350 MPa) in a continuous manner [3-6]. The technology is based on forcing a dispersion to flow at a high velocity through a narrow passage, resulting in turbulence, high shear, cavitation, and temperature increase [3].

The aim of this work was to explore possibility to alter structure of pectin by pilot-scale ultrahigh dynamic pressure treatment, as a physical modification approach, in relation to the impact on gel properties.

2. Material and Methods

2.1. Material

Apple and citrus unstandardized high esterified pectins (Pektin Classic CU-L 033/20 and Pektin Classic AU-L034/20), provided by Herbstreith & Fox GmbH & Co.KG were used for the experiments. Pectin powders had recorded moisture level of 10%.

2.2. Sample Preparation and UHPH Treatment

Sample preparation.

For UHPH treatment the 2.5% (m/m) CU and AU pectin solutions were prepared. Pectin powder was added and dispersed in water at temperature of around 80.0 °C and mixed with Kotthoff mixer for 5 minutes. Final temperature of pectin solution was around 75.0 °C and pH-value around 3,0 (for the study of the impact of UHPH on molecular weight and intrinsic viscosity the pH of the solution was also corrected to 6 using NaOH). The pectin solution was further cooled to around 20 °C with plate heat exchanger. The untreated sample was taken and put on ice while the rest of the solution was high pressure treated.

UHPH treatment.

Pectin solution intended for UHPH treatment was pumped by a screw feed pump (Seepex GmbH, Bottrop, Germany) to the pilot-scale, two-stage high pressure homogenizer (Modell FPG 11300:350, Stansted Fluid Power Ltd., Essex, UK). Chosen treatment pressure levels in first stage were 50, 150 and 250 MPa, while in the second stage pressure was 8 MPa. The samples were cooled down between first and second stage valve by tube-in-tube heat-exchanging system, using cold water as a cooling medium. The sample temperature was tracked just after the first valve and at second stage, as well as after the UHPH treatment. After the treatment, the samples were placed on ice and stored refrigerated overnight at temperature of 4±1 °C. The samples intended molecular characterization were freeze-dried on a pilot-scale freeze-drier (Gefriertrocknung Greven GmbH, Greven). The pectin sample were firstly frozen at -30 °C and then dried during 3 days at temperature of around 20 °C under vacuum conditions of 0.4 mbar. Final moisture content of freeze-dried samples was around 2.2%.

Gel preparation.

Pectin gels were prepared from pectin solutions, according to adapted IPPA °SAG method. According to the procedure, 60 g of the 2.5 % (m/m) pectin solution was added to 60 g of tap water in a tared beaker with magnetic stirrer. The sugar (193,5 g) was added slowly while heating and constant stirring at 310 rpm. After adding the sugar, the total solution weight was 313,5 g. The prepared solution was fast brought to boil and while boiling reduced to 301,5 g. During the boiling 0,3 mL 48 m/v % tartaric acid was added in each one of three 60 mL vessels (Ø48 mm, height 62 mm). After reaching a weight of 301,5 g the beaker was removed from the heating plate and the solution had time to rest until the weight dropped to 300 g. Then 50 g of the solution was poured fast and without interruption in each of the before prepared small vessels, then shortly stirred with a thin stainless-steel rod. The rest of the boiled solution was transferred to another vessel. Then all rested open for one hour at room temperature before they were closed and stored overnight at 4 °C. Final concentration of pectin in prepared gels was 0.5% m/m.

2.3. Pectin Characterization

Molecular weight, dispersity and intrinsic viscosity measurements.

Characterization of the UHPH treated pectins was obtained based on a previously described method [7]. Freeze-dried UHPH treated and untreated (0.1 MPa) pectins were dissolved in 100 mM $NaNO_3$ solution (0.1% w/w). Molecular weight, intrinsic viscosity and dispersity (Mw/Mn) of the pectins were measured by utilization of multi-detector system (multi angle laser light scattering detector (9 angles), RI Detector and on-line viscometer (Postnova analytics, Landsberg, Germany). Size exclusion separation was obtained at 35 C by a series of three Waters columns (Waters, Milford, MA), ° namely, Ultra-hydrogel 250, 1000, and 2000 eluted by a filtered 100 mM $NaNO_3$ eluent. The specific refractive index (dn/dc) of 0.147 mL/g was used for calculation of pectins concertation from the RI detector, the injection volume and flow rate were set on 100μl and 0.5 ml/min respectively. The molecular weight was calculated using the appropriate model by Auto fitting method by the MALLS detector software (Nova Mals, version 1.5.0.8, Postnova analytics, Germany).

Apparent viscosity.

Measurements of the flow properties were performed according to the guidelines of the German Institute of Standardisation Norm (DIN 53019) by using a cone-plate (cone-Ø 6 cm) rotary rheometer (AR 2000, TA Instruments, New Castle, DE) with a shear rate range from 0.1 to 1000 s^{-1} at measuring temperature of 20 °C.

For the evaluation of the viscosity functions and the flow properties the power-law model was used.

$$\tau = K\dot{\gamma}^n$$

with τ as a shear stress (Pa), K as a consistency index (Pas^n), $\dot{\gamma}$ as a shear rate (s^{-1}) and n as a flow index (dimensionless).

Gel strenght.

Gel strenght was measured by adapted standard BLOOM method with a TA XT2 Texture Analyzier (Stable Mirco Systems, Godalming, UK) [8]. A plunger (Ø 0,5 in) was pressed 4 mm deep in the surface of the sample, deforming it but not breaking it. During pressing, force applied and time were recorded. Pre-test and test-speed were 0,5 mm/s, post-test speed was 1 mm/s and the trigger-value was 0,04 N. Six measurements per sample were performed of which the average value was formed.

Gelation speed and melting point (Sol-gel-sol transition).

The 5 grams of prepared pectin-sugar solution was heated to 108 °C and placed on pre-heated rheometer plate at the same temperature. After one drop of the tartaric acid solution (48 m/v %) was added and shortly stirred, upper rheometer plate was lowered. To prevent evaporation of the water, a thin paraffin oil layer was applied around the sample. The sample was first cooled down from 108 °C to 30 °C (gelation) and then heated up again to 108 °C. The cooling rate was 5 K / min and the heating rate was 10 K / min. The measurements were performed with a plate-plate (Ø 4cm) oscillation rheometer (AR 2000, TA Instruments, New Castle, DE) the following parameter were chosen: plate gap - 1000 μm, torque - 0,001 Nm, frequency - 1 Hz.

3. Results and Discussion

3.1. Apparent viscosity

Apparent viscosity was measured in untreated and UHPH AU and CU samples treated at 50, 150 and 250MPa. It was observed that CU pectin had higher viscosity when compared to AU pectin (*Figure A1*). UHPH CU pectin exhibited lower viscosity compared to the untreated sample. The extend of viscosity decrease seemed to be correlated with intensity of applied pressure. Unlike the CU pectin, it seems that changes in viscosity of AU pectin were significantly less pronounced when different pressure levels (50, 150 and 250 MPa) were applied (*Figure A1*). Both, untreated and UHPH treated AU and CU pectin exhibited shear thinning behaviour (*Figure A2*; *Table 1*), which is considered typical pectin flow behaviour [3,5]. With increasing pressure, decrease in consistency index (K) and increase in flow index (n) were observed for both, CU and AU pectin. Consistency index of untreated and UHPH treated CU samples significantly differed (Tukey test, $p<0,001$) (Table 1A). However, in case of AU pectin, samples treated at 150 and 250 MPa showed statistically different consistency indexes compared to untreated sample. These finding are mainly aligned with findings of Chen et al. (2012), where apple pectin solutions were treated by Dynamic High Pressure Microfluidization (DHPM) and Xie et al. (2018), where potato pectin was treated with UHPH. In both studies, apparent viscosity decreased with applied dynamic high pressure treatments and flow behaviour shifted from pseudoplastic more towards Newtonian behaviour.

Table 1. Flowing properties of CU and AU pectin solution.

Sample	Consistency index K [Pas^n]	Flow index n [-]	R^2 [-]
CU untreated	8,659 ± 0,236	0,534 ± 0,004	0,996
CU 50	7,914 ± 0,23	0,542 ± 0,005	0,995
CU 150	5,507 ± 0,154	0,590 ± 0,005	0,996
CU 250	3,710 ± 0,098	0,633 ± 0,004	0,997
AU untreated	0,337 ± 0,005	0,816 ± 0,002	0,999
AU 50	0,291 ± 0,004	0,829 ± 0,002	0,999
AU 150	0,218 ± 0,004	0,855 ± 0,003	0,999
AU 250	0,171 ± 0,003	0,871 ± 0,003	0,999

3.2. Impact of UHPH at pH 3 and pH 6 on pectin molecular characteristics

The average molecular weight of the pectins differed depending on the source and the pH value (Figure 3A and B). As the differences were already observed at 0.1 MPa, this suggests that the incubation at the higher pH value increases the observed Mw of the apple pectin while decreasing (to a lesser extent) the Mw of the citrus pectin. As it is unlikely that the pH changes differently degraded the pectin backbone, the differences due to pH are likely the outcome of the impact of pH on the conformation of pectin in solution. The impact of UHPH and different pressures is also dependent on the source. Citrus pectin clearly showed the pressure dependent decrease while the impact on apple pectin was much smaller, in line with previously published work and with results from section 3.1. The dispersity (i.e. Mw/Mn, Figure 3 E, F) was much more impacted by UHPH pressure in the apple pectin, suggesting that the degradation of only larger chains of pectin had a larger impact for this pectin. Furthermore, the dispersity increased at 300 MPa for the CU. The intrinsic viscosity (Figure 3 C,D) decreased for both pectins at both pH levels again confirming the reduction of Mw and the degradation of pectin, likely of the backbone by UHPH.

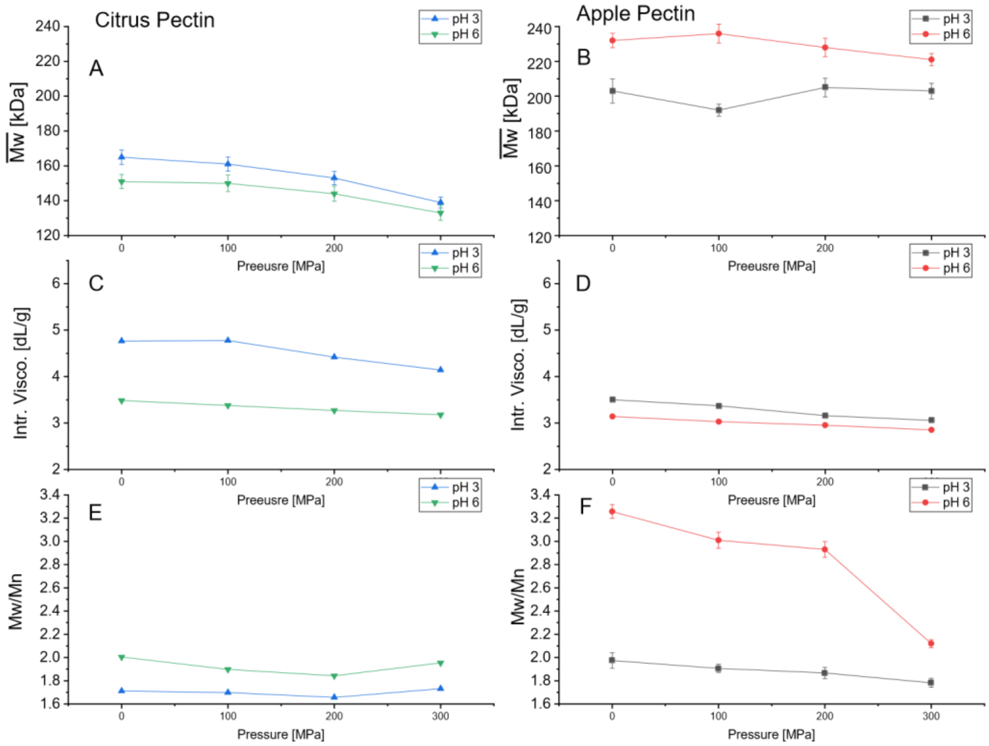
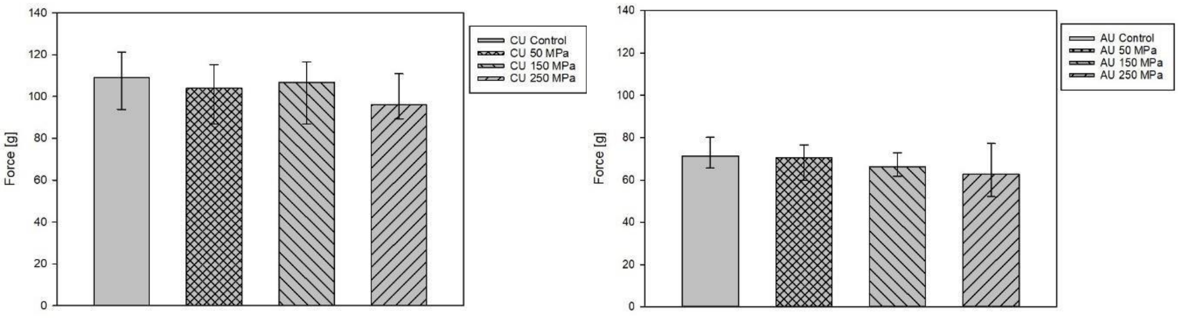


Figure 1. The impact of HPH at 3 levels (0.1 MPa, 100 MPa, 200 MPa, 300 MPa) on the mass-weighted average molecular weight (A, B), intrinsic viscosity (C, D) and dispersity (E, F) of citrus (CU) pectin (A, C, E) and apple (AU) pectin (B, D, F) when treated at pH 3 and pH 6.

3.3. Gel strenght

From Figure 2 it can be observed that at similar formation conditions CU pectin forms stronger gels than AU pectin, likely due to a larger content of neutral sugars in apple pectin compared to citrus one [9]. Although statistically significant differences were found between apparent viscosity of untreated and UHPH treated pectin samples, those differences were not observed in strength of the produced pectin gels. UHPH treatment did not have a statistically significant impact on gel strength of AU *(p = 0,462)* and CU *(p = 0,771)* under examined conditions.



(a) (b)

Figure 2. Gel strength of untreated and UHPH treated (a) CU and (b) AU pectin.

3.3. Gelation speed and melting point

All analyzed pectin gel samples exerted non-melting behavior (*Figure B1*). Although, UHPH treatment of both, CU and AU pectin, resulted in shorter gelation time and consequently higher gelation temperatures (*Table 2*, *Figure B2*), those differences were not significantly different and additional repetitions will be needed.

Table 2. Gelation temperature and speed of untreated and HPH treated CU and AU pectin.

Indicators	**Sample name**			
	CU Untreated	**CU 50**	**CU 150**	**CU 250**
Gelation Temperature [°C]	59,2 ± 1,10	63,2 ± 1,23	62,4 ± 0,87	62,5 ± 1,07
Gelation time [s]	586,4 ± 14,58	572,1 ± 28,12	547,7 ± 10,52	546,9 ± 13,29
	AU Untreated	**AU 50**	**AU 150**	**AU 250**
Gelation Temperature [°C]	54,8 ± 1,7	52,1 ± 0,066	57 ± 0,435	54,7 ± 0,528
Gelation time [s]	639,2 ± 21,02	656,5 ± 2,59*	616,4 ± 2,16	644,6 ± 2,885

5. Conclusion

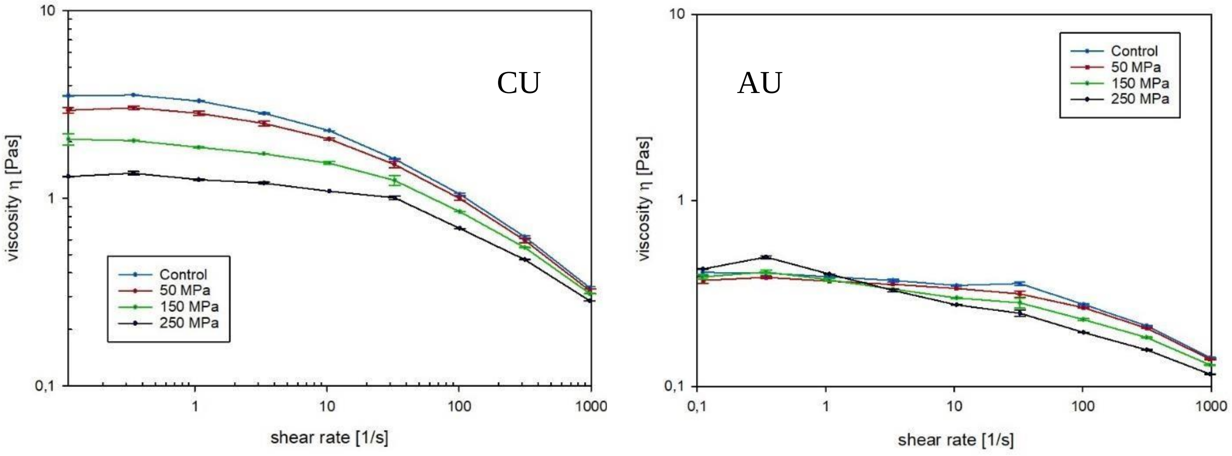
From the obtained results, it can be concluded that UHPH process has a potential to modify the structure and flowing behavior of pectins, but further research is needed in order to elucidate all the changes and potential benefits of this ubiquitous and multifunctional hydrocolloid treated by UHPH.

Author Contributions: supervision, K.A, A.S; funding acquisition, K.A, A.S, H-U.E; writing, M.Z, E.E; review and editing, K.A, A.S; conceptualization, K.A, A.S; methodology, M.Z, E.E, C.P, Z.O, H-U.E; formal analysis, M.Z, E.E, C.P; investigation, M.Z, E.E, C.P; project administration, K.A, Z.O, H-U.E.

Acknowledgments: This research has received funding from the European Institute of Innovation & Technology Food (EIT Food) under Activity code: 20032, Project full Title: “HPHC - Development and application of hydrocolloids functionalized by dynamic high pressure”.

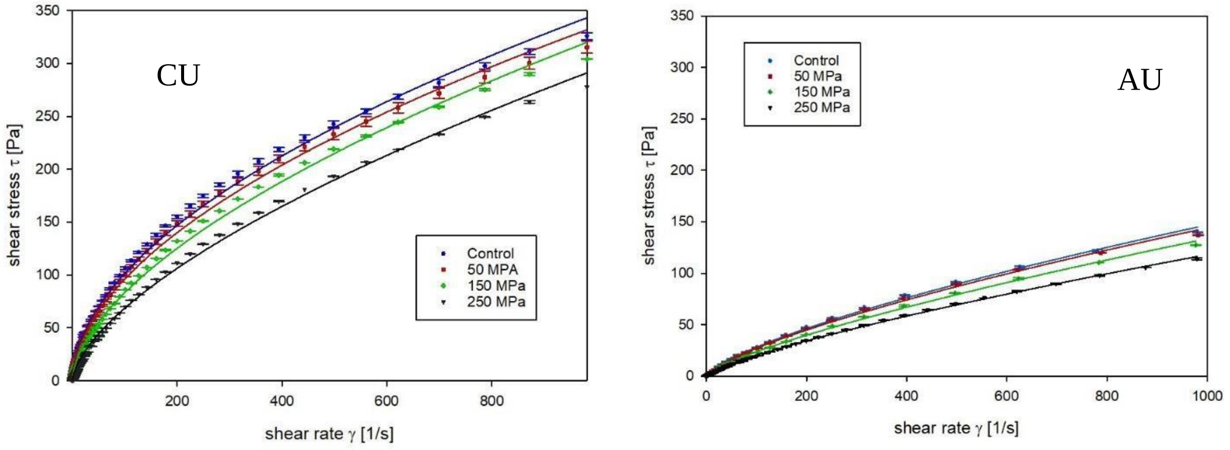
Conflicts of Interest: The authors declare no conflict of interest.

Appendix A



(a) (b)

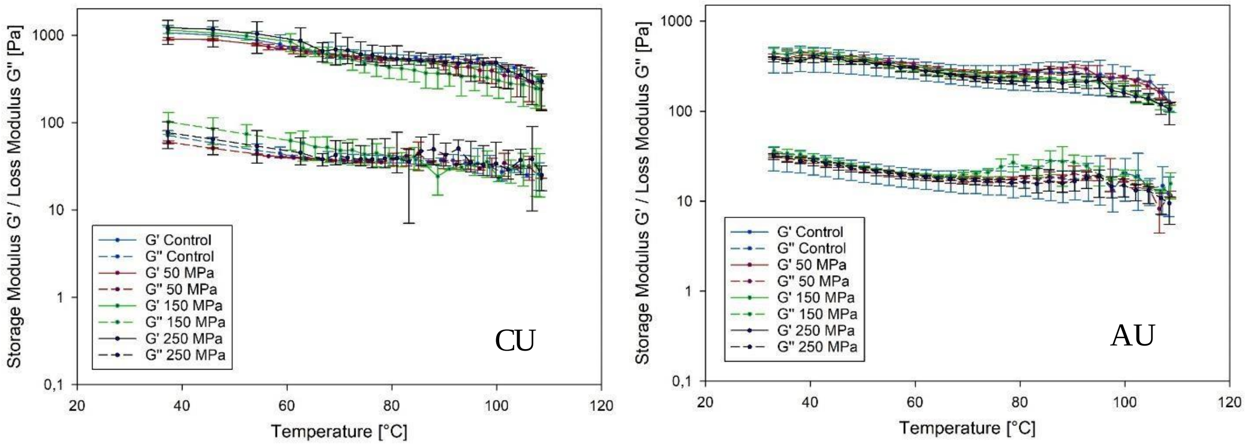
Figure A1. Apparent viscosity of untreated and UHPH treated (a) CU and (b) AU pectin solution.



(a) (b)

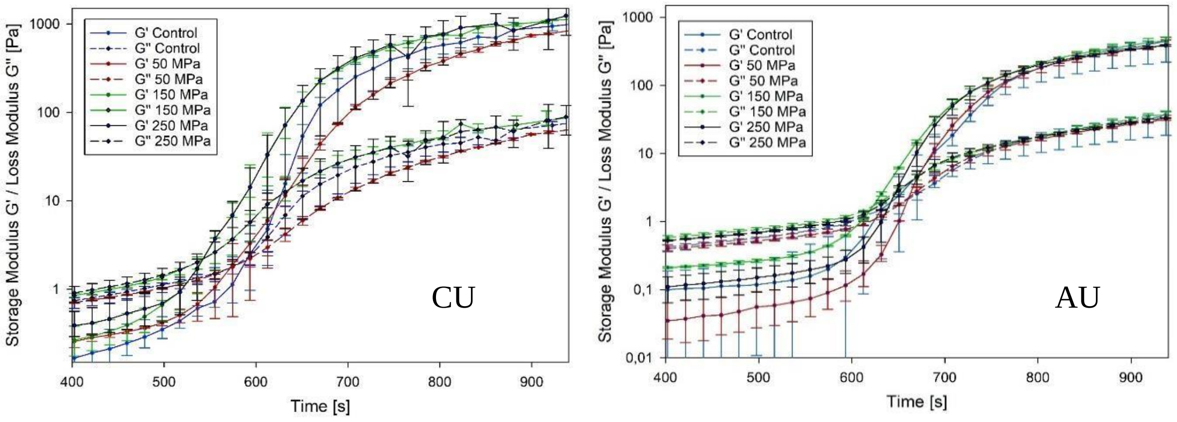
Figure A2. Flowing properties of untreated and UHPH treated (a) CU pectin and (b) AU pectin.

Appendix B



(a) (b)

Figure B1. Storage Modulus G' and Loss Modulus G'' of untreated and UHPH treated pectin (a) CU and (b) AU gels during heating step.



(a) (b)

Figure B2. Storage Modulus G' and Loss Modulus G'' of untreated and UHPH treated pectin (a) CU and (b) AU samples during cooling and gelation step.

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