Impact of high hydrostatic pressure on gel formation of low methoxylpectin

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Accepted 30 April, 2013

The effect of different treatment conditions (pressure, treatment time, and temperature), as well as composition of pectin solution (Pectin, Ca\textsuperscript{2+} ion and sugar concentration, pH) on gel formation of two types of low methoxylpectins were investigated. The results have shown that with increasing pressure, time or temperature during high hydrostatic pressure (HHP) treatment, the gel hardness increased continuously. Up to 2.5 times higher gel hardness could be achieved after HHP treatment (550 MPa, 35°C, 10 min, and 2% pectin) compared to untreated sample. Higher sugar concentration in pectin solution (about 30 to 60\%) resulted in drastically increasing the gel hardness for untreated as well as HHP treated samples but the overall gel hardness was, in the case of HHP treated samples, distinctly higher than the untreated. Similar results could be observed if the Ca\textsuperscript{2+} ion concentration increased up to 90 mg/g pectin. Increasing the pH of pectin solution from pH $= 3.4$ to 5.6 decreased the gel hardness in the case of untreated as well as HHP treated samples. The results of this study confirmed that it is possible to produce firm gel from low methoxylpectin at temperature of $\leq 35^\circ$C using high hydrostatic pressure technique.

Key words: Low methoxylpectin, gels, high hydrostatic pressure.

INTRODUCTION

Pectin is a naturally-sourced polysaccharide with wide range of application in food industries as thickener or gelling agent (Lootenes et al., 2003). Pectins are complex mixtures of polysaccharine with linearly connected $\alpha$ (1-4) D-galactronic acid residues interrupted occasionally by 1-2 linked rhamnose residue. Pectin is usually characterized by its degree of methylation (DM) of the carboxyl groups (Thakur et al., 1997). The degree of substitution of methyl esters determines the mechanism of formation of pectin gels and their conformational and rheological properties (Fishman et al., 1984). Pectin with a degree of methylation less than 50\%, so-called low-methoxylpectin, forms gel in the presence of Ca++ ions. The gel formation process of low methoxylpectin involves the simultaneous bounding of calcium ion to carbonyl groups of two adjacent pectin molecules and to two hydroxyl groups from one of the molecules (Smidsrod et al., 1972). Grant et al. (1973) suggested an egg-box like model to explain the structure of pectin molecules bound by the calcium ions.

Since the early 1980s, high hydrostatic pressure has been evaluated as an alternative to classical heat treatment food-processing technologies for energy-saving reason. One of the main focuses of high hydrostatic pressure applications has been food preservation at moderate temperatures (Dumoulin et al., 1988). Pressure affects molecules (for example, inactivation/activation of enzymes, protein denaturation), can change the state of molecular organization (for example, crystallization of fat, phase changes of phospholipids, denaturation-induced aggregation and gelation, gelatinization of crystalline starch, depolymerization) (Michel and Autio, 2005). The
The effect of pressure on hydrocolloids has been studied only to a limited extend. Yen and Lin (1998) reported that hydrocolloid solutions (colloids) are not affected by hydrostatic pressure. Pectin is affected as a mixture of heteropolysaccharides consisting predominantly of partially methoxylated galacturonic acid residue (Pilnik, 1990). Michel and Autio (2005) have reported that the degree of methyl esterification of the high methoxylpectin (HMP) (extracted from citrus fruits) did not change when the solutions were pressure treated at pH 5.0; when the pH was 7.0, high hydrostatic pressure treatment caused the degree of methyl esterification to fall from 65% to below 60%. Additionally, they found that the viscosity of a 1.5% HM-pectin solution could be increased up to 10 fold after pressure treatment at 400 MPa and 25°C.

An analysis of the elastic (\(G'\)) and viscous moduli (\(G''\)) showed that \(G'\) is much greater than \(G''\), indicating that the pectin solution shows a predominantly "solution-like" behavior at all pressure. Pressure increases both the elastic and the viscous moduli. Michel et al. (1998) observed a much more pronounced viscosity increase upon pressure treatment with 1.5% sugar-free high methoxyl apple pectin (degree of methyl esterification 70 to 75%). Those the pressure influenced might be dependent on the pectin source.

The aim of this study was to investigate the effect of different processing conditions (pressure level, treatment temperature and time) during high hydrostatic treatment as well as composition of pectin solutions (Ca\(^{2+}\), sugar, pectin concentration) on the firmness of two different low methoxylpectin gels.

**MATERIALS AND METHODS**

**Raw material**

Low methoxylpectin from company Kelco (Genu\(^{®}\) Pectin typ LM-102 AS, with degree of esterification of 35% and degree of amidation of 15%) and company Herbstreith and Fox (Pectin classic AB 902, with degree of esterification of 35 to 44% and without amidation) was used as raw material. We used the abbreviation "LM" for 15% amidated low methoxylpectin from company Kelco and "902" for low methoxylpectin without amidation for pectin from company Herbstreith and Fox, respectively.

**High-pressure treatments**

High hydrostatic pressure treatments were performed in a hydrostatic pressure unit (National forge, St. Niklass, Belgium) with an effective volume of 700 ml and a maximum working pressure of 600 MPa and a maximum working temperature of 90°C. Temperature within the pressure vessel was controlled by an external heat exchanger. The samples were sealed in polyethylene bags and placed in the high-pressure vessel. Pressure buildup was achieved within 2 min (600 MPa) and decompression time was about 10 s. For sample characterization, variation of treatment temperature (up to 65°C), time (0 to 20 min) and pressure (up to 550 MPa) was used.

**Texture analysis**

Texture (firmness of samples) was measured using a texture analyser (Model TA - XT2, Stable Micro System, Godalming, UK). The maximum deformation force required to compress the sample to a depth of 3 mm on a non-lubricated flat platform using a cylindrical probe (11.3 mm diameter) was recorded by the texture analyser and used as a measure of product firmness. The speed of compression during texture measurement was 1 mm/s. The firmness of gels was calculated as g/cm\(^2\).

**Sample preparation**

Pectin solution at different concentration of 0.5 to 2.0 was prepared using distilled water and adjusted to pH = 4.0. For investigation of the effect of sugar concentration on gel firmness, different concentration of calcium ion (0 to 90 mg calcium ion/g pectin) as calcium lactate was dissolved in pectin solution. To investigate the effect of sugar concentration on gel firmness, sucrose was mixed with pectin and dissolved in distilled water to achieve 0 to 60% sugar concentration in final pectin solution. For adjusting pH of pectin solution to pH = 3.4 to 5.6, diluted HCl or NaOH was used. The prepared pectin solutions were immediately packed in a polyethylene bag (without air inside the bag) and heat sealed.

**Statistics**

Statistical analyses were carried out using Plot-it 3.2. Experiments were run in quadruplicates or eight times. Average values were reported.

**RESULTS**

**High hydrostatic pressure (HHP) treatments**

*Effect of pressure level on firmness of pectin sample*

Increasing the pressure at constant treatment temperature (35°C) and treatment time of 10 min increased the firmness of pectin gel distinctly. Both of the investigated pectin solutions showed a linear increase on firmness whereas the “LM” pectin (15% amidated pectin) showed higher firmness value than pectin “902” (without amidation) (Figure 1). In general, the firmness of amidated pectin (pectin “LM”) was higher than the pectin without amidation (pectin “902”). It is not clear if the lower firmness of HHP treated “902” pectin compared to “LM” pectin is because of higher esterification degree or because of lack of amidation group in “902” pectin.

Dervisi et al. (2001) have investigated the effect of high hydrostatic pressure (up to 400 MPa) on gel formation of high methoxypectin. They found that in using high pressure, it is possible to produce jam at room temperature without heating. The best texture for jam was achieved when the pectin concentration was between 2.5 and 5% w/w. In our investigation, we have observed that the HHP treated pectin “LM” and “902” became solid after treatment at 400 MPa and 10 min at
35°C treatment temperature (Figure 2a to d). This indicates the advantage of HHP treatment for production of gels which is suitable for cutting in desired sizes.

Pressure could influence the apparent viscosity of high-methoxylpectin solution. After pressure treatment of 1.5% high-methoxylpectin solution (containing one-third sucrose) at 400 MPa and 25°C, an up to tenfold viscosity increase was observed. This is maybe because of changes in the interactions at the molecular level (Michel and Autio, 2005). Michel et al. (1998) observed a much more higher viscosity increase on 1.5% sugar free high methoxyl apple pectin (degree of methyl esterification 70 to 79%) after high pressure treatment. Thus, the pectin source (apple or citrus) could have effect on pressure induced viscosity increase. The mechanism of viscosity changes during pressure treatment is unknown. It may be because of intermolecular interaction of pectin during pressure treatment. Pressure might influence the hydrophobic interaction between the pectin molecules.

**Effect of treatment temperature during high hydrostatic pressure treatment on firmness of pectin sample**

In Figure 3 is the effect of different treatment temperatures during high hydrostatic pressure treatment at constant pressure of 550 MPa and 10 min on firmness of pectins demonstrated. A linear increase of gel firmness with increasing treatment temperature was observed. The “LM” pectin showed higher firmness at given temperature compared to “902” pectin. Increasing the treatment temperature from 35 to 65°C resulted in up to 2 times increase in the gel firmness. Eladouli (2001) and Bakki (2001) investigated the effect of high hydrostatic pressure on gel firmness and heat stability of gels from low methoxylpectin containing fruit puree. It could be stated that the high hydrostatic pressure treatment induced gel with distinct higher firmness compared to sample without high hydrostatic pressure treatment. Again, the firmness of HHP treated “LM” pectin was higher than “902” pectin for all investigated treatment time. Additionally, the high hydrostatic pressure treated gel provides higher heat resistant compared to untreated one. High hydrostatic pressure treated gel retained the shape after autoclave (120°C, 15 min), whereas the untreated gels loss the shape during heat treatment at 120°C (not published data).

**Effect of treatment time during high hydrostatic pressure treatment on firmness of pectin sample**

The treatment time during high hydrostatic pressure
Figure 2. (a) 2% “LM” pectin solution, adjust pH 4.0, Ca\(^{2+}\) concentration 60 mg/g pectin, without HHP. (b) 2% “LM” pectin solution, adjust pH 4.0, Ca\(^{2+}\) concentration 60 mg/g pectin, after HHP treatment at 400 MPa, 10 min at 35°C. (c) 2% “902” pectin solution, adjust pH 4.0, Ca\(^{2+}\) concentration 60 mg/g pectin, without HHP. (d) 2% “902” pectin solution, adjust pH 4.0, Ca\(^{2+}\) concentration 60 mg/g pectin, after HHP treatment at 400 MPa, 10 min at 35°C.

treatment (550 MPa, 35°C, 2% pectin solution and calcium concentration of 60 mg/g pectin) showed distinct effect on gel firmness of “LM” pectin as well as “902” pectin (Figure 4). With increasing treatment time, the firmness of high hydrostatic pressure treated pectin was increased. Similar to the effect of pressure and treatment time, the firmness of “LM” pectin was distinctly higher than the “902” pectin.

Effect of pH on firmness of high hydrostatic pressure treated sample

In addition to the effect of process parameters (pressure level, treatment temperature and time), the composition of pectin solution could affect the firmness of high hydrostatic pressure treated pectin solution. As demonstrated in Figure 5, the firmness of pectin gel was rapidly decreased with increased pH from 3.4 to 5.6. This was true for pectin “LM” and “902”. The overall firmness of high hydrostatic pressure treated pectin solution was at different pH and distinctly higher compared to untreated pectins. This indicates the positive effect of high hydrostatic pressure on gel formation of low methoxylpectin.

Effect of sugar concentration on firmness of high hydrostatic pressure treated sample

Grosso et al. (2000) reported the effect of sugar on the formation of low methoxylpectin gels. They suggested that pectin and sugar molecules could compete for cations. Depending on the sugar structure, a stable complex can be formed between the sugar and Ca\(^{2+}\). This interaction can be unfavorable to the formation of the gel, due to the decrease of Ca\(^{2+}\) available to associate with pectin molecules and therefore, decreasing the gel rigidity. In low methoxylpectin gels, the rigidity is essentially dependent on the capacity of the sugar to compete with the pectin for the calcium ions. The interaction between sugar and water has a secondary effect (Grosso et al., 2000). Similar to the effect of sugar concentration on gel firmness at ambient pressure, the gel firmness of the investigated pectin solution first increased slightly (up to 30% sugar concentration) and then very rapidly (at sugar concentration higher than 30%) with increasing sugar.
sugar concentration in pectin solution (Figure 6). The overall firmness of high hydrostatic pressure treated pectin at given treatment conditions (550 MPa, 35°C, 10 min) and composition of pectin solution (2% pectin concentration, 48 mg calcium ion/g pectin) was obviously higher than untreated samples.

**Effect of Ca**\(^{++}\) ion concentration on firmness of high hydrostatic pressure treated sample

Increasing the Ca\(^{++}\) ion concentration affected the firmness of high hydrostatic pressure treated pectins (Figure 7). Whereas, the firmness of untreated pectin
solution (2% pectin solution, pH = 4.0) linear with increasing of the calcium ion concentration up to 30 mg/g pectin increased; the high hydrostatic pressure treated pectin solution showed the linear firmness increasing up to calcium ions concentration of about 48 mg/g pectin. This showed that the high hydrostatic pressure treated pectin could bind higher amount of calcium ions compared to untreated pectin. In addition, the high hydrostatic pressure treated pectin showed distinct higher firmness at constant calcium ion concentration compared to untreated sample (Figure 7).

Lootens et al. (2003) investigated the effect of Ca$^{2+}$ concentration on gelation of low methoxylpectin. They found that at 20°C and pectin concentration of 10 g/l with increasing Ca$^{2+}$ concentration from 2 mM/g pectin to 7 mM/g pectin, the $G''$ storage shear modulus increased. The effects of pH, Ca$^{2+}$ concentration, temperature and amidation on the gelation of low methoxylpectin were investigated by Lootens et al. (2003). The shear modulus of pectin solution increased with added Ca$^{2+}$ ion. The shear modulus of partly amidated pectin gels was larger. In contrast, the pH does not strongly influence the shear modulus of pectin gels formed by Ca$^{2+}$ ion for pH > 3.5.

Capel et al. (2006) investigated the effect of calcium ion concentration on gelation of amidated methoxylpectin. Calcium concentration higher than 4 mM induced athermal
gelation of lomethoxylpectin. In our study, the concentration of Ca\(^{2+}\) (max. 90 mg/g pectin) was far less than the concentration for athermal gel formation.

**Effect of pectin concentration on firmness of high hydrostatic pressure treated sample**

With increasing pectin concentration (up to 1% pectin concentration) in pectin solution, the firmness of untreated pectin increased rapidly. Further increase of pectin concentration increased the firmness slightly. This was more obvious for "LM" pectin compared to "902" pectin (Figure 8). In contrast, the high hydrostatic pressure (550 MPa, 35°C, 10 min) treated "LM" pectin did not show any firmness increase at low pectin concentration (up to 1.5% pectin) with increased pectin concentration. Pectin concentration higher than 1.5% led to drastic increase of high hydrostatic pressure treated "LM" pectin. The high hydrostatic pressure (550 MPa, 35°C, 10 min) treated "902" pectin showed increasing firmness with increasing pectin concentration. In general, the high hydrostatic pressure treated pectin showed up to 2 times higher firmness compared to untreated pectin at a given composition of pectin solution. It is well known that pectin solution with viscosities higher than 10 mPas are above the so-called critical entanglement concentration. Pectin solutions with viscosities higher than 30 mPas, even at the highest shear rates, are in the concentration range where entanglements occur. It is assumed that pressure could have influence on the intermolecular entanglement, especially in the hairy regions. If pressure treatment increased the number of entanglements, the viscosity would consequently be higher (Michel and Autio, 2005).

**DISCUSSION**

There are only limited studies available about the effect of pressure on hydrocolloids. Yen and Lin (1998) reported that hydrocolloid solution could not be affected by hydrostatic pressure. Michel and Autio (2005) have studied the effect of pressure (up to 1000 MPa) on degree of methyl esterification on the high-methoxylpectin (extracted from citrus pectin). They have observed that the degree of methyl esterification did not change when the solution were pressurized at pH 5.0. In contrast, a slight decrease of (from 65% to below 60%) of degree of methyl esterification of the high methoxylpectin was observed at pH 7.0. They suggested that the observed reduction in degree of methyl esterification at pH 7.0 seems to be a purely pH driven demethylation. The demethylation by \(\beta\)-elimination could only occur at high pH and elevated temperature (Albersheim et al., 1960), so that pectin could not be affected chemically by pressure at pH 5.0.

Investigation of Naghshineh et al. (2013) led to the conclusion that high pressure treatment does not have any significant effect on molecular weight of pectin. Therefore,
high pressure treatment could not cause pectin polymer chain degradation. Gustin (1995) suggested that the carboxyl group in low methoxyl group of pectin will be dissociated under pressure. The dissociation of carboxyl group of low methoxylpectin is associated with negative activation volume and could be accelerated under pressure. During pressure release, the carboxyl group will be reorganized. This leads to inducing new connection between carboxyl group and calcium ions and increasing the firmness of low methoxylpectin gels. In addition, the dissociation of water molecules at high hydrostatic pressure decreased the pH in pectin solution.

Hinrichs (2000) have suggested that the salt and acids could be dissociated under high hydrostatic pressure. The induced charged ions are compact and have less volume. The ionization volume of water is about -22.2 ml/mol at 25°C (Kitamura and Itoh, 1987; Neuman et al., 1973) and could be promoted under pressure. This leads to a pH decrease of 0.7 units at 800 MPa (Heinz, 1997). The increasing firmness of low methoxylpectin investigated in this study could also be explained because of dissociation of carboxyl group during high hydrostatic pressure and rearrangement during pressure release as well as because of pH reduction during high hydrostatic pressure treatment.

**Conclusion**

High hydrostatic pressure treatment could affect the firmness of low methoxylpectin. Generally, high hydrostatic pressure treatment leads to increase in the firmness. The treatment conditions during high hydrostatic pressure treatment (pressure level, treatment time and temperature) can positively affect the firmness. In general, the firmness was of partially amidated pectin (Pectin “LM”) and distinctly higher after HHP treatment compared to pectin “902” at constant HHP processing conditions. Although, increasing of pH decreased the firmness of pectin solution drastically but the high hydrostatic pressure treated pectin solution showed overall higher firmness compared to untreated samples. The positive effect of sugar content (at higher concentration of 30%) in pectin solution was obvious for both untreated and high hydrostatic pressure treated samples. Both investigated low methoxyl “LM” and “902” showed higher Ca++ ion tolerance compared to untreated samples, and the firmness after high hydrostatic pressure treatment at given Ca++ concentration was up to two times higher for high hydrostatic pressure treated samples than untreated samples.

**ACKNOWLEDGEMENT**

The authors thanks Technical University of Berlin, Institute of food and Bioprocess Engineering, Germany for providing HHP and texture analyzer equipments.

**REFERENCES**

Technical University of Berlin, Germany.