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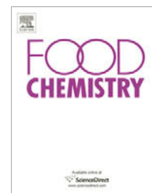

Effect of high-pressure/high-temperature processing on chemical pectin conversions in relation to fruit and vegetable texture

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ABSTRACT

Heat sterilization of plant derived food products entails considerable organoleptic and nutritional quality losses. For instance, texture loss of fruits and vegetables occurs, next to turgor pressure losses, mainly due to chemical changes in the cell-wall pectic polysaccharides. High-pressure sterilization, i.e. the combination of high temperature (⩾90 °C) with high pressure (⩾500 MPa), could present a positive alternative assuring safety while minimizing quality losses. In this study, the potential of high-pressure sterilization in preserving fruit and vegetable texture was evaluated by investigating the effect of combined high-pressure/high-temperature (HP/HT) treatments on two texture related chemical pectin conversions in model sytems. First, a protocol was developed to perform reproducible kinetic studies at HP/HT under constant processing conditions. Subsequently, apple pectin solutions at pH 6.5 were subjected to different HP/HT combinations (500, 600 and 700 MPa/90, 110 and 115 °C) and the extent of chemical demethoxylation and β-eliminative depolymerization was determined. At atmospheric pressure, both zero-order reaction rate constants increased with increasing temperature. At all temperatures, demethoxylation showed a higher rate constant than β-elimination. However, a temperature rise resulted in a stronger acceleration of β-elimination than of demethoxylation. When combining high temperature with high pressure, β-elimination was retarded or even stopped, whereas demethoxylation was stimulated. These results are very promising in the context of the texture preservation of high-pressure sterilized fruits and vegetables, as β-elimination is accepted to be one of the main causes of thermal softening and low methoxylated pectin can enhance tissue strength by forming cross-links with calcium ions present.

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1. Introduction

Consumer demand is increasing for high quality, fresh tasting foods free from additives, microbiologically safe and with an extended shelf life. The most commonly used food preservation method has been thermal processing, including pasteurization and sterilization. However, these processes adversely affect organoleptic, textural and nutritional qualities. Food scientists and the food industry are therefore continuously searching for novel, less degradative processing technologies (Mertens & Knorr, 1992). A technology that has shown such potential is high-pressure processing (Ludikhuyze, Van Loey, Indrawati, & Hendrickx, 2001). High-pressure processing applies pressures of 400–600 MPa at ambient temperature to inactivate enzymes and vegetative micro-organisms. At the same time, it offers an advantage in minimal deleterious effects on food quality attributes (e.g. colour, flavour, and nutritional value). Pressure is transmitted uniformly and instantaneously throughout the food, which results in a very homogeneous processing impact. Currently, high-pressure processing is successfully applied on a commercial scale for pasteurization of a whole range of food products, e.g. fruit juices, guacamole, oysters and ham. To achieve a complete inactivation of enzymes, vegetative micro-organisms, as well as spores, high pressure must be combined with a second inactivating factor. In high-pressure sterilization, this second factor is elevated temperature. In general, sterilization with high pressure is possible by starting high-pressure treatments at elevated temperatures, e.g. 60–90 °C, and using the compression heat for rapid and uniform heating to higher temperatures (de Heij et al., 2003). Depending on the nature of the product, the initial product temperature, and the applied pressure, the adiabatic temperature increase may vary from 3 to 9 °C/100 MPa. Various quality aspects of high-pressure sterilized food products are superior to conventionally heat sterilized products (Matser, Krebbers, van den Berg, & Bartels, 2004). However, data on the influence of combined high-pressure/high-temperature

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processing on the texture of fruits and vegetables are rather sparse.

Texture is probably one of the most important quality characteristics of edible fruits and vegetables. Texture is a characteristic at the plant organ level, depending on a structural hierarchy (Waldron, Parker, & Smith, 2003). The polysaccharides that make up the plant cell wall (cellulose, hemicellulose and pectin) form the basis of this hierarchy. Changes in texture during ripening, processing and storage are mainly related to (bio-)chemical conversions in pectin as pectin is principally abundant in the plant middle lamella, plays a crucial role there in cell–cell adhesion, and, moreover, is brought into solution more easily and is more chemically reactive than the other cell-wall polymers (Van Buren, 1979). One of the main structural components of pectin is homogalacturonan, a linear chain of α-(1,4)-linked galacturonic acid residues which can be methoxylated (Ridley, O'Neill, & Mohnen, 2001). Enzymes responsible for degradation of this pectin component include pectin methylesterases and polygalacturonases (Rexova-Benkova & Markovic, 1976). At elevated temperatures, high methoxylated pectin is prone to non-enzymatic conversions: depolymerization and demethoxylation. The β-eliminative depolymerization is mainly responsible for the extensive softening of low-acid fruits and vegetables during heat treatments (Sila, Smout, Elliot, Van Loey, & Hendrickx, 2006). This reaction proceeds on uronic acids, which possess a glycosidic linkage on C-4 in the β-position of the carboxyl group at C-5 (Kiss, 1974). A prerequisite is the presence of a methyl-ester group at C-6, rendering H-5 sufficiently acidic to be removed by an alkali. This results in the formation of unstable, intermediary anions that are stabilized by losing the C–O linkage in the β-position. Consequently, a double bond appears between C-4 and C-5 at the non-reducing end. This depolymerization leads to pectin solubilization and, consequently, to decreased cell–cell adhesion, resulting in tissue softening. The reaction rate is strongly dependent on the degree of methoxylation of pectin, pH and the presence of ions (Keijbets & Pilnik, 1974; Sajjaanantakul, Van Buren, & Downing, 1989; Sajjaanantakul, Van Buren, & Downing, 1993). Chemical demethoxylation takes place at the same time as it proceeds under the same temperature and pH conditions and influences the β-elimination (Kravtchenko, Arnould, Voragen, & Pilnik, 1992). In order to improve or preserve the texture of fruits and vegetables limited demethoxylation is desirable because demethoxylated pectin is less susceptible to β-elimination and can be ionically cross-linked by divalent cations such as calcium forming fortifying networks. The aim of this study was to investigate the effect of combined high-pressure/high-temperature treatments on the chemical demethoxylation and β-eliminative depolymerization of pectin to gain insight in the possible impact of HP/HT treatments on the texture of fruits and vegetables.

2. Materials and methods

2.1. Pectin

Apple pectin (degree of methoxylation 70–75%, Fluka, Switzerland) was used for all experiments.

2.2. Thermal treatments

A 0.3% (w/v) solution of apple pectin in 0.1 M Na-phosphate buffer pH 6.5, divided over screw-capped test tubes (2 ml per tube), was heated at 70, 80, 90, 100, 110 and 120 °C in a thermostated oil bath. After an equilibration period of 5 min, allowing the solution to reach the desired temperature, a first sample (treatment time 0 min) was withdrawn. The rest of the samples were removed after preset time intervals. The samples were immediately cooled in an ice water bath, and subsequently analyzed for unsaturated uronides and methanol content. Thermal treatments were carried out once.

2.3. High-pressure/high-temperature treatments

A 0.3% (w/v) pectin solution in 0.1 M MES–NaOH pH 6.5 (MES = 2-(N-morpholino)ethanesulfonic acid) was divided over flexible microtubes (500 μl), and subsequently treated at 90, 110 and 115 °C in combination with 500, 600 and 700 MPa for different time intervals. The HP/HT treatments were carried out in a custom-made laboratory scale high-pressure unit (Resato, The Netherlands), consisting of six individual vessels (6 × 43 mL, internal diameter = 20 mm), each surrounded by a heating coil connected to a thermostat. The pressure medium consisted of propylene glycol. This equipment allows computer-controlled pressure build-up, data logging of both pressure and temperature, and processing conditions up to 1000 MPa and 120 °C.

First, a protocol had to be developed to treat samples in a reproducible way at semi-constant HP/HT. As an example, in Fig. 1 the temperature and pressure history of a pectin sample during treatment at 110 °C and 600 MPa is shown (starting from pressure build-up). The samples were inserted in cylindrical, polyoxymethylene acetale (POM) sample holders (85 mm long, 12 mm internal diameter, and 3 mm thickness), filled up with water (excluding air), and closed with a movable stopper sealed with a ring. These sample holders were tailor-made to fill the vessels optimally, so the ratio of sample volume to pressure medium volume is as large as possible and constant. The sample holders (containing the samples) were cooled to 10 °C in a cryostat. Subsequently, the sample holders were transferred to the pressure vessels already equilibrated at the desired process temperature. The vessels were closed and the temperature in the sample holder was allowed to rise to an initial temperature (e.g. up to ~66 °C) which was dependent on the desired process temperature after pressure build-up. This preheating was the result of heat transfer from the pressure medium to the samples. This initial temperature was beforehand experimentally determined for each pressure/temperature combination under study. Sample holders with a 1.6 mm hole were used to allow temperature measurement inside the sample holder using a 36.8 mm type J thermocouple attached to the pressure vessel stopper. Subsequently, pressure was built up very fast; increasing in 5 s from 0.1 to 150 MPa and then from 150 MPa to the set pressure (e.g. 600 MPa) at a rate of 10 MPa/s. This was accompanied by a temperature rise (e.g. up to ~107 °C) due to compression heating. After attaining the desired pressure, the individual vessels were isolated.

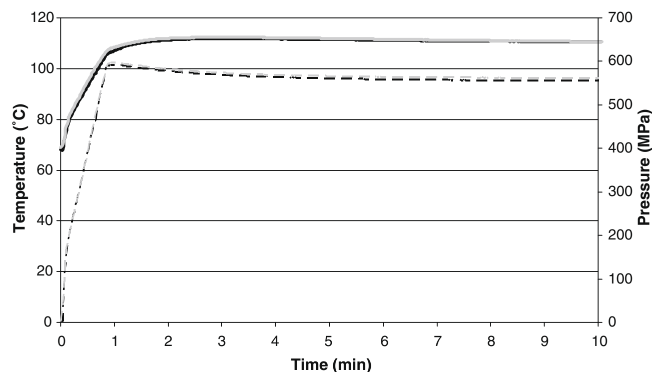

Fig. 1. Temperature and pressure history of a pectin sample during treatment at 110 °C and 600 MPa, starting from pressure build-up (—: temperature profile first run, - - -: pressure profile first run, —: temperature profile second run, - - -: pressure profile second run).

During pressure holding the temperature of the sample continued to increase up to 110 °C due to the higher temperature of the pressure medium. A constant temperature was reached within 1 min. It was preferred to attain a somewhat lower temperature than the process temperature at the end of pressure build-up, to avoid a temperature overshoot. During treatment the temperature remained constant, whereas there always was a small pressure loss. After preset time intervals the individual vessels were decompressed. The samples were immediately cooled in an ice water bath, and subsequently analyzed for unsaturated uronides and methanol content. In Fig. 1 the temperature and pressure evolution of a sample during two independent runs is represented, illustrating the reproducibility of the method. High-pressure/high-temperature treatments were carried out once.

2.4. Determination of unsaturated uronides

The extent of β-elimination was monitored by measuring the formation of unsaturated uronides during treatment, as indicated by absorbance changes at 235 nm. The concentration of unsaturated uronides was calculated using an average molar extinction coefficient of 5412 $M^{-1}\,cm^{-1}$ (Sajjaanantakul et al., 1989).

2.5. Determination of methanol

The amount of methanol released during treatment was determined using the colorimetric method of Klavons and Bennett (1986). Briefly, methanol is oxidized to formaldehyde by an alcohol oxidase and subsequently condensed with 2,4-pentanedione resulting in a coloured compound (3,5-diacetyl-1,4-dihydro-2,6-dimethylpyridine) of which the absorbance is measured at 412 nm.

2.6. Determination of degree of methoxylation of pectin (DM)

The DM of the apple pectin was determined by taking the ratio of moles of methoxyl groups to the moles of anhydrous galacturonic acid. To estimate the latter, pectin was hydrolyzed with concentrated sulfuric acid according to the method of Ahmed and Labavitch (1977). Subsequently, the galacturonic acid content was determined colorimetrically as described by Blumenkrantz and Asboe-Hansen (1973). The methoxyl content was determined by hydrolyzing the ester bonds of the pectin with NaOH (Ng & Waldron, 1997) and colorimetric quantification of the released methanol as described by Klavons and Bennett (1986).

2.7. Data analysis

Demethoxylation and β-elimination kinetic parameters were obtained using a two-step regression approach. First, the reaction rate constant k at a given temperature and pressure was determined by plotting the concentration of either methanol or unsaturated uronides as a function of time. Taking into account only the initial linear part of the curve, both reactions could be adequately modeled by pseudo zero-order reaction kinetics (Fraeye et al., 2007):

$$\frac{\partial C}{\partial t} = k$$

which can be integrated at isothermal or isothermal–isobaric conditions (k time independent) to $C_t = C_0 + k \cdot t$ with C_0 the concentration of methanol or unsaturated uronides at zero treatment time, C_t the concentration at treatment time t (min), and k the reaction rate constant (mM/min).

In the second step, the temperature and pressure dependence of the reaction rate constants was determined. The temperature dependence of the rate constant at constant pressure is expressed in terms of the activation energy E_a and estimated using the Arrhenius equation:

$$k_T = k_{ref} \cdot \exp\left[\frac{E_a}{R_T}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right],$$

where E_a is the activation energy (J/mol), k_T the reaction rate constant (mM/min) at temperature T (K), k_{ref} the reaction rate constant (mM/min) at reference temperature T_{ref} (K), and R_T the universal gas constant (8.314 J mol^{-1} K^{-1}). The E_a values were estimated by plotting the natural logarithm of the k value against the reciprocal of the respective absolute temperature. The pressure dependence of the rate constant at constant temperature is expressed in terms of the activation volume V_a and calculated using the Eyring concept:

$$k_P = k_{ref} \cdot \exp\left[\frac{V_a}{R_P T}(P_{ref} - P)\right]$$

where V_a is the activation volume (cm^3/mol), k_P the reaction rate constant (mM/min) at pressure P (MPa), k_{ref} the reaction rate constant (mM/min) at reference pressure P_{ref} (MPa), and R_P the universal gas constant (8.314 cm^3 MPa mol^{-1} K^{-1}). The V_a values were estimated by plotting the natural logarithm of the k value against pressure. A positive E_a or negative V_a value indicates that the reaction rate constant increases with respectively increasing temperature or pressure. A high absolute value of E_a or V_a signifies that the rate constant depends strongly on respectively temperature or pressure.

2.8. Calculation of pH value under high pressure and high temperature

Both the extreme pressures and temperatures involved in HP/HT treatments alter the pK_a of weak acids and bases within samples. This combined pressure and temperature effect can result in a reversible but significant shift in pH in samples undergoing HP/HT treatment. Therefore, to evaluate whether the observed phenomena were directly caused by temperature and pressure changes, or also by pH changes, the pH of MES at the different HP/HT conditions examined was calculated according to the method of Bruins, Matser, Janssen, and Boom (2007). For the calculation, the pH at atmospheric pressure, the reaction volume ΔV^0, the pK_a^0, and the ΔpK_a^0/°C of the buffer must be known. The superscript 0 denotes the value at atmospheric pressure. The pK_a^0 and ΔpK_a^0/°C can easily be found in text books (e.g. Dawson, Elliott, Elliott, & Jones, 1969). Kitamura and Itoh (1987) have measured reaction volumes for many protonic ionization reactions. First, the shift in pK_a due to the high temperature and high pressure has to be calculated. The pK_a at high temperature can be obtained using ΔpK_a^0/°C. The following relation described by El'yanov and Hamann (1975) was used to compute the pK_a at high pressure:

$$\ln\left(\frac{K_a}{K_a^0}\right) = -\frac{P\Delta V^0}{RT(1+bP)},$$

where P denotes pressure (MPa), R the universal gas constant (8.314 cm^3 MPa mol^{-1} K^{-1}), T the absolute temperature (K), and b a universal constant ($9.2 \cdot 10^{-4}$ MPa^{-1}). Irrespective of the parameter for which is corrected first, basic assumptions have to be made. In case the pK_a is corrected first for temperature, it is assumed that the ΔV^0 does not change with temperature. In the other case, it is believed that the ΔpK_a^0/°C does not change with pressure. The obtained K_a can then be used with the following equation:

$$K_a = \frac{[H^+][A^-]}{[HA]}.$$

As this equation has too many unknowns to be solved, the molarity of the buffer solution and a mass balance on the hydrogen atoms can be used as a boundary condition. The total amount of

hydrogen should be the same under high pressure and high temperature as under atmospheric pressure and room temperature. Finally, the $[H^+]$, and thus the pH can be calculated by iteration.

The calculations do not take into account the buffering capacity of the pectin itself, due to its many carboxyl groups. However, since the pK_a of galacturonic acid (the main building block of pectin) is 3.5, the buffering capacity of pectin at pH 6.5 will be rather limited. Nevertheless, it is not known in which way this pK_a is influenced by temperature and pressure.

3. Results and discussion

3.1. Influence of temperature on the rate of pectin degradation

A pectin solution in 0.1 M Na-phosphate buffer pH 6.5 was subjected to temperatures ranging from 70 to 120 °C at 0.1 MPa and the rate of pectin demethoxylation and β-elimination was monitored (Fig. 2). A pH of 6.5 was chosen as this is a pH value relevant for many vegetables, as well as a pH where demethoxylation and β-elimination occur at a significant rate. A phosphate buffer was selected because this buffer is, with a ΔpK_a^0/°C of −0.003, little temperature dependent. Table 1 shows for the different temperatures the theoretical pH of the solution and the reaction rate constants of the pectin conversions studied.

Rate constants of both reactions increased with increasing temperature. The temperature dependence of the reaction rate constants could be adequately modeled with the Arrhenius equation, resulting in activation energies of 96.6 and 70.8 kJ/mol, respectively for β-elimination and demethoxylation. The higher E_a value for β-elimination implies that any temperature rise results in a stronger acceleration of β-elimination than of demethoxylation. Fraeye et al. (2007) estimated similar activation energies, ranging from 80.2 to 123.9 kJ/mol for β-elimination and from 44.4 to 96.9 kJ/mol for demethoxylation, for apple pectin with different degrees of methoxylation at various pH values. The ratio of the rate constant for demethoxylation (k_d) to the rate constant for β-elimination (k_β) was calculated for the different temperatures. A ratio k_d/k_β from 2 to 9 was observed, indicating that at all temperatures demethoxylation was faster than β-elimination. The ratio decreased with increasing temperature, corresponding with the observation that the activation energy of β-elimination is higher than that of demethoxylation.

Table 1
Theoretical pH values, and experimentally determined reaction rate constants and activation energies (± standard error of regression) for β-elimination and demethoxylation of pectin treated in phosphate buffer at different temperatures and 0.1 MPa.

T (°C)	pH	β-elimination k_β (10^{-6} M/min)	Demethoxylation k_d (10^{-6} M/min)
70	6.37	0.69 ± 0.071	5.63 ± 0.268
80	6.34	1.81 ± 0.066	12.37 ± 0.306
90	6.31	7.83 ± 0.488	30.30 ± 1.570
100	6.28	14.70 ± 0.376	51.71 ± 3.449
110	6.25	29.95 ± 2.652	80.47 ± 6.739
120	6.22	45.03 ± 4.728	133.84 ± 9.642
	E_a (kJ/mol)	96.6 ± 7.8	70.8 ± 3.8

3.2. Influence of high temperature in combination with high pressure on the rate of pectin degradation

A MES buffer was chosen for combined HP/HT experiments. Amine buffers such as MES and TRIS (2-amino-2-hydroxymethyl-1,3-propanediol) are less sensitive to pressure ($\Delta V^0 \approx 4$ cm³/mol) than anionic buffers such as phosphate and citrate ($\Delta V^0 \approx -10$ to -20 cm³/mol). Within the pressure tolerant buffers is MES, with a ΔpK_a^0/°C of −0.011, also least sensitive to temperature. In comparison, the ΔpK_a^0/°C of TRIS is −0.028. In Fig. 3 the calculated shift in pH of MES within the P-T window examined is illustrated. As the pK_a is first corrected for temperature and then for pressure, it is assumed that ΔV^0 does not change with temperature. For the different HP/HT combinations, the pH values are ranging from 5.69 to 6.02. If the pK_a is first corrected for pressure and afterwards for temperature (assuming ΔpK_a^0/°C does not change with pressure) the pH estimations differ slightly, ranging from 5.74 to 6.08 (results not shown). The higher the temperature is, the lower the pH value. Conversely, applying high pressure increases the pH.

The pectin solution was treated in a P-T window of 500–700 MPa and 90–115 °C. The formation of unsaturated uronides and methanol at 110 °C is shown in Fig. 4. Similar trends were observed at 90 and 115 °C. The treatment time for the HP/HT treated samples (time 0 min) was started directly after pressure build-up. At 0.1 MPa, both β-elimination and demethoxylation occurred at a significant rate. However, the rate constants, $14.62 \cdot 10^{-6}$ M/min for β-elimination and $39.64 \cdot 10^{-6}$ M/min for demethoxylation, were remarkably lower as compared with treatment in phosphate buffer

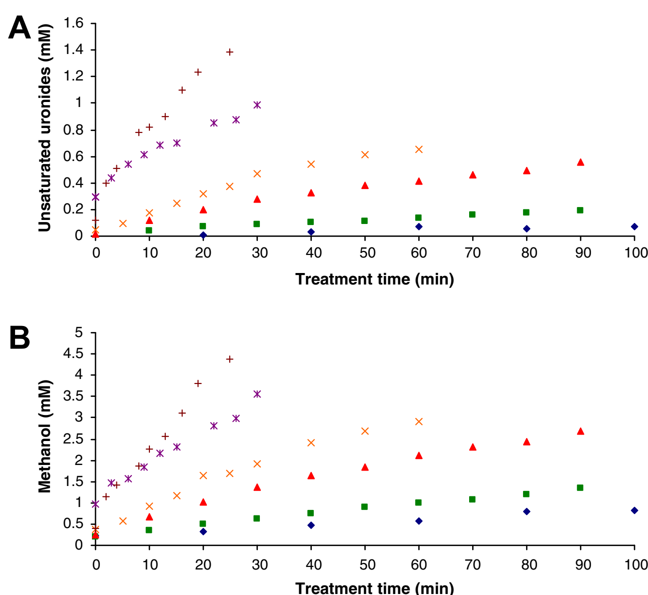

Fig. 2. Formation of unsaturated uronides (A) and methanol (B) as a function of treatment time when incubating pectin dissolved in 0.1 M phosphate buffer pH 6.5 at 0.1 MPa: (◆) 70 °C, (■) 80 °C, (▲) 90 °C, (×) 100 °C, (✳) 110 °C, and (+) 120 °C.

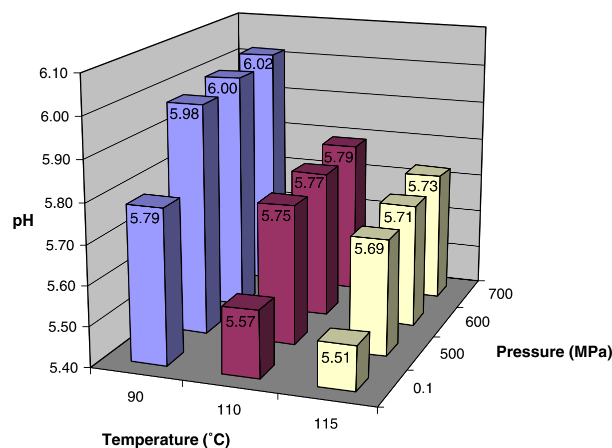

Fig. 3. Calculated pH of 0.1 M MES buffer pH 6.5 (at 0.1 MPa and 25 °C) under the assumption that ΔV^0 does not change with temperature.

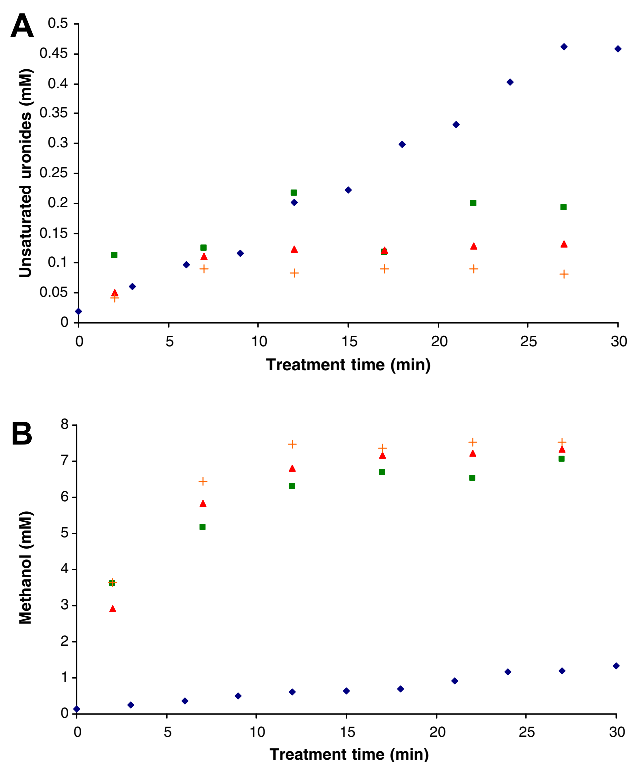

Fig. 4. Formation of unsaturated uronides (A) and methanol (B) as a function of treatment time when incubating pectin dissolved in 0.1 M MES pH 6.5 at 110 °C: (◆) 0.1 MPa, (■) 500 MPa, (▲) 600 MPa, and (+) 700 MPa.

(Table 1). A possible explanation could be the more pronounced temperature dependence of MES resulting in a pH of 5.57 at 110 °C (Fig. 3). However, differences in ion concentrations could also play a role as it has already been described that the β-elimination rate is affected by ions (Sajjaanantakul et al., 1993). High pressure slowed down or even inhibited the β-elimination reaction, whereas it had a pronounced stimulating effect on the demethoxylation. This stimulating effect has already been observed by Verlent et al. (2004) at pH 8.0 for pressures and temperatures up till 500 MPa and 65 °C. The enhancing effect of pressure on the demethoxylation can be explained by the principle of Le Chatelier. This principle states that any phenomenon accompanied by a decrease in reaction volume is enhanced by an increase in pressure, and vice versa (Cheftel, 1995). Solvation of the charged groups created by the pectin demethoxylation, is accompanied by a reduction in reaction volume resulting from electrostriction (i.e. the compact alignment of water dipoles owing to the coulombic field of the charged groups). As the curves were horizontal or leveled off quite rapidly, kinetic parameters (k, V_a) were difficult to estimate.

It is difficult to judge whether the inhibition of the β-elimination is due to a direct effect of pressure, or if other, indirect effects of the HP/HT treatment play a role. If the β-elimination is accompanied by an increase in reaction volume, direct inhibition of β-elimination by high pressure could be explained by the principle of Le Chatelier. However, other or additional mechanisms (i.e. changes in pH, degree of methoxylation during treatment) could be responsible for inhibition, which are discussed in the following two paragraphs.

3.3. Influence of pH on the rate of pectin degradation

As β-elimination is strongly dependent on pH, it is possible that β-elimination did not occur due to a shift in pH of the sample during treatment. Therefore, the effect of pH on the β-elimination and demethoxylation was investigated at atmospheric pressure. Pectin solutions in 0.1 M citrate buffer at pH values from 6.2 to 4.5 were treated at 100 °C and 0.1 MPa (Fig. 5). Decreasing the pH decelerates both β-elimination and demethoxylation, as also observed by Fraeye et al. (2007). At pH 4.5, both reactions hardly occurred. However, given the low extent of the pH drop during pressure treatment of MES buffer (Fig. 3), it is unlikely that the decrease in pH would be accountable for the inhibition.

3.4. Influence of degree of methoxylation on the rate of pectin degradation

Another possible cause for inhibition could be the concomitant and extensive pectin demethoxylation reducing the degree of methoxylation below a level at which β-elimination does no longer occur. Demethoxylation and β-elimination are two competitive reactions. With increasing treatment time, the rate of β-elimination decelerates as a result of the decreasing DM. To verify below which DM β-elimination is significantly retarded, pectin dissolved in 0.1 M MES pH 6.5 or in 0.1 M citrate buffer pH 6.2 was treated at 100 °C and 0.1 MPa for long times. Fig. 6 shows the formation of unsaturated uronides and changes in DM of pectin in citrate buffer. From 80 min on, the β-elimination slowed down remarkably. This corresponded to a DM of ~32%. In MES buffer, very long treatment times were required to obtain a change in β-elimination rate which was in addition less pronounced. This change was observed at a DM of ~40% (data not shown). One can imagine that, with further decrease of DM, the β-elimination rate approaches zero. The samples treated for 2 min at HP/HT (Fig. 4) had a DM of about 40%, probably not low enough to inhibit the β-elimination completely. However, the samples treated for longer times had DM values ranging from 26 down to 5% depending on the pressure level and treatment time. Consequently, it is plausible that the low DM

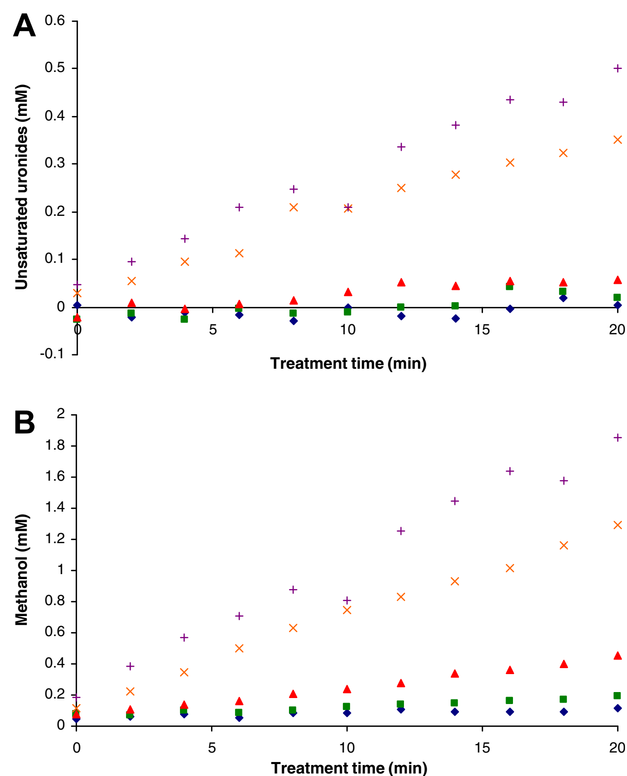

Fig. 5. Formation of unsaturated uronides (A) and methanol (B) during treatment of pectin dissolved in 0.1 M citrate buffer at 100 °C and 0.1 MPa: (+) pH 6.2, (×) pH 6.0, (▲) pH 5.5, (■) pH 5.0, and (◆) pH 4.5.

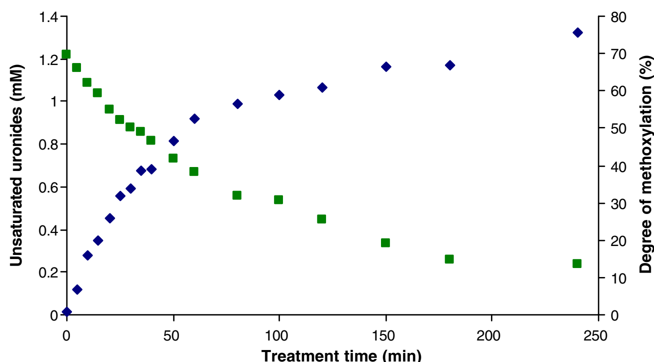

Fig. 6. Formation of unsaturated uronides (◆) and changes in degree of methoxylation (■) during treatment of pectin dissolved in 0.1 M citrate buffer pH 6.2 at 100 °C and 0.1 MPa.

caused inhibition of the β-elimination reaction during HP/HT treatments.

3.5. Importance of buffer choice: Comparison of Na-phosphate and MES buffer

To verify whether buffer type and its pH dependence on temperature and pressure can have an important influence on the results, a pectin solution in 0.1 M phosphate buffer pH 6.5 was treated at 90 °C and 500, 600 and 700 MPa. The β-elimination and demethoxylation were assayed (Fig. 7) and compared with the results obtained at 90 °C with MES buffer pH 6.5 (data not shown). The same observations could be made: high pressure inhibited the β-elimination reaction, whereas it stimulated the demethoxylation, although the latter was less pronounced. Neuman, Kauzmann, and Zipp (1973) established that the pH of a phosphate buffer shifts about −0.3 °C/100 MPa when applying high pressures. Under the assumption that the pH of the samples in phosphate buffer was around 4.5 to 5 during HP/HT treatment, this low pH could account for the retarded β-elimination (Fig. 5). However, at this low pH a deceleration of the demethoxylation would be expected too, contrary to what was observed. Possibly, demethoxylation is stimulated by pressure to that extent that it compensates for the inhibiting effect of the low pH.

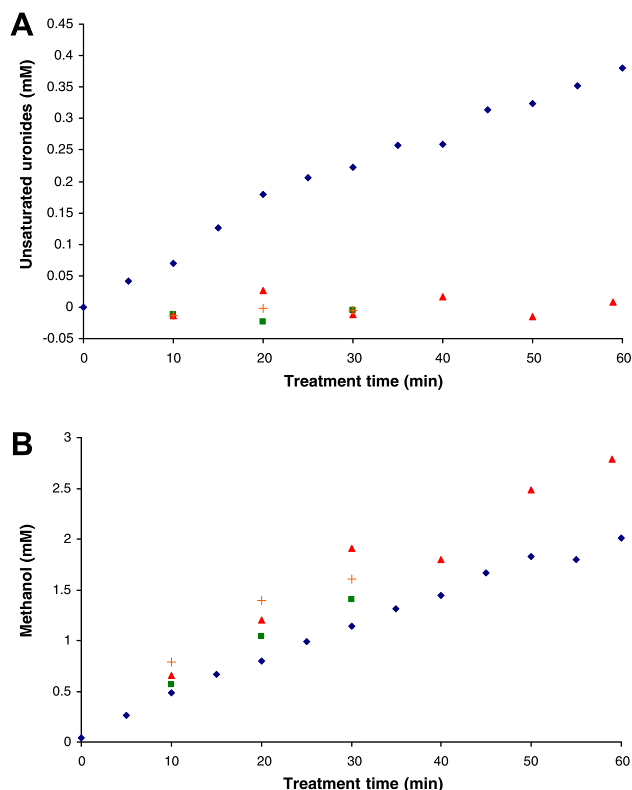

Fig. 7. Formation of unsaturated uronides (A) and methanol (B) during treatment of pectin dissolved in 0.1 M phosphate buffer pH 6.5 at 90 °C: (◆) 0.1 MPa, (■) 500 MPa, (▲) 600 MPa, and (+) 700 MPa.

Although both buffers yielded the same results, different mechanisms were probably responsible for inhibition. Phosphate buffers are considered temperature stable but pressure labile. Therefore, the observations made could be due to a combination of a direct pressure effect and a pH shift due to the pressure. In contrast, MES buffers are considered pressure stable and show less dependence on temperature than other pressure stable buffers. In this buffer, large changes in pH are less likely to occur. Here, it is probably the extensive demethoxylation, resulting in pectin with a very low degree of methoxylation that inhibited β-elimination, possibly in combination with a direct inhibiting pressure effect.

4. Conclusion

The effect of combined high-pressure/high-temperature treatments on two chemical conversions of pectin, namely β-eliminative depolymerization and demethoxylation, was investigated to evaluate the possible impact of HP/HT treatments on the texture of fruits and vegetables. In addition, attention was drawn to the importance of an intelligent buffer choice when investigating (bio-)chemical systems at high temperature and/or high pressure.

At 0.1 MPa and elevated temperature, β-elimination and demethoxylation occurred at a significant rate. Irrespective of the buffer type, the rate of β-elimination decreased when combining high temperatures with elevated pressures, whereas the rate of demethoxylation increased. These observations are very promising for the texture of high-pressure sterilized food products, since the β-eliminative depolymerization of pectin is accepted to be one of the main causes of softening of heat processed fruits and vegetables (Sila et al., 2006). Moreover, low methoxylated pectin was proven to enhance tissue strength by forming fortifying networks with divalent ions (e.g. calcium) present (Sila, Smout, Vu, & Hendrickx, 2004). The exact cause of inhibition of β-elimination (i.e. a direct pressure effect, an indirect effect of the pressure treatment (e.g. shift in pH or degree of methoxylation), or a combination of both) is difficult to establish as many parameters influence the reaction rate. Concerning buffer solutions, it has to be taken into account that the activity of hydrogen ions varies with pressure and temperature. As a consequence, the buffer pH can increase or decrease depending on the temperature, pressure, and buffer type. Generally, phosphate buffers are considered temperature stable but pressure labile. Hence, it could be that the observations made in phosphate buffer during pressure treatment were not (only) due to direct pressure effects but were due to a pH shift or to a combination of both. MES buffers are considered pressure stable and show less dependence on temperature than other pressure stable buffers. Here, it is probably the extensive demethoxylation, resulting in pectin with a very low degree of methoxylation that inhibited β-elimination. At present, buffers that are both pressure and temperature stable unfortunately do not exist.

As it is not unambiguous what causes inhibition of β-elimination in model systems and consequently, if the same inhibition occurs in real systems, it would be very interesting to investigate real systems and relate the observations made in the model systems to the real systems. This will be done in the future by subjecting fruits and vegetables to HP/HT treatments.

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