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Influence of pectin properties and processing conditions on thermal pectin degradation

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Abstract

Texture degradation of fruits and vegetables during thermal processing is partly due to pectin depolymerization. In this contribution we investigate the influence of pectin properties (degree and pattern of methoxylation) and processing conditions (pH 2.0, 3.0, 5.0 and 7.0; temperatures of 80 up to 110 °C) on pectin degradation rate constants. Pectins with different degrees and patterns of methoxylation were prepared and heated, the extent of acid hydrolysis, β-elimination and demethoxylation was assayed as a function of treatment time. As the degree of methoxylation and pH decreased, β-elimination rate constants decreased while acid hydrolysis rate constants increased. Demethoxylation rate constants were minimal at pH 3.0 and increased both at lower and higher pH. Reaction rate constants were not considerably influenced by the pattern of methoxylation. All reaction rate constants increased with increasing temperature. As β-elimination rates are reduced by competing demethoxylation, both rate constants were compared. The ratio of these reaction rate constants showed no trend with pH. However, an increase in degree of methoxylation or temperature resulted in a more pronounced increase in β-elimination rate constants than in demethoxylation rate constants.

Keywords: Pectin degradation; Depolymerization; β-Elimination; Acid hydrolysis; Demethoxylation; Kinetics

1. Introduction

Texture of fruit and vegetable based products is an important quality aspect. Firmness of plant tissues is partly determined by three types of polysaccharides present in the plant cell walls: cellulose, hemicellulose and pectin. From these polysaccharides, pectin is most water soluble and most subject to enzymatic and non-enzymatic changes during ripening, storage and processing (Van Buren, 1979). Pectin is a complex polysaccharide, the exact architecture is still under discussion (Vincken et al., 2003). One of the main structural components is homogalacturonan, a linear chain of α-(1,4)-linked galacturonic acid residues, part of which are methyl esterified (Ridley, O'Neill, & Mohnen, 2001). During thermal processing of plant tissues, pectin is subject to depolymerization reactions, which result in texture deterioration (Sila, Smout, Elliot, Van Loey, & Hendrickx, 2006; Van Buren, 1979). Depending on pH and degree of methoxylation (DM), β-elimination or acid hydrolysis may occur.

β-Elimination in uronic acid polymers such as pectin is known to proceed via an E1cB mechanism (BeMiller & Kumari, 1972). A prerequisite is the presence of a methyl ester group at C-6 (Keijbets & Pilnik, 1974). Hence, pectin with a high DM is more subject to β-elimination than pectin with a low DM (Krall & Mcfeeters, 1998; Sajjaanantakul, Van Buren, & Downing, 1989; Sajjaanantakul, Van Buren, & Downing, 1993). β-elimination has been measured at pH-values as low as 3.8 (Krall & Mcfeeters, 1998), reaction rates increase with increasing pH (Krall &

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Mcfeeters, 1998; Kravtchenko, Arnould, Voragen, & Pilnik, 1992; Sila et al., 2006). However, at neutral to alkaline pH, chemical demethoxylation can occur, generating pectin with a lower DM (Renard & Thibault, 1996). Hence, during thermal treatment at these pH values, DM of the pectin decreases, causing a decrease in β-elimination rates (Kravtchenko et al., 1992; Sajjaanantakul et al., 1989).

Pectin with a low DM is subject to acid hydrolysis during thermal treatment. The lower DM, the faster pectin is hydrolyzed (Krall & Mcfeeters, 1998). Slow acid hydrolysis of pectin has been measured at pH 6.0, reaction rates increase with decreasing pH (Krall & Mcfeeters, 1998; Smidsrod, Haug, & Larsen, 1966). During thermal processing in acidic conditions, some chemical demethoxylation can occur (Van Buren, 1979), increasing the sensitivity of pectin to acid hydrolysis.

Pectin depolymerization is one of the main causes of texture deterioration of fruits and vegetables during thermal processing (Sila et al., 2006). In case of porous plant materials, texture deterioration can be reduced by infusion of calcium ions and pectinmethylesterase (PME, EC 3.1.1.11) (Degraeve, Saurel, & Coutel, 2003; Duvetter et al., 2005a; Javeri, Toledo, & Wicker, 1991). The latter enzyme demethoxylates pectin, giving rise to negatively charged groups which crosslink with Ca^{2+}. This interaction induces texture firming. On the other hand, demethoxylation may also influence texture by reducing the sensitivity of pectin to β-elimination while increasing the sensitivity to acid hydrolysis, in particular at elevated temperature. The influence of calcium ions on β-elimination rates have been investigated by Keijbets and Pilnik (1974), Sajjaanantakul et al. (1993) and Sila et al. (2006), while Krall and Mcfeeters (1998) investigated the influence of Ca^{2+} on acid hydrolysis rates.

Different types of PME exist, each of which demethoxylate pectin according to a specific pattern. Most PMEs from plant origin demethoxylate pectin according to a blockwise pattern (Cameron, Luzio, Kauffman, & Grohmann, 2004; Catoire, Pierron, Morvan, du Penhoat, & Goldberg, 1998; Daas, Meyer-Hansen, Schols, De Ruiter, & Voragen, 1999; Duvetter et al., 2006; Grasdalen, Andersen, & Larsen, 1996; Kim, Teng, & Wicker, 2005). During the initial phase of demethoxylation, part of the pectin chains are strongly demethoxylated, while other chains remain intact (Denes, Baron, Renard, Pean, & Drilleau, 2000; Duvetter et al., 2006). On the other hand, most PMEs from microbial origin demethoxylate pectin in a more random way. These PMEs demethoxylate a limited number of subsequent galacturonic acid residues on a chain and subsequently attack another chain (Denes et al., 2000; Duvetter et al., 2006; Limberg et al., 2000). The resulting methoxylation pattern of pectin (blockwise or more random) may influence the texture of processed fruits or vegetables to a large extent, not only by modulating calcium binding properties of pectin (Ralet, Dronnet, Buchholt, & Thibault, 2001), but possibly also by influencing depolymerization rates of pectin. The distribution pattern of unesterified galacturonic acid residues, hence free carboxylic acid groups, along the pectic chains may influence the ability of H^+ and OH^--ions to approach the chains in order to catalyze acid hydrolysis or β-elimination reactions.

Up to present, quantitative information on the kinetics of depolymerization of pectin is limited. Moreover, to our knowledge the influence of methoxylation pattern on depolymerization rates has not yet been investigated. Therefore, the objective of this research was to quantify the influence of temperature, pH, degree and pattern of methoxylation on depolymerization rates (β-elimination and acid hydrolysis) of pectin. Since simultaneous chemical demethoxylation can occur and can influence the depolymerization rates, this reaction was assayed as well.

2. Materials and methods

2.1. Materials

Apple pectin was purchased from Fluka (Buchs, Switzerland). Neutral sugar composition, galacturonic acid content, degree of methoxylation and an indication of the molar mass distribution of this pectin were discussed in Fraeye et al. (2007). Starting from this parent pectin, several modified pectins with varying patterns and degrees of methoxylation (PM and DM) were produced by action of tomato PME or *Aspergillus aculeatus* PME.

To obtain tomato PME, fresh tomatoes (*Lycopersicon esculentum*) were cut into pieces, frozen using liquid nitrogen and stored at −40 °C until use. PME was extracted from these tomatoes and purified using affinity chromatography as described by Fachin et al. (2002).

Recombinant *A. aculeatus* PME was purified from a commercial liquid preparation (Novoshape, Novo Nordisk, Bagsvaerd, Denmark) by gel filtration chromatography as described by Duvetter et al. (2005b).

All chemicals were of analytical grade.

2.2. Pectinmethylesterase activity assay

Activity of the purified *A. aculeatus* and tomato PME solutions was determined by following the release of acid as a function of time (during 10 min) using an automatic pH stat titrator (Metrohm, Herisan, Switzerland) at constant temperature (22 °C) and pH (8.0 for tomato PME, 4.5 for *A. aculeatus* PME). The reaction mixture consisted of 30 ml of a 3.5 g l^{-1} apple pectin solution containing 0.117 mol l^{-1} NaCl to which 250 μl of PME-solution was added. A correction for incomplete dissociation of demethoxylated carboxyl groups was made by multiplying the activity with a factor $[1 + 10^{(pKa-pH)}]$, p*K*a being estimated as 3.5, the p*K*a of galacturonic acid (Christgau et al., 1996). One unit (U) of PME activity is defined as the amount of enzyme capable of catalyzing the release of 1 μmol methyl-ester bonds per minute under above mentioned standard assay conditions.

2.3. Pectin preparation

Pectins with different PM and DM were prepared by enzymatic demethoxylation of the parent apple pectin (P-pectin). Pectin was treated with tomato PME generating pectins with a blockwise PM (T-pectins) or with *A. aculeatus* PME generating pectins with a more random PM (A-pectins).

Apple pectin was dissolved (0.8% w/w) in Na-phosphate buffer (0.1 mol l^{-1}; pH 7.0) for preparation of T-pectins or Na-acetate buffer (0.1 mol l^{-1}; pH 4.5) for preparation of A-pectins. Large pyrex tubes with caps were filled with 25 ml of the pectin solution. After addition of 15 U of PME, the tubes were mixed and incubated in a thermostated waterbath at 30 °C. After a specific incubation time, tubes were withdrawn and were put in a waterbath at 85 °C during 4 min. This heat shock proved to be sufficient to inactivate the enzyme. Tubes were cooled in ice water. The demethoxylated pectin samples were dialyzed against demineralized water during 48 h with 10 changes of water, lyophilized and stored in a vacuum desiccator over phosphorous pentoxide until use.

2.4. Determination of degree of methoxylation of pectin

DM of all pectins were calculated as the ratio of the molar amount of methanol esters to the molar amount of galacturonic acid residues. To estimate the amount of methoxyl esters, a pectin solution was saponified to pectate and methanol according to the procedure by Ng and Waldron (1997). The amount of methanol released was measured using the spectrophotometric method of Klavons and Bennett (1986). To determine the galacturonic acid content, pectin was hydrolysed using sulfuric acid as described by Ahmed and Labavitch (1977). Next, the concentration of galacturonic acid was quantified using the spectrophotometric method described by Blumenkrantz and Asboe-Hansen (1973).

2.5. Thermal treatment

P-, A- and T-pectin was dissolved (3 g l^{-1}) in Na-phosphate buffer (0.1 mol l^{-1}; pH 2.0 or 7.0) or Na-citrate buffer (0.1 mol l^{-1}; pH 3.0 or 5.0). This solution was divided over several tubes with screw cap (2 ml per tube). The samples were incubated in a thermostated oil bath at 80, 90, 100 or 110 °C. A temperature equilibration period of 3 min was taken into account. From this moment the experiment was considered to be isothermal. At preset time intervals, the samples were withdrawn one by one and placed in an ice-water bath in order to stop all reactions.

2.6. Analytical methods

In order to determine the extent of chemical, non-enzymatic pectin demethoxylation during the thermal treatments, the amount of methanol formed in each sample was determined using the colorimetric method of Klavons and Bennett (1986).

To quantify the extent of β-elimination, the absorbance of diluted samples at 235 nm was measured. The concentration of 4,5-unsaturated uronides was calculated using an extinction coefficient of 5412 l mol^{-1} cm^{-1} (Krall & Mcfeeters, 1998; Sajjaanantakul et al., 1989; Sajjaanantakul et al., 1993).

The concentration of reducing end groups formed in each sample was quantified as a measure for depolymerization, both by β-elimination and acid hydrolysis. This assay was performed by heating the solutions (10 min, 100 °C) in presence of cyanoacetamide at pH 9.0, and measuring the absorbance at 276 nm as described by Gross (1982). The latter heating step at pH 9.0 caused additional β-elimination. The extent of this reaction depended strongly on DM of the sample, since β-elimination only occurs next to an esterified galacturonic acid residue (Keijbets & Pilnik, 1974). A correction for this additional β-elimination had to be made. Therefore, concentrations of reducing ends and unsaturated bonds measured in all samples that had been thermally treated at pH 7.0 were compared. As at this pH-value no acid hydrolysis occurs, the formation of reducing end groups during thermal treatment is uniquely due to β-elimination (Sajjaanantakul et al., 1989). As expected, in each sample the measured concentration of reducing ends was higher than the concentration of unsaturated bonds, due to the additional β-elimination during the heating step at pH 9.0 of the reducing ends assay. For each sample, this difference in concentration was plotted against DM of the sample. A strong correlation was observed. A straight line was fitted using linear regression analysis. The equation of this line was used to correct the measured concentration of reducing end groups in the samples which were thermally treated at pH 2, 3 and 5.

2.7. Data analysis

2.7.1. Acid hydrolysis

The concentration of reducing ends in each sample is a measure for the extent of depolymerization, both by acid hydrolysis and β-elimination. From this concentration, the amount of 4,5-unsaturated bonds (caused by β-elimination) in each sample was subtracted, in order to calculate the concentration of reducing ends formed by acid hydrolysis. This way, the extent of acid hydrolysis could be monitored as a function of heating time. The concentration of reducing ends increased linearly with time, indicating zero order reaction kinetics:

$$\frac{dR}{dt} = k_{AH} \quad (1)$$

$$R_t = R_0 + k_{AH}t \quad (2)$$

where R_0 = initial concentration of reducing ends (mmol l^{-1}), R_t = concentration of reducing ends formed by acid hydrolysis at time t (mmol l^{-1}), k_{AH} = zero order reaction rate constant of acid hydrolysis (mmol l^{-1} min^{-1}).

Hence, the reaction rate constant k_{AH} at a given temperature was estimated from the slope of the curve of reducing ends concentration vs. treatment time, which was determined by linear regression. As at conditions where acid hydrolysis of pectin was observed (pH 2 and 3) chemical demethoxylation of pectin was limited, this reaction did not influence hydrolysis kinetics to a great extent.

2.7.2. β-elimination

When the increase in 4,5-unsaturated bonds was followed as a function of treatment time, a non-linear curve was obtained at some conditions, particularly at pH 7.0 (an example is given in Fig. 1). It can be observed that the β-elimination rate decreased during thermal treatment. This can be explained by a strong decrease in DM due to chemical demethoxylation, for increasing amounts of methanol were measured during the thermal treatment. This non-enzymatic demethoxylation occurs according to a completely random pattern. Since the objective of this research is to investigate the influence of PM and DM on depolymerization reaction rates, only the initial linear part of the curve was modeled, based on samples whose DM value had not decreased more than 10%. Hence, β-elimination kinetics were simplified to a pseudo zero order model:

$$U_t = U_0 + k_\beta t \tag{3}$$

where U_0 = initial concentration of unsaturated bonds (mmol l^{-1}), U_t = concentration of unsaturated bonds at time t (mmol l^{-1}), k_β = pseudo zero order reaction rate constant of β-elimination (mmol l^{-1} min^{-1}).

2.7.3. Demethoxylation

Demethoxylation of pectin can occur in alkaline as well as in acidic conditions. Alkaline demethoxylation or saponification can be written as

$$RCOOCH_3 + OH^- \rightarrow RCOO^- + CH_3OH \tag{4}$$

This reaction follows second-order kinetics (Renard & Thibault, 1996)

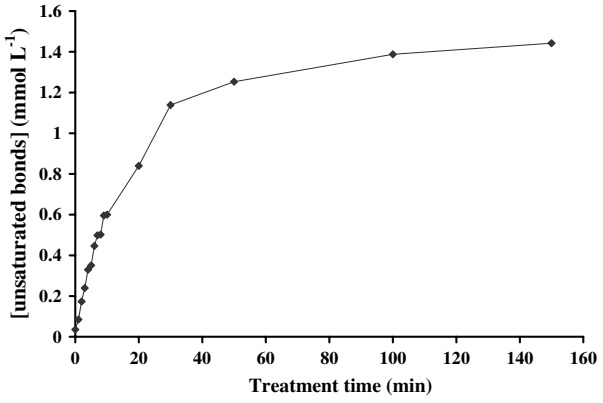

Fig. 1. Formation of unsaturated bonds during thermal treatment of P78 at 110 °C and pH 7.0.

$$-\frac{d[RCOOCH_3]}{dt} = \frac{d[CH_3OH]}{dt} = k_S^{2nd}[RCOOCH_3][OH^-] \tag{5}$$

During thermal treatments, $[OH^-]$ remained constant by the use of a buffer. Hence, the reaction can be simplified to a pseudo first order reaction with respect to the ester:

$$-\frac{d[RCOOCH_3]}{dt} = \frac{d[CH_3OH]}{dt} = k_{Sapp}^{1st}[RCOOCH_3] \tag{6}$$

where k_{Sapp}^{1st} = apparent first order reaction rate constant of saponification = $k_S^{2nd}[OH^-]$. Demethoxylation at acidic conditions is catalyzed by H_3O^+ and can be written as

$$RCOOCH_3 + H_2O \rightarrow RCOOH + CH_3OH \tag{7}$$

This reaction follows first order kinetics.

$$-\frac{d[RCOOCH_3]}{dt} = \frac{d[CH_3OH]}{dt} = k_{AD}^{1st}[RCOOCH_3] \tag{8}$$

However, experimental discrimination between alkaline and acidic demethoxylation was not possible. Hence, methanol liberated may originate from any of both demethoxylation reactions:

$$\frac{d[CH_3OH]}{dt} = \left(k_{Sapp}^{1st} + k_{AD}^{1st}\right)[RCOOCH_3] = k_D^{1st}[RCOOCH_3] \tag{9}$$

Eq. (9) implicates that the rate of demethoxylation declines as the reaction proceeds. However, for similar reasons as described above for β-elimination, only the initial linear part of the curve describing $[CH_3OH]$ against time was modelled, based on the samples whose DM had not decreased more than 10%. Hence, demethoxylation kinetics were simplified to pseudo zero order kinetics:

$$\frac{d[CH_3OH]}{dt} = k_D \tag{10}$$

$$[CH_3OH]_t = [CH_3OH]_0 + k_D t \tag{11}$$

where $[CH_3OH]_0$ = initial concentration of methanol (mmol l^{-1}), $[CH_3OH]_t$ = concentration of methanol at time t (mmol l^{-1}), k_D = pseudo first order reaction rate constant of demethoxylation (mmol l^{-1} min^{-1}).

To summarize, it is important to emphasise again that all calculated k-values, of acid hydrolysis, β-elimination and demethoxylation, indicate *initial* reaction rates.

2.7.4. Temperature dependence of rate constants

The temperature dependence of all reaction rate constants was determined using the Arrhenius model:

$$k = k_{ref} \exp\left(\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right) \tag{12}$$

where E_a = activation energy (J mol^{-1}), R = universal gas constant (8.3143 J K^{-1} mol^{-1}), k_{ref} = reaction rate constant at reference temperature T_{ref} (K) and k = reaction rate constant at temperature T (K). Activation energies were estimated by plotting the natural logarithm of the k-values against the reciprocal of the respective tempera-

ture (K^{-1}). The activation energy is a measure for the temperature dependence of the reaction rate constant. A positive E_a value indicates that reaction rate constants increase as temperature is increased. A high absolute value of E_a signifies that the rate constants depend strongly on temperature.

3. Results

3.1. Preparation of pectins with varying DM and PM

Pectins with varying DM and PM were produced by demethoxylation of an apple pectin using tomato PME, generating a blockwise PM, or *A. aculeatus* PME, generating a more random PM. DM-values for each of the pectins are shown in Table 1. In what follows, pectins will be denoted by a letter (P for parent pectin, T or A for pectin demethoxylated by tomato PME or *A. aculeatus* PME, respectively) followed by a number indicating DM of the pectin.

3.2. Pectin degradation reaction rate constants

All reaction rate constants are shown in Table 2. At some conditions (e.g. acid hydrolysis at pH 5 and 7), the reaction rate was too low to quantify, particularly at lower temperatures.

3.2.1. Influence of pH on rate of pectin degradation

Fig. 2 shows the influence of pH on reaction rate constants of acid hydrolysis of T19 and β-elimination and demethoxylation of P78 at 110 °C.

Acid hydrolysis rates of T19 were negligible at pH 5.0 and 7.0. At pH 3.0 some acid hydrolysis was observed, while at pH 2.0, the reaction rate was quite high. For all other pectins, similar trends were observed. These trends are explained by the fact that acid hydrolysis is catalyzed by H_3O^+-ions. The results are in accordance to observations of Krall and Mcfeeters (1998) who observed rather fast acid hydrolysis at pH 2.0, while at pH 4.5 until 6.0, reaction rates were very small.

An opposite trend was observed for the reaction rates of β-elimination of P78 at 110 °C. At pH 2 and 3 the reaction rate was negligible, while at pH 5.0 some β-elimination was detected. At pH 7.0 the rate was very high. Similar trends were observed for all other pectins. These findings are due to the fact that β-elimination is catalyzed by OH^-. Kravtchenko et al. (1992) also observed an increase in initial β-elimination rates when pH increased from 5 up to 6. Similary, Krall and Mcfeeters (1998) measured increasing reaction rates at pH values of 3.5 up to 6.0, while Sila et al. (2006) obtained similar results for pH values from 3.5 up to 9.0.

Demethoxylation rates of P78 at 110 °C were minimal at pH 3.0. Lowering the pH to 2.0 resulted in only a slight increase in reaction rate. At this low pH, demethoxylation was probably catalyzed by H_3O^+. At pH 5.0, some demethoxylation was detected, while at pH 7.0 the reaction rate was very high. At these pH-values, most likely saponification occurred. For most other pectins, similar trends were observed. T19 showed a slightly different trend, as can be seen in Table 2. However, because reaction rates were very close to the detection limit, standard errors of regression were rather large in this case, which reduces the reliability of this trend.

Table 1
DM-values of pectins ± standard deviation (%)

	DM
Parent pectin	77.9 ± 3.2
T-pectins	59.2 ± 3.7
	37.4 ± 1.9
	19.3 ± 1.6
A-pectins	59.4 ± 4.0
	31.0 ± 1.6

3.2.2. Influence of DM and PM on rate of pectin degradation

Fig. 3 shows the influence of DM and PM on reaction rate constants of acid hydrolysis at pH 2.0 and both β-elimination and demethoxylation at pH 7.0 and 110 °C.

A decrease in DM from 78% to 59% resulted in a two-fold increase in acid hydrolysis rate at pH 2.0. A further decrease in DM had less influence on the reaction rate. A similar tendency was observed at pH 3.0 (Table 2). Krall and Mcfeeters (1998) also demonstrated increasing hydrolysis rates with decreasing DM at pH 3.0 and 100 °C.

In Fig. 3, it is shown that below DM 59% the reaction rate of β-elimination at pH 7.0 decreased strongly. Previously, several authors observed that β-elimination rates decreased strongly with decreasing DM (Krall & Mcfeeters, 1998; Sajjaanantakul et al., 1989, 1993). When comparing the order of magnitude of the rate constants of acid hydrolysis and β-elimination, it can be observed that at the conditions investigated, β-elimination rates were higher than acid hydrolysis rates.

Reaction rates of demethoxylation at pH 7.0 declined as DM decreased. This is obvious, since pectin with a lower DM contains less methoxyl esters available for demethoxylation (see also Eqs. (9) and (10)).

Fig. 3 shows that acid hydrolysis, β-elimination and demethoxylation were not strongly influenced by PM, since T-pectins and A-pectins with comparable DM were degraded at similar rates.

3.2.3. Influence of temperature on rate of pectin degradation

The effect of temperature (80–110 °C) on the rate of acid hydrolysis, β-elimination and demethoxylation was determined for P78 and T-pectins at different pH-values. Activation energies are shown in Table 3. They were only calculated for conditions at which *k*-values were obtained for at least three different temperatures.

All E_a values were positive, indicating that acid hydrolysis, β-elimination and demethoxylation were accelerated by

Table 2
Reaction rate constants (10^{-6} mol l^{-1} min^{-1}) ± standard error of regression for acid hydrolysis, β-elimination and demethoxylation for different pectins and at different pH-values and temperatures

Pectin	T (°C)	Acid hydrolysis		β-elimination				Demethoxylation			
		pH 2	pH 3	pH 2	pH 3	pH 5	pH 7	pH 2	pH 3	pH 5	pH 7
P78	80	n.d	n.d	n.d	n.d	0.286 ± 0.0689	9.13 ± 0.580	1.58 ± 0.148	n.d	2.37 ± 0.285	60.2 ± 4.69
	90	<d.l	n.d	<d.l	<d.l	0.843 ± 0.0405	27.3 ± 0.894	2.50 ± 0.209	<d.l	3.97 ± 0.197	99.9 ± 10.7
	100	0.927 ± 0.208	n.d	0.128 ± 0.0279	0.670 ± 0.0773	2.31 ± 0.00798	56.0 ± 2.75	3.69 ± 0.0624	1.42 ± 0.100	9.04 ± 0.181	218 ± 9.34
	110	1.75 ± 0.372	<d.l	0.581 ± 0.0733	1.22 ± 0.113	3.98 ± 0.269	92.0 ± 1.17	6.20 ± 0.336	2.28 ± 0.198	14.6 ± 0.634	310 ± 20.2
T59	80	<d.l	n.d	n.d	n.d	<d.l	4.26 ± 0.451	1.71 ± 0.347	0.707 ± 0.193	0.931 ± 0.148	23.7 ± 2.33
	90	0.613 ± 0.181	<d.l	<d.l	n.d	0.340 ± 0.0257	12.7 ± 0.531	1.98 ± 0.315	1.15 ± 0.164	2.38 ± 0.152	73.0 ± 8.31
	100	1.53 ± 0.200	0.648 ± 0.110	0.283 ± 0.0187	n.d	0.491 ± 0.0319	37.7 ± 2.73	4.27 ± 0.257	1.85 ± 0.0652	3.86 ± 0.222	126 ± 7.24
	110	3.78 ± 0.241	2.13 ± 0.119	1.61 ± 0.159	<d.l	2.65 ± 0.0575	87.7 ± 8.07	8.61 ± 0.637	2.23 ± 0.427	7.46 ± 1.40	277 ± 12.0
T37	80	0.319 ± 0.117	n.d	n.d	n.d	<d.l	1.92 ± 0.219	n.d	n.d	0.528 ± 0.0663	18.8 ± 1.61
	90	0.973 ± 0.305	<d.l	<d.l	n.d	0.237 ± 0.0179	6.34 ± 0.232	<d.l	n.d	1.99 ± 0.146	45.1 ± 1.88
	100	1.59 ± 0.146	0.886 ± 0.0658	0.130 ± 0.0227	<d.l	0.491 ± 0.0319	12.8 ± 0.559	1.55 ± 0.338	<d.l	3.68 ± 0.229	53.0 ± 5.33
	110	4.20 ± 0.391	1.41 ± 0.258	0.675 ± 0.244	0.952 ± 0.0410	2.03 ± 0.127	48.9 ± 3.62	3.85 ± 0.351	1.96 ± 0.247	7.53 ± 0.398	192 ± 10.6
T19	80	0.141 ± 0.0867	<d.l	n.d	n.d	n.d	0.945 ± 0.0910	n.d	n.d	n.d	10.4 ± 0.674
	90	0.810 ± 0.0415	0.395 ± 0.0495	<d.l	n.d	<d.l	2.92 ± 0.175	n.d	n.d	n.d	19.0 ± 1.4
	100	1.14 ± 0.0781	0.903 ± 0.160	0.362 ± 0.0716	n.d	0.215 ± 0.0376	4.56 ± 0.169	<d.l	<d.l	<d.l	33.1 ± 1.36
	110	4.79 ± 0.258	2.32 ± 0.266	0.553 ± 0.134	<d.l	0.937 ± 0.0962	11.1 ± 0.425	1.67 ± 0.400	4.38 ± 1.39	2.65 ± 0.617	60.6 ± 0.493
A59	80	<d.l	n.d	n.d	n.d	n.d	3.46 ± 0.318	2.08 ± 0.205	n.d	n.d	24.4 ± 2.19
	90	0.512 ± 0.0744	n.d	<d.l	n.d	n.d	12.2 ± 0.355	2.82 ± 0.162	n.d	n.d	61.9 ± 2.04
	100	1.61 ± 0.0754	<d.l	0.186 ± 0.0304	n.d	<d.l	32.8 ± 1.88	5.47 ± 0.356	<d.l	<d.l	141 ± 4.55
	110	3.48 ± 0.221	2.07 ± 0.234	1.13 ± 0.174	<d.l	1.42 ± 0.124	78.9 ± 13.3	8.81 ± 0.594	2.91 ± 0.352	5.54 ± 0.480	269 ± 6.44
A31	90	n.d	n.d	n.d	n.d	n.d	4.15 ± 0.651	n.d	n.d	n.d	17.5 ± 2.89
	100	n.d	n.d	n.d	n.d	n.d	5.68 ± 0.915	n.d	n.d	n.d	39.8 ± 5.31
	110	3.71 ± 0.141	2.72 ± 0.115	0.966 ± 0.101	<d.l	0.512 ± 0.131	17.3 ± 1.76	3.66 ± 0.837	1.45 ± 0.966	1.67 ± 0.106	80.1 ± 4.78

n.d = Not determined.
d.l = Detection limit.

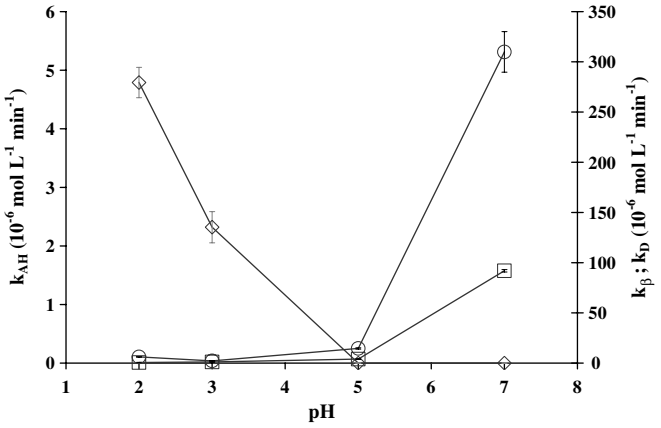

Fig. 2. Influence of pH on reaction rate constants (±standard error of regression) of acid hydrolysis of T19 (◇; primary *Y*-axis), β-elimination of P78 (□; secondary *Y*-axis) and demethoxylation of P78 (○; secondary *Y*-axis) at 110 °C.

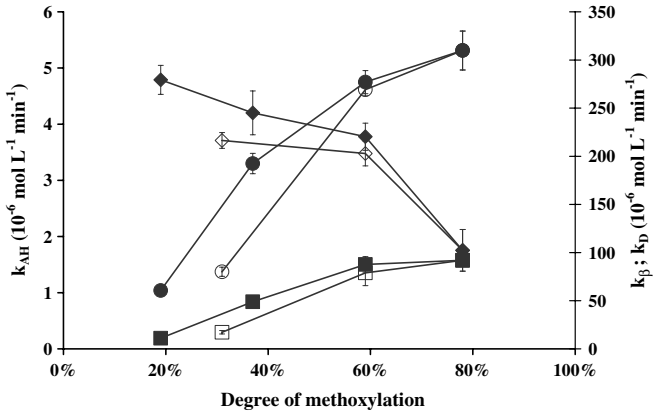

Fig. 3. Influence of DM and PM on reaction rate constants (±standard error of regression) of acid hydrolysis at pH 2.0 (♦: P78 and T-pectins; ◇: P78 and A-pectins; primary *Y*-axis), β-elimination at pH 7.0 (■: P78 and T-pectins; □: P78 and A-pectins; secondary *Y*-axis) and demethoxylation at pH 7.0 (●: P78 and T-pectins; ○: P78 and A-pectins; secondary *Y*-axis) at 110 °C.

temperature. E_a values of the two depolymerization reactions were of the same order of magnitude (102.3–122.9 kJ mol^{-1} for acid hydrolysis and 80.2–123.9 kJ mol^{-1} for β-elimination). No trends were observed as a function of DM. Sila et al. (2006) estimated similar activation energies ranging from 82.9 to 129.3 kJ mol^{-1} for β-elimination of water soluble carrot pectin at pH 6.5.

E_a values of demethoxylation ranged from 44.4 to 96.9 kJ mol^{-1}. The mechanism of demethoxylation of pectin depends on the pH. At pH 2.0, the reaction was probably catalyzed by H_3O^+, while at pH 5.0 and 7.0, most likely a saponification reaction occurred. E_a-values for the acid catalyzed demethoxylation were somewhat lower than for the saponification.

3.3. Comparison of β-elimination and demethoxylation reaction rates

At pH 5.0 and 7.0, both demethoxylation and β-elimination of pectin occurred. Demethoxylation of pectin results in a decrease of DM, hence in a decrease in sensitivity of pectin to β-elimination. The influence of temperature, pH and DM on the competition between these two reactions was examined. In order to do so, the ratio of *k*-value of demethoxylation to *k*-value of β-elimination (k_D/k_β) was calculated for P78 and T-pectins at all temperatures and pH 5.0 and 7.0. A ratio k_D/k_β from 3 to 10 was observed, indicating that at all conditions demethoxylation was faster than β-elimination.

With decreasing DM, k_D/k_β showed some tendency to increase (Fig. 4). Hence, decreasing DM induced a somewhat stronger decrease in β-elimination rate than in demethoxylation rate.

The ratio k_D/k_β showed no general trend as a function of pH (results not shown), indicating that the initial rate of both reactions was proportionally decreased by a decrease in pH from 7.0 to 5.0. The influence of pH on the ratio of demethoxylation to β-elimination has been investigated by Kravtchenko et al. (1992). However, they made conclusions based on data obtained after 24 h, while in this work, initial reaction rates are investigated. Kravtchenko et al. (1992) showed that the extent of β-elimination after 24 h was higher at pH 5.0 compared to pH 6.0. They concluded that a decrease in pH induced a stronger decrease in demethoxylation than in β-elimination rate. However, these experiments were subject to two important drawbacks. First of all, demethoxylation was not measured directly. Secondly, it was not taken into account that DM varied along the experiment, which lasted for 24 h. The influence of DM on both reaction rates may explain their

Table 3
Activation energies (kJ mol^{-1}) ± standard error of regression for acid hydrolysis, β-elimination and demethoxylation for different pectins and at different pH-values

Pectin	Acid hydrolysis		β-elimination		Demethoxylation			
	pH 2	pH 3	pH 5	pH 7	pH 2	pH 3	pH 5	pH 7
P78	n.d	n.d	100.5 ± 8.4	80.2 ± 12.6	50.5 ± 2.6	n.d	70.6 ± 5.2	64.1 ± 5.8
T59	105.2 ± 1.2	n.d	118.1 ± 45.8	114.5 ± 3.1	62.7 ± 12.3	44.4 ± 5.1	75.8 ± 6.3	89.3 ± 8.0
T37	105.9 ± 13.5	n.d	123.9 ± 25.0	117.0 ± 10.1	n.d	n.d	96.9 ± 11.3	80.0 ± 17.0
T19	122.9 ± 20.1	102.3 ± 5.6	n.d	88.3 ± 9.3	n.d	n.d	n.d	65.8 ± 1.3

n.d = Not determined due to low reaction rate.

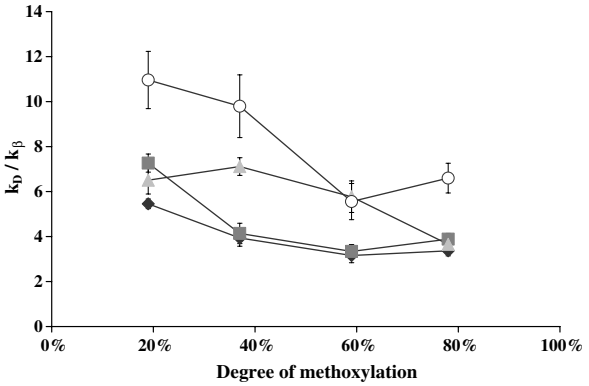

Fig. 4. Influence of DM on ratio k_D/k_β (±standard error) for P78 and T-pectins at pH 7.0 and different treatment temperatures (♦: 110 °C; ■: 100 °C; ▲: 90 °C; ○: 80 °C).

observations, rather than the influence of pH. At pH 6.0, both reactions proceeded very fast initially. Pectin was demethoxylated very quickly, giving rise to a larger decrease in β-elimination rates, compared to demethoxylation rate. Hence, after 24 h, the extent of depolymerization was limited. On the other hand, at pH 5.0, β-elimination rates were somewhat lower, but because of the slower demethoxylation, depolymerization proceeded at a significant rate during a longer time. As a result, after 24 h pectin was degraded to a larger extent.

As indicated above, activation energies of demethoxylation were lower compared to β-elimination. This indicates that any temperature increase resulted in a stronger increase in depolymerization rate than demethoxylation rate. In other words, the ratio k_D/k_β decreased with increasing temperature.

4. Conclusion

The results obtained can be used to evaluate pectin stability during processing in industrial applications and might contribute to a better understanding of the effect of processing on pectin related functional properties such as texture of processed fruits and vegetables.

As pH and DM increased, β-elimination rates increased. The opposite was observed for acid hydrolysis rates. The pH of plant cell walls is generally between 4 and 6 (Brett & Waldron, 1996). At this pH acid hydrolysis of pectin is negligible, while β-elimination rates are significant. Therefore, it may be beneficial to infuse PME in tissues prior to processing, lowering DM of pectin and hence inducing a decrease in sensitivity to β-elimination. This would be beneficial for texture of processed fruits and vegetables. Moreover, at pH 4–6, some chemical demethoxylation of pectin can occur, further reducing the extent of depolymerization.

Depolymerization rates were independent of PM. As a consequence the type of PME (plant PME or microbial PME) infused in fruits and vegetables will not influence the pectin depolymerization rate during processing. However, it is possible that the pattern according to which depolymerization occurs depends on PM. Pectin demethoxylated by tomato PME is composed of a mixture of chains with a high DM and chains with a low DM (Duvetter et al., 2006). When these pectins are heated, the sensitivity of each single chain to depolymerization depends on its DM. Some chains would be degraded while others remain unaffected. On the other hand, pectin demethoxylated by *Aspergillus* PME is more homogeneous, which probably leads to a more homogeneous depolymerization pattern. These depolymerization patterns might influence texture of the processed tissues.

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