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# Nonenzymatic Degradation of Citrus Pectin and Pectate during Prolonged Heating: Effects of pH, Temperature, and Degree of Methyl Esterification

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The underlying mechanisms governing nonenzymatic pectin and pectate degradation during thermal treatment have not yet been fully elucidated. This study determined the extent of nonenzymatic degradation due to $\beta$-elimination, acid hydrolysis, and demethylation during prolonged heating of citrus pectins and its influence on physicochemical properties. Solutions of citrus pectins, buffered from pH 4.0 to 8.5, were heated at 75, 85, 95, and 110 °C for 0−300 min. Evolution of methanol and formation of reducing groups and unsaturated uronides were monitored during heating. Molecular weight and viscosity changes were determined through size exclusion chromatography and capillary viscometry, respectively. Results showed that at pH 4.5, the activation energies of acid hydrolysis, $\beta$-elimination, and demethylation are 95, 136, and 98 kJ/mol, respectively. This means that at this pH, acid hydrolysis occurs more rapidly than $\beta$-elimination. Furthermore, the rate of acid hydrolysis is diminished by higher levels of methyl esterification. Also, citrus pectin (93% esterified) degrades primarily via $\beta$-elimination even under acidic conditions. Acid hydrolysis and $\beta$-elimination caused significant reduction in relative viscosity and molecular weight.

**KEYWORDS: Nonenzymatic degradation; $\beta$-elimination; acid hydrolysis; demethylation; pectin degradation; citrus pectin**

## INTRODUCTION

The textural properties of plant-derived food products have been closely associated with the ability of pectins to form gels and aggregates, thus modulating consistency of fluid products (*1*) and firmness of particulate foods (*2*). However, pectin solubilization and degradation occur during the processing of fruits and vegetables. This is caused by both enzyme-catalyzed and nonenzymatic mechanisms. In many cases, enzymes are completely inactivated by an initial heat treatment, such as the hot break process for tomatoes or pasteurization in juices. However, as the product is subjected to further thermal processing, pectin breakdown may occur via nonenzymatic mechanisms. This leads to a decrease in pectin molecular weight, resulting in a loss of pectin functionality. For this reason, much of the research describing textural modifications during industrial processing centers on characterization of pectin changes through the study of changes in molecular weight profiles (*3*). Although many studies have attempted to characterize pectin solubilization and degradation, only a few have presented data on the extent to which various nonenzymatic degradation mechanisms occur.

Chemical $\beta$-elimination is one of the mechanisms of nonenzymatic degradation in pectins. The rate of this reaction is accelerated with increasing degrees of methylation, temperature, and pH (*4*−*6*). This trans-elimination reaction results in the removal of the H atom at C-5 and the glycosidic residue at C-4 of galacturonic acid residues, leading to the formation of an unsaturated compound that absorbs at 235 nm (*6*). An increase in absorbance at 235 nm may be used to monitor the formation of $\beta$-eliminative degradation products (*7*).

Previous studies have shown that at pH >8, $\beta$-elimination occurs at a significant rate (*7*). Demethylation at pH 8−11 has also been reported to occur significantly (*8*). However, characterization of these reactions under acidic conditions has not been evaluated. It is relevant to determine degradation rates under acidic conditions because most food products formulated with pectins or those that inherently contain pectins are acidic in nature (e.g., citrus and grape juices, tomato products, yogurt, etc.). However, there is limited information in the literature on the rates of $\beta$-eliminative degradation and demethylation of pectins and the subsequent influence on physicochemical properties under acidic conditions. Likewise, the extent of $\beta$-elimination as compared to acid hydrolysis under acidic conditions has not been directly shown. Literature related to demethylation reactions under various conditions of industrial processing of plant-derived food products is also limited (*9*).

Smidsrod et al. (*10*) reported that at the pH range of acid foods (pH 2.5−4.5), pectate degraded more rapidly than pectin and that increases in pH resulted in decreased degradation rates. Similarly, Krall and McFeeters (*9*) reported that when pectin solutions were heated at pH 3.5 or lower, there was an increase in reducing sugar production as the pH was decreased. They

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also reported that higher degrees of pectin methyl esterification resulted in lower rates of reducing sugar formation. However, in both studies actual data differentiating $\beta$-elimination or acid hydrolysis reactions were not shown. If the mechanism of nonenzymatic degradation was $\beta$-elimination, there should not only be an increase in reducing sugars, but this should also be closely followed by a proportionate increase in unsaturated uronide formation. On the other hand, if acid hydrolysis occurs, there should be a significant increase in the evolution of reducing sugars without a significant formation of unsaturated uronides. Therefore, it should be possible to discriminate the two reactions by looking at end products.

The dynamics between $\beta$-elimination and acid hydrolysis reactions in food systems, as affected by various conditions of pH, temperature, and pectin methylation level, has not been fully examined. This study aimed to follow these nonenzymatic degradation mechanisms under a controlled system using citrus pectin and citrus pectate as model compounds. We also determined the influences of $\beta$-eliminative degradation, acid hydrolysis, and demethylation on the physicochemical changes in citrus pectin during prolonged heating under acidic conditions. Ultimately, the goal of this study was to improve the quality of food products containing pectins by understanding the mechanics of pectin solubilization and degradation and how they may be minimized.

## MATERIALS AND METHODS

**Materials.** Citrus pectin and citrus pectate [degree of esterification (DE) about 93, 69, 24, and <5%, respectively], alcohol oxidase (from *Pichia pastoris*), and Purpald (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole; catalog no. 162892) were obtained from Sigma Chemical Co., St Louis, MO. Dextran T40 and T200 were obtained from Pharmacia, Uppasala, Sweden.

**Pectin and Pectate Heat Treatments.** To investigate the influence of nonenzymatic mechanisms of degradation on the physicochemical properties of citrus pectins during prolonged heating, 1% solutions of citrus pectin (93% esterified) and citrus pectate (<5% esterified) were made at pH 4.5 and 8.5 using 100 mM Tris−acetate buffer. The treatment at pH 8.5 was used as a positive control for the occurrence of $\beta$-elimination. The presence of Tris does not affect the reaction rates. The resulting solutions were heated at 75, 85, 95, and 110 °C in screw-capped test tubes for 0−300 min. The pH of the solution was determined at the actual heating temperatures using a Tris-compatible double-junction Ag/AgCl reference combination eletrode. Samples were taken every 30 min and subsequently analyzed for reducing sugars, unsaturated uronides, methanol content, molecular weight, and relative viscosity. To investigate the effect of pH, 1% solutions of citrus pectin (93% esterified) and citrus pectate (<5% esterified) were made. The pH of the solutions were adjusted to from pH 4 to pH 7. The resulting solutions were heated at 95 °C for 2 h in screw-capped tubes. Samples were taken and analyzed for the formation of reducing sugars and unsaturated uronides.

The effect of methylation levels on the degradation rates of citrus pectins at pH 4.5 was also investigated. Solutions of 1% citrus pectin with 93, 69, 24, and <5% DE were made using 100 mM sodium acetate, pH 4.5. The resulting solutions were heated in screw-capped tubes at 95 °C for 2 h. Samples were obtained every 30 min and analyzed for the formation of reducing sugar and reduction in molecular weight. Heating experiments and analytical assays were conducted in triplicate.

**Determination of Reducing Sugars.** The reducing sugars were measured following a modification of the assay described by Milner and Avigard (*11*). Briefly, 100 $\mu$L of copper reagent consisting of 23.3 g of NaCl, 5.4 g of sodium acetate, and 0.5 g of $CuSO_4 \cdot 5H_2O$ at pH 4.8 was added to 100 $\mu$L of sample. The mixture was heated for 15 min at 100 °C. Subsequently, 0.8 mL of Folin−Ciocalteu reagent (diluted 1:40 with distilled water) was added, and the absorbance of the resulting solution at 750 nm was determined. A standard curve was made using galacturonic acid.

**Determination of Unsaturated Uronides.** The extent of $\beta$-elimination was monitored by measuring the formation of unsaturated uronides during heating, as indicated by absorbance changes at 235 nm according to the method of Kravtchenko et al. (*12*). The absorbance spectrum from 500 to 200 nm was monitored. Unsaturated uronides have an extinction coefficient of 5200 $M^{-1}$ $cm^{-1}$. All results obtained were correlated with the formation of reducing sugars during heating.

**Determination of Methanol.** The evolution of methanol was monitored following the assay described by Anthon and Barrett (*13*). Briefly, 30 $\mu$L of pectin solution was added to 90 $\mu$L of phosphate buffer (pH 7.5, 0.2 N); 10 $\mu$L of alcohol oxidase was then added to oxidize the methanol to formaldehyde. The resulting formaldehyde was reacted with Purpald to produce a stable colored product that was quantified at 550 nm against a methanol standard curve.

**Determination of Relative Viscosity.** The relative viscosity of samples heated for up to 210 min was measured using a standard Cannon-Fenske capillary viscometer setup supported in a Canon-Fenske visometer holder in a 30 °C water bath. The viscometer holder kept the viscometer in a vertical position. A 10 mL sample was pipetted into the viscometer, and the time, in seconds, for the fluid to pass through demarcation lines was recorded. Results were reported relative to the time water at 30 °C passes through the demarcation lines.

**Determination of Molecular Weights.** Changes in the molecular weight profile of the heated pectin solutions were determined using high-performance size exclusion chromatography equipped with a multiangle laser light scatter detector, HPLC-MALLS (DAWN DSP-F, Wyatt Technology Corp., Santa Barbara, CA) and a refractive index detector (ANSPEC Co., Tokyo, Japan). The chromatography was conducted with three Waters colums, namely, Ultrahydrogel 250, 1000, and 2000 with exclusion limits of $8 \times 10^4$, $1 \times 10^6$, and $7 \times 10^6$ g/mol, respectively. The columns were maintained at a constant temperature of 40 °C. Injections of 100 $\mu$L aliquots of sample were made using an automatic sampler. The eluent used was 0.01 N sodium nitrate (pH 6.8) with 0.01% sodium azide at a constant flow rate of 0.6 mL/min, resulting in 30 bar pressure. Dextrans T40 and T200 were used to verify the accuracy of the system. A value of 0.15 mL/g refractive index increment was used. Data collected were processed using ASTRA software, version 4.73.04 (Wyatt Technology Corp.) and were expressed as weight-average molecular weight.

## RESULTS

**Degradation of Citrus Pectin and Citrus Pectate during Prolonged Heating.** When heated at pH 4.5 and 95 °C, solutions of citrus pectate (degree of methyl esterification < 5%) produced reducing sugars more rapidly than a solution of citrus pectin (degree of methyl esterification = 93%), an indication that at this pH, citrus pectate is hydrolyzed more rapidly than citrus pectin (**Figure 1A**). Pectin solutions with intermediate levels of methyl esterification showed intermediate rates of reducing sugar formation (**Figure 2**). The hydrolysis of the pectate solution did not lead to the accumulation of comparable amounts of reducing sugars and unsaturated uronides, indicating that the formation of reducing sugars was mainly due to hydrolytic breakdown, not $\beta$-elimination. In contrast, whereas the rate of reducing sugar formation in the pectin solution was much lower than for the pectate, similar amounts of reducing sugars and unsaturated uronides were formed, indicating that the pectin breakdown was primarily due to $\beta$-elimination. When the same experiment was performed at pH 8.5, the relative stabilities of the pectate and pectin were reversed, with citrus pectin degrading more rapidly than citrus pectate (**Figure 1B**). Again, the pectin solution produced equal amounts of reducing sugars and unsaturated uronides, whereas the pectate solution did not. These results indicate that at both acid and alkaline pH pectin (93% DE) breakdown is due to $\beta$-elimination reaction. Under alkaline conditions pectin is less stable than pectate.

Heating pectin solutions also led to an accumulation of methanol, indicating pectin de-esterification. The rate of metha-

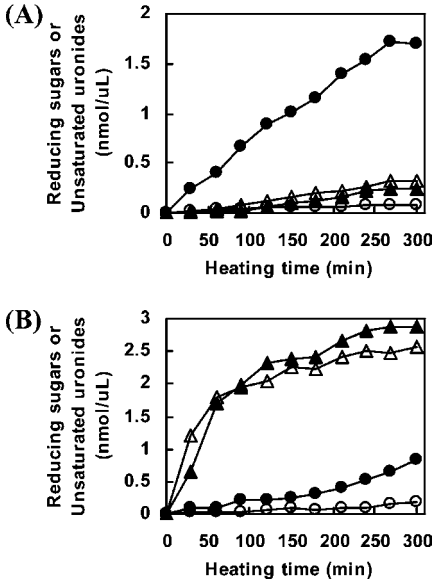


**Figure 1.** Formation of reducing sugars (solid symbols) and unsaturated uronides (open symbols) during the thermal treatment of citrus pectin (triangles) and citrus pectate (circles) at 95 °C at pH 4.5 (**A**) and pH 8.5 (**B**).

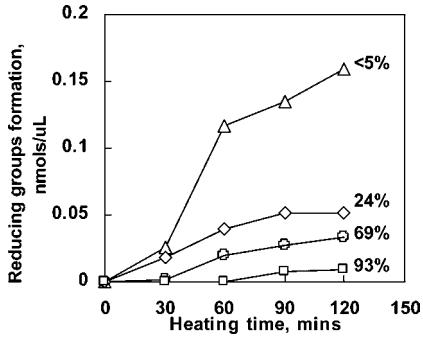


**Figure 2.** Formation of reducing sugars during thermal treatment of citrus pectins with various degrees of methyl esterification at 95 °C at pH 4.5.

nol formation was monitored during heating at pH 4.5 over the temperature range from 75 to 110 °C (**Figure 3A**). An Arrhenius plot of these rates is linear and continuous over this temperature range (**Figure 3B**). The extent of demethylation of citrus pectin under the conditions studied resulted in only about 1−3% of the total methyl ester available. The activation energy ($E_a$) based on the Arrhenius plot (**Figure 3B**) yielded 98 kJ/mol. Previous demethylation studies carried out from pH 8 to 11 and from 15 to 45 °C reported $E_a$ values of 42−49 kJ/mol, describing the reaction as pseudo-first-order (*8*).

The effect of pH on the degradation rates of citrus pectin and citrus pectate during heating at 95 °C is illustrated in more detail in **Figure 4**. Pectate hydrolysis, measured as the formation of reducing sugars, did not occur at pH values ≥6.0. Below pH 6.0, the degradation rate for pectate increased with decreasing pH. At all pH values the level of unsaturated uronides was low and did not show any correlation with the formation of reducing groups. These results indicate that pectate degrades primarily by acid hydrolysis and that this reaction only occurs at pH values of <6.0. The effect of pH on pectin breakdown showed the opposite trend. Reducing sugar formation increased with increasing pH, and the formation of reducing groups correlated with the formation of unsaturated uronides. This suggests that citrus pectin degrades primarily through a $\beta$-elimination reaction. Unsaturated uronides were detected at all

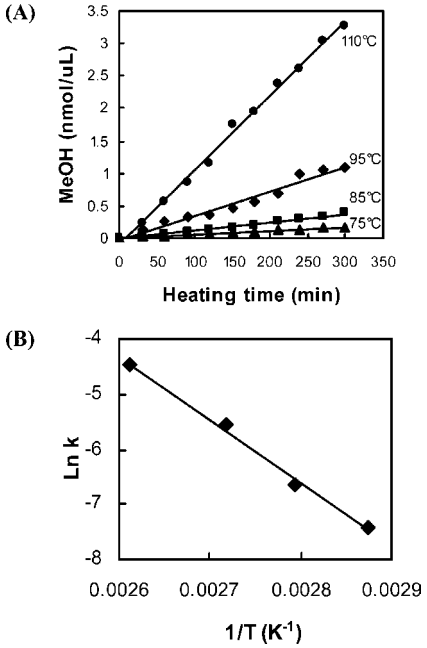


**Figure 3.** Evolution of methanol during thermal treatment of citrus pectin at pH 4.5 as affected by increasing heating temperatures (**A**). An activation energy of 98 kJ/mol was obtained from the corresponding Arrhenius plot (**B**).

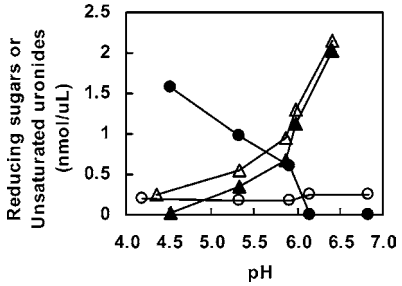


**Figure 4.** Effect of pH on the formation of reducing sugars (solid symbols) and unsaturated uronides (open symbols) during thermal treatment of citrus pectin (triangles) and citrus pectate (circles) at 95 °C for 120 min.

pH values, indicating that $\beta$-elimination can occur at pH 4.5, although the rate is very low (**Figures 1** and **4**).

The rates of reducing sugar and unsaturated uronide formation during the heating of pectin at pH 4.5 increased as the temperature was increased (**Figure 5A**). An Arrhenius plot of these rates is linear and continuous over the temperature range from 75 to 110 °C (**Figure 6**). There is an approximately 3.5-fold increase in $\beta$-eliminative reaction rate for every 10 degree rise in temperature ($Q_{10}$). The activation energy ($E_a$) for the $\beta$-elimination reaction was determined to be 136 kJ/mol. Several authors who described $\beta$-elimination at pH 6.8−11 found similar values of $Q_{10}$ and $E_a$ (*5*, *9*, *14*). For example, Sila et al. (*14*) reported $E_a$ values of 122.6 (± 11.0) kJ/mol in their study of nonenzymatic depolymerization of carrot pectin. When pectate is heated at pH 4.5, the rates of reducing sugar and unsaturated uronides did not increase proportionately (**Figure 5B**). An Arrhenius plot of these rates is linear and continuous over the temperature range from 75 to 110 °C (**Figure 6**). The $E_a$ for acid hydrolysis was determined to be 95 kJ/mol.

**Effect of $\beta$-Elimination and Acid Hydrolysis on Physicochemical Properties of Citrus Pectin and Citrus Pectate.** *Effect on Relative Viscosity*. Heating a solution of citrus pectin to 95 °C at pH 4.5 for 210 min resulted in a 20% decrease in relative viscosity (**Figure 7**). Under similar conditions, citrus

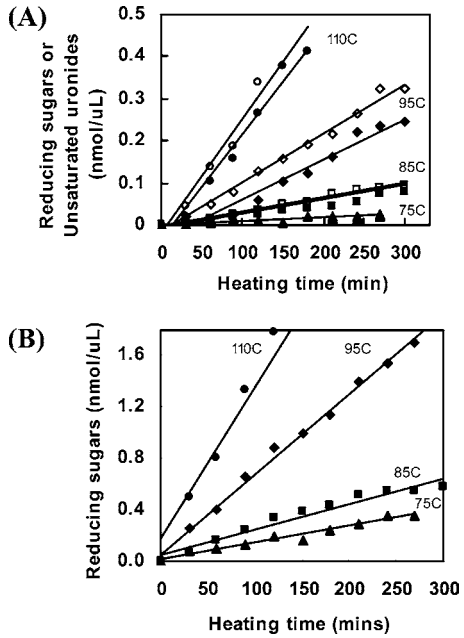


**Figure 5.** Formation of reducing sugars (solid symbols) and unsaturated uronides (open symbols) during thermal treatment of citrus pectin (**A**) and citrus pectate (**B**) at pH 4.5 as affected by increasing heating temperatures. Negligible amounts of unsaturated uronides (<0.1 nmol/uL) were determined at all time–temperature conditions for citrus pectate.

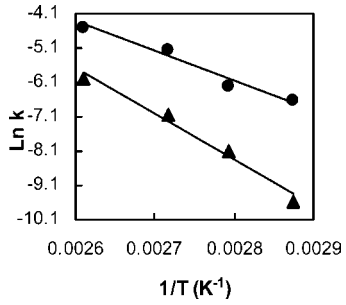


**Figure 6.** Arrhenius plot for $\beta$-elimination of citrus pectin (▲) and acid hydrolysis of citrus pectate (●) at pH 4.5 heated from 75 to 110 °C. Slopes (*k*) were obtained from panels **A** and **B** of **Figure 5**, respectively. The plot gave activation energies of 136 kJ/mol for $\beta$-elimination and 95 kJ/mol for acid hydrolysis.

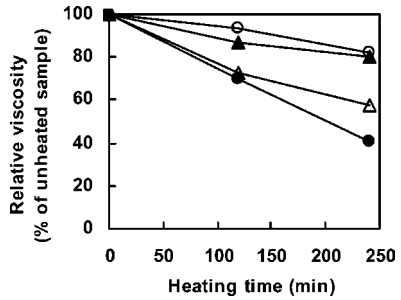


**Figure 7.** Time course for change in relative viscosity in 1% citrus pectin (circles) and 1% citrus pectate solutions (triangles) during thermal treatment at 95 °C and pH 4.5 (open symbols) and pH 8.5 (solid symbols).

pectate underwent about a 40% reduction in relative viscosity. The relatively greater loss in viscosity in the pectate versus the pectin solution at this pH is consistent with the results in **Figure 1A**. Under these conditions, the decrease in relative viscosity in citrus pectin may be attributed to $\beta$-eliminative degradation, whereas the decrease in relative viscosity in citrus pectate is due to acid hydrolysis. At pH 4.5, acid hydrolysis of pectate occurs more rapidly than $\beta$-elimination of pectin under the same conditions (**Figure 1A**).

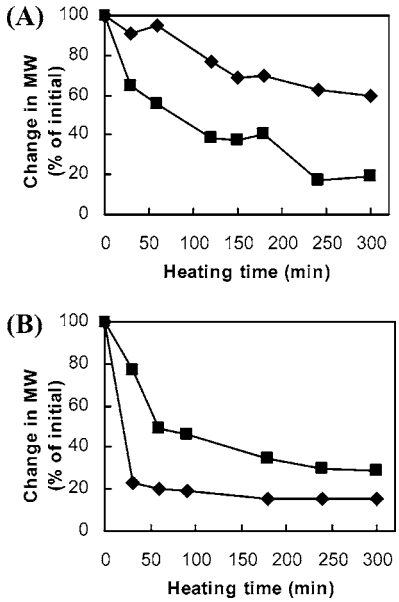


**Figure 8.** Changes in molecular weight of citrus pectin (◆) and citrus pectate (■) during thermal treatment at 95 °C and pH 4.5 (**A**) and pH 8.5 (**B**).

When heated at 95 °C at pH 8.5 for 210 min, a solution of 1% citrus pectin resulted in a 60% decrease in relative viscosity. Under similar conditions, a 1% solution of citrus pectate underwent about a 20% reduction in relative viscosity (**Figure 7**), which is a reversal of the trend when the solutions were heated at pH 4.5. At pH 8.5, citrus pectin degrades much more quickly than citrus pectate because $\beta$-elimination occurs significantly more quickly than acid hydrolysis (**Figure 1B**).

The rates of loss in relative viscosities of pectin and pectate solutions heated at 95 °C at pH 4.5 and 8.5 (**Figure 7**) are consistent with the trends in the formation of reducing sugars and unsaturated uronides (**Figure 1**).

*Effect on Molecular Weight.* Changes in molecular weights were monitored through HPLC-MALLS. The changes in molecular weight of pectin and pectate during heating to 95 °C at pH 4.5 and 8.5 are shown in **Figure 8**. At pH 4.5, there is a rapid decrease in the molecular weight in citrus pectate. After 30 min of heating at 95 °C and pH 4.5, there is a 40% decrease in the molecular weight of citrus pectate, from $6.47 \times 10^4$ to $4.16 \times 10^4$ g/mol. Under similar conditions, the molecular weight of citrus pectin decreased by only about 10% from $3.56 \times 10^4$ to $3.20 \times 10^4$ g/mol. After 300 min of heating at 95 °C and pH 4.5, there were 80 and 40% decreases in the molecular weight in citrus pectate and citrus pectin, respectively. During heating at 95 °C and pH 8.5, citrus pectin degraded much more rapidly than citrus pectate. After only 30 min of heating at 95 °C and pH 8.5, the molecular weight of citrus pectin decreased by about 80% from $3.56 \times 10^4$ to $8.27 \times 10^3$ g/mol. Under similar conditions, the molecular weight of citrus pectate decreased by only about 20%, that is, from $7.11 \times 10^4$ to $5.5 \times 10^4$ g/mol. After 300 min of heating at 95 °C and pH 8.5, there are approximately 85 and 70% decreases in the molecular weights of citrus pectin and citrus pectate, respectively. These molecular weight changes were consistent with the kinetics of pectin breakdown (**Figure 1**) and relative viscosity changes (**Figure 7**) at both pH levels.

A summary of changes in citrus pectin and pectate due to nonenzymatic degradative reactions is given in **Table 1**.

**Table 1.** Summary of Changes Due to Nonenzymatic Degradation of Citrus Pectin and Citrus Pectate during Prolonged Heating

| | | | | pH effects on reaction rates at 95 °C (nmol of reduced groups or unsaturated uronides ($\times 10^{-3}$)/min) | | | effect of pH and heat (95 °C) on loss of relative viscosity and mol wt | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | viscosity loss % | | wt av mol wt loss (%) | |
| | degree of esterification (%) | mechanism of nonenzymatic degradation | $E_a$ (kJ/mol) | pH 4.5 | pH 8.5 | critical pH for reaction | pH 4.5 | pH 8.5 | pH 4.5 | pH 8.5 |
| pectate | <5 | acid hydrolysis | 95 | 5.9 | 30 | ≤6.0 | 40 | 20 | 80 | 70 |
| pectin | 93 | $\beta$-elimination | 136 | 90 | 1.2 | >6.0 | 20 | 60 | 40 | 85 |

## DISCUSSION

The formation of reducing sugars and unsaturated uronides may be used to determine whether $\beta$-eliminative degradation or acid hydrolysis occurred in a given pectin system. An increase in reducing sugars with concomitant formation of unsaturated uronides indicates $\beta$-eliminative degradation. On the other hand, an increase in reducing sugars alone indicates degradation by acid hydrolysis. In past studies, only the formation of either reducing sugars (*9*) or unsaturated uronides (*14*) was determined to follow nonenzymatic reaction rates. The present study follows both reactions and therefore ascertains the conditions under which acid hydrolysis or $\beta$-elimination predominates.

Kravtchenko et al. (*12*) reported that the determination of unsaturated uronides at 235 nm is a good estimate of $\beta$-elimination. However, it was noted that at high temperatures (>110 °C) and long times, the amount of unsaturated uronides may be overestimated because browning products may interfere with their detection at 235 nm. Also, if only reducing sugars are determined, an accurate measure of $\beta$-elimination may not be obtained because acid hydrolysis also produces the same product. This study points out that pectins degrade primarily through $\beta$-elimination under alkaline and acidic conditions. During pectin degradation, the rate of reducing sugar formation is always accompanied by a proportionate increase in unsaturated uronides. A previous study suggests that pectin degrades via acid hydrolysis below pH 3.8 (*8*). However, this suggestion comes from data showing a difference in the rate of reducing sugar formation from pectin as compared to pectate. In the present study, the production of both reducing sugars and unsaturated uronides was monitored to determine the mechanism of degradation.

This study points out that at pH 4.5, citrus pectin is more stable than citrus pectate (**Figure 1**). This finding is corroborated by the results on the changes in relative viscosity and molecular weight (**Figures 7** and **8**, respectively). Furthermore, the degradation of citrus pectate decreased as the degree of methylation increased. Krall and McFeeters (*8*) previously reported that pectin hydrolyzed more slowly than pectate below pH 3.5. These authors also reported that increased methylation levels decreased hydrolysis rates under acidic conditions. In contrast, we found that pectate undergoes acid hydrolysis below pH 6.0. The pH levels we report here are the pH levels at the actual heating temperature. The pH was measured throughout the duration of the heating experiments.

At pH 4.5, $\beta$-elimination of pectin increased with increasing heating temperature, generating an $E_a$ of 136 kJ/mol to describe the temperature dependence of this reaction (**Figure 6**). This value was similar to previously reported activation energies for $\beta$-elimination (*5*, *9*, *14*) at pH levels between 7 and 11. The fact that the $E_a$ was similar at pH values ranging from pH 4.5 to 11.0 suggests that $\beta$-eliminative degradation is more temperature dependent than it is pH dependent. Under similar conditions, pectate degraded via acid hydrolysis. The lack of correlation between the formation of reducing groups and unsaturated uronides suggests this (**Figure 5B**). The $E_a$ of the reaction is calculated to be 95 kJ/mol (**Figure 6**). The $E_a$ between pectin and pectate varied largely, with acid hydrolysis of pectate much greater than $\beta$-elimination of pectin. This indicates that at pH 4.5, the hydrolytic breakdown of pectate more readily occurs than the $\beta$-elimination of pectin.

At pH 4.5, approximately 2% of the pectins are demethylated in solutions heated at 95 °C (**Figure 3**). Renard and Thibault (*9*) studied demethylation at pH values from 8 to 11 at 45 °C and reported an activation energy of 42−48 kJ/mol. In the present study an activation energy of about 98 kJ/mol was calculated for the demethylation reaction at pH 4.5. This $E_a$ is 2 times greater than that previously reported (*9*). The higher activation energy obtained may be due to the fact that the range of pH values evaluated was lower and the temperature range was much higher than in previous studies. Demethylation may be viewed as a base-catalyzed reaction, the rate of which is dependent on the amount of available hydroxyl groups for the reaction to proceed. Results of this work suggest that demethylation is dependent on both pH and temperature conditions. Renard and Thibault (*9*) noted that any increase is temperature will increase the rate of $\beta$-elimination, whereas any increase in pH increases the rate of demethylation.

These results have direct application to processes during which products are heated initially to inactivate enzymes and then thermally concentrated for prolonged times. For example, previous studies on tomato paste processing indicate that significant nonenzymatic viscosity loss occurs the during concentration from about 5 to 31 °Brix, which takes about 3−6 h (*3*). Under similar time and temperature conditions, solutions of 1% citrus pectate showed about a 40% decrease in relative viscosity, mainly due to acid hydrolysis. Because the rate of acid hydrolysis at pH 4.5 is slower at higher levels of methylation (**Figure 1**), it may be possible to preserve the consistency of pectin-containing products under similar conditions. Indeed, citrus pectin (DE 93%) heated at pH 4.5 showed only a 20% decrease in relative viscosity (**Figure 7**). These results suggest that in order to minimize loss in pectin viscosity during prolonged heat treatment of pH 6.0 or lower products, it may advantageous if the methyl esterification of pectin is conserved. This condition may be achieved through high-temperature (about 90−95 °C) treatments targeting enzyme inactivation such as blanching (*15*). Another specific process operation that may result in greater pectin methylation is the hot break process in tomato paste production. During this process, polygalacturonase and pectin methylesterase enzymes are quickly inactivated, resulting in more intact pectins (*16*). Furthermore, it was found that at pH 4.5 there is only about

2% demethylation occurring during heating of citrus pectin at 95 °C for 300 min (**Figure 2**). Therefore, an initial high degree of methyl esterification should retard acid hydrolysis during prolonged heating at pH 4.5.

Conversely, in citrus pectin solutions with pH >6.0, at which $\beta$-eliminative degradation is more significant than acid hydrolysis (**Figure 4**), citrus pectate (DE <5%) proved to be more stable than pectin, maintaining higher relative viscosity and molecular weight during heating. In products with pH >6.0, such as carrots and green beans (*17*, *18*), $\beta$-elimination may be the dominant nonenzymatic mechanism of pectin degradation. Results of this study suggest that it may be best to maintain low levels of methyl esterification in such relatively low acid products in order to maintain viscosity. One way that this may be achieved is through low-temperature (about 60−70 °C) blanching regimes (*14*, *15*), which activate pectin methyl esterase, thereby resulting in pectins with low degrees of methyl esterification. Any decrease in processing temperature during concentration may result in decreased $\beta$-eliminative rates because the reaction has a strong temperature dependence. Either low-temperature blanching or lower concentration process temperatures may result in more intact pectins in products with pH ≥6.0. The study implies that improved firmness observed in low-temperature-blanched pH ≥6.0 fruit and vegetable pieces may indeed be due at least in part to a decrease in $\beta$-eliminative degradation.

The nonenzymatic degradation of pectin during prolonged heating depends not only upon inherent pectin properties (for example, degree of methyl esterification and molecular weight) but also on processing regime applied, in particular, temperature. It may be necessary to characterize specific pectin systems that exist in various fruit and vegetable commodities to determine which nonenzymatic degradative mechanisms predominate. In particular, knowledge of the initial pH of the product will allow processors to optimize final viscosity by implementing either a hot break operation to maintain pectin methyl esterification and therefore reduce acid hydrolysis in products with pH ≤6.0 or a low-temperature-blanch operation and lower concentration temperatures to reduce pectin methyl esterification and therefore $\beta$-elimination in products with pH ≥6.0.

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