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Carbohydrate Research 286 (1996) 139–150

CARBOHYDRATE RESEARCH

# Degradation of pectins in alkaline conditions: kinetics of demethylation

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Received 18 November 1995; accepted 22 February 1996

**Abstract**

The demethylation of commercial citrus pectins (86% by weight galacturonic acid, degree of methylation 73) was studied between pH 8 and 11, for temperatures between 15 and 45 °C. The pectins were incubated in hermetically closed tubes in 0.2 M ethanolamine/HCl buffer. After stopping the reaction by adding acetic acid to reach pH ~ 4.5, the amount of methanol liberated was analysed by gas chromatography. At the higher pH values, the reaction showed marked deviation from the expected pseudo-first-order kinetics with respect to methyl-esterified carboxyl groups. This deviation could be accounted for by the changes of pH that occurred during saponification as the buffer salt was used up by the reaction. The rate constant of the reaction was calculated using the initial steps of the reaction and a constant pH approximation ($k = 25$ mol$^{-1}$ L min$^{-1}$) or using an integrated rate equation taking into account the pH variations ($k = 27$ mol$^{-1}$ L min$^{-1}$). The energy of activation was determined using the Arrhenius equation ($E_a = 42$–$49$ kJ mol$^{-1}$). © 1996 Elsevier Science Ltd.

*Keywords:* Pectin; Saponification; Kinetics; pH

## 1. Introduction

One of the difficulties in the use and study of pectins is their susceptibility to degradation in neutral and akaline conditions [1–4]. Two competitive reactions occur, both leading to loss of the functional properties of pectins. The methoxyl groups, carried by the galacturonic acid moieties, are saponified, and the polygalacturonic chain is split by β-elimination. This second reaction requires the presence of methoxyl groups, and is

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*PII* S0008-6215(96)00056-0

therefore inhibited by saponification. This competition is modulated by pH and temperature conditions: in alkaline conditions, any increase in temperature increases the rate of β-elimination more than that of demethylation, while an increase of pH increases demethylation more than β-elimination [4,5]. However, few attempts have been made to quantify these phenomena, most of them dating back to the 1940's [6–10].

Industrially, treatment of pectins in alkaline conditions is limited to production of amidated pectins, for which pectins are treated by ammonia in alcoholic medium at low temperatures in order to retain relatively high molecular weights [1,11]. In this heterogeneous system, the methyl-esterified galacturonic residues undergo saponification, at a slower rate than with sodium hydroxide in water, at the same time as amidation which give these modified pectins improved functionalities [8,9,12,13].

On the other hand, most studies of β-elimination have been carried out at pHs close to neutrality and elevated temperatures [2,3,5,10,14], where de-esterification is limited. In the present paper we have tried to use conditions where actual competition occurs, i.e. mild alkaline pH and temperatures between 15 and 45 °C. These conditions occur in the food industry in a few processes such as lye treatments of olives [15] (prior to lactic fermentation). They induce extensive modifications of the pectins that are extremely important for the texture of the final product. We report the kinetics constants of saponification of pectins by a study of the liberation of methanol between pH 8 and 11, and between 15 and 45 °C, in a buffered medium.

## 2. Experimental

*Pectins.*—Commercial Rapid Set (RS) citrus pectin was from Bio-Industries Systems (Baupte, France). Pectins with degrees of methylation of 55 and 24 were prepared from the initial pectin by partial saponification. Ten grams of RS pectin were dissolved in 500 mL of distilled water. The pH was first adjusted to 7 with 0.1 M NaOH then an amount of NaOH equal to the amount of methyl esters to saponify was added in the cold under stirring. All these operations were carried out in an ice bath in a cold room. After 48 h, the pH was acidified to pH 3–4 by 1 M HCl and the pectin precipitated by 3 volumes of 96% ethanol. Precipitates were collected on a sintered glass filter and dried by solvent exchange (ethanol 96% then acetone).

*Methods.*—Free methanol was measured by gas–liquid chromatography in 1 μL of a sample solution (100 μL containing 5 pL butanol as internal standard) on a DB-wax column (30 m × 0.32 mm) at 50 °C with hydrogen as a carrier gas at 0.85 bar. The injector and the flame ionisation detector were at 150 °C. After each third sample, the column was cleaned by heating to 150 °C. Ethanolamine/HCl buffer (initial buffer at 0.4 M) was prepared by diluting 2.42 mL of ethanolamine in water, adjusting the pH with 1 M HCl then adjusting the volume to 100 mL. The kinetics of demethylation were followed in hermetically closed vials in a water bath. Tubes containing 1 mL of pectin solution in distilled water were prepared; at $t = 0$, 1 mL of ethanolamine buffer was added (giving 0.2 M buffer during the reaction). One tube was used to read the initial pH of the pectin/ethanolamine buffer mixture, and determine the amount of acetic acid necessary to bring the pH to 4–4.5. In the other tubes, the reaction was stopped after a

given time by adding this predetermined amount of acetic acid. The evolution of pH during demethylation was followed in a parallel series of experiments, where the pH of 10 mL of pectin in 0.2 M ethanolamine buffer was measured at intervals for up to 6 h.

Pectins (5 mg/mL) were injected on a column of DEAE Sepharose CL-6B (20 × 1.3 cm) in sodium acetate buffer pH 4.8. The column was washed with 0.05 M buffer (90 mL) then eluted by a gradient up to 1 M in 90 mL followed by 45 mL at 1 M. Fractions (4.4 mL) were collected. GalA and neutral sugar concentrations were measured in the fractions by automated metahydroxybiphenyl [15] and orcinol [16] methods, respectively, with correction for the interference of the uronic acids in the orcinol method [17]. Individual neutral sugars were determined by GLC after derivatisation to alditol acetates [18]. Hydrolysis conditions were 2 M trifluoroacetic acid, 120 °C, 2.5 h for Rha [19] and 1 M $H_2SO_4$, 100 °C for 2 h for all other sugars. GalA and methanol contents were measured simultaneously by titrimetry [20]. Ash was measured gravimetrically after 16 h at 550 °C then 1 h at 900 °C.

## 3. Results

*Characterisation of the pectins.*—The original RS citrus pectin had a high GalA content and high degree of methylation (Table 1). Our results were in close agreement with the producer's data (84% GalA degree of methylation of 72.5). It contained less than 10% neutral sugars, mainly Gal, as expected in citrus pectin [21]. We found more Rha than Kravtchenko et al. [21], probably because we used a more drastic hydrolysis. With these conditions, it was possible to cleave the aldobiouronic acids resulting from the rhamnogalacturonan fraction of the backbone of pectins [19]. DEAE Sepharose CL-6B fractionation of the RS pectin (Fig. 1) showed one main peak with very little non-retained neutral polysaccharide (about 3% of the sugars) and it was therefore not further purified. De-esterification using known amounts of alkali in the cold was used to produce pectins with lower DMs. The values obtained (Table 1) were close to the expected values, which were of 50 and 27 (calculated from the producer's data).

*Kinetics of demethylation.*—We chose to follow the saponification of pectins by the release of methanol, for which more sensitive methods were available to us than for

Table 1
Composition of the pectins (mg/g)

| | RS pectin | DM 55 | DM 25 |
|---|---|---|---|
| GalA | 864 | 939 | 875 |
| Methanol (DM) | 114 (73) | 94 (55) | 38 (24) |
| Rha | 16 | nd [a] | nd |
| Ara | 28 | nd | nd |
| Man | 4 | nd | nd |
| Gal | 45 | nd | nd |
| Ash | 4 | nd | nd |

[a] nd: Not determined.

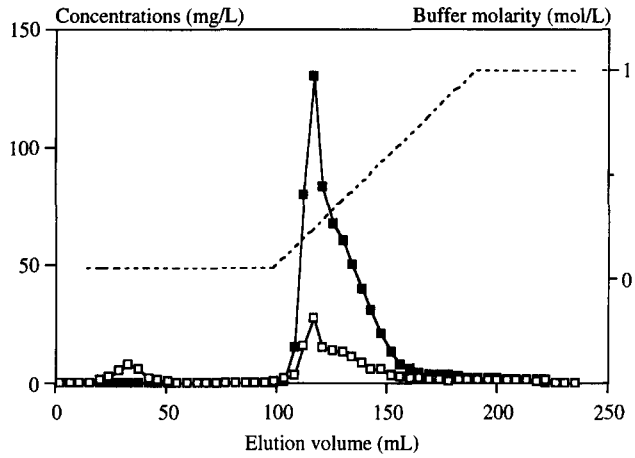


Fig. 1. DEAE Sepharose CL-6B (acetate buffer pH 4.8) chromatography of the initial RS citrus pectin. ■: galacturonic acid; □: neutral sugars; ---: molarity of the acetate buffer.

measure of the increase in free carboxyl groups. However, methanol being volatile, the reaction had to be carried out in hermetically closed vials. This precluded use of a pH-stat system and imposed the use of a buffer system to adjust the pH. Most common buffers available in the pH range 8–11 interfered with the GLC measurement of methanol. The buffer systems tested and rejected were: tris/HCl, sodium carbonate/hydrogen carbonate, glycine/NaOH, Clark and Lubs buffer ($KCl/H_3BO_3$). The buffer system finally chosen was ethanolamine/HCl ($pK_a$: 9.5). A complete series of demethylations (from 0 to 24 h) was carried out in duplicate. The variation between duplicate points was low obviating duplication of further experiments.

Classically [22], saponification reactions can be written as:

$$RCOOR' + OH^- \rightarrow RCOO^- + R'OH,$$

and follow second-order kinetics with

$$-\frac{d[RCOOR']}{dt} = \frac{d[R'OH]}{dt} = k[RCOOR'][OH^-] \quad (1)$$

It should therefore be possible, by keeping $[OH^-]$ constant throughout the reaction, to transform the reaction into a pseudo-first-order reaction with respect to the ester, with:

$$\frac{-d[RCOOR']}{dt} = \frac{d[R'OH]}{dt} = k_{app}[RCOOR'] \quad (2)$$

$k_{app}$: apparent reaction constant; $k$ is obtained by: $k_{app} = k[OH^-]$.

One of the characteristics of a pseudo-first-order reaction is that, at a given time, the extent of reaction is independant of the initial concentration of the reactant. Another characteristic feature is that the plot of logarithm of the concentration of reactant against time is a straight line. This results directly from the integration of eq. (2):

$$\ln\left(\frac{[RCOOR']_t}{[RCOOR']_0}\right) = -k_{app}t \quad (3)$$

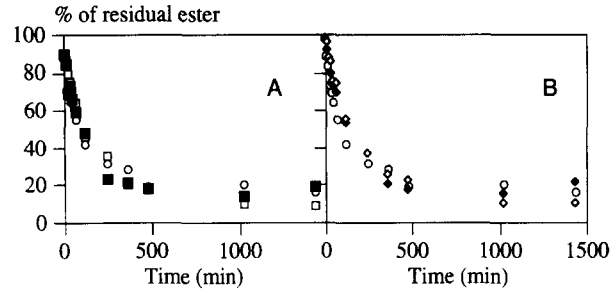


Fig. 2. Influence of pectin concentration (A) and initial degree of methylation (B) on the kinetics of demethylation at pH 10.75. RS pectin at approximately: ○: 5 g/L; ■: 2.5 g/L and □: 1.25 g/L; partially demethylated pectin: ■: DM = 55; □: DM = 25.

with: $[RCOOR']_0$, initial concentration of ester groups; $[RCOOR']_t$, concentration of remaining ester groups at the time $t$.

This holds true for an 'ideal' reaction. For real systems, deviation from linearity is expected after the first two or three half-lives [22].

*Effect of pectin concentration, degree of methylation and pH.*—The demethylation of the RS pectin at three concentrations ( ~ 5, 2.5 and 1.25 g/L) is shown in Fig. 2A. Plots of the extent of reaction versus time appeared independant of the concentration, which implied that this reaction followed a pseudo-first order with respect to concentration. The speed of the reaction also appeared independant of the DM of the pectin (Fig. 2B), at least for DMs between 75 and 25. For this experiment, all three pectins had been solubilised at the same initial methyl-ester concentration, so that the initial concentration of free carboxyl groups varied in a ratio of 1 to 3. Superposition of the demethylation curves indicated that the presence of negative charges in the vicinity of the methyl esters did not influence in any major way the reaction constant. The effect of pH on the reaction (Fig. 3) was studied between pH 11.25 and 8.25, though the most extreme pH

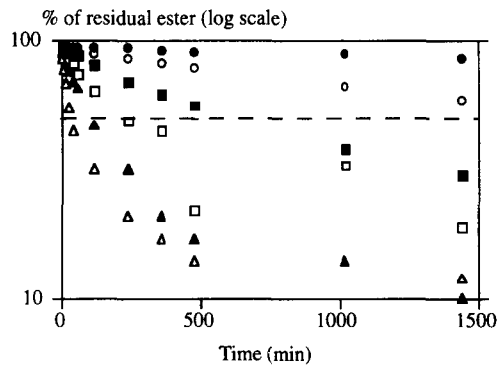


Fig. 3. Demethylation of RS citrus pectin (5 g/L) as a function of pH: evolution of the proportion of residual ester as a function of time (semi-logarithmic scale; the dotted line represents 50% demethylation). ●: pH 8.25; ○: pH 9.02; ■: pH 9.53; □: pH 10.05; ▲: pH 10.55; △: pH 11.25.

values exceeded the buffer range of the ethanolamine buffer. The reaction proceeded much faster at higher pH values. At pH 11.25, half of the methyl esters were saponified within 1 h, whereas at pH 8.25 less than 20% of the methyl esters have been saponified after 24 h. However, saponification did not reach completion after 24 h even for the highest pH values, which plateaued at about 90%. In earlier experiments [19], some methyl esters also persisted after 16 h at pH 13, giving residual DMs of 2–5.

*Determination of the rate constants.*—Though the extent of reaction was independant of the initial concentration of the pectin (Fig. 2A), the data did not give straight lines in a logarithmic plot (Fig. 3) but deviated markedly from linearity after the first half-life (represented by the dotted line at 50% demethylation), notably for the higher pHs. Though the reaction showed a pseudo-first order with respect to pectin concentration, it did not show a first order with respect to time. An explanation of this deviation from the expected behaviour was sought in a drift of the system away from the initial conditions. As the speed of reaction is directly proportional to $[OH^-]$, pH was measured at the end of the reaction: in the buffer area of ethanolamine (8.5 to 10.5), there was a decrease of of 0.1 to 0.2 pH units, not much higher than the precision of pH measurement, but which corresponds to a 20% to 40% decrease in $[OH^-]$.

The pH variations that would be caused by total de-esterification have been calculated from eq. (4):

$$\mathrm{pH} = \mathrm{p}K_a + \log_{10}\left(\frac{[\mathrm{EtNH_2}]_t}{[\mathrm{EtnH_3^+}]_t}\right) \tag{4}$$

with $[EtNH_2]_t$ and $[EtNH_3^+]_t$ as the concentrations at time $t$ of the base and salt forms of ethanolamine, respectively. At $t \neq 0$, electroneutrality leads to the following variations in the concentrations:

$$[\mathrm{EtNH_3^+}]_t = [\mathrm{EtNH_3^+}]_0 + [\mathrm{RCOO^-}]_t \text{ and } [\mathrm{EtNH_2}]_t = [\mathrm{EtNH_2}]_0 - [\mathrm{RCOO^-}]_t$$

with $[RCOO^-]_t$ the amount of carboxyl groups that have been liberated at time $t$ ($[RCOO^-]_t = [MeOH]$). For total de-esterification $[RCOO^-]_{100} = [RCOOMe]_0$, and:

$$\Delta\mathrm{pH} = \log_{10}\left(\frac{[\mathrm{EtNH_2}]_0 - [\mathrm{RCOOMe}]_0}{[\mathrm{EtNH_3^+}]_0 + [\mathrm{RCOOMe}]_0}\right) - \log_{10}\left(\frac{[\mathrm{EtNH_2}]_0}{[\mathrm{EtNH_3^+}]_0}\right) \tag{5}$$

A plot of ΔpH versus initial pH is given in Fig. 4 for a RS pectin concentration of 5 g/L. There was no pH value for which the pH was not influenced by liberation of the carboxyl groups of pectins and ensuing consumption of the base form of ethanolamine. The buffer was most efficient between pH 9.5 and 10, but the variation that would occur at complete demethylation was still close to 0.2 pH unit for an inital pectin concentration of 5 g/L. The actual pH variations were followed as a function of time. Variations of pH itself were limited for pH values up to ~ 10.5 (indeed about 0.1–0.2 pH units) and after 6 h $[OH^-]$ was about 70% of the initial concentration for pHs < 10.5, and 50% for pHs > 10.5. In Fig. 5, the measured pH variations are plotted together with the variations calculated from the amount of liberated MeOH (with the same initial pH and pectin concentration), using eq. (4) with $[EtNH_3^+]_t = [EtNH_3^+]_0 + [MeOH]_t$ and $[EtNH_2]_t = [EtNH_2]_0$-$[MeOH]_t$. Almost perfect superposition of the two curves was

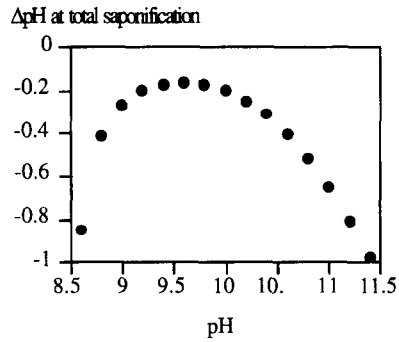


Fig. 4. pH Variation at 100% saponification calculated from eq. (5) for an initial RS pectin concentration of 5 g/L.

obtained, implying that the pH variations were indeed directly due to liberation of carboxyl groups. The decreased speed of reaction was due to the impossibility of compensating totally the use of $OH^-$ ions in the reaction. Use of a more concentrated buffer was considered but it would only diminish this effect and might cause decreased solubility of pectins. For pH $\geq$ 10.5, the buffering capacity of the ethanolamine was obviously insufficient to compensate for the consumption of $OH^-$ in the de-esterification reaction.

Due to this lack of linearity, linked to the pH change, we chose to use two parallel approaches to establish the rate constants:

(1) to use the equations of a pseudo-first-order reaction, extrapolating to the inital steps of the reaction, to calculate the $k_{app}$s;

(2) to take into account the pH changes, as calculated in eq. (5), in the calculation of the rate constant.

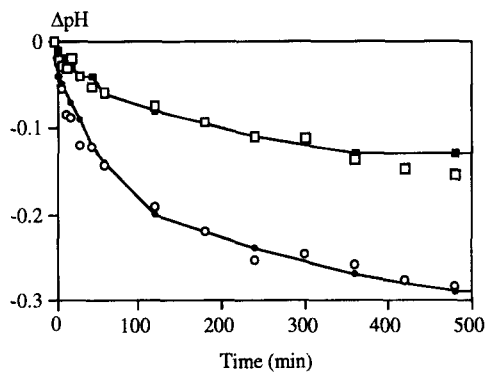


Fig. 5. Variations of pH during demethylation of RS citrus pectin: comparison between measured and calculated [from eq. (4) and measured methanol] variations. Measured pH: □: $pH_0 = 9.72$; ○: $pH_0 = 10.48$, and pH calculated from methanol data: ●: $pH_0 = 10.52$; ■: $pH_0 = 9.75$.

Table 2
Conditions of demethylation experiments of RS citrus pectin and rate constants calculated from the liberated MeOH data

| $pH_0$ | RS pectin concentration (g/L) | Temperature (°C) | Using the constant pH approximation | | Modelising the pH variation | |
|---|---|---|---|---|---|---|
| | | | $k_{app}$ | $k_{app}/[OH^-]$ | $k'$ | $k$ |
| 11.25 | 4.2 | 25 | 0.0170 | 9.6 | 0.00046 | 14.6 |
| 10.85 | 4.2 | 25 | 0.0117 | 16.5 | 0.00086 | 27.3 |
| 10.75 | 4.1 | 25 | 0.0084 | 14.9 | 0.00071 | 22.5 |
| 10.72 | 2.2 | 25 | 0.0074 | 14.1 | 0.00050 | 15.8 |
| 10.72 | 1.2 | 25 | 0.0061 | 11.6 | 0.00046 | 14.6 |
| 10.55 | 4.1 | 25 | 0.0062 | 17.6 | 0.00058 | 18.2 |
| 10.26 | 4.6 | 15 | 0.0017 | 9.5 | 0.00042 | 13.4 |
| 10.26 | 4.6 | 25 | 0.0043 | 23.7 | 0.00060 | 19.1 |
| 10.26 | 4.6 | 35 | 0.0078 | 43.1 | 0.00117 | 37.1 |
| 10.26 | 4.6 | 45 | 0.0094 | 51.5 | 0.00260 | 82.1 |
| 10.23 | 2.3 | 25 | 0.0038 | 22.3 | 0.00063 | 20.0 |
| 10.09 | 5.2 | 25 | 0.0028 | 22.4 | 0.00108 | 34.1 |
| 10.05 | 4.8 | 25 | 0.0032 | 28.5 | 0.00089 | 28.3 |
| 10.04 | 2.3 | 25 | 0.0024 | 21.9 | 0.00076 | 24.0 |
| 10.04 | 1.2 | 25 | 0.0027 | 24.6 | 0.00068 | 21.5 |
| 10.00 | 2.3 | 25 | 0.0028 | 27.8 | 0.00095 | 30.0 |
| 9.76 | 4.6 | 15 | 0.00069 | 11.5 | 0.00043 | 13.7 |
| 9.76 | 5.0 | 25 | 0.0011 | 18.6 | 0.00066 | 20.8 |
| 9.76 | 5.0 | 35 | 0.0027 | 46.6 | 0.00149 | 47.0 |
| 9.76 | 5.0 | 45 | 0.0041 | 71.1 | 0.00225 | 71.2 |
| 9.65 | 2.3 | 25 | 0.00145 | 32.5 | 0.00078 | 24.8 |
| 9.53 | 5.5 | 25 | 0.00110 | 32.5 | 0.00089 | 28.3 |
| 9.53 | 2.4 | 25 | 0.00113 | 33.4 | 0.00089 | 28.1 |
| 9.53 | 1.3 | 25 | 0.00117 | 33.7 | 0.00099 | 31.5 |
| 9.02 | 5.2 | 25 | 0.00033 | 31.4 | 0.00111 | 35.0 |
| 9.02 | 2.5 | 25 | 0.00042 | 40.4 | 0.00133 | 42.0 |
| 9.02 | 1.3 | 25 | 0.00044 | 41.3 | 0.00133 | 42.0 |
| 8.25 | 5.1 | 25 | 0.000072 | 40.5 | 0.00148 | 46.8 |

*Calculation of* $k_{app}$ *and* k *using a pseudo-first-order approximation.*—In this approach the values of $k_{app}$ (Table 2) are calculated from logarithmic plots of $[RCOOMe]_t/[RCOOMe]_0$ during the first half-life of the reaction (or 24 h if 50% demethylation is not reached in that time). All the $k_{app}$ thus obtained for saponifications at 25 °C are plotted in Fig. 6 as a function of $[OH^-]$. There appears to be a linear variation of the $k_{app}$ with $[OH^-]$ up to pH 10.85, confirming the direct effect of $OH^-$ in the reaction, in spite of the pH variation that occurs during the course of the reaction. A regression line could be drawn, with $r = 0.97$. The rate constant was calculated as the average $k_{app}/[OH^-]$ for this series of kinetics (Table 3).

*Determination of* k *taking into account the pH variations.*—As the pH changes could be predicted (Fig. 5) using eq. (5), the kinetic data were recalculated using at each point $[OH^-]$ calculated from the initial pH and the amount of reacted ester with:

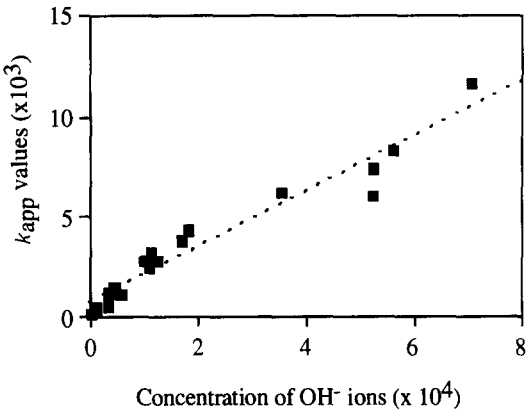


Fig. 6. Plot of $k_{app} = f([OH^-])$ using a constant pH approximation and the initial stages of the reaction.

$[EtNH_2]_t + [EtNH_3^+]_t = 0.2$, using the $pK_a$ value of ethanolamine (9.5) and $[EtNH_2]_t = [EtNH_2]_0 - [MeOH]_t$ therefore:

$$\log_{10}[OH^-] = -4.5 + \log_{10}\left(\frac{[EtNH_2]_0 - [MeOH]_t}{0.2 - [EtNH_2]_0 + [MeOH]_t}\right) \quad (6)$$

$$\frac{d[MeOH]}{dt} = k'([RCOOMe]_0 - [MeOH]_t)\frac{[EtNH_2]_0 - [MeOH]_t}{0.2 - ([EtNH_2]_0 - [MeOH]_t)} \quad (7)$$

with $k' = k \times 10^{-4.5}$.

An equation of the form:

$$\frac{0.2 - (a - x)}{(a - x)(b - x)}dx = k'dt \quad (8)$$

is thus obtained with $x = [MeOH]$, $a = [EtNH_2]_0$, and $b = [RCOOMe]_0$ and can be integrated as:

$$\frac{0.2}{(a - b)}\ln\left(\frac{(a - x)b}{(b - x)a}\right) + \ln\left(\frac{(b - x)}{b}\right) = k't \quad (9)$$

Table 3
Rate constant (at 25 °C) and activation energy for demethylation of pectins

| | Constant pH approximation | Correcting for pH variations |
|---|---|---|
| $k$ (mol$^{-1}$ L min$^{-1}$) | 25.4 ± 9.4 | 27.2 ± 9.0 |
| $E_a$ (kJ mol$^{-1}$) | | |
| at pH 9.76 | 41.2 ($r$ = 0.99) [a] | 48.7 ($r$ = 0.99) |
| at pH 10.26 | 46.3 ($r$ = 0.98) | 43.6 ($r$ = 0.97) |

[a] $r$: Correlation coefficient of the regression line.

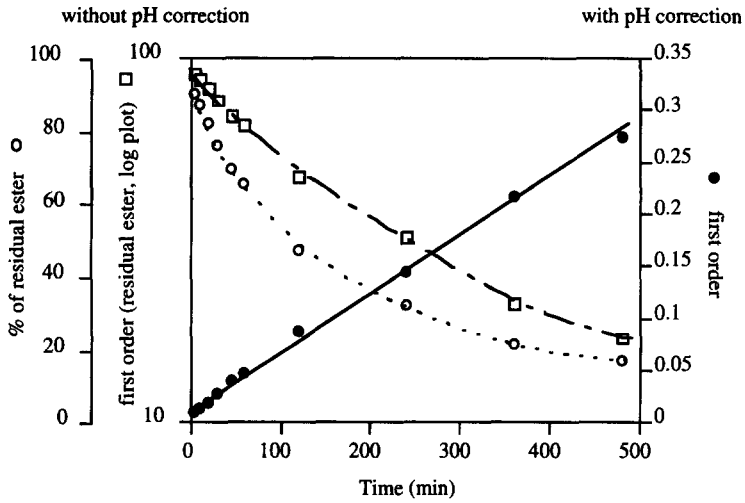


Fig. 7. Demethylation of RS citrus pectin at pH 10.55 expressed: ○: as percentage residual ester, linear plot; □: as percentage residual ester, logarithmic plot; ●: with a first-order kinetics, with correction for the pH variation.

or, expressed as a function of $p$, the advancement of the reaction ($p = x/b$):

$$\frac{0.2}{(a-b)} \ln\left(\frac{1-\frac{b}{a}p}{1-p}\right) + \ln(1-p) = k't \tag{10}$$

Fig. 7 shows demethylation at pH 10.5, plotted using either the raw data, or the logarithmic plot of the raw data (i.e. the first order, constant pH, approximation), or taking into account the pH variation with a pseudo-first-order hypothesis, i.e. eq. 10. A straight line was indeed obtained with the pseudo-first-order hypothesis corrected from the pH variation, confirming that the deviation form linearity observed in the $\log(c/c_0)$ plots was due to the pH variations.

Twenty-three kinetic experiments were carried out at 25 °C, between pH 11.25 and 8.25. The reaction constant $k$ (Table 3) was calculated as the average of the slopes of all the kinetics carried out at 25 °C, using eq. 10, up to 24 h or 80% reaction, whichever occurred first (Table 2). This value of $k$ was similar to the previous one.

*Determination of the activation energy.*—At pH 9.76 and 10.26, saponification was carried out at 15, 25, 35 and 45 °C (Table 2), and the activation energy $E_a$ has been calculated from a regression on the plot $\ln k = f(1/T)$ according to the Arrhenius equation (Fig. 8 and Table 3):

$$\ln k = \ln A - \frac{E_a}{RT} \tag{11}$$

with $T$ the temperature in Kelvin and $R = 8.3143$ J K$^{-1}$ mol$^{-1}$.

Similar activation energies were found for both approaches and both pHs (Table 3).

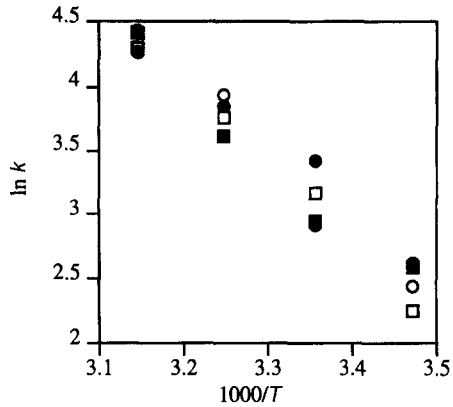


Fig. 8. Arrhenius plots of the demethylation reaction. With the constant pH approximation, ○: at pH 9.76; ●: at pH 10.26. With correction for the pH variation, □: at pH 9.76; ■: at pH 10.26.

Variations between the determined $E_a$ values was probably due to the limited temperature range and low number of points used. However, these values were in good agreement with the values obtained by Slavockova [8] and Lineweaver [7].

## 4. Discussion

In weakly alkaline conditions, pectins were demethylated following pseudo-first-order kinetics with respect to the concentration of methylated GalA moieties. In this pH range the methyl-ester concentrations were higher than the initial [$OH^-$]. Use of a buffer led to deviations from the ideal behavior as [$OH^-$] varied in proportions which could not be neglected in the equations of the kinetic; however these deviations could be totally accounted for by the pH variation. In spite of this limitation, we chose to use a buffer to carry out the reaction in hermetically closed tubes, allowing a reliable measurement of the liberated methanol. Similar rate constants and activation energies were found either using a constant pH approximation, and the inital steps of the reaction, or taking into account the variation of the $OH^-$ ions, validating this double approach. A rate constant of $\sim 25$ mol$^{-1}$ L min$^{-1}$ at 25 °C was obtained, lower than that obtained by Scamparini and Bobbio [9] at 20 °C ($k_{app} = 0.26$ min$^{-1}$ for 0.1 M NaOH), Lineweaver [7] (0.8 mol$^{-1}$ L s$^{-1}$ at 29.5 °C), and Black and Smit [23]: $k_{app} = 25.1 \times 10^{-3}$ min$^{-1}$ for NaOH de-esterification at pH 11 and 5–7 °C. This could be due to use, instead of additions of sodium hydroxide, of an organic salt. De-esterification of pectins with ammonia also proceeds slower than with sodium hydroxide at the same pH [9,11]. Values of the activation energies found were in agreement with previously reported data for demethylation of pectin with alkali in aqueous solutions (Lineweaver [7]: 10.7–10.9 kcal/mol; Slavockova [8]: 12.7 kcal/mol). With an activation energy of $\sim 45$ kJ mol$^{-1}$, the speed of the reaction will double between 20 and 32 °C.

Demethylation of pectins in ethanolamine buffer could be quantified and followed easily during incubations in hermetically closed tubes. Using the equations established in

this series of experiments, we now hope to be able to quantify $\beta$-elimination in similar conditions and establish the pattern of competition between these two reactions in mild alkaline conditions.

## Acknowledgements

The authors thank Dr. T.P. Kravtchenko, from SBI (France) for the citrus pectin and the gas chromatographic method of methanol measurement, and Miss J. Vigouroux for excellent technical assistance.

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