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Production of a low ester pectin by de-esterification of high ester citrus pectin

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The kinetics of de-esterification of high ester citrus pectin, using aqueous hydrochloric acid (HCl) solution or treatment with a mixture of ammonia in isopropanol were studied with respect to changes in methyl ester groups, molecular weight, and the formation of acid amide groups. Acid demethylation using HCl showed that higher concentrations of acid at low temperatures gave less depolymerization during de-esterification than when lower concentrations and higher temperatures were used. In a series of NH_4OH de-esterifications and amidations using different ammonia concentrations at different temperatures, apparent molecular weight was retained better with a certain decrease of ester groups, as the temperature was lowered and the concentration of ammonia increased.

INTRODUCTION

Commercial pectins, mainly derived from citrus peel, fall into two general groups: over 50% of the galacturonan units of high methoxyl or high ester pectins are esterified with methyl alcohol, while less than 50% of the carboxyl groups are esterified in low methoxyl pectin. High ester pectins require large amounts of sugar and low pH for gel formation, while low ester pectins form gels with or without sugar in the presence of divalent cations. They are, therefore, used in low calorie or dietetic foods. Gels made from low ester pectins are relatively stable during prolonged storage at room temperature (Owens *et al.*, 1949), and retain their shape at higher temperatures (Joseph, 1955).

There are four different methods for the preparation of low ester pectin from high ester pectin. They are: (a) acid demethylation; (b) alkali demethylation; (c) enzyme demethylation; and (d) ammonia in alcohol or concentrated aqueous ammonia demethylation and amidation.

Low methoxyl pectins produced by enzyme demethylation have been found to be inferior to those produced by other methods because of the nonrandom distribution of methyl ester groups among molecules of the pectin (Speiser & Eddy, 1946; Hills *et al.*, 1949), and because of the removal of very little ballast i.e. non-uronide materials (chiefly galactin and araban) (Kertesz, 1951). Acid demethylation removes ballast at high rates, leading to production of pectins having a higher percentage of polygalacturonic acid (Hills *et al.*, 1949). The main disadvantage of acid treatment is the slowness of the reaction (Kersetz, 1951). It may be speeded up by using higher temperatures, but this results in increased depolymerization of the pectin chains (Doesberg, 1965). Alkaline demethylation is rapid but the removal of methyl ester groups is accompanied by depolymerization of pectin chains (Kertesz, 1951), and the rate of depolymerization increases faster than the rate of demethylation as the temperature increases (Slavickova, 1961). Many workers showed that the rate of acid demethylation proceeds at less than 0.01% of the rate of alkaline demethylation at approximately the same temperature and concentration of reagents (Baker, 1948; Speiser *et al.*, 1945; McCready *et al.*, 1944; Black & Smit, 1972).

The use of ammonia in alcoholic systems or in concentrated aqueous ammonia systems results in a low methoxyl pectin that contains amide groups. It has been found that the percentage of amide groups in the pectin play a positive role in gel formation (Rees, 1972), and contribute to gel texture and strength (Kim *et al.*, 1978; Nelson, 1981). Demethylation of ammonia in alcoholic systems or in concentrated aqueous ammonia systems have a rate of reaction approximately half that of alkaline demethylation (McCready *et al.*, 1944; Black & Smit, 1972).

In this study, attempts were made to determine the best procedures to prepare low ester pectins using acid

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in alcohol or ammonia in alcohol. The kinetics of de-esterification, depolymerization, and the formation of amide groups was studied and related to the manufacture of the pectin.

MATERIALS AND METHODS

Sample preparation

Demethylated pectin samples were prepared from a high molecular weight pectin. Pectin has been extracted from lemon peel according to the method previously investigated (El-Nawawi & Shehata, 1987) at a mild extraction temperature of 70°C with aqueous hydrochloric acid solution at pH 2.5 to avoid depolymerization of pectin chains (Wiles & Smit, 1973). Pectin is then precipitated with 70% acidified alcohol V/V to wash out electrolytes and soluble non-pectin materials. The final precipitate is then washed with 60% isopropanol V/V to remove chloride ions, filtered, shredded, dried at 60°C and ground to pass 60 mesh screen. The pectin prepared by this method has 71.5% esterification and an apparent molecular weight of 167×10^3.

Acid demethylation

Acid demethylation may be carried out in suspension in an organic solvent acid mixture as recommended by Joseph (Kertesz, 1951), in a water–acid solution (Hills *et al.*, 1942; Speiser *et al.*, 1945) or in combination with the pectin extraction procedure (Woodmansee & Baker, 1949). Black (1970) found that the distribution of methoxyl groups and the solubility of the demethylated pectin are not only dependent on the temperature and concentrations used during demethylation, but also on particle size and degree of hydration of the pectin. He found that larger particle sizes would result in slower overall reaction rates and further demethylation of the outer layer.

In this experiment, samples of pectin having a mesh size of 60 or less were first hydrated in distilled water overnight before demethylation. After excess water was removed by pressing, a series of pectin samples kept at 5°C were diluted to 6% concentration with 60% isopropanol mixtures cooled to 5°C and containing 1, 3.5 and 4.5 N HCl, respectively. The pectin solutions were stirred continuously at 5°C during demethylation. To investigate the effect of temperature on the demethylation process, one pectin solution containing 1 N HCl was stirred continuously at 30°C. Portions of the reaction mixture were removed at intervals, precipitated with alcohol, filtered, washed with 60% isopropanol v/v until free of chlorides, pressed, dried at 60°C, ground to pass 60 mesh screen and analysed for carboxyl content, methoxyl content, apparent molecular weight and moisture content.

Demethylation with NH_4OH

A 60 g sample of purified dry pectin was rehydrated overnight in distilled water. After excess water was removed, stock pectin solutions of 1000 ml were made by dissolving the hydrated pectin samples in water and stored at 5°C. At the same time, 800 ml of NH_4OH and isopropanol mixtures containing 1 2 and 4 N NH_4OH were prepared and stored at 5°C. After adjustment of samples and solutions to the desired temperature, NH_4OH demethylation was started by mixing the samples and solutions in Erlenmeyer flasks and continuously stirring the reaction mixtures at 5°C. To investigate the effect of temperature, two experiments were carried out at 25°C with 1 and 2 N NH_4OH concentrations, respectively. Small portions of samples were taken at different time intervals up to 160 min. These samples were filtered immediately, and rapidly washed with 60% isopropanol followed by 1 N HCl in 60% isopropanol. The filter cake was then stirred with acid alcohol for 20 min to remove ammonia present in the salt form. Samples were then washed with isopropanol, dried at 60°C, ground and analysed.

Analytical procedure

Methoxyl content, galacturonic acid content, moisture, ash, amide level and apparent molecular weight by viscosity were determined as previously described (National Formulary, 1965; Nelson *et al.*, 1977; Smit & Bryant, 1967).

RESULTS AND DISCUSSION

Acid demethylation

The decrease in ester content of the high ester pectin during acid de-esterification at 5 and 30°C is shown in Fig. 1. Low temperature treatments were chosen to minimize molecular weight losses during acid demethylation. Demethylation with high concentrations of acid showed a tendency to slow down as the reaction progressed. This could be due to the availability of methoxyl groups in the pectin. With further demethylation, methyl ester groups are less available for reaction and this will eventually slow down the reaction rate. Table 1 shows reaction rate constants calculated for 120 and 200 h of acid demethylation at 30 and 5°C, respectively, using a first order reaction.

$$K = \frac{1}{t} \ln \frac{C_0}{C},$$

where C_0 is the initial degree of esterification and C is the degree of esterification at time t in minutes. The K value obtained in this work at 30°C agrees favourably with values reported by other workers in which different

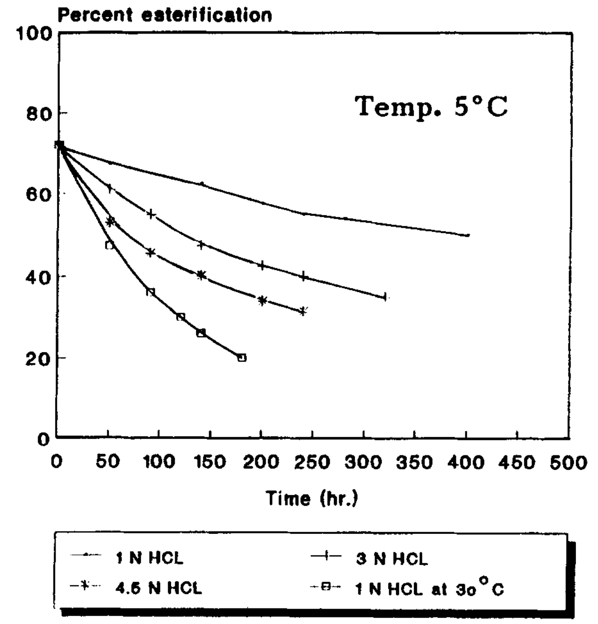

Fig. 1. Changes in per cent esterification during acid demethylation of pectin.

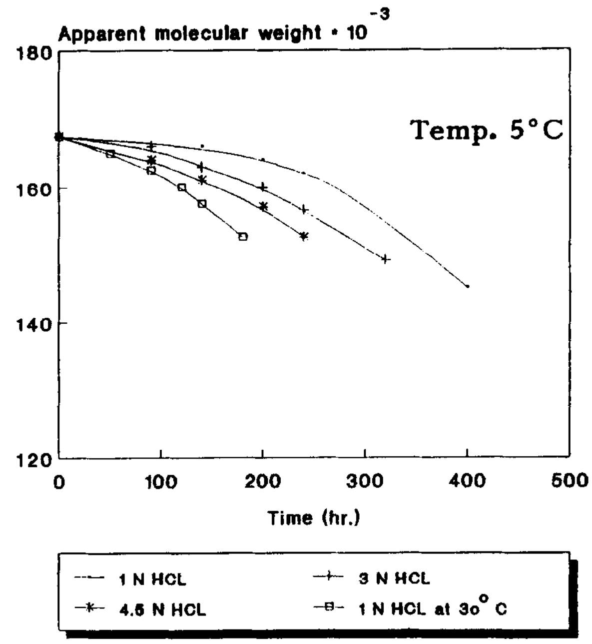

Fig. 2. Changes in apparent molecular weight during acid demethylation of pectin.

conditions of temperature and acid concentration were used e.g., 0.7×10^{-3} min^{-1} at pH 3 and 50°C (McCready *et al.*, 1944), 0.190×10^{-3} min^{-1} at 30°C with 0.87 N HCl (Speiser *et al.*, 1945), and 0.1×10^{-3} min^{-1} at 25°C with 1.4 N HCl (Black & Smit, 1972). The values obtained in this work at 5°C are much lower than the above reported data; this may be due to the low temperature used for demethylation, but they agree well with those obtained by Kim *et al.* (1978), e.g., 0.048×10^{-3} min^{-1} at 3°C with 3.5 N HCl and 0.061×10^{-3} min^{-1} at 3°C with 4.5 N HCl. Figure 2 shows the decrease in apparent molecular weight during acid demethylation. It was found that treatment with higher concentrations of acid caused more depolymerization in a given time. Apparent molecular weight was plotted against the per cent esterification (Fig. 3). At 5°C and low ester levels, higher concentrations of acid appeared to result in low apparent molecular weight losses. This figure also shows that apparent molecular weight decreases as de-esterification progresses.

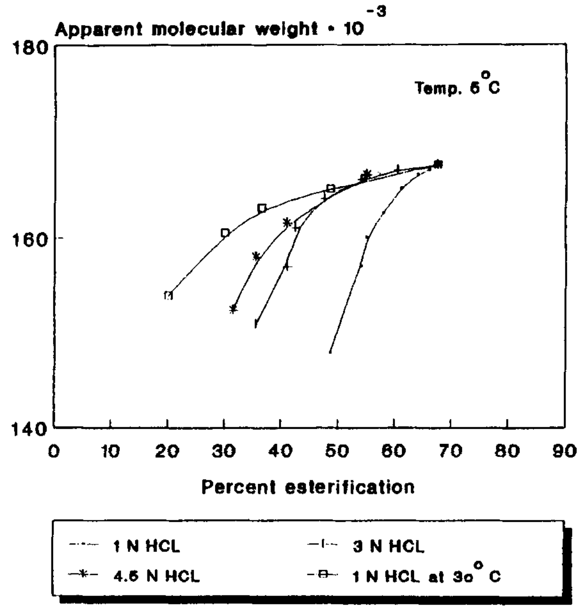

Fig. 3. Changes in apparent molecular weight and per cent esterification during acid acid demethylation of pectin.

NH_4OH Demethylation

Figure 4 shows the changes in per cent esterification of samples during NH_4OH treatment. Treatment with 4.0 N NH_4OH at 5°C produced the fastest reaction rate and this reduced esterification from 75.5% to 26% in 200 min. The reaction rate constants were calculated on the basis of 200 min demethylation, using the formula for a first order reaction (Table 1). These results show very similar reaction rates for treatments with 4.0 N NH_4OH at 5°C and 1.0 N NH_4OH at 25°C with *K* values of 5.13×10^{-3} and 4.76×10^{-3} min^{-1}, respectively. A higher reaction rate was observed with 2.0 N NH_4OH at 25°C than with 2.0 N NH_4OH at 5°C. McCready *et al.* (1944) reported *K* values of 5×10^{-3} min^{-1} for NH_4OH demethylation at pH 11 at 15°C which is within the range of *K* values obtained in these experiments. Kim *et al.* (1978) reported *K* values of 3.66×10^{-3} min^{-1} at 3°C and 2.0 N NH_4OH, 7.704×10^{-3} min^{-1} at 3°C with 4.0 N NH_4OH, 1.58×10^{-3} min^{-1} at 15°C and 1.0 N NH_4OH, 3.798×10^{-3} min^{-1} at 15°C and 2.0 N NH_4OH,

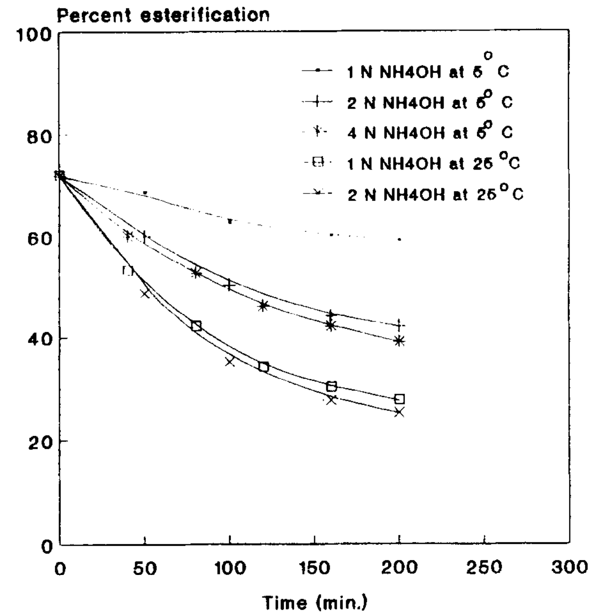

Fig. 4. Changes in per cent esterification during ammonia demethylation and amidation of pectin.

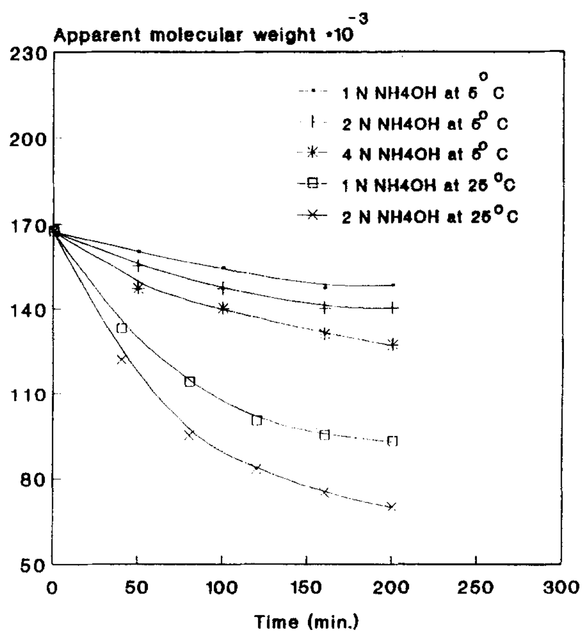

Fig. 5. Changes in apparent molecular weight during ammonia demethylation and amidation of pectin

Table 1. Specific reaction rate constants

Demethylation procedure	Temp (°C)	Time	K ($\times 10^{-3}$ min^{-1})
1 N HCl	30	120 h	0.123
1 N HCl	5	200 h	0.016
3 N HCl	5	200 h	0.048
4.5 N HCl	5	200 h	0.071
1 N NH_4OH	5	200 min	0.9462
2 N NH_4OH	5	200 min	2.612
4 N NH_4OH	5	200 min	5.1375
1 N NH_4OH	25	200 min	4.766
2 N NH_4OH	25	200 min	2.9713

1.967×10^{-3} min^{-1} at 23°C and 0.5 N NH_4OH and 7.303×10^{-3} min^{-1} at 23°C and 1.5 N NH_4OH. These values are different from those reported in Table 1, but it should be recognised that differences in starting material, experimental conditions and the presence of cations will all have an effect on the rate of demethylation (Merill & Weeks, 1946; Slavickova, 1961). A much higher value for K (25.1×10^{-3} min^{-1}) was given by Black & Smit (1972) for the treatment of pectin solution at pH 11 and 5–7°C. The major reason for this is probably the fact that demethylation was carried out in solution and that methoxyl groups were more accessible to the ammonia.

The apparent molecular weight during NH_4OH treatment was plotted against time (Fig. 5). After 200 min, the treatment with 4.0 N NH_4OH at 5°C reduced the apparent molecular weight by only 40×10^3 from the original value of 167×10^3 while the loss with 1.0 N NH_4OH at 25°C was approximately 76×10^3. Comparing the molecular weight loss between treatments having the same concentrations of NH_4OH and very similar K values, 2.0 N NH_4OH at 25°C caused 98×10^3 reduction in apparent molecular weight while 2.0 N NH_4OH at 25°C reduced it by only 27×10^3 for nearly the same reduction in per cent esterification. This agrees with Doesberg's statement (1965) that the alkaline breakdown of pectinic acids increases more rapidly with increasing temperature than does the concurrent saponification of methyl ester groups.

Figure 6 shows the increase in acid amide content during NH_4OH demethylation and amidation. Formation of amide groups was most rapid on treatment with 4.0 N NH_4OH at 5°C. In this treatment, more than 30% of the total groups were converted to amide groups in 120 min. This treatment was followed by 2.0 N NH_4OH at 5°C, 1.0 N NH_4OH at 5°C, 1.0 N NH_4OH at 25°C and 2.0 N NH_4OH at 25°C. From the data it was not clear, however, how temperature and ammonia concentration alone affect the rate of amide formation. As the temperature decreased and the NH_4OH concentration increased, the apparent molecular weight losses decreased. The most rapid depolymerization was observed on treatment with 1.0 N NH_4OH at 25°C, which was the lower concentration of NH_4OH and the higher temperature used. From a practical point of view, higher concentrations and lower temperatures during NH_4OH demethylation would be recommended to minimize apparent molecular weight losses. A similar effect of temperature and concentration of NH_4OH was observed when the increase in acid amide and decrease in apparent molecular weight were compared. De-esterification with 4.0 N NH_4OH at 5°C gave a maximum formation of

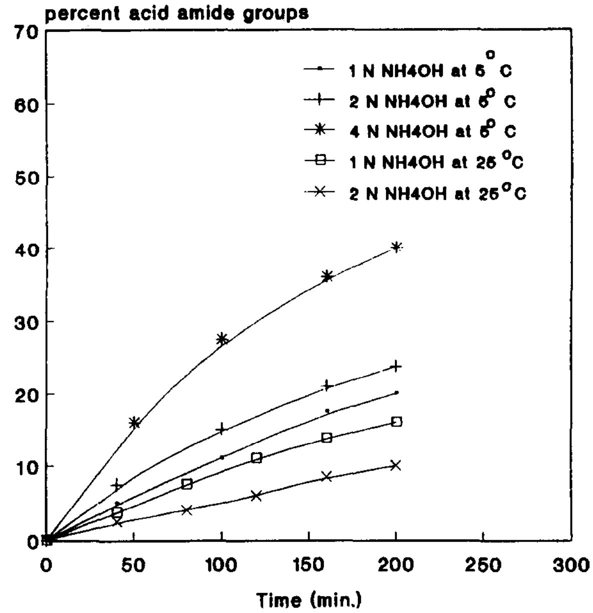

Fig. 6. Changes in pectin acid amide groups during ammonia demethylation and amidation of pectin.

amide groups with a minimum loss in apparent molecular weight. Further, with an increase in temperature and a decrease in NH_4OH concentration, the increase in amide levels was small in relation to the accompanying decrease in apparent molecular weight.

CONCLUSIONS

Acid demethylation using HCl showed that higher concentrations of acid at low temperatures gave less depolymerization during de-esterification than when lower concentrations and higher temperatures were used. In a series of NH_4OH de-esterifications and amidations using different ammonia concentrations at different temperatures, apparent molecular weight was retained better for a certain decrease of ester groups, and the conversion of methoxyl groups to acid amide groups increased as the temperature was lowered and ammonia concentration increased. A long acid treatment followed by a short NH_4OH demethylation would result in maximum retention of apparent molecular weight and low acid amide levels in the final product. To obtain a high amide level and also a relatively high molecular weight, higher concentrations of ammonia at low temperatures are required.

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