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Research Note

Production of Low Ester (LM) Pectin by De-esterification of High Ester (HM) Apple Pectin

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In this paper, LM pectin production from commercial apple pectin by using acid, alkali and ammonia methods in the presence of ethanol was studied. Also, the effect of different parameters such as, temperature, time, acid, alkali and ammonia treatment in LM pectin production were evaluated. In all the experiments, low temperature showed a better influence from a recovery, purity and low de-polymerization viewpoint. Optimum conditions in the acid method reached at pH = 2, $t = 17$ hrs and $T = 30$°C, in the alkaline method reached at pH = 11, $t = 3$ hrs and $T = 5$°C and in the ammonia method reached at 2 N ammonia and $T = 5$°C, were determined.

INTRODUCTION

Pectin is obtained mainly from the waste materials of apple, orange, grapefruit and lemon juice production [1]. Pectins with an ester level over 50% based on the total level of carboxylic acid and ester, are described as high ester pectin (HM). They are used to form jams and jellies having soluble solid levels near 65%. Formation of the gelled state requires high levels of dissolved solids and acidic pH. High methoxyl pectin does not contain sufficient acid groups of forming gel or precipitate with calcium ions. As the pH is gradually reduced, the pectin is capable of forming gel at first, at a very high sugar content, of around 80%. Pectins, modified so as to have an ester level equal to or under 50%, are described as low methoxyl or low ester pectins (LM), which are used to form gels having reduced soluble solid levels. They form gels with or without sugar in the presence of divalent cathions. They are, therefore, used in low calorie or dietetic foods [2]. Low methoxyl pectins are produced by further de-esterification to a point where less than 50% of the total carboxyl groups are esterified. If this process is carried out using acid or alkali, the balance exists as free acid groups. Low methoxyl pectins form gel by reaction with calcium and are believed to gel by the egg box mechanism [1].

There are four different methods for the preparation of low ester pectin from high ester pectin. HM pectin demethylation could be effected by: (a) Acid (b) Alkali; (c) Enzyme and (d) Ammonia in alcohol or concentrated aqueous ammonia demethylation and amidation [3,4].

Low methoxyl pectins produced by enzyme demethylation have been found to be inferior in quality to those produced by other methods, because of the non random distribution of methyl ester groups among molecules of the pectin and because of the removal of very small units, such as non-uronide materials. Acid demethylation removes special units at a high rate, leading to the production of pectin having a higher percentage of poly galacturonic acid. The main disadvantage of acid treatments is the slowness of the reaction. It may be speeded up by using higher temperatures, but this results in the de-polymerization of the pectin chains. Alkaline demethylation is rapid but the removal of methyl ester groups is accompanied by the de-polymerization of the pectin chains and the rate of de-polymerization is faster than the rate of demethylation as the temperature increases [5].

The use of ammonia in alcoholic or 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 plays a positive role in gel formation and contributes to gel texture and strength [6,7].

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In this study, attempts were made to determine the experimental procedures required to prepare low ester apple pectin, due to its high utility as a gelling agent and very versatile thickeners, with different treatment methods using acid, alkali and ammonia. The effect of different parameters on LM pectin production were also studied and compared.

MATERIALS AND METHODS

Raw Material

The experiments were effected using commercial apple pectin obtained from Fluka, Switzerland (Degree of Esterification (DE) 74.3% and viscosity average molecular weight of 32000 Dalton). All the chemical solutions utilized were analytically pure.

Acid Demethylation

Acid demonstration was studied in an aqueous solution of an acid alcohol mixture (heterorganic solution) while a mixture of water acid suspension was used as the homogenic solution [8,9]. After preparing a solution of pectin at the level of 2% in the case of an heterorganic mixture, an equal volume of the ethanol solution (60%) and 0.1 N hydrochloric acid were added to the pectin solution. The solution prepared as described was stirred continuously, while the temperature was kept constant at 30 and 50°C during the demethylation reaction. After the desired mixing time, the samples were separated with centrifugation at 3000 rpm. The separated LM pectin was washed with 60 and 96% ethanol v/v until it was free of chlorides and dried at 60°C, ground and analyzed. In the homogen method, a sample of 1 g of apple pectin was dissolved in 20 ml of distilled water and acidity was controlled by 0.1 N hydrochloric acid. After one night, the pectin precipitates was separated by centrifugation for 20-30 min at 3000 rpm. The precipitate was washed with 100 ml of 60% ethanol; final washing was done with 96% ethanol and then dried at 50°C [10].

Alkali Demethylation

To a 1% pectin solution, 1 N NaOH solution was added to give a pH of the desired value. The temperature was maintained at 5-15°C over a period of 2.5-3 hrs.

An equal volume of 95% ethanol was added to the reaction mixture, with enough 0.1 N HCl to adjust the pH value to 5. The mixture was stirred, allowed to stand for 20 min and separated by centrifugation at 2000 g. The separated cake was suspended in 50% ethanol at pH 5.2, in order to remove the undesired salt. It was, then, suspended in 95% ethanol for 20 min and was pressed and dried at 60°C and analyzed [5].

Demethylation with NH_4OH

Samples of 125 ml 1% pectin solution were prepared and stored at 5°C at 20 min. 100 ml of NH_4OH 1 N and ethanol mixtures with a ratio of 1:1 were added, mixed and stored at 5°C. NH_4OH demethylation was started by mixing the samples and solution and continuously stirring the reaction mixtures at the desired temperature. The separation and washing are similar to alkali demethylation and the amidation degree is determined [6].

Physico Chemical Analysis of Samples

The degree of esterification, methoxyl, galacturonic acid content, moisture and ash were determined according to the methods described by authors [11,12-14]. The intrinsic viscosity was measured by an ubbelholde viscometer, which is a glassware viscometer with 5.5 ml inherent volume and 0.5 mm capillary diameter. It was tested with 0.155 M of NaCl solution with flow time of 105s. This method was described by Hwang [13]. The viscosity average molecular weight of the pectin was calculated by the following Mark Houwink equation in NaCl solution [10,13]:

$$\text{Degree of Esterification (DE)} : D_E = 32 - 95\%$$

$$[\eta] = 9.55 \times 10^{-2} \times M_w^{0.73},$$

where $[\eta]$ is the intrinsic viscosity (ml/g) and M_w, is the viscosity average molecular weight.

Reaction rate constants of de-esterification were calculated by using a first order reaction, according to the data observed by other investigators [3]:

$$K = \frac{1}{t} \ell_n \frac{c_o}{c},$$

where c_o is the initial degree of esterification and c is the degree of esterification at time t in minutes.

RESULT AND DISCUSSION

In order to obtain information on the production of pectin with a low esterification degree, three different chemical treatments of commercial apple pectin were effected, using acid, alkali and ammonia under prescribed conditions. The experiments were effected in parallel and the data observed showed the average of the two parallel repeated tests carried out.

Acid Demethylation

Table 1 shows experimental conditions for the acid demethylation of pectin. De-esterification was effected under homogen and hetrogen conditions. For homogen

Table 1. Experimental conditions for acid demethylation of pectin.

Samples	Acid De-esterification	pH	T (°C)	Time (hrs)
1	Hemogen	1.5	30	17
2	"	2	"	"
3	"	1	"	"
4	"	3	"	"
5	Hetrogen	2	"	"
6	"	"	50	4
7	"	"	30	48

treatment, the temperature and time were constant at 30°C and 17 hrs, respectively, but pH was changed from 1 to 3. For hetrogen treatment, pH was constant, but the temperature and time were changed.

The characteristics of sample (LM) pectins produced under different treatments and conditions are presented in Table 2. Characteristics of the product are recovery, humidity, ash, methoxyl, galacturonic acid, esterification degree, average molecular weight and the reaction rate of demethylation. Comparing the data in Table 1 and the results in Table 2 shows that, homogen treatment of sample 4, with an 85% recovery and pectin with an average molecular weight of 32000, is more favorable. For hetrogen treatment, sample 5, with an 87.9% recovery average molecular weight of 32000, is more acceptable. Comparing the results of sample 2, using homogen treatment, with sample 5, using hetrogen treatment, shows that hetrogen treatment results in the production of LM pectin with a higher recovery and average molecular weight. For other hetrogen samples, such as sample 6, de-esterification is low (40%), due to a high temperature of 50°C. Also, the percent of recovery is low at 50% and ash content is high, which could be related to an impurity of the sample. So, conditions for acid de-esterification are related to higher recovery and purity viewpoints, which could be summarized as $T = 30$°C, pH = 2 and time = 17 hrs under hetrogen conditions. According to Table 1, viscosity, average molecular weight and rate of reaction are compared for all the samples; higher temperature resulting in higher rates of de-esterification (sample 6). The average Mw for all the samples is presented in Figure 1, which shows that sample 5 has an Mw of 32000 under hetrogen conditions.

The effect of pH could be seen from sample nos. 3, 4 and 5. The high concentrations of acid increase reaction rate but decrease viscosity average molecular weight by de-polymerization. Therefore, the highest de-polymerization is observed at pH 1 (sample 3).

The most recovery is obtained at intermediate

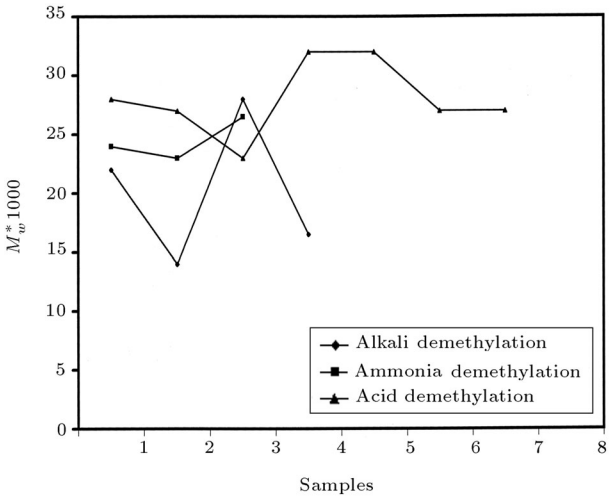

Figure 1. Viscosity average molecular weight (M_w) for pectin samples after acid, alkali and ammonia demethylation.

Table 2. Characteristics of samples after acid demethylation of pectin.

	Samples						
Specification	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Recovery %	84	80	80.3	85	87.9	66.5	50.6
Humidity %	16	18	18.6	13.46	14.02	10	5.2
Ash %	13	13	3.47	1.4	1.4	3.1	5.4
Methoxyl %	3.41	6.01	7.8	9	8.31	6.51	7.46
Galacturonic Acid %	31.9	42.7	78.65	74.46	78.88	78	95.8
Esterification Degree %	43.1	66.7	44	58.4	50.0	40	40
Intrinsic Viscosity (ml/g)	171	166	143	186	186	166	166
Viscosity Average (Mw)	28500	27500	22300	32000	32000	27500	27500
Reaction Rate (min^{-1})*0.001	0.54	0.125	0.52	0.24	0.40	0.62	0.20

concentrations of acid, due to the high degradation of pectin at high concentrations of acid. Also, it could be seen that the most rapid de-polymerization compared to de-esterification is observed at high concentrations of acid, for sample no. 3, which results in a lower average Mw.

The effect of temperature and time on the demethylation process is presented in Tables 1 and 2. Comparing the characteristics of samples 5 to 7, shows that by increasing temperature and decreasing time, recovery and viscosity average molecular weight decrease (sample 6). Also, it could be clearly found that by increasing time, methyl ester groups are less available for reaction and this will slow down the reaction rate. Increasing treatment time also increases purity. Thus, in order to reach favorable results in acid demethylation and a lower degree of esterification, longer treatment time and a lower temperature should be used.

Alkali Demethylation

For alkali demethylation, the effect of pH and temperature were studied at about equal de-esterification treatment times (Table 3). According to Table 3, for samples 2 and 3, temperature is significantly different but pH is constant. For samples 1 and 4, temperature and pH are varied. Table 4 shows the characteristics of the studied samples from alkali demethylation. The selected variables for the alkali demethylation treatment were temperature and pH as indicated in Table 4. The viscosity average molecular weight is plotted in Figure 1. Comparing the results in Tables 3, 4 and Figure 1, shows that temperature has a significant influence on the process of de-esterification. So that, using higher temperature can cause an increase in the reaction rate, but, in contrast, increases the rate of de-polymerization, which results in a decrease in the molecular weight. Therefore, high temperature is undesirable for the demethylation of pectin, as it causes molecular weight loss. For sample 3, at a lower temperature under alkaline conditions, higher purity and average molecular weight are observed, respectively. The most favorable conditions for alkali de-esterification is observed for sample 3 at: pH = 11, $T = 5$°C and $t = 3$ hrs.

Comparing data for acid and alkali demethylation, shows that the rate of acid demethylation is lower than the rate of alkali demethylation; although alkali demethylation requires more close control of operating conditions. The other disadvantage of alkali treatment is low purity and high de-polymerization of pectin chains, which results in pectin with a lower average molecular weight (Figure 1).

Table 3. Experimental conditions for alkaline demethylation of pectin.

Samples	pH	T (°C)	Time (hrs)
1	10	10	2.5
2	11	15	3
3	11	5	3
4	12	7	2.5

Table 4. Characteristics of samples after alkaline demethylation of pectin.

	Samples			
Specification	**1**	**2**	**3**	**4**
Recovery%	81.3	100	96	90
Humidity%	14.9	17.2	20	19.62
Ash%	8	10.3	6.2	4.3
Methoxyl%	5.89	2.24	2.71	5.86
Galacturonic Acid %	35.2	61.2	71.2	36.1
Esterification Degree %	70	15.1	16	38
Intrinsic Viscosity (ml/g)	138	100	170	114
Viscosity Average (Mw)	21300	13800	28300	16400
Reaction Rate (min^{-1})*0.001	0.46	8.94	8.58	4.53

NH_4OH Demethylation

To compare NH_4OH demethylation with the other two methods, similar experiments were carried out (Table 5). The results are presented in Table 6, which present the characteristics of pectin with ammonia treatment, under different conditions of temperature and ammonia concentration.

Treatment with 2 N NH_4OH at 30°C presented the fastest reaction rate (sample 2), but caused a high reduction in molecular weight. Therefore, it could be seen that as the temperature decreased and the NH_4OH concentration increased, the molecular weight losses decreased (sample 3). A similar effect of temperature and concentration of NH_4OH was observed in amidation. From Table 6, it is clear that low temperature in the ammonia treatment of native pectin results in LM pectin with higher molecular weight and amidation%, respectively (Figure 1). So,

Table 5. Experimental conditions for ammonia demethylation of pectin for 2.5 hrs.

Samples	Ammonia concentrations (N)	T (°C)
1	1	30
2	1	30
3	2	5

Table 6. Characteristics of samples after ammonia demethylation.

	Samples		
Specification	**1**	**2**	**3**
Recovery %	60.4	80.3	37.6
Humidity %	6.5	7.4	12
Ash %	10	5.8	2.4
Methoxyl %	3.61	2.42	4.2
Galacturonic Acid %	23.2	25.3	26.5
Degree of Esterification %	65	47	46.5
Inherent Viscosity (ml/g)	152	145	166
Viscosity Average Mw	24300	22800	27400
Reaction Rate (min^{-1})*0.001	0.95	3.1	2.61
Amidation %	1.6	4.2	7.5

more favorable conditions are observed for sample 3 under 2 N ammonium hydroxide at 5°C.

CONCLUSIONS

The effects of acid, alkali, and ammonia treatments on LM pectin production from commercial apple pectin were investigated. Each data point was the average of two parallel repeated experiments. In acid demethylation of pectin, higher yield is observed at hetrogen conditions, pH 2, 30°C and 17 hrs treatments, which resulted in pectin with an average molecular weight of 32000 with 50% esterification degree, 79% galacturonic acid and 88% recovery. For alkali treatment, the effect of pH, temperature and time was studied on yield, moisture, ash, galacturonic acid and degree of esterification. The significant results reached at DE = 16%, galacturonic acid 71%, recovery 96% and average molecular weight under these conditions was 28300. The results show that higher yield is observed at a lower temperature of 5°C, a longer treatment time of 3 hrs and pH of 11. For NH_4OH demethylation, the effect of NH_4OH normality, temperature and the same treatment time were studied on percent recovery, which shows that higher NH_4OH concentration and a lower temperature of 5°C resulted in lower percent recovery, pectin with higher purity, DE = 46%, amidation of 7.5%, galacturonic acid of 26.5%, percent recovery of 38% and average Mw of 27400. For a commercial pectin, an ester content of 34%, amide content of 11%, galacturonic acid content of 91%, with molecular weight of 100000, on an ash and moisture free basis, was obtained [3]. Under experimental conditions prescribed for acid, alkali and ammonia demethylation, LM pectin was produced with some different specifications.

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