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Characterization of orange peel pectin and effect of sugars, L-ascorbic acid, ammonium persulfate, salts on viscosity of orange peel pectin solutions

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Abstract

Anhydrogalacturonic acid, methoxyl, acetyl, ash contents and optical rotation of pectin obtained by a HCl extraction procedure (90°C, pH = 2.5, 90 min) were found to be 74.30, 12.15, 0.37, 6.07% (DM basis) and +252° respectively. The degree of methylation was found to be over 50%, indicating that the orange peel pectin had a high methoxyl content.

The reduced viscosities of orange peel pectin obtained by the HCl extraction procedure were measured in aqueous solutions and in the presence of dextrose, maltose and dextrin. Dextrose and maltose were found to increase the reduced viscosity of pectin solutions whereas dextrin decreased it. The viscosity-enhancing effect of dextrose and maltose can be interpreted in terms of the decrease in dielectric constant of the solvent, dehydration action, and hydrogen bonding formation. The specific fluidity of pectin solutions has been observed to be directly proportional to time through the depolymerization effect of L-ascorbic acid. When the concentrations of the pectin solutions were increased in the presence of ammonium persulfate, the increase in the ratio of reduced viscosity to initial reduced viscosity was higher. In addition, the effect of some salts on the viscosity of the pectin solution was examined. The viscosity of the pectin solutions decreased in the presence of salts. When the salt concentration increased, great decreases were observed in the low salt concentrations.

Keywords: Orange peel pectin; Anhydrogalacturonic acid; Viscosity

1. Introduction

The structure of pectic substances is based on a backbone of 1,4-linked α-D-galacturonic acid (or its methyl ester) interspersed by 2-linked L-rhamnosyl residues. Neutral sugars, as side-chains, are covalently linked to the rhamnogalacturonan via the C-3 of galacturonosyl and/or the C-4 of the rhamnosyl residues. Side-chain sugars are predominantly galactose and arabinose forming galactan, arabinan and arabinogalactan (Thibault, 1983).

Pectic substances are the major component of the middle lamella and of the primary cell walls of fruit tissues (Batisse, Fils-Lycaon, & Buret, 1994). Generally, citrus peels, apple pomace and sugar-beet pulp are good sources of high-methoxyl pectin (HMP) (Cohen, Sharon, Volman, Hoenig, & Saguy, 1984; Ma, Cervera, Sanchez, & Gaspar, 1993; Arslan & Toğrul, 1996; El-Nawawi & Heikal, 1996; Arslan & Kar, 1998). Orange peel has been reported to contain appreciable quantities (25% on a dry weight basis) of pectin (Akhtar & Uddin, 1971; Gregory, 1986).

Pectin is widely used in the food, cosmetic and drug industries, in confectionery, in jelly and jam making, and in medical treatments as a stabilizer and gelling agent (Koseki et al., 1986). Pectins are also used as stabilizers in the dairy industry, where the main function is to act as a protective hydrocolloid in complex with casein in low pH milk products (Gregory, 1986).

Viscosity is affected by molecular weight, degree of methylation, pH, and presence of counter-ions (Phatak, Chang, & Brown, 1988). Viscosity behavior also changes with concentration and temperature. Gelling is related to viscosity of pectin solutions. In view of the practical importance which oxidative–reductive depolymerizations may have in biological phenomena, it was of interest to investigate the influence of several autoxidants on the viscosity of several polysaccharides. The information on the viscosity is needed for a variety of engineering applications. The literature on the effect of sugars, autoxidants and oxidizing agents on the viscosity of the commercial and sugar beet pulp

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pectin solutions has been reviewed (Chen & Joslyn, 1967; Herp, Rickards, Matsumura, Jakosalem, & Pigman, 1967; Thibault & Rombouts, 1986), but the effect of the agents mentioned above and salts on viscosity of orange peel pectin solutions have not been studied.

The objectives of the this study is (1) to extract the pectin from the orange peel by the HCl extraction procedure; (2) to study the physicochemical properties of the orange peel pectin; and (3) to investigate the effect of different sugars, L-ascorbic acid, ammonium persulfate and some salts on the viscosity of orange peel pectin solutions.

2. Experimental

2.1. Extraction of pectin from orange peel

Washington oranges (citrus sinensis) used as the source of pectin were purchased from a local market and peeled (albedo plus flavedo). Sun dried orange peel was crushed, ground by a hammer mill to pass through a 50-mesh size screen, and stored at − 15°C until use. Before the orange peel was utilized for pectin production, the peel oil was removed by petroleum ether and dried. The peel was heated to 97°C in a water bath for about 10 min to inactivate the pectic enzymes, washed extensively with water to remove sugars and flavenoids, filtered through a suction filter, dried at 50°C and sieved. Dried orange peel (10 g) were mixed with 250 ml of 0.003 N HCl (pH: 2.5), preheated to 90°C in a water bath. The slurry was shaken in a thermostatted bath for 90 min at 90°C. After the extraction, the liquid phase was separated from the fruit mass by filtering. When it reached a temperature of about 37°C, the pH was brought to 7.5 by adding 0.1 M sodium phosphate buffer and 50 mg of protease enzyme (Sigma Chemical Co., P 5147) was added. The extract was incubated overnight at 37°C. The acid extract was separated by filtering and the pectin was precipitated with two volumes of ethanol. The gelatinous precipitate was filtered and dried at 50°C. The yield of raw pectin was determined gravimetrically. The word "pectin" stands for the pectin obtained in this study.

2.2. Chemical and physical determinations

The mixture was prepared in a ratio of 100 parts KBr powder per part of pectin obtained by the HCl extraction procedure. The mixture was pressed by means of a manually operated hydraulic press. The IR-spectrum of the pressed pellet was recorded (ATI UNICAM 1000 MATTSON Spectrometer) to find out the characteristic groups present in pectin and thereby to illuminate the orange peel pectin's structure.

The ash content was determined by incinerating the orange peel pectin overnight in a muffle furnace at 600°C (AOAC, 1984). The anhydrogalacturonic acid content of the orange peel pectin was determined by a colorimetric procedure using a *p*-hydroxydiphenyl color reagent (Blumenkrantz & Asboe-Hansen, 1973). The degree of methoxylation was determined by titration (McCready, 1970). Since the amount of methoxyl in 100% methoxylated pectin is 16.32%, the methoxyl percentage was calculated from the following equation (Gee, McComb, & McCready, 1958):

$$\text{Methoxyl\%} = (16.32/100) \times (\text{the degree of methylation}).$$

The reaction between the ester groups in pectin and alkaline hydroxylamine at room temperature produces pectin hydroxamic acid and acetohydroxamic acid. Pectin hydroxamic acid forms with ferric ions an insoluble red complex, whereas the acetohydroxamic acid produced from secondary acetyl groups forms a soluble red iron complex. These reactions, applied to pectic substances, serve as the basis for a specific and rapid colorimetric method for the determination of the acetyl amount (McComb & McCready, 1957).

Optical rotation was estimated on a polarimeter according to the procedure of McCready, Shepherd, Swenson, Erlandsen, and Maclay, (1951). Pectin samples (0.5%, w/v) were prepared in distilled water and centrifuged prior to measuring the optical rotation estimation at 25°C.

2.3. Viscosity determination of pectin solution

Dried pectin (0.25, 0.50, 1.0, 1.5 and 2.0 g) were dissolved in 100 ml of 0.1 M sodium phosphate (pH: 7.0). An Ubbelohde type viscosimeter (capillary no: Ic, ID: 0.84 mm) with a k constant of 0.03005 was used in the viscosity measurement performed at 20°C. A pycnometer was used to determine the density of the pectin solutions. All of the experiments were replicated and the average values were taken. Two consecutive measurements had a reproducibility of about 0.1% for viscosity.

The time for the sample to flow from one level indicator to another, known as flow time, was measured and converted to kinematic viscosity using the viscometer constant provided by the manufacturer for each viscometer.

Kinematic, relative, specific and reduced viscosity and specific fluidity were calculated by the following equations:

$$\text{Kinematic viscosity} = \nu = (t - \theta)$$

$$\text{Relative viscosity} = \eta_r = \eta/\eta_0 = \nu\rho/\nu_0\rho_0$$

$$\text{Specific viscosity} = \eta_{sp} = \eta_r - 1$$

$$\text{Reduced viscosity} = \eta_{red} = \eta_{sp}/c$$

$$\text{Specific fluidity} = 1/\eta_{sp}$$

where k is the viscosity constant, t the flow time of pectin solutions (s), θ the Hagenbach correction coefficient, η_0 the viscosity of solvent (mPa s), ν_0 the kinematic viscosity of solvent ($m^2 s^{-1}$), ρ the density of pectin solutions (kg m^{-3}) and ρ_0 the density of solvent (kg m^{-3}).

In the range of the dilute solutions (<1%) the difference

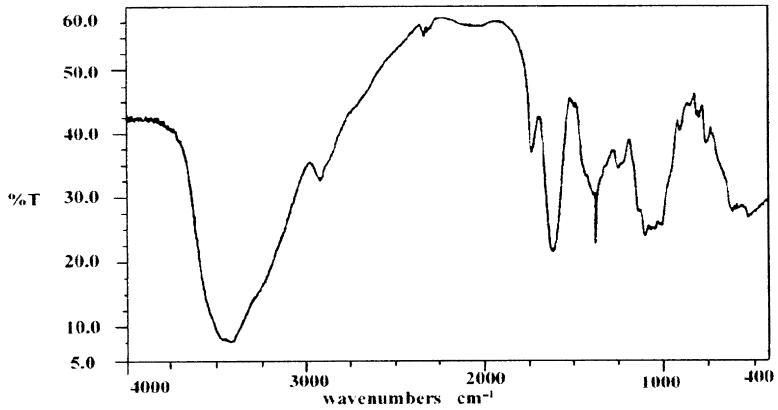

Fig. 1. The infrared spectrum of pectin produced by HCl extraction procedure.

in densities between the solution and solvent was insignificant, and density corrections were neglected.

2.4. Effect of sugars on viscosity of pectin solutions

To study the effect of sugars such as dextrose, maltose and dextrin on the viscosity of pectin solutions, all the sugars were dried at 70°C for 24 h before use in the experiments. Pectin was dissolved in a 0.1 M sodium phosphate buffer (pH = 7.0) and the pectin solutions were mixed with sugars to give solutions with the sugar content ranging from 0 to 50 g/100 g solution and with final concentrations of 0.25×10^{-2}, 0.5×10^{-2}, 1.0×10^{-2}, 1.5×10^{-2} and 2.0×10^{-2} kg/l pectin, in 0.1 M sodium phosphate buffer. The complete solution of the sugars at high concentrations required constant stirring and standing up to 2 days. Reduced viscosity was calculated by means of flow times of solution and solvent.

2.5. Depolymerization effect of L-ascorbic acid on viscosity of pectin solutions

For the study of the effect of autoxidants on the viscosity of pectin solutions, pectin was dissolved in a 0.1 M sodium phosphate buffer (pH = 7.0) and the pectin solutions were mixed with L-ascorbic acid to give solutions with final concentrations of 0.33 and 3.3 mM L-ascorbic acid and of 0.25×10^{-2}, 0.5×10^{-2}, 1.0×10^{-2}, 1.5×10^{-2} and 2.0×10^{-2} kg/l pectin, in a 0.1 M sodium phosphate buffer. Control solutions consisted of the same concentrations of pectin in the 0.1 M sodium phosphate buffer in the absence of L-ascorbic acid. For viscosity measurements, 15 ml of the final mixture was introduced into viscometers kept in a water-bath at 20 ± 0.01°C. The viscosities were measured at frequent time intervals over a period of 2 h and specific fluidity ($1/\eta_{sp}$) values were calculated. In addition, the specific viscosities were calculated from the viscosities measured after 2 h.

2.6. Effect of ammonium persulfate on viscosity of pectin solutions

Fifteen milliliters of the mixture containing 0.5×10^{-2} kg/l pectin solutions in 10, 15, 20 and 40 mM $(NH_4)_2S_2O_8$ and 0.25×10^{-2}, 0.5×10^{-2} and 1.0×10^{-2} kg/l pectin solutions in 10 mM $(NH_4)_2S_2O_8$ prepared at 20°C were introduced into the viscometer and the flow-times were recorded as a function of the time of reaction. Reduced viscosity was calculated and the results are expressed as the ratios of the reduced viscosity at time t to the initial reduced viscosity.

2.7. Effect of salts on viscosity of pectin solutions

In order to study the salt effect on viscosity, three salts (NaCl, Na_2SO_4 and Na_3PO_4) having anions in different valencies were added to pectin solution (with 0.25×10^{-2} kg/l concentration) to give solutions with the final concentrations of 0.5, 1.0, 1.5 and 2.0% salts.

3. Results and discussion

The IR-spectrum gives very typical peaks for a number of special groups. The IR-spectrum of pectin obtained by the HCl extraction procedure is given Fig. 1. The ample peak at 3200–3600 cm^{-1} shows that there are too many OH groups in the pectin molecule. The characteristic peak at 1625 cm^{-1} is the –O– tensile vibration band neighboring the H group. The peak at 1410 cm^{-1} is the C–O–H in plane bending vibration band. There is a very weak C–O tensile vibration band at 1280 cm^{-1}. The peak at 1245 cm^{-1} is asymmetric C–O–C tensile vibration band, and it indicates the abundance of $-O-CH_3$ (methoxyl) groups. That the strong peak at 1040 cm^{-1} is the symmetric C–O–C tensile vibration band supports this view. The peak at approximately 1735 cm^{-1} is the C=O tensile vibration band, and it indicates the acetyl ($COCH_3$) groups in pectin.

By understanding the physicochemical properties of pectin extracted from orange peels, food technologists

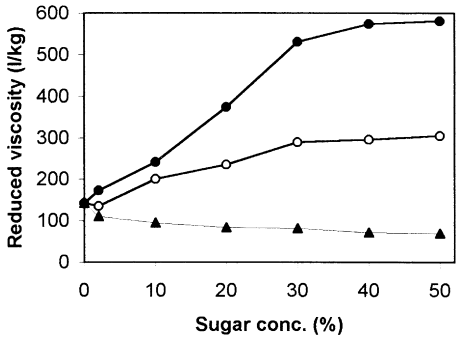

Fig. 2. Effect of sugar concentration on reduced viscosity of 0.5×10^{-2} kg/l pectin solution (●, dextrose; ○, maltose; ▲, dextrin).

would be able to better use orange peel pectin in food product development and processing. The higher galacturonic acid and the lower ash contents of pectin are the two criteria for its purity. Ash content affects the ability of pectin to gel (Miyamoto & Chang, 1992). Since anhydrogalacturonic acid (AGA) is the fundamental unit, or the backbone of pectins, its quantification is a primary method used to determine the pectin content in a sample. The degree of esterification (DE) is a key factor to determine conformation and rheological properties of pectins (Hwang, Roshdy, Kontominas, & Kokini, 1992). The physicochemical properties of pectin, as related to its function as food fiber, cell wall component in plants, and thickening agents in foods, are determined to a great extent by the degree of methylation of carboxylic acid groups (Barford, Magidman, Philips, & Fishman, 1986). A chemical characterization of pectic substances from a particular source material requires a determination of acetyl as well as determination of the methoxyl content because the poor gelling properties of pectin is related to its acetyl content.

Anhydrogalacturonic acid, methoxyl, acetyl and ash contents of orange peel pectin obtained by acid extraction and ethyl alcohol precipitation were found to be 74.30, 12.15, 0.37 and 6.07% (DM basis) respectively.

The methoxyl content was equivalent to the esterification degree of 74.46%. The degree of methylation in the sample analyzed was found to be over 50%, which indicates that the orange peel pectin was the high-methoxyl and rapid set pectin. Orange peel pectin can be used for jam and jelly manufacturing because the ability of pectin to gel depends largely on the degree of methylation. High methoxyl pectins form gels if the pH is below about 3.6 and if a cosolute is present, typically sucrose at a concentration of greater than 55% by weight (Oakenful & Scott, 1984). Acetyl groups inhibit gel formation of both high and low methoxyl pectins by steric hindrance of chain association (Rombouts & Thibault, 1986). Optical rotation of pectin was +252°, reflecting the characteristics of sugar compositions.

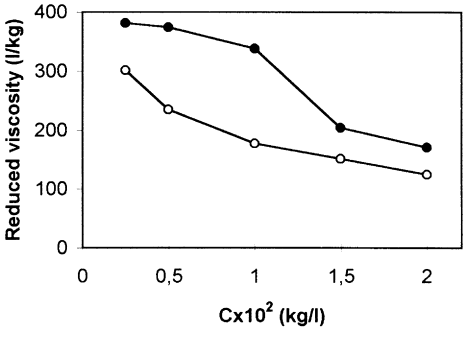

Fig. 3. Changes in reduced viscosity with concentration of pectin in the presence of 20% dextrose and maltose (●, dextrose; ○, maltose).

3.1. Effect of sugars, L-ascorbic acid, ammonium persulfate and salts on viscosity of pectin solutions

The effect of variations in sugar concentrations on the reduced viscosities of 0.5×10^{-2} kg/l pectin solution is given in Fig. 2. Dextrose and maltose increased the reduced viscosity of pectin, and this effect increased with increasing sugar concentration up to 30% dextrose and maltose concentrations. At higher sugar concentration, it decreased. In contrast, dextrin had a negative effect on the reduced viscosity of pectin solution. The maximum viscosity-depressing effect of dextrin on pectin solution seems to occur at 20% dextrin concentration, and further increase in dextrin concentration apparently had no additional effect (Fig. 2). The viscosity-depressing effect of dextrin on the reduced viscosity of the pectin could be related to the presence of ionic impurities in the dextrin preparations.

Pectin is a flexible linear polyelectrolyte consisting of galacturonic acid monomer units with some of the carboxyl groups esterified. The increase in the reduced viscosity on dilution of a salt-free polyelectrolyte solution has been attributed to the decreased screening of the fixed charges by the counter ions and the consequent coil expansion due to the mutual repulsion between the increasing number of like charges attached to the coil. The viscosity-enhancing properties of dextrose and maltose is caused by the aggregate formation of pectin molecules due either to polyhydroxyl combinations brought about by the formation of cross-linking hydrogen bonds between the hydroxyl groups of sugars and those of the pectin, or to the dehydrating action of sugars to prevent the water from participating in hydrogen bonding with pectin so that the pectin molecules could form cross-linked bonds. The reason why dextrose had a greater effect on pectin viscosity than maltose is that dextrose contains more primary alcohol groups per unit weight than maltose, and it is known that the reactivity of the primary alcohol group is greater than that of a secondary alcohol group (Chen & Joslyn, 1967).

To study the viscosity-enhancing effect of dextrose and maltose on viscosity of pectin solutions having different concentrations, the variations of reduced viscosity with pectin concentration were measured in 20% dextrose and

Table 1
Rate of increase in specific fluidity of pectin by L-ascorbic acid

Ascorbic acid concentration (mM)	Pectin concentration (%)					
	Time (min)	0.25	0.5	1.0	1.5	2.0
0.33	0	2.22	1.41	0.56	0.18	0.06
	30	2.33	1.59	0.76	0.26	0.09
	60	2.54	1.75	0.88	0.34	0.12
	90	2.75	1.88	1.10	0.43	0.15
	120	2.85	2.04	1.23	0.52	0.18
3.3	0	2.22	1.41	0.56	0.18	0.06
	30	2.41	1.68	0.78	0.28	0.10
	60	2.70	1.92	0.98	0.37	0.14
	90	2.95	2.13	1.21	0.46	0.17
	120	3.11	2.35	1.34	0.56	0.21

maltose solutions (Fig. 3). It was observed that the reduced viscosity of the pectin solutions decreased with increase in pectin concentrations in the presence of 20% dextrose and maltose.

Table 1 shows the rate of increase in specific fluidity of pectin solutions having different concentrations in the presence of 0.33 and 3.3 mM L-ascorbic acid.

Depolymerization by L-ascorbic acid resulted in a linear increase in specific fluidity with time. The specific fluidity increased with increase in L-ascorbic acid concentration. The higher concentration of L-ascorbic acid produced the greater increase in specific fluidity. The depolymerization of the pectin produced by the autoxidant was calculated as percentage reduction in specific viscosity after 2 h compared to that of the control solution. For example, the specific viscosity of the presence of 0.33 mM L-ascorbic acid, whereas it decreased by 66.7% in the presence of 3.3 mM L-ascorbic acid. The higher the concentration of ascorbic acid, the greater the decrease in specific viscosity. L-ascorbic acid was a strong degrading agent of pectin because it markedly reduced the viscosity of pectin.

The reduced viscosity values of 0.25×10^{-2}, 0.5×10^{-2} and 1.0×10^{-2} kg/l pectin solutions were found to be 180, 142 and 178 l/kg respectively. Variations of the reduced viscosity with time when pectin solutions having different concentrations was treated with 10 mM ammonium persulfate are shown in Fig. 4. The extent of the reaction with 10 mM ammonium persulfate was monitored by viscometry. Increases in reduced viscosity/initial reduced viscosity of pectin solutions of 0.25×10^{-2}, 0.5×10^{-2} and 1.0×10^{-2} kg/l in 10 mM $(NH_4)_2S_2O_8$ after an induction period of 8 h were 160, 320 and 420% respectively. When the

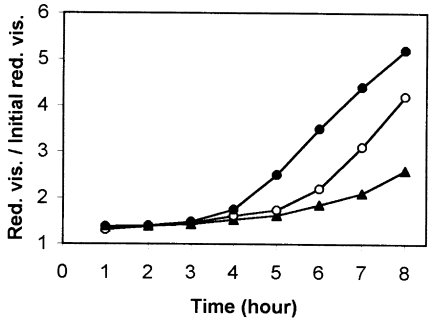

Fig. 4. Changes with time of the ratio of reduced viscosity to initial reduced viscosity of pectin solution at different concentrations in 10 mM ammonium persulfate (pectin concentration (%): ▲, 0.25; ○, 0.50; and ●, 1.0).

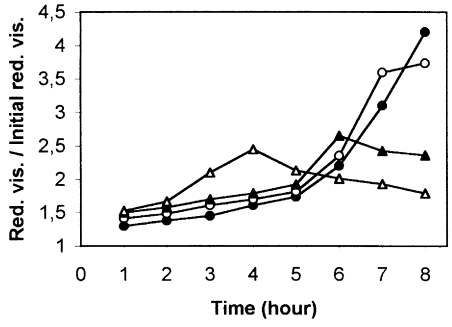

Fig. 5. Changes with time of the ratio of reduced viscosity to initial reduced viscosity of a 0.5×10^{-2} kg/l pectin solution in ammonium persulfate (ammonium persulfate concentration (mM): ●, 10; ○, 15; ▲, 20; and △, 40).

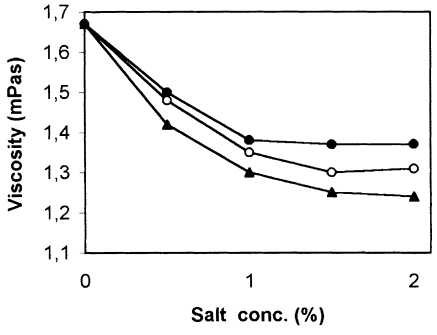

Fig. 6. Effect of salts on viscosity of a 0.25×10^{-2} kg/l pectin solution (●, NaCl; ○, Na_2SO_4; and ▲, Na_3PO_4).

concentration of pectin was 0.25×10^{-2} kg/l, only a slight increase in viscosity was observed after an induction period of 3 h. On increasing the concentration, the rate of the increase in viscosity increased. The maximum value of the reduced viscosity of a 1.0×10^{-2} kg/l solution of pectin was 926 l/kg.

The influence of the ammonium persulfate concentration on reduced viscosity of 0.5×10^{-2} kg/l pectin solutions is given Fig. 5. The maximum values of the reduced viscosity were obtained after 8, 8, 6 and 4 h for ammonium persulfate concentrations of 10, 15, 20 and 40 mM, respectively, but these values were lowered by the increase in the concentrations of ammonium persulfate (for 20 and 40 mM) and the reduced viscosities decreased more rapidly above these maxima.

The effect of some salts on the viscosity of the pectin solution is shown in Fig. 6. From the curves in Fig. 6, it was seen that the salts affected the viscosity in the same way. While the concentration increased, great decreases in dilute concentrations was attributed to the dissociation of the salts in large amounts causing remarkable decreases in electroviscous effect. It was considered that the decreases in the slopes of the curves in the higher salt concentrations might be due to the decrease in the dissociation of the salts in high concentrations and to the decrease in the tendency of the particles to adsorb oppositely charged ions because of the approach of zeta potentials to zero. It is known that the addition of salts in larger amounts can also cause charge reversal.

In conclusion, the formation of aggregates between the pectin molecules due to dehydration action and the hydrogen bond formation reaction of the sugars such as dextrose and maltose cause the increase in viscosity of pectin solutions. Orange peel pectin degrades by the depolymerization effect of L-ascorbic acid and thereby the viscosity of pectin solutions decreases. The autoxidation by reducing agents such as L-ascorbic acid in aqueous media is generally considered to be a free radical reaction. The free radicals produced is thought to be responsible for the depolymerization effect. The depolymerization reaction involves the abstraction of a hydrogen atom by the OH free radical, the attacking agent, and subsequent β-elimination. The presence of the carboxyl ion or ester would assist the elimination by its tendency to attract electrons from the neighboring C–C linkage. The attack would be at C-5 for (1 → 4)-linked materials, and a carboxyl group at C-6 might be expected to assist the elimination (Herp et al., 1967). Ammonium persulfate is known to generate free radicals and to cause the coupling reaction which may cross-link feruloyl groups linked to the side chains of the rhamnogalacturonan backbone (Thibault & Rombouts, 1986).The cross-linking of sulfate radicals produced by the decomposition of persulfate ions with feruloyl groups in pectin increases the molecular weight of pectin molecule, and thereby the viscosity. The persulfate ion decomposes in aqueous solution to give sulfate radicals, which probably leads to changes in ionic strength and pH, and consequently in viscosity.

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