

<연구논문>

펙틴 다당류의 측쇄가 점탄성에 미치는 영향

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The Branching Effects of Pectic Polysaccharides on Viscoelastic Properties

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요 약

중량비로 측쇄(side branches)의 함량이 각각 4.37%(시료 I)와 8.97%(시료 II)인 두 종류의 사과 펙틴을 이용하여 측쇄가 펙틴용액의 점탄성에 미치는 역할에 대하여 연구하였다. 시료 II가 시료 I보다 동일한 농도에서 더 높은 zero-shear viscosity(η_0) 값을 보였으며, 또한 전체적으로 점도의 전단속도 의존성이 큰 경향을 보여 높은 전단속도에서는 오히려 시료 I의 전단점도가 시료 II보다 높은 값을 보였다. 시료 I과 시료 II의 η_0 는 농도에 대하여 각각 $\eta_0 \propto C^{4.04}$, $\eta_0 \propto C^{4.23}$ 의 관계를 보여 시료 II가 더 높은 농도의존성을 보였다. 한편, 용액의 탄성특성을 나타내는 저장탄성계수(G')와 zero-shear recoverable compliance(J_e^0)의 경우에는 시료 II가 시료 I보다 높은 값을 보였는데, 이는 측쇄가 탄성과 밀접한 관계가 있다는 것을 의미한다. 펙틴용액의 전단점도(shear viscosity; η)와 복소점도(complex viscosity: η^*)를 비교했을 때 거의 비슷한 값을 보였으나, 예외적으로 고측쇄의 시료 II의 경우 고농도에서 서로 상이한 값을 보였다. 본 연구의 결과는 진한 용액하에서 측쇄가 분자간의 상호영킴(intermolecular entanglements)에 상당한 기여를 하고 있음을 나타내었다.

Abstract—Pectins with the varying branching degrees were prepared by two different metal precipitation techniques, i.e., copper precipitation of polygalacturonic pectins followed by either 1% acid alcohol treatment (lower branched due to acid action: 4.37%) or EDTA treatment (higher branched: 8.97%). Increasing side branches resulted in higher zero-shear viscosity (η_0) and increasing shear rate dependence of viscosity in steady shear measurements at the same concentration. The more branched pectins showed $\eta_0 \propto C^{4.04}$, and the less branched one $\eta_0 \propto C^{4.23}$, indicating the more concentration dependence of higher branched pectins. The small amplitude oscillatory measurements revealed that the storage modulus (G') representing the elastic properties was higher for pectin samples with higher branching degrees, and the zero-shear recoverable compliance (J_e^0), calculated from $\eta_0 J_e^0 \dot{\gamma}_{0.8} \sim 0.6$, showed the same trend. This indicates the positive contribution of side branches to the elastic properties of pectins. Comparison of steady shear viscosity (η) and dynamic complex viscosity (η^*) exhibited a good superposition except the highly branched pectin samples at high concentration. The results observed in this research strongly support the fact that side branches of pectins are involved in intermolecular entanglements in concentrated solutions.

Keywords : Biopolymer rheology, pectin, branch, viscoelasticity

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1. Introduction

Branching is an important factor affecting rheological properties of synthetic polymers [1, 2]. The typical rheological properties influenced by the presence of side branches are intrinsic viscosity, zero-shear viscosity, shear rate dependence of viscosity, zero-shear recoverable compliance, extensional viscosity, thermal sensitivity of viscosity, and so on [3]. In these cases, significant intermolecular entanglements through the branches in concentrated system act as the primary factor conferring unique rheological properties of branched synthetic properties [4-6].

The presence of side branches also greatly affect a variety of functional properties of carbohydrate polymers such as solubility, gelatinization, gelling, retrogradation, film formation, freeze-thaw stability and polymer-polymer interaction properties [3, 7]. The topological role of side branches, preventing intermolecular association of polymers, was considered to be the major mechanism determining these functional properties [8].

Pectin, present in the primary cell wall and middle lamellae of plant cell walls, is a complex carbohydrate polymer which is involved in the evolution of firmness and cohesiveness of the tissues of fruits and vegetables during maturation and ripening as well as in the texture and consistency of processed products [9, 10]. Industrially, pectins are extracted from fruits processing by-products, i.e., apple pomace and citrus peel, by using hot acid solution (pH 1.5~2.5, temperature 80~95°C) [11]. In food systems such as jam, jelly and dairy products, pectins are widely employed as gelling, stabilizing and thickening agents [12, 13].

Pectin consists of a linear α -(1,4)-linked D-galacturonic backbone, in which varying proportions of the galacturonic carboxyl groups are present as the methyl esters, and (1,2)-linked L-rhamnosyl residues are inserted at intervals [14]. The side branches,

consisting mainly of D-galactose, L-arabinose, D-xylose, and less frequently D-mannose, L-fucose, D-glucose, are covalently attached to the main backbone primarily through C-4 of the rhamnosyl residues, although substitution of the galacturonyl residues at either C-2 or C-3 is also found [15, 16]. Most of these sugars occur in short chains, but the more common neutral sugar constituents, L-arabinose and D-galactose, are also found in multiple units, i.e., arabinan, galactan and arabinogalactan of varying length [17]. It has been reported that the side branches of pectins amount to approximately 5~50% depending on the plant sources and the extraction methods [18]. Therefore, significant dimension and heterogeneity are two key factors of side branches of pectins.

The contribution of side branches to rheological properties of carbohydrate polymers was investigated first by Hwang and Kokini [19] using apple pectins which naturally possess significant branching size. It was found that side branches of pectins greatly influenced steady shear rheological properties such as zero-shear viscosity, shear rate dependence of viscosity and various rheological superposition patterns. The results suggested that side branches of pectins exist as significantly entangled states in concentrated solutions, based on the rheological theories developed in synthetic polymers.

Besides steady shear properties, branching can also affect elastic properties of polymers, and the positive relation between branching degrees and elasticity were reported previously for synthetic polymers [20-22]. However, no report has been issued to date for the branching role in the elastic properties of carbohydrate polymers. Therefore, in this research special emphasis will be placed on the branching effects on dynamic viscoelastic properties of pectins in conjunction with some complementary studies on steady rheological properties following our previous report [19].

2. Experimental

2.1. Preparation of Pectins with Different Degrees of Side Branches

Apple pectins of commerce were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Preparation of pectin samples having different degrees of side branches was performed by two different metal precipitation techniques, i.e., Cu(II) precipitation of polygalacturonic pectins followed by 1% acid alcohol treatment (lower branched due to the acid action on the neutral sugar side branches) or Cu(II) precipitation followed by EDTA treatment (higher branched). The detailed procedures can be found elsewhere [23]. These two pectin samples will be referred to as sample I and sample II. It was found that sample II possessed the side branches twice as much as sample I, i.e., 8.97% and 4.37% in weight, respectively [23].

2.2. Rheological Measurements

Steady shear viscosities were measured using Rheometrics Fluids Spectrometer (Rheometrics, Piscataway, NJ) over the shear rate range of 0.01~100 sec^{-1} . The cone and plate geometry was employed with a cone angle of 0.0196 radian and a plate radius of 25 mm. The gap between the cone and plate geometry was set at 50 microns.

Small amplitude oscillatory measurements were conducted under the same conditions as the steady measurements. Strain sweeps showed that all pectin solutions were linear in the 0~100% strain range, and thus a 40% strain was selected as being optimal for the best resolution of the stress signal. Storage modulus (G'), which is the elastic component, and loss modulus (G''), which is the viscous component, were measured in the frequency (ω) of 0.1~100 rad/s. From G' and G'' measurements, the complex viscosity can be obtained as follows :

$$\eta^* = \frac{[(G')^2 + (G'')^2]^{1/2}}{\omega} \quad (1)$$

Table 1. The zero-shear viscosity (η_0) and $\dot{\gamma}_{0.8}$ of apple pectin samples I and II as a function of concentration

C (% w/v)	I		II	
	η_0 (poise)	$\dot{\gamma}_{0.8}^*$ (1/s)	η_0 (poise)	$\dot{\gamma}_{0.8}$ (1/s)
6.0	50.8	24.2	63.3	0.5
5.0	19.5	63.3	25.7	0.8
4.0	6.4	210.9	8.0	3.3
3.0	2.1	463.2	2.5	10.6
2.0	0.6	NA**	0.6	119.8

* Shear rate at which $\eta = 0.8\eta_0$

** Not available

For steady and dynamic measurements, appropriate concentrations of pectin samples were first moisturized in 50 mM citrate phosphate buffer (pH 4.5) for 10 min, which were then completely dissolved by using the magnetic stirrer for 2 h at room temperature. Prior to measurements the resulting pectin solutions were sat for 30 min to get rid of foams. The sample sizes for the experiments were kept constant at 1.0 ml. All measurements were conducted at 25°C.

3. Results and Discussion

3.1. Steady Shear Viscosity

Based on our previous report [19], the general steady shear rheological characteristics are summarized in Table 1. It can be seen that the more branched sample II gives the higher zero-shear viscosity (η_0), but the lower $\dot{\gamma}_{0.8}$ than the less branched sample I. This indicates that increased branching degree resulted in the more shear rate dependence of viscosity. The more shearing dependence of intermolecular entanglements induced by the branches was also well recognized for synthetic polymers [1, 2, 24].

The concentration dependence of η_0 can be expressed in terms of the power-law type equation as follows [25] :

$$\eta_0 = k_1 C^a \quad (2)$$

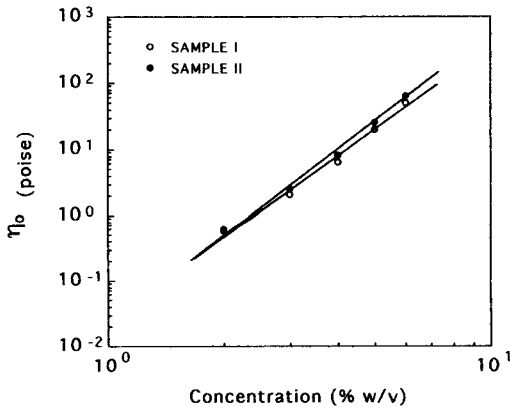


Fig. 1. Zero-shear viscosity (η_0) vs. concentration of apple pectin samples I and II.

where k_1 is the constant, C is the pectin concentration, and a is the exponent determining the concentration dependence of η_0 . Fig. 1 shows the double logarithmic plot of η_0 vs. concentration, where the slopes represent the a values of Eq. (1). The a values for pectin samples I and II were 4.04 and 4.23, respectively. These values are in good agreement with the previous works on other random-coil polysaccharides. However, they are higher than $\eta_0 \propto C^{3.75}$ predicted by DeGennes [26] for linear polymers, based on the interactions through purely topological entanglements. The slope of sample II is higher, despite its lower hydrodynamic intrinsic viscosity [19], than that of sample I. This is because more branched structure of sample II resulted in significant entanglements through the branches. This agrees well with the reports for synthetic polymers [27, 28].

Depending on the shear rate, the concentration dependence of viscosity can be generally expressed as follows :

$$\eta_{app} = k_2 C^b \tag{3}$$

where η_{app} is the apparent viscosity, k_2 is the constant, C is the concentration, and b is the constant which is calculated from the slope of a double logarithmic plot of η_{app} vs. concentration. Fig. 2 shows the concentration dependence of apparent viscosity

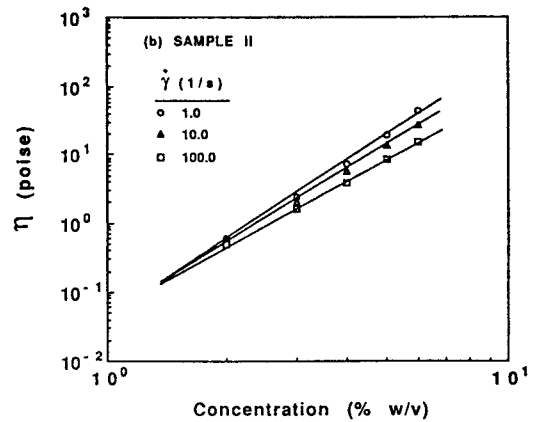
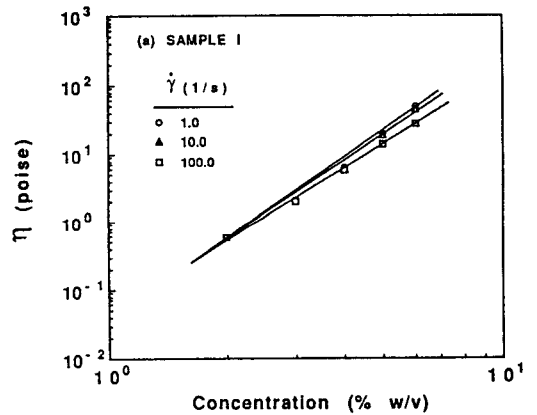


Fig. 2. Viscosity (η) vs. concentration of apple pectin (a) sample I and (b) sample II as a function of shear rate.

(η_{app}) for samples I and II at three different shear rates ($\dot{\gamma}=1.0, 10.0$ and 100.0 sec^{-1}). It is observed for both samples that the concentration dependence of η is more pronounced for lower shear rate values. It is also evident that sample II shows more significant decrease in slopes with increasing shear rates than sample I. The slopes of η_0 vs. concentration (Fig. 1) and η_{app} vs. concentration (Fig. 2) at different shear rates are summarized in Table 2. The slopes decreased with increasing shear rates for both pectin samples. This is because the polymer molecules are more and more oriented and disentangled by the shearing forces as the shear rate is increased [29]. It can be also seen that the slopes

Table 2. The slopes of zero-shear viscosity (η_0) vs. concentration and apparent viscosity (η_{app}) vs. concentration at different shear rates for apple pectin samples I and II*

Samples	<i>a</i>	<i>b</i>		
		$\dot{\gamma} = 1.0$	$\dot{\gamma} = 10.0$	$\dot{\gamma} = 100.0$
I	4.04	4.04	3.96	3.55
	(0.993)**	(0.993)	(0.995)	(0.998)
II	4.23	3.89	3.53	3.12
	(0.996)	(0.998)	(0.999)	(0.999)

* $\eta_0 \propto C^a$, $\eta_{app} \propto C^b$

** Correlation coefficients (R^2)

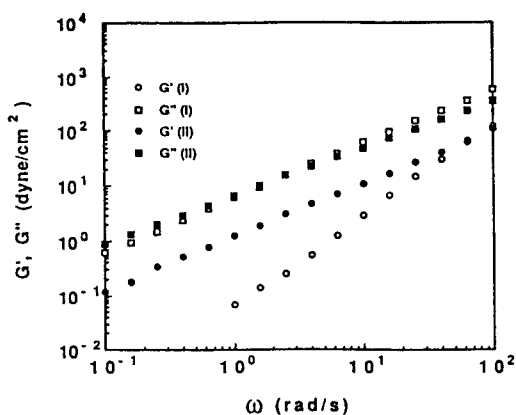


Fig. 3. Dynamic storage modulus (G') and loss modulus (G'') vs. ω of 4% apple pectin samples I and II.

of apple sample I range 4.04~3.55, while those of apple sample II 4.23~3.12, depending on shear rates. These results reflect that the non-covalently crosslinked intermolecular associations through the branches of sample II are more readily disentangled by the imposed shear forces. This is in good accordance with the report of Graessley [2] and Jacovic et al. [24] for synthetic polymers.

3.2. Dynamic Viscoelasticity

The storage modulus (G') and the loss modulus (G'') of apple pectin solutions were determined in the range of 2~6% pectin concentration, and a typical G' and G'' vs. frequency (ω) profile at 4% is plotted in Fig. 3. Both G' and G'' increase with in-

Table 3. The slopes of G' vs. ω and G'' vs. ω of apple pectins as a function of concentration*

C	I		II	
	α	β	α	β
6.0**	1.61	0.97	0.89	0.80
5.0	1.64	0.98	0.91	0.83
4.0	1.66	0.99	0.97	0.86
3.0	1.69	0.99	1.03	0.91
2.0	1.75	1.00	1.24	0.94

* $G' \propto \omega^\alpha$, $G'' \propto \omega^\beta$ at $0.1 \leq \omega \leq 100.0$ (rad/s) except 6%; ** $0.1 \leq \omega \leq 10.0$ (rad/s)

creasing pectin concentrations. This is consistent with the arguments of Nielsen [30] and Barnes et al. [31] that increasing intermolecular entanglements enhance not only the viscosity but also the elasticity. G'' is larger than G' at the same concentrations studied for both samples, indicating the predominant viscoelastic liquid-like behavior of pectin solutions. It is also clearly observed that G' of the more branched pectin sample II is higher than that of the less branched sample I. This reflects the positive contribution of side branches to the elasticity of pectin solutions. These observations consist with the report of Cogswell [1] and Han [32], showing that branched low-density polyethylene (LDPE) exhibited higher elasticity than linear high-density polyethylene (HDPE).

The frequency (ω) dependence of G' and G'' can be expressed in terms of a power-law formula as follows :

$$G' \propto \omega^\alpha \quad (4)$$

and

$$G'' \propto \omega^\beta \quad (5)$$

The constants α and β are the slopes of the log-log plots of G' vs. ω and G'' vs. ω , respectively. The results for α and β are presented in Table 3 as a function of concentration. The data show that at the same concentration pectin sample II gives the lower α and β values than sample I. This reflects that intermolecular entanglements of side branches re-

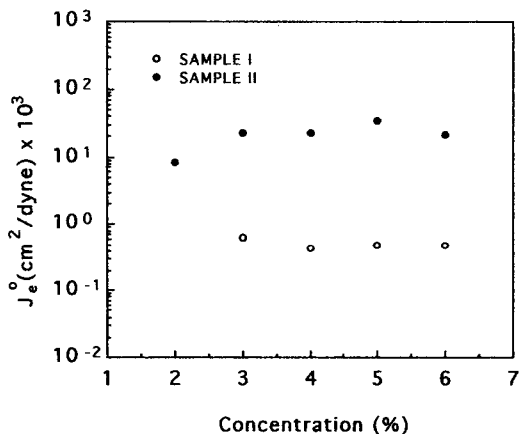


Fig. 4. Zero-shear recoverable compliance (J_e^0) vs. concentration of apple samples I and II.

sult in the lower frequency dependence of G' and G'' . Table 3 also demonstrates that α values between samples I and II are much more different than β values. This indicates that the branching effects are more clearly manifested on the elastic properties.

The zero-shear recoverable compliance (J_e^0), representing the elastic coil that occurs when the external forces producing the steady state flow are suddenly removed [5], has long been considered as a useful parameter of fluid elasticity [32, 33]. In reality, J_e^0 can be determined by the steady shear measurements [34]. Roovers [35] demonstrated that multiplication of η_0 , J_e^0 and $\dot{\gamma}_{0.8}$ was approximately constant, i.e., $\eta_0 J_e^0 \dot{\gamma}_{0.8} \sim 0.6$, regardless of concentration and branching degrees. Using the data represented in Table 1, J_e^0 of apple pectins were calculated and then plotted vs. concentration in Fig. 4. It can be seen that the more branched sample II shows the higher J_e^0 when compared with the less branched sample I. This is consistent with the results for dynamic measurements that more branched sample II shows the higher elastic modulus (G') than sample I as shown in Fig. 3.

3.3. Comparison of Steady Shear and Dynamic Complex Viscosity

For many polymer solutions the steady shear vi-

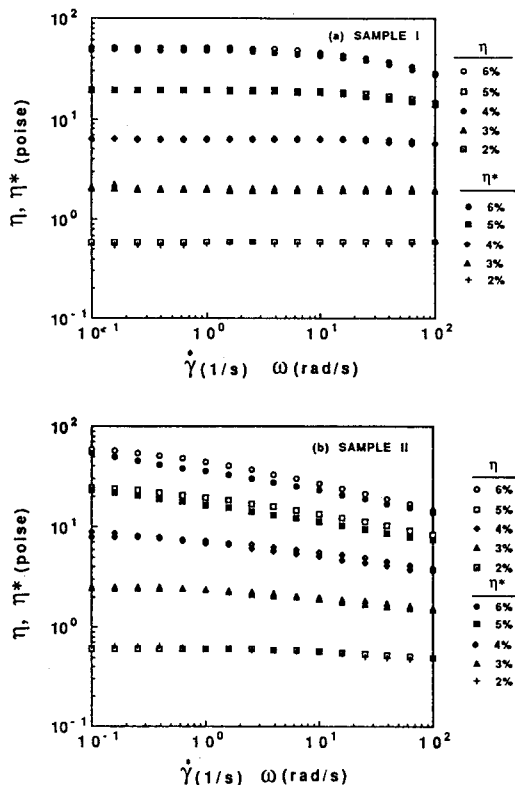


Fig. 5. Comparison of steady shear viscosity (η) and dynamic viscosity (η^*) of apple pectin (a) sample I and (b) sample II.

scosity (η) is very closely superimposed to the dynamic complex viscosity (η^*) when they are compared at the same values of $\dot{\gamma}$ and ω [36].

$$\eta(\dot{\gamma}) \approx \eta^*(\omega) \quad \text{at } \dot{\gamma} = \omega \quad (6)$$

This empirical correlation is referred to as the Cox-Merz rule [36], which holds for the vast majority of disordered polysaccharides [37] and synthetic polymers [34].

The relations of η vs. $\dot{\gamma}$ and η^* vs. ω are compared in Fig. 5. It is noted that η and η^* are almost identical with an exception of apple sample II at high concentrations. Intermolecular entanglements through the branches of sample II may be the source for this abnormality, which is brought about by structural destruction of intermolecular entanglements under steady shear force.

4. Conclusion

Branching is a key factor affecting rheological and other physicochemical properties of synthetic and carbohydrate polymers. In this research, we showed the significant contribution of side branches to the viscoelastic properties of pectins. In reality, gelling is the most important attribute for the practical application of pectins, where the elastic properties determine the overall quality of pectin gels in foods such as jams and jellies. Thus, clear understanding on the contribution of side branches to the elasticity would be valuable to understand and design the gelling systems constituted by pectins.

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