



# Calcium binding and calcium-induced gelation of normal low-methoxyl pectin modified by low molecular-weight polyuronate fraction



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## ABSTRACT

Functions of low molecular-weight polyuronate fractions in the calcium binding and calcium-induced gelation of normal low-methoxyl pectin (LMP) were investigated. The fractions from pectin with two different degrees of esterification (DE) and also from alginate with two different mannuronate/guluronate (M/G) ratios were prepared within the weight average molecular-weight range from ca. 40–65 kg/mol. In the mixtures of LMP and each polyuronate fraction, changes in the relative viscosity ( $\eta_r$ ) of dilute solutions and in rheological properties of gels were examined in the presence of calcium. Different results were indicated between the pectin and the alginate fractions. The addition of the pectin fractions, regardless of its DE, increased  $\eta_r$  of dilute solutions and increased dynamic storage modulus ( $G'$ ) of gels with showing greater effects at the lower DE. On the contrary, the addition of the alginate fractions, regardless of its M/G ratio, increased the critical threshold concentration of calcium required for the increase in  $\eta_r$  of dilute solutions and decreased  $G'$  of gels with showing greater effects at the lower M/G ratio (i.e. guluronate rich). Based on the results, molecular associations of the mixtures were presented schematically to compare the functions between the pectin and the alginate fractions.

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## 1. Introduction

Pectin is widely used in the food industry based on its gelling, thickening, and stabilizing properties (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). As common features of the pectin molecules (Voragen et al., 1995), the backbone consists of  $\alpha$ -(1–4)-linked D-galacturonic acid units interrupted by the insertion of (1–2)-linked L-rhamnopyranosyl residue in adjacent or alternate positions; whereas the side chains consist mainly of D-galactose and L-arabinose as found in galactan, arabinogalactan, and arabinan with a considerable chain length, linked glycosidically to O4 and/or O3 of

the L-rhamnopyranose. As an anionic polysaccharide with the polyuronate backbone, calcium-binding behaviors of pectin have been investigated extensively as in the case of alginate. For pectin, it has been reported that degree of esterification and weight average molecular-weight both influence the pectin gelation in terms of the gel strength and the kinetics of gel formation, and these functions relate to affinity and sensitivity to calcium (Hotchkiss et al., 2002; Luzio & Cameron, 2008; Ralet, Dronnet, Buchholt, & Thibault, 2001; Thibault & Rinaudo, 1985).

Studies on the molecular interactions (association and segregation) in mixed systems can always excite researchers' interest in the hydrocolloid field. Despite a great deal of progress, there are still arguments on synergistic behavior of the mixed systems. One example is the molecular interactions between pectin and alginate (Williams & Phillips, 1995), and synergistic gelation of alginate and high-methoxyl pectin under acidic

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conditions below pKa has been reported as a result of associations between protonated uronic acid blocks for each polysaccharide based on the configuration similarity of the monomer between  $\alpha$ -D-galacturonic acid in pectin and  $\alpha$ -L-guluronic acid in alginate (Toft, Grasdalen, & Smidsrod, 1986). From this perspective, our research team has investigated the calcium binding and calcium-induced gelation of normal sodium alginate modified by the addition of low molecular-weight polyuronate fractions (Nakauma et al., 2016). It was clarified that the addition of the alginate fractions increased the critical threshold concentration of calcium required for the increase in the relative viscosity ( $\eta_r$ ) of dilute solutions due to the competition with normal alginate for calcium and decreased dynamic storage modulus ( $G'$ ) of gels although these effects of the alginate fractions depended both on  $M_w$  and mannuronate/guluronate (M/G) ratio. It was also clarified in the same report that the addition of the G-rich alginate fraction improved the water holding capacity of calcium-induced alginate gels, making the gels more rheologically deformable represented by increased yield strain. These indicate potential usage of the alginate fraction as a novel texture modifier. On the other hand, low molecular-weight low-methoxyl pectin fraction, as a counterpart to the G-rich alginate fraction, showed quite different effects, and in the mixture with normal sodium alginate,  $\eta_r$  of dilute solutions was increased at a calcium concentration even below the stoichiometry of egg-box dimers and  $G'$  of calcium-induced alginate gels was increased by the addition of the pectin fraction. As a series of the study, functions of low molecular-weight polyuronate fractions (i.e. oligomers) in the calcium binding and calcium-induced gelation of normal low-methoxyl pectin were investigated in the present study. Effects of the polyuronate fractions from either pectin or alginate on the molecular associations with normal low-methoxyl pectin were compared, and the difference from or the similarity to the effects in the mixtures with normal alginate was presented.

## 2. Materials and methods

### 2.1. Materials

Pectin from citrus with two different degree of esterification DE values (SAN-SUPPORT<sup>®</sup> P-160 for high-methoxyl pectin and SAN-SUPPORT<sup>®</sup> P-161 for low-methoxyl pectin) and sodium alginate (SAN-SUPPORT<sup>®</sup> P-80) were provided as commercial products by San-Ei Gen F.F.I., Inc. (Osaka, Japan). Other materials were the same as those in the previous study (Nakauma et al., 2016), and the definition of the enzyme unit should be referred also to the previous study (Nakauma et al., 2016). The following abbreviations were used for convenience throughout this study:

LMP low-methoxyl pectin; HMP high-methoxyl pectin; SAL sodium alginate; MAN polymannuronate; GUL polyguluronate.

### 2.2. Preparation of low molecular-weight pectin fractions

Low molecular-weight pectin fractions were prepared using HMP (SAN-SUPPORT<sup>®</sup> P-160) as a starting material and by the combination of enzymatic hydrolysis and de-esterification as reported (Nakauma et al., 2016). In brief, HMP with pectinase treatment but without esterase treatment was identified as  $LM_w$ -HMP (high DE type), whereas HMP with both treatments was identified as  $LM_w$ -LMP (low DE type).

Macromolecular characteristics of the pectin fractions and normal LMP (SAN-SUPPORT<sup>®</sup> P-161), including weight average molecular-weight  $M_w$ , number average molecular-weight  $M_n$ , radius of gyration  $R_g$ , polydispersity index defined by  $M_w/M_n$ , and the Flory exponent  $\nu$ , were determined by size-exclusion chromatography coupled with a multiangle laser light scattering photometer (SEC-MALS) as reported (Nakauma et al., 2016). As physicochemical characteristics, constitutional sugars were identified by high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD), whereas DE was determined spectrophotometrically as reported (Nakauma et al., 2016). These characteristics were summarized in Table 1.

### 2.3. Preparation of low-molecular weight alginate fractions

Low molecular-weight alginate fractions were prepared using SAL (SAN-SUPPORT<sup>®</sup> P-80) as a starting material and by the combination of acid hydrolysis and pH-based fractionation as reported (Nakauma et al., 2016). Exceptions from previous report were heating condition for hydrolysis; 1 h instead of 5 h and pH conditions for recovery of G-rich fraction identified as  $LM_w$ -GUL; 3.8 instead of 3.5 and for recovery of M-rich fraction identified as  $LM_w$ -MAN; 2.4 instead of 2.6.

Macromolecular characteristics of the alginate fractions and normal SAL were determined by SEC-MALS as reported (Nakauma et al., 2016). As physicochemical characteristics, G content and average G-block length (i.e. the length of G-block larger than 1) were determined by a nuclear magnetic resonance NMR spectrometry as reported (Nakauma et al., 2016). These characteristics were summarized in Table 2.

### 2.4. Relative viscosity measurement of dilute solutions

For the mixture of normal LMP and each polyuronate fraction,  $\eta_r$  changes upon calcium addition were measured at 25 °C using an Ubbelohde type capillary viscometer as reported (Nakauma et al., 2016). Concentration of LMP in the mixture was fixed at 0.05%, whereas those of each polyuronate fraction were 0.01%, 0.02%, and 0.05%.  $\eta_r$  of dilute solutions was determined as  $t_s/t_0$ , where  $t_s$  is the flow time for test solutions (either the mixture or the LMP control)

**Table 1**  
Macromolecular characteristics and componential information for pectin samples used.

	$M_w^a$ (kg/mol)	$R_g^a$ (nm)	Polydispersity index <sup>a</sup>	$\nu^a$	Constituent sugars <sup>b</sup> (% w/w)					DE <sup>c</sup> (%)
					Rha	Ara	Gal	Glu	GaLA	
LMP	157.6	24.5	1.55	0.67	3.0	2.8	3.0	0.4	72.5	35.1
$LM_w$ -HMP	65.0	17.5	1.31	0.78	2.9	5.6	10.5	2.9	74.4	70.6
$LM_w$ -LMP	66.2	18.0	1.29	0.77	3.5	2.3	4.7	1.3	81.3	5.2

<sup>a</sup> Weight-average molecular weight  $M_w$ , radius of gyration  $R_g$ , and polydispersity index were determined by SEC-MALS. Polydispersity index was calculated as the ratio of weight-average molecular weight to number-average molecular weight. The Flory exponent  $\nu$  was determined from the relationship  $R_g = M_w^\nu$ .

<sup>b</sup> Constituent sugars were determined by HPAEC-PAD. Rha: Rhamnose, Ara: Arabinose, Gal: Galactose, Glc: Glucose, GaLA: Galacturonic acid.

<sup>c</sup> Degree of esterification DE was determined by spectrophotometry in combination with alcohol oxidase and 2, 4-pentanedione treatment. See the text for experimental details.

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