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

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Abstract

The functions of low molecular-weight polyuronate fraction in the calcium binding and calcium-induced gelation of normal low-methoxyl pectin (LMP) were investigated. Pectin fractions with different degrees of esterification (DE) and alginate fractions with different mannuronate/guluronate (M/G) ratios were prepared. Weight average molecular-weight (M_w) of each low molecular-weight polyuronate fraction ranged from ca. 40,000 to 65,000 g/mol. In the mixtures of LMP and each low molecular-weight polyuronate fraction, changes in the relative viscosity (η_r) of dilute solutions and in rheological properties of gels were examined in the presence of calcium. The addition of low molecular-weight pectin fraction, regardless of DE, increased η_r of dilute solutions and increased dynamic storage modulus (G') of gels with greater effects at lower DE. On the contrary, the addition of low molecular-weight alginate fraction, regardless of M/G ratio, shifted the critical threshold calcium concentration required to steepen η_r of dilute solutions higher and decreased G' of gels with greater effects at lower M/G ratio (i.e. rich in G). Gelation behavior of the mixture was schematically presented, and the functions of low molecular-weight polyuronate fraction were compared on the molecular level between pectin and alginate.

Keywords: Calcium binding; Egg-box dimer; Gelation; Low-methoxyl pectin; low molecular-weight polyuronate

1. Introduction

Calcium-binding behavior of polysaccharides with polyuronate backbone has been investigated extensively as in the case of pectin and alginate. For pectin, it has been reported that degree of esterification (DE) and weight average molecular-weight (M_w) both influence pectin gelation in terms of gel strength and the kinetics of gel formation, and the functions relate to affinity and sensitivity to calcium (Hotchkiss et al., 2002; Luzio & Cameron, 2008; Ralet, Dronnet, Buchholt, & Thibault, 2001; Thibault & Rinaudo, 1985). Our research team investigated previously (Nakauma et al., 2016) the calcium binding and calcium-induced gelation of normal sodium alginate modified by low molecular-weight polyuronate fractions. It was clarified that the addition of the alginate fraction shifted the critical threshold calcium concentration required to steepen the relative viscosity (η_r) of dilute solutions higher and decreases dynamic storage modulus (G') of gels and that these effects of the alginate fraction depended both on M_w and mannuronate/guluronate (M/G) ratio. It was also clarified in the same report that the addition of low molecular-weight G-rich alginate fraction improved the water

holding capacity of calcium-induced alginate gels and made the gels more rheologically deformable represented by increased yield strain. These results indicated the potential usage of the G-rich alginate fraction as a novel texture modifier. On the other hand, effects of low molecular weight low-methoxyl pectin fraction were quite different from those of the G-rich alginate fraction, and in the mixture of the pectin fraction and normal sodium alginate, viscosity increase of dilute solutions was detected at a calcium feed even below the stoichiometry of egg-box dimers. Also, mechanical strength of calcium-induced sodium alginate gels was increased by the addition of the pectin fraction as represented by increased G'. As a series of the study, the functions of low molecular-weight polyuronate fraction in the calcium binding and calcium-induced gelation of normal low-methoxyl pectin (LMP with M_w of ca. 150,000 g/mol) was investigated in the present study, and the effects of the polyuronate fraction on the molecular association with LMP were compared between pectin and alginate.

2. Materials and methods

2.1. Materials

Pectins from citrus with different DE values (SAN-SUPPORT® P-160 for high-methoxyl pectin and SAN-SUPPORT® P-161 for low-methoxyl pectin) and sodium alginate (SAN-SUPPORT® P-80) were provided as commercial products by

San-Ei Gen F.F.I., Inc. (Osaka, Japan). Other materials used and the definition of enzyme unit were the same as 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 pectin fractions

Low molecular-weight pectin fractions with different DE values was prepared using HMP (SAN-SUPPORT® P-160) as a starting material and combination of enzymatic hydrolysis and de-esterification in the same procedure as reported previously (Nakauma et al., 2016). In brief, HMP with pectinase treatment but without esterase treatment was identified as LM_w-HMP, whereas that with both treatments was identified as LM_w-LMP.

Macromolecular characteristics of the pectin fractions and LMP (SAN-SUPPORT® P-161), including 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 ν, were determined by size-exclusion chromatography coupled with a multiangle laser light scattering photometer (SEC-MALS) as reported previously (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 previously (Nakauma et al., 2016). These characteristics were summarized in Table 1.

2.3. Preparation of alginate fractions

Low molecular-weight alginate fractions with different M/G ratios were prepared using SAL (SAN-SUPPORT® P-80) as a starting material and combination of acid hydrolysis and pH-based fractionation in the same procedure as reported previously (Nakauma et al., 2016). Exceptions from previous report were heating condition for hydrolysis ; 1 h and pH conditions for recovery of G-rich fraction (identified as LM_w-GUL);3.8 and for recovery of M-rich fraction (identified as LM_w-MAN); 2.4.

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

2.4. Relative viscosity measurement of dilute solutions

For the mixture of LMP and each low molecular-weight polyuronate fraction,

109 changes in η_r by calcium addition were measured at 25 °C using an Ubbelohde type
110 capillary viscometer as reported previously (Nakauma et al., 2016). Concentration of
111 LMP in the mixture was fixed at 0.05%, whereas those of each low molecular-weight
112 polyuronate fraction were 0.01%, 0.02%, and 0.05%. η_r of dilute solutions was
113 determined as t_s/t_0, where t_s is the flow time for test solutions (either the mixture or
114 LMP alone) titrated by 7.5 mM $CaCl_2$ solution, and t_0 is the flow time for the solvent;
115 20 mM acetate buffer (pH 5.0). To eliminate the dilution effect by the addition of
116 $CaCl_2$ solutions during titration, η_r was normalized:

117 $$\eta_r^N = \eta_r^{Ca}/\eta_r^C$$

118 Here η_r^{Ca} is the relative viscosity in calcium titration, and η_r^C is the relative viscosity
119 in buffer titration (Fang et al., 2008). Data were presented as means ± SD of triplicate.

120 *2.5. Rheological measurements of gels*

121 For the mixture of LMP and each low molecular-weight polyuronate fraction,
122 rheological properties of gels were measured at 25 °C using a strain-controlled
123 rheometer in an oscillation shear mode as reported previously (Nakauma et al., 2016).
124 Concentration of LMP in the mixture was fixed at 0.8%, where as those of each low
125 molecular-weight polyuronate fraction were 0.2%, 0.4%, and 0.8%. Dynamic
126 viscoelasticity measurements, including frequency sweep and strain sweep tests, were

127 applied to gels formed by curing of the mixture at 25 °C for at least 20 min to reach to
128 pseudosaturation. From the frequency sweep test, some rheological parameters were
129 determined, including constant K_f and exponent n_f, based on the power-law relationship
130 between frequency ω and complex viscosity η^* (Keogh & O' Kennedy, 1998):

131 $\eta^*(\omega) = K_f \omega^{nf}$ $(0 < n_f < 1)$

132 From the strain sweep test, some rheological parameters were determined, including
133 constant for the higher modulus component K_{s1}, constant for the lower modulus
134 component K_{s2}, exponent for the higher modulus component n_{s1}, and exponent for the
135 lower modulus component n_{s2} in the following dual exponential equation:

136 $G'(\gamma) = K_{s1}\exp(-n^{s1} \times \gamma) + K_{s2}\exp(-n^{s2} \times \gamma)$

137 In addition, the yield strain was identified as a peak in the plot of the elastic stress (G'
138 multiplied by strain) as a function of strain (Walls, Caines, Sanchez, & Khan, 2003).
139 Data were presented as means ± SD of triplicate for each rheological parameter.

140 *2.6. Statistics*

141 Data were analyzed by t-test to know the statistical difference from the control with a
142 significance defined at $p < 0.05$ or 0.01 at both sides using Microsoft Excel 2013
143 (Redmond, WA).

3. Results and discussion

3.1. Relative viscosity measurement of dilute solutions

3.1.1. Mixture of LMP and low molecular-weight pectin fraction

η_r^N of LMP alone (i.e. control) increased monotonously with increased concentration of calcium (in mM), and this was also the case for the mixture with either LM_w-HMP or LM_w-LMP (Fig. 1a & b). For the mixture, increasing degree of η_r^N was larger with increased addition level of low molecular-weight pectin fraction in general, and the deviation from the control was enlarged with increased calcium feed. LM_w-LMP was more effective than LM_w-HMP in these regards. η_r^N was replotted as a function of $R_{\text{total fGal}}$; the molar ratio of fed calcium to free galactose residues from both LMP and the pectin fraction (Fig. 1c & d). In this plot, η_r^N reached peaked or saturated at $R_{\text{total fGal}}$ of 2.14 for the control, whereas η_r^N did so at 1.41 for the mixture with 0.05% LM_w-HMP and at 0.89 for the mixture with 0.05% LM_w-LMP. It is likely for each low molecular-weight pectin fraction, particularly LM_w-LMP, to associate with LMP due to molecular similarity from thermodynamic point of view. The addition of the pectin fraction, particularly LM_w-LMP, can act as a low molecular weight cross-linker to increase the hydrodynamic size of LMP. This contributes to increased exclusion volume and thus increased η_r^N. Differed from previous study using SAL (Nakauma et

al., 2016), decrease in η_r^N at low calcium feed (i.e. $R_{\text{total fGal}} < 0.25$) was not detected in the LMP control or the mixture with low molecular-weight pectin fraction. This indicates that monocomplexation should hardly occur for LMP at low calcium feed, and thus the pectin faction has no impact on that molecular event. Calcium-binding behavior of LMP is less critical than that of SAL due to sequential irregularity of calcium binding site (Winning, Viereck, Norgaard, Larsen, & Engelsen, 2007), and egg-box dimer formation can start even when theoretical calcium/galacturonate stoichiometry (i.e. 0.25; 1 mol calcium/4 mol galacturonate) is not achieved (Fang et al., 2008). This can be a cause for absence of the initial critical threshold concentration of calcium in the LMP control or the mixture with low molecular-weight pectin fraction. The second critical threshold concentration of calcium, which indicates the initiation of lateral associations of egg-box dimer starting theoretically at the calcium/galacturonate stoichiometry of 0.55 (Fang et al., 2007), was obscure in the LMP control or the mixture with low molecular-weight pectin fraction, which is another difference from previous study using SAL. If the peak in η_r^N corresponds to the second critical threshold concentration, it is anticipated that low molecular-weight pectin fraction, particularly LM_w-LMP, can promote the associations of LMP in some way.

179 *3.1.2. Mixture of LMP and low molecular-weight alginate fraction*

180 η_r^N increased monotonously with increased concentration of calcium (in mM)
181 followed by a peak in some cases for the mixture with either LM_w-MAN or LM_w-GUL
182 at each addition level (Fig. 2a & b). In contrast to low molecular-weight pectin
183 fraction, increasing degree of η_r^N was smaller with increased addition level of low
184 molecular-weight alginate fraction, and the deviation from the control was enlarged
185 with increased calcium feed. LM_w-GUL was more effective than LM_w-MAN in these
186 regards. η_r^N was replotted as a function of $R_{fGal+Gul}$; the molar ratio of fed calcium to
187 free galacturonate residues from LMP and free guluronate residues from alginate
188 fraction (Fig. 2c & d). No substantial difference was observed between plots for the
189 mixture with LM_w-MAN. On the other hand, η_r^N for the mixture with LM_w-GUL was
190 lower than that for the control when the stoichiometry was lower than 0.25 and almost
191 overlapped with the control within the stoichiometry range from 0.5 to 1.0 at each
192 addition level of LM_w-GUL. η_r^N for the mixture with LM_w-GUL was again lower than
193 that for the control when the stoichiometry was higher than 1.0, and this effect was
194 enhanced with increased addition level of LM_w-GUL. It is unlikely for low
195 molecular-weight alginate fraction, particularly LM_w-MAN, to associate with LMP from
196 thermodynamic point of view, and molecular associations can occur separately and

197 independently between LMP and the alginate fraction. It is thus anticipated that the
198 decrease in η_r^N by the addition of LM_w-MAN should be mainly due to its chelating
199 effect. In the mixture with LM_w-GUL, monocomplexation and subsequent egg-box
200 dimer formation of LM_w-GUL can occur prior to molecular associations of LMP. This
201 may be reasonable when differences in the chain length, sequential regularity of calcium
202 binding site, and molecular conformation between LMP and LM_w-GUL are considered.
203 Contribution of LM_w-GUL (even after self-associations) to η_r^N should be lower than that
204 of LMP, and this can explain the η_r^N behavior at low calcium feed. On the other hand,
205 macroscopic phase separation between LMP and LM_w-GUL can explain the η_r^N
206 behavior at high calcium feed.

207 *3.2. Rheological measurements of gels*

208 *3.2.1.Mixture of LMP and low molecular-weight pectin fraction*

209 Concentration of calcium fed to the system was 20 mM in theory, corresponding to
210 $R_{\text{total fGal}}$ = 1.06 for 0.8% LMP alone (i.e. control), and the addition of low
211 molecular-weight pectin fraction increased the content of free galacturonate residues in
212 the system and thus decreased $R_{\text{total fGal}}$ (Table 3). From the stoichiometry point of view,
213 calcium feed should be sufficient for LMP to form egg-box dimers and multimers
214 except for the mixture with 0.08% LM_w-LMP, in which $R_{\text{fGal+Gul}}$ was smaller than 0.55,

215 theoretical calcium/galacturonate stoichiometry for starting lateral associations of
216 egg-box dimer (Fang et al., 2007). From the strain sweep test, no difference was found
217 in the yield strain between the LMP control and the mixture with LM_w-HMP at each
218 addition level, whereas the sum of K_{s1} and K_{s2} (i.e. equilibrium G' in the linear
219 viscoelastic regime) for the mixture increased with increased addition level of
220 LM_w-HMP (Table 3). From the frequency sweep test, no difference was found in the
221 power-law exponent n_f between the LMP control and the mixture with LM_w-HMP at
222 each addition level (Table 3). Also, G' for the mixture with LM_w-HMP was almost
223 independent of frequency from 0.1 to 100 rad/s and increased with increased addition
224 level of LM_w-HMP in the whole frequency range tested (Fig. 3a). These results
225 indicate that the addition of LM_w-HMP should not alter the nature of inter-molecular
226 associations of LMP and strengthen the super-molecular structure of LMP. This
227 accords qualitatively with the η_r^N profile in dilute solutions. For the mixture with
228 LM_w-LMP, the yield strain decreased with increased addition level of LM_w-LMP in
229 general, whereas the sum of K_{s1} and K_{s2} increased with increased addition level of
230 LM_w-LMP. LM_w-LMP showed a greater effect in increasing the equilibrium G' than
231 LM_w-HMP even though the calcium feed per binding site (represented by $R_{\text{total fGal}}$) was
232 lower when compared at the same addition level (Table 3). No difference was found in

233 n_f between the LMP control and the mixture with LM_w-LMP at each addition level
234 (Table 3). Similar to the case of LM_w-HMP, G' for the mixture with LM_w-LMP was
235 almost independent of frequency from 0.1 to 100 rad/s and increased with increased
236 addition level of LM_w-LMP in the whole frequency range tested (Fig. 3b). LM_w-LMP
237 showed a greater effect in increasing G' than that LM_w-HMP when compared at the
238 same addition level. These results indicate that the addition of LM_w-LMP should not
239 alter the nature of inter-molecular associations of LMP and strengthen the
240 super-molecular structure of LMP, similar to LM_w-HMP. One marked difference is the
241 structural brittleness provided by the addition of LM_w-LMP with the LMP system as
242 presented by decreased yield strain. This also accords qualitatively with the η_r^N profile
243 in dilute solutions, and decreased yield strain of gels may correspond to the peak shift to
244 lower calcium concentration (Fig. 1d).
245 *3.2.2.Mixture of LMP and low molecular-weight alginate fraction*
246 From the stoichiometry point of view, calcium feed should be sufficient for LMP to
247 form egg-box dimers and multimers except for the mixture with 0.08% LM_w-GUL, in
248 which $R_{fGal+Gul}$ was smaller than 0.55 (Table 4). From the strain sweep test, the yield
249 strain for the mixture with LM_w-MAN increased with increased addition level of
250 LM_w-MAN in general, whereas the sum of K_{s1} and K_{s2} decreased with increased

addition level of LM_w-MAN (Table 4). From the frequency sweep test, n_f decreased with increased addition level of LM_w-MAN (Table 4). Also, G' for the mixture was more frequency dependent, particularly within the frequency range from 0.1 to 1.0 rad/s, with increased addition level of LM_w-MAN (Fig. 4a). These results indicate that the addition of LM_w-MAN should prevent inter-molecular associations of LMP and weaken the super-molecular structures. This accords qualitatively with the η_r^N profile in dilute solutions. Similar results were obtained for the mixture with LM_w-GUL, but these effects of LM_w-GUL were much larger than those of LM_w-MAN when compared at the same addition level. It is anticipated that LM_w-GUL should bind with calcium prior to LMP and should form microgels or clusters which can prevent the inter-molecular associations of LMP. Rheological data were obtained at a fixed calcium dose not at a fixed $R_{\text{total fGal}}$ or $R_{\text{fGal+Gul}}$ in the present study. It should be noted that the functions of low molecular-weight polyuronate fraction in calcium-induced gelation of LMP may be different at lower R values than theoretical stoichiometry of forming egg box dimer.

3.3. Molecular association mechanism between LMP and low molecular-weight pectin fraction in comparison with low molecular-weight alginate fraction

Molecular association during calcium-induced gelation of LMP alone was presented schematically in comparison with that of SAL alone (Fig. 5). For LMP,

intra-molecular and inter-molecular associations occur coincidently upon calcium addition, which is quite different from multiple steps and critical behaviors of SAL. Intra-molecular association leads to the reduction of molecular size and volume, while inter-molecular association leads to the expansion. It may depend on M_w of LMP and also the gelation step which association is dominant, but in the case of LMP used in the present study, inter-molecular association can be dominant over intra-molecular association even at low calcium feed, causing the increase in η_r (Ralet, Dronnet, Buchholt, & Thibault, 2001; Fang et al., 2007). This is quite different from SAL, in which intra-molecular association occurs dominantly at below the stoichiometry R = 0.25. Absence of the second critical threshold concentration for LMP, which is detected at R = 0.55 in the case of SAL, indicates that lateral association of egg-box dimers is more difficult to form than in SAL. This difference between LMP and SAL can be attributed to the degree of molecular homogeneity in terms of monomer composition and conformation. For SAL, very trace amount of monomers exists in the molecules other than guluronate and mannuronate, and the sequence of these monomers is regular with linear molecular conformation. Thus, calcium binding behavior of SAL is critical through a series of molecular event, including intra-molecular association (i.e. monocomplexation) and egg-box dimer formation, followed by lateral inter-molecular

287 association of the dimers. In contrast, LMP is characterized by a variety of monomers
288 and existence of the hairy region, making molecular associations more random and
289 super-molecular structures less regular than in SAL, thus preventing the lateral
290 associations (Fang et al, 2008). As a contribution to elasticity enhancement, it is
291 anticipated that energetic factor due to the strength of crosslinks should be dominant for
292 SAL, particularly G-rich one (Funami et al, 2009), whereas entropic factor due to the
293 number of crosslinks plays an additional role for LMP.

294 Molecular association during calcium-induced gelation of the mixture of LMP and
295 each low molecular-weight polyuronate fraction was also presented schematically (Fig.
296 6 for the mixture with LM_w-LMP and Fig. 7 for the mixture with LM_w-GUL). As
297 mentioned, for LMP used in the present study, inter-molecular association can be
298 dominant over intra-molecular association even at low calcium feed, and η_r increases
299 gradually without showing critical concentration boundary of calcium, both of which
300 are different from the behavior of SAL. Moreover, the pectin fraction added can act as
301 a low molecular weight cross-linker to increase the hydrodynamic size of LMP and can
302 promote the association of the long chain normal pectin since thermodynamic
303 incompatibility between LMP and the pectin fraction should not be high considering the
304 similarity of monomer composition and conformation. Low molecular-weight pectin

305 fraction, particularly LM_w-LMP, associates with free galacturonate in the LMP
306 molecules via calcium, and as a result, LMP has longer chain and larger number of
307 galacturonate site than the original LMP. It is anticipated that these changes should
308 increase the opportunity for molecular associations but decrease the structural
309 homogeneity of super-molecular structure at the same time, which may result in the
310 network formation of various pore sizes (Fig. 6). These may explain larger G' and
311 smaller yield strain for the mixture in a concentrated system. Effects of the pectin
312 fraction relate to the sequentiality of calcium binding site, and a certain length is
313 necessary for binding, for example consecutive 14-20 M free galacturonate (Rees, 1982;
314 Axelos, & Thibault, 1991). This is why LM_w-LMP is more effective than LM_w-HMP
315 in modifying the gelation behavior of LMP.

316 Thermodynamic incompatibility between LMP and low molecular-weight alginate
317 fraction can be higher than in the pectin fraction, and thus the alginate fraction prefers to
318 bind together rather than binding to LMP. Differed from the pectin fraction, dimers
319 and multimers of the alginate fraction cannot associate easily with LMP molecules (Fig.
320 7). It is clear from viscometry in dilute solutions that the addition of low
321 molecular-weight alginate fraction, particularly LM_w-GUL, decreases η_r of LMP at
322 below the stoichiometry R = 0.25. This may indicate the absence of inter-molecular

association between LMP and low molecular-weight alginate fraction, and the alginate fraction can compete with LMP for calcium at low calcium feed. Decrease in η_r for the mixture with LM_w-GUL at above the stoichiometry R = 0.55 can be attributed to macroscopic phase separation based on the incompatibility between LMP and LM_w-GUL. Super-molecules from LM_w-GUL are dispersed within the LMP system and can prevent molecular associations of LMP, making the pore size of the gel network larger and rheological nature of the system more flexible and plastic. Effects of low molecular-weight alginate fraction relate to the sequentiality of calcium binding site, and a certain length is necessary for binding, for example consecutive 20 M free guluronate (Kohn, 1975; Kohn & Larsen, 1972). This is why LM_w-GUL is more effective than LM_w-MAN in modifying the gelation behavior of LMP.

4. Conclusions

Low molecular-weight polyuronate fraction modifies the calcium binding and consequent molecular association behaviors of long chain normal low-methoxyl pectin and thus rheological properties of the gels. These effects of the polyuronate fraction are enhanced when the degree of methyl-esterification is low for pectin and the guluronate content or guluronate-block length is high for alginate, indicating a key role of calcium binding site. Low molecular-weight pectin fraction, particularly

low-methoxyl one, strengthens the gel structure of normal low-methoxyl pectin, whereas low molecular-weight alginate fraction, particularly guluronate-rich one, weakens the gel structure of normal low-methoxyl pectin. Different functions between low molecular-weight pectin and alginate fractions can be attributed to the molecular compatibility with normal low-methoxyl pectin.

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419

420 **Figure captions**

421 **Fig. 1**. Changes in normalized relative viscosity η_r^N (η_r^{Ca}/η_r^C) during titration of 7.5 mM $CaCl_2$ for

422 the mixture of 0.05% normal low-methoxyl pectin (LMP) and low molecular-weight pectin fraction

423 at 0% (closed circle), 0.01% (open triangle), 0.02% (closed square), and 0.05% (open circle) for

424 high-metoxyl pectin fraction (LM_w-HMP) (a & c) and low-mexthoxyl pectin fraction (LM_w-LMP) (b

425 & d). Data are plotted as a function of calcium concentration (a & b) and the molar ratio $R_{\text{total fGal}}$

426 (calcium/total free galacturonate from LMP and pectin fraction) (c & d). See the text for

427 experimental detail. Data are presented as means ± SD of triplicate.

428 **Fig. 2**. Changes in normalized relative viscosity η_r^N (η_r^{Ca}/η_r^C) during titration of 7.5 mM $CaCl_2$ for

429 the mixture of 0.05% normal low-methoxyl pectin (LMP) and low molecular-weight alginate

430 fraction at 0% (closed circle), 0.01% (open triangle), 0.02% (closed square), and 0.05% (open circle)

431 for mannuronate-rich alginate fraction (LM_w-MAN) (a & c) and guluronate-rich alginate fraction

(LM_w-GUL) (b & d). Data are plotted as a function of calcium concentration (a & b), and the molar ratio $R_{fGal+Gul}$ (calcium/the sum of free galacturonate from LMP and free guluronatefrom alginate fraction) (c & d). See the text for experimental detail. Data are presented as means ± SD of triplicate.

Fig. 3. Frequency-dependence of dynamic storage modulus G' for the mixture of 0.8% normal low-methoxyl pectin (LMP) and low molecular-weight pectin fraction at 0% (closed circle), 0.2% (open triangle), 0.4% (closed square), and 0.8% (open circle) for high-methoxyl pectin fraction (LM_w-HMP) (a) and low-methoxyl pectin fraction (LM_w-LMP) (b). Concentrations of both $CaCO_3$ and glucono-δ-lactone were fixed at 20 mM. See the text for experimental detail. Measurements were carried out in triplicate, and one representative datum is shown.

Fig. 4. Frequency-dependence of dynamic storage modulus G' for the mixture of 0.8% normal low-methoxyl pectin (LMP) and low molecular-weight alginate fraction at 0% (closed circle), 0.2% (open triangle), 0.4% (closed square), and 0.8% (open circle) for mannuronate-rich alginate fraction (LM_w-MAN) (a) and guluronate-rich alginate fraction (LM_w-GUL) (b). Concentrations of both $CaCO_3$ and glucono-δ-lactone were fixed at 20 mM. See the text for experimental detail. Measurements were carried out in triplicate, and one representative datum is shown.

Fig. 5. Schematic presentation of calcium-induced gelation for normal low-methoxyl pectin (LMP) (a) in comparison with normal sodium alginate (SAL) (b).

Fig. 6. Schematic presentation of calcium-induced gelation for the mixture of normal low-methoxyl pectin (LMP) and low molecular-weight low-methoxyl pectin fraction (LM_w-LMP).

Fig. 7. Schematic presentation of calcium-induced gelation for the mixture of normal low-methoxyl pectin (LMP) and low molecular-weight guluronate-rich alginate fraction (LM_w-GUL).