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# Chemical modification of citrus pectin to improve its dissolution into water

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ABSTRACT

Citrus pectin was modified with glycine, glycine methyl ester, or glycylglycine by the use of intramolecular associations in polar organic solvents. The modified pectins except glycine methyl ester significantly improved its dissolution in water, compared with unmodified pectin. The pectin modified with glycylglycine was the most soluble into water and was not influenced by pH range from 4.6 to 7.6 and ionic strength range from 1 mM to 50 mM phosphate buffer (pH 5.1), while the dissolution of the unmodified pectin decreased as pH and ionic strength increased. The required time at 45 °C at 95% hydration rate in the glycylglycine-modified pectin (2%, w/v) was toward 58% that in the unmodified pectin. In glycylglycine-modified pectin, the hydration rate was dependent on the bound amounts of the modifying agent to pectin. The potentiometric titration curves revealed that the glycylglycine-modified pectin would bear more negative charge than the unmodified pectin. From analysis of the water adsorption isotherms, the Brunauer–Emmett–Teller C constant as an index of hydrophilicity in the glycylglycine-modified pectin was 1.23-fold and 1.87-fold, respectively, higher than those in the unmodified and glycine methyl ester-modified pectins. These results suggested that the dissolution of pectin into water was preferably related to the net charge and the surface hydrophilicity in pectin molecules.



## 1. Introduction

Pectin is an ionic hydrocolloid that is mainly used as a gelling agent, thickening agent and stabilizer in food. The majority of the structure consists of homopolymeric partially methylated poly-(1→4)-α-D-galacturonic acid residue in commercial pectins (Lopes da Silva & Rao, 2006; Sriamornsak, 2003). The functional properties depend on the degrees of methyl esterification (DE) (Thakur, Singh, & Handa, 1997). In order to effectively process polysaccharides such as pectins, above all, uniformly dissolving the polysaccharides in a dispersion medium is a top priority. However, when many water-soluble polysaccharides in powder form are added to water, they are hydrated more quickly, on their surface contacted with water. They produce so-called "undissolved powder lumps" which are difficult to dissolve. Thus, the formation of lumps imposes a serious obstacle in the food industry.

In order to efficiently dissolve water-soluble polysaccharides without forming lumps and agglomerations, there are procedures in which water temperature is raised, stirring is conducted under reduced pressure, and mixing time is extended during dissolution. Consequently, there is an increased burden of time, energy and production cost by any corresponding facility in the food industry. As a means to solving the problem, some convenient and effective methods have been employed. The granulation of a water-soluble polysaccharide in powder using a mixed solution of a water-soluble polysaccharide and an emulsifier as a binder solution can produce a water-soluble polysaccharide excellent in handling behavior such as significantly improved dissolution in water of the granules (Hattori, Nagaoka, & Maeda, 2001). The particulate water-soluble or water-swellable polymer, which has been at least partially agglomerated by treatment with polyol hydrates in water-containing solvents, dissolves substantially faster than untreated water-soluble or water-swellable polymer (Graff-Andersen & Modi, 1999). To improve dispersion stability, pectin was modified by heating it at 50–80 °C for 10–360 min while adjusting the relative humidity to 45–85% (Hiroe, Kataoka, & Funami, 2009). However, these do not take into consideration as to whether dissolution of the water-soluble polysaccharides could be kept at broad pH and/or high ionic strength.

In the present study, we report that the glycylglycine-modified pectin is able to readily dissolve into water at pH range from acidic to neutral, even at high ionic strength. The modified pectin was prepared by the method independent of the solubility and viscosity of polysaccharides due to reactions in high concentrations of polar organic solvents at which polysaccharides can be precipitated, as described in our previous study (Kurita, Murakami, & Fujiwara, 2010). Chemical modification was based on reactions of dehydration-condensation between polysaccharides and modifying agents. In the event of formation of precipitates from alcohol used for fractionation of polysaccharides, the polysaccharides with

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increasing intramolecular hydrogen bonding can incorporate the modifying agents into their molecules in polar organic solvents. The co-precipitates acted as a precursor of the modified polysaccharides. Eventually, the modification was accomplished by simply drying of the precipitates. The chemically modified pectin with superior dissolution has not been reported as yet. In addition, a factor of dissolution in water-soluble polysaccharides which has not been adequately understood is addressed with regard to the hydrophilicity of surface structure in their molecules in this study.

## 2. Materials and methods

### 2.1. Materials

Citrus pectin (uronic acids, 60–65%) was purchased from Wako Pure chemicals (Osaka, Japan) and was used as a substrate chemically modified. Glycine, glycine methyl ester hydrochloride, and glycylglycine were used as modifying agents for chemical modification. All chemicals used were of the purest grade from Wako Pure Chemicals (Osaka, Japan). GENU pectin type AS-J (DE 54%), type BB (DE 73%), type DD-J (DE 65%), type LM-84AS (DE 32%) and SLENDID speciality pectin type 100 (DE 15%) using standards of DE (degree of esterification) were kindly provided by CP Kelco Japan Aps (Tokyo, Japan).

### 2.2. Modification of pectin

Dried powder of pectin was dissolved in distilled water (4%, w/v) containing various concentrations of modifying agents at pH 5.4 adjusted by NaOH or HCl with a laboratory dispersion device (Polytron Model K, Kinematica AG, Switzerland) at the maximum speed 30,000 rpm for 5 min. Then two volumes of acetone were promptly added to the solution. The mixtures were left for 1 h at room temperature. The acetone precipitates were collected and dried by air-drying at 60 °C for 18 h. The dried reaction mixtures were dissolved and dialyzed (membrane cut-off 14,000 Da) against distilled water at 7 °C. Finally, the modified pectins were obtained by the freeze-drying of dialyzed solutions. The control sample without chemical modification was directly dissolved in distilled water, adjusted at pH 5.4 and then followed the same procedure described above.

### 2.3. Measurement of dissolution

Dissolution of pectin into water or buffers with different pH values or ionic strengths was determined by the hydration rate by using a Rapid Visco Analyzer (RVA Super4, Newport Scientific Pty. Ltd., Narrabeen, Australia) interfaced with a personal computer equipped with Thermocline software. 25 mL of distilled water was placed into an aluminum canister including precisely weighed amounts of pectin (0.5 g) at final concentrations of 2% (w/v). The hydration rate was recorded by running the motor at a set speed (700 rpm) and temperature (45 °C). Once the viscosity reached a stable value, that value was considered the final viscosity corresponding to complete hydration. The hydration rate (%) for each time was obtained by dividing the viscosity read at time by the final viscosity, and multiplying by 100.

### 2.4. Determination of uronic acid, total sugars, and amino acids

Total uronic acid content of pectin was determined by the xylenol method (Walter, Fleming, & MacFeeters, 1993). Sample (500 μL) in 1% (w/v) NaCl was mixed with 4.0 mL of sulfuric acid in an ice bath, and then heated in a boiling water bath for 10 min. The solution was mixed with 200 μL of glacial acetic acid containing 0.1% (w/v) xylenol, and rested for 10 min at room temperature. The reaction mixture was then measured at absorbance 450–415 nm, while the galacturonic acid solutions (0–100 μg/mL) were used as a standard.

The content of total sugars of pectin was determined by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) using D-galactose as a standard.

Amino acid analysis was performed to determine the bound amounts of modifying agents (glycine, glycylglycine, and glycine methyl ester hydrochloride) to pectin by measuring the glycine contents. The amino acids were modified with phenylisothiocyanate and analyzed by HPLC (Waters 2690 Alliance with 996-photodiode array detector, Waters, Milford, MA, USA) using a Pico/Tag Hydrolysis column (3.9 × 150 mm) at 46 °C at an absorbance of 254 nm (Cohen & Strydom, 1988). Ten milligrams of pectin was dissolved in 6 N HCl (1.0 mL) and hydrolyzed at 110 °C for 24 h under nitrogen. The hydrolysates were used as a sample for HPLC analysis.

### 2.5. Determination of degree of esterification

Degree of esterification (DE) in pectin was determined by the ruthenium red method (Hou, Chang, & Jiang, 1999). To aliquots (1 mL) of pectins (0.1%, w/v) in 0.1 M MOPS buffer (pH 6.5) 0.5 mL of 0.02% ruthenium red was added, mixed, and left to stand for 5 min. 0.5 mL of 0.6 M calcium chloride was then added to precipitate pectin. The mixtures were centrifuged at 2000 × *g* for 15 min. The absorbance at 534 nm of the supernatants was measured. A blank with distilled water was used instead of pectin solution. Means of each value of absorbance at 534 nm with different DE pectins were plotted against corresponding calculated DE.

### 2.6. FT-IR spectroscopy

FT-IR spectrum of polysaccharide was obtained at a resolution of 1 $cm^{-1}$. Sample was incorporated with KBr (spectroscopic grade) and pressed into a 3 mm pellet. The 256 scans were entered before Fourier transformation. Spectra were recorded in the transparent mode from 4000 to 400 $cm^{-1}$, using a Spectrum 2000 (PerkinElmer Ink., MA, USA).

### 2.7. Potentiometric titrations

Potentiometric titrations were carried out using an Automatic Titrator COM-1700 (Hiranuma Sangyo Co. Ltd., Ibaragi, Japan) and a glass electrode calibrated with standard buffers at pH 4.01 and 6.86 (Sist et al., 2003). Aqueous solutions of pectin in the free form were prepared by dissolving dried samples in 0.1 M acetic acid and dialyzing against the same solution for 24 h at 7 °C. The sample solutions were subsequently dialyzed against distilled water at 7 °C until the pH of the dialyzing solvent was nearly neutral. The dialyzed solutions were freeze-dried and then used as measuring samples. Potentiometric titrations were performed at 25 °C with 0.1 M NaOH.

### 2.8. Adsorption isotherm

The adsorption isotherms in pectins were measured using a Hydrosorb 1000 (Quantachrome Instruments, FL, USA). Approximately 30 mg of the freeze-dried pectin sample was placed in glass cell with outside stem diameter of 9 mm, and predried and degased in a heating mantle at 50 °C for 18 h, until the sample reached a constant weight. The temperature was kept constant at 25 °C during the measurement. The water content in the pectins was represented by the weight of adsorbed water per weight of dried pectin as a function of relative vapor pressure. The experimental adsorption isotherms were approximated using the BET equation (Brunauer,

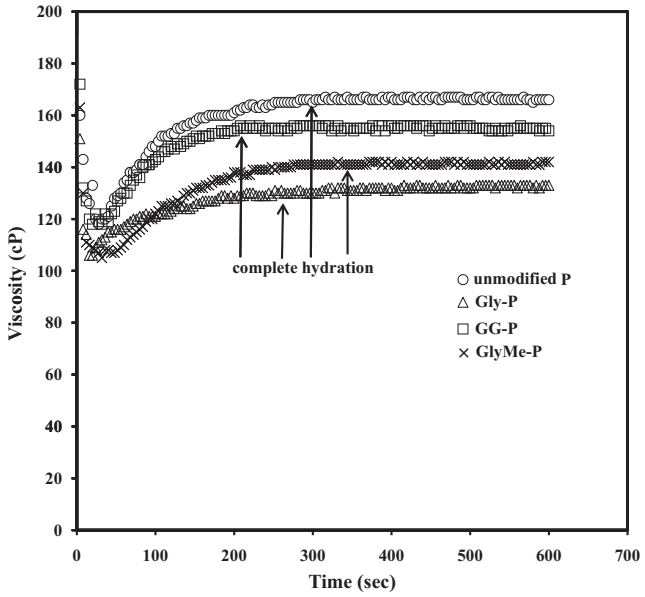


**Fig. 1.** RVA viscosity profiles of unmodified and modified pectins. Modifications at the concentrations of 0.2 M different modifying agents were used. Citrus pectins were dispersed into water at final concentrations of 2% (w/v). The suspensions were stirred by rotating the plastic paddle of the RVA at 700 rpm and 45 °C. Gly-P, GG-P, and GlyMe-P were designated as glycine-, glycylglycine-, and glycine methyl ester-modified pectins, respectively. Data shown are from a typical experiment that was reproduced at least three times.

Emmett, & Teller, 1938). The surface areas were determined from monolayer capacities assuming that the area occupied by water molecules is equal to $1.08 \times 10^{-19}$ m$^2$ (Orchiston, 1954).

### 2.9. Statistical analysis

Data represent mean ± standard error of the mean (SEM) of three independent experiments with two measurements each. The data were analyzed by one-way analysis of variance. Differences were considered statistically significant when the $p$ level was less than 0.05.

## 3. Results

### 3.1. Dissolution of the modified pectins

RVA viscosity profiles of pectins were shown in Fig. 1. As the sample is dissolved in water, the viscosity increases to a final viscosity, which can be attributed to the complete hydration. The glycine methyl ester-modified pectin was less soluble than the unmodified pectin, while the dissolutions of the glycine- and glycylglycine-modified pectins into water were improved. The time (s) of final viscosity for unmodified pectin, glycine-, glycine methyl ester-, and glycylglycine-modified pectins were 287 ± 13, 242 ± 14, 354 ± 14, and 200 ± 11, respectively. Additionally, the viscosity (cP) of the unmodified pectin, glycine-, glycine methyl ester-, and glycylglycine-modified pectins were 168 ± 3, 145 ± 13, 149 ± 9, and 163 ± 3, respectively, at the concentrations of 2% (w/v). The most soluble glycylglycine-modified pectin was chosen as the candidate for comparison of hydration properties to unmodified pectin.

In order to examine whether the improved dissolution was due to the bound amounts of modifying agent, pectin was modified with glycylglycine at the concentrations from 0 to 0.5 M. As the concentrations of glycylglycine increased, the hydration time decreased (Fig. 2). The glycine contents (mg/g) of unmodified pectin, 0.05, 0.1, 0.2, and 0.5 M modified-glycylglycine-pectins were 2.04 ± 0.008, 23.1 ± 2.5, 41.2 ± 1.2, 52.3 ± 0.6, and 141 ± 0.3, respectively. Thus, the dissolution of the modified pectins was dependent on the bound amounts of glycylglycine to the pectin.

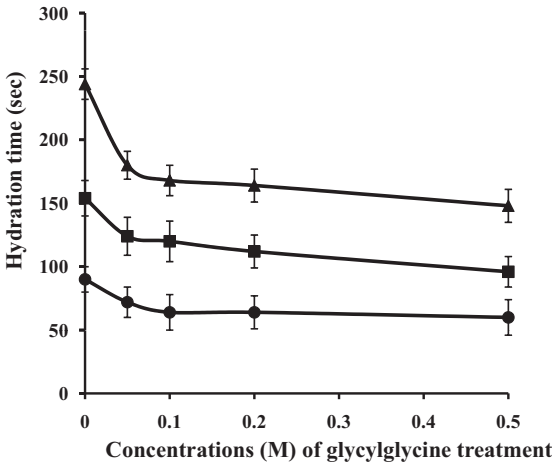


**Fig. 2.** Hydration time of glycylglycine-modified pectins with different concentrations at different hydration rates (80% (●), 90% (■), and 95% (▲)). Citrus pectins were dispersed into water at final concentrations of 2% (w/v). The suspensions were stirred by rotating the plastic paddle of the RVA at 700 rpm and 45 °C. The data are averages ± SE of three independent experiments with two measurements.

### 3.2. Effect of temperature, pH, and ionic strength on dissolution

As the temperature increased, just as expected, the hydration increased. In every instance, the glycylglycine-modified pectin had higher hydration than the unmodified pectin (Fig. 3). The required

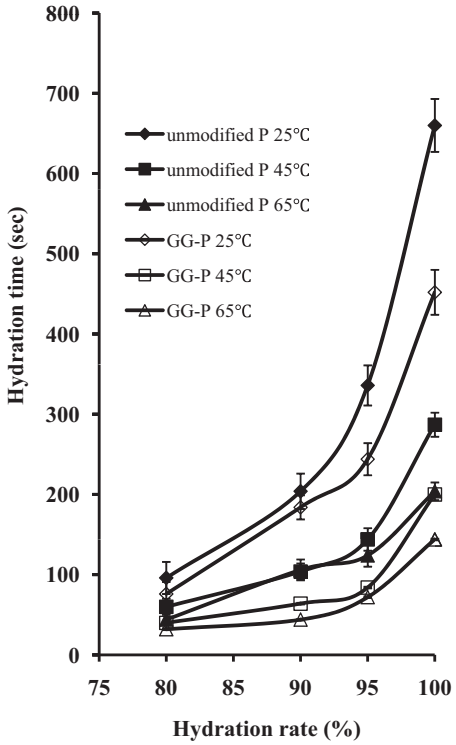


**Fig. 3.** Hydration rate of the unmodified and glycylglycine-modified pectins at three different temperatures. Modifications at the concentrations of 0.2 M different modifying agents were used. Citrus pectins were dispersed into water at final concentrations of 2% (w/v). The suspensions were stirred by rotating the plastic paddle of the RVA at 700 rpm. The data are averages ± SE of three independent experiments with two measurements.

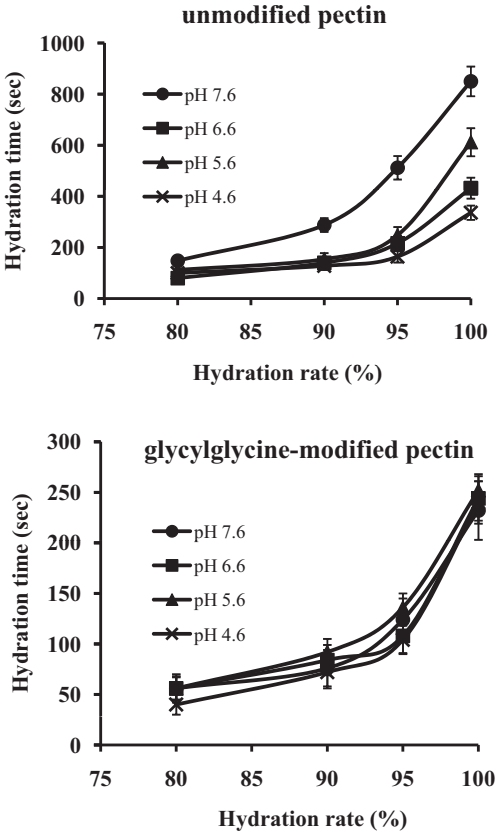


**Fig. 4.** Hydration rate of the unmodified and glycylglycine-modified pectins in 25 mM phosphate buffer at four different pHs. Modifications at the concentrations of 0.2 M modifying agents were used. Citrus pectins were dispersed into water at final concentrations of 2% (w/v). The suspensions were stirred by rotating the plastic paddle of the RVA at 700 rpm and 45 °C. The data are averages ± SE of three independent experiments with two measurements.

hydration times at 25, 45, and 65 °C at 95% hydration rate in the glycylglycine-modified pectin were 244 s, 84 s, and 72 s which were 72%, 58%, and 58%, respectively of the hydration times observed for the unmodified pectin.

In terms of weakly charged pectin of which polyelectrolyte character affects its dissolution, the hydration behavior was examined by the use of 25 mM phosphate buffer at four different pH values, 4.6, 5.6, 6.6, and 7.6. In the unmodified pectin, the hydration was the highest at acidic pH 4.6, though it reduced sharply at neutral pH 7.6 (Fig. 4). For 95% hydration rate at pH 7.6, the unmodified pectin required a 3.1-fold time compared with pH 4.6. However, in the glycylglycine-modified pectin the hydration behavior was almost uniform in the pH region from 4.6 to 7.6. In addition, there was no difference between unmodified and glycylglycine-modified pectins at the final pHs after hydration experiment, which were 4.2, 4.5, 6.2, and 7.1 for the buffered pH values 4.6, 5.6, 6.6, and 7.6, respectively. Like pH, ionic strength is one of the indispensable factors in using pectin as a food additive to various foods. In the unmodified pectin, as the ionic strength of soluble media increased, the hydration decreased (Fig. 5). The hydration behavior of the glycylglycine-modified pectin was not influenced by measure of ionic strengths. The glycylglycine-modified pectin had a complete hydration within 300 s from 1 to 50 mM phosphate buffer at the concentrations of 2% (w/v), while the unmodified pectin required 400 s at 10–50 mM phosphate buffer for complete hydration. In addition, 50 mM phosphate buffer was not completely dissociated and therefore not equivalent to 50 mM ionic strength due to the presence of the weakly charged pectin, although the effect of buffer concentration was conducted at pH 5.1.

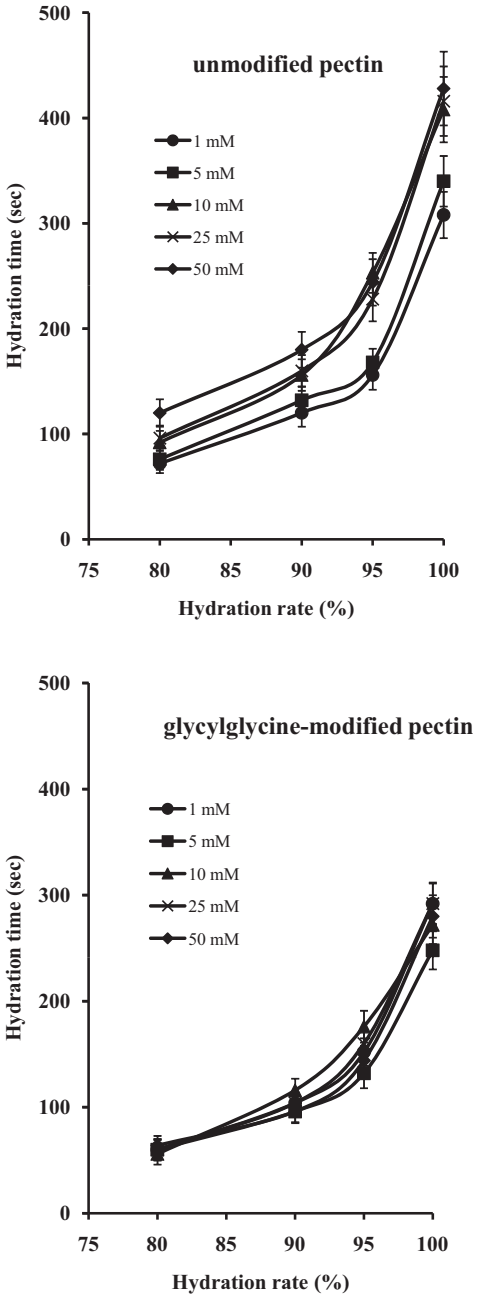


**Fig. 5.** Hydration rate of the unmodified and glycylglycine-modified pectins in five different concentrations of phosphate buffer at pH 5.1. Modifications at the concentrations of 0.2 M modifying agents were used. Citrus pectins were dispersed into water at final concentrations of 2% (w/v). The suspensions were stirred by rotating the plastic paddle of the RVA at 700 rpm and 45 °C. The data are averages ± SE of three independent experiments with two measurements.

### 3.3. Chemical analysis

As shown in Table 1, the glycylglycine- and glycine methyl-modified pectins had lower contents of total sugar compared to the unmodified pectin. In the contents of uronic acids, the glycine methyl-modified pectin was higher than the unmodified one. One of the uronic acids, D-galacturonic acid in pectin tightly binds some water molecules. The degrees of esterification (DE) which have a different effect on the structure of the surrounding water were examined in pectins modified with different agents. Significant difference between the unmodified and modified pectins could be detected in the degrees of esterification and the glycine contents

**Table 1**
Chemical properties of pectins with or without modification.

| | Unmodified-P | Gly-P | GG-P | GlyMe-P |
|---|---|---|---|---|
| Uronic acids (%, w/w) | 61.5 ± 0.4 | 61.9 ± 0.3 | 62.6 ± 0.9 | 64.1 ± 0.9* |
| Total sugar (%, w/w) | 88.8 ± 1.0 | 86.7 ± 0.8 | 84.4 ± 1.0* | 85.7 ± 0.8* |
| Degree of esterification (%) | 66.7 ± 0.4 | 67.9 ± 0.2* | 68.2 ± 0.4* | 69.1 ± 0.5* |
| Glycine (%, w/w) | 0.20 ± 0.0008 | 1.05 ± 0.06* | 5.23 ± 0.06* | 1.80 ± 0.04* |

The modified pectins were prepared with 0.2 M modifying agents. The values are averages ± SE of three independent experiments with two assays.
* $p < 0.05$, significant difference as compared to the unmodified pectin.

($p < 0.05$). It appeared that the increase of DE resulted from an incorporation of modifying agents into pectin molecules and that the modification had an influence on total charge in the molecules. Concerning the contents of glycine (0.20%, w/w) in the unmodified pectin, the glycylglycine was considerably more incorporated into the pectin molecules (glycine contents: 5.23%, w/w) when compared to the glycine-modified pectin (glycine contents: 1.05%, w/w). However, as a whole, it seemed that chemical analysis did not yield meaningful differences among different modified pectins in respect of their dissolution.

### 3.4. FT-IR

In order to confirm the presence of modified pectins by chemical analysis, both unmodified and modified pectins were analyzed by FT-IR (Fig. 6). No significant difference between the unmodified pectin and the glycylglycine-modified pectin was observed in the characteristic absorption bands related to anion carboxylate (1603 and 1412 $cm^{-1}$ (Gonzaga, Ricardo, Heatley, Soares, & de, 2005). In the glycine methyl ester-modified pectin, the intensities of the free carboxyl stretching band at 1412 $cm^{-1}$ decreased and those of amide III band (C–N) at 1250 $cm^{-1}$ increased (Yang & Yen, 2002). This represents the conversion of carboxyl groups into amide on the glycine methyl ester-modified pectin due to the reaction between the amino group in glycine methyl ester and the carboxyl group in pectin molecules. In addition, it seems that C=O stretching band at 1028 $cm^{-1}$ which can be attributed to some glycine methyl ester moieties that increases progressively in the glycine methyl ester-modified pectin (Gonzaga et al., 2005). It can at least be said that glycylglycine was distinct from glycine methyl ester with regard to the reaction mode into pectin molecules.

### 3.5. Potentiometric titration

Potentiometric titration gives information on possible pH-induced conformational transition. As it can be readily seen in Fig. 7, the glycine methyl ester-modified pectin showed a titration curve quite similar to that unmodified one, while the glycylglycine-modified pectin did not require a higher amount of NaOH to reach the isoelectric pH. This represents that the glycylglycine-modified pectin had more negative charge groups which was not much reflected in the degree of esterification. Considering that glycylglycine exhibits a zwitterionic character, the result was attributed to the presence of amino groups in pectin molecules as well as the possibility for the participation of carboxyl groups to modify pectins. On the other hand, the reason as to why the potentiometric titration curve of the glycylglycine-modified pectin was different from that of the glycine methyl ester-modified pectin seemed to be attributed to the fact that glycylglycine's carboxyl group increases the basicity of its amino group ($pK_2 = 8.17$) with respect to that of glycine methyl ester ($pK = 7.75$). Provided that the amino groups of modifying agents are involved in the modification, the glycylglycine-modified pectin will have more negative charge than the glycine methyl ester-modified one. However, as modification was conducted at pH 5.4, glycylglycine was of the zwitterionic form, and, by contrast, glycine methyl ester was of the cationic form. Hence, it cannot be said that the amino groups of modifying agents solely participated in the modification. Besides, there is no denying that the neutral sugars other than galacturonic acid in pectin molecules were not involved in the reaction.

### 3.6. Adsorption isotherm

According to the classification of Brunauer et al. (1938), the adsorption isotherms for the pectins were of a typical sigmoid shape of II type (Fig. 8). At water activities of the ranges from 0.05 to

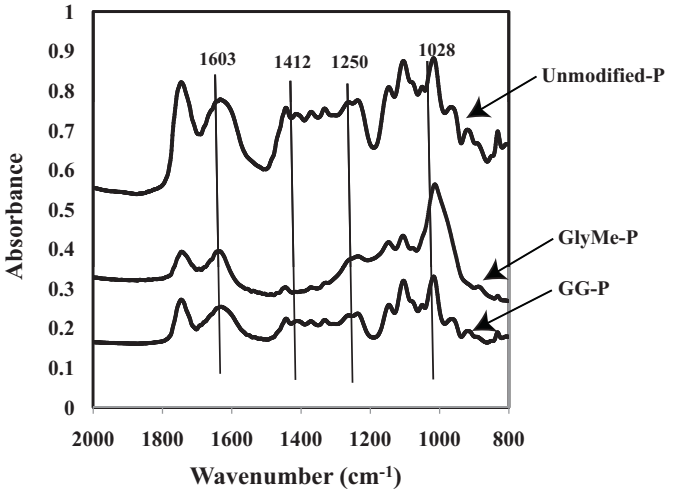


**Fig. 6.** Fourier transform infrared spectra of the pectins with and without modification. The modified sample was prepared with 0.2 M different modifying agents. Data shown are from a typical experiment that was reproduced at least three times.

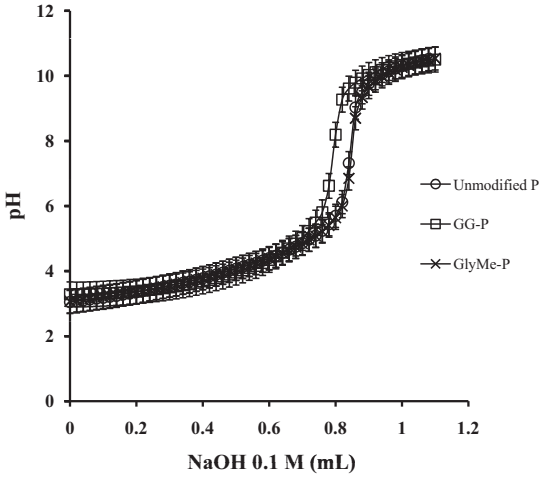


**Fig. 7.** Titration curves of the pectins with or without modification. The modified sample was prepared with 0.2 M different modifying agents. The data are averages ± SE of three independent experiments with two measurements.

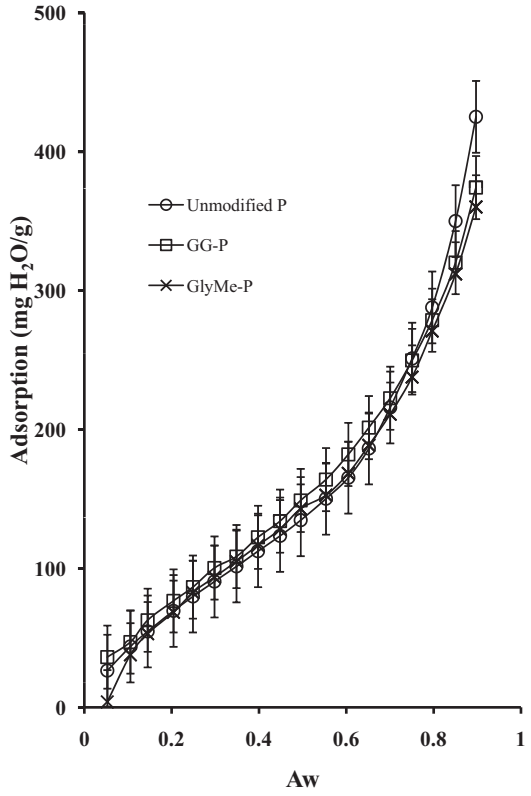


**Fig. 8.** Water vapour isotherms of the pectins with and without modification at 25 °C. The modified sample was prepared with 0.2 M different modifying agents. The data are averages ± SE of three independent experiments with two measurements.

0.30, the amounts of adsorbed water were higher in glycylglycine-modified pectin than in the unmodified pectin. Throughout the range of water activity, the glycine methyl ester-modified pectin had a lower adsorption. There was a sharp increase of the adsorbed water in the unmodified pectin for higher water activities (Aw > 0.7), which was consistent with the previous study (Tsami, Vagenas, & Marinos-Kouris, 1992).

Table 2 shows the effects of physical properties of the unmodified and modified pectins on their monolayer moisture capacity and surface area. In the glycine methyl ester-modified pectin, higher values of the monolayer capacity and surface area were observed ($p < 0.05$), while these values were not so different in both the unmodified and glycylglycine-modified pectins. Significant difference between the unmodified and modified pectins could be detected in the BET constant C ($p < 0.05$). It is to be noted that the estimated BET constant C was in the order of glycylglycine-modified pectin (100%) > unmodified pectin (81%) > glycine methyl ester-modified pectin (53%) which was consistent with the hydration behavior of the three samples (Fig. 1).

**Table 2**
Values of monolayer moisture capacity and surface area of the pectins with or without modification calculated by the BET equations.

| | Surface area ($m^2$/g) | Monolayer capacity (mg/g) | BET constant C |
|---|---|---|---|
| Unmodified P | 359.9 ± 6.9 | 86.0 ± 1.6 | 7.29 ± 0.21 |
| GG-P | 368.9 ± 8.8 | 88.2 ± 2.1 | 8.99 ± 0.35* |
| GlyMe-P | 418.1 ± 1.9* | 100.0 ± 0.5* | 4.80 ± 0.17* |

The modified sample was prepared with 0.2 M different modifying agents. The values are averages ± SE of three independent experiments with two measurements.
* $p < 0.05$, significant difference as compared to the unmodified pectin.

## 4. Discussion

In this study, there were two obvious approaches to meeting the demand for the application of water-soluble polysaccharides such as pectin to an industrially practical use: (i) pectin with superior dissolution, and (ii) its characterization with a solid comprehension of the dissolution into solvents involving water. The first approach could be overcome by using a chemical modification with intramolecular associations in polar organic solvents described in our previous study (Kurita et al., 2010). Glycylglycine was a promising agent for improving the hydration properties of pectin (Fig. 1). Glycylglycine has 3 H-bond donor and 4 H-bond acceptor (Pubchem Compound ID: 11163), while glycine has 2 H-bond donor and 3 H-bond acceptor (Pubchem Compound ID: 750). This is why glycylglycine-modified pectin was more soluble into water than glycine-modified pectin (Fig. 1). Induction of functional groups such as amino groups and carboxyl groups into pectin molecules increased the formation of hydrogen bonds which would be preferably attributed to the dissolution of pectins.

The amino group of glycine methyl ester is supposed to react with the carboxyl groups in pectin due to the presence of methyl ester protecting the reaction. In fact, the formation of amide bond III was identified by FT-IR analysis in the glycine methyl ester-modified pectin (Fig. 6). Considering that the solubility increases with increasing the degree of esterification in pectin (Iijima, Nakamura, Hatakeyama, & Hatakeyama, 2000), in other words, decreasing the carboxyl groups, increasing dissolution will be expected in the glycine methyl ester-modified pectin. On the contrary, the glycine methyl ester-modified pectin had a decreased hydration (Table 1 and Fig. 1). This implies that the incorporation of methyl group existing in glycine methyl ester into pectin molecules might lead to detrimental effect on the dissolution. Generally, the carboxyl groups of D-galacturonic acid residue in pectin determine the hydration properties (Ryden, MacDougall, Tibbits, & Ring, 2000) and tend to expand the structure of pectins as a result of their charge, unless they interact through divalent cationic bridging (Ralet, Dronnet, Buchholt, & Thibault, 2001). From the viewpoint of these findings, decreasing carboxyl groups may lead to a folding structure of pectin molecules and consequently water molecules cannot easily penetrate into the pectin molecules. This is concurrent with increasing hydrophobic interactions due to the induction of methyl group from glycine methyl ester. It is worth noticing a previous work (Ayers & Hunt, 2001) using chitosan–silica hybrid aerogels to comprehend the role of carboxyl groups in pectin molecules with regard to dissolution. It was found that as the amount of chitosan was increased, the shrinkage of the gel decreased significantly during drying and the BET surface area of the gel decreased. Chitosan is capable of forming hydrogen-bonded bridges between adjacent silica particles. Therefore, on the occasion of the formation of hydrogen-bonded bridges with difficulty, the molecules will tend to shrink and to have a more large BET surface area. In glycine methyl-modified pectin as well, the decreased carboxyl groups in the molecule will bring a poor dissolution into dispersion media due to the increased shrinkage corresponding with increased BET surface area. Meanwhile, higher values of the monolayer capacity and surface area in the glycine methyl ester-modified pectin could result from the changes in surface structure of the molecule at hydration (Jamroz, Sokolowska, & Hajnos, 1999).

Regarding the second approach in the characterization of the modified pectins, high methoxyl pectin (HM pectin) was to low methoxyl pectin (LM pectin) what glycylglycine-modified pectin was to glycine methyl ester-modified pectin in the adsorption isotherms. For lower water activities (Aw < 0.6) HM pectin is more hygroscopic than LM pectin (Tsami et al., 1992). The presence of the

methoxyl group leads to a loose packing of the pectin molecules, which allows the penetration of the water molecules. Low value of the BET constant, C (Table 2), indicates that glycine methyl ester-modified pectin is not a micropore adsorbent. Besides, the high value of the BET constant, C, in the glycylglycine-modified pectin is attributed to the higher energy of adsorption in the first adsorbed layer. The hydration enthalpies are 70.3 and 23.9 kJ/mol for the amino and hydroxyl groups, respectively (Gocho, Shimizu, Tanioka, Chou, & Nakajima, 2000). Induction of glycylglycine into pectin molecules can be interpreted as the presence of amino groups on the outer surface area. This is strongly supported by the result of potentiometric titration in the glycylglycine-modified pectin bearing more negative charges (Fig. 7). Thus, the improvement of dissolution in polysaccharides as well as biopolymer solubility such as protein, favors high charge density and low average hydrophobicity (Damodaran, 1997).

In hydration behavior, the glycylglycine-modified pectin was soluble at low temperature (Fig. 3). Hydrogen bonding is favored by low temperature. This indicates that the glycylglycine-modified pectin had lower intramolecular attractive forces by hydrogen bonding. Similarly, high ionic strength causes a decrease in the solubility of biopolymers by neutralizing the charged function groups or dehydrating the water molecules binding to their molecules (Rozenblat, Magdassi, & Garti, 1989), as well known as salting out. There is a phenomenon to estimate the contribution of intramolecular attractive forces in pectin molecule for the dissolution. At high water activity (Aw > 0.8) which was the so-called capillary condensation region, the unmodified pectin adsorbed more quantities of water than the modified pectins. In this case, the adsorbed moisture causes a subsequent swelling of the pectin, and there is an increasing availability of the polar groups to the water molecules (Tsami et al., 1992). The behavior is observed for the result that LM pectin is more hygroscopic than HM pectin at high water activity (Wallingford & Labuza, 1983). This implies that LM pectin, which has more intramolecular attractive forces than HM pectin, provides the carboxyl groups to adsorb the water at high water activity. The decreased degrees of esterification in the modified pectin could be involved in the adsorption behavior at high water activity. However, the answer as to why the dissolution of the glycylglycine-modified pectin did not change even at ionic strength above a critical maximum value in the unmodified pectin will be offered by conducting measurements of oscillatory and steady shear flows relevant with inter/intra-molecular interaction in aqueous solutions of pectin (Kjøniksen, Hiorth, & Nyström, 2005) and measurement of glass-transition temperature in pectin-water systems by differential scanning calorimetry (Iijima et al., 2000).

Compared with ionic strength, pH of dissolving solution greatly affects the hydration properties of pectin (Fig. 4). The carboxyl groups in pectin molecules should become ionized at pH 7.6 since the p$K_a$ of galacturonic acid is 3.3 (Ryden et al., 2000). At neutral pH the hydrogen bonds would be broken, electrostatic repulsion would arise between pectin molecules. As a result, water would be taken up and the dissolution would decrease. What should not be overlooked is that pectin becomes unstable as the pH increases due to the deesterification and degradation. The reason as to why the glycylglycine-modified pectin had stable dissolution at extensive pH is that its p$K_a$ is apparently higher than that of the unmodified pectin (Fig. 7).

The easily soluble pectin in this study is distinguished from modified pectin obtained by heat treatment at a relatively high humidity (Hiroe et al., 2009) due to the differences in physical and chemical modification. The utmost interest in our modified pectin is an answer to whether it is applicable as a thickener, a gelling agent and a colloidal stabilizer.

## 5. Conclusions

To our knowledge, this is the first evidence of citrus pectin with superior dissolution regardless of pH and ionic strength. The pectin was prepared by the modification with glycylglycine as a modifying agent. From the analysis of water vapor adsorption isotherm, the glycylglycine-modified pectin had a hydrophilic surface on its structure. Meanwhile, the glycine methyl ester bound to the carboxyl groups of pectin molecules which led to less dissolution of pectin. The net charge and carboxyl groups in pectin molecules have a great influence on the dissolution. To improve the dissolution, the reduction of intra/intermolecular attractive forces which are attributed to hydrogen bonds, electrostatic bonds, and hydrophobic interactions is imperative.

Certainly, improved dissolution of pectin contributes to alleviate time, energy and production cost in the food industry. However, although the soluble pectin was successfully produced, the effects of other processing parameters such as shearing rate and time, hydrostatic and dynamic high-pressures remain unknown. In view of the complexities in food industry, further intensive research about numerous combinations, for example, protein–polysaccharide complexes still wait to be tested.

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## References

Ayers, M. R. & Hunt, A. J. (2001). Synthesis and properties of chitosan–silica hybrid aerogels. *Journal of Non-Crystalline Solids*, *285*, 123–127.
Brunauer, S., Emmett, P. H. & Teller, E. (1938). Adsorption of gases in multimolecular layers. *Journal of American Chemical Society*, *60*, 309–314.
Cohen, S. A. & Strydom, D. J. (1988). Amino acid analysis utilizing phenylisothiocyanate derivatives. *Analytical Biochemistry*, *174*, 1–16.
Damodaran, S. (1997). Food proteins: An overview. In S. Damodaran, & A. Paraf (Eds.), *Food proteins and their application* (pp. 1–24). New York: Marcel Dekker, Inc.
Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.
Gocho, H., Shimizu, H., Tanioka, A., Chou, T. & Nakajima, T.-J. (2000). Effect of polymer chain end on sorption isotherm of water by chitosan. *Carbohydrate Polymers*, *41*, 87–90.
Gonzaga, M., Ricardo, L. C., Heatley, N. M. P. S., Soares, F. & de, S. A. (2005). Isolation and characterization of polysaccharides from *Agaricus blazai* Murill. *Carbohydrate Polymers*, *60*, 43–49.
Graff-Andersen, A. & Modi, J. J. (1999). Dispersible water-soluble or water-swellable polymers and process for making toothpastes containing them. *US Patent Office*, Pat. No. 5 869 029.
Hattori, M., Nagaoka, S., & Maeda H. (2001). Process for producing water-soluble polysaccharide. *US Patent Office*, Pat. No. 6 294 665 B1.
Hiroe, M., Kataoka, Y., & Funami, T. (2009). Method for modification of pectin and application thereof. *US Patent Application*. No. 20 090 110 799.
Hou, W. C., Chang, W. H. & Jiang, C. M. (1999). Qualitative distinction of carboxyl group distributions in pectins with ruthenium red. *Botanical Bulletin of Academia Sinica*, *40*, 115–119.
Iijima, M., Nakamura, K., Hatakeyama, T. & Hatakeyama, H. (2000). Phase transition of pectin with sorbed water. *Carbohydrate Polymers*, *41*, 101–106.
Jamroz, J., Sokolowska, Z. & Hajnos, M. (1999). Moisture sorption hysterisis in potato starch extrudates. *International Agrophysics*, *13*, 451–455.
Kjøniksen, A. L., Hiorth, M. & Nyström, B. (2005). Association under shear flow in aqueous solutions of pectin. *European Polymer Journal*, *41*, 761–770.
Kurita, O., Murakami, K. & Fujiwara, T. (2010). Chemical modification of polysaccharides by the use of intramolecullar associations in polar organic solvents. *Polymer Bulletin*, *65*, 443–454.
Lopes da Silva, J. A. & Rao, M. A. (2006). Pect. In Structure, uses. functionality, A. M. In:, G. O. Stephen, P. A. Phillips, & Williams (Eds.), *Food polysaccharides and their applications* (pp. 353–411). Boca Raton, FL: Taylor & Francis.
Orchiston, H. D. (1954). Adsorption of water vapor: II. Clays at 25 °C. *Soil Science*, *78*, 463–480.
Ralet, M.-C., Dronnet, V., Buchholt, H. C. & Thibault, J.-F. (2001). Enzymatically and chemically de-esterified lime pectins: Characterisation, polyelectrolyte behaviour and calcium binding properties. *Carbohydrate Research*, *336*, 117–125.
Rozenblat, J., Magdassi, S. & Garti, N. (1989). Effect of electrolytes, stirring and surfactants in the coacervation and microencapsulation process in presence of gelatin. *Journal of Microencapsulation*, *6*, 515–526.

Ryden, P., MacDougall, A. J., Tibbits, C. W. & Ring, S. G. (2000). Hydration of pectic polysaccharides. *Biopolymers*, *54*, 398–405.
Sist, P., Cescutti, P., Skerlavaj, S., Urbani, R., Leitão, J. H., Sá-correia, I., et al. (2003). Macromolecular and solution properties of Cepacian: The exopolysaccharide produced by a strain of *Burkholderia cepacia* isolated from a cystic fibrosis patient. *Carbohydrate Research*, *338*, 1861–1867.
Sriamornsak, P. (2003). Chemistry of pectin and its pharmaceutical uses: A review. *Silpakorn University International Journal*, *3*, 206–228.
Thakur, B. R., Singh, R. K. & Handa, A. K. (1997). Chemistry and uses of pectin—A review. *Critical Reviews in Food Science and Nutrition*, *37*, 47–73.
Tsami, E., Vagenas, G. K. & Marinos-Kouris, D. (1992). Moisture sorption isotherms of pectins. *Journal of Food Processing and Preservation*, *16*, 151–161.
Wallingford, L. & Labuza, T. P. (1983). Evaluation of the water binding properties of food hydrocolloids by physical/chemical methods and in a low fat meat emulsion. *Journal of Food Science*, *48*, 1–5.
Walter, W. M., Jr., Fleming, H. P. & MacFeeters, R. F. (1993). Base-mediated firmness retention of sweet potato products. *Journal of Food Science*, *58*, 813–816.
Yang, J. & Yen, H. E. (2002). Early salt stress effects on the changes in chemical composition in leaves of ice plant and Arabidopsis: A Fourier transform infrared spectroscopy study. *Plant Physiology*, *130*, 1032–1042.