

CHARGE MODIFICATION OF CITRUS AND SUGAR BEET PECTINS AND
PHYSICOCHEMICAL PROPERTIES OF PECTIN-WHEY PROTEIN COMPLEXES

by

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(Under the Direction of Louise Wicker)

ABSTRACT

The hypothesis was that pectinmethylesterase (PME) fractions with different peptides will have different deesterification action pattern. Also physicochemical and rheological properties of pectin-whey protein isolate (WPI) complexes will be affected by and intrinsic factor, such as degree of esterification (DE), as well as extrinsic factors, such as heat-treatment and pH. Based on this hypothesis, the objectives of this research were to deesterify citrus and sugar beet pectin by PME fractions with different peptides, to determine DE and distribution of deesterified groups of modified pectin, and to characterize gelling property of original/modified pectin in the presence of calcium. Also, the physicochemical properties of original/modified pectin-WPI were investigated with/without heat-treatment (at 75°C for 20 min) at two different pHs (4.5 and 7). Finally, the effect of different DE of pectin on rheological property of pectin-WPI complexes was investigated, as well as effects of heat-treatment and pH.

PME with 36 kDa peptide showed highest specific activity followed by 27/36 kDa peptides, 13/27/36 kDa peptides, and 27 kDa peptide. All PMEs showed block wise deesterification pattern in citrus and sugar beet pectin, which increased sequential structure of deesterified groups and decreased sequential structure of methyl-esterified groups. Elastic

property (G') of citrus and sugar beet pectin in the presence of calcium increased from 10.35 up to 1354.60 Pa and from 0.05 to 201.02 Pa by PME modification.

The following physicochemical properties of WPI were affected by the presence of pectin: surface hydrophobicity, zeta potential, and particle sizes. The presence of citrus and sugar beet pectin decreased surface hydrophobicity, zeta potential, and particle size of WPI at pH 4.5. Heat-treatment at 75°C for 20 min increased surface hydrophobicity of pectin-WPI complex. Zeta potential of WPI only at pH 7.0 was more negative than at pH 4.5. The presence of citrus or sugar beet pectin made zeta potential more negative at pH 4.5. The particle size of WPI only was increased with heat-treatment and at pH 4.5. The added citrus or sugar beet pectin decreased particle sizes of WPI at pH 4.5.

Regardless of pH and heat-treatment, elastic property (G') of modified citrus pectin-WPI were significantly ($p \leq 0.05$) higher than those of original citrus- or original/modified sugar beet pectin-WPI. Heat-treatment at 75°C for 20 min increased G' of modified citrus pectin-WPI complex. At the combination of pH 4.5 and heat-treatment, G' of modified sugar beet pectin-WPI mixture was significantly higher than that at pH 7 or without heat-treatment.

INDEX WORDS: Pectinmethylesterase, Citrus pectin, Sugar beet pectin, Degree of esterification, Distribution of deesterified groups, Whey protein isolate, Pectin-whey protein isolate complex, Surface hydrophobicity, Zeta potential, Particle size, Rheological property

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DEDICATION

I would like to dedicate this dissertation to my family for their love and support.

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CHAPTER 1

INTRODUCTION

Pectin is a complex structural polysaccharide found in cell wall of plants, which has been used in processed food as gelling agents (Nelson, Smit, & Wiles, 1995; Voragen, Pilnik, Thibault, Axelos, Renard & Stephen, 1995). Partially methyl-esterified α -1 \rightarrow 4-linked D-galacturonic acids form the backbone in pectin which are interrupted by 1 \rightarrow 2-linked L-rhamnose residue with side chains of neutral sugars (Voragen, Pilnik, Thibault, Axelos, Renard & Stephen, 1995).

Pectinmethylesterase (PME, E.C. 3.1.1.11) catalyses demethoxylation of esterified groups in pectin backbone. Plant PME deesterify high methoxyl pectin by block wise pattern, which yield block structures on the homogalacturonan backbone. This block structure can induce calcium cross linking of pectin chains (Hotchkiss, Savary, Cameron, Chau, Brouillette, Luzio, & Fishman, 2002)

Whey protein isolate (WPI) was selected as protein source in this study due to its well-defined characteristics and various applications, such as emulsifiers and gelling agents (de Wit, 1998; Kinsella & Whitehead, 1989a). Generally improved functionalities of protein-polysaccharide complex have been reported due to the combination of the functionality of each component (Tolstoguzov, 1997; Tolstoguzov, 1994; Tolstoguzov, 1991; Tolstoguzov, 1986, Ledward, 1994; Ledward, 1979; Samant, Singhal, Kulkarni, & Rege, 1993; Dickson, 1995).

The objectives of this research were to deesterify citrus and sugar beet pectin by PME, to characterize modified pectins, and to investigate the physicochemical/rheological properties of original/modified pectin-WPI. In the third chapter, we prepared PME fractions containing different peptides and used them to modify citrus and sugar beet pectin. NMR was used to determine DE and distribution of deesterified groups. Modified pectins were characterized by

viscoelastic properties in the presence of calcium. In the fourth and fifth chapters, the physicochemical properties of original/modified citrus or sugar beet pectin-WPI were investigated with treatments, such as heat and pH. In the sixth chapter, effect of different DE of pectin on rheological property of pectin-WPI complexes was investigated, as well as effects of heat-treatment and pH.

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CHAPTER 2

LITERATURE REVIEW

Pectin Methylesterase

Pectin methylesterase (PME, EC 3.1.1.11) hydrolyzes C₆-methylester groups in homogalacturonan regions of pectin. Depending on sources of PME, PMEs show different action pattern in removing methyl ester group (Ralet, Dronnet, Buchholt, & Thibault, 2001; Limberg, Korner, Buchholt, Christensen, Roepstorff, & Mikkelsen, 2000; Savary & Hotchkiss, 2002; Denes, Baron, Renard, Pean, Drilleau, 2000). Acidic microbial (*Aspergillus japonicus*, *Aspergillus niger*, and *Aspergillus foetidus*) PMEs have random deesterification pattern to form random distribution of free carboxyl groups (Thibault & Rinaudo, 1985; Ralet, Dronnet, Buchholt, & Thibault, 2001). Plant PMEs have been reported to have block wise deesterification. These PMEs start to proceed linearly along the backbone from a free carboxyl group or the chain reducing end and produce a block of deesterified pectin (Rexová Benková & Markovič, 1976; Speiser, Eddy, & Hill, 1945; Solms, & Deuel, 1955; Lee, & Macmillan, 1970; Bordenave, 1996).

PME from Valencia orange pulp was reported to have block wise deesterification pattern (Kim, Teng, & Wicker, 2005). Also, individual isozyme in orange can have different expression pattern, physical and biochemical properties (Bordenave, 1996). Kim et al (2005) reported that PME extract containing 36 and 27 kDa had higher PME activity than that containing 36 and 13 kDa. In the study about clarification of orange juice (Ackerley, Corredig, & Wicker, 2002), thermolabile PME containing 36 and 27 kDa protein bands caused rapid clarification of orange juice. In contrast, PME containing 36 and 13 kDa protein bands did not show clarification of

orange juice. Three protein bands were identified with PME activity and molecular weights were 34, 27, and 8 kDa, respectively (Savary, Hotchikiss, & Cameron, 2002).

Pectin Structure

Pectin is a complex structural polysaccharide in cell wall of plants, which has been used in processed food as gelling agents (Nelson, Smit, & Wiles, 1995; Voragen, Pilnik, Thibault, Axelos, Renard & Stephen, 1995). Partially methyl-esterified α -1 \rightarrow 4-linked D-galacturonic acids are main the chain in pectin structure. These main chains are interrupted by 1 \rightarrow 2-linked L-rhamnose residue with side chains of neutral sugars, such as galactose and arabinose (Voragen, Pilnik, Thibault, Axelos, Renard, & Stephen, 1995). The homogalacturonan portions of the polymer are referred to as the smooth regions, while the rhamnose-rich zones are called hairy regions, which carry neutral oligosaccharide side chains.

Mass spectrometer, and ^{13}C and ^1H NMR have been used to determine the primary structure of pectin. (De Vries, Rombouts, Voragen, & Pilnik, 1982; Limberg, Korner, Korner, Buchholt, Christensen, Roepstorff, & Mikkelsen, 2000). In addition to these techniques, various techniques have been reported to determine pectin structure, which can be applied to solid-state pectin or liquid-state pectin. Transmission electron microscopy (Fishman, 1993), fiber diffraction (Walkinshaw & Arnott, 1981), atomic force microscopy (Kiby, 1996), and ^{13}C NMR (Jarvis & Apperley, 1995) have been used to determine structure of solid-state pectin. The techniques for liquid-state include light scattering (Ousalem, Busnel, & Nicolai, 1993), viscometry (Cesaro, Ciana, Delben, Mazini, & Paoletti, 1982; Axelo, Lefebvre, & Thibault, 1987; Harding, Berth, Ball, Mitchell, & de la Torre, 1991), ^{13}C NMR (Catoire, Derouet, Redon, Goldberg, & du Penhoat, 1997), potentiometry and circular dichroism (Ravanat & Rinaudo, 1980), small-angle

neutron scattering (Cros, Ganier, Axelos, Imberty, & Perez, 1996), and small-angle X-ray scattering (Axelos, Garnier, Renard, & Thibault, 1996).

Degree of Esterification

Degree of esterification (DE) can be defined as the moles of methoxyl groups per 100 moles of galacturonic acid residues. Depending on DE value, pectins with DE>50%, which have more than one-half of the carboxyl groups in the methyl ester form (-COOCH₃), are known as high methoxyl pectins (HMP), and those with DE<50% are low methoxyl pectins (LMP). (Hill, Motern, Nutting, & Speiser, 1949; Rinaudo, 1996).

Chromatography techniques, such as gas-liquid chromatography (GLC) and high performance liquid chromatography (HPLC) have been used to determine DE in pectin. The GLC method has advantage in terms of small sample requirement (about 100 µg). The sample preparations are conducted before GLC analysis, which include reduction of galacturonic acid residue, methanolysis, and derivation (Mannes, Ryan, & Mort, 1990). This sample preparation procedure is time consuming mainly due to reduction of galacturonic acid residue. HPLC technique is widely used for determination of pectin structure. There are two disadvantages in HPLC technique. The first one is large sample requirement, approximately 30 mg. The second disadvantage is potential error in integration of methanol peak in chromatogram. Normally, a negative peak is present before a methanol peak, which could contribute potential error in integration (Levigne, Thomas, Ralet, Quemener, & Thibault, 2002). Alternatively, enzymatic methods and titration or colorimetry method can be used to determine pectin structure.

NMR and FT-IR also have been used as direct measurement methods of DE (Anderson, Larsen, & Grasdalen, 1995; Gnanasambandam & Proctor, 2000; Hunter, 2002). ¹H NMR has

been widely used to determine DE of pectin as well as pectin structure. In the ^1H NMR spectra of pectin, chemical shifts (δ) of protons of E (esterified galacturonic acid) and G (de-esterified galacturonic acid) are used for determination of %DE. With NMR method, it takes only a few minutes to obtain DE from small quantity of pectin sample, without external standards. It was demonstrated that there was no difference between DE values by NMR and those by titration method (Rosenbohm, Lundt, Christensen, & Young, 2003).

Distribution of de-esterified groups

As well as DE of pectin, distribution of de-esterified groups in pectins affects the functionalities of pectin, such as a stabilizing, gelling, and calcium binding properties. (Glahn, 1982; Christensen, Keiberg, Thorsøe, Buchholt, Rasmussen, & Nielsen, 1997; Wicker, Ackerley, & Hunter, 2003; Limberg et al., 2000; Ralet et al., 2001; Willats, Orfila, Limberg, Buchholt, van Alebeek, Voragen, Marcus, Christensen, Mikkelsen, & Murray, 2001). ^1H NMR is used to determine distribution of de-esterified groups as well as DE of pectin. Dyads, triads, and some tetrads in partially esterified galacturonic acid could be differentiated in ^1H NMR spectra, which measured and characterized block de-esterification (Anderson et al., 1995). Furthermore, action pattern of PME can be determined through the combination of ^1H NMR spectra with ion exchange column method. Different action pattern of apple PME was determined depending on pH through combination of ^1H NMR and ion exchange column method (Denes et al., 2000). They reported that higher frequency of F_{GGG} than the Bernoullian probabilities were blockwise distribution as DE decreased. High content of homogeneous triads (GGG and EEE) by enzymatic reaction was reported, which demonstrated production of sequential structure (Grasdalen et al., 1996). It was reported that stronger lines in the spectra, corresponding to contiguous

arrangements of esterified and de-esterified units denoted by EE, EEE, and GG, indicated block-type distribution in enzyme treated sample (Anderson, Larsen, & Grasdalen, 1995).

Pectin Gel

Depending on methyl ester group, expressed as degree of methyl esterification (DE), pectin can be classified as low methoxyl (DE < 50%, LMP) and high methoxyl pectin (DE > 50%, HMP), which have different gelling mechanism (Hill, Mottern, Nutting, & Speiser, 1949; Rinaudo, 1996). Acidic medium and water soluble solute, typically sucrose (65%) are required for HMP gelling through hydrogen bonds and hydrophobic interaction (Rinaudo, 1996). LMP gelling can be induced through ionic interactions of polyvalent cations, such as calcium, with free carboxyl groups in the pectin backbone without sucrose (Hill, Motern, Nutting, & Speiser, 1949; Rinaudo, 1996). It is similar manner to dimeric egg-box structure for alginate, in which the participating sequences adopt a two fold zigzag conformation, with chelation of calcium ions to carboxyl groups along inner faces of both chains (Grant, Morris, Rees, Smith, & Thom, 1973).

Normally, calcium binding strength of pectin increases as DE value of pectin decreases (Thibault & Rinaudo, 1985; Ralet, Dronnet, Buchholt, & Thibault, 2001; Kohn, 1975). Transition in calcium affinity toward randomly charged pectins occurs around 40% DE (Ralet et al., 2001). In addition to DE, other intrinsic and extrinsic parameters affect calcium binding, such as charge distribution, the number and size of side chain, molecular weight, ionic strength, pH, temperature, and cosolutes (Garnier, Axelos, & Thibault, 1993; Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). Different distribution free carboxyl groups attributed to different gelling properties in LMP with similar DE (Heri, Neukom, & Deuel, 1961; Kohn, Furda, & Kopec, 1968). It was reported that DE and de-esterification pattern affect elasticity of pectin gels in the presence of calcium (Willats et al., 2001). With two pectins, which had different DE but

same distribution of de-esterified groups, lower-DE pectin gel was stronger than higher-DE pectin gel. With pectins having similar DE and different distribution of de-esterified groups modified by plant PME and fungal PME, there was a 3-fold difference in yield point of gels. Pectin with block-wise distribution by enzymatic de-esterification showed linear relationship between calcium binding and fraction of free carboxyl groups. In contrast, random distribution of free carboxyl groups obtained by chemical de-esterification showed non-linear relationship (Powell, Morris, Gidley, & Rees, 1982).

Whey Protein Isolate

Whey is by-product of cheese and casein manufacture. Whey proteins are globular proteins with limited number of disulfide bonds (Fox & Mulvihill, 1982; Swaisgood, 1982). Compared to casein, whey proteins are more heat sensitive, less calcium sensitive, and can engage in thio-disulfide interchanges to form oligomeric structure (Fox & Mulvihill, 1982; Swaisgood, 1982). Whey protein isolate (WPI) contains over 90% of protein. Ion exchange can be used to prepare WPI. Whey proteins are loaded into the cellulose-based cation exchanger. Non protein components were eluted and proteins are absorbed onto the cation exchanger. The absorbed protein portions can be eluted by pH adjustment to >5.5 . Eluted proteins are concentrated by ultrafiltration, evaporated, and spray-dried (Palmer, 1981). Other ion exchange processes involving Spherosil-S and Spherosil-QMA were reported to result in whey protein concentrates with high ash contents and poor solubilities (Nichols & Morr, 1985; Barker & Morr, 1986).

Major proteins in whey protein are β -lactoglobulin, α -lactalbumin, and bovine serum albumin. Whey protein is about 50% β -lactoglobulin, which is responsible for the functional properties (de Wit, 1989; Ziegler & Foegeding, 1990). β -lactoglobulin exists as a dimer in

solution because of electrostatic interaction between Asp¹³⁰ and Glu¹³⁴ of one monomer with corresponding lysyl residues of another monomer (Creamer, Parry, & Malcolm, 1983). Heat treatment above 65°C induce time/temperature dependant denaturation of β -lactoglobulin. Through the induced denaturation, extensive conformational transition occurs resulting in exposure of highly reactive nucleophilic group in hydrophobic regions (Kella & Kinsella, 1988).

α -Lactalbumin accounts for 25% of whey protein. The ratio of α -lactalbumin to β -lactoglobulin in bovine milk is approximately 1:3. α -lactalbumin is a compact globular protein containing excellent essential amino acid profile rich in lysine, leucine, threonine, tryptophan, and cysteine. The biological function of α -lactalbumin is to modulate the substrate specificity of galactosyltransferase in the lactose synthesis complex, which is responsible for synthesis of lactose in lactating mammary tissue (Hill & Brew, 1975). The association of α -lactalbumin to the lactose synthesis complex catalyzes the addition to galactose in Gogi apparatus (Jones, 1978). α -Lactalbumin was reported to bind calcium, which may stabilize the molecule against irreversible thermal denaturation (Hiraoka & Sugai, 1984). Binding site for a zinc ion ions was also reported in α -lactalbumin (Murakami & Berliner, 1983).

Bovine serum albumin is a large globular protein with good essential amino acid profile. The protein consists of a single polypeptide chain containing about 580 amino acid residues with 17 intra-chain disulfide bonds and one free thiol group at residue 34. The distribution of disulfide bonds and location of specific residues throughout the polypeptide chain suggest that the albumin molecule folds to form three structural domains and nine subdomains (Brown, 1977). The multidomain structure of bovine serum albumin is responsible for the anomalous behavior of the protein under denaturing conditions (Pace, 1975). The domains of bovine serum albumin are dissimilar in hydrophobicity, net charge, and ligand binding sites and each has a distinct function

apart from the others (Peters & Reed, 1977). Bovine serum albumin binds free fatty acids, other lipids, and flavors which can stabilize the molecules somewhat against thermal denaturation (Damodaran & Kinsella, 1981a). Between 40 and 50°C, bovine serum albumin partially unfolds, exposing hydrophobic residue to molecular surface, facilitating reversible protein-protein interaction.

Intrinsic/Extrinsic Factors and Functionalities of WPI

WPI has been widely used in food systems as functional ingredients as emulsifier and gelling agents. (Dickson, 2003; McClements, 2004; de Wit, 1998; Kinsella & Whitehead, 1989). Intrinsic factors affect the functionalities of WPI such as amino acid composition, conformation, molecular size, shape, flexibility, net charge, hydrophobicity, substituent chemical groups, and sulfhydryl groups. In addition to intrinsic factors extrinsic factors, such as temperature, pH, and ion concentration, also affect the functionalities of WPI. The knowledge of relationship between intrinsic and extrinsic factors is required to control functional behavior in different applications and modify protein and process conditions to optimize desirable functions (Kinsella, 1982, 1984).

The folding behavior and reactivity of proteins are determined by the amino acid composition. Protein with a high content of polar and charged amino acid can be used as emulsion stabilizer due to its tendency to be exposed to aqueous phase and bind water. High content of hydrophobic amino acid residue is responsible for surface activity of protein. (Kato & Nakai, 1980). But these hydrophobic amino acid residues can negatively affect gelling properties because of extensive self-association by hydrophobic interaction resulting in coagulation (Fox & Mulvihill, 1982; Swaisgood, 1982; Morr, 1985).

The native structure of whey protein exhibits a thermodynamic equilibrium. Environmental conditions may affect protein conformation (Creighton, 1985; Karples &

McCammon, 1986). Noncovalent forces involved in stabilizing native protein structure are hydrogen binding, hydrophobic, van der Waals' and electrostatic interaction. Covalent disulfide bond is important in maintaining the structural integrity of extracellular proteins (Schulz & Schirmer, 1979). Changes in environmental factors affect these noncovalent forces which results in change in conformation of protein. Protein unfolding and protein-protein interaction also can be facilitated by cleavage of disulfide bonds, resulting in coagulation or gelation (Schmidt, 1981). Acidic conditions can affect calcium binding (Bernal & Jelen, 1984). Temperature treatments greatly affect whey protein conformation, which result in dissociation of β -lactoglobulin and exposure of the reactive thiol group of β -lactoglobulin upon heating (Watanabe & Klostermeyer, 1976).

The denaturation of whey protein occurs above 65 and 70°, which may result in coagulation after heat treatment (Patocka, Drathen, & Jelen, 1987). A number of factors can affect the nature, extent, and rate of denaturation, such as pH, ionic strength, protein concentration, time, and temperature (Kilara & Sharkasi, 1986). The thermal denaturation of whey protein is sensitive to pH. Isoelectric point of approximately 4.6 is used to recover heat-denatured whey protein. The pH affects the net charge of protein, which finally affects rate of denaturation and coagulation proteins (Harwalkar, 1986). Minimum coagulation occurs when whey proteins are heat-denatured above pH 6.5 or below critical pH range of 3.7 and 3.9 (deWit, 1981; Bernal & Jelen, 1985). The presence of calcium enhances protein aggregation following heating at particular pHs due to neutralization of electrostatic repulsions (deWit & Klarenbeek, 1981; deWit & Klarenbeek, 1984; Bernal & Jelen, 1984; Patocka, Drathen, & Jelen, 1987).

The extrinsic factors that affect functionalities are methods of preparation, isolation, drying, storage, extent of refining and purification, content and concentration of proteins,

temperature, pH, and solute concentration. The method and conditions of drying can affect the extent of denaturation, particle size, rehydration, and dispersibility (Kinsella, 1981b). The efficiency of lipid removal may affect many properties related to surface phenomena and sensory quality.

Gelation of Whey Protein

Gelation is the result of a balance between polymer molecules interacting with other polymer molecules and the interaction between polymer molecules and the solvent resulting in network. This network possesses the capacity for holding water and other materials. In terms of water holding capacity, gelation differs from coagulation. Coagulation is primarily a chain-chain interaction, and coagula are not capable of holding water. On heating above a critical temperature, protein solution undergo conformational changes and on cooling set to viscous, soft, opaque coagula or clear viscoelastic gels are formed depending on the type of protein, concentration, heating rate, and environmental conditions, especially pH and calcium (Mulvihill & Kinsella, 1988). Thermally induced gelation is a two-stage sequential process. First phase involves heat-induced conformational changes in a protein. On conformational changes in protein, some polypeptide segments undergo unfolding followed by exposure of reactive residue. Protein-protein interactions are induced through exposed reactive residues resulting in a progressive formation of a network structure when protein-protein interaction is limited (Bernal & Jelen, 1985). Thermal activation of protein molecules to expose interactive sites may involve only very minor changes in overall molecular conformation. Protein-protein interactions can lead to a three-dimensional network capable of entrapping water with adequate protein concentration and slow heating rate. If aggregation is more rapid than unfolding, a precipitate may form (Ferry, 1948). For the formation of an appropriate three-dimensional network, a balance between

attractive and repulsive forces is necessary. In the formation of a gel from whey proteins, limited unfolding exposes hydrophobic segments on proteins which tend to associate recooling, facilitating bonding between contiguous molecular groups. Networks formed vary considerably in structure depending on the number and relative reactivity of interactive sites, and the degree of electrostatic repulsion between the solute molecules. These are markedly affected by pH and ionic strength.

Whey proteins can be used as a gelling agent which exhibits various gelling properties from viscous fluid soft, smooth pastes or curds to stiff, rubbery gels. These gels also show various characteristics in terms of hardness, cohesiveness, stickiness, color, and mouthfeel (Hiller & Cheeseman, 1979; Schmidt, Cornell, & Illingworth, 1979; Hiller, Lyster, & Cheeseman, 1980; Schmidt, 1981; Kornhorst & Mangino, 1985; Zirbel, 1987). Whey protein gels also vary in visual appearance from firm elastic transparent gels to opalescent curdlike coagula. At low concentrations and low ionic strength, weak gels with gray or translucent appearance are obtained. Whey protein has excellent gelling characteristics, above pH 7.0. Undenatured, soluble whey proteins prepared under mild processing conditions form irreversible gels at the appropriate pH, ionic strength, and protein concentration. Heating β -lactoglobulin above pH 6.5 causes formation of gels, which become clearer as pH increases (deWit, 1984). Coagula are formed on heating β -lactoglobulin below pH 6.5 reflecting extensive enhanced attractive interactions. The higher gelation tendency in the region above pH 8 reflects some disulfide cross linking and matrix formation via thiol-disulfide interchange. Whey proteins can form clear gels or opaque gels. Clear gels tend to be more elastic and hold water more effectively. Apparently, they possess finer filaments, smaller mesh size, more even spacing of intermolecular linkage, and are generally formed at higher pH (Schmidt, 1981). Kornhorst & Mangino (1985) reported that

protein hydrophobicity and calcium content are important factors of whey protein gel strength. It was also demonstrated that pasteurizing milk used in cheese making affected the ability of whey protein gel at pH 6.5 but not pH 8.0 (Mangino, Liao, Harper, Morr, & Zadow, 1987). Heating ultrafiltration retentates significantly reduced gel strength. It was reported that whey protein isolates heated at 90 °C for 15 min at pH 6.5-8.5 and protein concentrations 9-10.5% form a reversible gel (Rector, Kella, & Kinsella, 1989). The melting temperature of gels at pH 8 ranged from 24.5 to 57.8 °C.

Protein – Polysaccharide Interaction

Protein-polysaccharide interaction dictates the structure and stabilization of many food systems through gelling, thickening, and surface stabilizing properties (Tolstoguzov, 1991). Since the texture and stability can be key factors for food quality, characterizing and controlling these two biopolymers interaction can be significant concern to develop new food product with the improved food quality (Sanchez, Schmitt, Babak, & Hardy, 1997). Also, functionalities of protein-polysaccharide complex generally are improved due to the combination of the functionality of each component (Tolstoguzov, 1997; Tolstoguzov, 1994; Tolstoguzov, 1991; Tolstoguzov, 1986, Ledward, 1994; Ledward, 1979; Samant, Singhal, Kulkarni, & Rege, 1993; Dickson, 1995).

Mixture of protein and polysaccharide, can lead phase separation through thermodynamic incompatibility (Zhang, Karlström, & Lindman, 1994). The mixture of these two biopolymers also can form complex coacervation (Tolstoguzov, 1991; Piculell & Lindman, 1992). In coacervation, interaction between two biopolymers is attractive and two phases can be formed, where one phase contains concentrated two biopolymers (Tolstoguzov, 1991; Piculell & Lindman, 1992).

Three types of interactions between biopolymers may be responsible for complex formation, which are interactions between the charged molecules, interactions between oppositely charged side group, and between other available side groups (Tolstoguzov, 1997). In another way, these interaction can be expressed by weak/strong, specific/not specific, and attractive/repulsive (Dickinson, 1993). Repulsive interaction can be induced in the system of nonionic or anionic polysaccharide and protein above isoelectric point of protein, where protein is negatively charged. Repulsive interaction is always nonspecific (Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998). Attractive interaction can be weak/strong, specific/not specific. Specific and strong attractive interaction can be caused by covalent bonds between protein and polysaccharide (Stainsby, 1980). The nonspecific attractive interaction between protein and polysaccharide can be induced by collective weak interactions, such as ionic, Van der Waals, hydrophobic interactions, and hydrogen bonding. Strong attractive interaction between protein and anionic polysaccharide can be induced below isoelectric point of protein. Below isoelectric point of protein, protein shows positive charge, which results in strong attractive interaction with anionic polysaccharide. Even above isoelectric point of protein, complex between protein and anionic polysaccharide can be induced due to the presence of positively charged regions on proteins (Xia & Dubin, 1994).

Effect of pH on Protein – Polysaccharide Interaction

Since pH affect ionization of functional side groups on biopolymers, it has a critical role in controlling protein-polysaccharide interactions (Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998). In the system with anionic polysaccharide and protein, maximum coacervation can be yielded below isoelectric point of protein. At this pH, protein is positively charged, which results

in strong electrostatic attraction between two biopolymers with opposite net charges (Xia & Dubin, 1994).

Maximum recovery of whey protein was found at pH 3.2 in whey protein purification process through complexation with carboxymethyl cellulose (Hansen, Hidalgo, & Gould, 1971). The complex coacervate of albumin and acacia gum was reported to be sensitive to pH. Maximum coacervate was obtained at pH 3.9 (Burgess & Singh, 1993).

Effect of Charge Density of Biopolymers on Protein – Polysaccharide Interaction

Charge density of biopolymers can be defined as the number of charges present on the biopolymer per unit length (Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998). Charge density of biopolymer is critical in complex coacervate. In the study about compatibility of bovine serum albumin with anionic synthetic polymer, poly(vinylalcohol sulfate) was used as anionic synthetic polymer, whose charge density can be controlled easily (Park et al., 1993). They reported coacervation formation above isoelectric point of bovine serum albumin, where two biopolymers carry negative net charge (Park, Muhoberac, Dubin, & Xia, 1993). Complexation of albumin with anionic polysaccharide was also reported above isoelectric point of albumin (Noguchi, 1956; Noguchi, 1959). The presence of patch, which is local positive charged region on protein, makes complexation possible. These positive patches overcome repulsion between negatively charged protein and another polymer (Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998).

Effect of Temperature on Protein – Polysaccharide Interaction

Temperature affects complex coacervation through a number of effects (Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998). Hydrogen bond is induced at low temperature, whereas hydrophobic interaction and covalent bonding are enhanced by increased temperature. This is because reactive sites of protein can be exposed through heat-denaturation of globular protein

and conformation of polysaccharide can be changed by increased temperature (Kelly, Gudo, Mitchell, & Harding, 1994). On increasing temperature from 20 to 90 °C, complex of bovine serum albumin and alginate was found up to 70 °C. This can be explained by exposure reactive site on heating and interaction of exposed reactive sites with alginate (Kelly, Gudo, Mitchell, & Harding, 1994). It was also reported that the number of complexes of whey protein and xanthan gum was increased by heat-treatment at 80 °C for 20 min (Le Hénaff, Turgeon, Sanchez, & Paquin, 1997). This result also can be explained by exposure of reactive site of whey protein and conformational change of xanthan gum by heat-treatment, which favors interactions of functional side groups of the biopolymers.

Gelation of WPI-Polysaccharide

WPI has been widely used in food system as gelling agents. (Dickson, 2003; McClements, 2004; de Wit, 1998; Kinsella & Whitehead, 1989a). In terms of gelling property, elevating temperature above room temperature induces whey protein gelling. On heating to approximately 70°C, β -lactoglobulin is dissociated from a dimer to monomer (McKenzie, 1971; Hambling, McAlpine, & Sawyer, 1992). Hydrophobic amino acid groups, which are originally located on the inside of β -lactoglobulin, can be exposed by partially unfolding of β -lactoglobulin by elevated temperature (Iametti, Cairoli, De Gregori, & Bonomi, 1995; Iametti, De Gregori, Vecchio, & Bonomi, 1996; Qi, Holt, Menulty, Clarke, Brownlow, & Jones, 1997). The exposed hydrophobic amino acid group can induce aggregation of proteins, which results in gelation of β -lactoglobulin (Totosaus, Montejano, Salazar, & Guerrero, 2002).

Polysaccharide and protein are the ingredients of many food products. These two biopolymers may dictate the textural characteristics of food products through gelling of individual ingredients and/or mixed gelling of both biopolymers (Tolstoguzov, 1991). Mixed

gelling with two different biopolymers can be formed by intermolecular attraction (complex) or intermolecular repulsion (incompatible). Both attractive and repulsive interactions have been reported to improve gelling properties (Cai & Arntfield, 1997; Ganz, 1974; Ould Eleya & Turgeon, 2000; Sanchez, Schmitt, Babak, & Hardy, 1997; Shim & Mulvaney, 2001; Smith, Nash, Eldridge, & Wolf, 1962; Wang & Qvist, 2000). Sodium alginate and sodium caseinate were reported to form complex gel under conditions where both biopolymers do not form gel (Tolstoguzov, 1986). Synergistic and antagonistic effects on gelling were observed in heat-denatured WPI-xanthan system after cold gelation (Bryant & McClements, 2000). The enhanced gelling strength at neutral pH condition was reported in high-pressure treatment of β -lactoglobulin with pectin (Dickson & James, 2000). A cloudy complex was formed in β -lactoglobulin - sodium polypectate system suggesting potential of sodium polypectate to modify β -lactoglobulin and/or whey protein gelling in various food systems (Ndi, Swanson, Dunker, & Luedecke, 1996; Ndi, Swanson, Barbosa-Canovas, & Luedecke, 1996). Gel formation was inhibited in a gelatin and κ -carrageenan system by strong electrostatic interaction (Watase & Nishinari, 1983). Antagonistic effect of adding xanthan on β -lactoglobulin gelling was also reported at higher protein concentration than 10% (Zasytkin, Dumay, & Cheftel, 1996). Therefore, overall gelling property of food protein can be modified by interaction of polysaccharides (Ndi, Swanson, Barbosa-Canovas, & Luedecke, 1996). Bertrand & Turgeon (2006) reported that increased G' of WPI by adding xanthan could be attributed to phase separation. They also reported that as pH decreases to pH 5.5, electrostatic interaction between WPI and xanthan could decrease the effect of phase separation on increase in G' of WPI (Bertrand & Turgeon, 2006). In the mixture of bovine serum albumin (BSA) and anionic polysaccharide, pectin, a one-phase viscous material was observed at pH 5.7 above isoelectric

point of BSA (Cai & Arntfield, 1997). They also reported that increase G' value by adding pectin could be explained by interaction between negatively charged free carboxyl groups in pectin molecules and positively-charged patch (NH_3^+) on BSA (Cai & Arntfield, 1997).

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CHAPTER 3

DEESTERIFICATION PATTERN OF VALENCIA ORANGE PECTINMETHYLESTERASE AND CHARACTERIZATION OF MODIFIED PECTINS¹

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Abstract

Pectinmethylesterase (PME) isozymes of differing isozyme distribution and specific activity were used to modify citrus and sugar beet pectin. HP7-10 showed highest specific activity followed by SPB+HPB, U-PME, SPU, CE, HP1-5, and HPU (no specific activity). All PMEs showed block wise de-esterification pattern in citrus and sugar beet pectin, which increased sequential structure of de-esterified groups and decreased sequential structure of methyl-esterified groups. The elastic property (G') of citrus and sugar beet pectin in the presence of calcium increased from 10.35 Pa to 1354.60 Pa and from 0.05 to 201.02 Pa, respectively by PME modification.

Keywords: Pectinmethylesterase; Block wise de-esterification; Elastic property (G')

1. Introduction

Pectin is a complex structural polysaccharide in cell wall of plants, which is used in processed food as a gelling and stabilizing agents (Nelson, Smit, & Wiles, 1995; Voragen, Pilnik, Thibault, Axelos, Renard & Stephen, 1995). Partially methyl-esterified α -1 \rightarrow 4-linked D-galacturonic acid groups form the backbone in pectin which are interrupted by 1 \rightarrow 2-linked L-rhamnose residue with side chains of neutral sugars (Voragen, Pilnik, Thibault, Axelos, Renard & Stephen, 1995). Depending on degree of methyl esterification (DE), pectin is classified as low methoxyl (DE < 50%, LMP) and high methoxyl pectin (DE > 50%, HMP) (Hill, Mottern, Nutting, & Speiser, 1949). Gelling of LMP is induced through ionic interactions of polyvalent cations, with free carboxyl groups without sucrose (Hill, Mottern, Nutting, & Speiser, 1949; Rinaudo, 1996). Normally, calcium binding strength of pectin increases as the DE value of pectin decreases (Thibault & Rinaudo, 1985; Ralet, Dronnet, Buchholt, & Thibault, 2001; Kohn, 1975).

In addition to total DE of pectin, distribution of carboxyl acid groups affects the strength of calcium binding (Thibault & Rinaudo, 1986; Tuerena, Tayler, & Mitchell, 1982). LMPs with similar DE values have different gelling properties due to the different distribution of free carboxyl groups (Heri, Neukom, & Deuel, 1961; Kohn & Furda, 1968). Calcium gelling ability of pectin increases with decreasing DE and a transition in calcium affinity for randomly distributed carboxylic acids of pectin occurs around 40% of DE (Thibault & Rinaudo, 1985; Ralet, Dronnet, Buchholt, & Thibault, 2001; Kohn, 1975). Calcium activity coefficient was defined as the ratio of the activity of calcium ions in the presence of pectins to the activity of calcium ions in ideal CaCl₂ solutions at the same ionic concentrations (Ralet, Crépeau, Buchholt, & Thibault, 2003). Pectins (DE < 60%) with block wise distribution of free carboxyl groups

were characterized by low calcium activity coefficient, which means stronger binding of calcium (Kohn, Markovic, & Machova, 1983; Thibault & Rinaudo, 1985; Ralet, Dronnet, Buchholt, & Thibault, 2001; Limberg, Körner, Buchholt, Christensen, Roepstoff, & Mikkelsen, 2000).

PMEs have different action patterns, which likely influence pectin and functionality. In citrus fruit, two PME extracts from Valencia orange pulp have block wise de-esterification patterns that differ in the extent of intra molecular de-esterification, number of molecule affected and gelling activity (Kim, Teng, & Wicker, 2005; Kim & Wicker, 2005). The PME fraction that created shorter block charge and affected more pectin molecules also clarified orange juice (Ackerley, Corredig, & Wicker, 2002). Whereas the PME fraction that created longer block and affected fewer pectin molecules did not clarify juice (Ackerley, Corredig, & Wicker, 2002; Kim, Teng, & Wicker, 2005). These PMEs are distinguished by containing 36 & 27 kDa and 36 & 13 kDa, respectively (Ackerley, Corredig, & Wicker, 2002; Kim, Teng, & Wicker, 2005).

Sugar beet pectin differs structurally from citrus pectin. Sugar beet pectin contains more acetyl groups at positions C-2 and C-3 position of galacturonic acid residues, higher protein content, higher arabinose content, and feruloyl groups attached to galactose and arabinose (Colquhoun, Ralet, Thibault, Faulds, & Williamson, 1994; Gullion & Thibault, 1988, 1989a,b; Ralet, Thibault, Faulds, & Williamson, 1994; Keenan, Belton, Matthew, & Howson, 1985). In contrast to citrus pectin, sugar beet pectin has poor gelling properties (Pippen, McCready, & Owens, 1950). However, sugar beet pectin has surface activity and emulsifying property compared to citrus pectin (Leoroux, Langedorff, Schick, Vaishnav, & Mazoyer, 2003), attributed to higher content of protein and acetyl groups in sugar beet pectin (Leoroux et al., 2003).

In this study, several PME fractions were isolated that were different in SDS-PAGE peptide pattern and specific activity. The hypotheses of this research is that PME fractions

containing different protein bands have different de-esterification patterns, and the modification pattern by PME is different for citrus and sugar beet pectin. The objectives of this study were to characterize modification pattern of PME fractions using NMR analysis, and to characterize calcium sensitivity using rheological analysis of modified pectin by PME fractions.

2. Materials and Methods

2.1. Materials

High methoxyl citrus pectin (CP Kelco, Lille Skensved, Denmark) and high methoxyl sugar beet pectin (Herbstrein & Fox Inc., Elmsford, NY) were used as pectin sources. PMEs were prepared from Valencia orange pulp (Citrus World, Lake Wales, FL).

2.2. Preparation of PME

PME was extracted and purified as described by Ackerley, Corredig, Wicker (2002). Chromatography technique was used to obtain purified and fractionated PMEs from crude PME using an Äkta Prime System (Amersham Pharmacia Biotech, Uppsala, Sweden). PME fractions were collected according to Fig. 3.1. The crude PME (CE) was loaded onto a 5 mL Hi-Trap SP cation exchange column (Amersham Pharmacia Biotech, Uppsala, Sweden). Unbound PME to Hi-Trap SP cation exchange column (SP-column), which was denoted as SPU, was loaded onto 5 mL Heparin affinity column (HP-column). Unbound PME to heparin column was collected and denoted as U-PME. Bound PME to heparin column was eluted by salt gradients with 10 mM Na phosphate, pH 7 and 1 M NaCl in 10 mM Na phosphate, pH 7. The fractions of eluted bound PME was collected and denoted as HP1-5 for first five fractions and HP7-10 for following four fractions. Bound PME to SP-column was eluted by 50 mM Na phosphate, pH 7 and 1 M NaCl in 50 mM Na phosphate, pH 7. Bound PME to SP-column was denoted as SPB. SPB was also

loaded into HP-column. Bound SPB to HP-column was eluted by 10 mM Na phosphate, pH 7 and 1 M NaCl in 10 mM Na phosphate, pH 7 and denoted as SPB/HPB. All buffers were filtered using 0.45 µm filters (Whatman, Clifton, NJ) and degassed before chromatography.

The activity of PME was measured by pH Stat Titrator (Brinkmann, Westbury, NY) at 30°C in 1% high methoxyl pectin (Genu Pectin Type B, CP Kelco, Lille Skensved, Denmark) and 0.1 M NaCl at a set pH of 7.5. A unit of PME activity was defined as the microequivalent of ester hydrolyzed/min at 30°C.

Protein contents in PMEs were determined by Bradford protein assay (Bradford, 1976) using a Microplate Reader (MPR Model 550, Bio-Rad Inc., Hercules, CA). Protein subunits of PMEs were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, Amersham Pharmacia Co., Uppsala, Sweden). PMEs (5 µl) were loaded and run on 8-25% gel. After separation, gels were silver stained according to manufacturer specification (Amersham Pharmacia Co., Uppsala, Sweden).

2.3. Modification of pectin by PME

Pectin dispersions (1%, w/v and 0.1 M NaCl) were prepared the day before the modification and stored at 4°C overnight to hydrate completely. Pectin was equilibrated to room temperature and pH was adjusted to 7.5 with NaOH. After adding PME fraction, the pH was maintained at pH 7.5 with 0.5 N NaOH for the calculated time to achieve target DE. PME activity was stopped by dropping the pH to 5.0 with 1 N HCl and boiling in 95% ethanol. After cooling to room temperature, the precipitated modified pectin was washed with ethanol and acetone. The washed pectin was dried overnight under the hood and stored at -20°C until analysis. Sugar beet pectin was modified by U-PME. Citrus pectin was modified by U-PME and other PME isolates. Modified citrus pectin/used PME for modification are U-CP/U-PME, U-

SP/U-PME, 63%/CE, 53%/CE, 43%/CE, CE-P/CE, SPU-P/SPU, HP1-5-P/HP1-5, HP7-10-P/HP7-10, SPB/HPB-P/ SPB/HPB, and HPU+HP7-10-P/ HPU+HP7-10. The modifications of U-CP, U-SP, 63%, 53%, and 43% was conducted at room temperature and other modifications were conducted at 30°C. CP and Con were unmodified citrus pectin, and SP was unmodified sugar beet pectin as controls. Controls underwent same procedures of pectin modifications without addition of PME fractions.

2.4. Degree of esterification and distribution of DE

¹H NMR spectroscopy was used to measure % DE and distribution of DE in pectin molecule. Pectin samples were lyophilized a total of five times with D₂O (6 mg pectin in 0.7 ml 50 mM phosphate buffer, pH 7.0 in D₂O and four times in 1 ml D₂O) to remove most solvent protons. The lyophilized samples were dissolved in 0.75 ml D₂O and diluted by 1 to 5 with D₂O in NMR tube. 500 Hz ¹H NMR spectra were recorded at 90°C on Varian Inova 500 spectrometer (Varian, Inc., Palo Alto, CA). ¹H NMR resonances were assigned according to the published literatures (Denes, Baron, Renard, Pean, & Drilleau, 2000; Grasdalen, Andersen, & Larsen, 1996; Kim, Teng, & Wicker, 2005). The value of DE was determined by $DE = I_E / (I_E + I_G)$, where I represents integration volume, and E and G represent esterified and deesterified resonances, respectively. The overlapped peaks were deconvoluted and integration volumes of peaks were calculated using OriginPro 7.5 software (OriginLab Co., Northampton, MA).

2.5. Rheological measurement

Rheological properties of pectin samples were measured by Controlled Stress Dynamic Rheometer™ (Rheometrics, Piscataway, NJ) equipped with cone and plate device (60 mm diameter, 0.0385°). The temperature of plate was maintained at 20°C on all measurements. Gelation of pectin sample was conducted on the plate by mixing 1.2 ml of 2% pectin and 500

mM CaCl₂ solution to have a final concentration of 35 mM. Dynamic frequency sweep test was conducted from 0.1 to 6.0 Hz at fixed stress of 1 Pa at 20°C.

3. Results and Discussions

3.1. Characterization of PME

Protein contents, PME activity, specific activity, and protein bands by SDS-PAGE are presented in Table 3.1. Protein contents of U-PME, CE, HP7-10, and SPU (0.22, 0.24, 0.24 and 0.17 µg/µl, respectively) were about 2-fold higher than those of HPU, SPB/HPB, and HP1-5 (0.07, 0.10 and 0.09 µg/µl, respectively). PME activity of HP7-10 was highest (40.00 PEU/ml) followed by U-PME (19.39 PEU/ml), CE (12.12 PEU/ml), SPB/HPB (9.09 PEU/ml), and HP 1-5 (4.24 PEU/ml) (Table 3.1). HP7-10 showed the highest specific PME activity (166.65 PEU/mg protein) followed by SPB/HPB (90.90 PEU/mg protein), U-PME (88.15 PEU/mg protein), SPU (62.27 PEU/mg protein), CE (49.67 PEU/mg protein), and HP1-5 (47.13 PEU/mg protein) (Table 3.1). Three protein bands, 36, 27 and 13 kDa were commonly found in PME fractions. HP7-10, which had the highest specific activity, had only 36 kDa protein band. U-PME and SPU showed 36 and 27 kDa protein bands. Only 27 kDa protein bands were observed in HP1-5, which had lowest specific activity. Savary et al. (2002) reported very similar protein bands from purified commercial orange PME, which were 34, 27, and 8 kDa. Partially purified PME from Valencia orange pulp containing 36 and 27 kDa protein bands was more likely to cause clarification of citrus juice than that containing 36 and 13 kDa protein bands (Wicker et al. 2003).

3.2. Degree of methyl esterification and distribution of de-esterified group

The %DE of original and modified citrus and sugar beet pectin are presented in Table 3.2. DE of citrus pectin and sugar beet pectin decreased to 47.8% from 58.3% and to 42.0% from

56.1% by U-PME, respectively. DE of Cont was 55.0%. After modification, %DE decreased to 45.3, 35.6, and 25.1% (Table 3.2). DE of CP and Cont were a little lower than Control (62% DE). In the case of CP and Cont, the pectin dispersions underwent same procedures of pectin modifications without PME fractions including adjusting pH 7.5 and dropping pH 5.0. In contrast, dispersion of Control did not undergo pH adjustment. During pH adjustment, saponification of pectin might occur, which resulted in lower DE of CP and Cont. The modified pectin by SPU showed the lowest %DE, 25.1% lower than targeted DE (Table 3.1). The %DE of SPB+HPB-P, CE-P, HP1-5-P, HPU+HP7-10-P, HP7-10-P and Israel ranged from 32.4 to 44.8% (Table 3.2). In the case of Cont, 63%, 53%, and 43%, the exact 10% reduction in %DE was obtained. Modified pectin that had lower experimental %DE than targeted ones may be due to cumulative experimental error in PME assay, pectin modification, and NMR analysis.

Signals and peak areas of G, GG and GGG of pectin in NMR spectra were increased as values of %DE decreased by modification. Citrus and sugar beet pectin modified by U-PME showed an increase in signals of G, GG, and GGG and the decrease in signals of E, EE, and EEE (Fig. 3.3). The peak areas of G, GG and GGG of CP were 13.031, 14.744 and 4.63 and increased to 15.09, 17.34 and 7.80 after modification by U- PME (Table 3.3). The decreases in peak areas of E, EE and EEE of modified citrus pectin were from 18.17, 11.50 and 13.57 to 14.26, 8.23 and 10.62 (Table 3.3). Sugar beet pectin also showed an increase in peak areas of G, GG and GGG from 11.73, 13.79 and 5.89 to 13.89, 18.44 and 9.17, and a decrease in peak areas of E, EE and EEE from 15.03, 8.19 and 12.20 to 10.07, 6.59 and 12.07 (Table 3.3). Among control, 63%, 53%, and 43%, the modified pectin with the highest peak areas of G, GG and GGG was 43%, which had lowest %DE after modification (Table 3.3). The signals of G, GG and GGG increased and those of E, EE and EEE decreased in NMR spectra in order of control, 63%, 53% and 43% (Fig.

3.2). This trend was also found in pectin samples modified by PME fractions. The highest peak areas of G, GG and GGG (21.41, 24.79 and 20.83) and lowest E, EE and EEE peak areas (7.18, 2.51 and 5.97), were observed in SPU-P, which had lowest %DE of 25.1 (Table 3.3). NMR spectra of SPU-P showed higher signals of G, GG and GGG, and lower signals of E, EE and EEE than other PME fractions modified pectins (Fig. 3.4 and 3.5). Signals and peak areas of G, GG and GGG of modified pectin by HP1-5-P, HP7-10-P, SPB+HPB-P, and HPU+HP7-10-P were higher than those of Cont (Table 3.2, and Fig. 3.3 and 3.5). Anderson et al. (1995) reported that a block-type distribution in the enzyme treated samples is indicated by stronger lines in the spectra corresponding to contiguous arrangement of esterified and de-esterified units denoted by EE, EEE, and GG, and corresponding weaker lines from residues characterizing block transitions, EG and GE. Grasdalen et al. (1996) reported that the enzyme reaction resulted in a high content of homogeneous triads (GGG and EEE) demonstrating the production of sequential structure. In this research, stronger lines corresponding GG and GGG was observed in NMR spectra, and increased GG and GGG integration volume were found after modifications by all PME fractions. This supports the conclusion that PMEs used in this research showed block-wise de-esterification.

3.3. Rheological properties of pectins with calcium

At all range of frequencies from 0 to 10 Hz, 2% citrus pectin with 35 mM CaCl₂ showed solid-like behavior but not sugar beet pectin. Sugar beet pectin showed liquid-like property at low frequency (below 0.8 Hz). G' values increased after PME modification. As peak areas of G, GG and GGG increased, G' values increased. The G' values of U-PME modified citrus pectin and sugar beet pectin at 0.4 Hz increased from 10.35 to 570.60 Pa and from 0.05 to 201.02 Pa, respectively (Table 3.4). The %DE of modified sugar beet pectin was 6% less than citrus pectin. However, G' value of modified citrus pectin was much higher than that of modified sugar beet

pectin. One of distinctive characters of sugar beet pectin is high acetyl content which hinders gelation with calcium (Pippen et al 1950). High acetyl content in sugar beet can explain that modified sugar beet pectin showed lower G' value than modified citrus pectin with calcium despite of lower %DE of modified sugar beet pectin than that of modified citrus pectin.

Among Cont, 63%, 53%, and 43%, modified pectins at 43% had highest G' , 1172.35 Pa and also had the highest peak areas of G, GG and GGG (Table 3.3 and 3.4). The same trend of higher G' with lower DE and higher peak areas of G, GG and GGG was also found in modified pectin by different PME fractions. SPU-P with lowest DE and highest peak areas of GG and GGG showed the highest G' of 1355 Pa at 0.4 Hz (Table 3.3 and 3.4). The data in Fig. 3.6a illustrates the relationship of G' value at 0.4 Hz with %DE values. A reverse sigmoid shape was obtained between G' and % DE values (Fig. 3.6a). There were two dramatic increases in G' values as % DE decreased. When % DE decreased from 55.0 to 47.8 and from 37.1 to 35.6, G' values dramatically increased from 21 to 571 Pa and from 614 to 745 Pa (Fig. 3.6a). The relationship between integration volumes of GG and GGG, and G' values is depicted in Fig. 3.6b and c. As peak areas of GG increased from 14.54 to 17.34, G' values dramatically increased from 21 to 571 Pa. Another increases in G' values were observed as peak area of GG increased from 21.44 to 22.48 (Fig. 3.6b). A similar relationship was observed between peak area of GGG and G' values (Fig. 3.6c). An increase in peak area of GGG from 4.56 to 7.80 was observed with an increase in G' values from 21 to 571 Pa. Another increase in G' value was also observed with increase in peak area of GGG from 14.00 to 15.34 (Fig. 3.6c). Schmelter et al. (2002) reported that G' -values of de-esterified pectin were increased 35-fold and the gel-like properties were markedly enhanced in the presence of calcium. Lopes da Silva et al. (1993) reported that different viscoelastic behavior can be due to the lesser extent of intermolecular association and

higher hydrodynamic volumes of low methoxyl pectin caused by the higher charge density in low methoxyl pectin (Lopes da Silva et al 1993).

4. Conclusions

PME fractions used in this study commonly had 13, 27, and 36 kDa protein. The different PME fractions from Valencia orange pulp show different enzyme activity. However, all PME fractions showed block wise de-esterification, which increased the integration volumes of block structure of free carboxyl groups. The block structure of free carboxyl groups in pectin molecules increased elastic property of pectin in the presence of calcium.

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Table 3.1. Protein content and enzyme activity of PME fractions

	Protein content ($\mu\text{g}/\mu\text{l}$)	PME activity (PEU/ml)	Specific activity (PEU/mg protein)	Protein bands by SDS-PAGE (kDa) ^b
U-PME ^a	0.22	19.39	88.15	36, 27
CE	0.24	12.12	49.67	36, 27, 13
SPU	0.17	3.64	21.52	36, 27
HPU	0.07	-	-	27
SPB/HPB	0.10	9.09	90.90	-
HP1-5	0.09	4.24	47.13	27
HP7-10	0.24	40.00	166.65	36

^a U-PME, unbound to SP cation exchange column and Heparin affinity column;

CE, crude PME extract; SPU, unbound to SP cation exchange column;

HPU, bound to SP cation exchange column and unbound to Heparin affinity column;

SPB/HPB, bound to SP cation exchange column and bound to Heparin affinity column;

HP1-5, unbound to SP cation exchange column and bound to Heparin affinity column;

HP7-10, unbound to SP cation exchange column and bound to Heparin affinity column.

^b Protein subunits of PMEs were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Gels were silver stained

Table 3.2. Degree of esterification of original and modified pectin by different fractions of PME

Pectin	Targeted degree of esterification (%)	Experimental degree of esterification (%) ^a	Pectin sources	PMEs used in modification
CP	-	58.3	Citrus	-
U-CP	48	47.8	Citrus	U-PME
SP	-	56.1	Sugar beet	-
U-SP	45	42.0	Sugar beet	U-PME
Cont	-	55.0	Citrus	-
63%	45	45.3	Citrus	CE
53%	35	35.6	Citrus	CE
43%	25	25.8	Citrus	CE
CE-P	45	40.0	Citrus	CE
SPU-P	45	25.1	Citrus	SP
HP1-5-P	45	40.5	Citrus	HP1-5
HP7-10-P	45	44.8	Citrus	HP7-10
SPB/HPB-P	45	37.1	Citrus	SPB/HPB
HPU+HP7-10-P	45	42.6	Citrus	HPU+HP7-10
Israel	52	32.4	Citrus	
Control	-	62.0	Citrus	

^a Obtained from ¹H NMR spectra.

Table 3.3. Relative peak areas of monad, dyad and triad of PME modified pectin in NMR spectra

Pectin ^a	Monad		Dyad				Triad				
	G	E	GG	GE	EG	EE	GGG	GGE	EGG	EGE	EEE
CP	13.03 ^b	18.17	14.74	9.04	7.43	11.50	4.63	3.14	5.56	1.01	13.57
U-CP	15.09	14.26	17.34	8.60	6.78	8.23	7.80	4.51	5.49	0.79	10.62
SP	11.73	15.03	13.79	12.57	8.80	8.19	5.89	3.26	3.87	5.12	12.20
U-SP	13.89	10.07	18.44	9.64	6.82	6.95	9.17	4.39	3.75	4.80	12.07
Cont	13.81	16.91	14.54	8.30	7.64	10.76	4.56	3.03	5.41	1.79	13.24
63%	16.05	13.32	16.74	9.69	6.11	8.04	8.62	3.68	5.68	1.27	10.81
53%	18.86	10.43	22.48	7.88	4.22	5.34	15.34	2.51	3.34	2.27	7.35
43%	22.69	7.88	26.80	6.08	3.15	4.09	17.28	3.38	2.11	1.34	5.21
CE-P	18.21	12.13	19.94	8.62	5.02	7.65	11.18	4.72	4.26	1.18	7.88
SP-P	21.41	7.18	24.79	7.54	2.02	2.51	20.83	2.92	3.39	1.44	5.97
HP1-5-P	16.84	11.48	19.94	8.62	5.02	7.65	11.11	3.81	4.49	1.17	9.87
HP7-10-P	15.68	12.71	18.50	7.90	6.71	9.61	8.27	3.87	4.05	1.84	10.86
SPB/HPB-P	17.71	10.43	21.44	8.21	4.56	6.60	14.00	4.21	3.55	1.09	8.22
HPU+HP7-10-P	16.31	12.10	18.66	7.30	6.43	7.92	10.09	4.04	4.64	1.27	11.24
Israel	17.00	8.16	20.10	10.31	3.01	4.44	17.13	5.28	4.85	2.52	7.20

^a CP and Cont, unmodified citrus pectin; U-CP, modified citrus pectin by U-PME;

SP, unmodified sugar beet pectin; U-SP, modified sugar beet pectin by U-PME;

63%, 53%, 43%, and CE-P, modified citrus pectin by CE;

SP-P, modified citrus pectin by SPU;

HP1-5-P, modified citrus pectin by HP1-5;

HP7-10, modified citrus pectin by HP7-10;

SPB/HPB-P, modified citrus pectin by SPB/HPB;

HPU+HP7-10-P, modified citrus pectin by HPU+HP7-10.

^b Average of 4 measurements.

Table 3.4. Storage (G') and loss (G'') modulus of pectins in the presence of 35 mM calcium chloride at 1 Pa

Pectin ^a	0.4 Hz		4 Hz	
	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)
CP	10	1.8	15	7.4
U-CP	571	33	609	42
SP	0.05	0.1	1.8	0.2
U-SP	201	14	224	16
Cont	21	2.1	26	6.3
63%	556	30	594	37
53%	748	83	851	95
43%	1172	144	1311	173
CE-P	584	41	653	35
SP-P	1355	197	1541	201
HP1-5-P	571	39	620	29
HP7-10-P	563	39	577	31
SPB+HPB-P	614	51	641	661
HPU/HP7-10-P	570	27	606	24

^a CP and Cont, unmodified citrus pectin; U-CP, modified citrus pectin by U-PME; SP, unmodified sugar beet pectin; U-SP, modified sugar beet pectin by U-PME; 63%, 53%, 43%, and CE-P, modified citrus pectin by CE; SP-P, modified citrus pectin by SPU; HP1-5-P, modified citrus pectin by HP1-5; HP7-10, modified citrus pectin by HP7-10; SPB/HPB-P, modified citrus pectin by SPB/HPB; HPU+HP7-10-P, modified citrus pectin by HPU+HP7-10.

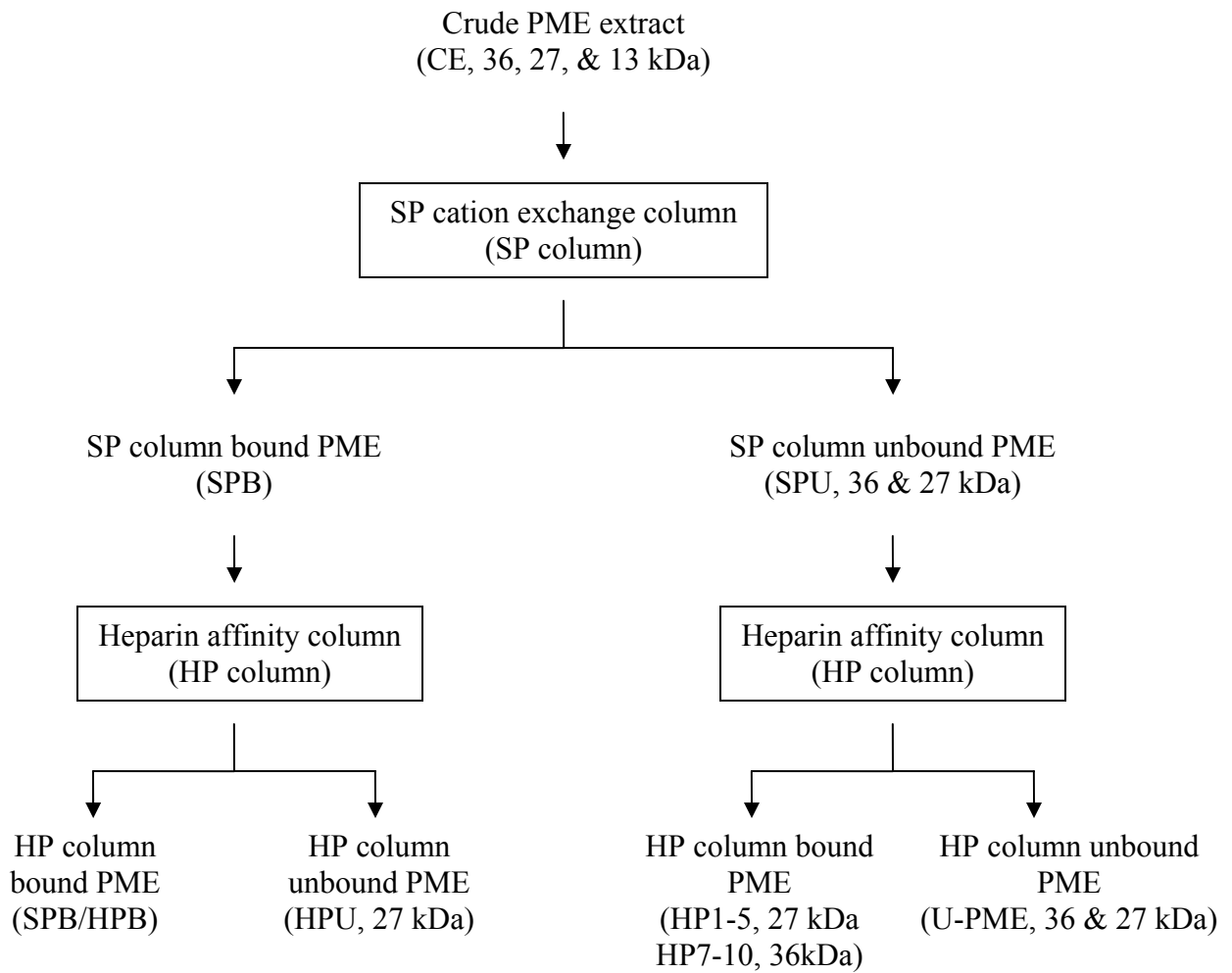


Fig. 3.1. Diagram for PME purification by SP cation exchange and Heparin affinity columns.

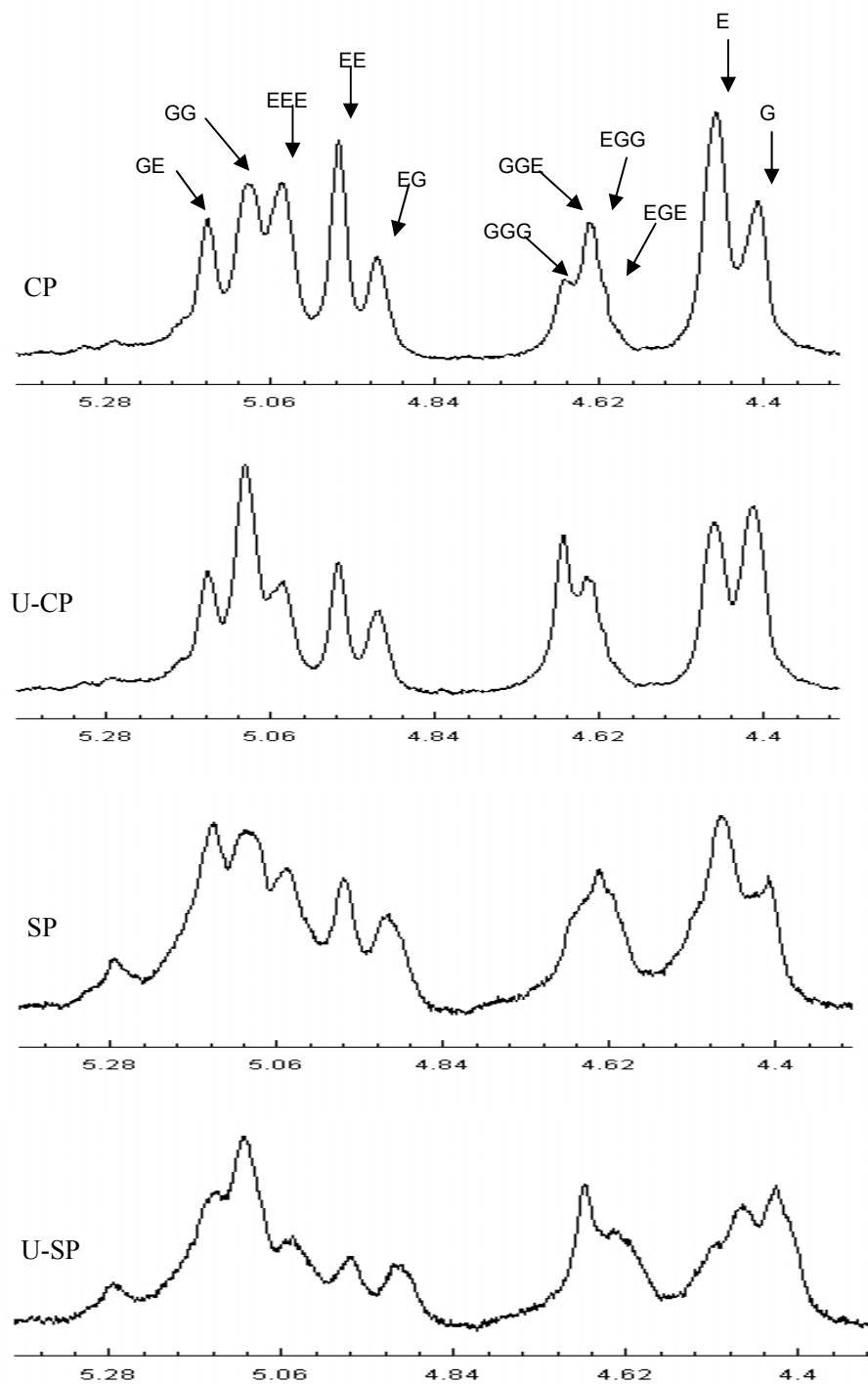


Fig. 3.2. ^1H NMR spectra of original/modified citrus and sugar beet pectin with different degree of methyl-esterification.

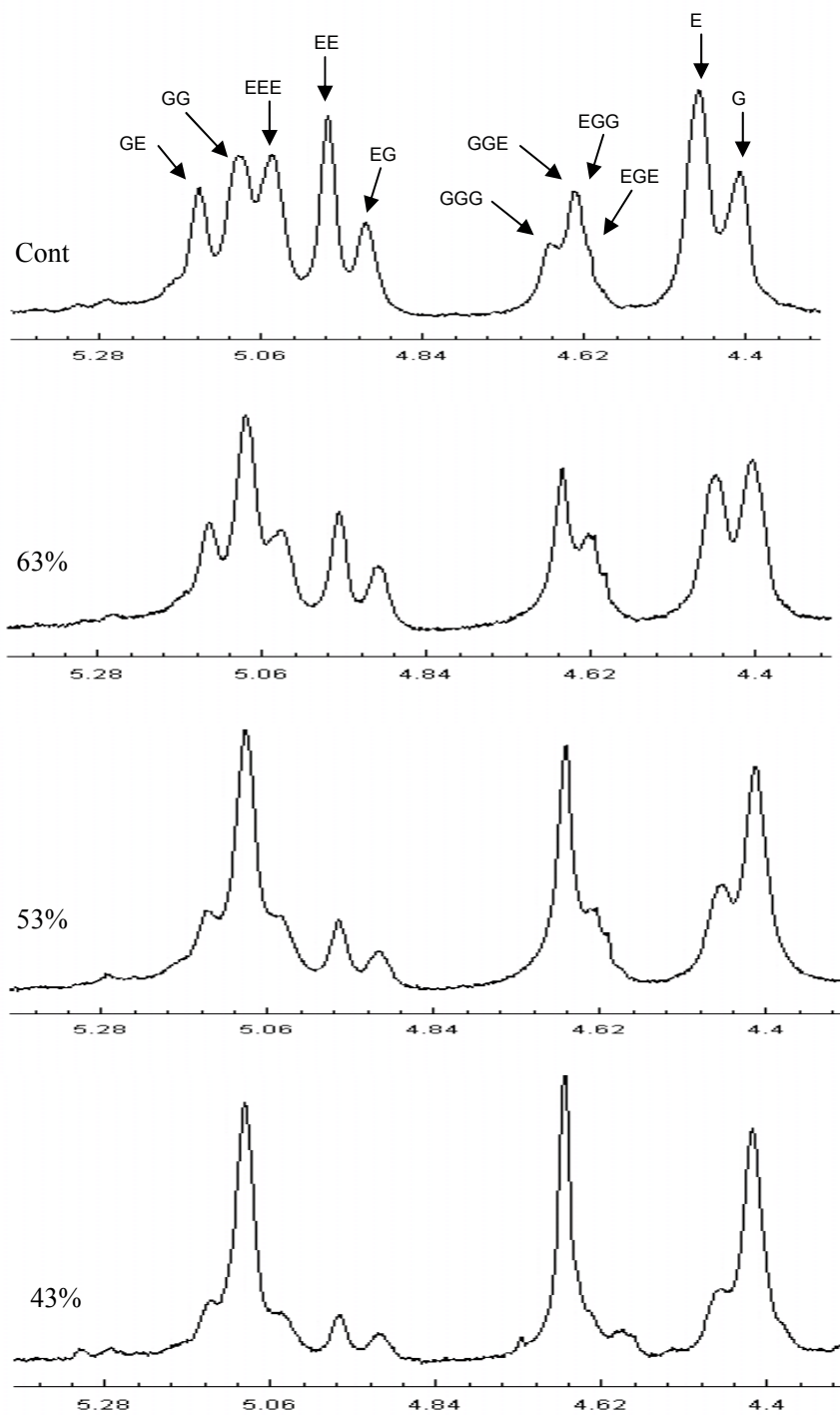


Fig. 3.3. ^1H NMR spectra of original/modified citrus pectin with different degree of methylation.

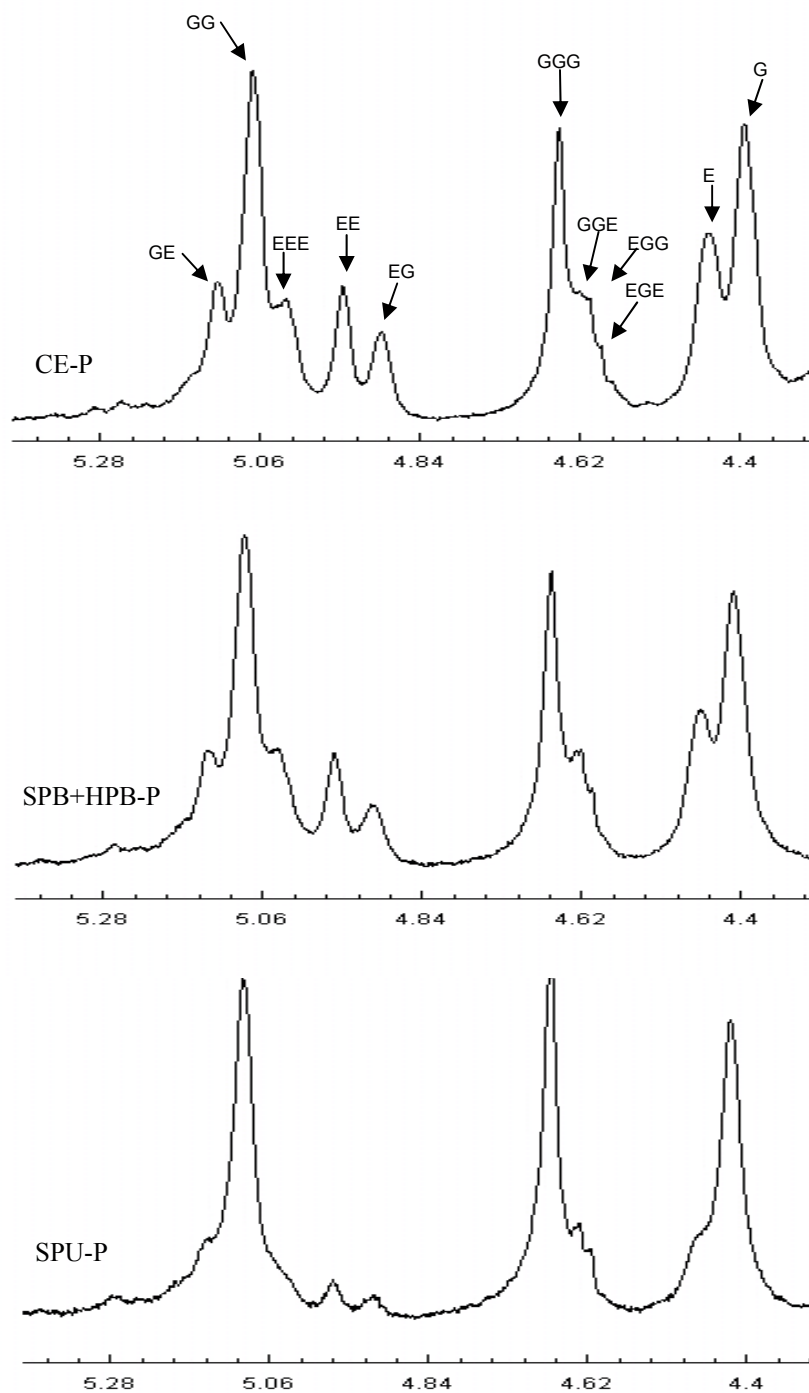


Fig. 3.4. ^1H NMR spectra of modified citrus pectin by different fractions of PMEs.

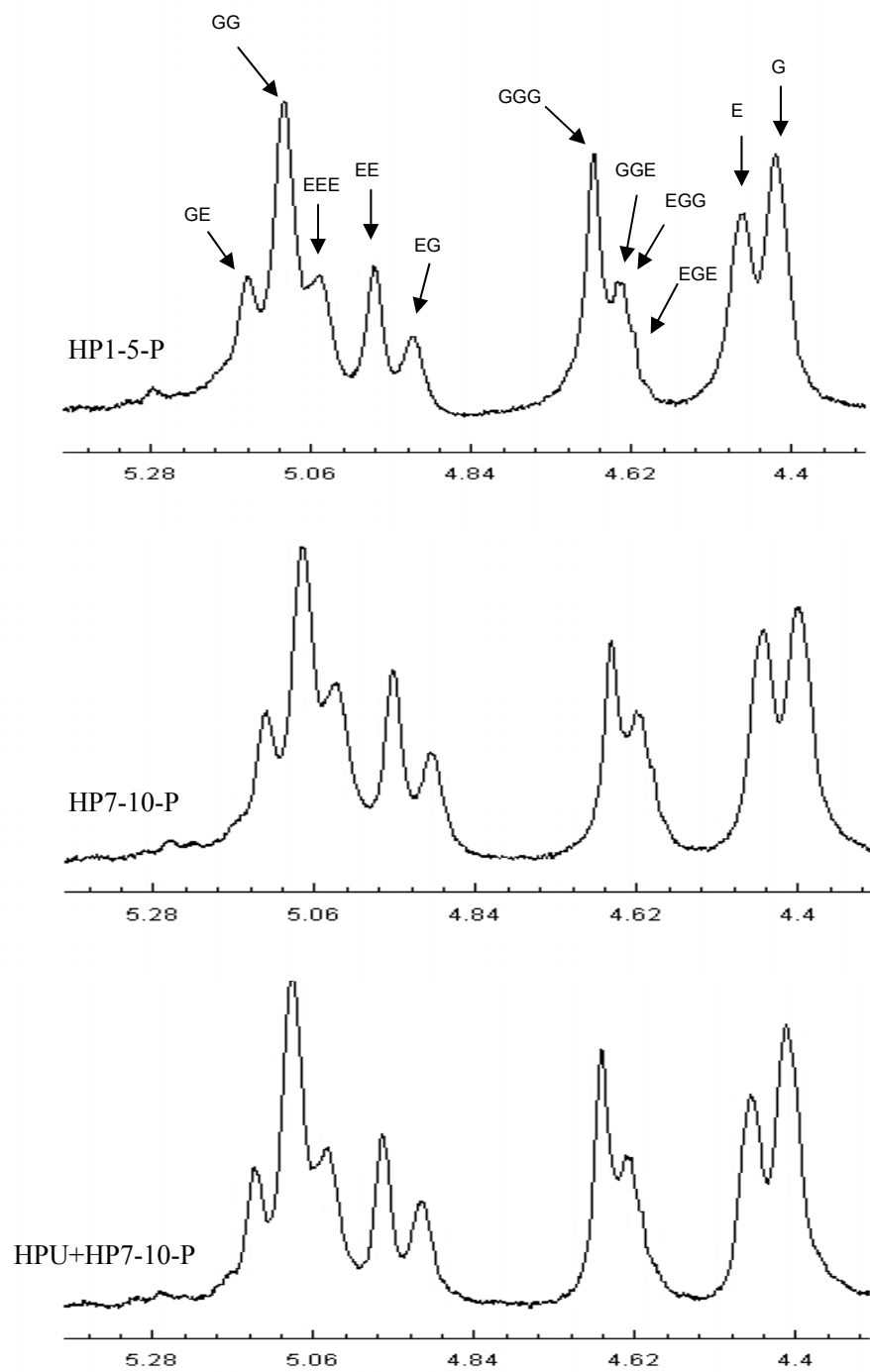
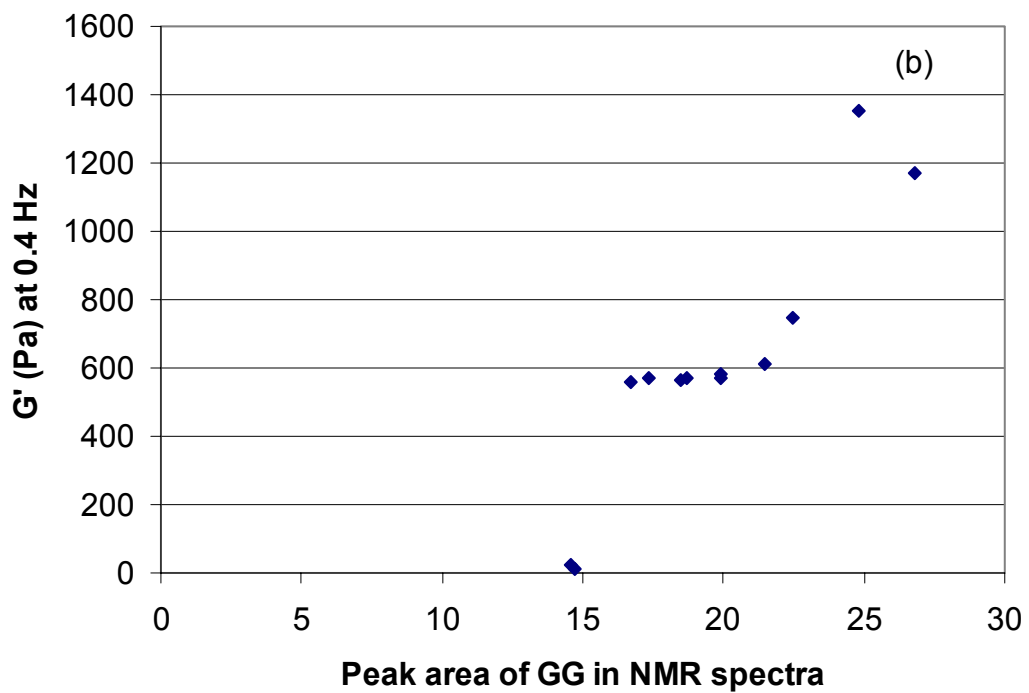
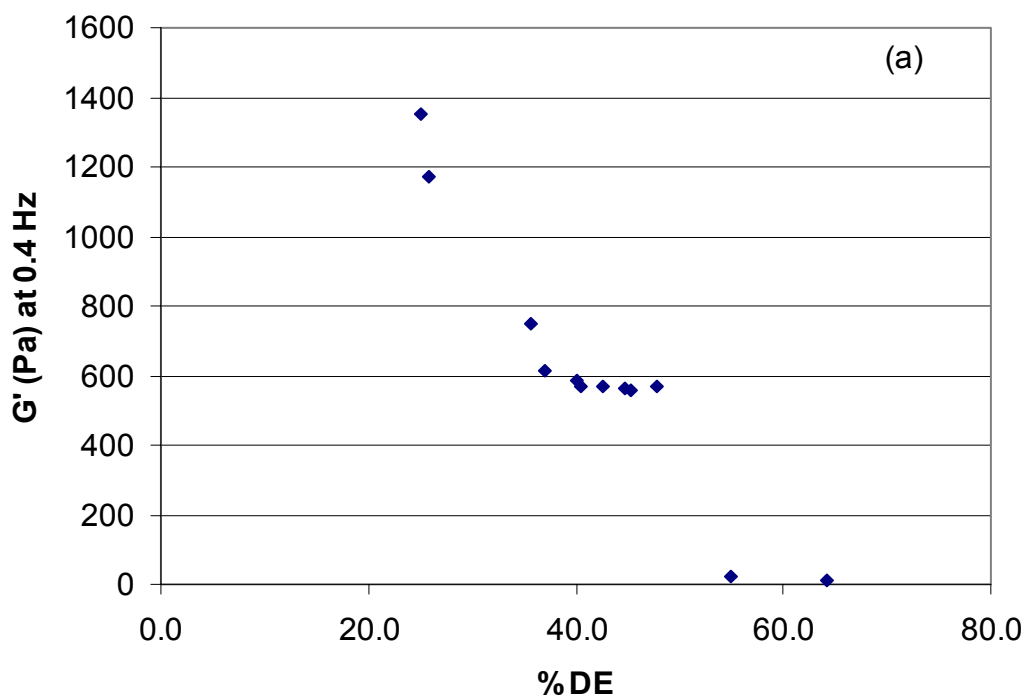


Fig. 3.5. ^1H NMR spectra of modified citrus pectin by different fractions of PMEs.



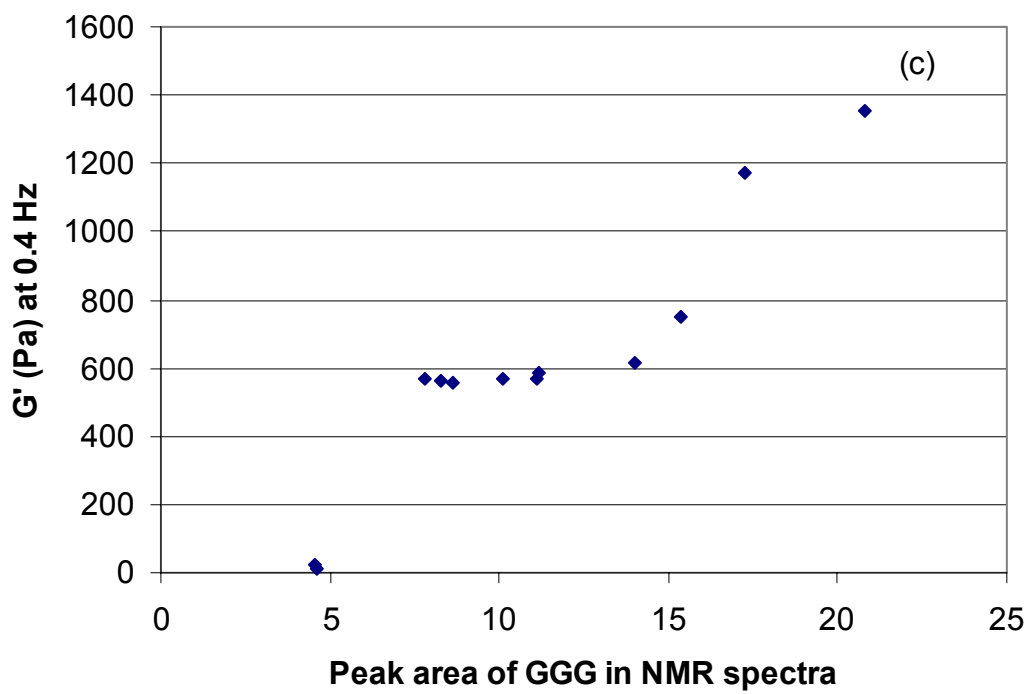


Fig. 3.6. Correlation of G' (Pa) at 0.4 Hz with %DE (a), peak areas of GG (b) and GGG (c) in NMR spectra.

CHAPTER 4

SURFACE HYDROPHOBICITY, ZETA POTENTIAL AND PARTICLE SIZE OF CITRUS PECTIN-WHEY PROTEIN ISOLATE COMPLEXES¹

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Abstract

Pectin-WPI complexes were prepared with original/modified citrus pectin with/without heat-treatment at pH 4.5/7.0 at 1:6/1:10 pectin to WPI ratios. Surface hydrophobicity, zeta potential, and particle size were investigated. The presence of citrus pectin decreased surface hydrophobicity, zeta potential, and particle size of WPI at pH 4.5. The surface hydrophobicity of WPI was higher at pH 4.5 than pH 7.0. Heat-treatment at 75°C for 20 min increased surface hydrophobicity of pectin-WPI complexes. Only at pH 4.5, surface hydrophobicity at 1:10 ratio was higher than that at 1:6. The added citrus pectin induced more negative zeta potential of WPI at pH 4.5. Particle sizes of WPI only at pH 4.5 were bigger than at pH 7 regardless of filtration or heat-treatment. The presence of citrus pectin decreased particle size of WPI at pH 4.5, not at pH 7.0.

Keywords: original/modified citrus pectin-WPI complex; surface hydrophobicity; zeta potential; and particle size

1. Introduction

Protein-polysaccharide interaction influences the structure and stabilization of many food systems through gelling, thickening, and surface stabilizing properties (Tolstoguzov, 1991). Since the texture and stability are key factors for food quality, characterizing and controlling these biopolymer interaction is of significant concern to develop new food products with improved food quality (Sanchez, Schmitt, Babak, & Hardy, 1997). Protein-polysaccharide complexes often show improved functionality compared to single component ingredients (Tolstoguzov, 1997; Tolstoguzov, 1994; Tolstoguzov, 1991; Tolstoguzov, 1986, Ledward, 1994; Ledward, 1979; Samant, Singhal, Kulkarni, & Rege, 1993; Dickson, 1995). Pectin interacts with food proteins on the interface between oil and water in emulsions and provides electrostatic and steric stabilization (Dickinson & Eriksson, 1991). In the study about stability of oil in water (O/W) emulsion (Guezy, Kim, & McClements, 2004), oil in water emulsion stabilized with β -lactoglobulin-pectin complex showed better stability than if stabilized with only β -lactoglobulin at intermediate pH values where food proteins fail to function (Guezy, Kim, & McClements, 2004). Whey protein concentrate-pectin complex prepared by a drying heat method improved emulsifying, gelling, and foaming properties (Mishira, Mann, & Joshi, 2001). The presence of pectin during high-pressure treatment of β -lactoglobulin resulted in enhanced gelling strength at neutral pH condition (Dickinson & James, 2000). Sodium polypectate could induce gelation of β -lactoglobulin at pH 6.5 (Ndi, Swanson, Barbosa-Canovas, & Luedecke, 1996). This study suggested that potential use of sodium polypectate to modify β -lactoglobulin and/or whey protein gelling in various food systems (Ndi, Swanson, Barbosa-Canovas, & Luedecke, 1996).

WPI has been widely used in food system as functional ingredients as emulsifier and gelling agent (de Wit, 1998; Kinsella & Whitehead, 1989a). β -Lactoglobulin was characterized

by globular and amphiphilic protein (Kinsella & Whitehead, 1989). This globular and amphiphilic protein can be used as emulsifier by adsorbing at the water-oil and lowering the interfacial tension (Hunt & Dalgleish, 1994). Hydrophobic amino acid groups, which are originally located on the inside of β -lactoglobulin, can be exposed by partially unfolding of β -lactoglobulin by elevated temperature (Iametti, De Gregori, Vecchio, & Bonomi, 1996; Qi, Holt, Menulty, Clarke, Brownlow, & Jones, 1997). The exposed hydrophobic amino acid group can induce aggregation of proteins, which results in gelation of β -lactoglobulin (Totosaus, Montejano, Salazar, & Guerrero, 2002).

Pectin is complex polysaccharide, which is composed of partially methyl-esterified α -1 \rightarrow 4-linked D-galacturonic acids interrupted by 1 \rightarrow 2-linked L-rhamnose residue with side chains of neutral sugars, such as galactose and arabinose (Voragen, Pilnik, Thibault, Axelos, Renard & Stephen, 1995). It has been reported that DE, distribution of DE and other extrinsic factors, such as pH, pectin concentration, and ionic strength affect functional properties of pectin, such as gelling, thickening and stabilizing properties (Rinaudo, 1996; Vorgen et al., 1995).

Surface hydrophobicity is an important physicochemical property of food protein, which has significant relationship with functionalities of food proteins, such as solubility and emulsifying ability (Nakai, 1983). Additionally, zeta potential can be also important physicochemical property in terms of stability of food protein and polysaccharides against flocculation and coalescence (Morrison & Ross, 2002). The objectives of this study were to investigate physicochemical properties of pectin-WPI, such as, surface hydrophobicity, zeta potential, and particle size and to investigate the effect of intrinsic or extrinsic factors, such as DE, pH, and heating on the physicochemical properties of WPI/pectin mixture.

2. Materials and Methods

2.1. Materials

WPI (Lot # CS43563E) was donated by Trega Foods Inc. (Little Chute, WI). Based on the company manual, protein content was 92.7% (dry basis) and protein fractions were β -lactoglobulin, 51.9-61.2%; α -lactalbumin, 13.7-17.9%; glycomacropeptide, 10.0-19.9%; immunoglobulin, 2.5-3.5%; bovine serum albumin, 2.3-2.8%; lactoferrin, 0.14-0.2%. High methoxyl citrus pectin was obtained from CP Kelco (Svenved, Denmark). Pectinmethylesterase (PME) was extracted from Valencia orange pulp (Citrus World, Lake Wales, FL). Crude PME was purified by chromatography technique with 5 mL Hi-Trap SP cation exchange and Heparin affinity columns (Amersham Pharmacia Biotech, Uppsala, Sweden) (Ackerley, Corredig, & Wicker, 2002). PME unbound to SP and Heparin columns was used to modify pectin. Pectin modification and characterization were performed by Kim, Teng, & Wicker (2005).

2.2. WPI/pectin mixture

Stock WPI solution (2%, w/v) was prepared by adding WPI to 5 mM Na phosphate buffer, pH 7 or 4.5 followed by 2 h stirring. After stirring, the stock WPI solution was stored at refrigerator overnight for full hydration. At pH 4.5, the stock WPI solution had precipitation and was filtered through 0.45 μ m syringe filter (Whatman, Clifton, NJ). Stock pectin solution (0.5%, w/v) was made by strong stirring for at least 2 h and stored at 4°C overnight to hydrate completely. WPI/pectin mixtures were prepared to obtain a final volume of 5 ml at two ratios of pectin to WPI (1:6 and 1:10). The mixtures were heated at 75°C for 20 min in water bath. Both filtered and non-filtered WPI solutions were used in preparing WPI-pectin mixture at same final WPI concentration. WPI solutions were used in preparing WPI-pectin mixture. All WPI/pectin mixtures were prepared freshly before measurements.

2.3. Surface hydrophobicity

8-Anilino-1-naphthalene-sulfonic acid (ANS, Lot# 45F-0562, Sigma, St. Louis, MO) was used to measure surface hydrophobicity of WPI/pectin mixtures. The concentration of ANS was 0.01 mg/ml in deionized water. The range of final five concentrations of WPI was from 0.05 to 0.5 mg/ml. Final ANS concentration was 0.0025 mg/ml on fluorescent measurements. Fluorescent intensity of WPI-pectin mixture was measured by Fluorometer (Fluorolog-2 Spectrofluorometer, Spex Industries Inc, Edison, NJ) at excitation 360 nm and emission 470 nm. Slits were set at 1.88 nm band path. Fluorescent intensity of 5 mM Na phosphate buffer, pH 7 or 4.5 was measured as blank, which was subtracted from those of WPI-pectin mixtures. Linear regression line was drawn fluorescent intensities at 470 nm versus WPI concentration. The slope of linear line was reported as surface hydrophobicity (Alizadeh-Pasdar & Li-Chan, 2000).

2.4. Particle size and zeta (ζ)-potential

Particle size analyzer adding the BI-Zeta option (90 Plus, Brookhaven Inst., Holtsville, NY) with 50 mW diode laser (90 angles) and BI-9000AT correlator was used to measure particle size and ζ -potential. The WPI/pectin mixtures were diluted to 0.0375% WPI. Particle size and ζ -potential were measured in triplicate with 5 measurements each.

2.5. Statistical analysis

Five variables were used to characterize physicochemical properties of pectin-WPI complexes and WPI according to factorial design, which were pectin, pectin:WPI ratio, pH, filtering, and heat-treatment. SAS system (Version 6.2, SAS Inc, Cary, NC) was used to conduct statistical analysis, such as analysis of variance (ANOVA) and Tukey's multiple comparison. Surface hydrophobicity were collected in duplicate. Particle size and ζ -potential were measured in triplicate with 5 measurements each.

3. Results and Discussions

3.1. Surface hydrophobicity

Surface hydrophobicity of original or modified citrus pectin-WPI complex is presented in Table 1. Surface hydrophobicity showed very wide range from 3097 to 1000000 %⁻¹ (Table 4.1). Surface hydrophobicity of pectin only ranged from 3097 to 7327 %⁻¹, which was much lower than pectin-WPI complex and WPI only (Table 4.1). Table 4.2 shows the ANOVA of surface hydrophobicity of pectin-WPI. The effects of heat, pH, pectin, and filtration were significant ($p \leq 0.001$), and explain 12.4, 44.2, 9.4, and 17.1% of total variance, respectively. A significant interaction effect of heat*pH was observed ($p \leq 0.001$).

The hydrophobicity of WPI at pH 4.5, filtered or not-filtered was significantly ($p \leq 0.05$) higher than WPI at pH 7 (Fig. 1a). Das & Kinsella (1989) reported that surface hydrophobicity of β -lactoglobulin at pH 2.8 was higher than at pH values between 2.8 and 9.7. Higher surface hydrophobicity of β -lactoglobulin was also reported at pH 3.0 than at pH 5 and 7 (Shimizu, Saito, & Yamauchi, 1985). In this study, higher surface hydrophobicity at pH 4.5 than at pH 7.0 was observed.

Hydrophobicity of complexes of WPI with original or modified citrus pectin was not significantly different from hydrophobicity of WPI alone at pH 7.0 (Fig. 4.1a). Surface hydrophobicity was higher at pH 4.5 than pH 7.0. Surface hydrophobicity of WPI at pH 4.5, filtered, was significantly higher than if complexed with original or modified citrus pectin (Fig. 4.1a). A similar trend was observed for unfiltered WPI-pectin complexes if higher ratio of pectin (1:6) was present. The results suggest that pectin protected WPI from the increase in hydrophobicity observed at the lower pH. Dextran sulfate, an anion polysaccharide significantly decreased surface hydrophobicity of bovine serum albumin (BSA) (Galazka, Smith, Dickson, &

Leward, 1999a). κ -carrageenan or ι -carrageenan decreased surface hydrophobicity of BSA (Galazka, Smith, Lewward, & Dickson, 1999b) and attributed to complexation of polysaccharide to the protein (Galazka et al., 1999b). Pectin is used in acid dairy drinks under acidic condition, which coats casein micelle and prevents casein from aggregation by steric stabilization and electrostatic repulsion of pectin located on the surface of casein micelle (Parker, Boulenguer, & Kravtchenko, 1994; Tromp, Kruif, Eijk, & Rolin, 2004). Citrus pectin-WPI complex may be formed by electrostatic interaction at pH 4.5, where WPI had positive charge and citrus pectin had negative charge (Leward, 1994; Weinbreck, Nieuwenhuijse, Robin, & de Kruif, 2003). Kazmierski et al. (2003) reported pH induced reversible aggregation of β -lactoglobulin-high methoxyl pectin with heat-treatment at 65° at pH 3.8 and dissociation by adjusting pH to 6.0, which suggests electrostatic interaction between β -lactoglobulin and high methoxyl pectin (Kazmierski, Wicker, & Corredig, 2003). Girad, Turgeon, & Gauthier (2002) also reported that electrostatic forces play key role in interaction between low- or high-methoxyl pectin and β -lactoglobulin.

The decrease in surface hydrophobicity of citrus pectin-WPI complex at pH 4.5 compared to WPI alone may be explained by masking of hydrophobic groups on WPI. Low pH induced aggregation may be prevented by pectin (Ibanoglu, 2005). Hydrophobic interaction between pectin and WPI also might contribute to the decrease in hydrophobicity of WPI. Methoxyl and/or acetyl groups in pectin molecules might induce hydrophobic interactions with WPI. These hydrophobic interactions between pectin and WPI also might protect WPI from increase in hydrophobicity.

On comparison of filtered vs. not-filtered WPI, only the hydrophobicity of filtered WPI was significantly higher than not-filtered WPI at pH 4.5 (Fig. 4.1a). Since the protein

concentration was adjusted higher to account for aggregation and filtration (see methods), the decrease in hydrophobicity of complexes at pH 4.5 is due to the protective effect of pectin. Further, the protective effect is observed more at the 1:6 ratio and is greater with charge modified pectin.

The heat-treatment at 75 °C for 20 min significantly ($p \leq 0.05$) increased hydrophobicity of WPI only or pectin/WPI complexes at all combinations (Fig. 4.1b). The increase was greater at pH 4.5 than at pH 7.0. Removal of aggregates by filtration affected hydrophobicity values that were observed. Higher turbidity was observed in heat-treated samples than in not heat-treated samples. On heating to approximately 70°C, β -lactoglobulin, major protein in WPI, is dissociated from a dimer to monomer (McKenzie, 1971; Hambling, McAlpine, & Sawyer, 1992). Hydrophobic amino acid groups, which are originally located on the inside of β -lactoglobulin, are exposed by partially unfolding of β -lactoglobulin by elevated temperature (Iametti, Cairoli, De Gregori, & Bonomi, 1995; Iametti, De Gregori, Vecchio, & Bonomi, 1996; Qi, Holt, Menulty, Clarke, Brownlow, & Jones, 1997). These exposed hydrophobic groups by heat could increase hydrophobicity of heat-treated samples.

Pectin protected against the effect of heat on hydrophobicity of WPI most likely by affecting aggregation of WPI. The more pectin present (1:6), the greater the protective effect. The protective effect was mixed with charge modified pectins and was greater at the 1:6 than 1:10 ratio.

3.2. Zeta potential

Zeta potentials of pectin-WPI complexes, and WPI only showed a range of charge from -30.91 to 4.75 mV (Table 4.1). Zeta potentials of pectin only ranged from -19.40 to -40.18. Zeta potentials of modified citrus pectin were more negative than original ones (Table 4.1). Single

sources of variation included pH and pectin ($p \leq 0.001$), which explained 60.1 and 11.0% of total variance, respectively (Table 4.3). Significant interactive effects ($p \leq 0.001$ or $p \leq 0.001$) included pectin:WPI ratio, pH*pectin, heat*pectin*ratio, pH*pectin*ratio, heat*pH*pectin*ratio, heat*filtered, and heat*pH*filtered. Interactive effects accounted for 1.3 to 6.5% of total variance.

Zeta potential of WPI at pH 7 was significantly ($p \leq 0.05$) more negative than at pH 4.5 (Fig. 2). Zeta potentials of pectin-WPI complexes were significantly more negative than those of WPI only at pH 4.5 (Fig 4.2). At pH 7.0, there was no significant difference between pectin-WPI complex and WPI alone. The zeta potential of soluble aggregates (pH 4.5, filtered) was not significantly different from zeta potential of insoluble aggregates (pH 4.5, not-filtered) (Fig. 4.2). After heating, the zeta potential remained negative for complexes. For complexes of WPI-pectin, pH 7.0, 1:6 ratio, zeta potential was significantly ($p < 0.05$) lower after heating (Fig. 4.2). In this study, positively charged WPI aggregates were detected at unfiltered WPI at pH 4.5. Filtration likely removed insoluble aggregates. Of soluble aggregates at pH 4.5, the surface charge is slightly negative.

Guzy et al. (2004) reported a slight positive zeta potential of β -lactoglobulin-stabilized O/W emulsion and aggregation of emulsion droplets at pH 4 and 5. It was reported that the addition of xanthan, anionic polysaccharide, decreased zeta potential of WPI below isoelectric point (Benichou, Aserin, & Garti, 2002). In the acidified milk drinks, zeta potential of casein aggregate decreased by addition of pectin, which was mainly due to adsorption of negatively charged pectin molecules onto positively charged protein aggregate (Sejersen, Salomonsen, Ipsen, Clark, Rolin, & Engelsen, 2006; Rolin, 2002). Surh, Decker, & McClement (2005) measured the zeta potentials of sodium caseinate (CAS) – coated O/W emulsion droplet in the

absence or presence of pectin. They also reported decreased zeta potentials in the presence of pectin below pH 5, which was caused by electrostatic interactions between positively charged CAS and negatively charged pectin. The decreased zeta potential below pH 5 was also observed in the β -lactoglobulin – stabilized O/W emulsion in the presence of pectin (Guezy et al., 2004). In this study, the decreases in zeta potential of WPI were observed by addition of citrus pectin at pH 4.5 (Fig. 4.2). This can be explained by adsorption of negatively charged pectin molecules onto positively charged WPI. At pH 7.0, there was no significant difference in zeta potentials between citrus pectin-WPI and WPI only (Fig. 2). Surh et al. (2005) reported that there was no significant effect of the pectin on the zeta potential of CAS – coated O/W emulsion droplet at pH 6 and 7. It was postulated that at pH 6 and 7, pectin molecules were not absorbed onto casein due to electrostatic repulsion between negatively charged pectin and negatively charged casein (Surh et al., 2005). This electrostatic repulsion can explain the no significant difference in zeta potentials between citrus pectin-WPI and WPI only at pH 7.0.

Zeta potentials of original pectin- and modified pectin-WPI complexes were not significantly different ($p \leq 0.05$) (Fig 4.2). In the study about the adsorption of synthetic polyelectrolytes onto oppositely charge surface, zeta potential was largely independent of the charge density of absorbing polyelectrolyte (Schonhoff, 2003). It was also reported that there was no significant difference in zeta potential of CAS – coated O/W emulsion droplet depending on pectin DE (Surh et al., 2005). Once the surface charge reaches at certain value by adsorption of polyelectrolytes, strong electrostatic repulsion can be induced between the surface and non-absorbed polyelectrolytes in the aqueous phase, limiting further adsorption of polyelectrolytes (Surh et al., 2005; Schonhoff, 2003).

3.3. Particle size

Particle sizes of WPI only, pectin-WPI complexes, and pectin only varied from 170 to 4085 nm (Table 4.1) and heat, pH, pectin, pectin:WPI ratio, and filtration had significant effects as shown by ANOVA ($p \leq 0.001$) (Table 4.4). The effects of pH, pectin, and filtration explain 47.8, 18.0 and 4.1% of total variance, respectively. Interactive effects were observed as described by the ANOVA ($p \leq 0.01$ or $p \leq 0.001$) (Table 4.4).

Particle sizes of WPI only at pH 4.5 not filtered or filtered were about ten fold greater than particle size of WPI-pectin complexes at pH 7.0 (Fig 4.3). At pH 7.0, particle size was small and there was no effect of pectin on particle size. Particle sizes of soluble pectin-WPI were smaller than un-filtered, insoluble aggregates of pectin-WPI complexes at pH 4.5 (Fig. 4.3). Heat-treatment increased particle sizes of WPI only and did not change particle size of WPI-pectin complexes (Fig. 4.3).

In the case of pectin-WPI complexes, there was no significant difference in particle size with heating except one source combination, OC/1:6. At pH 7, there was no significant difference in particle sizes among original citrus pectin-, modified citrus pectin-WPI and WPI only with heat (Fig. 4.3). On contrast, significant decrease in particles of WPI size was observed by the addition of original/modified citrus pectin at pH 4.5 (Fig. 4.3). There was no significant difference in particle sizes between original citrus pectin- and modified citrus pectin-filtered WPI at pH 4.5 (Fig. 4.3). Particle sizes of original citrus pectin-not filtered WPI were bigger than those of modified citrus pectin-filtered WPI (Fig. 4.3).

At pH 7, pectin molecules could not be absorbed onto WPI due to electrostatic repulsion between negatively charged pectin and negatively charged WPI. Therefore, the added original or modified citrus pectin could not affect particle size of WPI at pH 7. In acid dairy drinks under acidic condition, pectin coats casein micelle and prevents casein from aggregation by steric and

electrostatic stabilization of pectin located on the surface of casein micelle (Parker, Boulenguer, & Kravtchenko, 1994). In this study, negatively charged pectin molecules could be absorbed onto positively charged WPI at pH 4.5. The adsorbed pectin molecules could induce steric stabilization and electrostatic repulsion among WPI molecules and inhibit WPI aggregation. The induced steric stabilization and electrostatic repulsion by absorbed pectin and reduced hydrophobicity of WPI can contribute to smaller particle size of citrus pectin-WPI than WPI only. Once the surface charge of WPI reaches at certain value by adsorption of pectin, strong electrostatic repulsion can be induced between the surface of WPI and non-absorbed polyelectrolytes in the aqueous phase, limiting further adsorption of pectin molecules (Surh et al., 2005; Schonhoff, 2003). This can cause no difference in surface charges of citrus pectin-WPI depending on original and modified citrus pectin, which means no difference in induced electrostatic repulsion of original citrus- and modified citrus pectin-WPI. The same level of electrostatic repulsion of original citrus- and modified citrus pectin-WPI can contribute to no significant difference in particle sizes between original citrus pectin- and modified citrus pectin-WPI at pH. The exposed hydrophobic groups by heat-treatment could induce WPI aggregation resulting in the increase in particle sizes of heat-treated WPI (Iametti et al., 1995; Iametti, De Gregori et al., 1996; Qi et al., 1997).

4. Conclusions

Physicochemical properties of WPI can be changed by the presence of original or modified citrus pectin. Surface hydrophobicity, zeta potential and particle size can be decreased by adding original or charge modified citrus pectin below isoelectric point of WPI. The interaction can be electrostatic attraction and/or hydrophobic interaction between pectin and

positively WPI. The reduced surface hydrophobicity and zeta potential of pectin-WPI complexes can finally result in decrease in particle sizes of pectin-WPI complexes.

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Table 4.1. Hydrophobicity, zeta potential, and particle size of citrus pectin/WPI complex

Pectin ^a	Pectin:WPI ratio	pH	Filter ^b	Heat ^c	Hydrophobicity (1/%)		Zeta potential (mV)		Particle size (nm)	
					Avg ^d	SD ^e	Avg ^f	SD	Avg ^g	SD
Orig	1:6	4.5	NF	NH	215300	253	-9.44	3.22	582	18
Orig	1:6	4.5	F	NH	233600	3038	-19.37	2.28	210	8
Orig	1:6	7.0	-	NH	149500	2432	-17.55	4.25	366	5
Orig	1:6	4.5	NF	H	631900	3219	-9.26	0.97	394	11
Orig	1:6	4.5	F	H	698100	1677	-20.66	2.61	221	1
Orig	1:6	7.0	-	H	210100	2354	-30.91	8.15	351	23
Orig	1:10	4.5	NF	NH	254000	4755	-9.99	0.71	501	1
Orig	1:10	4.5	F	NH	265600	1089	-17.26	3.73	210	7
Orig	1:10	7.0	-	NH	145000	729	-15.58	2.69	342	31
Orig	1:10	4.5	NF	H	613100	3422	-18.31	6.57	493	14
Orig	1:10	4.5	F	H	783900	3980	-16.38	4.32	210	5
Orig	1:10	7.0	-	H	202800	2174	-15.67	3.51	278	9
Mod	1:6	4.5	NF	NH	182900	5296	-10.73	0.48	348	4
Mod	1:6	4.5	F	NH	195800	445	-14.62	6.26	170	2
Mod	1:6	7.0	-	NH	145800	4965	-12.74	2.73	365	2
Mod	1:6	4.5	NF	H	460200	21060	-12.75	2.90	377	26
Mod	1:6	4.5	F	H	724900	7975	-20.38	3.55	181	9
Mod	1:6	7.0	-	H	199800	3563	-27.34	2.30	332	8
Mod	1:10	4.5	NF	NH	243700	11510	-10.29	2.60	273	22
Mod	1:10	4.5	F	NH	264600	279	-19.81	2.58	178	4
Mod	1:10	7.0	-	NH	141100	946	-14.38	1.76	355	19
Mod	1:10	4.5	NF	H	647200	740	-11.71	1.32	226	6
Mod	1:10	4.5	F	H	910300	2744	-22.98	1.75	176	1
Mod	1:10	7.0	-	H	204300	1199	-19.07	5.51	289	10
-	WPI only	4.5	NF	NH	254500	13538	4.75	1.40	3714	102
-	WPI only	4.5	F	NH	301600	2062	-5.81	1.63	1502	20
-	WPI only	7.0	-	NH	154200	1855	-22.97	0.13	232	12
-	WPI only	4.5	NF	H	924500	33070	6.89	0.59	4085	45
-	WPI only	4.5	F	H	1000000	0	-2.41	0.60	3789	102
-	WPI only	7.0	-	H	190200	2676	-28.09	7.12	267	65
Orig	Pectin only	4.5	-	NH	7327	1687	-19.40	1.56	705	45
Orig	Pectin only	7.0	-	NH	3589	238	-34.27	3.55	665	14
Orig	Pectin only	4.5	-	H	6819	1267	-19.03	3.08	548	82
Orig	Pectin only	7.0	-	H	4617	1327	-26.20	6.94	459	51
Mod	Pectin only	4.5	-	NH	5015	159	-25.80	4.51	664	22
Mod	Pectin only	7.0	-	NH	4884	1109	-40.18	0.47	625	12
Mod	Pectin only	4.5	-	H	6024	636	-26.54	1.24	567	19
Mod	Pectin only	7.0	-	H	3097	1489	-34.85	0.71	434	18

^a Orig, original citrus pectin; Mod, modified citrus pectin.

^b WPI solution at pH 4.5 was filtered by 0.45 μm syringe filter.

^c Heat-treatment at 75° for 20 min.

^d Average of hydrophobicity, n=2.

^e SD, standard deviation.

^f Average of zeta potential, n=3.

^g Average of particle size, n=3.

Table 4.2. Analysis variance of surface hydrophobicity of citrus pectin/WPI complex

Source of variation ^a	DF ^b	SS ^c	F ^d
Heat	1	0.443	169.31***
pH	1	1.583	605.50***
Pectin	1	0.335	128.12***
Filtered	1	0.614	235.02***
Heat*pH	1	0.540	206.49***

^a The sources with significant effects were presented in this table. R^2 (coefficient of determination)=0.98632, $(MSE)^{1/2}$ =51143.22.

^b Degree of freedom, Model DF=18, Error DF=19, Corrected total DF=37.

^c Sum of squares. SS=Value presented* 10^{12} , Model SS= 3.533×10^{12} , Error SS= 0.049×10^{12} , Corrected total SS= 3.582×10^{12} .

^d Fisher ratio value, Model F=75.06***, Significant level: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

Table 4.3. Analysis variance of zeta potential of citrus pectin/WPI complex

Source of variation ^a	DF ^b	SS ^c	F ^d
pH	1	3323.956	344.29***
Pectin	1	610.441	63.23***
Pectin:WPI ratio	1	89.279	9.26**
pH*Pectin	1	248.987	25.79***
Heat*Pectin*Ratio	1	85.701	8.88**
pH*Pectin*Ratio	1	129.695	13.43***
Heat*pH*Pectin*Ratio	1	188.493	19.52***
Heat*Filtered	1	74.678	7.74**
Heat*pH*Filtered	1	360.992	37.39***

^a The sources with significant effects were presented in this table. R^2 (coefficient of determination)=0.947660, $(MSE)^{1/2}$ =3.107174.

^b Degree of freedom, Model DF=26, Error DF=30, Corrected total DF=56.

^c Sum of squares, Model SS=5244.125, Error SS=289.636, Corrected total SS=5533.761.

^d Fisher ratio value, Model F=20.89***, Significant level: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

Table 4.4. Analysis variance of the particle size of citrus pectin/WPI complex

Source of variation ^a	DF ^b	SS ^c	F ^d
Heat	1	8038.876	354.38***
pH	1	287158.476	12658.9***
Pectin	1	108714.350	4792.50***
Pectin:WPI Ratio	1	16058.605	707.92***
Filtered	1	24784.846	1092.60***
Heat*pH	1	266.588	11.75**
Heat*Pectin	1	17470.534	770.16***
pH*Pectin	1	15511.878	683.82***
Heat*pH*Pectin	1	4414.078	194.59***
Heat*Ratio	1	5025.957	221.56***
pH*Ratio	1	2324.535	102.47***
Heat*pH*Ratio	1	2303.888	101.56***
Pectin*Ratio	1	1282.106	56.52***
Heat*Pectin*Ratio	1	3574.743	157.59***
pH*Pectin*Ratio	1	836.567	36.88***
Heat*pH*Pectin*Ratio	1	1945.529	85.77***
Heat*Filtered	1	2630.778	115.97***
Heat*Pectin*Filtered	1	813.360	35.86***
pH*Pectin*Filtered	1	97605.573	4302.79***

^a The sources with significant effects were presented in this table. R^2 (coefficient of determination)=0.999585, $(MSE)^{1/2}$ =4.762799.

^b Degree of freedom, Model DF=26, Error DF=11, Corrected total DF=37.

^c Sum of squares, Model SS=600943.681, Error SS=249.527, Corrected total SS=601193.208.

^d Fisher ratio value, Model F=1018.91***, Significant level: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

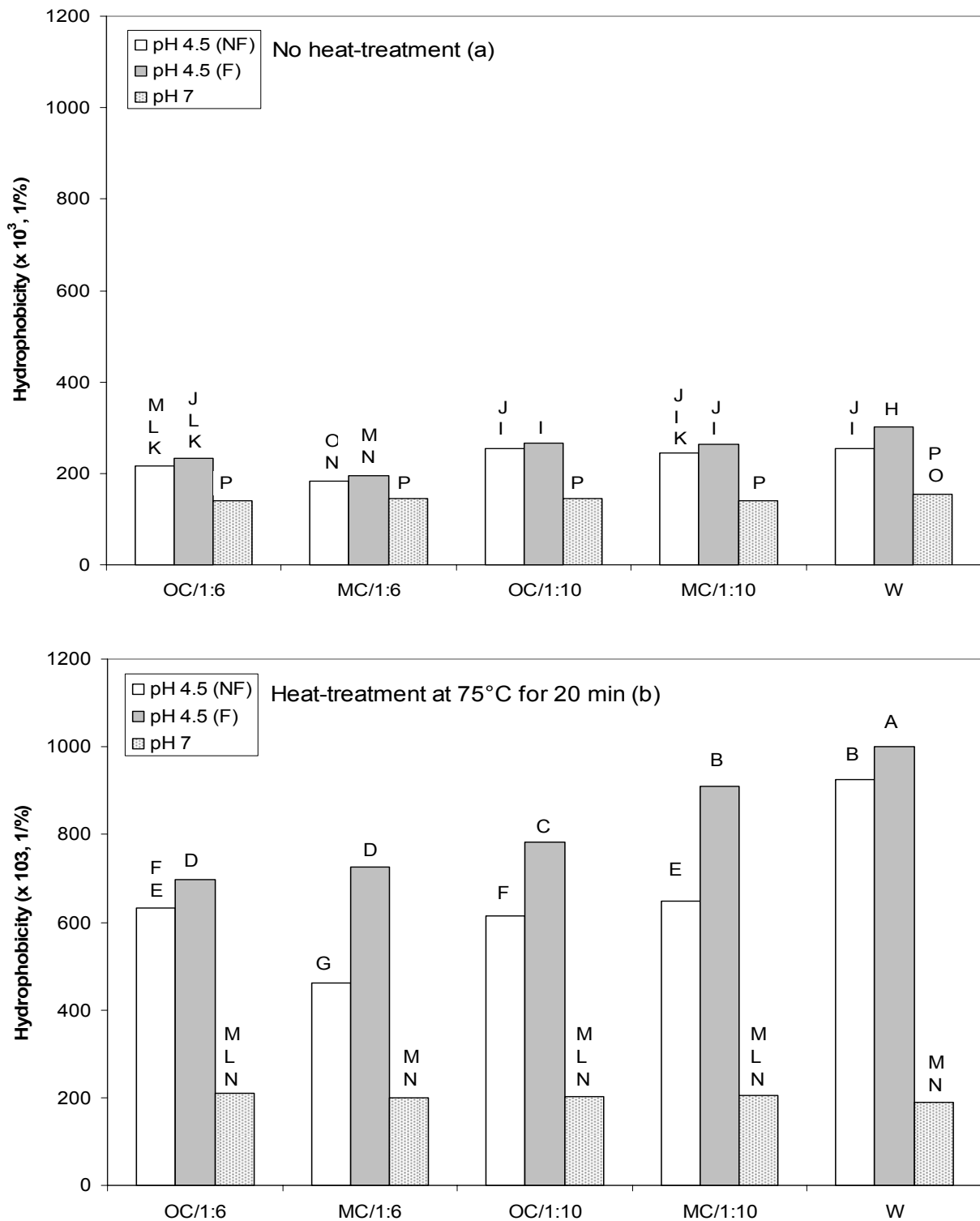


Fig. 4.1. Hydrophobicity of citrus pectin/WPI complexes. Hydrophobicity with same letter means no significant difference at 5% confidence level. The abbreviations: OC, original citrus pectin; MC, modified citrus pectin; 1:6 and 1:10, pectin to WPI ratio; NF, not filtered; F, filtered; W, WPI.

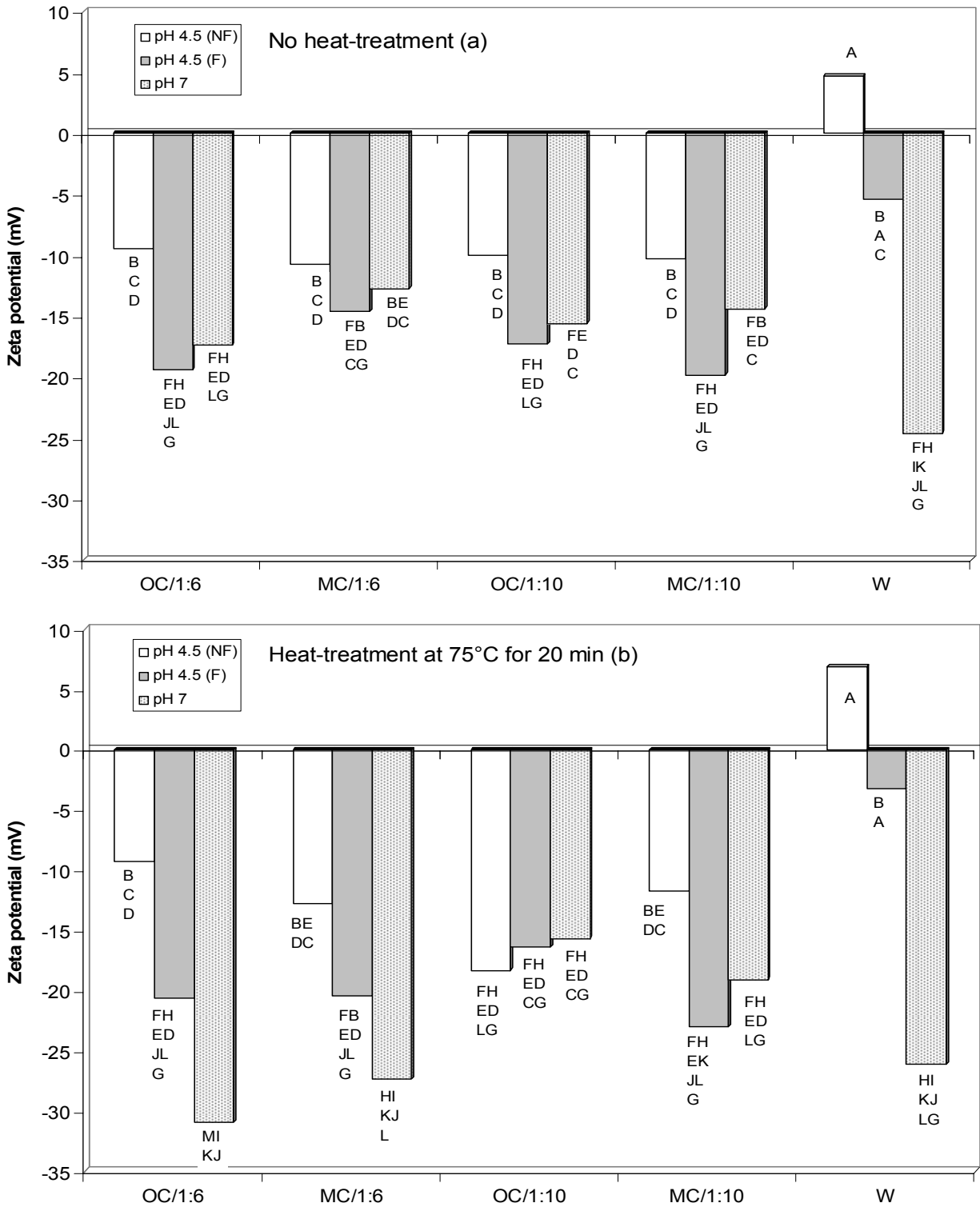


Fig. 4.2. Zeta potential of citrus pectin/WPI complexes. Zeta potentials with same letter mean no significant difference at 5% confidence level. The abbreviations: OC, original citrus pectin; MC, modified citrus pectin; 1:6 and 1:10, pectin to WPI ratio; 7 and 4.5, pH 7 and pH 4.5; NF, not filtered; F, filtered; W, WPI.

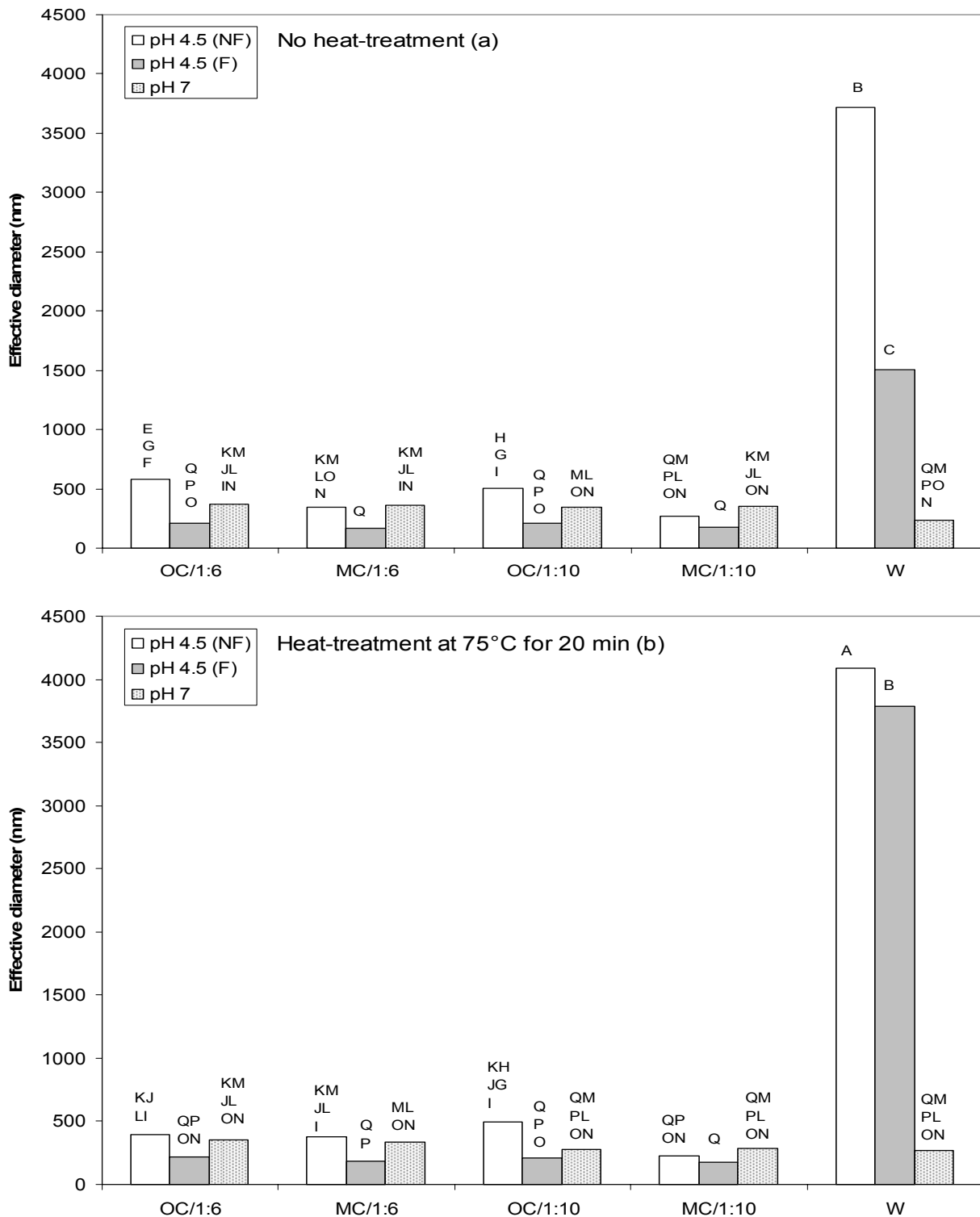


Fig. 4.3. Particle sizes of citrus pectin/WPI complexes. Particle sizes with same letter mean no significant difference at 5% confidence level. The abbreviations: OC, original citrus pectin; MC, modified citrus pectin; 1:6 and 1:10, pectin to WPI ratio; 7 and 4.5, pH 7 and pH 4.5; NF, not filtered; F, filtered; W, WPI.

CHAPTER 5

SURFACE HYDROPHOBICITY, ZETA POTENTIAL AND PARTICLE SIZE OF SUGAR BEET PECTIN-WHEY PROTEIN ISOLATE COMPLEXES¹

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Abstract

Surface hydrophobicity, zeta potential, and particle size of original/modified sugar beet pectin-WPI complex were investigated with heat-treatment (at 75°C for 20 min), two different pH (4.5 and 7.0), and two pectin to WPI ratio (1:6 and 1:10). Heat-treatment (at 75°C for 20 min) significantly ($p \leq 0.05$) increased surface hydrophobicity of sugar beet pectin-WPI complex. The hydrophobicity of sugar beet pectin-WPI complex at pH 4.5 was significantly ($p \leq 0.05$) higher than that at pH 7.0. At pH 4.5, surface hydrophobicity of WPI was significantly ($p \leq 0.05$) decreased by added sugar beet pectin. Zeta potential of WPI only at pH 7.0 was more negative than at pH 4.5. The added sugar beet pectin made zeta potential of WPI more negative. The particle size of WPI only was increased with heat-treatment and at pH 4.5. The presence of sugar beet pectin at pH 4.5 caused dramatic decrease in hydrophobicity, zeta potential and particle size of pectin-WPI complex.

Keywords: sugar beet pectin – WPI complex; surface hydrophobicity; zeta potential; particle size

1. Introduction

Pectin is anionic polysaccharide, which has been used in food systems, as stabilizer and gelling agent (Nelson, Smit, & Wiles, 1995; Voragen, Pilnik, Thibault, Axelos, Renard & Stephen, 1995). In its structure, linear chain of partially methyl-esterified α -1 \rightarrow 4-linked D-galacturonic is main feature. This linear chain is interrupted by the insertion of 1 \rightarrow 2-linked L-rhamnose residue with side chains of neutral sugars, such as galactose and arabinose (Voragen, Pilnik, Thibault, Axelos, Renard & Stephen, 1995). Compared to citrus and apple pectin, sugar beet pectin is characterized by higher acetyl groups located on C-2 and C-3 position of galacturonic acid residues, higher protein content, higher arabinose content, and feruloyl groups attached to galactose and arabinose (Colquhoun, Ralet, Thibault, Faulds, & Williamson, 1994; Gullion & Thibault, 1988, 1989a,b; Ralet, Thibault, Faulds, & Williamson, 1994; Keenan, Belton, Matthew, & Howson, 1985). The use of sugar beet pectin has been limited due to its poor gelling property (Pippen, McCready, & Owens, 1950). However, sugar beet pectin shows equal or superior properties to apple or citrus pectin in some applications, such as stabilization of oil in water emulsion in juice concentrate, and stabilization of drinkable yogurt (Weibel, 1991; Takahashi, Furuta, Tobe, & Kiwata, 1999). It was also reported surface tension of 2% sugar beet pectin was significantly lower than that of 2% citrus pectin (Leroux, Langendorff, Schick, Vanishav, & Mazoyer, 2003). Furthermore, water in oil emulsions stabilized by sugar beet pectin showed finer particle distribution and more stability than that stabilized by citrus pectin. This means that sugar beet pectin is more efficient in preparing oil in water emulsion than citrus pectin (Leroux et al., 2003).

Whey protein isolate (WPI) was selected as protein source in this study due to its well-defined characteristics and various applications, such as emulsifiers and gelling agents (de Wit,

1998; Kinsella & Whitehead, 1989a). The major component of whey protein is β -lactoglobulin, which is mainly responsible for the functional properties (de Wit, 1989; Ziegler & Foegeding, 1990). Generally improved functionalities of protein-polysaccharide complex have been reported due to the combination of the functionality of each component (Tolstoguzov, 1997; Tolstoguzov, 1994; Tolstoguzov, 1991; Tolstoguzov, 1986, Ledward, 1994; Ledward, 1979; Samant, Singhal, Kulkarni, & Rege, 1993; Dickson, 1995). Food protein can be located on the interface between oil and water in oil in water emulsion can absorb pectin. The absorbed pectin onto the proteins provides electrostatic and steric stabilization to the emulsion system (Dickson & Eriksson, 1991). Oil in water emulsion stabilized with β -lactoglobulin-pectin complex showed better stability than stabilized with only β -lactoglobulin at intermediate pHs where food proteins fail to function (Guezy, Kim, & McClements, 2004). It was reported that whey protein concentrate-pectin complex prepared by dry heat method showed improved emulsifying, gelling, and foaming properties (Mishira, Mann, & Joshi, 2001). The presence of pectin during high-pressure treatment of β -lactoglobulin enhanced gelling strength at neutral pH condition (Dickson & James, 2000).

Surface hydrophobicity has a significant relationship with functionalities of food proteins, such as solubility and emulsifying ability (Nakai, 1983). Zeta potential can be also an important physicochemical property in terms of stability of food protein and polysaccharides against flocculation and coalescence (Morrison & Ross, 2002). The objectives of this study were to investigate the physicochemical properties of original/modified sugar beet pectin-WPI complex, such as surface hydrophobicity, zeta potential, and particle size, and to determine the degree of esterification, pH, pectin to WPI ratio, and heat, on the physicochemical properties of original/modified sugar beet pectin-WPI complex. The sugar beet pectin-WPI complexes were

prepared with several different conditions and their physicochemical properties will be presented in this article. These all presented properties can be fundamental information for development and application of potential combination of sugar beet pectin and WPI with improved functionality.

2. Materials and Methods

2.1. Materials

WPI (Lot # CS43563E) was donated by Trega Foods Inc. (Little Chute, WI). Based on the company manual, protein content was 92.7% (dry basis) and protein fractions were β -lactoglobulin, 51.9-61.2%; α -lactalbumin, 13.7-17.9%; glycomacropeptide, 10.0-19.9%; immunoglobulin, 2.5-3.5%; bovine serum albumin, 2.3-2.8%; lactoferrin, 0.14-0.2%. Sugar beet pectin was obtained from Herbstreith & Fox Inc (Elmsford, NY). PME was extracted from Valencia orange pulp (Citrus World, Lake Wales, FL). Crude PME was purified by column chromatography technique (Ackerley, Corredig, & Wicker, 2002). Hi-Trap SP cation exchange and Heparin affinity columns (Amersham Pharmacia Biotech, Uppsala, Sweden) were used to purify crude PME. PME unbound to SP and Heparin columns was used to modify pectin. Pectin modification and characterization were performed by Kim, Teng, & Wicker (2005).

2.2. WPI/pectin mixture

Stock WPI solution (2%, w/v) was prepared by adding WPI to 5 mM Na phosphate buffer, pH 7 or 4.5 followed by 2 h stirring. After stirring, the stock WPI solution was stored at refrigerator overnight for full hydration. At pH 4.5, the stock WPI solution had precipitation and was filtered through 0.45 μ m syringe filter (Whatman, Clifton, NJ). Stock pectin solution (0.5%, w/v) was made by strong stirring for at least 2 h and stored at 4°C overnight to hydrate

completely. WPI/pectin mixtures were prepared to obtain a final volume of 5 ml at two ratios of pectin to WPI (1:6 and 1:10). The mixtures were heated at 75°C for 20 min in water bath. Both filtered and non-filtered WPI solutions were used in preparing WPI-pectin mixture at same final WPI concentration. WPI solutions were used in preparing WPI-pectin mixture. All WPI/pectin mixtures were prepared freshly before measurements.

2.3. Surface hydrophobicity

8-Anilino-1-naphthalene-sulfonic acid (ANS, Lot# 45F-0562, Sigma, St. Louis, MO) was used to measure surface hydrophobicity of WPI/pectin mixtures. The concentration of ANS was 0.01 mg/ml in deionized water. The range of final five concentrations of WPI was from 0.05 to 0.5 mg/ml. Final ANS concentration was 0.0025 mg/ml on fluorescent measurements. Fluorescent intensity of WPI-pectin mixture was measured by Fluorometer (Fluorolog-2 Spectrofluorometer, Spex Industries Inc, Edison, NJ) at excitation 360 nm and emission 470 nm. Slits were set at 1.88 nm band path. Fluorescent intensity of 5 mM Na phosphate buffer, pH 7 or 4.5 was measured as blank, which was subtracted from those of WPI-pectin mixtures. Linear regression line was drawn fluorescent intensities at 470 nm versus WPI concentration. The slope of linear line was reported as surface hydrophobicity (Alizadeh-Pasdar & Li-Chan, 2000).

2.4. Particle size and zeta (ζ)-potential

Particle size analyzer adding the BI-Zeta option (90 Plus, Brookhaven Inst., Holtsville, NY) with 50 mW diode laser (90 angles) and BI-9000AT correlator was used to measure particle size and ζ -potential. The WPI/pectin mixtures were diluted to 0.375 mg/ml WPI. Particle size and ζ -potential were measured in triplicate with five measurements each.

2.5. Statistical analysis

Five variables were used to characterize physicochemical properties of pectin-WPI complexes and WPI according to factorial design, which were pectin, pectin:WPI ratio, pH, filtering, and heat-treatment. SAS system (Version 6.2, SAS Inc, Cary, NC) was used to conduct statistical analysis, such as analysis of variance (ANOVA) and Tukey's multiple comparison. Surface hydrophobicity were collected in duplicate. Particle size and ζ -potential were measured in triplicate with 5 measurements each.

3. Results and Discussions

3.1. Surface hydrophobicity

Table 5.1. presents all surface hydrophobicity data of pectin-WPI hybrid, WPI only, and pectin only. Surface hydrophobicity showed very wide ranges from 10800 to 1000000 $1\%^{-1}$. Table 5.2 shows ANOVA of surface hydrophobicity of pectin-WPI. The effects of Heat, pH, Pectin, and Filtered were significant ($p \leq 0.001$), which, respectively, explain 12.6, 43.1, and 7.5, of total variance. A significant interaction effects of Heat*pH and pH* Pectin also were obtained ($p \leq 0.001$). Tukey's multiple comparison was conducted to determine which level shows different hydrophobicity.

The heat-treatment at 75 °C for 20 min significantly ($p \leq 0.05$) increased hydrophobicity at all combinations (Fig. 5.1). Heating WPI increased hydrophobicity at pH 3.0, 5.0, 7.0 and 9.0 (Mleko, Li-Chan, & Pikus, 1997). Hydrophobic amino acid groups, which are originally located on the inside of β -lactoglobulin, can be exposed by partially unfolding of β -lactoglobulin by elevated temperature (Iametti, Cairoli, De Gregori, & Bonomi, 1995; Iametti, De Gregori, Vecchio, & Bonomi, 1996; Qi, Holt, Menulty, Clarke, Brownlow, & Jones, 1997). The increased

surface hydrophobicity with heat-treatment can be explained by the exposed hydrophobic groups by heat.

At pH 4.5, surface hydrophobicity of pectin-WPI hybrid and WPI only were significantly ($p \leq 0.05$) higher than those at pH 7 (Fig. 5.1). The higher hydrophobicity of β -lactoglobulin was reported at lower pH ((Das & Kinsella, 1989; Shimizu, Saito, & Yamauchi, 1985). The highest surface hydrophobicity of β -lactoglobulin was at pH 3.0 hydrophobicity decreased as pH value increased (Shimizu et al., 1985). The data obtained in this study, which is higher surface hydrophobicity at pH 4.5 than that at pH 7.0, agree with other previous studies.

There was no significant difference in surface hydrophobicity among original sugar beet pectin-, modified sugar beet pectin-WPI, and WPI only at pH 7 (Fig. 5.1). Hydrophobicity with original/modified sugar beet pectin was significantly ($p \leq 0.05$) lower than that of WPI only at pH 4.5 (Fig. 5.1), which suggests that suggesting that the added pectin protected WPI from increase in hydrophobicity. Decrease in surface hydrophobicity of bovine serum albumin (BSA) was observed on adding dextran sulfate, an anion polysaccharide (Galazka, Smith, Dickson, & Leward, 1999a). Adding κ -carrageenan or ι -carrageenan to BSA was also reported to decrease surface hydrophobicity (Galazka, Smith, Lewward, & Dickson, 1999b). They assumed that complexation of polysaccharide mainly contributed to decrease in surface hydrophobicity (Galazka et al., 1999b). Pectin is used in acid dairy drinks under acidic condition, which coats casein micelle and prevents casein from aggregation by steric stabilization and electrostatic repulsion of pectin located on the surface of casein micelle (Parker, Boulenger, & Kravtchenko, 1994; Tromp, Kruif, Eijk, & Rolin, 2004). It was reported that aggregate of β -lactoglobulin-high methoxyl pectin with heat-treatment at 65° at pH 3.8 and dissociation by adjusting pH to 6.0, which suggests electrostatic interaction between β -lactoglobulin and high methoxyl pectin

(Kazmierski, Wicker, & Corredig, 2003). Girad, Turgeon, & Gauthier (2002) confirmed that electrostatic forces play key role in interaction between low- and high-methocyl pectin and β -lactoglobulin. In this study, negatively charged sugar beet pectin and positively charged WPI could interact with each other and form complex by electrostatic interaction at pH 4.5 (Girad, Turgeon, & Gauthier, 2002; Ledward, 1994; Weinbreck, Nieuwenhuijse, Robin, & de Kruif, 2003). The dangling pectin molecules can cause steric stabilization and electrostatic repulsion, and mask or cover hydrophobic groups of WPI. The decrease in surface hydrophobicity of sugar beet pectin-WPI complex could be explained by masked or covered by sugar beet pectin. The higher hydrophobicity at 1:10 of Pectin:WPI ratio than that at 1:6 could be explained by that lower content of sugar beet pectin might contribute to less masking or covering hydrophobic groups resulting in higher surface hydrophobicity. Protein in sugar beet pectin mainly interacted with oil phase in oil in water emulsion suggesting that proteins in sugar beet pectin molecules have hydrophobic property (Leroux, Langendorff, Schick, Vanishav, & Mazoyer, 2003). Also, sugar beet pectin contains higher content of acetyl groups than citrus pectin (Ralet, Thibault, Faulds, & Williamson, 1994). These hydrophobic groups in sugar beet pectin could interact with hydrophobic groups on the surface of WPI. These hydrophobic interactions between pectin and WPI also might protect WPI from increase in hydrophobicity. With heat-treatment, higher hydrophobicity with modified sugar beet pectin was observed than that with original sugar beet pectin (Fig. 5.1). In this case, modified sugar beet pectin might be adsorbed on to the surface of WPI mainly by electrostatic attraction rather than hydrophobic interaction due to more negative charge of modified sugar beet pectin. Therefore, modified sugar beet anchored on WPI mainly by electrostatic attraction was more likely to contain higher content of hydrophobic protein in its dangling chain than original sugar beet pectin adsorbed by electrostatic attraction and

hydrophobic interaction. The higher content of hydrophobic protein in dangling chain of modified sugar beet pectin might contribute to higher surface hydrophobicity of modified sugar beet pectin-WPI than original one.

On comparing hydrophobicity with not filtered and filtered WPI, pectin-Filtered WPI showed higher hydrophobicity than pectin-not filtered WPI (Fig. 5.1). At pH 4.5, WPI aggregation was observed in not filtered WPI solution. Hydrophobic association between aggregated whey protein molecules might reduce the number of hydrophobic amino acids access to ANS (Bonomi, Iametti, Pagliarini, & Peri, 1988). The aggregated WPI could be removed by filtering. Filtered WPI was used to prepare pectin-WPI mixture at same final protein concentration with not filtered WPI. This means that filtered WPI contained higher soluble protein content than not filtered WPI. This soluble protein with higher content might contain more number of hydrophobic amino acids access to ANS. With heat-treatment, soluble WPI protein residue might more likely to expose hydrophobic groups than aggregated WPI, which might explain higher surface hydrophobicity of filtered WPI than not filtered WPI with heat-treatment.

3.2. Zeta potential

Zeta potentials of pectin-WPI hybrids, WPI only, and pectin only ranged from -30.72 to 6.90 (Table 5.1). On ANOVA, effects of pH and Pectin were significant ($p \leq 0.001$) explaining 60.9 and 11.5% of total variance (Table 5.3). The following effects were also significant ($p \leq 0.05$, 0.001 or 0.001) explaining up to 5.8% of total variance: Pectin:WPI ratio, pH*Pectin, Heat*Pectin*Ratio, Heat*pH*Pectin*Ratio, Heat*Filtered, and Heat*pH*Filtered (Table 5.3).

WPI only at pH 7 showed more negative zeta potential than at pH 4.5 (Fig. 5.2). Interestingly, filtered WPI only at pH 4.5 showed slight negative zeta potential. Slight positive

zeta potential was observed in β -lactoglobulin – stabilized O/W emulsion at pH 4 and 5, which finally resulted in aggregation of emulsion (Guzy et al., 2004). At pH 4.5, zeta potential of not-filtered WPI was 4.75, which could result in WPI aggregation. The positively charged aggregated WPI could be removed by filtering and zeta potential of filtered WPI could be slight negative value, -5.81. At pH 4.5, zeta potentials of pectin-WPI were more negative than WPI only (Fig 2). At pH 7, zeta potentials of pectin-WPI were not significantly different from those of WPI only (Fig. 2). Below pH 5, the decreased zeta potential was reported in sodium caseinate (CAS) – coated O/W emulsion droplet by addition of pectin (Surh, Decker, & McClement, 2005). This was due to electrostatic interactions between positively charged CAS and negatively charged pectin below pH 5 (Surh et al., 2005). The presence of pectin decreased zeta potential of β -lactoglobulin – stabilized O/W emulsion below pH 5 (Guezy et al., 2004). The addition of xanthan, anionic polysaccharide, decreased zeta potential of WPI below isoelectric point (Benichou, Aserin, & Garti, 2002). The adsorption of negatively charged pectin molecules onto positively charged protein aggregate was reported to mainly cause the decrease in zeta potential of casein aggregate in acidified milk drink (Sejersen, Salomonsen, Ipsen, Clark, Rolin, & Engelsen, 2006; Rolin, 2002). The decrease in zeta potential of WPI by adding pectin at pH 4.5 can be explained by adsorption of negatively charged pectin molecules onto positively charged WPI. At pH 7.0, there was no significant difference in zeta potentials between sugar beet pectin-WPI and WPI only (Fig. 5.2). Surh et al. (2005) reported that there was no significant effect of the pectin on the zeta potential of CAS – coated O/W emulsion droplet at pH 6 and 7. It was postulated that at pH 6 and 7, pectin molecules were not absorbed onto casein due to electrostatic repulsion between negatively charged pectin and negatively charged casein (Surh et al., 2005). The electrostatic repulsion can be induced between negatively charged sugar beet pectin and

negatively charged WPI at pH 7. With the electrostatic repulsion, the presence of sugar beet pectin cannot affect zeta potential of WPI only at pH 7.0.

There was no significant ($p \leq 0.05$) difference in zeta potential of WPI complexes with original and modified sugar beet pectin (Fig. 5.2). Schonhoff (2003) reported that zeta potential was largely independent of the charge density of absorbing polyelectrolyte on the adsorption of synthetic polyelectrolytes onto oppositely charge. Once the surface charge reaches at certain value by adsorption of polyelectrolytes, strong electrostatic repulsion can be induced between the surface and non-absorbed polyelectrolytes in the aqueous phase, limiting further adsorption of polyelectrolytes (Surh et al., 2005; Schonhoff, 2003). In this study, original or modified sugar beet pectin molecules could be absorbed to WPI surface at certain zeta potential value, and the absorbed pectin molecules could induce electrostatic repulsion with non-absorbed pectin. This can explain no significant ($p \leq 0.05$) difference in zeta potential of WPI complexes with original and modified sugar beet pectin.

3.3. Particle size

Particle sizes of pectin-WPI hybrid, WPI only, and pectin only shoed wide range from 192 to 4085 nm (Table 5.1). The significant effects of Heat, pH, Pectin, Pectin:WPI ratio, and Filtered obtained in ANOVA ($p \leq 0.001$) (Table 5.4). The effects of Heat, pH, Pectin, and Filtered explain 1.0, 54.9, 4.1 and 19.1% of total variance, respectively. Interaction effects also were observed on ANOVA ($p \leq 0.01$ or $p \leq 0.001$) (Table 5.4).

Heat-treatment on WPI only increased particle sizes (Fig. 5.3). The exposed hydrophobic groups by heat-treatment could induce WPI aggregation resulting in the increase in particle sizes of heat-treated WPI (Iametti et al., 1995; Iametti, De Gregori et al., 1996; Qi et al., 1997). Particle sizes of pectin-WPI hybrid with heat-treatment at 75 °C for 20 min did not have

significant ($p \leq 0.05$) difference from those without heat-treatment (Fig. 5.3). Particle sizes of WPI only at pH 4.5 were bigger than those at pH 7 (Fig. 5.3). Filtered WPI only had smaller particle sizes than not Filtered WPI regardless of heat-treatment (Fig. 5.3). This is due to the removing WPI aggregate by filtering.

On comparing particle sizes with original or modified sugar beet pectin, there was no significant difference in particle size with original and modified sugar beet pectin (Fig. 5.3). At pH 4.5, particle sizes of pectin-WPI hybrid were significantly smaller than those of WPI only. On contrast, at pH 7, there was no significant difference in particle sizes of WPI only and pectin-WPI hybrid (Fig. 5.3). At pH 7, electrostatic repulsion between negatively charged pectin and negatively charged WPI could be induced resulting in no adsorption of pectin onto WPI. Therefore, the added original or modified sugar beet pectin could not affect particle size of WPI at pH 7. In acid dairy drinks under acidic condition, pectin coats casein micelle and prevents casein from aggregation by steric and electrostatic stabilization of pectin located on the surface of casein micelle (Parker, Boulenguer, & Kravtchenko, 1994). In this study, negatively charged pectin molecules could be absorbed onto positively charged WPI at pH 4.5. The adsorbed pectin molecules could induce steric stabilization and electrostatic repulsion among WPI molecules and decrease surface hydrophobicity of WPI. The induced electrostatic repulsion by absorbed pectin and reduced surface hydrophobicity can contribute to smaller particle size of citrus pectin-WPI than WPI only. Once the surface charge of WPI reaches at certain value by adsorption of pectin, strong electrostatic repulsion can be induced between the surface of WPI and non-absorbed polyelectrolytes in the aqueous phase, limiting further adsorption of pectin molecules (Surh et al., 2005; Schonhoff, 2003). This can cause no difference in surface charges of citrus pectin-WPI depending on original and modified sugar beet pectin, which means no difference in induced

electrostatic repulsion of original sugar beet- and modified sugar beet pectin-WPI. The same level of electrostatic repulsion of original sugar beet- and modified sugar beet pectin-WPI can contribute to no significant difference in particle sizes between original sugar beet- and modified sugar beet pectin-WPI at pH 4.5.

4. Conclusions

WPI and original or modified sugar beet pectin interact and change physicochemical properties. The surface hydrophobicity, zeta potential, and particle sizes can be reduced in the presence of original or modified sugar beet pectin below isoelectric point of WPI. The interaction between WPI and pectin can be electrostatic attraction and hydrophobic interaction. The reduced surface hydrophobicity and zeta potential of pectin-WPI complexes can finally result in decrease in particle sizes of pectin-WPI complexes.

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Table 5.1. Hydrophobicity, zeta potential, and particle size of sugar beet pectin/WPI mixture

Pectin ^a	Pectin:WPI ratio	pH	Filter ^b	Heat ^c	Hydrophobicity		Zeta potential (mV)		Particle size (nm)	
					Avg ^d	SD ^e	Avg ^f	SD	Avg ^g	SD
Orig	1:6	4.5	NF	NH	240300	5967	-11.97	0.53	342	4
Orig	1:6	4.5	F	NH	218100	151	-19.33	3.56	192	6
Orig	1:6	7.0	-	NH	154800	280	-14.15	2.32	348	1
Orig	1:6	4.5	NF	H	375500	8849	-14.83	2.04	321	25
Orig	1:6	4.5	F	H	714200	18581	-26.77	0.27	217	5
Orig	1:6	7.0	-	H	209500	9481	-30.72	6.97	276	20
Orig	1:10	4.5	NF	NH	270000	4088	-15.47	2.91	260	6
Orig	1:10	4.5	F	NH	289200	3663	-21.99	5.04	209	2
Orig	1:10	7.0	-	NH	151300	3956	-13.48	2.72	320	34
Orig	1:10	4.5	NF	H	580400	12978	-19.55	7.78	264	8
Orig	1:10	4.5	F	H	873600	4194	-23.14	1.54	233	0
Orig	1:10	7.0	-	H	202800	3130	-19.26	4.63	320	34
Mod	1:6	4.5	NF	NH	199900	11316	-13.54	3.02	403	35
Mod	1:6	4.5	F	NH	232500	2790	-21.55	7.36	346	62
Mod	1:6	7.0	-	NH	165700	5117	-22.72	0.88	378	29
Mod	1:6	4.5	NF	H	657500	6901	-15.88	3.81	367	24
Mod	1:6	4.5	F	H	952100	3125	-27.71	5.53	292	1
Mod	1:6	7.0	-	H	206300	1843	-26.50	4.13	352	13
Mod	1:10	4.5	NF	NH	240600	1374	-14.03	2.00	342	4
Mod	1:10	4.5	F	NH	296600	647	-21.64	3.97	482	30
Mod	1:10	7.0	-	NH	153300	4857	-16.52	3.93	358	12
Mod	1:10	4.5	NF	H	798100	1724	-21.93	7.66	318	9
Mod	1:10	4.5	F	H	1000000	0	-24.78	5.03	356	12
Mod	1:10	7.0	-	H	202500	853	-19.64	3.12	358	12
-	WPI only	4.5	NF	NH	254500	13530	4.75	1.40	3714	102
-	WPI only	4.5	F	NH	301600	2062	-5.81	1.63	1502	20
-	WPI only	7.0	-	NH	154200	1855	-22.97	0.13	232	12
-	WPI only	4.5	NF	H	924500	33070	6.89	0.59	4085	45
-	WPI only	4.5	F	H	1000000	0	-2.41	0.60	3789	102
-	WPI only	7.0	-	H	190200	2676	-28.09	7.12	267	65
Orig	Pectin only	4.5	-	NH	12840	585	-26.15	1.45	509	34
Orig	Pectin only	7.0	-	NH	48180	804	-38.54	2.20	464	43
Orig	Pectin only	4.5	-	H	11490	363	-23.49	1.24	385	37
Orig	Pectin only	7.0	-	H	45560	1805	-28.39	7.18	276	11
Mod	Pectin only	4.5	-	NH	14230	812	-33.45	7.11	607	68
Mod	Pectin only	7.0	-	NH	47480	684	-48.67	1.18	487	16
Mod	Pectin only	4.5	-	H	10800	1278	-28.39	7.18	476	50
Mod	Pectin only	7.0	-	H	43260	357	-30.17	0.18	298	54

^a Orig, original sugar beet pectin; Mod, modified sugar beet pectin.

^b WPI solution at pH 4.5 was filtered by 0.45 μm syringe filter.

^c Heat-treatment at 75° for 20 min.

^d Average of hydrophobicity, n=2.

^e SD, standard deviation.

^f Average of zeta potential, n=3.

^g Average of particle size, n=3.

Table 5.2. Analysis variance of surface hydrophobicity of sugar beet pectin/WPI complex

Sources ^a	DF ^b	SS ^c	F ^d
Heat	1	0.597	290.43***
pH	1	2.000	973.46***
Pectin	1	0.350	170.29***
Filtered	1	0.855	415.93***
Heat*pH	1	0.749	364.40***
pH*Pectin	1	0.019	9.61**

^a The sources with significant effects were presented in this table. R^2 (coefficient of determination)=0.991589, $(MSE)^{1/2}$ =45332.04.

^b Degree of freedom, Model DF=18, Error DF=19, Corrected total DF=37.

^c Sum of squares. SS=Value presented* 10^{12} , Model SS= 4.603×10^{12} , Error SS= 0.039×10^{12} , Corrected total SS= 4.642×10^{12} .

^d Fisher ratio value, Model F=124.44***, Significant level: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

Table 5.3. Analysis variance of zeta potential of sugar beet pectin/WPI complex

Sources ^a	DF ^b	SS ^c	F ^d
pH	1	5055.103	340.00***
Pectin	1	958.328	64.46***
Pectin:WPI Ratio	1	130.927	8.91**
pH*Pectin	1	404.104	27.18***
Heat*Pectin*Ratio	1	257.211	17.30***
Heat*pH*Pectin*Ratio	1	277.528	18.67***
Heat*Filtered	1	90.049	6.06*
Heat*pH*Filtered	1	478.260	32.17***

^a The sources with significant effects were presented in this table. R² (coefficient of determination)=0.9476289, (MSE)^{1/2}=3.855923.

^b Degree of freedom, Model DF=26, Error DF=30, Corrected total DF=56.

^c Sum of squares, Model SS=7858547, Error SS=446.044, Corrected total SS=8304.591.

^d Fisher ratio value, Model F=20.33***, Significant level: *, p≤0.05; **, p≤0.01; ***, p≤0.001.

Table 5.4. Analysis variance of the particle size of sugar beet pectin/WPI complex

Sources ^a	DF ^b	SS ^c	F ^d
Heat	1	9480.001	20.77***
pH	1	523451.816	1146.84***
Pectin	1	38665.263	84.71***
Pectin:WPI Ratio	1	6440.039	14.11**
Filtered	1	181812.322	398.34***
Heat*Pectin	1	21601.476	47.33***
pH*Pectin	1	12972.620	28.42***
Heat*pH*Pectin	1	12396.953	27.16***
Pectin*Ratio	1	3075.197	6.74*
Heat*pectin*Ratio	1	4159.728	9.11*
pH*Pectin*Ratio	1	4631.483	10.15**
Heat*Filtered	1	112287.874	246.01***
pH*Pectin*Filtered	1	14401.127	31.55***

^a The sources with significant effects were presented in this table. R^2 (coefficient of determination)=0.994732, $(MSE)^{1/2}=21.36422$.

^b Degree of freedom, Model DF=26, Error DF=11, Corrected total DF=37.

^c Sum of squares, Model SS=948093.2973, Error SS=5020.7311, Corrected total SS=953114.0284.

^d Fisher ratio value, Model F=79.89***, Significant level: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

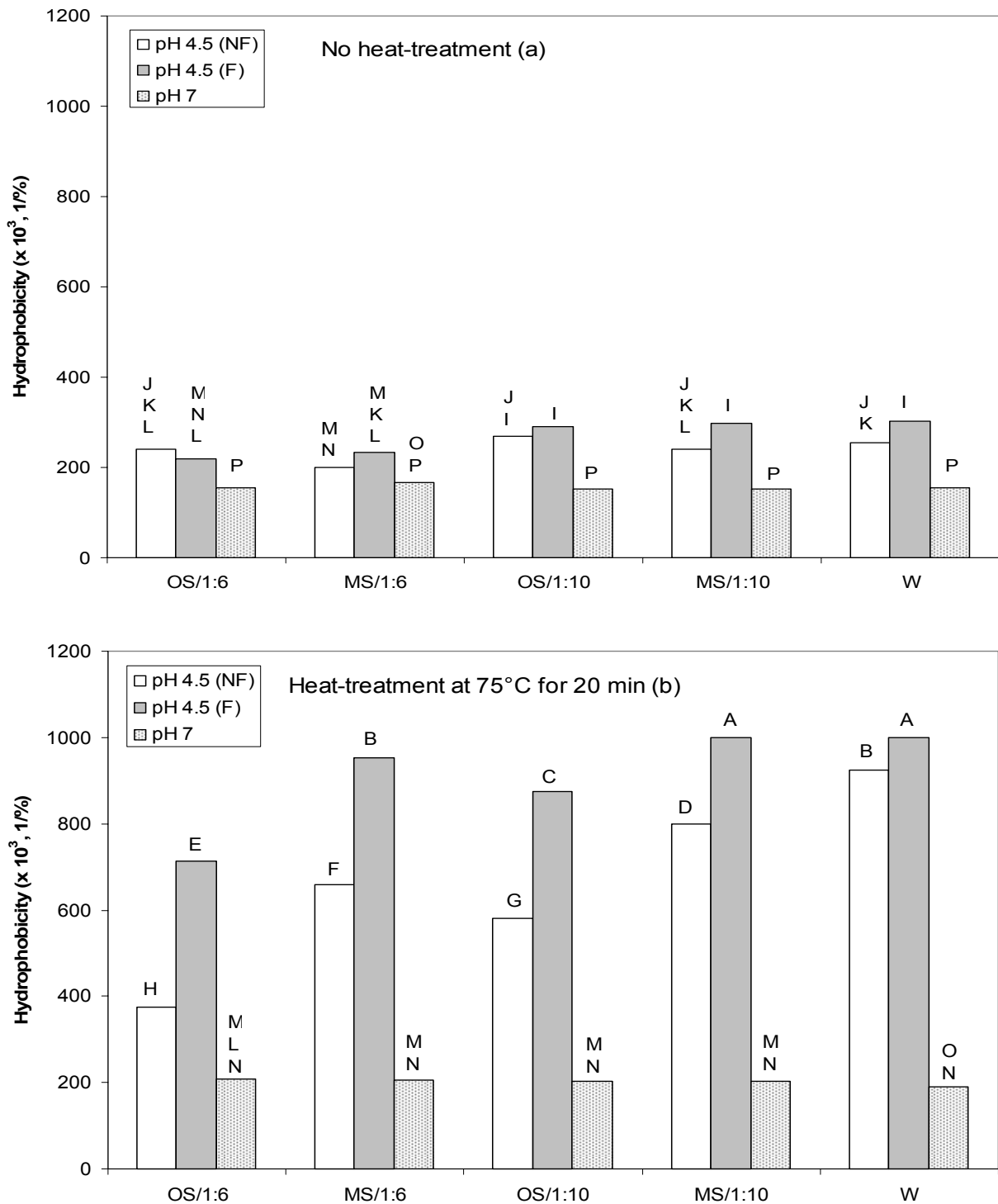


Fig. 5.1. Hydrophobicity of sugar beet pectin/WPI complexes. Hydrophobicity with same letter means no significant difference at 5% confidence level. The abbreviations: OC, original citrus pectin; MC, modified citrus pectin; 1:6 and 1:10, pectin to WPI ratio; NF, not filtered; F, filtered; W, WPI.

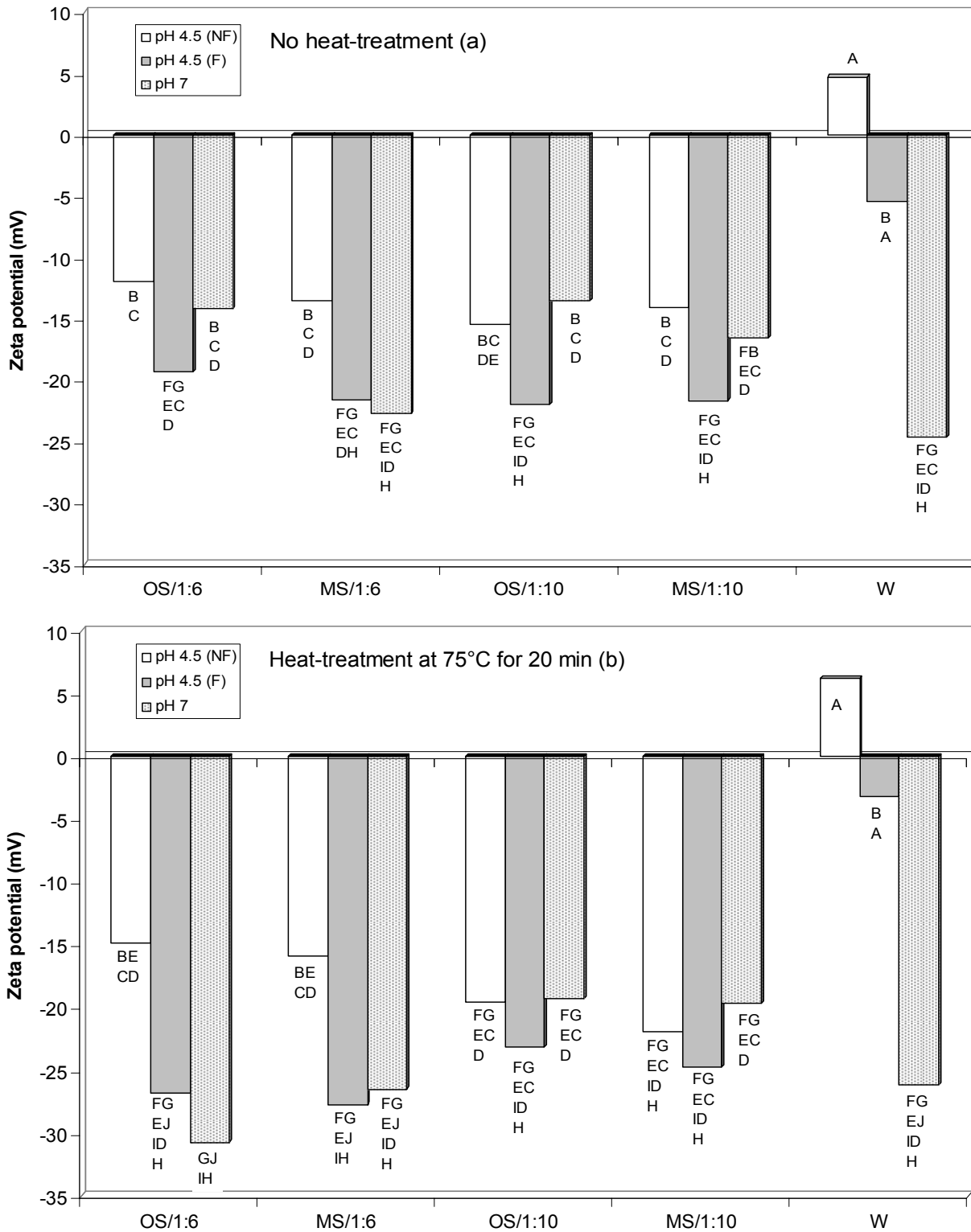


Fig. 5.2. Zeta potentials of sugar beet pectin/WPI complexes. Zeta potentials with same letter mean no significant difference at 5% confidence level. The abbreviations: OC, original citrus pectin; MC, modified citrus pectin; 1:6 and 1:10, pectin to WPI ratio; 7 and 4.5, pH 7 and pH 4.5; NF, not filtered; F, filtered; W, WPI.

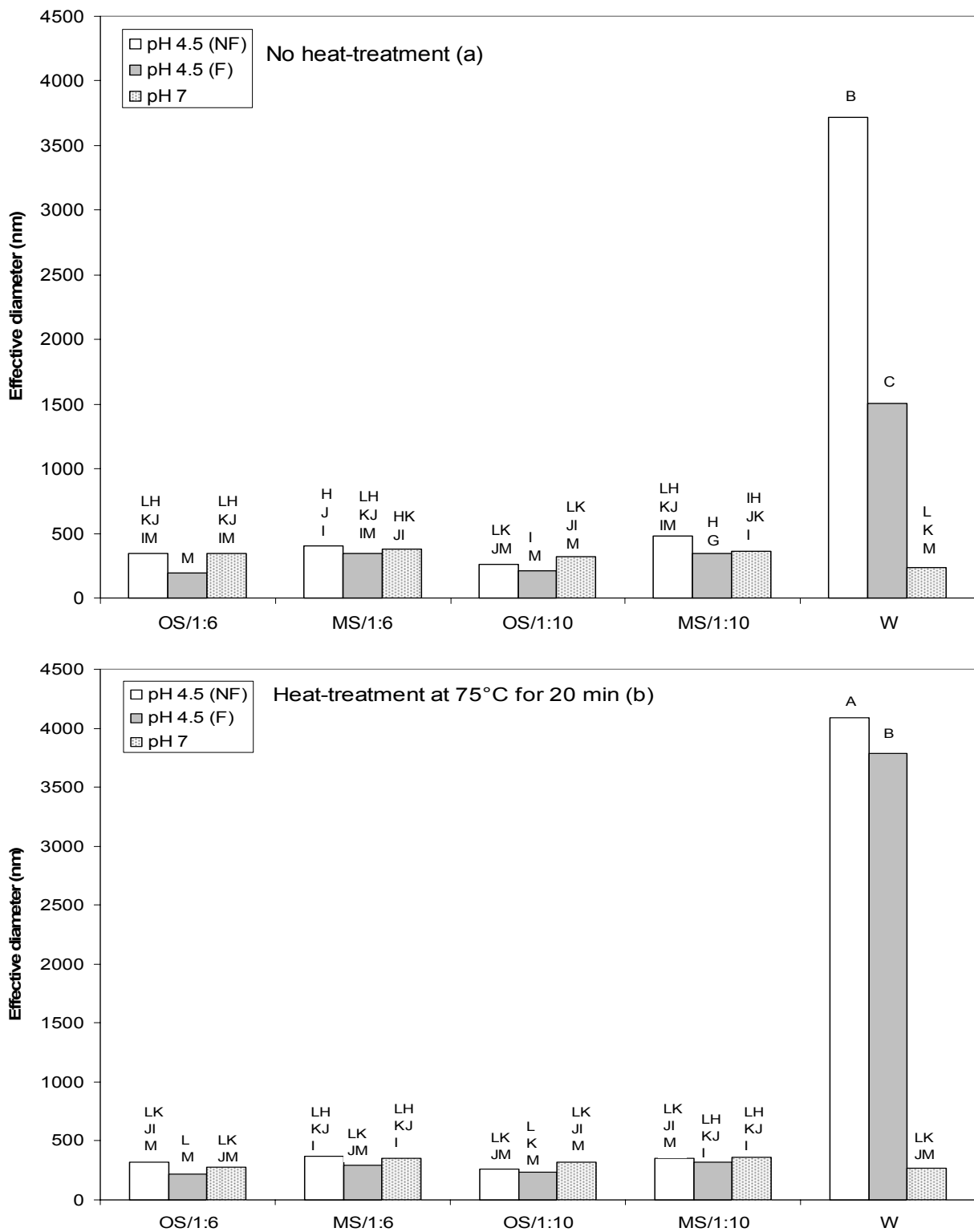


Fig. 5.3. Particle size of sugar beet pectin/WPI complexes. Particle sizes with same letter mean no significant difference at 5% confidence level. The abbreviations: OC, original citrus pectin; MC, modified citrus pectin; 1:6 and 1:10, pectin to WPI ratio; 7 and 4.5, pH 7 and pH 4.5; NF, not filtered; F, filtered; W, WPI.

CHAPTER 6

RHEOLOGICAL PROPERTIES OF PECTIN-WHEY PROTEIN ISOLATE COMPLEXES: EFFECTS OF SOURCES OF PECTIN, DEGREE OF ESTERIFICATION OF PECTIN, PH AND HEAT-TREATMENT¹

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Abstract

The effects of different pectin types (citrus and sugar beet pectin), original and charge modified pectin, heat-treatment (75°C for 20 min), and pH (4.5 and 7.0) on rheological properties of pectin-WPI mixture (1:6 ratio) were determined. Elastic (G') properties of pectin-WPI mixtures were measured by deformation oscillatory measurements. In dynamic time sweep test at 5 Hz and 1 Pa for 15 min, G' of modified citrus pectin-WPI mixture was highest followed by modified sugar beet pectin-WPI mixture all pH values and regardless of heat-treatment. The added original citrus and sugar beet pectin did not affect G' of pectin-WPI at all pH and heat-treatment. Regardless of pH and heat-treatment, elastic property (G') of modified citrus pectin-WPI were significantly ($p \leq 0.05$) higher than those of original citrus- or original/modified sugar beet pectin-WPI. G' of modified citrus pectin-WPI at pH 4.5 or with heat-treatment was higher than that at pH 7 or without heat-treatment. At the combination of pH 4.5 and heat-treatment, G' of modified sugar beet pectin-WPI mixture was significantly higher than that at pH 7 or without heat-treatment.

Keywords: citrus pectin, sugar beet pectin, modified/original pectin, pH, heat-treatment, elastic property, pectin-WPI mixture

1. Introduction

Polysaccharides and proteins are the ingredients of many food products. These two biopolymers influence the textural characteristics of food products through gelling of individual ingredients and/or mixed gelling of both biopolymers (Tolstoguzov, 1991). Mixed gelling with two different biopolymers can be formed by intermolecular attraction (complex) or intermolecular repulsion (incompatible). Both of attractive and repulsive interactions influence gelling properties (Cai & Arntfield, 1997; Ganz, 1974; Ould Eleya & Turgeon, 2000; Sanchez, Schmitt, Babak, & Hardy, 1997; Shim & Mulvaney, 2001; Smith, Nash, Eldridge, & Wolf, 1962; Wang & Qvist, 2000). Sodium alginate and sodium caseinate form complex gels under conditions where both biopolymers do not form gel (Tolstoguzov, 1986). Synergistic and antagonistic effects on gelling were observed in heat-denatured WPI-xanthan system after cold gelation (Bryant & McClements, 2000). The enhanced gelling strength at neutral pH condition was reported in high-pressure treatment of β -lactoglobulin with pectin (Dickson & James, 2000). A cloudy complex was formed in β -lactoglobulin - sodium polypectate system suggesting potential of sodium polypectate to modify β -lactoglobulin and/or whey protein gelling in various food systems (Ndi, Swanson, Dunker, & Luedecke, 1996; Ndi, Swanson, Barbosa-Canovas, & Luedecke, 1996). Gel formation was inhibited in a gelatin and κ -carrageenan system by strong electrostatic interaction (Watase & Nishinari, 1983). Xanthan had a negative effect on β -lactoglobulin gelling at higher protein concentration than 10% (Zasytkin, Dumay, & Cheftel, 1996). Overall gelling property of food protein can be favorably or unfavorably modified depending on the nature of interaction of polysaccharides (Ndi, Swanson, Barbosa-Canovas, & Luedecke, 1996).

WPI has been used as excellent functional food proteins in food products (Dickson, 2003; McClements, 2004; de Wit, 1998; Kinsella & Whitehead, 1989a). WPI is composed of β -lactoglobulin (about 50%), α -lactalbumin (about 20%), serum albumin, immunoglobulin and minor protein (de Wit, 1989; Ziegler & Foegeding, 1990). In terms of proportion of composition, β -lactoglobulin is mostly related to the functionalities (de Wit, 1989; Ziegler & Foegeding, 1990). Elevating temperature above room temperature induces the dissociation of β -lactoglobulin from a dimer to monomer (McKenzie, 1971; Hambling, McAlpine, & Sawyer, 1992). Hydrophobic amino acid groups, which are originally located on the inside of β -lactoglobulin, can be exposed by partially unfolding of β -lactoglobulin by elevated temperature (Iametti, Cairoli, De Gregori, & Bonomi, 1995; Iametti, De Gregori, Vecchio, & Bonomi, 1996; Qi, Holt, Menulty, Clarke, Brownlow, & Jones, 1997). The exposed hydrophobic amino acid group can induce aggregation of proteins (Totosaus, Montejano, Salazar, & Guerrero, 2002).

Pectin is complex polysaccharide, which is composed of α -1 \rightarrow 4-linked D-galacturonic acid esterified with methyl group. Degree of esterification (DE) and distribution of de-esterified group in α -1 \rightarrow 4-linked D-galacturonic acid backbone can affect gelling and calcium binding of pectin (Hill, Mottern, Nutting, & Speiser, 1949). This galacturonic acid backbone is interrupted by 1 \rightarrow 2-linked L-rhamnose residue carrying neutral sugar side chain (Voragen, Pilnik, Thibault, Axelos, Renard & Stephen, 1995). Pectin has been added to foods as gelling and stabilizing agents (Nelson, Smit, & Wiles, 1995; Voragen, Pilnik, Thibault, Axelos, Renard & Stephen, 1995). In terms of structure, sugar beet pectin is different from citrus pectin. Sugar beet pectin contains higher acetyl located on C-2 and C-3 position of galacturonic acid residues, higher protein content, higher arabinose content, and feruloyl groups attached to galactose and arabinose (Colquhoun, Ralet, Thibault, Faulds, & Williamson, 1994; Gullion & Thibault, 1988,

1989a,b; Ralet, Thibault, Faulds, & Williamson, 1994; Keenan, Belton, Matthew, & Howson, 1985). The functionalities of citrus and sugar beet pectin can be different due to the difference in structures. Citrus pectin was used to form gel in the presence of sugar or polyvalent cations (Hill, Mottern, Nutting, & Speiser, 1949; Rinaudo, 1996). In acid dairy drinks under acidic condition, citrus pectin coats casein micelle and prevents casein from aggregation by electrostatic and steric stabilization of pectin located on the surface of casein micelle (Parker, Boulenguer, & Kravtchenko, 1994). Sugar beet pectin was reported to have poor gelling property (Pippen, McCready, & Owens, 1950). However, sugar beet pectin was reported to have higher surface activity to show better emulsifying property than citrus pectin (Leoroux, Langedorff, Schick, Vaishnav, & Mazoyer, 2003). This is due to its higher content of protein in sugar beet pectin compared to citrus pectin (Leoroux et al., 2003).

The hypotheses of this research were that the structural difference between citrus pectin and sugar beet pectin may lead different rheological properties of WPI-pectin mixture and DE of pectin could affect rheological properties of WPI-pectin mixture as well as pH and heat-treatment. The objectives of these studies were to study fundamental rheological properties of WPI-pectin mixture with original/modified citrus and sugar beet pectins, and to determine the effects of different DE of pectin, pH and heat-treatment on rheological properties of citrus or sugar beet pectin – WPI system.

2. Materials and Methods

2.1. Materials

Original/modified citrus pectin (CP Kelco, Svenved, Denmark) and sugar beet pectin (Herbstreith & Fox Inc, Elmsford, NY) were used as pectin sources. WPI was obtained from

Trega Foods Inc (Little Chute, WI). Pectinmethylesterase (PME) was extracted from Valencia orange pulp (Citrus World, Lake Wales, FL). Crude PME was purified by Ackerley, Corredig, & Wicker (2002). Unbound PME to Hi-Trap SP cation exchange and Heparin affinity columns (Amersham Pharmacia Biotech, Uppsala, Sweden) was used for pectin modification. Pectin modification and characterization were performed by Kim, Teng, & Wicker (2005). DE values of original citrus pectin and sugar beet pectin were 58 and 56%. After modification, DE values of citrus pectin and sugar beet pectin decreased to 48 and 42%.

2.3. Preparation of WPI and pectin solutions

Stock WPI solution (20%, w/v) was prepared by adding WPI to 5 mM Na phosphate buffer, pH 7 or 4.5. The stock WPI solution was stirred at room temperature for 2 h and stored at 4°C overnight for complete hydration. Stock pectin solution (2%, w/v) was prepared by strong stirring at room temperature for 2 h followed by overnight hydration in 4°C. WPI-pectin mixtures were made by two ratio of pectin to WPI of 1:6. WPI solution at pH 4.5 was filtered through 0.45 µm syringe filter (Whatman, Clifton, NJ). Both of filtered and non-filtered WPI solutions were used in preparing WPI-pectin mixture. For heat-treated samples, the pectin-WPI mixture was heated at 75 °C for 20 min at the water bath and cooled it down in the ice.

2.3. Rheological measurement

Controlled Stress Dynamic RheometerTM (Rheometrics, Piscataway, NJ) was used to measure rheological property of pectin-WPI complex. The rheometer was equipped with Peltier temperature controller, and cone and plate device (60 mm diameter, 0.0385°). Temperature plate was maintained at 20°C on all measurements. An aliquot of 1.4 ml pectin-WPI complex was placed on the plate. Dynamic time sweep test was conducted at 5 Hz and 1 Pa for 15 min followed by dynamic frequency sweep test at 1 Pa from 0 to 10 Hz.

2.4. Statistical analysis

Four variables were used to characterize rheological properties of pectin-WPI complexes and WPI according to factorial design, which were pectin, pH, filtering, and heat-treatment. SAS system (Version 6.2, SAS Inc, Cary, NC) was used to conduct statistical analysis, such as analysis of variance (ANOVA) and Tukey's multiple comparison. Rheological properties were measured in triplicate.

3. Results and Discussions

3.1. Dynamic time sweep test of pectin-WPI hybrid

The effects of pectin, pH and heat-treatment on rheological property of pectin-WPI were observed in dynamic time sweep test. G' values of pectin only and WPI only were less than 6 Pa in all dynamic time sweep tests. Fig. 6.1. shows G' values of pectin-WPI hybrid (1:6 ratio), and WPI only at pH 7 over 1500 s at 1 Pa and 5 Hz. Without and with heat-treatment at 75 °C for 20 min, modified pectin-WPI hybrid had higher G' values than original pectin-WPI hybrid over 1500 s. Modified citrus pectin-WPI hybrid exhibited highest G' value followed by modified sugar beet pectin-WPI hybrid. G' value of modified citrus-, modified sugar beet, and original sugar beet pectin-WPI with heat-treatment were higher than those without heat-treatment (Fig. 6.1). At pH 4.5, same trends were observed (Fig. 6.2 and 3). Regardless of heat-treatment, G' of modified citrus pectin-WPI hybrid was highest followed by modified sugar beet pectin-WPI hybrid. Heat-treat increased G' values of modified citrus- and modified sugar beet pectin-WPI. When G' values of modified citrus pectin- and modified sugar beet pectin-WPI at pH 4.5 were compared to those at pH 7, G' values at pH 4.5 were higher than those at pH 7.

3.2. Effect of heat, pH, and pectin on viscoelastic properties of pectin-WPI complexes

Table 1 presents storage and loss modulus of pectin-WPI hybrid, WPI only, and pectin only at 1 Pa and 2.5 Hz. G' values of WPI only and pectin only were less than 1.00 Pa (Table 6.1). In contrast, G' values of pectin-WPI complexes exhibited wide range from less than 1.0 to 984 Pa depending on pH, heat-treatment, and pectin. Analysis of variance (ANOVA) was conducted to determine significant effect of G' values. From the ANOVA, there were significant effects of pH, heat and pectin on rheological properties of pectin-WPI ($p \leq 0.05$).

3.2.1. Effect of pH

Different G' values of modified citrus beet pectin-WPI were observed in pH 4.5 and 7.0 regardless of heat-treatment (Fig. 6.4a). The difference in G' of modified sugar beet pectin-WPI between pH 4.5 and 7 was observed only with heat-treatment (Fig. 6.4a).

Since protein-anionic polysaccharide interaction is mainly caused by electrostatic interaction, pH can be most important factor (Girard, Turgeon, & Gauthier, 2002). Below isoelectric point, positively charged protein can interact with anionic polysaccharide and form complexation of protein and anionic polysaccharide. In the mixture of bovine serum albumin (BSA) and anionic polysaccharide, pectin, positive charged BAS interacted with negatively charged pectin (Cai & Arntfield, 1997). This electrostatic attraction between two macromolecules with opposite charges can be referred as a particle model binding, which has been referred to as nonspecific interaction (Stainsby, 1980; Cai & Arntfield, 1997). It was reported that the amount of β -lactoglobulin interacted with low methoxyl pectin was higher than that with high methoxyl pectin at pH 4.5 (Girard, Turgeon, & Gauthier, 2002). The presence of κ -carrageenan, anionic polysaccharide, enhanced β -lactoglobulin gel strength at pH 4.0 by electrostatic attraction (Ould Eleya & Turgeon, 2000). At pH 4.5, below isoelectric point of WPI,

positively charged WPI molecules could interact with negatively charged modified citrus pectin, which contributed to elastic property of modified citrus pectin-WPI.

Above isoelectric point, phase separation could contribute to elastic property of anionic polysaccharide-protein complex (Bertrand & Turgeon, 2007). Mixed gel could be successfully prepared with WPI and LM pectin after heat-induced gelation (Beaulieu, Turgeon, & Doublier, 2001). They assumed that WPI could be main gelling agent in the mixed system. The added pectin could entrap water in the pectin phase, which could result in the concentration of WPI in the WPI phase. The concentration of WPI finally could increase elastic property of WPI (Beaulieu, Turgeon, & Doublier, 2001). Bertrand & Turgeon (2007) also reported that phase separation of WPI and xanthan at pH 6.0 and 6.5, which could contributed the increase in elastic property of xanthan-WPI complex. At pH 7.0, phase separation of modified citrus pectin and WPI could mainly contribute to elastic property of modified citrus pectin-WPI complex. Furthermore, the significant difference in elastic properties of modified citrus pectin-WPI between pH 4.5 and 7 might be explained by different mixing patterns, complexation by electrostatic attraction and phase separation by electrostatic repulsion.

3.2.2. Effect of pectin DE

At pH 4.5, G' values of modified citrus pectin were significantly ($p \leq 0.05$) higher than those of original citrus and original/modified sugar beet pectin (Fig. 6.4). It was reported that G' values of de-methoxylated pectin were increased 35-fold and the gel-like properties were markedly enhanced in the presence of calcium (Schmelter, Wientjes, Vreeker, & Klaffke, 2002). In our previous studies, remarkable increase in G' value of modified citrus pectin was observed in the presence of calcium. De-esterified groups, free carboxyl group, de-esterified group in low methoxyl pectin interact with polyvalent cations, such as calcium and induce gelling (Hill,

Mottern, Nutting, & Speiser, 1949; Rinaudo, 1996). In the case of pectin and WPI system, electrostatic interaction can be induced between negatively charged free carboxyl group in pectin and positively charged groups in WPI at pH 4.5. Higher content of free carboxyl groups in modified citrus pectin could induce more interaction with WPI at pH 4.5, which contributed to higher G' value than that of original citrus pectin-WPI. Although DE of modified sugar beet pectin was not different from that of modified citrus pectin, G' value of modified sugar beet pectin-WPI was significantly less. The high content of acetyl group is distinctive characteristic of sugar beet pectin and hinders gelling of sugar beet pectin in the presence of calcium (Pippen, McCready, & Owens, 1950). The low G' value of modified sugar beet pectin-WPI also can be explained by antagonistic effect of high acetyl content on gelling.

G' values of modified citrus pectin-WPI at pH 7.0 were significantly ($p \leq 0.05$) higher than G' values of original pectin-WPI (Fig. 6.4a). This could be explained by that modified citrus pectin with higher negatively charge could interact with more water and entrap more water than original citrus pectin. More entrapping water by modified citrus pectin could cause more concentration of WPI in the WPI phase, which contributed to increase in elastic property of WPI. Although DE of modified sugar beet pectin was slightly lower than modified citrus pectin, G' of modified sugar beet pectin-WPI was significantly lower than that of modified citrus pectin-WPI. Surface hydrophobicity of sugar beet pectin was higher than that of citrus pectin in our previous studies. Sugar beet pectin with higher surface hydrophobicity might entrap less water than citrus pectin, which resulted in less concentration of WPI in sugar beet pectin-WPI system. Therefore, lower G' of modified sugar beet pectin-WPI could be explained by less water-entrapment and less concentration of WPI.

3.2.3. *Effect of heat*

On comparing G' values with and without heat-treatment at 75 °C for 20 min, G' values of modified citrus with heat-treatment were significantly higher than those of original citrus pectin (Fig. 3). Modified sugar beet pectin showed significant increase in G' value by heat-treatment at pH 4.5, but not at pH 7.0 (Fig. 6.4). G' values of original citrus and sugar beet pectin with heat-treatment were not significantly different from those without heat-treatment at pH 4.5 and 7.0 (Fig. 6.4).

The increased G' value with heat-treatment can be explained by exposed hydrophobic groups and exposed reactive site of WPI by heat-treatment. Whey protein is about 50% of β -lactoglobulin, major protein in WPI, is responsible for the functional properties (de Wit, 1989; Ziegler & Foegeding, 1990). The dissociation of β -lactoglobulin from a dimer to monomer occurs at elevating temperature above room temperature (McKenzie, 1971; Hambling, McAlpine, & Sawyer, 1992). The elevated temperature cause partial unfolding of β -lactoglobulin. The partial unfolding of β -lactoglobulin induces the exposure of hydrophobic groups, which are originally located on the inside of β -lactoglobulin, can be exposed by partially unfolding of β -lactoglobulin by elevated temperature (Iametti, Cairoli, De Gregori, & Bonomi, 1995; Iametti, De Gregori, Vecchio, & Bonomi, 1996; Qi, Holt, Menulty, Clarke, Brownlow, & Jones, 1997). The exposed hydrophobic group can induce aggregation of proteins (Totosaus, Montejano, Salazar, & Guerrero, 2002). More reactive sites, which are originally located inside of protein, can be exposed by heat-denaturation of protein. These exposed reactive sites can interact with polysaccharides (Park, Muhoberac, Dubin, & Xia, 1992). Both of WPI aggregation and more interaction of reactive heat-treated WPI with heat-treatment may attribute to increase in G' value at pH 4.5. At pH 7.0, significant ($p \leq 0.05$) increase in G' value of modified citrus pectin-WPI was

also observed by heat-treatment, which could be explained by more WPI aggregation in concentrated WPI phase.

Interestingly, G' value of modified sugar beet pectin at pH 4.5 with heat-treatment was significantly ($p \leq 0.05$) higher than that without heat-treatment (Fig. 6.4a and b). The electrostatic interaction between free carboxyl groups in modified sugar beet pectin and more reactive sites in heat-treated WPI might overcome hindrance by high acetyl content in sugar beet pectin resulting in increase in G' value with heat-treatment. In addition, sugar beet pectin contains a protein moiety (Leroux et al. 2003) that contributes to the superior emulsifying capacity of sugar beet pectin. It is possible that the protein part of sugar beet pectin contributes to gelation.

4. Conclusions

The presence of higher negative charge on citrus and sugar beet pectin increases elastic properties of WPI mixed gels. Heat-treatment also increases elastic properties of pectin-WPI complexes. Rheological properties of WPI gels can be modified by charge modified pectins, heat-treatment and pH.

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Table 6.1. Storage (G') and loss (G'') modules of pectin-WPI, WPI only and pectin only at 2.5 Hz and 1 Pa

Pectin	pH	WPI		Storage module	Loss module
		Filtered ^a	Heat ^b	G' (Pa)	G'' (Pa)
Original citrus pectin	4.5	Not-filtered	No heat-treatment	6.89	4.01
Original citrus pectin	4.5	Filtered	No heat-treatment	3.52	3.48
Original citrus pectin	7.0	-	No heat-treatment	0.50	0.27
Modified citrus pectin	4.5	Not-filtered	No heat-treatment	220.75	37.55
Modified citrus pectin	4.5	Filtered	No heat-treatment	146.15	34.10
Modified citrus pectin	7.0	-	No heat-treatment	59.12	19.38
Original sugar beet pectin	4.5	Not-filtered	No heat-treatment	0.58	0.07
Original sugar beet pectin	4.5	Filtered	No heat-treatment	0.56	0.11
Original sugar beet pectin	7.0	-	No heat-treatment	0.59	0.06
Modified sugar beet pectin	4.5	Not-filtered	No heat-treatment	15.79	14.60
Modified sugar beet pectin	4.5	Filtered	No heat-treatment	14.99	13.03
Modified sugar beet pectin	7.0	-	No heat-treatment	3.91	2.80
Original citrus pectin	4.5	Not-filtered	Heat-treatment	3.54	4.37
Original citrus pectin	4.5	Filtered	Heat-treatment	8.49	7.36
Original citrus pectin	7.0	-	Heat-treatment	1.91	1.81
Modified citrus pectin	4.5	Not-filtered	Heat-treatment	984.23	216.55
Modified citrus pectin	4.5	Filtered	Heat-treatment	961.60	218.89
Modified citrus pectin	7.0	-	Heat-treatment	190.65	32.42
Original sugar beet pectin	4.5	Not-filtered	Heat-treatment	0.11	0.34
Original sugar beet pectin	4.5	Filtered	Heat-treatment	1.08	0.75
Original sugar beet pectin	7.0	-	Heat-treatment	12.05	6.12
Modified sugar beet pectin	4.5	Not-filtered	Heat-treatment	102.00	42.35
Modified sugar beet pectin	4.5	Filtered	Heat-treatment	129.37	54.90
Modified sugar beet pectin	7.0	-	Heat-treatment	21.77	8.33
-	4.5	Not-filtered	No heat-treatment	0.52	-0.01
-	4.5	Filtered	No heat-treatment	0.50	-0.05
-	7.0	-	No heat-treatment	0.57	0.07
-	4.5	Not-filtered	Heat-treatment	0.62	0.07
-	4.5	Filtered	Heat-treatment	0.32	0.10
-	7.0	-	Heat-treatment	0.64	0.05
Original citrus pectin	4.5	-	No heat-treatment	0.64	0.28
Original citrus pectin	7.0	-	No heat-treatment	0.50	0.27
Modified citrus pectin	4.5	-	No heat-treatment	0.64	0.28
Modified citrus pectin	7.0	-	No heat-treatment	0.50	0.31
Original sugar beet pectin	4.5	-	No heat-treatment	0.50	0.05
Original sugar beet pectin	7.0	-	No heat-treatment	0.59	0.06
Modified sugar beet pectin	4.5	-	No heat-treatment	0.72	0.22
Modified sugar beet pectin	7.0	-	No heat-treatment	0.74	0.25
Original citrus pectin	4.5	-	Heat-treatment	0.64	0.54
Original citrus pectin	7.0	-	Heat-treatment	0.21	0.15
Modified citrus pectin	4.5	-	Heat-treatment	0.64	0.30
Modified citrus pectin	7.0	-	Heat-treatment	0.21	0.26
Original sugar beet pectin	4.5	-	Heat-treatment	0.67	0.28
Original sugar beet pectin	7.0	-	Heat-treatment	0.21	0.19
Modified sugar beet pectin	4.5	-	Heat-treatment	0.78	0.29
Modified sugar beet pectin	7.0	-	Heat-treatment	0.32	0.26

^a At pH 4.5, WPI solutions were filtered through 0.45 μ m syringe filter.

^b Heat-treatment at 75 °C for 20 min.

Table 6.2. Analysis variance of G' values of citrus and sugar beet pectin/WPI complex

Source of variation ^a	DF ^b	SS ^c	F ^d
Heat	1	244591	7.52**
pH	1	189585	5.83**
Pectin	1	405317	12.46***
Heat*pectin	1	177278	5.45*

^a The sources with significant effects were presented in this table. R² (coefficient of determination)=0.255067, (MSE)^{1/2}=1803573.

^b Degree of freedom, Model DF=11, Error DF=126, Corrected total DF=137.

^c Sum of squares, Model SS=1403379, Error SS=4098624, Corrected total SS=5502004.

^d Fisher ratio value, Model F=75.06***, Significant level: *, p≤0.05; **, p≤0.01; ***, p≤0.001.

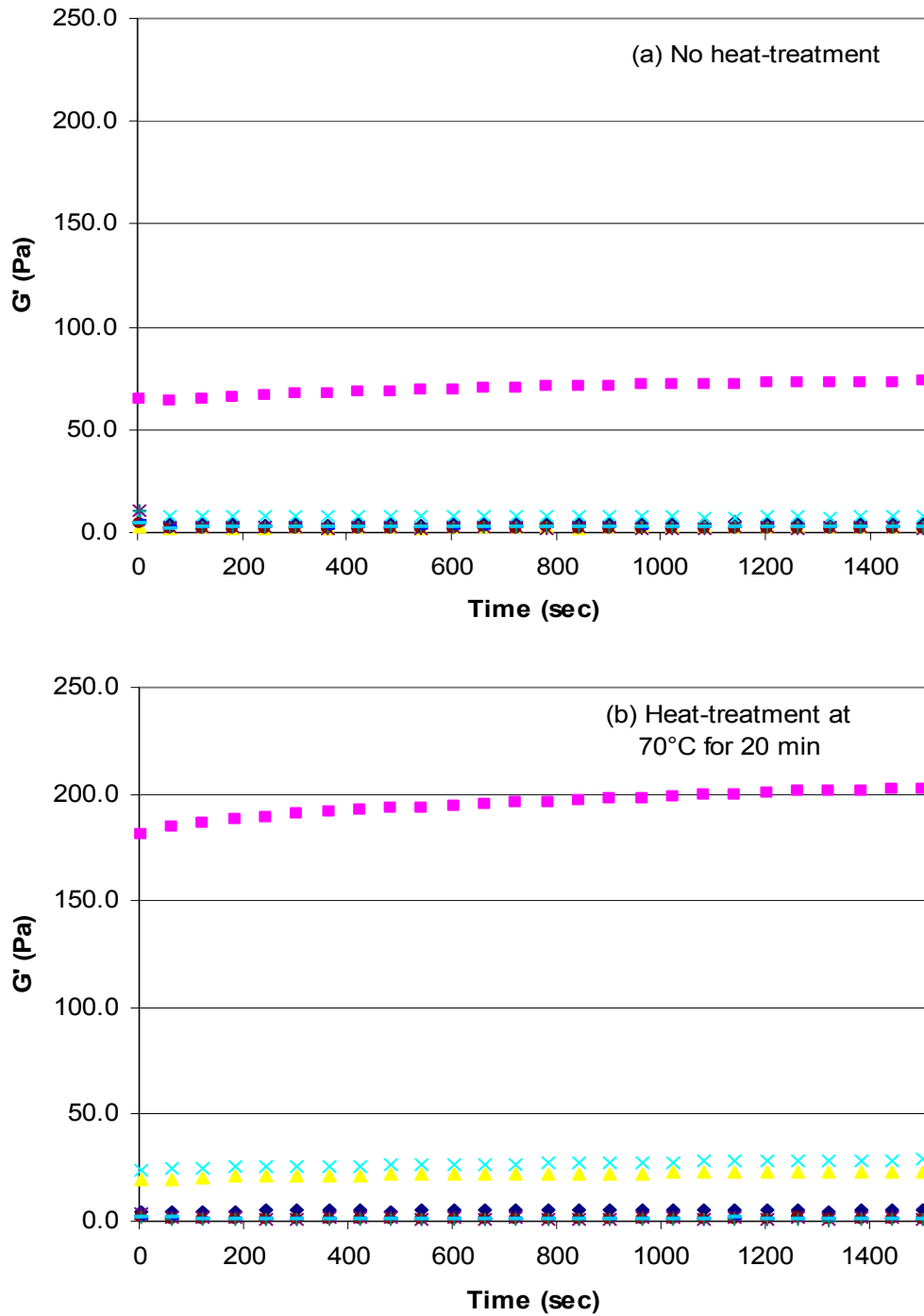


Fig. 6.1. Storage modulus (G') as a function of time of pectin-WPI hybrid (1:6 ratio), and WPI only, pectin only at pH 7 without (a) or with (b) heat-treatment at 75°C for 20 min at 1 Pa and 1 Hz, where \blacklozenge , citrus pectin-WPI; \blacksquare , modified citrus pectin-WPI; \blacktriangle , sugar beet pectin-WPI; \times , modified sugar beet pectin-WPI; $*$, WPI only; \bullet , citrus pectin only; $+$ modified citrus pectin only; $-$, sugar beet pectin only; — , modified sugar beet pectin only.

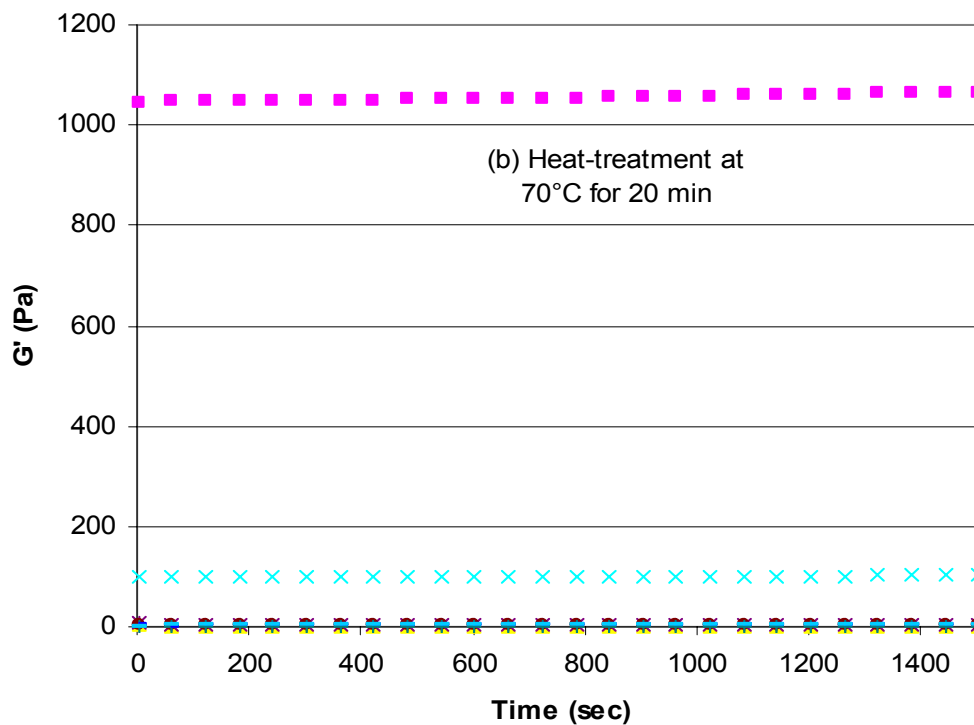
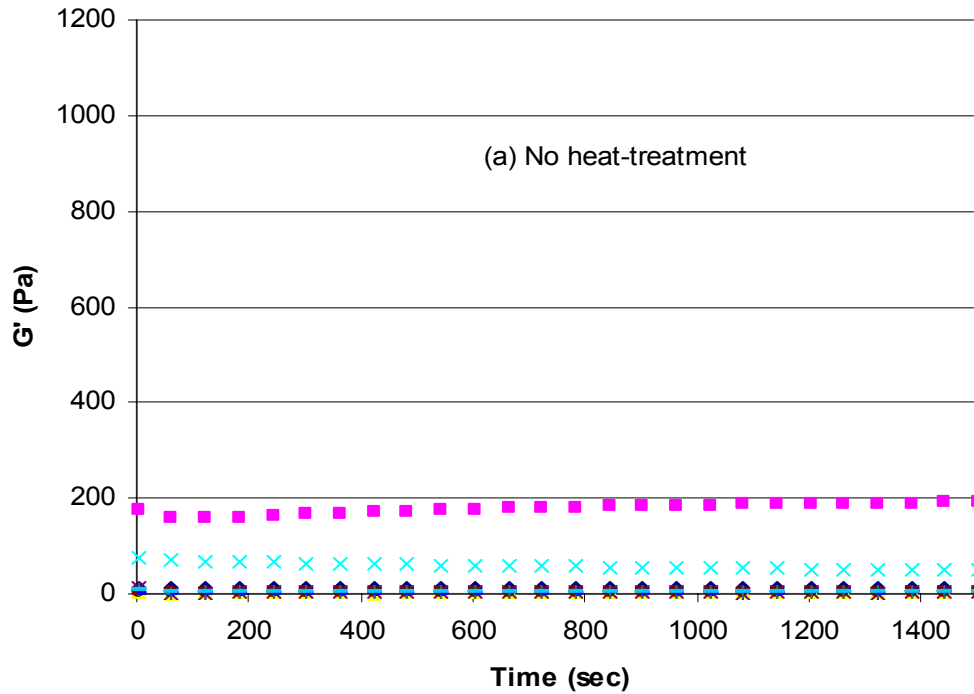


Fig. 6.2. Storage modulus (G') as a function of time of pectin-not filtered WPI hybrid (1:6 ratio), and WPI only, pectin only at pH 4.5 without (a) or with (b) heat-treatment at 75°C for 20 min at 1 Pa and 1 Hz, where \blacklozenge , citrus pectin-WPI; \blacksquare , modified citrus pectin-WPI; \blacktriangle , sugar beet pectin-WPI; \times , modified sugar beet pectin-WPI; $*$, WPI only; \bullet , citrus pectin only; $+$ modified citrus pectin only; $-$, sugar beet pectin only; $-$, modified sugar beet pectin only.

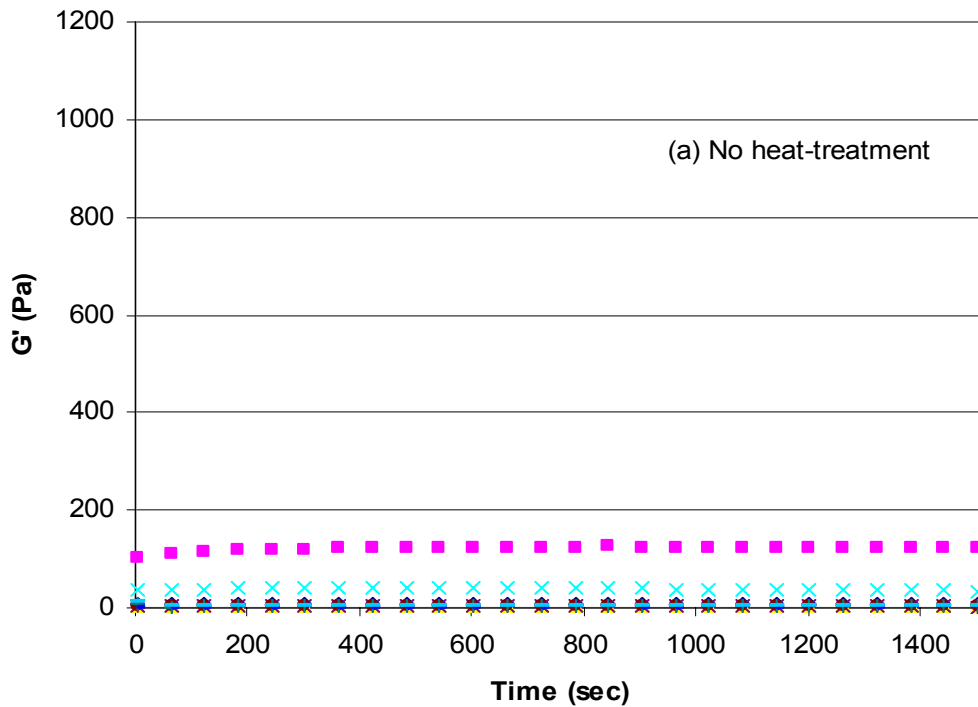
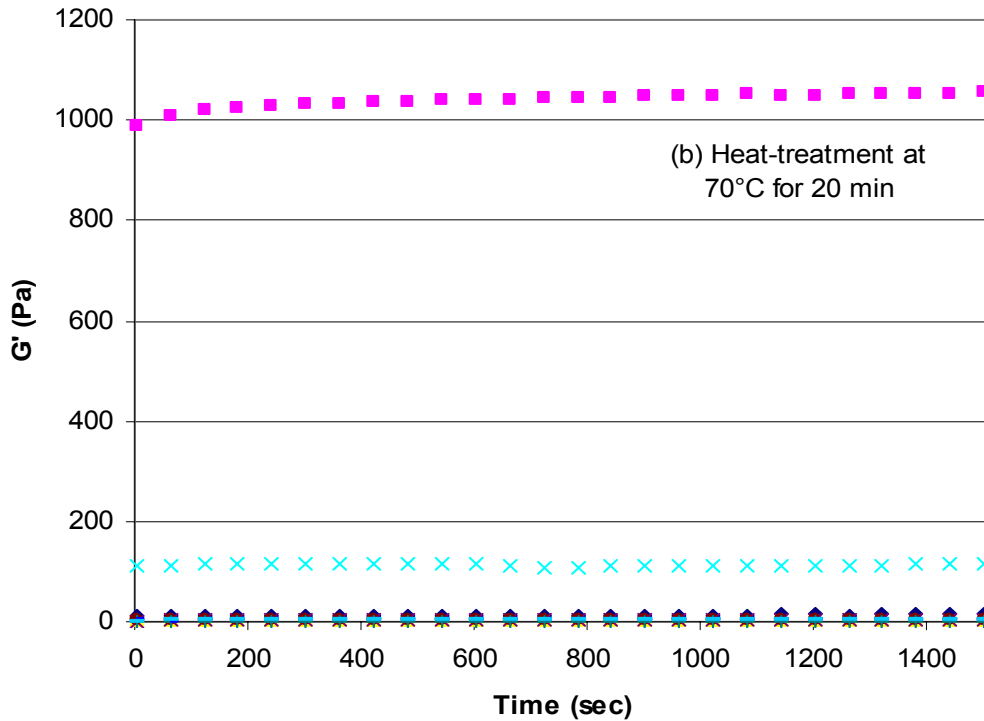


Fig. 6.3. Storage modulus (G') as a function of time of pectin-filtered WPI hybrid (1:6 ratio), and WPI only, pectin only at pH 4.5 without (a) or with (b) heat-treatment at 75°C for 20 min at 1 Pa and 1 Hz, where \blacklozenge , citrus pectin-WPI; \blacksquare , modified citrus pectin-WPI; \blacktriangle , sugar beet pectin-WPI; \times , modified sugar beet pectin-WPI; $*$, WPI only; \bullet , citrus pectin only; $+$ modified citrus pectin only; $-$, sugar beet pectin only; $-$, modified sugar beet pectin only.

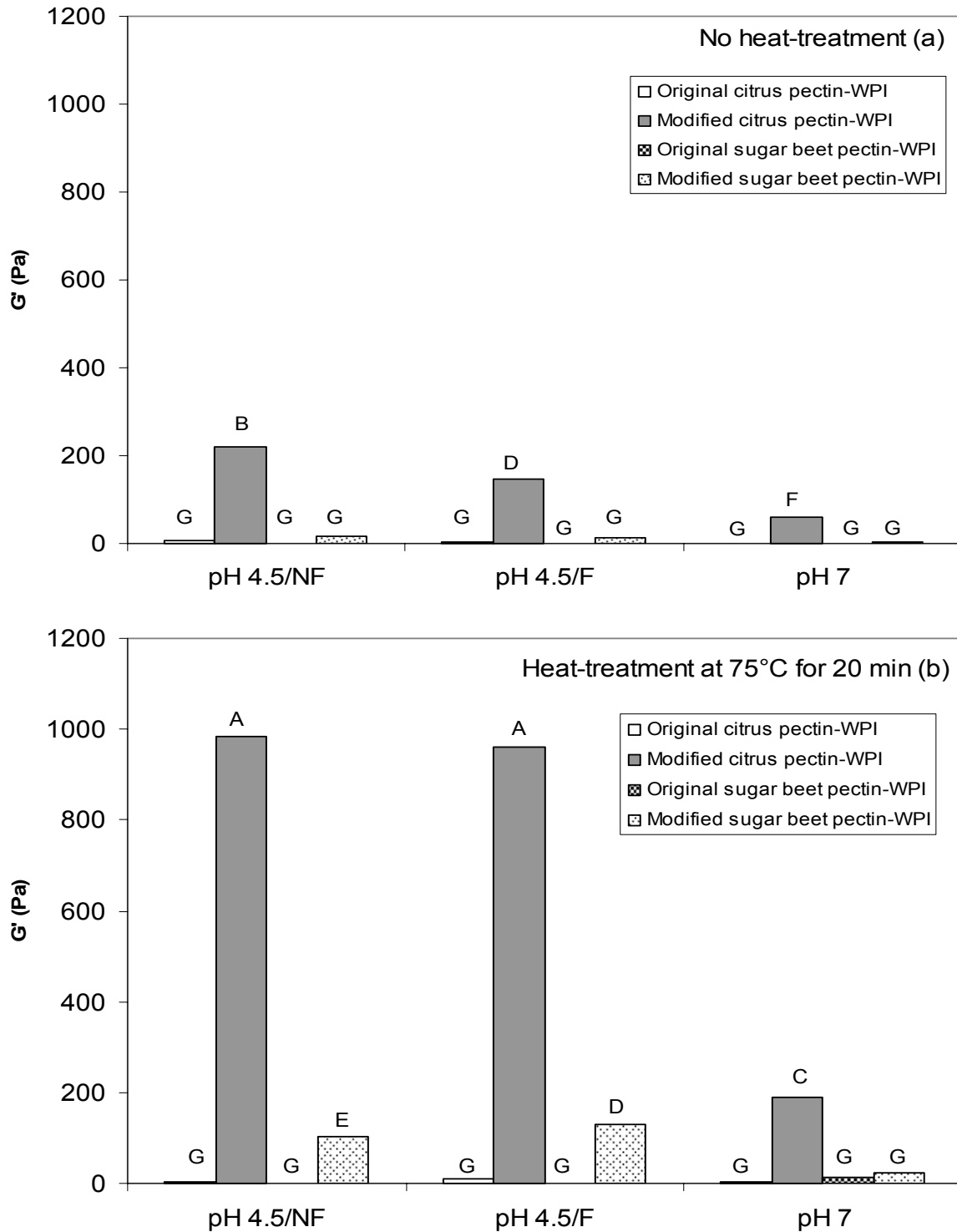


Fig. 6.4. Effect of original, modified or no pectin on storage modulus (G') of pectin-WPI Hybrid at 1 Pa and 2.5 Hz. G' values with same letter mean no significant difference at 5% confidence level. The abbreviations: NF, not filtered WPI; F, filtered WPI.

CHAPTER 7

CONCLUSIONS

The objectives of this research were to deesterify citrus and sugar beet pectin by PME fractions with different peptides, to determine DE and distribution of deesterified groups of modified pectin, and to characterize gelling property of original/modified pectin in the presence of calcium. Also, the physicochemical properties of original/modified pectin-WPI were investigated with/without heat-treatment (at 75°C for 20 min) at two different pHs (4.5 and 7). Finally, effect of different DE of pectin on rheological property of pectin-WPI complexes was investigated, as well as effects of heat-treatment and pH.

PMEs commonly had 13, 27, and 36 kDa protein. The different PME fractions from Valencia orange pulp show different enzyme activity. However, all PME fractions show block wise deesterification, which increase integration volumes of block structure of free carboxyl groups. The block structure of free carboxyl groups in pectin molecules increase elastic property of pectin in the presence of calcium.

Physicochemical properties of WPI can be changed by the presence of citrus or sugar beet pectin. Surface hydrophobicity and zeta potential can be decreased by adding pectin below isoelectric point of WPI. The interaction can be electrostatic attraction between negatively charged pectin and positively charged WPI below isoelectric point of WPI. The reduced surface hydrophobicity and zeta potential of pectin-WPI complexes can finally result in decrease in particle sizes of pectin-WPI complexes.

Rheological properties of WPI can be changed by addition of pectin. Charge on pectin molecules most significantly affects elastic property of WPI. The pectin with higher negative charge increases elastic property of WPI. Synergistic effect of charge on pectin on increase in

elastic property of WPI can be obtained with heat-treatment, and pH. The main interaction between WPI and pectin, which affect elastic property of WPI, can be electrostatic interaction between negatively charged pectin and WPI even above isoelectric point of WPI.