2000

Characterization of biopolymers: starch and soy protein

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Characterization of biopolymers: Starch and soy protein

by

Yongxia Song

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirement for the degree of

DOCTOR OF PHILOSOPHY

Major: Food Science and Technology

Major Professor: Jay-lin Jane

Iowa State University

Ames, Iowa

2000

Graduate College

Iowa State University

This is to certify that the Doctoral dissertation of

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has met the dissertation requirements of Iowa State Universtiy

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For the Major Program

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For the Graduate College

DEDICATION

The dissertation is dedicated to my beloved parents, C-F. Song and W-F. Xue.

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GENERAL INTRODUCTION

Starch and protein are the major functional ingredients in food systems. They are also the important natural biopolymers for industrial applications. Starch is the second most abundant carbohydrate and the main source of energy intake for human. Each starch is unique in its structure and properties. To fully understand the relationship between structures and properties will be helpful to guide starch applications and to design starches with desirable properties through genetic modifications. Because of the significant difference in thermal properties between barley starches and maize starches and less detailed information was available on barley starch structures, barley starches of different varieties were chosen as a model to study the relationship between starch structures and properties.

From the standpoints of nutrition and health, and the functionality, soy protein plays an important role in food industry. With concerns for environmental pollution caused by plastic waste, the development of biopolymers for plastics has gained increasing interest. Although research on soy plastics has a long history, soy plastics still have not been industrialized. More information is needed on the desirable properties of soy proteins for plastics with good quality. Different soy protein products were used to study the property difference among different compression-molded soy plastics.

The study of interaction between starch and soy protein is an important subject in food industry. It is important to understand the functions of these two biopolymers in food systems and to reveal how different starches and proteins interact.

Dissertation Organization

This dissertation includes three papers. The hypothesis for the study is that starch structures determine its properties, and soy protein physical properties and conformation affect its food and non-food applications. The first paper, "Characterization of barley starches of waxy, normal and high amylose varieties", has been published in Carbohydrate Polymers for publication. The second paper, "Characterization and comparison of mechanical properties and water absorption of compression-molded soy protein specimens made from different soy proteins", will be submitted for publication in Industrial Engineering and Chemistry Research. The third paper, "Effect of soy protein on pasting properties of different starches", will be submitted to Carbohydrate Polymers. The three papers follow the format of the journals submitted. The three papers are preceded by a General Introduction and followed by a General Conclusions. Three appendixes, showing additional research reports and data, follow the Acknowledgemens.

LITERATURE REVIEW

Starch

Starch is a major source of energy. Energy from the sun is converted into chemical energy by photosynthesis and accumulated in green plants. Starch is the major reserve polysaccharide and the second largest biomass, next to cellulose. Starch is synthesized mainly in the amyloplast and in a granular form. It is stored in cells of seeds, roots, tubers, stems, or leaves. Starch is the principal component in food for human and many animals. As a food ingredient, starch affects the texture, appearance, and shelf-life of food. Modifications improve the functions of starch in food products such as thickening, stabilizing, gelling, binding, film-formation, etc. Starch is a natural biopolymer material for industrial applications. The understanding of the relationships between starch structures and starch functions through fundamental studies provides basic information for genetic modifications to produce starches with desirable properties.

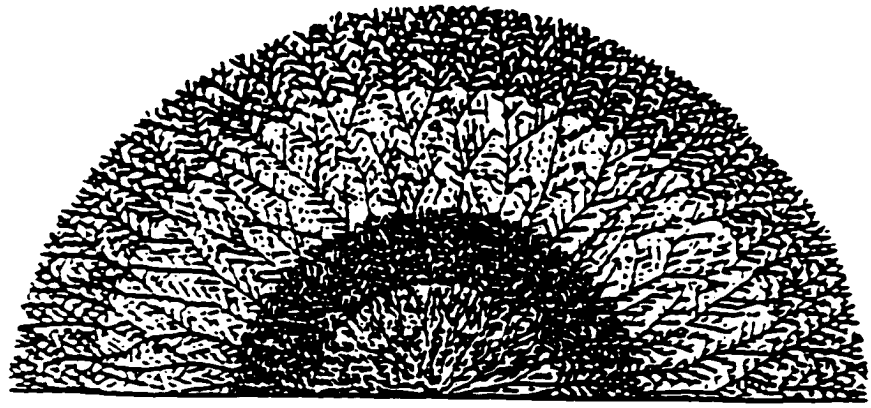
Granular structure

Starch granules vary in shapes and sizes among different botanical sources. Scanning electron micrographs of 54 starches were reported by Jane et al. (1994). The shapes of starch granules include polygonal, lenticular, spherical, oval, elliptical, disc, etc. Starch granule sizes differ widely (submicrometer to over 100 μm) from their botanical origins (eg. canan 100 μm, amaranth 0.5 μm) . Starches from tubers and roots are usually smooth, oval, and round shaped. Wheat, barley and rye starches are known for their bimodal size distributions with disc-shaped large granules (A granules) and spherical-shaped small granules (B granules).

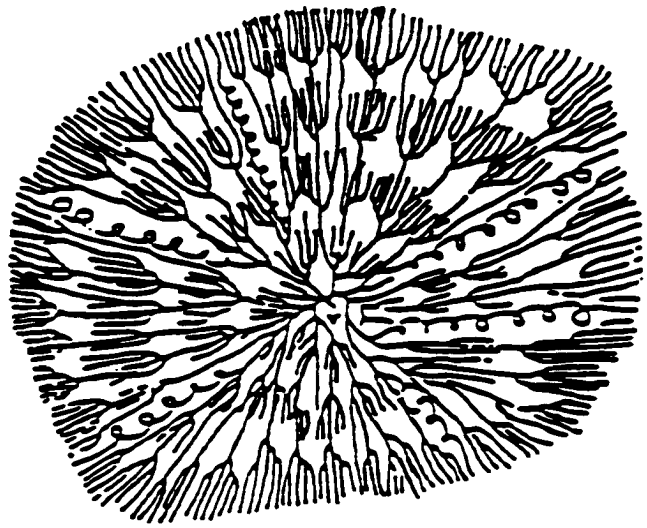
Small granule starches (such as amaranth, Chinese taro, cow cockle, dasheen, parsnip, and small pigweed) are irregular and polygonal shaped. Pores on the external surfaces of starch granules, which penetrate into the granule interior have been found by using scanning electron microscpy (SEM) and transmission electron microscopy (TEM) (Fannon et al., 1992, 1993).

Starch granules are classified into single granules (wheat and corn starch), compound granules (rice and oat starch), semi-compound granules, and pseudo-compound granules (wrinkled pea starch) according to the way granules grow inside the amyloplast or chloroplast of a cell (Lineback, 1984). High degree of molecular orientation within a granule causes optical birefringence and a "Maltese Cross" pattern under polarized light. The hilum at the center of the cross is the starting point of starch biosynthesis (French, 1984). Polarized microscope and dichroism have been used to study the arrangement and orientation of starch molecules within granules based on starch birefringence and the oriented amylose-iodine complex's linear dichroism, respectively (French, 1984). Starch molecules are oriented in a radial direction and perpendicular to the surface of the granule.

Based on the starch granule models (Figs. 1a and 1b) proposed by Myer (1895) and Nikuni (1969), Lineback (1984) presented a schematic model (Fig. 2). The model included amylose existing as random coil or amylose-lipid helical complex, and amylopectin with double helices in outer chains. The amylopectin arrangement within a growth ring (Fig. 3) was reported by Kainuma (1980). Growth rings can be observed by SEM or TEM when starches are fully hydrated or chemically hydrolyzed. Growth rings exist in concentric shells or layers and result in different starch properties, such as refractive index, density, crystallinity and resistance to chemical or enzymatic reactions (French, 1984; Lineback, 1984).



(a)



(b)

Figure 1. Starch granule models proposed by (a) Myer (1895) and (b) Nikuni (1969).

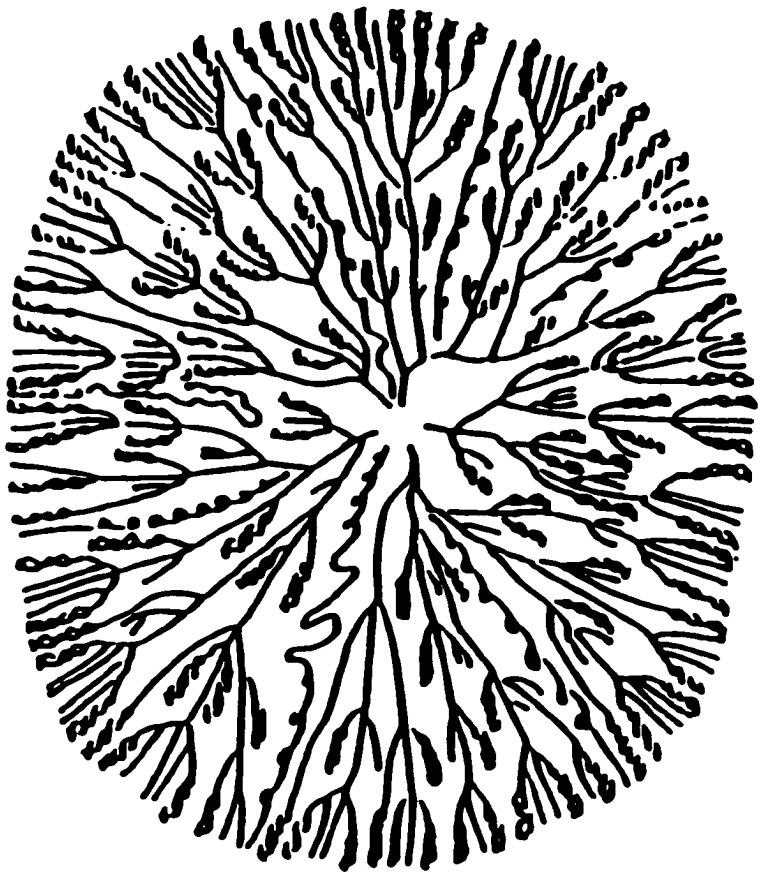


Figure 2. Schematic representationof the organization of a starch proposed by Lineback (1984).

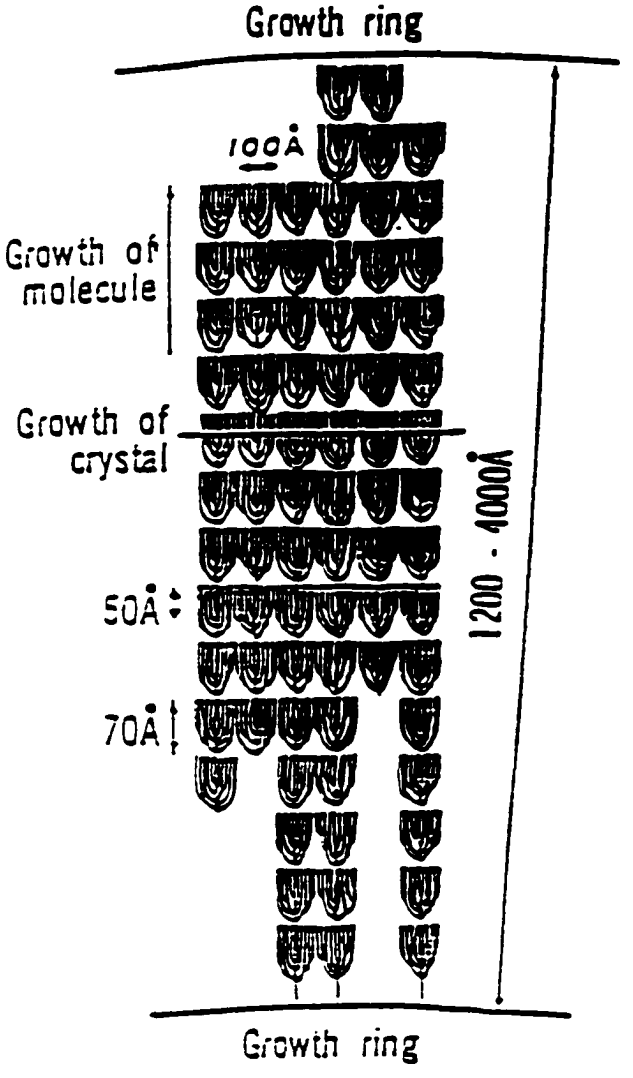


Figure 3. Schematic representation of the arrangement of amylopectin molecules in a waxy maize granule proposed by Kainuma (1980)

Starch is known as a semi-crystalline biopolymer. X-ray diffraction studies indicate that starch granules give A, B, C, and V patterns (Fig. 4) (Zobel, 1988; Sarko and Wu, 1978). The double helices in A-type structure are tightly packed into a monoclinic lattice. A hexagonal lattice is shown in B-type starch, and B-type starches have a large void in their crystal packing (Fig. 5) and therefore contain much more water than A-type starches (Sarko and Wu, 1978, Zobel, 1988, Imberty et al., 1991). A C-pattern is considered to be intermediate between A and B patterns or a result of mixture of A- type and B-type starches. Some root starches, seed starches and most legume starches have C patterns. A V pattern is due to the amylose complexation with alcohol, fatty acids, iodine or other complexing agents. The X-ray pattern of starch could be changed according to different treatments. Changes of A (or C)-pattern to B-pattern were observed after starch retrogradation (Eliassson and Gudmundsson, 1996; Zoble, 1973). A change of B (or C)-pattern to A-pattern was found after heat-moisture treatments (Eliasson and Gudmundsson, 1996; Wondovan et al., 1983; Hoover and Vasanthan, 1994). Heat and moisture treatment of cereal starches (such as maize, wheat, and rice) can produce helical complex structures, which derive a V pattern. Some starch genotypes give mixed patterns, such as ae su_1 du starch gives a B+V pattern.

Native starch granules have 15-45% crystallinity that varied with starch sources (Zobel, 1988). Yamaguchi et al (1979) and French (1984) reported that starch granules seem to be made up of alternating semi-crystalline and crystalline shells which are 120-400 nm thick. Solid-state ^{13}C-NMR is used to study ordered structures of starch and quantify the ratio of double helices to single chains (Biliaderis, 1998). Gidley and Bociek (1985) found

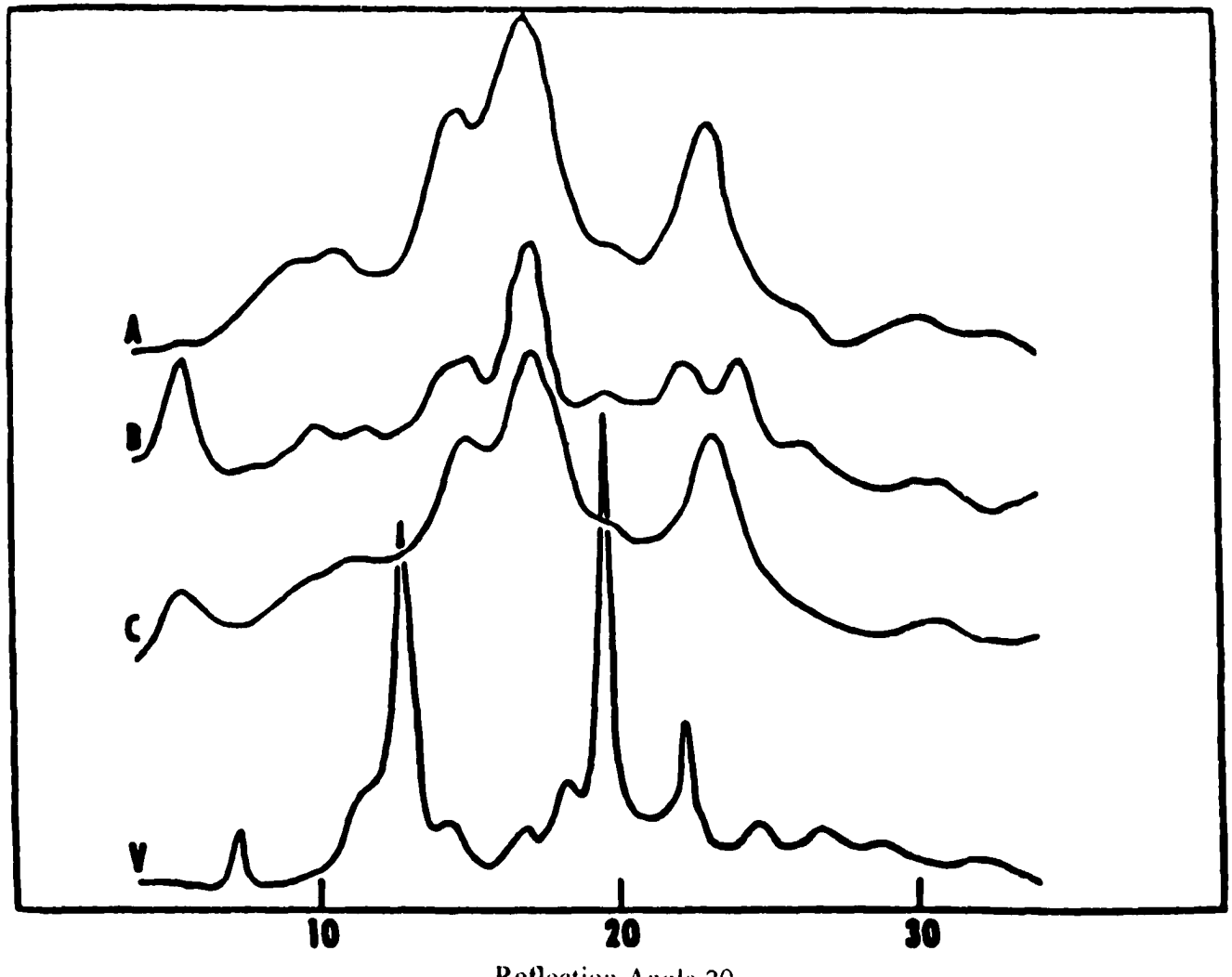


Figure 4 X-ray diffraction patterns from different starches (Zobel, 1988).

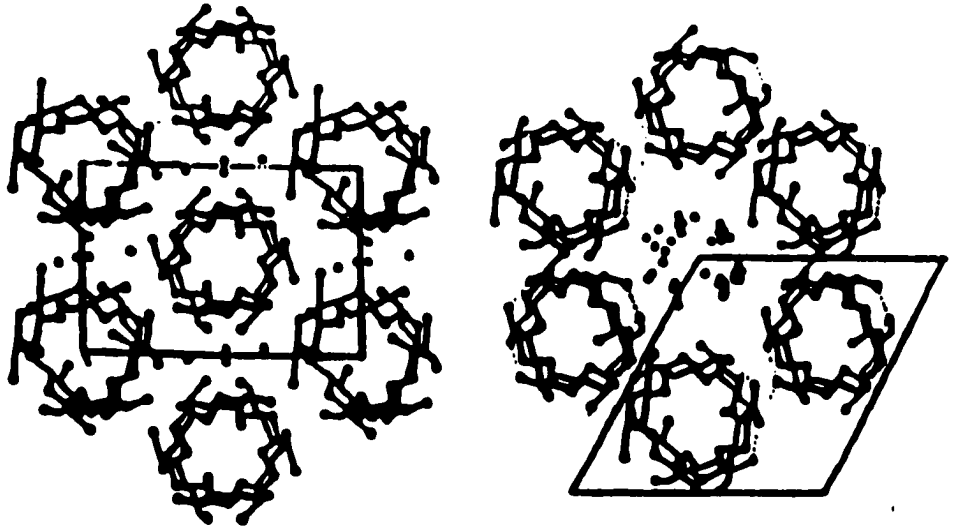


Figure 5. Helix packing of A-type (left) and B-type (right) (Zobel, 1988).

that the percentage of double helices obtained by using ^{13}C-NMR was never less than the percentage of crystallinity by X-ray diffraction method. The results indicate that many amylopectin molecules in semi-crystalline shells is in the double helical form, although it is not crystalline. However, the organization of molecules in starch semi-crystalline shells has not been intensively studied. In contrast, great effort has been focused on the study of the structure in crystalline shells by instrumental and enzymatic analysis methods. The structure has been investigated on four different length scales, the molecules (~ Å), the lamellae (~ 9 nm), the growth rings (~ 100 nm), and the whole granule (~ μm). Amylopectin molecules and the whole granule have been relatively well studied (Waigh et al., 1999). Three-dimensional lamellar and growth rings structures of starch are still actively studies in starch field.

It has been accepted with considerable evidence that the crystalline shells consist of alternating amorphous and crystalline lamellae (Oostergetel and van Bruggen, 1989; Jenkins et al., 1993; Gallant et al., 1997). Electron microscopy and small angle x-ray scattering demonstrate that the periodicity of the lamellar structure is 9-10 nm (Kassenbeck, 1978; Waigh, 1999). There are two different structure models for the lamellae in potato starch. In the first model proposed by Oostergetel and van Bruggen (1993), the lamella were predicted to be twisted into helical structures that merge together side by side. The second model based on the blocklet concept was proposed by Gallant et al. (1997), according to the TEM and atomic force microscopy (AFM) data. Recently, Waigh et al. (1999) reported that the lamellae is a product of the chiral side chain liquid-crystalline structure of amylopectin in the presence of chiral bending forces, based on the Oostergetel and van Bruggen's model. Further study in this area will combine biochemistry knowledge to investigate the structure

development and to correlate starch biosynthesis pathways with proposed models to fully understand starch granule structure.

The major components in starch granules are amylose and amylopectin. Double helical chains of amylopectin are located in the crystallline region (French, 1972). Different methods have been used to determine the location of amylose in starch granules. Microscopy suggests that amylose is located at the centre of the maize endosperm starch granules (Schwatz, 1982). Cross-linking reaction studies have demonstrates that amylose is cross-linked to amylopectin, which suggestes that amylose is interspersed among amylopectin instead of being in bundles in the granule (Jane et al., 1992). The surface gelatinization method was used to analyze starch granule stuctures by separating starch molecules from the periphery of the granule. Results show that amylose is more concentrated at the periphery and amylopectin at the periphery has shorter long-B chains than that at the core (Jane and Shen, 1993). Small-angle x-ray scattering technique was used to investigate the effect of amylose content on the internal structure of starches (Jenkins and Donald, 1995). Results show that amylose disrupts the structural order within amylopectin crystallites. Enzymatic treatment of maize starch granules reveales that amylose is located in the amorphous region (Helbert et al., 1996). The enzyme-gold labelling method indirectly visualized the location of amylose and amylopetin in starch granules by using histochemical stains and cytochemical markers (Atkin et al., 1999). Without amylose, dry waxy maize granules showed a concentric amylopectin layer. Potato starch with 21% amylose displayed alternating layers of densely packed amylopectin and amylose molecules. Amylomaize starch with 70% amylose was shown to have an amylopectin centre surrounded by an amylose periphery encapsulated by an amylopectin surface.

The minor components include cell-wall fragments, surface components, and internal components. Proteins, lipids, moisture, and ash are usually present in starch granules in very small quantities (Thomas and Atwell, 1999). Lipids are the most important minor components in starch granules. The location of the surface lipids (such as glycolipids and phospholipids) of starch granule is still unknown. The internal lipids in cereal starches are mainly free fatty acids and lysophospholipids, and form helical complexes with amylose or outer chains of amylopectin (Morrison, 1995). The internal lipids in tuber or root starches are phosphate monoesters, which are mainly located on the C-6 of amylopectin in the crystalline region of starch granules (Taketa and Hizukuri, 1982; Muhrbeck et al., 1991; Lim and Seib, 1993).

Molecular structure

Amylose is primarily a linear polysaccharide of D-glucose units with α-1,4 linkages. Amylose is heterogeneous in molecular size. The average of molecular weight is 10^6. The degree of polymerization is variety dependent and in the range of 500-5,000. Some amylose molecules contain a few branches with α-1,6 linkages, either very long or very short (Banks and Greenwood, 1975; Whistler and BeMiller, 1996). Peat et al. (1952) first suggested the presence of branches in amylose. They found that only 70% amylose was hydrolyzed by crystalline sweet potato β-amylase. The main barrier to β-amylase is α-1,6 linkage (French, 1975; Kjolberg and Manners, 1963). Banks and Greenwood (1967) demonstrated that amylose as well as amylopectin has branch structure by using enzyme (β-amylase and pullulanase) hydrolysis and ultracentrifugation. Hizukuri et al. (1981) also confirmed that amyloses from several plant sources showed multi-branched nature by quantitatively

determinating the reducing and non-reducing residues. The fine structures of branched amylose were studied by β-amylase, isoamylase, β-amylase degradation (β,*i*, β-degradation) and the results showed that branched amylose has some tiny clusters of short chains (Hizukuri and Maehara, 1990). Although there are no effective methods to separate linear and branched molecules, the structure of amylose molecules can be characterized by indirect analysis. Taketa et al. (1992) studied the structures of amylose subfractions with different molecular sizes and found that the subfractions with dp_n ~400 are in large amount and rich in linear molecules, and the subfractions with dp_n ~2500 contain mainly branched molecules. The branched molecules in each subfraction comprised the short-chain fraction and the larger branched molecules had a larger number of the short chains. The C chain of the larger branched molecules appeared to be longer. The tritium labelling method and gel permeation chromatography were used to study the structures of branched and linear rice amylose molecules (Taketa, 1993). The branched molecule is a structural intermediate between true linear amylose and amylopectin. Cura et al. (1995) concluded that amylose is not strictly linear by using chemical and enzymatic methods.

Amylose could form complexes with complexing agents. Kuge and Takeo (1968) examined over one hundreds organic compounds to study the precipitation ability for starch. They found that good complexing agents include all of the aliphatic and aromatic monhydroxy alcohols (except triophenylcarbinol), normal aliphatic carboxylic acids and halogenated aliphatic carboxylic acids, and all of the aliphatic and aromatic ketones without a conjugated system in its molecule. The structure of amylose V complex has been intensively studied since the 1960s. The V type conformation is due to amylose complexes precipitated from aqueous

solution with various alcohols. Yamashita (1965) reported that single crystals of amylose V complexes can be crystallized as rectangular-shaped lamellae, and the helix axes are oriented perpendicular to the lamellar surface. Electron microscopy and x-ray diffraction were used to study the morphology and structure on the lamellar crystals of amylose V complexes. The crystals consisted of lamellae about 100 Å in the thickness, confirming that the concept of chain folding in polymer crystals could be applied to lamellar crystals of amylose V complexes with large helix diameter. Single crystals of n-butyl alcohol complex gave the 6_1 helical structure (Rundle, 1943). Crystals of amylose V complexes with 7_1 (Yamashita, 1966) and 8_1 (Yamashita and Monobe, 1971) helical conformations can be obtained by using branched chain alcohols (isopropyl, isobutyl, sec-butyl and tert-butyl alcohols) and α-naphthol, respectively. The electron micrographs of different amylose V complexes were reported by Yamashita et al (1973). Their results revealed that the transformation between different helical conformations occurred when the crystals were dispersed in another complexing agent. A computer model (Fig. 6) of amylose helix was reported by French and Murphy (1977). Helical parameters are the pitch, *p*, and the number of residues per pitch, n. Depending on the size of the complex agent, helices could have six, seven, and eight monomers per pitch. It was reported that those helices have the repeating distance of 8 Å.

Enzyme (α-amylase) hydrolysis and acid hydrolysis were used to study the structures of amylose V complexes and retrograded amylose by Jane and Robyt (1984). α-Amylase hydrolyzes the amorphous, folding areas on the surfaces of the lamellae of packed helices, with the formation of resistant, amylodextrin fragments. The DP (degree of polymerization) corresponds to the diameter of the helices and the folding length of the chain. Acid hydrolysis

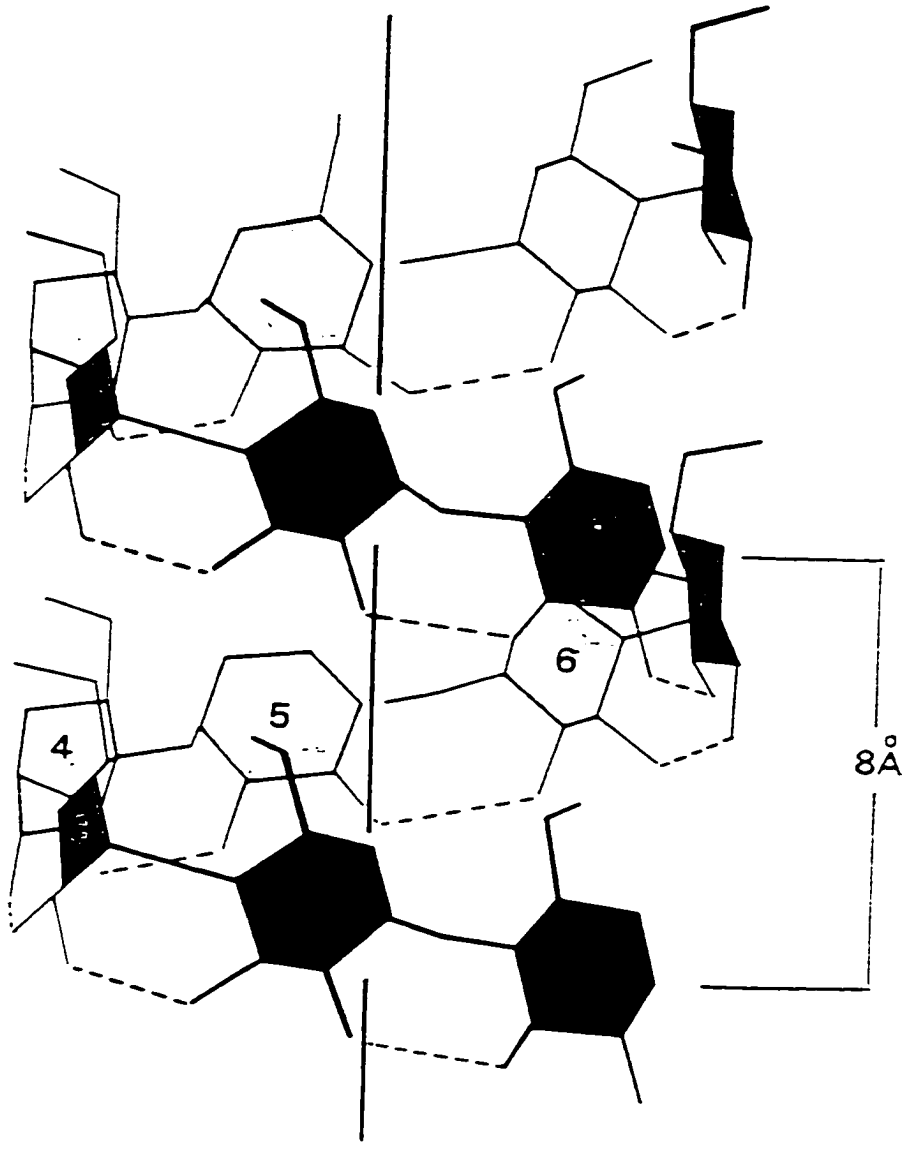


Figure 6. Amylose single helix model. p = 8Å, n = 7, h = p/n = 1.14Å (French and Murphy, 1977).

of retrograded amylose gave a resistant fragment with an average DP of 32, human-salivary and porcine-pancreatic α-amylase gave a resistant fragment with DP of 43, and *Bacillus subtilis* α-amylase gave a resistant fragment with DP of 50. The differences in the sizes of the resistant amylodextrins depend on the differences in the specificities of the hydrolyzing agents. Acid hydrolyzes up to the edge of the crystalline region, whereas α-amylase hydrolyzes up to some point, several D-glucosyl residues away from the crystalline region, leaving "stubs" on the ends of the amylodextrins whose sizes are dependent on the sizes of the binding sites of the individual α-amylase. The conformation of helical complexes of amylodextrin and of amylose in solution was studied by using ^{13}C-NMR (Jane and Robyt, 1985). Results showed that a downfield change in the chemical shifts of carbons 1 and 4 of glucose residues of the amylodextrin. The change was interpreted as a change in the torsion angle of the α-1,4 glycosidic linkage. Differences in the ratio of the downfield shifts of carbons 1 and 4 distinguish an extended helix from a compact helix. Temperature effects on the retrogradation of potato amylose solutions were studied (Lu et al., 1997). Their results showed that as incubation temperature increased from 5 to 45°C, the retrogradation rate decreased, and the chain length of resistant segments increased from DP_n 39 to 52 for α-amylolysis, and from DP_n 34 to 40 for acid hydrolysis.

Amylopectin is a branched macromolecule. The major chains are α-1,4-D-glucan chains with various lengths, and branched through α-1,6 linkages. It has about 5% branch linkages. Many models (Fig. 7) of amylopectin structure have been proposed since 1937(Hizukuri, 1996). The cluster model has been widely accepted. A super helical structure model for potato amylopectin was reported by Oostergetel and Bruggen (1993). Peat et al.

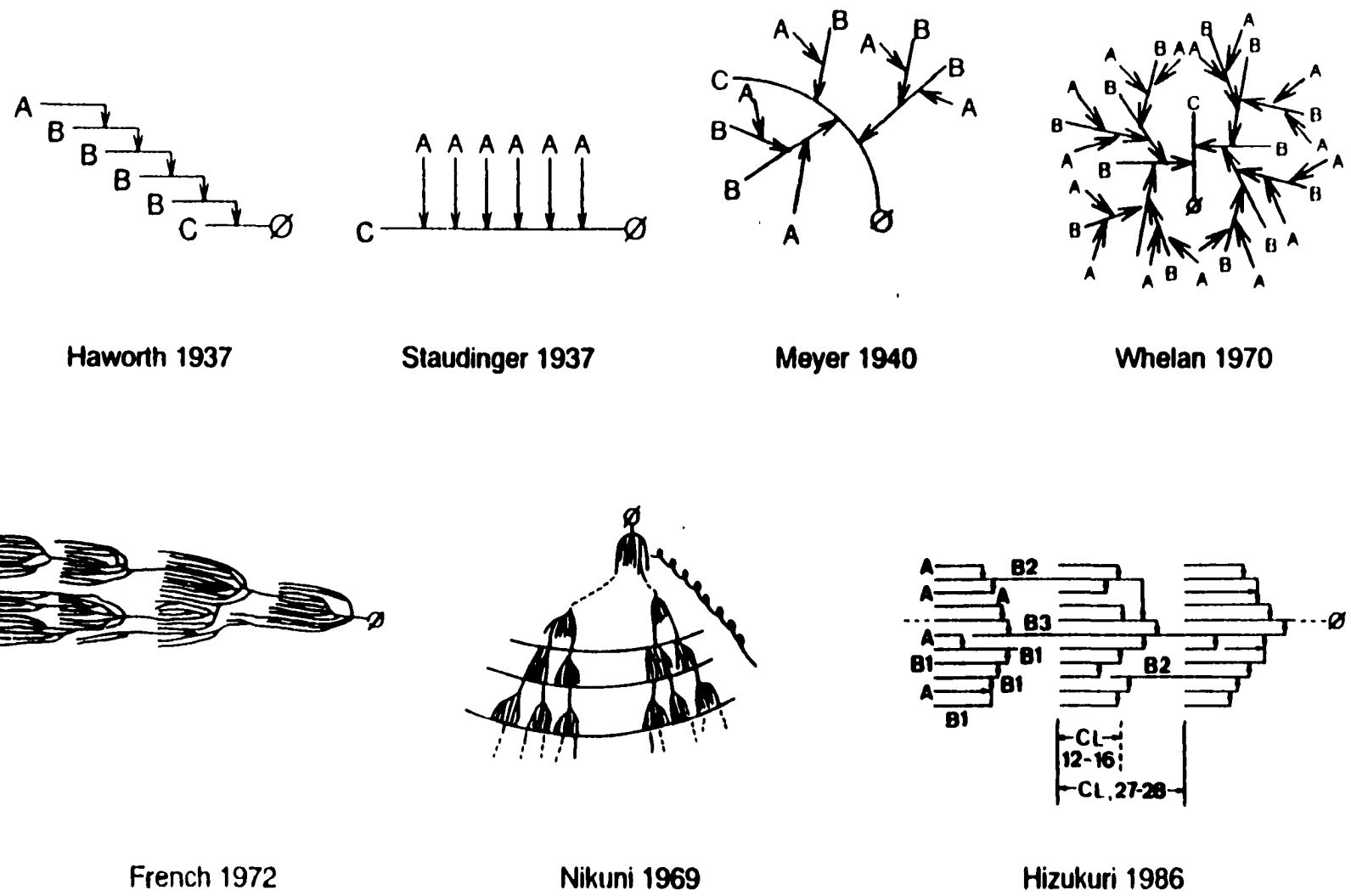


Figure 7. Amylopectin structure models (Hizukuri, 1996).

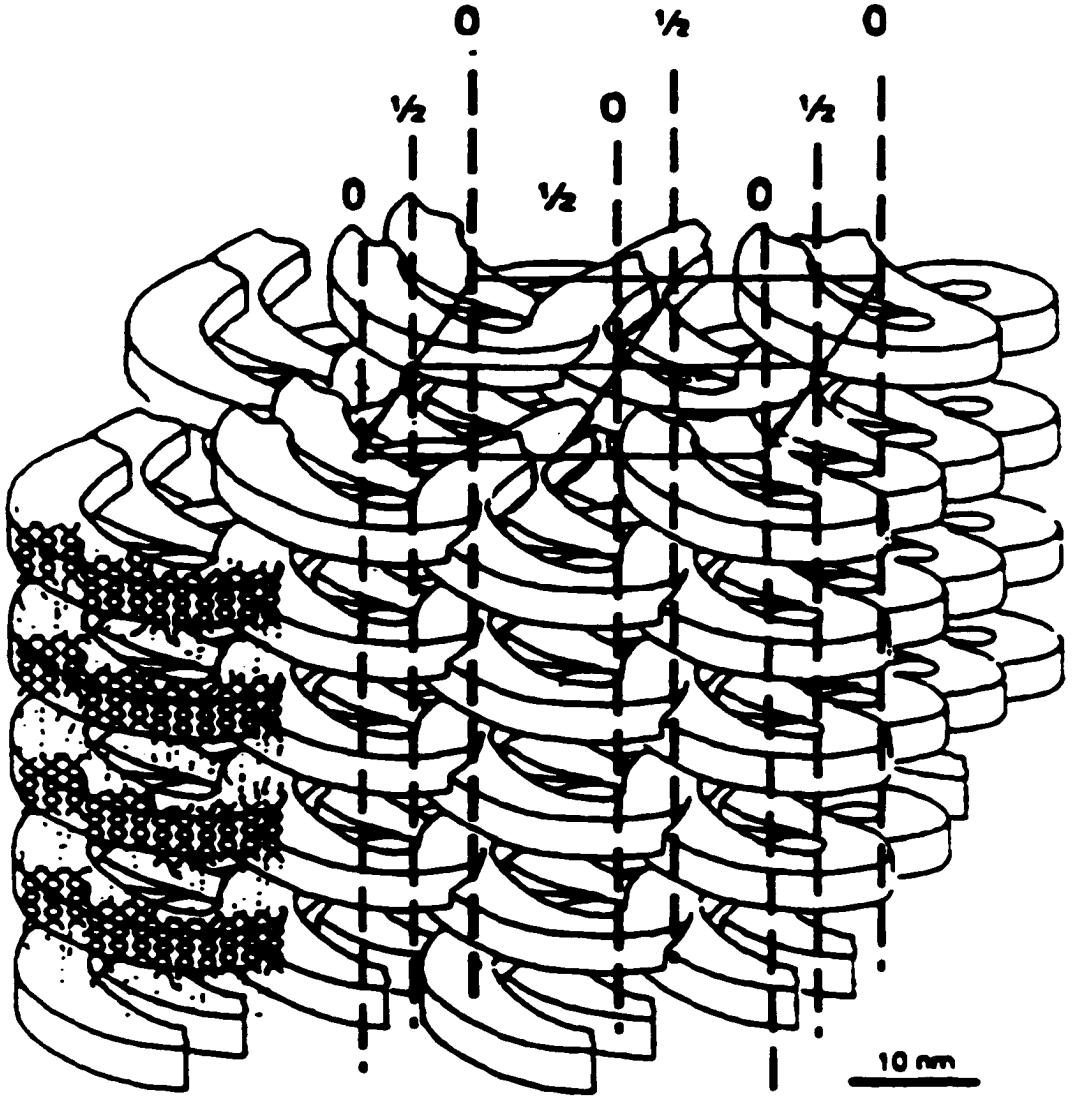


Figure 8. A super helical structure model of amylopectin in potato starch (Oostergetel and van Bruggen, 1993).

(1956) classified the chains of amylopectin into three types, A, B, and C. The A chain is linear and its reducing end links to other chains through α-1,6 linkage. The B chain carries A or other B chains at its C-6. The C chain is the only chain having a reducing end in the molecule. The ratio of A chains to B chains is one of characteristics of amylopectin structure. The calculation of A/B chain ratio was based on the reducing powers produced by hydrolysis of β-LD with isoamylase alone and the combined action of isoamylase and pullulanase (Marshall and Whelan, 1974). Although there are variations among different methods, the most acceptable A/B chain ratio for amylopectin is 1.0~1.4:1 (Hizukuri, 1996). Hizukuri (1986) classified B chains into B_1, B_2, B_3, and B_4 according to high-performance liquid charomatography (HPLC) results of polymodal distribution of amylopection chain lengths. The polymodal distribution supports the cluster structure. Gel permeation or size-exclusion chromatography and sequential enzymatic analyses were used to quantitatively study the cluster structure (Hizukuri et al., 1989; Takeda and Hizukuri, 1987). Chain length distribution is a major parameter to describe the structural characteristics of amylopectin molecules. Size exclusion chromatography (SEC) and HPLC with a refractive index (RI) detector have been used in this study, however, they could not separate chains well. On the contrary, high-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD) can separate amylopectin chains over DP 80 (Hanashiro et al., 1996). With an amyloglucosidase (AMG) reactor and nitrate as pushing agent, HPAEC-ENZ-PAD could give even better separation of debrached amylopectin chains (Wong and Jane, 1995, 1997).

The relationship between branching structure of amylopectin and crystalline structure

of starch granule has been studied by different methods. Hizukuri et al. (1983) studied the average chain length of amylopectin of 27 starches by a modified Park-Johnson's colorimetric method and the anthrone-sulfuric acid method. Result showed that the average chain length of A-type starch (17.1-20.6) is shorter than that of the B-type starch (21.6-30.9), and C-type starches with intermediate chain lengths of 20.3-21.3. HPLC was used to characterize the amylopectin chain length distributions of 20 starches by Hizukuri (1985). The weight-average chain lengths of the amylopectins of the A-, B-, and C-type starches are in the ranges of 23-29, 30-44, and 26-29, respectively. Results from HPAEC-PAD method showed that the amylopectins of A-type starches had more A-chains (DP 6-12) than that of B-type starches (Hanashiro et al., 1996). Jane et al. (1997) investigated structures of Naegeli dextrins of different starches by using HPAEC-ENZ-PAD. It is shown that the A- and B-type starches have different branch structures. The α-1,6 branch linkages of the A-type starch are more scattered and many linkages are located within the crystalline region. The linkages of B-type starch are clustered in the amorphous region and are susceptible to acid hydrolysis. A model of branching patterns for A- and B-type starches was proposed (Fig. 9). Jane et al. (1999) reported the branch-chain length distribution profiles of amylopectins from 21 different starches. The chain length distribution is characteristic for each starch variety.

Th intermediate components in starch have been observed based on the degree of branching and the molecular weight. There is no effective method to isolate the intermediate materials. Lansky et al. (1949) found a starch fraction had different properties from amylose and amylopectin and the fraction displayed iodine-binding capacity and β-amylolysis limit between that of amylose and amylopectin. Starches with different types and maturity had

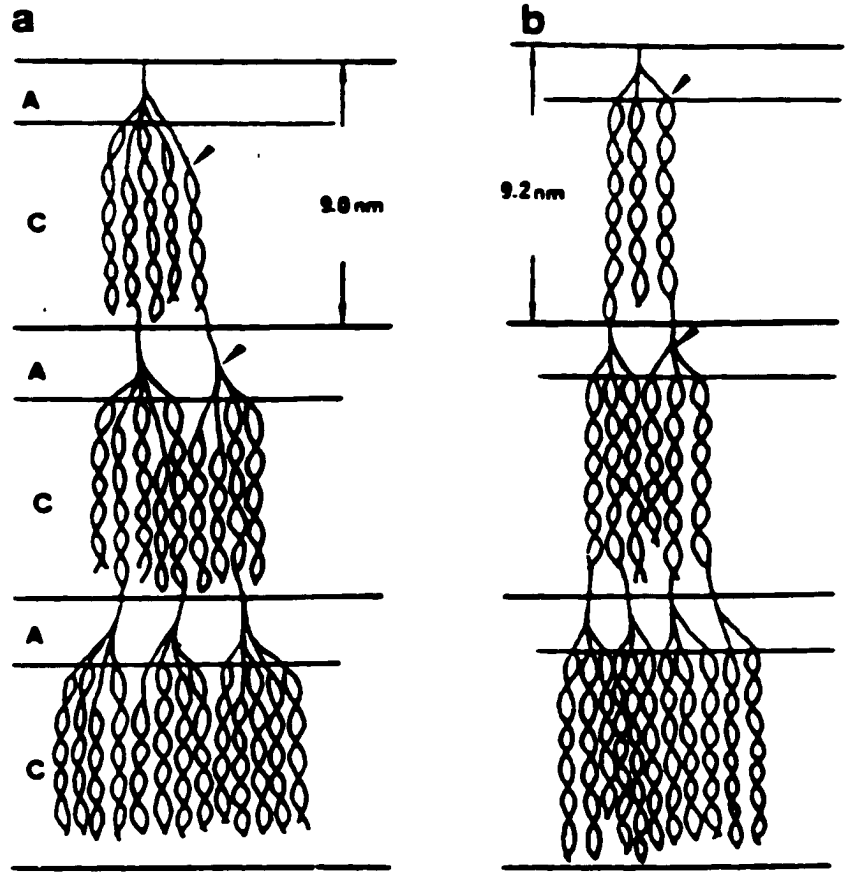


Figure 9. Models for branching patterns of (a) waxy maize starch and (b) potato starch (Jane et al., 1997).

different amount and structure of intermediate materials (Banks and Greenwood, 1975). Wolff et al. (1955) first suggested that the amylopetin of amylomaize starch had intermediate properties because of its longer outer and inner chains. Wang et al. (1993) studied 17 maize mutants with the Oh43 inbred line and found that starches having *ae*, *du1*, *su1*, *ae bt1*, *ae du1*, and *du1 su1* gene showed higher intermediate contents. Kasemsuwan et al. (1995) reported that the dominant mutant (*Ae1-5180*) maize starch had greater proportion of amylose and intermediate than normal maize starch and it was similar to *ae bt1* mutant maize starch. The blue value and total carbohydrate ratio of the intermediate fractions was similar to that of amylopectin. The intermediate components had a branched structure and smaller molecular weight than amylopectin.

Starch properties

Gelatinization occurs when starch is heated in water. It is an irreversible process. One of the definitions for gelatinization is that it is the collapse (disruption) of molecular orders within the starch granule along with concomitant and irreversible changes in properties such as crystallite melting, loss of birefringence, and starch solubilization. The point of initial gelatinization and the range over which it occurs is governed by starch concentration, method of observation, granule type, and heterogeneities within the granule population under observation (Atwell et al., 1988). Gelatinization occurs over a temperature range, and usually presented as onset temperature (T_o), peak temperature (T_p), and completion temperature (T_c). Differential scanning calorimetry (DSC) is often used to determine gelatinization temperature of starches based on the thermal transition of starch crystallite melting during heating.

Gelatinization temperature varies among starch varieties. Jane et al. (1999) summarized gelatinization properties of 21 different starches. Their results showed that waxy rice, wheat, barley and potato starches displayed low onset gelatinization temperature, and gelatinization ranges of starches varied from 6.6°C (barley) to 76.4°C (high-amylose maize V). Besides varieties and modification effects, water and heat are the other two major factors that affect gelatinization temperature. Heat-moisture treatment is referred to the treatment at low water contents and temperatures above gelatinization temperature range. Hoover and Vasanthan (1994) reported the effect of heat-moisture treatment on the structure and physicochemical properties of cereal, legume, and tuber starches. A broadening of gelatinization temperature range and a shifting of the endothermal transition towards higher temperatures were observed. Annealing occurs when water contents are high but temperatures are below the gelatinization temperature range. It has been reported that after annealing, gelatinization temperature range of starches has been more narrow and moved to high temperatures (Hoover and Vasanthan, 1994; Larsson and Eliasson, 1991; Seow and Teo, 1993; Knutson, 1990).

Pasting is an important starch property for food application because it increases viscosity. Pasting is defined as the phenomenon following gelatinization in the dissolution of starch. It involves granular swelling, exudation of the granular molecular components, and eventually, the total disruption of the granules (Atwell et al., 1988). Unmodified starch granules are generally insoluble in water below 50°C, however, after temperature reaches gelatinization temperature, starch granules absorb a large amount of water, swell to many times their original size, and viscosity increases. The temperature at the onset of the rise in viscosity is known as pasting temperature. Pasting temperature provides an indication of the

minimum temperature required to cook a given sample, which can have implications for the stability of other components in a formula, and also indicate energy costs. The pasting property of starches can be measured by using Brabender Visco Amylography or the Rapid Visco Analyser (RVA) according to the viscosity of starch pastes during heating and shearing. A typical RVA profile with commonly measured parameters is shown in Figure 10 (Newport Scientific Pty, Ltd., 1995). The peak viscosity occurs at the equilibrium point between swelling causing an increase in viscosity, and rupture and alignment causing its decrease. Peak viscosity indicates the water-binding capacity of the starch or mixture. A shear-thinning or breakdown indicates the ability of starch granules to withstand heating (at 95°C) and shear stress. Setback viscosity is measured as the difference between final viscosity and peak viscosity. Setback region involves retrogradation of starch molecules. Final viscosity is used to define a particular starch's quality, which indicates the ability of the starch to form a viscous paste or gel after cooking and cooling. Starch variety, modification of starch, interaction of starch with other components in the mixture, and test conditions are all the factors related to starch pasting profiles. Unique RVA profiles of different starches were reported by Jane et al (1999).

Retrogradation is defined as the event that occurs when starch molecules begin to reassociate into an ordered structure. In its initial phases, two or more molecules may form a simple juncture point that then may develop into more extensively ordered regions. Ultimately, under favorable conditions, a crystalline order appears and precipitation from "solution" occurs (Atwell et al., 1988). Factors affecting the rate of retrogradation include the molecular ratio of amylose and amylopectin, structures of the amylose and amylopectin

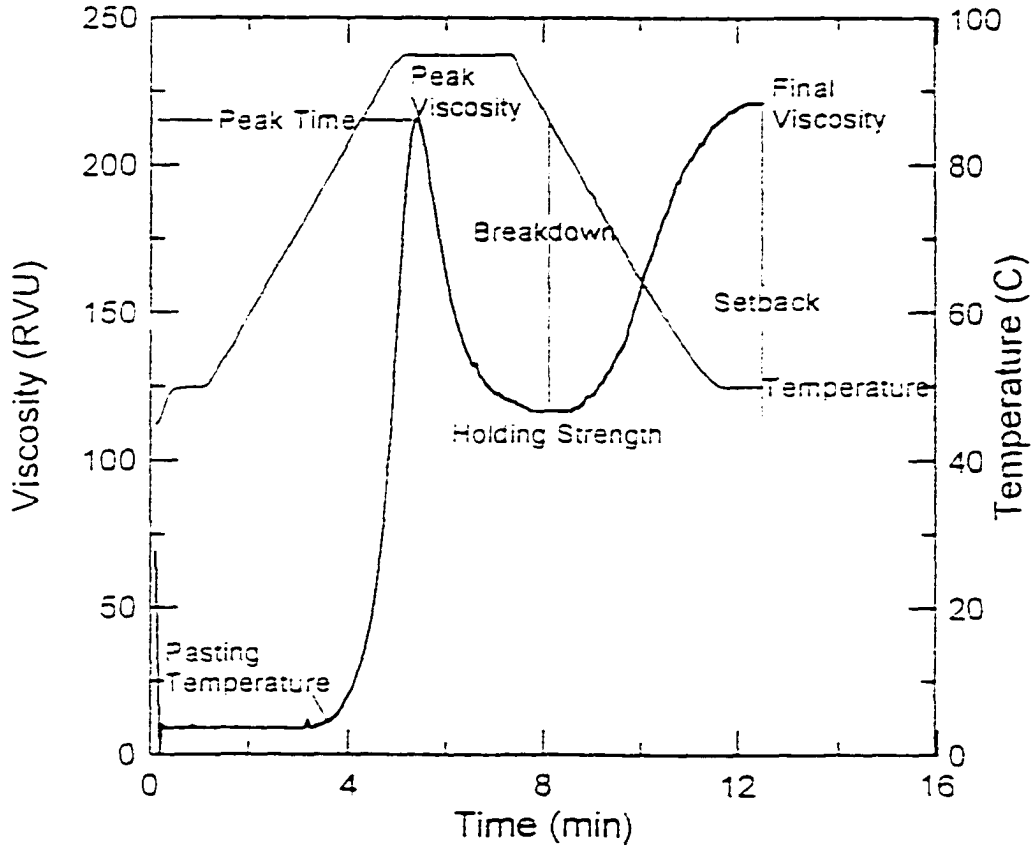


Figure 10. **Typical RVA pasting profile** (Newport Scientific Ltd., 1995).

molecules, which is dependent on the starch variety, temperature, starch concentration, and the presence and concentration of other ingredients, such as salts, lipids, and surfactants (Whistler and BeMiller, 1996). Common methods to measure retrogradation include x-ray diffraction analysis (crystalline property), DSC (the rate of retrogradation), and rheological techniques (such as gel firmness). The retrogradation properties of 21 different starches were studied by DSC analysis (Jane et al., 1999). It was first suggested by Schoch and French (1947) that the retrogradation of amylopectin, not amylose was essentially involved in the staling of bread. The kinetics of retrogradation are still not completely known.

Relationship between structures and properties

Each starch has its own unique properties and is suitable for different applications in food or non-food industries. To correlate properties to the structures of each starch will provide fundamental knowledge for utilization and helpful information for genetic modification to design structures of starches with desirable properties.

During gelatinization, starch granular structure is partially or completely disrupted. Therefore, gelatinization is an important step for any starch application. Starch structure disorder occurs when water causes starch granule swelling in the amorphous region. The gelatinization phase transition as the disordering of individual chains being separated from ordered regions (Donovan, 1979). Gelatinization temperature is correlated with the quantity (degree) of starch crystallinity and the quality (perfection) of crystallites (Zobel, 1984; Levine and Slade, 1987; Tester and Morrison, 1990). Studies showed that shorter long B-chains contributed to lower gelatinization temperatures (Jane et al., 1992; Shi and Seib, 1992; Yuan

et al., 1993). The branch chain length distributions of wheat and barley amylopectins displayed obvious shoulders at dp 18-21, which suggesting a defective crystalline structure and contributing to the low gelatinization temperature (Jane et al., 1999). Waxy starches display large gelatinization enthalpy changes, reflecting a higher percentage crystallinity of amylopectin. It was also reported that methods used for starch isolation affect gelatinization temperature (Jane et al., 1999). The change of gelatinization property after heat-moisture treatment was due to that heat-moisture treatment can cause a change in the type of crystallinity, from the less stable polymorphs (B) to the most stable one (C and A) (Lorenz and Kulp, 1982; Hoover and Vasanthan, 1994). Annealing can improve or change perfection of crystallites to a more stable crystal structures (Larsson and Eliasson, 1991). The effect of annealing decreases with the increasing amylose content and the effect may be related to different degrees of interaction between amylopectin and amylose in different starches (Knutson, 1990). Cold water-soluble starch can be produced with a high-temperature, aqueous alcohol system. Characterization of granular cold water-soluble starch was reported by Jane et al (1986). Their results showed that heating starch with aqueous alcohol to a high temperature can convert the native doulbe helical structure into single helices. The transformation occurs in both amylose and amylopectin molecules (Fig. 11). Cold water-soluble starch does not require heating for gelatinization and provides an instant starch paste.

Molecular mechanisms have been studied during retrogradation. Retrogradation is the process of molecule recrystallization. Studies showed that cereal starches have higher tendency to retrograde than tuber starches (Roulet et al., 1990). This is because that cereal starches have lipids and phospholipids that restrict starch granule swelling and dispersion and

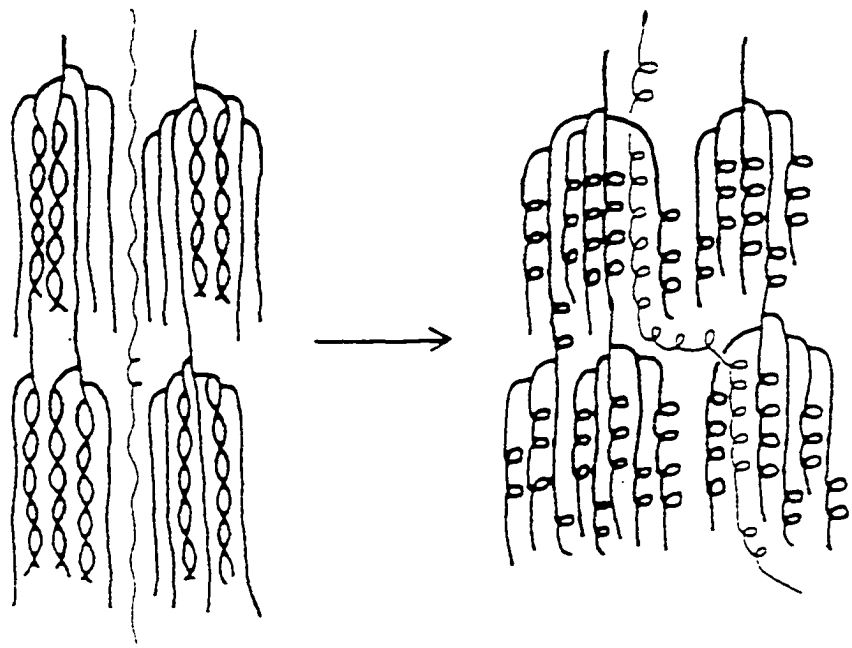


Figure 11. Proposed conversion of an A-type starch granule to a V-type granule ——, amylose; ——, amylopectin (Jane, et al., 1986).

accelerate retrogradation, but tuber and root starches contain phosphate monoesters that can retard retrogradation (Jane et al., 1999). Retrogradation rates of starches are inversely proportional to the amount of short chains of dp 6-9 (Shi and Seib, 1992; Jane et al., 1999). Amyloepectin molecules with branched structures could be effective for crystalline structure formation, depending on the structure of branch chains. Most waxy starches have lower retrogradation rates compared to the normal starch counterpart. However, *ae* waxy maize starch with very long branch chain length (average dp 29.5) displayed a higher retrogradation rate than normal maize starch, and *du* waxy maize starch with an average chain length of dp 23.1 and containing phospholipids also had high retrogradation rate (Jane et al., 1999).

Swelling is the first step in gelatinization and pasting. Intact swollen starch granules are major contributors to starch paste. Native starch granules are insoluble in cold water. When heated in water, the granule begins to swell in the amorphous region. It exerts tension on neighboring crystallites and tends to distort them. Further heating leads to uncoiling or dissociation of double helical regions and break-up of amylopectin crystallite structure. The liberated side chains of amylopectin become hydrated and swell laterally, further disrupting crystallite structure. The starch molecules are unable to stretch longitudinally in disk-shaped granules, and may have a tendency to contract to approach a random coil conformation. This provides a constraint against swelling in the chain direction (French, 1984). Amylose molecules hold the granule integrity and restrict swelling. Therefore, pasting properties of starch are affected by amylose and lipid contents and by branch chain-length distribution of amylopectin. Amylopectin contributes to swelling of starch granules and pasting, but amylose and lipids inhibit swelling (Tester and Morrison, 1990; Jane et al., 1999). Jane and Chen

(1992) studied that synergistic effect of combination of amylose and amylopectin on the pasting viscosity. In general, waxy cereal starches have clear paste, and display higher pasting viscosity than the normal starch counterparts, because waxy starches mainly contain amylopectin and granules can fully swell without the restriction of amylose-lipid complex (Jane et al., 1999). Because the long branch-chains of amylopectin also form helical complexes with lipids and intertwine other branch chains to hold the integrity of starch granules during heating and shearing, *ae* waxy and *du* waxy maize starches showed different profiles from other waxy starches. Wheat and barley starches had the highest pasting temperature and low peak viscosity due to high phospholipid contents and low amylopectin contents. Tuber and root starches had lower pasting temperature and lower resistance to shear-thinning and lower set-back viscosity than cereal starches (Jane et al., 1999). Granule size and isolation methods also affect the pasting properties (Chen, 1997; Jane et al., 1999).

Starch applications

As a basic ingredient in processed food industry, starch plays an important role as a nutritive stabilizer to provide viscosity, texture, and consistency of many food products (Moore et al., 1984). Uses of raw starch are restricted because of retrogradation and shear instability (Lillford and Morrison, 1997). Modified starches can provide better properties, but any chemically or genetically modified starches need to get FDA approval before they are used in food products. BeMiller (1997) reviewed starch modification and described the principal reasons for modification (Table 1) and listed the property changes of genetically modified starches. Opportunities for new modified starch products were suggested as to

Table 1. Principal reasons for starch modification

To modify cooking characteristics
To decrease retrogradation
To decrease gelling tendencies of pastes
To increase freeze-thaw stability of pastes
To decrease paste and/or gel syneresis
To improve paste and/or gel clarity and sheen
To improve paste and/or gel texture
To improve film formation
To improve adhesion
To add hydrophobic groups (for emulsion stabilization)

control reaction sites within granules, to control reaction sites on molecules, and to investigate new commerical starch sources, and to genetically modify existing starches. Resistant starch is physiologically defined as the sum of starch and starch degradation products which are not digested and absorbed in the small intestine of healthy individuals. Starch can be used as a dietary fiber resource. Starch has also been used as dusting agents for candy, carrying agents for baking powder. Small granular or small particle starch and microcrystalline starch (diameters $\leq$ ~2 μm) can be used as fat substitutes and fat mimetics (Jane, 1997).

Starch has been widely utilized in non-food industry (Koch and Roper, 1988; Roper, 1994). As processing aids, raw starch and modified starches have been used in paper manufacturing, textile, or oil well drilling industries, as binders, thickeners, sizing agents, or adhesives. As functional additives or fillers, starches and starch derivatives can be processed with synthetic polymers to improve material biodegradability. As reactive components, starches can be incorporated during production of synthetic polymers leading to synergistic effects. As barrier coatings, starch films can provide low oxygen permeability. As raw

materials, starches can be converted by chemical or fermentation processes into alcohols, polyols, acids or enzymes. As chiral structural elements, starches can be chemically modified into biologically active compounds (eg. surfactants, chiral building blocks for synthesing compounds for pharmaceutical applications).

Soy protein

Soybeans have been used as a food source in China for over thousands years because of its high protein contents. Soy proteins are important functional ingredients in many food products. Soybeans and its products also have long history in industrial applications, such as paper coatings, plastic polymers, wood adhesives, synthetic fibers, and fire-fighting foams, etc. Soybean utilization for industrial uses only accounts for less than 3% of total soybean use, the majority is for food and feed uses. Besides genetic modification through breeding to improve soy protein functional properties, research also includes the investigation of relationship between structures and properties of soy proteins to fully understand the materials and broaden their applications.

Composition and structure

Proteins in soybeans are predominantly albumins and globulins. Globulins are the dominant proteins and account for about 50-90% of seed proteins. According to sedimentation coefficients, the storage globulins in soy proteins are mainly 7s globulins and 11s globulins (Utsumi et al., 1997).

7s soy protein globulins include three major fractions, β-conglycinin, γ-conglycinin, and basic 7s globulin (Utsumi et al., 1997). β-Conglycinin is a vicilin-type protein with a molecular mass of 150-200 kDa. The amino acid composition of β-conglycinin is shown in Table 2 (Nielsen, 1985; Utsumi et al., 1997). There are more arginine and amide-containing amino acids, but less amino acids containing sulfur. β-Conglycinin is a trimer or a hexamer in solution and probably in both forms in the seed (Murphy, 1984). It has four subunits. Three major subunits are α' (72 kDa), α (68 kda), and β (52 kDa). One minor subunit is γ with the similar size to β subunit (Thanh and Shibasaki, 1977). The cysteine residues of α' and α subunits are near the N-terminal. No cysteine is in β subunit. β-conglycinin is a glycoprotein. It has been proposed that the α' and α subunits contain two carbohydrate moieties and the β subunit has one. β-Conglycinin subunits are N-glycosylated. Studies showed that β-conglycinin has molecular heterogeneity (Thanh and Shibasaki, 1978; Yamauchi et al., 1981). Subunit compositions as $\alpha'\beta_2$, $\alpha\beta_2$, $\alpha\alpha'\beta_2$, $\alpha_2\beta$, $\alpha_2\alpha'$, and α_3 were isolated and identified by Thanh and Shibasaki (1978). β_3 subunit, which associates with glycinin in the process of purification, was also observed and reported byYamauchi (1981). Each subunit has different amino acid compositions (Table 2) and displays different functional properties (Nakamura et al., 1986).

Heterogeneity of molecular species caused the difficulty in crystallization of soy protein β-conglycinin. However, three-dimentional structure of phaseolin was studied by using x-ray scattering (Plietz et al. 1983). Results showed that this β-conglycinin is consisting of three Y-shaped subunits. Circular dichroism and optical rotary dispersion were applied in protein secondary structure study (Koshiyama and Fukushima, 1973). Results suggested that

soy protein β-conglycinin had 5% α-helix, 35% β-sheet and 60% random coil. The disulfide crosslinking of β-conglycinin is limited because there are only 2 or 3 cystine groups per mole of protein (Kinsella, 1979). The crystals of soybean β-conglycinin were obtained by vapour diffusion technique using 7.3% PEG 10,000 and 0.1 M MES (pH 5.5) buffer for two weeks at 4°C (Maruyama et al., 1997). The structure analysis is still in progress.

The soybean 11s globulin, glycinin, is a hexamer with molecular mass of 300-380 kDa. Each subunit is composed of an acidic polypeptide with molecular mass of ~35 kDa and a basic polypeptide with molecular mass of ~20 kDa. The acidic and basic polypeptides are linked by a disulfide bond. The isoelectric point of basic subunits is between 8.0 and 8.5, and that of acidic subunits is 4.7-5.4 (Kinsella, 1979). The genes and cDNAs encoding subunits of glycinin have been cloned and sequenced (Utsumi, 1992). Sequence homology at the N-terminal in the subunits is observed. All subunits have Asn at the C-terminus of acidic polypeptide and Gly at the N-terminus of the basic polypeptide (Utsumi, 1992). The amino acid sequence of each subunit is different among soybean cultivars (Utsumi et al., 1987a; Utsumi et al., 1987b). According to the sulfur content, acidic and basic peptides produced from the same gene can be classified into sulfur-rich and sulfur-poor peptides, such as A_{1a}, A_{1b}, A_2 and A_3 contain sulfur amino acids, whereas A_4 and A_5 do not have. For basic subunits, B_{1a}, B_{1b} and B_2 are sulfur-rich amino acids, but B_3 and B_4 are sulfur-poor amino acids (Moreira et al., 1979). Five subunits were identified, as a constituent subunit, $A_{1a}B_{1b}$ (G1), A_2B_{1a} (G2), $A_{1b}B_2$ (G3), $A_5A_4B_3$ (G4), and A_3B_4 (G5) (Nielsen, 1985). The amino acid compositions and molecular masses of constituent subunits and polypeptides of glycinin are shown in Table 3 (Utsumi et al., 1997).

Table 2. Amino acid composition of β-conglycinin and its subunits[a]

Amino acid	α′ Subunit	α Subunit	β Subunit	β-Conglycinin
Asx	11.8	12.6	14.1	14.1
Thr	2.7	2.1	2.8	2.8
Ser	7.0	6.9	7.6	6.8
Glx	23.4	23.5	18.1	20.5
Pro	7.1	6.4	4.7	4.3
Gly	5.6	4.8	5.2	2.8
Ala	4.0	4.5	5.7	3.7
Val	3.4	3.4	4.1	5.1
Met	0.6	0.4	0.0	0.2
Ile	3.7	4.5	4.4	6.4
Leu	7.0	8.3	9.6	10.2
Tyr	2.1	1.5	2.6	3.6
Phe	4.7	5.0	6.1	7.4
His	3.6	1.1	1.8	1.7
Lys	7.0	6.0	5.8	7.0
Arg	6.9	8.0	7.2	8.8
Cys	0.2	0.4	0.0	0.3
Trp	ND[b]	ND	ND	0.3

[a]Expressed as molar percentage.
[b]ND, not determined.
Source: Nielson, 1985.

Table 3. Amino acid compositions and molecular masses of polypeptides of glycinin

Amino acids	$A_{1a}B_{1b}$ (Mol%)	$A_{1b}B_2$ (Mol%)	A_2B_{1a} (Mol%)	A_3B_4 (Mol%)	A_5 A_4B_3 (Mol%)
Ala	5.67	6.06	6.63	3.65	4.08
Arg	5.67	6.27	6.20	6.70	6.67
Asn	7.77	7.79	8.56	6.70	6.12
Asp	3.57	3.46	3.85	4.87	5.56
Cys	1.68	1.73	1.71	1.21	1.11
Gln	10.08	10.60	10.92	9.14	8.9
Glu	8.61	8.22	7.92	8.53	10.20
Gly	7.35	6.70	7.28	8.13	6.86
His	1.68	1.29	0.85	3.04	2.78
Ile	5.46	5.19	4.92	3.45	3.89
Leu	6.93	6.70	7.06	6.91	6.86
Lys	5.04	3.89	3.85	3.65	5.00
Met	1.26	1.08	1.49	0.81	0.37
Phe	4.20	5.62	4.06	3.04	2.59
Pro	6.09	6.27	5.56	7.52	6.86
Ser	6.72	6.92	6.42	7.72	7.97
Thr	4.20	3.89	3.85	4.06	3.71
Trp	0.84	0.64	0.85	0.81	1.11
Tyr	2.31	2.16	2.35	3.04	2.78
Val	4.83	5.41	5.56	6.91	6.49

Source: Utsumi, S. et al. (1997).

Circular dichroism (CD) spectroscopy and computerized sequence analysis were used to estimate the proportions of α-helix, β-sheet, β-turn and random coil for the secondary structure of glycinin (Pleitz et al., 1987; Wright, 1987). Different results could be given based on the method of data analysis. Typically, glycinin has 10% or less of α-helix, approximately 40-50% each of β-sheet and random coil (Lambert and Yarwood, 1992). The acidic subunits are composed of β and β-turns. The basic subunits are also mainly β-turn structure with an α-helix tail of varying lengths at the N-terminal end (Pernollet and Mosse, 1983; Murphy, 1984). Relationship between the amino acid sequence and the domain structure of the subunits of the 11s seed globulins was studied by Plietz et al. (1987). Results show that the N-terminal region of the acidic (α) and of the basic (β) polypeptide chain is much better than the corresponding variable C-terminal chain region. An alternating sequel of β-strands and β-turns dominates within the sequence regions of high similarity. A hypothetical model of the arrangement of the α-chain and β-chain of each subunit within the hexameric molecule of glycinin was proposed (Fig. 12). The more hydrophobic C-terminal β-chain is suggested close to the center of the molecule, and stabilize the oligomeric structure of glycinin molecules by hydrophobic interactions with the same sequence regions of the other five subunits. The radial-symmetrically arranged domains within the molecule are flanked by the strongly hydrophilic C-terminal α-chain region at the surface of the globulin molecule and by the more hydrophilic C-terminal β-chain region in the central part of the molecule. Molecular heterogeneity causes the difficulty in the crystallization of glycinin. Overall molecular shape and dimensions are still not clear (Lambert and Yarwood, 1992). X-ray scattering and electron microscopy were used to investigate the subunit arrangement within the molecule in

the absence of available detailed crystallographic structures. A favored model is a trigonal antiprism arrangement, i.e. two trimeric rings superimposed and twisted about 60°. However, the true structure is still uncertain (Lambert and Yarwood, 1992).

15S fraction is a minor component and accounts for less than 10% of protein in soybean (Nielsen, 1985). Partial purification and characterization of the 15s globulin of soybeans were studied by gel filtration, ultracentrifugation, and gel electrophoresis (Wolf and Nelson, 1996). Gel electrophoresis results suggested that 15s is an aggregate of glycinin. Molecular weight estimations by gel filtration indicated that 15s is a dimer of glycinin. Soybeans, like other plants, contain protease inhibitors. Kunitz inhibito is a trypsin inhibitor. Bowman-Birk inhibitor is a major trypsin and chymotrupsin inhibitor in soybean. The protease inhibitors could depress animal growth, therefore they are considered as antinutritional components. Inactivation of the protease inhibitors could increase protein nutritive value. Heat treatments were used to inactivate trypsin inhibitors from soybean (DiPieto and Liener, 1989). Sodium metabidulfite is an excellent inactivator of soybean trypsin inhibitor during chemical inactivation. Conformation changes of Bowman-Birk inhibitor were studied when heated in the presence and absence of sodium metabisulfite (Wu and Sessa, 1994). Results showed that disulfide bonds were broken by sodium metabisulfite, however, Bowman-Birk inhibitors have a stable conformation. Soybean contains three lipoxygenase isozymes, called L-1, L-2, and L-3 (Nielsen, 1985). Lipoxygenase catalyses the hydroperoxidation of unsaturated fatty acids and polyunsaturated lipids. The oxidation products, hydroperoxides, decompose to form the medium-chain-length aldehydes and alcohols that are responsible for the grassy-beany flavor, and the trihydroxy acids that cause

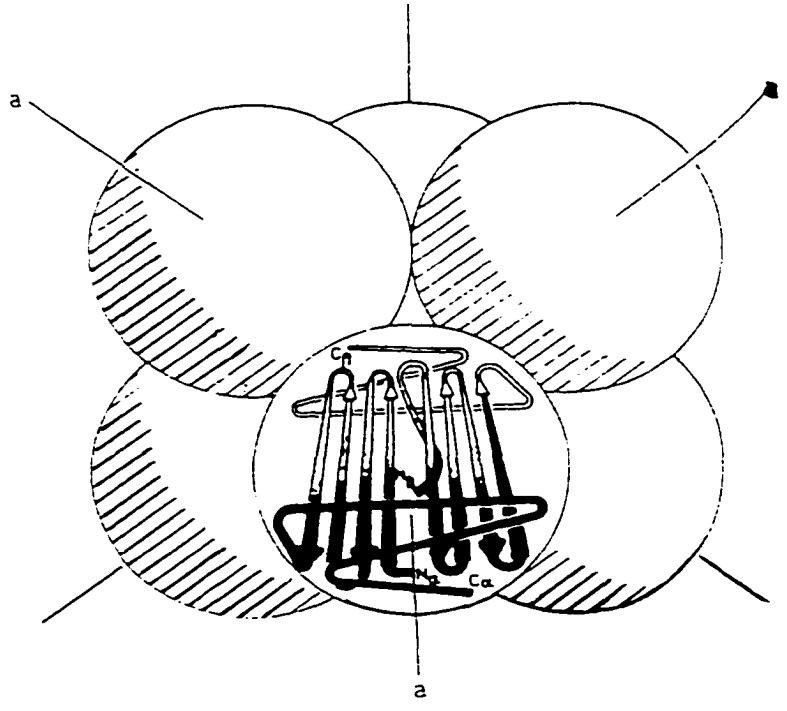


Figure 12. Hypothetical model of glycinin (Plietz et al., 1987). N_α and N_β are the N-terminal region of the α-chain and of the β-chain, respectively. C_α, C_β and a indicate the corresponding C-terminal regions and the twofold pseudosymmetry axes, respectively.

the bitter taste of soybeans, limiting a broad utilization of soy products. Besides using heat treatment to suppress the lipoxygenase activity, genetic modification has also been demonstrated that single recessive alleles operation resulted in the absence of the L-1, L-2, and L-3 isozymes, and reduced the brassy-beany flavor, which was indicated by sensory evaluation (Kitamura, 1993).

Physicochemical and functional properties

Due to the structural characteristics, soy protein displays diverse physicochemical properties and provides different functionalities in food systems. The basic physicochemical parameters related to soy protein functionality include purity, homogeneity, composition, conformation, total and available SH and SS groups, isoelectric points of subunits and polymers, denaturation temperature, optical rotation and dispersion properties, polarization of fluorescence properties, visible and UV spectroscopy properties (Morr, 1990). Typical functional properties of soy protein include solubility, water absorption and water binding, viscosity, gelation, cohesion-adhesion, elasticity, emulsification, fat absorption, flavor binding, and foaming (Kinsella, 1979). Factors influencing functional properties of soy protein are classified into intrinsic factors, environmental factors, and processing treatment factors and summerized in Table 4 (Kinsella et al., 1985).

Gelation is an important property of soy protein for food (eg. tofu) and non-food applications (eg. plastics). Gelation is a two-step process. The important initial step is protein denaturation by heating. The second step for tofu making is protein coagulation promoted by protons from glucono-δ-lactone or by calcium ions (Kaoru et al., 1995). The

Table 4. Factors that influence the functional properties of oilseed proteins in foods

Intrinsic	Environmental factors	Processing treatment
Composition of protein	pH	Heating
Conformation of protein	Oxidation-reduction status	Drying
Mono- or multicomponet	Salts	pH
	Water	Ionic strength
	Carbohydrates	Reducing agents
	Lipids	Storage conditions
	Surfactants	Physical modification
	Flavors	Chemical modification

second step for other heat-induced gelations is the formation of a three-dimensional network by protein-protein interaction (Wang and Damodaran, 1991). Transmission electron microscopy was used to visualize network structure of heat-induced gels at molecular level. The study of glycinin gelation at 100°C and 0.5 ionic strength suggested that aggregation of glycinin molecules resulted in the formation of strands, and the interaction of the strands contributed to form gel network (Nakamura et al., 1984). Nakamura et al (1985) isolated intermediary subunits of glycinin designated as IS I, IS II, and IS III by DEAE-Sephadex column chromatography. Results of glycinin gel properties showed that IS II is related to the generation of the gel turbidity and IS III plays an important role in increasing the gel hardness. The hardness of the gel seems to be determined by both the length and the extent of branching of the constitute strands of the gel network structure. Gelation property of glycinin differed significantly among cultivars (Nakamura et al., 1984). Their study also suggested that acidic subunit A_4 was linked to its basic subunit counterpart by noncovalent interactions, which could break during heating. Liberation of A_4 caused the conformation change of glycinin and

further resulted in the rapid gel formation. Gel hardness was different among cultivars too. High molecular weight acidic subunit A_3 contributed to the gel hardness.

The disulfide bond linking the acidic and basic polypeptides is interchanged to sulfhydryl bond during heat denatureation, which is responsible for the rapid cleavage of the interchain disulfide bond of glycinin (Wolf, 1993). Effects of disrupting disulfide bonds on the formation and maintenance of structure and on the functional properties of proglycinin were examined by replacing the cysteine residues (Cys12 and Cys88) through oligonucleotide-directed mutagenesis, giving mutant proglycinins Gly12, Ser88, and Gly12Ser88 (Utsumi et al., 1993). Results showed that the removal of both disulfide bonds resulted in a molecule that was less compact than native proglycinin, but the overall conformations are similar. The mutant proglycinin Gly12 could not form a gel at low protein concentration (<6%) as native proglycinin did, in the meantime, the gel hardness of the mutant proglycinin Ser88 was much higher than those of the native glycinin and the unmodified proglycinin. It conformed the fact that disulfide exchange plays an important role in the formation of heat induced gel (Mori et al., 1982), and the number and the arrangement of free sulfhydryl residues are closely related to the heat-induced gel-forming ability and the gel properties of glycinin (Nakamura et al., 1984). Genetics affects glycinin and β-conglycinin content in soybeans, however, environmental conditions seem to have a greater impact on glycinin concentration than gentics (Murphy and Resurreccion, 1984).

Nagano et al. (1996) studied the gelation properties of different soybean protein isolates by dynamic viscoelastic method. Results showed that an increase in the storage modulus G' was in the order of β-conglycinin-rich soy isolate > control soy isolate > glycinin-

rich soy isolate at the heating condition from 30 to 80°C. Their results suggested that β-conglycinin played an important role in the heat-induced gel formation of commercial soy protein isolates. Mechanism of heat-induced gelation and gel properties of β-conglycinin were studied by Nakamura et al. (1986). Results showed that the gel properties and gelation mechanism for β-conglycinin were different from those for glycinin. The proposed mechanism for β-conglycinin gelation includes two steps. When a β-conglycinin solution is heated, soluble aggregates with molecular weights of around 1 million are formed first, and associate with one another randomly to form a cluster, then a gel with aggregates of cluster is formed. A negative relationship was found between tofu hardness and the ratio of glycinin to β-conglycinin (Murphy et al., 1997). Thirteen soybean varieties were used to produce tofu by three different methods, bench, pilot plant, and scale-up production (Cai and Chang, 1999). Results showed that different processing methods gave different β-conglycinin and glycinin contents that contributed to tofu hardness, yield, and sensory quality. Heat-induced interactions between β-conglycinin and glycinin were studied by Utsumi et al. (1984). Two-dimensional gel electrophoretic analysis revealed that the macro complexes contained predominantly the basic subunits of glycinin and the β subunit of β-conglycinin; little α and α' subunits of β-conglycinin were present in the complexes, indicating that the basic subunits of glycinin have higher affinity for the β subunit. Results also showed that disulfide bonds between the basic subunits were invovled in the formation of soluble macro complexes. Utsumi and Kinsella further studied subunit interactions in heat-induced gelation of β-conglycinin, glycinin and soy isolate proteins by ultracentrifugation and two-dimentional gel electrophoresis (1985). Results indicate that acidic subunit A_3 is an integral component of

glycinin and soy isolate gels; the three subunits (α, α', and β) of β-conglycinin participate uniformly in gel matrix formation from β-conglycinin, and there is a preferential interaction between the β subunit of β-conglycinin and the basic subunits of glycinin in soy isolate gels.

Effect of protein conformation on gel strength was studied by using circular dichroism (Wang and Damodaran, 1991). Results showed that native soy protein mainly contains β-sheet and aperiodic structures. A reduction in the β-sheet content and an increase of aperiodic structure content were observed in protein gels. Fourier transfer infrared (FTIR) and differential scanning calorimetry (DSC) were also used to study the relationship between protein conformation and protein gelation and gel properties (Ker et al., 1993; Nagano et al., 1994). More α-helix and less random coil structures were indicated in heat-induced glycinin gels (Ker et al., 1993). More β-strands were exposed after β-conglycinin underwent denaturation with increasing protonation of its carboxyl groups. The expsoed β-strands intermolecularly bond to form gel netwroks (Nagano et al., 1994).

Protein engineering is an ever-increasing technique to modify the primary structure of proteins by gene manipulation. According to the relationship between protein structure and protein gel properties, genetically modified protein could be designed to improve functionality of protein in the end products. Structurally altered glycinin subunits were produced by modifying a Gy_4 glycinin cDNA (Dickinson et al., 1990). Results showed that alterations made in the acidic polypeptide changed the subunit solubility and modifications in the basic polypeptide eliminated assembly of subunits into trimers. Glycinin samples with different genetic background were studied in their biochemical properties (Yagasaki et al., 1997). The absence of subunits resulted in significant changes in the proportion of glycinin to β-

conglycinin. Decreasing glycinin/β-conglycinin ratios and structural changes could be caused by a lack of glycinin subunits.

Transgenic potato and soy-rice are two products of protein engineering (Hashimoto et al., 1999; Katsube et al., 1999). Glycinin gene was expressed in the transgenic potato (Hashimoto, 1999). There was no significant difference in compositions between transgenic and control potatoes, however, higher level of glycoalkaloids were found in transgenic potato tubers than in normal potato. It is known that rice glutelin subunits and glycinin subunits have a common fundamental structure, and rice glutelin exhibits no significant functional properties with deficiency in lysine, whereas glycinin is rich in lysine (Katsube et al., 1998). Therefore, transforming of glycinin gene into rice could result in conversion of the properties of rice storage protein from glutelin into globulin. Soy-rice will be one of the products in the development of improving rice nutritional quality (Momma et al., 1999).

Soy protein products

Soy protein products are classified into soybean meal, soy flour and grits, soy protein isolates, soy protein concentrates, textured soy protein products, and hydrolyzed soy proteins (Johnson, 1984). The flow chart (Fig.13) illustrates the processing of soy protein products from soybeans. The main purposes of most processing steps are to remove undesirable taste and odor components, to remove or inactivate nutritionally undesirable components, to prepare a suitable products for applications (Visser and Thomas, 1987). Fractionation of

glycinin and β-conglycinin were successfully scaled up to the pilot plant scale (15 kg soy flakes) and the process steps are shown in Figure 14 (Wu et al., 1999). However, soybean glycinin and β-conglycinin have not been commercialized yet.

Major differences among these soy protein products are the protein content and carbohydrate content (Table 5). Soy flour and grits are least refined products for human food consumption. According to oil type and content, soy flour and grits are commonly divided into full-fat flours, high-enzyme flours, defatted flours, defatted grits, and lecithinated/defatted flours. Most of soy flour and grits are used in baking industry. Soy protein concentrate products contain about 70% protein on a moisture-free basis. There are three major processes for preparation of soy protein concentrates: acid wash process at pH 4.5, extraction with 70-90% aqueous ethanol, and extraction with water after heat denaturation of the protein with moist heat. Heat treatment by steam injection or jet cooking can improve the solubility and functionality of soy concentrate products. Soy protein isolate products have high protein content of more than 90% and usually are costly. Most soy isolate products are neutralized to be more soluble and have different functionality. Isoelectric soy isolates are another type of products and usually used in infant formulas and for nutritional applications (Rhee, 1994). Textured soy proteins are produced by extrusion processing and used to resemble meat or seafood in structure and appearance when they are hydrated. Hydrolyzed soy protein ingredients are produced by proteolytic enzyme hydrolysis and they are used as flavor ingredients.

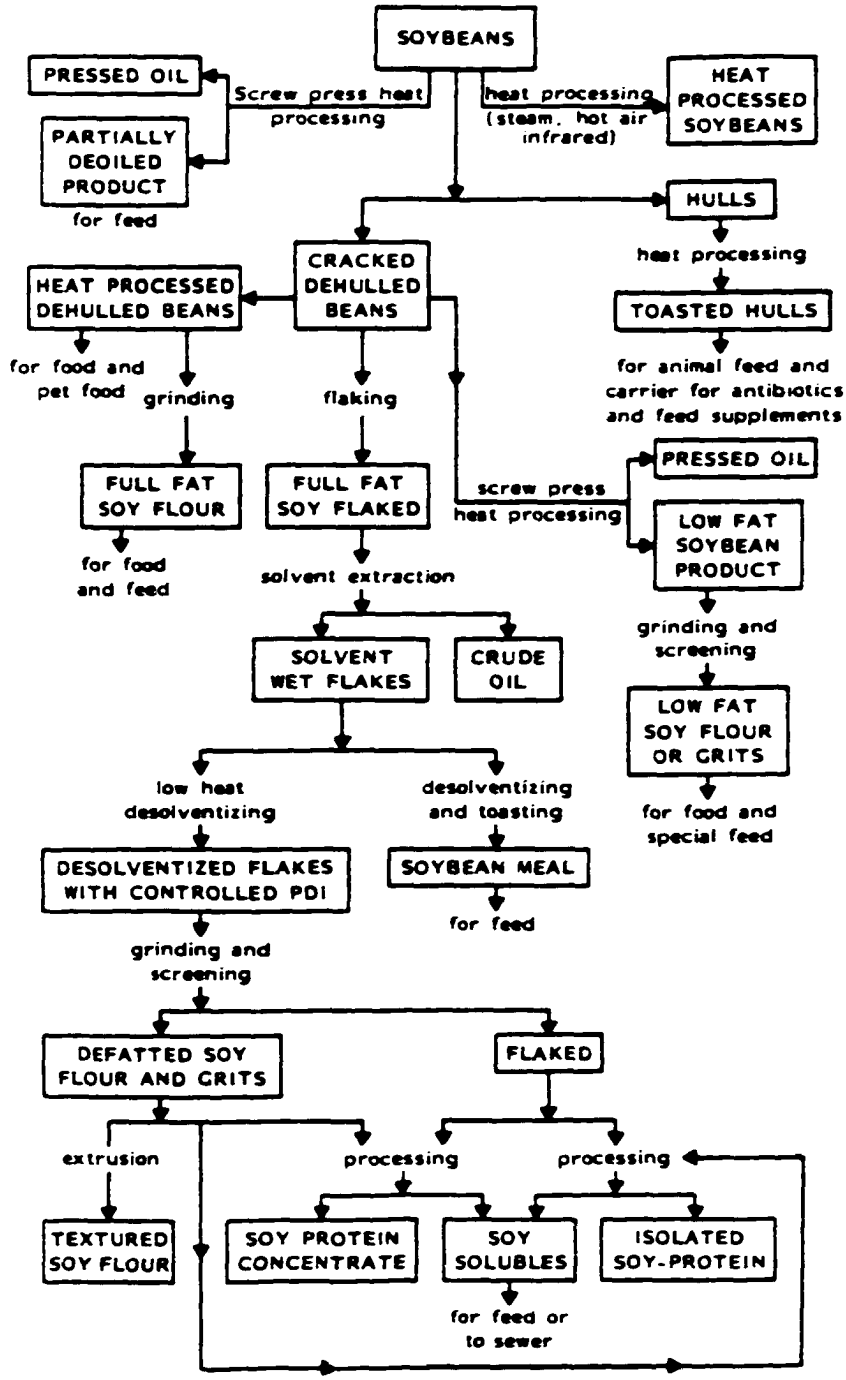


Figure 13. Flow chart of processing of soy products from soybeans (Johnson, 1984)

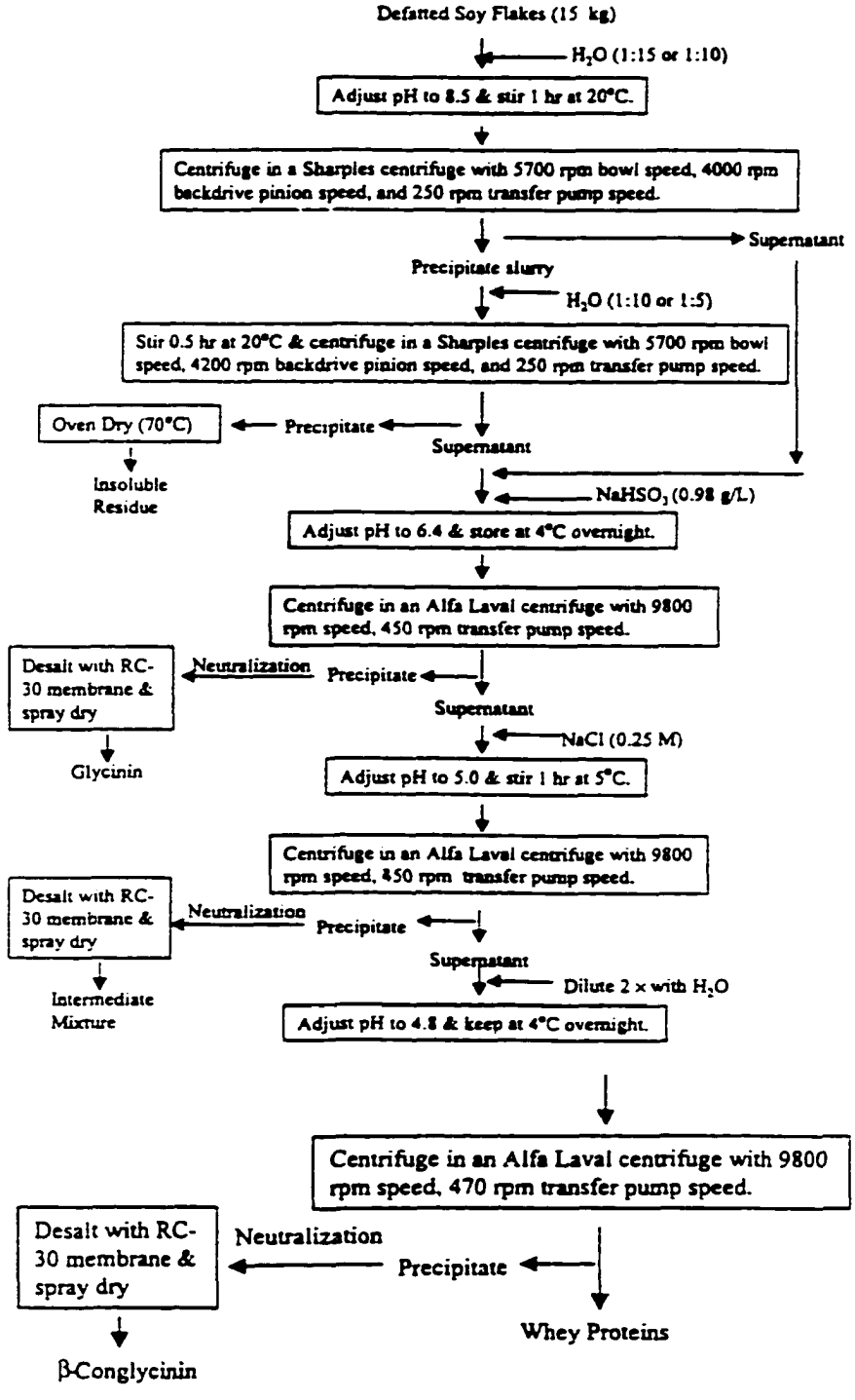


Figure 14. Flow chart of fractionation of glycinin and β-conglycinin from soy flakes (Wu et al., 1999).

Table 5. Compositions of soy protein products[a]

Properties	Meal	Flour	Concentrate	Isolate
Protein[b], %	50-51[c]	52-54[c]	68-72[c]	90-92[c]
Moisture, %	12	6-8	4-6	4-6
Crude free lipid, %	0.5-1.0	0.5-1.0	0.5-1.0	0.5-1.0
Crude fiber, %	5.0-6.9	2.5-3.5	3.4-4.8	0.1-0.2
Ash, %	5.0-6.2	2.5-6.0	3.8-6.2	3.8-4.8
Carbohydrate[d], %	40-48	30-32	19-21	3-4

[a] Sources: Soy protein Council, 1987; Lusas and Rhee, 1995
[b] Protein calculated by N × 6.25
[c] Moisture-free basis
[d] Carbohydrate calculated by difference

Soy plastics

The early documents on soy plastic material were the patents issued in France and Great Britain in 1913 and Satow's patent of 1919 (Johnson and Myers, 1995). Henry Ford was a great contributor in the development of soy plastics during 1930s and 1940s. His idea of "all agricultural" car promoted the application of soy proteins although the driving force at that time was to solve the economic depression problem. Since the1970s, the research on biodegradable materials by investigating natural biopolymers has been of importance to change the situation of shortage of petroleum products and solve the environmental pollution problem caused by synthetic material wastes.

The compression molding process has been used for fundamental studies of soy protein plastics to investigate the processing condition effects and characterize their properties. Paetau et al (1994a) reported the effect of preparation and processing on mechanical properties and water absorption of compression-molded soy plastics. Their results showed that both soy protein isolate and concentrate samples can be compression molded into rigid and brittle specimens. The molding temperature and moisture content of protein samples are important for the properties of molded specimens, such as at 140°C and moisture content of less than 10%, molded specimens displayed higher tensile strength than at other conditions. Acid treatment of protein can decrease the water absorption of molded specimens. Different plasticizer effect on the properties of soy protein specimens was reported by Wang et al. (1996). It is shown that polyhydric alcohols (ethylene glycol, glycerol, and propylene alcohols) as plasticizers are well compatible with soy protein and improve the flexibility and elasticity of soy protein plastics. Glass transition temperature (150°C) of dry soy protein

Specimens could be reduced to -50°C when glycerol was added as plasticizer. The combination of water and polyhydric alcohols can give the best plasticizing effect. Effects of cross-linking of soy protein isolate with formaldehyde and glyoxal were studied by Paetau et al. (1994b). Their results showed that cross-linking treatment with 5% formaldehyde increased the tensile strength and decreased water absorption whereas treatment with glyoxal decreased mechanical properties and water absorption. Huang (1994) modified soy protein isolate by esterification and acetylation, and results showed that acetylated soy protein isolate specimens displayed lower mechanical properties and the effect on water absorption was not significant. Esterified soy protein isolate specimens enhanced both mechanical properties and water absorption. It was also reported that short-fiber cellulose can increase the mechanical properties of soy protein specimens (Paetau et al., 1994b). Morphology and mechanical behaviors of soy plastics were investigated by Sue et al. (1997). They found that soy plastics displayed high modulus and high toughness, comparing to synthetic polymers (such as epoxy resin or polycarbonate). The fracture in dry soy plastics is mainly because of the formation of voids in the crack tip damage zone. Sun et al. (1999) reported the results of characterization of soy protein component specimens by compression molding process. It was shown that specimens made from the mixture of glycinin and β-conglycinin displayed higher tensile strength than those made from the fractions alone. Glycinin specimens had lower water absorption than β-conglycinin specimens.

Soy proteins can be processed by extrusion. Sheard et al. (1984) studied the role of carbohydrate in soy extrusion. Soy flour extrudates had a more regular and honeycombed structure than soy isolate extrudates although they had similar expansion ratios.

Macromolecular changes associated with the heat treatment of soy isolate were investigated by Sheard et al (1986a). Non-disulfide covalent links were involved in the formation of the aggregates after processing. There was a negative correlation between texture of extrudates and protein solubility in the feed and a positive correlation between protein solubility in the feed and in the extrudates (Sheard et al., 1986b). Flow behaviour of molten soy protein isolate at a relatively low moisture content by using a capillary tube extrusion rheometer (Fujio et al., 1991). The flow behaviour could be classified as pseudo-plastic when the moisture content was above 41%. Effects of glycinin and β-conglycinin on the extrusion performance of soy protein concentrates were reported by Ning and Villota (1994). Glycinin enhanced expansion and water holding capacity of the finished product, and the ratio of glycinin to β-conglycinin at 1.5 in the feed formulation gave good texture characteristics to extruded products. Pressure-volume-temperature relationships of soy protein isolate/starch plastic were investigated by Otaigbe and Jane (1997) to provide insights into biodegradable product development. Soy protein can also be used to produce foam products with various thermal insulation properties to replace styrofoam products.

Soy protein foams were successfully extruded using a single screw Brabender extruder (Mungara et al., 1998). The mount of water, the best plasticizer, and salts were studied for the best formulation which could be easily compounded, pelletized and used in the final extrusion process. Sodium tripolyphosphate dramatically enhanced the processability of soy protein and also increased densities of the resulting foams. Foams with 15% glycerol showed relatively good mechanical properties comparing with the foams with 10 or 20% glycerol. Lower amounts of glycerol decreased the densities of foams and increased the modulus. The

foams possess suitable properties which make them prime candidates for packaging or disposable utensils applications. Plasticization effects of water, glycerol, and sugars on soy protein sheet process were investigated (Zhang et al., 1998). Mechanical and dynamic mechanical properties of soy protein sheets with the above plasticizers were studied. Both glycerol and sugar could function as a plasticizer and reduce the glass transition temperature of the resulting sheets. With the increase of moisture content, the tensile strength and modulus dropped rapidly. The modulus increased with the increase of zinc sulfate or epichlorohydrin because of the cross-linking formation between soy protein side-chain functional groups.

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CHARACTERIZATION OF BARLEY STARCHES OF WAXY, NORMAL, AND HIGH AMYLOSE VARIETIES

A paper published in Carbohydrate polymers 41(2000), 365-377[1]

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ABSTRACT

Four varieties of barley starches, W.B. Merlin, glacier, high amylose glacier, and high amylose hull-less glacier, were isolated from barley seeds. Apparent and absolute amylose contents, molecular size distributions of amylose and amylopectin, amylopectin branch-chain-length distributions, and Naegeli dextrin structures of the starches were analyzed. W.B. Merlin amylopectin had the longest detectable chain length of DP 67, whereas glacier, high amylose glacier and high amylose hull-less glacier amylopectins had the longest detectable chain length of DP 82, 79, and 78, respectively. All the four starches displayed substantially reduced proportions of chains at DP 18-21. Amylopectins of high amylose varieties did not

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show significantly larger proportions of long chains than that of normal and waxy barley starch. Onset gelatinization temperatures of all four barley starches ranged from 55.0 to 56.5 °C. Absolute amylose contents of W.B. Merlin, glacier, high amylose glacier, and high amylose hull-less glacier were 9.1, 29.5, 44.7, and 43.4%, respectively; phospholipid contents were 0.36, 0.78, 0.79, and 0.97%, respectively.

INTRODUCTION

Barley is one of the major sources of cereal starch. Barley grains consist of up to 65% starch (MacGregor & Fincher, 1993). It has been reported that barley starches from different genotypes vary in chemical compositions and properties (Bathgate & Palmer, 1972; Schulman et al. 1995; Vasanthan & Bhatty, 1996; Oscarsson et al. 1997; Tester, 1997). The analyses of amylose contents and amylopectin chain-length profiles of normal, high amylose, and waxy barley have been analyzed by using gel permeation chromatography (GPC) on a Sepharose CL-6B column and by using ^{1}H-NMR spectroscopy after enzyme debranching (Salomonsson & Sundberg, 1994). The results showed that the average amylopectin chain length of high amylose barley starch was 5 glucose units longer than that of normal and waxy barley starches. Great differences in physicochemical properties of barley starches were reported among different varieties by Vasanthan and Bhatty (1996). Twelve varieties of waxy barley and six normal barley starches were studied for swelling and gelatinization (Tester & Morrison, 1990; Morrison & Tester, 1992; Morrison et al., 1993). The results showed that lipid-complexed amylose and free amylose affected the gelatinization properties of starch granules. Properties and chemical structures of Nupana and Titan barley starches have been

studied and reported by DeHass and Goering (1972). Their results show that Nupana starch, which displays a higher viscosity, has larger molecules of amylose and amylopectin than Titan starch. This result indicates that starch structure and properties differ between the two barley varieties. In contrast to maize starch counterparts, high amylose glacier and high amylose hull-less glacier barley starches have gelatinization temperatures similar to glacier (normal) and W.B. Merlin (waxy) starches. Relationships between the chemical structures and the thermal properties of these starches are of great interest. In this study, we analyzed the apparent and absolute amylose contents, phosphorus contents, branch-chain-length distributions, and branch structures of the amylopectins by examining the structures of Naegeli dextrins.

MATERIALS AND METHODS

Materials

Four varieties of barley starches, W.B. Merlin, glacier, high amylose glacier, and high amylose hull-less glacier, were provided by Dr. C.W. Newman, Montana State University. Protease from *Aspergillus sojae* was purchased from Sigma Chemical Co. (St. Louis, MO). Isoamylase from *Pseudomonas amyloderamosa* was purchased from Hayashibara Biochemical Laborataries, Inc. (Okayama, Japan). Other chemicals, all reagent grade, were used without purification.

Isolation of starch

Whole barley kernels were cracked slightly by a blender before steeping in 0.05% NaOH solution (pH 12) for 24 hr under propeller stirring. The steeping solution was discarded and the kernels were washed with water, then the sample was blended for 5 min by using an Osterizer blender at full speed (Radosavljevic et al., 1998). Protease (Type XIV, from *Aspergillus*, 0.35 units/mg solid) was added (5mg/g grain) to the sample at pH 7.5. The slurry was mixed in the blender for 1 min at full speed and then incubated in a shaker water bath at 37°C and 50 rpm for 2 hr. After incubation, the slurry was filtered through a nylon screen (30 μm) with additional distilled water for washing the fiber fraction. The fiber fraction was blended with additional water until no more starch was released. The solids obtained by centrifugation were purified by additional protease XIV treatment (5mg/g of starch in 30 ml of incubation mixture) and four times by the toluene shaking procedure (McDonald & Stark, 1988). The clean white layer was washed with water and methanol before drying in a convection oven at 40°C for 48 hr.

Fractionation of amylose and amylopectin

Fractionation of amylose and amylopectin was carried out by following the general procedure of Schoch (1942) and Jane and Chen (1992). The procedure consisted of heating and stirring a starch dispersion (1.33%, w/v in water) in a water bath at 100°C until starch gelatinized. Starch solutions were filtered to remove insoluble residues. The pH of the solution was adjusted to 5.9-6.3 with a phosphate buffer and then autoclaved at 121°C for 3 hr. The flask containing the starch solution was stirred in a boiling water bath for 2 hr to

disperse starch molecules. n-Butyl alcohol was added (20%, v/v), and the solution was stirred at 100°C for 1 hr under reflux. The sealed flask with mixture was placed in a Dewar flask that was filled with boiling water. The Dewar flask was then sealed and allowed to cool down to room temperature over a period of 24-36 hr. Amylose-butyl-alcohol complex crystals formed and precipitated during cooling. The crude amylose-butyl-alcohol complex was separated by centrifuging (8,700 × *g*, 30 min.). The amylopectin remaining in the supernatant was concentrated by using a rotary evaporator and then treated twice more with n-butyl-alcohol to remove amylose residues. The solution was further concentrated and precipitated with excess methyl alcohol.

Scanning electron microscopy

Scanning electron micrographs (SEM) of the starches were taken with a scanning electron microscope (JEOL JSM-35, Tokyo, Japan) at Bessey Microscopy Facility, Iowa State University. Starch samples were suspended in ethanol and placed on silver tape attached to a brass disk. The specimens were coated with gold-palladium (60:40). Micrographs of each starch sample were taken at 1,500x magnification (Jane et al., 1994).

X-ray diffraction pattern

Starch samples were equilibrated in a 100% relative humidity chamber for 24 h at room temperature. X-ray patterns of the starches were obtained with copper, nickel foil-filtered, K_α radiation using a diffractometer (D-500, Siemens, Madison, WI). The

diffractometer was operated at 27 mA and 50 kV. The scanning region of the two-theta angle (2θ) was from 4° to 40° with a 0.05° step size and a count time of 2 sec.

Gel-permeation chromatography (GPC)

Starch molecular-weight distribution profiles were determined by using a GPC column (2.6 cm i.d. × 90 cm) packed with Sepharose CL-2B gel (Pharmacia Inc., Piscataway, NJ), following the method of Jane and Chen (1992). Starch (0.5 g) was moistened with 5 ml of water, and dimethyl sulfoxide (DMSO) (45 ml) was added. The suspension was mechanically stirred while heating in a boiling water bath for 1 hr and then stirred for 24 hr at 25°C to prepare a starch solution (1%). An aliquot (2 ml) of the starch solution was mixed with absolute ethyl alcohol (8 ml) to precipitate the starch, followed by centrifugation. The precipitated starch was redissolved in boiling water (10 ml) and stirred for 30 min, and the solution was filtered to remove the insoluble residues. The supernatant (5 ml) containing starch (15 mg) and glucose (0.75 mg, as a marker) then was injected into the column. The column was run in the ascending mode. A solution made of distilled water containing 25 mM NaCl and 1 mM NaOH was used as an eluent at a flow rate of 30 ml/hr. Fractions of 4.8 ml per cup were collected and analyzed for total carbohydrate (anthrone-sulfuric acid method) and blue value (iodine staining) by using Autoanalyzer II (Technicon Instruments Corp., Elmsford, NY) at 630 nm and 640 nm, respectively (Jane & Chen, 1992).

Apparent and absolute amylose contents

Apparent amylose contents were determined by measuring iodine affinities of defatted starches using a potentiometric autotitritor (702 SM Titrino, Brinkmann Instrument, Westbury, NY). The analysis was based on the method of Schoch (1964) and Kasemsuwan et al (1995). Iodine affinities of the starch samples (I_{starch}) were analyzed in duplicate. Iodine affinity ($I_{amylose}$) of 20% for amylose was used for calculation (Takeda and Hizukuri, 1987). The iodine affinity of amylopectin ($I_{amylopectin}$) was determined by using pure amylopectin following the same method of Schoch (1964). "Absolute" amylose contents were calculated by using the formula $(I_{starch} - I_{amylopectin}) / (I_{amylose} - I_{amylopectin}) \times 100$ % (Takeda et al., 1983).

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Phosphorus analysis

The phosphorus contents of barley starches were determined by a colorimetric chemical method (Smith & Caruso, 1964). ^{31}P-NMR was used to characterize and quantify the phosphorus structures and contents in starch, using nicotinamide adenine dinucleotide (NAD) as an internal reference standard (Kasemsuwan & Jane, 1996). The total phosphorus content of each starch was calculated as the sum of all the phosphorus contents. Phosphorus contents of the starches were calculated by multiplying phosphorus contents in the form of phospholipids with 16.16 (Morrison & Tester, 1992).

Amylopectin branch chain-length distribution

Amylopectin (10 mg) in 90% DMSO solution (1 ml) was precipitated with methyl alcohol (4 ml). The precipitate was dissolved in distilled water (9 ml) by heating and stirring

in a boiling water bath for 1 hr. The solution was cooled to room temperature (25°C), and 1 ml of acetate buffer (0.1 M, pH 3.5) and isoamylase (150-300 units) were added. The mixture was incubated for 48 hr in a shaker bath at 40°C and 120 strokes/min. The sample solution was then adjusted to pH 6 by adding sodium hydroxide solution dropwise and heated in a boiling water bath for 15 min to inactivate the enzyme. Sodium azide was added to the sample solution (0.02%) to prevent microorganisms from growing.

The branch chain-length distributions of amylopectins were analyzed by using high-performance anion-exchange chromatography equipped with an amyloglucosidase reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD) (Dionex, Sunnyvale, CA) following the procedures reported by Wong and Jane (1997). Each sample was analyzed in duplicate.

Preparation and structure analysis of Naegeli dextrins

A rapid method of preparation of Naegeli dextrin was used (Umeiki & Kainuma, 1981). The starch sample (20 g, dsb) was suspended in a 15% (v/v) H_2SO_4 solution (400 ml) and held at 38°C in an incubator. Starch suspensions were gently shaken daily by hand. Samples were taken after 12 days. The supernatant was siphoned off. An aliquot of the supernatant was analyzed for total carbohydrate content to calculate the starch hydrolyzed. The starch residues were washed with ethanol and then dried at 38 °C. The chain-length distributions of Naegeli dextrins before and after isoamylase debranching were analyzed by using the HPAEC-ENZ-PAD system as described earlier.

Thermal properties

Gelatinization and retrogradation properties of starches were analyzed using a differential scanning calorimeter (DSC-7, Perkin-Elmer, Norwalk, CT) equipped with an intracooling II system. Aluminum pans (Perkin-Elmer) were used for the analysis. Starch samples (about 2 mg each, dsb) were weighed in the sample pans, mixed with distilled water (about 6 mg), and sealed. The heating rate was at 10°C per min over the temperature range of 25-120°C. Indium and zinc were used as the reference standards. Enthalpy change (ΔH), gelatinization onset temperature (T_0), peak temperature (T_p), and gelatinization ranges were measured and calculated by using Pyris software (Perkin-Elmer, Norwalk, CT). The data were averages of a minimum of three replicates of each starch sample. The retrogradation study was performed following the same method using the same gelatinized starch samples that had been stored at 4°C for 7 days.

Pasting properties

Pasting properties of the starches were determined by using a Rapid Visco-Analyzer (RVA) (Newport Scientific, Sydney, Australia). Each starch suspension (8%, dsb, w/w; 28 g total weight) was equilibrated at 50°C for 1 min and then heated at a rate of 6°C/min to 95°C and then maintained at that temperature for 5 min. The sample was then cooled to 50°C at a rate of 6°C/min. A rotating speed of the paddle (160 rpm) was used except the paddle speed was 960 rpm at the first 10 seconds.

RESULTS AND DISCUSSION

Granule morphology and crystalline structure

Barley starch granules showed bimodal size distributions (Fig. 1). Large (A) granules had diameters of 15-25 μm, and small (B) granules had 2-5 μm. The results were in agreements with those reported by Jane et al (1994) and by MacGregor and Fincher (1993). Scanning electron micrographs showed that W.B. Merlin and glacier starches had greater proportions of A granules, whereas high amylose and high amylose hull-less glacier starches had A granules with smaller diameters than did glacier and W.B. Merlin starches. W.B. Merlin starch had a greater proportion of B granules than did glacier starch. All barley starches displayed an A-type x-ray diffraction pattern (Fig. 2). High-amylose glacier, high-amylose hull-less glacier, and glacier barley starches also displayed a minor peak at $2\theta=20°$ which reflected the presence of amylose-lipid complex (Zobel, 1988).

Starch molecular size distribution and amylose and phosphorus contents

Gel permeation chromatograms of the four varieties of barley starches are shown in Fig. 3. The first peak in the profile corresponded to amylopectin. The second peak displaying substantial blue value corresponded to amylose. The last peak was glucose used as a marker. W.B. Merlin barley starch had a very small blue-value peak at around fraction number 65, indicating a small concentration of amylose content. High amylose glacier barley starch had a substantially broader second peak than the glacier and high amylose hull-less glacier starch samples, indicating that amylose had larger molecular weights and a broader molecular weight distribution.

Apparent and absolute amylose contents of barley starches were determined by using iodine potentiometric titration, and the results are shown in Table 1. Absolute amylose contents of W.B. Merlin, glacier, high amylose glacier, and high amylose hull-less glacier barley starches were 9.1, 29.5, 44.7, and 43.4%, respectively. W.B. Merlin amylopectin did not show any iodine affinity. GPC profiles of glacier (Fig. 3B) and high-amylose hull-less glacier (Fig. 3D) barley starches displayed molecules with smaller molecular-weight than amylopectin and little blue values (Fig. 3, marked with an arrow) indicating the molecules having short branches. This structural feature showed that these two barley starches consisted of intermediate component that had branched structures but smaller molecular weight than amylopectin (Kasemsuwan et al., 1995).

Chemical analysis showed that W.B. Merlin had 0.024% phosphorus; glacier, 0.052%; high amylose glacier, 0.057%; and high amylose hull-less glacier, 0.073% (Table 1). ^{31}P-NMR spectra (Fig. 4) showed signals mainly at the chemical shift between 0 and 2 ppm, indicating that barley starches had phospholipids (Lim et al., 1994). The phosphorus contents of phospholipids were 0.022, 0.048, 0.051, and 0.060%, corresponding to 0.36, 0.78, 0.79, and 0.97% of phospholipids (Morrison & Tester, 1992), in W.B. Merlin, glacier, high amylose glacier, and high amylose hull-less glacier barley starch, respectively. The spectra of the two high amylose barley varieties (Fig. 4C, and 4D) showed a signal with chemical shift at ~3 ppm, which indicated inorganic phosphate (Kasemsuwan & Jane, 1996). In contrary to that reported by Morrison and Tester (1992), no signals with chemical shift at ~3 ppm were found in the spectra of W.B. Merlin and glacier barley starches (Fig. 4A, and 4B). The signals of phospholipids in the two high-amylose varieties were substantially broader than those of W.B.

Merlin and glacier starches, which could be resulted from complex formation between the phospholipids and amylose (Morrison et al., 1993). There was a small proportion of the phospholipids remained uncomplexed and displayed a sharp peak at ~1 ppm.

Branch chain-length distributions

The bracn chain length distributions of the four varieties of amylopectins debranched by isoamylase and analyzed by HPAEC-ENZ-PAD are shown in Fig. 5. These barley starch amylopectins had similar chain length distributions. Amylopectin of W.B. Merlin barley starch had its longest detectable chain of DP 67, which was the shortest among the four (Table 2). This lack of very long chains (DP > 67) resulted in no detectable iodine affinity in the amylopectin. This result agreed with those reported by Jane et al (In press). Amylopectins of the high amylose varieties had similar peak chain lengths as the others (DP 12 and 48 for peak I and II, respectively) but had lower proportions of DP 6-12, especially for the very short chains (DP 6-9). In contrast to the high-amylose maize starch that had very large proportions (26.1% and 29.5% for high-amylose maize V and VII, respectively) of long branch chains (DP > 37) (Jane et al., In press), high amylose glacier and high amylose hull-less glacier barley starches had only 20.7% and 17.8% long branch chains of DP > 37, similar to W.B. Merlin and glacier.

Naegeli dextrins

After 12 days of acid hydrolysis, 87% of the W.B. Merlin barley starch, 67% of the glacier barley starch, 71% of the high amylose glacier barley starch, and 66% of the high

amylose hull-less glacier barley starch were hydrolyzed. It is plausible that W. B. Merlin barley starch, a waxy variety, swelled to a larger extent than other varieties and was more susceptible to acid hydrolysis, which resulted in a larger percentage hydrolysis.

Anion exchange chromatograms of the Naegeli dextrins obtained after 12 days of hydrolysis are shown in Fig. 6. For W.B. Merlin Naegeli dextrins, peaks were well separated, with clusters of peaks evident around DP 12 and 25 plus additional minor peaks at higher DP. Those clusters were also seen for the other varieties, but were not as well separated, and a cluster around DP 35 was readily visible. The peaks, at DP 12, 25, and higher DP, corresponded to linear, singly branched, double branched, and more highly branched molecules in the dextrin, respectively. After debranching with isoamylase (Fig. 7), W. B. Merlin contained only the cluster with a peak DP of 12; the other dextrins were also predominantly DP 12, but also contained significant fractions up to DP 40, which could be attributed to resistant fragments of retrograded amylose. Retrograded amylose contains crystalline and amorphous regions, and the crystalline region is resistant to acidic and amylolytic hydrolysis (Jane & Robyt, 1984; Lu et al., 1997). Compared with potato and maize counterparts, barley amylopectins had larger proportions of α-1, 6-branch linkages located within the crystalline region, which were protected from acid hydrolysis (Jane et al., 1997). Potato Naegeli dextrin consists primarily of linear molecules, whereas normal and waxy maize Naegeli dextrins consist mainly of linear and singly branched molecules.

Thermal properties

The gelatinization onset temperatures of barley starches varied from 55.0 to 56.5°C (Table 3). In contrast to their maize starch counterparts, high amylose barley starches did not show significantly higher gelatinization temperatures than did W.B. Merlin and glacier barley starches. High-amylose maize V and VII starches both display much higher gelatinization onset temperature (71.0°C, and 70.6°C, respectively) than waxy and normal maize starch (64.2°C and 64.1°C, respectively) (Jane et al., In press). This difference could be attributed to the fact that high-amylose maize starches had much longer branch chain lengths than waxy and normal maize starches. The longer branch chains of high-amylose maize amylopectin developed into larger crystallites, which required higher temperatures to gelatinize. All the barley starch varieties had relatively short branch chain length. The shoulder of the branch chain length distribution (DP 18-21) (Fig. 5), equivalent to distances of 6.3 to 7.35 nm, which are in the proximity of the distance of the crystalline region of amylopectin (6.65 nm) (Cameron & Donald, 1992). The low proportion of DP 18-21, as shown in the shoulder, suggested a defective crystalline structure. The defective structure might be responsible for the very low gelatinization temperature of barley starch (Jane et al., 1999). Retrogradation of these starches after storage at 4°C for seven days was also analyzed by DSC. High amylose barley starches displayed higher retrogradation rates (Table 3), which could be due to the crystallization involving amylose molecules.

Pasting property

RVA amylographs of the barley starch varieties showed distinctive differences in their pasting properties (Fig. 8). W.B. Merlin barley starch had a higher peak viscosity (221 RVU, compared with 205 RVU of waxy maize), and a lower pasting temperature (61.8°C, compared with 69.5°C of waxy maize) (Jane et al., In press). A similar result was found in zero amylose hull-less barley starch (Zheng et al, 1998). Glacier barley starch had a pasting temperature at 90.8°C, a lower peak viscosity of 77 RVU, and a substantially higher setback of 61 RVU. Two high-amylose barley starches showed similar pasting temperatures and very low peak viscosities (~5 RVU) (Table 4). The characteristic difference between barley starch varieties can be attributed to their amylose and phospholipid contents. With little amylose and being primarily amylopectin (>90%), W.B. Merlin barley starch could swell more freely and develop large peak viscosity at a low pasting temperature. Glacier barley starch contained 29.5% absolute amylose and 0.048% phosphorus corresponding to 0.78% phospholipids. Phospholipids could form helical complex with amylose and restricted granule swelling to a lower peak viscosity at a substantially higher pasting temperature. The high-amylose glacier barley starch, containing 43.4-44.7% absolute amylose and 0.049-0.060% phosphorus corresponding to 0.79-0.97% phospholipids, displayed the lowest peak viscosity. The very low granule swelling and viscosity were attributed to low amylopectin contents and amylose-lipid complexes. Amylopectin is primarily responsible for granule swelling (Tester & Morrison, 1990).

CONCLUSION

Four varieties of barley starches consisted of different proportions of amylose contents (9.1% to 44.7%), and total phosphorus contents (0.022% to 0.068%) that were mainly from phospholipids (0.36% to 0.97%). All the barley starch varieties had short branch chain lengths. Unlike high-amylose maize starch, high-amylose barley starches, as well as other varieties in this study, had low gelatinization temperatures. W.B. Merlin barley starch had a high peak viscosity, whereas high-amylose barley starches had very low peak viscosity.

ACKNOWLEDGEMENTS

The authors thank Dr. C.W. Newman for providing the barley seeds; Mr. B. Wager and Ms. T. Pepper of Bessey facilities for SEM study; Dr. S. XU and Mr. D. Scott for NMR spectroscopy assistance; Ms. N. Morain for editorial assistance.

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Table 1. Amylose and phosphorus contents of barley starches

Sample	Iodine Affinity (%)		Amylose Content (%)		Phosphorus Content (%)			
	Starch	Amylopectin	Apparent[a]	Absolute[b]	Chemical Method	^{31}P-NMR Method		
					Total Phosphorus	Phospholipids	Inorganic phosphate	Total Phosphorus[d]
W.B. Merlin	1.82 ± 0.03	nd[e]	9.1	9.1	0.024	0.022 (0.36%)[c]	nd	0.022
Glacier	5.95 ± 0.01	0.08 ± 0.03	29.8	29.5	0.052	0.048 (0.78%)	nd	0.048
High Amylose Glacier	9.60 ± 0.42	1.20 ± 2.13	48.0	44.7	0.057	0.049 (0.79%)	0.002	0.051
High Amylose Hull-less Glacier	9.30 ± 0.43	1.10 ± 1.82	46.5	43.4	0.073	0.060 (0.97%)	0.008	0.068

[a] Apparent amylose content (%) = $IA_{starch} / IA_{amylose} \times 100\%$

[b] Absolute amylsoe content (%) = $(IA_{starch} - IA_{amylopectin}) / (IA_{amylose} - IA_{amylopectin}) \times 100\%$

[c] Data in the parenthesis were phospholipid contents. Phospholipid contents = Phosphorus content in the form of phospholipids × 16.16 (Morrison & Tester, 1992).

[d] Total phosphorus content was the sum of the phosphorus contents of phospholipids and inorganic phosphate.

[e] Not detectable.

Table 2. Summary of amylopectin structures of barley starches

Sample	Peak DP		Average	%Distribution					Highest
	I	II	CL	DP6-9	DP6-12	DP13-24	DP 25-36	DP≥37	Detectable DP
W.B.Merlin	12	48	24.2	5.3	21.6	43.0	16.1	19.2	67
Glacier	12	50	26.6	4.1	18.0	40.9	17.2	23.7	82
High amylose glacier	12	48	25.5	3.1	16.5	44.9	17.9	20.7	79
High amylose hull-less glacier	12	48	24.5	3.2	17.4	47.5	17.2	17.8	78

Table 3. Thermal properties of barley starches

Sample[a]	Native starch			Retrograded starch[c]			
	T_o (°C)	T_p (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	ΔH (J/g)	%Rt.
W.B. Merlin	55.4 ± 0.0[b]	60.3 ± 0.1	13.0 ± 0.2	38.3 ± 0.0	47.8 ± 0.0	3.1 ± 0.2	23.6
Glacier	55.0 ± 0.0	59.0 ± 0.0	9.2 ± 0.1	38.9 ± 0.5	48.7 ± 1.3	3.3 ± 0.2	35.6
High amylose glacier	55.5 ± 0.2	62.8 ± 0.0	7.7 ± 0.5	38.3 ± 0.6	49.2 ± 0.7	6.0 ± 0.1	78.5
High amylose hull-less glacier	56.5 ± 0.1	63.2 ± 0.0	7.3 ± 0.0	39.4 ± 0.2	49.7 ± 0.0	5.1 ± 0.1	70.1

[a] Samples (~2 mg, dry starch basis) and distilled water (~6 mg) were used for analysis

T_o, T_p, and ΔH = onset, peak gelatinization temperature, and enthalpy change, respectively

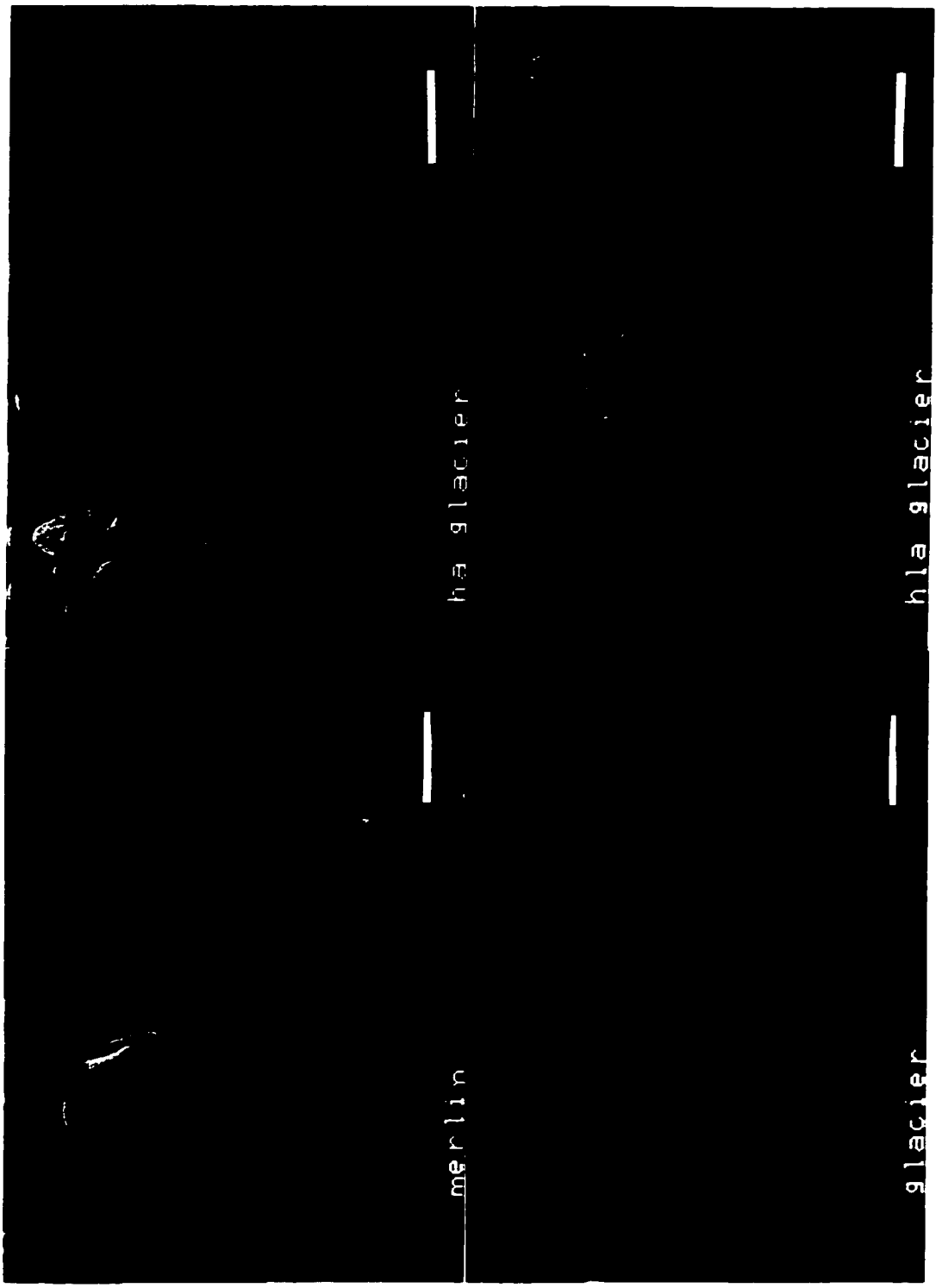
[b] Values were calculated from three replicates

[c] After storage at 4 °C for 7 days

Table 4. Pasting Properties of Starches

Sample[a]	Pasting Temp. (°C)	Peak Viscosity (RVU)	Final Viscosity (RVU)	Setback (RVU)
W.B.Merlin	61.8	221	102	32
Glacier	90.8	77	106	61
High amylose glacier	91.5	5	23	24
High amylose hull-less glacier	91.5	5	16	18

[a] Starch sample suspensions were 8%, w/w, on dry starch basis, in 28g total weigh.


marlin glacier
ha glacier
hla glacier
glacier

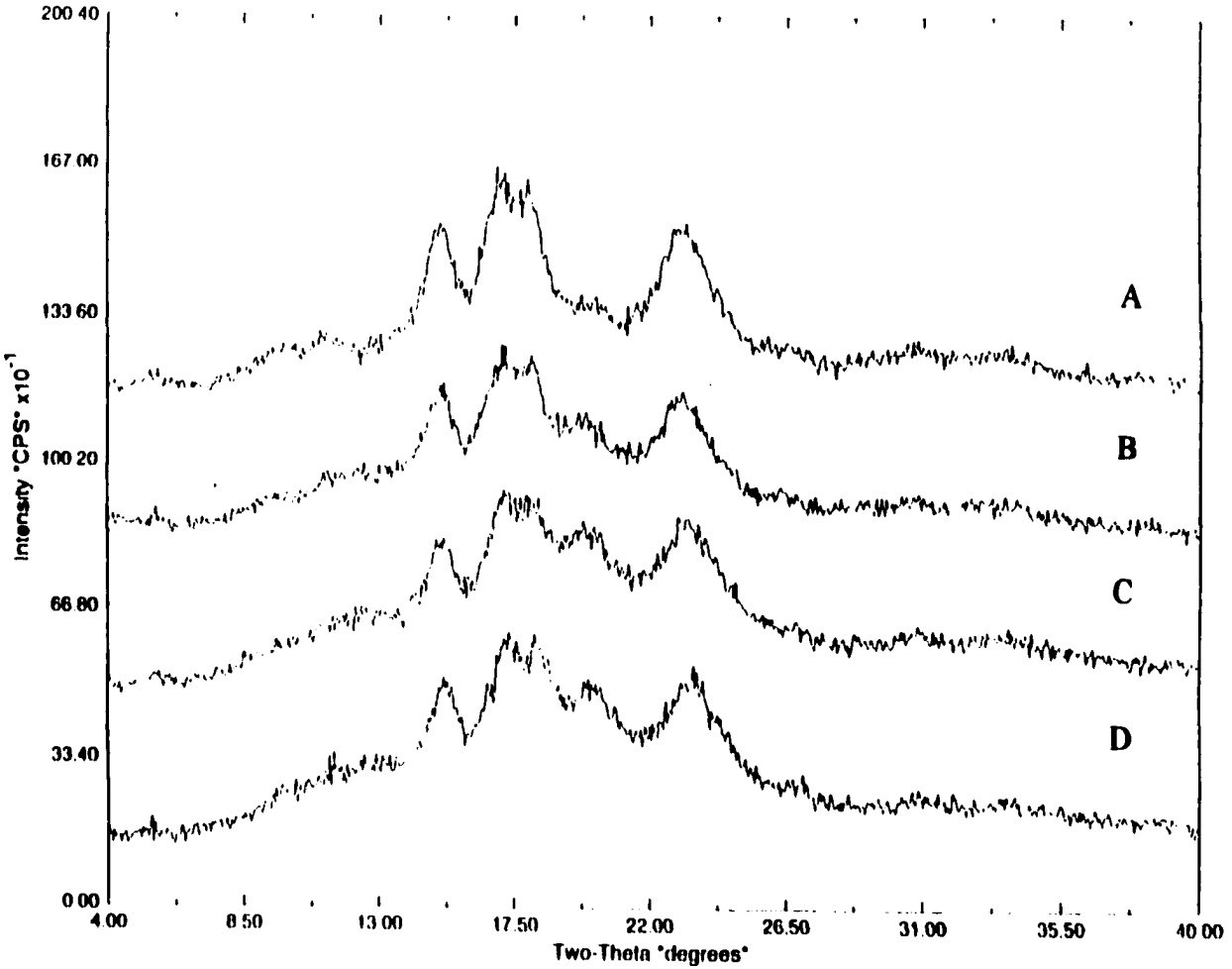


Figure 2. X-ray patterns of barley starches. A, W.B. Merlin; B, Glacier; C, High amylose glacier; D, High amylose hull-less glacier.

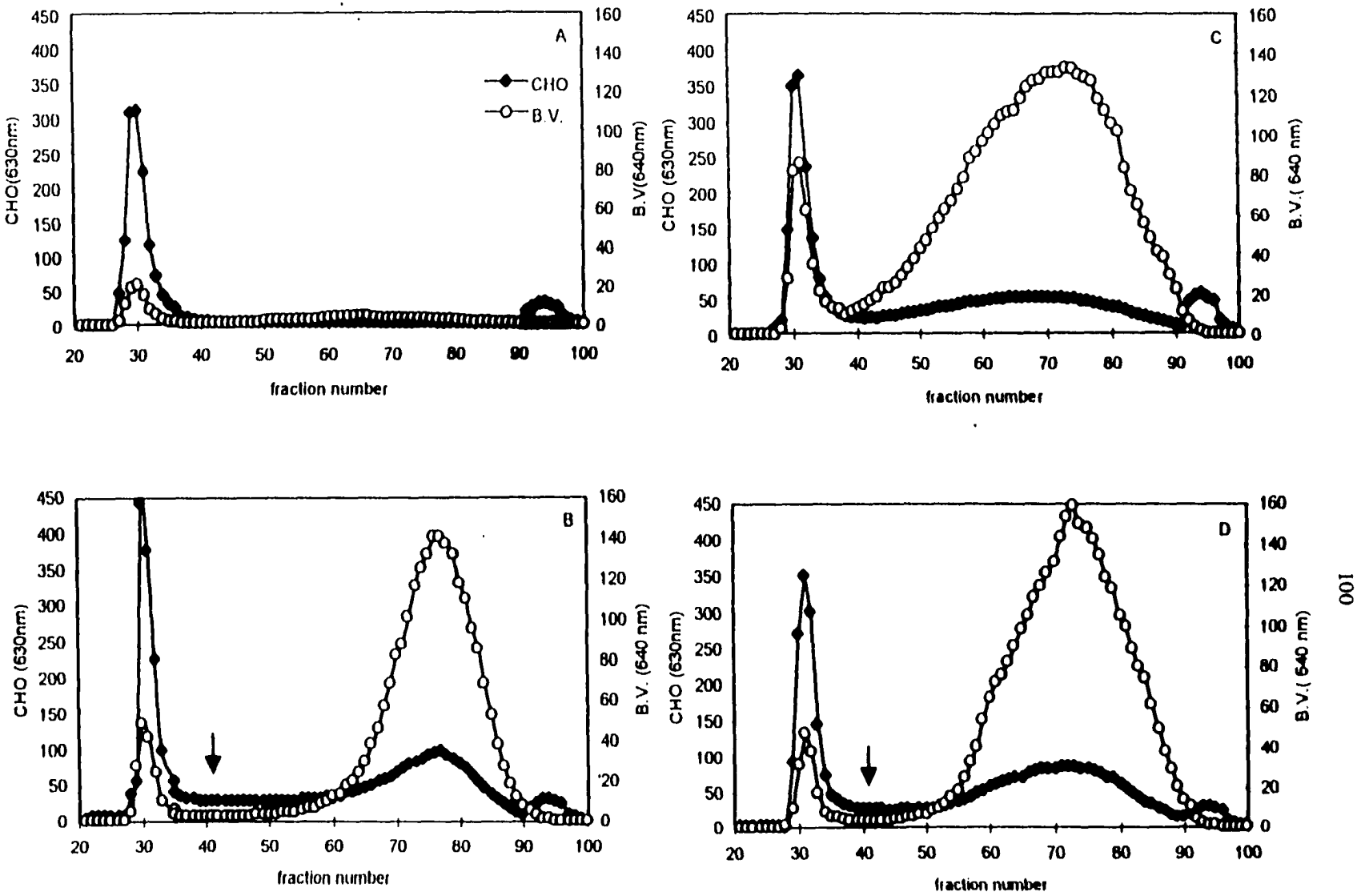


Figure 3. Sepharose CL-2B gel permeation chromatographic profile of barley starches.
CHO = total carbohydrate, BV = blue value.
A, W.B. Merlin; B, Glacier; C, High amylose glacier;
D, High amylose hull-less glacier. -♦-; total carbohydrate, -o-; blue value.

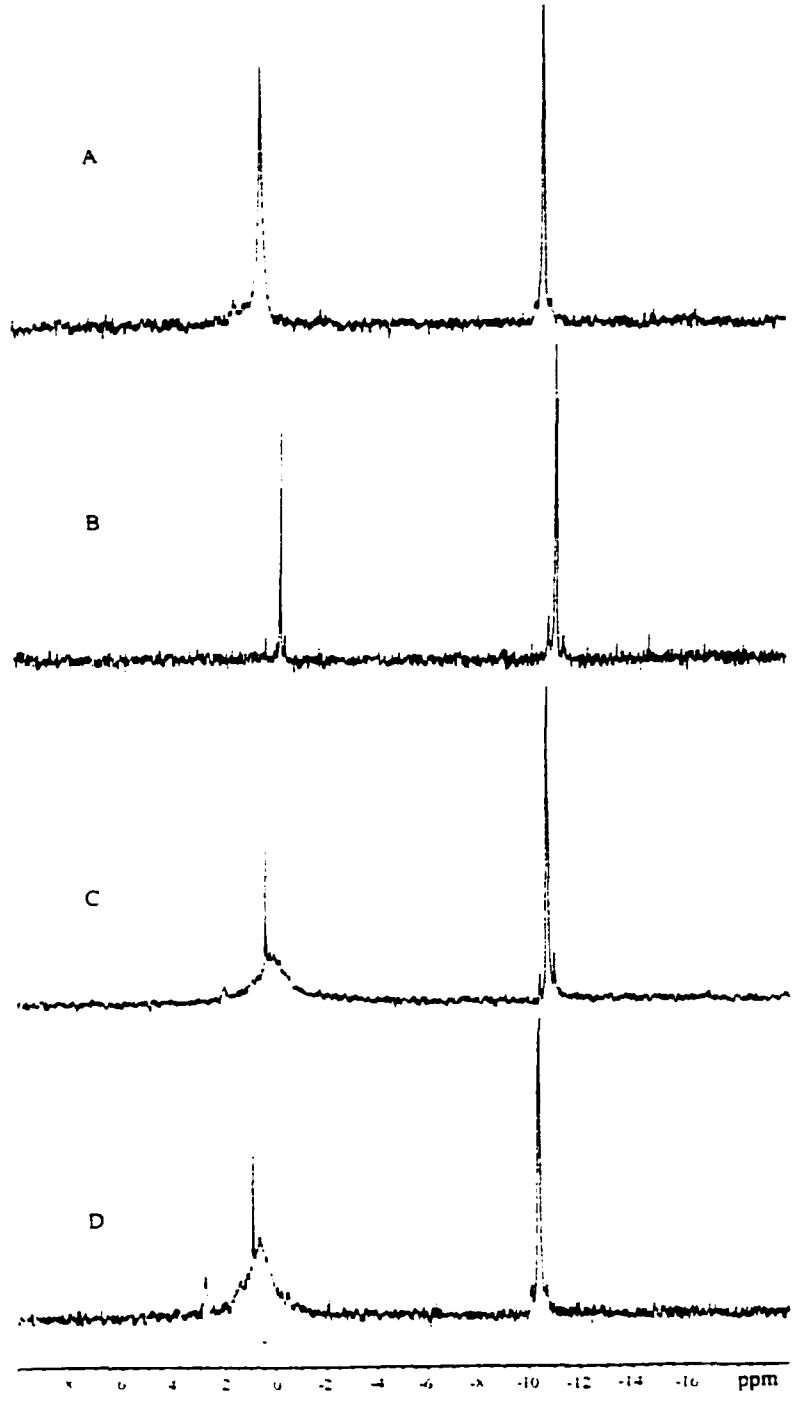


Figure 4. ^{31}P-nuclear magnetic resonance spectra of barley starches. A, W.B. Merlin; B, Glacier; C, High amylose glacier; D, High amylose hull-less glacier.

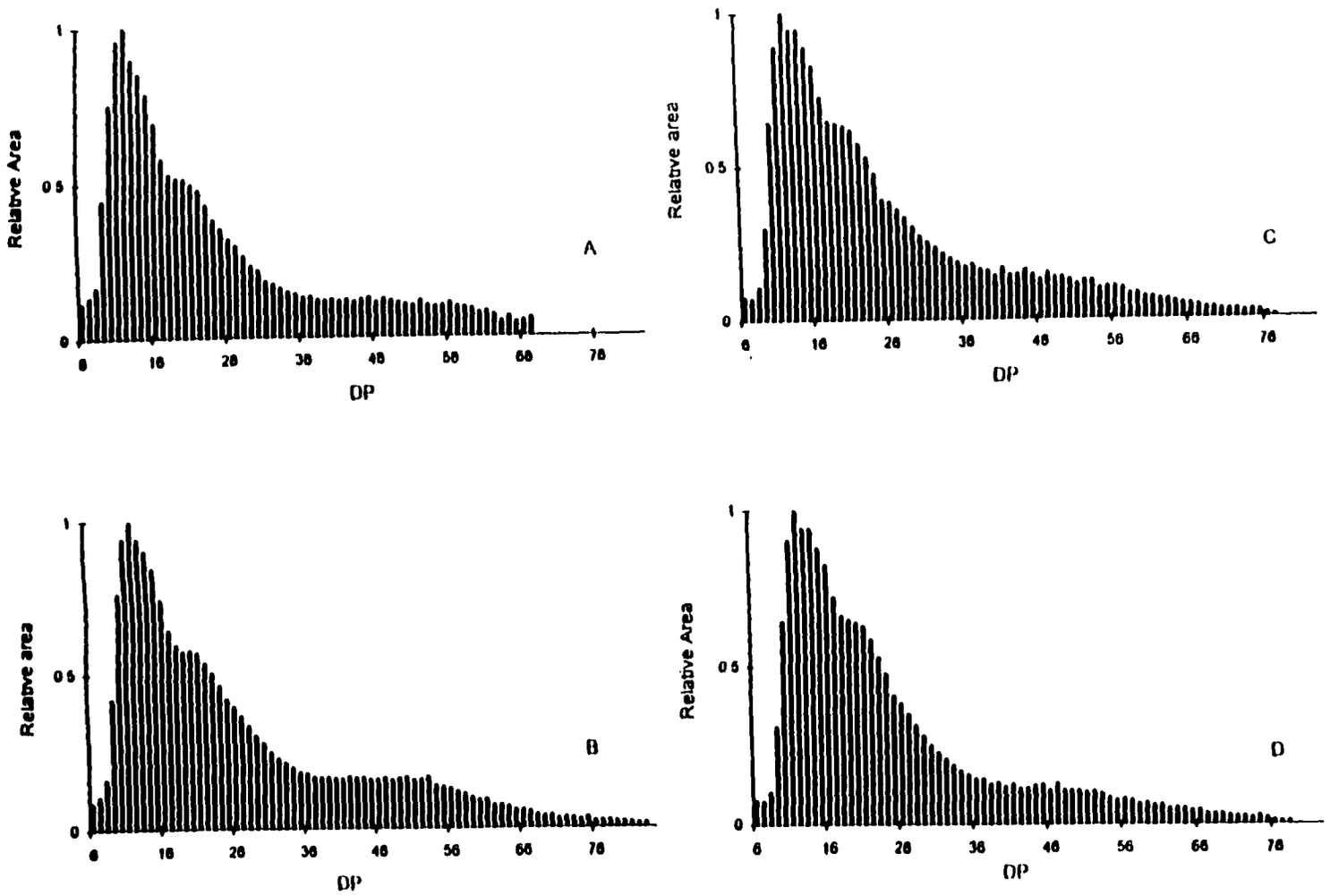


Figure 5. Branch chain-length distributions of barley amylopectins by HPAEC-ENZ-PAD. A, W.B. Merlin; B, Glacier; C, High amylose glacier; D, High amylose hull-less glacier.

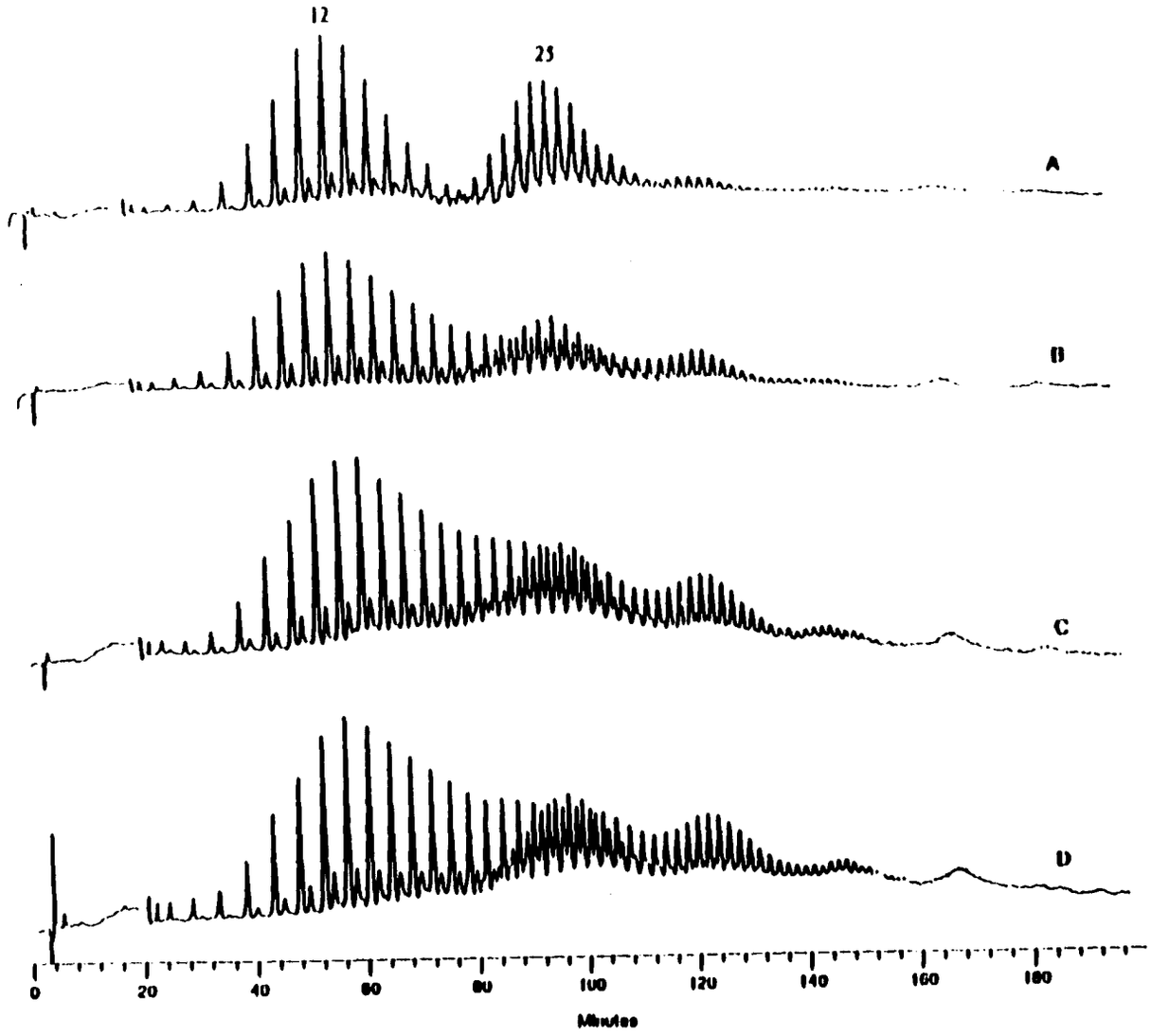


Figure 6. HPAEC-ENZ-PAD chromatograms of barley Naegeli dextrins (after 12 days hydrolysis). A, W.B. Merlin, B, Glacier; C, High amylose glacier; D, High amylose hull-less glacier.

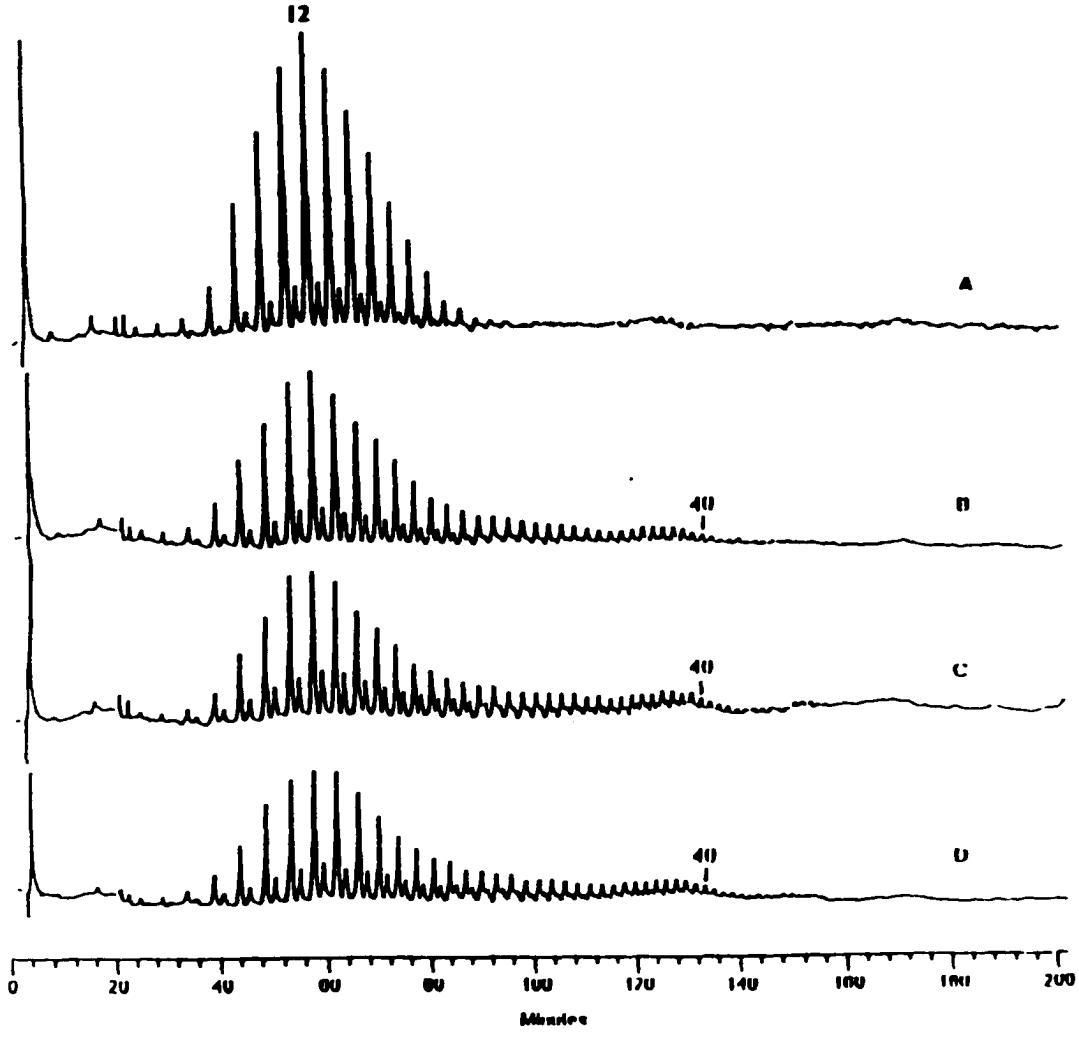


Figure 7. HPAEC-ENZ-PAD chromatograms of debranched barley Naegeli dextrins (after 12 days). A, W.B. Merlin; B, Glacier; C, High amylose glacier; D, High amylose hull-less glacier.

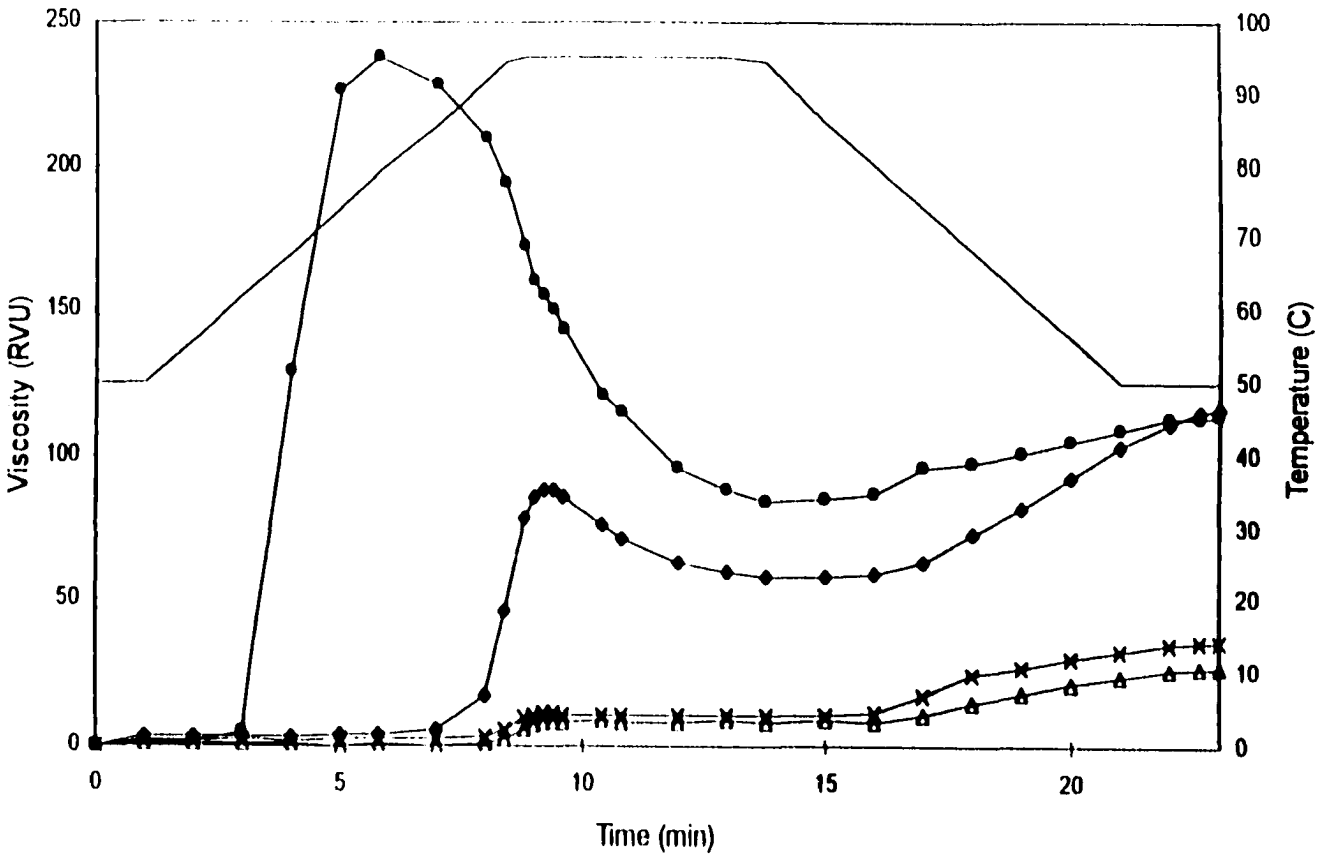


Figure 8. Pasting profile of barley starches measured by Rapid Visco-Analyzer. —, temperature; -●-, W.B. Mrelin; -♦-, Glacier; -×-, High amylose glacier; -▲-, High amylose hull-less glacier.

CHARACTERIZATION OF MECHANICAL PROPERTIES AND WATER ABSORPTION OF COMPRESSION-MOLDED SOY PREOTIN SPECIMENS[1]

A paper to be submitted to Industrial Engineering and Chemistry Research

Y. Song[2] and J. Jane[2,3]

ABSTRACT

Five commercial soy protein isolate samples and one pilot-plant-prepared soy protein isolate sample were studied for their efforts on mechanical properties and water absorption after compression molding. Molded Supro 760 specimens displayed better tensile strength than other isolate specimens. Molded EDI-Pro A specimens had the lowest water absorption among different soy products, and had better wet tensile strength than molded Supro 760. Pilot-plant-prepared protein isolate showed lower tensile strength and water absorption than did Supro 760. Scanning electron microscopy and transmission electron microscopy were used to reveal the surface structure of molded specimens, and the structure of protein aggregation, respectively.

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[1] Journal Paper No. J- #### of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA. Project No. ####.

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Introduction

In addition to being used as food ingredients, soy protein has also been used for industrial applications, such as paper coatings, plastics, and adhesives. Although an early patent on soy plastics was issued back in 1913, major developments in this field started in the 1930s to broaden the application of agricultural products (Johnson et al., 1992). Environmental and economic benefits inspired the chemurgic efforts again from late 1970s and early 1980s after most of the efforts were disbanded by 1955 (Johnson et al., 1992). Soy protein isolates and concentrates have been successfully demonstrated in manufacturing molded plastics, and the mechanical properties and water absorption of the molded specimens were reported (Paetau et al., 1994a and b). There is no soy protein product commercially available in the market place designated for soy plastics manufacturing. More efforts are needed to improve the properties of soy protein for plastic applications.

β-Conglycinin (7s) and glycinin (11s) are the two major storage protein globulins in soybeans. β-Conglycinin is a trimer with a molecular mass of 150-200 kDa. It contains three major subunits α' (72 kDa), α (68 kDa), and β (52 kDa) (Nielsen, 1985; Utsumi et al., 1997; Yamauchi et al., 1991; Wolf, 1970). Glycinin has a hexameric structure. It consists of acidic and basic subunits (~35 KDa and ~20 KDa, respectively), and the acidic and basic subunits are linked by a disulfide bond (Utsumi et al., 1997; Plietz et al., 1987). β-Conglycinin and glycinin have different properties due to their structure differences (Kinsella et al., 1985; Utsumi et al., 1997).

Objectives of this study were to investigate the mechanical properties and water absorption of compression-molded specimens made from different commercial soy protein isolate samples and from one pilot plant prepared soy protein isolate sample. Mechanical

and physical properties of the molded specimens were related to physical properties of protein raw materials.

Experimental Section

Materials. Commercial soy protein isolate samples (Supro 760, 710, 670, HD-90, EDI-Pro A) were purchased from Protein Technologies International (St. Louis, MO). Protein contents and pHs of the samples are shown in Table 1. One soy protein isolate sample was prepared in the pilot plant at the Center for Crops Utilization Reserarch, Iowa State University, following the procedures reported by Wu et al. (1998).

Specimen molding. Type I specimens (ASTM standard D638-92, dumbbell shaped, overall length of 165 mm) were made from 15.0 g of soy protein with 7% moisture by compression molding using a Wabash compression-molding machine (Wabash Metal Products, Inc., Wabash, IN). Compression molding was performed at 150°C and 10 MPa pressure for 12 min. After molding, the mold with specimen was cooled for 5 min before the specimen was removed from the mold. The edge of the specimen was carefully smoothed by sandpaper. Processing was replicated five times.

Mechanical properties. Tensile strength, elongation, and Young's modulus of the compression molded specimens were measured by using an Instron Model 4502 testing system (Canton, MA), following the standard test method for tensile properties of plastics (ASTM D638-92, 92). Specimens were equalibrated at 50% relative humidity for 48 hr before mechanical tests. Five specimens were analyzed.

Water absorption. A modified ASTM standard test method (D570-81, ASTM, 92) using bar-shaped specimens was used for water absorption tests of the specimens following the procedure of Peatau et al. (1994a).

Surface structure. The surface structures of the molded soy protein specimens and the specimens after submerging in water were examined by using scanning electron microscopy (JEOL JEM-35, Tokyo, Japan). Molded soy protein specimens were dried in the air for 3 hours after submerged in water for 24 hr. All samples were placed on silver tape attached to brass disks and sputtered with gold-palladium (60:40) for 3 min. Micrographs of the samples were taken at 500x magnification (Sun et al., 1999; Paetau, et al., 1994a).

Thermal properties of soy protein samples. Thermal properties of the soy protein isolate samples were analyzed by using a Perkin-Elmer differential scanning calorimeter DSC-7 (Norwalk, CT) equipped with an intracooling II system. About 10 mg of samples were weighed and sealed in stainless steel pans. The heating rate was 10°C per min.

Molecular weight of soy protein samples. Linear gradient urea-SDS-PAGE gels were prepared with equal volumes (16 ml) of 8% and 18% acrylamide solutions. The 8% acrylamide solution contained 1.5 M Tris-HCl (pH 8.8, 4.0 ml), urea (3.6 g), 10% SDS (0.16 ml), TEMED (10 µl), bis (acrylamide) (37.5:1, 3.2 ml), 10 % ammonium persulfate (100 µl), and deionized water (5.38 ml). The 18% acrylamide solution contained 0.38 M Tris-HCl (pH 8.8, 4.0 ml), urea (5.24 g), 10% SDS (0.16 ml), TEMED (10 µl), bis (acrylamide) (37.5:1, 7.2 ml), 10% ammonium persulfate (100 µl), and deionized water (0.93 ml). The

stacking gel was prepared by 4% acrylamide solution which contained 0.5 M Tris-HCl (pH 6.8, 2.5 ml), urea (1.65 g), 10% SDS (0.10 ml), TEMED (10 μl), bis (acrylamide) (37.5:1, 1.0 ml), 10% ammonium persulfate (100 μl), and deionized water (4.95 ml). The gel solutions were poured immediately after addition of ammonium persulfate to polymerize (Petruccelli and Anon, 1994; Wu et al., 1999).

Protein samples were dissolved in a protein extraction buffer solution that contained 50 mM Tris-HCl, 0.2% SDS, 5 M urea, and 0.2% 2-mercaptoethanol, and diluted with a sample buffer which contained 125 mM Tris-HCl (pH 6.8), 5 M urea, 20% (v/v) glycerol, 2% SDS, 0.4% bromophenol blue and 2% 2-mercaptoethanol. The gels were run at 135 v constant voltage for about 4.5 hr. Gels were stained with 0.22% Coomassie blue, and destained with the same solvent without Coomassie Blue (Wu et al., 1999).

Protein aggregation of soy protein samples. Negative staining was used for studying protein aggregation by using transmission electron microscopy. A drop of 5% soy protein solution was applied to a carbon-coated electron microscope grid, and the grid was floated on a top of 2% potassium phosphotungstate with 0.02% BSA. Excess liquid was drained off with a filter paper (Nakamura et al., 1984).

Results and Discussion

Specimens molded from Supro 760 displayed larger tensile strength, percentage elongation, and Young's modulus than specimens molded from other soy isolate proteins (Table 2). Specimens made from the pilot-plant-prepared soy isolate showed a good tensile strength next to Supro 760 specimens. Differences in water absorption were observed among

molded soy isolate specimens (Table 2). After being submerged in water for 24 hr, Supro 760 specimens displayed an average water absorption of 139%, which was significantly larger than EDI-Pro A specimens, having an average of water absorption of 28%.

Changes in color, shape, and strength of the molded specimens were observed after the specimens had been submerged in water for 24 hr. Supro 670 and 710 specimens showed similar phenomena of losing dark brown color, the shape and strength of the specimens. The specimens also showed separated layers. Supro 760 and HD-90 specimens displayed a slight color loss, but retained the shape and strength after submerging in water for 24 h and dried. In contrast, EDI-Pro A specimens retained the dark brown color, dumbbell shape, and 15% of original strength. The tensile strengths of Supro 760 and EDI-Pro A specimens after submerging in water for different periods of time were presented in Figure 1. EDI-Pro A specimens displayed better wet tensile strength than Supro 760 specimens. EDI-Pro A specimens retained 87, 60, and 15% of tensile strength and Supro 760 specimens retained 44, 17, and 4% of tensile strength after submerging in water for 6, 12, and 24 hr, respectively.

Significant differences in water absorption between EDI-Pro A specimens and Supro 760 specimens could be attributed to their pH difference (Table 1). EDI-Pro A specimens had pH of 4.3-5.0, close to the isoelectric point (4.5) of soy protein, whereas Supro 760 specimens had a neutral pH. The superior water resistance of EDI-Pro A specimens was in an agreement with the results of acid treatments of soy protein using different acids at pH close to the isoeletric point of the protein (Peatau et al., 1994). The minimal charge of protein at the isoelectric point contributed to the low water absorption.

Commercial soy protein isolate materials used in this study displayed a good processability. Supro 760, EDI-Pro A, HD-90, and pilot plant prepared soy isolate samples

displayed clear SDS-PAGE protein bands with different molecular weights, which indicated that those samples retained natural subunits of soy protein β-conglycinin and glycinin (Fig. 2). However, Supro 710 and Supro 670 samples did not show well separated bands, suggesting that these two protein samples did not have substantial amount of molecules with high molecular weights. It is supported by the fact that these two protein samples were hydrolyzed during manufacture. DSC results (Fig. 3) showed that EDI-Pro A sample (7.04% moisture) had obvious thermal transitions of β-conglycinin and glycinin at 145 and 169°C, respectively. The pilot-plant-prepared isolate sample (7.28% moisture) also showed two thermal transitions of β-conglycinin and glycinin at 135 and 161°C. No thermal transition peaks were observed in the other commercial soy protein isolate samples. Hermansson (1978) reported that pH affected thermal properties of soy protein dispersions (10%) by using DSC. Their results showed that two peaks were observed in the pH range 4-9, but only one peak was observed at low (2-3) and high (10) pH. Because the commercial soy protein products used in this study all had pH within the range of 4-9 (Table 2), the lack of thermal transition peaks suggested that most commercial soy protein samples were highly denatured during manufacture.

For polymers, molecular weight has a positive relationship with mechanical properties, especially tensile strength (Hall, 1985). Soy protein specimens made of Supro 710 and Supro 670 that had low molecular weight showed lower tensile strength than other specimens. The difference in pH between Supro 760 and HD-90 soy isolate samples may contribute to the different mechanical properties between their specimens. Higher mechanical properties of Supro 760 specimens may also be due to the higher protein content (Table 1), comparing to other protein samples. Thermal analysis results showed that

commercial soy isolate sample EDI-Pro A and that prepared in the pilot plant had obvious thermal transition peaks at 137°C and 163°C. At the processing temperature of 150°C (below the denaturation temperature of glycinin), EDI-Pro A and pilot-plant-prepared soy isolate sample might still partially possess the native conformation of protein molecules. This could be responsible for the low percentage elongation of specimens made from these two soy protein samples.

Scanning electron micrographs revealed that original specimens had smooth surfaces (Fig. 4a). After submerging in water for 24 h and dried, the Supro 760 specimen had a rough surface with obvious voids (Fig. 4b), but EDI-Pro A specimen displayed a flat surface with small voids (Fig. 4c). The pilot-plant-isolate specimen also showed a rough surface without voids (Fig. 4d). These results could be attributed to the fact that Supro 760 specimens absorbed more water than EDI-Pro A specimens; there was more water penetrated into Supro 760 protein matrix resulting in swelling. After drying, the matrix that absorbed large volume of water would lose the moisture resulting in the voids.

Transmission electron micrographs (Fig. 5) showed that Supro 760 and pilot-plant-prepared isolate samples had different patterns of protein aggregation in aqueous solution. Pilot-plant-prepared isolate displayed a continuous and more evenly dispersed phase, but Supro 760 sample displayed a discontinuous phase with more densed aggregates. During compression molding process, the dispersed pilot-plant-prepared isolate protein molecules were more easily molded into homogeneous specimens, which contributed to the uniform surface structure of water submerged specimens, compared with Supro 760 sample. Highly aggregated Supro 760 molecules produced less homogenous specimens. This might result in the large voids shown in SEM results after drying.

Conclusions

Commercial soy protein isolate products displayed abetter processability than the pilot-plant-prepared counterpart. Most of commercial soy protein isolate products were denatured except EDI-Pro A. Pilot-plant-prepared isolate sample was less denatured. Molded specimens made from soy protein isolate Supro 710 and 670 showed poor mechanical properties. Molded Supro 760 specimens had better tensile strength than specimens made from other soy isolate materials. EDI-Pro A specimens showed the lowest water absorption among all the specimens in the study. EDI-Pro A specimens with pH close to isoelectric point had a better wet strength than Supro 760 specimens.

Acknowledgement

The authors thank Dr. L. Johnson and Dr. P. Murphy 's group for providing the pilot plant protein samples, the United Soybean Board for financial support of this research, Dr. A. Fratzke for assisting in SDS-PAGE analysis and Ms. T. Pepper of Bessey facilities for SEM and TEM analysis.

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Table 1. Protein content and pH of soy protein isolate samples

Sample	Protein Content (%)	pH
Supro 760[a]	93	7.0[c]
Supro 710[a]	87	6.9-7.2
Supro 670[a]	87	7.3-7.7
HD-90[a]	90	6.2-6.6
EDI-Pro A[a]	92	4.3-5.0
Pilot plant prepared soy isolate sample[b]	90	6.8

[a] Sample information were provided by Protein Technologies International Company, St. Louis, MO.
[b, c] Samples were tested in the lab.

Table 2. Mechanical properties of different soy protein isolate specimens

Sample[a]	Elongation (%)	Tensile Strength (MPa)	Young's Modulus (MPa)	Water Absorption (%)
Supro 760	8.9±3.7	29.9 ± 4.0	793 ± 125	139.3 ± 12.0
Supro 710	4.1 ± 1.5	14.0 ± 1.8	410 ± 86	83.0 ± 3.5
Supro 670	6.9 ± 0.7	14.7 ± 1.1	393 ± 42	115.9 ± 4.0
HD-90	1.5 ± 0.2	16.2 ± 2.8	632 ± 50	89.0 ± 7.0
EDI-Pro A	3.0 ± 1.1	16.0 ± 4.5	445 ± 100	28.3 ± 0.5
Pilot plant isolate	3.9 ± 0.3	22.7 ± 2.5	610 ± 27	128.1 ± 4.2

[a]Sample were compression-molded at 150°C and 10 MPa for 12 min.

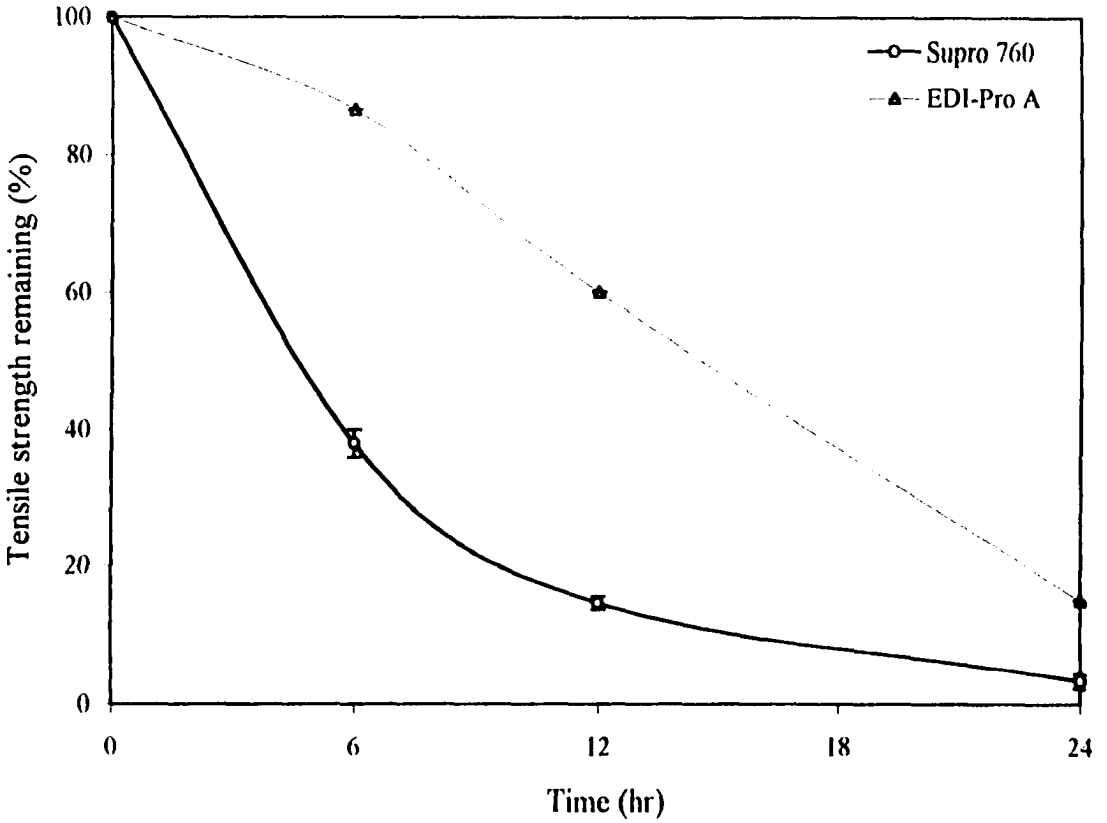
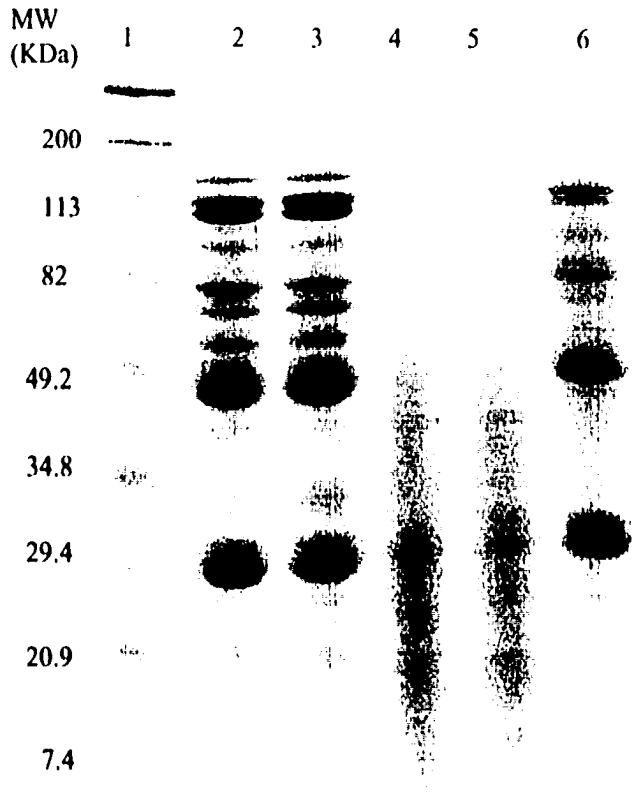
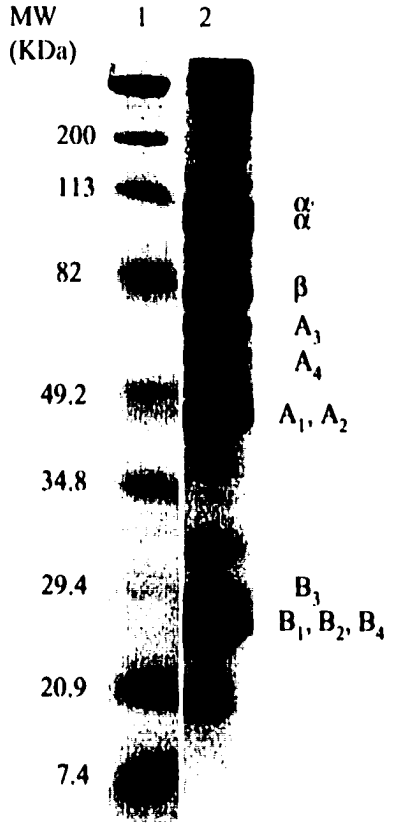


Figure 1. Effect of submerging time in water on the remaining tensile strength of compression molded soy isolate specimens


MW
(KDa)
1
2
3
4
5
6
200
113
82
49.2
34.8
29.4
20.9
7.4

(A)


MW
(KDa)
1
2
200
113
82
49.2
34.8
29.4
20.9
7.4
α
α
β
A3
A4
A1, A2
B3
B1, B2, B4

(B)

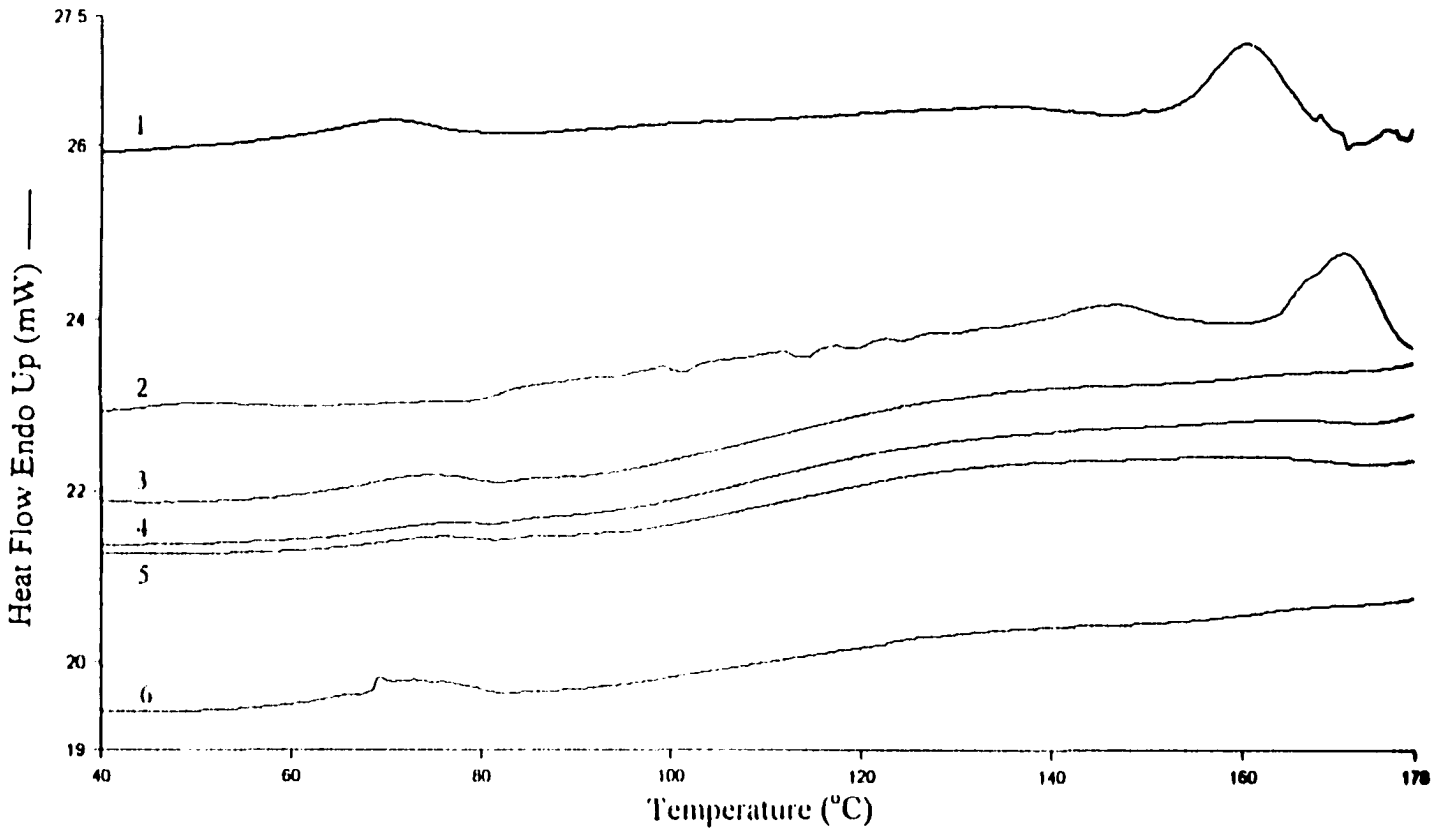
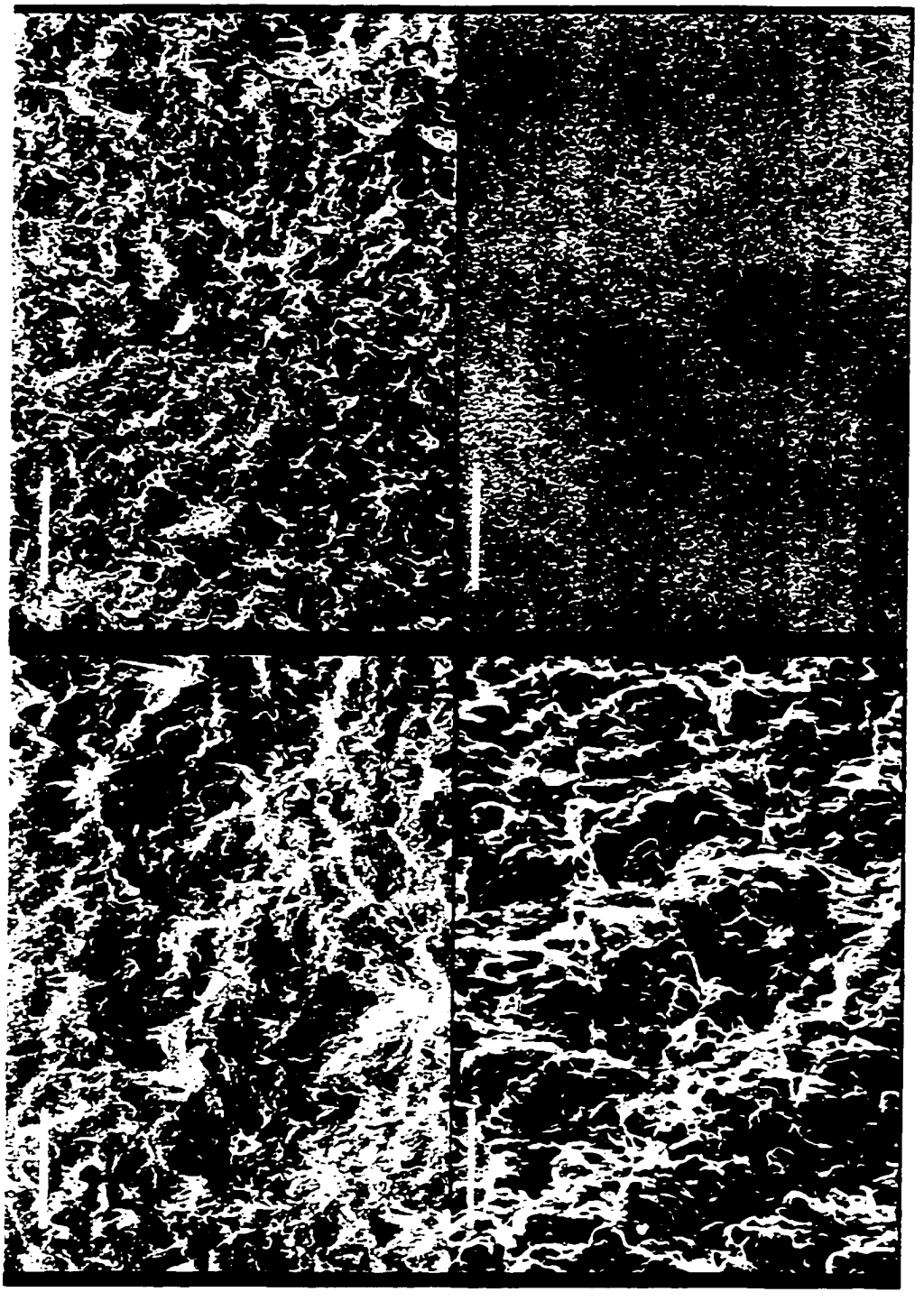
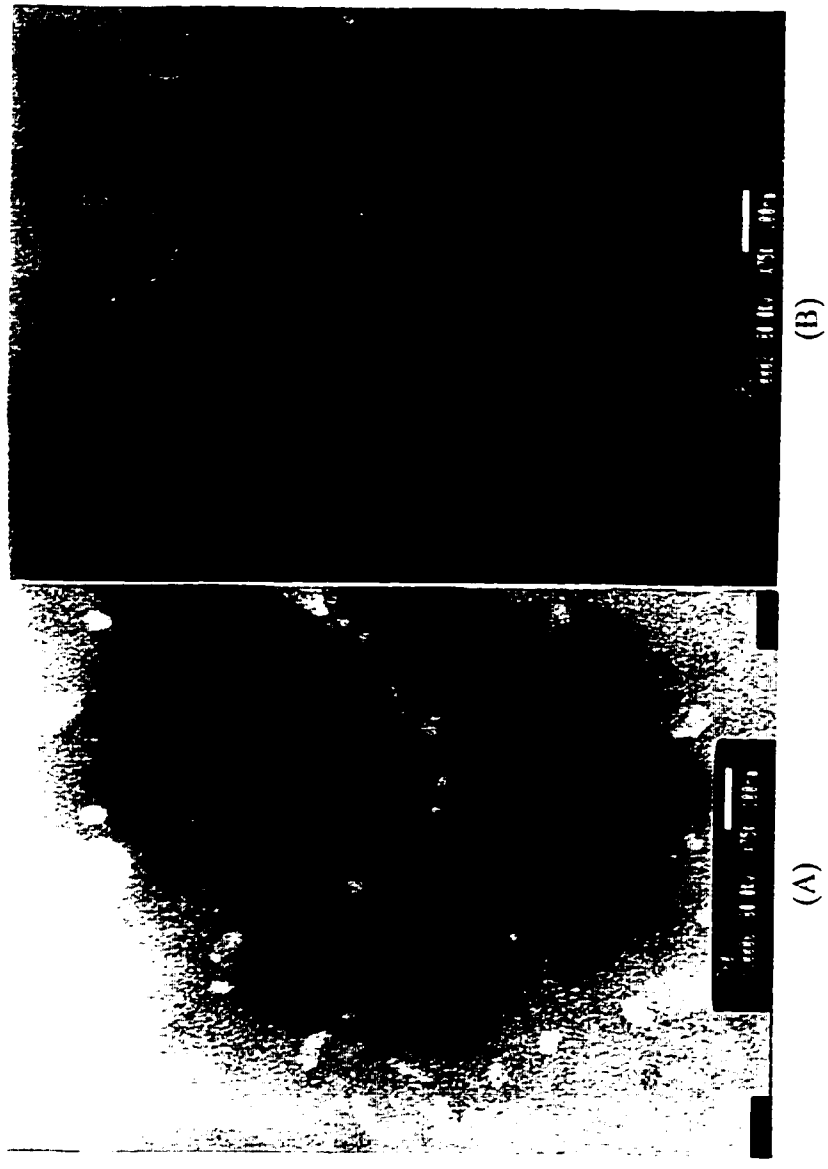


Figure 3. DSC thermograms of commercial soy protein isolate and pilot plant isolate. 1. Pilot plant isolate; 2. EDI-Pro A; 3. HD-90; 4. Supro 710; 5. Supro 670; 6. Supro 760. Moisture content was 7%




(A)
(B)

EFFECT OF SOY PROTEIN ON PASTING PROPERTIES OF DIFFERENT STARCHES[1]

A paper to be submitted to Carbohydrate Polymers

Y. Song[2] and J. Jane[2,3]

ABSTRACT

Soy protein isolate and soy protein fractions of β-conglycinin (7s) and glycinin (11s) were used to study their effects on the pasting properties of normal maize, waxy maize, and potato starches by using a Rapid Visco Analyser. Effects on the viscosity profiles varied with starch variety, type of protein, and concentration of protein in the mixture. Pasting temperature decreased when soy protein was added to a normal maize starch dispersion; however, pasting temperatures increased when soy protein was added to a mixture of waxy maize or potato starch. When the protein concentration increased from 2 to 8% in the mixtures of starch and protein, peak viscosity and final viscosity increased. Salt

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[1] Journal Paper No. J- #### of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA. Project No. ####.

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residues in soy protein reduced the viscosity of the mixtures containing potato starch, which was attributed to the suppression of charge interactions. Setback viscosities of the mixtures of soy proteins and starches were increased.

INTRODUCTION

Starch and protein are two major food biopolymers. They contribute to the texture and stability of food products through gelling, thickening, and other physicochemical properties (Tolstoguzov, 1991). Interactions between starch and protein play important roles in determining texture and quality of food products. Protein-polysaccharide interactions have been intensively reviewed by many researchers (Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998; Tolstoguzov, 1997; Dickson & McClements, 1995; Tolstoguzov, 1991). Attraction and repulsion are the two major inter-biopolymer interactions (Tolstoguzov, 1997). Interactions between proteins and polysaccharides could result in co-solubility, incompatibility, or complexing. In mixed solutions, proteins and polysaccharides are in either stable or phase-separated states in single-phase systems and two-phase systems (Tolstoguzov, 1991).

Interactions of corn starch with casein and casein hydrolysates were studied by using a Brabender amylograph (Goel, Singhal, & Kulkarni, 1999). Results showed that the pasting temperature of corn starch decreased after casein was added to the system. Hermansson (1979) patented protein (casein or caseinate) and starch complexes. A complex formation starts when starch molecules collide with caseinate molecules. Properties of protein and starch complex are significantly different from starch alone. Different proteins (zein, gliadin, glutelin, and glutenin) were used to study the effect of protein on rheological properties of

starches with various amylose contents (Chedid & Kokini, 1992; Madeka & Kokini, 1992). Interaction between starch and protein increase viscosity once a threshold temperature is exceeded. The amylose-amylopectin ratio, the type of protein and moisture content affect the level of increase in viscosity. Effect of *amaranthus* and buckwheat proteins on the rheological properties of maize starch was studied by using a Rapid Visco Analyser and an oscillatory rheometer (Bejosano & Corke, 1999). With the addition of protein concentrates, an increase in peak viscosity, a decrease in pasting temperature, and a weakened starch gel structure were obtained. Rheological properties of heated corn starch and soy proteins were investigated by using a rheometer (Chen, Liao, Okichukwu, Damodaran, & Rao, 1996; Liao, Okichukwu, Damodaran, & Rao, 1996). It has been reported that corn starch and β-conglycinin fraction (7s) of soy protein dispersions are classified as weak gels, whereas the viscoelastic behavior of corn starch and glycinin fraction (11s) of soy protein dispersions was as the behavior of true gels. Soy protein isolate dispersion showed gel-like behavior.

In this study, soy protein isolate and soy protein fractions (β-conglycinin and glycinin) were used to study effects of soy protein on pasting properties of starch. Different types of starches, normal maize, waxy maize, and potato starch, were used to investigate effects of starch structures on the interaction with soy protein.

MATERIALS AND METHODS

Materials

Normal maize starch and potato starch were purchased from Sigma Chemical Co. (St. Louis, MO). Waxy maize starch was a gift from Cerestar, USA (Hammond, IN). Soy protein isolate and soy protein fractions, glycinin (11s) and β-conglycinin (7s), were prepared

from the pilot plant at the Center for Crops Utilization and Research, Iowa State University (Wu, Murphy, Johnson, Fratzke, & Ruber, 1999)

Thermal properties

Thermal properties of starches (normal maize, waxy maize, and potato starch) and soy proteins (soy isolate, β-conglycinin, and glycinin) were determined by using a differential scanning calorimeter (DSC-7, Perkin-Elmer, Norwalk, CT) equipped with an intracooling II system. Twenty-five μl samples of starch dispersions (8%) and protein dispersions (8%) were sealed in stainless steel pans for analysis. The heating rate was 10°C per min over the temperature range of 25-100°C. Indium and zinc were used as the reference standards. Peak temperature range was measured and calculated by using Pyris software (Perkin-Elmer, Norwalk, CT). The data were averages of a minimum of duplicates of each analysis.

Pasting properties

Pasting profiles of starch, protein, and mixtures of starch and protein were determined by using a Rapid Visco Analyser (RVA) (Newport Scientific, Sydney, Australia). Each suspension contained 8% starch (dsb, w/w) in a total weight of 28g. Mixtures of starch and protein were prepared with three protein concentrations (2, 4, and 8%) and one starch concentration (8%). Each suspension was equilibrated at 50°C for 1 min, heated at a rate of 6°C/min to 95°C, held at 95°C for 5 min, and then cooled to 50°C at a rate of 6°C/min. A

rotating speed of the paddle of 960 rpm was used at the first 10 seconds, and a speed of 160 rpm was used through the analysis. Each sample was tested in triplicates.

Desalting of protein by dialysis

The dialysis tube was presoaked in deionized water for 10 min. Protein dispersion in the tube was placed in a beaker containing deionized water and a stir bar. Water was circulated with stirring and was changed with fresh water every three hours. The protein dispersion was dialyzed at 5°C for 24 hr and freeze-dried in a Virtis freeze-dryer (The Virtis, Inc., Gardiner, NY).

Salt content

Salt contents of protein samples with and without desalting were measured by following AOCS official method Bc 5-49 (1998). Protein samples (1~2g) in crucible was heated at 600°C for 2 hr, then ash residues were weighed after being cooled to room temperature in a desiccator. Salt content was calculated by mass of ash/mass of sample. Samples were tested in duplicates.

RESULTS AND DISCUSSION

Figure 1 shows viscosity profiles of soy protein dispersions (8%). A sharp peak (94.5°C) was observed in β-conglycinin (7s) dispersion and the viscosity remained at 2.6 RVU through the test. Glycinin (11s) dispersion displayed an increase in viscosity at 95°C and during cooling. The soy isolate dispersion had a higher viscosity than the other two fractions alone, and showed a sharp increase in viscosity at the same temperature (94.5°C) as

β-conglycinin dispersion reached its peak viscosity. The soy protein isolate dispersion (8%) had two thermal transition peaks at 75.9°C and 94.2°C (Table 1). The first peak (75.9°C) was attributed to the denaturation of β-conglycinin and the second peak was that of glycinin (Hermansson, 1978).

The viscosity profile of potato starch was significantly changed when soy proteins were added to the system (Fig. 2). The pasting temperature of the starch and protein mixture was higher than that of potato starch alone, and the peak viscosities of potato starch (8%) and soy protein (2% and 4%) mixtures were substantially lower than that of potato starch (8%) alone. Setback viscosity of the mixtures of potato starch and soy proteins increased when protein concentration increased. In the mixture of potato starch and β-conglycinin, two broad peaks at different temperatures, which had a lower peak viscosity than potato starch alone, were observed at protein concentration of 2% and 4%. The mixture of potato starch (8%) and β-conglycinin (8%) displayed a higher peak viscosity at higher temperature than potato starch (8%) alone (Fig. 2A). When glycinin concentration was at 2%, a broad peak with peak viscosity of 234.9 RVU was observed in the mixture (Fig. 2B). As glycinin concentration increased to 4% and 8%, the mixtures displayed a shoulder at low temperature (80°C) and a major peak at 95°C. The mixture of potato starch and soy protein isolate displayed a higher peak temperature than did potato starch, and its peak viscosity increased as soy isolate concentration increased from 2% to 8%. A splitted peak was observed at soy isolate concentration of 8%.

It is known that potato starch has high peak viscosity because potato starch contains high phosphate monoester (0.089%, dry basis) and no detectable lipids and phospholipids

(Lim et al., 1994; Jane et al., 1999). The negative charges of phosphate groups repel one another and enhance potato granule swelling. Potato starch swells faster and reaches its peak viscosity at lower temperature than soy proteins.

It has been reported that salt has a significant effect on peak viscosity of potato starch. At a low concentration of 0.04%, NaCl significantly reduced the peak viscosity of potato starch but not normal maize and waxy maize starches (Paterson, Mathtashim, Hill, Mitchell, and Blanshard, 1994; Muhrbeck and Eliasson, 1987). Viscosity decreased with the presence of salts in potato dispersion. There was no significant effect of salt on pasting temperatures of starch dispersions. The salt contents of β-conglycinin, glycinin, and soy isolate were 8.2, 7.1, and 4.2%, respectively. Sodium ions formed cation-ion layer on phosphate derivatives of potato starch and retarded the charge repelling between phosphate groups, which decreased viscosity. Peak viscosity of potato starch varied according to the salt concentration (Fig. 3). In order to study protein effect on pasting profile of potato starch, protein samples were dialyzed to remove salt. After dialysis, the salt content of soy isolate sample reduced to 1.7%. Pasting profiles of mixtures of potato starch with soy protein isolate with and without desalting are shown in Figure 4. Desalted soy protein produced higher peak viscosity and lower peak temperature compared with its counterparts without desalted soy protein isolate samples. The peak viscosities of potato starch mixtures containing desalted protein at 2% and 4% were lower than that of potato starch (8%) alone. This might be attributed to that potato starch contains 16.9% amylose (Jane et al., 1999). Formation of helical complexes between amylose and protein might have retarded the swelling of potato starch in the mixtures and caused the decrease in peak viscosity and the increase in pasting temperature of the mixtures.

The viscosity profiles of mixtures of protein (β-conglycinin, glycinin, and soy protein isolate) and waxy maize starch are shown in Figure 5A, 5B, and 5C, respectively. Pasting temperatures of the mixtures of waxy maize starch and soy protein were slightly higher than that of waxy maize starch alone. Peak viscosity, final viscosity and setback viscosity increased with the increase of soy protein content in the mixture. Slight increases in peak viscosity at protein concentration of 2% and 4%, and significant increase of peak viscosity at protein concentration of 8% were observed in mixtures of 8% waxy maize starch with β-conglycinin and with soy protein isolate. There were no significant peak viscosity changes observed in the mixtures of glycinin and waxy maize starch as glycinin concentration increased. In addition to the peak of waxy maize starch at 80°C, there was another peak appeared at 95°C in the mixture of waxy maize starch (8%) and glycinin(4% and 8%), and in the mixture of waxy maize starch (8%) and soy protein isolate (8%).

Because there was no amylose (Jane et al., 1999) and no phospholipids found in waxy maize starch (Lim, Kasemsuwan, & Jane, 1994; Kasemsuwan & Jane, 1996), there was no interaction between amylose and lipid and between amylose and protein in the mixtures. Waxy maize starch could easily swell and reached the peak viscosity at a lower temperature than swelling temperature of soy proteins. The second peak in the RVA profiles (Fig. 5B and 5C) appeared at 95°C coincided with the swelling temperature of glycinin. Because of the high swelling temperature of glycinin and without an interaction with amylose, it displayed a separate peak instead of merging with the pasting peak of waxy maize starch, as β-conglycinin did. Pasting temperatures of soy protein and waxy maize starch mixtures were mainly contributed by waxy maize starch.

Normal maize starch and soy protein mixtures displayed different pasting profiles (Fig. 6A, 6B, and 6C) from the mixtures of soy protein and the other two starches. Onset pasting temperatures of the mixtures of soy protein and normal maize starch were all lower than that of normal maize starch alone. Peak pasting temperature (95°C) of normal maize starch (8%) and glycinin (8%) mixture was higher than that (93°C) of normal maize starch (8%) and β-conglycinin (8%) mixture and that (90°C) of normal maize starch (8%) and soy protein isolate (8%). The viscosities of the protein samples (8%) were substantially lower than that of normal maize starch (8%, dsb). The viscosity profiles of the mixtures changed with the concentration of soy protein in the mixture. Mixtures of protein (2%) and starch (8%) displayed lower peak viscosities, lower onset and peak pasting temperatures, less shear thinning, and higher final viscosity than normal maize starch alone. The peak viscosities of the mixtures of protein (2%) and starch (8%) were 111.4, 120.7, and 120.0 RVU for mixtures containing β-conglycinin, glycinin, and soy isolate, respectively, which were lower than that of normal maize starch alone (8%, 151.3 RVU). No shear thinning was observed for mixtures of normal maize starch (8%) with β-conglycinin and with glycinin (4%). The peak viscosity of normal maize starch (8%) and soy isolate (4%) mixture (279.5 RVU) was higher than that of the mixture with β-conglycinin (165.0 RVU), and with glycinin mixture (204.2 RVU). Viscosities of the mixtures of protein (8%) and normal maize starch (8%) were significantly higher; 650.8 RVU, 485.8 RVU, and 605.5 RVU, for the mixture with β-conglycinin, glycinin, and soy isolate, respectively. Viscosities of starch and β-conglycinin mixtures were higher than that of starch and glycinin mixture counterpart (Fig. 6A and 6B).

Normal maize starch consists of 22.5% amylose (Jane, Chen, Lee, McPherson, Wong,

Rodosavljevic, & Kasemsuwan, 1999) and phospholipids and free fatty acids (Kasemsuwan & Jane, 1996; Morrison, Tester, Snape, Law, & Gidley, 1993). When soy proteins were mixed with normal maize starch, soy protein, as an emulsifier, could interact with and remove the lipids to prevent amylose-lipid complex formation, thus facilitated swelling of starch granules. Consequently, pasting temperatures of normal maize starch and protein mixtures were lower than that of normal maize starch alone. The result was in an agreement with the pasting result of normal corn and low-lipid corn reported by Takahashi and Seib (1988). Low pasting temperature was observed in the corn starch dispersion with lipid removed. Addition of 2% soy protein to 8% normal maize starch dispersion decreased the viscosity. This might be attributed to the interaction of amylose and protein molecules through hydrophobic interaction. Amylose could form a helical complex with protein, which retarded the swelling of starch granules. As the protein concentration increased to 8%, the viscosities of mixtures increased and were dramatically higher than the sum of viscosity of starch and of protein alone, which indicated formation of a network between starch and protein. Heating starch in water results in hydration of starch granules and swelling of granules.

According to the characteristics of the three starches and pasting results observed, a schematic diagram of pasting process of starch and protein mixture is proposed in Figure 7. With the addition of proteins, protein-lipid interaction facilitated starch swelling. As temperature reaches to starch gelatinization temperature, starch crystallites melt, granules swell dramatically, amylose leaches out of granule and viscosity of starch dispersion increases (French, 1984). As temperature reached protein denaturation temperature, protein molecules started unfold and more buried amino acid side chains could interact with water

and facilitated protein solubility. The dispersed starch molecules then formed three dimensional network matrix with denatured protein molecules, therefore the peak viscosity and final viscosity were significantly increased. It is suggested by results of amino acid composition (Nielsen, 1985) that β-conglycinin has larger proportions of amino acids with polar side chains than glycinin. After gelation, β-conglycinin displayed a lower hydrophobicity and higher solubility than did glycinin (Song and Jane, 1998a, and b). It resulted in a better compatibility between β-conglycinin and normal maize starch than between glycinin and normal maize starch. Therefore, viscosity of the mixture of β-conglycinin and normal maize starch was higher than that of the mixture of glycinin and normal maize starch counterpart (Fig. 6A and 6B).

CONCLUSION

Viscosity profiles of soy protein and starch mixtures differed from that of starch alone. Peak viscosity of mixtures of starch (8%) with soy protein (8%) significantly increased except the mixture of waxy maize starch and glycinin. High swelling temperature of soy protein, especially glycinin, caused the separated viscosity peaks in the pasting profiles of mixtures of waxy maize starch and glycinin, and of mixtures of potato starch and soy protein. Salt residue in soy protein decreased the viscosity of potato starch and soy protein mixtures. Interaction between amylose and soy protein retarded starch granule swelling and caused the decreased peak viscosity at low protein concentration in the mixtures containing normal maize starch or potato starch, and increased the pasting temperature of potato starch and protein mixtures. Protein-lipid interaction could facilitated starch granule swelling. Pasting temperature decreased when soy proteins were present in normal maize

starch dispersion. Mixtures of starch and soy protein displayed higher setback viscosity than starch alone. Viscosity of mixtures of starch and soy proteins significantly increased compared with soy protein dispersion alone.

ACKNOLEDGEMENTS

The authors thank Dr. Johnson and Dr. Murphy groups to provide soy protein samples.

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Table 1. Thermal properties of soy protein dispersions and starch dispersions

Sample	Peak I				Peak II			
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
β-Conglycinin (7s)	72.9	77.5	83.7	3.8	89.8	94.4	97.8	1.3
(8%)	(1.0)[a]	(0.5)	(0.8)	(0.5)	(0.3)	(0.3)	(0.7)	(0.1)
Glycinin (11s)	nd[b]	nd	nd		83.1	92.5	98.4	12.5
(8%)					(0.4)	(0.0)	(0.3)	(2.1)
Soy isolate	71.3	75.9	79.1	0.8	86.7	94.2	98.9	6.3
(8%)	(0.7)	(1.2)	(0.1)	(0.1)	(1.5)	(0.5)	(0.3)	(0.5)
Normal maize starch	66.9	70.5	75.7	16.3	81.8	86.2	90.7	2.5
(8%)	(0.1)	(0.2)	(0.7)	(2.0)	(0.6)	(0.0)	(0.4)	(0.2)
Waxy maize starch	66.4	71.7	76.1	23.8	nd	nd	nd	
(8%)	(0.5)	(0.9)	(1.0)	(2.0)				
Potato starch (8%)	60.4	64.5	71.0	37.5	nd	nd	nd	
	(0.2)	(0.0)	(0.1)	(4.2)				

[a] Data were the average of duplicates. Standard deviation was in the parenthesis.
[b] Not detectable

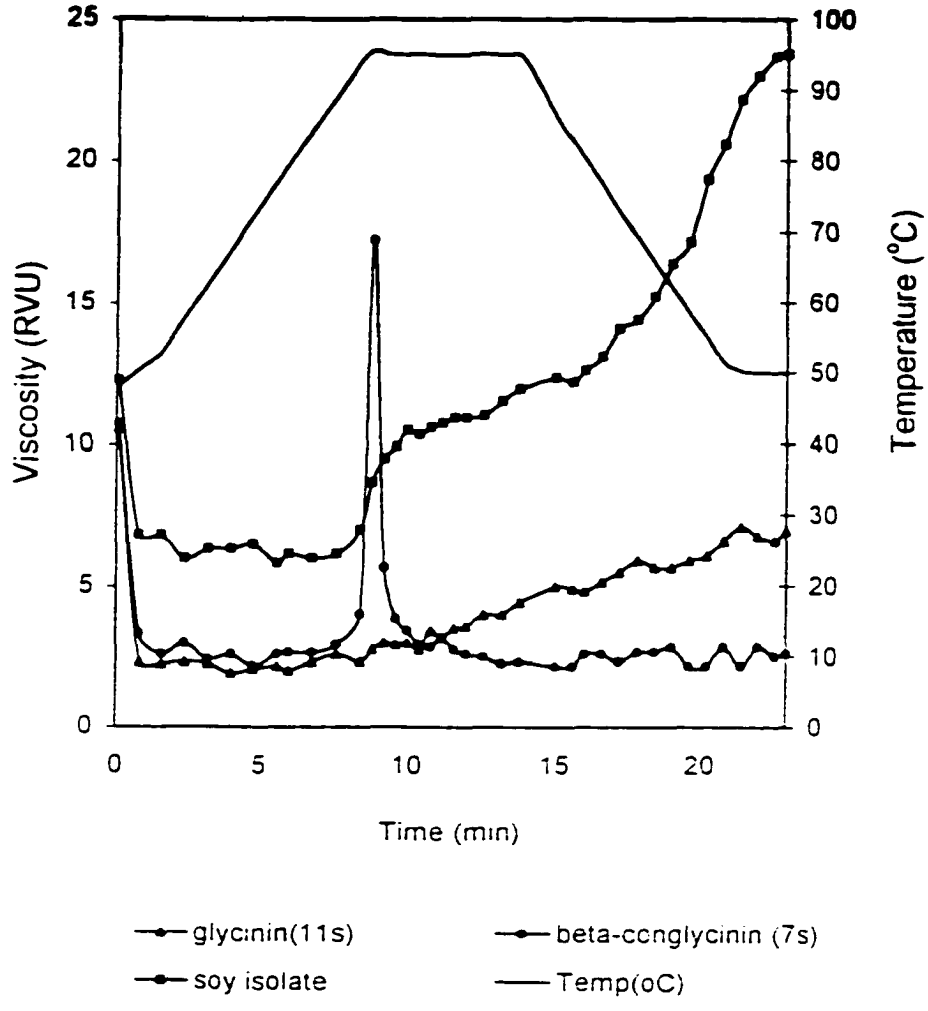


Figure 1. Viscosity profiles of soy protein (8%).

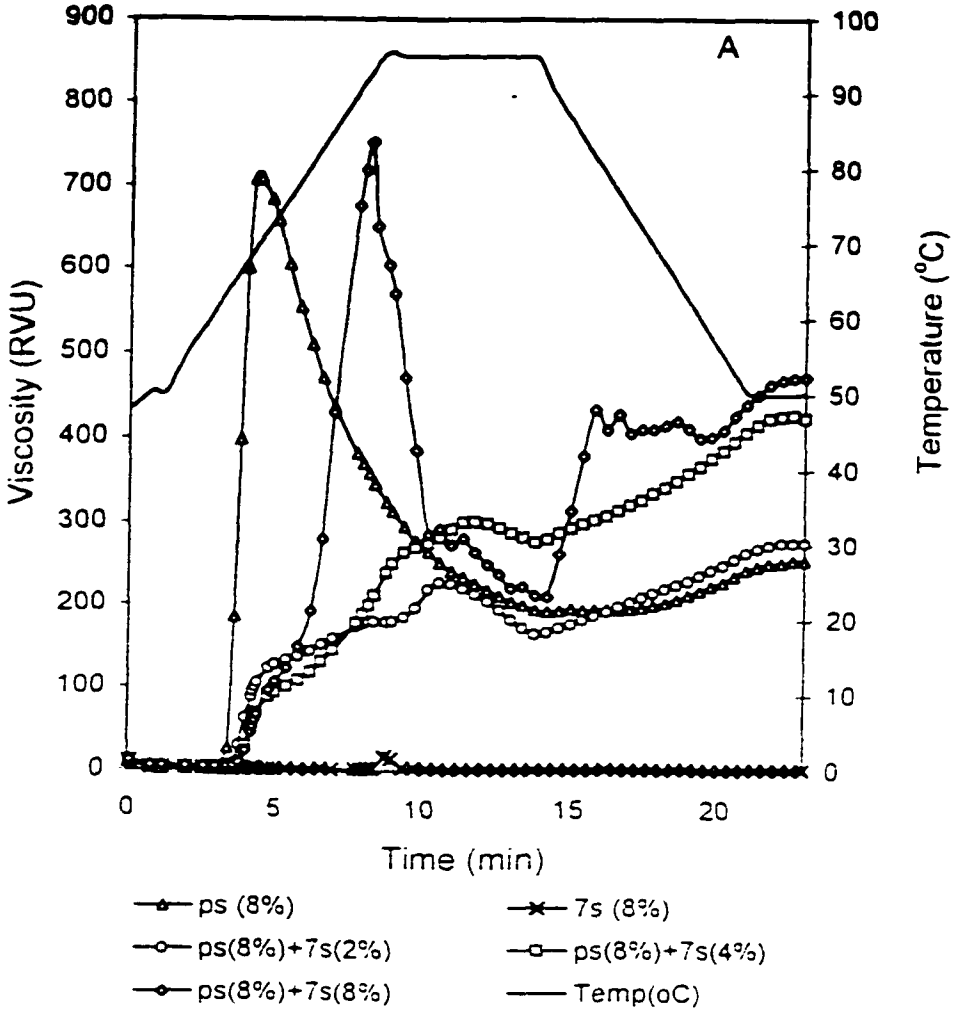


Figure 2A. Viscosity profiles of the mixture of potato starch with β-conglycinin. ps = potato starch.

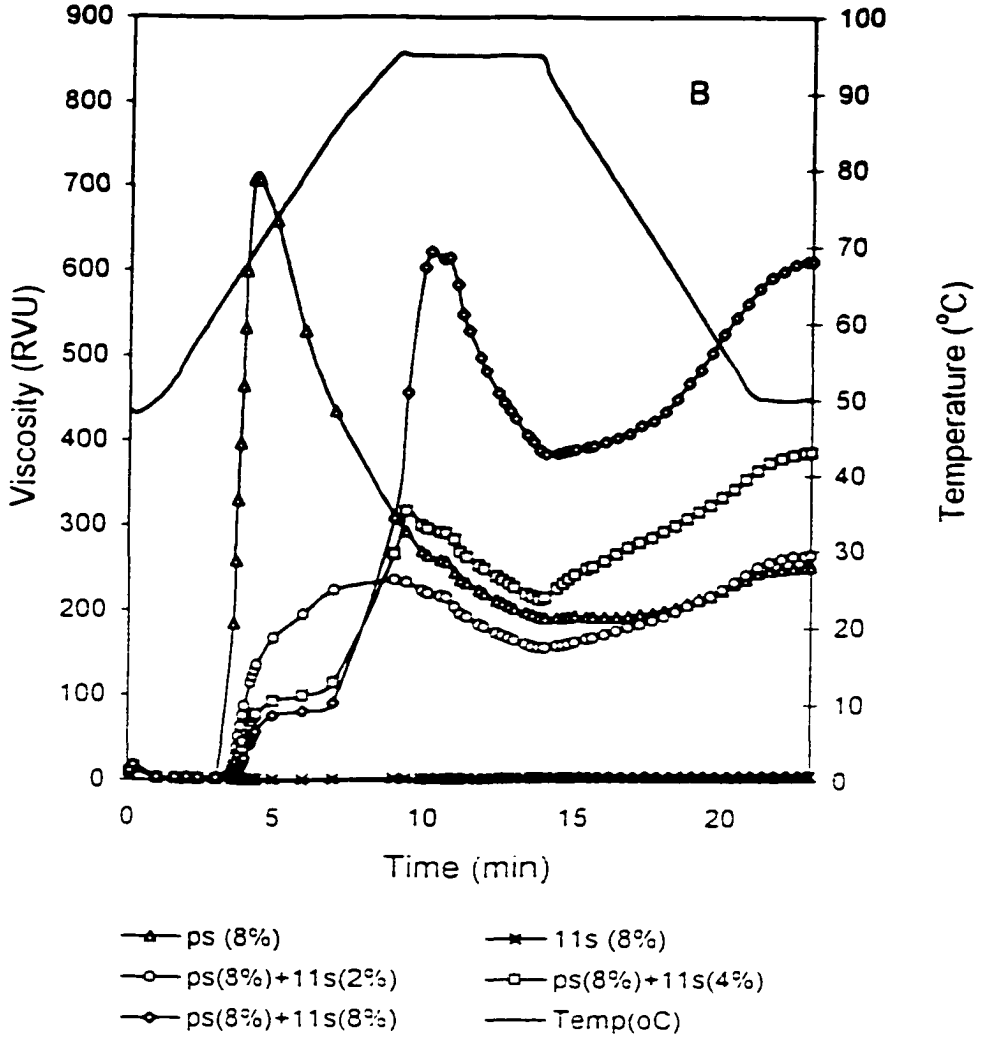


Figure 2B. Viscosity profiles of the mixture of potato starch with glycinin. ps = potato starch.

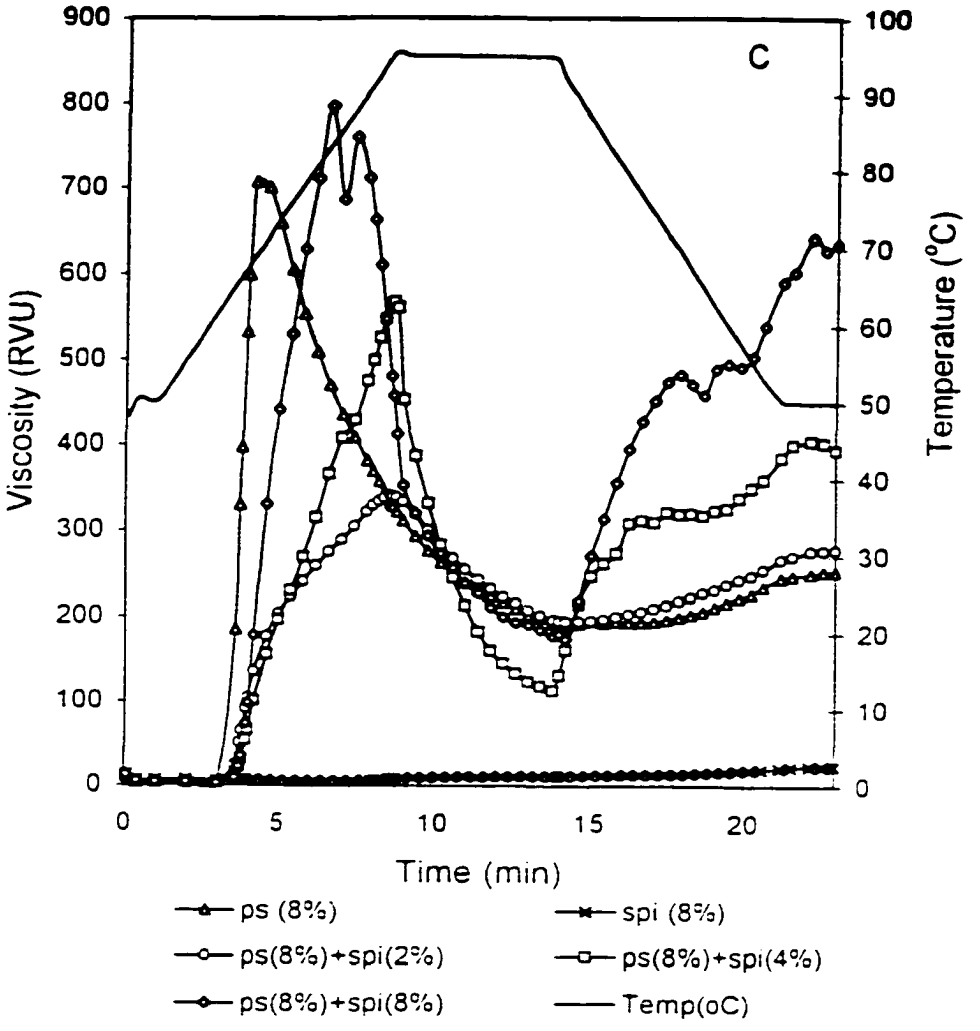


Figure 2C. Viscosity profiles of the mixture of potato starch with soy protein isolate. ps = potato starch.

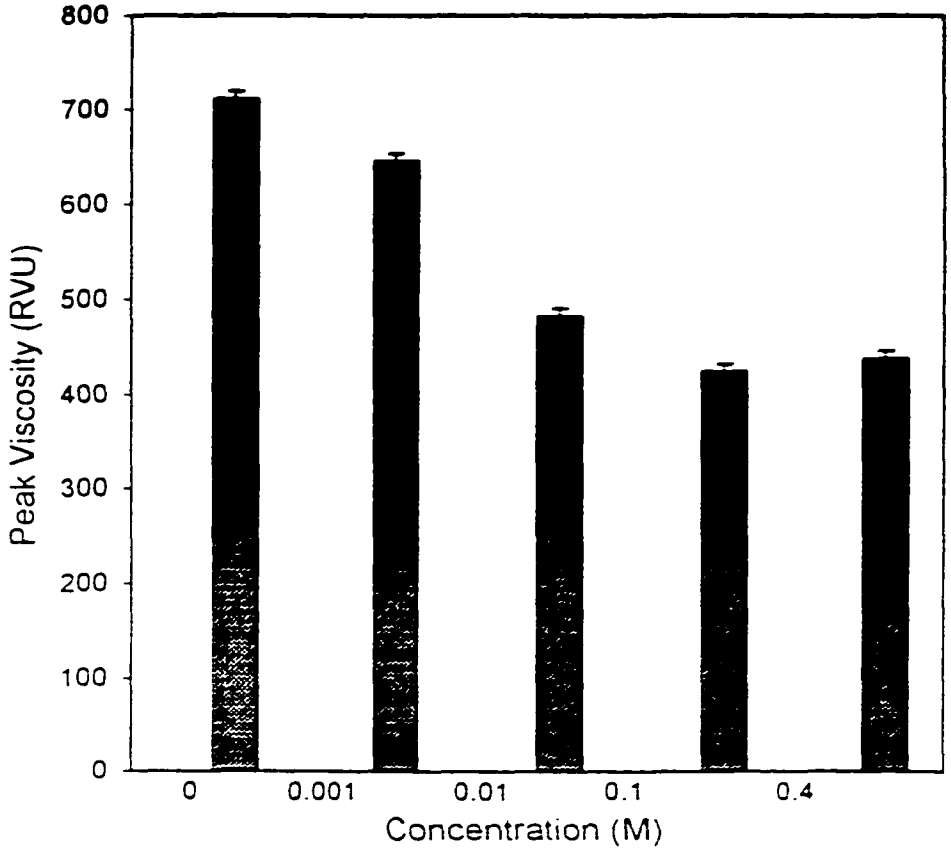


Figure 3. Peak viscosity of potato starch in sodium chloride solution.

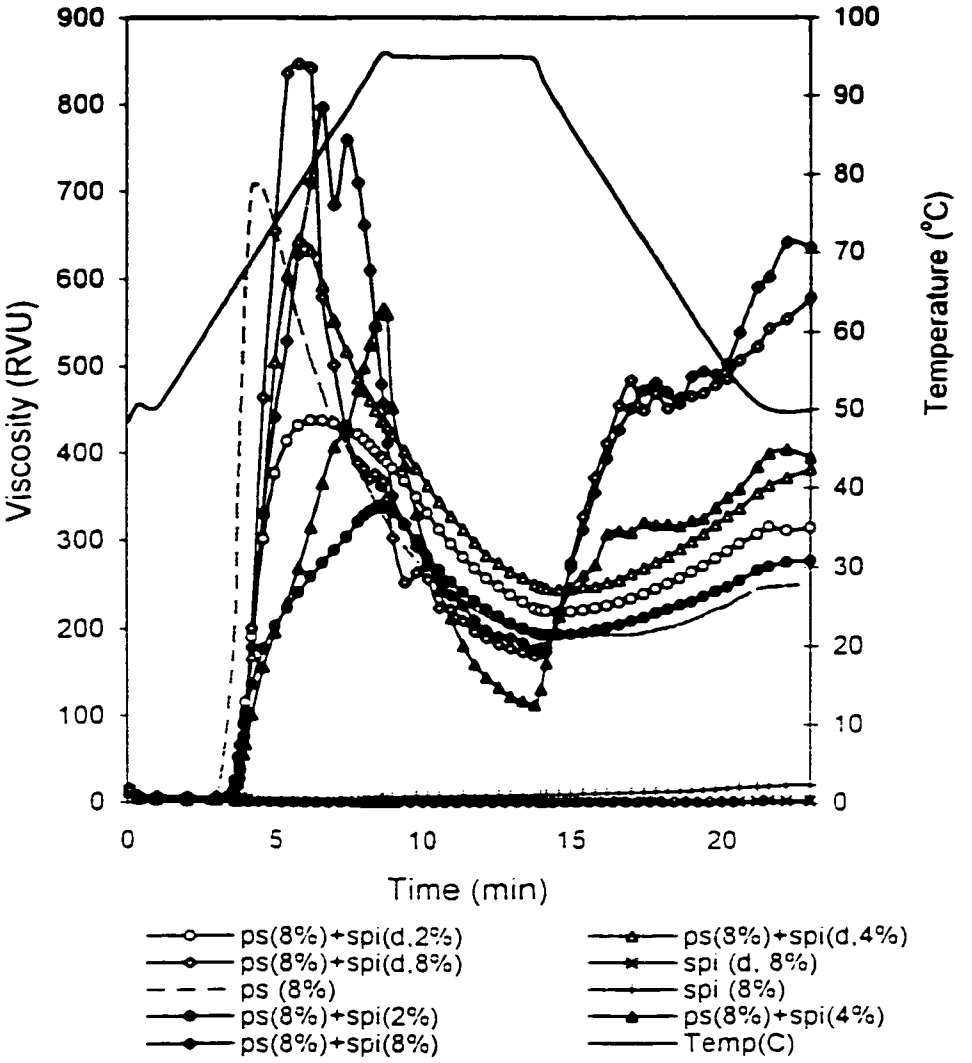


Figure 4. Salt residue effects on pasting properties of the mixture of potato starch with soy protein isolate. ps = potato starch, spi = undesalted soy protein isolate, spi (d) = desalted soy protein isolate.

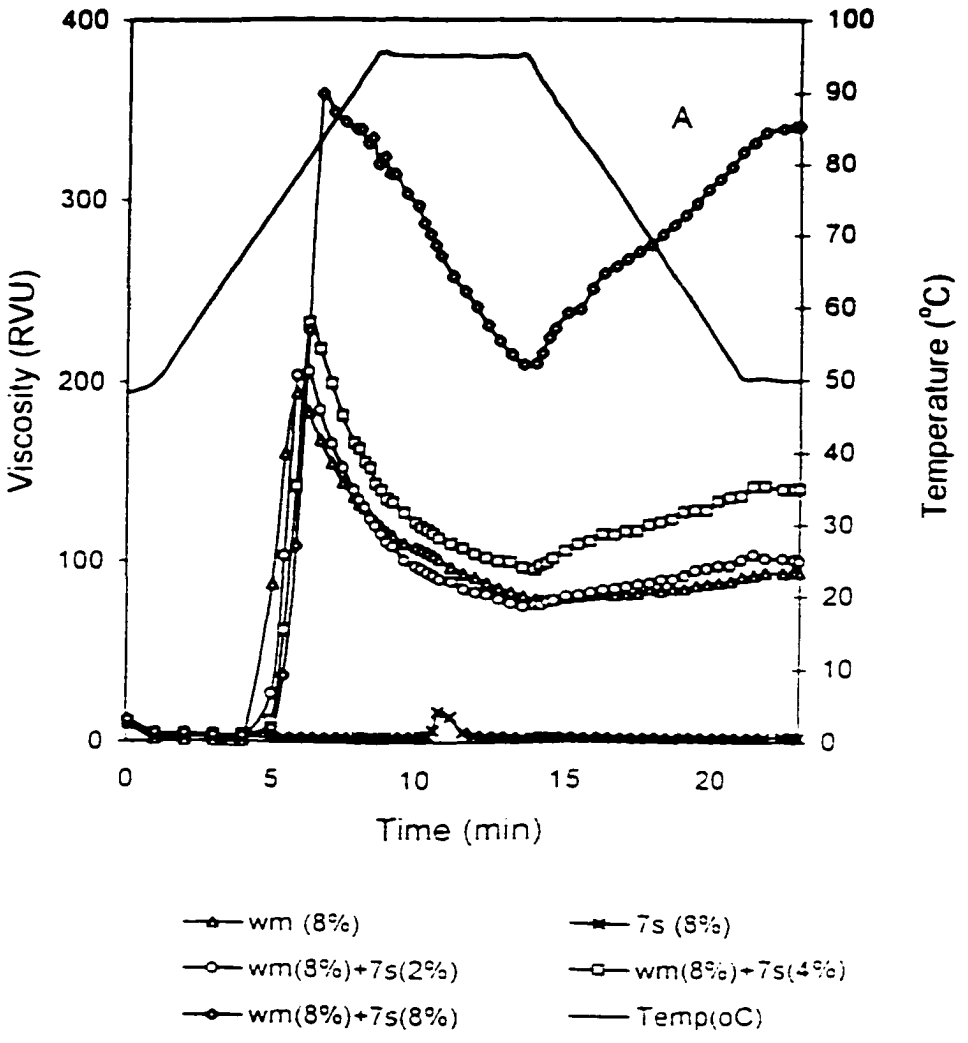


Figure 5A. Viscosity profiles of the mixture of waxy maize starch with β-conglycinin. wm = waxy maize starch.

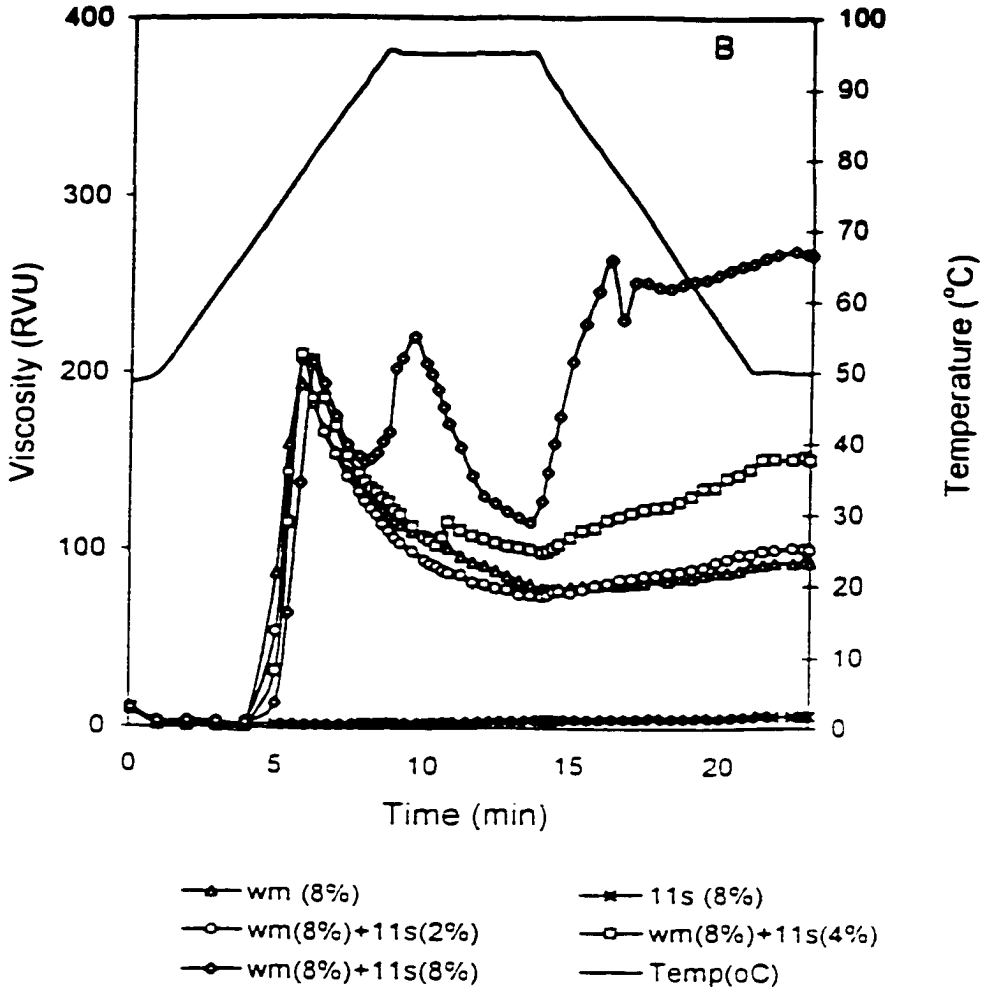


Figure 5B. Viscosity profiles of the mixture of waxy maize starch with glycinin. wm = waxy maize starch.

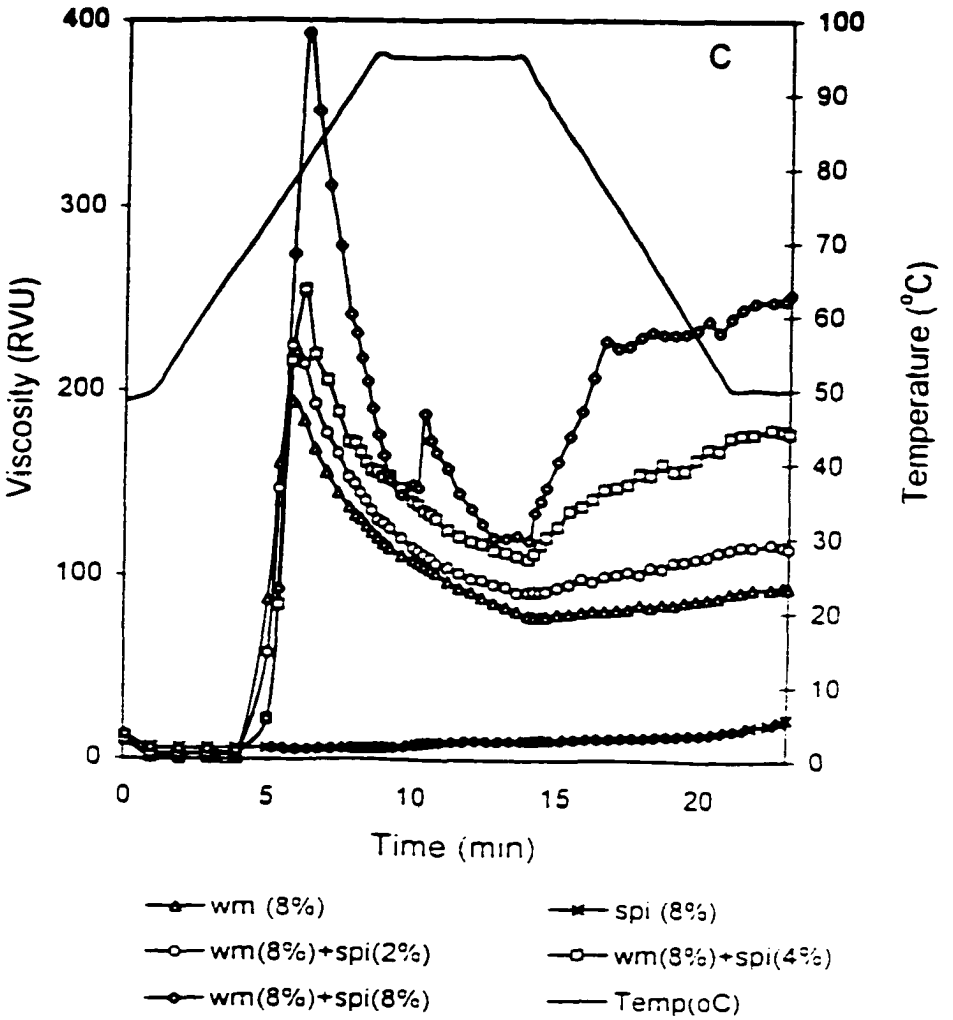


Figure 5C. Viscosity profiles of the mixture of waxy maize starch with soy protein isolate. wm = waxy maize starch.

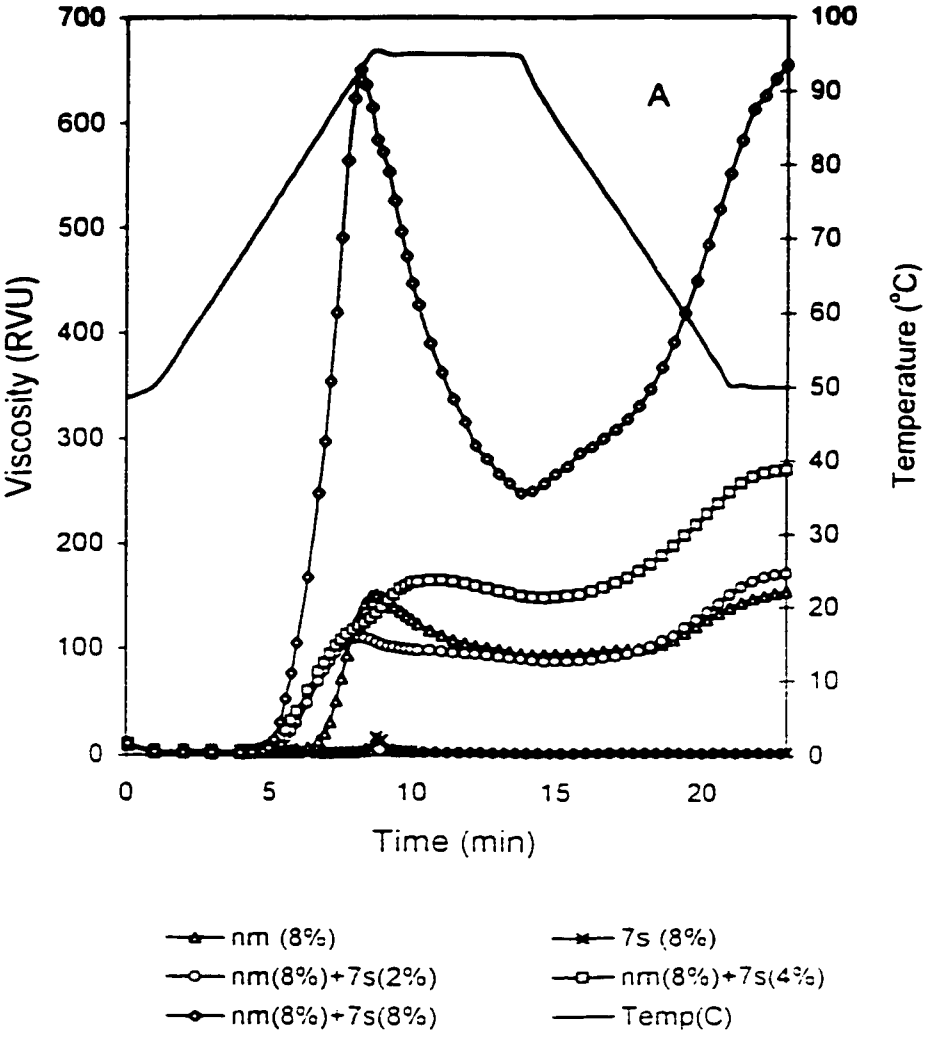


Figure 6A. Viscosity profiles of the mixture of normal maize starch with β-conglycinin. nm = normal maize starch.

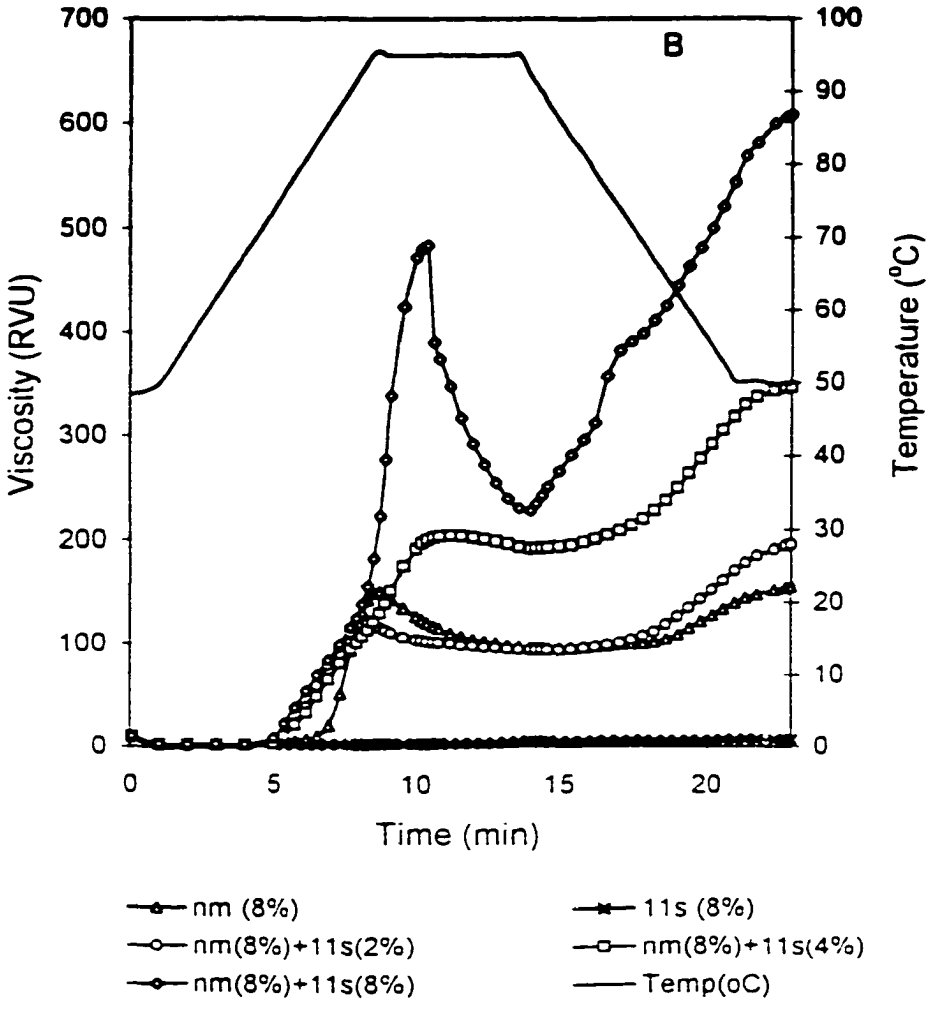


Figure 6B. Viscosity profiles of the mixture of normal maize starch with glycinin. nm = normal maize starch.

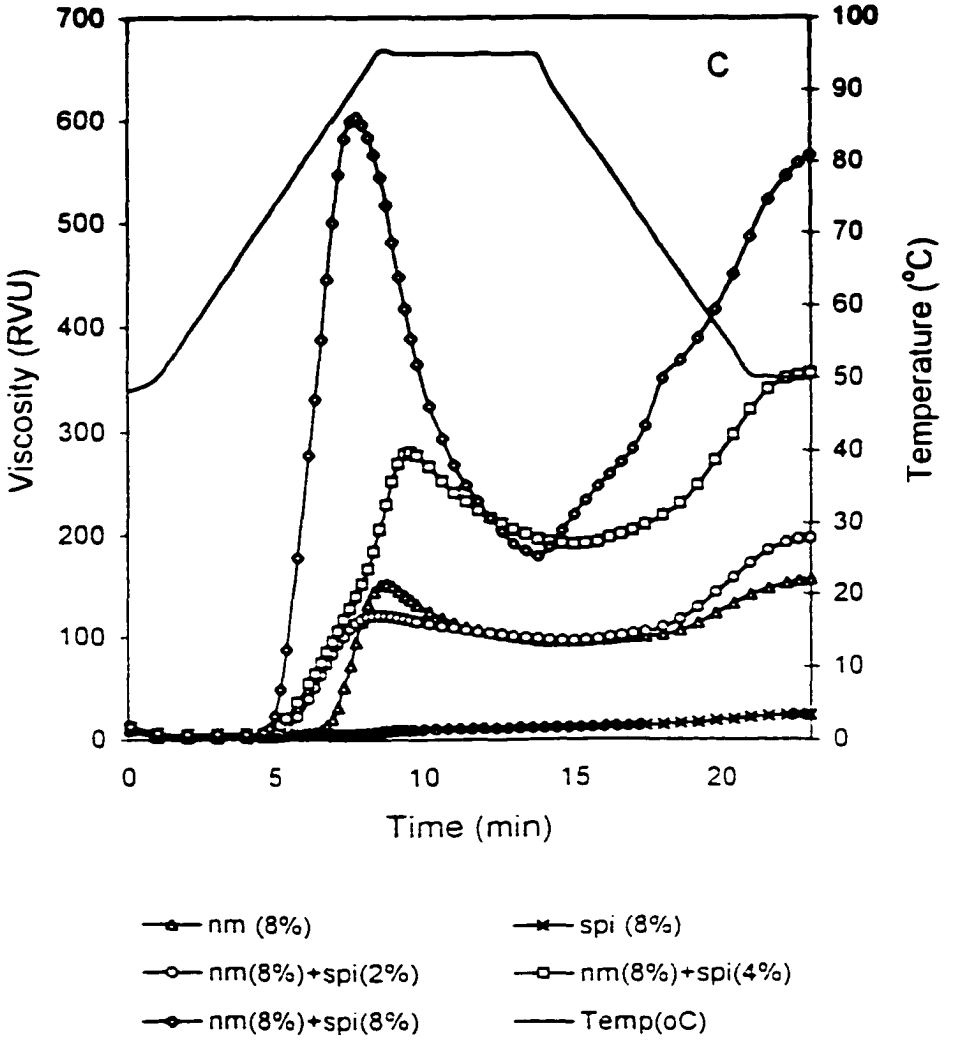
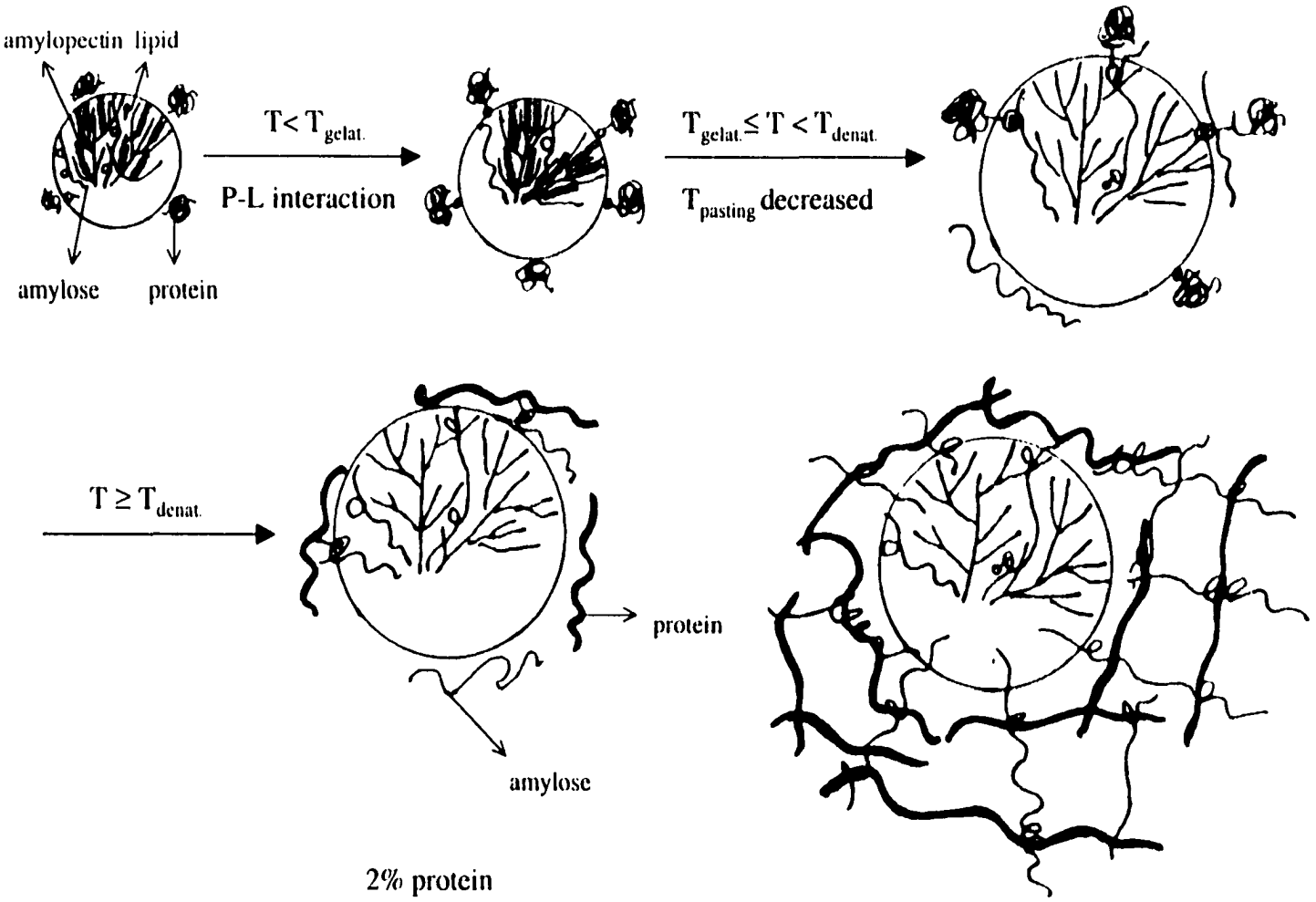


Figure 6C. Viscosity profiles of the mixture of normal maize starch with soy protein isolate. nm = normal maize starch.


amylopectin lipid
amylose
protein
T< T gelat.
P-L interaction
T gelat.≤ T < T denat.
T pasting decreased
T ≥ T denat.
protein
amylose
2% protein
4% or 8% protein

GENERAL CONCLUSIONS

Four different types of barley starches were isolated from seeds. Four barley starches displayed bimodal size distributions and A-type x-ray diffraction pattern. Unlike the significant difference in gelatinization onset temperatures between normal maize starch and high amylose maize starch, barley starches displayed similar onset gelatinization temperature ranging from 55.0 to 56.5°C. The difference was due to the difference of starch structure in amylopectin branch-chain-length distributions. Maize starches have more proportion of long branch chains, whereas barley starches showed more proportions of short chains. Barley starches also had a shoulder in the branch chain length distribution, which suggested a defective crystalline structure. Naegeli dextrins study showed that double branched and more highly branched molecules, besides linear molecules and singly branched molecules, were found in barley Naegeli dextrins. ^{31}P-NMR study showed that phosphorus in barley starches was mainly from phospholipids.

Different soy protein isolate products were compared on mechanical properties and water absorption property after compression molding process. Results showed that all the soy protein isolate samples used in this study had good processability by compression molding. Properties of molded soy protein specimens varied among soy protein samples. The specimens made from the protein isolate samples with less denaturation and pH close to isoelectric point gave better dry and wet mechanical properties and low water absorption property. Physical properties of soy protein isolate samples could affect the properties of molded specimen counterparts.

Starch pasting properties were changed after soy proteins were added in the system. It varied among starch varieties, type of soy proteins and protein concentrations. Generally,

mixtures displayed higher viscosity than soy protein dispersion alone, and higher setback than starch dispersion alone. Soy proteins, as emulsifiers, interacted with lipids in normal maize starch, which decreased pasting temperature of normal maize starch. Pasting temperatures were slightly increased in waxy maize and potato starch systems. Amylose in normal maize starch and potato starch could form helical complex with soy proteins, which retarded starch swelling. Salt in soy proteins had stronger effect on decreasing viscosity of potato starch than on that of normal maize starch and waxy maize starch.

APPENDIX I

CHARACTERIZATION OF SOY PROTEIN FRACTIONS

A report for USB project (1998)

Y. Song and J. Jane

ABSTRACT

β-Conglycinin and glycinin were made into specimens by compression-molding. Mechanical properties and water absorption property were tested. Effect of the ratio of these two soy protein fractions on properties was studied. Results showed that glycinin specimens displayed higher tensile strength and lower water absorption than did β-conglycinin specimens. Fractionation methods and soybean varieties affected the properties of protein specimens. Properties of specimens varied among different ratios of β-conglycinin to glycinin.

INTRODUCTION

Besides as food ingredients, soy proteins have also been used for industrial applications, such as paper coatings, plastics, and adhesives. Soy protein isolate and concentrate have been demonstrated suitable for manufacturing molded plastic specimens (Paetau et al., 1994). There is no soy product available in the market designated for soy

plastics manufacturing. More efforts are needed to investigate the properties of soy protein for plastic application.

β-Conglycinin (7s) and glycinin (11s) are the two major storage protein globulins in soybeans. β-Conglycinin is a trimer with a molecular mass of 150-200 kDa. It contains three major subunits α' (72 kDa), α (68 kDa), and β (52 kDa), and one minor subunit γ similar in the size to β subunit. Glycinin consists of two equal hexamers, each of which has a total molecular mass of 300-380 kDa. Each hexamer protein is composed of three pairs of acidic and basic subunits (~35 KDa and ~20 KDa, respectively), and the acidic and basic subunits are linked by a disulfide bond (Nielsen, 1985; Utsumi et al., 1997; Yamauchi et al., 1991; Wolf, 1970).

Objectives of the study are to compare mechanical properties and water absorption property of compression-molded specimens made from different soy protein fractions, to study the effect of processing condition on properties of specimens, and to investigate the effect of processing methods and soybean variety on properties of specimens.

MATERIALS AND METHODS

Samples. Glycinin (I, II and III) and β-conglycinin (I, II and III) were fractionated at the pilot plant in Iowa State University. Samples (I, and II) and the pilot plant soy isolate were prepared from the same soybean flakes (MBS-2795). Samples III were produced by using a different soy bean variety which was provided by Archer Daniels Midland Company (Decatur, IL). Samples (I) were fractionated by using modified Nagano's method. Samples (II, and III) were fractionated by a simplified method which was developed by Wu et al. (1999).

Specimen preparation. Type I specimens (ASTM standard D638-86, dumbbell shaped, overall length of 165 mm) were made from 15.0 g of soy protein with 7% moisture by compression molding using a Wabash compression-molding machine (Wabash Metal Products, Inc., Wabash, IN). Compression molding was performed at temperature of 120°C and 150°C, and 10 MPa pressure for 12min. After molding, the mold with specimen was cooled for 5 min before the specimen was removed from the mold. The edge of the specimen was carefully smoothed by sandpaper. All samples were replicated in five times.

Mechanical properties. Tensile strength, elongation and Young's modulus of the compression molded specimens were measured by using an Instron Model 4502 testing system (Canton, MA), following the standard test method for tensile properties of plastics (ASTM D638-92). Specimens were equalibrated at 50% relative humidity for 48 hr before mechanical tests.

Water absorption. A modified ASTM standard test method D570-81 using bar-shaped specimens was used for water absorption tests of the specimens following the procedure of Peatau et al. (1994a).

Solubility. Soy protein specimens were grounded into powder by using a cyclone sample mill (UDY Corporation, Fort Collins, CO) were dissolved in deionized water with stirring for 2 hr. The supernatant was obtained by centrifugation at 10,000g for 30 min. Protein content in the supernatant was determined according to the biuret method. Solubility was calculated based on the protein content and the original amount.

Molecular weight of soy protein samples. Linear gradient urea-SDS-PAGE gels were prepared with equal volumes (16 ml) of 8% and 18% acrylamide solutions. The 8% acrylamide solution contained 1.5 M Tris-HCl (pH 8.8, 4.0 ml), urea (3.6 g), 10% SDS (0.16 ml), TEMED (10 μl), bis(acrylamide) (37.5:1, 3.2 ml), 10 % ammonium persulfate (100 μl), and deionized water (5.38 ml). The 18% acrylamide solution contained 0.38 M Tris-HCl (pH 8.8, 4.0 ml), urea (5.24 g), 10% SDS (0.16 ml), TEMED (10 μl), bis(acrylamide) (37.5:1, 7.2 ml), 10% ammonium persulfate (100 μl), and deionized water (0.93 ml). The stacking gel was prepared by 4% acrylamide solution which contained 0.5 M Tris-HCl (pH 6.8, 2.5 ml), urea (1.65 g), 10% SDS (0.10 ml), TEMED (10 μl), bis(acrylamide) (37.5:1, 1.0 ml), 10% ammonium persulfate (100 μl), and deionized water (4.95 ml). The gel solutions were poured immediately after addition of ammonium persulfate to polymerize (Petruccelli and Anon, 1994; Wu et al., 1999).

Protein samples were dissolved in a protein extraction buffer solution that contained 50 mM Tris-HCl, 0.2% SDS, 5 M urea, and 0.2% 2-mercaptoethanol, and diluted with a sample buffer which contained 125 mM Tris-HCl (pH 6.8), 5 M urea, 20% (v/v) glycerol, 2% SDS, 0.4% bromophenol blue and 2% 2-mercaptoethanol. The gels were run at 135 v constant voltage for about 4.5 hr (Wu et al., 1999).

RESULTS AND DISCUSSION

Glycinin and β-conglycinin were fractionated by a modified Nagano's method and a simplified method (Wu et al., 1999). Three products (glycinin, β-conglycinin, and intermediate sample) were produced by the modified Nagano's method. Two products

(glycinin and β-conglycinin) were obtained by using the simplified method. The compression-molded glycinin and β-conglycinin specimens were more difficult to process than commercial soy isolate samples during compression molding under the same condition. Glycinin (III) and β-conglycinin (III) fractionated from a different soybean flake were used to investigate the process condition and also to compare the properties of the specimens between different soybean varieties. Mechanical properties of glycinin and β-conglycinin specimens were shown in Table 1.

Glycinin specimens displayed better tensile strength than did β-conglycinin specimens. Fractionation methods had a significant effect on the tensile strength. The purity and composition differed between the samples fractionated by different methods. The purity of β-conglycinin (I) and glycinin (I) was better by using the modified Nagano's method. SDS-PAGE results (Fig. 1) showed that β-conglycinin (II) had higher proportions of glycinin subunits than β-conglycinin (I). Results of compression-molded mixtures of β-conglycinin and glycinin showed that the molded mixtures had higher tensile strength than molded β-conglycinin and molded glycinin specimen alone (Fig. 2). Results differed between different soybean varieties. For samples (I), the molded mixture specimens with the ratio 2 to 1 of glycinin to β-conglycinin had the highest tensile strength. While for sample (III), the tensile strength of the molded mixture specimen increased as the ratio of glycinin to β-conglycinin increased.

Glycinin and β-conglycinin specimens displayed significantly higher water absorption than did soy protein isolate specimens (Fig. 3). Molded β-conglycinin specimens absorbed more water than did glycinin specimens. The effect of molding temperature on the

water absorption of the specimens showed that both glycinin and β-conglycinin specimens molded at 120°C displayed higher percentage of water absorption than those molded at150°C. Glycinin (III) and β-conglycinin (III) specimens absorbed water faster than glycinin (I, II) and β-conglycinin (I, II) specimens, respectively. It was observed that glycinin (II, and III) and β-conglycinin (III) specimens were dissolved in water from the surface to the center after submerging in water for 6 hr. Although the center of the specimens still had a hard core, the measurement could not continue. Glycinin (I) and β-conglycinin (I) absorbed water more homogeneously. The strength of glycinin (I) and β-conglycinin (I) specimens greatly decreased after water was absorbed within 24 hr, but the specimens still remained whole pieces. Results demonstrated that the fractionation method affected the properties of the products. The interesting water absorption phenomena in glycinin specimens and β-conglycinin specimens was due to the different structures, properties between these two proteins and the effect of processing condition on the conformation changes of the proteins. Effect of processing temperature on solubility of native and compression-molded soy proteins was shown in Figure 4. Results suggested that after compression molding, protein solubility decreased as processing temperature increased. Results varied among different protein samples. For more purified protein samples (I), β-conglycinin specimens displayed higher solubility than did glycinin specimens, which was in an agreement with water absorption results.

CONCLUSION

β-Conglycinin and glycinin could be processed into specimens by compression molding. Glycinin specimens showed better mechanical properties and lower water absorption than did β-conglycinin specimens. Fractionation methods affected the properties of specimens. Specimens displayed different properties between two different soybean varieties.

ACKNOWLEDGMENT

The authors thank Dr. L. Johnson and Dr. P. Murphy 's group for providing the pilot plant protein samples, and the United Soybean Board for a grant support of this research.

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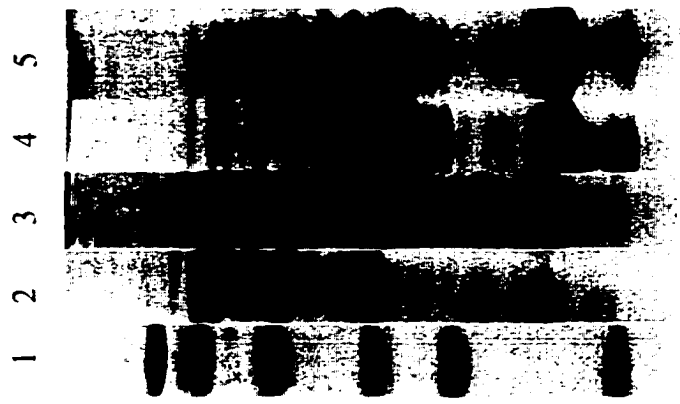
Wu, S., Murphy, P.A., Johnson, L.A., Fratzke, A.R., Ruber, M.S., Simplified pilot-plant process for soybean glycinin and beta-conglycinin fractionation, *J. Agric Food Chem., to be submitted, (1999).*

Table 1. Mechanical properties of different β-conglycinin and glycinin specimens

Sample[a]	Elongation (%)	Tensile Strength (MPa)	Young's Modulus (MPa)
β-conglycinin (I)	3.2 ± 1.0	11.4 ± 5.5	678 ± 100
β-conglycinin (II)	3.2 ± 0.1	16.3 ± 2.2	396 ± 76
β-conglycinin (III)	1.2 ± 0.4	8.3 ± 3.1	320 ± 55
glycinin (I)	1.2 ± 0.5	10.8 ± 2.5	288 ± 182
glycinin (II)	2.9 ± 1.0	17.4 ± 2.0	520 ± 100
glycinin (III)	1.7 ± 0.9	11.6 ± 3.5	325 ± 85

[a] Samples were processed at 150°C and 10 MPa for 12 min.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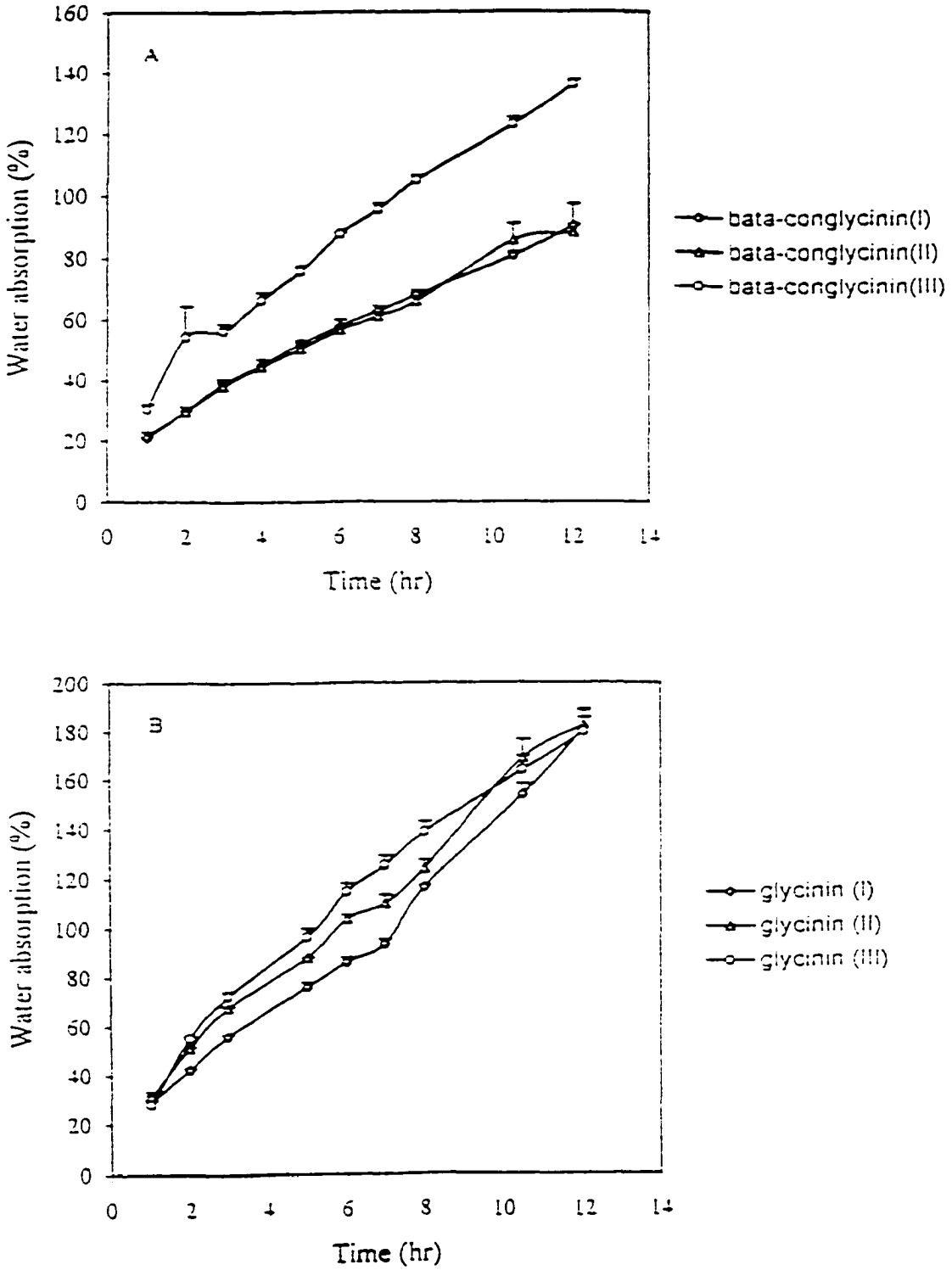

Figure 2. Water absorption of different β-conglycinin specimens and glycinin specimens (150oC). A, β-conglycinin; B, glycinin.

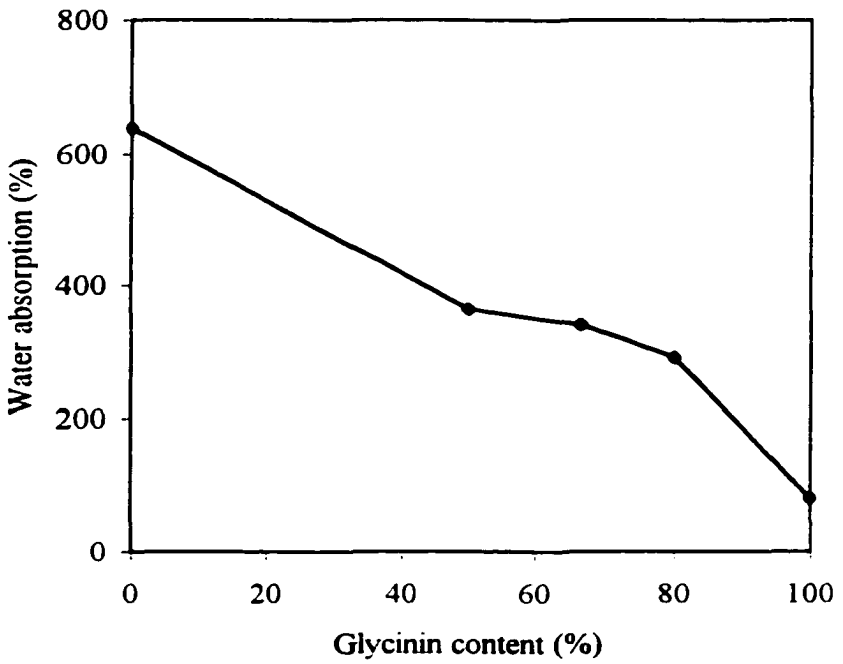

Figure 3. Effect of glycinin content on water absorption (24 Hr) of compression-molded soy protein fraction mixtures (150°C).

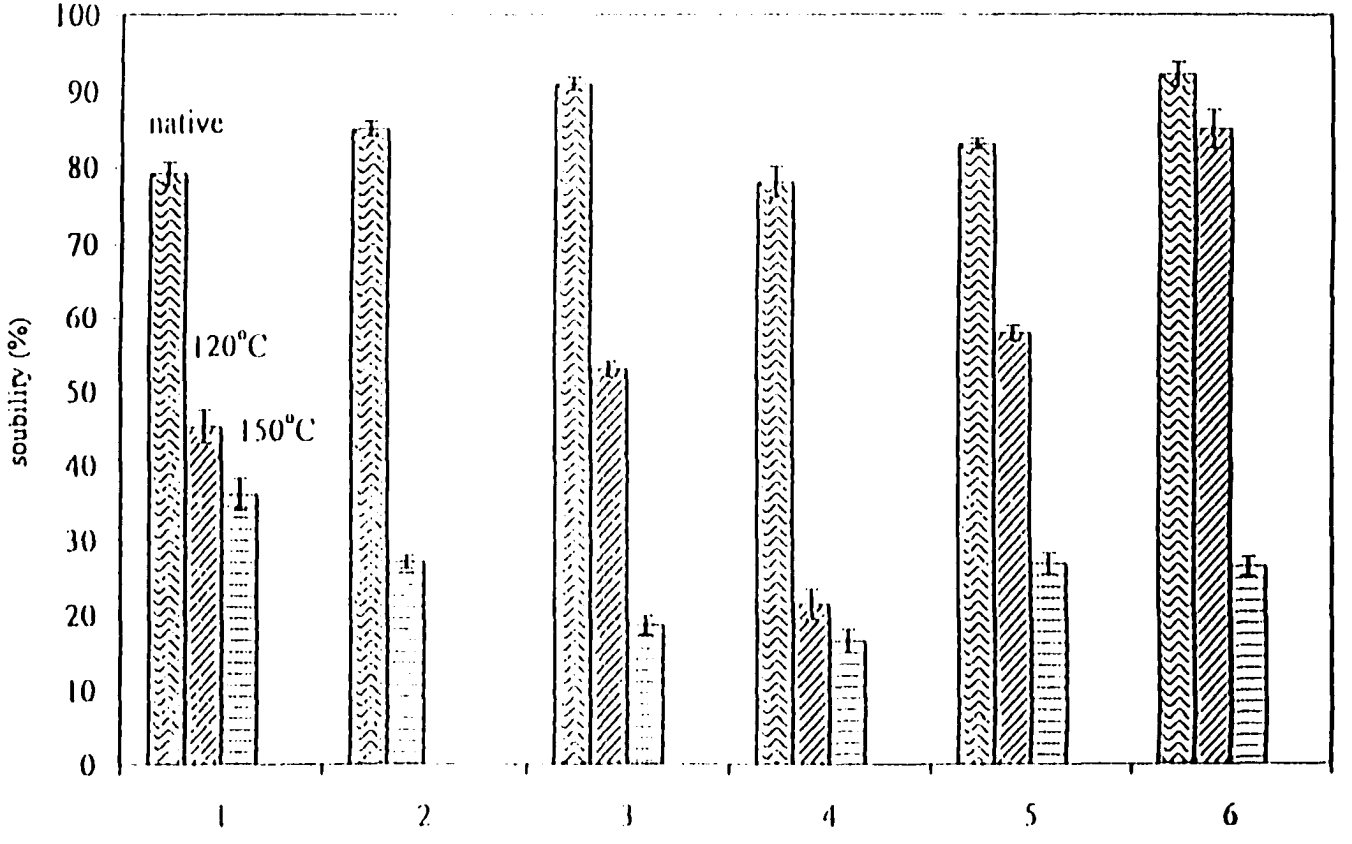

Figure 4. Solubility of native and compression-molded soy proteins.
1. β-conglycinin (I); 2. β-conglycinin (II); 3. β-conglycinin (III);
4. glycinin (I); 5. glycinin (II); 6. glycinin (III).

APPENDIX II

EFFECT OF COMPRESSION-MOLDING ON PROPERTY AND STRUCTURE OF SOY PROTEINS

A report for USB project (1998)

Y. Song and J. Jane

INTRODUCTION

Besides as food ingredients, soy proteins have also been used for industrial applications, such as paper coatings, plastics, and adhesives. Soy protein isolate and concentrate have been demonstrated suitable for manufacturing molded plastic specimens (Paetau et al., 1994). It has been reported that soy protein fractions (β-conglycinin and glycinin) are also suitable for compression molding process (Song and Jane, 1998; Sun et al., 1999). The appropriate property of soy products is still being investigated to industrialize soy plastics.

β-Conglycinin (7s) and glycinin (11s) are the two major storage protein globulins in soybeans. β-Conglycinin is a trimer with a molecular mass of 150-200 kDa. It contains three major subunits α' (72 kDa), α (68 kDa), and β (52 kDa), and one minor subunit γ similar in the size to β subunit. Glycinin consists of two equal hexamers, each of which has a total molecular mass of 300-380 kDa. Each hexamer protein is composed of three pairs of acidic and basic subunits (~35 KDa and ~20 KDa, respectively), and the acidic and basic

subunits are linked by a disulfide bond (Nielsen, 1985; Utsumi et al., 1997; Yamauchi et al., 1991; Wolf, 1970).

There is little information available on structure and property changes of soy protein after compression molding. Therefore, this investigation was conducted to study the conformation changes of soy proteins and soy protein specimens by instrumental analysis, and to examine the physical property changes of soy proteins after compression molding.

MATERIALS AND METHODS

Samples. Soy protein isolate Supro 760 was purchased from Protein Technologies Internationals (St. Louis, MO). A soy protein isolate sample was prepared from the pilot plant at Iowa State University (Ames, IA). β-Conglycinin and glycinin were fractionated at the same pilot plant.

Specimen preparation. Type I specimens (ASTM standard D638-86, dumbbell shaped, overall length of 165 mm) were made from 15.0 g of soy protein with 7% moisture by compression molding using a Wabash compression-molding machine (Wabash Metal Products, Inc., Wabash, IN). Compression molding was performed at 150°C and 10 MPa pressure for 12 min. After molding, the mold with specimen was cooled for 5 min before the specimen was removed from the mold. The edge of the specimen was carefully smoothed by sandpaper. All samples were replicated in five times.

Molecular weight of soy protein samples. Linear gradient urea-SDS-PAGE gels were used to study molecular weight changes of soy proteins before and after compression molding. The same method for gel preparation (Wu et al., 1999) was followed.

Solubility. Soy protein specimens were grounded into powder by using a cyclone sample mill (UDY Corporation, Fort Collins, CO) were dissolved in deionized water with stirring for 2 hr. The supernatant was obtained by centrifugation at 10,000g for 30 min. Protein content in the supernatant was determined according to the biuret method. Solubility was calculated based on the protein content and the original amount.

Surface Hydrophobicity(S_o). S_o was determined by the hydrophocity fluorescence probe 1-aniline-S-naphthalene-sulfonate (ANS). Protein dispersions (1mg/ml) in 0.01 M phosphate buffer (pH 7.5) were stirred for 2 hr at 20°C and centrifuged at 6500 rpm for 20 min. the supernatant was serially diluted with the same buffer to obtain protein concentrations ranging from 0.1 to 0.0005 mg/ml. An aliquot (40 μl) of ANS (0.8 mM in0.1 M phosphate buffer, pH 7.0) was added to 2 ml of sample. Fluorescence intensity (FI) of the sample mixture measured using SPEX FluoroMax spectrofluorometer (SPEX Industries, Inc., Edison, NJ), at wavelengths of 365 nm (excitation) and 484 nm (emission). The initial slope (S_o) of FI versus protein concentration plot was used as an index of the protein hydrophobicity (Kato, A., and Nakai, S., 1980; Boatright, W., and Hettiarachchy, N., 1995, and Wu, et al., 1999).

Conformation. FTIR spectroscopy was used to study the conformation change of soy proteins after processing. Diffuse reflectance Infrared Fourier Transform (DRIFT) spectra of

soy protein powder were recorded on a Nicolet 256 spectrophotometer equipped with a TGS detector in the main compartment and a MCT detector in the auxiliary experiment module (AEM). The AEM housed a Harrick diffuse reflectance accessory. Samples were put in a Harrick microsampling cup. Circular dichroism (CD) was also used to study the secondary structure of soy proteins by following the method of Koshiyama and Fukushima (1973).

RESULTS

The dark band on the top of each processed sample was observed in the molecular weight distribution results (Fig. 1). It indicated that protein molecules possibly aggregated during compression molding. This aggregation contributed to the increased molecular weight and decreased solubility. Solubility of soy protein decreased after processing, and varied among different pH conditions (Fig. 2). Decreased solubility after compression molding was also observed at different pH conditions. Solubility of soy proteins varies with pH. Above or below the isoelectric point, solubility increased for native proteins and molded specimens. Surface hydrophobicity results showed that native β-conglycinin was more hydrophobic than glycinin, however, after compression molding, the hydrophobicity of β-conglycinin specimens dramatically decreased and was lower than that of glycinin specimens (Fig. 3).

Infrared spectra of proteins can display amide bands that represent different vibrations of the peptide moiety. Most commonly used group is the amide I. The vibration mode originates from the C=O stretching vibration of the amide group (coupled to the in-phase bending of the N-H bond and the stretching of C-N bond) and gives rise to infrared bands in the region between approximately 1600-1700 cm^{-1}.

DRIFT spectra showed that soy concentrate had higher absorbance at wavenumbers 1800-1500 cm^{-1} (amide bands) than soy isolate, β-conglycinin, and glycinin. The absorbance significantly decreased after protein samples were compression-molded (Fig. 4). Soy isolate Supro 760 had the similar spectra pattern under processing temperature 120°C and 150°C, with native protein sample, however, at higher temperature (180°C), protein was denatured and lost the spectra pattern. Spectra differed among different proteins (shown in Fig. 5). Protein sample's pH could change spectra pattern (shown in Fig. 6).

CD was also used to investigate the secondary structure change of soy protein after compression molding. The primary result (shown in Fig. 7 and Fig. 8) displayed different CD spectra patterns, which could be further analyzed through calculation software to give quantitative data on the specific conformation of soy proteins.

CONCLUSION

Study of protein structure is an interesting and complicated area. This report covered some of primary analysis, which will be beneficial to further study.

ACKNOWLEDGEMENTS

The authors thank Dr. L. Johnson and Dr. P. Murphy's group for providing the pilot plant protein samples; Dr. J. Applequist for discussion on circular dichroism; Dr. R. Angelici for providing instrument to DRIFT test; and the United Soybean Board for a grant support of this research.

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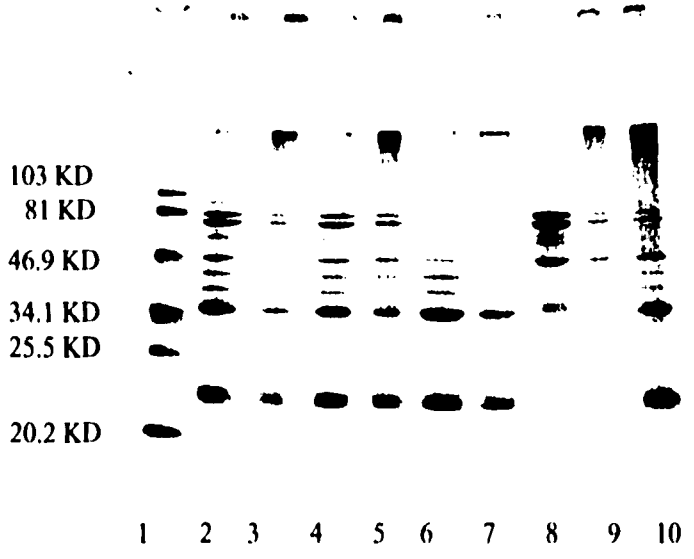
Wu, S., Murphy, P.A., Johnson, L.A., Fratzke, A.R., Ruber, M.S., Simplified pilot-plant process for soybean glycinin and beta-conglycinin fractionation, *J. Agric Food Chem., Accepted, (1999).*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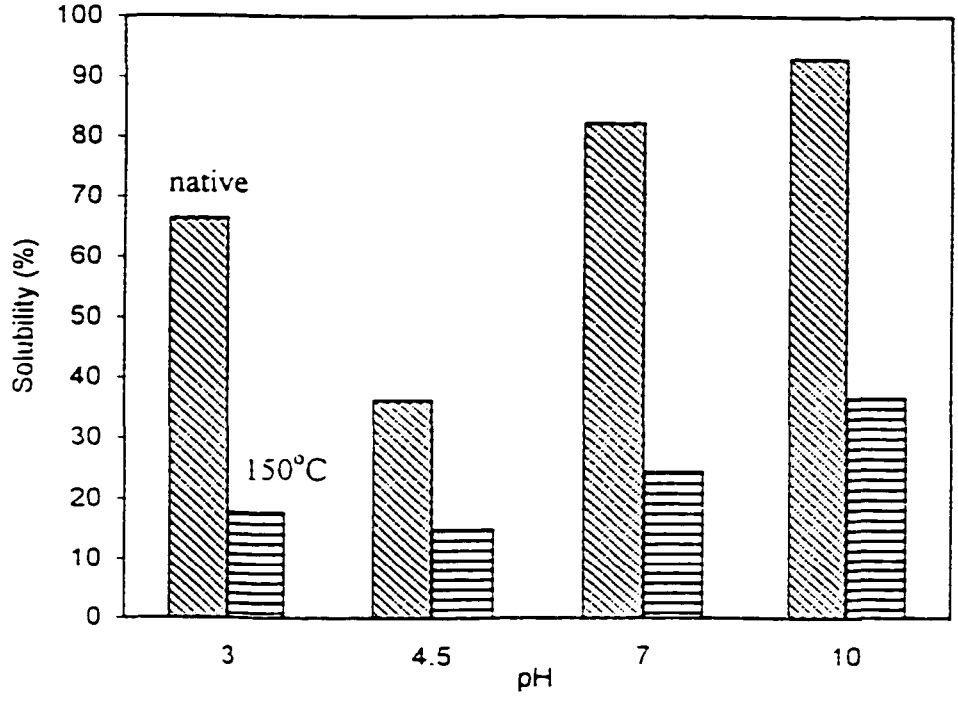

Figure 2. Effect of pH on the solubility of pilot-plant-prepared soy isolate samples and specimens.

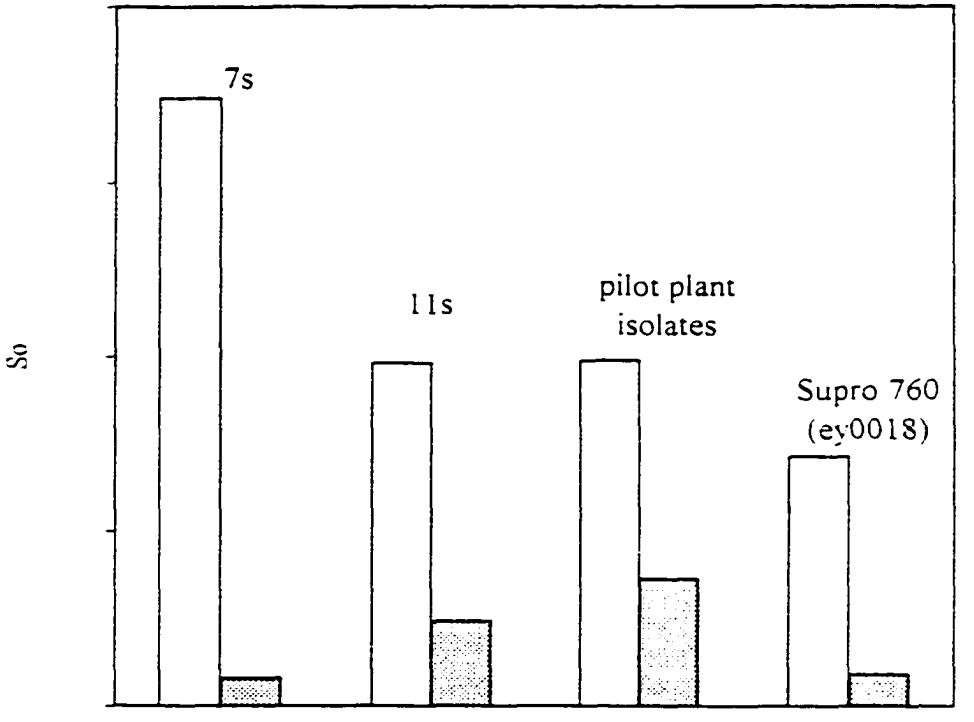

Figure 3. Effect of compression-molding process on the surface hydrophobicity of soy protein products.

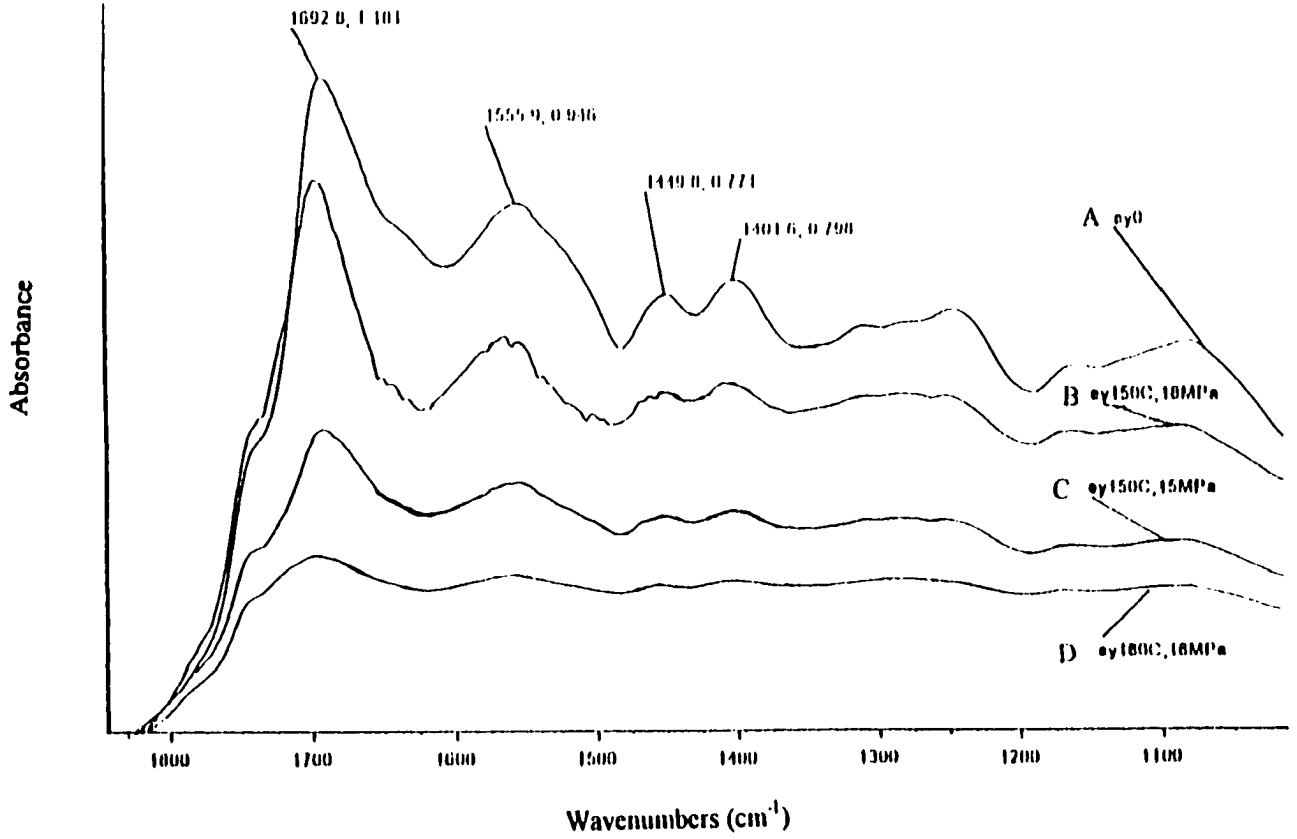

Figure 4. Effect of compression-molding process on Infrared spectra of soy protein isolate (Supro 760, Lot E7MEY0149). A. native; B. processed at 150°C and 18 MPa, C. processed at 150°C and 15 MPa; D. processed at 180°C and 18 MPa.

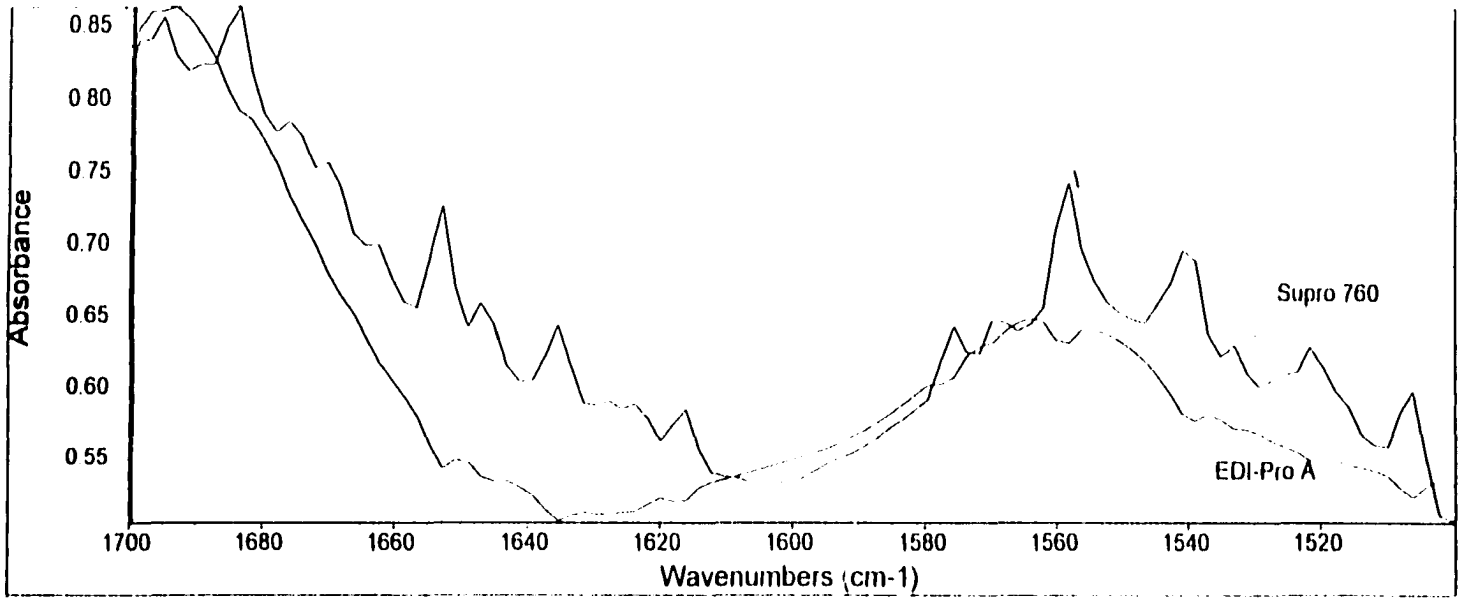

Figure 5. Infrared spectrum of different soy protein products.
A. Supro 760; B. EDI-Pro A.

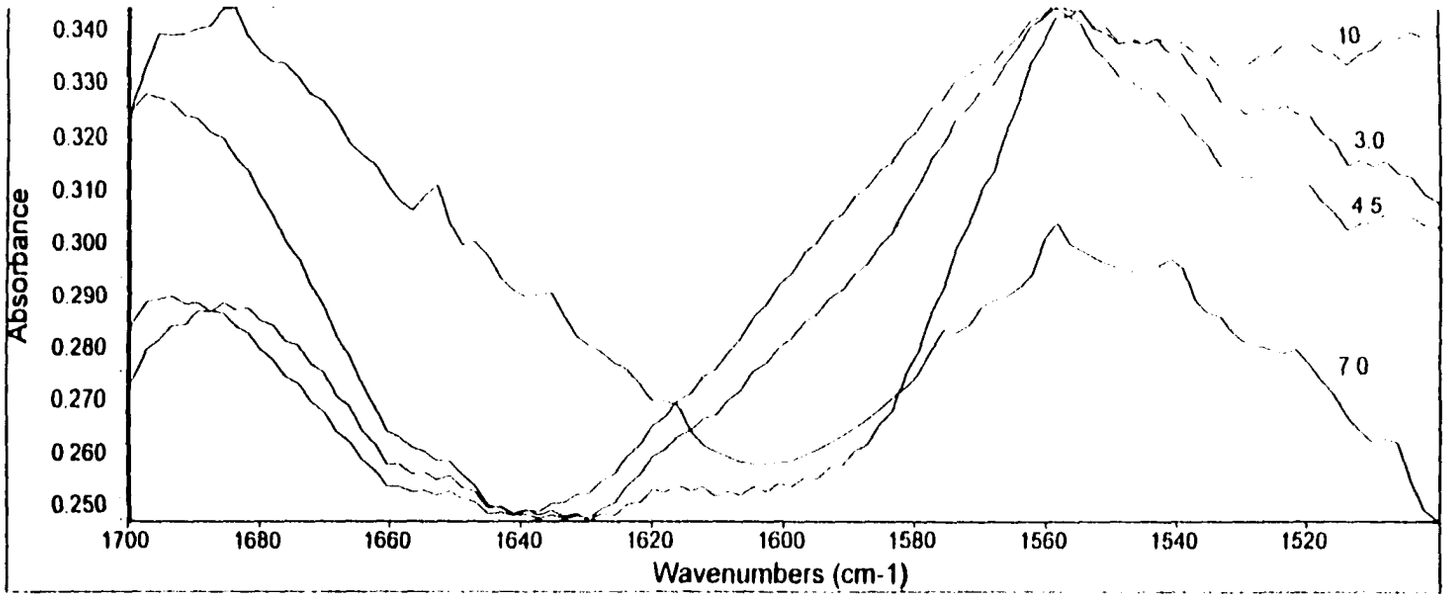

Figure 6. pH effect on Infrared spectrum of soy protein isolate made from the pilot plant. A. pH = 3.0; B. pH = 4.5; C. pH = 7.0; D. pH = 10.0.

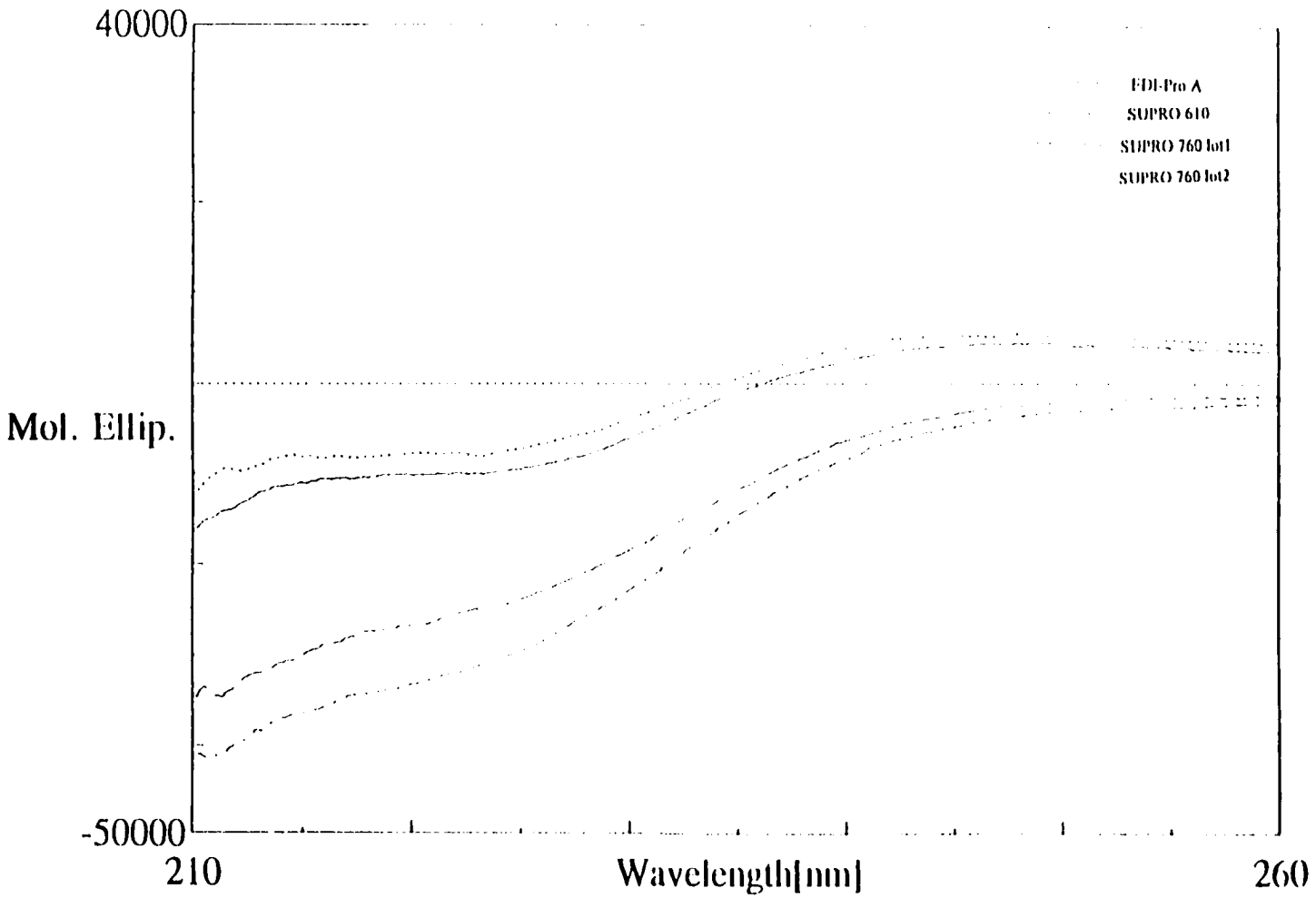

Figure 7. CD spectrum of soy protein products.

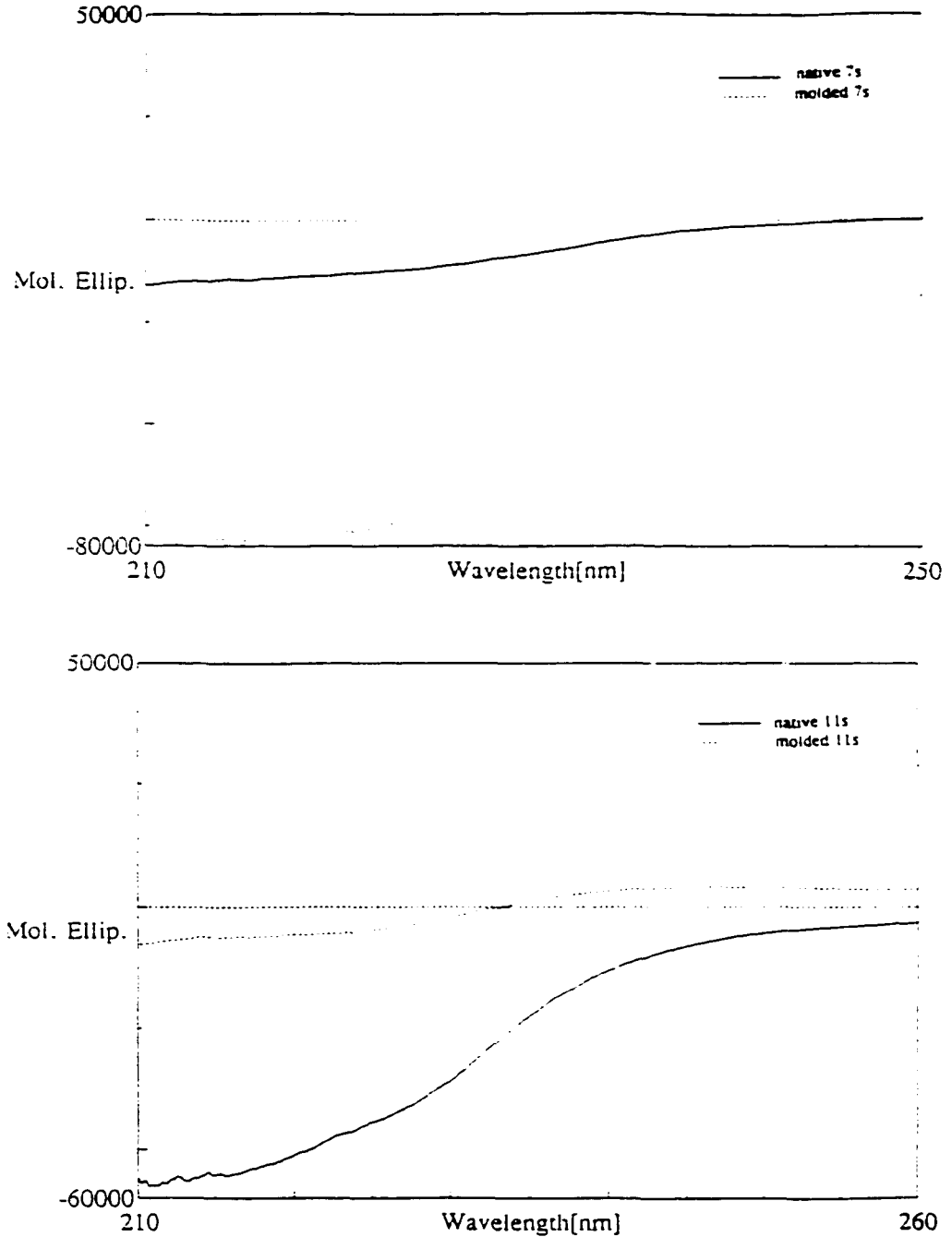

Figure 8. CD spectrum of native and compression-molded 7s and 11s.

APPENDIX III

PASTING PROPERTIES OF STARCH AND SOY PROTEIN MIXTURES

Table 1. Pasting properties of normal maize starch and soy proteins

Sample	Peak Viscosity (RVU)[a]	Final Viscosity (RVU)[a]	Setback (RVU)[a]	Pasting Temperature (°C)
Normal maize starch (8%)	151.3 ± 0.8[b]	155.1 ± 6.8	60.3 ± 5.8	83.6 ± 1.1
7s (8%)	17.4 ± 1.2	2.6 ± 0.7	0.7 ± 0.2	94.0 ± 1.5
7s+normal maize starch (2%+8%)	111.4 ± 3.8	172.5 ± 9.6	84.2 ± 2.2	75.9 ± 2.0
7s+normal maize starch (4%+8%)	165.0 ± 4.0	271.7 ± 8.9	123.2 ± 5.0	75.2 ± 0.5
7s+normal maize starch (8%+8%)	650.8 ± 1.0	654.4 ± 2.2	406.9 ± 1.4	74.0 ± 0.2
11s (8%)	4.1 ± 1.0	6.9 ± 1.2	3.0 ± 0.8	nd[c]
11s+normal maize starch (2%+8%)	120.7 ± 2.0	195.8 ± 0.8	102.6 ± 1.2	75.3 ± 1.4
11s+normal maize starch (4%+8%)	204.2 ± 2.8	345.8 ± 3.0	154.8 ± 2.6	74.4 ± 0.5
11s+normal maize starch (8%+8%)	485.8 ± 1.0	608.2 ± 1.0	381.6 ± 2.8	73.4 ± 0.2
Soy protein isolate (8%)	11.5 ± 2.0	23.8 ± 1.6	12.6 ± 1.0	nd
soy isolate+normal maize starch (2%+8%)	120.0 ± 1.8	195.5 ± 1.2	100.1 ± 1.2	76.7 ± 1.4
soy isolate+normal maize starch (4%+8%)	279.5 ± 8.6	354.5 ± 9.0	164.1 ± 5.4	74.8 ± 2.0
soy isolate+normal maize starch (8%+8%)	605.5 ± 1.0	567.1 ± 0.8	389.2 ± 2.0	72.4 ± 0.5

[a] Measured in Rapid ViscoAnalyzer units.
[b] Values were the average of three replicates of each sample.
[c] Not detectable.

Table 2. Pasting properties of waxy maize starch and soy proteins

Sample	Peak Viscosity (RVU)[a]	Final Viscosity (RVU)[a]	Setback (RVU)[a]	Pasting Temperature (°C)
Waxy maize starch (8%)	194.2 ± 2.2[b]	93.3 ± 1.0	15.5 ± 1.0	70.4 ± 0.2
7s (8%)	17.4 ± 1.2	2.6 ± 0.7	0.7 ± 0.2	94.0 ± 1.5
7s+waxy maize starch (2%+8%)	216.7 ± 2.8	99.4 ± 4.0	24.3 ± 2.0	72.3 ± 0.2
7s+waxy maize starch (4%+8%)	229.8 ± 1.2	135.7 ± 3.4	46.9 ± 3.0	73.1± 0.5
7s+waxy maize starch (8%+8%)	352.1 ± 1.0	317.3 ± 1.2	196.6 ± 2.0	73.7 ± 0.2
11s (8%)	4.1 ± 1.0	6.9 ± 1.2	3.0 ± 0.8	nd[c]
11s+waxy maize starch (2%+8%)	207.9 ± 1.5	100.3 ± 1.2	26.0 ± 2.0	71.2 ± 1.1
11s+waxy maize starch (4%+8%)	230.1 ± 1.8	151.1 ± 2.4	52.7 ± 2.1	72.0 ± 0.4
11s+waxy maize starch (8%+8%)	220.6 ± 2.2	266.3 ± 2.1	150.7 ± 2.0	72.7 ± 0.5
Soy protein isolate (8%)	11.5 ± 2.0	23.8 ± 1.6	12.6 ± 1.0	nd
soy isolate+waxy maize starch (2%+8%)	224.3 ± 3.0	114.4 ± 2.7	24.0 ± 2.3	71.7 ± 0.4
soy isolate+waxy maize starch (4%+8%)	254.8 ± 2.5	176.3 ± 3.0	67.8 ± 2.0	71.9 ± 0.3
soy isolate+waxy maize starch (8%+8%)	369.6 ± 1.1	341.9 ± 1.8	133.0 ± 1.5	74.0 ± 0.2

[a] Measured in Rapid ViscoAnalyzer units.
[b] Values were the average of three replicates of each sample.
[c] Not detectable.

Table 3. Pasting properties of potato starch and soy proteins

Sample	Peak Viscosity (RVU)[a]	Final Viscosity (RVU)[a]	Setback (RVU)[a]	Pasting Temperature (°C)
Potato starch (8%)	711.0 ± 4.0[b]	252.2 ± 8.6	61.6 ± 3.4	63.2 ± 0.3
7s (8%)	17.4 ± 1.2	2.6 ± 0.7	0.7 ± 0.2	94.0 ± 1.5
7s+potato starch (2%+8%)	226.3 ± 5.8	273.7 ± 6.3	111.2 ± 9.8	65.1 ± 1.0
7s+potato starch (4%+8%)	336.5 ± 6.0	283.6 ± 7.1	172.5 ± 8.8	66.0 ± 0.5
7s+potato starch (8%+8%)	753.0 ± 2.4	471.1 ± 5.0	262.7 ± 6.2	66.4 ± 0.4
11s (8%)	4.1 ± 1.0	6.9 ± 1.2	3.0 ± 0.8	nd[c]
11s+potato starch (2%+8%)	234.9 ± 6.0	265.0 ± 5.2	109.4 ± 6.0	64.4 ± 0.4
11s+potato starch (4%+8%)	322.3 ± 3.0	388.0 ± 4.8	175.3 ± 4.0	64.8 ± 0.4
11s+potato starch (8%+8%)	622.8 ± 2.0	613.1 ± 3.8	229.8 ± 2.5	66.0 ± 0.5
Soy protein isolate (8%)	11.5 ± 2.0	23.8 ± 1.6	12.6 ± 1.0	nd
soy isolate+potato starch (2%+8%)	341.8 ± 4.8	277.3 ± 3.8	83.8 ± 6.7	64.7 ± 0.4
soy isolate+potato starch (4%+8%)	567.8 ± 5.0	394.5 ± 4.2	282.2 ± 8.6	65.6 ± 0.5
soy isolate+potato starch (8%+8%)	859.0 ± 2.0	636.3 ± 9.2	464.6 ± 4.8	65.1 ± 0.2

[a] Measured in Rapid ViscoAnalyzer units.
[b] Values were the average of three replicates of each sample.
[c] Not detectable.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to my major professor Dr. Jay-lin Jane for her guidance, patience and invaluable training in science field and support through my study.

I thank my committee members, Dr. Tao Chang, Dr. Ted Bailey, Dr. Lawrence Johnson, and Dr. Patricia Murphy, for guiding me to be a good scientist, for their understanding and encouragement through my study.

Special thanks to my friend Milica and her family for their understanding and support during my hard times, and for cherishing our friendship. Many thanks to all lab-friends in our group, especially Vincent, Yungyung, Perminus, Kris, Hanming, Hsin-Chi, Herman, Ting-Jang, and Kit-sum to teach me the techniques on starch analysis, how to use the equipments in the pilot plant and how to use computer softwares. Thank all others taught me different culture backgrounds in each of their own ways. Many thanks to all my old classmates and friends in China, who gave me their great support in soul and trust all the time, and shared the experiences during oversea study.

I deeply thank my former Chinese professors for their trust and recognition, for providing positive suggestions to strengthen my courage to overcome difficulties, to understand people with a great heart, and to finish my study. Thank my relatives for their care and support. Also, a special thank to my dear young sister, for her forgiveness on me leaving her alone, for her understanding about my dreams, for supporting me with inspiring messages, and for sharing her pride with me.