Effects of amylose and amylopectin on the functional properties of starch

by

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ABBREVIATIONS

G	acceleration due to gravity
Ap	amylopectin
Am	amylose
B.V.	blue value
cps	centipoises
°C	degrees Celsius
DP	degree of polymerization
DMSO	dimethylsulfoxide
g	gram
h	hour
HA-5	high amylose corn starch V
HA-7	high amylose corn starch VII
HCl	hydrochloric acid
l	liter
µg	microgram
mg	milligram
ml	milliliter
mm	millimeter
mM	millimoles/liter
mV	millivolt
min	minute
M	moles/liter

nm	nanometer
N	normality
CHO	total carbohydrate
KOH	potassium hydroxide
KI	potassium iodide
K_2HPO_4	potassium phosphate, dibasic
KH_2PO_4	potassium phosphate, monobasic
rep	replication
rpm	revolutions per minute
H_2SO_4	sulfuric acid
U	units
WxM	waxy maize
w/w	weight/weight

INTRODUCTION

Starches of different botanical origins have different functional properties. For example, normal corn starch, after being cooked, produces an opaque paste which is not sticky and is referred to as a short paste. The paste eventually sets to a strong gel. Waxy corn and potato starches, on the other hand, produce clear but sticky long pastes, and the pastes have less tendency to set to gels (Swinkels, 1985). Both normal and waxy corn starches have gelatinization temperatures of about 62°-72°C which is significantly higher than those of wheat and potato starches (59°-68°C) (Lineback, 1984). Furthermore, chemical structures of amylose and amylopectin have been reported to be different among the starch species. Studying the molecular size of various amyloses showed that cereal amyloses appear to be smaller than tuber amyloses (Takeda and Hizukuri, 1987). The branch-chain-length distributions of various amylopectins were studied by using isoamylase and followed by high performance liquid chromatography (H.P.L.C). Results showed that the branch-chain-length distributions differed among amylopectins isolated from different botanical sources (Hizukuri, 1985).

Even though the differences in functional properties of starches of different varieties and the distinctions of starch molecular structures have been known for a long time, effects of structures of amylose and amylopectin on the functional properties of starch pastes are not known.

This study is to investigate the correlation between molecular structures and functional properties of starch. Reconstituted starches,

prepared by mixing various amyloses and amylopectins of different structures and at different proportions, were used in this study. Gel strength, viscosity, and clarity of the reconstituted starch pastes were investigated. The objectives of the study are to: 1) categorize the effects of structures on properties of starch, and 2) provide a guideline for molecular geneticists to develop starches with tailored properties.

LITERATURE REVIEW

General Properties of Starch

Starch is a storage polysaccharide in plants. It is initially formed in the amyloplast. The storage site of starch varies from plant to plant. It may be in the seed (cereal grains), in the root and tuber (tapioca and potato), in the stem-pith (sago), and in the fruit (banana). No matter where the starch is stored, it is laid down in a granule that is characteristic of its botanical origin (Lineback, 1984). Potato starch granules are large, oval in shape, 15-100 μm in diameter, with pronounced oyster-shell-like striations. Corn starch granules are medium sized, round or polygonal in shape, and 15 μm in diameter. Rice starch granules are small, polygonal, and 3-8 μm in diameter.

Gelatinization of starch granules

When starch granules are progressively heated above 60°C in water, the granules undergo irreversible swelling, and lose the birefringent properties (Guilbot and Mercier, 1985). The process is known as gelatinization of starch granules. Gelatinization is merely a process of breaking inter- and intra-molecular hydrogen bonds and hydrophobic interactions within the starch granule (Hari et al., 1989). Generally, it happens over a range of temperature and is a characteristic of the variety of starch (Lineback, 1984). The temperature range over which the swelling of all the granules occurs is known as the gelatinization temperature range. The gelatinization temperature range of potato starch, for

example, is 59°-68°C but that of corn starch is 62°-72°C. In the case of high amylose corn starch, the starch granule only swells to a limited degree at 100°C. It does not gelatinize until 125°C (Greenwood, 1976; French, 1975). The suggested reason was that the longer branches in the amylopectin of high amylose corn starch enable firmer association of the amylopectin molecules with each other and with amylose and, therefore, increase resistance of the granule to swelling and gelatinization (Montgomery et al., 1961).

The extent of gelatinization also depends on other factors. Sugar increases gelatinization temperature of starch and greatly extends the temperature range. Disaccharides are more effective in delaying gelatinization than are monosaccharides (Osman, 1972). Competition for available water between starch and sucrose (Derby et al., 1975; Hoseney et al., 1977), inhibition of granular hydration (D'Appolonia, 1972), and sucrose-starch interactions (Osman, 1975) have all been proposed to explain the suppression of starch gelatinization by sucrose. A study using ^{13}C nuclear magnetic resonance (NMR) suggested that sugar-starch interactions occurred during heating just before the onset temperature of starch gelatinization which was measured by differential scanning calorimetry (Hansen et al., 1989). The study also pointed out that potential interaction sites on sucrose were C-1, C-5, and C-6 carbons of the glucose moiety and C-1, C-3, and C-6 carbons of the fructose moiety. The addition of monoacylglycerols which have fatty acid components of 16-18 carbon atoms causes an increase in gelatinization temperature (Whistler and Daniel, 1985). Iodides and thiocyanates, on the other hand,

tend to lower the gelatinization temperature (Sterling, 1978). Under alkaline conditions, starch also gelatinizes at a lower temperature (Zobel, 1984).

Three different criteria have been used to determine the gelatinization temperature: loss of birefringence, increase in optical transmittance and rise in viscosity (Leach, 1965). More recently, differential scanning calorimetry (Eliasson and Karlsson, 1983; Nakazawa et al., 1984; Krueger et al., 1987) and hot stage microscopy (Leszczynski, 1987) methods have been employed to detect the gelatinization temperature of starch.

Clarity of starch pastes

As the starch granules swell in water, the opaque suspension gradually becomes clear. The clarity of paste is, therefore, related to the condition of dispersion of starch (Leach, 1965). Generally, pastes of potato, tapioca, and waxy maize starches are transparent. Corn and wheat starches, in contrast, give opaque pastes. Many factors affect the clarity of the paste. For example, sugars greatly increased clarity of pastes of cereal starches, such as corn starch, but surfactants, such as glyceryl monostearate, made the pastes much more opaque (Osman, 1967). Studies done by Craig et al. (1989) demonstrated that addition of sucrose to starch pastes increased %T and visual clarity, and decreased whiteness. The clarity of starch solution also relates to the retrogradation of the starch in that retrograded starch solutions are more opaque (Swinkels, 1985).

Viscosity of starch pastes

Further heating in water above gelatinization temperature causes greater swelling of starch granules. The swollen granules gradually disperse and form a viscous paste. The viscosity of the starch paste increases with the temperature until the "peak viscosity" is reached. This increase in viscosity is caused by the swollen granules being sufficiently close together to have their movement restricted (Osman, 1972). After the viscosity reaches its peak, the cohesive forces in the original granular structure become excessively weakened and the viscosity of the paste gradually breaks down as the integrity of the granules is lost (Greenwood, 1976). The change of the viscosity during the swelling and the subsequent disruption of the granule can be readily followed by using a Brabender Amylograph. Methods for measurement and interpretation of Brabender amylogram have been thoroughly studied to determine starch properties (Elder and Schoch, 1959; Greenwood, 1964; Sandstedt and Abbott, 1964; Zobel, 1984). Recently, use of a Rapid Visco-Analyzer method to study starch pasting characteristics was reported (Walker et al., 1988; Deffenbaugh and Walker, 1989a; 1989b).

The viscosity of starch paste is affected by the variety of starch, the preparation procedure, and the starch concentration. Generally, potato starch paste shows a higher viscosity than any other starches because of the polyelectrolyte behavior of the phosphate group on potato starch (Banks and Greenwood, 1975). Starch solutions of higher concentration also exhibit a higher viscosity.

Retrogradation

Some physical changes will occur when freshly gelatinized starch paste is aged. If a dilute starch solution stands for a prolonged time, it gradually becomes cloudy and eventually forms a white precipitate. If a concentrated starch dispersion, such as a hot 7% (w/w) corn starch paste, is allowed to cool, it will set to a gel. Both cases are the process of retrogradation (Schoch, 1969). As applied to starch, French (1975) defined the term retrogradation as a return from a solvated, dispersed, amorphous state to an insoluble, aggregated or crystalline condition.

Some changes to starch pastes occur during retrogradation. The changes include: 1) formation of crystallites which resist enzymatic hydrolysis; 2) decrease in light transmission; 3) loss of the ability to form a blue complex with iodine (Collison, 1968). These afford sensitive tools to follow the retrogradation (Matsunaga and Kainuma, 1986).

Rate of retrogradation depends on starch varieties, starch concentration, temperature, pH, and salts. Different starches retrograde at different rates. Normal cereal starches seem to retrograde more quickly than tuber starches (Collison, 1968). The rate of retrogradation also increases with increasing starch concentration because less swelling and structural disorientation happen when the starch concentration is increased (Collison and Elton, 1961). Temperature has a marked effect on the retrogradation rate. Retrogradation is greatly enhanced by a lower temperature at about 0° to 5°C (French, 1975). On the other hand, the existence of complexing agents, such as n-butyl alcohol, which react

mainly with the amylose molecule to form helical complexes during gelatinization, prevents retrogradation of starch (Matsunaga and Kainuma, 1986).

Fractionation of Starch

The most important progress in the study of starch was the separation of starch into its major components, amylose and amylopectin. Several methods have been developed to fractionate starches: selective precipitation of amylose (Schoch, 1942b; French et al., 1963), aqueous leaching (Banks and Greenwood, 1975), and chromatographic separation (Ebermann and Schwarz, 1975; Kobayashi et al., 1985; Jackson et al., 1988; Matheson and Welsh, 1988; Kamath et al., 1989).

Schoch (1942b) first reported the selective precipitation technique for starch fractionation. The method involved n-butyl alcohol (or other alcohol)-amylose complex formation during slow cooling of a hot aqueous starch solution saturated with n-butyl alcohol. Steps in the starch fractionation include: (1) dispersion of starch by autoclaving, (2) selective precipitation of amylose through a complex formation with n-butyl alcohol or other alcohol, (3) separation of the complex by centrifuge, (4) purification of the complex by washing and recrystallization. Repeated crystallization from aqueous butyl alcohol gives a product of amylose with high purity. The material remaining in solution consists primarily of amylopectin which can be recovered by adding an excess of methyl alcohol. The efficiency of a complexing agent for starch fractionation can be evaluated on the basis of yields and

purities of the fractions. Since the iodine reaction is a very sensitive qualitative and quantitative test for amylose (Bates et al., 1943; Lansky, 1949), the potentiometric iodine titration has been widely used to evaluate the efficiency of starch fractionation.

Minor components of starch, such as fatty acids, affect the efficiency of starch fractionation. Because of the acidity imparted by the presence of free fatty acid, raw corn starch pastes were found to undergo slight hydrolysis during autoclaving (Schoch, 1942a). Also, lipids in native starches increased the resistance of starch granules to swell and then affected dispersion of starch granules (Morrison and Coventry, 1989). Therefore, it was suggested that starches be defatted by successive extraction with hot 85% methanol. In addition, lipid extraction was reported to exceed 90% of total lipids with one two-hour extraction of maize starch at 100°C when using not less than 14 ml of 75-85% methanol per g of starch (Morrison and Coventry, 1985). Studies on lipids of rice starch demonstrated that the starch lipids of non-waxy rice were mainly free fatty acids and lysophospholipids, and defatting with water saturated with butanol at 25°-27°C could reduce phosphorus content (Maniñgat and Juliano, 1980). It was also reported that lysophospholipids in wheat starch were extracted with 65% butyl alcohol (Acker and Schmitz, 1967a; 1967b). Pretreatment of cereal starches with DMSO prior to fractionation will also remove fatty acids (Greenwood, 1976).

Fractionation of high amylose corn starch would be difficult by the Schoch's method. The problem is that this starch is difficult to disperse by autoclaving (French, 1975). Montgomery et al. (1961) reported that the

problem was caused by the molecular structures of amylopectin. The study done by Mercier et al. (1970) also demonstrated that the swelling properties of high amylose corn starch granules decreased with increasing amylose content. As a result, fractionation of high amylose corn starch requires a special pretreatment to disrupt the granular organization and to form aqueous dispersions. Several pretreatments have been developed to achieve this goal. Those methods include solubilization in alkali (Potter et al., 1953), treatment with liquid ammonia (Wolff et al., 1955), and solubilization in DMSO (Adkins and Greenwood, 1969).

The solubilization of granular starches in DMSO not only disrupts the granular structure, but also has the advantage of eliminating lipid material (Greenwood, 1976). The solubility of granular starches in DMSO is greatly increased by the presence of small amounts of water. Leach and Schoch (1962) suggested that 90% DMSO solution was the best solvent. In proof of this view, they demonstrated that viscometric studies showed no molecular degradation of corn starch solution in 90% DMSO after prolonged standing.

Major Components of Starch

Starch is composed of two major types of polysaccharide molecules, amylose and amylopectin. Studies have shown the existence of the third component in some starches (Lansky et al., 1949; Peat et al., 1952; Perlin, 1958; Erlander and French, 1958). Banks and Greenwood (1975) have isolated five to seven percent of such material from cereal and potato starches. Based on the physicochemical properties of these

fractions, they suggested that the fractions have an intermediate range of structures between the structures of amylose and amylopectin.

Amylose content is different with different starch types (Lineback, 1984). For example, corn starch contains about 28% amylose and potato starch has about 23%. In general, normal starches contain 17 to 28% amylose; high-amylose starches contain 45 to 69% amylose (Greenwood and Thomson, 1962; Banks et al., 1973). Waxy starches, however, contain nearly 100% amylopectin (Schannon and Garwood, 1984).

Amylose

Amylose used to be known as a linear macromolecule containing glucose units with α-1,4 linkages. Each macromolecule bears one reducing end and one non-reducing end. However, enzymatic studies indicated that the amylose molecule has a minor degree of branching by α-1,6 linkages as in amylopectin (French, 1975; Hizukuri et al., 1981; Takeda and Hizukuri, 1986). The slightly branched amylose molecules may contain 3-20 chains (Hizukuri et al., 1981; Takeda and Hizukuri, 1987) that are characteristic of their origins. Amylose covers a range of degree of polymerization (DP), which is defined as the number of glucose residues per reducing end group and is dependent on the starch varieties. Amylose of potato starch has a DP about 6000 glucose units (Hizukuri et al., 1981). Amylose of high-amylose corn starch, on the other hand, has a DP about 700 (Takeda et al., 1989). In general, the cereal amyloses appear to be smaller than other amyloses.

Amylose can form complexes with certain organic compounds. Schoch

(1942b) demonstrated that amylose can form a precipitate with n-butyl alcohol. Whistler and Hilbert (1945) proposed that any water-soluble compound possessing either donor or acceptor groups capable of hydrogen-bond formation was a suitable complexing agent. They found that nitropropane, nitroethane, amyl acetate, methyl ethyl ketone, butyl mercaptan, and pyridine could form complexes with amylose. Cyclic alcohols and phenols were also able to form complexes with amylose (Haworth et al., 1946; Bourne et al., 1948). Some non-polar molecules, such as benzene and aliphatic hydrocarbon were also reported to form complexes with amylose (French et al., 1963).

Amylose can also form complexes with DMSO, fatty acids, various surfactants, and iodine. It is believed that the structure of these amylose complexes, known as amylose-V complexes, are helical, with the polysaccharide enclosing the complexing agent (Rundle and French, 1943; Osman et al., 1961; Hoover and Hadziyev, 1981; Eliasson and Krog, 1985; Jane et al., 1985). Besides, the amylose-KOH complex is an extended amylose helical complex (Sarko, 1985). Its structure is based on a more stretched-out helix in which there is no channel in the center of the helix. On the other hand, the amylose-DMSO complex was proposed to be a more compact helix (Jane et al., 1985).

Amylose-iodine complex gives a characteristic blue color. It is a very sensitive qualitative and quantitative test for the amylose component (Bates et al., 1943; Lansky et al., 1949). Consequently, potentiometric iodine titration was widely used to evaluate the efficiency of starch fractionation. Moreover, the iodine reaction can give some clues as to

the relative chain length of amylose (Bailey and Whelan, 1961). Under the standard conditions, chains less than 12 glucose units long give no colors. Those from 14 to 38 units give a red to purple color with a peak at 490 nm to 555 nm. Followed by transitional colors, the color becomes blue with a peak at 568 nm when a length of 45 units is reached. In addition, chains as long as 400 glucose units gave a peak absorption spectra closely at 645 nm.

Amylose has a tendency to retrograde and is considered primarily responsible for retrogradation of starch. The retrogradation reaction is characterized by a lag period followed by a markedly accelerated phase, then by a slow approach to a limit (Loewus and Briggs, 1957). The retrogradation rate of amylose is largely dependent on chain length. A maximum rate of retrogradation was found at DP of 80-100 (Pfannemüller et al., 1971; Gidley and Bulpin, 1989). Pfannemüller et al. (1971) also suggested the presence of a well ordered, rigid structure in retrograded amylose at DP around 80. Jane and Robyt (1984) proposed the presence of crystalline and amorphous regions in retrograded amylose. The crystalline regions are double helices and are interspersed with amorphous regions.

Amylopectin

Amylopectin is the branched component of starch. The D-glucopyranose residues of amylopectin are connected mainly by α-1,4 linkages with 5-6% of α-1,6 linkages at the branch points. The average branched-chain length is 20 to 25 glucose units (Manners, 1985). However, the average branched-chain length of high amylose corn amylopectin is above 30 (Greenwood and

Mackenzie, 1966; Hizukuri et al., 1983; Hizukuri, 1985). Studies have shown the relationship between the molecular structure of amylopectin and the crystalline structure of the starch granule (Hizukuri et al., 1983; Hizukuri, 1985). It was found that A-type starch had shorter branched-chain length than B-type starch.

The organization of chains of amylopectin is conveniently divided into three types (Peat et al., 1956). The A chains, without carrying any other chain, are linked to the molecule only by the reducing end, while B chains are similarly linked but also carry one or more A chains or other B chains. The C chain is the one that bears the reducing end group. The ratio of A chains to B chains is one method being used to characterize the structure of amylopectin. The ratio in rice amylopectins was reported to be 1.1 to 1.5 (Marshall and Whelan, 1974; Umeki and Yamamoto, 1977; Asaoka et al., 1985; Enevoldsen and Juliano, 1988).

Structures of amylopectin have been studied extensively with specific enzymes and the gel filtration chromatographic method since the 1970s. The exact arrangement of chains within the amylopectin molecule is still not clear. A cluster type structure for amylopectin has been proposed by French (1973) to account for the high viscosity of amylopectin (Figure 1). Since then, somewhat similar cluster structure models were reported, based on studies of chain length distribution of amylopectin (Figure 2) (Robin et al., 1974; Manners and Matheson, 1981; Hizukuri, 1986). The general features of these models are that amylopectin is composed of clusters which are randomly or somewhat regularly branched, and the clusters are connected by long B chains which extend into two or more clusters. At

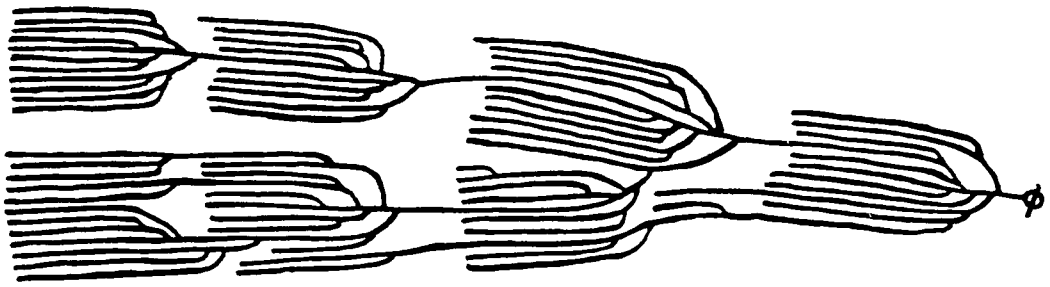

Figure 1. A cluster structure of amylopectin (French, 1973)

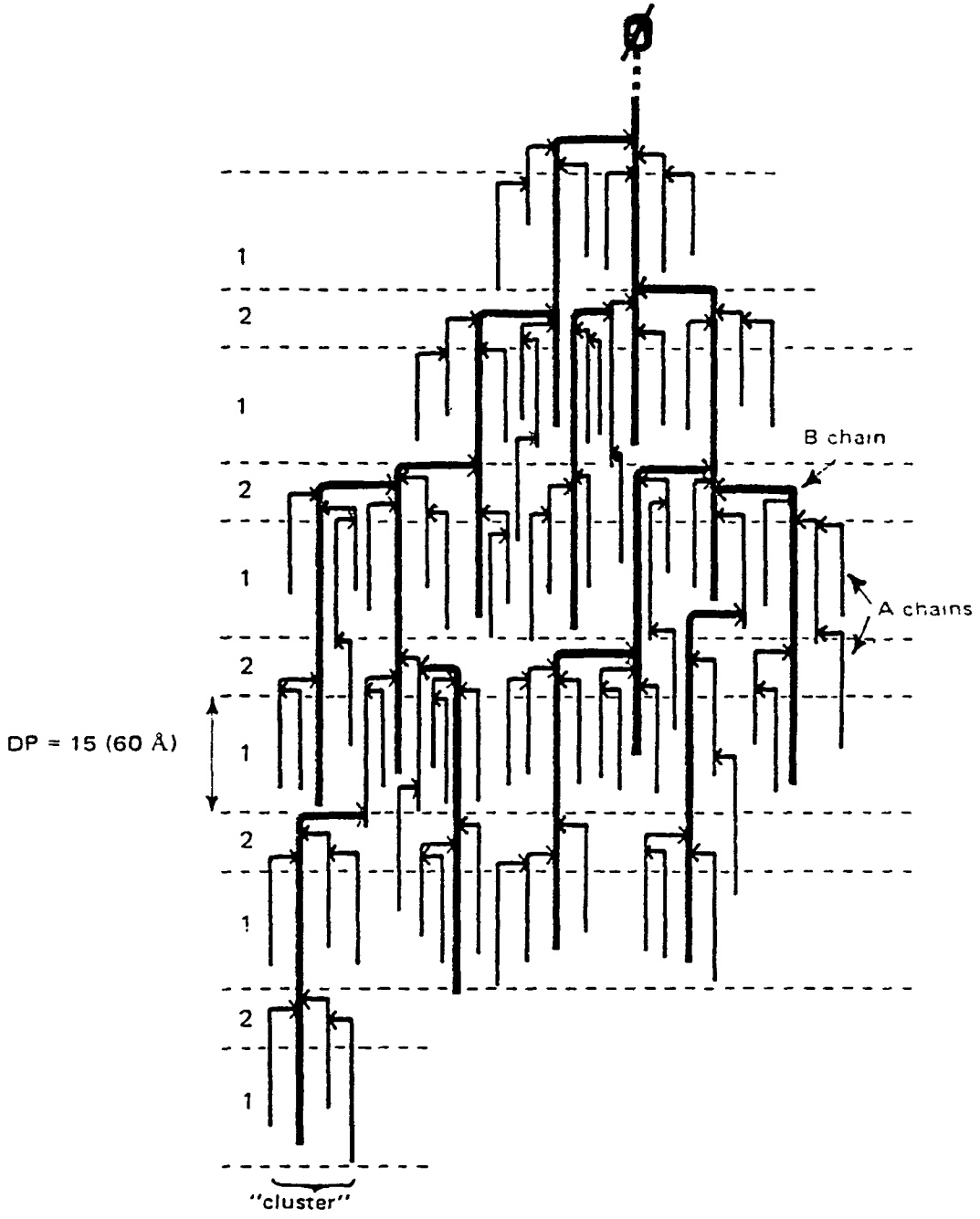

Figure 2. Proposed structure for potato amylopectin. 1 = compact area; 2 = less compact area, rich in branching points; ϕ = reducing unit (Robin et al., 1974)

present, the cluster model is the most widely accepted one.

Because of short branch chain-lengths, amylopectin gives only a weak violet-red color when it reacts with iodine. Under normal potentiometric titration conditions, the iodine binding capacity of amylopectin is very small. However, the uptake of iodine by the branched amylopectin is increased at low temperature (Banks and Greenwood, 1975). For example, the iodine binding capacity of waxy maize amylopectin is 0.1% at 20°C, and 0.15% at 1.5°C.

Some starches contain a measurable phosphate content, most of which is found as an ester in the amylopectin fraction. Studies on phosphate derivatives of Japanese rice starch indicated that the phosphorus content of waxy rice starch granules is mainly glucose 6-phosphate ester and phospholipid in non-waxy granules (Tabata et al., 1975). Potato amylopectin contains more ester phosphate than any other amylopectins. The distribution of esterified phosphate in potato amylopectin was estimated to be 38% on the C-3, and 61% on the C-6 of glucose residues (Tabata and Hizukuri, 1971). The ester phosphate confers the properties of a polyelectrolyte on amylopectin and affects the viscosity of starch solutions (Greenwood, 1960; Banks and Greenwood, 1975).

MATERIALS AND METHODS

Materials

Starches

Normal corn, potato, and rice starches were purchased from Sigma Chemical Company (St. Louis, MO). Waxy maize starch was a gift of A. E. Staley Manufacturing Co. (Decator, IL). HA-5 corn and HA-7 corn starches were gifts of National Starch and Chemical Co. (Bridgewater, NJ).

Enzyme

Crystalline *Pseudomonas* isoamylase (EC 3.2.1.68) was a product of Hayashibara Shoji, Inc. (Okayama, Japan). The specific activity was about 59,000 units per mg protein. The enzyme was used directly without further purification.

Gel chromatography

Bio-Gel P-6 and Sepharose CL-2B were purchased from Bio-Rad Laboratories (Richmond, CA) and Pharmacia Inc. (Piscataway, NJ), respectively.

Chemical reagents

Other chemicals were all reagent grade and were used without further treatment.

Methods

Defatting of starches

Various starch samples were defatted by the method of Schoch (1942a). Starches were extracted with 85% methyl alcohol for 24 h in the Soxhlet extractor. Rice starch was defatted with 65% n-butyl alcohol because of its lysophospholipids (Acker and Schmitz, 1967a; 1967b). After extraction, starch was washed with methyl alcohol and dried in an oven at 40°C.

Pretreatment of high amylose corn starch

High amylose corn starch (20 g) was wetted with 40 ml distilled water and dispersed with 360 ml DMSO. The DMSO solution was heated in a boiling water bath with stirring for 1 h and kept stirring at room temperature for another 24 h. Two volumes of methyl alcohol (800 ml) was added to precipitate the starch from the DMSO solution. The precipitant was removed by centrifuging at 4400 G for 20 min and washed twice with methyl alcohol.

Fractionation of starch

Fractionation of starches followed primarily the method of Schoch (1942b). Although waxy maize starch contains nearly 100% amylopectin (Schannon and Garwood, 1984), it was treated with the same fractionation procedure to be consistent with other samples. Amyloses were fractionated from normal corn, potato, and HA-7 corn starches. Amylopectins were isolated from normal rice, waxy maize, and HA-5 corn starches. The method involved following steps:

Dispersion of the starch Twenty grams of defatted starch or pretreated high amylose corn starch were suspended with 1500 ml water in a

2000 ml-flask. The starch suspension was heated in a boiling water bath with stirring until the starch was gelatinized. The solution was then filtrated with a Whatman No.4 filter paper to remove undissolved residues. The pH of the starch suspension was then adjusted to 5.9-6.3 by adding a phosphate buffer solution which consists of anhydrous KH_2PO_4 (16.4%) and anhydrous K_2HPO_4 (3.6%) before autoclaving (Lansky et al., 1949). The flask containing the gelatinized starch sample was covered with aluminum foil and autoclaved for 3 h at 121°C.

Flocculation After being autoclaved, the starch suspension was heated under reflux in a boiling water bath with stirring for 2 h to completely disperse the starch granules. Fifteen to twenty percent (by volume) of n-butyl alcohol was carefully added and stirred for another hour. The mixture was then transferred to a duwar flask previously warmed up with boiling water. The duwar flask was sealed with a lid and the hot starch-butyl alcohol mixture inside slowly cooled down to room temperature over a period of 24 to 36 h. The slowly cooling period allowed the amylose-butyl alcohol complex to grow and precipitate.

Separation Separation of crude amylose-butyl alcohol complex was achieved by centrifuging at 8700 G, 5°C for 30 min. The precipitant was the crude amylose-butyl alcohol complex. Amylopectin remained in supernatant was recovered by treating with methyl alcohol.

Purification of amylose and amylopectin

Amylose Purification of amylose is achieved by recrystallization. The crude amylose-butyl alcohol precipitate was rinsed with cold water

saturated with n-butyl alcohol. The rinsed precipitant was then suspended in boiling water, stirred under reflux, and followed the procedures of flocculation and separation described above. After being recrystallized twice, the precipitant was treated with methyl alcohol, stirred for 30 min, and then centrifuged at 4400 G, 0°C for 20 min. This washing procedure was repeated twice. The isolated amylose was then dissolved in 90% DMSO. Concentration of the amylose in DMSO solution was determined by measuring total carbohydrate. Purity of the amylose was examined by the gel-filtration chromatography on Sephrose CL-2B (Colonna and Mercier, 1984) and the potentiometric titration method (Schoch, 1964).

Amylopectin After the amylose-butyl alcohol complex was removed, the supernatant in which the amylopectin was dissolved was concentrated 2 times using a rotary vacuum evaporator. The concentrated amylopectin solution was then treated following the procedures of flocculation and separation. After being recrystallized twice, the supernatant was mixed with 2 volumes of methyl alcohol and was kept at 4°C overnight to precipitate the amylopectin. The precipitant was removed by centrifuging at 8700 G, 5°C for 30 min. The pure amylopectin was then washed twice with methyl alcohol and solubilized in DMSO. Concentration of the amylopectin was also determined. The purity of amylopectin was determined by gel-filtration chromatography on Sephrose CL-2B. The measurement of branch-chain length was achieved by enzymatic hydrolysis. After being debranched, the branch-chain length distribution was examined by gel-filtration on Bio-Gel P-6.

Preparation of reconstituted starches

Exact amounts of amylose and amylopectin in DMSO solutions were mixed and stirred overnight at room temperature. The mixture was precipitated with 2 volumes of methyl alcohol. The precipitant was removed from the solution by centrifuging at 8700 G for 15 min and washed twice with methyl alcohol. The isolated reconstituted starch was then redissolved in 0.5M KOH solution and stirred at constant speed for 1 h at 4°C. After being neutralized with 6N HCl to pH 5.8-6.0, the starch sample was diluted to a desired concentration for analysis.

Viscosity

A paste (3%, w/w) of reconstituted starch was used for this analysis. The paste was prepared by heating a starch suspension in a boiling water bath with stirring for 20 min and then cooled to 30°C. The heating rate and the stirring speed were kept constant. After heating, the paste was cooled and kept in a water bath at 30°C. After the temperature was equilibrated, the viscosity of the sample was determined by using the Brookfield viscometer (model LV) (Brookfield Engineering Laboratories, Inc., Stoughton, MA). The U.L. Adapter which consists of a cylindrical spindle rotating inside a tube was used for the analysis. The spindle and the tube were pre-equilibrated at 30°C by immersing in a water bath. An aliquot (20 ml) was transferred into the tube and dipped directly in the bath at 30°C. The viscosity was recorded at the spindle speed of 30 rpm. The viscosity of a blank which contains only neutralized KOH solution was also measured.

Light transmittance (%T)

Solutions (1%, w/w) of reconstituted starches were used for this study. Measurements of light transmittance of starch solutions were carried out by the method of Craig et al. (1989). The starch solution was kept in a screwcap tube and heated in a boiling water bath for 30 min. The tube was thoroughly shaken every 5 min. After cooling to room temperature, the transmittance at 650 nm was measured against a water blank using a Spectronic 21 spectrophotometer (Milton Roy Company, Rochester, NY).

Gel strength

Pastes (8%, w/w) of reconstituted starches were used for the gel strength determination. The paste was prepared by heating and stirring a starch suspension in a boiling water bath for 20 min. The heating rate and the stirring speed were kept consistent. After heating, the sample was transferred into aluminum pans which were wrapped with aluminum foil around the wall to increase the depth of the pans. The starch gel was set at room temperature for 7 h. Before measuring the gel strength, the aluminum foil was removed and the gel above the top of the pan was sliced off carefully to prepare a smooth surface. Gel strength of the reconstituted starch was determined by using the Voland Texture Analyzer (Voland Corp., Hawthorne, NY). The TA 53 punch probe was used and the test distance was 3 mm. On operating, the probe descended and the force (in grams) was recorded on the chart recorder when the probe started to touch the sample. The probe continuously penetrated into the gel until the pre-set distance was reached. The peak recorded reflected the gel strength.

The strength of starch gel which had been set at 5°C for 72 h was also measured and compared with that of the gel set for 7 h at room temperature. The difference of the strength was used as an index of retrogradation of starch gel.

Determination of amylose content by potentiometric titration

Analysis of amylose contents using potentiometric titration was done by the procedure of Schoch (1964). An appropriate amount of amylose (approximately 50 mg) was precipitated from DMSO solution by using methyl alcohol. The precipitate was washed twice with methyl alcohol. The sample was transferred to a 250 ml beaker which had been tared. Five ml of 1.0N KOH solution was added. The mixture was kept at 4°C for 30 min, and then neutralized with 0.5N HCl. To the mixture were added 10 ml of 0.5N KI solution, and sufficient water at 30°C to give a total weight of 100.9 g over the weight of the empty beaker. The sample was then titrated with iodine at 30°C with mechanical stirring. An Orion Research digital pH/mV meter (model 501) (Orion Research Inc., Boston, MA) was used and millivolt readings were recorded at 12 to 15 different points which had values between 230 to 280 mV. From the millivolt readings, the concentration of free iodine in the sample solution was determined with a standard curve which was constructed by titrating a mixture of KCl (373 mg) and KI (830 mg) in 100 ml of distilled water. The bound iodine was calculated from the difference between the total amount of iodine used as titratant and the free iodine. The iodine affinity of the sample at each point was estimated by multiplying the bound iodine by 100, and dividing it by the dry weight

of the sample. Free iodine (X) was then plotted against iodine affinity (Y). The upper linear portion of this curve was extrapolated back to intersect the Y axis. This value was the iodine affinity of the sample. The purity of the sample was calculated by dividing the iodine affinity of the sample by 20% which is the theoretical value of iodine affinity of pure amylose (Banks and Greenwood, 1975).

Enzymatic debranching of amylopectin

Amylopectin (about 50 mg) dissolved in DMSO was precipitated with methyl alcohol. After centrifuging, the precipitant was suspended in 9 ml of distilled water and heated with stirring in a boiling water bath for 1 h. The suspension was cooled to room temperature, and 1 ml of 0.1M acetate buffer (pH 3.5), and 9000 U of crystalline Pseudomonas isoamylase were added. The mixture was incubated for 48 h in a shaker bath (Versa-Bath S, model 236, Fisher Scientific) at 40°C, 120 strokes/min for debranching. Completion of debranching reaction was confirmed by gel-permeation chromatography on Bio-Gel P-6. The debranched sample was subjected to total carbohydrate and reducing end-groups analyses. The average chain length was then calculated by dividing the total carbohydrate by reducing value.

Determination of total carbohydrate by phenol-sulfuric acid method

The determination of total carbohydrate was carried out by the method of Dubois et al. (1956). One ml of debranched amylopectin sample solution was immersed in a boiling water bath for a few minutes to terminate the enzyme reaction and then diluted to 50 ml. An aliquot (1 ml) of the

diluted solution was mixed with 5% phenol solution (1 ml). Five ml of concentrated sulfuric acid was quickly added to generate heat. After being properly mixed, the solution was kept for 30 min at room temperature. The absorbance at 470 nm was determined by using Spectronic 21 spectrophotometer (Milton Roy Company, Rochester, NY). A standard curve was constructed with solutions containing 10-100 μg of glucose.

Analysis of reducing end-groups using modified Park-Johnson's method

An aliquot (1 ml) as described above was mixed with 0.5 ml of sodium carbonate-sodium hydrogencarbonate buffer containing potassium cyanide (4.8 g of Na_2CO_3, 9.2 g of $NaHCO_3$, and 0.65 g of KCN/l of water) and 1 ml of ferricyanide solution (0.5 g/l of water) (Hizukuri et al., 1981). Reaction would not be completed without the ferricyanide solution which was not cited by Hizukuri et al. (1981). The mixture was then heated for exactly 15 min in a vigorously boiling water bath. After cooling to room temperature under running tap-water, 2.5 ml of ferric ammonium sulphate solution (3 g/l of 50 mM H_2SO_4) was added under effective ventilation and kept for 20 min at room temperature. The absorbance of the resulting blue solution was measured at 715 nm by using Spectronic 21 spectrophotometer (Milton Roy Company, Rochester, NY). A standard curve was plotted by measuring solutions containing 1-5 μg of glucose.

Gel-permeation chromatography on Sepharose CL-2B

Amylose or amylopectin (about 30 mg) was precipitated from DMSO solution with 2 volumes of methyl alcohol. After centrifuging, the precipitant was suspended in 10 ml of water and heated in a boiling water

bath with stirring for 30 min. The sample solution was then cooled to room temperature and glucose (about 1.5 mg) was added as a marker. Five ml of aliquot was injected into a 2.6x80 cm column (Pharmacia Inc., Piscataway, NJ) packed with Sepharose CL-2B gel. The sample was eluted in an ascending direction and distilled water containing 0.02% sodium chloride was used as an eluent with a flow rate of about 30 ml/h. Fractions of 4.8 ml per cup were collected and analyzed by using the Autoanalyzer II (Technicon Instruments Corp., Elmsford, NY). Responses of amylose-iodine blue value and total carbohydrate (anthrone-sulfuric acid reaction) were measured at 640 nm and 630 nm, respectively. Solutions containing 3, 4, and 5 mg of glucose per 100 ml solutions were also analyzed as standards of total carbohydrate. The purity of amylose and amylopectin was calculated on the basis of total carbohydrate.

Gel permeation chromatography on Bio-gel P-6

The debranched sample solution (2 ml) was heated in a boiling water bath to terminate the enzyme reaction. After cooling to room temperature, the solution was neutralized with KOH (0.75M). Glucose solution (160 μg) was added as a marker. The sample was then injected into a 1.5x80 cm Bio-Rad Econo-column (Bio-Rad Laboratories, Richmond, CA) packed with Bio-gel P-6. Samples were eluted in a descending direction with deionized water as an eluent. Fractions of 2.3 ml per cup were collected and analyzed by the Autoanalyzer II for total carbohydrate content. Response of total carbohydrate was determined at 630 nm. The chain length distribution was measured by the method described above.

RESULTS AND DISCUSSION

Measurement of Physical Properties of Corn Starch as a Reference

To evaluate the effects of amylose and amylopectin on functional properties of starch, corn starch pastes with different treatments were prepared and used as references. Our analysis showed that native normal corn starch consisted of 27% amylose and 73% amylopectin. Reconstituted corn starch paste was, therefore, prepared by mixing 27% corn amylose and 73% corn amylopectin. Partially fractionated corn starch paste was prepared by fractionating native corn starch; however, the amylose-butyl alcohol complex was not removed from the amylopectin. After removing the n-butyl alcohol by vacuum evaporating, the amylose and amylopectin suspension was treated with methyl alcohol to precipitate the mixture and separated by centrifuging. The precipitant was then washed twice with methyl alcohol and dissolved in DMSO solution. Native corn starch paste was prepared by dissolving the starch in 90% DMSO solution. Viscosity, transmittance, and gel strength of the pastes were determined following the procedures described previously. The results are shown in Table 1.

Table 1. Viscosity, transmittance, and gel strength of corn starch pastes with different treatments

	Reconstituted	Partial fractionated	Native
Viscosity (cps)	11.97±0.20	12.70±0.17	>19
Transmittance (%)	80.5±0.5	79.7±0.3	80.3±0.3
Gel strength (g)[a]	2.7±0.4	2.9±0.2	6.0±0.8
Gel strength (g)[b]	7.1±0.3	7.0±0.3	9.0±0.5

[a]Fresh gel set at room temperature for 7 h.

[b]Aged gel set at 4°C for 72 h.

Amylose and Amylopectin

Potentiometric titration curves of isolated amylose samples are shown in Figures 3 to 5. By extrapolating the curve back to intersect the Y axis, the iodine affinities of potato, corn, and HA-7 amyloses are 19.3%, 18.6%, and 19.0%, respectively. The purity of samples was then calculated as 96.5%, 93%, and 95%, respectively. The gel-permeation column chromatography on Sepharose CL-2B was also used to examine the purity of the amyloses (Colonna and Mercier, 1984). Any contaminating amylopectin would be in the void volume. The elution profiles of amylose samples are shown in Figures 6 to 8. In comparison with the response of amylose, the peak of contaminating amylopectin was very small. The purity of amyloses,

based on the calculation of total carbohydrate response under amylose peak and void volume, are 90.2%, 96.2%, and 96.2% for potato, corn, and HA-7 amyloses, respectively. The measured purities of amyloses were thus somewhat different with these analytical methods. The low purity of potato amylose calculated from gel-permeation chromatography may be attributed to some large amylose molecules being eluted at void volume. The samples, however, were considered to contain sufficiently high percentages of the linear fraction and were used as pure amyloses in this study.

Molecular sizes of the amyloses used in this study are different. This is shown in Sepharose CL-2B gel filtration profiles (Figures 6 to 8). Since a large molecule will be eluted first from the column, the ratio of fraction number of amylose peak to that of glucose marker can be used to compare the molecular sizes of amyloses. The larger the ratio, the smaller the molecule. The ratios of potato, normal corn, and HA-7 amyloses were 0.77, 0.82, and 0.85, respectively. The molecular sizes of the amyloses were reported to have a DP of 4000 to 6000 (Hizukuri et al., 1981), a DP of 930 to 990 (Takeda and Hizukuri, 1987; Takeda et al., 1988), and a DP of 690 (Takeda et al., 1989) for potato, normal corn, and HA-7 amyloses, respectively. Therefore, they were used as large, intermediate, and small molecular amyloses, respectively.

Figure 3. Potentiometric titration curve of potato amylose. The iodine affinity is 19.3%

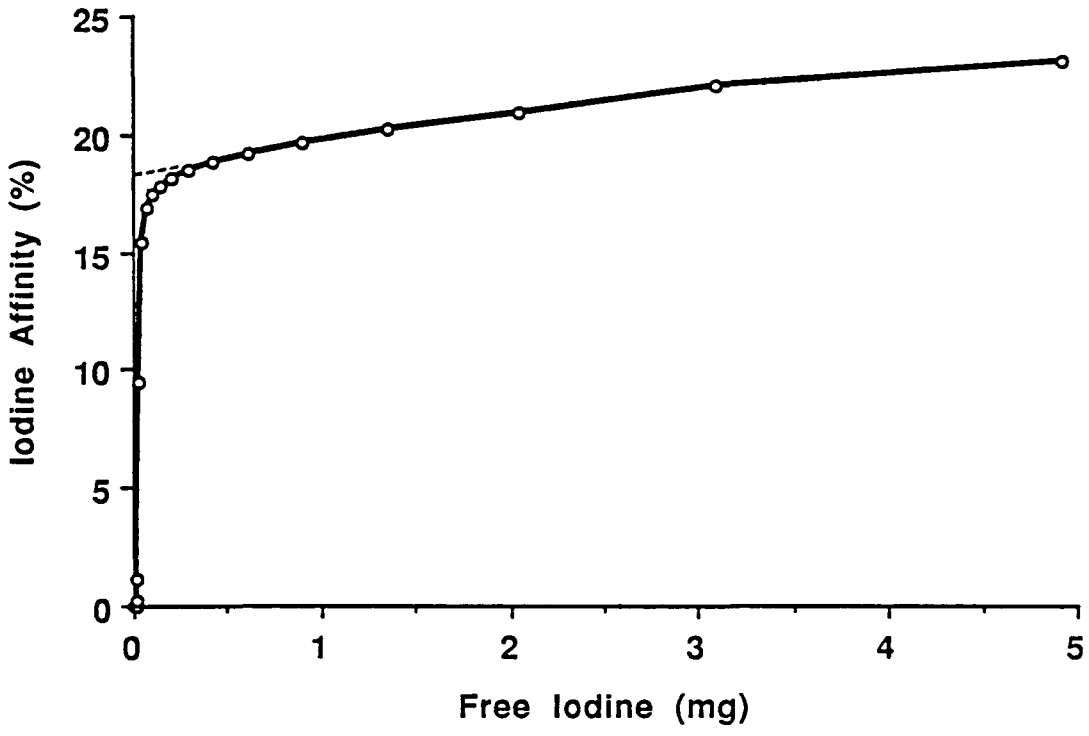

Figure 4. Potentiometric titration curve of normal corn amylose. The iodine affinity is 18.6%

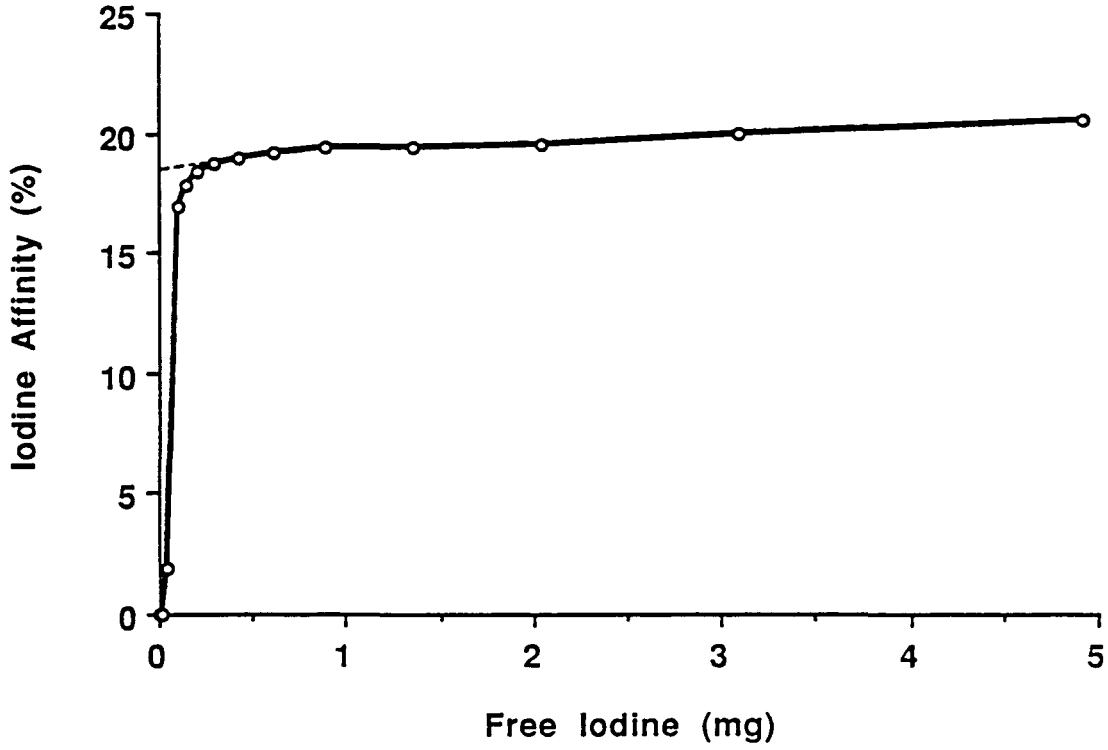

Figure 5. Potentiometric titration curve of HA-7 amylose. The iodine affinity is 19.0%

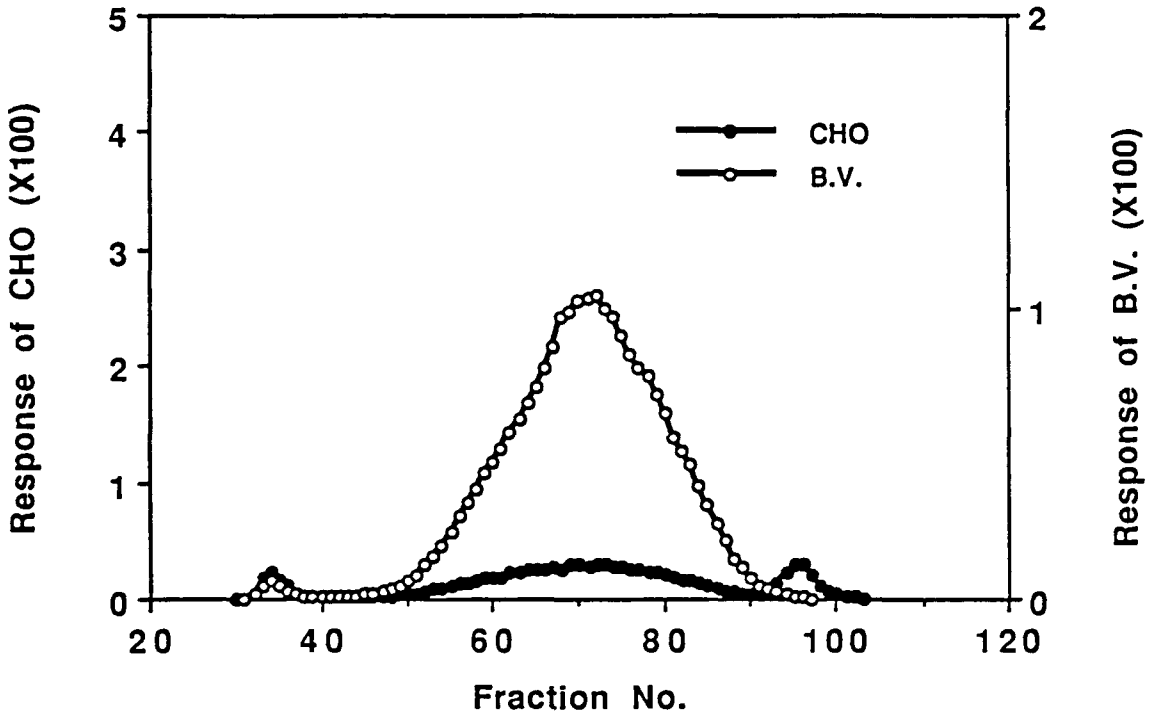

Figure 6. Sepharose CL-2B gel filtration profile of potato amylose

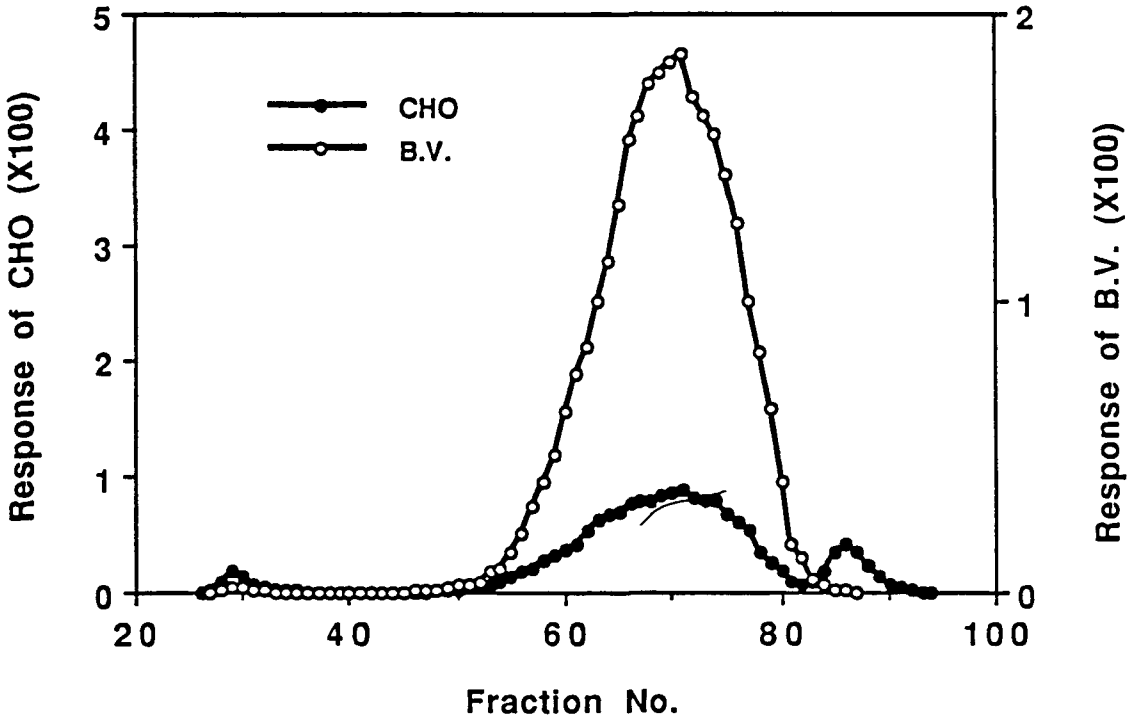

Figure 7. Sepharose CL-2B gel filtration profile of normal corn amylose

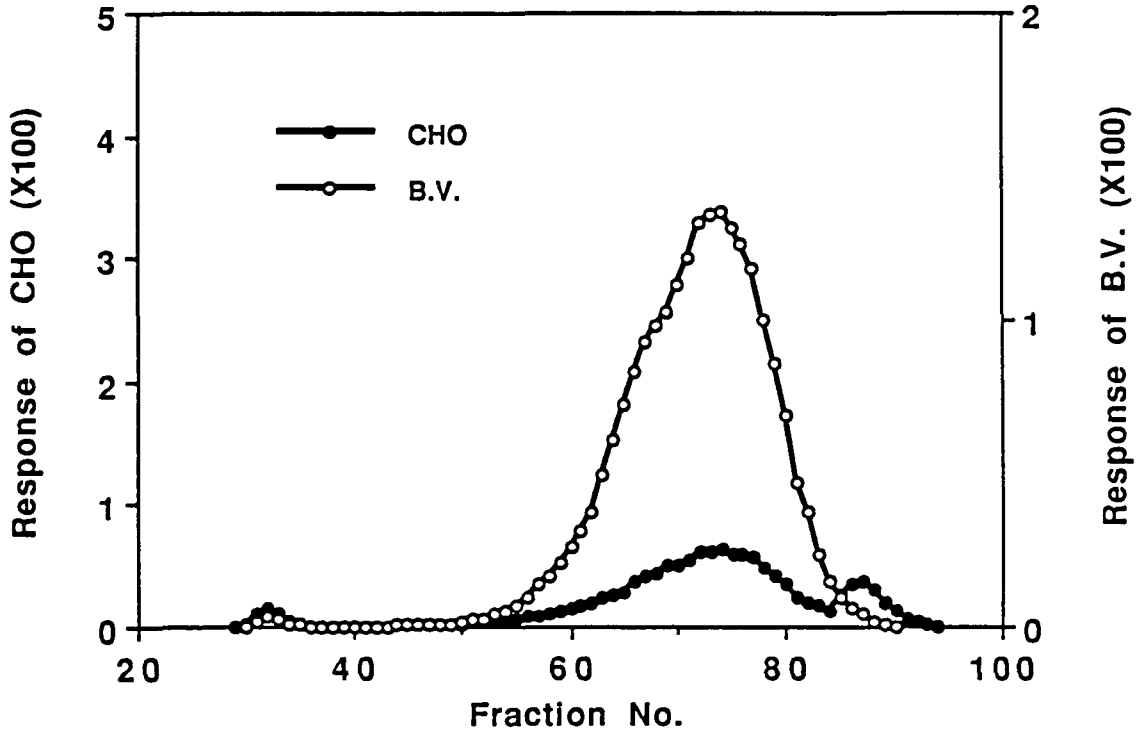

Figure 8. Sepharose CL-2B gel filtration profile of HA-7 amylose

Purity of the amylopectin was also examined by gel-permeation column chromatography on Sepharose CL-2B (Figures 9 to 11). The elution profiles revealed that some oligosaccharides were present with amylopectin. The branch-chain lengths of amylopectin were determined by debranching enzyme hydrolysis. Table 2 shows average chain lengths of amylopectins debranched by Pseudomonas isoamylase.

Table 2. Average branch chain-length[a] of amylopectins debranched by Pseudomonas isoamylase

Sample	Incubation time (h)		
	24	48	72
HA-5 amylopectin	47.8	33.1	32.2
Waxy maize starch	26.9	23.3	22.8
Rice amylopectin	29.8	22.5	22.8

[a]Average branch chain-length was reported as DP.

Debranching reaction was completed within 48 h because the branch chain-lengths did not decrease significantly after 48 h incubation. The average branch chain-lengths were consistent with those reported elsewhere (Greenwood and Mackenzie, 1966; Hizukuri et al., 1983; Hizukuri, 1985). Gel-permeation chromatograms of debranched amylopectins showed bimodal distributions (Figures 12 to 14).

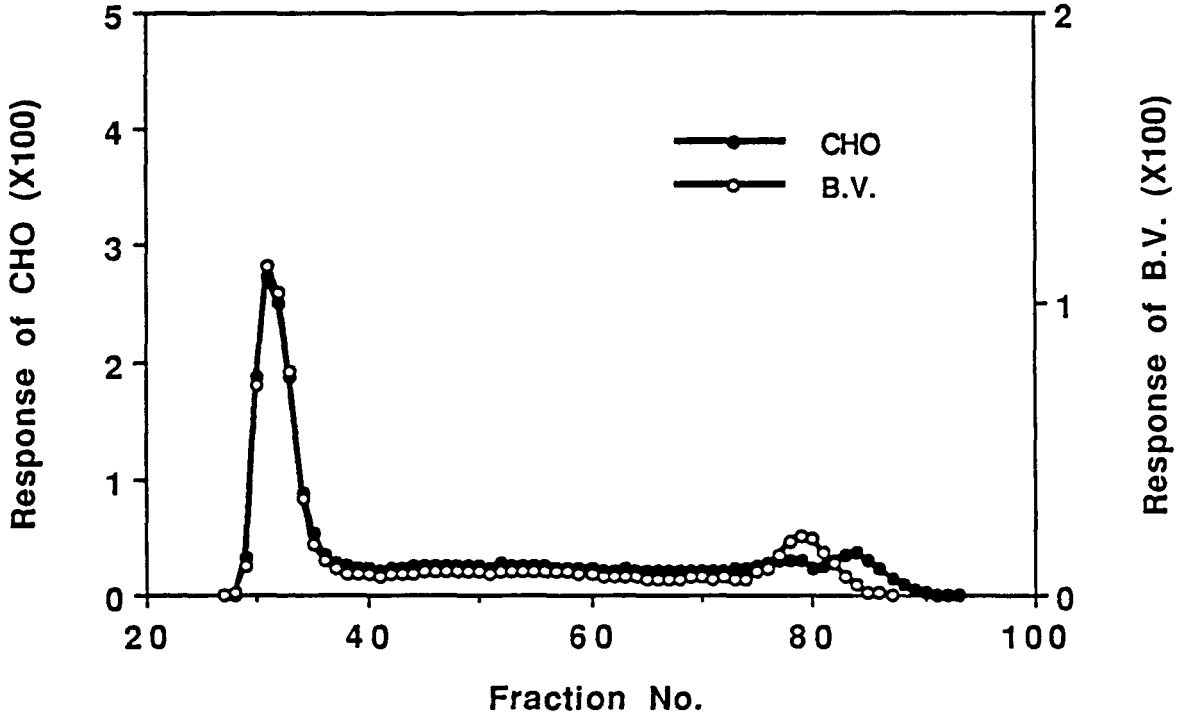

Figure 9. Sepharose CL-2B gel filtration profile of HA-5 amylopectin

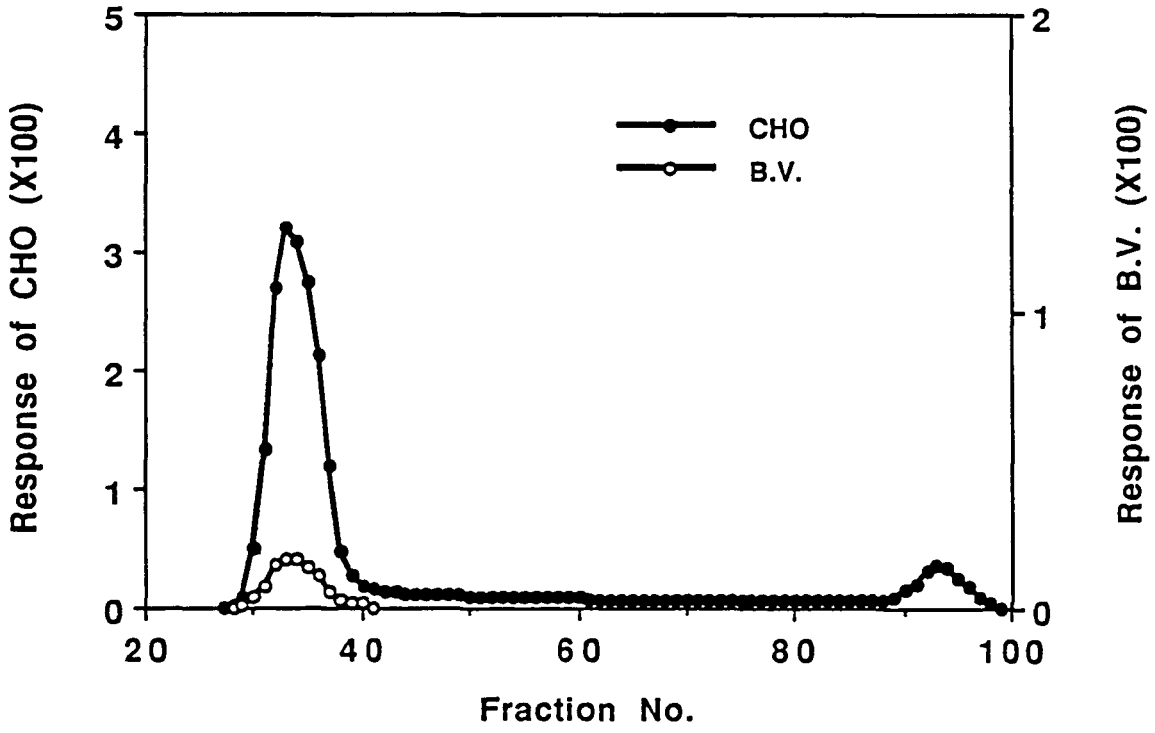

Figure 10. Sepharose CL-2B gel filtration profile of waxy maize starch

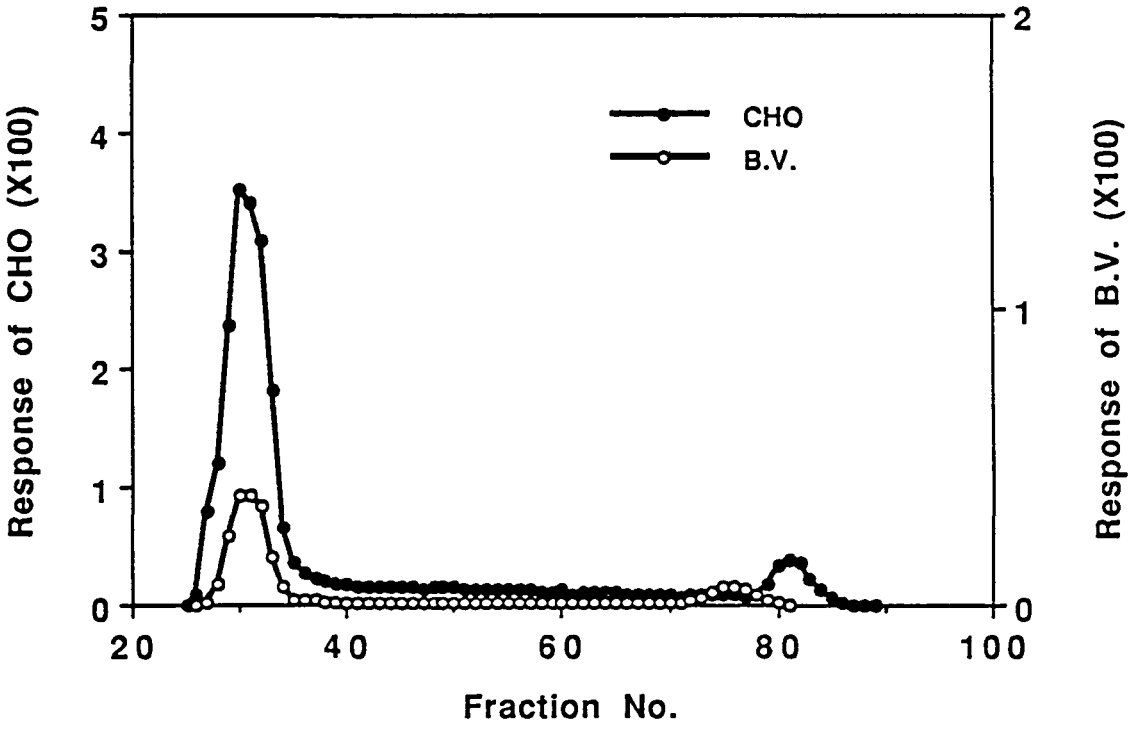

Figure 11. Sepharose CL-2B gel filtration profile of rice amylopectin

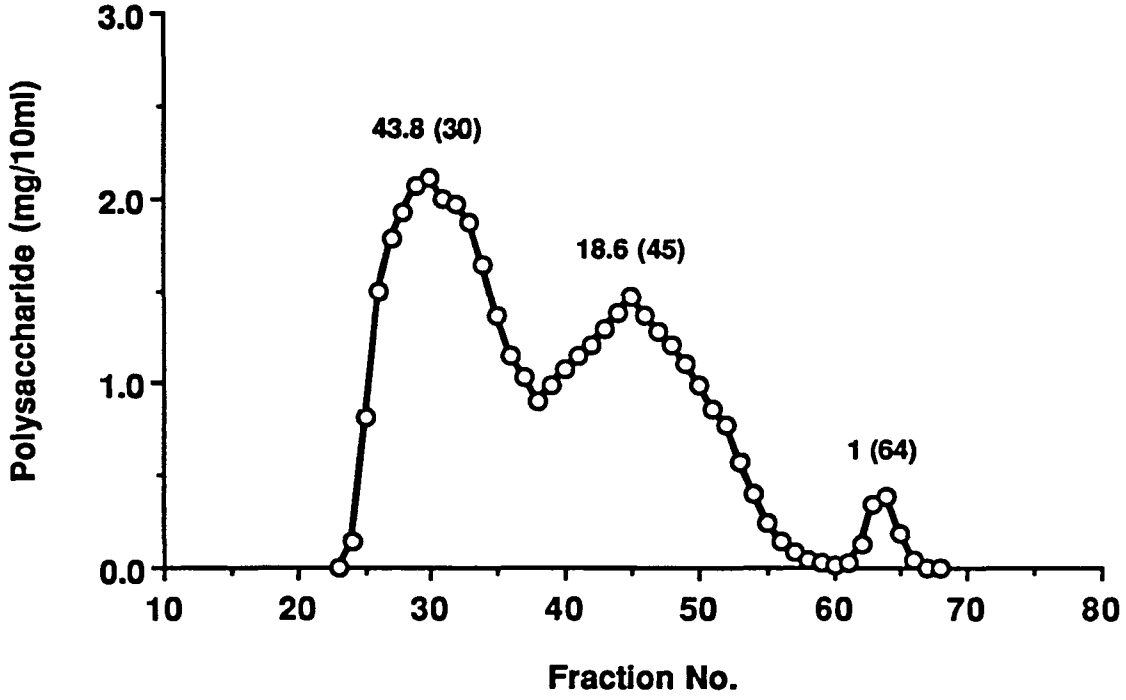

Figure 12. Bio-gel P-6 gel filtration profile of debranched HA-5 amylopectin. The chain length of a peak is given right above the peak. The fraction number is given in the parenthesis. The peak of fraction 64 is glucose maker

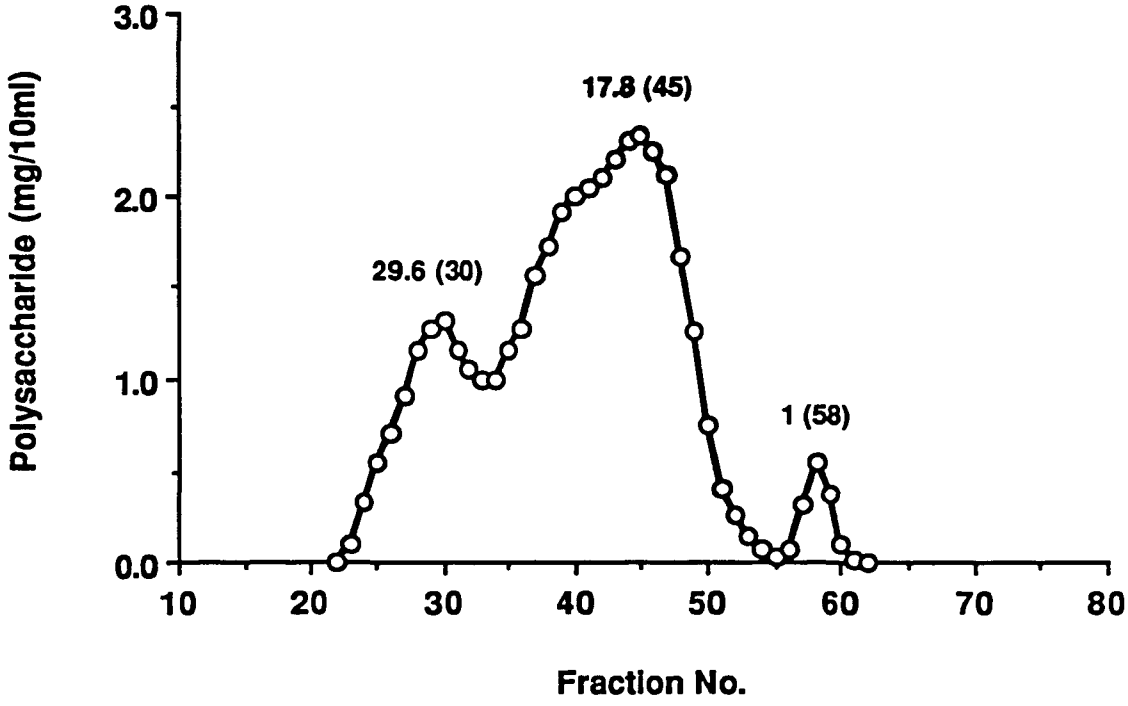

Figure 13. Bio-gel P-6 gel filtration profile of debranched waxy maize starch. The chain length of a peak is given right above the peak. The fraction number is given in the parenthesis. The peak of fraction 64 is glucose maker

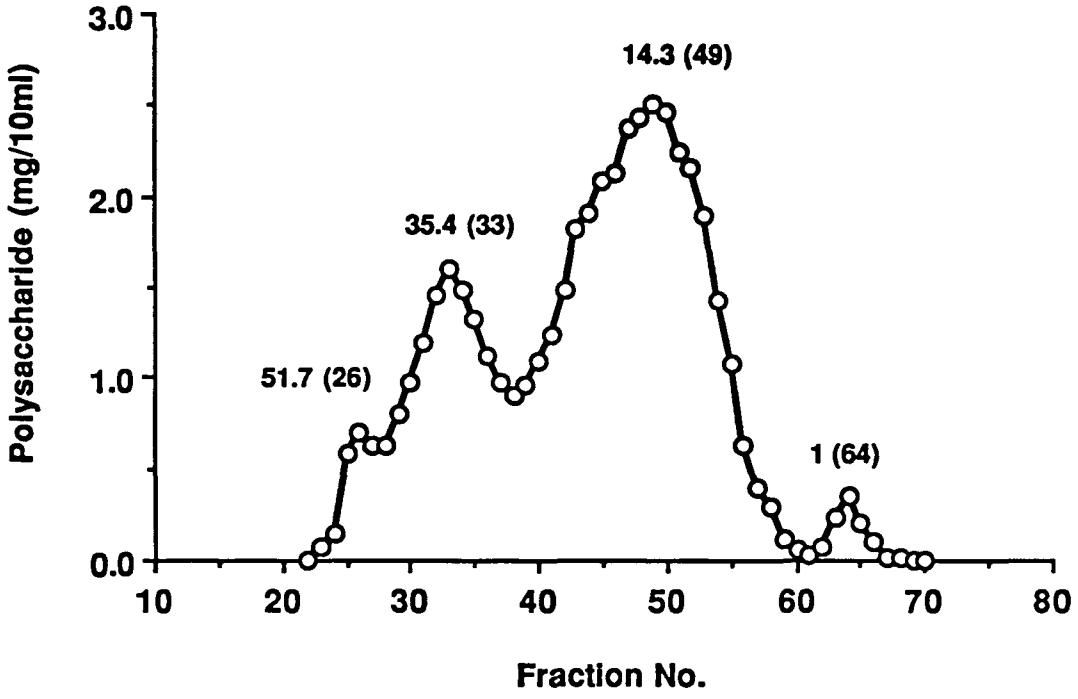

Figure 14. Bio-gel P-6 gel filtration profile of debranched rice amylopectin. The chain length of a peak is given right above the peak. The fraction number is given in the parenthesis. The peak of fraction 64 is glucose maker

Similar distributions have been reported by Hizukuri (1985). However, the profile of rice amylopectin showed a peak at the void volume (4% of total carbohydrate) (Figure 14). This might be the contaminating amylose which contained a long linear chain or some amylopectin which was more resistant to debranching and could not be hydrolyzed by isoamylase. As a result, it was eluted at the void volume.

Elution profiles showed that the branch-chain lengths of rice amylopectin are shorter than those of waxy maize starch. Thus, HA-5 amylopectin, waxy maize starch, and rice amylopectin were used as long branch-chain, intermediate branch-chain, and short branch-chain amylopectins, respectively.

Viscosity of Reconstituted Starches

Viscosities of 3% (w/w) reconstituted starch samples were measured. Ratios of amylose to amylopectin in the reconstituted starch were 1 to 9 and 2 to 8. Viscosities of amylose (0.3% and 0.6%) and amylopectin (2.4% and 2.7%) alone were also measured separately. Results showed that differences of the viscosities among the various amylose solutions alone were not significant ($P > 0.05$) (Table 3). However, the differences among the various amylopectin pastes were significant. The viscosity of rice amylopectin is significantly higher than those of HA-5 and waxy maize amylopectins. This might be partially attributed to the higher phosphorous content in rice amylopectin (Greenwood, 1960; Banks and Greenwood, 1975). An analysis showed that phosphorous contents were 0.033%, 0.011%, and 0.014% in rice, waxy maize, and HA-5 amylopectin samples, respectively.

In comparison with the viscosity of pastes containing amylopectin alone, the reconstituted starches in which amylopectin was partially replaced by amylose show lower viscosities with an exception of a substitution of HA-5 amylopectin by corn amylose (Table 3). Results showed that corn amylose significantly increased viscosity when mixed with HA-5 amylopectin, and this is attributed to a synergistic effect between amylose and amylopectin molecules.

Table 3. Viscosity of reconstituted starch pastes at 3% concentration measured by Brookfield viscometer at 30°C, spindle speed of 30 rpm

Ratio (%) Ap/Am	Source		Viscosity (cps)				
	Ap	Am	rep.1	rep.2	rep.3	mean	S.D
0/10	--	Potato	0.35	0.30	0.40	0.35	0.05
	--	Corn	0.30	0.40	0.30	0.33	0.06
	--	HA-7	0.25	0.30	0.30	0.28	0.03
100/0	HA-5	--	8.95	9.00	8.80	8.92	0.10
90/0	HA-5	--	6.85	6.70	6.70	6.75	0.09
90/10	HA-5	Potato	8.60	8.10	8.15	8.28	0.28
	HA-5	Corn	8.80	8.90	8.90	8.87	0.06
	HA-5	HA-7	8.20	7.80	8.30	8.10	0.26
100/0	WxM	--	7.30	7.50	7.40	7.40	0.10
90/0	WxM	--	5.90	5.80	6.00	5.90	0.10
90/10	WxM	Potato	6.90	7.00	7.00	6.97	0.06
	WxM	Corn	7.10	6.80	7.20	7.03	0.21
	WxM	HA-7	6.80	6.80	6.70	6.77	0.06
100/0	Rice	--	11.75	12.00	11.95	11.90	0.15
90/0	Rice	--	9.40	9.60	9.70	9.57	0.15
90/10	Rice	Potato	10.50	10.40	10.70	10.53	0.15
	Rice	Corn	11.50	11.20	11.40	11.37	0.15
	Rice	HA-7	11.00	10.95	10.90	10.95	0.05
0/20	--	Potato	0.65	0.60	0.55	0.60	0.05
	--	Corn	0.70	0.70	0.70	0.70	0.00
	--	HA-7	0.45	0.49	0.50	0.48	0.03
80/0	HA-5	--	5.40	5.55	5.60	5.52	0.10
80/20	HA-5	Potato	8.10	8.00	7.95	8.02	0.08
	HA-5	Corn	9.70	9.00	8.80	9.17	0.42
	HA-5	HA-7	9.00	8.70	7.90	8.53	0.57
80/0	WxM	--	4.80	4.75	4.70	4.75	0.05
80/20	WxM	Potato	6.60	6.40	6.60	6.53	0.12
	WxM	Corn	6.90	6.90	6.90	6.90	0.00
	WxM	HA-7	6.50	6.45	6.40	6.45	0.05
80/0	Rice	--	7.40	7.40	7.40	7.40	0.00
80/20	Rice	Potato	9.80	9.60	9.60	9.73	0.12
	Rice	Corn	10.70	10.60	10.75	10.68	0.08
	Rice	HA-7	9.90	10.05	10.00	9.98	0.05

The synergistic effects on viscosity were observed on all the reconstituted starches prepared in this study. It was found that when amylopectins were mixed with amyloses, the viscosity of the mixtures were greater than that expected from the sum of the viscosity of amylose and amylopectin measured alone. The percent of increase in viscosity was taken as a percent of synergistic effect (Tables 4, 5 and Figures 15, 16). It was also found that the pastes containing 20% amylose had greater synergistic effects compared to those containing 10% amylose. In addition, the synergistic effect of the pastes containing potato amylose decreased with decreasing branch chain-length of amylopectin. On the other hand, the pastes which comprised corn or HA-7 amylose gave lowest synergistic effect when mixed with waxy maize amylopectin which has an intermediate branch chain-length. Moreover, corn amylose resulted in the highest synergistic effect among the three amyloses when mixed with amylopectin, especially with HA-5 amylopectin. Among all combinations, the mixture of HA-5 amylopectin and corn amylose gave the highest synergistic effect. This fact suggests that amylopectin with long branch-chains interacts better with amylose of intermediate molecular size, perhaps through an entanglement, to give a higher viscosity. In contrast, potato amylose which has a large molecular size and HA-7 amylose which has a small molecular size do not interact strongly with amylopectin.

Table 4. Percent increase in viscosity of reconstituted starch pastes in which the ratio of amylopectin to amylose is 90% to 10%

Source		Viscosity (cps)			Percent increase in viscosity $\frac{C - (A + B)}{A + B}$ x100
Ap	Am	Ap (A)	Am (B)	Reconstituted starch (C)	
HA-5	Potato	6.75±0.09	0.35±0.05	8.28±0.28	16.62
	Corn	6.75±0.09	0.33±0.06	8.87±0.06	25.28
	HA-7	6.75±0.09	0.28±0.03	8.10±0.26	15.22
WxM	Potato	5.90±0.10	0.35±0.05	6.97±0.06	11.52
	Corn	5.90±0.10	0.33±0.06	7.03±0.21	12.84
	HA-7	5.90±0.10	0.28±0.03	6.77±0.06	9.55
Rice	Potato	9.57±0.15	0.35±0.05	10.53±0.15	6.15
	Corn	9.57±0.15	0.33±0.06	11.37±0.15	14.85
	HA-7	9.57±0.15	0.28±0.03	10.95±0.05	11.17

Table 5. Percent increase in viscosity of reconstituted starch pastes in which the ratio of amylopectin to amylose is 80% to 20%

Source		Viscosity (cps)			Percent increase in viscosity $\frac{C - (A + B)}{A + B}$ x100
Ap	Am	Ap (A)	Am (B)	Reconstituted starch (C)	
HA-5	Potato	5.52±0.10	0.60±0.05	8.02±0.08	31.05
	Corn	5.52±0.10	0.70±0.00	9.17±0.42	47.49
	HA-7	5.52±0.10	0.48±0.03	8.53±0.57	42.17
WxM	Potato	4.75±0.05	0.60±0.05	6.53±0.12	22.06
	Corn	4.75±0.05	0.70±0.00	6.90±0.00	26.61
	HA-7	4.75±0.05	0.48±0.03	6.45±0.05	23.33
Rice	Potato	7.40±0.00	0.60±0.05	9.73±0.12	21.63
	Corn	7.40±0.00	0.70±0.00	10.68±0.08	31.85
	HA-7	7.40±0.00	0.48±0.03	9.98±0.05	26.65

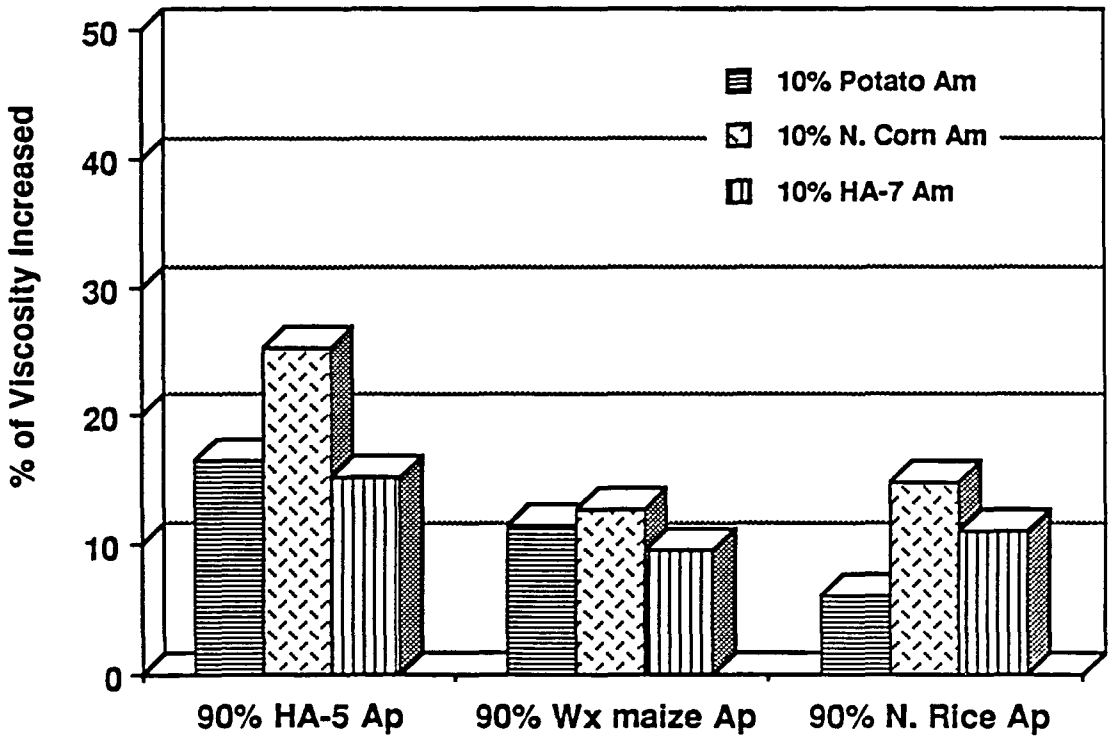

Figure 15. Percent increase in viscosity of reconstituted starches consisting of 90% amylopectin and 10% amylose

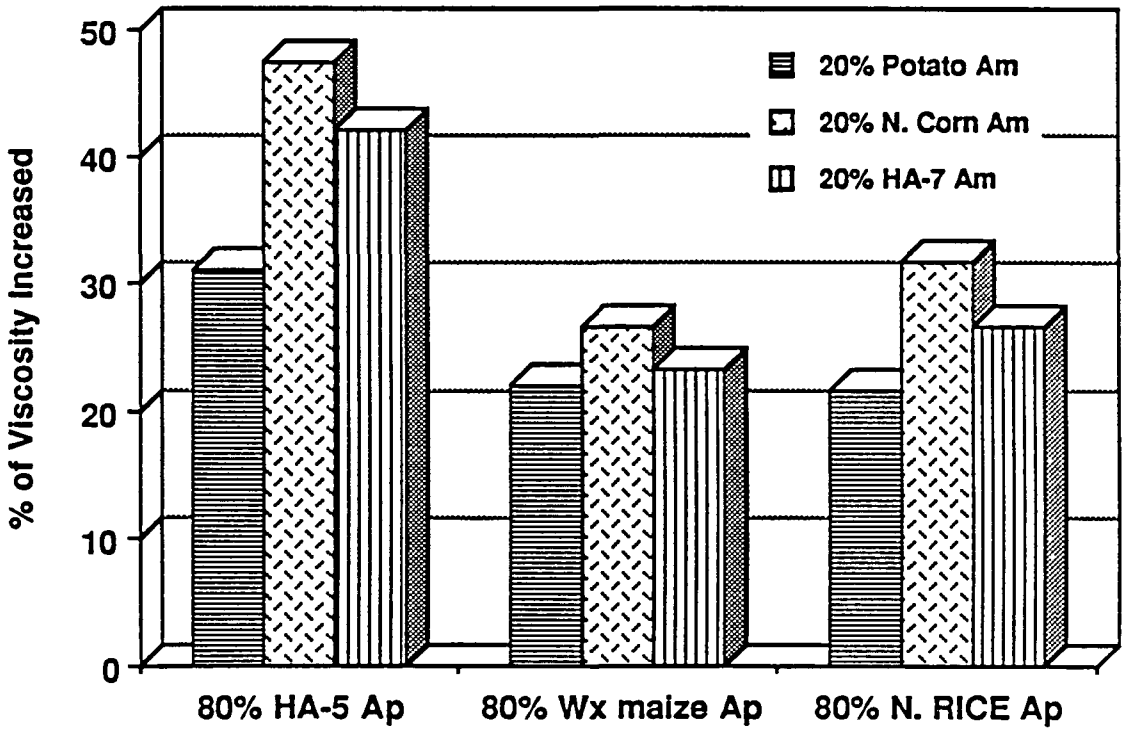

Figure 16. Percent increase in viscosity of reconstituted starches consisting of 80% amylopectin and 20% amylose

An attempt was done to measure the viscosity of the reconstituted starch containing 25% amylose (in 3% sample solution). However, retrogradation of amylose solution (0.75%) happened right after the solution was neutralized. Therefore, the viscosity of the reconstituted starch containing more than 20% amylose was not reported.

Transmittance of Reconstituted Starches

Table 6 summarizes the transmittance of reconstituted starch pastes. It has been reported that the paste clarity depends on the state of dispersion (Leach, 1965; Swinkels, 1985). Lipids in native cereal starches increase the resistance of starch granules to swell (Morrison and Coventry, 1989) and thus affect dispersion of starch granules. When lipids were added to a starch paste, the transmittance of the paste decreased (Hoover and Hadziyev, 1981). The starch samples subjected to fractionation were defatted, and granular integrity was destroyed during fractionation. Therefore, the transmittances of reconstituted starch solutions were, in general, higher than the data reported by Craig et al. (1989).

Table 6. Light transmittance of 1% reconstituted starch pastes[a]

Source		Transmittance (% at 650 nm)				
Ap	Am	rep.1	rep.2	rep.3	mean	S.D
HA-5	Potato	91.5	92.0	91.0	91.5	0.61
	Corn	88.0	86.0	86.5	86.8	1.04
	HA-7	85.5	87.5	88.0	87.0	1.32
WxM	Potato	90.0	89.0	88.5	89.2	0.76
	Corn	82.0	82.5	82.0	82.2	0.26
	HA-7	77.5	79.0	77.0	77.8	1.04
Rice	Potato	84.0	84.0	85.0	84.3	0.58
	Corn	80.0	81.5	82.0	81.2	1.04
	HA-7	69.0	67.5	69.0	68.5	0.87

[a]The ratio of amylopectin to amylose in pastes is 80% to 20%.

The results showed a trend that the transmittance decreased with the decrease in the molecular size of amyloses and the branch-chain length of amylopectins (Figure 17). Therefore, the solution of HA-5 amylopectin mixed with potato amylose showed the highest light transmittance. The mixture of rice amylopectin and HA-7 amylose, on the other hand, demonstrated the lowest transmittance. It has been reported that development of turbidity for amylose solutions depends on the molecular size of amyloses (Gidley and Bulpin, 1989). For the amyloses of DP higher than 90, both the initial rate of turbidity development and the absorbance value after setting for 400 min decreased with increasing chain length.

value after setting for 400 min decreased with increasing chain length. The increase in turbidity was the result of aggregation or retrogradation of amylose. Pfannemüller et al. (1971) also reported that retrogradation of amylose was highly dependent on molecular size. In general, amylose with DP 80 to 100 exhibits the maximum retrogradation rate. The rate decreased with increased chain length when DP is above 100. Therefore, HA-7 amylose retrogrades faster than potato amylose. Consequently, the transmittances of pastes decreased with decreasing chain length of amylose.

Takeda et al. (1986) reported that amylopectin with long branch-chains, such as HA-5 amylopectin, tended to retrograde rapidly. Rice amylopectin, on the other hand, was found to have a higher phosphate content. In light of this, the transmittance was expected to be higher with rice amylopectin and lower with HA-5 amylopectin. However, the experimental results were contrary to this conclusion. The fact suggests that some other factors affect the transmittance of the solution when amylose is mixed with amylopectin.

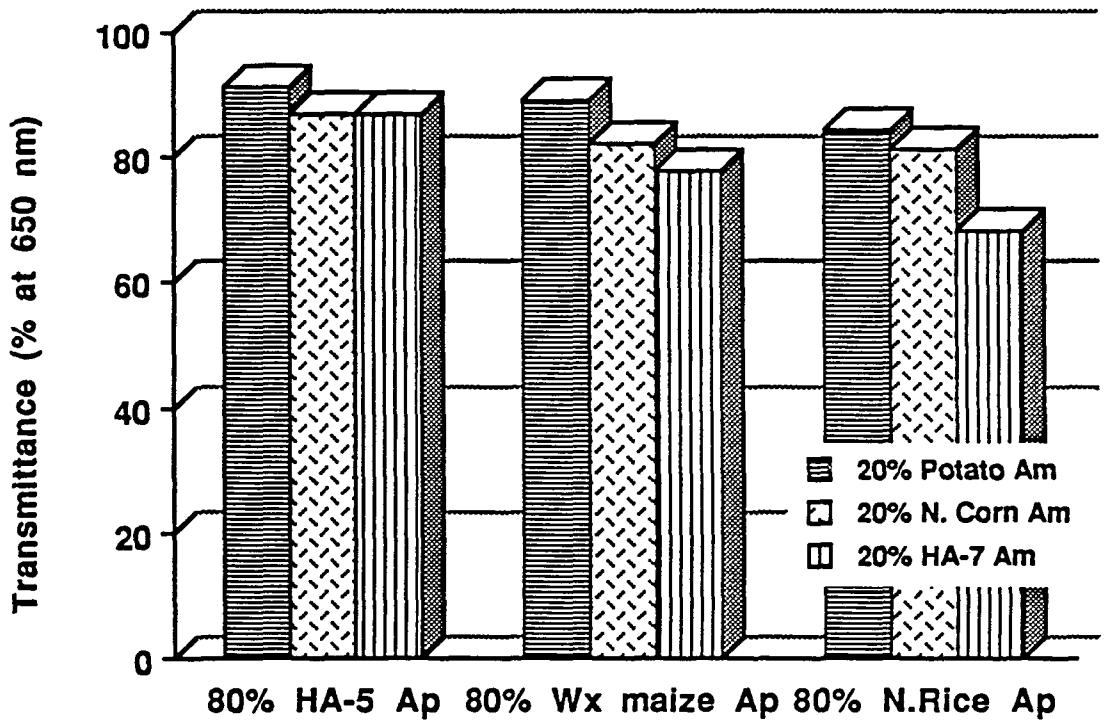

Figure 17. Light transmittance of reconstituted starches consisting of 80% amylopectin and 20% amylose

Gel Strength of Reconstituted Starches

Gel strengths of reconstituted starches are presented in Tables 7 and 8. The concentration of the gels used for this study was 8% (w/w). Results showed that the reconstituted starches containing HA-5 amylopectin exhibited a high gel strength. Those containing waxy maize amylopectin did not gel with the exception of the mixture of HA-7 amylose. The reconstituted starch consisting of waxy maize amylopectin and HA-7 amylose did not gel until setting at 4°C for 72 h, and the gel was very weak. Those pastes containing waxy maize amylopectin and potato or corn amylose never gelled even after setting at 4°C for 72 h.

It was also noticed that the gel strengths increased after setting at a refrigerating temperature for 72 h (Figures 18 to 20). This can be interpreted in terms of the retrogradation of starch gel, since starch gels became more rigid after being retrograded (Hoseney, 1986). The gels containing HA-7 amylose showed much greater increases in gel strength after the storage at 4°C. Those containing corn amylose, on the other hand, only increased a little.

Table 7. Gel strengths of reconstituted starches set for at room temperature for 7 h

Source		Gel strength (g)				
Ap	Am	rep.1	rep.2	rep.3	mean	S.D
HA-5	Potato	2.9	3.1	2.8	2.9	0.16
	Corn	7.6	8.5	7.4	7.8	0.59
	HA-7	4.4	3.4	4.1	4.0	0.51
WxM	Potato	--[a]	--	--	--	--
	Corn	--	--	--	--	--
	HA-7	--	--	--	--	--
Rice	Potato	--	--	--	--	--
	Corn	1.0	1.1	0.9	1.0	0.10
	HA-7	0.7	0.8	0.9	0.8	0.10

[a]No gel formed.

Table 8. Gel strengths of reconstituted starches set at 4°C for 72 h

Source		Gel strength (g)				
Ap	Am	rep.1	rep.2	rep.3	mean	S.D
HA-5	Potato	8.7	9.0	8.4	8.7	0.30
	Corn	8.3	8.9	7.8	8.3	0.55
	HA-7	14.8	12.7	14.3	13.9	1.10
WxM	Potato	--[a]	--	--	--	--
	Corn	--	--	--	--	--
	HA-7	1.9	1.3	1.6	1.6	0.30
Rice	Potato	0.8	0.8	0.8	0.8	0.00
	Corn	1.5	1.6	1.4	1.5	0.10
	HA-7	6.7	7.1	7.8	7.2	0.56

[a]No gel formed.

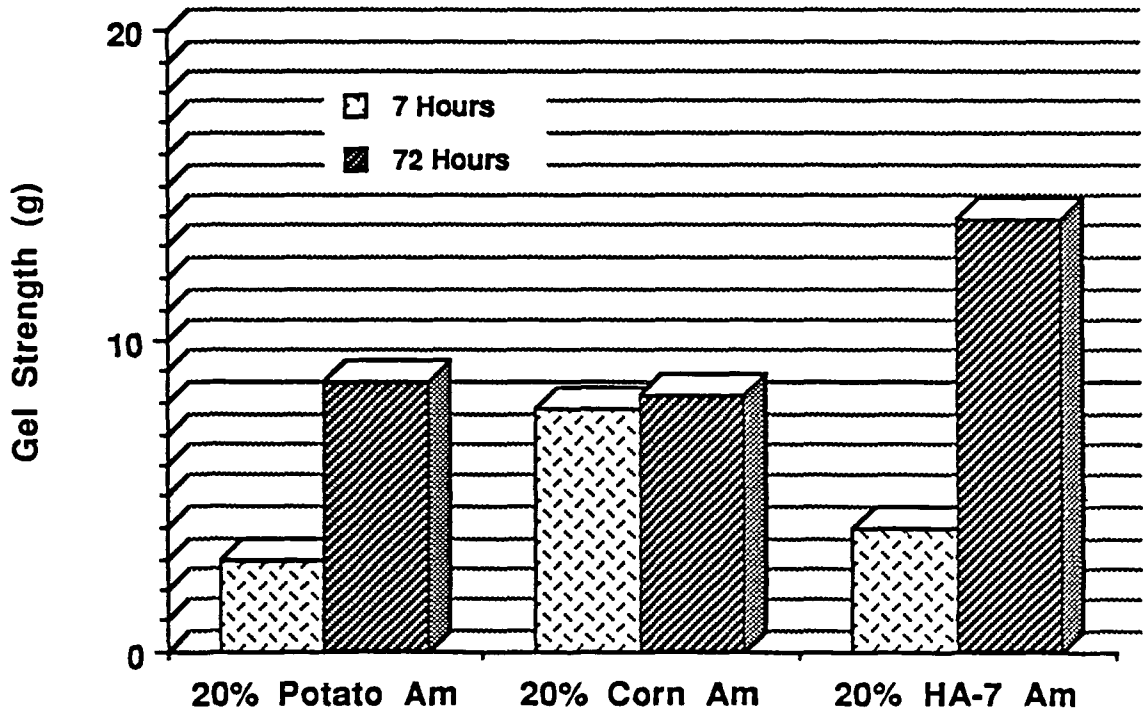

Figure 18. Gel strength of reconstituted starches consisting of 80% HA-5 amylopectin and 20% amylose

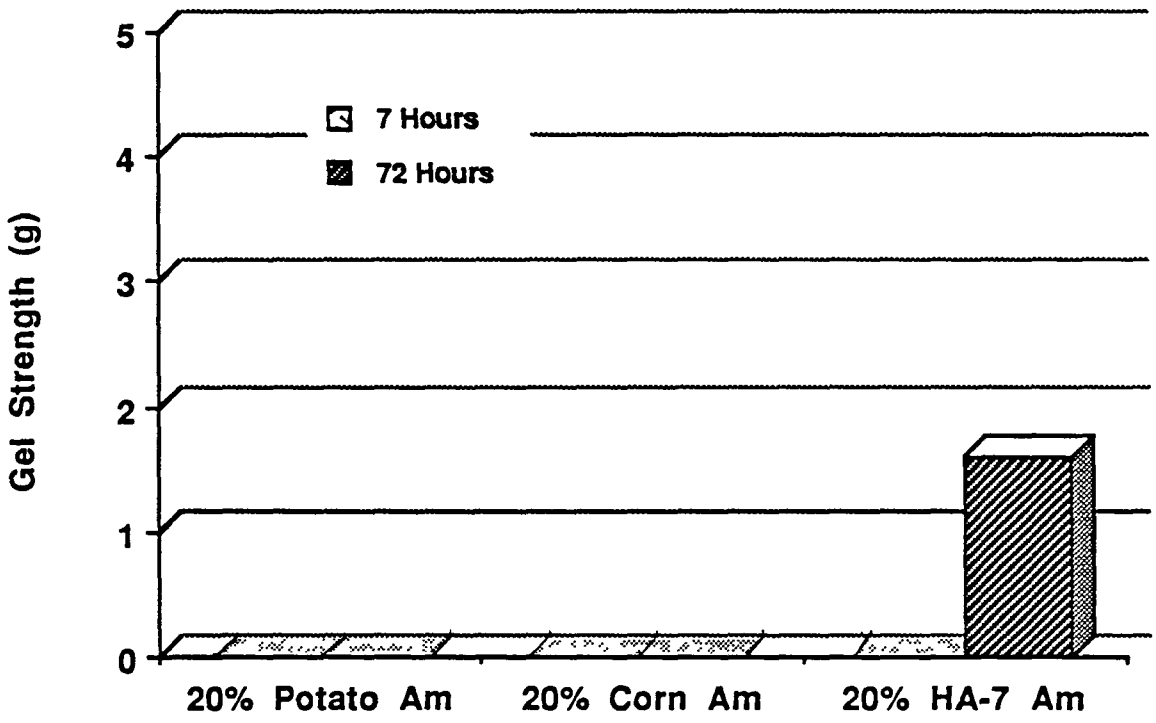

Figure 19. Gel strength of reconstituted starches consisting of 80% waxy maize amylopectin and 20% amylose

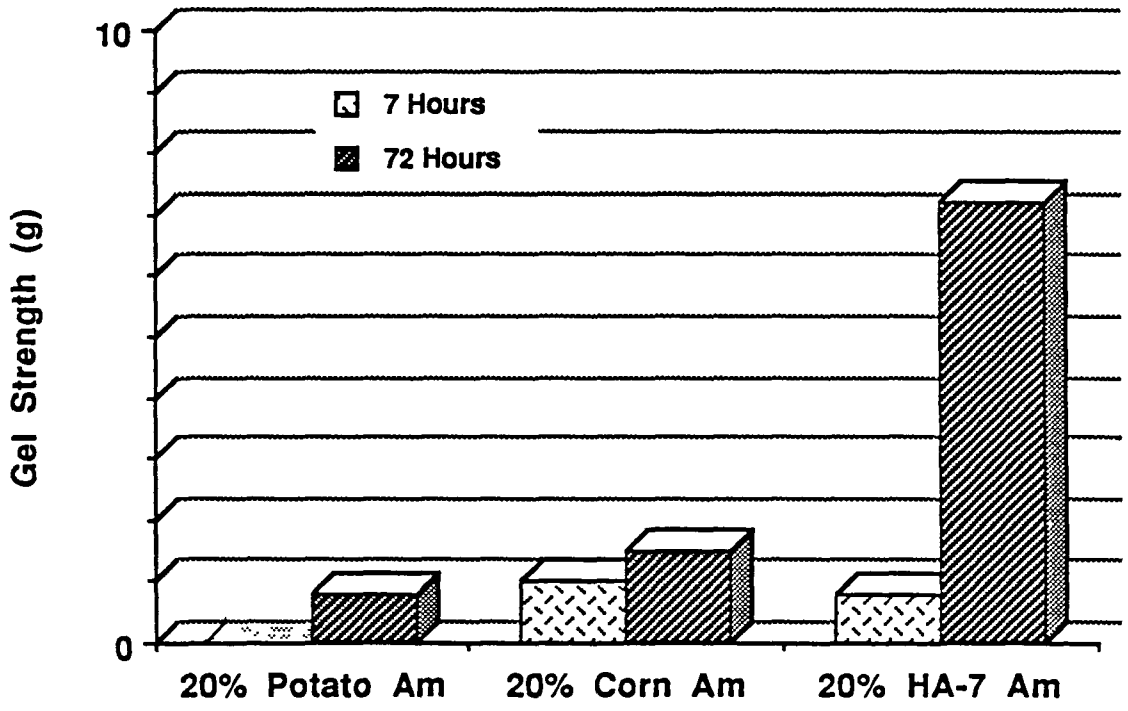

Figure 20. Gel strength of reconstituted starches consisting of 80% rice amylopectin and 20% amylose

SUMMARY

Effects of the molecular size of amylose, the branch chain-length of amylopectin, and the proportion of amylose to amylopectin on the functional properties of starch were studied by using reconstituted starches. The reconstituted starches were prepared by mixing amyloses and amylopectins of different structures. Amyloses of large, intermediate, and small molecular sizes were prepared from potato, normal corn, and HA-7 starches, respectively, by using a modified Schoch's method. Amylopectins with long, intermediate, and short branch-chain lengths were isolated from HA-5, waxy maize, and rice starches, respectively. Purities of amyloses and amylopectins were examined by using the potentiometric titration and the gel-filtration column chromatography.

Proportion of amylose to amylopectin in the reconstituted starches were 1 to 9 and 2 to 8. Viscosity, clarity, and gel strength of the reconstituted starch pastes were studied and compared. Results showed that the viscosity of the mixture was higher than that expected from the sum of the viscosity of each measured alone when amylose was mixed with amylopectin. The difference was attributed to the synergistic effect which varied with compositions of the mixtures. Samples of reconstituted starches which consisted of long branch-chain amylopectins, such as HA-5 amylopectin, showed a greater effect compared to those which consisted of rice and waxy maize amylopectins. The reconstituted starch containing amylose with an intermediate molecular size and amylopectin with a long branch chain-length exhibited the greatest synergistic effect.

Furthermore, higher concentration of amylose gave greater synergistic effect.

Because of the removal of lipids and fractionation treatment, the transmittance of reconstituted starch paste was higher than that of native starch paste. The results also showed a trend that the transmittances decreased with the decline in molecular sizes of amyloses and branch chain-lengths of amylopectins. This trend is consistent with the retrogradation of amylose. The amylose with small molecular size isolated from HA-7 corn starch has the highest retrogradation rate among all the amyloses selected. Since retrogradation of amylose results in turbidity, the higher the retrogradation, the lower the transmittance is expected. Reasons for the correlation between transmittance and branch chain-length of amylopectin are not clear except that rice amylopectin is more highly branched than others.

In comparison with the gels of reconstituted starches containing an intermediate or a short branch-chain amylopectin, those containing long branch-chain amylopectins displayed higher gel strength. Those pastes containing waxy maize amylopectin and large or intermediate amylose did not gel after setting at 4°C for 72 h. Gels containing amyloses with small molecular sizes, such as HA-7 amylose, demonstrated higher strengths than any other gels after setting. This was attributed to the retrogradation of amylose.

In conclusion, these results reveal that different molecular sizes and structures of amyloses and amylopectins, indeed, affect the functional properties of starches. The results are summarized in Table 9. A score

Table 9. Summary of functional properties of reconstituted starches

Ap	Am	Synergistic effect on viscosity		Gel strength		T%	Total score
		90:10	80:20	7h	72h		
Long	Large	8	15	5	10	9	47
	Intermediate	12	18	8	9	6	53
	Small	7	17	6	11	7	48
Intermediate	Large	4	10	0[a]	0	8	22
	Intermediate	5	13	0	0	4	22
	Small	2	11	0	4	2	19
Short	Large	1	9	0	1	5	16
	Intermediate	6	16	2	3	3	30
	Small	3	14	1	7	1	26

[a]No response.

from 0 to 18 is given for each combination: higher scores suggest higher synergistic effect, gel strength, and transmittance. Therefore, the total score will serve as a reference to those who are interested in manipulating the functional properties of starch, especially to food technologists and molecular geneticists.

RECOMMENDATIONS FOR FUTURE STUDIES

Future explorations might include: 1) analyzing the fine structure of amylopectin and correlating the structure to the functional property of reconstituted starch; 2) explorating in vitro biosynthesis of amylose and amylopectin and correlating these synthesized materials with tailored structures to the functional property of reconstituted starch; 3) introducing phosphate groups into amylopectins to study their effects on the functional property of reconstituted starch.

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