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Characterization and food applications of high amylose and other varieties of starch

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**Characterization and food applications of high amylose
and other varieties of starch**

by

Tunyawat Kasemsuwan

**A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY**

**Department: Food Science and Human Nutrition
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Approved:

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In Charge of Major Work

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**Iowa State University
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1995

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GENERAL INTRODUCTION

Starch is generally considered the second most abundant biopolymer in higher plants. Starch is widely distributed as the reserve carbohydrate in the leaves, seeds, stems, roots, and fruits. Commercial starches are found in cereal grains (maize, rice, sorghum, and wheat) and in root crop (potato, tapioca, and taro). Starch is an important ingredient in foods and also has many industrial applications. Starch from various botanical origins, degree of maturity, growing conditions, and genetic mutations provides different functional properties, as a result of differences in molecular structure and minor constituents, such as lipids and phosphate derivatives.

Starch consists mainly of two polysaccharides: amylose and amylopectin. Amylose is an essentially linear polymer consisting of glucose units in an $\alpha(1-4)$ linkage with few branching. Amylopectin is a high molecular-weight branched polysaccharide ($DP \approx 10^5$) consisting of glucose units in $\alpha(1-4)$ linkage for the backbone chains and linked together by $\alpha(1-6)$ glucosidic bonds for branch points. The third component, found in some starches, is known as the "intermediate component". The intermediate component possesses iodine-binding capacity and a β -amylolysis limit that are between those of amylose and amylopectin. The amount and structure of the intermediate component varies with the variety and maturity of starch.

Phosphorus in starch significantly affects the functional properties of starch, e.g., phosphate monoester in potato starch gives a clear paste with high viscosity, phospholipids in wheat starch reduce the paste clarity and viscosity. Determination of the phosphorus content in starch is critical because it helps predict the physical properties of the starch. P-31 NMR spectroscopy can detect the structures and amounts of phosphorus in starch. In contrast, the traditional chemical method only provides the total phosphorus content.

Because of a growing interest in using native starches in the food industry and due to the difficulty in achieving regulatory approval for chemically modified starches, there is demand for new alternative starch sources, especially from mutant genotypes that provide desired properties. Mutations in maize have been well identified, for example high amylose maize starch was developed from the mutation of the *amylose-extender genes (ae)*. Mutations have been shown to alter the levels of starch and amylose and amylopectin proportions and their structures. Therefore, mutations provide potential for developing novel starches for special uses. Some current applications of mutant-derived starches include: high amylose maize for film forming and waxy maize starch for improving freeze thaw stability in foods. The availability of these mutants also has enabled us to study the correlation between the molecular structure and functional properties of starch.

Starches are used as components in the manufacture of many products such as adhesives, textiles, paper, food, pharmaceuticals, and building materials.

Utilization of starch is based on its functional properties (thickening, gelling, adhesive, and film forming properties), its low cost, and availability. In order to understand the functional properties of the starch, molecular structures need to be revealed.

Dissertation Organization

This dissertation consists of three papers. All of the three papers follow the format of the journal, *Cereal Chemistry*. The first paper, "Characterization of the Dominant Mutant Amylose-Extender (*Ae1-5180*) Maize Starch," is in press. The second paper, "A quantitative method for the analysis of phosphorus structures and contents in starch by P-31 NMR spectroscopy," has been submitted to *Cereal Chemistry*. The third paper entitled "Manufacture of clear noodles with mixtures of tapioca and high-amylose starch" will be submitted to *Cereal Chemistry*. The three papers are preceded by a General Introduction and a Literature Review and followed by a General Conclusion. Literature Cited in the General Introduction and in the Literature Review are listed in alphabetical order according to author's name and follow the General Conclusion.

LITERATURE REVIEW

General Properties of Starch

Starch is often the main form of energy storage for plants. Starch can be found in stems, roots, leaves, fruits, and seeds (Lineback 1984, Swinkels 1985). The structures and physical properties vary with botanical origin, growing conditions, mutations, and maturity (Boyer et al. 1976, Lineback 1984, Golachowski 1985, Swinkels 1985, Asaoka et al. 1985, Morrison and Gadan 1987, Kolodziej 1993). Starch consists of two major forms of polysaccharides: amylose and amylopectin; it also consists of minor components such as lipids, proteins, and phosphorus. These minor components significantly affect the functional properties of starch.

Amylose

Amylose is essentially a linear α -1,4 glucan with some molecules having α (1,6) (Greenwood 1964, French 1973, Banks and Greenwood 1975). The molecular weights of amylose in various starches range from 178,000 (d.p.920-1100) in maize, 734,000 (d.p 4350) in lily and 1,080,000 (d.p. 6680) in tapioca (Hizukuri and Takagi 1984, Takeda et al. 1984, Takeda et al. 1988). The β -amylolysis of amylose ranges from 70-82%; but, with the addition of pullulanase,

the hydrolysis is almost completed (Banks and Greenwood 1967, Banks et al. 1973, Greenwood 1976, Hizukuri et al. 1981, Hizukuri et al. 1983a, Takeda et al. 1986, Takeda and Hizukuri 1987). The main barriers to β -amylolysis are the α -1,6 branch linkages (Peat et al. 1952, Hizukuri et al. 1981, Takeda and Hizukuri 1989, Takeda et al. 1989a, Takeda et al. 1993). Amylose in neutral, aqueous salt solution forms random coils; but, with a complexing agent, it forms a helix with 6-8 glucose units per turn (Rundle and French 1943, Bank et al. 1971a, Yamashita and Monobe 1971, French and Murphy 1977, Wu and Sarko 1978, Bank and Muir 1980, Davies et al. 1980, Biliaderis and Galloway 1989). French (1972) suggested that the strands of the double-helix are left-handed.

When amylose forms a complex with iodine, it gives a dark blue color, a feature which is used in the analysis of amylose (Bates et al. 1943, Lansky et al. 1949, Banks et al. 1971a, Banks et al. 1974, Pfannemuller 1978). Potentiometric iodine titration was widely used to evaluate the quantity of amylose in starch (Schoch 1964). Moreover, the iodine reaction can indicate the relative chain length of amylose (Bailey and Whelan 1961). The formation of complexes with polar and nonpolar organic compounds has been used to fractionate starch into amylose and amylopectin (Kuge and Takeo 1968). These complexes were also used to study the conformation of the helical complexes and affect the properties imparted by starch to complex systems containing these materials, such as food systems containing fatty acids (Schoch 1942, Winter and Sarko 1972, Winter and Sarko 1974, Rutenberg 1980, Jane et al. 1985, Biliaderis and Galloway 1989).

Amylose has a tendency to retrograde or come out of solution by forming inter chain associations and is considered to be responsible for the retrogradation of starch (Miles et al. 1984, Biliaderis and Seneviratne 1990). Jane and Robyt (1984) reported the presence of amorphous and crystalline regions in retrograded amylose. The crystalline regions have been postulated to be double helices that are interspersed with amorphous regions. The rate of starch retrogradation are depended on many parameters, such as storage temperature and chain-length. The formation of resistant starch attributed to the crystallization of amylose in a partially crystalline polymer system. The crystallinity of the resistant starch also increases with storage time at higher storage temperature (68 and 100°C) (Eerlingen et al 1993a, Eerlingen et al 1993b).

Amylopectin

Amylopectin is a branched molecule found in starch. In linear chain, the D-glucopyranose units are connected by α -1,4 linkages with 5-6% of its bonds being α -1,6 branch linkages. Studies have shown the relationship between the molecular structure of amylopectin and the crystalline structure of the starch granule (French 1972, Hizukuri et al. 1983a, Lineback 1984, Hizukuri 1985, Manners 1989). French (1972) proposed a well accepted molecular structure of amylopectin, the cluster model. On the basis of the cluster structure, the

physical properties of amylopectin can be explained, including viscosity and acid resistance (French 1972). The average branch chain length is 20-25 glucose units, but those of high amylose starches are longer than 30 glucose units; the degree of polymerization of amylopectin ranges from 10^4 to 10^5 glucose units (Hizukuri et al. 1983a, Hizukuri 1986, Zobel 1984, Manner 1985, Takeda et al. 1988). The structure of amylopectin contains three types of chains: A, B, and C. The A chains are the chains that are linked to the B and C chains through the reducing group by an α -1,6 linkage and do not have any other chains attached. The B chains are similarly linked but carry one or more A chains or other B chains. The C chain is the only chain of the molecule which has a free reducing group. The ratio of A chains to B chains is one method used to characterize the structure of amylopectin (Atwell et al. 1980, Manners and Matheson 1981, Enevoldsen and Juliano 1988, Hizukuri and Maehara 1990). Hizukuri (1986) proposed a polymodal distribution in which the B chains were further classified into B1-B4 fractions (Fig. 1). He suggested that amylopectin is composed of many clusters that are randomly or regularly distributed, and linked by long chains extending to two or more clusters.

Intermediate Component

Many reports have indicated the presence of a material having properties different from those of amylose and amylopectin so called the intermediate

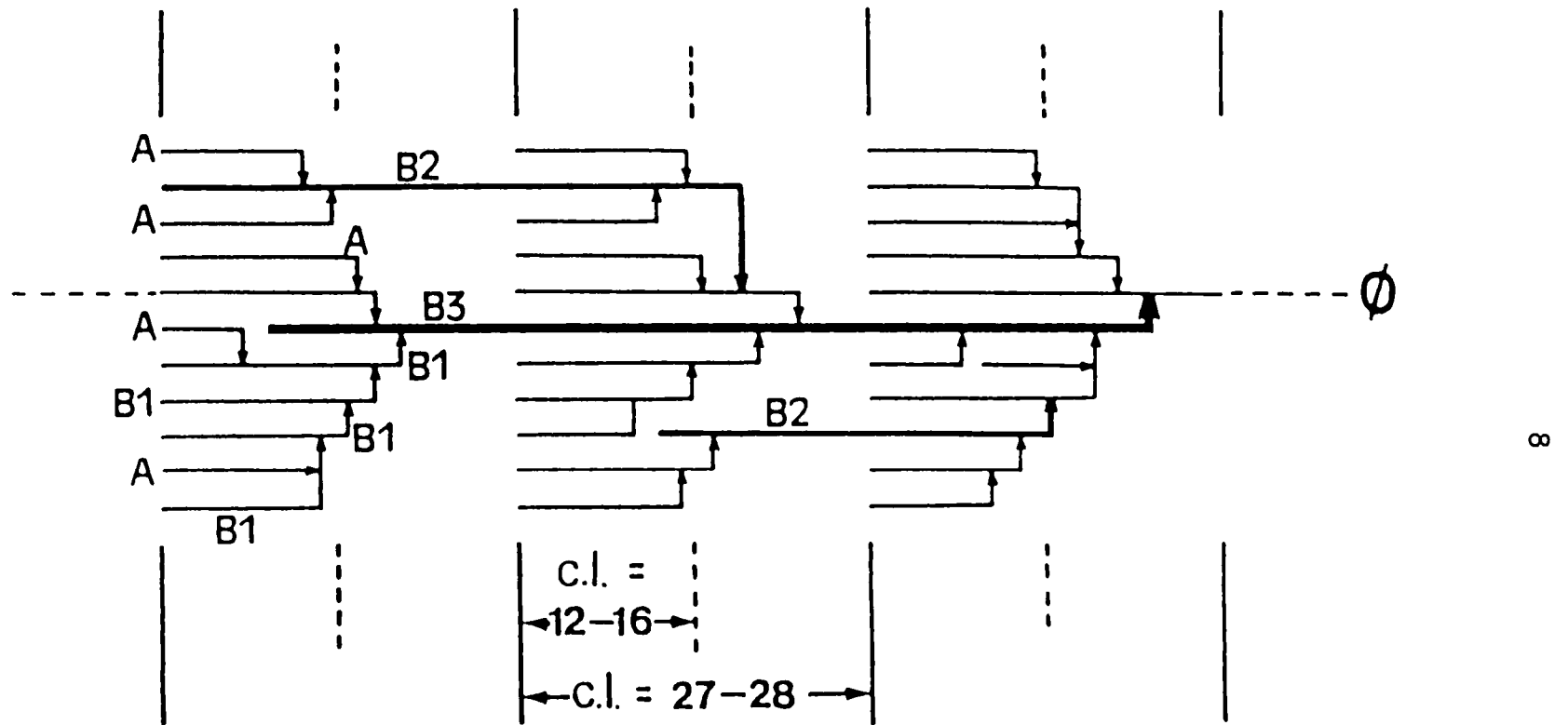


Figure 1. Cluster model for amylopectin. (For definition of A and B1-B3, see text: ∅, reducing chain-end; __, (1-4)- α -D-glucan chain; \rightarrow , α -(1-6) linkage.)

component (Lansky et al. 1949, Peat et al. 1952, Wolff et al. 1955, Greenwood 1979, Takeda et al. 1989b). The intermediate components displayed iodine-binding studies and by a β -amylolysis limit between those of amylose and amylopectin. Lansky et al. (1949) reported that maize starch contained 5-7% of the intermediate component. Erlander and French (1958) also reported the presence of a component with a lower molecular weight than amylopectin in waxy-maize starch. Many reports also suggest the presence of an intermediate component in high amylose starch (Greenwood and Mackenzie 1966, Banks et al. 1974, Colonna et al. 1982, Colonna and Mercier 1984, Baba and Arai 1984). Whistler and Doane (1961) reported that the isolation of an intermediate amylose component by sequential precipitation with 1-butanol followed by 2-nitropropane. Dais and Perlin (1982) studied the intermediate component (amylopectin C) by using high field C-13 NMR. They claimed that the intermediate component has a chain length that is 20-25% shorter than that of normal amylopectin.

Starch Granules

Starch molecules are organized into quasi-crystalline macromolecular aggregates granules (French 1972, Hood 1982). Starch granule size and shape vary with botanical origin {such as potato-starch granules are oval shaped (15-80 μm), normal-maize starch-granules are round or polygonal shaped (5-20 μm), and tapioca-starch granules are round shaped (3-18 μm)}, maturity, and growing

condition (Banks et al. 1974, Greenwood 1979, Golachowski 1985, Morrison and Gadan 1987). Scanning electron microscopy (SEM) has been used to study the morphology and surface of starch granules. The micrographs provided important evidence for identifying the starch variety, hydrolysis effects, and changes during different treatments (Mussulman and Wagoner 1968, Hall and Sayre 1969, Hall and Sayre 1970, Nikuni 1978, Ikawa and Fuwa 1980, Liu and Zhao 1990, Baldwin et al. 1994, Jane et al. 1994, Morrison et al. 1994, Tester et al. 1994).

Starch granule-surface enzyme-hydrolysis has been studied by SEM and revealed the mechanism of the enzyme hydrolysis (Fuwa et al. 1978, Takaya et al. 1978, Sargeant 1980, Ikawa and Fuwa 1980, Salomonsson et al. 1989, Gallant et al. 1992, Planchot et al. 1995).

During maturation of starch granules, starch contents and granule size increased, the ratio of amylose content also increased (Adkins et al. 1970, Boyer et al. 1976, Greenwood 1979, Asaoka et al. 1985, Morrison and Gadan 1987). Morrison and Gadan (1987) also reported that the lysophospholipid content generally decreased with granule size, but increased with granule maturity. The starch granule is insoluble in cold water but dispersible after gelatinized in boiling water, and it also can be dissolved completely in 80-95% dimethyl sulfoxide (Leach and Schoch 1962, Wolf et al. 1970). Amylopectin forms the clusters and lines up perpendicular to the growth rings and grows from the hilum to the surface of the granule in a radial arrangement (French 1972, Nikuni 1978, French 1984, Lineback 1984). A starch granule consists of two alternating

regions, amorphous and crystalline. Amylopectin clusters are present in the amorphous and the crystalline regions (French 1984). The amylose is located in both amorphous and crystalline regions of the starch granule (Kainuma and French 1971, French 1972, Nikuni 1978, Blanshard 1986, Jane et al. 1992, Kasemsuwan and Jane 1994). X-ray diffraction analysis of starch crystallinity has been studied extensively. X-ray diffraction provides information about the crystalline structure of the starch granule and the relative amounts of crystalline and amorphous phases within the starch granules. Starches are known to give three X-ray diffraction patterns: A type, B type, and C type (Sarko and Wu 1978, Zobel 1988), with the C type is considered to be an intermediate between the A type and B type. It is well known that amylopectin is responsible for the crystallinity of native starch granule. Waxy maize starch, which is almost 100% amylopectin, shows an x-ray diffraction pattern similar to that of normal maize starch, whereas high amylose maize starch shows different x-ray diffraction pattern and a poor crystallinity. The A-chains and the exterior parts of the B-chains of the amylopectin are mainly responsible for the crystallinity of the starch granules. Thus, the branch-chain length of amylopectin is responsible for the type of the x-ray diffraction pattern (Hizukuri et al. 1983a, Hizukuri 1985, Wild and Blanshard 1986). They found that in A-type starches, the amylopectin-branch chain-lengths are short, whereas in B-types, they are long, and in the C-type starches, they have intermediate chain-lengths.

Mutant Effects on Starch

Mutations often change the development of the maize kernel that results in a change of the composition and structure of the starch. The mutation effect on maize has been studied extensively. Some mutations affect the endosperm protein production, which affects as opaqueness and flouriness, and these mutations reduce the zein content and increase the globulin proteins. Mutations also affect polysaccharide composition, a mutation in maize has been shown to change the percentage of amylose content, the structures of amylose and amylopectin, the degree of branching, the chain length, and the amount of the intermediate component. Starch from the amylose-extender mutant shows an increase in amylose content, and also an increase in the chain length. The kernel size of this mutant is reduced with the increase in amylose content. Because of the high amylose content, the starch gel exhibits high gel strength. The amylose-extender gene is also responsible for increasing the intermediate content, and the irregular shape of the starch granules (Wolf et al. 1964, Banks et al. 1971a, Banks et al. 1974). Wolf et al. (1955) reported that both the inner and outer branches of the new amylopectin are longer than those of normal maize amylopectin. Baba and Arai (1984) reported that amylo maize intermediate-component was mainly composed of branches with average chain-length of 50. All reported amylose-extender mutants have been simple recessives, requiring the mutants to be homozygous in order to produce high

amylose starch. A dominant mutant allele at the *ae* locus has been used to accelerate the development of high amylose inbred or hybrid lines because such a dominant genetic trait, which produces the high amylose starch, expresses itself in every generation of crossing and, thus, is readily followed (Robertson and Stinard 1991, Stinard et al. 1993). The recessive *ae* mutant alleles are not as readily followed in a crossing regime.

Waxy mutants also have been isolated in maize, rice, sorghum, rice, amaranth, and barley (Shannon and Garwood 1984). These mutants produce starches that contain essentially 100% amylopectin. Waxy mutant starch exhibits birefringence similar to normal starch, and both give A type x-ray diffraction patterns. Shi and Seib (1995) and Wong et al (1993) reported that waxy and du-waxy starches have a large proportion of short chains and show A type x-ray patterns, whereas the *ae*-waxy and *ae*-du-waxy starches have a large proportion of long chains and show B type x-ray pattern. By studying mutant starches, we can gain an insight into the pathways and the enzymes that are involved in starch biosynthesis and thus, understand the fine structure of starch.

Lipids in Starch

Morrison (1981) proposes that there are two types of lipids found in starch: surface lipids that are derived from non-starch lipids, and internal starch lipids, that are exclusively monoacyl lipids. In the starchy endosperm of mature wheat,

non-starch lipids occur as spherosomes and are found in the aleurone region of the cell. The non-starch lipids are mostly triglycerides and their derivatives, with only small amounts of free fatty acids and monoacyl lipids (Morrison et al. 1975, Morrison 1988). In cereal starches the internal lipids are mostly monoacyl lipids (free fatty acids and lysophospholipids) (Schoch 1942, Morrison 1981, Morrison et al. 1984, Morrison 1988), whereas the root and tuber starches carry only a small amount of lipids which are probably not internal lipids (Swinkels 1985, Morrison 1988). In wheat starch the lipids are at least 90% lysophospholipids and account for most of the phosphorus in wheat starch (Meredith et al. 1978, Lim et al 1994). They also reported that the amount of lipids per granule tend to be proportional to the granular surface areas of the larger granules but proportional to the volumes of the smaller granules. It has been well established that all non-waxy cereal starches contain lipids in proportion to their amylose content (Morrison et al. 1984, Soulaka and Morrison 1985). These lipids affect starch properties such as granular swelling, gelatinization and solubilization, gel viscosity, retrogradation, and susceptibility to amylolytic degradation (Maningat and Juliano 1983, Swinkels 1985, Soulaka and Morrison 1985, Biliaderis and Seneviratne 1990, Seneviratne and Biliaderis 1991, Morrison et al. 1993). The rate and extent of enzyme hydrolysis were inversely related to the degree of organization of helices into larger domains of ordered chains; complexes with greater crystallinity were more resistant to enzyme degradation (Seneviratne and Biliaderis 1991, Morrison et al 1993). Amylose-lipid complex, determined by

differential scanning calorimetry, is a reversible endothermic transition and occurred at about 100°C. These complexes can be formed in an exothermic reaction when starch is gelatinized in the presence of lipid (Kugimiya et al. 1980, Kugimiya and Donovan 1981). Morrison and Coventry (1989) proposed that the barrier to lipid extraction is the structure of the granule rather than the resistance of inclusion complex to lipid extraction, because rigorous conditions are required for extraction of lipids from native cereal starch granules.

Phosphorus in Starch

Phosphorus in the cereal starches is mainly present as phospholipids (Schoch 1942, Gracza 1965, Morrison 1981, Hizukuri et al. 1983b, Morrison et al. 1984, Soulaka and Morrison 1985, Morrison 1988, Lim et al. 1994). Root starches contain very low amounts of phosphorus and mostly in the phosphate monoester forms. Phosphate monoester derivatives are found in many varieties of starch such as maize, potato, wheat, and rice (Tabata et al. 1975, Hizukuri et al. 1983b, Lim et al. 1994). Tabata et al. (1975) also reported that normal maize starch contains phosphorus (about 170 ppm), most of which is in the phospholipid form and 7% of the total is in the phosphate monoester form. Potato starch is the only commercial starch that contains a significant amount of phosphate monoester covalently bound to starch (Posternak 1935 and 1951, Hodge et al. 1948, Hizukuri et al. 1970, Swinkels 1985, Lim and Seib 1993, Lim

et al. 1994). The repulsion of negatively charged phosphate groups of individual polymer chains in potato starch accounts, in part, for the more rapid hydration and swelling and the higher viscosities of potato starch pastes, compared with cereal starches (Galliard and Bowler 1987). Rice starches are also known to be esterified with low levels of phosphate (Hizukuri et al. 1983b). They also reported that glucose-6 phosphate is the major form of phosphorus in waxy rice starch granules, whereas choline as lysophosphatidyl choline is the main phosphorus form in nonwaxy rice granules. Tabata and Hizukuri (1971) identified phosphodextrins in the hydrolysate from potato starch. They found 1, 38, and 61% of the endogenous phosphates in potato starch were located on C-2, C-3, and C-6, respectively. Tabata et al. (1975) also reported similar results with waxy rice starch, but the phosphates were located primarily at C-6 (82-94%) with the remainder on C-2 and C-3 of the glucose residues. Radomski and Smith (1963) reported that potato starch fractionated into amylopectin, intermediate component, and amylose had phosphorus contents of 0.165, 0.083, and 0.008%, respectively. They also concluded that the phosphates in starch are in close proximity to the branch points, because after β -amylolysis of corn and potato starches, the phosphorus content increased. Takeda and Hizukuri (1982), and Tabata et al. (1978) reported that no phosphate ester is located at the nonreducing or reducing terminal end, and the phosphate esters are located mostly in B-chains and are distributed over the length of the B-chains, except in the vicinity of branch points. About 1/3 of the phosphate groups are present in

the inner sections of B-chains and 2/3 are in the A-chain and outer section of B-chain amylopectin (Takeda and Hizukuri 1982). The phosphorus content in starch is also affected by many parameters, such as steeping conditions (which significantly affects the nitrogen, lipid and phosphorus contents of the starch) (Takeda et al. 1988) and maturity (Nielsen et al. 1994). Nielsen et al. (1994) also reported that the degree of phosphorylation is found to be almost constant during the development of the potato tuber.

Nuclear Magnetic Resonance in Starch Analysis

Nuclear magnetic resonance (NMR) is a powerful spectroscopic technique which detects the magnetic properties of atomic nuclei. Proton (^1H), ^{13}C and ^{31}P sensitivities of NMR are dependent on nuclei, ^1H is the most sensitive followed by ^{31}P , and ^{13}C NMR (McIntyre et al. 1990a, 1990b). NMR has been widely used to study the structure of starch polysaccharides (Seymour et al. 1979, Gidley 1985, Jane and Robyt 1985, Horii et al. 1987, Gidley and Bociek 1988, Colquhoun et al. 1990, Marsman et al. 1990, McIntyre et al. 1990a, 1990b, Jane 1993, Lim and Seib 1993, Lim et al. 1994). Molecular organization in starch, conformations of glycosidic linkages, branching in starch chain, helical complexes, and the structure of the polymorphs were also studied by NMR spectroscopy (Gidley and Bociek, 1985, Jane et al. 1985, Horii et al. 1987). Quantitative analyses are also done by NMR spectroscopy such as the degree of

polymerization and reduction of residues (Gidley 1985, McIntyre et al. 1990a). McIntyre et al. (1990a) proposed that proton NMR spectroscopy can determine the degree of polymerization and the degree of branching without recourse to the chemical or the enzymatic method. Jane et al. (1985), using C-13 NMR, reported amylose and Nagaeli dextrin changed conformations when they complexed with various complexing agents. Carbon-13 NMR can also be used to study the structure of each position of carbon in the sugar ring. Phosphorus-31 NMR is becoming more widely used to study the phosphorus in native and modified starches (Lim 1990, McIntyre et al. 1990a, Muhrbeck and Tellier 1991, Lim and Seib 1993, Bay-Smidt et al. 1994, Kasemsuwan and Jane 1994, Lim et al. 1994). P-31 NMR spectroscopy was used to determine variations between potato varieties in the degree of phosphorylation on C-3 and C-6 of the glucosidyl units of amylopectin molecules (Muhrbeck and Tellier 1991). Lim and Seib (1993) also used P-31 NMR to locate the phosphate groups on phosphorylated wheat starch. They found phosphorylation mainly at the C-6 position, and lower levels at C-3 and C-2, respectively. Kasemsuwan and Jane (1994) reported the locations of phosphodiester cross-linkage in cross-linked corn starch. P-31 NMR can be used to detect the phosphate ester in a low concentration (1-10mM) (McIntyre et al. 1990). The pH and temperature affect the chemical shifts of the P-31 signal because of the change in the nuclei environment (Bock and Sheard 1975, Barany and Glonek 1982, Jame 1985, McIntyre et al. 1990a, Tebby and Glonek 1991, Bay-Smidt et al. 1994). Barany

and Glonek (1982) and Lim (1990) claimed that the resolution of the spectra can be improved by using a chelating agent (e.g., EDTA) to reduce the broadening of the signals caused by cation interactions. Kasemsuwan (1991) reported that some of the broadening of the P-31 NMR signals are caused by molecules which give signals with a similar chemical shift, such as lecithin, which has a mixture of fatty acids. These signals overlap and cannot be resolved by the spectroscopy. At pH 8-12 most of the sugar phosphate monoesters show signals at the chemical shift between 2 and 5 ppm. Inorganic phosphate produces signals at the chemical shift between 2 and 3 ppm, and phosphodiester produce signals between -1 and 1 ppm (85% phosphoric acid as external reference at 0.0ppm) (Barany and Glonek 1982, James 1985, Tebby and Glonek 1991, Lim and Seib 1993, Kasemsuwan and Jane 1994). Barany and Glonek (1982) also reported the chemical shift of phosphatidylcholine at -0.9 ppm, and phosphatidylethanolamine at -0.3 ppm. Lim (1990) and Lim and Seib (1993) reported that the spectra of D-glucose 2-, 3-, and 6-phosphate produce signals at 1.38, 1.85, and 2.0 ppm (inorganic phosphate as internal reference at 0.0 ppm, at pH 8.0), respectively. They also reported that the α,γ -phosphodextrin of phosphorylate potato amylose produced signals at 1.95 and 1.62 ppm, respectively, which indicated phosphate monoesters at C-6 and C-3 on the non-reducing end. Signals at 1.03 and 0.8 ppm indicated phosphate monoesters at C-6 and C-3 in the internal chain of repeating anhydroglucose units, respectively.

Modifications of Starch

Modified starches were developed to overcome the functionality limitations and, thus, expand the usefulness of starch in a myriad of industrial applications. Any products in which the chemical and/or physical properties of native starch have been altered might be considered to be modified (Wurzburg 1986a). Chemically modified starches cover many products such as converted starches (acid fluidities, chlorinations, pyroconversions), cross-linked starches, and derivatized starches. Physically modified starch includes pregelatinized starches, redried starches, extruded starches, and moist, heat-treated starches (Wurzburg 1986b) and granular cold-water soluble starch (Chen and Jane 1995). Starch conversions involve the treatment of granular starch by chemical and/or physical means that cause rupturing of some or all of the starch molecules, thus weakening the granules, decreasing their capacity to swell on pasting or cooking in water, and decreasing the size of the molecules. The viscosities of the converted starches are reduced permitting them to be dispersed at higher concentrations than unmodified starch (Rohwer and Klem 1984).

Acid-modified starches have been used in many industries, such as textile manufacturing, building products manufacturing, starch gum candy manufacturing, and paper manufacturing.

Oxidized or hypochlorite-modified starches are starches that have been oxidized by an oxidizing agent such as hydrogen peroxide, peracetic acid,

permanganate, and persulfate. In general, oxidized starch granules still retain birefringence and the characteristic iodine staining is still the same as unmodified granules; however, oxidized starch is whiter than unmodified, as a result of the bleaching action of hyperchlorite on the impurities (pigments) present in the starch (Wurzburg 1986b). The mechanisms used to obtain the oxidation reactions are the introduction of carboxyl and carbonyl groups and also the cleavage of glycosidic linkages. The molecular weight and the viscosity both decrease and the amounts of carbonyl and/or carboxyl contents increase with an increase in the level of hypochlorite treatment (Mellies et al. 1961). The main use of oxidized starches are as wet-end additives in the paper industry (Mentzer 1986).

Cross-linked starches occur by the intermolecular bridges between molecules increasing the average molecular weight. Cross-linking granular starch reinforces the holding of the granule together. Most cross-linking reagents also produce monoester derivatives such as phosphate monoester. The degree of cross-linking in starch is very low and is difficult to determine directly (Hullinger 1967, Rutenberg and Solarek 1984, Wurzburg 1986c). Cross-linking produces considerable changes in physical properties such as gelatinization and swelling of the starch granule (Chilton and Collison 1974, Rutenberg and Solarek 1984, Wurzburg 1986c). The physical properties, such as viscosity (detected by visco/amylograph) and swelling power, are generally used for determining the degree of cross-linking (Hullinger 1967, Jarowenko 1971, Rutenberg and Solarek

1984). The ratio of the diester (cross-linking) and monoester derivatives can be controlled by the pH of the reaction. At high alkalinity, the cross-linking reaction is predominant, whereas at low alkalinity reaction favor the production of monoester derivatives (Felton and Schopmeyer 1943, Wetzstein and Lyon 1956, Patten et al. 1969, Lloyd 1970, Rogols and Salter 1979). After starch granules are cross-linked, the granules are resistant to swelling and rupturing. The water sorption isotherm for cross-linked granular starch is similar to that of native starch (Felton and Schopmeyer 1943, Rogols and Salter 1979, Chilton and Collison 1974). One cross-link per hundred anhydro glucose units can retain the integrity of the swollen starch granule, eliminate cohesiveness, and stabilize hot paste viscosity. Cross-linked starches also resist acid and enzymatic hydrolysis and mechanical shear (Mellies 1961, Hullinger 1967, Hood et al. 1974, Abraham et al. 1979). Cross-linked starches are used in baby foods, salad dressings, and fruit pie fillings in which a stable, high viscosity starch paste is needed (Rutledge et al. 1974, Whistler and Daniel 1985). While one type of modification is limited, the combination of more than one type of modification could give additional properties.

Functional Properties and Food Applications

Gelatinization

Starch granules are insoluble in cold water. Heating provides sufficient energy to disrupt the weak hydrogen bonding between crystalline micelles and the granule starts to hydrate and swell. Starch is solubilized into solution; amylose leaches out from swollen granular (Banks et al. 1971b), and the viscosity increases, followed by a loss in birefringence. Further increasing temperature eventually causes the granule to collapse and rupture. The degree of swelling depends on the type of starch, temperature, pH, shear rate, solute, and starch concentration (Wong 1989, Yamada et al. 1986). Atwell et al. (1988) proposed the definition of gelatinization as "the phenomena of collapse (disruption) of molecule orders within the starch granule manifested in irreversible changes in properties such as granular swelling, native crystalline melting, loss of birefringence, and starch solubilization." The gelatinization process is affected by the structure and components of starch such as chain-length of amylopectin, phosphate monoester, and lipids. The longer branch-chains in amylopectin of high-amylose maize starch provide a firmer association of the amylopectin molecules with each other and with amylose and, therefore, increase the resistance of the granule to swelling and gelatinization (Montgomery et al. 1961). Liu and Zhao (1990) found a honey-comb-like structure within starch granules at the gelatinization temperature (using SEM). They believed that formation of this

structure caused the irreversible, profound swelling and loss of the birefringence. Gelatinization can be measured by many methods such as light microscopy, viscometry, nuclear magnetic resonance, differential scanning calorimetry, x-ray diffraction, laser light scattering, and enzymatic analysis (Zobel 1984).

Retrogradation

Upon cooling a dilute gelatinized starch solution, the linear molecules realign themselves into an insoluble precipitate. This association of the linear molecules resulting in decreased solubility is commonly referred to as retrogradation. Atwell et al. (1988) described retrogradation as "a process which occurs when the molecules comprising gelatinized starch begin to reassociate in an ordered structure. In its initial phases, two or more starch chains may form a simple juncture point that then may develop into more extensively ordered regions. Ultimately, under favorable conditions, a crystalline order appears." Amylopectin associates relatively slowly after cooling, forming tighter amylopectin micelles, because of the highly branched structures (Wong 1989).

Retrogradation of starch pastes or starch solutions may have the following effects: increased viscosity, development of opacity and turbidity, formation of insolubility on hot paste, precipitation of insoluble starch particles, formation of gels, and syneresis of water from paste. Retrogradation is a complex process and depends on many factors, such as the type of starch, starch concentration, cooking method, post cook temperature, storage time, pH, cooling method, and

the presence of other compounds. One example, which exemplifies retrogradation clearly, is the staling of bread (Hellman et al. 1954, Eerlingen et al 1994).

Applications of Starches in Foods

Food starches perform two basic roles: (1) nutritive stabilizers, providing the characteristic viscosity, texture, mouth-feel, and consistency of food products, i.e., in sauces, pudding, gum drops; (2) processing aids to facilitate manufacturing, i.e., in dusting surfaces to prevent sticking (Moore et al. 1984). Starch is used in food applications two kinds: native starch and modified starch. Processed foods generally require the following characteristics: pH stability, viscosity stability, processing tolerance, texture properties, shelf stability, particulate integrity, emulsification stability, and good surface appearance. These characteristics can be provided by choosing the proper starch. Production of shelf-stable, refrigerated, frozen, hot-filled, retorted, or aseptically processed food generally requires use of modified starch. Many starch types and modifications may be required for product formulation, and considerable knowledge of the starches, modification techniques, and functional properties is necessary to make the correct choice. Selection and use of starches in food products for consistent production quality and for innovative new products require understanding basic food processing technology and starch properties (Moore et al. 1984). There is a growing interest in using natural starches, i.e., those starches derived from

mutation, which can provide special properties. These starches have an advantage over the chemically modified starch which need FDA approval and are difficult to achieve consumer acceptance.

CHARACTERIZATION OF THE DOMINANT MUTANT AMYLOSE-EXTENDER (*Ae1-5180*) MAIZE STARCH

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ABSTRACT

Maize plants homozygous for the dominant amylose-extender allele *Ae1-5180* were self-pollinated and crossed onto wild type *Ae* plants, and vice versa, to obtain endosperms with 0-3 doses of the *Ae1-5180* allele. Starches from dominant amylose-extender mutant kernels were isolated and analyzed with respect to molecular size distribution, amylose contents (iodine affinity), thermal properties (DSC), and microscopic structure (SEM). Gel-permeation chromatograms of these starches showed greater proportions of amylose and intermediate fractions than those from normal maize starch. The iodine-staining blue-value of the amylopectin peak on the profile results revealed longer amylopectin branch-chain-lengths than those of normal corn, which was confirmed by enzyme debranching. The structures of the intermediate component and amylopectin were also revealed by gel-permeation chromatography and high-performance-anion-exchange chromatography. Greater dosage of the *Ae1-5180* gene did not increase amylose content (ca.

33%). The DSC thermograms indicated that all the mutant starches with different dosages had similar thermal properties. The gelatinization onset temperatures (T_g) ranged from 65.7 to 67.0°C and the gelatinization range (R) was 54.0 to 109.7°C. The SEM showed that the mutant starches had irregular and round shaped granules with diameters that ranged from 4 to 18 μm .

INTRODUCTION

Next to cellulose, starch is the most abundant carbohydrate in plants. Starch is an important ingredient in food and has many industrial applications. The versatility of starch-utilization (e.g., an agent for thickening, gelling, and sizing paper and textiles) is related to its chemical structure and granular size. Starch consists mainly of two primary components: amylose and amylopectin. Amylose is essentially a linear molecule containing glucose units linked by $\alpha(1-4)$ linkage with a small number of branches (Greenwood 1964, French 1973, French 1975, Hizukuri et al 1981, Takeda and Hizukuri 1987). Amylopectin is a branched molecule with $\alpha(1-4)$ linked-glucose-unit linear chains and $\alpha(1-6)$ linked branch points (Greenwood 1964, French 1973, Lineback 1984). Some studies have shown the existence of a third component in some starches, known as intermediate material (Lansky et al 1949). The amounts and structures of the intermediate material differ with starch type and maturity (Banks and Greenwood

1975, Wang et al 1993). Because of the different functionalities of these components, characterization of the molecular structure of starch is important.

Starch is the major component of maize kernels (72% of the kernel dry weight) (Boyer and Shannon 1987) and is the primary product obtained from the wet milling maize. Most of the starch is in the endosperm, but a significant amount is also in the embryo, bran, and tip cap fractions due to imperfect separation (Watson 1984, Boyer and Shannon 1987). Mutations that alter the levels of starch, amylose and amylopectin proportions, and starch structure in the maize endosperm have been identified (Shannon and Garwood 1984). The starches with "new" properties and functionalities have expanded the possible food and industrial applications and have led to increased research into the roles of starch in various applications. The availability of these mutants has also enabled us to correlate molecular structures with functional properties of starch.

One of the mutants, *ae1*, increases the proportion of amylose relative to amylopectin and elongates the branch-chain lengths of amylopectin. Starch obtained from lines homozygous for this mutant can be used to produce tough, edible, and biodegradable films and gels. Recently, a dominant mutant allele of the *ae1* locus has been reported (Stinard et al. 1993). A dominant mutant allele at the *ae1* locus can be used to accelerate the development of high-amylose inbred or hybrid lines because such a dominant genetic trait, which produces the high-amylose starch, expresses itself in every generation of crossing and thus is

readily followed. The recessive *ae* mutant alleles are not as readily followed in a crossing regime (Robertson and Stinard 1991).

In this study, we investigated structures and properties of the dominant mutant amylose-extender (*Ae1-5180*) maize starch. X-ray diffraction was used to reveal the crystalline structure of starch granules and the relative amounts of crystalline and amorphous phases within the starch granules (Zobel 1964). Size distribution and shape of the starch granules were recorded by using a scanning electron microscope. Molecular size distributions of the starch were determined by gel-permeation chromatography. Branch-chain lengths of amylopectin were determined by using high-performance-anion-exchange chromatography and gel-permeation chromatography. The intermediate component structure was also determined in the same manner as that of amylopectin. Thermal properties of the starch at the gelatinization and the retrogradation stages were investigated by using differential scanning calorimetry.

MATERIALS AND METHODS

Enzyme and Chemicals

Crystalline *Pseudomonas* isoamylase (EC 3.2.1.68) was a product of Hayashibara Shoji, Inc. (Okayama, Japan). The specific activity was about 66,000 units per mg protein. The enzyme was used directly without further

purification. All the chemicals were reagent grade and were used without further treatment.

Maize Kernel Samples and Chemicals

Ae dominant mutant mature maize kernels (*Ae1-5180/Ae1+/Ae1+*, *Ae1-5180/Ae1-5180/Ae1+*, *Ae1-5180/Ae1-5180/Ae1-5180*) and wild type (*Ae1+/Ae1+/Ae+*) mature kernels were obtained from maize plants grown in field plots during 1991 at Iowa State University, Ames, IA. Maize plants homozygous for the dominant mutant *amylose-extender1* (*ae*) allele *Ae1-5180* were self-pollinated and crossed onto wild type *Ae1* plants, and *vice versa*, to obtain endosperms with 0, 1, 2, and 3 doses of the *Ae1-5180* allele. The *Ae1-5180* source had been backcrossed for six generations to the inbred A636 and self-pollinated for two generations to achieve homozygosity for *Ae1-5180* in an A636 background (Robertson and Stinard 1991). The inbred A636 was used as the source for the wild type *Ae* allele. Ears were harvested at physiological maturity and then dried for seven days at 39°C. Kernels were shelled off the ears, weighed, and processed for endosperm starch analysis.

Starch Isolation

Starches were isolated following a slight modification of Badenhuizen's method (1964). The kernels were soaked in 0.01 M mercuric chloride solution, ground, and strained through a 30 μ m screen. The starch was then isolated by

centrifugation and resuspended in 0.1 M NaCl with 10% volume of toluene. The mixture was stirred for at least 1 hr and allowed to stand until the starch precipitated. The protein-toluene layer was removed, and the procedure was repeated until the starch sediment became clean. After washing with distilled water the starch was dried in a forced-air oven at 40°C.

Scanning Electron Microscopy (SEM)

The starch sample was suspended in 100% methanol. The suspension was stirred up, and a drop of the suspension was placed on aluminum tape (non-sticky side) attached to a brass disc. The specimens were coated in a Polaron E5100 sputter coater with gold-palladium (60:40). The mounted specimens were observed using a JEOL JSM-35 scanning electron microscope at 10 kV (Tokyo, Japan). Micrographs were taken at 1500x magnification of each starch sample (Jane et al 1994). The diameters of the granular starch were determined by measuring 20 granules from the micrographs.

X-ray Diffraction Pattern

The x-ray patterns of the starches were obtained with copper, nickel foil-filtered, $K\alpha$ radiation using a Siemens D-500 diffractometer (Siemens, Madison, WI) located at the Engineering Research Institute, Iowa State University. Operation was at 25 μ A and 50 kV with a medium resolution and a step-scan mode of 0.05° per step, with a counting time of 2 sec.

Molecular Size Distribution by Gel-Permeation Chromatography (GPC)

One gram of starch was suspended in 100 ml of 90% DMSO. The suspension was boiled and stirred at 95°C for 2 hr followed by continuous stirring at room temperature for 24 hr. Fifteen milliliters of the solution (about 150 mg of sample) was precipitated with approximately 100 ml of 100% methyl alcohol. The precipitate was separated by centrifuging at, 5000 x g for 20 min. The precipitate was then redissolved in 50 ml of hot distilled water with 10 mg of glucose added as a marker. The solution was then boiled and stirred at 95°C for 1 hr. A 5 ml sample (containing 15 mg of starch) was quickly cooled and injected into a 2.6 x 80 cm column (Pharmacia Inc., Piscataway, NJ) packed with Sepharose CL-2B gel. Distilled, deionized water containing 10 mM NaOH and 50 mM sodium chloride (NaCl) was used to elute the sample in an ascending direction at the flow rate of 30 ml/hr. Fractions of 4.8 ml were collected and analyzed by using an Autoanalyzer II (Technicon Instrument Corp., Elmsford, NY). The total carbohydrate (anthrone-sulfuric acid reaction) and amylose-iodine blue values of the fractions were measured spectrophotometrically at 630 nm and 640 nm, respectively. Molecular size distributions were calculated on the basis of total carbohydrate. The blue value was used to identify locations of the amylose and amylopectin in the chromatograms. The chromatograms were replicated at least five times (Jane and Chen 1992).

Fractionation

Amylose was separated from the mixture of amylopectin and intermediate by following the methods of Schoch (1942) and Jane and Chen (1992). The fraction containing a mixture of amylopectin and intermediate was recrystallized five times. The absence of amylose in the mixture of amylopectin and intermediate was confirmed by gel-permeation chromatography (Sephacose CL-2B).

Amylose Contents

Amylose contents were determined by measuring iodine affinities of defatted starches using a potentiometric autotitrator (702 SM Titrino, Brinkmann Instrument, Westbury, NY). The analysis was based on Schoch's method (1964). The starch samples and mixtures of amylopectin and intermediate component were determined, and the iodine affinities of the samples were replicated four times.

Branch Chain Lengths of Amylopectin and the Intermediate Components

Amylopectin fractions, collected from gel-permeation chromatography (Sephacose CL-2B) of the amylopectin and intermediate components mixture (fractionated by Schoch's method), were concentrated by using a vacuum evaporator at 45°C. An amylopectin solution containing 20 mg amylopectin in 3.2 ml of distilled water was prepared. An acetate buffer of pH 3.5 (0.1 M, 0.4

ml) was added and thoroughly mixed before adding Pseudomonas isoamylase (900 units). The mixture was incubated in a water bath shaker at 40°C for 48 hr. The enzyme reaction was stopped by heating the mixture in a water bath at 95°C for 30 min. Branch-chain-length distribution was analyzed by using a Bio-Gel P-6 gel-permeation column. The chain length of each peak was determined by measuring the reducing value using a modified Park-Johnson method (Park and Johnson 1949, Hizukuri et al 1981, Jane and Chen 1992) and total carbohydrate content using phenol-sulfuric analysis (Dubois et al 1956) of the peak fractions. The chain length determinations were repeated at least three times.

The intermediate components were also collected from gel-permeation chromatography (Sephacrose CL-2B) from amylopectin and intermediate components mixtures (fractionated by Schoch's method). Branch-chain length of the intermediate was analyzed in the same way as for amylopectin.

High Performance Anion Exchange Chromatography (HPAEC)

The branch-chain-length distribution of debranched amylopectins also was analyzed by using a Dionex DX-300 system (Sunnyvale, CA) equipped with a pulsed amperometric flow-through cell with a gold-working electrode, a silver-silver chloride reference electrode, and a potentiostat. A debranched sample solution (1.5 mg/ml) was filtered through a 0.45 μm membrane (Supor 450, Gelman Sciences, Ann Arbor, MI). The debranched samples were analyzed by following the method of Wong and Jane (1995). The filtrate (25 μl) was injected

and analyzed using a Dionex CarboPac™ PA1 guard column. The pulsed potentials and durations were $E_1 = 0.05 \text{ V}$ ($t_1 = 480 \text{ msec}$), $E_2 = 0.60 \text{ V}$ ($t_2 = 120 \text{ msec}$), and $E_3 = -0.60 \text{ V}$ ($t_3 = 60 \text{ msec}$) at range 2 (sampling periods, 200 msec). The eluents A and B were 150 mM sodium hydroxide solution and 150 mM sodium hydroxide in 500 mM sodium nitrate solution, respectively. The solutions were degassed with helium by using a Dionex degas module. The following gradient was used: 0-5 min, 94% of eluent A and 6% of eluent B; 5-10 min, linear gradient to 8% eluent B; 10-30 min, linear gradient to 13% eluent B; 30-80 min, linear gradient to 20% eluent B; and 80-100 min, linear gradient to 25% eluent B. The eluent gradient was operated at 1 ml/min flow rate. The HPAEC chromatograms were repeated six times.

Thermal Properties by Differential Scanning Calorimetry (DSC)

Thermal properties of starches were determined by following a slight modification of the procedure described by Wang et al (1992). The DSC studies were performed using a Perkin-Elmer DSC-7 analyzer equipped with a thermal analysis data station (Perkin-Elmer Corp., Norwalk, CT). Starch samples (~10.0 mg) were weighed into stainless steel pans (Perkin Elmer Corp., Norwalk, CT) using a microbalance (Metler AE240, Hightstown, NJ), and distilled water (~21.0 mg) was added. The pan was hermetically press-sealed and allowed to equilibrate for about 12 hr before analysis. The samples were heated at 10°C/min over the temperature range of 25-150°C. Indium was used as the

reference standard. Enthalpy (ΔH), onset temperatures (T_o), peak temperatures (T_p), and gelatinization ranges (R) were computed automatically. The data were calculated from at least three tests of each genotype. The retrogradation was done by the same method with the gelatinized samples being stored at 4°C for 7 days.

RESULTS AND DISCUSSION

Isolation of starch was difficult because of tightly bound protein on the surfaces of starch granules. Starch contents in the kernels were ca. 43-51%, and the starch yields were ca. 30-40%, equivalent to recovery rates of 65 to 80%. The contamination of protein would be high if the starch were isolated extensively.

Scanning electron micrographs of starch granules, isolated from the wild type ($Ae+/Ae+/Ae+$) and *Ae1-5180* dominant mutant starches (*Ae1-5180/Ae+/Ae+*, *Ae1-5180/Ae1-5180/Ae+* and *Ae1-5180/Ae1-5180/Ae1-5180*) are shown in Figures 1a-1d. The diameters of the granular wild type ($Ae+/Ae+/Ae+$) starch ranged from 5 to 20 μm and the diameter of the *Ae1-5180* dominant mutant starch with 1-3 doses of dominant mutant genes ranged from 4 to 18 μm . Granule shapes of the wild type starch were similar to that of normal maize starch which were polygonal shaped (Banks et al 1974, Wang 1993, Jane et al

1994). The granular shapes of the *Ae1-5180* dominant mutant starches with 1-3 doses were all similar, typically spherical or oval, with some containing bud-like attachments, but none showed a polygonal shape. The shapes of the *Ae1-5180* dominant mutant starches were similar to the high amylose maize starch (*ae*) (Banks et al 1974, Wang 1993), but *Ae1-5180* dominant mutant did not have long rod-like granules.

X-ray diffraction of the wild type starch (*Ae+/Ae+/Ae+*) showed that the A-pattern (Figure 2a), was the same as normal maize starch (Zobel 1964). The *Ae1-5180* dominant mutant starches (*Ae1-5180/Ae+/Ae+*, *Ae1-5180/Ae1-5180/Ae+*, *Ae1-5180/Ae1-5180/Ae1-5180*) showed that the B-pattern (Figures 2b-2d) was the same as high amylose maize starch (*ae*). The X-ray patterns also indicated low degrees of crystallinity in the *Ae1-5180* dominant mutant starch granules. These results were consistent with x-ray data of the high-amylose starches (French 1984). The difference can be attributed to amylopectin being the main component responsible for the crystallinity of starch. Crystalline domains of starch granules are composed of both A-chains and the exterior parts of B-chains of amylopectin.

Molecular size distributions of the wild type starch and *Ae1-5180* dominant mutant starches were determined by gel-permeation chromatography (Sephacose CL-2B). The chromatograms are shown in Figures 3a-3d. In the chromatograms, the first peak, which eluted out early at the void volume, corresponded to amylopectin. The second major peak, displaying a high blue

value peak, corresponded to amylose, and the last peak was glucose added as a marker for the profile. In the *Ae1-5180 dominant* starch, there was another broad peak between the amylopectin and amylose peaks, the intermediate fraction. The chromatogram of the wild type starch showed an elution profile similar to that of the native normal-maize starch (Wang et al 1993). The chromatograms of the *Ae1-5180* dominant mutant starch with different doses of dominant mutant gene (*Ae1-5180/Ae+/Ae+*, *Ae1-5180/Ae1-5180/Ae+*, and *Ae1-5180/Ae1-5180/Ae1-5180*) showed similar profiles. The dominant mutant starch chromatograms showed greater total carbohydrate peaks of the intermediate and amylose fractions, but the blue value of the amylose fractions was similar or slightly lower than that of the wild type. The blue values of the amylopectin peak were comparably higher (relative to the amylopectin carbohydrate peak) than that of the wild type, indicating long-branch-chain lengths of the amylopectin. The blue value/total carbohydrate ratio of the intermediate fractions was similar to that of the amylopectin; evidently the intermediate components were smaller-sized amylopectin molecules. The elution profiles of the *Ae1-5180* dominant mutant starches resembled that of *ae bt1* mutant maize starch (Wang et al 1993), but differed from that of high amylose (*ae*) starch (Wang et al 1993).

Butanol-soluble fraction obtained by the Schoch's method (contained both amylopectin and intermediate component) were analyzed by gel-permeation chromatography (Sephacrose CL-2B). The chromatograms are shown in Figure 4a-4c. The Sephacrose CL-2B chromatograms showed the high ratio of the blue

value to the total carbohydrate, which indicated long branch-chain lengths in both the intermediate component and amylopectin molecules. It also showed that the mixtures were free from contamination of amylose. The peak that appeared before the glucose marker was attributed to maltodextrin, which could not be removed by precipitating with n-butyl alcohol. The weight ratio of amylopectin to intermediate component was 1 : 2.6.

Amylose contents of the starches were analyzed by measuring iodine affinity. The amylose content and iodine affinity are shown in Table I. The iodine affinity of the wild type starch (*Ae+ / Ae+ / Ae+*) was similar to that of the normal maize starch. Iodine affinities of the *Ae1-5180* dominant mutant starches were similar to that of the high amylose V starch, which contained about 55% amylose contents, but the high amylose V starch did not contain as high an amount of the intermediate component (Jane and Chen 1992) as did the *Ae1-5180* dominant mutant maize starch. The real amylose contents of *Ae1-5180* were calculated by the following equation:

$$C = (IA_S - IA_{AP+IC}) / \{0.19 - (IA_{AP+IC} / 100)\}$$

C is the percentage of the real amylose content

IA_S is the iodine affinity of the whole defatted starch

IA_{AP+IC} is the iodine affinity of the amylopectin and intermediate component mixture.

The high iodine affinity of the amylopectin and intermediate component mixture also confirmed the presence of long branch-chains that could interact with iodine.

Amylopectin structure was investigated by determining branch-chain length. Gel-permeation chromatography of isoamylase-debranched amylopectin samples by Bio-gel P-6 column displayed three major peaks (Figure 5). The first peak eluted at the void volume of the column contained some very long B chains (which could be B3 or longer) (Hizukuri 1986). The second peak corresponded to the B2 chains of amylopectin (long chains), and the last peak contained A chains and short B chains of amylopectin (short chains). The fractions collected from the second and third peaks were analyzed for their peak chain lengths. The ratio of the long chain to short chain contents and the peak chain lengths of the long and short chains are shown in Table II. These results confirmed those of GPC, iodine affinity, and X-ray diffraction, which indicated longer chain length in the mutant amylopectin than that of the wild type starch. The amylopectin chain length for wild type starch was similar to that of normal maize. The chain length of the *Ae1-5180* dominant mutant for different doses of dominant mutant genes was shorter than the branch-chain lengths of the high amylose (*ae*) V and VII starches (Wang et al 1993, Chen and Jane 1995).

Intermediate component structure was also investigated following the same procedure as amylopectin. Gel-permeation chromatography (Biogel P-6) of debranched intermediate component (Figure 6) displayed two major peaks,

compared with the chromatograms of debranched amylopectin, which contained three peaks. The intermediate component consisted of long chains (B2) (the first peak) and short B and A chains (the second peak). There were no B3 and longer branch-chains. The peak chain length and the ratio of long-chain-to-short-chain total carbohydrate contents are shown in Table III. These results indicated that the intermediate component did not have B3 and longer chains, but had longer B2 and short branch-chains.

High-performance anion exchange chromatogram of the debranched *Ae1-5180* dominant mutant amylopectin is shown in Figure 7a, and HPAEC chromatograms of debranched *Ae1-5180* dominant mutant intermediate component is shown in Figure 7b. The profiles were limited to about DP 65 because of the signal/noise ratio. The chromatograms of the *Ae1-5180* dominant mutant amylopectin and the intermediate component with different doses of dominant genes showed similar bimodal distributions. The HPAEC results were consistent with those of the gel-permeation chromatograms showing that the different doses of the dominant mutant genes did not differ from those of amylopectin and intermediate branch-chain length. It also indicated that both amylopectin and intermediate component have similar molecular structure, except the intermediate component had more very short chains (DP 9-14) than amylopectin, and amylopectin had more chains of DP 16-30 than did the intermediate component (calculated from the peak area of the normalized chromatograms)

Gelatinization thermograms of the *Ae1-5180* dominant mutant starches determined by a differential scanning calorimeter (DSC) showed two peaks (Figure 8b-8d), which were different from that of the wild type starch (Figure 8a). The second peaks in the *Ae1-5180* dominant mutant starch was referred to as amylose-lipid complex. The thermograms of the *Ae1-5180* dominant starches also were different from those of high amylose starches (*ae*). The gelatinization temperature and enthalpy changes are reported in Table IV. The gelatinization temperatures of *Ae1-5180* with different doses of mutant gene were similar, and they were all higher than that of the wild type starch (T_p 69.5). The onset temperatures and peak temperatures for gelatinization of the *Ae1-5180* dominant maize starches were lower than those of the high amylose starches (*ae*). The gelatinization enthalpy of the wild type starch (14.7 ± 1.1 J/g) was greater than that of the dominant mutant starches (ca. 13.7 J/g).

Retrogradation of the starch sample after being stored at 4°C for 7 days was analyzed by using the DSC with the same parameters as those for gelatinization. The thermogram (Figure 9a) of the retrograded wild type starch (*Ae+/Ae+/Ae+*) was similar to that of normal starch. The thermograms of retrograded *Ae1-5180* dominant mutant starches showed broad peaks (Figures 9b-9d). The thermal transition temperatures and enthalpy changes for the retrograded starches are shown in Table V. Thermal transition ranges of the retrograded starches were broader than the gelatinization of native starches. The enthalpy change of the retrograded wild type starch was lower than those of

the mutant starches, which could be attributed to the greater tendency of reassociation of the longer branch-chains of the *Ae1-5180* dominant mutant starches. The thermograms of the high amylose 50 and 70% starches showed very broad peaks, and it is difficult to identify the peaks. Thermograms of native and retrograded starches for all the varieties showed a peak at about 100°C, which was attributed to the amylose-lipid complex. The amylose-lipid complex peak was confirmed by rescanning, which showed the peak occurring at the same position. The enthalpies of the dominant mutant *Ae1-5180* amylose-lipid complex (0.9 ± 0.3 J/g) were higher than that of wild type amylose lipid complex (0.6 ± 0.1 J/g). This indicated that the dominant mutant *Ae1-5180* starch contained more amylose-lipid complex than did wild type starch.

SUMMARY

The characteristics of the *Ae1-5180* dominant mutant maize starches with different doses of genes were not significantly difference. The scanning electron micrographs showed that some protein attached to the surface of starch granules. Results from SEM, X-ray diffraction patterns, GPC, structure of amylopectin, HPAEC, and thermal properties indicated that *Ae1-5180* dominant mutant maize starches were different from the common high amylose maize starch isolated from recessive (*ae*) mutants. The analysis of amylopectin and intermediate component

suggested that the amylopectin branch-chain length was shorter than for the intermediate component. The real amylose contents of *Ae1-5180* dominant mutant maize starch was less than that of high amylose 50% starch and high amylose 70% starch, but the *Ae1-5180* dominant mutant starch contained very high amounts of intermediate components.

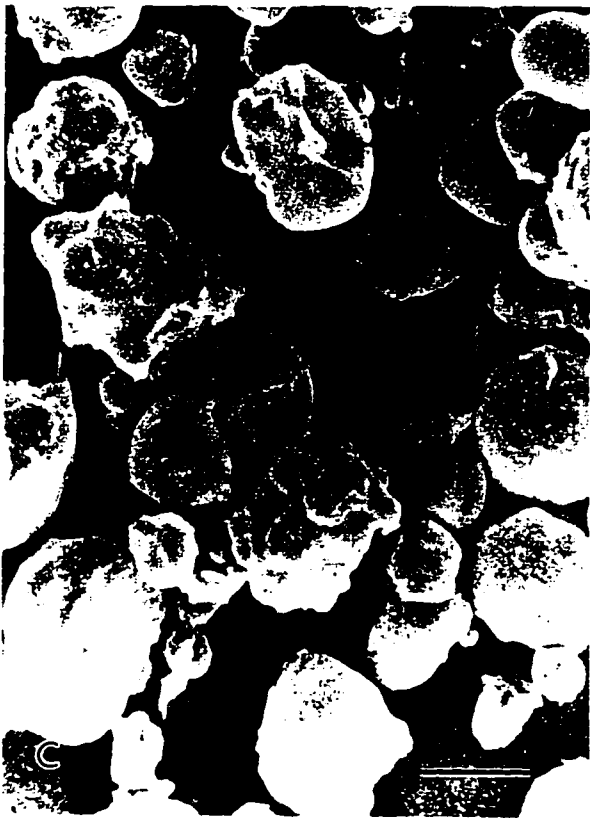
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Figure 1 Scanning electron micrographs of maize starch granules (1,500x). A. wild type ($Ae^{+}/Ae^{+}/Ae^{+}$), B. dominant mutant $Ae1-5180$ with one dose ($Ae1-5180/Ae^{+}/Ae^{+}$), C. with two doses ($Ae1-5180/Ae1-5180/Ae^{+}$), and D. with three doses ($Ae1-5180/Ae1-5180/Ae1-5180$). Bar = 10 μm .



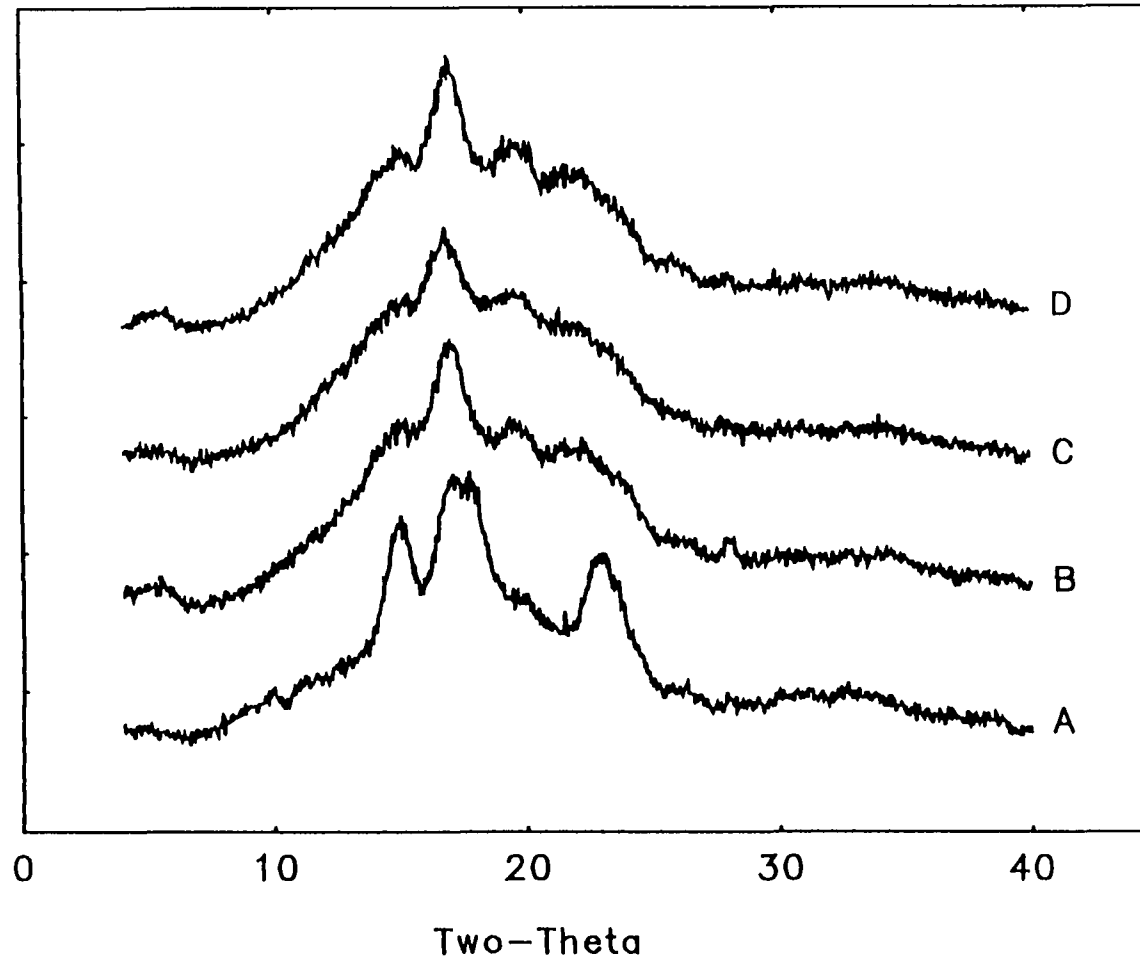


Figure 2 X-ray diffraction patterns of A. wild type maize starch ($Ae^+/Ae^+/Ae^+$), B. Dominant mutant $Ae1-5180$ maize starch with one dose ($Ae1-5180/Ae^+/Ae^+$), C. with two doses ($Ae1-5180/Ae1-5180/Ae^+$), D. with three doses ($Ae1-5180/Ae1-5180/Ae1-5180$).

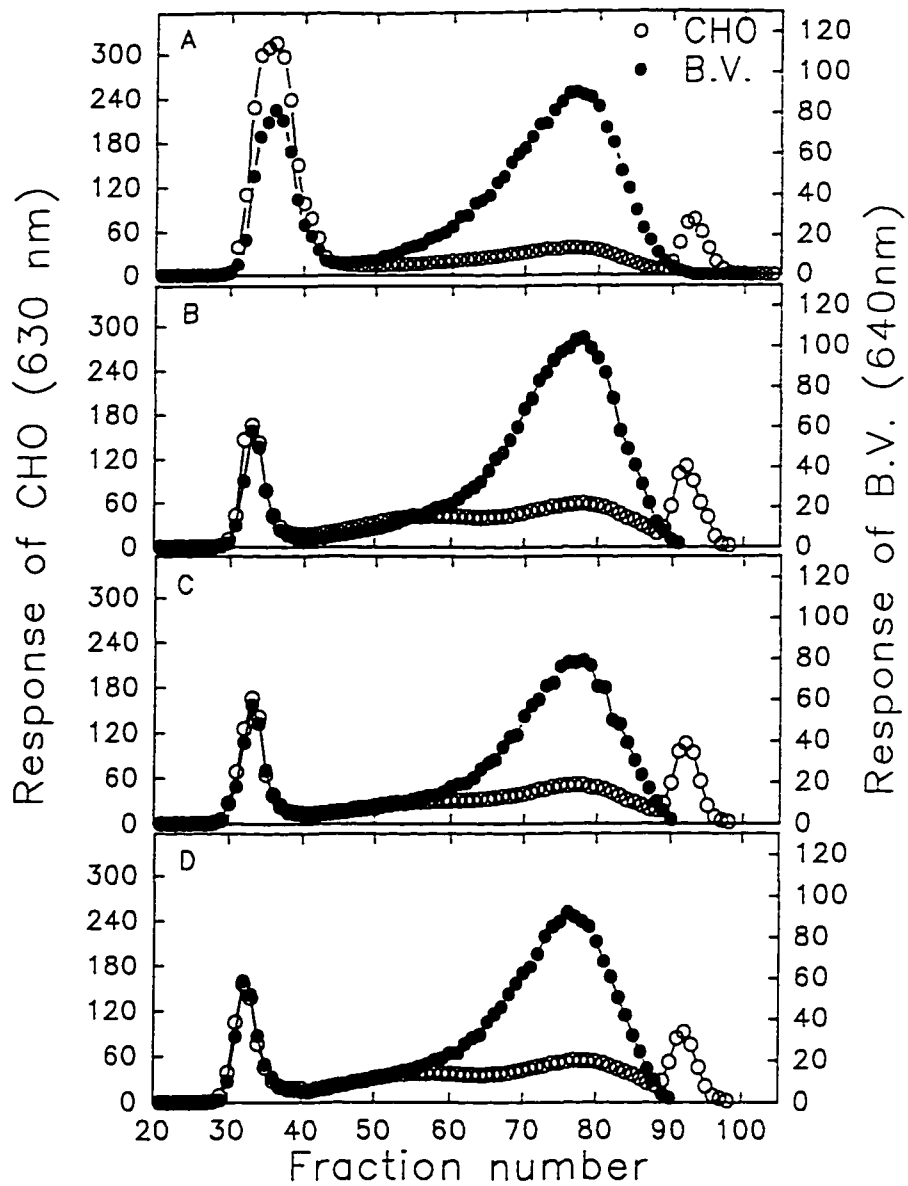


Figure 3 Sepharose CL-2B column (2.6 I.D. x 90 cm) profiles of native starches: A. wild type maize starch ($Ae^+/Ae^+/Ae^+$), B. dominant mutant $Ae1-5180$ maize starch with one dose ($Ae1-5180/Ae^+/Ae^+$), C. with two doses ($Ae1-5180/Ae1-5180/Ae^+$), and D. with three doses ($Ae1-5180/Ae1-5180/Ae1-5180$). The column was eluted with 50mM sodium chloride aqueous solution with 10mM NaOH, and flow rate was 0.5 ml/min. Fractions (4.8 ml) were analyzed for total carbohydrate (anthrone-sulfuric acid procedure): $\circ - \circ$, and blue value (amylose-iodine complex): $\bullet - \bullet$. Glucose was used as the marker.

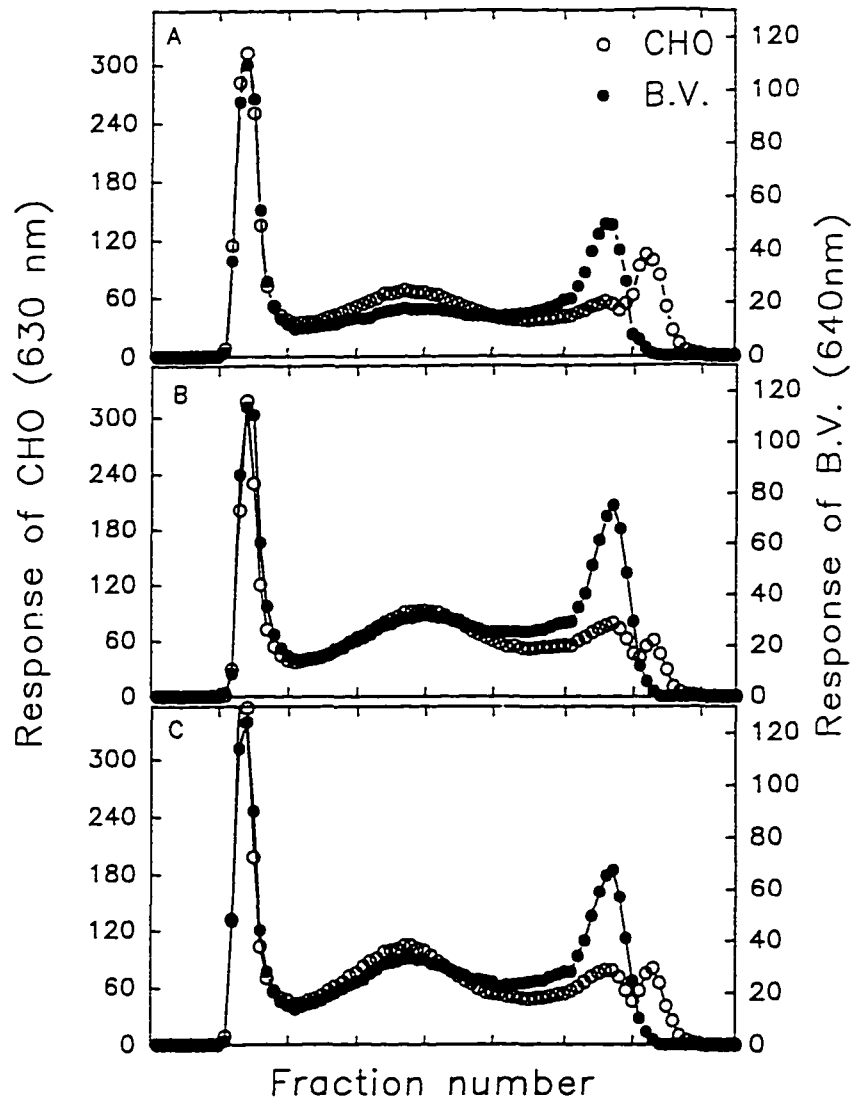


Figure 4

Sepharose CL-2B column (2.6 I.D. x 90 cm) profile of mixture of amylopectin and intermediate: A. dominant mutant *Ae1-5180* maize starch with one dose (*Ae1-5180/Ae+/Ae+*), B. with two doses (*Ae1-5180/Ae1-5180/Ae+*), and C. with three doses (*Ae1-5180/Ae1-5180/Ae1-5180*). The column was eluted with 50mM sodium chloride aqueous solution with 10mM NaOH and flow rate was 0.5 ml/min. Fractions (4.8 ml) were analyzed for total carbohydrate (anthrone-sulfuric acid procedure): ○ - ○, and blue value (amylose-iodine complex): ● - ●. Glucose was used as the marker.

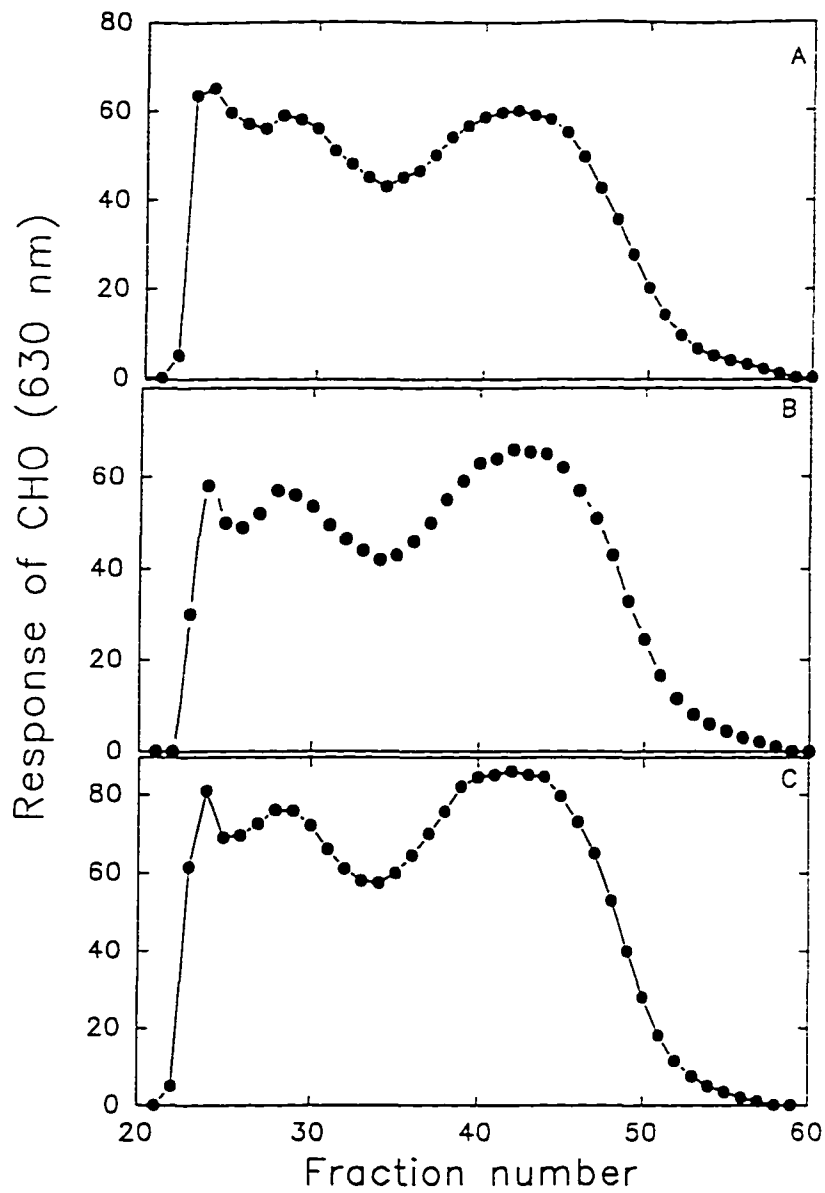


Figure 5 Bio-Gel P-6 column (1.5 I.D. x 80 cm) profiles of debranched amylopectins: A. dominant mutant *Ae1-5180* maize starch with one dose (*Ae1-5180/Ae+/Ae+*), B. with two doses (*Ae1-5180/Ae1-5180/Ae+*), and C. with three doses (*Ae1-5180/Ae1-5180/Ae1-5180*). The column was eluted with distilled deionized water. Fractions (2.3 ml) were analyzed for total carbohydrate (anthrone-sulfuric acid procedure): ● - ●.

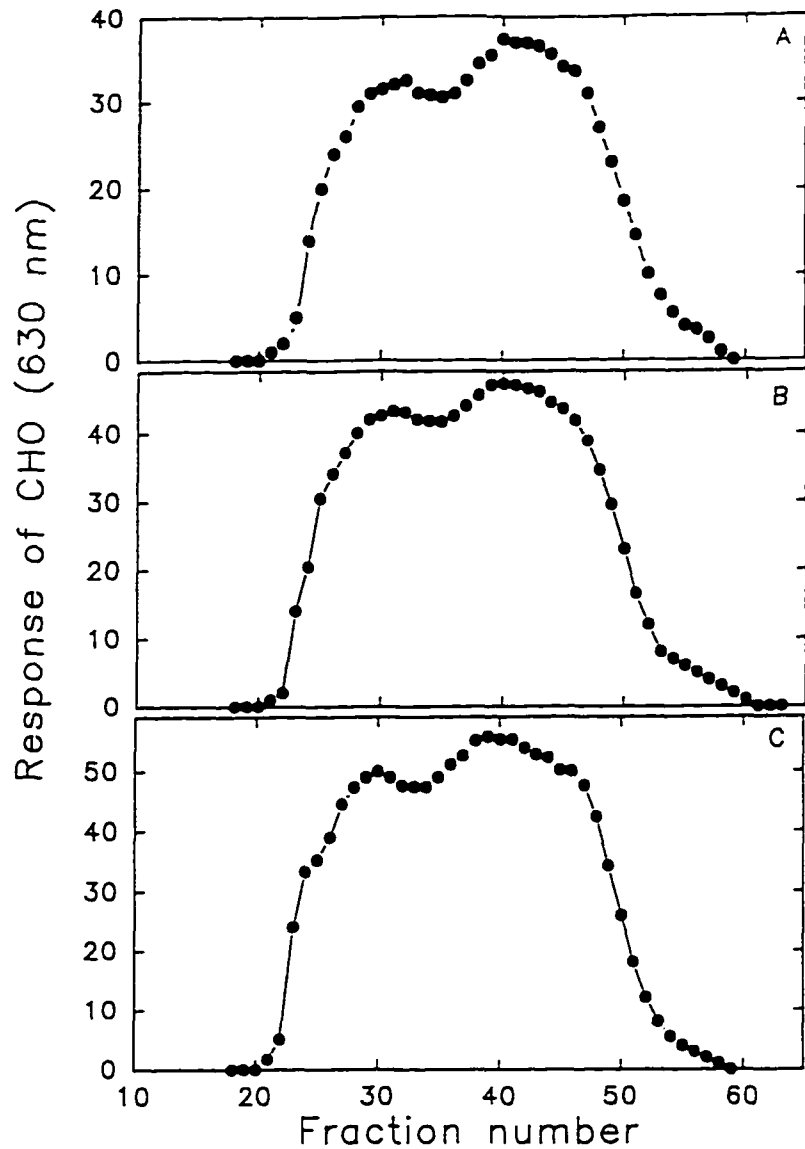


Figure 6 Bio-Gel P-6 column (1.5 I.D. x 80 cm) profiles of debranched intermediate components: A. dominant mutant *Ae1-5180* maize starch with one dose (*Ae1-5180/Ae+/Ae+*), B. with two doses (*Ae1-5180/Ae1-5180/Ae+*), C. with three doses (*Ae1-5180/Ae1-5180/Ae1-5180*). The column was eluted with distilled deionized water. Fractions (2.3 ml) were analyzed for total carbohydrate (anthrone-sulfuric acid procedure): ● - ●.

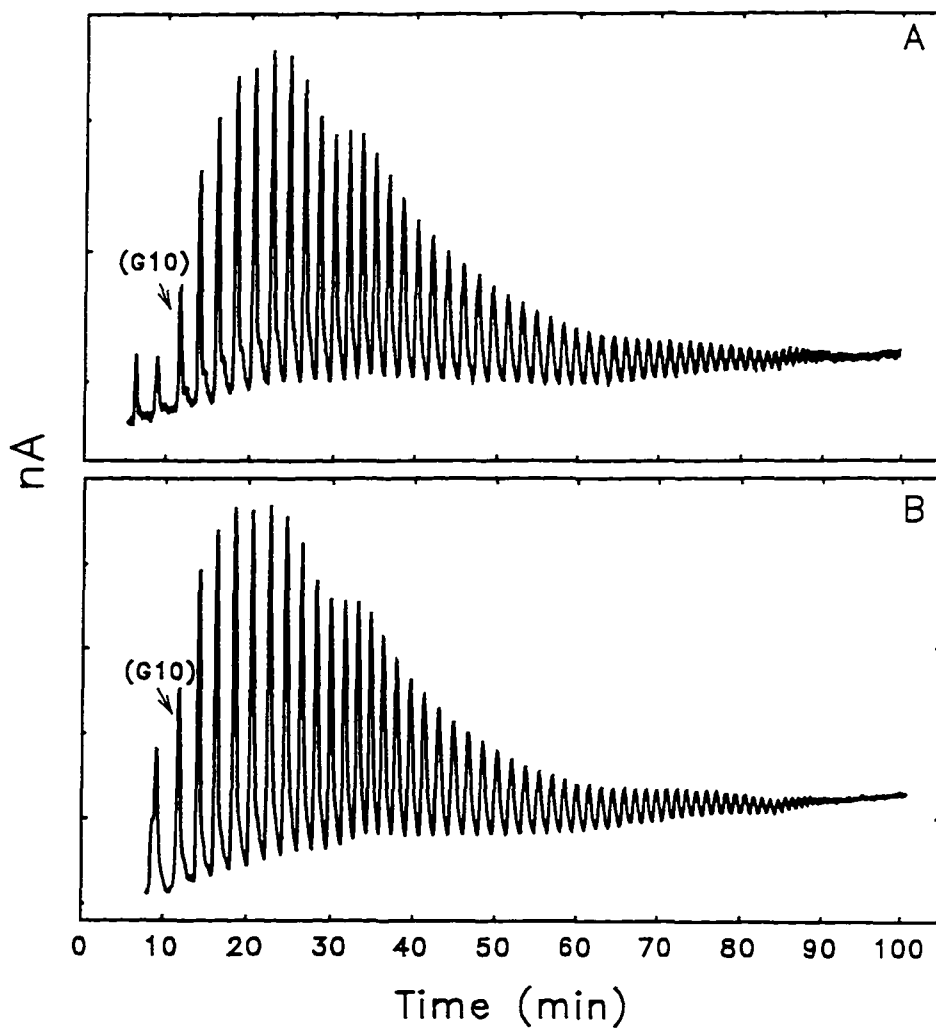


Figure 7 High performance anion exchange chromatograms: A. the *Ae1-5180/Ae1+/Ae1+* dominant mutant debranched amylopectin, and B. the *Ae1-5180/Ae1+/Ae1+* dominant mutant debranched intermediate component. The chromatograms were detected by pulse amperometric detector with gold electrode.

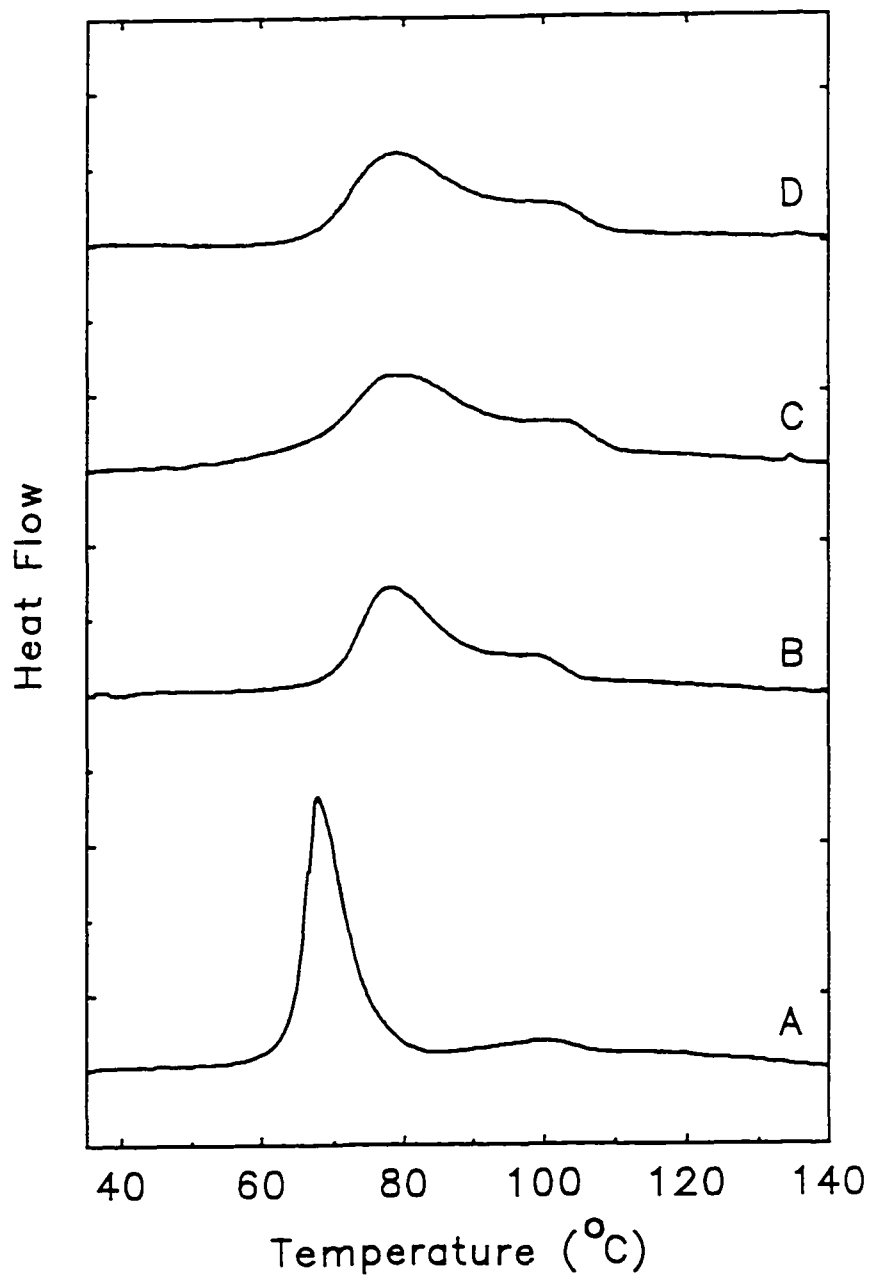


Figure 8 DSC thermograms of native starches: A. wild type maize starch ($Ae+/Ae+/Ae+$), B. dominant mutant $Ae1-5180$ with one dose ($Ae1-5180/Ae+/Ae+$), and C. with two doses ($Ae1-5180/Ae1-5180/Ae+$), D. with three doses ($Ae1-5180/Ae1-5180/Ae1-5180$).

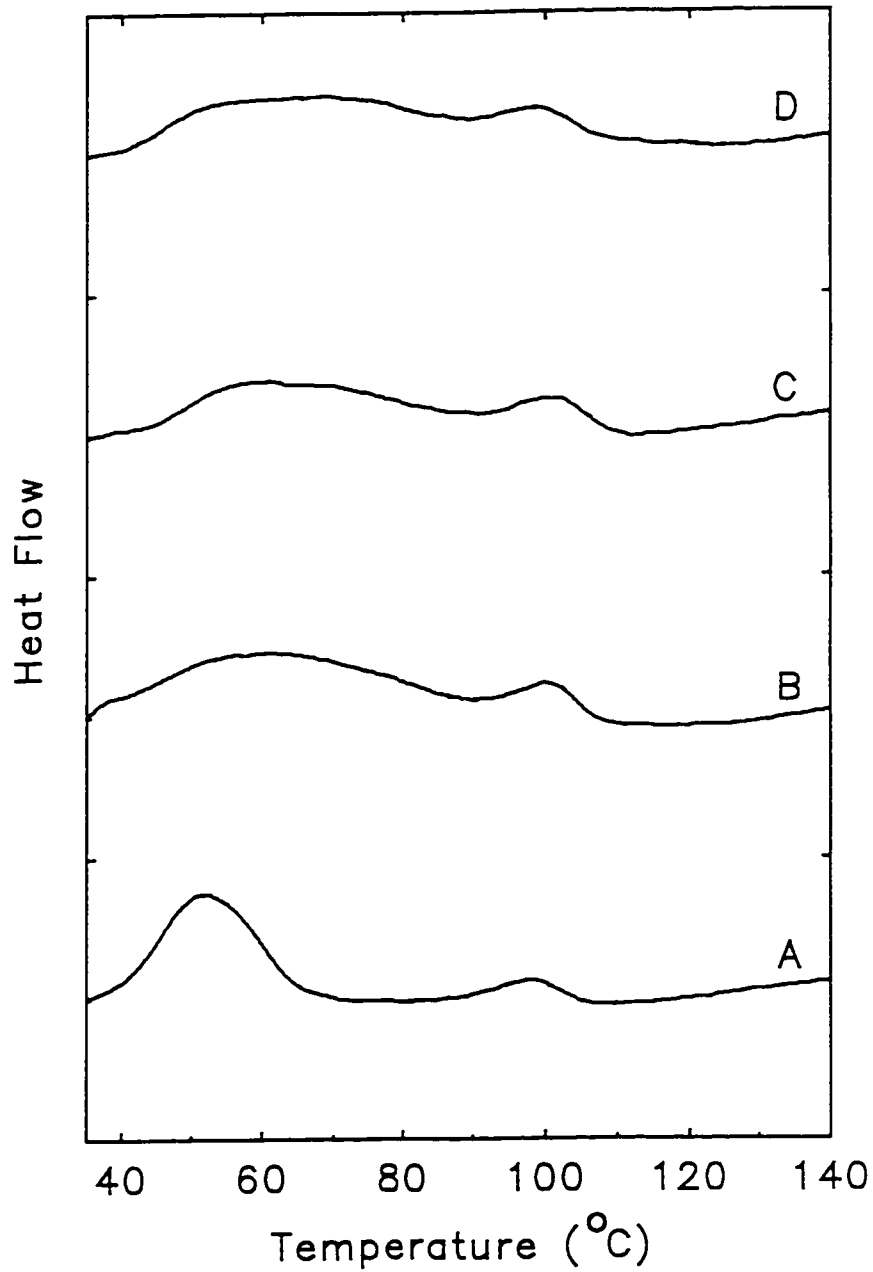


Figure 9 DSC thermograms of retrograded starches: A. wild type maize starch ($Ae^+/Ae^+/Ae^+$), B. dominant mutant $Ae1-5180$ maize starch with one dose ($Ae1-5180/Ae^+/Ae^+$), C. with two doses ($Ae1-5180/Ae1-5180/Ae^+$), and D. with three doses ($Ae1-5180/Ae1-5180/Ae1-5180$).

Table I. Percentage of amylose contents and iodine affinity in starches.

Type	Apparent Amylose Content(%) ^a (Iodine Affinity ^b)		Real Amylose Content (%) ^c
	Defatted Starch	Mixture of Amylopectin and Intermediate Component	
<i>Ae+</i> / <i>Ae+</i> / <i>Ae+</i>	29.0 ± 0.4 (5.5 ± 0.1)	6.4 ± 0.2 (1.2 ± 0.1)	24.1 ± 0.6
<i>Ae1-5180/Ae+</i> / <i>Ae+</i>	58.3 ± 0.5 (11.1 ± 0.1)	34.7 ± 0.4 (6.6 ± 0.1)	36.3 ± 0.9
<i>Ae1-5180/Ae1-5180/Ae+</i>	53.8 ± 1.0 (10.2 ± 0.2)	34.5 ± 0.2 (6.6 ± 0.1)	29.0 ± 1.2
<i>Ae1-5180/Ae1-5180/Ae1-5180</i>	55.5 ± 0.9 (10.6 ± 0.2)	33.5 ± 0.9 (6.4 ± 0.2)	33.3 ± 1.8

^a Amylose contents were determined by iodine potentiometric titration. The amylose contents were calculated by dividing iodine affinity by a factor of 0.19.

^b Iodine affinities were averaged from at least 2 starch samples with at least 4 replications of each sample.

^c Real amylose contents were calculated from the following equation:

$$C = (IA_S - IA_{AP+IC}) / \{0.19 - (IA_{AP+IC}/100)\}$$

C is the percentage of the real amylose content

IA_S is the iodine affinity of the whole defatted starch

IA_{AP+IC} is the iodine affinity of the amylopectin and intermediate component mixture.

Table II. Summary of amylopectin branch-chain-length distribution.^a

Type	Chain Length (glucose units)		Ratio of Total Carbohydrate Contents Long Chains: Short Chains
	Long Chains ^b	Short Chains ^c	
<i>Ae+/Ae+/Ae+</i>	42.3 ± 0.9	13.9 ± 2.1	1 : 3.0
<i>Ae1-5180/Ae+/Ae+</i>	45.7 ± 2.8	19.0 ± 0.4	1 : 1.8
<i>Ae1-5180/Ae1-5180/Ae+</i>	44.5 ± 3.5	19.1 ± 0.7	1 : 1.7
<i>Ae1-5180/Ae1-5180/Ae1-5180</i>	46.6 ± 3.3	19.1 ± 0.6	1 : 1.6

^a The amylopectin branch-chain-lengths were averages of at least two starch samples with at least three replications of each sample.

^b Peak chain-length of the long chain (B2) of amylopectin.

^c Peak chain-length of the short B and A chains of amylopectin.

Table III. Summary of intermediate branch-chain-length distribution.^a

Type	Chain Length (glucose units)		Ratio of Total Carbohydrate Contents Long chains: Short Chains
	Long Chains ^b	Short Chains ^c	
<i>Ae1-5180/Ae+/Ae+</i>	51.1 ± 4.0	21.8 ± 0.8	1 : 1.6
<i>Ae1-5180/Ae1-5180/Ae+</i>	51.6 ± 0.5	20.2 ± 1.7	1 : 1.7
<i>Ae1-5180/Ae1-5180/Ae1-5180</i>	52.9 ± 4.4	21.8 ± 0.9	1 : 1.7

^a The amylopectin branch-chain-lengths were averages of at least two starch samples with at least three replications of each sample.

^b Peak chain-length of the long chain (B2) of intermediate.

^c Peak chain-length of the short B and A chains of intermediate.

Table IV. Thermal properties of starch gelatinization determined by differential scanning calorimetry.^a

Type	T _o ^b (°C)	T _p ^c (°C)	R (°C)	Δ H _{S+A.L} ^f (J/g)	Δ H _{A.L} ^g (J/g)	Δ H _S ^h (J/g)
<i>Ae+</i> / <i>Ae+</i> / <i>Ae+</i>	65.7 ± 1.5	69.5 ± 1.9	56.1 ± 0.5 to 85.0 ± 2.6 ^d	15.1 ± 1.1	0.4 ± 0.1	14.7 ± 1.1
<i>Ae1-5180/Ae+</i> / <i>Ae+</i>	65.7 ± 1.8	78.1 ± 1.0	58.3 ± 4.4 to 109.2 ± 1.3 ^e	14.6 ± 0.8	1.0 ± 0.1	13.5 ± 0.8
<i>Ae1-5180/Ae1-5180/Ae+</i>	66.7 ± 0.8	78.9 ± 1.7	54.0 ± 1.3 to 109.7 ± 1.4 ^e	14.6 ± 0.7	0.7 ± 0.2	13.9 ± 0.9
<i>Ae1-5180/Ae1-5180/Ae1-5180</i>	67.0 ± 3.3	78.9 ± 2.1	57.3 ± 3.4 to 108.8 ± 1.3 ^e	14.4 ± 1.2	0.6 ± 0.2	13.8 ± 1.4
high amylose 50%	67.3 ± 1.2	75.0 ± 1.7	62.5 ± 0.5 to 109.5 ± 1.3 ^e	17.1 ± 0.4	1.0 ± 0.1	16.1 ± 0.4
high amylose 70%	69.3 ± 0.4	75.3 ± 0.7	65.7 ± 0.9 to 109.4 ± 0.5 ^e	12.7 ± 0.8	1.1 ± 0.1	11.6 ± 0.8

^a The values are averages of at least three starch samples with at least three replications of each sample (mean ± standard deviation) .

^b Onset temperature.

^c Peak temperature.

^d Gelatinization range

^e Gelatinization and melting amylose-lipid complex range.

^f Enthalpy of starch gelatinization and melting of amylose-lipid complex.

^g Enthalpy of melting amylose-lipid complex.

^h Gelatinization enthalpy of starch (Δ H_{S+A.L} - Δ H_{A.L}).

Table V. Thermal properties of retrograded starch determined by differential scanning calorimetry.^a

Type	T _o ^b (°C)	T _p ^c (°C)	R (°C)	Δ H _{S+A.L} ^f (J/g)	Δ H _{A.L} ^g (J/g)	Δ H _S ^h (J/g)
<i>Ae+/Ae+/Ae+</i>	41.1 ± 0.3	51.5 ± 0.1	36.9 ± 1.9 to 67.6 ± 2.3 ^d	7.8 ± 1.0	0.8 ± 0.1	7.0 ± 1.1
<i>Ae1-5180/Ae+/Ae+</i>	42.8 ± 2.0	59.9 ± 1.0	42.3 ± 1.7 to 106.8 ± 1.5 ^e	8.8 ± 0.4	1.0 ± 0.1	7.8 ± 0.4
<i>Ae1-5180/Ae1-5180/Ae+</i>	43.7 ± 1.6	64.2 ± 4.2	43.0 ± 2.6 to 107.9 ± 1.2 ^e	9.2 ± 0.8	0.9 ± 0.1	8.3 ± 0.8
<i>Ae1-5180/Ae1-5180/Ae1-5180</i>	46.9 ± 2.9	69.4 ± 1.0	44.5 ± 2.6 to 109.3 ± 1.8 ^e	9.2 ± 1.4	1.0 ± 0.1	8.2 ± 1.5
high amylose 50%	40.0 ± 1.6	N/A ^k	36.1 ± 0.4 to 111.1 ± 2.6 ^e	10.1 ± 1.0	1.2 ± 0.1	9.0 ± 1.1
high amylose 70%	43.4 ± 0.3	N/A	39.5 ± 0.4 to 110.1 ± 1.2 ^e	6.8 ± 1.1	1.2 ± 0.1	5.6 ± 1.1

^a The values are averages of at least three starch samples and at least three replications of each sample (mean ± standard deviation).

^b Onset temperature.

^c Peak temperature.

^d Melting range.

^e Melting and amylose-lipid complex range.

^f Enthalpy of melting retrograded starch and melting of amylose-lipid complex.

^g Enthalpy of melting of amylose-lipid complex.

^h Enthalpy of melting retrograded starch (Δ H_{S+A.L} - Δ H_{A.L}).

^k not available

A QUANTITATIVE METHOD FOR THE ANALYSIS OF PHOSPHORUS STRUCTURES AND CONTENTS IN STARCH BY P-31 NMR SPECTROSCOPY

A paper submitted to journal of Cereal Chemistry

Tunyawat Kasemsuwan and Jay-lin Jane

ABSTRACT

Phosphorus of different chemical structures (e.g., phospholipids, starch phosphate monoester, and inorganic phosphate) are found in starch. In contrast to the colorimetric chemical method (Smith and Caruso 1964) which determines total phosphorus content in starch without differentiating phosphate monoester from phospholipids, P-31 NMR spectroscopy determines phosphorus chemical structures and their individual contents. The relaxation times of starch phosphate monoesters, phospholipids, inorganic phosphate, and phosphate derivatives in nicotinamide adenine dinucleotides (NAD) (internal reference standard) ranged from 1.0 to 2.1 sec. To ensure full relaxation between pulses for quantitative results, a relaxation delay of 11 sec was programmed between data acquisitions. Dimethyl sulfoxide solution (45%) (DMSO) was used to improve α -limit dextrin solubility. P-31 NMR spectroscopy of this solution provided quantitative results. P-31 NMR results showed that potato starch contained mainly phosphate

monoester (0.0859%), wheat starch contained mostly phospholipids (0.0576%). mung bean starch contained mainly phosphate monoester (0.0083%) and phospholipids (0.0006%), tapioca starch contained mainly phosphate monoester (0.0065%), high amylose (50%) maize starch contained mainly phospholipids (0.0169%) and phosphate monoester (0.0057%), and waxy maize starch contained only a trace of phosphate monoester. The total phosphorus contents in starches obtained by P-31 NMR spectroscopy agreed with those obtained by using the colorimetric chemical method. Acid hydrolysis of starch and high temperature operation were attempted to improve the α -limit dextrin solubility, but the amylose-phospholipid complexes remained insoluble in aqueous solution.

INTRODUCTION

Phosphorus in starch is found in three major forms: starch phosphate monoester, phospholipids, and inorganic phosphate. Phosphorus structures and contents in starches vary with the botanical source, maturity, and growing conditions of the plant (Muhrbeck and Tellier 1991, Bay-Smidt et al. 1994, Lim et al. 1994, Nielsen et al. 1994). Most normal cereal starches contain phosphorus in the form of phospholipids (Schoch 1942, Morrison 1981, Hizukuri et al. 1983, Morrison and Gadan 1987, Morrison 1988, Lim et al 1994), whereas phosphorus

in root and tuber starches is in the form of starch phosphate monoesters (Schoch 1942, Hizukuri et al. 1970, Tabata et al. 1975, Lim et al. 1994).

Lim et al. (1994) characterized phosphorus in a variety of starches by using P-31 NMR. They reported that root and tuber starches (i.e., potato, sweet potato, tapioca, lotus, arrow root, and water chestnut) contain mainly starch phosphate monoesters with some inorganic phosphate; no phospholipids were found in these starches. Normal cereal starches (i.e., maize, wheat, rice, oat, and millet) contain mainly phospholipids; normal rice starch also contains a small amount of phosphate monoester. Legume starches (i.e., green pea, lima bean, mung bean, and lentils) contain mainly starch phosphate monoester. Waxy starches (i.e., waxy maize, waxy rice, *du-waxy* maize, and amaranth) contain mainly phosphate monoester; *du-waxy* maize and waxy rice starches also contain small amounts of phospholipids. Total contents of phosphorus in starches were determined by the chemical method of Smith and Caruso (1964).

Phosphorus in starch plays important roles in starch functional properties. For example, phosphate monoester in potato starch accounts for paste clarity, high pasting viscosity, low gelatinization temperature, and slow retrogradation rate. Phospholipids in wheat starch reduce the paste clarity and pasting viscosity (Schoch 1942, Swinkels 1985, Lim 1990).

Quantification of phosphorus in organic matter has been analyzed by using numerous methods (Telep and Ehrlich 1958, Cincotta 1960, Morrison 1964, Smith and Caruso 1964, Kovacs 1986, Singh and Ari 1987). These

methods are based on the destruction of organic matter by incineration or by wet oxidation and converting the phosphorus into its inorganic form. These methods have several disadvantages; namely, requiring large samples, being time consuming, having color instability, and only providing total phosphorus content.

P-31 nuclear magnetic resonance (NMR) has been used to characterize phosphorus in starches and also has been used to identify phosphorylation in modified starches (McIntyre et al. 1990, Muhrbeck and Tellier 1991, Lim and Seib 1993, Bay-Smidt et al. 1994, Kasemsuwan and Jane 1994, Lim et al. 1994). The objective of this study was to develop a quantitative method using P-31 NMR spectroscopy, and to analyze phosphorus chemical structures and contents in starches. Such a method may help reveal the chemical structures of starch phosphorus as well as understand the relation between structure and functional properties of starch. Total phosphorus content obtained by P-31 NMR spectroscopy were compared with those obtained by the colorimetric chemical method.

MATERIALS AND METHODS

Maize, wheat, and potato starches, and crystalline α -amylase of *Bacillus* species were purchased from the Sigma Chemical Company (St. Louis, MO); tapioca and high amylose maize (Hylon-5) starches were donated by National

Starch and Chemical Company (Bridgewater, NJ); waxy maize starch was donated by the American Maize-Products Company (Hammond, IN); and mung bean starch was purchased from the Srithinun Company (Bangkok, Thailand). Dimethyl- d_6 sulfoxide was purchased from Cambridge Isotope Laboratories (Andover, MA).

α -Limit Dextrins Preparation

Starch (2 g, dry starch base, dsb) was suspended in 6.0 ml of acetate buffer (0.01 M, pH 6.9). α -Amylase (0.5 mg) was added, and the suspension was heated and stirred in a boiling water bath for about 10 min. High amylose maize starch was autoclaved for 1 hr (with α -amylase). Additional enzymes (1 mg) were added, and the digestion was continued by incubation in a water-bath shaker at 70°C for 2 hrs. The hydrolysate was heated in a boiling water bath for 10 min to stop the enzyme reaction. The hydrolysate was frozen (-85°C) and dried in a freeze dryer (Unitrap II, Vistis, Gardiner, NY).

P-31 NMR Spectroscopy

The freeze-dried α -limit dextrins were resuspended in 90% deuterate dimethyl sulfoxide (1.5 ml) and heated in a boiling water bath for 10 min. To develop an internal reference standard, the solution was mixed with 1.0 ml of deuterium oxide and 0.5 ml of nicotinamide adenine dinucleotide (NAD) at a

concentration proportional to the phosphorus content in the starch analyzed. The solution was adjusted to pH 8.0 ± 0.1 .

To improve the solubility of α -limit dextrans in the aqueous solution, the α -limit dextrans (3 ml) were further hydrolyzed by adding 4 N hydrochloric acid (HCl) to a final concentration of 0.7 N. The solution was stirred in a boiling water bath for 4 hr, and the hydrolysate was neutralized with 4.0 N sodium hydroxide (NaOH) (Bay-Smidt et al. 1994). The hydrolysate was adjusted to pH 8.0 ± 0.1 before NMR analysis.

P-31 NMR spectra were acquired by using a Bruker AC-200 NMR spectrometer (USA Bruker Instruments, Billerica, MA) at a frequency of 81 MHz, flip angle 90° (24 msec), sweep width 31.18 ppm, 8 k data points, and temperature at 298° K. A relaxation delay of 11 sec was inserted to ensure full relaxation between pulses. The Waltz-16 sequence was used for proton decoupling, and 5000 scans were collected for each spectrum. All chemical shifts were recorded in parts per million (ppm) with 85% phosphoric acid as an external reference (as 0.0 ppm).

Phosphorus Analysis

For colorimetric chemical analysis, total phosphorus content in starch was determined following the Smith and Caruso method (1964). P-31 NMR spectroscopy was undertaken based on the phosphorus contents of starch phosphate monoester, phospholipids, and inorganic phosphate. They were

calculated on the basis of the ratio of their peak areas compared with the peak area of a known concentration of the internal reference compound (NAD). The total phosphorus content of each starch was calculated as the sum of all the phosphorus contents.

Statistical Methods and Analysis

Treatment design was a 2-factor factorial. The first factor was method of determination of phosphorus contents (colorimetric chemical method and P-31 NMR spectroscopy method). The seven starches contributed the second factor. Thus, there were 14 treatment combinations. The experiment was conducted by using a randomized complete block design with two blocks. Each block contained a complete set of 14 treatment combinations and was obtained at different times. For the chemical method, three determinations were obtained and averaged for each of the 14 treatment combinations in each block. A single measurement of each starch sample was obtained using P-31 NMR spectroscopy. Treatment means and standard errors were determined and least significant difference, LSD ($\alpha = 0.05$) was calculated to compare the phosphorus contents of the starches.

RESULTS AND DISCUSSIONS

Phosphorus was found in only small quantities of most native starches; therefore, we needed to increase the concentration of starch for the analysis. In using P-31 NMR spectroscopy (liquid state), because the sample must be in solution, starch was hydrolyzed by α -amylase to increase solubility. Enzymatic hydrolysis decreased the viscosity of the starch paste to prevent peak broadening and concentrated the starch solution to speed up data acquisition.

P-31 NMR spectroscopy of starch samples conducted in aqueous solutions gave only qualitative results because of the limited solubility of α -limit dextrins. Fine particles were observed in the hydrolysate, which was attributed to the starch-phospholipid complex (Jane et al. 1995). To obtain quantitative results, the α -limit dextrins were solubilized in DMSO solution, and a long pulse relaxation interval (11 sec) was used (five times the relaxation interval of the starch monoester). The relaxation times (T1) of phosphorus with different chemical structures are shown in Table 1.

P-31 NMR spectrum of potato starch in 45% DMSO solution (Figure 1) registered signals mainly at chemical shifts 4.1 and 5.2 ppm, which indicated phosphate monoester, and a minor peak at the chemical shift 3.0 ppm, which indicated inorganic phosphate (Kasemsuwan and Jane 1994, Lim et al. 1994). Potato starch contains a significant amount of phosphorus; thus, the spectrum of potato starch was acquired with only 500 scans. Wheat starch (Figure 2)

registered the signals mainly at chemical shifts between 0.0 and 1.5 ppm, which indicated phospholipids (Kasemsuwan and Jane 1994, Lim et al. 1994). The P-31 NMR signal of phospholipids showed a broad peak because the phospholipids consisted of a mixture of compounds with similar molecular structures, such as phosphatidylethanolamine and phosphatidylcholine. Those compounds gave similar chemical shifts that overlapped and could not be resolved by spectroscopy. Mung bean starch (Figure 3) registered signals mainly at 4.0 and 5.5 ppm, which indicated phosphate monoester; the signal at 1.8 ppm indicated phosphoprotein (which usually appeared at the chemical shift range from 1.5 to 3.0 ppm, data not shown), and the signal at 0.7 ppm indicated phospholipids. Tapioca starch (Figure 4) registered broad signals at the chemical shift between 3.5 and 5.5 ppm which indicated phosphate monoesters. Tapioca starch contained a very small amount of total phosphorus (Tables 2 and 3), thus P-31 NMR spectroscopy required at least 8000 scans to get a spectrum of a low noise to signal ratio. Maize starch (Figure 5) registered a broad signal at the chemical shift between 0.5 and 1.5 ppm, which indicated phospholipids, and a minor peak at 3.0 ppm, which indicated a small amount of inorganic phosphate.

High amylose maize starch (50% amylose) (Figure 6) registered signals mainly at the chemical shift between 0.5 and 2.0 ppm, which indicated phospholipids. A large peak at 3.0 ppm indicated a large amount of inorganic phosphate, which might have been derived from the hydrolysis of other

derivatives as a result of the autoclaving preparation of the starch sample. It also showed a small signal at 4.0 ppm, which indicated phosphate monoester. Waxy maize starch (Figure 7) registered a very small signal at chemical shift 5.5 ppm, which indicated phosphate monoester. The spectrum displayed a high noise baseline because of the low concentration of phosphorus in the waxy starch. The high noise level in the P-31 NMR spectra limited identification and measurement of the peak area. P-31 NMR spectra of the sample in DMSO solution displayed broader peaks than those prepared in aqueous solution. For example, the spectrum of potato starch in an aqueous solution displayed split peaks at chemical shifts 4.2 and 4.5 ppm (Figure 8a), but that obtained in DMSO solution displayed a broad peak (Figure 1).

The P-31 NMR spectra showed both the structure (Figures 1-7) and the quantity of each form of phosphorus in starch. The quantity of phosphorus in each form is shown in Table 2. Tuber starches (e.g., potato and tapioca) contained mainly phosphate monoester derivatives. Cereal starches (e.g., wheat, maize, and high amylose maize) contained mainly phospholipids. Waxy maize starch contained only phosphate monoester. Mung bean starch contained mainly phosphate monoester and a small amount of phospholipids.

To reduce the deviation of the measurement caused by the large differences between peak sizes, the concentration of the reference compound (NAD) was adjusted to a range similar to the phosphorus content of starch. Potato and wheat starch samples each had 7.5 mg of internal reference standard

(NAD) added into a 3 ml sample solution; high amylose maize, normal maize, and mung bean starch each had 1.25 mg of NAD; the tapioca starch sample had 0.625 mg of NAD, and waxy maize starch had 0.3125 mg of NAD. The spectra (Figures 1-7) revealed that each starch contained different phosphorus structures and contents that related to its physical properties. The spectra also revealed the phosphorylation location of the phosphate monoester (C-6, C-3, and C-2), which will help us understand more about starch structure (Lim and Seib 1993).

The total phosphorus content determined by chemical analysis was compared with that calculated from the total peak area of P-31 NMR spectra (Table 3). Data obtained for potato, wheat, mung bean, and waxy maize were in good agreement; however, maize and high amylose maize had fairly large deviations, perhaps, because of the broad peaks and a low signal-to-noise ratio in the spectra. The statistical analysis indicated that the phosphorus contents varied between sources of starch, but the phosphorus contents obtained by the two methods of analysis did not vary. There was no significant difference (at $\alpha \leq 0.05$) in phosphorus contents between these two analyses.

Because α -limit dextrins were not completely soluble in the aqueous solution, a quantitative analysis by liquid-NMR spectroscopy was not possible. Acid hydrolysis was attempted to improve the solubility of α -limit dextrins. The α -limit dextrins was hydrolyzed with hydrochloric acid (0.7 N) in a boiling water bath for 4 hr (Bay-Smidt et al. 1994). P-31 NMR Spectra of the aqueous acid hydrolysates were different from those of aqueous α -limit dextrins. The spectrum

of potato starch acid hydrolysate (Figure 8b) showed high intensity phosphate monoester signals and increased intensity of the inorganic phosphate signal. Compared with the α -limit dextrins spectrum (Figure 8a), the phosphate monoesters, which occurred at different positions (C-6, C-3, and C-2) of α -limit dextrins, were hydrolyzed and produced glucose-6-phosphate (4.9 ppm) (Kasemsuwan and Jane 1994). The signal at 3.0 ppm (inorganic phosphate) also increased. The result suggested that the phosphate monoesters at C-2 and C-3 (which were unstable) had been hydrolyzed.

The spectrum of wheat starch acid hydrolysate (Figure 9b) showed a phosphate monoester signal at 4.6 ppm, which coincided with the chemical shift of β -glycerophosphate and a mixture of α - and β -glycerophosphates (4.6 ppm, data not shown). The results indicated that phospholipids were hydrolyzed during the acid hydrolysis and produced glycerophosphate (Christie 1982). The phosphate diesters of phospholipid signals in the α -limit dextrins spectrum (-1.0 to 1.0 ppm) (Figure 9b) disappeared after the acid hydrolysis. The inorganic phosphate signal remained the same. The spectrum of rice starch acid hydrolysate also showed a high intensity of phosphate monoester signal at 4.6 ppm, whereas the phospholipid signals of the α -limit dextrins disappeared, indicating hydrolysis of phospholipids. The phosphoprotein signals (1-3 ppm) remained at the same chemical shifts, but the signal intensities reduced, which indicated some hydrolysis had occurred (Figure 10a, b). The results suggested that acid hydrolysis cannot be used to prepare starch samples for the

quantitative analysis by P-31 NMR spectroscopy. Structures of the phosphorus in starches were obviously changed by acid hydrolysis, and some dextrin-lipid complex still remained insoluble.

P-31 NMR spectra of the acid hydrolysate showed that the chemical shift of the pyrophosphate was changed because of the high salt concentration (which came from the neutralization). This was confirmed by the chemical shift of the pyrophosphate peak of the potato α -limit dextrins moving from -6.6 to -5.8 ppm with addition of $\approx 1\%$ sodium chloride, whereas the chemical shifts of inorganic phosphate and phosphate monoesters remained the same (data not shown).

High temperature (70°C) P-31 NMR spectroscopy also was attempted to improve the solubility of the α -limit dextrins. The spectra showed high noise, and the insoluble precipitant was still present (data not shown).

CONCLUSIONS

P-31 NMR spectroscopy revealed the chemical structures and contents of each form of phosphorus in starch. P-31 NMR spectra of those starch samples solubilized in DMSO solution provided quantitative results, but those prepared in aqueous solution contained amylose-phospholipid complexes, which were insoluble and did not provide quantitative results. Results of starch total phosphorus contents in DMSO solution obtained by P-31 NMR spectroscopy

were in good agreement with those obtained from the chemical method. Acid hydrolysis and the high temperature operation did not improve quantitative analysis. The acid hydrolysate changed the structures of the phosphorus in the starch.

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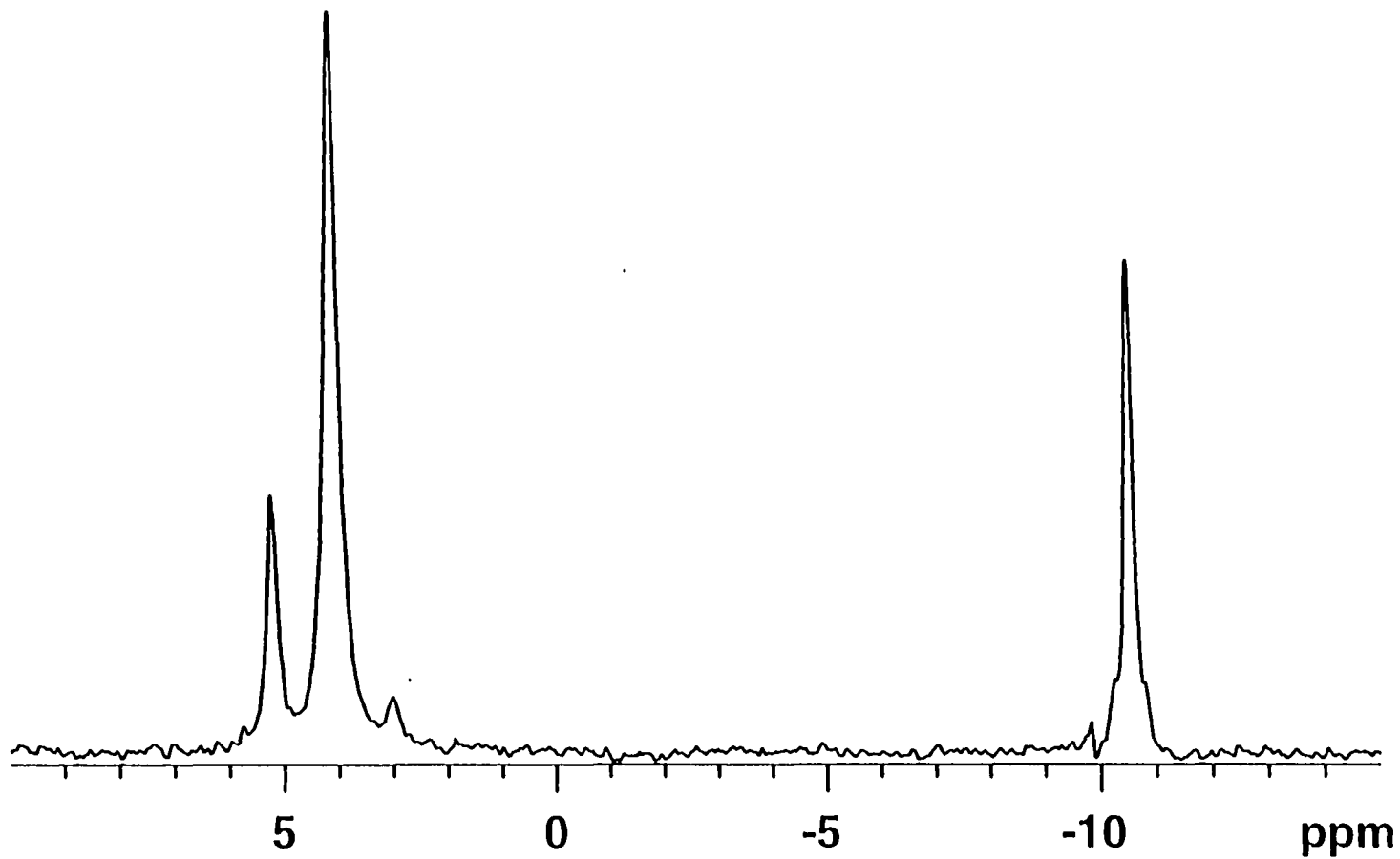


Figure 1 P-31 NMR spectrum of α -limit dextrins prepared from potato starch. The signal at 10.5 ppm is NAD (7.5 mg) as an internal reference.

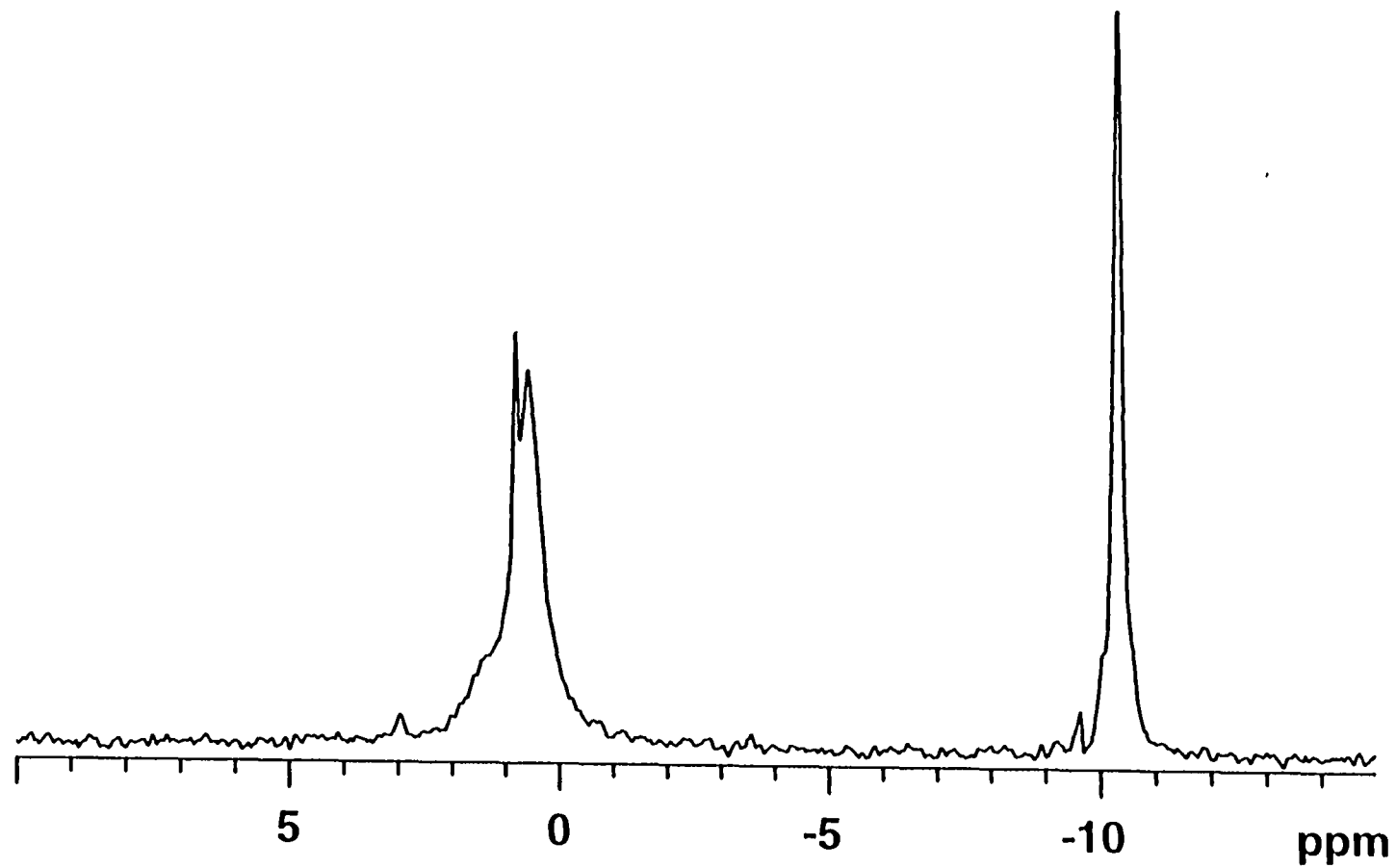


Figure 2 P-31 NMR spectrum of α -limit dextrins prepared from wheat starch. The signal at 10.2 ppm is NAD (7.5 mg) as an internal reference.

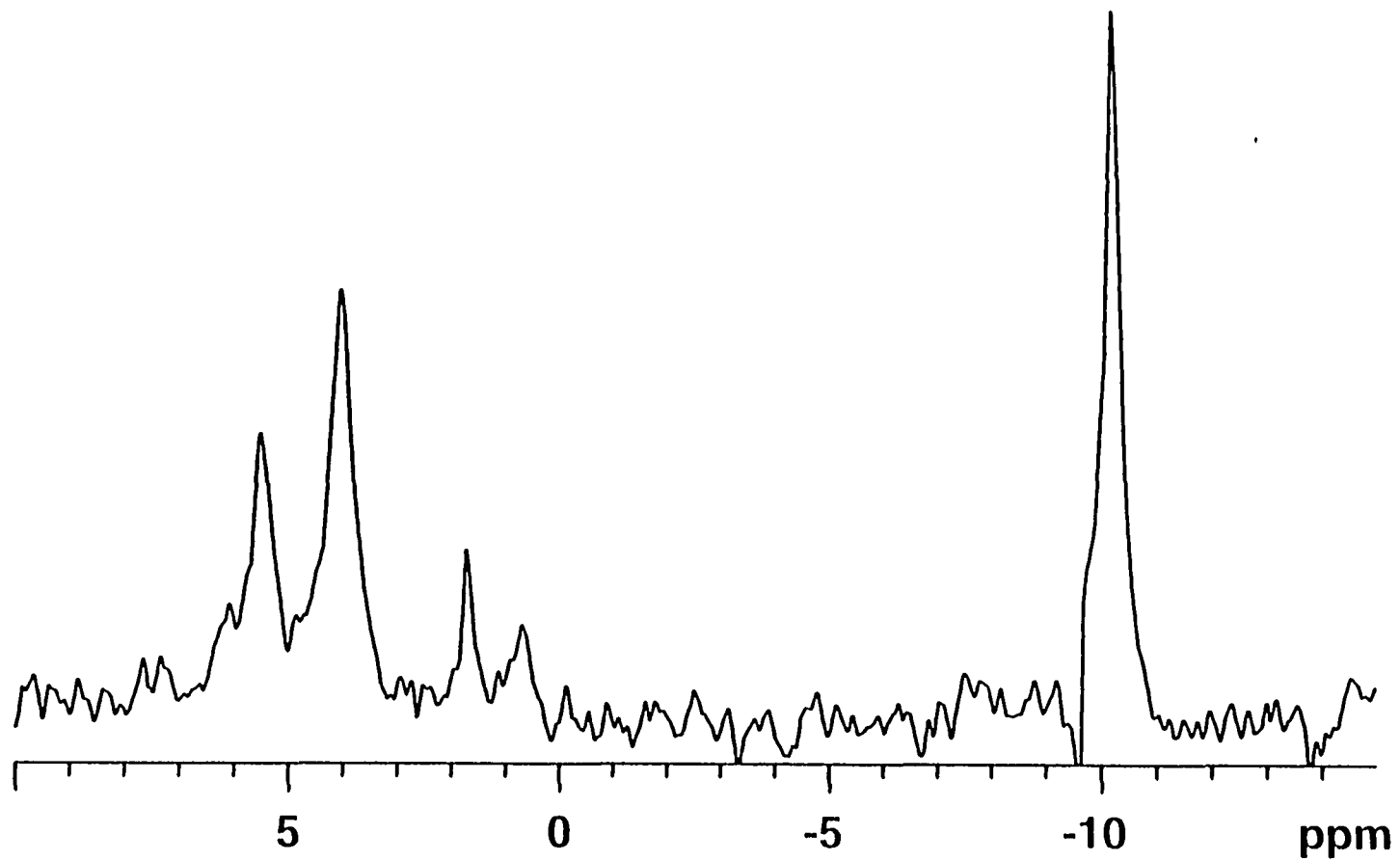


Figure 3 P-31 NMR spectrum of α -limit dextrins prepared from mung bean starch. The signal at 10.2 ppm is NAD (1.25 mg) as an Internal reference.

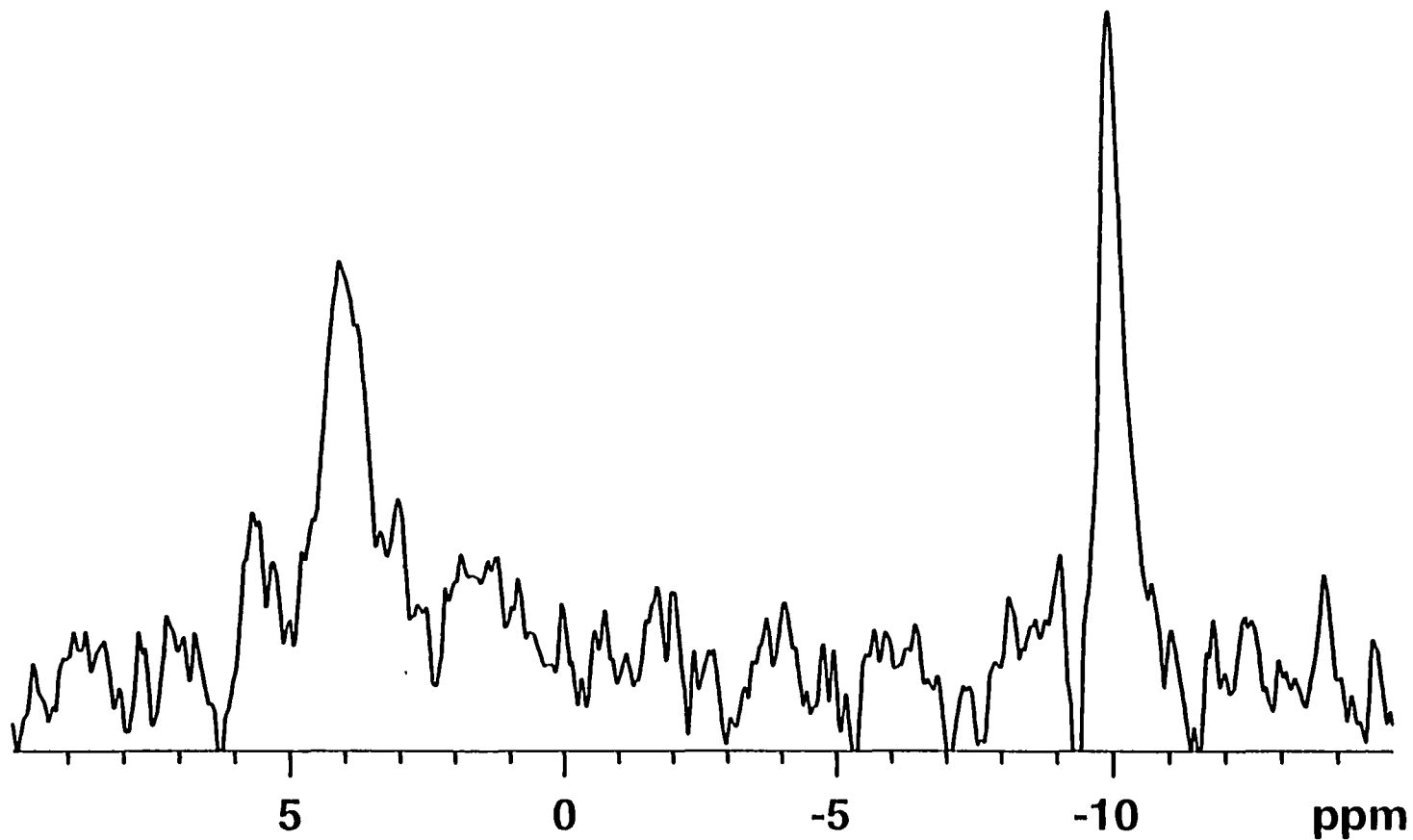


Figure 4 P-31 NMR spectrum of α -limit dextrins prepared from tapioca starch. The signal at 10.1 ppm is NAD (0.625 mg) as an internal reference.

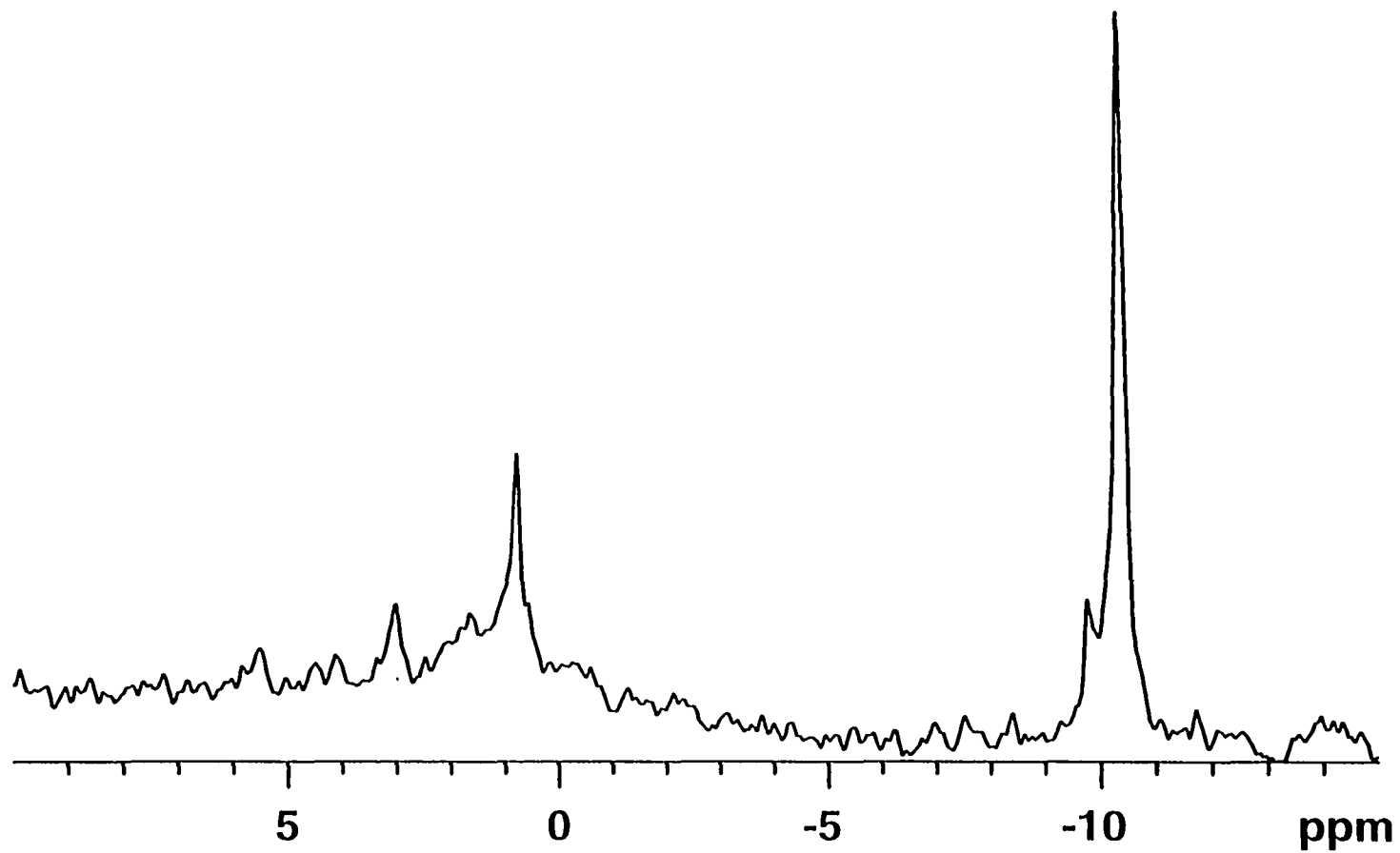


Figure 5 P-31 NMR spectrum of α -limit dextrans prepared from normal maize starch. The signal at 10.4 ppm is NAD (1.25 mg) as an internal reference.

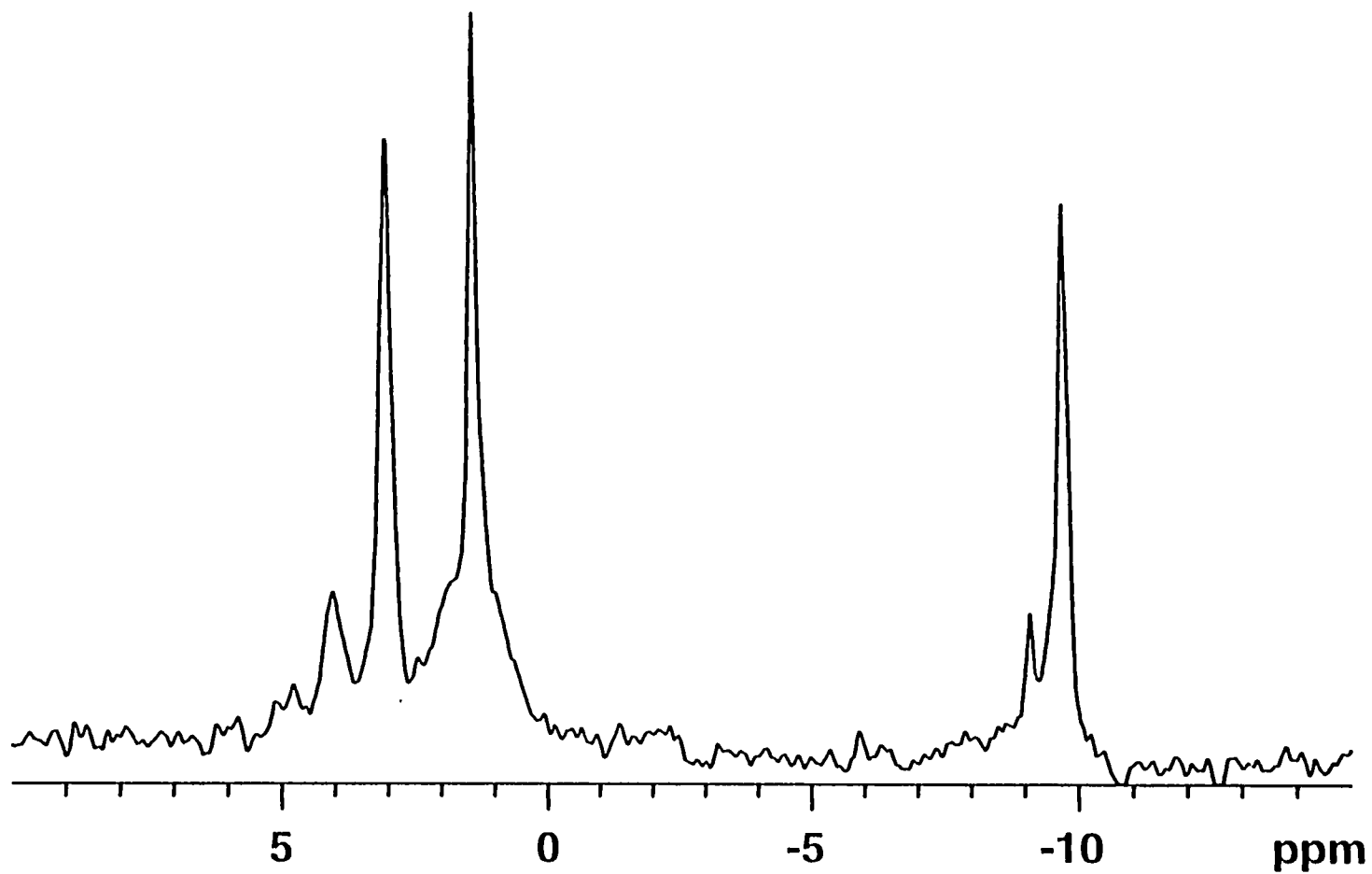


Figure 6 P-31 NMR spectrum of α -limit dextrins prepared from high amylose maize starch (50% amylose). The signal at 9.8 ppm is NAD (1.25 mg) as an internal reference.

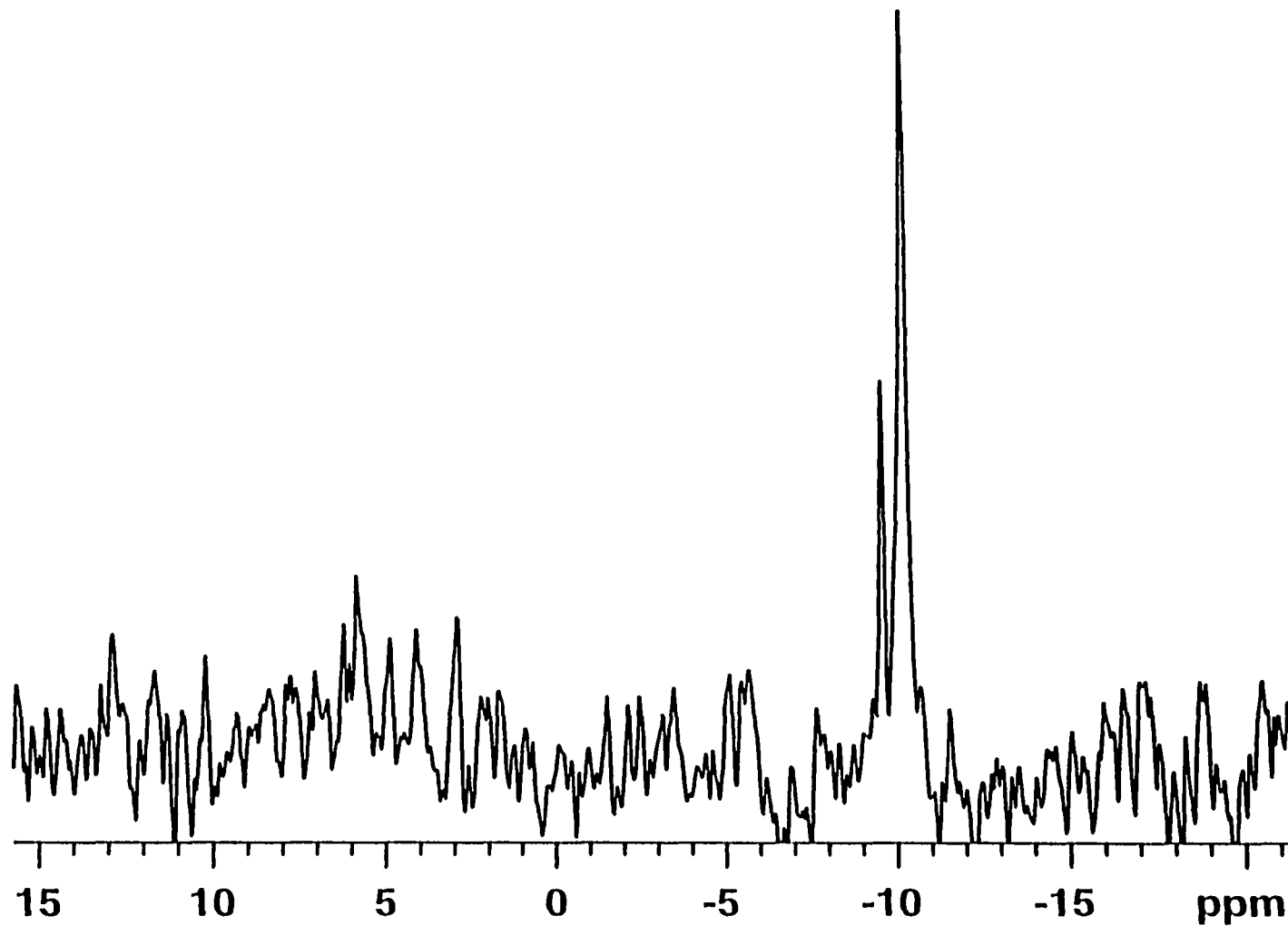


Figure 7 P-31 NMR spectrum of α -limit dextrins prepared from waxy maize starch. The signal at 10.2 ppm is NAD (0.3125 mg) as an internal reference.

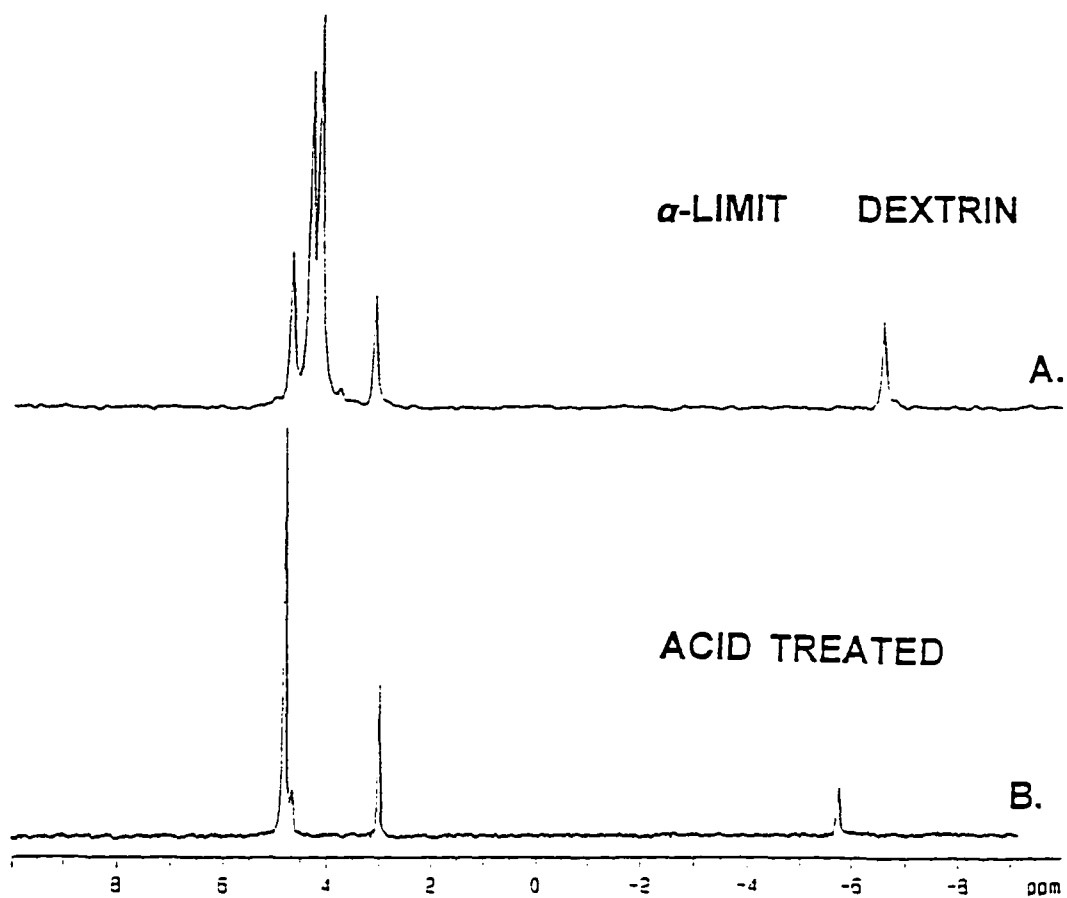


Figure 8 P-31 NMR spectra of α -limit dextrins prepared from potato starch: A. without acid hydrolysis; B. with acid hydrolysis. Signals between 3.5 to 5.0 ppm are phosphate monoesters; 4.9 ppm is glucose-6-phosphate; and 3.0 ppm is inorganic phosphate.

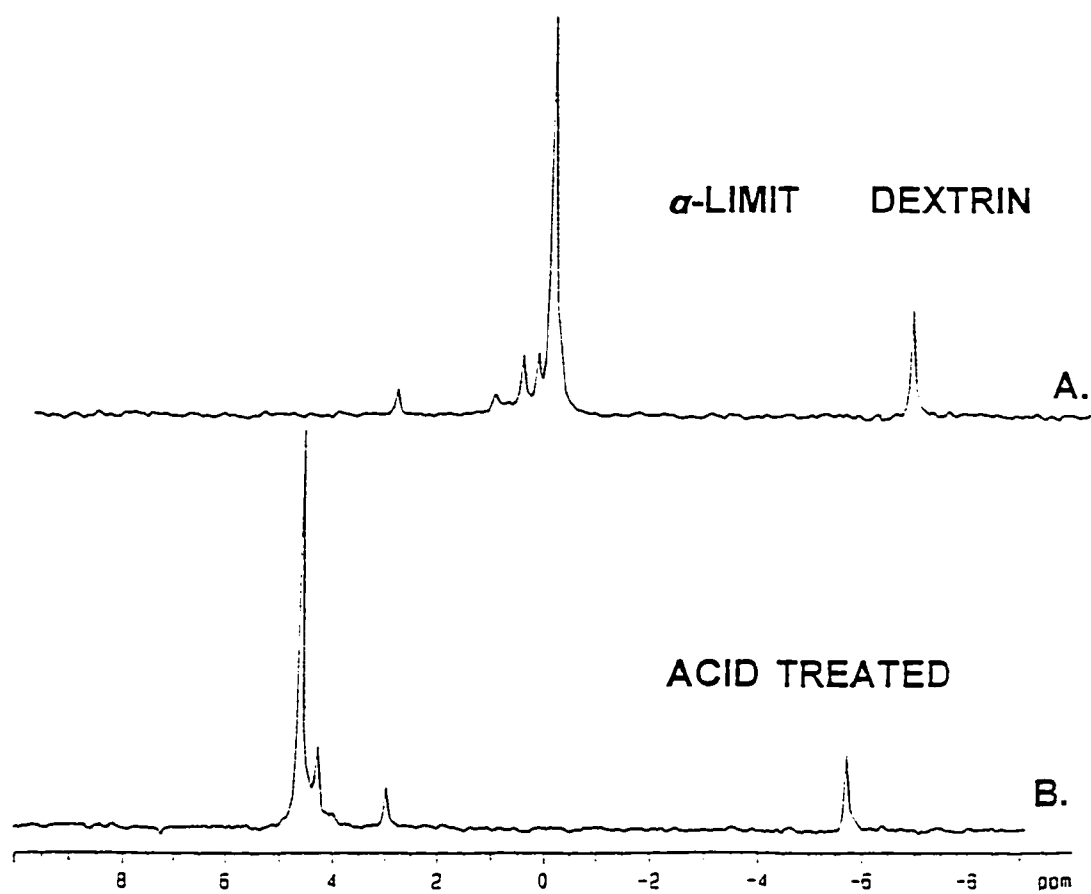


Figure 9 P-31 NMR spectra of α -limit dextrins prepared from wheat starch: A. without acid hydrolysis; B. with acid hydrolysis. Signals between -1.0 to 1.0 ppm are phospholipids; 4.6 ppm is glycerophosphate; and 3.0 ppm is inorganic phosphate.

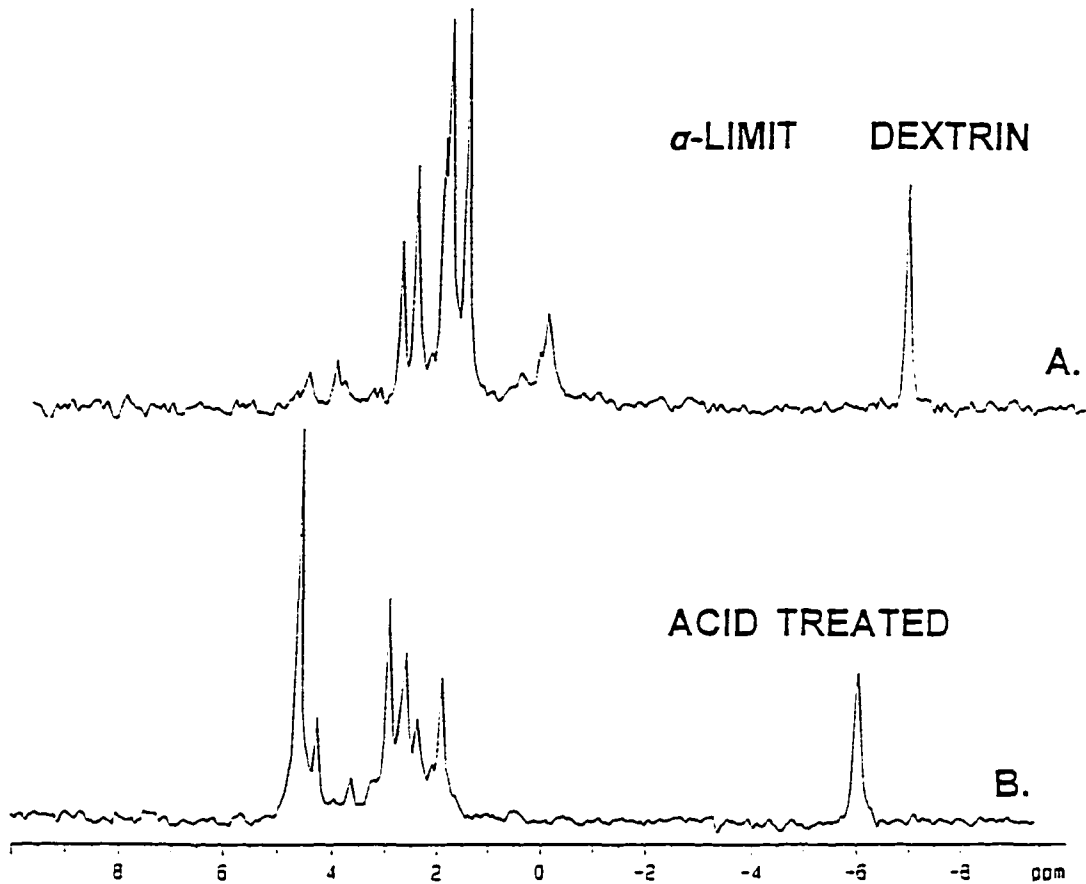


Figure 10 P-31 NMR spectra of normal rice starch α -limit dextrans: A. without acid hydrolysis. B. with acid hydrolysis. Signals between -1.0 to 1.0 ppm are phospholipids. The signal at 4.6 ppm is glycerophosphate; between 1.0 to 3.0 ppm are phosphoproteins; 3.0 ppm is inorganic phosphate, and between 3.5 to 5.0 ppm are phosphate monoester.

Table 1. Relaxation Times and Chemical shifts¹

Types	Chemical Shift, ppm	T1, sec ²
Phosphate Monoester	5.386	2.127 (0.006)
	4.077	1.784 (0.004)
Inorganic Phosphate	2.999	1.422 (0.009)
Phospholipids	0.766	0.851 (0.020)
	0.539	1.030 (0.030)
NAD (Internal Reference)	10.37	1.079 (0.004)

¹ These data were obtained in 45% DMSO solution.

² Data in parenthesis are standard deviation.

Table 2. Phosphorus Contents in Starches^{1,2}

Starches	Phosphate Monoester	Phospholipids	Inorganic Phosphate
Potato	0.0859 ± 0.0070 ³	N/D ⁴	0.0048 ± 0.0003
Wheat	N/D	0.0576 ± 0.0020	trace
Mung bean ⁵	0.0083 ± 0.0002	0.0006 ± 0.0001	N/D
Tapioca	0.0062 ± 0.0004	N/D	trace
Maize	0.0031 ± 0.0010	0.0097 ± 0.0001	0.0013 ± 0.0007
High Amylose Maize (50%)	0.0049 ± 0.0012	0.0150 ± 0.0026	0.0076 ± 0.0006
Waxy Maize	0.0012 ± 0.0006	N/D	0.0005 ± 0.0001

¹ Percentage of phosphorus in starch (dsb, w/w).

² The analysis was replicated twice.

³ Individual standard deviation.

⁴ Not detectable.

⁵ Mung bean starch also contained phosphoproteins (0.0028 ± 0.0015).

Table 3. Total Phosphorus Contents^{1,2}

Starch	Method ³		Starch Mean ⁴
	Chemical Analysis	P-31 NMR Analysis	
Potato	0.0901	0.0906	0.0903
Wheat	0.0569	0.0580	0.0574
Mung bean	0.0121	0.0119	0.0120
Tapioca	0.0071	0.0062	0.0067
Maize	0.0189	0.0141	0.0165
High Amylose Maize (50%)	0.0295	0.0275	0.0285
Waxy Maize	0.0021	0.0021	0.0021
Method Mean (Standard error)	0.0310 (0.0006)	0.0300 (0.0006)	

¹ Percentage of phosphorus in starches (dsb, w/w)

² The analysis was replicated twice.

³ Standard error of a starch-method means is 0.0016.

⁴ Least significant difference for starch means is LSD ($\alpha = 0.05$) = 0.0035.

MANUFACTURE OF CLEAR NOODLES WITH MIXTURES OF TAPIOCA AND HIGH-AMYLOSE STARCHES

A paper to be submitted to journal of Cereal Chemistry

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ABSTRACT

Molecular structures of mung bean and tapioca starches were analyzed. Scanning electron micrographs showed that tapioca starch granules were smaller than that of mung bean starch. X-ray diffraction patterns of mung bean and tapioca starch were A- and C_A- patterns, respectively. Iodine affinity studies indicated that mung bean starch contained 37% of apparent amylose and tapioca starch contained 24%. Gel permeation chromatograms showed that mung bean amylopectin had longer long-branch chains (DP 40) than that of tapioca starch (DP 35) but shorter short-branch chains (DP 16) than that of tapioca starch (DP 21). P-31 NMR spectroscopy showed that both starches contained monoester phosphate, but only mung bean starch contained phospholipids. The physical properties, including pasting viscosity, gel strength, and thermal properties (gelatinization), were determined. The results of the molecular structure and physical properties were used to develop compositions of using tapioca and high-amylose maize starches in making clear noodles.

Tapioca starch was cross-linked by sodium trimetaphosphate (STMP) with various reaction times, pHs, and temperatures. The correlation between those parameters and the pasting viscosity were studied by using a visco/amylograph. Starches, which were cross-linked with 0.1% STMP, pH 11.0, 3.5 hr reaction time at 25, 35, and 45°C (reaction temperature), were used for making the noodles. High-amylose maize-starch (70% amylose) was mixed at varying ratios (9, 13 and 17%) with cross-linked tapioca starches. The properties of the noodles were analyzed, including tensile strength, water absorption, and soluble loss. Trained panelists were used to evaluate the noodles sensory properties. The noodles made from a mixture of cross-linked tapioca starch and 17% of high-amylose starch were comparable to the clear noodles made from mung bean starch.

INTRODUCTION

Clear noodles made from mung bean starch are a popular in Oriental food. The noodles are uniquely translucent and resilient after cooking and have bland taste (Chiu and Chua 1989). The clear noodles are expensive compared with other types of noodles because mung bean starch is expensive as result of its low production supply and tedious processing methods (Haase et al. 1991, Zhu et al. 1990). The molecular structure of mung bean starch and its contribution to

noodle quality have been previously reported (Metres et al. 1988, Singh et al. 1989, Oates 1990, Xu and Seib 1993). Research has been conducted to produce clear noodles with other starches such as canna, red bean, sweet potato, pigeonpea, and modified starches (Lii and Chang 1981, Singh et al. 1989, Chiu and Chua 1989). Noodles prepared from those starches were too soft and not acceptable replacement for mung bean starch noodles (Chiu and Chua 1989). Tapioca starch is a good candidate to manufacture clear noodles because of its low cost and clarity of the starch paste.

The relation between starch structure and physical properties has been partially understood, such as high-amylose starch providing strong gel-strength (Hullinger et al. 1973), and the effect of amylose molecular size and amylopectin branch-chain length on paste properties (Jane and Chen 1992). Characterization of the molecular structures and the physical properties of mung bean starch and tapioca starch could provide the necessary information for revealing the relation between the starch molecular structure and the quality of the noodle.

The purposes of this study were to investigate and compare the molecular structures and the physical properties of mung bean starch and tapioca starch, and to simulate physical properties of mung bean starch by using a mixture of chemically modified tapioca starch and high-amylose maize starch to make the clear noodles.

MATERIALS AND METHODS

Materials

Tapioca and high-amylose maize starches were donated by National Starch and Chemical Company (Bridgewater, NJ). Mung bean starch was purchased from Srithinun Company (Bangkok, Thailand). All the chemicals were reagent grade and used without further treatment. Crystalline Pseudomonas isoamylase (EC 3.2.1.68) was purchased from Hayashibara Shoji, Inc. (Okayama, Japan).

Starch Structure Analyses

Scanning electron microscopy (SEM) followed the Jane et al. (1994) method. The micrographs were obtained with a JEOL JSM-35 scanning electron microscopy.

X-Ray diffraction patterns of starches were obtained with an x-ray diffractometer (D-500, Siemens, Madison, WI) following the method of Jane et al. (1992).

Apparent amylose contents were calculated from iodine affinities which were determined (after the starch was defatted with methanol for 24 hr) by potentiometric titration (Schoch 1964) using a potentiometric autotitrator (702 SM Titrimo, Brinkmann Instruments, Westbury, NY). The real amylose contents were

calculated following Kasemsuwan and Jane (1995a) method. The analysis was replicated at least three times.

Molecular size distributions were determined by Sepharose CL-2B gel (Pharmacia LKB, Uppsala, Sweden) permeation chromatography (GPC), following the method of Jane and Chen (1992). Molecular size distributions were determined on the basis of total carbohydrate (anthrone-sulfuric acid reaction) measured at 630 nm. The blue value was used to identify locations of amylose and amylopectin in the chromatograms (measured at 640 nm). The chromatograms were done in duplicate.

Amylose was separated from amylopectin using the methods of Schoch (1942) and Jane and Chen (1992). The amylopectin fraction was recrystallized at least 4 times. The amylopectin fraction was debranched by isoamylase enzyme following the method of Jane and Chen (1992). The amylopectin branch-chain length distributions were determined by Bio-gel P-6 (Bio-Rad Laboratories, Hercules, CA) gel permeation chromatography. Peak chain-lengths were also analyzed by following Jane and Chen 1992, Kasemsuwan and Jane 1995a methods.

Phosphorus structures and contents were determined by P-31 NMR spectroscopy (Bruker AC200, USA Bruker Instruments, Billerica) following the method of Kasemsuwan and Jane (1995b).

Starch Physical Properties Analyses

Viscosity and pasting properties of starches were determined by using a visco/amylograph (model VA-5, 700 cm-g, Brabender, Hackensack, NJ), following the methods of Smith (1964) and Kasemsuwan and Jane (1994). The amylograms were replicated at least twice.

Gel strength was measured with a Voland texture analyzer (model TA, Scarsdale, NY) following the method of Jane et al. (1992). The starch paste (8%, from the visco/amylograph) was stored at 5°C for 24 hr before analysis. A 2-mm diameter probe and a 3-mm distance of penetration were used throughout the analysis. The measurement was replicated ten times.

Gel strength of the mixture of modified tapioca starch and high-amylose starch (70% amylose content) with the same ratios as that in the noodles was determined. The paste (10% dsb) of the starch mixture was prepared by mixing the amylograph-cooked, modified tapioca starch with high-amylose starch cooked by using a pressure reactor. The gel strength was measured by using the same method as previously described.

Thermal properties of starches were determined following the methods of Wang et al. (1992) and Kasemsuwan and Jane (1995). Gelatinization properties of starches were analyzed by using a Perkin-Elmer DSC-7 analyzer equipped with an Intra-Cooling II system and a thermal analysis data station (Perkin-Elmer Corp., Norwalk, CT). Onset temperature, peak temperature, temperature range,

and enthalpy change were computed automatically. The thermal analyses were repeated at least three times.

Starch Cross-linking with Sodium Trimetaphosphate (STMP)

Tapioca starch (200 g, dry starch base, dsb) was suspended in distilled water (400 g). Sodium sulfate (4 g) was added into the starch slurry, and the slurry was adjusted to a designated pH (11.0 or 11.5) with 4.0 M NaOH. STMP (0.2 g in 5 ml of distilled water) was slowly added to the slurry. The mixture was mechanically stirred, and the pH was controlled by using a pH controller (Chemcadet, Cole Parmer Instrument Company, Chicago, IL). The temperature was controlled by using a water-bath at a designated temperature (25, 35, or 45°C). After a selected reaction time (1.5, 2.5, or 3.5 hr), the slurry mixture was adjusted to pH 5.5 to stop the reaction. The starch was washed three times with distilled water and dried in a forced-air oven at 40°C. The cross-linked starches were analyzed by using the visco/amylograph. Pasting temperature, peak temperature, peak viscosity, viscosity after shear-thinning, set-back viscosity (at 50°C), and gel strength were determined.

Statistic Design and Analysis

The experiment included the study of the effect of three treatment factors: pH (11.0 and 11.5), reaction time (1.5, 2.5, and 3.5 hr), and reaction temperature (25, 35, and 45°C). All combinations of these factors were used in the

experiment, resulting in a total of 18 treatment combinations. A split-plot experimental design (Steel and Torrie 1980) was chosen with temperatures randomly allocated to the whole-plot. A randomized complete block design was used for the whole-plot treatment, with two replications. The reaction time and pH treatment combinations were randomly allocated to each temperature. Analyses of variances were carried out for all dependent variables (paste temperature, peak temperature, peak viscosity, viscosity after shear-thinning, setback viscosity, and gel strength).

Noodle Preparation

The process for making mung bean noodles involves mixing dry and gelatinized starch to form a dough, extruding it directly into boiling water to cook, cooling the cooked noodles in cold water, holding them at refrigerated or freezing temperatures, washing them in cold water, and drying the washed noodles (Lii and Chang 1981, Galvez et al. 1994, Chiu and Chua 1989). In this study, high-amylose maize starch (70% amylose content) was gelatinized in a pressure reactor (model 4522) with a controller (model 4843, Parr Instrument Company, Moline, IL). The high-amylose starch slurry (10, 15, or 20%, dsb) was first heated on a hot plate with continuous stirring for 10 min, and the heated slurry was transferred to the pressure reactor. The starch slurry (in the pressure reactor) was stirred at 100 rpm and heated to 150°C, then it was held at 150°C for 10 min. The slurry was then cooled to 90°C and held at 90°C for 1 hr. The

dry tapioca starch (200 g, dsb) and gelatinized high-amylose starch pastes (200 g, starch paste weight) were mixed for 10 min by using a dough hook and kitchen mixer at speed 3 (Model K5SS, Kitchen Aid Mixer, St. Joseph, MI). The dough was extruded by using an extruder attachment with hole openings of 2-mm diameter (Model SNPA, Kitchen Aid, St. Joseph, MI), into boiling water containing a small amount ($\approx 0.001\%$) of soybean oil. The noodles were cooked in the boiling water for selected times (10, 20, 30, 40, 50, and 60 sec) and were immediately transferred into ice cold water and held for about 5-10 min. The noodles were hung on racks and placed in a cold room (5°C) for approximately 24 hr. They were then transferred into water and soaked at $\approx 25^{\circ}\text{C}$ for 24 hr. The noodles were air-dried on the rack at room temperature. The dry noodles were stored in plastic bags at room temperature until analysis.

Noodle Analyses

Instrument analysis

The analyses followed the method of Lii and Chang (1981) with modification. The dried noodles were cut into 10-15 cm long segments. They were equilibrated for moisture content in a 54% RH humidity chamber (containing a saturated solution of calcium nitrate). Tensile strength was measured using an Instron Universal Testing System (UTM, model 4502) with a 100N load cell and pneumatic-action grips No. 2712-002 (Instron Corporation, Canton, MA). For each treatment, at least five specimens were tested (at a crosshead speed of 50

mm/min and a distance between the two grips of 50 mm) and the values were averaged.

Physical properties

Water absorption was determined following the method of Paetau et al. (1994) with modification. The noodles (approximately 2 g) were conditioned by drying in an oven at 110°C for 24 hr, and then cooled in a desiccator and weighed. The noodles were then soaked in distilled water for 24 hr at 25°C. The surface water was wiped off, and the noodles were weighed. The 24-hr water absorption was calculated as a percentage of the dry noodle weight.

Soluble loss was also determined following the method of Paetau et al. (1994) with modification. The noodles were weighed after soaking for 24 hr and dried in the same manner (110°C, 24 hr) before they were re-weighed. The weight difference between before- and after-soaking was calculated as the percentage soluble loss of dry-weight noodles.

Sensory evaluation

Freshly cooked noodles were prepared by boiling them in water for 10 min and then cooling them in tap water. The noodles were evaluated by ten trained panel members (the sensory properties were explained to the panel members before the evaluation) for firmness, chewiness, clarity, flavor, and general acceptability, using an unstructured 6-inch line-scale. The panelists tasted the

noodles under red lights (to mask possible color differences). The noodles were evaluated in sets of five samples per plate and each set was replicated twice. The scores of each characteristic were averaged.

RESULTS AND DISCUSSIONS

Scanning Electron microscopy

SEM micrographs of mung bean and tapioca starches indicated that mung bean starch granules were oval shaped with an indent in the middle, and the diameter ranged from 10-21 μm . Tapioca starch granules were irregularly shaped. The diameter ranged from 5-20 μm . SEM micrographs of the starches were reported by Jane et al. (1994). The diameter of tapioca starch was smaller than that of mung bean starch (Table 1).

X-ray Diffraction Pattern

The X-ray diffraction pattern of the mung bean starch showed an A-pattern, but that of tapioca starch showed a C_A pattern. The results of the X-ray diffraction patterns (Table 1) indicated that tapioca starch had longer A-chains than mung bean starch. X-ray diffraction patterns also showed higher crystallinity of tapioca starch than that of mung bean starch which contained more amylose.

Amylose Contents

The potentiometric titration results showed that mung bean starch contained more apparent amylose (37.0%) than tapioca starch (24.3%, Table 1). Because long branch-chains (B2 and longer) of amylopectin had greater iodine affinity which increased the apparent amylose content, the real amylose content needed to be determined. The real amylose content in mung bean starch was $30.7 \pm 0.5\%$, significantly greater than that of tapioca starch (19.6 ± 0.7) (Table 1). Tapioca starch showed less of a difference between the apparent amylose content and the real amylose content than mung bean starch. This could be attributed to mung bean starch having longer long-branch-chains than tapioca starch (DP 40 versus DP 35 at peak). The maximum extinction coefficient (E_{\max}) of amylose-iodine complex is proportional to log DP of amylose up to DP 100 (Szejtli et al. 1967). Amylose is the most important factor affecting the starch gel-strength because of its prompt retrogradation and its interaction with lipids and amylopectin to give strong gel networks.

Gel Permeation Chromatography

The molecular-size distribution of starch molecules was determined by GPC. The chromatogram (Figure 1) showed three peaks: the first peak, eluted at the void volume, represented amylopectin fraction; the second peak (broad peak), represented amylose fraction; and the last peak was the glucose marker. The chromatogram also showed that mung bean starch contained smaller sized

amylose than the tapioca starch. The results agreed with those reported previously (Hizukuri et al. 1987, Biliaderis et al. 1981, Xu and Seib 1993). The greater blue value ratio of the mung bean amylopectin was consistent with that it had longer long-branch-chains than tapioca amylopectin. The chromatograms also showed that there were no intermediate components.

Amylopectin's Branch-Chain length

Amylopectins were studied for their branch chain-length distribution (by GPC), the chain lengths of the short and long branch-chains, and the ratio between the long and short chains (Table 1). Chromatograms of debranched amylopectins isolated from both starches are shown in Figure 2. They both contained more short chains (A and B1) than long chains (B2 and longer). The chromatogram of the debranched mung bean amylopectin also showed that it contained very long chains (possible B3, B4) which eluted out at the void volume. The chemical analysis indicated that the long chains of mung bean amylopectin (DP 40 at peak) were longer than that of tapioca starch (DP 35 at the peak); but the short chains of mung bean amylopectin (DP 16) were shorter than that of tapioca starch (DP 21). The ratio of long chains to short chains was greater in tapioca starch than in mung bean starch, indicating that tapioca starch contained more long chains than mung bean starch.

P-31 NMR Spectroscopy

P-31 NMR spectroscopy (Kasemsuwan and Jane, submitted) indicated that mung bean starch contained more total phosphorus than tapioca starch (Table 1). The spectrum also indicated that mung bean starch contained phospholipid, whereas tapioca starch did not. The phospholipids in mung bean starch might affect the clarity of the starch paste. Tapioca starch paste was more clear than mung bean starch paste. However, both starches contained starch phosphate monoester, which enhanced the clarities and the viscosities of these starches. The repulsion of negatively charged phosphate groups in these starches accounted, in part, for the more rapid hydration and swelling. The higher viscosities of the starch pastes compared with those starches which do not contain phosphate monoester, such as maize and wheat starches (Galliard and Bowler 1987).

Thermal Properties

Thermal properties determined by DSC are shown in Table 2. The gelatinization peak of mung bean starch ranged from 63.3°C to 83.5°C, and its amylose-lipid complex peak ranged from about 95°C to 105°C. The gelatinization peak of the tapioca starch was similar (61.5°C to 82.2°C). No amylose-lipid complex peak was found in the tapioca thermogram. Both onset and peak gelatinization temperatures of the mung bean starch were higher than those of tapioca starch. The enthalpy change of the mung bean starch (8.0 cal/g) was

also higher than that of tapioca starch (7.6 cal/g). The results were consistent with that proposed: the longer long B chains of mung bean starch resulted in a higher gelatinization temperature and required a higher temperature and more energy to cook (Montgomery et al. 1961, Jane and Chen 1992).

Viscosity and Pasting Properties

Tapioca starch had a lower pasting temperature (the temperature at which viscosity began to increase) than mung bean starch (Figure 3). The higher pasting temperature was primarily attributed to the lipid and higher amylose contents of the mung bean starch. The peak viscosities (the highest viscosity during heating) of both starches were similar. During the holding period, the tapioca starch underwent a significant shear-thinning (Figure 4). The viscosity of the tapioca starch dropped from 1100 to 400 BU, whereas mung bean starch did not show much shear thinning. The mung bean starch showed good shear resistance, similar to those cross-linked starches. The significant shear-thinning of tapioca starch was attributed to the break-down of swollen granules. This was caused by the high temperature and shear force. The swollen granules of mung bean starch withstood the shear force and high temperature because of its longer amylopectin branch-chains (B3 and B4) and greater amylose and lipid contents, which prevented the disruption of the granules. During setback (cooling period), both starches increased in viscosity, especially mung bean starch.

One way to increase shear resistance of tapioca starch is to cross-link the starch. In this study, we selected sodium trimetaphosphate, which provided a more controlled reaction than phosphorus oxychloride, to be the cross-linking agent. The cross-linking reaction was influenced by numerous factors such as pH, starch concentration, reagent concentration, temperature, and reaction time. To select the optimum product, tapioca starch (50% suspension) was cross-linked with 0.1% sodium trimetaphosphate (w/w = STMP/starch) at various pHs, reaction times, and temperatures. We selected pH 11.0 and 11.5 for the study because the cross-linking reaction predominated at high alkalinity (Felton and Schopmeyer 1943), and it was difficult to control the reaction of the pH above 11.5. The relations between the pasting temperature, peak temperature, peak viscosity, viscosity after shear-thinning, setback viscosity, and gel strength (24 hr at 5°C) with reaction time, reaction pH, and reaction temperature are shown in Figures 4-9.

Pasting temperatures were affected by reaction pH (significant at $P \geq 0.0001$), reaction time (significant at $P \geq 0.0007$), reaction temperature (significant at $P \geq 0.033$), and the combinations of reaction temperature and three different reaction times (significant at 0.0118). The relationships between these factors are shown in Figures 4a-4d.

Peak temperatures were affected by reaction pH (significant at $P \geq 0.0156$), reaction time (significant at $P \geq 0.0001$), and reaction temperature

(significant at $P \geq 0.0095$). The relationships between these factors are shown in Figure 5a-5c.

Peak viscosities were affected by reaction pH (significant at $P \geq 0.0237$) and the combinations of reaction temperature and reaction pH values (significant at 0.0155). The relationships between these factors are shown in Figures 6a-6b.

Viscosities after shear-thinning were affected by reaction pH (significant at $P \geq 0.0033$), reaction time (significant at $P \geq 0.0001$), and reaction temperature (significant at $P \geq 0.025$). A significant relationship was demonstrated between reaction temperatures and three different reaction times (significant at 0.0452), and the combinations of reaction times and reaction pHs (significant at 0.0315). The relationships between these factors are shown in Figures 7a-7e.

Setback viscosities at 50°C were affected by reaction pH (significant at $P \geq 0.0011$), reaction time (significant at $P \geq 0.0001$), reaction temperature (significant at $P \geq 0.0124$), and the combinations of reaction times and reaction pHs (significant at 0.0120). The relationships between these factors are shown in Figures 8a-8d.

Gel strengths were affected by reaction pH (significant at $P \geq 0.0001$), reaction time (significant at $P \geq 0.0001$), reaction temperature (significant at $P \geq 0.0273$), the combinations of reaction temperatures and reaction pHs values (significant at 0.0009), and the combinations of reaction temperatures and reaction times (significant at 0.0009). The relationships between these factors are shown in Figures 9a-9e.

The gel strength of the mung bean starch paste after 24 hr storage at 5°C was much higher than that of tapioca starch paste (Table 3). The mung bean starch gel was very strong and less sticky compared with tapioca starch gel which was weak and very sticky. The high gel strength of mung bean starch could be attributed to its high amylose content and longer long-branch-chains of amylopectin. Both starches were clear as pastes and gels, which is likely the result of their phosphate monoester derivatives. The gel strengths of the cross-linked tapioca starches, with different degrees of cross-linking, were still much lower than that of mung bean starch, but they were less sticky than the native tapioca starch. The gel strength of the cross-linked tapioca starch prepared at different conditions indicated that the starches that having higher degree of cross-linking exhibited higher gel strength. The higher amount of the high-amylose starch in the mixture of starch gel also increased the gel strength, which could be attributed to the gel network resulting from amylose retrogradation. A starch mixture, prepared by mixing 13% (dsb) high-amylose starch and 87% (dsb) tapioca starch, contained 30.2% apparent amylose, and the one prepared by mixing 17% high-amylose starch and 83% tapioca starch contained 32.1% apparent amylose. Both of the mixtures contained less apparent amylose than mung bean starch (37%) and displayed less gel strength than mung bean starch (Table 3).

Properties of Mung Bean Starch Noodles

Clear noodles were made from dough derived from different ratios of dry and gelatinized mung bean starch at ratios of 1:1, 2:3, and 1:2 (w/w). Lower and higher ratios of gelatinized starch were attempted, but the dough did not form or the dough was too soft to extrude.

SEM micrographs of the clear noodle surface and cross-section are shown in Figure 10. The cross-section of the noodle showed that the starch granules were gelatinized and ruptured. There were no intact starch granules. The micrograph of the cross-section also indicated that the starch molecules were formed into networks which resulted from the retrogradation process. The pattern of the networks was similar to the retrograded amylose (at 5°C) micrographs reported by Lu and Jane (1995).

Tensile strength of clear noodles of mung bean starch prepared from the lower ratios of gelatinized starch was higher than that of noodles prepared from higher ratios of gelatinized starch (Table 4). Water absorption of the noodles after 24 hr soaking in water at room temperature (25°C) increased slightly as the ratio of the gelatinized starch increased. The ratio of the gelatinized starch did not significantly affect the amount of soluble-matter loss ($\approx 5\%$).

Properties of Clear Noodles Made from Mixtures of Tapioca and High-Amylose Starches

Many starch compositions were tested to make clear noodles. Native and cross-linked tapioca starch alone produced noodles which were too soft and could not be separated into single strands. Mixtures of tapioca starches (native or cross-linked starch) and high-amylose starch were also used for making clear noodles, which was more successful. The high-amylose starch was gelatinized and mixed with dry tapioca starch. Noodles were made from the mixtures which contained 13 and 17% high-amylose starch (equivalent to 30% and 32% apparent amylose content, respectively). Lower percentages of high-amylose starch were also tested, but the noodles were not consistent in diameter and broke into short strands during extrusion. Higher levels of high-amylose starch were also attempted, but we were unable to make a stable high-amylose-starch paste with a solids concentration above 20% (dsb).

The tensile strength, water absorption, and the soluble loss of the noodles made from mung bean starch and from mixtures of tapioca starch and high-amylose starch, boiled for the same lengths of time (10-60 sec) after extrusion were all similar (data not shown). This indicated that the length of boiling time between 10 and 60 sec after extrusion did not affect the tensile strength, water absorption, and the soluble loss of the noodles. The tensile strengths of noodles made from mixtures of tapioca starch (native or cross-linked starches) and high-amylose starch (13 or 17%, dsb) were also similar (Figure 11). This indicated

that the different mixtures of starch did not affect the tensile strength of the dry noodles. The tensile strengths of the dry noodles did not relate with the eating property of the cooked noodles.

Water absorptions, after soaking at 25°C for 24 hr, of noodles made from mixtures of tapioca starch and high-amylose starch are shown in Figure 12. Noodles made from mixtures of tapioca starches and 13% of high-amylose starch absorbed more water than the noodles made from mixtures of tapioca starches and 17% high-amylose starch. Noodles made from mixtures of cross-linked tapioca starches and high-amylose starches absorbed less water than those made from native tapioca starch and high-amylose starch mixtures. Noodles made from tapioca starch with higher degrees of cross-linking (higher reaction temperature) also absorbed less water. Water absorption of the noodles could be used to predict the quality of the noodles because the less water the noodles absorbed, the stronger the texture of the noodles. Mung bean starch noodles absorbed a similar amount of water (127.7%, w/w) compared with noodles made from the mixtures of cross-linked tapioca starches (prepared at 35 and 45°C reaction temperature) and 17% high-amylose starch (133.2 and 126.1%, respectively).

The soluble losses of those noodles made from the mixtures of the cross-linked tapioca starches and 13% high-amylose starch were less than those made from the mixtures of 17% high-amylose starch counterparts (Figure 13). The higher soluble loss of the noodles which contained higher proportions of high-

amylose starch might have resulted from the leaching of amylose. The degree of cross-linking of tapioca starch in noodles did not affect the degree of soluble loss. The soluble loss of mung bean starch noodles (4.9%) was similar to that of the noodles made from mixtures of cross-linked tapioca starch and 13% high-amylose starch (4.9-5.3%).

Sensory evaluations (Figure 14) indicated that the firmness of the cooked mung bean starch noodles was higher than that of clear noodles made from the mixtures of tapioca starches (native or cross-linked) and high-amylose starch. The firmness increased as the degree of cross-linking increased. The noodles containing 13 and 17% high-amylose starch did not show significant differences in firmness. Chewiness also increased as the degree of cross-linking increased. The amount of high-amylose starch in noodles (13 and 17%) did not affect chewiness. The clarity of the noodles made from mixtures of tapioca starch and high-amylose starch was lower than the mung bean starch noodles. The noodles made from mixtures of tapioca starch and high-amylose starch were more opaque because of the high-amylose starch. The noodles made from mixtures of high-amylose starch (13 and 17%) and tapioca starch did not differ in the clarity. The degree of cross-linking also did not affect the clarity of the noodles. The degree of off-flavor of mung bean starch noodles was the same as that of noodles made from mixtures of tapioca starch and high-amylose starch. Even though lower in firmness, chewiness, and clarity, the noodles made from mixtures of tapioca starch and high-amylose starch were ranked higher in

general acceptability by the panelists than noodles made from mung bean starch. The higher acceptability of the noodles made from the mixtures of tapioca starch and high-amylose starch was due to the panelists' overall preference of the noodles.

CONCLUSIONS

The results of this research of the fine molecular structures and physical properties of starch generated the relations between these structures and properties. These relations will help to understand starch behavior and utilization. Tapioca starch cross-linked by using STMP with varying reaction temperature, reaction time, and reaction pH; viscosities and pasting properties indicated that the different cross-linked treatments affected the functional properties of starch. Native and cross-linked tapioca starch alone produced noodles which is unacceptable. The noodles prepared from mixtures of cross-linked tapioca starch and high-amylose starch indicated good quality at both the dry and cooked stages. The sensory evaluation indicated that panelists preferred the noodles made from the mixtures of tapioca and high-amylose starch than mung bean noodles.

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Table 1 Comparison of the molecular structures between tapioca starch and mung bean starch.

	Tapioca starch	Mung bean starch
Granular diameter (micron)	5-20	10-21
X-ray diffraction pattern	C _A -type	A-type
Apparent amylose ¹ content, % (Iodine affinity) ¹	24.3 ± 1.8 (4.9 ± 0.4)	37.0 ± 0.5 (7.4 ± 0.1)
Real amylose content ^{1,2} (%)	19.6 ± 0.7	30.7 ± 0.5
Total phosphorus content (% dsb)	0.008	0.012
Phospholipids content (% dsb)	N/D ³	0.0033
Phosphate monoester (% dsb)	0.0065	0.0083
Amylopectin peak Short chain-length ¹	21.4 ± 2.7	15.9 ± 3.5
Amylopectin peak Long chain-length ¹	35.4 ± 4.3	40.0 ± 2.8
Ratio of amylopectin's Long-chain/short-chain	0.77 : 1.0	0.56 : 1.0

¹ Data were reported in means ± standard deviation.

² Real amylose contents were calculated following Kasemsuwan et al. (1995) method.

³ Not detected

Table 2 Comparison of the gelatinization property between tapioca starch and mung bean starch¹.

	Tapioca starch	Mung bean starch
Onset temperature, T_o (°C)	63.8 ± 0.2	68.0 ± 0.9
Gelatinization range, R (°C)	61.5 ± 0.8 to 82.2 ± 1.4	63.3 ± 0.1 to 85.5 ± 3.3
Gelatinization enthalpy, ΔH (cal/g)	7.6 ± 0.3	8.0 ± 0.01

¹ Data were reported in means \pm standard deviation.

Table 3 Gel strength¹ (10% dsb) of tapioca starch, mung bean starch, cross-linked tapioca starches, and the mixture of the cross-linked starches and high amylose starch.

Starches	Gel strength (gram force)
Native tapioca starch	1.5 ± 0.2
Native mung bean starch	49.1 ± 2.3
Cross-linked tapioca starch ² at 25°C reaction temperature	1.6 ± 0.4
Cross-linked tapioca starch ² at 35°C reaction temperature	2.6 ± 0.2
Cross-linked tapioca starch ² at 45°C reaction temperature	4.8 ± 0.9
Mixture of native tapioca starch and 13% (w/w) high-amylose starch ³	2.6 ± 0.5
Mixture of the cross-linked starch (25°C reaction temperature) and 13% high- amylose starch ³	3.5 ± 0.8
Mixture of the cross-linked starch (35°C reaction temperature) and 13% high- amylose starch ³	7.9 ± 1.5
Mixture of the cross-linked starch (45°C reaction temperature) and 13% high- amylose starch ³	11.7 ± 1.4
Mixture of the native tapioca starch and 17% high-amylose starch ³	3.2 ± 0.6
Mixture of the cross-linked starch (25°C reaction temperature) and 17% high- amylose starch ³	3.9 ± 0.6
Mixture of the cross-linked starch (35°C reaction temperature) and 17% high- amylose starch ³	10.8 ± 1.2
Mixture of the cross-linked starch (45°C reaction temperature) and 17% high- amylose starch ³	15.2 ± 2.1

¹ Starch paste (10% dsb) was stored at 5°C for 24 hr before measured with a Voland texture analyzer (Model TA, Scarsdale, NY). A 2-mm diameter probe and a 3-mm distance of penetration were used. The measurement was replicated at least ten times, and reported in means ± standard deviation.

² Cross-linked tapioca starch with 0.1% sodium trimetaphosphate at pH 11.0, 3.5 hr reaction time.

³ High-amylose maize-starch, contained 70% amylose.

Table 4 Properties of mung bean starch noodles, prepared with different ratios of dry and gelatinized starches¹.

Ratio between dry : gelatinized starches ² (moisture content, % ³)	Tensile strength (MPa)	Water absorption at 24 hr (%)	Soluble loss (%)
1 : 1, (45%)	54.1 ± 4.0	127.7 ± 5.4	5.0 ± 0.3
2 : 3, (54%)	48.6 ± 4.1	133.5 ± 5.1	5.0 ± 0.4
1 : 2, (60%)	24.5 ± 1.8	138.4 ± 6.4	5.8 ± 0.8

¹ Data are reported in means ± standard deviation.

² dry starch base weight : starch paste weight (from 10% dsb slurry).

³ percentage based on the total weight of the starch dough.

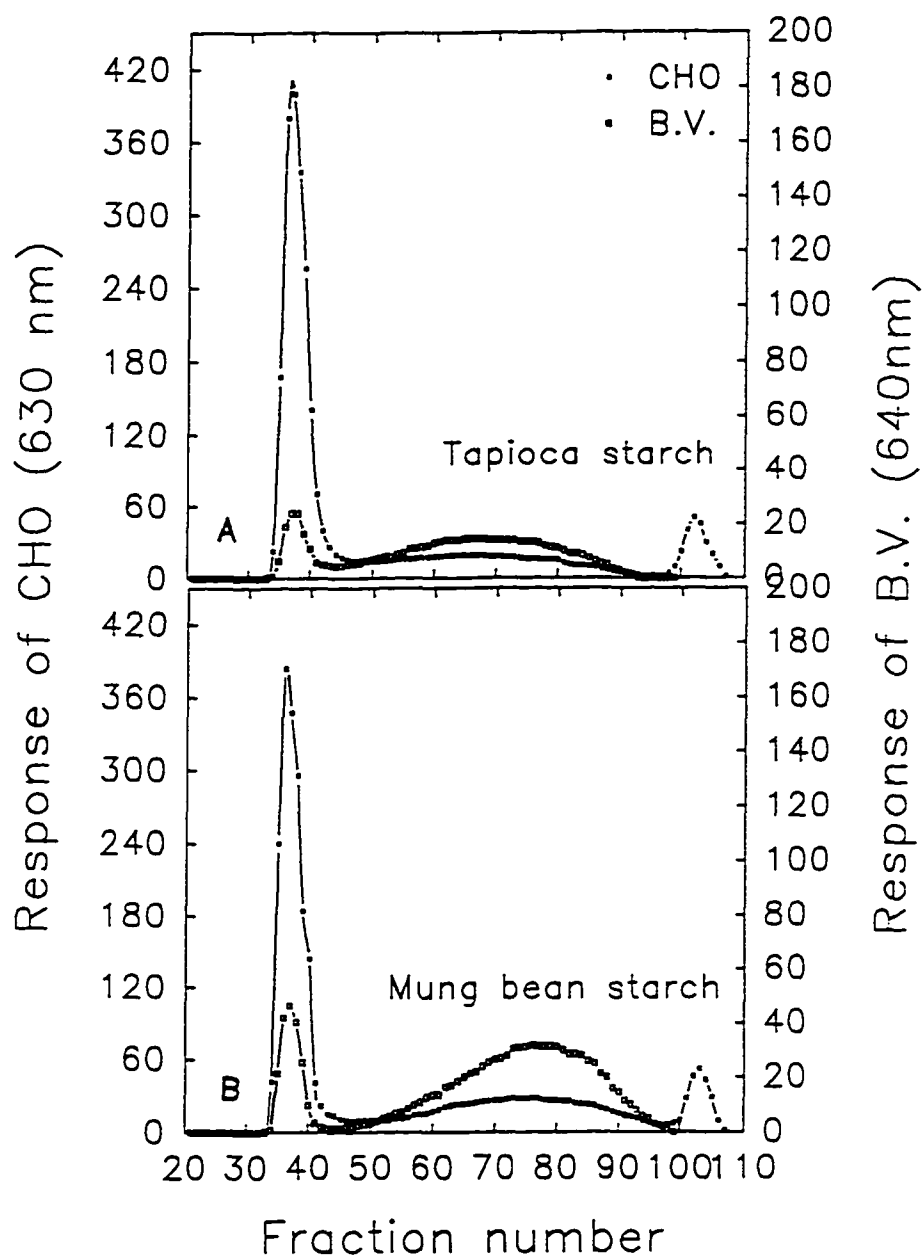


Figure 1 Sepharose CL-2B column (2.6 I.D. x 90 cm) profiles of native starches: A. tapioca starch, B. mung bean starch. The column was eluted with 50mM sodium chloride aqueous solution with 10mM NaOH, and flow rate was 0.5 ml/min. Fractions (4.8 ml) were analyzed for total carbohydrate (anthrone-sulfuric acid procedure): ○ - ○, and blue value (amylose-iodine complex): ● - ●. Glucose was used as the marker.

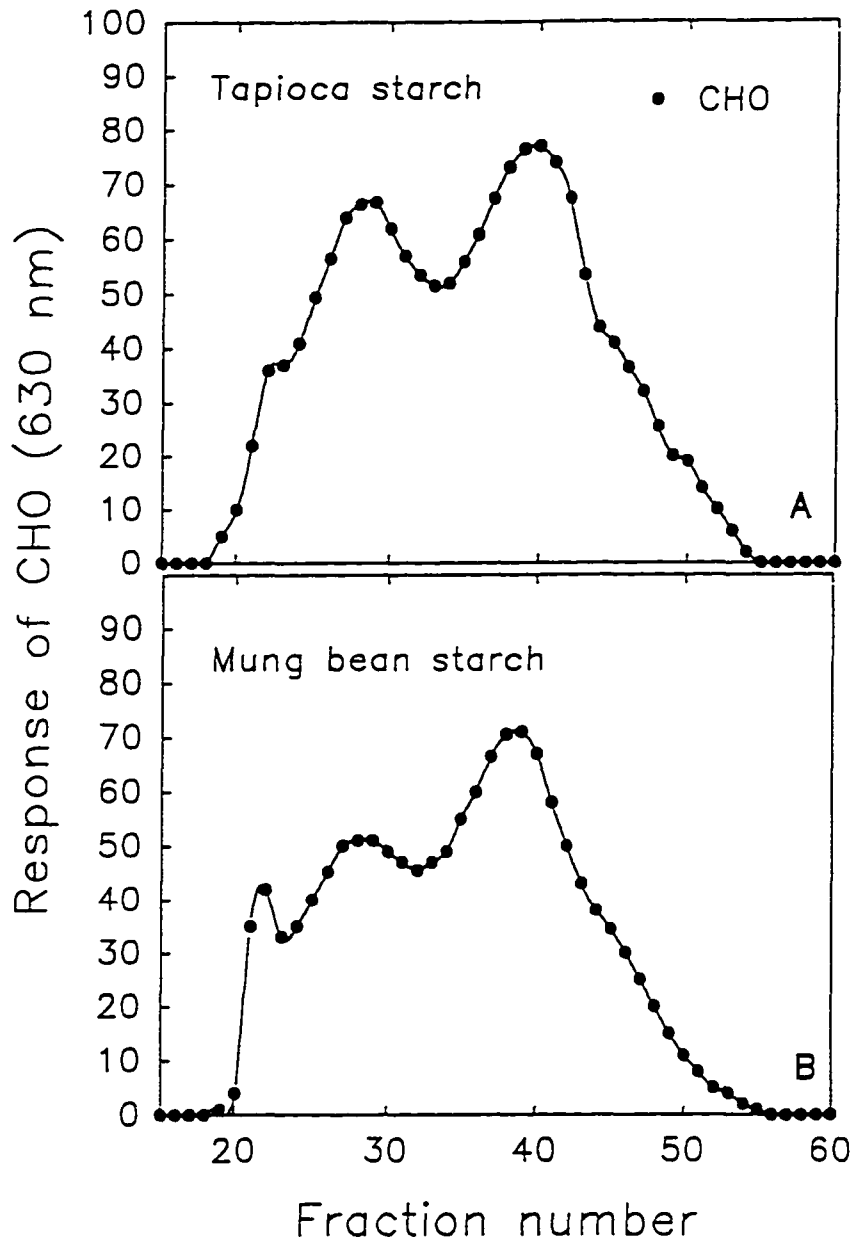


Figure 2 Bio-Gel P-6 column (1.5 I.D. x 80 cm) profiles of debranched amylopectins: A. tapioca starch. B. mung bean starch. The column was eluted with distilled deionized water. Fractions (2.3 ml) were analyzed for total carbohydrate (anthrone-sulfuric acid procedure): ● - ●.

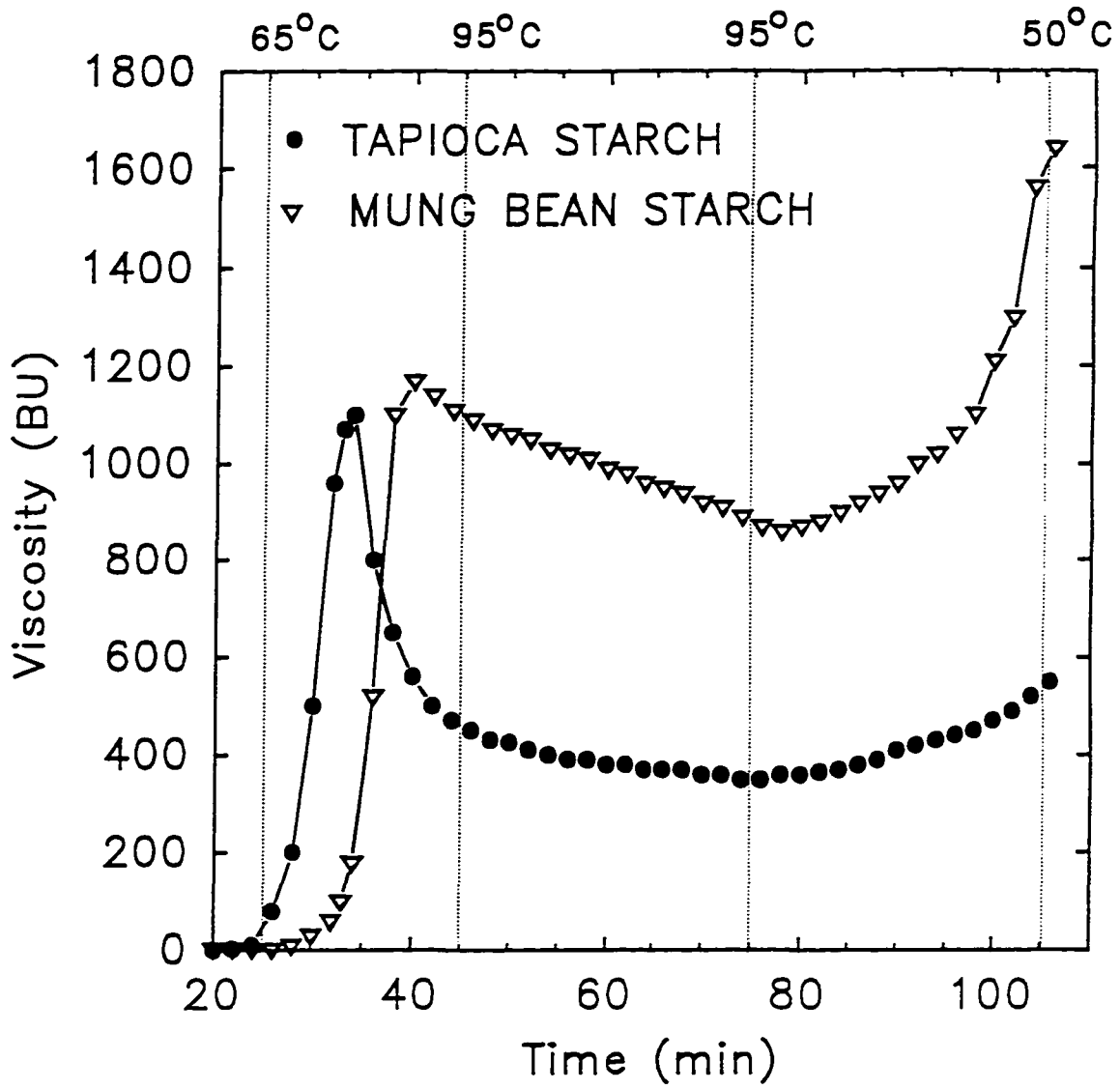


Figure 3 Brabender amylogram of slurries (8% dsb) of mung bean starch: ▽ - ▽, tapioca starch: ● - ●.

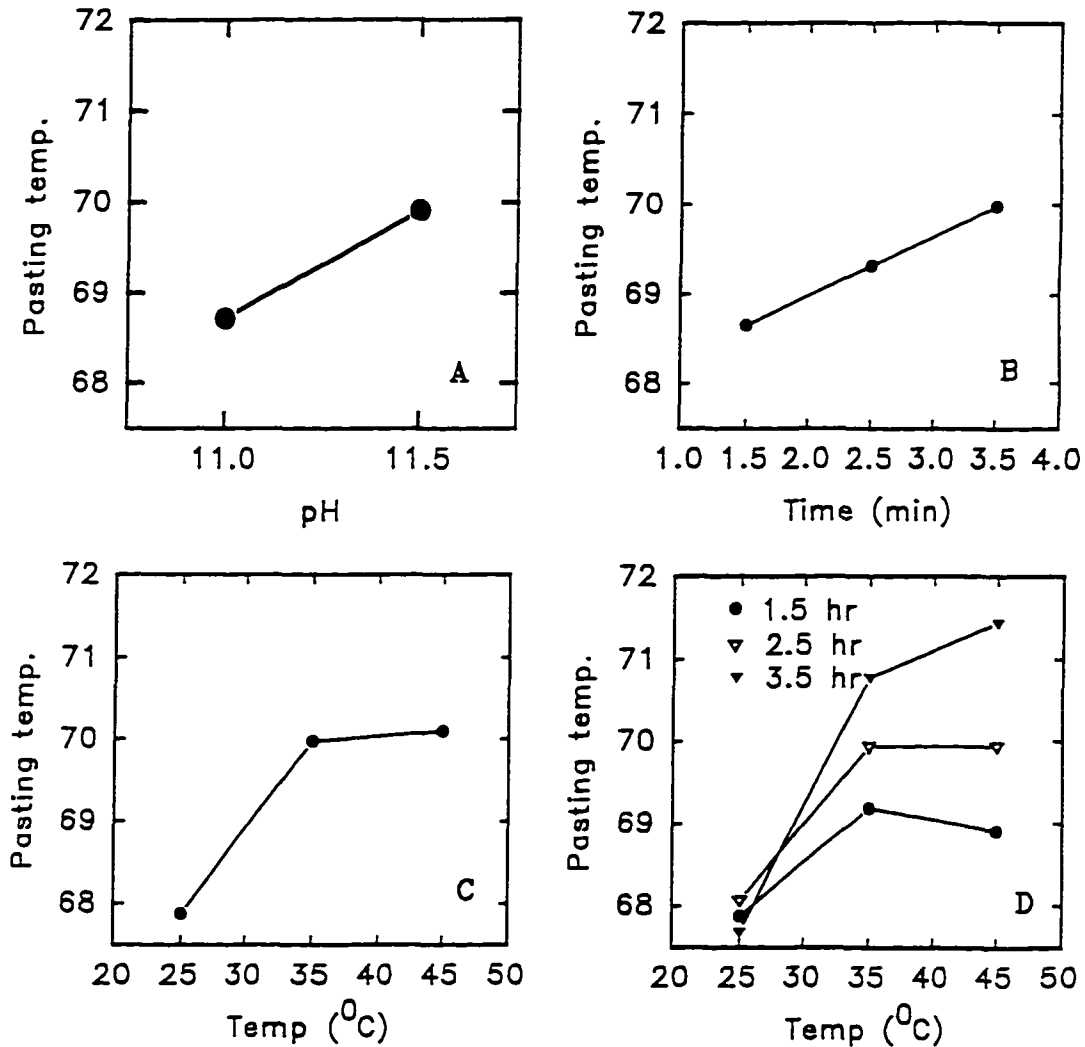


Figure 4 Pasting temperatures of the modified tapioca starch (8%) were measured by using the visco/amylograph. A. The effect of reaction pH (averaged over reaction times of 1.5, 2.5, 3.5 hr and reaction temperatures of 25, 35, 45 °C) on pasting temperature. B. The effect of reaction time (averaged over reaction temperatures of 25, 35, 45°C and pHs of 11.0, 11.5) on pasting temperature. C. The effect of reaction temperature (averaged over reaction times of 1.5, 2.5, 3.5 hr and pHs of 11.0, 11.5) on pasting temperature. D. The combined effect of reaction temperature and reaction time (average over pHs 11.0 and 11.5) on pasting temperature.

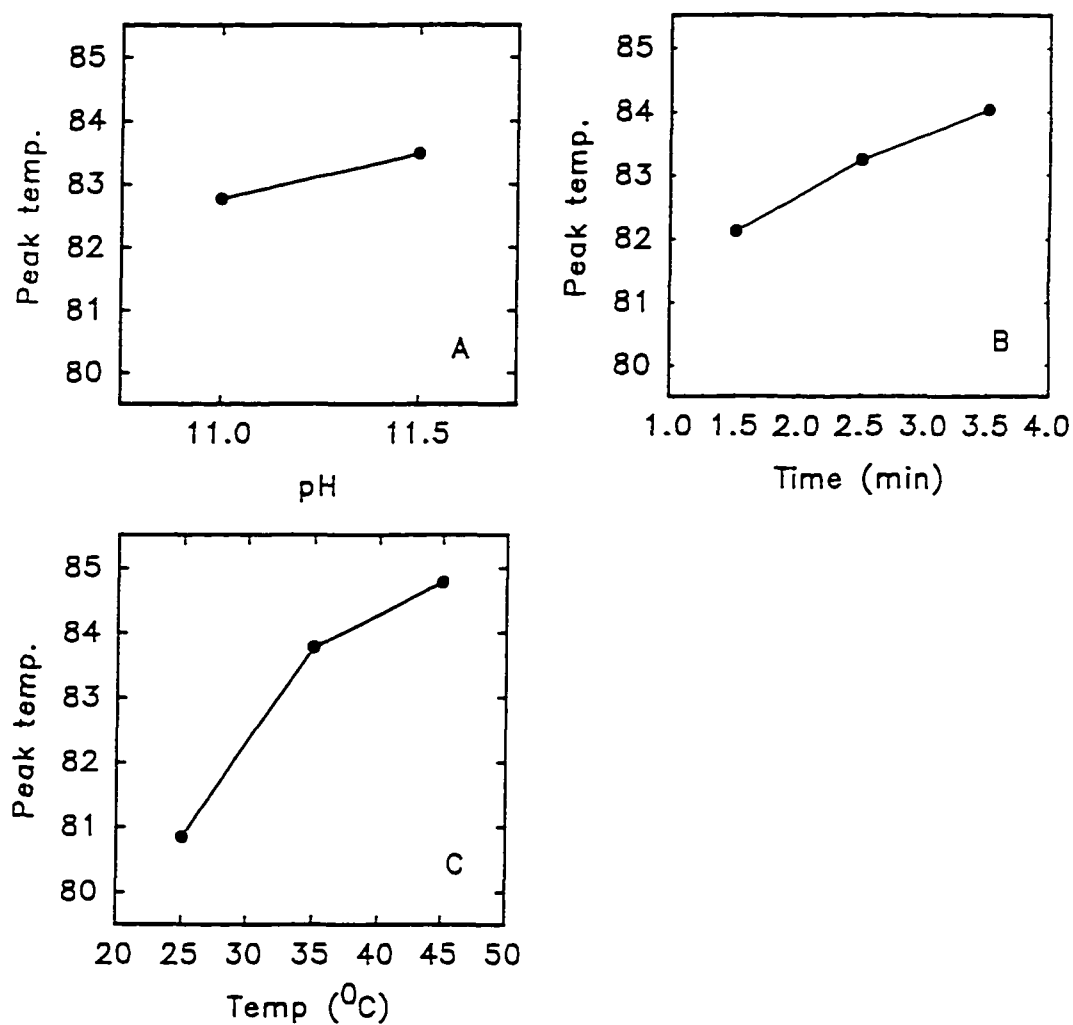


Figure 5 Peak temperatures of the modified tapioca starch (8%) were measured by using the visco/amylograph. A. The effect of reaction pH (averaged over reaction times of 1.5, 2.5, 3.5 hr and reaction temperatures of 25, 35, 45 $^{\circ}$ C) on peak temperature. B. The effect of reaction time (averaged over reaction temperatures of 25, 35, 45 $^{\circ}$ C and pHs 11.0, 11.5) on peak temperature. C. The effect of reaction temperature (averaged over reaction times of 1.5, 2.5, 3.5 $^{\circ}$ C and pHs of 11.0, 11.5) on peak temperature.

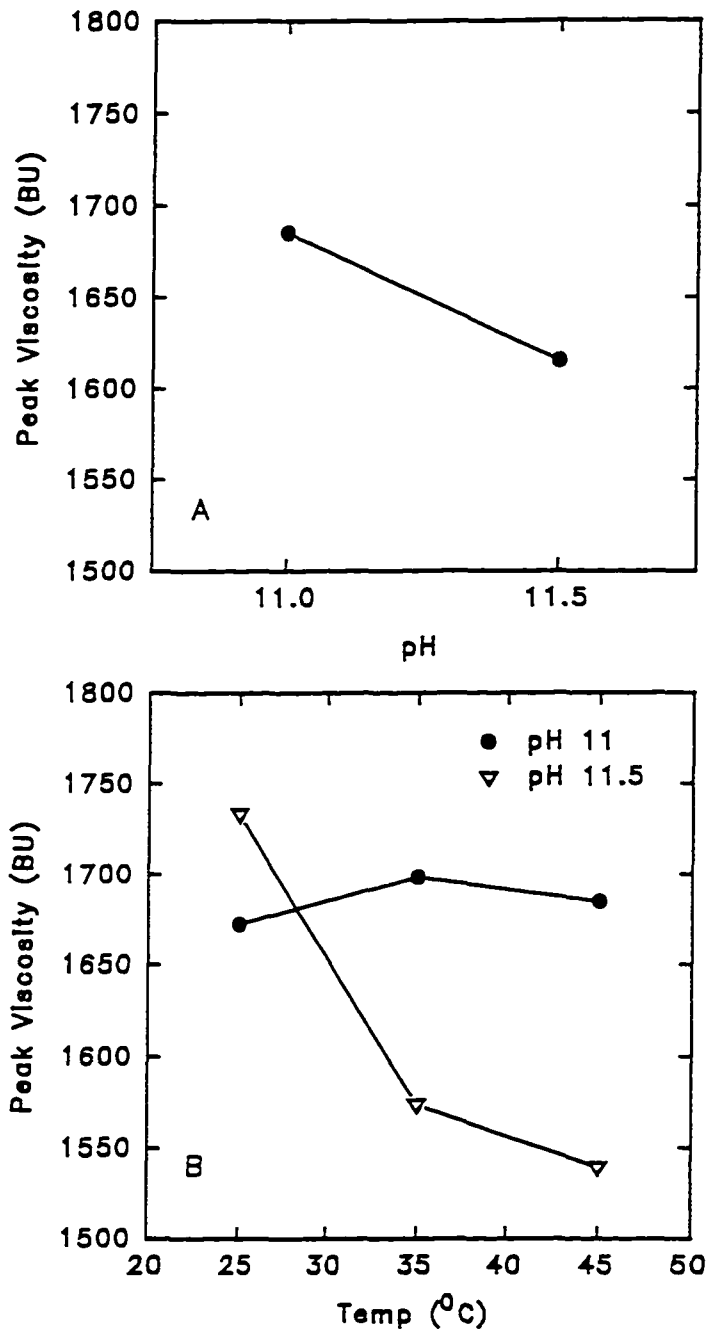


Figure 6 Peak viscosity of the modified tapioca starch (8%) were measured by using the visco/amylograph. A. The effect of reaction pH (averaged over reaction times of 1.5, 2.5, 3.5 hr and reaction temperatures of 25, 35, 45 $^{\circ}$ C) on peak viscosity. B. The combined effect of reaction temperature and reaction pH (averaged over reaction times of 1.5, 2.5, 3.5 hr) on peak viscosity.

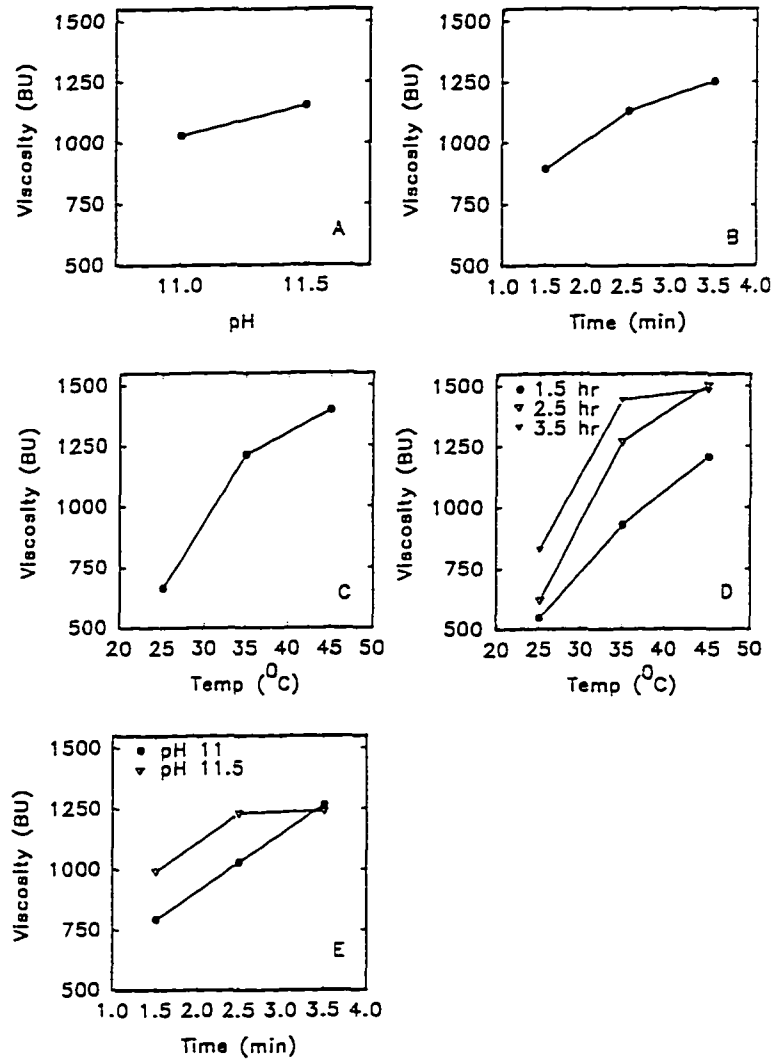


Figure 7 Viscosity after shear thinning of the modified tapioca starch (8%) were measured by using the visco/amylograph. A. The effect of reaction pH (averaged over reaction times of 1.5, 2.5, 3.5 hr and reaction temperatures of 25, 35, 45 $^{\circ}$ C) on shear viscosity. B. The effect of reaction time (averaged over reaction temperatures of 25, 35, 45 $^{\circ}$ C and pHs of 11.0, 11.5) on shear viscosity. C. The effect of reaction temperature (averaged over reaction times of 1.5, 2.5, 3.5 hr and pHs of 11.0, 11.5) on shear viscosity. D. The combined effect of reaction temperature and reaction time (averaged over pHs 11.0 and 11.5) on shear viscosity. E. The combined effect of reaction time and reaction pH (averaged over reaction temperatures of 25, 35, 45 $^{\circ}$ C) on shear viscosity.

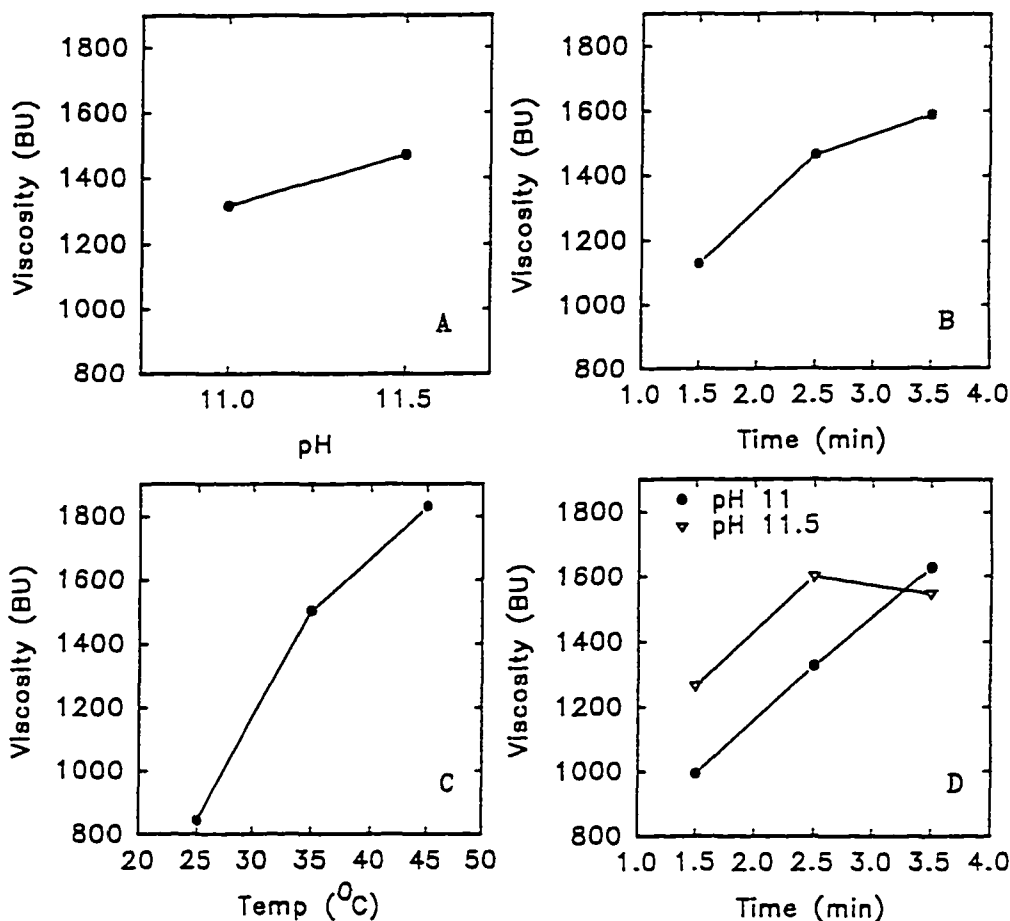


Figure 8 Setback viscosity of the modified tapioca starch (8%) were measured by using the visco/amylograph. A. The effect of reaction pH (averaged over reaction times of 1.5, 2.5, 3.5 hr and reaction temperatures of 25, 35, 45°C) on the setback viscosity. B. The effect of reaction time (averaged over reaction temperatures of 25, 35, 45°C and pHs of 11.0, 11.5) on setback viscosity. C. The effect of reaction temperature (averaged over reaction times of 1.5, 2.5, 3.5 hr and pHs of 11.0 and 11.5) on setback viscosity. D. The combined effect of reaction time and pH (averaged over reaction temperatures of 25, 35, 45°C) on setback viscosity.

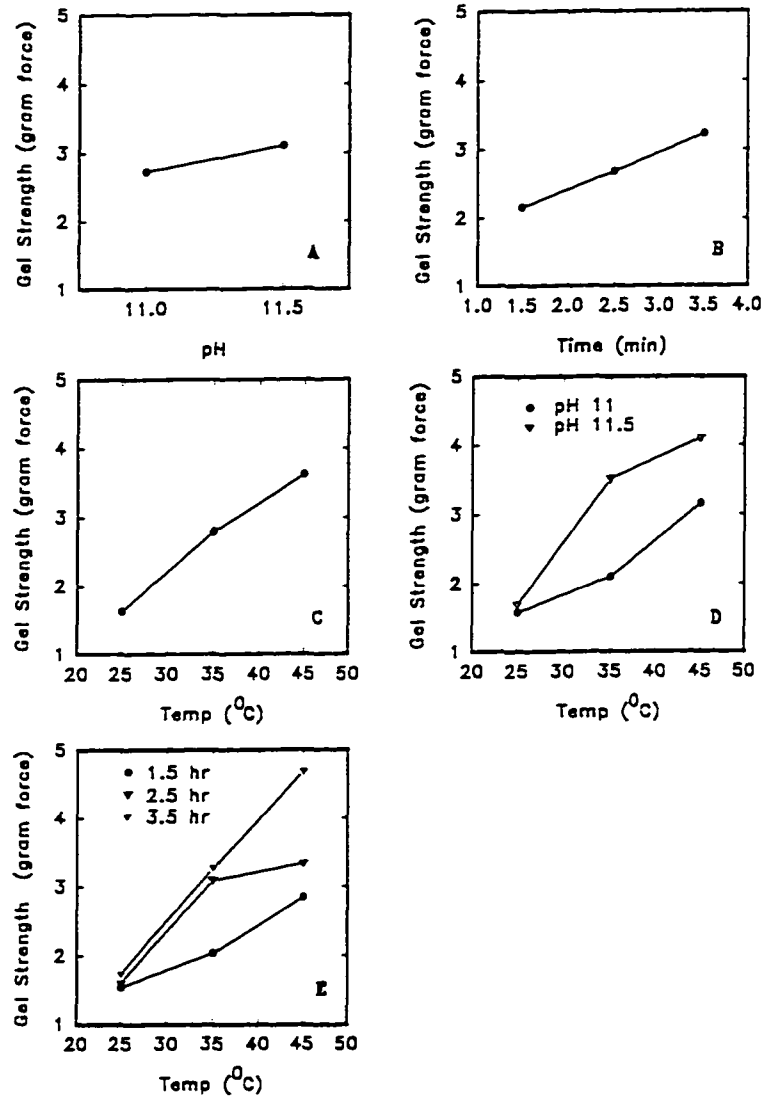
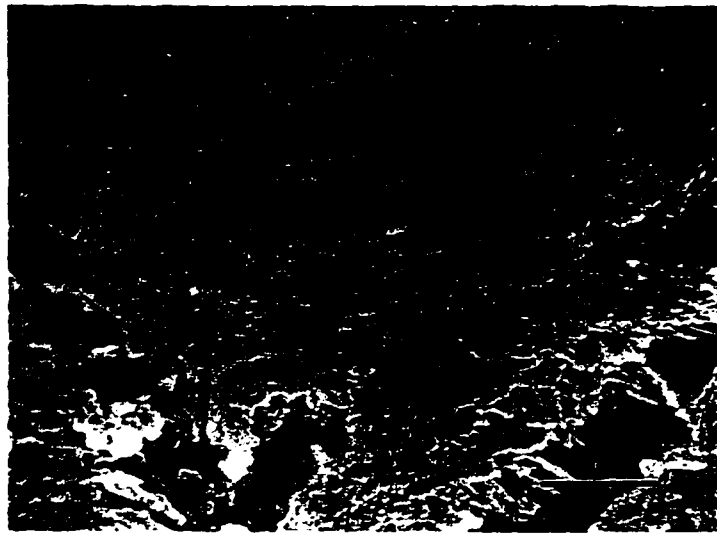
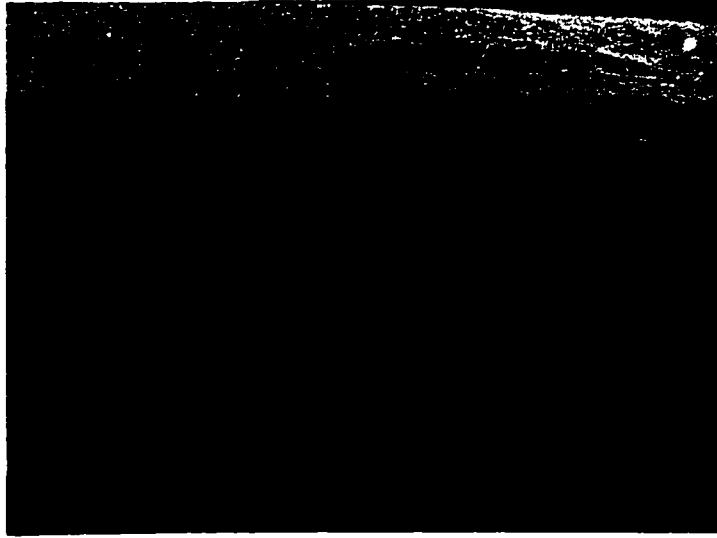


Figure 9 Gel strength of 8% (dsb) modified tapioca starch paste kept at 5°C for 24 hr were measured by using Voland Texture Analyzer. A. The effect of reaction pH (averaged over reaction times of 1.5, 2.5, 3.5 hr and reaction temperatures of 25, 35, 45°C) on gel strength. B. The effect of reaction time (averaged over reaction temperatures of 25, 35, 45°C and pHs of 11.0, 11.5) on gel strength. C. The effect of reaction temperature (averaged over reaction times of 1.5, 2.5, 3.5 hr and pHs of 11.0, 11.5) on gel strength. D. The combined effect of reaction temperature and pH (averaged over reaction times of 1.5, 2.5, 3.5 hr) on gel strength. E. The combined effect of reaction temperature and reaction time (averaged over pHs of 11.0 and 11.5) on gel strength.

Figure 10 Scanning electron micrograph: A. the surface of the noodle (bar = 300 μm), B. the surface and the cross-section of the noodle (bar = 3 μm), and C. the cross-section of the noodles (bar = 8 μm).



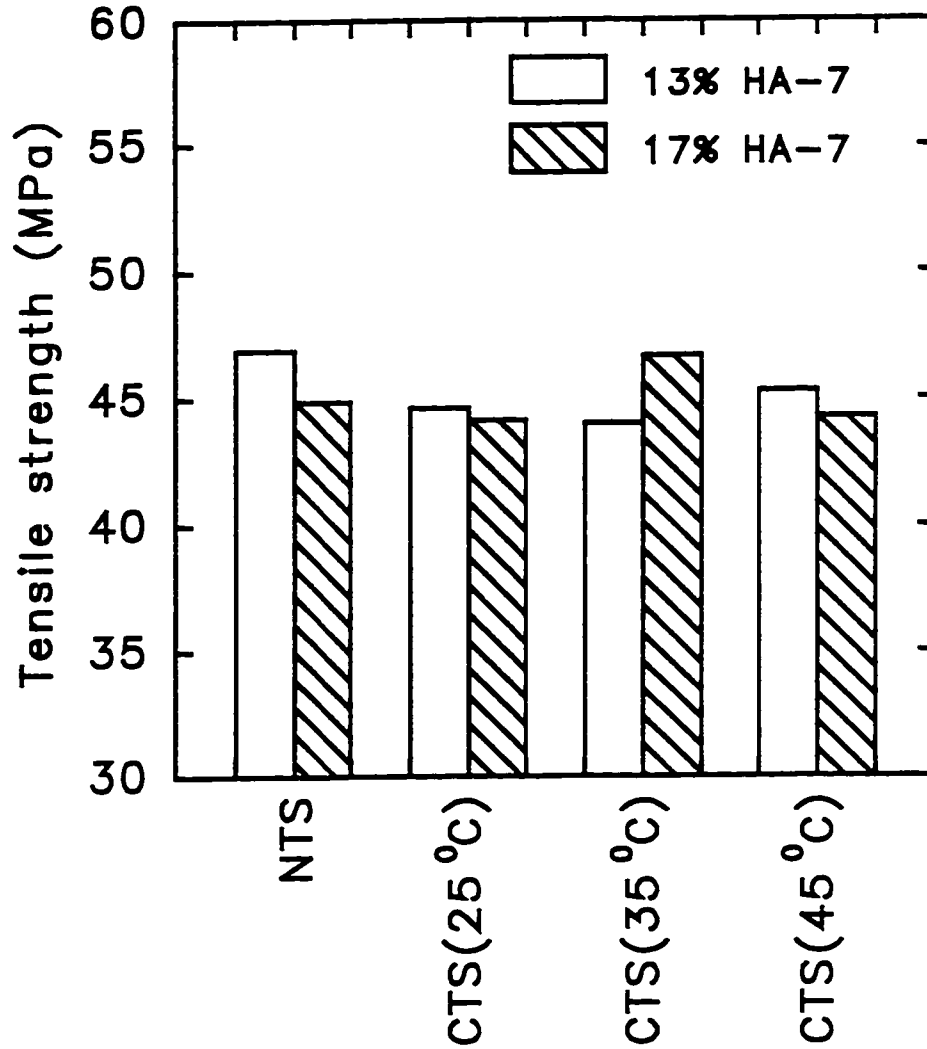


Figure 11 The tensile strengths of the noodles (54% moisture) prepared from the native tapioca starch (NTS) contained high-amylose starch (13 and 17%, dsb), and cross-linked tapioca starch (CTS) prepared at 25, 35, and 45°C reaction temperature contained high amylose starch.

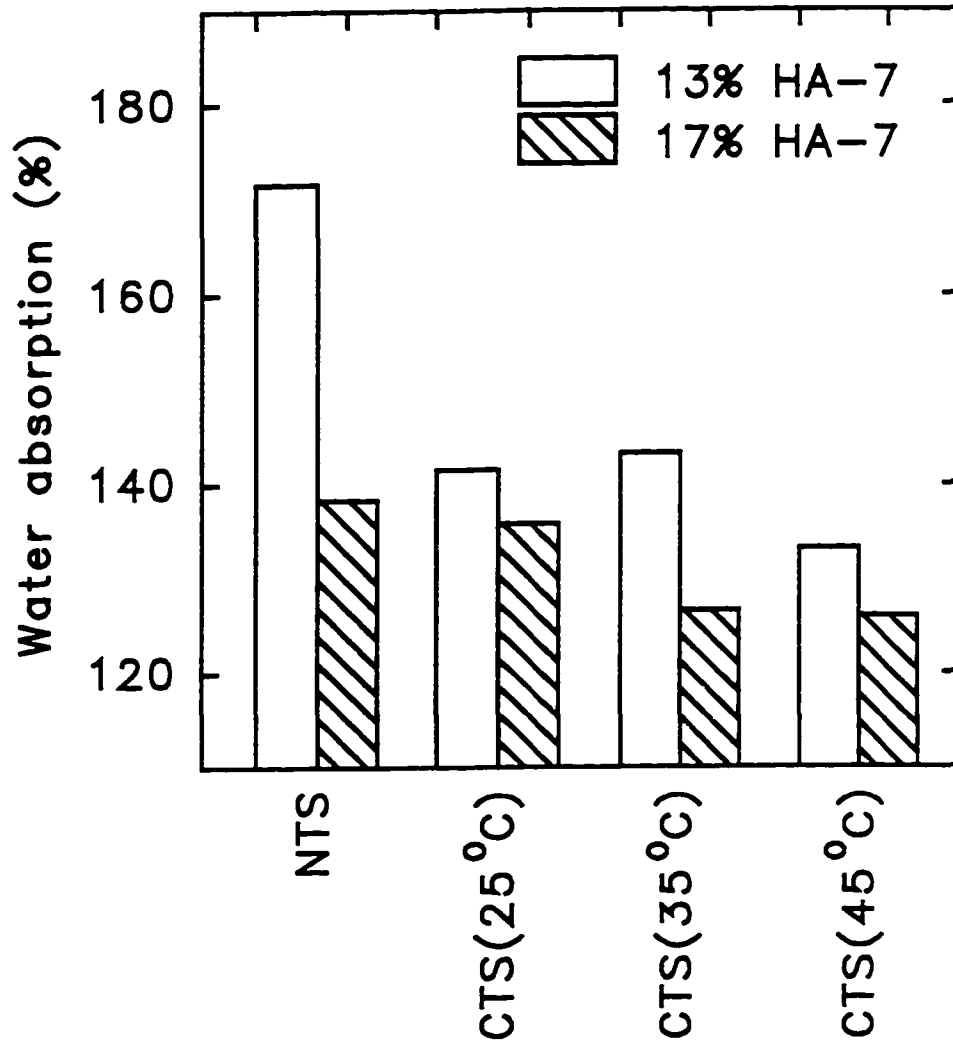


Figure 12 The water absorptions of the dry noodles after being soaked in water at 25°C for 24 hr. The noodles prepared from mixtures of native tapioca starch (NTS) and high-amylose starch (13 and 17%, dsb), and cross-linked tapioca starch (CTS) prepared at 25, 35, and 45°C reaction temperatures and same amount of high-amylose starch.

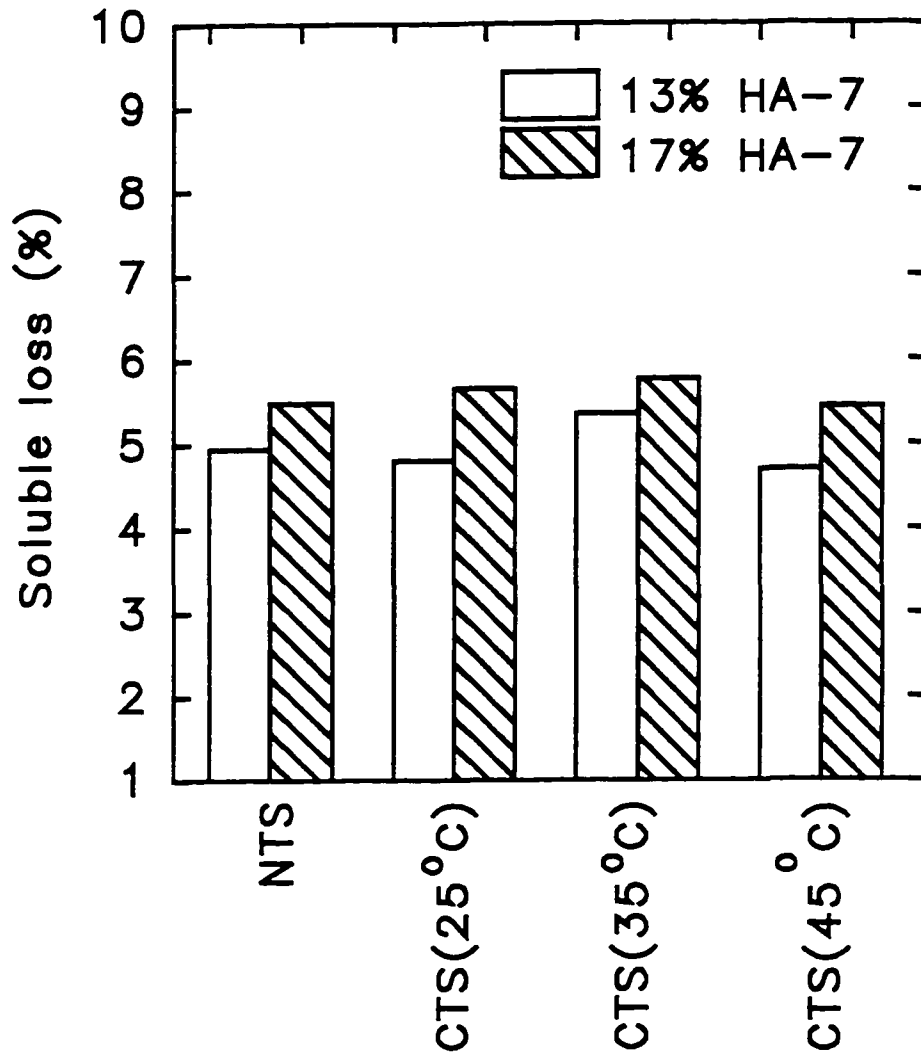


Figure 13 The soluble loss of the noodles prepared from mixtures of native tapioca starch (NTS) and high-amylose starch (13 and 17%, dsb), and cross-linked tapioca starch (CTS) prepared at 25, 35, and 45°C reaction temperature and high amylose starch (13% and 17%, dsb).

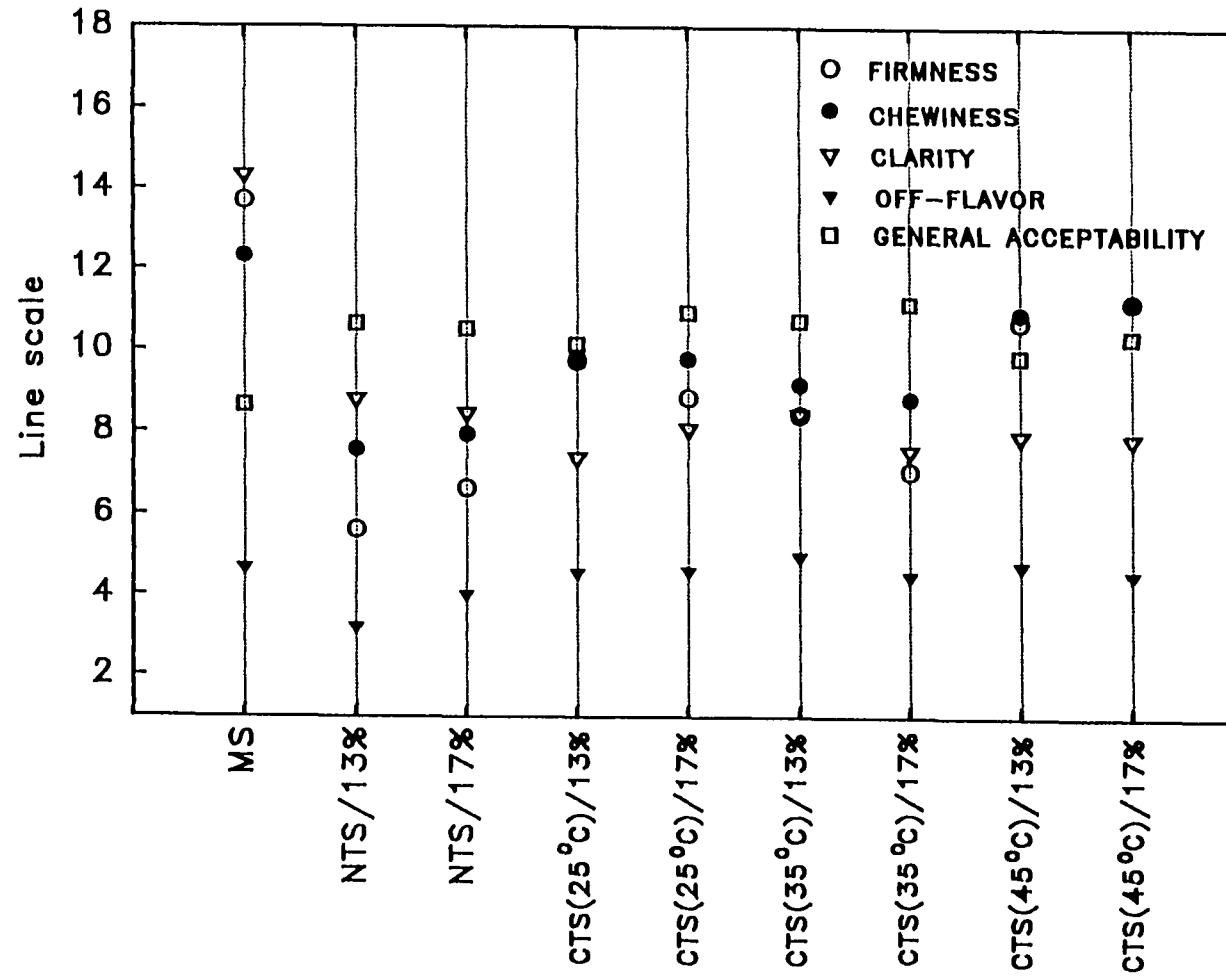


Figure 14 The sensory evaluations which were determined by line scale: firmness (○-○), chewiness (●-●), clarity (▽-▽), off-flavor (▼-▼), and general acceptability (□-□). (Line scale, 1 = least, 18 = most), MS = mung bean starch noodles, NTS = native tapioca starch, and CTS = cross-linked tapioca starch.

GENERAL CONCLUSIONS

Characterization of the dominant mutant *Ae1-5180* maize starch with different doses showed similar structures and properties. SEM, x-ray diffraction pattern, GPC, amylose content, amylopectin and intermediate component branch-chain-length distribution, and thermal properties (gelatinization and retrogradation) showed that the dominant mutant starch contained different structures and properties from the recessive mutant amylose-extender maize starch. The GPC profiles showed that dominant starch contained a high amount of intermediate component. The real amylose content indicated that the dominant mutant starch contained a smaller amount of amylose than the recessive, high amylose starches. The chain-length of the branches of amylopectin were shorter than that of the intermediate component. Gelatinization of the dominant mutant starch also indicated that onset temperature was lower than that of recessive high-amylose starches.

There are non-carbohydrate components found in starches, e.g., lipids, proteins, and trace elements. Phosphorus is one of the non-carbohydrate components. The phosphorus in starch is found in many forms, such as phospholipids, starch phosphate monoesters, phosphoproteins, and inorganic phosphate. The phosphorus content in starch varies from very trace amounts to significantly high amounts in potato starch (0.1%). The starch properties were affected by the phosphorus structure and contents. Phospholipids reduced

clarity and also reduced the pasting viscosity of the starch. Whereas, starch phosphate monoester increased both clarity and viscosity of the starch. The traditional method for determining phosphorus in starch involves a tedious ashing method, that is time consuming. The traditional method only provides the total phosphorus content without differentiating the different structures. A P-31 NMR Spectroscopic method was developed which provided the contents of each of phosphorus structures in starch. This can help starch scientists to understand the relation between the non-carbohydrate phosphorus and the phosphorus covalently linked to starch.

The relation between the fine structures and the properties of starch, provided a better understanding for using starch in food applications. Mung bean starch noodles (clear noodles) are expensive primarily because of the high cost for the production of mung bean starch. Replacing mung bean starch with a cheaper starches is desirable. Since tapioca starch is cheap and plentiful, and the paste clarity of both mung bean starch and tapioca starch is similar, tapioca starch was used to replace mung bean starch in the production of clear noodles. Tapioca starch was cross-linked by using sodium trimetaphosphate (STMP) with varying reaction temperatures, reaction times, and reaction pH. Pasting temperature, peak temperature, peak viscosity, viscosity after shear-thinning, set-back viscosity (by using visco/amylograph), and gel strength (by using Volland texture analyzer) indicated that the different cross-linked treatments affected the pasting properties of the starches. Selected cross-linked starches were used for

the making of clear noodles. Only the noodles that were made from the mixture of cross-linked tapioca starch and high-amylose maize starch, however, showed a quality similar to the clear noodles made from mung bean starch.

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APPENDIX

Clear Noodles Sensory Evaluation

NAME _____ DATE _____
 AGE _____ SEX _____

FIRMNESS: amount of force needed to bite through the noodles on
 the first two or three chew

SOFT FIRM

CHEWINESS: number of chews needed before swallowing

NOT CHEWINESS CHEWINESS

CLARITY: visual ability to see through

OPAQUE CLEAR

FLAVOR: flavor characteristic of freshly boiled noodles

NONE INTENSE

GENERAL ACCEPTABILITY

NOT ACCEPTABLE ACCEPTABLE

COMMENTS _____