1992

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Iowa State University, 1992

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# Characterization of starch structures and properties of maize mutants from the Oh43 inbred line

by

Ya-Jane Wang

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
Major: Food Science and Technology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1992

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# GENERAL INTRODUCTION

Next to cellulose, starch is the most abundant carbohydrate present in higher plants. A high proportion of food energy intake in the world is provided by starch. In addition, starch is also important in the paper and textile industries among others. Starch exists in a particle, a starch granule, and accumulates in many organs of plants. Only a few plants are economically and commercially used for starch production. Among the major starches produced in the United States, maize (*Zea mays* L.) is the most important crop, providing 95% of manufactured starch.

Starch consists mainly of two components: amylose and amylopectin. Amylose is an essentially linear polymer of (1→4)-linked α-D-glucopyranosyl units with some minor branching. Amylopectin is a high-molecular-weight branched polymer linked together mainly through α-(1→4) linkages but with about 5 to 6% of the linkages having α-(1→6) bonds, thus creating branch points. Also present in starch is a third component, termed intermediate materials, which possess iodine-binding capacity and a ß-amylolysis limit that are between those of amylose and amylopectin (Banks and Greenwood 1975). The structures and amounts of the intermediate materials vary with the source and maturity of starch.

Maize is unique among higher plants for its number of mutants which have been identified and examined. Some mutants modify the quantity and quality of starch granules in the endosperm, altering such features as percentage distribution of amylose and amylopectin, the molecular weight, and the degree of branching. With the natural diversity of properties that exist, maize mutants may be potential natural alternatives as modified starches for use in the food industry.

Although much work has been done on maize mutant starches, few studies have focused on a comprehensive evaluation of starch structure and properties. The objectives of this study were to characterize the structures and physicochemical properties of maize mutants from one genotypic background, the Oh43 inbred line, and to determine the relationships between structures and properties of mutant corn starches in this genetic background.

## A. Explanation of Dissertation Format

This dissertation consists of four papers. The first paper, "Thermal and gelling properties of maize mutants from the Oh43 inbred line", has been published in the Cereal Chemistry journal. The second paper, "Characterization of starch structures of 17 maize endosperm mutant genotypes with Oh43 inbred line background", and the third paper, "Physicochemical properties of starches from mutant genotypes of the Oh43 inbred line", have been accepted for publication in the Cereal Chemistry journal. The fourth paper is entitled "Characterization of amylopectin and intermediate materials in starches from mutant genotypes of the Oh43 inbred line", and will be submitted to Cereal Chemistry journal. The four papers follow the format of the Cereal Chemistry journal. The four 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

## A. Biosynthesis of Starch

Starch is composed of two main components: a fraction which is essentially linear with some minor branching, amylose, and a highly branched fraction, amylopectin. A third component, the intermediate fraction, exists in various percentages depending upon the source of starch, and its structures and properties lie between those of amylose and amylopectin. Starch types vary in their proportions of these components and in their fine structures.

### 1. Starch-synthesizing enzymes in normal starch

Three types of enzymes are required for starch biosynthesis, including those enzymes 1) to form a primer, 2) to add glucose units by α-(1→4) bonds to that primer, and 3) to introduce α-(1→6) branch points (Banks and Greenwood 1975). A primer is a necessary molecule whose ends are required and are the sites where new monomers are added (Robyt 1984). The disproportionating enzyme (D-enzyme) is suggested to form priming molecules containing approximately 50 glucose units (Banks and Greenwood 1975). There are three enzymes involved in catalyzing the synthesis of α-(1→4) glucosidic bonds: phosphorylase, starch-granule-bound starch synthase, and soluble starch synthase (Shannon and Garwood 1984). Phosphorylase uses glucose-1-phosphate (G-1-P) as substrates to catalyze the elongating reaction. Bound synthase uses either adenosine diphosphate glucose (ADPG) or uridine diphosphate glucose (UDPG) as the D-glucosyl donors but prefers ADPG. Soluble starch synthase, however, exclusively uses ADPG as the D-glucosyl donors.

By studying the waxy maize mutant which has only amylopectin in its endosperm, it was found that bound synthase was not involved, suggesting that it makes only amylose (Tsai 1974). It is not clear what proportion of α-(1→4) linkages are produced by the synthases relative to the action of phosphorylase. There are two types of branching enzymes existing in plants, one that converts amylose into amylopectin and another that converts amylopectin into a glycogen-like product (Robyt 1984). The branching enzyme catalyzes an interchain α-(1→4)-α-(1→6) transfer of one chain to that of another.

*In vivo*, amylose and amylopectin are produced side-by-side in the starch granule. Whelan (1963) proposed that amylose and amylopectin might be synthesized on opposite sides of a semi-permeable barrier which allows the diffusion of glucoses but not the debranching enzymes. Geddes and Greenwood (1969) proposed a multipathway biosynthesis of starch in which the enzymes are absorbed at the surface of a nucleation site and the starch granule grows by apposition at the granule surface. French (1972) proposed that starch is synthesized at the granule surface but that amylose and amylopectin are oriented in opposite directions, thus preventing the action of the debranching enzyme on the amylose molecules. These hypotheses still remain to be proven.

## 2. Mutant effects

Maize is the most important crop in the U.S. and most of the corn grown in the U.S. Corn Belt is dent corn which is characterized by the presence of vitreous, horny endosperm at the sides and back of the kernel, while the central core to the crown of the kernel is soft and floury (Zuber and Darrah 1987).
Corn is genetically the most accessible and the most characterized among the higher plants. There are mutants affecting endosperm protein production, such as opaque (*o*) and floury (*fl*). The horny (*h*) mutant results in a loose starch packing without altering starch composition and structure (Fuwa et al 1978). Several recessive mutant genes have been identified which alter the quality and quantity of starch in the kernel, in addition to modifying the kernel development and kernel phenotype. These mutant genes include amylose-extender (*ae*), brittle (*bt*), dull (*du*), shrunken (*sh*), sugary (*su*), and waxy (*wx*). Mutant genes causing the same effect but which are controlled by different genes on different chromosomes are given a number after the named genotype, for example *su1* and *su2*. The effects of various mutants on kernel phenotype, starch granule size and amylose percentage are summarized in Tables I, II, and III, respectively, and the color plates of *ae*, *bt1*, *fl1*, *o2*, *sh1*, *su1* and *wx* mutants have been published (Neuffer et al 1968).

Amylose-extender (*ae*): The amylose extender (*ae*) gene produces starch with increased apparent amylose percentage of up to 75% (Banks and Greenwood 1975), and a kernel of reduced size. Variations in amylose

Table I. Mature kernel phenotype of normal and selected single, and double recessive maize genotypes[a]

| Genotype | Kernel phenotype[b] |
|---|---|
| Normal | Translucent |
| *ae* | Tarnished, translucent, or opaque; sometimes semi-full |
| *du* | Opaque to tarnished; S.C[c].: Semi-collapsed, translucent with some opaque sectors |
| *su* | Wrinkled, glassy; S.C.; Not as extreme as shrunken genotype |
| *su2* | Slightly tarnished, often etched at base |
| *wx* | Opaque |
| *ae du* | Translucent, not as full as *ae*; S.C.: Etched, translucent, or tarnished |
| *ae su* | Not quite as full as *ae*, translucent, may have opaque caps |
| *ae su2* | Translucent or opaque, etched base |
| *ae wx* | Semi-full to collapsed, translucent or glassy, may have opaque caps; S.C.: Slightly fuller, etched, translucent to glassy |
| *du su* | Wrinkled, glassy; S.C.: Extremely wrinkled, glassy |
| *du su2* | Translucent, etched |
| *du wx* | Semi-collapsed, opaque; S.C.: Shrunken, opaque |
| *su wx* | Wrinkled, glassy to opaque |
| *su2 wx* | Opaque, often etched |
| *su su2* | Wrinkled, glassy |

[a]Adapted from Garwood and Creech (1972).

[b]Kernels approach full size unless indicated as semi-full, semi-collapsed, shrunken, or wrinkled.

[c]S.C. means the phenotype observed in sweet corn inbreds.

Table II. Starch granule size of 14 maize genotypes at 24 days postpollination[a]

| | Granule size, μm | |
|---|---|---|
| Genotype | Minimum | Maximum |
| Normal | 7.99 | 8.53 |
| *ae* | 5.56 | 6.32 |
| *du* | 5.19 | 5.98 |
| *su* | 3.06 | 3.52 |
| *su2* | 7.68 | 9.14 |
| *wx* | 8.61 | 9.41 |
| *ae du* | 5.42 | 6.54 |
| *ae su* | 5.34 | 8.20 |
| *ae su2* | 5.57 | 6.46 |
| *ae wx* | 5.67 | 6.03 |
| *du su* | 3.11 | 3.85 |
| *du su2* | 5.97 | 8.79 |
| *du wx* | 6.18 | 6.86 |
| *su su2* | 2.56 | 2.98 |

[a]Adapted from Brown et al (1971).

Table III. Apparent amylose percentages of various maize genotypes determined by using iodine binding procedure[a]

| Genotype | Reference | | |
|---|---|---|---|
| | Kramer et al (1958) | Seckinger and Wolf (1966) | Holder et al (1974) |
| Normal | 27 | 27 | 29 |
| *ae* | 61 | 57 | 60 |
| *du* | 38 | 35 | 34 |
| *su* | 29 | ---[b] | 33 |
| *su2* | 40 | 28 | 38 |
| *wx* | 0 | — | <1 |
| *ae du* | 57 | 50 | 45 |
| *ae su* | 60 | — | 51 |
| *ae su2* | 54 | 45 | 56 |
| *ae wx* | 15 | 26 | 26 |
| *du su* | 63 | 13 | 40 |
| *du su2* | 47 | — | 46 |
| *du wx* | 0 | — | 2 |
| *su wx* | 0 | — | 0 |
| *su2 wx* | 0 | — | 0 |
| *su su2* | 55 | 30 | 41 |

[a]Adapted from Shannon and Garwood (1984) Table VII. Colorimetric measurement of starch-iodine complex used to estimate apparent amylose percentages. Genotypes were not incorporated into an isogenic background.
[b]Genotype not included in study.

percentage within inbred lines containing the *ae* recessive mutant gene result from differences in the genetic background, and environmental factors such as growing location, climate, moisture, etc. It was found that *ae* starch has more free starch synthase relative to the branching enzyme-starch synthase complex (Schiefer et al 1973). Boyer and Preiss (1978) reported that one of the branching enzyme fractions (fraction IIb) is absent in *ae* starch. Because of the high amylose content, *ae* starch gels rapidly and forms high-strength gels which make it useful in the confectionery industry.

The blue value test and the IA method are usually used to determine the apparent amylose content. These two methods, however, may overestimate the amylose percentage of *ae* starch because of the presence of the long external chains associated with the *ae* gene. Yeh et al (1981) employed gel permeation chromatography

(GPC) to fractionate starch components of maize mutant starches. They found a considerable amount of intermediate materials between the elutions of amylopectin and amylose of *ae* starch. These intermediate materials were similar to the loosely branched amylopectin reported for *ae wx* starch (Boyer et al 1976, Yamada et al 1978). The *ae* gene is evidently associated with increased amounts of intermediate materials. Also, there is an increased proportion of irregularly shaped starch granules in *ae* starch (Wolf et al 1964, Banks et al 1974).

Brittle (*bt*): The dried endosperm of brittle (*bt*)-type kernels is greatly collapsed because little starch has developed. On drying, the kernel shrinks and collapses into an angular structure with marked concaves and a brittle texture. ADPG pyrophosphorylases was found absent (Tsai and Nelson 1966) or low (Dickison and Preiss 1969) in *bt2* starch. Among the characteristics of gelatinization studied by differential scanning calorimetry (DSC), *bt1* starch exhibited lower onset and peak temperatures, and lower enthalpy than those of normal corn starch (Ninomya et al 1989). The *bt1* starch had an amylose content similar to that of normal corn but the ratio of A and short B chains to long B chains of amylopectin was higher in *bt1* starch than in normal starch (Ninomya et al 1989).

Dull (*du*): The appearance of the dull (*du*) kernel is similar to that of normal corn, but the amylose percentage of *du* starch was reported to be 5-25% higher than normal starch depending upon the background of the corn (Krammer et al 1958, Seckinger and Wolf 1966, Holder et al 1974, Yeh et al 1981, Boyer and Liu 1985). The ratio of A and short B chains to long B chains of amylopectin also was increased when the *du* gene was present (Inouchi et al 1983, 1987).

Shrunken (*sh*): The kernel characteristic of shrunken (*sh*) is similar to but less severely collapsed than that of the *bt* genotype. The *sh2* starch is lacking (Tsai and Nelson 1966) or low (Dickinson and Preiss) in ADPG pyrophosphorylase activiety. The *sh2* mutant originally was suggested as an alternative for sweet corn because the sugar content of *sh2* remains high longer than does that of the sweet corn (Laughnan 1953).

Sugary (*su*): The traditionally commercial sweet corn is homogeneous recessive for sugary-1 (*su1*), which, when dried, shows a glassy, wrinkled, and irregular appearance. The main effect associated with the *su1*

gene is the accumulation of large amounts (up to 25% or more of the dry kernel weight) of phytoglycogen, a highly branched and water-soluble polysaccharide (Creech and McArdle 1966). A branching enzyme (phytoglycogen branching enzyme) is present in *su1* starch, which is capable of producing a phytoglycogen-like polysaccharide from amylose *in vitro* (Black et al 1966). The amylose percentage of *su1* starch is higher than that of normal starch but the exact amount varies widely with different methods of analysis and investigators (Kramer et al 1958, Holder et al 1974, Yeh et al 1981).

The *su2* genotype has a starch granular size similar to that of normal starch granules but is 10-15% higher in amylose content than is normal starch (Kramer and Whistler 1949). However, genetic background, genotypes incorporated with the *su2* gene, and year of production all affect the apparent amylose percentage (Shannon and Garwood 1984). The thermal properties, including onset tempersture, peak temperature, and enthalpy, for both gelatinization and retrogradation measured by DSC of *su2* starch are lower than those of normal starch (Inouchi et al 1991a, b). The properties of amylose and amylopectin from *su2* starch were reported to be similar to those of normal starch (Dvonch et al 1951).

Waxy (*wx*): The waxy (*wx*) mutant is unique relative to all other known mutants in blocking the accumulation of amylose, and is very low in bound starch synthase activities. The *wx* kernel displays a uniform, marble-like opacity and stains reddish brown with iodine solution rather than blue like nonwaxy starches. The *wx* starch exhibits birefringence that is similar to that of normal starch, and both starches have an A-type x-ray diffraction pattern. However, *wx* starch exhibits a higher gelatinization temperature and peak viscosity than does normal starch as measured by DSC (Inouchi et al 1991a) and the viscoamylograph, respectively.

Cooked *wx* starch paste shows high viscosity and good clarity, and, being essentially free of amylose, is resistant to syneresis. The *wx* starch also constitutes a mjor part of market for all-purpose modified thickeners (Moore et al 1985).

Other mutants: Crossing single mutants to create double, triple, and quadruple mutants results in many more unusual corn mutant types. The starch properties resulting from these crosses has been the subject of

many recent research papers (Yeh et al 1981, Boyer and Liu 1985, Fuwa et al 1987, Sanders et al 1990, Inouchi et al 1991a, b).

## B. Molecular Structure of Starch

### 1. Fine structure of amylose

Amylose is essentially a linear macromolecule which is composed of only α-D-glucose residues which are linked together by (1→4) bonds with a few (1→6) bonds. The purely linear nature of amylose was generally accepted until Peat et al (1949) reported that crystalline sweet potato ß-amylase, an enzyme that removes maltose units from the non-reducing end of the molecule, only hydrolyzed about 70% of amylose in potato starch. It was Peat et al (1952) who first suggested that the barrier to ß-amylase in the incomplete hydrolysis of amylose was a minor degree of branching by α-(1→6) linkages, the linkages that cause branching in amylopectin. By using a mixture of ß-amylase and pullulanase, an enzyme that specially cleaves α-(1→6) linkages, Banks and Greenwood (1966,1967) proved the presence of branching in amylose as well as in amylopectin. They showed quantitative convertion of amylose of potato and wheat starches into maltose by observing the reduction of the limiting viscosity number and the increase of ß-amylolysis limit to near 100%. With the concurrent action of pullulanase and ß-amylase on the amylose ß-limit dextrins, they further confirmed the presence of some α-(1→6) linkages in amylose. Hizukuri et al (1981) also supported the theory for the multi-branched nature of amylose after examining starches from several plant sources.

### 2. Fine structure of amylopectin

Amylopectin is a branched molecule with 5-6% of its bonds being α-(1→6) linkages. The organization of chains within the amylopectin molecule has been the subject of intense research. The amylopectin chains can be classified into A, B, and C chains to illustrate the fine structure of amylopectin (Peat et al 1956). The A chains are linked to another chain at the reducing ends by α-(1→6) linkages and without carrying other chains. The B chains bear other chains as branches and are linked to other chains at the reducing

ends by α-(1→6) linkage. There is a single C chain per amylopectin molecule which carries the only reducing group in the starch molecule and to which A or B chains may be attached.

A cluster model, proposed in similar fashions by French (1973) (Fig. 1) and Robin et al (1974) (Fig. 2), is widely accepted to elucidate the chain arrangement of amylopectin. The cluster model shows many branch chains running parallel to each other and can account for the high viscosity and high crystallinity of amylopectin. Additional support for the cluster model of amylopectin was obtained based on chain length distribution studies (Manners and Matheson 1981, Hizukuri 1986).

The fine structure of amylopectin can be deduced through successive degradation by the action of debranching enzymes (pullulanase and isoamylase), ß-amylase, and amyloglucosidase followed by fractionation by gel filtration of the debranched materials. Two major populations were observed for most amylopectins debranched by isoamylase. The first fraction (F1) corresponded to long B chains with an average chain length (CL) of about 50 glucose units; the second fraction (F2) included short B chains and A chains with CL of about 20 glucose units (Akai et al 1971). A trimodal or a polymodal distribution, however, was proposed by Hizukuri (1986) after improving the resolution by using high performance liquid chromatography (HPLC). In this work, the B chains were further divided into B1-B4 fractions. The previous fraction F1 then was composed of fractions B2, B3 and B4, and fraction F2 contained fractions A and B1. He suggested that the results showed amylopectin being composed of many oriented clusters which were randomly or regularly distributed , and linked by long chains extending to two or more clusters.

### 3. Intermediate material

The presence of a starch fraction having properties different from amylose and amylopectin was postulated by Lansky et al (1949). The materials exhibited iodine-binding capacity and had a ß-amylolysis limit between those of amylose and amylopectin. The amount and structure of intermediate materials differed with starch types and maturity of starch (Banks and Greenwood 1975).

Dais and Perlin (1982) studied this anomolous fraction from wheat by using $^{13}C$-NMR and the results

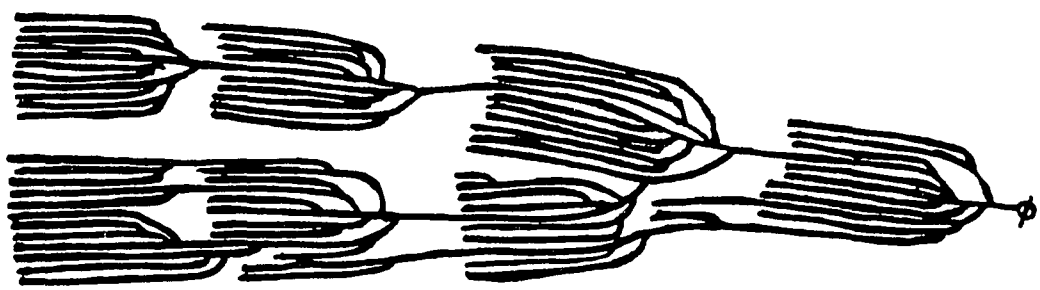


Figure 1. A cluster structure of amylopectin (French 1973). ϕ = reducing unit. The model is in accord with the structural properties of amylopectin, including the relatively high viscosity, the fibrillar and partial crystalline structure, and the relative resistance to degradation by acids and enzymes.

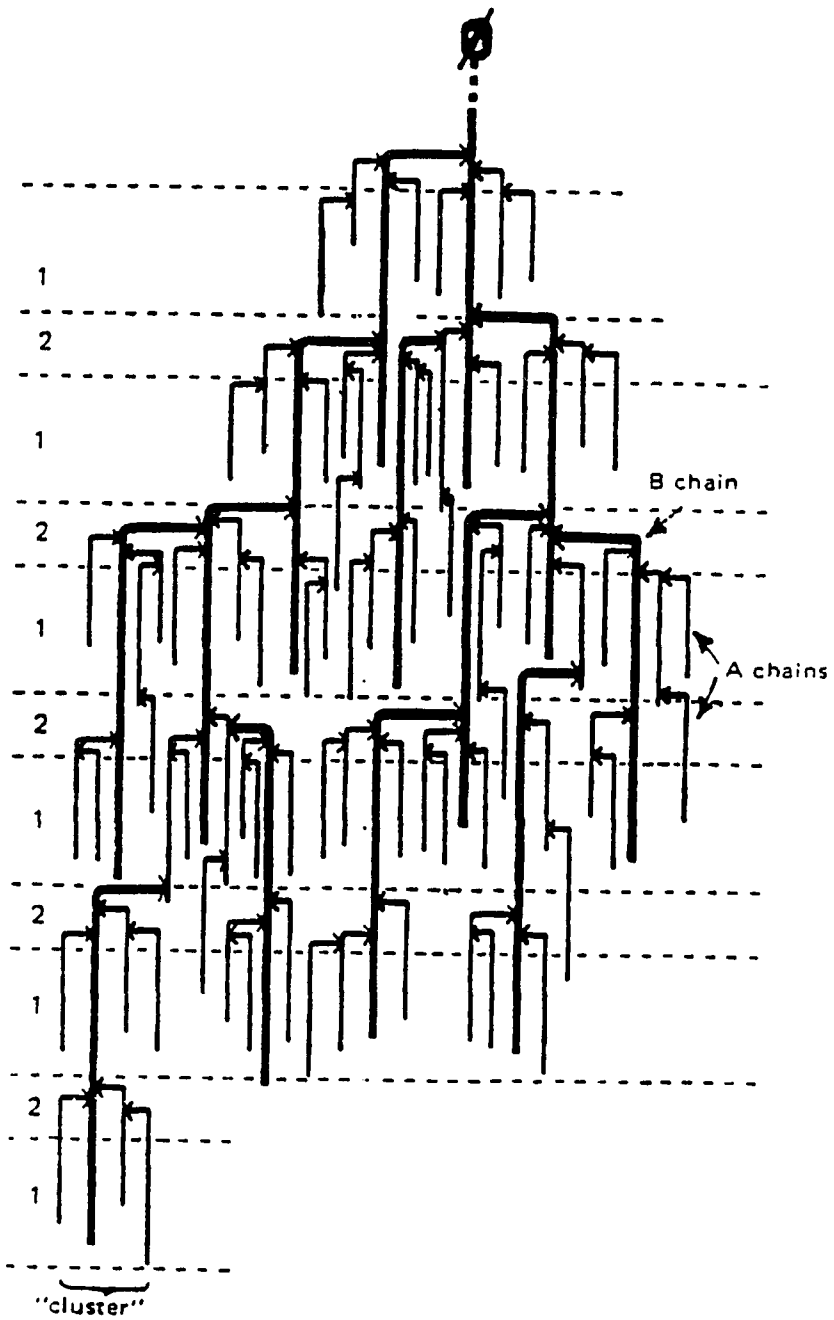


Figure 2. Proposed structure for potato amylopectin. 1 = compact area, 2 = amorphous area, rich in branching points; $\phi$ = reducing end group (Robin et al 1974). The B chains would form the backbone of the amylopectin molecule and should extend over two or more cluster, each cluster resulting from the association of two or three A-chains.

indicated that the intermediate fraction had more branch points than did normal amylopectin and had a CL that was 20-25% shorter than that of normal amylopectin. Colonna et al (1982, 1984) characterized the intermediate materials from wrinkled pea starch as a branched fraction with a low molecular weight (MW), but which was very polydisperse. Baba and Arai (1984) investigated the intermediate materials from amylomaize starch containing 50% amylose and found that the average degree of polymerization (DP) was 250 to 300 glucose units per molecule, with four or five branches having a CL of around 50 glucose units.

## C. Organization of the Starch Granule

Under polarized light, starch shows birefringence and a "maltese cross" pattern where the hilum, the growing point of the starch granule, is at the center of the granule. Birefringence implies a high degree of molecular orientation within a granule without any indication of crystallinity. Fresh starch granules exhibit growth rings which may originate from the concentric deposition of starch. Each ring represents shells of high and low starch contents which varied in rate or mode of starch deposition during growth.

### 1. Crystalline structure of starch

X-ray diffraction can provide information about the crystalline structure of starch granules, and the relative amount of crystalline and amorphous phases within starch granules. Native starch granules can be classified as three types according to their x-ray diffraction patterns: A type, characteristic of cereal starch, such as maize, rice, and wheat; B type, characteristic of tuber, fruit and high amylose maize; and C type, with characteristics between those of A and B types with examples including bean and pea starches. The characteristics displayed by the x-ray pattern depend on the source of the starch, the temperature, and the water content (Sair 1967).

It is generally accepted that amylopectin is responsible for the crystallinity of starch. The waxy maize starch, being nearly 100% amylopectin, exhibits birefringence and an x-ray diffraction pattern similar to those of normal maize starch. In contrast, high amylose maize starch shows poor crystallinity and weak birefringence. The crystallinity domains of starch granules are composed of A-chains and the exterior parts of B-chains of

amylopectin.

## 2. Fractionation of starch

Several methods may be used to separate amylose from amylopectin. The methods are all based on differential solubility of the fractions in various aqueous media.

Selective leaching: Amylose molecules diffuse out of the starch granule when starch is subjected to temperatures at or slightly above the gelatinization temperature for a period of time while the swollen granule still remains intact. With successive leaching at higher temperatures, small MW amylopectin also may leach out and contaminate the amylose portion. Amylose then can be obtained by precipitating with a suitable alcohol (Whistler 1965).

Selective precipitation: The most widely adopted method to fractionate starch is selective precipitation. The starch granule is first completely dispersed into solution, then amylose is precipitated from solution as an insoluble complex by adding a complexing agent. The most important criterion leading to a successful fractionation is to disperse the starch as completely as possible, which can be achieved either by liquid ammonia treatment followed by dispersion in boiling water or by dissolving in dimethyl sulfoxide (DMSO). A DMSO pre-treatment allows the dispersion of starches which are hard to disperse in boiling water and removes contaminating fatty acids (Banks and Greenwood 1975). Dispersed starch is thereafter fractionated with butanol or thymol (Schoch 1945) which complexes with amylose and forms insoluble crystal. The complex is recovered by centrifugation, whereas the amylopectin fraction is recovered from the supernatant. If the initial pre-treatment of dispersing starch granules is incomplete, subsequent efforts to purify the amylose fraction will be unsuccessful: that is, the amylopectin cannot be removed from the amylose.

Chromatographic separation: The basis for chromatographic separation of amylose from amylopectin is the differences in solubility and/or preferential adsorption of one of the components or its complexes with a suitable ligand. Patil and Kale (1973) achieved separation of amylose and amylopectin by adsorbing amylose

onto a cellulose column through ethanol ligands. Colonna et al (1985) fractionated of amylopectin by using affinity chromatography based on the interaction between concanavalin A (Con A) and amylopectin. Con A is a lectin which specifically binds the non-reducing ends of amylopectin to form insoluble complexes. Two structural features of amylopectin leads to the special binding with Con A, namely, the hydrodynamic volume and the external chain-length.

### 3. Arrangement of amylose and amylopectin in the starch granule

The arrangement of amylose and amylopectin within starch granules relative to each other still needs investigation. Nikuni (1978) demonstrated that the amylodextrin of sweet potato starch exhibited various types of x-ray diffraction patterns depending on the environmental conditions during the growth period. He proposed a model of a starch granule in which amylose exists separate from amylopectin and that is present in the amorphous regions. A similar model was proposed by Lineback (1984) in which part of the amylose in the starch granule is complexed with naturally occurring lipids, forming a helical amylose-lipid complex, and the outer chains of amylopectin exist as double helices. Recently, Kasemsuwan and Jane (1991), Kasemsuwan (1991), and Jane et al (1992) suggested that amylose, instead of being isolated from amylopectin, is distributed among amylopectin molecules and is located in both crystalline and amorphous regions. But how the arrangement of chains gives either the A or B x-ray diffraction pattern is not clear.

### 4. Granule morphology

The shapes and sizes of starch granules differ from each other and are unique characteristics of the sources of the starch. Granules from tuber starches are generally large and ellipsoidal with a hilum that is off center, whereas cereal-starch granules are heterogeneous in shape. Granules from normal maize starch are polyhedral and range in size from 5 to 25 microns in diameter. Amylomaize starch produces irregular and filamentous granules with a size smaller than that of granules from normal maize starch.

Scanning electron microscopy (SEM) is widely used to elucidate the surface structure of starch granules. Light microscopy (LM) also may be used for this purpose but because of the transparent

characteristics of starch the interpretation of the internal and surface structures of the starch granules may be difficult. Hall and Sayre (1969, 1970, 1971) systematically studied the shapes and surface structures of various starches by using SEM and concluded that SEM had great advantages in studying the morphology of starch. Beside studying the shapes and surface structures of starch granules, SEM also can be used to study the changes during the gelatinization process, interactions between starch and water, and granular changes caused by different treatments.

**5. Susceptibility to enzyme degradation**

Starch-degrading enzymes that can hydrolyze α-D-glycosidic bonds and release energy may be divided into three types: exo-acting, endo-acting, or debranching enzymes. Exo-enzymes, including ß-amylase, phosphorylase, and glucoamylase, degrade amylose and amylopectin by the successive removal of low-MW products from the non-reducing ends. Beta-amylase and phosphorylase cannot bypass branching points, thus producing dextrins of both high and low MW. Glucoamylase additionally is able to hydrolyze α-(1→6) glucosidic linkages and, thus, converts starch to glucose. Endo-enzymes, applied exclusively to α-amylases, hydrolyze α-(1→4) glucosidic bonds in a random pattern. The end products from α-amylolysis of amylose are mainly maltose and glucose, and of amylopectin are residual branched α-limit dextrin as well as linear oligosaccharide (Roberts and Whelan 1960, Brammer et al 1972). The α-amylase works on both raw and gelatinized starches, whereas ß-amylase transforms gelatinized starch into about 60% maltose but has no action on raw starch (Guilbot and Mercier 1985). The class of debranching enzymes is composed of R-enzyme, pullulanase, and isoamylase, and is responsible for degrading α-(1→6) glucosidic linkages in amylopectin and the outer chains of the α- and ß-limit dextrins. The susceptibility of starch granules to α-amylase depends on the source of the starch and on the α-amylase. Among the granular starches investigated by Leach and Schoch (1961), waxy maize starch was the most susceptible to bacterial α-amylase and amylomaize starch was the least susceptible.

## D. General Properties of Starch

### 1. Gelatinization

Starch is insoluble in cold water but swells reversibly to a limited extent through hydrogen bonding. For some applications it is necessary to disrupt the granular structure, which leads to swelling, hydration and solubilization of starch molecules which is referred to as gelatinization. Gelatinization is described as " the phenomena of collapse (disruption) of molecular orders within the starch granule manifested in irreversible changes in properties such as granular swelling, native crystalline melting, loss of birefringence, and starch solubilization" (Atwell et al 1988). The temperature range in which gelatinization occurs is different for each starch type, with the following examples noted: wheat, 58-64°C; potato, 59-68°C; maize, 62-72°C; and rice, 68-78°C (Lineback 1984). The gelatinization process is affected by the fine structure of starch components and the percentage distribution of amylose and amylopectin. Different starch genotypes, therefore, gelatinize at different temperatures. For example, high-amylose starches gelatinize at higher temperatures than do normal and waxy starches.

There are many methods for measuring gelatinization and they are described as follows:

Light microscopy: The loss of birefringence, a characteristics of the gelatinization, can be followed by using an optical microscope equipped with crossed polarizers and a heating stage (Watson 1964). During gelatinization, starch absorbs water and swells, thus the orientation within the starch granule is destroyed resulting in loss of granule birefringence.

Scanning electron microscopy: The transformation of starch granular shape occurring during gelatinization has been studied by using SEM (Hill and Dronzek 1973, Miller et al 1973, Hoseney et al 1977, Lineback and Wongsrikasem 1980). The SEM can show in detail the changing structure of the granule throughout the gelatinization process.

Viscometry: The changes in viscosity of a starch slurry subjected to a programmed cycle of heating and

cooling cycle with the Brabender viscoamylograph can provide information on gelatinization and on the properties of the cooled paste (Zobel 1984). The temperature of the first rise in viscosity is called pasting temperature, which is highly dependent on starch concentration and generally is higher than the temperature determined from the loss of birefringence. The peak viscosity, the stability of the starch paste, and the set-back produced by cooling are also points of interest.

Swelling and solubility determination: The use of swelling power and a solubility curve to characterize starch was first developed by Leach et al (1959). The curves are derived by plotting the swelling power or solubility against temperature at certain degree intervals over the entire pasting temperatures. It is usually used to determine swelling power and solubility at 85°C, which is suitable for single-point characterization. Each starch has a characteristic pattern of swelling and solubility.

Nuclear magnetic resonance: The gelatinization of starch can be followed at the molecular level by using either high-resolution or wide-line proton magnetic resonance (pmr) techniques. At the initiation of gelatinization there is an increase in chain mobility of starch granules which decreases the spectral line-width and causes an abrupt change in line width (Jaska 1971).

Differential scanning calorimetry: Differential scanning calorimetry (DSC) monitors the heat change associated with the gelatinization process through a programmed cycle and records heat flow as thermal events occur. The difference in heat flow between a sample and a reference is recorded as a peak. Stevens and Elton (1971) first applied DSC to determine the enthalpies of gelatinization of several starches. The DSC is easy to operate, requires only a small sample size, and can record both gelatinization temperature and enthalpy (Nakazawa et al 1985).

X-ray diffraction: The starch crystallites yield reflection from crystal plane as determined by X-ray diffraction. After gelatinization, the melting of starch crystallites, these reflections disappear and a broad halo appears, indicating a change from a crystalline state to an amorphous state (Zobel et al 1988).

Other methods: Light transmission (Longley and Miller 1971), enzymatic analysis (Shetty et al 1974), and laser light scattering (Wilkes 1974) also may be used to measure starch gelatinization.

## 2. Pasting

Pasting is "the phenomenon following gelatinization in the dissolution of starch. It involves granular swelling , extrudation of molecular components from the granule, and eventually, total disruption of the granules" (Atwell et al 1988). The formation of a starch paste is accompanied by a large increase in viscosity because of the effects of the dissolved swollen starch granules. Therefore, the properties of starch pastes are commonly measured by using methods for determining viscosity, such as the Brabender viscoamylograph (Shuey and Tipple 1980), the Brookfield viscometer (Smith 1964), and rheometers.

## 3. Retrogradation

Retrogradation of a starch paste is "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 which then may develop into more extensively ordered regions. Ultimately, under favorable conditions , a crystalline order appears" (Atwell et al 1988). Retrogradation is caused by the formation of hydrogen bonding between hydroxyl groups and by hydrophobic interactions. The chain length or MW of amylose is related to the tendency to retrograde (Suzuki et al 1981, Takeda et al 1983). Amylose molecules, with a DP of around 80, are mainly responsible for retrogradation (Guilbot and Mercier 1985). However, a phenomenon related to retrogradation, bread staling, involves mainly amylopectin rather than amylose (Schoch and French 1947). Bread staling causes bread to become firm due to the recrystallization of amylopectin, but there may be some interaction with amylose during recrystallization (D'Appolonia and Morad 1981).

The rate of retrogradation is affected by many factors, such as temperature, source and concentration of starch paste, pH and the presence of salt and other chemical agents. In general, cereal starches retrograde more quickly than do tuber starches, and the retrogradation rate is enhanced at a temperature of about 0-4°C and at pH 6 (Collison and Elton 1961).

## E. Methods for Measuring Amylose, Amylopectin and Intermediate Materials

### 1. Iodine affinity (IA)

The linear fraction of starch, amylose, can interact with iodine to generate a helical inclusion complex with iodine in the central cavity and to give a blue color. In contrast, the branched fraction, amylopectin, has a low affinity for iodine and gives a red color. Because of the distinct feature that amylose binds about 20% of its own weight of iodine whilst amylopectin binds none, the ratio of amylose and amylopectin present in a starch sample can be determined by using a potentiometric technique, iodine affinity (IA) (Schoch 1964). The amylose percentage calculated from the IA method, however, is considered as "apparent amylose" (Montgomery et al 1961). The presence of branched molecules with long external chains overestimates amylose content; likewise, the presence of amylose with short chains underestimates amylose percentage.

### 2. Gel permeation chromatography (GPC)

Based on the difference in mloecular size, starch can be fractionated into two major fractions, amylopectin and amylose, by using GPC. The amylopectin fraction, being a large molecule, is eluted earlier than the amylose fraction and, thus, can be separated from amylose. The amylopectin and amylose fractions can further be subfractionated into a graded series of molecules with a broad distribution of MW (Erlander and French 1958, Banks and Greenwood1968) because both fractions are polymolecular and polydisperse.

The presence of intermediate materials is evident by comparing the GPC profile of a mixture of purified amylopectin and amylose with that of normal corn starch (Boyer et al 1976, Yeh et al 1981). Baba and Arai (1984) separated intermediate materials from amylopectin by precipitating amylose out from starch with butanol, then, fractionating butanol non-precipitating materials by using GPC.

### 3. High-performance size-exclusion chromatography (HPSEC)

More recently, high-performance size-exclusion chromatography (HPSEC) has been employed in starch

research. Size exclusion chromatography (SEC) is a liquid chromatographic technique to separate molecules by differences in size and to obtain MW distribution (Poole and Schuette 1986). Traditional (low-pressure) SEC requires long time for fraction collection and chemical analyses. The combination of high-performance liquid chromatography (HPLC) with SEC gives separations similar to those from low-pressure column but with less time and with the potentially high resolution. Most of the work has been done on the structure of amylose (Hizukuri and Takagi 1984, Takeda et al 1986, Takeda et al 1989a, Takeda et al 1989b) and on the chain length distribution of amylopectin (Hizukuri et al 1986, Takeda et al 1987, Hizukuri and Maehara 1990, Koizumi et al 1991). HPSEC has proven to be a fast and accurate technique for determining the structures of starch components.

## F. Chemical Modifications of Starches

The use of native starches is limited in many commercial applications because of many shortcomings which include unrestricted swelling after cooking, instability of the starch paste to shear or low pHs, and the tendency of starch sols to retrograde during cooling (Wurzburg 1986). Modified starches are developed to modify the gelatinization and cooking characteristics for expanding the use of starches to provide desired functionality such as thickening, gelling, binding, adhesive, and film-forming properties.

Oxidized starches are used primarily in the paper industry and are also widely used in breaded foods because they improve the adhesion of starch batters (Rutenberg and Solarek 1984). Cross-linked starches are used in baby foods, salad dressings, and fruit pie fillings in which a stable, high-viscosity starch paste is needed (Whistler and Daniel 1985). Starch acetates with a low degree of substitution (DS) are used in canned, frozen, baked, and dry foods because of their stable viscosity which prevents syneresis and development of cloudiness at low temperatures (Rutenburg abd Solarek 1984). Hydroxypropyl-starches impart a smooth, thick, clear, and non-granular texture to be used in gravies and sauces. Starch monophosphates are useful in frozen foods because they have excellent freeze-thaw stability (Whistler and Daniel 1985). Cationic starches are often used as additives in paperboard packaging. Acid-modified starches are used in the manufacture of confections because they can form firm gels on cooling. Through these chemical modifications, the use of starches can be increased

in the food industry.

## G. Applications of Starches in Foods

Starches used in the food industry perform two basic functions: as a stabilizer in food and as a processing aid to facilitate manufacturing (Moore et al 1984). As a stabilizer, starches provide characteristic viscosity, texture, mouth-feel, and consistency in either a native granular or gelatinized form. As a processing aid, starches prevent materials from sticking together to improve processibility.

Several characteristics about the foods themselves, such as cooking temperature and time, moisture and dissolved solids content, pH, and physical agitation must be considered in making the proper starch selection for food production (Moore et al 1984). The type of equipment, order of addition of other influencing ingredients, and processing requirements also should be taken into consideration (Langan 1986). Native starches in various forms are used in large volumes throughout the food industry such as coatings, candy jellies, and salad dressings. The canning industry requires both a retort temperature of 120°C for 20-60 min and processing at 145°C for a few seconds. Desired starches, thus, should gelatinize at elevated temperatures allowing canned materials to remain fluid in the early heating stages to facilitate heat penetration. Some processes require the application of heat in an acid environment over a period of time. Modified starches are usually employed for this purpose. The frozen industry requires starches with good freeze-thaw stability. High-amylose starch improves the manufacture of jelly candy because it gels rapidly and forms high-strength gels, whereas native waxy starch, with chemical or physical modification, is suitable for thickening fruit pies and many prepared canned, and frozen foods because it develops heavy viscosity and good clarity, and is resistant to syneresis (Moore et al 1984). Pregelatinized or cold-water swelling starches are gaining popularity in convenience package mixes and in the manufacturing process that extrudes and cuts doughs for baking (Moore et al 1984). Limited availabilty and high prices of natural ingredients have stimulated the investigation and development of new suitable alternatives for the diverse use of starches in foods.

There is a growing interest in studying maize mutants because they offer alternatives to modified starches in many applications. How starch biosynthesis is affected by the presence of mutant genes still is not

completely clear. It would be a great advantage to understand the relationships between starch structure and function. Mutants of corn with many possible variations in starch structure could provide materials for such a study. By placing all these mutants in the same dent corn background the effects of the mutants on starch structure and function can be compared.

# PAPER I

# THERMAL AND GELLING PROPERTIES OF MAIZE MUTANTS FROM THE OH43 INBRED LINE

# THERMAL AND GELLING PROPERTIES OF MAIZE MUTANTS FROM THE OH43 INBRED LINE[1]

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1This is joint contribution of Journal paper no. J-14604 of the Iowa Agriculture and Home Economics Experiment Station at Iowa State University, Ames, IA and Field Crops Research Unit, USDA-ARS. Project no. 2568 and 2778.

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## ABSTRACT

Starches were isolated from the maize (*Zea may* L.) inbred line, Oh43, and its single mutants [amylose extender (*ae*), brittle-1 (*bt1*), brittle-2 (*bt2*), dull-1 (*du1*), floury-2 (*fl2*), horny (*h*), shrunken-2 (*sh2*), sugary-1 (*su1*), and waxy (*wx*)], and the double-mutant combinations within Oh43. Differential scanning calorimetry (DSC) was used to determine the onset temperature ($T_O$), range, enthalpy ($\Delta H$) of gelatinization and retrogradation, and percentage of retrogradation (r%). The gel strength was measured by using a Voland-Stevens texture analyzer. For gelatinization, the starches of *wx du1* and *sh2 du1* had the highest $T_O$. Double-mutants *ae bt2* and *bt2 du1* had the highest $T_O$ of retrogradation. The highest $\Delta H$ of gelatinization was observed for *h wx*. The gelatinization enthalpy peak for *bt1* starch had a characteristic low temperature shoulder and wide range. Compared with the respective single mutants, most double-mutant combinations had higher $T_O$ and $\Delta H$ for gelatinization and lower $T_O$ for retrogradation. For gel strength, the *du1* starch gave the lowest values for firmness and stickiness among the samples. Double mutants generally had gel strength measurements lower than those of the single mutants *bt1*, *bt2*, *fl2*, *h*, and *sh2*, but higher than those of *du1*.

# INTRODUCTION

Several endosperm mutants that are genetically recessive have their primary effect on the synthesis of starch or on a particular protein in maize (*Zea mays* L.) (Ikawa et al 1981, Yeh et al 1981, Inouchi et al 1983, 1987, Fuwa et al 1987, Sanders et al 1990). Identified recessive mutant genes include: amylose extender (*ae*), brittle (*bt*), dull (*du*), floury (*fl*), horny (*h*), opaque (*o*), shrunken (*sh*), sugary (*su*), and waxy (*wx*). These mutants cause variation in amylose percentage or the total amount of starch accumulation. The nomenclature of these mutants is, in part, based on the effect that these mutant genes exert on the appearance or phenotype of the kernel. Some genotypes that cause the same effect but are controlled by different genes on different chromosomes are given a number after the named genotype (for example, sugary-1 [*su1*], and sugary-2 [*su2*]).

Because of the diverse applications of starch in industries, chemical and/or physical modifications are often made to the starches to meet the needs of the users. However, with the increasing difficulty in achieving regulatory approval of chemically modified starches in the food industry (Sanders et al 1990), there is a great potential for novel starches from mutant genotypes that bear desired properties. Furthermore, such novel starches might replace chemically modified starches, thereby providing economic advantages by reducing the cost of processing.

The mutant genes can influence the total starch content and the amylose-amylopectin ratio. The *ae* mutant is associated with a high amylose content of the endosperm starch, whereas the *wx* starch has essentially no amylose (Shannon and Garwood 1984). In differential scanning calorimetry (DSC) analyses, the *wx* starch showed thermal behavior similar to that of normal corn starch. The *ae* starch, however, did not exhibit a clear peak, and the endotherm extended beyond 100°C (Stevens and Elton 1971). The special properties of different mutants, such as gelatinization characteristics and susceptibility to enzymes, have been described elsewhere (Inouchi et al 1984, Boyer and Liu 1985, Krueger et al 1987b, Brockett et al 1988, Ninomya et al 1989, Sanders et al 1990).

The double-mutant combinations create additional modifications in the structure and properties of starch granules (Ikawa et al 1981, Yeh et al 1981, Fuwa et al 1987, Brockett et al 1988, Ninomya et al 1989, Sanders

et al 1990). For example, when the *ae* gene was introduced as a double mutant, amylose content increased and an intermediate fraction and amylopectin with longer branches were found (Ikawa et al 1981). The DSC thermograms of double mutant starches with the *wx* gene shifted to a narrower temperature range (R) compared with those of their respective single mutants (Sanders et al 1990).

Important physical properties of starches include the thermal requirements for gelatinization, the susceptibility of gelatinized starch to retrogradation, and the shear modulus of the starch gel. The temperature of gelatinization can be studied by using DSC or by loss of birefringence under a polarized light microscope equipped with a hot stage. DSC has been widely used to study the thermal behavior of starch because it requires only a small sample size, both gelatinization temperature and enthalpy can be obtained, and it is easy to operate (Nakazawa et al 1985). DSC also can be applied to retrograded starches to measure transition temperature and enthalpy.

The objectives of the present work were to examine the thermal properties of native and retrograded starches and gelling properties using single and double mutants of Oh43.

# MATERIALS AND METHODS

## Materials

Mature kernels of Oh43 and its single and double mutants (Table I) were used in this study and were identified according to their kernel phenotypes (Garwood and Creech 1972). Single mutants were obtained from the Maize Genetics Cooperation Stock Center at Urbana, IL. Single mutants were crossed in all combinations and self-pollinated. The double mutants were selected on the basis of having kernel phenotypes different from Oh43 and their respective single mutants. They were grown either in a winter nursery in Puerto Rico during 1989-1990 or near Ames, IA, in 1990. Plants were self-pollinated or crossed as appropriate, and ears were harvested at full maturity. After harvest, corn ears were dried at 38°C for five days to 13% moisture content. The samples were stored in a cold room at 4°C and 45% relative humidity until analyzed.

## Single-Kernel Starch Isolation

Starches were isolated as described by White et al (1990) except that a 30-μm sieve was used and starch from two kernels was extracted at a time. Two separate extractions per starch type were run, and starch from a single isolation was used to determine both thermal and gel properties.

## Differential Scanning Calorimetry

The DSC studies were performed by using a Perkin-Elmer DSC 7 analyzer equipped with a thermal-analysis data station (Perkin-Elmer Corp., Norwalk, CT). The gelatinization of starch was accomplished as previously described by White et al (1990), and refrigerated-storage retrogradation was done by the procedure of White et al (1989). Approximately 3.5 mg (dry-weight basis [dwb]) of starch was weighed accurately into an aluminum pan, and 8 mg of distilled water was added. The pan was hermetically sealed and allowed to equilibrate at least 1 hr before analysis. Samples were heated from 30 to 110°C at a rate of 10°C/min. Enthalpy ($\Delta H$), onset ($T_o$), and peak ($T_p$) temperatures were computed automatically. At the water level used, the endotherms were essentially symmetrical, which allowed the total gelatinization range to be computed as $2(T_p$-

$T_O$) as described by Krueger et al (1987a). The results are the average of three scans each for two extractions from one sample. Enthalpies were calculated on a starch dry-weight basis. The peak height index (PHI), which is the ratio ΔH/(Tp-To), was calculated to allow a quantitative evaluation of variations in peak shape (Krueger et al 1987a).

### Gel Properties

Limited quantities of starches were available, so the preparation of starch gels was adapted to a small size as follows. Starch (60.0 ± 0.1 mg, dwb) was put in a vial (4.7 cm high and 1.5 cm diameter), and distilled water was added to a total weight of 1.00 g to make a starch gel of 6% (w/w). A half-inch stirring bar was inserted into the vial, the vial was placed on a cold hot plate stirrer and stirred slowly until the starch was dispersed. The sample then was heated to boiling with stirring, held for 20 sec, and removed from the hot plate stirrer. High amylose starches were boiled for 2 min to ensure complete gelatinization. The stirring bar was carefully removed, and the vial was tapped gently on a hard surface to redistribute the gel to the bottom of the vial. The vial was covered with Parafilm® and placed at 25°C for 4 hr to allow the gel to set and cool before analysis.

The resistance to penetration of the gel was determined by using a model TA-100 Voland-Stevens texture analyzer (Voland Corp., Hawthrone, NY) fitted with an L6512 series flat-bed recorder. The gel was compressed at a speed of 0.2 mm/sec to a distance of 3 mm with a punch probe (TA53, 3 mm diameter) with the chart recorder speed at 10 cm/min. The peak height at 3-mm compression was termed firmness, and the negative peak height during retraction of the probe was termed stickiness (Fig. 1) according to Takahashi and Seib (1988). One gel was measured for each starch extraction.

### Statistical Analyses

Analysis of variance and data and starch group comparisons were computed with the General Linear Model Program (SAS 1990). Multiple comparisons were done by least significant difference (LSD) after a preliminary F test (Steel and Torrie 1960). Correlation analyses were done on the enthalpy data of DSC and on

the gel-strength data.

# RESULTS AND DISCUSSION

## Gelatinization Properties

The DSC properties of starches of Oh43 and its single mutants and double mutants are summarized in Table I, and LSDs are listed for each property. A summary of significant differences among DSC properties of single- and double-mutant starches is presented in Table II, and some representative thermograms are shown in Figures 2 and 3. Mutants that did not grow in Puerto Rico during 1989-1990 were grown in Ames, IA, in 1990. This environmental effect may affect their DSC properties (White et al 1991). Among the single mutants, the onset temperature of gelatinization ($T_o$) was highest for *ae*, at 68.7°C, and lowest for *bt1*, at 63.4°C. The temperature range (R) and enthalpy of gelatinization ($\Delta H_g$) of *ae* were larger in this study than in previous studies (Krueger et al 1987b, Brockett et al 1988, Sanders et al 1990) but smaller than in other studies (Wootton and Bamunuarachchi 1979, Biliaderis et al 1980). The reported differences may be attributed to environmental effects (White et al 1991). The *wx* genotype produced higher $T_o$ and $\Delta H_g$ for gelatinization than did other single mutants, which was similar to previous reports (Inouchi et al 1984, Fuwa et al 1987).

The PHI, ($\Delta H/[T_p-T_o]$), was developed by Krueger et al (1987a) to differentiate raw and annealed starches. The PHI provides a numerical value that describes the relative shape of the endotherm; e.g., a tall, narrow endotherm has a higher PHI than does a short, broad endotherm. The thermogram of *bt1* exhibited an unusual low-temperature shoulder, that gave *bt1* starch the lowest $T_o$, the broadest R, and the lowest PHI (excluding *ae*) among single mutants (Fig. 2). The *du1* starch had the lowest $\Delta H_g$ (2.1 cal/g), which was lower than the same genotype (2.9 cal/g) reported in earlier studies performed at the same starch/water ratio (Inouchi et al 1984, Fuwa et al 1987). The $\Delta H_g$ values of *bt1*, *du1*, *sh2*, and *su1* were lower than that of the normal starch ($P<0.05$). The normal, *ae*, *sh2*, and *wx* starches had higher PHI than those reported by Krueger et al (1987a, b). The PHI values for normal starch (Oh43) varied from 0.32 to 0.43 in their study (1987a), compared with 0.67 in our study.

Starches from the double mutants had $T_o$ values for gelatinization that ranged from 65.0°C for *sh2 h* to 70.9°C for *wx du1*. The R ranged from 4.9°C for *h wx* to 10.9°C for *sh2 wx*. The *h wx* starch showed a

very sharp and well-defined endotherm, giving it the narrowest R, the highest $\Delta H_g$, and the highest PHI among double mutants (Fig. 3). The *wx su1* also exhibited a sharp endothermic peak and a high $\Delta H_g$ similar to that of the *h wx* (Fig. 3). The double-mutant combinations containing the wx gene (*h wx*, *wx su1*, and *wx du1*) had higher $\Delta H_g$ values than that of the normal starch ($P<0.05$), which agreed with results of Sanders et al (1990). The PHI values for single and double mutants were higher than reported previously (Krueger et al 1987a, b) with *h bt1*, *h wx, and wx su*1 having PHI values larger than one.

When the mutants containing the same recessive mutant gene were grouped and compared with other mutants, some trends were noted (Table II). For gelatinization, the double-mutant combinations had significantly higher $T_0$ and $\Delta H_g$, and lower R values than the single mutants ($P<0.01$). No significant difference was found, except $\Delta H_g$ for the Oh43 versus double mutants comparison, when Oh43 was compared with either single or double mutants. When the *ae* gene was introduced, a broad R for the gelatinization peak was seen. The mutants containing the *bt1* or the *du1* gene exhibited significantly lower $T_0$ and higher R than other mutants. In contrast, the *h* or *wx* gene produced mutants with high $T_0$ and low R values. As indicated earlier, the mutants with the *wx* gene produced significantly higher $\Delta H_g$ ($P<0.01$) than did the other mutants. Most mutants containing the same recessive mutant gene possessed distinctive thermoproperties, which may be useful as an index or reference in the mutant screening process.

Correlation coefficients (r values) were determined among all DSC parameters; however, few r-values were greater than 0.5. The r value between $T_0$ and $\Delta H_g$ for single mutants was 0.72, indicating some correlation between $T_0$ and $\Delta H_g$. But the r value between these same parameters was only 0.29 for the double mutants. These different r values for the same parameters support the idea that the influence of a particular gene on thermal properties varies according to the presence of other mutant genes (Sanders et al 1990). Furthermore, the thermal properties are influenced by structural characteristics of the starch, such as amylose-amylopectin ratio, differences in fine structure, and degree of crystallinity.

Gelatinization is a semicooperative process (Donovan 1979, French 1983) in which the amorphous regions take up water and swell to a gel phase, generating strain on the crystalline regions. This action stresses

the crystallites so that they cooperatively melt at a lower temperature than when not associated with the gel phase. The structural relationship between amorphous regions and crystallites in a starch granule is responsible for the shape and $T_o$ of the endotherm (Krueger et al 1987b). The *wx* starch, being primarily amylopectin, possesses a different amorphous-crystalline structural relationship than does the normal starch granule. Mutant combinations with the *wx* gene produce endosperm starch with no amylose (Boyer et al 1976, Ikawa et al 1981, Yeh et al 1981, Boyer and Liu 1985, Fuwa et al 1987, Sanders et al 1990). Both Stevens and Elton (1971) and Inouchi et al (1984) reported higher $\Delta H_g$ and R for *wx* starch than for normal starch and concluded that there is a more important contribution from amylopectin than from amylose in gelatinization.

In our study, the *wx* starch showed a sharper endotherm and narrower R than did the normal starch and, therefore, a higher PHI value. The narrowed R for gelatinization of *wx* starch might suggest that the melting of starch is highly cooperative and that more energy is needed for initiation in the absence of the amylose-rich amorphous regions (Krueger et al 1987b). Some double mutants containing the *wx* gene (*fl2 wx*, *h wx*, *su1 wx*, *wx du1*, and *wx su1*) had higher PHI than that of normal starch, but *ae wx*, *bt1 wx*, and *bt2 wx* had lower PHI values. Although the *su1 wx* and *wx su1* starches contained the same recessive mutant genes, they exhibited different thermograms (Fig. 3), which may be attributed to the different contributions originating from female (pistil) or male (pollen) (Yamada et al 1978). These observations suggest that the fine structure of amylopectin among different double mutants containing the wx gene may differ and that amylopectin plays a complex role in determining the thermal properties of starch, as suggested by Sanders et al (1990).

The *ae*, *du1*, and *su1* genotypes are reported to increase amylose content of starch (Ikawa et al 1981, Yeh et al 1981, Inouchi et al 1983, Boyer and Liu 1985). This increase in amylose may dilute the crystalline regions. Consequently, the crystallites may be so far apart that cooperative melting is not possible. Low $\Delta H$ and PHI were observed in the *ae*, *du1*, and *su1* starches, perhaps because of this dilution theory. Inouchi et al (1984) also reported that the $\Delta H$ values of the starches increased with decreasing apparent amylose contents. Boyer et al (1976) showed that the *ae wx* starch possessed longer outer chains that did the *wx* starch. In the present study, the longer exterior chains of *ae wx* starch may be responsible for a broader R, lower $\Delta H$, and lower PHI than those for *wx* starch. In a similar work by Yeh et al (1981), most of the mutant combinations

containing the *ae* gene produced long exterior chains of amylopectin and, thus, relatively broad endotherms as indicated by their low PHI values. The results suggest that the ratio of amylose to amylopectin in the starch granule, the distribution of amorphous and crystalline regions, and the fine structure of amylopectin are all important in determining the gelatinization properties of the starch.

**Refrigerated-Storage Retrogradation**

The DSC properties of the starch samples stored at 4°C for 7 days (retrogradation) are reported in Table I and summarized group comparisons are listed in Table II. The endothermic transition for all recrystallized starches occurred at a lower temperature than that of gelatinization ($P<0.01$), with values ranging from 38.2°C for *h bt2* to 44.4°C for *ae bt2*. Also, the R for the enthalpy peak of retrogradation was broader than that of the native starch ($P<0.01$). When the gelatinized starch molecules reassociated during storage at 4°C, they formed a weaker structure than in the native molecules, as indicated by the smaller enthalpy values of retrogradation ($\Delta H_r$). The $\Delta H_r$ for all samples ranged from 0.9 for *su1* to 1.9 cal/g for *wx*. The $\Delta H_r$ of *ae* was difficult to determine because its broad range extended beyond 100°C, so these data were omitted. For most samples, the ratio of $\Delta H_r$ to $\Delta H_g$ (r%) was close to 50%, meaning that the energy required to regelatinize the starches after 7 days of storage at 4°C was about half of its original value. The r% for the *du1* starch was far higher than the others, at 73.5, which simply reflected its low $\Delta H_g$ value.

The *wx* starch displayed the highest retrogradation tendency, as shown by its highest $\Delta H_r$, which supports the idea that amylopectin is responsible for the retrogradation as measured by using DSC (Russell 1983, Eliasson 1985, Eliasson and Ljunger 1988). All the double mutants containing the *wx* gene had lower $\Delta H_r$ than did the *wx* starch ($P<0.05$). Although the *wx* gene is epistatic in its ability to produce amylopectin, these molecules may vary in structure once the *wx* gene is combined with another mutant gene. Thus, although amylopectin plays an important role in the retrogradation of starch during storage (White et al 1989), the fine structure of amylopectin may play an even more important role in determining the thermal behaviors of starch.

There were no significant differences between Oh43 and single mutants or between Oh43 and double mutants for the retrogradation properties (Table II). The double mutants had significantly ($P<0.05$) lower $T_o$

and broader R than those of the single mutants for retrogradation. The mutants containing the *bt1* gene had higher $T_o$ and mutants containing the *fl2* or *h* or *sh2* gene had lower $T_o$ than other mutants ($P<0.01$). No significant difference in $\Delta H_r$ was found for all comparisons.

The major variations in the fine structure of amylopectin are the chain length, the distribution of chain lengths, and the ratio of short to long chains (Kalichevsky et al 1990). The branching chain length of amylopectin may have an important effect on the rate of aggregation. As mentioned earlier, starches containing the *ae* gene have longer exterior chains, which may result in a steric effect that decreases the association of starch molecules and lowers $\Delta H_r$ compared with that of the *wx* starch ($P<0.05$). On the other hand, the double-mutant combinations containing the *wx* gene did not exhibit higher $\Delta H_r$ compared with double mutants not containing the *wx* gene in the present study (Table II). These results suggest that, although DSC evidently is sensitive to the amylopectin fraction of the retrograded starches, another type of molecular interaction may also be involved, such as an interaction between amylose and amylopectin (Miles et al 1985a).

**Gel Properties**

Table III lists the gel properties of all samples as measured by the texture analyzer. A typical load-penetration curve of commercial corn starch at 6% (w/w) solid is shown in Fig. 1. Because of the limited sample size available, it was not possible to make large gels in which a freshly cut surface could be exposed, as described by Takahashi and Seib (1988). Therefore, all the load-penetration curves in this study showed a drop in force after the probe penetrated the gel surface (noted at about 1.8-mm distance on Fig. 1) likely resulting from the break through the "skin" on the gel surface. Nonetheless, the peak height at 3-mm compression (noted at about 3.8-mm distance on Fig. 1) was an accurate measure of inside gel firmness as measured by Takahashi and Seib (1988). All of the *wx*-containing starches and a few others formed weak gels not measurable under the test conditions because the gels were too soft. The present conditions required a force of 0.5 g to be reached before the probe traveled its 3 mm through the gel. Thus, the probe hit the bottom of the vial before traveling the required distance through the soft gels. The firmness of the starch gels ranged from 0.8-g force for the *du1* starch to 3.3-g force for the *sh2* and *ae sh2* starches. The *ae sh2* exhibited the highest stickiness at 1.2-g force,

whereas *du1, fl2 bt1, sh2 su1*, and *su1 h* had the lowest stickiness scores of 0.4-g force.

Correlations between gel strength parameters and all DSC thermal behavior parameters were run, with most correlation values being less than 0.6, so the data are not shown. Firmness and stickiness values correlated somewhat with $\Delta H_r$ with r values of -0.74 and -0.62, respectively. These negative correlations suggest that starches with greater tendency to retrograde produced less firm and less sticky gels. Much of this behavior could be explained by the effects of *wx* versus *ae* starch.

Initial gel formation has been reported to correlate with the amylose fraction, which, being linear, has the ability to quickly form junction zones, reassociate, and reestablish intermolecular hydrogen bonds (Howling 1980). The increase in the firmness of a starch gel after the initial cool down is related to the crystallization of amylopectin within the gelatinized starch granule (Ring et al 1987). Because it is a branched molecule, amylopectin cannot form junction zones and, thus, maintains a poor resistance to penetration. Some researchers propose that gelation of an amylose dispersion occurs only after exceeding a certain concentration (C*) (Miles et al 1985a, Ring et al 1987). The gel formation arises as a result of a phase separation that produces polymer-rich and polymer-deficient regions. If the amylose concentration is sufficiently high, the polymer-rich regions form an interconnected gel network (Miles et al 1985a). The C* for amylose of molecular weight $5x10^5$ was ~1.5% (Miles et al 1985a). At a fixed molecular weight, the branching of amylopectin reduced the hydrodynamic volume, resulting in a C* that was shifted toward a higher value than for a linear chain.

By studying the gelation of amylose and amylopectin, Miles et al (1985a) and Ring et al (1987) found that the formation of a network, as measured by the shear modulus, lagged behind the development of crystallinity, as detected by X-ray diffraction and DSC. The low correlations between TA and DSC results in the current study support these results. Miles et al (1985b) also showed that the formation of a starch gel could be separated into two processes, short term and long term. The short-term process was dominated by irreversible gelation within the amylose matrix, and the long-term one was linked to a reversible crystallization involving amylopectin. The negative correlations between $\Delta H_r$ and firmness and stickiness measurements in the current work supports their observations. Increased formation of a retrograded gel ($\Delta H_r$) did not mean increased firmness and stickiness, suggesting more than one development process.

# CONCLUSION

Amylose and amylopectin both are important to the thermal properties and firmness of starch gels; however, the various responses of the samples to DSC analyses suggest that structural differences beyond those of amylose and amylopectin also influence these characteristics. The data should be verified by studing the mutant effect in other varieties and under different growing conditions. Other work has shown an environmental effect on DSC properties of starches grown in two environments (White et al 1991). The $T_0$ were higher and R were lower in starches grown in a tropical rather than temperate environment, however, there was a cultivar by location interaction. These observations should be considered when evaluating the few samples in our work that were grown near Ames, IA rather than in Puerto Rico. But, for the most part, the starches grown near Ames were *ae* single and double mutants that can be compared within one environment. Also, averaged over all samples, the double mutants had higher $T_0$ and $\Delta H$ and lower R values than did the single mutants. To understand these relationships, future work will involve studying the effect of single and double mutants of Oh43 on the structures of starch components. In some cases, the fine structures of amylose and amylopectin will be determined to relate the physical properties to the chemical structures.

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Table I. Differential scanning calorimetry properties of starches[a]

| Starch | Gelatinization | | | | Refrigerated-storage retrogradation | | | |
|---|---|---|---|---|---|---|---|---|
| | $T_o$[b] (°C) | R[c] (°C) | $\Delta H_g$[d] (cal/g) | PHI[e] | $T_o$ (°C) | R (°C) | $\Delta H_r$[f] (cal/g) | r%[g] ($\Delta H_r/\Delta H_g$) |
| Oh43 | 67.2 | 8.8 | 2.9 | 0.67 | 42.6 | 16.9 | 1.5 | 49.5 |
| Single mutants | | | | | | | | |
| *ae*[h] | 68.7 | 31.3 | 3.7 | 0.24 | ...[i] | — | — | — |
| *bt1* | 63.4 | 14.9 | 2.5 | 0.33 | 42.7 | 15.4 | 1.2 | 46.8 |
| *bt2* | 66.8 | 10.6 | 2.9 | 0.55 | 42.7 | 16.6 | 1.3 | 45.1 |
| *du1* | 67.2 | 9.1 | 2.1 | 0.46 | 42.9 | 17.7 | 1.5 | 73.5 |
| *fl2* | 66.2 | 9.1 | 2.9 | 0.64 | 44.2 | 15.1 | 1.4 | 49.7 |
| *h* | 65.6 | 9.4 | 2.7 | 0.57 | 44.1 | 15.8 | 1.4 | 52.7 |
| *sh2* | 64.3 | 8.5 | 2.4 | 0.57 | 42.1 | 16.5 | 1.1 | 46.7 |
| *su1* | 64.6 | 7.1 | 2.1 | 0.59 | 43.4 | 14.1 | 0.9 | 45.1 |
| *wx*[h] | 68.6 | 9.0 | 3.6 | 0.80 | 39.9 | 21.2 | 1.9 | 52.8 |
| Double mutants | | | | | | | | |
| *ae bt2*[h] | 67.1 | 10.3 | 2.9 | 0.57 | 44.4 | 14.0 | 1.3 | 44.2 |
| *ae h*[h] | 69.6 | 9.5 | 2.7 | 0.58 | 40.7 | 20.7 | 1.4 | 49.4 |
| *ae sh2*[h] | 68.2 | 9.7 | 2.9 | 0.60 | 42.2 | 17.5 | 1.3 | 44.6 |
| *ae su1*[h] | 65.4 | 10.7 | 2.6 | 0.49 | 43.3 | 15.5 | 1.3 | 47.7 |
| *ae wx*[h] | 70.1 | 9.2 | 2.8 | 0.61 | 43.7 | 14.9 | 1.5 | 51.7 |
| *bt1 du1*[h] | 67.3 | 8.5 | 2.8 | 0.66 | 44.0 | 14.6 | 1.3 | 47.1 |
| *bt1 su1*[h] | 67.8 | 9.4 | 3.0 | 0.63 | 43.4 | 15.7 | 1.3 | 44.4 |
| *bt1 wx*[h] | 68.2 | 10.3 | 3.1 | 0.60 | 41.6 | 19.3 | 1.6 | 51.1 |
| *bt2 du1* | 66.9 | 9.5 | 2.8 | 0.59 | 44.2 | 14.9 | 1.4 | 50.0 |
| *bt2 sh2*[h] | 67.8 | 9.8 | 2.9 | 0.59 | 42.6 | 17.1 | 1.4 | 49.3 |
| *bt2 wx*[h] | 69.4 | 9.4 | 3.0 | 0.64 | 42.9 | 16.5 | 1.5 | 50.9 |
| *fl2 ae* | 67.1 | 10.9 | 3.2 | 0.59 | 42.6 | 18.1 | 1.3 | 42.1 |
| *fl2 bt1* | 68.1 | 7.0 | 3.0 | 0.87 | 39.6 | 18.3 | 1.5 | 49.7 |
| *fl2 bt2* | 68.1 | 7.7 | 3.3 | 0.84 | 40.7 | 20.8 | 1.5 | 46.5 |
| *fl2 du1* | 67.8 | 6.9 | 3.3 | 0.94 | 41.8 | 18.2 | 1.5 | 45.3 |
| *fl2 h* | 68.3 | 6.9 | 3.1 | 0.89 | 42.3 | 18.2 | 1.4 | 46.0 |
| *fl2 su1* | 67.1 | 7.8 | 3.2 | 0.81 | 41.3 | 19.4 | 1.5 | 48.2 |
| *fl2 wx* | 67.4 | 8.1 | 3.1 | 0.77 | 41.4 | 19.0 | 1.6 | 53.9 |
| *h bt1* | 68.1 | 5.8 | 3.2 | 1.09 | 41.3 | 16.9 | 1.4 | 45.4 |
| *h bt2* | 68.5 | 8.7 | 3.0 | 0.69 | 38.2 | 19.9 | 1.6 | 51.7 |
| *h du1* | 67.2 | 7.8 | 2.9 | 0.74 | 41.1 | 18.6 | 1.6 | 53.6 |
| *h fl2* | 67.7 | 7.9 | 3.2 | 0.80 | 41.4 | 19.4 | 1.5 | 47.0 |
| *h sh2* | 68.4 | 6.7 | 3.1 | 0.93 | 42.0 | 18.2 | 1.4 | 45.6 |
| *h su1* | 67.5 | 7.5 | 2.8 | 0.75 | 42.5 | 17.8 | 1.4 | 50.3 |
| *h wx* | 69.7 | 4.9 | 3.6 | 1.45 | 42.2 | 17.0 | 1.5 | 42.7 |

Table I (continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *sh2 bt1* | 67.7 | 9.0 | 2.8 | 0.63 | 41.5 | 19.1 | 1.6 | 58.1 |
| *sh2 du1* | 70.3 | 5.1 | 3.3 | 1.31 | 40.3 | 18.7 | 1.6 | 48.3 |
| *sh2 fl2* | 69.3 | 7.1 | 3.0 | 0.85 | 41.5 | 18.5 | 1.4 | 47.0 |
| *sh2 h* | 65.0 | 10.1 | 3.0 | 0.58 | 42.4 | 18.6 | 1.4 | 48.0 |
| *sh2 su1* | 68.9 | 9.0 | 3.0 | 0.66 | 40.5 | 20.5 | 1.6 | 52.5 |
| *sh2 wx*[h] | 68.1 | 10.9 | 3.0 | 0.55 | 42.0 | 18.0 | 1.5 | 51.0 |
| *su1 bt2* | 67.0 | 8.6 | 3.1 | 0.71 | 43.4 | 16.9 | 1.6 | 51.1 |
| *su1 du1* | 68.2 | 8.1 | 2.5 | 0.62 | 39.7 | 16.6 | 1.4 | 57.9 |
| *su1 h* | 68.7 | 6.6 | 2.7 | 0.82 | 42.2 | 20.2 | 1.4 | 53.9 |
| *su1 sh2* | 67.5 | 7.5 | 3.0 | 0.79 | 41.3 | 18.3 | 1.4 | 47.0 |
| *su1 wx* | 67.4 | 7.9 | 3.1 | 0.78 | 42.6 | 17.1 | 1.4 | 47.0 |
| *wx du1* | 70.9 | 7.5 | 3.3 | 0.87 | 42.4 | 18.9 | 1.5 | 44.9 |
| *wx su1* | 68.3 | 5.4 | 3.3 | 1.23 | 42.2 | 17.3 | 1.5 | 45.3 |
| $LSD_{0.05}$ | 0.70 | 0.72 | 0.2 | | 1.06 | 1.78 | 0.1 | 4.58 |

[a] Values are the average of three determinations each from two separate extractions. *ae* = Amylose extender, *bt* = brittle, *du* = dull, *fl* = floury, *h* = horny, *sh* = shrunken, *su* = sugary, and *wx* = waxy.

[b] Onset temperature.

[c] Gelatinization range calculated as 2 ($T_p$-$T_o$) as described by Krueger et al (1987a).

[d] Enthalpy of gelatinization.

[e] Peak height index = $\Delta H/(T_p-T_o)$ as described by Krueger et al (1987a).

[f] Enthalpy of retrogradation.

[g] Ratio of enthalpy of retrogradation to enthalpy of gelatinization.

[h] Mutants grown in Ames, IA. Other mutants were grown in Puerto Rico.

[i] Data are omitted because its broad thermogram extended beyond 100°C.

Table II. Summary of significant differences among differential scanning calorimetry properties of single and double mutant starches[a]

| | Gelatinization | | | Retrogradation | | | |
|---|---|---|---|---|---|---|---|
| Starch Group Comparison | $T_O$[b] | R[c] | $\Delta H_g$[d] | $T_O$ | R | $\Delta H_r$[e] | r%[f] |
| Oh43 vs mutants | ns[g] | ns | ns | ns | ns | ns | *[h] |
| Oh43 vs single mutants | ns | ns | ns | ns | ns | ns | ns |
| Oh43 vs double mutants | ns | ns | * | ns | ns | ns | ns |
| Single vs double mutants | ** | ** | ** | ** | ** | ns | ns |
| *ae*[i] vs other mutants | ns | ** | ns | ns | ns | ns | ns |
| *bt1* vs other mutants | ** | ** | ns | ** | ns | ns | ns |
| *bt2* vs other mutants | ns | ** | ns | ns | ns | ns | ns |
| *du1* vs other mutants | ** | ** | ns | ns | ns | ns | ** |
| *fl2* vs other mutants | ns | ** | ** | ** | ns | ns | ** |
| *h* vs other mutants | * | ** | ns | ** | * | ns | ns |
| *sh2* vs other mutants | ** | ns | ns | ** | * | ns | ns |
| *su1* vs other mutants | ns | ** | * | ns | ns | ns | ns |
| *wx* vs other mutants | ** | * | ** | ns | ns | ns | ns |

[a] *ae* = Amylose extender, *bt* = brittle, *du* = dull, *fl* = floury, *h* = horny, *sh* = shrunken, *su* = sugary, and *wx* = waxy.

[b] Onset temperature.

[c] Gelatinization range calculated as 2 ($T_p$-$T_O$) as described by Krueger et al (1987a).

[d] Enthalpy of gelatinization.

[e] Enthalpy of retrogradation.

[f] Ratio of enthalpy of retrogradation to enthalpy of gelatinization.

[g] Not significant at $P < 0.05$.

[h] * and ** = Significant at $P < 0.05$ and $P < 0.01$ levels of probability, respectively.

[i] All single and double mutants containing this recessive mutant gene.

Table III. Firmness and stickiness of starch gels

| Mutant[a] | Firmness (g)[b] | Stickiness (g)[b] |
|---|---|---|
| Oh43 | 2.6 | 0.8 |
| Single mutants | | |
| *æ* | 2.4 | 1.1 |
| *bt1* | 2.1 | 0.6 |
| *bt2* | 2.3 | 0.9 |
| *du1* | 0.8 | 0.4 |
| *fl2* | 2.6 | 0.8 |
| *h* | 2.3 | 0.7 |
| *sh2* | 3.3 | 0.8 |
| *su1*[c] | – | – |
| *wx*[c] | – | – |
| Double mutants | | |
| *ae bt2* | 3.0 | 1.0 |
| *ae h* | 2.3 | 0.8 |
| *ae sh2* | 3.3 | 1.2 |
| *ae su1* | 2.4 | 0.8 |
| *ae wx*[c] | – | – |
| *bt1 du1* | 2.1 | 0.8 |
| *bt1 su1* | 2.9 | 0.8 |
| *bt1 wx*[c] | – | – |
| *bt2 du1* | 2.1 | 0.6 |
| *bt2 sh2*[c] | – | – |
| *bt2 wx*[c] | – | – |
| *fl2 ae* | 2.7 | 0.7 |
| *fl2 bt1* | 1.8 | 0.4 |
| *fl2 bt2* | 1.6 | 0.6 |
| *fl2 du1* | 2.4 | 0.5 |
| *fl2 h* | 1.4 | 0.5 |
| *fl2 su1* | 1.6 | 0.6 |
| *fl2 wx*[c] | – | – |

Table III (continued)

| | | |
|---|---|---|
| *h bt1* | 2.0 | 0.7 |
| *h bt2* | 1.4 | 0.7 |
| *h du1* | 1.4 | 0.5 |
| *h fl2* | 1.3 | 0.5 |
| *h sh2* | 2.0 | 0.5 |
| *h su1*[d] | – | – |
| *h wx*[c] | – | – |
| *sh2 bt1*[d] | – | – |
| *sh2 du1*[c] | – | – |
| *sh2 fl2* | 1.3 | 0.5 |
| *sh2 h* | 1.8 | 0.5 |
| *sh2 su1* | 0.9 | 0.4 |
| *sh2 wx*[c] | – | – |
| *su1 bt2* | 1.6 | 0.6 |
| *su1 du1* | 2.4 | 0.7 |
| *su1 h* | 1.5 | 0.4 |
| *su1 sh2* | 2.1 | 0.5 |
| *su1 wx*[c] | – | – |
| *wx du1*[d] | – | – |
| *wx su1*[d] | – | – |

[a] *ae* = Amylose extender, *bt* = brittle, *du* = dull, *fl* = floury, *h* = horny, *sh* = shrunken, *su* = sugary, and *wx* = waxy.

[b] Gram-force recorded by the Voland-Stevens instrument. Values are the average of two determinations from two separate extractions.

[c] Gel too weak to support the probe.

[d] Insufficient sample.

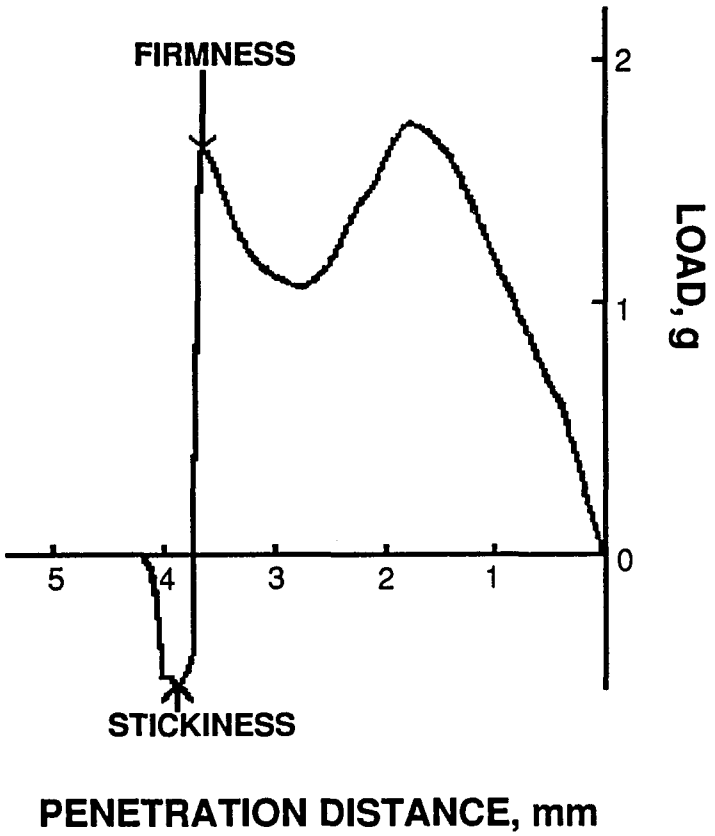


Figure 1. Load penetration curve of 6% (w/w) commercial corn starch gel measured by the Voland-Stevens texture analyzer. The gel was aged for 4 hr at 25°C before measurement.

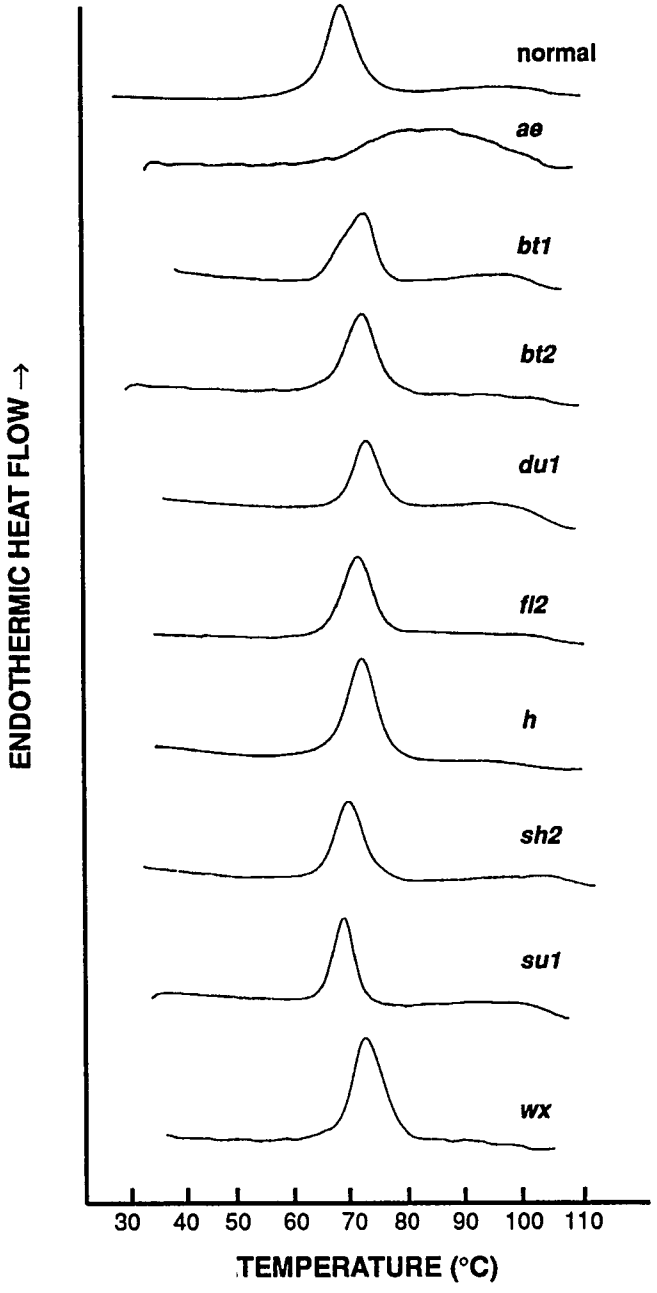


Figure 2. Differential scanning calorimetry thermograms of selected single-mutant starches within the Oh43 inbred line.

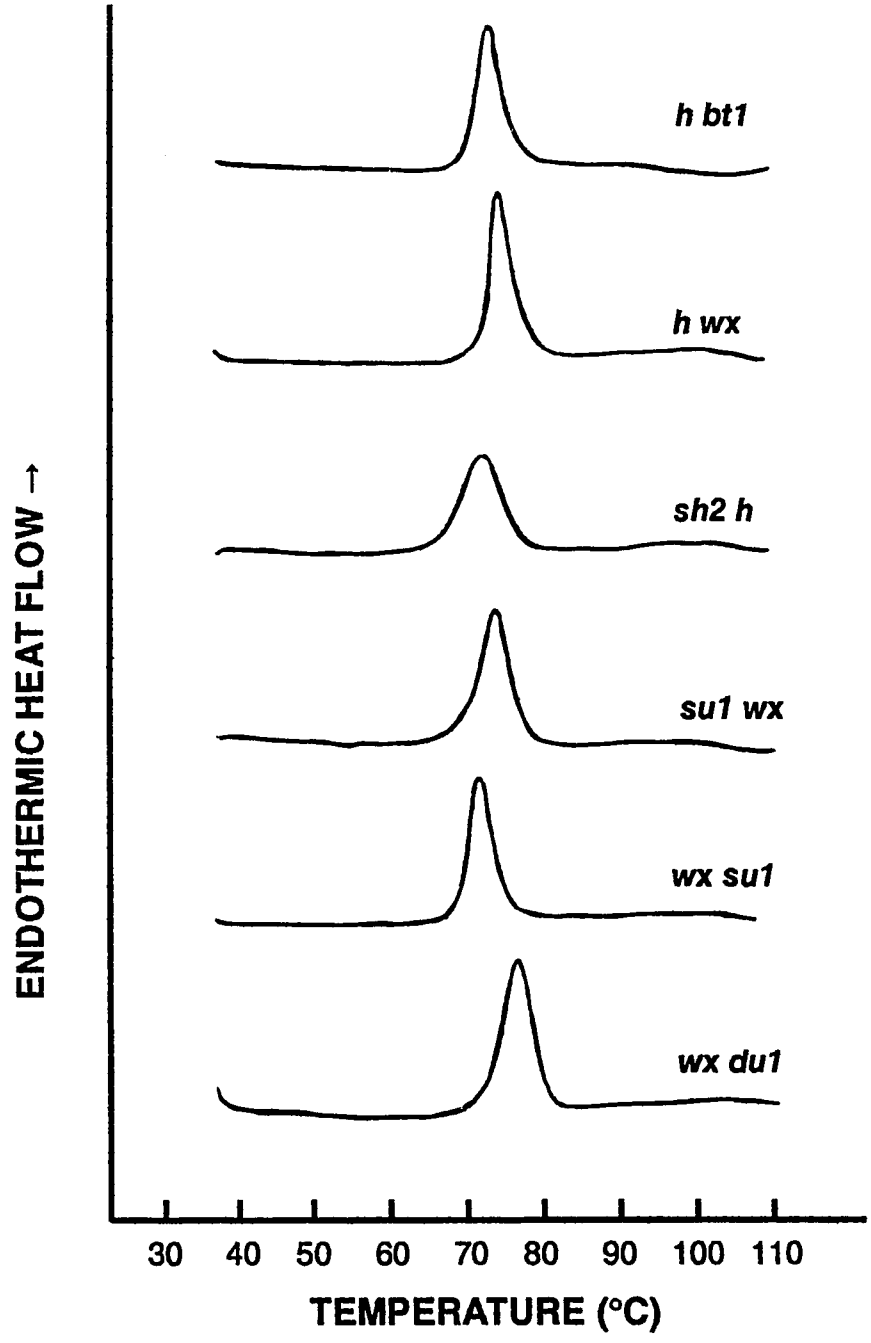


Figure 3. Differential scanning calorimetry thermograms of selected double-mutant starches within the Oh43 inbred line.

# PAPER II

## CHARACTERIZATION OF STARCH STRUCTURES OF 17 MAIZE ENDOSPERM MUTANT GENOTYPES WITH OH43 INBRED LINE BACKGROUND

# CHARACTERIZATION OF STARCH STRUCTURES OF 17 MAIZE ENDOSPERM MUTANT GENOTYPES WITH OH43 INBRED LINE BACKGROUND[1]

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## ABSTRACT

The characteristics of starches from 17 endosperm mutant genotypes in a common Oh43 inbred background were examined by using gel permeation chromatography (GPC), iodine affinity (IA), and scanning electron microscopy (SEM). The chain length distributions of amylopectins were determined by using an enzymatic-chromatographic method. Each genotype exhibited distinctive GPC elution patterns of its native and isoamylase-debranched starches and distinctive morphology as noted by SEM. The amylose-extender (*ae*), dull-1(*du1*), and sugary-1(*su1*) genes were associated with increased amounts of amylose and intermediate fraction compared with normal starch. The waxy (*wx*) gene was epistatic to other genes relative to the accumulation of amylopectin, which was consistent with work done elsewhere. The discrepancy in amylose percentage determined by using GPC and IA in some genotypes may have resulted from the presence of a large amount of intermediate materials in those genotypes, which could not always be distinguished from amylose by the IA method. For example, in *ae* starch, most of the intermediate materials were measured as amylose by the IA procedure, whereas in *du1*, *ae bt1* and *ae du1* starches, most of the intermediate materials were excluded from IA measurements. The intermediate fractions from each genotype in the GPC elution profiles also differed from each other, suggesting differences in molecular weight and/or branching. The proportions of long B chains and the average chain length of amylopectins were increased when the *ae* gene was present. In contrast, the *du1* gene decreased the proportions of the long B chains of amylopectins. The mutants containing the *ae* gene showed low degrees of branching in amylopectin; mutants containing the *du1* and/or *su1* genes had high degrees of branching. Genetic background played a major role in determining the fine structure of starch components. The effects of interactions between recessive mutant genes on the structures and morphology of different starch genotypes were evident.

# INTRODUCTION

Normal maize (*Zea mays* L.) starch is composed primarily of essentially linear (amylose) and branched (amylopectin) components. The amylose content in normal maize starch ranges from 25 to 30% but can vary among cultivars and especially with the presence of mutant genes.

Many recessive mutant genes of maize have been identified that alter the quality and quantity of starch in the endosperm. The amylose-extender (*ae*) genotype produces high apparent amylose content from 50 to 80% (Banks and Greenwood 1975). In contrast, the waxy (*wx*) gene is epistatic to all other known recessive genes for blocking the accumulation of amylose (Ikawa et al 1981, Yeh et al 1981, Inouchi et al 1983, 1987, 1991, Boyer and Liu 1985). The brittle-1 (*bt1*) mutant increased sugar content at the expense of starch accumulation (Creech 1965) and the shrunken-2 (*sh2*) genotype reduced the starch content to about 30% of the normal amount and dramatically increased the sucrose content (Holder et al 1974). The sugary (*su*) mutant synthesizes and accumulates a highly branched polysaccharide, phytoglycogen, to 25% or more of the kernel dry weight (Shannon and Garwood 1984). In other work, Yeh et al (1981) reported 55% amylose in the dull (*du*) starch from a sweet corn background.

Moreover, additional variations can be generated when several recessive genes are combined. Fuwa et al (1987) found the fine structure of amylopectins to be affected by the recessive gene (*ae* or *du*) coupled with the *wx* gene. Holder et al (1974) reported that the presence of *sh2* in multiple recessive genotypes inhibited the effect of the *ae* gene at increasing amylose content.

Elucidation of the fine structure of amylopectin is the subject of many current investigations, involving measurements such as average chain length, the A:B chain ratio, and the chain-length of the exterior and interior chains. Fuwa et al (1987) showed that, although the *wx* gene was epistatic to others for amylopectin production, the fine structure of the starches varied with the presence of other recessive genes. Inouchi et al (1983) proposed that the fine structure of amylopectin was under genetic control. For example, the *ae wx* starch had increased long B chains and decreased short B chains, and the *du wx* starch had decreased long B chains and increased short B chains compared with starch of the *wx* genotype.

A potentiometric titration method measuring iodine affinity (IA) has long been employed to determine the amylose content in the endosperm starch. The estimated amylose is termed "apparent amylose" because the occurrence of short chain-length amylose underestimates the amylose content and amylopectin with long external chains overestimates the amylose content (Shannon and Garwood 1984). More recently, gel permeation chromatography (GPC) has been employed to elucidate the profiles of starch components. The elution profile of native starch disclosed that there was no sharp separation between amylopectin and amylose (Yeh et al 1981, Boyer and Liu 1985). An intermediate material, consisting of a branched molecule with a lower molecular weight than amylopectin, was present and eluted between amylopectin and amylose fractions. Whistler and Doane (1961) obtained intermediate materials ranging from 4.5% of the total starch amount for normal commercial corn starch to 6.6-8.7% for high-amylose corn starches. They demonstrated that the properties of the intermediate fractions from different starch types were similar to each other and were between those of amylose and amylopectin. The presence of intermediate materials in amounts greater than just a few percentage points usually is associated with the presence of the *ae* gene (Whistler and Doane 1961, Ikawa et al 1978, 1981, Yeh et al 1981, Inouchi et al 1983, Baba and Arai 1984). The intermediate materials of amylomaize (50% apparent amylose) were characterized as having 4 or 5 branches with an average chain length (CL) of approximately 50 glucose units, which were linked to a main linear chain containing 100 to 150 glucose units (Baba and Arai 1984).

The structure of starch should be considered at both molecular and granular levels (Banks et al 1973). It is the physical and mechanical properties of granular starch that determine many industrial applications. Many researchers have studied elucidating the morphology of various starches by using light microscopy (LM) (Alsberg 1938, Badenhuizen 1965). Because starch granules are translucent crystals, they often give images that are hard to define with LM. The greatest error comes from the diffraction effect of light, which may make the interpretation of the internal and surface structures of starch granules difficult. By using scanning electron microscopy (SEM), only the surface structure of the starch granule is revealed. Hall and Sayre (1969, 1970, 1971) studied the shapes and surface structures of various starches by using SEM, demonstrating the advantages of SEM in determining the shape and detailed surface characteristics of starch granules.

Although much information is now known about the effects of some recessive mutant genes of maize on starch properties, few studies have done a comprehensive evaluation of the visual and structural properties of the starch granules from many mutant genotypes in a common maize background. The objectives of this study were to characterize the structure and morphology of starches from 17 maize endosperm mutant genotypes in a common Oh43 inbred background to help in understanding the influences of recessive mutant genes on the maize starches.

# MATERIALS AND METHODS

## Materials

Mature kernels of Oh43 inbred (normal) and its single and double mutants (Table I) were harvested from a summer nursery near Ames, Iowa, in 1991. Development of the genotypes, and sampling and storing of the kernels have been previously described (Wang et al 1992).

## Isolation and Preparation of Starch Samples

Starches were isolated according to a small-scale wet-milling procedure described by White et al (1990). Approximately 5 g of kernels from each genotype was used for starch extraction.

After extraction from the kernels, starch was preliminarily defatted by refluxing in 85% methanol for 24 hr and dried at 45°C overnight. Defatted starch granules were then dispersed in 90:10 (v/v) dimethyl sulfoxide (DMSO)/deionized water and stirred in a boiling water bath for 1 hr and at room temperature for another 24 hr to ensure complete dispersion.

## Gel Permeation Chromatography (GPC)

GPC of native starch. An amount of 75 mg of starch was precipitated from 1.5 mL DMSO-dispersed starch solution (50 mg starch/mL) with 10 volumes of absolute ethanol and was collected by centrifugation at 8,700 X *g* for 20 min at 4°C. Precipitated starch was redissolved in 25 mL boiling water, stirred in a boiling water bath for 30 min, and filtered through Whatman No. 1 filter paper for further purification.

Five milliliters of starch solution (containing 15 mg starch and 0.8 mg glucose marker) was loaded onto a Pharmacia column (2.6 i.d. x 79 cm) packed with Sepharose CL-2B gel (Pharmacia LKB Biotech., Uppsala, Sweden). The procedure followed that of Jane and Chen (1992) except that the column was eluted with degassed 20-mM NaCl solution adjusted to pH 11 with 1 N NaOH in the ascending mode with a flow rate of 30 mL/hr. Fractions of 4.9 mL effluent were collected every 9.5 min and subjected to total carbohydrate and

amylose content analyses by using the anthrone-sulfuric acid method (Wright and Gann 1966) and the iodine staining tests (Juliano 1971), respectively. The minimum value from iodine staining was used to identify the end of the eluted amylopectin fraction; thus, the starch profile could be identified as to amylopectin and amylose fractions. The amylose percentage was calculated by dividing the amount of starch under the amylose peak by the total starch in all fractions according to Jane and Chen (1992). Starch from each genotype was fractionated twice and the values from the data were averaged. Replicates were very similar to one another.

**GPC of isoamylase-debranched starch.** The starch was prepared, debranched, and fractionated on a Bio-Gel P-6 column (Bio-Rad Laboratories, Richmond, CA) by following the method of Jane and Chen (1992) except that native starch was used instead of amylopectin. Crystalline *Pseudomonas* isoamylase was used (Hayashibara Shoji, Inc., Olayama, Japan).

Fractions (2.3 mL) from the elution were collected and assayed for total carbohydrate by using the anthrone-sulfuric acid method (Wright and Gann 1966). The eluted materials were separated into three fractions with divisions being made at minimum points according to the total carbohydrate values. Fig. 1A shows the elution pattern and fractions of normal starch. The waxy starches, containing 100% amylopectin, consisted only of Fr. II and Fr. III. Three portions (2.3 mL each) of effluent at the peaks of Fr. II and Fr. III were assayed for total carbohydrate by using the phenol-sulfuric acid method (Dubois et al 1956) and for reducing value by using a modified Park-Johnson method (Hizukuri et al 1981, Jane and Chen 1992). The average chain length (CL) of debranched amylopectin was calculated by dividing total carbohydrate by its reducing value.

**Iodine Affinity (IA)**

Iodine affinities (IA) for the defatted starches, expressed as mg of iodine bound to 100 mg of starch, were determined in duplicate with amperometric titration (Schoch 1964) at 30°C and the results were averaged. Amylose percentages were extrapolated from the inflection points and calculated by assuming that pure maize amylose has an iodine affinity of 19.0%.

### Scanning Electron Microscopy (SEM)

Each starch sample was stirred carefully to obtain a homogeneous mixture from which a small amount of sample was removed for SEM. Starch granules were sprinkled onto double-stick tape attached to specimen stubs and coated with gold-palladium. The mounted specimens were examined with a JEOL JSM-35 scanning electron microscope (JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 10 kV. Representative micrographs were taken on each genotype at a magnification of X 1,000. The starch granule diameter was estimated by averaging the largest dimension of 15 random starch granules from duplicate micrographs for each starch type.

### Statistical Anaylses

Duplicate results were obtained and analyzed for correlations among percentage compositions and chain length distribution of polysaccharides by using the SAS program (SAS Institute 1990).

# RESULTS AND DISCUSSION

## Characteristics of Native Starches of 17 Genotypes from GPC

The elution profiles of native starches presented in Figs. 1B, 2 and 3 show the total carbohydrate content and the blue value response derived from the iodine staining test. In the elution profiles, the first peak (Fr. I) corresponds to amylopectin, which, because of its large molecular weight, was excluded from the gel and appeared at the void volume. The second peak (Fr. II) is considered to be amylose, and the peak at fraction number 88 is glucose, added to mark the end of the elution. Intermediate material eluted between Fr. I and Fr. II was detected either as a small hump or, more likely, by an elevated baseline between Fr. I and Fr. II that did not allow clear separation between the two fractions.

The normal starch exhibited typical amylopectin and amylose peaks (Fig. 1B). There was no baseline separation between amylopectin and amylose, which also was observed by Yeh et al (1981) and Boyer and Liu (1985), but the amount of intermediate materials was very little. The *ae* starch (Fig. 2A) had a highly elevated baseline between Fr. I and II, indicating a large proportion of intermediate materials, and the blue value response was relatively high in the amylose region. The elution profiles of *bt1*, *bt2* and *h* starches (Figs. 2B, 2C and 2E) were similar to that of normal starch. The *du1* starch (Fig. 2D) contained an amount of intermediate materials similar to that of *ae* starch; however, the blue value response in the amylose region was less than that of *ae* starch. A large amount of intermediate materials in *du1* starch in a dent corn inbred (W64A) also was observed by Boyer and Liu (1985), but Yeh et al (1981) did not observe this in a sweet corn background (Ia5125). It is likely that the genetic background plays a role in determining the fine structure of starch components. The chromatogram of the *sh2* starch (Fig. 2F) was similar to that of normal starch at the amylose portion but had a higher blue value response at the amylopectin portion than that of normal starch. Although *su1* starch (Fig. 2G) had a lesser amylose content than did *du1* starch as measured by GPC of the native starch, its blue value response was greater than that of the *du1* starch, indicating the amylose portion of *su1* starch bound more iodine than did the amylose portion of *du1* starch. There was no amylose peak observed in the profile of *wx* starch (Fig. 2H), indicating the presence only of amylopectin.

An amylopectin peak and a broad, two-component polysaccharide response in the region normally associated with amylose were noted in the profile of *ae bt1* starch (Fig. 3A). The amylose percentage of *ae bt1* starch (54.9% when calculated according to the GPC data) was overestimated because it included both intermediate and amylose components as noted in the Materials and Methods section. Similarly, the *ae du1* and *du1 su1* starches (Figs. 3B and 3C) contained a large amount of intermediate materials as evidenced by the elevated baseline between Fr. I and Fr. II. High proportions of intermediate fractions in the elution profiles of *ae du* and *du su2* also were reported by Yeh et al (1981). The blue value response of *ae du1* in the region of amylose was the greatest among all the starches, indicating the high amylose content of *ae du1* starch. The elution profiles of *h sh2* and *sh2 bt1* starches (Figs. 3D and 3F) were similar to that of normal starch for polysaccharide response, but the blue value response of *sh2 bt1* starch was higher in the amylopectin region and lower in the amylose region than was that of normal starch. A small response of blue value was noted for amylose in the profile of *h wx* starch (Fig. 3E), suggesting the presence of low molecular-weight (MW) molecules in *h wx* starch. Starches from the double-mutant genotypes of *sh2 wx* and *wx du1* (Figs. 3G and 3H) exhibited elution profiles similar to that of the *wx* starch.

The amylose percentages of starch from the 17 genotypes, determined from Fr. II of GPC profiles of native starches, are summarized in Table I. The normal starch contained 29.9% amylose, which is in the range (25% to 30%) usually found in normal maize starch. The amylose contents of *bt1*, brittle-2 (*bt2*), horny (*h*), and *sh2* starches were within the range of that of normal starch. The single- and double-mutant genotypes containing *ae*, *du1*, and *su1* (except when present with the *wx* gene) exhibited amylose percentages higher than in normal maize, which agrees with previous reports (Yeh et al 1981, Inouchi et al 1983, 1987, Boyer and Liu 1985). The *ae* starch had the highest amylose percentage, 61.3%, among single mutants. The *du1* and *su1* genotypes produced starch with 45.7% and 40.5% amylose, respectively, which were less than those reported by Yeh et al (1981). Boyer and Liu (1985) reported that dent corns produced mutants with more amylose content than did sweet corns. Their findings were in contrast to results shown in the present study in which amylose contents of *du1* and *su1* starches from a dent background were less than those from a sweet corn background studied by Yeh et al (1981). Results from these two research groups and from our work suggest that the genetic

background produces specific variations in the starch properties of maize mutants.

The waxy (*wx*) genotype and the double mutants containing the *wx* gene (*h wx*, *sh2 wx*, and *wx du1*) produced starches consisting of 100% amylopectin, which is in agreement with previous reports that the *wx* gene blocked the accumulation of amylose (Ikawa et al 1981, Yeh et al 1981, Inouchi et al 1983, 1987, 1991, Boyer and Liu 1985). When the *ae* gene was present with another gene, the amylose percentages of the combined mutants increased considerably. The double-mutant combinations of *ae bt1* and *ae du1* resulted in higher amylose percentages (54.9% and 76.2%, respectively) than did normal, *bt1*, or *du1* starches, which is consistent with other results (Boyer and Liu 1985, Inouchi et al 1991). An additive effect was noted when the *ae* and *du1* genes were combined, which resulted in *ae du1* starch having the greatest amylose content among all starches in this study. This effect, however, was not noted by Yeh et al (1981). The *du1 su1* starch also had a large amylose content, 46.2%, which was more than that of the *du1* or *su1* starches. The amylose contents of the *h sh2* and *sh2 bt1* starches were similar to those of their respective single mutants and to normal starch.

**Amylose Percentage Determined by Iodine Affinity (IA)**

The amylose percentages of the 17 starch genotypes determined by using iodine potentiometric titration are presented in Table I. In general, the amylose percentage calculated from IA was similar to that measured by using column chromatography for all genotypes except for those of *du1*, *ae bt1*, *ae du1*, and *du1 su1*. The amylose content derived from the IA method was significantly lower than that calculated from the GPC profile of the native starches of *du1*, *ae bt1*, *ae du1*, and *du1 su1*. These starches, at the same time, showed a great amount of intermediate materials by means of GPC, suggesting that the IA method did not measure the intermediate components of the *du1*, *ae bt1*, *ae du1*, and *du1 su1* starches. The *ae* starch also contained a significant amount of intermediate component, but there was little difference in amylose percentage determined by using the two methods. It is suspected that the branched chains of intermediate materials in *ae* starch were longer than those of *du1*, *ae bt1*, *ae du1*, and *du1 su1* starches; therefore, they were detected as amylose by the IA method. The results suggest that the intermediate materials in starch from each genotype may be different and should not be grouped as similar materials, which conflicts with the earlier belief that intermediate materials

from different starch types were similar (Whistler and Doane 1961). Furthermore, it is hard to predict the amount of intermediate materials simply by using the IA or GPC methods, perhaps because of the diverse nature and, thus, behavior of intermediate materials among the different genotypes. Several research groups demonstrated that the presence of intermediate materials caused a discrepancy in determining amylose content by different methods (Kramer et al 1958, Seckinger and Wolf 1966, Holder et al 1974, Ikawa et al 1978, Yeh et al 1981).

**GPC Properties of Debranched Starches**

The chromatograms of isoamylase-debranched starch from the 17 maize genotypes are presented in Figs. 1A, 4 and 5. The eluted profile was divided into three fractions, and the CL from material collected at the apices of peaks from fraction II (Fr. II) and fraction III (Fr. III) were determined (see Fig. 1A and Table I). Fraction I (Fr. I) was composed mostly of amylose, Fr. II included long B chains of amylopectin, and Fr. III contained A and short B chains of amylopecitn (Hizukuri 1986). The ratio of Fr. III to Fr. II, as shown, may be used as an index of the extent of branching of amylopectin; the higher the ratio, the higher the degree of branching (Biliaderis et al 1981).

The elution profiles of *bt1*, *bt2*, *h*, *sh2*, ae *bt1*, *h sh2* and *sh2 bt1* starches (Figs. 4B ,4C ,4E, 4F, 5A, 5D and 5F) are similar to that of normal starch (Fig. 1A) which exhibited three fractions with clear division. The *ae* and *ae du1* (Figs. 4A and 5B) had similar elution profiles in which Fr. II is hard to separate from Fr. I. The starches of *du1*, *su1* and *du1 su1* (Figs. 4D, 4G and 5C) did not show clear Fr. II in the chromatograms. All of the waxy starches- *wx*, *h wx*, *sh2 wx* and *wx du1* (Figs. 4H, 5E, 5G and 5H)- had similar elution profiles where no Fr. I was present.

Among single mutants, the ratios of Fr. III to Fr. II were similar for normal, *bt1*, *h*, and *wx* starches (ratios ranging from 2.6 to 3.0) and close to those of previous reports (Inouchi et al 1983, 1987, Hizukuri 1985, Ninomya et al 1989). The ratios for *ae* and *bt2* starches were lower than those for normal starch at 1.0 and 2.4, respectively, whereas *du1*, *sh2*, and *su1* starches had ratios higher than 3.8. The *du1* starch had the highest ratio (5.6) among all starches, which was higher than those in previous reports (Inouchi et al 1983, 1987). Among

single mutants, the peak CL for Fr. II ranged from 39 for *wx* to 51 for *du1*, and the peak CL for Fr. III ranged from 13 to 15 except for the *ae* genotype, which had a peak CL of 20 glucose units. These results are consistent with those of Inouchi et al (1987) in that, compared with the amylopectin from normal or *wx* starch, *ae* starch had longer branches and more long B chains (Fr. II) and *du1* starch had more A and short B chains of amylopectin (Fr. III). In addition, the longest peak CL for Fr. II (51 glucose units) among all starches was found in *du1* starch, a feature which is usually associated with *ae* starch.

The ratios of Fr. III to Fr. II for double mutants ranged from 1.2 for *ae du1* starch to 4.0 for *du1 su1* starch. When the *ae* gene was combined with the *bt1* or *du1* gene, the double-mutant combinations (*ae bt1* and *ae du1*) showed ratios and peak CL similar to those of *ae* starch, suggesting that the *ae* gene is more important than are the *bt1* and *du1* genes in determining the fine structure of amylopectin of *ae bt1* and *ae du1* starches. The starches of *du1 su1* and *sh2 bt1* possessed longer peak CL at Fr. II than did the normal starch. The results of the ratios of Fr. III to Fr. II for *bt1* (3.0), *h* (2.8), *sh2* (3.8), *h sh2* (2.7) and *sh2 bt1* (3.7) suggest that the *h* gene is more important than the *sh2* gene and that the *sh2* gene is more important than is the *bt1* gene in determining the Fr. III/Fr. II ratios of *h sh2* and *sh2 bt1* starches. However, *bt1*, *h*, and *sh2* starches had similar peak CL at both Fr. II and Fr. III.

The starches with high amylose content (*ae*, *ae bt1* and *ae du1*) had low ratios of Fr. III to Fr. II, indicating low degrees of branching. They also had long peak CL at both Fr. II and Fr. III. After being debranched by isoamylase, the starch genotypes exhibited chromatograms that were different from each other, demonstrating that the chromatograms of debranched starches are characteristic of the starch genotype in addition to providing fine structure information of amylopectin.

### Estimation of Intermediate Fraction from GPC Profiles

Recently, South et al (1991) reported that a more accurate amylose content can be obtained from GPC data of debranched starches than by using IA or GPC of native starch. The IA procedure gave reliable estimates of amylose content only when amylopectin had a normal low iodine-binding capacity. When anomalous types of amylopectin with extended external chains were present, false high values for amylose contents were obtained.

Similarly, high amylose values were calculated from the elution profile of native starch because the anomalous amylopectin (intermediate materials) was included in the amylose fraction. South et al (1991) estimated that the low-MW (anomalous) amylopectin could be calculated as the difference between Fr. II of the native starch and Fr. I of the debranched starch GPC elution profiles. Accordingly, the intermediate materials corresponding to the anomalous amylopectins in the present study were quantified (Table I).

The *ae*, *du1*, *su1*, *ae bt1*, *ae du*1, and *du1 su1* starches had large amounts of intermediate materials as also qualitatively noted from GPC of the native starches. When starches containing the *wx* gene were excluded, the amounts of intermediate materials were weakly correlated with the peak CL of Fr. II from GPC of debranched starches ($r = 0.73$, $P < 0.01$). These results support the statement of South et al (1991) that the intermediate materials are a type of low-MW amylopectin with extended chains and, through the action of the *ae* gene, can give enhanced IA values for starches. The *du1* gene, in contrast, increased amylose and intermediate materials contents but did not increase iodine-binding capacities, which also is consistent with the observation of South et al (1991). The peak CL of Fr. II from GPC of debranched *du1* starch was longer than that of normal starch, which, according to South et al (1991), should cause a marked difference between the IA value and the amylose value as measured in Fr. I of debranched starch. The discrepancy in amylose content between the IA method and that calculated from Fr. I in GPC of the debranched starch was observed for *ae* starch, but not for *du1* starch. The *ae bt1* and *ae du1* starches also showed little difference between the two values. The results indicate that other factors besides chain length also may be important in controlling the binding between iodine and starch molecules.

**Scanning Electron Micrographs of Starch Granules**

The morphology of native starch granules from the 17 maize genotypes was captured by means of scanning electron microscopy (SEM) (Figs. 6-8). All micrographs were taken at a magnification of X 1,000. Table II summarizes the size distribution of starch granules from different genotypes.

Normal starch granules had typical angular and spherical shapes (Fig. 6A), and the diameters of granules varied from 6 to 17 μm, with an average of 11.6 μm (Table II). Starch granules in the floury

endosperm are more rounded, whereas those in the horny endosperm are more angular (Watson 1967). The *ae* starch granules were generally spherical with some elongated shapes and had smooth outlines in contrast to the facets of normal starch, which suggests a loose arrangement of granules in the endosperm of the *ae* genotype (Wolf et al 1964) (See Fig. 6B). The appearance of irregular and elongated shaped granules in *ae* starch is well documented (Deatherage et al 1954, Wolf et al 1964, Sandstedt et al 1968, Hall and Sayre 1970, Banks et al 1974, Boyer et al 1976, Gallant and Bouchet 1986). The average size of *ae* starch granules, 7.0 μm, was smaller than that of normal starch, which is in agreement with previous reports (Wolf et al 1964, Boyer et al 1976, Cluskey et al 1980). Granules of *bt1*, *du1*, *sh2*, and *su1* starches (Figs. 6C, 6E, 7B and 7C, respectively) were generally smaller than those of normal starch (see Table II). The granular size (range and average) for *bt2* and *wx* starches (Figs. 6D and 7D, respectively) was similar to that for normal starch. The *h* starch granules were large with a smooth surface (Fig. 7A), and the average size (13.8 μm) was the largest among all genotypes. The small granules of *su1* starch (Fig. 7C) were agglomerated with distinct divisions, which first was observed by Sandstedt et al (1968).

The morphology of the starch granules is influenced by the presence of another mutant gene, and additional complexity is created by the interactions of the two genes. When the *ae* gene was combined with the *bt1* or *du1* gene, abnormally shaped granules, characteristic of *ae* starch, were found (Fig. 7E and F). Many researchers have suggested that, as the amylose content increases, the irregularity of starch granule shape increases (Wolf et al 1964, Banks et al 1974, Gallant and Bauchet 1986). On the other hand, Boyer et al (1977) suggested that high amylose content was not a requirement for abnormal starch granule formation in genotypes containing the *ae* gene and that the abnormal granule was indeed a result of a high ratio of linearity to branching in the total starch. No conclusion regarding this relationship can be drawn from the present results.

One of many examples illustrating the effects between two genes comes from *du1 su1* starch (Fig. 8A). The *du1 su1* starch looked similar to *su1* starch with respect to the aggregations of small granules, but the size of the granules (6.9 μm) was between those of *du1* (7.8 μm) and *su1* (5.4 μm) starches. In contrast, the average size of *ae bt1* was slightly smaller than that of *ae* and *bt1* starches, suggesting a combined effect on granular size. Similarly, *sh2 bt1* starch had an average granular size smaller than that of either *sh2* or *bt1* starches. The

*h* gene seemed to have more influence than did the *sh2* gene relative to the size of the starch granules when the *h sh2* starch was examined (Fig. 8B). The *wx* gene seemed to be more important than *du1*, *sh2*, and, perhaps, *h* genes in the *h wx*, *sh2 wx* and *wx du1* genotypes (Figs. 8C, E, and F, respectively) relative to the morphology and size of the starch granules. The granular size of the *h sh2* starch (11.2 μm) (Fig. 8B) was between that of *h* and *sh2* starches. Banks et al (1974) and Cluskey et al (1980) reported an inverse relationship between the size of starch granules and the apparent amylose content of amylomaize starches with different amylose contents and normal maize starch. Only a weak relationship ($r = -0.29$, $P < 0.01$) was found in the present study between the apparent amylose content (from IA) and the average granular size of the 13 maize genotypes (excluding waxy starches).

## CONCLUSION

By using GPC and SEM, the structure and morphology of 17 maize endosperm mutant genotypes of Oh43 inbred were elucidated. The starches containing the *ae* gene showed high amylose content, low degree of branching, long branch chain length of amylopectin, and high intermediate materials content, suggesting the important role of the *ae* gene in determining the fine structure of starch. The *du1* starch had increased amylose and intermediate materials contents with the longest peak CL at Fr. II of debranched amylopectin among all starches. The *su1* and *du1 su1* genotypes also produced starches with higher amylose and intermediate materials contents than did the normal genotype. The starches of *wx*, *h wx*, *sh2 wx*, and *wx du1* had similar structures as measured by GPC of native and isoamylase-debranched starches although minor differences existed. In general, starches from each genotype were different from each other, and the combination of two genes created additional variations in structure and shape of starch granules. How these variations affect the starch properties needs further investigation.

### Acknowledgment

The authors thank Mr. Luzhen Shen for technical assistance.

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Table I. Percentage compositions of polysaccharides[ab] and chain length distribution[b] of starches from 17 mutant genotypes of Oh43 inbred

| Genotype | GPC, native | | IA[e] | GPC, debranched | | | | | | Intermediate materials[j] % |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | CL[i] at peak of | | |
| | Fr. I[c] % | Fr. II[d] % | % | Fr. I[f] % | Fr. II[g] % | Fr. III[h] % | Fr. III / Fr. II | Fr. II[g] | Fr. III[h] | |
| normal | 70.1 (0.6) | 29.9 (0.6) | 27.8 (1.1) | 26.7 (0.3) | 19.2 (0.1) | 54.1 (0.1) | 2.8 (0) | 43 (2) | 15 (1) | 3.2 |
| *ae* | 38.7 (0.1) | 61.3 (0.1) | 56.4 (1.1) | 46.0 (0.6) | 27.2 (0.2) | 26.8 (0.4) | 1.0 (0) | 48 (1) | 20 (1) | 15.3 |
| *bt1* | 73.3 (1.3) | 26.7 (1.3) | 23.2 (0.8) | 24.9 (0.7) | 18.9 (0.6) | 56.2 (0.1) | 3.0 (0.1) | 42 (3) | 14 (0) | 1.8 |
| *bt2* | 73.2 (0.6) | 26.8 (0.6) | 26.9 (0) | 24.7 (0.6) | 22.1 (0.3) | 53.2 (0.3) | 2.4 (0) | 41 (1) | 15 (0) | 2.1 |
| *du1* | 54.3 (0.1) | 45.7 (0.1) | 31.4 (0.4) | 30.5 (0.2) | 10.6 (0.4) | 58.9 (0.3) | 5.6 (0.2) | 51 (1) | 14 (0) | 15.2 |
| *h* | 71.3 (2.5) | 28.7 (2.5) | 26.4 (0.8) | 28.1 (0.1) | 19.2 (1.0) | 52.7 (1.1) | 2.8 (0.2) | 41 (1) | 15 (1) | 0.6 |
| *sh2* | 72.6 (0.6) | 27.4 (0.6) | 28.8 (0) | 30.1 (0.6) | 14.7 (0) | 55.2 (0.6) | 3.8 (0.1) | 40 (3) | 13 (1) | -2.7 |
| *su1* | 59.5 (0.4) | 40.5 (0.4) | 37.4 (1.5) | 31.2 (0.6) | 12.3 (0.1) | 56.5 (0.7) | 4.6 (0.1) | 41 (4) | 14 (0) | 9.3 |
| *wx* | 100.0 (0) | 0 (0) | 0 (0) | 0 (0) | 27.7 (0.4) | 72.3 (0.4) | 2.6 (0) | 39 (1) | 15 (1) | 0 |
| *ae bt1* | 45.1 (0.1) | 54.9 (0.1) | 30.5 (0.1) | 32.4 (0) | 24.5 (0.4) | 43.1 (0.4) | 1.8 (0) | 49 (2) | 16 (1) | 22.5 |
| *ae du1* | 23.8 (0.1) | 76.2 (0.1) | 57.8 (1.4) | 57.3 (0.1) | 19.8 (0.1) | 22.9 (0.1) | 1.2 (0) | 49 (0) | 19 (1) | 18.9 |

Table I (continued)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *du1 su1* | 53.8 | 46.2 | 39.9 | 34.5 | 13.0 | 52.5 | 4.0 | 47 | 13 | 11.7 |
| | (0.4) | (0.4) | (0.7) | (1.3) | (0.4) | (0.9) | (0.1) | (1) | (0) | |
| *h sh2* | 70.5 | 29.5 | 25.4 | 26.4 | 20.0 | 53.6 | 2.7 | 44 | 14 | 3.1 |
| | (0.4) | (0.4) | (0.1) | (0.2) | (0.2) | (0.4) | (0) | (2) | (0) | |
| *h wx* | 100.0 | 0 | 0 | 0 | 29.2 | 70.8 | 2.4 | 43 | 16 | 0 |
| | (0) | (0) | (0) | (0) | (0.1) | (0.1) | (0) | (2) | (1) | |
| *sh2 bt1* | 71.7 | 28.3 | 27.6 | 27.7 | 15.4 | 56.9 | 3.7 | 49 | 13 | 0.6 |
| | (0.2) | (0.2) | (0.4) | (0.2) | (0) | (0.1) | (0) | (1) | (0) | |
| *sh2 wx* | 100.0 | 0 | 0 | 0 | 30.6 | 69.4 | 2.3 | 42 | 13 | 0 |
| | (0) | (0) | (0) | (0) | (0) | (0) | (0) | (2) | (1) | |
| *wx du1* | 100.0 | 0 | 0 | 0 | 26.3 | 73.7 | 2.8 | 39 | 15 | 0 |
| | (0) | (0) | (0) | (0) | (0.4) | (0.4) | (0) | (0) | (0) | |

[a]The division of each fraction (Fr.) is described in the section on Materials and Methods.

[b]Values are the average of two separate determinations. Standard deviations (SD) are listed immediately below.

[c]Amylopectin.

[d]Amylose and intermediate materials.

[e]Amylose percentage calculated from iodine affinity (IA).

[f]Amylose.

[g]Long B chains of amylopectin.

[h]A and short B chains of amylopectin.

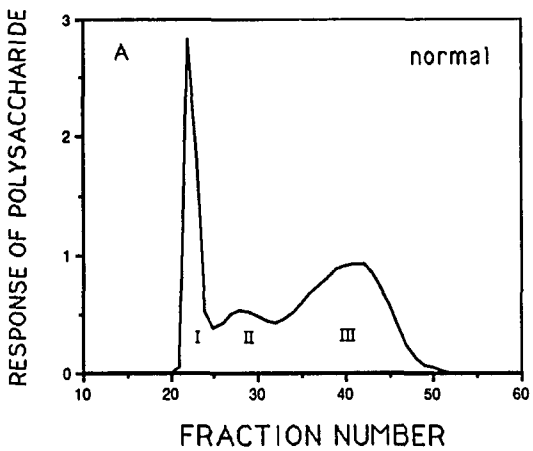
[i]Average chain length of isoamylase-debranched starch measured at the apex of the peak from each fraction. Expressed as number of glucose units.

[j]Calculated as the difference between Fr. II of the native starch and Fr. I of the debranched starch GPC elution profiles.

Table II. Size distribution of starch granules of 17 maize genotypes from Oh43 inbred measured from scanning electron micrographs

| Genotype | Range (μm) | Average ± SD[a] (μm) |
|---|---|---|
| normal | 6-17 | 11.6 ± 4.5 |
| *æ* | 4-11 | 7.0 ± 2.1 |
| *bt1* | 4-9 | 6.1 ± 1.3 |
| *bt2* | 6-19 | 10.8 ± 3.4 |
| *du1* | 4-11 | 7.8 ± 2.1 |
| *h* | 8-22 | 13.8 ± 3.6 |
| *sh2* | 2-9 | 6.3 ± 1.9 |
| *su1* | 2-10 | 5.4 ± 2.6 |
| *wx* | 6-14 | 10.3 ± 2.6 |
| *ae bt1* | 3-10 | 5.6 ± 1.6 |
| *ae du1* | 4-14 | 7.4 ± 2.3 |
| *du1 su1* | 3-12 | 6.9 ± 2.6 |
| *h sh2* | 6-23 | 11.2 ± 3.8 |
| *h wx* | 3-20 | 11.0 ± 4.4 |
| *sh2 bt1* | 3-9 | 5.4 ± 1.6 |
| *sh2 wx* | 4-16 | 10.2 ± 3.7 |
| *wx du1* | 4-19 | 10.2 ± 3.6 |

[a]Average ± SD of 30 starch granules, 15 each from two micrographs.



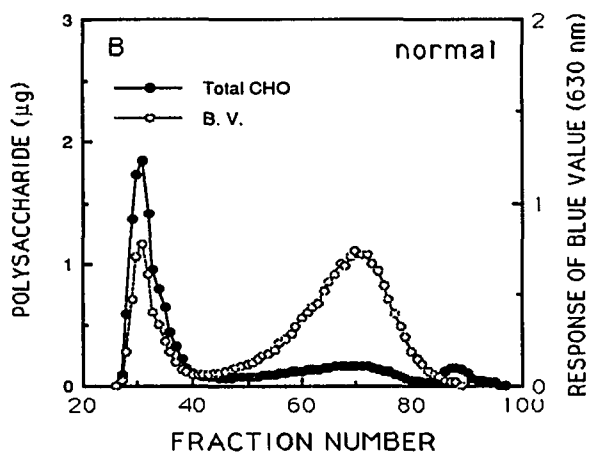




Figure 1. GPC elution profile of Oh43 starch. A. isoamylase-debranched starch. Fraction (Fr.) I was composed of amylose. Fr. II included long B chains of amylopectin. Fr. III contained A and short B chains of amylopectin. B. native starch.

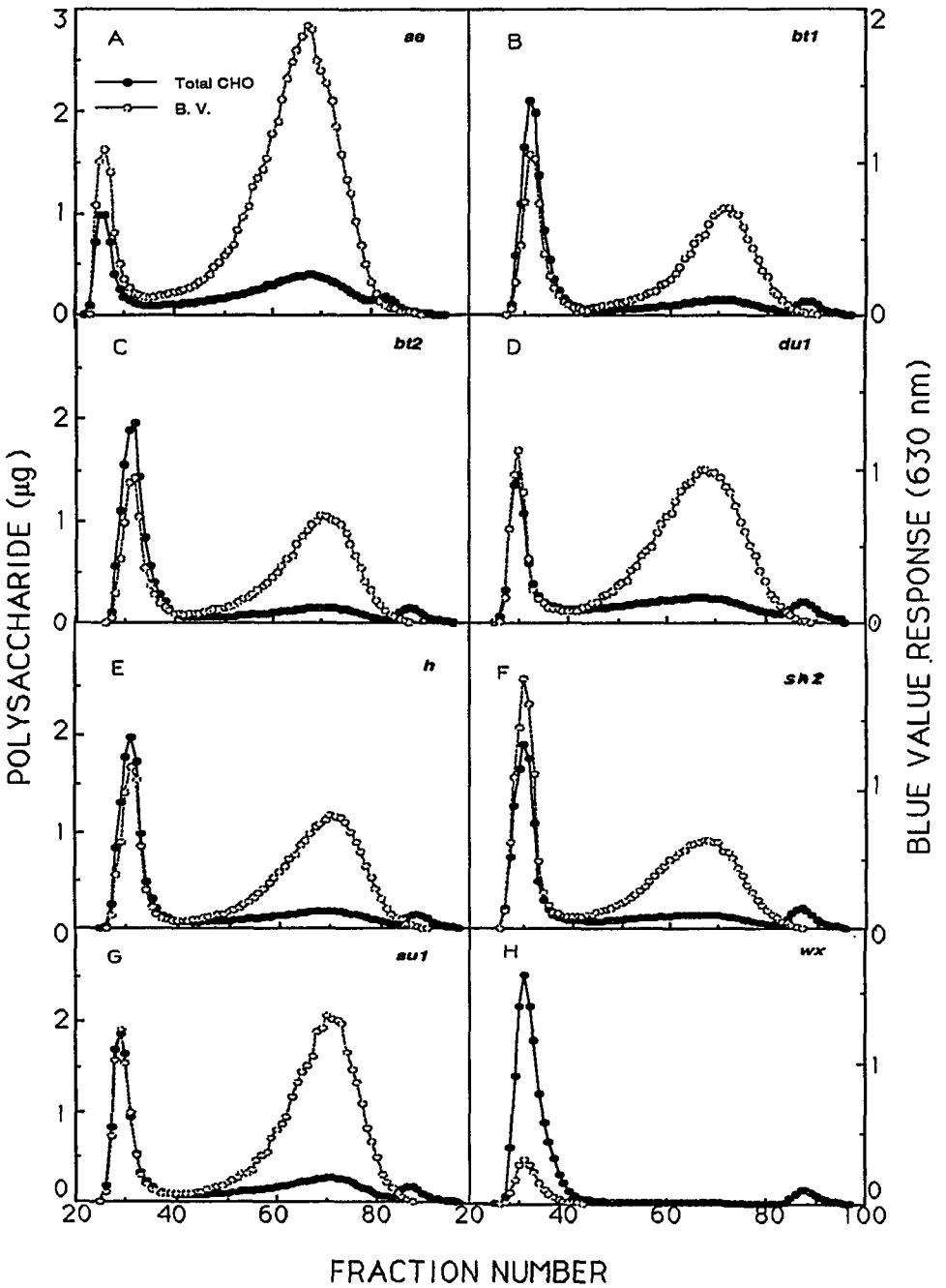



Figure 2. Elution profile of native starch from single mutant genoytypes on Sepharose CL-2B. A. *ae* starch, B. *bt1* starch, C. *bt2* starch, D. *du1* starch, E. *h* starch, F. *sh2* starch, G. *su1* starch, H. *wx* starch. BV = blue value response; total CHO = total polysaccharide value.

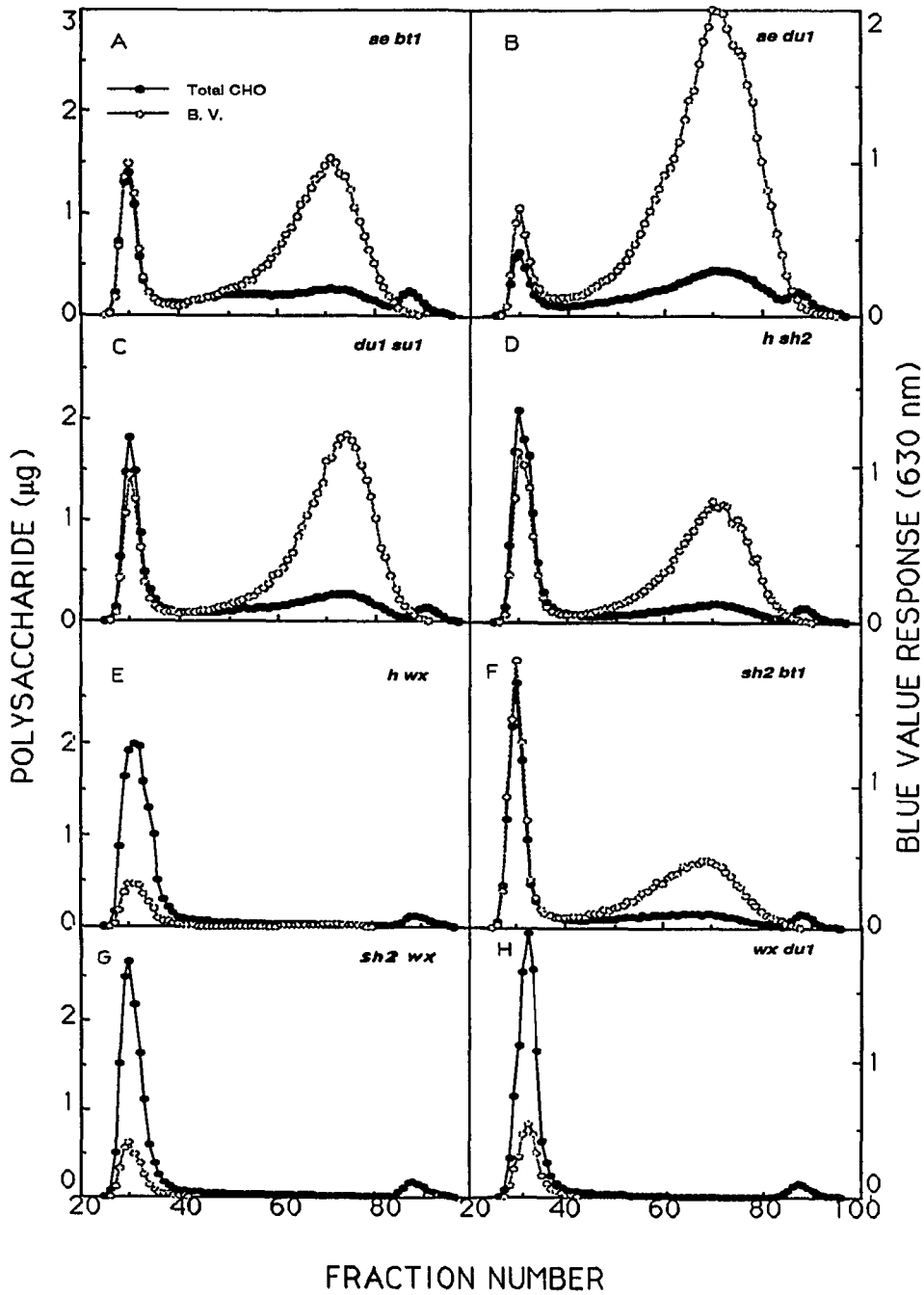



Figure 3. Elution profile of native starch from double mutant genoytypes on Sepharose CL-2B. A. *ae bt1* starch, B. *ae du1* starch, C. *du1 su1* starch, D. *h sh2* starch, E. *h wx* starch, F. *sh2 bt1* starch, G. *sh2 wx* starch, H. *wx du1* starch. See Fig. 2 for abbreviation of symbols.

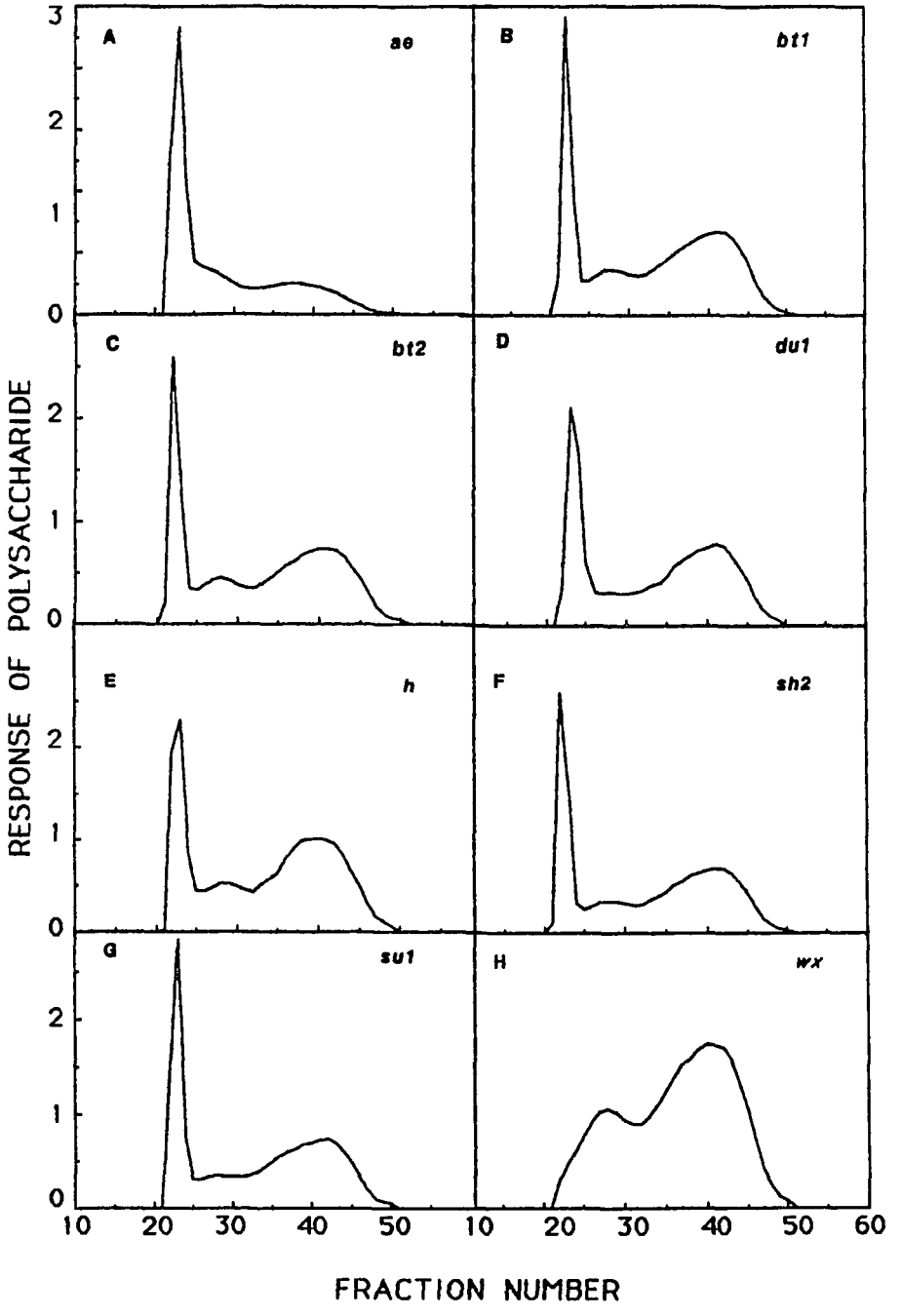



Figure 4. GPC elution profile of isoamylase-debranched starch from single mutant genoytypes on Bio-Gel P-6. A. *ae* starch, B. *bt1* starch, C. *bt2* starch, D. *du1* starch, E. *h* starch, F. *sh2* starch, G. *su1* starch, H. *wx* starch.

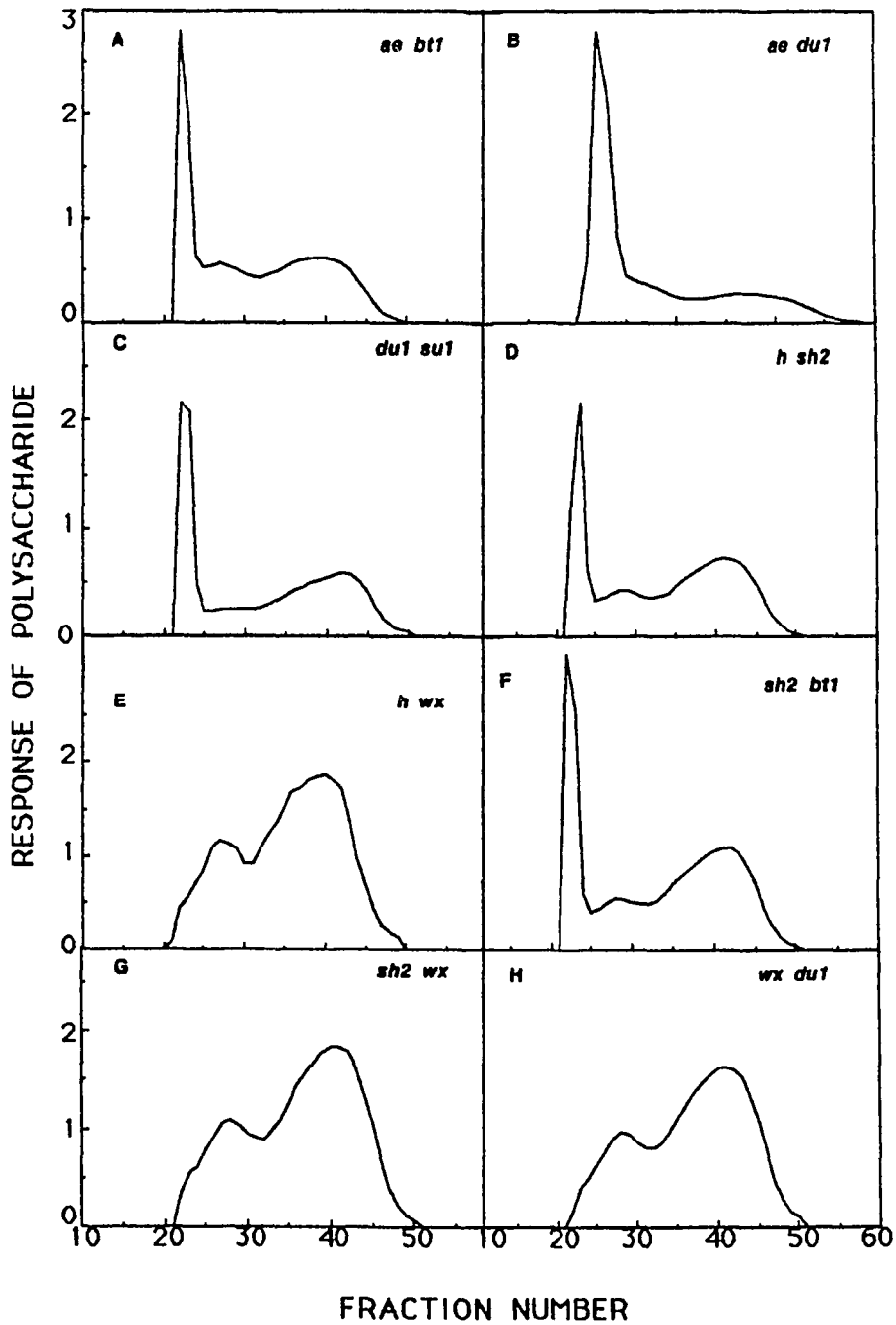
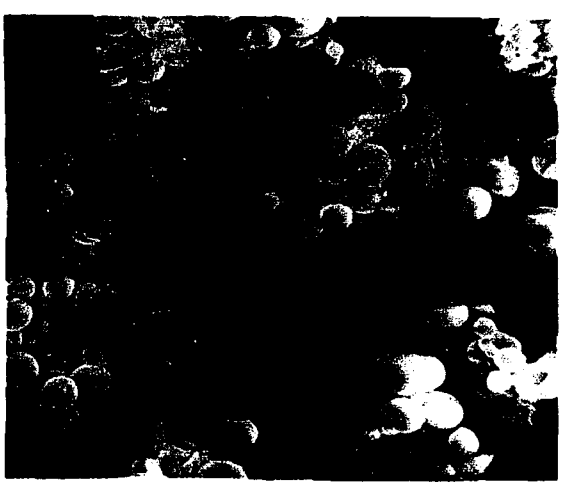



Figure 5. GPC elution profile of isoamylase-debranched starch from double mutant genoytypes on Bio-Gel P-6. A. *ae bt1* starch, B. *ae du1* starch, C. *du1 su1* starch, D. *h sh2* starch, E. *h wx* starch, F. *sh2 bt1* starch, G. *sh2 wx* starch, H. *wx du1* starch.

Figure 6. SEM of maize starch granules (1,000 X). A. normal starch, B. *ae* starch, C. *bt1* starch, D. *bt2* starch, E. *du1* starch.



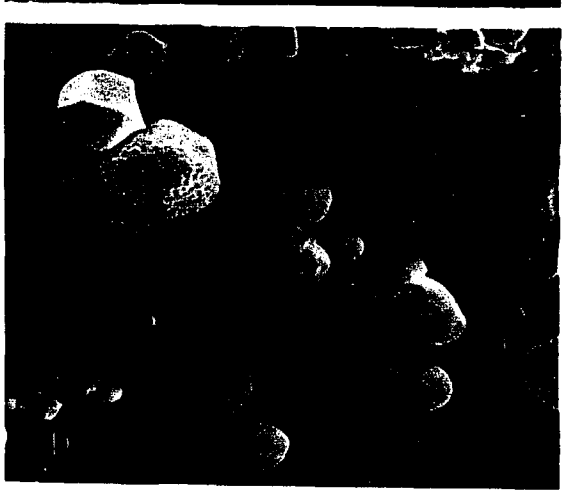


Figure 7. SEM of maize starch granules (1,000 X). A. *h* starch, B. *sh2* starch, C. *su1* starch, D. *wx* starch, E. *ae bt1* starch, F. *ae du1* starch.

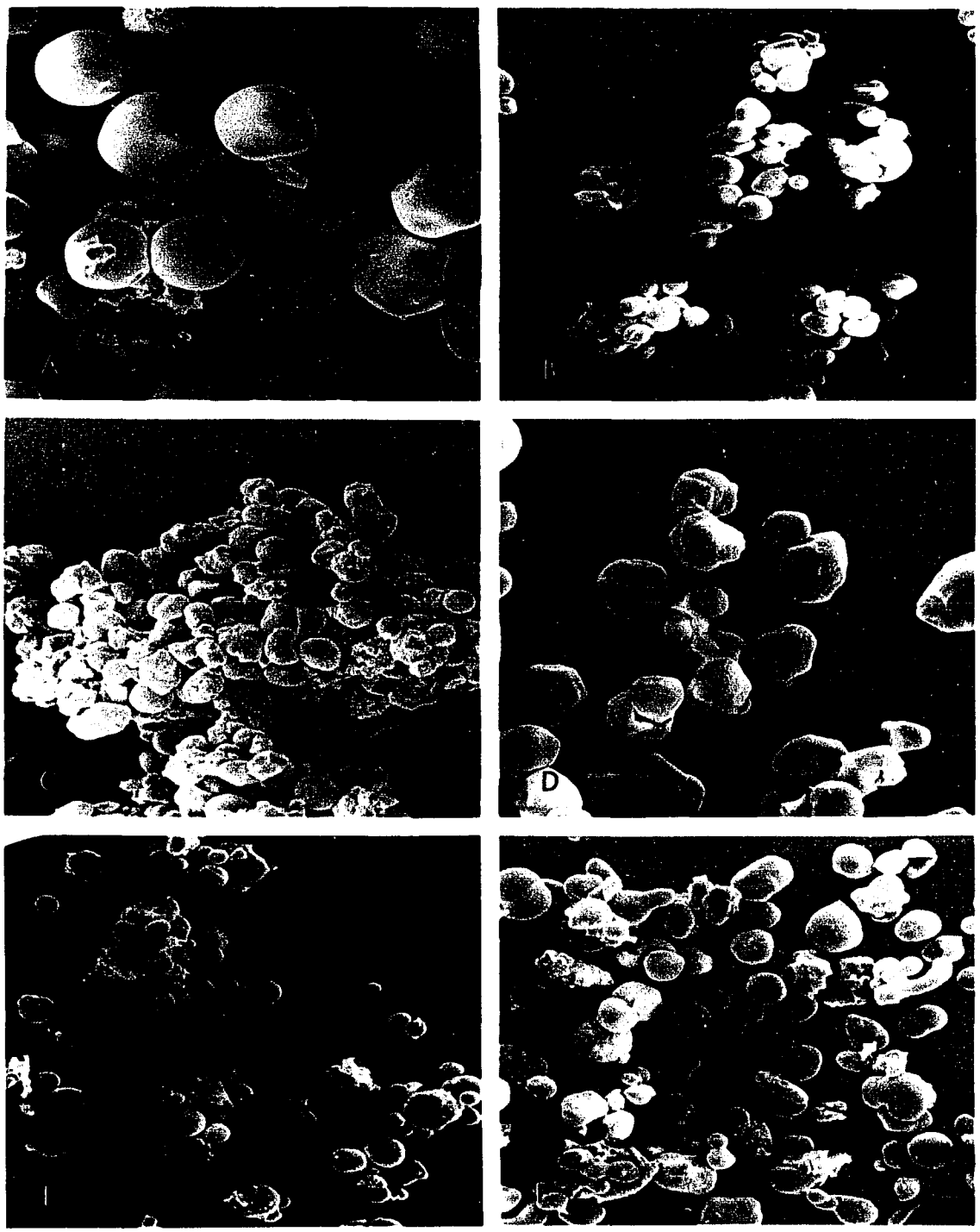
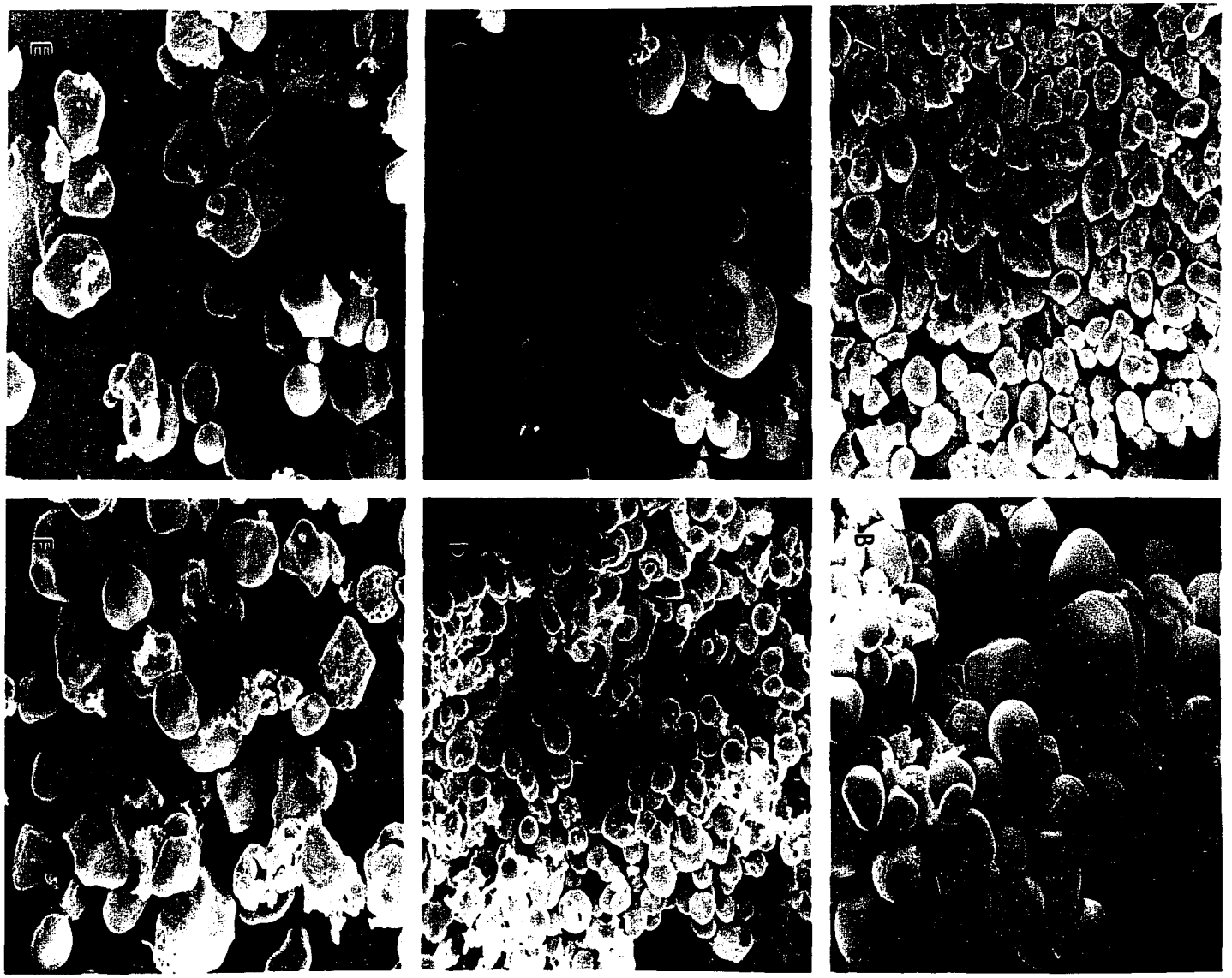

A
B
D

Figure 8. SEM of maize starch granules (1,000 X). A. *du1 su1* starch, B. *h sh2* starch, C. *h wx* starch, D. *sh2 bt1* starch, E. *sh2 wx* starch, F. *wx du1* starch.



# PAPER III

## PHYSICOCHEMICAL PROPERTIES OF STARCHES FROM MUTANT GENOTYPES OF THE OH43 INBRED LINE

# PHYSICOCHEMICAL PROPERTIES OF STARCHES FROM MUTANT GENOTYPES OF THE OH43 INBRED LINE[1]

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[1]This is Journal Paper no. J.-14977 of the Iowa Agriculture and Home Economics Experimental Station at Iowa State University, Ames, IA (Project no. 2568 and 2778), a joint contribution with the Field Crops Research Unit, USDA-ARS.

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## ABSTRACT

The physicochemical properties of 17 mutant genotypes of the inbred line Oh43 were investigated to clarify the relations between structural characteristics and physicochemical properties, and among the properties themselves. These physicochemical properties included blue value (BV), maximum absorbance wavelength ($\lambda_{max}$), limiting viscosity number ([η]), swelling power and solubility at 85°C, and percentage transmittance (%T) of starch paste at 650 nm. Pasting properties were determined by means of Brabender viscoamylography; gel strength, by texture analysis; and thermal properties, by differential scanning calorimetry. Amylose content was the most important structural characteristic affecting the physicochemical properties of starch. Amylose content was significantly ($P < 0.01$) correlated with BV ($r = 0.96$) and $\lambda_{max}$ ($r = 0.81$) and was negatively correlated with [η] ($r = -0.83$) , %T ($r = -0.88$), swelling power ($r = -0.86$), and peak viscosity ($r = -0.97$). Other structural characteristics, including intermediate material content, average chain length of debranched amylopectin, and ratio of long B chains to short B chains plus A chains of amylopectin, were weakly correlated with properties. Some significant correlations were found among properties, including BV , %T, swelling power, and peak viscosity.

## INTRODUCTION

Starch is the most important reserve carbohydrate in the plant world and is used widely in the food processing and other industries. Because of a growing interest in using native starches in the modern food industry, there is a demand for new alternative sources of starch.

Maize is unique among higher plants in its number of genetically accessible mutants and in the degree to which it had been characterized. Much work has helped determine starch structures of maize mutants (Boyer et al 1976, Ikawa et al 1978, 1981, Yeh et al 1981, Inouchi et al 1983, 1987, Boyer and Liu 1985, Fuwa et al 1987), yet little emphasis has focused on the physicochemical properties of these mutants. In general, blue value, iodine absorption spectrum, and ß-amylolysis limit are used to distinguish amylopectin from amylose in mutant starches (Boyer et al 1976, Yeh et al 1981, and Fuwa et al 1987). No other physicochemical properties, except limiting viscosity number, have been studied to characterize the starches of maize mutants.

In a previous study (Wang et al in press), 17 maize mutant genotypes of the Oh43 inbred line were characterized for their starch fine structures. The objectives of the present study were to examine the physicochemical properties of starches from 17 maize genotypes and to clarify the relations between these properties and starch structures.

# MATERIALS AND METHODS

## Materials

Mature kernels of Oh43 inbred and its single and double mutants (Table I) were harvested from a summer nursery near Ames, Iowa, in 1991. Development of the genotypes and sampling and storing of the kernels were described previously (Wang et al 1992).

Starches were isolated by using a wet-milling procedure (Steinke and Johnson 1991). The isolated starches were purified by treating with 5 volumes of 0.2 M sodium chloride-toluene (5:1, v/v) at least five times, and after each treatment the starch granules were sedimented by centrifugation. The final sediment was washed three times with distilled water and dried at 45°C for 24 hr. All the starches used in this study were obtained from a single isolation and purification. The amount of starch isolated depended upon the amount of available corn of each genotype, but ranged from 5 to 100 g.

## Blue value (BV) and Iodine-absorption Spectrum

The blue value (BV) was determined according to Gilbert and Spragg (1964), and the same sample was used to measure the wavelength of maximum absorption ($\lambda_{max}$) from 700 to 500 nm. Two separate determinations were done on each starch genotype.

## Limiting Viscosity Number [$\eta$]

The limiting viscosity number [$\eta$] (mL/g) was determined with an Ostwald viscometer at 22.5°C by following the method of Myers and Smith (1964) except that starch was dissolved in 1 N KOH. The flow time was 133 sec for 1 N KOH. Three separate measurements were performed for each starch type.

## Swelling Power and Solubility

Swelling power and solubility were performed at 85°C according to Leach et al (1959), but with these modifications. Starch (0.5 g dry-weight basis [dwb] for non-waxy starches and 0.25 g [dwb] for waxy starches)

was added to 8 mL distilled water in a 85°C water bath for 30 min and mixed with a stirring bar at moderate speed. The swelling power of amylose-extender (*ae*) and of amylose-extender dull1 (*ae dul*) starches was measured at 100°C because of their poor swelling at 85°C from a preliminary test. After 30 min of heating, the stirring bar was removed and rinsed with distilled water, and additional water was added to make the total water weight 10.0 g. The starch paste was centrifuged at 1,500 X g for 20 min, after which 5 mL of supernatant was pipetted into a weighing dish and dried at 120°C for 2 hr to determine the soluble content. The remaining supernatant was carefully removed by suction and weighed to determine the amount of water absorbed by starch granules. Swelling power (%) was calculated with correction for solubles. The results are the average of two determinations.

Both swelling power and solubility techniques have been used to reflect the arrangement of molecules within the starch granules. A starch with an extensive and strongly bonded structure, such as high amylose starch, exhibits restricted swelling and dispersion. In contrast, waxy starch shows unrestricted swelling.

### Light Transmittance (%T)

Light transmittance of starch solutions in water (1%, w/w) was determined according to the method of Craig et al (1989). The solutions were heated in a boiling water bath and stirred for 30 min. After solutions were cooled to room temperature, the percentage transmittance (%T) at 650 nm was measured against a water blank by using a Hitachi U-2000 spectrophotometer. The results are the mean of two replicate samples.

Clarity of a starch paste is one of the important attributes in food systems and is a characteristic of starch source. Craig et al (1989) used the percentage transmittance (%T) as a measure of clarity. They proposed that when a beam of light passes through the native starch granules, most of the light is reflected back and the starch seems white and opaque because the surface of the granule is larger than the wavelength of light.

### Pasting Properties

Pasting charactersitics of starch suspensions (6% w/w, dwb), with the pH adjusted to 5.5, were measured by using the Brabender Viscoamylograph (C.W. Brabender Instruments, Inc., S. Hackensack, NJ)

equipped with a 700 cmg sensitivity cartridge operated at a bowl speed of 75 rpm. The temperature was raised from 30°C to 95°C at a rate of 1.5°C/min, maintained at 95°C for 30 min, and lowered to 50°C at the same rate and held for 30 min. Because of a limited sample size, only one measurement was made on selected starch genotypes.

### Gel Strength

The starch paste prepared with the Brabender Viscoamylograph was used to measure the gel strength after storing for 1 and 7 days at 4°C. The paste from each starch genotype was poured into four aluminum dishes (27 mm i.d. x 27 mm), which were taped around the rims with aluminum foil to increase the depth of the gel to 1 cm above the rims (Takahashi et al 1989). The gel strength of the starch paste was measured at five different locations on each gel sample, and two gel samples per starch type were measured after 1 or 7 days of storage by using a Voland Texture Analyzer (Texture Technologies, Scarsdale, NY), as previously described (Wang et al 1992).

### Thermal Properties

The thermal properties of starches were determined, according to the method of Wang et al (1992 ), by using a Perkin-Elmer DSC 7 analyzer equipped with a thermal analysis data station (Perkin-Elmer Corp., Norwalk, CT). Three determinations were done on each starch genotype.

### Statistical Analyses

Data of physicochemical properties among 17 maize genotypes were analyzed by using the SAS program (SAS Institute 1990), and correlations were computed among properties and structural characteristics previously characterized (Wang et al in press). Least significant differences were computed at a significance level of $P < 0.05$.

## RESULTS AND DISCUSSION

### General Physicochemical Properties

The general properties of 17 mutant genotypes of the Oh43 inbred line and their least significant differences are summarized in Table I. Normal starch had an amylose content of 26.7%, a blue value (BV) of 0.371, and a maximum absorbance ($\lambda_{max}$) at 608 nm. The starches with higher amylose content, i.e., *ae* (46.0%), *du1* (30.5% ), *ae du1* (57.3%), and *du1 su1* (34.5%) (Wang et al in press), had significantly greater BVs than did normal starch, with the exception of *ae bt1* starch, which had an amylose content of 32.4% and a BV of only 0.306. The $\lambda_{max}$ of nonwaxy starches ranged from 598 nm to 617 nm. All waxy starches exhibited similar blue values and maximum absorbance values, except *h wx* starch, which had a significantly ($P < 0.01$) greater BV and $\lambda_{max}$ than did the other waxy starches. According to previous results (Wang et al in press), starch from *h wx* contained little amylose, as shown on the elution profile from gel permeation chromatography (GPC), which might account for the different BV and $\lambda_{max}$ of *h wx* starch. The relatively small BV and $\lambda_{max}$ of *ae bt1* starch might be caused by the presence of either a great amount of intermediate material (22.5%) (Wang et al in press) or structural differences. Except for *sh2* starch, the starches with amylose contents greater than 30% (*ae*, *du1*, *su1*, *ae bt1*, *ae du1*, and *du1 su*1) had limiting viscosity numbers, [η], of less than 200 mL/g, whereas waxy starches had [η]s greater than 250 mL/g. Again, *h wx* starch showed different [η] from those of other waxy starches. The standard deviation of the [η] of all starches was less than 2. The shear rate was not determined nor were the [η] values verified to be in the Newtonian region.

All starches in this study were heated at 85°C to measure swelling power and solubility, with the exception of *ae* and *ae du1* starches, which were heated at 100°C because of their high amylose content and their consequent resistance to gelatinization (Wang et al in press). Even when heated at 100°C, *ae* and *ae du1* starches exhibited less swelling power (9.7% and 11.9%, respectively) than did normal starch (15.6%) heated at 85°C. The *ae* and the *ae du1* starches showed significantly ($P < 0.01$) higher solubility than did normal starch, presumably because the amylose, having a small molecular weight, leached out at 100°C. In contrast, the waxy starches showed unrestricted swelling and great solubility, likely because of the absence of a network structure

from amylose molecules to hold the starch molecules together. The *h wx* starch had less swelling power and solubility than did other waxy starches, results corresponding to those for BV, $\lambda_{max}$, and [η], and thus suggesting the presence of associative bonding in *h wx* starch.

Results of the percentage transmittance (%T) measurements differed greatly among samples. The *ae* starch had the smallest %T (0.62), and *wx* starch the greatest (56.83) among all starches. In general, nonwaxy starches had a %T smaller than 10, whereas waxy starches had a %T greater than 30. Craig et al (1989) proposed that the disassociation of starch molecules during gelatinization diminished the reflecting ability of a starch granule and thus increased the %T of a starch paste. Swinkles (1985) suggested that lipid present naturally in the starch granules affects the clarity of starch pastes and that the presence of amylose-lipid inclusion compounds makes starch pastes opaque or cloudy. In the present study, among waxy starches, which generally contain almost no lipid, there were different %T values, and therefore factors other than starch granular integrity and lipid content may also be important. Results from the present study confirm the hypothesis of Craig et al (1989), which states that the more starches swell, the greater the %T will be. Waxy starches, which exhibited unrestricted swelling, had greater %T than did nonwaxy starches. Moreover, high amylose starches, which evidenced restricted swelling and dispersion, also evidenced relatively small %T.

**Pasting Properties**

The pasting properties of starches from some mutant genotypes are listed in Table II. Because of the limited sample size, these tests were not performed on all starches. All waxy starches exhibited a high peak viscosity, followed by a rapid decrease in viscosity due to the thinning effect from mechanical shearing, and their viscosities after cooling to 50°C (set-back viscosities) were less than the viscosity of normal starch. Their pasting temperatures also were lower than the temperature of normal starch. The high content of amylose in *ae* starch restricted the swelling of starch granules, and for this reason no viscosity was observed under the conditions of the test. The *du1* starch had a low peak viscosity, which increased slightly during both heating and cooling. The pasting pattern of *du1* starch was attributed to its relatively high amylose content (30.5%) (Table I), which reinforced the bonding force within starch granules by the long chains of amylose (Howling

1980) and resulted in a high pasting temperature (90.3°C) for *du1* starch. Normal, *h*, and *h sh2* starches had similar pasting patterns.

Schoch and Maywald (1968) classified four types of starch, according to pasting behavior. Type A starches swelled unrestrictedly under cooking and were unstable towards shearing forces while heating. Waxy starches in the present study, *wx*, *h wx*, *sh2 wx*, and *wx du1*, belonged to Type A, which were high-swelling starches. The normal, *h*, and *h sh2* starches were Type B starches, which did not swell extensively and were not fragile towards mechanical shearing. They showed high set-back viscosities, which indicate a degree of reassociation during cooling. Type C starch, a restricted swelling starch, showed no pasting peak but, rather, exhibited a constantly high or an increasing viscosity during cooking. The *du1* starch was a Type C starch, but with quite a low viscosity, which increased consistantly during cooking and cooling. Type D starch, containing high amylose content, evidenced very limited swelling; therefore, no viscosity was observed at normal concentrations. The *ae* starch in this test was of Type D.

**Gel Strength**

The starch paste resulting from cooking in the Brabender was used to evaluate the gel strength during refrigerated storage. Results are listed in Table III. In general, the gel strength increased during storage for 7 days, but the extent of increase depended upon the starch source. Normal, *h*, and *h sh2* starches showed similar values for both firmness and stickiness after 1 and 7 days of storage. The waxy starches did not form gels after 1 day of storage and only formed very weak gels even after 7 days storage probably because of a lack of amylose to form the network structure (Howling 1980, Ring et al 1987, Wang et al 1992). The very limited firmness and stickiness of the waxy starches after 7 days indicated occurrence of some reassociation. The *ae* starch, not being fully gelatinized, had very small values for both firmness and stickiness. The *du1* starch was less firm than was normal starch after 1 day of storage, but after 7 days the *du1* starch gel became significantly firmer ($P < 0.01$) than all other starches, likely because of its high amylose content. Syneresis of water from the starch gel was observed for *ae* and *du1* starches after 7 days of storage, a fact reflecting their high amylose contents.

## Thermal Properties

The thermal properties of *ae bt1*, *ae du1*, and *du1 su1* starches measured by using differential scanning calorimetry (DSC) are listed in Table IV, and the data for other starches were published elsewhere (Wang et al 1992). Perhaps because of their high intermediate contents, the *ae bt1*, *ae du1*, and *du1 su1* starches had lower onset temperatures ($T_O$), broader gelatinization ranges (R), lower enthalpies ($\Delta H_g$), and lower peak height indices (PHI) for gelatinization than did other starches reported previously (Wang et al 1992).

## Correlation Analyses

Table V lists starches of 17 mutant genotypes and the correlations among their structural characteristics determined previously (Wang et al in press) and their physicochemical properties measured in the present study. Amylose content was the most important attribute determining the physicochemical properties of starches. Amylose content was positivly correlated with BV ($r = 0.96$) and $\lambda_{max}$ ($r = 0.81$) and was negatively correlated with [η] ($r = -0.83$), swelling power ($r = -0.86$), %T ($r = -0.88$), and peak viscosity on the viscoamylogram ($r = -0.97$), at a significance level of $P < 0.01$. The intermediate material amount was negatively correlated both with [η] ($r = -0.92$) and peak viscosity on the viscoamylogram ($r = -0.87$) ($P < 0.01$). The average chain length (CL) at Fr. II of debranched amylopectin (long B chain of amylopectin) was negatively correlated with peak viscosity on the viscoamylogram ($r = -0.81$, $P < 0.01$). No other significant correlations with r values greater than 0.8 were found among other structural characteristics and physicochemical properties.

The high correlations between intermediate materials and [η] and between intermediate materials and peak viscosity can be explained if the intermediate materials are indeed small molecules of amylopectin. Whistler and Doane (1961) reported that intermediate materials from starches of *du su2*, *ae su1*, and *ae ae* maize mutants had similar properties, such as BV, iodine sorption capacity, and rate of retrogradation. More recently, the intermediate materials from amylomaize starch were characterized as having both low-molecular-weight branched molecules with four or five branches and a CL of 50 glucose units linked to a main linear chain of 100 to 150 glucoses (Baba and Arai 1984). Mercier (1973) suggested that differences in the chain length distribution

within a population modified the solubility and other physical properties of a polysaccharide, without necessarily altering the CL or the iodine-staining properties, so correlations among these properties would be unexpected.

The correlations among selected physicochemical properties of starches from mutant genotypes of the Oh43 inbred line are summarized in Table VI. Swelling power was significantly ($P < 0.01$) correlated with both %T ($r = 0.99$) and peak viscosity on the viscoamylogram ($r = 0.92$) and was negatively correlated with blue value ($r = -0.88$) and $\lambda_{max}$ ($r = -0.98$). Crosbie (1991) reported that wheat starch swelling power was significantly ($P < 0.01$) correlated with starch paste peak viscosity ($r = 0.81$) and with total sensory score of boiled noodles made from the wheat starch ($r = 0.88$). He suggested that swelling power may be used as an alternative to starch paste viscosity for predicting noodle eating quality because of the relatively large sample size and long analysis time needed for determining paste peak viscosity with Brabender viscoamylography. The present data suggest that %T may be an appropriate alternative. Moreover, %T is easy to determine and needs only a small amount of sample. Peak viscosity also significantly correlated with BV ($r = -0.97$), $\lambda_{max}$ ($r = -0.87$), and [$\eta$] ($r = 0.93$). Firmness and stickiness of gels were significantly correlated ($r = 0.98$) to each other.

# CONCLUSION

Amylose content was the most important structural characteristics affecting the physicochemical properties of starch from maize mutant genotypes. Amylose content significantly correlated with BV, $\lambda_{max}$, swelling power, %T, and peak viscosity. Although amylose content alone was the most important predictor of physicochemical properties, however, it did not explain the behavior of all starches. Among waxy starches, the *h wx* starch exhibited physicochemical properties different from those of other waxy starches. These differences suggest that the small amount of amylose present in *h wx* starch may be quite important in determining the properties of starch or the fine structure of amylopectin in *h wx* starch.

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Table I. Properties of starches from 17 mutant genotypes of the Oh43 inbred line[a]

| Genotype | Amylose content[b] | Blue value | $\lambda_{max}$ (nm) | [η] (mL/g) | Swelling power (%) at 85°C | Solubility (%) at 85°C | %T at 650 nm |
|---|---|---|---|---|---|---|---|
| normal | 26.7 | 0.371 | 608 | 241 | 15.6 | 9.4 | 8.97 |
| *ae* | 46.0 | 0.677 | 610 | 125 | 9.7[c] | 23.1[c] | 0.62 |
| *bt1* | 24.9 | 0.369 | 615 | 218 | 12.0 | 7.0 | 3.41 |
| *bt2* | 24.7 | 0.323 | 613 | 237 | —[d] | —[d] | —[d] |
| *du1* | 30.5 | 0.434 | 611 | 157 | 11.8 | 13.6 | 9.23 |
| *h* | 28.1 | 0.388 | 616 | 234 | 14.9 | 10.0 | 8.55 |
| *sh2* | 30.1 | 0.351 | 610 | 238 | 10.6 | 3.6 | 2.37 |
| *su1* | 31.2 | 0.397 | 616 | 160 | 12.4 | 10.4 | 3.00 |
| *wx* | 0 | 0.063 | 542 | 268 | 62.1 | 25.7 | 56.83 |
| *ae bt1* | 32.4 | 0.306 | 598 | 139 | —[d] | —[d] | 1.72 |
| *ae du1* | 57.3 | 0.619 | 604 | 119 | 11.9[c] | 37.8[c] | 0.85 |
| *du1 su1* | 34.5 | 0.477 | 615 | 132 | 9.8 | 9.1 | 1.67 |
| *h sh2* | 26.4 | 0.394 | 614 | 228 | 15.3 | 9.8 | 8.86 |
| *h wx* | 0 | 0.109 | 560 | 257 | 40.7 | 15.1 | 31.09 |
| *sh2 bt1* | 27.7 | 0.367 | 617 | 228 | 9.6 | 3.3 | 1.95 |
| *sh2 wx* | 0 | 0.067 | 540 | 267 | 57.6 | 23.8 | 48.79 |
| *wx du1* | 0 | 0.061 | 540 | 266 | 56.2 | 25.4 | 49.29 |
| $LSD_{0.05}$ | 1.0 | 0.004 | 10 | 2 | 3.8 | 5.0 | 0.30 |

[a]Values are the average of two determinations,except for [η] which is the average of three determinations. *ae* = amylose extender, *bt* = brittle, *du* = dull, *h* = horny, *sh* = shrunken, *su* = sugary, *wx* = waxy.

[b]Amylose percentage calculated from fraction I of gel permeation chromatogram of isoamylase-debranched starch (Wang et al in press).

[c]Measured at 100°C.

[d]Sample not available for measurement.

Table II. Pasting properties of starches from mutant genotypes of the Oh43 inbred line[a]

| Genotype | Pasting temperature (°C) | Peak viscosity (BU) | Viscosity (BU) | | | |
|---|---|---|---|---|---|---|
| | | | at 95°C | at 95°C for 30 min | at 50°C | at 50°C for 30 min |
| normal | 84.5 | 359 | 340 | 274 | 550 | 483 |
| *æ* | ---[b] | ---[b] | ---[b] | ---[b] | ---[b] | ---[b] |
| *dul* | 90.3 | 101 | 70 | 80 | 101 | 100 |
| *h* | 85.2 | 300 | 300 | 280 | 535 | 522 |
| *wx* | 73.2 | 680 | 320 | 230 | 318 | 293 |
| *h sh2* | 86.0 | 315 | 314 | 290 | 535 | 520 |
| *h wx* | 72.0 | 620 | 340 | 229 | 352 | 310 |
| *sh2 wx* | 72.3 | 673 | 330 | 228 | 326 | 294 |
| *wx dul* | 74.6 | 622 | 311 | 212 | 312 | 290 |

[a]Starch concentration 6% (w/w, db). Values are from one determination.

[b]No value observed.

Table III. Gel strength of some starches from mutant genotypes of theOh43 inbred line[a]

| | Firmness (g force) | | Stickiness (g force) | |
|---|---|---|---|---|
| Genotype | Day 1 | Day 7 | Day 1 | Day 7 |
| normal | 5.4 | 6.1 | 0.5 | 1.0 |
| *ae* | 0.8 | 1.8 | 0.2 | 0.5 |
| *dul* | 3.4 | 10.4 | 1.2 | 2.1 |
| *h* | 6.3 | 7.8 | 0.7 | 1.8 |
| *wx* | ---[b] | 0.8 | ---[b] | 0.4 |
| *h sh2* | 6.4 | 8.0 | 0.8 | 1.7 |
| *h wx* | ---[b] | 0.8 | ---[b] | 0.5 |
| *sh2 wx* | ---[b] | 0.9 | ---[b] | 0.4 |
| *wx dul* | ---[b] | 0.8 | ---[b] | 0.4 |
| $LSD_{0.05}$ | 2.0 | 2.0 | 2.0 | 2.0 |

[a]Values are the average of 10 determinations from two separate samples.

[b]Gel too weak to support the probe.

Table IV. Thermal properties of starches from mutant genotypes of the Oh43 inbred line[a]

| | Gelatinization | | | | Refrigerated-storage retrogradation | | | |
|---|---|---|---|---|---|---|---|---|
| Genotype | $T_o$[b] (°C) | R[c] (°C) | $\Delta H_g$[d] (cal/g) | PHI[e] | $T_o$ (°C) | R (°C) | $\Delta H_r$[f] (cal/g) | r%[g] ($\Delta H_r/\Delta H_g$) |
| *ae bt1* | 63.7 | 13.3 | 2.0 | 0.30 | 38.7 | 23.3 | 1.4 | 70.0 |
| *ae du1* | 65.6 | 16.0 | 1.2 | 0.15 | 41.2 | 25.6 | 0.8 | 66.7 |
| *du1 su1* | 62.3 | 11.2 | 1.7 | 0.31 | 38.1 | 17.5 | 1.1 | 64.7 |

[a]Values are the average of three determinations. *ae* = amylose extender, *bt* = brittle, *du* = dull, *su* = sugary.

[b]Onset temperature.

[c]Range of peak calculated as 2 ($T_p$ - $T_o$), as described by Krueger et al (1987).

[d]Enthalpy of gelatinization.

[e]Peak height index = $\Delta H/(T_p - T_o)$, as described by Krueger et al (1987).

[f]Enthalpy of retrogradation.

[g]Ratio of enthalpy of retrogradation to enthalpy of gelatinization.

Table V. Correlations between structural characteristics[a] and physicochemical properties of starches from 17 mutant genotypes of the Oh43 inbred line

| | Amylose content[b] | Intermediate material content | Average chain length at fraction II of debranched amylopectin | Average chain length at fraction III of debranched amylopectin | Fraction III /fraction II of debranched amylopectin |
|---|---|---|---|---|---|
| Blue value | **0.96**[c] | 0.61** | 0.56** | 0.47** | -0.05 |
| $\lambda_{max}$ | **0.81**** | 0.25 | 0.43* | 0.05 | 0.25 |
| [η] | **-0.83**** | **-0.92**** | -0.68** | -0.46** | 0.02 |
| Swelling power | **-0.86**** | -0.47 | -0.54** | -0.13 | -0.25 |
| Solubility | -0.02 | 0.03 | 0.62** | -0.60** | 0.40 |
| %T | **-0.88**** | -0.49 | -0.55** | -0.18 | -0.14 |
| Peak viscosity | **-0.97**** | **-0.87**** | **-0.81**** | -0.45 | -0.18 |
| Firmness of gel | 0.61 | 0.40 | 0.56 | -0.31 | 0.68* |
| Stickiness of gel | 0.55 | 0.35 | 0.50 | -0.33 | -0.15 |
| $T_o$[d] | -0.55* | -0.36 | -0.31 | 0.27 | -0.25 |
| $\Delta H_g$[e] | 0.54* | 0.51* | 0.35 | 0.63** | -0.52* |

[a]Values and description are listed in Wang et al (in press).

[b]Values are from Fr. I of gel permeation chromatogram of isoamylase-debranched starch (Wang et al in press).

[c]* and ** = Significant at $P < 0.05$ and $P < 0.01$ levels of probability, respectively.

[d]Onset temperature of gelatinization.

[e]Enthalpy of gelatinziation.

Table VI. Correlations among selected physicochemical properties of starches from mutant genotypes of the Oh43 inbred line

| | Swelling power | Solubility | Peak viscosity | Firmness of gel | Stickiness of gel |
|---|---|---|---|---|---|
| Blue value | **-0.88**[a] | -0.07 | **-0.97**** | 0.55 | 0.50 |
| $\lambda_{max}$ | **-0.98**** | -0.55** | **-0.87**** | **0.80**** | 0.76* |
| [η] | 0.66** | -0.17 | **0.93**** | -0.40 | -0.37 |
| %T | **0.99**** | 0.46* | **0.90**** | -0.71* | -0.66 |
| Swelling power | — | 0.50** | **0.92**** | -0.76* | -0.71* |

[a]* and ** = Significant at $P < 0.05$ and $P < 0.01$ levels of probability, respectively.

# PAPER IV

# CHARACTERIZATION OF AMYLOPECTIN AND INTERMEDIATE MATERIALS IN STARCHES FROM MUTANT GENOTYPES OF THE OH43 INBRED LINE

# CHARACTERIZATION OF AMYLOPECTIN AND INTERMEDIATE MATERIALS IN STARCHES FROM MUTANT GENOTYPES OF THE OH43 INBRED LINE[1]

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## ABSTRACT

The amylopectin (AP) and intermediate materials (IM) from five endosperm mutant genotypes in a common Oh43 inbred line were isolated and examined by using gel permeation chromatography (GPC), iodine affinity, blue value, and viscosity. The chain-length distributions of AP and IM were determined by using an enzymatic-chromatographic method. Each isoamylase-debranched AP and IM fraction exhibited characteristic GPC elution patterns. The degree of branching in the AP and IM was decreased when the amylose-extender (*ae*) gene was present, whereas the dull-1 (*du1*) gene produced AP and IM with the highest degrees of branching among the samples. The *ae* starch had significantly ($P < 0.01$) longer long-B chains in the IM fraction than other starches, and the peak average chain length (CL) of long-B chains in the IM of *ae* starch was much longer than that of the AP fraction. There was no significant difference in the AP and IM fractions between the peak CL of long-B chains and A and short B-chains of brittle-1 (*bt1*), *du1*, *ae bt1* and *ae du1* starches. Physicochemical property measurements showed that the AP and IM from the same starch exhibited different properties in addition to different structural characteristics. It is evident that many facets of genetic background contribute to the fine structure and properties of AP and IM of different starches.

# INTRODUCTION

Starch is composed primarily of two components: amylose (AM), an essentially linear molecule; and amylopectin (AP), a highly branched molecule. The existence of a third component in normal maize starch with properties different from those of AM and AP, termed intermediate materials (IM), was first postulated by Lansky et al (1949). Banks and Greenwood (1975) suggested that the type and amount of IM depended primarily on the AM percentage of the starch, although it varied considerably among starches.

The AM content in normal maize (*Zea mays* L.) starch ranges from 25 to 30% but can vary among cultivars and, especially, with the presence of mutant genes. Amylomaize, which contains the recessive mutant gene amylose-extender (*ae*), has an apparent AM content of up to 80% (Banks and Greenwood 1975) and is associated with the presence of abnormal AP (Mercier 1973, Ikawa et al 1978, Boyer et al 1980, Ikawa et al 1981). Wolff et al (1955) demonstrated that the AP fraction in a 50% amylomaize starch had longer inner and outer chains than did chains of normal AP. Some investigators (Banks and Greenwood 1968, Adkins and Greenwood 1969), however, proposed that the resulting abnormal AP came from contaminating short-chain AM. In contrast, Montgomery et al (1964), and Banks and Greenwood (1975) confirmed the proposal of Wolff et al (1955) that the AP in amylomaize starch was less highly branched than that of normal AP.

More recently, Baba and Arai (1984) examined the fine structures of AP and IM in a 50% amylomaize starch. They found that the AP of amylomaize possessed an average chain-length (CL) that was 10 glucose units longer than that of waxy (*wx*) maize, and the IM had an average degree of polymerization (DP) of 250 to 300 glucose units per molecule with four or five branches having a CL of around 50 glucose units.

In addition to the *ae* gene, the combinations of a dull (*du*) or sugary-1 (*su1*) gene with other recessive mutant genes (except the *wx* gene) also produced starches with increased AM contents (Ikawa et al 1981, Yeh et al 1981, Inouchi et al 1983, 1987, Boyer and Liu 1985, Wang et al 1992). In a previous report (Wang et al 1992), several mutant genotypes (*ae*, *du1*, *ae* brittle-1 [*bt1*] and *ae du1*) were found to have increased AM content as well as increased IM. This study was undertaken to characterize the structures and the physicochemical properties of AP and IM from several mutant genotypes in an Oh43 inbred background to illustrate the

influences of recessive mutant genes on the starches.

# MATERIALS AND METHODS

## Materials

Mature kernels of single mutants [amylose-extender (*ae*), brittle-1 (*bt1*), and dull-1 (*du1*)] and double mutant combinations (*ae bt1* and *ae du1*) from a common Oh43 inbred line were harvested from a summer nursery near Ames, Iowa, in 1991. Development of the genotypes and sampling and storing of the kernels were described previously (Wang et al 1992).

## Isolation of Starch

Starch was isolated by using a wet-milling procedure (Steinke and Johnson 1991). The isolated starches were purified by treating with five volumes of 0.2 M sodium chloride-tolune (5:1, v/v) at least five times, and, after each treatment, the starch granules were sedimented by centrifugation (8,700 X g). The final sediment was washed three times with distilled water and dried at 45°C for 24 hr.

## Separation of Amylopectin and Intermediate Materials

Starch was fractionated according to the general procedure of Schoch (1942) and Jane and Chen (1992), and modified by Wang et al (1992). The fraction which did not complex with 1-butanol (butanol noncomplexing fraction, BNF) during fractionation was considered to include amylopectin (AP) and intermediate materials (IM) and was further purified by recrystallizing three times to remove contaminating amylose (AM). The supernatant precipitated with methanol was collected and redissolved in 90: 10 dimethyl sulfoxide (DMSO)/deionized water (Wang et al 1992) for further study. The amount of BNF collected from different starch genotypes varied from 0.5 g to 4.0 g depending upon the availability of starch.

An amount of 75 mg of BNF was fractionated on a Sepharose CL-2B column by following the procedure of Wang et al (1992) except that 75 mg starch was loaded onto the column. Fractions (4.9 mL of effluent) were collected every 9.5 min and subjected to total carbohydrate and amylose content analyses by using the anthrone-sulfuric acid method (Wright and Gann 1966) and the iodine staining test (Juliano 1971).

respectively. The minimum value from iodine staining after elution of the first major fraction was used to identify the end of the eluted amylopectin and the rest of the effluent was considered to be IM. The corresponding fractions from AP and IM were pooled, condensed by vacuum evaporation, and precipitated with five volumes of methanol. The precipitates were separated by centrifuging at 8,700 X g for 20 min at 4°C, and then redissolved in 90: 10 (v/v) dimethyl sulfoxide (DMSO)/deionized water. The same procedure was repeated many times to successively purify the IM from the AP fraction for all BNF samples. Previously, the separation of IM from AP was achieved by ultracentrifugation (Adkins and Greenwood 1966), by complexing with iodine (Adkins and Greenwood 1969), or by leaching (Banks et al 1971). It has been suggested that AP can be better separated from IM without interference from each other by using GPC (Baba and Arai 1984).

**Gel Permeation Chromatography of Isoamylase-Debranched AP and IM**

The starch was prepared, debranched by isoamylase, fractionated on a Bio-Gel P-6 column, and assayed for total carbohydrate by using the anthrone-sulfuric acid method (Wright and Gann 1966) as described by Wang et al (1992). Crystalline *Pseudimonas* isoamylase was used (Hayashibara Shoji, Inc., Olayama, Japan). The eluted materials were separated into two fractions with the division being made at minimum points between the two major peaks according to the response of the polysaccharide. The average chain length (CL) of debranched starch also was calculated (Hizukuri et al 1981, Jane and Chen 1992).

**Physicochemical Properties of AP and IM**

The isolated AP and IM in DMSO of different mutant genotypes were characterized for their physicochemical properties. The blue value (BV) was determined according to Gilbert and Spragg (1964). Two separate determinations were done on each sample.

The limiting viscosity number [$\eta$] was determined in 1 N KOH by using an Ostwald viscometer at 22.5°C. The flow time was 124 sec for 1 N KOH. Three measurements were performed for each sample.

Iodine affinity (IA) , expressed as mg of iodine bound to 100 mg of starch, was determined with amperometric titration (Schoch 1964) at 30°C. Because of a limited sample size, only one determination was

made on some samples.

### Statistical Analyses

Correlations were computed on the data of physicochemical properties among starch samples, and among starch properties and structural characteristics by using the SAS program (SAS Institute 1990). Least significant differences were computed at a significance level of $P < 0.05$.

## RESULTS AND DISCUSSION

### Separation of Amylopectin and Intermediate Materials

The butanol noncomplexing fraction (BNF) of starches from five maize mutant genotypes of Oh43 inbred line were eluted on the Sepharose CL-2B column and the profiles are presented in Fig. 1. The amylose extender (*ae* ) starch (Fig. 1A) showed two major fractions, the brittle-1 (*bt1*) and dull-1 (*du1*) starches (Figs. 1B and 1C, respectively) showed only one major fraction, and the *ae bt1* and *ae du1* starches (Figs. 1D and 1F, respectively) exhibited three fractions. The first, and also the major, fraction in the elution profile was composed of highm molecular-weight (MW) molecules, which were considered to be amylopectin (AP), whereas the rest of the material was regarded as intermediate materials (IM), mainly containing low-MW molecules (Baba and Arai 1984). For *ae bt1* and *ae du1* starches, the third fraction exhibited a greater blue-value (BV) response than did the second fraction, although the second fraction had a greater polysaccharide content than did the third fraction. This data suggests that the third fraction possessed longer branch chain lengths than did the second fraction (Bailey and Whelan 1961). The amounts of IM present in these five mutant genotypes were determined in a previous study (Wang et al 1992), and are listed in Table I.

### Individual Characterization of AP and IM

The chromatograms of isoamylase-debranched AP and IM from the five maize mutant genotypes are presented in Fig. 2. To calculate the amount of materials in low- and high-MW fractions, each elution profile was divided into two fractions at the point of minimum BV between the two major peaks, and the average chain-length (CL) at the apices of peaks from fraction I (Fr. I) and fraction II (Fr. II) and the percentage of Fr. I and Fr. II were determined (Table I). In the chromatograms for AP, Fr. I was assumed to include long B chains and Fr. II was composed of A and short-B chains (Hizukuri 1986). In the present study, the same designation of Fr. I and Fr. II was adopted for the IM. The ratio of Fr. II to Fr. I may be used as an index of the extent of branching, with the higher the ratio, the higher the degree of branching (Biliaderis et al 1981). Materials eluting at the beginning of all chromatograms, except for those of AP from *ae* and *bt1* starches, was suspected to be

contaminating amylose remaining after fractionation and was omitted from calculations of Fr. I and II.

The elution profiles of debranched AP from *ae*, *ae btl* and *ae dul* starches were similar to each other (Figs. 2A, 2G and 2I) with similar percentage compositions of Fr. I and Fr. II (Table I), whereas the AP of *btl* and *dul* had chromatograms similar to each other (Figs. 2C and 2E) in which the percentage of Fr. II was much greater than that of Fr. I (Table I). The elution patterns of debranched IM from the five samples were all different from each other (Figs. 2B, 2D, 2F, 2H and 2J), suggesting a structural difference among IM of different starch genotypes. The elution patterns of the AP and IM from the same sample were similar to each other for *btl*, *dul*, and *ae btl* samples (Figs. 2 C & D, 2E & F and 2G & H, respectively), but were different for *ae* and *ae dul* samples (Figs. 2A & B and 2I & J, respectively). The *ae* gene decreased the proportion of Fr. II in the IM more than in AP for *ae* and *ae dul* starches but this effect was not seen in *ae btl* starch.

The ratios of Fr. II to Fr. I of debranched AP ranged from 1.5 for *ae dul* starch to 4.6 for *btl* starch, and of debranched IM from 0.6 for *ae dul* starch to 5.2 for *dul* starch (Table I). These low ratios for AP and IM from *ae*, *ae btl* and *ae dul* starches indicated low degrees of branching in these polysaccharides, resulting from the effect of the *ae* gene (Wang et al 1992). Moreover, the *ae* gene decreased the degree of branching more on the IM than on the AP. In contrast, the high ratios (Fr. II to Fr. I) of AP and IM of *dul* starch suggested the presence of highly branched molecules, which agrees with a previous report (Wang et al 1992). The ratio of Fr. II to Fr. I in the AP was slightly higher than that in the IM for the same starch, except for *dul* starch, indicating that the IM was generally more lightly branched than was the AP for the same starch. These findings agree with previous reports (Banks and Greenwood 1975, Baba and Arai 1984)

The peak CL of Fr. I for the AP varied from 37 for *dul* starch to 73 for *ae* starch, and for the IM ranged from 38 for *btl* starch to 177 glucose units for *ae* starch (Table I). The peak CL of Fr. II for the AP and the IM were similar to each other, ranging from 14 to 26 glucose units. For the *ae* starch, the peak CL at Fr. I of the IM was significantly ($P < 0.01$) longer than that of the AP. No significant differences ($P < 0.05$) were noted between the peak CL (Fr. I) of the IM and of the AP for *btl*, *dul*, *ae btl* and *ae dul* starches. For Fr. II, no significant differences ($P < 0.05$) were noted between the peak CL of the AP and the IM for all starches. The *ae* starch had the longest peak CL at Fr. I for both AP and IM of all starches.

The influence of the *ae* gene on the fine structure of AP has been extensively studied. It is evident that the AP from amylomaize has a unique structure, with a longer CL than does the AP of normal and waxy maize (Wolff et al 1955, Montgomery et al 1964, Mercier 1973). When combined with other genes, however, (in *ae btl* and *ae dul*) the *ae* gene had less impact on the fine structure of the starches. Similarly, the high degree of branching in AP usually associated with the *dul* gene was not apparent in *ae dul* starch. These results suggested that the combination of two mutant genes created additional modifications in starch structure.

**Physicochemical Properties of AP and IM**

The physicochemical properties of isolated AP and IM, including BV, limiting viscosity number [η], and iodine affinity (IA), are summarized in Table II. The *ae* and *ae dul* starches exhibited higher BV than did other starches in both AP and IM components, reflecting the strong binding of AP and IM with iodine and suggesting their long branch chains. The *ae btl* starch did not have a high BV although it previously exhibited high BV response during fractionation on GPC (Fig. 1D). The IM gave a higher BV than did the AP in *ae* and *ae dul* starches, there was no difference in the BV of AP and IM for *btl* starch, and the AP had a higher BV than did the IM for *dul* and *ae btl* starches ($P < 0.05$). The results indicate that structural differences resulting in different iodine binding abilities exist among the AP and among the IM, and also between the AP and IM.

Differences between the [η] of AP and IM fractions were observed. All AP fractions exhibited significantly ($P < 0.01$) greater [η] than did the IM fractions, suggesting that the IM contained smaller molecules than did the AP. The AP and IM fractions of *btl* and *dul* starches had greater [η] values than did the respective fractions of *ae* and *ae dul* starches. There were no significant differences ($P < 0.05$) in IA between AP and IM for *btl*, *dul* and *ae btl* starches. The IM of *ae* starch had a much greater IA than did the AP of the same starch. Although some contaminating amylose appeared in the AP and IM, the great discrepancy in IA between AP and IM fractions of *ae* starch suggested structural differences in the CL of the AP and IM of *ae* starch. Baba and Arai (1984) also observed similar results in 50% amylomaize starch.

Correlations were run among structural characteristics, including ratios of Fr. II to Fr. I, peak CL at Fr. I and peak CL at Fr. II, and physicochemical properties (Table III). Although some correlations were expected,

e.g. between IA and peak CL of Fr. I, and between IA and BV, the correlations did not account for all of the variation and low correlations among other parameters may be important. The results suggest that other structural characteristics such as the molecular arrangement of AP and IM and the distribution of AP and IM within the starch granule, may also have a great effect on the physicochemical properties of starches.

# CONCLUSIONS

The different physicochemical properties observed in the AP and IM of five mutant genotypes likely reflected the structural differences in these starch fractions. Each AP and IM fraction exhibited a characteristic GPC chromatogram for the native and debranched starches. The results also suggest that the fine structures of AP and IM within a starch type are different from each other and are affected by the genetic background. The presence of another recessive mutant gene influenced the expression of the existing gene with respect to both structure and properties of the starch components.

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Table I. Percentage compositions of polysaccharides[a] and chain length distribution[a] of debranched amylopectin and intermediate materials from five mutant genotypes of the Oh43 inbred line

| Starch component | Intermediate materials (%)[b] | Fraction I (Fr. I) | Fraction II (Fr. II) | Ratio of Fr. II/Fr. I | Peak average chain length of Fr. I | Peak average chain length of Fr. II |
|---|---|---|---|---|---|---|
| *ae*-AP[c] | | 39.2 (0.3) | 60.8 (0.3) | 1.6 (0) | 73 (3) | 26 (4) |
| *ae*-IM[d] | 15.3 | 60.1 (0.2) | 39.9 (0.2) | 0.6 (0) | 177 (8) | 26 (7) |
| *bt1*-AP | | 21.2 (0.5) | 78.8 (0.5) | 3.7 (0.1) | 55 (10) | 16 (0) |
| *bt1*-IM | 1.8 | 22.3 (0.2) | 77.7 (0.2) | 3.5 (0) | 38 (8) | 20 (0) |
| *du1*-AP | | 17.8 (0.2) | 82.2 (0.2) | 4.6 (0.1) | 37 (2) | 17 (5) |
| *du1*-IM | 15.2 | 16.0 (0.2) | 84.0 (0.2) | 5.2 (0.1) | 51 (2) | 14 (3) |
| *ae bt1*-AP | | 35.9 (0.1) | 64.1 (0.1) | 1.8 (0) | 50 (4) | 18 (0) |
| *ae bt1*-IM | 22.5 | 37.6 (0.3) | 62.4 (0.3) | 1.7 (0) | 52 (19) | 21 (5) |
| *ae du1*-AP | | 39.9 (1.2) | 60.1 (1.2) | 1.5 (0.1) | 56 (3) | 23 (3) |
| *aedu1*-IM | 18.9 | 56.9 (2.0) | 43.1 (2.0) | 0.8 (0.1) | 55 (5) | 23 (4) |
| $LSD_{0.05}$ | | | | 0.1 | 18 | 8 |

[a]Values are the average of two separate determinations. Standard deviations (SD) are listed immediately below.

[b]Percentage composition of intermediate materials in starch. Data are adapted from Wang et al (1992) Table I.

[c]Amylopectin.

[d]Intermediate materials.

Table II. Physicochemical properties[a] of amylopectin and intermediate materials from five mutant genotypes of the Oh43 inbred line background

| Sample | Blue value | [η] (mL/g)[b] | Iodine affinity |
|---|---|---|---|
| *ae*-AP[c] | 0.277 ± 0.006 | 124 ± 3 | 2.8 |
| *ae*-IM[d] | 0.318 ± 0.007 | 70 ± 4 | 6.1 |
| *bt1*-AP | 0.164 ± 0.002 | 137 ± 2 | 1.6[e] |
| *bt1*-IM | 0.162 ± 0.001 | 82 ± 0 | 2.0[e] |
| *du1*-AP | 0.186 ±0.005 | 142 ± 2 | 1.7 |
| *du1*-IM | 0.155 ± 0.010 | 80 ± 1 | 2.2 |
| *ae bt1*-AP | 0.189 ± 0.004 | ---[f] | 1.0[e] |
| *ae bt1*-IM | 0.159 ± 0.001 | ---[f] | 1.1[e] |
| *ae du1*-AP | 0.252 ± 0.008 | 127 ± 1 | ---[f] |
| *ae du1*-IM | 0.304 ± 0.001 | 50 ± 1 | ---[f] |
| $LSD_{0.05}$ | 0.011 | 6 | ---[g] |

[a]Values are the average ± standard deviation of two separate determinations except for [η] which is the average of three determinations.

[b]Limiting viscosity number.

[c]Amylopectin.

[d]Intermediate materials.

[e]One measurement.

[f]Not determined.

[g]Value not available due to lack of replication.

Table III. Correlations between structural characteristics and physicochemical properties of amylopectin and intermediate materials from five mutant genotypes of the Oh43 inbred line

| | Peak CL[a] of fraction I | Peak CL of fraction II | Blue value | [η][b] | Iodine affinity |
|---|---|---|---|---|---|
| Ratio of fraction II/ fraction I | -0.35*[c] | -0.70** | -0.69** | 0.38** | -0.26 |
| Peak CL of fraction I | — | 0.26* | 0.45** | -0.21 | 0.89** |
| Peak CL of fraction II | — | — | 0.62** | -0.21 | 0.55** |
| Blue value | — | — | — | -0.45** | 0.87** |
| [h] | — | — | — | — | -0.58** |

[a]Average chain length.

[b]Limiting viscosity number.

[c]* and ** = Significant at $P < 0.05$ and $P < 0.01$ levels of probability, respectively.

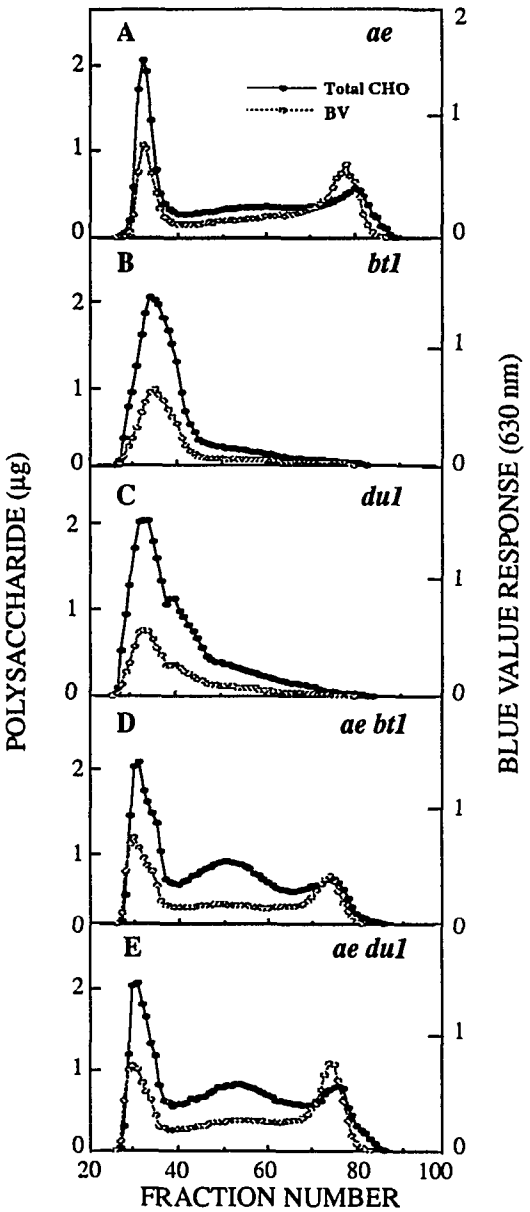


Figure 1. Elution profile on Sepharose CL-2B column of butanol noncomplexing fractions from five mutant genotypes. A. *ae* starch, B. *btl* starch, C. *dul* starch, D. *ae btl* starch, E. *ae dul* starch. Where BV = blue value response and total CHO = total polysaccharide value.

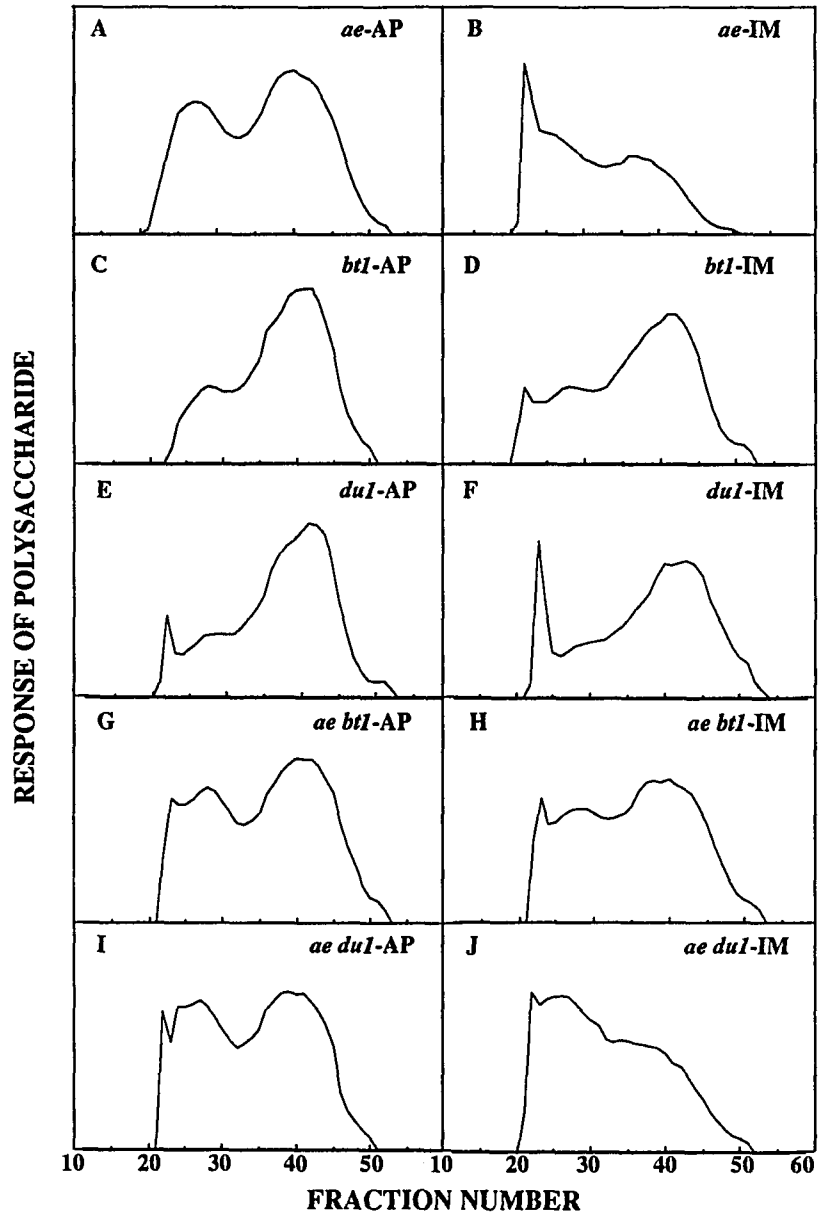


Figure 2. Elution profile of isoamylase-debranched starch from amylopectin (AP) and intermediate materials (IM) of five mutant genoytpes om Bio-Gel P-6 column. A. AP of *ae* starch, B. IM of *ae* starch, C. AP of *btl* starch, D. IM of *btl* starch, E. AP of *dul* starch, F. IM of *dul* starch, G. AP of ae btl starch, H. IM of *ae btl* starch, I. AP of *ae dul* starch, J. IM of *ae dul* starch.

# GENERAL CONCLUSIONS

The starches isolated from various mutant genotypes of the Oh43 inbred line were characterized and correlated for their structures and physicochemical properties. Each genotype exhibited different structural characteristics as well as physicochemical properties. The characteristic elution patterns from GPC were observed for native and isoamylase-debranched starches from the different starches. Distinctive morphology and properties further supported the existence of differences in fine structure. When one recessive mutant gene was combined with another mutant gene, additional modifications in structure and property were created.

For thermal properties determined by using DSC, most double mutants showed higher onset temperature and enthalpy for gelatinization, and lower onset temperature for retrogradation than those of their respective single mutants. The DSC might be used as a rapid screening method to select mutants with special thermal properties. For gel strength, double mutants generally gave lower gel strength measurements than single mutants. The starches with low $T_O$ (*bt1*) and/or $\Delta H$ (*du1* and *su*1) could be used as alternatives to save energy. The starches with high or low gel strength measurements might be potentially used in paper or confectionery industry, respectively.

Starches containing the *ae* gene had high amylose contents and were also associated with increased intermediate material contents. Low degrees of branching in the amylopectin and intermediate materials were observed in *ae*-containing starches. In contrast, a high degree of branching was found in starches with *du1* and/or *su1* genes. The proportion of long B chains and the average chain lengths of amylopectin and intermediate materials were increased when the *ae* gene was present, whereas the presence of the *du1* gene decreased the proportions of the long B chains. The starch components from different genetic backgrounds may be unique although they all are polymolecular and polydisperse. The SEM micrographs demonstrated the differences in size and morphology among starches. Some starches had an average granule size of around 5 μm (similar to that of rice starch granule), which might have special applications in specialized photographic paper, and in the laundry industry (Juliano 1984).

Amylose content was the most important structural characteristic affecting the physicochemical

properties; however, other structural characteristics such as the arrangement of amylopectin molecules, and the distribution of amylose, intermediate materials and amylopectin relative to each other within the starch granule also affected the physicochemical properties. The *du1* starch showed lower viscosities than those of normal starch on viscoamylograms, which has not been previously reported and might have practical potential. The %T correlated well with peak viscosity on the viscoamylograms and might be used as an easy screening technique for starch with desired textural characteristics.

Some genes had more important roles than others in determining the structures and properties of starches, although the interactions between different mutant genes were clear. Naturally occurring maize mutants provide a variety of novel starches which potentially may be used without further processing or with minimal modification to replace generally expensive, chemically-modified starches.

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## ACKNOWLEDGMENTS

I am most grateful to my major professor, Dr. Pamela J. White, for her guidance, encouragement and patience that made my studies at Iowa State University an enjoyable and memorable experience. She helped me to see the best no matter in what situations and to appreciate what I am. Special thanks are to Dr. Jay-lin Jane for her advice and kindly allowing me to use her equipment throughout my study, and to Dr. Linda Pollak for her support and providing me the corn samples. Thanks also go to my committee members, Dr. Lawrence Johnson and Dr. Patricia Murphy, for their guidance and supervision.

Sincere appreciation to all my friends, especially to Jen-Fang Chen and Luzhen Shen, for sharing the good times and the bad times in the laboratory together. Hsin-Chi Huang also deserves a credit for his support and love.

My deepest thanks are to my parents whose unconditional love and support strengthened me to have the confidence to complete my graduate study.