Targeting of an Appropriate Amylose Type Starch for Specific Product Applications

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SUMMARY: Some varieties of maize are known to produce starches with different chemical and physical properties. These characteristics have been recognized over the years to provide different functional properties. Amylose, in particular, has been used to provide unique functionality in numerous applications where gel strength and rigidity, as well as film forming capability, have been desired qualities. In recent years, researchers have begun to recognize that amylose is not a single chemical substance, but rather a diverse group of glucans differing in molecular size, and branching characteristics. The main shared attribute of all "amylose" polymers is their common ability to bind iodine. This binding ability is still the basis for most routine amylose determination assays. We have shown that there are uniquely different amylose polymers, and our laboratory has reported on some of the differences in physical properties. This paper reports how these unique physical properties can be applied effectively to practical applications.

Introduction

Starch is a biopolymer. It can be defined as a homopolymer whose sole monomeric component is *alpha*-D-glucose. Starch biopolymers are packaged in nature in spherulitic cellular sub-organelles, called granules. Granular structure usually reflects the source of that starch in nature [1]. Starches are found in a broad array of plant systems and generally represent the energy storage biopolymer used by germinating seeds. The focus of this paper is on starch biopolymers extracted from varieties of maize. Starch granular structure must first be disrupted before the full polymeric potential of the starch polysaccharides can be completely utilized.

The glucose monomers are linked together through glucosidic bonds between hydroxyls on neighboring glucose molecules and the carbonyls of the aldehyde functional group, called Carbon number one, of the adjacent glucose residue. Chemically speaking, the glucosidic linkage is a hemiacetal bond. Most of these hemiacetals are formed between the hydroxyl group on the fourth carbon of the adjacent glucose residue. The resulting linkages, between

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Carbon number one and Carbon number four of adjacent glucose moieties, are thought to result in more linear or helical chains. A substantially smaller number of linkages also exist between the primary hydroxyl groups on the sixth carbon and the reducing ends, Carbon number, one of neighboring glucose residues (Figure 1). These *alpha*-1→6 D glucosidic linkages are believed to be the basis for branch points in starch. Generally, those portions of starches that are more highly branched are called "amylopectin", while the less branched components are called "amylose". Normal, or common, maize starch is reported to contain about twenty seven percent amylose, while *waxy* maize starch contains no measurable amylose. The branches of amylopectin are arranged in clusters rather than evenly distributed across the molecule.

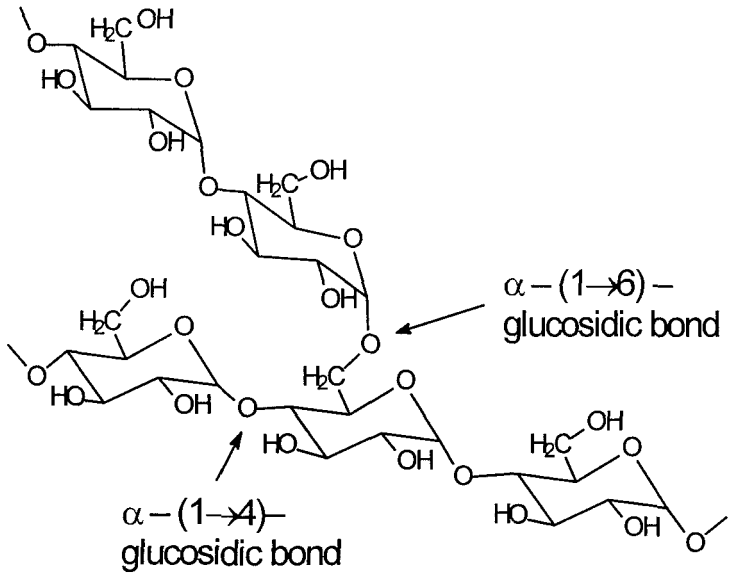

Figure 1. Chemical linkages in starch between glucose monomers. Linear portions are linked through alpha-(1→6)-glucosidic bonds. Branch points are the alpha-(1→6)-glucosidic bonds.

Amylose is most commonly measured using some sort of binding assay, usually with iodine. There is no convenient method for a direct determination of amylopectin, though nuclear magnetic resonance, NMR, has been reported[2] to have some benefit here. Amylopectin is commonly "determined" by the absence of amylose. Some question has been raised recently[3] regarding the adequacy of amylose to amylopectin ratios determined by iodine binding

measurement alone, for predicting or assessing starch biopolymer functionalities. Various levels of amylose have been found in different agricultural products (Table I).

Table I. Amylose Content of Starches from Various Plant Sources[a]

Plant Source	Apparent Amylose Content
Maize	25-28%
Wheat	17-27%
Sorghum	23-28%
Barley	24-27%
Oats	23-24%
Rice	16-17%
Rye	27%
Smooth Pea	30-34%
Wrinkled Pea	66-70%

[a]Not Bred for Altered Amylose Content

Some varieties of maize are known to produce starches with different chemical and physical properties[3] from conventional starches. These characteristics have been recognized over the years to provide different functional properties. Amylose, in particular, has been used to provide unique functionality in numerous applications where gel strength and rigidity, as well as film forming capability, have been desired qualities[4]. In recent years, researchers have begun to recognize that amylose is not a single chemical substance, but rather a diverse group of glucans differing in molecular size, and branching characteristics. The main shared attribute of all "amylose" polymers is their common ability to bind iodine. This binding ability is still the basis for most routine amylose determination assays. Broad differences have been found among different types of maize starches (Table II). We have shown that these amylose biopolymers are uniquely different polymers[3], and our laboratory has reported[5] some of the differences in physical properties. This paper reports how these unique physical properties can be exercised effectively in practical applications.

Table II. High Amylose-Containing Starches

Amylose by Iodine Binding	
Starch	% Amylose Content
Amylomaize 7	66 – 73
Amylomaize 5	51 – 55
amylose extender sugary-2	55 – 65
sugary-2	35 – 45
dull sugary-2	40 – 58
dull h	30 – 35

Experimental

High amylose Starches: Until recently, commercial production of high amylose-containing starches from corn was limited to a mutation in maize called *amylose extender*. Depending on background effects and other modifier genes in the maize plant, this mutation will produce high amylose starches with varying apparent amylose contents, of 45 percent or higher. One commercial form of high amylose-containing starch measured about 50% amylose, while another measured roughly 70% amylose. Higher levels of amylose have also been reported in the literature[6]. In recent years, however, additional varieties of high amylose-containing maize starches have become commercially available. These have shown distinctive properties that are inconsistent with amylose content based on iodine binding, alone[7].

For purposes of this study, high amylose starches were selected that contained approximately fifty percent amylose as measured by iodine binding. Starch samples from maize, some containing the *amylose extender* gene and some lacking the *amylose extender* gene, were utilized. In the present study, starch from maize, containing the mutations *dull* combined with *sugary2*, was employed to represent the latter category. Starch from normal maize was used as control.

Analytical methods: Rheological evaluation of gelatinized starch pastes was performed as described earlier[8]. Solubilization of intact starches for chromatography was carried out using the microwave solubilization method of Delgado et al.[9]. Enzyme-catalyzed debranching of gelatinized starches was performed essentially as reported[10]. Size Exclusion Chromatography

of enzyme de-branched starches was carried out as described[9]. Standard methods for assessing starch gum candy and potato coatings were employed.

Results and Discussion

Chromatography: Chromatographic separation of high amylose starches was carried out after the starches were solubilized using microwave energy. Figure 2 shows the Size Exclusion Chromatographic (SEC) pattern of normal (common) maize starch compared to the patterns of *amylose extender 5* (*ae5*) and *dull sugary2* (*dusu2*). It is evident that the normal maize starch contains a greater proportion of high molecular weight material. This high molecular weight component is usually ascribed to an amylopectin component. Normal maize contains roughly twenty-seven percent amylose by iodine binding. The remainder is assumed to be amylopectin. The amylose component is usually attributed to a chromatographic fraction that is lower in molecular weight than that of amylopectin. This lower molecular weight fraction is relatively reduced in quantity in the case of *waxy* maize.

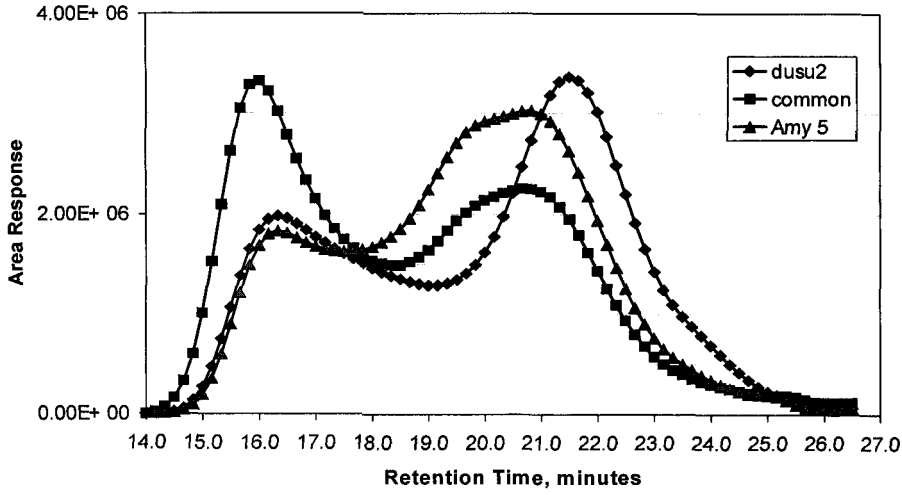

Figure 2. SEC traces comparing normal (common) maize starch to starches from *ae* 5 and *dusu2*.

Enzyme Debranching Studies: Exhaustive treatment of gelatinized starch with the enzyme iso-amylase is believed to remove all the branches (*alpha*-1→6 linkages) from starch. Chromatography of this material can then be employed as a tool to separate material that is resistant to the iso-amylase enzyme activity. For example, the all-amylopectin-containing starch from *waxy* maize will yield only short oligosaccharide chains from the branches from the amylopectin clusters. Figure 3 compares the chromatographic pattern obtained from the debranched reaction mixture of normal maize with the patterns obtained from *amylose extender 5* and *dull sugary2*. With amylose-containing starches, apparently, there are roughly three fractions. The lowest molecular weight component, called S3, constitutes branches liberated from the amylopectin clusters by the enzyme action. The intermediate molecular weight fraction, called S2, contains materials in the D.P. range of between 60 and 200. The highest molecular weight fraction, called S1, represents materials with an average D.P. range between about 700 and 1000. We have suggested in the past[1,3] that fractions S1 and S2 both satisfy the definitions for amylose. Both S1 and S2 produce a blue colour with iodine and are precipitated with *n*-butanol. S2 is thought to arise from long chains in the amylopectin clusters. Figure 4 is a schematic representation of the three fractions. The actual distribution of fractions is shown in Table III.

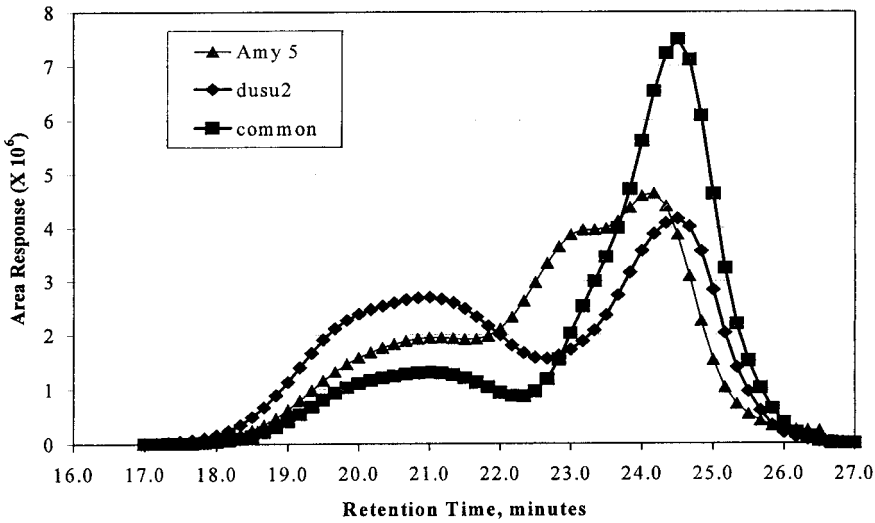

Figure 3. SEC chromatograms comparing the patterns of maize starches after treatment with *iso*-amylase enzyme.

Table III. Debranched Structural Data for Various High Amylose Starches

Starch	%S3	DP avg	%S2	DP avg	%S1	DP avg
dusu2	40	19	8	66	51	807
ae5	37	19	33	76	30	735
ae7	28	21	38	93	34	742
common	60	16	16	52	23	790

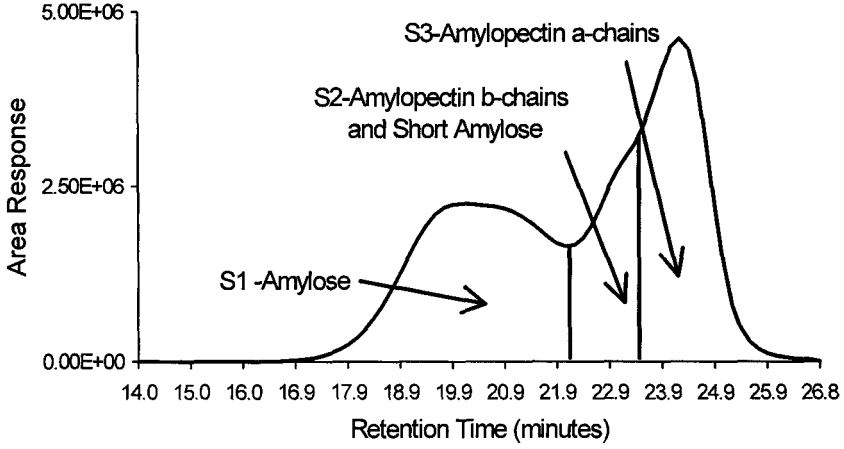

Figure 4. Schematic representation of the three major fractions of isoamylase-treated amylose-containing starches.

Applications

Gum Candies: Assuming that starch structure and function relationships are valid criteria for selection of ingredients in complex food systems, it becomes important that interactions be evaluated in a systematic manner. For the purposes of the present study, the relative influence of the two "amylose" fractions on G' of starch gels was compared. The rigidity (or storage) modulus, G', is an indication of the firmness of a gel[11]. Its dimensions can be depicted by the ratio of stress in phase with the dimensionless strain. We have also coined a term G'_{max} to indicate the highest G' value obtained for a particular starch by optimizing the cooking conditions[5]. The crucial indicator selected in this study is the relative contribution of S2 to S1. This relative contribution was initially organized by comparing relative chromatographic

areas under those peaks. Figure 5 shows one way to demonstrate these relationships. It is a three dimensional chart in which the vertical "y" axis represents the firmness of the starch gel, or G', and the "x" and "z" axes represent the relative contributions of S1 and S2.

The role played by starches in starch gum candies is to provide rapid setting and the formation of a firm gel. If one uses the level of G' as an indicator of firmness of the gel, one sees from Figure 5 that a higher contribution of S2 is more important. Conversely, the contribution of a high S1 results in a stronger and more brittle film.

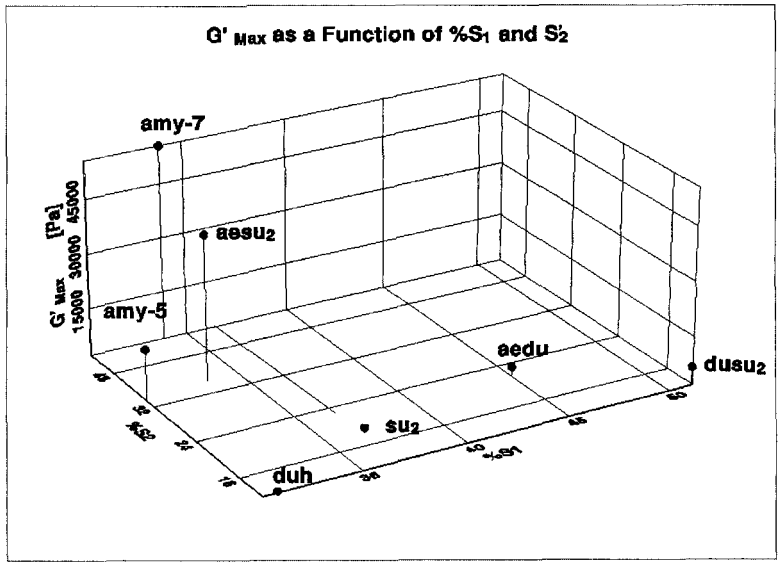

Figure 5. Graphic representation of the influence of the percentage of various amylose fractions on the G' value of the starch-water gels.

Comparing the amylose distributions with a practical assessment of firmness of starch gel candies, one finds that there exists a close relationship between the percentage of the S2 fraction and the Voland Gel Strength data for the test starch gel candies. The three highest values are derived from the three amylose extender starches in proportion to their S2 composition (Figure 6).

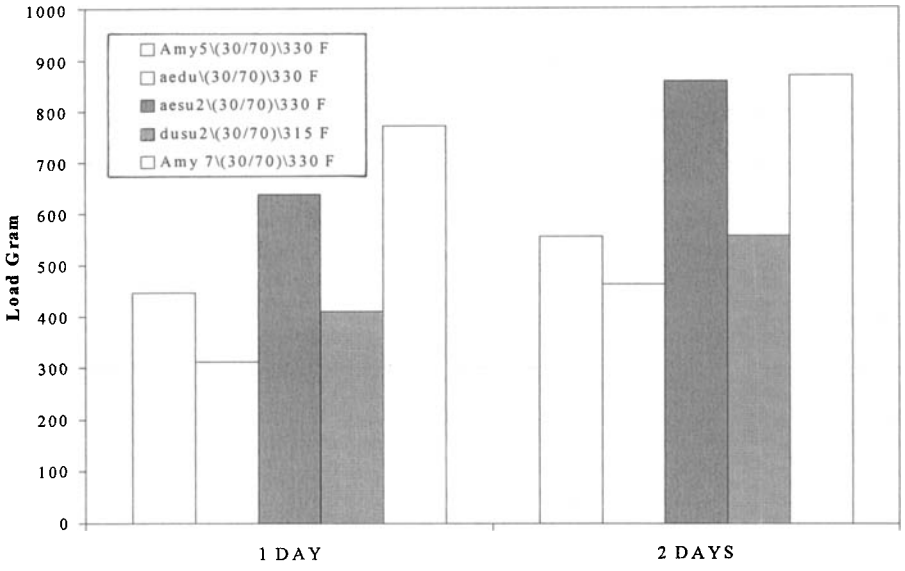

Figure 6. Comparison of the Voland gel strength data for the test starch gel candies on the first and second days after manufacture.

Starch Based Coatings: Starches have been used over the years as important components in coatings for various food products. These coatings function as effective barriers on food product surfaces and as adhesives to hold the barrier film on the surface of the food product. The role played by starch components in flexible but firm films, is to provide the maintenance of an intact crisp film. From Figure 5 it is apparent that the S1 fraction contributes flexibility and firmness. Figure 7 is a graphic representation of the influence of the percentage of various amylose fractions in a french fry coating mix on the force necessary to fracture the coating. Here one finds good correlation between the S1 fraction and the resistance of the coating to fracture. The stronger film is found with the starch with the greater S1 component. The time in the x axis refers to the duration of holding of the french fries under a heat lamp.

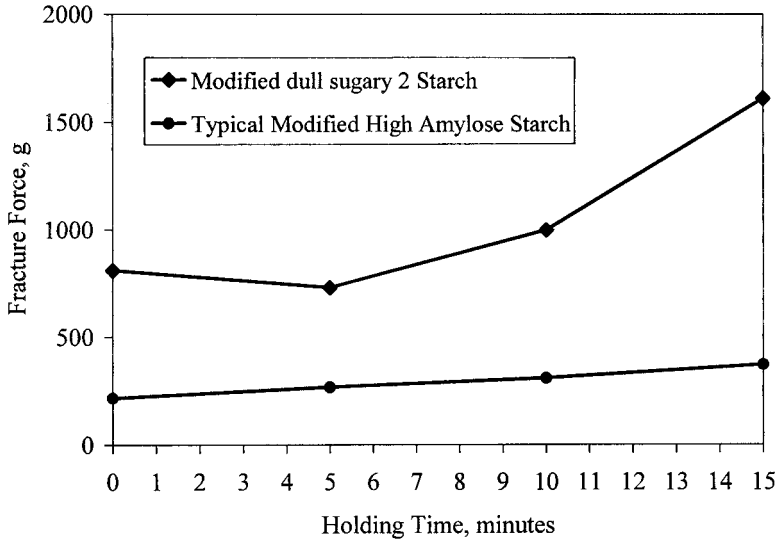

Figure 7. Influence of various amylose fractions in a french fry coating mix on the force necessary to fracture the coating.

Conclusions

1. Evidence has been presented to show that amylose biopolymers can be fractionated using biochemical and chromatographic tools to yield useful diagnostic information. This information can be applied to help select the appropriate amylose containing starch to a food formulation.
2. It has been shown that the firmness of gels is controlled by the lower molecular weight amylose component of starch, as is seen in starch gel candies.
3. It has also been shown that the higher molecular weight amylose component influences the firmness and flexibility of gels. This is shown by the performance of the higher molecular weight amylose component in food product barrier films.

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