1993

Granular cold-water-soluble starch: preparation, characterization, and its use on controlled release of atrazine

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Iowa State University, 1993

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Granular cold-water-soluble starch: Preparation, characterization, and its use on controlled release of atrazine

by

Jen-fang Chen

A Dissertation Submitted to the
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Iowa State University
Ames, Iowa

1993

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GENERAL INTRODUCTION

Next to cellulose, starch is the principal carbohydrate in plants. Starch is important not only for its role as an energy source but also a leading role in determining the texture of many foods. The hydrocolloidal properties of starch make starch suitable for a great variety of food and industrial applications. This property together with its low cost promise a tremendous growth in the use of starch.

The demands for the starches in food industries are based on their functional properties, such as viscosity, gel formation, and paste clarity. However, the physical properties of the native starches sometimes limit the application of starch, such as the lack of solubility at low temperature, the loss of viscosity during cooking at low pH, and so on. These shortcomings may be overcome by using modified starches.

Cold-water-soluble starches or pregelatinized starches, which are important functional ingredients for many instant and microwave foods, were developed to expand the usefulness of starch for industrial applications. Most commercial food-quality pregelatinized starches are manufactured by drum drying, extrusion, or cooking followed by spray drying (Powell 1967). The products are cold-water-dispersible. But the quality of the dispersions does not match that of the freshly cooked pastes. The pregelatinized starch pastes showed more graininess, less sheen and less flexibility to processing conditions. These

disadvantages are caused by the drying process which caused some breakdown of the swollen granules as well as with some retrogradation of gelatinized starches (Osman 1967).

In the past decade, efforts have been put on developing granular cold-water-soluble (GCWS) starches. The GCWS starch pastes have smooth textures and cook-up-like properties which are similar to fresh prepared starch pastes. Several patents have been issued (Pitchon et al 1981, Eastman and Moore 1984, Eastman 1987, Rajagopalan and Seib 1991). However, none of them can be applied on high-amylose or waxy starch alone. Recently, Jane and Seib (1991) demonstrated an alcoholic-alkaline treatment for preparing GCWS starches which could be used on waxy and high-amylose starches.

The objectives of this study were to improve the alcoholic-alkaline treatment for the preparation of GCWS starches, to investigate the mechanisms of the method and characterize the resulting GCWS starches, and to study the use of GCWS starches on controlled release of atrazine.

Explanation of Dissertation Format

This dissertation consists of three papers. The three papers follow the format of the Cereal Chemistry journal and will be submitted to the journal. The first paper studies the method for preparing the granular cold-water-soluble (GCWS) starches, the second paper investigates the properties of the resulting GCWS starches and derives a mechanism of the GCWS

starch formation, and the third paper studies the use of the GCWS starches as a controlled release material. The three papers are preceded by a General Introduction and a Literature Review and are followed by a General Conclusion. Literature cited both in General Introduction and in Literature Review are listed in alphabetical order and follow the section of General Conclusions.

LITERATURE REVIEW

General Properties of Starch

Starch is produced by photosynthesis in higher plants and stored as granules in seeds, roots, stems, leaves, and fruits. The size and the shape of starch granules depend on their botanical origin (Lineback 1984, Fitt and Snyder 1984). Potato starch granules are large, oval in shape, 15-100 μm in diameter. Corn starch granules are medium sized, round or polygonal in shape, and an average of about 15 μm in diameter. Rice starch granules are small, polygonal, and 3-8 μm in diameter (Lineback 1984).

Starch is composed of two major components, amylose and amylopectin. Amylose is essentially a linear polymer of repeating glucose units connected by α-1,4 glycosidic linkages. Amylopectin is a branched polymer of repeating glucose units connected by α-1,4 linkages and branched with α-1,6 linkages. Enzymatic studies reveal that the amylose molecule has a minor branching by α-1,6 linkages like that in amylopectin (French 1975, Hizukuri et al 1981, Takeda and Hizukuri 1986). The average branch-chain length of amylopectin is 20-25 glucose units (Manners 1985). In contrast, the average branch-chain length of amylopectin from high-amylose maize starch is above 30 (Jane and Chen 1992, Hizukuri 1985, Hizukuri et al 1983).

Gelatinization of starch granules

When an aqueous suspension of starch is progressively heated, an order/disorder transition process known as gelatinization takes place (Zobel 1992). Gelatinization of starch happens over a range of temperature known as the gelatinization temperature range and is a characteristic of the starch variety (Lineback 1984). The gelatinization temperature range of potato starch is 59°-68°C, and that of normal maize starch is 62°-72°C. High-amylose starches, however, do not gelatinize until 125°C (Greenwood 1976, French 1975).

It has been reported that the marked increase in gelatinization temperature of high-amylose starches may be caused by the structural difference of amylopectin as well as the higher level of amylose (Montgomery et al 1961). Studies have shown that the increase of amylose content is not responsible for the increase of gelatinization temperature. Instead, increase in long-branch-chain length of amylopectin resulted in an increase of gelatinization temperature (Lu et al 1993, Jane et al 1992). Tester and Morrison (1990) reported that, other than amylopectin, lipids affected the swelling of wheat starch rather than the amylose itself. The swelling of starch granules is inhibited when amylose-lipid complexes are formed. Morrison and co-workers (1993) reported that small fraction of amylose existed as lipid-complexed amylose and majority is in lipid-free amylose in native starch granules.

The lipid-complexed amylose increases the gelatinization temperature but the free-amylose decreases it. Increase of gelatinization temperature by 1.407°C per gram of lipid-complexed amylose or decrease of gelatinization temperature by 0.39°C for each gram of lipid-free amylose has been reported (Morrison et al 1993). Therefore, the high gelatinization temperature of high-amylose starches is attributed to the long branches in the amylopectin which enable firmer association of the amylopectin molecules with each other and with amylose and to the amylose-lipid complexes. It has been reported that high-amylose starches have elevated levels of lipids (Morrison 1988).

Measurement of gelatinization temperature is an important parameter in starch characterization. Different methods have been used to determine the gelatinization temperature: increase in optical transmittance (Longly and Miller 1971, Beckford and Sandstedt 1947), loss of birefringence (Watson 1964), rise in viscosity (Shuey and Tipples 1980), change of starch granules monitored by the scanning electron microscope (Liu and Zhoa 1990, Hoseney et al 1977), enzymatic analysis (Kainuma et al 1981), x-ray diffraction (Zobel et al 1988), NMR spectroscopy (Hennig et al 1976, Jaska 1971), and differential scanning calorimetry (DSC) (Krueger et al 1987, Bilideris et al 1980, Wootton and Bamunuarachchi 1979).

Gelatinization temperature of starch is affected by other solutes or ingredients. Sugar increases gelatinization temperature of starch and greatly extends the temperature range.

Disaccharides are more effective in delaying gelatinization than are monosaccharides (Kim and Walker 1992, Osman 1972). Competition for available water between starch and sucrose (Hoseney et al 1977, Derby et al 1975), inhibition of granular hydration (Wootton and Bamunuarachchi 1980, D'Appolonia 1972), and sucrose-starch interactions (Johnson et al 1990, Oosten 1984, Osman 1975) have all been proposed to explain the suppression of starch gelatinization by sucrose. Eliasson (1992) found a change of DSC endotherm from a double-peak into a single-peak in limited water/starch systems when sucrose was added. The author proposed that the effects of sucrose on gelatinization were both to restrict gelatinization and to make the gelatinization more easily. Water has been interpreted as a role of plasticizer which depresses the glass transition temperature (T_g) of starch and the melting of crystallites. The effect of sugars was described as anti-plasticizing which diminished the depressing effect of water on T_g (Slade and Levine 1988). The result is an increase in T_g, which in turn increases the melting temperature. At the same time, sucrose may cause a lower local viscosity during gelatinization, resulting in a shorter time interval necessary for the transitions to occur (Eliasson 1992).

Various salt solutions also exert effects on starch gelatinization temperature. Potassium iodide and potassium thiocyanate tend to lower the gelatinization temperature (Jane 1993). Sodium sulfate, on the other hand, increases

gelatinization temperature (Evans and Haisman 1982). Jane (1993) proposed that starch gelatinization in salt solutions can be attributed to water structure and to electrostatic interaction between salts and hydroxyl groups of starch.

Many salts at elevated concentration, such as potassium thiocyanate (>2M) and potassium iodide(>2.5M) solutions, may gelatinize starches at room temperature (Jane 1993). Aqueous solutions of alkali also have the same effects on starch gelatinization. The cold gelatinization of starch in aqueous alkali depends on water, alkali, and starch (Oosten 1982, Leach 1965).

Retrogradation of starch pastes

Some physical changes occur when a gelatinized starch paste or solution is aged at an appropriate temperature. In a dilute starch solution, a white precipitate eventually forms. In a concentrated starch dispersion, a gel will be set. Both cases are termed as starch retrogradation (Schoch 1969). French (1975) defined the retrogradation as a return from a solvated, dispersed, and amorphous state to an insoluble, aggregated and crystalline structure.

A feasible structure of retrograded starch gel has been elucidated (Matsukura et al 1983) (Fig. 1). The structure is considered as a mixture of domain A, B, and C in various proportions. It is proposed that domain A is primarily composed of retrograded amylopectins and a combined form with amylose.

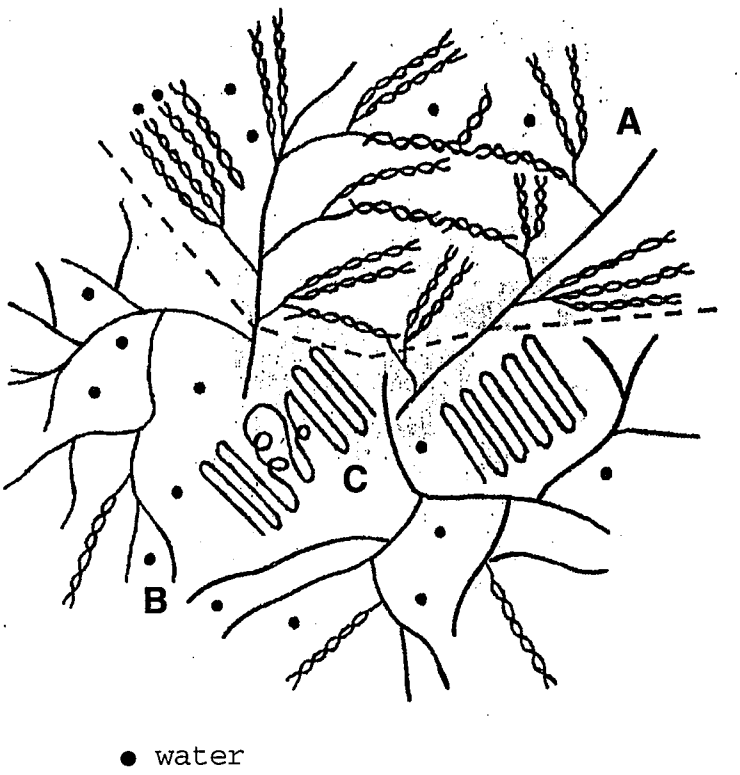


Fig. 1. A model of retrograded starch. A, B, C domains were described in text (Matsukura et al 1983).

Domain B is a slightly retrograded structure of gelatinized amylopectin which is still well hydrated and relatively well dispersed. Domain C is mainly from retrograded amylose molecules which is drawn as a folding model. In addition, crystalline double-helix structures of retrograded amylose have been proposed (Eerlingen et al 1993, Jane and Robyt 1984). The proposed structures of retrograded amylose are double helices interspersed with amorphous regions.

Physical changes on aged starch solution or paste provide useful tools to follow the retrogradation (Collison 1968, Matsunaga and Kainuma 1986). The changes include: 1) formation of crystallites which resist enzymatic hydrolysis; 2) decrease in light transmission of the solution; 3) loss of the ability to form a blue complex with iodine; 4) the progressive increase in gel firmness; 5) formation of crystallites with a B-type X-ray diffraction.

Retrogradation of amylopectin is slow because of its highly branched structure. It may be accelerated by a freeze-thaw treatment or an increase of concentration. Amylose, the linear fraction, is more prompt to retrograde. It has been found that the molecular weight (degree of polymerization, DP) of amylose and the branch-chain length (DP) of amylopectin affect the retrogradation rate of starches (Suzuki et al 1985). The result showed that the rate of starch retrogradation, in a descending order, was kuzu (DP 2810), potato (DP 5170), and tapioca (DP 7710). It has been proved that retrogradation of amylose was further increased by partial acid hydrolysis (Whistler and Johnson 1948). A maximum rate of retrogradation is found at DP of 80 to 100 (Pfannemüller et al 1971, Gidley and Bulpin 1989). In addition, the retrogradation of starch is greatly enhanced by temperature at about 0° to 5°C (French 1975).

X-Ray analysis of starch granules

X-Ray diffraction studies on crystallinity of starch have been done for several decades. All common native starches give well-defined X-ray diffraction patterns which are classified as A-, B-, and C-types (Zobel 1988) (Fig. 2). The C-type is considered to be a mixture of the A- and the B-types. Each type has its own feature. The A-type shows three strong peaks at 5.8, 5.2, and 3.8 Angstrom (Å). The B-type shows a peak at 15.8-16.0 Å (line 1), a broad medium intensity line at about 5.9 Å (lines 3a, 3b), a strong line at 5.2 Å (line 4) and a medium intensity doublet at 4.0 and 3.7 Å (lines 6a, 6b). The C-type is the same as A except for the addition of a medium to strong peak at about 16.0 Å.

It has been proved that the crystallinity of starch is essentially due to amylopectin. The relationship between the branch-chain length of amylopectin and the X-ray diffraction patterns has been proposed by Hizukuri and co-workers (Hizukuri 1985, Hizukuri et al 1983). On the basis of their finding, the chain lengths of amylopectins of A-type starches are short, whereas than those of B-type starches are long. The C-type starches have intermediate chain lengths. In addition, retrograded starches or retrograded amyloses give a B-type X-ray diffraction.

Models of crystalline structure of starch have been proposed (Sarko and Wu 1978). In crystallites of both A and B

starches, two right-handed, parallel stranded, antiparallel packed helices are proposed. The differences between A and B starches arise from water content and the manner in which these pairs are packed in the respective crystals. The unit cells of both A and B starches contain 12 glucose residues, and 8 and 36 molecules of water, respectively. The A-type starch crystallizes in an orthogonal unit cell while B-type is hexagonal. Cell dimensions of B starch were: a = b = 18.5 Å, and c = 10.40 Å. For A starch, the size was: a = 11.90 Å, b = 17.70 Å, and c = 10.52 Å.

French (1984) has suggested that, from biosynthetic considerations of amylose double helices, a parallel packing of parallel-stranded double helices would be more reasonable. In 1988, Pérez and co-workers proposed new three-dimensional structures of the crystalline parts of A and B starches (Imberty and Pérez 1988, Imberty et al 1988). Their models suggest that the unit cell of A and B starches contain 12 glucose residues located in two left-handed, parallel-stranded double helices packed in a parallel fashion. Four molecules of water are located between these helices in A-type structure while 36 water molecules are located in B-type starch. Crystallization of A starch is a monoclinic lattice with a = 21.24 Å, b = 11.72 Å, and c = 10.69 Å. The B-type starch is packed in an hexagonal array where a = b = 18.5 Å and c = 10.4 Å.

For the both models, the lattice of B starch has a large void in which numerous water molecules can be accommodated

(Fig.2). This void is not present in A starch. As a result, a more stable structure of A starch is suggested (Lii and Lee 1993, Zobel 1992, Pérez et al 1990). In addition, B starch can be transformed to A when it is subjected to a heat-moisture treatment (Donovan et al 1983, Lorenz and Kulp 1982).

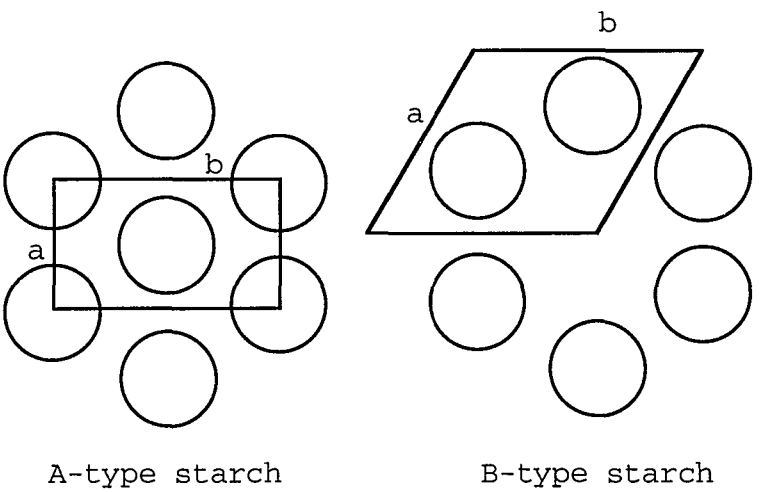


Fig. 2. Helix packing of A-type and B-type starches. The double helices are shown as circles (a top view) and the unit cell base planes are indicated.

A model of the polymorphic transition from the B-type to the A-type starch has been proposed (Pérez et al 1990). The integrity of the duplex of double-helices is maintained during the conversion of B-type packing to A-type packing. On the other hand, the transformation of A-type to B-type starch can not take

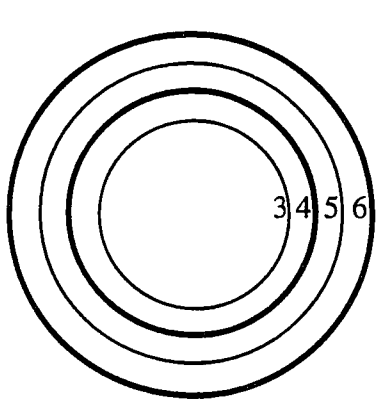
place, unless the A-type structure is destructured and followed by a recrystallinzation of B-type structure (Zobel 1988).

Other than A-, B-, and C-types, a V-type crystallinity has also been found in granular starches. Natural occurring V-type structures have not been observed in starches with less than 30% amylose. However, V-structures can be found in native high-amylose starches with the amylose extender (*ae*), dull (*du*), and sugary (*su*) genotypes in single or multiple combinations (Zobel 1993). The V-type shows peaks at 12, 6.8, and 4.4 Å (Zobel 1988). Figure 3 shows photographic X-ray diffraction patterns. Correspondence between photographic and diffractometer patterns is described in Figure 4 (Zobel 1964).

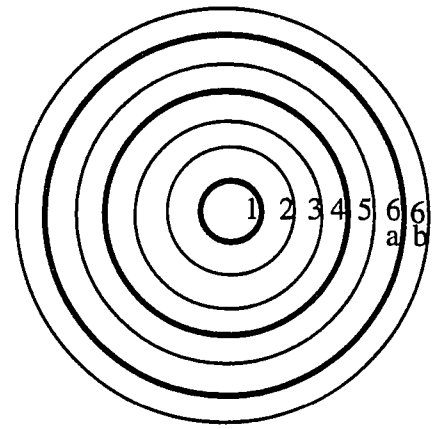
A practical way to introduce V structure into granular, amylose-bearing starches can be achieved by using selected heat-moisture treatment (Zobel 1988). In general, the V conformation is a result of amylose being complexed with complexing agents, such as aliphatic fatty acid and surfactants.

Amylose-V complexes

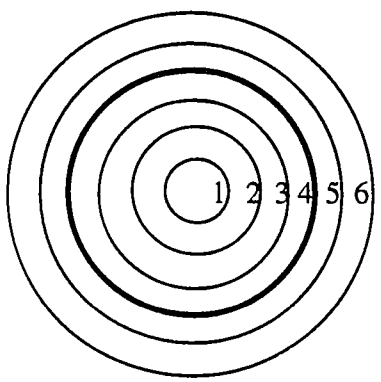
Amylose can form complexes with certain organic and inorganic compounds, and these are known as amylose-V complexes. Schoch (1942) demonstrated that amylose can form a complex with n-butyl alcohol. Thereafter, the selective precipitation of amylose with n-butyl alcohol has been widely used for



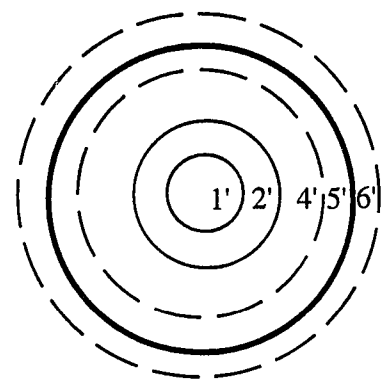
A-Spectrum



B-Spectrum



C-Spectrum



V-Spectrum

Fig. 3. X-ray diffraction patterns of starch (Zobel 1988).

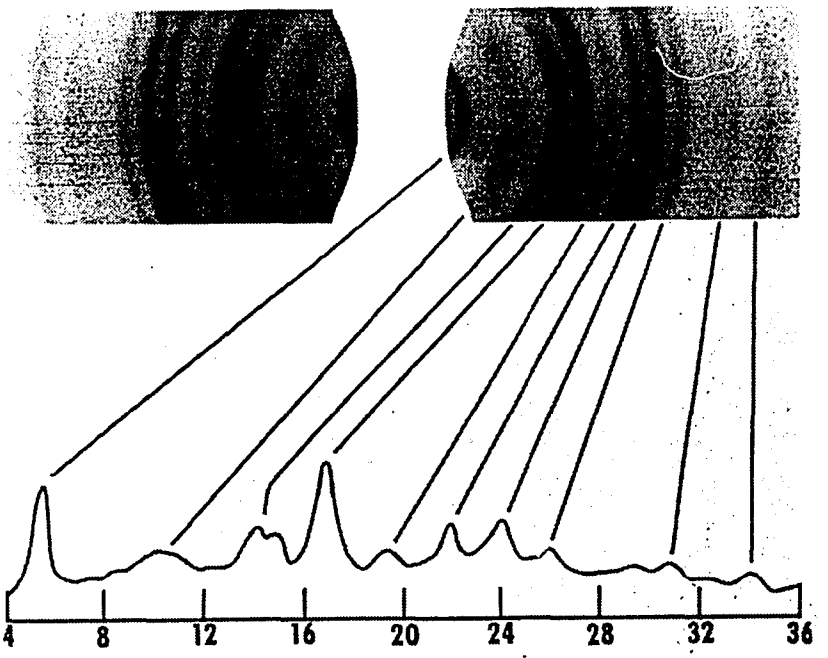


Fig. 4. Correspondence between photographic and diffractometer patterns of X-ray diffraction. Diffractometer pattern is smoothed trace of original record (Zobel 1964).

fractionation of starch. Whistler and Hilbert (1945) proposed that any water-soluble compound possessing either donor or acceptor groups capable of hydrogen-bond formation was a suitable complexing agent. They found that nitropropane, nitroethane, amyl acetate, methyl ethyl ketone, butyl mercaptan, and pyridine could form complexes with amylose. Benzene and aliphatic hydrocarbon were also reported to form complexes with amylose (French et al 1963).

Mechanisms of the amylose-V complex formation is believed to be driven by hydrophobic interaction (French 1975). X-Ray diffraction data of amylose-V complexes indicated that the guest molecules occupy the central axis of a long left-handed helix of amylose consisting of six (Yamashita 1965, French et al 1963), seven (Yamashita and Hirai 1966), or eight (Yamashita and Monobe 1971) glucosyl units per turn. Electron microscopy, and alpha-amylolysis followed by gel filtration indicated that the amylose-V complexes formed lamella-like crystals with a lamellar folding length of approximately 10 nm for all the amylose helices (Jane and Robyt 1984, Yamashita and Monobe, 1971). Jane and Robyt (1984) proposed a feasible structure of the lamella-like amylose helices. The amylose helices are folded back and forth and packed as a crystalline structure. The folding points which are the amorphous parts expose on the surface of the crystals and are accessible to enzyme hydrolysis. It has been reported that the change in lamellar thickness is a function of the crystallization temperatures (Welland and Donald 1991).

Granular Cold-water-soluble Starches

Modified starches are developed to expand the usefulness of the starches in industries. Pregelatinized starch or cold-water pasting starch is one of important physically modified starches. The pregelatinized starches are used in precooked or instant-type foods. However, the pregelatinized starch, upon dispersing in cold water, shows more graininess, less sheen and less flexibility to processing conditions. As a result, granular cold-water-soluble (GCWS) starches are developed to overcome the shortcomings of pregelatinized starches.

Pitchon et al (1981) have described a process for preparation of GCWS starch by using a spray-dryer fitted with a two-fluid nozzle. The starch slurry (35-45%, w/w) was atomized into an enclosed chamber from an atomization aperture within the nozzle, and steam was injected into the chamber from the second aperture in the nozzle at the same time to cook or gelatinize the starch. The gelatinized starch granules were moving rapidly and exited the chamber to a subsequent spray-drying process. The resulting starch granules having indented spheres swelled upon rehydration.

GCWS starches have also been produced by a heat treatment (Eastman and Moore, 1984). The starch slurry (12-20%, w/w), in aqueous alcohol solutions (18-26%, w/w), was heated to about 325-340°F for about 2 to 5 minutes. The slurry was then cooled, washed with alcohol, and dried. The resulting GCWS starch

exhibited at least 50% of cold-water solubility. In addition, Eastman (1987) disclosed a similar treatment with a starch composition comprised at least two starches. One starch component is an essentially amylose free starch, a preferred waxy maize starch. The other starch typically contains 20% to 28% amylose and 72% to 80% amylopectin. The waxy component composes from about 15% to about 85% of the combined starch weight. The slurry of the combined starch in an aqueous alcohol is heated to about 155 to 178°C for about 1 to 10 minutes. The slurry was then cooled, washed with alcohol, and dried. The resulting GCWS starches display 50-90% of cold-water solubility

An atmospheric pressure process for preparing GCWS starches has been disclosed (Rajagopalan and Seib 1991, 1992). The process involved heating of a starch slurry in a mixture of water-polyhydric alcohol at atmospheric pressure. The polyhydric alcohol can be selected from ethylene glycol, glycerol, 1,2- or 1,3-propanediol, and the four positional isomers of butanediol. The heating temperature was about 80-130°C for 3-30 minutes. The resulting products have cold water-solubility of 70-95%.

However, the methods described above can not be applied on high-amylose or waxy starch alone. Recently, Jane and Seib (1991) demonstrated an alcoholic-alkaline treatment for preparing GCWS starches which can be used on waxy and high-amylose starches. The method is carried out by treating starches with mixtures of ethanol and alkali, primarily NaOH, to

swell starch granules. The treated starches are then neutralized with HCl, washed, and dried at 80°C. The resulting starches displayed about 50-94% cold-water solubility.

It has been reported that the GCWS starch prepared by a high-temperature treatment displayed a V-type X-ray diffraction pattern (Jane et al 1986a, 1986b). The amylose-V complexes were water-soluble at 25°C (French and Murphy, 1977).

Starch As A Carrier In Controlled Release

During the past 20 years, controlled release technology has received increasing attention due to a growing understanding that substances ranging from drugs to agricultural chemicals are generally toxic and sometimes ineffective when administered or applied by conventional means. When agricultural chemicals, such as pesticides, fertilizers, herbicides, and fumigants, are applied directly, losses of these chemicals through volatilization, drifting, leaching and surface run-off are severe. Leaching of herbicides to groundwater also causes health problems.

A basic controlled-release formulation includes an active agent and a carrier. The common characteristics of carriers include chemical and physical stability, non-toxicity or biological inertness, and processability (Langer and Peppas 1983). Natural polymers and synthetic polymers are two major categories of polymeric materials used as carriers. Because of

a chemical pollution concern, naturally occurring, biodegradable polymers are preferred.

Choudary et al (1989) reported a method for encapsulation pesticides via complexation with metal in the interlamellars of montmorillonite, which was a naturally abundant and cheaply available smectite clay. Polymers with crystallizable side chains have been developed for the controlled-release of pesticides (Carter et al 1992). The polymers have an acrylic backbone, with side chains of long chain fatty alcohols esterified to it. The side chains have the ability to crystallize and then melt over a very narrow temperature range (5°C). Therefore, the controlled-release is activated as a function of temperature. Polymeric microsphere formulations have been used to control the release of an herbicide, Dicamba (DA, 3,6-dichloro-2-methoxybenzoic acid) (Tefft and Friend 1993). The microspheres were produced from ethyl cellulose or polyarylsulfone in the size range of 20-40μm by solvent evaporation or spray drying.

Starch is a natural polymer, which is inexpensive and renewable. Its degradation products are nontoxic. As a result, starch is ideal for using as a carrier in a controlled release system. Several processes have been developed to encapsulate chemicals within a starch matrix, including cross-linked starches and gelatinized starches (Trimnell et al 1991, Wing et al 1987, Trimnell et al 1982, Shasha et al 1981, Shasha et al 1976).

Granular formulations made with cross-linked starches

Early starch encapsulation methods involved the use of chemicals to crosslink the starch. Shasha et al (1976) invented a process for formulating pesticides trapped within a starch xanthate granule, formed by crosslinking a starch xanthate with an oxidant, sodium nitrite. Biologically active chemicals encapsulated by the starch xanthate may be liquids or solids. The rate of release of active ingredients depends largely on the nature of the ingredient, the texture of the encapsulated product, and moisture content. Schreiber et al (1978) reported that the selection of the starch xanthate and oxidant used in formulation process could cause a difference in controlled-release rate. The slower release characteristics are associated with stronger oxidants (Schreiber and White 1980). Greenhouse and field studies with the starch xanthate products showed improved efficiency over unencapsulated pesticides (Schreiber et al 1978). A preliminary toxicological evaluation suggests that the starch-xanthate formulation is an improved granular formulation from the standpoint of pesticide handler safety (Riley 1983).

In 1981, a simple method which involved adding the active agent to alkali-gelatinized starch and then crosslinking with calcium chloride was introduced (Shasha et al 1981). The precipitated starch-alkaline earth adduct contained the pesticide trapped in small cells within granular particles. The

exact structures of the adducts were believed to be held together by hydrogen bonding. Pesticide encapsulation by using a starch-borate complex has been developed (Shasha et al 1984, Trimnell et al 1982). The method involved in mixing starch, pesticide, and water, adding alkali to gelatinize the starch, and treating the mixture with boric acid.

Granular formulations made with cooked starches

The methods were based on the use of gelatinized starch without any chemicals. Starch was gelatinized either in a jet cooker at temperatures ranging from 90-143°C (Wing et al 1987) or in a twin-screw extruder (Trimnell et al 1991). Better mixing in the twin-screw extruder allowed the use of a high level starch (65%) in the mixture and provided for more efficient encapsulation, and of a continuous process (Wing et al 1991). In addition, encapsulated products prepared by the extruder had smaller cells of active agent, decreased swellability and slower release of active agent than the samples prepared by the jet-cooker.

Retrogradation of the gelatinized starch is the most important character in controlling encapsulation and rate of release (Wing et al 1988, Wing et al 1987, Wing and Doane 1987). The release rate followed the decrease of amylopectin contents. A decrease in particle size of encapsulated products increased the release rate. Studies has shown that controlled release

starch granule formulations reduced herbicide leaching (Boydston 1992, Fleming et al 1992).

Adherent granular formulation made with pregelatinized starches:

Granular baits formulated by mixing pregelatinized starch with a water-organic solvent solution for controlling most leaf-feeding pests have been developed (McGuire and Shasha 1992). The benefits of the invention include: 1) decreasing the loss of baits because of their adherent nature; 2) extending the effect of the baits. On the other hand, the disadvantage for the use of adherent formulations is the need of free water at the site of application for development of adherent nature.

Sprayable formulation made with pregelatinized starches:

McGuire and Shasha (1990) developed a sprayable self-encapsulating starch formulations for *Bacillus thuringiensis*. The formulations consist of a pregelatinized starch that forms an insect-digestible film around *B. thuringiensis* spores and crystals. Soluble dyes or UV absorbing compounds were incorporated into the formulations to protect *B. thuringiensis* from sunlight. Sugar was also added to the formulations to increase the adherent property.

PAPER I

PREPARATION OF GRANULAR COLD-WATER-SOLUBLE STARCHES BY ALCOHOLIC-ALKALINE TREATMENT

Preparation of Granular Cold-Water-Soluble Starches By Alcoholic-Alkaline Treatment[1]

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ABSTRACT

Granular cold-water-soluble (GCWS) starches were prepared by an alcoholic-alkaline treatment. The method was carried out by treating starches with mixtures of ethanol and NaOH solution to swell starch granules. The treated starches were then neutralized with HCl, washed, and dried at 80°C for 3 h. The method was effective with wide varieties of starches, including normal, high-amylose, and waxy starches. The efficacy of the method depended mainly on starch varieties, concentrations of ethanol and NaOH, and reaction temperature. Different reaction conditions gave products different properties. Lower concentration of ethanol, higher concentration of NaOH, or higher reaction temperature gave the resulting starches better cold-water solubility. The resulting starches displayed about 70-90% cold water solubility. Trace amount of small molecule, mainly amylose, was leached out in the supernatant during the preparation. Total weight losses were about 0.01%, 0.4%, and 1.9% for waxy maize, normal maize, and high-amylose maize starches, respectively.

INTRODUCTION

Cold-water-soluble starches, which provide important functional properties to many instant foods, have been developed to expand the usefulness of starch for industrial applications. Pregelatinized starch is an example. Traditional methods of preparing pregelatinized starches include drum drying, extrusion, and cooking followed by spray drying (Powell 1967). However, compared with fresh prepared starch pastes, the pregelatinized starch pastes showed more graininess, less sheen and less flexibility to processing conditions. Therefore, the textures of instant foods made with pregelatinized starches can not match the quality of those made with cook-up starches.

In the past decades, efforts have been put on developing granular starches with an ability to dissolve/swell in cold water. The granular cold-water-soluble (GCWS) starches exhibit a smooth texture and have cook-up-like properties. Pitchon et al (1981) have described a process for preparation of GCWS starch by using a spray-dryer fitted with two-fluid nozzles. The starch slurry was atomized into an enclosed chamber from one of the nozzles, and steam was injected into the chamber from the other nozzle at the same time to cook the starch. The cooked starch granules were moving rapidly and exited the chamber to a subsequent spray-drying process. The starch granules with indented spheres swelled upon rehydration. GCWS starches have also been produced by subjecting normal starch, slurried in

aqueous alcohol, to conditions of a high temperature and a high pressure (Eastman and Moore 1984, Jane et al 1986). The resulting GCWS starch exhibited at least 50% of cold-water solubility. The method failed to convert waxy starch into GCWS starch because waxy starch tended to gelatinize during the treatment. However, Eastman (1987) successfully conducted a similar treatment with a starch composition comprised at least two starches: waxy and normal starches. Rajagopalan and Seib (1991, and 1992) describe a method of preparing GCWS starches by heating a starch slurry in a mixture of water-polyhydric alcohol at atmospheric pressure. The resulting products have cold-water solubility of 70-95%.

However, the methods described above can not be applied on high-amylose or waxy starch alone. Recently, Jane and Seib (1991) proposed an alcoholic-alkaline treatment for preparing GCWS starches which was applicable to waxy and high-amylose starches. The method is carried out by treating starches with mixtures of ethanol and alkali, primarily NaOH, to swell starch granules. The treated starches are then neutralized with HCl, washed, and dried at 80°C.

The objectives of this study were to investigate mechanisms of the alcoholic-alkaline treatments and to improve the method for industrial application. Parameters of alcohol and alkali concentration, and temperature effects were studied.

MATERIALS AND METHODS

Materials

Normal maize starch was purchased from Sigma Chemical Company (St. Louis, MO). Waxy maize starch was a gift of American Maize-Products Company (Hammond, IN). High-amylose maize starches of Hylon V (HA5) and Hylon VII (HA7) were gifts of National Starch and Chemical Company (Bridgewater, NJ). Crystalline *Pseudomonas* isoamylase (EC 3.2.1.68) was a product of Hayashibara Shoji, Inc. (Okayama, Japan). The enzyme was used without further purification. Other chemicals were all reagent grade and were used without further treatment.

Methods

Preparation of GCWS starches

GCWS starches were prepared by treating starches with mixtures of ethanol and NaOH (3M) at different proportions and at different temperatures, 25 and 35°C. Amylose is reported to intertwine with amylopectin (Jane et al 1992, Jane et al 1986) and to preserve the integrity of starch granules. Without amylose, waxy starch is fragile and tends to disperse during processing. Therefore, different processes were developed for waxy starch and other starches. Detailed processes are described below:

A. GCWS normal maize, HA5, and HA7 starches: Starches (100 g, dry-starch base, dsb) were suspended in 400-700 g of

ethanol solution (40%, w/w) in a 3000-ml beaker equipped with a mechanical stirrer. NaOH solution (3M, 220-500g) was added at a rate about 4 g/min. The mixtures were then allowed to stand for 15 min with gentle stirring. Additional ethanol solution (40%, w/w) was then added slowly to precipitate the starch granules. After a 10-minute stirring, the slurry was set at room temperature until the starch granules were settled down on the bottom. The supernatant was carefully separated and subjected to analyses of total soluble starch and molecular size distribution of the soluble starch. Fresh ethanol solution (40%, w/w) was used to wash the precipitated starch. After washing, the starch was resuspended in aqueous ethanol solution (40%, w/w) and neutralized with HCl (3 M in absolute ethanol). The neutralized starch was washed with 60% and then 95% ethanol solutions, dehydrated with absolute ethanol, and dried in an oven at 80°C for 3 h. The dried starch was sieved with a 212-micron screen and stored in a bottle at room temperature for further analyses.

B. GCWS waxy maize starch: Starch (100 g, dsb) was suspended in 400-700 g of absolute ethanol in a 3000-ml beaker equipped with a mechanical stirrer. After addition of 200 g of aqueous NaOH (3M) solution followed by adding 100 g of ethanol solution (80%, w/w), an extra 120 g of NaOH solution (3M) was slowly added. The mixtures were stirred for 15 min, and an additional ethanol solution (80%, w/w) was added slowly. The

slurry was allowed to stand for another 10 min with gentle stirring, and followed by washing. The modified starch was collected and washed with ethanol solution (80%, w/w). After resuspended in sufficient ethanol solution (80%, w/w) and neutralized with HCl (3 M in absolute ethanol), the starch was rinsed with 95% ethanol solution, dehydrated with absolute ethanol, and dried in an oven at 80°C for 3 h. The dried starch was sieved with a 212-micron screen and stored in a bottle at room temperature for further analyses.

Different treatments with changes of the relative amounts of alkali and alcohol were used in this study to investigate the effects of the parameters on the resulting GCWS starches. A summary of the treatments are listed in Table 1. The reaction temperatures were controlled at 25°C or 35°C to investigate the temperature effects on the preparation of GCWS starches.

Gel permeation chromatography (GPC) by Sepharose CL-2B

The supernatant from the preparation process was neutralized and concentrated. Total carbohydrate content of the concentrate was then determined (Dubois et al 1956). Molecular weight of the soluble starch in the supernatant was determined by GPC on Sepharose CL-2B (Jane and Chen, 1992). The samples were solubilized by using dimethyl sulfoxide (DMSO, 90%), precipitated with alcohol, redissolved in boiling water, and injected into a 2.6 x 80 cm column packed with Sepharose CL-2B gel (Pharmacia Inc., Piscataway, NJ). The sample was eluted in

an ascending direction. Distilled water containing 25 mM NaCl and 1 mM NaOH was used as an eluent at a flow rate of 30 ml/hr. Fractions of 4.8 ml per cup were collected and analyzed total carbohydrate and blue value by using an Autoanalyzer II (Technicon Instruments Corp., Elmsford, NY).

Isoamylase debranching reaction of soluble starch

Adequate amount of the concentrate of the supernatant was subjected to an isoamylase debranching reaction by the method reported by Jane and Chen (1992). Molecular size distribution of the soluble starch before and after the enzyme treatment was determined by GPC on a Bio-gel P-6 column (Jane and Chen 1992).

Cold-water solubility (CWS)

Cold-water solubility of the GCWS starch was determined following the method of Eastman and Moore (1984). Distilled water (100ml) was precisely measured and transferred into a blender jar (Hamilton Beach, model 609-4). Starch sample (1g, dry-starch base, dsb) was carefully weighed and gradually added into the blender operated at low speed. After sample had all been added, the blender was switched to a high speed for 2 minutes. The starch suspension was then transferred to a 250-ml centrifuge bottle and centrifuged at 3100 rpm for 15 minutes. A 25-ml aliquot of the supernatant was transferred to a tared petri dish and dried in an oven at 110°C for 4 hours. The cold-water solubility was calculated by the following equation:

$$\text{CWS (\%)} = \frac{\text{g of solids in supernatant x 4}}{\text{g of sample}} \times 100\%$$

Fractionation of amylopectins of HA5 and HA7 starches

Starch samples (7.5 g) wetted with distilled water (12.5 ml) were dissolved in dimethyl sulfoxide (DMSO, 112.5 ml). The solution was heated at 96°C for 1 h with a constant stirring and was stirred for additional 24 h at room temperature. The dissolved starch was then precipitated with ethanol and redispersed in distilled water to make a 1.33% aqueous solution and followed by fractionation (Schoch 1942, Jane and Chen 1992). The fractionated amylopectin was subjected to a debranching reaction by isoamylase as described earlier.

Amylopectin branch-chain length analysis

Branch-chain length distributions of the debranched amylopectins were analyzed by using a Bio-gel P-6 GPC column (Jane and Chen 1992) and a Dionex high-performance anion exchange chromatography (HPAEC) (DX-300 system, Sunnyvale, CA) equipped with a pulsed amperometric detector (PAD). The HPAEC system consists of an amperometric flow-through cell with a gold working electrode and a silver-silver chloride reference electrode and a potentiostat. The debranched sample solution was filtrated through a 0.45-μm membrane (Supor 450, Gelman Sciences, Ann Arbor, MI). The filtrate (25 μl) was injected and analyzed. A Dionex CarboPac™ PA1 column (4 x 250 mm) with a Dionex CarboPac™ PA1 guard column was used for the analysis.

The pulsed potentials and durations were E_1 = 0.05 V (t_1 = 480 msec), E_2 = 0.60 V (t_2 = 120 msec), and E_3 = -0.60 V (t_3 = 60 msec) at range 2 (sampling periods, 200 msec). An eluent gradient with flow rate at 1 ml/min was as follows: 75% of A and 25% of B at 0 minutes, and 100% of B at 70 minutes. The eluents A and B were 150 mM sodium hydroxide solution and 150 mM sodium hydroxide in 500 mM sodium acetate solution, respectively, and were degassed by a Dionex degas module with helium gas. The system was equilibrated with 75% of A and 25% of B for 10 minutes. Total run-time for collecting data was 80 minutes.

Scanning electron microscopy

Scanning electron micrographs (SEM) were taken by a JEOL JSM-35 scanning electron microscope (JEOL Ltd., Tokyo, Japan). The starch sample was sprinkled on a 3M metallic tape mounted on a brass discs and coated with platinum/palladium alloy (60/40).

RESULTS AND DISCUSSION

Cold-water solubility of the GCWS starches was dependent upon the variety of starches. The results showed that the cold-water solubility of the GCWS normal maize, HA5, and HA7 starches treated by the A3 treatment at 35°C, which 7 X of 40% ethanol solution and 5 X of 3M NaOH solution were used, were about 84%, 93%, and 78%, respectively (Table 2). This could be attributed to the differences of amylose contents and of crystalline structure of the starches.

Amylose has been proposed to intertwine with amylopectin to prevent dispersion of starch granules during heating in aqueous alcohol solution (Jane et al 1986, Lindqvist 1979). Jane et al (1992) also reported that amylose interspersed among amylopectin in normal starch granules. Banks et al (1973) reported that the forces holding the molecules within the starch granule tend to increase as the apparent amylose content increases. As a result, waxy maize starch, containing no amylose, dispersed when it was treated with the A3 treatment at 25 °C (Table 2). Tapioca starch, containing 17% amylose, also dispersed under the same treatment. Normal maize starch, consisting of 28% amylose, retained its granular structure through the process.

At 25°C, the GCWS high-amylose starches (HA5 and HA7) exhibited higher cold-water solubilities than GCWS normal maize starch prepared by the same treatment. This could be attributed to their crystalline structures. It was well documented that

the crystalline structure of A-type starches has a close-packed arrangement, whereas that of B-type starches has more space available for water in the unit cell (Imberty and Pérez 1988, Sarko and Wu 1978). Therefore, the structure of the A-type starches was suggested to be more stable and more resistant to the treatment (Lii and Lee 1993, Zobel 1992, Gidley 1987). Consequently, at a given condition, GCWS starch prepared from normal maize starch that has an A-type crystalline structure has a lower cold-water solubility than those prepared from high-amylose maize starches.

The solubility of GCWS HA7 was significantly lower than that of GCWS HA5. The difference of cold-water solubility between the two starches was of interest. Molecular structures of both starches were investigated to reveal the solubility difference. The branch-chain length distributions of both amylopectins were investigated by HPAEC with a PAD detector after isoamylase hydrolysis. Because of the continuous decrease in response of the PAD with increasing molecular weight (Suzuki et al 1992), a close view on distributions of branch-chain length would be better for short branched chain. Both debranched amylopectins were first analyzed by GPC on Bio-gel P-6 (Fig. 1). Three cups before and after the peaks of fraction II were collected and concentrated. The concentrated samples containing small branch-chain length molecules were then analyzed by HPAEC. The results were shown in Figure 2. The response was based on the proportion of the area under each peak

to the total area of the peaks. The branch-chain length up to DP 7 was identified by comparing to known standards, and the other peaks for longer retention times were counted as higher homologues (Suzuki et al 1992). The profile showed that HA5 had more long branch-chains than HA7. It has been reported that long branch-chain contributed to a B-type X-ray diffraction pattern of the starch (Hizukuri 1985, Hizukuri et al 1983). As discussed earlier, HA5 starch should have more favorable crystalline structure for conversion during preparation. As a result, the resulting GCWS HA5 starch has a greater cold-water solubility than GCWS HA7 starch.

Cold-water solubility of the GCWS starch varied with the concentration of ethanol in the reaction mixture. When normal maize starch was subjected to both A3 and B treatments in which the proportion of ethanol increased from 2.8 X to 4.2 X (equivalent to 40% to 60% of alcohol, w/w) at 25°C, the cold-water solubility of the resulting GCWS starches decreased from 23% to 12% (Fig 3). A similar trends were also found on GCWS HA5 and HA7 starches, which decrease form 90% to 78% and 50% to 37%, respectively. High concentration of ethanol in the reaction mixture restricted starch granule swelling and retarded destruction of native, double helical crystalline structure. Consequently, the cold-water solubility of the resulting starches decreased. The mechanism and the function of ethanol solution will be reported elsewhere (Chen and Jane, unpublished).

Alkalinity of the reaction mixture was also an important factor for the cold-water solubility of the resulting starches. Results showed that treatments with greater volume of NaOH solution (3M), while other conditions were held constant, produced GCWS starches with a greater cold-water solubility (Fig 4). When normal maize starch was subjected to the D1 treatment at 25°C, which 4 X of 40% ethanol solution and 2.2 X of 3M NaOH solution were used, the cold-water solubility of the resulting GCWS starch was about 42%. However, with the D2 treatment which alkaline solution was increased from 2.2 X to 5.0 X, the cold-water solubility increased to 60%. Similar effects were shown on the samples produced at 35°C. Treatments with a higher concentration of NaOH increased the swelling of the granules (Leach, 1965; Lancaster and Conway, 1968). Therefore, the resulting GCWS starches had a higher cold-water solubility.

Raising the reaction temperature had a progressive effect on the cold-water solubility of the GCWS starches (Fig. 5). Lancaster and Conway (1968) reported an exponential relationship between temperature and the swelling rate of starch granules in NaOH solution. Because raising the reaction temperature caused starch granules to swell to a greater extent, the cold-water solubility of the starch granules increased. When reaction temperature increased from 25°C to 35°C, the solubility of resulting normal maize starch treated with the A treatment increased from 23% to 84%, whereas, the temperature effect on the solubility of high-amylose starches was much less. It may

be attributed to the relatively low gelatinization temperature of normal maize starch compared with those of high-amylose starches. Therefore, normal maize starch has a prompt response to temperature increase.

Scanning electron microscopy (Fig. 6 and 7) showed that the GCWS starches prepared by the alcoholic-alkaline treatment retained intact granules. However, the granule size of the GCWS starches is bigger than that of the original granules. In addition, the GCWS starch granule showed an indented appearance. This seemed to be caused by a change of granular structure from swelling. The GCWS waxy starch displayed a highly indented granule structure. In contrast, the GCWS normal corn, HA5, and HA7 starches showed less indented granules. This could be attributed to both amylose and amylopectin contents. Amylose served as a connector to hold the starch granules during the treatment (Jane et al 1993, Jane et al 1986, Lindqvist 1979). According to Tester and Morrison (1990), swelling is primarily a property of amylopectin. Therefore, waxy starch, with high amylopectin content (>99%), swelled much more than other starches during the preparation, resulted in a surface of granules with more dimple. In addition, it was noticed that large starch granules swelled first during the alcoholic-alkaline treatments. The small granules were more resistant to swell or even unswelled under the reaction condition.

During preparation, small amount of soluble starch was leached out in the supernatant. Total weight losses of the

soluble starches were 0.01%, 0.4%, and 1.9% for waxy maize, normal maize, and high-amylose maize starches, respectively. A study of GPC on Sepharose CL-2B showed that the soluble starches were mainly small, linear molecules which displayed high blue value (Fig 8). The molecular size of the soluble starches determined by GPC on Sepharose CL-2B (Jane and Chen, 1992) was about 1.02×10^4 Dalltons. Bio-gel P-6 gel permeation chromatograms of the soluble starch before and after the isoamylase hydrolysis showed a slight decrease of the peak and an increase of a shoulder as a result of enzyme treatment (Fig. 9). These results indicated that the soluble starch was a slightly branched molecule.

CONCLUSIONS

GCWS starches can be prepared by an alcoholic-alkaline treatment. Various reaction conditions were applied to different starches. Results showed that treatments at a lower concentration of ethanol solution, a higher concentration of NaOH solution, and a higher reaction temperature produced GCWS starches with greater cold-water solubilities. When amylose contents reached a certain level, the influence of crystalline structure of starches was dominant. Normally, A-type starches, such as normal maize starch, will be more stable than B-type starches, such as HA5 and HA7 starches, at a given condition in this study.

The study is especially useful for preparing GCWS waxy and high-amylose starches. This will improve the use of waxy or high-amylose starch in instant or microwave foods.

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Table 1. Treatments for preparation of GCWS starches

Treatment	Weight Proportions Starch[a] : H_2O : 100% EtOH : 3M NaOH
A1	1.0 : 4.2 : 2.8 : 3.5
A2	1.0 : 4.2 : 2.8 : 4.5
A3	1.0 : 4.2 : 2.8 : 5.0
B	1.0 : 2.8 : 4.2 : 5.0
C	1.0 : 0.0 : 7.0 : 3.2
D1	1.0 : 2.4 : 1.6 : 2.2
D2	1.0 : 2.4 : 1.6 : 5.0

[a] Dry starch base.

Table 2. Cold-water solubility of GCWS starches prepared under various conditions

Starch	Treatment[a]	Reaction temperature (°C)	Cold-water solubility (%)[b]
Waxy maize	A3	25	gelatinized
Tapioca	A3	25	gelatinized
Normal maize	A3	25	22.5 ± 3.5
HA5	A3	25	90.3 ± 2.1
HA7	A3	25	50.0 ± 2.0
Normal maize	B	25	11.7 ± 3.1
Hylon V	B	25	78.3 ± 2.5
Hylon VII	B	25	37.0 ± 3.6
Normal maize	A3	35	84.3 ± 0.4
Hylon V	A3	35	93.3 ± 2.4
Hylon VII	A3	35	78.1 ± 1.6
Waxy maize	C	25	93.7 ± 2.8
Normal maize	D1	25	41.9 ± 2.3
Normal maize	D2	25	60.4 ± 2.4

[a] Conditions of the treatments were described in Table 1.
[b] Data are shown as mean ± standard deviation of duplicate samples.

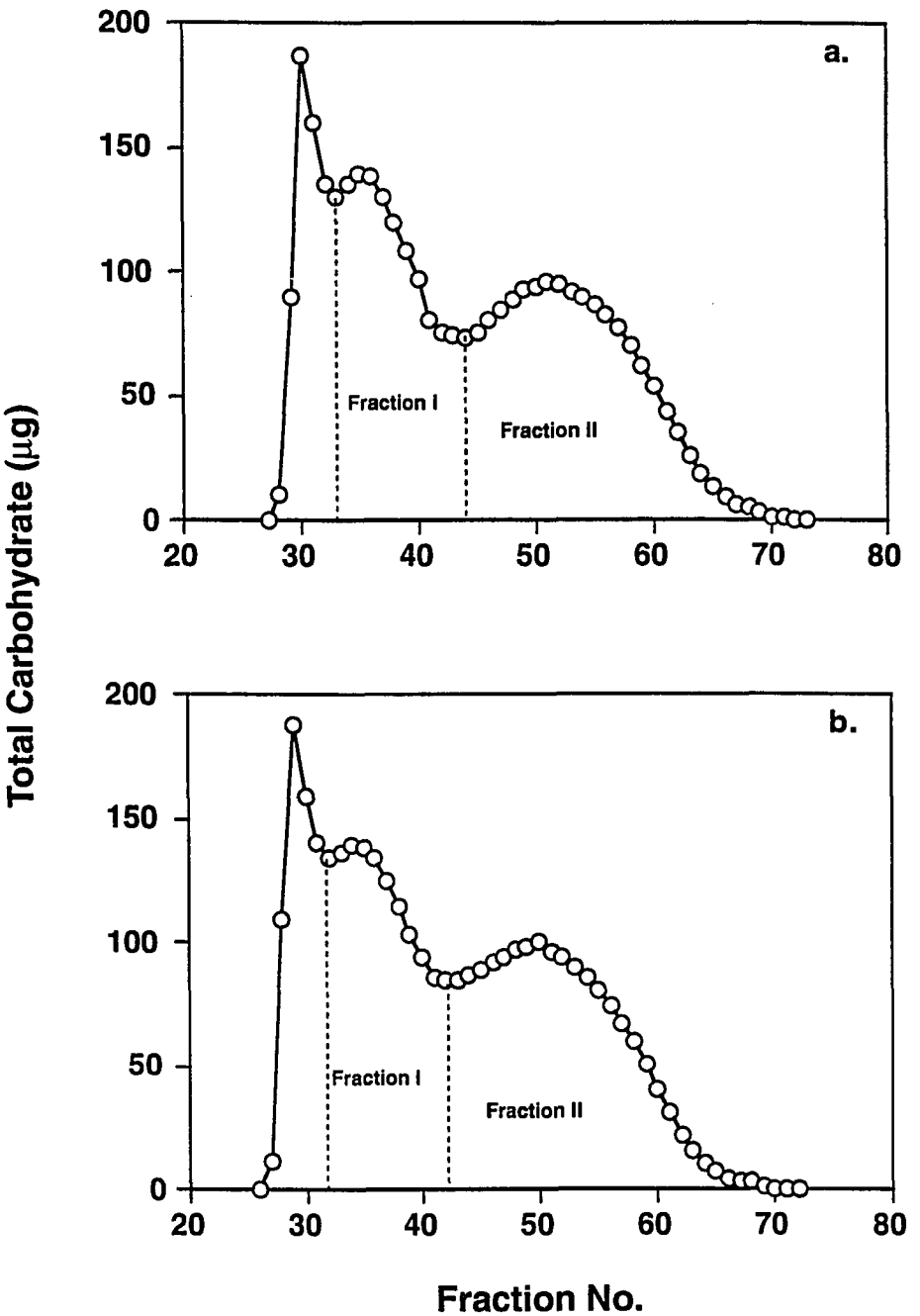

Fig. 1. Bio-gel P-6 gel permeation chromatograms of debranched amylopectins: **A**, HA5; **B**, HA7. Deionized water was the eluant at a flow rate of 21 ml/hr.

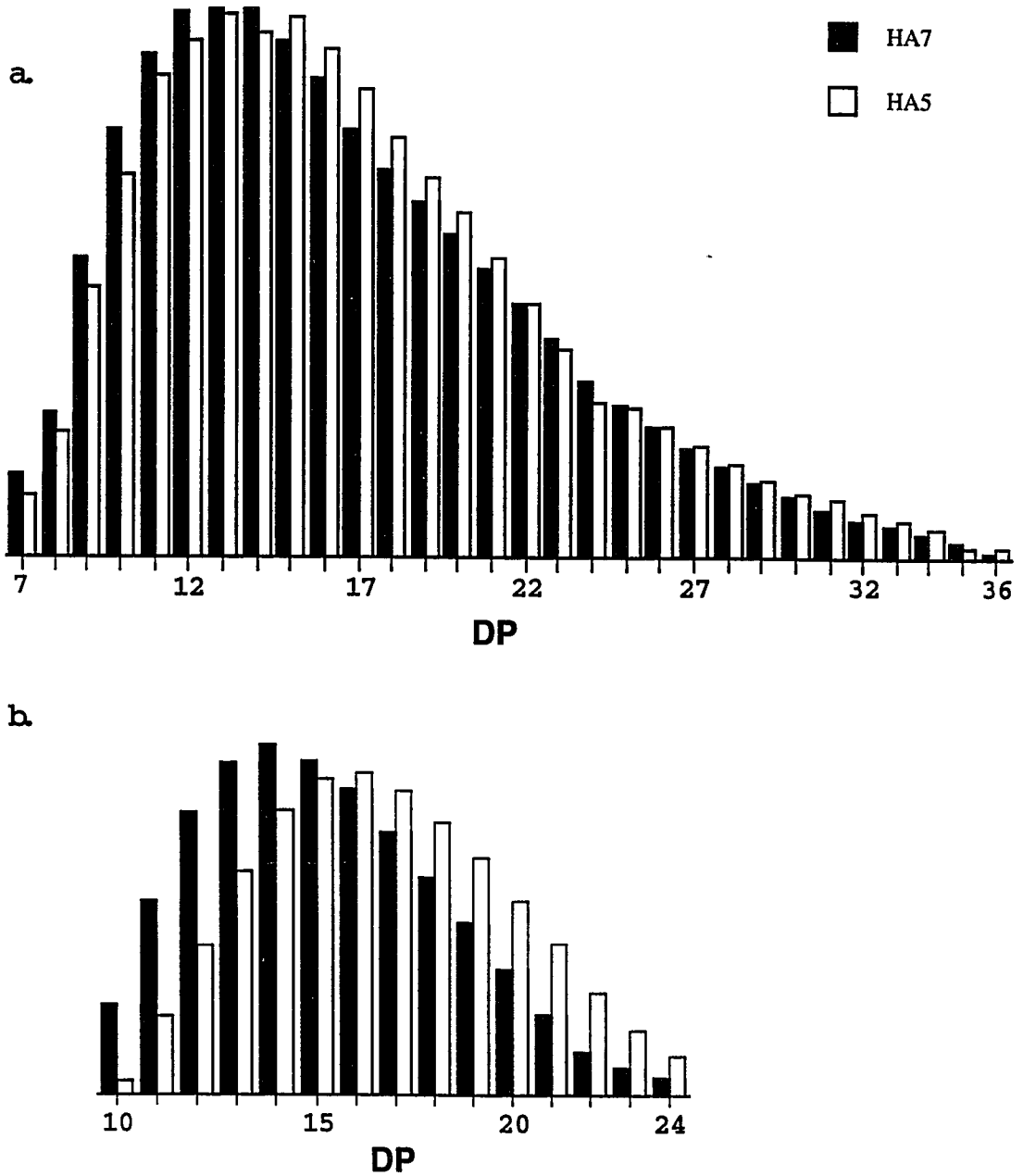

Fig. 2. High-performance anion-exchange chromatograms of debranched amylopectins: **a**, full profile; **b**, fraction II from Bio-gel P-6 column.

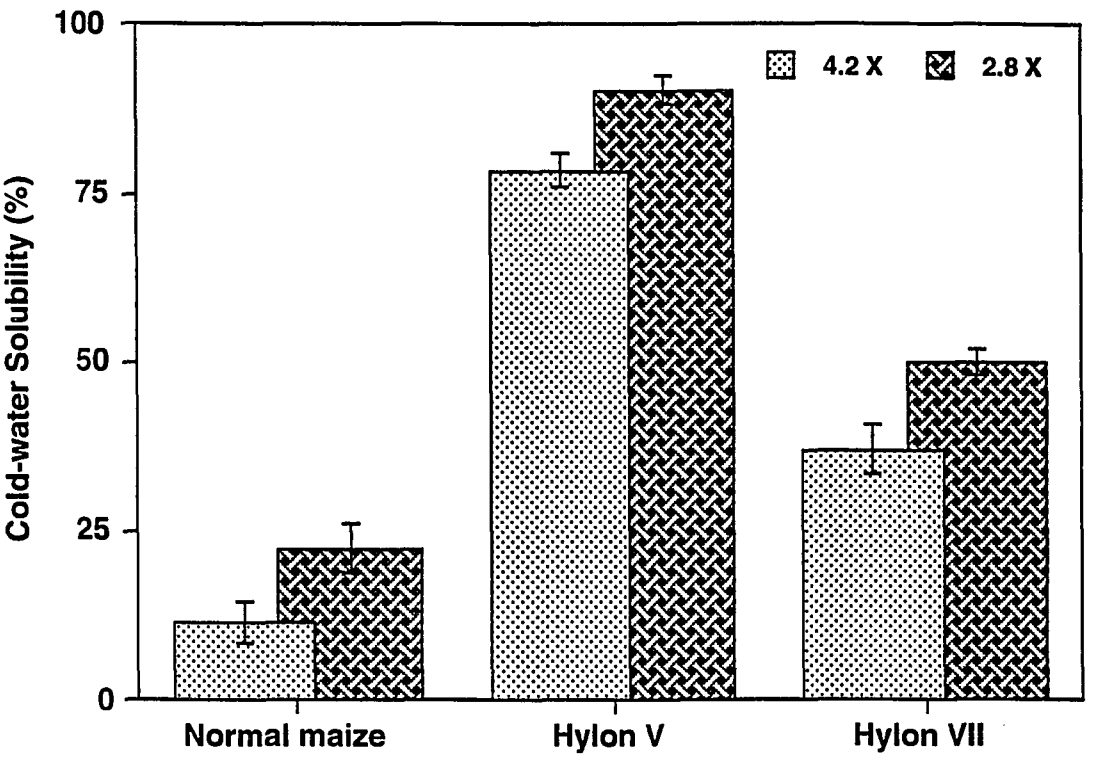

Fig. 3. Effect of alcohol on cold-water solubility of GCWS starches. Samples were prepared by the A3 and B treatments at 25°C. Concentrations of alcohol in both treatments were 2.8x and 4.2x of starch dry weight, respectively.

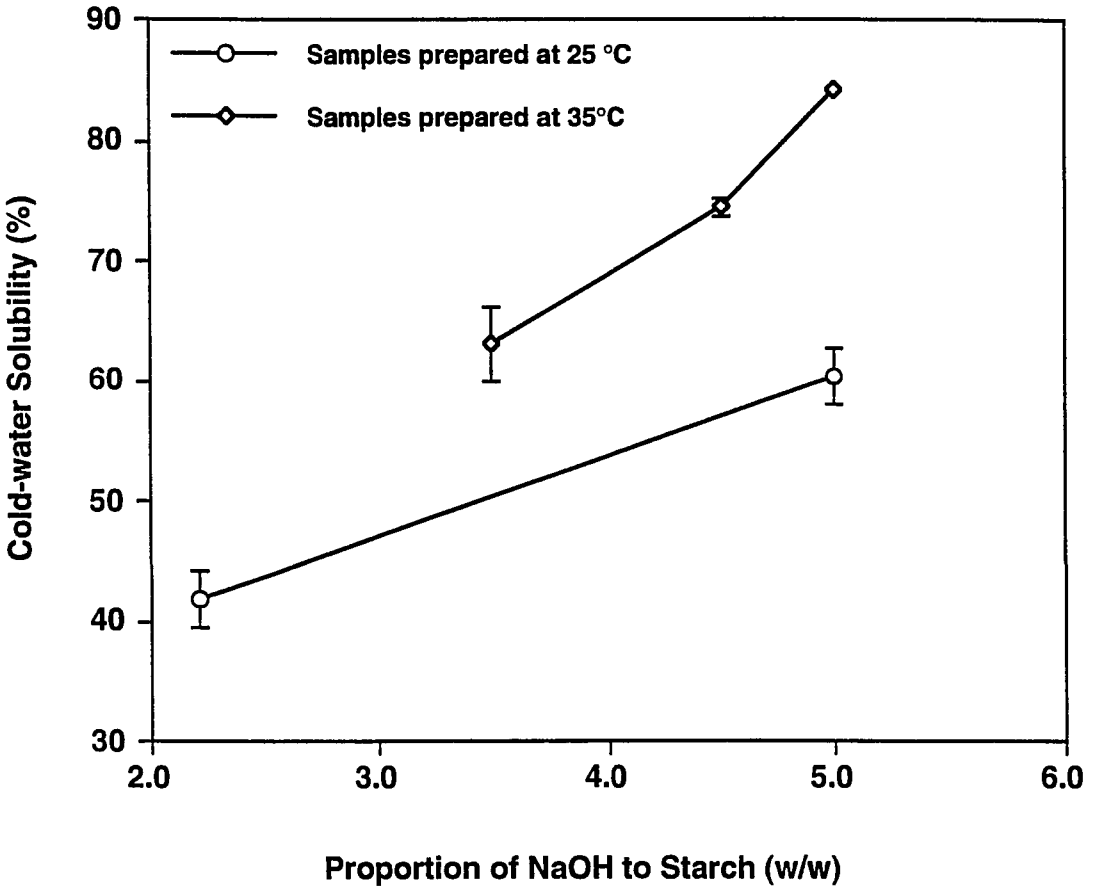

Fig. 4. Effect of alkalinity on cold-water solubility of GCWS normal maize starch. Samples were prepared by D1 and D2 treatments at 25°C and by A1, A2, and A3 treatments at 35°C. The concentration of NaOH is 3M.

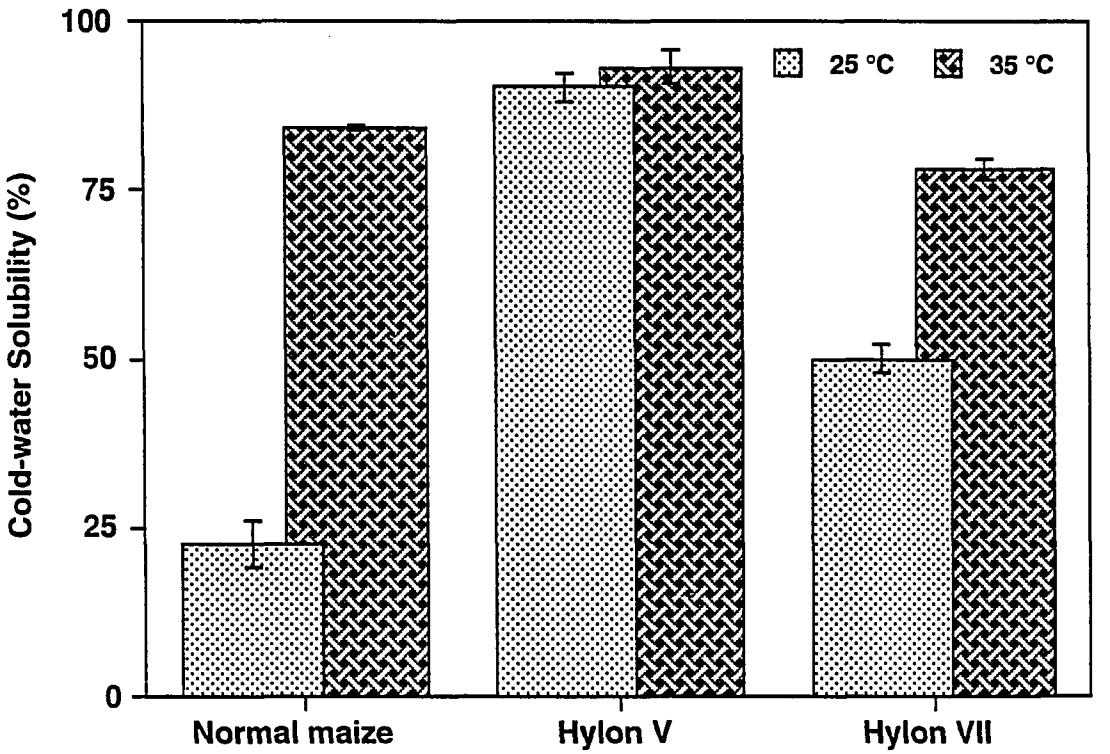

Fig. 5. Effect of temperature on cold-water solubility of GCWS starches. Samples were prepared by A3 treatment at 25°C and 35°C.

Fig. 6. SEM of GCWS starch granules. A. native waxy maize, B. GCWS waxy maize, C. native normal maize, D. GCWS normal maize treated with A3 treatment at 25°C, E. GCWS normal maize treated with A3 treatment at 35°C. The size bar represents 10μm.

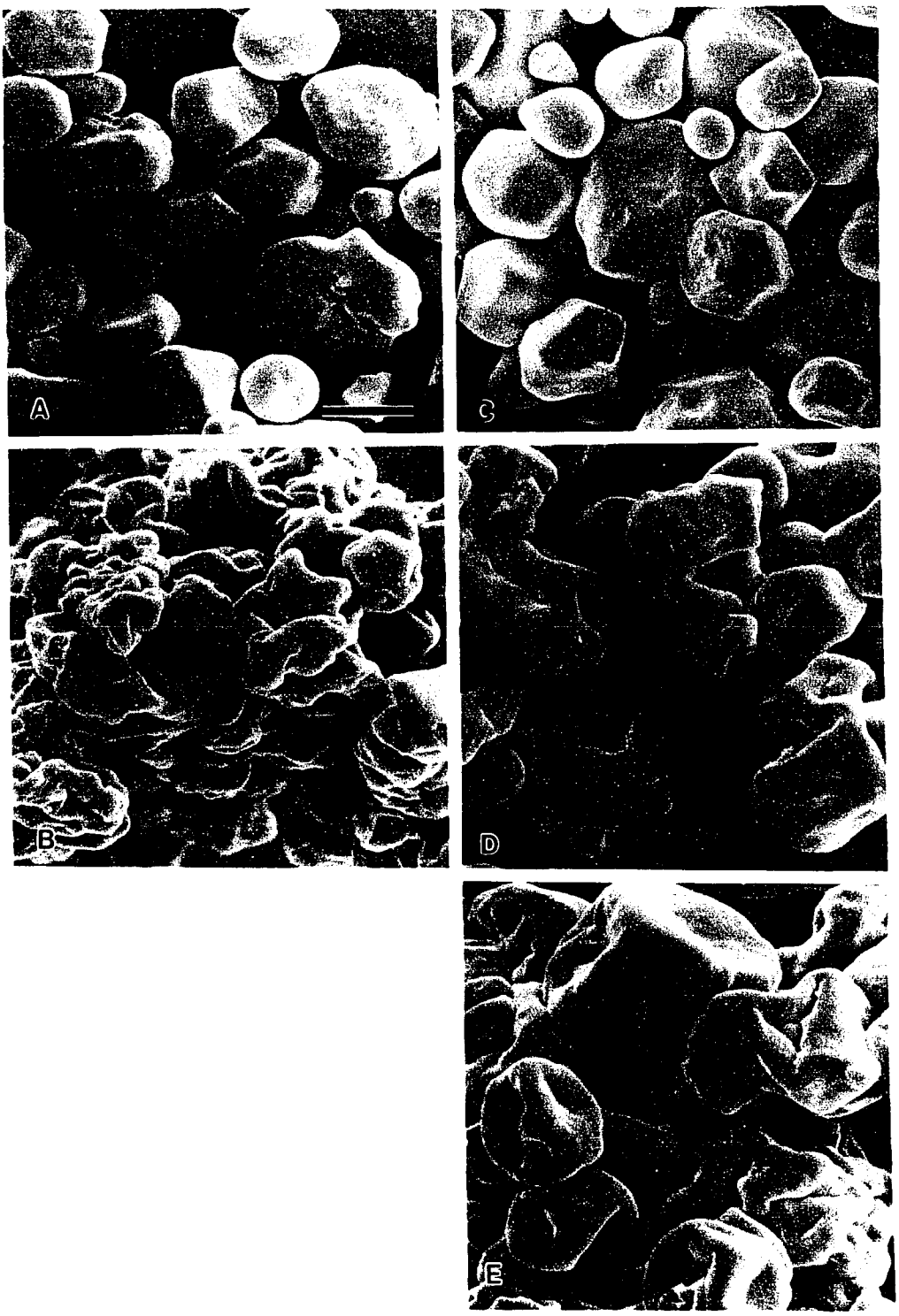
A
C
B
D
E

Fig. 7. SEM of GCWS starch granules. A. native HA5, B. GCWS HA5 treated with A3 treatment at 25°C, C. GCWS HA5 treated with A3 treatment at 35°C, D. native HA7, E. GCWS HA7 treated with A3 treatment at 25°C, F. GCWS HA7 treated with A3 treatment at 35°C. The size bar represents 10μm.

A
B
C
D
E
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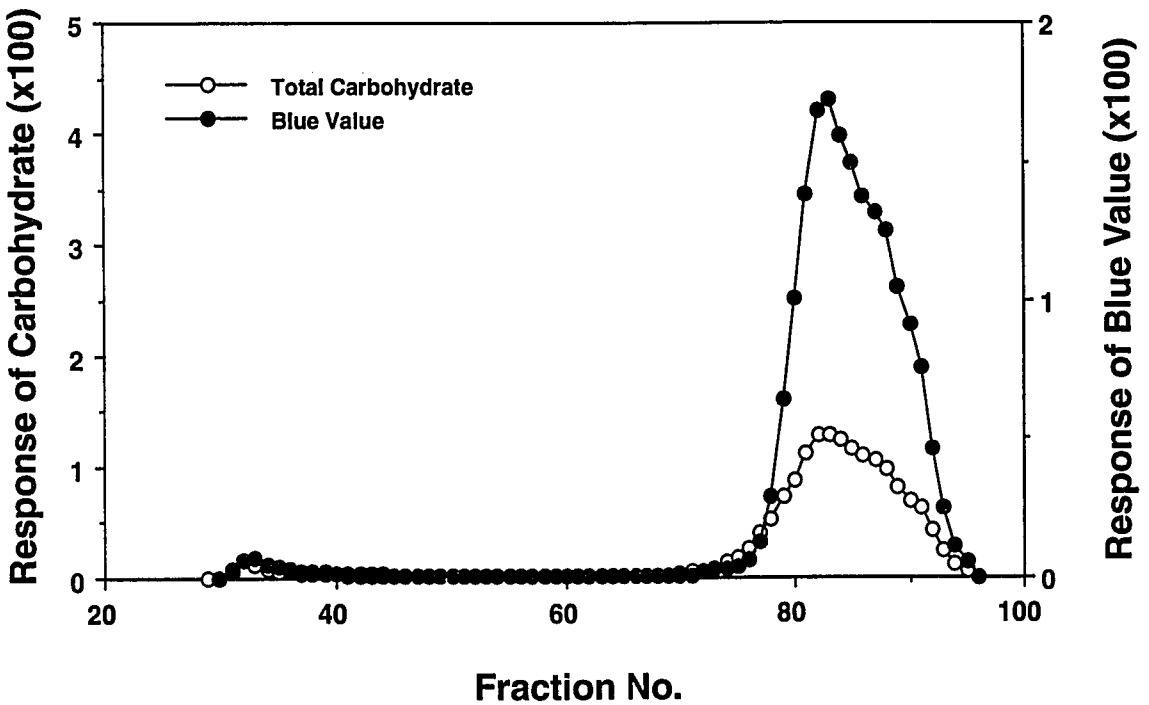

Fig. 8. Sepharose CL-2B gel permeation chromatogram of soluble starches leached in the supernatant during the preparation of GCWS starches.

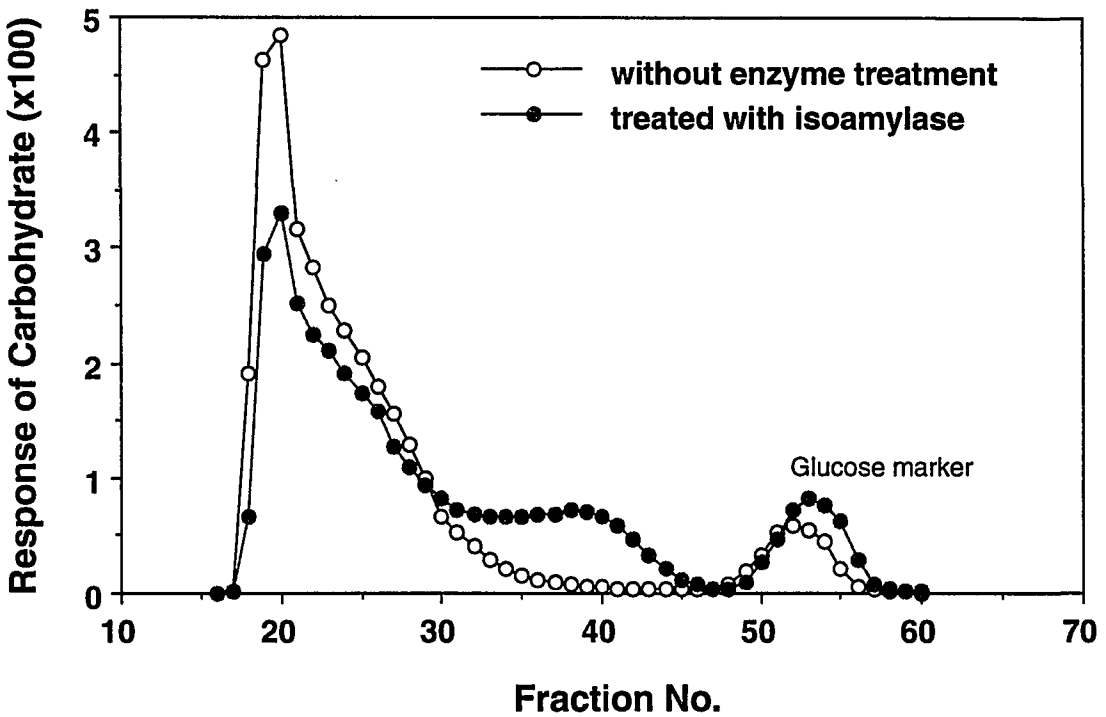

Fig. 9. Bio-gel P-6 gel permeation chromatogram of soluble starches before and after isoamylose hydrolysis.

PAPER II

PROPERTIES OF GRANULAR COLD-WATER-SOLUBLE STARCHES PREPARED BY ALCOHOLIC-ALKALINE TREATMENTS

Properties of Granular Cold-Water-Soluble Starches Prepared by Alcoholic-Alkaline Treatments[1]

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[1]Journal paper no.

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ABSTRACT

Granular cold-water-soluble (GCWS) starches were prepared from normal maize, Hylon V (HA5), Hylon VII (HA7), and waxy maize starches by treating the starches with mixtures of ethanol and NaOH solutions at a controlled temperature. The GCWS starches prepared by this method had no Maltese cross when examined by a microscope under polarized light, indicating the changes of crystalline structures. Gel permeation chromatograms of the GCWS starches were identical with those of their native counterparts, indicating no detectable degradation of starch molecules during the preparation. The X-ray diffraction of the resulting GCWS starches from normal maize, HA5, HA7 starches were V-type X-ray patterns, but the pattern of GCWS waxy maize starch was amorphous. The GCWS starches showed fully swollen granules when dispersed in cold water and exhibited about 70% to 90% cold water solubility. Most of the GCWS starches displayed higher viscosities and better freeze-thaw stabilities than their native counterparts.

INTRODUCTION

Granular cold-water-soluble (GCWS) starches provide instant and greater viscosity and a smoother texture compared with drum-dried pregelatinized starch. Therefore, GCWS starches are desirable as ingredients of instant foods. GCWS starches also show more processing tolerance than traditional pregelatinized starches (Light 1990). GCWS starches can be prepared by: (1) spray-drying (Pitchon et al 1981); (2) heating native starch in aqueous monohydric alcohol at 149-177°C under elevated pressure (Eastman and Moore 1984); (3) heating native starch in aqueous polyhydric alcohol at atmospheric pressure (Rajagopalan and Seib 1992a and 1991); (4) treating a slurry of native starch and a monohydric alcohol with an alkaline solution (Jane and Seib 1991, Chen and Jane, submitted).

The molecular structure and the property of GCWS starch are of interest and have been investigated. GCWS starch prepared by high-temperature treatment of a starch-aqueous alcohol suspension displayed a V-type X-ray diffraction pattern (Jane et al 1986a and 1986b). The mechanism was proposed as that treating native starch with aqueous alcohol at high-temperature converted the native double helical structure into single helices. Removal of alcohol by drying left an empty cavity on the center of the helices, which resulted in starch granules meta-stable and cold-water-soluble. It has been demonstrated that the GCWS starch is not chemically modified, but the

processing caused a mild degradation of starch molecules. The paste viscosity of the GCWS starch is similar to that of its native starch counterpart prepared by an amylograph.

Rajagopalan and Seib (1992b) reported a V-type X-ray pattern of GCWS starches prepared by heating native starches in aqueous propan-1,2-diol at atmospheric pressure. However, the X-ray pattern of hydroxypropylated cross-linked wheat starch was amorphous. Thickening and gelling properties of the GCWS starches prepared by heating starches in aqueous propan-1,2-diol at atmospheric pressure were reported to be similar to their native counterparts.

Objectives of this study were to characterize the GCWS starches prepared by alcohol/alkaline treatments (Chen and Jane, submitted) and to derive a mechanism of the GCWS starch formation. Molecular size distribution, viscosity, ash content, X-ray diffraction pattern, pasting property, and freeze-thaw stability of the resulting starches were investigated.

MATERIALS AND METHODS

Materials

GCWS normal maize, HA5, and HA7 starches were prepared by treating native starches with aqueous ethyl alcohol and NaOH (3M) solution (starch : H_2O : absolute ethyl alcohol : 3M NaOH = 1.0 : 4.2 : 2.8 : 5.0, by weight) at 35°C (Chen and Jane, submitted). GCWS waxy starch was produced with different proportion of starch to solvents (starch : H_2O : absolute ethyl alcohol : 3M NaOH = 1.0 : 0.0 : 7.0 : 3.2, by weight) at 25°C (Chen and Jane, submitted). Amyloglucosidase (EC 3.2.1.3), from *Rhizopus* mold, was a product of Sigma Chemical Co. (St. Louis, Missouri). The enzyme activity was 11,600 units/g solid. One unit (U) of the enzyme is defined as release 1 mg of glucose from starch in 3 min at pH 4.5 and 55°C. The enzyme was used without further purification.

Methods

Gel permeation chromatography (GPC) by Sepharose CL-2B

Molecular size distribution of GCWS starches was determined by gel permeation chromatography on Sepharose CL-2B (Chen and Jane, submitted).

Viscosity

Viscosity of starch pastes at concentration of 6% (w/w, dry-starch base, dsb) was measured by a Brabender

Visco/Amylograph (model VA-5, 700 cm-g, Hackensack, NJ). Starch paste was prepared by mixing 27 g (dsb) of GCWS normal maize or waxy maize starch with sufficient water to make total weight of 450 g. Four hundred grams of the paste were then transferred into a Brabender Amylograph cup and subjected to viscosity measurement at 30°C and 75 rpm. The final viscosity after stirring for 1 h at 30°C was used to compare with amylograph-cooked native starch pastes. Native starch pastes were prepared by following a standard cooking procedure using the amylograph (Smith 1964a). The final viscosity was recorded after the temperature was cooled to 30°C. For high-amylose starch, a paste at concentration of 3% (w/w, dsb) was prepared by a high pressure cooker (Model 4522 bench top reactor, Parr Instrument Co., Moline, Illinois) at 140°C for 30 minutes. Viscosity was measured by using the Brookfield Viscometer (model LVF, Brookfield Engineering Laboratories, Inc., Stoughton, MA) with #1 spindle, at 30°C, 60 rpm.

Enzyme susceptibility of GCWS starch

GCWS normal maize starch was subjected to amyloglucosidase hydrolysis. Starch sample (0.1 g) was dissolved in 9 ml acetate buffer (0.1 M, pH 4.5). One ml of enzyme solution (in 0.1 M acetate buffer, pH 4.5) containing 20 U of amyloglucosidase was added. The mixture was then incubated for 12 h in a shaker bath (Versa-Bath S, model 236, Fisher Scientific) at 55°C, 100 strokes/min. The efficiency of the enzyme hydrolysis was

determined by measuring the proportion of reducing sugars to total sugars. Measurement of reducing sugars was accomplished by the use of modified Park-Johnson's method (Hizukuri et al 1981, Jane and Chen 1992). Total sugars was determined by a phenol-sulfuric acid method (Dubois et al 1956).

Lipid analysis

Total lipid content of native starches and GCWS starches were determined by following the Smith's method (1967).

X-Ray diffraction

X-Ray diffraction patterns of starches were recorded on a Simens D500 diffractometer with Cu X-ray tube operated at 40 KV and 25 mv. A step-scan was set at an angle of 0.05° per step with a counting of 2 seconds.

Differential scanning calorimetry (DSC)

DSC analyses of starches were conducted by using a Perkin-Elmer DSC-7 (Norwalk, CT) equipped with an intracooling I system. Starch (2.0 ± 0.1 mg, dsb) was weighed into an aluminum pan (Perkin-Elmer), and distilled water (about 6 mg) was added to the starch sample. The pan was sealed and allowed to equilibrate for 2 to 3 hr at ambient temperature. The sample was then heated from 25 to 100°C at a rate of 10°C/min using an empty pan as a reference.

Determination of ash contents

Ash contents of starches were determined by following the Smith's method (Smith 1964b).

Freeze-thaw stability

To measure freeze-thaw stability, native starch pastes (6%, w/w) were prepared following a standard cooking process by using the Brabender Visco/Amylograph. Preparation of GCWS starch pastes were conducted to disperse adequate amount of GCWS starches in distilled water at room temperature, and then transferred to the amylograph operated at 30°C, 75 rpm for 1 hour. Fifteen grams of each starch paste were transferred into each of ten disposable petri dish (60 x 15 mm). The petri dishes were covered and sealed with a Scotch tape to prevent moisture loss while froze at -20°C. On each freeze-thaw cycle, samples were frozen at -20°C 24 h and thawed at room temperature for 3 h. The thawed samples were then subjected to a vacuum filtration after 1, 2, 3, 5, and 10 freeze-thaw cycles (Lim 1990). Water of syneresis from duplicate gel samples was collected and weighed. Freeze-thaw stability was expressed by the percent of water lost.

RESULTS AND DISCUSSION

All the GCWS starch granules prepared in this study had no Maltese cross when examined by a microscope under polarized light. The X-ray diffraction of the GCWS starches showed V-type patterns (single helical conformation) except GCWS waxy maize starch which gave an amorphous pattern (Fig. 1). The amylose helical complexes and amorphous starch have been reported to be water-soluble at 25°C (French and Murphy 1977). DSC thermograms also confirmed the cold-water-soluble properties of GCWS starches that none of the GCWS starches in this study gave any gelatinization endotherm between 25 and 100°C (Fig. 2).

In characterization of GCWS starch prepared by superheating in aqueous ethanol solution under pressure, Jane et al (1986a) proposed a mechanism for the transformation of commercial GCWS normal maize and wheat starches. They proposed that amylopectin, as well as amylose, formed a V-complex with the alcohol when the native double helical structure was dissociated by heating. Removal of the alcohol leaves the starch in a metastable state that is soluble in cold water.

A similar mechanism was applied for the formation of the GCWS starches prepared by alcoholic-alkaline treatment. Starch is a weak ion-exchanger (Oosten 1982). When starch molecules were placed in a strong alkaline solution, protons of the -OH group were dissociated and left negative charges on starch molecules. The repulsion between negative charges resulted in

swelling of starch granules. The swelling of the granules exerted a tension on neighboring crystallites of starch molecules and tended to distort them (French 1984). Further swelling led to uncoiling or dissociation of double helical regions and break-up of amylopectin crystalline structure. As a result, the order of crystallites was destroyed. However, the entanglement of amylose with amylopectin molecules inside the granules retained the swollen granules in one entity (Jane et al 1993, Jane et al 1986a). After neutralization of the treated starches, the starch molecules formed single-helix complexes with ethanol (V-complex). GPC profiles of the GCWS starches showed no detectable degradation of starch molecules (Fig. 3).

The function of alcohol in the reaction mixture was not only to restrict the swelling of starch granules by decreasing the effective water concentration but also to serve as a complexing agent. Rajagopalan and Seib (1992b) reported that, immediately after heating starches in aqueous polyhydric alcohol, X-ray diffraction pattern of the treated starches was amorphous. Following solvent exchange with ethanol, the diffraction pattern was changed to a V-type pattern.

A study of enzyme hydrolysis showed no significant differences between GCWS normal maize starch and its native counterparts. The result indicated that the GCWS starch was not chemically modified, since modified starch would interfere with enzyme hydrolysis (Chan et al 1984). It confirmed that the cold-water solubility was rendered by a physical change of

crystalline structure of starch, from double helix (A-type) to single helix (V-type).

Paste viscosities of the GCWS starches prepared at 30°C and their native counterparts prepared by a normal cooking process and cooled to 30°C are shown in Table 1. Because of high gelatinization temperature of high-amylose starches, pastes of HA5 and HA7 starches were prepared by a high pressure cooker at 140°C for 30 min. After cooking, the starch pastes were cooled to 30°C and transferred into a 600ml-beaker. The beaker was then placed in a water bath set at 30°C, and viscosity of the paste was measured by the Brookfield Viscometer. Results showed that most GCWS starches exhibited higher viscosities than their native counterparts.

Figure 4 showed typical pasting curves for GCWS normal maize and waxy maize starches (Fig. 4a) and their native counterparts (Fig. 4b) at 6% (w/w) concentration. The viscosities of the GCWS starches instantly reached to about 300 B.U., and then gradually reached to their maximum consistencies of 400 B.U. after stirring 15 min at 30°C. The viscosity was kept on plateau and remained unchanged for 1 hr under a continuous stirring at 75 rpm. GCWS normal maize and waxy maize starches, under mechanical shearing force, showed better stabilities than their native counterparts. When GCWS starch was dispersed in cold water, starch granules swelled instantaneously and produced viscous pastes. The swollen granules were maintained integral because of the entanglement of

amylose with amylopectin molecules inside the granules; therefore, GCWS starch paste showed a stable viscosity (Jane et al 1993).

Dispersion of the GCWS starch granules prepared by alcoholic-alkaline treatment in water did not produce lumps like the paste of GCWS starch prepared by heating starch in aqueous alcohol. The smoothness of the texture and easy preparation of paste were attributed to the trace amount of salt residues in the GCWS starch granules. Ash contents of the starches had been measured as a reference of salt residues in the GCWS starch granules. The ash contents of the GCWS starches produced by this method were 2-5 times higher than those of their native counterparts (Table 2). The lipid contents of the GCWS starches were reduced about 20 to 40% (Table 3).

Freeze-thaw stability studies revealed that the pastes of the GCWS starches prepared by alcoholic-alkaline treatment have improved their freeze-thaw stabilities (Fig. 5). The paste of GCWS normal maize starch didn't reach its maximum syneresis up to 5 freeze-thaw cycles, whereas the paste of native normal maize starch reach its maximum syneresis after the first freeze-thaw cycle. The water loss of GCWS waxy maize starch was not detectable till second cycle. Both GCWS normal and waxy maize starches displayed better freeze-thaw stabilities than their native counterparts. This could be attributed to the integrity of the swollen granules, which did not have a complete

dispersion of starch granules. Consequently, the tendency of starch retrogradation was decreased.

CONCLUSIONS

Granular cold-water-soluble starches prepared by alcoholic-alkaline treatment exhibited V-type X-ray patterns, except GCWS waxy maize starch which showed an amorphous type. The changes of the crystalline structures from double helices to single helical structures resulted in the changes of X-ray diffraction patterns. Molecular size distributions of the GCWS starches on Sepharose CL-2B chromatograms were identical with those of their native counterparts, indicating no detectable degradation of starch molecules. Results of enzyme hydrolysis indicated that the GCWS starch was not chemically modified. All the GCWS starch granules prepared in this study showed no Maltese cross when examined by a microscope under polarized light. The GCWS starches swelled instantaneously when rehydrated in cold water and the pastes had better viscosities and freeze-thaw stabilities. The smoothness of the texture and easy preparation of paste are attributed to the trace amount of salt residues in the GCWS starch granules.

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Table 1. Viscosity of Various Starch Pastes

Sample	Viscosity[a]
Waxy maize[b]	320 ± 28 B.U.
GCWS waxy maize[b]	375 ± 7 B.U.
Normal maize[b]	570 ± 28 B.U.
GCWS normal maize[b]	405 ± 7 B.U.
Hylon V[c]	9.5 ± 0.7 cps
GCWS Hylon V[c]	11.0 ± 0.7 cps
Hylon VII[c]	5.5 ± 2.1 cps
GCWS Hylon VII[c]	10.0 ± 2.8 cps

[a] Data are shown as mean ± standard deviation of duplicate samples. B.U.= Brabender Unit. cps = centipoises.

[b] Viscosity was measured by Brabender Amylograph at 6% (w/w) starch concentration and was recorded at the end of the measurement.

[c] Viscosity was measured by Brookfield Viscometer at 3% (w/w) starch concentration with #1 spindle, at 30°C, 60 rpm.

Table 2. Ash Contents of Various Starches

Sample	Ash content (%)[a]
Waxy maize	0.09 ± 0.02
GCWS waxy maize	0.40 ± 0.07
Normal maize	0.08 ± 0.01
GCWS normal maize	0.14 ± 0.00
Hylon V	0.16 ± 0.02
GCWS Hylon V	0.81 ± 0.00
Hylon VII	0.22 ± 0.01
GCWS Hylon VII	0.81 ± 0.08

[a] Data are shown as mean ± standard deviation of duplicate samples.

Table 3. Lipid Contents of Various Starches

Sample	Lipid content (%)[a]
Waxy maize	0.05 ± 0.01
GCWS waxy maize	0.04 ± 0.01
Normal maize	0.46 ± 0.04
GCWS normal maize	0.35 ± 0.01
Hylon V	0.74 ± 0.06
GCWS Hylon V	0.45 ± 0.02
Hylon VII	0.86 ± 0.06
GCWS Hylon VII	0.64 ± 0.01

[a] Data are shown as mean ± standard deviation of duplicate samples.

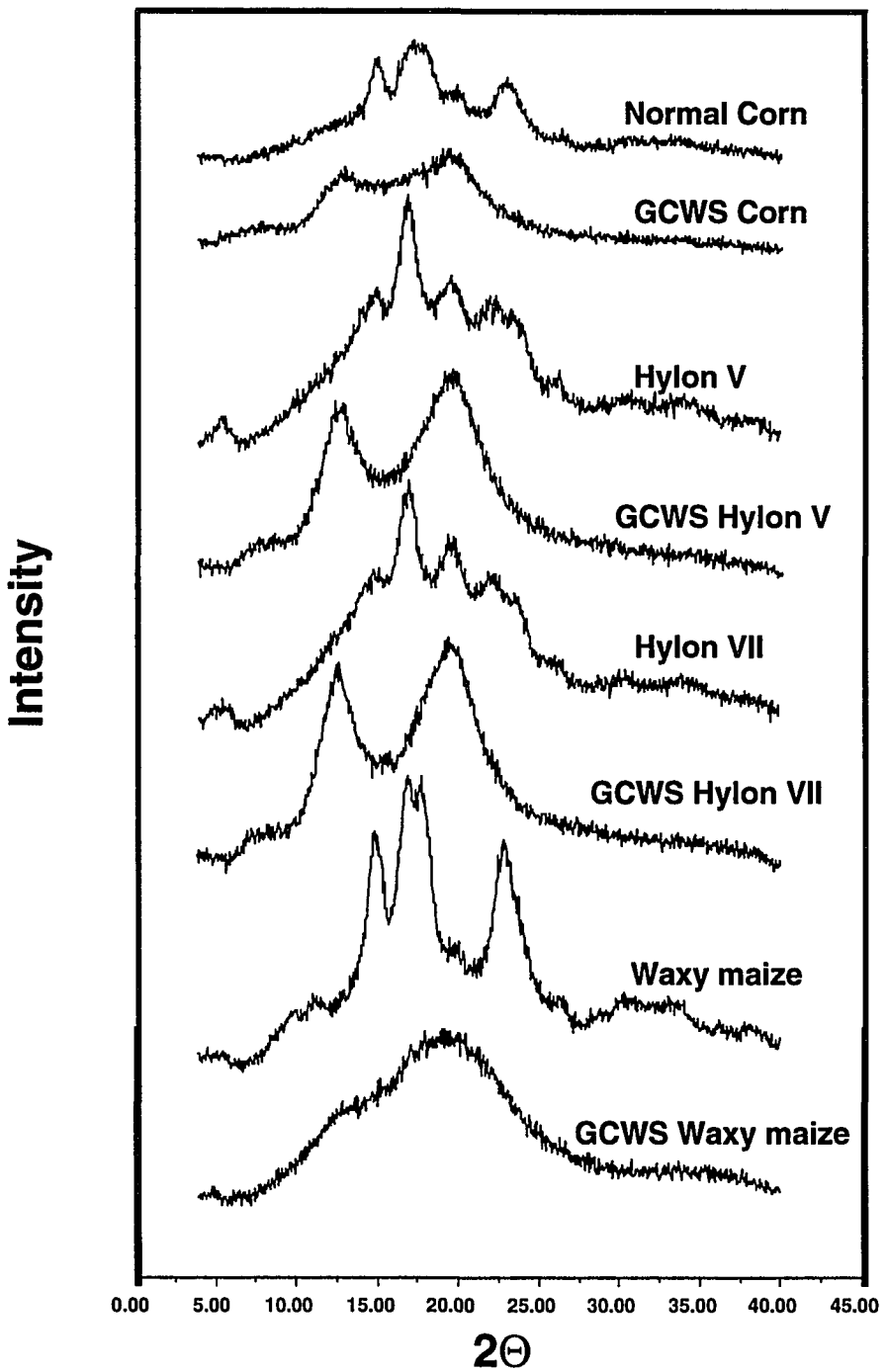

Fig. 1. X-Ray diffraction profiles of GCWS starches and their native counterparts. GCWS starches displayed V-type X-ray diffraction patterns, except GCWS waxy maize starch which exhibited an amorphous type.

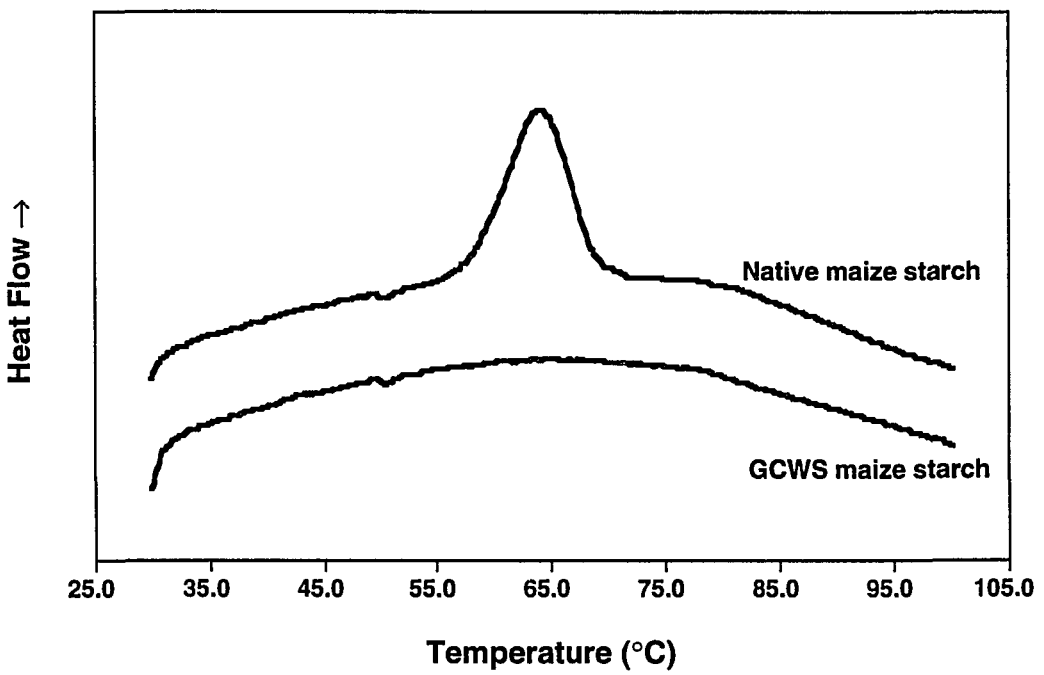

Fig. 2. DSC Thermograms of GCWS normal maize and its native counterpart. Samples (25%, w/w) were heated from 25 to 100°C at a rate of 10°C/min.

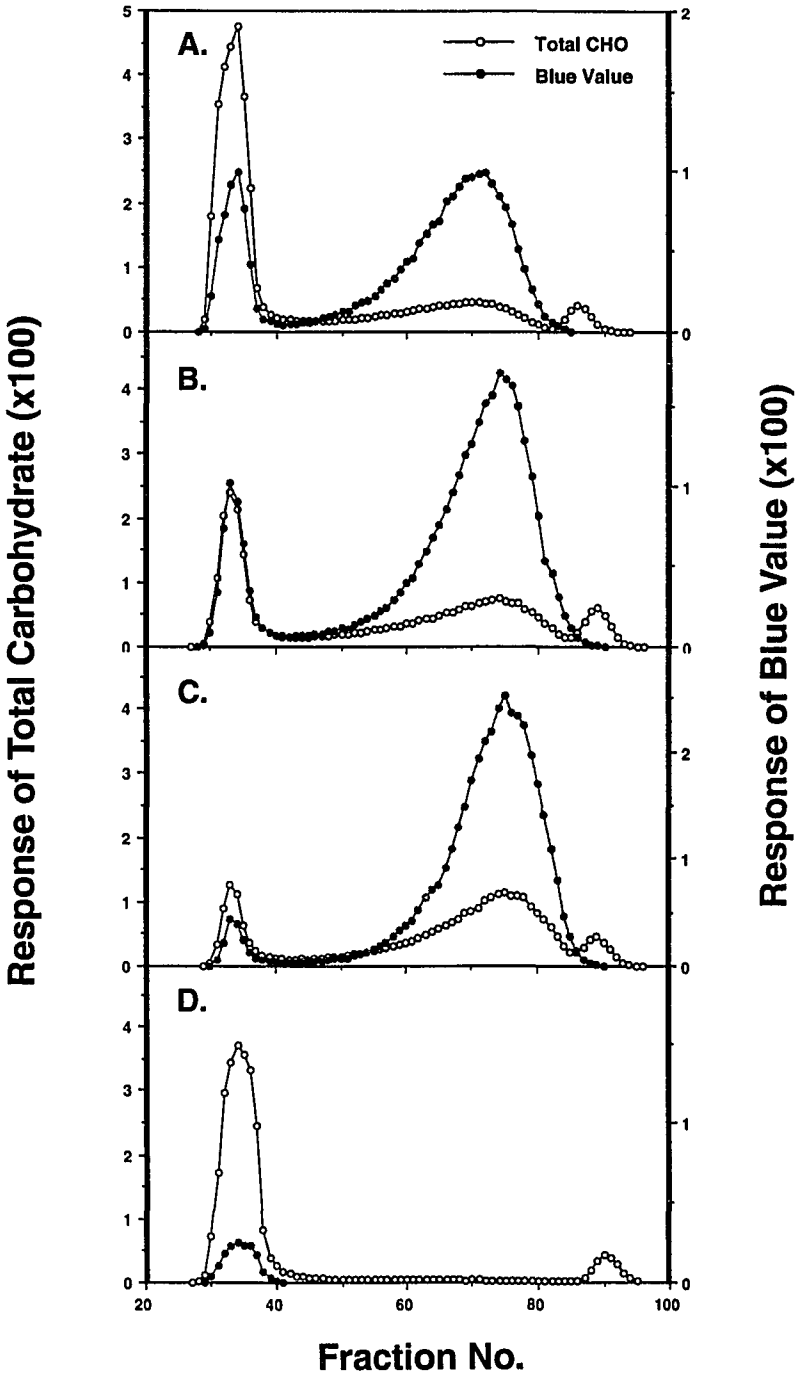

Fig. 3. Sepharose CL-2B gel permeation chromatograms of GCWS starches: **A**, normal maize starch; **B**, HA5 starch; **C**, HA7 starch; **D**, waxy maize starch. No degradation of starch molecules was found.

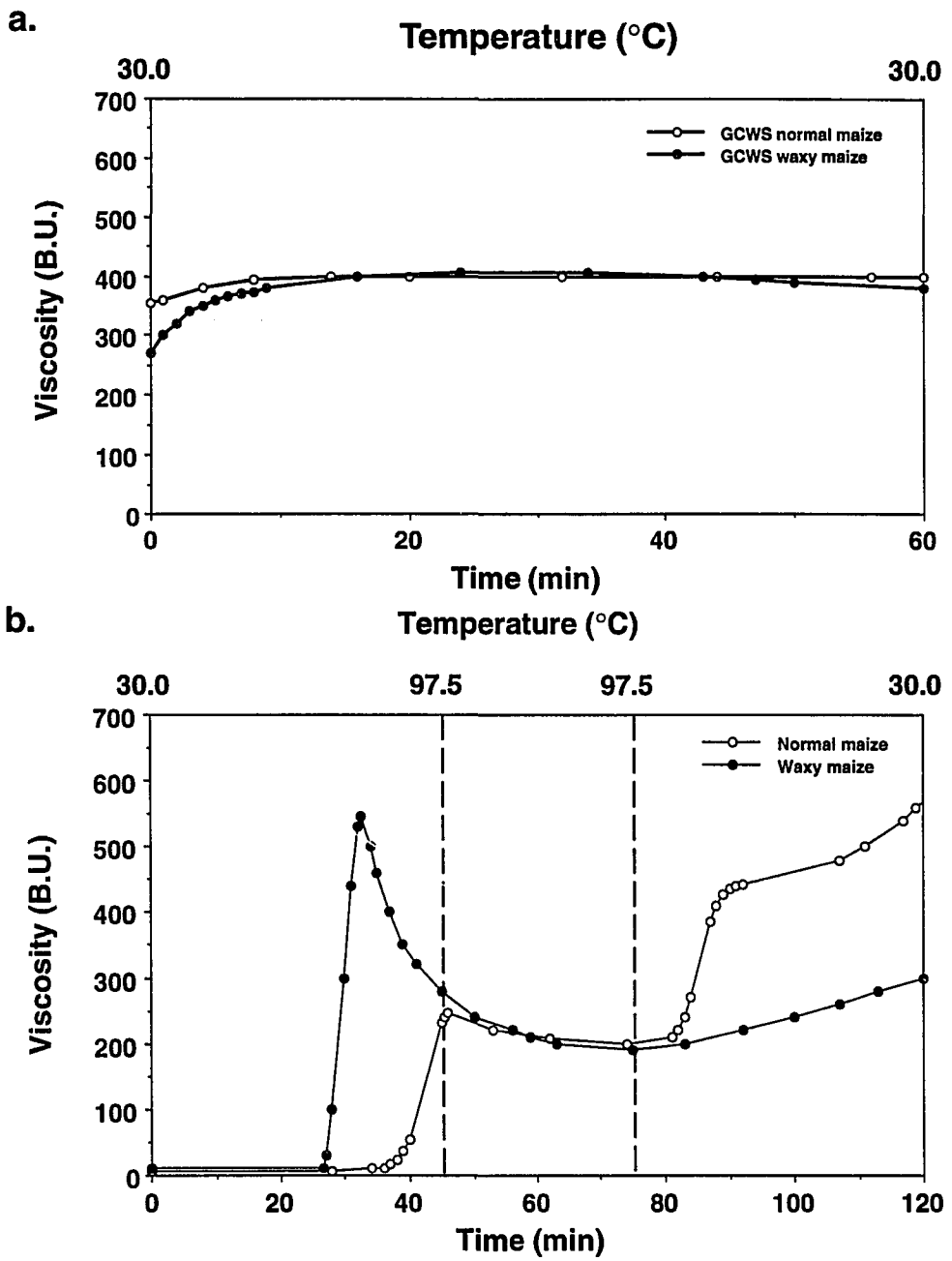

Fig. 4. Amylograms of GCWS normal maize and waxy maize starches and their native counterparts (6%, w/w); **a**, pastes were prepared by dispersing starches in cold water and transferred to the Brabender Amylograph operated at 30°C, 75 rpm for 60 min; **b**, pastes were subjected to a regular cooking process.

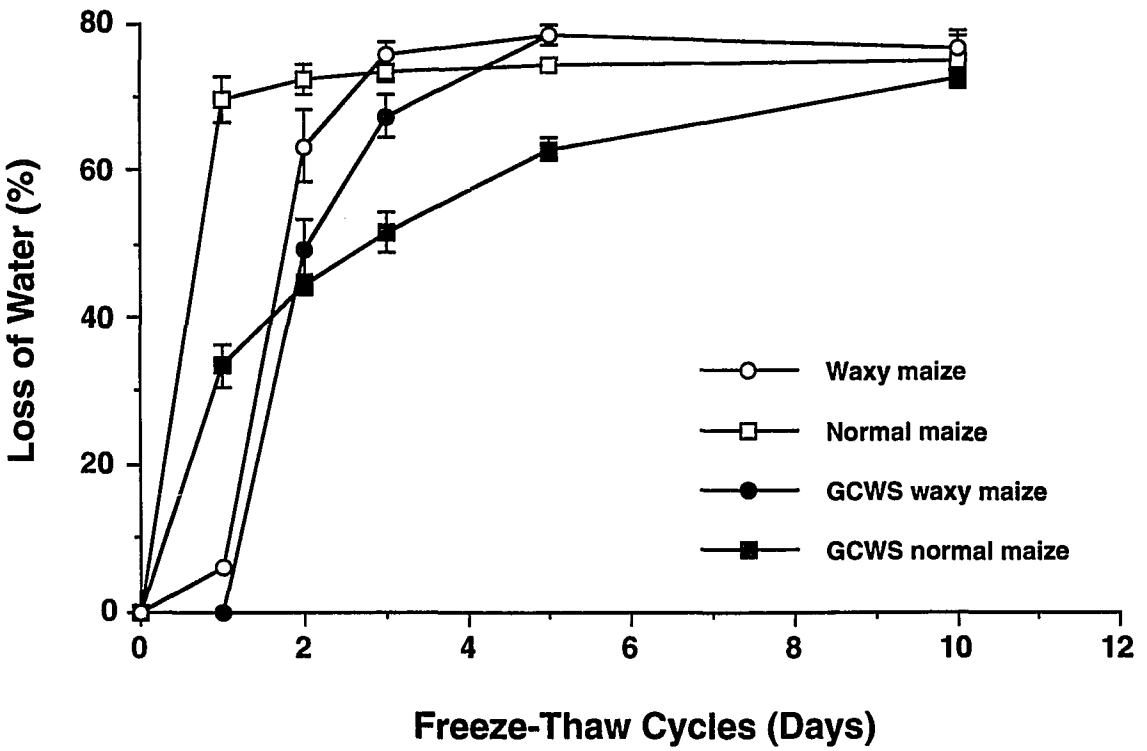

Fig. 5. Freeze-thaw stabilities of the GCWS normal maize and waxy maize starches and their native counterparts. Pastes (6%, w/w) of GCWS starches were prepared by dispersing starches in distilled water at room termperature and then transferred to amylograph operated at 30°C, 75 rpm for 1 hour. Pastes (6%, w/w) of native starches were prepared by the Brabender amylograph with a regular cooking cycle. Fifteen grams of each sample were taken for the study at the end of sample preparation. Data were means of four replicates.

PAPER III

EFFECTIVENESS OF GRANULAR COLD-WATER-SOLUBLE STARCH AS A CONTROLLED RELEASE MATRIX

Effectiveness of Granular Cold-Water-Soluble Starch As A Controlled Release Matrix[1]

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[1]Journal paper no.

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ABSTRACT

Granular cold-water-soluble (GCWS) starches (waxy maize, normal maize, Hylon V, and Hylon VII) prepared by alcoholic-alkaline treatment are potential encapsulation materials. The starch is soluble in cold water and is desirable for encapsulation of volatile and toxic chemicals. Controlled release of atrazine encapsulated in GCWS starch matrices was selected for the study. Results showed that atrazine was physically embedded in starch matrices. GCWS Hylon VII starch had the best encapsulation efficiency among all the starch types. The release rate of atrazine in aqueous ethanol solution (10%, v/v) was affected by starch varieties, particle sizes, and release temperature. Changes of pH between 5 and 9 in this study had no significant effect on the release rate of atrazine. Amylose content of a starch was related to release rate. Release rate of atrazine encapsulated in the GCWS starch decreased with the increase of amylose content of the starch, decreased with the increase of particle size, but increased with raising release temperature.

INTRODUCTION

During the past decades, controlled release technology has received increasing attention. A basic controlled release formulation includes an active agent (drugs, fertilizers, etc.) and a carrier (commonly a polymer). The active agent arranged in the carrier can be released at the target over a period of time at a controlled rate. Since release of active agents at a controlled rate would provide just enough concentration at all time, a much smaller amount would be needed to offer the same activity for a period of time. The loss of the active agents by degradation, leaching, evaporation, or surface run-off is also minimized (Fleming et al 1992, Boydston 1992).

Controlled release of bioactive agents encapsulated in starch-based matrices by different methods has been found effective. The encapsulation ability of some methods was based on chemical crosslinking (Wing et al 1987a, Shasha et al 1984, Trimnell et al 1982, Shasha et al 1981, Shasha et al 1976). However, use of a crosslinking agent sometimes confines applications of the encapsulated product in food or feed. In addition, environmental pollution of the crosslinking agents has been a matter of concern. Use of steam injection or twin-screw extrusion for preparing starch-based encapsulation without chemical modification were thus developed (Wing et al 1987b, Trimnell et al 1991, Carr et al 1991). The encapsulation ability of both methods were controlled by retrogradation of the

starch. The methods encountered some disadvantages. Both processes were heat required for gelatinizing starch, especially, for high amylose maize starch. As a result, both processes may not be suitable for those active agents which are heat labile. In addition, loss of agent by heating and inhalation of vapor were also concerned.

The objectives of present work were to evaluate the effectiveness of encapsulation of atrazine, a herbicide, by using GCWS starches prepared by alcoholic-alkaline treatment and to investigate the effects of environmental variables on releasing rate of herbicide.

MATERIALS AND METHODS

Materials

GCWS starches were prepared by the methods of Chen and Jane (Chen and Jane, submitted). GCWS normal maize, HA5, and HA7 starches were produced by treating native starches with aqueous alcohol (40%, w/w) and NaOH (3M) solution (starch : H_2O : absolute ethyl alcohol : 3M NaOH = 1.0 : 4.2 : 2.8 : 5.0, by weight) at 35°C. GCWS waxy maize starch was produced with weight proportions of starch to reagents as starch : absolute ethyl alcohol : 3M NaOH solution = 1.0 : 7.0 : 3.2, at 25°C. Dried potato amylose was purchased from Sigma Chemical Company (St. Louis, MO). Powdered atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) was a gift of CIBA-GEIGY Corporation (Greensboro, NC). Other chemicals were all reagent grade and were used without further treatment.

Methods

Complex formation between atrazine and amylose

The test was conducted by following Kuge and Takeo's method (1968). Potato amylose (1 g) was dispersed into boiling water (150 ml) in a 500-ml flask with vigorous stirring to give a clear solution. Atrazine (1 g) was added into the solution. The mixture was boiling for 10 min with stirring. The flask was then covered with aluminum foil and placed in a dewar flask filled half with boiling water. The dewar flask was sealed with

a lid and the hot potato amylose-atrazine mixture inside slowly cooled to room temperature over a period of 24 to 36 h. The cooled mixture was centrifuged at 8700 G for 30 min. The precipitant was rinsed with ethanol twice and dried in a vacuum oven at 60°C for at least 4 h. Two control samples, one without any complexing agent and the other with n-butyl alcohol as a complexing agent, were also prepared in the same way. The dried samples were kept in a sealed bottle for further X-ray diffraction analysis.

X-Ray diffraction

X-Ray diffraction patterns of the amylose samples were recorded on a Simens D500 diffractometer with Cu X-ray tube operated at 40 KV and 25 mv. A step-scan was set at an angle of 0.05° per step with a counting of 2 seconds.

Encapsulation of atrazine

Three grams of atrazine (10% of starch base) were weighed and added into a 1000-ml beaker containing about 70 g of distilled water. The suspension was stirred constantly to disperse atrazine evenly. GCWS starch (30 g, dry weight) was carefully mixed with atrazine suspension. The mixture was thoroughly mixed by a hand mixer. Following mixing, the semi-solid mass was dried at 60°C overnight in a forced-air oven. The dried solid was then ground and sieved to 9-20 mesh and 20-35 mesh.

Scanning electron microscopy

Scanning electron micrographs (SEM) were taken with a JEOL JSM-35 scanning electron microscope (JEOL Ltd., Tokyo, Japan). The starch sample was sprinkled on a 3M metallic tape mounted on a brass discs and coated with platinum/palladium alloy (60/40).

Recovery and Encapsulation efficiency (EE) of atrazine

The measurements were conducted by following the method of Carr et al (1991). Sieved samples (0.5 g) were subjected to a nitrogen determination by Kjeldahl method to quantify atrazine in the samples. Recovery of atrazine was then calculated by following equation:

$$\text{Recovery (\%)} = \frac{\text{mg of atrazine in product}}{88.8} \times 100\%$$

The value of 88.8 was a theoretical value of atrazine in the product which was calculated from encapsulation process. Chloroform-washed samples (0.5 g) were also analyzed by the same procedure. Atrazine on the surface of the samples but encapsulated in the samples was washed off with $CHCl_3$. The total nitrogen of washed samples were determined by Kjeldahl method to quantify encapsulated atrazine. The encapsulation efficiency (EE) was calculated by following equation:

$$\text{EE (\%)} = \frac{\text{mg of atrazine in chloroform-washed product}}{\text{mg of atrazine in the unwashed product}} \times 100\%$$

Swellability

Swellability was determined by following the method of Carr et al (1991). Sieved samples (1 g) were placed in a 10-ml graduate cylinder and the dry volume was recorded. Ten ml of aqueous alcohol solution (10%, v/v) were added to each sample. The graduate cylinders were placed in a water bath which was kept at a designated temperature. Two temperatures, 30°C and 40°C, were chosen for studying their effects on the swellability of starch-encapsulated atrazine products. The percent increase in volume of the swollen samples was calculated after 24 hr.

Release of encapsulated products

Release of atrazine was conducted by monitoring the concentration of atrazine in the supernatant of the media (Carr et al 1991). Chloroform-washed samples containing about 10.0 mg of atrazine were added into a 125-ml polyethylene bottle filled with 75 ml of aqueous ethanol solution (10%, v/v). The bottles sealed with caps were then agitated in a water bath (Versa-Bath S, model 236, Fisher Scientific) with shaking at 100 strokes/min for up to 72 hr. The temperature of the water bath was controlled at 20, 30, or 40°C to study effects of temperature on releasing rate. Samples of supernatant were taken at 1, 2, 4, 24, 48, and 72 hr intervals, and the concentration of released atrazine was determined by measuring its absorbance at 230 nm using a spectrophotometer (U-2000, Hitachi, Japan). A

calibration curve with standard solutions was also prepared. Release of atrazine was then calculated in percentage.

Statistical analyses

The duplicate data were analyzed statistically by using the SAS program (SAS Institute 1990). The Duncan's multiple range test was applied to compared mean values.

RESULTS AND DISCUSSION

GCWS starches prepared by alcoholic-alkaline treatment provide an effective controlled release of atrazine. When the GCWS starch was added into atrazine-distilled water suspension at room temperature, the mixture became a viscous paste. After vigorous mixing, all starch-atrazine mixtures but the one with GCWS waxy maize became cake-like semi-solids. When the semi-solid mixtures were cut with a spatula, the cut edges were very smooth, especially the mixtures containing GCWS high-amylose starches. It could be attributed to the retrogradation of starches. A marked physical feature of retrograded starch gels was the progressive increase in gel firmness. This feature was advantageous for preparing the GCWS starch-encapsulated products.

The mechanism which hold atrazine in starch matrices is of interest. Figure 1 shows a scanning electron micrograph of GCWS starch-encapsulated atrazine product. It appeared that atrazine particles were embedded in starch matrices. An attempt to grow amylose-atrazine helical complex has been made by incubating amylose with atrazine in an aqueous suspension. X-Ray study of the incubated amylose showed a B-type diffraction. It was well known that retrograded amylose, as well as retrograded starch, normally exhibited the B-type X-ray diffraction pattern. As a result, it was concluded that atrazine could not form a complex with amylose at the specified condition. The inability of the

complex formation of atrazine might be affected by its low solubility in water, which was about 70 ppm. It might be necessary that the concentration of a complexing agent was large enough to force complex formation against the entropy of coiling of amylose (Kuge and Takeo 1968).

Figure 2 shows that encapsulation efficiency was significantly affected by amylose content. The result showed that higher amylose content of the GCWS high-amylose starches, including HA5 and HA7 starches, increased the encapsulation efficiency of atrazine ($\alpha = 0.05$). However, recovery of atrazine was not significantly different ($\alpha = 0.05$). It has been reported that higher amylose content lowered the encapsulation efficiency of butylate encapsulated by jet-cooked corn starch (Wing et al 1988). The contrary results might be caused by the differences of the preparation between a jet-cooked starch and a GCWS starch.

When GCWS starch-encapsulated atrazine products were placed in aqueous alcohol solution (10%, v/v), they displayed different extent of swelling (Fig. 3). The data clearly showed two distinct groups. One group included the products made with GCWS waxy maize and normal maize starches, and the other group was those made with GCWS HA5 and HA7 starches. The former group which has lower amylose content swelled more than the latter group which has higher amylose content. The result was consistent with the data reported elsewhere (Carr et al 1991, Wing et al 1988). It was interpreted as a tight association of

amylose network, which restricted the ability of starch matrices on rehydration. Increase of temperature of the medium could improve the swellability of GCWS starch-encapsulated atrazine products (Fig. 3).

Data of release studies showed a linear relationship when plotted as cumulative percent of released atrazine versus square root of time (Fig. 4). The results followed the equation for matrix diffusion of a controlled release (Higuchi 1963):

$$\text{Cumulative percent of released atrazine (\%)} = k(t)^{1/2}$$

where t was time (hr) and k was a constant. The k constants calculated by linear regression analyses were used as references for comparing release rate. The greater the k constant, the faster the release rate. Effects of variables, such as particle size, temperature, and pH, on the release rate of GCWS starch-encapsulated atrazine products were investigated. Table 1 summarized the k constant for each sample under different conditions. In general, the k constants of the products followed the swellability. The higher the swellability, the faster the release rate. Figure 5 shows effects of release temperature and particle size on release rate. The release rate increased with increase of temperature and decrease of particle size. The result was consistent with the data previously reported (Trimnell et al 1991, Trimnell and Shasha 1990). However, the effect of particle size on the release rate was not significant at 20°C and 30°C in this study ($\alpha = 0.05$). Effects

of pH changes were also studied. The changes of pH between 5 and 9 were chose for the study because most crops were grew within this range. Therefore, the controlled release of atrazine, which was used to control weeds and some grasses, might be used within this range. The result showed that changes of pH between 5 and 9 in this study had no significant effects on release rate ($\alpha = 0.05$) (Fig. 6).

CONCLUSIONS

The scanning electron micrograph and the study of complex formation between amylose and atrazine revealed that atrazine appeared to be simply entrapped in starch matrices. The release rate followed the swellability of the encapsulated products. The release rate can be controlled by amylose contents of GCWS starches, by particle sizes, or by release temperatures. As the amylose content increased, the release rate decreased due to the retrogradation of amylose. Small particle sizes or higher temperature promoted the swelling of the starch-encapsulated atrazine product, and resulted in a faster releasing rate. The release rate was not affected by pH changes between 5 and 9.

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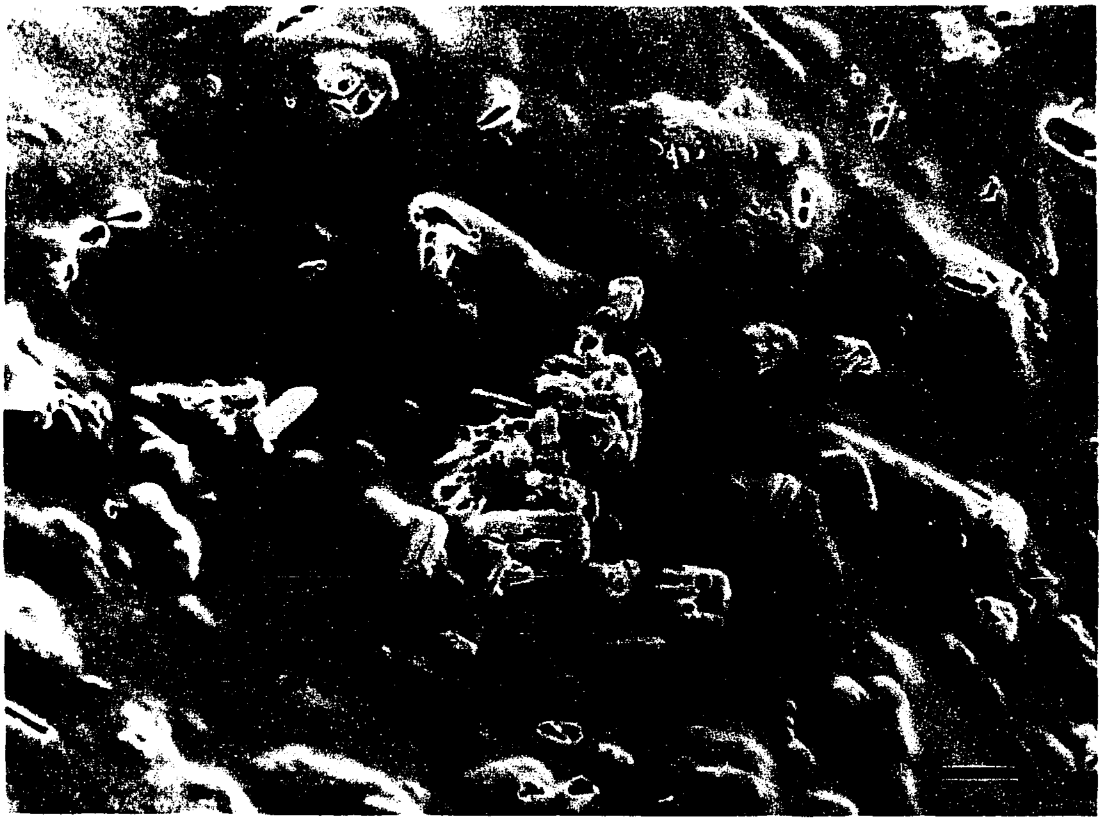
Table 1. K constants of controlled release of GCWS starch-encapsulated atrazine product under different conditions[a]

GCWS Starch Matrix	pH			Size: 9-20 mesh, Temp. (°C)			Size: 20-35 mesh, Temp. (°C)		
	5	7	9	20	30	40	20	30	40
GCWS waxy maize	7.4^{a}	7.2^{ab}	7.5^{a}	5.3^{j}	7.4^{fg}	10.1^{c}	5.6^{ij}	8.0^{ef}	16.6^{a}
GCWS normal maize	6.8^{b}	6.8^{b}	7.1^{ab}	5.1^{j}	6.8^{h}	9.3^{d}	5.6^{ij}	7.4^{fg}	16.9^{a}
GCWS HA5	6.0^{c}	6.0^{c}	6.1^{c}	4.0^{k}	6.0^{i}	8.6^{e}	5.4^{ij}	7.2^{gh}	11.0^{b}
GCWS HA7	5.4^{d}	5.4^{d}	6.0^{c}	3.7^{k}	5.4^{ij}	8.0^{f}	5.4^{ij}	6.8^{h}	11.0^{b}

[a] Means not sharing the same letter are significantly different within column of pH or size (Duncan's multiple range test, $\alpha = 0.05$).

Fig. 1. Scanning electron micrograph of GCWS starch-encapsulated atrazine products. The size bar represents 10 μm.



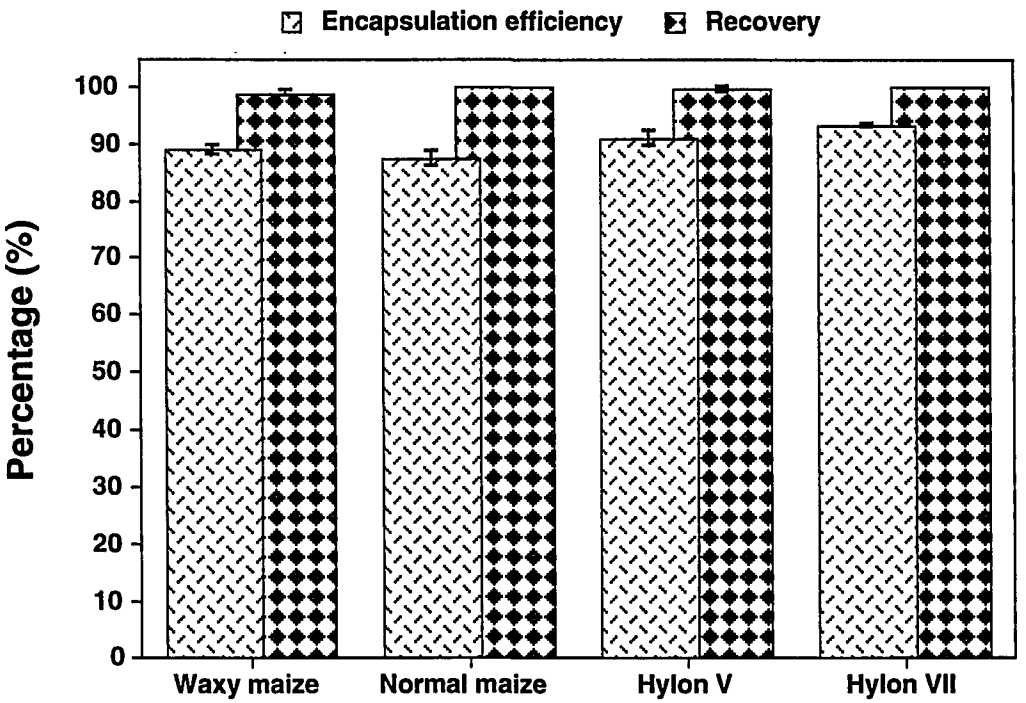


Fig. 2. Encapsulation efficiency and recovery of atrazine in GCWS starch-encapsulated atrazine products. Values were means of two replicates.

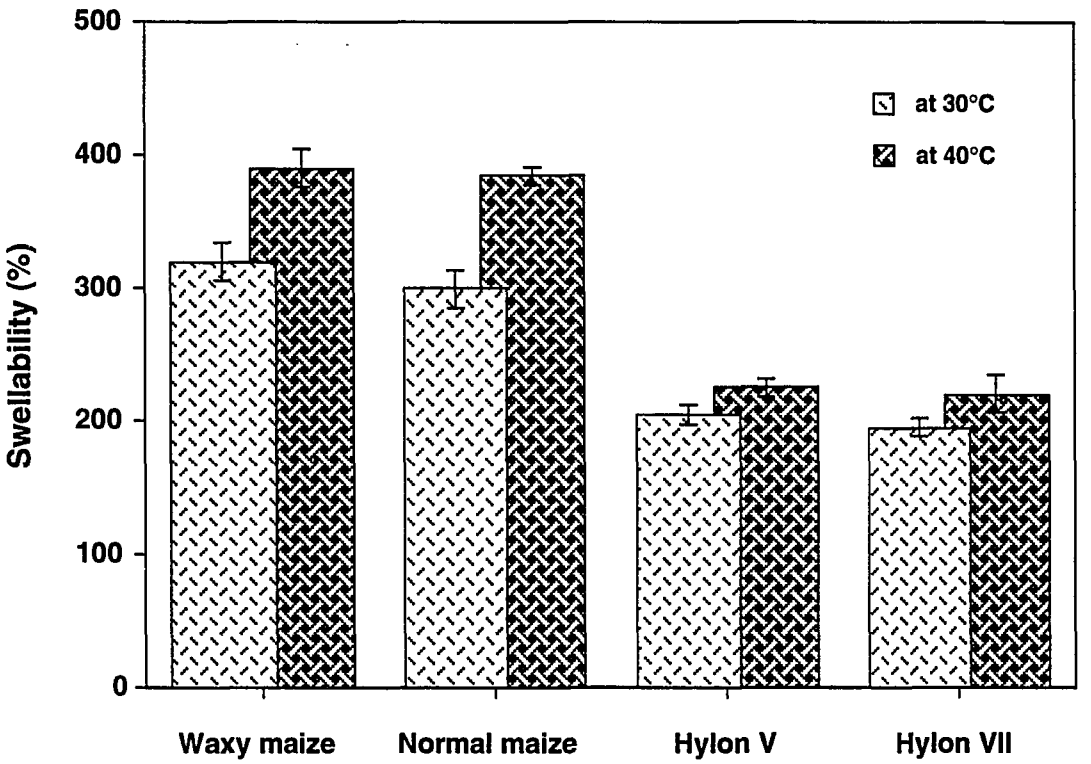


Fig. 3. Effect of temperature on swellability of GCWS starch-encapsulated atrazine products. Products of 9-20 mesh were soaked in aqueous alcohol solution (10%, v/v) for 24 h. Values were means of two replicates.

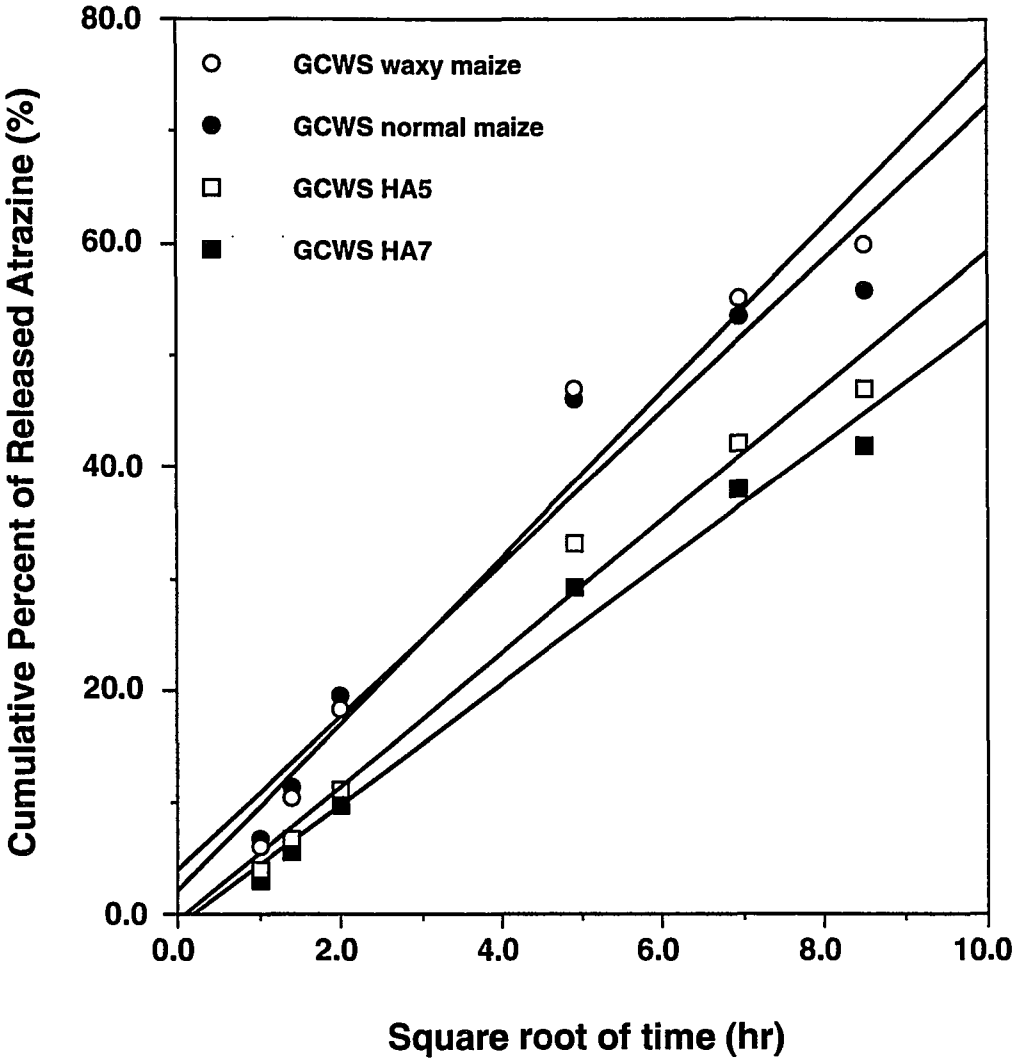


Fig. 4. Controlled release of GCWS starch-encapsulated atrazine products. Linear relationship was found after a square root-transformation. Samples of 9-20 mesh were used for the study at 30°C, pH 5. Data were means of two replicates. Statistical analyses of K constants were summarized in Table 1.

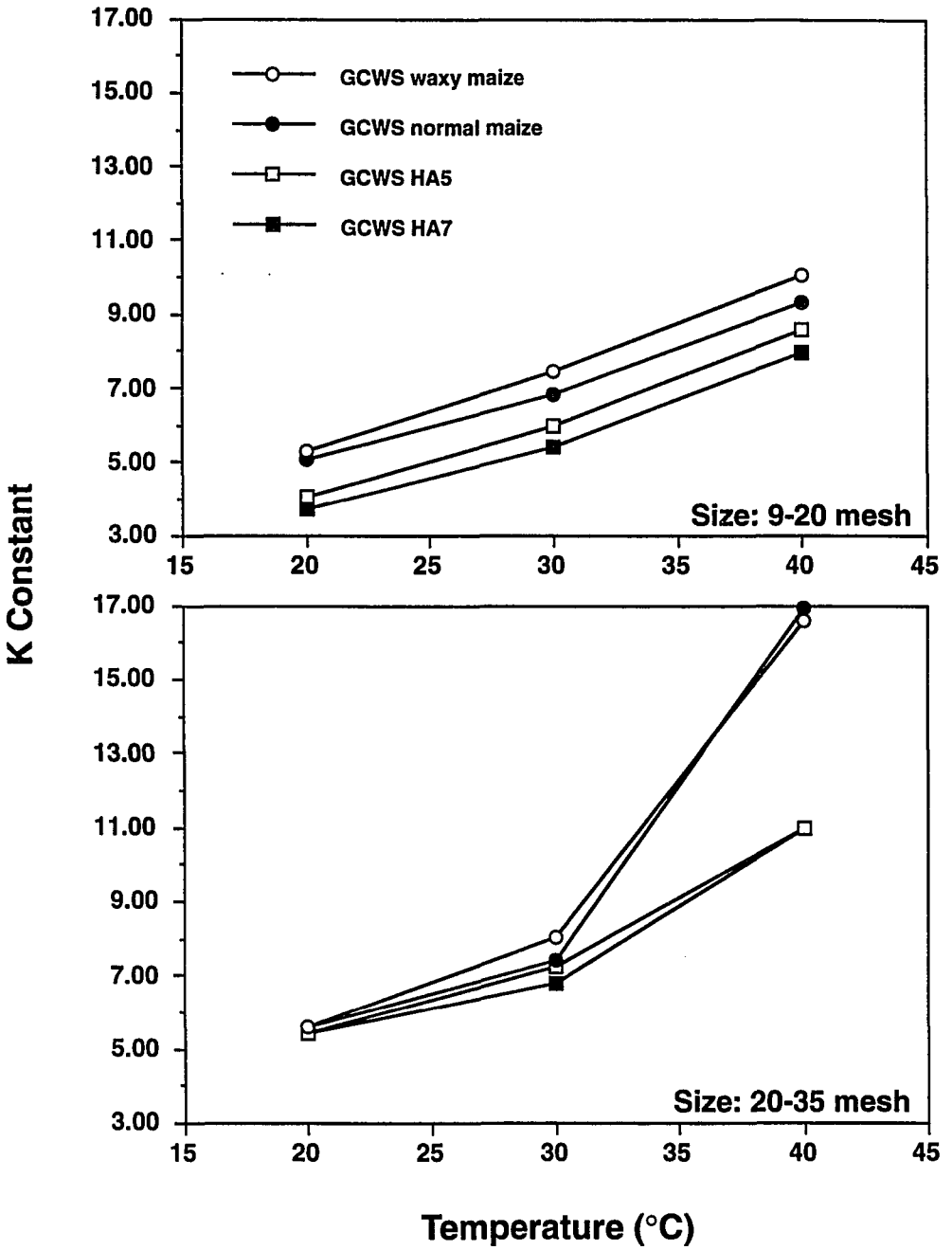


Fig. 5. Effects of temperature and particle sizes on release rate of GCWS starch-encapsulated atrazine products. Data were means of two replicates. Statistical analyses of K constants were summarized in Table 1.

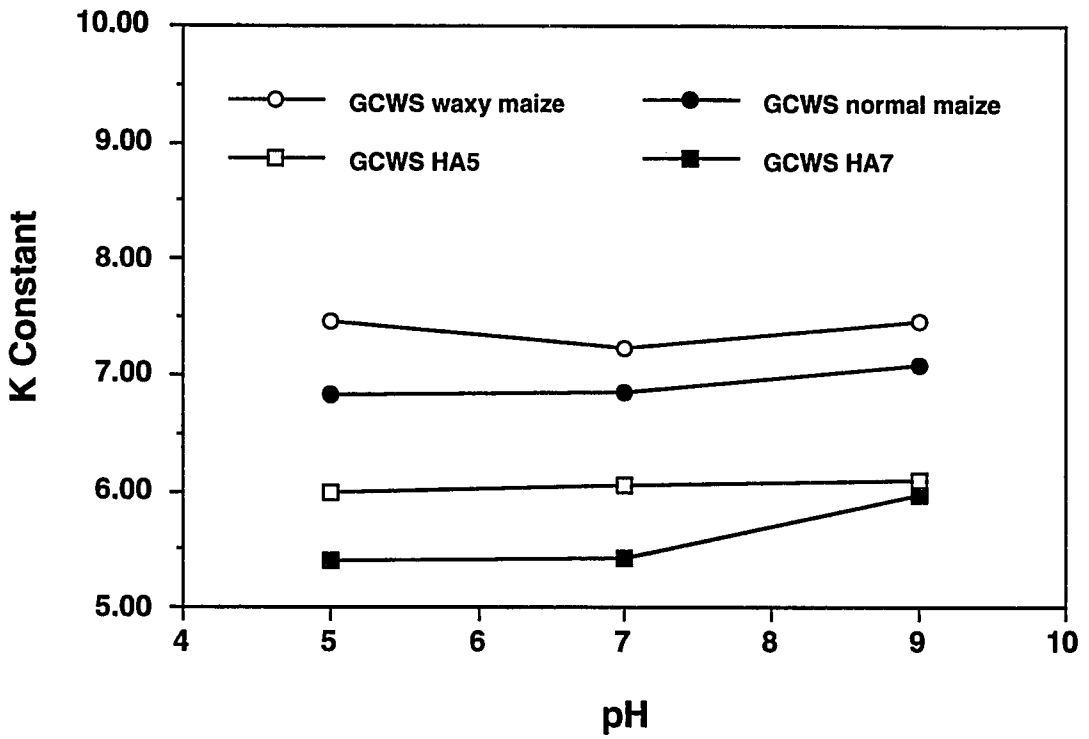


Fig. 6. Effect of pH on release rate of GCWS starch-encapsulated atrazine products. Products of 9-20 mesh were used for the study. The temperature was controlled at 30°C. Data were means of two replicates. Statistical analyses of K constants were summarized in Table 1.

GENERAL CONCLUSIONS

In a study to prepare GCWS starches, the alcoholic-alkaline treatment provides an effective method for the preparation of GCWS starches, especially GCWS high-amylose and waxy starches. From the study, it is concluded that the efficacy of the method depends mainly on the relative amounts of each of the three components present, alkali, alcohol, starch, and on the reaction temperature. Because of the differences on amylose contents, crystalline structures, and branch-chain length of amylopectins, different species of the starch response differently to the treatment.

A mechanism has been proposed to explain the formation of GCWS starches. The starch crystalline structures are changed from double helices to single helices by the alcoholic-alkaline treatment. The change of the crystalline structure results in the GCWS starches soluble/swollen in cold water. Meanwhile, the entanglement of amylose with amylopectin molecules inside the granules retains the swollen granules in one entity.

The GCWS starches can provide an effective controlled-release of bioactive agents. Potential benefits of using GCWS starches prepared by alcoholic-alkaline treatment for controlled release include: using high-amylose starches with high tendency of slow releasing rate, processing at room temperature, enhancing the application for heat-labile active agents, and safety to operators.

Although current study has provided a clear picture on GCWS starches prepared by alcoholic-alkaline treatments, further studies are recommended. Future explorations may include: 1) minimizing the use of ethanol and NaOH solutions; 2) recycling ethanol and NaOH solutions; 3) further exploring the temperature effects; 4) testing the herbicidal effects of encapsulated products; 5) field tests. In addition, the complex formation between amylose and an active agent may have some effects on controlled release. Therefore, development on advanced applications of GCWS high-amylose starches is of interest.

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