


1999

Physicochemical properties of selected root and tuber starches and characterization of extruded, chemically modified corn starches

Andrew Edward McPherson
Iowa State University

Follow this and additional works at: <https://lib.dr.iastate.edu/rtd>

 Part of the [Agricultural Science Commons](#), [Agriculture Commons](#), [Agronomy and Crop Sciences Commons](#), [Food Science Commons](#), and the [Plant Biology Commons](#)

Recommended Citation

McPherson, Andrew Edward, "Physicochemical properties of selected root and tuber starches and characterization of extruded, chemically modified corn starches " (1999). *Retrospective Theses and Dissertations*. 12592.
<https://lib.dr.iastate.edu/rtd/12592>

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

**A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600**

**Physicochemical properties of selected root and tuber starches and characterization of
extruded, chemically modified corn starches**

by

Andrew Edward McPherson

**A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of**

DOCTOR OF PHILOSOPHY

Major: Food Science and Technology

Major Professor: Jay-lin Jane

Iowa State University

Ames, Iowa

1999

UMI Number: 9924746

**UMI Microform 9924746
Copyright 1999, by UMI Company. All rights reserved.**

**This microform edition is protected against unauthorized
copying under Title 17, United States Code.**

UMI
300 North Zeeb Road
Ann Arbor, MI 48103

Graduate College
Iowa State University

This is to certify that the Doctoral dissertation of

Andrew Edward McPherson

has met the dissertation requirements of Iowa State University

Signature was redacted for privacy.

Committee Member

Signature was redacted for privacy.

Committee Member

Signature was redacted for privacy.

Committee Member

Signature was redacted for privacy.

Committee Member

Signature was redacted for privacy.

Major Professor

Signature was redacted for privacy.

For the Major Program

Signature was redacted for privacy.

For the Graduate College

TABLE OF CONTENTS

GENERAL INTRODUCTION	1
Dissertation Organization	3
LITERATURE REVIEW	4
General Properties of Starch	4
Characteristics of Amylose	4
Characteristics of Amylopectin	6
Intermediate Component	9
Organization of the Starch Granule	10
Minor Components of Starch	11
Gelatinization and Retrogradation	14
Modification of Starches	16
Applications of Starch in Foods	21
References	22
COMPARISON OF WAXY POTATO WITH OTHER ROOT AND TUBER STARCHES	35
ABSTRACT	35
INTRODUCTION	36
MATERIALS AND METHODS	37
RESULTS AND DISCUSSION	42
REFERENCES	49
EFFECTS OF EXTRUSION PARAMETERS ON CROSS-LINKED HYDROXYPROPYLATED CORN STARCHES. I. PASTING PROPERTIES	68
ABSTRACT	68
INTRODUCTION	68

MATERIALS AND METHODS	70
RESULTS AND DISCUSSION	74
CONCLUSIONS	80
REFERENCES	80
EFFECTS OF EXTRUSION PARAMETERS ON CROSS-LINKED HYDROXYPROPYLATED CORN STARCHES. II. MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION	95
ABSTRACT	95
INTRODUCTION	96
MATERIALS AND METHODS	97
RESULTS AND DISCUSSION	100
CONCLUSIONS	108
REFERENCES	108
GENERAL CONCLUSIONS	128
ACKNOWLEDGMENTS	130

GENERAL INTRODUCTION

Starch is the primary energy reserve in the plant kingdom and is used for growth of the plant. In green plants starch is found in roots, tubers, stems, leaves, fruits, and seeds. Starch is largely composed of two polymers of glucose: the essentially linear amylose and the branched amylopectin. In general, starches contain 20-30% amylose and 70-80% amylopectin. The ratio and molecular structures of these two polymers greatly influence the physical properties of starch. Plants synthesize and deposit starch in the form of granules. Starch granules are typically classified according to botanical origin, size, shape, and x-ray diffraction pattern. Within starch granules, the molecular structures and arrangement of amylose and amylopectin produce alternating concentric layers of crystalline and amorphous material. The crystalline layers are composed almost entirely of highly ordered amylopectin branches which are arranged into double helices. The packing arrangements of double helices within the crystalline layers give rise to the three known x-ray diffraction patterns of native starches: A, B, and C. The amylopectins of A-type starches are known to have relatively short average branch chain lengths (dp 23-29) whereas those of B-type starches have amylopectins of relatively long average branch chain lengths (dp 30-44). The amylopectins of C-type starches display intermediate average branch chain lengths (dp 26-30). The molecular structures, granular characteristics, and crystalline arrangements depend greatly on botanical source, maturity, growth conditions, and sample history.

The properties of native starches may not achieve the specific functionalities needed in various food and industrial applications. To overcome these limitations chemical, physical and genetic modifications have been utilized to enhance or modify the properties of starches. With the suitable modification(s) starch paste clarity is increased, retrogradation is retarded, and stability to acid, shear and heat are increased. Modification of starches can yield customized starches for specific applications

Extrusion of starch and starch-based foods has become a well accepted processing method. Starch extrusion variables including temperature, moisture, shear, pressure, reagents, and processing time allow for production of novel products. Using extrusion, the potential exists for production of physically and/or chemically modified starches which have unique properties and functionalities from those of conventionally modified starches.

A fundamental understanding of the relationship between the molecular structures of starch and its physical properties is critical in choosing starches for specific applications. Similarly, an understanding of the effect of extrusion processing parameters on starch molecular structures and the physical properties of resulting extruded starches will guide development of processing parameters to produce specific physical properties.

The objectives of this study are to investigate and relate the molecular structures of normal potato, waxy potato, yam, and sweet potato starches to their physical properties, to examine the effects of extrusion variables on the pasting properties of cross-linked hydroxypropylated corn starches, and to examine the molecular structures of extruded, cross-linked hydroxypropylated corn starches.

Dissertation Organization

This dissertation consists of three papers. The first paper, "Comparison of Waxy Potato with Other Root and Tuber Starches", has been accepted for publication by Carbohydrate Polymers. The second paper, "Effects of Extrusion Parameters on Cross-Linked Hydroxypropylated Corn Starches. I. Pasting Properties", will be submitted to Cereal Chemistry for publication. The third paper, "Effects of Extrusion on Cross-Linked Hydroxypropylated Corn Starches. II. Morphological and Molecular Characterization," will be submitted to Cereal Chemistry for publication. The three papers are preceded by a General Introduction and Literature Review and followed by a General Conclusion and Acknowledgments.

LITERATURE REVIEW

General Properties of Starch

Starch, produced via photosynthesis, is the main energy reserve in higher plants. Starch is found throughout nature in leaves, stems, fruits, nuts, roots, and tubers (Lineback, 1984, Swinkels, 1985, Robyt, 1998). In nature, starch is synthesized in granules. Starch granules are composed mainly of two polysaccharides: amylose and amylopectin. Minor amounts of lipids, proteins, and phosphorous are also contained. The composition of the granule and the molecular structure of its components are greatly affected by maturity, mutation, growth conditions, and cultivar (Boyer et al. 1976, Lineback 1984, Swinkels 1985; Asaoka et al. 1985, Morrison and Gadan 1987, Noda et al. 1996). Starch comprises a large portion of the human diet and contributes important functional properties to food products. Starch offers great functional diversity in its properties through cultivar differences and various chemical and physical modifications which contribute to the importance of starch in food and industrial applications.

Characteristics of Amylose

Amylose is a primarily linear polysaccharide of D-glucose units linked α -1 \rightarrow 4 with a very low amount of α 1 \rightarrow 6 linked branches (Greenwood 1964, French 1973, Banks and Greenwood 1975, Robyt 1998). The molecular size of the amylose fraction of starches range from a number average degree of polymerization (dp_n) of 2110 for potato to 990 for maize

(Takeda et al. 1988, Suzuki et al. 1994). Amylose from cereal starches is generally smaller than that from other sources (Hizukuri 1996). The linear nature of amylose was generally accepted until Peat et al. (1949) found that crystalline sweet potato β -amylase hydrolyzed only 70% of amylose to maltose. The β -amylolysis limit of a truly linear amylose molecule would be 100%. However, the β -amylolysis of amylose ranges from 70-82% and increases to 100% with addition of pullulanase (Banks and Greenwood 1967, Banks et al. 1973, Greenwood 1976, Hizukuri et al. 1981, Hizukuri et al. 1983a, Takeda and Hizukuri, 1989, Cura et al. 1995). In solution, amylose exists in random coils but rapidly complexes with itself or appropriate complexing agents to form imperfect crystallites that precipitate rapidly from solution (Whistler 1965, Miles et al. 1984, Colonna et al. 1992). Amylose, in neutral aqueous solution, forms a helix with 6-8 glucose units per turn (Rundle and French 1943, Banks et al. 1971, Yamashita and Monobe 1971, French and Murphy 1977, Wu and Sarko 1978, Davies et al. 1980, Billiaderis and Galloway 1989). French (1972) suggested that the strands of the double helix of retrograded amylose are left handed. In solution, the retrogradation of amylose is rapid, producing large and imperfect crystallites (Flory 1953, Greenwood 1964). The precipitate is a mixture of amorphous and crystalline regions and can display A or B-type x-ray diffraction patterns (Kainuma and French 1971, Wu and Sarko 1978, Kitamura et al. 1984, Buleon et al. 1984, Jane and Robyt 1984, Ring et al. 1987, Le Bail et al. 1993, Colonna et al. 1992, Cairns et al. 1995). It has been observed that amylose from different sources with varying chain lengths retrograde at different rates, with amylose of dp 80-100 retrograding most readily (Whistler and Johnson 1964, Whistler 1953,

Pfannenuller et al. 1971, Gidley et al. 1986, Gidley et al. 1989, Eerlingen et al. 1993). The rate and extent of amylose retrogradation is affected by many factors including temperature, pH, salt or sugar content, and storage time. The inter-chain associations or retrogradation of amylose is thought to be responsible for the retrogradation of starch gels (Miles et al. 1984, Billiaderis and Seneviratne 1990).

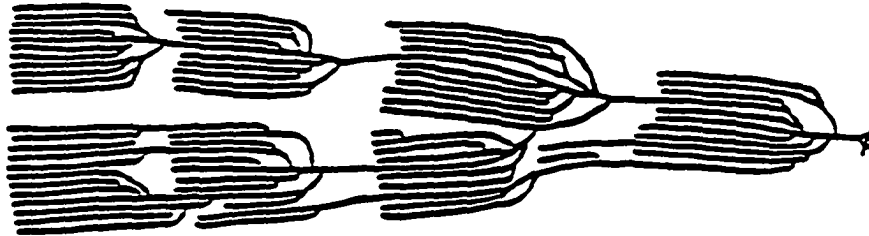
Amylose and iodine form a complex which is dark blue in color and is conveniently used to measure the amylose content of starches (Bates et al. 1943, Lansky et al. 1949, Banks et al. 1971, Banks et al. 1974, Pfannemuller 1978). The intensity of the blue color from the iodine-amylose gives information about the relative length of the amylose chains (Bailey and Whelan 1961). Potentiometric titration of amylose with iodine has been used to determine the amylose content of starches (Schoch 1964, Takeda et al. 1983, Kasemsuwan and Jane 1995). Long-chain alcohols and lipids can form complexes with amylose that are able to prevent or retard retrogradation (Schoch 1942, Lansky et al. 1949, Kuge and Takeo 1968, Hibi and Kuge 1987, Gudmundson and Eliasson 1990, Eerlingen et al. 1994). These complexes can be used to separate amylose from amylopectin and to modify the properties of amylose in a system containing complexing agents (Schoch 1942, Winter and Sarko 1972, Winter and Sarko 1974, Rutenberg 1980, Jane et al. 1985, Biliaderis and Galloway 1989).

Characteristics of Amylopectin

Amylopectin is a branched polysaccharide of D-glucose units linked α -1 \rightarrow 4 containing approximately 5% α -1 \rightarrow 6 branch points. The average branch-chain length is

approximately dp 20 (Whistler and Daniel 1984, Robyt 1998). High amylose maize starch has an average branch-chain length of above dp 30 (Hizukuri et al. 1983a, Hizukuri 1985, Jane and Chen 1992). Molecular structures, including average branch chain length of amylopectin, have been correlated with starch x-ray diffraction type (French 1972, Hizukuri et al. 1983a, Lineback 1984, Hizukuri 1985, Manners 1989, Jane et al. 1997). Shorter average amylopectin branch-chain lengths are well correlated to A-type starches whereas longer average branch-chain lengths are correlated to B-type starches and intermediate average branch-chain lengths correlated to C-type starches (Hizukuri et al. 1983, Hizukuri 1985, Chen and Jane 1994, Hanashiro et al. 1996). Increasing branch-chain length results in higher gelatinization temperatures (Jane et al. 1992), and higher paste viscosity and turbidity of starch solution (Jane and Chen 1992). The conceptual model of amylopectin contains three types of chains: A, B, and C. A chains are connected to B and C chains through an α 1 \rightarrow 6 branch point at their reducing end and have no other branch chains. B chains are similarly connected to other B and C chain and may have A and B branch chains. The C chain is the only branch in the molecule to carry a free reducing group. The ratio of A:B chains has been used to characterize the structure of amylopectin (Atwell et al. 1980, Manners and Matheson 1981, Enevoldsen and Juliano 1988, Hizukuri and Maehara 1990). Many models of amylopectin structure have been proposed and reviewed (Wolfram and Khadem 1965, Banks and Greenwood 1975, Whistler and Daniel 1984, Hizukuri 1996). The current model (Figure 1a) was first proposed by Nikuni (1969) and further modified by French (1972) and Robin et al. (1974). Hizukuri (1986) noted a polymodal distribution of

a



b

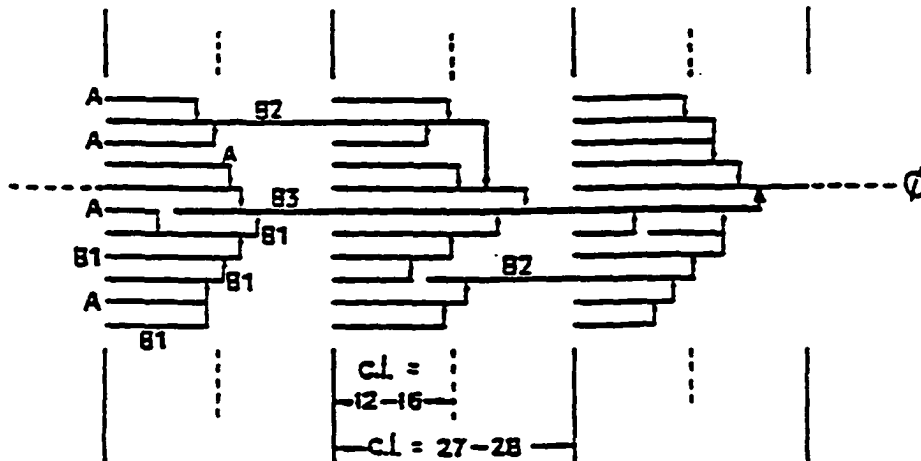


Figure 1. A cluster structure of amylopectin as proposed by French (1973) (a) and further defined with B-chain classification by Hizukuri (1986) (b).

amylopectin branch chains, and further modified the model by classification of B chains into B1-B4 fractions (Figure 1b). The Hizukuri model (1986) suggested that amylopectin is composed of numerous clusters that may be randomly or regularly distributed and linked by long chains extending through two or more clusters. The B chains have also been classified as Ba or Bb chains if A chains were bound (Ba) or not bound (Bb) (Hizukuri and Maehara 1990, Hizukuri and Maehara 1991). Jane et al. (1997) proposed models showing that branch points in A-type starches are scattered in both the amorphous and crystalline regions whereas branch points in B-type starches are located more in the amorphous regions. Those α -1 \rightarrow 6 linkages present in the crystalline regions were preserved in Naegeli dextrans of the starches. Hence, greater amounts of branches were preserved in the Naegeli dextrans of A-type starches than for B-type starches.

Intermediate Component

Several studies have reported material fractionated from starch with properties intermediate between those of amylose and amylopectin (Lansky et al. 1949, Peat et al. 1952, Greenwood 1975, Takeda et al. 1989). The intermediate material had iodine binding values and β -amylolysis limits between those of amylose or amylopectin. Maize starch (Lansky et al, 1949) and high amylose maize starch (Greenwood and Mackenzie 1966, Banks et al. 1974, Colonna and Mercier 1984, Baba and Arai 1984) have both been shown to possess intermediate material.

Organization of the Starch Granule

Amylose and amylopectin are arranged in nature in semi-crystalline macromolecular structures called granules (French 1972, Hood 1982, Robyt 1998). Starch granule size and shape vary with botanical origin, maturity, and growing conditions (Banks et al. 1974, Greenwood 1979, Morrison and Gadan 1987, Jane et al. 1994). The granules of potato starch are oval (15-80 μ m) whereas the granules of normal maize are round or polygonal (5-20 μ m) (Jane et al. 1994). Scanning electron microscopy has proven to be an effective tool for identification of starch, extent of starch damage, and extent of enzymic attack. Under polarized light microscopy, native starch granules display a birefringence pattern known as the Maltese cross. The Maltese cross indicates a high degree of order (crystallinity) within the granule. At the center of the Maltese cross is the hilum, which is believed to be the starting point of biosynthesis (French 1984). Starch granules exhibit alternating amorphous and crystalline growth rings encircling the hilum. Amylopectin forms clusters, lines up perpendicular to the growth rings, and grows from the hilum to the surface of the granule in a radial arrangement (French 1972, Nikuni 1978, French 1984, Lineback 1984). Amylopectin clusters are present in both amorphous and crystalline regions (French 1984). Amylose is also located in both crystalline and amorphous regions (Kainuma and French 1971, French 1972, Nikuni 1978, Blanshard 1986, Jane et al. 1992, Kasemsuwan and Jane 1994).

X-ray diffraction can be used to examine the crystalline nature of the starch granule and to define the relative amounts of crystalline and amorphous phases within the granule. Double helices of amylopectin branch chains are ordered into three arrangements giving

three x-ray diffraction patterns of starch: A, B, and C-types (Wu and Sarko 1978, Zobel 1988). The C-type pattern is intermediate between those of A- and B-types and is proposed to be a mixture of A- and B-type unit cells (Wu and Sarko 1978, Imberty et al. 1988). These double helices of amylopectin branch chains are stabilized by hydrogen and Vander Waals bonds (Imberty et al. 1988). The double helices for A-type starches are packed into a monoclinic lattice whereas for B-type starches double helices are packed in a hexagonal arrangement (Figure 2).

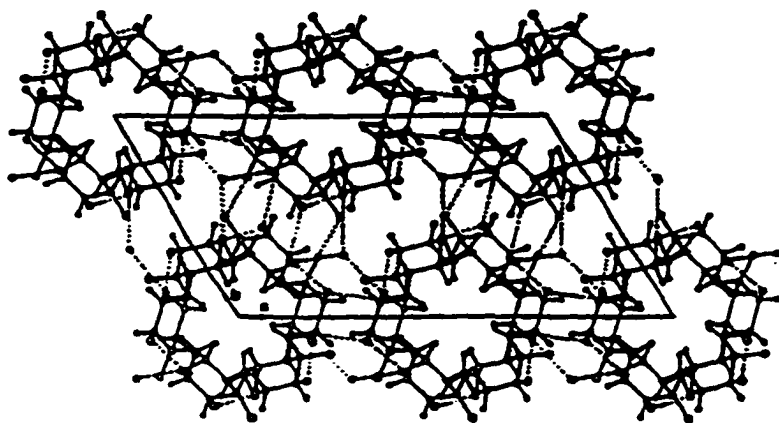
The A chains and outer B chains of amylopectin are mainly responsible for the crystallinity of starch granules. The crystalline packing arrangement has great significance to the properties of starches. Those of the B-type have lower gelatinization temperatures and thermal stability, greater granular swelling and increased enzymatic resistance to hydrolysis.

Minor Components of Starch

Starch lipids

Starches contain two types of lipids: surface lipids which are derived from non-starch lipids and internal starch lipids that are monoacyl lipids (Morrison 1988). The non-starch lipids in mature wheat starch occur in the aleurone region and are mostly triglycerides with small amounts of free fatty acids and monoacyl lipids (Morrison et al. 1975, Morrison 1988). In cereal starches the internal lipids are primarily monoacyl lipids consisting of free fatty acids and lysophospholipids (Schoch 1942, Morrison et al. 1984, Morrison 1988). In contrast, the starches of roots and tubers do not have significant amounts of lipids (Swinkels

a



b

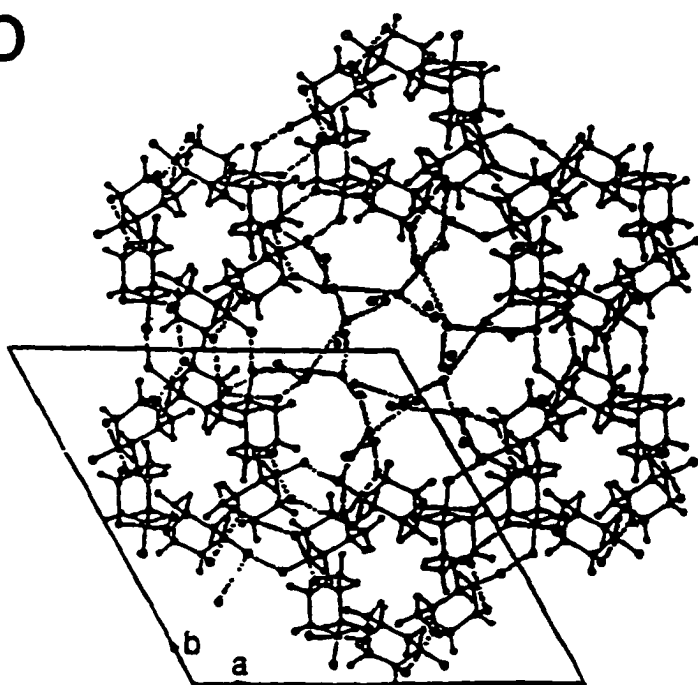


Figure 2. The arrangement of the double helices in starch crystalline structure in A-type (Imberty et al. 1987) (a) and B-type (Imberty and Perez 1988) (b) polymorphs.

1985, Morrison 1988). In non-waxy cereal starches the amounts of lipids are proportional to the amounts of amylose (Morrison et al. 1984, Soulaka and Morrison 1985). Starch lipids are thought to exist in the amorphous regions of the starch granule. ^{13}C MAS-NMR has shown there to be a weak shift for the amylose-lipid complex in barley starch (Morrison et al. 1993). The difficulty in extraction of lipids from the granule is thought to be due to the structure of the granule rather than resistance of the inclusion complex (Morrison and Coventry 1989). These internal starch lipids are intimately involved in modifying the properties of starch, including granular swelling, gelatinization, gel viscosity, retrogradation, and susceptibility to amylolytic degradation (Maningat and Juliano 1983, Swinkels 1985, Soulaka and Morrison 1985, Biliaderis and Seneviratne 1990, Morrison et al. 1993).

Phosphorous derivatives

Naturally occurring phosphorous is found in starches in three main forms: inorganic phosphate, phosphate monoesters, and phospholipids. Phospholipids are the major source of phosphorous in cereal starches with minor amounts occurring as monoesters and inorganic phosphate (Schoch 1942, Gracza 1965, Tabata et al. 1975, Morrison 1981, Hizukuri et al 1983b, Morrison et al. 1984, Soulaka and Morrison 1985, Morrison 1988, Lim et al. 1994, Kasemsuwan and Jane 1995). Root and tuber starches contain significant amounts of monophosphate esters covalently bound to starch (Posternak 1935, Hodge et al. 1948, Hizukuri et al. 1970, Swinkels 1985, Lim and Seib 1993, Lim et al. 1994, Kasemsuwan and Jane 1995). Phosphate monoesters have been examined closely in potato starch and found to

be located exclusively on the amylopectin and more densely located at the core of the granule (Gracza 1965, Jane and Shen 1993). Tabata and Hizukuri (1971) identified 1, 28, and 61% of phosphate monoesters were located on C-2, C-3 and C-6, respectively in potato starch hydrolysate. Tabata et al. (1975) reported similar results for the phosphate derivatives in waxy rice starch. Fractionated potato starch was reported to contain 0.165, 0.083 and 0.008% phosphorous in amylopectin, intermediate component, and amylose, respectively (Radomski and Smith, 1963). Tabata et al. (1978) and Takeda and Hizukuri (1982) reported the phosphate monoesters of potato amylopectin are located mostly in B-chains and are located more than nine glucose units away from the branch points. The phosphate monoesters are located in the inner sections of the B-chains (33%) and in the outer sections of B-chains and A-chains of amylopectin (Takeda and Hizukuri 1982). The phosphorous content and form are affected by many factors including, growing condition, temperature, and storage (Hizukuri et al. 1970, Nielsen et al. 1994, Muhrbeck and Tellier 1991). The phosphate monoesters on amylopectins greatly influence the properties of starch. The gelatinization and pasting temperatures are decreased, whereas the paste, gel clarity, and gel stability are increased due to charge repulsion of the phosphate groups in (Galliard and Bowler 1987).

Gelatinization and Retrogradation

Starch granules are insoluble in cold water but swell reversibly to a limited extent through hydrogen bonding. With additional heat, the granules continue to swell. As the

temperature is increased, the thermal energy overcomes the inter- and intra-molecular hydrogen bonds and hydrophobic interactions, resulting in irreversible disruption of granular order. In the gelatinization process, the point at which the native crystalline regions are melted, birefringence is lost (French 1984). Continued heating will result in a colloidal solution. Generally, the gelatinization of starch granules occurs over a temperature range that differs for each starch type. Examples include: normal maize, 64.4 to 80.4°C; waxy maize, 64.2 to 80.4°C; and normal potato, 59-68°C (Jane et al. 1997). Gelatinization temperatures of starch are affected by granule size (Banks and Greenwood 1975), degree of crystallinity (Zobel, 1984), relative amounts of amylose and amylopectin (Inouchi 1983), presence of phosphorous derivatives (Lim and Seib 1993), lipids (Morrison 1988) and the amylopectin branch-chain length (Kasemsuwan and Jane 1995).

Pasting follows gelatinization and was defined by Atwell et al. (1988) as “the phenomenon following gelatinization in the dissolution of starch. It involves the granular swelling, extrudation of molecular components from the granule and eventually total disruption of the granules.” In both food and industrial applications, pasting is an extremely important attribute of the functionality of starch. Starch pastes are extensively used as thickeners, stabilizers and binders. Paste viscosity of starch, as measured by Brabender Visco-amylograph or Rapid Visco Analyzer, is often used as a critical acceptance measurement in quality control processes by both starch manufacturers and end-use industries.

With cooling, the solution of gelatinized and dispersed starch will form a gel at concentrations above 6% solids. Starch gels are generally regarded as composites of swollen gelatinized granular remnants of amylopectin existing in three dimensional amylose gel network (Miles et al. 1985). With storage, molecular rearrangement, known as retrogradation, of amylose occurs. Atwell et al. (1988) defined retrogradation as the process of reassociation of gelatinized starch molecules into an ordered structure. Retrogradation is more prevalent in amylose due to its linear structure having a greater tendency to align and reassociate as compared to amylopectin. Long-term retrogradation, as with bread staling, is primarily caused by amylopectin branches (Schoch and French 1947, D'Appolonia and Morad 1981).

Modification of Starches

Native starches have great application in food and industrial applications. However, starches may be modified to give products with unique and/or improved functionality, thus increasing their utility. Three general methods of modification are currently used to improve starch functionality: genetic modification, chemical modification, and physical modification.

Genetic modification

Genetic modification through traditional cross-breeding or by transgenic processes can produce starches with unique compositions and molecular structures. Genetic mutants of the cereal starches are well known and have been studied extensively. Maize, barley, rice,

sorghum, amaranth, and wheat are all known to have waxy, or 100% amylopectin varieties (Shannon and Garwood 1984, Watson 1984, Robyt 1998). High-amylose varieties are known for maize and barley. Maize mutants have been screened and studied extensively. Mutation of protein production in maize can affect physical characteristics, such as hardness and opaqueness of the endosperm, and can change the protein type. Mutation in maize can affect amylose and amylopectin ratios, branching structure, branch chain length, and amount of intermediate component. Currently, waxy potato starch is the only root or tuber mutant known (Kortstee et al. 1997). Genetic modifications to starch offer many advantages over normal starches in terms of utility, functionality, and consumer acceptance.

Chemical modification

Chemical modification has been used for many years to overcome the functional limitations of native starches. Chemically modified starches include acid thinned starches, oxidized starches, derivatized starches, and cross-linked starches. Acid-modified starches (thin boiling starches), which are produced by limited acid hydrolysis of starch, have applications in candy manufacturing, textile sizing, paper coatings, and wall board industries. Oxidized starches are produced by the action of oxidizing agents, such as hydrogen peroxide or peracetic acid on starch, and introduces carboxyl and carbonyl groups and cleaves glycosidic linkages while retaining granular birefringence and increasing whiteness (Mellies et al. 1961, Wurzburg 1986). Oxidized starches are mainly used as wet-end additives in the paper-making industry (Mentzer 1986) and as textile sizings (Kirby 1986). Derivatized

starches are those starches onto which a substituent group is placed. Examples include, phosphorylated starches, acetylated and succinylated starches, and ethylated and hydroxypropylated starches. The addition of covalent phosphate groups to cereal starches gives them similar properties to potato starch. Phosphorylation of starches increases paste clarity and viscosity, and imparts freeze-thaw stability (Solarek 1986). Phosphorylated starches are widely used in paper manufacturing, corrugating adhesives, textile warp sizings, and numerous food applications. Hydroxypropylated starches produced by reaction with propylene oxide have improved cold storage stability, freeze-thaw stability, cold water swelling, paste clarity (Tuschoff 1986). Hydroxypropylated starches are used mainly as thickeners in refrigerated or frozen low-acid food systems.

Cross-linked starches are prepared by several methods. Cross-linking produces diester bridges between molecules, increasing the average molecular weight and reinforcing the granule during processing. The cross-linking process also produces monophosphates. Physical properties, such as viscosity and swelling power, are used for determining the degree of cross-linking as it is difficult to monitor otherwise (Hullinger 1967, Rutenburg and Solarek 1984, Wurzburg 1986). The ratio of diester to monoester derivative can be controlled by the pH of reaction. At high pH the cross-linking reaction is predominant whereas at low pH the monoester is favored (Patten et al 1969, Rogols and Salter 1979). Cross-linking of starches improves acid and enzyme stability, and shear resistance (Hullinger 1967, Hood et al. 1974, Abraham 1974). Cross-linked starches are widely used to provide stable, high-viscosity pastes in processed foods where stability to high pH, thermal extremes,

and shear (Whistler and Daniel 1984). In practice, one modification may not achieve the characteristics needed in a starch. In such cases a second modification is often used to obtain an appropriate product (Tuschoff 1986).

Extrusion modification

Physical modification of starch includes pre-gelatinization and extrusion processing. Methods for pre-gelatinization of starch include drum drying, spray drying, alcoholic-alkaline treatment, pressurization, and extrusion. Pre-gelatinized starches are used in instant foods such as pudding where a cold swelling starch is needed to impart viscosity to the product. Physically modified starches, like genetically modified starches, are advantageous in that little or no chemical modification may be needed to give the desired physical property.

Extrusion cooking is useful for processing starch and starch based-food products. Extrusion processing is used extensively in the production of ready-to-eat breakfast cereals, snack foods, pet foods, and confectionery products. Extrusion processing of starch involves the starch being conveyed, heated, sheared or mixed, and pressurized during processing. This results in significant chemical, physical, and functional changes to the starch prior to discharge through the die (Harper 1992). Cooking extruders typically consist of a segmented, heated barrel through which one or two flighted screws are rotated by an electric motor. The flights on the screws fill with starch and convey the feedstock material from the feed port to the die. The heat and pressure generated facilitates temperatures above 100°C without the production of steam and subsequent loss of moisture. At elevated temperatures

and pressures starch is rapidly gelatinized. In extrusion processing, the screws perform four functions: conveying, mixing, heating, and pressurizing (Harper 1989). Mixing or kneading elements are often inserted to dissipate mechanical energy by mixing the product (Harper 1992, Chang and Halek 1991). In the extrusion process, starch is fed into the screws and progressively compressed into a dense solid material (Colonna et al. 1984). The starch loses crystallinity during heating, shearing, and pressurizing and is transformed into a hot amorphous mass (Colonna et al. 1989). At low moistures and low mechanical energy inputs, starch granules are deformed, whereas at higher temperatures the granules are totally disrupted (Mercier et al. 1979, Guy and Horne 1988). However at high moisture content, low temperature and low mechanical energy input starch granules can remain relatively intact (Richmond and Smith 1985). During extrusion, starch can experience substantial molecular degradation. Intrinsic viscosity and size exclusion chromatography have shown extrusion at low moisture and high shear to cause the most molecular degradation in native starches (Mercier 1977, Colonna et al. 1984, Diosady 1985, Colonna et al. 1989, Jackson et al. 1990, Wasserman and Timpa 1991, Rodis et al. 1993, Politz et al. 1994). Extrusion of starch has been shown to yield large fragments instead of small molecules, which suggests the mode of degradation is random chain cleavage, with amylopectin being more susceptible than amylose (Diosady 1986, Colonna et al. 1989). For extrusion processed starches, properties such as water solubility, absorption, paste viscosity, expansion, and density of the final product, can be altered based upon processing variables. The high temperatures and pressures used in extrusion processing of starches are beneficial in reactive extrusion to produce

derivatized, grafted, and hydrolyzed products (Carr et al. 1992, Chang and Lii 1992, Gimmler and Meuser 1994, Wang et al. 1997).

Applications of Starch in Foods

Starches play major roles in the physical properties of foods. In food systems they function as thickeners, binders, coatings, water holders, and stabilizers. The textural attributes of food products such as cook-up and instant puddings and sauces are based almost entirely on starches. Starches may be used in their native state or may be chemically or physically modified. Processed foods require many characteristics such as low pH stability, viscosity stability, processing tolerance, shelf stability, and good surface appearance. Production of high acid, shelf-stable, frozen, refrigerated, retorted, or aseptically packed foods requires selection of at least one starch. Selection and use of starches in food products require basic knowledge of food processing and starch properties (Moore et al. 1984). Therefore, the study of starch and its molecular structures and functional relationships is important. The combination of genetic modification of starches and understanding of starch molecular structures give direction to breeders and starch producers and processors. Today, there is growing interest in natural starches with unique properties for use in foods to avoid a “food starch modified” label statement.

References

- Asaoka, M. Okuno, K., and Fuwa, H. 1985. Effect of environmental temperature at milky stage on amylose content. XI Some physico-chemical properties of amylo maize starch, and observations on the nature of high-amylose starches. *Cereal Chem.* 47:13-18.
- Abraham, T. E., Raja, K.C. M., Sreemulana, H., and Matthew, A.G. 1979. Improvement of texture of cassava flour by chemical treatment. *J. Root Crops* 5:11-18.
- Atwell, W. A., Hood, L. F., Lineback, D. R., Varriano-Marston, E., and Zobel, H. F. 1988. The terminology and methodology associated with basic starch phenomena. *Cereal Foods World* 33:306-310.
- Atwell, W. A., Milliken, G. A., and Hosney, R. C. 1980. A note on determining the ratio of A to B chain ratios. *Starch/Staerke* 32:362-364.
- Baba, T., and Arai, Y. 1984. Structural characterization of amylopectin and intermediate material in amylo maize starch granules. *Ag. Biol. Chem.* 48:1763-1775.
- Bailey, J. M., and Whelan, W. J. 1961. Physical properties of starch. I. Relationship between iodine stain and chain length. *J. Biol. Chem.* 48:1763-1775.
- Banks, W., and Greenwood, C. T. 1967. Physicochemical studies on starches. Part XXXII The incomplete β -amylolysis of amylose: A discussion of its cause and implications. *Starch/Staerke* 7:197-206.
- Banks, W., and Greenwood, C.T. 1975. *Starch and its components*. University Press, Edinburgh, UK.
- Banks, W., Greenwood, C. T., and Khan, K.M. 1971. The interaction of linear amylose oligomers with iodine. *Carbohydr. Res.* 17:25-33.
- Banks, W., Greenwood, C. T., and Muir, D. D. 1973. The structure of starch. Pages 179-194 in G. G. Birch and L. F. Green, eds. *Molecular Structure and Function of Food Carbohydrates*. John Wiley and Sons, New York.
- Banks, W., Greenwood, C. T., and Muir, D. D. 1974. Studies on starches of high amylose content. Part 17: A review of current concepts. *Starch/Staerke* 26:289-300.

- Bates, F. L., French, D., and Rundle, R. E. 1943. Amylose and amylopectin content of starches determined by their iodine complex formation. *J. Am. Chem. Soc.* 65:142-148.
- Billiaderis, C. G., and Galloway, G. 1989. Crystallization behavior of amylose-V complexes: Structure-property relationships. *Carbohydr. Res.* 189:31-48.
- Billiaderis, C. G., and Seneviratne, H. D. 1990. Solute effects on the thermal stability of glycerol monostearate-amylose complex super structures. *Carbohydr. Res.* 208:199-213.
- Blanshard, J. M. V. 1986. The significance of the structure and function of the starch granule in baked products. Pages 1-13 in J. M. V. Blanshard, ed. *Chemistry and Physics of Baking*. Royal Society of Chemistry, London.
- Boyer, C. D., Shannon, J. C., Garwood, D. L., and Creech, R. G. 1976. Change in starch granule size and amylose percentage during kernel development in several *Zea mays* L. genotypes. *Cereal Chem.* 53:327-337.
- Buleon, A., Duprat, F., Booy, F. P., and Chanzy, H. 1984. Single crystals of amylose with a low degree of polymerization. *Carbohydr. Polym.* 4:161-173.
- Cairns, P., Sun, L., Morris, V. J., and Ring, S.G. 1995. Physicochemical studies using amylose as an in vitro model for resistant starch. *J. Cereal Sci.* 21:37-47.
- Carr, M. E. , Kim, K. J., Yoon, K. J., and Stanley, K. D. 1992. Graft polymerization of cationic methacrylate, acrylamide, and acrylonitrile monomers onto starch by reactive extrusion. *Cereal Chem.* 69:70-75.
- Chang, K. L. B., and Halek, C. W. 1991. Shear and thermal history during co-rotating twin-screw extrusion. *J. Food Sci.* 56:518-524.
- Chang, Y. H., and Lii., C. Y. 1992. Preparation of starch phosphates by extrusion. *J. Food Sci.* 57:203-205.
- Chen, J., and Jane, J. 1994. Preparation of granular cold water soluble starches by alcoholic-alkaline treatment. *Cereal Chem.* 71:618-622.
- Colonna, P., Doublier, J. L., Melcion, J. P., DeMonredon, F., and Mercier, C. 1984. Extrusion cooking and drum drying of wheat starch I. Physical and macromolecular modifications. *Cereal Chem.* 61:538-543

- Colonna, P., Leloup, V., and Buleon, A. 1992. Limiting factors of starch hydrolysis. *Eur. J. of Clin. Nutr.* 46:S17-S32.
- Colonna, P., and Mercier, C. 1984. Macromolecular structure of wrinkled- and smooth- pea starch components. *Carbohydr. Res.* 126:233-240.
- Colonna, P., Tayeb, J., and Mercier, C. 1989. Extrusion cooking of starch and starchy products. Pages 247-319 in C. Mericer, P. Linko, and J. Harper, eds., *Extrusion Cooking*. American Association of Cereal Chemists, St. Paul, MN.
- Cura, J. A., Jansson, P., and Krisman, C. R. 1995. Amylose is not strictly linear. *Starch/Staerke* 47:207:209.
- D'Appolonia, B. L., and Morad, M. M. 1981. Bread staling. *Cereal Chem.* 58:186-190.
- Davies, T., Miller, D. C., and A. A. Porter. Inclusion complexes of free fatty acids with amylose. *Starch/Staerke* 32:149-158.
- Diosady, L. L., Paton, D., Rosen, N., Rubin, L. J., and Athanassoulis, C. 1985. Degradation of wheat starch in a single screw extruder: mechanico-kinetic breakdown of cooked starch. *J. Food Sci.* 50:1697-1699, 1706.
- Diosady, L. L. 1986. Review of recent studies in the mechanism of starch extrusion. Pages 143-172 in M. Le Mauger, and P. Jelen, eds., *Engineering and Process Application*. Vol. 2. Unit Operations. Elsevier Applied Science Pub., New York.
- Eerlingen, R. C., Jacobs, H., and Delcour, J. A. 1994. Enzyme resistant starch VI. Effect of endogenous lipids and added sodium dodecyl sulfate on formation of resistant starch. *Cereal Chem.* 61:351-355.
- Eerlingen, R. C., Deceunick, M., and Delcour J. A. 1993. Enzyme resistant starch II. Influence of amylose chain length on resistant starch formation. *Cereal Chem.* 70:345-350.
- Enevoldsen, B. S., and Juliano, B. O. 1988. Ratio of A chains to B chains in rice amylopectins. *Cereal Chem.* 65:424-427.
- Flory, P. J. 1953. *Principles of Polymer Chemistry*. Pages 266-316. Cornell University Press, Ithaca, NY.
- French, D. 1972. Fine structure of starch and its relationship to the organization of starch granules. *Denpun Kagaku* 19:8-25.

- French, D. 1973. Chemical and physical properties of starch. *J. Animal Sci.* 37:1048-1061.
- French, D. 1984. Organization of starch granules. Pages 183-247 in R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. *Starch: Chemistry and Technology*, 2nd Edition, Academic Press, New York.
- French, D., and Murphy, V. G. 1977. Computer modeling in study of starch. *Cereal Foods World* 22:61-70.
- Galliard, T., and Bowler, P. 1987. Morphology and composition of starch. Pages 55-78 in T. Galliard, ed. *Critical Reports on Applied Chemistry*. Vol. 13. *Starch: Properties and Potential*. John Wiley & Sons, London, UK.
- Gidley, M. J., and Bulpin, P. V. 1989. Aggregation of amylose in aqueous systems: The effect of chain length of phase behavior and aggregation kinetics. *Macromolecules* 22:341-346.
- Gidley, M. J., Bulpin, P. V., and Kay, S. 1986. Effect of chain length on amylose retrogradation. Pages 167-176 in G. O. Phillips, D. J. Wedlock, and P. A. Williams eds., *Gums and Stabilizers for the Food Industry*, Elsevier Applied Science, London.
- Gimmler, N., and Meuser, F. 1994. Influence of extrusion cooking conditions on the efficiency of the cationization and carboxymethylation of potato starch granules. *Starch/Staerke* 46:268-276.
- Gracza, R. 1965. Minor components of starch. Pages 105-131 in R. L. Whistler and E. F. Paschall, eds. *Starch: Chemistry and Technology*, Vol. I, Academic Press, New York.
- Greenwood, C. T. 1964. Structure, properties and amyolytic degradation of starch. *J. Food Technol.* 18:138-144.
- Greenwood, C. T. 1976. Starch. Pages 119-157 in Y. Pomerantz, ed. *Advances in Cereal Science and Technology*. Vol. 1. American Association of Cereal Chemists, St. Paul, MN
- Greenwood, C. T. 1979. Observations on the structure of the starch granule. Pages 129-138 in J. M. V. Blanshard and J. R. Mitchell, eds. *Polysaccharides in Food*. Butterworth, London, UK.

- Greenwood, C. T., and Mackenzie, S. 1966. Studies on starches with high amylose content. Part IV. The fractionation of amylo maize starch: A study of the branched component. *Carbohydr. Res.* 3:7-13.
- Gudmundsson, M., and Eliasson, A. C. 1990. Retrogradation of amylopectin and the effects of amylose and added surfactants/emulsifier. *Carbohydr. Polym.* 13:295-315.
- Guy, R. C. E., and Horne, A. W. 1988. Extrusion and co-extrusion of cereals. Pages 331-349 in J. M. V. Blanshard and J. V. Mitchell eds., *Food Structure: Its Creation and Evaluation*. Butterworth, London.
- Hanashiro, I., Abe, J., and Hizukuri, S. 1996. A periodic distribution of the chain length of amylopectin as revealed by high performance anion-exchange chromatography. *Carbohydr. Res.*, 283, 151-159.
- Harper, J. M. 1989. Food extruders and their applications. Pages 1-16 in C. Mercier, P. Linko, and J.M. Harper, eds. *Extrusion Cooking*. American Association of Cereal Chemists, St. Paul, MN.
- Harper, J. M. 1992. Extrusion processing of starch. Pages 37-64 in R.J. Alexander and H. Zobel, eds., *Developments in Carbohydrate Chemistry*. AACC. St. Paul, MN.
- Hibi, Y., and Kuge, T. 1987. Effects of fatty substances on the early process of retrogradation of starch. *J. Jpn. Soc. Starch. Sci.* 34:271-278.
- Hizukuri, S. 1985. Relationship between the distribution of the chain length of amylopectin and the crystalline structure of starch granules. *Carbohydr. Res.* 141:295-3106.
- Hizukuri, S. 1986. Polymodal distribution of the chain lengths of amylopectin, and its significance. *Carbohydr. Res.* 147:342-347.
- Hizukuri, S. 1996. Starch: analytical aspects. Pages 347-429 in A. C. Eliasson, ed. *Carbohydrates in Foods*. Marcel Dekker, Inc., New York.
- Hizukuri, S., Kaneko, T., and Takeda, T. 1983a. Measurement of the chain length of amylopectin and its relevance to the origin of crystalline polymorphism. *Biochimica Et Biophysica Acta* 760:188-191.
- Hizukuri, S., Tabata, S., and Nikuni, Z. 1970. Studies on starch phosphates. Part I. Estimation of glucose-6-phosphate residues in starch and the presence of other bound phosphate(s). *Starch/Staerke* 22:338-343.

- Hizukuri, S., and Maehara, Y. 1990. Fine structure of wheat amylopectin: The mode of A to B chain binding. *Carbohydr. Res.* 206:145-149.
- Hizukuri, S., and Maehara, Y. 1991. Distribution of the binding of A chains to a B chain in amylopectin. Pages 213-218 in R. B. Friedman, ed. *Biotechnology of amylopectin oligosaccharides*, American Chemical Soc. Washington, D. C.
- Hizukuri, S., Shirasaka, S., and Juliano, B. O. 1983b. Phosphorous and amylose branching in rice starch granules. *Starch/Staerke* 35:348-350.
- Hizukuri, S., Takeda, Y., and Yasuda, M. 1981. Multi-branched nature of amylose and the action of debranching enzymes. *Carbohydr. Res.* 134:1-10
- Hodge, J. E., Montgomery, E. M., and Hilbert, G. E. 1948. Hydrolysis of the amylopectin from various starches with beta-amylase. *Cereal Chem.* 25:19-24.
- Hood, L. F. 1982. Current concept of starch structure. Pages 217-236 in D. R. Lineback and G. E. Inglett eds. *Food Carbohydrates*. AVI Publishing Co., Westport, CT.
- Hood, L. F., Seigfried, A. S., and Meyer, R. 1974. Microstructure of modified tapioca starch-milk gels. *J. Food Sci.* 39:117-120.
- Hullinger, C. H. 1967. Production and use of cross-linked starch. Pages 445-450 in: R. L. Whistler and E. F. Paschall eds., *Starch: Chemistry and Technology*, Vol II. Academic Press, Inc., New York.
- Imberty, A., Chazny, H., and Perez, S. 1987. New three-dimensional structure for A-type starch. *Macromolecules* 20:2634-2642.
- Imberty, A., and Perez, S. 1988. A revisit to the three dimensional structure of B-type starch. *Biopolymers* 27:1205-1213.
- Inouchi, N., Glover, D.V., Sugimoto, Y., and Fuwa, H. 1984. Developmental changes in starch properties of several endosperm mutants of maize. *Starch/Starke* 36(1):8-12.
- Jackson, D. S., Gomez, M. H., Waniska, R. D., and Rooney, L. W. 1990. Effects of single screw extrusion cooking on starch as measured by aqueous high-performance size-exclusion chromatography. *Cereal Chem.* 67:529-532.
- Jane, J., and Chen, J. F. 1992. Effect of amylose molecular size and amylopectin branch chain length on paste properties of starch. *Cereal Chem.* 69:60-65

- Jane, J., Kasemsuwan, T., Leas, S., Zobel, H., and Robyt, J. F. 1994. Anthology of starch granule morphology by scanning electron microscopy. *Starch/Staerke* 46:212-129.
- Jane, J., Shen, L., Chen, J., Lim, S., Kasemsuwan, T., and Nip, W. K. 1992. Physical and chemical studies of taro starches and flours. *Cereal Chem.* 69:528-535.
- Jane, J., and Robyt, J. F. 1984. Structure studies of amylose-V complexes and retrograded amylose by action of alpha amylases, and a new method for preparing amyloextrins. *Carbohydr. Res.* 132:105-118.
- Jane, J., Robyt, J. F., and Huang, D. H. 1985. ^{13}C -NMR study of the conformation of helical complexes of amyloextrin and amylose in solution. *Carbohydr. Res.* 140:21-25.
- Jane, J., and Shen, J. J. 1993. Internal structure of the potato starch granule as revealed by chemical gelatinization. *Carbohydr. Res.* 247:279-290.
- Jane, J., Wong, K., and McPherson, A. 1997. Branch-structure difference in starches of A- and B-type x-ray patterns revealed by their Naegeli dextrans. *Carbohydr. Res.*, 300, 219-227.
- Kainuma, K., and French, D. 1971. Naegeli amyloextrin and its relationship to starch granule structure. I. Preparation and properties of amyloextrins from various starch types. *Biopolymers* 10:1673-1680.
- Kirby, K. W. 1986. Textile industry. Pages 229-251, in O. B. Wurzburg, ed., *Modified Starches: Properties and Uses*. CRC Press, Boca Raton, FL.
- Kitamura, S., Yoneda, S., and Kuge, T. 1984. Study on the retrogradation of starch. I. Particle size and its distribution of amylose retrograded from aqueous solution. *Carbohydr. Polym.* 4:127-136.
- Kasemsuwan, T., and Jane, J. 1995. Location of amylose in normal starch granules. II. Location of phosphodiester cross-linking revealed by phosphorous-31 nuclear magnetic resonance. *Cereal Chem.* 71:282-287.
- Kasemsuwan, T., Jane, J., Schanble, P., Stinard, P., and Robertson, D. 1995. Characterization of the dominant mutant amylose-extender (Ae1-5180) maize starch. *Cereal Chem.* 72:169-176.

- Kortstee, A. J., Suurs, L. C. J. M., Vermeesch, A. M. G., and Visser, R. G. F. 1997. Characterization of starch from genetically modified potato after transformation with the bacterial branching enzyme of *Anacyctis nidulans*. Pages 239-247 in P. J. Frazier, P. Richmond, and A. M. Donald, eds. *Starch : Structure and Functionality*, the Royal Society of Chemistry, Cambridge, UK.
- Kuge, T., and Takeo, K. 1968. Complexes of starchy materials with organic compounds Part II. Complex formation in aqueous solution and fractionation of starch by 1-methanone. *Ag. Biol. Chem.* 32:1230-1238.
- Lansky, S., Kooi, M., and Schoch, T. J. 1949. Properties of the fractions and linear subfractions from various starches. *J. Am. Chem. Soc.* 71:4065-4075.
- Le Bail, P., Bizot, H., and Buleon, A. 1993. "B" to "A" type phase transition in short amylose chains. *Carbohydr. Polym.* 21:99-104.
- Lim, S. T., Kasemsuwan, T., and Jane, J. 1994. characterization of phosphorous in starch by ³¹P-nuclear magnetic resonance spectroscopy. *Cereal Chem.* 71:488-493.
- Lim, S. T., and Seib, P. A. 1993. Location of phosphate esters in a wheat starch phosphate by ³¹P-nuclear magnetic resonance spectroscopy. *Cereal Chem.* 70:145-152.
- Lineback, D. R. 1984. The starch granule organization and properties. *Baker's Dig.* 16-21.
- Manners, D. J. 1989. Some aspects of the structure of starch and glycogen. *Denpun Kagaku* 36:313-323.
- Manners, D. J., and Matheson, N. 1981. The fine structure of amylopectin. *Carbohydr. Res.* 90:99-110.
- Manningat, C. C., and Juliano, B. O. 1983. Starch lipids and their effect on rice starch properties. *Starch/Staerke* 35:348-350.
- Mellies, R. L., Yeates, T. E., Mehtretter, C. L., and Senti, F. R. 1961. Preparation and physical characteristics of cross-linked hypochlorite-oxidized corn starch. *Starch/Staerke* 4:114-116.
- Mentzer, M. J. 1984. Starch in the paper industry. Page 543-574 in R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. *Starch Chemistry and Technology*. Second Edition. Academic Press, London.
- Mercier, C. 1977. Effect of extrusion cooking on potato starch using a twin screw French extruder. *Starch/Staerke* 23:48-52.

- Mercier, C., Charbonniere, R., Gallant, D., and Guilbot, A. 1979. Structural modifications of various starches by extrusion cooking with a twin screw French extruder. Pages 153-170 in: Polysaccharides in Food. J. M. V. Blanshard and J. R. Mitchell, eds. Butterworths, London.
- Miles, M. J., Morris, V.J., and Ring, S. G. 1984. Some recent observations of the retrogradation of amylose. *Carbohydr. Polym.* 4:73-77.
- Moore, C. O., Tuschoff, J. V., Hastings, C. W., and Schanefelt, R. V. 1984. Applications of starches in foods. Pages 575-591 in R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. *Starch Chemistry and Technology*. Second Edition. Academic Press, London.
- Morrison, W. R. 1981. Starch lipids: A reappraisal. *Starch/Staerke* 33:408-410.
- Morrison, W. R. 1988. Lipids in cereal starches: A review. *J. Cereal Sci.* 8:1-15.
- Morrison, W. R., and Coventry, A. M. 1989. Solvent extraction of fatty acids from amylose inclusion complexes. *Starch/Staerke* 41:24-27.
- Morrison, W. R., and Gadan, H. 1987. The amylose and lipid contents of starch granule in developing wheat endosperm. *J. Cereal Sci.* 5:263:275.
- Morrison, W. R., Mann, D. L., Soon, W., and Coventry, A. M. 1975. Selective extraction and quantification analysis of non-starch and starch lipids from wheat flour. *J. Cereal Sci.* 5:263-275.
- Morrison, W. R., Milligan, T. P. and Azudin, M. N. 1984. A relationship between the amylose and lipid contents of starches from diploid cereals. *J. Cereal Sci.* 2:257-271.
- Morrison, W. R., Tester, R. F., Snape, C. E., and Gidley, M. J. 1993. Swelling and gelatinization of cereal starches. IV. Some effects of lipid-complexed amylose and free amylose in waxy and normal barley starches. *Cereal Chem.* 70:385-391.
- Muhrbeck, P., and Tellier, C. 1991. Determination of the phosphorylation of starch from native potato varieties by ³¹P-NMR. *Starch/ Staerke*, 46:25-27.
- Nielsen, T. H., Wischman, B., Eneroldsen, K. and Moller, B. J. 1994. Starch phosphorylation in potato tubers proceeds concurrently with de novo biosynthesis of starch. *Plant Physiol.* 105:111-117.

- Nikuni, Z. 1978. Studies on starch granules. *Starch/Staerke* 30:105-111.
- Noda, T., Takahata, Y., and Sato, T. 1996. Distributions of the amylopectin chain length of sweet potatoes differing in stages of development, tissue zone and variety. *Nippon Shokuhin Kagaku Kogaku Kaishi* 42:200-206.
- Patten, E. M. V., Paek, T., and Boswell, E. L. 1969. Process for the manufacture of cross-linked granular starch products. US Patent 3,422,089.
- Peat, S., Whelan, W. J., and Thomas, G. J. 1949. The amylolytic enzymes of soya bean. *Nature* 164:499
- Peat, S., Pirt, S. J., and Whelan, W. J. 1952. Enzymic synthesis and degradation of starch. Part XV. B-amylose and the constitution of amylose. *J. Chem. Soc.* 3:705-713.
- Pfannemuller, B. 1978. Ordered arrangements in solutions of amylose-iodine complexes derived from free and terminally fixed amylose chains. *Carbohydr. Res.* 61:41-52.
- Pfannemuller, B., Mayerhofer, H., and Schulz, R. C. 1971. Conformation of amylose in aqueous solution: Optical rotatory dispersion and circular dichroism of amylose-iodine complexes and dependence on chain length of retrogradation of amylose. *Biopolymers* 10:234-261.
- Politz, M. L., Timpa, J. D., and Wasserman, B. P. 1994. Quantitative measurement of extrusion-induced starch fragmentation products in maize flour using nonaqueous gel-permeation chromatography. *Cereal Chem.* 71:531-536.
- Posternak, T. 1935. Phosphorous in starch. *Helv. Chim. Acta.* 18:1351-1356.
- Radomski, M. W., and Smith, M. D. 1963. Location and possible role of esterified phosphorous in starch fractions. *Cereal Chem.* 40:31-36.
- Richmond, P., and Smith, A. C. 1985. The influence of processing on biopolymeric structures. *Br. Polym.* 17:246-250
- Ring, S. G., Miles, M. J., Morris, V. J., and Turner, R. 1987. Spherulitic crystallization of short chain amylose. *Int. J. Macromol.* 9:158-160.
- Robin, J. P., Mercier, C., Charbonniere, R., and Guilbot, A. 1974. Lintnerized starch. Gel filtration and enzymatic studies of insoluble residues from prolonged acid treatment of potato starch. *Cereal Chem.* 51:389-406.

- Roby, J. F. 1998. Polysaccharides I. Structure and function. Pages 157-227 in J. F. Roby ed. *Essentials of Carbohydrate Chemistry*. Springer, New York.
- Rodis, P., Wen, L. F., and Wasserman, B. P. 1993. Assessment of extrusion-induced fragmentation by gel-permeation chromatography and methylation analysis. *Cereal Chem.* 70:152-157.
- Rogols, S., and Salter, J. W. 1979. Enlarged granules starch in the starch-iodine complex. III. X-ray diffraction studies of the starch-iodine complex. *J. Am. Chem. Soc.* 65:1707-1710.
- Rundle, R. E., and French, D. 1943. The configuration of starch in the starch-iodine complex. III X-ray diffraction studies of the starch-iodine complex. *J. Am. Chem. Soc.* 65:1707-1710.
- Rutenberg, M. W. 1980. Starch and its modifications. Pages 22-1 to 22-73 in R. L. Davidson, ed. *Handbook of Water-Soluble Gums and Resins*. McGraw-Hill Book Co., New York.
- Rutenberg, M. W., and Solarek, D. B. 1984. Starch derivatives: Production and uses. Pages 312-388 in R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. *Starch: Chemistry and Technology*, 2nd Edition, Academic Press, New York.
- Schoch, T. J. 1942. Fractionation of starch by selective precipitation with butanol. *J. Am. Chem. Soc.* 63:2957-2961.
- Schoch, T. J. 1964. Starch fractions: Iodimetric Determination of amylose potentiometric determination: Standard method. Pages 157-160 in R. L. Whistler, ed. *Methods in Carbohydrate Chemistry Vol 4: Starch*. Academic Press, London.
- Schoch, T. J., and French, D. 1949. Studies on bread staling. I. *Cereal Chem.* 24:231-249.
- Shannon, J. C., and Garwood, D. 1984. Genetics and physiology of starch development. Pages 25-79 in R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. *Starch: Chemistry and Technology*, 2nd Edition, Academic Press, New York.
- Solarek, D. B. 1986. Phosphorylated starches and miscellaneous organic esters. Pages 97-112 in O. B. Wurzburg, ed. *Modified Starches: Properties and Uses*. CRC Press, Boca Raton, FL.
- Soulaka, A. B., and Morrison, W. R. 1985. The amylose and lipid contents, dimensions, and gelatinization characteristics of some wheat starches and their A- and B-granule fractions. *J. Sci. Food Agric.* 36:709-718.

- Suzuki, A., Shibamura, K., Takeda, Y., Abe, J., and Hizukuri, S. 1994. Structures and pasting properties of potato starches from Jaga Kids Purple '90 and Red '90. *Oyo Toshitsu Kagaku* 41:425-436.
- Suzuki, A., Kaneyama, M., Abe, J., and Hizukuri, S. 1987. Relationship between temperature for retrogradation and gelatinization temperature of the retrograded soluble starch. *J. Jpn. Soc. Starch Sci.* 34:286-291.
- Swinkels, J. J. M. 1985. Source of starch, its chemistry and physics. Pages 15-46 in G. M. A. Van Beynum, J. A. Roels, eds. *Starch Conversion Technology*, Marcel Dekker Inc., New York.
- Tabata, S., and Hizukuri, S. 1971. Studies on starch phosphate. *Starch/Staerke* 23:267-272.
- Tabata, S., Hizukuri, S. and Nagata, K. 1978. Action of sweet potato β -amylase on phosphodextrin of potato starch. *Carbohydr. Res.* 67:189-195.
- Tabata, S., Nagata, K., and Hizukuri, S. 1975. Studies on starch phosphates. Part 3. On the esterified phosphates in some cereal starches. *Starch/Staerke* 27:333-335
- Takeda, Y., and Hizukuri, S. 1982. Re-examination of the action of sweet potato beta-amylase on phosphorylated (1-4)- α -D-glucan. *Carbohydr. Res.* 89:174-178.
- Takeda, C., Takeda, Y., and Hizukuri, S. 1983. Physicochemical properties of Lily Starch. *Cereal Chem.* 60:212-216.
- Takeda, Y. and Hizukuri, S. 1989. Structures and amounts of branched molecules in rice amylose. *Carbohydr. Res.* 186:163-166.
- Takeda, Y., Murua, N., Hizukuri, S., and Juliano, B. O. 1989. Structures of indica rice starches (IR48 and IR64) having intermediate affinities for iodine. *Carbohydr. Res.* 187:287-294.
- Takeda, Y., Shitaozono, and Hizukuri, S. 1988. Molecular structure of corn starch. *Starch/Staerke* 40: 51-59.
- Tuschoff, J. V. 1986. Hydroxypropylated starches. Pages 89-96 in O.B. Wurzburg, ed. *Modified Starches: Properties and Uses*. CRC Press, Boca Raton, FL.
- Wang, L., Shogren, R. L., and Willett, J. L. 1997. Preparation of starch succinates by reactive extrusion. *Starch/Staerke* 3:116-120.

- Wasserman, B. P., and Timpa., J. D. 1991. Rapid quantitative measurement of extrusion induced starch fragmentation by automated gel permeation chromatography. *Starch/Staerke* 43:389-392.
- Watson, S. A. 1984. Corn and sorghum starches: production. Pages 417-464 in R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. *Starch: Chemistry and Technology*, 2nd Edition, Academic Press, New York.
- Whistler, R. L. 1953. Starch retrogradation. Pages 213-228 in *Starch and Its Derivatives*, J. A. Radley, ed., Chapman and Hall Ltd., London, England.
- Whistler, R. L. 1965. Fractionation of starch. pages 331-347 in R. L. Whistler and E. F. Paschall eds., *Starch: Chemistry and Technology*. Vol I. Academic Press, New York.
- Whistler, R. L., and Johnson, C. 1965. Effect of acid hydrolysis on the retrogradation of amylose. *Cereal Chem.* 25:418-424.
- Whistler, R. L., and Daniel, J. R. 1984. Molecular structure of starch. Pages 153-182 in R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. *Starch: Chemistry and Technology*, 2nd Edition, Academic Press, New York.
- Winter, W. T., and Sarko, A. 1972. The crystal structure of amylose-DMSO complex. *Biopolymers* 11:849-852.
- Winter, W. T., and Sarko, A. 1974. Crystal and molecular structure of V-anhydrous amylose. *Biopolymers* 13:1447-1460.
- Wu, H. C. H. and Sarko, A. 1978. The double helical molecule structure of crystalline B-amylose. *Carbohydr. Res.* 61:7-25.
- Wurzberg, O. B. 1986. Cross-linked starches. Pages 42-53 in O.B. Wurzberg, ed. *Modified Starches: Properties and Uses*. CRC Press, Boca Raton, FL.
- Yamashita, Y., and Monobe, K. 1971. Single crystals of amylose V complexes. III Crystals with a helical configuration. *J. Polymer Sci. A-2*:1471-1481.
- Zobel, H. F. 1984. Gelatinization of starch and mechanical properties of starch pastes. Pages 285-311 in R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. *Starch: Chemistry and Technology*, 2nd Edition, Academic Press, New York.
- Zobel, H. F. 1988. Molecules to granules: A comprehensive starch review. *Starch/Staerke* 40:44-67.

COMPARISON OF WAXY POTATO WITH OTHER ROOT AND TUBER STARCHES

A paper accepted by Carbohydrate Polymers.

A. E. McPherson and J. Jane

ABSTRACT

The physicochemical properties of normal potato, waxy potato, yam and sweet potato starches were examined and compared. Normal potato and waxy potato starches displayed the B-type x-ray diffraction pattern, whereas yam and sweet potato displayed the C_A- and C-type, respectively. X-ray diffraction patterns of Naegeli dextrans of normal potato and waxy potato remained the B-type, but those of yam and sweet potato changed to the A-type. ³¹P-NMR showed the phosphorus contents of the starches to be primarily phosphate monoesters with no detectable phospholipid in any of the four starches. The chain-length distributions of debranched amylopectins of the starches were analyzed using high performance anion-exchange chromatography equipped with a post-column amyloglucosidase reactor and a pulsed amperometric detector. Normal potato and waxy potato starches displayed lower proportions (13 and 14.8%, respectively) of short branch chains of chain length dp 6-12 than did yam and sweet potato starches (17.1 and 19.0%, respectively). Normal potato displayed a larger proportion of long branch chains than did waxy potato amylopectin. The average amylopectin branch chain lengths of normal potato, waxy potato, yam and sweet potato starches were dp 28.6, 25.8, 25.8 and 26.3, respectively. The Naegeli dextrans of all four starches displayed linear and singly branched chains but no multiply branched chains. The

Naegeli dextrans of normal potato, waxy potato, yam, and sweet potato starches displayed ratios of branched to linear branch chains of 0.31, 0.36, 0.42 and 0.51, respectively. The absolute amylose contents of the four starches were normal potato, 18.3%; waxy potato, 0%; yam, 17.7%; and sweet potato, 22.8%.

INTRODUCTION

Normal potato starch is widely used in food and industrial applications and is economically important in the United States and Europe (Mitch, 1984). Normal potato starch has been well-studied and is known to give a B-type x-ray diffraction pattern. Waxy potato starch is a recently developed potato mutant that is devoid of amylose content and has not been extensively studied. Sweet potato starch is widely used in Asia in a variety of food and industrial applications (Tian *et al.*, 1991). Yam starch is used in parts of Africa for food and limited industrial applications (Coursey, 1967; Emiola and DeLarosa, 1981). Sweet potato starch has been reported to be of the A-, C_A- and C-type x-ray diffraction pattern (Watanabe *et al.*, 1982; Takeda *et al.*, 1986; Hanashiro *et al.*, 1996). The x-ray diffraction pattern for many starches is affected by sample preparation (Nara *et al.*, 1978) and by growth conditions and maturity of the parent plant at the time of harvest (Sugimoto *et al.*, 1987; Noda *et al.*, 1995). These effects may be more profound in C-type starches because they are reported to be a mixture of A- and B-type crystalline polymorphs (Wu and Sarko, 1978; Watanabe *et al.*, 1982; Zobel, 1988).

Average branch chain lengths of amylopectins are highly correlated to the crystalline polymorphs observed in the native starch (Hizukuri, 1985). A-type starches contain shorter average branch chain lengths of amylopectins, whereas B-type starches contain longer average branch chain lengths (Hizukuri *et al.*, 1983; Hizukuri, 1985; Hanashiro *et al.*, 1996). C-type starches contain amylopectins with both long and short branch-chain lengths. Recently, Hanashiro *et al.*, (1996) investigated the branch chain distributions of a number of starches by use of high performance anion exchange chromatography with pulsed amperometric detection. The authors examined starches of the A-, B-, and C-types and divided the proportions of branch chains into DP 6-12, 13-24, 25-36 and ≥ 37 .

In this study, we investigated chemical structures, including apparent and absolute amylose contents, amylopectin branch chain lengths and distributions, phosphate contents, Naegeli dextrin structures and physical properties including x-ray diffraction patterns, gelatinization and retrogradation properties. The results helped us understand how chemical structures affect the properties of starch.

MATERIALS AND METHODS

Normal potato starch was purchased from Sigma Chemical Corp. (St. Louis, MO, USA). Waxy potato starch was a gift from Lykkeby Stakelsen Food and Fiber AB, Lykkeby, Sweden. Yams (*Dioscorea*) and sweet potatoes (*Ipomoea*) were purchased from local markets, and their starches were isolated following the method of DeWilligen (1964).

Scanning Electron Microscopy

Scanning electron microscopy was performed by the method of Jane *et al.*, (1994). Starches were suspended in absolute methanol, and a drop of the suspension was placed on silver tape, sticky side down, attached to a brass disk and sputter coated with gold/palladium (60/40). The mounted specimens were observed using a scanning electron microscope (JEOL model 1850, Tokyo, Japan).

X-Ray Diffraction Patterns

The x-ray patterns of the starches and their Naegeli dextrans were obtained with copper, nickel foil filtered, $K\alpha$ radiation using a diffractometer (D-500 Siemens, Madison, WI, USA) following the method of Jane *et al.*, (1997). The diffractometer was operated at 27 mA and 50 kV. The scanning region of the diffraction angle (2θ) was from 4 to 40 at 0.05 step size with a count time of 2 s. Starch and Naegeli dextrans were equilibrated at 100% relative humidity for 24 h at 25°C prior to examination.

Phosphorus Content

Total phosphorus contents were determined chemically by the method of Smith and Caruso (1964). Samples were examined in triplicate. Structures and quantities of phosphorus derivatives also were determined with ^{31}P -nuclear magnetic resonance (NMR) following the method of Kasemsuwan and Jane (1996).

Preparation of Naegeli Dextrins

Naegeli dextrins were prepared following the method of Umeki and Kainuma (1981). The starch (20 g, dry starch basis(dsb)) was suspended in 15.3% (vol/vol) H₂SO₄ and held at 38°C in an incubator. Starch suspensions were shaken daily by hand. Samples were taken on days 3, 6, 9 and 12, and the supernatant was siphoned off. An aliquot of the supernatant was analyzed for total carbohydrate content to calculate the percentage starch hydrolyzed (Dubois *et al.*, 1956). The starch residues were washed with water until the washings reached pH 7. The samples then were dehydrated with absolute ethanol and dried at 30°C. Naegeli dextrins of normal potato starch also were prepared at 25°C for 3 mo. by the method of Kainuma and French (1971).

Molecular Size Distribution by Gel Permeation Chromatography

An aliquot (5 ml) containing 15 mg starch with a glucose marker (0.5 mg) was injected into a column (2.6 cm, I.D. x 80 cm) packed with Sepharose CL-2B gel (Pharmacia, Inc., Piscataway, NJ, USA). Distilled and deionized water containing 10 mM NaOH and 50 mM NaCl was used to elute the sample in an ascending direction at 30 ml/h flow rate. Fractions of 4.8 ml were collected and analyzed using an Autoanalyzer II (Technicon Instrument Corp, Elmsford, NY, USA). The total carbohydrate by anthrone-sulfuric acid reaction and amylose-iodine blue value were measured at 630 and 640 nm, respectively (Jane and Chen, 1992). The blue value was used to identify the locations of the amylose and amylopectin in the chromatograms.

Amylose Contents

Apparent amylose contents were determined by measuring iodine affinities of defatted starches by use of a potentiometric autotitrator with Metrodata recording software (702 SM Titrino, Brinkman Instrument, Westbury, NY, USA). The analysis was based on Schoch's method (1964). Iodine affinities were measured in triplicate. Amylose was separated from amylopectin by the methods of Schoch (1942) and Jane and Chen (1992). The amylopectin fraction was purified by recrystallization five times. The iodine affinities of purified amylopectins were also determined and then were used to correct the overestimation of amylose content (Takeda and Hizukuri, 1983; Kasemsuwan *et al.*, 1995). Absolute amylose contents were assessed by subtracting the iodine affinity of amylopectin from that of the defatted starch (Kasemsuwan *et al.*, 1995).

Chain-Length Analysis by Anion-Exchange Chromatography

Isolated amylopectins and Naegeli dextrans were subjected to enzymatic debranching by isoamylase following the method of Jane and Chen (1992). Chain-length distributions of the debranched amylopectins, the Naegeli dextrans and debranched Naegeli dextrans were quantitatively analyzed using a high-performance anion-exchange chromatograph equipped with a post column amyloglucosidase reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD) (Wong and Jane, 1997). A mixture of homologous maltodextrans (dp 1-7) that contained equivalent concentrations (0.1 mg/ml) of each sugar was used to monitor the activity of the enzyme reactor and to calibrate the eluting volumes of the maltodextrans. The

separation of a sample (25 μ l, 1 mg/ml) with the system employed a PA-100 anion-exchange analytical column and a PA-100 guard column (Dionex, Sunnyvale, CA, USA) with a gradient composed of eluent A (100 mM NaOH) and eluent B (100 mM NaOH, 300 mM NaNO₃) at a flow rate of 0.5 ml/min. The separation gradient was: 0-5 min, 99% A and 1% B; 5-30 min, linear gradient to 8% B; 30-150 min, linear gradient to 30% B; 150-200 min, linear gradient to 45% B.

Thermal Properties of Starches Determined by Differential Scanning Calorimetry

Thermal properties of the starches were analyzed using a differential scanning calorimeter (DSC) (Perkin Elmer DSC-7) equipped with an Intracooler II System and Pyris thermal analysis software (Perkin-Elmer Corp., Norwalk, CT, USA). Starch and water suspensions (1:3) were sealed in aluminum pans and equilibrated at room temperature for 2 h before analysis. An empty aluminum pan was used as the reference. The samples were heated at 10°C/min over a temperature range of 25 to 100°C. Indium and naphthyl ethyl ether were used as reference standards. The gelatinization temperature and enthalpy change were determined following the procedure of Jane and Chen (1992). Enthalpy change (ΔH), onset temperature, (T_o), peak temperature (T_p) and conclusion temperature (T_c) were recorded. The analysis of the retrograded starches was done using the same method with the gelatinized samples having been stored at 4°C for 7 d. The data were calculated from at least three replications.

Pasting Properties

Pasting properties of starches (8% dsb; 30 g total weight) were determined by using a Rapid ViscoAnalyzer-4 RVA-4 (Newport Scientific Pty. Ltd., Warriewood, NSW, Australia). A heating profile was programmed as follows: 1 min at 50°C, heat to 95°C at 6°C/min, hold for 5 min and cool to 50°C at 6°C/min. The rotating speed of the paddle was kept at 160 rpm throughout the measurement.

RESULTS AND DISCUSSION

Scanning electron micrographs of the four starches are shown in Fig. 1. Normal potato and waxy potato starches both showed large, rounded or oval granular shapes with axial diameters of 12-70 μm and 12-37 μm for normal potato and 12-72 μm and 14-44 μm for waxy potato starch. Yam and sweet potato starches had angular granules with diameters of 4-20 and 4-15 μm , respectively. The results were consistent with those reported by Jane *et al.*, (1994). The presence of angular granule features may indicate the presence of compound starch granules as reported by Shannon and Garwood (1984) for sweet potato.

X-ray diffraction patterns of the four starches are shown in Fig. 2. Normal potato and waxy potato starches both gave B-type x-ray diffraction patterns, whereas yam starch gave a C_A-type and sweet potato starch gave a C-type pattern. The x-ray diffraction patterns of the Naegeli dextrins of the starches after 12-days of acid hydrolysis are shown in Fig. 3. Naegeli dextrins of normal potato and waxy potato retained the same type x-ray diffraction pattern as their respective native parent starches with increased peak intensity, as previously reported by

Kainuma and French (1971) and Jane *et al.*, (1997). Whereas, the x-ray diffraction patterns of the 12-day Naegeli dextrins of yam and sweet potato displayed pronounced A-type characteristics (Fig. 3). Because the C-polymorph is a combination of the A- and B-polymorphs, the results suggest that the B-polymorph of the starches is preferentially hydrolyzed. Bogracheva *et al* (1998) have reported that C-type pea starch has both the A- and B-polymorphs present in a single granule and the B-polymorph being at the hilum and the A-polymorph at the periphery. We also observed that more birefringence was retained at the periphery of acid treated starch granules. More studies are needed to reveal if the B-polymorph is located at the hilum of the sweet potato and yam starches and is hydrolyzed during the acid treatment.

Phosphorus contents of the starches are shown in Table 1. Both normal potato and waxy potato starches contained more phosphorus than yam and sweet potato starches. ^{31}P -NMR spectra showed the majority of the phosphorus in the starches was phosphate monoester with minor amounts as inorganic phosphate (Table 1), which was in agreement with those reported in the literature (Muhrbeck and Eliasson, 1991; Muhrbeck and Tellier, 1991; Lim and Seib, 1993; Bay-Smidt *et al.*, 1994; Lim *et al.*, 1994; Kasemsuwan and Jane, 1996). None of these tuber and root starches contained phospholipids. The Naegeli dextrin of potato starch prepared by acid hydrolysis at 25°C for 3 mo retained 65.1% of the total phosphorus and 45.2% of the original carbohydrate of the native starch. ^{31}P -NMR spectra of potato starch and potato Naegeli dextrin, in aqueous solutions, showed that the structures of phosphate monoesters were preserved (δ 4.08 and δ 4.25 ppm for C-6 phosphate and δ 4.70

ppm for C-3 phosphate) as shown in Fig 4. A small peak of free glucose 6-phosphate (δ 4.97 ppm), generated during acid hydrolysis, appeared in the spectra of the Naegeli dextrin. These results were consistent with the phosphate monoesters being present on amylopectin long B-chains at least nine glucose residues away from α -1,6 branch points (Takeda and Hizukuri, 1982) and located within the crystalline region (Muhrbeck and Eliasson, 1991; Muhrbeck *et al.*, 1991). Thus, the phosphate monoesters were protected from acid hydrolysis.

Iodine titration of the defatted starch showed that normal potato starch contained more apparent amylose (37.8%) than sweet potato starch (33.1%), yam starch (29.2%) and waxy potato starch (19.4%) (Table 2). The apparent amylose content of sweet potato starch has been reported to range from 28 to 38% (Martin and Deshpande, 1985), and that of yam starch from 21.6 to 25.4% (Emiola and Delarosa, 1981) and 22% (Suzuki *et al.*, 1986). After subtraction of the iodine affinities of the amylopectins from those of the whole starches the absolute amylose contents were normal potato, 18.3%; waxy potato, 0%; sweet potato, 22.8%; and yam, 17.7% (Table 2). Molecular size distributions of the starches determined by gel permeation chromatography confirmed that waxy potato starch contained no amylose (Fig. 5). The proportions of amylose to amylopectin, calculated by the total carbohydrate in each peak, arbitrarily cut at the minimum points of both blue value and total carbohydrate, showed the amylose fraction of normal potato starch, 27.3%; yam starch, 25.0%; and sweet potato starch, 21.1%. The differences between these and the absolute amylose contents obtained by iodine titration may be attributed to amylopectin of smaller molecular weight present in the second peak. The molecular weight distributions also showed normal potato

starch to have amylose of larger molecular weight than that of yam and sweet potato starches. The ratios of blue value to total carbohydrate peak intensities showed the amylopectins of normal potato and waxy potato starches developed less blue color than yam and sweet potato amylopectins, which may be attributed to the interference of phosphate monoesters.

The normalized branch chain length distributions of debranched amylopectins of the starches are shown in Fig. 6. The first peak in the bimodal peak distribution had a peak chain length of dp 14 for normal potato and waxy potato starches and dp 13 for the yam and sweet potato starches, while the second peak varied from chain length of dp 48 to 52 for all four starches (Table 3). Among the four starches, normal potato had the largest average chain length of dp 28.6. Sweet potato had an average chain length of dp 26.3, and yam and waxy potato both had an average chain length of dp 25.8. The yam and sweet potato amylopectins had larger proportions (19.09 and 17.05%, respectively) of short branch chains (dp 6-12), in comparison to normal and waxy potato amylopectins (13.07 and 14.75%, respectively) (Table 3). This result was consistent with that reported by Takeda *et al.*, (1986). Normal potato amylopectin had a larger proportion (28.5%) of long branches (dp \geq 37) than did waxy potato (22.43%), yam (21.80%) and sweet potato (23.44%) (Table 3). All four starches had maximum detectable chain lengths at dp 85. These results are in agreement with previous work that B-type starches have longer branch chains than do A- and C-type starches (Hizukuri, 1985).

Acid hydrolysis rates of the starches differed (Fig. 7). After 6 d waxy potato starch had a higher extent of hydrolysis than did normal potato, yam and sweet potato starches (Fig.

7). HPAEC-ENZ-PAD chromatograms of the Naegeli dextrans after 12 d hydrolysis showed a peak chain length at dp 15 (65.0 min retention time) for the normal and waxy potato starches and dp 16 (66.4 min retention time) for the yam and sweet potato starches. A second peak was observed in each chromatogram, which corresponded to singly branched molecules, and occurred at dp 25-26 (~100 min retention time) (Fig 8). After isoamylase debranching, the singly branched molecules were hydrolyzed to two linear molecules and the second peak disappeared (Fig. 9). The ratios of the peak heights of the branched molecules to those of the linear molecules were 0.31, 0.36, 0.42 and 0.52 for normal potato, waxy potato, yam and sweet potato Naegeli dextrans, respectively, and indicated that more branch chains were present in the Naegeli dextrans of yam and sweet potato starch. These results agreed with the models proposed by Jane et al. (1997), in which branch points in A-type starches are scattered throughout the amorphous and crystalline regions. Those α 1-6 linkages present in the crystalline region were preserved in Naegeli dextrans of A-type starches. The majority of branch linkages of B-type starches are clustered in the amorphous regions, which were susceptible to acid hydrolysis. Thus, fewer branches were found in the Naegeli dextrans of B-type starches.

Thermal analysis by DSC (Table 4) showed gelatinization onset temperatures to be 60.8, 62.5, 64.6 and 57.9°C for normal potato, waxy potato, yam and sweet potato starches, respectively. Phosphate monoesters on amylopectin are known to decrease the gelatinization temperatures. Therefore, normal potato and waxy potato starches, despite their long amylopectin average branch chain lengths, had low onset gelatinization temperatures because

of their large phosphate monoester contents. Yam starch, having a low concentration of phosphate monoester on its amylopectin, longer long B chains (peak dp 52) and more characteristics of the A-type polymorph, displayed a higher onset gelatinization temperature. Sweet potato starch had relatively short B2 chains (peak chain length of dp 48), substantial phosphate derivatives (0.020%), more α 1-6 branch linkages in its amylopectin crystalline region and a more pronounced shoulder at dp 18-20 indicating a defective crystalline structure, which may account for its lower onset gelatinization temperature. Normal potato and waxy potato starches had narrower ranges of gelatinization temperatures (8-10°C) than did yam and sweet potato starches (13-14°C). Enthalpy changes for the normal potato, waxy potato, yam and sweet potato starches were 17.3, 18.2, 13.5 and 13.3 J/g, respectively. Increasing amylose content decreases the enthalpy change (Inouchi *et al.*, 1984). The large enthalpy changes in normal potato and waxy potato starches result from long amylopectin branch chains packing into large crystallites and waxy potato being composed of only amylopectin. The percentages of retrogradation of the four starches were similar.

Pasting properties of the starches determined by Rapid Visco Analyzer -4 (RVA) are shown in Fig. 10. In contrast to most normal and waxy cereal starches, normal potato starch displayed a larger peak viscosity than waxy potato starch. This difference between normal potato and waxy potato starch was attributed to amylose, which by physically interacting with amylopectin, maintained the integrity of normal potato starch granules and allowed it to swell to a greater degree and to achieve a higher pasting peak viscosity than waxy potato starch. Without amylose, waxy potato starch granules were rapidly dispersed as the granules

imbibed water and swelled, which resulted in a substantially lower pasting peak viscosity, more rapid shear thinning and less set-back viscosity. The fewer long branch chains and less phosphate monoesters also contributed to the lower pasting peak viscosity of waxy potato starch. The difference in pasting behavior between normal cereal and potato starches can be attributed to starch lipids and phospholipids in normal cereal starches, which complex with amylose and long branch-chains of amylopectin, and severely retard swelling and inhibit amylose leaching (Larson, 1980). By comparison, waxy cereal starches, which have negligible lipid contents, swell rapidly and achieve a higher pasting peak viscosity. Sweet potato and yam starches were similar in pasting profiles except that sweet potato had a stepwise increase in viscosity. Reasons for this phenomenon are not known and are of interest. Pasting temperatures were 64.3, 64.4, 72.0 and 70.3°C for normal potato, waxy potato, yam and sweet potato starches, respectively. The large phosphate monoester contents of normal potato and waxy potato starches resulted in the lower pasting temperatures. The high amylose content of sweet potato starch might contribute to its lower peak viscosity and resistance to shear thinning in comparison to yam. Sweet potato starch showed a final viscosity similar to that of normal potato starch, whereas yam and waxy potato were somewhat lower. Waxy potato starch gave a jagged pasting profile which was also observed for waxy maize starch at a lesser extent. This could be results of the highly stringy (long) pastes of the starches.

REFERENCES

- Bay-Smidt, A. M., Wischman, B., Olson, C. E., and Nielsen, T. H. (1994). Starch bound phosphate in potato as studied by a simple method for determination of organic phosphate and ^{31}P -NMR. *Starch/Staerke* 46,167-172.
- Bogracheva, T., Morris, V. J., Ring, S. G., and Hedley, C. L. (1998). The granular structure of C-type pea starch and its role in gelatinization. *Biopolymers* 45, 323-332.
- Coursey, D. G. (1967). *Yams*. Longmans, Green and Co. Ltd, London.
- DeWilligen, A. H. A. (1964). Potato starch isolation. In *Methods in Carbohydrate Chemistry*, Vol.4, Starch, R. L. Whistler, R. J. Smith, J. N. BeMiller, and M. L. Wolfram, eds. Academic Press, London, U.K.
- Dubois, M., Gilles, K. A., Hamilton, P. A. Rebers, P. A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, 28, 350-356.
- Emiola, L. O. and Delarosa, L. C. (1981). Physicochemical properties of yam starches. *J. Food Biochem.*, 5, 115-130.
- Hanashiro, I., Abe, J, and Hizukuri, S. (1996). A periodic distribution of the chain length of amylopectin as revealed by high performance anion-exchange chromatography. *Carbohydr. Res.*, 283, 151-159.
- Hizukuri, S. (1985). Relationship between the distribution of the chain length of amylopectin and the crystalline structure of starch granules. *Carbohydr. Res.*, 141, 295-306.
- Hizukuri, S., Kaneko, T., and Takeda, Y. (1983). Measurement of the chain length of amylopectin and its relevance to the origin of crystalline polymorphism of starch granules. *Biochim. Biophys. Acta*, 760, 188-191.
- Inouchi, N., Glover, D. V., Sugimoto, Y., and Fuwa, H. (1984). Developmental changes in starch properties of several endosperm mutants of maize. *Starch/Staerke*, 36, 8-12.
- Jane, J. and Chen, J-F. (1992). Effect of amylose molecular size and amylopectin branch chain length on paste properties of starch. *Cereal Chem.*, 69, 60-65.

- Jane, J., Kasemsuwan, T., Leas, S., Zobel, H., and Robyt, J. F. (1994). Anthology of starch granule morphology by scanning electron microscopy. *Starch/Staerke*, 46, 121-129.
- Jane, J., Wong, K., and McPherson, A. (1997). Branch-structure difference in starches of A- and B-type x-ray patterns revealed by their Naegeli dextrans. *Carbohydr. Res.*, 300, 219-227.
- Kainuma, K. and French, D. (1971). Naegeli amyloextrin and its relationship to starch granule structure. I. Preparation and properties of amyloextrins from various starch types. *Biopolymers*, 10, 1673-1680.
- Kasemsuwan, T. and Jane, J. (1996). Quantitative method for the survey of starch phosphate derivatives and starch phospholipids by ^{31}P -nuclear magnetic resonance spectroscopy. *Cereal Chem.*, 73, 702-707.
- Kasemsuwan, K., Jane, J., Schnable, P., Stinard, P., and Robertson, D. (1995). Characterization of the dominant mutant amylose-extender (Ae1-5180) maize starch. *Cereal Chem.*, 72, 457-464.
- Larson, K. (1980). Inhibition of starch gelatinization by amylose-lipid complex formation. *Starch/Staerke*, 32, 125-129.
- Lim, S. T., Kasemsuwan, T., and Jane, J. 1994. Characterization of phosphorus in starch by ^{31}P -nuclear magnetic resonance spectroscopy. *Cereal Chem* 71:488-493.
- Lim, S. and Seib, P. A. (1993). Location of phosphate esters in a wheat starch phosphate by ^{31}P -nuclear magnetic resonance spectroscopy. *Cereal Chem.*, 70, 145-152.
- Martin, F. W. and Deshpande, S. N. (1985). Sugars and starches of non sweet potato compared to those of conventional cultivars. *J. Agric. Univ. P. R.*, 69, 169-176.
- Mitch, E. L. (1984). Potato starch: production and utilization. In *Starch Chemistry and Technology*, 2nd ed., eds. R. L. Whistler, J. N. BeMiller, and E. F. Paschall. Academic Press, London, UK, pp. 479-490
- Muhrbeck, P. and Eliasson, A. C. (1991). Influence of the naturally occurring phosphate esters on the crystallinity of potato starch. *J. Sci. Food Agric.*, 55, 13-18.
- Muhrbeck, P., Svensson, E., and Eliasson, A. C. (1991). Effect of the degree of phosphorylation of the crystallinity of native potato starch. *Starch/ Staerke*, 43, 466-468.

- Muhrbeck, P., and Tellier, C. (1991). Determination of the phosphorylation of starch from native potato varieties by ^{31}P -NMR. *Starch/Staerke*, 46, 25-27.
- Nara, S., Mori, A., and Komiya, T. (1978). Study on relative crystallinity of moist potato starch. *Starch/Staerke*, 30, 111-114.
- Noda, T., Takahata, Y., and Sato, T. (1995). Distributions of the amylopectin chain length of sweet potatoes differing in stages of development, tissue zone and variety. *Nippon Shokuhin Kagaku Kogaku Kaishi*, 42, 200-206.
- Schoch, T. J. (1942). Fractionation of starch by selective precipitation with butanol. *J. Am. Chem. Soc.*, 64, 2957-2961.
- Schoch, T. J. (1964). Iodometric determination of amylose. Potentiometric titration: Standard method. In *Methods in Carbohydrate Chemistry*, Vol. 4 Starch, eds. R. L. Whistler, R. J. Smith, J. N. BeMiller, and M. L. Wolfram, Academic Press, London, U.K., pp. 157-160
- Shannon, J. C. and Garwood, D. L. (1984). Genetics and physiology of starch development. In *Starch Chemistry and Technology*, 2nd ed., eds. R. L. Whistler, J. N. BeMiller, and E. F. Paschall, Academic Press, London, UK., pp. 25-60.
- Smith, R. J. and Caruso, J. L. (1964). Determination of phosphorus. In *Methods in Carbohydrate Chemistry*, Vol. 4. Starch, eds. R. L. Whistler, R. J. Smith, J. N. BeMiller, and M. L. Wolfram, Academic Press, London, U.K., pp. 42-46
- Sugimoto, Y., Yamamoto, M., Abe, K., and Fuwa, H. (1987). Developmental changes in starch properties of the Chinese yam. *J. Jpn. Soc. Starch Sci.*, 34, 11-20.
- Suzuki, A., Kanayama, M., Takeda, Y., and Hizukuri, S. (1986). Physicochemical properties of Nagiamo (yam) starch. *J. Jpn. Soc. Starch Sci.*, 33, 191-198.
- Takeda, Y., and Hizukuri, S. (1982). Location of phosphate groups in potato amylopectin. *Carbohydr. Res.*, 102, 321-327
- Takeda, C., Takeda, Y., and Hizukuri, S. (1983). Physicochemical Properties of Lily Starch. *Cereal Chem.*, 60, 212-216.
- Takeda, Y., Tokunaga, N., Takeda, C., and Hizukuri, S. (1986). Physicochemical properties of sweet potato starches. *Starch/Staerke*, 38, 345-350.

- Tian, S. J., Rickard, J. E., and Blanshard, J. M. V. (1991). Physicochemical Properties of Sweet Potato Starch. *J. Sci. Food Agric.*, 57, 459-491.
- Umeki, K. and Kainuma, K. (1981). Fine structure of Naegeli amylopectin obtained by acid treatment of defatted waxy-maize starch-structural evidence to support the double helix hypothesis. *Carbohydr. Res.*, 96, 143-159.
- Watanabe, T., Akiyama, Y., Takahashi, H., Adachi, T., Matsumoto, A., and Matsuda, K. (1982). Structural features and properties of Naegeli amylopectin from waxy maize, sweet potato, and potato starches. *Carbohydr. Res.*, 109, 221-232.
- Wong, K. S. and Jane, J. (1997). Quantitative analysis of debranched amylopectin by HPAEC-PAD with a postcolumn enzyme reactor. *J. Liq. Chrom. & Rel. Technol.*, 20:297-310.
- Wu, H.-C. H. and Sarko, A. (1978). The crystal structures of A-, B- and C-type polymorphs of amylose and starch. *Starch/Staerke*, 30, 73-78.
- Zobel, H. F. (1988). Starch crystal transformations and their industrial importance. *Starch/Staerke*, 40, 1-7.

Table 1. Phosphorus contents of starches and potato Naegeli dextrans

	Chemical method^a	³¹P-NMR method^b			Phospholipid
	Total phosphorus (%)	Total phosphorus(%)	Monoester phosphate	Inorganic phosphate	
Normal potato	0.075 ± 0.001	0.075 ± 0.006	0.073 ± 0.005	0.001 ± 0.001	ND ^c
Waxy potato	0.066 ± 0.001	0.069 ± 0.003	0.069 ± 0.003	0.001 ± 0.000	ND
Yam	0.012 ± 0.000	0.012 ± 0.005	0.011 ± 0.005	0.001 ± 0.000	ND
Sweet potato	0.020 ± 0.000	0.021 ± 0.007	0.020 ± 0.007	0.000 ± 0.000	ND

^aDetermined by the method of Smith and Caruso (1964).

^bDetermined by the method of Kasemsuwan and Jane (1995).

^cND not detected.

Table 2. Percentage of amylose contents and iodine affinity in starches^a

Starch	Iodine Affinity of Starch ^b	Iodine Affinity of Amylopectin and Intermediate Component ^b	Apparent Amylose Content (%)	Absolute Amylose Content (%) ^c
Normal Potato	7.2 ± 0.3	4.6 ± 0.1	37.8 ± 1.4	18.3
Waxy Potato	3.6 ± 0.4	3.6 ± 0.3	19.2 ± 2.0	0
Yam	5.4 ± 0.2	2.7 ± 0.3	29.2 ± 0.9	17.7
Sweet Potato	6.3 ± 0.4	2.5 ± 0.4	33.1 ± 1.8	22.8

^aAmylose contents were determined by iodine potentiometric titration. The amylose contents were calculated by dividing iodine affinity by a factor of 0.20.

^bIodine affinities were calculated from at least three replications of each sample.

^cAbsolute amylose contents were calculated from the following equation:

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

C is the percentage of the real amylose content

IA_S is the iodine affinity of the whole defatted starch

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

Table 3.**Branch chain length (CL) distributions^a**

	First peak	Second peak	Percent Distribution				Average CL	Maximum Detectable DP
			DP 6-12	DP 13-24	DP 25-36	DP ≥37		
Normal potato	14	51	13.07 ± 0.02	44.39 ± 0.05	14.00 ± 0.02	28.54 ± 0.13	28.6	85
Waxy potato	14	49	14.75 ± 0.02	48.43 ± 0.04	14.38 ± 0.10	22.43 ± 0.03	25.8	85
Yam	13	52	19.09 ± 0.01	44.81 ± 0.09	14.32 ± 0.03	21.80 ± 0.05	25.8	85
Sweet potato	13	48	17.05 ± 0.02	48.10 ± 0.02	13.56 ± 0.02	23.40 ± 0.02	26.3	85

^a Results are the means of three replicates from the high performance anion-exchange chromatograph with pulsed amperometric detection (Fig. 5). The total relative peak area was used to calculate percent distribution.

Table 4. Thermal properties of starch gelatinization for native and retrograded starch as determined by differential scanning calorimetry ^a

	Native starch				Retrograded starch				%Retr ^f
	T _o ^b (°C)	T _p ^c (°C)	T _c ^d (°C)	ΔH ^e (J/g)	T _o ^b (°C)	T _p ^c (°C)	T _c ^d (°C)	ΔH ^e (J/g)	
Normal potato	60.8 ± 0.1	65.2 ± 0.0	70.6 ± 0.4	17.3 ± 0.3	41.1 ± 0.3	56.1 ± 0.1	66.4 ± 0.1	6.9 ± 0.5	40.1
Waxy potato	62.5 ± 0.2	66.6 ± 0.2	70.2 ± 0.4	18.2 ± 0.5	38.6 ± 0.3	56.0 ± 0.2	65.2 ± 0.2	7.8 ± 0.3	42.0
Yam	64.6 ± 0.2	70.9 ± 0.3	77.8 ± 0.4	13.3 ± 0.3	39.2 ± 0.1	51.9 ± 0.5	61.5 ± 0.2	5.0 ± 0.1	37.5
Sweet potato	57.9 ± 0.2	63.1 ± 0.1	71.9 ± 0.3	13.5 ± 0.6	39.9 ± 0.3	52.7 ± 0.4	63.2 ± 0.4	6.1 ± 0.3	44.8

^a The values are the averages of at least three replications (mean ± standard deviation)

^b Onset temperature.

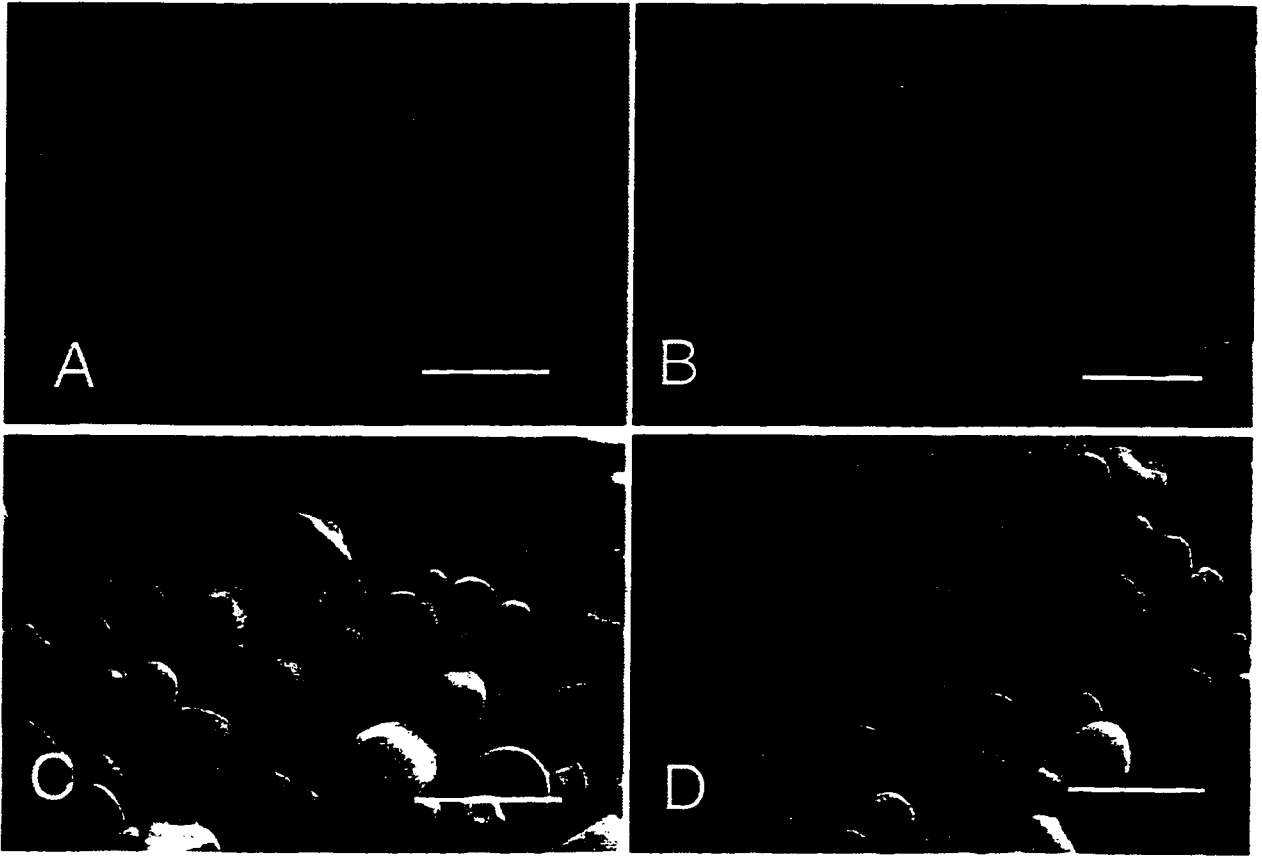
^c Peak temperature.

^d Conclusion temperature.

^e Gelatinization enthalpy of starch.

^f Percent retrogradation (retrograded starch enthalpy/native starch enthalpy)

Fig. 1. Scanning electron micrographs of normal potato (A), waxy potato (B), sweet potato (C) and yam (D) starch granules. (Bars are 20 and 10 μm for A and B, and C and D, respectively).



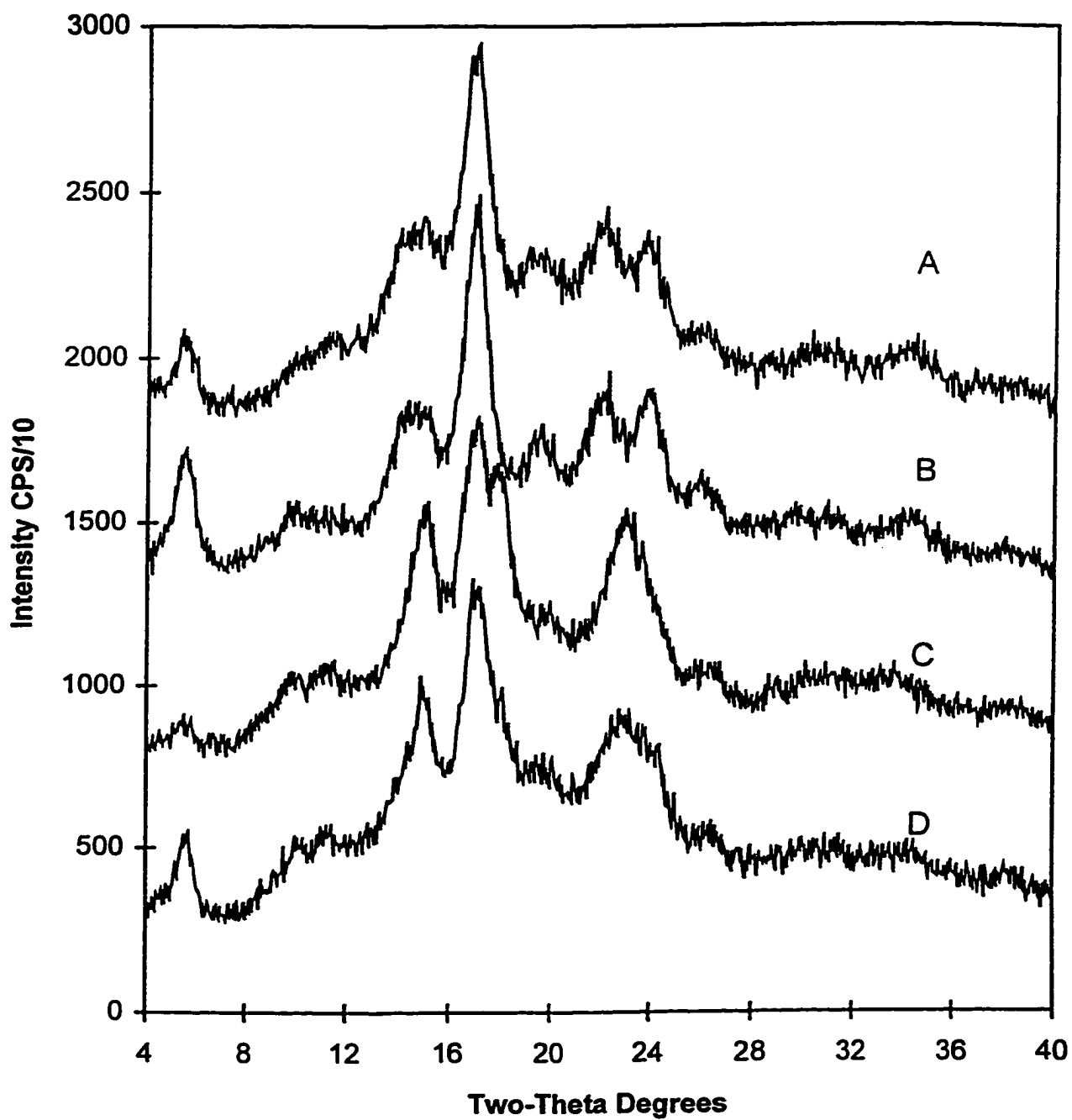


Fig. 2. X-ray diffraction patterns of normal potato (A), waxy potato (B), yam (C) and sweet potato (D) starches.

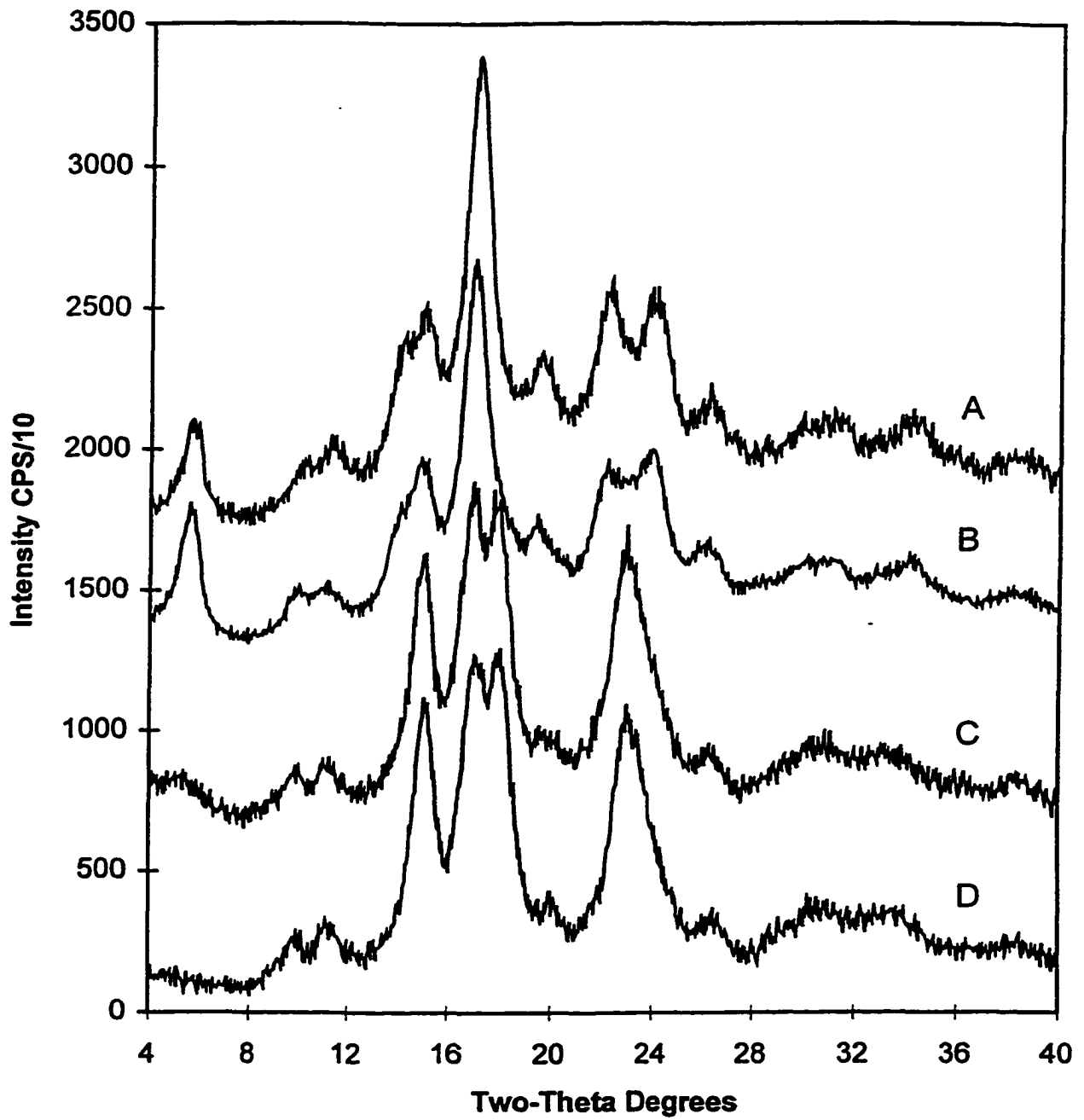


Fig. 3. X-ray diffraction patterns of 12-day Naegeli dextrins of normal potato (A), waxy potato (B), yam (C), and sweet potato (D).

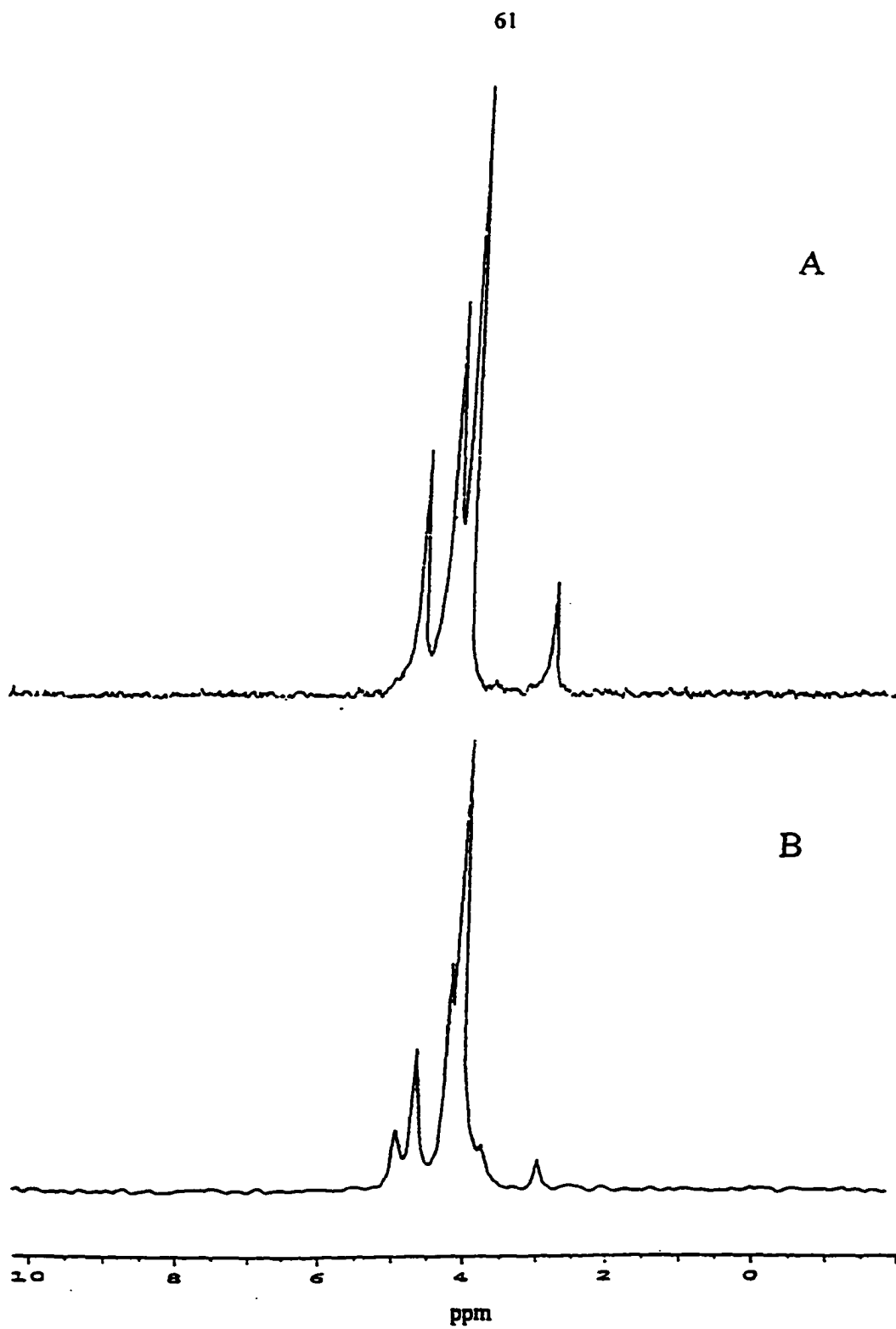


Fig. 4. The ^{31}P -nuclear magnetic resonance spectra of (A) normal potato starch and (B) the Naegeli dextrin of potato starch.

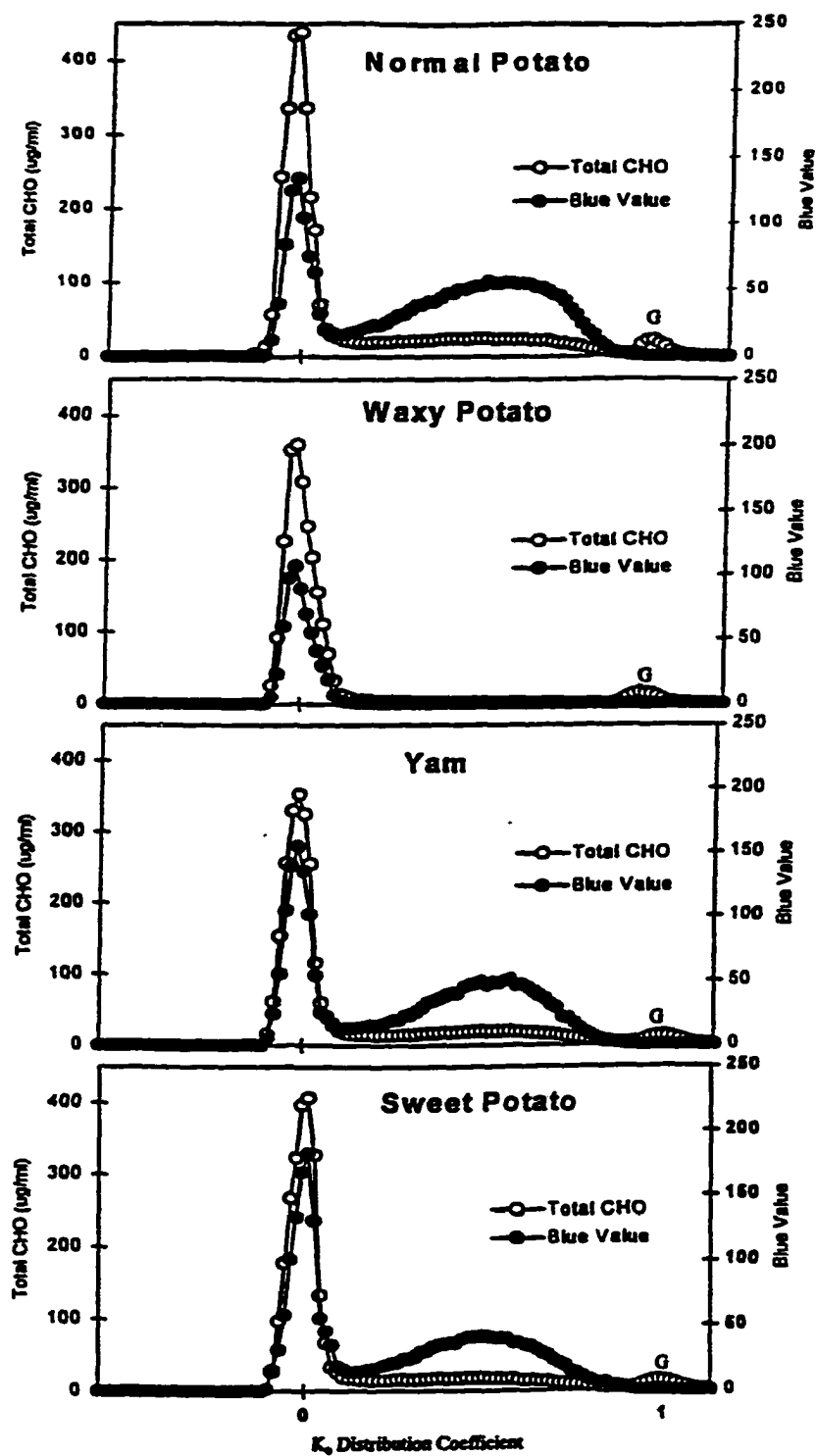


Fig. 5. Sepharose CL-2B column profiles of normal potato, waxy potato, yam and sweet potato starches.

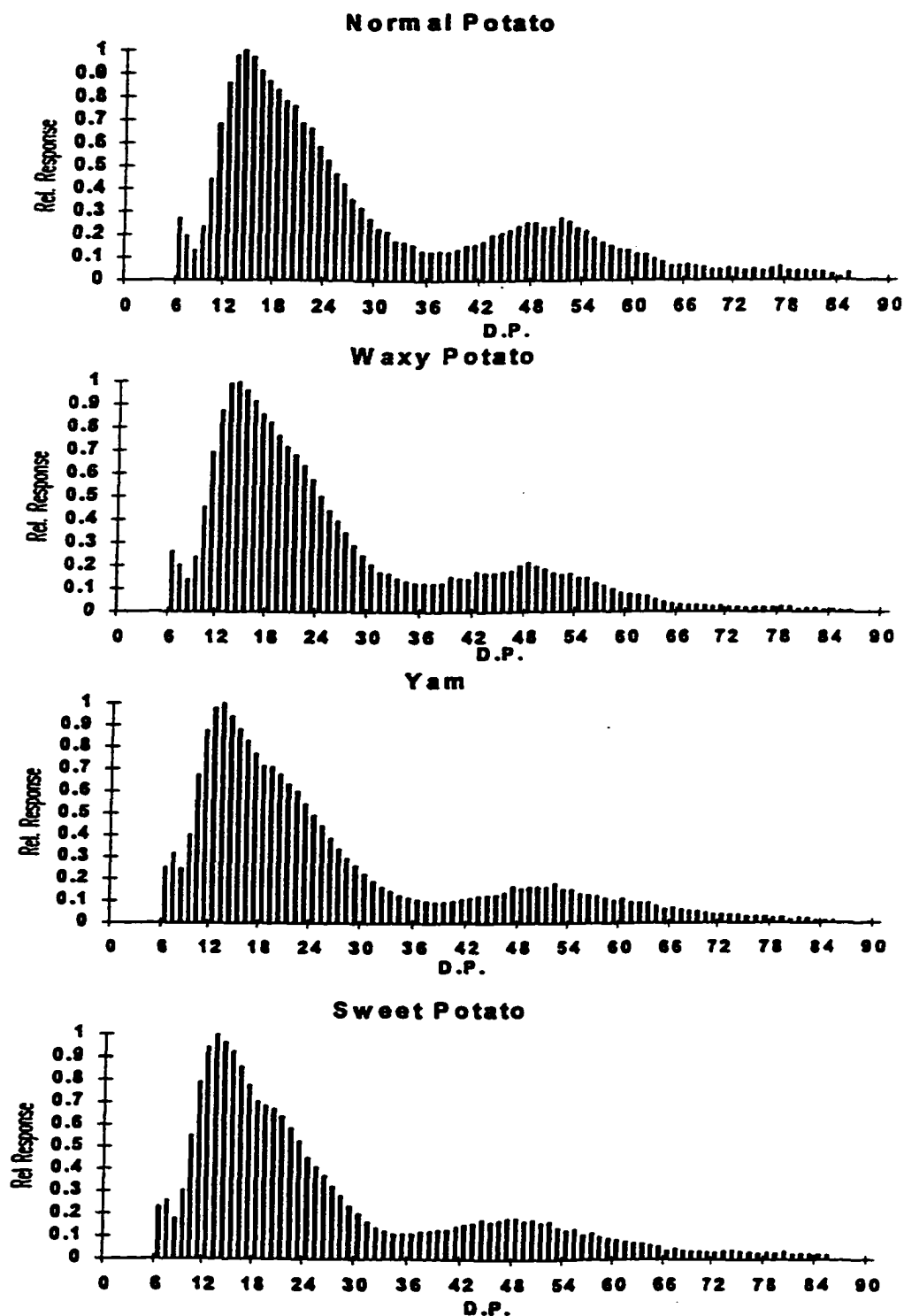


Fig. 6. Normalized peak area chromatograms of isoamylase debranched amylopectins of normal potato, waxy potato, yam and sweet potato starches produced by using high performance anion exchange chromatography equipped with an on-line amyloglucosidase reactor and a pulsed amperometric detector.

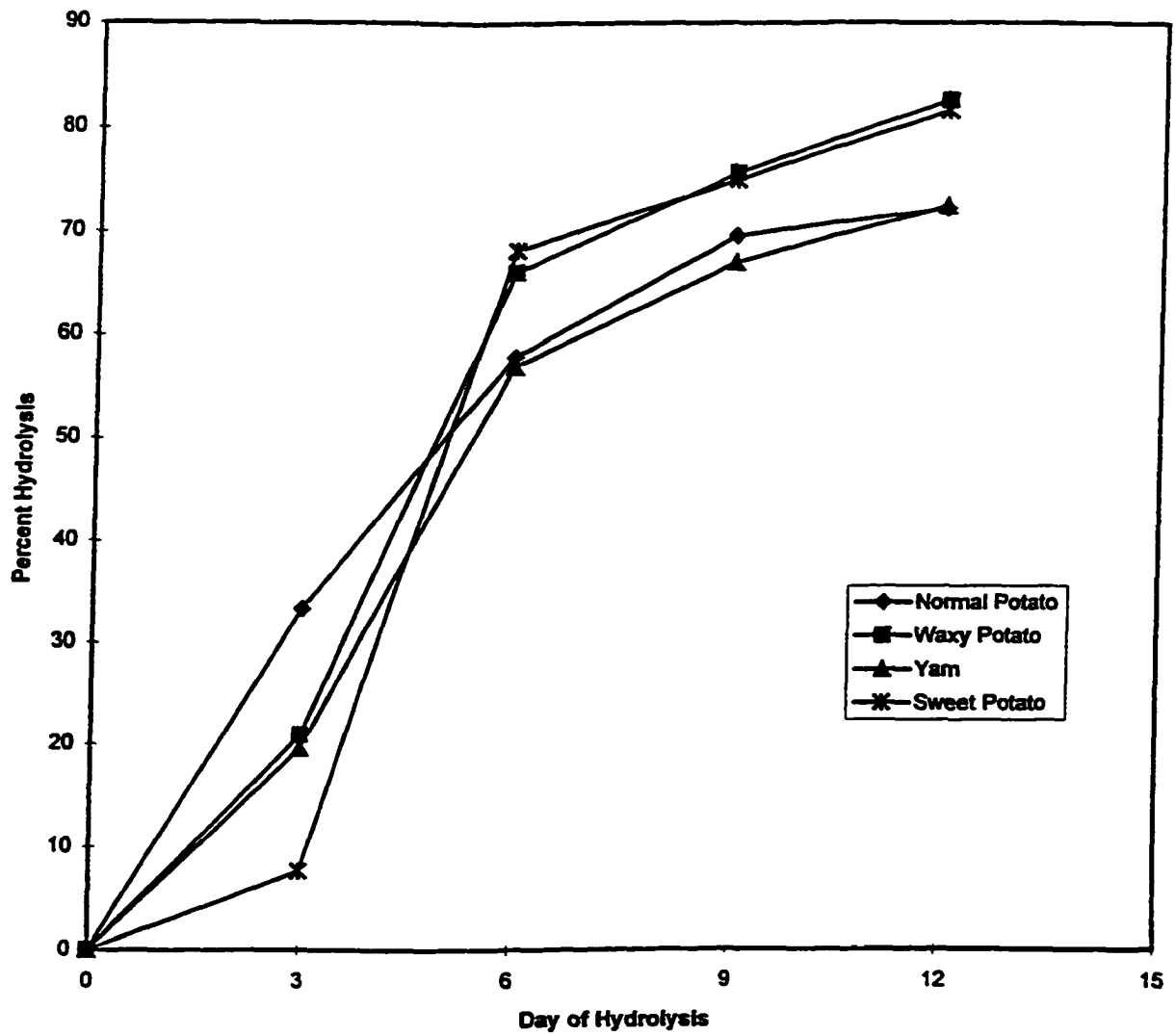


Fig. 7. Acid hydrolysis rates (15.3% H_2SO_4 v/v) of normal potato, waxy potato, yam and sweet potato starches.

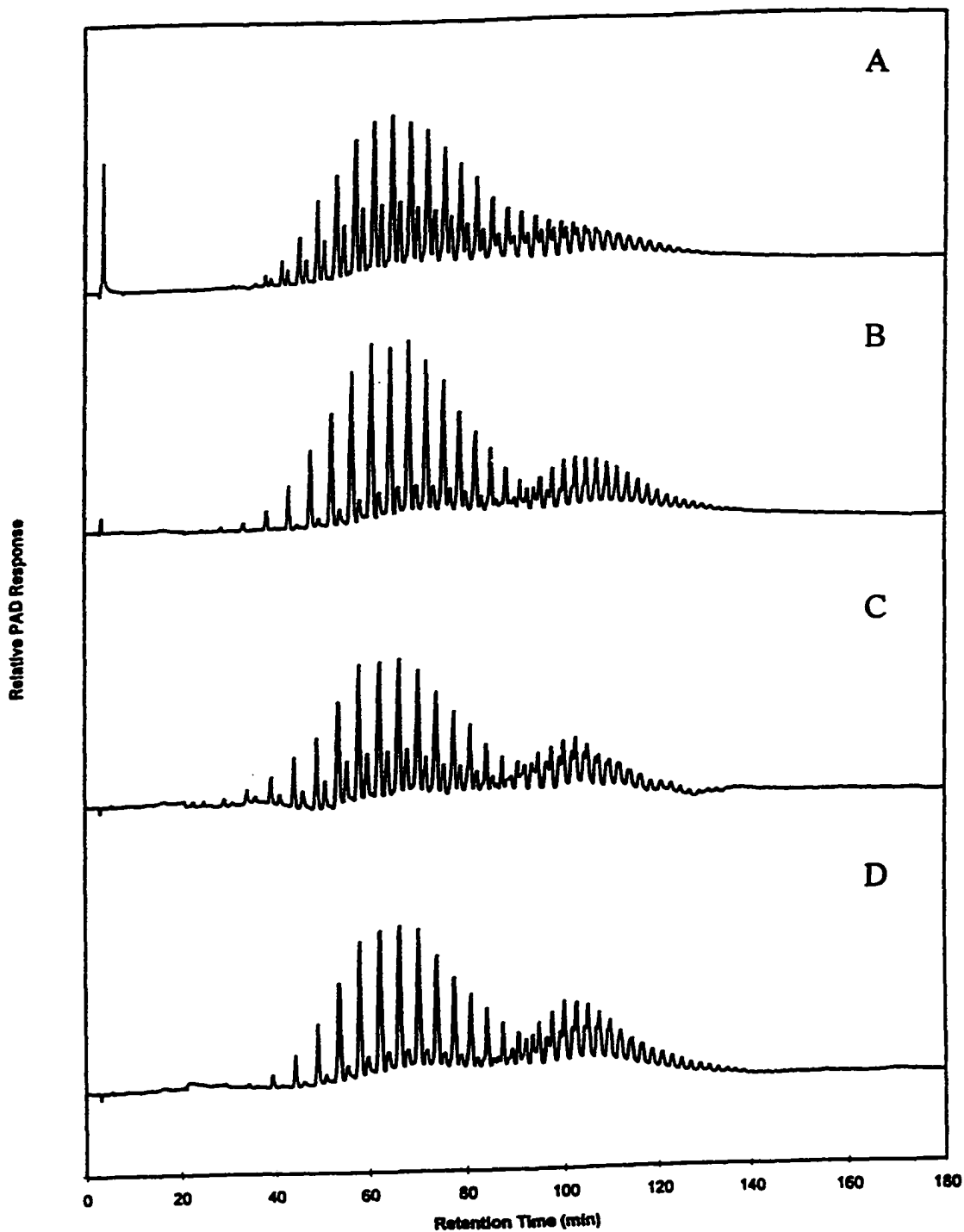


Fig. 8. Chromatograms of normal potato (A), waxy potato (B), yam (C) and sweet potato (D) 12-day Naegeli dextrans analyzed by use of high performance anion exchange chromatography equipped with an on-line amyloglucosidase reactor and a pulsed amperometric detector. Dextrin of dp 15 is eluted at 65 min.

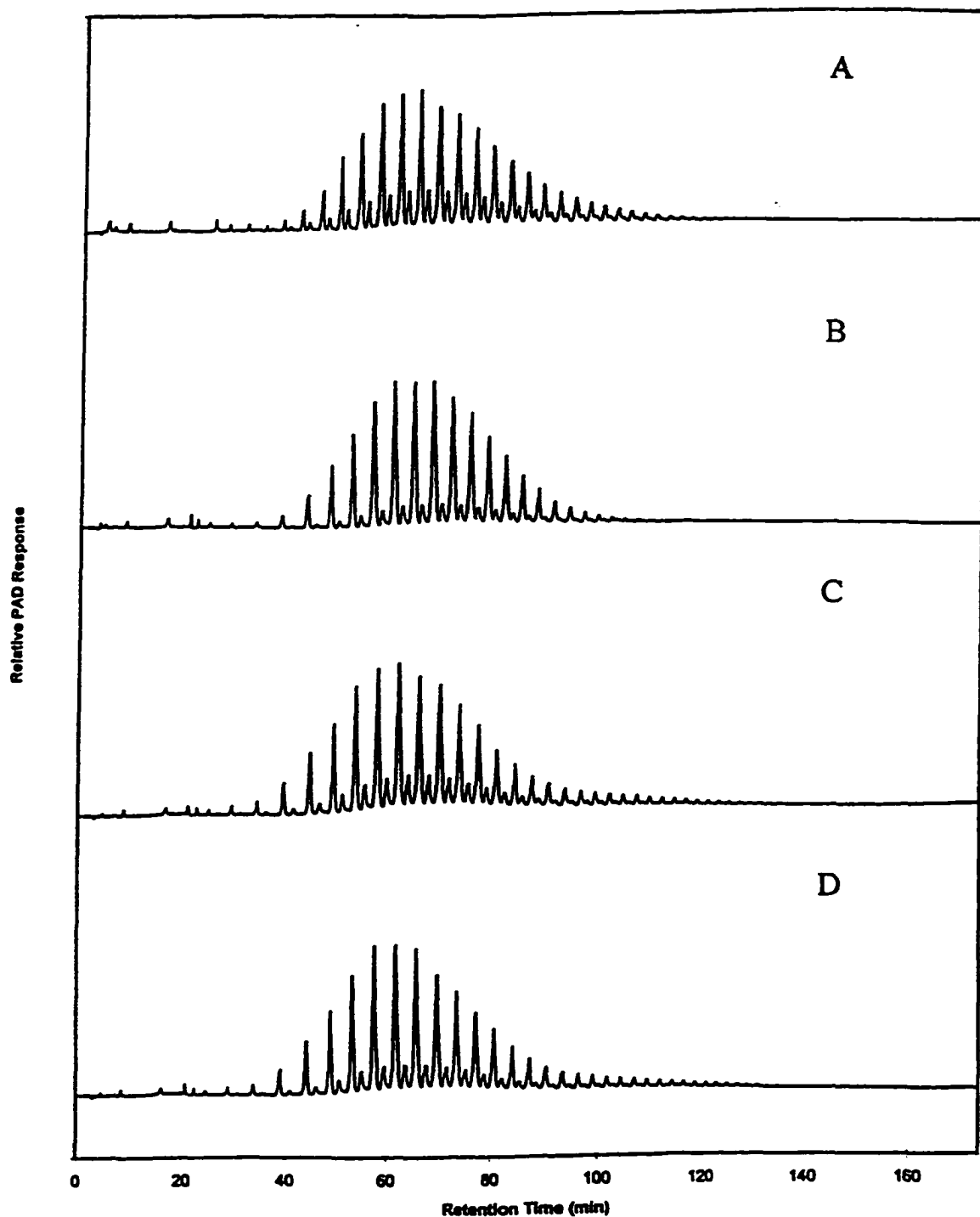


Fig. 9. Chromatograms of isoamylase debranched normal potato (A), waxy potato (B), yam (C) and sweet potato (D) 12-d Naegeli dextrans analyzed by use of high performance anion exchange chromatography equipped with an on-line amyloglucosidase reactor and a pulsed amperometric detector. Dextrin of dp 15 is eluted at 65 min.

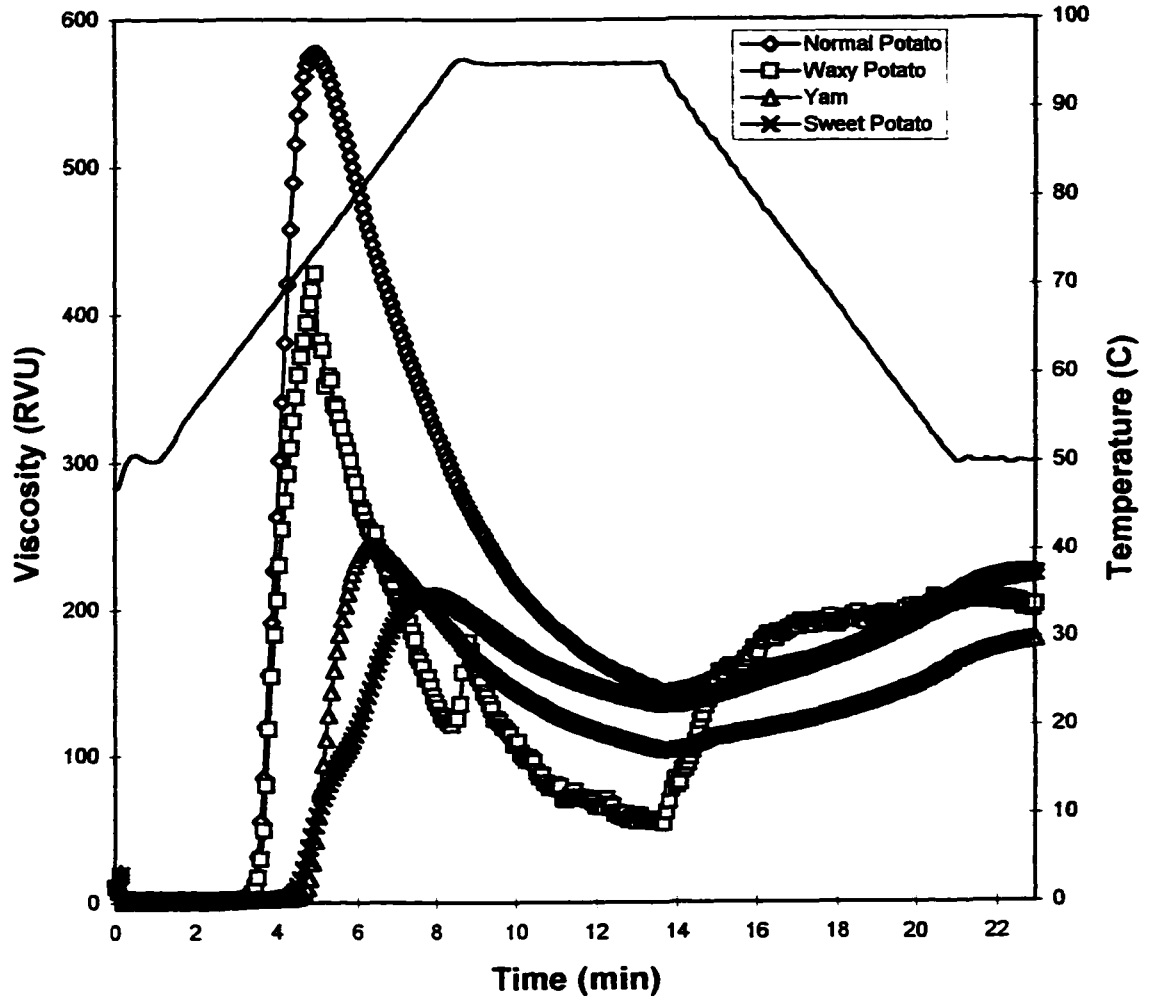


Fig.10. Rapid Visco Analyzer pasting profiles of normal potato, waxy potato, yam and sweet potato starches (8% dsb).

EFFECTS OF EXTRUSION PARAMETERS ON CROSS-LINKED HYDROXY-PROPYLATED CORN STARCHES. I PASTING PROPERTIES

A paper to be submitted to Cereal Chemistry

A. E. McPherson, T. B. Bailey, and J. Jane

ABSTRACT

A series of cross-linked (0, 0.014, 0.018, 0.024, and 0.028% POCl₃, dry starch basis) hydroxypropylated (8%) corn starches were extruded using a Leistritz micro-18 co-rotating extruder. Process variables included moisture, barrel temperature, and screw design. Differential scanning calorimetry and x-ray diffraction studies showed the level of starch crystallinity decreased with increasing severity of extrusion conditions. Pasting properties of the extruded starches were examined using a Rapid Visco Analyzer. Pasting profiles of starches extruded at different conditions displayed different hot paste viscosity and final viscosity. Increasing starch moisture content during extrusion and level of cross-linking increased starch viscosity ($p < 0.0001$). Whereas, increasing extrusion temperature and shear decreased starch viscosity ($p < 0.0001$). Interactions were found between level of cross-linking and screw design and between extrusion temperature and starch moisture content ($p < 0.0001$).

INTRODUCTION

Extrusion is an important method for processing starch and starch-based products (Colonna et al 1984, Colonna et al 1989, Harper 1989). Extrusion cooking has been studied

for the production of pre-gelatinized, unmodified starches (Anderson et al 1970, Mercier and Feillet 1975, Mercier 1977, Gomez and Aguilera 1984, Doublier et al 1986, Chinnaswamy and Hanna, 1990, Ryu and Walker 1995). Traditionally, pre-gelatinized starches have been produced by drum drying (Powell 1967). Pre-gelatinized starches display an “instant” viscosity upon dispersion in water without heating, and their properties are dependent upon cooking and drying conditions (Colonna et al 1984)

The characteristics of extruded native starches are well documented, whereas relatively little is known of extruded chemically modified starches. Extrusion processing is used to produce pre-gelatinized starches which have been reported to display different pasting properties from those produced via conventional cooking and drum drying (Colonna et al 1984, Pan et al 1998). The differences are attributed to varying degrees of depolymerization and molecular entanglement resulting from extrusion (Colonna et al 1984, Diosady 1986, Harper 1992).

Extruded starches have been characterized using differential scanning calorimetry (DSC), intrinsic viscosity, pasting profiles, and chromatography. When large numbers of samples are generated, however, a rapid method of analysis, such as a Rapid Visco Analyzer (RVA) pasting profile, is advantageous and has been used for starch mixtures and extruded starches (Walker et al 1988, Deffenbaugh and Walker 1989, Deffenbaugh and Walker 1990, Harper 1992, Whalen et al 1997).

The objectives of this study were to characterize a series of hydroxypropylated (8%) and cross-linked (0.0, 0.014, 0.018, 0.024, 0.028% POCl₃) normal corn starches extruded at

different conditions using DSC, x-ray diffraction, and RVA pasting profiles. Extrusion variables included: screw design of the extruder, starch moisture content, extrusion temperature, and level of starch cross-linking.

MATERIALS AND METHODS

Normal corn starch was obtained from Grain Processing Corporation, Muscatine, IA. Chemicals used were reagent grade and were obtained as follows: propylene oxide, Dow Chemical Company (Midland, MI); phosphorous oxychloride, FMC (Princeton, NJ); sodium chloride, Cargill Salt (Minneapolis, MN); and hydrochloric acid and sodium hydroxide, Harcross Chemical Inc. (Kansas City, KS).

Chemical Modifications of Starch

Hydroxypropylation: an aqueous slurry of unmodified dent corn starch was heated to 40-50°C with agitation and purged with nitrogen gas. Sodium hydroxide and salt (sodium chloride/sodium sulfate) were added to the slurry under vigorous agitation followed by the addition of propylene oxide (8%, dry starch basis (dsb)). The reaction was allowed to proceed for 16 hr until terminated by pH adjustment to 6.0 (Hjermstad 1967, Tuschoff 1986, Rutenberg and Solarek 1984).

Cross-linking: NaOH (0.5-1.0%, dsb) and phosphorous oxychloride were slowly added (0.01-0.03%, dsb) to the agitated slurry (Hullinger 1967, Rutenberg and Solarek 1984, Solarek 1986) and the reaction was run for 2 h. The reaction was terminated by adjusting the

pH to 5.0-6.0 using hydrochloric acid. After pH adjustment, the reaction slurry was thoroughly washed to remove salts and reaction by-products.

Starch Extrusion

Starch extrusion was carried out using a Leistritz Micro-18 co-rotating extruder (American Leistritz Extruder Company, Somerville, NY) with a 30:1 screw length to diameter ratio and a 3.175 mm die opening. Extruder screws were designed with an increasing number of kneading blocks to impart increasing shear to the extrudates (Fig. 1). The extruder barrel was composed of 6 programmable heating zones (Table 1). Starches with varying moisture contents were prepared using a Kitchen Aid mixer (Kitchen Aid, St. Joseph, MI) and a spray bottle to add water on a weight basis. Mixtures were sealed in polyethylene bags and allowed to equilibrate for at least 2 h before extrusion. Starch was fed into the extruder at a rate of 1.6 kg/hr. Residence time in the extruder barrels, using dyed starch, were 49, 56, and 68 s, respectively, for low-, medium-, and high-shear screw designs.

Extruded starch was collected as continuous strand after torque, barrel temperature, and die pressure reached steady state. Upon completion of extrusion, the extrudate strands were immediately placed in a 100°C forced air convection oven and dried for 8 hr. Dried extrudates were milled with a hammer mill, followed by a Udy Cyclone mill (Udy Corp., Ft. Collins, CO) and then sieved through a 160 mesh screen. Sieved starches were sealed in polyethylene bags and stored until analysis.

Experimental Design

The treatment design was a four factor factorial with a total of 162 treatments. Two replicates of the 162 treatments were carried out. Variables and their levels used as treatments are presented in Table I. The experiment was conducted following a split, split plot design. Split plot treatments were the moisture and temperature combinations. Screw designs were randomly set at low, medium, or high and the starches were extruded in random order. A single experimental error term was used because the three error terms in the split, split plot analysis of the hot paste viscosity and final viscosity were similar.

Thermal Properties of Starches Determined by DSC

Thermal properties of the starches were analyzed by using a differential scanning calorimeter (DSC-7, Perkin Elmer Corp., Norwalk, CT) equipped with an Intracooler II System and Pyris thermal analysis software (Perkin-Elmer Corp., Norwalk, CT). Starch and water mixtures (1:3) were sealed in aluminum pans and equilibrated at room temperature for 2 hr before analysis. An empty aluminum pan was used as the reference. The samples were heated at 10°C/min over a temperature range of 25-100°C. Indium and zinc were used as reference standards. The gelatinization temperature and enthalpy change were determined following the procedure of Kasemsuwan et al (1995). Enthalpy change (ΔH), onset temperature (T_o), peak temperature (T_p), and conclusion temperature (T_c) were computed.

X-Ray Diffraction Patterns

The x-ray patterns of the starches were obtained with copper, nickel foil filtered, $K\alpha$ radiation using a diffractometer (D-500 Siemens, Madison, WI, USA) following the method of Jane et al (1997). The diffractometer was operated at 27 mA and 50 kV. The scanning region of the diffraction angle (2θ) was from 4° to 40° at a 0.05° step size with a count time of 2 sec. Starches were equilibrated at 100% relative humidity for 24 hr at 25°C prior to examination.

Pasting Properties of Extruded Starches

Pasting profiles of extruded starches were examined using a Rapid Visco Analyzer (RVA-4, Newport Scientific, NSW, Australia). Starch suspension (15%, dsb) was prepared by weighing milled, extruded starch (4.50 g, dsb) into a RVA canister and adjusting the total weight to 30g with distilled water. A manual premixing step with a spatula was required for the pre-gelatinized starch samples to ensure homogeneity of the sample mixture. After premixing, the starch paste was removed from the spatula with the RVA paddle. The heating profile was run as follows: hold at 30°C for 1 min, heat to 95°C at $6.5^\circ\text{C}/\text{min}$, hold at 95°C for 5.5 min, cool to 50°C at $6^\circ\text{C}/\text{min}$, and hold at 50°C for 2 min. Paddle speed was set at 960 rpm for the first 5 sec and then 160 rpm for the remainder of the analysis. Viscosity values were collected after holding at 95°C , at 16 min (hot paste), and cooling to 50°C , at 26 min (final).

RESULTS AND DISCUSSION

The onset gelatinization temperature and enthalpy change of native corn starch determined by DSC were 66.1°C and 13.2 J/g, respectively. The hydroxypropylated (8%) maize starches with cross-linking levels of 0, 0.014, 0.018, 0.024, and 0.028% POCl₃, had onset gelatinization temperatures of 50.6, 52.6, 50.6, 51.9, and 50.8°C, and enthalpy changes of 6.5, 9.2, 8.5, 8.1, and 8.3 J/g, respectively (Table II). The decreased gelatinization temperatures and enthalpy changes of the chemically modified starches from that of the native corn starch indicated the crystalline structure of the native starch granules was destabilized and possibly reduced during the cross-linking and hydroxypropylation reactions at alkaline pH (Table II). DSC thermograms of extruded starches showed those extruded at 60 and 80°C displayed a smaller gelatinization endotherm than their respective unextruded counterparts. The onset gelatinization temperatures of the extruded starches generally were somewhat higher than those of the parent starches (Table II). The magnitude of the enthalpy change decreased when the extrusion temperature increased. Increased onset gelatinization temperatures of the extruded starches indicated that starch granules with lower gelatinization temperatures (such as damaged starches) were gelatinized during extrusion; those with higher gelatinization temperatures were more resistant to extrusion. Annealing of remaining crystalline material during drying may have contributed to the increased gelatinization temperatures of the extruded starches. Starches subjected to higher extrusion temperatures

(100°C) showed no gelatinization peak, indicating total gelatinization of the starch during high-temperature extrusion.

The x-ray diffraction patterns of the extruded starches showed that crystallinity decreased as the temperature of extrusion increased. Native corn starch displayed an A-type x-ray diffraction pattern (Fig. 2). Native corn starch extruded at 30% moisture and high shear at 60°C displayed reduced crystallinity. At extrusion temperatures of 80°C and 100°C the starch was gelatinized as indicated by the absence of crystalline peaks (Fig. 2). X-ray diffraction patterns of extruded hydroxypropylated and cross-linked hydroxypropylated starches showed similar decreases in crystallinity as extrusion temperature increased (data not shown). DSC showed a small thermal transition peak for the native corn starch extruded at 80°C, whereas x-ray analysis showed no diffraction pattern. It is possible that the remaining crystallites were too small to display an x-ray diffraction pattern. The x-ray pattern of the native corn starch extruded at 100°C displayed a peak at ~20° and a small bumps at 13°, indicating a small amount of V-type amylose-lipid complexes.

The pasting profiles of the native and chemically modified starches (8% dsb) are shown in Figure 3. The starch pasting temperatures were 76.4, 57.3, 59.1, 59.0, 59.5, and 59.9°C for native corn starch and cross-linked (0.0, 0.014, 0.018, 0.024, and 0.028% POCl₃) hydroxypropylated (8%) corn starches, respectively (Fig. 3). The pasting temperatures of the starches increased with increasing level of cross-linking. The final viscosity of the starches also increased with increasing level of cross-linking, 98.3, 131.7, 253.8, 307.4, 325.8, and 380.42 RVU, for native and cross-linked (0.0, 0.014, 0.018, 0.024, and 0.028% POCl₃)

hydroxypropylated (8%) corn starches, respectively (Fig. 3). The pasting peak viscosity increased with levels of cross-linking up to 0.018% POCl₃. With further increases in cross-linking level in normal starches, the peak viscosity decreased and the final viscosity increased (Fig. 3), which agreed with those reported by Rutenberg and Solarek (1984) and Kasemsuwan and Jane (1994).

The experimental design for extrusion processing consisted of 2 replications of 162 treatment combinations that included: screw design (shear), extrusion temperature, starch cross-linking level, and starch moisture content. Pasting profiles were generated and analyzed for all treatment combinations. Because the extruded starches displayed low hot paste viscosity and final viscosity, a solids content of 15% (dsb) was used for the pasting study, which resulted in reproducible RVA pasting profiles with adequate viscosity levels for detection. Most of the extruded starches displayed substantial instant viscosity after stirring for 1 min at 30°C, indicating that the starches had been pre-gelatinized by extrusion (Figs. 3 - 7).

Average hot paste viscosity and final viscosity of extruded starches were calculated for each of the treatments and are summarized in Tables III and IV. Differences between the means of the treatments are discussed in terms of significant variables and variable interactions. Extruded starches displayed different pasting profiles from their respective parent starches (Figs. 3-7). Representative pasting profiles of extruded starches from various treatment combinations are shown in figures 4-7.

The extrusion temperature affected the hot paste viscosity and final viscosity of extruded starches (Table III). Figure 4 shows cross-linked (0.028% POCl_3) hydroxypropylated (8%) corn starches extruded at 40% moisture, high shear, and increasing temperature (60, 80, and 100°C). Starches extruded at low temperature (60°C) displayed a lower instant viscosity and higher hot paste and final viscosity than those extruded at 80°C and 100°C. Similar extrusion temperature effects on instant and final viscosities have been reported for wheat flour (Seiler et al 1980) and wheat starch (Colonna et al 1984). Cross-linked (0.028% POCl_3) hydroxypropylated (8%) corn starch extruded at low temperature (60°C) and high moisture (40%) displayed a small pasting peak with a pasting temperature of 60.0°C (Fig. 4). This small pasting peak occurred at a similar temperature range to the pasting peak of the unextruded, parent starch (Fig. 3). Those extruded starches displaying a small pasting peak also displayed a DSC gelatinization peak with a reduced enthalpy change and residual A-type x-ray diffraction pattern. These results confirmed the presence of remaining crystalline structure in the starches extruded at low temperature (60°C) and were in agreement with the scanning electron microscopy results which showed distorted and fractured granule residues present in these starches (McPherson and Jane 1999).

Viscosity of extruded starches decreased as temperature of extrusion increased from 60°C to 100°C (Table III) ($p < 0.0001$) except those extruded at 30% moisture content. The increased extrusion temperature gelatinized the starches to a greater extent. Passage of the gelatinized starch through the extruder barrel resulted in shear degradation of the molecules and subsequent decreases in hot paste and final viscosity (Bhattacharya and Hanna 1987).

Viscosity of the extruded starches increased as starch moisture content increased from 30% to 40% (Table III) ($p < 0.0001$). An example of the effect of varying moisture content during extrusion on starch pasting profiles is shown in Fig. 5, cross-linked (0.014% POCl_3) hydroxypropylated (8%) corn starch extruded at 100°C and medium shear with moisture levels of 30, 35, and 40% (Fig. 5). Moisture acts as a plasticizer during extrusion of starches and lowers the extent of shear degradation (Lai and Kokini 1991). This effect has been reported for hot paste and final viscosity in unmodified wheat starch (Colonna et al 1984, Mason and Hosenev 1986) and corn starch (Chinnaswamy and Hanna 1990). Amylopectin molecular weights of extruded cross-linked hydroxypropylated (8%) starches decreased as the moisture content decreased during extrusion (McPherson and Jane 1999). Starch extruded with 30% moisture content displayed increased viscosity with increasing temperature, which was opposite to those extruded with 35% and 40% moisture ($p < 0.0001$) (Table III). This difference may be attributed to the starch with lower moisture content having higher glass transition temperature. Thus, being extruded at higher temperature, the starch became rubbery and resulted in less friction and less degradation as supported by SEM structures reported by McPherson and Jane (1999).

As the level of starch cross-linking increased, the average viscosity of the chemically modified starches increased to a greater degree with extrusion at low shear than did those extruded at medium and high shear (Table IV) ($p < 0.0001$). In general, the instant, hot paste, and final viscosity of starch pastes increased with increasing the level of starch cross-linking as shown in Figure 6 for starches extruded at 30% moisture, 100°C , and high shear. Cross-

linked starches retained more structure when extruded at low shear which resulted in greater hot paste and final viscosity than did those extruded at medium and high shear. The extruded native corn starches had higher average viscosity than did the extruded chemically modified starches (Table IV). This was caused by the retention of crystalline material in the native starch as shown by DSC, x-ray diffraction, and RVA pasting profiles, which was a result of native corn starch having a higher gelatinization temperature (Table II). Level of starch cross-linking had a significant effect on the viscosity of the extruded starches ($p < 0.0001$). As the level of starch cross-linking increased from 0.0% POCl_3 to 0.028% POCl_3 the average viscosities of the starches also increased (Table IV) ($p < 0.0001$). This is in agreement with the results reported by McPherson and Jane (1999) showing that amylopectin molecular weights of extruded hydroxypropylated (8%) starches with cross-linking were higher than those without cross-linking. Cross-linking is used to impart shear, acid, and thermal stability to starches in various conventional processing applications such as retorting, continuous mixing, and pumping (Hullinger 1967, Rutenberg and Solarek 1984). Viscosity of extruded starches decreased as the level of shear increased from low to high (Table IV) ($p < 0.0001$). Figure 7 shows an example of the effect of shear on cross-linked (0.028% POCl_3) hydroxypropylated (8%) corn starch extruded at 100°C and 30% moisture. Similar findings have been reported for extruded corn grits (Barres et al 1990) and wheat flour (Seiler et al 1980).

CONCLUSIONS

Extrusion of hydroxypropylated (8%) corn starch with varying levels of cross-linking (0, 0.014, 0.018, 0.024, and 0.028% POCl₃) at different levels of moisture, barrel temperature, and shear resulted in a range of partially to totally pre-gelatinized starches.

Differential scanning calorimetry and x-ray diffraction both showed remaining crystallinity in starches extruded at low temperature (60°C). Starch moisture content, extrusion temperature, level of starch cross-linking, and screw design were shown to affect pasting characteristics as measured by RVA ($p < 0.0001$). Increasing the starch moisture content level and cross-linking resulted in extruded starches with increased hot paste and final viscosity. The interactions between cross-linking level and level of shear and between starch moisture content and extrusion temperature were significant.

REFERENCES

- Anderson, R. A., Conway, H. F., and Peplinski, A. J. 1970. Gelatinization of corn grits by roll cooking, extrusion cooking and steaming. *Starch/Staerke* 22:130-135.
- Barres, C., Vergnew, B., Tayeb, J., and Della Verde, G. 1990. Transformation of wheat flour by extrusion cooking: Influence of screw configuration and operating conditions. *Cereal Chem.* 67:427-433.
- Bhattacharya, M., and Hanna, M. A. 1987. Kinetics of corn meal gelatinization at high temperature and low moisture. *J. Food Sci.* 52:764-766.
- Chinnaswamy, S., and Hanna, M. 1990. Macromolecular and functional properties of native and extrusion-cooked corn starch. *Cereal Chem.* 67:490-499.

- Colonna, P., Doublier, J. L., Melcion, J. P., DeMonredon, F., and Mercier, C. 1984. Extrusion cooking and drum drying of wheat starch I. Physical and macromolecular modifications. *Cereal Chem.* 61:538-543.
- Colonna, P., Tayeb, J., and Mercier, C. 1989. Extrusion cooking of starch and starchy products. In *Extrusion cooking*, C. Mercier, P. Linko, and J.M. Harper, eds. American Association of Cereal Chemists, St. Paul, MN.
- Deffenbaugh, L. B., and Walker, C. E. 1989. Use of the rapid visco analyzer to measure starch pasting properties. Part I: Effect of sugars. *Starch/Staerke* 41:461-467.
- Deffenbaugh, L. B., and Walker, C. E. 1990. Use of the rapid visco analyzer to measure starch pasting properties. Part I: Effects of emulsifiers and sugar-emulsifiers interactions. *Starch/Staerke* 42:89-95.
- Diosady, L. L. 1986. Review of recent studies in the in the mechanism of starch extrusion. In *Food Engineering and Process Applications*. Vol. 2. M. Lemauger and P. Jelen, eds. Elsevier Applied Science., New York.
- Doublier, J. L., Colonna, P., and Mercier, C. 1986. Extrusion cooking and drum drying of wheat starch. II. Rheological characterization of starch pastes. *Cereal Chem.* 63:240-246.
- Gomez, M. H., and Aguilera, J. M. 1984. A physicochemical model for extrusion of corn starch. *J. of Food Sci.* 49:40-43.
- Harper, J. M. 1989. Food extruders and their applications. Pages 1-16 in: *Extrusion Cooking*. C. Mercier, P. Linko, and J. M. Harper, eds. AACC , St. Paul, MN.
- Harper, J. M. 1992. Extrusion processing of starch. Pages 37-64 in: *Developments in Carbohydrate Chemistry*. R. J. Alexander and H. Zobel, eds. AACC. St. Paul, MN.
- Hjermstad, E. T. 1967. Production and uses of hydroxyethyl starch. Pages 423-430 in: *Starch: Chemistry and Technology*, Vol II. R. L. Whistler and E. F. Paschall eds. Academic Press, Inc., New York.
- Hullinger, C. H. 1967. Production and use of cross-linked starch. Pages 445-450 in: *Starch: Chemistry and Technology*, Vol. II. R. L. Whistler and E. F. Paschall eds. Academic Press, Inc., New York.

- Jane, J., Wong, K., and McPherson, A. (1997). Branch-structure difference in starches of A- and B-type x-ray patterns revealed by their Naegeli dextrans. *Carbohydr. Res.*, 300:219-227.
- Kasemsuwan, T., and Jane, J. 1994. Location of amylose in normal starch granules. II. Locations of phosphodiester cross-linking revealed by phosphorous-31 nuclear magnetic resonance. *Cereal Chem.* 71:282-287.
- Kasemsuwan, T., Jane, J., Schnable, P., Stinard, P., and Robertson, D. 1995. Characterization of the dominant mutant amylose-extender (Ae1-5180) maize starch. *Cereal Chem.* 72:457-463
- Lai, L. S., and Kokini, J. L. 1991. Physicochemical changes and rheological properties of starch during extrusion: a review. *Biotechnol. Prog.* 7:251-266.
- Mason, W. R., and Hosney, R.C. 1986. Factors affecting the viscosity of extrusion-cooked wheat starch. *Cereal Chem.* 63:436-441.
- McPherson, A.E., and Jane, J. 1999. Extrusion of cross-linked, hydroxypropylated starches. II. Morphological and molecular characterization. Submitted to *Cereal Chem.*
- Mercier, C. 1977. Effect of extrusion-cooking on potato starch using a twin screw French extruder. *Starch/Staerke* 29:48-52.
- Mercier, C., and Feillet, P. 1977. Modification of carbohydrate components by extrusion-cooking of cereal products. *Cereal Chem.* 52:283-297.
- Pan, Z., Zhang, S., and Jane, J. 1998. Effects of extrusion variables and blowing agents on the pasting properties of starch based binders and extrusion operation parameters. *Cereal Chem.* 75:541-546.
- Powell, E. L. 1967. Production and uses of pregelatinized starch. Pages 523-536 in: *Starch: Chemistry and Technology Vol. II.* R. L. Whistler, and E. F. Paschall, eds. Academic Press, New York.
- Rutenberg, M. H. and Solarek, D. 1984. Starch derivatives: Production and uses. Pages 311-366 in: *Starch: Chemistry and Technology, 2nd edition.* R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. Academic Press, New York.
- Ryu, G. H., and Walker, C. E. 1995. The effects of extrusion conditions of the physical properties of wheat flour extrudates. *Starch/Staerke* 47:33-36.

- Seiler, K., Weipert, D., and Seibel, W. 1980. Viscosity behavior of ground extrusion products in relation to different parameters. Pages 808-819 in: *Food Process Engineering* vol. 1. P. Linko, Y. Malkki, J. Olkku, and J. Larinkari eds. Elsevier Applied Science Publ., London.
- Solarek, D. 1986. Phosphorylated starches and miscellaneous inorganic esters. Pages 98-108 in: *Modified Starches: Properties and Uses*. O. B. Wurzburg, ed. CRC Press, Boca Raton, FL.
- Tuschoff, J. V. 1986. Hydroxypropylated starches. Pages 90-96 in: *Modified Starches: Properties and Uses*. O. B. Wurzburg, ed. CRC Press, Boca Raton, FL.
- Walker, C. E., Ross, A. S., Wrigley, C. W., and McMaster, G. J. 1988. Accelerated starch paste characterization with the rapid visco analyzer. *Cereal Chem.* 33: 491-494.
- Whalen, P. J., Bason, M. L., Booth, R. I., Walker, C. E., Williams, P. J. 1997. Measurement of extrusion effects by viscosity profile using the rapid visco analyzer. *Cereal Foods World* 42:469-475.

Table 1. Experimental variables and levels of use.

Factor	Factor Levels				
Cross-Linking (%POCl ₃)	0	0.014	0.018	0.024	0.028
Moisture (% dsb)		30	35		40
Screw		low	medium		high
Temp. Profile of Barrel (°C) feed→ die	40, 45, 50, 55, 60, 60	45, 55, 65, 75, 80, 80	60, 70, 80, 90, 100, 100		

Table 2. Thermal properties of starch gelatinization of native starches and selected extruded starches by differential scanning calorimetry^a.

Starch	Moist. Cont. (%)	Temp (°C)	Screw Shear	Onset (T _o)	Peak (T _p)	Conclusion (T _c)	Enthalpy (ΔH)
native				66.1 ± 0.3 ^b	70.7 ± 0.2	75.2 ± 0.3	13.2 ± 0.8
HP, 0.0% POCl ₃				50.6 ± 0.1	55.6 ± 0.1	62.0 ± 0.2	6.5 ± 0.6
HP, 0.014% POCl ₃				52.6 ± 0.1	57.4 ± 0.2	63.3 ± 0.1	9.2 ± 0.9
HP, 0.018% POCl ₃				50.6 ± 0.2	56.0 ± 0.1	62.0 ± 0.3	8.5 ± 0.4
HP, 0.024% POCl ₃				51.9 ± 0.0	57.2 ± 0.0	63.3 ± 0.0	8.1 ± 0.1
HP, 0.028% POCl ₃				50.9 ± 0.3	56.5 ± 0.3	62.8 ± 0.1	8.3 ± 0.3
native	30	60	low	69.8 ± 0.1	73.7 ± 0.2	77.3 ± 0.5	1.9 ± 0.0
	30	80	low	66.4 ± 0.0	70.7 ± 0.0	73.6 ± 0.1	0.4 ± 0.0
	30	100	low	ND			
	30	60	med	63.2 ± 0.1	69.3 ± 0.0	74.3 ± 0.3	2.0 ± 0.3
	30	80	med	67.9 ± 0.2	72.3 ± 0.2	76.2 ± 0.1	1.4 ± 0.1
	30	100	med	ND			
	30	60	high	65.5 ± 0.1	70.4 ± 0.5	74.5 ± 0.0	0.9 ± 0.2
	30	80	high	66.7 ± 0.4	71.6 ± 0.2	75.6 ± 0.0	1.6 ± 0.2
	30	100	high	ND			
	40	60	low	68.2 ± 0.3	72.2 ± 0.2	76.2 ± 0.4	4.3 ± 0.2
	40	80	low	73.3 ± 0.4	76.9 ± 0.4	80.3 ± 0.3	0.6 ± 0.1
	40	100	low	ND			
	40	60	high	65.2 ± 0.1	69.8 ± 0.2	74.0 ± 0.3	5.3 ± 0.2
	40	80	high	71.0 ± 0.2	74.2 ± 0.2	77.5 ± 0.2	0.9 ± 0.4
	40	100	high	ND			
HP, 0.0% POCl ₃	30	60	low	52.9 ± 0.4	58.1 ± 0.4	65.0 ± 0.5	0.9 ± 0.1
	30	80	low	66.4 ± 0.1	70.7 ± 0.2	73.6 ± 0.1	0.4 ± 0.1
	30	100	low	ND			
	30	60	high	51.0 ± 0.1	56.5 ± 0.2	63.5 ± 0.7	1.7 ± 0.1
	30	80	high	ND			
	40	60	low	55.6 ± 0.0	60.0 ± 0.2	64.4 ± 0.7	0.3 ± 0.1
	40	80	low	ND			
	40	60	high	51.2 ± 0.0	56.5 ± 0.2	63.4 ± 0.8	1.7 ± 0.0
	40	80	high	61.8 ± 0.9	65.1 ± 0.6	66.7 ± 0.9	0.4 ± 0.2
	40	100	high	ND			
HP, 0.028% POCl ₃	30	60	low	54.4 ± 0.2	59.4 ± 0.6	64.2 ± 0.0	0.5 ± 0.0
	30	80	low	59.3 ± 0.0	60.8 ± 0.1	63.2 ± 0.1	0.2 ± 0.0
	30	100	low	ND			
	30	60	med	50.3 ± 0.6	56.1 ± 0.1	58.8 ± 0.0	0.2 ± 0.0
	30	80	med	ND			
	30	60	high	66.9 ± 0.6	69.0 ± 0.9	71.2 ± 0.3	0.3 ± 0.1
	30	80	high	ND			
	35	60	high	50.7 ± 0.2	56.3 ± 0.1	62.2 ± 0.3	0.9 ± 0.1
	35	80	high	ND			
	40	60	low	54.7 ± 0.2	60.3 ± 0.7	65.0 ± 0.9	0.5 ± 0.0
	40	80	low	ND			
	40	60	high	52.5 ± 0.1	57.3 ± 0.0	62.8 ± 0.1	1.8 ± 0.2
	40	80	high	ND			

^a Results are the mean of at least three replications

^b ± Standard deviation.

ND no peak was detected

Table 3. Average viscosities (RVU) for combinations of starch moisture content and extrusion temperature (Standard error = 12.93).

Starch Moisture Content (%)	Extrusion Temperature (°C)			Starch Moisture Content Mean ^a
	60	80	100	
30	94.61	102.08	104.71	100.20
35	170.35	160.01	133.79	154.71
40	267.32	230.42	198.59	232.11
Extrusion Temp. Mean ^b	177.43	164.14	145.46	

^a Standard error of the starch moisture content means = 7.46.

^b Standard error of the extrusion temperature means = 7.46.

Table 4. Average viscosities (RVU) for combinations of the level of starch cross-linking and shear (Standard error = 5.08).

Shear	Native Corn Starch	% POCl ₃ in Cross-Linked and Hydroxypropylated (8%) Corn Starches					Shear Mean ^a
		0	0.014	0.018	0.024	0.028	
Low	347.24	89.95	167.31	226.52	230.61	299.91	226.92
Medium	292.14	72.3	97.23	120.51	130.19	174.19	147.76
High	260.77	56.08	80.23	96.68	101.91	135.69	121.89
Starch Cross- Linking Mean ^b	300.05	72.78	114.92	147.90	154.24	203.26	

^a Standard error of shear means = 2.28.

^b Standard error of starch cross-linking means = 2.93.

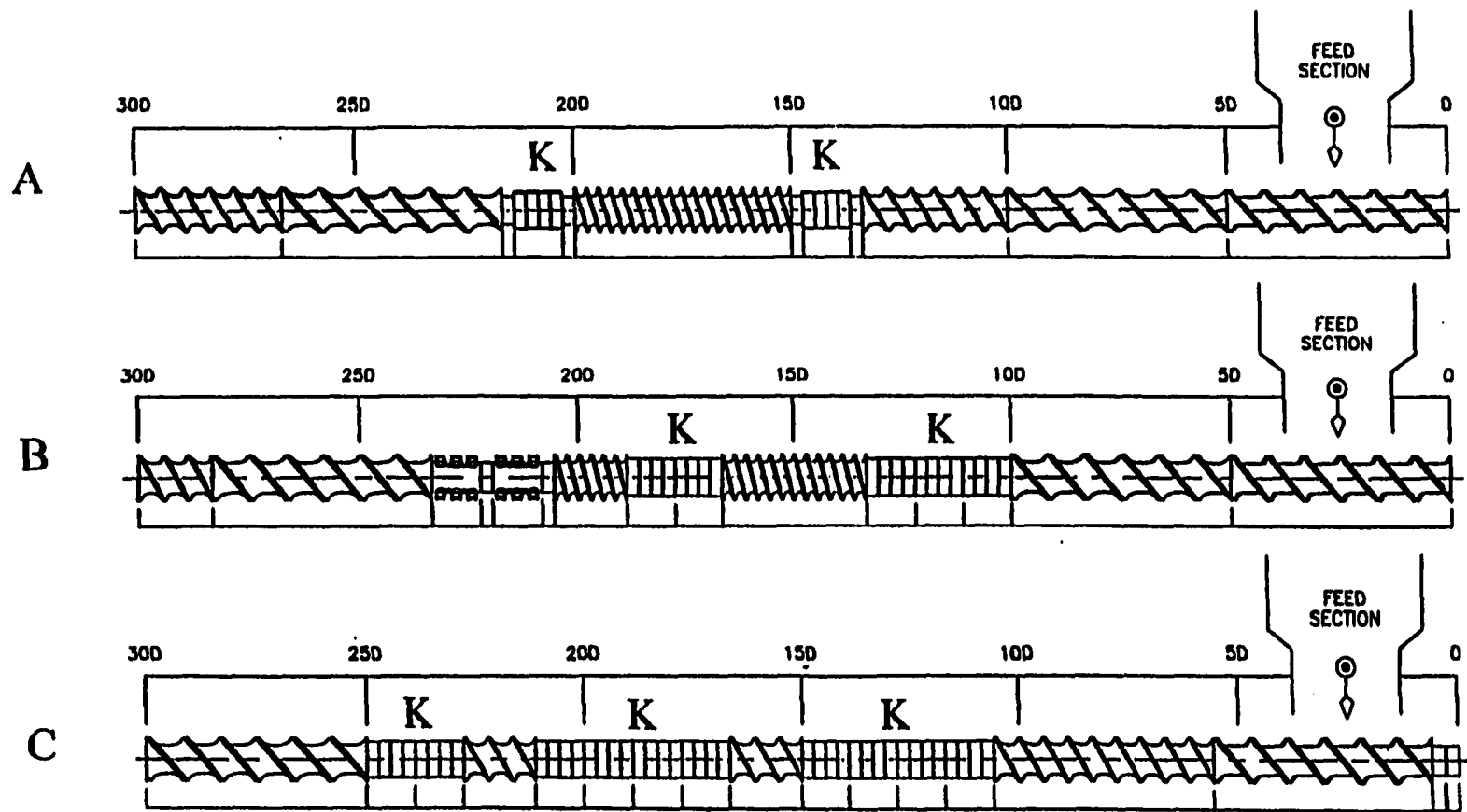


Figure 1. Designs of screws. Number of kneading elements increase from low (A), medium (B), to high (C) shear. K denotes sections of kneading blocks.

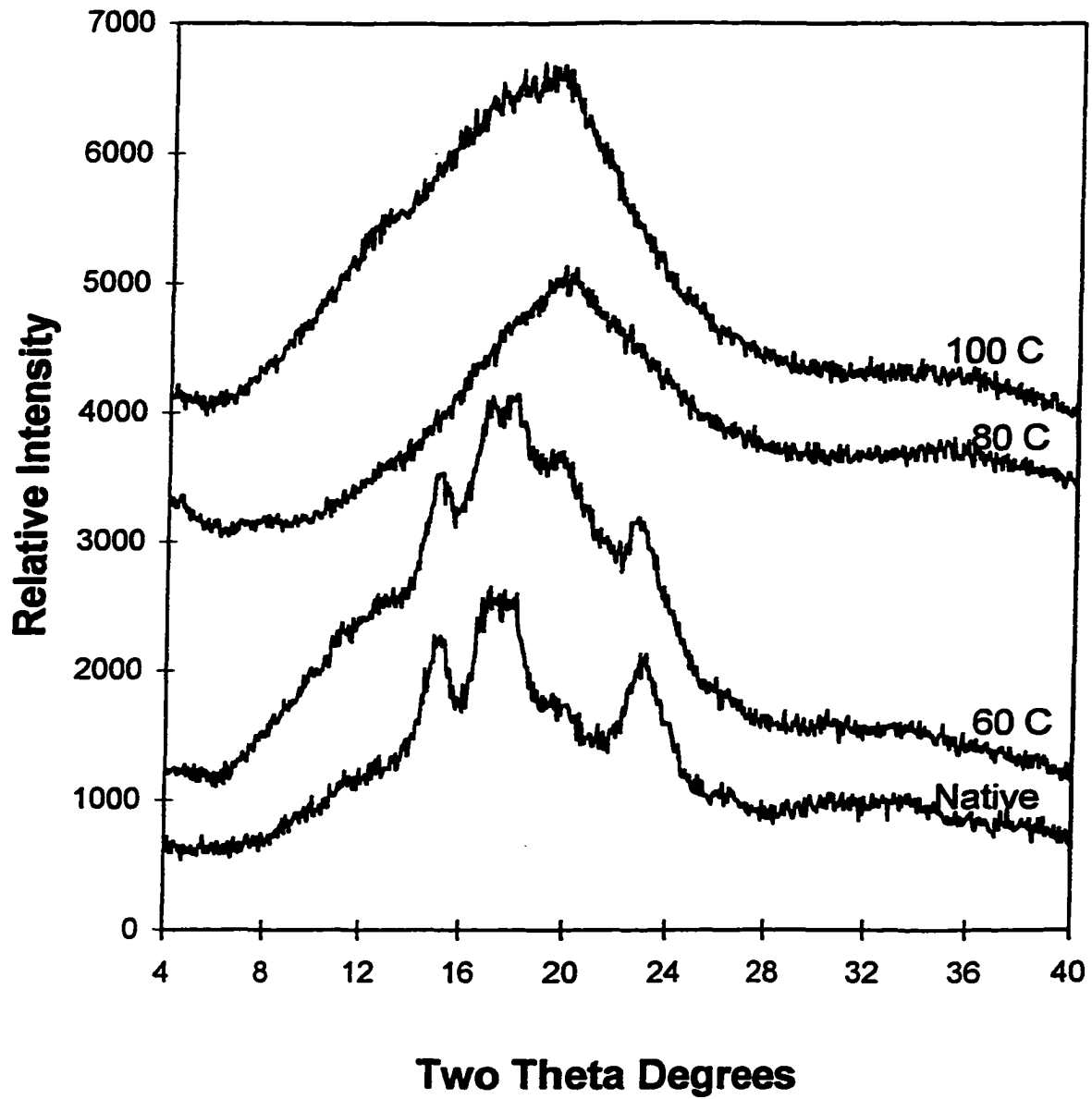


Figure 2. X-ray diffraction patterns for native corn starch and native corn starch extruded at 30% moisture and high shear at 60, 80, and 100°C.

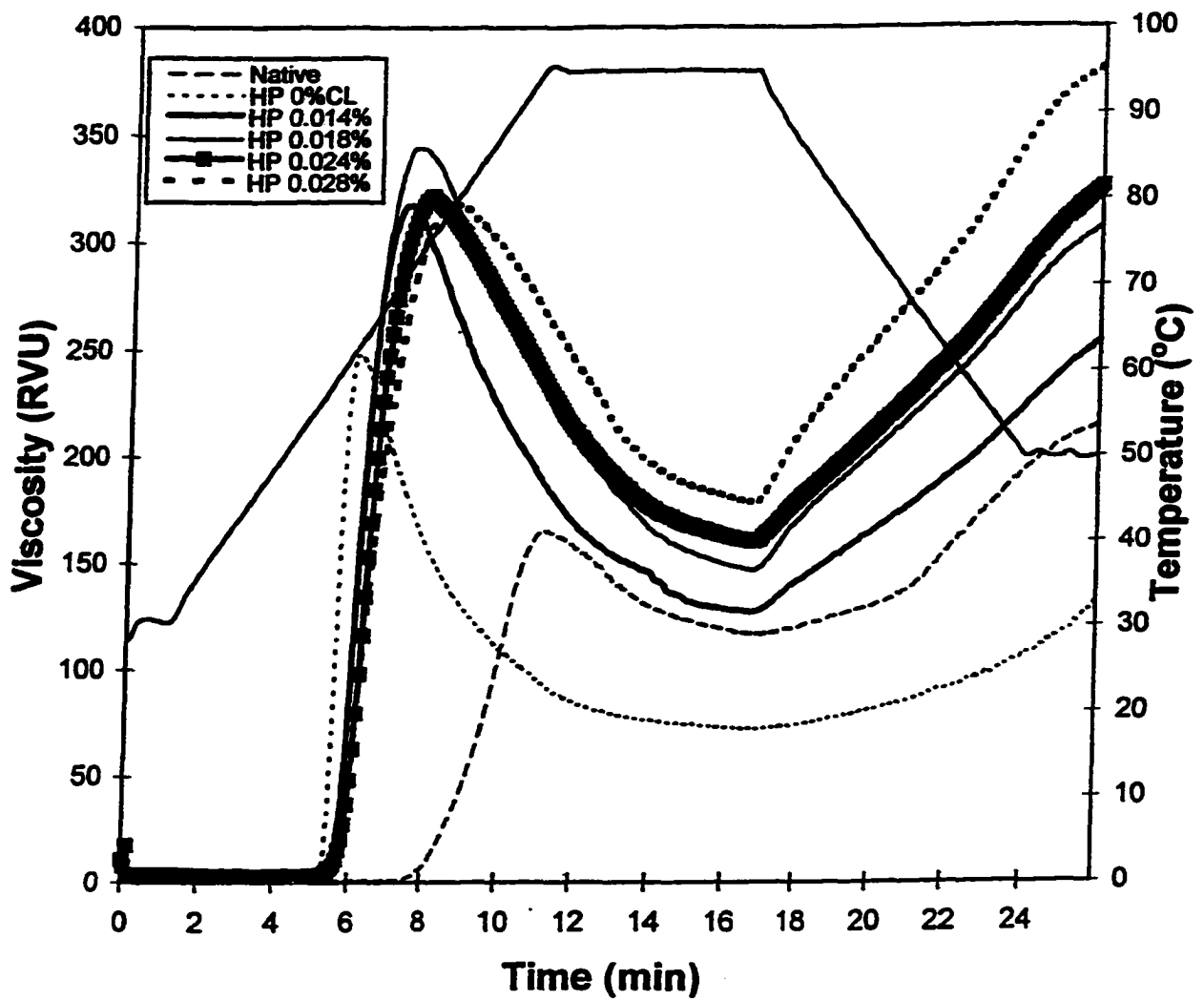


Figure 3. Rapid Visco Analyzer pasting profiles of unextruded native corn and cross-linked (0, 0.014, 0.018, 0.024, and 0.028% POCl_3) hydroxypropylated (8%) corn starches at 8% solids (dsb).

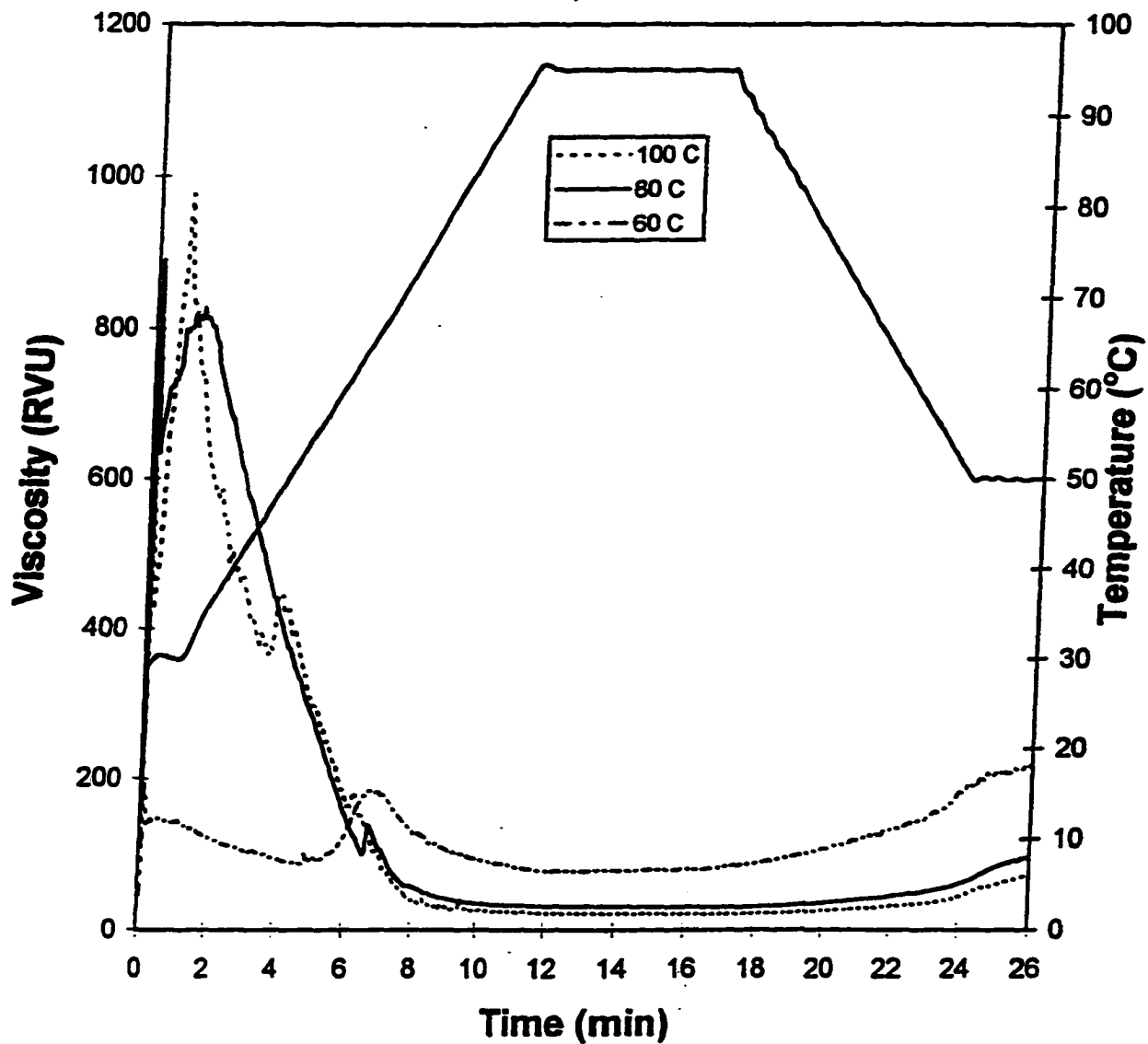


Figure 4. Rapid Visco Analyzer pasting profiles (15% solids, dsb) of cross-linked (0.028% POCl₃) hydroxypropylated (8%) corn starch extruded at 40% moisture and high shear at 60, 80, and 100°C.

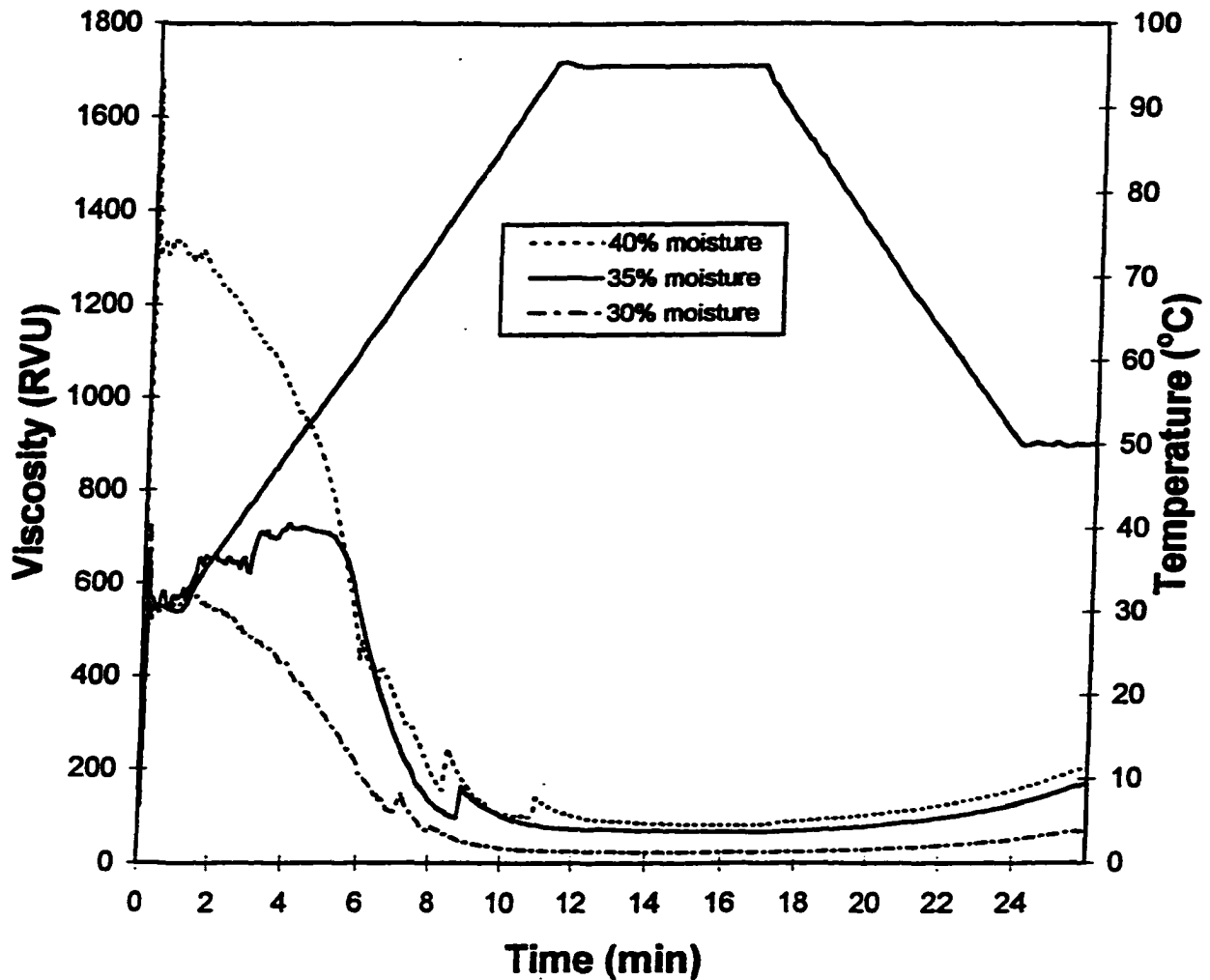


Figure 5. Rapid visco analyzer pasting profiles (15% solids, dsb) of cross-linked (0.014% POCl_3) hydroxypropylated (8%) corn starch extruded at 100°C and medium shear at 30, 35, and 40% moisture.

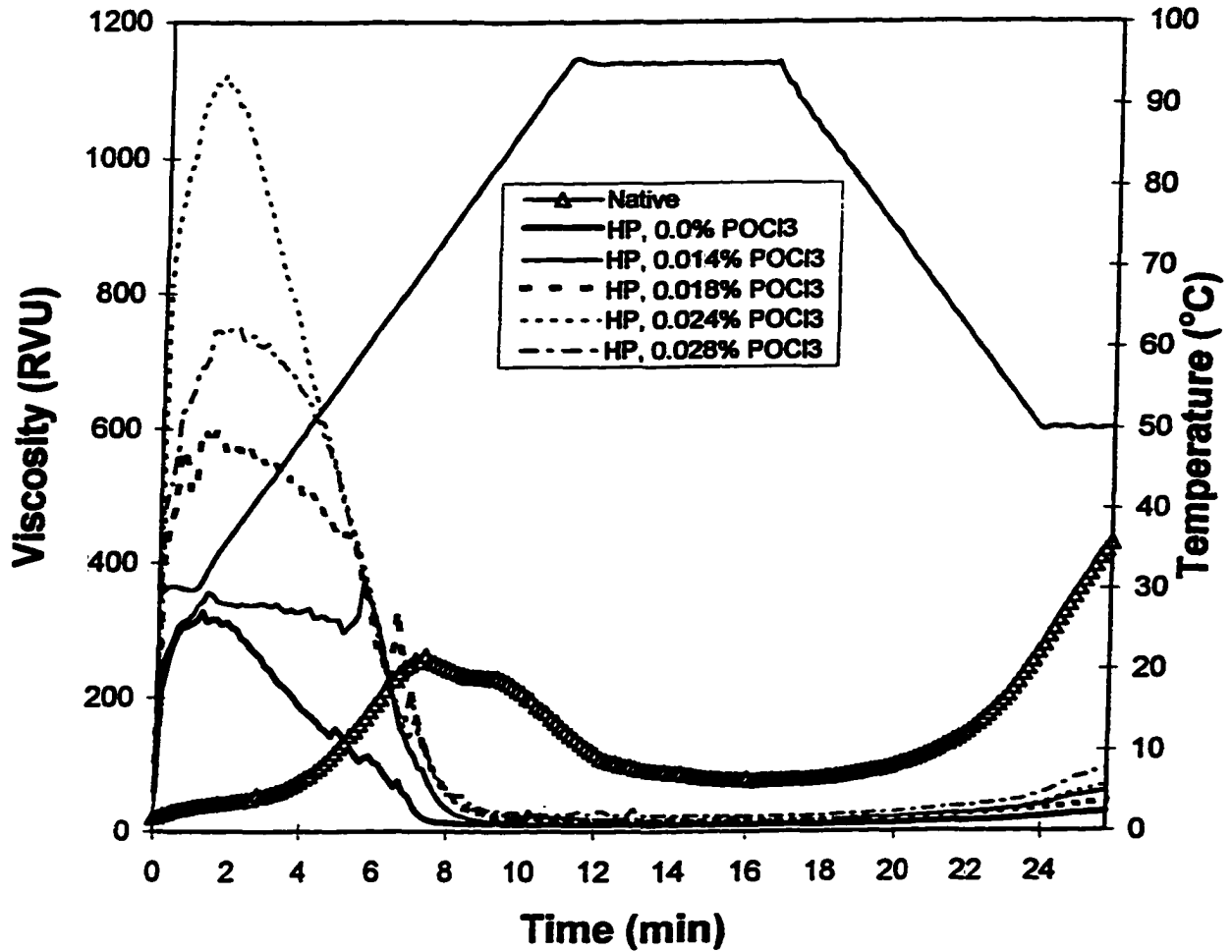


Figure 6. Rapid visco analyzer pasting profiles (15% solids, dsb) of native corn and cross-linked (0, 0.014, 0.018, 0.024, and 0.028% POCl₃) hydroxypropylated (8%) corn starch at 30% moisture, 100°C, and high shear.

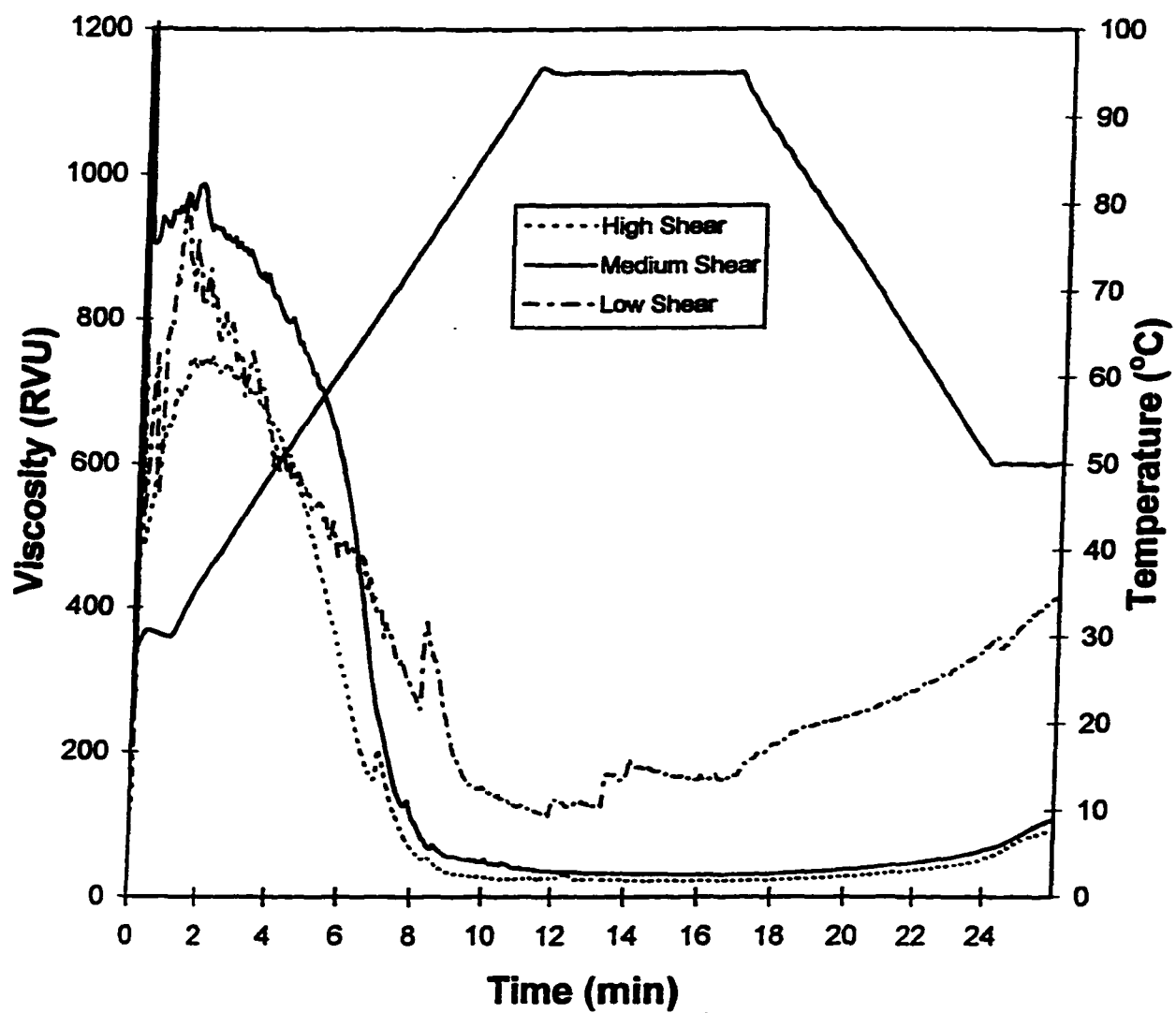


Figure 7. Rapid visco analyzer pasting profiles (15% solids, dsb) of cross-linked (0.028% POCl_3) hydroxypropylated (8%) corn starch extruded at 100°C, 30% moisture at low, medium and high shear.

**EFFECTS OF EXTRUSION PARAMETERS ON CROSS-LINKED
HYDROXYPROPYLATED CORN STARCHES.
II. MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION**

A paper to be submitted to Cereal Chemistry

A.E. McPherson and J. Jane

ABSTRACT

A series of cross-linked hydroxypropylated corn starches were extruded with a Leistritz micro-18 co-rotating extruder. Extrusion process variables including moisture (30, 35, and 40%), barrel temperature (60, 80 and 100°C), and screw design (low, medium, and high shear) were investigated. Scanning electron microscopy (SEM) of extruded starches showed a gel phase with distorted granules and granule fragments after extrusion at 60°C. After extrusion at 100°C only a gel phase was observed with no granular structures remaining. High performance size exclusion chromatography (HPSEC) equipped with multi-angle laser light scattering (MALLS) and refractive index (RI) detectors showed extruded starches degraded to different extents, depending on extrusion conditions. The average molecular weight of the amylopectin of unextruded native corn starch was 7.7×10^8 . Extrusion at 30% moisture, 100°C, and high shear reduced the molecular weight of amylopectin to 1.0×10^8 . Hydroxypropylated normal corn starch extruded at identical conditions showed greater decreases in amylopectin molecular weight. With the addition of cross-linking, the amylopectin fraction of the extruded starches was less degraded than those of their native and hydroxypropylated corn starch counterparts. Similarly, increasing

moisture content during extrusion lowered amylopectin degradation in the extruded starches. Increasing temperature during extrusion of cross-linked hydroxypropylated starches at high moisture content (e.g. 40%) lowered amylopectin molecular weights of the extruded starches, whereas increasing extrusion temperature at low moisture content (30%) resulted in less degraded molecules. This difference was attributed to the higher glass transition temperatures of the cross-linked starches.

INTRODUCTION

Extrusion has become a common processing method for starch-based foods and for producing pre-gelatinized starches (Snyder 1984, Mercier et al 1989, Harper 1992, Linko 1992, Govindasamy et al 1997). The effects of extrusion on native starches have been well studied. Extrusion of native starches has been reported to cause decreases in crystallinity (Mercier et al 1979), intrinsic viscosity (Colonna and Mercier 1983, Colonna et al 1984, Diosady et al 1985), and paste viscosity (Seiler et al 1980, Colonna et al 1984, Gomez and Aguilera 1984, Mason and Hosney 1986). During the extrusion process thermal energy, shear, and pressure cause reductions in molecular size (Mercier 1977, Colonna and Mercier 1983, Colonna et al 1984, Davidson et al 1984a, Davidson et al 1984b, Diosady et al 1985, Jackson et al 1990, Wen et al 1990, Mitchell and Areas 1992). Extrusion variables including starch moisture content, barrel and die temperatures, screw design, screw speed, and starch type have been shown to affect the molecular weight of extruded starches (Colonna and

Mercier 1983, Colonna et al 1984, Diosady et al 1985, Jackson et al 1990, Wen et al 1990, Tayeb et al 1992, Politz et al 1994).

Molecular degradation in extruded native starches has been examined using intrinsic viscosity, gel permeation chromatography (GPC), and high performance size exclusion chromatography (HPSEC) (Chinnaswamy and Hanna 1990, Jackson et al 1990, Wasserman and Timpa 1991, Harper 1992, Politz et al 1994). HPSEC, equipped with light scattering and refractive index detectors (RI), is a method for determination of absolute molecular weight and size of synthetic polymers and biopolymers such as starches (Podzimek 1994, Fishman et al 1996, Yokoyama et al 1998).

In this study, we examined the effects of extrusion variables on granular morphology and molecular weight of the starch components of native and cross-linked (0.0 to 0.028% POCl_3) hydroxypropylated (8%) corn starches. Extrusion variables, including starch moisture content, screw design, extrusion temperature, and the level of cross-linking in the modified corn starches, were investigated.

MATERIALS AND METHODS

Preparation of cross-linked (0, 0.014, 0.024, and 0.028% POCl_3) hydroxypropylated (8% propylene oxide) starches and extrusion variables (moisture, screw designs, and temperature profiles) have been reported elsewhere (McPherson et al 1999).

Scanning Electron Microscopy (SEM)

Extruded starches (0.5 g) were suspended in water (10 ml) and gently agitated at 20°C (20 min) to disperse the gelatinized material. A drop of the suspension was placed on a glass slide, spread with a spatula, and examined under light microscopy (Nikon Labophot, Nikon, Garden City, NY). The remaining granules were counted in 10 fields and compared to that in the respective unextruded parent starch to arrive at an approximate percentage of granules remaining after extrusion. A drop of the starch suspension was placed on a glass cover slip, spread with a spatula, and allowed to dry. The cover slip was then attached to an aluminum stub with double-sided tape and colloidal silver applied between the edge of the cover slip and the aluminum stub. The specimens were then sputter coated with gold/palladium (60/40). The mounted specimens were observed using a scanning electron microscope (JEOL model 1850, Tokyo, Japan) at the Bessey Microscopy Facility, Iowa State University (Ames, IA).

High Performance Size Exclusion Chromatography

Starch (0.5 g, dsb) was solubilized in dimethyl sulfoxide solution (90%) (50.0 ml) in a boiling water bath for 1 h with constant stirring, and continuously stirred for 24 h at room temperature. Starch was precipitated from an aliquot of DMSO solution (1.0 ml) with excess absolute ethyl alcohol and centrifuged at 6750 x g for 15 min. The precipitated amorphous starch pellet, which had been defatted, was resolubilized in deionized water (10 ml, 95°C)

and stirred with a magnetic stirrer in a boiling water bath for 30 min. Starch solutions were then filtered using a 5.0 μm syringe filter. The filtrate was injected (100 μl) into a high performance size exclusion chromatography (HPSEC) system. This system consisted of a HP 1050 isocratic pump (Hewlett Packard, Valley Forge, PA), refractive index (RI) detector (model HP1047A, Hewlett Packard, Valley Forge, PA), and multi-angle laser light scattering (MALLS) detector (model Dawn F, Wyatt Tech., Santa Barbara, CA) with a helium-neon laser light source ($\lambda=632.8$ nm) and a K-5 flow cell. The columns used were Shodex OH pak KB-G, KB-806, and KB-804 (Shodex Denko, Tokoyo, Japan) HPSEC columns connected in series and kept at 55°C. The mobile phase was distilled, deionized, and degassed water passed through in-line filters (0.2 μ and 0.1 μ) in series, at a flowrate of 0.7 ml/min.

The electronic outputs of the RI and MALLS detectors were collected by ASTRA software (version 4.10, Wyatt Tech., Santa Barbara, CA). Peaks were assigned using the RI chromatograms. A second order fit in the Berry analysis method (ASTRA) was found to have the least statistical error for determination of amylopectin weight average molecular weight. Because of the reduced sensitivity of MALLS for small molecular weight species, the molecular weight of the peak two (amylose and degraded amylopectin fragments) of extruded starches was calculated from the refractive index signal using a calibration curve constructed from a series of pullulan molecular weight standards (22.0, 47.3, 112.0, 212.0, 404.0, and 788.0 $\times 10^3$) (Viscotek, Houston, TX).

Glass Transition Temperature of Starches Determined by DSC

The glass transition temperature of the extruded starch was analyzed by using a differential scanning calorimeter (DSC-7, Perkin Elmer Corp., Norwalk, CT) equipped with an Intracooler II System and Pyris thermal analysis software (Perkin-Elmer Corp., Norwalk, CT). Starch and water mixtures (0.85:0.15 and 0.74:0.26) were prepared in screw capped glass vials at 20°C and equilibrated for 48 h. The mixtures were sealed in aluminum pans and equilibrated at room temperature for 2 h before analysis. An empty aluminum pan was used as the reference. Indium and zinc were used as reference standards. The initial scan was made from 25-120°C at 10°C/min followed by cooling to 5°C. The second scan was made from 5-120°C at 5°C/min. Moisture content of the starch samples was determined by puncturing the sealed pans after scanning and drying at 100°C for 8 h. The glass transition temperature was determined from the midpoint of the baseline shift (Zeleznaek and Hosney 1987). All samples were examined in triplicate.

RESULTS AND DISCUSSION

The scanning electron micrographs (SEM) of native corn starch and representative extruded starches are shown in fig. 1. Native corn starch granules were polyhedral in shape and had axial diameters of between 5-20 μ (Fig. 1a) as has been reported by Jane et al (1994). Starches extruded at low temperature (60°C) were found to have relatively small amounts of distorted and broken granular fragments. These results agreed with those of differential scanning calorimetry (DSC) and x-ray diffraction patterns of starches extruded at 60°C,

which showed remaining crystallinity (McPherson et al 1999). Native corn starch extruded at 40% moisture, 60°C, and low shear (Fig. 1b) showed remaining distorted granules and some fractured granules ($\sim 42 \pm 8\%$ granules remained). That extruded at 30% moisture, 60°C, and high shear (Fig 1c) showed substantially fractured granules ($\sim 21 \pm 6\%$ granules remained). The hydroxypropylated (8%) corn starch extruded at 30% moisture, 60°C, and high shear showed residual swollen, distorted and fractured granules (data not shown). The highly cross-linked (0.028% POCl₃) hydroxypropylated (8%) corn starch extruded at 40% moisture, 60°C, and low shear levels showed disrupted swollen granules (Fig 1d) ($\sim 14 \pm 2\%$ granules remained). Extrusion of this starch at 40% moisture, 100°C, and low shear displayed only a continuous gel phase with no remaining granular structure (Fig. 1e). All starches showed no remaining granular structure after extrusion at 100°C. Extrusion of starch at high temperatures ($>100^\circ\text{C}$) has been reported to completely destroy granular structure of native starches (Mercier et al 1979, Richmond and Smith 1985).

The HPSEC chromatogram of the unextruded native starch showed two major peaks corresponding to amylopectin and amylose, with retention volumes of 7.6-11.8 and 11.9-20.7 ml, respectively (Fig. 2). The extruded starches displayed multiple peaks, resulting from degradation during extrusion. Degradation has been reported in both the amylopectin and amylose fractions of extruded starches (Colonna et al 1984, Wen et al 1990). As a consequence, the molecular weight of amylopectin decreased and shifted to higher retention volumes. Therefore, the second major peak in the HPSEC chromatograms of the extruded starches is referred to as peak two because it is composed of a mixture of amylose and

degraded amylopectin molecules. The weight average molecular weights of native corn amylopectin and amylose were 7.7×10^8 and 5.5×10^5 , respectively (Table I). Previously reported molecular weight values for amylopectin and amylose using HPSEC vary with sample preparation, SEC columns, mobile phase, and detection systems. The molecular weights of corn amylopectin and amylose from various sources have been reported to range from 1.5×10^7 to 2.27×10^8 and 1.36 to 4.89×10^5 , respectively (Politz et al 1994, Fishman et al 1996, Mua and Jackson 1997, Bello-Perez 1998, Yokoyama et al 1998).

Extrusion of native corn starch at 30% moisture and 100°C with increasing shear degraded the amylopectin and caused the peak to broaden and shift to higher retention volumes (8.5-12.5 ml) (Fig. 2). Residence time of the starch in the extruder barrel increased as shear increased and likely contributed to molecular degradation. The amylopectin molecular weight decreased as shear increased (1.5 , 1.1 , and 1.0×10^8 for low, medium, and high, respectively) (Table I). As a result of increasing shear and degraded amylopectin molecules shifting to higher elution volumes (Fig. 2) peak two molecular weight increased (5.9 , 7.0 , and 7.3×10^5 for low, medium, and high shear, respectively) (Table I) and developed a pronounced shoulder at an elution volume of 13.2 ml (Fig. 2). Increasing extrusion temperature of native corn starch at 30% moisture decreased the amylopectin molecular weight at low shear (3.2 , 2.6 , and 1.5×10^8 , for 60, 80, and 100°C, respectively) and high shear (2.6 , 1.4 , and 1.0×10^8 , for 60, 80, and 100°C, respectively) (Table I). Increasing extrusion temperature of native corn starch also increased peak two molecular weight (Table I). Amylopectin peaks of starches extruded at 30% moisture were broad,

indicating the molecules were severely degraded during extrusion (Table I and Fig. 2), whereas those extruded at 40% moisture content were less degraded and had a narrower molecular weight distribution, close to that of the unextruded corn starch amylopectin (Fig. 3). These results agreed with those of starches extruded at 40% moisture that displayed greater viscosity than those extruded at 30% moisture (McPherson et al 1999). For native corn starch, the greatest degradation of amylopectin occurred with extrusion at high shear, 30% moisture, and 100°C. This is in agreement with previous findings that the amylopectin of extruded corn starch is degraded most readily at low moisture and high shear conditions (Wen et al 1990, Orford et al 1993, Politz et al 1994, Pan et al 1998).

Unextruded hydroxypropylated (8%) corn starch with no cross-linking displayed retention volumes of 7.8-12.4 ml and 12.4-19.9 ml for amylopectin and amylose, respectively (Fig. 4). The molecular weights of amylopectin and amylose were 6.5×10^8 and 5.5×10^5 , respectively (Table II). Extrusion of hydroxypropylated (8%) starch at 30% moisture content and 100°C with increasing shear produced amylopectins with lower molecular weight of 1.3, 0.6, and 0.4×10^8 for low, medium, and high shear, respectively (Fig. 4 and Table II), compared with 1.5, 1.1, and 1.0×10^8 for low, medium, and high shear, respectively, of their native corn starch counterparts. The lower onset gelatinization temperature of hydroxypropylated starch (50.6°C) than that of native corn starch (66.1°C) (McPherson et al 1999), resulted in a higher degree of gelatinization and dispersion during extrusion and subsequently more molecular degradation from shear. Increasing extrusion temperatures at 30% moisture content resulted in decreased amylopectin molecular weight at both low shear

(2.7, 2.4, and 1.3×10^8 for 60, 80, and 100°C , respectively) and high shear (0.8, 0.5, 0.4×10^8 for 60, 80, and 100°C , respectively) (Table II). Similar temperature effects have been reported in native wheat starch (Diosady et al 1985) and in native corn (Chinnaswamy and Hanna 1990). Like that observed in native corn starch, extrusion at 40% starch moisture content resulted in less degraded amylopectin molecular weights (Table II) and a narrower distribution of molecular weight (data not shown).

Dispersed, unextruded cross-linked (0.014% POCl_3) hydroxypropylated (8%) starch could not be filtered through a $5.0 \mu\text{m}$ syringe filter. Therefore, the molecular weight of the unextruded cross-linked starches could not be obtained. After extrusion the cross-linked starches could be filtered and analyzed by HPSEC. Amylopectins of the extruded cross-linked starches had larger molecular weight than those of the native and hydroxypropylated starches extruded at the same conditions (Figs. 2, 4, and 5). Extrusion of cross-linked (0.014% POCl_3) hydroxypropylated (8%) starch at 30% moisture and 100°C yielded amylopectin molecular weights of 5.0, 1.2, and 1.3×10^8 for low, medium, and high shear, respectively (Fig. 5 and Table III), compared with 1.3, 0.6, and 0.4×10^8 for low, medium, and high shear, respectively (Table II) of their hydroxypropylated corn starch without cross-linking counterparts. The amylopectin peaks of the extruded cross-linked (0.014% POCl_3) hydroxypropylated (8%) corn starches were not as broad as those of hydroxypropylated (8%) and native corn starches extruded at the same conditions (Figs. 2, 4, and 5), indicating the cross-linking prevented the amylopectin molecules from being highly degraded as those of the non-cross-linked starch counterparts (Figs. 2 and 4). The substantially lower molecular

weight of the peak two in the extruded cross-linked (0.014% POCl₃) hydroxypropylated (8%) corn starch than that of the amylose of the unextruded native corn starch might be a result of large-molecular-weight amylose being preferentially cross-linked to the amylopectin and eluted with amylopectin (Jane et al 1992). Cross-linked starches are produced for food and industrial applications in which shear resistance is needed (Hullinger 1967, Wurzburg 1986).

The amylopectin molecular weights were 6.9, 5.3, and 1.7 x 10⁸ for low, medium, and high shear, respectively, for cross-linked (0.024% POCl₃) hydroxypropylated (8%) corn starch extruded at 30% moisture and 100°C (Table IV). The amylopectin of extruded cross-linked (0.024% POCl₃) hydroxypropylated (8%) corn starch displayed decreased molecular weight with increasing shear (Fig. 6). Extrusion of cross-linked (0.024% POCl₃) hydroxypropylated (8%) corn starch at 40% moisture also yielded less degraded amylopectin compared with their counterparts extruded at 30% moisture (Table IV). Increases in starch moisture content during extrusion at 100°C and high shear resulted in increasing amylopectin molecular weights (1.7, 3.6, and 6.7 x 10⁸ for 30, 35, and 40% moisture content, respectively) (Table IV and Fig. 7). This agreed with the positive effect of the moisture content on the hot paste viscosity and final viscosity of extruded cross-linked hydroxypropylated (8%) starches (McPherson et al 1999). The differences are attributed to a decrease in friction during extrusion at increased moisture content (Lai and Kokini, 1991).

In contrast to the results of extruded native and hydroxypropylated corn starches, extrusion of cross-linked hydroxypropylated corn starches at 30% moisture yielded increased amylopectin molecular weight as temperature increased at both low and high shear (Tables I-

V). Extrusion of cross-linked (0.024% POCl₃) hydroxypropylated (8%) corn starch at 30% moisture yielded increasing amylopectin molecular weights as temperature increased at low shear (3.3, 4.3, and 6.9 x 10⁸ for 60, 80, and 100°C, respectively) and high shear (0.7, 0.7, and 1.7 x 10⁸ for 60, 80, and 100°C, respectively) (Table IV). This agreed with the reported increased average viscosity of starches extruded at 30% moisture with increasing temperature of extrusion (60°C to 100°C) (McPherson et al 1999). However, extrusion of cross-linked (0.024% POCl₃) hydroxypropylated (8%) corn starch at 40% moisture and low shear yielded decreased amylopectin molecular weight with increasing temperature (9.0, 7.7, and 3.4 x 10⁸ for 60, 80, and 100°C, respectively) (Table IV). Amylopectin molecular weight of cross-linked (0.028% POCl₃) hydroxypropylated corn starch decreased with increasing shear (12.8, 4.1, and 2.5 x 10⁸ for low, medium, and high shear, respectively) during extrusion at 100°C and 30% moisture (Table V). Increasing the temperature of extrusion at 30% moisture yielded increased amylopectin molecular weights of the cross-linked (0.028% POCl₃) hydroxypropylated corn starch (Table V).

Extrusion of the starches at 100°C and 30% moisture decreased amylopectin molecular weight as shear increased (Tables I-V and Fig. 8). The amylopectin of native corn starch was not degraded to the same magnitude as that of the hydroxypropylated (8%) corn starch (Fig. 8). This difference was attributed to the lower gelatinization temperature of the hydroxypropylated (8%) starch which resulted in higher degrees of gelatinization and dispersion during extrusion. The highly dispersed starch molecules were more susceptible to shear degradation. The magnitude of amylopectin molecular weight change, from low to

high shear, became more drastic as the level of cross-linking increased (Fig. 8). At low shear the chemical cross-links were able to prevent severe molecular degradation, whereas at medium and high shear substantial degradation occurred to the large cross-linked amylopectin molecules. These results agreed with the average viscosity of extruded starches decreasing more for chemically modified starches than for the native starch counterparts as shear increased (McPherson et al 1999).

Extrusion of native corn and hydroxypropylated corn starches at 30% moisture yielded decreased amylopectin molecular weights as temperature increased (Tables I and II) (Figs. 9 and 10). Extrusion of cross-linked (0.024% POCl_3) hydroxypropylated corn starch at 30% moisture showed that amylopectin molecular weights were less degraded when the extrusion temperature increased (60°C to 100°C), whereas at 40% moisture amylopectin molecular weight were more degraded with increasing temperature (Table IV). This effect can be attributed to the higher glass transition temperatures of the cross-linked starches. The glass transition temperatures at 26% moisture were 29.1, 28.3, 43.2, and 67.2°C for native, hydroxypropylated, and cross-linked (0.014 and 0.028% POCl_3) hydroxypropylated (8%) corn starch, respectively. The glass transition temperature of polymers is known to increase with the addition of cross-linking (Nielsen 1974). At 60°C, the cross-linked starch was in the glassy state and the rigid molecules were more susceptible to shear degradation. When the temperature of extrusion increased, the cross-linked molecules became rubbery, and degradation decreased.

CONCLUSIONS

Extrusion of starches produced substantial morphological changes in granular structure. Starches extruded at 60°C showed distorted and fragmented granules, whereas those extruded at 100°C showed no granular structure and were completely amorphous. Extrusion conditions showed large effects on the molecular weights of the extruded starches. Increasing starch moisture content reduced amylopectin degradation during extrusion. Cross-linking prevented amylopectin degradation. However, the magnitude of amylopectin degradation increased at higher levels of cross-linking as shear increased. Increasing temperature of extrusion decreased amylopectin molecular weight of extruded native and hydroxypropylated corn starches, whereas the opposite effect was observed in cross-linked hydroxypropylated starches as a result of glass transition temperature.

ACKNOWLEDGMENTS

The authors acknowledge the Bessey Microscopy Facility for its assistance with scanning electron microscopy.

REFERENCES

- Bello-Perez, L.A., Roger, P., Baud, B., and Colonna, P. 1998. Macromolecular features of starches determined by aqueous high-performance size exclusion chromatography. *J. Cereal Sci.* 27:267-278.
- Chinnaswamy, R., and Hanna, M. A. 1990. Macromolecular and functional properties of native and extrusion cooked corn starch. *Cereal Chem.* 67:490-499.
- Colonna, P. and Mercier, C. 1983. Macromolecular modifications of manioc starch components by extrusion cooking with and without lipids. *Carbohydr. Polymers* 3:87-108.

- Colonna, P., Doublier, J. L., Melcion, J. P., DeMonredon, F., and Mercier, C. 1984. Extrusion cooking and drum drying of wheat starch. I. Physical and macromolecular modifications. *Cereal Chem.* 61:538-543.
- Davidson, V. J., Paton, D., Diosady, L. L., and Laroque, G. J. 1984a. Degradation of wheat starch in a single screw extruder: Characteristics of extruded starch polymers. *J. Food Sci.* 49:453-458.
- Davidson, V. J., Paton, D., Diosady, L. L., and Rubin, L. J. 1984b. A model for mechanical degradation of wheat starch in a single screw extruder. *J. Food Sci.* 49:1154-1157.
- Diosady, L. L., Paton, D., Rosen, N., Rubin, L. J., and Athanassoulis, C. 1985. Degradation of wheat starch in a single screw extruder: mechanico-kinetic breakdown of cooked starch. *J. Food Sci.* 50:1697-1699, 1706.
- Fishman, M. L., Rodriguez, L., and Chau, H. K. 1996. Molar masses and sizes of starches by high-performance size-exclusion chromatography with on-line multi-angle laser light scattering detection. *J. Agric. Food Chem.* 44:3182-3168.
- Gomez, M. H., and Aguilera, J. M. 1984. A physicochemical model for extrusion of corn starch. *J. Food Sci.* 49:40-43.
- Govindasamy, S., Campanella, O.H., and Oates, C.G. 1997. High moisture twin screw extrusion of sago starch. II. Saccharification as influenced by thermomechanical history. *Carbohydr. Polym.* 30:267-272.
- Harper, J.M. 1992. Extrusion processing of starch. Pages 37-62 in: *Developments in Carbohydrate Chemistry*. R.J. Alexander and H. Zobel, eds. AACC. St. Paul, MN.
- Hullinger, C.H. 1967. Production and use of cross-linked starch. Pages 445-450 in: *Starch: Chemistry and Technology, Vol II*. R. L. Whistler and E. F. Paschall eds. Academic Press, Inc., New York.
- Jackson, D. S., Gomez, M. H., Waniska, R. D., and Rooney, L. W. 1990. Effects of single screw extrusion cooking on starch as measured by aqueous high-performance size-exclusion chromatography. *Cereal Chem.*, 67(6):529-532.
- Jane, J., and Chen, J-F. (1992). Effect of amylose molecular size and amylopectin branch chain length on paste properties of starch. *Cereal Chem.*, 69, 60-65.

- Jane, J., Kasemsuwan T., Leas, S., Zobel, H., and Robyt, J. 1994. Anthology of starch granule morphology by scanning electron microscope. *Starch/Staerke* 4:121-129.
- Jane, J., Xu, A., Radosavljevic, M., and Seib, P.A. 1992. Location of amylose in normal starch granules. I. Susceptibility of amylose and amylopectin to cross-linking reagents. *Cereal Chem.* 69:405-409.
- Lai, L. S., and Kokini, J. L. 1991. Physicochemical changes and rheological properties of starch during extrusion: a review. *Biotechnol. Prog.* 7:251-266.
- Linko, P. 1992. Twin-screw extrusion cooker as a bioreactor for starch processing. Pages 335-343 in: *Food Extrusion Science and Technology*. J.L. Kokini, C.T. Ho, and M.V. Karwe, eds. Marcel Dekker, New York.
- Mason, W. R., and Hosney, R.C. 1986. Factors affecting the viscosity of extrusion-cooked wheat starch. *Cereal Chem.* 63:436-441.
- McPherson, A. E., Bailey, T. B., and Jane, J. 1999. Extruded hydroxypropylated and cross-linked corn starches. I. Pasting properties. Submitted to *Cereal Chem.*
- Mercier, C. 1977. Effect of extrusion cooking on potato starch using a twin screw French extruder. *Starch/Staerke* 29:48-52.
- Mercier, C., Charbonniere, R., Gallant, D., and Guilbot, A. 1979. Structural modification of various starches by extrusion cooking with a french twin screw extruder. Pages 153-170 in *Polysaccharides in Foods*. J. M. V. Blanshard and J. R. Mitchell, eds. Butterworths, London.
- Mitchell, J. R., and Areas, J. A. G. 1992. Structural changes in biopolymers during extrusion. Pages 345-360 in: *Food Extrusion Science and Technology*. J. L. Kokini, C. T. Ho, and M. V. Karwe, eds. Marcel Dekker, New York.
- Mua, J. P., and Jackson, D. S. 1997. Fine structure of corn amylose and amylopectin fractions with various molecular weights. *J. Agric. Food Chem.* 45:3840-3847
- Nielsen, L. E. 1974. *Mechanical properties of polymers and composites*, 2nd ed. Marcel Dekker, New York.
- Pan, Z., Zhang, S., and Jane, J. 1998. Effects of extrusion variables and blowing agents on the properties of starch-based binders and operational extrusion parameters. *Cereal Chem.* 56:541-546.

- Podzimek, S. 1994. The use of GPC coupled with a multiangle laser light scattering photometer for the characterization of polymers. On the determination of molecular weight, size, and branching. *J. Applied Polymer Sci.* 54:91-103.
- Politz, M. L., Timpa, J. D., and Wasserman, B.P. 1994. Quantitative measurement of extrusion-induced starch fragmentation products in maize flour using nonaqueous gel-permeation chromatography. *Cereal Chem.* 71:531-536.
- Richmond, P., and Smith, A. C. 1984. The influence of processing on biopolymeric structures. *British Polymer J.* 17:246-250.
- Seiler, K., Weipert, D., and Seibel, W. 1980. Viscosity behavior of ground extrusion products in relation to different parameters. Pages 808-819 in: *Food Process Engineering* vol. 1. P. Linko, Y. Malkki, J. Olkku, and J. Larinkari eds. Elsevier Applied Science Publ., London.
- Synder, E. M. 1984. Industrial microscopy of starches. Pages 661-673 in: *Starch: Chemistry and Technology*, 2nd Ed., R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. Academic Press, New York
- Tayeb, J., Della Valle, G., Barres, C., and B. Vergnes. 1992. Simulation of transport phenomena in twin screw extruders. Pages 41-70 in: *Food Extrusion Science and Technology*. J.L. Kokini, C.T. Ho, and M.V. Karwe, eds. Marcel Dekker, New York.
- Wasserman, B. P., and Timpa, J.D. 1991 Rapid quantitative measurement of extrusion induced starch fragmentation by automated gel permeation chromatography. *Starch/Staerke* 43(10):389-392.
- Wen, L., Rodis, P., and Wasserman, B. P. 1990. Starch fragmentation and protein insolubilization during twin-screw extrusion of corn meal. *Cereal Chem.* 67:268-275.
- Wurzburg,, O.B. 1987. Cross-linked starches. Pages 42-53 in: *Modified starches: properties and uses*. O.,B. Wurzburg, ed. CRC Press, Boca Raton, FL.
- Yokoyama, W., Renner,-Nantz, J. J., and C.F. Shoemaker. 1998. Starch molecular mass and size by size-exclusion chromatography in DMSO-LiBr coupled with multiple laser light scattering. *Cereal Chem.* 75:530:535.
- ZeleznaK, K. J., and HoseneY, R. C. 1987. The glass transition in starch. *Cereal Chem.* 64:121-124.

Table I. Weight average molecular weights M_w of amylopectin and peak two (amylose and degraded amylopectin) and gyration radii of amylopectins of extruded native corn starches^a.

Starch Moisture Content (%)	Temp. of Extrusion (°C)	Level of Shear	Amylopectin Weight Av M_w ($M_w \times 10^6$) ^b	Gyration Radii of Amylopectin (R_z) (nm) ^b	Peak Two Weight Av M_w ($M_w \times 10^6$) ^c
	unextruded		7.7 ± 0.6^d	368.6 ± 16.5	5.5 ± 0.2
30	60	LOW	3.2 ± 0.2	296.1 ± 14.2	6.3 ± 0.1
30	80	LOW	2.6 ± 0.0	226.7 ± 33.1	6.7 ± 0.0
30	100	LOW	1.5 ± 0.0	223.8 ± 6.6	5.9 ± 0.2
30	100	MED.	1.1 ± 0.0	240.4 ± 7.1	7.0 ± 0.0
30	60	HIGH	2.6 ± 0.2	296.1 ± 58.0	7.0 ± 0.1
30	80	HIGH	1.4 ± 0.1	242.1 ± 13.5	7.0 ± 0.2
30	100	HIGH	1.0 ± 0.1	229.2 ± 0.9	7.3 ± 0.2
40	60	LOW	6.0 ± 0.2	363.5 ± 67.2	4.1 ± 0.2
40	100	LOW	4.4 ± 0.3	300.0 ± 20.5	5.9 ± 0.2
40	100	HIGH	1.9 ± 0.0	268.2 ± 2.5	5.2 ± 0.2

^a Results are the average of at least two replications.

^b Molecular weight determined by light scattering and refractive index detectors.

^c Molecular weight determined by pullulan standard curve with refractive index detector.

^d Standard error.

Table II. Weight average molecular weights M_w of amylopectin and peak two (amylose and degraded amylopectin) and gyration radii of amylopectins of extruded hydroxypropylated (8%) corn starches^a.

Starch Moisture Content (%)	Temp. of Extrusion (°C)	Level of Shear	Amylopectin Weight Av. M_w ($M_w \times 10^3$) ^b	Gyration Radii of Amylopectin (R_z) (nm) ^b	Peak Two Weight Av. M_w ($M_w \times 10^3$) ^c
	unextruded		6.5 ± 0.6^d	369.6 ± 27.0	5.5 ± 0.2
30	60	LOW	2.7 ± 0.0	308.0 ± 29.3	5.8 ± 0.1
30	80	LOW	2.4 ± 0.0	242.1 ± 11.3	6.3 ± 0.0
30	100	LOW	1.3 ± 0.1	208.1 ± 11.9	5.3 ± 0.2
30	100	MED.	0.6 ± 0.0	178.9 ± 3.4	6.6 ± 0.1
30	60	HIGH	0.8 ± 0.0	253.5 ± 0.7	7.3 ± 0.2
30	80	HIGH	0.5 ± 0.2	222.5 ± 4.2	7.1 ± 0.2
30	100	HIGH	0.4 ± 0.0	131.8 ± 6.4	6.9 ± 0.1
40	60	LOW	3.7 ± 0.3	357.4 ± 44.5	5.2 ± 0.1
40	100	HIGH	1.3 ± 0.1	194.0 ± 10.8	6.1 ± 0.0

^a Results are the average of at least two replications.

^b Molecular weight determined by light scattering and refractive index detectors.

^c Molecular weight determined by pullulan standard curve with refractive index detector.

^d Standard error.

Table III. Weight average molecular weights M_w of amylopectin and peak two (amylose and degraded amylopectin) and gyration radii of amylopectins of extruded cross-linked (0.014% POCl_3) and hydroxypropylated (8%) corn starches^a.

Starch Moisture Content (%)	Temp. of Extrusion (°C)	Level of Shear	Amylopectin Weight Av. M_w ($M_w \times 10^3$) ^b	Gyration Radii of Amylopectin (R_z) (nm) ^b	Peak Two Weight Av. M_w ($M_w \times 10^3$) ^c
30	100	LOW	5.0 ± 0.4 ^d	381.7 ± 4.6	3.6 ± 0.0
30	100	MED	1.2 ± 0.1	292.3 ± 6.3	3.7 ± 0.1
30	60	HIGH	1.1 ± 0.0	687.5 ± 69.7	4.5 ± 0.1
30	80	HIGH	1.0 ± 0.3	424.6 ± 68.4	4.7 ± 0.3
30	100	HIGH	1.3 ± 0.2	286.3 ± 25.2	3.9 ± 0.0
40	100	HIGH	4.5 ± 0.2	450.5 ± 34.4	3.9 ± 0.0

^a Results are the average of at least two replications.

^b Molecular weight determined by light scattering and refractive index detectors.

^c Molecular weight determined by pullulan standard curve with refractive index detector.

^d Standard error.

Table IV. Weight average molecular weights M_w of amylopectin and peak two (amylose and degraded amylopectin) and gyration radii of amylopectins of extruded cross-linked (0.024% $POCl_3$) and hydroxypropylated (8%) corn starches^a.

Starch Moisture Content (%)	Temp. of Extrusion (°C)	Level of Shear	Amylopectin Weight Av M_w ($M_w \times 10^5$) ^b	Gyration Radii of Amylopectin (R_g) (nm) ^b	Peak Two Weight Av. M_w ($M_w \times 10^5$) ^c
30	60	LOW	3.3 ± 0.3 ^d	472.1 ± 49.0	3.1 ± 0.0
30	80	LOW	4.3 ± 0.2	421.0 ± 12.7	3.2 ± 0.0
30	100	LOW	6.9 ± 0.6	434.3 ± 15.3	3.5 ± 0.3
30	100	MED.	5.3 ± 0.4	465.4 ± 13.4	3.7 ± 0.0
30	60	HIGH	0.7 ± 0.1	337.6 ± 10.6	4.0 ± 0.0
30	80	HIGH	0.7 ± 0.0	411.1 ± 11.6	3.7 ± 0.0
30	100	HIGH	1.7 ± 0.0	460.5 ± 51.6	4.0 ± 0.1
35	100	HIGH	3.6 ± 0.1	400.1 ± 5.7	3.4 ± 0.0
40	60	LOW	9.0 ± 0.8	410.6 ± 33.0	2.7 ± 0.1
40	80	LOW	7.7 ± 0.1	340.7 ± 12.2	2.6 ± 0.0
40	100	LOW	3.4 ± 0.1	379.5 ± 2.1	3.7 ± 0.0
40	100	HIGH	6.7 ± 0.5	417.9 ± 10.6	3.4 ± 0.1

^a Results are the average of at least two replications.

^b Molecular weight determined by light scattering and refractive index detectors.

^c Molecular weight determined by pullulan standard curve with refractive index detector.

^d Standard error.

Table V. Weight average molecular weights M_w of amylopectin and peak two (amylose and degraded amylopectin) and gyration radii of amylopectins of extruded cross-linked (0.028% POCl_3) and hydroxypropylated (8%) corn starches^a.

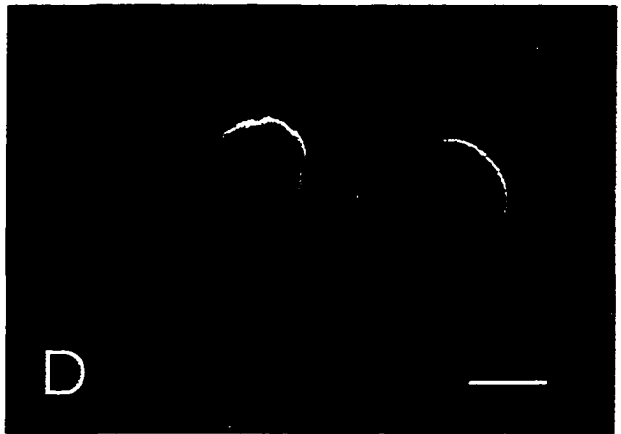
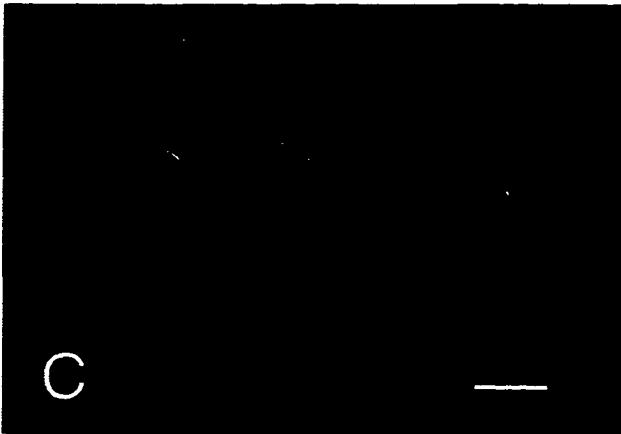
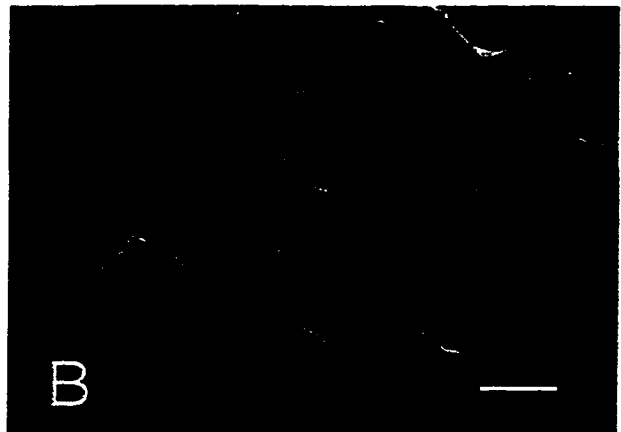
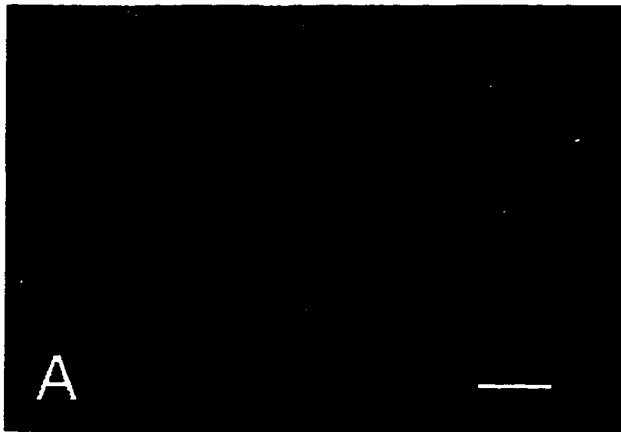
Starch Moisture Content (%)	Temp. of Extrusion (°C)	Level of Shear	Amylopectin Weight Av M_w ($M_w \times 10^3$) ^b	Gyration Radii of Amylopectin (R_z) (nm) ^b	Peak Two Weight Av M_w ($M_w \times 10^3$) ^c
30	60	LOW	7.9 ± 0.2	417.4 ± 22.3	2.9 ± 0.0
30	80	LOW	9.9 ± 0.3	474.5 ± 39.7	2.8 ± 0.1
30	100	LOW	12.8 ± 0.8 ^d	575.9 ± 34.5	3.2 ± 0.0
30	100	MED.	4.1 ± 0.0	381.4 ± 4.2	3.4 ± 0.1
30	60	HIGH	1.5 ± 0.0	353.2 ± 29.7	3.1 ± 0.0
30	80	HIGH	1.7 ± 0.1	382.1 ± 32.1	3.3 ± 0.1
30	100	HIGH	2.5 ± 0.1	422.3 ± 14.1	3.5 ± 0.1

^a Results are the average of at least two replications.

^b Molecular weight determined by light scattering and refractive index detectors.

^c Molecular weight determined by pullulan standard curve with refractive index detector.

Figure 1. Scanning electron micrographs of native corn starch (A), native corn starch extruded at 40% moisture, 60°C and low shear (B), native corn starch extruded at 30% moisture 60°C and high shear (C), cross-linked (0.028% POCl₃), hydroxypropylated corn starch at 30% moisture, 60°C and high shear (D), and cross-linked (0.028% POCl₃), hydroxypropylated corn starch at 40% moisture, 100°C and low shear(E).



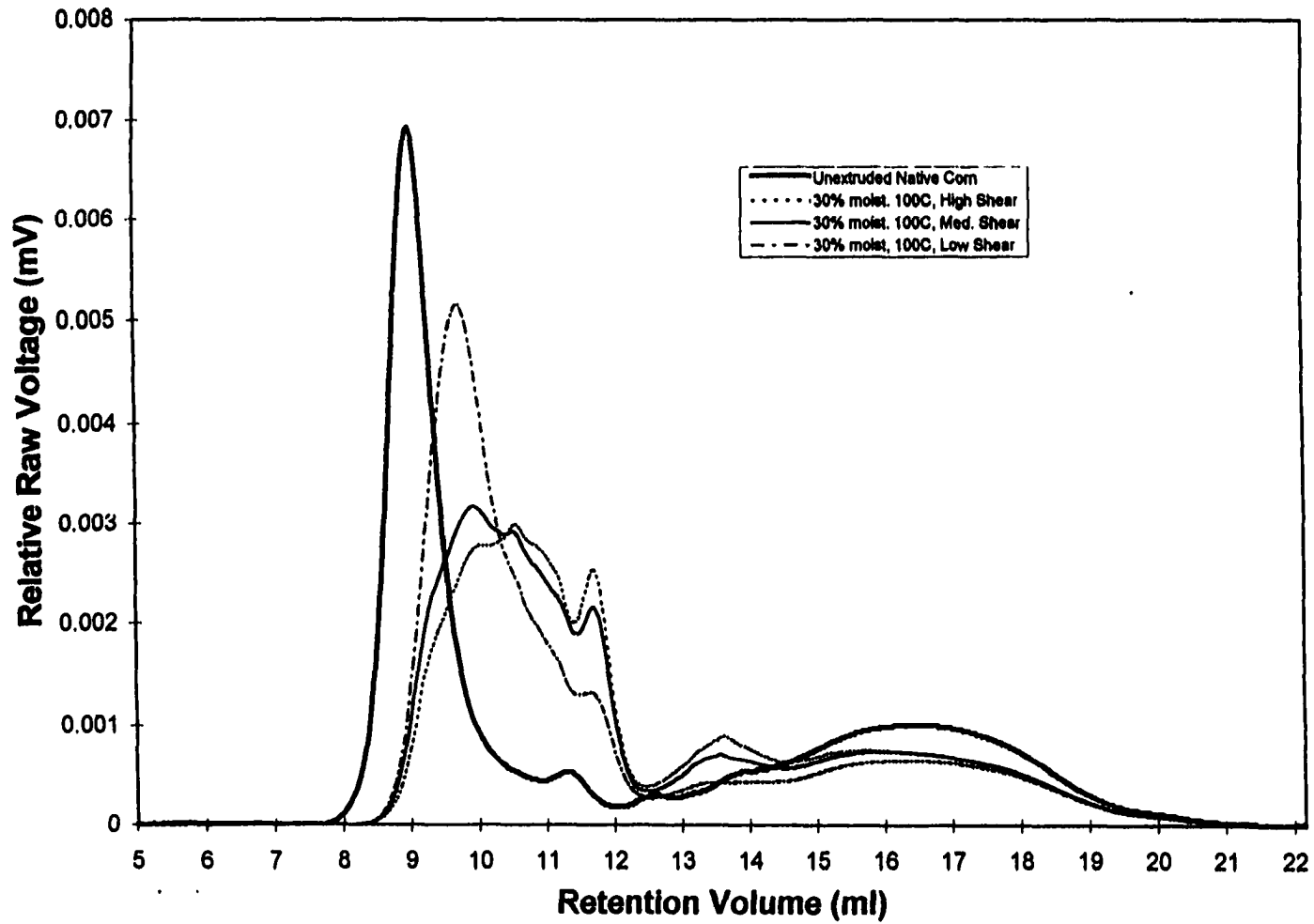


Figure 2. Normalized chromatograms of unextruded native corn starch and extruded native corn starches at 30% moisture and 100°C at low, medium, and high shear (detected by RI).

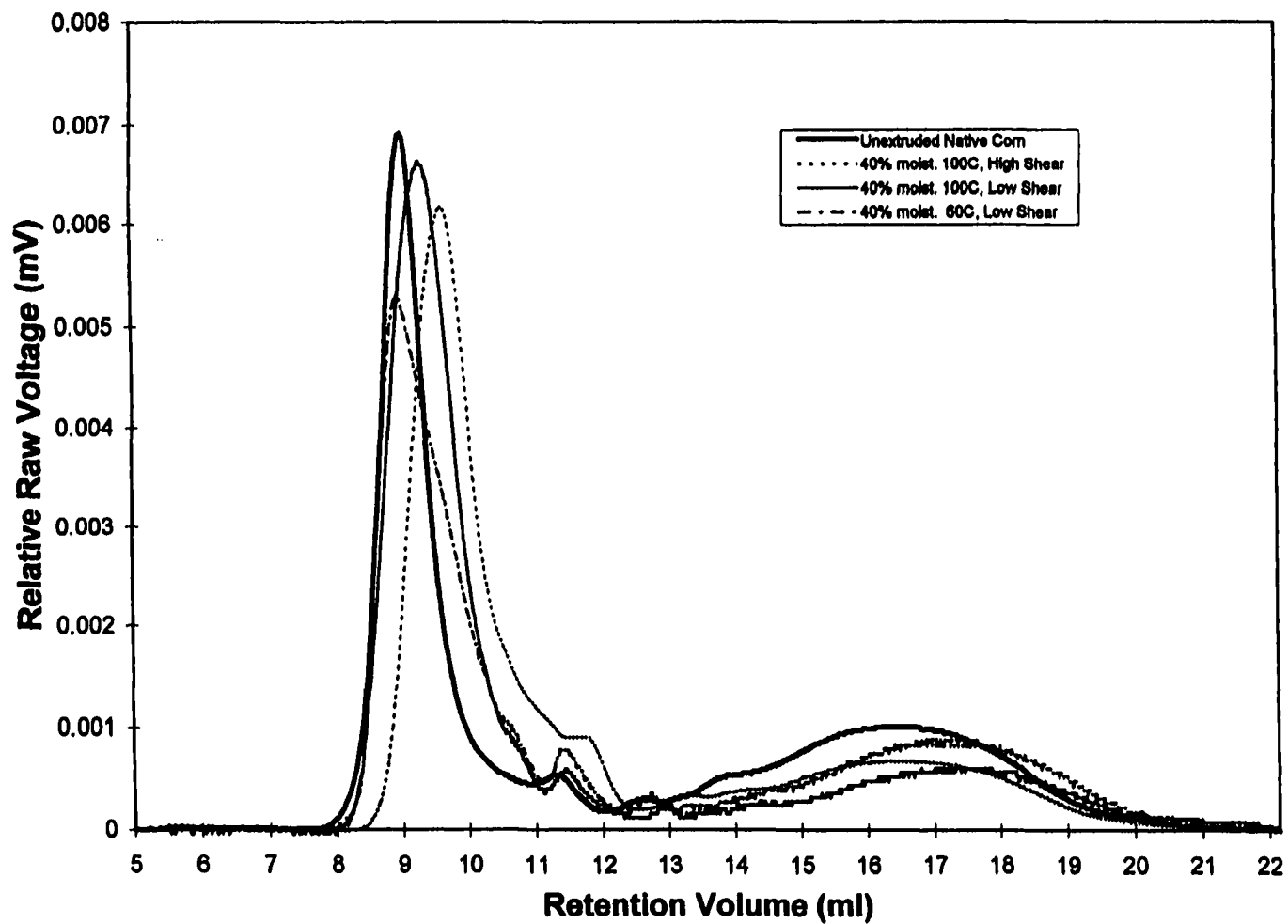


Figure 3. Normalized chromatograms of unextruded native corn starch and extruded native corn starches at 40% moisture and low shear at 60 and 100°C and at 40% moisture and high shear at 100°C (detected by RI).

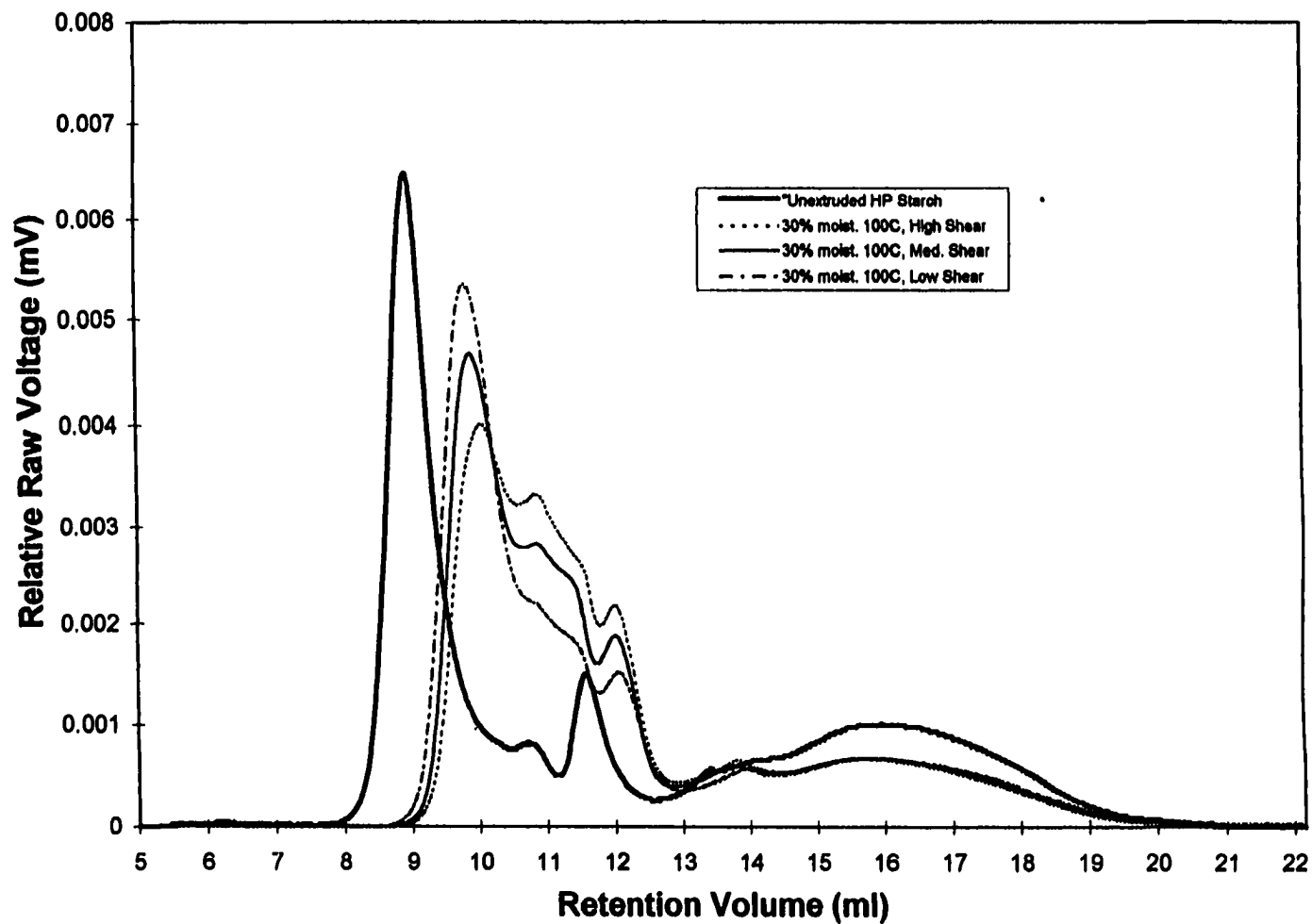


Figure 4. Normalized chromatograms of the unextruded hydroxypropylated (8%) and extruded hydroxypropylated (8%) corn starches at 30% moisture and 100°C at low, medium, and high shear (detected by RI).

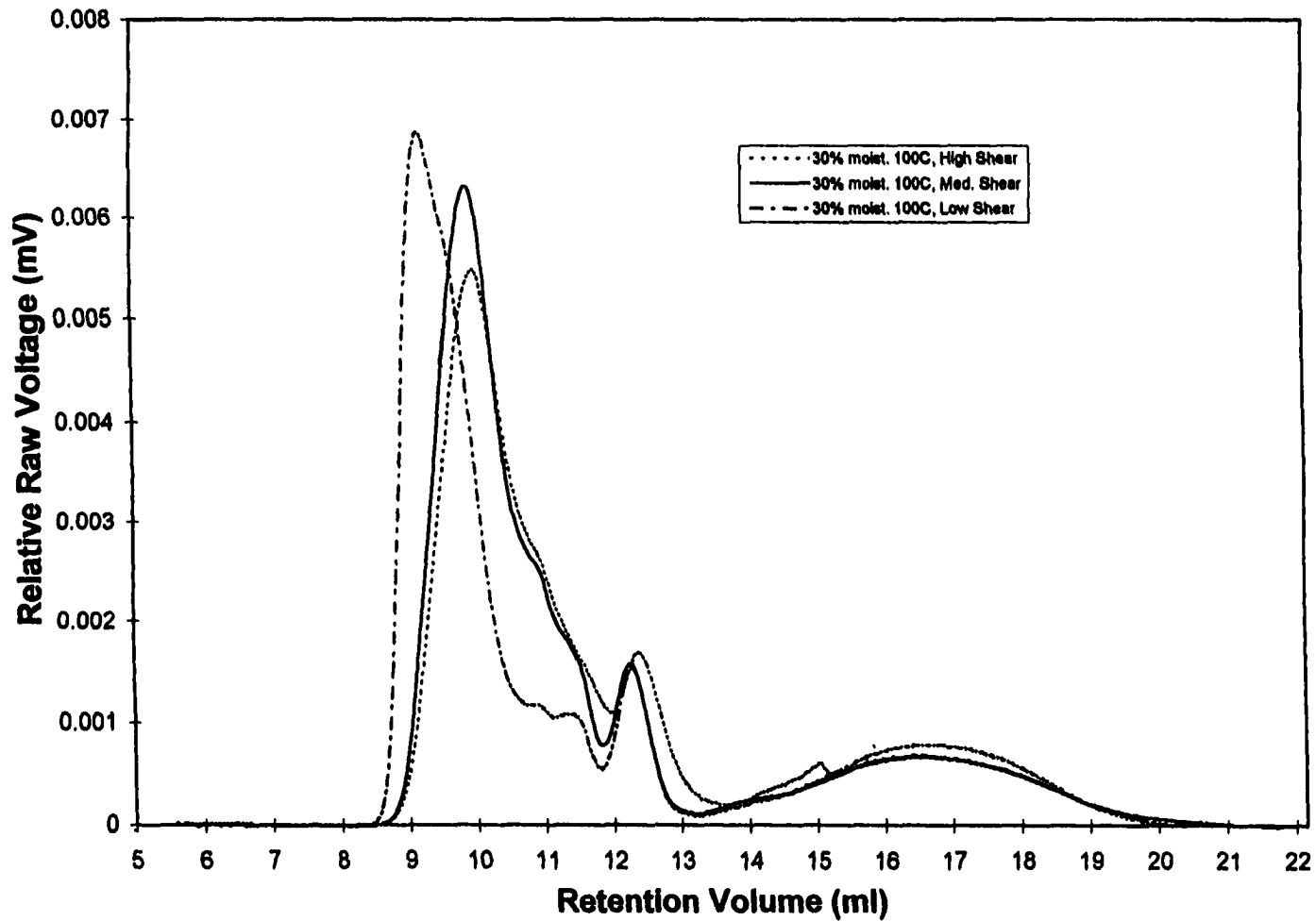


Figure 5. Normalized chromatograms of extruded cross linked (0.014% POCl_3) and hydroxypropylated (8%) and cross linked (0.014% POCl_3) corn starches at 30% moisture, 100°C and low, medium, and high shear (detected by RI).

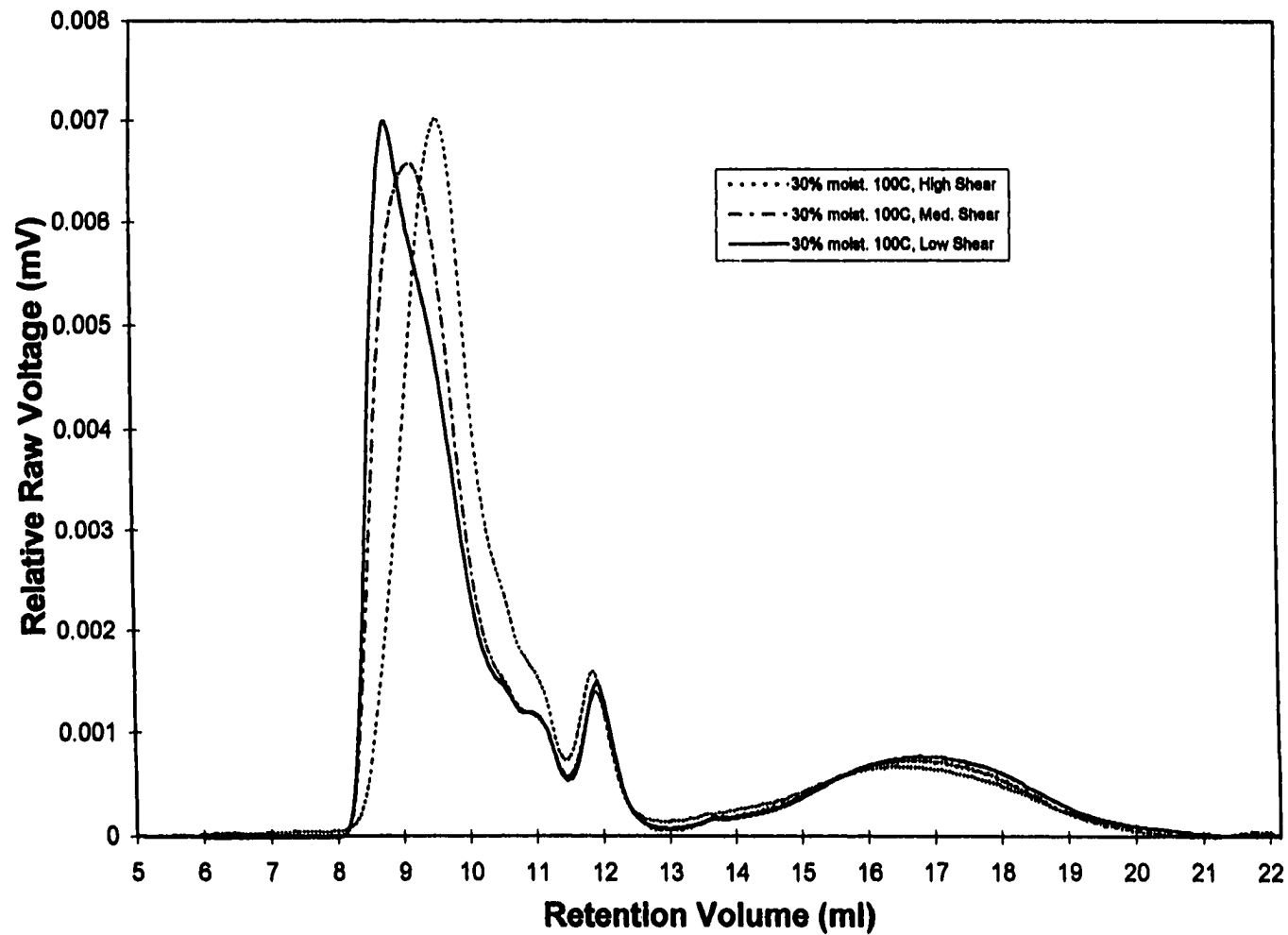


Figure 6. Normalized chromatograms of extruded cross-linked (0.024% POCl_3) and hydroxypropylated) corn starches at 30% moisture, 100°C and low, medium, and high shear (detected by RI).

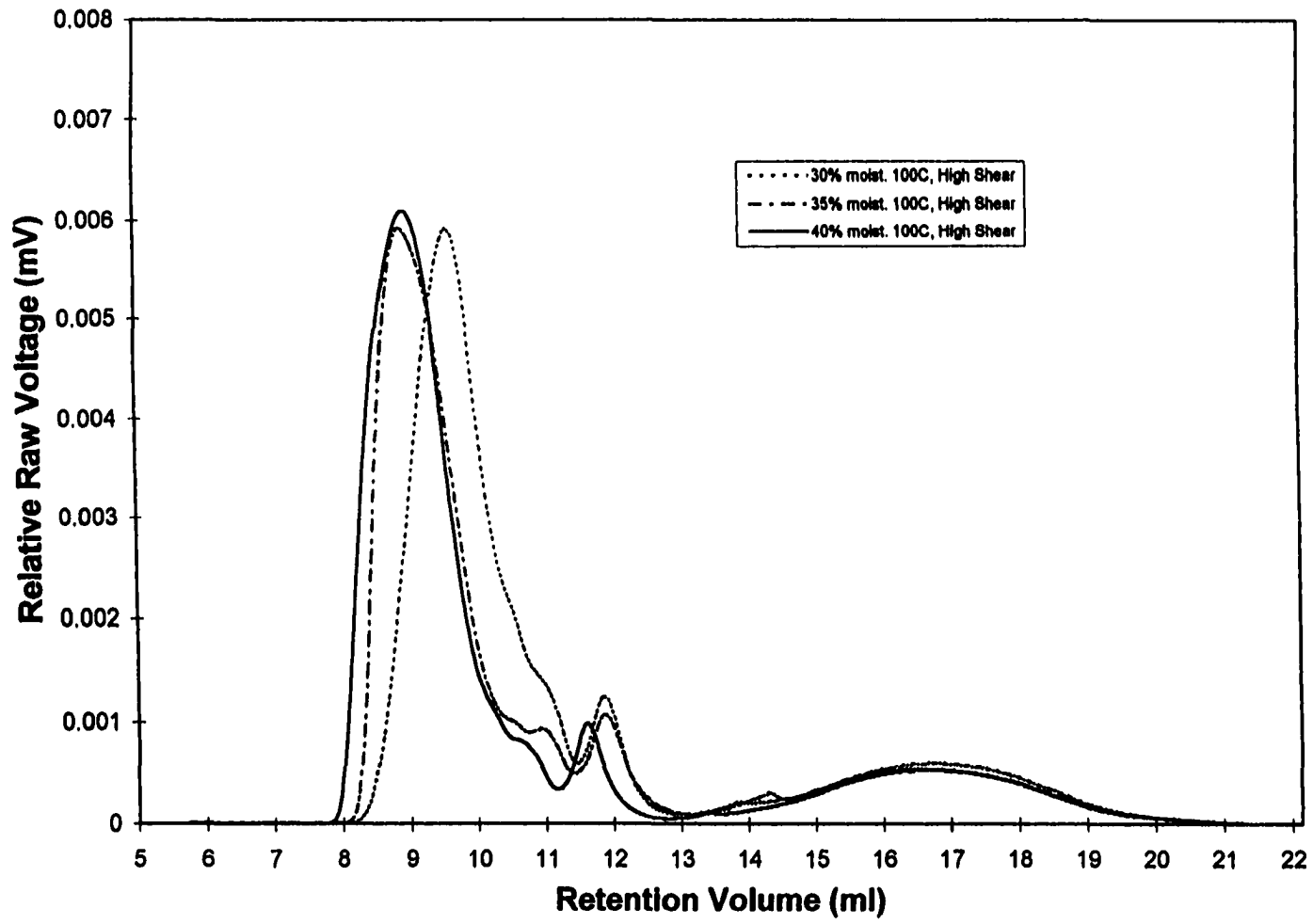


Figure 7. Normalized chromatograms of extruded cross-linked (0.024% POCl_3) and hydroxypropylated corn starches at high shear, 100°C and 30, 35, and 40% moisture (detected by RI).

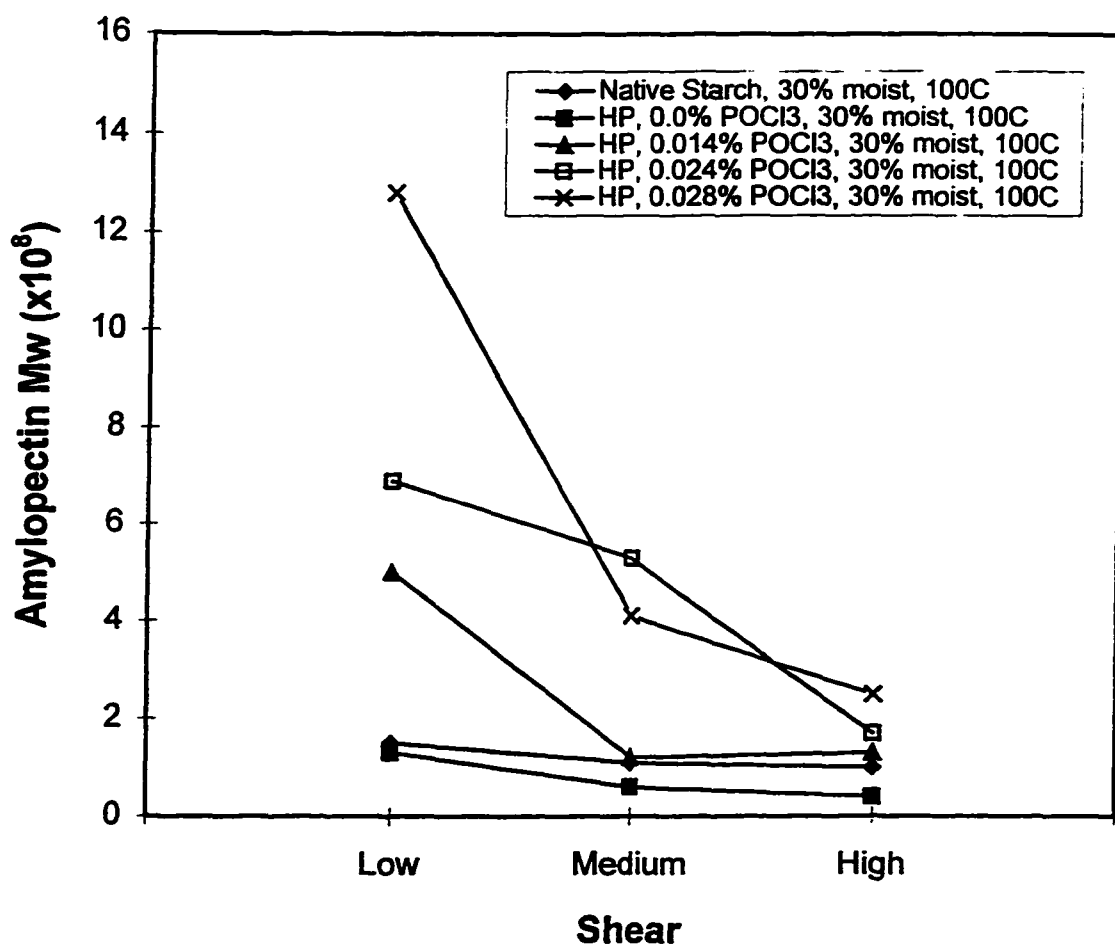


Figure 8. Amylopectin molecular weights of starches extruded at 30% moisture and 100°C at low, medium, and high shear.

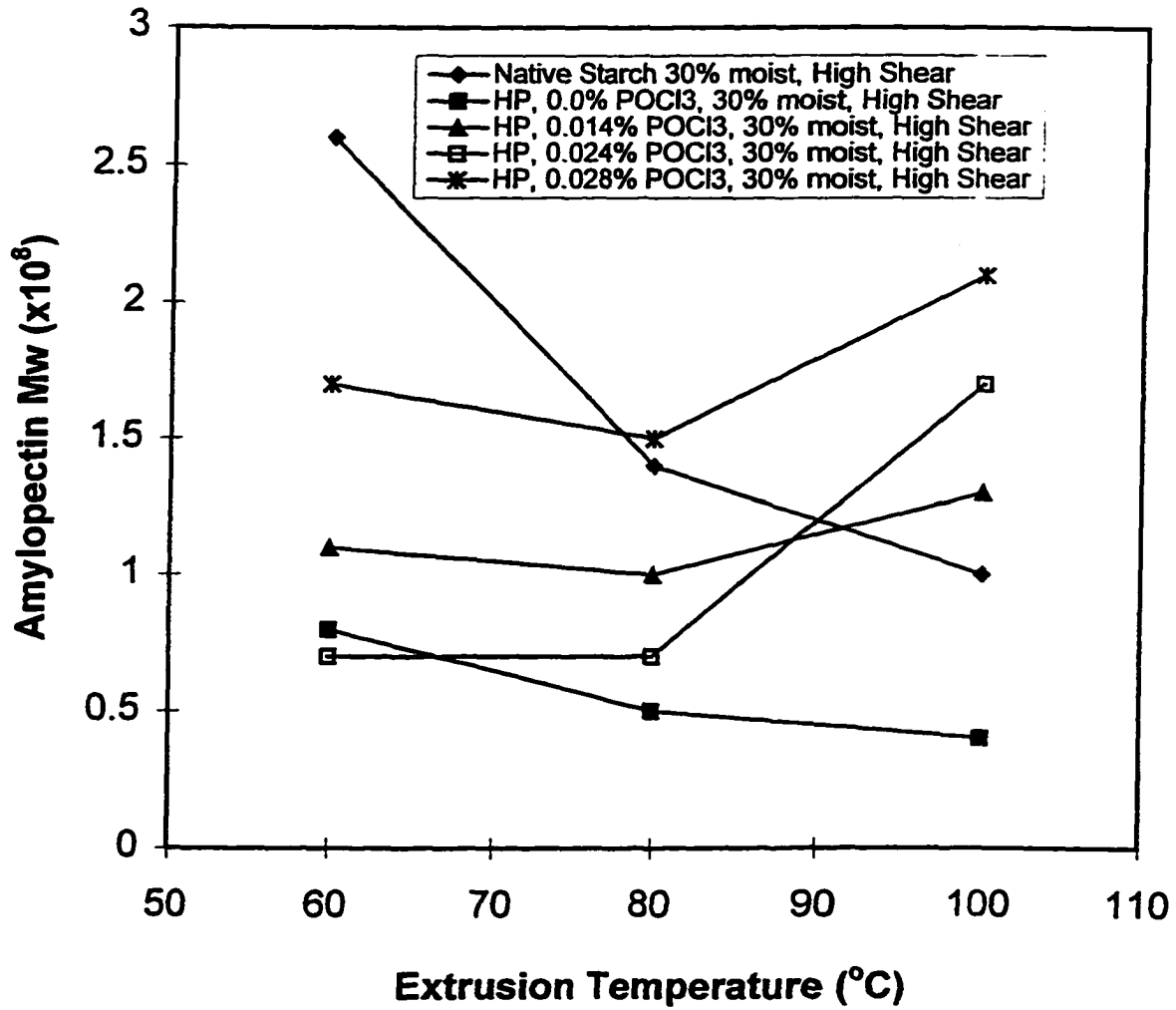


Figure 9. Amylopectin molecular weights of starches extruded at 30% moisture and high shear at 60, 80, and 100°C.

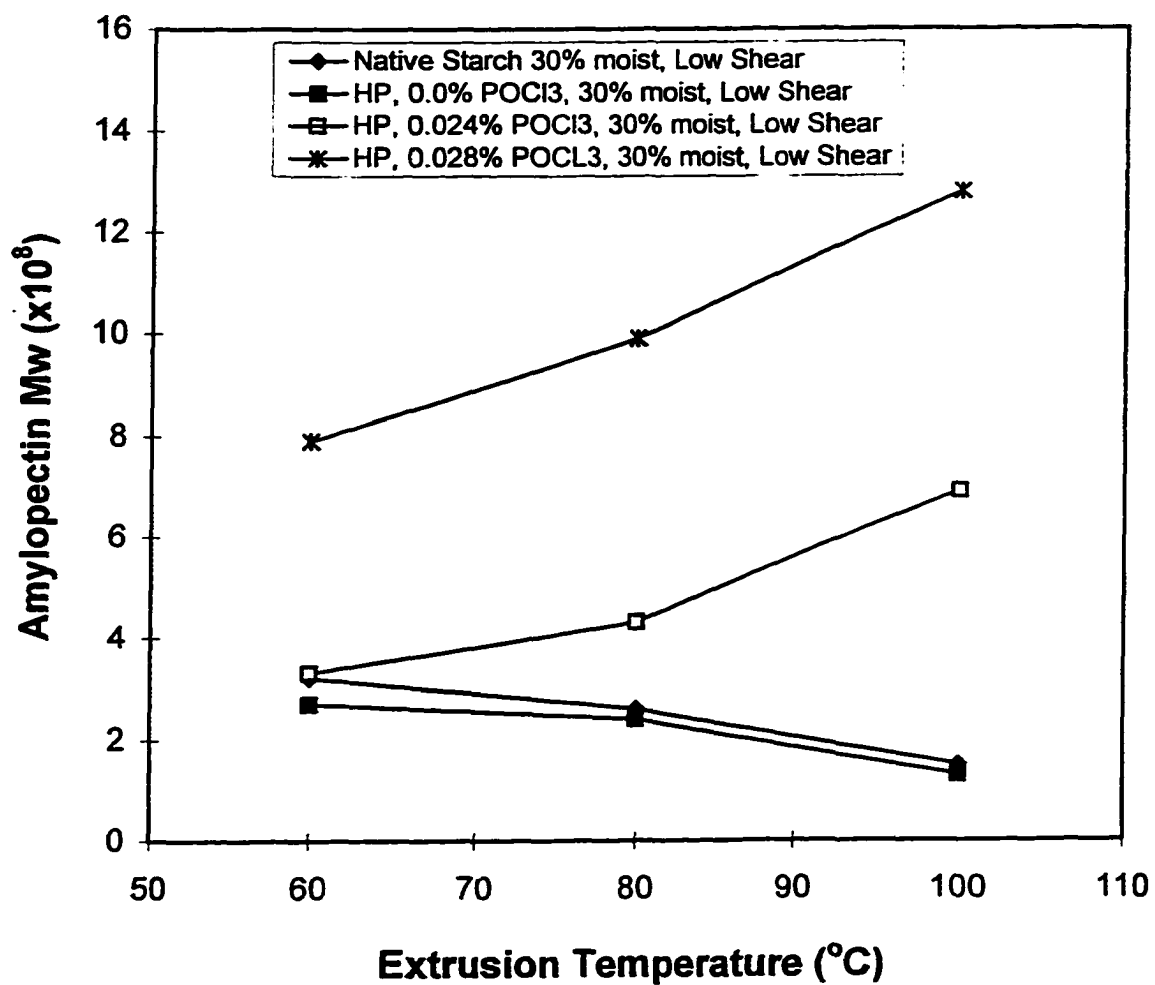


Figure 10. Amylopectin molecular weights of starches extruded at low shear at 60, 80, and 100°C (moisture content is given in parentheses)

GENERAL CONCLUSIONS

Starch is utilized in a wide variety of food, pharmaceutical, and industrial applications. An understanding of the fundamental starch structure - function relationships is crucial to optimizing current uses as well as development of new applications. This work encompassed three areas of starch structure function relationships. First, the contribution of the unique molecular structures to different varieties of root and tuber starches. Second, the role of extrusion processing variables on the viscosity of extruded starches. Finally, the relationship of extrusion processing variables to molecular degradation of extruded starches.

The physical and chemical properties of waxy potato starch were examined and compared to those of normal potato, yam, and sweet potato starches. Normal potato and waxy potato starches and Naegeli dextrins of both displayed the B-type x-ray diffraction pattern. Yam and sweet potato starches displayed the C_A- and C-type x-ray diffraction patterns. The Naegeli dextrins of yam and sweet potato starches displayed the A-type x-ray diffraction pattern. ³¹P NMR showed the phosphorous contents of the starches and the Naegeli dextrin of normal potato to be primarily phosphate monoesters. Normal and waxy potato starches displayed lower proportions of short branch chains than did yam and sweet potato starches. Normal potato starch was found to have a higher proportion of long branch chains than waxy potato.

Extrusion variables of starch moisture, barrel temperature, and screw design were examined in terms of thermal and pasting profile analysis in a series of cross-linked and hydroxypropylated corn starches. Pasting profiles were shown to be an effective method to

follow extrusion variable effects. Increasing starch moisture content and level of cross-linking were shown to significantly increase starch viscosity. Increasing shear and barrel temperature significantly decreased starch viscosity. Significant interactions were found between level of cross-linking and screw design and between extrusion temperature and starch moisture content.

Although pasting profile analysis was effective tool to monitor viscosity changes due to extrusion variables high performance size exclusion chromatography (HPSEC) was shown to be a powerful tool to follow variables on a molecular basis. HPSEC, with multi angle laser light scattering and refractive index detection, revealed the molecular weights of both amylose and amylopectins in the non-cross-linked, unextruded starches and the changes in molecular weight with extrusion. The addition of cross-linking both increased the molecular weight of the amylopectin molecules and prevented severe degradation. Increasing starch moisture content also lessened amylopectin degradation, whereas increasing temperature had the opposite effect.

ACKNOWLEDGMENTS

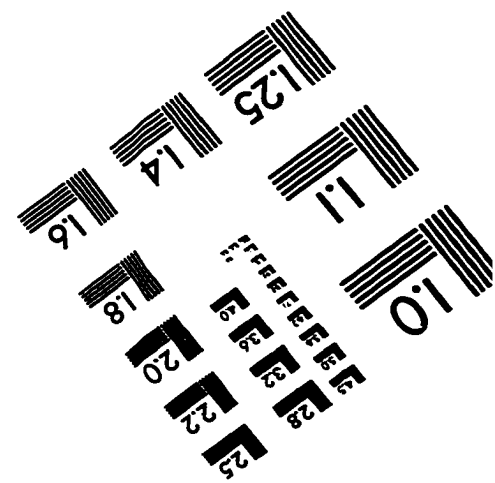
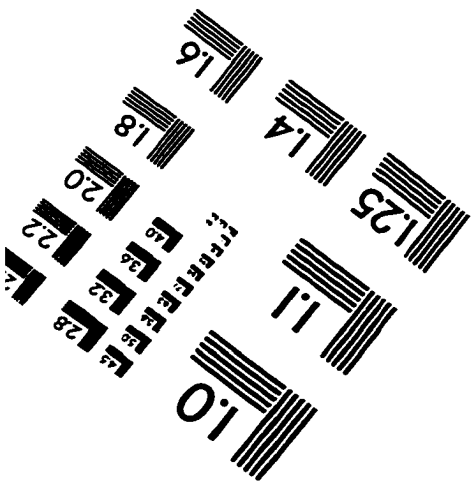
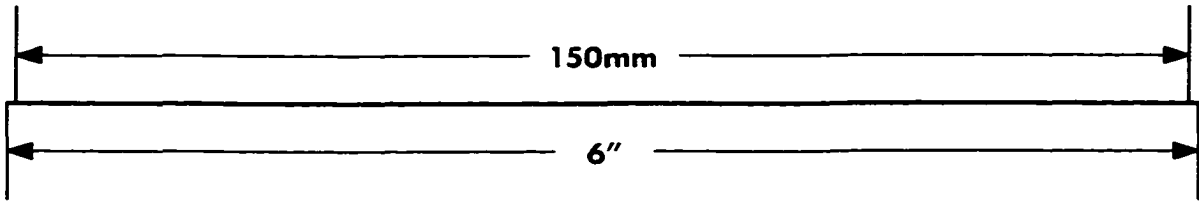
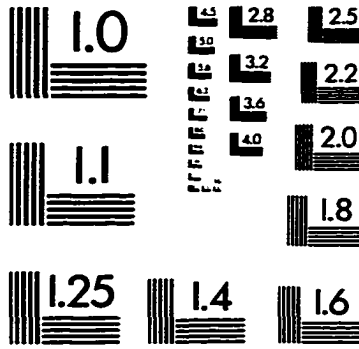
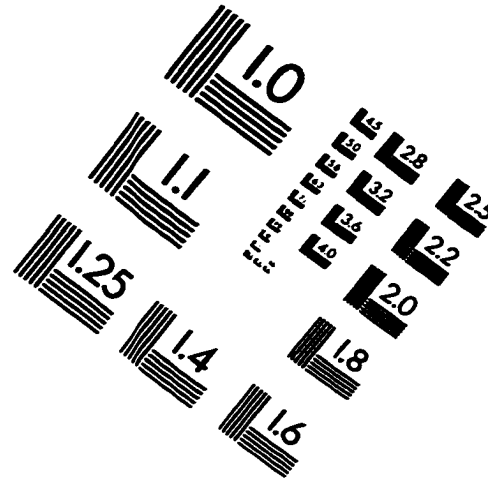
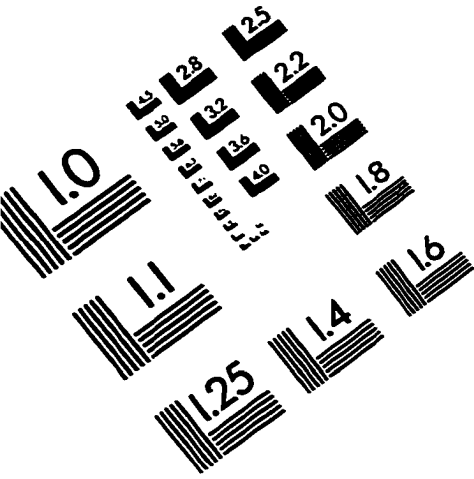
I must express my sincere gratitude to my major professor, Dr. Jay-lin Jane, for her thoughtful advice and guidance throughout the course of this project. I have truly enjoyed participating in the innovative basic and applied research conducted by Dr. Jane's research group. Also worth mentioning is the collection of instrumentation assembled by Dr. Jane's research laboratory. The majority of this study could not have been accomplished without the superb instrumentation support.

I also wish to thank the members of my committee, Dr. John Robyt, Dr. Ted Bailey, Dr. Larry Johnson, and Dr. Zivko Nikolov for their interest in my research and their valuable advice.

I thank my comrades: Kris, Ting-jang, Milica, Lolita, Lisa, Vincent, Yung-yung, Kitsum, Perminus, Sushe, and Jingwen for their assistance and friendship. I also thank the faculty and staff of the Food Science and Human Nutrition Department for their support and assistance.

Finally, I wish to thank my wife, parents, and family for their unfailing love, patience, and support without which this endeavor would have been impossible.

IMAGE EVALUATION TEST TARGET (QA-3)



APPLIED IMAGE, Inc
 1653 East Main Street
 Rochester, NY 14609 USA
 Phone: 716/482-0300
 Fax: 716/288-5989

© 1993, Applied Image, Inc., All Rights Reserved