WET-MILLING OF WAXY WHEAT FLOURS AND CHARACTERISTICS OF WAXY WHEAT STARCH

by

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Abstract

Waxy wheat starch contains almost all amylopectin and is relatively new. Currently, advanced lines of hard winter waxy wheats are being bred through genetic elimination of waxy proteins. To realize the full potential of waxy wheat, the wet-milling of waxy wheat flour to produce gluten and waxy wheat starch was investigated. Flours of six advanced lines of waxy hard wheats and two normal hard wheats cultivars, Karl '92 and Trego, were fractioned by the dough-washing method. Doughs prepared from the waxy flours were found to be weaker than those of from normal wheats. All the waxy wheat and normal wheat flours were wet-milled by the dough-washing (Martin) process and the yield and recovery of starch and gluten were compared. One waxy wheat flour, NWX02Y2459, was sticky during the early stages of dough washing, and it gave relatively poor gluten and starch recoveries with low purity. By mixing the dough with 2% NaCl solution or by adding hemicellulase, the stickiness of the dough subsided during the washing step, and thereby recoveries of the gluten and starch fractions were improved. Waxy wheat starch offers unique functional properties. Waxy wheat starches gelatinize and cook at a relatively low temperature compared to maize starches, and their pastes retrograde more slowly and to a lower extent than waxy maize starch. Pasting curves showed that waxy wheat starch generated a much higher viscosity at a lower temperature, and a lower setback viscosity than normal wheat starch and waxy maize starch. Changes in the morphology of waxy and normal wheat starch granules were determined by using a hot-stage microscope, and those changes were related to their pasting properties. After waxy wheat starch was cross-linked in an aqueous slurry at about 37% starch solids with 0.01% phosphoryl chloride (starch basis), visco-amylograms showed that viscosity breakdown was eliminated and that the cooked paste became non-cohesive (less "stringy"). Increasing levels of phosphoryl chloride at 0.03% and 0.06% caused a steady decline in the peak and final paste consistencies of cross-linked waxy wheat starch, whereas the consistencies of waxy maize starch proceeded through an optimum. Waxy maize starch cross-linked with 0.03% phosphoryl chloride had a higher peak and final consistency at 7% solids than when cross-linked with 0.01% and 0.06% phosphoryl chloride.

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CHAPTER 1 - Introduction

Production and Uses of Wheat

Wheat is a worldwide cultivated cereal crop. The combination of improved agricultural methods and higher yielding varieties has resulted in rapid growth of the world wheat crop (Knight and Olson 1984). According to recent statistics of the Food and Agriculture Association (FAO) of the United Nations, wheat production in 2007 approached 619 million tones. That level is up 3.6 percent from the previous year, and contributed to the 2007 record production of cereals in the world (FAO 2007).

The main use for wheat is food, while corn (maize) is mainly used as animal feed (Knight and Olson 1984). An industrially important and growing use of wheat is for production of starch and vital gluten (FAO 2007). Wheat starch manufacturing is competitive with that of corn starch because of the value of the co-product, vital wheat gluten (Knight and Olson 1984).

Wheat is a major source of nutrition for people in many regions of the world because of its agronomic adaptability, ease of storage, nutritional goodness, and the ability of its flour to produce a variety of foods (Orth and Shellenberger 1988). Wheat provides substantial amounts of nutrients to the human diet, such as food energy, protein, vitamins, minerals and dietary fiber (Hemery 2007). Nutrients are generally found in the highest concentrations in the germ or embryo and in the aleurone cells surrounding the starchy endosperm, although a significant proportion of starch (food energy), minerals, and vitamins are located in the endosperm of wheat (Hemery 2007). Researchers and consumers have focused on a more integrated approach to the nutritional value and health implications of wheat foods.

Composition of Wheat

Davis et al (1981) evaluated wheats from five market classes, with four subclasses of white wheat, comprising 406 samples of 231 varieties and representing three crop years and 49 growing locations. Moisture, carbohydrate (nitrogen-free

extract), protein, and ash content, respectively, had overall mean values of 11.4±0.23%, 72.4 ± 0.18%, 13.85±0.16%, and 1.8±0.23%. All values except moisture are expressed on a dry weight basis.

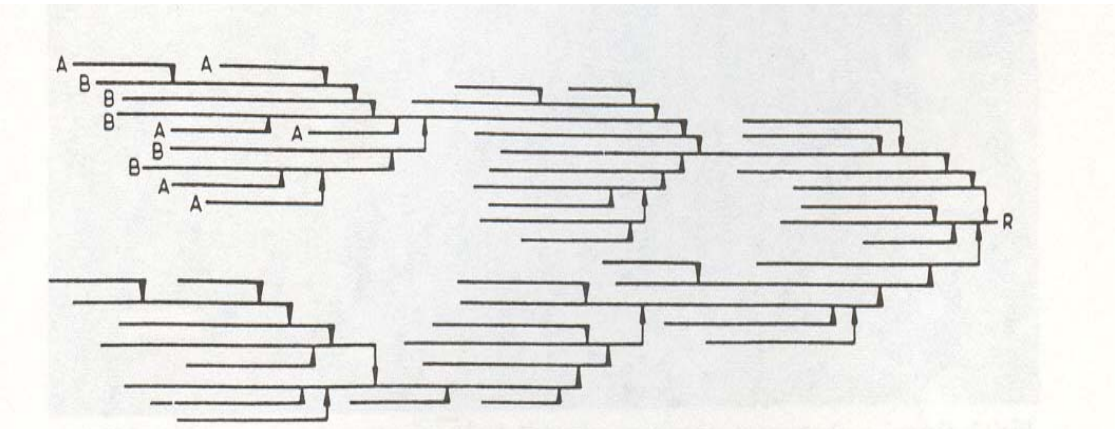
Composition of Wheat Starch

Starch, being 75~80% of the wheat ((*Triticum aestivum L*.) endosperm, is the major component in wheat. Normal wheat starch consists mainly two types of glucose polymers, amylose and amylopectin, but contains minor levels of protein and lipids (Lillford and Morrison 1997) (Table 1.1).

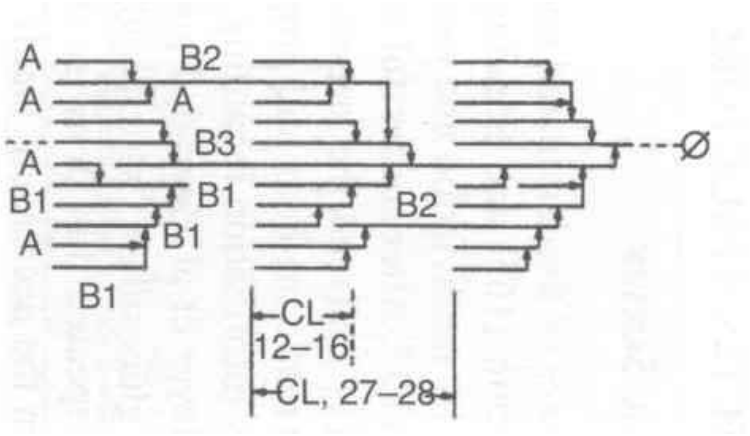
Table 1.1 Composition of Wheat Starch (Wild Type) Granules (Lillford and Morrison 1997).

Component		Level (%, db)
Amylose		23-27
Amylopectin		73-77
Lipids	surface	0.02-0.6
	interior	0.1-0.2
Proteins	surface	0.006-0.5
	interior	0.07

Amylose is an essentially linear polymer consisting of α- (1-4) linked D-glucosyl units. There are two properties that dominate the solution behavior of amylose in water: the ability to form inclusion complexes with surfactants, iodine, lipids, and primary aliphatic alcohols, and the ability to participate in strong intermolecular interactions leading to precipitation or gelation (Lineback and Rasper 1988). Amylose forms an intense blue complex with iodine which has been used as the basis for quantitation of amylose. Pure amylose has an iodine-binding capacity (iodine affinity) of 20% on a weight basis (Lineback and Rasper 1988).



Manners and Matheson 1981



Hizukuri 1986

Figure 1.1 Structural model for amylopectin proposed by Manners and Matheson (1981) and by Hizukuri (1986), respectively. A=A-chain; B=B-chain; R=reducing group. The average chain length (CL) of one cluster (linear plus branched region) is 27~28 glucose units in the Hizukuri model.

Amylopectin consists of approximately 95% α- (1-4) linked D-glucosyl units and 5% α- (1-6) branch points. The average chain length of wheat amylopectin was reported in the range of 17-23 which calculates to 5.9-4.4% branching (Lineback and Rasper 1988). A model for amylopectin (Fig 1.1) proposed by Manners and Matheson (1981) is compatible with observations made from studies concerning the structure of amylopectins, including an A-chain/B-chain ratio of approximately 1:1, and incorporates the concept of "clusters" of branch points (interchain linkages) and linear chains in certain regions of the molecule. An A-chain is an unsubstituted chain linked

α-1,6 to an amylopectin molecule, whereas a B-chain carries one or more chains attached to its primary hydroxyls and is attached to amylopectin by an α-1,6-linkage. The clustering of interchain α-1,6 linkages creates an amorphous region, whereas the clustering of linear chains creates a crystalline region. Figure 1.1 represents only a small portion of the amylopectin molecule. X-ray crystallography of starch indicates the linear A-chains and B-chains probably exist in a double-helical conformation (Young 1984). The most popular and recent model of the structure of amylopectin by Hizukuri (1986) shows that amylopectin B-chains can occur as B4, B3, B2, and B1 chains, where B4, B3 and B2 chains traverse 4, 3 and 2 clusters, and B1 chains and A-chains occur in one cluster.

Intermolecular aggregation owing to hydrogen bonding is inhibited in amylopectin, probably because of the relative abundance of α-1,6 bonds. So, aqueous solutions of amylopectin molecules do not readily retrograde to form a gel or precipitate (Young 1984). Highly concentrated solutions of amylopectin above ~10% have a slow retrogradation rate (Young 1984). The high cost of energy for fractionation of starch into amylose and amylopectin makes high-amylose starch mutants and waxy starch mutants more economical compared with isolated amylose or amylopectin (Young 1984).

All native starches occur as granules. Starch granules have a partially crystalline character. About 70-80% of the mass of a normal starch granule is in the amorphous states and that state contains almost all the amylose and the clustered branch points of amylopectin. The remaining 20-30% is crystalline, which consists primarily of the linear portion of the B1 chains and the A-chains in amylopectin (Sajilata et al 2006) (Figs. 1.2 and 1.3).

Wheat starch shows a biomodal size-distribution of two types of granules, which are called the A- and B- type granules (Hoseney 1986). The A-type granules in mature kernels are disk-shaped with diameter 10-35μm and thickness ~5μm, whereas the B-type granules appear as spheres with diameter 1-10μm (Hoseney 1986). Granules have highly organized architecture of concentric rings (Sajilata et al 2006) (Figs. 1.2 and 1.3) and exhibits birefringence under polarized light (Hoseney 1986).

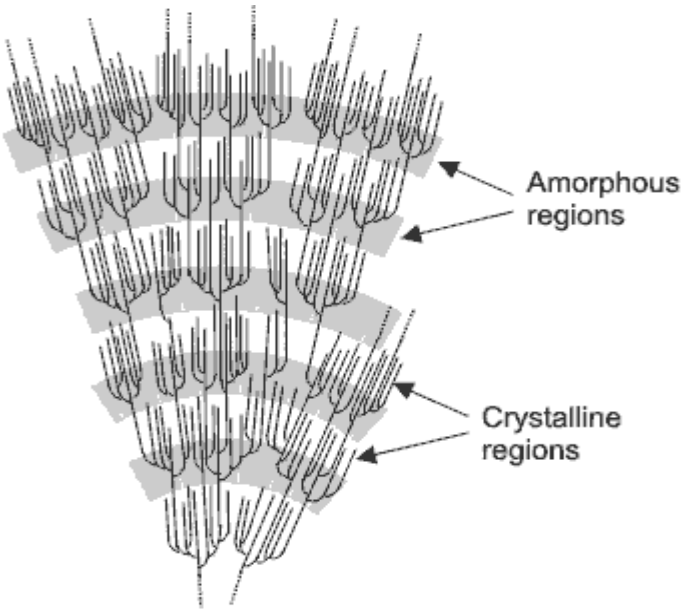


Figure 1.2 The organization of the amorphous and crystalline regions (or domains) of a starch granule (Adapted from Sajilata et al 2006).

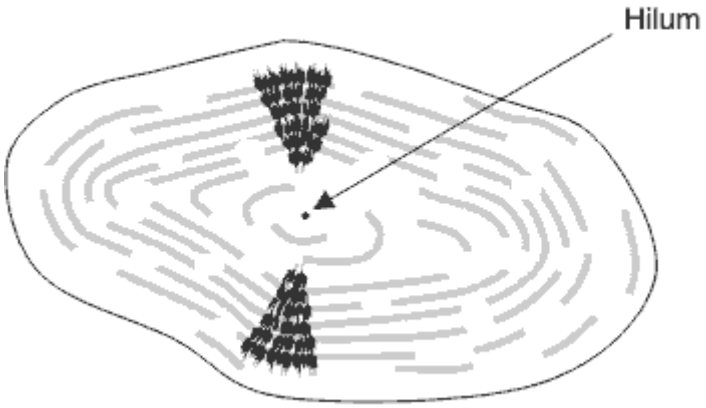


Figure 1.3 The orientation of the amylopectin molecules in a cross section of an idealized starch granule. Linear amylose molecules are assumed also to orient perpendicular to the granules surface (Adapted from Sajilata et al 2006).

Uses of wheat starch and derivatives

Wheat starch produced in the United States and Canada is often considered a by-product in the manufacture of wheat gluten because gluten has unique elastic properties among plant proteins (Knight and Olson 1984). Wet processing of wheat flour yields approximately 5 parts of starch for every 1 part of gluten. The highest proportion of wheat starch is consumed by the paper industry, where it is used as a wet-end adhesive, surface coating, and adhesive for manufacture of corrugated board (Mentzer 1984). The lower gelatinization temperature of wheat starch offers an advantage over corn starch for corrugating adhesive uses (Mentzer 1984). Other uses are in laundry sizing and cotton finishing, where wheat starch provides a superior finish (Knight and Olson 1984). The thickening power of wheat starch is less than that of corn starch, but paste texture, clarity, and strength are about the same (Mentzer 1984). However, the high pentosan and soluble protein contents of wheat flour cause wheat starch to be more difficult to isolate and purify than corn starch (Knight and Olson 1984).

Wheat starch is preferred over other starches in baked products. Its principal use in baking is to replace portions of wheat flour and thereby dilute flour protein content (Moore et al 1984). Wheat starch has several functional advantages over other starches added to flour, including increased volume and tenderness of cakes, and reduction of fat absorption in doughnuts (Moore et al 1984). Modified wheat starches have better emulsifying property over other starches when used in some food products perhaps because wheat starch has a higher monoacyl lipid content (Moore et al 1984). Limited usages has occurred in the confectionery and canning industry (Moore et al 1984).

Dry-Milling of Wheat

Generally, the objective of milling is to separate the anatomical parts of cereals to make them more palatable in foods and to increase their storage stability (Hoseney 1986). Milling is essentially a process of grinding and separating (Bass 1988). Grinding is done on break rolls, sizing rolls, and reduction rolls, and separation

is made using machines called sifters and purifiers (Bass 1988). The "gradual reduction system" is the most popular approach to mill wheat. Wheat kernels are first tempered to a moisture content of ~15% to toughen the bran. Wheat kernels are initially cracked on corrugated break rolls, and then sifted. The broken kernels are recovered and then ground to a smaller particle size on another set of roller mills, and the break-process is repeated on 5 or 6 break rolls. The two rolls of a break mill are moving at different speeds, and the corrugated rolls scrap a endosperm from the bran skin. The severity of scraping (grinding) is controlled consistent with obtaining flour of the required ash or color specifications. After each break roll the stock is sifted and chunks of endosperm are purified from broken bran and ground to flour on smooth-surfaced reduction rolls (Bass 1988). All mills use the same basic principles of grinding and separating, which can be divided into the following seven distinct operating systems: break, grading, purification, sizings (scratch), reduction, flour dressing, and millfeed systems (Bass 1988). Flour is created in all the grinding steps, and it is separated from many intermediate streams by sifting though an opening of ~100μm. Straight-grade flour is a composite of all the flours produced on the mill and represents about 72% by weight of the total products (Hoseney 1986).

Wet-Milling of Wheat Flour

Generally, wet milling attempts to make a clean separation of bran and germ from the endosperm, and furthermore to separate the endosperm into its chemically distinct components (Hoseney 1986). Starch and gluten have been fractionated from wheat flour or whole wheat kernels by a number of processes. But only a few processes are used in industry today, and all start from wheat flour (Van Der Borght et al 2005). The reasons that whole-wheat processes have not been commercialized are mainly due to the bran-contamination of gluten and the poor color of starch (Sayaslan 2004).

Flour fractionation processes can be classified by the consistency of the starting mixture of flour and water. They are called the dough, dough-batter, and batter-based processes (Van Der Borght et al 2005). Another classification of wet-

processes for wheat flour is based on the size of agglomerated gluten in the mixing stage. The degree of gluten agglomeration is high in a mixed dough but low in a mixed batter. Nowadays, the dough (Martin) process, the Alfa-Laval/Raisio process and the hydrocyclone process are widely used in North America whereas the High-pressure Disintegration (HD) process is popular in Europe (Sayaslan 2004; Sayaslan et al 2006). The gluten obtained from all the above processes can be kept vital if it is properly dried (Van Der Borght et al 2005).

Straight-grade or high-yield flours from hard (in North America) or soft wheats (in Europe), not bleached nor malted nor enriched and containing high protein content (>11%), strong aggregation property, and low starch damage, ash, and α-amylase activity are preferred for wet-milling (Sayaslan 2004; Sayaslan et al 2006). Protein content and strength are controlled by genetic and environmental factors, and are important factors in the functional use of a flour. Unlike low-protein maize, low-protein wheat flour is difficult to fractionate because its protein lacks proper coagulation and separation during wet-processing, which results in contaminated starch that is not suitable for modification (Weegels et al 1992).

Several studies showed that hydrolases that act on non-starch polysaccharides can be added as a processing aid in the separation of wheat flour into starch and gluten. Weegels et al (1992) found that cellulases and hemicellulases improved gluten yield, protein recovery in gluten, gluten coagulation and starch yield of flour with intermediate processing properties. Protease and amylase is unfavorable for gluten-starch separation since their actions increase the amount of soluble protein and carbohydrate, which in turn increases the viscosity of the starch slurry and results in a less efficient separation of starch, protein and water-solubles. Redgwell et al (2001) suggested that xylanase has the beneficial effect by decreasing the interaction between gluten protein and water-extractable arabinoxylans, which is one component of non-starch polysaccharides.

The Martin ("dough-washing") process was first applied by Beccari in Italy in 1728 (Anderson 1967). Washing a dough ball under a small stream of water washes away the starch and solubles from a visoelastic ball of gluten. This process, which is

the oldest wet-milling process (Anderson 1967), was further developed by Martin and reported in Paris in 1745, But it was not commercialized for the production of wheat starch until the 20th century (Sayaslan 2004). Improvements in the traditional Martin process were made to reduce the fresh water consumption through recycling water and more efficient equipment (Sayaslan 2004).

After Hilber and co-workers in the USA and Shewfelt and Adams in Canada developed the Batter process (Seib, 1994), it was used by several plants in the 1940s and early 1950s in North American and Australia (Sayaslan 2004). A typical vital gluten fraction isolated by the Batter process contained ~ 80% protein (db) and 75~85% of flour protein was recovered in the gluten fraction (Anderson 1967).

The Hydrocyclone process is one of the new wet-miling processes for wheat flour which was developed by the KSH company of Holland in the late 1970s (Sayaslan 2004). This process has several advantages, such as low cost, absence of moving parts in equipment, a wide range of operating conditions, less dependence on protein strength and low water usage (Sayaslan 2004).

In 1984, the High-Pressure Distintegration (HD) process technology was applied to wheat flour wet-milling by three plants in Germany (Witt 1997). It became the most popular process in the Europe for wet-milling of wheat flour and the most recent industrial method to separate wheat starch and vital wheat gluten from wheat flour (Maningat and Bassi 1999, Sayaslan 2004). A sheared flour-water batter is created in the HD process by rapidly moving (pumping) a slurry through a stationary valve (Sayaslan 2004). The HD process includes a three-phase decanter centrifuge which removes viscous pentosans and water-solubles from the batter early in the process (Witt 1997). The densest stream from the decanter centrifuge is almost pure A-starch granules, while the medium-dense stream is swollen protein with the B-starch granules and some A-granules. Removal of the pentosans and most of the A-granules allows a more efficient separation of gluten and starch from the middle-dense phase. Therefore, less fresh water is needed in starch purification, and that reduces the effluent stream. Since the centrifugal separation is based on density

difference of hydrated components in flour, a “strong” protein flour is not required as starting material in the HD process (Sayaslan 2004).

Similar to HD process, the Alfa-Laval/Raisio process begins with shear-mixing of wheat flour in water to form a flowable batter (Seib, 1994). But a two-phase centrifugal separation gives the dense stream containing mostly the A-type starch, and the light stream containing the remaining starch, gluten, pentosans, and water-solubles. The light stream is further processed to separate gluten, starch, and effluent.

Wheat Gluten Quality

Wheat gluten quality (strength) in flour can be assessed or screened by several methods, such as 1) resistance to dough mixing recorded on the Mixograph, 2) resistance to extension of dough on the Alveograph, 3) solvent retention by high molecular weight glutenins measured by lactic acid solvent-retention-capacity (SRC) and sodium dodecyl sulfate (SDS) sedimentation volume, and the 4) the Gluten Index obtained from Glutomatic instrument. (Kulkarni et al 1987, Gaines et al 2006).

Mixograms

The Mixograph is a useful tool for evaluating dough performance. A mixogram is the recorded output of the Mixograph. The mixogram from a modern Mixograph shows midline and envelope curves. The envelope curves define the top and bottom of the mixogram center (Hazelton and Walker 1997). The mixogram is evaluated to give peak times, heights, ascending and descending slopes (left and right of peak), band widths, and curve areas (representing work input) (Hazelton and Walker 1997).The ascending slope is associated with of the dough development rate and the descending slope in an indicator of the rate of dough breakdown (Hazelton and Walker 1997). As the dough develops, the mobility of dough decreases and the pull on the pins revolving through the dough increases (Finney and Shogren 1972). The point of minimum mobility is taken to be Mixograph mixing time (Finney and Shogren 1972). After the mixing peak is reached, the curve tails off to varying

degrees depending on the rate of mechanical breakdown of the gluten protein (Finney and Shogren 1972). A wide angle approaching the limit of 180^0 between the developing and weakening slopes indicates a greater mixing tolerance (Hazelton and Walker 1997).

Flour mixing properties with water are a function of protein quantity and quality (Finney and Shogren 1972). The rate of dough development (ascending slope and peak time), resistance of the dough to mixing (band width), and tolerance of the dough to over-mixing (range of stability) all relate to gluten characteristics (Pitz 1997). Therefore, flour protein content should be taken into consideration when evaluating flour quality by the mixogram. The Mixograph is also used to determine optimum water absorption of the flour. Flour absorption is a function of protein content, protein quality, variety, flour moisture, and growing environment (Finney and Shogren 1972) and probably amylose level in the starch. Water absorption influences dough stiffness and the work needed to mix the dough.

Baig and Hoseney (1977) varied Mixograph absorption over 6 percentage points (+3 to -3) from the optimum level for a series of flours with different mixing times and protein contents. The authors found that as absorption is increased, mixogram peak height decreased and time to peak generally increased for a given cultivar. Finney and Shogren (1972) also studied the effects of decreasing or increasing water absorption from the optimum level. In contrast with stiff doughs which showed wild swings on the Mixograph, slack doughs generally produced narrow mixograms with a characteristic "swayback" during hydrating and developing periods before reaching the peak (Swanson and Jonson 1943, Finney and Shogren 1972). The narrowness and the "swayback" extent of mixograms were a function of the degree of slackness (Finney 1997). A reasonable index of relative dough stability, elasticity, stickiness and mixing tolerance can be obtained from the mixing time in the Mixograph. Both very short and long mixing times are undesirable in a bread dough. A short mixing time indicates the dough is less stable, less elastic, and more extensible than an ideal dough, whereas a dough with a long mixing time increases

production costs and complicates scheduling in the bakery (Finney and Shogren 1972).

Gluten Isolation by Glutomatic

Gluten isolated from a flour is an index of the protein content in flour and its extent of agglomeration when mixed with water. The physical properties of isolated gluten provide an index of flour "strength". The Glutomatic gluten washing system is a mechanical device developed for improving the reproducibility of gluten recovery when washing a dough, since hand washing contributes personal factors due to variations in the gluten isolation process (Kulkarni 1987). This rapid and simple instrumental test--Gluten Index involves separating gluten from wheat flour using the Glutomatic instrument followed by centrifuging the wet gluten on a special sieve in the Glutomatic instrument (Fig1.4). The amount of wet gluten remaining on the centrifuge sieve divided by the total wet weight of gluten is the Gluten Index. Taylor and Randall (1994) investigated the relationship between Gluten Index and a wide range of commonly used direct and indirect measures of bread making quality on South African wheats. They suggested that the Gluten Index is a measure of gluten elasticity, and that wet total gluten yield is an indication of gluten viscosity or cohesiveness.

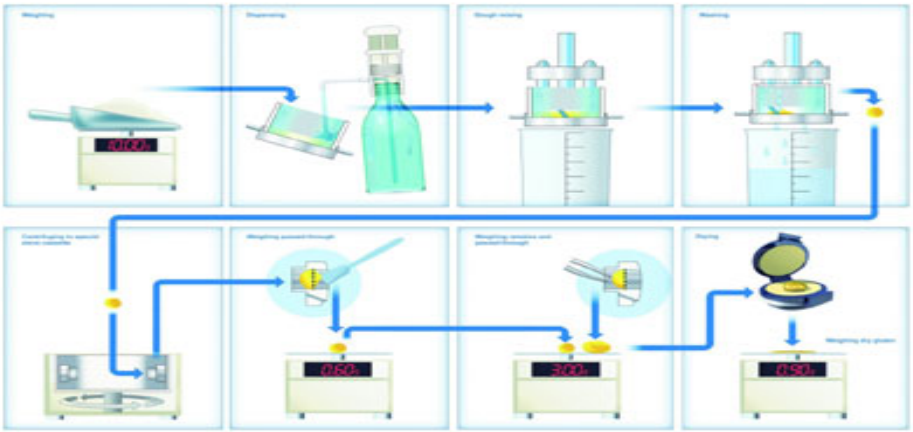


Figure 1.4 Schematic diagram of Glutomatic washing system (Accessed from http://www.perten.com/pages/ProductPage418.aspx?epslanguage=EN).

Development of Waxy Wheat Starch

Hexaploid wheat (*Triticum aestivum*) contains threes sets of chromosomes (A, B and D genomes) and each genome consists of seven pairs of homoeologous chromosomes. Therefore, the chromosomes can be divided into seven homoeologous groups because each chromosome pair is genetically similar to certain chromosome pairs of the two remaining genomes, which means the location and structure of genes from these homoeologous groups are identical (Graybosch 1998).

The granule-bound starch synthase I (GBSS I), also known as the "waxy protein" with a molecular weight of about 60 kDa, is the major enzyme associated with amylose biosynthesis in endosperm (Miura et al., 2000). The genetic loci containing the genes encoding for waxy (*wx*) proteins in hexaploid wheat were found on chromosome arms 7AS (*wx-A1*), 4AL (*wx-B1*) and 7DS (*wx-D1*) (Graybosch 1998; Miura et al 2000) (Fig 1.5). Common wheat lines with a non-functional mutation at any single *wx* locus would still exhibit mainly wild-type character because the GBSS would be produced by the remaining functional *wx* loci (Graybosch 2005). Reduced amylose wheats, termed "partial waxy", were produced as the result of the presence of one or two GBSS null alleles (Graybosch 1998). The possible combinations of *wx-A1*, *wx-B1*, and *wx-D1* produce eight types of isogenic lines differing in starch properties (Seib 2000) (Table1.2).

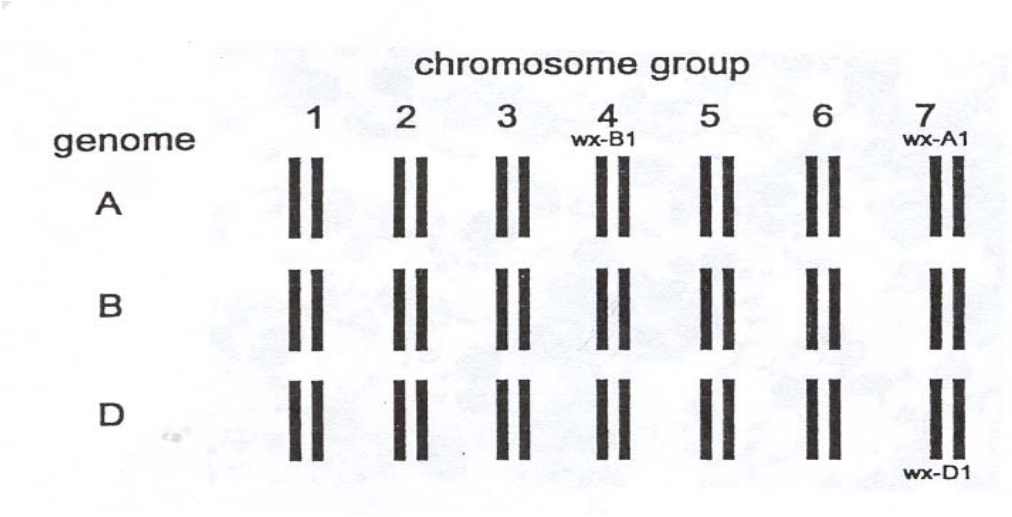


Figure 1.5 Organization of bread wheat chromosomes and location of waxy loci (Graybosch 1998).

Nakamura and collegues in Japan first separated these isozymes, *wx-A1*, *wx-B1*, and *wx-D1* in hexaploid wheat by two-dimensional gel electrophoresis (Seib 2000; Graybosch 2005) (Fig1.6). Subsequently, a number of wheat lines from 1,960 cultivars were assayed for *wx* proteins to determine null alleles (Seib 2000; Graybosch 1998). A high frequency of null mutations at *wx-A1* and *wx-B1* loci was detected among the wheats while the null mutation at *wx-D1* locus was rarely found except for two lines from China, Bai Huo and Bai Huo Mai. (Graybosch 2005).

Table 1.2 Granule-Bound Starch Synthase I (*wx-A1*, *wx-B1*, and *wx-D1* Proteins) and Amylose Levels in Isogenic Lines of Wheat (Miura et al 2002, Seib 2000).

Type	Wx-A1	Wx-B1	Wx-D1	Apparent Amylose(%)[a]	
				Near-Isogenic Lines	Isolines
Wild-type					
1	+	+	+	25	28
Single null					
2	--	+	+	23	27
3	+	--	+	22	26
4	+	+	--	23	27
Double null					
5	+	--	--	16	20
6	--	+	--	19	24
7	--	--	+	19	22
Waxy					
8	--	--	--	3	<1

(a) Apparent amylose content determined on lipid-extracted starch by iodimetric method.

In 1994, the world's first waxy wheat was produced by M. Yamamori and T. Nakamura in Japan using conventional breeding techniques by crossing two partial waxy wheats. Bai Huo from China, which lacks the *wx-D1* protein, was crossed with Kanto 107 from Japan, which lacks the *wx-A1* and *wx-B1* proteins (Seib 2000; Chibbar and Chakraborty, 2005). A small proportion of the progeny was waxy wheat carrying null alleles at all three *wx* loci, and its endosperm contained no detectable amylose.

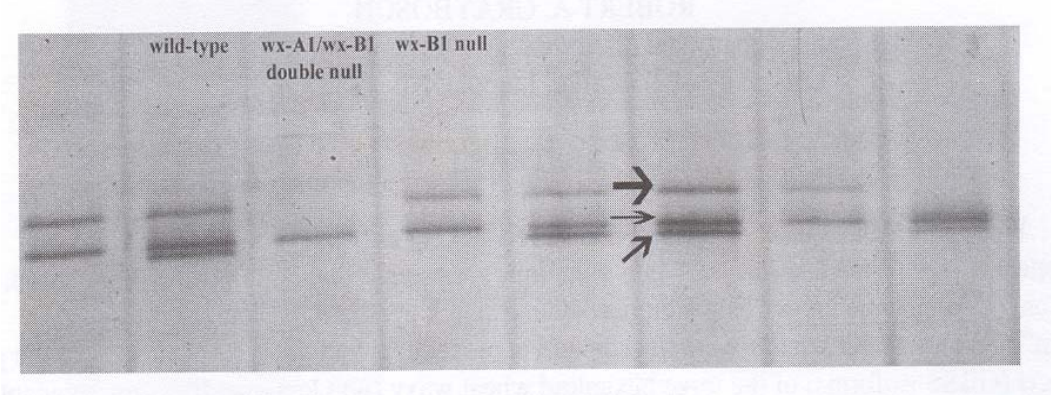


Figure 1.6 SDS-PAGE separation of granule-bound starch synthases of various wheat lines. GBSS wild-type isoforms arise from *wx-A1* locus (large arrow), *wx-D1* locus (small arrow) and *wx-B1* locus (medium arrow) (Graybosch 2005).

Wheat GBSS I and II are encoded by separate genes that are expressed in different tissues. In contrast with GBSS I expression which is high in endosperm tissue, GBSS II is found to be expressed in leaf, culm, and pericarp tissue (Vrinten and Nakamura 2000). In these years, genetic elimination of GBSS I alleles through conventional breeding and chemical mutagenesis of partial waxy wheat was applied to produce waxy wheat in North America and Japan (Reddy and Seib 2000; Chibbar and Chakraborty 2005). A new wheat, Penawawa-X, developed by Craig Morris, ARS Western Wheat Quality Laboratory, would be one of the first commercial soft white spring wheats with 100% amylopectin starch (Banasiak 2005)

Fractionation of Waxy Wheat

Dry-Milling of Waxy Wheat

Yasui and colleges (1999) reported that the waxy wheat from two Japanese waxy mutant lines had reduced flour flowability and gave about 20% lower flour yield than the non waxy parent, which was attributed to 20% higher grain fat and β-glucan contents. Lower flour yield also has been reported for waxy soft wheat and waxy spring wheat compared to their wild types (Kim et al 2003; Graybosch et al 2003). Bettge et al (2000) reported that waxy starch granules were less resistant to

mechanical damage than normal starch granules. The increased crystallinity in waxy starch (Chakraborty et al 2004) may cause more fragility to crushing forces. Subsequently, an increase in starch damage during milling was observed for a hard waxy wheat (Bettge et al 2000). Therefore, developing waxy wheat with softer kernels may help reduce the damaged starch content of waxy wheat flour. However, milling yield is usually higher for hard-textured wheat grain (Graybosch et al 2003).

Wet-Milling of Waxy Wheat Flour

Sayaslan et al (2006) investigated the wet-milling properties of waxy wheat flours by dough washing and by a dough-batter process on the laboratory bench. They found that the gluten proteins of waxy flours appeared to be weaker than those of nonwaxy wheats. Five of seven waxy flours failed in the dough-washing method as their doughs lost cohesiveness and elasticity during kneading and washing under water. Those waxy flours were instead wet-milled by a modified dough-washing method, where the doughs were gently hand-rubbed over a sieve with 125μm openings. The wet-milling properties of waxy flours by the dough-batter process were comparable to those of control flours. In the dough-batter process, most of the starch is removed before an impure gluten mass is formed during centrifugation. The components in waxy flour that cause stickiness of its dough may be in the water-solubles fraction.

Gluten Fraction

The gluten fraction isolated by wet-processing of wheat flour is enriched in gluten proteins (~80%, db) with the remainder being ash, lipids, and carbohydrate. The carbohydrates are mainly starch and a small amount of non-starch polysaccharides (Hoseney 1986; Van Der Borght et al 2005). Gluten proteins are the storage proteins of wheat. They are primarily responsible for the uniqueness of wheat flour, which is to be able to form a strong and cohesive dough that retains gas. And "vital" gluten is defined by its retention of the viscoelastic properties associated with wheat storage proteins that have not been denatured.

Gluten proteins are comprised of two main components: gliadin and glutenin, both of which are insoluble in water but water-swellable. Gliadin and glutenin can be easily separated by stirring in 70% ethanol because gliadins dissolve and glutenins do not. Gliadins are single polypeptide chains and have an average molecular weight of about 40,000 kDa (Hoseney 1986). They are sticky when hydrated and contribute to a dough's cohesiveness. Glutenins are composed of polypeptide (glutenin) subunits which are cross-linked by disulfide bonds. Glutenins vary in molecular weight from about 100,000 to several million (average MW of ~ 3 million) (Hoseney 1986) and their great size gives dough its resistance to extension and elastic recovery.

Characteristics of Waxy Wheat Starch

Waxy wheat starch contains little, if any, detectable amylase. It also has low lipid-binding capacity. Therefore, waxy wheat starch contains no detectable amylose-lipid inclusion complex, which was confirmed by x-ray diffraction studies (Abdel-Aal et al 2002). X-ray diffraction of waxy and normal wheat starches both show typical A-type diffraction patterns, but waxy wheat starch has somewhat higher cystallinity (Chakraborty et al 2004). The A-type pattern gives strong reflections at 2θ ~15° and 23°.

Yasui and coworkers (1996) reported that the waxy trait in wheat has little effect on the chain-length distribution of amylopectin molecules. Debranched waxy wheat starch shows a chain length (CL) distribution the same as that of the amylopectin from its non-waxy parents, both of which give a average CL of 22.

Starch swells and is gelatinized when it is heated with excess water (Sasaki et al 2000). Amylose leached from gelatinized starch reassociates in an ordered structure to form a viscoelastic gel during storage; that process is known as retrogradation (Sasaki 2005). This structural change effects eating quality in starch-based products (Sasaki 2005). The ratio of amylose and amylopectin has a major influence on the physicochemical properties of starch and food (Sasaki et al 2000, Sasaki 2005).

The gelatinization temperature of several rice starches in excess water correlated with in their degree of starch crystallinity, which is a property of their amylopectin (Shi and Seib 1992). Thermal transition temperatures of starches with various level of amylopectin have been measured by differential scanning calorimetry (DSC). Hayakawa et al (1997) reported that waxy wheat starch had a higher gelatinization temperature (T_o, T_p, and T_c) and enthalpy (ΔH) than normal wheat starch. Sasaki (2005) also reported that final gelatinization temperature and gelatinization enthalpy correlated negatively with amylose content because amylopectin is the component in starch associated with granule crystallinity. It is hypothesized that the presence of amylose in the amorphous phase of starch lowers the melting temperature of the crystalline regions of amylopectin as well as the energy (ΔH) for gelatinization.

Waxy wheat starch swells rapidly in hot water and reaches a much higher pasting peak viscosity at a lower temperature in the Rapid Visco Analyzer (RVA) than normal wheat starch, and gives a high breakdown and low setback viscosity (Lumdubwong and Seib 2001, Chakraborty et al 2004). Waxy wheat and waxy maize starch at ~10% concentration in water have similar pasting peaks, but waxy wheat starch forms a paste at a temperature of 10^0C lower than that of corn starch (Lumdubwong and Seib 2001).

Applications of Waxy Wheat Starch

The retrogradation of amylose in starch pastes and the shear loss of viscosity by amylopectin greatly restrict the uses of normal starches as thickening agents. Often native starches are modified chemically to correct these deficiencies. However, chemically modified starch is unpopular with sophisticated consumers and is restricted by legislation (Lillford and Morrison 1997). Waxy wheat starch lacks detectable amylose and provides broad diversity in its potential applications. The desire for novel kinds of starch that possesses versatile physical and chemical properties to meet the demands of markets and consumers has been achieved by altering the amylose/amylopectin ratio based on plant-breeding techniques, which

reduces the need for expensive and environmental-unfavored chemical treatments of starch (Miura et al 2002; Chibbar and Chakraborty 2005). Like normal starches, unmodified waxy starches are not usually used in food but often are cross-linked and substituted (Reddy and Seib 1998).

In baked products, the availability of waxy wheat provides an opportunity to study bread staling in an amylose-free environment without introducing the undesirable flavor and color factors associated with nonwheat cereals (Chibbar and Chakraborty 2004). Starch regrogradation is associated with bread crumb firming that occurs during bread staling. However, there are conflicting conclusions about the staling being caused by amylose or amylopectin (Krog et al 1989; Hug-Iten et al 1999; Chibbar and Chakraborty 2004), perhaps because of variation of moisture contents in baked foods. No direct relationship was found between bread aging and overall crystallinity of starch (Chibbar and Chakraborty 2004). Biliaderis (1992) stated that wheat starch retrogradation was a biphasic phenomenon, involving early complexing of amylose followed by a slow recrystallization of amylopection. Waxy wheat can be used to eliminate amylose from base flour, which may help us study the functionality of amylose and amylopectin in baked products. Waxy wheat starch could be useful in retaining higher moisture in bread crumb which helps retard staling and extends the shelf life of baked products (Graybosch 1998; Bhattacharya et al 2002; Chibbar and Chakraborty 2004). Studies showed that some amylose is essential to baked goods produced with present-day methods (Graybosch 1998). Bread made from waxy wheat flour resulted in an open-crumb structure with excessive shrinkage of the loaf upon cooling, which is caused by the lack of amylose and by excessive damaged starch (Lee et al 2001; Chibbar and Chakraborty 2004). Therefore, optimum amylose-to-amylopectin ratios need to be identified for a specific bakery product.

Another potential application of waxy wheat starch is as a fat replacer due to its slow retrogradation rate (Graybosch 1998; Bhattacharya et al 2002; Chibbar and Chakraborty 2004).

The use of waxy wheat starch could result in extruded puffed snacks with higher expansion, lower fat, and higher fiber contents. It also can be used in “easy-to-

cook" instant food products due to its rapid gelatinization at a relatively low temperature. Tortilla processing, textural characteristics and shelf stability could be improved by blending with 10-20% waxy wheat flour, which correlated with a 24-26% amylose content (Chibbar and Chakraborty 2004). If waxy wheat flour can be wet-processed successfully, the most likely application of waxy wheat starch is its chemical modification and substitution for chemically modified waxy corn starch not only in the food industry but also nonfood industries.

There has been much speculation about the use of fully waxy wheat flour in various food products which depend on the unique performance properties of amylopectin. However, many of its applications are still under investigation due to relatively limited understanding of the relationship between waxy starch structure and functional properties (Chibbar and Chakraborty 2004).

Objectives

The objectives of this study were to obtain samples of U.S. varieties of waxy wheat and advanced breeding lines, and (i) mill the wheats to flour, then determine the suitability to wet-mill the flours by the Martin dough-washing procedure, (ii) examine the factors affecting the wet-milling of waxy wheat flours, and (iii) study the basic structure and properties of waxy wheat starch.

CHAPTER 2 - Materials and Methods

Materials

Six advanced waxy hard wheats (NX03Y2114, NX03Y2115, NX03Y2205, NX03Y2315, NWX03Y2459, NX03Y2489) and two normal hard wheats (Karl, Trego) were provided by Dr. Robert Graybosch (USDA/ARS, University of Nebraska). The pedigrees for his 6 waxy wheat samples are: NX03Y2315--BaiHuoMai/Ike (97GC1015wx)//KSSB-3697/NE88584; NX03Y2114--Cimarron/RioBlanco//BaiHuo4/L910145/3/Colt/Cody//Stozher/NE86582; NX03Y2205--BaiHuo/Kanto107//Ike/4/KS831672/3/Rannaya12/Bez.4/2/Lancota/f9-67; NX03Y2489--BaiHuo/Kanto107//Ike/3/KS91H184/3*RBL//N87V106; NWX03Y2459--BaiHuoMai/Ike//KS91H184/3*RBL//N87V106; NX03Y2115--Cimarron/RioBlanco//BaiHuo4/L910145/3/Colt/Cody//Stozher/NE86582. A soft waxy wheat (Waxy-Pen waxy wheat) was obtained from Dr. Craig Morris (USDA, ARS, Western Wheat Quality Laboratory, Washington State University).

Wheat kernels were tempered to 15.5% moisture for 20h and roller-milled into straight-grade flour on a Buhler experimental mill (Buhler Co., Uzwil, Switzerland). The flour was stored in plastic bags at room temperature. Starch was isolated from the flour samples by the AACC gluten hand-washing method 38-10 with modification according to Sayaslan et al. (2002). The purified starch was oven dried at 40^{o}C for 2 days, gently ground with motor and pestle and stored in Ziploc bags. Waxy maize starch (Amioca) was obtained from National Starch (Batch NO. MD8230).

Hemicellulase (SEBake-X) was obtained from Specialty Enzymes and Biochemicals Co., Chino, CA. Isoamylase (EC 3.2.1.68) was obtained from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). Heat-stable α-amylase was obtained from Sigma Chemical Co., St. Louise, MO. Commercial vital wheat gluten (>72%) was obtained from MGP ingredients Inc., Atchison, KS. Phosphoryl chloride was obtained from Aldrich Chemical Co., Milwaukee, WI.

General Assays

Moisture, ash and lipid were determined and repeated in duplicates by American Association of Cereal Chemists (AACC International) Methods 44-15A, 08-01, 30-25, respectively (AACC, 2000). Protein contents (N x 5.7) were measured and repeated in duplicates by the combustion method on an FP protein/Nitrogen Analyzer (Leco Corp., St. Joseph, MI). Total starch was determined and repeated in duplicates by AACC Method 76-13 using an assay kit from Megazyme International Ltd. (Wicklow, Ireland). Single kernel characteristics of wheat samples were determined on the Single Kernel Characterization System (SKCS4100, Perten Instrument North America, Inc., Reno, NV).

Water Absorption Capacity

Saturated $MgCl_2 \cdot 6H_2O$, saturated NaCl solutions, and water were put into desiccators at 25^{o}C to obtain 32%, 75% and 100% relative humidity atmospheres, respectively (Karel and Lund 2003). Three grams (db) of kernels, flour and starch of waxy wheat and normal wheat were spead out in weight pans, placed in the desiccators, and the weight gained was measured with time. The flour and starch were milled or isolated from the same kernels placed in the desiccators. The water-absorption experiments were terminated when weight gain ceased (~4days). Each sample was repeated in duplicate.

Protein Fraction Analysis

Size-exclusion chromatography (SEC) of solubilized gluten was done through the courtesy of Dr. Scott Bean (Grain Marketing and Production Research Center, ARS-USDA, Manhattan, KS) Wheat proteins were extracted from flours with aqueous 50% 1-propanol and analyzed by SEC. The proteins not extracted were measured using nitrogen combustion.

Wet gluten remaining on the centrifuge sieve and those passing through the sieve on the Glutomatic instrument were collected separately. Isolated wheat gluten was extracted on an equal protein basis and then analyzed on SEC. The total peak

area, soluble polymeric protein (SPP) area, gliadin (GLI) area, and albumin-globulin (AG) area were obtained. Total protein for the isolated glutens was analyzed by using conbustion assay on the LECO.

Gluten Isolation by Glutomatic

Gluten Index of flour samples was determined and repeated in triplicate using the Glutomatic system (Perten Instruments AB) which comprises a gluten washer (Glutomatic 2200), a centrifuge (Centrifuge 2015), and a dryer (Glutork 2020). Flour (10.0 g± 0.01 g) and 2% sodium chloride solution (4.8 ml) was placed into the Glutomatic wash chamber fitted with an 88 micron polyester sieve. After gluten separation, the wet gluten pieces were subjected to centrifugation, which forced a fraction of the gluten through the sieve and into a specially designed sieve cassette. Gluten Index was calculated as the amount of wet gluten remaining on the centrifuge sieve divided by the total weight of wet gluten. The wet gluten was dried in the dryer to obtain dry gluten content and water binding of the wet gluten.

Mixograms

Mixograms of flours were determined by AACC Method 54-40A using a 10-g scale mixograph (National Manufacturing Co., Lincoln, NE). A model-dough system formula was balanced to produce constant protein content (db) from commercial wheat gluten and 7.3g starch content (db) for each test. The addition of water was adjusted to produce acceptable mixograms. Mixograms of waxy wheat and normal wheat flours were recorded at pH4 and pH6 with optimum amount of water which was determined previously. Mixograms of waxy wheat and normal wheat flours mixed with 2% sodium chloride solution at optimum absorption level also were recorded.

Dough Washing: Isolation of Wheat Starch and Gluten

Flour (100g, 14%mb) was put in a 200g bowl pin mixer (National Manufacture Co., Lincoln, NE) and mixed for 1min. Based on the absorption determined with the mixograph, flour was mixed with an optimal amount of water

until it reached peak time. In a separate experiment with NWX02Y2459, hemicellulase (0.5g) was first added to distilled water (65.8 ml), and then the enzyme solution mixed with flour. The formed dough was covered by laboratory wrap film and allowed to rest for 1 hour at room temperature. After mixing, a formed dough ball was gently hand-kneaded under a stream of water over a wire-mesh sieves with 125μm opening. Washing was continued until the water squeezed from the gluten ball was clear, which indicated the majority of the starch and soluble materials had been washed away. One drop of the wash water was transferred onto a microscope slide and covered by the a glass coverslip, then viewed by microscope under 40x magnification. The final wash water showed a small number of B-type granules of starch. The gluten fraction (~40g) was covered and stored at room temperature for 1h. Wet gluten was weighed and partially frozen in a freezer and then placed in a jar which was attached to a freeze-dryer. The gluten expanded approximately two-fold due to the reduced pressure, which created more surface area for drying. The remnants on the 75μm opening sieve were collected after being washed with 50mL water, while the material (starch "milk") passing through the 75μm opening sieve was centrifuged at 2,500 xg for 20min. The supernatant was carefully decanted and saved, and the upper gray layer which is the tailing fraction, was carefully removed with a spatula. The starch in the bottom layer was resuspended in 200mL water, and the mixture centrifuged at 2,500g for 20min. Once again the tailings were carefully removed with a spatula. The fibrous residues on the 75μm sieve and the tailings removed from the first and second centrifugation steps were combined and freeze-dried. The starch was weighed after oven-drying at 40^{o}C for 2 days. The dried starch was analyzed for moisture and protein after being gently ground with a motor and pestle. The starch yield was calculated based on the total starch in the flour and on the weight of flour.

Fourier Transform Horizontal Attenuated Total Reflectance (FT-HATR) Spectroscopy

Waxy wheat (NWX03Y2459) and normal wheat (Trego) flour were mixed into a dough on the Mixograph (10 gram scale) to peak time with optimal water. Dough was carefully removed and mounted onto a clean, dry ZnSe FT-HATR cell with minimal stress to the dough, and then scanned using an interferometer. After scanning, the FT-HATR sample cell was removed and cleaned with a fine damp cloth followed by a thorough cleaning with methanol (Spectranal, Sigma-Aldrich Corp., St. Louis, MO) to remove dough residue and allowed to dry. A Nicolet Nexus 870 FT-IR spectrometer (Thermo Electron Corp., 5225 Verona Rd., Madison, WI, 53711) equipped with a FT-HATR accessory (Thermo Electron Corp., 5225 Verona Rd., Madison, WI, 53711), ZnSe sample cell, mercury-cadmium-telluride (MCT/A) liquid nitrogen-cooled detector, and a KBr beam splitter, was used to record spectra at room temperature in the mid-IR range at 4000-700 cm^{-1}. A 128-scan co-added interferogram was collected for each dough sample at a resolution of 2 cm^{-1} using Thermo Electron's Omnic software (v7.3, Thermo Electron Corp., 5225 Verona Rd., Madison, WI, 53711)). The single beam spectrum of the dough was divided by the background single beam spectrum and then converted to an absorbance spectrum. Each spectrum was manually corrected with a linear baseline correction at five points (approximately 4000, 3990, 2500, 1880 and 700 cm^{-1}), followed by an Advanced ATR Correction algorithm that further corrected for the wavelength dependence of penetration depth and refractive index dispersion around the absorbance peak. Spectral data were processed to quantify the peak areas in the frequencies for protein secondary structure. In a separate experiment, 0.05 g hemicellulase was added and mixed into a dough. For Trego flour, a delayed peak time occurred when hemicellulase was added. Therefore, Trego flour was tested at two peak times when the enzyme was added. Spectral data was collected in triplicate.

Viscometry

The viscosity of a flour suspension (30% solid basis) was measured at varying spindle speeds using a rotating viscometer (Brookfield DV-II +PRO).The speed independence of viscosity, shear stress and shear rate was investigated over range from 0.5 to 5 rpm. When viscosity, shear stress and shear rate appeared stable, data was recorded.

Microscopy of Starch Granules with Iodine Staining

Starch (0.1g) was added to distilled water (10ml) and dispersed by using a vortex mixer. A small amount of high-vacuum grease (Dow Corning Corporation, Midland, MI.) was spread evenly as an extremely thin layer around the edge of a square-shaped coverslip. One drop of the starch suspension was transferred onto a slide and covered by the coverslip. The starch was viewed with an Olympus BX51 microscope (Olympus America Inc., Melville, NY) equipped with a STC200 hot stage. Images and photographs were captured under 40x magnification using SPOT Insight camera and SPOT 4.6. Windows software (Diagnostic Instrument Inc., Sterling Heights, MI, USA). The heating rate was set at 5°C/min and 25°C/min, and control temperature by 1/16 DIN temperature controller using Intect Wintemp as Windows software.

Contamination of waxy starch granules with normal starch granules was examined by iodine staining. Starch (30mg) was added to 15ml of distilled water, and in a separate beaker, 0.1N iodine solution (19g potassium iodine and 6.4g iodine crystals were dissolved in 500ml distilled water) was mixed with 85ml distilled water. Then the iodine mixture was rapidly transferred into the starch suspension. A drop of the stained starch mixture was placed on a microscope slide, covered by a coverslip, and the starch was viewed at 40x with the Olympus BX51 microscope (Olympus America Inc., Melville, NY) .

Pasting Curves by Rapid ViscoAnalyser (RVA)

Pasting properties of 1.96 g (db) starch in 26.0ml water (7% solid basis of 28g) were determined by the Rapid ViscoAnalyser (RVA) (Newport Scientific, Narrabeen, Australia). In a separate experiment for enzyme susceptibility, 25µl α-amylase solution (0.1ml α-amylase was diluted to 100ml distilled water) was added to each starch suspension. Each starch suspension was held at 50^{o}C for 1 min, heated at a rate of 6^{o}C/min to 95^{o}C, maintained for 5 min, and then cooled to 50^{o}C at a rate of 6^{o}C/min. Pasting curves and parameters including peak viscosity (PV), breakdown (PV-HPV) and set-back (CPV-HPV) were recorded on the instrument.

Pasting properties of cross-linked starch (6.90g, 8.05g and 9.20g, equal to 0.06, 0.07 and 0.08 wt % solid basis of 115g) were determined in a Visco/Amylo/Graph (C.W.Brabender Instruments Inc., Hackensack, NJ) by heating from 40^{o}C to 95^{o}C at a rate of 6.0^{o}C/min, holding the paste at 95^{o}C for 5min, and then cooling to 50^{o}C at 6.0^{o}C/min, and holding for 2min. The same temperature profile and the heating rate of 12^{o}C/min were both used for determining the pasting properties of normal wheat starch, waxy wheat starch and waxy maize starch (8.05g, db) in 106.95g water (7% solid basis of 115g). Viscoamylogram curves were recorded on the instrument.

Differential Scanning Calorimetry (DSC) of Starch/Water Mixtures

Thermal analysis of starch samples were performed in duplicate with a DSC instrument (PerkinElmer, Waltham, MA). Starch (15mg) was weighed with 45µL of water in a DSC aluminum pan (1:3 starch-to-water ratio), and the mixture was heated from 20^{0}C to 140 ^{0}C at a rate of 10^{0}C/min. The onset of gelatinization (T_o), temperature at the peak (T_p), temperature of completion (T_c), and enthalpy of gelatinization (ΔH) were obtained using Pyris Software (Version 7.0) supporting the DSC instrument. For retrogradation, samples were stored for one week at 4^{0}C and then rescanned.

Gel permeation chromatography (GPC) of Debranched Starches

Starch (20mg) was added to 10ml 0.01M acetate buffer in a 12ml glass vial with a micro-stir bar. The vial was placed in a boiling water bath on a stir plate for one hour. Isoamylase (50µl) was added to the solution after it was cooled to room temperature. The vial was placed at 50^0C in a water bath overnight, frozen by using a dry ice/acetone bath, and then freeze-dried. Debranched starch (4 mg, db) was dissolved in 4ml dimethyl sulfoxide (DMSO) solution by placing in a boiling water bath for 24 h with constant stirring. Each starch solution was filtered through a 45um filter (MILLEX AP 20 PREFILTER, Millipore, Carrigtwohill, Co. Cork, Ireland), and then the filtrate was injected into a PL – GPC 220 by an autosampler. The system is equipped with differential refractive index (DRI) detector and phynogel 00H-0646-KO, 00H-0644-KO, 00H-0642-KO columns (Phenomenex, Torrance, CA) connected in a series. The mobile phase in the column was DMSO with 5mM NaNO3 at a flow rate of 0.8 mL/ min. The column oven temperature was controlled at 80℃. A series of dextran standards (American Polymer Standards Corporation, Mentor Ohio) representing molecular weights of 6.30x106, 4.00x106, 2.60x106, 846x103, 348x103, 131x103, 85.0x103, 36.7x103, 18.5x103, 3.40x103 were used for comparing the retention time with the test samples for the molecular weight calculations. The electronic outputs of the DRI detectors were collected by GPC software (version. 3.0, Polymer Laboratories, A Varian, Inc. Company).

Cross-Linking Modification for Waxy Starches

Granular waxy wheat starch and waxy corn starch (Amioca) (30g, dry basis) were cross-linked in water (45mL) at 25^oC. Sodium sulphate (0.2g) was added to starch slurry followed by dropwise addition of a mixture of 1.5M sodium hydroxide to pH 11.5. Phosphoryl chloride (2.04µL, 6.12µL and 12.24µL, equal to 0.01, 0.03 and 0.06 wt % on dry starch basis) was added with stirring. After 60min, the slurry was adjusted to pH 5.5 with 1M hydrochloric acid, and the cross-linked starch was

isolated by centrifuging (2,500 xg for 20min), washing with water (100mL), and oven-drying at 30^{o}C for 2 days.

CHAPTER 3 - Results and Discussion

Wheat Kernel Properties

Single kernel characteristics of wheat kernels are listed in Table 3.1. Weights of individual waxy wheat kernels ranged from 25.6~30.3 mg, compared to 28.7 to 31.6 for normal wheat kernels. Diameters of the waxy wheats were close to those of normal wheats (~2.3mm). A variation was observed in terms of hardness index (62.82~76.23).

Flour yields of wheat kennels are listed in Table 3.2. Flour yields of waxy hard wheat ranged from 68.8~73.3% compared to 70.8% to 72.5% for normal hard wheat. The same flour yield was obtained from waxy hard wheat as from normal hard wheat by roller-milling on a Buhler experimental mill, but the feed rate of waxy wheat kernels must be reduced or the waxy flour would clog the system during dry milling (S. Garimella, personal communication, 2007).

Table 3.1 Single Kernel Characteristics of Wheat Kernels Determined on the Single Kernel Characterization System.

Wheat Kernel	Weight (mg)		Diameter (mm)		Hardness Index		Moisture (%)	
	Ave	StD	**Ave**	StD	**Ave**	StD	**Ave**	StD
NX03Y2114	**27.6**	6.7	**2.29**	0.37	**66.7**	16.1	**9.45**	0.56
NX03Y2115	**29.0**	6.8	**2.37**	0.36	**62.8**	13.9	**9.36**	0.63
NX03Y2205	**25.6**	6.4	**2.22**	0.35	**73.0**	15.9	**9.13**	0.60
NX03Y2315	**30.3**	9.2	**2.38**	0.46	**72.5**	18.3	**9.55**	0.65
NWX02Y2459	**28.5**	7.3	**2.35**	0.37	**67.5**	15.4	**9.77**	0.45
NX03Y2489	**27.5**	7.2	**2.27**	0.39	**76.2**	16.4	**9.49**	0.57
Karl	**31.6**	7.3	**2.43**	0.35	**64.8**	15.8	**8.27**	0.98
Trego	**28.7**	8.0	**2.31**	0.45	**75.9**	17.5	**9.51**	0.75

Table 3.2 Flour Yields of Waxy Hard Wheat and Normal Hard Wheat by Dry Milling.

Wheat	Flour Yield (%)
NX03Y2114	71.0
NX03Y2115	73.3
NX03Y2205	69.3
NX03Y2315	68.8
NWX02Y2459	73.0
NX03Y2489	71.4
Karl	70.8
Trego	72.5

Water Absorption Capacities of Wheat Kernels, Flours and Starches

At 100% relative humidity and 25^0C waxy soft wheat kernels absorbed more water than waxy hard wheat, normal soft wheat and normal hard wheat. (Table 3.3). At 32% humidity, normal hard wheat and soft wheat kernels lost moisture, whereas both waxy wheats gained ~1.5% moisture in 7 days.

Table 3.3 Water Absorption (%) of Wheat Kernels under 100% and 32% Relaive Humidity Atmospheres at 25^0C [a].

Humidity	Time ,d	Normal Hard		Normal Soft		Waxy Hard		Waxy Soft	
		Ave	StD	Ave	StD	Ave	StD	Ave	StD
100%	0	**9.80**	0.75	**12.7**	0.89	**7.80**	0.78	**7.70**	0.82
	1	**14.9**	0.12	**16.2**	0.15	**13.4**	0.13	**15.0**	0.09
	2	**18.3**	0.07	**18.7**	0.10	**17.6**	0.10	**18.9**	0.09
	3	**20.8**	0.08	**21.0**	0.09	**20.6**	0.07	**22.0**	0.07
	4	**21.4**	0.00	**22.1**	0.07	**22.2**	0.00	**23.4**	0.00
	5	**22.2**	0.00	**23.0**	0.00	**23.4**	0.00	**24.6**	0.00
	6	**22.8**	0.00	**23.6**	0.00	**24.1**	0.00	**25.7**	0.06
	7	**23.5**	0.00	**24.1**	0.00	**25.9**	0.07	**26.3**	0.00
32%	0	**9.80**	0.75	**12.7**	0.89	**7.80**	0.78	**7.70**	0.82
	1	**9.80**	0.06	**11.3**	0.09	**8.42**	0.70	**8.55**	0.12
	2	**9.80**	0.00	**11.0**	0.07	**8.72**	0.00	**8.85**	0.00
	3	**9.80**	0.00	**11.0**	0.00	**9.02**	0.00	**9.15**	0.00
	4	**9.80**	0.00	**10.9**	0.00	**9.17**	0.00	**9.15**	0.00
	5	**9.80**	0.00	**10.4**	0.00	**9.17**	0.00	**9.30**	0.00
	6	**9.80**	0.00	**10.4**	0.00	**9.17**	0.00	**9.30**	0.00
	7	**9.70**	0.06	**10.4**	0.00	**9.31**	0.08	**9.30**	0.00

[a] Normal hard wheat – Karl; Normal soft wheat – 03' Yuma;

Waxy hard wheat – NWX02Y4529; Waxy soft wheat – Waxy-Pen.

Table 3.4 Water Absorption (%) of Wheat Flours under 100%, 75% and 32% Relative Humidity Atmospheres.

Humidity	Time, d	Normal Hard		Normal Soft		Waxy Hard		Waxy Soft	
		Ave	StD	Ave	StD	Ave	StD	Ave	StD
100%	0	**12.0**	0.65	**13.3**	0.78	**11.3**	1.25	**10.7**	0.77
	1	**20.0**	0.12	**23.5**	0.13	**21.8**	0.14	**21.2**	0.15
	2	**24.6**	0.09	**25.7**	0.06	**26.1**	0.12	**23.4**	0.09
	3	**24.6**	0.00	**27.8**	0.07	**28.1**	0.00	**25.6**	0.00
75%	0	**12.0**	0.65	**13.3**	0.78	**11.3**	1.25	**10.7**	0.77
	1	**14.8**	0.00	**16.1**	0.00	**16.9**	0.00	**13.5**	0.00
	2	**14.8**	0.00	**16.1**	0.00	**16.9**	0.00	**13.5**	0.00
	3	**14.8**	0.00	**18.7**	0.07	**16.9**	0.00	**13.5**	0.00
32%	0	**12.0**	0.65	**13.3**	0.78	**11.3**	1.25	**10.7**	0.77
	1	**12.0**	0.00	**13.3**	0.00	**11.3**	0.00	**10.7**	0.00
	2	**12.0**	0.00	**13.3**	0.12	**11.3**	0.00	**10.7**	0.00
	3	**12.0**	0.00	**13.3**	0.00	**11.3**	0.00	**10.7**	0.00

At 100% humidity, waxy hard wheat flour and normal soft wheat flour showed greater absorption capacities compared to waxy soft and normal hard wheat flours (Table 3.4), but at75% humidity, the order of absorption was normal soft >waxy hard >normal hard > waxy soft. At 32% humidity, none of the flours changed moisture content (Table 3.4)

Baik and Lee (2003) reported that water retention capacity of waxy wheat starches (80-81%) after soaking in water was much higher than that of regular wheat starch (55-62%). At 100% humidity, waxy hard wheat starch has a higher absorption than that of normal hard, but it has approximately the same absorption after three days at 75% humidity. At 32% humidity, both waxy hard and normal hard wheat starches had nearly identical moisture contents which remained constant for 1~3 days (Table 3.5).

Table 3.5 Water Absorption (%) of Wheat Starches under 100%, 75% and 32% Relative Humidity Atmospheres.

Humidity	Time, d	Normal Hard		Waxy Hard	
		Ave	StD	Ave	StD
100%	0	**9.00**	1.23	**8.70**	1.35
	1	**19.7**	0.07	**21.7**	0.14
	2	**22.0**	0.14	**23.9**	0.08
	3	**24.2**	0.00	**25.9**	0.00
75%	0	**9.00**	1.23	**8.70**	1.35
	1	**14.7**	0.12	**14.4**	0.00
	2	**14.7**	0.00	**14.4**	0.00
	3	**17.3**	0.00	**14.4**	0.00
32%	0	**9.00**	1.23	**8.70**	1.35
	1	**9.00**	0.00	**8.70**	0.00
	2	**9.00**	0.00	**8.70**	0.00
	3	**9.00**	0.00	**8.70**	0.00

Composition and Gluten Indexes of Wheat Flours

Flour composition is listed in Table 3.6. The protein contents of the waxy flours ranged from 12.00%~15.10% (db) compared to 14.04% to 15.47% for the normal flours. Starch in the flours ranged from 77-82%, and ash from 0.49-0.67%. Free lipids, extracted from flour with hexane or ethyl ether, were elevated 40% in the two samples of waxy wheat flours (Table 3.6).

The level of gliadins in the flours, based on total protein content, ranged between 31-44%, and insoluble polymeric protein (IPP) between 36-55% (Table 3.7). Three of the waxy wheat flours (NX03Y2315, NWX02Y2459, NX03Y2489), contained 41-46% IPP, which indicates weak gluten characteristics, but the three other waxy wheat flours contained 49-55% IPP indicating strong gluten character.

Hard-winter waxy wheat flours generally had lower gluten indeces (Table 3.8) than normal hard-winter wheat flours because they had less elasticity. Waxy wheat flours gave less reproducible results, especially NWX02Y2459, because doughs were sticky and their proteins had poor aggregation properties. Although NWX02Y2459 had high protein content, it had the lowest insoluble polymer protein level and a high free-lipid content (Table 3.6 and Table 3.7).

Table 3.6 Composition of Waxy and Normal (Karl and Trego) Wheat Flours

Wheat	Level[a], %				
Flour	Moisture	Protein	Starch	Ash	Free-Lipid
NX03Y2114	12.40	13.88	75.0	0.50	0.34
NX03Y2115	12.02	13.78	81.7	0.52	0.49
NX03Y2205	11.82	15.10	78.3	0.49	0.48
NX03Y2315	11.63	12.00	81.7	0.58	0.41
NWX02Y2459	12.03	13.33	78.3	0.57	0.66
NX03Y2489	11.70	12.82	80.0	0.64	0.68
Karl	13.20	15.47	76.7	0.67	0.40
Trego	12.47	14.04	76.7	0.54	0.49

[a] Moisture level on wet basis, but other levels on a dry-weight basis.

Table 3.7 Protein Fractions in Waxy and Normal Wheat Flours [a]

Wheat	Protein Fraction [b], % of protein			
Flour	Gli	Alb/Glob	SPP	IPP
NX03Y2114	32.1	4.0	12.3	52.6
NX03Y2115	34.5	4.5	12.5	48.1
NX03Y2205	31.3	3.5	11.7	54.8
NX03Y2315	41.8	4.8	9.2	43.3
NWX02Y2459	38.7	4.9	13.1	40.9
NX03Y2489	37.1	4.7	12.4	45.6
Karl	36.4	3.8	11.6	47.2
Trego	43.6	5.0	11.7	35.7

[a] Data obtained from Dr. Scott Bean.

[b] Gli — Gliadin, Alb/Glob — Albumin and Globulin, SPP — Soluble polymeric protein, IPP— Insoluble polymeric protein; % protein (14% mb)

Table 3.8 Gluten Index of Waxy Hard Wheat and Normal Hard Wheat Flours Determined on Glutomatic 2200 System.

Flour		NX03Y 2114	NX03Y 2115	NX03Y 2205	NX03Y 2315	NWX02Y 2459	NX03Y 2489	Karl	Trego
Gluten Index (%)	**Avg.**	**77.1**	**83.9**	**71.0**	**45.8**	**53.9**	**79.5**	**94.4**	**95.6**
	St. Dev	2.55	3.38	1.3	--*	7.62	1.42	0.17	0.35

***** Sample failed to have triplicate tests.

Two of the waxy wheat flours (NX03Y2315 and NWX02Y2459) had gluten indexes (~46 and 54) that were 41~49 units lower than the average (~95) of the two

control flours of normal wheats (Table 3.8). Three other waxy wheat flours (NX03Y2114, NX03Y2115, NX03Y2205) were 11~18 units lower, and the NX03Y2489 was intermediately lower by ~24 units. The value of the gluten indexes among the waxy wheat flours corresponded to a flour's insoluble polymeric property, because insoluble polymeric proteins are more likely to remain atop the filter screen during centrifugation. However, the control flours had intermediate levels of insoluble polymeric protein, yet they had the highest gluten indexes, indicating the formation of more insoluble polymeric protein during dough mixing.

Isolated wheat gluten was extracted on an equal protein basis and then analyzed on SEC. Since these samples were all extracted on an equal protein basis, the more total SEC peak area, the more soluble protein there was. However, the samples were similar in terms of their SPP, GLI, and AG contents. Total SEC areas were all similar, which suggests that the molecular weight distribution of the isolated glutens were not different.

Table 3.9 Isolated Waxy and Normal Wheat Gluten Analysis[a]

Wheat Gluten		Total Area	SPP[b] %	GLI %	AG %	Total Protein[b] %
NX03Y2114	**t[c]**	$1.22x10^5$	26.3	68.4	5.3	64.8
	o	$1.29 x10^5$	27.4	67.7	4.9	72.2
NX03Y2115	**t**	$1.29 x10^5$	29.2	65.1	5.7	46.8
	o	$1.30 x10^5$	30.4	64.7	4.9	77.2
NX03Y2205	**t**	$1.07 x10^5$	23.0	71.6	5.4	79.1
	o	$1.12 x10^5$	23.8	71.0	5.2	80.8
NX03Y2315	**t**	$1.18 x10^5$	20.8	73.6	5.6	51.2
	o	$1.19 x10^5$	21.0	73.3	5.7	45.3
NWX02Y2459	**t**	$1.28 x10^5$	30.2	65.2	4.6	78.9
	o	$1.28 x10^5$	29.6	65.8	4.6	77.3
NX03Y2489	**t**	$2.33 x10^5$	32.0	62.4	5.7	29.0
	o					76.3
Karl	**t**					75.3
	o	$1.17 x10^5$	24.3	70.5	5.1	77.6
Trego	**t**					75.4
	o	$1.31 x10^5$	28.2	66.7	5.1	77.8

[a] Data obtained from Dr. Scott Bean.

[b] SPP — Soluble Polymeric Protein, GLI — Gliadin, AG — Albumin and Globulin; Total Protein in "t" or "o" fractions by combustion assay.[c] t — The fraction passed through the sieves, o — The fraction remaining over the sieve

Mixograms

Mixograms of Wheat Flours

Mixograms are used to indicate the mixing quality of bread flours, which is an indirect measure of gluten quality. The strength of gluten in a dough is related to the spread between the high and low traces on a mixogram curve determined at optimum absorption. The greater the spread, the stronger the gluten. In addition, the lower the slope of the curve ("breakdown") following the mixing peak, the stronger the gluten.

Water absorption is crucial when recording a mixogram since it influences dough stiffness and the work needed to mix the dough (Baig and Hoseney 1977). Optimum water absorption was predicted (Fig 3.1) determined experimentally (Fig 3.2) for each wheat flour tested on the Mixograph. The mixogram of waxy wheat NWX02Y2459 in Fig 3.2 showed weak gluten as indicated by a short mixing time (2.48 min), narrow mixing curve, and large breakdown. This mixograph data agreed with the low gluten index and low insoluble polymeric protein of that waxy wheat. On the other hand, mixograms of NX03Y2114, NX03Y2115 and NX03Y2205 showed an increased mixing time (3.33~4.22 min), broad mixing curve and limited dough breakdown, similar to the mixograms of the control flour of Karl and Trego (mixing time 4.7~4.8 min). The mixogram of waxy flour NX03Y 2315 showed good strength of its gluten during early mixing, but considerable weakening appeared upon overmixing. The insoluble polymeric protein and gluten index of NX03Y 2315 were both low.

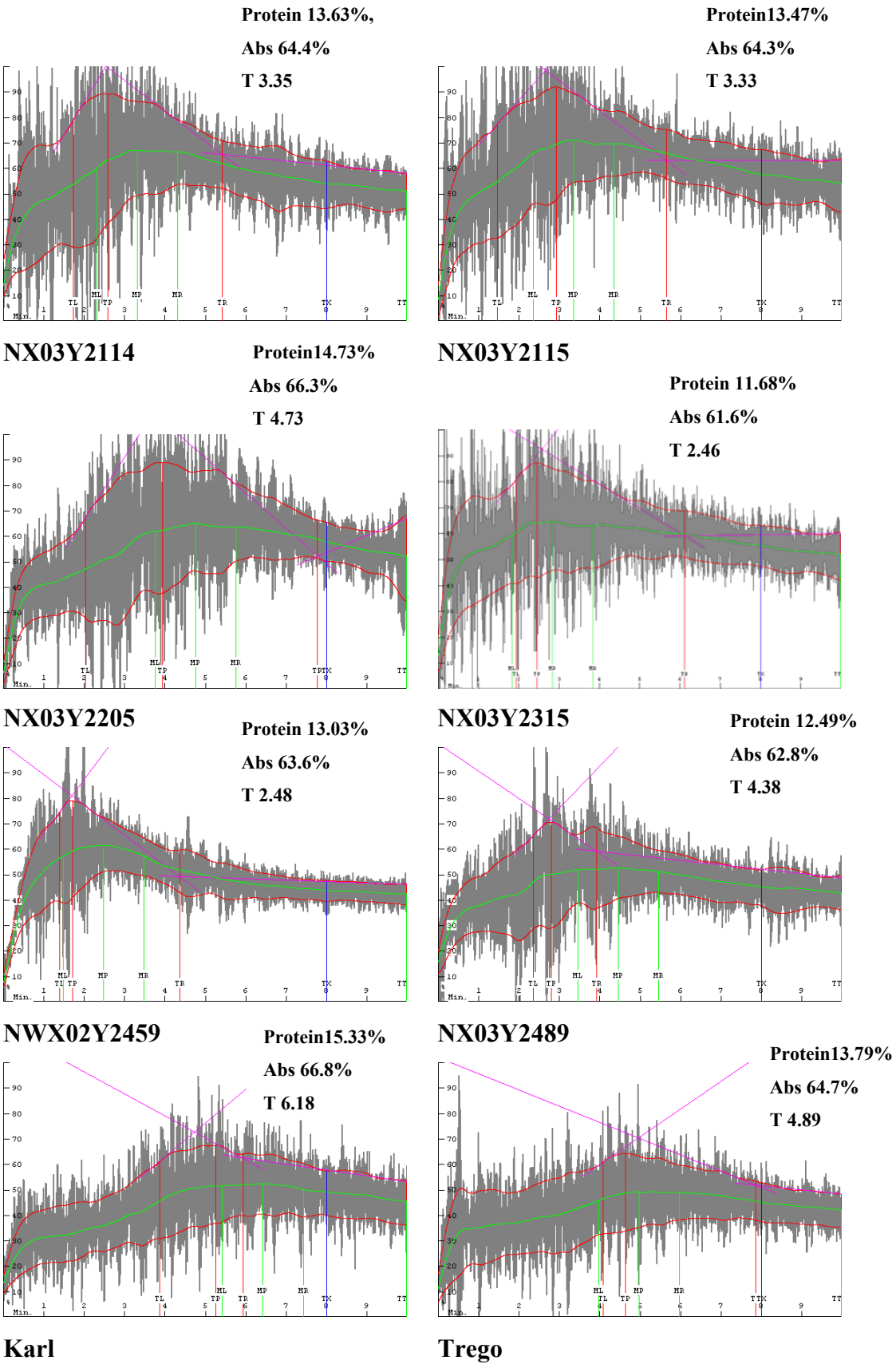


Figure 3.1 Mixograms of waxy wheat and normal wheat flours determined at predicted optimum water absorption levels. Protein contents of flours given on 14 % mb. T=mixing time.

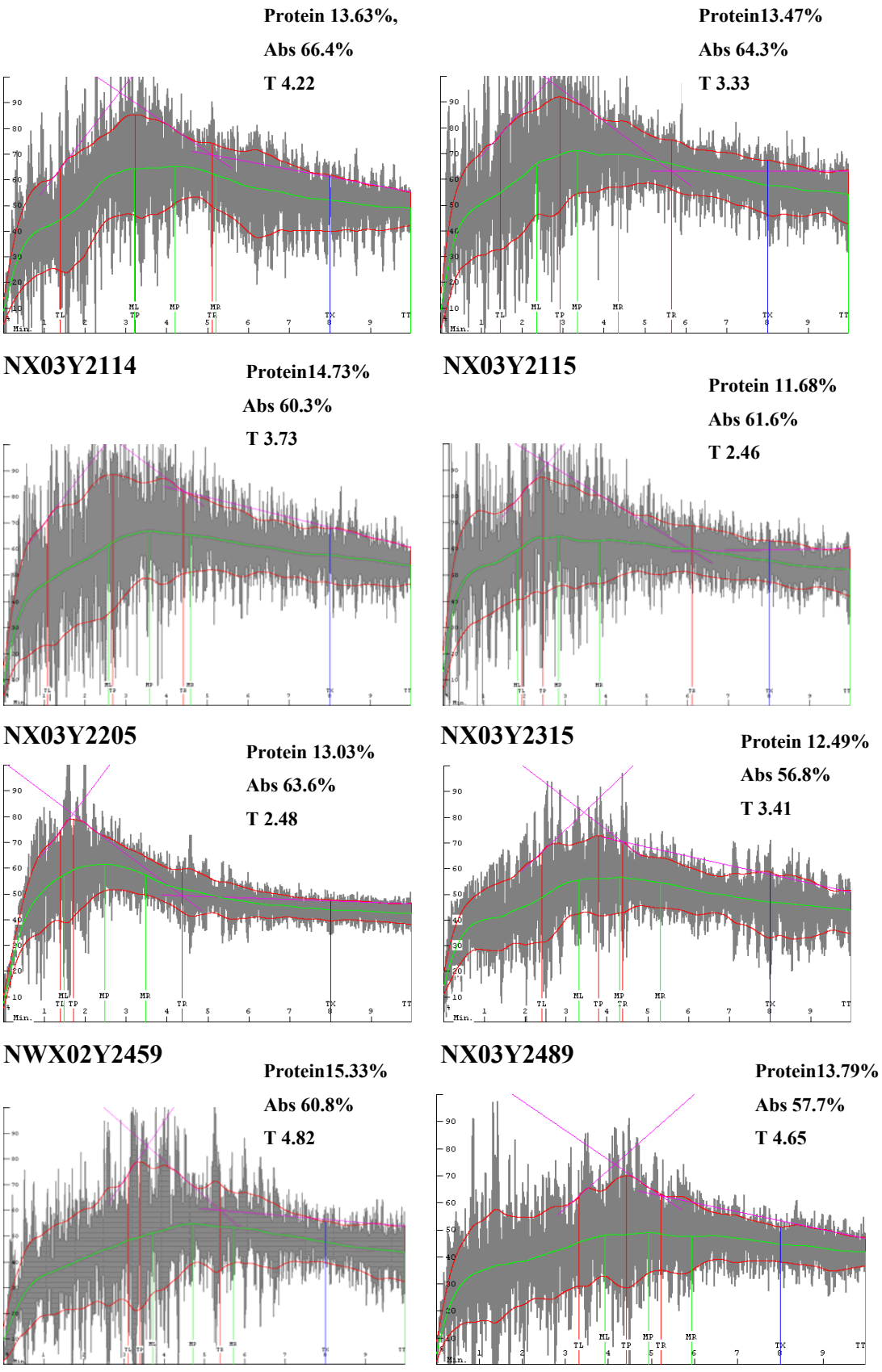


Figure 3.2 Mixograms of waxy wheat and normal wheat flours determined at optimum water absorption level. Protein contents of flours given on 14 % mb.

Effect of pH on Mixogram

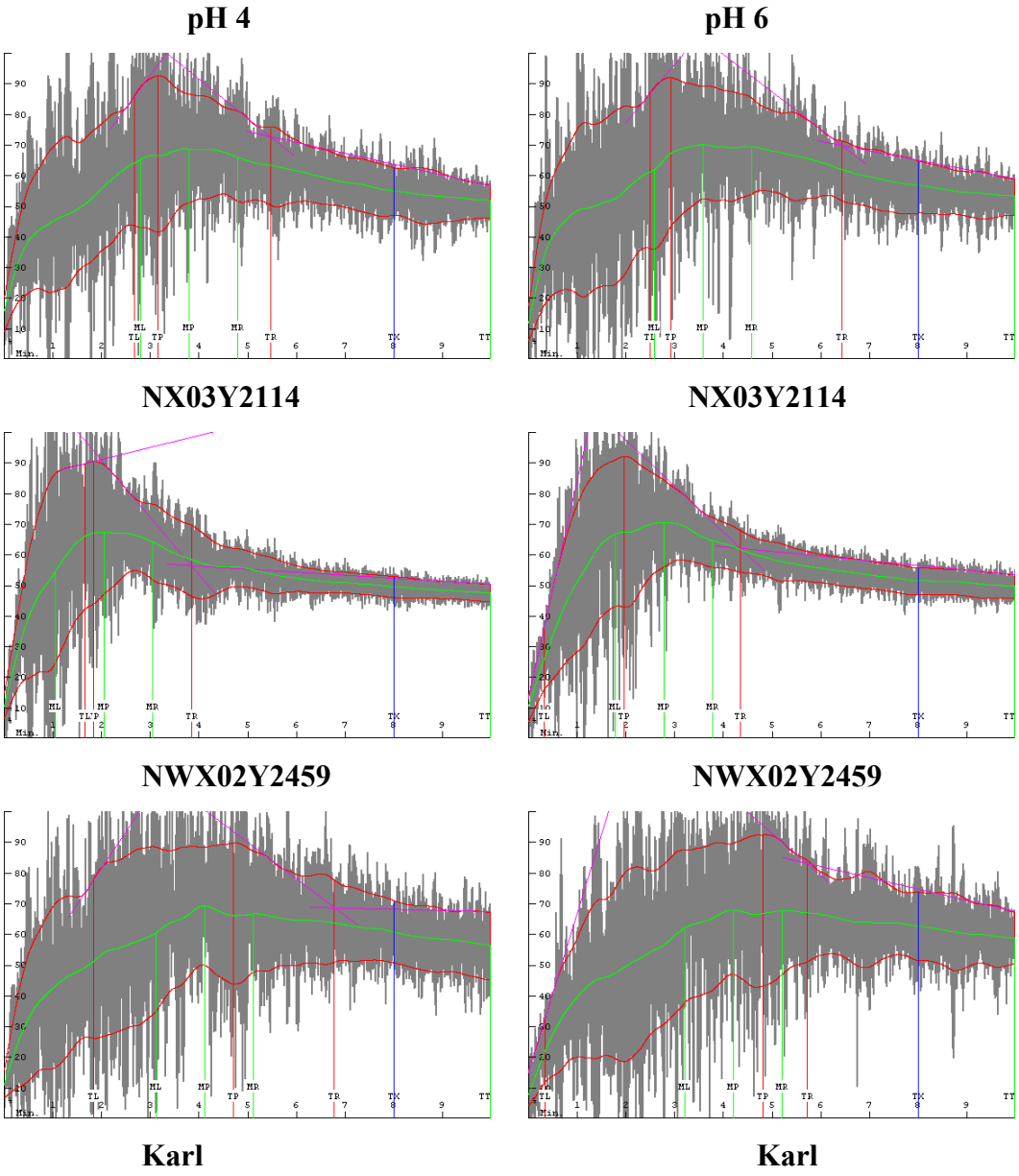
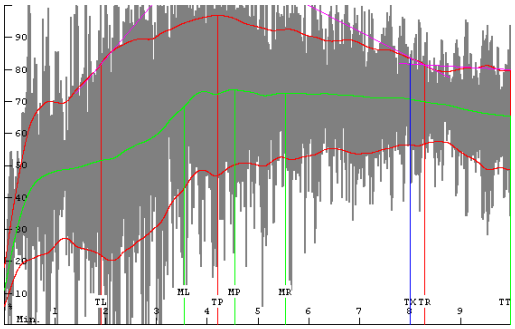


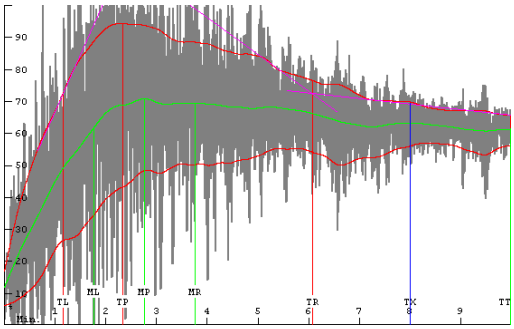
Figure 3.3 Mixograms of waxy wheat and normal wheat flours mixed with pH 4 and pH 6 water at optimum absorption level.

Mixograms of waxy wheat and normal wheat flours were recorded at pH4 and pH6 with optimum amount of water which was determined previously. Not much of effect of pH on the mixograms was observed.

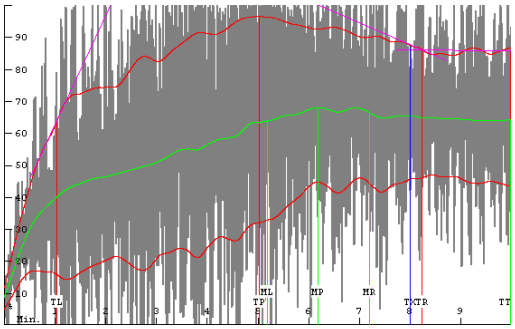
Effect on mixogram of Using Salt Solution



NX03Y2114



NWX02Y2459

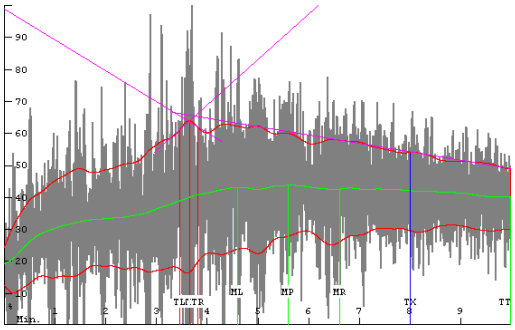


Karl

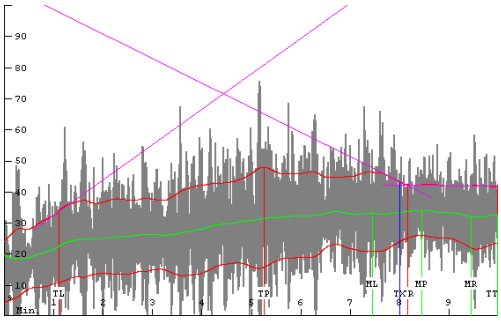
Figure 3.4 Mixograms of waxy wheat and normal wheat flours mixed with 2% NaCl solution at optimum absorption level.

The mixogram of waxy wheat and normal wheat flours mixed with 2% sodium chloride solution showed longer mixing time, broader mixing curve and significantly reduced breakdown compared with those flours mixed with water. However mixing a hard waxy wheat flour with 2% sodium chloride solution did not improve the cohesiveness of the dough during dough washing to isolate starch, although the waxy wheat flour dough was less sticky on its surface.

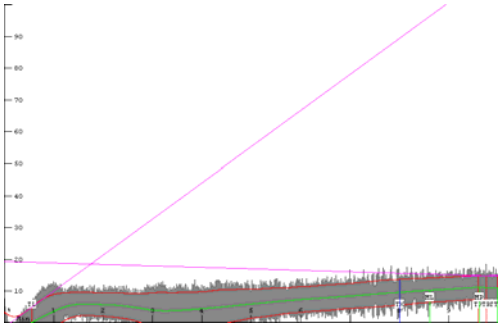
Blends of Vital Wheat Gluten with Starches



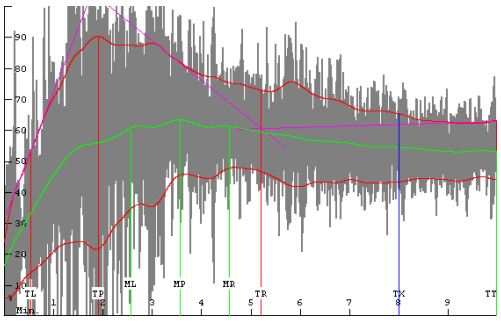
Karl +6ml H_2O



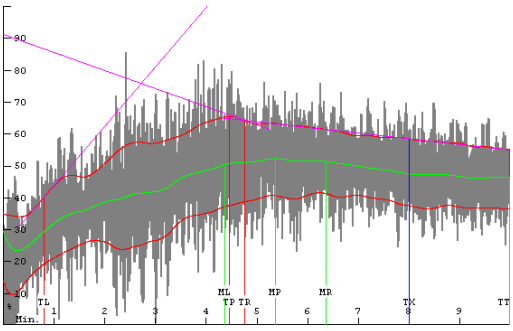
Karl +6.8ml H_2O



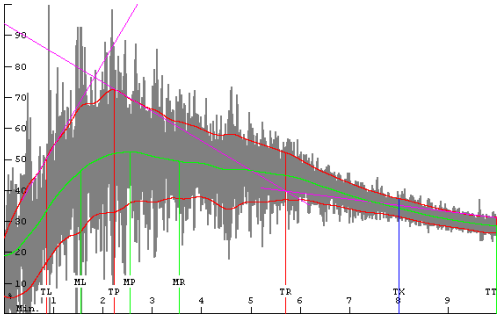
Karl +7.5ml H_2O



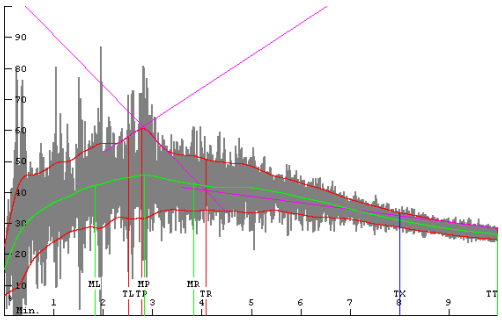
NWX02Y2459+6.8ml H_2O



NWX02Y2459+7.5ml H_2O



NX03Y2114+6.8ml H_2O



NX03Y2114+7.5ml H_2O

Figure 3.5 Effect of starch on mixogram absorption. Mixograms measured on blends of 7.3g (db) of various starches with 1.8g commercial vital wheat gluten (68% protein).

Waxy starch absorbs more water than normal starch as indicated by a mixograph experiment (Fig 3.5). A blend of 7.3g (db) of normal starch from Karl wheat with 1.8g commercial vital wheat gluten at ~66% absorption failed to give a developed curve. In comparison, the same blend with waxy wheat starch (NWX02Y2459 + 7.5mL H_2O or NWX03Y2114 +7.5mL H_2O) (Fig 3.5) at the same absorption gave a developed curve. At the lower water content (~51%), the blend of normal wheat starch and wheat gluten developed a dough and gave a mixogram curve but the blend of waxy wheat starches and wheat gluten did not form a cohesive dough but remained mostly a moistened flour. It was probably due to waxy wheat starch had bigger water absorption capacity which competed with wheat gluten. Therefore, there was too little water for wheat gluten to hydrate and to form protein network when the blend was mixed at lower water content.

In water absorption experiment, wheat starch (10g, db) and 100mL H_2O were placed into a 200ml centrifuge tube, mixed and rested at room temperature for 1h. After centrifugation at 2,500xg for 15min, the supernatant was decanted and the wet weight of the starch was determined.

Waxy wheat starches (NX03Y2114 and NWX02Y2459) absorbed 106% and 119% water (w/w), respectively, whereas normal wheat starch (Karl) gained 98%, indicating that waxy starch absorbs more water than normal starch (Table 3.10). These results suggest that the difference in water absorption between waxy wheat starch and normal wheat starch may affect the water distribution between starch and wheat gluten, which in turn affects the hydration and gluten development in the mixograms of the flours.

Table 3.10 Water Retention Capacities of Waxy Wheat Starches (NX03Y2114 and NWX02Y2459) and Normal Wheat Starch (Karl).

Wheat Starch	Wet Weight (g)	Weight Gained (%)
NX03Y2114	20.62	106.2%
NWX02Y2459	21.92	119.2%
Karl	19.84	98.4%

Starch Isolation by Dough Washing

Based on absorption-optimized mixograms, optimal absorption was used when evaluating the wet-milling performance of waxy wheat flours by the dough washing procedure. Wet gluten, prime starch, tailings, and water solubles were measured for waxy wheat and normal wheat flours, as well as the protein content of the gluten and starch fractions (Table 3.11). Compared to the control doughs from Karl or Trego wheat, washing the waxy wheat doughs under a stream of water caused a dough to become slack, to spread out more on the sieve and to break apart into several pieces (Fig 3.6). However, the slack dough pieces did not block the screen and when approximately two-third of starch had been washed away, the small dough pieces coalesced into one elastic dough that behaved like the controls. These results suggested the advanced lines of hard waxy wheats can be wet-processed in commercial operations. The dough of the waxy wheat flour NWX02Y2459 was especially weak and sticky during the early stages of dough washing, and gave relatively poor gluten and starch recoveries with low purity (Table 3.11). Adding hemicellulase to the dough of waxy wheat flour NWX02Y2459 improved gluten recovery, but not starch recovery. On the other hand, waxy wheat flours NX03Y2114, NX03Y2205 and NX03Y2315 gave 85~88% recovery of protein in their gluten fractions and 58~67% recovery of starches, and each starch fraction was contaminated with just 0.16~0.17% protein (Table 3.11). The total recovery of solids in the wet-milling of a flour ranged from 94-101%, except for waxy flour NWX02Y2459, where 89% solids recovery was found (Table 3.11).

Figure 3.6 The doughs from waxy wheat (NX03Y2114) and normal wheat washed under a stream of water (600ml).

Table 3.11 Wet-Milling Data for Wheat Flours by Dough-Washing Method on 100g (14%mb) of Flour

Wheat Flour	Flour Protein (g)	Flour Starch (g)	Wet Gluten (g)	Dry Gluten Fraction			Starch Fraction				Soluble Solids (g)	Tailings (g)	Total Solids Recovery (g) (%)
				Wt (g)	Protein Content (%)	Recovery (%)	Wt (g)	Protein Content (%)	MC (%)	Recovery (%)			
NX03Y2114	13.6	73.7	44.9	14.1	82.2	85.2	51.4	0.16	4.7	66.5	9.4	11.5	84 (97)
NX03Y2115	13.5	79.9	48.8	15.2	71.3	80.3	44.3	0.16	11.2	58.2	12.7	15.2	82 (96)
NX03Y2205	14.7	76.4	43.9	16.3	79.6	88.3	46.8	0.21	5.3	58.0	11.9	12.3	85 (99)
NX03Y2315	11.7	79.5	39.7	13.1	75.8	84.9	46.4	0.17	12.3	66.0	17.0	15.4	86 (101)
NWX02Y2459	13.0	76.5	48.0	15.0	66.8	77.1	46.1	0.05	9.3	56.4	10.0	9.4	76 (89)
NX03Y2489	12.5	77.9	37.3	13.3	78.0	83.0	48.6	0.05	4.6	59.5	10.9	13.0	83 (97)
Karl	15.3	76.0	43.7	13.8	85.8	77.4	52.4	0.27	4.8	65.6	6.0	10.9	81 (94)
Trego	13.8	75.4	33.8	10.8	82.3	64.4	59.0	0.14	6.4	73.2	8.7	10.6	86 (100)
NWX02Y2459+0.5gEn	13.0	76.5	50.5	17.2	67.0	88.6	45.3	0.04	5.9	55.7	11.0	10.9	82 (95)
NWX02Y2459+2%NaCl	13.0	76.5	49.6	16.3	72.4	90.0	60.0	0.33	6.5	73.3	9.57	4.0	86(100)

Effects of Hemicelluase on Rheology of Waxy Wheat Flour Dough

Adding hemicellulase to the relatively poorly performing waxy wheat flour (NWX02Y2459) reduced the dough stickiness during the early stage of washing and therefore improved gluten recovery but not starch (see Table 3.10).

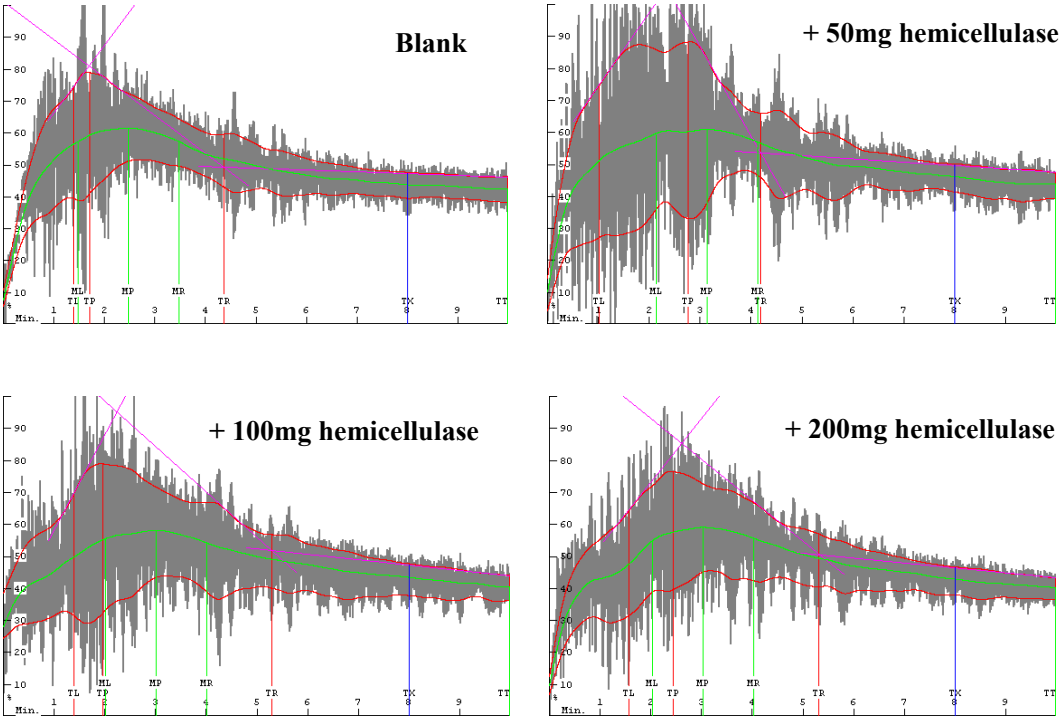


Figure 3.7 Effect of hemicellulase on mixogram of waxy flour dough (NWX02Y2459) at optimum absorption of 63.6% and flour protein of 13.03% (14%mb).

It appears addition of 50 mg hemicellulase to 8.6g (db) flour made some insoluble hemicellulose become soluble and compete for water, and the dough mixing curve strengthened. But adding 100mg or 200mg addition of hemicellulase depolymerized the hemicelluloses excessively, it appears, and the dough became less stiff. The overall strength of the dough was not decreased, so little protease was in the hemicellulase.

Effects of Hemicelluase on Secondary Protein Structure in Gluten

The area under mid-infrared peaks associated with secondary protein structures in gluten was measured by Fourier transform horizontal attenuated total reflectance (FT-HATR) spectroscopy. As hemicellulase was added to the flour, some changes occured in the gluten

structure of doughs wherein pentosans had likely undergone hydrolysis. Upon addition of hemicellulase, there was a significant decease in peak area at 1265 cm^{-1} both for waxy and normal wheat flour doughs, indicating a loss of random coil configuration. For the normal wheat flour doughs, peak area also decreased at 1336 cm^{-1} which indicates loss of α-helix structure.

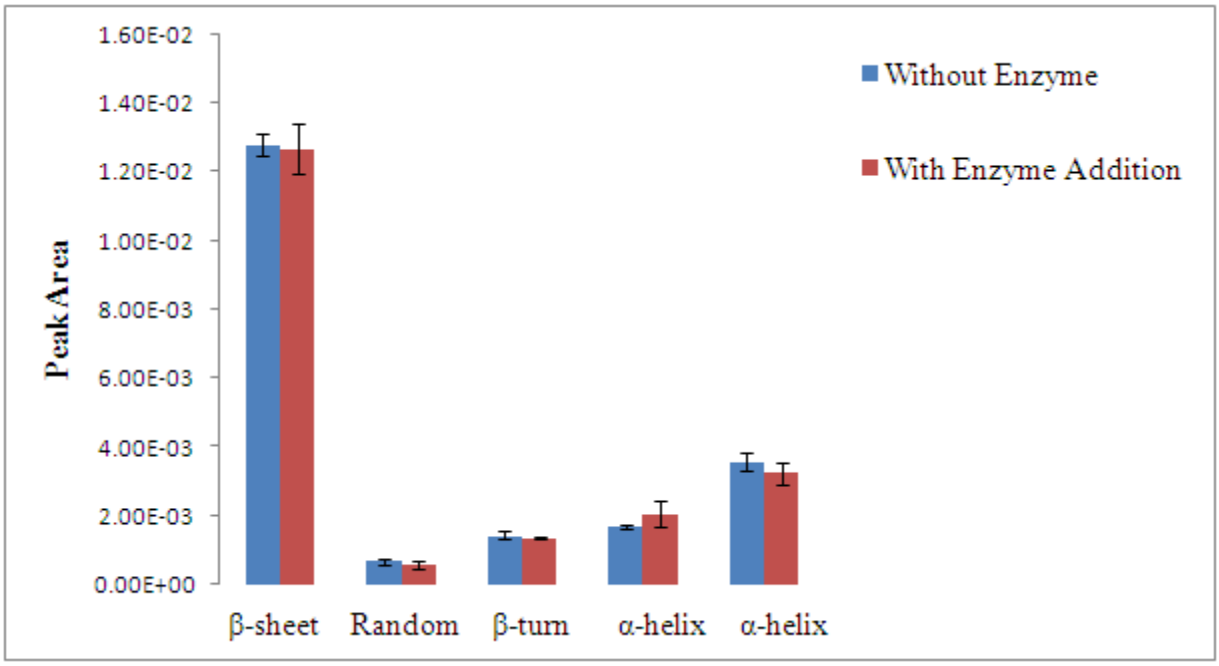


Figure 3.8 Changes in peak area of the spectra for waxy wheat flour dough with hemicellulase addition (Data expressed as scientific notation).

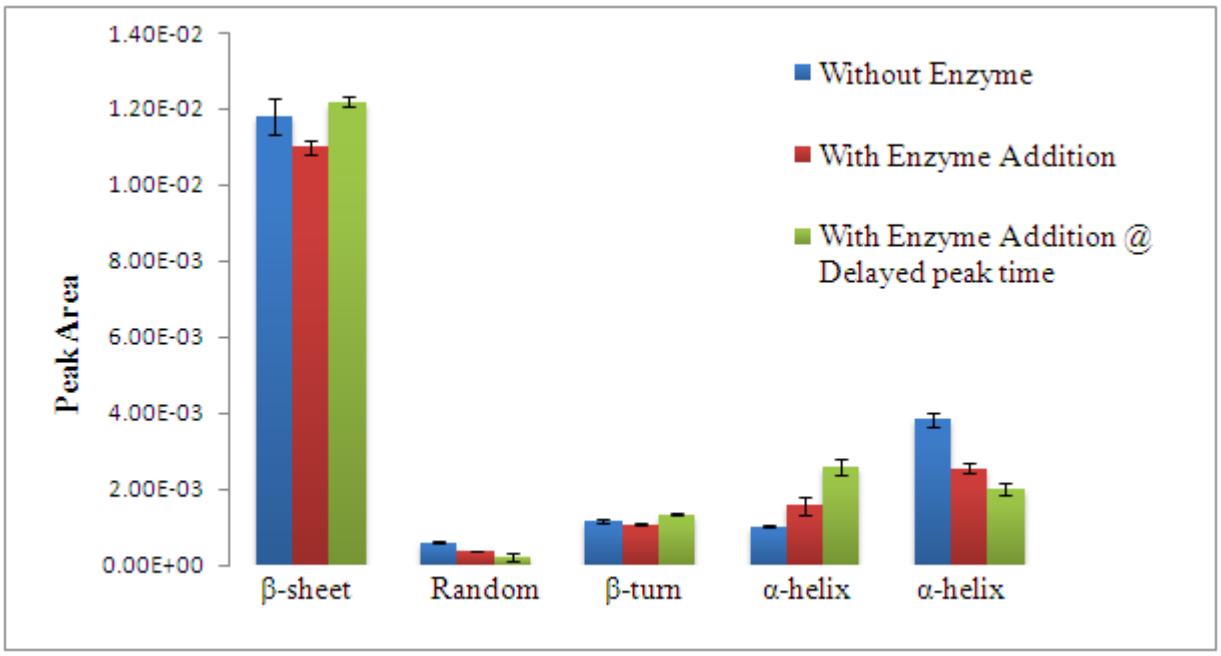


Figure 3.9 Changes in peak area of the spectra for normal wheat (Trego) dough with hemicellulase addition (Data expressed as scientific notation).

Viscosity of Flour-Water (3/7, g/g) Mixtures

The stirring-rate independence of viscosity, shear stress and shear rate was measured by rotational viscometry at 25°C (Table 3.12). Waxy wheat flours (NX03Y2114, NX03Y2115, NX03Y2205, and NX03Y2315) at 30% solids showed a higher viscosity compared with normal wheat flours. The viscosities decreased with the increasing stirring speeds which is called "shear thinning", the characteristic property of solutions of polysaccharides. Flour samples at certain speeds failed to produce a stable reading, probably because flour particles in those suspensions were settling during the time of the test. If the flour settles below the spindle, the spindle is measuring a lower solids concentration as settling progresses.

Table 3.12 Viscosity Parameters for Waxy Wheat and Normal Wheat Flour Suspensions (30% solids) at Various Speed.

V(rpm)	2.5			2		
	CP[a]	SS	SR	CP	SS	SR
NX03Y2114	2960	68.8	2.33	3150	58.6	1.86
NX03Y2115	3680	85.6	2.33	4000	74.4	1.86
NX03Y2205	3040	70.7	2.33	3800	70.7	1.86
NX03Y2315	2500	58.6	2.33	2775	51.6	1.86
NWX02Y2459	1140	26.5	2.33	1150	21.4	1.86
NX03Y2489	1520	35.3	2.33	1675	30.7	1.86
Karl	2280	53.5	2.33	2600	48.4	1.86
Trego	2300	53.5	2.33	2525	47.0	1.86

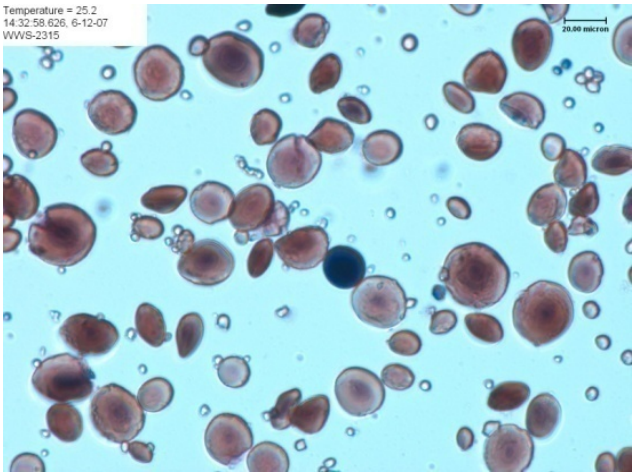
[a] CP- centipoise, SS-shear stress (N/m^2), SR-shear rate (s^{-1}).

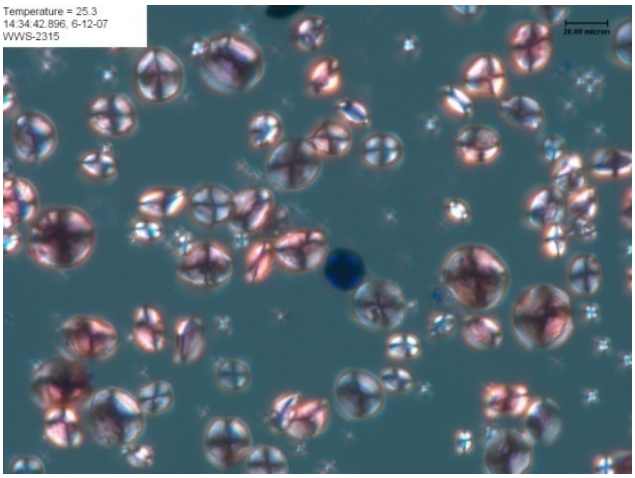
Characteristics of Waxy Wheat Starch

Waxy wheat starches from six advanced lines and from two normal wheat starches (Trego and Karl) were suspended in water near 25°C and stained with a low concentration of polyiodide ion and viewed under light and dark field microscopy. Fig 3.10 shows the starch granule of one (NX03Y2315) waxy line and one of normal (Karl) wheat starch. Both waxy and normal wheat starches showed two types of granules, the large disk-shaped or Type A, or the small spherical shaped granules, or Type B. Under light field illumination, amylose-containing granules of starch gave a blue color. In contrast, granules contain amylopectin granules gave a red-brown color. The waxy wheat starches showed a clear birefringence under crossed-polarization (dark-field) while the normal wheat starches showed a faint birefringence with bluish surrounding area. The waxy wheat starch NX03Y2315 showed a very low level of contamination with normal wheat starch granules, as did the other isolated waxy wheat starches (not shown).

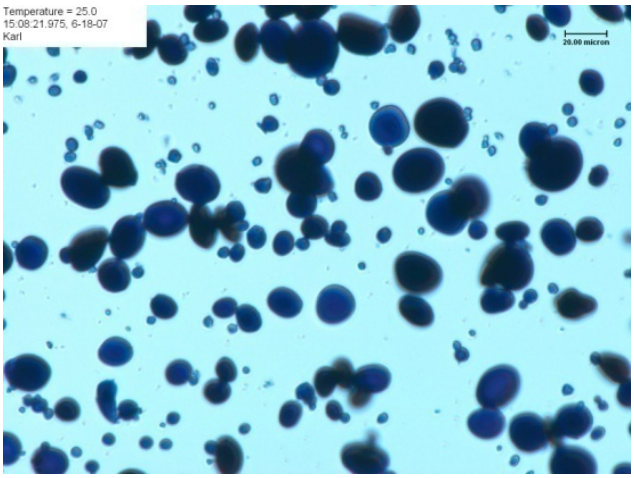
Amylose Contamination

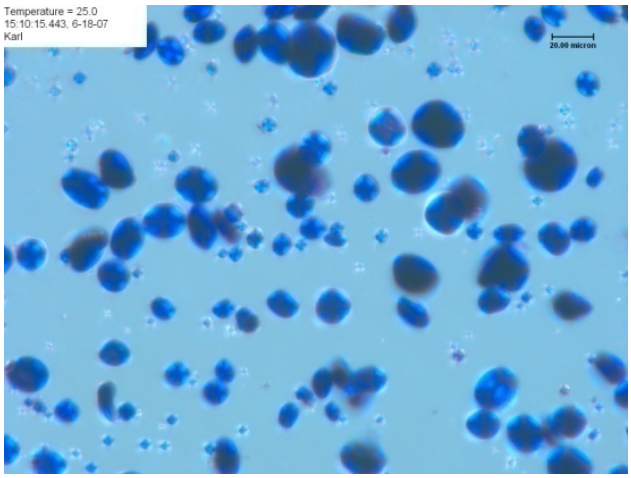
Bright Field **Dark Field**





NX03Y2315 NX03Y2315





Karl Karl

Figure 3.10 Photomicrographs of iodine-stained starch samples viewed under light and dark-field respectively (left and right side, respectively).

Gelatinization and Retrogradation Properties

Table 3.13 shows the gelatinization properties of starches in excess water determined using DSC. The two waxy wheat starch samples showed higher enthalpy values than normal wheat starch. If normal wheat starch contains ~25% amylose, all its crystallinity is in the amylopectin fraction, and the structure of amylopectin in normal and waxy wheats are the same, one would predict a gelatinization enthalpy of 15.5J/g/(0.75)=20.6J/g. The ΔH for waxy wheat starch calculated from that of normal wheat starch was lower than predicted, possibly because of

lower hydration of amylose in the amorphous phase. An endothermic peak at about 100^0C which is responsible for the melting transition of the amylose-lipid complex was observed in normal wheat starch, but no corresponding peak was found in waxy wheat starch (curves not shown). The gelatinization temperature of waxy and normal wheat starches were the same, except for the concluding T_c temperature. The gelatinization temperature of waxy corn was 7-11^{0}C higher than for waxy wheat starch.

Table 3.13 Gelatinization of Starches in Three Parts Water Determined by Differential Scanning Calorimeter.

Starch		**Transition Temperature**			**Enthalpy**
		T_o(°C)	**T_p(°C)**	**T_c(°C)**	**ΔH(J/g)**
Normal	**AV**	**60.4**	**65.1**	**69.9**	**15.5**
Wheat	SD	0.86	0.79	1.42	0.79
Waxy	**AV**	**59.7**	**64.6**	**73**	**17.6**
Wheat I	SD	0.26	0.30	0.73	1.08
Waxy	**AV**	**60.3**	**64.5**	**72**	**18.2**
Wheat II	SD	0.53	0.15	0.42	0.70
Waxy	**AV**	**67.1**	**74.4**	**81.2**	**16.7**
Corn	SD	0.32	0.32	0.52	1.59

Table 3.14 Retrogradation (4^0C, 7days) of Starch Pastes (33.3% solids) Determined by Differential Scanning Calorimeter.

Starch		Transition Temperature			Enthalpy
		T_o(°C)	T_p(°C)	T_c(°C)	ΔH(J/g)
Normal	**AV**	**40.2**	**51.8**	**63.2**	**4.8**
Wheat	SD	1.65	0.26	0.31	0.21
Waxy	**AV**	**42.1**	**52.8**	**62.9**	**2.0**
Wheat I	SD	4.23	2.04	0.63	0.67
Waxy	**AV**	**50.8**	**55.6**	**62.9**	**1.3**
Wheat II	SD	7.39	2.93	1.68	1.21
Waxy	**AV**	**54.3**	**53.4**	**65.5**	**5.9**
Corn	SD	1.84	1.21	0.49	1.49

The melting temperature and enthalpy for retrograded starch was analyzed after the gelatinized samples were stored at 4^0C for one week. The initiation temperature for melting of retrograded waxy starch was imprecise. Retrograded waxy wheat starches showed lower gelatinization temperature and ~2/3 less enthalpy than retrograded waxy corn starch (Table 3.14). Corn amylopectin has a greater proportion of chains with dp ~22 compared to wheat amylopectin, and has fewer chains of dp~10. Those differences in chain length apparently caused greater retrogradation of waxy corn compared with waxy wheat, which agrees with data of Reddy and Seib (2000). After storage, amylose had a higher tendency to retrograde due to its long linear structure, while amylopectin with its short chain length impedes retrogradation and gel formation (Chakraborty et al 2004).

Pasting Properties

The pasting properties of isolated starches were measured with RVA (Fig 3.11). Since starch swelling is mainly a property of amylopectin (Sasaki 2005), the higher amylopectin content of a starch the greater the swelling ability. Greater swelling reduces the quantity of free water and leads to higher pasting viscosity (Sasaki 2005).

Waxy wheat starch had a higher increase in viscosity at lower temperature than normal wheat starch and waxy maize starch, indicating that waxy wheat starch rapidly developed viscosity. Starches isolated from NX03Y2115, NX03Y2205, NWX02Y2459 had slightly higher peak viscosity than the other waxy wheat starches. Waxy wheat starches had lower set back than that of normal wheat starches and waxy maize starch. The branched structure of amylopectin impedes retrogradation and slows down starch gel formation after cooking and cooling (Hayakawa et al 1997). The cooked paste of waxy wheat starch remains cohesive with a gummy texture.

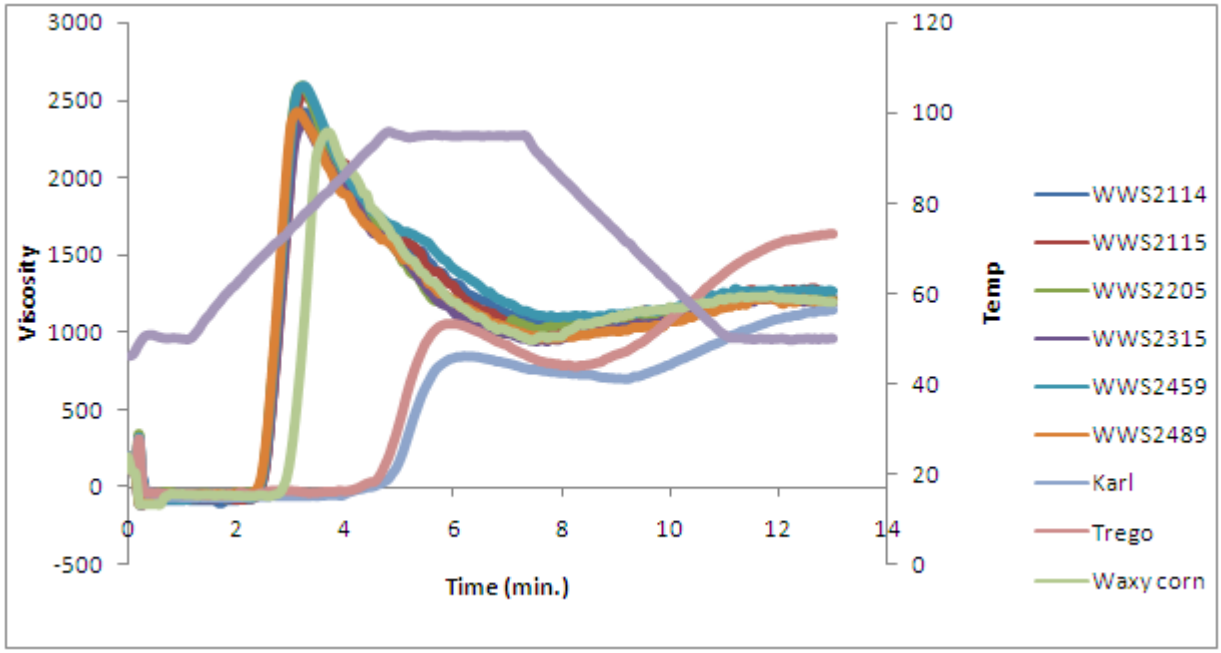


Figure 3.11 Pasting curves of waxy wheat starch (NX03Y2114, NX03Y2115, NX03Y2205, NX03Y2315, NWX02Y2459, and NX03Y2489) and normal wheat starch (Karl, Trego) and Amioca determined at 7% starch solids on the RVA.

Pasting curves of waxy wheat starch (NWX02Y2459) and normal wheat starch (Trego) and waxy maize starch (Amioca) were also measured with visco-amylograph at the heating rate of 6^{o}C/min (Fig 3.12). Unlike the results from RVA in Fig 3.11, waxy maize starch had a higher peak viscosity than waxy wheat starch. At the heating rate of 12^{o}C/min (Fig 3.13), waxy wheat starch had a higher peak viscosity at lower temperature than waxy maize starch, indicating that the heating rate affects waxy starch developing its viscosity.

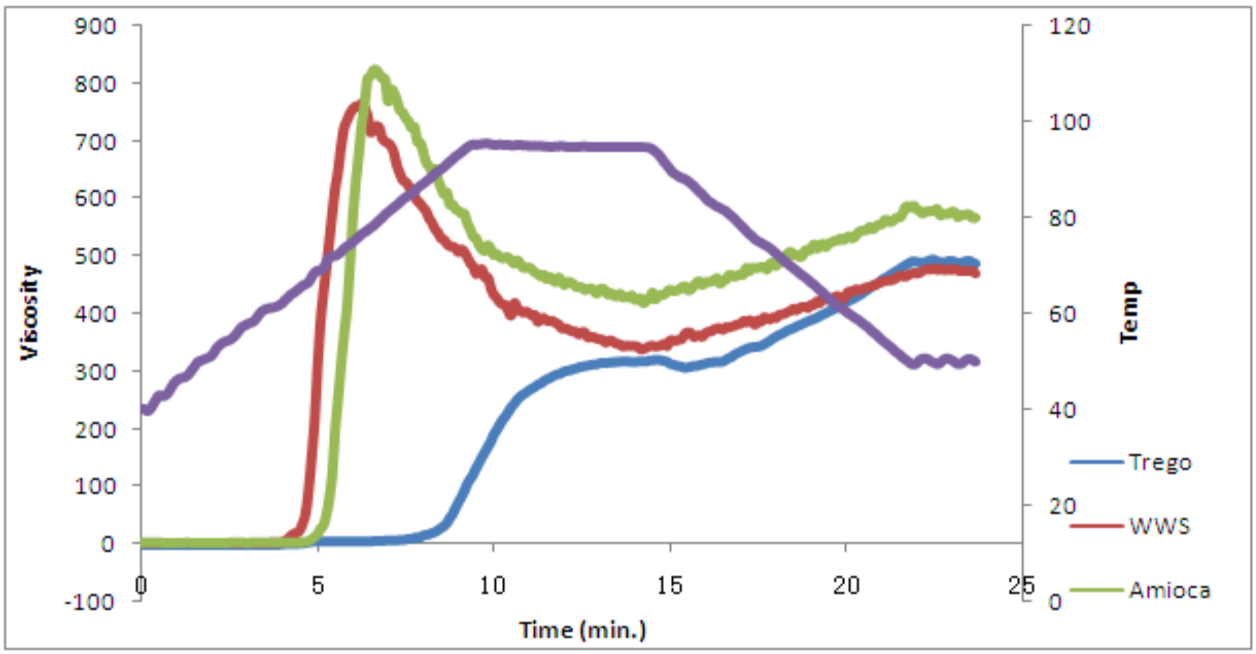


Figure 3.12 Viscoamylogram curves of waxy wheat starch (NWX02Y2459) and normal wheat starch (Trego) and Amioca determined at 7% starch solids on the visco-amylograph (Heating rate 6^{o}C/min). .

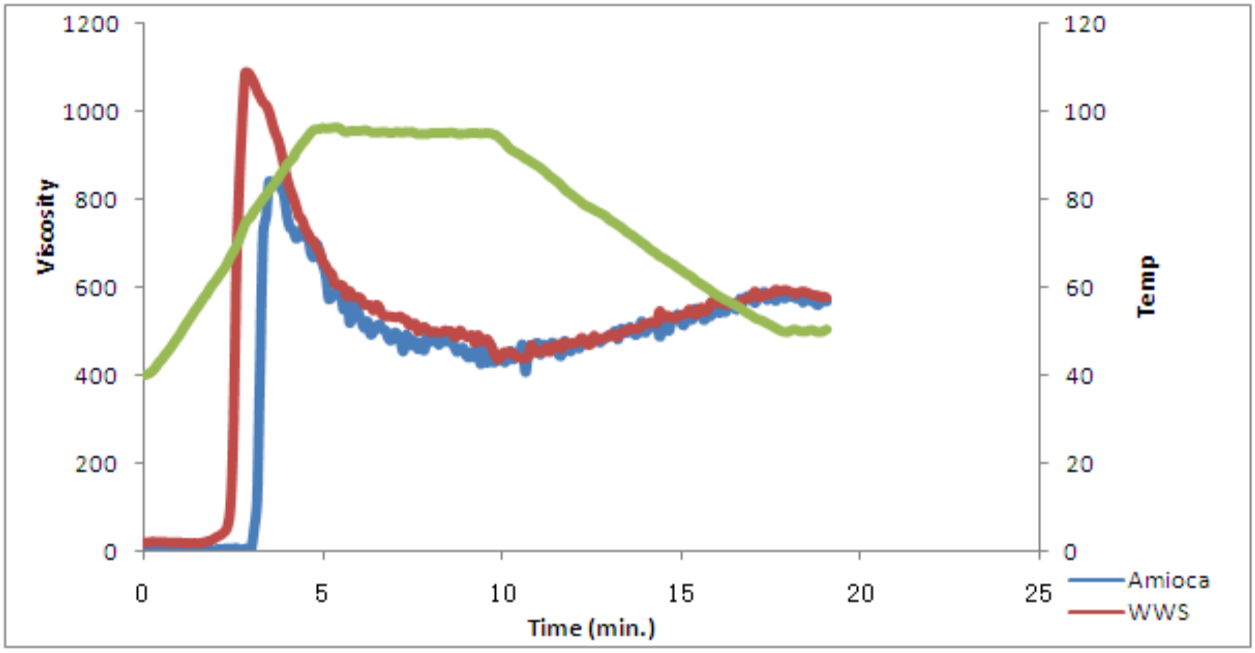
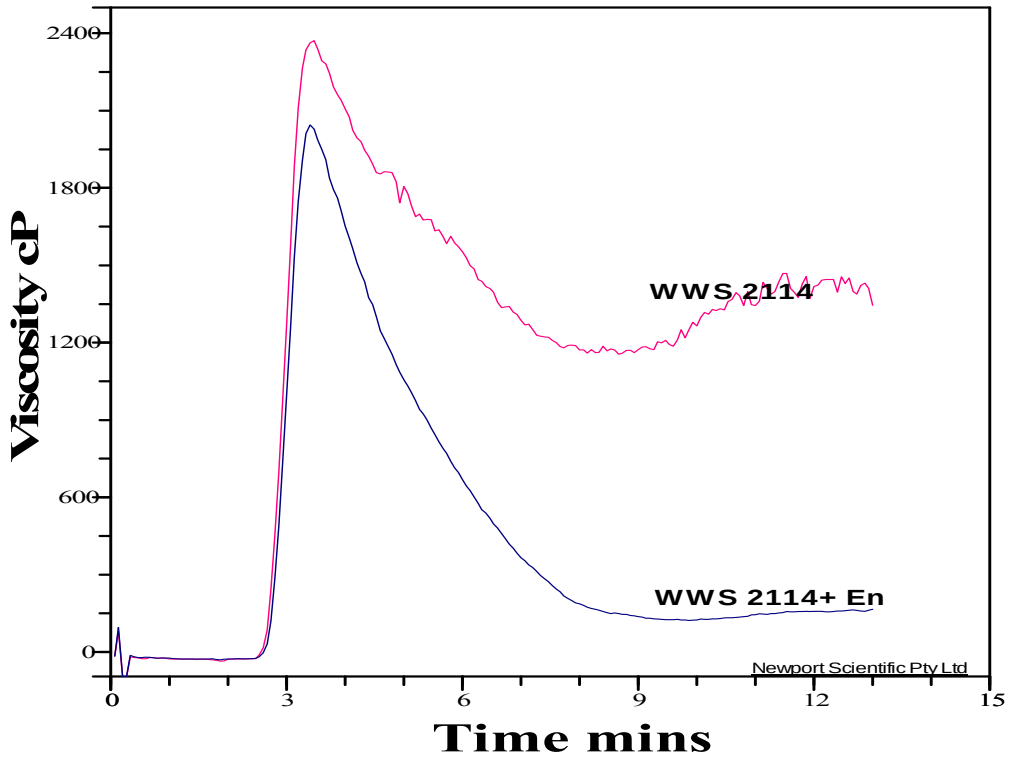


Figure 3.13 Viscoamylogram curves of waxy wheat starch (NWX02Y2459) and Amioca determined at 7% starch solids on the visco-amylograph (Heating rate 12°C/min).

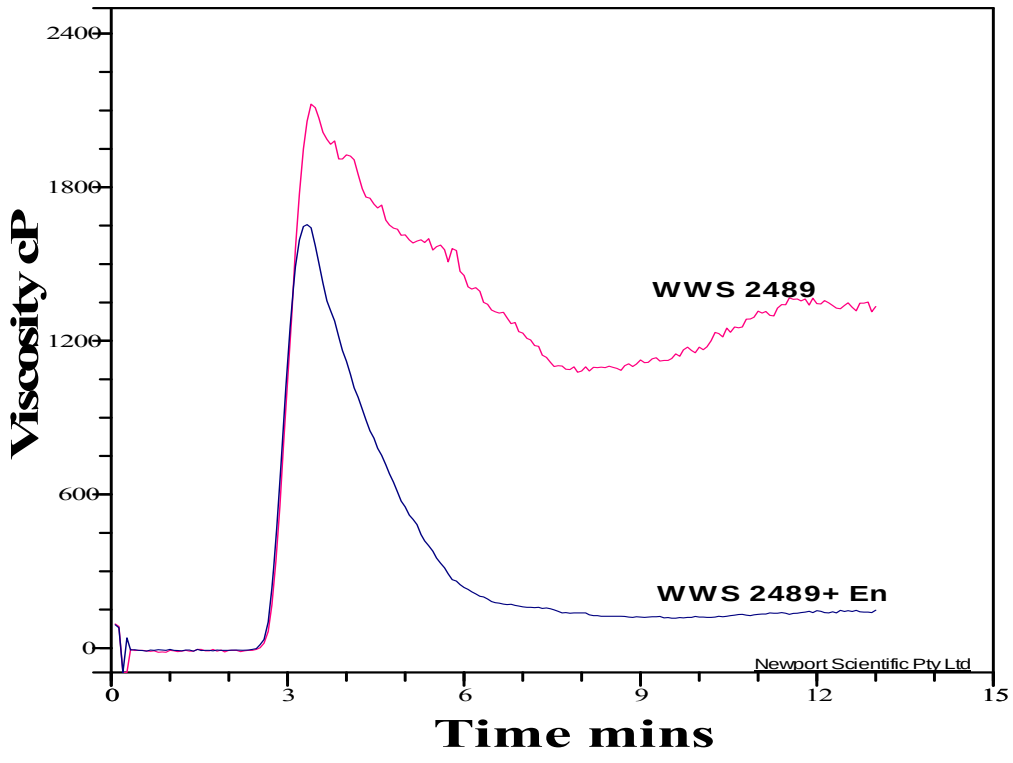
Enzyme Susceptibilities

The enzyme susceptibilities were determined on RVA by adding 25µl α-amylase to waxy wheat starches (NX03Y2114 and NX03Y2489), waxy maize starch (Amioca), and normal wheat starch (Karl) suspensions. The pasting curves (Fig 3.14) showed that waxy wheat starches and Amioca was more susceptible to α-amylase degradation compared to normal wheat starch.

Waxy Wheat Starch (NX03Y2114)



Waxy Wheat Starch (NX03Y2489)



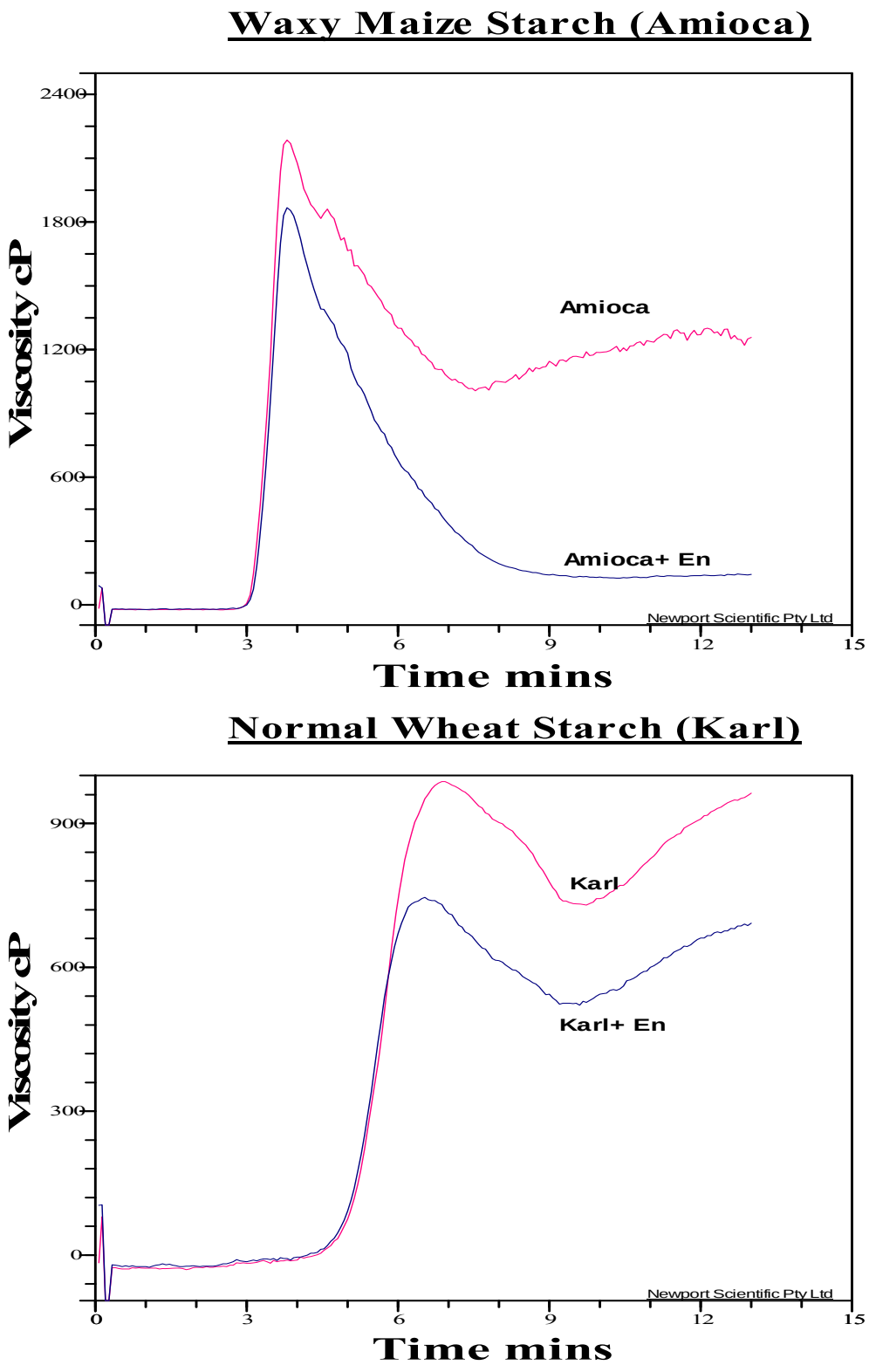
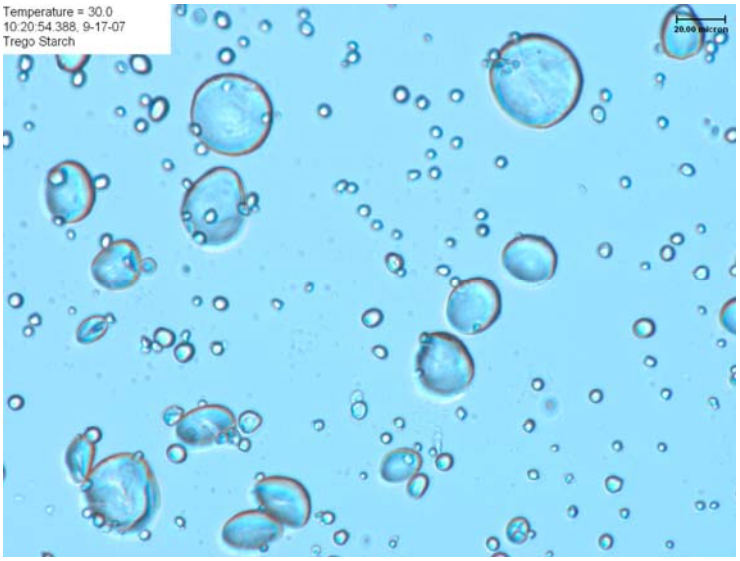


Figure 3.14 Pasting curves of waxy wheat starch (NX03Y2114, NX03Y2489) and waxy corn starch (Amioca) added with one/two drops of 0.1% α-amylase determined at 7% starch solids on the RVA.

Morphology Changes

Changes in morphology of waxy wheat, normal wheat and waxy maize starch granules were recorded continuously during heating in water on a hot stage. Images (Fig 3.15) showed that normal wheat starch granules started increasing in size at 57°C and continued up to 90°C. Normal wheat starch granules retained a rounded shape even after being heated to 90°C. In contrast, waxy wheat starch granules started swelling at 47°C and swelled greatly at 60-70°C but then disintegrated into many small fragments between 70-80°C depending on heating rate (Fig 3.16). Waxy maize starch started swelling at 50°C and swelled greatly at 60-80°C, then each granule disintegrated into small fragments 80~85°C (Fig 3.17). At the lower heating rate, more swelling was observed before granule disintegration than a high heating rate. The observations could be used to explain pasting property differences between waxy and normal wheat starches. It is possible the amylose-lipid complex suppresses swelling and maintains the integrity of swollen starch granules. Normal wheat granules developed viscosity at a higher temperature and started approaching peak viscosity when they were almost fully swollen. In contrast with normal wheat starch granules, waxy wheat starch granules rapidly swelled at a lower temperature, developed a higher peak viscosity, but then disintegrated and viscosity decreased rapidly.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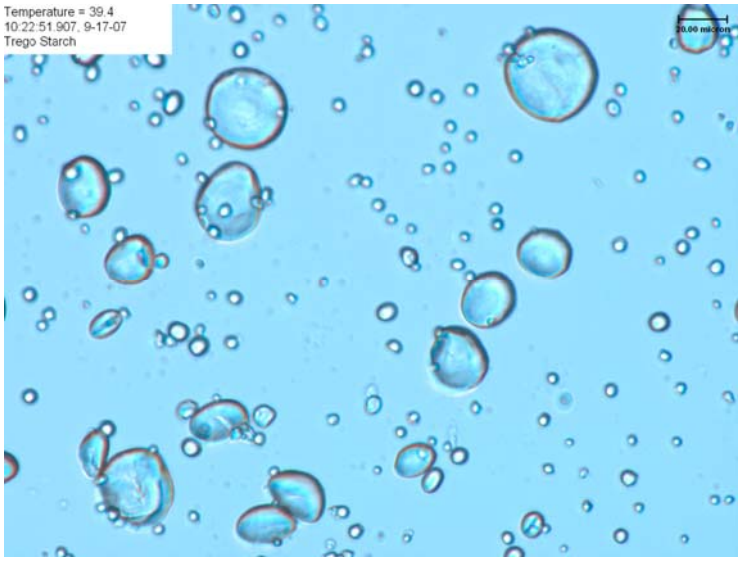
Trego Starch
20.00 micron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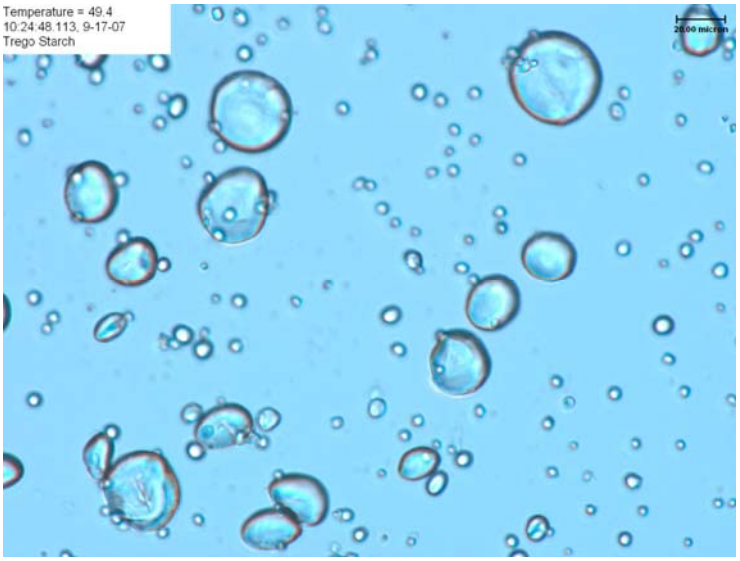
20.00 micron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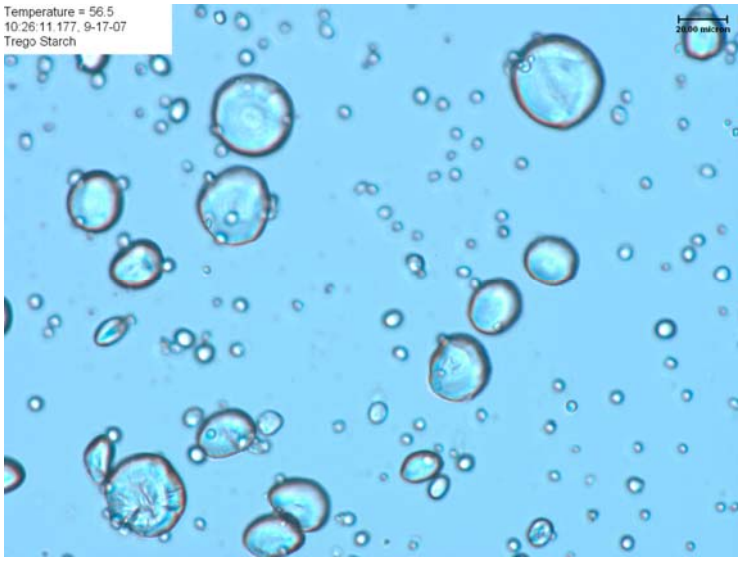
Trego Starch
20.00 micron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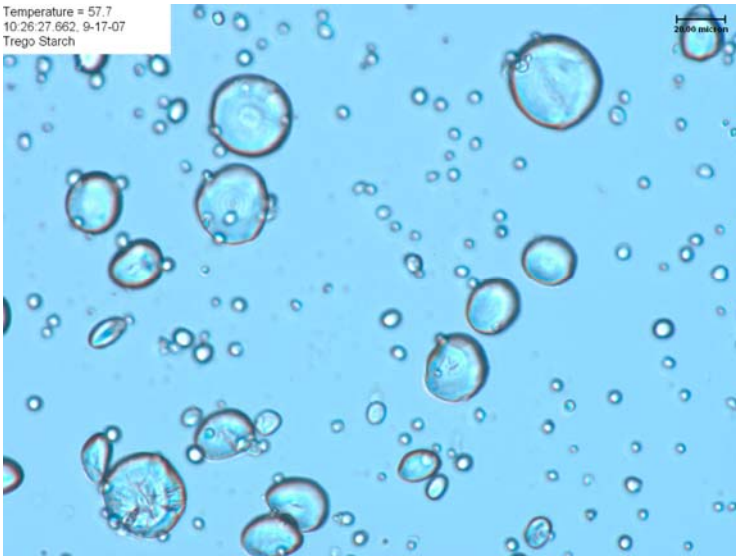
20.00 micron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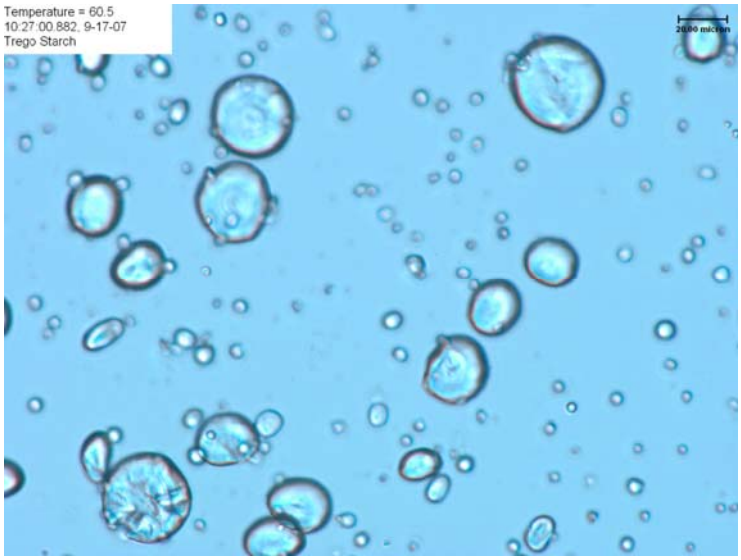
20.00 micron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Trego Starch
20.00 micron

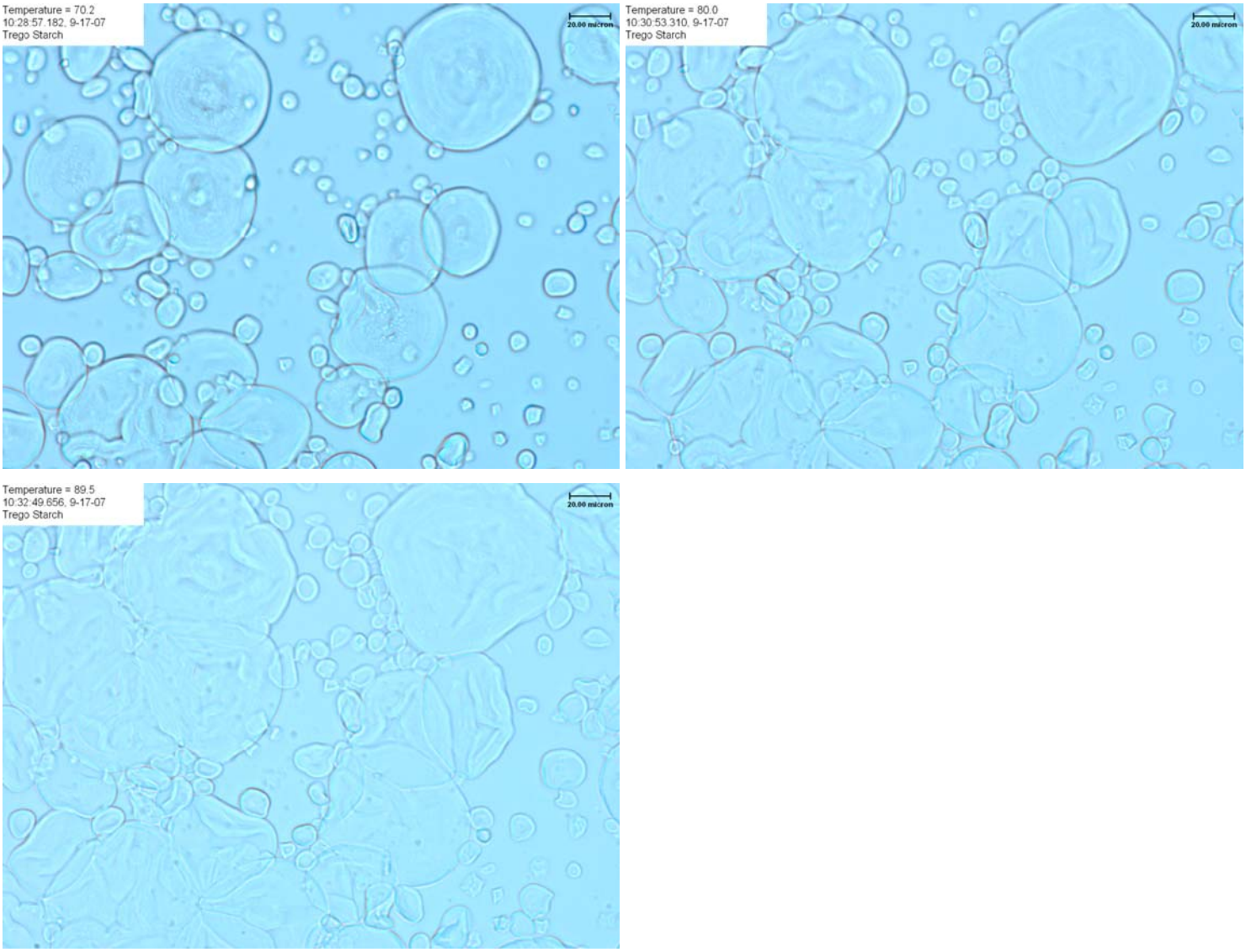
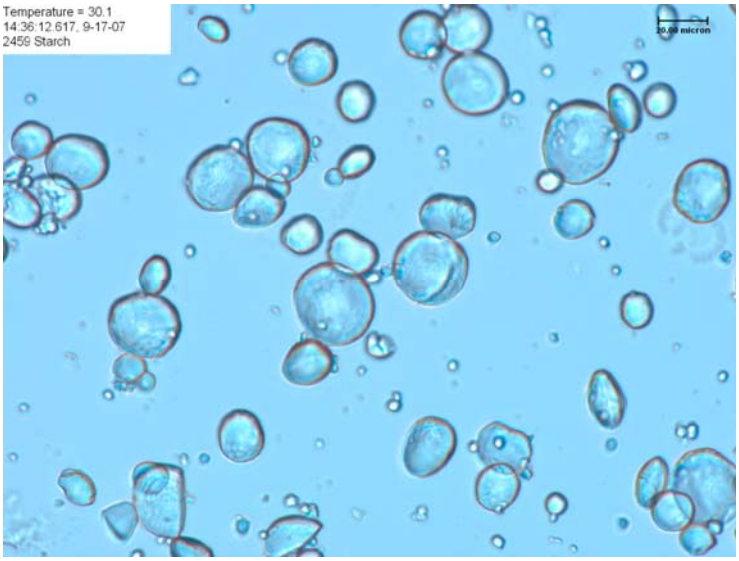


Figure 3.15 Microphotographs of morphology changes of normal wheat starch (Trego) suspension heated at 5^{o}C/min.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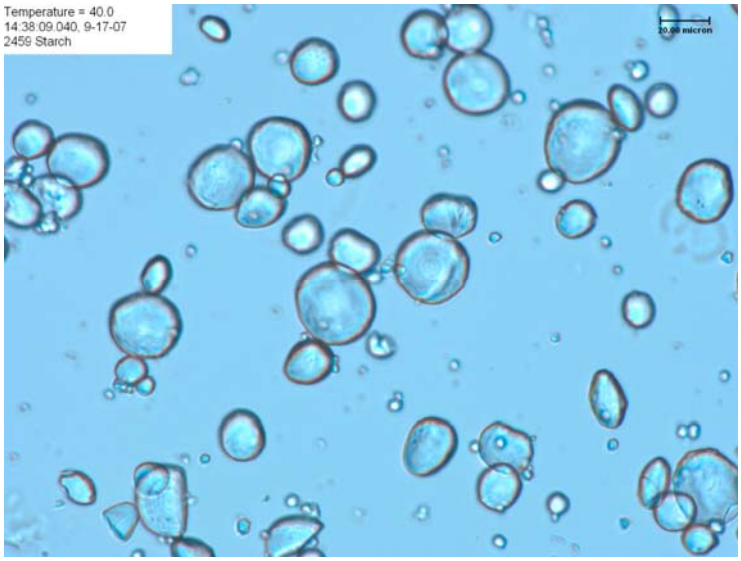
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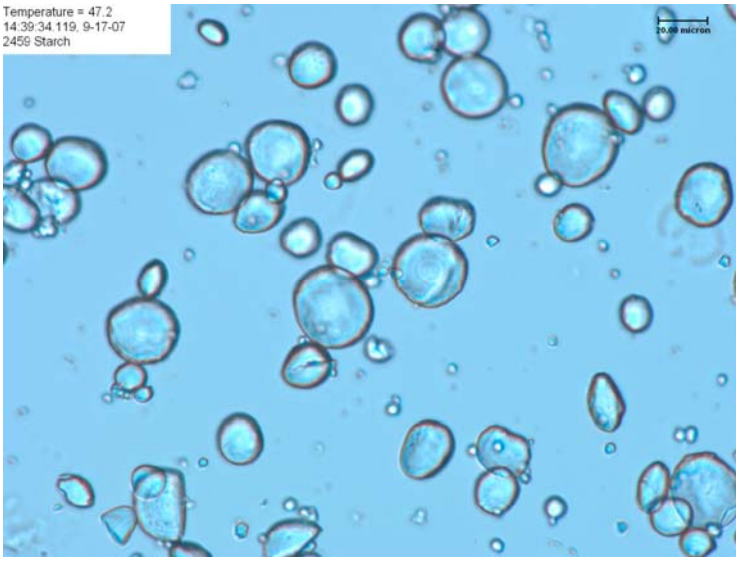
20.00 micron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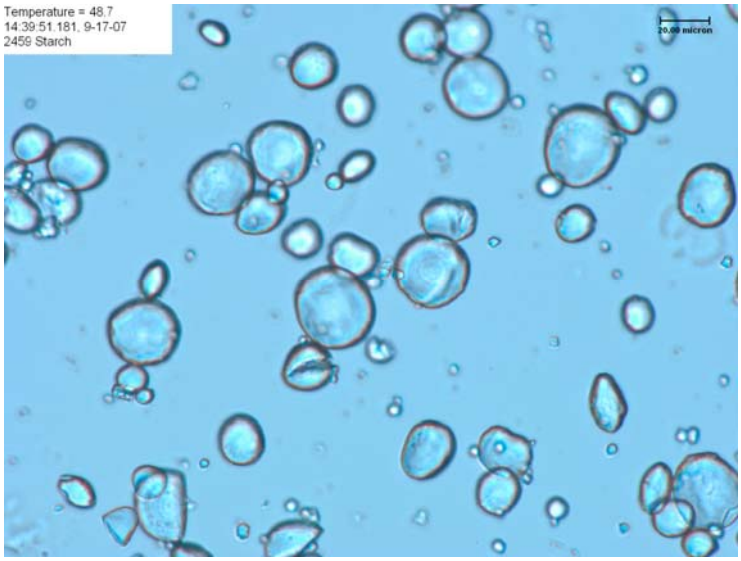
20.00 micron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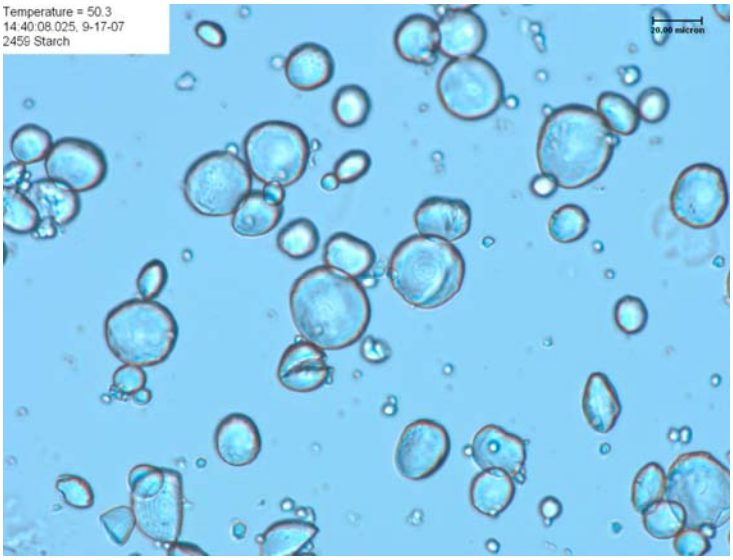
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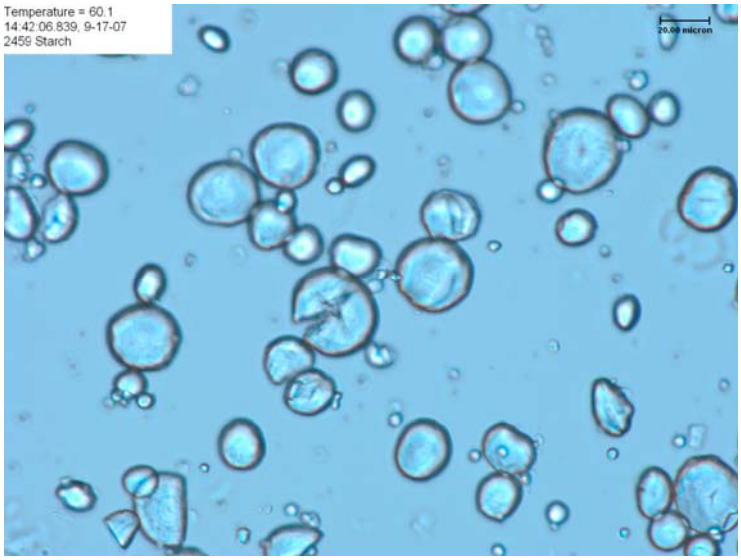
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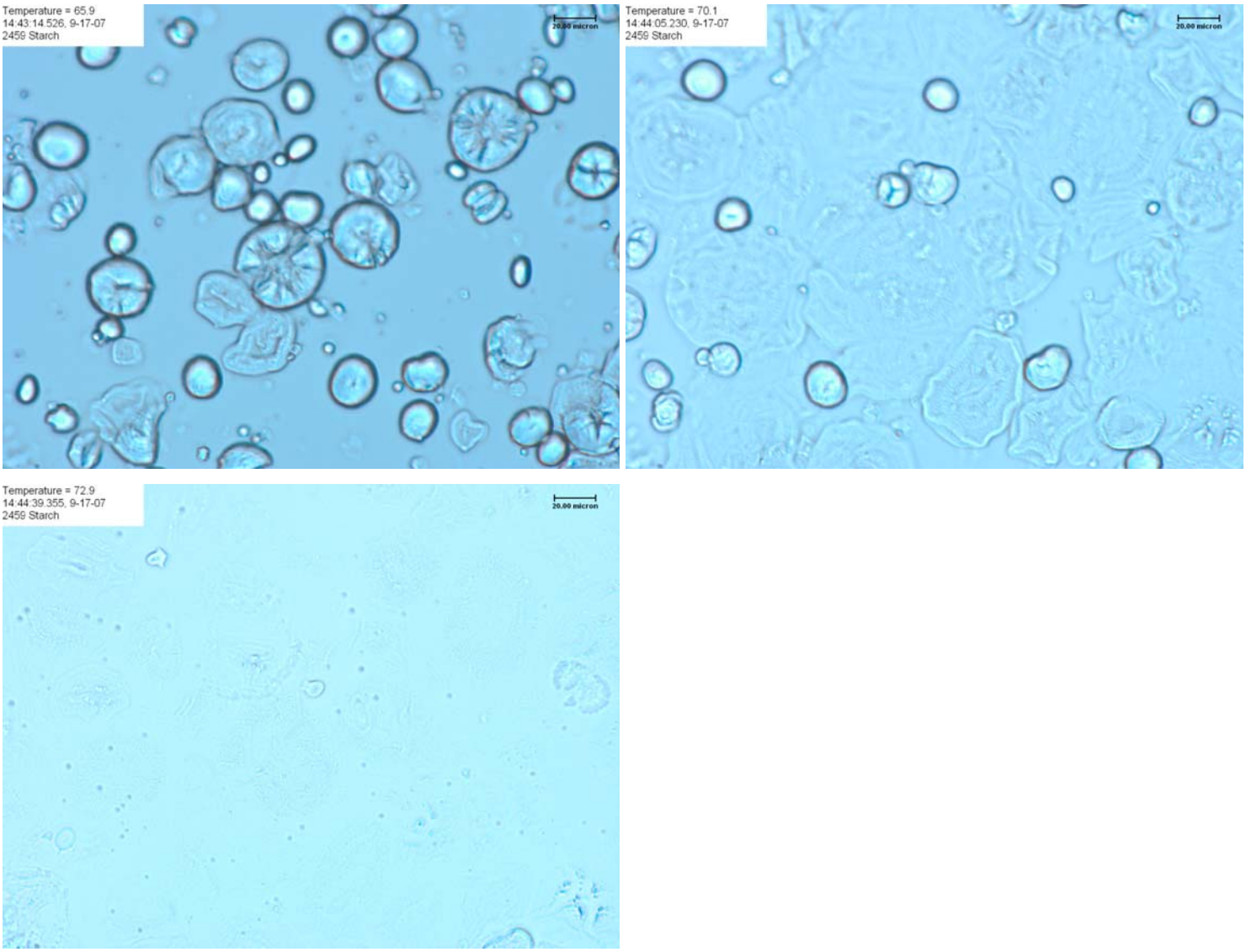
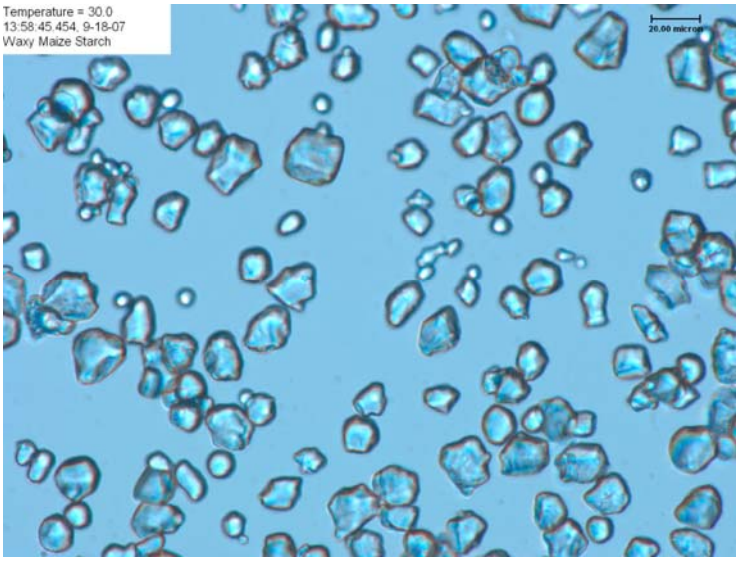


Figure 3.16 Microphotographs of morphology changes of waxy wheat starch (NWX03Y2459) suspension heated at 5°C/min.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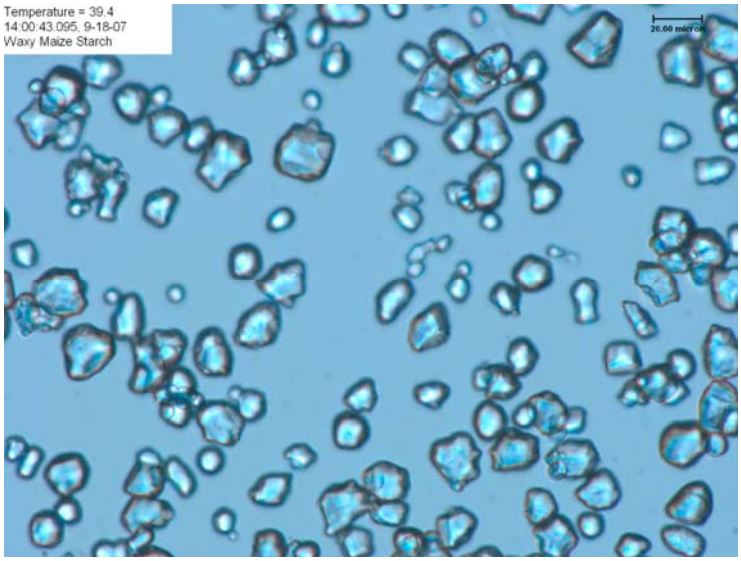
Waxy Maize Starch
20.00 micron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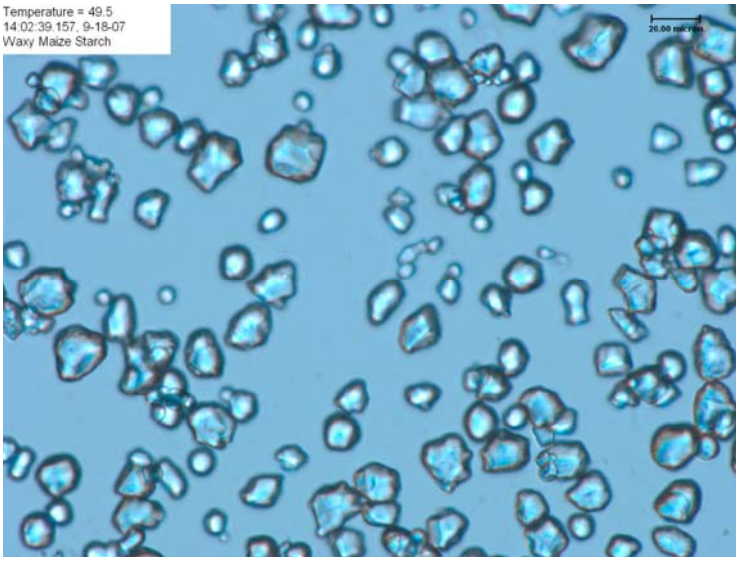
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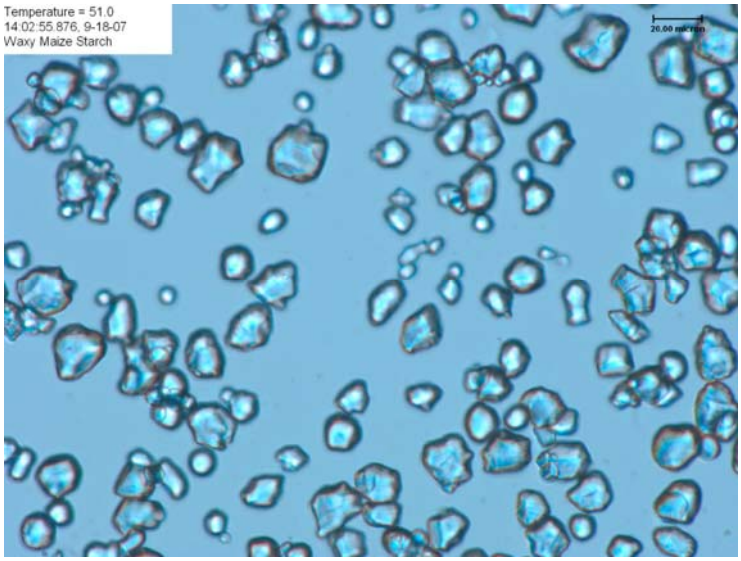
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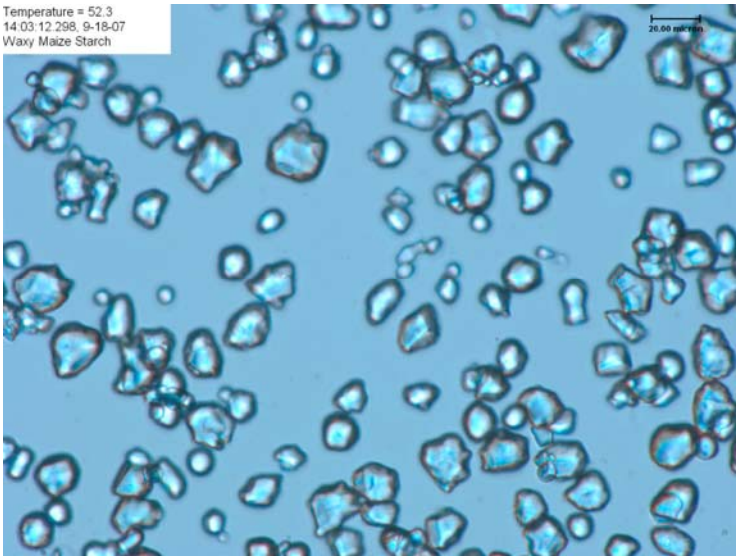
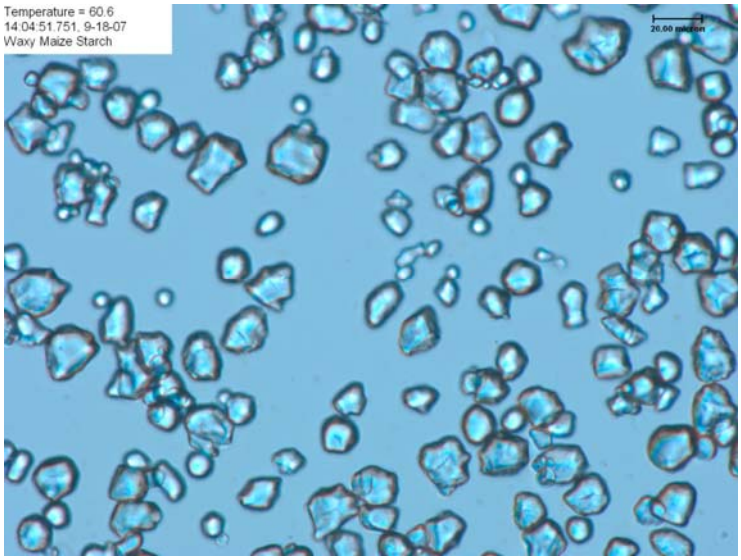
Waxy Maize Starch
20.00 micron


Temperature = 52.3
14:03:12.298, 9-18-07
Waxy Maize Starch
20.00 micron


Temperature = 60.6
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Waxy Maize Starch
20.00 micron

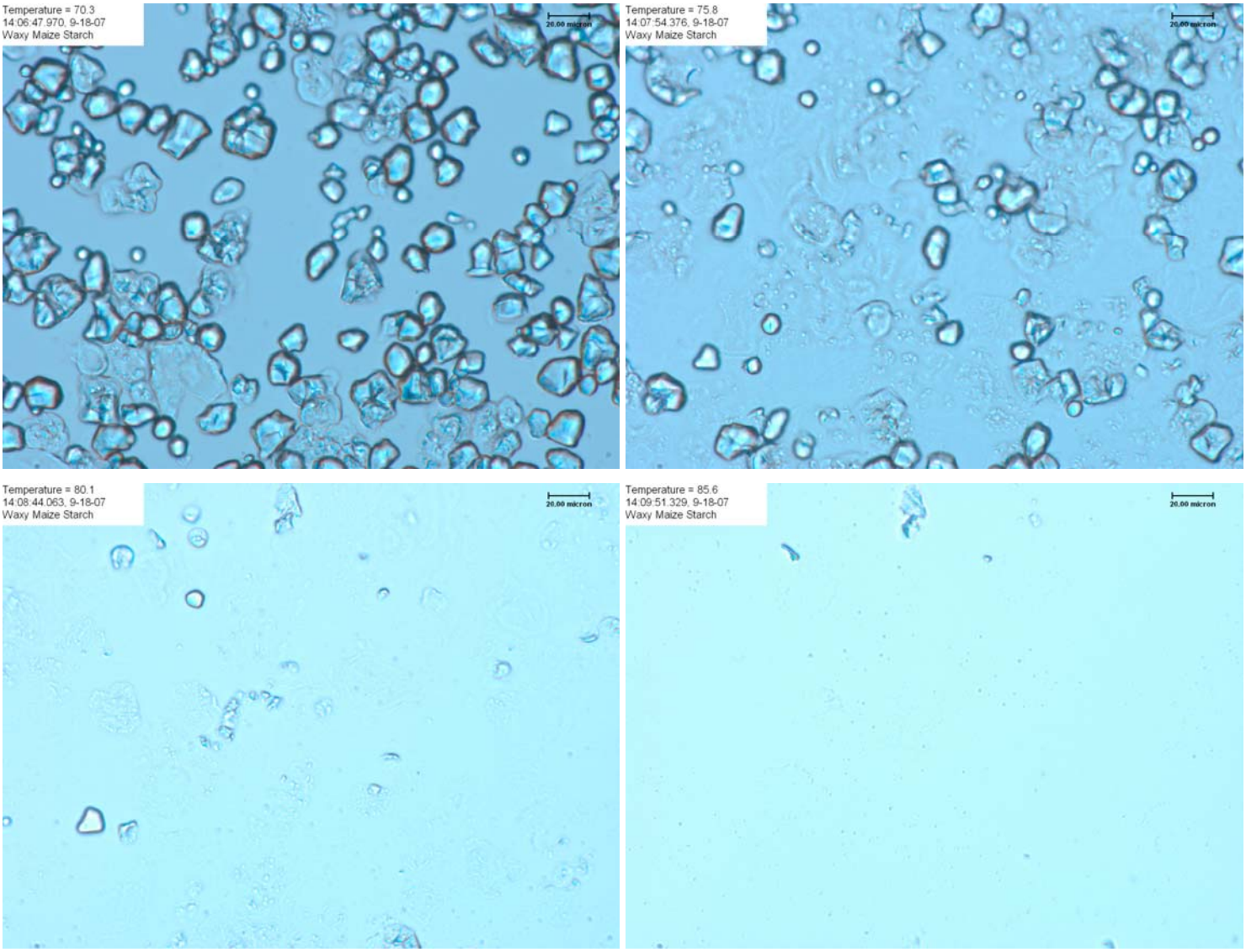


Figure 3.17 Microphotographs of morphology changes of waxy maize starch (Amioca) suspension heated at 5°C/min.

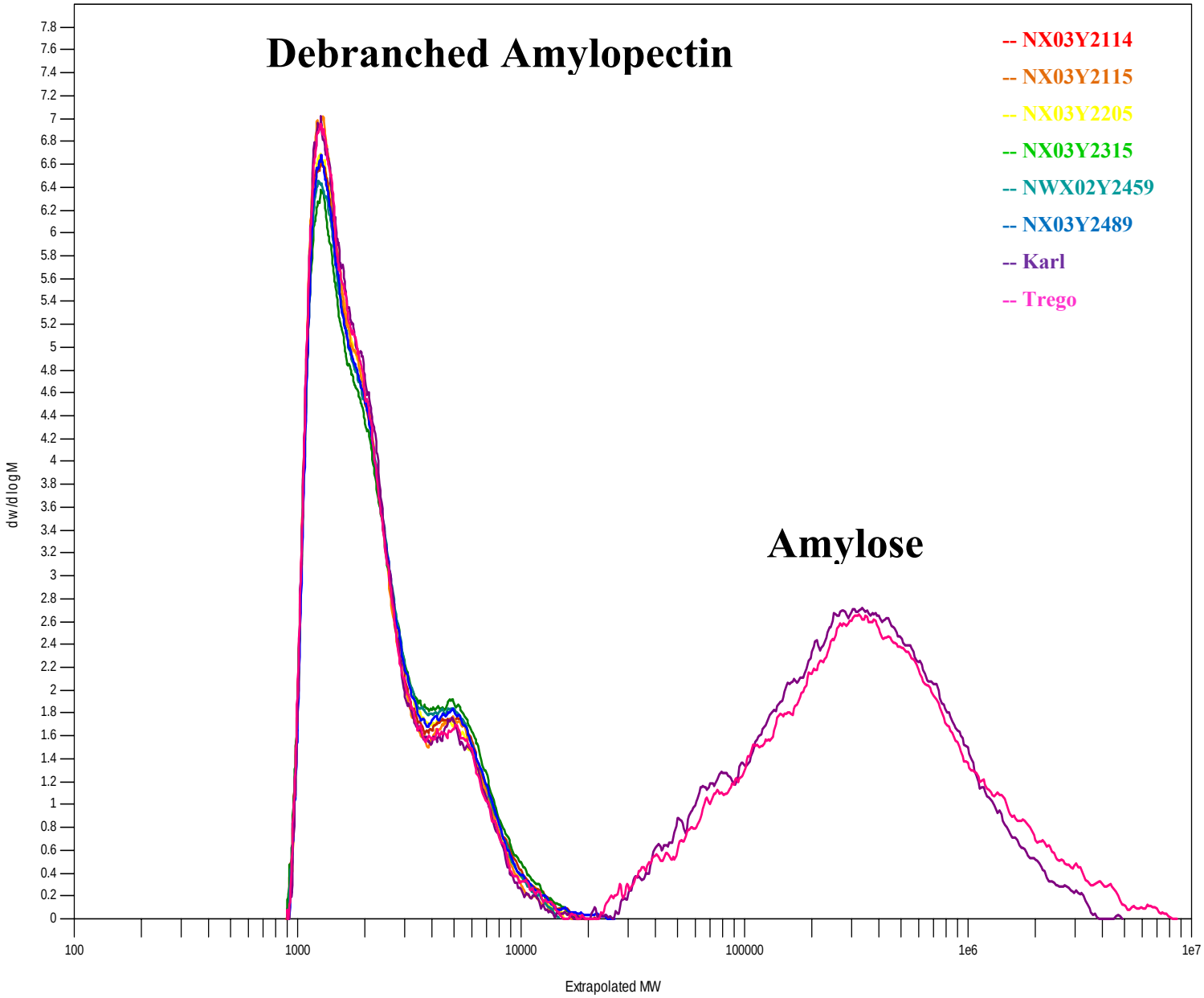


Figure 3.18 Molecular weight distributions of debranched waxy wheat starches (NX03Y2114, NX03Y2115, NX03Y2205, NX03Y2315, NWX02Y2459, and NX03Y2489) and normal wheat starch (Karl, Trego) determined on GPC.

The molecular weight distribution of debranched waxy wheat starches and normal wheat starches determined on GPC were compared in Fig 3.18. Debranched waxy wheat starch showed

a bimodal distribution and it had similar proportions compared to that of debranched normal wheat starch which indicated that the molecular structure of the branched component which is mainly amylopectin in waxy wheat starch was close to that of amylopectin in normal wheat starch.

Pasting Properties of Cross-Linked Waxy Wheat Starch

After waxy wheat starch was cross-linked in an aqueous slurry at about 37% starch solids with 0.01% phosphoryl chloride (starch basis), visco-amylograms showed that viscosity breakdown was eliminated and that the cooked starch became non-cohesive. Increasing levels of phosphoryl chloride at 0.03% and 0.06% caused a steady decline in the peak and final paste consistencies at 6% and 7% solids of cross-linked waxy wheat starch (Fig 3.19 and Fig 3.20). At 8% solids basis, waxy wheat starch cross-linked with phosphoryl chloride at 0.03% and 0.06% had the same final consistency and was higher than that of waxy wheat starch cross-linked with 0.01% phosphoryl chloride (Fig 3.21).

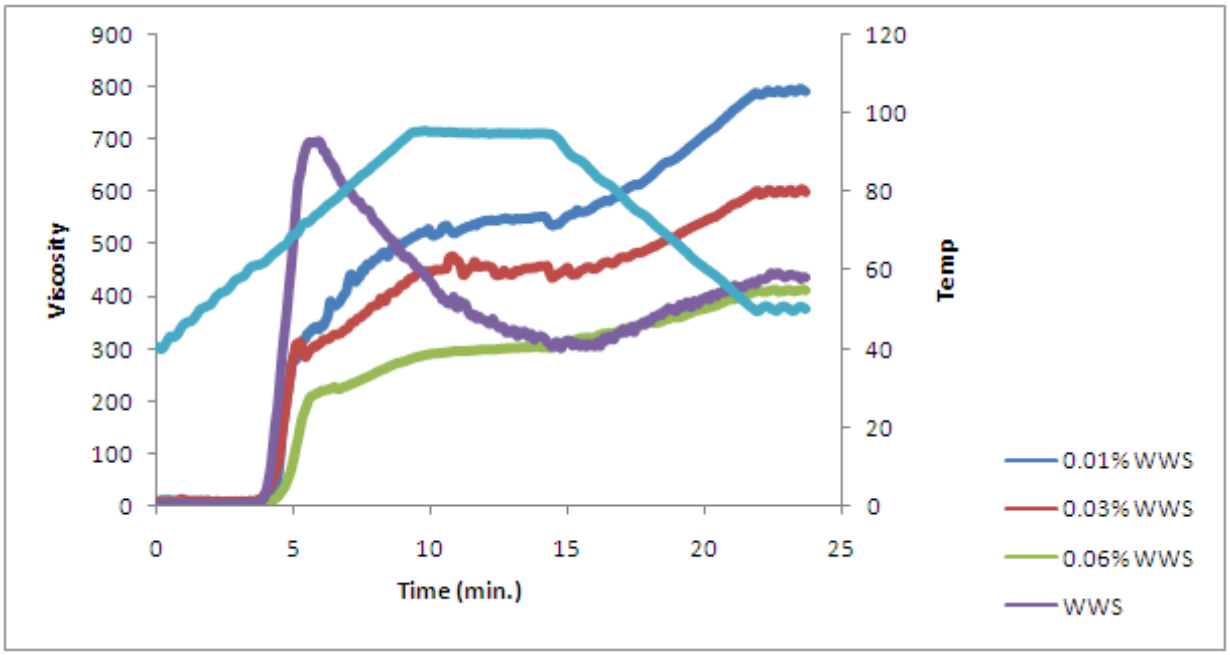


Figure 3.19 Viscoamylogram curves (6.0% starch solids basis) of cross-linked waxy wheat starch (NWX02Y2459). Starch was reacted with 0.01%, 0.03% and 0.06% phosphoryl chloride (starch basis) (Heating rate 6^{o}C/min).

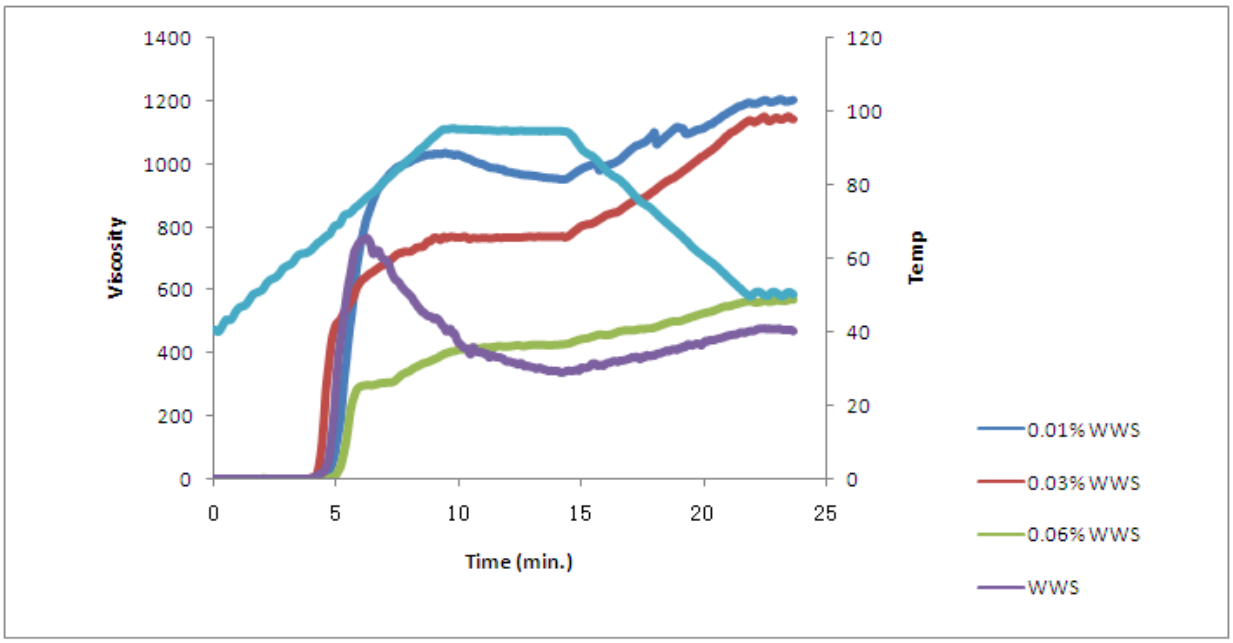


Figure 3.20 Viscoamylogram curves (7.0% starch solids basis) of cross-linked waxy wheat starch (NWX02Y2459). Starch was reacted with 0.01%, 0.03% and 0.06% phosphoryl chloride (starch basis) (Heating rate 6°C/min).

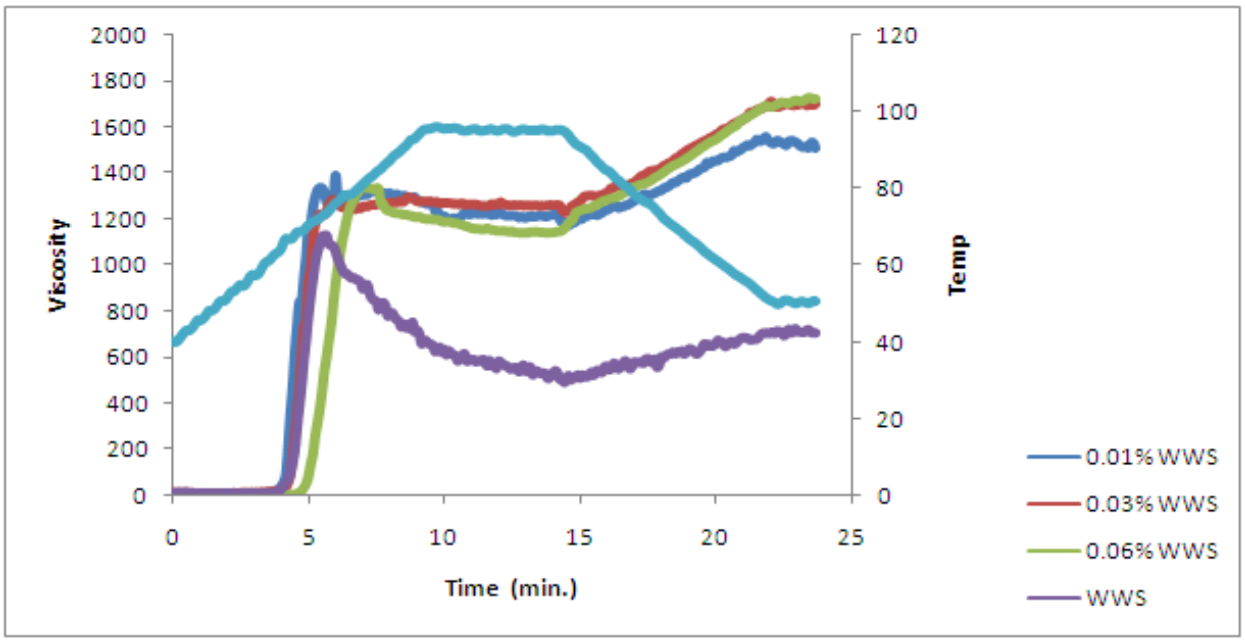


Figure 3.21 Viscoamylogram curves (8.0% starch solids basis) of cross-linked waxy wheat starch (NWX02Y2459). Starch was reacted with 0.01%, 0.03% and 0.06% phosphoryl chloride (starch basis) (Heating rate 6°C/min).

Waxy maize starch cross-linked with 0.01% phosphoryl chloride had a higher peak and final consistency at 6% solids than when cross-linked with 0.03% and 0.06% phosphoryl chloride (Fig 3.22). However, when pasting at 7% starch solids, the consistencies of cross-linked waxy maize starch proceeded through an optimum as the cross-linking level increased. At 7% solids basis, waxy maize starch cross-linked with 0.03% phosphoryl chloride had the highest peak and final consistency (Fig 3.23). At 8% solids basis, waxy maize starch cross-linked with phosphoryl chloride at 0.03% and 0.06% had the same final consistency and was higher than that of waxy maize starch cross-linked with 0.01% phosphoryl chloride (Fig 3.24)

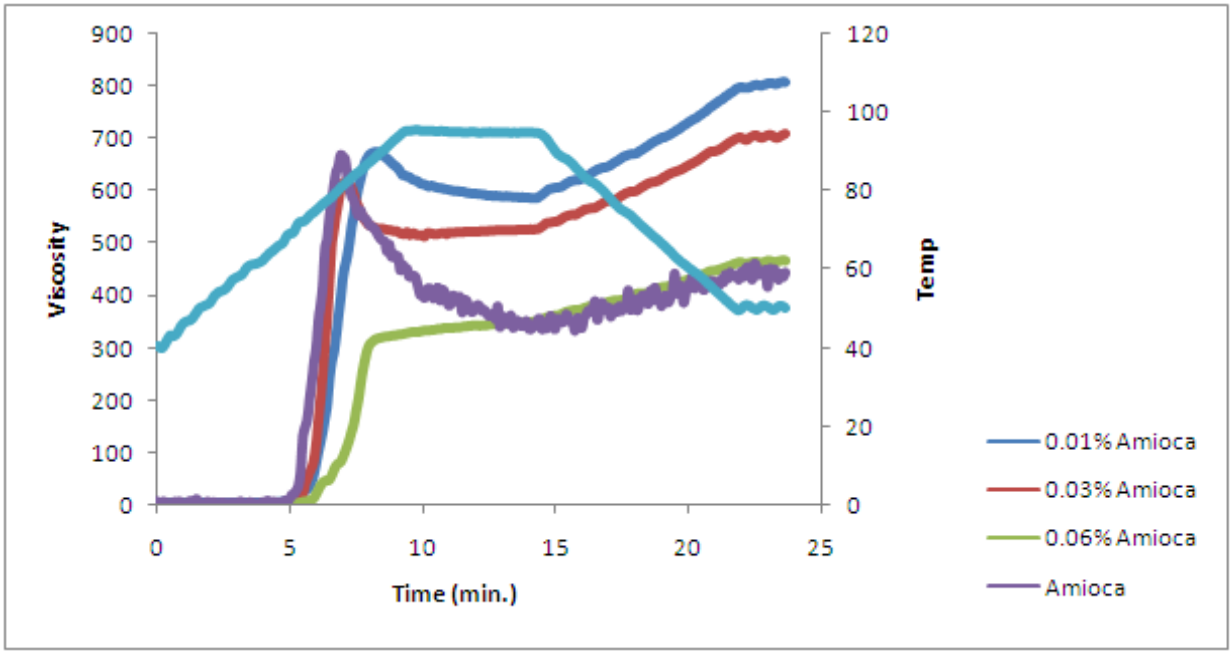


Figure 3.22 Viscoamylogram curves (6.0% starch solids basis) of cross-linked waxy corn starch (Amioca). Starch was reacted with 0.01%, 0.03% and 0.06% phosphoryl chloride (starch basis) (Heating rate 6^{o}C/min).

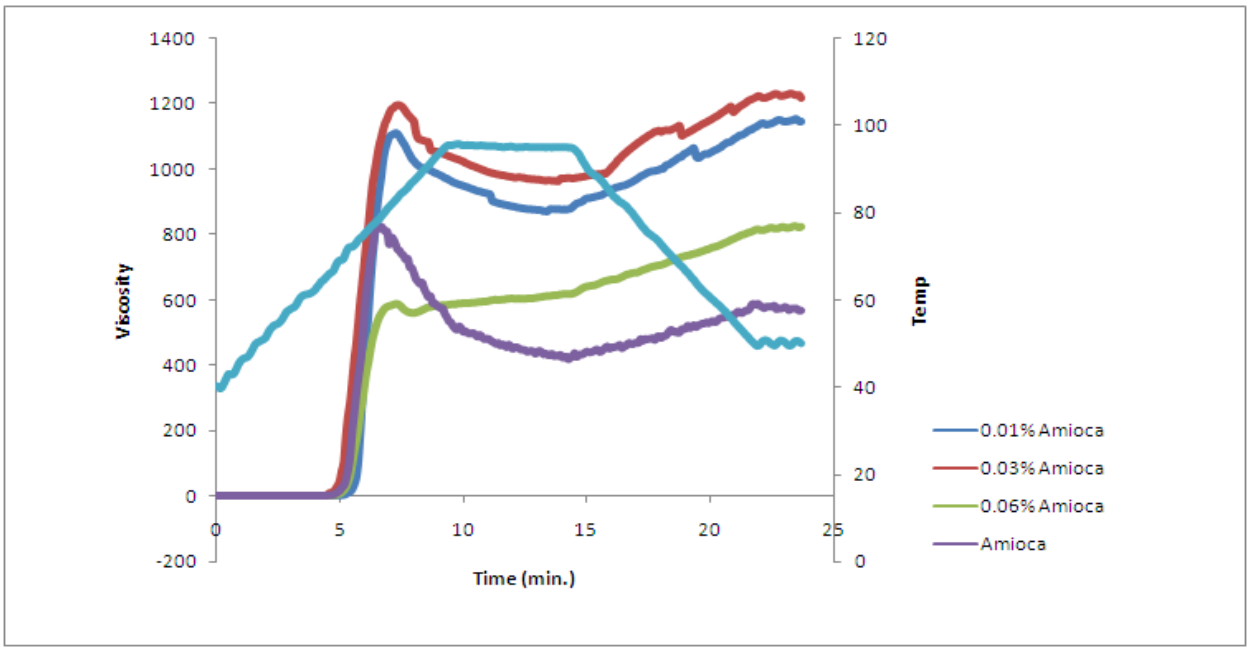


Figure 3.23 Viscoamylogram curves (7.0% starch solids basis) of cross-linked waxy corn starch (Amioca). Starch was reacted with 0.01%, 0.03% and 0.06% phosphoryl chloride (starch basis) (Heating rate 6°C/min).

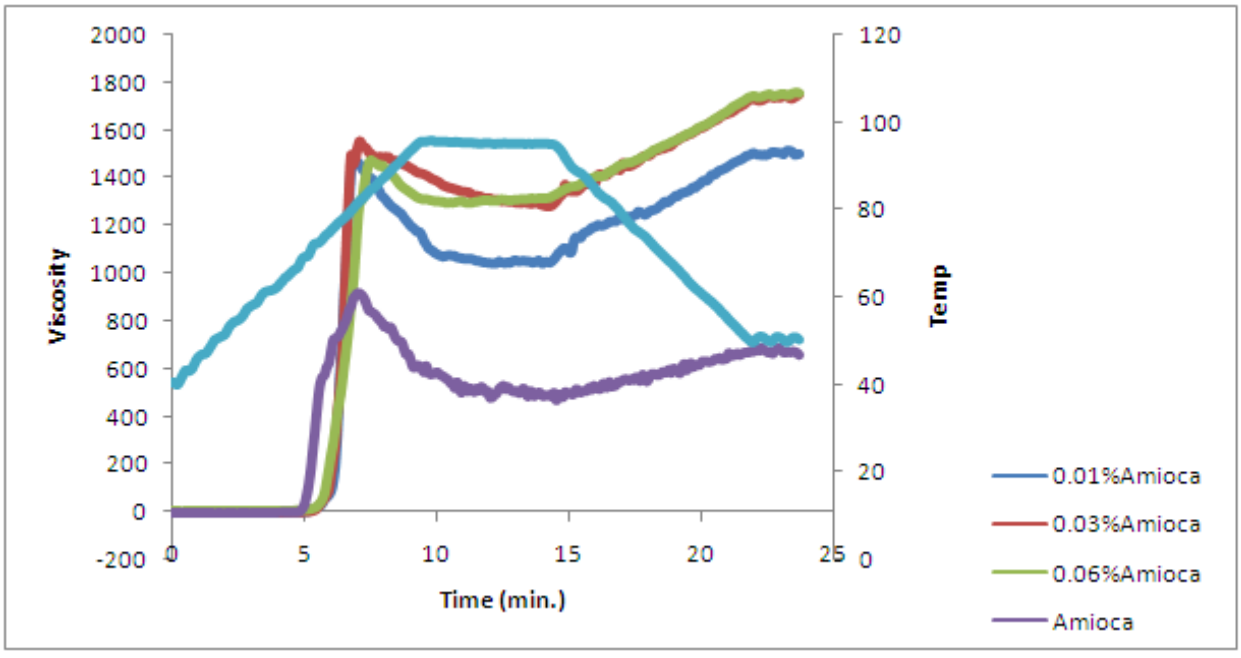


Figure 3.24 Viscoamylogram curves (8.0% starch solids basis) of cross-linked waxy corn starch (Amioca). Starch was reacted with 0.01%, 0.03% and 0.06% phosphoryl chloride (starch basis) (Heating rate 6°C/min).

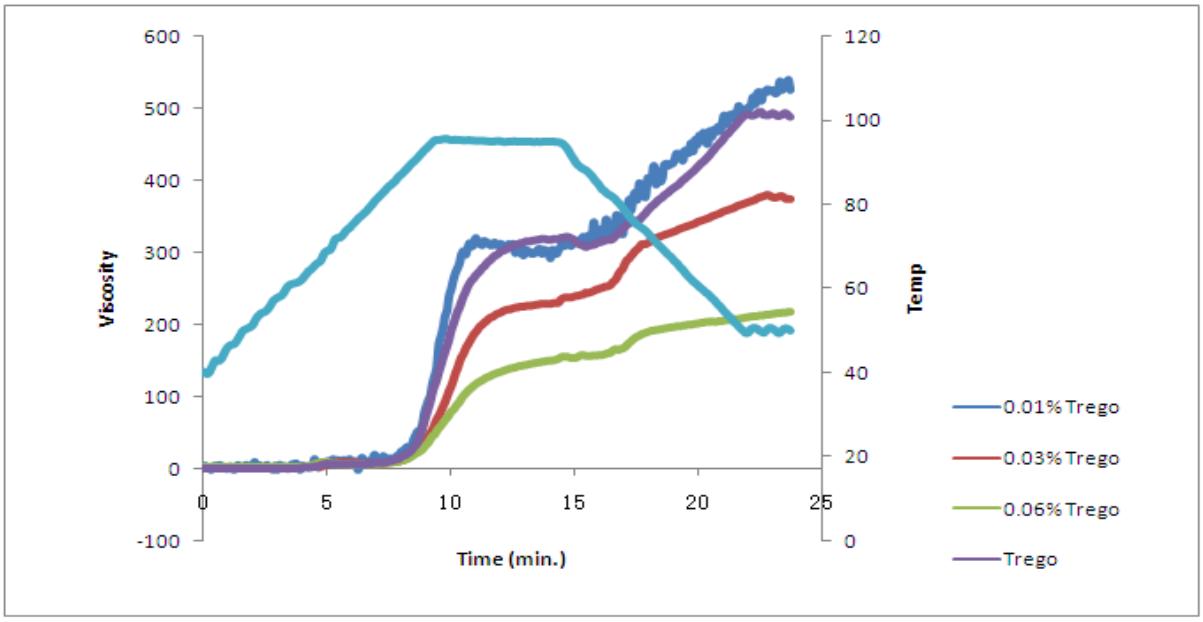


Figure 3.25 Viscoamylogram curves (7.0% starch solids basis) of cross-linked normal wheat starch (Trego). Starch was reacted with 0.01%, 0.03% and 0.06% phosphoryl chloride (starch basis) (Heating rate 6^{o}C/min).

Normal wheat starch was cross-linked in an aqueous slurry at about 37% starch solids with 0.01%, 0.03% and 0.06% phosphoryl chloride (starch basis). Visco-amylograms showed that increasing levels of phosphoryl chloride caused a steady decline in the peak and final paste consistencies at 7% solids of cross-linked normal wheat starch (Fig 3.25)

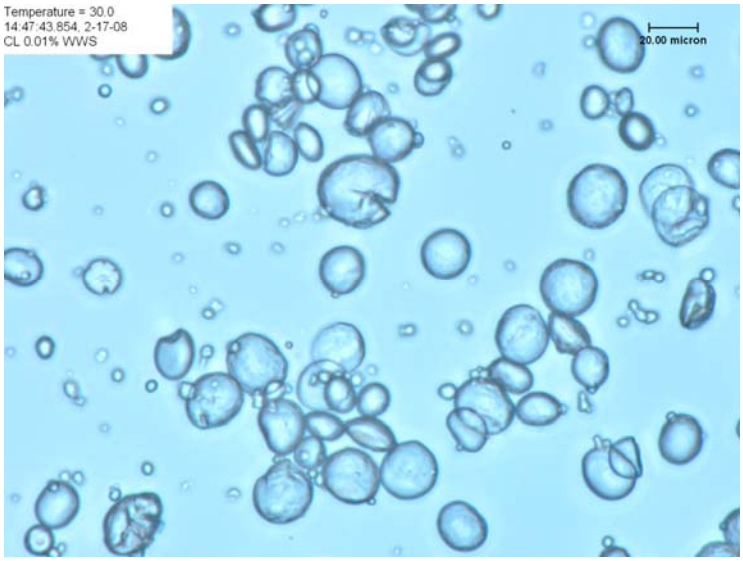
Swelling capacities of cross-linked starches were determined. Cross-linked starch (1g, db) and 50ml distilled water were placed in a beaker covered with parafilm and placed in a 75°C water bath for an hour. The suspension was transferred to a cylinder and diluted to100ml, and then rested at room temperature overnight. The volume of precipitate was recorded (Table 3.15).

Cross-linked waxy maize starches showed higher swelling capacities compared to cross-linked waxy wheat starches. As cross-linking level increase, both cross-linked waxy wheat and waxy maize starches have decreased swelling capacities.

Table 3.15 Swelling Capacities of Cross-linked Waxy Wheat Starch (NWX02Y2459) and Waxy Maize Starch (Amioca).

Cross-linked Starch	Volume (ml)
waxy wheat starch	
0.01% $POCl_3$	27
0.03% $POCl_3$	26
0.06% $POCl_3$	21
waxy maize starch	
0.01% $POCl_3$	32
0.03% $POCl_3$	28
0.06% $POCl_3$	25

Changes in morphology of cross-linked waxy wheat and waxy maize starch granules were recorded continuously during heating in water on a hot stage. Images (Fig 3.26) showed that waxy wheat starch cross-linked with 0.01% phosphoryl chloride (starch basis) started increasing in size at 50°C and continued up to 90°C. Cross-linked waxy wheat starch granules retained shapes even after being heated to 90°C. In contrast, waxy wheat starch granules disintegrated into many small fragments between 70-80°C depending on heating rate (Fig 3.16).


Temperature = 30.0
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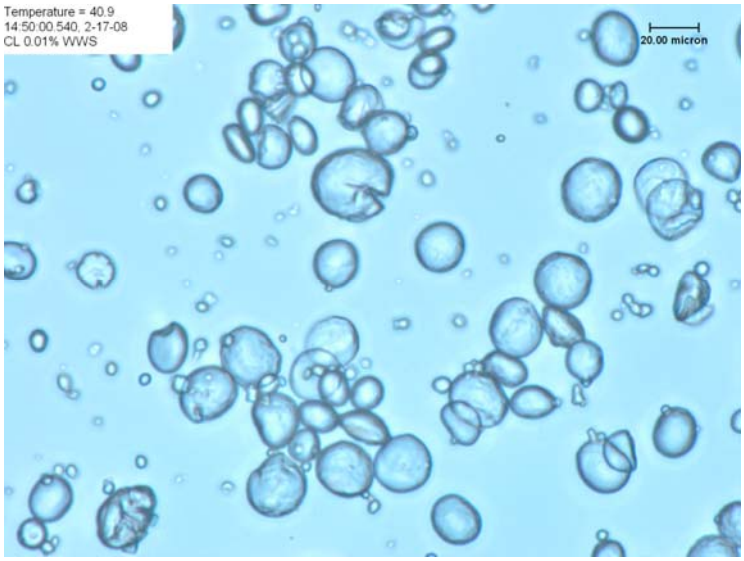
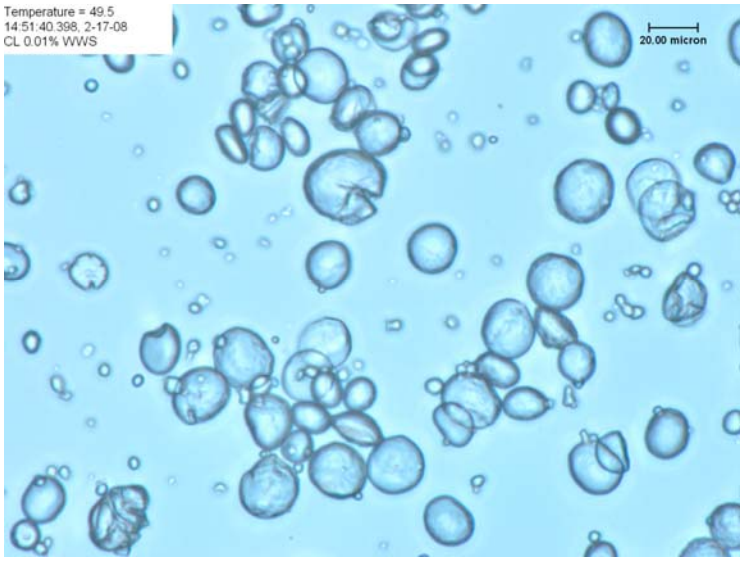
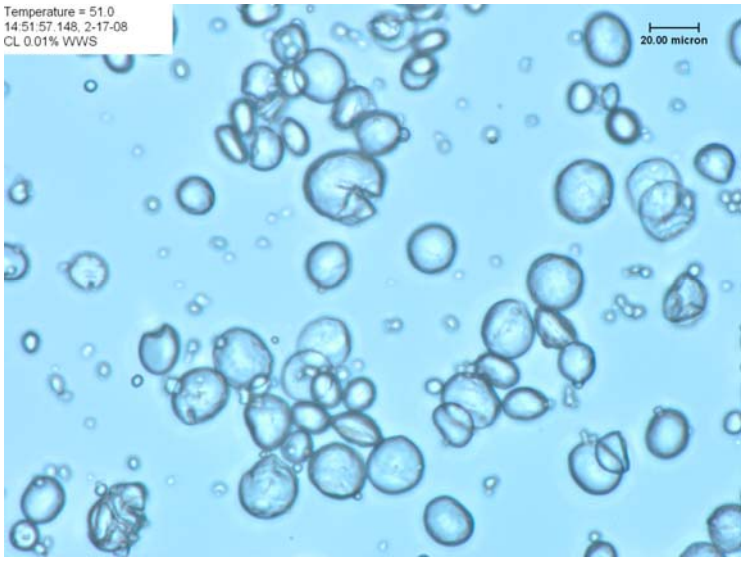
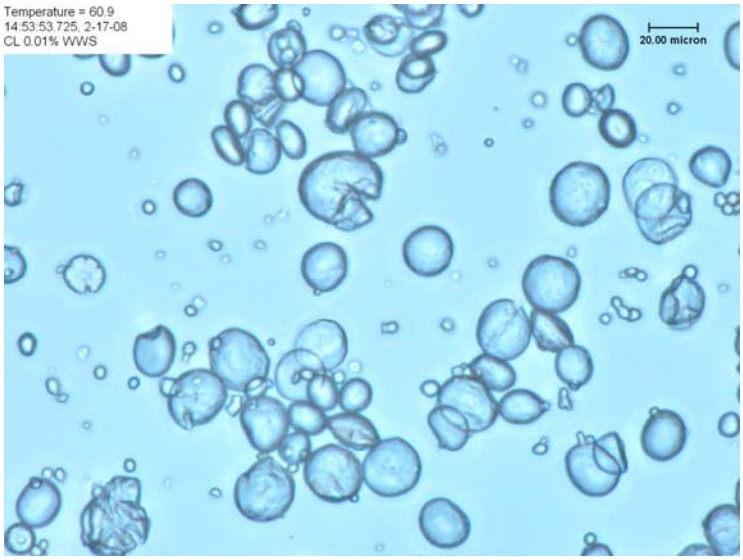
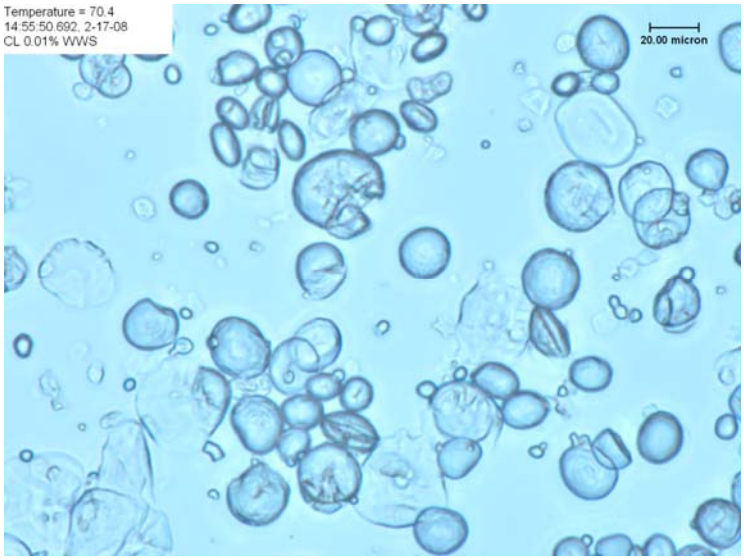
CL 0.01% WWS
20.00 micron


Temperature = 40.9
14:50:00.540, 2-17-08
CL 0.01% WWS
20.00 micron


Temperature = 49.5
14:51:40.398, 2-17-08
CL 0.01% WWS
20.00 micron


Temperature = 51.0
14:51:57.148, 2-17-08
CL 0.01% WWS
20.00 micron


Temperature = 60.9
14:53:53.725, 2-17-08
CL 0.01% WWS
20.00 micron


Temperature = 70.4
14:55:50.692, 2-17-08
CL 0.01% WWS
20.00 micron

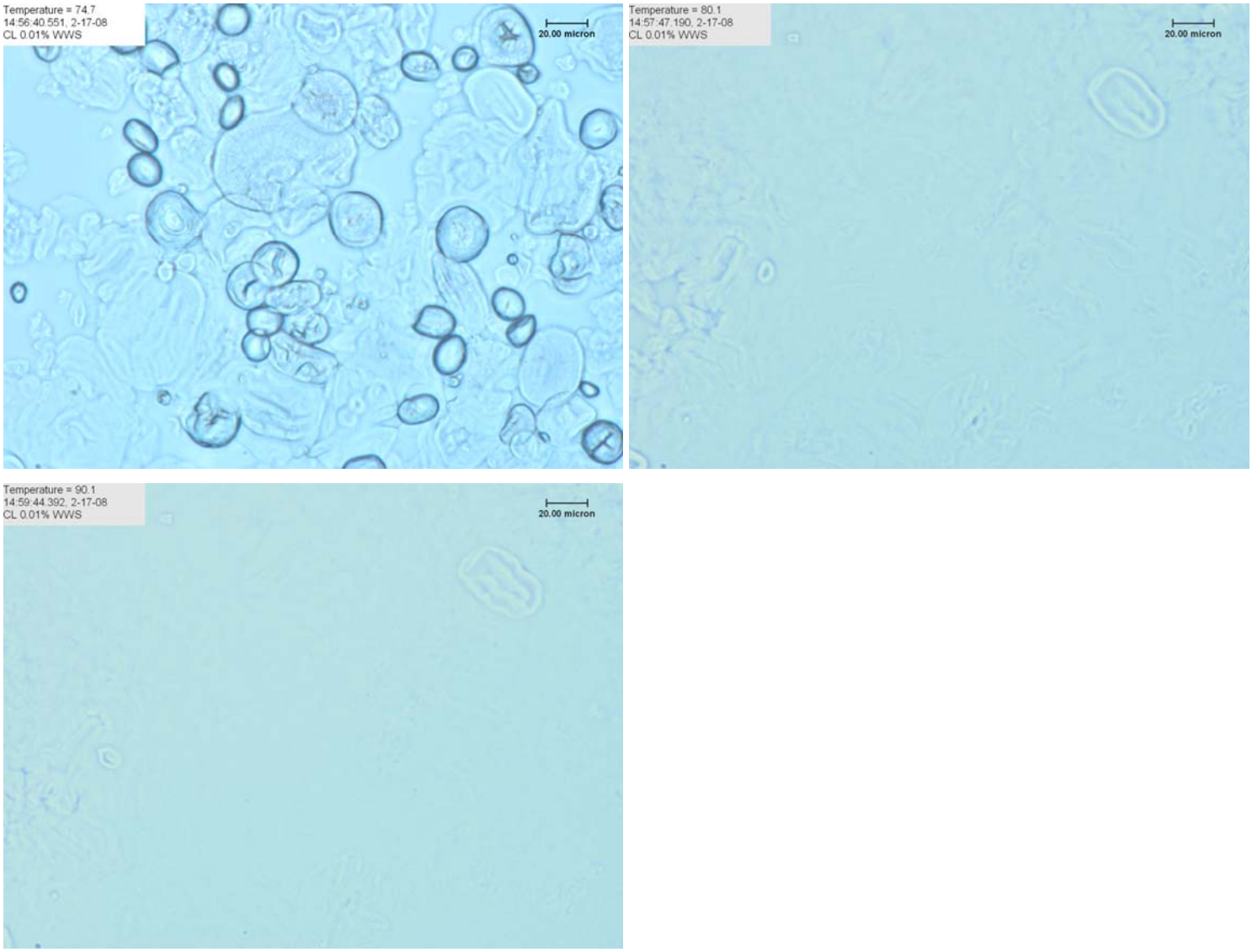


Figure 3.26 Microphotographs of morphology changes of waxy wheat starch cross-linked with 0.01% phosphoryl chloride (starch basis) suspension heated at 5°C/min.

CHAPTER 4 - Conclusions

(i) Six hard waxy wheats, all advanced breeding lines, give 68.8~73.3% of straight-grade flours. Compared to wet-milling (Martin process) the control doughs from Karl or Trego normal wheat varieties, washing any of the waxy wheat doughs under a stream of water causes a dough to become slack, to spread out more on the sieve and to break apart into several pieces. However, the slack dough pieces do not "blind" the screen and when approximately two-third of starch has been washed away, the small dough pieces coalesce into one elastic dough that behaves like the controls. These results suggest that the advanced lines of hard waxy wheats can be wet-processed in commercial operations.

(ii) A model-dough system and a water absorption experiment both suggest that waxy starch absorbs more water than normal starch. Increased water absorption by starch can affect the water distribution between starch and gluten, which in turn affects wheat dough rheology. By mixing a weak dough with 2% NaCl solution or by adding hemicellulase, the stickiness of a waxy wheat dough subsides during the washing step.

(iii) Waxy wheat starch, or its cross-linked form, gelatinizes and cooks to a thick paste at a relatively low temperature, and their pastes retrograde more slowly than those of waxy maize starch. Granule morphology recorded continuously on a hot-stage microscope indicates that waxy wheat starch granules swell rapidly at a ~47°C, but then disintegrated at ~72°C. Cross-linked waxy wheat starch also swells rapidly starting at ~50°C, but its granules do not disintegrate.

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