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Title

Effect of amylose content and gluten on gelatinization and retrogradation of starch blends and starch/gluten blends and on bread staling

By

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

CEREAL SCIENCE

ABSTRACT

Matković, Kornelija; Ph.D.; Program: Cereal Science; College of Agriculture, Food Systems, and Natural Resources; North Dakota State University; November 2010. Effect of Amylose Content and Gluten on Gelatinization and Retrogradation of Starch Blends and Starch/Gluten Blends and on Bread Staling. Major Professor: Dr. Frank A. Manthey.

Effect of amylose content and gluten on starch gelatinization and retrogradation properties, and consequently bread staling, still is not clear. In the case of starch and starch/gluten blends, information on the relationship between functional properties of starch blends and amylose and gluten contents is scarce. Effects of amylose content on baking and staling properties of bread were investigated by using 20, 30, and 40% blends of waxy spring (WS) or waxy durum (WD) wheat flour with non-waxy wheat flour. Crumbs with 30% and 40% waxy flour exhibited very open, porous structure. Retrogradation enthalpies and bread firmness were higher for waxy than for non-waxy crumbs and higher for WD than for WS crumbs at the end of storage (5 days), although waxy crumbs had a higher amount of soluble starch (especially WD crumbs) than non-waxy crumb. Results indicated that retrogradation and staling are complex processes that depend not only on amylose content, but also possibly on interactions of starch with other crumb components or interactions between two starches in a blend. To elucidate the effect of amylose content and gluten on properties of starch, blends of WD starch (0, 12.5, 25, 50, 75, and 100% w/w) and non-waxy starch, as well as starch blends combined with 30% gluten were studied. Gelatinization and retrogradation properties, as well as properties of soluble starch isolated from gels after 5, 10, 15, 20 days of storage and fractionated by gel permeation chromatography (GPC), were studied. Gelatinization enthalpy (ΔH) was higher for blends with low than for blends with high amylose content. However, ΔH was not significantly different between each consecutive blend although their amylose contents were different. Retrogradation enthalpy of starch blends (ΔH_{BR}) increased during 20 days

of storage. On each storage day, ΔH_{BR} was lower for low amylose blends than for high amylose blends, showing that low amylose content in starch blends slowed the process of retrogradation. Similar to ΔH, ΔH_{BR} was not significantly different between each consecutive blend. Apparently, gelatinization and retrogradation properties of starch blends with different amylose contents were more complex than in single starches and could not be interpreted as a simple sum of contributions of individual components. Gluten did not affect gelatinization enthalpy of starch blends due to excess amount of water in the system. However, it significantly lowered the ΔH_{BR} of low amylose blends (50, 75, 100% WD) compared to that of high amylose blends, especially on day 15 and day 20, which was interpreted as the result of gluten interacting with branched starch molecules. Analysis of GPC fractions of soluble starch showed that retrogradation patterns of 0 wx, 12.5 wx, and 25 wx blends were different, although their ΔH_{BR} were similar. Low proportion of branched fraction in 0 wx soluble starch after day 5 and low ratio of blue value/total peak carbohydrate on days 15 and 20 indicated retrogradation due to reassociation of branched molecules with long chains. In 12.5 wx and 25 wx soluble starch, low values for the wavelength of maximum iodine absorption (λ_{max}) of linear fraction indicated that some amylopectin fragments eluted with the linear fraction. Recrystallization of these molecules could have been facilitated by the presence of amylose in the fraction. Gluten affected retrogradation pattern of starch by promoting reassociation of branched molecules (reduction in λ_{max}) at the beginning of storage. All starch/gluten blends had similar retrogradation patterns. Overall, amylose content affected gelatinization and retrogradation properties of starch significantly; however, in starch blends these properties were not simple averages of properties of two starches. In addition to the amylose content, properties of blends also could be governed by specific interactions between two starches or between starch and gluten.

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LIST OF SYMBOLS/ABBREVIATIONS

AACC: American Association of Cereal Chemists International

ABS: Farinograph absorption

$\mathbf{a_w}$: Water activity

BD: Breakdown

BKD: Farinograph time to breakdown

BU: Brabender units

BV: Blue value

BV/CHO: Ratio of blue value and total carbohydrate

CHO: Carbohydrate

CPV: Cold paste viscosity

db: Dry basis

DDT: Farinograph dough development time

DMSO: dimethyl sulfoxide

DSC: Differential scanning calorimetry

g: Gram

GBSS: Granule bound starch synthase

GOPOD: Glucose oxidase/peroxidase reagent

GPC: Gel permeation chromatography

HP-SEC: High performance size exclusion chromatography

HPV: Hot paste viscosity

HRSW: Hhard red spring wheat

J/g: Joules/gram

L: Liter

M: Molar

mb: moisture basis

mg: miligram

min: minute

mL: mililiter

m*M* : milimolar

µm: micrometer

MTI: Farinograph mixing tolerance index

nm: nanometer

PT: Peak time

PV: Peak viscosity

RVA: Rapid Visco Analyzer

RVU: Rapid visco units

STB: Setback

STAB: Farinograph stability

T_c: Completion temperature of gelatinization

T_{cr} : Completion Temperature of Melting Retrograded

T_o: Onset temperature of gelatinization

$T_o - T_c$: Gelatinization temperature range

T_{or} : Onset Temperature of Melting Retrograded Starch

T_p: Peak temperature of gelatinization

T_{pr} : Peak Temperature of Melting Retrograded Starch

v/v: volume on a volume basis

WD: Waxy durum

WS: Waxy spring

wx: waxy starch blends

wxg: waxy starch/gluten blends

w/w: weight on a weight basis

ΔH: Enthalpy of gelatinization

ΔH_{BR}: Enthalpy of retrogradation normalized to amylopectin cntent

ΔH_{calc1}: Enthalpy of gelatinization calculated based on the percent of starch in blends.

ΔH_{calc2}: Enthalpy of gelatinization calculated based on % of starch in starch/gluten blends

ΔH_r: Enthalpy of retrogradation

λmax: Wavelength of maximum iodine absorption

°C: degree Celsius

GENERAL INTRODUCTION

Bread is a product that undergoes a complex process of staling during storage. Staling has been defined most commonly in terms of all changes, except microbiological spoilage, that occur during storage of a baked product, and that make the product less acceptable for consumers (Zobel and Kulp 1996; Baik and Chinachoti 2000; Schiraldi and Fessas 2001). One of the key quality criteria for consumers' acceptance of bread is freshness of crumb, which is manifested most obviously through the ability of bread to maintain crumb softness. Staling represents a significant loss for the baking industry (Zobel and Kulp 1996). Baking industry deals with the problem of bread staling by using a variety of antistaling ingredients such as alpha-amylases and different lipids (e.g. glycerol monostearate), that prolong the shelf-life of bread, but also increase the production cost.

Starch retrogradation has been recognized as the main cause of bread staling (Kim and D'Appolonia 1977b; Variano-Marston et al. 1980; Hug-Iten et al. 2001). Reassociation of amylose molecules starts within several hours after starch has been gelatinized, followed by much slower reassociation of amylopectin branch chains, formation of double helices, and eventually recrystallization (Miles et al. 1985). Since amylose retrogradation is believed to be complete within several hours after bread is baked, bread staling often has been attributed to amylopectin retrogradation (Eliasson and Larsson 1993; Zobel and Kulp 1996; Gray and BeMiller 2003; BeMiller 2007). Therefore, a reduction in content of either amylose or amylopectin could have significant effect on starch retrogradation and

staling of bread (Fredriksson et al. 1998; Abdel-Aal 2002; Martin et al. 2004; Mariotti et al. 2008). Several studies showed that wheat starch that contains very little or no amylose, known as waxy wheat starch, had high gelatinization enthalpy due to high amylopectin content and therefore high degree of relative crystallinity, but it also had low retrogradation enthalpy, which was an indication of slow retrogradation. Consequently, a potential use of waxy starch in retardation of bread staling has been proposed (Yasui et al. 1996; Hayakawa et al. 1997; Graybosch 1998; Yasui et al. 1999; Abdel-Aal et al. 2002).

Blends of waxy and normal (non-waxy) wheat flour, resulting in different amylose contents, have been tested for their effect on bread staling, but the results were inconsistent. Some studies showed retardation of staling (Bhattacharya et al. 2002; Morita et al. 2002b), showed no difference between waxy and normal flour in bread (Park and Baik 2007), or showed an increase in retrogradation enthalpy in bread that contained certain amount of waxy flour (Lee et al. 2001; Baik et al. 2003). Studies of starch blends with different amylose contents showed that gelatinization and retrogradation properties of starch blends were affected by amylose content, but the results also often were inconsistent. Differences between single and mixed wheat starches in their gelatinization properties were found in several studies (Fredriksson et al. 1998; Sasaki et al. 2000; Gupta et al. 2009) and were attributed to the lack of homogeneity in starch granules, and to specific associations between amylose and amylopectin in mixed starches that are different from those in single starches (Hagenimana et al. 2005; Hagenimana and Ding 2005). The effect of amylose content on retrogradation properties of starch

has been attributed to different phenomena such as phase separation of amylose and amylopectin in pastes and gels (Kalichevsky and Ring 1987; Leloup et al. 1991; Kim and Willet 2004), and more often to interactions between amylose and amylopectin (Jane and Chen 1992; Boltz and Thompson 1999; Klucinec and Thompson 1999; Klucinec and Thompson 2002; Sasaki et al. 2007; Yu et al. 2009). Studies on retrogradation properties of starch blends are scarce and results varied from increased retrogradation of low amylose starch blends (Sasaki et al. 2000) to no measurable retrogradation endotherm of blends (Obanni and BeMiller 1997), and eventually to low amylose content suppressing retrogradation (Yu et al. 2009). Often, the experimental conditions (moisture level, % amylose, types of starch) are different among studies, which causes an additional difficulty in interpretation of the results. Overall, current findings indicate that blending two wheat starches to manipulate amylose content may not be a simple solution to retrogradation and bread staling. However, if starch with lower amylose content than that of normal wheat starch was proven to be efficient in staling retardation, the only viable way for baking industry to obtain certain amylose content would be by blending normal and waxy wheat flours. Therefore, research is needed to understand the effect of amylose content on gelatinization and retrogradation properties of starch blends.

Gluten, as the second most abundant component in wheat flour, could possibly affect the process of starch gelatinization and retrogradation. Martin and Hoseney (1991) and Martin et al. (1991) pointed out that bread firming, which is one of the main characteristics of bread staling, was not synonymous with starch

recrystallization, and attributed staling to a possible gluten and starch interaction. However, the nature and mechanism of this interaction still remains unclear. A small number of studies investigated the effect of gluten on gelatinization (Eliasson 1983a; Erdogdu et al.1995; Chevallier and Colonna 1999; Mohamed and Rayas-Duarte 2003) and retrogradation (Eliasson 1983b; Lindahl and Eliasson 1986; Champenois et al. 1998; Ottenhof and Farhat 2004; Wang et al. 2004), but the results often were contradictory.

Bread presents a very complex system to study a possible interaction between two components, or to elucidate the effect of a single component without interference of others. In order to study the functionality of a single flour component or its interaction with other components, gel model systems often are used. A gel system made of starch and gluten can be considered as a simplified model of bread dough (Eliasson 1993; Petrofsky and Hoseney 1995; Eliasson and Gudmundsson 1996; Champenois et al. 1998).

The objectives of this study were: 1) to investigate the effect of amylose content on baking and staling properties of bread by using blends of waxy spring (WS) and waxy durum (WD) wheat flour with a single hard red spring wheat flour; 2) to compare the effect of WD and WS flour on quality and staling properties of bread; 3) to investigate the effect of amylose content on gelatinization and retrogradation properties of starch blends and starch/gluten blends by using gel model systems; and 4) to determine possible differences in retrogradation patterns during storage of soluble starch isolated from starch and starch/gluten gels.

LITERATURE REVIEW

Properties of Wheat Starch

Starch is a reserve carbohydrate of many plants. It is the most abundant component of wheat grain. Unique properties of starch determine its functionality in many food products, particularly baked products. Native starch occurs in the form of cold water insoluble granules that are semi-crystalline with varying polymorphic types and degrees of crystallinity depending on the botanical source. Wheat starch granules have a bimodal distribution: spherical B-granules (1 - 10μm) and lenticular A-granules (15 – 35 μm) (Biliaderis 1998).

Wheat starch is a polymer composed of α-D-glucose molecules that are linked by α – (1→4) glycosidic linkages to form linear chains, whereas branched chains are formed through the connection of α – (1→4) chains via α – (1→6) linkages. The number of monosaccharide molecules in a polysaccharide is known as the degree of polymerization (DP). Starch granules are composed of two main polysaccharides, amylose and amylopectin that differ in the degree of polymerization and branch frequency (BeMiller 2007; Maningat et al. 2009). Normal wheat starch contains on average 25% amylose and 75% amylopectin (Maningat et al. 2009), and its molecular composition and semi-crystalline structure determine its behavior during granule swelling, gelatinization, and retrogradation in bread baking and subsequent storage.

Amylose is the linear component of starch; however, it is slightly branched having 0.2 - 0.8% α – (1→6) branch points per molecule. The number average DP of wheat amylose is 1000 – 5000, while the side chains are mainly small with

DP~18 (Maningat et al. 2009; Pérez et al. 2009).

Amylopectin is a highly branched molecule with number average DP of 10,000 or higher, and with 4 – 6% branch points of total linkages. Amylopectin molecules consist of A, B, and C chains. The A chains are the outermost chains and have no branching points. B chains carry one or more A and/or B chains that are linked via α – (1→6) linkages, while the C chain is the chain that carries the only reducing end and to which B chains are linked through α – (1→6) linkages. Unit chains of amylopectin consist on average of 18 -25 glucose units linked by α – (1→4) linkages. The A:B chains ratio in wheat starch is 1.2:2.0. In wheat starch, amylopectin has a polymodal chain length distribution with A chains having a DP of 6-12, B1 chains 13-24, B2 chains 25-36, and long B chains (B3-B5) having a DP >37. Short chains (A+B1) are prevalent with distribution of 85-93% by number (Hanashiro et al. 1996).

Amylopectin molecules are responsible for the crystallinity in wheat starch granules. Short branch chains of amylopectin intertwine into double helices that can form ordered structures (crystallites). According to the cluster model of amylopectin, crystalline lamellae in starch granule alternate with amorphous lamellae at a periodicity of 9 nm (Manners and Matherson 1981; Manners 1989). Crystalline part of wheat starch is in a form of A polymorph where double helices are arranged in a monoclinic lattice with approximately 4% water (BeMiller 2007; Maningat et al. 2009). Large proportion of short branch chains (A and B1) in wheat amylopectin causes these chains to be located within one cluster (Gidley 1987), unlike in some other starches (e.g. potato) where one B chains can

participate in more than one crystalline cluster (Hizukuri 1986).

Starch Gelatinization

Heat and water induced transformations of starch during baking and aging of bread determine the structure and texture of the final baked product (Zobel and Kulp 1996). During mixing of dough, starch is in its native granule form and absorbs only a small amount of water due to the semi-crystalline nature of starch granule and due to hydroxyl groups being involved in hydrogen bonding (Eliasson and Gudmundsson 1996). During baking starch undergoes gelatinization. Gelatinization is a process that is induced by heat and water and that results in irreversible changes of the granule morphology and molecular arrangement.

When starch is heated in water, the granules absorb water and swell. Swelling of the granule is thought to be primarily a property of amylopectin molecules (Tester and Morrison 1992). For wheat starch, the volume of swollen granules was found to be over 20 mL/g dry starch (Eliasson 1986). Swelling is facilitated by breaking of intermolecular hydrogen bonds in starch and increased binding of water by available hydrogen bonds. Due to their semi-crystalline nature, starch granules undergo characteristic phase transitions during heating in water: 1) glass transition that causes transition of amorphous region from glassy to rubbery form, and 2) melting of crystalline region (Billiaderis 2009).

Gelatinization is a collective term used to describe irreversible changes that involve loss of birefringence, granular swelling, melting of crystallites, disruption of granules and solubilization of starch that results in leaching of amylose and some

amylopectin from granule (Atwell et al. 1988). These events result in an increase in viscosity of starch paste (Waniska and Gomez 1992; Eliasson and Gudmundsson 1996; Zobel and Kulp 1996). The process of gelatinization is monitored by measuring the rheological properties of starch paste, enthalpy of gelatinization (energy needed to melt the crystalline region of starch), and the changes in the X-ray diffraction pattern of gelatinized starch.

Several models have been proposed that describe the processes in granules during gelatinization (Donovan 1979; Blanshard 1987; Jenkins and Donald 1998; Waigh et al. 2000a,b). The current interpretation of macromolecular changes during gelatinization presents gelatinization as a granule swelling driven process (Donovan 1979; Blanshard 1987). Amorphous growth rings in starch granule expand as a result of water absorption. Blanshard (1987) postulated that heating starch in excess water causes mobilization of starch chains in amorphous region that leads to extensive swelling and disruption of crystallites. According to Jenkins and Donald (1998), the amorphous region is connected with amylopectin molecules at the edges of lamellar stacks, thus providing backbone to the granule. Therefore, expansion of the amorphous region imposes a stress upon the amylopectin crystallites and causes disruption of the semi-crystalline lamellae, reduction of granule crystallinity, and loss of birefringence.

Cooke and Gidley (1992) provided an important insight into the mechanism of gelatinization. They proposed that disruption of molecular (double-helical) and crystalline order occurs simultaneously, but that the endothermic enthalpy (measured by differential scanning calorimetry) during gelatinization reflects the

loss of double helical order rather than loss of crystallinity. Further elaboration of gelatinization was provided by Waigh et al. (2000 a, b) in a liquid crystalline model. According to this model, gelatinization is a two stage process where amylopectin double helices disassociate from their lamellar crystallites followed by unwinding of helices and transformation into a coil form. In excess amounts of water (> 40%, w/w), these two stages occur at the same time and give a rise to a single endotherm measured by DSC. Recent research conducted by Ratnayake and Jackson (2007) shows that amylose molecules in the amorphous region rearrange and form new intermolecular bonds prior to the initiation of the granule breakdown. These new molecular arrangements and intermolecular bonds come with an array of different thermal stabilities that could have a significant effect on the course of gelatinization and gelatinization temperatures.

Gelatinization temperature is related to crystallite size and perfection (Tester and Morrison 1990; Biliaderis 2009). For pure starches, large crystalline regions in the starch granule are created with high number of hydrogen bonds that do not break until high temperatures are reached (Kohyama et al. 2004). When heated in excess water, wheat starch shows endothermic peak at around 60°C (Eliasson 1980).

The material that leaches out of starch granules is composed mainly of amylose. Doublier (1981) suggested that in wheat starches most of the amylose is solubilized before the leaching of amylopectin starts. The solubilized material becomes more branched and increases in molecular weight with increased temperature (Ellis and Ring 1985; Prentice and Stark 1992). Microscopy of

gelatinized starch has shown that amylose leaches out of the granule into intergranular phase or into the center of granule, while amylopectin and amylopectin fragments are concentrated in the outer layers of granule (Langton and Hermansson 1989; Conde-Petit et al. 1998; Hug-Iten et al. 1999).

Effect of Amylose Content on Gelatinization

Studies of the effect of amylose content on gelatinization of wheat starch are hindered to a certain degree by the fact that wheat starch does not occur naturally with a wide range of amylose contents (Maningat et al. 2009). Therefore, the majority of studies have been done on corn starch because corn has a variety of mutants with different amylose contents (Campbell et al. 1999). The recent introduction of wheat with no/very low amylose content, known as waxy wheat, enabled blending of starches to obtain different amylose contents. Gelatinization properties of waxy wheat starch in relation to amylose content are discussed in the section "Waxy Wheat: properties and Applications in Bread". However, blends may exhibit specific properties, not necessarily related to amylose content, which have to be taken into consideration.

The gelatinization enthalpy of corn starches with different amylose contents (single starches, not blends) increased when the amount of amylose decreased (Liu et al. 2006). Sasaki et al. (2000) compared the gelatinization properties of starch blends made of waxy and nonwaxy wheat. The enthalpy and completion temperature of gelatinization correlated negatively with amylose content. However, they also pointed out that the association between amylose and amylopectin in

mixed starch could be different from that in a single starch. According to Lu et al. (2009), mixing two different types of starch (high-amylose rice and waxy rice) caused an increase in peak temperature of gelatinization compared to single starches, regardless of the amylose content. Gupta et al. (2009) reported that mixed starches had lower onset and peak temperatures of gelatinization than single starches with the same amylose content. Hagenimana et al. (2005) studied the gelatinization properties of single and mixed rice starches with different amylose contents, and found that waxy rice had the highest enthalpy, which was attributed to the high amylopectin content. Some mixed starches exhibited double endothermic peaks, which was interpreted as the results of two separate gelatinization processes in mixed starches.

In a study of different starches (wheat, rye, barley, corn, pea, and potato) amylose content was negatively correlated with the onset and the peak temperatures of gelatinization (Fredriksson et al. 1998). Jane et al. (1999) analyzed starches from a variety of botanical sources with different amylose contents, but no direct correlation was found between amylose content and gelatinization enthalpy; most likely because of different molecular properties of starches. The results showed that amylopectin branch chain length affected gelatinization temperature and gelatinization enthalpy of starch. Gelatinization temperature of starch increased with increasing branch chain length. Gelatinization is affected by molecular properties of starch. Yuan et al. (1993) studied different waxy corn genotypes, and reported the highest gelatinization temperature and enthalpy for starches with high proportion of long amylopectin

chains. The authors suggested that these chains could form long double helices that would require higher temperature to dissociate than short double helices.

Effect of Gluten on Gelatinization

The effect of gluten on starch gelatinization has been investigated in several studies, and the results do not lead to a uniform conclusion. Gluten increased the gelatinization temperature of the starch in the study of Eliasson (1983a). The effect of gluten was attributed to a small amount of water available to the starch in the presence of gluten. The author also postulated that another effect of gluten could be that its presence on the starch granule reduced the leaching of amylose from granules during gelatinization. Increase in onset and peak temperatures of gelatinization in the presence of gluten also was observed by Mohamed and Rayas-Duarte (2003) (60% water content) and by Li et al. (2007) for corn starch/soy protein blends (80% water content).

Lower enthalpies of gelatinization for starch/gluten blends than for pure starch were reported by Eliasson (1983a) and by Mohamed and Rayas-Duarte (2003). According to Eliasson (1983a), the lesser amount of water available for starch gelatinization decreased the degree of gelatinization and consequently the enthalpy of gelatinization, which was attributed to competition for water between starch and gluten. Mohamed and Rayas-Duarte (2003) proposed that gluten fibrils formed a network that restricted access of water to starch granules and caused incomplete gelatinization (hence low enthalpy). Hamaker and Griffin (1993) showed that proteins with disulfide bonds in the rice flour restricted starch granule

swelling during gelatinization and made the swollen granules less susceptible to disruption by shear. When the disulfide bonds were cleaved, the degree of gelatinization increased. Contrary to these results, Chevallier and Colonna (1999) and Erdogdu et al. (1995) found no evidence of the effect of gluten on starch gelatinization. Chevallier and Colonna (1999) reported that the endotherm had the same shape as the endotherm of starch gelatinized in 80% water.

Starch Retrogradation

Gelatinized starch undergoes significant transformations upon cooling and aging of bread starch pastes. The term retrogradation was introduced in 1902 by Lindet to describe changes in starch that decreased the soluble starch content in stale bread. Retrogradation has been defined by Atwell et al. (1988):

> Retrogradation of starch is the event which occurs when starch molecules begin to reassociate into ordered structures. In its initial phase, two or more molecules may form a simple juncture point which then can develop into more extensively ordered regions. Ultimately, under favorable conditions, crystalline order appears.

According to this definition, retrogradation involves both gelation and crystallization of gelatinized starch. As explained by Zobel and Kulp (1996), two starch molecules can intertwine into a double helix and form a juncture point, but this does not necessarily result in immediate formation of crystalline structure. Starch gel can be regarded as a hydrated polymer composite, where dispersed phase (swollen granules mainly containing amylopectin) is embedded in a

continuous matrix (mainly entangled amylose that leached out of the granules) (Ring 1985). The thermal and mechanical properties of such complex system depend on the properties of the dispersed and continuous phases (Eliasson 1986). The rate and extent of retrogradation depends on many factors such as ratio of amylose and amylopectin, structure of amylose and amylopectin and their degree of polymerization (DP), starch concentration, time, temperature, method of cooking, botanical source of starch, and type and concentration of added ingredients (Jacobson and BeMiller 1998; Biliaderis 2009).

Miles et al. (1985) described two stages of starch retrogradation. First, the short-term development of gel structure has been attributed to the irreversible re-association of amylose molecules into long double-helical structures. The second stage involves the long term processes of reversible re-association of amylopectin chains (shorter than amylose chains) into short double helices that eventually organize into crystallites (Miles et al. 1985; Ring et al. 1987). Miles et al (1985) suggested that partial crystallization within granules resulted in an increase in rigidity of the granules, which enhanced their reinforcement of the amylose matrix, and consequently of the whole gel network. Retrogradation process is facilitated by low temperatures because of reduced Brownian motion of molecules and therefore more intense intermolecular hydrogen bonding between amylopectin molecules (Tako and Hizukuri 2000).

During retrogradation, the amylose gel formation progresses through two stages (Morris 1990; Biliaderis 1992). First, a molecular aggregation takes place due to double helical associations of 40-70 glucose units (Jane and Robbyt 1984;

Liu et al 1997). In the second stage, double helices associate and organize into crystallites (Morris 1990; Biliaderis 1992). Gidley (1989) and Clark et al. (1989) studied amylose gelation. Gidley (1989) suggested that gelation of amylose occurs through association of amylose chains in double helices followed by aggregation of helices that form junction zones. Clark et al. (1989) found that DP of 1,100 is required for the formation of gel, while short chains, with DP below 300, results in turbidity and heterogeneous gel structure.

The concentration of amylose in the continuous phase appears to be the most important factor affecting the gel forming capacity of a heated starch dispersion. The critical concentration of amylose below which a gel cannot form is 0.8-1.1% (Hayashi et al. 1983; Doublier and Choplin 1989; Biliaderis 1992). Critical concentration for amylopectin to form a gel is much higher than for amylose (10% and above) (Biliaderis and Zawistowski 1990; Kalichevsky et al.1990). The gel formation rate is very slow, and unlike stiff amylose gels, amylopectin gels are initially less cohesive. Low temperatures are also required for gelation. Amylopectin forms shorter double helices than amylose due to the restrictions imposed by the branching structure of the molecules and the chain length of the branches. Studies of the mechanism of amylopectin gels indicate that amylopectin gels may form through the formation of short intermolecular aggregates (Miles et al. 1985; Ring et al. 1987). Ring et al. (1987) suggested that the small chains involved in the gel network were the exterior chains of the amylopectin molecule with the DP 10-20.

Role of Amylose Content and Amylose - Amylopectin Interaction in Retrogradation

Although much is known about the structure and behavior of amylose and amylopectin during gelatinization and retrogradation of starch, their possible interaction in gel network formation still is the subject of research. Some authors proposed the concept of phase separation of amylose and amylopectin in gel to explain the effect of amylose content on properties of gel (Kalichevsky and Ring 1987; Russell 1987; Leloup et al. 1991). Kalichevsky and Ring (1987) found that amylose and amylopectin are thermodynamically incompatible, and therefore immiscible in aqueous solutions. This leads to phase separation in retrograding gels, where one phase is rich in amylose and the other is rich in amylopectin. Phase separation leads to increase of effective concentrations of amylose and amylopectin in their microdomains, which leads to higher potential of re-association of molecules within each domain at concentrations at which they would not be able to interact and form a gel network alone.

Polymer composition of dispersed and continuous phase depends on amylose/ amylopectin ratio. At a certain ratio of amylose/amylopectin, an inversion of phases occurs and the continuous phase becomes discontinuous. Leloup et al. (1991) found that if amylose/amylopectin ratio is smaller than 0.43 (phase inversion point), the starch gel behaves amylopectin-like, while for ratios higher than 0.43 it behaves amylose-like. The results were explained in terms of supramolecular organization of gel, suggesting a phase-separated structure, with a continuous matrix of one polymer embedding microdomains of the other polymer. Another study found that the phase inversion point was 0.17 (Doublier and Llamas

1993). In normal wheat starch, most of the amylose leaches out from the granule during gelatinization. Coleaching of amylopectin is cited as responsible for the weakening of starch gels (Svegmark and Hermansson 1990; Hansen et al. 1991).

Studies of the effect of amylose content on retrogradation of wheat starch have similar limitations as described in the 'Starch Gelatinization' section discussed above. Therefore, most studies were conducted on blends of different starches. Obanni and BeMiller (1997) reported that after two weeks of storage at 4°C the majority of blends did not have a measurable retrogradation endotherm. Sasaki et al. (2000) found that waxy and non-waxy wheat starches and their blends that had low amylose (high amylopectin) content recrystallized to a high degree during 4 weeks of storage at 4°C. High amylose content was showed to facilitate retrogradation of rice starch, while low amylose content suppressed retrogradation (Yu et al. 2009). Ortega-Ojeda and Eliasson (2001) also observed increased retrogradation enthalpy during storage of gels made of different blended starches. Rheological properties of blends made of waxy and non-waxy wheat starch were investigated (Sasaki et al. 2002), and reported that blends with low amylose content produced weak gels that contained higher amount of solubilized starch than gels with high amylose content.

Several authors proposed an association between amylose and amylopectin during retrogradation (Obanni and BeMiller 1997; Tako and Hizukuri 2000; Klucinec and Thompson 2002). Obanni and BeMiller (1997) proposed that a possible reason for the lack of retrogradation endotherms in starch blends was related to the association of amylose with amylopectin and consequently to the

unavailability of these molecules for recrystallizaton. The authors speculated that the interaction between amylose and amylopectin in blends could be more intensive than in a single starch.

Klucinec and Thompson (2002) proposed a model of starch gelation where physical junction zones are formed between amylose molecules, amylose and amylopectin, and between amylopectin molecules. High proportion of amylose (50% w/w) in gel model system facilitated inclusion of amylopectin molecules into the network within one day, through the formation of internal elements from amylose-amylopectin junction zones (Klucinec and Thompson 2002). At low amylose content, the formation of amylose-amylopectin junction zones did not contribute to the gel network. The retrogradation enthalpy was higher for gels that contained amylose than for gels that contained the same concentration of amylopectin but no amylose. The study suggested that when amylose was present in the system, both small amylopectin molecules and amylopectin molecules with long side chains could form more internal elements due to the formation of amylose-amylopectin junction zones than the single waxy starch (high amylopectin starch).

Besides amylose content, other factors have been found to affect the process of retrogradation. Jane and Chen (1992) reported that retrogradation of amylose and amylopectin mixture cannot be explained based on simple combinations of gelation abilities of each component in the mixture. Klucinec and Thompson (1999) suggested that other factors, such as chain length distribution and molecular size of branched molecules, also are important for retrogradation of

starch gels. They suggested that the branched molecules in continuous phase influenced gel properties by inhibiting or altering amylose-amylose interactions.

Several studies showed that starch with large proportion of short amylopectin chains retrogrades slower than starch with a large proportion of long chains. Shi and Seib (1992) showed that starches with high mole fraction of DP 14-24 branches had higher tendency to retrograde than starches with high mole fraction of DP 6-9. High percent of short branch chains of amylopectin increased the proportion of non-crystalline regions in amylopectin and consequently slowed retrogradation. Waxy corn starches with a high proportion of fraction with DP 20-30 had pronounced gelling and retrogradation properties (Yuan and Thompson 1998).

Role of Gluten in Starch Retrogradation

The effect of gluten on starch retrogradation has been investigated mainly because of its possible relation to bread staling. Different results showed either increase or decrease in retrogradation in the presence of gluten, or no effect at all. Eliasson (1983b) measured the crystallization of starch in the presence of different amounts of gluten using differential scanning calorimeter (DSC), and found that gluten caused a decrease in the extent of starch crystallization during aging at 21°C, and an increase in the temperature that was needed to melt retrograded starch. Change in the amount of water available for crystallization was speculated to be one of the possible reasons for this effect. In the same study, the source of gluten (different wheat varieties) did not influence the enthalpy of retrogradation. Wang et al. (2004) used ^{1}H NMR relaxometry and found that gluten retarded

starch retrogradation by retarding water loss from granule remnants.

Champenois et al. (1998) and Chanvrier et al. (2005) studied the rheological properties of starch/gluten gels and found that gluten weakened the gel network. Champenois et al. (1998) examined the difference in rheological behavior of starch and starch/gluten blends upon heating and cooling. They found that gluten strongly changed the viscoelastic properties of the blends. Furthermore, gluten delayed the temperature at which the storage and loss moduli, began to increase upon heating of starch suspension. At the end of heating, storage modulus was lower when gluten was present in the starch suspension. At the end of cooling, the storage modulus of the formed paste decreased with increase in the amount of added gluten. The overall conclusion was that gluten weakened the strength of starch gels. The phenomenon was explained by gluten fibrils reducing the contact between the starch components and hindering the formation of starch network. According to Champenois et al. (1998), the rheological behavior of pure starch pastes and gels is determined by granules, i.e. by their ability to deform and pack in a network. In the presence of gluten, rheological properties are mostly influenced by gluten because its fibrils form "cells", which are filled with starch granules. The effect was attributed to phase separation of starch and gluten. Starch and gluten are thermodynamically incompatible polymers and therefore they tend to phase separate in aqueous mixtures (Tolstoguzov 1997). Champenois et al. (1998) hypothesized that gluten diluted the starch and therefore increased the critical concentration of starch needed for the formation of gel network.

Opposite to these findings, Lindahl and Eliasson (1986) found that addition

of 1% (dry basis) gluten to 6.5% (w/w) starch suspension increased the storage modulus of wheat starch, which was related to increase in starch retrogradation. Ottenhof and Farhat (2004) found no evidence of gluten effect on starch retrogradation when extruded wheat starch/gluten (10:1) blends were analyzed by DSC, X-ray diffraction and NMR relaxometry. However, the study was done with low gluten content and under limited water conditions that could have lead to non-uniform partitioning of water in the system.

Bread Staling

Bread staling has been researched extensively for more than a century and a half, but it still remains a topic of research due to its complexity. Bread staling presents a complex phenomenon, which is not understood completely at the molecular level. Both bread and staling processes are affected by multiple factors such as flour constituents and added baking ingredients, processing and storage conditions, which all together make it difficult to precisely elucidate the mechanism (or possibly mechanisms) of bread staling (Gray and BeMiller 2003). The currently accepted definition by Bechtel et al. (1953) describes staling as "a term which indicates decreasing consumer acceptance of bakery products caused by changes in crumb other than those resulting from the action of spoilage organisms". The first study on staling was reported by Boussingault in 1852. This study showed that the difference between fresh and aged bread was not due to the loss of moisture. Bread was kept in sealed containers to prevent moisture loss, but it still became stale.

Most researchers agree that retrogradation of starch is the main cause of bread staling. Lindet (1902) was the first researcher who attributed bread staling to starch retrogradation, which was assumed to be the cause of the decrease of the soluble starch content in stale bread. Lindet (1902) also stated that loss of moisture from starch during retrogradation was the result of the recrystallization of amorphous gelatinized starch. Katz (1928) proposed that a fundamental cause of bread staling was the transformation of starch from one physical form to another. The evidence presented in this work suggested that linear molecules in retrograded starch formed associations that lead to B-type crystallinity. Using X-ray diffraction technique, Katz et al. (1934 a, b) showed that the starch in bread and pastes underwent parallel changes during ageing. The X-ray diffraction patterns of fresh bread and freshly gelatinized starch were similar (V-type), while the X-ray pattern of stale bread was similar to that of retrograded starch (B-type). This work was followed by the work of Hellman et al. (1954) who showed that the crystallinity type in starch gels depended on the moisture level. In concentrated starch gels, the rate of development of crystallinity was similar to the rate of bread firming.

More in-depth studies of the staling mechanism and the role of amylose and amylopectin were made possible after Schoch (1945) succeeded in separating and characterizing the properties of amylose and amylopectin. Following this work, Schoch and French (1947) studied the water soluble material that was isolated from crumb at 30°C, and found that it consisted mainly of amylopectin. The amylopectin content of soluble starch decreased with aging of bread. The authors proposed that the staling of bread was due to spontaneous intermolecular

aggregation between side chains of amylopectin. The gradual association of amylopectin molecules caused firming of bread. The contribution of amylose to bread staling was suggested to be minor since amylose retrograded at such a high rate that most of it became insoluble during cooling of bread. The role of amylopectin in bread staling also was demonstrated in reheating experiments. Bread that was re-heated at 95°C regained its initial firmness due to melting of retrograded amylopectin. Similar results were reported by Pisesookbunterng et al. (1983) for the first re-heating of bread.

Kim and D'Appolonia (1977b) also studied the soluble starch isolated from bread during storage, and presented evidence that supported the findings of Schoch and French (1947). Kim and D'Appolonia (1977b) found that the soluble starch contained mainly amylopectin that progressively decreased during storage. The amount of amylose in soluble starch from fresh crumb was small, and its content sharply decreased during the first five hours after baking. The authors concluded that most of the amylose retrograded during bread cooling, but the remaining small amount of amylose that could be extracted during the first day could contribute to the staling of bread during the first day. However, the crystallization of amylopectin was found to be the main reason for bread staling during further storage, as indicated by the kinetic studies. Ghiasi et al. (1984) found that amylose contributed to retrogradation of waxy barley starch paste during the first day of storage.

Role of amylopectin in bread staling was further elaborated in DSC studies by Eliasson (1985). All treatments that were aged and that contained amylopectin,

such as bread, starch gels, and amylopectin gels, showed a distinct DSC endothermic peak between 50°C and 60°C. The area of the DSC endotherms depended on the amount of amylopectin in the treatment. Pure aged amylose gels did not show any endotherm when reheated below 100°C. Eliasson (1985) concluded that the DCS endotherm measured melting of recrystallized starch, which was responsible for bread staling.

The role of amylose in bread staling has been shown to be minor compared to the role of amylopectin. Retrograded amylose does not change during reheating of bread and it does not show an endothermic peak at temperatures between 50°C and 60°C since temperatures in the range of 120°C are required to reverse ordered structures in retrograded amylose (Zobel and Kulp 1996). However, rapid retrogradation of amylose is responsible for the initial setting of crumb structure (Hoseney et al. 1978), partially by 'cementing' together the swollen granules in bread crumb (Zobel and Kulp 1996). Hug-Iten et al. (1999) studied the structure of bread and starch gels by light microscopy and provided further evidence on the changes of amylose during bread staling. During baking, starch gelatinizes and due to phase separation amylose and amylopectin are not homogeneously distributed in the granules. The microstructure of bread showed that amylose was concentrated in the granule center, while outer layers of gelatinized granules were rich in amylopectin. The most intensive birefringence during storage was noticed in the granule center. The authors suggested that amylose in granule center reorganizes during storage, forms lateral chain associations, and eventually partially recrystallizes. These changes in amylose fraction were hypothesized to

enhance the rigidity of starch granules during bread staling.

Starch retrogradation and recrystallization often are used interchangeably in literature in discussions of bread staling. However, Zobel and Kulp (1996) pointed out that retrogradation of starch in bread does not always result in the formation of three-dimensional crystalline order. While crystallization is a form of retrogradation, retrogradation can be synonymous with crystallization only if the presence of crystallinity is proven by analysis. Taking into consideration current understanding of the role of amylose and amylopectin in bread staling, Zobel and Kulp (1996) proposed a model of staling, which is a modification of models by Schoch (1965) and Lineback (1984). This model shows molecular structures of starch during several stages: dough, fresh bread, stale bread, and bread refreshened by heating. In the dough stage, gluten is presented as a continuous phase that surrounds starch granules that contain amylopectin in crystalline form and amylose in amorphous form. Polar lipids are presented as components of starch granules that can interact with starch upon gelatinization. During baking, starch granules swell and the crystalline structure of amylopectin melts. Amylopectin is converted into an amorphous form, and while most of amylopectin is still located in the granules, it can also protrude in the intergranular space. Amylose leaches to intergranular space but due to its fast retrogradation it forms double helices in the fresh bread. This way amylose forms juncture points that facilitate gelation in the intergranular space, and consequently it is responsible for the initial firmness of bread after baking. According to the model, part of amylose that remains in the granule can form inclusion complexes with polar lipids. In stale bread, the model

presents formation of double-helices by amylopectin molecules and reorganization of amylopectin into crystalline regions. These ordered structures impart rigidity to the swollen granules and also act like cross-links in the gel network. At the same time, amylose develops cross-links with remaining granules. All the cross-links, whether they are double-helices or crystallites, promote continuity of the gel network; they interlock the neighboring granules and promote the firming of crumb. Gluten in this model has been presented as a continuous network between swollen starch granules. According to Zobel and Kulp (1996) gluten does not change during aging of bread and does not participate in any major interaction with starch granules.

Crumb Firmness and Retrogradation

Crumb firming is considered to be the most obvious manifestation of bread staling, and often staling is determined by correlating amylopectin recrystallization with crumb firmness. However, several studies have presented evidence that shows that bread firmness and starch crystallization do not necessarily occur concurrently, i.e. recrystallization of starch may or may not result in crumb firming. As early as in 1928, Alsberg proposed that retrogradation in starch pastes is slower than in bread, and therefore staling could not be attributed completely to starch retrogradation. Ghiasi et al. (1984) showed that the firmness of bread increased during seven days of storage, but retrogradation enthalpy did not change much after three days of storage. After reheating bread at 80°C, the firmness and retrogradation enthalpy were the same as for the one day old bread,

but subsequent storage resulted in firming rate increasing at a higher rate than the enthalpy of retrogradation. The authors concluded that the degree of retrogradation was not closely related to the rate of staling.

Hallberg and Chinachoti (1992) studied the glass transition temperature (T_g) of the amorphous phase during aging of bread. After storing bread in hermetically sealed pouch at room temperature for up to three days the T_g did not increase. Slade and Levine (1991) suggested previously that the T_g of bread may increase as the results of staling. The conclusion of Hallberg and Chinachoti (1992) was that firming of bread could be minimized by keeping the amorphous phase plasticized. Following this study, Hallberg and Chinachoti (2002) studied long shelf-life military bread and found that bread could retain its softness when it was stored in sealed pouches and when humectants were added to keep starch plasticized despite extensive recrystallization of amylopectin. Based on the results in this study, the authors proposed that in some cases the firmness of bread could be influenced by factors other than recrystallization of amylopectin, namely by controlling the changes in the amorphous regions. Besides amylopectin recrystallization, other factors have been found to impact bread firmness. Among the most obvious reasons is the migration of moisture from crumb to crust (Baik and Chinachoti 2000).

Role of Gluten in Bread Staling

The role of starch in bread staling has been studied extensively, and much of the evidence shows that starch plays a major role in bread staling. The role of

gluten in bread staling has been studied to a much lesser extent than the role of starch, and majority of studies disagree either on the role of gluten or its possible mechanism of action in bread staling. However, gluten is the second most abundant polymer in wheat flour, and its possible effect on bread staling deserves consideration. The effect of gluten on bread staling has been studied by using different approaches: using flours with different protein quantity and quality; reconstitution studies where flour fractions were interchanged; and using gel model systems made of starch and gluten.

Erlander and Erlander (1969) postulated that the retrogradation of starch could be inhibited by protein. They proposed that starch and gluten interact possibly via hydrogen bonds between amide groups of protein and hydroxyl groups of starch. According to these authors, the ratio of protein and starch is critical for bread firming. Kim and D'Appolonia (1977a) investigated the effect of flour protein content on bread staling, at two storage temperatures, and found that the staling rate of bread decreased as the protein content increased. However, kinetics study of starch recrystallization showed similar crystallization process in bread regardless of the protein content in the flour. The conclusion was that the primary effect of proteins in reducing bread staling rate was dilution of starch.

Opposite to the findings of Kim and D'Appolonia (1977a), Martin and Hoseney (1991) and Martin et al. (1991) proposed a possible model of bread firming, which involves significant role of gluten. This model involves protein fibrils, which represent the continuous gluten phase, and starch remnants and partially leached amylose, which represent discontinuous phase. The authors proposed

that bread firming was primarily due to hydrogen bonds forming cross-links between continuous and discontinuous phase.

Every et al. (1998) presented results that did not support either findings of Kim and D'Appolonia (1977a,b), or the model proposed by Martin et al. (1991) and Martin and Hoseney (1991). According to Every et al. (1998), gluten does not have a significant role in bread firming. The authors hypothesized that increase in bread firmness results from partially leached amylose and amylopectin chains, attached to swollen starch granules, forming hydrogen bonds with other starch granule remnants, and to a lesser degree with gluten fibrils.

Maleki et al. (1980) fractionated and exchanged components of flours of poor and good breadmaking quality to examine the effect of protein quality on bread staling. The authors postulated that gluten quality might affect firming rates of bread, and this phenomenon was explained with interactions among swollen starch granules, partial solubilization of starch molecules, and protein. In another study (He and Hoseney 1991), showed that poor quality gluten had more hydrophilic properties than gluten in flours of good quality. Therefore, poor quality gluten would interact more strongly with starch granules in dough, and these interactions would also be stronger during and after baking. The conclusion was that bread made with weak gluten flour firmed at a faster rate than bread made of strong gluten flour.

Water migration between components of bread also was considered as a possible reason for bread staling and/or bread firming. Senti and Dimler (1960) studied changes in starch and gluten during aging of bread, and found that

migration of water from starch to gluten occurred during bread staling. Majority of studies suggested the opposite, i.e. migration of water from gluten to starch. According to Willhoft (1973), changes that occur in gluten and starch during aging of bread (or gel) are due to formation of cross-links between gluten-gluten molecules and also between starch-starch molecules. The formation of cross-links could be associated with the release of water initially bound to the polymer chains (Schiraldi et al. 1996). Breaden and Willhoft (1971) and Willhoft (1973) hypothesized that water expelled from cross-linked gluten migrates to starch during bread staling, and that the crumbliness of stale bread depends on the partial dehydration of gluten. Other authors (Leung et al. 1983; Slade and Levine 1991; Chen et al. 1997) confirmed that starch takes up water released from gluten during bread staling, and that water mobility decreases due to its incorporation into crystalline structure of starch.

Waxy Wheat: Properties and Applications in Bread

Wheat starch typically contains 25-28% amylose and 72-75% amylopectin. Waxy wheat starch contains very little or no amylose. Amylose synthesis in the endosperm is controlled by the enzyme granule bound starch synthase (GBSS), also known as the 'waxy' protein (Tsai 1974; Graybosch 1998). Amylose content in normal, non-waxy wheat starch varies between 20 and 35%, whereas waxy starches contain less than 15% of amylose, depending on the number of null alleles (Tester et al. 2004). Chakraborty et al. (2004) reported amylose contents of 2.1 – 2.6% in several lines of waxy hard red spring and waxy durum wheat. In

hexaploid wheat, the GBSS is encoded by three loci (*Wx-A1, Wx-B1, Wx-D1*). Wheat lines carrying null alleles at one or two loci (partial waxy wheat) have reduced amylose content, whereas wheat carrying null alleles at all three loci ('full waxy wheat') is characterized by near complete absence of amylose (Graybosch 1998; Gaines et al. 2000).

Waxy wheat has been studied by several research groups and its unique properties have been characterized. X-ray diffraction analysis showed that waxy wheat starch has the same A-type crystalline pattern as the normal (non-waxy) wheat starch, and higher relative degree of crystallinity than normal wheat starch due to high amount of amylopectin (Fujita et al. 1998). Due to the lack of amylose, waxy starch has specific gelatinization, pasting, and retrogradation properties. Waxy starches were found to develop higher peak viscosity in a shorter time and at lower temperature than normal starches (Kiribuchi-Otobe et al. 1997; Hayakawa et al. 1997; Gaines et al. 2000; Sasaki et al. 2000; Abdel-Aal et al. 2002; Chakraborty et al. 2004; Hayakawa et al. 2004). Amylose was reported to suppress swelling of starch granule and to help reduce the loss of granular rigidity of swollen granules (Tsai et al. 1997). According to Tester and Morrison (1990), amylopectin is mainly responsible for starch granule swelling. Tester and Morrison (1990) attributed differences in pasting properties between normal and waxy starches to high levels of phospholipids in normal wheat starches that complex with amylose and restrict swelling of granules. Intensive swelling of starch granules reduces the quantity of free water in the starch/water system and causes development of high peak viscosity (Ming et al. 1997). Compared to normal wheat

starch, waxy starch granules easily disintegrate when heated in water, which results in low stability of paste viscosity, i.e. high breakdown and low setback as measured by Rapid Visco Analyzer (RVA) (Sasaki et al. 2000; Abdel-Aal et al. 2002; Chakraborty et al. 2004).

Thermal characteristics of waxy wheat were studied mostly by DSC. Yasui et al. (1996), Hayakawa et al. (1997), Fujita et al. (1998), Yasui et al. (2002), and Hung et al. (2007) reported that waxy wheat starch had higher gelatinization enthalpy than normal wheat starch, as well as higher gelatinization temperatures. Waxy wheat starch showed a single peak in DSC endotherm (Hung et al. 2004), which can be explained by the absence of the amylose-lipid complex in waxy starch. According to Eliasson (1980), the first endothermic peak corresponds to the gelatinization of starch, and the second peak corresponds to the melting of amylose-lipid complex (provided the starch suspension is heated above 100°C). High gelatinization enthalpy of waxy wheat starch was attributed to the high amount of amylopectin, i.e. lack of amylose, and consequently higher crystallinity than in normal starch (Hung et al. 2007). Contrary to these results, Kim et al. (2003) reported lower onset and peak temperatures of gelatinization for waxy than for normal wheat starch. Chakraborty et al. (2004) studied the properties of waxy hexaploid (hard red spring wheat) and waxy tetraploid (durum) wheat and found that waxy tetraploid starches had higher gelatinization enthalpy and higher gelatinization temperatures than the waxy hexaploid starches. Although these starches did not differ significantly in amylose content, their thermal properties were different, which lead to a conclusion that wheat class (hexaploid, tetraploid)

could play a significant role in properties of waxy starches.

The potential of waxy wheat flour in retardation of bread staling was investigated by several researchers, and different results were reported depending on the percent of waxy flour used, type of bread, bread-making procedures, as well as preferences for bread texture in different parts of the world. One of the rationale of using waxy flour to retard staling was the fact that amylopectin retrogrades more slowly than amylopectin, as showed by Miles et al. (1985).

Dough made of waxy flour exhibits different characteristics than dough made of normal wheat flour. One of the characteristics of waxy flours is unusual high Farinograph water absorption (Lee et al. 2001; Bhattacharya et al. 2002; Guo et al. 2003). This property of waxy flour often is attributed to high amylopectin content of waxy flour. In addition to high water absorption, Sahlstrom et al. (2006) and Park and Baik (2007) also reported short mixing times and weak doughs made of waxy flours. Waxy doughs exhibited lower stability during mixing than doughs made of normal wheat flour, and increased stickiness (Morita et al. 2002a; Hung et al. 2005). Results of several studies showed that waxy flour produced bread of inferior quality compared to the bread made of normal wheat. Texture of waxy bread crumb often is described as glutinous and sticky (Morita et al. 2002b; Baik et al. 2003). Hayakawa et al. (2004) provided an elaborate description of bread made of waxy wheat flour. Bread made of hexaploid waxy wheat flour had low volume and open crumb grain, with significant cave-in problem. Eating quality of waxy bread also was low, and the crumb was described as lumpy and sticky. Bread made of 100% waxy wheat flour often had a distorted shape, or could not retain its

shape and collapsed during cooling (Morita et al. 2002a; Hayakawa et al. 2004). The authors reported that stickiness of crumb was detected even at 5% level of waxy flour. At 50% level of waxy flour, the quality of bread crumb significantly deteriorated. Levels between 10% and 30% were suggested as acceptable.

Several studies reported improved softness of bread that was made with waxy flour at different levels. Martin et al. (2004) found that bread made from partial waxy wheat missing *Wx-B1* allele had higher loaf volume and lower firmness than bread made of control non-waxy wheat. Park and Baik (2007) also reported improved crumb softness of French bread with the use of partial waxy wheat flour; however, the loaf volume was smaller or comparable to the loaf volume of bread made with hard red spring wheat flour. The loaves made of waxy flour resulted in higher loaf volume and lower firmness during storage than the non-waxy ones. Partial substitution of common wheat flour with waxy wheat flour resulted in improved softness of bread crumb and retardation of staling at 20% and 40% level in the study of Morita et al. (2002b) and at 15% level in the study of Qin et al. (2009). Hayakawa et al. (2004) reported that bread with 5-30% waxy flour had soft crumb, but they also emphasized that bread containing high percent of waxy flour may become more stale than regular bread after long storage times (more than six days). Most studies on the effect of waxy wheat starch on bread quality involved hexaploid waxy wheat. Bhattacharya et al. (2002) conducted a study with full waxy durum (durum) wheat (null alleles at both *Wx-A1* and *Wx-B1* loci). Replacing 20% of the normal flour with waxy durum flour resulted in bread of equal quality to control and increased softness over a 5 day storage period.

Several studies on bread made with partial substitution of normal wheat flour with waxy flour showed that waxy flour lowered the retrogradation enthalpy of crumb, and therefore had the ability to retard bread staling (Bhattacharya et al. 2002; Morita et al. 2002b) Contrary to these findings, some studies showed that the retrogradation enthalpy of waxy and non-waxy bread did not differ (Park and Baik 2007), or that bread with waxy flour had higher retrogradation enthalpy than non-waxy bread (Lee et al. 2001; Baik et al 2003). Lee et al. (2001) reported that bread baked with 25-50% waxy starch and gluten had reduced crumb firmness compared to control bread (normal starch); however, the retrogradation enthalpy was higher for waxy breads than for normal breads. Park and Baik (2007) indicated that waxy flours could be expected to have high retrogradation enthalpies during storage due to their high proportion of amylopectin. Evidently, the effect of waxy flour on bread staling still is not clear.

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CHAPTER 1: EFFECTS OF WAXY WHEAT FLOUR BLENDS ON BREAD BAKING AND STALING PROPERTIES

ABSTRACT

Bread staling mainly is attributed to the retrogradation of starch. Therefore, different amylose contents of starch possibly could have different effects on the staling process of bread. Effects of blends of 20, 30, and 40% waxy spring (WS) wheat flour, waxy durum (WD) wheat flour, and non-waxy durum (Ben) with hard red spring wheat flour (Gunner) on baking and staling properties of bread were evaluated. Although amylose contents of two waxy blends were similar, their pasting properties were different and were very different from pasting properties of non-waxy flours. Rapid Visco Analyzer (RVA) showed dual viscosity peaks with all waxy blends. Waxy blends had higher enthalpy of gelatinization than non-waxy flours. WS and WD had similar enthalpies of gelatinization, but WD had higher gelatinization temperatures than WS blends and Ben blends. Crumbs made with 30% and 40% waxy flour exhibited very open, porous structure, which is unacceptable by standards of white panned bread. Moisture content of all crumbs was similar at the end of storage (5 days). Differences between RVA pasting profiles of WS and WD crumbs were even more pronounced than differences between their flours. Retrogradation enthalpies were higher for waxy than non-waxy crumbs and higher for WD than WS crumbs at the end of storage; although starch in waxy crumbs regained less of its initial crystallinity during storage than starch in Gunner crumb. Waxy crumbs had higher amount of soluble starch (especially WD crumbs) than Gunner crumb, yet their crumb firmness was higher indicating that crumb firmness does not depend only on starch retrogradation. Results indicate that WS and WD starch might have some structural differences

that impacted their functionality in bread. Differences in pasting properties, crumb firmness, soluble starch content, retrogradation enthalpy between WD and non-waxy durum (Ben) blends indicate that the functionality of WD flours in bread baking was not due to their durum nature but rather due to starch properties.

INTRODUCTION

Starch is the most abundant component of wheat flour and has unique functional properties that are crucial for the structure and shelf-life of bread. Major changes to starch occur during bread baking. Starch granules swell and gelatinize during baking due to the effect of moisture and heat. During gelatinization, starch granules lose their ordered crystalline structure and some of the starch leaches from the granules to intergranular space. Partially solubilized starch and swollen and fragmented granules are essential structural elements of bread (Keetels et al. 1996; Hug-Iten et al. 1999; Goesaert et al. 2004).

Bread staling is attributed mainly to the changes in starch structure upon cooling and aging of bread. These starch transformations include the reassociation of starch molecules that eventually results in recrystallization and retrogradation of starch. Solubilized amylose retrogrades within several hours after baking. Therefore, amylose retrogradation presents a determining factor for crumb setting and initial bread firmness. Amylopectin retrogradation requires several days. In several studies, bread staling has been attributed to amylopectin retrogradation, specifically the formation of double helical structures and crystalline regions (Miles et al. 1985; Eliasson and Larsson 1993; Zobel and Kulp 1996; Gray and BeMiller 2003). On the other hand, Hug-Iten et al. (2003) proposed that both formation of structured network, consisting of interlinked crystallites, and the molecular reorganization of the amylopectin-rich and amylose-rich regions in starch granules contribute to bread staling.

Gelatinization and retrogradation properties of starch are affected greatly by

starch amylose content. Therefore, a reduction in content of either amylose or amylopectin has significant effect on quality (mainly textural properties) and shelf-stability of food products (Fredriksson et al. 1998; Abdel-Aal 2002; Martin et al. 2004; Mariotti et al. 2008). Thermal properties determined by DSC showed that low amylose (waxy wheat) starches had high gelatinization enthalpy and high peak temperature of gelatinization (Yasui et al. 1996; Hayakawa et al. 1997); however; they also showed that waxy starches were more resistant to retrogradation. Therefore, the potential use of waxy starch in retardation of bread staling has been proposed (Graybosch 1998; Yasui et al. 1999; Abdel-Aal et al. 2002).

Amylose synthesis in the endosperm is controlled by the enzyme granule bound starch synthase (GBSS), also known as the 'waxy' protein (Tsai 1974; Graybosch 1998). Amylose content in normal, non-waxy wheat starch varies between 20 and 35%, whereas waxy starches contain less than 15% of amylose, depending on the number of null alleles (Tester et al. 2004). In hexaploid wheat, the GBSS is encoded by three loci (*Wx-A1, Wx-B1, Wx-D1*). Wheat lines carrying null alleles at one or two loci (partial waxy wheat) have reduced amylose content, whereas wheat carrying null alleles at all three loci ('full waxy wheat') is characterized by a near complete absence of amylose (Graybosch 1998; Gaines et al. 2000).

The potential of waxy wheat flour in retardation of bread staling was investigated by several researchers, and different results were reported depending on the percent of waxy flour used, type of bread, and bread-making procedures, as well as preferences for bread texture in different parts of the world. Martin et al.

(2004) found that bread made from partial waxy wheat missing *Wx-B1* allele had higher loaf volume and lower firmness than bread made of control non-waxy wheat. Park and Baik (2007) also reported improved crumb softness of French bread with the use of partial waxy wheat flour; however, the loaf volume was smaller or comparable to the loaf volume of bread made with hard red spring wheat flour. Using full waxy wheat in bread baking, Morita et al. (2002a) found that waxy flour had higher water absorption than non-waxy flour. The loaves made of waxy flour resulted in higher loaf volume and lower firmness during storage than the non-waxy ones. However, the 100% waxy flour bread collapsed during storage due to its sticky glutinous crumb structure. Partial substitution (20% and 40%) of common wheat flour with waxy wheat flour resulted in improved softness of bread crumb and retardation of staling at 20% and 40% substitution level in the study of Morita et al. (2002b) and at 15% level in the study of Peng et al. (2009).

Most studies on the effect of waxy wheat starch on bread quality involved hexaploid waxy wheat lines. Bhattacharya et al. (2002) conducted a study with full waxy durum (durum) wheat (null alleles at both *Wx-A1* and *Wx-B1* loci). Replacing 20% of the normal flour with waxy durum flour resulted in bread of equal quality to control and increased softness over a 5 day storage period. Following this study, it was shown that starches derived from waxy spring (WS) and waxy durum (WD) wheat exhibited some significant differences (Chakraborty et al. 2004). Although WD and WS starches did not differ significantly in amylose content, WD starches exhibited higher DSC enthalpy and transition temperatures, as well as lower RVA breakdown and setback than WS starches. These results suggest that both the

nature (waxy, non-waxy) and class (hexaploid, tetraploid) of wheat starch could play a significant role when using waxy flour blends in bread baking.

Tetraploid wheat is not used commonly for white panned bread, but a study by Boyacioglu and D'Appolonia (1994a) showed that tetraploid wheat could be used for bread, although the crumb texture and loaf volume were somewhat inferior compared to bread made with hexaploid wheat flour. Hareland and Puhr (1998) reported acceptable bread quality when durum flour was incorporated at the 60% level in bread formula using the sponge and dough baking method, which is a standard baking method in most commercial bakeries in the U.S.A. The external properties and crumb grain and texture of bread with 60% durum flour were similar to those of bread with 100% spring wheat flour whereas the crumb color was slightly yellow and the loaf volume was lower than that of spring wheat bread. Firming rate of the 60% durum crumb was higher than that of spring wheat bread; however, the enthalpy changes during storage were significantly slower for 60% durum bread than for spring wheat bread. Hareland and Puhr (1998) indicated that this difference between durum and spring wheat flour may be attributed to possible differences in amylose content, molecular structure of amylopectin, and pentosan content between two classes of wheat.

In order to further investigate the functional properties of WD and WS wheat starch in bread, a study was conducted using blends of full WD and non-waxy hard red spring flour and blends of full WS flour and non-waxy hard red spring flour. The objectives of this study were to determine the effect of amylose content on baking and staling properties of bread, to compare the effect of WD and WS starch on

quality and staling properties of bread, and to determine if the staling properties of waxy durum bread are due to waxy or durum nature of wheat.

MATERIALS AND METHODS

Wheat

An experimental full waxy spring wheat line (WS) (PI 619375, experimental line designation 99ID594) was used as a source of waxy spring flour. The line was developed and released in September 2002 by the USDA-ARS and the Nebraska Agricultural Experiment Station in cooperation with the Agricultural Experiment Stations of North Dakota and Idaho. The line was developed from Asian and North American sources of the *Wx* null alleles together with 18 other waxy spring wheat lines (Graybosch et al. 2004).

A waxy durum wheat line (WD) was used as source of waxy durum flour. This line was derived from an initial cross of hard red winter wheat, 'Ike', which carried null alleles at *Wx-A1* and *Wx-B1 loci,* and durum wheat cultivar 'Ben'. Subsequently, full waxy durum wheat lines were developed by backcrossing to Ben while selecting among backcross progeny for the full waxy genotype. The full waxy durum line, derived from the fourth backcross to the recurrent durum parent, Ben, was provided by Dr. Douglas Doehlert (USDA-ARS, Cereal Crops Research Unit, Fargo, ND).

Non-waxy commercial hard red spring wheat (HRSW) cultivar, 'Gunner' (developed by AgriPro Wheat, North Dakota) was used as a source of base flour. Durum cultivar Ben was selected as a source of durum flour.

Flour

All cultivars and lines were tempered and milled into a straight grade flour

using a Bühler laboratory mill according to AACC Approved Methods 26-10 and 26-21 (2000). Flour was stored 1 week at room temperature before analyses and baking test. Flour blends were made by combining 20, 30 and 40% (w/w) WS flour with non-waxy HRSW flour (Gunner); 20, 30 and 40% (w/w) WD flour with non-waxy HRSW flour (Gunner); and 20, 30 and 40% (w/w) durum flour (Ben) with non-waxy HRSW flour (Gunner).

Flour Analyses

Rheological properties of dough and water absorption for baking were determined using a Brabender Farinograph according to AACC Approved Method 54-21 (2000).

Moisture content of flour was determined by the air oven method (AACC Approved Method 44-15A, 2000). Protein content of flour was determined by the crude protein combustion method (AACC Approved Method 46-30, 2000) using Leco FP428 nitrogen analyzer (Leco Corporation, St. Joseph, MI).

The amount of damaged starch in flour was determined using the Megazyme assay kit (Megazyme International Ireland Ltd. Wicklow, Ireland) (AACC Approved Method 76-13, 2000). Activity of α-amylase was determined using Megazyme assay kit (Ceralpha Method, Megazyme International Ireland Ltd. Wicklow, Ireland) (AACC Approved Method 22-02, 2000).

Amylose and amylopectin content of starch in flours were determined using High-Performance-Size-Exclusion-Chromatography (HP-SEC) according to the method of Grant et al. (2002). Starch (20 mg) was solubilized by 4.5 mL of 1*M*

KOH and 0.5 mL of 6*M* urea and heated at 100°C, under nitrogen, for about 90 min. Solubilized starch (1 mL) was neutralized with1 mL of HCl and filtered through a 13-mm diameter, 0.45 µm hydrophilic polyvinylidine fluoride syringe filter. Amylose and amylopectin were separated on a Waters Ultrahydrogel Linear 6-13 µm, 7.8 x 300 mm column, and Ultrahydrogel guard column (Waters, Milford, MA) by using a Hewlett Packard (HP 1090) high-performance-liquid-chromatograph (Agilent Technologies, Wilmington, DE) with an autosampler. Refractive index (RI) detector (Hewlett Packard 1074A) and a PC with chemstation (HP ChemStation for LC Rev. A.04.01) were used to quantify amylose and amylopectin content in the sample. Samples were analyzed at 45°C with filtered deionized water as eluent. Flow rate was 0.3 mL/min, and the injection volume was 20 µL.

Pasting Properties of Flour

Pasting properties of flour blends and corresponding bread crumbs were determined by Rapid Visco Analyzer (RVA) (Newport Scientific, Narrabeen, Australia), as described by Bhattacharya et al. (1997, 1999). Flour (3.5 g, 14% mb) was weighed directly into an aluminum RVA sample canister, and 25 mL distilled water (14% mb) was added and mixed thoroughly with the sample. Flour samples were run in 1 mM solution of silver nitrate in order to inhibit possible α-amylase activity (Hutchinson 1966; Meredith et al. 1971). A programmed heating and cooling cycle (13 min) was used, where the samples were held at 50°C for 1 min, heated to 95°C in 3.5 min, held at 95°C for 2.5 min before cooling to 50°C, and holding at 50°C for 1 min. Peak viscosity (PV), time from onset of pasting to

peak viscosity (P_{time}), hot paste viscosity at the end of holding at 95°C (HPV), breakdown (BD) (PV – HPV), final viscosity at 50°C or cool paste viscosity (CPV), and setback (STB) (CPV – HPV) were recorded. Results were reported in Rapid Visco Units (RVU).

Thermal Properties of Flour

Thermal properties of flour were analyzed using DSC according to the method described by Bhattacharya et al. (1999). A sample (3.0 mg, db) was weighed directly into a tared aluminum crucible and distilled water was added to obtain a flour-to-water ratio of 1:3 (w/w, db). The crucible was hermetically sealed and allowed to equilibrate for 1 hr in order to obtain uniform water distribution in the flour before analysis. The sample was heated from 10°C to 110°C at the rate of 10°C/min. An empty crucible was used as a reference. The onset temperature of gelatinization (T_o), the temperature at peak (T_p), the temperature at the end of gelatinization (T_c), and the enthalpy of gelatinization (ΔH) were obtained using the data processing software supplied with the DSC instrument. No measurements were made on the amylose-lipid endotherm in the region 95–120°C. Indium was used to calibrate the calorimeter.

Bread Baking

A straight dough procedure (AACC Approved Method 10-09, 2000) was used to evaluate the effect of 20, 30, and 40% (w/w) Ben, WS and WD flour on the physicochemical and staling properties of bread. The bread formula and water

used were optimized after a series of trials with different levels and types of oxidizing agents, baking with and without amylase and shortening, varying amounts of water, and testing 2 vs 3 hr of fermentation. The baking formula (flour basis) consisted of 100 g flour (14% mb), 5 g sugar, 1 g salt (both added in a solution), 2 g shortening, 0.1 mL solution of fungal α-amylase (17 SKB units, American Ingredients, Co., Kansas City, MO), 10 ppm potassium bromate as oxidizing agent, 1 mL solution of ammonium phosphate (10%) as yeast food, and 1 g instant dry yeast (Lallemand, USA). The amount of added water for optimum dough consistency was determined as Farinograph water absorption minus 1.5 mL for Gunner and Ben blends. The Farinograph water absorption was reduced by 2.5 mL for waxy flour blends, since the waxy flour dough exhibited extreme stickiness and softness that resulted in difficult dough handling. All samples were mixed to optimum dough development using a pin mixer. A two-step punching procedure was adopted using 3 hr of fermentation. Proofing was done at 30°C for 55 min at 85% rh before baking. Bread was baked at 220°C for 25 min. Loaves were allowed to cool to room temperature before testing (fresh bread) or storage in plastic bags.

Bread Quality Evaluation

Analyses of bread quality were done on fresh loaves and loaves stored for 1, 3, and 5 days. A subsample of bread crumb was freeze-dried after each storage day. After grinding in Falling Number mill (Perten Instruments, Sweden), the freeze-dried samples were used for analysis of thermal and pasting properties of

crumb, as well as properties of crumb soluble starch.

Moisture content of bread crumb was determined according to a two-stage drying method for samples containing 13% or more moisture (AACC Approved Method 44-15, 2000). Bread crumb was placed in open dishes and air-dried over night, followed by drying in the oven. Moisture was calculated based on the moisture loss from both drying stages.

Water activity of bread crumb was measured using a water activity meter (Series 3 model, Decagon Devices, Pullman, WA). Water activity was measured immediately after taking a piece of crumb out of plastic bag and manually reducing it to the size that fitted in the chamber of the water activity meter.

Bread firmness was determined using the texture analyzer (TA-XT2, Texture Technologies Corp, Scarsdale, NY) according to Approved Method 74-09 (AACC 2000). Two central slices (total sample thickness 25 mm) from each loaf were used to determine bread firmness. An aluminum plunger (20 mm diameter) was used to compress bread slices at a rate of 100 mm/min. The compression force value was recorded at 25% compression (6.25 mm of sample thickness).

RVA pasting properties of crumb were determined according to the same method as the pasting properties of flour. Freeze-dried, ground, crumb samples were analyzed in distilled water since the α-amylase was inactivated during baking. Parameters evaluated were hot paste viscosity (HPV), cold paste viscosity (CPV) and setback (SB). Clear reading of peak viscosity from RVA pasting profiles was not possible.

Thermal properties of bread crumb were determined according to the same

method as for the flour. The data obtained from DSC of crumb is related to endothermic changes due to starch retrogradation during bread staling.

To determine the soluble starch content, freeze-dried crumb was homogenized with distilled water (1:10 w/v) and centrifuged at 2,000 x *g* for 10 min. The supernatant was used for the soluble starch measurement using the Total Starch Assay kit (Megazyme International, Wicklow, Ireland) (AACC Approved Method 76-13, 2000).

Statistical Analysis

The experimental design for flour blends was a randomized complete block (RCBD). Three sets of blends were prepared, and each set was considered a replication (block). Data were analyzed using the general linear model procedure (GLM) of the Statistical Analysis Systems (version 9.1, SAS Institute, Cary, NC). The baking experiment and bread analyses were conducted using RCBD with factorial arrangement of ten blends (Gunner control and 20%, 30%, and 40%, (w/w) blends of Ben, WS, and WD with Gunner) and four storage days (0, 1, 3, and 5). Two sets of blends were prepared (due to the limited amount of waxy flours), and each set was considered a replication (block). Data were subjected to analysis of variance using Statistical Analysis Systems (version 9.1, SAS Institute, Cary, NC). Means were separated by Fisher's protected least significant difference test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Characteristics of Flour Blends

All base flours (Gunner, Ben, WS, WD) differed significantly in protein content, with Ben having the lowest (11.2%) and WD the highest (15.7%) protein content (Table 1.1). Protein content of flour blends reflected the differences in protein content of base flours. WS flour blends exhibited significantly lower protein content than did WD blends.

Starch damage of Gunner flour was 7.4%, whereas the starch damage of WS flour was only 4.0%. Ben flour and waxy durum flour did not differ significantly in the starch damage content (Ben 10.2%, WD 10.4%). Higher starch damage of WD than WS flour was reported by Chakraborty et al. (2004). They found that starch damage was lower for WS flour than for non-waxy spring flour, and reported similar values for starch damage between WD and non-waxy durum flour. Based on these results, it is difficult to conclude how much of the starch damage is due to waxy nature of wheat and how much is due to the type of wheat (spring and durum). Chakraborty et al. (2004) suggested that structural differences between waxy spring and waxy durum starch might contribute to the difference in milling properties of two types of waxy wheat. On the other hand, the results of our study show that the high starch damage in waxy durum flours might also be attributed to the higher kernel hardness typical for durum wheat than for hard red spring wheat (Pomeranz et al. 1988).

One of the characteristics of waxy flours is unusual high Farinograph water absorption (Lee et al. 2001; Bhattacharya et al. 2002; Guo et al. 2003). This

Table 1.1. Protein Content, Farinograph Properties and Loaf Volume of Flour Blends

Blends[a]	Protein content (%db)	ABS (%)[b]	DDT (min)[c]	STAB (min)[d]	MTI (BU)[e]	BKD (min)[f]	Loaf volume (ml)
Gunner	14.3	68.0	8.8	15.9	19.0	20.0	1057
Ben	11.2	69.2	3.8	4.8	70.0	6.8	na[g]
WS	12.7	73.3	5.0	3.2	130.0	6.3	na
WD	15.7	71.2	4.2	3.6	115.0	5.8	na
20% Ben	13.6	68.5	7.4	9.8	29.5	12.8	975
30% Ben	13.4	68.7	7.2	8.2	33.5	11.1	914
40% Ben	13.1	68.7	6.5	6.8	44.5	9.9	905
20% WS	14.0	68.8	7.2	12.1	20.0	15.8	969
30% WS	13.8	69.5	7.5	9.6	30.0	14.4	913
40% WS	13.8	70.4	7.0	8.7	36.0	11.8	884
20% WD	14.4	68.5	5.8	7.5	42.0	9.8	995
30% WD	14.3	68.7	5.5	5.7	57.0	8.2	1005
40% WD	14.5	69.2	5.2	5.1	48.5	7.9	1023
LSD (0.05)	0.2	0.9	0.7	0.8	12.7	1.0	32

[a]WS =100% waxy spring wheat flour; WD = 100% waxy durum wheat flour
20%, 30%, 40% WS = Blends of waxy spring wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour
20%, 30%, 40% WD = Blends of waxy durum wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour
[b] ABS = Farinograph water absorption
[c] DDT = Farinograph dough development time
[d] STAB = Farinograph stability
[e] MTI = Farinograph mixing tolerance index
[f]BKD = Farinograph time to breakdown
[g] na= not applicable

property of waxy flour often is attributed to high amylopectin content of waxy flour.

Water absorptions of WS and WD flours were 73.3% and 71.2% (Table 1.1), However, when WS and WD flours were blended with Gunner flour, water absorptions of blends were reduced (Table 1.1). Water absorption of flour blends

reflected the water absorption of the base flours.

Besides high water absorptions, WS and WD flours produced weak and sticky dough that required short mixing times (Table 1.1). These doughs were more susceptible to overmixing and breakdown than other doughs as indicated by their low Farinograph stability and breakdown values and high mixing tolerance indices (MTI) (Table 1.1). Similar findings for high water absorption, short mixing times, and weak doughs of waxy flours were reported by Sahlstrom et al. (2006) and Park and Baik (2007). Waxy flour blends produced generally weaker doughs than Gunner flour, as indicated by lower Farinograph stability and breakdown values and higher MTI values (Table 1.1), but stronger doughs than the pure WS and WD flours. Among waxy blends, WD blends produced significantly weaker dough than WS blends. Durum wheat generally has weaker gluten strength than bread wheat. This might be one of the reasons for weaker dough response of WD blends than WS blends. Ben was one of the parents of the WD line, and Ben has moderate gluten strength that is lower than the gluten strength of bread wheat. Farinograph data in Table 1.1 shows the difference in dough quality between Ben durum and Gunner bread wheat, which is greatly due to the difference in their gluten strength. Although WD blends had higher protein content than corresponding WS blends (Table 1.1), WD blends showed significantly lower Farinograph dough stability, shorter dough development time and shorter time to breakdown than WS blends. Due to these properties, the processing of WD dough during mixing, punching, and sheeting was difficult. Waxy durum flours also produced weaker dough than corresponding Ben flours, despite the higher protein

content of WD flours. Ben was one of the parents of WD line, therefore some similarity in protein quality was expected. Properties of dough made of durum blends were related to the properties of corresponding base flours (Ben and WD). Waxy durum flour had the highest protein content and Ben flour had the lowest protein content of all flours; however, Ben flour had better Farinograph mixing properties than WD. Waxy durum flour had significantly lower Farinograph stability and much poorer mixing tolerance index than Ben flour. A similar relationship was found between WS and Gunner flour; however, Gunner also had significantly higher protein content than WS.

The hypothesis that could be derived from these results is that dough properties of Ben and WD flours might have been affected by some differences in starch properties. Differences between dough properties of WD and WS flours might be attributed to the difference in the bread-making quality between spring and durum wheat proteins (Dexter et al. 1981; Dick and Matsuo 1988; Boyacioğlu and D'Appolonia 1994a). Both WS and WD reduced Farinograph dough quality.

The 30 and 40% WD flour blends resulted in higher loaf volume than corresponding Ben flour blends, probably as a result of higher protein content in WD flours. Loaf volume was affected by protein content ($r=0.76$, $p<0.001$) more than by starch properties, since the protein content and loaf volume of WS blends decreased with increasing amount of WS flour in a blend and protein content and loaf volume of WD blends increased (Table 1.1). However, loaf volume alone cannot be considered a reliable quality factor in the case of waxy flours and their blends since most of these flours produced bread with poor crumb texture, which is

discussed in the section "Cross Section of Bread" on page 78.

Amylose content of flour blends is presented in Table 1.2. Amylose contents of Gunner flour and Ben flour blends were similar; except for 40% Ben blend, which had lower amylose content than other mentioned blends. Amylose contents of base waxy flours were 2.1% for WS flour, and 2.3% for WD flour. The amylose content of blends generally decreased with increased WD or WS content and it was significantly lower than the amylose content of Gunner and Ben flour. Since amylose contents of WD and WS were similar, no significant differences in amylose content were found between WS and WD blends with the same concentrations of waxy flour in a blend. Among all WS blends, 40% blend had significantly lower amylose content than the 20% blend. Larger differences were found among WD blends; 40% blend had significantly lower amylose content than both 20% and 30% blends.

Pasting Properties of Flour

Pasting properties of waxy and non-waxy base flours differed significantly (Table 1.2, Figure 1.1). Waxy flours produced high RVA peak viscosity as a result of extensive starch granule swelling, followed by significantly higher breakdown and smaller setback than non-waxy flours. Waxy flours attained peak viscosity at lower temperature than non-waxy flours and took less time to reach peak viscosity than the non-waxy flours. Similar differences in pasting properties between waxy and non-waxy flours were reported in other studies (Hayakawa et al. 1997; Kiribuchi- Otobe et al. 1997; Gaines et al. 2000; Sasaki et al. 2000; Chakraborty et

Table 1.2. Amylose Content and RVA Pasting Properties of Flour

Blends[a]	AM (%db)[b]	PV (RVU)[c]	HPV (RVU)[d]	BKD (RVU)[e]	CPV (RVU)[f]	STB (RVU)[g]	PT (min)[h]
Gunner	27.9	226	149	77	260	111	6.3
Ben	26.2	219	142	77	258	116	6.2
WS	2.3	372	131	241	167	36	3.1
WD	2.1	288	112	177	155	43	3.6
20% Ben	28.6	225	154	71	266	113	6.4
30% Ben	27.9	220	144	77	260	116	6.3
40% Ben	24.8	230	145	85	265	120	6.2
20% WS	21.8	210	141	69	233	92	6.2
30% WS	21.0	196	131	65	225	94	6.2
40% WS	19.3	189	123	66	212	89	6.1
20% WD	23.2	213	129	84	229	100	6.1
30% WD	22.7	198	124	74	223	99	6.1
40% WD	20.1	187	116	71	210	94	5.9
LSD (0.05)	2.3	6	6	5	6	5	0.2

[a]WS =100% waxy spring wheat flour; WD = 100% waxy durum wheat flour 20%, 30%, 40% WS = Blends of waxy spring wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour; 20%, 30%, 40% WD = Blends of waxy durum wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour
[b] AM = Amylose content
[c] PV = Peak viscosity
[d] HPV = Hot paste viscosity
[e] BKD = Breakdown
[f] CPV = Cold paste viscosity
[g] STB = Setback
[h] PT = Peak time

al. 2004). Characteristic pasting properties of waxy flours were attributed to the low amylose content of waxy flours and unique properties of waxy starch granules. Intensive swelling of starch granules reduces the quantity of free water in the starch/water system and causes development of high peak viscosity (Ming et al. 1997). Amylose was reported to suppress swelling of starch granule and to help

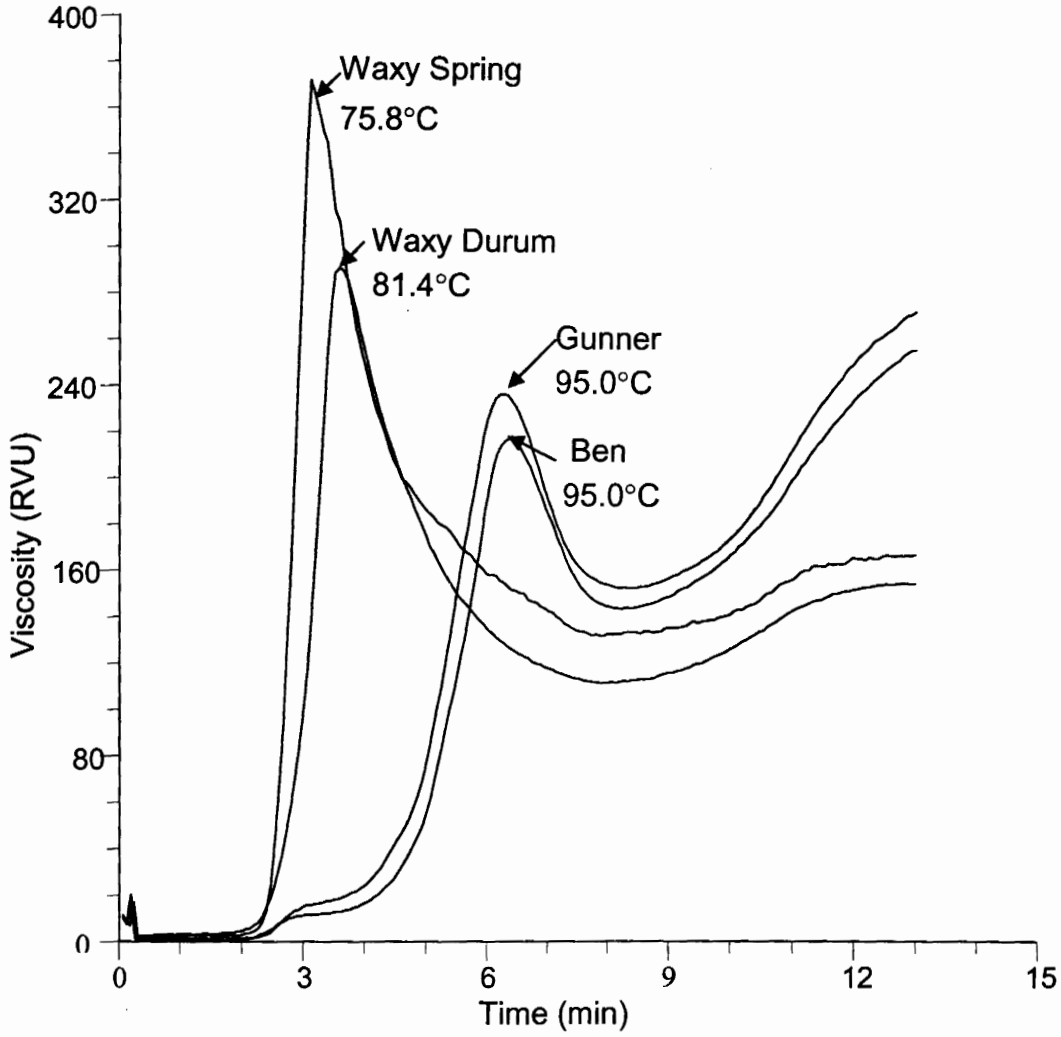

Figure 1.1. Rapid Visco Analyzer (RVA) pasting curves of waxy spring flour, waxy durum flour, Gunner flour, and Ben flour

reduce the loss of granular rigidity of swollen granules (Hermansson et al. 1997; Mei-Lin et al. 1997). Conversely, amylopectin is mainly responsible for starch granule swelling (Tester and Morrison 1990). Therefore, heat easily disrupted the structure of starch granule containing low amylose. Waxy starch granules swell rapidly, which results in high paste viscosity. However, when compared to normal wheat starch, waxy starch granules disintegrate at lower temperature, which results in low stability of paste viscosity (high breakdown) (Sasaki et al. 2000).

The effect of proteins on pasting properties is difficult to elucidate in a complex system like flour. Starch and gluten in dough or any dough model system compete for water during heating (Ghiasi et al. 1982; Mohamed and Rayas-Duarte 2003). Although Gunner and Ben had significantly different protein contents and protein quality (as shown by Farinograph mixing properties, Table 1.1), these two flours did not exhibit significant differences in RVA pasting properties, with the exception of slight difference in peak viscosity (Table 1.2). Also, the amylose content of these two flours was not different; therefore pasting properties of non-waxy flours probably were due solely to starch properties of flour.

Although amylose contents of two waxy starches were similar, differences in their pasting properties were observed (Table 1.2). WS flour developed a peak viscosity of 372 RVU, whereas the peak viscosity of WD flour was 288 RVU. A significantly lower breakdown and significantly higher setback occurred with WD flour, compared to WS flour (Figure 1.1). All RVA tests were done using 1mM $AgNO_3$ instead of water to suppress possible α-amylase activity (Hutchinson 1966; Meredith et al. 1971). While non-waxy flours (Gunner and Ben) reached peak viscosity at the same temperature (95.0°C) (Figure 1.2), WS flour reached its peak viscosity at 75.8°C and WD reached its peak viscosity at 81.8°C. WS flour also had significantly shorter peak time than WD flour (Table 1.2). Limited information can be found about differences between waxy wheat starches. In a previous study (Chakraborty et al. 2004), WD starches were found to be more resistant to granule swelling than WS starches. Differences between WS and WD starch might be due to differences in amylopectin structure that affect gelatinization, pasting and

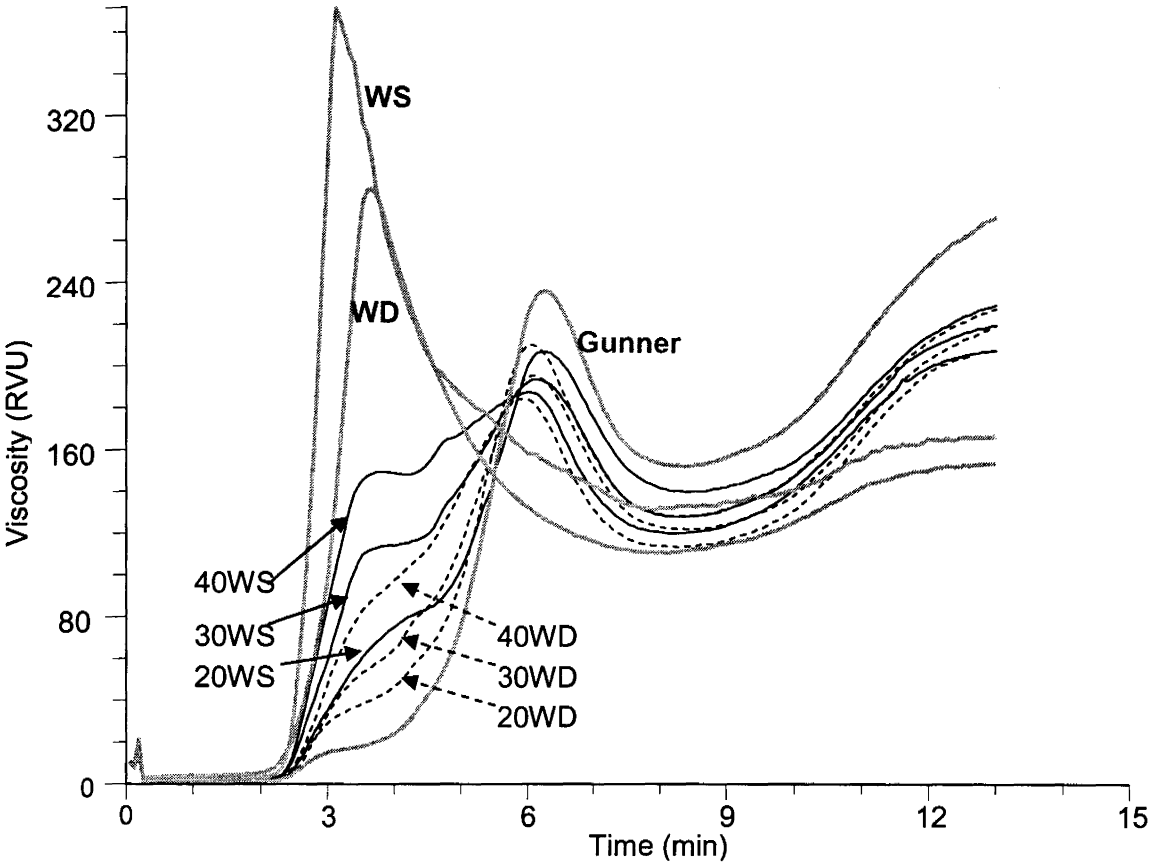

Figure 1.2. RVA pasting curves of Gunner flour, WS and WD flours, and waxy blends

retrogradation properties of starch (Shibanuma et al. 1996; Jane et al. 1999). Baik et al. (2003) also found some differences in pasting properties of two waxy wheat starches and hypothesized that the differences might be due to different granular size distribution, amylopectin branch chain length distribution, crystallinity, or minor constituents.

Blends of waxy and non-waxy flours had both waxy and non-waxy RVA viscosity peaks (Figure 1.2). The second peak was much more pronounced than the first peak; the first peak could not be determined accurately for some blends. The double viscosity peak was most distinct for 40% waxy blends and less distinct

for 20% blends. All blends reached the second (non-waxy) peak viscosity somewhat earlier than Gunner flour (Table 1.2). Since there were two different types of starch granules in blends, each granule type exhibited a particular viscosity profile. Blends with 40% waxy flour clearly showed the first viscosity peak at lower temperature corresponding to the peak temperature of waxy flour, and the second peak at about 95.0°C, corresponding to the peak temperature of non-waxy flour. (Figure 1.2). Similar findings were reported by Obanni and BeMiller (1997) and Sasaki et al. (2000) for starch blends and by Guo et al. (2003) for flour blends.

The peak viscosities (viscosity measured at the second peak) of waxy blends were significantly lower than those of waxy and non-waxy base flours (Table 1.2). This might be an indication of two separate granule swelling processes, where the starch that swelled later had limited water available for swelling and eventually developed lower peak viscosity. However, a possible effect of dilution of one starch with another also cannot be excluded as a possible cause for lower peak viscosities of flour blends. Most likely, lower peak viscosities of blends compared to that of base flours might be the result of combination of both mentioned effects. The two peaks indicate that waxy starch in the blend started to collapse before the non-waxy starch reached its peak viscosity. Non-waxy starch granules developed viscosity while waxy starch granules disintegrated (broke down) (Sasaki et al. 2000). Obanni and BeMiller (1997) also suggested that starch with a lower gelatinization temperature might affect the pasting properties of a starch with higher gelatinization temperature in the blend.

All waxy blends had significantly lower HPV than Gunner flour (Table 1.2).

While CPV values for all waxy blends were between those of Gunner and corresponding waxy flours, HPV of blends exhibited different behavior. Hot paste viscosity of 30% WS blend was the same as that of WS flour, whereas 20% WS had significantly higher and 40% WS blend had significantly lower HPV than WS flour (Table 1.2). Among WD blends, HPV of 40% WD blend was similar to that of WD flour while both 20 and 30% WD blends had significantly higher HPVs than WD flour. These results show that the pasting behavior of mixed starches (flours) might be influenced by interaction between amylopectin and amylose that is different than in a simple starch. Sasaki et al. (2000) and Obanni and BeMiller (1997) indicated that the paste properties of blends might be attributed to specific interactions among soluble starch, swollen granules, and fragmented granules of starch. Breakdown values for waxy blends were significantly lower than those of fully waxy flours but also lower than for Gunner flour, with exception of 20 and 30% WD blends. All blends also exhibited significantly higher setback than waxy flours but significantly lower than Gunner flour (Table 1.2).

The peak viscosities of WD and WS blends with the same percentage of waxy flour were similar (Table 1.2), which was expected since this peak viscosity actually corresponded to the peak viscosity of Gunner flour (second peak). All WS blends developed higher initial viscosity (first peak) than WD blends (Figure 1.2), which might be the result of more intensive granule swelling (more intensive water uptake) in WS than in WD flour. Peak viscosities decreased with increased amount of waxy flour in blend. The amount of water available for swelling of non-waxy starch (the second swelling starch) probably decreased as the amount of waxy

flour in the blend Increased, which resulted in lower peak viscosity.

WS and WD blends containing the same percent of waxy flour differed in their HPV, breakdown, and setback even though their amylose contents were similar (Table 1.2). WS blends had significantly higher HPV than waxy durum blends showing that swollen starch granules in WS blends ruptured to a lesser extent than swollen WD granules. Perhaps an opposite effect could have been expected with WS starch swelling more (and developing higher peak viscosity) than the WD starch. The results clearly show the difference between waxy starch in WS and WD wheat. Cold paste viscosities were similar between WS and WD blends with the same percent of waxy flour (Table 1.2) indicating that end of the RVA cooling cycle starch in WS and WD blends retrograded in a similar fashion. The HPV and CPV decreased with increased amounts of waxy flour in blends. This was expected since waxy starch granules have a greater tendency to disintegrate than do non-waxy granules and have a lesser tendency for their starch molecules to reassociate upon cooling (Hung et al. 2007).

Thermal Properties of Flour

Gelatinization properties of flours and flour blends are presented in Table 1.3. Gelatinization temperatures and enthalpies of gelatinization varied among flours. Significant differences in onset temperature of gelatinization (T_o) were observed among all base flours. Although Ben and Gunner flours had similar amylose contents, their thermal properties were different. Ben flour had the lowest onset and peak temperature of gelatinization of all analyzed flours, and was

Table 1.3. DSC Thermal Properties of Flour Blends

Blends[a]	ΔH (J/g)[b]	T_o (°C)[c]	T_p (°C)[d]	T_c (°C)[e]
Gunner	7.48	54.9	61.5	66.9
Ben	8.27	48.4	58.6	66.8
WS	11.61	54.0	60.7	68.3
WD	10.82	56.0	65.6	72.2
20% Ben	8.36	53.1	60.9	67.1
30% Ben	8.51	51.8	60.6	67.0
40% Ben	8.30	51.0	60.7	67.3
20% WS	9.54	54.9	61.6	66.9
30% WS	9.65	54.6	61.1	66.4
40% WS	10.32	53.9	61.0	66.9
20% WD	9.22	55.3	62.4	68.6
30% WD	9.73	55.0	62.5	68.8
40% WD	9.79	55.1	63.0	69.6
LSD (0.05)	0.74	0.8	0.5	0.9

[a]WS =100% waxy spring wheat flour; WD = 100% waxy durum wheat flour 20%, 30%, 40% WS = Blends of waxy spring wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour; 20%, 30%, 40% WD = Blends of waxy durum wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour.
[b] ΔH = Enthalpy of gelatinization
[c] T_o = Onset temperature of gelatinization
[d] T_p = Peak temperature of gelatinization
[e] T_c = Completion temperature of gelatinization

significantly lower from those of Gunner flour. Ben flour also exhibited significantly higher enthalpy of gelatinization than Gunner flour.

When Gunner and Ben flours were compared with their corresponding waxy flours, different behavior with respect to gelatinization temperatures was observed. The onset and peak temperatures of gelatinization were significantly higher for Gunner than for WS flour, while the opposite was observed for Ben and WD flour (Table 1.3). Both Gunner and Ben flour had lower final temperatures of

gelatinization than their corresponding waxy flours. Gunner flour showed narrower gelatinization temperature range ($T_o - T_c$ = 12.0°C) than WS flour ($T_o - T_c$ = 14.3°C), while the opposite was found between Ben flour ($T_o - T_c$ = 18.4°C) and WD flour ($T_o - T_c$ = 16.2°C) (Table 1.3). Previous studies (Yasui et al. 1996; Fujita et al. 1998) reported that waxy starch had higher gelatinization temperature than non-waxy starch. In the case of WS flour, only the final temperature of gelatinization (T_c) was higher than that of Gunner flour. On the other hand, WD flour exhibited characteristics typical for waxy flours, i.e. WD flour had higher temperatures of gelatinization than Ben flour. Both waxy flours had significantly higher enthalpies of gelatinization than Gunner and Ben flours, which was expected since waxy flours had significantly lower amylose content, i.e. higher amylopectin content, than Gunner and Ben flours. Gelatinization has been known as the loss of double-helical (molecular) order of amylopectin (Cooke and Gidley 1992). Double helices of amylopectin are responsible for the crystallinity of starch; therefore the endothermic energy recorded by DSC often is related to the crystallinity of starch. Gelatinization temperature is considered to be related to crystallite perfection (Tester and Morrison 1990).

A strong negative correlation was found between the enthalpy of gelatinization and amylose content ($r = -0.88$, $p < 0.0001$), showing that flours with low amylose content of starch (waxy flours) require high gelatinization enthalpies. Also, the final temperature of gelatinization showed certain association with the amylose content ($r = -0.57$, $p = 0.042$), indicating that flours with low amylose content have to be heated to high temperatures in order to disrupt the crystalline

regions of starch. These results are consistent with those reported by Russel (1987) and Flipse et al. (1996). These researchers reported that amylose-rich amorphous regions facilitate melting of crystalline regions, and therefore starches with high amylose content require less energy for gelatinization than starches with low amylose content.

Thermal properties of WS and WD flour were different although their amylose contents did not differ significantly (Table 1.3); implying that factors other than amylose/amylopectin ratio were responsible for this difference between two waxy flours. The reason for this difference might be due to the effect of gluten on gelatinization properties of starch as shown in the study of Eliasson et al. (1983) or it could be due to some structural differences in amylopectin. Matsuki et al. (2003) showed that gelatinization properties of starch were related with amylopectin chain length distribution. Starches with high proportions of long amylopectin branch chains exhibited high peak temperatures of gelatinization (Yuan et al. 1993; Kohuyama et al. 2004).

Waxy flour blended with non-waxy Gunner flour affected the gelatinization properties of flour blends (Table 1.3). The enthalpy of gelatinization (ΔH) increased with increased amounts of waxy flour in blends (both in WS and WD), and fell between ΔH of the two individual components. All ΔH values of flour blends were significantly higher than ΔH of Gunner flour and significantly lower than ΔH of corresponding waxy flours. Although ΔH of WS and WD flour were significantly different, it did not reflect the gelatinization enthalpies of flour blends. The ΔH of WS and WD blends with the same amount of waxy flour were similar (Table 1.3).

None of the blends showed two endotherms that would correspond to the separate melting of two individual components, as suggested by Liu and Lelièvre (1992). Obanni and BeMiller (1997) also did not observe two endotherms in starch blends, attributing this to specific interactions between components of two cooked starches. In general, the onset temperatures of gelatinization of all waxy blends fell between those of Gunner and waxy flour, with WD blends having somewhat higher T_o than WS blends. The peak temperatures of gelatinization followed the same pattern, with exception of 20% WS blend that had a slightly higher T_o than Gunner flour. All WD blends had significantly higher peak and final temperatures of gelatinization than the same percent WS blends, which was expected considering the difference in gelatinization temperatures between WS and WD flours (Table 1.3). These results as well as results for T_o and T_p of individual components of the blends confirmed the findings of Sasaki et al. (2000) who reported that T_o and T_p were not related to the amylose content.

Final temperatures of gelatinization (T_c) of WS blends did not follow a pattern of increase or decrease in relation to the amount of WS flour, and they were all the same or close to the T_c of Gunner flour (Table 1.3). Waxy durum blends, on the other hand, showed increasing T_c with increasing amounts of WD flour in blends, with all values being significantly higher than that of Gunner flour. Ben and Gunner flour blends tended to result in ΔH and T_c that were higher than those of Ben flour, whereas T_o and T_p values were between the values of Ben and Gunner flour.

Physical Properties of Bread

Cross Section of Bread

Visual appearance of bread, along with flavor and texture, are important quality factors for consumer acceptance of the product. Desired quality traits of white panned bread are softness of crumb with uniform distribution and size of cells (Figure 1.3 a, b – 100% Gunner). Waxy flour in Gunner flour blends caused changes in crumb structure. Increasing the proportion of waxy flour resulted in more open grain of bread crumb (Figure 1.3 a) than the crumb grain of Gunner bread. Besides open crumb grain, loaves made of waxy flour also exhibited non-uniform size and distribution of cells. Some very large cells with non-uniform shape could be observed in bread made of 40% waxy flour (Figures 1.3 c and 1.4). Although baking and staling properties of 100% WS and WD flour were not evaluated in this experiment, the cross section of their loaves is presented for comparison with non-waxy bread and breads made of waxy blends (Figure 1.3 a, b and c). Bread made with 100% WS or WD flour had unacceptable crumb quality, characterized by very open grain, large holes, and sticky texture. Bread made with 100% waxy flour could not hold its structure and often collapsed acquiring a distorted or keyhole shape (Figure 1.3 b) and very soft crumb that caused the loaf to become compressed during slicing. The texture of waxy bread is described often as glutinous (Morita et al. 2002; Baik et al. 2003). Loaves made with 40% WS flour were in general whiter with somewhat less open structure than loaves made with 40% WD flour; however, both blends produced bread of inferior quality compared to the bread made with Gunner flour. Flour blends with 20% waxy flour

resulted in crumb structure that was comparable to the structure of Gunner bread (Figures 1.3 and 1.4). These results show that amylose is involved in controlling the crumb grain structure. One of the reasons for inferior crumb quality of waxy flours may be insufficient amount of amylose in waxy flours. Gelation of amylose that leached into intergranular phase during baking is an essential element for the formation ("setting") of the crumb structure upon the first few hours of bread cooling (Eliasson and Larsson 1993; Hug-Iten et al. 1999). Schoch and French (1947) proposed a model of bread staling that described quick post-baking amylose retrogradation as a main cause for initial bread firmness. Kim and D'Appolonia (1977 b) confirmed that the most intensive gelation of amylose in fresh bread occurred during the first day of storage. Since waxy flours and their blends have no or reduced amounts of amylose, starch does not undergo a necessary amount of retrogradation immediately after baking, and therefore bread crumb lacks structure and desirable firmness. Large holes in waxy bread crumb caused reduced crumb density, which consequently reduced the ability of crumb to withstand changes in internal and external pressure upon cooling and the sides of the loaves collapsed (Cauvain and Young 2000).

Moisture Content and Water Activity

The effect of blend composition, storage days, and interaction of blend by storage day on crumb moisture (Figure 1.5) was analyzed. Blend by storage day interaction was significant for moisture content of crumb (Table A-2). Moisture content of crumb of most blends decreased during storage (Figure 1.5) with the

Table 1.4. Moisture Content of Bread Crumb (%) as Affected by Interaction of Blends and Storage

	Days			
Blends[a]	**0**	**1**	**3**	**5**
Gunner	42.6	37.3	34.9	36.7
20% Ben	43.3	37.3	37.1	36.0
30% Ben	43.0	38.1	35.9	35.1
40% Ben	42.5	37.9	36.1	35.9
20% WS	42.5	36.5	35.4	34.5
30% WS	42.7	38.7	36.8	36.3
40% WS	42.9	39.8	35.4	35.2
20% WD	40.8	37.2	34.8	34.7
30% WD	40.5	38.0	31.2	34.5
40% WD	39.9	38.1	34.6	34.9
LSD (0.05)	---------- 2.0 ----------			

[a] 20%, 30%, 40% WS = Blends of waxy spring wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour; 20%, 30%, 40% WD = Blends of waxy durum wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour

spring wheat flour and double-null partial waxy wheat during storage. WD breads had somewhat lower moisture content right after baking (day 0) than other samples (Figure 1.5). This could be attributed to more open crumb of waxy durum loaves than other loaves, which contributed to faster moisture loss.

Major difference between two types of waxy crumbs was observed on day 0 while WS and WD crumbs with the same percent of waxy flour did not differ in later stages of storage. Moisture contents of all WS blends on the same storage day were similar, and the same was observed for WD blends; although higher moisture content could have been expected in blends with higher amount of waxy flour due to higher water absorption of dough. Reduction of crumb moisture content due to

the moisture migration from crumb to crust during storage, and consequent changes in bread texture, is a well known phenomenon (Czuchajowska et al. 1989). However, the reason for the difference in crumb moisture content on day 0 between WD and WS crumbs is not completely clear. Several factors could have contributed to this difference. WS flours had somewhat higher water absorption than WD flours. Higher moisture in bread crumbs made with waxy flour was attributed to the higher water absorption of flour (Morita et al. 2002a). However, since no correlation was found between flour water absorption and crumb moisture, difference in water absorption between WS and WD flours most likely cannot be used as an explanation for the difference in crumb moisture content. The more intensive moisture loss of waxy crumbs could be attributed also to more open and porous structure of crumb than the crumb of non-waxy bread. This phenomenon could explain some differences in moisture content between WD and non-waxy crumbs, but does not explain completely the difference between two types of waxy crumbs, since they all had open crumb grain. To what extent the difference in crumb openness between WS and WD bread affects moisture content is not clear. Finally, the difference in crumb moisture between WD and WS samples might be due to some structural differences between their starches. Structural differences between starches, such as difference in branch chain length of amylopectin, could cause differences in amylopectin recrystallization, and consequently, differences in the amount of water incorporated in amylopectin crystalline structure during staling of bread. Leung et al. (1983), Wyne-Jones and Blanshard (1986), and Lin et al. (2001) suggested that during storage of bread,

amylopectin re-crystallizes, incorporating water into the starch crystalline structure and decreasing the water mobility in bread.

Change of crumb water activity (a_w) during storage did not parallel the change of moisture content. The blend by storage day interaction was not significant for crumb water activity (Table A-2). Water activity decreased during storage, although the differences between storage days were not statistically significant. Water activity was between 0.974 on day 0 and 0.970 on day 5. Decrease in water activity was registered between day 3 (0.973) and day 5 (0.970), whereas during this period moisture content reached a constant level (Table 1.4). While moisture content of Gunner crumb was not significantly different from majority of other blends throughout the storage period, the water activity of Gunner crumb was the lowest of all samples (Table 1.5). Waxy spring crumbs with 30 and 40% waxy flour had the highest a_w of all analyzed blends, while their waxy durum counterparts had the lowest a_w. Water activity in bread is related to the ability of water to move in the crumb (Schiraldi and Fessas 2001). The inconsistency between moisture content and water activity in analyzed crumbs clearly shows that the relationship between water content and water activity in bread crumb is not linear. Since flour and bread contain multiple components with hydrophilic parts (starch, proteins, pentosans), the water activity is governed most likely by complex interactions between water and these components. Some authors proposed that the water detected in a_w should be the most mobile fraction of water, which is located within the amorphous regions in starch in bread (Leung et al. 1983, Kim-Shin et al. 1991). During storage, water moves from a less-bound

Table 1.5. Water Activity of Bread Crumb as Affected by the Composition of Blends

Blends[a]	Water activity
Gunner	0.968
20 Ben	0.972
30 Ben	0.972
40 Ben	0.973
20 WS	0.972
30 WS	0.975
40 WS	0.976
20 WD	0.972
30 WD	0.970
40 WD	0.972
LSD (0.05)	0.004

[a] 20%, 30%, 40% WS = Blends of waxy spring wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour; 20%, 30%, 40% WD = Blends of waxy durum wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour

to more-bound state (from amorphous to crystalline region) and this decreases the a_w.

Bread Firmness

Crumb firmness was significantly affected by blend by storage day interaction (Table A-2). Firmness of all samples increased during storage in a sigmoidal pattern (Figure 1.6). The highest crumb firmness increase occurred between day 1 and day 3 of storage. Firmness values leveled off or increased at a slower rate between days 3 and 5 (Figure 1.6). The initial firmness of Ben and Gunner crumbs was comparable, which was similar to the findings of Boyacioğlu and D'Appolonia (1994b). On day 0, WD and WS crumbs had similar firmness

0 and 1; while for WD blends, firmness was reduced with increasing amount of WD flour in blend (Table 1.7). The increase in crumb firmness was most pronounced between the first and third day of storage. Although waxy flour in blends was expected to reduce crumb firming during storage, as reported by some researchers (Baik et al. 2003; Morita et al. 2004; Qin et al. 2009), the firming rate of most waxy crumbs actually was significantly higher than the firming rate of Gunner crumb and crumb of bread made with blends of Gunner and Ben flour. Firming of crumb significantly slowed down between the third and fifth day of storage, although the results show that bread made with waxy blends continued firming more intensively than Gunner crumb between days 3 and 5 (Table 1.7). While WS crumbs did not show any clear trend of firming depending on the percent of substitution, WD crumbs showed clearly that firming decreased with higher level of substitution of Gunner flour with WD flour.

The mechanism responsible for the firming behavior is not completely clear. Crumb firming is a complex process that can depend on many factors. Large loaf volume and softer crumb commonly are associated with high protein content of flour (Maleki et al. 1980). The relationship between flour protein content, loaf volume, and crumb firmness, was opposite from that reported by Maleki et al. (1980). Crumb firmness did not correlate with loaf volume neither did it correlate with flour protein content, indicating that other factors might have influenced crumb firming. Negative correlation ($r=-0.75$, $p<0.001$) was found between moisture content of crumb and firmness, indicating that part of crumb firming could be explained by moisture loss. Moisture content and migration during aging of bread

Table 1.7. Percent Increase in Crumb Firmness during Storage as Affected by Interaction of Blends and Storage Days

Blends[a]	Day 0 - Day 1	Day 1 - Day 3	Day 3 - Day 5
Gunner	49	138	16
20% Ben	88	103	13
30% Ben	70	127	26
40% Ben	82	96	44
20% WS	73	162	41
30% WS	68	246	25
40% WS	86	197	49
20% WD	72	189	45
30% WD	50	257	33
40% WD	31	301	30
LSD (0.05)	----------	43	----------

[a] 20%, 30%, 40% WS = Blends of waxy spring wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour; 20%, 30%, 40% WD = Blends of waxy durum wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour

has been recognized as important factor in crumb firming (Chen et al. 1997; Baik et al. 2001; Vodovotz et al. 2002). However, this correlation does not provide a complete explanation of factors that can affect crumb firmness since the moisture of waxy crumbs was not significantly different from that of Gunner crumb at the end of the storage period (Table 1.4); yet waxy crumbs firmed much more during storage than Gunner crumb (Table 1.6). Besides moisture content, moisture migration together with the state of bread polymers (amorphous vs. crystalline) during aging of bread have been recognized as important factors in crumb firming (Chen et al. 1997; Baik et al. 2001; Vodovotz et al. 2002). Moisture redistribution among components of bread and between amorphous and crystalline regions of starch in bread is still rather speculative. WD and WS wheat starch could possibly

differ in structural and retrogradation properties (Chakraborty et al. 2004), which could cause differences in final bread firmness. If water migrates from less-bound (amorphous) to more bound (crystalline) state (Leung et al. 1983; Wyne-Jones and Blanshard 1986; Lin et al. 2001), differences in starch structure also could affect mobility of water.

Pasting Properties of Bread Crumb

Freeze-dried bread crumbs were analyzed for their RVA pasting properties using the same RVA heating and cooling cycle as for the flour samples. Tables 1.8 – 1.10 present the hot paste viscosity (HPV), cold paste viscosity (CPV), and setback (STB) values, respectively, for bread crumbs. Peak viscosity, and consequently, breakdown values for bread crumbs were not reported, because precise determination of peak viscosity was not possible for non-waxy crumbs (Gunner and Ben). Part of the non-waxy crumb RVA curve where the peak viscosity was expected was mostly flat without a distinct peak and breakdown (Figures 1.7 and 1.8). Also, the HPV for Gunner and Ben was not apparent (Figures 1.3 and 1.4) and therefore was determined at the end of 95.0°C heating period. Because of these factors, explanation of some of the processes during pasting of bread crumb was done based on the pasting profiles (Figures 1.7 and 1.8) and not actual numerical data.

Pasting profiles of non-waxy and waxy crumbs were very different (Figures 1.7 – 1.10), suggesting that starch in these crumbs responded differently to heat and moisture treatment during baking, and also to processes occurring during

Table 1.8. RVA Hot Paste Viscosity (HPV, RVU) of Bread Crumb as Affected by Interaction of Blends and Storage Days

	Days			
Blends[a]	**0**	**1**	**3**	**5**
Gunner	138	118	125	125
20% Ben	124	113	115	120
30% Ben	127	116	116	116
40% Ben	117	109	118	109
20% WS	142	153	164	149
30% WS	143	157	161	154
40% WS	137	151	171	151
20% WD	106	102	104	108
30% WD	101	102	103	105
40% WD	95	101	106	105
LSD (0.05)	---------- 9 ----------			

[a] 20%, 30%, 40% WS = Blends of waxy spring wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour; 20%, 30%, 40% WD = Blends of waxy durum wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour

storage of bread. Although the peak viscosity of Gunner crumbs could not be read precisely, their pasting profiles showed that they developed much lower paste viscosities than Gunner flour during the whole RVA cycle (Figure 1.7). RVA profiles of Gunner crumb had the same shape for all storage days. The crumb obtained from fresh bread (Day 0) developed somewhat higher viscosity than crumbs from other storage days.

Blend by storage day interaction was significant for all measured RVA parameters (HPV, CPV, and STB) (Table A-1). The HPV of fresh Gunner crumb was significantly higher than HPV of crumb stored for 1, 3, and 5 days (Table 1.8), whereas STB values (Table 1.9) and CPV (Table 1.10) did not change significantly

Table 1.9. RVA Setback (STB, RVU) of Bread Crumb as Affected by Interaction of Blends and Storage Days

	Days			
Blends[a]	**0**	**1**	**3**	**5**
Gunner	32	41	41	40
20% Ben	27	37	33	38
30% Ben	33	35	34	40
40% Ben	29	28	34	42
20% WS	29	27	34	42
30% WS	32	28	21	41
40% WS	40	27	30	46
20% WD	31	38	34	44
30% WD	30	34	32	38
40% WD	35	33	37	41
LSD (0.05)	---------- 8 ----------			

[a] 20%, 30%, 40% WS = Blends of waxy spring wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour; 20%, 30%, 40% WD = Blends of waxy durum wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour

during storage. Pasting profiles of bread crumb made with Ben blends had the same pattern (Figure 1.8) as the pasting profiles of Gunner crumb (Figure 1.7). Figure 1.8 represents only 40% Ben crumbs; however, 20 and 30% Ben crumbs exhibited the same pasting profiles with somewhat different values (profiles not presented). No distinct peak viscosity or breakdown could be detected on RVA pasting profiles of Ben crumbs. The HPV was similar for all Ben blends within the same storage day and comparable to the HPV of Gunner crumbs (Table 1.8). Ben crumbs exhibited somewhat lower CPVs than Gunner crumbs on each storage day (Table 1.10), but this did not affect the setback values (Table 1.9).

Bread crumb is a much less frequent subject of pasting analyses than flour

Table 1.10. RVA Cold Paste Viscosity (CPV, RVU) of Bread Crumb as Affected by Interaction of Blends and Storage Days

	Days			
Blends[a]	**0**	**1**	**3**	**5**
Gunner	170	159	166	165
20% Ben	152	150	148	158
30% Ben	160	152	150	156
40% Ben	146	137	152	150
20% WS	171	180	197	192
30% WS	174	186	182	196
40% WS	176	177	201	196
20% WD	138	139	138	151
30% WD	131	136	135	143
40% WD	129	134	143	146
LSD (0.05)	---------- 12 ----------			

[a] 20%, 30%, 40% WS = Blends of waxy spring wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour; 20%, 30%, 40% WD = Blends of waxy durum wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour

or starch, but several studies in the past attempted to provide explanation for the specific pasting profile of bread crumbs. The RVA pasting profiles obtained for Gunner crumbs (Figure 1.7) and also Ben crumbs (Figure 1.8) were very similar to those reported by Kim and D'Appolonia (1977a), Morad and D'Appolonia (1980), and Xu et al. (1992a,b). Although these experiments were performed with Brabender amylograph that uses a heating cycle different from that used in RVA method, the underlying processes during heating and stirring of crumb in water can be expected to be the same in amylograph and RVA. According to Xu et al. (1992a), differences in pasting properties between flour and crumb could be attributed to two main factors: 1) bread contains ingredients that are not present in

Table 1.11. DSC Enthalpy of Retrogradation (ΔH, J/g) of Bread Crumb as Affected by Interaction of Blends and Storage Days

	Days			
Blends[a]	**0**	**1**	**3**	**5**
Gunner	1.08	2.36	2.93	3.12
20% Ben	0.92	2.36	2.48	3.43
30% Ben	0.98	2.54	2.50	2.87
40% Ben	1.03	2.56	2.36	3.06
20% WS	1.01	3.09	3.19	3.27
30% WS	1.22	2.59	3.20	3.07
40% WS	0.83	1.92	3.46	3.13
20% WD	0.94	2.64	2.70	3.35
30% WD	1.13	1.92	3.12	3.48
40% WD	0.98	2.11	3.41	3.86
LSD (0.05)	---------- 0.28 ----------			

[a] 20%, 30%, 40% WS = Blends of waxy spring wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour; 20%, 30%, 40% WD = Blends of waxy durum wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour

crumbs might have a 'multistage' retrogradation pattern (Table 1.12). All crumbs had similar retrogradation enthalpies on day 0, with the exception of 40% WS that had lower enthalpy than some other samples. Gunner and Ben crumbs had similar enthalpies on day 1. On day 3, the enthalpy of Gunner was higher, but at the end of the storage all non-waxy crumbs had similar retrogradation enthalpies.

WS and WD blends containing the same percent of waxy flour did not differ in their gelatinization enthalpies (Table 1.3), but their crumbs exhibited some differences in retrogradation enthalpies (Table 1.11). After 1 day of storage, 20 and 30% WD crumbs had lower retrogradation enthalpies than corresponding WS crumbs, and on day 3 the same behavior was detected between 20% WD and WS

Table 1.12. Percent Increase in Retrogradation Enthalpy of Bread Crumb during Storage as Affected by Interaction of Blends and Storage Days

Blends[a]	Day 0 - Day 1	Day 1 - Day 3	Day 3 - Day 5
Gunner	124	24	6
20% Ben	157	5	38
30% Ben	159	1	15
40% Ben	148	0	30
20% WS	207	3	3
30% WS	119	24	1
40% WS	128	86	0
20% WD	181	6	24
30% WD	71	63	11
40% WD	115	63	9
LSD (0.05)	----------	42	----------

[a] 20%, 30%, 40% WS = Blends of waxy spring wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour; 20%, 30%, 40% WD = Blends of waxy durum wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour

crumbs. However, at the end of storage, all WD crumbs showed higher enthalpies than WS crumbs, especially 30 and 40% WD crumbs. Furthermore, based on these results WD crumbs seemed to retrograde faster than other analyzed crumbs since WD crumbs had the highest retrogradation enthalpies. Difference in retrogradation enthalpies between WS and WD crumbs could possibly originate from some structural differences between two classes of waxy starch. Retrogradation kinetics of amylopectin has been shown to depend on its fine structure, i.e. branch chain length distribution (Shibanuma et al. 1996; Yuan and Thompson 1998; Jane et al. 1999; Matsuki 2003; Kohyama et al. 2004).

Bhattacharya et al. (2002); Morita et al. (2002b) reported results that waxy flour lowered the retrogradation enthalpy and therefore had the ability to retard

bread staling. In contrast, the retrogradation enthalpy of waxy and non-waxy bread did not differ (Park and Baik 2007), or bread with waxy flour had higher retrogradation enthalpy than non-waxy bread (Lee et al. 2001; Baik et al. 2003). Retrogradation enthalpy describes the enthalpy of melting re-crystallized amylopectin since amylose retrogradation is irreversible at temperatures below 100°C (Miles et al. 1985; Biliaderis and Zawistowski 1990; Morita et al. 2002a; Biliaderis 2009). The moisture level (35-45%) of bread crumb and many other bakery products was shown to be favorable for starch recrystallization and hence for deteriorative processes that occur during storage of bread. Water is needed to mobilize long starch chains and bring them in close proximity for reassociation, as well as for inclusion in crystalline structure of starch (Chinachoti and Steinberg 1986; Ring et al. 1987; Slade and Levine 1987).

Waxy flour has been researched for its potential to retard starch retrogradation and bread staling because it contains high amounts of amylopectin that retrogrades in later stages of storage due to the branched nature of molecules. The retrogradation enthalpy results in this study did not indicate that WS or WD blends would have different effect on bread staling than non-waxy wheat flour after 5 days of storage. The reason could be that amylopectin recrystallization took place by this time. In earlier stages of storage, 40% WS and 30 and 40% WD crumbs had lower retrogradation enthalpies than Gunner and Ben crumbs (day 1), but their enthalpies increased thereafter (Table 1.11). Park and Baik (2007) indicated that waxy flours could be expected to have high retrogradation enthalpies during storage due to their high proportion of

amylopectin. The onset of amylopectin recrystallization most likely depends on multiple factors besides amylopectin molecular structure such as moisture content of bread, presence of other ingredients in bread formula, interaction between starch and other ingredients, and it is conceivable that amylopectin recrystallization starts at different times in different bread formulations.

Retrogradation enthalpy of bread crumbs also was analyzed relative to the enthalpy of gelatinization of flour blends (Table 1.13). The results in Table 1.13 represent retrogradation enthalpy as the percent of the gelatinization enthalpy. The assumption was that during 5 days of storage, starch did not regain its total initial crystallinity. The goal was to investigate whether different starch compositions would re-crystallize to different extent compared to their initial crystallinity (as measured by the retrogradation enthalpy of crumb). These results show somewhat different situation than the absolute values of retrogradation enthalpy. Fresh waxy crumbs had lower retrogradation/gelatinization enthalpy values than Gunner crumb; on day 1, waxy crumbs with higher percent of waxy flour (30 and 40%) showed the same behavior. Based on these results the conclusion could be that starch in waxy crumbs did re-crystallize to lesser extent at the beginning of storage than starch in Gunner crumb. This difference between Gunner and waxy crumbs was even more pronounced on day 3 of storage. On the fifth day of storage, all waxy crumbs had significantly lower ratio of retrogradation/gelatinization enthalpy than Gunner crumb. The discrepancy between these results and absolute values of retrogradation enthalpy (Table 1.11) remains unclear. One possible explanation is that high retrogradation enthalpy of waxy bread crumbs could be an artifact of

Table 1.13. Percent Retrogradation of Bread Crumb Based on the Enthalpy of Gelatinization of Flour Blends

	Days			
Blends[a]	**Day 0**	**Day 1**	**Day 3**	**Day 5**
Gunner	14.5	31.6	39.1	41.7
20% Ben	11.0	28.3	29.6	41.0
30% Ben	11.6	29.9	29.3	33.7
40% Ben	12.4	30.8	28.4	36.8
20% WS	10.6	32.4	33.4	34.2
30% WS	12.6	26.8	33.2	31.8
40% WS	8.1	18.6	33.6	30.4
20% WD	10.2	28.6	29.3	35.4
30% WD	11.6	19.6	32.1	35.6
40% WD	10.0	21.6	34.9	36.2
LSD (0.05)	---------- 3.6 ----------			

[a] 20%, 30%, 40% WS = Blends of waxy spring wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour; 20%, 30%, 40% WD = Blends of waxy durum wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour

incomplete gelatinization of starch during baking. If some starch granules in bread remain nongelatinized during baking, then those granules could gelatinize during heating of crumb in excess water in DSC and contribute to final enthalpy, which is considered to be the retrogradation enthalpy.

Soluble Starch Content of Crumb

Soluble starch content in bread crumbs (Table 1.14) followed the same pattern like the percent retrogradation presented in Table 1.13. Soluble starch is starch that leached from granules into an intergranular space during gelatinization,

Table 1.14. Soluble Starch Content (% db) of Bread Crumb as Affected by Interaction of Blends and Storage Days

	Days			
Blends[a]	**Day 0**	**Day 1**	**Day 3**	**Day 5**
Gunner	5.4	4.6	4.8	4.6
20% Ben	6.2	5.4	5.6	5.5
30% Ben	5.7	5.9	5.8	5.8
40% Ben	6.9	6.5	6.0	6.3
20% WS	14.2	7.6	5.6	5.8
30% WS	19.7	9.5	6.6	6.0
40% WS	26.1	11.0	6.5	6.3
20% WD	15.2	9.1	7.9	7.3
30% WD	18.8	10.2	9.3	8.6
40% WD	24.3	12.5	10.3	9.4
LSD (0.05)	---------- 1.2 ----------			

[a] 20%, 30%, 40% WS = Blends of waxy spring wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour; 20%, 30%, 40% WD = Blends of waxy durum wheat flour (20%, 30%, 40%, respectively) and non-waxy spring wheat flour

and therefore can be extracted with water (Kim and D'Appolonia 1977b; Bello et al. 1995). Soluble starch content decreased in all crumbs during storage, which was expected because of starch recrystallization. A sharp decrease occurred between days 0 and 1, and it continued to decrease much more gradually through the rest of the storage period. Waxy crumbs had the highest soluble starch content over the storage period, and at the end of the storage it was significantly higher than soluble starch content of Gunner crumb. Also, a difference was detected between WS and WD crumb; WD crumbs had higher soluble starch content on day 5 than the corresponding WS crumbs, showing again a possible difference in retrogradation pattern between waxy starch originating from two different classes

of wheat.

Partial solubilization of starch during gelatinization plays an important role in the textural characteristics of starch-based food (Atwell et al. 1988; Waniska and Gomez 1992). In studies of bread staling, retrogradation enthalpy often is correlated with firmness of bread since amylopectin recrystallizaton is believed to be a major cause of bread firming (Ring et al. 1987; Yuan et al. 1993). Since soluble starch represents the part of starch that has not been entrapped in the crystalline gel structure during retrogradation, it seems reasonable to expect that high content of soluble starch could result with low enthalpy of retrogradation and low firmness of crumb. Therefore, the high soluble starch content could easily lead to the conclusion that waxy crumbs would have lower enthalpies and lower firmness than Gunner crumbs. Nevertheless, the firmness of waxy crumbs was higher than the firmness of Gunner crumb (Table 1.6). Several authors also reported discrepancy between bread firmness and retrogradation enthalpy (Sahlström and Bråthen 1997; Hallberg and Chinachoti 2002; Park and Baik 2007). It should be noted that even though waxy crumbs had higher soluble starch content than Gunner crumb at the end of storage, the decrease of soluble starch content in these crumbs was much larger than in Gunner crumb (Table 1.14). This raises the possibility that in spite of higher soluble starch content at the end of storage, waxy crumbs still might have developed higher crystallinity during storage than Gunner crumbs.

Considering the complexity of bread, firmness should be viewed as a complex process that cannot be explained simply by recrystallization of starch.

While firmness is one of the (and probably most obvious) manifestations of starch retrogradation and bread staling, it is not caused only by retrogradation. Many other factors can influence bread firmness, such as moisture content, possible evaporation and moisture migration, structure of crumb cells, cell wall properties, and presence of emulsifiers and fats (Rao et al. 1992; Halberg and Chinachoti 2002).

CONCLUSIONS

Blending WS and WD flour with non-waxy flour significantly affected the quality of white pan bread and its staling properties. Overall, the addition of waxy flour to non-waxy bread flour did not improve softness of bread and did not retard staling of bread that was stored for 5 days. Addition of waxy flours to non-waxy flour did have some positive impact on firmness reduction and staling reduction, as measured by the retrogradation enthalpy, in early stages of bread storage, i.e. up to 1 and in some cases up to 3 days of storage. Waxy blends produced bread of lower or even inferior quality than non-waxy flours, especially at 40% blend. Waxy bread had very open crumb grain, and it was prone to collapsing and shrinking upon cooling. Therefore, waxy flours may not be suitable for production of white pan bread, especially at high concentrations. Waxy flour might have some potential for use at levels up to 20% in white pan bread, provided that the bread is well formulated with enzymes and conditioners that are traditionally used in industrial bread formulations. At higher levels of waxy flour, higher levels of conditioners in bread formula probably would be needed to compensate for deterioration of crumb done by the waxy flour (mainly to manage crumb cell size). This probably would not be cost effective for commercial bakeries. On the other hand, waxy flour could have a potential of being used in products that require open, porous structure and that are consumed fresh, like puff pastry or different types of 'artisan bread'. Many of these products (especially 'artisan bread' products) often do not allow the use of traditional bread conditioners that retard staling. In these products, waxy flour could impart short-term softness and open

structure to the product with possible reduction of fat that is used in puff pastry.

Waxy crumbs (especially from waxy durum) exhibited higher retrogradation enthalpy and higher firmness than Gunner and Ben crumbs, yet had higher content of soluble starch in crumb. These results indicate that bread firming is a complex process influenced by not only starch retrogradation, but possibly by moisture loss and migration and by interactions of starch with other crumb components.

Clearly, a blend of two starches or flours such as waxy and non-waxy is a more complex system than a single starch (flour). Properties of blends most likely are not a mathematical average of the properties of single components, but rather a result of their interactions. Therefore, properties such as gelatinization, retrogradation, bread firming should be analyzed and interpreted carefully. The best example in this study is the complex relationship between retrogradation enthalpy, soluble starch content, and bread firmness. Although waxy crumbs restored a lower percentage of their initial crystallinity than non-waxy Gunner and Ben blends (measured by the ratio of retrogradation and gelatinization enthalpy, Table 1.13), they also had higher retrogradation enthalpy at the end of storage and produced firmer bread than non-waxy flours.

While WS and WD blends had similar amylose contents, they differed in pasting and gelatinization properties, as well as in retrogradation enthalpy of starch in crumb, pasting properties of crumb, amount of soluble starch in crumb, and firming rate of crumb. These results indicate that WS and WD flours might have structural differences between their starches that caused differences in functional properties of these flours and their blends.

Finally, a question emerged throughout this study on whether properties of WD flour and starch are more attributable to their durum or waxy nature. The results show that Ben and WD blends differed in every property that was analyzed in this study. Properties of Ben blends were much more similar to properties of Gunner flour than they were to WD blends. Differences in dough properties, RVA pasting properties of blends and crumbs, crumb firmness, gelatinization properties, soluble starch content, and retrogradation enthalpy between WD and Ben blends indicate that the difference in functionality of these flours in bread baking is clearly governed not by their durum nature but rather by their starch properties.

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CHAPTER 2: EFFECT OF AMYLOSE CONTENT AND GLUTEN ON GELATINIZATION AND RETROGRADATION PROPERTIES OF WHEAT STARCH BLENDS AND WHEAT STARCH/GLUTEN BLENDS

ABSTRACT

The ratio of amylose and amylopectin is thought to have an important effect on gelatinization and retrogradation of starch. Gluten as the second most abundant polymer in bread flour could potentially affect behavior of starch. Therefore, waxy durum (WD) starch (2.4% amylose content) was blended with non-waxy hard red spring wheat starch (25.1% amylose content) at levels 0, 12.5, 25, 50, 75, and 100% (w/w) to obtain blends with different amylose content. Gluten was added to these blends at 30% (w/w). All blends were analyzed for their pasting, gelatinization, and retrogradation properties. Amylose contents of blends were significantly different. Blends with 50 and 75% WD, low amylose content, exhibited dual RVA pasting peaks, faster swelling, lower hot paste viscosity, and lower cold paste viscosity, while high amylose blends (0-25% WD) had only one RVA peak. Gluten delayed swelling and pasting of blends with low amylose content. Gluten did not affect starch gelatinization enthalpy (ΔH), but did increase the onset and peak temperatures of gelatinization. ΔH of starch blends fell between ΔH of the two individual components, but the difference in ΔH between blends with 25 and 50% WD and between 50 and 75% WD was not significant. Retrogradation enthalpy of starch blends, based on the proportion of amylopectin in blend (ΔH_{BR}) increased during the whole storage period. On each storage day, ΔH_{BR} was lower for low amylose blends than for high amylose blends. Gluten significantly lowered the ΔH_{BR} of low amylose blends (50, 75, 100% WD level) compared to that of starch blends, especially on day 15 and day 20. The results indicate that pasting, gelatinization, and retrogradation properties of starch depend on amylose content

of starch. When gluten is present in the system, these properties often are affected by complex interactions between amylose, amylopectin, and gluten.

INTRODUCTION

Texture and shelf-life of cereal-based baked products often are related to starch. In many food products, the semi-crystalline structure of starch is subjected to conversion by thermal processing in the presence of different amounts of water and mechanical shear (Farhat et al. 1999). Functional properties of starch in food products greatly depend on its chemical composition, presence of other food components, and availability of water (Kim and D'Appolonia 1977a,b; Eliasson 1983; Leloup 1991; Slade and Levine 1991; Fredriksson 1998).

Gelatinization and retrogradation are major physical properties of starch that determine its functionality in food products. During gelatinization, starch granules swell due to the heating of starch in the presence of water. Swelling is facilitated by breaking of intermolecular hydrogen bonds in starch and increased binding of water by available hydrogen bonds. Starch granules undergo irreversible changes such as loss of birefringence, melting of starch crystals, and solubilization of starch (Atwell et al. 1988; BeMiller 2007; Biliaderis 2009). As a result of gelatinization, linear amylose molecules and some amylopectin leach from the granules and form a continuous phase outside the granules, while the remaining swollen granules and granule ghosts (gelatinized starch granules from which the majority starch polymers leached into intergranular space) are rich in amylopectin (Hermansson and Svegmark 1996).

Several models of gelatinization have been proposed (Donovan 1979; Evans and Haisman 1982; Biliaderis et al. 1986; Blanshard 1987). The current interpretation of macromolecular changes that take part in granules during

gelatinization is based on the model of Donovan (1979) and Blanshard (1987), later refined by Jenkins and Donald (1998), who proposed that gelatinization is a granule swelling driven process. According to Jenkins and Donald (1998), the amorphous region is connected with amylopectin molecules at the edges of lamellar stacks, thus providing backbone to the granule. Expansion of the amorphous region imposes a stress upon the amylopectin crystallites and causes disruption of the semi-crystalline lamellae, reduction of granule crystallinity, and loss of birefringence. A liquid crystalline approach to gelatinization, proposed by Waigh et al. (2000 a,b), presents gelatinization as a two stage process; the first stage is the dissociation of amylopectin double helices from their lamellar crystallites and the second stage is unwinding of helices and transformation into a coil form.

Retrogradation is a process of starch recrystallization that occurs when starch molecules reassociate into an ordered state after gelatinization (Biliaderis 2009). Ordered structure can develop into crystalline structure under favorable conditions (Atwell et al. 1988). In starch pastes, retrogradation results in gel formation. The initial stage of gelation is dominated by the rapid and irreversible reassociation of solubilized amylose, followed by slower and reversible recrystallization of amylopectin (Miles et al. 1985; Biliaderis and Zawistowski 1990). According to Gidley (1989), gelation is due to interchain associations in the form of double helices and aggregation of helices that form junction zones and eventually a three-dimensional network. Partial crystallization of amylopectin within granules has been suggested to increase the rigidity of granules and impart

reinforcement to the gel amylose matrix (Miles et al. 1985).

Gelatinization and retrogradation of starch are affected by amylose content, amylose and amylopectin structures, as well as by the presence of other ingredients that interact with starch and compete for water (Fredriksson et al. 1998; Koch et al. 1998; Mohamed and Rayas-Duarte 2003). Different amylose contents can be obtained by blending non-waxy and waxy wheat starch. For practical applications in food products, a blend of non-waxy and waxy wheat starch could be more suitable than the waxy starch alone, since waxy starch can impart undesirable processing and textural characteristics to a food product (Lee et al. 2001; Morita et al. 2002; Baik et al. 2003).

Studies on gelatinization and retrogradation properties of wheat starch blends with different amylose contents are scarce. Sasaki et al. (2000) found differences between native and mixed wheat starches with the same amylose content. Mixed and native starches with the same amylose content differed in onset and peak gelatinization temperature.The DSC endotherm was broader and the RVA peak viscosity was much lower in mixed starches than in native starches. Hagenimana and Ding (2005) reported that blends of 25% waxy and non-waxy rice starch showed RVA profiles with two peaks, with the second peak being higher than that of non-waxy starch but lower than that of waxy starch. Fredriksson et al. (1998) and Gupta et al. (2009) reported that starch blends showed different gelatinization properties than native starches with the same amylose content due to the lack of homogeneity.

The effect of amylose and amylopectin and their ratio on retrogradation

properties of starch has been attributed to different phenomena such as phase separation of amylose and amylopectin in pastes and gels (Kalichevsky and Ring 1987; Leloup et al. 1991; Kim and Willet 2004), and more often to interactions between amylose and amylopectin (Jane and Chen 1992; Boltz and Thompson 1999; Klucinec and Thompson 1999; Klucinec and Thompson 2002; Sasaki et al. 2007; Yu et al. 2009). Exterior chains of amylopectin, especially long chains, could form double helices with amylose upon retrogradation of a gel (Klucinec and Thompson 1999), while highly branched amylopectin molecules inhibit formation of long amylose-amylose double helices (Jane and Chen 1992; Klucinec and Thompson 1999). Gelation is proposed to be due to the formation of physical junction zones between amylose molecules, amylose and amylopectin molecules, and between amylopectin molecules (Klucinec and Thompson 2002); therefore the amylose content of starch can be expected to have a crucial effect on retrogradation properties of starch.

Effect of gluten, the second most abundant polymer in wheat flour, on starch properties has been investigated mainly from the perspective of bread staling (Willhoft 1973; Kim and D'Appolonia 1977a,b; Martin and Hoseney 1991; Martin et al. 1991). Several studies investigated the effect of gluten on starch gelatinization and retrogradation; however, the results were inconsistent. DSC studies of starch and gluten blends conducted by Erdogdu et al. (1995) and Chevallier and Colonna (1999) showed no interaction of starch and gluten and no influence of gluten on gelatinization properties of starch. Opposite to these studies, wheat gluten (Eliasson 1983a) as well as proteins with disulfide bonds in the rice

flour (Hamaker and Griffin 1993) were found to reduce the swelling of starch granule during gelatinization and to reduce leaching of amylose from starch granules. Eliasson (1983a) and Mohamed and Rayas-Duarte (2003) showed that gluten increased the gelatinization temperature of starch and decreased the enthalpy of gelatinization. Gluten was found to retard retrogradation (Eliasson 1983b) by reducing water loss from granule remnants (Wang et al. 2004), or by hindering the formation of starch network and weakening the strength of starch gels (Champenois et al. 1998). Opposite to those findings, Lindahl and Eliasson (1986) found that addition of 1% (dry basis) gluten to 6.5% (w/w) starch suspension increased the storage modulus (G') of wheat starch, which was related to increase in starch retrogradation. However, Ottenhof and Farhat (2004) found no evidence of gluten effect on starch retrogradation when extruded wheat starch/gluten (10:1) blends were analyzed by DSC, X-ray diffraction and NMR relaxometry.

Based on the available information, the effect of amylose content and gluten on gelatinization and retrogradation properties of starch blends still is not clear. Therefore, the objective of this research was to study the effect of amylose content and gluten on pasting, gelatinization, and retrogradation properties of starch blends. Starch blends with different amylose contents were prepared by blending waxy and non-waxy wheat starch and subsequently gluten was added to these blends. All blends were subjected to testing of their structural composition, pasting, gelatinization, and retrogradation properties.

MATERIALS AND METHODS

Wheat

Non-waxy commercial hard red spring wheat cultivar, 'Alsen' was used as a source of non-waxy starch and gluten. A waxy durum wheat line (WD) was used as a source of waxy starch. This line was derived from an initial cross of hard red winter wheat, 'Ike', which carried null alleles at *Wx-A1* and *Wx-B1 loci,* and durum wheat cultivar 'Ben'. Subsequently, full waxy durum wheat lines were developed by backcrossing to Ben while selecting among backcross progeny for the full waxy genotype. The full waxy durum line, derived from the fourth backcross to the recurrent durum parent, Ben, was provided by Dr. Douglas Doehlert (USDA-ARS, Cereal Crops Research Unit, Fargo, ND).

Waxy durum wheat was used as a source of waxy starch since waxy common wheat was not available in sufficient quantity for the experiments. Current information available on similarities and differences between durum and common wheat starch are presented in the 'Results and Discussion' section.

Isolation of Starch and Gluten

Waxy and non-waxy wheat were tempered and milled into a straight grade flour using a Bühler laboratory mill according to AACC Approved Methods 26-10 and 26-21 (2000). Waxy and non-waxy starch were isolated using a dough washing method according to Kim and Seib (1993), which was a modification of the method of Wolf (1964). Flour (approx. 400 g) and distilled water (60-65% w/w based on flour) were mixed in a pin mixer for a short time just to obtain a cohesive

mass but prevent gluten development as much as possible. Starch was washed out by adding small amounts of distilled water, at least five times in succession, and separated from gluten by sieving through the US 70 sieve (212 µm). Starch suspension was centrifuged at 2,000 x *g* for 15 min. Supernatant was discarded and the upper pigmented sediment (consisting of tailings, water soluble proteins) was removed by careful scraping with a spatula. Starch was re-suspended in distilled water and the process was repeated two times. The third washing was done in ethanol in order to remove non-starch lipids, i.e. lipids that are not associated with amylose in the starch granule. Removal of endogenous starch lipids was not done since it would require starch to be gelatinized and then to extract of lipids with water saturated butanol (Morrison 1980; Morrison 1985). These conditions cause swelling and partial disruption of starch crystallinity and consequently change the functional properties of starch. Native properties of starch had to be preserved for this study. Prime starch was air dried overnight at room temperature, and ground using mortar and pestle (to avoid damaging starch granules by more abrasive grinding technique) and sieved through US 70 sieve. Starch was stored in tightly closed containers to prevent moisture absorption.

Gluten was isolated from the cultivar 'Alsen' simultaneously with starch isolation (from the same dough). After starch was isolated, gluten was continuously washed until the wash water did not contain any starch (clear wash water, also tested with iodine solution). Gluten was dried by freeze-drying and milled using a ball mill to avoid heating of gluten. Ground gluten was sieved through the US 70 sieve.

Preparation of Blends

Starch blends for pasting and gelatinization studies were prepared by mixing non-waxy starch and waxy durum (WD) starch (on dry basis) so that the blends contained 12.5, 25, 50, and 75% waxy durum starch (w/w). Pure non-waxy and waxy starches were also analyzed, and labeled as samples with 0% and 100% WD (w/w), respectively. All blends for pasting and textural analyses were prepared by weighing required amounts of two starches directly into RVA canisters to obtain replicates. Starch blends that were used for DSC analysis were prepared by mixing waxy and non-waxy starch with distilled water at room temperature, and stirring the dispersion for 30 min on a magnetic plate. The mixture was centrifuged to remove water, and starch was dried overnight at room temperature. This procedure was selected in order to ensure thorough blending of two starches. The amount of sample used for DSC measurements is very small (3 mg) and inadequate blending could affect the results to great extent.

Gluten/starch blends consisted of 30% (w/w) gluten isolated from hard red spring wheat cultivar 'Alsen' and 70% (w/w) starch blends described above. Blending of starch and gluten for pasting and textural analyses was done the same way as described for starch blends, while an alternative method had to be used for DSC analyses. Required proportions of gluten, WD, and non-waxy starch were weighed (dry basis) in a dry form into glass tubes and mixed first with spatula. Following this, glass tubes were capped, wrapped in a protective material to prevent breakage, and transferred to a V-blender for 15 min to thoroughly blend starch and gluten. This method enabled blending of small amount of material

needed for DSC.

This procedure was replicated three times for each blend. The identification for starch blends in the text is 0 wx, 12.5 wx, 25 wx, 50 wx, 75 wx, and 100 wx and for starch/gluten blends is 0 wxg, 12.5 wxg, 25 wxg, 50 wxg, 75 wxg, and 100 wxg.

Chemical Analyses

Moisture content of starch, gluten, and blends was determined by the air oven method (AACC Approved Method 44-5A, 2000). Nitrogen content of isolated starches was determined by the crude protein combustion method (AACC Approved Method 46-30, 2000) using Leco FP428 nitrogen analyzer (Leco Corporation, St. Joseph, MI). The quality of gluten isolated from hard red spring wheat cultivar 'Alsen' was determined using gluten index method, according to the AACC Approved Method 38-12 (2000). Amylose and amylopectin content of starch were determined using the Megazyme amylose/amylopectin assay kit (Megazyme International Ireland Ltd. Wicklow, Ireland). The method is based on complex formation and precipitation of amylopectin with lectin concanavalin A (Con A), and subsequent determination of amylose content by enzymatically hydrolyzing amylose to glucose and analyzing glucose content using glucose oxidase/peroxidase reagent (GOPOD). Concentration of amylose was obtained as the ratio of GOPOD absorbance at 510 nm of the supernatant of the Con A precipitated sample, to that of the total starch sample (Gibson et al. 1997).

Pasting Properties of Blends

Pasting properties of base starches and starch/gluten blends were determined by Rapid Visco Analyzer (RVA) (Newport Scientific, Narrabeen, Australia), as described by Bhattacharya et al. (1997, 1999). Starch (or starch and gluten) (3.0 g, 14% mb) was weighed directly into an aluminum RVA sample canister, and 25 mL deionized water was added and mixed thoroughly with the sample. A programmed heating and cooling cycle (13 min) was used, where the samples were held at 50°C for 1 min, heated to 95°C in 3.5 min, held at 95°C for 2.5 min before cooling to 50°C, and holding at 50°C for 1 min. Peak viscosity (PV), time from onset of pasting to peak viscosity (P_{time}), hot paste viscosity at the end of holding at 95°C (HPV), breakdown (BD; PV – HPV), final viscosity at 50°C or cool paste viscosity (CPV), and setback (SB; CPV – HPV) were recorded. Results were reported in Rapid Visco Units (RVU).

Thermal Properties of Blends

Thermal properties were analyzed using a differential scanning calorimeter (DSC) (DSC 7, Perkin-Elmer Corp., Norwalk, CT in conjunction with a digital DEC-425 thermal analysis data station) according to the method described by Bhattacharya et al. (1999). Starch blends and starch/gluten blends for DSC measurements were prepared as described in "Preparation of Blends". A sample (3.0 mg db) was weighed directly into a tared aluminum pan and deionized water was added to obtain a dry material-to-water ratio of 1:3 (w/w, db). The pan was hermetically sealed and allowed to equilibrate overnight at room temperature to

obtain uniform water distribution in the sample before analysis. Samples were heated from 10°C to 110°C at the rate of 10°C/min. An empty DSC pan with deionized water was used as a reference. The onset temperature of gelatinization (T_o), the temperature at peak (T_p), the temperature at the end (completion) of gelatinization (T_c), and the enthalpy of gelatinization (ΔH) were obtained using the data processing software supplied with the DSC instrument. No measurements were made on the amylose-lipid endotherm in the region 95–120°C. Indium was used to calibrate the calorimeter.

Thermal Properties of Gels

Gelatinized starch and starch/gluten blends were stored in DSC pans at 4°C, and analyzed after 0, 5, 10, 15, and 20 days of storage. Samples were allowed to equilibrate to room temperature for 2 hr before measurement. The same heating regime was used as for gelatinization. In order to differentiate DSC data for retrograded starch from those for gelatinization, DSC values for retrograded starch will be labeled as T_{or} (onset temperature of meting retrograded starch), T_{pr} (peak temperature of melting retrograded starch), T_{cr} (temperature at the end of melting retrograded starch), and ΔH_r (enthalpy of melting retrograded starch).

Fractionation of Starch

Gel Permeation Chromatography (GPC) was used to fractionate starch into amylose and amylopectin. Gel permeation chromatography of starch generally followed the method of Jane and Chen (1992) and Klucinec and Thompson (1998).

Native (granular) starch (6 mg, dry weight) was dispersed in 90% (v/v) dimethyl sulfoxide (DMSO) (3 mL) by heating the mixture in boiling water bath with constant stirring for 1 hr. Mixture was stirred for 24 hr at room temperature. Starch was precipitated by adding three volumes of ethanol (9 mL) and centrifuged at 6000 x *g* for 15 min at 20°C. The supernatant was discarded carefully to minimize the amount of ethanol in the centrifuge tube. Pellets were redissolved in hot distilled water (3 mL) and boiled in a water bath with constant stirring for 30 min. Following this, the starch solution was cooled quickly to room temperature, and 2 mL of the solution was loaded onto the GPC column 1.0 cm x 50 cm, Pharmacia Inc., Piscataway, NJ) packed with Sepharose CL-2B gel (Sigma-Aldrich Co, St. Louis, MO). The mobile phase was deionized water containing 25 m*M* NaCl and 1 m*M* NaOH. Sodium azide (NaN_3) was added (0.02%) to preserve the column packing. Mobile phase was filtered through nylon membrane filter (0.2 µm) and degassed before use. Eluent was run through the column overnight to condition the column. Elution of starch fraction was done by gravity flow of the mobile phase, and 1 mL of each fraction was collected. The end of run of a sample was determined by adding glucose to the starch solution (glucose eluted at the end).

A subsample (0.1 mL) of each fraction was loaded into a 96-well microplate and tested for the blue value by adding the same volume of I_2/KI solution (0.2 g I_2 + 2.0 g KI in 100 mL 0.1 M Acetate buffer pH 5.0, diluted 10 x). Blue value was determined according to the general method of Schoch (1964). Absorbance of each fraction was read by using the Dynex MRX microplate reader (Dynex Technologies, Chantilly, VA). The blue value was used to identify locations of

amylose and amylopectin in the chromatograms and also in the fractions. The amylose and amylopectin solutions were further used for the determination of the wavelength of maximum iodine absorption (λ_{max}).

Total carbohydrate content in each fraction was determined using the phenol-sulfuric method, following the procedure of Dubois et al. (1956). A sample (0.2 mL) was mixed with 0.2 mL of 5% phenol solution, and 1 mL of concentrated H_2SO_4 was added in a form of a rapid stream to facilitate mixing and develop heat necessary for the reaction. The sample was cooled down to room temperature for 30 min and 0.2 mL was transferred to microplate reader. Absorbance was read at 470 nm.

Iodine Binding λ_{max}

The wavelength of maximum iodine absorption (λ_{max}) of starch, amylose, and amylopectin was determined following the general method of Morrison and Laignelet (1983) with slight modification introduced by Klucinec and Thompson (1998). The λ_{max} of starch is defined as the peak absorbance over the range of wavelengths examined.

Starch solution for λ_{max} determination was prepared as described under the Gel Permeation Chromatography method. A subsample (0.5 mL) of 0.2% starch solution was mixed with deionized water (2.0 mL) and 0.1 mL of I_2/KI solution (2.0 mg of I_2/mL and 20.0 mg of KI/mL) was added, mixed with starch solution immediately, and scanned using the spectrophotometer (Hach DR/4000U, Hach Company, Loveland, CO) from 400 nm and 800 nm. Equal volumes of each GPC

fraction of amylose and amylopectin were collected in two separate tubes and 2.5 mL of each amylose and amylopectin fraction were mixed with 0.1 mL I_2/KI solution and scanned following the same procedure as for the starch solution. The volumes of starch solution, amylose and amylopectin solution, and I_2/KI solution used in this experiment were determined based on several trials in order to obtain adequate readings on the spectrophotometer.

Statistical Analysis

The experimental design for the analysis of amylose content of blends and for iodine binding of blends was a randomized complete block (RCBD). Data were analyzed using the general linear model procedure (GLM) of the Statistical Analysis Systems (SAS) (version 9.1, SAS Institute, Cary, NC). Three sets of blends were prepared, and each set was considered a replication (block).

The experimental design for pasting and gelatinization properties of blends was a randomized complete block (RCBD) with factorial arrangement of six levels of waxy durum starch in blends (0, 12.5, 25, 50, 75, and 100%) and two levels of gluten (0 and 30%). The experimental design for the retrogradation study (DSC of gels) was conducted using a RCBD with factorial arrangement of twelve blends (0 wx, 12.5 wx, 25 wx, 50 wx, 75 wx, 100 wx, 0 wxg, 12.5 wxg, 25 wxg 50 wxg, 75 wxg, and 100 wxg) and five storage days. All variables were fixed. In each design, three sets of blends were prepared, and each set was considered a replication (block). All data were subjected to analysis of variance using Statistical Analysis Systems (SAS) (version 9.1, SAS Institute, Cary, NC). F-test was significant at $P<$

0.05. Means were separated by Fisher's protected least significant difference test ($P \leq 0.05$). Pearson's correlation coefficients were calculated using SAS (version 9.1, SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Chemical Analyses

Non-waxy starch (isolated from wheat cultivar Alsen) contained 0.08% nitrogen (0.46% protein) and waxy starch (isolated from waxy durum line) contained 0.07% nitrogen (0.4% protein). These values were between those reported for prime starch by Reddy and Seib (2000) (0.2% protein) and Czuchajowska and Pomeranz (1993) (0.8% protein). Gluten index of 'Alsen' gluten was 97 which indicated sound strong gluten without damage caused by proteases or excessive temperatures during the growing season.

Amylose contents of blends and iodine binding properties of starch and its fractions are presented in Tables 2.1 and 2.2. The amylose content of blends decreased when the amount of WD starch increased in blends (Table 2.1), which was expected because WD (100 wx) had the lowest amylose content of all starch samples. Amylose content was significantly different among all starch blends and also among all starch/gluten blends. Starch/gluten blends contained less amylose than starch blends due to the part of starch being substituted by gluten. The 25 wx blend and 0 wxg blend had similar amylose contents, which can be attributed to 30% dilution of starch with gluten in starch/gluten samples. This was the only case where similarity in amylose content was observed between two samples with different percent of WD starch. All other samples that differed in percent WD also differed in amylose content (Table 2.1). The similarity in amylose content between 25 wx and 0 wxg samples will be discussed later with regard to pasting and gelatinization properties of these two blends.

Table 2.1. Amylose Content (%, w/w) of Starch Blends and Starch/Gluten Blends

Blends	Amylose content (%, w/w)
0 wx	25.1
12.5 wx	22.3
25 wx	17.4
50 wx	13.8
75 wx	8.1
100 wx	2.4
0 wxg	17.5
12.5 wxg	15.6
25 wxg	12.2
50 wxg	9.6
75 wxg	5.7
100 wxg	1.7
LSD (0.05)	1.3

Table 2.2. Maximum Wavelength of Iodine Binding (λ_{max}) of Starch, Amylose, and Amylopectin

Waxy durum starch (%)	λ_{max} starch	λ_{max} amylose	λ_{max} amylopectin
0	645	642	567
12.5	637	640	563
25	628	640	557
LSD (0.05)	10	1.6	1.2

Iodine binding properties of starch, amylose, and amylopectin were examined on samples that contained 0%, 12.5%, and 25% WD starch. Maximum wavelength of starch iodine binding (λ_{max}) was significantly different between 0 wx and 25 wx blends (Table 2.2), and it can be attributed to higher amounts of amylopectin in 25 wx blend than in waxy starch. The ability of starch to complex

with iodine is primarily due to long, unbranched chains of amylose. Fales (1980) found a linear relationship between the wavelength of maximum absorbance (λ_{max}) and the chain length of amylose. Long, unbranched chains of amylose have high λ_{max} because they are able to include more iodine molecules in the amylose helix than shorter amylopectin chains (Klucinec and Thompson 1998). Although the amylose content of 12.5 wx sample was significantly different from amylose content of 0 wx and 25 wx (Table 2.1), 12.5 wx had similar λ_{max} to both 0 wx and 25 wx. The λ_{max} of amylose in all blends was the same (640 nm). This result indicates that the structure of amylose in non-waxy and waxy starch was not different. Although iodine mainly binds to amylose, Morrison and Laignelet (1983) found that some linear chains in amylopectin can complex with iodine but with lower λ_{max} than that of amylose. Unlike the λ_{max} of amylose, the λ_{max} of amylopectin decreased when the amount of WD starch in analyzed blends increased. In fact, the λ_{max} of WD wheat amylopectin and WD starch was found to be 530 nm (data not shown in .2 2), while the λ_{max} of non-waxy wheat amylopectin was 567 nm (Table 2.1). Fujita et al. (1998) also found low values (between 524 and 534 nm) for waxy wheat starches. These results suggest a difference in the structure of waxy and non-waxy amylopectin. Low value of λ_{max} indicates that WD starch amylopectin contains shorter iodine-complexible branch chains than amylopectin of non-waxy wheat starch (Klucinec and Thompson 1998; Tziotis et al. 2004).

An uncertainty exists as to whether the difference in λ_{max} between two amylopectins is a true difference between waxy and non-waxy wheat starch, or a

difference between common and durum wheat. Several studies compared durum and common wheat starch. In studies of Medcalf and Gilles (1965) and Lii and Lineback (1977), durum wheat starch began to swell and gelatinize at lower temperature than common wheat starch. Medcalf and Gilles (1965) attributed this behavior to less compact granule structure of durum starch than common wheat starch, while Vansteelandt and Delcour (1999) attributed it to a lower relative double helix content of durum than common wheat starch. Opposite to these findings, Soulaka and Morrison (1985) reported that durum and common wheat starch had similar gelatinization properties. The starch granule size distribution of durum and hard red spring wheat was similar in the study of Berry et al. (1971) and Vansteelandt and Delcour (1999), as well as the relative proportions of A to B granules (Vansteelandt and Delcour 1999). Soulaka and Morrison (1985) and Vansteelandt and Delcour (1999) found higher lipid content in durum starch than in common wheat starch, while Berry et al. (1971) found similar lipid contents in these starches. Evidently, findings about differences between durum and common wheat starch disagree to certain extent. Also, all the presented results apply to non-waxy durum and common wheat, while waxy wheat could exhibit different behavior. However, since the impact of the durum nature of waxy durum starch on experimental results cannot be excluded, it is important to emphasize that all the results in this study were obtained using blends of common wheat starch and waxy durum starch.

Literature does not provide direct comparison of λ_{max} between durum and common wheat. However, a study (unpublished data, Matkovic) in which soluble

starch was isolated from fresh bread made of common (hard red spring) wheat and combination of common and durum wheat showed similar λ_{max} values (between 582 nm and 586 nm). These results suggest that amylopectins of common and durum wheat do not differ. However, λ_{max} of soluble starch isolated from fresh bread was affected by the presence of waxy common or waxy durum wheat. Both waxy wheats resulted in lower λ_{max} of soluble starch than non-waxy wheats, indicating that waxy wheat amylopectins have shorter chains than those of corresponding non-waxy wheats. In addition, soluble starch isolated from bread containing waxy durum flour had λ_{max} between 530 and 556 nm depending on its concentration in bread, while waxy common flour in bread produced λ_{max} of soluble starch between 546 and 568 nm, showing some structural differences between waxy durum and waxy common starch. Additional research is needed to better define structural properties of two waxy starches; however, based on presented results the low λ_{max} value of waxy durum amylopectin and starch blends containing waxy durum starch (Table 2.2) most probably is due to the waxy rather than durum nature of waxy durum starch.

The results for λ_{max} were confirmed by gel permeation chromatography (GPC) profiles of waxy durum and non-waxy starches and their blends (Figure 2.1). The first GPC peak corresponded to amylopectin, whereas the second peak, which had a high blue value (relative to the carbohydrate peak), corresponded to amylose. As expected, waxy wheat starch eluted mostly in the amylopectin peak, and the amylose peak was almost not detectable. In addition, the GPC profiles of waxy and non-waxy starches showed differences in blue value of amylopectin

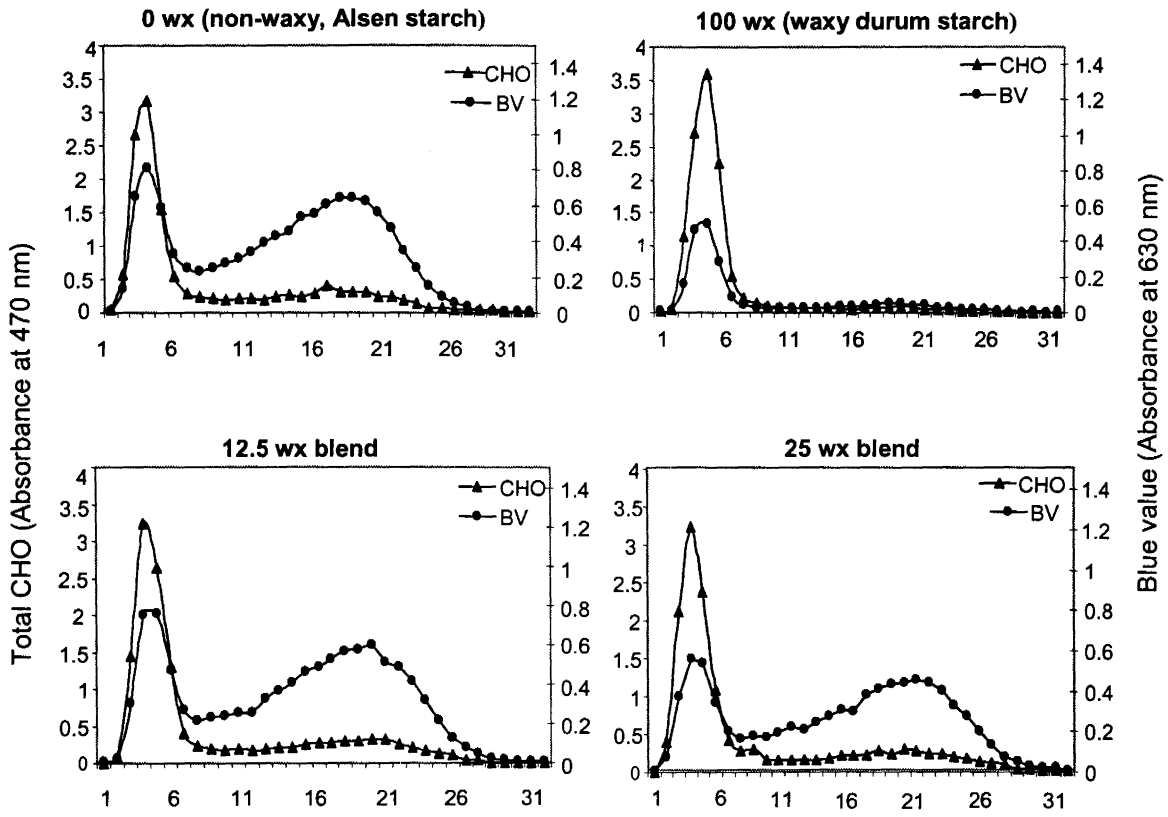

Figure 2.1. Gel Permeation Chromatography (GPC) profiles of 0 wx (non-waxy, Alsen starch), 100 wx (waxy durum starch), 12.5 wx blend, and 25 wx blend

fraction. The ratio of blue value peak and amylopectin carbohydrate peak of the waxy durum starch was lower (0.16) than that of non-waxy starch amylopectin (0.38), indicating that waxy amylopectin had shorter branch chains than the non-waxy amylopectin. Similar results were reported by Yoo and Jane (2002) who found that high blue value of normal (non-waxy) starch amylopectin was due to the extra-long chains present in non-waxy and absent in waxy amylopectin.

RVA Pasting Properties of Blends

Non-waxy and WD starch (labeled as 0 wx and 100 wx, respectively)

showed RVA pasting behavior typical for these starches (Figure 2.2). Pure WD starch developed peak viscosity (PV) at lower temperature (78.5°C) and in a shorter time (3.4 min) than non-waxy starch that peaked at 95 °C after 6.9 min. Swelling of starch granules primarily is a property of amylopectin (Tester and Morrison 1990). Due to reduced amylose content, waxy wheat starch is very susceptible to swelling and shear deformation. As a result, waxy starch develops maximum paste viscosity at lower temperatures than non-waxy starch.

WD starch was not able to maintain the stability of developed viscosity as shown by rapid breakdown. Similar behavior of waxy starch also was observed by Hermansson and Svegmark (1996), Hayakawa et al. (1997), and Sasaki et al. (2000). Its granules started disintegrating even before the non-waxy starch reached its peak viscosity (Figure 2.2). This resulted in high breakdown (BKD) of 182 RVU for waxy starch vs. 37.2 RVU for non-waxy starch. The cold paste viscosity (CPV) and setback (STB) of waxy starch were much lower than those of non-waxy starch (Figure 2.2, Table 2.3), which was shown also in some previous studies that involved waxy starches from different sources (Hayakawa et al. 1997; Jane et al. 1999; Sasaki et al. 2000; Hagenimana and Ding 2005). Upon cooling of starch paste, amylose molecules start to aggregate through hydrogen bonds and form a gel network (Jane et al. 1999; Blazek and Copeland 2007). Since CPV is part of RVA profile in which starch paste was subjected to cooling, it is reasonable to conclude that CPV forms as a result of partial aggregation and gelation of amylose molecules. The 100 wx (WD) starch contained very low amount of amylose and formed a weak gel that reflected on low CPV value.

The temperature at which the peak viscosity was reached remained the same (95.0°C) for blends with low amounts of WD starch (0 wxg, 12.5 wxg, 25 wxg). Peak times of these blends were significantly shorter than those for corresponding starch blends, probably due to lower amount of starch in starch/gluten blends than in starch blends (Table 2.3). Peak temperatures for 50 wxg, 75 wxg, and 100 wxg were significantly higher than those of corresponding starch blends, showing that gluten possibly delayed swelling of waxy starch granules (that would normally swell first) and pasting of starch in these blends. Peak times for these starch/gluten blends, however, were only slightly longer than those of starch blends. These results indicate that gluten may affect differently the swelling of starch that contains high proportions of waxy component and starch with low proportions of waxy component.

HPVs of starch/gluten blends were significantly lower than those of corresponding starch blends (which was expected considering the lower PVs of starch/gluten blends compared to those of starch blends); however, a different trend in HPV behavior between starch blends and starch/gluten blends was noticed. HPV for starch/gluten blends behaved exactly opposite from HPV of starch blends. The HPV for starch blends was the highest for non-waxy starch (0 wx) and decreased for each subsequent blend, while the HPV of starch/gluten blend was the highest for 100 wxg and lower in blends with lower amount of WD starch (Table 2.3, Figure 2.3). This behavior is exactly opposite from the usual behavior of waxy starch, which normally has low HPV due to low resistance of granules to disintegration caused by heat and shear. This specific HPV behavior of

starch/gluten blends was followed by the BKD values. Interestingly, the BKD values for 75 wxg and 100 wxg were lower than those of corresponding starch blends while it was opposite for all other investigated blends. The RVA profiles of 0 wxg, 12.5 wxg, 25 wxg, and 50 wxg indicated that starch in these blends started breaking down faster than in starch blends (Figures 2.2 and 2.3). These results show that gluten altered the pasting properties of starch.

The effect of gluten on pasting behavior of starch could be the attributed to several effects: a) reduction of contact and consequently reduction of interaction among starch granules, b) increased starch granular rigidity in starch/gluten blends due to the swelling restriction caused by gluten (and therefore less susceptibility to breakdown), and c) entanglements of leached starch molecules with gluten. Under conditions applied in RVA analysis, gluten was transformed from its brittle glassy state into rubbery state before starch started swelling and gelatinizing. According to Hoseney and Zeleznak (1986) glass transition of gluten occurs at room temperature at water contents above 13%. Gluten proteins are insoluble in water due to the very large molecular weight and lack of ionizable groups. As a result of these properties, gluten forms fibrils and a cohesive network in an aqueous environment (Singh and MacRitchie 2001). Consequently, the gluten fibrils might have surrounded the starch granules and hindered the interaction among granules. Gluten fibrils also could have restricted hydration of starch granules and reduced the PV; however, this effect is not completely clear since starch/gluten blends contained less starch than the pure starch blends. Nevertheless, proteins that form disulfide bonds, such as gluten, can restrict swelling of starch granules and their

disruption by high shear as suggested by Hamaker and Griffin (1993). Although gluten could have restricted swelling of starch granules, waxy granules were probably still able to swell faster than non-waxy granules and release some amylopectin fragments before the gelatinization of non-waxy granules took place. The leached amylopectin could probably interact with gluten fibrils. Although the nature of the interaction is unlikely to be through covalent binding (Martin and Hoseney 1996; Martin et al. 1991; Champenois and Walaker 1998), or any plasticizing effect upon each other (Blanshard 1995), entanglements could have been formed between gluten fibrils and amylopectin and facilitated by the branched structure of amylopectin. These entanglements could be responsible for increase in HPV viscosity and lower BKD of 75 wxg and 100 wxg than BKD of corresponding starch blends.

The CPV for starch/gluten blends also exhibited a different behavior than that of starch blends, and showed less difference among starch/gluten blends than among starch blends (Figures 2.2 and 2.3). CPV of starch/gluten blends appeared to be 'clustered' around similar values. Development of cold paste viscosity in starch/gluten blends is more complex than in starch blends due to the role of gluten. Amylose chains undergo rapid reassociation that contributes to an increase in RVA viscosity during cooling stage, but gluten might alter this chain reassociation process. The STB results indicate that gluten might hinder the interaction between starch molecules. It is also reasonable to assume that gluten becomes embedded between starch granules that normally reinforce the firmness of the gel. Champenois et al. (1998) suggested that gluten created fault zones in

starch network and affected the rheological behavior of the paste and gel. While STB values for 0 wxg, 12.5 wxg and 25 wxg did not differ significantly, they decreased from 50 wxg to 100 wxg (Table 2.2), indicating again that gluten might not have interacted in a same way with low-amylose and high-amylose blends.

DSC Gelatinization Properties of Blends

Gelatinization properties of blends, measured by DSC, were affected significantly by the amylose x gluten content interaction (Table A-5). The only exception was the peak temperature of gelatinization (T_p) that was affected by only the amylose content, i.e. by the % of waxy starch in blend. Effect of amylose content on gelatinization properties of starch has been studied mainly in pure starches and much less data is available on gelatinization properties of starch blends. However, starch blends often acquire properties different from those of their constituting single starches, and therefore could be used to obtain starches with functional properties needed for specific food applications (Liu and Lelièvre 1992; Obanni and BeMiller 1997; Karam et al. 2006).

Enthalpy of gelatinization (ΔH) of starch blends was negatively correlated with amylose content ($r = -0.96$, $p<0.01$) (Table A-7), showing that starch blends with high percent of waxy starch in blend, i.e. low amylose content, had high ΔH (Table 2.4). Low amylose content in starch has been associated with high enthalpy of gelatinization in wheat (Yasui et al. 1996; Fujita et al. 1998; Chakraborty et al. 2004; Hung et al. 2007), in rice (Hagenimana et al. 2005), and in corn (Liu et al. 2006). High ΔH in blends with high amount of waxy starch is the result of higher

Table 2.4. DSC Gelatinization Properties of Starch Blends and Starch/Gluten Blends as Affected by Interaction of % Waxy Starch and % Gluten in Blends

		Waxy durum starch (%)					
	Gluten (%)	0	12.5	25	50	75	100
ΔH [a] (J/g)	0	10.98	12.09	14.98	15.50	16.08	17.90
	30	8.06	9.40	10.03	10.5	11.5	12.10
	LSD (0.05)	----------	----------	0.92	----------	----------	----------
ΔH_{calc1} [b] (J/g)	0	10.98	12.09	14.98	15.50	16.08	17.90
	30	11.51	13.43	14.33	14.98	16.45	17.28
	LSD (0.05)	----------	----------	1.03	----------	----------	----------
ΔH_{calc2} [c] (J/g)	0	7.69	8.46	10.59	10.85	11.25	12.53
	30	8.06	9.40	10.03	10.5	11.5	12.10
	LSD (0.05)	----------	----------	0.72	----------	----------	----------
T_o [d] (°C)	0	54.1	52.8	52.5	52.7	52.7	53.8
	30	54.2	53.2	53.4	53.4	53.8	55.5
	LSD (0.05)	----------	----------	0.6	----------	----------	----------
T_c [e] (°C)	0	67.0	66.4	67.8	68.6	69.2	70.7
	30	66.2	66.5	67.0	67.5	67.7	67.3
	LSD (0.05)	----------	----------	1.2	----------	----------	----------
$T_c - T_o$ [f] (°C)	0	13.0	13.7	15.2	15.9	16.5	16.9
	30	12.0	13.3	13.5	14.1	13.9	11.8
	LSD (0.05)	----------	----------	1.6	----------	----------	----------

[a] ΔH = Measured enthalpy of gelatinization

[b] ΔH_{calc1} = Enthalpy of gelatinization calculated based on the percent of starch in blends. For starch/gluten blends $\Delta H_{calc1} = \Delta H / 0.7$

[c] ΔH_{calc2} = Enthalpy of gelatinization calculated based on % of starch in starch/gluten blends ($\Delta H_{calc2} = \Delta H \times 0.7$)

[d] T_o = Onset temperature of gelatinization

[e] T_c = Completion temperature of gelatinization

[f] $T_c - T_o$ = Gelatinization temperature range

relative crystallinity in these blends than in blends with low waxy starch content. Previous research (Chakraborty et al. 2004) has shown that WD starch had higher relative crystallinity, measured by X-ray diffractometry, than non-waxy starch. Therefore, starch granules in blends with high amount of waxy starch, i.e. low amylose content, can be expected to have higher apparent degree of crystallinity than granules in blends with low amount of waxy starch.

Although there are several different explanations of the sequence of phenomena during gelatinization, it is generally defined as a loss of double-helical (molecular) order of amylopectin and crystalline melting (Cooke and Gidley 1992). Since crystalline regions in starch are formed by double helices of amylopectin (Blanshard 1987; Donald 2001), the endothermic energy recorded by DSC is related to the crystallinity of starch, and therefore starch blends with higher amount of amylopectin (lower amylose content) were expected to have higher ΔH. Also, amylose was shown to have a crucial role in the initial disruption of crystalline structure during gelatinization. Gelatinization is a swelling-driven crystallite disruption (Jenkins and Donald 1998) in which swollen amorphous region imparts stress upon the crystalline region in granule. This process is facilitated by sufficient plasticization of amorphous growth rings by water (Slade and Levine 1989; Waigh et al. 2000a,b). Since amorphous regions are made of amylose, starches with low amylose content need more energy to initiate crystallite melting due to insufficient swelling of amorphous region.

ΔH of starch blends fell between ΔH of the two individual components: 0 wx (non-waxy Alsen starch) and 100 wx (WD starch) (Table 2.4). According to Liu and

Lelièvre (1992), at low starch concentration (less than 30% w/w) in suspension, ΔHs of starch blends are the sum of the proportional contributions of ΔH of each constituent. Although amylose content differed significantly among all blends and although ΔH increased with an increase in the amount of WD starch in blend, the difference in ΔH between 25 wx and 50 wx and between 50 wx and 75 wx was not significant. The 100 wx had significantly higher ΔH than all other starch blends (Table 2.4).

DSC traces of starch blends are presented in Figure 2.4. All starch blends showed a single DSC endotherm. When starch blends are gelatinized, the occurrence of a single or double endothermic peak depends on the amount of water available and botanical origin of starch. Literature often reports different results on the occurrence of one or two DSC gelatinization endotherms for starch blends in excess water. Two endotherms are observed usually when starch blends are gelatinized in limited water (below 70% w/w) (Liu and Lelièvre 1992; Liu et al. 2006) due to the competition for water between granules. In excess water, two DSC endotherms were recorded also in blends of corn and yam starch (Karam et al. 2006), waxy and non-waxy rice starch suspension (Lu et al. 2009), in blends of wheat starch with potato, cassava and yam starch (Zaidul et al. 2008), in blends of waxy corn-potato starch and waxy corn-barley starch (Ortega-Ojeda and Eliasson (2001).

The reason for the occurrence of two endotherms in excess water is a large difference in granule stability between two starches, as well as large difference in their gelatinization temperature ranges (Fredriksson et al. 1998). Hagenimana et

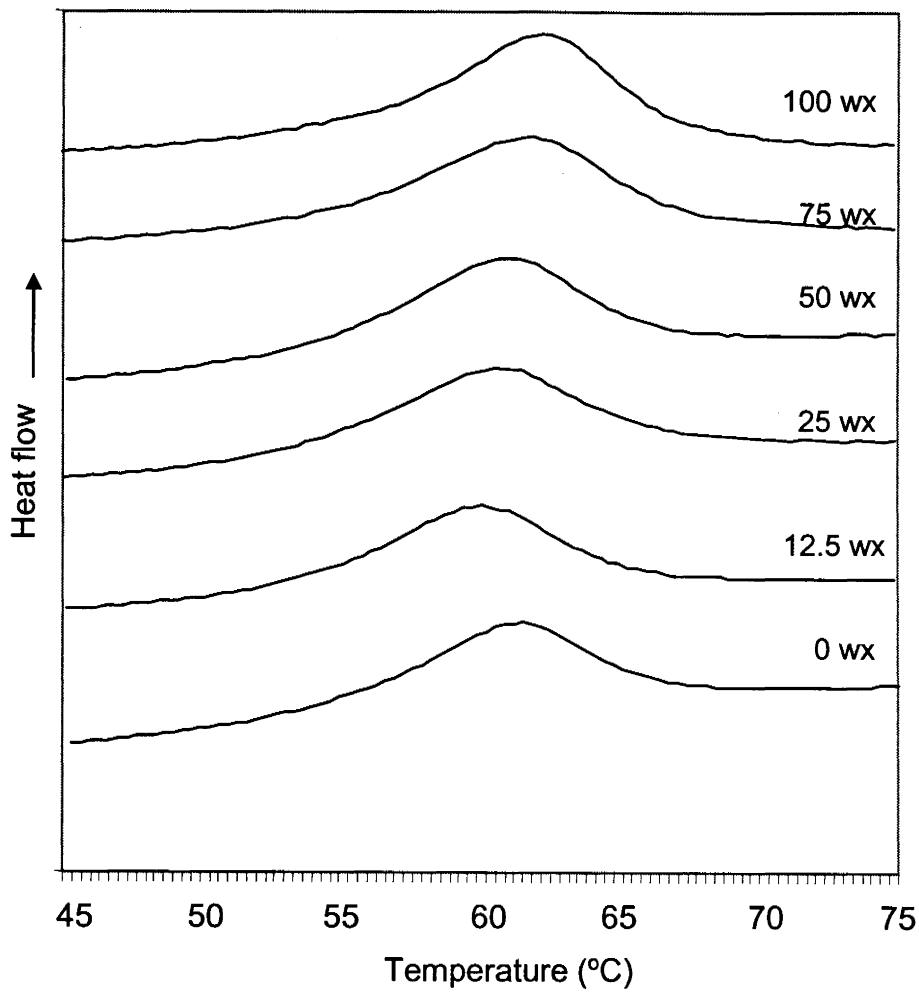

Figure 2.4. DSC gelatinization endotherms of starch blends

al. (2005) and Hagenimana and Ding (2005) postulated that the association between amylose and amylopectin in mixed starches is different than in single starches, causing specific interactions between starches during gelatinization. According to these authors, each starch in a blend gelatinizes independently of the other starch in the mixture, and therefore the resulting DSC thermogram shows two peaks. While it is not certain whether the waxy and non-waxy wheat starch in studied blends gelatinize independently or interact in some form, the single DSC endotherm most likely was a result of close gelatinization range for the two

starches. The T_o of 0 wx and 100 wx samples was very similar, the T_p was 60.9°C and 63.0°C for 0 wx and 100 wx respectively, while the T_c of 0 wx and 100 wx was also in close range (Table 2.4). A similar finding was reported by Hagenimana et al. (2005) for blends of waxy rice with non-waxy rice with varying amylose contents. Information on gelatinization properties of wheat starch blends is scarce, but Sasaki et al. (2000) also reported a single endotherm for a blend of waxy and non-waxy starch, and higher ΔH in blends that had lower amylose content. According to Gunaratne and Corke (2007), endotherms of two starches will overlap if the starch crystals have similar thermal stability and consequently similar thermal transition temperatures, regardless of their botanical origin. Nevertheless, according to these authors, gelatinization of each component in a blend, even at the same temperature, still may affect the interaction between two components and affect swelling of starch. Obanni and BeMiller (1997) also did not observe two endotherms in starch blends, attributing this to specific interactions between components of two cooked starches.

The T_o of 0 wx and 100 wx was not different (Table 2.4) although the amylose content of 100 wx was significantly lower than the amylose content of 0 wx. This result was in disagreement with some of results reported in literature because waxy starch and low amylose content usually are associated with high DSC transition temperatures (Shi and Seib 1992; Hayakawa et al. 1997; Fujita et al. 1998; Demeke et al. 1999; Yasui et al. 2002). The discrepancy between results reported in literature and results of this research could be due to some structural differences between non-waxy Alsen starch and WD starch (i.e., 0 wx and 100 wx

samples). Difference in λ_{max} between WD wheat amylopectin (530 nm) and non-waxy Alsen amylopectin (567 nm) indicates that Alsen amylopectin had longer chains than the WD amylopectin. This could have caused the Alsen starch to have T_o as high as the WD starch.

Molecular structure of starch has been shown to affect the transition temperatures during gelatinization. Jane et al. (1999) studied gelatinization properties of starches from different botanical origin and found that starches with short average amylopectin branch chain lengths had lower gelatinization temperatures than starches with longer amylopectin chain length. In another study, wheat starch containing high ratios of amylopectin with long side chains had higher gelatinization temperatures than starch with low ratios (Kohyama et al. 2004). These results confirmed findings of Yuan et al. (1993) who proposed that long chains of amylopectin form long double helices that require high temperature to dissociate.

The T_o of 12.5 wx, 25 wx, 50 wx, and 75 wx starch blends were similar and not related to the amylose content. No correlation was found between T_o and amylose content of starch blends (Table A-7). Moreover, the T_o of these starch blends was lower than the T_o's of 0 wx and 100 wx starch, which were the components of the starch blends (Table 2.4). Gupta et al. (2009) reported that mixed starches had lower onset and peak temperatures of gelatinization than single starches with the same amylose content. The exact reason for this unusual behavior of starch blends is not clear, but it indicates that gelatinization in starch blends may be more complex than in single starches and that not each

gelatinization parameter depends solely on amylose content.

The completion temperature of gelatinization (T_c) was significantly higher for 100 wx than for 0 wx starch (Table 2.4), which was in agreement with results reported in literature that show that waxy starch requires higher temperature for complete melting of crystalline structure (Sasaki et al. 2007). Although the T_c was higher in blends with lower amylose content (Table 2.4) and it was negatively correlated with amylose content ($r=-0.83$, $p<0.05$; Table A-7). Furthermore, T_c was not significantly different between each consecutive blend. For example, the only significant difference in T_c between two consecutive blends was between 12.5 wx and 25 wx and between 75 wx and 100 wx although the amylose content was significantly different between each consecutive blend (Table 2.1). In blends of waxy and non-waxy wheat starch, Sasaki et al. (2000) also found a negative correlation between amylose content and T_c, but at the same time, amylose did not have a significant effect on T_o and T_p.

Peak temperature of gelatinization (T_p) showed similar behavior like the completion temperature (T_c). The T_p was highest for the 100 wx sample and it was significantly higher than the T_p of the 0 wx sample (Table 2.5). This was in agreement with literature data (presented earlier in the text), that shows that waxy starch requires higher temperature for gelatinization than non-waxy starch. Overall, the T_p in starch blends increased when the amylose content decreased (with the exception of 0 wx), but similar to T_c, the T_p also was not significantly different among all bends although amylose content was significantly different. Besides 100 wx, the only 75 wx had significantly higher T_p than 0 wx, while the

Table 2.5. DSC Peak Temperature of Gelatinization as Affected by % Waxy Durum Starch

Waxy durum starch (%)	T_p (°C) [a]
0	60.9
12.5	60.4
25	60.6
50	61.1
75	61.4
100	63.0
LSD (0.05)	0.5

[a] T_p = Peak temperature of gelatinization

Tp of other starch blends was lower or similar to the T_p of 0 wx starch.

Literature data on the effect of amylose on peak temperature of gelatinization in starch blends is equally scattered as data for the onset and completion temperatures of gelatinization. According to Lu et al. (2009), mixing two different types of starch (high-amylose rice and waxy rice) caused an increase in T_p compared to single starches regardless of the amylose content. Obanni and BeMiller (1997) studied gelatinization properties of potato-corn, wheat-tapioca, normal rice-potato starch blends and found that the T_p of corn-potato and wheat-tapioca blends fell between T_p of individual components, while for the rice-potato blend, the T_p was higher than that of either component. The authors concluded that each transition temperature was specific to the blend. In addition, the complex behavior of blends was interpreted as a result of interactions between starches in the blend rather than each starch gelatinizing independently.

Gelatinization temperature is considered to be related to crystallite size and

perfection (Tester and Morrison 1990; Biliaderis 2009). For pure starches, large crystalline regions in the starch granule are created with high number of hydrogen bonds that do not break until high temperatures are reached (Kohyama et al. 2004). However, starch blends appear to deviate from this rule to a certain extent, as shown by some non-significant differences in T_p between some starch blends in this study (Table 2.5). Gelatinization transition temperatures were found to depend not only on amylose content but also on thermal stability of starch crystals. More closely packed crystals possess a more stable structure and therefore require a higher gelatinization temperature (Oostergetel and van Bruggen 1989). Overall, the effect of amylose content on DSC transition temperatures of starch blends appears to be even more complex than its effect on ΔH.

The results in this study show that both the ΔH and the transition temperatures of starch blends depended only partially on amylose content. While the exact reason for gelatinization properties of studied starch blends is not understood completely, it could be attributed to similar factors that affect pasting properties of blends, as discussed in the previous section. Starch blends are composed of two populations of starch granules that are structurally different and therefore possibly possess different thermal stability. According to Fredriksson et al. (1998) and Gupta et al. (2009) starch blends have different gelatinization properties than single starches due to difference in homogeneity. According to these authors, blended starches contain crystals with varied heat stability and therefore they have broader range of gelatinization temperatures (T_c - T_o) than single starches. Lack of homogeneity of ordered structures in granules can cause

broad range of gelatinization temperatures (Yuan et al. 1993; Hagenimana et al. 2005).

It has to be taken into consideration that blends are not mixtures of pure amylose and amylopectin but mixes of two different granule types in which amylose and amylopectin are contained. The measured amount of amylose in starch blends is not distributed evenly in all starch granules and therefore the resulting gelatinization properties cannot be a mathematical average of the gelatinization properties of two granule types and this is probably the reason for an often non-linear relationship between amylose content and gelatinization properties of blends. Gelatinization occurs over a temperature range and granules in different blends could possibly undergo gelatinization changes at a different rate during the heating period. Since all starch blends were gelatinized in excess water (75% water), gelatinization was not affected by the availability of water; however, it could have been affected by specific interactions between amylose and amylopectin and also by solubilized amylose and amylopectin molecules. It is conceivable that these interactions were specific to each blend, which would confirm the hypothesis of Obanni and BeMiller (1997) that starch blends behave like new starches, and that the interactions between solubilized amylose and amylopectin molecules and starch granules in a blend are different from interactions in a single starch.

Recent studies of the mechanism of gelatinization provide new insight that could help understand the processes occurring during gelatinization of starch blends. Crochet et al. (2005) studied dissolution and gelatinization of starch and

found that gelatinization is related to the connectivity of the crystallites through the amylopectin chain since each amylopectin molecule is involved in many different crystals. When amylopectin chain transitions from double helix to coil form (upon heating in water) inside the granule, it contributes to further swelling of the granule, and eventually granule disruption. Based on this finding, Crochet et al. (2005) concluded that differences in molecular structure of amylopectin between two starches in the blend could affect the temperature at which the helix-coil transition occurs and therefore affect further water uptake by the granule and gelatinization. Vermeylen et al. (2006) showed that, during gelatinization, melting of amylopectin crystals (an endothermic reaction) is accompanied by the exothermic formation of amorphous network by reassociation of amylose and outer branches of amylopectin. The authors proposed that formation of such network is initiated at the onset of gelatinization and that these structures can withstand dissolution at higher temperatures.

The role of amylose and difference in gelatinization properties between waxy and non-waxy starch were further elaborated by Ratnayake and Jackson (2007). The authors proposed that gelatinization is more complex than order-to-disorder transition since it involves a series of events in which amylose molecules play a significant role at low temperatures. During gelatinization, significant structural rearrangements of amylose in the amorphous regions occur at low temperatures. These events occur at temperatures lower than those that initiate morphological changes and granular disruption during gelatinization. These new molecular arrangements and intermolecular bonds come with an array of different

thermal stabilities and it seems reasonable to assume that they could have a significant effect on the course of gelatinization and gelatinization temperatures. This molecular rearrangement was not observed in waxy starch. Taking into consideration these finding, the specific gelatinization behavior of starch blends could be in part attributed to the formation of rearranged amylose molecular structures before actual gelatinization.

It is possible that heating at low temperature could have induced structural changes in 0 wx starch (Alsen starch) and brought about the formation of new amylose structures that were more resistant to breakdown by heat and moisture. Since 0 wx starch had the highest amylose content, it required higher temperature to onset the gelatinization (T_o) than the 12.5 wx, 25 wx, 50 wx, and 75 wx starch blends (Table 2.4). In 100 wx starch, gelatinization was governed by amylopectin due to very low amylose content. Due to the presence of waxy and non-waxy starch granules in blends, gelatinization in starch blends most likely occurred as two gelatinization processes with different mechanisms and different time/temperature for certain events. However, it is reasonable to assume that the two gelatinization processes affected each other through interaction of leached amylose and amylopectin molecules or interaction of leached starch with granules.

Obanni and BeMiller (1997) hypothesized that starches in blends interact and that some of the interactions occuring even before gelatinization include interactions between leached amylose molecules from one starch and granules from another starch. Evidently, waxy and non-waxy starch granules gelatinize in a different way and release solubilized molecules from their granules. Therefore, at a

given time, each starch blend could have a different composition of its soluble part (i.e., starch that leached from the granule), or a different concentration of certain amylose and amylopectin molecules in the soluble portion and these molecules can either interact or affect the gelatinization by coating the remaining granules, since not all granules gelatinize at the same time.

Effect of Gluten

Gelatinization properties of starch blends were affected by gluten (Table A-5, A-6). All starch/gluten blends had lower ΔH values than the corresponding starch blends (Table 2.4), which was expected because of the 30% dilution of starch with gluten. ΔH values for all starch/gluten blends fell between those of 0 wxg and 100 wxg blends (Table 2.4) and blends with higher amylose content had lower ΔH. Although amylose content differed significantly among all starch/gluten blends and although ΔH increased with an increase in the amount of WD starch in blend, the difference in ΔH was not significant between each consecutive blend. Significant differences in ΔH were observed between 0 wxg and 12.5 wxg blends and between 50 wxg and 75 wxg blends (Table 2.4). Based on the measured ΔH between two consecutive blends, gelatinization pattern of starch/gluten blends was different from that of pure starch blends. Both starch blends and starch/gluten blends showed significant difference between levels 0 and 12.5%, non-significant difference between levels 25 and 50%, while for all other levels the differences were of opposite significance between two consecutive starch blends and corresponding two starch/gluten blends (Table 2.4). Comparison of measured ΔH

values between starch blends and starch/gluten blends with same amylose content does not provide valuable information because of obvious effect of dilution by gluten. However, when the ΔH ranges ($\Delta H_{100wx\text{-}0wx}$ and $\Delta H_{100wxg\text{-}0wxg}$) were calculated, starch/gluten blends had a narrower range (4.04 J/g) than the starch blends (6.92 J/g). The reason for this phenomenon is not clear, but it indicates that gluten could have affected starch gelatinization. Moreover, gluten could have interacted differently with blends with different amylose content. The difference in ΔH between starch blends and their corresponding starch/gluten blends was 2.92 J/g and 2.69 J/g for 0% and 12.5% blends, respectively, while the difference for 25%, 50%, 75%, and 100% blends was higher, ranging from 4.58 J/g to 5.80 J/g (Table 2.4). Based on these results an assumption can be made that interaction of gluten and starch in low amylose blends could be different from the interaction in high amylose blends. Chedid and Kokini (1992) proposed that gluten and starch interaction could be facilitated by high amylopectin content because the potential of protein entanglement with branched polymer is higher than the potential of entanglement with linear polymer such as amylose.

Enthalpy of gelatinization (ΔH) of starch/gluten blends was negatively correlated with amylose content ($r = -0.98$, $p<0.01$) (Table A-8), showing that blends with high percent of waxy starch, i.e. low amylose content, had high ΔH (Table 2.4). This result implies that thermal properties of starch/gluten blends mainly are related to starch and depend on amylose content of blends. However, when discussing ΔH for blends made of starch and gluten, thermal properties of gluten also have to be considered. Eliasson and Hegg (1980) and Arntfield and

Murray (1981) studied thermal properties of gluten by DSC and found that gluten proteins had very little to no endothermic response in excess water within the temperature range of 30 - 130°C. Eliasson and Hegg (1980) proposed that the lack of denaturation endotherm of gluten was either due to the lack of ordered structures or due to unusual thermal stability of ordered structures. Subsequent studies (Hoseney et al. 1986; Noel et al. 1995; Pouplin et al. 1999) have shown that gluten is a highly amorphous polymer, and as such it undergoes glass transition in the presence of a plasticizer. Gluten does not show endotherm of crystalline melting since it lacks ordered structures. Hoseney et al. (1986) and Pouplin et al. (1999) showed strong plasticizing effect of water on gluten. Hoseney et al. (1986) also showed that at water content higher than 13%, the glass transition of gluten occurs at room temperature. Since gelatinization of starch/gluten blends in this study was performed at 3:1 water:solids ratio (75% water), gluten can be expected to have transitioned from glassy to rubbery state by the time starch gelatinization started. Based on these findings, the ΔH of starch/gluten blends most likely originated fully from starch with no contribution from gluten.

DSC traces of starch/gluten blends are presented in Figure 2.5. All starch/gluten blends showed a single DSC endotherm, which is most likely due to gelatinization in the excess amount of water. Also, all starch/gluten blends had the same shape of the endotherm as their corresponding starch blends with a slight shift in gelatinization temperatures (Figure 2.6). At 47% water content in a starch/gluten system, Eliasson (1983) observed two endotherms that were

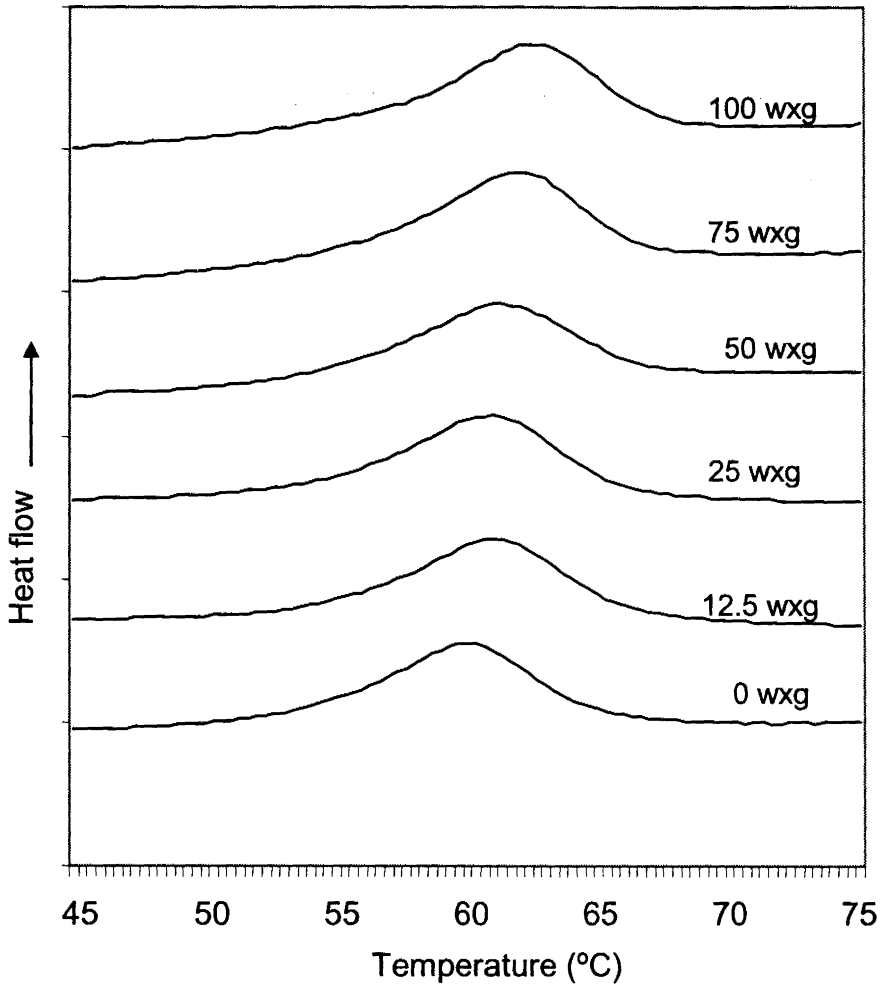

Figure 2.5. DSC gelatinization endotherms of starch/gluten blends

attributed to starch thermal transitions and that were affected by the limited amount of water. However, in the study of Chevallier and Colonna (1999) and Huerta-Abrego et al. (2010), a single gelatinization endotherm was recorded when starch/gluten mixture was gelatinized. Chevallier and Colonna (1999) reported that the endotherm had the same shape as the endotherm of starch gelatinized in 80% water. The authors concluded that gluten did not have an effect on starch gelatinization endotherm. Erdogdu et al. (1995) also reported no effect of gluten on starch gelatinization at 1:1:4 gluten/starch/water ratio.

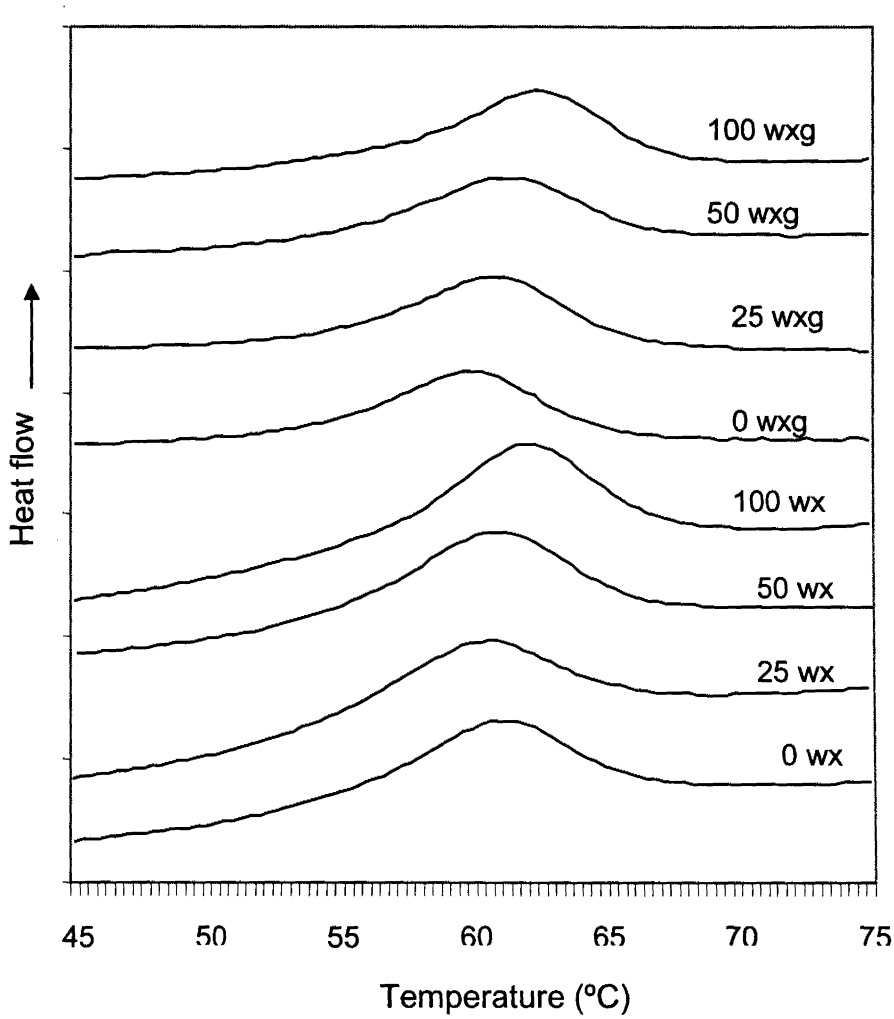

Figure 2.6. Comparison of DSC gelatinization endotherms of starch blends and starch/gluten

In order to compare ΔH of starch blends and starch/gluten blends, ΔH was calculated in two different ways; both ways essentially calculated ΔH based on the equal percent of starch in blends. Calculated ΔHs provide more realistic comparison of blends because they account for the dilution with gluten. First, ΔH of starch/gluten blends was converted to that of starch, i.e. it was calculated based on the amount of starch in starch/gluten blends ($\Delta H_{calc1} = \Delta H_{starch/gluten}/0.7$). Second, ΔH of starch blends was calculated based on the percent of starch in

starch/gluten blends ($\Delta H_{calc2} = \Delta H_{starch} \times 0.7$). The rationale for the latter calculation was that ΔH of blends originates only from starch, and therefore a linear relationship exists between ΔH of starch blends and ΔH of starch/gluten blends. Both ΔH_{calc1} and ΔH_{calc2} provided similar information about the differences in gelatinization enthalpy between starch blends and starch/gluten blends (Table 2.4). The only significant difference was between 12.5 wx and 12.5 wxg blend, while calculated ΔHs were similar for all other starch and starch/gluten blends that were made with the same percent of waxy starch. Based on these results, the conclusion is that gluten did not affect starch gelatinization in the majority of blends. A possible explanation for this behavior could be excess moisture (solids:water = 1:3). According to Chedid and Kokini (1992), polymers are completely hydrated in excess moisture environment, which reduces the potential for their hydrophilic interaction and entanglement. The reason for the difference in calculated ΔHs between 12.5 wx and 12.5 wxg is not known. Among all blends of waxy and non-waxy starch (levels 12.5% - 75%), 12.5 wx and 12.5 wxg had the largest difference in amylose content (6.7%); however, whether this difference caused the difference in calculated ΔHs is uncertain. The results for the majority of blends were in agreement with findings of Chevallier and Colonna (1999) and Erdogdu et al. (1995) who found no evidence of the effect of gluten on starch gelatinization. Opposite to these findings, Huerta-Abrego et al. (2010) reported higher ΔH for starch/lima bean proteins than for pure starch at 70% water level. However, it can be argued that these were lima bean proteins which are structurally different than gluten proteins, and thus affect starch differently. Lower

ΔHs for starch/gluten blends than for pure starch were detected in studies of Eliasson (1983) and Mohamed and Rayas-Duarte (2003). This effect was attributed to competition for water between starch and gluten. Migration of water from gluten to starch reduced the amount of water available for gelatinization of starch, and consequently lowered the ΔH (Eliasson 1983; Mohamed and Rayas-Duarte 2003). In addition, gluten can interact with starch by adhering to the surface of starch granules and delaying diffusion of water into granules (Eliasson and Tjerneld 1990). These could be plausible explanations in limited water systems; however, most likely not satisfactory for excess water systems.

Since gelatinization of analyzed starch/gluten blends in DSC does not include mixing or any type of mechanical force applied to gluten, it is unlikely that gluten developed sufficient amount of disulfide bonds and a full network. The lack of gluten network could have also contributed to the lack of interaction between starch and gluten. According to Mohamed and Rayas-Duarte (2003) the extension of gluten polypeptides and formation of network during mixing increases potential for interaction of starch and gluten. Water also was not a limiting factor because it was present in excess amount. Champenois et al. (1998) studied the influence of gluten on rheological properties of starch pastes and concluded that even in excess water gluten can still affect behavior of starch by surrounding starch granules and reducing contact between them. While this phenomenon may have more important effect on gel properties, it also could be related to starch gelatinization. During heating of analyzed starch/gluten blends in DSC, gluten most likely underwent glass transition before starch gelatinization started. Since water

was present in excess amount, the amount of water that was absorbed by gluten at low temperatures probably did not cause such a decrease in available water for starch gelatinization that would have affected ΔH.

However, presence of gluten and development of gluten fibrils due to hydration (although not a full gluten network) could have affected gelatinization temperatures. Starch/gluten blends 25 wxg, 50 wxg, 75 wxg, and 100 wxg had significantly higher T_o than their corresponding starch blends (Table 2.4). Two blends with high amylose content (0 wxg and 12.5 wxg) had T_o similar to T_o of their corresponding starch blends (Table 2.4). This phenomenon could be attributed to gluten fibrils "coating" starch granules and delaying diffusion of water into granules. Since water was available still in excess amount, the ΔH was not affected, but the T_o increased because gelatinization started at higher temperature. T_o showed this behavior for blends with low amylose contents, but not for two blends with highest amylose contents. Most probable reason for this is the cooperative effect of gluten and low amylose content in 25 wxg, 50 wxg, 75 wxg, and 100 wxg blends. As mentioned earlier, plasticization of amorphous regions(consisiting of amylose) of starch granule is necessary for the onset of gelatinization (Slade and Levine 1989; Waigh et al. 2000a,b). Increase in T_o in the presence of gluten also was observed by Mohamed and Rayas-Duarte (2003) (60% water content), Eliasson (1983) (47% water content), as well as by Li et al. (2007) for corn starch/soy protein blends (80% water content). T_o of 12.5 wxg – 75 wxg blends was between the T_o of 0 wxg and 100 wxg samples, which was the same for starch blends. Peak temperature of gelatinization (T_p) was not affected by the presence of gluten; it was affected only

by amylose content of blends (Table 2.5).

The completion temperature of gelatinization (T_c) was higher for starch/gluten blends that contained low amylose content than for blends with high amylose content (Table 2.4), which was the same behavior as for the starch blends. T_c showed negative correlation with amylose content ($r=-0.83$, $p<0.05$; Table A-7), but it was not significantly different between each consecutive blend (Table 2.4).

Retrogradation Properties of Blends

Retrogradation enthalpy (ΔH_r) of all blends was significantly affected by the blend by storage day interaction (Table A-9). All analyzed blends displayed a single endotherm (not shown) that corresponded to melting of retrograded starch after 5 – 20 days of storage at 4°C. Retrogradation endotherms followed the same monomodal pattern like the gelatinization endotherms. Similar results were reported by Ortega-Ojeda and Eliasson (2001) for 20% blends of barley starches, by Sasaki et al. (2000) for wheat starch blends, and by Guanaratne and Corke (2007) for blends of potato and amaranth starch, while two endotherms were observed for 50% starch blends by Ortega-Ojeda and Eliasson (2001).

Mono- or bimodal shape of retrogradation endotherms depend on the amount of water in the system and also on homogeneity of starch crystals in recrystallized starch and their thermal stability (Fredriksson et al. 1998; Ortega-Ojeda and Eliasson 2001; Gupta et al. 2009). Both factors also are related to the botanical origin of starch. Bimodal shape also can be the result of reorganization of

amylopectin that takes place during thermal scanning in DSC. Amylopectin chains can rearrange within crystallites at temperatures above onset temperature due to increased mobility of chains during heating (Biliaderis et al. 1986). In the case of analyzed starch blends, water was in excess amount (75%) and both components of the blend were wheat starches with most likely higher crystal uniformity than in blends of different botanical origins. Consequently, all blends had single endotherms.

ΔH_r of all starch blends increased during storage (Table 2.6). ΔH_r measured on day 0 was not considered because the endothermic response of freshly gelatinized samples was very small and not measurable. In majority of cases, differences between two consecutive days for the same blend were significant, which means that recrystallization of starch progressed throughout the whole storage period at considerable rate. The rate of retrogradation appeared to be highest between 5 and 10 days of storage for all blends (Table 2.6). The rate of retrogradation decreased after 10 days of storage, but it did not level off. Therefore, retrogradation most likely would have continued beyond 20 days of storage.

Retrogradation of analyzed starch blends can be explained by applying the generally accepted model by Miles et al. (1985). Reassociation of amylose molecules into long double-helical structures takes place shortly after gelatinization and it is responsible for irreversible gelation of amylose. Long term processes involve reversible reassociation of amylopectin chains (shorter than amylose chains) into short double helices that eventually organize into crystallites (Miles et

Table 2.6. Retrogradation Enthalpy (ΔH_r, J/g) of Starch Blends and Starch/Gluten Blends as Affected by Interaction of Blends and Storage Days

	Days			
Blends	**5**	**10**	**15**	**20**
0 wx	4.44	5.27	5.97	6.09
12.5 wx	4.02	5.58	5.82	6.28
25 wx	4.43	5.59	6.04	6.50
50 wx	3.95	5.55	5.80	6.45
75 wx	3.73	5.44	6.14	6.45
100 wx	2.89	5.71	6.36	7.07
0 wxg	2.55	3.20	4.10	4.49
12.5 wxg	2.90	3.51	4.03	4.25
25 wxg	2.91	3.40	3.92	4.26
50 wxg	3.22	3.27	3.26	3.91
75 wxg	2.18	2.55	3.56	3.71
100 wxg	1.42	1.82	2.42	2.90
LSD (0.05)	---------- 0.45 ----------			

al. 1985; Ring et al. 1987). The formation of short amylopectin double helices is controlled by the restrictions imposed by the length of branches and branching structure of amylopectin. Retrogradation process is facilitated by low temperatures because of a reduced Brownian motion of molecules and therefore more intense intermolecular hydrogen bonding between amylopectin molecules (Tako and Hizukuri 2000). Taking this model into consideration, the high rate of retrogradation between days 5 and 10 could be due to amylopectin recrystallization that became intensified after 5 days of storage. When difference in ΔH_r between days 5 and 10 was calculated for each blend, it was smallest for 0 wx blend (0.83 J/g) and largest for 100 wx (2.82 J/g). Further calculation showed that between days 5 and 20, the

ΔH_r of 0 wx blend increased only by 37% while ΔH_r of 100 wx increased by 89%. These results show that the highest amylose containing blend (0 wx) underwent considerable retrogradation by day 5, while blends with lower amylose (higher amylopectin) content had slower retrogradation.

ΔH_r exhibited different behavior on different storage days. On day 5, ΔH_r was lower in samples with lower amylose content. Although the difference between two consecutive blends was not significant in most cases but between 75 wx and 100 wx, two distinct groups of values could be observed. Blends with high amylose content (0 wx – 25 wx) had significantly higher ΔH_r than blends with low amylose content (50 wx – 100 wx). At this early stage of retrogradation, perhaps the phenomenon could be explained by thermodynamic incompatibility of amylose and amylopectin and their phase separation in gels, where one polymer represents a continuous phase that embeds microdomains of the other polymer (discontinuous dispersed phase) (Kalichevsky and Ring 1987; Leloup et al. 1991). At a certain ratio of amylose:amylopectin, an inversion of phases occurs and continuous phase becomes discontinuous. Leloup et al. (1991) found that if amylose:amylopectin ratio is smaller than 0.43 (phase inversion point), the starch gel behaves amylopectin-like, while for ratios higher than 0.43 it behaves amylose-like. Another study found that the phase inversion point was 0.17 (Doublier and Llamas 1993). Analyzed starch blends with high amylose content (0 wx – 25 wx) had the phase inversion point above 0.17, while this parameter for low amylose blends (50 wx – 100 wx) was 0.16 and lower. While this concept is perhaps more important for the discussion of gel rheology than discussion of ΔH_r, it could possibly be applied to

explain the difference between two groups of blends on day 5, i.e. to explain the reason for amylose-like vs amylopectin-like retrogradation of blends. If 0.17 is adopted as a phase inversion point, then in 0 wx, 12.5 wx, and 25 wx amylose presents the continuous phase, while in blends 50 wx, 75 wx, and 100 wx continuous phase is amylopectin.

After 10 days of storage, all blends had similar ΔH_r, most likely because retrogradation of amylose slowed down and retrogradation of amylopectin intensified. Consequently both low- and high amylose blends had similar ΔH_r (Table 2.6). Similar behavior was observed on day 15. At the end of storage period (20 days), ΔH_r of the 100 wx blend was significantly higher than ΔH_r of other blends. This could be attributed to high amylopectin content of 100 wx and consequently development of high crystallinity during long storage period.

Different studies provided inconsistent information on retrogradation of starch blends. The inconsistency is probably the result of different types of starches used, different water contents (excess vs. limited water), different storage temperatures, and different ΔH_r reported (measured vs. normalized to amylopectin content). Sasaki et al. (2000) studied retrogradation properties of waxy and non-waxy wheat starches and their blends, and found that starches with higher amylopectin content recrystallized to a higher degree during 4 weeks of storage at 4°C. Obanni and BeMiller (1997) studied properties of blends of different starches, and reported that after two weeks of storage at 4°C, the majority of blends did not have a measurable retrogradation endotherm. High amylose content was shown to facilitate retrogradation of rice starch, while low amylose content suppressed

retrogradation (Yu et al. 2009). Ortega-Ojeda and Eliasson (2001) also observed increase in ΔH_r during storage of starch blends, and it was the function of the blend composition.Retrogradation enthalpies of blends were the sum of the enthalpy contributions of each component of the blend. This did not apply to ΔH_r presented in Table 2.6. While some of the blends had ΔH_r similar to a calculated ΔH_r, none of the blends showed a distinct pattern. The conclusion was that ΔH_r in blends behaved in the same way as gelatinization enthalpy (discussed in the previous section), i.e. it was not a simple sum of contributions of individual components. The reason for this behavior is not clear, but it could be the result of some interactions between amylose and amylopectin from waxy and non-waxy wheat starches. Sasaki et al. (2000) proposed that interactions in mixed starches could occur between starch molecules, swollen granules, fragmented granules, and intact starch granules.

Besides amylose content, a possible co-crystallization of two starches in blends also could be considered. Amylopectin is considered to be the component that is solely responsible for the reversible crystallinity of starch (and therefore recordable by DSC as ΔH_r), but some amylopectin molecules could co-crystallize with amylose (Miles et al. 1985). Several authors proposed association between amylose and amylopectin during retrogradation (Obanni and BeMiller 1997; Tako and Hizukuri 2000; Klucinec and Thompson 2002). Obanni and BeMiller (1997) suggested a possible association of amylose with amylopectin ghosts (gelatinized starch granules from which the majority starch polymers leached into intergranular space) and consequently unavailability of these molecules for recrystallizaton. This

was suggested as an explanation for a lack of retrogradation endotherms in starch blends. Obanni and BeMiller (1997) suggested that retrogradation properties of starch blends could be affected by some specific interactions between starch molecules from two different starches. They also suggested that these interactions could be even more intensive than interactions between molecules of a single starch. Tako and Hizukuri (2000) reported that amylose and amylopectin in gelatinized starch can form intermolecular hydrogen bonds. Amylose molecule may associate with two or more short amylopectin chains. Once the intermolecular hydrogen bonding between amylose and amylopectin is saturated, amylopectin molecules associate with each other.

If any interaction between starch molecules took place in analyzed starch blends, it was probably more complex than in single starches. First, the gelatinization pattern of waxy and non-waxy starch was showed to be different, which may have caused different composition of leached (soluble) starch and fragmented granules (that contain amylopectin fragments) available for retrogradation. Second, amylopectin from waxy durum starch contained shorter iodine-complexible branch chains than amylopectin of non-waxy wheat starch (Table 2.2), which was an indication of different structure of waxy and non-waxy amylopectin. Amylopectin molecules from one starch could interact and recrystallize with amylopectin molecules from the other starch, and the same applies to amylose. This could possibly lead to development of crystallites that are different from those in single starches, and with different thermal stability.

To better assess the impact of amylose content on blends retrogradation,

ΔH_r was normalized to the percent of amylopectin in blends (ΔH_{BR}) (Table 2.7). The rationale for this calculation was the fact that thermally reversible crystallinity (at temperatures below 100°C) of retrograded starch is developed only by the branched portion of starch, i.e. by amylopectin (Miles et al. 1985). This approach was used also by several authors (Fredriksson et al. 1998; Sasaki and Matsuki 1998; Klucinec and Thompson 2002).

ΔH_{BR} of all blends exhibited behavior similar to ΔH_r with respect to increase during the whole storage period. Again, the increase was largest between days 5 and 10 and less pronounced in later stages of storage (Table 2.7). Four of six analyzed blends had even similar ΔH_{BR} between day 15 and 20, which means that, for most blends, retrogradation presented by ΔH_{BR} started leveling off after 15 days of retrogradation. An important feature of ΔH_{BR} was that it showed lower values for low amylose blends than for high amylose blends for each storage day, which was opposite for ΔH_r on days 10, 15, and 20. The difference between days was the largest between day 5 and day 10, which was the same for measured enthalpy (ΔH_r) indicating that retrogradation was most intensive between 5 and 10 days of storage.

ΔH_r and ΔH_{BR} were compared for each blend at the same storage day in order to understand the effect of amylose on retrogradation in different blends. The difference between ΔH_{BR} and ΔH_r on each storage day appeared to be larger for blends that contained higher amylose content (0 wx, 12.5 wx, and 25 wx) than for blends with lower amylose content (50 wx, 75 wx, and 100 wx) (Tables 2.6 and 2.7). The smallest difference was for the 100 wx sample, i.e. the sample with the

Table 2.7. Retrogradation Enthalpy Based on Branched Fraction of Starch (ΔH_{BR}, J/g) of Starch Blends and Starch/Gluten Blends as Affected by Interaction of Blends and Storage Days

	Days			
Blends	**5**	**10**	**15**	**20**
0 wx	5.94	7.04	7.98	8.13
12.5 wx	5.17	7.18	7.50	8.09
25 wx	5.37	6.77	7.31	7.88
50 wx	4.58	6.43	6.73	7.48
75 wx	3.83	5.92	6.68	7.02
100 wx	3.14	5.85	6.52	7.25
0 wxg	4.86	6.10	7.81	8.55
12.5 wxg	5.34	6.46	7.42	7.81
25 wxg	5.03	5.89	6.78	7.12
50 wxg	5.34	5.42	5.40	6.47
75 wxg	3.38	3.96	5.54	5.76
100 wxg	2.07	2.67	3.55	4.24
LSD (0.05)	----------	----------	0.65	----------

lowest amylose and highest amylopectin content. Based on these observations an assumption can be made that high amylose content in 0 wx – 25 wx blends accelerated recrystallization of amylopectin, while in 50 wx – 100 wx blends, low amylose content contributed less to amylopectin recrystallization. These results emphasize the importance of the ratio of amylose and amylopectin for starch retrogradation. A possible reason for this behavior lies in the polymeric nature of amylose and amylopectin. Kalichevsky and Ring (1987) found that amylose and amylopectin are thermodynamically incompatible (and therefore immiscible in aqueous solutions). This leads to phase separation in retrograding gels, where one

phase is rich in amylose and the other in amylopectin. Phase separation leads to increase of effective concentrations of amylose and amylopectin in their microdomains, which leads to higher potential of reassociation of molecules within each domain at concentrations at which they would not be able to interact and form a gel network alone. This concentration was found to be around 1% for amylose (Clark et al. 1989; Doublier and Choplin 1989) and much higher for amylopectin (around 10%) (Biliaderis and Zawistowski 1990; Kalichevsky et al. 1990). Taking this into consideration, high amylose content in 0 wx – 25 wx blends could have "helped" amylopectin to become more concentrated in its domain and amylopectin chains could have been in closer proximity to each other for faster recrystallization. The importance of amylose for recrystallization of amylopectin was shown also in the study of Klucinec and Thompson (2002). Retrogradation enthalpy of gels with amylose and amylopectin were higher than enthalpies of corresponding amylopectin alone. The results were obtained by normalizing the ΔH_r to the percent of amylopectin in starches. Same observation was made by Gudmundsson and Eliasson (1990), but only at amylose contents higher than 50%.

Since ΔH_{BR} is related to amylopectin retrogradation, inferences can be made about the relationship between ΔH_{BR} and length (degree of polymerization DP) of amylopectin branch chains. As mentioned earlier, waxy durum starch contained shorter iodine-complexible branch chains than amylopectin of non-waxy wheat starch (Table 2.2), indicating that waxy durum starch may have shorter branch chains of amylopectin than non-waxy starch. Consequently, blends with

higher percent of WD starch can be expected to have higher percent of short chains. Results for ΔH_{BR} showed that blends with high amylopectin content, i.e. low amylose content, had a tendency to retrograde less than high amylose blends. In addition to amylose content, high proportion of short amylopectin chains in blends with high amylopectin content could have brought about slow retrogradation and lower ΔH_{BR} values (Table 2.7). In order to form a double helix, two amylopectin chains have to be in close proximity. In addition, long chains are more likely to form double helices because they can form sufficient number of bonds to result in a stable helix. Double helices further aggregate eventually resulting with crystallinity, and likelihood of interaction between two helices is higher for long helices than for short ones (Klucinec and Thompson 1999).

Several studies showed that starch with large proportion of short amylopectin chains retrogrades slower than starch with high proportion of long chains. Shi and Seib (1992) showed that retrogradation enthalpy depended on the distribution of branch chain length of amylopectin. Starches with high mole fraction of DP 14-24 branches had higher tendency to retrograde than starches with high mole fraction of DP 6-9. High percent of short branch chains of amylopectin increased the proportion of non-crystalline regions in amylopectin and consequently slowed retrogradation. Yuan et al. (1993) showed that high percent of long DP chains (DP 20-30) and long B chains of amylopectin promoted retrogradation that resulted in one of the waxy starches retrograding to a greater extent than other starches.

Transition temperatures related to melting retrograded starch (onset T_{or},

peak T_{pr}, completion T_{cr}) (Tables 2.8, 2.9, and 2.10) were lower than corresponding gelatinization temperatures (Tables 2.4 and 2.5). This was in agreement with results of Sasaki et al. (2000) who attributed this phenomenon to weaker starch crystallinity in retrograded starch than in native starch. During retrogradation, starch molecules reassociate in a less ordered and hence less stable state than in native starch granules, and therefore the transition temperatures are lower (Yuan et al. 1993).

On each storage day, 0 wx blend had the lowest T_{or} (Table 2.8) and 100 wx in general had the highest T_{or}. The temperature in general increased with lower amylose content in blends, although the difference between two consecutive blends was not different in most cases. The need for higher temperature to start crystalline melting could be connected with the occurrences during gelatinization, as described earlier. Plasticization of amorphous region, made of amylose, is necessary to destabilize and melt crystals. Therefore, low amylose blends may need higher temperature to initiate melting because of lesser effect from amorphous region than in high amylose starches. Yu et al. (2009) did not observe any difference in transition temperatures between waxy and non-waxy rice starches (although this study was done on single starches, not blends). Ortega-Ojeda and Eliasson (2001) reported slight increase of T_{or} during storage, while T_{pr} and T_{cr} did not change throughout the whole storage period. The widest range of T_{cr}-T_{or} (data calculated from Tables 2.8 and 2.10) between 0 wx and 100 wx was on day 5 (3.6°C) and the narrowest range was on day 20 (1.2°C). The range of melting temperatures is related to the quality and homogeneity of recrystallized

Table 2.8. Onset Temperature of Melting Retrograded Starch (T_{or},°C) as Affected by Interaction of Blends and Storage

	Days			
Blends	**5**	**10**	**15**	**20**
0 wx	39.5	36.4	37.2	40.0
12.5 wx	40.1	36.7	38.4	39.8
25 wx	40.5	37.7	39.0	40.1
50 wx	40.7	38.4	39.7	40.5
75 wx	43.5	39.0	40.4	40.5
100 wx	43.1	39.8	40.9	41.2
0 wxg	39.1	39.7	38.0	37.4
12.5 wxg	38.4	39.4	38.6	38.3
25 wxg	40.9	40.0	40.1	38.3
50 wxg	41.5	40.6	40.9	39.0
75 wxg	42.5	41.0	41.7	39.6
100 wxg	41.5	39.4	41.6	39.2
LSD (0.05)	----------	----------	0.9	----------

amylopectin. During retrogradation, crystals of different stability can be formed and T_{or} is the temperature at which the least stable crystals start melting (Fredriksson et al. 1998). The results indicate that the homogeneity of crystallites in retrograded starch increased as the storage progressed.

The peak temperature, T_{pr}, showed a decrease on day 20 for all blends compared to day 5 (Table 2.9). Similar behavior was observed for the completion temperature (T_{cr}) (Table 2.10). The reason for this phenomen is not clear but it can be hypothesized that it is related to crystalline perfection and stability. Crystallites formed at the beginning of storage may be very heterogeneous, requiring higher temperatures to start melting. In later stages of storage, crystallites are probably

Table 2.9. Peak Temperature of Melting Retrograded Starch (T_{pr},°C) as Affected by Interaction of Blends and Storage

	Days			
Blends	**5**	**10**	**15**	**20**
0 wx	50.4	47.4	47.8	49.8
12.5 wx	50.4	47.6	48.7	49.5
25 wx	51.2	48.1	48.9	49.9
50 wx	51.1	48.4	49.7	49.8
75 wx	52.0	49.1	49.6	49.8
100 wx	52.3	49.4	50.4	50.1
0 wxg	49.8	49.7	48.4	47.8
12.5 wxg	49.8	49.9	48.8	48.7
25 wxg	50.9	50.2	50.1	48.5
50 wxg	51.9	50.6	50.3	49.1
75 wxg	52.5	50.7	50.8	49.4
100 wxg	52.6	51.0	50.8	50.3
LSD (0.05)	----------0.5----------			

more uniform and maybe melt cooperatively. Melting of one region may help to destabilize other regions and promote melting that results in lower T_{cr}.

Effect of Gluten

ΔH_r of all starch/gluten blends was lower than ΔH_r of starch blends (Table 2.7), which was expected due to dilution of starch with gluten. For this reason, ΔH_r cannot be used to evaluate the effect of gluten on retrogradation of starch blends. ΔH_r of starch/gluten blends increased during storage, but the increase between days 5 and 10 was not so pronounced as in starch blends. No specific pattern in ΔH_r increase was detected for starch/gluten blends. Between days 5 and 20, the

Table 2.10. Completion Temperature of Melting Retrograded Starch (T_{cr},°C) as Affected by Interaction of Blends and Storage Days

	Days			
Blends	**5**	**10**	**15**	**20**
0 wx	58.5	56.1	56.4	57.5
12.5 wx	58.2	56.3	56.5	57.0
25 wx	59.1	56.7	56.6	57.4
50 wx	58.5	56.3	56.9	57.0
75 wx	58.2	56.5	56.1	57.0
100 wx	58.4	56.7	57.3	56.8
0 wxg	57.5	57.3	56.1	56.4
12.5 wxg	57.8	57.4	56.4	56.6
25 wxg	58.3	57.7	57.6	56.6
50 wxg	58.9	57.8	57.0	56.8
75 wxg	58.9	57.2	57.4	56.7
100 wxg	59.9	58.1	57.1	57.4
LSD (0.05)	---------- 0.6 ----------			

ΔH_r of 0 wxg blend increased by 37% while ΔH_r of 100 wxg increased by 104%. The results show that starch/gluten blends retrograded more between 5 and 20 days of storage than the corresponding starch blends.

A major difference between starch blends and starch/gluten blends was that ΔH_r of starch gluten/blends decreased with increase in the proportion of waxy starch on each storage day; although the difference between two consecutive blends was not always significant. The opposite was observed for starch blends, with the exception of day 5 (Table 2.6). Similar to starch blends, after 10 days of storage, ΔH_r was significantly different between blends 50 wxg and 75 wxg and also between 75 wxg and 100 wxg. After 15 and 20 days of storage, ΔH_r of 100

wxg blend was significantly lower than ΔH_r of other blends, while the opposite was observed for starch blends. Apparently, gluten delayed development of crystallinity in starch/gluten blends, especially in blends with low amylose content.

In order to compare retrogradation of starch/gluten blends and starch blends and to assess the effect of gluten, ΔH_{BR} was compared between blends (Table 2.7). This allows a comparison of blends on an amylopectin basis and eliminates the affect of dilution. ΔH_{BR} of all blends exhibited similar behavior to ΔH_r with respect to increase during storage. However, unlike in starch blends, the increase was not larger between 5 and 10 days of storage, i.e. there was no steady pattern in ΔH_{BR} increase. ΔH_{BR} was lower for blends with low amylose content for each storage day, which was the same as for starch blends. However, on day 20, ΔH_{BR} was significantly different between each consecutive blend, which was not the case with starch blends.

ΔH_{BR} of starch/gluten blends was significantly lower than ΔH_{BR} of starch blends for many blend combinations. On day 5, significant difference was observed for blends 0 wxg, 50 wxg, and 100 wxg (Table 2.7). On day 10, all starch/gluten had significantly lower ΔH_{BR} than starch blends, while on days 15 and 20 a distinct pattern was observed where blends with low amylose content (50 wxg, 75 wxg, and 100 wxg) had significantly lower ΔH_{BR} than their corresponding starch blends. ΔH_{BR} of high amylose starch/gluten blends (0 wxg, 12.5 wxg, and 25 wxg) was similar to ΔH_{BR} of their corresponding starch blends on days 15 and 20 (Table 2.7).

The measured enthalpy (ΔH_r) and enthalpy normalized to amylopectin

content (ΔH_{BR}) indicate that gluten had an effect on starch retrogradation. In later stages of storage, the effect was especially pronounced with low amylose blends. Therefore, the hypothesis is that gluten could interfere more with retrogradation of branched molecules (amylopectin) than with retrogradation of long linear molecules of amylose. An uncertainty exists as to why the effect of gluten was not the same on days 5 and 10 like on days 15 and 20.

Findings of different studies were inconsistent. The inconsistencies are most likely the result of different gluten and water level in systems, or use of different models to study the interactions (bread vs. gel models). Several authors proposed possible mechanisms of the effect of gluten on starch retrogradation. A model of hydrogen bond cross-linking between granule remnants and protein matrix was proposed as a reason for bread firming by Martin et al. (1991) and Martin and Hoseney (1991). Gluten was found to have anti-firming effect in bread (which could be related to retrogradation) and the mechanism was believed to be simple dilution of starch with gluten (Kim and D'Appolonia 1977a).

Ottenhof and Farhat (2004) studied the effect of gluten on starch retrogradation in extruded product and found no evidence of the effect of gluten on recrystallization. However, the study was done with low gluten content and under limited water conditions that could have lead to non-uniform partitioning of water in system. Several authors have presented results that showed that gluten retarded recrystallization of starch, which was in agreement with findings of this study, at least for low amylose blends. Eliasson (1983b) attributed the effect to the lower amount of water available for starch recrystallization in the presence of water.

Another hypothesis was that gluten affected leaching of amylose during gelatinization and therefore affected the amylose/amylopectin composition of granule remnants and eventually the retrogradation.

Chanvrier et al. (2005) and Champenois et al. (1998) studied the rheological properties of starch/gluten gels and found that gluten weakened the gel network. The effect was attributed to phase separation of starch and gluten. Starch and gluten are thermodynamically incompatible polymers and therefore they tend to phase separate in aqueous mixtures (Tolstoguzov 1997). Champenois et al. (1998) hypothesized that gluten diluted the starch and therefore increased the critical concentration of starch needed for the formation of gel network. This hypothesis potentially could be applied to studied starch/gluten blends. As mentioned before, starch molecules have to be in a certain close proximity to form double helices, which is a necessary prerequisite for the formation of crystallinity. Since gluten diluted starch (and the dilution can be expected to be significant due to 30% of gluten in system), it may have retarded recrystallization by reducing the number of chains that are in proximity of each other to form double helices. Since amylopectin retrogrades slower than amylose, the effect of retardation was even more pronounced in high amylopectin blends. An alternative mechanism could be based on simple steric hindrance of starch recrystallization by gluten. Gluten fibrils could reduce the contact between starch chains and lower the chance of double helix formation. The effect was even more pronounced in blends with low amylose content in later stages of storage because of gluten could have interfered more with amylopectin molecules due to their branched nature. Chedid and Kokini

(1992) stated that interactions between starch and gluten are avoided in excess water conditions. Gluten did not have major effect on gelatinization enthalpies of blends, but it affected retrogradation enthalpies even though water was in excess in analyzed systems.

Eliasson (1983b) reported that gluten increased the transition temperatures needed for melting the retrograded starch. Transition temperatures of most starch/gluten blends decreased during the entire storage period (Tables 2.8 – 2.10). The completion temperature, T_{cr}, was higher for some starch/gluten blends than for corresponding starch blends, but no distinct pattern was found (Table 2.10). T_{cr} showed an increase in low amylose blends for each storage day, while this behavior was not characteristic for starch blends. Starch blends on day 20 even exhibited opposite behavior (Table 2.10). T_{cr} also showed a decrease during storage period for almost all starch/gluten blends. Also an increase in T_{or} was observed on days 15 and 20 (Table 2.8) and it could be attributed to more homogeneous crystallites. The same effect was noticed for starch blends. However, on day 20, most starch/gluten blends had lower T_{or} than their corresponding starch blends (Table 2.8). The reason for this behavior is not clear since gluten would be expected to interfere with diffusion of water to starch. It is possible that presence of gluten did not allow starch molecules to crystallize into structures similar to those in starch blends by the end of the storage period, i.e. gluten could have prevented formation of homogeneous crystallites.

CONCLUSIONS

Starch blends with high (12.5 wx and 25 wx) and low (50 wx and 75 wx) amylose content had significantly different pasting properties. Low amylose starch blends exhibited two RVA pasting peaks, faster swelling (at lower temperature), lower HPV, and lower CPV than high amylose blends. However, RVA peak viscosities did not change linearly with amylose content; each blend appeared to develop peak viscosity differently than the rest of the blends. Thus, the conclusion was that pasting properties of starch blends most likely were influenced not only by amylose content, but also by the occurrence of two separate granule swelling processes and specific interactions between two starches. Therefore, blending two starches to obtain certain amylose level could result in a 'new' starch with pasting properties that cannot be predicted by averaging pasting properties of its constituents.

Pasting profiles of starch/gluten blends differed significantly from those of starch blends, swelling and pasting of low amylose blends was delayed, and the HPV exhibited exactly opposite behavior compared to the HPV of starch blends. These results showed that the effect of gluten was not just simple dilution of starch, but either interaction of gluten fibrils with starch molecules or by hindering the interaction between starch granules.

Gelatinization properties of starch blends were affected by amylose content, with the exception of T_o, but differences were not significant between each consecutive blend. The results indicate that gelatinization properties of starch will not change necessarily for every combination of waxy and non-waxy starch or flour

even when their amylose contents are different. The measured amylose content in starch blends is not distributed evenly between granules of two starches in the blend. Gelatinization of blends can be seen as two separate gelatinization processes of waxy and non-waxy granules that could affect each other through interaction of leached amylose and amylopectin molecules or interaction of leached starch with granules. Hence, the resulting gelatinization properties of blends are not the average of gelatinization properties of two starches.

Due to the presence of gluten fibrils, gelatinization temperatures of starch/gluten blends were more affected than the enthalpy. The absence of an effect of gluten on gelatinization enthalpy of starch blends was attributed to the excess water and lack of complete gluten network. Although gluten fibrils most likely surrounded starch granules, the excess amount of water still enabled undisturbed gelatinization.

Recrystallization of all starch blends progressed through the whole storage regardless of amylose content, as shown by the retrogradation enthalpy normalized to amylopectin content of blends. Retrogradation enthalpy on each storage day was lower for low amylose blends (50 wx, 75 wx, 100 wx) than for high amylose blends (0 wx, 12.5 wx, 25 wx), showing that high amylopectin content in starch blends slowed the process of retrogradation. However, retrogradation enthalpy was not significantly different between each consecutive blend, and it was not a simple sum of contributions of individual components. These results indicate that, similar to pasting and gelatinization, retrogradation of starch blends was affected not only by amylose content but also possibly by other factors such as

phase separation tendency between amylose and amylopectin, structural differences between waxy and no-waxy starch, and co-crystallization of two starches in gelatinized blend.

Based on the retrogradation enthalpies normalized to amylopectin content, the conclusion was that gluten interacted more intensively with branched than with linear molecules either by reducing the contact between amylopectin chains or by diluting the starch to such an extent that prevents branched molecules to be in close enough proximity for helix formation. This behavior was especially pronounced at the end of storage (days 15 and 20) for low amylose starch/gluten blends.

Inferences based on the results obtained in this study for gel model systems, can be made about behavior of starch in food systems such as bread, or other wheat flour based bakery products. First, flour blends with different amylose contents can be used to alter pasting, gelatinization, and retrogradation properties of starch in dough/bread, but these properties do not change always linearly with amylose content due to specific interactions between two starches. Second, starch is never an isolated ingredient in bread; gluten is always present not only as an inherent flour constituent but also very often as added vital wheat gluten in different amounts. The effect of gluten on starch properties in bread could be even more pronounced than in studied gel model systems due to limited water conditions in dough and bread and competition for water between starch and gluten.

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CHAPTER 3: PROPERTIES OF SOLUBLE STARCH ISOLATED FROM RETROGRADED WHEAT STARCH GELS AND WHEAT STARCH/GLUTEN GELS

ABSTRACT

Retrogradation properties of starch blends are affected by amylose content, but they do not always change linearly with a change in amylose content. Furthermore, retrogradation properties of starch/gluten blends often are different from retrogradation properties of corresponding starch blends. In order to investigate possible reasons for these phenomena and possible differences in retrogradation patterns, soluble starch was isolated from gels (10.7% solids) made of starch blends with 0, 12.5, and 25% waxy durum starch and non-waxy hard red spring wheat starch, as well as from starch/gluten blends that were made with the addition of 30% gluten to starch blends. Soluble starch was subjected to fractionation by gel permeation chromatography and blue value (BV), total carbohydrate content (CHO), and the wavelength of maximum iodine absorption (λ_{max}) of each peak (linear and branched fraction) were determined. All blends, with the exception of 12.5 wx, had fast reassociation of linear starch molecules between 0 and 5 days. An increase in retrogradation enthalpy of 0 wx starch during storage most likely was the combination of two effects: retrogradation of branched molecules in later stages of storage, and reassociation of branched molecules with long chains. Lower λ_{max} of linear fractions in 12.5 wx and 25 wx than in 0 wx indicated presence of amylopectin fragments that probably eluted with linear fraction and that could have recrystallized and contributed to the retrogradation enthalpy. Soluble starch from all starch/gluten blends had intensive reassociation of branched molecules with long chains at the beginning of storage (between days 0 and 5), which could have contributed to the development of

crystallinity during storage. The 0 wxg and 0 wx soluble starches had different retrogradation patterns. Retrogradation of starch and starch/gluten blends did not depend only on amylose content; blends had different retrogradation patterns and they differed in the dynamics of reassociation of different chain lengths of both linear and branched fractions.

INTRODUCTION

Starch retrogradation is a process by which starch molecules begin to reassociate after gelatinization, and return into a more ordered state. Under favorable conditions, ordered structures of starch can recrystallize (Atwell et al. 1988). During retrogradation, amylose molecules reassociate rapidly by forming double-helical structures of 40-70 glucose units (Jane and Robyt 1984; Liu et al. 1997), while amylopectin reassociates slowly by forming short intermolecular helices (Miles et al. 1985; Ring et al. 1987; Biliaderis and Zawistowski 1990). Retrogradation of amylopectin is hindered by its branched structure that results in outer chains shorter than ~25 glucose units (Chung et al. 2008). In starch pastes, retrogradation results with the formation of gel, which consists of an amylose matrix (formed through junction zones of double helices) with starch granules embedded in it (Miles et al. 1985; Gidley 1989).

Retrogradation properties of starch have been studied using different methods. One of the ways to investigate the changes in starch molecules during retrogradation is by studying soluble starch. Soluble starch is a fraction that can be isolated from starch gel or paste and that contains starch molecules that became solubilized due to processing. The composition of soluble starch depends on the cooking time and temperature and the concentration of starch (Zhong and Hamaker 2000). Soluble starch has been investigated mainly for its relation to product texture. It was related to sorghum couscous stickiness (Aboubacar and Hamaker 2000), rheological properties of rice starch (Tsai and Lii 2000), corn masa adhesiveness (Miklus and Hamaker 2003), and staling of corn tortillas

(Fernandez et al. 1999). In bread, soluble starch was shown to be degraded amylopectin (Ghiasi et al. 1979) and the amount of soluble starch was related directly to bread stickiness (Every et al. 1996), as well as studied for the amount of dextrins produced by alpha-amylases in relation to bread staling (Palacios et al. 2004). In a sorghum gel, the reduction of soluble starch content (both amylose and amylopectin) was related to increase in retrogradation (Bello et al. 1995). Soluble starch is expected to contain mainly amylose since amylose leaches from granules during cooking; but studies have shown that it also can contain amylopectin, often in its fragmented form (Aboubacar and Hamaker 2000; Desse et al. 2009).

Process of starch retrogradation can be affected by various factors, such as the ratio of amylose and amylopectin, chain length of amylose and amylopectin, presence of other molecules in the system (e.g. gluten), and conditions under which the starch is gelatinized and stored (Fredriksson et al. 1998; Koch et al. 1998; Mohamed and Rayas-Duarte 2003; Chung et al. 2008). Some studies have shown that waxy starch, i.e. starch with no or very little amylose, retrogrades slower than normal starch and therefore could potentially be used to retard staling in bread (Bhattacharya et al. 2002; Morita et al. 2002b). Some studies showed no difference between waxy and normal starch in bread (Park and Baik 2007) while other studies showed an increase in retrogradation enthalpy in bread that contained certain amount of waxy starch (Lee et al. 2001; Baik et al. 2003). In starch blends with different amylose contents, Sasaki et al. (2000) found that low amylose content leads to a higher degree of recrystallization. Obanni and BeMiller (1997) reported no measurable retrogradation endotherm of starch blends after

two weeks of storage at 4°C, while high amylose content facilitated retrogradation of starch blends in the study of Yu et al. (2009). Many authors attributed retrogradation properties of blends to specific interactions between components of gelatinized starch.

The effect of gluten on starch retrogradation appears to be even more complex than the amylose/amylopectin ratio, and different mechanisms have been proposed in literature. Martin and Hoseney (1991) and Martin et al. (1991) proposed that bread firming was the result of cross-linking between granule remnants and protein matrix. Some authors proposed that gluten acts through reduction of water availability for starch recrystallization (Eliasson 1983) or through retardation of water loss from granule remnants (Wang et al. 2004). A model of phase separation of starch and gluten during retrogradation also was proposed (Champenois et al. 1998; Chanvrier et al. 2005)

The exact events in a starch paste/gel that lead to recrystallization of starch are not known. Klucinec and Thompson (1999) suggested that exterior chains of amylopectin could form double helices with amylose upon retrogradation of gel and therefore alter the interaction between amylose molecules. Klucinec and Thompson (2002) showed that amylopectin retrogrades more in the presence of amylose. The authors proposed that formation of physical junction zones between amylose molecules, amylose and amylopectin molecules, and between amylopectin molecules leads to the formation of gel. Jane and Chen (1992) also proposed that amylose and amylopectin interact during retrogradation and gel formation. Tako and Hizukuri (2000) proposed formation of intermolecular

hydrogen bonds between amylose and amylopectin, where amylose molecule may associate with two or more short amylopectin chains. Considering these models of retrogradation, amylose content of starch proves to have an important role.

Besides amylose content, the chain length of amylose and amylopectin also determines the pattern and rate of retrogradation. Amylopectin with large proportion of long side chains was shown to retrograde faster than amylopectin that has more short chains (Shi and Seib 1992; Yuan and Thompson 1998; Kohyama et al. 2004). Yuan et al. (1993) and Matalanis et al. (2008) found that these were the long B-chains of amylopectin. Longer amylopectin chains are more likely to form double helices between themselves and also with amylose than shorter chains (Jane and Chen 1992; Klucinec and Thompson 1999).

Changes in starch that occur during retrogradation of a complex system such as a starch blend or starch/gluten blend are difficult to study. The research reported in this chapter was done as a continuation of the study in Chapter 2 with the objective to investigate the composition of soluble starch isolated from gel during storage. The goal was to investigate the possible differences in retrogradation patterns during storage of starch blends and starch/gluten blends having different amylose contents.

MATERIALS AND METHODS

Wheat

Non-waxy commercial hard red spring wheat cultivar, 'Alsen' was used as a source of non-waxy starch and gluten. A waxy durum wheat line (WD) was used as source of waxy starch. This line was derived from an initial cross of hard red winter wheat, 'Ike', which carried null alleles at *Wx-A1* and *Wx-B1 loci,* and durum wheat cultivar 'Ben'. Subsequently, full waxy durum wheat lines were developed by backcrossing to Ben while selecting among backcross progeny for the full waxy genotype. The full waxy durum line, derived from the fourth backcross to the recurrent durum parent, Ben, was provided by Dr. Douglas Doehlert (USDA-ARS, Cereal Crops Research Unit, Fargo, ND).

Waxy durum wheat was used as a source of waxy starch since waxy common wheat was not available in sufficient quantity for the experiments. Current information available on similarities and differences between durum and common wheat starch are presented in the 'Results and Discussion' section

Isolation of Starch and Gluten

Waxy and non-waxy wheat were tempered and milled into a straight grade flour using a Bühler laboratory mil according to AACC Approved Methods 26-10 and 26-21 (2000).I. Waxy and non-waxy starch were isolated using a dough washing method according to Kim and Seib (1993), which was a modification of the method of Wolf (1964). Flour (approx. 400g in one batch) and distilled water (60-65% w/w based on flour) were mixed in a pin mixer for a short time to obtain a

cohesive mass with minimum gluten development. Starch was washed out by adding small amounts of distilled water, at least five times in succession, and separated from gluten by sieving through the US 70 sieve (212 µm). Starch suspension was centrifuged at 2,000 x *g* for 15 min. The supernatant was discarded and the upper pigmented sediment (consisting of tailings, water soluble proteins) was removed by careful scraping with spatula. Starch was re-suspended in distilled water and the process was repeated two times. The third washing was done in ethanol to remove non-starch lipids, i.e. lipids that are not associated with amylose in the starch granule. Removal of endogenous starch lipids was not done since it would require starch to be gelatinized and then to extract of lipids with water saturated butanol (Morrison et al. 1980; Morrison and Coventry 1985). These conditions cause swelling and partial disruption of starch crystallinity and consequently change the functional properties of starch. Native properties of starch had to be preserved for this study. Prime starch was air-dried overnight at room temperature, ground using mortar and pestle (to avoid damaging starch granules by more abrasive grinding technique), and sieved through US 70 sieve (212 µm). Starch was stored in tightly closed containers to prevent moisture absorption.

Gluten was isolated from the cultivar 'Alsen' simultaneously with starch isolation (from the same dough). After starch was isolated, gluten was continuously washed until the wash water did not contain any more starch (clear wash water, also tested with iodine solution). Gluten was dried by freeze-drying and milled using a ball mill to avoid heating of gluten. Ground gluten was sieved through the

US 70 sieve.

Preparation of Gels

Starch and starch/gluten gels were prepared using Rapid Visco Analyzer (Newport Scientific, Narrabeen, Australia). To prepare starch gels, non-waxy starch and waxy durum (WD) starch (on dry basis) were weighed directly in the RVA canister so that the blends contained 0, 12.5, and 25% WD starch (w/w). For the preparation of starch/gluten blends, non-waxy and waxy starch were weighed directly into a canister in the ratio 0, 12.5, and 25% of WD starch, and gluten was added. Starch/gluten blends consisted of 30% (w/w) gluten isolated from hard red spring wheat cultivar 'Alsen' and 70% (w/w) starch blends. All ingredients were blended first in a dry form by mixing with spatula.

Deionized water (25 mL) was added to 3.0 g of starch (or starch+gluten) (14% mb) and the slurry was mixed thoroughly. A programmed heating and cooling cycle (13 min) was used, where the samples were held at 50°C for 1 min, heated to 95°C in 3.5 min, held at 95°C for 2.5 min before cooling to 50°C, and holding at 50°C for 1 min. The paste was cooled to room temperature, the canister was sealed with parafilm to prevent moisture loss and stored at 4°C for 0, 5, 10, 15, and 20 days. All gels were prepared in three replications. Starch blends (gels) were labeled as 0 wx, 12.5 wx, and 25 wx, and the starch/gluten gels were labeled as 0 wxg, 12.5 wxg, and 25, wxg.

Isolation of Soluble Starch

Isolation of soluble starch from gels was done following the method of Zhong and Hamaker (2000) with some modifications. Gel (20 g) was combined with 100 mL water in plastic centrifuge bottles and gently mixed with a magnetic stir bar for 30 min. The slurry was centrifuged at 5,000 x *g* for 20 min, and the clear supernatant was separated from the solid phase. The supernatant was immediately frozen with liquid nitrogen, freeze-dried, and placed in plastic containers with tight caps in a dessicator to prevent moisture absorption.

Fractionation of Soluble Starch

Gel Permeation Chromatography (GPC) was used to fractionate soluble starch into amylose and amylopectin, i.e. into linear and branched fractions. Gel permeation chromatography of starch generally followed the method of Jane and Chen (1992) and Klucinec and Thompson (1998). Freeze-dried soluble starch (6 mg, dry weight) was dispersed in 90% (v/v) dimethyl sulfoxide (DMSO) (3 mL) by heating the mixture in boiling water bath with constant stirring for 1 hr. The mixture was stirred for 24 hr at room temperature. Starch was precipitated by adding three volumes of ethanol (9 mL) and centrifuged at 6,000 x *g* for 15 min at 20°C. The supernatant was discarded carefully so that no ethanol remained in the centrifuge tube. Pellets were redissolved in hot distilled water (3 mL) and boiled in a water bath with constant stirring for 30 min. Following this, starch solution was cooled quickly to room temperature, and 2 mL of the solution was loaded onto the GPC column (1.0 cm x 50 cm, Pharmacia Inc., Piscataway, NJ) packed with Sepharose

CL-2B gel (Sigma-Aldrich Co, St. Louis, MO). The mobile phase was deionized water containing 25 m*M* NaCl and 1 m*M* NaOH. Sodium azide (0.02%) was added to preserve the column packing. Mobile phase was filtered through nylon membrane filter (0.2 μm) and degassed before use. Eluent was run through the column overnight to condition the column. Elution of starch fraction was done by gravity flow of the mobile phase, and 1 mL of each fraction was collected. The end of a separation was determined by adding glucose to the starch solution (glucose eluted at the end). A subsample (0.1 mL) of each fraction was loaded into a 96-well microplate and tested for the blue value by adding the same volume of I_2/KI solution (0.2 g I_2 + 2.0 g KI in 100 mL 0.1 M Acetate buffer pH 5.0, diluted 10 x). Blue value (BV) was determined according to the general method of Schoch (1964). Absorbance of each fraction was read by using the Dynex MRX microplate reader (Dynex Technologies, Chantilly, VA). The blue value was used to identify locations of amylose and amylopectin in the chromatograms and also in the fractions.

Total carbohydrate content (CHO) in each fraction was determined using the phenol-sulfuric method following the procedure of Dubois et al. (1956). A 0.2 mL sample was mixed with 0.2 mL of 5% phenol solution, and 1 mL of concentrated H_2SO_4 was added in a form of a rapid stream to facilitate mixing and develop heat necessary for the reaction. The sample was cooled for 30 min and 0.2 mL was transferred to microplate reader. Absorbance was read at 470 nm.

Parameters calculated based on the BV and CHO of GPC fractions were the ratio of BV/CHO for two major GPC peaks determined as the linear and

branched fraction, and the distribution of carbohydrate content in two major peaks (Peak CHO/Total CHO).

Iodine Binding λ_{max}

The wavelength of maximum iodine absorption (λ_{max}) of GPC fractions was determined following the general method of Morrison and Laignelet (1983) with slight modification introduced by Klucinec and Thompson (1998). The λ_{max} of starch is defined as the peak absorbance over the range of wavelengths examined.

Starch solution for λ_{max} determination was prepared as described under the Gel Permeation Chromatography method. Equal volumes of each GPC fraction of amylose and amylopectin were collected in two separate tubes and 2.5 mL of each amylose and amylopectin fraction were mixed with 0.1 mL I_2/KI solution and scanned immediately using the spectrophotometer (Hach DR/4000U, Hach Company, Loveland, CO) from 400 nm and 800 nm. The volumes of GPC fractions and I_2/KI solution used in this experiment were determined based on several trials in order to obtain adequate readings on the spectrophotometer.

Statistical Analysis

The experimental design for the preparation of gels was a randomized complete block (RCBD) with factorial arrangement of six blends (0 wx, 12.5 wx, 25 wx, 0 wxg, 12.5 wxg, and 25 wxg) and five storage days (0, 5, 10, 15, and 20). All variables were fixed. Three sets of blends were prepared, and each set was

considered a replication (block). The ratio of blue value and carbohydrate content of fractions (BV/CHO), distribution of fractions in soluble starch (Fraction CHO/Total CHO), and wavelength of maximum iodine absorption (λ_{max}) of amylose and amylopectin were analyzed for two sets of blends where each set was considered a replication (block). All data were subjected to analysis of variance using Statistical Analysis Systems (SAS) (version 9.1, SAS Institute, Cary, NC). F-test was significant at $P< 0.05$. Means were separated by Fisher's protected least significant difference test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Limitations and Assumptions of Methods and Results

In this study, soluble starch was isolated from retrograded gel in order to determine the change in soluble starch content after each storage period. Although this method did not provide exact molecular weight distribution or chain length profile of branched fraction, it served as an assessment as to whether any difference existed between retrogradation patterns of studied blends.

Blends with higher amount of waxy starch (50, 75, and 100%) that were discussed in Chapter 2 could not be tested in this study. These blends produced gels that were very soft, especially gels containing gluten, and could not be successfully separated into solid and liquid phase. Even centrifugation at higher speeds than used in this study could not yield good separation. The issue was more pronounced with increased amount of waxy starch in the blends.

All blends had two of soluble starch GPC peaks (Figures 3.1 - 3.6). Fractions included in the first peak stained purple/red with iodine, which is a characteristic of short chains of amylopectin; fractions in the second peak stained blue with iodine, which is characteristic of longer linear chains that have higher ability to complex with iodine, such as amylose (Gérard et al. 2001). Thus, based on the blue values during analysis of each GPC fraction, it was concluded that the first peak contained branched molecules and the second peak contained linear molecules.

In further discussion, these fractions will be referred to as branched and linear fraction rather than amylopectin and amylose because the structural

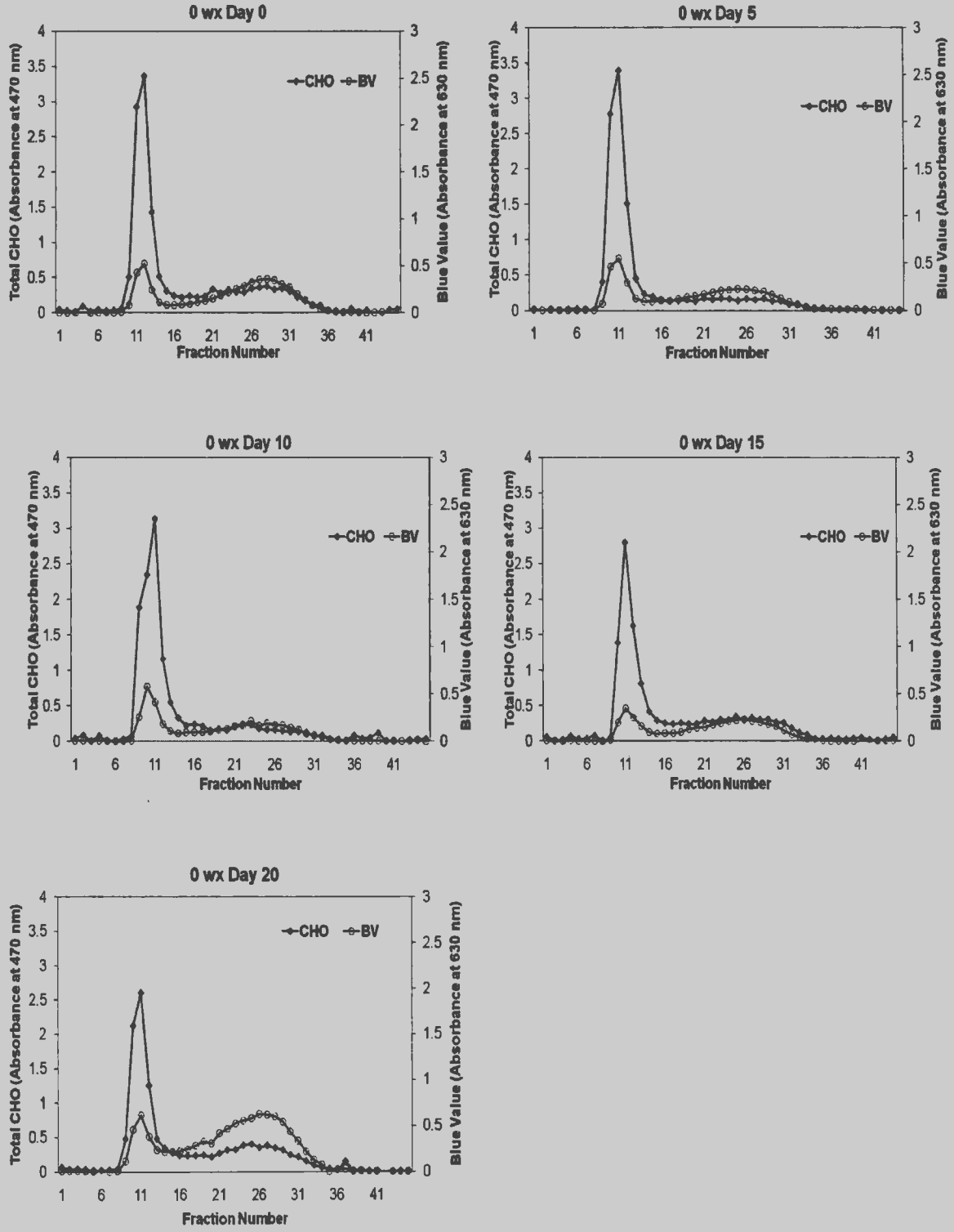

Figure 3.1. GPC profiles of soluble starch isolated from 0 wx starch gels

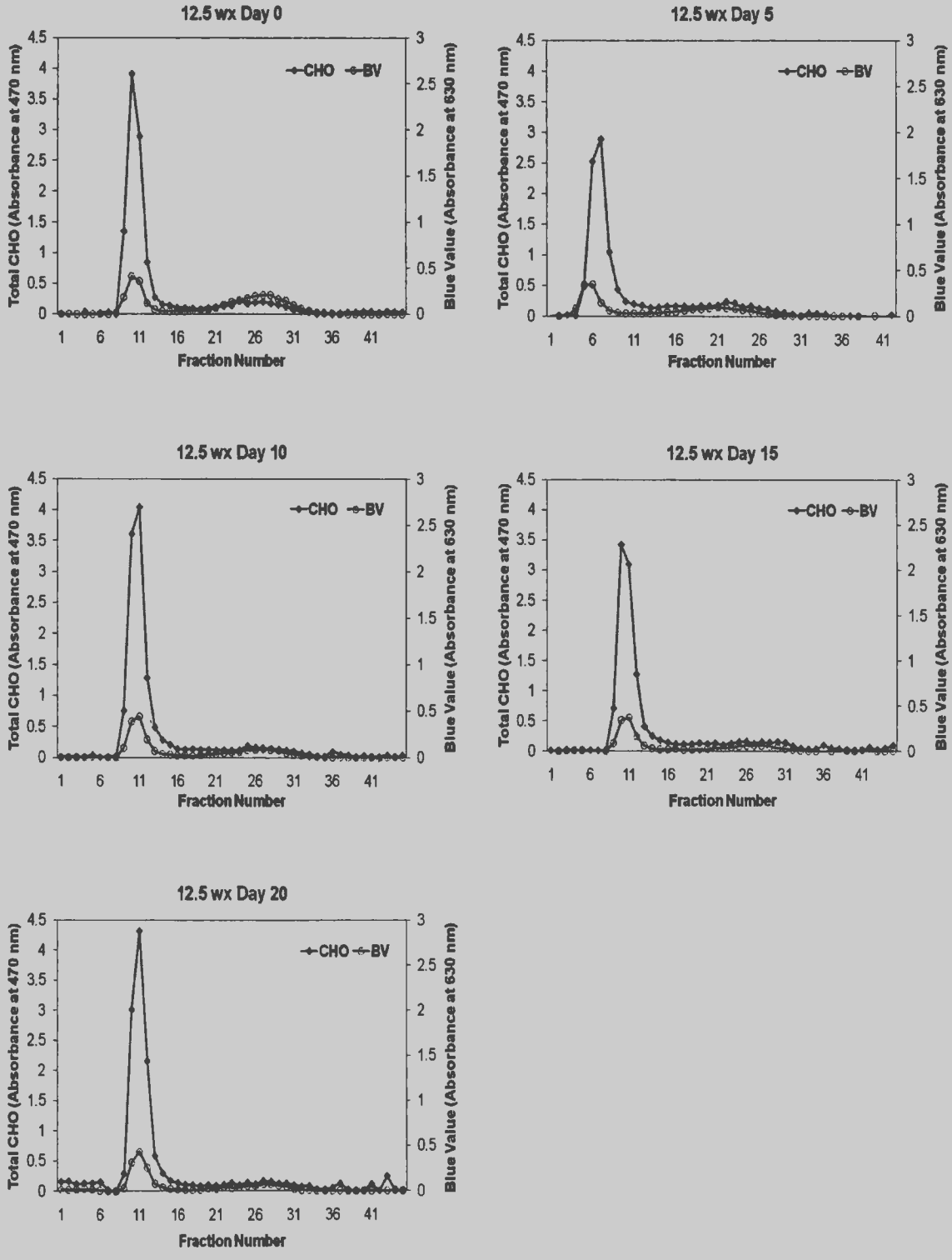

Figure 3.2. GPC profiles of soluble starch isolated from 12.5 wx starch gels

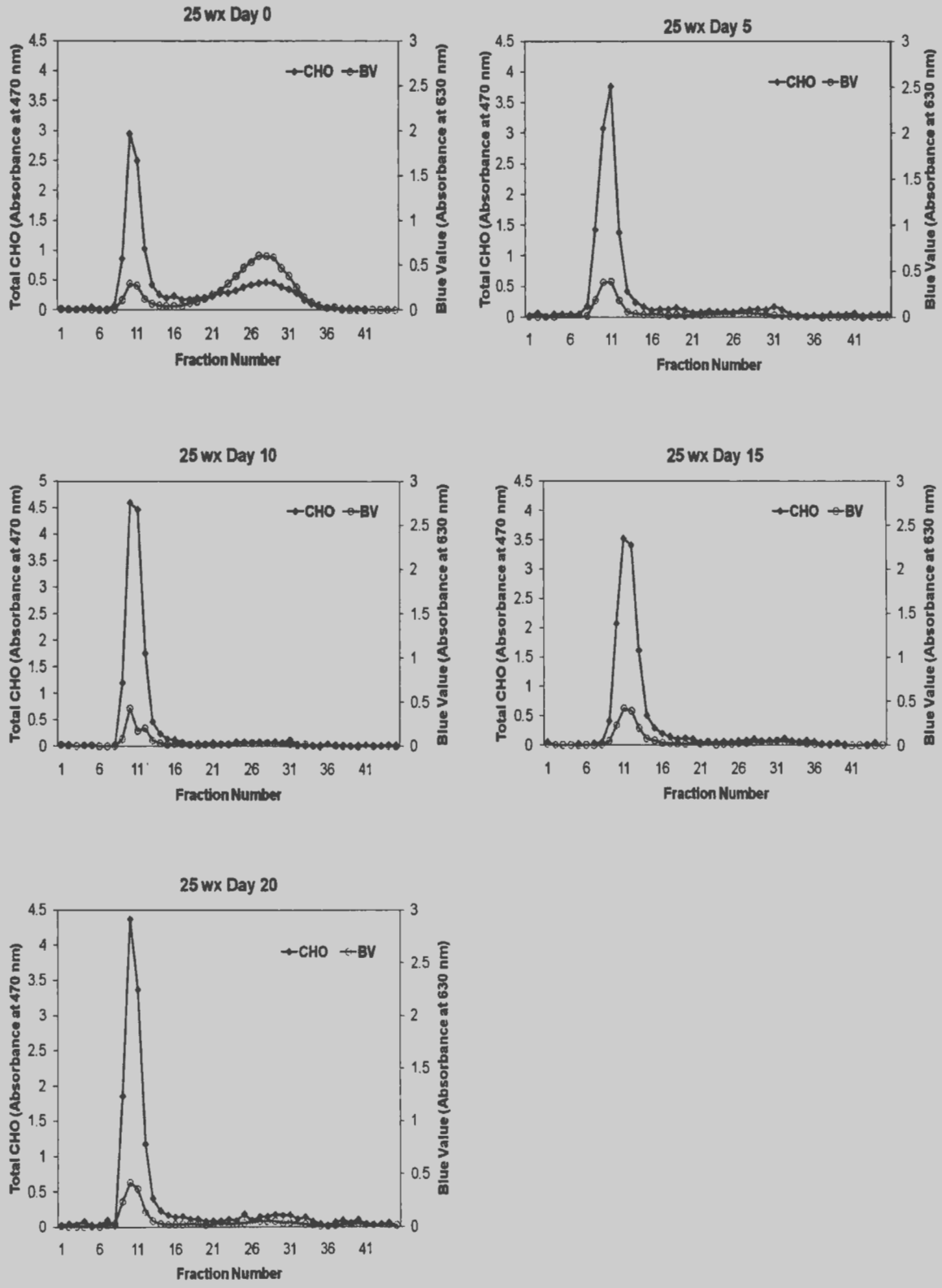

Figure 3.3. GPC profiles of soluble starch isolated from 25 wx starch gels

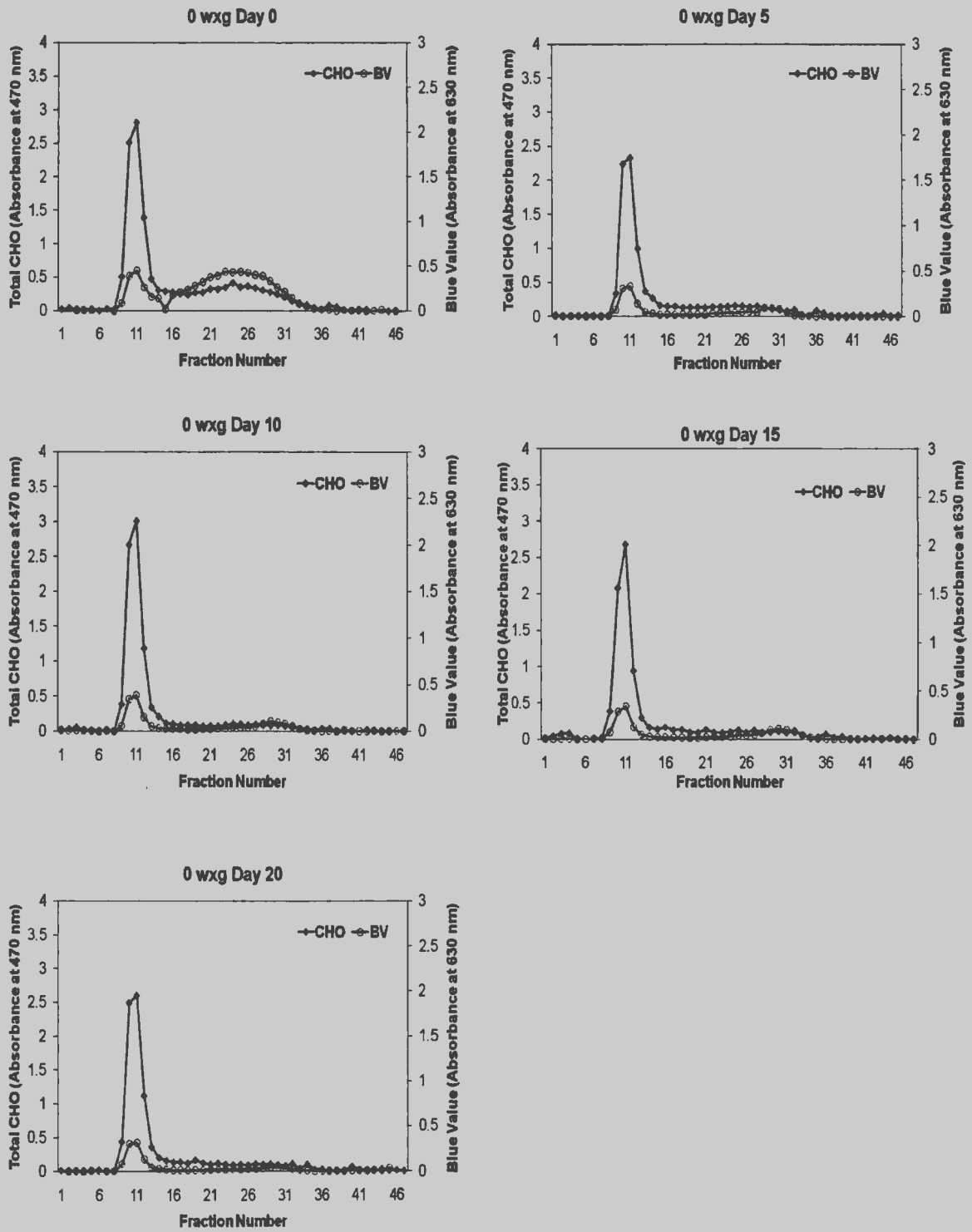

Figure 3.4. GPC profiles of soluble starch isolated from 0 wxg starch gels

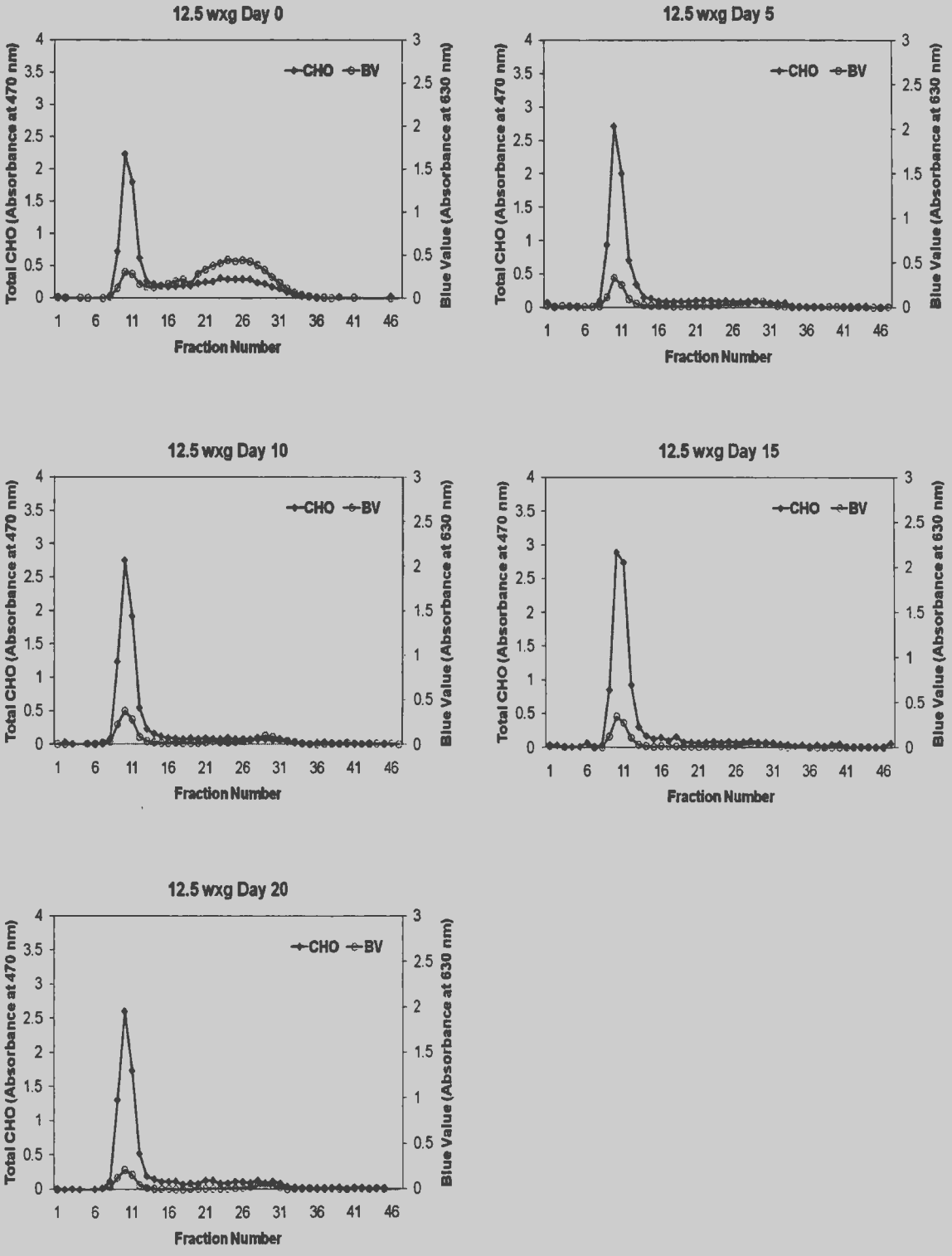

Figure 3.5. GPC profiles of soluble starch isolated from 12.5 wxg starch gels

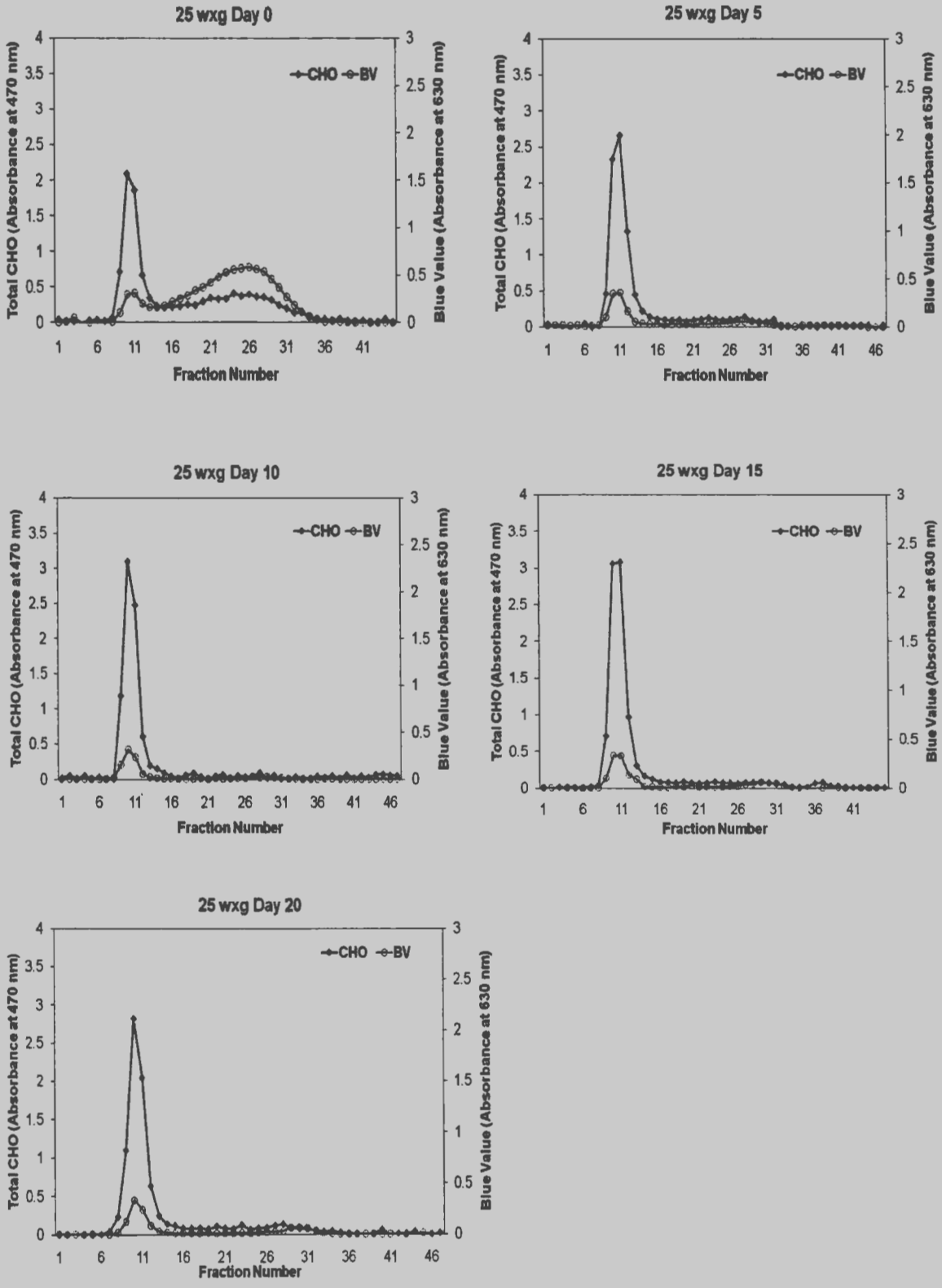

Figure 3.6. GPC profiles of soluble starch isolated from 25 wxg starch gels

properties of amylopectin and amylose most likely changed during preparation of gels, and they are not the same as in raw starch. Amylopectin molecules appear to be susceptible to partial depolymerization due to treatment with heat and shear (Jackson 1991). Partial depolymerization of amylopectin also was detected during aqueous leaching of starch at temperatures that fall within the gelatinization temperatures (Mua and Jackson 1995). Bowen et al. (2006) analyzed the molecular weight of extruded starches during storage and found that amylopectin chains broke in extruded products due to shear and increased temperatures. Hence, amylopectin molecules could have depolymerized partially during cooking with shear in RVA. The assumption is that some of the short branch chains broke-off the amylopectin molecule. These amylopectin chains behaved similar to short linear amylose chains and probably eluted in the second peak with amylose. Therefore, the sheared linear amylopectin chains would be measured as amylose by techniques used in this study.

Two chromatographic peaks also were observed by Bello et al. (1995) for the soluble starch isolated from sorghum gel. In another study (Aboubacar and Hamaker 2000), three peaks were revealed after debranching of soluble starch. The second and third peaks were identified as carbohydrates with intermediate and short chain length; the material in the third peak even did not stain with iodine. The high molecular weight fraction was defined as branched material, probably fragmented amylopectin. An uncertainty exists as to whether soluble starch analyzed in this study may have an intermediate fraction with molecular weight between the first and second peak material. If it existed, most likely it eluted and

was counted with the second peak.

All GPC profiles (Figures 3.1 - 3.6) had a dominant first peak for the CHO content, which suggested higher amount of branched fraction in each soluble starch on each storage day than linear fraction. On day 0, all blends had higher BV (relative to the CHO peak) for the second peak than on following days. For many soluble starches, the second peak was very small as storage time progressed, and any attempt to make conclusions based on visual evaluation of these peaks would be unsuccessful. Therefore, for each peak a BV/CHO ratio was calculated and it was used as an indication of chain length in the peak. Also, the ratio of peak CHO and total CHO was calculated to determine the amount of branched and linear fractions in soluble starch after each day of storage. This parameter was interpreted with caution because it shows the relative amounts of branched and linear fractions in soluble starch on a given storage day, and not their absolute amounts compared to the initial composition of soluble starch on day 0. This ratio is related to the composition of soluble starch on a previous day, and reflects the relative amounts of two components left in soluble starch after previous day. Therefore, the proportion of one fraction compared to the other can go up or down during storage, depending on the dynamics at which the starch molecules in fractions reassociate between two storage days. The BV/CHO values and the proportion of branched and linear fraction in total CHO (Peak CHO/Total CHO) should be interpreted simultaneously since they are equally important for the retrogradation of starch.

Properties of Soluble Starch from Starch Blends and Starch/Gluten Blends

The BV/CHO values for branched and linear fractions are presented in Tables 3.1 and 3.2, and the proportions of branched and linear fraction in total CHO (Peak CHO/Total CHO) are presented in Tables 3.3 and 3.4. On day 0, the proportion of branched and linear fraction in the soluble starch was similar between blends with and without gluten, with the exception of 12.5 wx and 12.5 wxg blends (Tables 3.3 and 3.4). This result shows that gluten did not affect the initial composition of soluble starch in blends containing 0 and 25% waxy starch; the reason for the difference in 12.5 wxg blend is not known. The proportion of branched fraction in soluble starch was significantly higher and the proportion of linear fraction significantly lower on day 5 than on day 0 (Tables 3.3 and 3.4). As storage progressed from day 0 to day 5, linear molecules started to reassociate and to become unavailable for extraction with water. Hence, the proportion of linear fraction in the soluble starch decreased compared to day 0, and consequently the proportion of branched fraction increased in the soluble starch (Tables 3.3 and 3.4). This is in agreement with the generally accepted model of retrogradation according to which linear molecules of amylose reassociate rapidly at the beginning of storage (Miles et al. 1985).

The proportion of branched fraction in 0 wx soluble starch decreased between each storage period after day 5 (Table 3.3), which was an indication of reassociation of branched molecules. The low proportion of branched fraction in the soluble starch on a particular storage day can be interpreted as the result of intensive reassociation of branched molecules between two storage days, which

Table 3.1. BV/CHO Ratio of Branched GPC Fraction as Affected by Interaction of Blends and Storage

	Days				
Blends	**0**	**5**	**10**	**15**	**20**
0 wx	0.174	0.176	0.172	0.144	0.144
12.5 wx	0.122	0.120	0.117	0.122	0.120
25 wx	0.130	0.118	0.100	0.116	0.117
0 wxg	0.203	0.148	0.154	0.138	0.124
12.5 wxg	0.194	0.122	0.130	0.106	0.100
25 wxg	0.140	0.160	0.114	0.121	0.123
LSD (0.05)	---------- 0.025 ----------				

Table 3.2. BV/CHO Ratio of Linear GPC Fraction as Affected by Interaction of Blends and Storage Days

	Days				
Blends	**0**	**5**	**10**	**15**	**20**
0 wx	0.587	1.212	0.618	0.584	0.365
12.5 wx	0.594	0.386	0.369	0.290	0.279
25 wx	1.243	0.215	0.497	0.300	0.272
0 wxg	1.006	0.372	0.568	0.469	0.228
12.5 wxg	1.306	0.379	0.398	0.276	0.197
25 wxg	0.718	0.436	0.234	0.358	0.322
LSD (0.05)	---------- 0.249 ----------				

Table 3.3. Proportion of Branched GPC Fraction in Total CHO as Affected by Interaction of Blends and Storage Days

	Days				
Blends	**0**	**5**	**10**	**15**	**20**
0 wx	64.28	79.04	67.52	59.61	50.82
12.5 wx	77.88	75.38	86.15	82.94	84.10
25 wx	61.68	87.54	93.04	89.55	87.94
0 wxg	57.64	79.80	85.58	83.92	81.06
12.5 wxg	63.90	83.16	84.13	86.78	80.70
25 wxg	62.55	82.04	89.24	91.57	88.56
LSD (0.05)	---------- 9.17 ----------				

Table 3.4. Proportion of Linear GPC Fraction in Total CHO as Affected by Interaction of Blends and Storage Days

	Days				
Blends	**0**	**5**	**10**	**15**	**20**
0 wx	35.72	20.96	32.46	40.39	49.10
12.5 wx	22.12	24.62	13.85	17.06	15.90
25 wx	38.32	12.46	6.96	10.45	12.19
0 wxg	42.36	20.20	14.42	16.08	18.94
12.5 wxg	36.10	16.84	15.87	13.21	19.31
25 wxg	37.37	17.96	10.76	8.43	11.44
LSD (0.05)	---------- 9.18 ----------				

leads to their insolubility and eventually their lower proportion in soluble starch compared to the previous day. Based on the composition of soluble starch, a retrogradation pattern of 0 wx blend can be hypothesized. On day 0, retrogradation was not intensive and the composition of soluble starch probably reflected the composition of the whole gelatinized system. The amount of amylose in the soluble starch was even higher than in raw starch (Table 2.1, Chapter 2), which could mean that some branch chains of amylopectin broke-off during cooking and became part of the linear fraction. After intensive reassociation of linear molecules between days 0 and 5, retrogradation of branched molecules became intensified as shown by the lower proportion of branched fraction in the soluble starch on day 10 than on day 5 (Table 3.3). The branched fraction continued to decrease from day 10 through day 20, which was an indication of amylopectin or amylopectin fragments retrograding in later stages of storage. However, the proportion of branched fraction in the soluble starch was not significantly different between each consecutive day after 10 days of storage, which means that the rate of retrogradation leveled off. These results were in agreement with the results for the retrogradation enthalpy normalized to amylopectin content (Chapter 2) that also showed intensive retrogradation between 5 and 10 days of storage. While enthalpy provides information about the degree of retrogradation, it does not provide sufficient information about the changes on molecular level of starch during storage.

During the first five days of storage, the long linear chains appeared to have participated in reassociation less than on the following days. The BV/CHO value

for the linear fraction was the highest on day 5 (Table 3.2), which means that the long chains remained in the soluble starch after 5 days of storage. High ratio of BV and CHO for a GPC fraction (i.e. area measured under the whole GPC peak) is the indication of the presence of long chains that have a strong ability to complex with iodine. Instead of reporting just the BV values, the BV/CHO ratio usually is reported to exclude the possible effect of concentration differences on the BV (Kasemsuwan et al. 1995; Klucinec and Thompson 1998; Yoo and Jane 2002; Tziotis et al. 2004). Significant decrease in the BV/CHO for linear fraction occurred between days 5 and 10. This was the result of intensive retrogradation of long linear starch chains, and consequently their amount in the soluble starch decreased (Table 3.2). The BV/CHO for linear fraction gradually decreased thereafter, but with no significant differences between two consecutive storage days. Apparently, by the end of the storage period, 0 wx soluble starch contained on average shorter linear chains than on day 5.

Changes in branched fraction occurred differently than in linear fraction. For the first 10 days of storage, the BV/CHO for branched fraction did not change (Table 3.1), although a significant retrogradation of branched fraction started on day 5. Longer chains started re-associating more intensively between 10 and 15 days of storage, as shown by significantly lower BV/CHO value on day 15 than on day 10 (Table 3.1).

Evidently, linear molecules of 0 wx soluble starch (amylose and maybe some broken linear chains of amylopectin) incorporated first into gel, followed by branched (amylopectin) molecules on day 5. This was shown by the proportion of

branched and linear fraction in soluble starch between days 0 and 5 (Tables 3.3 and 3.4). Most likely, short or intermediate length chains both of linear and branched fraction reassociated first between days 0 and 5, while long chains started retrograding later, after day 5. The increase in retrogradation enthalpy of 0 wx sample during storage (Table 2.8, Chapter 2) most likely was the combination of two effects: retrogradation of branched molecules in later stages of storage, and also reassociation of branched molecules with long chains, as shown by BV/CHO values (Table 3.1). Thermally reversible crystallinity of starch that produces the enthalpy of retrogradation is known to originate from recrystallized amylopectin (Miles et al. 1985). Amylopectin with large proportion of long side chains can develop retrogradation enthalpy faster than amylopectin with more short chains (Shi and Seib 1992; Yuan and Thompson 1998; Kohyama et al. 2004).

Retrogradation of non-waxy starch was significantly affected by gluten. Significant difference in retrogradation between 0 wx and 0 wxg started between days 5 and 10; the percent of branched fraction in the soluble starch decreased for the 0 wx blend, while in 0 wxg it increased and remained unchanged thereafter (Table 3.3). Apparently, gluten interfered with the retrogradation of branched molecules in 0 wxg soluble starch, and therefore their proportion in the soluble starch relative to the linear fraction increased compared to 0 wx starch. Based on the ratio of branched and linear fraction in soluble starch, the 0 wxg starch could be expected to develop lower retrogradation enthalpy than the 0 wx starch since the 0 wxg had much higher proportion of branched molecules remaining in soluble starch, especially during the later stages of storage (Table 3.3). However, the

enthalpy of retrogradation of both 0 wx and 0 wxg blend was similar during later stages of storage (day 15 and 20) (Chapter 2, Table 2.8). A possible explanation for this phenomenon was found in the chain length of the branched fraction. Values for λ_{max} decreased significantly between days 0 and 5 for all starch/gluten blends (Table 3.5). These results were in agreement with the results for BV/CHO value for branched fraction although the BV/CHO did not show significant difference for 25 wxg (Table 3.1). The λ_{max} values for starch/gluten blends were similar to those of starch blends on day 0. The significant drop in λ_{max} values between days 0 and 5 indicated more intensive reassociation of long chain branched molecules in starch/gluten blends than in starch blends. These reassociated starch chains most likely were able to develop crystallites during storage, and eventually resulted in retrogradation enthalpy comparable to that of starch blends. This was particularly true for the 0 wxg blends that had the highest drop in λ_{max} between days 0 and 5.

Retrogradation of starch blends that contained 12.5 and 25% waxy starch followed a different pattern than the retrogradation of 0 wx starch, as shown by the BV/CHO values (Tables 3.1 and 3.2) and by the ratio of branched and linear fraction in the soluble starch (Tables 3.3 and 3.4). The main difference compared to 0 wx was in the ratio of branched and linear fraction during storage. After day 5, the proportion of branched fraction in 12.5 wx and 25 wx soluble starch increased or remained unchanged between each storage day, while in 0 wx it decreased (Table 3.3). Also, the relative amount of branched fraction in the soluble starch was significantly higher in 12.5 wx and 25 wx samples than in 0 wx on days 10, 15,

Table 3.5. λ_{max} (nm) of Branched GPC Fraction as Affected by Interaction of Blends and Storage

	Days				
Blends	**0**	**5**	**10**	**15**	**20**
0 wx	541	539	538	535	530
12.5 wx	531	530	529	530	533
25 wx	530	530	525	528	530
0 wxg	542	530	532	531	528
12.5 wxg	538	528	529	531	524
25 wxg	532	526	529	527	528
LSD (0.05)	---------- 6 ----------				

and 20 (Table 3.3). This result indicates that the reassociation of branched molecules in later stages of storage probably was more intensive in 0 wx than in 12.5 wx and 25 wx blends. With all three starch blends, the proportion of branched fraction in soluble starch did not differ between days 10 and 15 and between days 15 and 20, which was similar behavior observed for the enthalpy of retrogradation (Chapter 2, Table 2.8).

A significant change in the proportion of branched and linear fraction occurred between days 5 and 10 for the 12.5 wx blend, and between days 0 and 5 for the 25 wx blend. The ratio of branched and linear fraction at these storage times increased in favor of the branched fraction. The opposite was true for the 0 wx soluble starch between days 5 and 10. The simple explanation of this

phenomenon could be the lower amylose, i.e. higher amylopectin content of 12.5 wx and 25 wx blends than the 0 wx blend, which resulted in higher content of branched fraction in the soluble starch. However, this would be a misleading conclusion since no difference was found between 0 wx and 25 wx soluble starch in the initial (day 0) composition of the soluble starch. The high proportion of branched fraction in the soluble starch in later stages of storage could be an indication of slow retrogradation of branched fraction, which would eventually result in low enthalpy of retrogradation. However, the results for retrogradation enthalpy (Chapter 2, Table 2.8) showed that 0 wx, 12.5 wx, and 25 wx starch blends had similar ΔH_{BR}, which lead to a conclusion that factors other than the difference in amylose content also may have a role in starch retrogradation, especially in retrogradation of starch blends.

Starch/gluten blends that contained 12.5 and 25% waxy starch behaved similarly to 0 wxg blend during storage, and their ratios of branched and linear fractions were similar to those of their corresponding starch blends on each storage day (Table 3.3 and 3.4). In later stages of storage (days 15 and 20), no major differences were found between 12.5 wxg and 25 wxg blends with regard to their branched BV/CHO value or branched λ_{max} value. Also, there were no major differences in the same parameters between 12.5 wxg and 12.5 wx blends, as well as between 25 wxg and 25 wx blends on days 15 and 20.

The λ_{max} values for linear fraction were lower for starch/gluten blends than corresponding starch blends on many storage days, although the initial λ_{max} on day 0 was higher for blends with gluten than for blends without gluten (Table 3.6).

Table 3.6. λ_{max} (nm) of Linear GPC Fraction as Affected by Interaction of Blends and Storage Days

	Days				
Blends	**0**	**5**	**10**	**15**	**20**
0 wx	613	621	606	599	600
12.5 wx	600	572	570	576	582
25 wx	588	584	583	576	570
0 wxg	626	576	589	586	566
12.5 wxg	626	574	558	554	555
25 wxg	603	561	561	561	545
LSD (0.05)	---------- 16 ----------				

Therefore, gluten appeared to interfere less with retrogradation of linear than branched fraction. While the mechanism is uncertain, gluten appeared to promote reassociation of linear starch chains. λ_{max} values showed this behavior more clearly than the BV/CHO values.

Two main factors were considered in this study in order to explain the retrogradation pattern of blends: the difference in average chain length of two fractions measured by BV/CHO and λ_{max} values, and interactions between two starches in the blend. The BV/CHO value of branched fraction for 12.5 wx and 25 wx soluble starches was lower than BV/CHO for 0 wx (Table 3.1), indicating that the soluble starch from two starch blends had lower average chain length of branched fraction than a single non-waxy starch. Both 12.5 wx and 25 wx soluble

starches had similar BV/CHO values for each storage period (Table 3.1). When the maximum wavelength of starch iodine binding (λ_{max}) was analyzed (Table 3.5), it also showed similar low values for the branched fraction of 12.5 wx and 25 wx soluble starch, while values of λ_{max} for 0 wx were somewhat higher, with the exception of day 20.

Maximum wavelength of starch iodine binding (λ_{max}) is defined as the peak absorbance of starch-iodine complex over the wavelengths range (in this case 400-800 nm), and it is directly related to the length of linear starch chains (Fales 1980). In native wheat starch, both waxy and non-waxy, amylose has higher λ_{max} than amylopectin due to longer iodine-complexible chains of amylose than amylopectin (Van Hung et al. 2007). High λ_{max} values also were found to be related to starch fractions that contained high percent of long chains in the studies of Klucinec and Thompson (1998) and Tziotis et al. (2004) for corn starch and in the study of Shibanuma et al. (1996) for wheat starch. The λ_{max} values for the branched fraction of 12.5 wx and 25 wx (Table 3.5) were very close to the λ_{max} value for the waxy starch amylopectin (530 nm) (Chapter 2). The λ_{max} for the branched fraction of 0 wx soluble starch was slightly higher than the λ_{max} for the 12.5 wx and 25 wx branched fractions, but lower than the λ_{max} of non-waxy starch (567 nm) (Chapter 2), which means that some amylopectin chains broke-off during pasting of starch, and therefore the average λ_{max} was reduced.

The λ_{max} of the linear fraction was on average lower for 12.5 wx and 25 wx soluble starches than for 0 wx soluble starch on most storage days (Table 3.6).The λ_{max} of amylose from waxy durum wheat starch was 640 nm (data not shown in

Table 2.2, Chapter 2) and for non-waxy wheat starch it was 642 nm (Chapter 2). Therefore, the λ_{max} for the linear fraction of all analyzed soluble starches would be expected to be similar, but that was not the case. This finding was in agreement with the BV/CHO values (Table 3.2). Only on day 0, the BV/CHO values for the linear fraction of 12.5 wx and 25x were similar or higher than that of 0 wx, and they were lower for subsequent storage days. The same behavior was noticed for the 25 wxg blend. The λ_{max} for the linear fraction of 25 wxg soluble starch was significantly lower during storage (including day 0) than the λ_{max} of 0 wxg soluble starch. A possible explanation for this behavior could be that some of the small amylopectin fragments, most likely originating from waxy starch, co-eluted with the linear fraction, and the low BV/CHO and λ_{max} values of linear fraction may be the result of "contamination" of the linear fraction with small amylopectin fragments. Waxy amylopectin fragments can be expected to have even lower λ_{max} than the native waxy amylopectin, and therefore they reduced the average iodine binding ability of the linear fraction in which they had eluted. Presence of amylopectin fragments in amylose fraction of soluble starch also was reported by Miklus and Hamaker (2003) for corn masa. The authors reported intermediate λ_{max} values for the soluble amylose fraction, which was lower than the λ_{max} of native amylose and higher than the λ_{max} of native amylopectin. Aboubacar and Hamaker (2000) studied the soluble starch from couscous, and also found fragmented amylopectin in the fraction that contained long linear chains. Desse et al. (2009) found low molecular weight amylopectin chains in the soluble starch of modified waxy corn starch, and hypothesized that that these amylopectin molecules were able to leach

from the granule because of their small size.

Waxy starch granules are more susceptible to swelling than non-waxy granules (as discussed in Chapters 1 and 2) and therefore their amylopectin could be more susceptible to fragmentation than the amylopectin of the non-waxy starch granules. Reassociation of starch molecules, their recrystallization and development of retrogradation enthalpy in a described system could have occurred by different mechanism than in the system made of a single starch. Development of retrogradation enthalpy in 12.5 wx and 25 wx gels similar to the enthalpy of 0 wx gels could be attributed to possible crystallization of amylopectin that eluted with the linear fraction, in addition to the crystallization of amylopectin that eluted as branched fraction. While the reassociation and crystallization of amylopectin molecules containing short chains is slow (Shi and Seib 1992; Yuan et al. 1993; Klucinec and Thompson 1999), it cannot be neglected. Presence of linear amylose chains in the same fraction could have promoted reassociation of amylopectin fragments due to the effect of phase separation (Kalichevsky and Ring 1987; Klucinec and Thompson 2002). Although the recrystallization of these amylopectin chains could be slow, by the end of the storage period, it was able to develop the same retrogradation enthalpy as found for the retrograded starch in 0 wx gels (Chapter 2, Table 2.8).

Another observation regarding the retrogradation enthalpy was the significantly lower BV/CHO ratio for 25 wx branched fraction than for 0 wx branched fraction on day 20 (Table 3.1), yet the retrogradation enthalpies for both blends were similar (Table 2.6, Chapter 2). The hypothesis is that in 25 wx more of

the long chains of soluble amylopectin reassociated during storage and became unavailable for the extraction, leaving amylopectin with short chains in the soluble starch. Therefore, even though the proportion of branched fraction in soluble starch was high (Table 3.3), recrystallization of long chains of amylopectin resulted in retrogradation enthalpy comparable to that of 0 wx starch. While the mechanism that governs the specific retrogradation pattern of starch blend is not clear, the analysis of GPC fractions of soluble starch showed that starch blends composed of waxy and non-waxy starch had different retrogradation pattern than a single starch even when the retrogradation enthalpy was not significantly different from that of a single non-waxy starch (0 wx).

In Chapter 2, a hypothesis was made, based on the results for retrogradation enthalpy that gluten could interfere more with retrogradation of branched molecules (amylopectin) than with retrogradation of long linear molecules of amylose, especially in later stages of storage of low amylose blends. This is probably more applicable to blends with lower amylose contents than in blends containing 12.5% and 25% waxy starch. The proportion of branched and linear molecules in soluble starch was similar between starch/gluten blends and corresponding starch blends with 12.5 and 25% waxy starch. Also, the λ_{max} of 12.5 wxg and 25 wxg was similar to the λ_{max} of corresponding starch blends in later stages of storage.

CONCLUSIONS

In Chapter 2, a hypothesis was made that starch blends retrograded differently than single starches and that, besides the amylose content, the retrogradation may be impacted by specific interactions between two starches. Multiple authors have presented different hypotheses on interaction between two starches during retrogradation; however, in most cases, hypotheses are uncertain since the mechanism of retrogradation proved to be challenging for any analytical procedure. The results of analysis of GPC fractions of soluble starch provided proof that blends of two starches and also blends of starches with gluten exhibited different retrogradation pattern than a single starch. The development of retrogradation enthalpy was not impacted solely by amylose content, but also by the chain length of linear and branched starch molecules present in the soluble starch that reassociate and recrystallize, and by the composition of soluble starch fractions.

The retrogradation pattern of single starch and starch blends was especially different, which was visible through the changes in BV/CHO ratio and λ_{max} of different blends during storage. It is uncertain why different chain lengths of both linear and branched fraction retrograded with different dynamics in different blends, but the results show that both the composition of each blend and the chain length of branched and linear fractions determined the retrogradation pattern.

Most likely, the observed differences also were due to the interaction of two starches and the interaction of starches with gluten could have imparted unique behavior to each blend. These findings are important because they could help to

understand why sometimes functional properties of blends do not change in a linear fashion with the amylose content. Gluten did not change significantly the retrogradation enthalpy at the levels of waxy starch included in this study. However, gluten affected the retrogradation pattern of starch. In the presence of gluten, the retrogradation pattern of starch blends appears to be different than the retrogradation pattern of single starches.

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OVERALL CONCLUSIONS

The overall objective of this research project was to investigate the effects of amylose content and gluten on retrogradation of starch. Understanding the factors that affect the process of starch retrogradation has been an area of research interest since it is necessary for finding ways to retard retrogradation and staling in commercial baking. The first chapter studied the potential of practical application of waxy wheat flour, i.e. wheat flour with no or very low amylose content, in blend with normal flour in retardation of bread staling. The second chapter used gel model systems to study the fundamental effects of different amylose contents and gluten on pasting, gelatinization, and retrogradation properties of starch blends. In the third chapter, the possible mechanism of retrogradation of starch blends with different amylose and gluten content was investigated in order to better understand the phenomena observed in the first two chapters.

Overall, lowering the amylose content of flour by blending waxy flour, either waxy durum (WD) or waxy spring (WS), with non-waxy wheat flour did not improve softness of bread and did not retard staling of bread that was stored for 5 days. Some positive impact of waxy flour on the reduction of crumb firmness and retrogradation enthalpy was observed in early stages of storage, i.e. up to 1 and in some cases up to 3 days of storage. The 30% and 40% WS and WD crumbs had lower retrogradation enthalpy at this stage than the non-waxy Gunner crumb, but their enthalpy was significantly higher than that of Gunner crumb at the end of the storage period (day 5). The assumption was that amylopectin recrystallization took

place by the end of the storage time, resulting in high retrogradation enthalpy of waxy crumbs. The onset of amylopectin recrystallization most likely depends on multiple factors such as amylopectin molecular structure, moisture content of bread, presence of other ingredients in bread formula, and interaction between starch and other ingredients.

Waxy crumbs, especially WD crumb, had inferior quality compared to non-waxy crumb, with very open crumb grain that was prone to collapsing and shrinking upon cooling. Therefore, waxy flour may not be suitable for the production of regular panned bread, but could have potential in bakery products that require open crumb structure and that are consumed fresh. Differences in pasting and gelatinization properties, retrogradation enthalpy of starch in crumb, pasting properties of crumb, amount of soluble starch in crumb, and firming rate of crumb between WS and WD blends with similar amylose contents could be attributed to structural differences between WS and WD starches. High retrogradation enthalpy and crumb firmness of waxy crumbs in spite of their high soluble starch content indicated that recrystallization of starch in blends of two flours is a complex process that most likely is the results of interactions between two starches.

Study of model systems (starch blends and starch/gluten blends) enabled the determination of the effect of amylose and gluten content on functional properties of starch, without interference of other ingredients present in bread. Although amylose contents of starch blends were different, their gelatinization enthalpies were not different between each consecutive blend. Gelatinization

properties of starch blends were complex and not a simple mathematical average of properties of two starches in the blend, but the result of interactions between two starches. Gluten did not have any effect on gelatinization of starches with different amylose contents; the absence of effect can be attributed to the excess water in the system.

Retrogradation enthalpy increased during storage for all analyzed blends, which showed that starch recrystallization progressed during the whole storage regardless of the amylose content. However, retrogradation enthalpy was not significantly different between each consecutive blend, and it was not a simple sum of contributions of individual components, indicating that besides amylose content other factors could have affected the process of retrogradation. Significantly lower enthalpy of retrogradation (normalized to amylopectin content) than that of non-waxy starch started at 50% waxy starch level in blend, indicating that low amylose contents i.e. high amylopectin content of starch slowed the process of retrogradation. Blends with lower waxy starch levels had enthalpy similar to that of non-waxy starch. This may be the reason for the similar retrogradation enthalpies observed in bread crumb at the end of storage, since the highest level of waxy flour was 40%. Gluten reduced the retrogradation enthalpy of starch blends, especially the enthalpy of starch blends with low amylose contents (50 wxg, 75 wxg, and 100 wxg). In late stages of storage, gluten appeared to interact more intensively with branched than with linear molecules either by reducing the contact between amylopectin chains or by diluting the starch to such an extent that prevents branched molecules to be in close enough proximity for

helix formation. At the end of the storage (day 20), the enthalpy was significantly different between each consecutive starch/gluten blend (not the case with starch blends), which was an indication of interaction of gluten and starch blends by the end of the storage.

Results in Chapter 1 showed that high soluble starch content in bread was related to low amylose content of flour; however, the retrogradation enthalpy of crumbs with low amylose content was also high, which was contrary to what was expected. Gel model systems, i.e. Chapter 2, showed that the retrogradation enthalpy of starch blends did depend on amylose content, but it did not always change linearly with amylose content. These observations lead to a conclusion that other factors may influence starch retrogradation, in addition to amylose content.

Analysis of the GPC fractions of soluble starch, isolated from gels, confirmed that the retrogradation pattern of a single starch (0 wx) and starch blends (12.5 wx and 25 wx) was different. Development of retrogradation enthalpy in a gel made of a single non-waxy starch (0 wx) could be attributed to retrogradation of branched starch molecules (originating from amylopectin) in later stages of storage, and also to reassociation of branched molecules with long branch chains. In gels made of starch blends (12.5 wx and 25 wx), retrogradation appeared to follow a different mechanism, but with the same resulting retrogradation enthalpy.

While soluble starch had high proportions of branched fraction, the λ_{max} and BV/CHO values indicated that retrogradation could have been a result of reassociation of amylopectin that eluted with linear fraction, in addition to

retrogradation of amylopectin that eluted in branched fraction. In the case of 25 wx blend, reassociation of higher proportion of long branch chains of soluble amylopectin could be an additional mechanism that contributed to retrogradation.

Gluten appeared to cause an early onset of amylopectin retrogradation. The significant drop in λ_{max} values between days 0 and 5 indicated more intensive reassociation of long chain branched molecules in starch/gluten blends than in starch blends. These reassociated starch chains most likely were able to develop crystallites during storage, and eventually resulted in retrogradation enthalpy comparable to that of starch blends. This effect was particularly applicable to 0 wxg and 12.5 wx blends, while the retrogradation mechanism of the 25 wxg blend could be attributed in part to the same effect as in the 25 wx blend.

The importance of this research for real food systems such as bread is that it shows that gelatinization and retrogradation of starch does not change necessarily in a linear fashion with the change of amylose content. Combination of low amylose (waxy) flour and regular flour results in starch blends that can have unique retrogradation patterns. Retrogradation patterns of starch blends can be affected not only by amylose content but also by the molecular composition of soluble starch (as a result of gelatinization) and possible interactions between molecules of two starches. In addition, retrogradation properties of starch (flour) blends, and consequently staling of bread, will always be affected also by gluten content.

COMMERCIAL APPLICATION AND FUTURE RESEARCH

Commercial Application

Based on the results of this study, following potential commercial applications of waxy wheat flour, as well as commercial applications of wheat starch blends are proposed:

- Waxy flour could be used at levels up to 20% in pan bread, provided that the bread is well formulated with of enzymes and conditioners that are traditionally used in commercial bread formulations.
- Best application of waxy wheat flour could be in bakery products that require open, porous structure and that are consumed fresh, like puff pastry or different types of 'artisan bread'. Many of these products (especially 'artisan bread' products) often do not allow use of traditional ingredients that retard staling. This research showed that waxy flours retarded staling for one to three days. Therefore, waxy flour could be used to prolong freshness of bakery products which are discarded usually the day after they are baked, provided that these products allow for more open crumb structure than in pan bread.
- The results of this research could be useful for baking industry where currently a considerable number and variety of ingredients are used to retard staling. A potential of flour components, starch and gluten in right ratio, being able to delay the retrogradation process would be beneficial for baking industry.
- Application of starch blends could be useful in different food products to obtain certain product texture, cooking properties, gelling properties, etc, since starch blends often behave like a new starch with unique properties.

Future Research

Based on the results obtained in this study, following future research is proposed:

- The efficiency of waxy flour in retarding staling at levels lower than 20% could be tested in a baking study. The rationale for this is that waxy flour at 20% level did not have the detrimental effect on bread crumb like at 30% and 40% levels.
- Research with different amounts of gluten would be useful to determine how retrogradation properties of starch are affected by the levels of gluten that are used commercially.
- Further investigation could include ingredients that are used commercially to improve gluten properties, such as oxidizers and enzymes that act upon gluten, to see how these ingredients may affect gluten's functionality in starch retrogradation.
- The study could be repeated with starch/gluten model systems in limited water conditions, which is a case in bread. A study designed with conditions similar to those in bread could provide further insight into role of interactions on starch properties in bread. Also, preparation of gels should be done without any mixing to avoid possible depolymerization of starch molecules due to shear. These conditions would be more similar to conditions in real dough and bread since starch in dough gelatinizes during baking, which does not involve mixing.
- Future research can be conducted using high-performance anion exchange chromatography with pulsed amperometric detector (HPAEC-PAD) to determine the chain length of branched fraction in soluble starch. This analysis

could provide further insight into molecular changes of starch during retrogradation. Also, analysis of the linear fraction before and after debranching would show whether some amylopectin fragments eluted in this fraction.

- Study of small-scale (i.e. nano-scale) rheological properties of gels with atomic force microscopy could provide a thorough insight into possible interactions between starch molecules or starch and gluten molecules.

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APPENDIX

Table A-1. Analysis of Variance for RVA Hot Paste Viscosity, Setback, Cold Paste Viscosity, and Enthalpy of Retrogradation of Bread Crumbs

	Source	DF[a]	Mean Square	F Value
Hot paste viscosity	Rep	1	59.44	2.99
	Blends	9	3528.80	177.51**
	Days	3	143.31	7.21**
	Blends*Days	27	102.21	5.14**
	Error	39	19.88	
Setback	Rep	1	5.22	0.33
	Blends	9	41.10	2.61*
	Days	3	384.11	24.35**
	Blends*Days	27	33.34	2.11*
	Error	39	15.77	
Cold paste viscosity	Rep	1	99.88	2.70
	Blends	9	3265.38	88.35**
	Days	3	530.36	14.35**
	Blends*Days	27	78.15	2.11*
	Error	39	36.96	
Enthalpy of retrogradation	Rep	1	0.065	2.19
	Blends	9	0.14	4.60**
	Days	3	19.32	654.88**
	Blends*Days	27	0.19	6.43**
	Error	39	0.03	

*Significant and $P \leq 0.05$
**Significant and $P \leq 0.01$
[a]DF = degrees of freedom

Table A-2. Analysis of Variance for Crumb Soluble Starch Content, Crumb Moisture, Water Activity of Crumb, and Crumb Firmness

	Source	DF[a]	Mean Square	F Value
Soluble starch	Rep	1	1.34	3.71
	Blends	9	83.63	230.83**
	Days	3	257.34	710.28**
	Blends*Days	27	21.92	60.50**
	Error	39	0.36	
Crumb moisture	Rep	1	0.27	0.28
	Blends	9	5.69	5.85**
	Days	3	204.37	209.78**
	Blends*Days	27	1.83	1.88*
	Error	39	0.97	
Water activity	Rep	1	0.0000021	0.14
	Blends	9	0.0000383	2.48*
	Days	3	0.0000578	3.74*
	Blends*Days	27	0.0000256	1.66
	Error	39	0.0000154	
Crumb firmness	Rep	1	137.31	0.69
	Blends	9	1717.81	8.59**
	Days	3	308187.34	1540.94**
	Blends*Days	27	1254.45	6.27**
	Error	39	200.00	
Percent firming of crumb	Rep	1	126.15	0.28
	Blends	9	2616.45	5.81**
	Days	2	122127.22	271.20**
	Blends*Days	18	3956.48	8.79**
	Error	29	200.00	

*Significant and P ≤0.05
**Significant and P ≤0.01
[a]DF = degrees of freedom

Table A-3. Analysis of Variance for RVA Properties of Starch Blends and Starch/Gluten Blends I

	Source	DF[a]	Mean Square	F Value
Peak viscosity	Rep	2	1.44	0.52
	Starch	5	5684.53	2061.03**
	Gluten	1	71574.08	25950.40**
	Starch*Gluten	5	102.21	218.54**
	Error	22	2.7581	
Hot paste viscosity	Rep	2	6.60	1.29
	Starch	5	1098.85	214.39**
	Gluten	1	72765.06	14196.60**
	Starch*Gluten	5	2472.35	482.75**
	Error	22	5.12	
Breakdown	Rep	2	4.82	1.39
	Starch	5	10140.94	2933.50**
	Gluten	1	4.99	1.44
	Starch*Gluten	5	2870.93	830.48**
	Error	22	3.46	
Cold paste viscosity	Rep	2	5.98	4.23
	Starch	5	5317.82	3758.04**
	Gluten	1	94054.67	66467.40**
	Starch*Gluten	5	4119.84	2911.44**
	Error	22	1.42	
Setback	Rep	2	4.54	0.92
	Starch	5	1877.92	382.33**
	Gluten	1	1362.84	277.47**
	Starch*Gluten	5	573.64	116.79**
	Error	22	4.91	

Table A-4. Analysis of Variance for RVA Properties of Starch Blends and Starch/Gluten Blends II

	Source	DF[a]	Mean Square	F Value
Peak time	Rep	2	0.03	0.84
	Starch	5	10.09	334.94**
	Gluten	1	2.78	92.20**
	Starch*Gluten	5	1.03	34.32**
	Error	22	0.03	
Peak temperature	Rep	2	0.04	0.62
	Starch	5	324.22	5461.21**
	Gluten	1	9.92	167.13**
	Starch*Gluten	5	2.27	38.28**
	Error	66	0.06	

**Significant at P<0.01
[a]DF = degrees of freedom

Table A-5. Analysis of Variance for DSC Thermal Properties of Starch Blends and Starch/Gluten Blends I

	Source	DF[a]	Mean Square	F Value
ΔH	Rep	2	0.34	0.46
	Starch	5	56.30	76.60**
	Gluten	1	392.26	533.65**
	Starch*Gluten	5	5.42	7.36**
	Error	66	0.74	
ΔH_{calc1}	Rep	2	0.47	0.51
	Starch	5	74.96	81.00**
	Gluten	1	0.13	0.14^{NS}
	Starch*Gluten	5	2.26	2.45*
	Error	66	0.93	
ΔH_{calc2}	Rep	2	0.24	0.52
	Starch	5	36.73	80.89**
	Gluten	1	0.06	0.14
	Starch*Gluten	5	1.10	2.43*
	Error	66	0.45	
T_o	Rep	2	0.35	1.34
	Starch	5	7.22	27.61**
	Gluten	1	15.39	58.78**
	Starch*Gluten	5	1.07	4.09**
	Error	66	0.26	

ΔH = Measured enthalpy of gelatinization
ΔH_{calc1} = Enthalpy of gelatinization converted to that of starch in blends. For starch/gluten blends it was calculated as $\Delta H_{calc1} = \Delta H / 0.7$
ΔH_{calc2} = Enthalpy of gelatinization based on % of starch in starch/gluten blends ($\Delta H_{calc2} = \Delta H \times 0.7$)
T_o = Onset temperature of gelatinization

Table A-6. Analysis of Variance for DSC Thermal Properties Starch Blends and Starch/Gluten Blends II

	Source	DF[a]	Mean Square	F Value
T_p	Rep	2	0.38	0.92
	Starch	5	12.32	29.65**
	Gluten	1	1.33	3.21
	Starch*Gluten	5	0.50	1.21
	Error	66	0.42	
T_c	Rep	2	0.49	0.40
	Starch	5	14.67	11.83**
	Gluten	1	33.52	27.02**
	Starch*Gluten	5	4.68	3.77**
	Error	66	1.24	
T_c - T_o	Rep	2	1.10	0.52
	Starch	5	14.60	6.93**
	Gluten	1	94.25	44.72**
	Starch*Gluten	5	9.54	4.50**
	Error	66	2.11	

T_p = Peak temperature of gelatinization
T_c = Completion temperature of gelatinization
T_c - T_o = Gelatinization temperature range
*Significant at P<0.05
**Significant at P<0.01
[a]DF = degrees of freedom

Table A-7. Correlation Coefficients for Amylose Content, RVA Pasting and DSC Thermal Properties of Starch Blends[a]

	PV	HPV	BKD	CPV	STB	P Time	P temp	AM	ΔH	T_o	T_p
HPV	-0.64**										
BKD	0.90***	-0.89***									
CPV	-0.52*	0.95***	-0.81***								
STB	-0.32	0.74**	-0.58*	0.91***							
P Time	-0.68**	0.99***	-0.92***	0.91***	0.66**						
P Temp	-0.58**	0.97***	-0.86***	0.89***	0.66**	0.97***					
AM	-0.64**	0.97***	-0.89***	0.97***	0.83***	0.95***	0.93**				
ΔH	0.47	-0.91*	0.76	-0.97**	-0.91*	-0.84*	-0.85*	-0.96**			
T_o	0.61	0.01	0.34	0.23	0.49	-0.09	-0.05	0.03	-0.20		
T_p	0.86	-0.76	0.90	-0.62	-0.36	-0.79	-0.77	-0.77	0.66	0.60	
T_c	-0.05	0.49	-0.29	0.39	0.21	0.53	0.44	-0.83*	0.74	0.28	0.81

[a] PV = Peak viscosity; HPV = Hot paste viscosity; BKD = Breakdown; CPV =Cold paste viscosity; STB = Setback; P Time = Peak time; P Temp = Peak temperature; ΔH = Measured enthalpy of gelatinization; T_o = Onset temperature of gelatinization; T_p = Peak temperature of gelatinization; T_c = completion temperature of gelatinization
*Significant at P < 0.05, **Significant at P < 0.01, ***Significant at P < 0.001

Table A-8. Correlation Coefficients for Amylose Content, RVA Pasting and DSC Thermal Properties of Starch/Gluten Blends[a]

	PV	HPV	BKD	CPV	STB	P Time	P temp	AM	ΔH	ΔH_{calc1}	ΔH_{calc2}	T_o	T_p
HPV	0.96***												
BKD	0.99***	0.93***											
CPV	0.69**	-0.57*	-0.72***										
STB	-0.96***	-0.92***	-0.95***	0.84***									
P Time	-0.85***	-0.84***	-0.84***	0.81***	0.93***								
P Temp	-0.84***	-0.81***	-0.84***	0.90***	0.95***	0.90***							
AM	-0.91***	-0.93***	-0.88***	0.74***	0.95***	0.88***	0.94***						
ΔH	0.86*	0.90*	0 .83*	-0.65	-0.90*	-0.83*	-0.89*	-0.98***					
ΔH_{calc1}	0.86*	0.91*	0.83*	-0.65	-0.90*	-0.83*	-0.89*	-0.98***	0.99***				
ΔH_{calc2}	0.85	0.90	0.82*	-0.56	-0.85*	-0.75	-0.84*	-0.96**	0.96**	0.96**			
T_o	0.80	0.72	0.81*	-0.65	-0.77	-0.76	-0.62	-0.60	0.44	0.44	0.47		
T_p	0.98***	0.93**	0.98***	-0.81	-0.98***	-0.93**	-0.89*	-0.91*	0.83*	0.83*	0.81	0.86*	
T_c	0.55	0.62	0.52	-0.55	-0.66	-0.61	-0.80	-0.83*	0.87*	0.87*	0.88*	0.11	0.55

[a] PV = Peak viscosity; HPV = Hot paste viscosity; BKD = Breakdown; CPV =Cold paste viscosity; STB = Setback; P Time = Peak time; P Temp = Peak temperature; ΔH = Measured enthalpy of gelatinization; ΔH_{calc1} = Enthalpy of Gelatinization converted to that of starch in blends (for starch/gluten blends it was calculated as $\Delta H_{calc1} = \Delta H / 0.7$); ΔH_{calc2} = Enthalpy of gelatinization based on % of starch in starch/gluten blends ($\Delta H_{calc2} = \Delta H \times 0.7$)
T_o = Onset temperature of gelatinization; T_p = Peak temperature of gelatinization; T_c = completion temperature of gelatinization
*Significant at $P < 0.05$, **Significant at $P < 0.01$, ***Significant at $P < 0.001$

Table A-9. Analysis of Variance for Retrogradation Properties of Starch Blends and Starch/Gluten Blends

	Source	DF[a]	Mean Square	F Value
ΔH_r	Rep	2	0.002	0.03
	Blends	11	18.56	240.47**
	Days	3	26.28	340.57**
	Blends*Days	33	0.53	6.83**
	Error	94	0.08	
ΔH_{BR}	Rep	2	0.05	0.29
	Blends	11	16.29	100.67**
	Days	3	47.48	293.50**
	Blends*Days	33	0.60	3.70**
	Error	94	0.16	
T_{or}	Rep	2	1.20	4.28*
	Blends	11	13.67	48.85**
	Days	3	24.42	87.28**
	Blends*Days	33	3.36	12.01**
	Error	94	0.28	
T_{pr}	Rep	2	0.86	7.62**
	Blends	11	7.10	63.07**
	Days	3	30.17	267.87**
	Blends*Days	33	1.69	15.0**
	Error	94	0.11	
T_{cr}	Rep	2	0.28	1.85
	Blends	11	1.59	10.63**
	Days	3	23.63	157.54**
	Blends*Days	33	0.67	4.45**
	Error	94	0.15	

ΔH_r = Retrogardation enthalpy; ΔH_{BR} = Retrogradation enthalpy based on branched fraction of starch; T_{or} = Onset temperature of melting retrograded starch; T_{pr} = Peak temperature of melting retrograded starch; T_{cr} = Completion temperature of melting retrograded starch; **Significant at P<0.01; [a]DF = degrees of freedom

Table A-10. Analysis of Variance for the BV/CHO and Peak CHO/Total CHO of Soluble Starch

	Source	DF[a]	Mean Square	F Value
BV/CHO Branched Fraction	Rep	1	0.000058	0.39
	Blends	5	0.003379	22.56**
	Days	4	0.003067	20.48**
	Blends*Days	20	0.000635	4.24**
	Error	29		
BV/CHO Linear Fraction	Rep	1	0.0054	0.37
	Blends	5	0.1034	6.99**
	Days	4	0.7022	47.45**
	Blends*Days	20	0.1088	7.35**
	Error	29	0.0148	
Peak CHO/Total CHO Branched Fraction	Rep	1	25.91	1.29
	Blends	5	521.78	25.95**
	Days	4	741.56	36.88**
	Blends*Days	20	108.11	5.38**
	Error	29		
Peak CHO/Total CHO Linear Fraction	Rep	1	25.45	1.26
	Blends	5	521.01	25.84**
	Days	4	740.58	36.72**
	Blends*Days	20	107.85	5.35**
	Error	29		

BV = Blue value
Peak CHO = Carbohydrate content of a soluble starch fraction
Total CHO = Total carbohydrate content of all soluble starch fractions
**Significant at P<0.01
[a]DF = degrees of freedom

Table A-11. Analysis of Variance for the λ_{max} of Soluble Starch

	Source	DF[a]	Mean Square	F Value
λ_{max} Branched Fraction	Rep	1	52.27	6.43*
	Blends	5	94.99	11.69**
	Days	4	84.57	10.40**
	Blends*Days	20	17.89	2.20*
	Error	29	8.13	
λ_{max} Linear Fraction	Rep	1	3.75	0.06
	Blends	5	2064.18	35.16**
	Days	4	3259.10	55.51**
	Blends*Days	20	298.46	5.08**
	Error	29	58.72	

λ_{max} = Wavelength of maximum iodine absorption
*Significant at P<0.05
**Significant at P<0.01
[a]DF = degrees of freedom