UNDERSTANDING AND IMPROVING FUNCTIONALITY OF WAXY WHEAT FLOURS

by

SHIVANANDA KUMAR GARIMELLA PURNA

B. Tech, Osmania University, 2002
M.S., University Of Minnesota, 2006

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry
College of Agriculture

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Abstract

To realize the full potential of waxy wheat flours in food applications, six advanced hard waxy wheat lines were studied. Pasting properties of waxy wheat flours as well as factors governing the pasting properties were investigated. Waxy wheat starch granules swelled more extensively and were more prone to α-amylase degradation than normal wheat starch. A combination of endogenous α-amylase activity and protein matrix contributed to a large variation of pasting properties of waxy wheat flours. Bi-axial extension properties classified dough from waxy wheat as in-elastic. Waxy wheat flour had higher water absorption and lower mixing time than normal wheat flour. Waxy wheat starch affected protein hydration but not protein extractability after optimum dough mixing. Presence of some non-protein free thiol contents and some gliadins acting as chain terminators could be the underlying reasons for waxy wheat flours producing slack dough.

In an effort to improve functionality of waxy wheat flours, hydro-thermal processing was used. Two temperatures (140 and 160°C), three moisture contents (0, 12.4 and 20%), and four exposure times (0, 5, 15, 30 and 60 min) were employed. Hydrothermal processing resulted in non-cohesive waxy wheat flours with high viscosity and greater acid stability than native waxy wheat flour. A closer investigation revealed the possible role of endosperm proteins in improving pasting properties of waxy wheat flours. Upon thermal processing, waxy wheat flours demonstrated a long hydration time before forming dough. Heating decreased protein solubility while no changes in starch molecular weight distribution were observed. Our results indicate that hydro-thermal processing results in increased starch protein interaction.

As part of application of waxy wheat, bread was baked by replacing normal wheat flour with two hard waxy wheat flours at 15, 30, and 45% levels. Substitution with waxy wheat flour resulted in higher loaf volume and softer loaves. However, substitution at > 30% resulted in excessive post-bake shrinkage and a ‘key-hole’ shape with an open crumb structure. Bread crumb microstructure indicated a loss of starch granule rigidity and fusing of starch granules. Soluble starch content was significantly higher in bread 1-day old crumb containing waxy wheat flour than in control bread.

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Major Professor
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Dedication

To his graciousness Sri Sri Sri Sri Sri God Garu, Vijaya Durga Pitadhipati, Vedurupaka, Andhra Pradesh.

CHAPTER 1 - STUDY OF HARD WAXY WHEAT FLOURS DIFFERING IN PASTING PROPERTIES

ABSTRACT

To realize the full potential of waxy wheat, the pasting properties of hard waxy wheat flours as well as factors governing the pasting properties were investigated and compared with normal and partial waxy wheat flours. Starches isolated from six hard waxy wheat flours had similar pasting properties, yet their corresponding flours had very different pasting properties. The differences in pasting properties were narrowed once endogenous α-amylase activity in waxy wheat flours was inhibited by silver nitrate. Upon action of protease, the extent of protein digestibility determined viscosity profile in waxy wheat flours. Waxy wheat starch granules swelled more extensively and were more prone to α-amylase degradation than normal wheat starch. A combination of endogenous α-amylase activity and protein matrix contributed to a large variation in pasting properties of waxy wheat flours.

INTRODUCTION

Starch in endosperm of normal wheat consists of 25% amylose (mostly linear) and 75% amylopectin (highly branched), whereas starch in waxy wheat endosperm is comprised of essentially all amylopectin (Graybosch, 2005). It has been suggested that amylopectin is responsible for swelling of starch granules (Tester and Morrison, 1990) while amylose –lipid complex was negatively correlated to swelling (Grant et al., 2001; Sasaki and Matsuki, 1998). When wheat flours containing waxy starches are heated in excess water, they exhibit different properties compared to flours containing normal starches (Kim et al., 2003; Graybosch, 2005). Waxy wheat flours exhibit peak viscosity at lower temperature compared to normal wheat flour (Kim et al., 2003), and this property can be used as an indicator to identify waxy wheat flours from normal wheat flours (Hayakawa et al., 1997; Sasaki et al., 2000; Grant et al., 2001; Zeng et al., 1997, Graybosch, 2005; and Yasui et al., 1996). In addition, waxy wheat flours have lower final viscosity compared to pasting of normal wheat flours (Hayakawa et al., 1997; Zeng et al., 1997 and Yasui et al., 1996). However, there are conflicting reports on the effect of waxy character on peak viscosity when analyzed in distilled water. Some reports suggest that waxy wheat flours have higher peak viscosity than normal wheat flours (Zeng et al., 1997; Abdel-Aal et al., 2002; Yoo and Jane, 2002; Kim et al., 2003), whereas other studies found normal flours having higher peak viscosity than waxy wheat flours (Hayakawa et al., 1997; Morris et al., 1998; Yasui et al., 1996). The discrepancies are not well investigated.

When examining hard waxy wheats from advanced breeding lines, we noted that different waxy wheat flours have different dough mixing properties and gluten indices (Guan et al, 2009). Our continued investigations revealed that there are large variations in pasting properties among hard waxy wheat flours. It is not clear how the inconsistency in pasting

properties would affect the use of waxy wheat flours in different applications. In light of the conflicting reports in the literature and our own observations, the objective of this study was to investigate and determine the factors governing the pasting properties of waxy wheat flours and compare their pasting properties with those of normal and partial waxy wheat flours.

MATERIALS AND METHODS

Materials

Six waxy wheat samples, one partial waxy wheat (Trego), and one normal hard red winter wheat (Karl 92) were procured from USDA-ARS, Lincoln, NE. Trego was hard white wheat with null at Wx-B loci. Pedigree of the waxy wheat lines were reported by Guan et al. (2009) and the last four digits in each sample identification were used in this paper. Kernels for each wheat were tempered to 16% moisture for 18 h and were roller-milled into straight-grade flour on a MLU 202 Bühler experimental mill (Bühler Co., Uzwill, Switzerland). Flour yield for all wheat samples was previously reported (Guan, 2008). Starches were isolated by a dough washing method (Guan et al., 2009).

Proximate analysis and analytical tests on flours

Moisture and protein content of the eight flour samples were measured by AACC 44-15A and AACC 46-30, respectively (AACC International, 2000) and were previously reported by Guan et al. (2009). Total and damaged starch content was determined by AACCI 76-13 and AACCI 76-30A (AACC International, 2000) respectively using assay kits from Megazyme (Wicklow, Ireland). Alpha- amylase activity of flours was determined by AACCI 22-02 (AACC

International, 2000) using a Megazyme assay kit. Enzyme activity was reported in Cerealpha Units (CU), where one unit of activity is defined as the amount of enzyme, in the presence of excess thermostable amyloglucosidase, required to release one micromole of *p*-nitrophenol from end blocked *p*-nitrophenyl maltoheptaoside in one minute under the defined assay conditions**.** Arabinoxylan content expressed as D- xylose was determined by using phloroglucinol colorimetric reagent (Douglas, 1981).

Particle size analysis

Particle size distribution for each flour was determined by Multisizer™ 3 COULTER COUNTER® (Beckman Coulter, CA). Each sample was analyzed in duplicates and the mean particle size (in µm), on the assumption that all particles were spherical in shape, was reported.

Pasting properties

Flour pasting properties were determined using a Rapid Visco Analyzer (RVA, Foss North America, Inc., Eden Prairie, MN). A mixture of flour (10% solids) in one of three solvents was prepared in an RVA canister. Final total weight in the RVA canister was 28 g. An RVA paddle was inserted into the canister, and the mixture was gently agitated to disperse flour lumps. The RVA canister was then subjected to a 13-min RVA test to determine flour pasting properties (Deffenbough and Walker, 1989). The RVA pasting curve profile included holding the sample at 50°C for 1 min followed by heating the sample from 50 to 95°C in 3 min, holding the sample at 95°C for 3 min, cooling the sample back to 50°C in 4 min, and holding the sample at 50°C for 2 min. The RVA curves were analyzed for pasting properties i.e. pasting temperature, peak viscosity, viscosity at trough, final viscosity and set back using Thermocline for Windows 3

(TCW3) software provided with the RVA. Pasting temperature was obtained using function TempAtViscRate(1,6,.1,50). Setback viscosity was calculated as difference between final viscosity and viscosity at trough.

The three solvents used were distilled water, 1 mM silver nitrate solution ($AgNO_3$) and 1 mM silver nitrate solution plus protease from *Streptomyces griseus* (Siga P-5147, 4.5 units/mg of protein, St. Louis, MO; Unit Definition: One unit hydrolyzes casein to produce color equivalent to 1.0 μmole (181 μg) of tyrosine per min at pH 7.5 at 37°C (color by Folin-Ciocalteu reagent)). The effect of protease on pasting properties of flours was measured as described by Zhu et al. (2009). Flours (2.8 g, % db) were suspended in water (12.5 g) containing 18 mg of protease and incubated at 37°C for 30 min. Subsequently, 2 mM $AgNO_3$ was added to the protease hydrolyzed flour to a total weight of 28 g. In addition, pasting properties of isolated starches were determined at 7% solids in water or a solution containing 0.01% α- amylase from porcine pancreas (A-3176, Siga Chemicals, St. Louis, MO; Unit Definition: One unit liberates 1.0 mg of maltose from starch in 3 minutes at pH 6.9 at 20 °C).

In a separate study, flour samples (10% solids) were treated with a mixture of protease in $AgNO_3$ solution (containing 18 mg of protease) for 30 min at 30°C. The final weight of the flour sample and protease containing $AgNO_3$ solution was 28 g. Subsequently, an aliquot (1 mL) of the flour suspension was centrifuged at 10,000 x *g* and supernatant was discarded. The residual protein in the pellet was analyzed using LECO™ FP-428 nitrogen determinator (LECO, St. Joseph, MI) and expressed at % of the original protein content of the samples. Each sample was analyzed in duplicate.

Gel permeation chromatography (GPC) of debranched starches

Starch (20 mg) was added to 10 ml 0.01 M acetate buffer at pH 4.0 in a 12 ml glass vial with a micro-stirring bar. The vial was placed in a boiling water bath on a stir plate for 1 h. Isoamylase (50 µl) (EC 3.2.1.68, Hayashibara Biochemical Laboratories, Inc., Okayama, Japan) was added to the solution after it was cooled to room temperature. The vial was placed at 50°C in a water bath overnight, frozen in a dry ice/acetone bath, and freeze-dried. The freeze-dried debranched starch (4 mg, db) was dissolved in 4 ml dimethyl sulfoxide (DMSO) by heating in a boiling water bath for 24 h with constant stirring. Each starch solution was filtered through a 45 µm filter (MILLEX AP 20 PREFILTER, Millipore, Carrigtwohill, Co. Cork, Ireland) prior to GPC analysis. The GPC analysis was performed with a Polymer Laboratory (Amherst, MA) PL-GPC 220 Integrated GPC/SEC fully automated system as previously described (Cai et al., 2010).

Light Microscopy

A method described by Guan et al. (2009) was used to stain starch granules using iodine solution. The mixture of starch in iodine solution (10µL) was loaded on a microscope slide and the number of iodine stained granules were counted using a 40X objective lens mounted on Olympus BX 51 microscope (Olympus Optical Co. Ltd., Shinjuku-ku, Tokyo, Japan). Each sample was analyzed in duplicate and % of granules stained blue was reported.

Thermal properties of flours

Thermal properties of flours were determined by differential scanning calorimetry (DSC) (Q100 DSC, TA Instruments, New Castle, DE). For gelatinization properties, each flour sample and distilled water were added to the DSC pan in a 1:2 ratio (w/w). The pan was hermitically

sealed and allowed to equilibrate at 25°C for 1 h. The samples were then heated from 10° C to 140° C at 10° C/min. An empty DSC pan was used as a reference. Onset, peak and end temperature and enthalpy were determined. Each sample was analyzed in duplicate and mean values were reported.

Statistical analysis

Macanova 4.12 (School of Statistics, University of Minnesota, Minneapolis, MN) was used to perform ANOVA and Tukey's honest significance difference (HSD) analysis. The level of significance was $P < 0.05$ throughout the paper.

RESULTS AND DISCUSSION

Composition and particle size of flours

Protein content of waxy wheat flours ranged from 12.0 to 15.1% (db), while Karl 92 and Trego had 15.47 and 12.47% (db), respectively (Guan et al., 2009) (**Table 1.1**). The protein content of Karl 92 was significantly higher than most of the waxy wheat flours except for sample 2205. Total starch content of six waxy wheat flours was between 75.0 and 81.7% (db), while Karl 92 and Trego had 76.7% (db) as reported by Guan et al. (2009). Although there were variations in starch content, we did not observe the correlation between starch content and pasting properties as discussed in the next section.

Damaged starch values for flours ranged from 6.20 to 11.38% (**Table 1.1**). Most waxy wheat flours had significantly higher damaged starch content as compared to the normal wheat flour (Karl 92) and partial waxy wheat flour (Trego). This could be due to greater susceptibility

of waxy wheat to mechanical damage during milling (Bettge et al., 2000). Hardness index of kernels from wheat samples used in this study are given in **Table 1.1**. There were no significant differences in arabinoxylan content between Karl 92 and waxy wheat flours, while Trego had significantly lower arabinoxylan content (**Table 1.1**). Our results on new waxy wheat samples are different from Sayaslan et al. (2006) who reported 20-30% higher arabinoxylan (pentosan) content in waxy wheat samples as compared to the normal wheat flour (Karl 92).

Waxy wheat flours exhibited significantly higher α- amylase activity as compared to normal wheat flour (Karl 92) and partial waxy wheat flour (Trego). Among waxy wheat flours, sample 2115 had the highest α- amylase activity (0.199 Cerealpha units/g) and sample 2489 had the lowest α-amylase activity (0.075 Ceralpha units/g). Karl 92 had the lowest α- amylase activity (0.033 Ceralpha Units/g) among all the wheat flours.

The mean particle size varied within a narrow range of 34-54 μm (**Table 1.1**). Although there were significant differences among all wheat samples the range of particle size was within the medium fraction for flours (Wang and Flores, 2000).

Debranched waxy wheat starch showed a bimodal distribution with similar proportions of the two fractions compared to that of debranched normal wheat starch (data not shown) indicating that the molecular structure of the waxy wheat starch was close to that of amylopectin in the normal wheat starch. There have been conflicting reports on the amylopectin structure of waxy wheat and their parent lines. Hayakawa et al (1997) have reported that amylopectin in waxy wheat starch had significantly higher DP values compared to amylopectin in starch isolated from their parent lines (non-waxy wheat). In a more recent study, Yoo and Jane (2002) have shown that waxy wheat amylopectin lacked the extra long branched chains that are more present in normal wheat, whereas Yasui et al. (1996) have reported similar amylopectin chain length

distribution profiles for both waxy and parent lines. In our study, we did not observe any differences in amylopectin average chain length among the advanced waxy wheat lines.

Pasting properties of flours

Pasting properties of flours in different solutions are given in **Table 1.2**. In all solvents, the waxy wheat flours exhibited lower pasting temperature (~70°C) as compared to the normal wheat flour (~90°C). In **Figure 1.1**, waxy wheat flours with similar pasting profiles were represented by one representative sample

Distilled water

Waxy wheat flours had a significantly lower peak temperature (~67°C) in water as compared to normal (93°C) and partial waxy wheat flours (91°C) (**Figure 1.1**). There were no significant differences in the pasting temperatures of waxy wheat flours. The low pasting temperature and peak temperature for our waxy wheat flours were in agreement with previous findings of other researchers (Hayakawa et al., 1997; Sasaki et al., 2000; Grant et al., 2001; Kim et al., 2003).

Remarkable variations in peak viscosity were observed for waxy wheat flours in water (**Figure 1.1a**). Sample 2114 and sample 2205 had a peak viscosity similar to Karl 92 but the rest of the waxy wheat flours had a significantly lower peak viscosity than Karl 92. Among waxy wheat flours, sample 2489 exhibited the lowest peak viscosity (472 cP) despite low α-amylase activity and damaged starch content, while sample 2205 exhibited highest peak viscosity (2011 cP) despite high damaged starch content.

Waxy wheat flours had significantly lower hot paste viscosity and exhibited shear thinning as compared to normal (Karl 92) and partial waxy (Trego) flours. When heated in excess water, waxy starches lose their granular rigidity upon swelling (Guan, 2008), which explains the shear thinning behavior of waxy wheat flours. Final viscosity of waxy what flours was significantly different as compared to Trego and Karl 92 flours, which could be due to lack of amylose and inability to form a gel matrix (Lecoup et al., 1991).

Silver nitrate ($AgNO_3$) solution

To examine the influence of α- amylase activity on pasting properties, pasting properties of flours were analyzed in the presence of $AgNO_3$ (**Figure 1.1b**). Previous researchers have utilized silver nitrate solution to inhibit α- amylase activity in sprouted and normal intact wheat flours (Yasui et al., 1999; Abdel-Aal, 2001; Crosbie et al., 2001).

Peak viscosity of all wheat flours increased when α-amylase activity was inhibited but the overall increase in pasting properties was greater in waxy wheat flours as compared to Karl 92 and Trego flours (**Table 1.2, Figure 1.1b**). In fact, all waxy wheat flours had higher peak viscosity than normal and partial waxy wheat flours. The differences in peak viscosity among waxy wheat flours were narrowed when α-amylase activity was inhibited. Our results suggest that waxy flours were more susceptible to amylolytic degradation as compared to normal wheat flour.

Pasting properties of isolated starches and their susceptibility to α- amylase

Because starch is the major flour component and largely responsible for pasting properties of a flour, we isolated starches from flours and determined their pasting properties.

Interestingly, starches isolated from six waxy wheat flours had similar pasting properties (**Figure 1.2**). Waxy wheat starch had a greater increase in viscosity at lower temperature than normal wheat starch, indicating that waxy wheat starch developed viscosity rapidly. Waxy wheat starches had a lower set back than that of normal wheat starches.

To further understand the susceptibility of waxy wheat starches to enzyme activity, isolated starches from waxy wheat and normal wheat were spiked with low levels of exogenous α- amylase (0.01% v/w). A significant decrease in pasting properties of waxy wheat starch was observed (**Figure 1.3**), while there was only a small change in pasting properties of normal wheat starch. This further validates the point that waxy wheat starches are relatively more susceptible to α-amylase activity as compared to normal wheat starch.

Our results (**Figure 1.4)** demonstrated the fate of starch granules of waxy and normal wheat upon heating in excess water. At 73°C, normal wheat starch granules still maintained granular integrity, whereas waxy starch granules swelled and lost their granular integrity. Our findings suggest that waxy wheat flours are prone to α-amylase degradation and explain the aberrant falling numbers of waxy wheat flours observed by Graybosch et al. (2000).

Silver nitrate and protease solution

To assess the role of protein on flour pasting properties, flours samples were treated with protease and then pasted in $AgNO_3$ solution **(Figure 1.1c)**. Peak viscosity of waxy wheat flours in $AgNO_3$ solution was significantly higher than normal wheat flour, whereas peak viscosity for all flours digested with protease and heated in $AgNO_3$ solution was lower. Debet and Gidley (2007) have suggested that starch swelling properties are affected due to protein adsorption on their surface. In cereal flour, protein coats the starch granules and could protect the starch

granules from mechanical damage during pasting (Hamaker and Griffin, 1993). Our results suggest that in flours, the action of protease could destroy the protein coat, and facilitate mechanical destruction of waxy starch granules.

When waxy wheat flours were treated with protease and pasted in $AgNO_3$ solution, their peak viscosities became similar (**Table 1.2; Figure 1.1b**). However, there were still differences in viscosity profiles of waxy wheat flours. To understand the effect of protein on pasting properties residual protein in the pastes was determined. For instance, ~60%of flour protein in sample 2115 was solubilized by protease in the 30 min digestion period, while only 52% of the flour protein was solubilized in sample 2489. The extensive proteolysis could have weakened the interaction between starch granules and protein, making the starch more susceptible to shear thinning.

Additionally, light microscopy data showed that samples 2114 and 2489 had significantly higher amylose contamination (counted as %dark granules in 10 μL of 10 mg/ml starch suspension) as compared to samples 2115 and 2459 (**Table 1.1**). The apparent differences in pasting curves in protease and silver nitrate solution could be due to combination of amylose contamination and residual protein. Samples with high amylose contamination and low protein digestion show low and broad peak viscosity.

Thermal properties of flour

Gelatinization temperature for Karl 92 and Trego flours was slightly lower than the waxy wheat flours as determined by DSC **(Table 1.3)**. For Karl 92 and Trego samples, there was apparent amylose-lipid complex peak at ~100°C, which was absent in waxy wheat samples. It is known that amylose and lipid inhibit swelling of starch granules (Tester and Morrison, 1990;

Grant et al., 1997; Sasaki and Matsuki, 1998), and as a result, normal wheat flours had a higher pasting temperature than waxy wheat flours (**Figure 1.1)** even though the gelatinization temperature of the normal wheat flour as determined by DSC was slightly lower (**Table 1.3**).

CONCLUSIONS

At least two factors, protein matrix and susceptibility to α-amylase activity, contribute to the wide range of pasting properties of waxy wheat flours. Waxy wheat flours are more prone to endogenous α-amylase degradation than normal wheat flours. Isolated waxy wheat starches which no longer contain α-amylase activity, had very similar pasting properties. Amylose contamination and protein matrix affect the swelling of starch granules and in turn causes variations in pasting properties of waxy wheat flours.

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Table 1.1 Damaged starch, arabinoxylan and α- amylase activity of wheat flours‡

Sample	Protein content† (%)	Total Starch content† (%)	% Damaged Starch	% Arabinoxylan Content (as D-(+)-xylose)	α- amylase activity (Cerealpha units/g)	Mean flour Particle size (µm)	Hardness Index†	Amylose contamination (% dark granules)
2114	13.88	75.0	6.02 ± 0.10 ^{e}	1.73 ± 0.15 ^{a}	0.12 ± 0.03 ^{bc}	34 ± 0.6 ^{f}	66.7	5
2115	13.78	81.7	8.98 ± 0.07 ^{bc}	1.78 ± 0.29 ^{a}	0.20 ± 0.02 ^{a}	37 ± 0.2 ^{e}	62.8	2
2205	15.10	78.3	10.08 ± 0.03 ^{b}	1.36 ± 0.09 ^{a}	0.09 ± 0.01 ^{abc}	54 ± 0.1 ^{a}	73.0	5
2315	12.00	81.7	8.52 ± 0.25 ^{c}	2.05 ± 0.42 ^{a}	0.14 ± 0.02 ^{ab}	47 ± 0.0 ^{b}	72.5	5
2459	13.33	78.3	11.38 ± 0.69 ^{a}	1.65 ± 0.09 ^{a}	0.13 ± 0.00 ^{bc}	42± 0.0 ^{d}	67.5	3
2489	12.82	80.0	9.33 ± 0.04 ^{bc}	1.59 ± 0.09 ^{a}	0.07 ± 0.01 ^{cd}	41 ± 0.2 ^{d}	76.2	7
Karl 92	15.47	76.7	7.19 ± 0.35 ^{d}	1.69 ± 0.05 ^{a}	0.03 ± 0.00 ^{d}	45 ± 0.1 ^{c}	64.8	96
Trego	14.04	76.7	6.77 ± 0.06 ^{de}	1.26 ± 0.08 ^{a}	0.04 ± 0.00 ^{d}	46 ± 0.5 ^{c}	75.9	-

* mean ± standard deviation values are reported
‡means not sharing the same superscript within each column are significantly different ($p < 0.05$)
† values reported by Guan et al., 2009.

Table 1.2 Pasting properties of wheat flours in different solvents (N = 2)*‡

Sample	Peak Viscosity (cP)	Hot Paste Viscosity (cP)	Final Viscosity (cP0	Setback Viscosity (cP)
Distilled water				
2114	1884 ± 137 ^{b}	734 ± 32 ^{b}	1038 ± 28 ^{b}	305 ± 4 ^{b}
2115	1592 ± 6 ^{c}	616 ± 6 ^{c}	852 ± 8 ^{c}	236 ± 14 ^{c}
2205	2011 ± 15 ^{b}	731 ± 15 ^{b}	1019 ± 25 ^{b}	289 ± 11 ^{bc}
2315	1461 ± 6 ^{c}	626 ± 6 ^{c}	920 ± 11 ^{c}	294 ± 5 ^{bc}
2459	1450 ± 1 ^{c}	392 ± 1 ^{d}	550 ± 6 ^{d}	159 ± 6 ^{d}
2489	472 ± 13 ^{d}	38 ± 13 ^{e}	73 ± 4 ^{e}	35 ± 9 ^{e}
Karl 92	1971 ± 15 ^{b}	1125 ± 15 ^{a}	2118 ± 25 ^{a}	994 ± 11 ^{a}
Trego	2389 ± 11 ^{a}	1151 ± 11 ^{a}	2168 ± 47 ^{a}	1018 ± 36 ^{a}
Silver nitrate (1 mM)				
2114	3488 ± 12 ^{bc}	1180 ± 3 ^{bc}	1668 ± 19 ^{b}	488 ± 16 ^{c}
2115	3319 ± 38 ^{c}	1148 ± 20 ^{cd}	1548 ± 20 ^{bc}	400 ± 0 ^{de}
2205	3623 ± 51 ^{ab}	1208 ± 23 ^{bc}	1633 ± 45 ^{b}	426 ± 22 ^{cde}
2315	2924 ± 31 ^{d}	1084 ± 6 ^{d}	1549 ± 21 ^{bc}	465 ± 14 ^{cd}
2459	3713 ± 63 ^{a}	1098 ± 22 ^{d}	1462 ± 41 ^{cd}	365 ± 19 ^{e}
2489	2810 ± 103 ^{d}	1014 ± 25 ^{e}	1380 ± 45 ^{d}	367 ± 21 ^{e}
Karl 92	2222 ± 19 ^{e}	1242 ± 8 ^{b}	2871 ± 28 ^{a}	1629 ± 20 ^{a}
Trego	2764 ± 30 ^{d}	1365 ± 2 ^{a}	2775 ± 17 ^{a}	1411 ± 19 ^{b}
Flours digested with protease (80 U) pasted in silver nitrate (1 mM)				
2114	1929 ± 26 ^{b}	835 ± 23 ^{c}	1230 ± 27 ^{d}	396 ± 4 ^{cde}
2115	2270 ± 12 ^{a}	510 ± 8 ^{e}	782 ± 17 ^{e}	273 ± 9 ^{e}
2205	2238 ± 24 ^{a}	599 ± 6 ^{d}	895 ± 2 ^{e}	296 ± 4 ^{de}
2315	1778 ± 6 ^{b}	739 ± 3 ^{c}	1138 ± 5 ^{d}	399 ± 8 ^{c}
2459	2262 ± 41 ^{a}	872 ± 7 ^{b}	1192 ± 24 ^{cd}	320 ± 17 ^{cde}
2489	2276 ± 21 ^{a}	901 ± 6 ^{b}	1268 ± 11 ^{c}	367 ± 5 ^{cd}
Karl 92	1652 ± 19 ^{b}	886 ± 7 ^{b}	1987 ± 18 ^{b}	1101 ± 11 ^{b}
Trego	2333 ± 12 ^{a}	1123 ± 11 ^{a}	2455 ± 4 ^{a}	1332 ± 14 ^{a}

* mean ± standard deviation values are reported

‡means not sharing the same superscript within each column are significantly different ($p < 0.05$)

Table 1.3 Gelatinzation properties of flours (33.3% solids) determined by differential scanning calorimeter (N =2)* ‡

Sample	Onset Temperature (°C)	Peak Temperature (°C)	End Temperature (°C)	Enthalpy (ΔH, J/g)
2114	61.3 ± 0.37 [a]	69.3 ± 0.52 [d]	81.1 ± 0.78 [ab]	8.0 ± 0.19 [a]
2115	61.2 ± 0.49 [a]	69.6 ± 0.55 [a]	80.8 ± 0.06 [bc]	8.2 ± 0.55 [a]
2205	62.0 ± 0.18 [ab]	70.0 ± 0.46 [a]	82.6 ± 0.17 [ab]	8.3 ± 0.38 [a]
2315	62.0 ± 0.10 [ab]	70.6 ± 0.42 [a]	81.7 ± 0.47 [ab]	6.0 ± 0.24 [b]
2459	61.8 ± 0.60 [a]	70.6 ± 0.69 [a]	84.7 ± 0.68 [a]	7.6 ± 0.61 [ab]
2489	61.9 ± 0.01 [a]	70.0 ± 0.08 [a]	82.9 ± 0.88 [ab]	7.1 ± 0.15 [ab]
Karl 92	60.4 ± 0.22 [b]	66.1 ± 0.17 [b]	78.2 ± 0.00 [cd]	6.2 ± 0.52 [b]
Trego	60.1 ± 0.16 [b]	66.1 ± 0.01 [b]	79.0 ± 0.40 [d]	6.8 ± 0.35 [ab]

* mean ± standard deviation values are reported

‡means not sharing the same superscript within each column are significantly different ($p < 0.05$)

Figure 1.1 RVA pasting properties of waxy and normal wheat flours in (a) distilled water, (b) 1mM silver nitrate solution, and (c) flours digested with protease and pasted in 1mM silver nitrate

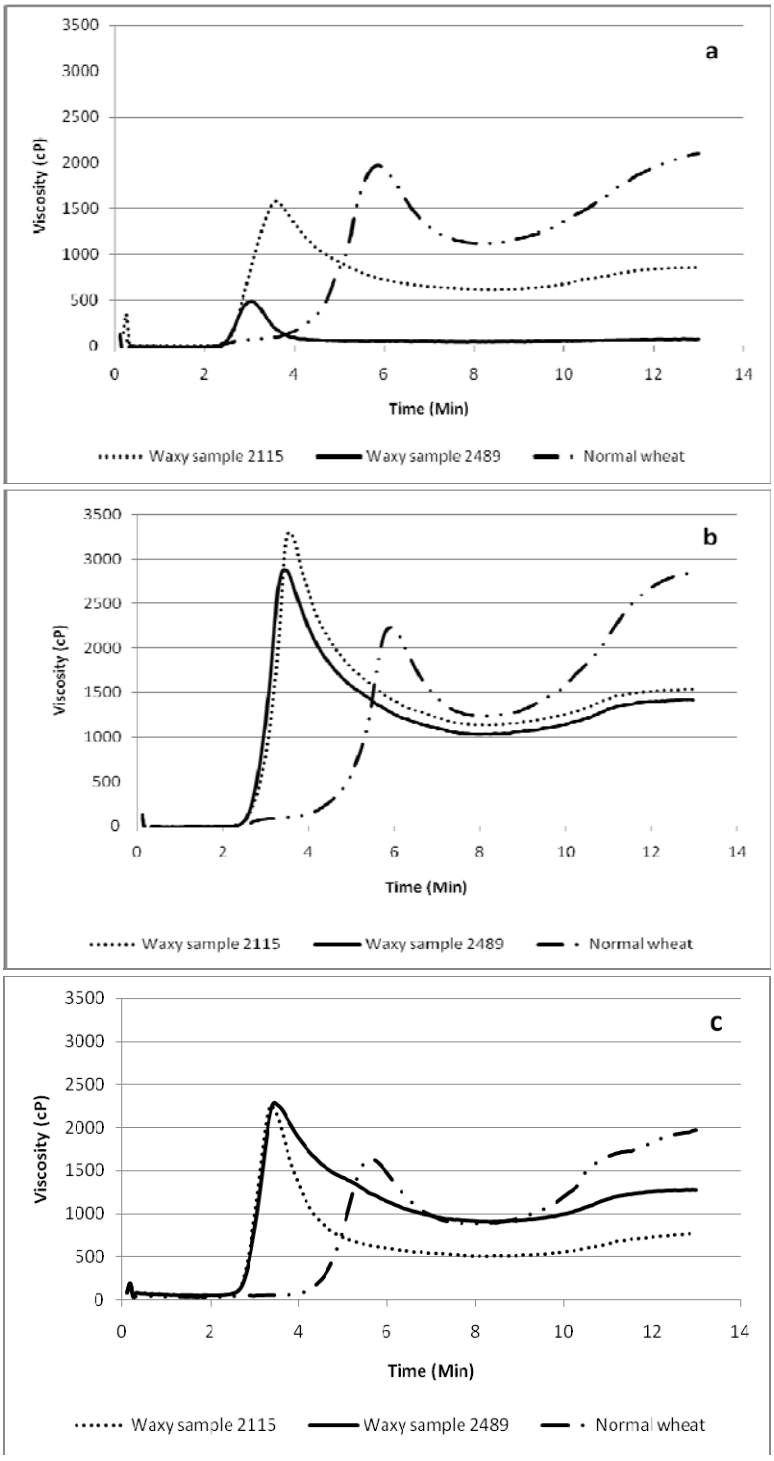


Figure 1.2 RVA pasting properties of waxy wheat starch (sample 2114, 2115, 2205, 2315, 2459 and 2489), normal wheat starch (Karl 92) and partial waxy wheat starch (Trego) determined at 7% solids.

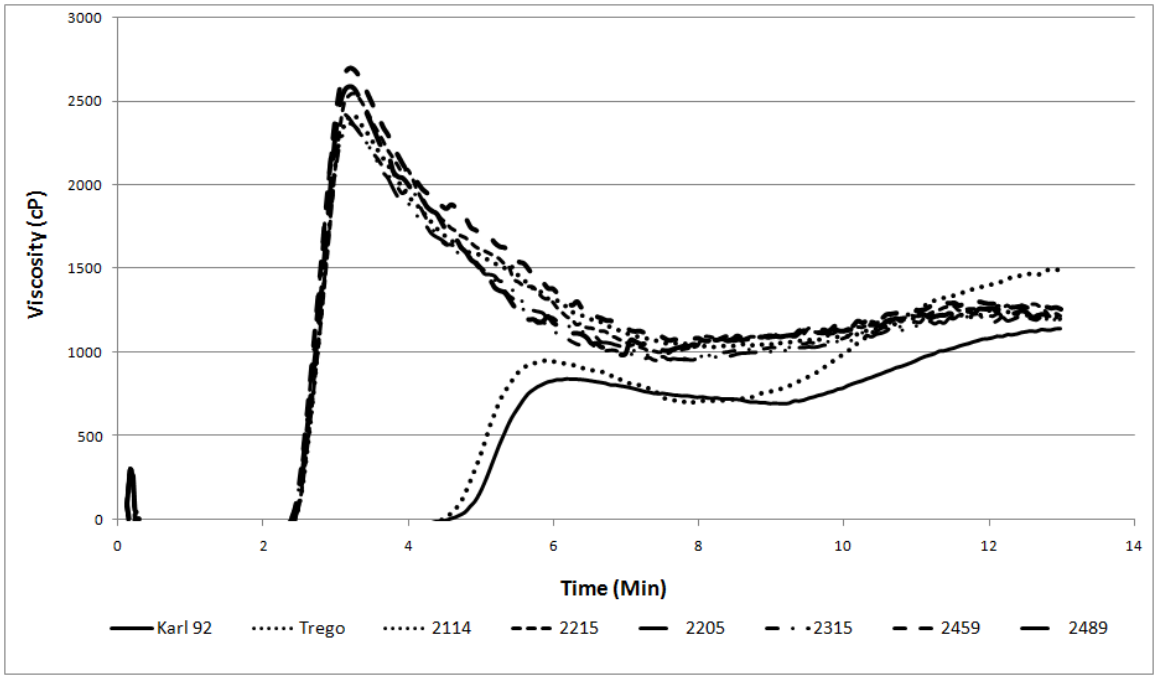


Figure 1.3 RVA pasting properties of isolated starches from waxy and normal wheat flour with and without addition of 0.01% α- amylase.

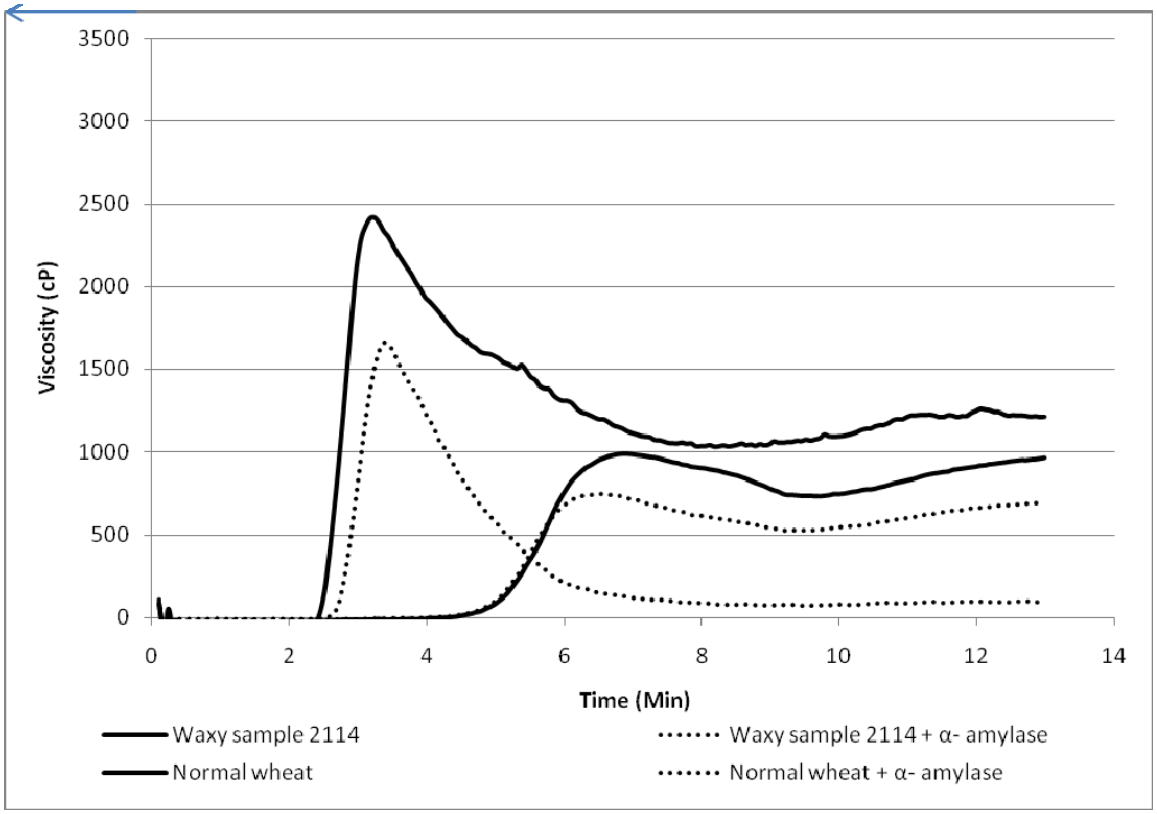
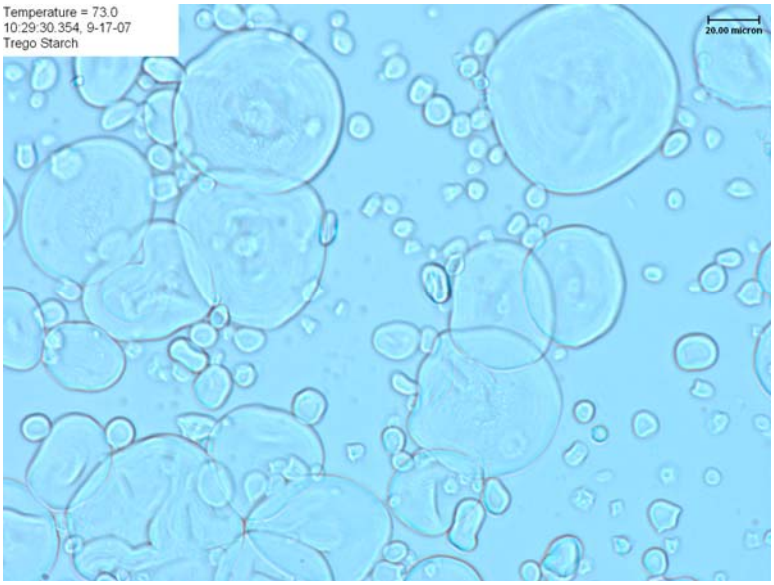
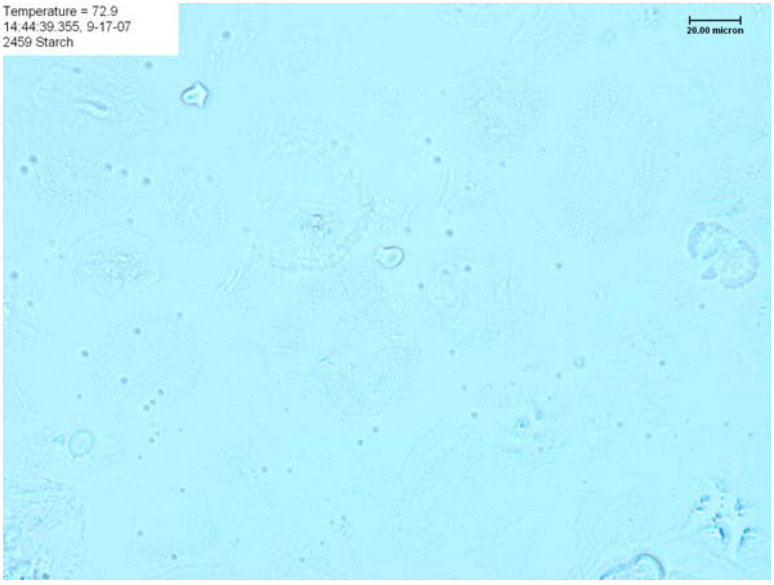


Figure 1.4 Starch granule morphology at 73°C for normal (above) and waxy (below) wheat starches





CHAPTER 2 - ENDOSPERM PROTEIN CHARACTERISTICS IN NORMAL AND HARD WAXY WHEAT AND THEIR CHANGES FROM FLOUR TO DOUGH

ABSTRACT

The objective of this work is to investigate the composition and solubility of hard waxy wheat flour proteins and their changes from flour to dough. Six waxy wheat flours, one normal wheat flour and one partial waxy wheat flour were examined. Flours were also analyzed for their mixing and biaxial extensional properties. Waxy wheat flour had higher water absorption and lower mixing time than did normal wheat flour. Biaxial extension properties classify a dough from waxy wheat flour as in-elastic. Free thiol content, protein composition of flour and dough were analyzed. Flour proteins (i.e. gliadins and glutenins) extracted using various solvents and sonication, were analyzed using size exclusion high performance chromatography. Waxy wheat flours had higher free thiol content, less of 50% propanol soluble proteins and lower soluble polymeric proteins than did normal wheat flour. Additionally, waxy wheat flours had higher residual protein after detergent and sonication extraction. The measurable gliadin content of waxy wheat samples significantly decreased from flour to dough, while remaining constant in normal wheat. Waxy starch affects protein hydration but not protein extractability after optimum dough mixing. The presence of high non protein free thiol content and some gliadins acting as chain terminators could be the underlying reason for waxy wheat flours producing slack dough.

INTRODUCTION

Wheat flour proteins upon hydration interact with each other to form a viscoelastic mass (Shewry et al., 2002). The role of gluten proteins in dough formation for normal wheat has been extensively studied and reviewed (Cornish et al., 2006; Shewry et al., 2009). The role of different protein fractions on dough strength has been studied by selectively isolating proteins using various solvents such as alcohol (Bean et al., 1998) and detergent solvents (Aussenac et al., 2001). Gluten proteins can be classified into monomeric gliadins and polymeric glutenins (Sapirstien and Fu, 1998; Singh and Macritchie, 2001; Kuktaite et al., 2004). The variations in the monomeric and polymeric gluten proteins among various cultivars have been previously discussed (Gupta et al., 1996; Johansson et al., 2001; Singh and MacRitchie, 2001). Most of the monomeric proteins and some polymeric protein from flour can be extracted by using 50% propanol, while the remaining polymeric protein is positively correlated to dough strength (Bean et al., 1998). Furthermore, detergent solvents along with reducing agents and sonication methods have been used to study the function of polymeric glutenin subunits (Singh et al., 1990; Aussenac et al., 2001). Polymeric glutenins are further classified into high molecular weight subunits (HMW-GS) and low molecular weight subunits (LMW-GS). During mixing certain HMW-GS typically undergo repolymerization and depolymerization (Weegles et al., 1996). Formation of dough and, subsequently, its strength are attributed to the polymers formed by intermolecular disulfide bond between HMW-GS and LMW-GS (Wrigley, 1996). Dough strength is positively correlated to insoluble high molecular weight protein fractions which are extracted using certain detergent solvents (MacRitchie, 1972).

Wheat flour is termed waxy, when its endosperm starch is primarily composed of amylopectin. Full waxy wheat has no or only traces of amylose. Waxy wheat has potentials in

various food applications (Graybosch, 1998). Guan et al. (2009) reported that hard waxy wheat flours tend to produce slack dough with low gluten index values. Currently, there is no available literature on the effect of waxy wheat trait on flour proteins and consequently their effect on dough mixing. The changes in molecular weight distribution of gluten proteins can be studied using high performance liquid chromatography (HPLC) in conjunction with size exclusion columns (Bean et al., 1998; Aussenac et al., 2001). Typically, protein solubility is affected by hydration during mixing. Waxy starches swell rapidly and absorb more water than do native starch counterparts (Guan, 2008). In a limited water system like dough, waxy starches could compete with proteins for available water and result in lower protein hydration. Consequently, the objectives of this study are to evaluate (i) protein composition of hard waxy wheat and (ii) changes in protein composition and solubility of hard waxy wheat from flour to dough (ii) effect of waxy wheat starch on protein extractability.

MATERIALS AND METHODS

Materials

Six waxy wheat samples, one partial waxy wheat (Trego), and one wild type hard wheat (Karl 92) were procured from USDA-ARS, Lincoln, NE. Pedigree of the waxy wheat lines were reported by Guan et al. (2009). The waxy wheat samples will be identified by the last four digits throughout this paper.

Proximate analysis

Moisture, protein and ash content of the eight flour samples were obtained from Guan (2008) and measured by AACC 44-15A; AACC 46-30; and AACC 08-01, respectively (AACC International, 2000).

Mixing and bi-axial extension properties

Flour samples were analyzed for mixing properties using a 10 g mixograph (AACC 54-40 A, AACC International, 2000) and were reported by Guan (2008). A series of mixograms were analyzed to determine the optimum % absorption for each flour. Analyses were performed in duplicate. Optimum absorption and time to reach peak were reported.

Flour samples were analyzed for their biaxial extension properties using Alveograph (AACC 54-30 A, AACC International, 2000). Each flour sample was analyzed as five replicates and the mean values were reported.

Dough samples were prepared in a pin mixer using optimum absorption and optimum mixing time calculated using Mixograph (**Table 2.1**). They were immediately immersed and liquid nitrogen and freeze dried overnight. The samples were ground using Thomas® Wiley® cutter mill (Thomas Scientific, Swedesboro, NJ) on a 40 mesh sieve, prior to further analysis.

Free thiol content

Free thiol estimation was determined by a method described by Chen and Wasserman (1993). Standard curve was developed using reduced glutathione (G4251, Sigma Aldirch, St.

Louis, MO) at 100, 200, 300, 500 and 100 nM repectively. The amount of free –SH in flour was reported as per gram of flour.

Free elemental sulphur analysis

Two samples, normal wheat (Karl 92) and waxy wheat (sample 2114) flours were analyzed for elemental sulphur analysis. Each flour (1 g) was suspended with 10 mL distilled water and mixed for 10 min. The tubes were centrifuged and the pellets were analyzed. Flours and water washed counterparts were analyzed for elemental sulphur content using perchloric digestion (Geiseking et al., 1935). Analysis of elemental sulphur from perchloric digest was done by an Inductively Coupled Plasma (ICP) Spectrometer, Model 720-ES ICP Optical Emission Spectrometer (Varian Austrailia Pty Ltd, Mulgrave, Vic Australia). Analysis was performed in duplicate and the average values were reported as % dry basis.

Vital wheat gluten (VWG) and isolated starch blends

Commercial VWG (King Arthur, Norwich, Vermont) (82% protein (db), 8.1% moisture) was blended with either normal wheat starch (Karl 92, 99.5% starch (db), 7.0% moisture) or waxy starch (sample 2114, 99.6% starch (db), 7.5% moisture). VWG and starches were mixed to obtain 14.5% protein in the final blend. The blends were mixed at two different absorption levels (54% and 66%). They were immediately immersed in liquid nitrogen and freeze dried overnight. The samples were ground using Thomas® Wiley® cutter mill (Thomas Scientific, NJ) through a 40 mesh sieve prior to further analysis.

Soluble and insoluble protein extraction

Sequential protein extraction was performed on flour and dough samples to obtain extractable protein (EP), insoluble polymeric protein (IPP) and residual protein (RP).

EP analysis was done according to Bean et al. (1998) method with following modifications. Each sample (100 mg) was suspended in 1.0 ml 50% isopropanol, vortexed for 5 min and centrifuged at 10,000 X *g* for 5 minutes. The sediment was extracted for a second time with 1.0 ml fresh 50% propanol. Extraction was repeated twice. Supernatant (total 1 ml, 0.5 ml from each extraction) was filtered through 0.45 µm filter and analyzed by size exclusion – high performance liquid chromatography (SE-HPLC). The pellet after two extractions with 50% isopropanol was analyzed for protein content by LECO™ FP-428 nitrogen determinator (LECO, MI). The protein in the pellet was reported as IPP.

To extract IPP, pellet after two 50% iso-propanol extractions (as described above) was mixed in 1.0 ml of sodium dodecyl sulphate (SDS) buffer (0.5% SDS + 0.05M sodium phosphate buffer at pH 6.9), vortexed for 1 min, sonicated at 10W for 20 sec, and centrifuged at 10,000 X *g* for 10 minutes. Supernatant (0.5 ml) was filtered through 0.45 µm filter and analyzed by SE-HPLC using the protocol described below. The pellet after SDS buffer extraction was analyzed for protein content by LECO™ FP-428 nitrogen determinator. The protein in pellet was reported as RP.

SE- HPLC analysis

EP and IPP extractions were analyzed by SE-HPLC (Agilent 1100 Series, Agilent Technologies, Palo Alto, CA) using Bio-Sep-SEC- 4000 column 300 x 7.80 mm (Phenomenex, Torrance, CA). The following parameters were used for SEC-HPLC - column temperature:

30°C; injection volume: 20 µL; eluting solvent : acetonitrile (ACN) : water (1:1) containing 0.1% trifluoroacetic acid (TFA); run time: 30 min; flow rate: 0.5 ml/min.

Extraction of gliadin fractions from flour for RP-HPLC

Gliadins were extracted by using the method of Weiser et al. (1998) with following modifications. Each flour sample (100 mg) was suspended in 1 mL 60% ethanol, vortexed for 5 min and centrifuged at 10,000 x *g* for 5 minutes. Extraction was repeated three times. The supernatant (total 1.5 ml, 0.5 ml from each extraction) was filtered through 0.45 µm filter and analyzed using reverse phase – high performance liquid chromatography (RP-HPLC) (Agilent 1100 Series, Agilent Technologies, Palo Alto, CA).

The samples were analyzed using Phenomenox® Jupiter™ C-18 column (Phenomenex, Torrance, CA). The following parameters were used for RP-HPLC - column temperature: 50°C; injection volume: 50 µL; eluting system- solvent A: TFA (0.1% v/v); solvent B: ACN + TFA (99.0/0.1%, v/v); linear gradient: 0 min 27% solvent B, 20 min 55% solvent B; run time: 30 min; flow rate: 1.0 ml/min. Detection was done by UV absorbance at 210 nm.

Statistical analysis

Macanova 4.12 (School of Statistics, University of Minnesota, Minneapolis, MN) was used to perform ANOVA and honest significance difference (HSD) analysis. The level of significance was $P < 0.05$ for all analyses.

RESULTS AND DISCUSSION

Proximate Analysis

Moisture, protein and ash values for all the flour samples were reported by Guan et al. (2009). Waxy wheat flours had lower moisture and ash content as compared to Karl 92. Among waxy wheat flours, sample 2114 had highest protein (12.4%), while sample 2315 had lowest % protein (11.63%).

Mixing and bi-axial extension properties

The mixograph results were previously reported in figures by Guan et al. (2009) and are summarized in **Table 2.1**. At optimum water absorption, the peak time for waxy wheat flours was lower as compared to Karl 92 and Trego. The optimum water absorption levels were higher for waxy wheat flours as compared to Karl 92 and Trego.

Alveograph results (**Table 2.1**) indicate that dough from waxy wheat flours required high pressure (P) but had very low extension (L) values. The P/L ratio of waxy wheat flours was about 1.5 times greater than that of Karl 92. P/L values are positively correlated to the ability of wheat flours to retain gas during baking (Walker et al., 1996). However, dough with both high pressure (P) and short time (L) to burst is classified as an in-elastic dough; doughs prepared from all the waxy wheat flours fall under that category. Work values for waxy wheat dough were significantly higher than those of Karl 92 except for sample 2489. The AACC 50-30 A method calls for addition of sodium chloride solution based on initial flour moisture to analyze dough bi-axial extension properties at constant hydration. Our mixograph results suggest that waxy wheat flours had higher water absorption as compared to normal wheat flour. Therefore the differences

in P/L values between waxy and normal wheat flours could be due to the effect of starch on dough hydration and consequently its biaxial properties.

Changes in Free –SH Content

Free thiol (-SH) content for both flour and dough samples is reported in **Figure 2.1**. Waxy wheat flour 2459 had highest –SH content, while Trego has the lowest –SH content. Waxy wheat samples (flour and dough) had significantly ($P < 0.05$) higher free –SH content than did Karl 92 and Trego samples. Except for sample 2459, waxy wheat samples had no significant ($P > 0.05$) differences in free –SH content between flour and dough samples. In contrast, free –SH content decreased in both Karl 92 and Trego flours when mixed into doughs. Free –SH could potentially be involved in sulfhydryl –disulphide exchange among gluten proteins during dough formation (Frater et al., 1960). However, not all free –SH in proteins are actively involved in dough formation process. S-rich prolamins such as α-, and γ- gliadins aid in formation of intra-chain disulphide bonds (depending on number of cysteine residues); while free –SH present in the LMW-GS subunits form inter-chain bonds between HMW-GS and LMW-GS (Shewry and Tatham, 1997). HMW-GS and LMW-GS form interchain bonds and contribute as chain extenders, typically there is only one crosslink between HMW- and LMW-GS via a disulphide bond involving cysteine residues in domain B of HMW-GS and C-terminal domain of LMW-GS. Additionally, the free –SH could also be contributed by reduced glutathione (GSH) that is endogenous to flour. The total GSH in flour can vary from 19 to 127 nmol/g of flour (Huttner and Weiser, 2001).

From the free –SH results and the changes from flour to dough, we note that Karl 92 and Trego samples had a decrease in free –SH groups from flour to dough, while waxy wheat

samples did not. The apparent no change in free –SH in waxy wheat samples from flour to dough indicates lack of sulfhydryl-disulfhydryl exchange in waxy wheat samples during mixing, which could be due to presence of chain terminators (such as gliadins with odd number of cysteine residues and GSH). Additionally, these chain terminators could also be contributing to the initial high free –SH content of waxy wheat flours.

The presence of GSH in flour can be indirectly measured by comparing flour sulphur content before and after washing with water. GSH has a solubility of 50 mg/ml (Product information sheet, Sigma-Aldrich, St. Louis, MO) in water and because GSH is present in much lower quantities in flour, it is possible to wash out free GSH with distilled water. By washing flour with water, we could potentially eliminate the sulphur contribution from GSH. Our results indicate that waxy wheat flours had higher initial elemental sulphur content, however when the flour was washed with distilled water, the sulphur content significantly decreased (from 0.17% in flour to 0.134% in flour washed with water), while there were no significant differences in elemental sulphur content in normal wheat flour and flour washed with water (0.145% in flour to 0.141% in flour washed with water).

Because there was no change in free –SH from flour to dough and decrease in sulphur content upon washing, we hypothesize that there are water soluble sulphur compounds (such as glutathione) in waxy wheat flours that may contribute to high free SH but that inhibit polymeric gluten formation during mixing.

Extractable protein (EP) in flour

Composition of EP analyzed by SE-HPLC is given in **Table 2.2 and Table 2.3**. Area under the SE-HPLC curve was divided into three regions as described by Bean et al. (1998)

soluble polymeric protein (SPP, > 70kDa), gliadins (Gli, 13-70 kDA) and albumins and globulins (Alb/Glb, <13 kDA). A representative curve for normal (Karl 92) and waxy wheat (sample 2315) flour is shown in **Figure 2.2**.

Karl 92 and Trego flours had significantly higher EP as compared to waxy wheat flours (**Table 2.2**). Compared to Karl 92, waxy wheat flours had significantly lower Alb/Glb and SPP values. Among waxy wheat samples, sample 2315 had lowest SPP content. There was no trend in gliadin content between waxy and normal wheat samples. Among the waxy wheat flours, samples 2315 and 2459 had significantly higher gliadin content.

In dough, Karl 92 had significantly higher EP than did waxy wheat. Karl 92 had significantly higher gliadin content as compared to most of the waxy wheat samples, except for sample 2459. Among doughs from waxy wheat samples, sample 2315 had the lowest SPP value. Representative curves for normal (Karl 92) and waxy wheat (sample 2315) flour dough are shown in **Figure 2.3**. In all cases, dough contained more EP than flour. This was particularly evident in SPP values. The increased level of soluble polymeric protein could be due to de-polymerization and consequently increased protein solubility during mixing (MacRitchie, 1975). Additionally, the amount of change in EP among waxy wheat samples was closely related to their genetic background of parent lines of waxy wheat lines, which were previously reported by Guan et al. (2009). Samples 2114 and 2115 which had identical parent background had similar changes in EP from flour to dough (~20%). Similarly samples 2459 and 2489 with similar genetic backgrounds had similar smaller changes in EP from flour to dough (~13%) and other two waxy wheat samples (2315 and 2205) had only 5% change. Sample 2315 had a high RP content.

RP-HPLC results

Three main gliadin sub-units, ω-, α-, γ- gliadins, were identified for each flour based on their surface hydrophobicity. They are reported as % peak area as shown in **Figure 2.4**. There was no trend in any of the three gliadin subunits measured using RP-HPLC that could differentiate waxy and normal wheat samples. Among waxy wheat flours, flour 2315 had significantly ($p < 0.05$) more ω- gliadins and less α- gliadins (**Table 2.4**). Previous researchers have demonstrated the weakening effect of various gliadin components in polymer formation during dough processing (Khatkar et al., 2002; Fido et al., 1997). Weakening effects of gliadins on farinograph and extensograph properties were highest for ω – gliadins followed by α- and γ-gliadins (Fido et al., 1997). Typically, α- and γ-type gliadins contain cysteine residues (Shewy and Tantham, 1997) and could play a key role in forming cross-links with glutenin polymers during dough mixing via disulphide/sulphydryl exchange (Bushuk, 1998), while ω-gliadins lack cysteine residues (Shewy and Tantham, 1997). Flour 2315 with almost more than double the % ω - gliadins than normal wheat had the lowest glutomatic value (Guan et al., 2009).

In dough, waxy wheat samples contained a higher α- gliadin content and lower γ- gliadin content as compared with the normal wheat flour. Overall, there was an increase in α- gliadin in all samples from flour to dough (**Table 2.4)**. Sample 2315 showed the highest change in α-gliadin content from flour to dough. The changes in α- and γ- gliadin fractions from flour to dough and its difference between normal and waxy wheat samples could be due to the differences in number of cysteine residues in them. Typically, gluten polymers (glutenin and gliadin) with at least two cysteine residues potentially act as chain extenders by forming inter and intra molecular disulphide bonds, while gluten polymers with one or odd cysteine residues can be potential chain terminators, forming intermolecular disulphide bond (Kasarda, 1989). It is

possible that α- and γ-type gliadins contain one or odd number of cystiene residues due to an apparent mutation of a serine codon to a cysteine codon in ancestral α- and γ-gliadin genes (Tao and Kasarda, 1989; Kasarda, 1989; Lew *et al*., 1992).

In-soluble polymeric proteins (IPP) composition

Composition of IPP analyzed by SE-HPLC for flour and dough is given in **Table 2.2** and **Table 2.3,** respectively. Area under the SE-HPLC curve was divided into two regions based on molecular weight Peak 1(< 70 kDa) as low molecular weight (LMW-GS) and Peak 2 (> 70kDa) as high molecular weight(HMW-GS). The area under < 13 kDa was negligible (less than 0.5%) in all cases so these proteins were omitted in all calculations.

Typically, HMW-GS have been positively correlated to dough strength and baking quality (Cornish et al., 2006). Flours with null alleles to express HMW-GS gave poor loaf characteristics and low dough development time (Payne et al., 1987; and Lawrence et al., 1988). The ratio of HMW-GS to LMW-GS in flour was demonstrated to be positively correlated with dough characteristics (Lawrence et al., 1988). In this study, the ratio of HMW-GS and LMW-GS could not be used to differentiate waxy wheat samples from normal wheat samples. However, there was an overall decrease in both HMW-GS to LMW-GS from flour to dough, which is in conjunction with previous researchers (Aussenac et al., 2001), who have shown a decrease in extractability of glutenin polymers upon mixing. The decrease in extractability of HMW-GS could be due to increase in HMW-GS due to their interactions with smaller gluten fractions such as LMW-GS, gliadins, and albumins/globulins (Lee et al., 2002). Our SEC-HPLC results for HMW-GS and LWM-GS (**Table 2.2**) in conjunction with mixograph results from Guan et al. (2009) do not exhibit the relationship between the ratio of HMW-GS to LMW-GS and dough

characteristics, which suggests the role of other factors such as starch composition in endosperm on dough properties.

Role of starch in protein hydration and solubilization

To better understand the role of starch in dough development and consequent solubilization of protein, blends of gluten and starch were investigated. VWG and starches (normal wheat starch or waxy wheat starch) were mixed to obtain 14.5% protein in the final blend. The blends were mixed at two different absorption levels (54% and 66%). The rationale to use two different absorption levels was due to the fact that waxy wheat flours had higher water absorption as compared to normal wheat flour (Guan, 2008). The results for mixograph for blending were shown by Guan et al. (2009). At 54% absorption, blends of VWG and normal wheat starch formed dough, while blends of VWG and waxy wheat starch remained very dry. At 66% absorption the blends of VWG and waxy wheat starch formed dough, while blends of VWG and normal wheat starch formed a very wet mass. In blends with VWG and waxy wheat starch, it is possible that at lower water absorption there was not enough water for protein to hydrate and form dough due to higher water absorption of waxy wheat starch granules (Guan et al., 2009). Our results of 50% propanol extracted proteins indicate no changes between blends of waxy and normal wheat starches when no water was added (**Figure 2.6A**). However, at lower absorption blends containing waxy starch showed a decrease in SPP (area under the curve from 10 to 16 min) as compared to blends with normal wheat starch (**Figure 2.6B**). At higher absorption, the differences between blends containing waxy and normal wheat starch were minimal (**Figure 2.6C**). Our results suggest that waxy starch affects protein hydration. However, under optimal

hydration conditions there was no effect of waxy starch on protein extractability using 50% propanol.

CONCLUSIONS

Waxy wheat flours were reported to result in dough with low gluten index (Guan et al., 2009). Waxy wheat flour had higher free –SH content, and yet produced slack dough which could be due to contribution of free –SH from GSH and other non protein sulphur containing moieties in flour. In all samples, the changes in protein solubility from flour to dough are marked by decrease in gliadin content, increase in SPP and RP. Waxy wheat samples showed significant decrease in γ- gliadins from flour to dough, suggesting the possible role of γ- gliadins as chain terminators in waxy wheat samples. Waxy starch affects protein hydration and gluten formation at lower % water absorption. However, at optimum water absorption waxy starch does not influence protein extractability. Flour 2315 had low protein content, high levels of ω-gliadins, low amount of HMW-GS, higher RP in both flour and dough and gave low gluten index and short mixing times.

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Table 2.1 Mixing properties[a] and Bi-axial extension (Alveograph) results for all flours (N = 5)*.

Flour Sample	% Optimum Absorption [a]	Mixing time (Min) at Optimum Absorption [a]	P (mmH2O)*	L (mm)†	P/L	W (10^{-4} J)‡
Karl 92	60.8	4.82	61 ± 4 ^{e}	134 ± 10 ^{a}	0.46 ± 0.05 ^{c}	270 ± 26 ^{d}
Trego	57.7	4.65	125 ± 3 ^{d}	74 ± 13 ^{bc}	1.69 ± 0.39 ^{b}	345 ± 2 ^{bc}
2114	66.4	4.22	121 ± 2 ^{d}	83 ± 7 ^{b}	1.46 ± 0.12 ^{b}	348 ± 1 ^{bc}
2115	64.3	3.33	141 ± 5 ^{c}	82 ± 8 ^{b}	1.72 ± 0.22 ^{b}	406 ± 1 ^{b}
2205	60.3	3.73	228 ± 11 ^{a}	48 ± 5 ^{d}	4.75 ± 0.47 ^{a}	475 ± 1 ^{a}
2315	61.6	2.46	182 ± 6 ^{b}	42 ± 4 ^{d}	4.33 ± 0.51 ^{a}	323 ± 2 ^{cd}
2489	56.8	3.41	113 ± 4 ^{d}	68 ± 7 ^{c}	1.66 ± 0.17 ^{b}	269 ± 1 ^{d}

[a] previously reported by Guan et al (2009);

* mean ± standard deviation values are reported

mean values with different letters within each column are significantly different ($p < 0.05$)

* P is maximum pressure required to burst the gluten bubble in mm of water

† L is extensibility

‡ W is work done i.e. area under the curve

Table 2.2 Composition of proteins‡ from waxy and normal wheat flour (N=2) analyzed using SE-HPLC*.

Flour							
Flour Sample	Protein Content (%, db)	Extractable Protein (EP)			Insoluble Polymeric Protein (IPP)		
		SPP	Gliadins	Alb/Glb	HMW-GS†	LMW-GS±	RP¥
Karl 92	15.47 ± 0.0	10.37 ± 0.26 ^{a}	38.27 ± 0.72 ^{b}	15.44 ± 0.27 ^{a}	21.45 ± 0.27 ^{c}	10.44 ± 0.52 ^{a}	4.03 ± 0.06 ^{e}
Trego	14.04 ± 0.2	8.25 ± 0.06 ^{cd}	41.70 ± 0.21 ^{a}	14.71 ± 0.32 ^{a}	23.30 ± 0.70 ^{bc}	5.57 ± 0.18 ^{e}	6.47 ± 0.41 ^{d}
2114	13.88 ± 0.1	7.62 ± 0.11 ^{de}	38.08 ± 0.68 ^{b}	10.96 ± 0.26 ^{c}	26.45 ± 0.22 ^{a}	5.77 ± 0.11 ^{e}	11.12 ± 0.71 ^{ab}
2115	13.78 ± 0.1	8.37 ± 0.08 ^{c}	37.23 ± 0.23 ^{b}	13.30 ± 0.07 ^{b}	25.13 ± 0.37 ^{ab}	7.83 ± 0.17 ^{bc}	8.14 ± 0.01 ^{d}
2205	15.1 ± 0.0	9.54 ± 0.01 ^{b}	38.26 ± 0.94 ^{b}	13.16 ± 0.39 ^{b}	22.14 ± 1.59 ^{c}	10.48 ± 0.76 ^{a}	6.42 ± 1.03 ^{cd}
2315	12.00 ± 0.0	4.36 ± 0.16 ^{f}	40.72 ± 0.14 ^{a}	12.43 ± 0.11 ^{b}	22.34 ± 0.70 ^{c}	7.45 ± 0.16 ^{cd}	12.70 ± 0.45 ^{a}
2459	13.33 ± 0.2	8.24 ± 0.06 ^{cd}	41.78 ± 0.95 ^{a}	10.36 ± 0.07 ^{c}	23.59 ± 0.43 ^{bc}	6.21 ± 0.09 ^{de}	9.81 ± 0.60 ^{bc}
2489	12.82 ± 0.1	7.43 ± 0.29 ^{e}	34.35 ± 0.15 ^{b}	14.65 ± 0.19 ^{a}	25.97 ± 0.04 ^{ab}	8.99 ± 0.24 ^{b}	8.62 ± 0.25 ^{c}

‡ expressed as percent of total protein content

* mean ± standard deviation values are reported

mean values with different letters within each column are significantly different ($p < 0.05$)

†HMW-GS – high molecular weight gluten subunits

±LMW-GS – low molecular weight gluten subunits

¥RP – residual protein

Table 2.3 Composition of proteins‡ from waxy and normal wheat dough (N=2) analyzed using SE-HPLC*.

Dough

Flour Sample	Protein Content (%, db)	Extractable Protein (EP) ‡			Insoluble Polymeric Protein (IPP) ‡		
		SPP	Gliadins	Alb/Glb	HMW-GS†	LMW-GS±	RP¥
Karl 92	15.47 ± 0.0	15.44 ± 0.18 ^{ab}	41.93 ± 0.23 ^{a}	13.65 ± 0.12 ^{b}	18.06 ± 0.69 ^{ab}	4.53 ± 0.05 ^{b}	6.40 ± 0.09 ^{a}
Trego	14.04 ± 0.2	14.74 ± 0.96 ^{ab}	39.16 ± 0.29 ^{ab}	14.38 ± 0.30 ^{b}	18.38 ± 0.49 ^{ab}	5.87 ± 0.16 ^{b}	7.46 ± 1.02 ^{a}
2114	13.88 ± 0.1	16.63 ± 0.69 ^{ab}	37.82 ± 0.48 ^{bc}	13.53 ± 0.16 ^{b}	18.60 ± 1.37 ^{ab}	5.52 ± 0.68 ^{b}	7.90 ± 2.10 ^{a}
2115	13.78 ± 0.1	17.80 ± 0.17 ^{a}	37.42 ± 0.53 ^{bc}	15.75 ± 0.32 ^{a}	15.54 ± 0.51 ^{b}	6.26 ± 0.42 ^{b}	7.24 ± 3.13 ^{a}
2205	15.1 ± 0.0	13.12 ± 0.44 ^{b}	36.93 ± 0.97 ^{bc}	13.77 ± 0.28 ^{b}	20.41 ± 0.58 ^{a}	6.37 ± 0.03 ^{b}	9.40 ± 1.41 ^{a}
2315	12.00 ± 0.0	8.55 ± 0.58 ^{c}	37.46 ± 0.79 ^{bc}	13.85 ± 0.40 ^{b}	20.41 ± 1.10 ^{a}	9.03 ± 0.89 ^{a}	10.70 ± 0.38 ^{a}
2459	13.33 ± 0.2	15.29 ± 0.53 ^{ab}	41.32 ± 0.28 ^{a}	11.51 ± 0.10 ^{c}	19.77 ± 1.22 ^{a}	5.53 ± 0.33 ^{b}	6.58 ± 1.69 ^{a}
2489	12.82 ± 0.1	14.74 ± 0.92 ^{ab}	35.70 ± 0.72 ^{c}	14.43 ± 0.39 ^{b}	20.97 ± 0.74 ^{a}	6.42 ± 0.55 ^{b}	7.74 ± 1.15 ^{a}

‡ expressed as percent of total protein content; * mean ± standard deviation values are reported
mean values with different letters within each column are significantly different ($p < 0.05$)
†HMW-GS – high molecular weight gluten subunits
±LMW-GS – low molecular weight gluten subunits
¥RP – residual protein

Table 2.4 RP-HPLC results for gliadin fractions‡ (N = 2)*.

Flour Sample	Flour			Dough		
	Omega	Alpha	Gamma	Omega	Alpha	Gamma
Karl 92	18.27 ± 0.10 ^{b}	36.50 ± 0.30 ^{d}	45.23 ± 0.20 ^{a}	15.38 ± 0.82 ^{a}	39.59 ± 0.25 ^{c}	45.02 ± 1.09 ^{a}
Trego	8.84 ± 0.06 ^{d}	50.38 ± 0.62 ^{a}	40.78 ± 0.67 ^{bc}	9.18 ± 0.01 ^{d}	52.04 ± 0.11 ^{ab}	38.77 ± 0.10 ^{bcd}
2114	15.83 ± 0.44 ^{bc}	45.21 ± 0.03 ^{b}	38.96 ± 0.48 ^{c}	12.92 ± 0.35 ^{b}	49.22 ± 0.19 ^{bc}	37.87 ± 0.16 ^{bcd}
2115	13.90 ± 1.01 ^{c}	44.99 ± 0.06 ^{bc}	41.10 ± 0.94 ^{bc}	12.35 ± 0.92 ^{bc}	48.28 ± 0.39 ^{c}	39.37 ± 0.54 ^{bc}
2205	9.82 ± 0.11 ^{d}	49.75 ± 0.84 ^{a}	40.43 ± 0.95 ^{bc}	9.60 ± 0.18 ^{d}	53.80 ± 0.01 ^{a}	36.61 ± 0.19 ^{cd}
2315	21.62 ± 0.63 ^{a}	37.61 ± 0.25 ^{d}	40.77 ± 0.89 ^{bc}	18.02 ± 0.43 ^{a}	45.90 ± 0.44 ^{c}	36.08 ± 0.01 ^{d}
2459	14.50 ± 0.56 ^{c}	43.07 ± 0.47 ^{c}	42.43 ± 0.09 ^{b}	10.33 ± 0.51 ^{cd}	48.89 ± 2.37 ^{bc}	40.77 ± 1.86 ^{b}
2489	8.30 ± 1.46 ^{d}	46.36 ± 0.69 ^{b}	45.35 ± 0.77 ^{a}	11.88 ± 0.19 ^{bc}	48.91 ± 0.15 ^{bc}	39.21 ± 0.33 ^{bcd}

‡ expressed as % of total peak area; * mean ± standard deviation values are reported
mean values with different letters are significantly different ($p < 0.05$)

Figure 2.1 Changes in free –SH levels in flour and dough among waxy wheat lines and control wheat flour.

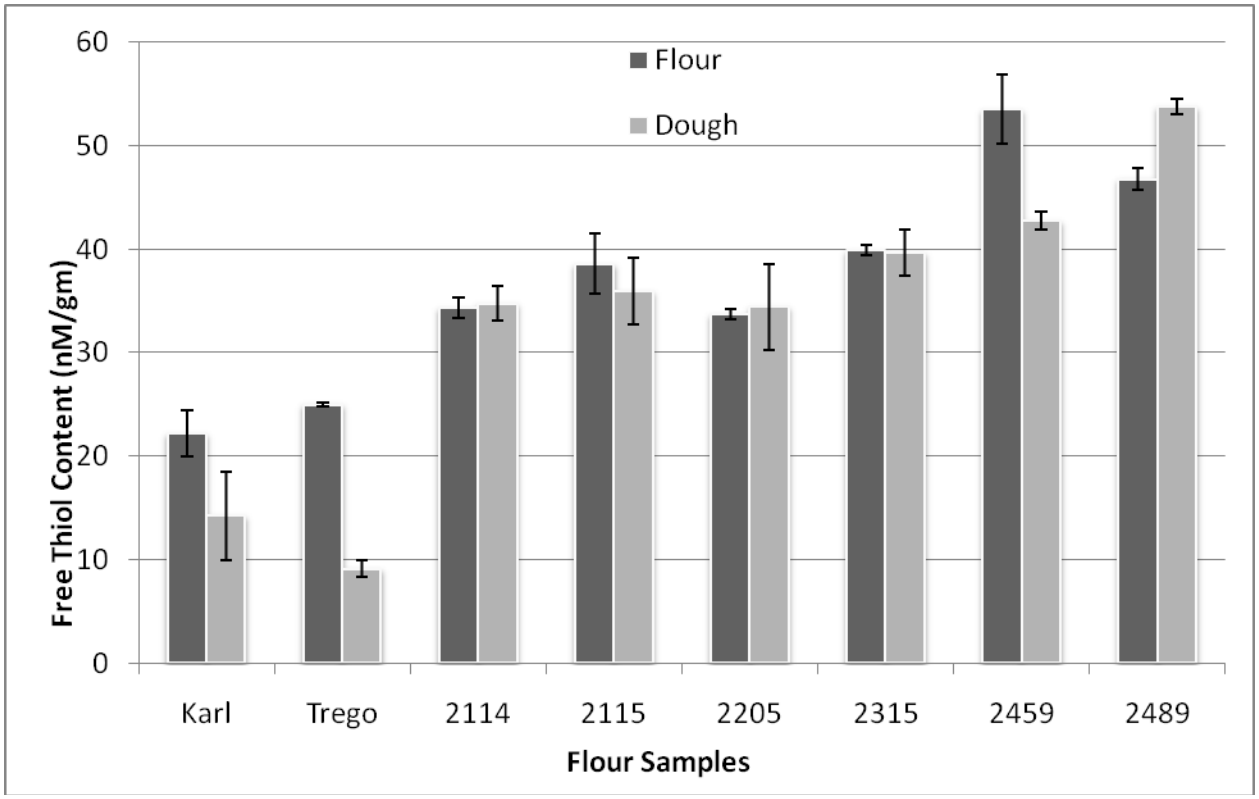


Figure 2.2 SE-HPLC for 50% isopropanol extractable proteins for normal (Karl) and waxy (2315) wheat flour samples.

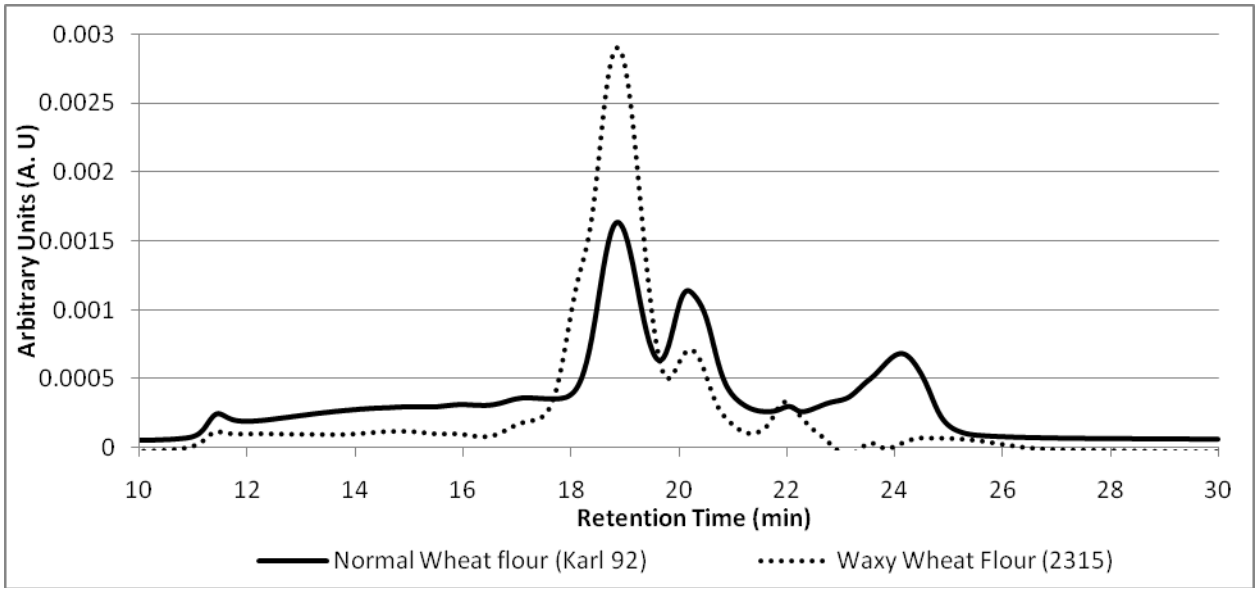


Figure 2.3 SE-HPLC for 50% isopropanol extractable proteins for normal (Karl) and waxy (2315) wheat flour dough samples.

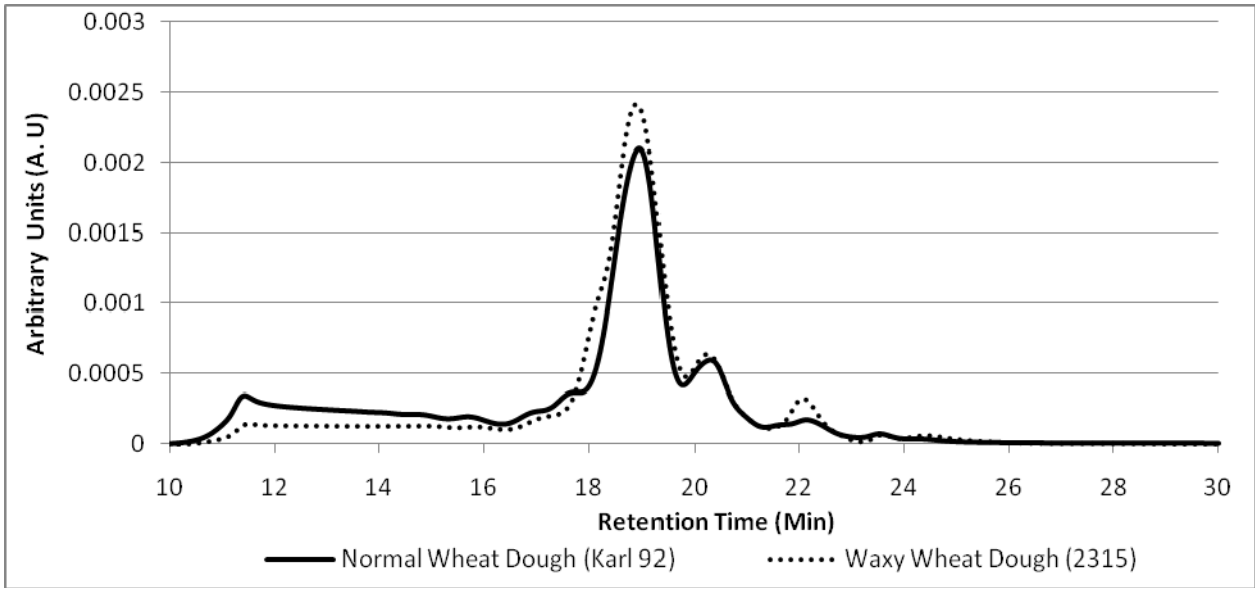


Figure 2.4 RP-HPLC curve for 60% ethanol extractable gliadins for normal wheat flour (solid line) and dough (dotted line).

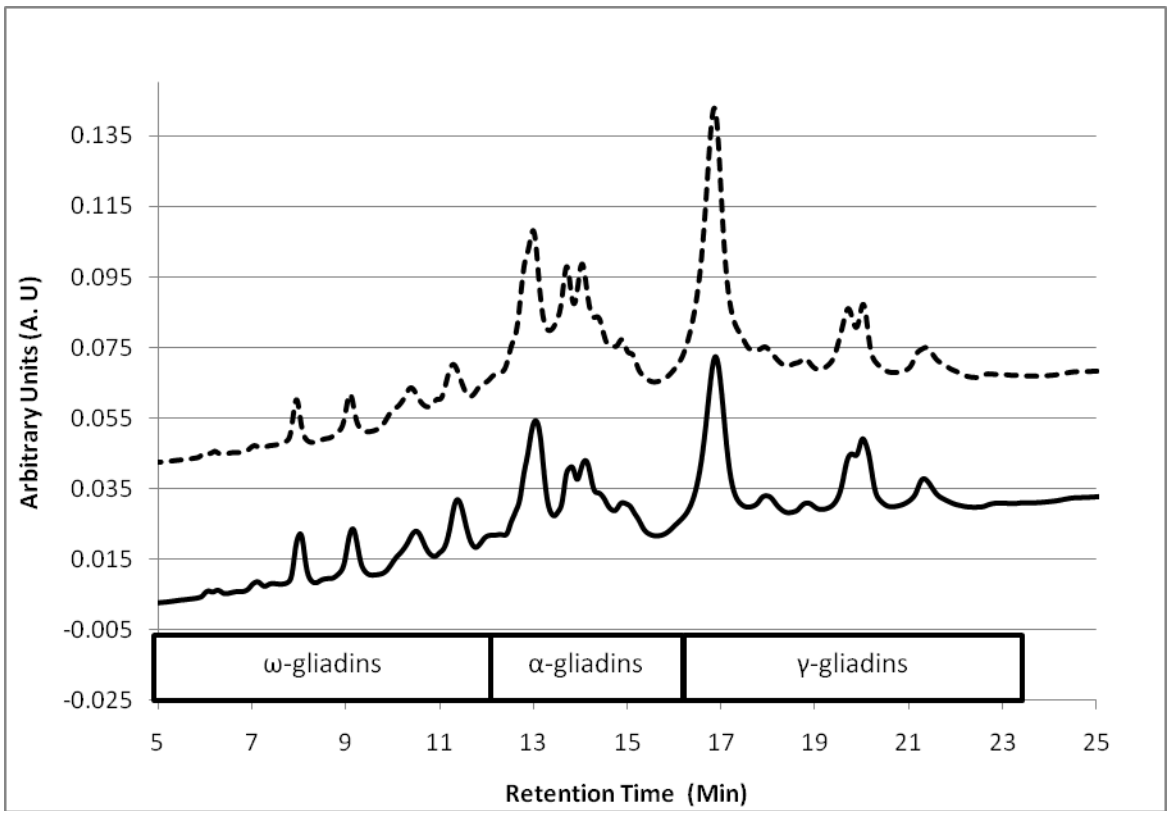


Figure 2.5 RP-HPLC curve for 60% ethanol extractable gliadins for waxy wheat (2315) flour (solid line) and dough (dotted line).

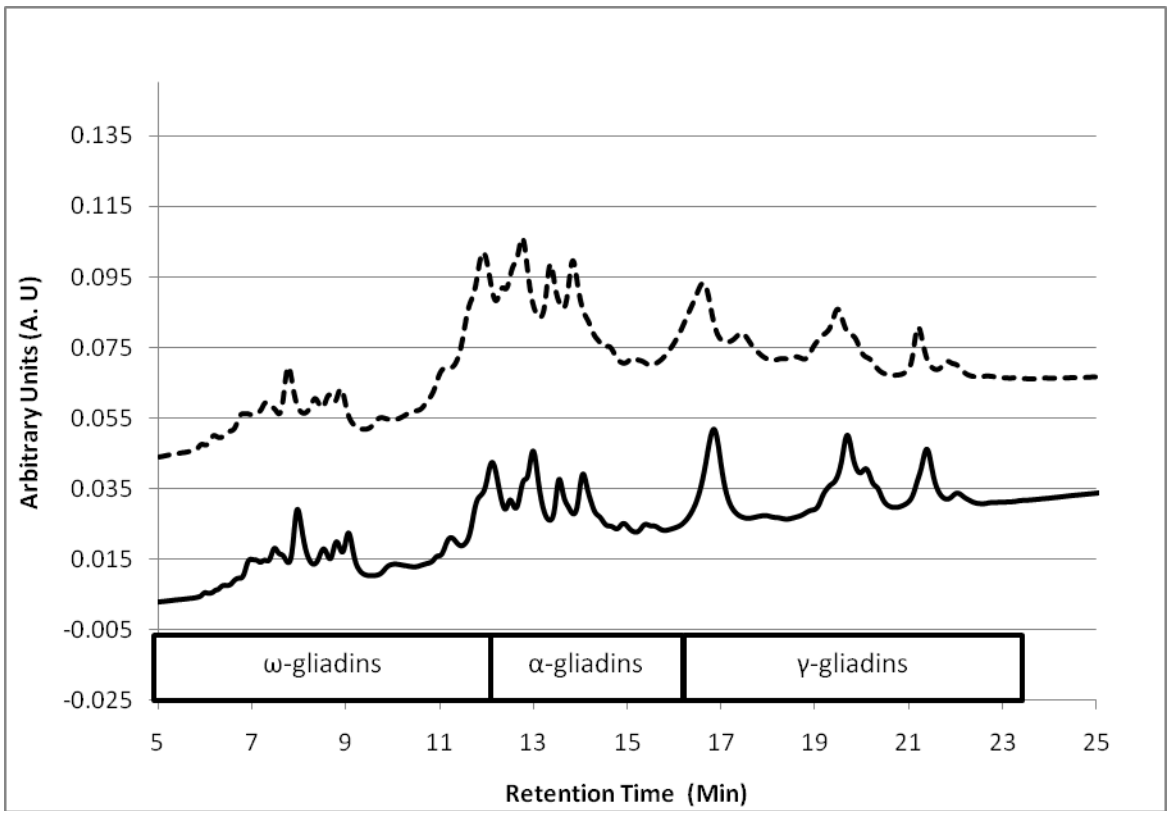
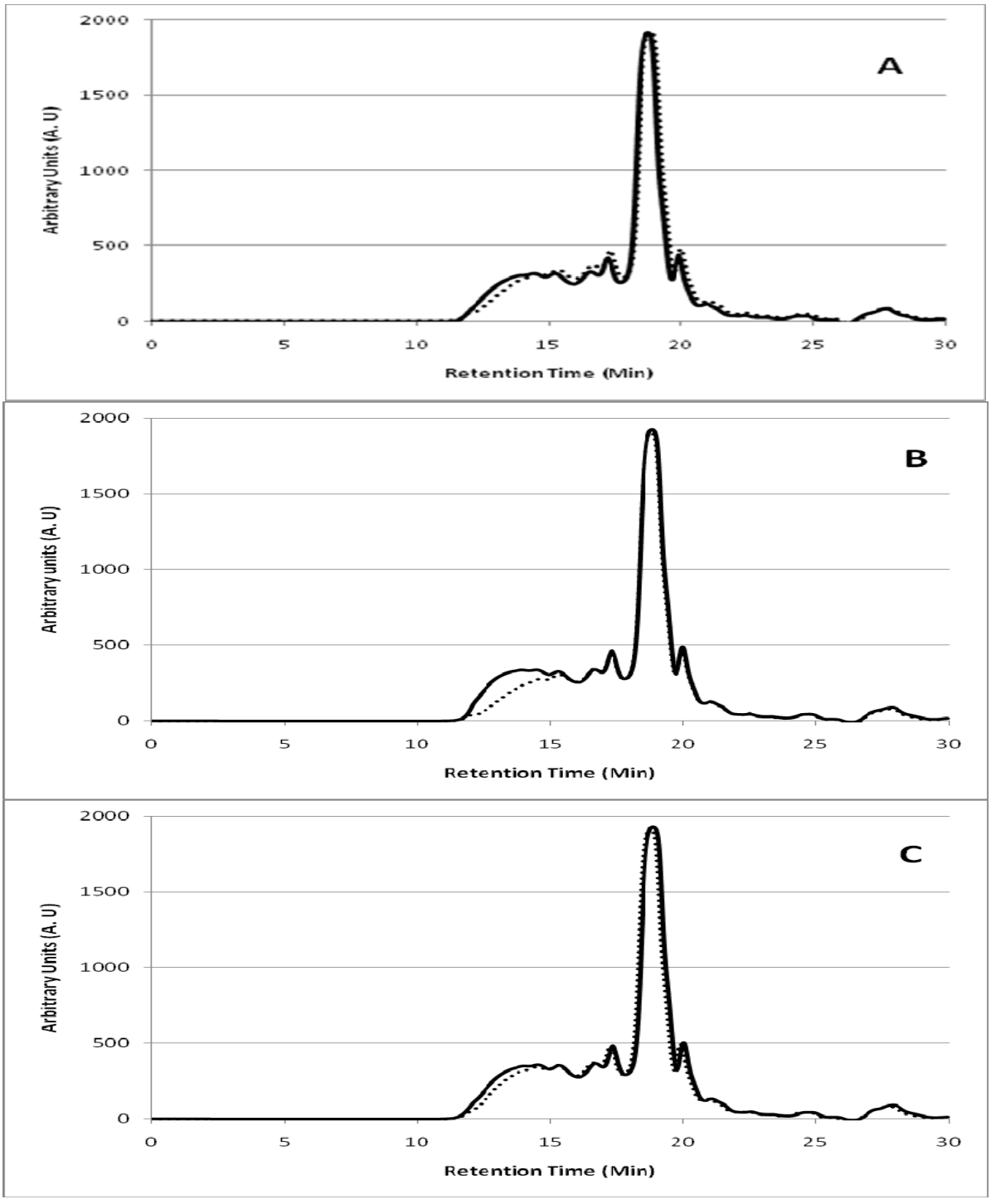


Figure 2.6 Changes in 50% propanol extractable proteins in blends and dough made with vital wheat gluten (VWG) and waxy wheat starch (dotted line) or normal wheat starch (solid line) at (A) no water added, (B) 54% absorption and (C) 66% absorption.



CHAPTER 3 - HEAT MEDIATED CHANGES IN NORMAL AND WAXY WHEAT FLOURS

ABSTRACT

Heat related changes in flour mixing and pasting properties of normal and waxy wheat flours were investigated. Additionally, changes in flour protein and starch were evaluated to understand changes at the molecular level due to thermal processing. Two temperatures (140 and 160°C) and four exposure times (0, 5, 15, 30 and 60 min) were employed. Pasting properties analyzed by Rapid Visco Analyzer (RVA) demonstrated that pasting properties of waxy wheat flours increased upon thermal processing, while pasting properties of normal wheat flour increased to a maximum and then decreased upon further processing. The effects of protease on pasting properties of thermally processed normal flour were lower as compared to waxy wheat flour, suggesting a possible interaction between protein and starch in waxy wheat samples. Mixing properties analyzed by Mixograph indicated lower breakdown of thermally processed samples as compared to their native counterparts. Upon thermal processing, waxy wheat flours had a long hydration time before forming a dough. Heating decreased protein solubility in 50% isopropanol of both normal and waxy wheat flours. However, the decrease was greater for waxy wheat samples. Upon thermal processing, there were no changes in starch molecular weight distribution. The differences in pasting properties between starches isolated from thermally processed flours and thermally processed isolated starches suggest a possible role of flour components in improving pasting properties of starch upon heating. Thermal processing resulted in non-cohesive waxy wheat flours with high peak and end viscosity.

INTRODUCTION

Dry heating of wheat flour has been proposed as an alternative to chlorination of cake flour and as a technique to improve flour and dough functionality (Russo and Doe, 1970; Johnson and Hoseney, 1979; Wolt et al., 1995; Gelinas et al., 2001; Seguchi, 1990). The concept of dry flour heating was investigated for its potential in various other baked goods using both soft and hard wheat flours (Table 1).

Heating of flour induces changes in flour components, i.e. starch, protein and lipid components (Seguchi 1990; Guerriera et al., 1996). Initial studies on the effect of heating on starch were conducted on isolated starch by heating the isolated wheat starch at 120°C for various time periods (Seguchi and Yamada, 1988). Heating improved the ability of starch to bind oil (Seguchi, 1984; Seguchi and Yamada, 1988). Similar results were observed for waxy wheat starch (Hayashi and Seguchi, 2004). Starch was isolated from heated flour and was further classified into prime starch and tailings using an acetic acid fractionation (Sollars, 1958). Heating decreased prime starch and increased tailings fraction of starch (Seguchi et al., 1998; and Sollars, 1958). After heating at 120°C for 3 h all prime starch was incorporated into the tailings fraction, i.e. both these fractions could not be separated using centrifugation (Ozawa and Seguchi, 2006). Additionally, examination of starch granules from heated treated flours indicated no difference in size and appearance from starch granules from untreated flour (Ozawa et al., 2009). Further examination into the changes in prime starch and tailings fraction of starch using differential scanning calorimetery (DSC) and rapid visco analyzer (RVA) elucidated the point that heating at 120°C for up to 30 min did not result in any structural changes in either starch fraction. It is possible that heating at 120°C has not changed the starch structure, however higher temperatures could result in degradation of starch and subsequently lead to unacceptable color

formation (Thomasson et al., 1995). Heat treatment was hypothesized to split some chains of amylose or amylopectin (Seguchi, 1990). The short chain oligosaccharides could undergo "peeling off" mechanism and subsequently converted into α-dicarbonyl compounds (Hollnagel and Kroh, 2000).

Thermally induced modifications of gluten proteins have been extensively studied both on native gluten as well as gluten in flour (Schofield et al., 1983; Guerrieri et al., 1996; Guerrieri and Cerletti, 1996). Studies on thermal treatment of native gluten demonstrated that thermal modifications of gluten are different for different gluten subunits (Booth et al., 1980; Schofield et al., 1983). Glutenin macromolecules and other large polymeric proteins unfold at lower temperature (55 to 75°C) and are locked into denatured state due to di-sulphydryl interchange, while gliadins undergo similar repolymerization at higher temperatures (> 75°C) which could be restored by the action of reducing agent (Schofield et al., 1983). Hansen et al. (1975) proposed that at lower temperatures the gluten protein interact via cross linking through di-sulphide mechanism and at higher temperatures (> 150°C) are degraded into lower peptides.

Flour protein when heated in native flour environment acts as a binder to improve the interactions between prime starch and tailings (Kusunose et al., 2002; Hayashi and Seguchi, 2004). Seguchi (1984) hypothesized that a thin protein film envelopes starch granules and that changes in the protein hydrophobicity plays a vital role in transforming starch granule surface behavior from hydrophilic to hydrophobic. Flours with higher protein content demonstrated complete loss of easily isolated prime starch at shorter time, at elevated temperatures, as compared to flours with lower protein content (Kusunose et al., 2002). When starch granules which were previously heat treated were treated with trypsin, the resulting starch granules showed decreased oil binding capacity, which indicates the modification of starch surface

granule proteins could be facilitating the changes in hydrophobicity of starch granules (Seguchi, 1993; Kusunose et al., 2002; and Hayashi and Seguchi, 2004). Additionally, when gluten fraction was removed from heated flour, the viscosity profiles starch fractions remained unchanged indicating the possible role of gluten in promoting the interaction between prime starch and tailings (Ozawa et al., 2009).

Waxy wheat is relatively new and consists essentially 100% amylopectin. Previous studies show the ability of waxy wheat flours to swell faster and reach peak viscosity at lower temperature (Guan, 2008). Additionally, waxy wheat flour pastes upon cooling do not form gels, and have a potential to be applied in a wide range of food products including soups and thickeners. However, the drawbacks of waxy wheat flours are low cold paste viscosity, low acid stability, high susceptibility to enzyme activity and cohesive texture (Garimella Purna, 2010). Shi (2009) disclosed methods to improve cooked texture of waxy wheat flour. Currently there is lack of information about the changes in various flour components during heating of hard waxy wheat flour. The objectives of this research were to (i) improve pasting properties of waxy wheat flour, i.e. to eliminate cohesive texture and produce waxy wheat flour with short texture (ii) investigate flour dough properties and mixing characteristics of normal and waxy wheat flours change after heat treatment, and (iii) examine the changes in starch and protein induced by heating of wheat flours

MATERIALS AND METHODS

Materials

A normal hard wheat (Karl 92) and a hard waxy wheat (Pedigree: Cimmaron/Rioblanco//Baihou4/L910145/3/Colt/Cody//Stozher/NE86582) were procured from

USDA-ARS, Lincoln, NE. Wheat kernels were tempered to 16% moisture for 18 h and were roller-milled into straight-grade flour on a MLU 202 Bühler experimental mill (Bühler Co., Uzwill, Switzerland).

General methods

Moisture content of native and thermally processed samples was measured by AACC 44-15A (AACC International, 2000). Color measurements (L*, a*, b* color space) were performed using a MINOLTA CR-310 (Minolta, Tokyo, Japan) color meter. *L** is the luminance or lightness component, which ranges from 0 to 100 (black to white), and parameters *a** (from green to red) and *b** (from blue to yellow) are the two chromatic components, which range from −60 to 60 (Papadakis et al., 2000). Protein content of normal and waxy wheat flours were previously reported by Guan et al. (2009) and were measured by AACC 08-01 (AACC International, 2000). The protein content of the flours was 11.44 and 13.01 (%db) for the normal and waxy wheat, respectively.

Heat treatment of flours

Two flours (normal and waxy) were subjected to eight different heating conditions. Heating conditions were two temperatures (140 and 160°C) and four different heating periods, (0, 5, 15, 30 or 60 min) i.e. after the sample has reached the targeted temperature. For each temperature-time combination 10 g of sample was placed in a 12 ounces Quilted Crystal® Jelly Jars (Ball®: 14400-81200) with no cap. After heating the samples were equilibrated to 25°C for

18 hours. The samples were ground using mortar and pestle and were passed through a 425 μm screen.

In separate experiments, moisture content of flours at the end of heating was determined. Each flour (2 g) was weighed into moisture pan the sample was subjected to thermal profile discussed below. At the end of heating, the pan was covered with lid and cooled to 25°C. Moisture content of sample was calculated based on the weight loss of the sample. The experiment was done in duplicate and average values were reported.

Mixing properties

Native and thermally processed flour samples were analyzed for mixing properties using 10 g mixograph (AACC 54-40 A, AACC International, 2000). Water absorption of native flours (both normal and waxy wheat) were optimized for each native flour sample based on series of mixograms. The same water absorption was used to analyze mixing properties of thermally processed samples. Analyses were performed in duplicate.

Rheological Properties

A stress controlled rheometer (Stress Tech HR, ATS Rheosystems, Bordentown, NJ) equipped with a 25mm serrated parallel plate system was used to characterize the rheology of flour dough. The gap between the two plates was set at 2.0 mm. A method described by Summer (2010) was used for creep recovery and stress relaxation tests. The temperature kept constant at 30°C for all creep recovery and stress relaxation tests.

Sample preparation

Dough samples were prepared using flour and water. Optimum absorption and mixing times were calculated using series of 10 g mixograms (**Table 3.1)**. The dough samples were then gently kneaded into ball and placed in a airtight container. The sample was allowed to rest for 30 min prior to measuring rheological properties. Creep recovery and stress relaxation were performed on two freshly prepared dough samples. A 2.0 g sample was then taken from dough and mounted on the bottom plate of the parallel plate measuring system and the gap was adjusted to 2.0 mm. The excess sample (over the edge of the top plate) was trimmed using a sharp blade. Silicone oil was used to prevent sample from drying during analysis. In a separate experiment, time sweep (total of 40 min) was performed, on the normal wheat and a waxy wheat dough sample, to monitor the normal force on the dough sample during resting. Normal force was recorded for every 5 seconds. For both the samples the normal force value between 28 - 32 minutes were within 2% of the final normal force value after 40 minutes. Hence 30 minutes was selected as resting time for all the samples.

Stress relaxation

Stress was measured when the dough samples were subjected to a constant strain of 0.001 for 250 seconds. Temperature was kept constant at 30 °C during the test. Stress (G(t)) was collected and G(t)/G(0) curves for all the curves were calculated. Each analysis was performed in in duplicate (on separately prepared dough samples) and the mean values were reported.

Creep recovery

Dough pieces were relaxed for 30 minutes prior to creep recovery tests. A shear stress of 50 Pa was applied over a creep time of 1200 seconds and recovery time of 1200 seconds. Data for maximum creep strain (MCS), maximum recovery strain (MRS) and precent recovery (recovery strain expressed as percent of MCS) were calculated from each curve. Each analysis was performed in duplicate (on separately prepared dough samples) and mean values are reported.

Free thiol contents

Free thiol estimation was performed using the method by Chan and Wasserman (1993). Standard curve was developed using reduced glutathione (G4251, Sigma Aldirch, St. Louis, MO) at 100, 200, 300, 500 and 100 nmol/ml respectively. The amount of free –SH in flour was reported as nmol/g of flour basis.

Protein extraction using 50% isopropanol

Extractable protein **(**EP) and insoluble polymeric protein (IPP) analysis was done using a method by Bean et al. (1998) with following modifications. Each sample (100 mg) was dissolved in 1 mL 50% isopropanol, vortexed for 5 min and centrifuged at 10,000 x *g* for 5 minutes. Extraction was repeated twice. Supernatant (total 1 ml, 0.5 ml from each extraction) was filtered through 0.45µm filter and analyzed by size exclusion – high-performance liquid chromatography (SE-HPLC). The pellet after two extractions with 50% isopropanol was freeze-dried and analyzed for protein content using LECO™ FP-428 nitrogen determinator (LECO, MI).

SE- HPLC analysis

The supernatants filtered through 0.45 μm filter were analyzed by SE-HPLC analysis (Agilent 1100 Series, Agilent Technologies, Palo Alto, CA). The samples were analyzed using Bio-Sep-SEC- 4000 column 300 x 7.80 mm (Phenomenex, Torrance, CA), kept at 30°C, with injection volume of 20 μL; eluting solvent : acetonitrile : water (1:1) containing 0.5% tri-floro acetic acid ; run time: 30 min; flow rate: 0.5 ml/min.

Starch isolation

Thermally treated flours (50 g, db) were suspended in distilled water (450 mL). The pH of the suspension was adjusted to 10.0 using 0.25M NaOH. Protease enzyme (Protex 6L, Genencor International, Palo Alto, CA) was added at 0.05% based on the weight of flour and the suspension was incubated at 40°C for 24 hours under gentle agitation. The mixture was passed through 200 mesh sieve and the throughs were centrifuged at 2,000 X g for 15 min. The top layer (tailings portion) of the pellet was scrapped and discarded. The sediment containing prime starch was re-suspended in water mixed for 10 min, centrifuged 2,000 X g for 15 min. The process was repeated twice. The final pellet was dried in an oven at 30°C for 24 hours. Dried starch was analyzed for protein and moisture content.

Synchrotron Wide-Angle X-ray Diffraction (WAXD) measurements

WAXD experiments were carried out at the Advanced Polymers Beamline (X27C) in the National Synchrotron Light Source (Brookhaven National Laboratory, Upton, NY). The details of the experimental setup at the X27C beamline have been reported elsewhere (Chen et al, 2006; and Chen et al, 2007). The wavelength used was 0.1371 nm. The sample-to-detector distance

was 129 mm. A 2D MAR-CCD (MAR USA, Inc.) X-ray detector was used for data collection.

Starch debranching and analysis

Non-granular defatted starch was prepared using method a by Kong et al. (2008) with following modifications. Granular starch (200 mg) was dissolved in 10 mL of di-methyl sulphoxide (DMSO) by heating the mixture in a boiling water bath for 3 hrs. The dispersion was cooled to 25 °C for 30 min and subsequently 50 ml of 95% ethanol was added with continuous stirring, a further 50 ml of 95% ethanol was added and the mixture was left at 25°C for 30 min and then centrifuged at 2500 x *g* for 10 min. The supernatant was discarded and the sediment was re-suspended in 20 ml of 95% ethanol, centrifuged at 2500 x *g* for 10 min. The process was repeated using 20 ml of 95% ethanol and once using 20 ml of acetone. The pellet was then vacuum dried. The non-granular starch (40 mg) was then dissolved in 4 ml of DMSO by heating the mixture in a boiling water bath for 1 hr and subsequently cooled to 25 °C for 30 min. To the mixture, 14 ml of 0.01M sodium acetate buffer at pH 4.0 and 4.0 µl of isoamylase (from *Psuedomonas amyloderamosa*, 250 U/ml, EC 3.2.1.68, Hayashibara Shoji Inc., Okayama, Japan) were added to debranch the starch. Debranching was carried out overnight by constant stirring the samples in a water bath at 50°C. The reaction was stopped by heating the mixtures for 1 hour in a boiling water bath. The mixture was cooled to 25°C for 30 min and subsequently starch was precipitated by adding 200 ml of acetone under constant agitation. The mixture was stirred for another 30 min and stored in a freezer overnight. The mixture was centrifuged at 2500 x *g* for 10 min, the supernatant was discarded and the sediment was re-suspended in 25 ml acetone and centrifuged at 2500 x *g* for 10 min. The sediment was then vacuum dried. Dried debranched starch (4 mg) was dissolved in DMSO (4 ml) by heating the mixture in a boiling water bath for 3

hrs. The mixture was subsequently cooled to 25 °C for 30 min, filtered using 2μm filter prior to analyzing using gel permeation chromatography (GPC).

Molecular weight distribution of debranched starch was determined by GPC. The GPC analysis was performed with a PL-GPC 220 Integrated GPC/SEC fully automated system (Polymer Laboratory, Amherst, MA). The system was equipped with an auto sampler, a differential refractive index (DRI) detector and Phynogel 00H-0646-KO, 00H-0644-KO, 00H-0642-KO columns (Phenomenex, Torrance, CA) connected in a series. The mobile phase in the column was DMSO with 5mM $NaNO_3$. The flow rate of 0.8 mL/min and the column oven temperature was controlled at 80°C. A series of ten dextran standards (American Polymer Standards Corporation, Mentor Ohio) with different molecular weights were used to calibrate the retention time with molecular weight. The electronic outputs of the DRI detectors were collected by GPC software (version 3.0, Polymer Laboratories, Amherst, MA).

Heat treatment of blends of vital wheat gluten and isolated starches

Commercial vital wheat gluten (VWG) (King Arthur, Norwich, Vermont) (82% protein (db), 8.1% moisture) and isolated waxy starch from thermally processed waxy wheat flour (sample 2114, 99.6% starch (db), 7.5% moisture) were heated individually as well as in a blend at 160°C for 30 min. VWG and starch were mixed to give blends of (i) unheated VWG and unheated starch, (ii) unheated VWG and heated starch, (iii) heated VWG and unheated starch and (iv) heated VWG and heated starch. The blends contained at final protein content of 14.5% (db) (VWG – 1.63 g and starch – 7.62 g). In addition, unheated starch and heated starch were also obtained. Pasting properties of blends and starches were determined at the same starch content (7% dry basis).

Pasting properties

Flour pasting properties were determined with a Rapid Visco Analyzer (RVA, Foss North America, Inc., MN). A 25-g mixture of flour and pH 3.0 (citrate buffer) or water (10% solids level) was prepared in an RVA canister. Citrate buffer was prepared by mixing 3:2 volumes of 1M tri-sodium citrate (W302600, Sigma-Aldrich, St. Louis, MO) and 0.4M of citric acid (C1857 Sigma-Aldrich, St. Louis, MO) respectively. Final weight in the RVA canister was 28 g. An RVA paddle was inserted into the canister, and the mixture was gently agitated to disperse flour lumps. The RVA canister was then subjected to a 13-min RVA test to determine flour pasting properties (Deffenbough and Walker, 1989). After RVA analysis the cooked pastes were cooled at 25°C for 6 hours and subsequently analyzed visually for their texture. They were classified as gel, cohesive or non-cohesive pastes. Isolated starch samples were analyzed for their pasting properties at 7% solids. The RVA curves were analyzed for pasting properties i.e. peak, hot paste, breakdown and cold paste viscosity using Thermocline for Windows 3 (TCW3) software provided with the RVA.

To understand the role of protein on pasting properties, each flour (2.8 g on dry basis) was suspended in water (12.5 g) containing 18 mg of protease (Sigma P-5147, 4.5 units/mg of protein, St. Louis, MO) and incubated at 37°C for 30 min. Subsequently, 2 mM silver nitrate solution was added to the protease hydrolyzed flour to a total weight of 28 g (10% solids).

Thermal properties of flours

Thermal properties of thermally processed flours were measured by differential scanning calorimetry (DSC) (Perkin-Elmer, Norwalk, CT). The DSC was calibrated with indium and zinc.

Flour samples were mixed with water prior to analyzing thermal properties. Mixtures of flour and water were prepared in small beakers using 100 mg of flour (dry basis) and adjusting the final weight to 300 mg using distilled water. A portion of the mixture (40 mg) was then transferred to a DSC pan. The pan was hermitically sealed and allowed to equilibrate at 25°C overnight. The samples were then heated from 10°C to 140°C at 10°C /min to determine the gelatinization properties of flours. The pans were stored for 7 days at 4°C and rescanned using the same conditions described above to determine the retrogradation properties of flour. Each sample was analyzed in duplicate and mean values were reported.

Statistical analysis

Macanova 4.12 (School of Statistics, University of Minnesota, Minneapolis, MN) was used to perform ANOVA and Tukey's honest significance difference (HSD) analysis. The level of significance was $P < 0.05$ for all the analyses.

RESULTS AND DISCUSSION

Moisture changes due to thermal processing

Normal and waxy wheat samples were subjected to similar heating profiles and the changes in moisture immediately after thermal processing were reported (**Table 3.2**). Initial flour moisture was about 13%, and most of the moisture was lost upon heating (140 and 160°C). As expected, moisture content of the samples decreased with increasing time of heat treatment. At the most extreme exposure conditions (60 min at 140°C; and 30 min at 160°C) both the samples had less than 0.5% moisture.

The samples were equilibrated at 25°C for 24 hours, during which samples absorbed some moisture. Heating profile had a significant effect on moisture absorbed. Samples with prolonged exposure had significantly lower moisture as compared to samples at lower exposure time (**Table 3.3**).

Effect of thermal processing on flour pasting properties

Distilled water (neutral conditions)

Thermal processing induced significant changes in pasting properties of both waxy and normal wheat flours (**Table 3.4**). Pasting properties of normal wheat samples analyzed in neutral conditions indicate that peak viscosity increased to a maximum and then decreased with subsequent increase in the time of the heat treatments (**Table 3.4**). The changes in pasting properties were different for samples processed at two temperatures. At 140°C, there was an increase in peak viscosity, hot paste viscosity and cold paste viscosity (CPV) up to 30 min heating followed by a decrease at longer times. At 160°C there was an increase in peak viscosity up to 15 min heating followed by a decrease. After waxy wheat flour was treated at 140°C for 30 min, the peak viscosity doubled and the CPV increased from 305 cP to 2328 cP (**Table 3.4**).

For waxy wheat samples, trends in peak viscosity were similar to normal wheat samples, i.e. peak viscosity increased and then decreased upon further heating at both temperatures. At 140°C, peak viscosity reached its maximum after 30 min, whereas at 160°C it reached at 15 min heating period. However, CPV increased with increasing thermal processing. Increases in CPV were higher at 160°C as compared to 140°C.

All normal wheat flours resulted in gel texture, while texture of native waxy wheat was cohesive. However, upon heating (> 15 min at 140°C and > 5min at 160°C) waxy wheat flours

resulted in cooked pasted with short texture (non-cohesive) with high viscosity. Consequently, heating improved the texture of pastes from waxy wheat flours from cohesive (low viscosity) to non cohesive (high viscosity) without gel formation. To further evaluate the stability of the pastes from thermally processed samples, the flour pasting properties were analyzed in acidic conditions.

Citrate buffer pH 3.0 (acidic conditions)

Pasting properties of normal wheat samples analyzed in acidic conditions (pH 3.0) showed very slight changes in pasting properties (**Table 3.4**). It is interesting to note that there were minimal differences between the pasting profiles of normal wheat at two different temperatures (**Table 3.4**). Both native normal and thermally processed normal wheat flour samples show higher pasting profile in acidic conditions as compared to distilled water.

Pasting properties of waxy wheat samples in acidic conditions (pH 3.0) processed at both temperatures indicate an increase in overall pasting properties with increase in heating time. Previous studies have shown stronger association of protein and starch at acidic pH (Dahle, 1971). At lower pH it was postulated that starch and protein contain opposite charges and thus could have increased interactions (Dahle, 1971).

Our results indicate that upon thermal processing, waxy wheat flours result in increased viscosity and result in pastes that are more acid stable as compared to native waxy wheat flours. The changes in pasting properties of flour upon thermal processed could be due to (i) the changes in the starch itself or (ii) the impact of other components such as protein on starch. Both the protein and starch were isolated from the heat treated samples and were studied for the possible changes occurring in those moieties.

Previous researchers have hypothesized that protein adsorbs on to starch surface upon thermal processing (Seguchi, 1993). In order to determine the role of protein, we used protease enzyme to hydrolyze protein and compared the pasting properties of flours before and after the protein matrix was disrupted. Two heating conditions i.e. 160°C for 0 min and 30 min were used. Pasting curves for native samples are shown in Figures 1A (normal wheat) and 1D (waxy wheat). For native samples (in both normal and waxy wheat flours) there was an increase in breakdown viscosity upon the action of protease. This could be the role of starch surface granule proteins in restricting the swelling of granules and thereby preventing shear induced breakdown of starch pastes (Debet and Gidley, 2007).

Thermally processed normal wheat samples showed no difference in pasting properties due to the action of protease (**Figures 3.1B and 3.1C**). However, there were significant changes in pasting properties of thermally processed waxy wheat flours upon action of protease (**Figures 3.1E and 3.1F**). For thermally processed waxy wheat samples under mild conditions (160°C for 0 min) there was a small decrease in peak viscosity, followed by an increase in the peak time. In contrast, samples processed at 160°C for 30 min showed significant decrease in pasting properties upon action of protease.

Our results clearly suggest a possible interaction between protein and waxy wheat starch. To further evaluate the role of protein on altering pasting properties of waxy wheat starches, blends of waxy starch and VWG were prepared (14.5% protein in final blend) and were analyzed for their pasting properties. Heating was carried at 160°C for 30 min. RVA curves (**Figure 3.2A and 3.2B**) of blends made from individually heated VWG and starch show no effect of heating VWG on pasting properties of blends. The pasting properties were mainly influenced by the presence of heated or unheated starch. Unheated starch showed higher peak viscosity as

compared to heated starch. However, when VWG and starch were blended prior to thermal treatment, pasting curve demonstrated lower shear thinning of pastes during heating and holding and higher cold paste viscosity (**Figure 3.2C**). Our results show the effect of protein on pasting properties, and the improved cold paste viscosity is possible only through interaction of protein and starch. Additionally, when isolated starch was heated (i.e. without presence of VWG) it showed a decrease in peak viscosity but increase in breakdown and cold paste viscosities (**Figure 3.2D**).

Pasting properties of starch isolated from thermally processed flours

For starches isolated from thermally processed normal wheat flours, protein content was 0.66 and 0.58% (db) for samples processed at 160°C for 0 and 30 min respectively; while for starches isolated from thermally processed waxy wheat flours, protein content was 0.55 and 0.53% (db) for samples processed at 160°C for 0 and 30 min respectively. Pasting properties of isolated starch samples (at 7% solids) from thermally processed normal wheat flours showed a shift in the peak viscosity and a decrease in pasting properties with an increase in exposure time (**Figure 3.3**). Starch isolated from waxy wheat samples show a decrease in pasting properties with increase in processing time (**Figure 3.3**). The changes in starch pasting properties were different to the changes in flour pasting properties due to thermal processing, indicating the role of other flour components on flour pasting properties.

Characteristics of starch isolated from thermally processed waxy wheat flours

Three starch samples were isolated from native waxy wheat flours and the flours processed at 160°C for 0 and 30 min and studied for thermally induced modifications of starch. Debranched starches from thermally processed waxy wheat flours were evaluated for changes in

molecular structure distribution, while WAXD was used to evaluate crystallinity changes in flour. GPC analysis shows no effect of thermal processing on molecular weight distribution of debranched starch (**Figure 3.4**). Additionally, WAXD results (**Figure 3.5**) show no changes in starch crystallinity due to thermal processing of waxy wheat flour. To further evaluate changes in starch, thermal analysis was conducted to obtain gelatinization and retrogradation properties of thermally processed flours (**Table 3.5**). There were no changes in gelatinization properties of waxy wheat samples. However, thermal processing resulted in lower peak temperature, end temperature and lower delta H values for normal wheat flour.

Our results of initial increase in peak viscosity of wheat flours upon thermal processing are in agreement with previous reports by Kusunose et al. (2002) and Ozawa et al. (2009), who have observed similar increase in peak viscosity of heating wheat flours at 120°C. They have demonstrated the effect of the gluten fraction on increased viscosity of thermally processed normal wheat samples, with no changes in starch granule structure. Our blend study in conjunction with GPC analysis agree with previous studies (Kusunose et al. (2002) and Ozawa et al. (2009)), that improving both peak viscosity and cold paste viscosity of waxy wheat samples can only be possible due presence of protein and other native flour environment. However, the decrease in delta H values for normal wheat samples, along with a decrease in peak viscosity suggest that starch related changes could be a possibility in normal wheat samples.

When isolated starches were heated alone, they resulted in decrease in their peak viscosity while increasing their end viscosity data (**Figure 3.2.D**). We hypothesize that adsorption of protein onto starch during hydrothermal treatment is a key part to improving starch pasting properties.

Mixing and rheological properties of thermally processed samples

Mixing properties

A series of mixograms were performed to determine the optimum % absorption for the native wheat flours. The optimum water absorption for waxy and normal wheat was 66.8% and 64.4% respectively. The absorption levels were kept constant for both native and thermally processed samples. Upon thermal processing all samples showed improved tolerance to breakdown upon mixing (**Figure 3.6**). As the time of exposure increased, the peak time increased for both normal and waxy wheat samples.

Width of the mixing curve of thermally processed normal wheat samples was significantly lower compared to native normal wheat flour. Heating at any of the temperatures (i.e. 140 or 160°C) resulted in similar mixograph profiles, where tolerance to mixing increased with increase in time of heating. The final dough (after 10 min mixing) for thermally processed samples ($\geq$ 30 min at 140°C; and $\geq$ 15 min at 160°C) resembled at in-elastic mass.

Mixing curves of waxy wheat samples were influenced by the temperature of heating. At lower temperature (140°C), with increase in time of exposure, the width of mixographs increased. The flours became tolerant to mixing and showed lower breakdown during mixing. At higher temperature (160°C) and prolonged heating time mixograph curves demonstrate a hydration stage during initial mixing and upon continued mixing form an elastic mass with no breakdown. The hydration stage/time increased with increase in thermal processing time (**Figure 3.3**).

The changes are different for normal and waxy wheat samples suggesting possible thermo susceptibility of waxy wheat samples as compared to normal wheat samples. In normal wheat samples, the flour tended to hydrate fast but fail to form visco-elastic dough; however in

waxy wheat samples there is a long hydration time followed by very strong elastic mass. To further test fundamental rheological properties, dough samples mixed using same water absorption to same mixing time were analyzed using dynamic mechanical analyzer.

Rheological properties

Creep recovery and stress relaxation tests were performed to evaluate rheological characteristics of dough. From creep recovery tests, MCS, MRS and % recovery for both normal and waxy wheat samples at various thermal processing times were calculated and are reported in **Table 3.6**. The data for % recovery indicates a gradual decrease with increase in processing time for both normal and waxy wheat samples. Samples with higher percent recovery are termed more elastic (Wang and Sun, 2002). Consequently, flours became more in-elastic upon thermal processing. The stress relaxation data (**Figure 3.7**) suggests that dough samples from thermally processed samples showed lower strain at constant strain, further validating the loss of elasticity in dough upon thermal processing of flours.

Changes in protein composition

With an increase in temperature, there was a decrease in free –SH content for both waxy and normal wheat flours (**Figure 3.8**). The decrease in free –SH was drastic in waxy wheat samples compared to normal wheat samples. Our results indicate that protein in waxy wheat samples is prone to thermal susceptibility as compared to normal wheat sample.

IPP content increased with thermal processing for both waxy and normal wheat samples (**Table 3.7**). The increase in %IPP with thermal processing was greater in waxy wheat

samples as compared to normal wheat samples. For samples processed at 160°C for 30 min, about 70% of the protein was unextractable using 50% propanol.

In our study, protein was extracted using 50% isopropanol and analyzed by SE- HPLC. The area under the SE-HPLC curve was divided into three regions as described by Bean et al. (1998) soluble polymeric protein (SPP, > 70kDa), gliadins (Gli, 13-70 kDA) and albumins and globulins (Alb/Glb, <13 kDA).

Extractability profiles were different for normal and waxy wheat flours (**Table 3.7**). For normal wheat flours, similar extractability profiles were observed at 140 °C and 160 °C. There were no significant changes in SPP at both temperatures. For waxy wheat samples, changes in SPP were different at different temperatures. At a higher temperature, there was significant decrease in SPP upon prolonged heating.

Extracted gliadins were significantly higher in normal wheat samples at both temperatures and over various heating times as compared to waxy wheat counterparts. For normal wheat samples, extractable gliadins were higher at 160°C compared to 140°C. For waxy wheat samples extractable gliadins decreased upon heating (at both temperatures) and the rate of decrease was greater at higher temperature (**Table 3.7**).

Changes in protein composition plays a major role in controlling the mixing properties of flours. During mixing gluten proteins interact via di-sulphide/sulphydryl exchange to form large protein aggregates and bestow the unique visco-elastic property to dough. However, heating results in failure of gluten proteins to form dough upon excessive heating (Geedes, 1929), which could be due to greater thermal susceptibility of high molecular weight proteins than low molecular weight protein and gliadins (Schofield et al., 1983). From our results of reduction in free –SH, the failure of proteins to form dough could be due to irreversible cross-linking of

proteins during thermal processing. More recent studies on normal wheat have indicated that upon heating flours at 120°C for 120 min, the flours show a long hydration phase in mixograph followed by very low breakdown of dough, which was attributed to increased hydrophobicity of proteins and starch (Ozawa and Seguchi, 2006).

Color

There was decrease in lightness (L) and increase in yellowness (b*) for the samples upon exposure to thermal treatments (**Table 3.3**). The L values decreased with an increase in the length of the exposure time, while b* values increased with increase in exposure time. At 140°C, there were no differences in color between waxy and normal wheat flours. However when heated at 160°C, the waxy wheat flours had lower L values as compared to the normal wheat flour. Additionally, at 160°C decrease in L values was greater in waxy wheat samples as compared to normal wheat samples. Formation of Maillard browning compounds has been demonstrated to cause decrease in L values and increase in a* and b* values, when flours were heated at > 150°C (Gokmen and Senyuva, 2006).

CONCLUSIONS

Thermal processing affected pasting and mixing properties of waxy and normal wheat flours differently. Thermal processing of waxy wheat flour can be successfully utilized to increase cold paste viscosity in neutral conditions and increase acid stability of hot pastes. Additionally, thermal processing of waxy wheat flour resulted in cooked paste with non-cohesive texture and high viscosity. Consequently, thermal processing can enhance use of waxy wheat flour in food applications. Isolated waxy wheat starch heated in presence of gluten protein

displayed higher cold paste viscosity than starches heated in absence of protein. Moreover, upon digesting protein in thermally processed waxy wheat flour, the resulting paste displayed lower peak and cold paste viscosity, which indicate that protein plays an important role in altering pasting properties of waxy wheat flour during thermal processing.

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Table 3.1 Application of dry heat treatment in various food applications

Flour type	Product	Temperature (°C) range	References
Soft wheat flour	Cake	50-125	Russo and Doe, 1970; Cuvain et al., 1976; Hanamoto and Bean, 1979; and Thomasson et al., 1995
	High ratio cake	120	Fustier and Gelinas, 1998
	Pancake	60-140	Seguchi, 1990; Seguchi, 1993; Ozawa and Seguchi, 2006; Ozawa and Seguchi, 2008; and Seguchi et al., 1998
	Kasutera cake (Japanese sponge cake)	120	Nakumura et al., 2007
Hard wheat flour	Dough stability	80°C	Gelinas and McKinnon, 2004
	Bread	50-130	Wolt et al., 1995; and Gelinas et al., 2001
	Non-cohesive pastes	100-160	Shi, 2009

Table 3.2 Moisture content of sample after heating[*] (N =2)[‡]

Heating Condition (°C/Min)	Normal Wheat	Waxy Wheat
140/0	1.40 ± 0.06 [b]	1.32 ± 0.03 [a]
140/15	1.28 ± 0.08 [c]	1.11 ± 0.01 [b]
140/30	0.26 ± 0.01 [e]	0.41 ± 0.00 [d]
140/60	0.09 ± 0.01 [f]	0.00 ± 0.01 [e]
160/0	1.70 ± 0.06 [a]	1.35 ± 0.03 [a]
160/5	1.49 ± 0.06 [b]	1.28 ± 0.04 [a]
160/15	0.98 ± 0.06 [d]	0.77 ± 0.06 [c]
160/30	0.21 ± 0.01 [e]	0.08 ± 0.03 [e]

[*] mean ± standard deviation values are reported

[‡] mean values with different superscripts within each column are significantly different ($p < 0.05$)

Table 3.3 Moisture content and color values[‡] (L, a*, b*) for normal and waxy samples (N = 2)

Heating Condition (°C/Min)	% Moisture	L	a*	b*	% Moisture	L	a*	b*
Sample	**Normal Wheat**				**Waxy Wheat**			
Native	13.20	90.43	-2.22	+8.91	12.40	90.56	-2.44	+9.36
140/0	7.96	90.63	-2.08	+8.48	6.39	89.54	-1.65	+8.18
140/15	3.62	90.39	-2.08	+8.59	6.68	90.03	-1.89	+7.86
140/30	3.09	90.15	-2.08	+8.68	6.34	89.72	-1.82	+8.42
140/60	2.57	89.24	-1.69	+9.32	6.25	89.43	-1.78	+8.82
160/0	8.09	89.20	-1.52	+7.08	6.15	88.59	-1.45	+10.16
160/5	6.38	89.86	-1.54	+7.19	5.08	89.67	-2.03	+8.52
160/15	5.16	89.28	-2.09	+8.64	5.44	88.22	-1.42	+10.52
160/30	4.77	88.79	-1.49	+10.22	5.44	85.51	-0.48	+12.81

[‡] the standard deviation for all samples was less than 0.001 and hence not reported

Table 3.4 RVA pasting properties (10% solids) for normal and waxy samples in neutral and acidic conditions[*] (N =2)[‡]

Heating Condition (°C/Min)	Normal Wheat (pH 7.0)				Normal Wheat (pH 3.0)			
	Peak Viscosity	Hot Paste Viscosity	Break Down	Cold P	Peak Viscosity	Hot Paste Viscosity	Break Down	Cold P
Native	1971 ± 15 ^{ef}	1125 ± 15 ^{de}	847 ± 25 ^{de}	994 ± 11 ^{e}	2230 ± 11 ^{c}	865 ± 14 ^{e}	1365 ± 25 ^{d}	1786 ± 20 ^{cd}
140/0	2196 ± 39 ^{d}	1498 ± 29 ^{a}	698 ± 10 ^{f}	2567 ± 52 ^{c}	2680 ± 39 ^{ab}	948 ± 15 ^{cd}	1732 ± 24 ^{ab}	1746 ± 28 ^{cd}
140/15	2204 ± 16 ^{cd}	1422 ± 8 ^{b}	782 ± 25 ^{e}	2548 ± 25 ^{c}	2567 ± 42 ^{b}	903 ± 14 ^{de}	1664 ± 28 ^{b}	1665 ± 42 ^{d}
140/30	2448 ± 8 ^{a}	1443 ± 17 ^{ab}	1005 ± 25 ^{ab}	2775 ± 23 ^{b}	2778 ± 54 ^{a}	984 ± 21 ^{bc}	1795 ± 33 ^{a}	1828 ± 36 ^{c}
140/60	2057 ± 10 ^{e}	1171 ± 5 ^{d}	887 ± 5 ^{cd}	2701 ± 18 ^{b}	2581 ± 43 ^{b}	1046 ± 19 ^{ab}	1535 ± 24 ^{c}	2164 ± 8 ^{a}
160/0	2260 ± 19 ^{cd}	1285 ± 13 ^{c}	975 ± 6 ^{ab}	2917 ± 1 ^{a}	2592 ± 8 ^{b}	1036 ± 6 ^{ab}	1556 ± 13 ^{c}	2032 ± 31 ^{b}
160/5	2307 ± 9 ^{bc}	1279 ± 11 ^{c}	1028 ± 1 ^{a}	2942 ± 2 ^{a}	2568 ± 21 ^{b}	1018 ± 9 ^{ab}	1551 ± 12 ^{c}	1990 ± 41 ^{b}
160/15	2411 ± 28 ^{ab}	1465 ± 3 ^{ab}	946 ± 25 ^{bc}	2771 ± 30 ^{b}	2616 ± 46 ^{b}	918 ± 30 ^{cde}	1698 ± 16 ^{b}	1755 ± 33 ^{d}
160/30	1927 ± 54 ^{f}	1068 ± 36 ^{e}	860 ± 8 ^{d}	2338 ± 65 ^{d}	2604 ± 4 ^{b}	1066 ± 14 ^{a}	1538 ± 11 ^{c}	2169 ± 24 ^{a}
	Waxy Wheat (pH 7.0)				**Waxy Wheat (pH 3.0)**			
Heating Condition (°C/Min)	**Peak Viscosity**	**Hot Paste Viscosity**	**Break Down**	**Cold P**	**Peak Viscosity**	**Hot Paste Viscosity**	**Break Down**	**Cold P**
Native	1809 ± 31 ^{f}	734 ± 32 ^{f}	1038 ± 28 ^{e}	305 ± 4 ^{h}	3104 ± 32 ^{g}	594 ± 31 ^{h}	2610 ± 1 ^{f}	751 ± 3 ^{i}
140/0	1993 ± 36 ^{e}	715 ± 29 ^{f}	1278 ± 21 ^{e}	1074 ± 16 ^{h}	3367 ± 41 ^{f}	596 ± 22 ^{h}	2771 ± 19 ^{e}	930 ± 19 ^{h}
140/15	3471 ± 27 ^{b}	1368 ± 36 ^{d}	2103 ± 20 ^{a}	1945 ± 9 ^{f}	4311 ± 60 ^{d}	977 ± 16 ^{f}	3334 ± 44 ^{b}	1344 ± 36 ^{f}
140/30	3639 ± 41 ^{a}	1610 ± 21 ^{c}	2029 ± 21 ^{ab}	2328 ± 11 ^{e}	4574 ± 51 ^{c}	1203 ± 26 ^{e}	3371 ± 15 ^{b}	1613 ± 24 ^{e}
140/60	3577 ± 69 ^{ab}	1936 ± 19 ^{b}	1641 ± 10 ^{d}	2965 ± 8 ^{c}	5111 ± 36 ^{a}	1705 ± 7 ^{c}	3406 ± 29 ^{b}	2241 ± 11 ^{c}
160/0	3061 ± 14 ^{c}	1121 ± 41 ^{e}	1940 ± 15 ^{bc}	1562 ±21 ^{g}	3909 ± 21 ^{e}	724 ± 14 ^{g}	3185 ± 7 ^{c}	1047 ± 13 ^{g}
160/5	3711 ± 12 ^{a}	1832 ± 18 ^{b}	1879 ± 21 ^{c}	2645 ± 32 ^{d}	4878 ± 18 ^{b}	1355 ± 16 ^{d}	3523 ± 2 ^{a}	1813 ± 13 ^{d}
160/15	2953 ± 13 ^{c}	2066 ± 21 ^{a}	887 ± 17 ^{f}	3566 ± 6 ^{a}	5245 ± 71 ^{a}	2310 ± 30 ^{b}	2935 ± 41 ^{d}	3129 ± 19 ^{b}
160/30	2638 ± 32 ^{d}	1939 ± 19 ^{b}	699 ± 4 ^{g}	3390 ± 7 ^{b}	4754 ± 60 ^{bc}	2471 ± 21 ^{a}	2283 ± 39 ^{d}	3493 ± 11 ^{a}

[*] mean ± standard deviation values are reported

[‡]means not sharing the same superscript within each column are significantly different ($p < 0.05$)

Table 3.5 Gelatinization properties of thermally processed normal and waxy wheat flours* (N=2)‡.

SAMPLE	Heating Condition (°C/Min)	Gelatinization				Retrogradation			
		Onset Temp (°C)	Peak Temp (°C)	End Temp (°C)	Enthalpy (ΔH) (J/g)	Onset Temp (°C)	Peak Temp (°C)	End Temp (°C)	Enthalpy (ΔH) (J/g)
Normal wheat	Native	61.1 ± 0.3 ^{a}	68.5 ± 0.7 ^{b}	77.6 ± 0.9 ^{b}	6.3 ± 0.3 ^{a}	50.4 ± 0.5 ^{ab}	59.4 ± 0.7 ^{a}	77.4 ±1.5 ^{a}	1.8 ± 0.7 ^{a}
	160/0	61.0 ± 0.5 ^{a}	67.8 ± 1.0 ^{bc}	75.9 ± 0.9 ^{bc}	5.3 ± 0.9 ^{ab}	51.1 ± 0.2 ^{ab}	58.9 ± 0.5 ^{a}	68.7 ± 1.7 ^{c}	1.1 ± 0.5 ^{ab}
	160/30	62.0 ± 0.2 ^{a}	65.4 ± 0.5 ^{c}	73.2 ± 1.8 ^{c}	3.8 ± 0.3 ^{b}	50.9 ± 1.5 ^{ab}	58.4 ± 0.3 ^{a}	73.5 ± 0.2 ^{ab}	1.5 ± 0.3 ^{ab}
Waxy wheat	Native	62.3 ± 1.4 ^{a}	72.0 ± 1.3 ^{a}	83.5 ± 0.7 ^{a}	7.0 ± 0.4 ^{a}	52.5 ± 1.0 ^{a}	59.9 ± 0.1 ^{a}	72.4 ± 0.7 ^{bc}	0.4 ± 0.1 ^{ab}
	160/0	62.6 ± 0.2 ^{a}	73.4 ± 0.1 ^{a}	83.4 ± 0.9 ^{a}	6.6 ± 0.3 ^{a}	49.1 ± 0.2 ^{b}	58.3 ± 0.9 ^{a}	73.5 ± 1.2 ^{ab}	0.3 ± 0.1 ^{b}
	160/30	61.6 ± 0.4 ^{a}	71.8 ± 0.1 ^{a}	84.1 ± 0.7 ^{a}	6.3 ± 0.4 ^{a}	52.3 ± 0.6 ^{a}	60.3 ± 0.0 ^{a}	71.8 ± 0.7 ^{bc}	0.4 ± 0.2 ^{ab}

* mean ± standard deviation values are reported

‡means not sharing the same superscript within each column are significantly different ($p < 0.05$)

Table 3.6 Creep recovery[*] data for thermally processed normal and waxy wheat samples[‡]

Heating Condition (°C/Min)	Normal Wheat			Waxy Wheat		
Property	MCS[†] (10^{-2})	MRS[¥] (10^{-2})	% Recovery	MCS (10^{-2})	MRS (10^{-2})	% Recovery
Native	3.63 ± 0.07 [b]	1.95 ± 0.03 [b]	53.5 ± 1.9 [a]	4.71 ± 0.26 [b]	2.64 ± 0.29 [b]	56.0 ± 3.2 [a]
160/0	10.2 ± 2.34 [a]	5.42 ± 1.26 [a]	52.9 ± 0.2 [a]	17.8 ± 0.33 [a]	9.08 ± 0.06 [a]	50.9 ± 0.6 [b]
160/30	2.15 ± 0.21 [b]	1.08 ± 0.07 [b]	50.2 ± 1.5 [a]	4.44 ± 0.06 [b]	2.11 ± 0.10 [b]	47.4 ± 3.0 [c]

[*] mean ± standard deviation values are reported
[‡]means not sharing the same superscript within each column are significantly different ($p < 0.05$)
[†]MCS – Maximum creep strain
[¥] MRS – Maximum recovery strain

Table 3.7 Composition of soluble and insoluble proteins[‡] extracted using 50% propanol from normal and waxy wheat flours[*] (N=2)[†].

Normal Wheat				
Protein extracted	**Soluble protein content**			**Insoluble protein content (IPP %)**
Heating Condition (°C/Min)	**>70 kDa**	**13-70 kDa**	**<13kDa**	
Native	10.4 ± 0.26 [a]	38.3 ± 0.72 [abcd]	15.4 ± 0.27 [a]	35.9 ± 0.72 [e]
140/0	11.7 ± 2.33 [a]	41.3 ± 2.27 [a]	13.8 ± 0.29 [b]	33.2 ± 0.24 [g]
140/15	11.5 ± 2.06 [a]	40.8 ± 2.44 [ab]	13.5 ± 0.37 [b]	34.1 ± 0.75 [fg]
140/30	11.3 ± 1.74 [a]	40.3 ± 1.56 [ab]	13.5 ± 0.03 [bc]	35.0 ± 0.21 [ef]
140/60	9.8 ± 1.95 [a]	37.7 ± 1.55 [abcd]	12.9 ± 0.42 [c]	39.6 ± 0.03 [d]
160/0	11.3 ± 0.85 [a]	38.6 ± 0.82 [abc]	11.4 ± 0.22 [d]	38.8 ± 0.25 [d]
160/5	10.5 ± 0.76 [a]	37.3 ± 1.54 [bcd]	11.0 ± 0.03 [d]	41.2 ± 0.75 [c]
160/15	10.1 ± 1.04 [a]	35.8 ± 1.86 [cd]	11.2 ± 0.27 [d]	42.8 ± 1.10 [b]
160/30	8.4 ± 1.77 [a]	34.7 ± 0.55 [d]	11.3 ± 0.29 [d]	45.6 ± 0.93 [a]
Waxy Wheat				
Heating Condition (°C/Min)	**>70 kDa**	**13-70 kDa**	**<13kDa**	**%IPP**
Native	7.6 ± 0.11 [abc]	38.1 ± 0.68 [a]	11.0 ± 0.26 [a]	43.3 ± 1.04 [e]
140/0	9.7 ± 1.15 [a]	32.4 ± 0.71 [b]	10.1 ± 0.01 [ab]	47.9 ± 0.44 [d]
140/15	8.3 ± 1.31 [ab]	29.5 ± 0.70 [cd]	10.0 ± 0.50 [ab]	52.2 ± 0.11 [c]
140/30	7.8 ± 1.77 [abc]	27.9 ± 1.50 [de]	10.2 ± 0.30 [ab]	54.0 ± 0.57 [c]
140/60	5.6 ± 0.03 [c]	26.9 ± 0.95 [e]	9.9 ± 0.46 [ab]	57.6 ± 1.38 [b]
160/0	9.1 ± 1.59 [ab]	30.4 ± 0.38 [bc]	10.7 ± 0.39 [a]	49.9 ± 0.81 [d]
160/5	6.6 ± 1.12 [bc]	26.6 ± 1.09 [e]	9.4 ± 0.64 [b]	57.4 ± 0.60 [b]
160/15	3.9 ± 0.88 [d]	20.5 ± 0.72 [f]	8.5 ± 0.75 [c]	67.1 ± 0.59 [a]
160/30	3.4 ± 0.69 [d]	19.5 ± 1.25 [f]	8.6 ± 1.01 [c]	68.5 ± 1.38 [a]

[*] mean ± standard deviation values are reported

[‡]Expressed as % total protein. Initial protein content of normal wheat (15.47% db); waxy wheat (13.88% db)

[†] samples with different superscripts within each column are significantly different ($p < 0.05$)

Figure 3.1 Effect of protease (dashed line) on normal wheat flour (A, B, C) and waxy wheat flour (D, E, F) for native (A, D) and thermally processed samples (160°C for 0 min – B, E; 160°C for 30 min – C, F)

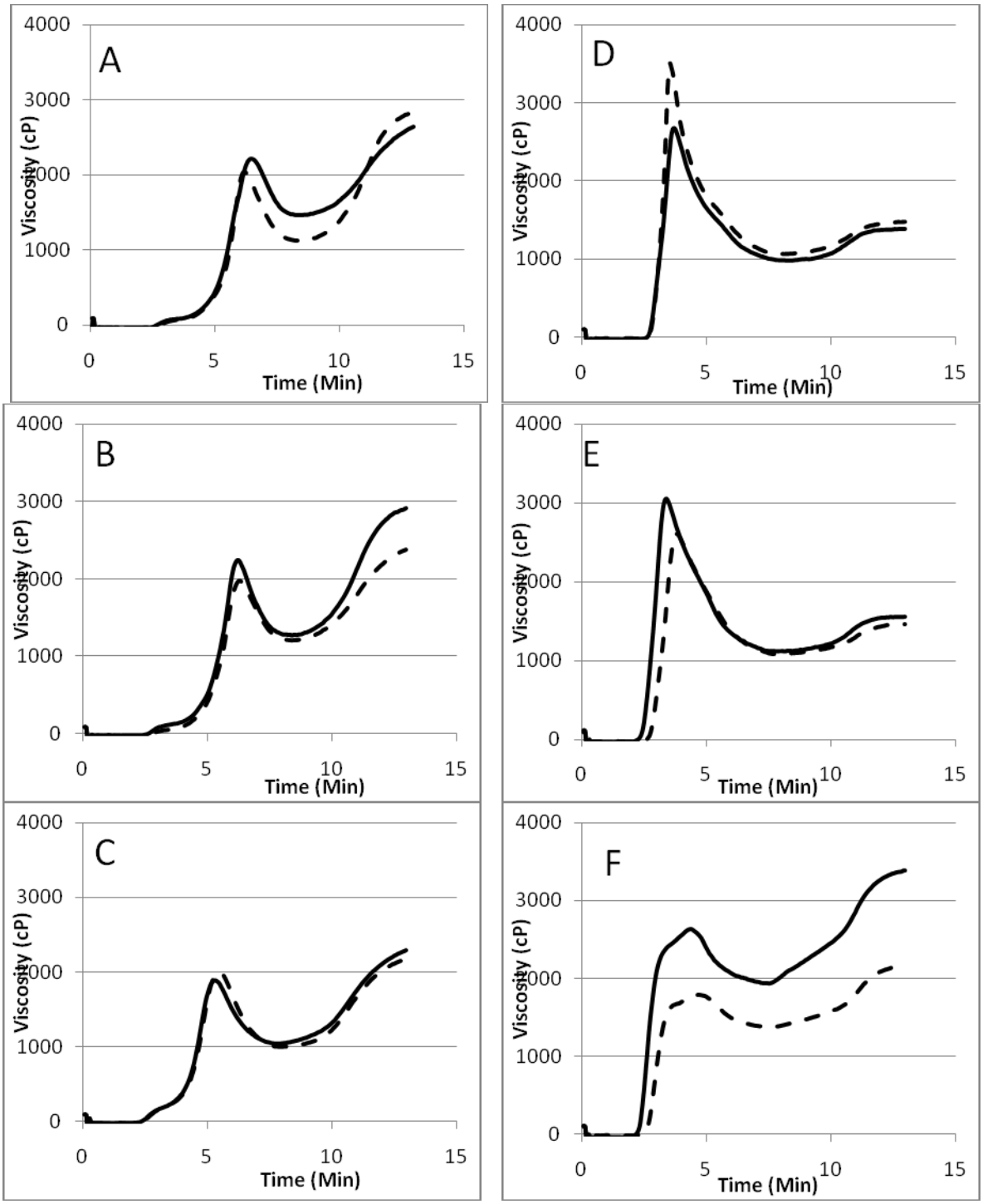


Figure 3.2 Rapid Visco Analyzer (RVA) pasting properties of blends containing vital wheat gluten (VWG) and waxy wheat starch at 10% solids.(A) unheated VWG blended with unheated starch (solid line) and heated starch (dashed line); (B) heated VWG (160°C for 30 min)blended with unheated starch (solid line) and heated starch (160°C for 30 min) (dashed line); (C) heated blend (160°C for 30 min) (dashed line) and unheated blend (solid line); (D) unheated starch (solid line) and heated starch (160°C for 30 min) (dashed line)

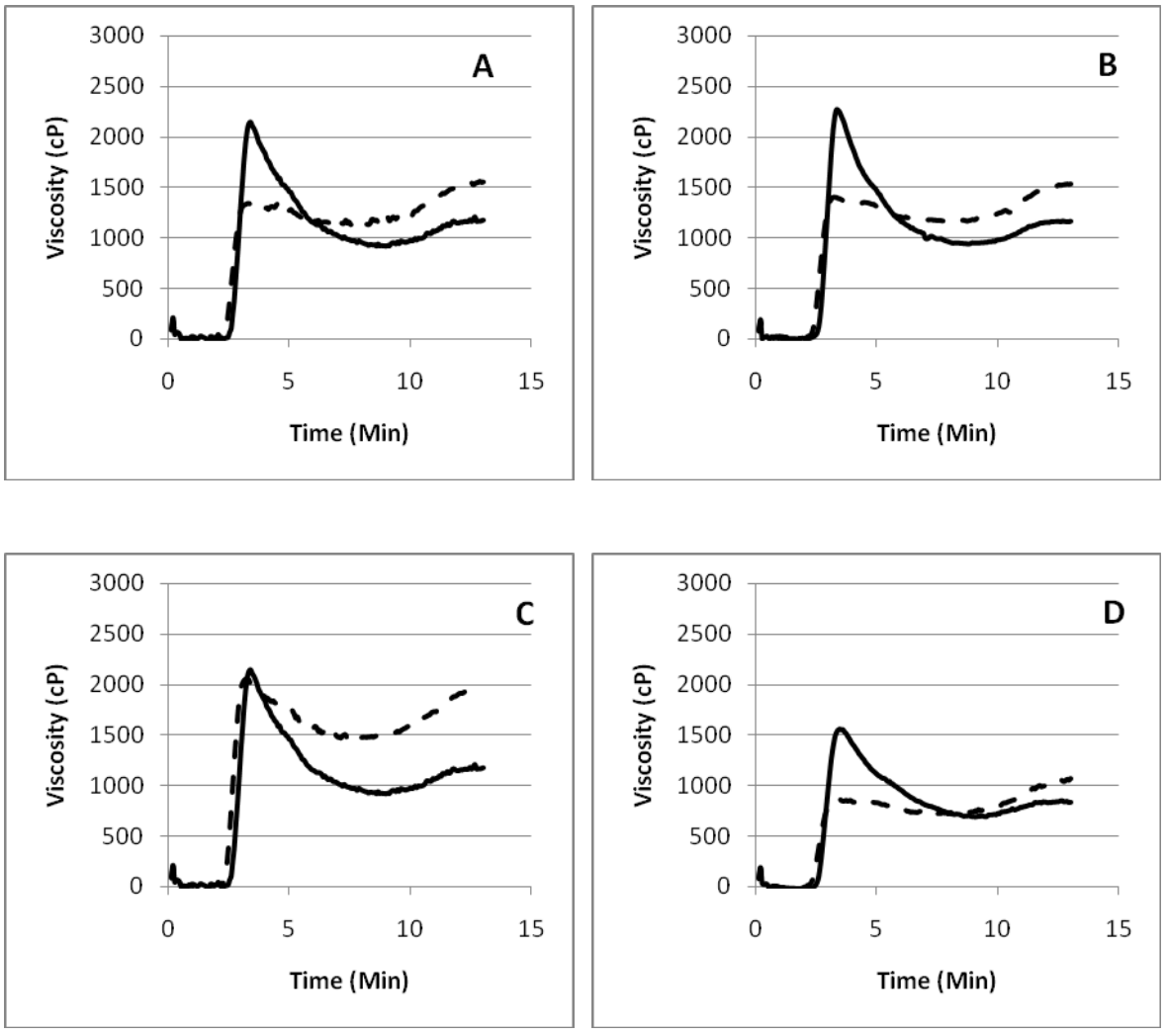


Figure 3.3 RVA pasting properties of starches isolated from native (solid line) and thermally processed (160°C for 0 min – dotted line; 160°C for 30 min – dashed line) normal wheat (A) and waxy wheat (B) flours.

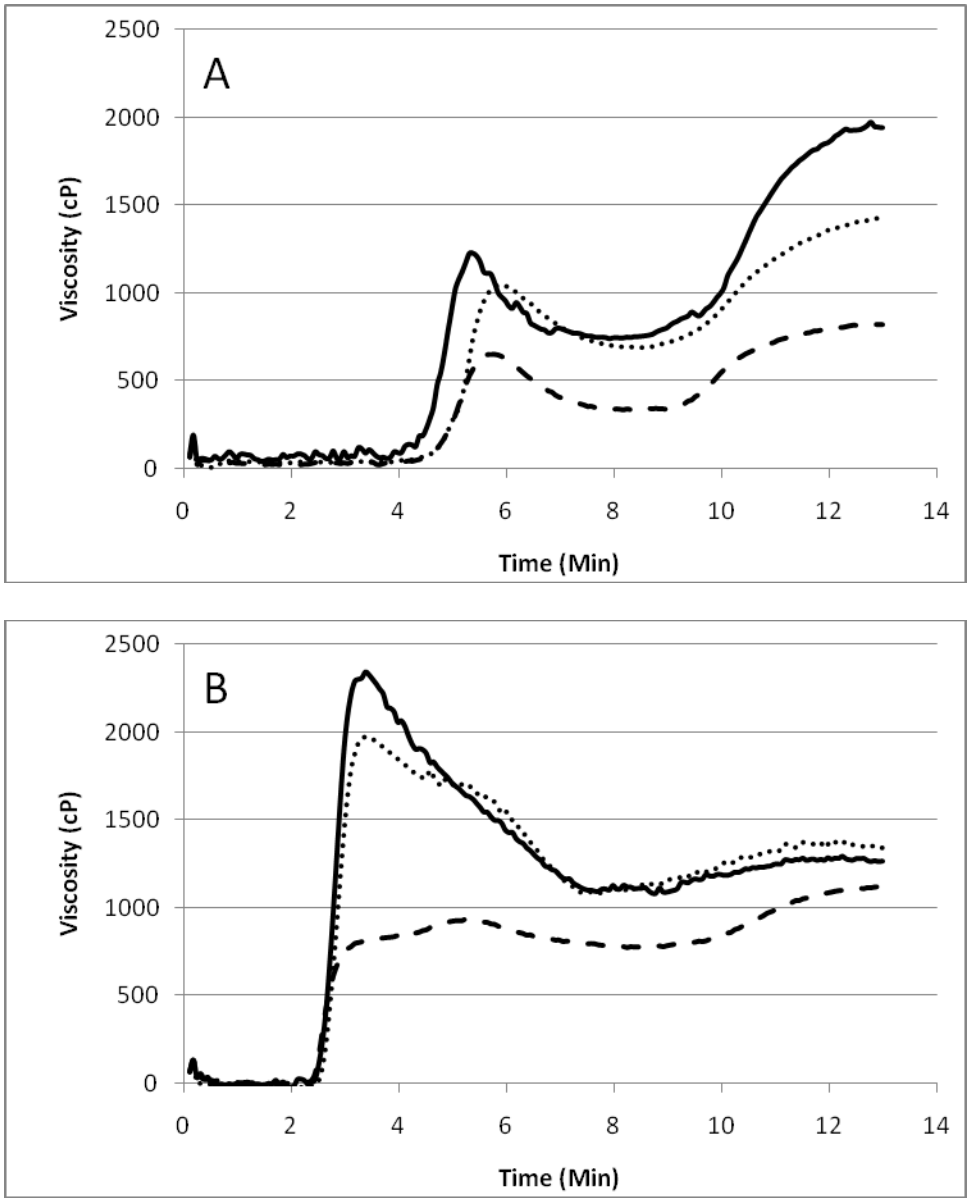


Figure 3.4 Normalized gel permeation chromatography (GPC) retention curves of isolated starch for native (solid line) thermally processed (160°C for 0 min – dotted line; 160°C for 30 min – dashed line) waxy wheat samples.

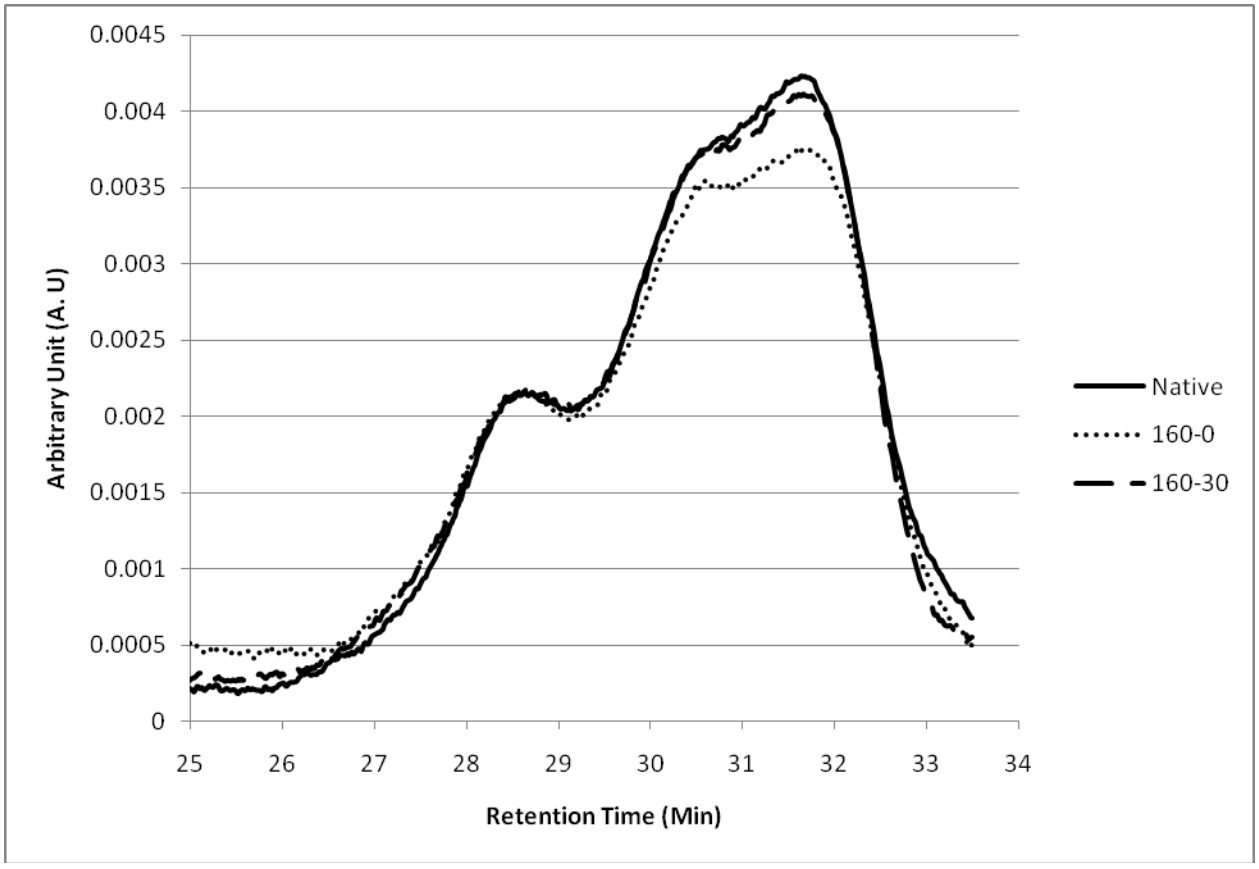
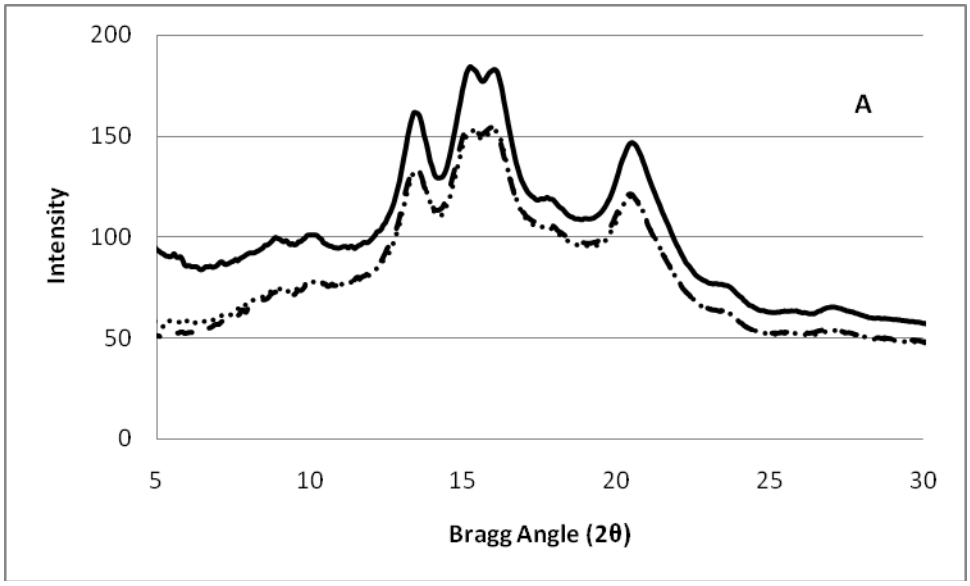


Figure 3.5 Synchrotron Wide-Angle X-ray Diffraction (WAXD) measurements on (A) waxy wheat and (B) normal wheat flour samples. Native (solid line) thermally processed (160°C for 0 min – dotted line; 160°C for 30 min – dashed line).



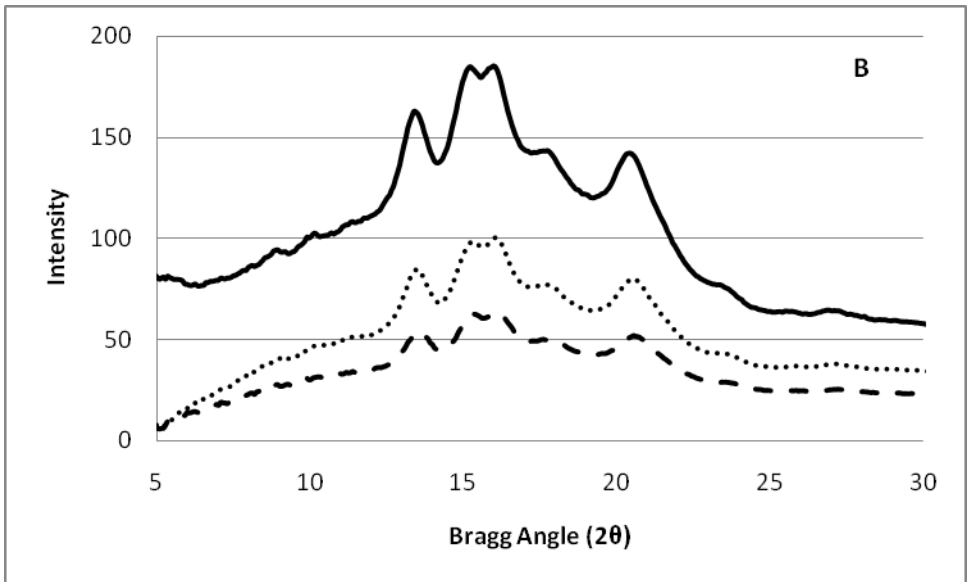
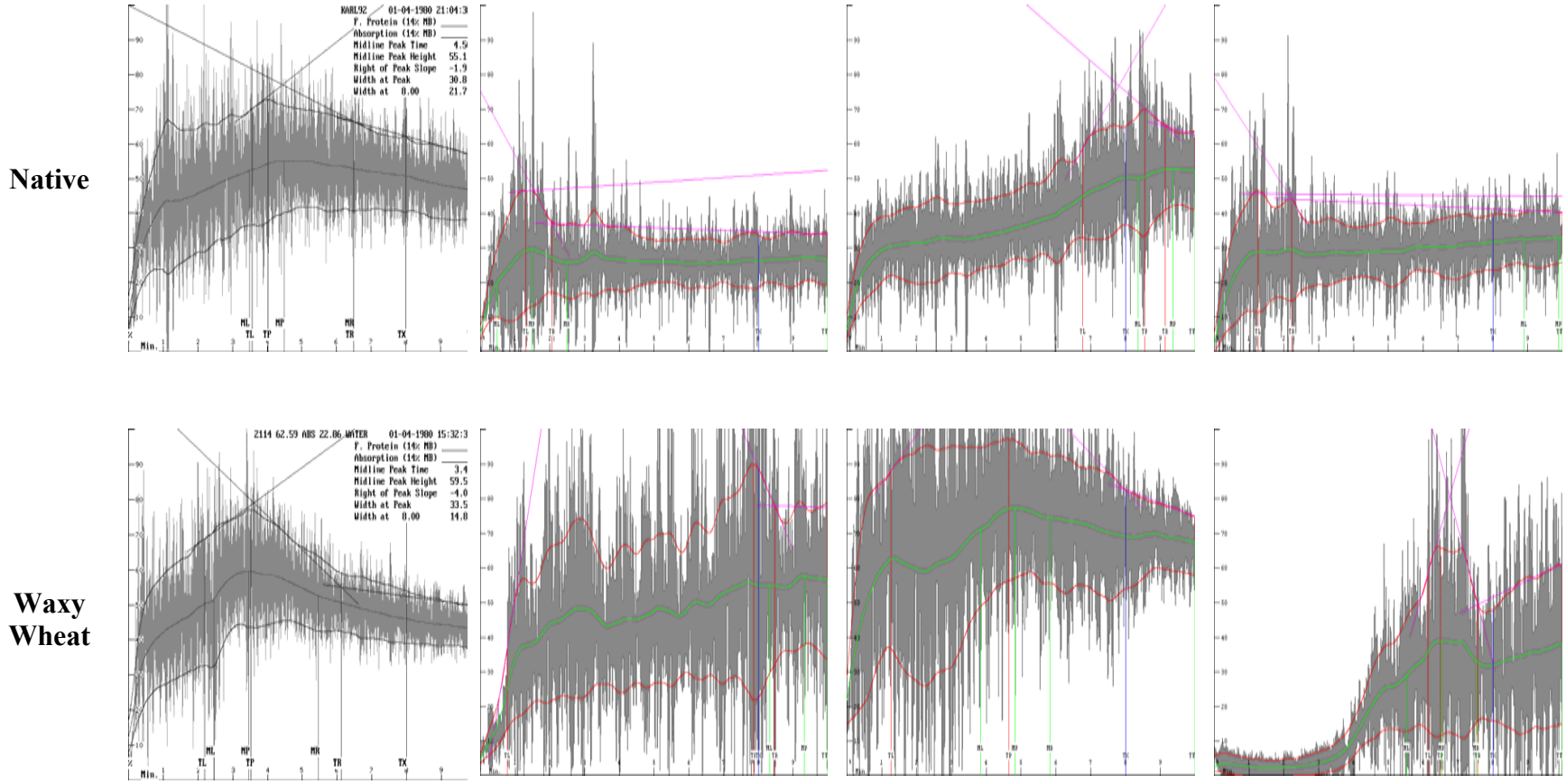


Figure 3.6 Mixograph curves for heat treated normal and waxy wheat samples



† x-axis is time (0-10 min; each marking represents one minute) and y-axis is % absorption (0-100 scale; each marking represents 10%).

Figure 3.7 Stress relaxation (G(0)/G(t)) curves for (A) normal wheat flour and (B) waxy wheat flour for native (solid line) and thermally processed samples (160°C for 0 min (dotted) and 160°C for 30 min (dashed))

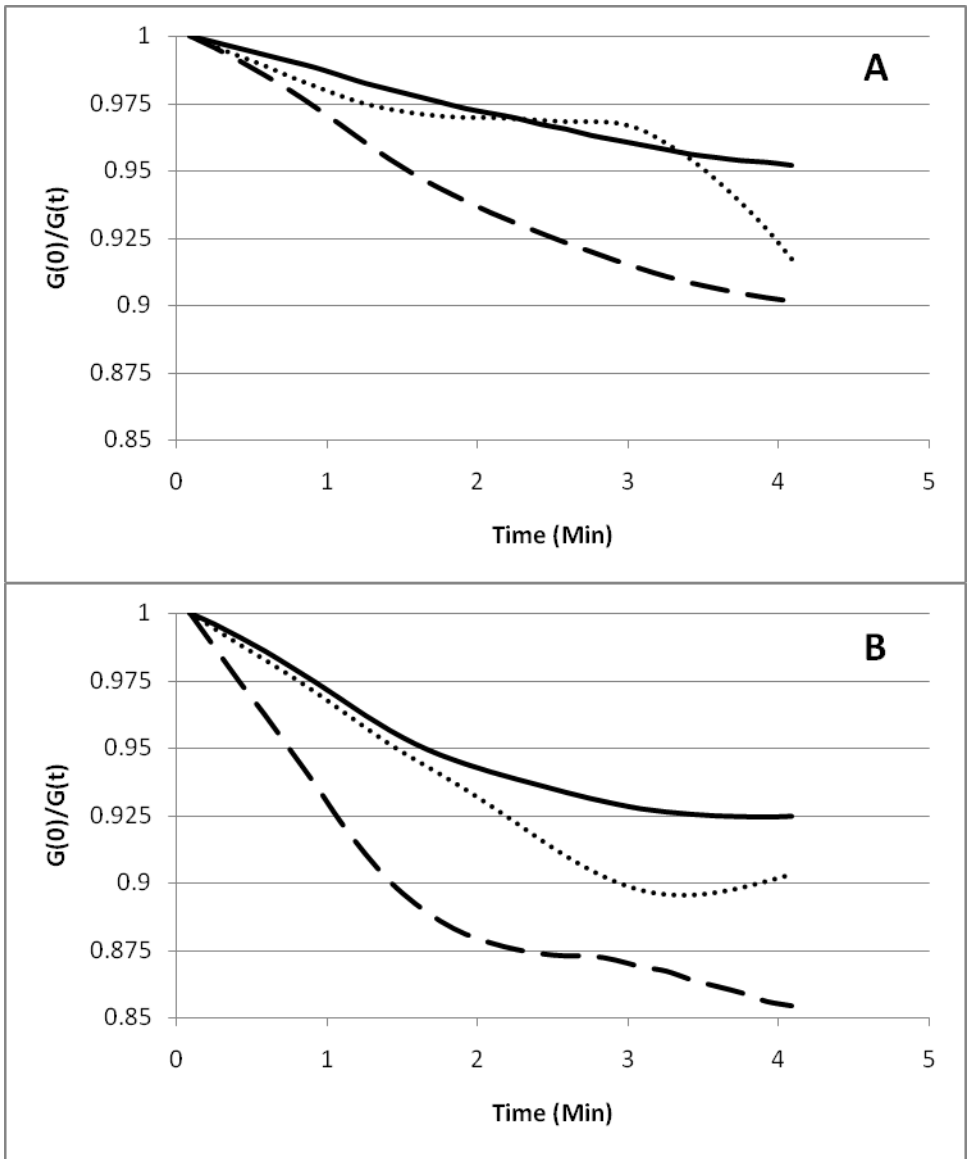
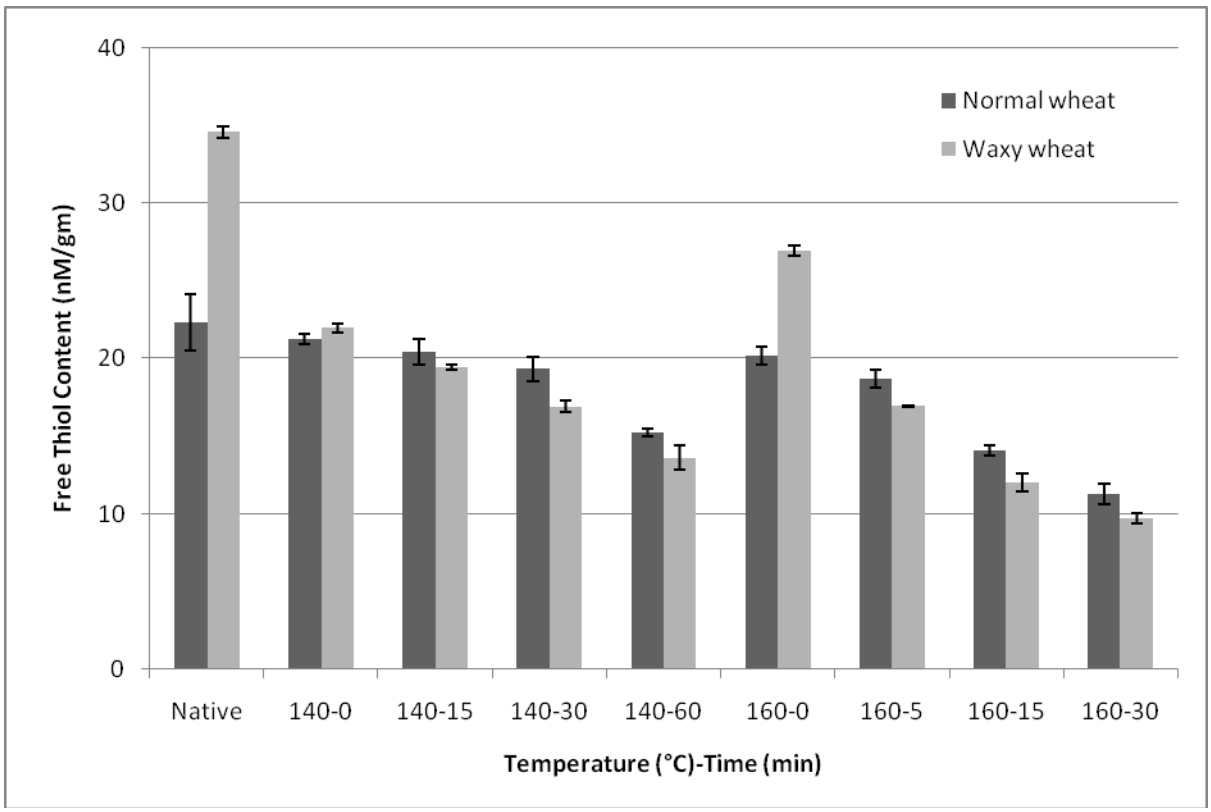


Figure 3.8 Changes in free –SH in normal and waxy wheat samples(N = 2)



CHAPTER 4 - HYDROTHERMAL PROCESSING OF FLOURS: EFFECT ON PROTEIN AND PASTING PROPERTIES

ABSTRACT

The objective of this study was to evaluate the effects of hydrothermal processing on protein solubility and pasting properties of normal and waxy wheat flours. A total of sixteen hydrothermal processing conditions, including two moisture conditions (as-is and 20%), two temperatures (140 and 160°C) and four times of heating periods (0, 5, 15, 30 or 60 min) were employed. Increase in moisture content resulted in decreased protein solubility in 50% propanol. Protein solubility was lower in waxy wheat samples than that in normal wheat. There was an increase in soluble polymeric protein content with an increase in initial flour moisture content during hydrothermal processing. Free thiol content decreased due to thermal processing, suggesting cross-linking of proteins during heating. Hydrothermally processed waxy wheat samples resulted in non-cohesive pastes with high viscosity. Pasting temperature of waxy wheat samples increased with increase in temperature and initial moisture content during processing; For waxy wheat samples, changes in pasting temperature were observed in both acidic and neutral conditions, while for normal wheat samples changes in pasting temperature were observed only in acidic conditions. Pasting properties, especially peak viscosity, reached a maximum and then decreased upon prolonged heating at both moisture levels. Hydrothermal processing of waxy wheat flours resulted in non-cohesive pastes that are more resistant to acid hydrolysis as compared to normal wheat flour.

INTRODUCTION

Thermal processing of flours has been shown to increase acid stability of thermally processed waxy wheat flours (Garimella Purna, 2010a). However, the effect of initial flour moisture content on flour pasting properties is largely unknown. Previous researchers have focused on effect of heat-moisture treatment on isolated gluten and starch (Weegles et al., 1994a &b; Lorenz and Kulp, 1982; Hoover and Vasanthan, 1994). Upon heating, wheat proteins tend to cross link and aggregate via disulphide bonding and hydrophobic interactions (Schofield et al., 1983; Weegles et al., 1994a&b), consequently lowering their extractability with detergent solvents (Singh and MacRitchie, 2004). Glutenin proteins aggregate at lower temperatures, while gliadins participate in protein aggregation at temperatures greater than 120°C (Guerrier and Cerletti, 1996). However, not all gliadins participate equally in the protein aggregation process (Schofield et al., 1983). The non-involvement of ω-gliadins in this process suggests that the protein aggregation between gliadins and other high molecular weight proteins is mediated by disulphide bonds and sulphydryl exchange (Schofield et al., 1983). The aggregation of proteins increased with increased moisture content of gluten prior to heat treatment (Weegles et al., 1994b). Additionally, at higher temperatures, insolubility of proteins is not reversed by addition of reducing agents (Guerriere and Cerletti, 1996).

The effect of heat moisture treatment on isolated starches from various sources is reviewed by Jacobs and Delcour (1998). In cereal starches, heat moisture treatment (18-27% moisture content; heating up to 100°C; up to 16hrs) has shown to drastically alter starch physic-chemical properties (Hoover and Vasanthan, 1994). Typically, hydrothermal processing of cereal starches results in increased enzyme susceptibility, higher paste stability, and broadened gelatinization temperatures (Lorenz and Kulp, 1981; Lorenz and Kulp, 1982). Jacobs and

Delcour (1998) detailed three possible mechanisms that occur during hydro-thermal processing i.e. changes with respect to starch crystallinity, changes with respect to amorphous fraction and alterations between crystalline and amorphous parts of starch. Additionally, starch chains within the amorphous and crystalline regions tend to associate during hydrothermal treatment (Hoover and Vasanthan, 1994). The re-crystallization could mainly be attributed to amylose-amylose and amylose-lipid interaction within the amorphous region of starch granule (Hoover and Manuel, 1996).

Most of the studies mentioned above using hydrothermal treatments discussed individual flour components in isolation, and employed temperatures that are below starch gelatinization at the given moisture level. High temperature and limited moisture content of flour and its effect on flour functionality are not well discussed. This study was aimed at evaluating the effects of different initial flour moisture content and thermal processing on proteins aggregation and changes in flour pasting properties of normal and waxy wheat flours. The secondary objective of the study was to evaluate acid stability of pastes derived from hydrothermally processed waxy wheat flours.

MATERIALS AND METHODS

Materials

A normal hard wheat (Karl 92) and a hard waxy wheat (Pedigree: Cimmaron/Rioblanco//Baihou4/L910145/3/Colt/Cody//Stozher/NE86582) were procured from USDA-ARS, Lincoln, NE. Wheat kernels were tempered to 16% moisture for 18 h and were roller-milled into straight-grade flour on a MLU 202 Bühler experimental mill (Bühler Co.,

Uzwill, Switzerland). The flour yields were 70.8 and 71.0% for normal and waxy wheat respectively.

Hydrothermal processing of flours

Two Flours (waxy and normal wheat) were subjected to eight different heating conditions at two different initial moisture levels (as-is and 20% moisture content). Heating conditions were two temperatures (140 and 160°C) and four different heating periods (0, 5, 15, 30 or 60 min). For each temperature-time combination, 10 g of flour was placed in a 12 ounces Quilted Crystal® Jelly Jars (Ball®: 14400-81200) and capped. After heating, the jars were cooled to 25°C, opened and left at 25°C for 18 hours. The samples were ground using mortar and pestle and were passed through 425 μm screen.

General methods

Moisture content of native and thermally processed samples was measured by AACC 44-15A (AACC International, 2000). Moisture content of normal and waxy wheat flours were 13.20 and 12.40%, respectively. Color measurements (L*, a*, b* color space) were performed by using a MINOLTA CR-310 (Minolta, Tokyo, Japan) model spectrophotometer. *L** is the luminance or lightness component, which ranges from 0 to 100 (black to white), and parameters *a** (from green to red) and *b** (from blue to yellow) are the two chromatic components, which range from −60 to 60 (Papadakis et al., 2000). Protein content of normal and waxy wheat flours was previously reported by Guan et al. (2009) and was measured by AACC 08-01 (AACC

International, 2000). The protein content of normal and waxy wheat flours were 15.5 and 13.9% (db) respectively.

Free thiol contents

Free thiol estimation was performed by a method of Chan and Wasserman (1993). A standard curve was developed by using reduced glutathione (G4251, Sigma Aldirch, St. Louis, MO) at 100, 200, 300, 500 and 100 nmol/ml respectively. The amount of free –SH in flour was reported as nmol/g of flour.

Protein extraction using 50% isopropanol

Extractable protein **(**EP) analysis was done according to a method by Bean et al. (1998) with following modifications. Each sample (100 mg) was dissolved in 1 ml 50% isopropanol, vortexed for 5 min and centrifuged at 10,000 x *g* for 5 minutes. Extraction was repeated twice. Supernatant (total 1 ml, 0.5 ml from each extraction) was filtered through 0.45µm filter and analyzed using size exclusion – high performance liquid chromatography (SE-HPLC). The pellet after two extractions with 50% isopropanol were freeze-dried and analyzed for protein content using LECO™ FP-428 nitrogen determinator (LECO, MI).

SE- HPLC analysis

The supernatants filtered through 0.45µm filter were analyzed by using SE-HPLC analysis (Agilent 1100 Series, Agilent Technologies, Palo Alto, CA). The samples were analyzed using Bio-Sep-SEC- 4000 column 300 x 7.80 mm (Phenomenex, Torrance, CA), kept

at 30°C, with injection volume of 20 µL; eluting solvent : acetonitrile : water (1:1) containing 0.5% trifluoroacetic acid ; run time: 30 min; flow rate: 0.5 ml/min.

Pasting properties

Flour pasting properties were determined with a Rapid Visco Analyzer (RVA, Foss North America, Inc., MN). A 25-g mixture of flour and water or pH 3.0 citrate buffer (10% solids level) was prepared in an RVA canister. Citrate buffer was prepared by mixing 3:2 volumes of 1M tri-sodium citrate (W302600, Sigma-Aldrich, St. Louis, MO) and 0.4M of citric acid (C1857 Sigma-Aldrich, St. Louis, MO) respectively. Final weight in the RVA canister was 28 g. An RVA paddle was inserted into the canister, and the mixture was gently agitated to disperse flour lumps. The RVA canister was then subjected to a 13-min RVA test to determine flour pasting properties (Deffenbough and Walker, 1989). Isolated starch samples were analyzed for their pasting properties at 7% solids. Pasting properties reported in this study are pasting temperature, peak viscosity, viscosity at trough and final viscosity.

After RVA analysis, cooked pastes of flours were cooled at 25°C for 6 hours and subsequently evaluated visually for their texture. They were classified as gel, cohesive or non-cohesive pastes.

Thermal properties of flours

Thermal properties of hydrothermally processed flours were measured using method previously described (Garimella Purna, 2010a). Each sample was analyzed in duplicate and mean values were reported.

Wide angle X-ray diffraction (WAXD) measurements

X-ray diffraction was conducted with a Philips X-ray diffractometer with Cu-Ka radiation at 35 kV and 20 mA, a theta-compensating slit, and a diffracted beam monochromator. The moisture of all samples was adjusted to about 18% in a sealed dessicator at room temperature before analysis. The diffractograms were recorded between 2 and 35° (2θ).

Statistical analysis

Macanova 4.12 (School of Statistics, University of Minnesota, Minneapolis, MN) was used to perform ANOVA and Tukey's honest significance difference (HSD) analysis. The level of significance was $P < 0.05$ throughout the paper.

RESULTS AND DISCUSSION

Changes in protein composition

With an increase in temperature, there was a decrease in free –SH (**Table 4.1**) content for both waxy and normal wheat flours. Native waxy wheat flour had higher free –SH content than did native normal wheat flour. However, upon hydrothermal processing there was a rapid and drastic decrease in free –SH content of waxy wheat samples. Loss of free –SH suggests cross linking of proteins. The extent of cross linking was higher in waxy wheat samples.

To further evaluate the change in proteins, the composition of proteins extracted by 50% propanol was studied using SE-HPLC. Proteins extracted using 50% propanol were termed extractable proteins (EP) and the protein remaining in the pellet was termed as insoluble polymeric protein (IPP). Extractable proteins were analyzed using SE-HPLC, the area under the SE-HPLC curve was divided into three regions as described by Bean et al. (1998) i.e. soluble

polymeric protein (SPP, > 70kDa), gliadins (Gli, 13-70 kDA) and albumins and globulins (Alb/Glb, <13 kDA).

Total IPP content increased with thermal processing for both waxy and normal wheat samples (**Tables 4.2 and 4.3**), but increase in IPP content was significantly greater in waxy wheat samples. The increase in %IPP with processing time for normal wheat samples was greater at higher temperature.

In our study, extractable protein (EP) was calculated as the difference between total protein and IPP. Hence the overall EP decreased with increase in IPP, which was a consequence of hydrothermal processing. Therefore the overall content of SPP, Gli and Alb/Glb decreased with increase in hydrothermal processing. The changes in EP profile were significantly influenced by moisture content. At similar heating profile, higher initial moisture content resulted in lower EP values, which could be a consequence of increase in IPP (**Tables 4.2 and 4.3**). At similar processing conditions, samples processed at higher moisture content had lower SPP values. Additionally, flours processed at higher moisture conditions demonstrated greater decrease in SPP as compared to as-is moisture condition. Similar trends were observed in both waxy and normal wheat flours.

Our results suggest that protein matrix in flour is affected by heat and moisture content during thermal processing. Previous researchers have studied the effect of higher moisture levels on protein degradation (Weegles et al., 1994a & b). At higher moisture levels there is a decrease in exposure of hydrophobic regions of protein and hence more hydrophobic protein-protein interactions could occur (Weegles et al., 1994b). Additionally, large gluten molecules are more susceptible to heat mediated aggregation (Schofield et al., 1983; Weegles et al., 1994a; Singh and MacRitchie, 2004). These large gluten aggregates could be a mixture of 50% soluble and

insoluble polymeric proteins and glutenins. The apparent increase in %IPP in our study indicates that some of the previously soluble polymeric protein (in 50% propanol) could interact with other polymeric protein and become insoluble. Additionally, at temperatures above 120°C, gliadins tend to interact with glutenin molecules via disulphide bonding (Singh and MacRitchie, 2004).

Changes in pasting properties during thermal treatment

Distilled water (neutral conditions)

The effect of thermal processing on pasting properties was governed by moisture content of sample as well as waxy trait. For normal wheat samples, pasting temperature decreased gradually with increasing temperature and heating time (**Tables 4.4 and 4.5**). However, for waxy wheat samples, pasting temperature significantly decreased upon heating and remained unchanged at all time periods of heating. For both waxy and normal wheat samples, initial flour moisture content affected the changes in pasting temperature. For normal wheat samples, there was a decrease in pasting temperature when flours were heated at increased moisture level compared to flour native moisture content. In contrast, for waxy wheat samples, pasting temperature increased with an increase in initial flour moisture content.

Peak viscosity for both waxy and normal wheat samples, heat-treated at both temperatures, increased to a maximum and then decreased upon further heating (**Tables 4.4 and 4.5**). The changes in peak viscosity were greater at lower initial flour moisture content. Increase in peak viscosity is similar to increase in peak viscosity of normal and waxy wheat flours in silver nitrate solution (as reported by Garimella Purna, 2010b). Our results suggest that initial increase in peak viscosity during heating could be due to inactivation of α-amylase in flour

(**Figure 4.1a**). In a separate study, pasting properties of waxy wheat sample (20% mc, 160°C for 30 min) were compared in two different solutions, i.e. distilled water and 1mM silver nitrate. There were no differences in peak viscosity (**Figure 4.1a**), which indicate inactivation of indigenous flour α-amylase upon hydrothermal processing. However, the pasting profile of heat treated sample in silver nitrate solution was different from native waxy wheat flour in silver nitrate (**Figure 4.1b**), which indicates that apart from enzyme inactivation there were other macro level changes in flour that helps improve viscosity profile of waxy wheat flour upon thermal processing.

Trends in hot paste and final viscosity values were different for waxy and normal wheat samples (**Tables 4.4 and 4.5**). For normal wheat samples, hot paste viscosity and cold paste viscosity values increased during initial heating and decreased upon prolonged heating. However, for waxy wheat samples, these values increased with increasing time of heating. The trends were similar at both low and high initial flour moisture content. Hot paste viscosity and cold paste viscosity values were lower at higher moisture content as compared to lower moisture content.

All normal wheat flours resulted in a gel texture, which is primarily attributed to presence of amylose. Texture of native waxy wheat was cohesive, while texture of hydrothermally processed samples (> 15 min at 140°C and > 5min at 160°C) was highly viscous non-cohesive paste. However, there was no effect of initial moisture content on the non-cohesive texture of waxy wheat. To further evaluate the stability of the pastes from hydrothermally processed samples, the flour pasting properties were analyzed in an acidic buffer.

Citrate buffer pH 3.0 (acidic conditions)

Pasting temperature of normal wheat flour had decreased upon thermal processing. Similar results were observed at both the moisture levels. However, the decrease in pasting temperature was greater in normal wheat samples processed at 20% moisture. The results were similar to RVA pasting properties observed using distilled water. In waxy wheat samples, pasting temperature decreased upon initial heating and did not during subsequent heating. However, the changes in pasting temperature at 20% initial moisture were different from waxy wheat flours processed in native flour moisture (12.40%). Pasting temperature increased to a maximum and then decreased upon further heating (**Tables 4.4 and 4.5**).

Peak viscosity of both native and hydrothermally processed flours had higher peak viscosity in citrate buffer (**Tables 4.4 and 4.5**). Previous studies have shown stronger association of protein and starch at acidic pH (Dahle, 1971). At lower pH, it was postulated that starch and protein contain opposite charges and thus could have increased interactions (Dahle, 1971). Waxy wheat samples displayed a consistent increase with increase in temperature and heating time during flour processing (**Table 4.5**).

Cold paste viscosity (CPV) for both normal and waxy wheat flours was lower in acidic conditions as compared to neutral conditions (**Tables 4.4 and 4.5**). For normal wheat samples, processed at initial flour moisture, cold paste viscosity reached a maximum (30 min at 140C and 0 min at 160C) and then decreased upon subsequent heating (**Table 4.4**). For waxy wheat samples the CPV values increased with increase in temperature and time of heating (**Table 4.5**). The same trends were observed for waxy wheat samples at both moisture conditions. However, viscosity values of the waxy wheat flour treated at higher initial moisture conditions were lower as compared to the flours processed at 20% moisture conditions. Importantly, the acid stability of waxy wheat samples improved upon hydrothermal processing.

X-ray diffraction results suggest no changes in starch crystallinity due to thermal processing in both normal wheat samples (**Figure 4.2**) and waxy wheat samples (**Figure 4.3**). Additionally, no changes in thermal properties (i.e. both gelatinization and retrogradation) were observed for of normal wheat flours upon hydrothermal processing (**Table 4.6**). For waxy wheat samples, there was no difference in gelatinization properties between native and hydrothermally processed samples (**Table 4.7**). However, for retrogradation delta H values for samples thermally processed at 20% initial moisture content had higher delta H values than samples processed at 12.4% initial moisture content.

Color

Lightness (L) values for all samples (both normal and waxy wheat flours) decreased, while a* (green-red scale) and b* (blue-yellow scale) values increased due to an increase in processing temperature, exposure time or moisture content (**Table 4.8**). At similar moisture and processing conditions, normal wheat had lower L and higher a* values as compared to waxy wheat flour. In case of b* values, native waxy wheat flour was significantly higher as compared to native normal wheat flour, which could have resulted in higher b* values in subsequent treatments. However, at longer exposure times the b* values of normal wheat were comparable to waxy wheat flours. Formation of Maillard browning compounds was demonstrated to cause decrease in L values and increase in a* and b* values, when flours were heated at >150°C (Gokmen and Senyuva, 2006).

CONCLUSIONS

Initial moisture conditions are critical to thermal processing of flours. Higher initial flour moisture content resulted in flours with low brightness values and lower pasting viscosity profiles. Protein cross-linking occurred during hydrothermal processing leading to decreased solubility of protein. Although initial flour moisture content determined the extent of protein insolubility in normal wheat samples, it did not affect protein insolubility in waxy wheat flours. Thermal processing rendered protein from waxy wheat flours more insoluble as compared to normal wheat flours. Thermal processing of waxy wheat flours resulted higher viscosity profiles at both moisture conditions, with lower initial moisture conditions giving higher pasting viscosities. Initial moisture conditions did not affect the acid stability values of normal wheat samples, however, waxy wheat samples processed at lower initial moisture conditions displayed greater acid stability. Hydrothermal processing of waxy wheat flours results in non-cohesive cooked paste with high acid stability and high cold paste viscosity.

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Table 4.1 Changes in free –SH content (nmol/g of flour) in normal and waxy wheat samples* (N=2)

Flour	Normal wheat†		Waxy wheat	
Flour Moisture content (%)	**13.2**	**20**	**12.4**	**20**
Heating Condition (°C/Min)	**Free –SH content (nmol/g of flour)**			
Native	22.47 ± 2.07 [a]	22.38 ± 1.53 [a]	34.91 ± 0.52 [a]	34.40 ± 0.76 [a]
140/0	20.92 ± 0.17 [ab]	19.61 ± 0.35 [ab]	21.10 ± 1.42 [c]	19.56 ± 0.80 [b]
140/15	18.72 ± 0.31 [bc]	17.41 ± 0.86 [abc]	17.59 ± 0.26 [d]	16.97 ± 0.51 [c]
140/30	16.58 ± 0.38 [c]	14.73 ± 0.81 [de]	15.46 ± 0.17 [de]	14.88 ± 0.24 [cd]
140/60	12.74 ± 0.45 [de]	11.34 ± 0.78 [ef]	12.30 ± 0.41 [fg]	10.94 ± 0.57 [e]
160/0	19.66 ± 0.08 [abc]	18.14 ± 0.52 [bc]	24.84 ± 0.93 [b]	19.52 ± 0.57 [b]
160/5	17.31 ± 0.64 [c]	15.51 ± 0.94 [cd]	14.57 ± 0.76 [ef]	14.02 ± 0.14 [d]
160/15	13.05 ± 0.60 [d]	11.43 ± 0.82 [ef]	10.64 ± 0.20 [ef]	10.28 ± 0.37 [ef]
160/30	9.67 ± 0.54 [e]	8.75 ± 0.63 [f]	8.17 ± 0.46 [f]	8.47 ± 0.58 [f]

* mean ± standard deviation values are reported

†within each column means with different superscript are significantly ($p < 0.05$) different

Table 4.2 Insoluble polymeric protein† and 50% propanol soluble protein composition† of hydrothermally processed normal wheat samples* (total protein content was 15.47 %, db)

Initial Moisture Content	13.2%				20%			
	Protein fraction, % of protein				Protein fraction, % of protein			
Heating Condition (°C/Min)	IPP‡	SPP±	Gli⫫	Alb/Glb¥	IPP‡	SPP±	Gli⫫	Alb/Glb¥
Native	5.56 ± 0.11 ^{f}	1.60 ± 0.04 ^{a}	5.92 ± 0.11 ^{ab}	2.39 ± 0.04 ^{a}	5.56 ± 0.11 ^{g}	1.60 ± 0.04 ^{a}	5.92 ± 0.11 ^{b}	2.39 ± 0.04 ^{a}
140/0	5.10 ± 0.08 ^{g}	1.96 ± 0.34 ^{a}	6.29 ± 0.28 ^{a}	2.12 ± 0.02 ^{b}	7.61 ± 0.16 ^{e}	1.14 ± 0.21 ^{a}	5.23 ± 0.07 ^{bc}	1.49 ± 0.02 ^{bc}
140/15	5.26 ± 0.10 ^{fg}	1.87 ± 0.32 ^{a}	6.25 ± 0.37 ^{a}	2.10 ± 0.05 ^{bc}	8.85 ± 0.04 ^{d}	0.20 ± 0.12 ^{b}	5.16 ± 0.17 ^{c}	1.26 ± 0.01 ^{bc}
140/30	7.17 ± 0.11 ^{d}	0.73 ± 0.27 ^{c}	5.69 ± 0.16 ^{ab}	1.89 ± 0.00 ^{cd}	10.52 ± 0.09 ^{bc}	0.07 ± 0.05 ^{b}	3.66 ± 0.22 ^{de}	1.23 ± 0.18 ^{bc}
140/60	9.09 ± 0.05 ^{b}	0.27 ± 0.10 ^{c}	4.19 ± 0.05 ^{d}	1.91 ± 0.11 ^{bcd}	10.78 ± 0.06 ^{ab}	0.05 ± 0.03 ^{b}	3.29 ± 0.19 ^{e}	1.35 ± 0.28 ^{bc}
160/0	6.31 ± 0.14 ^{e}	1.59 ± 0.20 ^{ab}	5.83 ± 0.11 ^{ab}	1.74 ± 0.05 ^{de}	6.35 ± 0.01 ^{f}	0.52 ± 0.23 ^{b}	6.82 ± 0.24 ^{a}	1.78 ± 0.00 ^{b}
160/5	8.09 ± 0.04 ^{c}	0.75 ± 0.15 ^{bc}	5.16 ± 0.14 ^{bc}	1.47 ± 0.03 ^{f}	10.00 ± 0.01 ^{c}	0.12 ± 0.07 ^{b}	4.17 ± 0.02 ^{d}	1.17 ± 0.06 ^{c}
160/15	9.13 ± 0.10 ^{b}	0.41 ± 0.12 ^{c}	4.48 ± 0.22 ^{cd}	1.44 ± 0.00 ^{f}	10.99 ± 0.20 ^{ab}	0.05 ± 0.04 ^{b}	3.17 ± 0.01 ^{e}	1.25 ± 0.25 ^{bc}
160/30	10.63 ± 0.10 ^{a}	0.17 ± 0.08 ^{c}	3.14 ± 0.10 ^{e}	1.53 ± 0.09 ^{ef}	11.23 ± 0.38 ^{a}	0.11 ± 0.08 ^{b}	2.96 ± 0.34 ^{e}	1.16 ± 0.04 ^{c}

†within each column means with different superscript are significantly ($p < 0.05$) different; * mean ± standard deviation values are reported

‡ IPP – Insoluble polymeric protein; ± SPP – Soluble polymeric protein; ⫫Gli – Gliadins; ¥Alb/Glb – Albumins and globulins

Table 4.3 Insoluble polymeric protein† and 50% propanol soluble protein composition† of hydrothermally processed waxy wheat samples* (total protein content was 15.47 %, db)

Initial Moisture Content	12.4%				20%			
Heating Condition (°C/Min)	Protein fraction, % of protein				Protein fraction, % of protein			
	IPP‡	SPP±	Gli╬	Alb/Glb¥	IPP‡	SPP±	Gli╬	Alb/Glb¥
Native	6.02 ± 0.14 ^{g}	1.06 ± 0.01 ^{ab}	5.28 ± 0.09 ^{a}	1.52 ± 0.04 ^{a}	6.02 ± 0.14 ^{g}	1.06 ± 0.01 ^{a}	5.28 ± 0.09 ^{a}	1.52 ± 0.04 ^{a}
140/0	6.50 ± 0.05 ^{f}	1.31 ± 0.14 ^{a}	4.78 ± 0.19 ^{ab}	1.29 ± 0.00 ^{b}	5.63 ± 0.04 ^{g}	1.37 ± 0.23 ^{a}	5.53 ± 0.25 ^{a}	1.35 ± 0.02 ^{ab}
140/15	8.15 ± 0.04 ^{d}	0.56 ± 0.17 ^{bcd}	4.14 ± 0.19 ^{c}	1.03 ± 0.01 ^{c}	9.92 ± 0.09 ^{d}	0.05 ± 0.05 ^{b}	3.12 ± 0.04 ^{c}	0.80 ± 0.10 ^{d}
140/30	10.11 ± 0.00 ^{b}	0.20 ± 0.11 ^{d}	2.79 ± 0.06 ^{d}	0.78 ± 0.05 ^{d}	10.72 ± 0.17 ^{bc}	0.07 ± 0.06 ^{b}	2.18 ± 0.11 ^{de}	0.90 ± 0.12 ^{cd}
140/60	11.45 ± 0.13 ^{a}	0.10 ± 0.04 ^{d}	1.72 ± 0.09 ^{e}	0.62 ± 0.08 ^{e}	11.67 ± 0.10 ^{a}	0.04 ± 0.03 ^{b}	1.41 ± 0.13 ^{f}	0.75 ± 0.06 ^{d}
160/0	6.59 ± 0.05 ^{f}	1.20 ± 0.20 ^{a}	4.82 ± 0.16 ^{ab}	1.27 ± 0.01 ^{b}	7.80 ± 0.12 ^{f}	0.25 ± 0.01 ^{b}	4.60 ± 0.09 ^{b}	1.22 ± 0.02 ^{abc}
160/5	7.45 ± 0.02 ^{e}	0.79 ± 0.19 ^{abc}	4.47 ± 0.20 ^{bc}	1.17 ± 0.01 ^{bc}	9.21 ± 0.11 ^{e}	0.15 ± 0.10 ^{b}	3.45 ± 0.12 ^{c}	1.07 ± 0.09 ^{bcd}
160/15	9.76 ± 0.10 ^{c}	0.30 ± 0.16 ^{cd}	3.03 ± 0.04 ^{d}	0.79 ± 0.02 ^{d}	10.37 ± 0.07 ^{cd}	0.08 ± 0.06 ^{b}	2.50 ± 0.02 ^{d}	0.94 ± 0.10 ^{cd}
160/30	11.39 ± 0.12 ^{a}	0.10 ± 0.05 ^{d}	1.71 ± 0.09 ^{e}	0.68 ± 0.02 ^{de}	11.20 ± 0.20 ^{ab}	0.07 ± 0.05 ^{b}	1.82 ± 0.10 ^{ef}	0.79 ± 0.15 ^{d}

†within each column means with different superscript are significantly ($p < 0.05$) different; * mean ± standard deviation values are reported

‡ IPP – Insoluble polymeric protein

± SPP – Soluble polymeric protein

╬ Gli - Gliadins

¥ Alb/Glb – Albumins and globulins

Table 4.4 Pasting properties† of normal wheat processed under different conditions

Normal wheat flour processed at 13.20% initial moisture content								
			Pasting properties analyzed at pH 7.0			Pasting properties analyzed at pH 3.0		
Heating Condition (°C-min)	Pasting temp (°C)	Peak visc. (cP)	Hot paste visc‡ (cP)	cold paste visc (cP)	Pasting temp (°C)	Peak visc. (cP)	Hot paste visc‡ (cP)	cold paste visc (cP)
Native	95.4 ^{a}	1971 ^{d}	1125 ^{d}	2118 ^{g}	95.0 ^{a}	3429 ^{a}	839 ^{de}	1522 ^{de}
140-0	95.3 ^{a}	2195 ^{c}	1401 ^{b}	2584 ^{e}	93.6 ^{a}	2780 ^{cde}	991 ^{bc}	1818 ^{bc}
140-15	92.9 ^{bc}	2425 ^{ab}	1487 ^{ab}	2879 ^{d}	92.4 ^{b}	2981 ^{c}	1030 ^{ab}	1872 ^{b}
140-30	90.4 ^{de}	2583 ^{a}	1565 ^{a}	3158 ^{ab}	91.9 ^{c}	3209 ^{b}	1069 ^{ab}	1945 ^{b}
140-60	90.0 ^{e}	1831 ^{d}	1179 ^{d}	2301 ^{f}	91.9 ^{c}	2534 ^{f}	906 ^{cd}	1676 ^{cd}
160-0	95.1 ^{a}	2229 ^{c}	1277 ^{c}	2906 ^{cd}	92.4 ^{b}	2751 ^{de}	1096 ^{a}	2151 ^{a}
160-5	93.3 ^{b}	2510 ^{ab}	1457 ^{b}	3232 ^{a}	92.4 ^{b}	2609 ^{ef}	1038 ^{ab}	1982 ^{ab}
160-15	90.8 ^{de}	2357 ^{bc}	1558 ^{a}	3049 ^{bc}	92.4 ^{b}	2861 ^{cd}	1054 ^{ab}	1935 ^{b}
160-30	91.6 ^{cd}	1434 ^{e}	956 ^{e}	1795 ^{h}	91.6 ^{c}	1966 ^{g}	780 ^{e}	1465 ^{f}
Normal wheat flour processed at 20% initial moisture content								
			Pasting properties analyzed at pH 7.0			**Pasting properties analyzed at pH 3.0**		
Heating Condition (°C-min)	Pasting temp (°C)	Peak visc. (cP)	Hot paste visc‡ (cP)	cold paste visc (cP)	Pasting temp (°C)	Peak visc. (cP)	Hot paste visc‡ (cP)	cold paste visc (cP)
Native	95.0 ^{a}	1388 ^{c}	798 ^{c}	1612 ^{c}	94.9 ^{a}	2539 ^{b}	736 ^{cd}	1426 ^{c}
140-0	94.9 ^{ab}	1413 ^{c}	834 ^{c}	1682 ^{bc}	92.1 ^{b}	2534 ^{b}	693 ^{d}	1332 ^{d}
140-15	92.0 ^{b}	1791 ^{a}	1178 ^{ab}	2228 ^{a}	91.1 ^{b}	2881 ^{a}	935 ^{a}	1647 ^{a}
140-30	86.2 ^{b}	1715 ^{a}	1229 ^{a}	2326 ^{a}	87.1 ^{c}	2756 ^{a}	910 ^{a}	1626 ^{a}
140-60	85.0 ^{b}	1486 ^{bc}	1179 ^{ab}	2240 ^{a}	84.3 ^{d}	2852 ^{a}	896 ^{a}	1637 ^{a}
160-0	95.1 ^{b}	1812 ^{a}	1087 ^{ab}	2103 ^{a}	93.2 ^{ab}	2831 ^{a}	811 ^{b}	1459 ^{bc}
160-5	92.5 ^{b}	1489 ^{bc}	1035 ^{b}	1998 ^{ab}	92.3 ^{ab}	2504 ^{b}	779 ^{bc}	1425 ^{c}
160-15	88.0 ^{b}	1637 ^{ab}	1174 ^{ab}	2222 ^{a}	87.1 ^{c}	2721 ^{a}	884 ^{a}	1595 ^{a}
160-30	87.9 ^{b}	1344 ^{c}	1037 ^{b}	1998 ^{ab}	87.1 ^{c}	2423 ^{b}	806 ^{b}	1495 ^{b}

‡Hot Paste Viscosity – is lowest viscosity while holding at 95°C

†within each column for each moisture content means with different superscript are significantly ($p < 0.05$) different

Table 4.5 Pasting properties† of waxy wheat processed under different conditions

Waxy wheat flour processed at 12.40% initial moisture content								
			Pasting properties analyzed at pH 7.0			Pasting properties analyzed at pH 3.0		
Heating Condition (°C-min)	Pasting temp (°C)	Peak visc. (cP)	Hot paste visc‡ (cP)	cold paste visc (cP)	Pasting temp (°C)	Peak visc. (cP)	Hot paste visc‡ (cP)	cold paste visc (cP)
Native	75.2 ^{a}	1884 ^{g}	734 ^{g}	1038 ^{h}	75.2 ^{a}	2803 ^{g}	542 ^{e}	827 ^{f}
140-0	67.8 ^{bc}	2129 ^{f}	773 ^{fg}	1156 ^{g}	72.7 ^{b}	3607 ^{f}	598 ^{de}	948 ^{e}
140-15	67.8 ^{bc}	3605 ^{abc}	1213 ^{d}	1766 ^{e}	72.7 ^{b}	4237 ^{d}	696 ^{c}	1087 ^{cd}
140-30	67.8 ^{bc}	3803 ^{a}	1454 ^{c}	2215 ^{c}	72.7 ^{b}	4654 ^{b}	817 ^{b}	1248 ^{b}
140-60	67.8 ^{bc}	3536 ^{bc}	1827 ^{a}	2835 ^{a}	72.7 ^{b}	4900 ^{a}	958 ^{a}	1431 ^{a}
160-0	67.9 ^{b}	2459 ^{e}	850 ^{e}	1249 ^{g}	72.7 ^{b}	3987 ^{e}	651 ^{cd}	1034 ^{de}
160-5	66.8 ^{d}	3281 ^{d}	1083 ^{f}	1595 ^{f}	72.7 ^{b}	3998 ^{e}	647 ^{cd}	1002 ^{de}
160-15	67.9 ^{b}	3659 ^{ab}	1363 ^{c}	2022 ^{d}	72.7 ^{b}	4323 ^{d}	769 ^{b}	1139 ^{c}
160-30	67.8 ^{bc}	3413 ^{cd}	1719 ^{b}	2588 ^{b}	72.7 ^{b}	4483 ^{c}	916 ^{a}	1329 ^{b}
Waxy wheat flour processed at 20% initial moisture content								
			Pasting properties analyzed at pH 7.0			**Pasting properties analyzed at pH 3.0**		
Heating Condition (°C-min)	Pasting temp (°C)	Peak visc. (cP)	Hot paste visc‡ (cP)	cold paste visc (cP)	Pasting temp (°C)	Peak visc. (cP)	Hot paste visc‡ (cP)	cold paste visc (cP)
Native	69.5 ^{c}	1288 ^{f}	634 ^{e}	913 ^{f}	72.7 ^{e}	2918 ^{bc}	621 ^{def}	949 ^{cd}
140-0	68.7 ^{c}	1830 ^{e}	883 ^{d}	1339 ^{d}	73.1 ^{de}	2956 ^{ab}	568 ^{f}	870 ^{d}
140-15	72.7 ^{b}	2352 ^{b}	1296 ^{c}	1913 ^{c}	76.0 ^{ab}	2558 ^{e}	654 ^{de}	972 ^{bcd}
140-30	72.7 ^{b}	2515 ^{a}	1407 ^{b}	2098 ^{b}	75.1 ^{bc}	2728 ^{d}	731 ^{bc}	1096 ^{ab}
140-60	72.7 ^{b}	2510 ^{a}	1512 ^{a}	2282 ^{a}	75.6 ^{bc}	3045 ^{a}	827 ^{a}	1212 ^{a}
160-0	69.5 ^{c}	2216 ^{c}	818 ^{d}	1094 ^{e}	74.2 ^{cd}	3017 ^{ab}	601 ^{ef}	873 ^{d}
160-5	69.5 ^{c}	2162 ^{cd}	869 ^{d}	1200 ^{e}	76.3 ^{ab}	2381 ^{f}	612 ^{def}	897 ^{cd}
160-15	74.6 ^{a}	2069 ^{d}	1280 ^{c}	1919 ^{c}	77.5 ^{a}	2520 ^{e}	681 ^{cd}	1007 ^{bc}
160-30	73.1 ^{b}	2462 ^{ab}	1491 ^{a}	2245 ^{a}	75.6 ^{bc}	2841 ^{c}	772 ^{ab}	1164 ^{a}

‡Hot Paste Viscosity – is lowest viscosity while holding at 95°C

†within each column for each moisture content means with different superscript are significantly ($p < 0.05$) different

Table 4.6 Gelatinization properties[ǂ] of thermally processed normal wheat flours[†] (N=2)[‡].

Initial Moisture Content	Heating Condition (°C/Min)	Gelatinization				Retrogradation			
		Onset Temp (°C)	Peak Temp (°C)	End Temp (°C)	Enthalpy (ΔH) (J/g)	Onset Temp (°C)	Peak Temp (°C)	End Temp (°C)	Enthalpy (ΔH) (J/g)
13.2%	Native	61.1 ± 0.3 [a]	68.5 ± 0.7 [a]	77.6 ± 0.9 [a]	6.3 ± 0.3 [a]	50.4 ± 0.5 [a]	59.4 ± 0.7 [a]	76.4 ± 2.9	1.8 ± 0.3 [a]
	160/0	61.0 ± 0.7 [a]	68.1 ± 0.6 [a]	76.5 ± 0.5 [a]	5.1 ± 0.4 [a]	49.1 ± 1.1 [a]	60.3 ± 2.0 [a]	69.5 ± 3.8	1.6 ± 0.3 [a]
	160/30	60.2 ± 0.4 [a]	67.1 ± 0.8 [a]	75.4 ± 0.7 [a]	4.8 ± 0.4 [a]	50.4 ± 0.1 [a]	59.1 ± 1.2 [a]	71.1 ± 4.7	1.5 ± 0.3 [a]
20%	160/0	61.8 ± 1.6 [a]	69.2 ± 1.7 [a]	77.5 ± 0.2 [a]	4.3 ± 1.5 [a]	50.4 ± 0.5 [a]	59.6 ± 1.0 [a]	71.1 ± 1.4	2.2 ± 0.4 [a]
	160/30[*]					51.0 ± 1.0 [a]	59.2 ± 0.4 [a]	68.4 ± 0.5	1.9 ± 0.3 [a]

[ǂ] Gelatinization properties were determined on flour pastes with 1:2 ratio of flour solids to water

[†] mean ± standard deviation values are reported

[‡] mean values with different superscripts within each column are significantly ($p < 0.05$) different

* The sample yielded no peaks during gelatinization and hence the values were not reported

Table 4.7 Gelatinization properties[╬] of thermally processed waxy wheat flours[†] (N=2)‡.

Initial Moisture Content	Heating Condition (°C/Min)	Gelatinization				Retrogradation			
		Onset Temp (°C)	Peak Temp (°C)	End Temp (°C)	Enthalpy (ΔH) (J/g)	Onset Temp (°C)	Peak Temp (°C)	End Temp (°C)	Enthalpy (ΔH) (J/g)
13.2%	Native	62.3 ± 1.4 [a]	72.0 ± 1.3 [a]	84.5 ± 2.1 [a]	7.0 ± 0.4 [a]	52.5 ± 1.0 [a]	59.9 ± 0.1 [a]	70.9 ± 2.9 [a]	0.4 ± 0.1 [b]
	160/0	63.5 ± 0.1 [a]	73.4 ± 0.5 [a]	85.1 ± 1.2 [a]	6.9 ± 0.2 [a]	52.9 ± 1.4 [a]	61.1 ± 0.6 [a]	72.5 ± 1.4 [a]	0.5 ± 0.0 [b]
	160/30	63.0 ± 1.3 [a]	71.4 ± 1.1 [a]	81.5 ± 0.9 [a]	6.8 ± 0.3 [a]	52.1 ± 0.9 [a]	59.5 ± 0.6 [a]	68.3 ± 1.5 [a]	0.8 ± 0.0 [b]
20%	160/0	55.2 ± 1.4 [a]	60.5 ± 1.4 [a]	66.6 ± 1.4 [a]	8.4 ± 0.7 [a]	51.2 ± 0.2 [a]	60.5 ± 1.5 [a]	66.6 ± 0.3 [a]	1.5 ± 0.3 [a]
	160/30	50.2 ± 0.9 [a]	59.5 ± 1.5 [a]	69.6 ± 1.5 [a]	7.3 ± 1.1 [a]	50.2 ± 0.3 [a]	59.5 ± 0.4 [a]	67.6 ± 2.0 [a]	1.3 ± 0.4 [ab]

[╬]Gelatinization properties were determined on flour pastes with 1:2 ratio of flour solids to water

[†] mean ± standard deviation values are reported

‡ mean values with different superscripts within each column are significantly ($p < 0.05$) different

Table 4.8 Color values (L, a*, b*) of normal and waxy wheat samples processed under different moisture conditions

Normal wheat						
Heating Condition (°C/Min)	**L**	**a***	**b***	**L**	**a***	**b***
Initial Moisture Content	**13.2%**			**20%**		
Native	90.43	-2.22	8.91	86.22	-0.67	9.67
140/0	90.43	-2.91	8.84	85.44	-0.72	10.41
140/15	89.81	-2.37	9.23	85.52	-1.31	11.15
140/30	89.28	-2.24	10.21	85.58	-1.27	11.81
140/60	87.34	-1.34	12.93	84.43	-0.92	14.03
160/0	89.94	-1.58	7.40	85.76	-1.00	10.77
160/5	89.19	-1.85	8.05	85.76	-1.20	10.97
160/15	89.02	-2.16	10.44	85.64	-1.16	11.53
160/30	86.06	-0.85	14.28	84.57	-1.86	12.87
Waxy wheat						
Heating Condition (°C/Min)	**L**	**a***	**b***	**L**	**a***	**b***
Initial Moisture Content	**12.4%**			**20%**		
Native	90.56	-2.44	9.36	88.69	-1.80	10.11
140/0	90.53	-2.46	9.41	87.65	-1.79	10.94
140/15	89.87	-2.70	10.10	87.63	-2.26	11.56
140/30	89.30	-2.60	10.93	87.37	-2.15	12.15
140/60	88.36	-2.12	12.84	86.33	-1.60	14.09
160/0	90.37	-2.58	9.80	87.88	-1.96	11.37
160/5	89.82	-2.65	10.20	87.51	-2.15	11.65
160/15	89.40	-2.71	10.89	87.24	-2.12	12.18
160/30	88.53	-2.25	12.49	86.82	-1.81	13.27

Note: The standard deviation values for all samples were zero

Figure 4.1 RVA curves representing pasting curve of (A) heat treated (20% initial moisture processed at 160°C for 30 min) and (B) native flour samples in distilled water (solid line) and 1mM silver nitrate solution (dotted line).

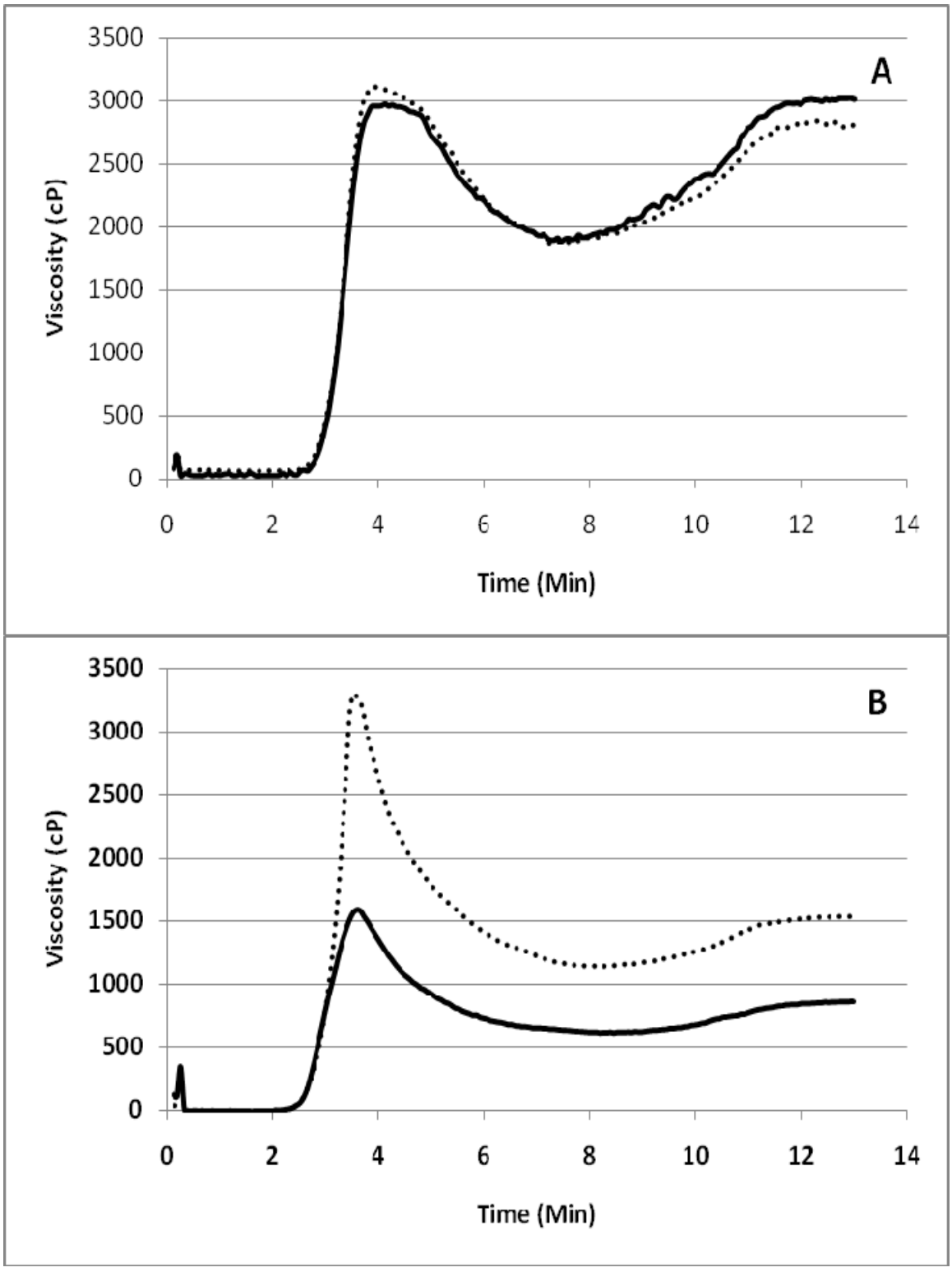


Figure 4.2 Wide-Angle X-ray Diffraction (WAXD) curves for native normal wheat flour (solid line) and normal wheat flours processed at at 160 C for 0 min (dotted line) and 160 C for 30 min (dashed line) at two different moisture conditions (A) 12.4% moisture content and (B) 20% moisture content.

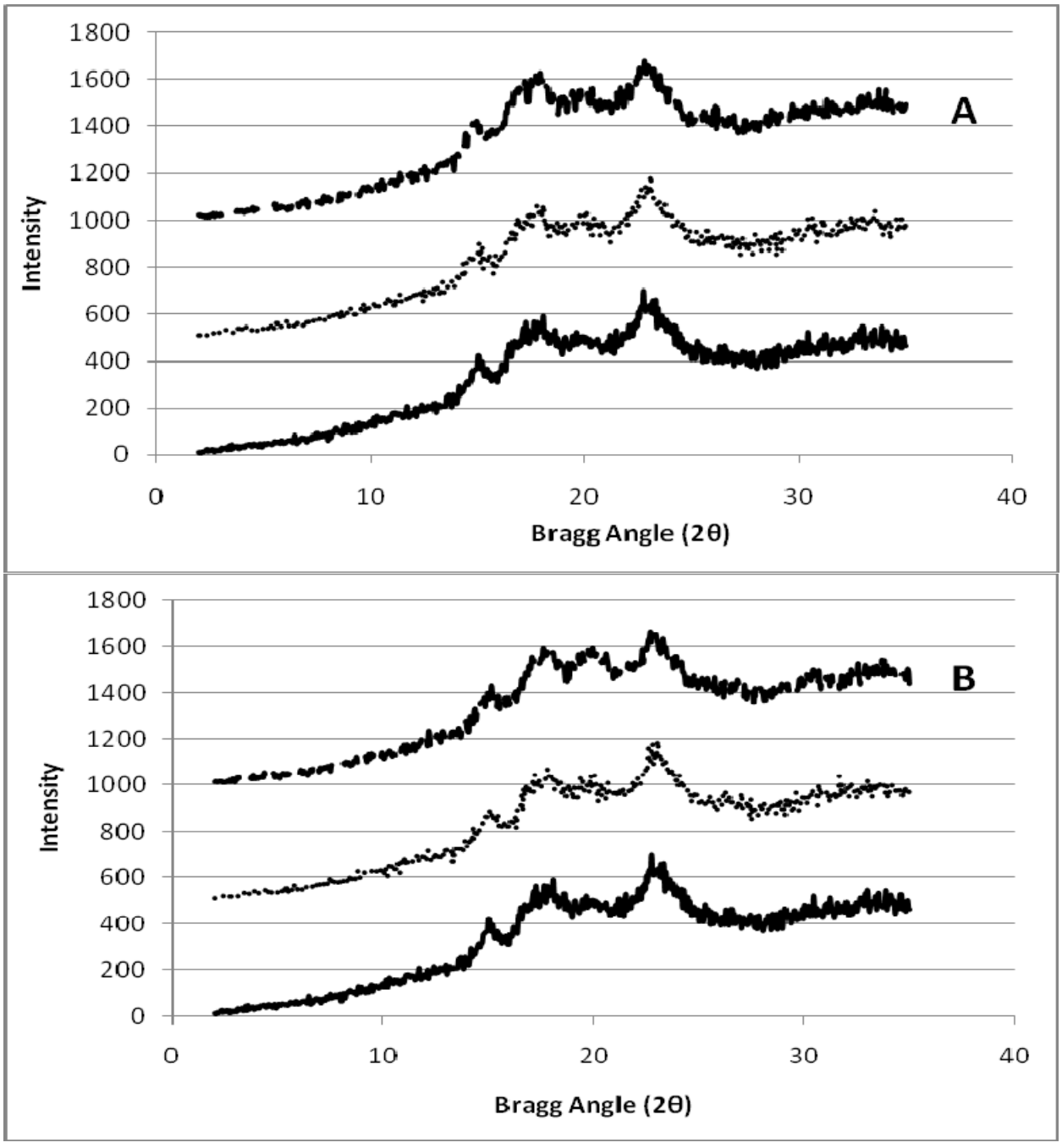
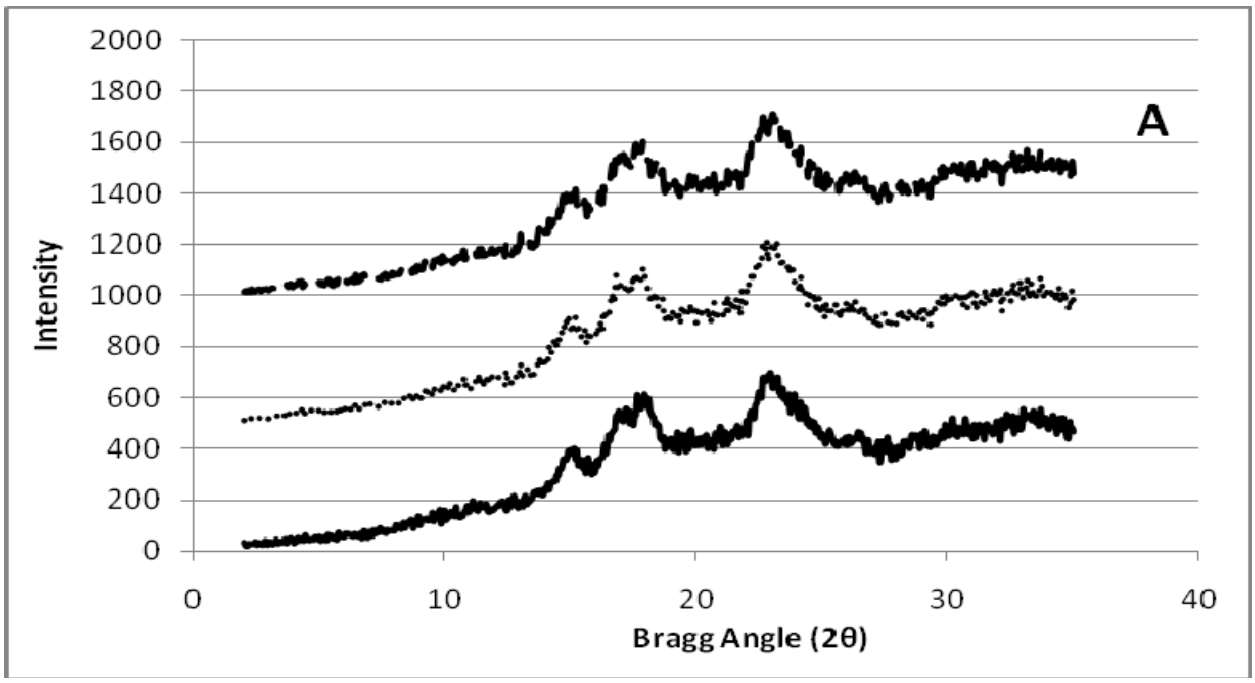
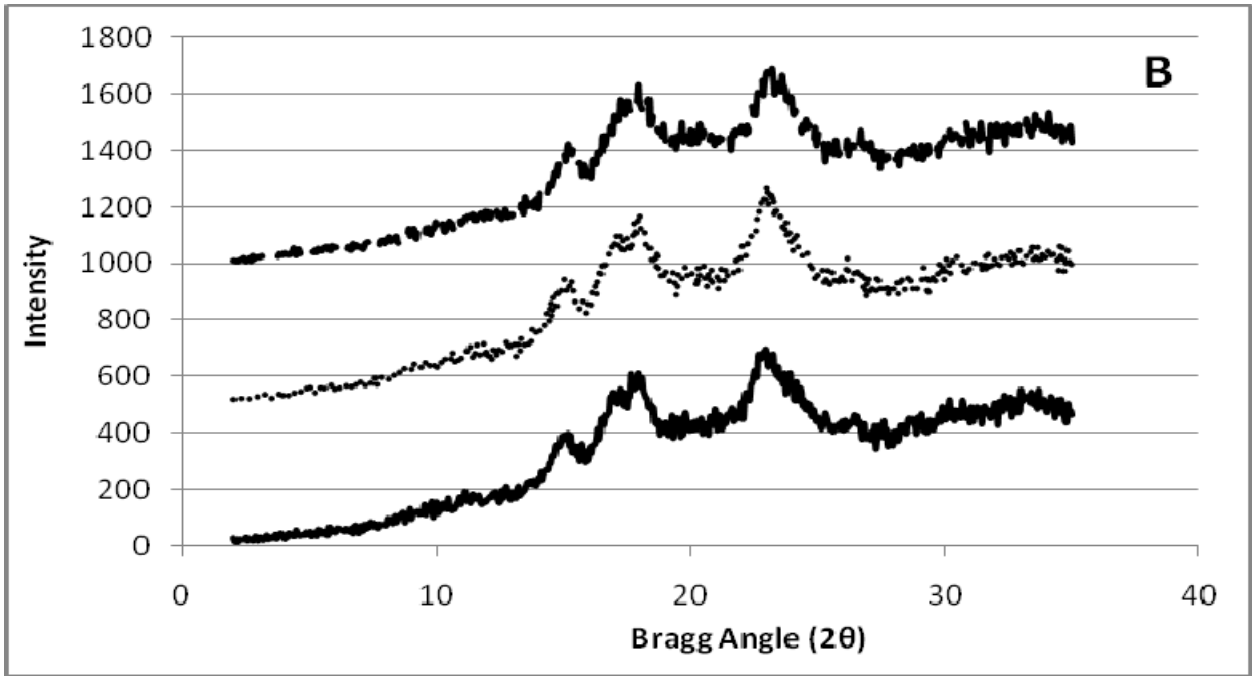


Figure 4.3 Wide-Angle X-ray Diffraction (WAXD) curves for native waxy normal wheat flour (solid line) and waxy wheat flours processed at at 160 C for 0 min (dotted line) and 160 C for 30 min (dashed line) at two different moisture conditions (A) 12.4% moisture content and (B) 20% moisture content.





CHAPTER 5 - VOLUME, TEXTURE, AND MOLECULAR MECHANISM BEHIND THE COLLAPSE OF BREAD MADE WITH DIFFERENT LEVELS OF HARD WAXY WHEAT FLOURS

ABSTRACT

Physico-chemical properties of bread baked by partially replacing normal wheat (*Triticum aestivum* L.) flour (15, 30, and 45%) with two hard waxy wheat flours were investigated. Substitution with waxy wheat flour resulted in higher loaf volume and softer loaves. However, substitution at > 30% resulted in excessive post-bake shrinkage and a 'key-hole' shape with an open crumb structure. Bread crumb microstructure indicated a loss of starch granule rigidity and fusing of starch granules. The cells in the interior of the bread did not become gas continuous and as a result, shrunk as the loaf cooled. Soluble starch content was significantly higher in bread crumb containing waxy wheat flour than in control bread. Debranching studies indicated that the soluble starch made with 30-45% hard waxy wheat flour was mostly amylopectin. Incorporation of waxy wheat did not retard staling upon storage.

INTRODUCTION

Based on the level of amylose in its endosperm starch, wheat (*Triticum aestivum* L.) varieties are classified as full waxy, partial waxy, normal (wild-type) wheat and high-amylose wheat (Nakumura et al., 1993; Nakumura et al., 1995; Graybosch, 1998). Full waxy wheat has little, if any amylose. A change in the ratio of amylose to amylopectin can result in altered textural attributes in food products, primarily because of differences in swelling and gelling properties. Because of its lack of amylose, waxy wheat can potentially reduce the initial phase of retrogradation i.e. rapid association of amylose molecules (Graybosch, 1998). A number of studies have been conducted to understand the potential of waxy wheat as a shelf-life extender of baked goods. Bread containing waxy wheat was reported to be softer than bread made with wild type wheat immediately after baking (Morita et al., 1998; Graybosch, 2001; Morita et al., 2002; Yi et al., 2009). Reduced amylose wheat used in a French bread formulation resulted in a soft crumb structure (Park and Baik, 2007). Incorporation of waxy wheat flour into a white pan bread formulation resulted in a high loaf volume immediately after baking (Morita et al., 1998; Graybosch, 2001; Bhattacharya et al., 2002); however, the loaves collapsed upon storage and shrunk excessively within 24 h after baking (Morita et al., 1998; Graybosch, 2001; Lee et al., 2001). The crumb structure of bread containing waxy wheat flour displayed a more open and porous structure compared to the control (Graybosch, 2001; Lee et al., 2001; Hung et al., 2007a and b).

Previous reports on the inclusion of waxy wheat flour in bread and its impact on staling have been inconsistent. When flour from near-isogenic waxy wheat lines was substituted (up to 40%) for wild type flour in a white pan bread formulation the bread showed lower firmness for up to 7 days of storage as compared to the control (Morita et al., 2002). When durum waxy

wheat flour was used (up to 30%), the resulting loaves showed lower firmness than the control (Bhattacharya et al., 2002). In contrast to those studies, when flours from waxy wheat lines were substituted for stronger hard red winter wheat flour (up to 50%), the rate of crumb firming was higher than the control (Graybosch, 2005). In a separate study, incorporation of waxy wheat flour in bread was reported to increase the moisture retention capacity of crumb during storage (Park and Baik, 2007).

In addition to the inconsistent conclusions on the impact of waxy wheat flour on bread staling the reasons why waxy wheat flour causes the collapse of bread loaves upon storage 6are not clearly understood. Objectives of this study were to (i) evaluate the impact on white pan bread of incorporating 15-45% of total flour weight with hard waxy wheat flour from advanced breeding lines; (ii) understand and explain the underlying mechanism of loaf collapse in bread containing high levels of waxy wheat flour; and (iii) clarify the impact of waxy wheat flour on bread staling.

MATERIALS AND METHODS

Materials

Control wild-type wheat (Karl 92) and two waxy wheats, NX03Y2114 (sample 2114) and NX03Y2489 (sample 2489) from advanced breeding lines were procured from USDA-ARS, Lincoln, NE. The pedigree of sample 2114 was Cimarron/Rio Blanco//Baihou4/L910145/3/Colt/Cody//Stozher/NE86582 and that of sample 2489 was BaiHuo/Kanto107//Ike/3/KS91H184/3*RBL//N87V106. Wheat kernels were tempered to 16% moisture for 18 h and were roller-milled into straight-grade flour on a MLU 202 Bühler experimental mill (Bühler Co., Uzwill, Switzerland). The protein content of the flours was

11.44, 13.01, and 13.25 (%db) for Karl 92, sample 2114 and sample 2489, respectively, and the starch content was 76.7, 75.0, and 80.0 (%db) for Karl 92, sample 2114 and sample 2489, respectively, as previously reported (Guan et al., 2009).

Dough mixing characteristics

Dough characteristics were measured using a 10 g mixograph according to AACC 54-40 A (AACC International, 2000). Water absorption was initially calculated based on protein content by using AACC 54-40A, but was finally optimized for each sample based on series of mixograms (Guan et al., 2009).

Gas generation from flours using Risograph

Gas generated from liquid ferment of flours was measured by using a modified AACC 89-01 method (AACC International, 2000). Instant yeast (0.4 g) (Lesaffre Yeast Corp., Milwaukee, WI) and distilled water (15 mL) were added to each flour (10 g) and mixed for 1 min in Risograph (RDesign, Pullman, WA) containers by using a glass rod, which was left in the container. The containers were connected to the Risograph and the rate and the total amount of carbon dioxide released from liquid ferment over a 90-min period.

Enzyme digestion of flours and release of D-glucose

Enzyme digestion of flours was done using a modified Englyst method (Englyst et al., 1992). The enzyme mixture was prepared by adding 3.0 g of Pancreatin (P-7545, Sigma Aldirch, St. Louis, MO) to 20 mL of distilled water, mixing for 10 min and centrifuging at 4000 x *g* for 10

min. An aliquot (15 mL) of supernatant was transferred into a solution of 60 mg of Amyloglucosidase (A-7255, Sigma Aldrich, St. Louis, MO) in 1.7 mL distilled water. Flours samples (0.60 g) were suspended in 10 mL of distilled water and incubated for 30 min at 37° C. Subsequently, 10 mL of 0.25 N sodium acetate and 5 mL of the enzyme mixture were added to the suspension which was then incubated up to 180 min at 37°C with continuous mixing. At time intervals of 20, 40, 60, 90, 120 and 180 min, 0.25 mL of solution was transferred into 25 mL glass tubes containing 10 mL of 66% ethanol. The tubes were centrifuged at 4500 x *g* for 10 min. The supernatant (0.1 mL) was transferred into a 10 mL glass tubes and 3.0 mL glucose oxidase – peroxidase (GOPOD, Megazyme Kit, Wicklow, Ireland) was added immediately. The tubes were incubated at 40° C for 20 min, and the absorbance was measured against a reagent blank at 510 nm.

Bread baking

Pup-loaf bread was baked using the AACC 10-10B (AACC International, 2000) straight dough method with 90-min fermentation time. The baking formula (flour basis) was 100.0 g flour (14% mb), 6.0 g sucrose, 3.0 g shortening (Crisco®, Orville, OH), 2.0 g yeast, 1.5 g salt, 50 mg of L-ascorbic acid 50 mg (Merck, Darmstadt, Denmark) and 0.5 g diastatic malt (King Arthur Flour, Norwich, VA). For breads made with 15-45% levels of waxy wheat flour, Karl 92 flour was partially replaced on a dry weight basis with one of the two hard waxy wheat flours (2114 or 2489). Additionally, pup-loaf breads were also baked for 100% waxy wheat flour. Four loaves of bread were baked for each formulation.

Loaf weight and loaf volume (rapeseed displacement AACC 10-05, AACC International, 2000) were measured immediately, 1 h and 24 h after removal from the oven, and specific

volume data were reported. The loaves were double bagged in polypropylene bags and stored at room temperature. On day 1 and day 7 after baking, two loaves of each formulation were sliced into 1" thick slices. The two slices from the middle were further analyzed. Characteristics of bread crumb were determined using C-Cell (Calibre Control Intl., Warrington, UK), an image analysis instrument, to obtain image of the slice and data on no. of gas cells, gas cell volume, cell wall thickness and slice brightness. Moisture content (AACC 44-15A, AACC International, 2000) of the slices was analyzed on days 1 and day 7.

Texture analysis

Firmness was measured by a modified AACC 74-09 method (AACC International, 2000). Bread slices were tested using a TA.XT2 texture analyzer (Texture Technologies Corp., Scarsdale, N.Y.) with a 36 mm cylindrical probe. Each slice was compressed to a 7 mm distance and held for 30 s. Firmness was calculated as the peak force at 7mm. Firmness values reported were the average of three measurements.

Soluble carbohydrate in bread crumbs

Bread samples were analyzed for soluble carbohydrate (starch) content and molecular weight distribution.

Soluble carbohydrate content was determined by a modified AACC 76-13 method (AACC International, 2000) (Megazyme Kit, Wicklow, Ireland). Soluble starch was extracted by mixing 100 mg of freeze-dried bread in 1.5 mL of water in a 2.0 mL microcentrifuge tube. The sample was vortexed for 45 min and centrifuged at 12,000 x *g*. The supernatant (1.0 mL) was immediately transferred to a test tube containing 3.0 mL of thermostable α-amylase (300 U) in

MOPS buffer (50 mM, pH 7.0). The contents of the test tube were vigorously mixed and incubated in a boiling water for 6 min with intermediate stirring at 2 and 4 min intervals. The test tube was placed in a 50° C water bath and sodium acetate buffer (4.0 mL, 200 mM, pH 4.5), followed by amyloglucosidase (0.1 mL, 20 U) were added. The contents were thoroughly mixed and the test tube was incubated in a 50°C water bath for 30 min. The volume of the test tube contents was adjusted to 10.0 mL with distilled water and centrifuged at 3000 x *g* for 10 min. An aliquot (0.1 mL) of the supernatant was transferred to a test tube to which 3.0 mL of glucose oxidase peroxidase (GOPOD) reagent was added. The tubes were incubated in a 50°C water bath for 30 min. Absorbance of the samples was taken at 510 nm against the reagent blank and D-glucose was used as the reference standard. Percent soluble starch was calculated based on the starch content of the flour. An average of three replicates was reported as total soluble carbohydrate (%).

Molecular weight distribution of soluble carbohydrate was determined by using gel permeation chromatography (GPC). Freeze dried soluble starch was dissolved in 1.0 mL of dimethyl sulphoxide (DMSO) in 2.0 mL microcentrifuge tubes to obtain a final concentration of 0.1% starch. The GPC analysis was performed with a PL-GPC 220 Integrated GPC/SEC fully automated system (Polymer Laboratory, Amherst, MA). The system was equipped with an auto sampler, a differential refractive index (DRI) detector and phynogel 00H-0646-KO, 00H-0644-KO, 00H-0642-KO columns (Phenomenex, Torrance, CA) connected in a series. The mobile phase in the column was DMSO with 5mM $NaNO_3$. The flow rate of 0.8 mL/min and the column oven temperature was controlled at 80°C. A series of dextran standards (American Polymer Standards Corporation, Mentor Ohio) with different molecular weights were used to calibrate the retention time with molecular weight. The electronic outputs of the DRI detectors were collected

by GPC software (version 3.0, Polymer Laboratories, Amherst, MA). Aliquots (20 μL) of solube starch dissolved in DMSO were injected into GPC and analysis was done in duplicate.

Thermal properties of bread

Thermal properties of bread crumbs were determined by using differential scanning calorimetry (DSC) (Q100 DSC, TA Instruments, New Castle, DE). Freeze-dried bread samples (10 mg) from days 1 and day 7 and distilled water were added to the DSC pan in a 1:2 ratio (w/w). The pan was hermitically sealed and allowed to equilibrate at 25 °C for 1 h. The samples were then heated from 10°C to 140°C at 10°C/min. An empty DSC pan was used as a reference. Onset, peak and completion temperatures along with enthalpy were determined. Each sample was analyzed in duplicate and average values were reported.

Scanning electron microscopy (SEM)

A small piece (< 1 mm^3) of freeze-dried bread crumb was fixed on specimen stubs using carbon paste. The samples were coated with gold-palladium by a sputter coater (Denton Vaccuum, LLC., Moorestown, NJ). The samples were viewed at 300X and 1000X resolution with a scanning electron microscope (S-3500N, Hitachi Science Systems, Ltd., Japan) operating at an accelerating voltage of 20 kV. Each sample was analyzed two times.

Confocal laser scanning microscopy (CLSM)

Slides of freeze-dried bread samples were prepared based on the methods of Lee et al. (2001) and Schrober et al. (2004). A small piece (< 1 mm^3) of freeze-dried bread crumb was placed on a

microscopic slide and a weakly alkaline solution of flourescein 5(6)- isothiocyanate (FITC) (Sigma-Aldrich, St. Louis, MO) was added to the sample. The slide was air-dried at room temperature in a dark environment for about 1 h. Prior to analysis, immersion oil was dropped on the sample and the sample was covered with a cover slip. A Zeiss LSM 5 Pascal CLSM (Ziess, Gottingen,Germany) was used to view the microstructure of the bread crumbs. Fluorescence emission imaging of FITC was done using the 488 nm line of a 458/488/514 argon gas ion laser to excite FITC. Overlaid images of birefringent starch granules and florescent protein matrix were used to compare the internal structures of different bread samples. Each sample was analyzed two times.

Statistical analysis

Macanova 4.12 (School of Statistics, University of Minnesota, Minneapolis, MN) was used to perform ANOVA and honest significance difference (HSD) analysis. The level of significance was $P < 0.05$ for all data analyses.

RESULTS AND DISCUSSION

Flour and dough properties

Protein content of Karl 92 and waxy wheat samples 2114, 2489 are 15.47, 13.88 and 12.82%, respectively (previously reported by Guan et al., 2009). As previously reported (Guan, 2008), the optimized mixograph data indicated that compared with a wild type bread wheat flour (Karl 92 which had a peak time – 4.82 min and a peak height – 55%), the two waxy wheat samples had a shorter mixing time (4.22 and 3.41 min for samples 2114 and 2489 respectively). Additionally,

sample 2114 and higher peak height (59.5%) and sample 2489 had lower peak height (47.4%) when compared to wild-type wheat flour. Water absorption capacity for Karl 92, sample 2114 and 2489 were 60.8, 66.4, and 57.7%, respectively. In this study, the blends of Karl 92 wheat flour and each of the waxy wheat flour were further examined by mixograph and the optimum water absorption and mixing time were determined from a series of mixographs (**Table 5.1**). As the amount of waxy wheat flour increased in the dough formulation, the water absorption increased for when sample 2114 was added to the blend; while it remained the same for sample 2489. The optimum mixing time decreased with increasing incorporation of waxy wheat.

Bread volume and texture

Volume

Changes in specific loaf volume (cc/gm) are given in **Figure 5.1**. Immediately after baking, the specific volume of bread loaves containing waxy wheat flour was significantly higher ($P < 0.05$) than the control, and was highest for bread loaves containing 45% waxy wheat flour. These results are consistent with findings by previous researchers (Morita et al., 2002; Yi et al., 2009) who reported an increase in volume of breads baked with waxy wheat. The higher loaf volume in waxy wheat breads could be due to higher gas (carbon dioxide) release during fermentation of waxy wheat flours (**Figure 5.2A**). In our study, the starch in waxy wheat flour was more readily digestible than starch in wild type flour (**Figure 5.2B**). Liquid ferment from waxy wheat flour released more carbon dioxide than wild type flour the during the 90-min fermentation time (**Figure 5.2A**). Probably higher amounts of damaged starch in waxy wheat (Bettge et al., 2000 and Garimella Purna, 2010) provided readily fermentable sugars during yeast fermentation (Lee et al., 2001). The differences in specific loaf volumes were not significantly different ($P > 0.05$)

between the waxy wheat samples at all substitution levels, although there were differences between the two waxy wheat samples with respect to gas production in a liquid ferment and their dough mixing properties.

"Keyhole" effect.

There was a considerable decrease in specific volume from 0 min (immediately after baking) to 24 h after baking for all formulations. The decrease in specific volume with time was higher in formulations containing higher levels of waxy wheat flour. Excessive shrinkage of loaves containing 45 and 100% waxy wheat flour resulted in a "keyhole" effect (**Figure 5.3**). Previous researchers who studied the use of tapioca and waxy barley starch in bread attributed excessive post-baking shrinkage in breads to lower pasting temperature and fusing of starch granules into a continuous network (Ghiasi et al., 1984; Kusunose et al., 1999). Waxy starch granules have a lower pasting temperature than wild-type starch granules (Guan, 2008). Moreover, waxy wheat starch swells rapidly and the granules lose structural integrity and disintegrate at temperatures around 70°C (Guan, 2008). Pictures from SEM showed that starch granules maintained integrity in control bread crumb (**Figure 5.3**) but waxy wheat bread crumb, especially those containing 100% waxy wheat, had a fused starch granule network (**Figure 5.3**), similar to the microstructure of bread made from tapioca and waxy barley (Ghiasi et al., 1984; Kusunose et al., 1999). The fusing of starch granules became more evident as the level of waxy wheat in bread crumb increased. Additionally, the protein network in bread crumbs made with high levels of waxy wheat appears to be elongated between the starch granules (**Figure 5.3**).

From dough to bread, there is a phase inversion during which foam structure of dough is converted into sponge i.e. bread (Bloksma, 1981). During mixing, air is incorporated in the form

of small nuclei/cells into the dough (Baker and Mize, 1946, MacRitchie, 1976). The gas cells are surrounded by starch gluten matrix, and this matrix acts as a cell wall. These gas cells expand during proofing along with incorporation of fermentation gases, and during baking the gas cells expand with increasing temperature (He and Hoseney, 1991). Up to this point, dough is considered to be a closed cell foam that retains CO_2 (Hoseney, 1986) During the later stages of baking, cross linking of proteins along with gelatinization of starch leads to a rupture in the cell wall, allowing the gas to escape from crumb to crust (Bloksma, 1981). After baking, during cooling the leached amylose forms a gel between the swollen starch granules and could be responsible for the setting or rigidity of loaf. Baked bread is considered to be a an open celled sponge that is permeable to air (Baker and Mize, 1946). We postulate that when dough consisting of waxy wheat is baked, fusing of starch granules makes the cell walls impermeable. Consequently, during baking, when high amounts of carbondioxide is corporated, the cell walls expand to their maximum but fail to rupture thereby continuing to maintain their foam structure. During cooling, the cell walls collapse due to negative internal pressure and result in keyhole effect.

C-Cell.

C-Cell results (**Table 5.2**) showed an open crumb grain structure in bread with high levels of waxy wheat flour. As the level of waxy wheat flour in the bread formulation increased, gas cell volume increased and the number of cells decreased. C-Cell results indicate that control bread (Karl 92) had more small cells than bread containing 45% waxy wheat (**Table 5.2**), which is clearly evident in **Figure 5.3**. Enzyme digestibility data indicated that compared with the control (Karl 92) flour, the starch in waxy wheat flours was more readily digestible by enzymes which could have contributed to the increased gas (carbon dioxide) released by the yeast in the liquid

ferment. Gas produced during fermentation is typically transported to gas nuclei that were formed during dough mixing (Gan et al., 1990), and the greater gas production in dough systems with waxy wheat flour could result in large gas cells. Those large gas cells expand during baking, creating an "open" crumb structure in the resultant bread. Alternatively, gas cell can coalescence during bread making with waxy wheat flour could due to the excessive swelling of waxy wheat starch granules which increases the moisture content and flour of the gas cell walls. Guan (2008) noted that waxy starch granules swell excessively and lose granule integrity upon heating in excess water. Excessive swelling of waxy starch could be the cause of open crumb structure.

Texture.

Firmness values of all bread loaves are shown in **Figure 5.4A**. On day 1, loaves with 30 and 45% waxy wheat were significantly ($P < 0.05$) less firm than the control. The lower firmness could be due to the lower amylose content in waxy wheat bread formulations. Previous researchers (Biliaderis, 1992; Hug-Iten et al., 2003) have attributed the initial firmness of bread crumb to rapid re-association of the amylose fraction. It was not possible to get consistent firmness values from 100% waxy wheat loaves. The crumb of the 100% waxy wheat loaves was too fragile and the texture analysis probe was touching the sides and the upper crust of the samples. Hence those measurements were not representative of crumb and are not reported.

Soluble Starch and Structure

The solubility of starch increased as the percentage of waxy wheat increased (**Figure 5.4B**). However, the profiles of increasing starch solubility were different for the two waxy wheat samples. For sample 2114, there was no significant difference in soluble starch between the 15

and 30% replacement levels, but soluble starch at these levels was significantly greater than the control. For sample 2489, there was a significant and gradual increase in the percentage of soluble starch with the increase in waxy wheat flour replacement level. This could be because amylopectin has greater solubility than amylose in 1- and 7-day old bread. The control formulation had more amylose than the formulations containing waxy wheat flour. Data from GPC showed differences between control and waxy wheat samples. **Figure 5.5** shows the data for sample 2114 only, but similar results were obtained for sample 2489. Soluble starch from control bread (Karl 92) had a prominent peak in the low molecular weight plus a shoulder peak in the higher molecular weight region. As the level of waxy wheat flour was increased from 15 to 45%, the distribution became bimodal, with the peaks being almost equally intense in the low and high molecular weight regions. The same phenomenon was observed for both waxy varieties. An increase in the replacement level of waxy wheat flour resulted in an increase in soluble starch in the bread crumb. The increase in soluble starch content could be due to the ease of fragmentation of waxy starch granules (Guan, 2008). Our results indicate that most of the soluble starch observed at high molecular weight in **Figure 5.5** was amylopectin, which agrees with previous studies (Schoch and French, 1947; Ghiasi et al., 1979). Overall, amylose content (the low molecular weight peak) decreased when wild-type wheat flour was partially replaced with waxy wheat flour; therefore, the amount of amylose leached was reduced. Leached amylose forms a gel between swollen starch granules (He and Hoseney, 1991) and is thought to be responsible for the setting or rigidity of loaf (Hug-Iten et al., 2003; Ghiasi et al., 1979). The combination of less amylose and more soluble starch from amylopectin could result in a soft crumb structure on day 1 and shrinkage after baking for bread that contains a high level of waxy wheat flour.

From day 1 to 7, the percentage of soluble starch decreased (**Figure 5.4B**), which could be due to the eventual retrogradation of amylopectin in bread. On day 7, there was no difference in the percentage of soluble starch between the control, 15% replacement, and 30% replacement, which contained, respectively, ~ 75, 79 and 83% amylopectin in starch. However, the 45% replacement with ~ 86% amylopectin in its starch had slightly more soluble starch.

Effect of waxy wheat on staling

On day 1, bread slices from loaves containing waxy wheat flour were much softer than control bread (**Figure 5.4A)**. Firmness decreased as the level of waxy wheat flour in the formulation increased. Firmness results from day 1 are consistent with previous studies (Graybosch, 2001; Hung et al., 2007a and b), which reported that loaves made from formulations containing waxy wheat flour were softer than loaves from formulations with wild-type wheat flour. On day 7, there were no significant differences in firmness between bread crumbs containing waxy wheat and control samples. Our results are contrary to two previous studies (Morita et al., 2002; Bhattacharya et al., 2002). Bhattacharya et al. (2002) reported a decrease in firmness over 5 days when waxy durum wheat flour was substituted at low levels (up to 30%), and Morita et al. (2002) reported a decrease in firmness over 7 days when wild-type waxy wheat was substituted at low levels (up to 40%). Our results show a trend of increasing firmness with increase in level of waxy wheat, however they are not significant. Some previous studies (Graybosch, 2001, Graybosch, 2005; Hung et al., 2007b) reported an increase in firmness upon storage for bread crumbs made with partial replacement with waxy wheat flour. The differences in staling results could be due to different control and waxy flours used in the baking formulations. It should be noted that in the present study, bread made with high levels of waxy wheat was softer than

control on day 1 but became as firm as control upon storage, which correlates with the change in soluble starch. These results are consistent with previous findings by other researchers (Ghiasi et al., 1984), who reported that bread loaves containing waxy barley starches were softer than control on day 1 but had equal firmness after three or five days of storage.

Thermal properties of bread crumb were shown in **Table 5.3**. After baking, starch retrogradation is a biphasic phenomenon of starch retrogradation, with rapid association of amylose followed by less rapid recrystallization of amylopectin (Biliaderis, 1992; Hug-Iten et al., 2003). The endothermic peak observed in DSC at onset temperature (T_o) 43.0 to 46.1°C was due to the melting of retrograded amylopectin. On day 1, bread crumb containing 45% waxy wheat had higher enthalpy, presumably due to the increased level of amylopectin. However, crumb firmness decreased on day 1 as the level of waxy wheat flour increased, because of the reduced contribution of amylose retrogradation. From day 1 to day 7, there was a smaller increase in enthalpy in bread crumbs containing 45% waxy wheat flour compared with bread crumbs made with wild wheat flour, despite the fact that bread crumb containing 45% waxy wheat flour had a higher level of amylopectin. The low retrogradation from waxy wheat flour is consistent with previous researchers (Hayakawa et al., 1997) and with our earlier experimental evidence from DSC analysis of starch based gels, which indicated a marked resistance of waxy wheat starch to retrogradation (Guan, 2008). Overall, there was no differences in enthalpy values between the bread crumbs containing waxy wheat flours and the control wheat (Karl 92) on day 7, and all the breads had similar firmness and starch solubilities.

In conclusion, substituting waxy wheat flour in a white-pan bread formulation resulted in increased loaf volume, but significant post-bake shrinkage occurred in formulations with higher

levels (> 30%) of waxy wheat flour. Disintegration and fusing of starch granules was observed in bread containing high levels of waxy wheat flour. The cells in the interior of the bread did not become gas continuous, which explains the excessive loaf volume and high post-bake shrinkage. Partial replacement of waxy wheat flour resulted in softer fresh bread immediately after baking but did not retard staling during storage (7 days).

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Table 5.1 Mixing and absorption conditions used for dough making (based on series of four mixographs).

Replacement	Waxy flour 2114		Waxy flour 2489	
	absorption(%)	Time (min)	absorption(%)	Time (min)
0% (Control)	62.0	5.5	62.0	5.5
15%	62.0	5.0	62.0	5.0
30%	63.0	5.0	61.0	4.75
45%	63.5	4.75	61.0	4.5

Table 5.2 C- cell results for bread slices 24 hours after baking (N=2)

Sample	Number of Cells *	Cell Volume (cc)	Cell Wall Thickness (μm)	Slice Brightness
Karl 92	4811 ± 496 [a]	5.94 ± 0.09 [d]	3.1 ± 0.0 [a]	146 ± 1 [a]
15_2114	4469 ± 216 [abc]	7.22 ± 0.30 [bc]	3.3 ± 0.0 [ab]	138 ± 1 [b]
30_2114	4282 ± 7 [abc]	7.67 ± 0.09 [b]	3.3 ± 0.0 [a]	128 ± 1 [d]
45_2114	3919 ± 49 [bc]	8.53 ± 0.52 [a]	3.4 ± 0.1 [a]	118 ± 2 [f]
15_2489	4542 ± 392 [ab]	6.45 ± 0.00 [cd]	3.2 ± 0.0 [ab]	137 ± 3 [b]
30_2489	3841 ± 366 [c]	7.08 ± 0.43 [bc]	3.3 ± 0.2 [ab]	132 ± 1 [c]
45_2489	3826 ± 6 [c]	8.54 ± 0.33 [a]	3.3 ± 0.0 [a]	124 ± 1 [e]

* Different letters within each column denote significant differences among the samples ($p < 0.05$)

Table 5.3 Thermal properties of bread samples measured by differential scanning calorimetry (DSC) (N = 2)

Sample	T_{onset} (°C) *	T_{peak}(°C)	T_{end}(°C)	ΔH (J/g)
		Day 1		
Karl 92	46.1 ± 0.4 [a]	58.2 ± 2.0 [a]	69.4 ± 0.5 [bc]	1.9 ± 0.1 [c]
15% 2114	45.8 ± 1.1 [a]	57.0 ± 0.1 [ab]	71.4 ± 1.1 [a]	2.2 ± 0.3 [abc]
30% 2114	45.6 ± 0.8 [ab]	56.7 ± 0.2 [ab]	69.5 ± 0.7 [bc]	1.9 ± 0.1 [c]
45% 2114	43.9 ± 1.6 [ab]	57.3 ± 0.4 [ab]	72.0 ± 0.5 [a]	2.4 ± 0.1 [a]
15% 2489	44.2 ± 0.3 [ab]	56.2 ± 0.2 [b]	71.3 ± 1.0 [ab]	2.5 ± 0.1 [ab]
30% 2489	45.6 ± 0.9 [ab]	56.5 ± 0.3 [ab]	69.2 ± 1.0 [c]	2.0 ± 0.1 [bc]
45% 2489	43.0 ± 1.8 [b]	55.9 ± 0.9 [b]	72.0 ± 0.2 [a]	2.5 ± 0.3 [a]
		Day 7		
Karl 92	44.4 ± 1.7 [a]	55.4 ± 0.7 [b]	70.5 ± 2.1 [ab]	4.0 ± 0.8 [ab]
15% 2114	44.7 ± 1.1 [a]	58.0 ± 0.5 [a]	72.4 ± 0.4 [a]	3.8 ± 0.5 [ab]
30% 2114	43.1 ± 1.5 [a]	54.9 ± 0.6 [b]	71.1 ± 0.7 [ab]	4.4 ± 0.6 [a]
45% 2114	45.2 ± 1.1 [a]	55.8 ± 1.2 [b]	70.6 ± 0.3 [ab]	3.6 ± 0.3 [ab]
15% 2489	44.9 ± 0.5 [a]	55.5 ± 1.0 [b]	70.0 ± 0.2 [b]	3.1 ± 0.1 [b]
30% 2489	44.7 ± 0.2 [a]	55.4 ± 0.7 [b]	70.8 ± 1.1 [ab]	4.2 ± 0.4 [a]
45% 2489	45.4 ± 0.5 [a]	55.4 ± 0.1 [b]	69.9 ± 0.8 [b]	3.4 ± 0.1 [ab]

* Different letters within each day and each column denote significant differences among the samples ($p < 0.05$)

Figure 5.1 Changes in bread specific volume after baking (N = 4)

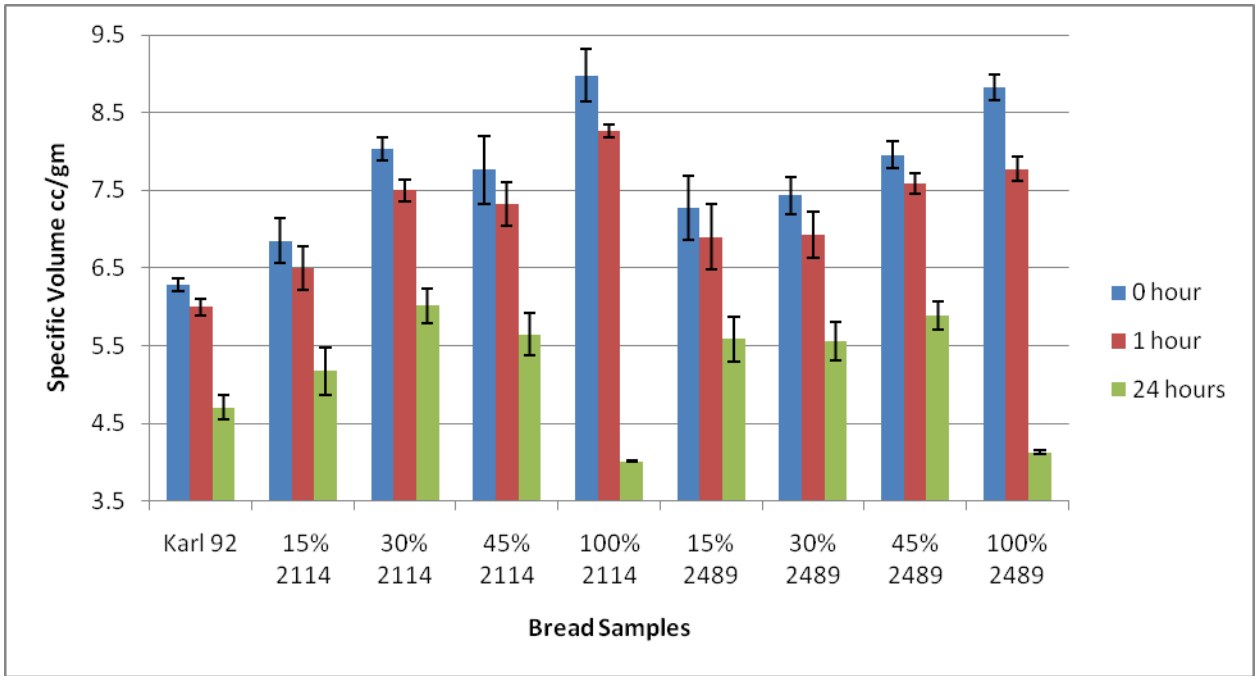
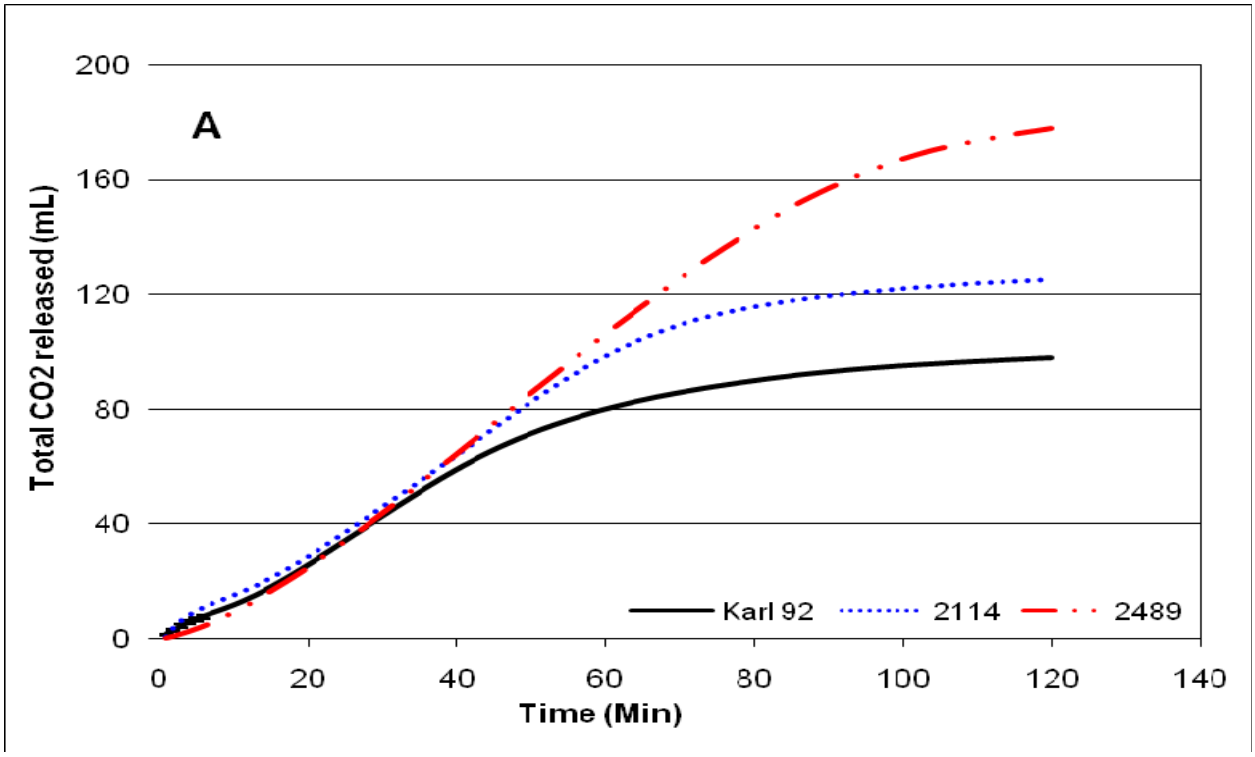


Figure 5.2 (A) Total carbon dioxide released from dough systems from control and waxy wheat flours as measured by a Risograph™ and (B) Enzyme digestion analysis for control and waxy flour samples (N = 3)



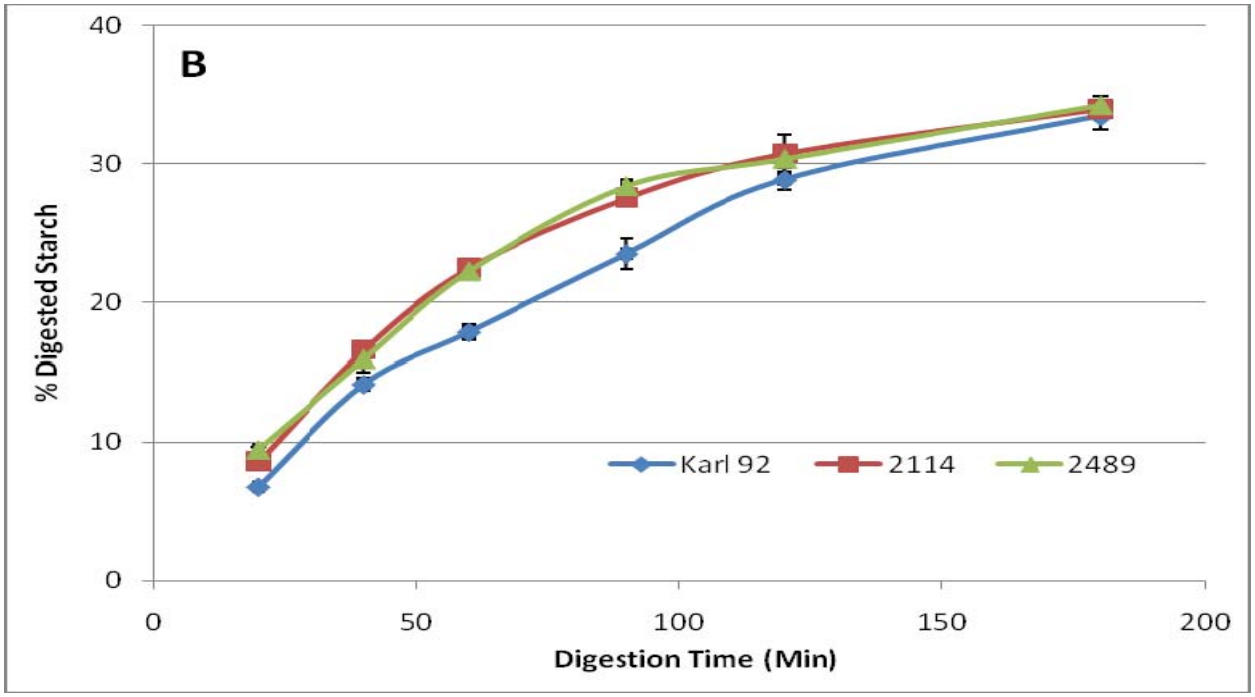
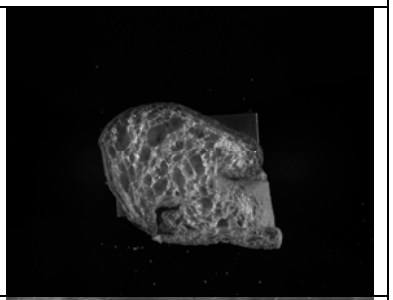
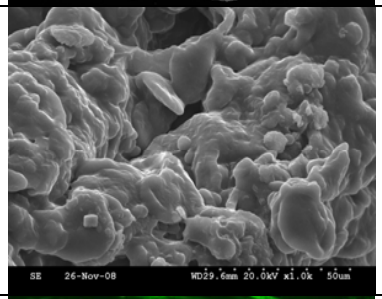
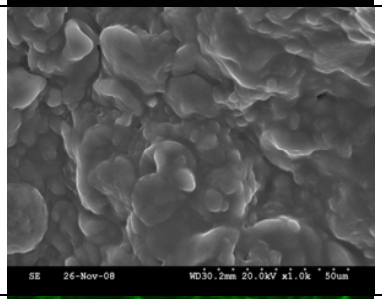
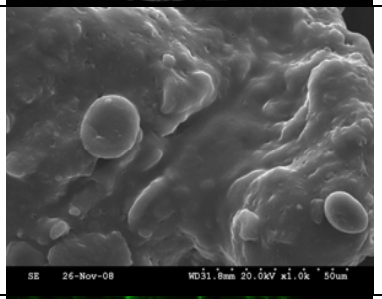
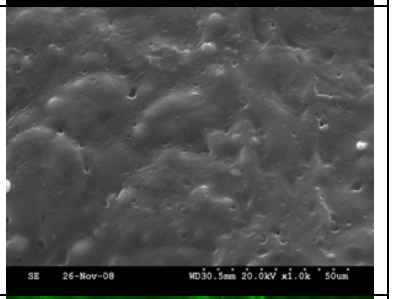
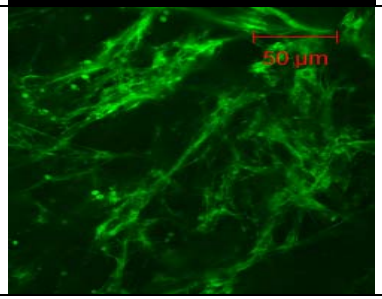
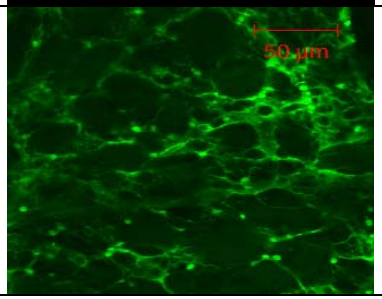
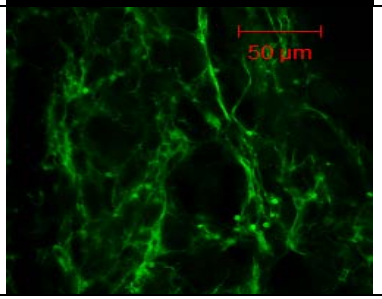
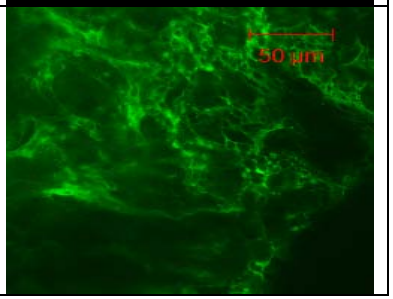


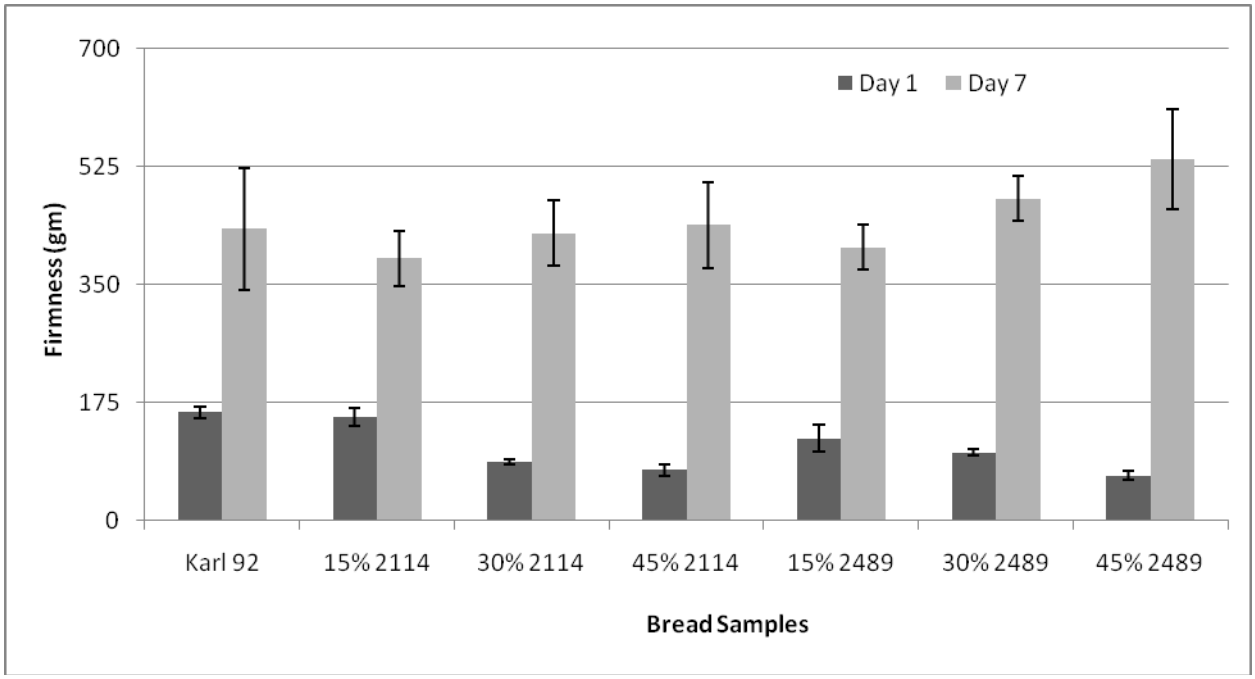
Figure 5.3 Changes in bread structure with inclusion of waxy wheat samples (24hrs after baking).

Sample/ Matrix	Karl	15% Waxy	45% Waxy	100% Waxy
C-Cell				
SEM†				
CLSM‡				

† Scanning Electron Microscopy representing the microstructure of bread crumb

‡ Confocal Laser Scanning Microscopy representing the protein matrix in bread crumb

Figure 5.4 Changes in (top) firmness and (bottom) soluble starch of bread samples (N = 4) day 1 and day 7 after baking



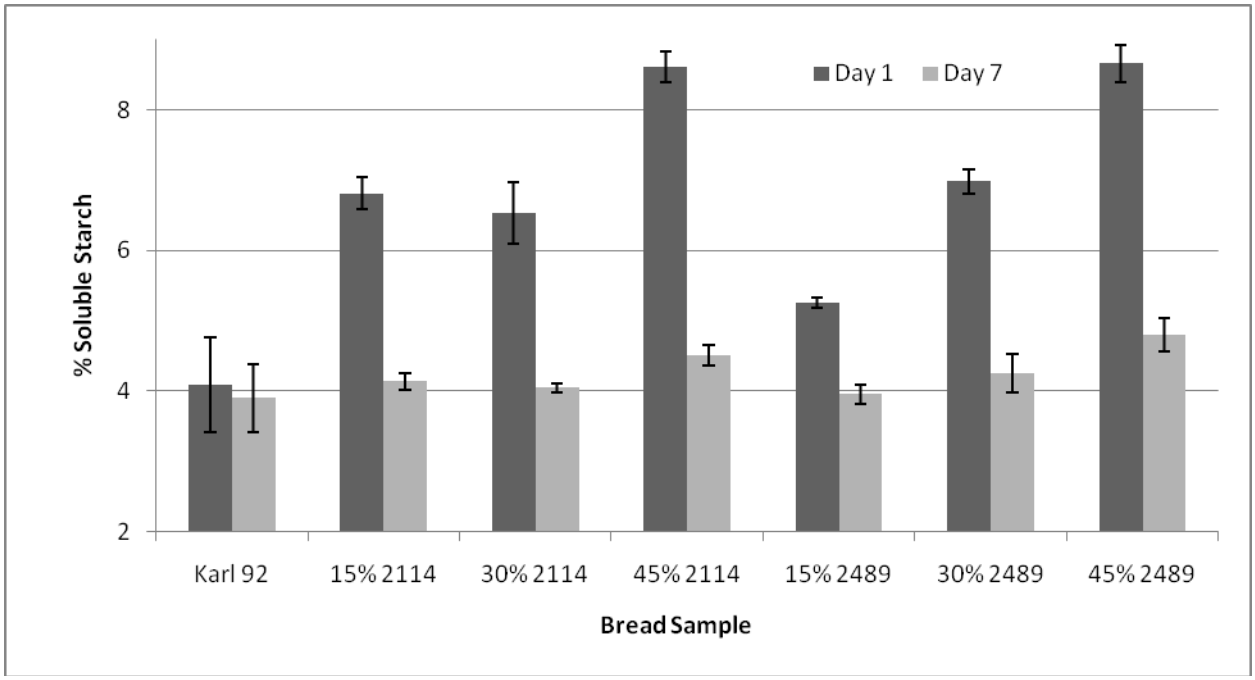
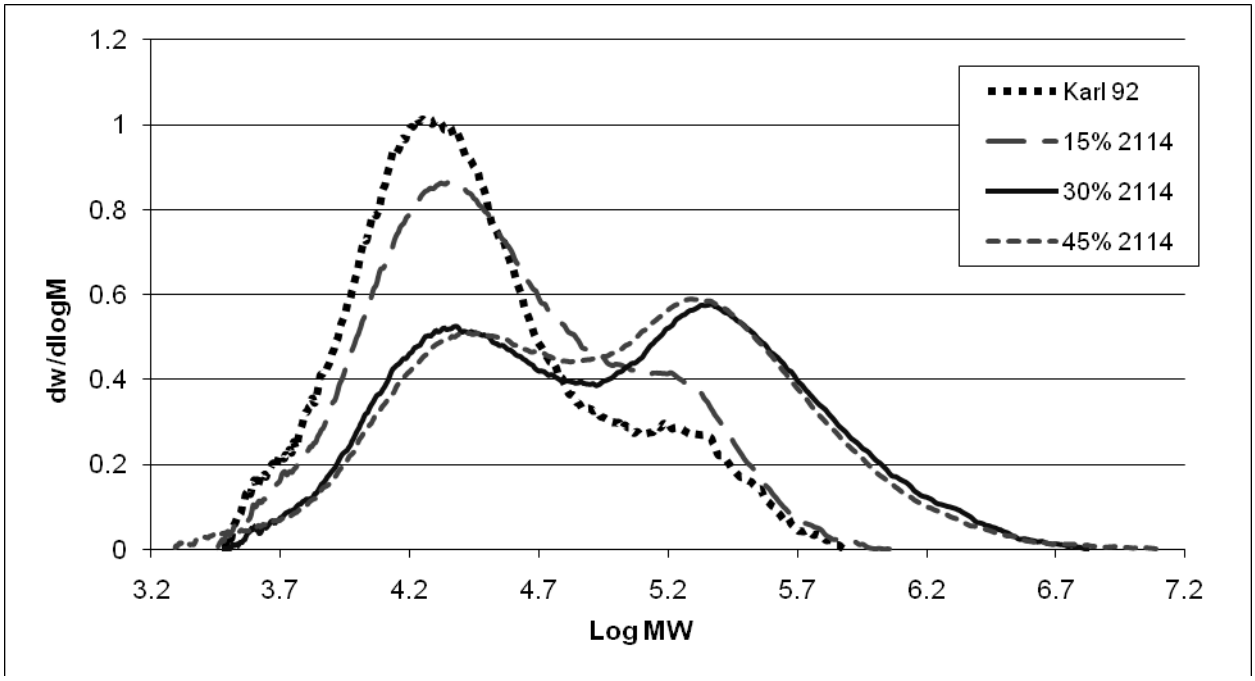


Figure 5.5 Molecular weight distribution of soluble starch profile in breads made with partial waxy wheat (2114) after 1 day of storage.



Appendix A - RHEOLOGICAL PROPERTIES OF WAXY AND NORMAL WHEAT FLOUR DOUGH

INTRODUCTION

Wheat flour dough is classified as visco-elastic material with high degree of viscous component and considerable elastic component (Schofield and Blair, 1932; Schofield and Blair, 1933). According to polymer science, visco-elastic behavior of polymer is goverened by molecular weight, structure and interactions of polymers (Graessley, 1982). It is widely accepted that wheat gluten polymers are mainly responsible for visco-elastic nature of flour dough. In which case, dough resistance to deformation and slippage rate between gluten proteins will depend on gluten protein molecular weight, their interactions through entanglements, disulfide bonding and non-bonding forces such as Van de Waals interactions (Belton, 1998; Singh and MacRitchie, 2001). Although most of the studies have focused on explaining changes in rheological properties as a consequence of gluten proteins, role of starch and effect of starch-protein interactions on adhesive and cohesive properties of dough cannot be undermined.

It is widely accepted that initial stage of dough formation constitutes protein hydration and that starch and protein compete for available water in a limited water system like dough. In such cases, nature and hydration rate of starch could play a vital role. Waxy starches contain mostly amylopectin and swell faster and more as compared to normal starch granules (Guan et al., 2009). Consequently, flour dough made from waxy wheat flours displayed higher water absorption as compared to normal wheat flours (Gaun et al., 2009). Additionally, Edwards et al (2002) have demonstrated the importance of starch granule surface characteristics and the nature of protein-starch bonding on visco-elastic behavior of dough from durum wheat. When starch was replaced by inert filler, such as glass beads of equal particle size distribution, at > 50% the resultant dough had lower adhesion between protein and filler (Edwards et al., 2002).

Currently there is lack of information on the effect of waxy trait on rheological properties of wheat flours. Guan et al (2009) has shown that dough from waxy wheat flours results were slack and that they had low glutomatic index. The formation of slack dough could not be correlated to gluten solubility in various detergents (Garimella Purna, 2010). The objective of this research is to utilize rheological properties to understand the differences between waxy and normal wheat flour dough.

MATERIALS AND METHODS

Materials

Six waxy wheat samples, one partial waxy wheat (Trego), and one wild type hard wheat (Karl 92) were procured from USDA-ARS, Lincoln, NE. Pedigree of the waxy wheat lines were reported by Guan et al. (2009). The waxy wheat samples will be identified by the last four digits throughout this paper.

Proximate analysis

Moisture, protein and ash content of the eight flour samples were obtained from Guan (2008) and were measured by AACC 44-15A; AACC 46-30; and AACC 08-01 respectively (AACC International, 2000).

Rheological Properties

A stress controlled rheometer (Stress Tech HR, ATS Rheosystems, Bordentown, NJ), equipped with a 25mm serrated parallel plate system was used. The gap between the two plates was set at 2.0 mm. The temperature kept constant at 30°C for all creep recovery and stress relaxation tests.

Sample preparation

Dough samples were prepared using flour and water. Optimum absorption and mixing times were calculated using series of mixograms obtain using 10-g mixograph, and were taken from our previous study (Garimella Purna, 2010). The dough samples were then gently kneaded into ball and placed in an airtight container. The sample was allowed to rest for 30 min prior to measuring rheological properties. Creep recovery and stress relaxation were performed on two freshly prepared dough samples. A 2.0 g dough sample was then taken from dough and mounted on the bottom plate of the parallel plate measuring system and the gap was adjusted to 2.0 mm. The excess sample (over the edge of the top plate) was trimmed using a sharp blade. Silicone oil was used to prevent sample drying during analysis. In a separate experiment, time sweep (total of 40 min) was performed, on the normal wheat and a waxy wheat dough sample, to monitor the normal force during of dough sample during resting. Normal force was recorded for every 5 seconds. For both the samples the normal force value between 28 - 32 minutes were within 2% of the final normal force value after 40 minutes. Hence 30 minutes was selected as resting time for all the samples.

Stress relaxation

A method proposed by Steeples (2010) was used with following modifications. Stress was measured when the dough samples were subjected to a strain of 0.001 for 250 seconds. Temperature was kept constant at 30C during the test. Stress (G(t)) was collected and G(t)/G(0) curves for all the curves were calculated. Each analysis was performed in duplicate (on separately prepared dough samples) and the mean values were reported.

Creep recovery

A method proposed by Steeples (2010) was used with following modification. Dough pieces (2.0 g) were relaxed for 30 minutes prior to creep recovery tests. A shear stress of 50 Pa was applied over a creep time of 1200 seconds and recovery time of 1200 seconds. Data for maximum creep strain (J_{max}), maximum recovery strain (J_m) and precent recovery (recovery strain expressed as percent of J_{max}) were calculated from each curve. Each analysis was performed at least in duplicate (on separately prepared dough samples) and mean values were reported.

RESULTS AND DISCUSSION

Creep Recovery

Flours were blended with optimum water and mixed to an optimum time. The creep-recovery curves of dough from waxy, partial waxy and normal wheat flours exhibited visco-elastic behavior, where in the deformation strain did not approach a constant value and the non-recoverable viscous proportion was larger than the recoverable elastic proportion (Steffe, 1992; Hibberd and Parker 1978; Carson and Sun 2001). In creep stage, the strain increased rapidly during first few minutes and then reached a steady state. In the recovery stage, the dough strain slowly recovered with time when the force from maximum creep strain was removed. From our results we can classify dough from waxy wheat fall into three categories (**Figure A.1.1**) based on creep strain values such as dough with high, intermediate and low creep strain values. Dough from waxy wheat sample 2115 had high creep profile similar to normal wheat samples, while samples 2114, 2315, 2459 had intermediate creep profiles; and samples 2205, 2489 and partial waxy wheat had low creep profiles. Maximum strain recovery (MCS) values for all samples are reported in **Table A. 1**. Typically, samples exhibiting large changes in strain upon application of

constant stress have higher viscous component than elastic component (Hibberd and Parker, 1978). According to Wang and Sun (2002) creep-recovery can be divided into six zones, three during the creep, and three during recovery stages. The three zones during creep represent instantaneous, retarded and equilibrium stages of deformation, while three zones during recovery represent instantaneous, delayed and steady stages of recovery. To understand the changes in our samples during creep and recovery phase, strain rate was calculated individually for both the phases and are given in **Figure A.1.2 and Figure A.1.3.** Normal wheat dough exhibited higher initial deformation and did not reach a steady stage until later in creep phase, while most of the waxy dough and partial waxy wheat dough reached the equilibrium stage earlier as compared to normal wheat samples (**Figure A.1.2**). Bockstaele et al. (2008) have attributed the initial higher maximum creep values to stronger flours, consequently dough from most of the waxy wheat flours can be classified as weak dough. **Figure A.1.3** represents recovery phase of all flour dough samples. There is no trend that can separate normal wheat flour from partial and waxy wheat flours.

Stress relaxation

Stress relaxation is used to demonstrate time dependence change in visco-elastic properties of dough (Li et al., 2003). Normalized stress relaxation curves plotted as G(t)/G(0) versus time) for all flour dough samples are shown in **Figure A.1.4**. The curves can be classified into two groups. Group one with minimal changes in stress with time consists samples of normal wheat and waxy wheat flours 2114, 2205 and 2315; while group two with very large changes in strain with time consists of waxy wheat flours 2115, 2459, and 2489 and the partial waxy wheat flour. There were no trends that could separate waxy wheat flour dough from partial waxy or

normal wheat samples. Rao and Dexter (2000) have shown the differences in stress relaxation curves between extra strong and moderately strong wheat flours. Strong dough has shown slower relaxation rate as compared to moderately strong dough. We have calculated the rate of in G(t)/G(0) (**Figure A.1.5**). Our results indicate that normal wheat and some waxy wheat samples (2114, 2205 and 2315) had very slow relaxation rate while partial waxy wheat and few of the waxy wheat samples (2115, 2459 and 2489) had very high relaxation rate. Moreover, the samples with high relaxation rate showed a peak at 60-70 seconds, while samples with slow relaxation rate had no characteristic peak. Previous researchers have carried out stress relaxation tests at small strain amplitude (0.1%) and found no correlation between dough strength (as measured using mixograph) and stress relaxation measurements (Safari-Ardi and Phan-Thien, 1998). They proposed strain levels at the order of 20% to be applied inorder to observe differences among various flour dough systems. It is possible that at small strain used in our study it was not possible to differentiate between waxy and normal wheat flour doughs.

CONCLUSIONS

Most of the waxy wheat dough were classified ‘slack’ as measured using creep tests. However, recovery tests and stress relaxation measurements could not differentiate dough from waxy wheat flours and normal wheat samples. Future studies should be conducted by mixing dough at various water absorptions as well as using higher strain rates.

ACKNOWLEDGEMENTS

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Table A.1 Creep recovery data for all flour dough samples [†]

Sample	Maximum creep recovery[*] (%)	Maximum recovery strain (%)	Recovery strain (%)
2114	4.58 ± 0.23^{ab}	2.16 ± 0.05^{a}	47.26 ± 1.15^{a}
2115	6.47 ± 1.42^{a}	2.07 ± 0.49^{a}	34.12 ± 0.17^{d}
2205	2.50 ± 0.41^{b}	0.94 ± 0.17^{b}	37.61 ± 0.44^{c}
2315	3.33 ± 0.49^{b}	1.49 ± 0.23^{ab}	44.58 ± 0.23^{b}
2459	4.18 ± 0.03^{ab}	1.48 ± 0.01^{ab}	35.29 ± 0.45^{cd}
2489	2.45 ± 0.05^{b}	1.16 ± 0.05^{b}	47.19 ± 0.99^{a}
Karl 92	6.17 ± 0.28^{a}	2.14 ± 0.09^{a}	34.66 ± 0.06^{d}
Trego	2.86 ± 0.22^{b}	1.23 ± 0.07^{b}	42.99 ± 0.66^{b}

[†] mean $\pm$ standard deviation values are reported
[*] within each column means with different superscript are significantly ($p < 0.05$) different

Figure A.1.1 Creep and recovery compliance curves for normal and waxy wheat dough

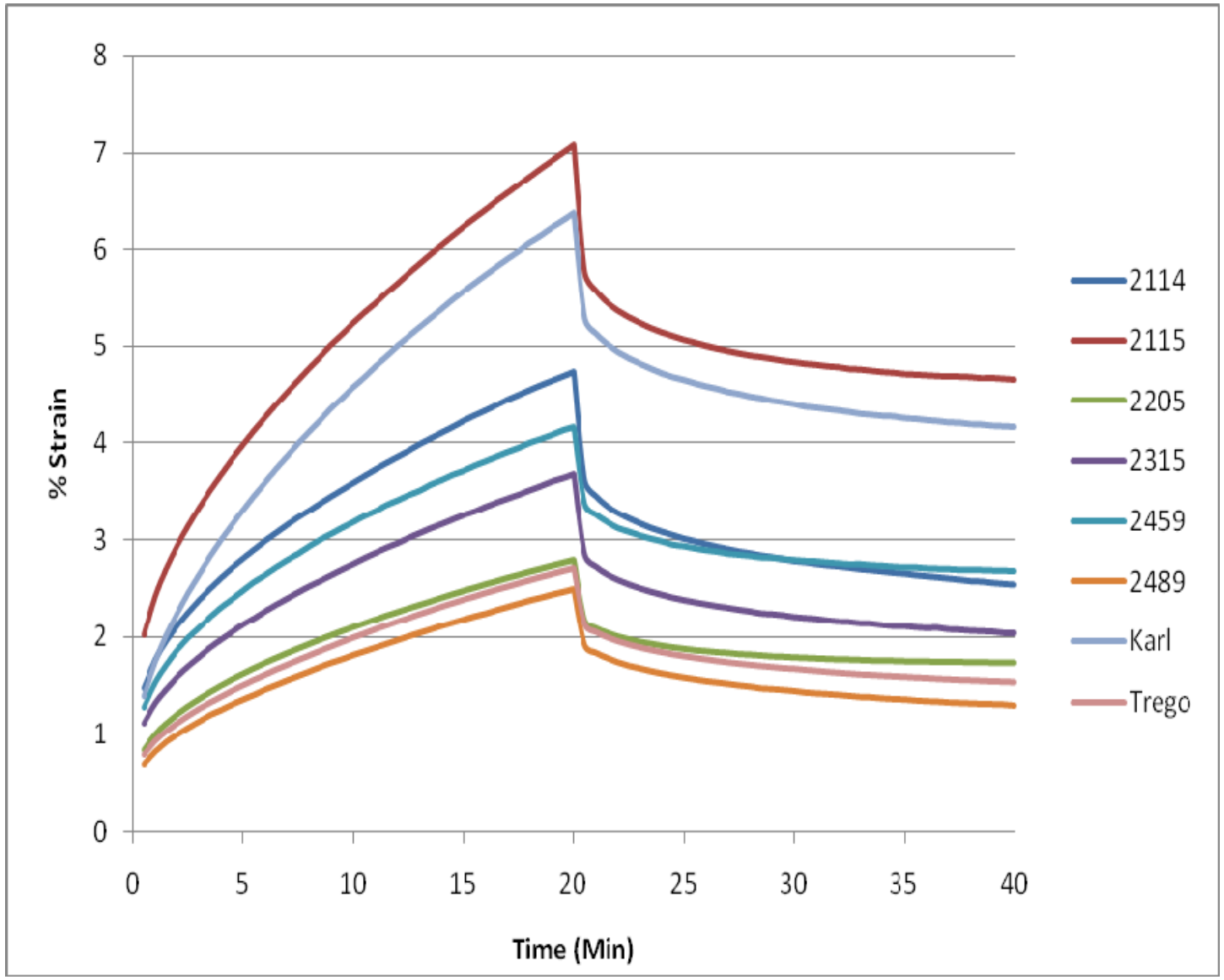

Figure A.1.2 Strain rate of normal and waxy wheat dough during creep-phase.

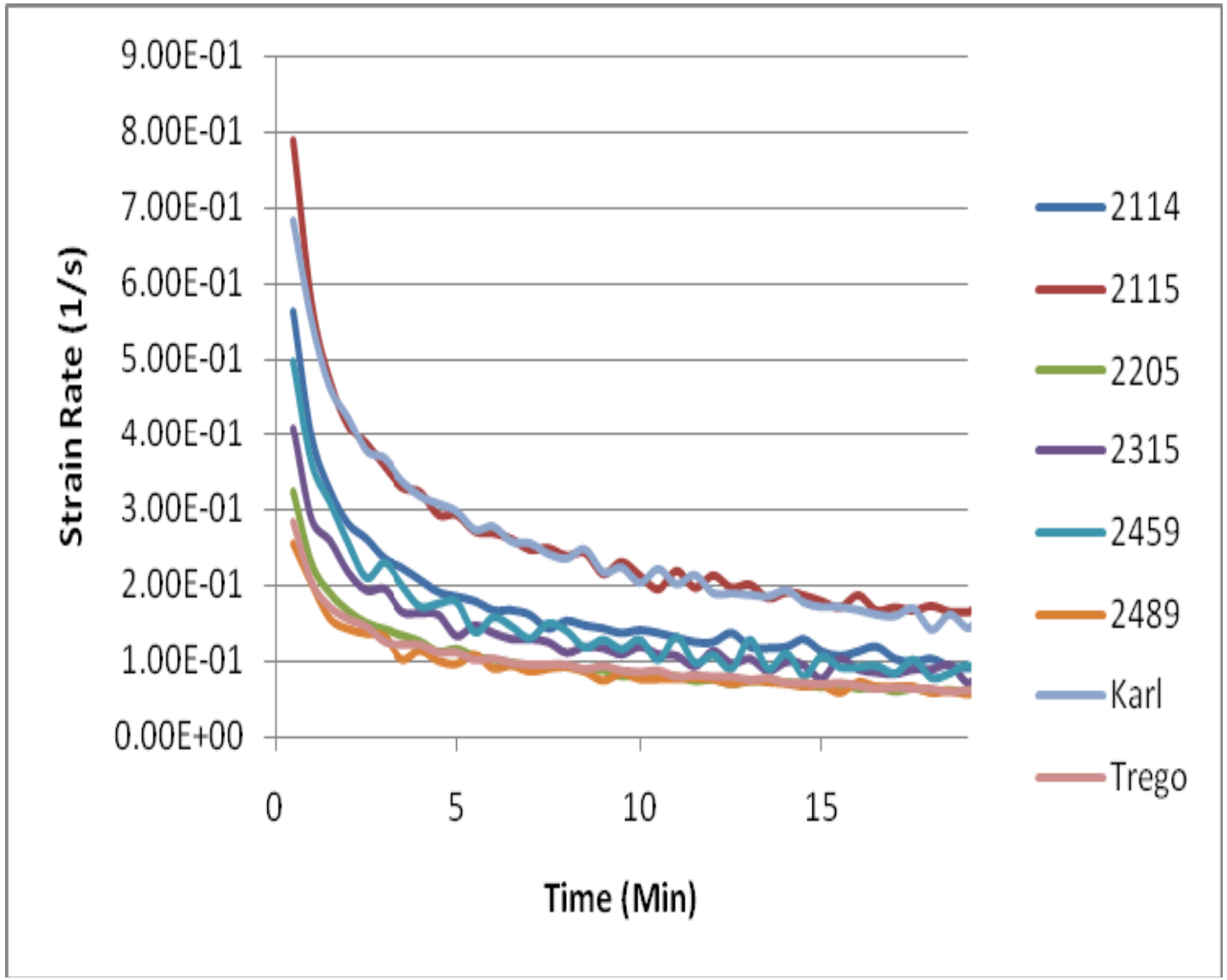

Figure A.1.3 Strain rate of normal and waxy dough during recovery phase.

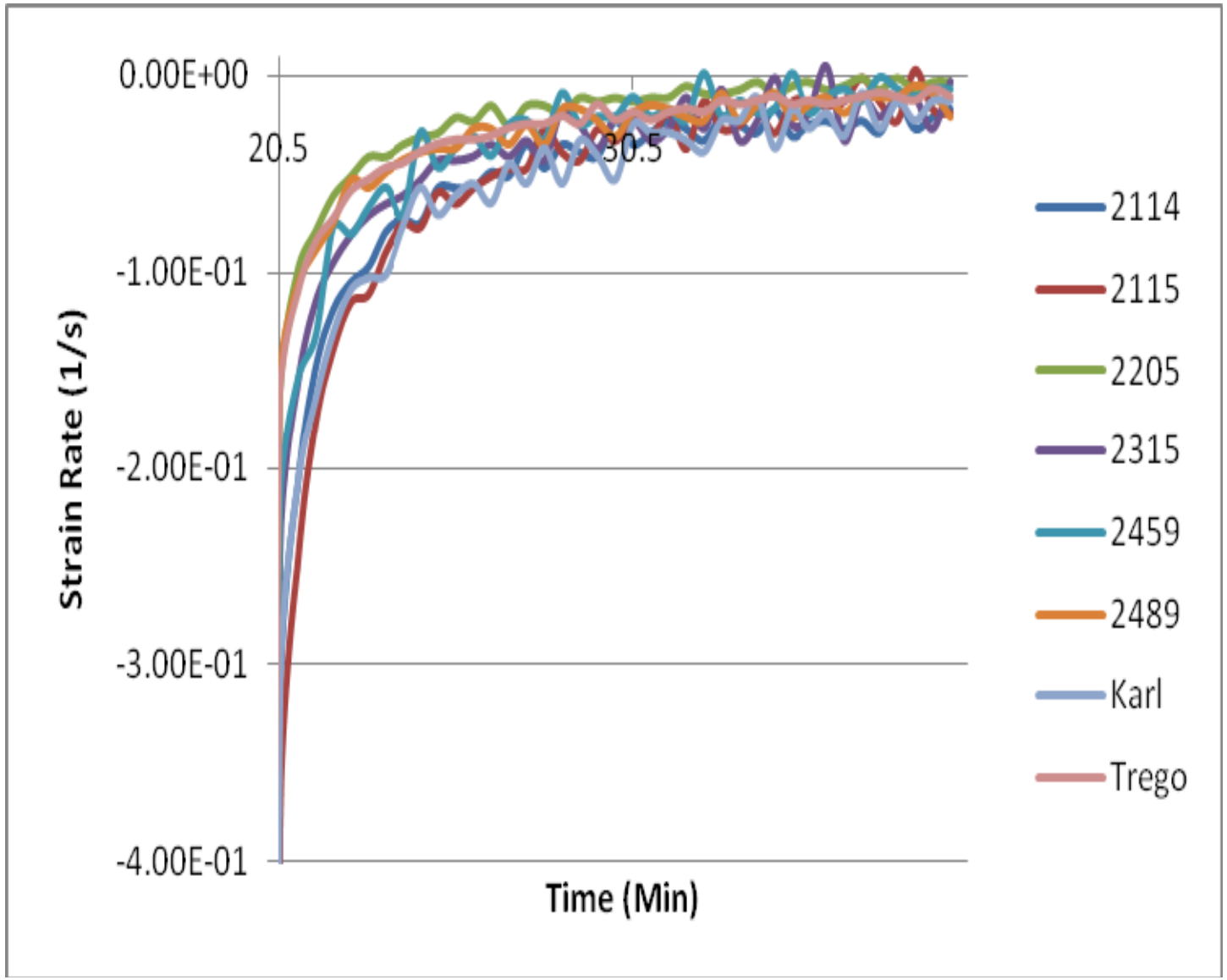

Figure A.1.4 Stress relaxation curves for normal and waxy wheat dough

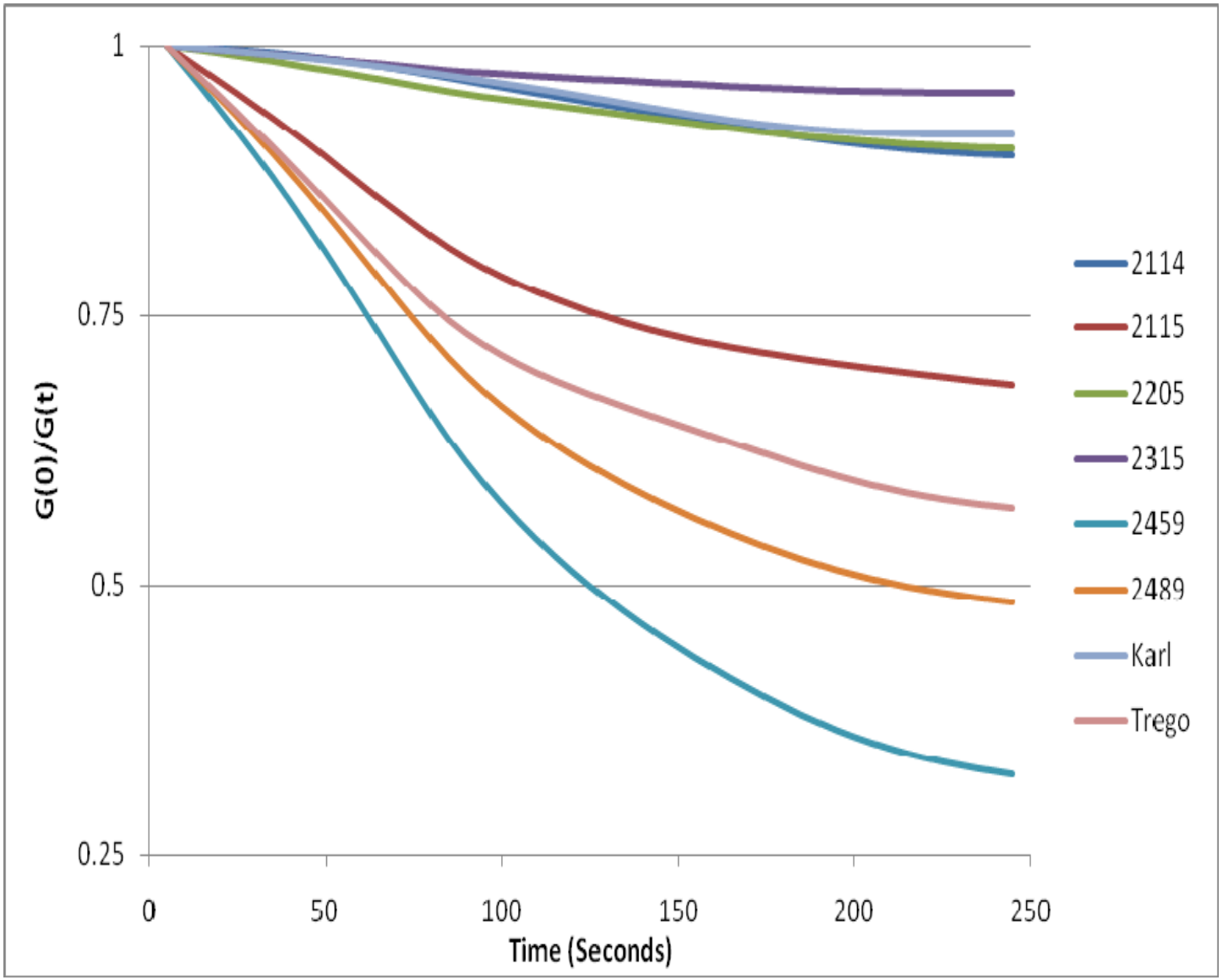

Figure A.1.5 Rate of change in % strain derived from stress relaxation curves for normal and waxy wheat dough