PHYSICOCHEMICAL PROPERTIES OF PRE-HARVEST SPROUTED HARD SPRING WHEAT

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ABSTRACT

Lu, Haiyan, M.S., Cereal Science Program, College of Agriculture, Food Systems, and Natural Resources, North Dakota State University, March 2011. Physicochemical Properties of Pre-Harvest Sprouted Hard Spring Wheat. Major Professor: Dr. Senay Simsek.

Pre-Harvest Sprouting (PHS) can cause severe economic loss in wheat grown across many areas of the world. Prolonged rainfall before harvest and high temperatures can contribute to the occurrence of PHS. Grain growers prefer wheat genotypes with low susceptibility to PHS. The objective of this study was to examine the physicochemical properties of Hard Red Spring Wheat (HRSW) and Hard White Spring Wheat (HWSW) affected by PHS.

Physicochemical properties of the starch and protein in HRSW and HWSW were significantly affected by PHS. α-Amylase was determined using an Azurine-crosslinked amylose substrate (AZCL-Amylose; Megazyme Co., Ltd). Endo-protease activity was determined using an Azurine-crosslinked substrate (Protazyme AK tablet; Megazyme Co., Ltd). Mean value of α-amylase of PHS damaged wheat (2.00 CU/g) was 17 times greater than sound wheat (0.12 CU/g). Mean value of endo-protease of PHS damaged wheat (2.30 A_{590}/g/h) was 1.6 times higher than sound wheat (1.44 A_{590}/g/h). PHS increased both α-amylase and endo-protease activities, resulting in the hydrolysis of starch and protein molecules. However, the increased endo-protease activity was not as significant as the increase in the α-amylase activity in PHS wheat. In a scale of 1.0-9.0, the wheat genotypes had significantly different sprouting scores ranging from 2.5 to 7.8, which had positive correlations with α-amylase and endo-protease activities ($P < 0.001$). Consequently, genotypes showed differences in degradation of starch and protein molecules. The endo-protease activity of PHS samples had greater correlation ($r = 0.78$) with protein degradation measured by High

Performance Size Exclusion Chromatography (HPSEC) than with sprouting score (r = 0.57). The degree of protein degradation was better estimated by the endo-protease activity than sprouting score in PHS samples. The pasting properties of starch were measured by Rapid Visco Analyzer (RVA). Mean value of peak viscosity of PHS damaged wheat decreased up to 96% compared to that of sound wheat sample. HPSEC was used to detect the starch molecular weight distribution. PHS damaged wheat had lower molecular weight for high molecular weight amylopectin (HMW-AP) and higher molecular weight for low molecular weight amylopectin (LMW-AP) and amylose. This result indicated that PHS had changed the molecular weight distribution of starch. Sodium Dodecyl Sulfate (SDS) buffer extractable proteins (EXP) and un-extractable proteins (UNP) were analyzed by HPSEC. Some portion of UNP had changed to EXP. The result indicated that the molecular weight distribution of protein had been changed due to PHS.

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LIST OF ABBREVIATIONS

A%	Area percentage
AM	Amylose
AP	Amylopectin
BD	Breakdown
EXP	Extractable proteins
FV	Final Viscosity
HMW	High Molecular Weight
HPSEC	High Performance Size Exclusion Chromatography
HPV	Hot Paste Viscosity
HRSW	Hard Red Spring Wheat
HSW	Hard Spring Wheat
HWSW	Hard White Spring Wheat
LMW	Low Molecular Weight
LSD	Least Significant Difference
MWD	Molecular Weight Distribution
NS	Not Significant
PHS	Pre-Harvest Sprouting
PV	Peak Viscosity
RVA	Rapid Visco Analyzer
RVU	Rapid Viscosity Unit
Sb	Setback
SDS	Sodium Dodecyl Sulfate
SEM	Scanning Electron Microscope
UNP	Un-extractable proteins

GENERAL INTRODUCTION

Due to unfavorable environmental conditions before harvest, mature grain may undergo germination in the spike, causing Pre-Harvest Sprouting (PHS). Prolonged rainfall and high humidity contribute to PHS damage to grain (Nielson et al 1984). PHS is relatively common in major wheat growing areas, occurring three to four years out of ten (Derera et al 1989). PHS causes producers to suffer losses when PHS damaged wheat is purchased at a discount. Millers have to face the problem of reduced flour yields; while bakers encounter problems in processing and end-product quality due to starch and protein degradation. In the U.S., PHS could lead to increasing wheat prices for food and feed use because of lower production levels. The incidence of PHS affects producers, the local, region, and world economy (Wahl et al 1992).

The ability to utilize grain for human food largely depends on wheat harvesting condition, storage, shipment and transformation into a number of highly desirable products (Wahl et al 1992). High enzymatic activity in PHS damaged wheat kernels affects the starch and protein fractions of the flour and therefore the quality of end products. The subsequent breakdown of starch and protein can lead to loss of wheat quality reducing the value of wheat throughout the production and utilization process (Walker-Simmons et al 1992). To effectively help breeders develop new genotypes with lower PHS susceptibility and quality attributes, it was important to understand the physicochemical properties of PHS damaged Hard Spring Wheat (HSW). Susceptibility to PHS may vary among different wheat classes and even among different genotypes within the same class.

Limited research has been conducted in this area, especially on the effect of PHS on starch and protein molecular distribution. It appears that there is no clear understanding of the PHS effect on physicochemical changes of HSW. Thus, this

research aims to understand the PHS effect on starch and protein characteristics of HSW. The specific objectives of the present research are to determine the variation of starch and protein characteristics in HSW genotypes damaged by PHS and their association with PHS.

LITERATURE REVIEW

Pre-Harvest Sprouting

Pre-Harvest Sprouting (PHS) is defined as the premature germination of wheat kernels in the spike under unfavorable environmental conditions. Premature germination causes embryo growth in the wheat kernel while still on the head in the field (Groos et al 2002). Periods of prolonged rainfall and high humidity after the grain has ripened and before it can be harvested contribute to PHS, which can be recognized as a premature germination (Thomason et al 2009). PHS is a serious problem in many wheat growing areas of the world. Most of the world's major wheat producing areas suffer economic loss due to PHS damage. PHS damaged wheat poses severe problems for the processing industry; because of these problems, local elevators largely discount PHS damaged grain (Sorenson et al 2004). The price of PHS damaged wheat is reduced by 20% to 50%. If the grain contains over four percent damaged kernels, it cannot be used for human food products and severely PHS damaged grain is usually used for animal feed (Sorenson et al 2004).

PHS in wheat kernels can occur once the kernel has almost reached maturity in the field; therefore, when mature wheat is subjected to proper moisture levels, temperature and time, it begins to sprout. If the moisture levels in wheat decreases prior to threshing, the sprouting process stops; if the sprouting stops before there are any visible signs of sprouted kernels, this is called incipient sprouting (Sorenson et al 2004). PHS damage can also be classified in terms of its severity with a continuum from very minor to very severe. PHS damage can be measured by the percentage of sprouted wheat kernels, starch degradation, or other indicators. The severity of PHS may cause economic consequences in several stages (Thomas et al 1992). Visible indications of PHS include swelling of the kernel, germ discoloration, seed- coat splitting, and the root

and shoot emerging (Thomason et al 2009). Three to four weeks following flowering, wheat grains start to lose water once the ripening process begins. Though the starchy endosperm cells have died at this time, those of the seed-coat layer and embryo remains alive but dormant. Dormancy prevents the seeds from PHS while still in the seed head. While, environmental conditions during grain development affect susceptibility to PHS, grain is less likely to exhibit PHS under low temperatures. High temperatures and rain prior to harvest during the later stages of grain filling can lead to low grain dormancy, leading to PHS (Thomason et al 2009).

The physiological changes needed to produce a new plant require energy and nutrients, which is the reason why the PHS damaged wheat kernel needs to produce enzymes to breakdown starch (amylases), oil (lipases) and protein (proteases). The impact of PHS wheat on foods depends on the amount of enzyme activity present and breakdown of macromolecules in the kernel (Sorenson et al 2004). Due to high enzyme activity in PHS damaged wheat kernels, starch and protein are degraded, resulting in major economic loss to grain producers.

The quality problems created by PHS are the most important issue regarding PHS damaged wheat. PHS can decrease the wheat's agronomic quality, particularly reducing the test weight, and functional quality of wheat for milling and baking (Swanson 1946). PHS damaged wheat flour loses its thickening power and some products cannot be produced from PHS damaged wheat flour (Groos et al 2002). Flour from the degraded wheat seed produces products which are porous, sticky, off-color and generally very poor quality. The impact of PHS damage on baking quality is viewed by lower absorption, reduced mixing strength and tolerance, and sticky dough. Loaf volume, crust strength and crumb texture are also affected; a wet and gummy crumb causes problems with slicing and shelf life (Sorenson et al 2004).

Thus, it is very critical to perform research to understand the physicochemical effects of PHS on Hard Spring Wheat (HSW). This research can help plant breeders to develop new wheat genotypes with lower PHS susceptibility. Many previous studies have shown that PHS susceptibility is a complex trait affected by many factors, such as temperature, genotype and rainfall. Xu et al (1993) has concluded that, when red and white hard wheat genotypes have the same degree of seed dormancy red wheat genotypes have more resistant to PHS damage in the field than white wheat genotypes. In the occurrence of PHS, the red seed coat plays an important part in deepening dormancy and decreasing or delaying PHS in the field. Lowering susceptibility of wheat to PHS is one of the main objectives of breeding programs in many important wheat-growing regions of the world. The study of Xu et al (1993) also demonstrated that highly significant correlations occurred between all germination parameters, indicating that genotypes having low susceptibility to PHS could be segregated.

Effect of PHS on Starch

Structure and Physicochemical Properties of Starch

Starch constitutes the major component of wheat flour and has unique properties. Cereal grains store energy primarily in the form of starch. The amount of starch in a cereal grain is not always the same but is generally around 60-75% of the weight of the grain (Hoseney 1986). In addition to its nutritive value, starch performs many important roles in the production and quality of baked goods and noodles (Khan et al 2009). Wheat starch granules demonstrate a bimodal size distribution of A and B type granules: The A type granule is large and lenticular with a diameter of more than 10 μm; the B type granule is the small and spherical with a diameter of 1-5 μm (Jane et al 1994). The starch granules consist of a mixture of two polymers: amylose a basically linear polysaccharide (α-(1→4) linked glucose), and amylopectin a highly branched (α-

(1→6) branches) polysaccharide (Zhang et al 2009). The molecular weight of amylose is around 250,000 but varies quite widely, not only between species of plants but also within a species, and depends on the plants' stage of maturity. Unlike amylose, amylopectin is a much larger and more complex molecule with a molecular weight of about 10^8. Generally, amylopectin is the major component of starch, and its fine structure is well related to starch characteristics.

Wheat starches obtain an X-ray diffraction pattern (A-type pattern) that is indicative of parallel, double helices separated by interstitial water. Starch granules have both polycrystalline and amorphous regions in alternating layers. The clustered branches of amylopectin show as packed double helices. The double-helices form many small crystalline regions in the dense layers of starch granules, which alternate with the less-dense layers, generally called amorphous layers. Amylose molecules in amorphous region can diffuse from water-swollen granules. Waxy starches (100% amylopectin) have the same degree of crystallinity and X-ray type as do normal starches. The length of branch chain of amylopectin is related with the starch crystalline structure (Hannashiro et al 1996).

The relative distribution of different types of starch granules is considered to influence the pasting and gelatinization characteristics of starch (Jane et al 1992). Starch gelatinization is a procedure that disrupts the intermolecular bonds of starch molecules in the presence of water and heat, absorbing more water at the site of the hydrogen bonding (the hydroxyl hydrogen and oxygen). The structure of amylose and amylopectin and their relative ratios in starch granules play an important role in pasting, gelation, and retrogradation properties of starch and the end product quality and stability (Jane et al 1992). The gelatinization and pasting properties of starch are primary providers to the textural properties of Asian noodles. As well as being

influenced by enzymatic activity, these properties are affected by the amylose-amylopectin ratio in starch (Khan et al 2009).

Impact of PHS on Starch Properties

Starch is the major storage polysaccharide in wheat kernels. It has unique physical and chemical properties compared to other carbohydrates. Many studies have been conducted on the damage caused by PHS in wheat. The influence of PHS on flour characteristics has been examined by falling number, amylograph (MacGregor et al 1972; Meredith et al 1985; MacGregor et al 1989). The detrimental effect of PHS on baking quality of flour milled from PHS damaged wheat has been identified for many years. Gelin et al (2007) also reported that PHS in durum wheat reduces seed quality and causes a loss of starch gel viscosity, which had a negative correlation with pasta quality. This unfavorable effect is generally ascribed to high α-amylase activity, which develops in the wheat kernel during PHS (Hwang et al 1973).

α-Amylase is an enzyme that hydrolyses α-1, 4 bonds of large polysaccharides such as starch and glycogen, generating small sugar molecules like glucose and maltose, thus, it can lead to a decrease in the size of starch molecules. High α-amylase activity results in the degradation of starch in wheat. Each grain kernel exhibits some level of α-amylase and the α-amylase activity levels at the different stages of growth are different for the same kind of grain kernels. Stoy et al (1976) have concluded that sprouting before harvest drastically increases the level of α-amylase activity. Some researchers believe that the main reason for the loss of quality due to PHS is the high α-amylase activity (Lunn et al 2001). The increased α-amylase activity decreases the viscosity of a flour solution or slurry (Hoseney et al 1998).

α-Amylase, which is well associated with PHS, is the enzyme most associated with wheat processing quality. Falling number, Rapid Visco Analyzer (RVA), and

amylograph are the most commonly used methods to indicate of the level of α-amylase activity in wheat or flour. α-Amylase has a significant effect on bread-making quality. A small amount of α-amylase activity is desirable to break down starch to provide sugars for yeast. However, the presence of excessive enzymatic (α-amylase) activity accelerates the starch damage and can lead to sticky dough with difficult handling properties, especially for products with long fermentation processes (Khan et al 2009). Bread made from PHS damaged wheat flour exhibits a very sticky crust, which can negatively influence slicing. Excess sugars metabolized by yeast, create too much gas, resulting in "key-holing" in Pullman bread, coarser crumb, or collapse in extreme cases; and residual sugars turn the crust dark. The amount of α-amylase activity in PHS damaged wheat kernels can vary extremely; a difference of up to several thousand-fold is possible (Kruger 1994). Generally, the more severely PHS damaged the wheat, the larger the amount of activity likely to be present.

Effect of PHS on Protein

Role of Protein Component

Proteins are one of the crucial signs of wheat quality and are probably the most important factor in bread flour quality (Zhang et al 2009). Protein quality is commonly believed by processors to be equal with dough strength, with baking quality being the ultimate test. Generally, high protein content is related to increased dough strength and better baking quality of bread wheat (Johansson et al 2001).

There are different criteria used to classify wheat proteins; those founded on chemical properties and those based on functionality of proteins. From a chemical point of view, wheat proteins can be divided into two groups: water soluble proteins and insoluble gluten proteins. The soluble groups, which are made up of albumins, globulins, and peptides, can dissolve in the natural aqueous mediums. The insoluble

gluten proteins consist of glutenins and gliadins which represent 80-85% of wheat storage protein. During the mixing procedure in the bread-making process, they can form gluten, which is believed to be primarily responsible for the unique viscoelastic and gas-retaining properties of dough. Gluten plays an important part in determining the quality of wheat flour and its end products (Veraverbeke et al 2002).

From a functional point of view, wheat proteins can be divided into two main groups, monomeric and polymeric. Monomeric proteins are comprised of three main groups: albumins, globulins and gliadins; intrachain disulphide bonds form in monomeric proteins. Polymeric proteins, which are held together by interchain disulphide bonds, are comprised of three main protein groups: glutenins, High Molecular Weight (HMW) albumins, and triticins (MacRitchie et al 1997).

The gliadins are a large group of proteins with similar properties and a molecular weight ranging from 25 to 100 kDa when analyzed by gel filtration (Hoseney 1998). Gliadins are soluble in 60-70% aqueous ethanol and provide viscosity of dough. Gliadins have little or almost no resistance to extension and perhaps are responsible for dough's viscosity (Hoseney 1998). The glutenin proteins are heterogeneous, varying in molecular weight from 100 kDa to several thousand kDa with an average of around 3 thousand kDa. These proteins supply dough with resistance to extension, and are believed to be the key wheat flour proteins providing strength and elasticity to the dough.

High Performance Size Exclusion Chromatography (HPSEC) was developed in the mid-1980s for molecular weight distribution analysis of wheat proteins (Bietz 1986). The proportions of monomeric and polymeric components and the proportions of large polymers can be detected by HPSEC, which is currently the most important tool used to quantitatively characterize the overall protein composition of wheat proteins

(Southan and MacRitchie 1999). Preston et al (2003) separated the total extracted proteins into three peaks consisting of polymeric gluten protein, monomeric gluten proteins, and non-prolamin proteins. The Sodium Dodecyl Sulfate (SDS) buffer, extractable protein (EXP) and un-extractable protein (UNP) can be examined using a two-step extraction procedure, followed by HPSEC separation of the monomeric and polymeric proteins (Gupta et al 1993). The amounts of the monomeric and polymeric components in the EXP and UNP fractions are related to wheat quality. Specifically, the UNP % provides a useful indicator of the proportions of large polymeric proteins in the flour sample and dough strength (Khan et al 2009).

Impact of PHS on Protein Properties

In addition to the significant increase of α-amylase activity during PHS, there is also some evidence that the characteristics of storage proteins are changed by PHS damage (Kruger 1971; Bushuk et al 1987; Lukow et al 1984; Janssen et al 1996; Weegels et al 1996). Proteolytic enzymes hydrolyze the peptide bonds that link amino acids together in the polypeptide chain forming the protein. Studies show that increase in endo-protease activities results in the degradation of protein in wheat kernels (Kiribuchi et al 1973; Preston et al 1979; Meredith et al 1985; Salomonsson et al 1989; Sun et al 1991; Jones et al 1993). Comparison of levels of enzyme activities for six enzymes (amylase, protease, catalase, peroxidase, lipoxygenase and phenol oxidase) from germinated grain shows that changes in flour properties and product defects have close relationships to levels of flour protease and amylase (Rosemary et al 1989). The increased endo-protease activity in PHS damage wheat kernel greatly deteriorated protein. The studies of Rosemary (1989) conclude that PHS can also soften the grain with consequent effects on milling properties. This is generally reflected by reduced flour water absorption and reduced milling yields.

Several previous studies report that increased endo-protease activity in PHS damaged wheat can result in degradation of endosperm protein of flour and the deterioration in bread making quality (Prestone et al 1979). However, the possible negative effect of endo-protease activity on bread making quality is obvious, while in general it is not believed to be as serious as that of α-amylase. Additionally, gluten from PHS damaged wheat is too extensible for optimal baking quality (Hwang et al 1973). Beresh (1969) and Redman et al (1971) offer evidence that the rapid softening of gluten washed from flour milled from grist that included small amount of PHS damaged wheat is due to proteolytic hydrolysis of the gluten proteins. Shorina (1967) suggested that the reduction of disulfide cross-linkages by protein disulfide reductase that develops during PHS results in the softening of gluten in PHS damaged wheat. Further, from the studies of Rosemary (1989), it can be concluded that with protein contents between 11% and 14%, the amount and quality of protein may influence the effects seen.

Endo-protease activity is generally related to increased α-amylase activity during PHS. Excessive endo-protease activity has a negative effect on dough handling and baking properties. The presence of α-amylase and endo-protease is also undesirable for noodle production. Endo-protease can lead to deterioration of protein and stretching of noodles during drying and poor cooked texture (Khan et al 2009). Proteolytic enzyme activity may play a key role during PHS by affecting the early breakdown and subsequent deterioration of gluten protein in situ (Kruger et al 1980). Excessive endo-protease activity can greatly contribute to the deterioration of bread making quality.

Hypothesis

The hypothesis of this research is: PHS affects starch and protein properties in Hard Spring Wheat kernels, and has significant correlation with α-amylase activity, endo-protease activity and size distribution of starch and protein molecules.

References Cited

Beresh, I.D. 1969. Proteolysis of gluten during sprouting of wheat. Trudy VNIIZ. 66: 111.

Bietz, J.A. 1986. High performance liquid chromatography for cereal proteins. Pages105-170 in: Advances in Cereal Science and Technology. Y. Pomeranz, eds. American Association of Cereal Chemists. St.Paul, MN.

Bushuk, W. and Lukow, O.M. 1987. Effects of sprouting on wheat proteins and baking properties. Pages 188-196 in: Fourth International Symposium on Pre-harvest sprouting in Cereals. D.J. Mares, eds. Westview Press: Boulder, Co, USA.

Derera, N. 1989. Pre-harvest Field Sprouting in Cereals. CRC Press, Inc., Boca Raton, FL. Page 176.

Gelin, J.R., Eias, E.M., Manthey, F.A., and Grant, L. 2007. Study of the relationship between sprouting score and sprout damage in durum wheat. Cereal Research Communicaitons. 35: 53-61.

Groos, C., Gay, G., Perretant, M-R., Gervais, L., Bernard, M., Dedryver, F., and Charmet, G. 2002. Study of the relationship between pre-harvest sprouting and grain color by quantitative trait loci analysis in white x red grain bread wheat cross. Theor. Appl. Genet.104: 39-47.

Gupta, R.B., Khan, K., and MacRitchie, F. 1993. Biochemical basis of flour properties in bread wheats. I. Effect of variation in the quality and size distribution of polymeric protein. J. Cereal Sci. 18:23-41.

Hanashiro, I., Abe, J., and Hizukuri, S. 1996. A periodic distribution of the chain length of amylopectin as revealed by high-performance anion-exchange chromatography. Carbohydrate Research: 283: 151-159.

Hoseney, R.C. 1998. Principles of Cereal Science and Technology. 2nd Ed. American Association of Cereal Chemists. St. Paul, MN.

Hwang, P. and Bushuk, W. 1973. Some changes in the endosperm proteins during sprouting of wheat. Cereal Chem. 50:147.

Jane, J. and Chen, J. 1992. Effects of amylase molecular size and maylopectin branch chain length on paste properties of starch. Cereal Chem. 69: 60-65.

Jane, J., Kasemsuwan, T., Leas, S., Zobel, H., & Robyt, J. 1994. Anthology of starch granule morphology by Scanning Electron Microscopy. Starch/Staerke. 46: 121-129.

Janssen, A.M., van Vliet, T. and Vereijken, M. 1996. Rheological behavior of wheat glutens at small and large deformations. Comparison of two glutens differing in bread making potential. J. Cereal Sci. 23: 19–31.

Johansson, E., Prieto-Linde, M.L., and Jönsson, J.Ö. 2001. Effects of wheat variety and nitrogen application on storage protein composition and breadmaking quality. Cereal Chem. 78: 19-25.

Jones, B.L. and Wrobel, R. 1993. The endoproteinases of germinating barley. Pages 262–269 in: Pre-harvest Sprouting in Cereals. M.K. Walker-Simmons, and J.L., Ried, eds. AACC, MN, USA.

Khan, K. and Shewry, P.R. 2009. Extraction and Analysis of Protein Fractions. Pages 233-236 in: Wheat Chemistry and Technology. AACC International Press, MN, USA.

Kiribuchi, S. and Nakamura, M. 1973. An analytical study on starch degrading enzymes and composition of carbohydrates in the endosperm during germination of barley seed. Nougei Kagaku Kaishi. 5: 333–340 (in Japanese).

Kruger, J.E. 1971. Effect of proteolytic enzymes on gluten as measured by a stretching test. Cereal Chem. 48: 121–132.

Lukow, O.M. and Bushuk, W. 1984. Influence of germination on wheat quality. I. Functional (breadmaking) and Biochemical Properties. Cereal Chem. 61: 336–339.

Lunn, G.D., Major, B.J., Kettlewell, P.S., and Scott, R. K. 2001. Mechanisms leading to excess alpha-amylase activity in wheat (Triticum aestivum L.) grain in the UK. J. Cereal Sci. 33:313-329.

MacGregor, A.W. and Dushnicky, L. 1989. Starch degradation in endosperms of developing barley kernels. J. Inst. Brew. 95: 321–325.

MacRichie, F. and Lafiandra, D. 1997. Structure-function relationships of wheat proteins. Pages 293-324 in: Food Proteins and Their Application. S. Damodaran and A. Paraf, eds. Marcel Dekker. Inc: New York. Basel. Hong Kong.

MacGregor, A.W. and Matsuo, R.R. 1972. Starch degradation in endosperm of barley and wheat kernels during initial stages of germination. Cereal Chem. 59: 210–216.

Meredith, P. and Pomeranz, Y. 1985. Sprouted grain. Pages 239-320 in: Advances in Cereal Science and Technology, vol. 7. Y. Pomeranz, eds. American Association of Cereal Chemist, Inc. St. Paul, MN, USA.

Nielson, M. T., McCrate, A. J., Heyne, E. G., and Paulsen, G. M. 1984. Effect of weather variables during maturation on pre-harvest sprouting of hard white winter wheat. Crop Sci. 24:779-782.

Prestone, K.R. and Kruger, J.E. 1979. Physiological control of exo and endoproteolitic activities in germinating wheat and their relationship to storage protein hydrolysis. Plant Physiol. 64: 450–454.

Redman, D.G. 1971. Softening of gluten by wheat proteases. J. Sci. Food Agr. 22:75.

Salomonsson, L., Heneen, K., L-Raznikiewicz, M., Carlsson, M., and Karlsson, R. 1989. In vitro degradation of starch granules by α-amylase isomers from mature Triticale. Starch. 41:340–343.

Shorina, O.S., Vakar, A.B., and Kretovich, V.L. 1967. Physicochemical changes during sprouting of wheat. III. Disulfide bonds and sulfhydryl groups. Prikl. Biokhim. Mikrobiol. 3:379.

Sorenson, B. and Wiersma, J. 2004. Sprout damaged wheat, crop insurance, and quality concerns. Minnesota Crop New Archive. University of Minnesota.

Stoy, V. and Sundin, K. 1976. Effect of growth regulating substances in cereal seed germination. Cereal Res. Commun. 4:157-163.

Sun, Z. and Henson, CA. 1991. A quantitative assessment of the importance of barley seed α-amylase, β-amylase, debranching enzyme, and α-glucosidase in starch degradation. Arch. Biochem. Biophysics. 284: 298–305.

Swanson, C.O. 1946. Effects of rains on wheat during harvest. Kansas Agric. Exp. Stn. Tech. Bul. 60: 92.

Thomason, W.E., Hughes, K.R., Griffey, C.A., Parrish, D.J., and Barbeau, W.E. 2009. Understanding Pre-harvest Sprouting of Wheat. Virginia Cooperative Extension: 424-060.

Veraverbekev, W.S. and Delcour, J.A. 2002. Wheat protein composition and properties of what glutenin in relation to bread making functionality. Critical Review in Food Science and Nutrition. 42: 179-208.

Wahl, T. I. and O'Rourke, A.D. 1992. The economics of sprout damage in wheat. Pages 10-17 in: Pre-harvest Sprouting in Cereals. M. K.Walker-Simmons and J. L. Ried, eds. AACC International Press.

Walker-Simmons, M. K., and Ried, J. L. 1992. Pre-harvest Sprouting in Cereals. AACC International Press, Inc., Minnesota, U.S.A. Page iii.

Weegels, P.L., van de Pijpekamp, A.M., Graveland, A., Hamer, R.J., and Schofield, J.D. 1996. Depolymerisation and repolymerisation of wheat gluten during dough processing 1. Relationships between GMP content and quality parameters. Cereal Science 23, 103–111.

Xu, F. and Xiao, W.Y. 1993. Pre-harvest sprouting susceptibility in wheat in the Jianghuai and Northern Huaihe region. Pages 91-97 in: Pre-harvest Sprouting in Cereals 1992. M.K. Walker-Simmons, and J.L., Ried, eds. AACC, MN, USA.

Zhang, Y. and Simsek, S. 2009. Changes of starch structural and physical properties in refrigerated dough system during storage. Carbohydrate Polymers. 78: 268-274.

PAPER 1. PHYSICOCHEMICAL CHANGES OF STARCH IN PHS DAMAGED WHEAT SAMPLES

Abstract

Pre-harvest sprouting (PHS) occurs when there is rainfall before harvest. Physiologically mature kernels start to sprout in the field. The objective of this study was to examine the physicochemical changes of starch due to PHS in Hard Red Spring Wheat (HRSW) and Hard White Spring Wheat (HWSW). The mean values of α-amylase activity of sound and PHS damaged wheat were 0.12 CU/g and 2.00 CU/g. respectively. PHS can increase α-amylase activity, which results in the degradation of starch. The pasting profile was analyzed by Rapid Visco Analyzer (RVA). Mean values of peak viscosity and final viscosity of sound wheat were 203.1 RVU and 219.6 RVU, respectively. Compared to sound wheat, the PHS damaged wheat samples exhibited very low peak viscosity (mean value = 8.4 RVU) and final viscosity (mean value = 2.8 RVU) compared to sound wheat. Starch granule morphology was detected by Scanning Electronic Microscopy (SEM). Images showed that starch granule in PHS damaged wheat kernels had been hydrolyzed and the protein matrix was absent. PHS changed the molecular weight distribution of starch. Based on High Performance Size Exclusion Chromatography (HPSEC) profiles, PHS damaged wheat samples had lagged retention time (2 min later) and lower molecular weight. There was a molecular weight shift from High Molecular Weight Amylopectin (HMW-AP) to Low Molecular Weight Amylopectin (LMW-AP) and amylase (AM). The percentage of HMW-AP was decreased and the percentage of LMW-AP and AM was increased. These results indicated PHS greatly changed the physicochemical properties of starch.

Introduction

Pre-harvest sprouting (PHS) is the germination of wheat kernels before harvest so that the embryo begins to grow while the kernel is still in the field. PHS can occur when wet conditions like rainfall postpone harvest (Groos et al 2002). PHS damage can also be classified in terms of its severity with a continuum from very minor to very severe. The severity and distribution of PHS differs from year to year depending on the weather. PHS damage can be measured by the percentage of PHS damaged wheat kernels, starch degradation.

The PHS damage may result in economic consequences for the grower, miller and baker (Thomas et al 1992). Growers suffer loss when the PHS damaged wheat kernels are bought at a discount. The price of PHS damaged wheat is reduced by 20% to 50%. If the grain contains over four percent damaged kernels then it is unacceptable for human food products and severely PHS damaged grain is often used for animal feed. Millers undergo loss because of the reduced flour yield and quality of PHS damaged wheat. Bakers meet problems during the bread making process and the poor end product quality. Flour from the PHS damaged wheat kernels produces product that are porous, sticky, off-color and generally poor quality.

Starch is the main storage polysaccharide and energy provider in wheat plants, and wheat flour is composed of 70-80% dry matter of starch. Compared with other carbohydrates, it possesses unique physical and chemical properties. In starch granules, there are two constituent polymers: amylose a basically linear polysaccharide (α-1→4 linked glucose), and a highly branched polysaccharide termed amylopectin (α-1→4 linked glucose and α-1→6 linked glucose) (Whistler et al 1997). There are two main types of starch granules in wheat endosperm: the large, lenticular (A type) and small,

spherical (B type). Amylopectin is the major component of starch and normal wheat starch is comprised of around 75% amylopectin. The relative distribution of different types of starch granules is considered to influence pasting and gelatinization characteristics. The structure of amylose and amylopectin and their relative ratios in starch granules play an important role in pasting, gelation, and retrogradation properties of starch and the end product quality and stability (Jane et al 1992).

The physiological changes needed to produce a new plant require energy and nutrients, which is the reason why the PHS damaged wheat kernel produces enzymes to breakdown starch (amylases), oil (lipases) and protein (proteases). The impact of PHS damaged wheat on foods depends on the amount of enzymes present and breakdown of the kernel (Sorenson et al 2004). Higher activity of the enzymes can result in more damage to starch and protein molecular, which leading to worse end product attributes and quality.

α-Amylase is an enzyme that hydrolyses α-1, 4 bonds of large polysaccharides such as starch and glycogen, generating small sugar molecules like glucose and maltose, leading to a decrease in the size of starch molecules and reduction of the water-holding capacity of dough. Due to high α-amylase activity in PHS damaged wheat kernels, starch is degraded, which is the main reason for major economic loss due to PHS. PHS has become a main constraint to the production of high quality cereal end products, such as bread and sponge cake prepared from PHS damaged wheat display undesirable quality characteristics. There has been limited research conducted in the area of the physicochemical changes of starch due to PHS in wheat kernels. The changes of physicochemical properties of starch can significantly affect the end product quality such as Asian noodle, sponge cake and bread, because of the altered pasting, gelation, and retrogradation properties of starch (Bean et al 1974).

Materials and Methods

Materials

Wheat samples were kindly provided by Mr. Mory Rugg and Dr. Mergoum, at the Department of Plant Science, North Dakota State University. Genotypes (24) were grown at three locations (Casselton, Carrington, and Prosper, ND) in 2008, utilizing randomized complete block design with four replications. Wheat samples consist of 12 Hard Red Spring Wheat (HRSW) and 12 Hard White Spring Wheat (HWSW) genotypes that were adapted to the U.S. Spring Wheat region (Table 1.1). Wheat samples harvested from two replications were combined together and treated as a block. In this research, both sound and PHS damaged wheat samples were analyzed. Thus, a total of 288 samples were analyzed in the present research.

Wheat samples were evaluated and scored for tolerance to PHS by Mr. Mory Rugg at the Department of Plant Science, North Dakota State University. (see Appendix Table A.1) These procedures were followed: at plant physiological maturity, 30 wheat spikes were randomly harvested from each experiment unit. The spikes were immediately stored at 10°C to inhibit additional α-amylase activity and placed in a mist chamber and misted for a period of 48h. Following the misting, a humidifier was placed in the chamber for 3 days. Visual observations of the spike were made degree of sprouting induced by artificial wet conditions was scored visually 0-9. Score of 0 represented no visible sprouting and score of 9 represented very severe sprouting with average coleoptiles length greater than 2 cm (Mr. Mory Rugg et al, non-published data).

α-Amylase Activity

All the wheat samples were dried and then ground in a cyclone sample mill (Udy, Fort Collins, CO) with a 1-mm sieve. Sample of ground wheat (0.5 g) was weighted into a test tube containing a stir bar.

Table 1.1 Hard Red Spring (HRS) and Hard White Spring (HWS) wheat genotypes, PVP number, Year released, Origin and Pedigree

Class	Genotype	PVP Number	Year	Origin	Pedigree
HRS	Alsen	200100066	2000	NDSU	ND674//ND2710/ND688,ND2710=ND2603(Sumai3/Wheaton)/ Grandin
HRS	Briggs	200300142	2002	SDSU	AC PASQUA/BERGEN//SD3097
HRS	Freyr	200400165	2004	AgriPro	Sonja/Vance/3/Sumai3/
HRS	Glenn	200500280	2005	NDSU	ARINA//FO/2791/ND694/3/ND706
HRS	Granite	200100197	2002	WPB	ACSS4m-k/3/LNL/TG/312S
HRS	Hanna	200100277	2002	AgriPro	MN70170/ECM403//KATEPWA/3/BENITO/4/AC DOMAIN
HRS	Ingot	9900208	1998	SDSU	SD3080/Dalen (SD3080 = Butte86 / SD3004)
HRS	Kelby	200600237	2005	AgriPro	N97-0117/3/N92-0098//SUMAI#3/DALEN
HRS	Norpro	200000226	1999	AgriPro	N88-0436/DALEN
HRS	Knudson	200100278	2001	AgriPro	N96-0144
HRS	Reeder	200000211	1999	NDSU	IAS20*4/HH567.71//Sota/3/ND674
HRS	Steele-ND	200400188	2004	NDSU	PARSHALL/ND706
HWS	AC Snowbird	200300350	2004	Canada	RL4137*6//TC/POSP48//AC DOMAIN
HWS	AC Vista	NA	1996	Canada	HY344/7915-QX76B2/HY358*3/BT10
HWS	Argent	9900320	1998	NDSU	Grandin * 5 / ND614
HWS	CS3100L	NA	2005	Canterra	AUS1408//Kokako / CSW1889 // Endeavour
HWS	CS3100Q	NA	2005	Canterra	Otane / AC Karma
HWS	Explorer	200300182	2001	MSU	MT8182/'Fortuna')//'Pondera'/MT8182
HWS	Lolo	NA	1997	U of Idaho	A9158S//'Oasis 86'/IDO377
HWS	MT9420	NA	2001	MSU	MT8182/MT8289
HWS	NDSW0602	NA	Exp	NDSU	N97-0117//MT9420/3/971//IDO533/9747
HWS	Otis	200500312	2005	WSU	(PI 591045)/3/"Tanager 3'/'Torim 73'
HWS	Pristine	200000180	2001	WPB	Fergus/Golden 86'
HWS	99S0155-14W	NA	Exp	AgriPro	IVAN/3/HAMER//SUMAI3/DALEN

NDSU: North Dakota State University; SDSU: South Dakota State University; MSU: Michigan State University; WSU: Washington State University.
The data in this table is provided by Mr. Mory Rugg at the Department of Plant Science, NDSU.

The test tube containing the sample was placed into a stirring heat block at 60°C and stirred at medium high speed. Sodium maleate buffer (5 mL, 100 mM, pH 6.0) was heated to 60°C and added to each tube, stirred for 5 min and then an amylazyme tablet was added. The reaction was stopped by adding 6 mL Trizma base (2% w/v, pH 9.5) after 5 min. Subsequently, the sample was left at room temperature for 5 min, then stirred and filtered. The absorbance of the filtrate at 590 nm was measured against the reaction blank and α-amylase activity was calculated by reference to a standard curve.

Pasting Properties

Pasting properties of the samples were evaluated by an RVA (Newport Scientific, Narrabeen, Australia) according to AACC approved method 76-21 (AACC, 2000). Sample of ground wheat (3.5 g, 14% moisture basis) was added to pre-weighed de-ionized distilled water in an RVA canister. Parameters of peak viscosity (PV), breakdown (BD), Hot Paste Viscosity (HPV), setback (Sb) and final viscosity (FV) were recorded.

Scanning Electron Microscopy (SEM)

SEM analysis was done at the NDSU EM laboratory. Four samples of ground wheat were chosen to conduct SEM analysis to see if there was any change in starch granule-size distribution or granule morphology between PHS damaged wheat and sound wheat. Two PHS damaged wheat samples with the highest sprouting scores were chosen from both the HRSW and HWSW genotypes. The other two were from their sound wheat samples. Wheat kernels were cracked open longitudinally through the crease, fixed to microscope stubs with Dotite silver paint. Then the samples were coated with gold using a Hummer II sputter coater (Technics/Anatech Ltd., Alexandria, Virginia USA) (MacGregor et al 1972). Images were obtained using a JEOL JSM-6490LV Scanning Electron Microscope (SEM) (JEOL, Peabody, Massachusetts, USA).

Accelerating voltages, magnification, and micron bars were listed on each photo.

Molecular Weight of Starch Components

Eight samples of ground wheat were chosen to conduct the HPSEC analysis. Among the eight samples, four samples with each of the HWSW and HRSW (one sprouted sample has the highest sprouting score and its sound sample, the other sprouted sample has the lowest sprouting score and its sound sample) were included. The modified method of Grant (2002) was used to analyze the percentage of amylose and amylopectin of starch in all samples. HPSEC of starch was performed using Agilent 1200 series High Performance Liquid Chromatography (Agilent Technologies, Wilmington, DE) equipped with an auto sampler. Waters Ultrahydrogel 1000 column was run in sequence with a Waters Ultrahydrogel Linear column and Ultrahydrogel guard column (Waters, Milford, MA). An Agilent refractive index detector and PC with chemstation (HP ChemStation for LC Rev. A.04.01) were used for control and integration. Before analysis, 30 mg of ground wheat samples were solubilized by adding 4.5 mL of 1.0 M KOH and 0.5 mL of 6.0 M urea solution and heating at 100°C for 90 min. After heating, samples were neutralized with hydrogen chloride and filtered through hydrophilic nylon syringe filter. The samples were analyzed at 50°C with filtered HPLC grade water as the mobile phase. The flow rate was 0.5 mL/min and injection volume was 20 μL. Weight-averaged molecular weights of starch samples were calculated using a series of gel permeation chromatography grade dextran as standards. The amylose to amylopectin ratio was examined by HPSEC analysis and calculated by determining the percentage of peak area.

Statistical Analysis

Statistic analysis was performed using the SAS System for Windows (V. 9.2, SAS Institute, Cary, NC). Bartlett's test was used to analyze the homogeneity of error

variance across the three locations. When error were homogenous, analysis of variance (ANOVA) was performed using the "Mixed" procedure in SAS assuming location as a random effect and genotype as a fixed effect. The difference between the HRSW and HWSW mean value was analyzed using the "Contrast" option. Bartlett's test indicated that error variance of pasting properties for PHS damaged wheat were heterogeneous across the three locations. Correlation coefficient was calculated across genotype means using the "Corr" procedure in SAS.

Results and Discussion

Physical Characteristics of Sound and PHS damaged Wheat Seeds

Close examination of sound and PHS damaged wheat seeds, black areas in the PHS damaged wheat kernels could be observed (Figure 1.1). The germ end of the PHS damaged wheat kernel was in general opened by germination and exhibited a sprout. The embryo started to grow while still on the spike. Sprouts of some PHS damaged wheat kernels had been broken off with only the socket left.

Sound

Pre-Harvest
Sprout Damaged

Figure 1.1 Physical characteristics of sound and PHS damaged wheat seeds

α-Amylase Activity

In a sound wheat kernel, α-amylase activity was mostly located in the seed coat, aleurone layer and scutellum (Rani et al 2001). Endogenous α-amylase is present in

flour at very low levels (Rani et al 2001). Low α-amylase activity indicates grain soundness. Based on Bartlett's test, variances across the samples of three locations were homogeneous for sprouting score, α-amylase and endo-protease activity of sound, sprouted samples and the difference between sound and PHS damaged wheat (△D), thus three locations can be combined for analysis.

α-Amylase and endo-protease activity of PHS damaged, sound wheat and △D were given in Table 1.2. The mean value of α-amylase of PHS damaged wheat was 2.00 CU/g; while this value for sound wheat was 0.12 CU/g. Endo-protease activity of PHS damaged wheat had a mean of 2.30 A_{590}/g/h compared to the sound wheat of 1.44 A_{590}/g/h. All the PHS damaged wheat had higher α-amylase and endo-protease activity than sound wheat. Endo-protease activity of all genotypes in the present study increased due to PHS damage, while the ratio of increase was smaller than that of α-amylase activity. These results were in agreement with the findings of Yasunori et al (2001) who reported that the α-amylase activity of wheat increase rapidly as germination progresses. Previous report (Yasunori et al 2001) also indicated that the starch in the PHS damaged wheat of each genotype degraded rapidly as the α-amylase activity increased during PHS. Many other studies (Hwang et al 1973; Yasunori et al 2001) also have shown that sprouting beyond 2 days produced a rapid increase in proteolytic activity.

Genotype tested showed different sprouting scores ranging from 2.5 to 7.8 (Table 1.2). Hanna had the lowest sprouting score (2.8) in PHS damaged HRSW genotypes, while Ingot had the highest sprouting score (7.0). The α-amylase and endo-protease activities of Hanna (sprouted) were 1.32 CU/g and 2.00 $_{A590}$/g/h. The α-amylase and endo-protease activity of Ingot (sprouted) were 2.37 CU/g and 2.44 A_{590}/g/h. Hanna (sprouted) with lowest sprouting score in HRSW, had lower α-amylase

and endo-protease activity than Ingot (sprouted). Genotype of 99S0155-14W had the lowest sprouting score of 2.5, while Otis had the highest sprouting score (7.8) in PHS damaged HWSW. The α-amylase and endo-protease activity of 99S0155-14W (sprouted) were 1.36 CU/g and 1.92 A_{590}/g/h. Otis (sprouted) had higher α-amylase (2.47 CU/g) and endo-protease activity (2.65 A_{590}/g/h) than 99S0155-14W. These results agreed with the findings of Huang (1979) who reported that genotypes with lower susceptibility to PHS had lower enzyme activity.

Wheat genotypes may exhibit high α-amylase activity without considerable PHS damage. Thus, the relationship between PHS damage and α-amylase and endo-protease activity of each genotype was determined. There was significant difference among genotypes for α-amylase activity and for endo-protease activity in PHS damaged wheat ($P < 0.001$). Significant difference also existed among genotypes for α-amylase activity, and for endo-protease activity in sound wheat ($P < 0.001$).

The △D values of α-amylase activity and endo-protease activity were significantly different among genotypes ($P < 0.001$). These results indicated that varietal differences were highly significant for enzyme activity ($P < 0.001$), and genotypes with low susceptibility to PHS may be able to be segregated on the basis of enzyme activity. Although α-amylase and endo-protease existed at very low activity in sound wheat kernels, wheat genotypes exhibited significantly different levels of α-amylase and endo-protease activity ($P < 0.001$). These results were similar to the results of Huang et al (1980) who reported that there is significant difference occurred among Kansas Hard White Wheat genotypes for α-amylase activity (see Appendix Table A.2).

α-Amylase and endo-protease activity were significant differences between three locations for sound wheat, PHS damaged wheat, and △D ($P < 0.05$). No significant difference was found between three locations for endo-protease activity in

Table 1.2 Sprouting score, α-amylase and endo-protease activity of sprouted, sound sample and their difference

Genotype	Sprouting Score[1]	α-Amylase Activity (CU/g)			Protease Activity (A590/g/h)		
		Sprouted	Sound	△D[2]	Sprouted	Sound	△D
HRSW							
Hanna	2.8	1.32	0.11	1.20	2.00	1.51	0.49
Ingot	7.0	2.37	0.10	2.27	2.44	1.56	0.88
Alsen	4.8	1.82	0.09	1.57	2.16	1.40	0.76
Briggs	5.7	2.16	0.11	2.06	2.28	1.50	1.07
Freyr	4.4	1.79	0.09	1.70	2.06	1.76	0.31
Glenn	4.0	1.68	0.08	1.61	2.08	1.43	0.66
Granite	5.3	2.09	0.13	1.96	2.27	1.42	0.85
Kelby	3.4	1.56	0.13	1.43	2.03	1.39	0.64
Norpro	6.0	2.18	0.09	2.09	2.28	1.35	0.94
Reeder	4.4	1.76	0.08	1.68	2.15	1.51	0.65
Steele-ND	5.0	1.93	0.08	1.85	2.27	1.32	0.95
Knudson	5.4	2.12	0.10	2.02	2.23	1.51	0.72
Mean	4.8	1.90	0.10	1.79	2.19	1.47	0.74
HWSW							
99S0155-14W	2.5	1.36	0.12	1.24	1.92	1.49	0.44
Otis	7.8	2.47	0.11	2.37	2.65	1.43	1.22
AC Snowbird	2.8	1.39	0.08	1.31	2.00	1.50	0.50
AC Vista	5.8	2.13	0.09	2.04	2.34	1.26	1.08
Argent	4.8	1.98	0.16	1.83	2.56	1.39	0.90
CS3100L	6.8	2.33	0.18	2.16	2.67	1.48	1.19
CS3100Q	6.8	2.44	0.14	2.3	2.48	1.40	1.08
Explorer	6.9	2.37	0.16	2.21	2.44	1.32	1.12
Lolo	5.7	2.17	0.12	2.05	2.48	1.46	1.02
MT9420	6.9	2.33	0.14	2.19	2.59	1.41	1.19
NDSW0602	6.3	2.37	0.12	2.24	2.47	1.41	1.06
Pristine	5.0	1.99	0.18	1.81	2.43	1.47	0.97
Mean	5.7	2.11	0.13	1.98	2.40	1.42	0.98
LSD[3]	1.4	0.39	0.05	0.40	0.14	0.20	0.20

[1]Sprouting scores were provided by Mr. Mory Rugg at the Department of Plant Science, NDSU.
[2]△D: Difference between sound and PHS damaged wheat.
[3] LSD: least significant difference (α=0.05), used to detect difference between genotypes.

sound samples. However, the differences of enzyme activity ($P < 0.05$) between three locations were not as highly as that between genotypes ($P < 0.001$). Interaction between genotypes by locations significantly affected enzyme activity. These results indicated that environments can affect the PHS damage level and enzyme activity, but the effect

of locations on PHS was less significant as that of genotypes (see Appendix Table A.2).

Huang et al (1980) reported that α-amylase activity was positively correlated with degree of PHS damage, and the major contribution to the high correlations was varietal difference. Correlations between sprouting score and α-amylase and endo-protease activities were determined (Table 1.3). There was significant and positive correlation between sprouting score and α-amylase activity (correlation coefficient value r = 0.98) and endo-protease activity (r = 0.88) in PHS damaged wheat. The correlations between sprouting score and the difference of α-amylase and endo-protease activity between PHS damaged and sound wheat were also significant (r = 0.98, 0.86). These results indicated that genotypes with greater sprouting score exhibited higher α-amylase and endo-protease activities, and starch and protein of those genotypes would been degraded more seriously. These results were in agreement with the finding of Huang et al (1980). There was significant and positive correlation between α-amylase and endo-protease activity (r = 0.88) in PHS damaged wheat, further indicating that PHS significantly increased both α-amylase and endo-protease activities. However, the correlation between endo-protease activity and sprouting score were lower than the correlation between α-amylase activity and sprouting score in PHS damaged wheat, which suggested α-amylase activity can be associated more to sprouting score than endo-protease activity.

Pasting Properties

In PHS damaged HRSW genotypes, Hanna with a very low sprouting score had a much higher viscosity than Ingot, which had a much higher sprouting score. Figure 1.2 shows the pasting profiles of Hanna and Ingot (sprouted) from Prosper, ND. PHS damaged Hanna with low sprouting score (2.8) exhibited a peak viscosity (≈16 RVU) which is relatively high for the PHS wheat samples. PHS damaged Ingot with very high

Table 1.3 Correlation coefficients between sprouting score and enzyme activities for 24 genotypes grown at Carrington, Casselton and Prosper in North Dakota

	Sprouting	Sound		Sprouted		ΔD[2]
	Score[1]	α-Amylase	Protease[3]	α-Amylase	Protease	α-Amylase
Sound						
α-Amylase	NS					
Protease	NS	NS				
Sprouted						
α-Amylase	0.98***	NS	NS			
Protease	0.88***	0.57**	NS	0.88***		
ΔD						
α-Amylase	0.98***	NS	NS	0.99***	0.86***	
Protease	0.86***	0.46*	0.57**	0.86***	0.89***	0.85***

[1]Sprouting scores were provided by Mr. Mory Rugg at the Department of Plant Science, North Dakota State University.
[2]ΔD: difference between sound and PHS damaged wheat.
[3]Protease: Endo-protease activity.
*, **, ***: significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; NS, not significant.

sprouting score (7.0) exhibited a low peak viscosity (≈ 5 RVU). This result indicated that the peak viscosity decreased as the PHS damage increased α-amylase activity. The pasting profile of starch determined by RVA had a direct relationship with its microstructure. Amylose, which has an important contribution to high gel consistency upon cooling, perhaps resulted in the initial rigidity to the swollen starch granules (Tsai et al 1997). The branch chain length of amylopectin has an effect on the gelatinization, retrogradation and pasting properties of starch (Jane & Chen 1992).

The pasting parameters of sound and PHS damaged wheat were shown in Table 1.4, 1.5 and 1.6. All RVA parameters changed dramatically due to PHS, which indicated that PHS had significant effects on starch pasting properties. All PHS damaged wheat had much lower peak viscosity, breakdown viscosity, setback viscosity and final viscosity than the sound wheat. Peak viscosity of sound wheat was ranging from 138.9 to 289 RVU. Peak viscosity of PHS damaged wheat was ranging from 5 to 18 RVU, which is significantly lower than that of sound wheat. Sound wheat had a final

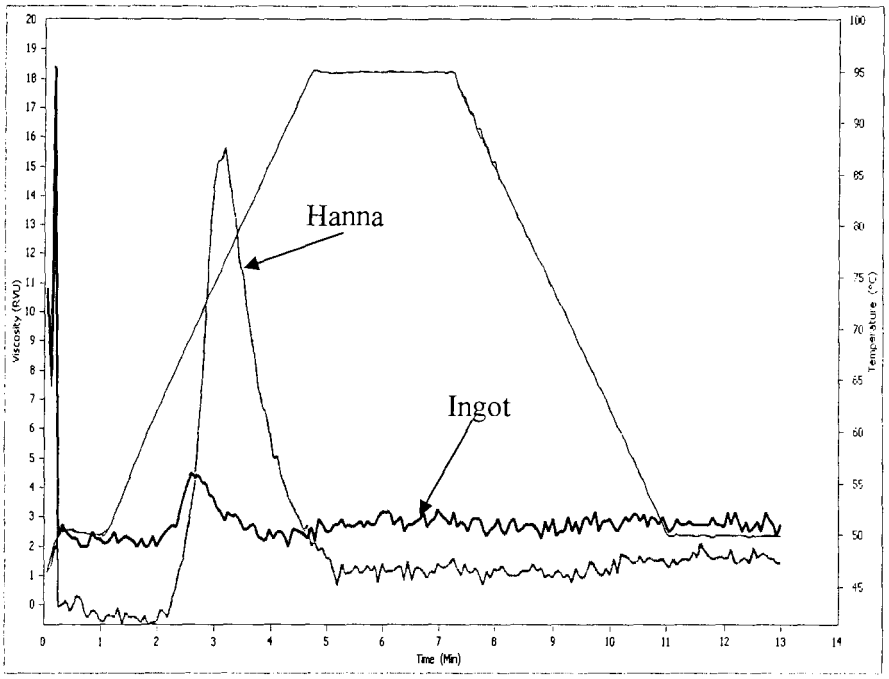

Figure 1.2 Pasting profile of genotypes Hanna and Ingot (sprouted) from Prosper, ND

viscosity ranging from 114 to 247 RVU; and PHS damaged wheat had a lower final viscosity than sound wheat ranging from 1.2 to 5.9 RVU. Results showed that the PHS damaged wheat samples exhibited very low peak viscosity, breakdown viscosity, setback viscosity and final viscosity compared to sound wheat. These results suggested that the water binding capacity of starch and starch paste stability decreased due to PHS. The decreased water binding capacity of starch and starch paste stability can result in the deterioration of end product quality of wheat flour such as bread and Asian noodle (Bean et al 1974).

In the PHS damaged HRSW genotypes, Hanna had the lowest sprouting score and highest paste viscosity; Ingot had the highest sprouting score and lowest paste viscosity. In the PHS damaged HWSW genotypes, 99S0155-14W had the lowest sprouting score and highest paste viscosity; Otis had the highest sprouting score and

lowest paste viscosity. Hanna (sprouted) had a sprout score of 2.8 and a peak viscosity of 12.27 RVU. Ingot, which had the highest sprout score (7.0) had the lowest peak viscosity of 6.97 RVU. The genotype 99S0155-14W (sprouted) had sprouting score of 2.5 and peak viscosity of 15.27 RVU, while Otis (sprouted) with sprouting score of 7.8, had a peak viscosity of 7.67 RVU, which is lower than that of 99S0155-14W. This means that those genotypes with different degrees of PHS damage exhibited different pasting properties; the peak viscosity, breakdown viscosity, setback viscosity and final viscosity decreased markedly as the degree of PHS damage increased. The results obtained in the present work appeared to reflect the effect of increased α-amylase activity on the starch granules. The starch granules may have lost some of their resistance to swelling due to the activity of the α-amylase in the PHS damaged wheat samples (Morad et al 1983). The reduced resistance to swelling may play an important role in the lower paste viscosity of the PHS damaged wheat. Genotypes in the present study with lower PHS susceptibility had better water binding capacity of starch and starch paste stability. PHS was a complex trait, and could be affected by many factors. Very highly significant genotypic difference ($P < 0.001$) existed among different genotypes for paste viscosity of sound and PHS damaged wheat and the $\triangle D$. These results mean that sound wheat genotypes exhibited significantly different pasting properties, and the changes of pasting properties of different genotypes due to PHS were significantly different ($P < 0.001$). HRSW and HWSW genotypes showed significantly difference for the $\triangle D$ of pasting parameters ($P < 0.05$), which indicated that the changes of the pasting parameters of HRSW and HWSW genotypes were significantly different due to PHS damage (see Appendix Table A.3, A.4 and A.5).

To relate pasting properties to sprouting score and enzyme activity in the present study, correlations between pasting characteristics and enzyme activities among 24 genotypes

in three locations were estimated in Table 1.7, 1.8 and1.9. The correlations between pasting characteristics and sprouting score and enzyme activity were similar in three locations. Sprouting score had significant and negative correlation with peak viscosity, breakdown viscosity and setback viscosity of PHS damaged wheat in all three locations. Furthermore, it had significant correlation with the △D of hot paste viscosity in Carrington and Casselton ($P < 0.05$). These results indicated that genotypes with high sprouting score had higher susceptibility to PHS and exhibited low peak viscosity, breakdown viscosity and setback viscosity. Significant and negative correlations existed between α-amylase of sound wheat and peak viscosity, hot paste viscosity and final viscosity of sound wheat sample in three locations ($P < 0.05$). α-Amylase existed in sound wheat kernels and had very low activity. These results indicated that the sound wheat genotypes with higher levels of α-amylase exhibited lower peak viscosity, hot paste viscosity and final viscosity. There was no correlation between endo-protease activities with pasting characteristic of the sound sample. In the sound wheat, the level of endo-protease activity had no relationship with pasting properties of starch. There was negatively significant correlation between enzyme activities (α-amylase and endo-protease activities) with peak viscosity ($P < 0.01$), breakdown viscosity ($P < 0.01$) and setback viscosity ($P < 0.05$) of PHS damaged wheat. The correlations between the difference of enzyme activities and hot paste viscosity, final viscosity were also significant and negative in Carrington. PHS increased α-amylase and endo-protease activities of wheat. The increased α-amylase activity hydrolyzed the starch granules and changed the molecular weight distribution of amylose and amylopectin, which largely changed the water binding capacity of starch and starch paste stability during PHS. Thus, the pasting viscosity of PHS damaged wheat had been decreased and exhibited significant and negative correlations with α-amylase and endo-protease activity.

Table 1.4 Mean value of pasting profile of 24 genotypes in Carrington

Genotype	Sprout Score[1]	Peak Viscosity			Breakdown Viscosity			Hot Paste Viscosity			Final Viscosity			Setback Viscosity		
		Sprout	Sound	ΔD	Sprout	Sound	ΔD	Sprout	Sound	ΔD	Sprout	Sound	ΔD	Sprout	Sound	ΔD
HRSW																
Hanna	2.8	11.2	222.7	212.8	9.0	86.0	77.3	2.3	134.8	133.2	3.5	258.5	255.4	1.2	122.4	121.2
Ingot	7.0	3.5	233.4	230.4	1.0	93.5	92.8	2.3	139.9	137.6	2.9	256.7	253.8	0.5	116.8	116.1
Alsen	4.8	4.1	190.6	186.9	3.3	97.8	94.5	0.9	92.6	92.0	1.4	181.9	180.8	0.3	89.2	88.7
Briggs	5.7	4.2	230.5	227.5	4.1	87.7	84.1	0.3	140.8	141.I	0.4	265.3	265.6	0.2	124.1	124.I
Freyr	4.4	4.9	224.3	220.7	1.6	81.2	79.9	3.1	141.0	138.3	3.0	264.8	262.1	0.1	122.6	122.7
Glenn	4.0	6.9	247.2	240.6	6.1	122.6	116.5	0.9	124.0	123.4	1.1	226.2	225.3	0.2	102.0	101.8
Granite	5.3	2.6	194.3	193.0	1.8	79.8	78.5	0.8	112.6	112.3	1.2	231.3	230.6	0.5	118.3	117.9
Kelby	3.4	6.1	226.9	221.8	4.6	94.3	89.9	1.8	130.6	129.5	2.4	259.7	258.1	0.6	128.7	128.3
Norpro	6.0	1.2	187.7	187.9	-0.2	76.1	76.6	1.5	109.9	109.1	1.5	226.9	225.8	0.1	115.8	115.7
Reeder	4.4	-1.2	249.5	250.9	-1.4	125.1	126.1	0.3	124.1	124.2	0.8	228.9	228.4	0.6	104.6	104.1
Steele-ND	5.0	9.3	232.2	224.5	7.6	85.5	79.1	1.9	144.8	143.4	2.4	278.0	276.1	0.6	132.8	132.3
Knudson	5.4	6.2	260.5	260.7	3.7	113.3	115.5	2.6	145.0	142.7	3.3	281.4	278.3	0.7	135.1	134.6
Mean	4.8	4.4	224.4	221.4	3.1	96.4	94.1	1.4	126.5	125.6	1.7	244.5	243.1	0.4	117.3	117.0
LSD	1.7	1.8	19.3	19.5	1.1	5.7	5.8	1.3	18.1	18.6	1.3	28.2	28.6	0.5	11.6	11.5

Table 1.4 Mean value of pasting profile of 24 genotypes in Carrington (Continued)

Genotype	Sprout Score[1]	Peak Viscosity			Breakdown Viscosity			Hot Paste Viscosity			Final Viscosity			Setback Viscosity		
		Sprout	Sound	ΔD	Sprout	Sound	ΔD	Sprout	Sound	ΔD	Sprout	Sound	ΔD	Sprout	Sound	ΔD
HWSW																
99S0155-14W	2.5	10.9	210.9	195.8	8.7	96.6	83.8	2.4	112.5	110.0	3.2	237.6	234.0	0.8	123.9	123.0
Otis	7.8	2.5	202.0	200.8	-0.7	115.6	117.8	3.2	86.0	82.4	3.5	196.2	192.2	0.3	110.1	109.8
AC Snowbird	2.8	11.4	252.4	239.3	9.4	126.9	114.7	1.9	125.2	124.2	3 0	237.8	235.5	1.2	112.4	111.2
AC Vista	5.8	2.3	235.7	237.4	1.6	98.6	100.2	0.7	135.3	134.8	0.8	281.3	280.8	0.2	145.6	145.6
Argent	4.8	-0.9	202.7	200.6	-2.4	98.8	97.6	1.6	101.6	100.5	1.8	220.6	218.5	0.4	117.6	116.9
CS3100L	6.8	5.5	202.8	205.3	3.1	111.0	114.7	2.4	91.6	89.9	2.7	216.5	214.8	0.2	124.8	124.9
CS3100Q	6.8	-4.4	210.3	203.3	-5.8	100.0	96.2	1.3	108.2	105.0	1.4	238.9	235.1	0.2	130.3	129.8
Explorer	6.9	8.2	164.6	166.2	4.6	103.1	102.9	3.3	59.5	60.9	3.9	140.8	141.8	0.6	79.9	79.7
Lolo	5.7	2.2	210.4	205.4	2.1	116.9	114.0	0.6	93.0	90.8	0.6	202.9	200.3	0.1	109.9	109.6
MT9420	6.9	4.3	192.7	193.3	1.9	110.2	111.9	2.4	80.5	79.1	2.8	173.9	172.1	0.5	92.8	92.6
NDSW0602	6.3	0.5	203.1	202.3	-1.9	113.0	114.0	2.5	88.5	86.2	3.0	201.8	198.7	0.5	112.2	111.7
Pristine	5.0	2.5	218.3	217.8	-1.0	107.8	108.5	3.4	108.2	106.8	3.6	242.8	241.3	0.2	134.1	134.1
Mean	5.7	3.1	209.3	207.1	1.2	108.6	107.5	2.0	99.2	97.8	2.4	215.7	213.9	0.4	116.0	115.6
LSD	1.7	1.8	19.3	19.5	1.1	5.7	5.8	1.3	18.1	18.6	1.3	28.2	28.6	0.5	11.6	11.5

[1]Sprouting scores were provided by Mr. Mory Rugg at the Department of Plant Science, NDSU.
The unit is expressed by Rapid Viscosity Unit (RVU).
LSD: least significant difference ($\alpha = 0.05$).

Table 1.5 Mean value of pasting profile of 24 genotypes in Casselton

Genotype	Sprout Score	Peak Viscosity			Breakdown Viscosity			Hot Paste Viscosity			Final Viscosity			Setback Viscosity		
		Sprout	Sound	ΔD	Sprout	Sound	ΔD	Sprout	Sound	ΔD	Sprout	Sound	ΔD	Sprout	Sound	ΔD
HRSW																
Hanna	2.8	20.5	197.9	177.4	17.4	69.7	52.3	3.1	128.6	125.4	4.8	237.5	232.4	1.6	107.7	105.9
Ingot	7.0	10.6	210.1	198.6	7.9	78.7	70.6	2.5	133.6	130.6	3.1	233.2	229.6	0.6	99.6	99.0
Alsen	4.8	14.8	196.3	180.6	13.3	88.4	74.9	1.6	109.4	107.5	2.2	191.5	188.9	0.5	81.9	81.3
Briggs	5.7	7.9	199.8	191.5	7.2	73.3	65.8	0.8	126.7	125.9	1.3	227.6	226.3	0.5	100.6	100.2
Freyr	4.4	14.2	187.4	173.4	9.3	64.2	54.8	4.5	123.7	119.0	4.9	228.6	223.3	0.4	103.8	103.3
Glenn	4.0	16.0	229.7	212.7	13.8	107.6	93.4	2.1	123.7	121.2	2.5	208.4	205.4	0.5	84.6	84.1
Granite	5.3	8.0	174.8	166.7	6.7	70.4	63.5	1.2	104.6	103.3	2.1	205.7	203.5	0.9	100.9	100.0
Kelby	3.4	18.0	209.2	191.0	13.6	82.9	69.3	4.3	126.3	121.9	5.5	238.1	232.3	1.2	111.5	110.2
Norpro	6.0	6.0	167.0	160.9	4.8	61.5	56.4	1.3	106.0	104.8	1.8	207.6	205.7	0.5	100.6	100.0
Reeder	4.4	2.6	229.3	214.9	2.0	112.7	100.8	0.7	118.4	116.3	0.9	208.3	205.0	0.4	89.8	88.7
Steele-ND	5.0	25.7	208.8	199.2	21.6	74.2	67.5	4.0	134.7	131.8	6.0	247.7	243.9	1.6	112.7	111.8
Knudson	5.4	10.8	242.4	232.4	7.8	96.2	89.4	3.1	146.3	143.2	4.1	267.9	263.8	1.0	120.5	119.7
Mean	4.8	12.4	204.5	192.3	10.0	83.1	73.6	2.3	122.0	119.5	3.1	223.1	219.8	0.7	100.7	99.9
LSD	1.7	5.3	25.5	25.4	3.9	7.3	8.4	2.1	20.4	19.9	2.8	37.9	37.4	1.2	18.2	18.2

Table 1.5 Mean value of pasting profile of 24 genotypes in Casselton (Continued)

Genotype	Sprout Score	Peak Viscosity			Breakdown Viscosity			Hot Paste Viscosity			Final Viscosity			Setback Viscosity		
		Sprout	Sound	ΔD	Sprout	Sound	ΔD	Sprout	Sound	ΔD	Sprout	Sound	ΔD	Sprout	Sound	ΔD
HWSW																
99S0155-14W	2.5	18.2	179.1	154.9	14.5	83.3	64.5	3.8	96.4	91.0	4.8	200.7	193.8	1.1	103.3	102.0
Otis	7.8	14.1	190.9	179.0	8.5	97.5	90.2	5.3	95.1	90.9	5.9	195.6	190.5	0.6	100.3	99.5
AC Snowbird	2.8	14.8	242.0	228.6	12.9	114.0	102.1	1.9	129.8	128.3	3.1	236.1	233.8	1.3	106.2	105.5
AC Vista	5.8	10.2	218.5	194.0	8.6	87.2	67.0	1.6	131.4	127.8	2.3	259.3	253.6	0.6	127.6	125.6
Argent	4.8	8.5	168.5	164.9	5.9	82.3	80.4	2.6	86.4	84.5	3.3	179.7	177.4	0.7	92.2	91.9
CS3100L	6.8	11.9	191.0	178.1	8.8	97.6	88.2	3.0	95.1	91.9	3.4	207.0	203.2	0.3	111.8	111.2
CS3100Q	6.8	10.8	186.1	177.5	8.3	86.0	80.0	2.4	100.3	97.6	2.9	210.1	206.9	0.5	109.6	109.1
Explorer	6.9	8.3	138.9	129.6	5.7	89.8	82.5	2.5	49.3	47.2	3.2	114.6	111.6	0.7	64.2	63.4
Lolo	5.7	10.6	213.9	200.0	7.8	103.6	93.8	2.8	112.0	108.4	3.5	216.2	212.0	0.7	104.2	103.6
MT9420	6.9	10.5	164.5	154.7	7.4	99.3	93.2	3.0	65.2	61.6	3.9	139.5	135.1	0.9	74.0	73.3
NDSW0602	6.3	6.5	166.1	156.8	4.3	96.4	90.0	2.4	70.2	67.1	3.2	158.4	154.0	0.8	87.2	86.1
Pristine	5.0	14.3	199.3	191.0	10.0	98.4	92.4	4.1	100.6	98.3	4.8	215.9	213.2	0.6	115.0	114.6
Mean	5.7	10.6	188.9	177.5	8.0	95.5	87.0	2.6	94.0	91.3	3.3	193.7	190.1	0.7	99.2	98.4
LSD	1.7	5.3	25.5	25.4	3.9	7.3	8.4	2.1	20.4	19.9	2.8	37.9	37.4	1.2	18.2	18.2

Sprouting scores were provided by Mr. Mory Rugg at the Department of Plant Science, NDSU.
The unit is expressed by Rapid Viscosity Unit (RVU).
LSD: least significant difference ($\alpha = 0.05$).

Table 1. 6 Mean value of pasting profile of 24 genotypes in Prosper

Genotype	Sprout Score[1]	Peak Viscosity			Breakdown Viscosity			Hot Paste Viscosity			Final Viscosity			Setback Viscosity		
		Sprout	Sound	ΔD	Sprout	Sound	ΔD	Sprout	Sound	ΔD	Sprout	Sound	ΔD	Sprout	Sound	ΔD
HRSW																
Hanna	2.8	14.7	200.2	183.2	13.1	71.5	57.9	1.8	129.1	125.6	3.0	240.8	236.2	1.2	112.2	110.9
Ingot	7.0	6.8	236.2	227.6	4.7	83.6	79.1	2.0	153.7	150.4	2.6	274.4	270.6	0.6	121.8	121.2
Alsen	4.8	11.8	157.9	144.1	9.9	82.7	72.3	1.9	77.5	74.3	2.1	152.3	148.9	0.2	76.5	76.1
Briggs	5.7	6.0	204.4	198.7	5.2	74.3	69.7	0.9	130.3	129.2	1.2	238.6	237.6	0.3	109.6	109.4
Freyr	4.4	8.6	221.4	212.8	5.7	69.1	63.1	2.8	152.0	149.7	3.0	280.5	278.3	0.2	128.8	128.8
Glenn	4.0	14.4	237.8	222.8	11.8	108.3	95.9	2.5	131.0	128.9	2.9	238.3	236.1	0.4	108.7	108.3
Granite	5.3	4.9	164.6	160.9	3.9	68.1	65.1	0.9	96.8	96.2	1.5	196.2	195.4	0.6	100.8	100.3
Kelby	3.4	10.9	160.8	147.6	8.5	70.8	60.9	2.6	91.0	87.4	3.3	183.1	179.1	0.8	93.6	92.9
Norpro	6.0	6.5	189.5	185.3	4.1	69.0	65.8	2.4	121.1	120.3	2.4	245.1	244.3	0.1	124.8	124.7
Reeder	4.4	10.2	280.4	269.1	8.5	121.1	112.7	1.6	159.9	157.9	2.4	287.5	284.8	0.7	128.8	127.8
Steele-ND	5.0	4.7	151.3	147.3	2.9	61.0	58.1	1.9	92.0	90.4	2.2	168.0	166.3	0.4	77.8	77.4
Knudson	5.4	6.3	248.9	240.2	3.7	101.4	96.5	2.7	147.5	143.8	3.3	292.1	288.0	0.6	145.0	144.4
Mean	4.8	8.4	201.7	192.9	6.4	82.6	76.0	2.0	119.9	117.8	2.4	228.2	225.9	0.5	109.4	109.0
LSD	1.7	2.3	24.1	24.3	2.3	7.0	7.4	1.2	19.5	19.3	1.3	30.9	30.8	0.5	13.2	13.1

Table 1.6 Mean value of pasting profile of 24 genotypes in Prosper (Continued)

Genotype	Sprout Score[1]	Peak Viscosity			Breakdown Viscosity			Hot Paste Viscosity			Final Viscosity			Setback Viscosity		
		Sprout	Sound	ΔD	Sprout	Sound	ΔD	Sprout	Sound	ΔD	Sprout	Sound	ΔD	Sprout	Sound	ΔD
HWSW																
99S0155-14W	2.5	16.7	178.6	161.6	12.8	81.2	67.6	3.9	98.1	94.6	4.9	215.9	211.4	1.0	118.3	117.2
Otis	7.8	6.4	182.5	179.4	3.0	101.5	101.9	3.4	82.8	79.4	3.9	179.2	176.2	0.5	98.0	98.1
AC Snowbird	2.8	17.9	289.0	268.2	16.0	125.6	108.4	1.9	163.7	160.9	3.3	294.6	290.5	1.4	132.1	130.6
AC Vista	5.8	9.2	174.4	170.1	6.5	77.4	74.3	2.4	98.4	97.0	2.5	206.9	205.6	0.2	110.2	110.1
Argent	4.8	2.6	165.7	159.3	1.5	80.8	76.4	1.2	85.5	83.5	1.7	197.4	194.9	0.5	112.4	111.8
CS3100L	6.8	8.4	222.3	210.1	6.1	108.1	100.9	2.4	115.1	110.7	2.8	242.0	238.1	0.3	128.4	128.5
CS3100Q	6.8	6.4	175.6	172.7	5.1	85.7	84.1	1.4	90.3	89.0	1.9	203.5	202.3	0.4	114.6	114.4
Explorer	6.9	4.3	118.3	112.4	2.9	79.8	75.4	1.5	39.4	37.9	2.0	109.9	107.7	0.6	71.1	70.2
Lolo	5.7	8.6	203.5	193.8	4.9	102.0	95.5	3.5	103.5	100.8	3.7	209.6	206.7	0.3	107.9	107.4
MT9420	6.9	1.2	140.3	134.5	-0.8	93.0	89.8	2.0	48.0	45.4	2.2	115.3	112.4	0.3	68.8	68.2
NDSW0602	6.3	13.0	200.8	193.1	9.0	104.6	101.3	4.0	95.9	91.7	4.8	212.9	208.3	0.8	117.3	116.8
Pristine	5.0	9.9	208.4	193.9	6.8	96.9	87.1	3.1	111.3	107.0	3.5	240.8	236.3	0.4	130.8	130.3
Mean	5.7	8.1	189.8	180.8	5.8	95.4	89.3	2.3	95.1	92.4	2.8	203.3	200.3	0.5	109.3	108.8
LSD	1.7	2.3	24.1	24.3	2.3	7.0	7.4	1.2	19.5	19.3	1.3	30.9	30.8	0.5	13.2	13.1

[1]Sprouting scores were provided by Mr. Mory Rugg at the Department of Plant Science, NDSU.
The unit is expressed by Rapid Viscosity Unit (RVU).
LSD: least significant difference ($\alpha = 0.05$).

Starch Granule Morphology

In the sound wheat endosperm, starch granules are usually embedded in dense protein matrix. Laboratory sprouting of barley and corn for seven days showed extensive damage to starch granules due to the increased α-amylase activity (Lorenz et al 1981). Dronzek et al (1972) and Bean et al (1974) have shown extensive damage to wheat starch granules due to PHS of the wheat kernels. Two genotypes Steel-ND and Pristine were chosen to conduct SEM analysis because of their high sprouting scores for the PHS damaged wheat. SEM images of sprouted sample and sound sample were compared: the sound sample contained intact starch granules embedded in a very dense protein matrix; however, starch granule had been degraded, and the protein matrix was absent in the sprouted sample (Figure 1.3). There was also pitting appeared on the starch granule due to the attack of the increased α-amylase activity.

Huang et al (1979) found similar results and reported that matrix protein decreased in PHS damaged wheat. The results of the study by Huang et al (1979) indicated that proteolytic enzymes broke down the matrix protein and thereby produced a loose structure around the starch granules, which made them more accessible to α-amylase attack. Starch is deposited in the endosperm of the wheat kernel and exists as discrete granules. The endosperm of wheat kernels contains two types of granules: a larger type, mostly about 20-35 micrometers diameter (A-starch), being lenticular in shape; a smaller spherical type, ranging from 2-8 micrometers in diameter (B-starch) (Cornell, 2004). Scanning electron microscopy (SEM) can be used to see the distribution of the A and B type granules as well as degradation of the granules due to high α-amylase activity attack. Furthermore, the loose of protein matrix made the starch granule much more easily to be attacked and protein properties would be changed a lot. (see Appendix Figure A.1 & A.2)

Table 1.7 Correlation coefficients between enzyme activities and pasting characteristics among 24 genotypes in Carrington

		Sound		Sprouted		ΔD	
	Sprout Score	α-Amylase	Protease	α-Amylase	Protease	α-Amylase	Protease
Carrington							
Sound							
PV	NS	-0.45 *	NS	NS	-0.54 **	NS	-0.44 *
BD	NS	NS	NS	NS	NS	NS	NS
HPV	-0.47 *	-0.50 *	NS	-0.51 *	-0.60 **	-0.45 *	-0.54 **
FV	NS	NS	NS	-0.43 *	-0.46 *	NS	NS
Sb	NS	NS	NS	NS	NS	NS	NS
Sprouted							
PV	-0.64 ***	NS	NS	-0.61 **	-0.43 *	-0.60 **	-0.46 *
HPV	NS	NS	NS	NS	NS	NS	NS
BD	-0.70 ***	NS	NS	-0.68 ***	-0.51 *	-0.65 ***	-0.48 *
FV	NS	NS	NS	NS	NS	NS	NS
Sb	-0.56 **	NS	NS	-0.49 *	NS	-0.47 *	-0.50 *
ΔD							
PV	NS	-0.45 *	NS	NS	-0.45 *	NS	NS
HPV	-0.48 *	-0.50 *	NS	-0.51 *	-0.62 **	-0.45 *	-0.54 **
BD	NS	NS	NS	0.41 *	NS	NS	NS
FV	NS	NS	NS	-0.43 *	-0.47 *	NS	NS
Sb	NS	NS	NS	NS	NS	NS	NS

ΔD: difference between sound and PHS damaged wheat; PV: Peak Viscosity; BD: Breakdown Viscosity; HPV: Hot Paste Viscosity; Sb: Setback; FV: Final Viscosity.
*, **, ***: correlation coefficient is significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; NS, not significant.

Table 1.8 Correlation coefficients between enzyme activities and pasting characteristics among 24 genotypes in Casselton

		Sound		Sprouted		ΔD	
	Sprout Score	α-Amylase	Protease	α-Amylase	Protease	α-Amylase	Protease
Casselton							
Sound							
PV	-0.41 *	-0.67 ***	NS	NS	-0.45 *	NS	NS
HPV	-0.44 *	-0.68 ***	NS	NS	-0.49 *	NS	-0.44 *
BD	NS	NS	NS	NS	NS	NS	NS
FV	NS	-0.54 **	NS	NS	NS	NS	NS
Sb	NS	NS	NS	NS	NS	NS	NS
Sprouted							
PV	-0.56 **	NS	NS	-0.54 **	NS	-0.54 **	NS
HPV	NS	NS	NS	NS	NS	NS	NS
BD	-0.60 **	NS	NS	-0.60 **	-0.42 *	-0.60 **	NS
FV	NS	NS	NS	NS	NS	NS	NS
Sb	-0.54 **	NS	NS	-0.54 **	NS	-0.53 **	NS
ΔD							
PV	NS	-0.68 ***	NS	NS	NS	NS	NS
HPV	-0.43 *	-0.69 ***	NS	NS	-0.49 *	NS	-0.43 *
BD	NS	NS	NS	NS	NS	NS	NS
FV	NS	-0.55 **	NS	NS	NS	NS	NS
Sb	NS	NS	NS	NS	NS	NS	NS

ΔD: difference between sound and PHS damaged wheat; PV: Peak Viscosity; BD: Breakdown Viscosity; HPV: Hot Paste Viscosity; Sb: Setback; FV: Final Viscosity.
*, **, ***: correlation coefficient is significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; NS, not significant.

Table 1.9 Correlation coefficients between enzyme activities and pasting characteristics among 24 genotypes in Prosper

		Sound		Sprouted		ΔD	
	Sprout Score	α-Amylase	Protease	α-Amylase	Protease	α-Amylase	Protease
Prosper							
Sound							
PV	NS	-0.64 ***	0.51	NS	NS	NS	-0.43 *
HPV	NS	-0.68 ***	0.59	NS	NS	NS	-0.57 **
BD	NS	NS	NS	NS	NS	NS	NS
FV	NS	-0.57 **	0.58	NS	NS	NS	-0.52 **
Sb	NS	NS	0.50	NS	NS	NS	NS
Sprouted							
PV	-0.76 ***	NS	NS	-0.69 ***	-0.49 *	-0.68 ***	-0.63 **
HPV	NS	NS	NS	NS	NS	NS	NS
BD	-0.80 ***	NS	NS	-0.73 ***	-0.55 **	-0.73 ***	-0.66 ***
FV	NS	NS	NS	NS	NS	NS	NS
Sb	-0.61 **	NS	NS	-0.57 **	-0.45 *	-0.55 **	-0.52 **
ΔD							
PV	NS	-0.66 ***	0.49	NS	NS	NS	NS
HPV	NS	-0.69 ***	0.58	NS	NS	NS	-0.57 **
BD	NS	NS	NS	NS	NS	NS	NS
FV	NS	-0.57 **	0.58	NS	NS	NS	-0.51 *
Sb	NS	NS	0.50	NS	NS	NS	NS

ΔD = PHS damaged wheat – sound wheat; PV: Peak Viscosity; BD: Breakdown Viscosity; HPV: Hot Paste Viscosity; Sb: Setback; FV: Final Viscosity.
*, **, ***: correlation coefficient is significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; NS, not significant.

HPSEC of Starch

HPSEC chromatography had been widely used to detect the fractions of starch because of its high efficiency recently. There were three peaks detected in the HPSEC chromatogram of starch in sound wheat sample (Figure 1.4): high molecular weight amylopectin (HMW-AP), low molecular weight amylopectin (LMW-AP), amylose (AM) (Simsek et al 2009).

Through comparison of HPSEC chromatography of sound and PHS damaged samples of four genotypes Hanna, Ingot, 99S0155-14W and Otis, it was concluded that sprouted samples had later retention time than sound wheat sample (for the sound sample, the retention time was around 21 min; while, for the PHS damaged wheat, the retention time was around 24 min); furthermore, PHS damaged wheat sample showed lower average molecular weight of HMW-AP than sound wheat sample. There was a molecular weight shift from HMW-AP to LMW-AP and AM, in the end shift to apparent amylose. Thus, the apparent amylose content seemed to increase because of PHS (Figure 1.5).

The average molecular weight of starch of sprouted samples decreased due to this shift in the Figure 1.5. This resulted in that low molecular weight starch fraction was higher with PHS damaged wheat samples. These results indicated that PHS can hydrolyze the HMW-AP and change it to LMW-AP and amylase. Furthermore, for the genotypes which had higher sprouting score, their retention time would be delayed more; and more HMW-AP had been changed to LMW-AP, which resulting in more change of starch properties.

Percent of starch fractions was shown in Table 1.10. There was a significant decrease in HMW-AP percentage for the sprouted samples of Hanna (61.5% to 31.6%), Ingot (60.6% to 34%), Otis (62.8% to 32.9%) and 99S0155-14W (64.5% to 28.9%);

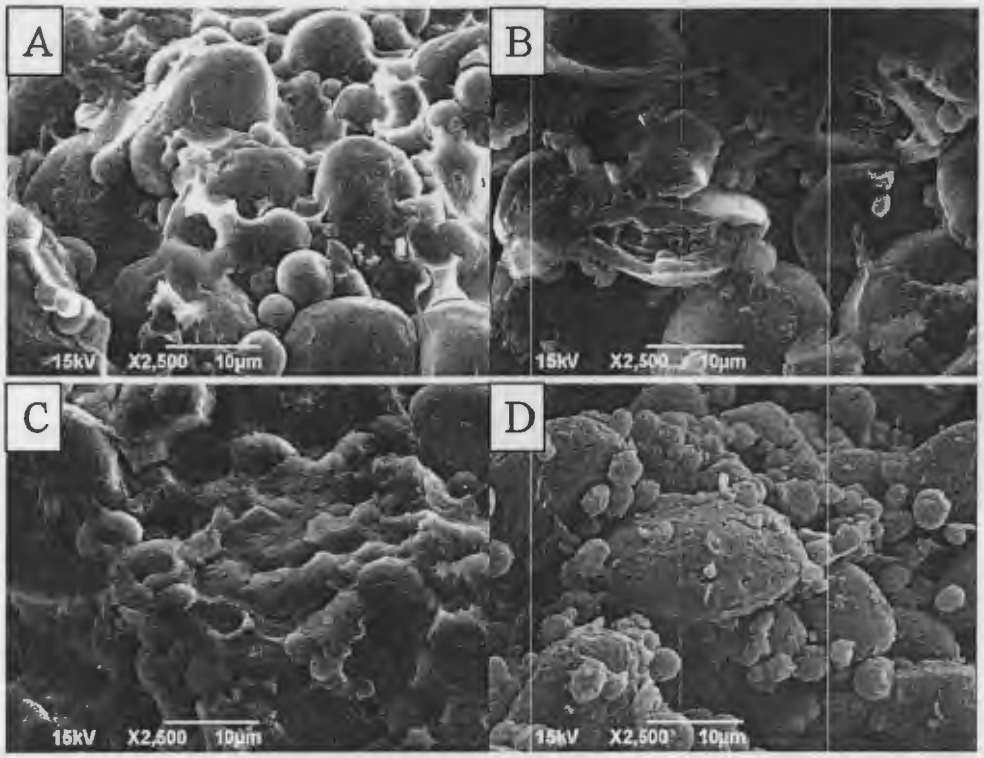

Figure 1.3 Scanning Electron Microscopy (SEM) images of starch from genotypes Steel-ND and Pristine

A: Sound1: Image from HRSW genotype Steel-ND sound sample; B: Sprouted 1: Image from HRSW genotype Steel-ND sprouted sample; C: Sound 2: Image from HWSW genotype Pristine sound sample; D: Sprouted 2: Image from HWSW genotype Pristine sprouted sample.

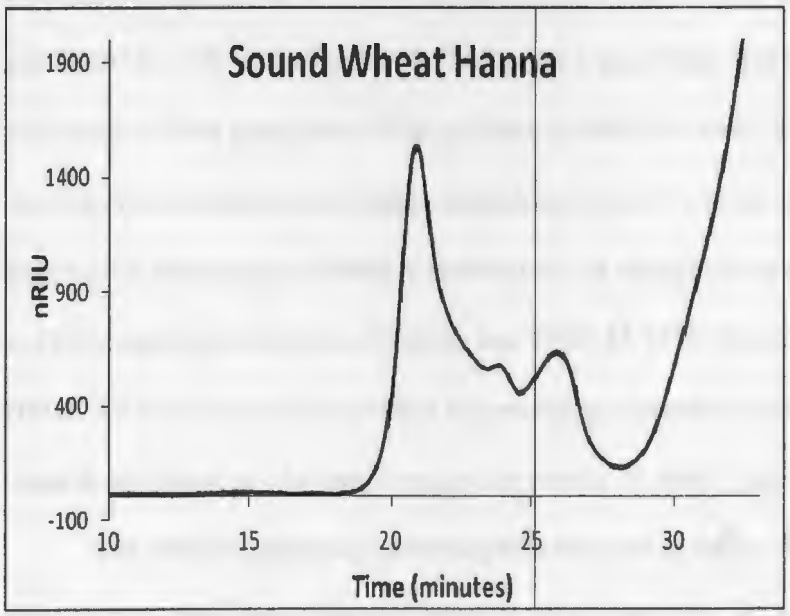

Figure 1.4 Typical HPSEC profiles of starch from genotype Hanna (sound)

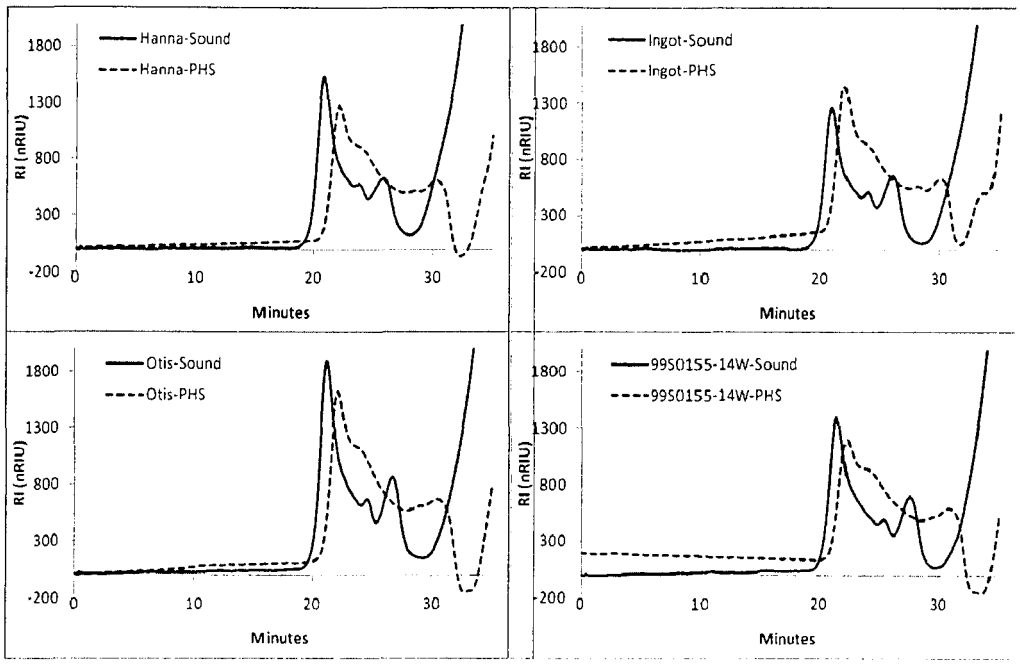

Figure 1.5 HPSEC profiles of sound and PHS damaged wheat samples of genotypes Hanna & Ingot (HRSW), Otis and 99S0155-14W (HWSW)

Dashed line represents the HPSEC chromatography of PHS sample; solid line represents the HPSEC chromatography of sound sample.

and there is a significant increase in LMW-AP percentage for the sprouted samples of genotypes Hanna (11.8% to 40.8%), Ingot (12.8% to 38.2%), Otis (11.1% to 37.5%) and 99S0155-14W (11% to 42.4%). There was a significant increase in the amylose percentage for the genotypes of Ingot, Otis and 99S0155-14W (PHS damaged). There was significant difference in apparent amylose content between sound and sprouted samples for the genotype Hanna. Furthermore, the change of percent of starch of Ingot and Otis was larger than that of Hanna and 99S0155-14W. This result indicated that the HMW-AP had been hydrolyzed and its percentage decreased due to PHS, which would result in the decrease of average molecular weight of starch during PHS.

The PHS damaged wheat had significantly lower molecular weight for the HMW-AP for genotypes Hanna (1.68x107 Da to 0.95x107 Da) Ingot (1.57x107 Da to 0.10x107 Da), 99S0155-14W (1.26x107 Da to 0.92x107 Da) and Otis (1.41x107 Da to

Table 1.10 Percent of starch fractions of HMW-AP, LMW-AP and AM from genotypes Hanna, Ingot, Otis and 99S0155-14W in sound and PHS damaged wheat, and the ΔD

	Sample	HMW-AP(%)	LMW-AP(%)	AM (%)
Sound	Hanna	61.5	11.8	26.8
	Ingot	60.6	12.8	26.6
	Otis	62.8	11.1	26.1
	99S	64.5	11.0	24.6
	LSD	1.2	1.1	0.1
Sprouted	Hanna	31.6	40.8	27.5
	Ingot	34.0	38.2	27.8
	Otis	32.9	37.5	29.7
	99S	28.9	42.2	28.9
	LSD	0.8	1.7	1.1
ΔD	Hanna	29.9	29.0	0.7
	Ingot	26.6	25.4	1.2
	Otis	29.9	26.4	3.6
	99S	35.6	31.2	4.3
	LSD	1.5	2.0	1.1

HMW: High Molecular Weight; LMW: Low Molecular Weight;
AP: Amylopectin; AM: Amylose;
99S: genotype 99S0155-14W.
LSD: least significant difference (α= 0.05), respectively.

$0.95x10^7$ Da) (Table 1.11). The molecular weight of the LMW-AP seemed to increase for all of these four genotypes Hanna, Ingot, 99S0155-14W and Otis. However, the LMW-AP in the PHS damaged wheat was a portion of what was HMW-AP in the sound wheat. The LMW-AP in the sound wheat had combined with the apparent amylose fraction. The average molecular weight of the apparent HMW-AP had decreased, respectively. Ingot and Otis had very high sprouting score, while, Hanna and 99S0155-14W had very low sprouting score. Furthermore, the change of molecular weight of genotype Ingot and Otis was larger than that of Hanna and 99S0155-14W. Based on the changes in relative molecular weight of starch, it was concluded that starch had been hydrolyzed during PHS due to the increased α-amylase activity. Some portion of HMW-AP changed into LMW-AP and amylase.

Table 1.11 Molecular Weight Distribution (MWD) of HMW-AP, LMW-AP and AM from genotypes Hanna and Ingot (HRSW), Otis and 99S0155-14W (HWSW)

	Sample	HMW-AP (Da)	LMW-AP (Da)	AM (Da)
Sound	Hanna	$1.68x10^7$	$4.61x10^6$	$1.82x10^6$
	Ingot	$1.57x10^7$	$4.08x10^6$	$1.60x10^6$
	Otis	$1.41x10^7$	$2.99x10^6$	$1.15x10^6$
	99S	$1.26x10^7$	$2.10x10^6$	$0.80x10^6$
	LSD	$1.03x10^6$	$6.25x10^5$	$1.71x10^5$
Sprouted	Hanna	$0.95x10^7$	$5.21x10^6$	$0.24x10^6$
	Ingot	$1.01x10^7$	$5.46x10^6$	$0.27x10^6$
	Otis	$0.95x10^7$	$4.90x10^6$	$0.24x10^6$
	99S	$0.92x10^7$	$4.88x10^6$	$0.19x10^6$
	LSD	$7.60x10^4$	$1.89x10^5$	$7.71x10^3$
ΔD	Hanna	$0.73x10^7$	$0.60x10^6$	$1.58x10^6$
	Ingot	$0.56x10^7$	$1.38x10^6$	$1.33x10^6$
	Otis	$0.46x10^7$	$2.09x10^6$	$0.91x10^6$
	99S	$0.34x10^7$	$2.78x10^6$	$0.61x10^6$
	LSD	$1.01x10^6$	$4.80x10^5$	$1.66x10^5$

HMW-AP: High Molecular Weight Amylopectin; LMW-AP: Low Molecular Weight Amylopectin; AM: amylase. 99S: genotype 99S0155-14W.

Conclusion

PHS damaged wheat kernels exhibited different physical characteristics with sound wheat kernels. There were black areas in the PHS damaged wheat kernels that were not found on the sound wheat kernels. The embryo of the PHS damaged wheat started to grow while still on the spike. As the PHS damage of the kernels continued, some of the kernels exhibited radicle emergence from the kernel.

PHS increased both α-amylase and endo-protease activity, resulting in the hydrolysis of starch and protein molecules. Genotypes exhibited significantly different α-amylase and endo-protease activity, which had positively correlation with sprouting score. Subsequently, genotypes showed significantly different degrees of starch and protein degradation. Significant difference existed between HRSW and HWSW

genotypes for the α-amylase and endo-protease activity, and the mean values of α-amylase and endo-protease activity in HRSW genotypes were lower than that in HWSW genotypes. The results suggested that the HRSW genotypes in general exhibited lower susceptibility to PHS than HWSW genotypes. However, some HWSW genotypes still showed as low of susceptibility to PHS as HRSW genotypes, such as the genotypes AC Snowbird and 99S0155-14W.

The increase in α-amylase activity has significant impacts on the physical and chemical properties of starch in PHS damaged wheat. The hydrolysis of starch by α-amylase activity can cause changes to granule morphology, starch molecular weight and functional properties of the wheat starch. SEM was employed to observe the starch morphology in sound and PHS damaged wheat kernels. In sound wheat kernels, the starch granules had a smooth surface free from pitting and were embedded in very dense protein matrix. However, in the PHS damaged wheat kernels, the starch granules had been hydrolyzed by the increased α-amylase activity resulting in holes and pits in the granule surface. Also, the protein matrix was absent in the PHS damaged wheat due to the attack endo-protease activity. The molecular weight distribution of starch was determined using HPSEC. PHS damaged wheat had later retention times and lower average molecular weights of HMW-AP. While, the average molecular weight of LMW-AP and AM increased. These results indicated that the HMW-AP has been hydrolyzed and appear as increased LMW-AP and AM. The increased α-amylase activity hydrolyzed the starch granules and changed the pasting properties of starch during in the PHS damaged samples. Varietal difference existed for the pasting properties; genotypes with higher sprouting score exhibited lower peak viscosity, breakdown viscosity and final viscosity. There were negatively significant correlations between α-amylase activity and the pasting viscosity in PHS damaged wheat.

In summary, PHS increased both α-amylase and endo-protease activity, resulting in the degradation of starch and protein molecules. Furthermore, the pasting properties, starch morphology and starch molecular weight distribution were changed due to PHS. Thus, PHS changed the physicochemical properties of starch in wheat.

References Cited

AACC. 2000. Approved methods of the AACC (10th ed.). St. Paul, MN: American Association of Cereal Chemists.

Bean, M. M., Keagy, P. M., Fullington, J. G., Jone, F. T., and Mecham, D. K. 1974. Dried Japanese noodles. I. Properties of laboratory prepared noodle doughs from sound and damaged wheat flours. Cereal Chem. 51: 416.

Cornell, H. 2004. The functionality of wheat starch. Starch in Food. Pages 221-238: A.C. Eliasson. Cambrige, UK: Woodhead Publishing Limited.

Dronzek, B. L., Hwang, P., and Bushuk, W. 1972. Scaning electron microscopy of starch from sprouted wheat. Cereal Chem. 49: 232.

Grant, L. A., Ostenson, A. M., and Rayas-Duarte, P. 2002. Determination of amylose and amylopectin of wheat starch using high performance size-exclusion chromatography (HPSEC). Cereal Chem. 79: 771-773.

Groos, C., Gay, G., Perretant, M-R., Gervais, L., Bernard, M., Dedryver, F., and Charmet, G., 2002. Study of the relationship between pre-harvest sprouting and grain color by quantitative trait loci analysis in white x red grain bread wheat cross. Theor. Appl. Genet.104: 39-47.

Huàng, G. R. 1979. A study of alpha amylase activity in Kansas hard white wheats. Master's thesis. Kansas State University. Manhattan.

Hwang, P. and Bushuk, W. 1973. Some changes in the endosperm proteins during sprouting of wheat. Cereal Chem. 50:147.

Jane, J. and Chen, J. 1992. Effects of amylase molecular size and maylopectin branch chain length on paste properties of starch. Cereal Chem. 69: 60-65.

Kruger, J. E. 1980. Progress in chemistry of some quality-affecting enzymes resulting from pre-harvest sprout damage. Cereal Res. Comm. 8: 39-47.

Lorenz, K., and Kulp, K. 1981. Sprouting of cereal grains. Effects on starch characteristics. Staerke 33: 183-216.

MacGregor, A.W. and Matsuo, R.R. 1972. Starch degradation in endosperm of barley and wheat kernels during initial stages of germination. Cereal Chem. 59: 210–216.

Morad, M. M. and Rubenthaler, G. L. 1983. Germination of soft white wheat and its effect on flour fractions, breadmaking , and crumb firmness. Cereal Chem. 60: 413-417.

Simsek, S. and Ohm, J. B. 2009. Structural changes of arabinoxylans in refrigerated dough. Carbohydrate Polymers. 77: 87-94.

Sorenson, B. and Wiersma, J. 2004. Sprout damaged wheat, crop insurance, and quality concerns. Minnesota Crop New Archive. University of Minnesota.

Tsai, M. L., Li, C. F., and Lii, C. Y. 1997. Effects of granular structures on the pasting behaviors of starches. Cereal Chemistry. 74: 750-757.

Wahl, T.I. and O'Rourke, A.D. 1992. The economics of sprout damage in wheat. Pages 10-17 in: Pre-harvest Sprouting in Cereals. M. K.Walker-Simmons and J. L. Ried, eds. AACC International Press.

Whistler, R. L. and BeMiller, J. N. 1997. Starch. Pages 117-152 in: Carbohydrate Chemistry for Food Scientists. R.L. Whistler, & J. N. BeMiller, eds. American Association of Cereal Chemists. St. Paul, MN, USA.

PAPER 2. PHYSICOCHEMICAL CHANGES OF PROTEIN IN PHS DAMAGED WHEAT SAMPLES

Abstract

Rainfall before harvest can cause the sprouting of the wheat kernel, which is termed as Pre-harvest Sprouting (PHS). The aim of this study was to examine the physicochemical properties of protein in PHS damaged Hard Red Spring Wheat (HRSW) and Hard White Spring Wheat (HWSW). Results showed that protein content of sprouted wheat samples were lower than that of sound wheat samples and did not show significant correlation with other parameters. Protein content of Carrington of PHS sprouted wheat had decreased up to 12.50% compared to sound wheat. Increased endo-protease activity due to Pre-Harvest Sprouting (PHS) resulted in the degradation of protein. Molecular Weight Distribution (MWD) was analyzed by High Performance Size Exclusion Chromatography (HPSEC). SDS buffer extractable proteins (EXP) and un-extractable proteins (UNP) were obtained. EXP showed significant and positive correlation with sprouting score (correlation coefficient (r) = 0.57) and endo-protease activity (r = 0.79). UNP showed significant and negative correlation with sprouting score (r = - 0.56) and endo-protease activity (r = -0.78). Endo-protease activity (r = 0.78) showed higher correlation than sprouting score (r = 0.57) with protein degradation detected by HPSEC, and can better represent degree of degradation of protein.

Introduction

Pre-harvest sprouting (PHS) is the germination of wheat kernels before harvest so that the embryo begins to grow while still on the head in the field. PHS happens when wet conditions like rainfall postpone harvest (Groos et al 2002). PHS damage can be classified in terms of its severity with a continuum from very minor to severe. PHS

damage can be measured by percentage of sprouted wheat kernels, starch degradation, or other indicators (Thomas et al 1992).

Protein is one of the crucial criteria of wheat quality and is possibly the most important factor in bread flour quality. Protein quality is commonly believed by processors to be well correlated with dough strength, with baking quality being the ultimate test. From a chemical point of view, wheat proteins can be separated into two groups: the soluble proteins and the insoluble gluten proteins. The soluble groups are made up of albumins, globulins, and peptides, and can dissolve in the natural aqueous mediums. The insoluble gluten proteins consist of glutenins and gliadins which represent 80-85 % of the wheat storage protein. During the mixing procedure in bread-making process, they can form gluten, which is believed to be primarily responsible for unique viscoelastic and gas-retaining properties of dough. Gluten protein plays a key role in determining the quality of wheat flour and its end products.

The physiological changes needed to produce a new plant require energy and nutrients, which is the reason why the PHS damaged wheat needs to produce enzymes to breakdown starch (amylases), oil (lipases) and protein (proteases). The impact of PHS wheat on foods depends on the amount of enzymes present and breakdown of the kernel (Sorenson et al 2004). Due to high endo-protease activity in PHS damaged wheat, protein is degraded, which is another important reason for economic loss due to PHS damage. Beresh (1969) and Redman et al (1971) offer evidence that the rapid softening of gluten washed from flour milled from grist that included small amount of PHS damaged wheat is because of proteolytic hydrolysis of the gluten proteins. Endo-protease activity is generally related to increased α-amylase activity during PHS; excessive endo-protease activity has a negative effect on dough handling and baking properties. The presence of α-amylase and endo-protease is also undesirable for noodle

production. Endo-protease can lead to deterioration of protein and stretching of noodles during drying and poor cooked texture (Khan et al 2009). PHS has become a main constraint to the production of high quality cereal end products; for example, bread and sponge cake prepared from PHS damaged wheat display undesirable quality characteristics (Thomas et al 1992).

There have been limited researches conducted in the area about the physicochemical changes of protein due to PHS in PHS damaged wheat, especially about the changes of protein molecular weight distribution (MWD). The MWD of wheat proteins is recognized as the main factor to determine the physical properties of dough. There are two main ways to vary the MWD of wheat proteins: one is the ratio of monomeric-to-polymeric, the other one is the MWD of polymeric protein. Both of two factors are genetically controlled but can be affected by environmental conditions such as PHS (Southan et al 1999). The changes of MWD of proteins can significantly affect the end product quality such as sponge cake and bread (Thomas et al 1992). The aim of the present work was to determinate the change of physicochemical properties of protein in PHS damaged wheat samples.

Materials and Methods

Materials

Wheat samples were kindly provided by Mr. Mory Rugg and Dr. Mergoum at the Department of Plant Science, North Dakota State University (Table 1.1). 24 genotypes were grown at three locations (Casselton, Carrington, and Prosper, ND) in 2008. Randomized complete block design with four replications was utilized. Wheat samples consist of 12 Hard Red Spring Wheat (HRSW) genotypes and 12 Hard White Spring Wheat (HWSW) genotypes that are adapted to the U.S. Spring Wheat region (Table 1.1). Wheat samples harvested from two replications were combined together

and treated as a block. In this research, both sound and PHS damaged wheat samples were analyzed. Thus, a total of 288 samples were analyzed in the present research.

Wheat samples were evaluated and scored for susceptibility to PHS by Mr. Mory Rugg at the Department of Plant Science, North Dakota State University. These samples were subjected to the following procedure, at plant physiological maturity, 30 wheat spikes were randomly harvested from each experiment unit. The spikes were immediately stored at 10°C to inhibit additional α-amylase activity and placed in a mist chamber and misted for a period of 48 h. Following the misting, a humidifier was placed in the chamber for 3 days. Visual observations of the spike were made, and spikes were rated for germination using a 1 to 10 scale, with 1 having no visual germination and 10 having nearly 100% of the seed in the spike exhibiting germination. Finally, degree of sprouting induced by artificial wet conditions was scored visually 0-9. Score of 0 represents no visible sprouting and score of 9 represents very severe sprouting with average coleoptiles length greater than 2 cm. (Mr. Mory Rugg et al, non-published data)

Protein Content

All the wheat samples were ground in a cyclone sample mill (Udy, Fort Collins, CO) with a 1-mm sieve at the NDSU-HRSW quality laboratories. Nitrogen content (14%, moisture basis) of each sample was determined by the combustion method (Approved Method 46-30, AACC International 2004) using a LECO FP428 nitrogen analyzer (LECO Corporation, St. Joseph Michigan). The calculation "P=N * 5.7" was used to convert nitrogen content to protein content.

Endo-protease Activity

Endo-protease activity was determined using an Azurine-crosslinked casein substrate (Protazyme AK tablet; Megazyme Co., Ltd). The method of Yasunori (2001)

about the endo-protease activity was used with some modification. 0.50 g of sample was weighted and added into a test tube. Extraction buffer (5.0 mL, 100 mM sodium phosphate buffer, pH 7.0) was added and stirred at room temperature for 30 min. Sample was centrifuged at 2000 rpm for 10 min. One tablet was added into 1.0 mL of reaction buffer (100 mM, pH 6.9, sodium phosphate buffer with 1% SDS (w/v)) and stirred at 40°C for 5 min. 1.0 mL of enzyme extract was added and stirred at 40°C for 2 h. 10 mL of 2 % Trisodium Phosphate was added to terminate the reaction and filtered through qualitative filter paper into Hach spectrophotometer tubes. The absorbance (590 nm) of the filtrates was measured using Hach spectrophotometer. One unit of enzyme activity was defined as the change in absorbance for 1 h per 1 g of sample (Yasunori et al 2001).

High Performance Size Exclusion Chromatography (HPSEC) Procedure

Proteins of samples were extracted following the method of Gupta (1993) with minor modification (Ohm et al 2006). SDS extractable proteins (EXP) and un-extractable proteins (UNP) were obtained based on the procedure of Gupta (1993). Extraction buffer was 0.5 % SDS and 0.1M sodium phosphate buffer (pH 6.9). Flour (10 mg, 14% moisture basis) was suspended in 1 mL of extraction buffer and stirred for 5 min at 2,000 rpm using a pulsing vortex mixer (Fisher Scientific) to solubilize EXP. Then the mixture was centrifuged for 15 min at 17,000 × g (Eppendorf Centrifuge 5424). The supernatant was filtered through a 0.45 μm PVDF membrane (Sun Sri, Rockwood, TN). After filtering, the sample was immediately heated for 2 min at 80°C to kill endo-protease activity (Larroque et al 2000). The UNP was solubilized from the residue by 30 sec of sonication in 1 mL of extraction buffer solution with the power setting of 10W output (Sonic Dismembrator 100, Fisher Scientific). The mixture was centrifuged and filtered, and the filtered solution was heated as the same method with

EXP. HPSEC was performed using an Agilent 1100 Series chromatographer (Agilent Technologies, Santa Clara, CA). The 10 μL of EXP and UNP were separated by a narrow-bore size exclusion column (BIOSEP SEC S4000, Phenomenex, 300 × 4.5 mm, Torrance, CA) with guard cartridges (BIOSEP SEC S4000) (Batey et al 1991; Ohm et al 2009b). Proteins were eluted by 50 % acetonitrile in water with 0.1 % trifluroacetic acid at a flow rate of 0.5 mL/min and determined at 214 nm using a photodiode array detector (1200, Agilent Technologies, Santa Clara, CA). These experiments were duplicated and the mean values were used for data analyses.

HPSEC Data Collection

Absorbance data from HPSEC of protein extracts was analyzed using MATLAB 2008 program (MATLAB 2008, The MathWorks, Natick, MA) (Ohm et al 2006). Absorbance values were interrupted to 0.002 min intervals by a split method in MATLAB. Absorbance Area (AA) was calculated by mean absorbance by time interval of 0.002 min using the interrupted absorbance values. Data collection was performed using the sum of AA for each retention time interval of 0.01 min between 3.6 and 9 min of run time. The AA values for total proteins were mathematically estimated by adding AA values of EXP and UNP (Ohm et al 2009). Absorbance area percentage (A%) values were calculated for each retention interval of 0.01 min over the total AA (Ohm et al 2006). Simple Linear correlation coefficients (r) were calculated between wheat parameters and A% values, and presented as a continuous spectrum over retention time.

HPSEC profiles were divided into six fractions: F1 (3.6–5.0 min), F2 (5.0–6.0 min), F3 (6.0–6.9 min), F4 (6.9–7.6 min), F5 (7.6–8.2 min) and F6 (8.2–9.0 min) (Morel et al 2000; Samson et al 2006; Ohm et al 2009b). Larroque et al (1997) showed electrophoresis patterns of protein fractions separated by HPSEC. Primary components of each fraction were high molecular weight polymeric protein for F1; low molecular

weight polymeric proteins for F2; gliadins for F3; albumin and globulins for F4; and hydrolyzed polymeric protein for F5 & F6 (Larroque et al 1997; Morel et al 2000; Samson et al 2005).

Statistical Analysis

Statistic analysis was performed using the SAS System for Windows (V. 9.2, SAS Institute, Cary, NC). Bartlett's test was used to analyze the homogeneity of error variance across the three locations. When errors were homogenous, analysis of variance (ANOVA) was performed using the "Mixed" procedure in SAS assuming location as a random effect and genotype as a fixed effect. The difference between red and white means was analyzed using the "Contrast" option. The error variances of protein content across the samples of three locations were heterogeneous, thus, protein content of three locations were analyzed separately. Correlation coefficient was calculated across genotype means using the "Corr" procedure in SAS except for protein content.

Results and Discussion

Change in Protein Content

Protein is considered one of the key flour components, although it is not the single factor affecting the end product quality of wheat flour (Finney 1943; Khan and Bushuk 1979). High protein content is commonly related to increased dough strength and improved baking quality of the wheat (Johansson et al 2001). Quantitative and qualitative changes of protein in the wheat endosperm occurred during PHS (Hwang et al 1973). In the present study, nitrogen content was converted into protein content.

Based on Bartlett's test, variances across the samples of three locations were heterogeneous for protein content, thus, the analysis of variance was calculated separately for the three locations. Protein content of sound and PHS damaged wheat in

three locations Carrington, Casselton and Prosper was shown in Table 2.1. The mean values of protein content of sound wheat and PHS damaged wheat in Carrington were 13.74 % and 12.51 %, respectively; the least significant difference (LSD) value of PHS damaged wheat was 1.00. The protein content of PHS damaged wheat had decreased by 7.13% compared to sound wheat. Genotypes MT9420 and 99S0155-14W (sprouted) had the lowest protein content of 10.65 % and 10.89 %; while, genotypes Alsen and Granite (sprouted) had the highest protein content of 13.67 % and 14.25 %, respectively. The mean value of protein content of sound wheat and PHS damaged wheat in Casselton were 13.93 % and 12.57 %; the LSD value of PHS damaged wheat was 1.20. The mean value of protein content of PHS damaged wheat had decreased up to 9.76% compared to sound wheat. Genotypes MT9420 & 99S0155-14W (sprouted) had the lowest protein content of 11.11 % and 11.38 %; while, Granite (sprouted) has the highest protein content of 14.65 %. For Prosper, the mean values of protein content of sound wheat and PHS damaged wheat were 13.83 % and 12.32 %; the LSD value of PHS damaged wheat was 0.70. The mean value of protein content of PHS damaged wheat had decreased up to 10.92 % compared to sound wheat. Genotypes of MT9420 & 99S0155-14W (sprouted) had the lowest protein content of 11.54 % and 11.09 %; while, Glenn (sprouted) had the highest protein content of 13.65 %. For all the three locations, the protein content of sound wheat was always higher than PHS damaged wheat; however, the mean value of protein content of PHS damaged wheat of Carrington had the most decrease compared to the other two locations of Casselton and Prosper. This result was different with the result of Yasunori et al (2001) who reported that germination did not affect the protein content in any of the genotypes in their research. Perhaps the decrease of protein content was due to the removal of shoot and root growing during PHS in wheat cleaning stage.

Protein content of sound wheat was significantly different among different genotypes ($P < 0.001$) and replications ($P < 0.05$). There was strongly significant difference between HRSW and HWSW genotypes for protein content of sound wheat for all the three locations (Carrington, Casselton, and Prosper) ($P < 0.001$). These data explained that varietal difference existed in the protein content of sound wheat. Protein content was significantly different among different genotypes for PHS damaged wheat ($P < 0.01$). There was significant difference between HRSW & HWSW genotypes for the protein content of PHS damaged wheat ($P < 0.01$). However, there was no significant difference in replications of protein content in PHS damaged wheat for Carrington and Casselton. Genotypes had significant ($P < 0.05$) variation in the difference between sound and PHS damaged wheat samples ($\triangle D$) of protein content, but it was not as significant as the variation in protein content of sound wheat genotypes ($P < 0.001$). $\triangle D$ of protein content was significantly different between HRSW and HWSW genotypes for Carrington and Casselton ($P < 0.01$); however, HRSW and HWSW did not show significant difference for $\triangle D$ of protein content for genotypes in Prosper. There was no significant difference among replications for $\triangle D$ of protein content. These results suggested that different genotypes had significantly different protein content for both sound and PHS damaged samples ($P < 0.01$). $\triangle D$ of protein content was significantly different among different genotypes, which indicated that some genotypes suffered more protein content loss because of PHS damage. High protein content represents better baking quality of wheat flour, thus, these results indicated that genotypes which had high protein content of sound and PHS damaged wheat and had lower susceptibility can be segregated such as genotype Granite. Samples of sound and PHS damaged wheat for HRSW and HWSW genotype showed

significant difference of protein content. The mean value of protein content of HRSW genotypes of sound and PHS damaged wheat was larger than that of HWSW genotypes of sound and PHS damaged wheat. However, some HWSW genotypes such as AC Snowbird and 99S0155-14W had high protein content as HRSW genotypes. (see Appendix Table A.6)

Correlations between protein content and other parameters were shown in Table 2.2. Protein content of sound and PHS damaged sample, and △D, did not show significant correlation with most of the parameters such as enzyme activities and pasting profiles in the present research. However, there were significant and positive correlations between protein content of sound wheat and PHS damaged wheat in all three locations ($P < 0.001$). Genotypes with high protein content in sound wheat would have high nitrogen content in PHS damaged wheat. There were significant and negative correlations between endo-protease activity of sprouted samples and protein content of sound wheat at Carrington ($r = -0.52$) and Prosper ($r = -0.45$). This indicated that genotypes with low protein content in sound wheat would have high endo-protease activity at Carrington and Prosper in 2008. For Casselton, there was significant negative correlation between △D of protein content between sprouted and sound samples and endo-protease activity of sprouted samples ($r = -0.45$). The pasting parameters of PHS damaged wheat did not show any correlation with protein content of sound and PHS damaged wheat in all three locations. Enzyme activities of sound wheat did not show have any correlation with protein content of sound and PHS damaged wheat in all three locations. These results indicated that the protein content of sound and PHS damaged wheat cannot be an effective indicator of the susceptibility of PHS of a genotype.

Change in Molecular Weight Distribution

HPSEC has been extensively conducted to analyze molecular weight

Table 2.1 Protein contents of 24 genotypes of sound and PHS damaged wheat in three locations (Carrington, Casselton, and Prosper)

Genotype	Sprouting	Sound			Sprouted			ΔD		
	Score	Carrington	Casselton	Prosper	Carrington	Casselton	Prosper	Carrington	Casselton	Prosper
HRSW										
Alsen	4.8	14.63	14.68	14.26	13.67	12.12	12.78	0.16	0.44	0.25
Briggs	5.7	13.97	14.67	14.10	12.99	13.12	12.45	0.17	0.27	0.28
Freyr	4.4	13.45	14.10	14.14	12.19	12.44	12.56	0.22	0.28	0.27
Glenn	4.0	14.51	14.94	14.58	13.21	13.59	13.65	0.22	0.23	0.16
Granite	5.3	15.13	16.00	14.67	14.25	14.65	12.78	0.15	0.23	0.32
Hanna	2.8	14.24	13.42	13.76	13.26	11.63	12.71	0.17	0.31	0.18
Ingot	7.0	14.05	13.86	14.29	12.71	12.20	12.71	0.23	0.28	0.27
Kelby	3.4	13.94	14.15	14.80	12.86	13.19	13.29	0.19	0.17	0.26
Norpro	6.0	13.65	14.45	14.68	12.38	13.35	13.13	0.22	0.19	0.27
Reeder	4.4	14.32	14.74	14.03	13.31	12.89	12.42	0.17	0.32	0.28
Steele-ND	5.0	14.18	14.44	14.58	13.08	13.47	13.56	0.19	0.17	0.17
Knudson	5.4	13.07	14.42	13.08	12.62	12.09	11.96	0.08	0.40	0.19
Mean	4.8	14.09	14.49	14.25	13.04	12.89	12.83	0.18	0.27	0.24
LSD	1.7	0.50	1.20	0.70	1.00	1.70	0.60	0.15	0.20	0.14

Table 2.1 Protein contents of 24 genotypes of sound and PHS damaged wheat in three locations (Carrington, Casselton, and Prosper) (Continued)

Genotype	Sprouting	Sound			Sprouted			ΔD		
	Score	Carrington	Casselton	Prosper	Carrington	Casselton	Prosper	Carrington	Casselton	Prosper
HWSW										
AC Snowbird	2.8	14.44	14.91	14.12	13.46	13.00	12.96	0.17	0.33	0.20
AC Vista	5.8	12.94	12.60	12.40	11.37	11.62	11.25	0.27	0.17	0.20
Argent	4.8	14.45	15.21	15.09	13.77	14.28	13.48	0.12	0.16	0.28
CS3100L	6.8	13.12	11.89	12.82	11.69	10.97	10.98	0.25	0.16	0.32
CS3100Q	6.8	13.04	13.72	13.48	12.05	12.71	11.98	0.17	0.17	0.26
Explorer	6.9	13.79	14.05	13.69	12.69	12.09	12.06	0.19	0.34	0.28
Lolo	5.7	12.90	12.35	12.55	11.58	11.31	11.54	0.23	0.18	0.17
MT9420	6.9	12.63	12.44	12.84	10.65	11.11	10.86	0.34	0.23	0.34
NDSW0602	6.3	13.93	13.44	14.03	11.14	13.27	11.70	0.48	0.03	0.40
Otis	7.8	12.73	12.46	12.63	12.17	12.26	12.16	0.10	0.04	0.08
Pristine	5.0	13.40	14.15	13.50	12.26	12.90	11.97	0.20	0.21	0.26
99S0155-14W	2.5	13.17	13.15	13.78	10.89	11.38	11.09	0.39	0.30	0.46
Mean	5.7	13.38	13.36	13.41	11.97	12.24	11.83	0.24	0.19	0.27
LSD	1.7	0.50	1.20	0.70	1.00	1.70	0.60	0.15	0.20	0.14

LSD: least significant difference ($\alpha = 0.05$).
△D: difference between sound and PHS damaged wheat.

distribution of proteins (Bietz, 1984; Ohm et al 2008). The analysis of wheat proteins using HPSEC chromatogram exhibited six main protein fractions corresponding to molecular weights (F1-F6). The six fractions are: F1: high molecular weight polymeric protein (retention time of 3.6-5.0 min), F2: low molecular weight polymeric protein (5.0-6.0 min), F3: gliadins (6.0-6.9 min), F4: albumins and globulins (6.9-7.6 min), and F5 & F6: hydrolyzed polymeric protein (7.6-9.0 min) fractions. Gluten proteins are a heterogeneous class of a mixture of polymeric glutenin with molecular weight from 80 kDa to several million Da and monomeric gliadins having molecular weight ranging from 30 kDa to 80 kDa. Albumins and globulins belong to non-gluten proteins with molecular weight less than 25 kDa (Veraverbeke et al 2002). The UNP have a strong effect on dough strength parameters because of the larger associations between high molecular weight glutenin subunits (Gupta et al 1993). Researches with HPSEC of protein in wheat indicated that UNP could enhance dough strength; however, EXP fractions were associated with weak dough characteristics (Gupta et al 1993; Ohm et al 2009a; Toi et al 2010). HPSEC profiles of EXP and UNP obtained from sound and PHS damaged wheat were shown in Figure 2.1. When the retention time reached 6.9 min, area values of F4, F5 and F6 fractions of PHS damaged wheat was larger than that of sound wheat. This indicated that PHS damaged wheat had more EXP than sound samples. While at the earlier retention time, area value of F1 and F2 of UNP in sound samples was larger than that of sprouted samples. This indicated that sound samples had more UNP than sprouted samples. Through comparison of figure A and figure B in Figure 2.1, it can be observed that during PHS, some portion of UNP had shifted to EXP which has negative relation with bread making quality. This result was in agreement with the founding of Hwang et al (1973) who reported that solubility fractionation shows a marked decrease in the amount of insoluble residue protein, and

Table 2.2 Correlation between protein content and α-amylase activity, endo-protease activity and pasting viscosity

	Carrington					Casselton					Prosper				
	Sound		Sprouted		ΔD	Sound		Sprouted		ΔD	Sound		Sprouted		ΔD
Nitrogen Content															
Sprouted	0.84	***				0.83	***				0.83	***			
ΔD	NS		-0.71	***		NS		NS		1.00	NS		NS		
Sprout score	0.5	*	NS		NS	NS		NS		NS	NS		NS		NS
Sound															
Amylase	NS		NS		NS	NS		NS		NS	NS		NS		NS
Protease	NS		NS		NS	NS		NS		NS	NS		NS		NS
Sprouted															
Amylase	NS		NS		NS	NS		NS		NS	NS		NS		NS
Protease	-0.52	*	-0.41	*	NS	NS		NS		-0.45	*	-0.45	*	NS	NS
ΔD															
Amylase	NS		NS		NS	NS		NS		-0.42	*	NS		NS	NS
Protease	-0.43	*	NS		NS	NS		NS		-0.51	*	-0.45	*	NS	NS

Table 2.2 Correlation between protein content and α-amylase activity, endo-protease activity and pasting viscosity (Continued)

	Carrington			Casselton			Prosper		
	Sound	Sprouted	ΔD	Sound	Sprouted	ΔD	Sound	Sprouted	ΔD
Sound RVA									
PV	NS	NS	NS	NS	-0.45 *	NS	NS	NS	NS
BD	NS	NS	NS	NS	NS	0.42*	NS	NS	NS
HPV	-0.57**	-0.49*	NS	-0.52**	NS	NS	NS	-0.43*	NS
Sb	NS	NS	NS	NS	NS	NS	NS	NS	NS
FV	-0.45	NS	NS	-0.53**	NS	NS	NS	NS	NS
Sprouted RVA									
PV	NS	NS	NS	NS	NS	NS	NS	NS	NS
BD	NS	NS	NS	NS	NS	NS	NS	NS	NS
HPV	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sb	NS	NS	NS	NS	NS	NS	NS	NS	NS
FV	NS	NS	NS	NS	NS	NS	NS	NS	NS

ΔD: difference between sound and PHS damaged wheat.
RVA: Rapid Visco Analyzer. PV: peak viscosity, BD: breakdown viscosity, HPV: hot paste viscosity, Sb: setback viscosity, FV; final viscosity.
*, **, ***: correlation coefficient is significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; NS, not significant.

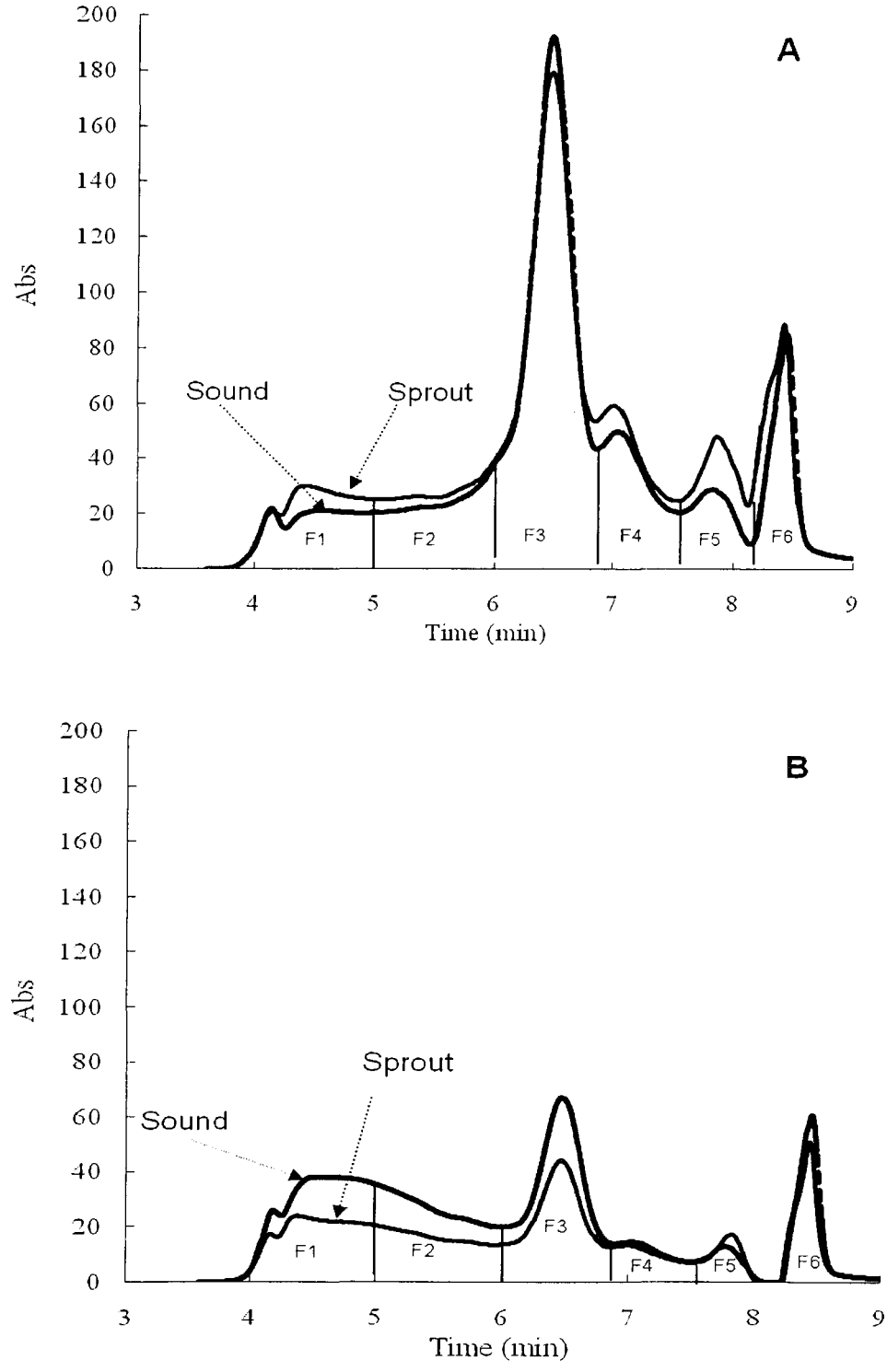

Figure 2.1 Typical HPSEC profiles of (A) SDS buffer EXP and (B) SDS buffer UNP from sound and PHS damaged wheat

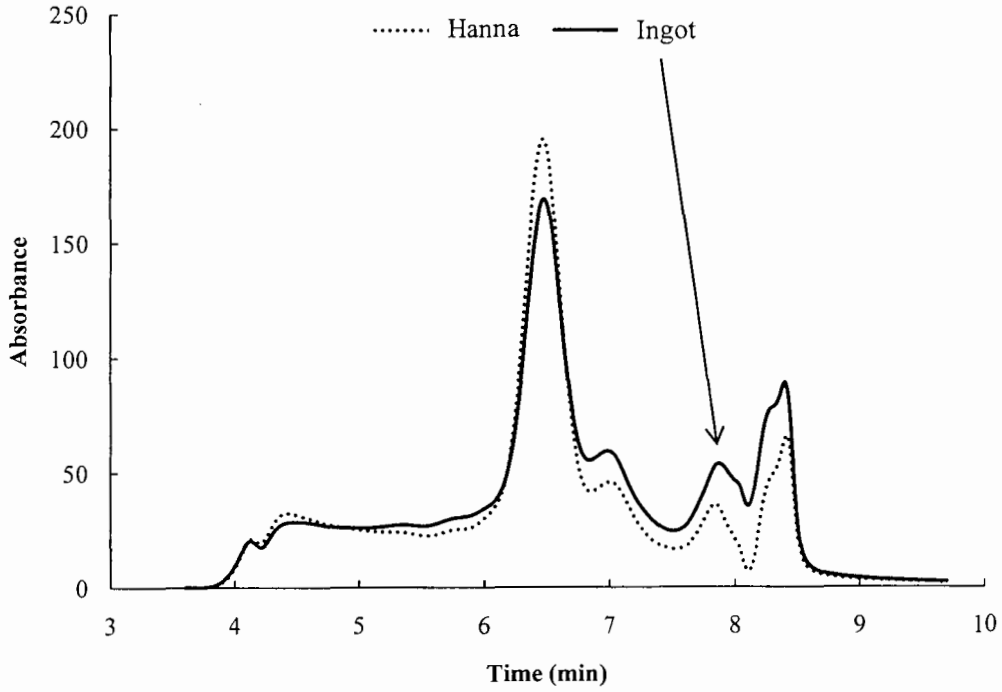

Figure 2.2 HPSEC profiles of total proteins extracted from Hanna and Ingot (Sprouted)

ANOVA for HPSEC A% of SDS buffer UNP was shown in Table 2.4. There were strongly significant difference of HPSEC A% of UNP among different genotypes of PHS damaged wheat ($P < 0.001$). There were significant differences among different genotypes of HPSEC A% of UNP in sound wheat and the difference of HPSEC A% between sound and PHS damaged wheat except the fractions of U5 ($P < 0.05$). These results indicated that different genotypes of PHS damaged wheat exhibited significantly different HPSEC profiles of UNP and the degree of change of HPSEC chromatogram due to PHS of different genotypes was significantly different. There was significant difference of HPSEC A% of UNP among different locations of fraction U1, U2 and U3 in PHS damaged wheat ($P < 0.001$), which indicated that locations played a role on PHS and the fraction U1, U2 and U3 in HPSEC A% of SDS buffer UNP of PHS

damaged wheat. There was no significant difference between three locations for HPSEC profiles of UNP in sound wheat, which indicated that the three locations did not have effect on the HPSEC profiles of sound wheat. There was no significant difference between three locations for the difference of HPSEC A% of UNP between sound and PHS damaged wheat except fraction of U1, indicating that locations did not affect the change of HPSEC profiles of UNP except fraction of U1. There was significant difference between interactions of genotypes and locations for fractions of U1, U2 and U3 of UNP in PHS damaged samples. The difference of HPSEC profiles of sound wheat between interactions of genotypes and locations was not significant. There was no significant difference between interactions of genotypes and locations for the HPSEC profiles of ΔD except fraction of U1. The correlations between interaction of genotypes and locations and HPSEC A% of UNP were similar with the correlations between locations and HPSEC A% of UNP. There was significant difference between HRSW and HWSW genotypes regarding to HPSEC A% data of PHS damaged wheat except fractions of U2 and U3 ($P < 0.05$). There was no significant difference between HRSW and HWSW genotypes regarding to HPSEC profiles of sound wheat except for fraction of U6. The differences between HRSW and HWSW genotypes regarding to ΔD were significant except fractions of U2 and U3 ($P < 0.05$). There were significant differences between HRSW and HWSW genotypes for the HPSEC A% of UNP in PHS damaged wheat ($P < 0.001$) except fractions of U2 and U3. These results indicated that there were significant differences regarding to HPSEC profiles of PHS damaged wheat among different genotypes. Locations and interactions of genotypes by locations had the similar correlation with HPSEC A% of UNP, and did not show any significant difference for the fractions of U4, U5 and U6. There was significant difference between HRSW and HWSW genotypes regarding to the degradation of UNP.

Correlation coefficients (r) between HPSEC area percentage (A %) and enzyme activities were given in Table 2.5. There were significant and positive correlations between sprouting score and enzyme activities and HPSEC A% of fractions E4, E5 and E6 of EXP in PHS damaged wheat ($P < 0.001$). There were significant and negative correlations between fraction of U1 of UNP in PHS damaged wheat ($P < 0.01$), and there was significant correlation between HPSEC A% of fraction U2 of UNP in PHS damaged wheat and sprouting score ($P < 0.05$). There were few significant correlations between fractions of EXP and UNP in sound wheat and sprouting score, and enzyme activity. However, there were significant and negative correlations between HPSEC A% of fraction E3 of EXP in sound wheat and endo-protease activity of PHS damaged wheat, and the difference of endo-protease activity between PHS damaged wheat and sound wheat ($P < 0.05$). There were significant and positive correlations between the difference of HPSEC A% of fractions E1, E2 and E3 of EXP and sprouting score, and enzyme activity ($P < 0.001$). The correlations between the difference of HPSEC A% of fractions U1 and U2 of UNP and sprouting score, and enzyme activity were negatively significant ($P < 0.01$). These results indicated that HPSEC profiles of sound wheat did not show much correlation with sprouting score and enzyme activities. PHS increased the α-amylase and endo-protease activity, and the excessive endo-protease activity hydrolyzed the UNP in the earlier retention time and increased the percentage of EXP.

Correlations between HPSEC absorbance area % (A%) values of EXP and UNP, and sprouting score are shown in Figure 2.3. The bold line represented the correlation coefficient value, and the thinner line represented the HPSEC chromatogram of protein. The correlation coefficients (r) for sprouting score against A% of EXP & UNP were determined. EXP fractions eluted at 6.9-8.5 min were positively correlated with sprouting score ($r > 0.58$). High molecular weight polymeric proteins in UNP eluted at

Table 2.3 Mean square values for HPSEC area percentage of SDS buffer extractable protein fractions

	E1			E2			E3		
	Sprouted	Sound	ΔD	Sprouted	Sound	ΔD	Sprouted	Sound	ΔD
Location	14.37**	NS	NS	14.73*	NS	16.40*	NS	NS	NS
Rep(Loc)	0.9*	NS	NS	1.46***	NS	NS	NS	NS	50.24*
Genotype	14.04***	3.21***	12.26***	3.61***	4.58***	2.99*	53.20***	45.48**	48.21**
W vs R	11.92***	NS	43.64***	NS	NS	NS	179.57***	NS	108.60*
Genotype*Loc	1.54***	NS	2.08*	0.82***	NS	NS	NS	NS	NS
Residual	0.29	0.85	1.31	0.17	1.21	1.36	1.90	13.54	15.21

	E4			E5			E6		
	Sprouted	Sound	ΔD	Sprouted	Sound	ΔD	Sprouted	Sound	ΔD
Location	102.18***	NS	13.99***	257.74***	NS	218.19***	204.35***	13.56***	114.26***
Rep(Loc)	NS	NS	NS	1.77*	NS	NS	NS	NS	NS
Genotype	9.75***	2.66***	6.14***	10.79***	1.66***	8.29**	13.01***	2.15***	7.18***
W vs R	12.23***	8.14***	14.55***	14.33***	4.91***	10.72***	21.20***	7.89***	8.26***
Genotype*Loc	1.03***	NS	1.42***	2.82***	NS	2.93***	1.86***	0.34*	1.89***
Residual	0.18	0.53	0.61	0.50	0.23	0.69	0.40	0.20	0.38

HPSEC: high performance size exclusion chromatography.
SDS: sodium dodecyl sulfate.
E1, E2, E3, E4, E5 and E6 represent SDS buffer extractable protein fractions.
W: Hard White Spring Wheat; R: Hard Red Spring Wheat.
Loc: Location; R: Replication.
ΔD: difference between sound and PHS damaged wheat.
*, **, ***: significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; NS, not significant.

Table 2.4 Mean square values for HPSEC area percentage of SDS buffer unextractable protein fractions

	U1			U2			U3		
	Sprouted	**Sound**	**ΔD**	**Sprouted**	**Sound**	**ΔD**	**Sprouted**	**Sound**	**ΔD**
Location	277.20***	NS	383.62***	72.92***	NS	NS	126.91***	NS	NS
Rep(Loc)	1.59*	NS	NS	NS	NS	8.30*	NS	NS	NS
Genotype	16.48***	10.26***	13.51***	4.74***	5.26**	5.46*	9.29***	35.94**	37.94**
W vs R	20.44***	NS	31.42***	NS	NS	NS	NS	NS	NS
Genotype*Loc	1.70***	NS	3.50**	1.28***	NS	NS	2.57***	NS	NS
Residual	0.53	1.34	1.76	0.55	2.44	2.95	1.03	12.85	13.69

	U4			U5			U6		
	Sprouted	**Sound**	**ΔD**	**Sprouted**	**Sound**	**ΔD**	**Sprouted**	**Sound**	**ΔD**
Location	NS	NS	NS	NS	NS	NS	NS	NS	NS
Rep(Loc)	0.14*	NS	NS	NS	NS	NS	NS	NS	NS
Genotype	0.68***	0.88**	1.32***	0.15***	NS	NS	2.56***	0.37***	2.04***
W vs R	2.68***	NS	2.98*	0.95***	NS	1.91**	21.03***	2.09***	5.01***
Genotype*Loc	NS	NS	NS	NS	NS	NS	NS	NS	NS
Residual	0.05	0.34	0.40	0.03	0.15	0.17	0.16	0.11	0.28

HPSEC: high performance size exclusion chromatography.
SDS: sodium dodecyl sulfate.
U1, U2, U3, U4, U5 and U6 represent SDS buffer un-extractable protein fractions.
W: Hard White Spring Wheat; R: Hard Red Spring Wheat.
Loc: Location; R: Replication.
ΔD: difference between sound and PHS damaged wheat.
*, **, ***: significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; NS, not significant.

3.6-6.0 min had significant and negative correlation with sprouting score ($r < -0.58$).

This means that high molecular weight polymeric proteins in UNP had been hydrolyzed more for the genotypes which showed larger PHS damage by increased endo-protease activity due to PHS damage and shift to EXP. Correlation coefficients (r) between HPSEC absorbance A% of EXP and UNP, and endo-protease activities in PHS damaged wheat were determined in Figure 2.4. At the early retention time (3.6-6.0 min), endo-protease activity of PHS damaged wheat and HPSEC absorbance A% of UNP had significant and negative correlations ($r < -0.70$); at the later retention time (6.9-8.2 min), endo-protease activity had significant and positive correlations with EXP ($r > 0.70$). This result indicated that polymeric proteins had been hydrolyzed by increased endo-protease activity due to PHS damage. Correlations between HPSEC absorbance A% of EXP and UNP, and endo-protease activity of difference between sound sample and sprouted sample were analyzed (Figure 2.5). The correlation between EXP and UNP with endo-protease activity of $\triangle D$ is very similar with the correlation between EXP and UNP with endo-protease activity in sprouted samples, but not as significant as the correlation between EXP and UNP with endo-protease activity in sprouted sample. These results in the present study reflects that the genotypes with greater sprouting score showed higher endo-protease activity, and more UNP had been hydrolyzed and shift to EXP which is undesirable for bread making quality.

Comparison of endo-protease activity of PHS wheat and sprouting score, the correlation coefficient ($r = 0.78$) of EXP and UNP with endo-protease activity was larger than the correlation coefficient ($r = 0.57$) of EXP and UNP with sprouting score, thus, endo-protease activity in PHS damaged wheat can represent the degree of protein degradation measured by HPSEC better.

Table 2.5 Correlation coefficients between high performance size exclusion chromatography (HPSEC) area percentage (A %) and enzyme activities

			α-Amylase				Endo-protease			
HPSEC A%	**Sprout score[1]**		**Sprouted**		**ΔD**		**Sprouted**		**ΔD**	
Sprouted										
Extractable										
E1	NS		NS		NS		NS		NS	
E2	NS		NS		NS		NS		NS	
E3	NS		-0.41	*	NS		-0.50	*	-0.44	*
E4	0.74	***	0.77	***	0.75	***	0.82	***	0.74	***
E5	0.81	***	0.81	***	0.79	***	0.87	***	0.76	***
E6	0.72	***	0.71	***	0.69	***	0.81	***	0.69	***
Unextractable										
U1	-0.69	***	-0.67	***	-0.66	***	-0.69	***	-0.63	**
U2	-0.44	*	NS		NS		NS		NS	
U3	NS		NS		NS		NS		NS	
U4	NS		NS		NS		NS		NS	
U5	NS		0.41	*	0.42	*	0.51	*	0.45	*
U6	NS		NS		NS		NS		NS	
Sound										
Extractable										
E1	NS		NS		NS		NS		NS	
E2	NS		NS		NS		NS		NS	
E3	NS		NS		NS		-0.44	*	-0.41	*
ΔD										
Extractable										
E1	NS		NS		NS		0.41	*	NS	
E2	NS		NS		NS		NS		NS	
E3	NS		NS		NS		NS		NS	
E4	0.79	***	0.81	***	0.80	***	0.91	***	0.84	***
E5	0.86	***	0.86	***	0.84	***	0.91	***	0.81	***
E6	0.84	***	0.83	***	0.83	***	0.89	***	0.78	***
Unextractable										
U1	-0.73	***	-0.76	***	-0.74	***	-0.88	***	-0.78	***
U2	-0.52	**	-0.54	**	-0.52	**	-0.57	**	-0.58	**

1. Sprouting scores were provided by Mr. Mory Rugg at the Department of Plant Science, NDSU.
ΔD: difference between sound and PHS damaged wheat
Extractable: SDS buffer extractable proteins; Unextractable: SDS buffer un-extractable proteins.
E1, E2, E3, E4, E5 and E6 represent SDS buffer extractable protein fractions; U1, U2, U3, U4, U5 and U6 represent SDS buffer un-extractable protein fractions.
*, **, ***: correlation coefficient is significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; NS, not significant.

Conclusion

PHS damage of the wheat samples resulted in changes to the protein component of the wheat. PHS damaged wheat showed lower protein content than sound wheat due

to the removal of root and shoot during cleaning of wheat samples. Varietal difference existed in sound and PHS damaged wheat for protein content. There was significant difference ($P < 0.05$) in the protein content between HRSW and HWSW genotypes, and the mean value of protein content in HRSW genotypes was higher than that in HWSW genotypes. However, protein content did not show significant correlation with enzyme activity or pasting properties of starch, indicating that protein content cannot be an indicator of the susceptibility of PHS of a wheat genotype.

SDS buffer EXP and UNP were analyzed with HPSEC of wheat proteins. PHS damaged wheat exhibited more EXP and less UNP than sound wheat, suggesting that UNP had been hydrolyzed becoming EXP. Varietal difference existed for the HPSEC area percentage of UNP due to the difference in level of endo-protease activity in PHS damaged wheat.

There were positively significant correlations between EXP fractions in the later retention time and sprouting score, α-amylase and endo-protease activity. However, negatively significant correlations existed between UNP fractions in the earlier retention time and sprouting score, α-amylase and endo-protease activity. Genotypes with higher sprouting score exhibited larger degree of UNP degradation and had a higher percentage of EXP. Through comparison of the correlation between HPSEC area percentage of protein and sprouting score, endo-protease activity and the difference of endo-protease activity between sound and PHS damage wheat, the correlation coefficient between HPSEC area percentage of protein and endo-protease activity was largest. These results suggest that endo-protease activity in PHS damaged wheat can represent the degree of protein degradation measured by HPSEC better than other parameters. In summary, the protein physicochemical properties had been changed by PHS due to the increased endo-protease activity.

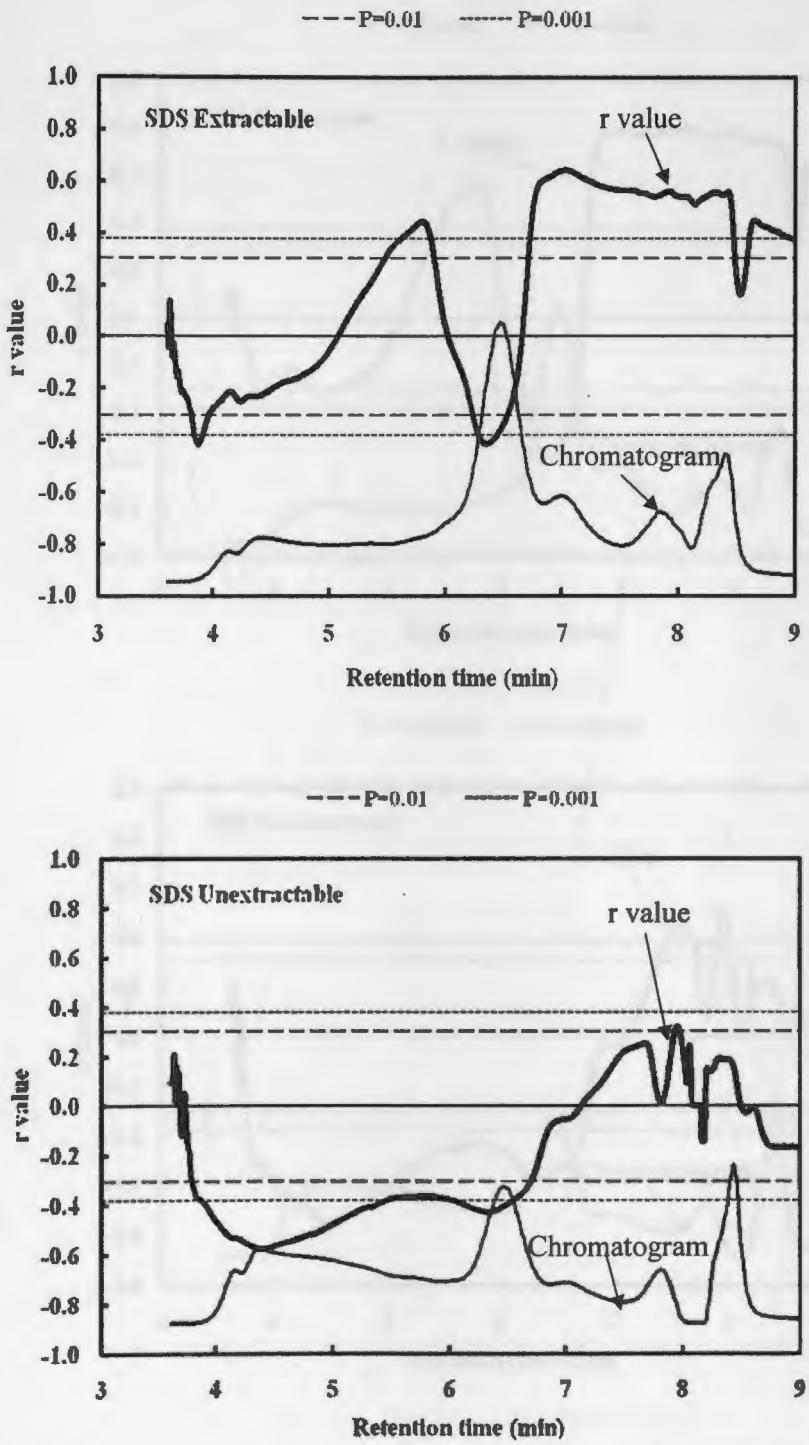

Figure 2.3 Spectrum of correlation coefficient (r) between HPSEC absorbance area percentage (A %) values of SDS buffer extractable proteins and unextractable proteins, and sprouting score

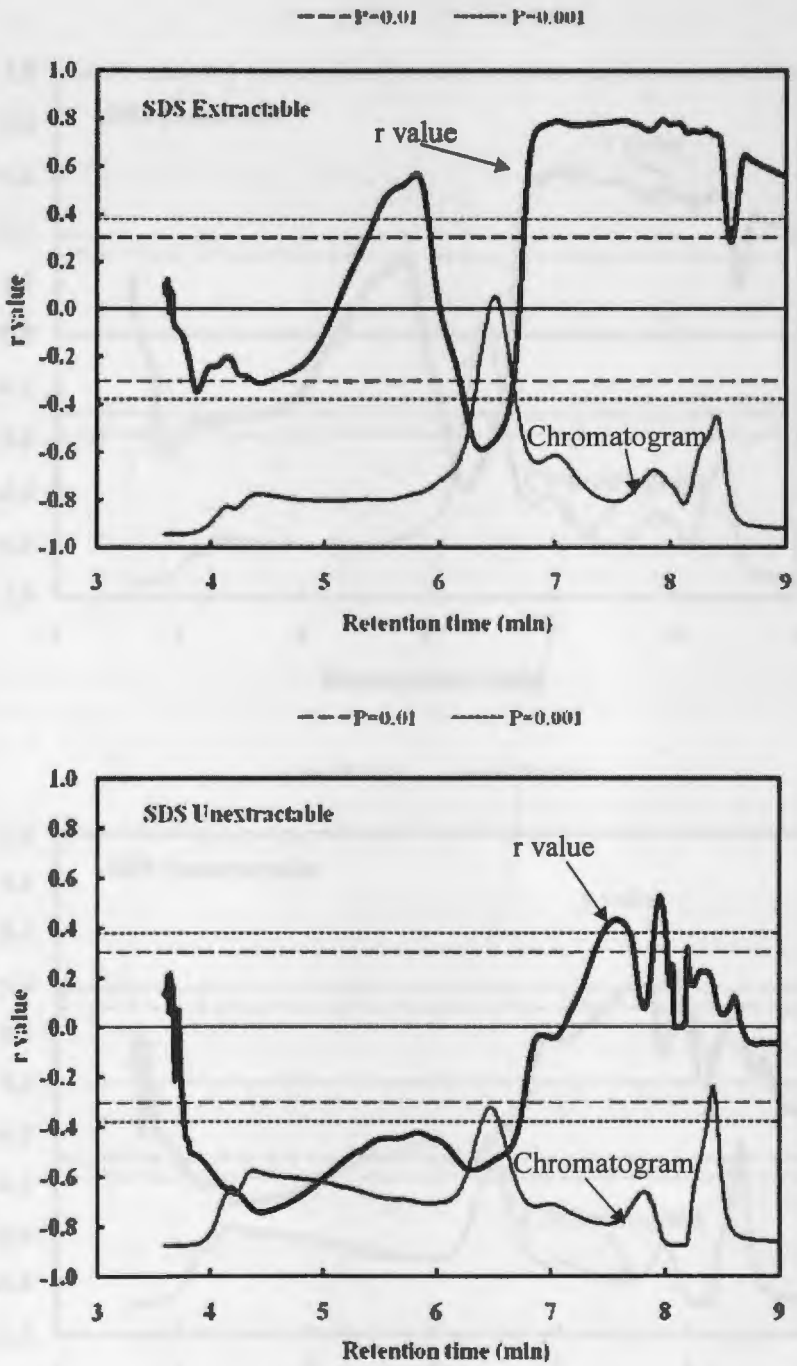

Figure 2.4 Spectrum of correlation coefficient (r) between HPSEC absorbance area percentage (A %) of SDS buffer extractable proteins and unextractable proteins, and endo-protease activity of sprouted sample

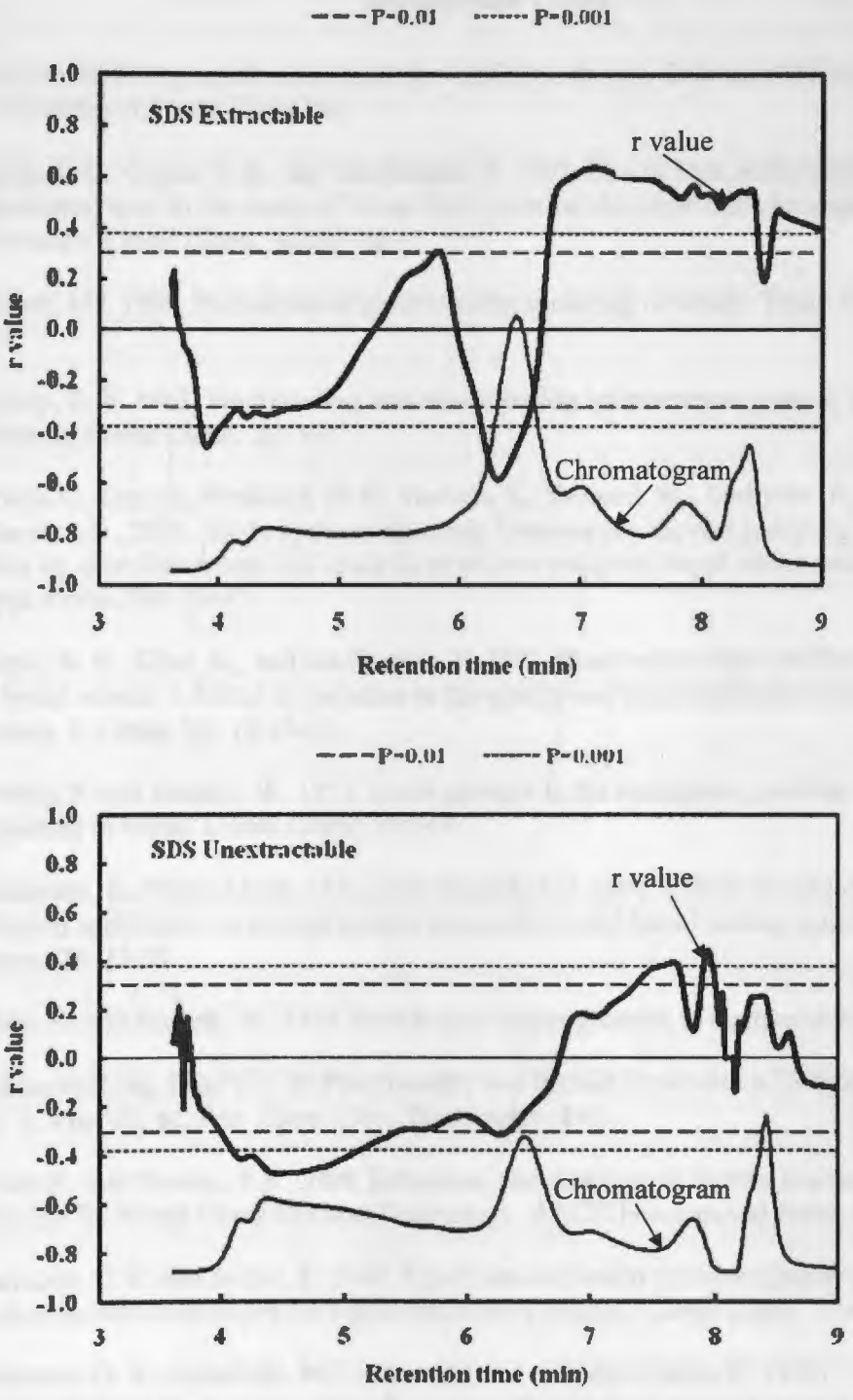

Figure 2.5 Spectrum of correlation coefficient (r) between HPSEC absorbance area percentage (A %) of SDS buffer extractable proteins and unextractable proteins and difference of endo-protease activity between sound sample and sprouted sample

References Cited

AACC. 2004. Approved methods of the AACC (11th ed.). St. Paul, MN: American Association of Cereal Chemists.

Batey, 1. L.. Gupta. R. B., and MacRitchie. F. 1991. Use of high performance liquid chromatography in the study of wheat flour proteins: An improved chromatographic procedure. Cereal Chem. 68:207-209.

Beresh, I.D. 1969. Proteolysis of gluten during sprouting of wheat. Trudy VNIIZ. 66: 111.

Finney, K. F. 1943. Fractionating and reconstituting techniques as tools in wheat flour research. Cereal Chem. 20: 381.

Groos, C., Gay, G., Perretant, M-R., Gervais, L., Bernard, M., Dedryver, F., and Charmet, G., 2002. Study of the relationship between pre-harvest sprouting and grain color by quantitative trait loci analysis in white x red grain bread wheat cross. Theor. Appl. Genet.104: 39-47.

Gupta, R. B., Khan, K., and MacRitchie, F. 1993. Biochemical basis of flour properties in bread wheats. I. Effect of variation in the quality and size distribution of polymeric protein. J. Cereal Sci. 18:23-41.

Hwang, P. and Bushuk, W. 1973. Some changes in the endosperm proteins during sprouting of wheat. Cereal Chem. 50:147.

Johansson, E., Prieto-Linde, M.L., and Jönsson, J.Ö. 2001. Effects of wheat variety and nitrogen application on storage protein composition and bread making quality. Cereal Chem. 78: 19-25.

Khan, K. and Bushuk, W. 1979. Structure of wheat glutenin in realtion to functionality

in breadmaking. Page 191 in: Functionality and Protein Structure, ACS Symp. Ser., No. 92. A. Pour-El, ed. Am. Chem. Soc., Washington, DC.

Khan, K. and Shewry, P.R. 2009. Extraction and Analysis of Protein Fractions. Pages 233-236 in: Wheat Chemistry and Technology. AACC International Press, MN, USA.

Larroque, O. R. and Bekes, F. 2000. Rapid size-exclusion chromatography analysis of molecular size distribution for wheat endosperm protein. Cereal Chem. 77:451-453.

Larroque, O. R., Gianibelli, M. C., Batey, I. L., and MacRitchie, F. 1997. Electrophoretic characterisation of fractions collected from gluten protein extracts subjected to size-exclusion high-performance liquid chromatography. Electrophoresis 18:1064-1067.

Larroque. O. R., Gianibelli, M. C., Gomez Sanchez M., and MacRitchie. F. 2000. Procedure for obtaining stable protein extract of cereal flour and whole mean for size-exclusion HPLC analysis. Cereal Chem. 77:448-450.

Morel, M.-F1., Dehlon. P., Autran, J. C., Leygue. J. P., and Bar I. L'Helgouac'h, C. 2000. Effects of temperature, sonication time, and power settings on size distribution and extractability of total wheat flour proteins as determined by size-exclusion high-performance liquid chromatography. Cereal Chem. 77:685-691.

Ohm, J. B., Hareland, G., Simsek, S., and Seabourn, B. 2009a. Size Exclusion HPLC of protein using a narrow-bore column for evaluation of breadmaking quality of hard spring wheat flours. Cereal Chem. 86: 463-469.

Ohm, J. B., Ross, A. S., Ong. Y.L., and Peterson, C. J. 2006. Using multivariate techniques to predict wheat-flour dough and noodle characteristics from size exclusion HPLC and RVA data. Cereal Chem. 83:1-9.

Ohm, J. B., Ross, A. S., Peterson, C. J., and Morris, C. F. 2009b. Relationships of quality characteristics with size-exclusion HPLC chromatogram of protein extract in soft white winter wheats. Cereal Chem. 86:197-203.

Park, S. H., Bean, S. R.. Chung. O. K., and Seib. P. A. 2006. Levels of protein and protein composition in hard winter wheat flours and relationship to breadmaking. Cereal Chem. 83:418-423.

Redman, D.G. 1971. Softening of gluten by wheat proteases. J. Sci. Food Agr. 22:75.

Samson, M. F., Mabille, F., Chéret, R., Abécassis, J. A., and Morel, M.H. 2005. Mechanical and physicochemical characterization of vitreous and mealy durum wheat endosperm. Cereal Chem. 82:81-87.

Sorenson, B. and Wiersma, J. 2004. Sprout damaged wheat, crop insurance, and quality concerns. Minnesota Crop New Archive. University of Minnesota.

Southan, M. and MacRitchie, F. 1999. Molecular Weight Distribution of Wheat Proteins. Cereal Chem. 76(6): 827-836.

Tsilo, T. J., Ohm, J. B., Hareland, G. A., and Anderson, J. A. 2010. Association of size exclusion HPLC of endosperm proteins with dough mixing and breadmaking characteristics in a recombinant inbred population of hard spring wheat. Cereal Chem. 87: 104-111.

Wahl, T.I. and O'Rourke, A.D. 1992. The economics of sprout damage in wheat. Pages 10-17 in: Pre-harvest Sprouting in Cereals. M. K.Walker-Simmons and J. L. Ried, eds. AACC International Press.

Yasunori, I., Kanenori, T., Tatsuo, K., Norio, I., Toshiyuki, A., and Hiroaki, Y. 2001. Effects of Increase in α-Amylase and Endo-Protease Activities during Germination on the Breadmaking Quality of Wheat. FSTR. Vol. 7: 214-219.

GENERAL CONCLUSION

This study investigated: 1) the effect of Pre-Harvest Sprouting (PHS) on enzyme activities (α-amylase and endo-protease) in wheat, 2) physicochemical properties of starch and protein molecules, and 3) the correlations between HPSEC profiles of protein, sprouting score and enzyme activity. The overall aims of this research were to study the effect of PHS on physicochemical properties of starch and protein in HRSW and HWSW genotypes.

PHS increased both α-amylase and endo-protease activities, resulting in the hydrolysis of starch and protein molecules. However, increased endo-protease activity was not as significant as the increased α-amylase activity. Hydrolysis of starch by the increased α-amylase activity lead to the change in pasting properties of starch. Scanning Electronic Microscopy (SEM) was employed to detect the morphology of starch granules. In sound wheat kernels, starch granules were integrated and embedded in very dense protein matrix. However, in PHS damaged wheat, the starch granules had been partially hydrolyzed and the protein matrix was absent. The Molecular Weight Distribution (MWD) of starch, measured by HPSEC, was significantly altered in PHS wheat samples. Some portion of HMW-AP had been degraded and became part of the LMW-AP and AM fractions. Furthermore, the average molecular weight of HMW-AP decreased, while the average molecular weight of LMW-AP and AM increased.

Varietal difference existed in sprouting score, which had positively significant correlations with α-amylase and endo-protease activity. Consequently, genotypes showed differences in degradation of starch and protein molecules. SDS buffer EXP and UNP were analyzed in this research. EXP fractions that eluted at the later retention time had significant positive correlation with sprouting score. However, UNP fractions which eluted at the earlier retention time exhibited negatively significant correlation

with sprouting score ($P < 0.05$). These results indicated that some portion of UNP had been hydrolyzed and become EXP. Through comparison of the correlations between HPSEC profiles of protein and sprouting score, endo-protease activity and the difference of endo-protease activity between sound and PHS damaged wheat, endo-protease activity in PHS damaged wheat had greater correlation coefficient with protein degradation measured by HPSEC. These results suggested that endo-protease activity in PHS damaged wheat can represent degree of protein degradation better than other parameters. Overall, genotypes with high sprouting score exhibited high enzyme activities, and the physicochemical properties of starch and protein molecules had been considerably altered by the PHS damage.

α-Amylase and endo-protease existed in many forms in PHS damaged wheat. Future research can focus on detection of the main form of α-amylase and endo-protease existing in PHS damaged wheat. Furthermore, there are many types of other isozymes existing in the PHS damaged wheat. Some isozymes may be not present in sound wheat kernel, but they will be present in PHS damaged wheat kernel. Thus, the future research direction is to detect the creation of other isozymes by PHS and their relationship with the PHS damage.

APPENDIX

Table A.1 Scale scores used in the study and their description

Score	Description
1	Visible radicles emerging from approximately 10% of spikelets.
2	Visible radicles emerging from approximately 20% of spikelets.
3	Visible radicles emerging from approximately 30% of spikelets.
4	Visible radicles emerging from approximately 40% of spikelets.
5	Visible radicles emerging from approximately 50 to 100% of spikelets. Coleoptiles emerging from 10 to 20% of spikelets.
6	Visible radicles emerging from approximately 50 to 100% of spikelets. Coleoptiles emerging from 30 to 40% of spikelets.
7	Visible radicles emerging from approximately 50 to 100% of spikelets. Coleoptiles emerging from 50 to 100% of spikelets. Average coleoptile length less than 1 cm.
8	Visible radicles emerging from approximately 50 to 100% of spikelets. Coleoptiles emerging from 50 to 100% of spikelets. Average coleoptile length 1 to 2 cm.
9	Visible radicles emerging from approximately 50 to 100% of spikelets. Coleoptiles emerging from 50 to 100% of spikelets. Average coleoptile length greather than 2 cm.

This table is cited from Mr. Mory Rugg's thesis, at the department of Plant Science, NDSU.

Table A.2 Mean square values for sprouting score, α-amylase and endo-protease activity of sound, PHS damaged wheat and ΔD

	DF	Sprouting	α-Amylase			Protease Activity		
		Score	Sprouted	Sound	ΔD	Sprouted	Sound	ΔD
Location	2	25.58*	1.82*	0.01*	2.32*	3.85**	NS	1.12**
Rep(Loc)	3	NS	0.16**	NS	0.23**	0.28**	NS	NS
Genotype	23	12.83***	0.74***	0.01***	0.73***	6.62***	0.06***	0.41***
W vs R	1	7.56***	0.63***	0.02***	0.55**	32.27***	0.04*	1.42***
Genotype*Loc	46	1.21***	0.09***	0.01***	0.10**	2.96***	0.02***	NS
Residual	69	0.45	0.04	0.01	0.05	1.34	0.01	0.05

[1]Sprouting scores were provided by Mr. Mory Rugg at the Department of Plant Science, NDSU.
Rep: Replication; Loc: Location; W: Hard White Spring Wheat; R: Hard Red Spring Wheat.
ΔD: difference between PHS damaged wheat and sound wheat.
*, **, ***: significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; NS, not significant.

Table A.3 Mean square values for pasting parameters of 24 genotypes of sound and PHS damaged wheat in Carrington

		Sound				
	DF	**PV**	**HPV**	**BV**	**FV**	**SV**
Genotype	23	1420***	738***	531***	1630***	340***
Rep(Loc)	1	1018**	598*	54*	1549**	NS
Error	23	87	76	7	186	31
R vs W	1	NS	1979***	2803***	NS	951***
		Sprouted				
	DF	**PV**	**HPV**	**BV**	**FV**	**SV**
Genotype	23	5.63***	2.99***	1.96**	3.34***	NS
Rep(Loc)	1	NS	NS	NS	NS	NS
Error	23	0.77	0.40	0.26	0.39	0.05
R vs W	1	NS	5.27**	2.34**	3.98**	NS
		ΔD				
	DF	**PV**	**HPV**	**BV**	**FV**	**SV**
Genotype	23	1392***	733***	543***	1599***	339***
Rep(Loc)	1	933**	565*	44*	1498*	223*
Error	23	89	81	8	191	31
R vs W	1	NS	2188***	2697***	NS	970***

*, **, ***: significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; NS, not significant.
ΔD: difference between sound and PHS damaged wheat.
PV: peak viscosity; HPV: hot paste viscosity; BV: breakdown viscosity; FV: final viscosity; SV: setback viscosity.
W: hard white spring wheat; R: hard red spring wheat.

Table A.4 Mean square values for pasting parameters of 24 genotypes of sound and PHS damaged wheat in Casselton

		Sound				
	DF	**PV**	**HPV**	**BV**	**FV**	**SV**
Genotype	23	2970***	2546***	443***	6598***	1120***
Rep(Loc)	1	1051*	685*	NS	2249*	452*
Error	23	152	97	13	335	77
R vs W	1	13309***	23369***	1390***	42960***	2956***
		Sprouted				
	DF	**PV**	**HPV**	**BV**	**FV**	**SV**
Genotype	23	139***	2.81***	114**	5.93***	0.89*
Rep(Loc)	1	NS	NS	NS	NS	NS
Error	23	7.00	0.99	3.56	1.88	0.35
R vs W	1	NS	NS	NS	NS	NS
		ΔD				
	DF	**PV**	**HPV**	**BV**	**FV**	**SV**
Genotype	23	2436***	2521***	393***	6523***	1113***
Rep(Loc)	1	1027**	649*	46*	2231*	473*
Error	23	150	93	16	327	78
R vs W	1	13040***	23776***	1582***	43661***	2998***

*, **, ***: significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; NS, not significant.
ΔD: difference between sound and PHS damaged wheat.
PV: peak viscosity; HPV: hot paste viscosity; BV: breakdown viscosity; FV: final viscosity; SV: setback viscosity.
W: hard white spring wheat; R: hard red spring wheat.

Table A.5 Mean square values for pasting parameters of 24 genotypes of sound and PHS damaged wheat in Prosper

		Sound				
	DF	**PV**	**HPV**	**BV**	**FV**	**SV**
Genotype	23	1623***	1281***	495***	2555***	444***
Rep(Loc)	1	NS	NS	57*	NS	NS
Error	23	136	88	11	223	41
R vs W	1	3744***	11160***	1985***	9707***	NS
		Sprouted				
	DF	**PV**	**HPV**	**BV**	**FV**	**SV**
Genotype	23	26***	2.71***	23**	2.41***	0.22***
Rep(Loc)	1	NS	NS	NS	NS	NS
Error	23	1	0.33	1	0.38	0.05
R vs W	1	7*	3.60**	21**	3.05**	NS
		ΔD				
	DF	**PV**	**HPV**	**BV**	**FV**	**SV**
Genotype	23	1616***	1335***	518***	2612***	447***
Rep(Loc)	1	NS	NS	NS	NS	NS
Error	23	138	87	13	222	40
R vs W	1	3421***	11566***	2416***	10054***	NS

*, **, ***: significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; NS, not significant.
ΔD: difference between sound and PHS damaged wheat.
PV: peak viscosity; HPV: hot paste viscosity; BV: breakdown viscosity; FV: final viscosity; SV: setback viscosity.
W: hard white spring wheat; R: hard red spring wheat.

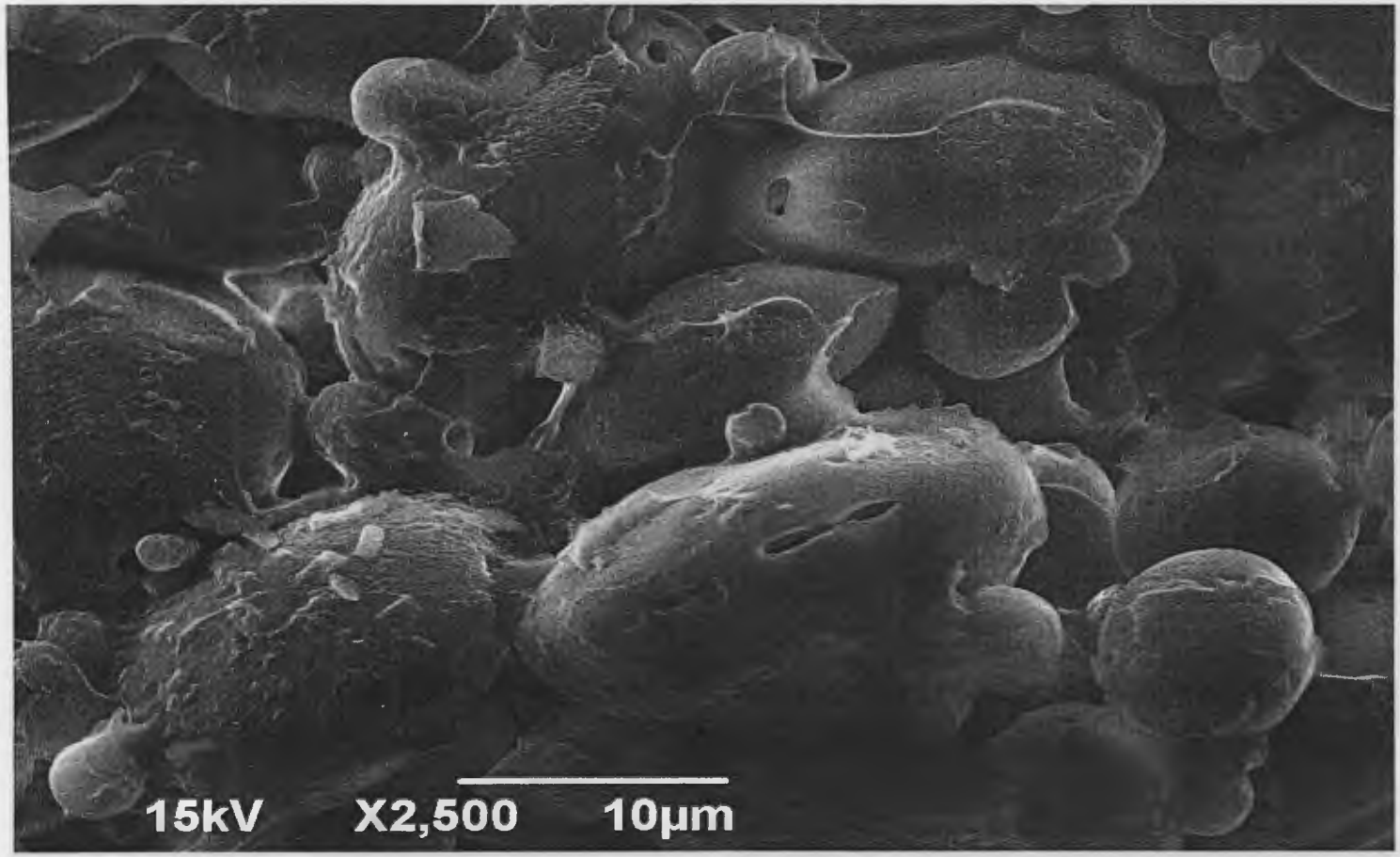

Figure A.1 SEM images of PHS damaged wheat kernel of genotype Steel-ND

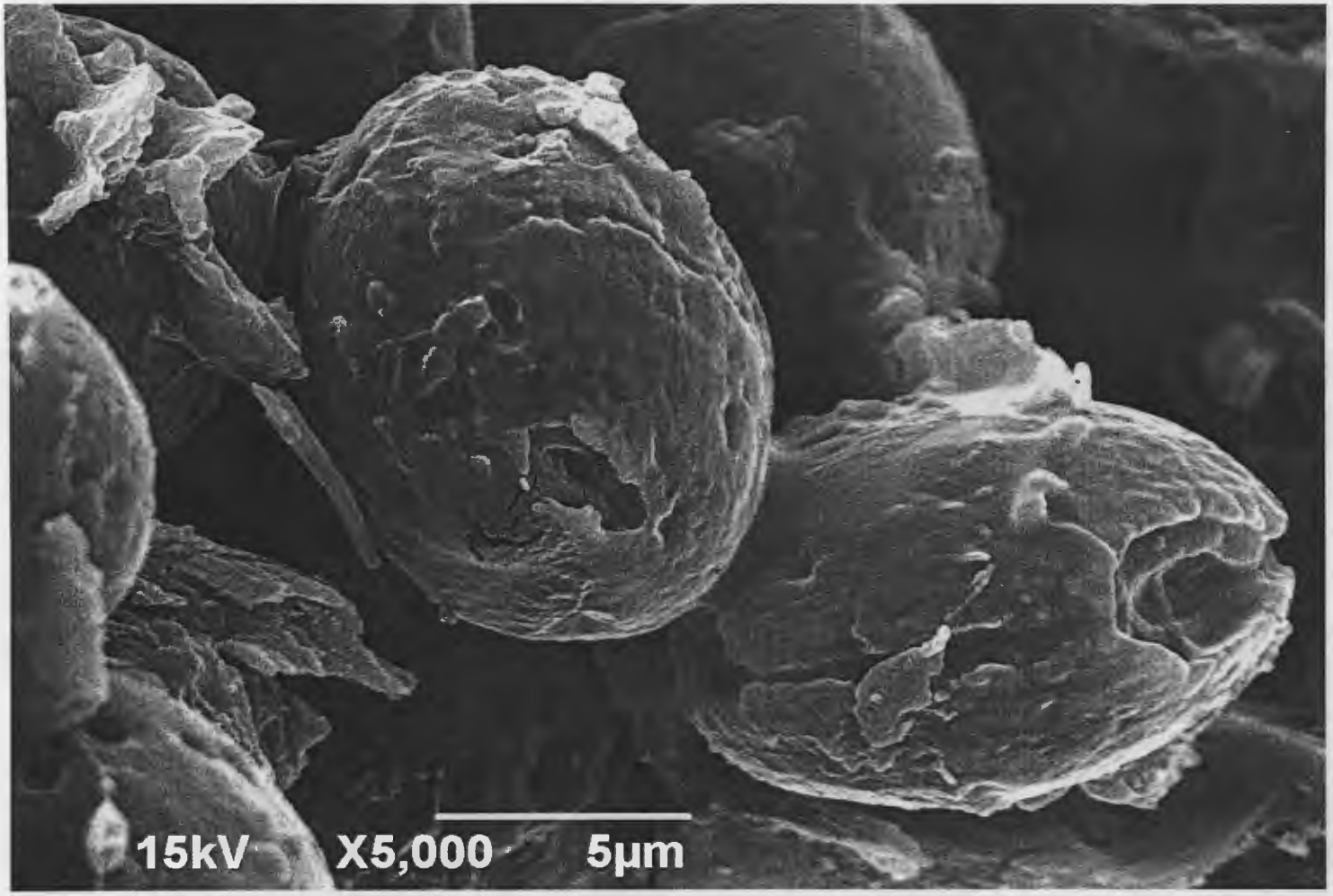

Figure A.2 SEM images of PHS damaged wheat kernel of genotype Pristine

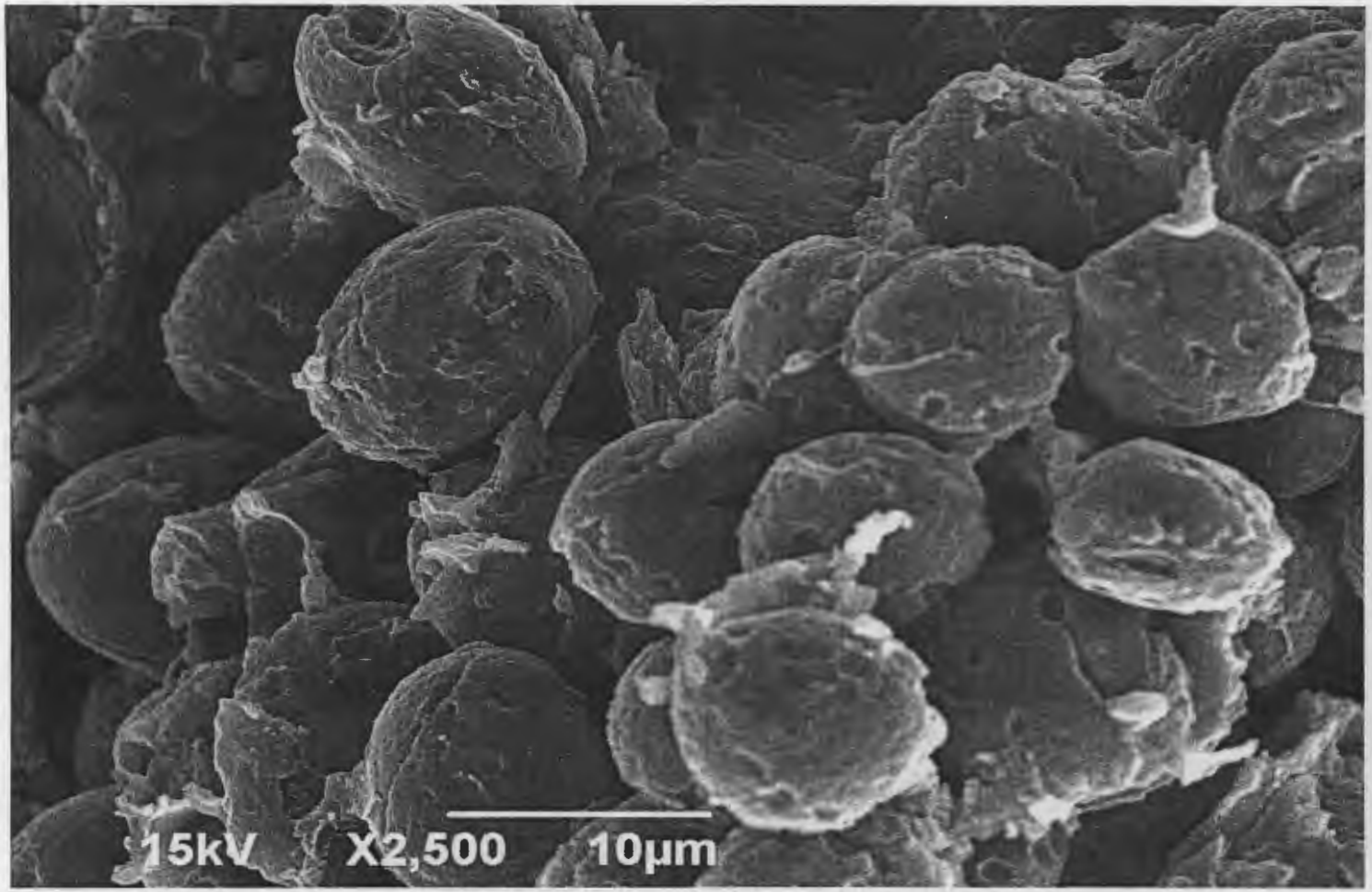

Figure A.2 SEM images of PHS damaged wheat kernel of genotype Pristine (Continued)

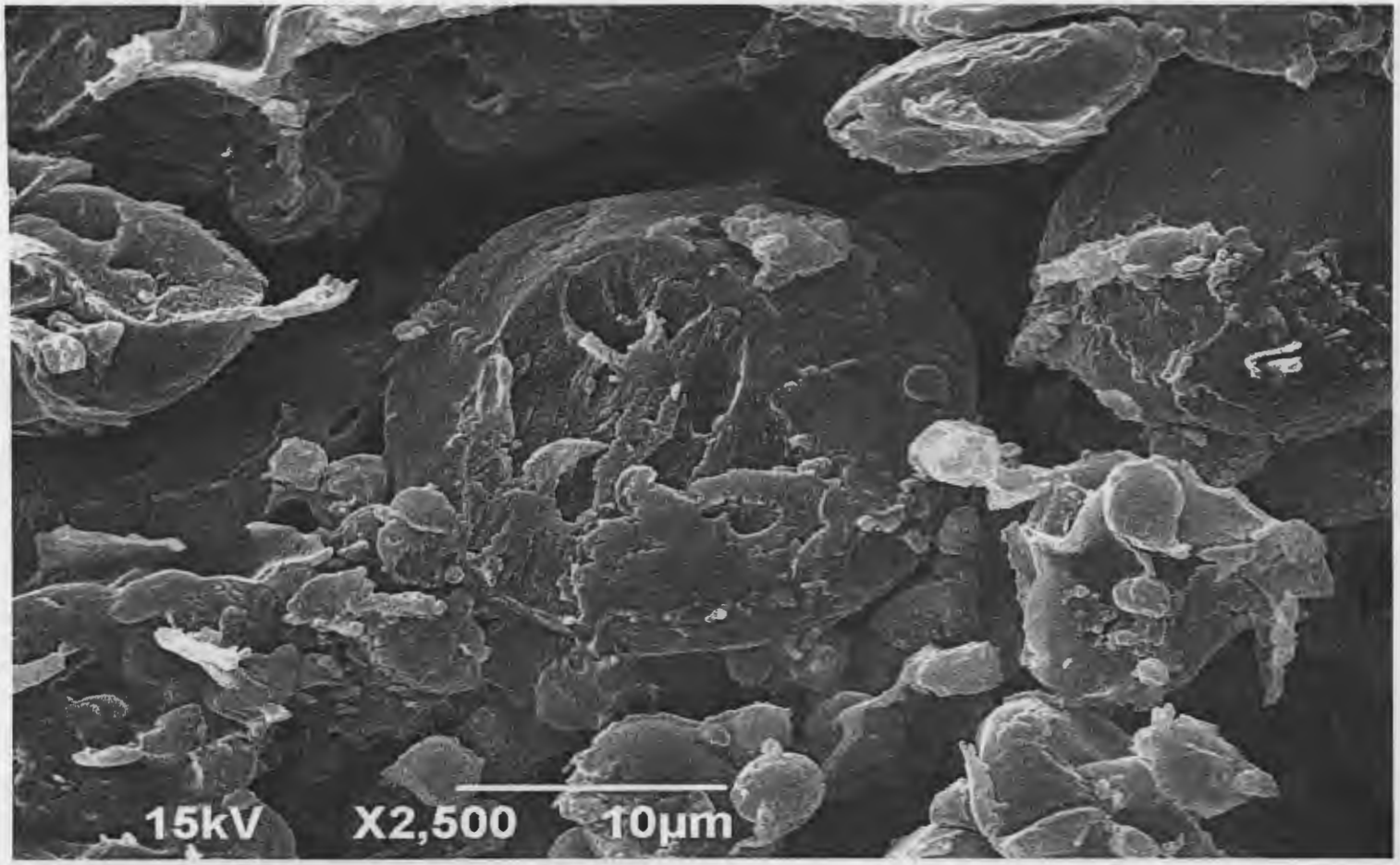

Figure A.2 SEM images of PHS damaged wheat kernel of genotype Pristine (Continued)

Figure A.2 SEM images of PHS damaged wheat kernel of genotype Pristine (Continued)

Table A.6 Mean square values for protein content of sound and PHS damaged wheat in three locations (Carrington, Casselton, and Prosper)

	Sound			Sprout			Difference		
	Carrington	**Casselton**	**Prosper**	**Carrington**	**Casselton**	**Prosper**	**Carrington**	**Casselton**	**Prosper**
Genotype	0.934***	2.13***	1.197***	1.79***	1.88**	1.35***	0.54**	0.67*	0.44*
Rep	0.385*	1.96*	0.99**	NS	NS	1.74***	NS	NS	NS
R vs W	6.07***	15.13***	8.24***	13.66***	5.15**	12.26***	2.66**	2.66**	NS
Residual	0.05	0.35	0.11	0.24	0.64	0.09	0.33	0.33	0.15

R: Hard Red Spring Wheat; W: Hard White Spring Wheat; Rep: Replication
ΔD: difference between sound and PHS damaged wheat.
*, **, ***: significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; NS, not significant.