PATTERNS OF ACCUMULATION OF WHEAT GLUTEN PROTEINS DURING KERNEL DEVELOPMENT IN RESPONSE TO WEATHER VARIATION

BY

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A Thesis Submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfilment of the requirement of the degree of

MASTER OF SCIENCE

Department of Food Science

University of Manitoba

Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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Abstract

Qiao, Lini, M.Sc., The University of Manitoba, 2010

Patterns of Accumulation of Wheat Gluten Proteins During Kernel Development in Response to Weather Variation

Advisor: Dr. H. D. Sapirstein

Investigating the patterns of variation of wheat gluten proteins during kernel development in relation to weather parameters acquired concurrently, should generate considerable worthwhile new knowledge on the environmental influences and biochemical nature of wheat quality variation for breadmaking. That investigation represented the focus of this thesis research.

Two hard spring wheat cultivars (Superb and AC Vista) were grown at two locations (Winnipeg, MB and Swift Current, SK) in replicated plots under optimal soil fertility conditions in two consecutive seasons (2003 and 2004). Wheat heads were sampled at 3-4 day intervals during kernel development from anthesis to maturity. Heads were preserved by freezing at field sites and subsequent freeze drying, and were threshed by hand and finally ground to a standard particle size for protein analysis. Ground wheat was extracted in 50% 1-propanol producing two fractions: 50% 1-propanol soluble protein (mainly gliadins) and 50% 1-propanol insoluble protein (i.e. insoluble or HMW polymeric glutenin). A different fractionation isolated soluble glutenin from the 50% 1-propanol soluble protein. Reduced soluble and insoluble glutenin was analyzed by reversed-phase HPLC resulting in quantification of individual and total HMW glutenin subunits (GS) and total LMW-GS. Thousand kernel weight

(TKW), a measure of grain dry matter accumulation was also measured. Meteorological data acquired concurrently with measurement on the kernels included hourly measurements of solar radiation, air temperature, humidity, precipitation, useful heat (GDD5, growing degree days > 5 ^{o}C), evapotranspiration, and modeled water use, demand and deficit.

Site-years produced very different weather conditions during the growing season and grain development periods resulting in substantial differences in grain filling duration and accumulation patterns of total protein and protein fractions. For the most part, there was relatively little difference in response between the two genotypes used in the study, despite differences in their HMW-GS composition. The very large range in total protein content at maturity across site-years (~ 9-17%) was very compelling and indicated the considerable influence that crop season weather can have molecular mechanisms of grain development and wheat quality in general.

Grain filling duration (hence time of protein accumulation) was negatively and positively associated with temperature related variables and rainfall, respectively. Grain filling duration was also strongly negatively correlated with the rate of grain filling.

Accumulation of total protein, and constituent protein fractions (gliadin, small polymeric glutenin, large polymeric glutenin, and residue) during kernel development were substantially affected by site-year differences in weather, although a common pattern of variation emerged confirmed the asynchronous nature of wheat protein synthesis. A continuously increasing ratio of glutenin to gliadin during kernel development also indicated a basic difference in regulation of gliadin and glutenin synthesis. Averaged across growing sites and years, protein by type started accumulating in the following order: gliadin, soluble glutenin, insoluble glutenin. Gliadin synthesis

began as early as 7 DAA for one growing location (2003 Swift Current that experienced relatively warm and dry conditions) and was clearly underway for all site-years by 15 DAA. Synthesis of small glutenin polymers lagged slightly behind that for gliadins by about 3 days, accumulated at a comparable or somewhat slower rate compared to gliadins, and reached a peak at least one week later. After peak absolute accumulation (mg/kernel), gliadins remained at generally constant levels in all site-years, while soluble glutenin comprising small glutenin polymers, decreased significantly for another 10 to 20 calendar days until maturity depending on growing location.

Insoluble glutenin (large glutenin polymers) started to form in general in significant amounts much later than that for gliadins, beginning around 25 DAA, but at a higher rate. However, for one growing location (2003 Swift Current) where kernel development was accelerated, insoluble glutenin began to form at a high rate at about 15 DAA, but still later than that for soluble glutenin at that location. Formation of insoluble glutenin invariably lagged behind that of soluble glutenin from 3 to 12 days depending on genotype and growing location. No peak accumulation was observed for insoluble glutenin, which continued to increase, but at a slower pace, until maturity. As well, the proportion of insoluble glutenin increased at the same time that the proportion of soluble glutenin decreased towards the latter part of kernel development, suggesting that the two events were mechanistically related, i.e. aggregation of smaller polymers (soluble glutenin) leads to formation of larger polymers (insoluble glutenin).

Like the parent glutenin fraction, accumulation patterns for constituent HMW glutenin subunit composition were highly influenced by weather-induced site-year effects and some different trends were observed for individual HMW-GS loci. Most notable was over-expressed Bx7 subunit of AC Vista as it accumulated at a much higher

rate compared to the other four HMW-GS in its complement. HMW-GS Dx5 and Bx7* of Superb also accumulated at a faster rate compared to the other three HMW-GS. These effects were consistent among site-years. Small but apparently significant differences in relative rates of synthesis of Superb HMW-GS among site-years towards the end of the kernel development were observed.

Identifying site-year independent trends in weather relationships to protein accumulation patterns during kernel development was challenging. Weather factors that were site characteristics included solar radiation (but not air temperature), wind speed (but not evapotranspiration), water demand, and water deficit; all had higher values in one location (Swift Current) compared to another (Winnipeg) averaged across years. In contrast, precipitation and air temperature were growing season characteristics. Rainfall was a poor predictor of protein accumulation patterns. Poor results were obtained were found when protein accumulation was examined for weather parameters varying by calendar days (i.e. DAA). When protein accumulation was expressed as percent of total protein and was analyzed in response to cumulative temperature-related weather parameters, such as thermal time (e.g. GDD5) strong site-year independent relationships were observed for gliadin, soluble glutenin and insoluble glutenin fractions. For insoluble glutenin, those relationships followed a linear trend. For gliadin and soluble glutenin, bell-shaped patterns of protein accumulation were evident, with soluble glutenin showing a more pronounced profile.

The influence of growing season weather in western Canada on hard spring wheat grown in optimally fertilized fields was striking in its large effects on protein content and composition. The strong relationships found between GDD5 and related weather parameters, and protein fraction accumulation in total protein could potentially be used

in developing models for predicting wheat breadmaking quality before harvest. Those models could be used for example, to improve the ability of the market place to match wheat quality requirements of specific customers to wheat grown in specific regions of Western Canada.

Acknowledgements

I would like to acknowledge the following parties who dedicated their great help to my M. Sc. project.

First, I would like to thank the Manitoba Agriculture, Food and Rural Initiatives (MAFRI) and ARDI funding program and the Canadian Wheat Board for providing financial support for this study. I am also very grateful to Dr. Hamid Naeem, Mr. Wayne Johnson, Dr. Akhtar Hussain and Ms. Alison Ser of the Department of Food Science for the technical support. Many thanks are due to Mr. Gordon Finlay, formerly of the Department of Soil Science who acquired and organized the collection of the agro-meteorological data I used in my study. I would also like to thank to Dr. Ron DePauw (AAFC-SPARC) and other field staff of the Department of Plant Science who supervised the wheat growing locations of Swift Current and Winnipeg, respectively.

Thank you to all the members of my thesis committee: Dr. Harry Sapirstein, Dr. Paul Bullock and Dr. Nancy Edwards for being very supportive and monitoring the progress of my project. I really appreciate the opportunity provided by my major advisor Dr. Harry Sapirstein to work on this comprehensive project in such a strong academic group. A very special thank you to Dr. Arnie Hydamaka and Dr. Greg Blank of the Department of Food Science for supporting my initial graduate study at University of Manitoba. Also deserving thanks is my landlord, Mr. On Chan of MAFRI, for providing me with a family-like residence in Winnipeg for the past years.

Last but not least, I would like to give a very big thanks to my family and friends in China, especially my mum who was so patient and supportive of my study in Canada. This thesis is also a late gift to my dear grandfather whom I lost recently. Thank you very much.

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List of Abbreviations

AACC: American Association of Cereal Chemists
AAFC-SPARC: Agriculture and Agri-Food Canada Semiarid Prairie Agriculture Research Centre
CPSW: Canada Prairie Spring White
CV: coefficient of variation
CWRS: Canada Western Red Spring
DAA: days after anthesis
DTT: dithiothreitol
ER: estrogen receptor
ET: reference evapotranspiration
FAO: Food and Agriculture Organization of the United Nation
GDD5: growing degree days at the base temperature of 5 °C
GMP: glutenin macropolymer
GS: glutenin subunit
HMW-GS: high molecular weight glutenin subunit
IG: insoluble glutenin
LMW-GS: low molecular weight glutenin subunit
NSERC: Natural Sciences and Engineering Research Council of Canada
R_{max}: maximum resistance to mixing
RP-HPLC: reversed-phase high performance liquid chromatography
S-C: Swift Current
SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis
SE-HPLC: size-exclusive high performance liquid chromatography
SEC: size exclusion chromatography
SG: soluble glutenin
TG: total glutenin
TKW: thousand kernel weight
TP: total crude protein
UPP: unextractable polymeric protein
UV-B: ultraviolet radiation-B
WIP: work input to peak
WPG: Winnipeg
50PI : 50% (v/v) 1-propanol insoluble protein fraction
50PS: 50% (v/v) 1-propanol soluble protein fraction
70PI : 70% (v/v) 1-propanol insoluble protein fraction
70PS: 70% (v/v) 1-propanol soluble protein fraction

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Chapter 1

Introduction

Many published studies have shown that wheat breadmaking quality for a particular sample of wheat or flour is highly dependent on both genotypic and environmental factors related mainly to the gluten forming protein component of endosperm tissue. Wheat gluten proteins consist of two main fractions, gliadin and glutenin, present in approximately equal amounts. The gliadins are monomeric proteins stabilized by intra-molecular disulfide bonds, whereas glutenins represent the polydisperse polymeric fraction of gluten protein comprised of subunits stabilized by intermolecular disulfide bonds. High molecular weight (HMW) subunits account for about 25-35% of the total glutenins, and the remainder are LMW-GS. The molecular weight of polymeric glutenin varies considerably and is considered one of the key fundamental properties of wheat in relation to its functionality for breadmaking and other uses. The qualitative composition and concentration of insoluble or HMW polymeric glutenin in wheat or flour are widely considered to be the most important variables that affect the molecular size distribution of glutenin.

The most important genotype-related variable of gluten proteins is the high molecular weight subunit composition of glutenin. On the other hand, environmental factors during crop development can have very substantial effects on wheat protein content and, in turn, on the proportions of all subfractions of wheat protein, which in turn can affect breadmaking quality. While there exists a large body of knowledge on the effects of environment on gluten proteins of mature wheat, a much smaller subset of information has been reported for wheat protein content and compositional changes during grain development. Moreover, studies which go further to quantify both

environmental factors as well as detailed gluten protein fraction accumulation patterns during kernel development are relatively few. Surprisingly, there are essentially no reports in the literature detailing these sorts of results for western Canadian bread wheat cultivars. Investigating the patterns of variation of wheat gluten proteins during kernel development in relation to weather parameters acquired concurrently, should generate considerable worthwhile new knowledge on the environmental influences and biochemical nature of wheat quality variation for breadmaking. That investigation represented the focus of this thesis research which involved two adapted hard spring wheat genotypes grown in replicated and fertilizer-optimized field trials in two western Canadian locations over two years.

1.1 Objectives

The main objectives of this M.Sc. project are as follows:

- To evaluate the effects of growing season weather during kernel development on the grain filling duration and rate patterns. This was done to develop a basic understanding of the effects of weather differences growing sites and years, and their impact on an easily measured and basic physical parameter of wheat prior to undertaking more compex determinations of environmental effects on the pattern of accumulation of wheat protein and constituent protein fractions during grain development.
- To characterize the patterns of accumulation during kernel development of total wheat protein and protein fractions including gliadin, glutenin, glutenin subunits, and properties related to protein molecular size considerations including ratio of HMW-

to-LMW glutenin subunits, ratio of insoluble-to-soluble glutenin, and ratio of total glutenin-to-gliadin. Determine if differences in accumulation patterns during kernel development of HMW-GS (esp. *Glu-D1* encoded subunits 5+10, 2+12) of wheat cultivars Superb and AC Vista, and other factors, translate into differences in molecular size of glutenin as estimated by solubility fractionation techniques.

- To investigate the influence of growing season weather conditions on protein accumulation patterns during kernel development to better understand the nature of environment effects on wheat protein composition in mature wheat and end-use quality.

Chapter 2

Literature Review

2.1 Accumulation of Wheat Gluten Protein during Kernel Development

Wheat (*Triticum aestivum L.*) gluten proteins consist of two main fractions, gliadin and glutenin, present in approximately equal amounts (Kasarda, 1989; Wieser, 2007). The gliadins are monomeric proteins, soluble in aqueous alcohol solutions with a molecular weight range of 30-60 kDa. The α-, β-, and γ-gliadins have intra molecular disulfide bonds but some γ-gliadins types may be similar to LMW-GS, but with one or two cysteine residues in the N-terminal region that are involved in intermolecular disulfide bonds (Köhler et al., 1993). In contrast, ω-gliadins, also called sulfur-poor prolamins due to very low content of sulfur-containing amino acids, do not contain disulfide bonds (Shewry et al., 1986). Glutenins comprise the polymeric fraction of gluten protein whose subunit composition is stabilized by intermolecular disulfide bonds. High molecular weight (HMW) subunits account for about 25-35% of the total glutenins, and the remainder are LMW-GS (Shewry et al., 2001a). The molecular weight of glutenin subunits varies considerately. HMW-GS range from 67-160 kDa, and LMW-GS range from 23-68 kDa (Kasarda, 1999; Wieser, 2007). Gliadins and glutenins represent wheat storage proteins which account for 85% of the total endosperm protein (Carceller and Aussenac, 1999). Storage proteins are defined as proteins that accumulate progressively during the grain-filling period and act as nitrogen sources for wheat grain germination (Dell'Aquila et al., 1983; Shewry and Halford, 2002). Endosperm storage proteins are considered synonymous with gluten proteins. They are synthesized during grain development and some (most notably glutenins) undergo modifications mainly during the grain desiccation phase, with increased level of aggregation (Bénétrix et al.,

1994). In contrast, albumin and globulins in general constitute non-storage proteins, and are typically metabolic proteins such as enzymes.

DuPont and Altenbach (2003) outlined the process of biosynthesis of wheat grain proteins during kernel development. They are synthesized on ribosomes attached to the endoplasmic reticulum (ER) with the assistance of specific enzymes (e.g. protein disulfide isomerase), and pass into the lumen with the cleavage of an N-terminal signal peptide. The gluten proteins are deposited in the developing endosperm cells in discrete protein bodies, which disappear during the advanced stages of kernel development. At this point, a continuous protein matrix surrounding the starch granules is formed and this is the stage of the mature kernel. The synthesis of all the storage proteins including SDS-unextractable protein was reported to be completed as early as 25 days after anthesis (El Haddad et al., 1997), whereas the most rapid accumulation of SDS-insoluble polymers was observed after 32 DAA by others (Aussenac and Carceller, 2000).

According to Martín del Molíno et al. (1988), albumin and globulins are the dominant proteins throughout the milky phase, when protein fractions related to the formation of the gluten network are absent. Iametti et al. (2006) also demonstrated that wheat grains in the milk-phase (15 DAA) do not contain gliadins. It was reported by Kim et al. (1988) that storage protein first appears in wheat endosperm at 9 DAA and is located within membrane-bound protein bodies. Zhu and Khan (1999) also reported that polymerization of glutenin occurred at 10 DAA or earlier and increased significantly throughout the grain-filling period until maturity. Hao et al. (2006) reported that no glutenin subunits were evident within 8 DAA and appeared around 12 DAA, and the peak amount of the subunits occurred around or after 20 DAA and fluctuated before maturity. Yue et al. (2007) observed that HMW-GS accumulation in grain started at

about 14 and 21 DAA for high and low protein content cultivars, respectively. The aforementioned studies indicate that there is general agreement among researchers that formation of glutenin starts no earlier than about 10 DAA. However, some researchers have come to different conclusions. Skerritt et al. (1988) detected HMW-GS at 8 DAA based on studies of monoclonal antibodies, and Gupta et al. (1996) reported that deposition of HMW-GS, LMW-GS and HMW albumin subunits began as early as 7 DAA, with all accumulating at different rates until maturity. That study showed that the absolute amount of albumins and globulins increased up to 19 DAA and then remained constant, while the absolute amounts of gliadins and glutenin increased with grain development until 31 DAA and 40 DAA, respectively.

Variation between cultivars has been observed. Deng et al. (2006) found that initial formation time of HMW-GS was completed by 10 DAA with strong gluten cultivars but was still only partially formed at this time in cultivars having weak gluten. Moreover, strong gluten cultivars had higher rates of accumulation of HMW-GS than weak cultivars.

Differences in patterns of accumulation of glutenin subunits have also been reported. Panozzo et al. (2001) found that after initial high rate of synthesis of HMW-GS, at approximately the midpoint of grain filling, the rate of synthesis was reduced, and this was followed by a period of more rapid synthesis of HMW-GS in the latter stages of grain development. In contrast, LMW-GS accumulated at a faster rate than HMW-GS. Although the initial synthesis of LMW-GS was delayed by about 7 days, synthesis was continuous and almost linear until physiological maturity, and eventually was present at levels higher than those for HMW-GS at maturity.

Gliadin and glutenin proteins were reported to accumulate together beginning at 10 DAA (Reeves et al., 1986). Contradictory results were reported later (Skerritt et al., 1988) indicating that gliadins started accumulating 4 days after glutenin. Different techniques and protein extracting agents are a likely explanation for different observations of different researchers. Stone and Nicolas (1996a) found that the accumulation of wheat kernel proteins during development was highly asynchronous. They observed that synthesis of albumin/globulin started first, followed by gliadins, then sodium dodecyl sulfate (SDS) soluble glutenin, and finally SDS-insoluble glutenin. Carceller and Aussenac (1999) had very similar observation. More recently, Abonyi et al. (2007) reported that accumulation of glutenin occurred later than the synthesis of gliadins. Panozzo et al. (2001) observed that the maximum rate of synthesis of glutenin occurred approximately 6-8 days after the maximum rate of gliadin synthesis, with its duration extended by a similar period about 10 days. In that study, HMW-GS were detected before LMW-GS.

Interestingly, Skerritt et al. (1988) reported that the development of gliadin and glutenin proteins could be altered by sulfur deficiency. In the condition of sulfur-deficient soil, glutenin was detected a few days after gliadins as observed by many other researchers. However, under non-limiting soil sulfur conditions, glutenin developed before gliadins. Shewry et al. (2001b) also concluded that changes in storage protein composition occur when sulfur is deficient. With the limitation in sulfur availability, total synthesis of gliadin and glutenin decreased. But sulfur-poor ω-gliadins and proportions of HMW-GS have been reported to increase (Moss et al., 1981; Wrigley et al., 1984; Fullington et al., 1987).

While the deposition of different classes of wheat protein is highly asynchronous, the timing of accumulation among various genotypes was found to be very similar (Stone and Nicolas, 1996a), leading to the conclusion that different rates of accumulation of the protein fractions determined genotypic differences in mature wheat protein content and composition. It is agreed by many researchers that the process of protein accumulation is very dynamic and follows an aggregative mechanism (Flint et al., 1975; Cressey et al., 1987; Stone and Nicolas, 1996a; El Haddad et al., 1997; Carceller and Aussenac, 1999; Abonyi et al., 2007). In other words, a decrease in one or more fractions of proteins is compensated by increase in other fractions (Stone and Nicolas, 1996a). Zhu and Khan (1999) proposed that there is a switch in mechanism in the rate of synthesis and incorporation of HMW-GS to form polymeric glutenin. With regard to the individual HMW-GS, Abonyi et al. (2007) found that all of the glutenin subunits are synthesized at the same time with differences in relative quantities, typically having lower concentration of subunits encoded by loci on chromosome A and higher amounts for subunits encoded by chromosomes B and D.

Tao and Kasarda (1989) established the presence of α- and γ-gliadin type protein subunits in glutenin polymers. It was hypothesized that those subunits, which have only one cysteine available for intermolecular crosslink formation may serve as chain terminators during formation of glutenin polymers and this would result in a shift of the size distribution of glutenin polymers to lower molecular weight. Lew et al. (1992) provided additional evidence for the possibility that α- and γ-type gliadins are incorporated into glutenin because of mutations that create an extra cysteine residue which confers the ability to form inter-molecular disulfide bonds.

The process of moisture content reduction at the desiccation phase during kernel development may be important in determining the stage at which disulfide bond formation and therefore glutenin occurs. As early as 1924, it was reported (Woodman and Engledow, 1924) that the first signs of gluten formation corresponded with the commencement of grain desiccation. In many studies, the period of most rapid formation of unextractable polymeric protein (UPP), i.e. insoluble glutenin, was found to coincide with the period of rapid water loss (Jones and Carnegie, 1971; Stone and Nicolas, 1996a; Aussenac and Carceller, 2000; Rhazi et al., 2003; Naeem and MacRitchie, 2005). Carceller and Aussenac (1999) reported that grain dehydration induced the formation of SDS-unextractable polymers, i.e. insoluble glutenin. Popineau et al. (1994a) hypothesized that the substantial loss of water during dessication induces the insolubilisation of accumulated glutenin and promotes further aggregation of glutenin structure by facilitating in particular the interaction between the repetitive domains (intermolecular β-sheets) of glutenin subunits. According to Gupta et al. (1996), SDS-insoluble glutenin formation would be induced only when a certain amount of total polymers was accumulated, i.e. 60-75%. In contrast, Aussenac and Carceller (2000) reported that insoluble glutenin formation was observed quite early in kernel development and that it was not necessary to reach a certain amount of total polymers for the accumulation of insoluble glutenin to occur.

Pérez et al. (1989) observed that accumulation of wheat storage protein is influenced more greatly and more directly by factors of supply of nutrient, mainly nitrogen than was the case for accumulation of starch. Similar results were reported by Jenner et al. (1991). They pointed out that the rate and duration of protein deposition are determined mainly by factors of supply of nitrogen external to the grain, as contrast to

starch deposition that determined mainly by factors that operate within or close to the grain itself. At the stage of grain filling, grain accumulation can be resolved into two components, rate and duration. Both components are variable and display genetic and environmental influences.

However, the two components appear to be quite distinct physiologically. Rate appears to reflect the speed of biochemical reactions involved in the synthesis of starch and protein, while duration is a reflection of the grain's development programming (Sofield et al., 1977a), and is mainly controlled by environmental conditions (Santiveri et al., 2002). Most recently, Dias and Lidon (2009) also concluded that genotype determines grain filling rate, whereas environmental factors, such as temperature affect the duration of grain filling period. Regarding the relationship between grain filling duration and grain filing rates, several authors proposed that longer grain filling periods are associated with lower grain filling rates (Nicolas et al., 1984; Wardlaw and Moncur, 1995; Motzo et al., 1996; Calderini et al., 1999; Santiveri et al., 2002).

2.2 Environmental Effects on Changes of Wheat Protein Quantity and Composition

Generally, environmental conditions significantly affect quantity and quality of wheat proteins and can have major effects on end-use quality. Many environmental factors have been reported to significantly modify grain quality. Those factors include soil fertilization (nitrogen and sulfur), wind speed, temperature, humidity, precipitation etc. Some of these factors have been studied individually, others not. Often the factors interact to cause an effect. It is difficult to generalize about effects of environmental factors which vary in regards to soil fertility, weather and timing and magnitude of the

effect during the growing season. The effects of nitrogen, sulfur, precipitation and temperature are reviewed below.

2.2.1 Nitrogen fertility

Numerous studies on positive effect of nitrogen fertility on wheat production have been reported with grain yield being the principal response factor. Wheat yield and protein content have also long been recognized as being negatively and linear related (Haunold et al., 1962; Campbell et al., 1977; Halloran, 1981; Campbell et al., 1997). However, investigations of relationships between environment and accumulation of storage proteins during kernel development are relatively few. Positive relationship between application of nitrogen (N) fertilizer and wheat protein content have been established by many researchers (Finney et al., 1957; Dubetz, 1972; Bole and Dubetz, 1986; Fowler et al., 1990; Gauer et al., 1992; Gupta et al., 1992; Scheromm et al., 1992; Peltonen and Virtanen, 1994; Pechanek et al., 1997; Zhao et al., 1999b; Daniel and Triboi, 2000; Luo et al., 2000; Triboi et al., 2000; Boehm et al., 2003; Yue et al., 2007; Johansson et al., 2008; Saint Pierre et al., 2008; Ma et al., 2009). However, only when N is no longer the limiting factor to grain yield, is there a positive response in protein content. I. E. if yield is limited due to N deficiency, small additions of N will increase crop yield without increasing protein, and a greater amount of N application is required for genotypes with higher potential grain yield to obtain the same extent of protein increase for genotypes with lower potential grain yield (Fowler et al., 1990). In addition, compared to wheat gluten proteins, some researchers have reported that quantities of albumins and globulins were hardly influenced by different levels of applied nitrogen fertilizer (Pechanek et al., 1997; Wieser and Seilmeier, 1998), and the effect of N fertilizer on increasing content of gliadins was significantly higher than that for

glutenins. It is generally agreed in the literature that the ratio of gliadin/glutenin increases with high N fertilizer level. The observation was seen by increase of gliadin content with no change in glutenin content after N fertilization (Levy et al, 1985), or mainly due to the more pronounced increase of gliadin (Doekes and Wennekes, 1982; Gupta et al., 1992; Prieto et al., 1992; Triboi and Leblevenec, 1995; Jia et al., 1996a; Pechanek et al., 1997; Wieser and Seilmeier, 1998; Zhu et al., 1999; Daniel and Triboi, 2000; Triboi et al., 2000; Saint Pierre et al., 2008), Cultivar-dependent effects of N fertility have also been observed (Prieto et al., 1992; Scheromm et al., 1992; Pechanek et al., 1997). The ratio of gliadin/glutenin was reported to increase in one wheat cultivar whereas a decrease in this ratio was found in another (Pechanek et al., 1997). Also, within the group of gliadins, some researchers have reported that the proportions of ω- and γ-gliadin in total gliadin were increased by N fertilization whereas α- and β-gliadin were decreased (Daniel and Triboi, 2000). According to some other researchers, the proportions of α- and ω-gliadin in total gliadin were increased by high levels of N and proportion of β- and γ-gliadin were decreased (Timms et al., 1981; Prieto et al., 1992; Wieser and Seilmeier, 1998). The decrease of concentration of γ-gliadin could be due to its disproportionate increase of content relative to other gliadins in response to environmental variation. Therefore, as γ-gliadins increased with increasing total protein and gliadin, the relative percentage of γ-gliadins in total gliadin was actually reduced (Huebner et al., 1997). Furthermore, the accumulation of ω-gliadin was relatively more affected by N supply than was the case for α-, β- and γ-gliadins (Wieser and Seilmeier, 1998; Daniel and Triboi, 2001).

Considering glutenin, the composition of glutenin subunits was reported to be remained unchanged as total protein content increased following increased N fertilizer

treatment (Scheromm et al., 1992), but some researchers found the ratio of LMW-/HMW-GS decreased with increasing N fertilization either by increasing relative amount of HMW-GS and decreasing LMW-GS (Pechanek et al., 1997), or by increasing more of HMW-GS than LMW-GS (Wieser and Seilmeier, 1998).

Rate of nitrogen application has also been reported to be one of the most important factors influencing the accumulation pattern of wheat gluten protein (Altenbach et al., 2003; Johansson et al., 2004). Johansson et al. (2008) reported that raising nitrogen fertilizer rate from 160 to 200 kg/ha resulted in significant (20%) increase in the amount of SDS-unextractable protein. Yue et al. (2007) also showed that amount of glutenin macropolymer (GMP) and HMW-GS was increased with increasing nitrogen rate between 0 and 225 kg/ha. Content of GMP was reported to have a positive relationship with flour protein content (Spiertz et al., 2006) and baking quality (Weegels et al., 1996; 1997), however, there seemed to be an optimum point for the N enhancement effect. Yue et al. (2007) observed that the percentage of GMP in flour and content of HMW-GS per grain decreased when the nitrogen fertilization rate reached at a very high level of 300 kg/ha. Genotypic differences have also been observed. The synthesis of HMW-GS in a low protein content cultivar was more sensitive in N response than that for a high protein content cultivar (Yue et al., 2007). In addition, Spiertz and De Vos (1983) pointed out that the later the nitrogen is applied, the greater the effect on protein percentage and the less the influence on yield. Luo et al. (2000) also reported that late N application produced higher absolute amounts of LMW- and HMW-GS, and glutenin quantity. Some studies also showed that addition of nitrogen under high temperature regimen (37 °C) had almost no effect on rate of protein accumulation as opposed to the

increasing effect under moderate temperature regimen (24 °C) (Zahedi et al., 2004; DuPont et al., 2006b).

Molecular weight distribution of gluten protein has also been demonstrated to be one of the main determinants of physical dough properties (Southan and MacRitchie, 1999). In theory, the molecular weight distribution can be altered from one wheat sample to another by changes in the relative proportions of gliadin and glutenin or by changes in the size distribution of glutenin (MacRitchie and Lafiandra, 1997), or by changing both the gliadin to glutenin protein ratio and the molecular weight distribution of glutenin (Lemelin et al, 2005). It has been reported that the decrease of gluten strength (Johansson et al., 2004), and reduced values of mixograph peak time, stability and tolerance as N fertilization rate increased (Saint Pierre et al., 2008) was likely due to a higher ratio of gliadin to glutenin. The weakening effect of dough could be the consequence of the shift in the molecular size distribution under N fertilization treatment.

2.2.2 Sulfur fertility

Based on the sulfur content, wheat proteins can be classified into sulfur-rich i. e. albumins, globulins, α-, β-, and γ-gliadins and LMW-GS, and sulfur-poor i. e. ω-gliadins and HMW-GS (Wrigley et al., 1984; Shewry et al., 1986). Of these, ω-gliadins were reported to be very low in S-containing amino acids, with no cysteine or methionine residues (Shewry et al., 1997).

As the wheat protein content increases with nitrogen fertilization, the levels of sulfur relative to nitrogen decrease, correlating with a lower proportion of methionine as well as cysteine, which is an essential amino acid in forming disulfide bonds and therefore influencing the polymerization of glutenin. Evidence of decreased protein

quality with insufficient sulfur relative to nitrogen has been established (Byers and Bolton, 1979; Moss et al., 1981; Haneklaus et al., 1992). Insufficient S supply also increased the proportion of non-gluten nitrogen and decreased the amount of cysteine and methionine in wheat (Yoshino and McCalla, 1966; Wrigley et al., 1984; Byers et al., 1987). According to Castle and Randall (1987) and Skerritt et al. (1988), the synthesis and accumulation of gluten protein occurred earlier in S-deficient grain than in the S-sufficient grain. This may be due to the effect of S deficiency on shortening initial seed development, which is characterized by a high rate of cell division and a low rate of protein accumulation (Zhao et al., 1999a).

When S was over supplied alone, reduction of glutenin quantity was observed (Luo et al., 2000). However, supply of S nutrition generally had little effect on the concentration of total protein (Zhao et al., 1999a), but the proportion of S-poor to S-rich gluten protein was reported to be dependent on S availability (Moss et al., 1981; Wrigley et al., 1984; Fullington et al., 1987). Under conditions of sulfur deficiency, sulfur-poor proteins increased in amounts at the expense of the sulfur-rich proteins, resulting in increasing the ratio of HMW-GS/LMW-GS and portion of polymeric proteins unextractable in SDS-buffer solution, causing the molecular weight distribution of glutenin to be skewed towards higher molecular weight. This imbalance can lead to over-strong dough with low extensibility (MacRitchie and Gupta, 1993). This result agreed with several other researchers' observations regarding the correlation between S concentration and S-rich or S-poor protein, and the general effects of varying S supply on dough functional properties. A positive correlation to dough quality was found between S-rich protein and S concentration, and a negative correlation was found between S-poor protein and S concentration (Wrigley et al., 1984; Castle et al., 1987;

Fullington et al., 1987). It was also reported that decreasing S supply resulted in increased dough strength and reduced extensibility (Moss et al., 1981; Timms et al., 1981; Wrigley et al., 1984). More recently, Wieser et al. (2004) reported that the amount of ω-gliadin was increased considerably as S availability was reduced, with moderate increase of the other S-poor protein fraction of HMW-GS. On the other hand, the amount of S-rich γ-gliadins and LMW-GS were significantly decreased and smaller reduction was found for α-gliadins (Wieser et al., 2004). The ratio of polymeric to monomeric proteins (Zhao et al., 1999b) and percentage of polymeric protein in total protein (MacRitchie and Gupta, 1993) were also reported to be decreased with lower S availability due to the significant decrease of LMW-GS. Because LMW-GSs are the major components of glutenin, the net effect of decreasing S level would be to decrease the proportion of glutenin in total protein.

The effects of S on dough rheology are consistent with its effects on the gluten composition. Zhao et al. (1999b) reported that application of sulfur fertilizer significantly decreased dough resistance, and increased dough extensibility and loaf volume. Decreased dough resistance due to S fertilization may be explain by a decrease in the ratio of HMW-GS/LMW-GS and a consequent shift to lower molecular weight (MacRitchie and Gupta, 1993), whereas S deficiency decreased dough extensibility probably due to the decreases in the proportion of low molecular weight glutenin and some gliadins (Flate et al., 2005).

It has been frequently reported that there is a positive interaction between sulfur and nitrogen fertilization reflected in increased grain yield (Byers and Bolton, 1979; Randall et al., 1981; Reneau et al., 1986; Salvagiotti and Miralles, 2008), higher nitrogen use efficiency by increasing the nitrogen recovery from the soil (Salvagiotti and Miralles,

2008; Salvagiotti et al., 2009), and improved dough properties such as higher Pelshenke values and mixograph peak heights (Luo et al., 2000). This synergistic effect between sulfur and nitrogen likely caused because sulfur is an essential constituent of enzymes involved in nitrogen metabolism i. e. nitrate reductase and nitrite reductase (Mendel, 1997; Campbell, 1999; Swamy et al., 2005).

It has been reported that breadmaking quality correlated more closely with S fertility than with N fertility (Zhao et al., 1999b). They found that loaf volume was significantly increased by S application up to 100 kg/ha but there was no significant effect by applying additional N.

2.2.3 Precipitation/water stress

In general, in semi-arid environments, crop yield and nitrogen uptake are typically limited by available water (Soon et al., 2008). While in non-dryland growing location, Greaves and Carter (1923) found that the nitrogen, hence protein content of the wheat decreased as the quantity of irrigation water increased. Correll et al. (1994) also found that growing conditions involving excess rainfall was associated with a decrease in wheat protein content. On the other hand, Palta et al (1994) found that water deficit increased transfer of pre-anthesis N from the plant to the grain, however it also decreased the pos-anthesis uptake of N, resulting in no general effect on grain protein accumulation, but total starch accumulation was reduced. Plaut et al. (2004) also reported that the rate of dry matter accumulation was decreased considerably by water deficit. Brooks et al. (1982) reported that water stress significantly decreased the number of B-type starch granules in grain endosperm. More recently, Singh et al. (2008) found that B-type and C-type granules decreased in response to water stress whereas A-type granules increased.

The content of wheat protein and total starch content (Kim et al., 2003), as well as protein accumulation rate and starch accumulation rate (Fernandez-Figares et al., 2000), were shown to be inversely related to each other, indicating competition in the transport of proteins and carbohydrates to the grain. It has been consistently reported that drought condition increases grain protein content at maturity while waterlogging reduces the amount (Altenbach et al., 2003; Ozturk and Aydin, 2004; Jiang et al., 2009). Jiang et al. (2009) also found that contents of GMP and HMW-GS did not increase with increasing protein content under drought; they were both reduced by experimentally produced drought and waterlogging conditions. In addition, the ratios of GMP and HMW-GS to total protein were also decreased by the conditions, but there were genotypic difference in ratio of HMW-GS/GMP in responding to drought and waterlogging conditions. The cultivar with high grain protein content had similar ratios of HMW-GS/GMP with various conditions whereas the ratio was reduced by water treatments in cultivar with low grain protein content. A genotypic effect of water stress on accumulation of glutenins, HMW-GS and LMW-GS, was also observed (Singh et al., 2008) i.e. water stress increased the parameters in some cultivars but decreased in others. Zhao et al. (2009) reported that proper water deficit (45% of soil water capacity) favored protein formation, increased the percentage of total protein, gliadin and glutenin, as well as the ratio of glutenin/gliadin compared to 85% of soil water capacity. Water stress (deficit) decreased the amount of albumins and globulins (Konopka et al., 2007).

Interestingly, Panozzo and Eagles (1999) showed that grain protein contents on a per kernel basis were similar in both irrigated and non-irrigated environments, but grain weights were much higher from the irrigated environment, which likely resulted from greater maximum rate and duration of grain filling.

Generally, irrigation treatments had less impact on protein content and composition than N fertilization treatment (Saint Pierre et al., 2008). A significant irrigation and fertilization interaction for flour protein was observed. Increase in flour concentration due to N fertilization was higher under moisture stress than under well-irrigated situations. (Saint Pierre et al., 2008). Flour protein content is significantly increased under water deficit, mainly due to higher rates of accumulation of grain N and lower rates of accumulation of carbohydrates (Guttieri et al., 2000; Ozturk and Aydin, 2004). The increase in protein was also likely to be a function of yield reduction induced by water stress (Saint Pierre et al., 2008). Irrigation, on the other hand, may decrease flour protein content by dilution of N with starch in the grain (Guttieri et al., 2000; Ozturk and Aydin, 2004).

2.2.4 Temperature

2.2.4.1 General effects

Temperature effect is one of the most comprehensively studied environmental factors with respect to wheat development. However, it is not always possible to directly compare published results due to the differences in experimental approaches and designs, e.g. different cultivars and temperature regimes used. The first publishing documenting a relationship between high temperature and gluten quality (Mangels, 1925) observed that conditions producing high protein content did not always result in high quality gluten for breadmaking. Numerous studies have shown that increase of temperature after anthesis results in higher grain protein content (Sofield et al., 1974; Kolderup, 1975; Sofield et al., 1977b; Schipper, 1991; Stone et al., 1997; Uhlen et al., 1998; Daniel and Triboi, 2002; Asseng and Milroy, 2006; DuPont et al., 2006b; Spiertz et al., 2006). However, some researchers reported negligible to slightly negative

responses of grain protein to high temperature growing conditions (Bhullar and Jenner; 1985; Stone and Nicolas, 1995c), as well as negative effect on glutenin polymerization (Motzo et al., 2007). Furthermore, high temperature may also cause lower nutritional properties due to reduced albumin/globulin content, despite an increased protein concentration (Stone and Nicolas, 1996b).

DuPont et al. (2006a) reported that high temperatures (24-40 °C) during grain development increased protein content in general and altered the proportion of protein fractions; higher accumulation rate for S-poor proteins (ω-gliadin) than for S-rich proteins (LMW-GS, α- and γ-gliadins). One possible reason for protein percentage tends to increase with rising growing temperature could be because the accumulation of protein is less sensitive to high temperature compare to starch (Sofield et al., 1974; Chowdhury and Wardlaw 1978; Bhullar and Jenner 1985; Jenner et al. 1991; Rao et al. 1993; Stone et al., 1996). Ciaffi et al. (1996) further suggested that very high temperature (>35 °C) induced decrease in HMW-GS synthesis. Don et al. (2005) observed that with increasing heat stress (from 30 °C to 40 °C), less GMP (i. e. insoluble glutenin) was formed; in addition, the amount of GMP was more sensitive to temperature than for total protein, i.e. heat stress caused reduction in GMP to a greater extent than total protein. It is somewhat in accordance with the previous conclusion made by Stone et al. (1996), and Stone and Nicolas (1996b) that the accumulation of SDS-insoluble polymer tended to be more sensitive to heat stress than gliadins. However, Carceller and Aussenac (2001b) found that heat stress during kernel development induced more rapid disulphide bond formation and favoured formation of

large sized glutenin. This may explain why Don et al. (2005) observed larger particles formed from a smaller amount of glutenin.

One of the other major effects of temperature stress on protein content in mature wheat is its effect on accumulation of starch and ultimately grain yield. Under non-limiting water and nutrient supply, grain yield is a function of the amount of light energy intercepted and utilized in carbon assimilation, and the partitioning of the carbon between above-ground biomass and the grain (Evans and Fischer, 1999). Several studies conducted in Australia indicated that annual crop production decreases about 10-15%, mostly because of high temperatures during anthesis (Wardlaw and Wrigley, 1994). Wardlaw et al. (1989) earlier pointed out that a global reduction in crop production of about 3-4% occurs when the mean temperature increases by 1 °C above the optimum value. The optimal temperature to achieve maximum yields is generally considered to be between 15 and 20 °C (Herzog, 1986), with 20 °C being the best temperature for grain filling (Jenner, 1991). Temperature in this range gives the longest duration of grain fill and the greatest accumulation of starch per grain (DuPont and Altenbach, 2003). With further increases to 30 °C, the extent of both protein and starch synthesis seems to be reduced, with starch being more affected than proteins (Kolderup, 1975). Accordingly, high temperature during grain filling tends to lead to high protein contents due to reduction in starch accumulation.

Altenbach et al. (2002, 2003) proposed that under the high temperature regimes, the overall grain development was accelerated and compressed, resulting the earlier accumulation of starch and protein, as well as the shortened time to reach maximum fresh and dry weight. Additionally, the combination of high temperature and drought affected more than either treatment alone.

2.2.4.2 Heat stress

When temperatures rise over 35 °C during kernel development, specific responses are induced, which are not prevalent under moderately high temperatures. For example, it was reported that moderately high temperatures affected only protein percentage (Wrigley et al., 1994), whereas the response to very high temperature was related to deleterious changes in protein composition (Blumenthal et al., 1991b). Even a few days of very high temperature (35-40 °C) during grain development could have a negative effect on grain quality (Ciaffi et al., 1996).

It has frequently been reported that glutenin/gliadin ratio decreases with increased grain protein percentages under heat stress (Abrol et al., 1971; Dubetz et al., 1979; Doekes and Wennekes, 1982; Stenram et al., 1990; Stone et al., 1996). Some researchers (Abrol et al., 1971; Dubetz et al., 1979; Blumenthal et al., 1990b) ascribed the decrease in this ratio to a concomitant increases in both gliadin and glutenin accumulation, where gliadin accumulation was more rapid, others believe a greater reduction in accumulation of glutenin was the main factor (Stone et al., 1996; 1997) because accumulation of glutenin was more sensitive to heat stress than that for gliadin (Stone and Nicolas, 1996b).

Stone and Nicolas (1994) suggested that gluten protein synthesis does not immediately and/or fully recover from short (3 days), severe heat stress. Treatment such as post-shock cooler temperatures also did not alleviate the heat shock effects such as reduction in grain yield (Stone et al., 1995). In addition, heat stress effects on protein content appeared to become evident much later than the time when heat stress happened. For example, there were no significant decreasing effects of heat stress on gliadin and glutenin content until 10 and 25 days, respectively when heat stress was terminated

(Stone et al., 1996). It was also suggested that the synthesis of intermediate products rather than the mature proteins themselves is interrupted by very high temperature (Stone et al., 1996). In the reaction to sudden as opposed to gradual rise to a very high temperature (>40 °C), the deposition of glutenin was more responsive to sudden heat shock than to gradual heat stress, although both heat treatments increased the accumulation of SDS-insoluble glutenin (Stone and Nicolas, 1998a). Also reported are significant genotypic differences in response to heat stress. Sudden heat treatment significantly reduced the accumulation of gliadin in a heat-sensitive cultivar, but not a tolerant one (Stone and Nicolas, 1995b; 1998a). Deng et al. (2008) also reported that accumulation of HMW-GS from a genotype with weaker gluten strength appeared to be less tolerant to different timing of heat treatment than the stronger one. Content of HMW-GS in genotype with weaker gluten strength was significantly higher when heat treatment was applied at 20 DAA compare to 15 DAA and 25 DAA, whereas influence of different timing of heat treatment was not significant in stronger one. Some researchers reported that genotypes possesses "5+10" HMW-GS alleles was more sensitive to heat shock (Spiertz et al., 2006), whereas others concluded that cultivars with "2+12" HMW-GS alleles were more sensitive to heat shock (Anderson and Green, 1989; Blumenthal et al., 1995b; Don et al., 2005; Irmark et al., 2008). The greater resistance to heat stress of genotype with "5+10" may be related to the greater number of cysteine residues in HMW-GS 5 (Anderson and Green, 1989). The higher concentration of S-H groups in genotype with higher amount of cysteine residues resulted in faster rate of S-S formation, and then faster rate of protein polymerization (Irmark et al., 2008). Also genotypes with "5+10" were reported to have glutenin polymerize at an earlier time than for "2+12" type genotypes (Carceller and Aussenac, 1999; Gupta et al., 1996;

Naeem and MacRitchie, 2005). Earlier formation of larger glutenin polymers may help alleviate the detrimental heat stress effect, i. e. making those genotypes more heat tolerant (Irmark et al., 2008).

Various mechanisms of heat stress influence on grain development, and at which stage the effects manifest, have been proposed. Bernardin et al. (1995) reported that synthesis of HMW-GS was suppressed by heat stress while there was still a continuous synthesis of both gliadin and LMW-GS. Ciaffi et al. (1996) also observed changes in composition of glutenin (soluble/insoluble glutenin) without changes in synthesis of gliadin in heat-shocked samples, and suggested that heat stress (>35 °C) may affect the mechanism by which intermolecular disulphide bonds are formed during the deposition of glutenin. Stone and Nicolas (1996b) reported that heat stress affected both synthesis of intermediates and the process governing the formation of mature protein. Disulfide-bond formation in wheat gluten occurs in endoplasmic reticulum with the assistance of specific enzymes (e.g. protein disulphide isomerase) (DuPont and Altenbach, 2003). Heat-shock may cause a selective destabilization of certain protein mRNAs, therefore affecting the assembly of glutenins into polymers (Brodl and Ho, 1991). Panozzo et al. (2001) showed that high temperatures could impede the synthesis of gluten proteins from non-prolamin precursors. Treglia et al. (1999) proposed that wheat plants respond to heat stress by increasing the synthesis of different heat-shock proteins. In Australia where heat stress is a relatively common occurrence, many studies have investigated these effects. Blumenthal et al. (1990a; 1991a) identified multiple heat-shock elements in gliadin genes (but not glutenin genes) and defined those proteins as heat-shock proteins. They presumed that the heat-shock elements of the gliadin genes permit continued gliadin synthesis under heat stress, while glutenin synthesis was decreased.

According to Pelham (1982), synthesis of heat-shock protein accompanied by repressing of other proteins, was an ancient and evolutionarily conserved response to thermal stress, which served as a protective function to protein synthesis. Further study also showed that synthesis of heat-shock protein was related to protective effects, i. e. reducing synthesis of HMW-GS but continuing synthesis of gliadin, which was acting as heat-shock proteins, and a preliminary heat shock improved thermal tolerance therefore provided a degree of protection against a later lethal shock (Blumenthal et al., 1994). It was suggested that very high temperature (>35 °C) during grain filling could activate heat shock elements in gliadin genes which would result in reduced dough strength (Blumenthal et al., 1991b). Synthesis of heat-shock proteins was reported to be correlated with heat tolerance in wheat (Nguyen et al., 1994).

On the other hand, Corbellini et al. (1998) observed that the most sensitive periods to heat stress was between early and mid grain filling. This differs from other reports that heat shock during early stage caused small effects, while damage was greater between midpoint to the final stage of grain filling (Randall and Moss, 1990; Borghi et al., 1995; Corbellini et al., 1997). These differing observations suggest that heat stress outcomes depend on the time, rate and intensity of exposure to high temperature conditions during kernel development. Wheat plants can gradually alter their metabolism to adjust to heat stress temperatures, thereby acquiring some measures of tolerance (Porter and Gawith, 1999).

Additionally, how temperature is measured in all the aforementioned studies may also contribute to the different conclusions. Triboi et al. (2003) showed that at moderate temperature (15-20 °C) during grain-filling increased 40% and 60% of the rate of N accumulation with an increase in temperature of 7 °C and 9 °C, respectively. Meanwhile

duration of N accumulation was reduced from 48 days to 28 days when temperature was raised from 15 °C to 24 °C. However, when the temperature was measured in thermal time or degree-days, no above differences were observed for the protein accumulation. This was consistent with previous reports that the rates and duration of accumulation of gliadins and glutenin expressed in degree-days were not by influenced by moderate temperature (<25 °C) (Daniel and Triboi, 2001; 2002). Johansson et al. (2005) similarly reported that the rate of accumulation of glutenin differed with varying temperature (15-25 °C) if time was measured in days after anthesis, but did not change if time was measured in degree-days. More, it needs to be understood about the mechanism and effects of temperature and heat stress on gluten protein accumulation patterns.

All the effects of genotypes and especially environment on wheat protein content and composition during kernel development clearly translate into effects on breadmaking quality. Many excellent reviews exist that cover this topic (i.e. effects on breadmaking quality) comprehensively (Finney, 1943; Finney and Barmore, 1948; Finney and Fryer, 1958; Webb et al., 1971; Orth and Bushuk, 1972; Dexter and Matsuo, 1978; Payne et al., 1979; 1987; Bushuk, 1984; Odenbach and Mahgoub, 1988; MacRitchie et al., 1990; Randall and Moss, 1990; Blumenthal et al., 1991a; 1993; 1995a; Gupta et al., 1992; 1993; 1994; 1995; Kolster and Vereijken, 1993; Fenn et al., 1994; Gupta and MacRitchie, 1994; Popineau et al., 1994b; Wrigley et al., 1994; Borghi et al., 1995; Graybosch et al., 1995; Weegels et al., 1996; Corbellini et al., 1997; Huang and Khan, 1997; Corbellini et al.,1998; Gibson et al., 1998; Panozzo and Eagles, 2000; Johansson et al., 2000; Skylas et al., 2002; Zhu and Khan, 1999; 2002; 2004; Altenbach et al., 2003; DuPont et al., 2006a; 2006b; Stathopoulos et al., 2006; Tahir et al., 2006; Finlay 2006; Finlay et al., 2007; Jarvis et al., 2008; Labuschagne et al., 2009; Zhang et

al., 2009). This topic will not be reviewed here given the focus of this thesis research on protein content and compositional changes during kernel development.

Chapter 3

Relationship between Weather and Patterns of Accumulation of Wheat Grain Dry Matter during Kernel Development

3.1 Abstract

Studies on genotype and environmental effects on wheat gluten protein and wheat quality for breadmaking have been going on for decades. Analyzing basic grain filling patterns (duration and rate) is, in principle, the first step needed to understand the environmental effect on outcomes such as wheat protein content and protein compositional changes during kernel development which was the focus of this thesis research. Two hard spring wheat cultivars (Superb and AC Vista) were grown at two locations (Winnipeg, MB and Swift Current, SK) in replicated plots under optimal soil fertility conditions in two consecutive seasons (2003 and 2004). Wheat heads were sampled at 3 or 4 day intervals during kernel development from anthesis to maturity. Heads were preserved by freezing at field sites and subsequent freeze drying, and were threshed by hand.

Meteorological information used in the study was acquired concurrently with measurement on the kernels. Environmental variables included hourly measurements of solar radiation, air temperature, humidity, precipitation, useful heat (GDD5, growing degree days > 5 ^{o}C), evapotranspiration, and modeled water use, demand and deficit.

All four site-years provided very different weather conditions during the growing season and grain development periods resulting in substantial differences in grain filling duration and accumulation patterns of total protein and protein fractions. The relationships between grain yield and grain filling duration and grain filling rate, as well

as the relationships between weather parameters and grain filling duration, and thousand kernel weight (TKW) were studied.

Results showed that longer grain filling duration was strongly associated with smaller grain filling rate (r=-0.77) and higher grain yield (r=0.81). But grain yield was not associated with grain filling rate (r=-0.30) and kernel weight accumulating rate (r=0.25). The strongest positive relationship between grain filling duration and weather conditions was observed for total rainfall (r=0.91), and strongest negative relationships were observed in average daily air temperature, average daily ET and average daily GDD5 with correlation coefficient r=-0.88, -0.92, -0.90, respectively. Total solar radiation, total water use and total water deficit had weaker positive relationships with correlation coefficients, r = 0.56, 0.64, 0.56, respectively. The least correlated weather parameters were average daily wind speed (r=-0.33) and total water demand (r=0.13). In addition, cumulative GDD5 and ET had the strongest interpretation power regarding the difference of accumulation pattern of TKW.

3.2 Introduction

Extensive research on the genotype and environmental effects on wheat quality for breadmaking has shown significant genotypic effects, and in most cases even more significant environmental effects (Baker et al., 1971; Fowler and De La Roche, 1975; Baker and Kosmolak, 1977; Randall and Moss, 1990; Marchylo et al., 1990; Peterson et al., 1992; 1998; Stone and Nicolas, 1995c; 1996b; Ames et al., 1999; Landau et al., 2000; Panozzo and Eagles, 1999; 2000; Preston et al., 2001; Zhang et al., 2004; Tahir et al., 2006; Finlay et al., 2007; Jarvis et al., 2008).

Studies of environmental effects on wheat growing and accumulation pattern have shown that temperature is the main factor determining growth rate after anthesis and the time period available for grain filling (Sofield et al., 1977a; Slafer and Rawson, 1994; Wheeler et al., 1996). Many reports have also revealed that high temperatures during kernel development significantly shorten grain filling for bread and durum wheat (Sofield et al., 1977a; Shpiler and Blum, 1986; Gibson and Paulsen, 1999; Dias and Lidon., 2009). Another important factor influencing the duration of growth stage of cereals may be water supply and moisture stress. Long grain filling periods in grains have been associated with relatively low temperatures and high levels of precipitation (Schelling et al., 2003). Whereas temperature has a direct effect on the dynamics of kernel development processes, the relationships between precipitation and crop growth are more complex because they interact with other factors of soil water balance such as water demand. It seems to be the case that plants respond to absolute rather than relative changes in temperature but their response to water deficit is an ongoing and resilient processing (Tanner and Sinclair, 1983). According to Plaut et al. (2004), the rate of grain dry matter accumulation decreased considerably by water deficit. Thousand-kernel weight and weight of kernels per spike also decreased. Solar radiation was reported to have no or minor effect on grain dry matter growth rate (Sofield et al., 1974; 1977a). Evapotranspiration (ET), the sum of transpiration by the crop (T) and evaporation from the soil (E), was found to have positive and curvilinear relationship to grain dry matter production, and water use efficiency (Zhang et al., 2008). Wind speed is believed to be an important factor driving evapotranspiration.

The objective of this initial study of the thesis research was to evaluate the effects of growing season weather during kernel development on the grain filling patterns. This

was done to develop a basic understanding of the effects of weather differences growing sites and years, and their impact on an easily measured and basic physical parameter of wheat prior to undertaking more compex determinations of environmental effects on the pattern of accumulation of wheat protein and constituent protein fractions during grain development.

3.3 Materials and Methods

3.3.1 Wheat samples

Two genotypes were selected for this study, Superb (Canada Western Red Spring (CWRS) wheat) and AC Vista (Canada Prairie Spring White wheat (CPSW)), each grown in two locations (Winnipeg and Swift Current) and in two different years (2003, 2004). Plots at each location for each genotype were triplicated. The fields were optimally fertilized based on standardized soil tests. Details concerning the field setup, as well as yield and mature wheat quality information for Superb and AC Vista have been published (Finlay et al., 2007). In brief, AC Vista was higher yielding by 16% (site-year average), had 1.1% lower grain protein content (14.2 vs. 13.1%) and had kernels of higher average weight (by 9%), had kernels of higher average thousand kernel weight (by 9%), and 15% higher starch damage (6.74% for AC Vista, 5.87% for Superb) indicating that kernels were harder.

From a glutenin protein composition perspective, these two genotypes had significantly different HMW glutenin subunit compositions at all three gene loci (Table 3.1), most notably at the *Glu-D1* locus. Superb possesses subunits 5+10 while AC Vista has subunits of 2+12. This is a classically recognized contrast in HMW-GS composition suggesting that Superb had a theoretical advantage in breadmaking quality based on

higher molecular size of glutenin (Gupta and MacRitchie, 1994; Carceller and Aussenac, 2001b). There were a few other notable differences between these genotypes (Finlay, 2006) such as grain yield (4069.6 kg/ha for Superb and 4712.5 kg/ha for AC Vista), averaged across seven environments grown in 2003 and 2004. There was relatively little practical difference in breadmaking quality for the two cultivars, however loaf volume (long fermentation bake test) of Superb was higher (1002 cc) compared to AC Vista (855 cc). That difference appears to be due to the protein content difference between the two wheat samples.

Table 3.1. Commercial class and high- and low-molecular weight glutenin subunit composition of wheat cultivars

Cultivar	Wheat Class	Glu A1	Glu B1	GluD1	Glu A3	Glu B3	Glu D3
Superb	CWRS	2*	7*+9	5+10			
AC Vista	CPSW	1	7+8	2+12	G	i	c

3.3.2 Sampling of wheat heads

The date of anthesis for the first field replication triggered the start of sequential wheat head sampling in all plots at each site. Starting at 7-10 days after 50% anthesis, head samples were taken from each plot twice weekly at 3-4 day intervals (e.g. Monday and Thursday) until harvest maturity. Heads were clipped from the outside 4 rows on either side of the plot and within 30 cm of the end of the plot. The center 8 rows and inside 3 m were left for yield determination.

In order to maintain the biochemical integrity of the immature wheat, heads immediately after cutting in the field were chilled in coolers containing dry ice and within 2 hr were placed in frozen storage at -20 °C. It was not practical to freeze the heads when cut in the field using liquid nitrogen, as is the preferred technique in the

context of a green house study of kernel development, which would involve a much smaller number and quantity of wheat heads. Following harvest of all the plots, the frozen heads at the Swift Current site were transported to Winnipeg in the frozen state by truck in chest freezers containing dry ice. Head samples were subsequently stored at -20 °C at the University of Manitoba, and subsequently freeze-dried, threshed and thoroughly cleaned in preparation for analysis.

3.3.3 Growing season weather

Several key meteorological parameters were monitored such as average daily air temperature, average daily ET, average daily windspeed and precipitation (Table 3.2). All meteorological data used in this study were obtained from an existing database as previous described (Finlay, 2006; Finlay et al., 2007). The 2003 and 2004 crop years in Western Canada provided a wide range of growing conditions across the study sites, leading to a very diverse set of wheat quality characteristics for the mature wheat, which has already been established in other studies. As shown in Table 3.2, the 2003 season had warmer and dryer conditions for crop growth, with similar average growing season temperatures across the two sites of about 21-22 °C. The 2004 season was much cooler and wetter, with average growing season temperatures of about 16°C. Compare to the Winnipeg site, weather conditions in Swift Current were more variable between two crop seasons. In 2003, Swift Current had very low precipitation, high evapotranspiration, high solar radiation, and high water deficit (Table 3.2). The most distinct weather condition for Swift Current was wind speed, which was about 2.6 times higher than that for Winnipeg. It was expected that wheat growing in Swift Current site would have

different protein accumulation patterns compared to the Winnipeg. Detailed weather conditions in four study sites are presented in Appendix A. 4.

Table 3.2. Growing season weather conditions at the two study sites

		Growing Season Mean [a]							
Site	Year	Temp [b]	ET [c]	Rad [d]	Wind [e]	Prec [f]	Watdem [g]	Watuse [h]	Watdef [i]
Swift Current	2003	22.5	7.11	21.35	4.00	0.70	2.15	0.28	-1.87
Swift Current	2004	15.7	3.28	18.53	4.68	1.61	2.77	1.89	-0.87
Winnipeg	2003	21.7	3.90	16.23	1.07	1.14	1.25	1.17	-0.08
Winnipeg	2004	15.6	2.58	12.28	2.14	3.90	1.07	0.88	-0.19

[a] Mean daily value between anthesis and maturity
[b] Air temperature (°C)
[c] Evapotranspiration measured as standard method of Food and Agriculture Organization of the United Nation (FAO), Irrigation and Drainage Paper No. 56 using Penman-Monteith (PM) equation (mm/d)
[d] Solar radiation (MJ/m^2)
[e] Wind speed (m/s)
[f] Precipitation (mm)
[g] Water demand measured as standard method of FAO (mm)
[h] Water use measured as standard method of FAO (mm)
[i] Water deficit measured as standard method of FAO (mm)

3.3.4 Thousand kernel weight

The objective of this study was to investigate the patterns of the accumulation of grain dry matter during the kernel development and the effect of weather on grain weight. Wheat kernels were threshed by hand from freeze dried spikes. Cleaned samples were subsequently freeze dried and kept at -20 °C freezer in polypropylene bags until analysis. Samples were thawed prior to analysis. One hundred sound kernels were randomly picked from each bag and the subsample was weighed using a three-place analytical balance (Fisher Scientific, Mettler PJ 300). The kernel weight (dry basis) was converted to thousand kernel weight (TKW).

3.3.5 Grain filling duration, grain filling rate and kernel accumulation rate

Duration of grain filling was measured as the time from anthesis to physiological maturity. Average rate of grain filling was estimated as maximum kernel weight divided by duration, assuming that grain weight is zero at anthesis. Kernel weight accumulating rate was evaluated by taking the slope of the linear relationship between TKW and time span measured as days after anthesis (see Fig 3.2).

3.4 Results and Discussion

3.4.1 Grain filling patterns

The growth of a wheat grain followed a recognized pattern of three distinct phases (Loss et al., 1989). The first one, usually referred to as the initial lag phase, is a short period of exponential growth following anthesis, during which rapid division of endosperm cells occurs and the potential size of the grain is determined (Brocklehurst, 1977). The second phase is characterized by a constant rate of grain growth and has been reported to vary from 0.5 mg/grain·day to 2.3 mg/grain·day, depending on growing conditions (Sofield et al., 1977a; Simmons and Crookson, 1979) and genotype (Gleadow et al., 1982). Grain growth ceases at the final phase, which is initiated when lipids are deposited in the phloem; strands supplying the grain with assimilates (Zee and O'Brien, 1970). This phase coincides with maximum dry matter in the grain and is also referred to as physiological maturity (Hanft and Wych, 1982).

Duration and timing of the development stage, depending on existing environmental conditions, may have remarkable differences from site to site and year to year. Results showed that cultivars Superb and AC Vista had significant environment-caused differences in growth patterns as reflected by the duration of the phenological phases,

but no genotypic difference in grain filling characteristics (Fig 3.1). This result differs from Sofield et al. (1977a) where substantial differences among genotypes were found in grain filling patterns, and from Santiveri et al. (2002) where a significant genotype and environment interaction was detected for the duration of grain filling. In this study, 2004 was a much longer growing season than 2003, which was due to the former's much cooler and wetter condition. No significant difference was observed between different locations for the entire growing period. However, differences were observed between locations for growing periods before and after anthesis (Table 3.3). Wheat grown in Winnipeg had a development period from seeding to anthesis about 9 days shorter than grain grown in Swift Current in 2003 and 16 days shorter in 2004. However, for the anthesis to maturity phase, Swift Current was about 10 days shorter than Winnipeg in 2003, and 15 days shorter in 2004. Accordingly, grain developed much more quickly in Swift Current with most of that difference occurring during the anthesis to soft dough stage (Fig 3.1, Table 3.3). Grain accumulation started later in Winnipeg than in Swift Current by 2 days and 4 days in 2003 and 2004, respectively.

Duration of grain filling is typically measured as the time from anthesis to physiological maturity (Dias and Lidon, 2009). As shown in Fig 3.1 and Table 3.3, crops grown in 2004 Winnipeg had the longest grain filling even though the total period from seeding to maturity were shorter than crops grown in 2004 Swift Current. The main cause was the slow accumulation between the soft dough stage to maturity, which took almost double the time in 2004 Winnipeg compare to 2004 Swift Current. The shortest duration of grain filling was observed in 2003 Swift Current. This was probably due to the distinct environmental condition in 2003 Swift Current which experienced very high evapotranspiration and low precipitation as mentioned above.

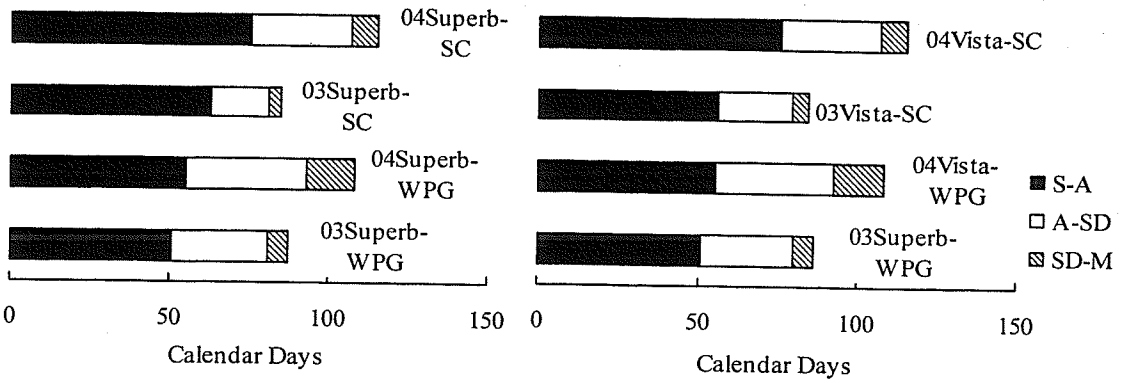

Figure 3.1. Duration of the phenological phases during the growth cycle for the different genotypes in different location and year. S=Seeding; A=Anthesis; SD=Soft dough; M=Maturity; WPG=Winnipeg; SC=Swift Current.

Table 3.3. Calendar days for main crop maturity stages for Superb and AC Vista

Year	Location	Variety	Calendar days			
			S-A	A-SD	SD-M	A-M
2003	Swift Current	Superb	63	18	4	22
2003	Swift Current	AC Vista	56	23	5	28
2003	Winnipeg	Superb	51	30	6	36
2003	Winnipeg	AC Vista	51	29	6	35
2004	Swift Current	Superb	75	32	8	40
2004	Swift Current	AC Vista	75	32	8	40
2004	Winnipeg	Superb	59	40	15	55
2004	Winnipeg	AC Vista	59	39	16	55

Kernel weight accumulation patterns for cultivars Superb and AC Vista are shown in Fig 3.2. There was an initial linear phase for both cultivars in which grain development proceeded at a fairly constant rate, reaching a maximum or plateau. Duration of grain development in Winnipeg was longer than that in Swift Current, and grain development duration was greatly reduced in 2003 compare to 2004, probably because of the higher temperature and lower precipitation in that crop year.

Summary results for grain filling duration, and grain dry matter accumulation rates are shown in Table 3.4. For Winnipeg, results agree with the notion that longer grain filling periods are associated with lower grain filling rates (Nicolas et al., 1984; Wardlaw and Moncur, 1995; Motzo et al., 1996; Calderini et al., 1999; Santiveri et al., 2002). However, for AC Vista in Swift Current, this inverse relationship between grain filling duration and grain filling rates did not occur.

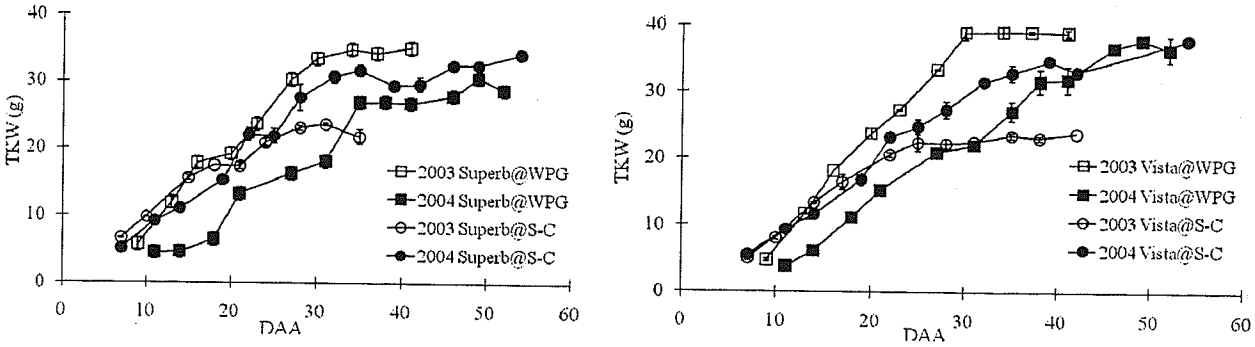

Figure 3.2. Increase in thousand kernel weight with time in Superb and AC Vista. Vertical bars indicated standard deviation. DAA: day after anthesis, defined as the date when 50% of the spikes reached anthesis.

Further analysis of daily increase in kernel weight gave more detailed information about the different patterns of dry matter accumulation for different sites (Fig 3.3). In 2003, for both genotypes, kernels had a growth rate peak at about 15 DAA and 16 DAA at Swift Current and Winnipeg, respectively, after which kernels accumulated dry matter at a slower pace. In 2004, kernels achieved growth rate maximas at a much late stage i.e., at about 22 DAA and 21 DAA at Swift Current and Winnipeg, respectively.

As shown in Figure 3.3, in 2003, the maximum daily increase in kernel dry weight in the Winnipeg site was 1.73 mg/day and 1.91 mg/day for Superb and AC Vista, respectively, which was 1.8 times corresponding value in 2004. However, for the Swift Current site, results were differed. In 2003, the maximum daily increase in kernel dry weight was 1.13 mg/d and 1.19 mg/d for Superb and AC Vista, respectively. The

corresponding values for 2004 was almost identical, i. e. 1.13 mg/d and 1.18 mg/d for Superb and AC Vista, respectively.

In general, crops grown in 2004 had more stable daily increases in kernel dry weight. Kernel dry weight in 2003 accumulated in a more variable manner.

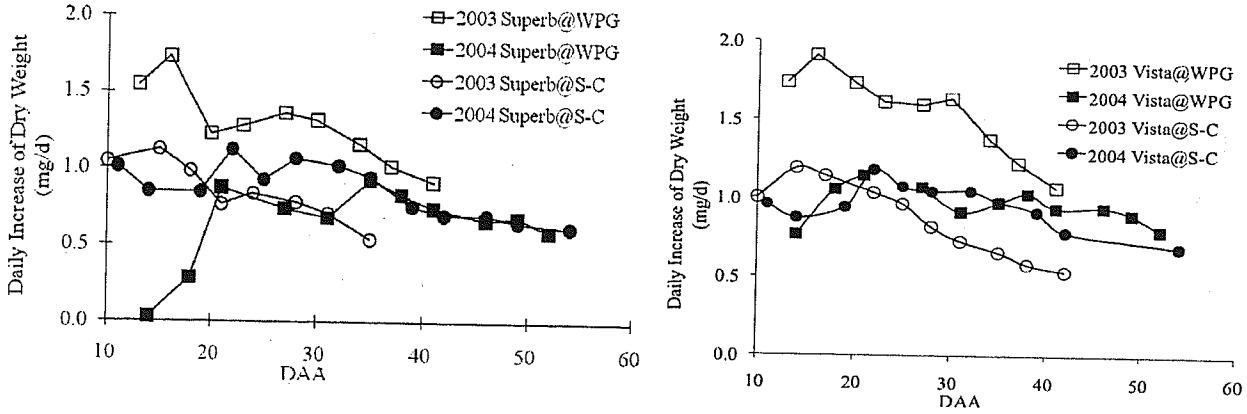

Figure 3.3. Daily increase in kernel dry weight in Superb and AC Vista.

A longer grain filling period has been proposed by many researchers to be related to lower grain filling rates (Nicolas et al., 1984; Wardlaw and Moncur, 1995; Motzo et al., 1996; Calderini et al., 1999). This observation was confirmed in this study. A negative relationship between grain filling duration and grain filling rate was observed (Fig 3.4a). Other studies have observed a synergistic interaction between duration of grain filling and grain weight, i. e. the longer grain filling periods, the higher grain weights, independent of temperature (Fokar et al., 1998; Dias and Lidon, 2009). However, according to Santiveri et al. (2002), grain yield was not associated with grain filling duration but strongly positively correlated to the maximum grain filling rate. A similar result was reported by Hunt et al. (1991) that the final grain weight correlated only with the grain filling rate. Both parameters have been studied for many years in an attempt to improve grain yields. Also some suggestions have been made that the grain filling rate

might be a selection criterion to increase final grain weight and subsequently improve grain yield (Bruckner and Frohberg, 1987; Whan et al., 1996).

In the present study, relationships between grain yield, grain filling duration, grain filling rate and kernel weight accumulating rate were evaluated. These data are presented in Table 3.4, and a few basic observations can be made. While grain yield was generally higher in 2004, the strikingly low grain yield at Swift Current in 2003 was the main reason for this outcome. Also grain duration in 2004 was longer. It can be seen clearly (Fig 3.4b) that there was a strong positive correlation between yield and grain filling duration across site years. Grain yield was not associated with grain filling rate (Fig 3.4c) and kernel weight accumulating rate (Fig 3.4d).

Table 3.4. Grain yield, grain filling duration, grain filling rate and kernel weight accumulating rate for Superb and AC Vista

Year	Location	Variety	Grain Yield (kg/ha)	Grain Filling Duration [a] (days)	Grain Filling Rate [b] (mg/d)	Accumulation Rate [c] (mg/d)
2003	Swift Current	Superb	1031.84	22	1.24	1.10
2004	Swift Current	Superb	3985.28	40	0.98	1.18
2003	Winnipeg	Superb	4191.92	36	1.12	1.42
2004	Winnipeg	Superb	4733.45	53	0.67	0.92
2003	Swift Current	Vista	1485.49	28	0.98	1.17
2004	Swift Current	Vista	5222.37	40	1.10	1.30
2003	Winnipeg	Vista	4957.34	35	1.29	1.92
2004	Winnipeg	Vista	5315.70	53	0.83	1.15

[a] grain filling duration is defined as the time span between anthesis and maturity
[b] grain filling rate is estimated as maximum grain weight divided by duration, assuming that grain weight is zero at anthesis
[c] kernel weight accumulating rate is evaluated by taking the slope of the linear relationship between TKW and time span measured as day after anthesis (see Fig 3.2)

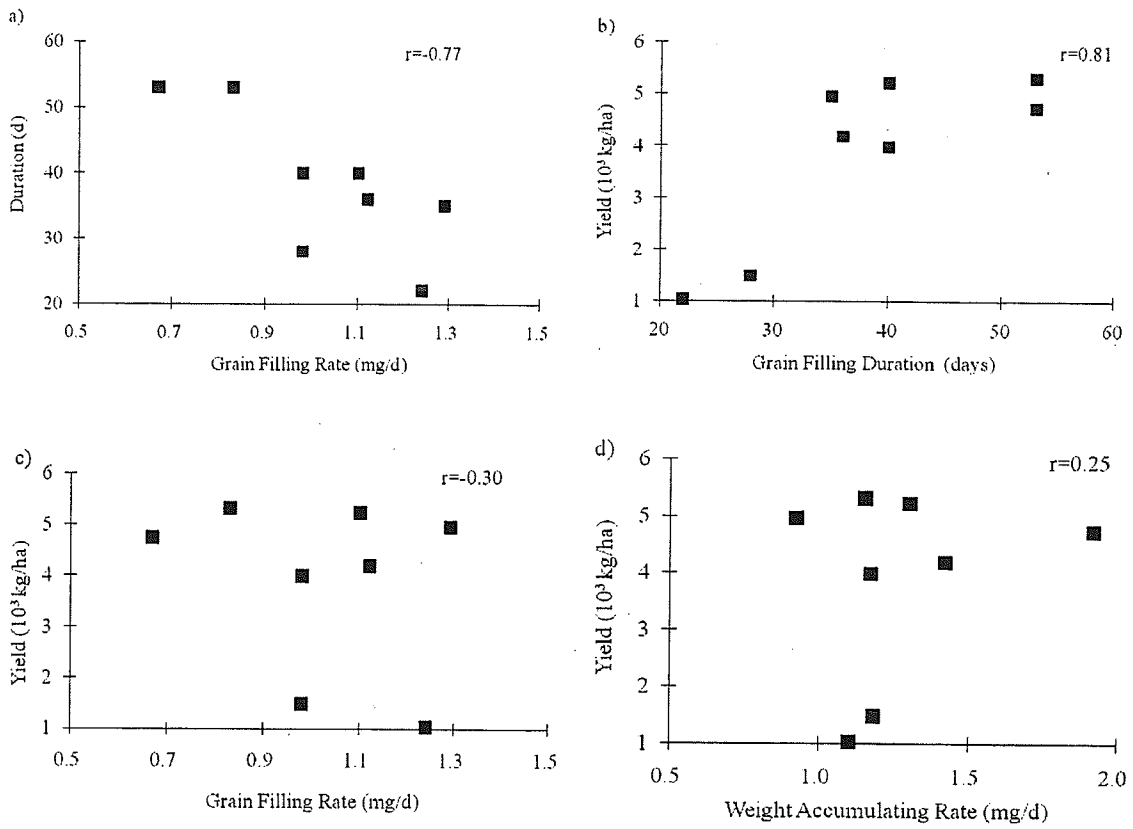

Figure 3.4. Relationship between a) grain filling duration and grain filling rate; b) grain yield and grain filling duration; c) grain yield and grain filling rate; d) grain yield and kernel weight accumulation rate.

3.4.2 Environmental factor impacts

Numerous studies have shown that relationships between environmental parameters and plant growth response factors are often not linear. Nevertheless, useful indications about these relationships can be derived from scatter plots. Nine major meteorological parameters in relation to grain growing duration were analyzed (Fig 3.5). While the sample size was low (8 site years of data), some correlations are compelling and plausible given previous results in the literature. Four weather parameters showed strong relationships to grain filling duration: average daily air temperature (r=-0.88); total rainfall (r=0.91); average daily ET (r=-0.92); and average daily GDD5 (r=-0.90). Lower but still compelling correlation to grain filling duration were observed for total solar

radiation (r=0.56); total water use (r=0.64); and total water deficit (r=0.56). The lowest correlations were average daily wind speed (r=-0.33) and total water demand (r=0.13). The result confirms the conclusion drawn by many other researchers that temperature is negatively associated with grain filling period (Sofield et al., 1977a; Shpiler and Blum, 1986; Gibson and Paulsen, 1999; Dias and Lidon, 2009). At the same time, several moisture-related parameters i.e. total rainfall, total water use; total water deficit and total water demand were positively correlated with the duration of grain growth. This result agrees with the observation of Schelling et al. (2003) that high moisture conditions lead to long grain filling periods. Wind speed is usually considered a driving factor for evapotranspiration. Figure 3.5(d) showed that there was a weak correlation (r=-0.33) between average daily wind speed and filling duration. However, evapotranspiration was highly correlated with grain filling period. Total solar radiation was positively correlated with the filling duration, but the correlation was very low (r=0.33).

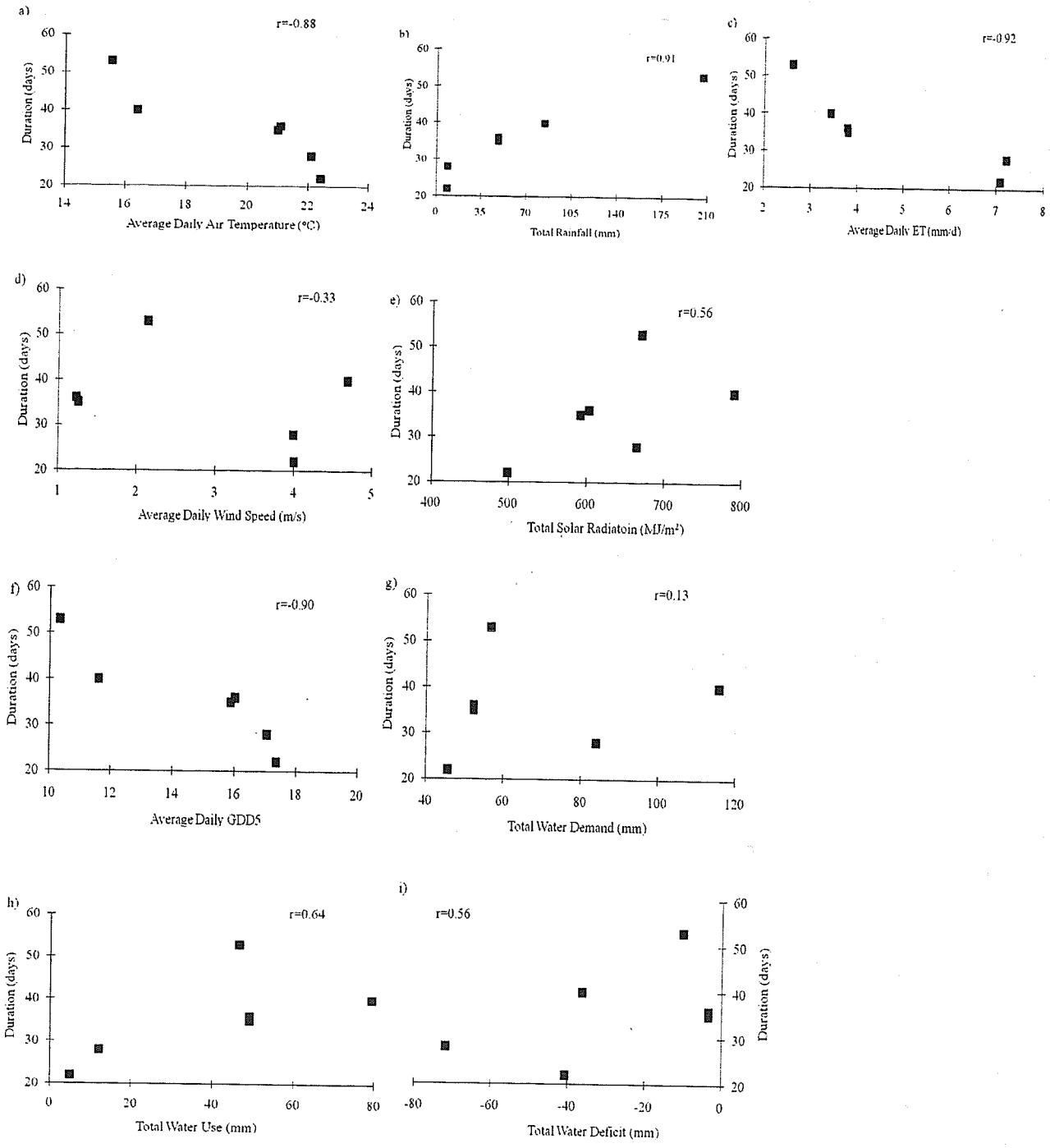

Figure 3.5. Relationship between grain growing duration and weather variables. Grain growing duration defined as the time span between seeding day and maturity day. a) average daily air temperature; b) total rainfall; c) average daily evapotranspiration; d) average daily wind speed; e) total solar radiation; f) average growing degree days, 5 °C as the base temperature; g) total water demand; h) total water use; i) total water deficit.

The effect of weather conditions on kernel development patterns was not only reflected by the duration of grain filling, but also in accumulation of dry matter. TKW results are presented in Table 3.5 and show that during kernel development, dry weight was largely affected by environmental influence. For both genotypes, the maximum TKW and maximum average TKW were observed in 2003 Winnipeg. The minimum TKW and minimum average TKW were observed in 2003 Swift Current.

Table 3.5. Thousand kernel weight (TKW) for Superb and AC Vista

Year	Location	Variety	Ave. TKW [a] (g)	Max.TKW [b] (g)
2003	Swift Current	Superb	23.12	31.49
2003	Swift Current	AC Vista	24.39	31.79
2003	Winnipeg	Superb	32.73	46.59
2003	Winnipeg	AC Vista	36.73	52.17
2004	Swift Current	Superb	31.62	45.43
2004	Swift Current	AC Vista	32.10	50.60
2004	Winnipeg	Superb	25.33	40.68
2004	Winnipeg	AC Vista	30.74	50.63

[a] Ave. TKW is measured as average of kernel dry weight during kernel development
[b] Max. TKW is measured as the maximum kernel dry weight during kernel development

Table 3.6 summarizes coefficient of variation (CV) of average TKW and maximum TKW during kernel development in different growing sites. The higher the CV, the more variable is the parameter. Genotype related CVs were relatively similar, as were location CVs. However, growing season, i.e. year showed considerable variation in TKW. The 2003 crop year was much more variable in TKW response compared to 2004. The distinct weather condition in 2003 especially as it related to the Swift Current site likely contributed to this higher CV.

Table 3.6. Coefficient of variation (%) of average TKW and maximum kernel weight during kernel development in different growing sites

	Genotype		Year		Location	
	Superb	AC Vista	2003	2004	Swift Current	Winnipeg
Ave. TKW	16.66	16.43	22.45	10.45	16.94	15.11
Max. kernel weight	16.72	20.95	25.90	10.20	24.32	10.79

Grain yield in wheat is the product of the number of grains produced per unit area and the mass per grain, which is the integral of the growth rate over the duration of grain growth (Loss et al., 1989). It is considered as one of the key agronomic properties of a genotype. Traits that are correlated with the grain yield may be useful for indirect selection for breeding or screening purpose. The most interesting trait for use as an indirect selection for yield is TKW, because this character is a yield component and is easier to determine than yield itself (Sadeghzadeh and Alizadeh, 2005). The relationship between TKW and several complementary weather parameters was examined (Fig 3.6). Previous results indicated that there were high correlations between grain filling duration and several meteorological parameters. Instead of using the mean value of the corresponding weather parameter during the development period, a cumulative model was used. The weather parameters were calculated as the accumulated amount between the anthesis date to the corresponding post anthesis day. The cumulative growing degree day parameter, often referred to as thermal time, is commonly used as a measure of the amount of warmth that plants or a crop experience over a period of time. In the case of wheat grown in Western Canada, temperature in the early spring is usually the limiting factor for crop development. Growing degree days, which measures useful heat for plant growth, gives a direct measure of the "driving" factor for growth (Klepper, 2009). Because developing kernels accumulate dry matter during grain filling, a cumulative calculation of weather parameters seemed appropriate.

Relationships between TKW and cumulative weather parameters (GDD5, rainfall, ET, and solar radiation) are presented in Fig 3.6. Depending on the weather variable, one or more site-years revealed a deviated trend in the relationship. For cumulative GDD5 and ET, 2003 Swift Current was an obvious outlier, with the other three site years

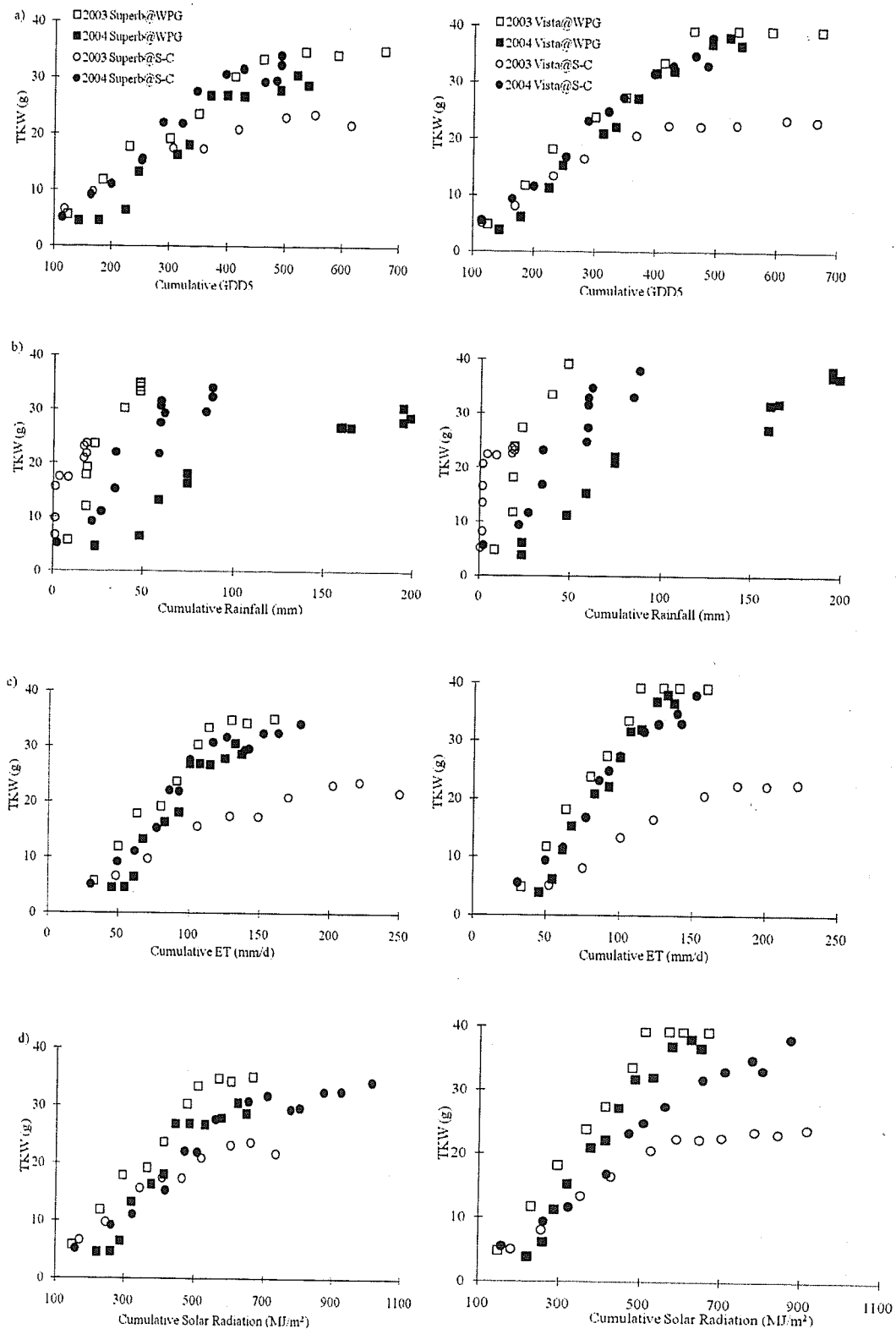

Figure 3.6. Relationship between TKW and weather parameters. Left side of the figure is Superb and right side is AC Vista; a) cumulative GDD5; b) cumulative total rainfall; c) cumulative ET; d) cumulative solar radiation.

producing non-distinguishable response in TKW throughout the kernel development period. In contrast, TKW responded very differently in relation to cumulative rainfall in three of four site years from the outset of grain filling. A different response was observed for cumulative solar radiation, which did not manifest site-year differences in TKW until above 15-20 DAA. Thereafter three of four site years showed different response, and a GxE interaction was evident. In 2003, both Superb and AC Vista had different TKW responses to solar radiation in Winnipeg and Swift Current. In 2004, only AC Vista was differentiated in TKW response to solar radiation between growing sites.

3.5 Conclusions

Numerous studies have shown that environmental factors can have a large impact on grain properties. The current study confirmed several observations from previous work. In general, a warm and dry season favoured rapid kernel development resulting in relatively shorter grain filling duration at the cost of reduced grain yield. Longer crop seasons with higher precipitation usually produce higher yields. Furthermore, compared to kernel filling rates, grain filling duration was more positively correlated to grain yield. Analysis of relationships between meteorological parameters and grain duration and grain weight (TKW) were also made. Results showed that a few factors including average daily air temperature, average daily GDD5, average daily ET and total rainfall had strong relationships to grain filling duration. Cumulative GDD5 and ET could well explain the TKW variation. However, due to the complex processes and interactions of environmental factors controlling grain development, there was no single weather parameter that could explain both kernel weight and grain filling duration.

These results emphasize the complex relationships between weather conditions during grain development and a seemingly "simple" response variable such as kernel weight. Results indicated for example that cumulative GDD5 and ET were generally good predictors of TKW in most growing environments except those like 2003 Swift Current where very low seasonal rainfall and high ET (Table 3.2) adversely affected grain filling which was likely as a result of restricted starch accumulation. In contrast, cumulative rainfall was a very poor predictor of kernel weight.

Chapter 4

Relationship between Weather and Patterns of Accumulation of Grain Protein, Gliadin and Insoluble Glutenin during Kernel Development

4.1 Abstract

Considerable experimental work has been undertaken in order to understand how wheat protein content and composition responds to different environmental conditions as a means to understand how environmental variation affects wheat end-use quality. However, knowledge in this area is incomplete and unclear, particularly as it relates to western Canadian genotypes grown in the Prairie region. Two hard spring wheat cultivars (Superb and AC Vista) were grown at two locations (Winnipeg, MB and Swift Current, SK) in replicated plots under optimal soil fertility conditions in two consecutive seasons (2003 and 2004). Diverse environmental conditions across site-years were experienced. Wheat heads were sampled at 3 or 4 day intervals during kernel development from anthesis to maturity. Heads were appropriately preserved and threshed grain was ground to a standard particle size for protein analysis. Environmental variables included hourly measurements of air temperature, solar radiation and precipitation in addition to derived parameters including useful heat (GDD5, growing degree days > 5 °C), standard evapotranspiration, crop water demand, water use, and crop water deficit. Response variables during grain development included total grain protein, 50% propanol soluble (50PS) protein (mainly gliadins) and 50% propanol insoluble (50PI) protein (insoluble glutenin), and residue protein (mainly non storage albumins and globulins). Protein variables were quantified in three ways: per kernel, per 100 mg grain, and fractions in total protein.

Accumulation patterns of total protein, and different protein fractions (50PS , 50PI and residue) were analyzed as a function of kernel development in calendar days (DAA), and in response to environmental parameters expressed cumulatively. Results showed that gluten protein (mainly gliadins) started forming as early as 7 DAA. The initial rate of total protein accumulation was mainly affected by the accumulation of gliadin protein whose accumulation started at a higher rate and much earlier than that for insoluble glutenin. Gliadins accumulated at a rapid rate until about 20-25 DAA (peak accumulation) depending on growing location, and thereafter declined marginally until maturity. Insoluble glutenin, i.e. HMW glutenin, on the other hand started to form in general, in significant amounts much later than that for gliadins, beginning around 25 DAA, and continued to accumulate, essentially until grain maturity. However, for one growing location (2003 Swift Current), whose rate and duration of kernel filling was relatively high and short, respectively, insoluble glutenin synthesis in significant amounts began much earlier, about 15 DAA. Residue protein (mainly albumins and gliadins) in contrast, decreased in concentration throughout the initial phase of grain development until about 20-25 DAA, and thereafter remained at a more or less constant level until maturity.

Relationships between protein accumulation patterns and weather parameters were complex. However, patterns of protein accumulation between genotypes Superb and AC Vista were essentially identical. Year effects shortened considerably the time for grain filling duration in 2003 compared to 2004. Higher temperatures (~ 6 °C) and much lower precipitation (by ~ 3.5X) during kernel development in 2003 growing sites are the likely reasons for this outcome. Weather factors that were site, but not year, characteristics included solar radiation (but not temperature), wind speed (but not

evapotranspiration), water demand, and water deficit; all had higher values in Swift Current compared to Winnipeg. Wheat grown in 2003 Swift Current accumulated the highest levels of protein, whereas the lowest levels were observed for 2003 Winnipeg. Across all four growing sites, total protein accumulation appeared to closely correspond to the initial rate of protein accumulation up to about 20 DAA which was in the following order: 2003 Swift Current > 2004 Swift Current > 2004 Winnipeg > 2003 Winnipeg. That initial rate of total protein accumulation was largely influenced by the accumulation of gliadin protein. A very strong linear relationship existed between total protein accumulation (constant grain weight basis) and cumulative water deficit across the site years, and pointed to an underlying effect of water stress on accumulation of starch. For both gliadins and insoluble glutenin (constant grain weight basis), cumulative GDD5, cumulative standard evapotranspiration and cumulative solar radiation, were closely related to protein accumulation during the early phase of kernel development, however, unexplained site-year variation became apparent at about 300 ^{o}C days of GDD5 (~ 40-45% of total grain development) and progressively increased in influence until maturity. However, when accumulation of gliadin and insoluble glutenin in total protein was charted, a very coherent relationship was found for some weather parameters. For gliadin accumulation as a function of cumulative GDD5, or cumulative evapotranspiration or cumulative solar radiation, the same curvilinear pattern existed that mirrored gliadin synthesis by calendar days, but without site-year effects. Both cumulative GDD5 and cumulative solar radiation had an excellent relationship to accumulation of insoluble glutenin in total protein. The relationship was linear and showed relatively little unexplained site-year variation, which was marginally greater for AC Vista compared to Superb.

4.2 Introduction

Variation in wheat breadmaking quality is largely related to variation in wheat protein content and composition. These characteristics are determined by genetic factors (Finney and Barmore, 1948; Payne et al., 1987; Gupta et al., 1993; Johansson et al., 2001), and environmental influences especially those related to weather where wheat is grown (Graybosch et al., 1995; Johansson and Svensson, 1998; Johansson et al., 2002).

Numerous studies of environment effects on wheat protein quantity and composition have shown that factors such as nitrogen (N) fertilization, rainfall and temperature have significant influences. Nitrogen fertilization has been frequently reported to positively correlated to wheat protein content (Finney et al., 1957; Dubetz, 1972; Bole and Dubetz, 1986; Fowler et al., 1990; Gauer et al., 1992; Gupta et al., 1992; Scheromm et al., 1992; Peltonen and Virtanen, 1994; Pechanek et al., 1997; Zhao et al., 1999b; Daniel and Triboi, 2000; Luo et al., 2000; Triboi et al., 2000; Boehm et al., 2003; Yue et al., 2007; Johansson et al., 2008; Saint Pierre et al., 2008; Ma et al., 2009). In contrast, the effect of N fertility on albumins and globulins is relatively negligible (Pechanek et al., 1997; Wieser and Seilmeier, 1998). High N fertility leads to high ratio of gliadin to glutenin, mainly by increasing gliadin content to a relatively greater extent compare to glutenin (Doekes and Wennekes, 1982; Gupta et al., 1992; Prieto et al., 1992; Triboi and Leblevenec, 1995; Jia et al., 1996a; Pechanek et al., 1997; Wieser and Seilmeier, 1998; Zhu et al., 1999; Daniel and Triboi, 2000; Triboi et al., 2000; Saint Pierre et al., 2008). Among gliadin protein types, accumulation of ω-gliadin appears to be more affected by N supply (and Seilmeier, 1998; Daniel and Triboi, 2001). It is generally agreed that proportion of ω-gliadin in total gliadin is increased by N fertilization, while proportion

of β-gliadin decreases (Timms et al., 1981; Prieto et al., 1992; Huebner et al., 1997; Wieser and Seilmeier, 1998; Daniel and Triboi, 2000).

Precipitation conditions during wheat growth have contrasting effects, depending on level. Drought generally increases total wheat gluten protein content due to restriction of starch accumulation (Guttieri et al., 2000; Altenbach et al., 2003; Ozturk and Aydin, 2004; Jiang et al., 2009). The content of wheat protein and total starch content (Kim et al., 2003), as well as protein accumulation rate and starch accumulation rate (Fernandez-Figares et al., 2000), were shown to be inversely related to each other, indicating competition in the transport of proteins and carbohydrates to the grain. However, albumins and globulin content decreased under drought condition (Konopka et al., 2007). On the other hand, excessive rainfall has been associated with lower wheat protein content (Greaves and Carter, 1923; Correll et al., 1994; Altenbach et al., 2003; Ozturk and Aydin, 2004; Jiang et al., 2009).

A positive relationship between moderate temperature (<30 °C) and grain protein content has been established in literature (Sofield et al., 1974; Kolderup, 1975; Sofield et al., 1977b; Schipper, 1991; Stone et al., 1997; Uhlen et al., 1998; Daniel and Triboi, 2002; Asseng and Milroy, 2006; DuPont et al., 2006b; Spiertz et al., 2006). The relationship arises from relative responses of protein and starch to rising temperature. Protein has been proposed to be less sensitive to high temperature conditions during grain development than starch (Sofield et al., 1974; Chowdhury and Wardlaw 1978; Bhullar and Jenner 1985; Jenner et al. 1991; Rao et al. 1993; Stone et al., 1996). However, higher temperatures are not always associated with positive effects on protein content. When temperature exceeds moderate levels (~ 30 °C), reaching temperature > 35 °C, even short periods of exposure during grain development can cause negative

effects on wheat protein composition (Blumenthal et al., 1991b; Ciaffi et al., 1996). It has frequently been reported that glutenin/gliadin ratio decreases with increased grain protein percentages under heat stress (Abrol et al., 1971; Dubetz et al., 1979; Doekes and Wennekes, 1982; Stenram et al., 1990; Stone et al., 1996).

Such conditions of heat stress are rare in Western Canada. Still variation in wheat protein content and breadmaking quality exists in the Prairie region due mainly to weather effects (Campbell and Davidson, 1979; Campbell et al., 1997; Finlay et al., 2007; Jarvis et al, 2008). As reviewed by these workers, there have been many studies that examined Canadian genotypes for end-use quality responses to seasonal environmental conditions. Surprisingly, apart from Finlay et al (2007), not a single Canadian study has been published on specific weather effects of wheat quality in relation to protein composition in a field-replicated experiment. Moreover, there is no published science on the effects of weather variation in the Canadian Prairie region on wheat protein content and composition effect during grain development.

The objective of this study was to complement the research of Jarvis et al (2008) by investigating the influence of growing season weather conditions on protein accumulation patterns during kernel development to better understand the nature of protein composition in mature wheat as affected by environment.

4.3 Materials and Methods

4.3.1 Wheat samples, growing locations a weather data collection

Two hard spring wheat cultivars from two Canadian commercial classes were grown in two environments in the Canadian prairies during the 2003 and 2004 growing season to provide a very diverse range of growing environments. Cultivars were Superb

(Canada Western Red Spring (CWRS) wheat) and AC Vista, a white seed coat wheat belonging to the Canada Prairie Spring wheat class. Growing locations were Winnipeg, MB and Swift Current, SK. The fields were optimally fertilized based on standardized soil tests. Details concerning the field setup, as well as yield and mature wheat quality information for Superb and AC Vista have been published (Finlay et al., 2007). In brief, AC Vista was higher yielding by 16% (site-year average), had 1.1% lower grain protein content (14.2 vs. 13.1%) and had kernels of higher average weight (by 9%). The glutenin composition of these two genotypes differed (Table 3.1) most notably at the *Glu-D1* locus; Superb possesses subunits 5+10 while AC Vista possesses subunits 2+12. The only practical difference in breadmaking quality for the two cultivars was loaf volume, where Superb, presumably because of its higher protein content produced higher loaf volumes (1002 cc) compared to AC Vista (855 cc).

4.3.2 Weather data and collection

Automated weather stations (Campbell Scientific, Logan, UT; Spectrum, Plainfield, IL) were installed at each growing location to collect on an hourly and daily basis the following: air temperature (°C), rainfall (mm), wind speed (m s^{-1}), and solar radiation (Watts m^{-2} d^{-1}) at 2.3 m. Growing degree days with base temperature of 5 °C was calculated from daily maximum and minimum temperatures. Evapotranspiration, and modeled crop demand, use, and deficit were determined as described by Finlay (2006).

4.3.3 Sampling of wheat heads and grain preparation for analysis

Details of wheat head sampling, storage and grain threshing in preparation for protein analysis are provided in section 3.3.2. An independent sample of CWRS wheat

flour was chosen as to be a reference flour to verify the stability of all protein tests. Sample wheat material was ground to flour using a Retsch ZM 200 ultra-centrifugal mill (Retsch Inc., Newtown, PA) operated at 14,000 rpm using a 0.5 mm sieve. Wheat (2-3g) was ground at each developmental stage to ensure sufficient samples for analysis. Sample recovery in the mill was 85-90%. Ground wheat was immediately transferred to clear glass vials. Ground samples were freeze-dried and kept under desiccation conditions at room temperature throughout the experimental period.

4.3.4 Moisture content

All protein analytical results were reported on a 14% moisture basis. Accordingly, accurate moisture determinations of ground wheat samples were a prerequisite for accurate determinations of protein content and composition during kernel development. As quantity of wheat kernel samples from growing sites were limited, moisture determinations were made on the CWRS wheat reference flour and a reference sample of ground mature wheat as proxy results, with both materials stored along with the experimental ground wheat samples. Moisture was checked monthly during the experiment period (February to December 2008) according to approved Method 44-15A (AACC International, 2000). The reference CWRS wheat flour had average moisture content of 11.36%. The reference ground wheat had average moisture content of 0.79% throughout the experiment period. All protein measurements for experiment samples were adjusted to 14% moisture content based on the average moisture content of reference ground wheat.

4.3.5 Total protein content and protein fractionation

Wheat protein content (%N × 5.7) was determined by micro-Kjeldahl method with a few modifications based on approved Method 46-13 (AACC International, 2000), using Kjeldahl 1002 distilling unit (Tecator). Flour protein composition was determined according to the method of Sapirstein and Johnson (2000). This procedure quantifies three fractions, i.e., protein soluble in 50% 1-propanol (mainly gliadins, 50PS protein), 50% 1-propanol insoluble protein (mainly high molecular weight polymeric glutenin, 50PI protein) and residue protein that contains mainly non-gluten protein (Fu and Sapirstein, 1996).

Ground wheat (50 mg) was extracted twice with 1 ml 50 % (v/v) 1-propanol (solution "A") for 15 min at room temperature (23 °C) in a 1.5 ml microcentrifuge tube with intermittent vortexing (every 5 min for 5 sec). After the first extraction, the mixture was centrifuged for 3 min at 3180 x g in a tabletop centrifuge (Biofuge A, Heraeus-Christ). The supernatant was collected in a 2 ml microcentrifuge tube. A micro-spatula was used to facilitate disruption of the starchy pellet, which was quite dense and hard. After the second extraction, the mixture was centrifuged for 3 min at 15,000 x g. The supernatant was combined with the first extraction.

A 1 ml aliquot of solution "A" was used as the blank for absorbance measurements at 214 nm. A calibration curve was prepared from 50PS protein from different wheat flours (Sapirstein and Johnson, 2000). Initial measurements of 50PS protein of ground wheat obtained from early stages of kernel development produced results that were clearly overestimated. Wheat at this stage of kernel development with very little gluten protein content contains relatively high amounts of mainly non-storage protein, i.e. metabolic enzymes (Martín del Molíno et al., 1988; Triboi et al., 2003; Abonyi et al.,

2007) that were evidently soluble in 50% 1-propanol (Fu and Sapirstein, 1996). As calibration of this UV spectroscopic determination of gluten proteins was developed based on flour from mature wheat which contains a preponderance of gliadin and glutenin protein, it was evident that the protein calibration was not appropriate to determine gliadin proteins at an early stage of wheat development when gliadins are in relatively low concentrations and 50PS protein may contain relatively high concentrations of metabolic albumins and globulins. Accordingly, the protein content of 50PS fractions was re-determined by micro-Kjeldahl method (AACC International, 2000).

To determine insoluble glutenin content of kernel development material, the 50PS protein residue was reduced with 1 ml of solution "A" containing 0.1% (w/v) dithiothreitol (DTT) for 30 min at 55 °C in a heating block. Freshly prepared solution of DTT (on a daily basis) was used for all the experiments. Samples were vortexed at 5 min, and 10 min intervals during extraction at 55 °C, and just prior to centrifugation to facilitate complete suspension of the 50PI residue. Subsequently, the mixture was centrifuged for 3 min at 15,000 x g. The microcentrifuge tube was inverted once to obtain a homogeneous supernatant, and placed in a rack. An aliquot of the supernatant was diluted 100-fold in a fresh microcentrifuge tube with solution "A", and the solution was thoroughly mixed by vortexing (5 sec) to obtain the sample for UV absorbance measurement. As this method extracts 50% 1-propanol insoluble polymeric protein only, no complication with UV absorbance measurements was found for any of the wheat kernel development material, in contrast to the problem observed for the 50PS protein extracts.

Protein remaining in the 50PI residue after reduction with DTT (i.e. residue protein), was determined by difference: residue protein = total protein – (50PS +50PI protein).

All analytical determinations of protein fractions were carried out at least in duplicate starting with ground wheat.

4.4 Results and Discussion

4.4.1 Accumulation pattern of protein fractions

4.4.1.1 Total protein

Site years showed a very wide variation in total protein accumulation (Fig. 4.1). The result clearly reflects the major differences in growing season weather which shortened considerably the time for grain filling duration in 2003 compared to 2004. As discussed in Chapter 3, higher temperatures and lower precipitation during kernel development in 2003 growing sites are the likely reasons for this outcome. This result confirms previous work that temperature (Sofield et al., 1977a; Shpiler and Blum, 1986; Gibson and Paulsen, 1999; Dias and Lidon., 2009) and total rainfall (Schelling et al., 2003) are negatively and positively associated with grain filling period, respectively. As can be seen in Fig. 4.1, patterns of total protein accumulation between genotypes Superb and AC Vista were essentially identical among growing sites.

Wheat grown in 2003 Swift Current accumulated the highest levels of protein, whereas the lowest levels were observed for 2003 Winnipeg. Across all four growing sites, total protein accumulation appeared to closely correspond to the initial rate of protein accumulation up to about 20 DAA (Table 4.1) which was in the following order: 2003 Swift Current > 2004 Swift Current > 2004 Winnipeg > 2003 Winnipeg.

Correlation analysis confirmed this observation, i.e. the correlation between initial rate of total protein accumulation and final total protein content was r=0.80. That initial rate of total protein accumulation was largely influenced by the accumulation of gliadin protein. As is discussed below, gliadin protein accumulation started at a higher rate and much earlier than that for insoluble glutenin.

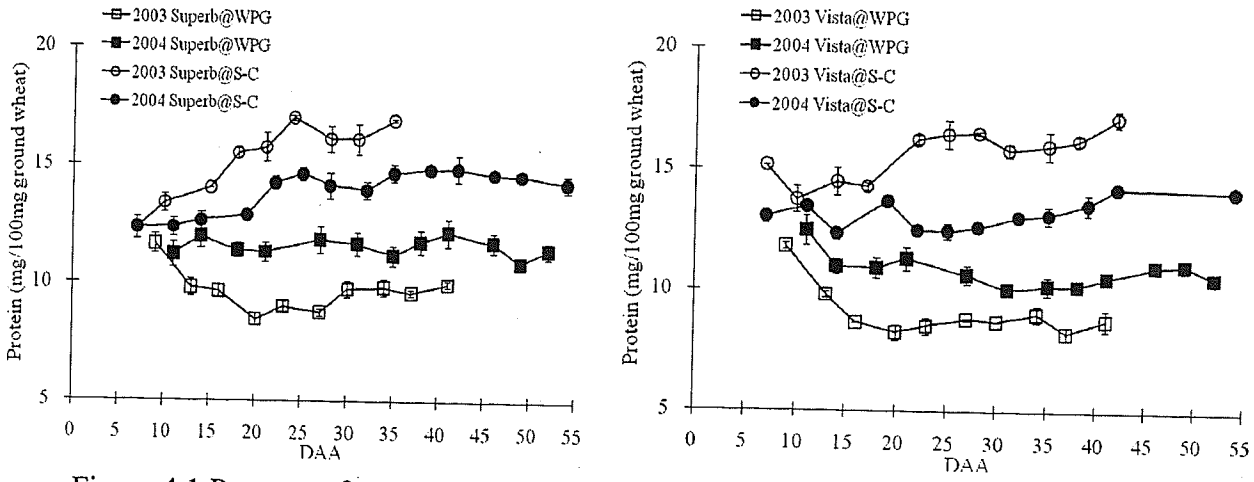

Figure 4.1 Patterns of accumulation of total protein content for Superb and AC Vista

Table 4.7. Accumulation rates of different protein fractions during the initial phase of kernel development [a]

Cultivar	Location	Year	Total Protein (mg/d)	50PS (mg/d)	50PI (mg/d)
Superb	Swift Current	2003	0.26	0.36	0.08
Superb	Swift Current	2004	0.05	0.27	0.04
Superb	Winnipeg	2003	-0.27	0.20	0.01
Superb	Winnipeg	2004	-0.01	0.32	0.04
AC Vista	Swift Current	2003	-0.05	0.18	0.06
AC Vista	Swift Current	2004	0.03	0.19	0.01
AC Vista	Winnipeg	2003	-0.33	0.06	-0.01
AC Vista	Winnipeg	2004	-0.10	0.20	0.02

[a] Accumulation rate was evaluated by the slope of the relationship between protein accumulation and DAA, up to 20 DAA.

4.4.1.2 Gliadin protein (50PS), insoluble glutenin (50PI), and residue protein

Total protein accumulation during kernel development is an aggregate of protein accumulation of underlying fractions. As was expected, the latter varied in an asynchronous fashion temporally, and showed very different patterns of accumulation. The trends of accumulation of 50PS and 50PI protein in calendar days (DAA) are shown in Figs. 4.2 and 4.3, respectively. 50PS protein comprises mainly gliadins (Fu and Sapirstein, 1996) but also contains some LMW or (50PS) soluble polymeric glutenin. 50PI protein corresponds to insoluble glutenin, i.e. glutenin polymers of largest molecular size. In the discussion below, the terms 50PS and gliadins, and 50PI and insoluble glutenin are used interchangeably.

Results showed distinct patterns of protein synthesis for gliadins and insoluble glutenin. The accumulation of 50PS was characterized by a bell-shape pattern, with a rapid initial increase starting from 7 to 12 DAA depending on the site or year. Gliadin synthesis reached a peak at about mid-point of kernel development ranging from 18 DAA to 25 DAA depending again on site-year. Thereafter, gliadin accumulation, basis constant grain weight, gradually decreased until the end of the grain filling period.

In general, site-year effects for gliadin accumulation for both Superb and AC Vista were relatively large throughout the entire grain filling period as reflected in the separation of curves in Fig. 4.2.

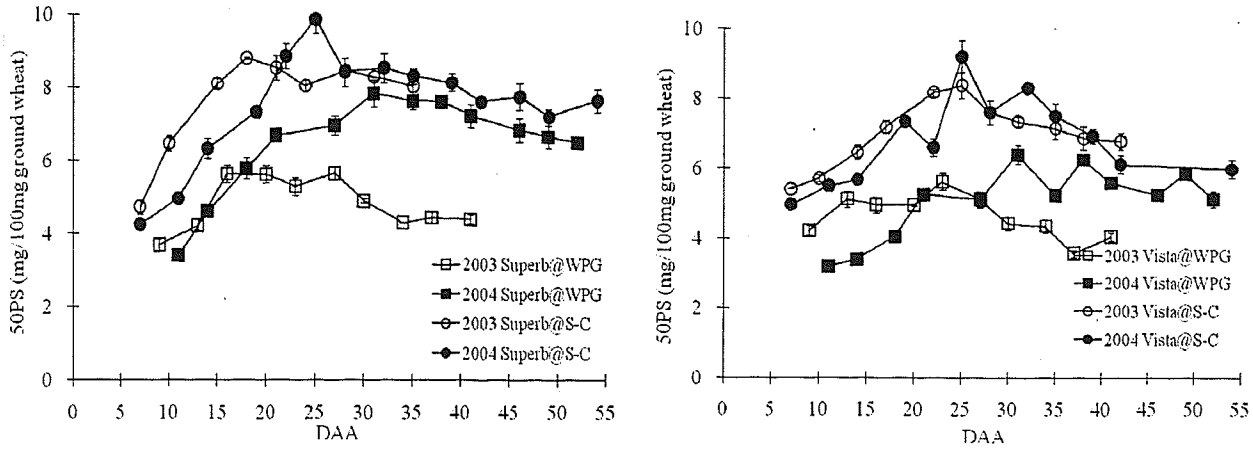

Figure 4.2 Patterns of accumulation of 50PS protein for Superb and AC Vista

Insoluble glutenin or 50PI protein had a very different pattern of protein accumulation (Fig. 4.3). Compared to gliadins, insoluble glutenin in general started to form in significant amounts much later (beginning around 25 DAA), and accumulated initially until 25 DAA much more slowly. However, the 2003 Swift Current location was a notable exception as insoluble glutenin synthesis was very apparent beginning at about 15 DAA, and was at a maximum rate by about 20-25 DAA. Excluding the 2003 Swift Current site, after the initial lag phase in 50PI protein synthesis, the rate of accumulation of insoluble glutenin increased markedly; insoluble glutenin continued to accumulate until the very end of grain development for most site-years for both Superb and AC Vista. In that respect, insoluble glutenin synthesis continued much later than that for gliadins.

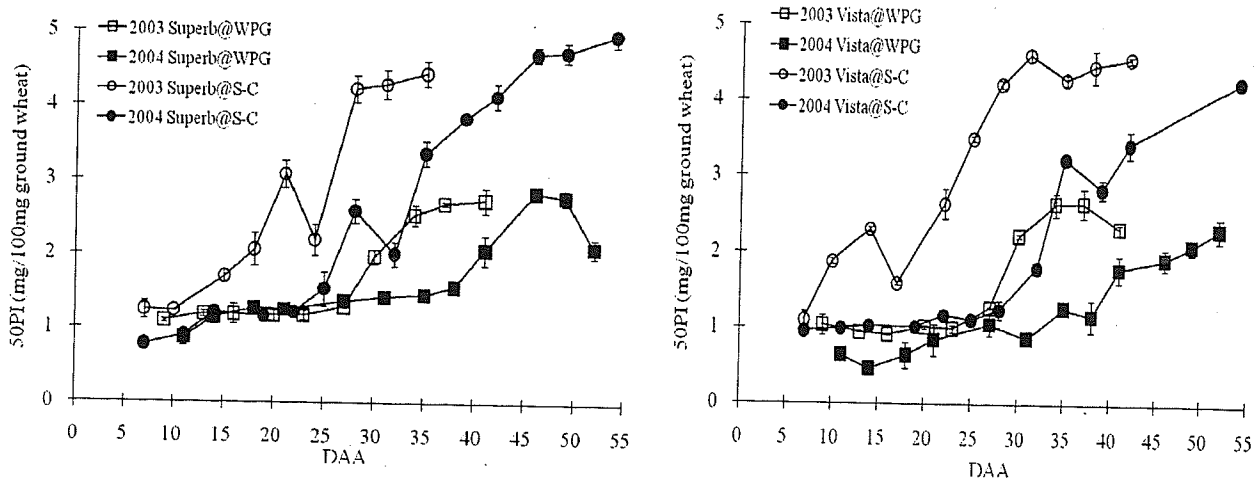

Figure 4.3 Patterns of accumulation of 50PI protein for Superb and AC Vista

In contrast to gliadin and insoluble glutenin protein, residue protein (Fig 4.4) was at its highest level (basis constant grain weight) at a very early stage in kernel development. Residue protein decreased in concentration throughout the initial phase of grain development until about 20-25 DAA, and thereafter remained at a more or less constant level until maturity. The result is in line with the identity of the residue protein as albumins and globulins having mainly regulatory and metabolic functions in the developing seed especially during the early cell division phase (Triboi et al., 2003).

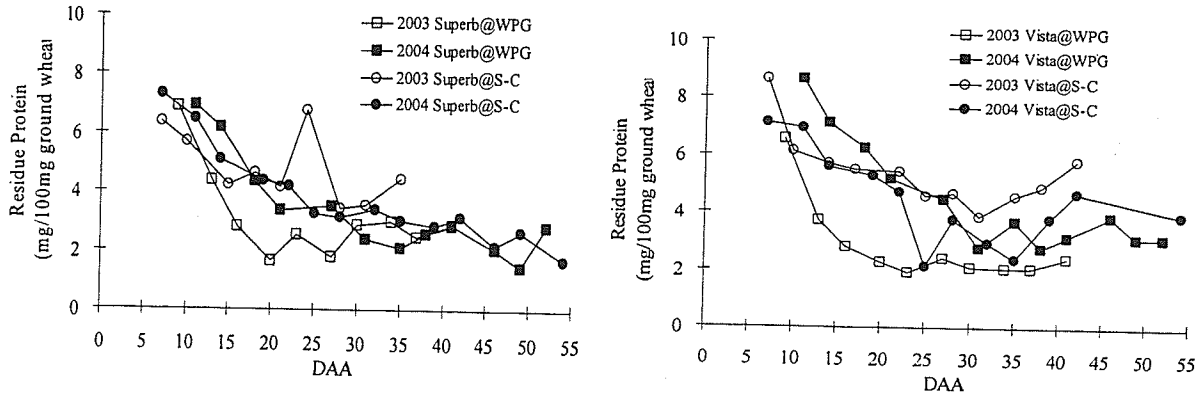

Figure 4.4 Patterns of accumulation of residue protein for Superb and AC Vista

4.4.2 Impacts of environmental factors

The impact of environment on patterns of accumulation of total protein and constituent subfractions was very large in this study. Summary information of environmental parameters that were measured (Table 3.2, in Chapter 3) indicated that 2004 growing sites were much cooler and wetter compared to those of 2003. The former had average daily temperatures 5.8 °C lower and 3.5 times the average daily precipitation than in 2003 during the grain filling period.

Weather parameters were assessed on their effects on accumulation of total protein, and solubility fractions, 50 PS (mainly gliadins) and 50PI (insoluble glutenin). Residue protein (mainly albumins and globulins) was not included in this analysis as this non-storage protein fraction declined in concentration across site-years during kernel development and levels seemed largely unrelated to weather influences (Fig. 4.4).

Four basic weather parameters were initially examined on their effects on protein accumulation patterns, viz. temperature (in thermal time as useful heat, i.e. GDD5 or ^{o}Cdays> 5 ^{o}C), rainfall, evapotranspiration and solar radiation. Further, total protein, gliadin and insoluble glutenin content were evaluated both in absolute terms (mg/100 mg wheat) and, for solubility fractions, as a percentage of total protein, thus eliminating the confounding effects of starch accumulation on the concentration of protein per unit weight of grain. Such an approach has been used in many previous studies (Sofield et al., 1977b; Stone et al., 1997; Asseng and Milroy, 2006; DuPont et al., 2006a; Spiertz et al., 2006; Tahir et al., 2006).

These three different ways of expressing protein accumulation as a function of kernel development time in calendar days (DAA) are shown in Fig. 4.5 using 50PI protein as an example. Weather effects on protein accumulation levels across site years,

for both cultivars Superb and AC Vista, were shown most clearly when 50PI protein was expressed as a constant grain mass, i.e. mg/100 mg of wheat (Fig. 4.5a). Quantifying 50PI protein on a per kernel basis (Fig. 4.5b) substantially altered the pattern of variation for 2003 Swift Current, by decreasing the absolute level of protein accumulated relative to other site-years. The considerably smaller kernel size of 2003 Swift Current grown wheat (Table 3.5) was the underlying reason for this result. Compared to Fig. 4.5a, both Figs. 4.5b (50PI expressed as mg/kernel) and 4.5c (50PI expressed as % protein) revealed a different pattern of protein accumulation especially in relation to responses between 2003 growing locations Swift Current and Winnipeg. While 2003 Winnipeg 50PI protein content (as well as total protein content) was much lower on a kernel weight basis (either per kernel or per 100 mg wheat), when expressed on a protein percentage basis, that difference was essentially eliminated. For example, on a protein percentage basis, both 2003 Swift Current and Winnipeg locations produced similar levels of protein at maturity. What is missing in this percentage calculation is starch accumulation, which can represent from 65-75% of kernel weight at maturity (Rahman et al., 2000). As protein and starch contents on a percentage basis are inversely related, starch content was much higher in 2003 Winnipeg compared to 2003 Swift Current where wheat matured very quickly under the influence of higher temperatures, higher evapotranspiration and greater water deficit (Table 3.2).

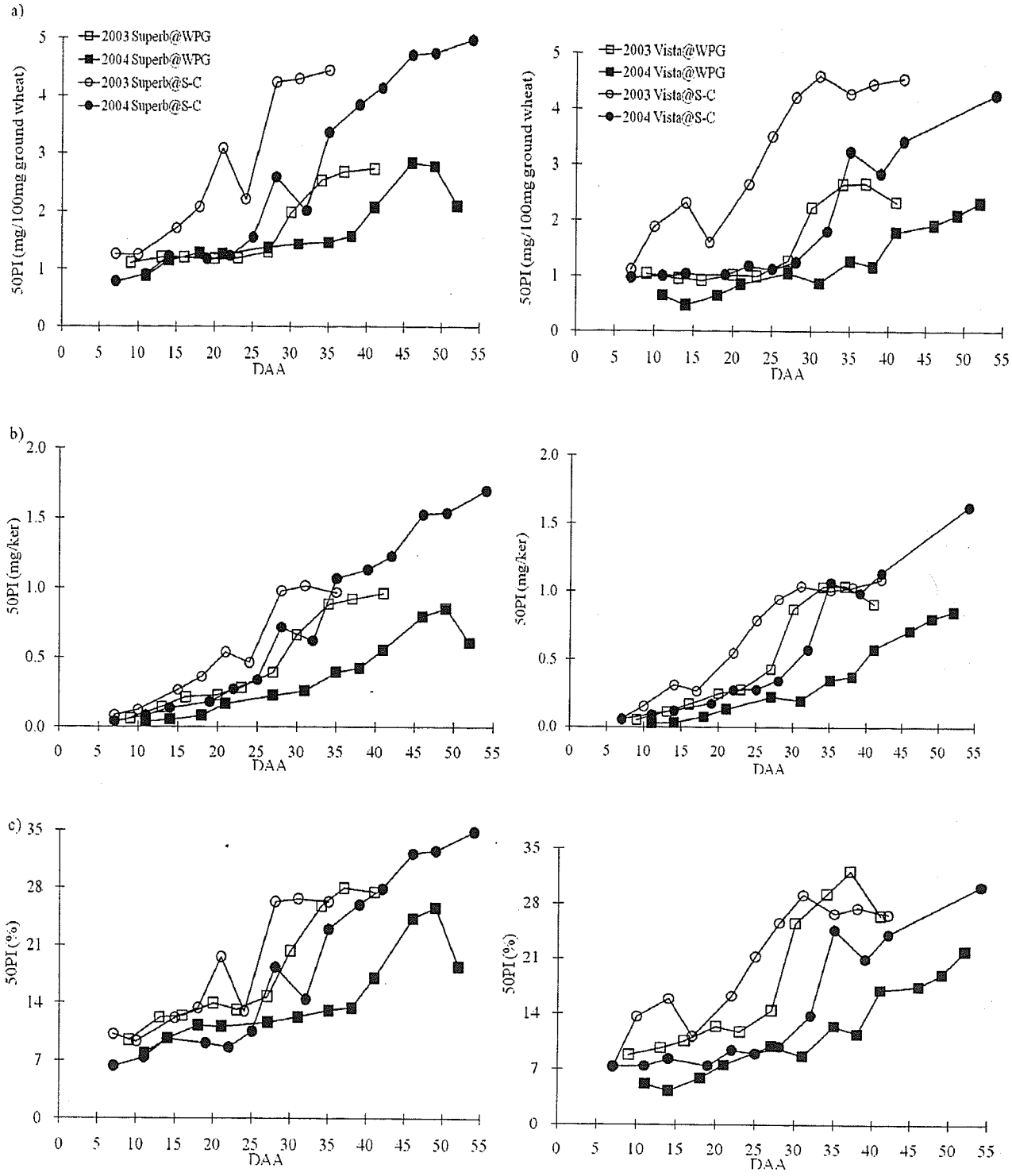

Figure 4.5. Pattern of accumulation of 50PI protein expressed at a constant grain basis (a); per kernel (b); and as percentage in total protein (c). Results for Superb and AC Vista are presented on the left and right, respectively.

Preliminary analysis of protein relationships to the basic weather data on identical days of data acquisition, i.e. in calendar time (DAA) produced no useful trends (results not shown). In principle, an ideal model of environmental or weather relationship to protein accumulation during kernel development should take into account both duration and rate of accumulation. For example, it is known that duration of grain filling is constant in thermal time (Triboi et al., 2003; Martre et al., 2006). As well, the rates and durations of accumulation of both gliadins and glutenins expressed in thermal time were found not to be affected by moderately high temperature (<35 °C) (Stone and Nicolas, 1998; Daniel and Triboi, 2001; Triboi et al., 2003). Accordingly, the analysis reported below examined the relationships between protein accumulation and four weather parameters (GDD5, rainfall, evapotranspiration, and solar radiation) calculated in a cumulative fashion, like thermal time, during the kernel development period.

Relationships between selected weather parameters and total protein, gliadin and insoluble glutenin, expressed basis mg per 100 mg wheat, are presented in Figs. 4.6, 4.7 and 4.8, respectively. No compelling relationship between total protein accumulation and weather was found (Fig. 4.6), as in general, site-year responses were well separated for cumulative GDD5, ET and solar radiation, i.e. these weather parameters were reflecting site-year effects on total protein, but no trend across site-years. In this respect, the result (Fig. 4.6) appears to closely correspond to the levels of total protein accumulated according to kernel development time after anthesis (Fig. 4.1) in the following order: 2003 Swift Current > 2004 Swift Current > 2004 Winnipeg > 2003 Winnipeg. Accordingly, no coherent relationship across site years existed between total protein accumulated during kernel development and cumulative weather effects for variables such as GDD5, precipitation, evapotranspiration and solar radiation. This

outcome, as is discussed below, is partly due to heterogeneous composition of "total protein" expressed on a mg per 100 mg wheat basis; there exists an inverse relationship between accumulation of residue protein (albumins and globulins) and storage protein fractions, combined with the asynchronous timing and different rates of accumulation of subfractions (gliadin, glutenin) as previously discussed (Figs. 4.1, 4.2 and 4.3).

When weather-related responses of storage protein fractions, 50PS (mainly gliadins) and 50PI (insoluble glutenin) were viewed separately (Figs 4.7 and 4.8), a more coherent response to weather emerged. For both gliadins (Fig. 4.7) and insoluble glutenin (Fig. 4.8), all three temperature related weather parameters (GDD5, evapotranspiration and solar radiation), but not precipitation, were closely related to protein accumulation during the early phase of kernel development as evidenced by the linear response of protein accumulation as a function of cumulative GDD5, ET and solar radiation. Gliadin accumulation rate was much higher than that for insoluble glutenin as previously observed (Table 4.1). Accumulation of insoluble glutenin was in fact very marginal during this early stage of grain development, except for the 2003 Swift Current site.

During the latter half or more of kernel development, these temperature related weather parameters were unable to provide an explanation for the variation of gliadin and insoluble glutenin protein as a proportion of constant grain weight. As can be seen in Figs. 4.7 and 4.8, gliadin and insoluble glutenin protein began to show site-year effects beginning at about 300 GDD5 units or 300 ^{o}Cdays>5 ^{o}C. Interestingly, for insoluble glutenin (and more so for cultivar Superb than for AC Vista), the accumulation rate was consistent in thermal time (GDD5) for different years, i.e. wheat grown in Swift Current, and separately in Winnipeg, showed similar respective patterns of insoluble

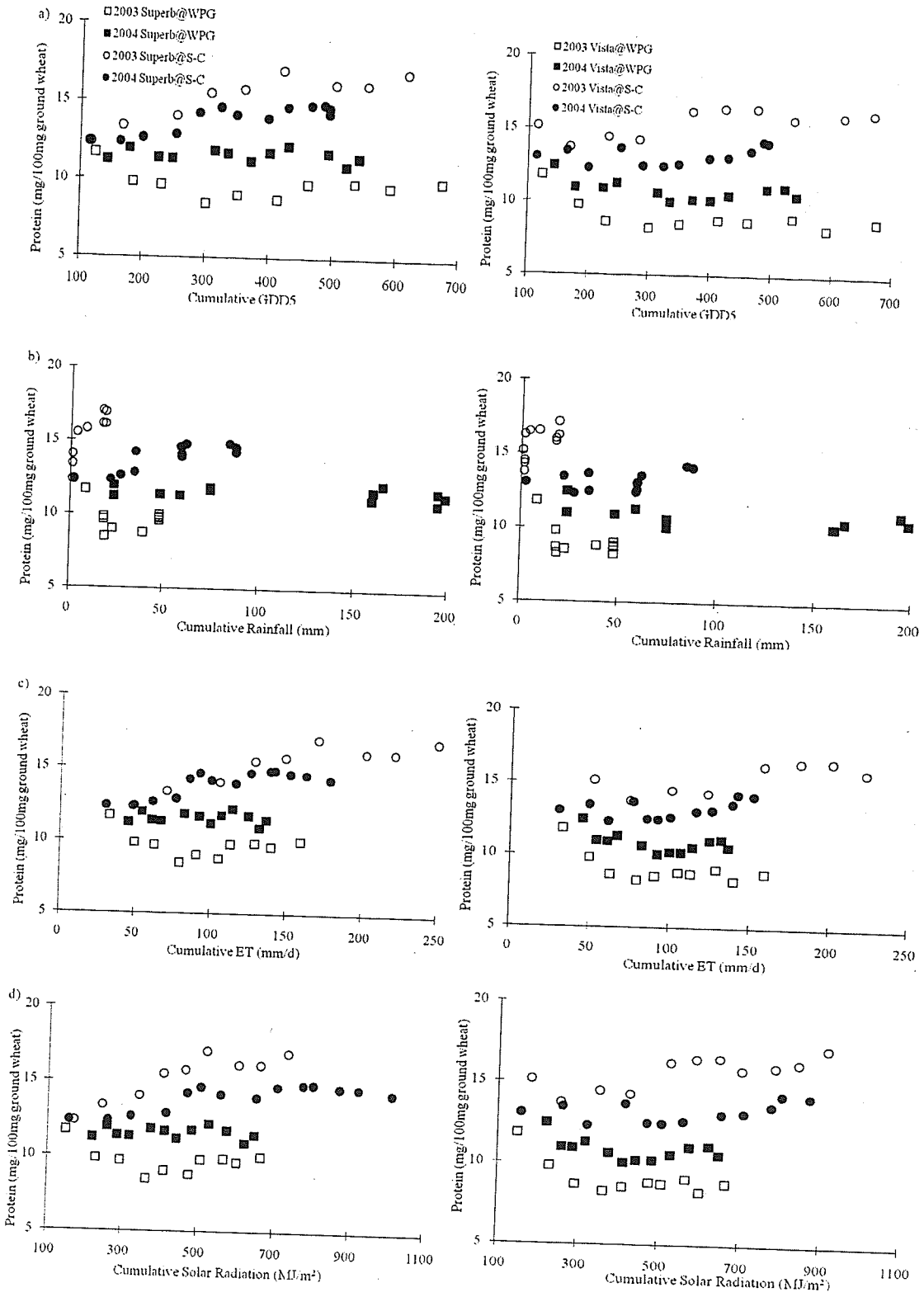

Figure 4.6. Relationship between total protein and weather parameters; a) cumulative GDD5; b) cumulative total rainfall; c) cumulative ET; d) cumulative solar radiation. Results for Superb and AC Vista are presented on the left and right, respectively.

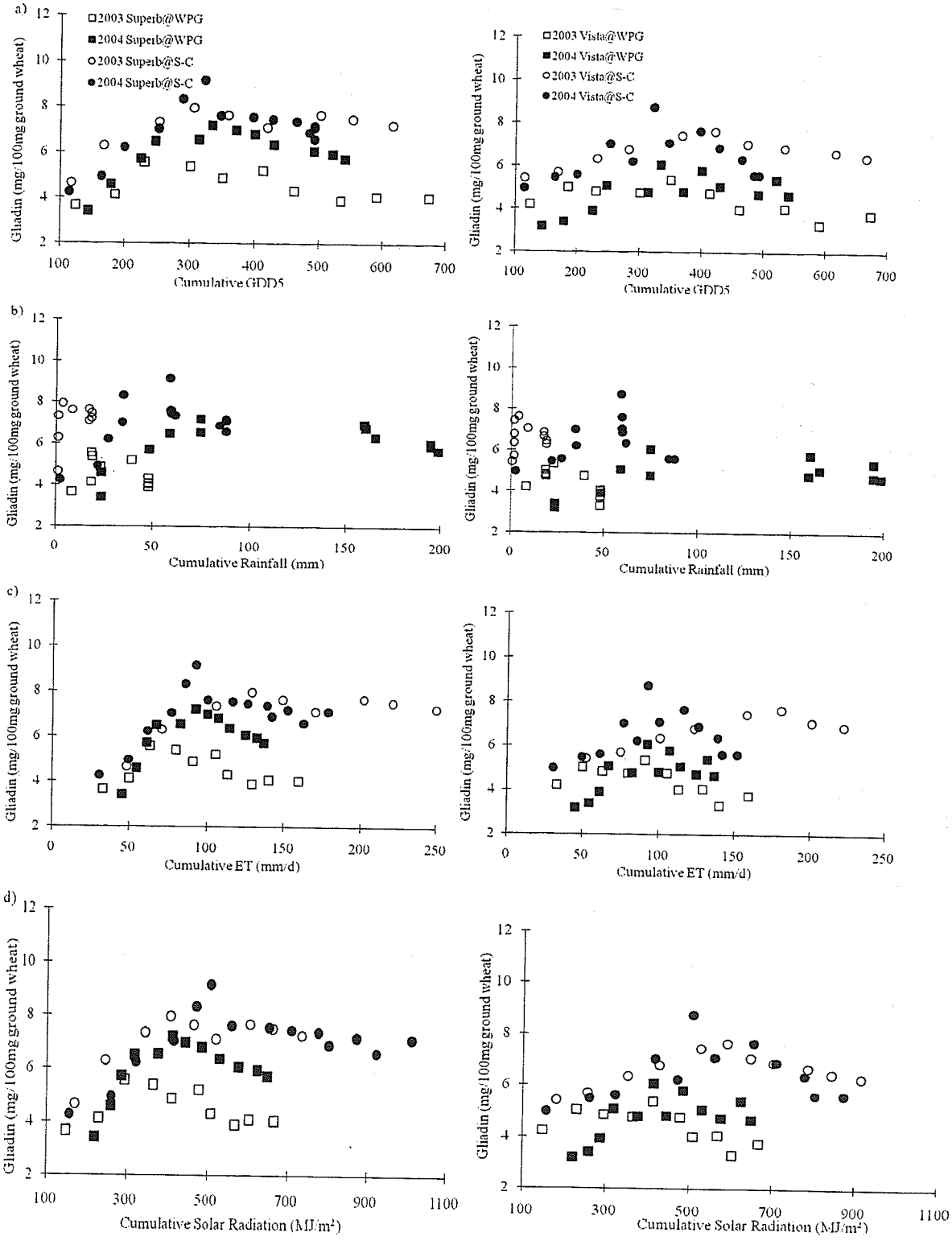

Figure 4.7. Relationship between gliadin and weather parameters; a) cumulative GDD5; b) cumulative total rainfall; c) cumulative ET; d) cumulative solar radiation. Results for Superb and AC Vista are presented on the left and right, respectively.

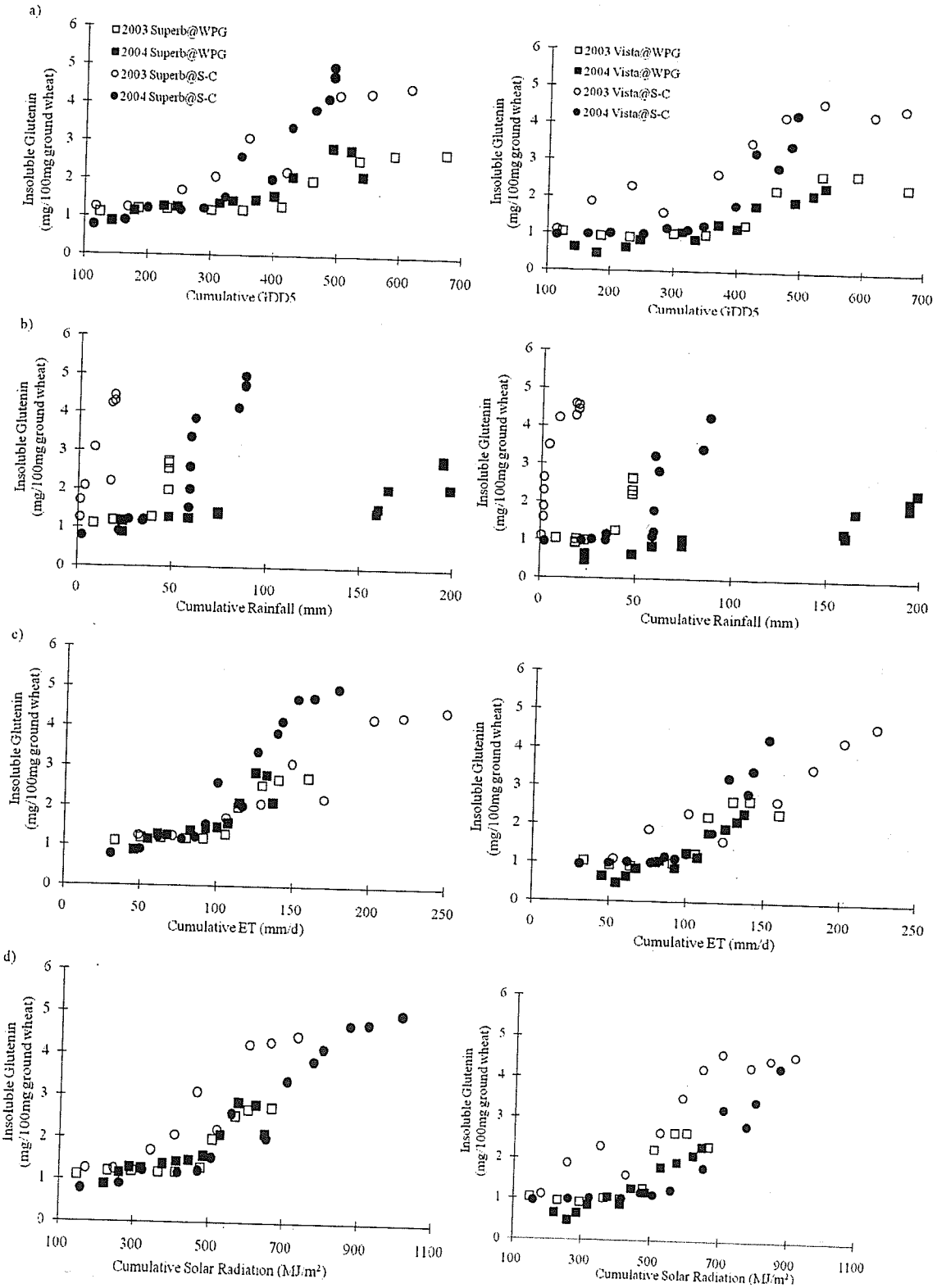

Figure 4.8. Relationship between insoluble glutenin and weather parameters; a) cumulative GDD5; b) cumulative total rainfall; c) cumulative ET; d) cumulative solar radiation. Results for Superb and AC Vista are presented on the left and right, respectively.

glutenin accumulation for the same growing location (higher in Swift Current) in the two different crop years. Weather factors that appeared to be site characteristics included solar radiation (but not air temperature), wind speed (but not ET), water demand, and water deficit; all had higher values in Swift Current averaged across years (Table 3.2). In contrast, precipitation and temperature were growing season characteristics.

Evidently, the relatively higher average water demand and deficit for Swift Current compared to the Winnipeg site, appear to be significant contributing factors in the higher levels of accumulation of total protein and insoluble glutenin in each of years 2003 and 2004. Crop water demand refers to the amount of moisture a crop would use given an unlimited supply of water. When water demand exceeds supply, a crop is theoretically in a water deficit status. Water deficit is a characteristic of an arid or semi-arid environment and south-western Saskatchewan, where Swift Current is located is often characterized as having a semi-arid climate. A very strong relationship existed between protein accumulation (expressed in mg per 100 mg as with Figs 4.6 – 4.8) and cumulative water deficit (Fig. 4.9) for both cultivars Superb and AC Vista; the relationship appeared marginally stronger for the former than for the latter. Total cumulative water deficit was higher for AC Vista because this cultivar took longer to reach maturity in 2003 Swift Current where water deficit increased noticeably towards the latter stages of kernel development.

On a constant grain mass basis, this increasing profile of protein accumulation versus cumulative water deficit likely reflects an underlying effect of water stress on starch accumulation. Experimentally induced drought has been shown to have very significant effects on wheat starch during grain development. Total numbers of granules are reduced considerably in endosperm under water stress (Brooks et al. 1982; Nicolas et

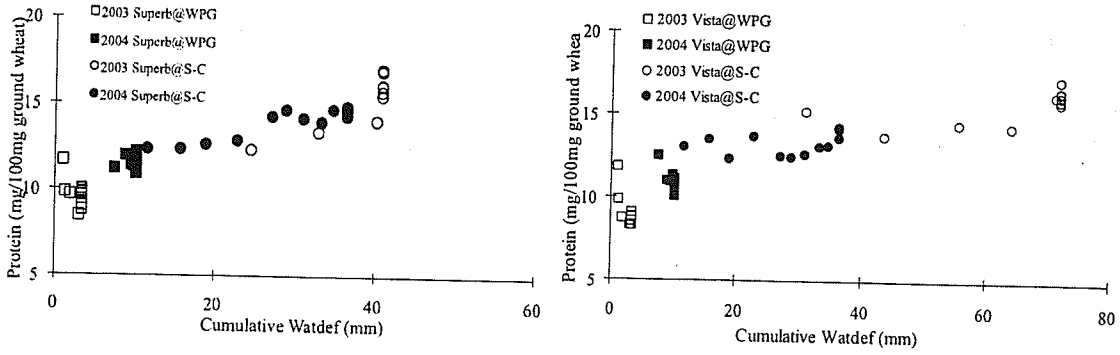

Figure 4.9 Pattern of accumulation of total protein as a function of cumulative water deficit for cultivars Superb (left) and AC Vista (right).

al., 1984). Water stress under very high daytime temperatures (37 °C) was found to shorten the duration of wheat starch accumulation and lead to a substantial decrease in the amount of starch and protein in mature grain (Altenbach et al 2003). While these studies (Brooks et al. 1982; Nicolas et al., 1984) did not measure protein responses to water stress conditions, or protein responses to moderate growing temperatures consistent with the present study (Altenbach et al 2003), one could reasonably conclude that decreased starch accumulation during grain development translates into higher protein contents. The incoherent nature of protein accumulation versus weather relations across site years previously discussed for Figs. 4.6-4.7 is therefore likely due at least in part to the confounding effects of starch accumulation when protein accumulation is expressed on a constant grain mass basis. If true, expressing separate protein fractions (50PS or gliadins, and 50PI or insoluble glutenin) on a protein percentage basis should result in improved trends of basic weather-protein accumulation relationships. This was exactly what was found (Figs. 4.10 and 4.11), with the exception of rainfall, which was largely unrelated to protein accumulation during grain development as previously noted.

Percent gliadin and insoluble glutenin accumulation in response to cumulative GDD5, evapotranspiration and solar radiation showed clear trends during kernel development; for cultivar Superb, more so than for AC Vista, site-year effects diminished very considerably compared to corresponding results (Figs. 4.7, 4.8) where protein fractions were expressed on a constant grain weight basis. This improvement in weather vs. protein relationships was especially evident in the latter half of kernel development, where cumulative weather parameters were unable to provide an explanation for the variation of gliadin and insoluble glutenin protein as a proportion of constant grain weight.

Because results for accumulation of gliadin and insoluble glutenin in total protein as a function of cumulative evapotranspiration and solar radiation (Figs. 4.10c,d, and Figs 4.11c,d, respectively) were very similar to those obtained for cumulative GDD5 (Fig. 4.10a and Fig. 4.11a), the discussion below is focused on only one of these weather parameters (GDD5) as an example of outcomes as a whole. Gliadin accumulation showed a biphasic response in relation to cumulative GDD5 (essentially thermal time) (Fig. 4.10a). Gliadin proteins accumulated at a characteristically high rate, reaching a maximum in protein concentration (about 58% on average) at approximately 300-350 ^{o}Cdays, depending on site-year and cultivar. Thereafter, gliadin as a percentage of total protein moderately decreased in concentration (to about 50% on average at maturity) due to the concomitant increase in concentration of insoluble glutenin (Fig. 4.11a), which increased from about 12% of total protein on average at 325^{o}Cdays (GDD5) to about 28% of total protein at maturity. Insoluble glutenin (HMW polymeric glutenin), sometimes referred to as unextractable polymeric protein or SDS-insoluble protein, is known to accumulate in significant amounts later than gliadins, and to continue to

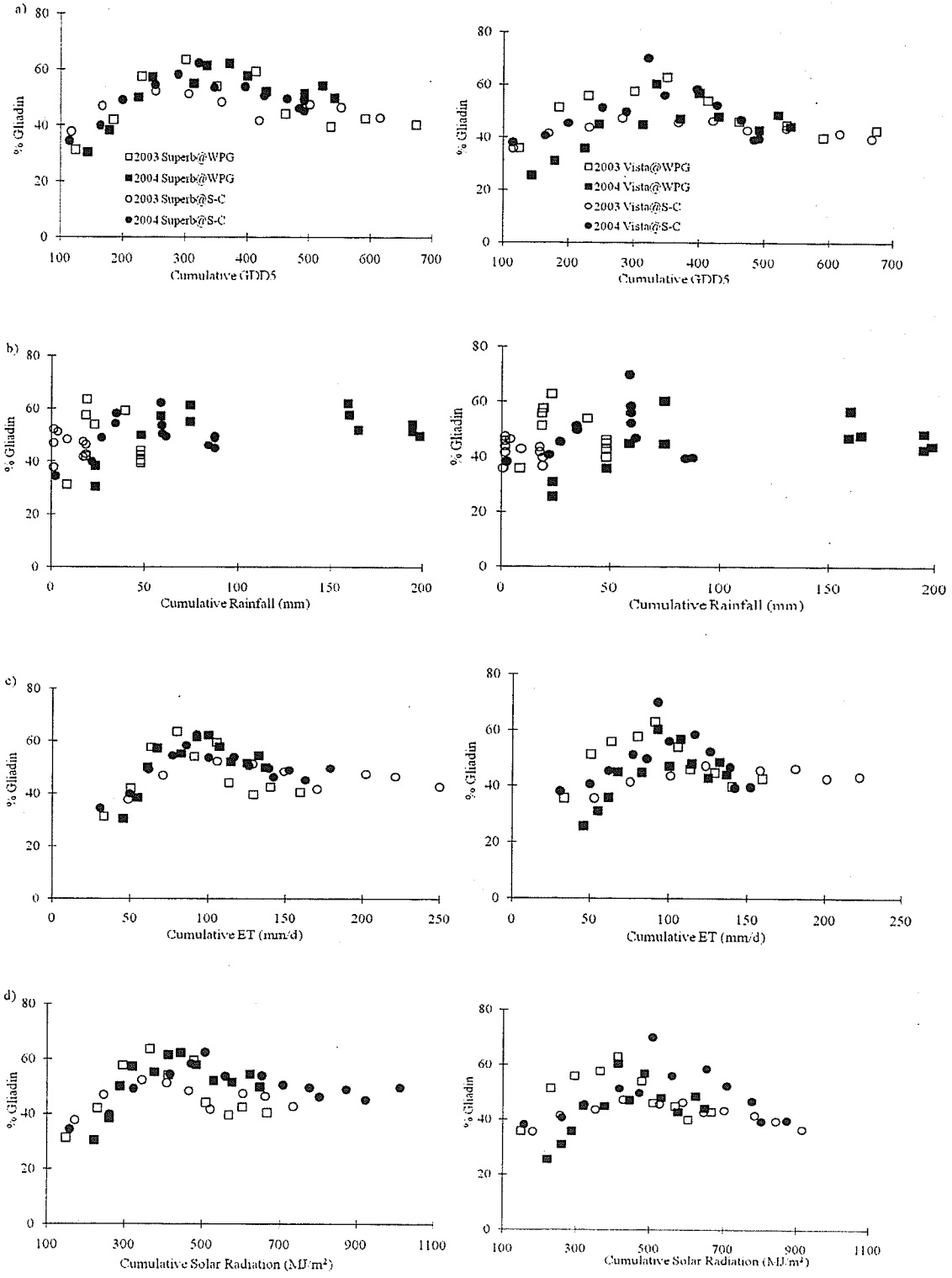

Figure 4.10. Pattern of accumulation of gliadin in total protein and weather parameters; a) cumulative GDD5; b) cumulative total rainfall; c) cumulative ET; d) cumulative solar radiation. Results for Superb and AC Vista are presented on the left and right, respectively.

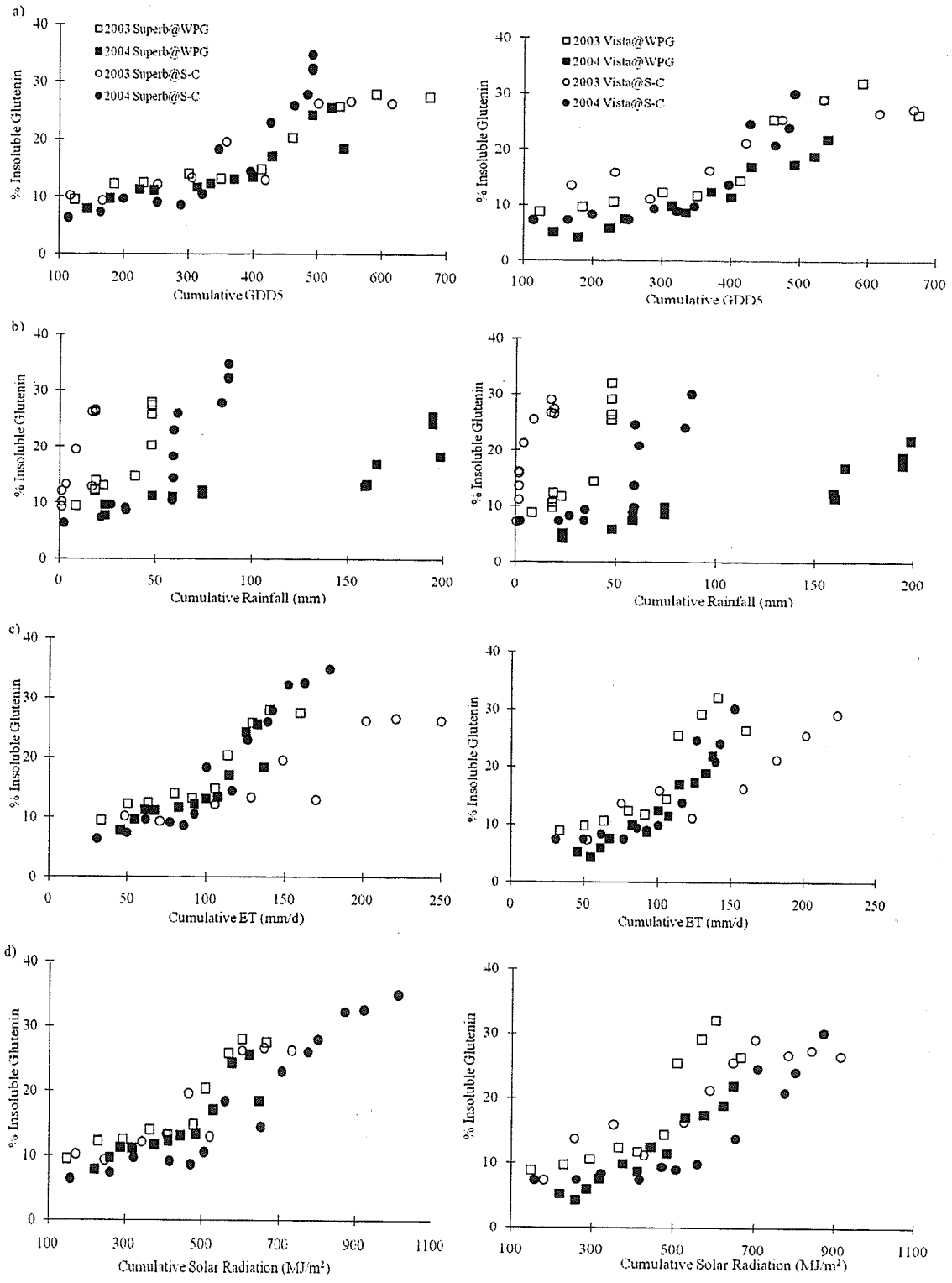

Figure 4.11. Pattern of accumulation of insoluble glutenin in total protein and weather parameters; a) cumulative GDD5; b) cumulative total rainfall; c) cumulative ET; d) cumulative solar radiation. Results for Superb and AC Vista are presented on the left and right, respectively.

increase during the later stages of kernel development (Stone and Nicolas, 1996; Gupta et al., 1996; Pannozzo et al., 2001).

4.5 Summary and conclusions

This chapter of the thesis research has documented accumulation patterns of total protein and major protein solubility fractions during kernel development in a field based study carried out in a western Canadian Prairie environment for two adapted hard spring bread wheats. Extensive analysis of weather relationships to protein accumulation patterns was carried out. Many of the results as they relate to wheat protein accumulation patterns during grain development are consistent with previously established knowledge.

Results showed that gluten protein (mainly gliadins) started forming as early as 7 DAA. Gliadin protein accumulation started at a higher rate and much earlier than that for insoluble glutenin. Gliadins accumulated at a rapid rate until about 20-25 DAA (peak accumulation) depending on growing location, and rate of synthesis thereafter declined marginally until maturity. Insoluble glutenin, i.e. HMW glutenin, on the other hand started to form in general, in significant amounts much later than that for gliadins, beginning around 25 DAA, and continued to accumulate, essentially until grain maturity. However, for one growing location (2003 Swift Current), whose rate and duration of kernel filling was relatively high and short, respectively, insoluble glutenin synthesis in significant amounts began much earlier, about 15 DAA. Residue protein (mainly albumins and gliadins) in contrast, decreased in concentration throughout the initial phase of grain development until about 20-25 DAA, and thereafter remained at a more or less constant level until maturity.

Year effects shortened considerably the time for protein accumulation in 2003 compared to 2004. Higher temperatures (~ 6°C) and much lower precipitation (by ~ 3.5X) during kernel development in 2003 growing sites are the likely reasons for this outcome. Wheat grown in 2003 Swift Current accumulated the highest levels of protein, whereas the lowest levels were observed for 2003 Winnipeg. Across all four growing sites, total protein accumulation appeared to closely correspond to the initial rate of protein accumulation up to about 20 DAA which was in the following order: 2003 Swift Current > 2004 Swift Current > 2004 Winnipeg > 2003 Winnipeg. That initial rate of total protein accumulation was largely influenced by the accumulation of gliadin protein. A very strong linear relationship existed between total protein accumulation (constant grain weight basis) and cumulative water deficit across the site years, and pointed to an underlying effect of water stress on accumulation of starch.

Understanding the levels and timing of protein accumulation in relation to widely varying weather conditions in growing locations and years was very challenging. Water deficit was found to be a key parameter that very well explained the accumulation of total protein across site years. This result appears to be a by-product of water stress effects on starch accumulation, which was not directly measured in this study. Nevertheless, it still represents a significant contribution to knowledge, as no similar result has been reported in a field-based study of wheat kernel development. The significant effect of water deficit on total protein accumulation rate and final concentrations at maturity, points to a need for much more knowledge that integrates soil-water relations together with more commonly acquired weather parameters in studies of environmental effects on wheat protein content.

Another important outcome of this chapter's research are results showing a close correspondence of accumulation of gliadin and insoluble glutenin (expressed as percent of total protein to eliminate confounding effects of starch accumulation) during kernel development to cumulative growing degree days (thermal time essentially), cumulative evapotranspiration and cumulative solar radiation, all temperature related parameters. Because site-years varied in grain filling (i.e. accumulation of protein) duration measured in calendar days, using cumulative weather parameters in this way, effectively corrected or normalized the time frame for the relationships. It has been previously observed that grain filling duration is constant in so-called "thermal time" (Triboi et al., 2003; Martre et al., 2006).

Effects of cumulative evapotranspiration and solar radiation on wheat protein accumulation during grain development have not been previously reported. Because of the expected high degree of covariance between thermal time (growing degree days), evapotranspiration and solar radiation, it is not clear from the results presented which of these three weather parameters was most influential. However, a subsequent analysis of protein accumulation expressed on a per kernel basis (Appendix 5) indicates that, compared to all other environmental parameters monitored in this study, solar radiation was most predictive in explaining site-year effects on accumulation of wheat protein and subfractions.

Chapter 5

Relationship between Weather and Patterns of Accumulation of Gliadin, Total Glutenin, and Soluble and Insoluble Glutenin during Wheat Kernel Development

5.1 Abstract

The objective of this chapter is to complete the study of accumulation patterns of wheat endosperm protein in relation to weather during kernel development using more precise and comprehensive protein fractionation and analysis compared to that described in Chapter 4. Wheat kernel development samples as described in Chapter 4 were used. A protein fractionation strategy was used that enabled separation of LMW- or soluble polymeric glutenin from 50PS protein which was enriched in gliadin content. Soluble and insoluble glutenin was subsequently chemically reduced to subunits and separately quantified by RP-HPLC. In this way, soluble and insoluble glutenin (i.e. small and large polymers) could be directly quantified from integrated peak areas of constituent subunits and patterns of accumulation of protein during kernel development could be analyzed.

Results showed that the isolation of gliadin (50PS protein) as described in Chapter 4 was very efficient and could be used as a quick estimation of gliadin content in wheat protein. The ratio of HMW/LMW-GS for insoluble glutenin at maturity was 36% higher on average than for soluble glutenin confirming that the molecular size distribution of glutenin is directly related to this key protein fraction ratio. Despite very

large effects of environment on protein fraction accumulation patterns, there was essentially no effect on the relative proportions of individual HMW-GS at maturity. HMW-GS also accumulated at very similar rates throughout grain development indicating common regulation of subunit synthesis. The regulation of gliadin and glutenin synthesis appeared to be different on the basis of continuously increasing ratio of glutenin to gliadin during kernel development.

Results indicated that formation of wheat protein fractions was in the order: gliadin, followed by soluble glutenin, followed by insoluble glutenin. Gliadin synthesis began as early as 7 DAA for one growing location (2003 Swift Current) and was clearly underway for all site-years by 15 DAA. Synthesis of small glutenin polymers (soluble glutenin) lagged slightly behind that for gliadins by about 3 days and reached a peak at least one week later than that for maximum gliadin accumulation. Thereafter the rates of synthesis of both gliadins and soluble glutenin declined in general towards the end of the grain filling period. Maximum rates of formation of insoluble glutenin lagged behind that of soluble glutenin from 3 to 12 days depending on genotype and growing location. Also, towards the latter part of kernel development, the proportion of IG increased at the same time that the proportion of SG decreased. These results suggest that larger polymeric glutenin is formed from smaller polymers glutenin, and this process begins at a relatively early stage of grain development. It is also likely, based on site-year differences, that the conversion of smaller polymers into larger

aggregates is concentration dependent and does not begin appreciably until a certain threshold concentration of constituent subunits are formed.

Accumulation of different protein fractions in response to weather parameters was very similar to results presented in Chapter 4. The keys to establishing cohesive site-year independent relationships between select weather parameters and protein accumulation patterns was to 1) express weather parameter in thermal time (which largely eliminates confounding effects of different grain filling durations in different environments) and 2) express protein fraction accumulation as a percentage of total protein (to eliminate confounding effects of starch accumulation). Cumulative GDD5, cumulative solar radiation and cumulative evapotranspiration had very good relationships to accumulation of gliadin and insoluble glutenin despite very large difference in site-year growing environments. For soluble glutenin, these weather parameters were capable of explaining protein accumulation patterns only for the initial phase of kernel development.

This study which reflects results derived from spring wheat grown in the Canadian Prairie region, using a different protein fractionation scheme not previously reported in the context of protein accumulation, has served to solidify understanding of the dynamic nature of wheat protein formation during grain filling that is the basis of wheat end-use quality.

5.2 Introduction

Wheat end-use quality can vary due to varying genotype and environmental factors. Depending on the nature of the wheat genotypes and growing conditions, environmental influences can often exceed those of genotype (Peterson et al., 1986; Stone and Nicolas, 1996; Panozzo and Eagles, 2000; Finlay et al., 2007). As reviewed in Chapter 4, the growing environment can affect both total wheat protein content and the content and relative proportions of the key constituent gluten protein fractions, gliadin and glutenin. Wheat gluten proteins consist of two main fractions, monomeric gliadin and polymeric glutenin. The polymeric glutenin fraction constitutes about 35-45% of the total proteins in flour (Jia et al., 1996b; Panozzo and Eagles, 2000) and in wheat kernels (Panozzo et al., 2001; Triboi et al., 2003; Rhazi et al., 2003b). The gliadin fraction is usually reported to represent 30-45% of total protein in flour (Jia et al., 1996b; DuPont and Altenbach, 2003; Metakovsky and Graybosch, 2006) and in wheat (Bénétrix et al., 1994; Stone and Nicolas, 1996a).

Glutenin protein is the major determinant of dough elasticity, while gliadins act as plasticisers and confer viscosity (Dimler, 1963; Shewry et al., 2001a). Accordingly, variation in relative amounts of glutenin and gliadin can have major effects on wheat dough rheology and end-use quality in general, by altering the molecular weight distribution of gluten proteins (Southan and MacRitchie, 1999).

A more important factor in the variation of wheat end-use quality is variation in the molecular weight distribution of polymeric glutenin. It is well established that the physical properties of polymers in general are for the most part due to their average molecular weight and molecular weight distribution (Southan and MacRitchie, 1999). That wheat glutenin proteins are among the largest protein molecules in nature (Wrigley, 1996) complements considerable published science that glutenin is the main determinant of the unique technological properties of wheat flours for breadmaking.

The molecular size distribution of glutenin can vary due to several factors, both genotypic and environmental. The basic component which is fixed for any genotype is the qualitative composition of subunits (Singh and Shepherd, 1985; Payne, 1987) which are linked by inter-molecular disulfide bonds. Glutenin is composed of two basic types of subunits which differ not only in amino acid composition but also in molecular weight. HMW-GS range from 67-160 kDa, and LMW-GS range from 23-68 kDa (Kasarda, 1999; Wieser, 2007). The relative contribution of specific subunits of HMW-GS and LMW-GS to wheat end-use quality and the molecular size distribution of glutenin have been extensively studied; see Shewry et al. (2006) and Juhász and Gianibelli (2006) for comprehensive reviews of HMW-GS and LMW-GS, respectively.

Non-genetic, i.e. environmental, contributors to varying the molecular size distribution of glutenin include the relative proportion of individual subunits and the

ratio of HMW- to LMW-GS. HMW-GS accounts for about 25-35% of total glutenins, with the balance comprised of LMW-GS (Shewry et al., 2001a). The environmental effects on glutenin molecular size distribution is ultimately influenced by the absolute and relative concentrations of individual subunits that accumulate during wheat kernel development.

The body of knowledge of accumulation patterns of wheat glutenin subunits is smaller than that for polymeric glutenin and storage protein fractions in general. It is well accepted that gliadin and glutenin protein accumulate in an asynchronous fashion (Stone and Nicolas, 1996a; Carceller and Aussenac, 1999; Triboi et al., 2003; Abonyi et al., 2007). The earliest formation of gluten proteins was proposed to be in the range of 7 DAA (Gupta et al., 1996; Panozzo et al., 2001) to 10 DAA (Shewry et al., 2009). Formation of gliadins has been reported to occur before glutenin (Gupta et al., 1996; Panozzo et al., 2001; Abonyi et al., 2007; Shewry et al., 2009), but glutenin synthesis has been reported to start as early as 7 DAA (Bushuk and Wrigley, 1971; Dell'Aquila et al., 1983; Skerritt et al., 1988; Huebner et al., 1990; Rubin et al., 1992; Gupta et al., 1996). Other research pegs the start of glutenin synthesis at about 10-14 DAA (Zhu and Khan, 1999; Deng et al., 2006; Yue et al., 2007). Insoluble or larger aggregates of polymeric glutenin are known to accumulate in significant amounts later than gliadins, and to continue to increase during the later stages of kernel development until maturity (Stone and Nicolas, 1996; Gupta et al., 1996; Pannozzo et al., 2001).

Results in Chapter 4 are in general agreement with these reports. It was observed that the 50PS protein fraction (i.e. gliadin enriched) started forming as early as 7 DAA, and accumulated at a rapid rate until about 20-25 DAA and thereafter declined marginally until maturity. Insoluble glutenin, i.e. HMW glutenin, started to form in general in significant amounts much later than that for gliadins, beginning around 25 DAA, and continued to accumulate much later than for gliadins, essentially until grain reached maturity. However, for one growing location (2003 Swift Current), insoluble glutenin synthesis began much earlier, at about 15 DAA.

Regarding changes in molecular size of glutenin during kernel development, direct evidence does not exist, as preparing total polymeric glutenin in a soluble form amenable for measurement has not been achieved. However, based on solubility fractionation of glutenin combined with size-exclusion HPLC or multistacking SDS-PAGE there is general agreement that glutenin increases its molecular size during kernel development (Gupta et al., 1996; Stone and Nicholas, 1996a; Carceller and Aussenac, 1999, 2001; Zhu and Khan, 1999; Panozzo et al., 2001; Daniel and Triboi, 2002), and that the size increase is related to an increase in the ratio of HMW- to LMW-GS (Gupta et al., 1996; Stone and Nicholas, 1996a; Carceller and Aussenac, 1999, 2001; Zhu and Khan, 1999). Concerning the differential rates and timing of accumulation of soluble (or small) polymeric glutenin compared to insoluble (or large) polymers, there has been very little reported. Daniel and Triboi (2002) observed that

compared to insoluble glutenin, soluble glutenin accumulated in a linear fashion earlier and faster, reached a maximum sooner, and finally decreased in total protein close to the end of kernel development. In contrast, insoluble glutenin showed an initial slow rate of synthesis that increased markedly past the mid-point of grain development. A similar pattern of accumulation of total or insoluble (larger polymeric) glutenin has been reported by others (Hussain and Lukow, 1994; Gupta et al., 1996; Stone and Nicholas, 1996a; Carceller and Aussenac, 1999, 2001; Zhu and Khan, 1999). These observations are consistent with the suggestion (Stone and Nicholas, 1996a) that glutenin formed during the earlier stages of grain development are mostly smaller polymers which aggregate into larger polymers as the grain matures. The rapid increase in formation of insoluble or large polymeric glutenin later in kernel development has been associated with, or attributed to effects of, grain dessication (Stone and Nicholas, 1996a; Carceller and Aussenac, 1999, 2001; Daniel and Triboi, 2002).

The effect of growing conditions including soil fertility factors on wheat protein content and composition has been comprehensively studied and documented, and the topic has been reviewed in Chapters 2 and 4. It can be summarized that supply of nitrogen, sulfur, and the effects of temperature and water stress during kernel development can highly influence the concentration and proportions of different proteins in wheat (Dupont et al., 2007). One extra observation that deserves mention is

the effect of environment on the proportions of individual HMW-GS. Based on several greenhouse experiments, Dupont et al. (2006a,b, 2007) concluded that while the concentrations of individual HMW-GS are strongly affected by varying N or temperature, synthesis during kernel development was coordinately regulated resulting in constant relative amounts of subunits under a range of growing conditions. Wieser and Zimmerman (2000) similarly reported that that there was little effect of growing conditions on the proportions of HMW-GS for genotypes with the same HMW-GS composition. On the other hand, it has been reported that the proportions of HMW-GS are affected by environment (Carcellar and Aussenac, 2001; Triboi et al., 2000).

The objective of this study was to complete the investigation started in Chapter 4 of accumulation patterns of wheat endosperm protein in relation to weather during kernel development using more precise protein fractionation, facilitated in part by RP-HPLC. A protein fractionation strategy was implemented that enabled separation of LMW- or soluble polymeric glutenin from both gliadins and HMW or insoluble glutenin. Soluble and insoluble glutenin was subsequently chemically reduced to subunits and separately quantified by RP-HPLC. This study represents the first time that accumulation patterns during kernel development of glutenin subunits of soluble and insoluble glutenin have been examined for Canadian wheats. Also there is no published science on the effects of weather variation in western Canada on wheat protein composition during kernel development. Results should contribute to a much

better understanding of environment effects on wheat protein composition and end-use quality.

5.3 Materials and Methods

5.3.1 Wheat sampling, growing locations and weather data collection

Details of wheat sampling, growing locations and environmental data collection have been described (refer to chapter sections 3.3.2, 4.3.1-4.3.3, 4.3.4).

5.3.2 Fractionation and quantification of gliadins and soluble and insoluble glutenin

Glutenin fractionation was carried out according to Fu and Sapirstein (1996) and Naeem and Sapirstein (2007) with modifications. 50% (v/v) 1-propanol soluble (50PS) and propanol insoluble (50PI) fractions were first produced as described in Chapter 4. Two subfractions were produced from the 50PS fraction, i.e. 70% (v/v) 1-propanol soluble protein and 70% (v/v) 1-propanol insoluble protein, denoted as 70PS and 70PI, respectively. Glutenin was precipitated from the 50PS fraction by addition of 1-propanol to bring the final 1-propanol concentration to 70% (v/v). 340 µl of 1-propanol was added to 500 µl of 50PS. Samples were vortexed and left at room temperature for 30 min, followed by 10 min of centrifugation at 15,000 x g. The residue, i.e. 70PI protein, represents soluble glutenin including a small quantity of ω-gliadins (Fu and Sapirstein, 1996). That residue was prepared for quantification using RP-HPLC. It was

first mixed with 200 µl of extraction buffer (described in Chapter 4) containing 1% (w/v) dithiothreitol (DTT) and extracted for 30 min at 60 °C. After reduction, the extract was alkylated with 45 µl of extraction buffer containing 5 µl of 4-vinylpyridine at 60 °C for 30 min. The extract was centrifuged for 3 min at 15,000 x g. Aliquots (120 µl) were transferred to auto-sampler vials without filtration for analysis by RP-HPLC (Agilent 1100 Series, Agilent Technologies Inc. Wilmington, DE). 50 PI protein representing insoluble glutenin was prepared for RP-HPLC analysis in an identical way to that described for the 70PI fraction.

A standard narrow bore RP-HPLC column Zorbax 300SB-C_8 (3.5 µm, 300 Å, 2.1x100 mm) was used for analysis. HPLC conditions and quantification of proteins were adopted from Naeem and Sapirstein (2007) with some modifications. Gradient conditions and peak area integration parameters are shown in Tables 5.1 and 5.2, respectively. Typical chromatograms of reduced soluble and insoluble glutenin are shown in Fig. 5.1. HMW-GS subunits were quantified according to integration areas of discrete subunits (Dy10, By9, Dx5, Bx7*, Ax2*) for Superb and (Dy12, By8, Dx2, Bx7, Ax1) for AC Vista.

Table 5.1 Gradient conditions for RP-HPLC of reduced glutenin

	Gradient type	Time (min)	%*B*
Initial hold	Isocratic	0-7	23
	Linear	7-54	23-44
	Linear	54-55	44-23
Stop time	Isocratic	55-65	23
Post-run time	Isocratic	10	23

Table 5.2 Integration parameters for quantification of glutenin RP-HPLC results

Time	Integration Events	Value
Initial	Slope sensitivity	2
Initial	Peak width	0.4
Initial	Area reject	5
Initial	Height reject	3
Initial	Shoulders	OFF
0.000	Integration	OFF
20.500	Integration	ON
35.000	Integration	OFF
35.000	Baseline next valley	ON
37.000	Integration	ON
54.000	Integration	OFF

HMW-GS retention times ranged from 21-35 min. LMW-GS subunits were quantified by integration of peaks separating between 37-54 min. All analytical determinations of glutenin fractions were carried out at least in duplicate starting with ground wheat. Soluble glutenin was determined as the sum of HMW-GS plus total LMW-GS in 70PI chromatograms. Total glutenin content was measured as sum of IG and SG. Gliadin content was determined as the difference between 50PS protein and 70PI protein excluding some ω-gliadins (Fig. 5.1) which are contained within 70PI protein, i.e. they have differential solubility properties similar to soluble glutenin as has been previously observed (Fu and Sapirstein, 1996).

A calibration curve was prepared to convert peak areas (mAU*s) to protein concentration (mg/ml). Ribonuclease A from bovine pancreas (Sigma R5125-50MG, type III-A, lyophilized powder) was used to produce the standard curve at 11 concentration points in the range 0.01 to 5.0 mg/ml. This was repeated three times over the eight week period of analysis and data were averaged to produce the final standard

curve used for protein calibration. Coefficients of variation for protein concentration were invariably below 5% indicating very acceptable reproducibility. A highly linear and tight relationship was obtained between peak areas and protein concentration as reflected by an essentially perfect correlation (R^2=0.9999).

5.4 Results and Discussion

5.4.1 RP-HPLC of soluble and insoluble glutenin and ratio of HMW-to-LMW-GS

Representative chromatograms of insoluble and soluble glutenin for cultivars Superb and AC Vista at maturity (34 DAA) for the 2003 Winnipeg location are presented in Fig. 5.1. What is most noteworthy about these results is the absolute different in protein concentration between insoluble and soluble glutenin; the former contains much more protein as evidenced by the difference in absolute scale. Also

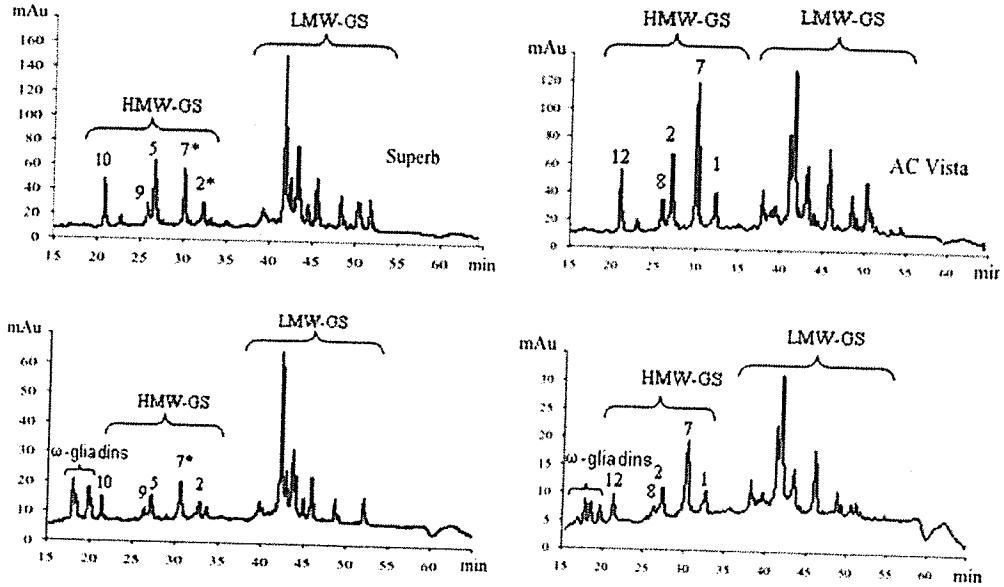

Figure 5.1 Chromatograms of insoluble (top) and soluble (bottom) glutenin for Superb (left) and AC Vista (right) at maturity (34 DAA) at 2003 Winnipeg

noteworthy is the relative difference in the ratio of HMW-to-LMW GS which was visibly greater in insoluble glutenin compared to soluble glutenin. Results in Table 5.3 confirmed the higher ratio for insoluble glutenin, which averaged about 36% higher than that for soluble glutenin. The result is consistent with the expectation that the

Table 5.3. Ratio of HMW-GS to LMW-GS in soluble, insoluble and total glutenin at maturity [1]

Cultivar	Location [2]	Year	HMW-/LMW-GS in Soluble Glutenin	HMW-/LMW-GS in Insoluble Glutenin	HMW-/LMW-GS in Total Glutenin
Superb	SC	2003	0.43	0.56	0.54
Superb	SC	2004	0.36	0.56	0.53
Superb	WPG	2003	0.30	0.52	0.48
Superb	WPG	2004	0.51	0.51	0.51
AC Vista	SC	2003	0.42	0.62	0.59
AC Vista	SC	2004	0.43	0.70	0.66
AC Vista	WPG	2003	0.39	0.50	0.48
AC Vista	WPG	2004	0.55	0.61	0.60
		Average	0.42	0.57	0.55
		Coefficient of Variation (%)	18.7	11.8	11.5

[1] Coefficients of variation across all site years and cultivars ranged from 0.2-5.3%.
[2] SC: Swift Current; WPG: Winnipeg

molecular size distribution of glutenin is directly related to the HMW-GS/LMW-GS ratio (Southan and MacRitchie, 1999) and that insoluble glutenin is associated with higher ratios (Gupta et al., 1996; Zhu and Khan, 1999; Carcellar and Aussennac, 2001). It also underscores the importance of HMW-GS as the key fraction in gluten formation and structure. Data used to calculate these ratios are documented in Table 5.4. The degree to which the HMW/LMW ratio varied due to environment was considerable;

soluble glutenin was much more variable in this regard compared with insoluble glutenin, but a genotype by environment interaction was apparent. For Superb and AC Vista soluble glutenin, the HMW/LMW-GS ratio ranged from 0.30 to 0.51, and 0.39 to 0.55, respectively. For Superb and AC Vista insoluble glutenin, the HMW/LMW-GS ratio ranged from 0.51 to 0.56, and 0.50 to 0.70, respectively. Accordingly, AC Superb was much more stable across growing locations and years for this important protein molecular factor compared with AC Vista. As expressed previously (Dupont et al., 2007), stability of wheat composition and quality is a desirable trait, and it is important to understand which wheat components are more or less affected by environment.

Table 5.4. Proportion of LMW- and HMW-GS in LMW and HMW glutenin at maturity [1]

Cultivar	Location [2]	Year	LMW-GS in Soluble Glutenin (%)	HMW-GS in Soluble Glutenin (%)	LMW-GS in Insoluble Glutenin (%)	HMW-GS in Insoluble Glutenin (%)	HMW-GS in Total Glutenin (%)
Superb	SC	2003	69.8	30.2	63.9	36.1	35.2
Superb	SC	2004	73.5	26.5	64.0	36.0	34.8
Superb	WPG	2003	76.8	23.2	66.0	34.0	32.6
Superb	WPG	2004	66.3	33.7	66.2	33.8	33.8
AC Vista	SC	2003	70.6	29.4	68.1	31.9	31.7
AC Vista	SC	2004	70.0	30.0	65.8	34.2	33.8
AC Vista	WPG	2003	71.7	28.3	72.2	27.8	27.7
AC Vista	WPG	2004	64.6	35.4	68.3	31.7	32.5
		Average	70.4	29.6	66.8	33.2	32.8

[1] Average coefficient of variation: 0.2-5.3%
[2] SC: Swift Current; WPG: Winnipeg

5.4.2 Protein solubility fractions at maturity

Table 5.5 summarizes the protein content results at maturity for the fractions that were studied. The very large variation among site-years and range in total protein percentages is a reflection of the concomitantly very large influence that the growing environment had on wheat protein content and composition, as emphasized in Chapter 4. In this respect it is worth pointing out that soil fertility was optimized at each growing site (Finlay et al., 2007) based on fertility testing. The protein fraction results are more or less similar, depending on the specific protein fraction, than those reported in an earlier study (Sapirstein and Fu 1998) using a similar protein fractionation scheme. In that study, refined flour samples were used and protein contents for gliadins, soluble glutenin, insoluble glutenin and residue were about 50%, 14%, 21%

Table 5.5. Total protein percentage at maturity and proportion of different protein solubility fractions in total protein at maturity [1]

Cultivar	Location [2]	Year	Total Protein (%)	Gliadin (%)	Soluble Glutenin (%)	Insoluble Glutenin (%)	Total Glutenin (%)	Residue Protein (%)
Superb	SC	2003	16.9	42.8	4.6	26.4	31.1	26.1
Superb	SC	2004	14.3	49.6	3.9	29.6	33.5	16.9
Superb	WPG	2003	10.0	40.6	3.5	23.5	27.0	32.4
Superb	WPG	2004	11.5	49.9	6.8	15.9	22.7	27.4
AC Vista	SC	2003	17.2	36.6	3.0	27.2	30.2	33.2
AC Vista	SC	2004	14.2	39.7	2.7	27.5	30.2	30.1
AC Vista	WPG	2003	8.8	42.8	3.2	24.4	27.6	29.6
AC Vista	WPG	2004	10.6	44.2	4.5	16.7	21.3	34.6
		Average	12.9	43.3	4.0	23.9	27.9	28.8

[1] Average coefficient of variation: 0.2-5.3%
[2] SC: Swift Current; WPG: Winnipeg

and 16%, respectively. In the present study, by comparison, gliadin protein content was lower on average by about 6%, while insoluble glutenin was marginally higher at 23.9%. However, soluble glutenin content was much lower in the present study (by about 10% protein) and residue protein was much higher (by about 12% protein). The whole grain nature of samples in this study can account for some of the difference, i.e. non-gluten protein from bran and germ tissue are likely contained in residue protein and cause percentage decreases in other fractions. Also, residue protein in the present study was calculated by difference and not directly as done by Sapirstein and Fu (1998), and that could account for the higher residue protein content that is reported in Table 5.5. As well, genotypes were different, and soluble glutenin varied widely in the earlier paper (from 9.6 to 19.4%). From a methodology perspective in the present study, soluble glutenin was separated from 50PS protein by precipitation with 70% 1-propanol for 30 min. In the earlier study, a 60 min precipitation period was used. It is possible that 30 min was too short a time to sufficiently fractionate the soluble glutenin, leading to the lower values as documented.

Results showed (Table 5.5) that cultivars Superb and AC Vista followed the same pattern by site-year for insoluble glutenin; for example, 2004 Swift Current and 2004 Winnipeg were associated with the highest and lowest protein contents for insoluble glutenin. This consistency between genotypes did not occur for gliadin and soluble glutenin. However, 2004 Winnipeg consistently had the highest proportion of gliadin

and soluble glutenin in total protein. Also, Swift Current was associated with the highest percentages of insoluble glutenin compared to the Winnipeg site.

Quantification of proportions of individual HMW-GS for cultivars Superb and AC Vista at maturity for the different site-years in this study are presented in Tables 5.6A and 5.6B, respectively. Average results for Superb are very similar to those reported for a related cultivar (Katepwa) possessing the identical HMW-GS (Naeem and Sapirstein, 2007). Results show, that with the exception of subunits 1By9 (Superb) and 1By8 (ACVista), there was essentially no environmental effect on subunit

Table 5.6A. Proportion of individual HMW-GS in total HMW-GS at maturity for Superb

Cultivar	Location	Year	Dy10 (%)	By9 (%)	Dx5 (%)	Bx7* (%)	Ax2* (%)
Superb	Swift Current	2003	14.2	14.7	27.5	28.0	15.6
Superb	Swift Current	2004	17.3	14.8	24.3	27.8	15.8
Superb	Winnipeg	2003	16.2	12.3	28.7	27.6	15.2
Superb	Winnipeg	2004	15.4	11.6	25.3	31.0	16.7
		Average	15.8	13.3	26.4	28.6	15.8
	Coefficient of Variation (%)		8.3	12.3	7.6	5.6	4.0

Table 5.6B. Proportion of individual HMW-GS in total HMW-GS at maturity for AC Vista

Cultivar	Location	Year	Dy12 (%)	By8 (%)	Dx2 (%)	Bx7 (%)	Ax1 (%)
AC Vista	Swift Current	2003	10.4	10.5	19.9	42.7	16.5
AC Vista	Swift Current	2004	11.5	10.3	19.5	42.7	16.0
AC Vista	Winnipeg	2003	11.9	6.8	22.0	45.3	14.0
AC Vista	Winnipeg	2004	12.4	8.2	20.7	44.5	14.2
		Average	11.5	8.9	20.5	43.8	15.2
	Coefficient of Variation (%)		7.4	19.8	5.4	3.0	8.3

proportions, despite the very large effects observed for site-years on protein fraction accumulation patterns (Chapter 4) and protein content at maturity (Table 5.5). In this respect, the results are in agreement with those reported previously (Wieser and Zimmermann, 2000; Dupont et al., 2007). On the other hand, Glu-1By subunits 9 and 8 in particular, showed very large variation in proportions as reflected in coefficient of variation results.

5.4.3 Accumulation pattern of protein fractions during kernel development

5.4.3.1 Gliadin, total glutenin, soluble and insoluble glutenin

Accumulation patterns of four different protein fractions as a function of kernel development time in calendar days (DAA) are shown in Fig. 5.2 and Fig. 5.3 for Superb and AC Vista, respectively. These fractions include gliadin, total glutenin, soluble and insoluble glutenin.

Distinct accumulation patterns of protein synthesis for 50PS protein and insoluble glutenin expressed as mg per 100 mg wheat have been discussed in Chapter 4. Gliadin content in this study was calculated as difference between 50PS protein and soluble glutenin, and represents a more purified fraction of monomeric protein compare to 50PS. Likewise, quantification of insoluble glutenin in this study compared to 50PI protein in Chapter 4, was more accurate based on RP-HPLC of reduced glutenin subunits. However, similar trends were expected.

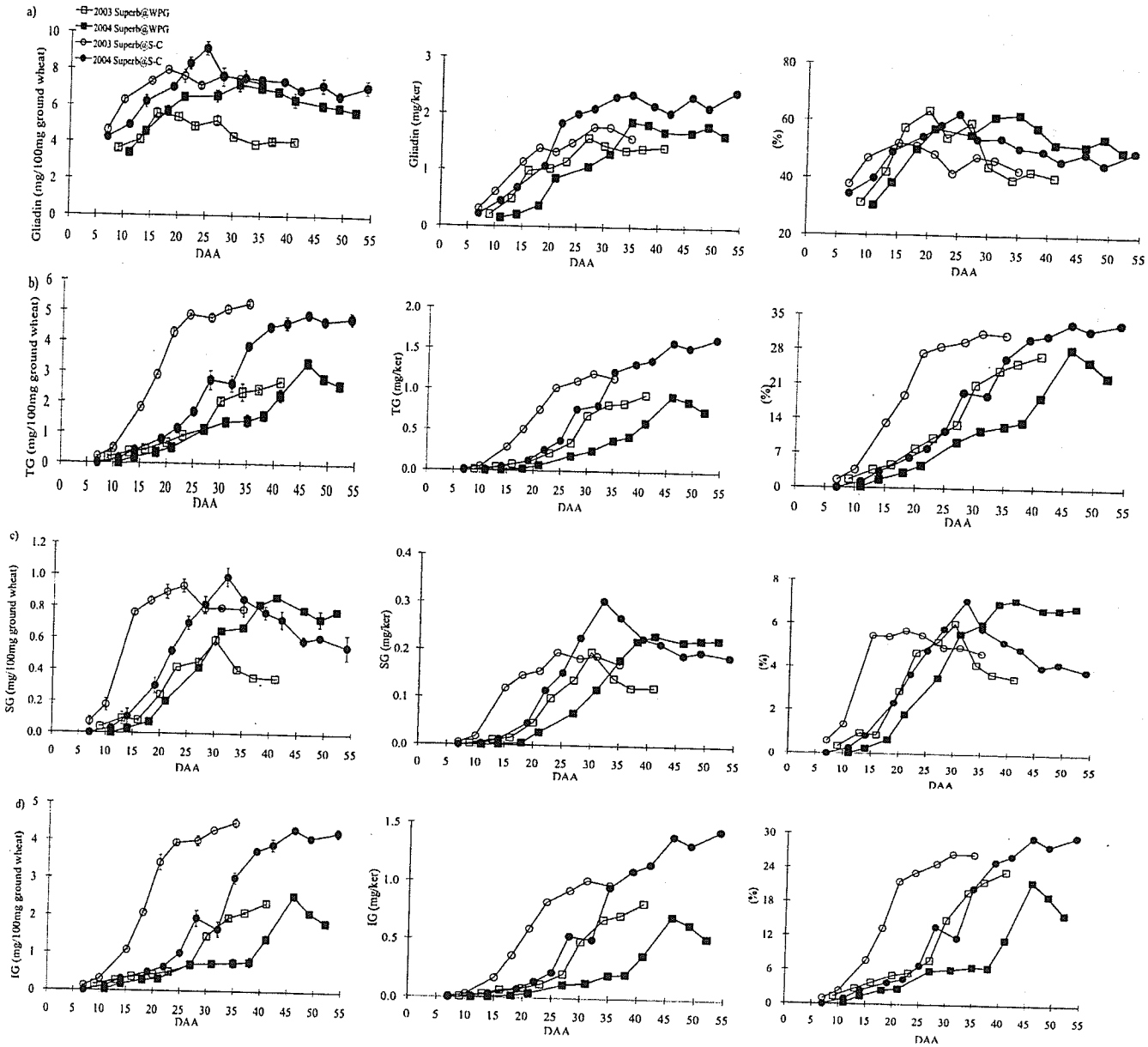

Figure 5.2 Pattern of accumulation of protein solubility fractions: a) gliadin; b) total glutenin (TG); c) soluble glutenin (SG); d) insoluble glutenin (IG) for Superb expressed based on constant grain mass (left), per kernel (middle) and as percentage in total protein (right).

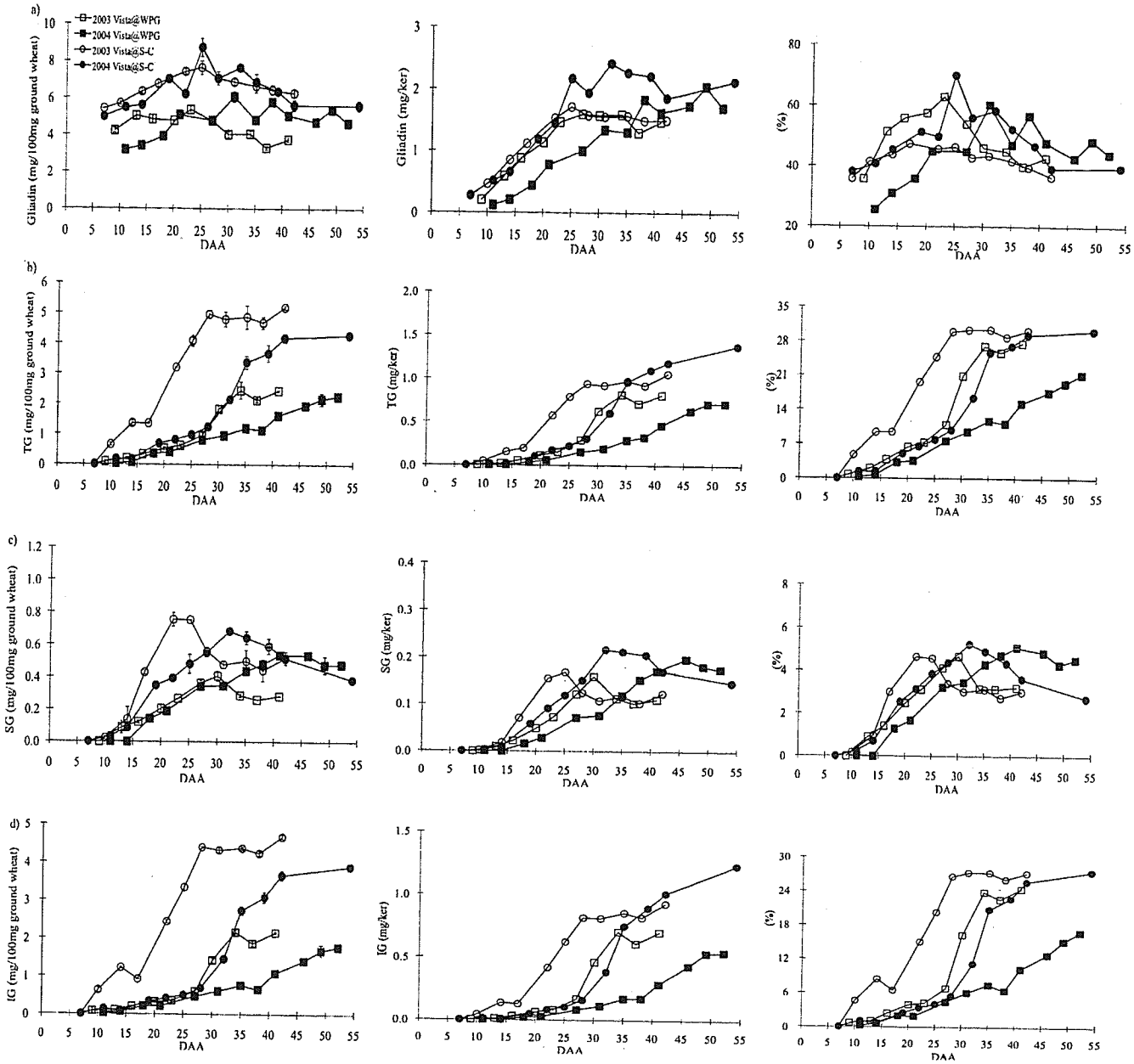

Figure 5.3 Pattern of accumulation of protein solubility fractions: a) gliadin; b) total glutenin (TG); c) soluble glutenin (SG); d) insoluble glutenin (IG) for AC Vista expressed based on constant grain mass (left), per kernel (middle) and as percentage in total protein (right).

Results in Fig 5.2 (a) and 5.3 (a) for gliadin expressed as mg/100 mg wheat are essentially identical to those in Fig 4.2 indicating that 50PS protein contains mainly gliadin proteins. Corresponding results for accumulation of insoluble glutenin during

kernel development (Figs. 5.2d and 5.3d), showed the same trends as in Fig. 4.3, but appeared to be qualitatively better or clearer. This is likely because the RP-HPLC method is obviously more specific and accurate for quantification of HMW-GS and LMW-GS compared to the UV spectrophotometric method used in Chapter 4. Still, the close correspondence in patterns of accumulation of both gliadins and glutenin between methods used indicate that the simpler and faster UV spectrophotometric approach (Sapirstein and Johnson 2000) for quantifying these protein fractions was highly effective.

Figs. 5.2b and 5.3b showed accumulation patterns of total glutenin (TG) during kernel development for Superb and AC Vista. Regardless of the protein expression method, the result is almost identical to Figs. 5.2d and 5.3d, but quantifies higher levels of protein. This is because TG is calculated as sum of both soluble (SG) and insoluble (IG) glutenin, and IG comprised about 86% of TG at maturity across sites. As observed above, applying differential solubility and RP-HPLC (Fu and Sapirstein 1996) to quantify and analyze glutenin, supplies better and more precise information of patterns of accumulation during kernel development. It also accommodates quantification of individual HMW-GS.

Accumulation patterns of SG for Superb and AC Vista (Figs 5.2c and 5.3c) showed some similarities to accumulation pattern of gliadin (Figs. 5.2a and 5.3a) during kernel development. Across all site-years, formation of SG reached a peak at

least one week later than that for maximum gliadin accumulation. Based on percentage metrics (protein expressed as mg/100 mg wheat or % of total protein), maximum gliadin synthesis occurred between 15-30 DAA, whereas SG synthesis peaked from 20-42 DAA. Thereafter, both gliadins and SG decreased in proportion until the end of the kernel development period.

As observed in Chapter 4, IG (Figs. 5.2d and 5.3d) had a very different pattern of protein accumulation compared to gliadins and SG. With the exception of 2003 Swift Current, insoluble glutenin in general started to form in significant amounts much later (beginning around 25 DAA), and accumulated initially much more slowly. Subsequently, IG significantly increased in proportion and continued to accumulate until the very end of grain development for both Superb and AC Vista. The different pattern of IG accumulation for 2003 Swift Current can be attributed to environment, i.e. the significantly higher temperatures, higher evapotranspiration and in particular, greater water deficit of this location (Table 3.2) which resulted in much quicker wheat maturation compared to other site-years. That quicker maturation was undoubtedly accompanied by rapid grain desiccation which is believed to be associated with formation of IG or large polymeric glutenin (Stone and Nicholas, 1996a; Carceller and Aussenac, 1999, 2001; Daniel and Triboi, 2002).

Regarding the association between IG and SG formation during kernel development, two observations merit attention. First, IG accumulation during the early

stages of kernel development always appeared to lag behind that of SG (compare Fig. 5.2c to Fig. 5.2d and Fig. 5.3c to Fig. 5.3d). This was true for all site-years and for both cultivars. Figure 5.4 presents this result more clearly by overlaying accumulation plots of IG and SG, each optimized to full scale. It can be seen that IG formation lags behind that of SG at respective points of maximum rates, by time periods depending on site year and genotype. For Superb and AC Vista, the range in lag time was 6-10 days and 3-12 days, respectively. A related observation is that towards the end of grain filling, and sooner for some growing sites or genotypes (Figs. 5.4d and 5.5b), the proportion of IG increased at the same time that the proportion of SG began to decrease.

These observations are similar to those reported by Stone and Nicholas (1996a) who studied analogous protein fractions, SDS-soluble and SDS-insoluble protein. Clearly, the formation of glutenin, given its highly polydisperse makeup of polymers ranging widely in molecular size (Southan and MacRitchie 1999), is very dynamic in nature. It would appear highly likely, from results of this study, that larger polymeric glutenin (IG) is formed from smaller polymers, and that this process begins at a relatively early stage of grain development. The notion that there is a continuous polymerization of smaller glutenin into larger aggregates during grain filling has been previously expressed (Pannozo et al., 2001). It is also likely, based on site-year

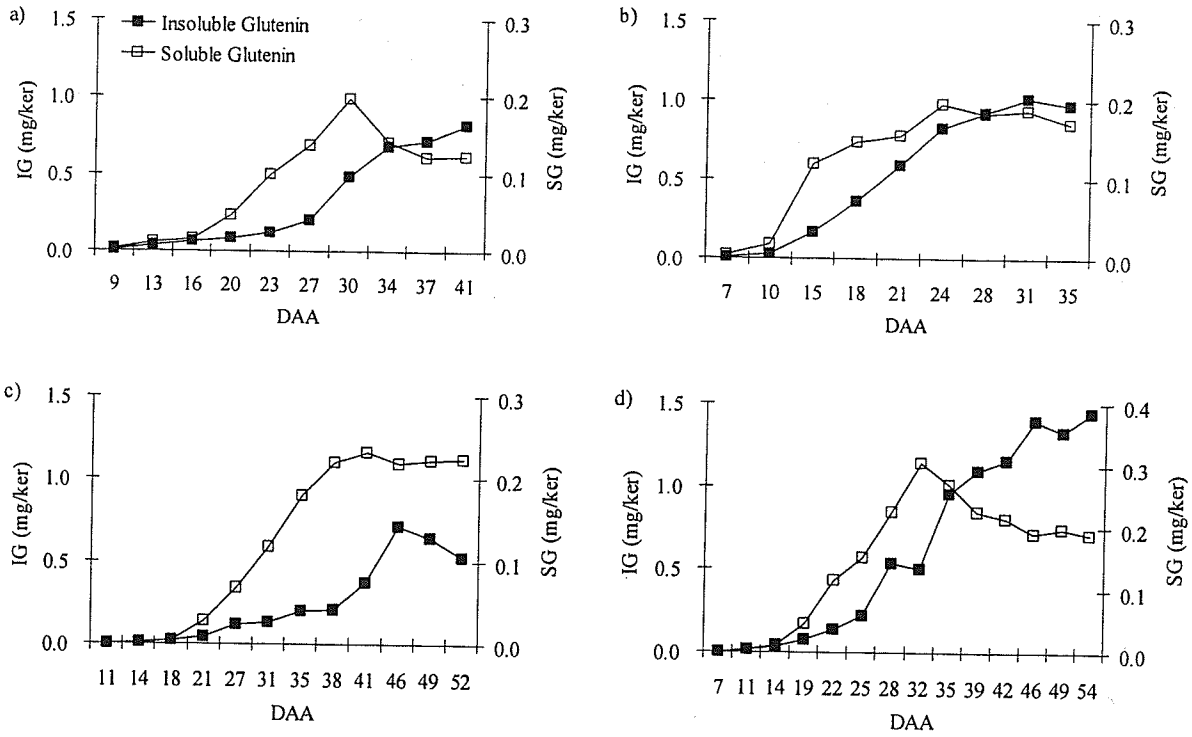

Figure 5.4. Accumulation pattern of insoluble glutenin (IG) and soluble glutenin (SG) for Superb at different site: 2003 Winnipeg (a); 2003 Swift Current (b); 2004 Winnipeg (c); 2004 Swift Current (d).

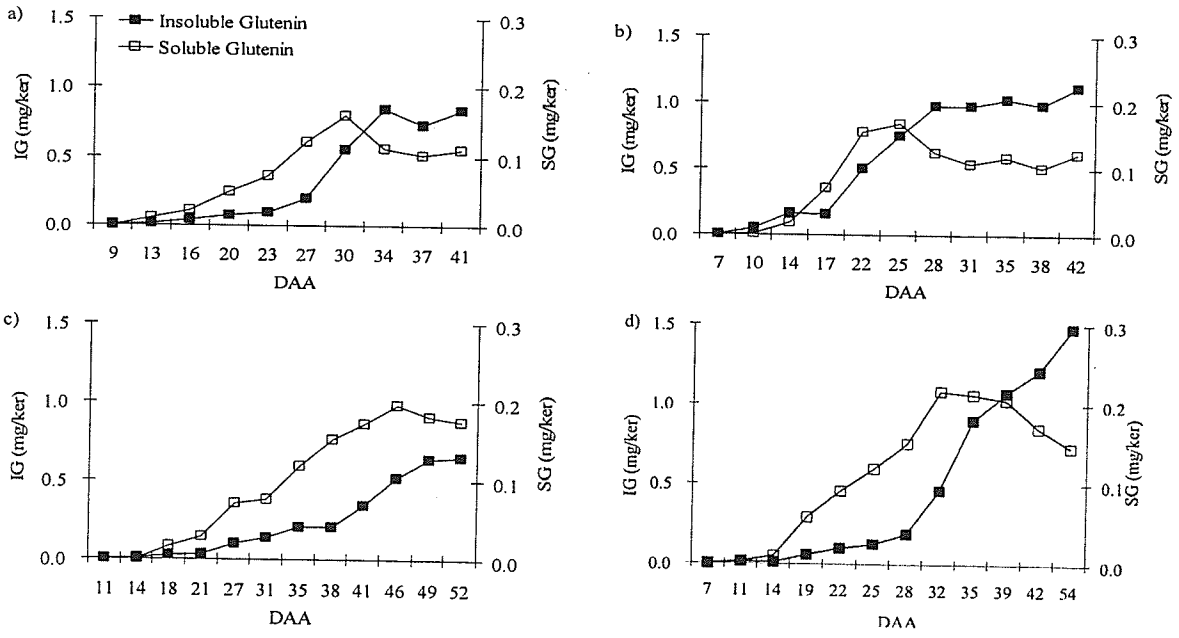

Figure 5.5. Accumulation pattern of insoluble glutenin (IG) and soluble glutenin (SG) for AC Vista at different site: 2003 Winnipeg (a); 2003 Swift Current (b); 2004 Winnipeg (c); 2004 Swift Current (d).

differences, that the conversion of smaller polymers into larger aggregates is concentration dependent and does not begin appreciably until a certain threshold concentration of constituent subunits are formed, a view expressed previously (Gupta et al., 1996) although the threshold value suggested ("2/3 of the total subunits or polymers") is clearly too high based on results presented in this study.

5.4.3.2 Accumulation of HMW-GS during kernel development

The two genotypes use in this study (Superb and AC Vista) had significantly different HMW-GS compositions most notably at the *Glu-D1* locus (Table 3.1); Superb possesses subunits 5+10, while AC Vista has subunits 2+12. Another notable difference concerns the Bx7 subunits; Superb having the normally expressed subunit 7* (average 29% of total HMW-GS across site-years, Table 5.6A) while AC Vista possesses an allelic form of the subunit that expresses considerably more protein (average 44% of total HMW-GS across site-years, Table 5.6B). The accumulation patterns of individual HMW-GS for the different site years are shown in Fig. 5.6. One of the most obvious features is the similarity of accumulation profiles within any given site year. This has been observed previously (Wieser and Zimmermann, 2000; Abonyi et al., 2007; Dupont et al., 2007) and appears to be directly related to similar rates of accumulation of RNA transcripts for HMW-GS genes (Altenbach et al., 2002). The

distinctly higher levels of over-expressed subunit Bx7 compared to other HMW-GS in AC Vista was also very noticeable.

Another compelling feature in the data were the different time frames for accumulation of HMW-GS, which reflects environment effects. As noted above in this chapter and previous ones, the duration of grain fill in 2003 was significantly shorter than in 2004, and this also translated into higher percent protein contents at maturity, likely due to interruption in starch accumulation. The result also at least partially explains the pattern of accumulation of IG as presented in Fig. 5.3d. With the exception of 2003 Swift Current where synthesis of HMW-GS was apparent at 15 DAA, significant accumulation of individual HMW-GS did not begin until about 25 DAA for other site-years.

It has been reported that genotypes with HMW-GS "5+10" are associated with glutenin polymerization at an earlier time stage than for "2+12" types (Carceller and Aussenac, 1999; Gupta et al., 1996; Naeem and MacRitchie, 2005). Results (Fig. 5.6) did not support this conclusion. The higher levels of protein expression of subunits 5 and 10 in Superb, compared to subunits 2 and 12 in AC Vista could lead to a misinterpretation of when polymeric glutenin forms.

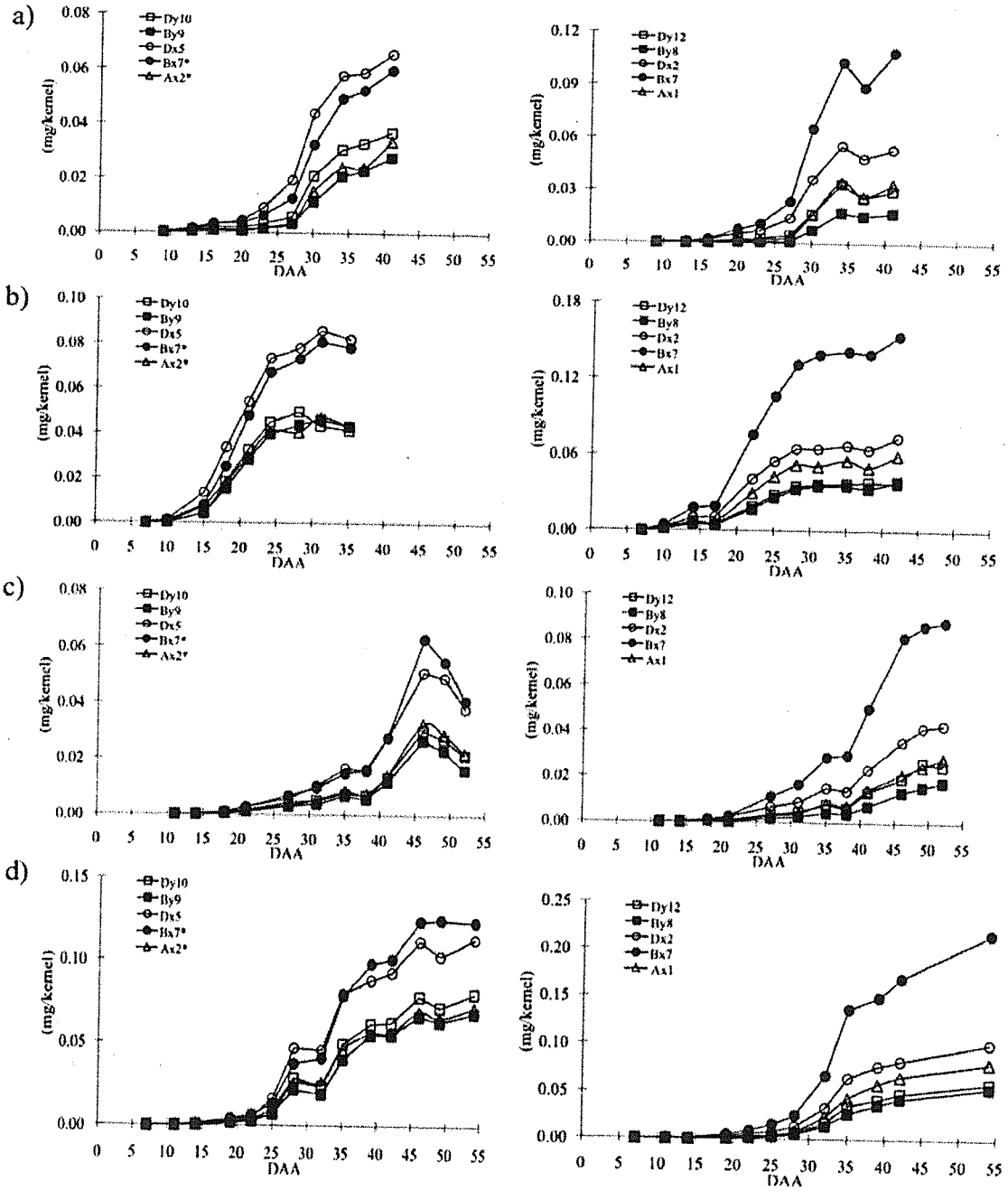

Figure 5.6 Patterns of accumulation of HMW-GS (mg per kernel) in total glutenin for Superb (left) and AC Vista (right) at different site: Winnipeg (a); 2003 Swift Current (b); 2004 Winnipeg (c); 2004 Swift Current (d).

5.4.3.3 Ratio of total polymeric glutenin to gliadin

The ratio of total glutenin to gliadin is a measure of the overall molecular size distribution of wheat storage proteins (Southan and MacRitchie, 1999). This ratio (Fig. 5.7) increased during the entire grain filling period reaching a maximum close to maturity. It tended to increase in a non-linear fashion somewhat similar to the relationship observed by Carcellar and Aussenac (1999), but was highly variable across site-years. This ratio was found to be more linear by Stone and Nicholas (1996a). The rate of increase in this ratio during the early phase of kernel development bears close similarity to the pattern of variation observed for total or insoluble glutenin across site years (Fig. 5.3). For sites excluding 2003 Swift Current, an initial lag in the ratio up until 25 DAA was followed by a rapid increase that corresponded to rapid increase in formation of insoluble glutenin. That this ratio varies in this manner indicates that the biochemical or regulatory mechanisms that control the synthesis of gliadins and glutenin are different. It was interesting that despite very large environmental effects, three of the four site-years (excluding 2004 Winnipeg) had very similar ratios of glutenin to gliadin at maturity. That is not to say that the end use quality of the wheat for these three sites would be equivalent, as protein contents at maturity varied widely (Table 5.5). Still, as an index of overall molecular size distribution of wheat gluten protein, this ratio has good information value (Southan and MacRitchie, 1999).

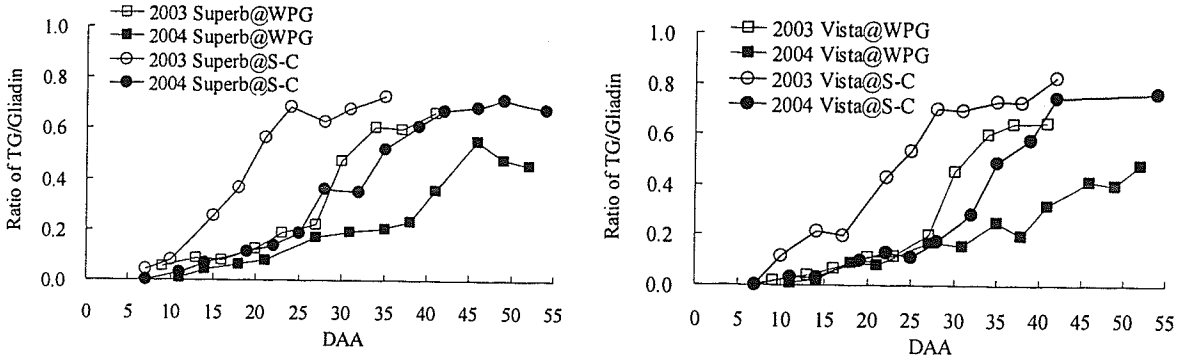

Figure 5.7. Accumulation pattern of ratio of total glutenin (TG) to gliadins for Superb (left) and AC Vista (right) at different site.

5.4.4 Impacts of environmental factors

The analysis in this chapter used the same basic approach as used in Chapter 4 to study the influence of environment across site-years on four protein solubility fractions (gliadin, total glutenin, soluble and insoluble glutenin expressed in different ways (mg/100 mg of wheat, mg/kernel, and percent of total protein). Since it was clearly shown that cultivars Superb and AC Vista produced very similar results, results for only Superb are discussed below. Appendix 6 documents corresponding results for AC Vista. It needs to be noted that in Chapter 4, a very strong relationship existed between total protein accumulation (mg per 100 mg) and cumulative water deficit (Fig. 4.9) for both cultivars Superb and AC Vista. In that study, the relationship between water deficit and 50PS protein or 50PI protein was not examined. Water deficit was examined in this study in relation to the various protein fractions that were produced,

however, no compelling relationship was found (results not shown). As well, rainfall data was found to be unrelated to protein accumulation during grain development, as was observed in Chapter 4; those relationships are excluded from the discussion below.

Results for gliadin (Fig 5.8), total glutenin (Fig 5.9), insoluble glutenin (Fig 5.10) and soluble glutenin (Fig. 5.11) as a function of four cumulative environmental parameters (GDD5 or useful heat, evapotranspiration (ET), solar radiation, and water demand) are shown in panels (a), (b), (c), (d), respectively. The ideal relationship (linear or not) between a given environmental parameter and protein variable would not show site-year effects. Several noteworthy relationships were found.

Thermal related parameters, cumulative GDD5 and cumulative solar radiation, as expected, had very similar trends for all protein fractions, regardless of whether those trends were favourable or not. Cumulative ET, also influenced by air temperature and indirectly by solar radiation, also produced some compelling relationships to some protein fractions. In contrast, cumulative water demand was only marginally related to the pattern of variation of gliadin protein only (Fig. 5.8). Crop water demand refers to the amount of moisture a crop would use given an unlimited supply of water.

Another general observation is that the best relationships between accumulation of a given protein fraction and weather variable were found when protein was expressed as a percentage of total protein, i.e. it produced the fewest site-year differences. For example, site-year effects became evident in gliadins expressed on a constant grain

weight basis (mg/100 mg wheat) after about 300 GDD5 units, 300 solar radiation units, and 80 ET units. In contrast, site-year effects were largely eliminated when gliadin was expressed as percentage of total protein. The latter calculation eliminates the confounding effects of starch accumulation in establishing a coherent predictive model of weather effects on accumulation of protein during kernel development. Clearly starch accumulation during grain filling would influence protein accumulation measures when the latter is quantified as mg protein per 100 mg wheat, or mg/kernel.

Taking each protein fraction in turn, gliadin accumulation as a percent of total protein showed strong relationships to all three temperature related weather parameters (Fig. 5.8). It can be seen that these relationships were generally site-year independent, more so for cumulative GDD5 and ET. This result was similar to that reported in Chapter 4 for 50PS protein (Fig. 4.10) which is enriched in gliadin protein.

Not surprisingly, total glutenin (Fig. 5.9) and insoluble glutenin (Fig. 5.10) responded very similarly to all the weather parameters; total glutenin was largely composed of insoluble glutenin. For total glutenin in total protein, cumulative evapotranspiration appeared to provide the best predictive relationship, as some site-year effects marginally degraded the relationships to cumulative GDD5 and solar radiation.

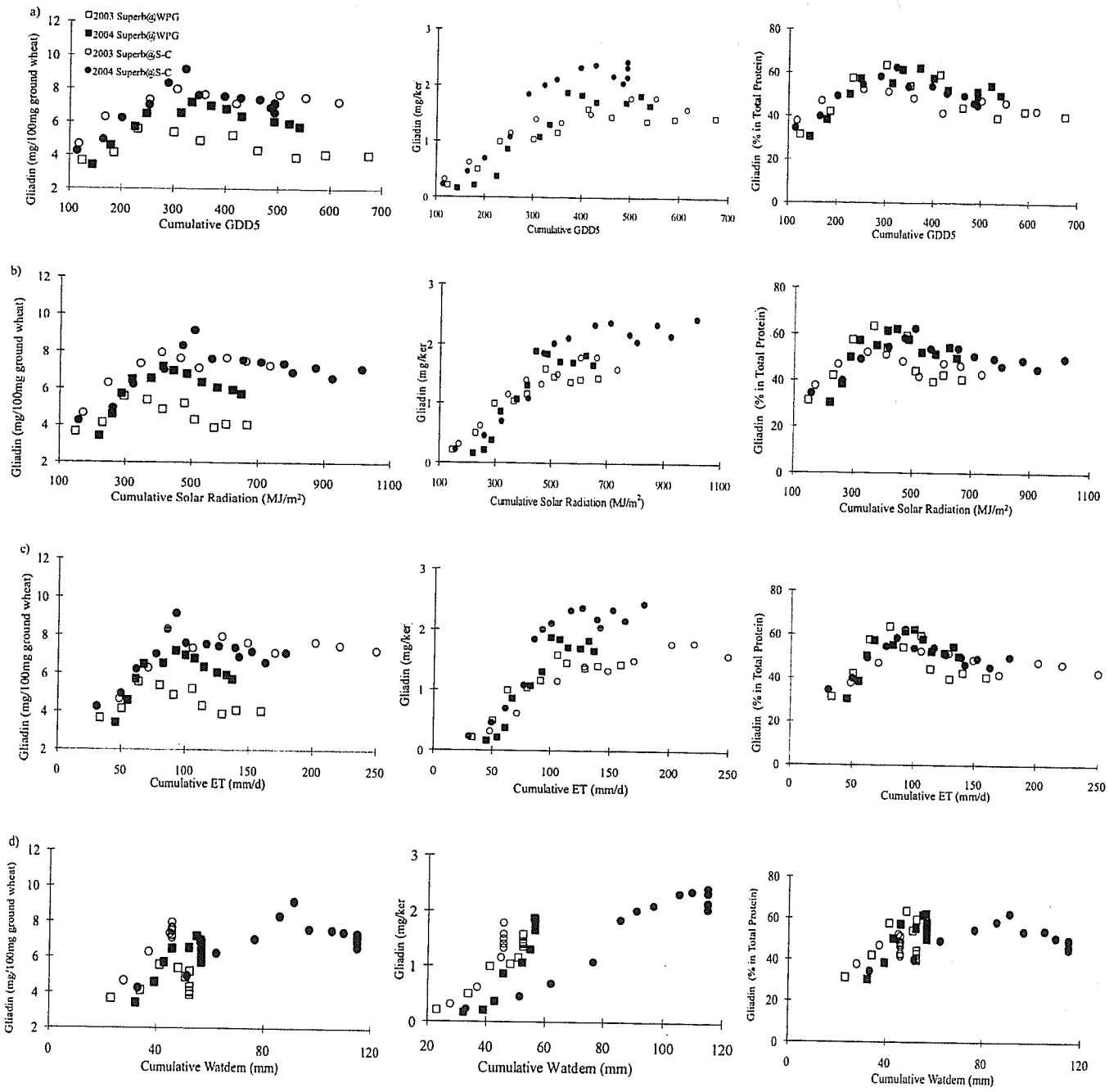

Figure 5.8. Relationship between gliadin and weather parameters; a) cumulative GDD5; b) cumulative solar radiation; c) cumulative ET; d) cumulative water demand, expressed at a constant grain basis (left), per kernel (middle) and as percentage in total protein (right) for Superb.

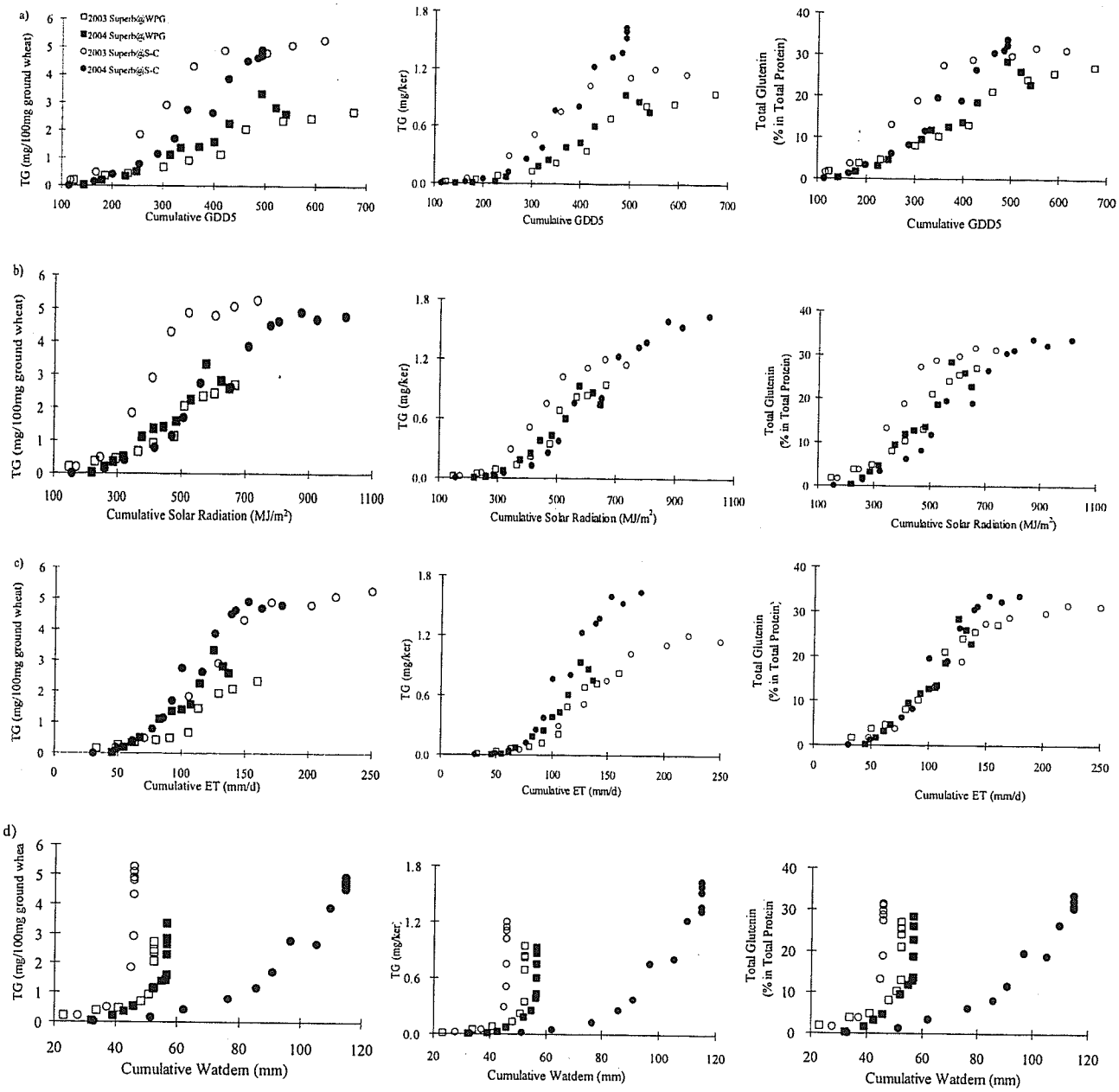

Figure 5.9. Relationship between total glutenin and weather parameters; a) cumulative GDD5; b) cumulative solar radiation; c) cumulative ET; d) cumulative water demand, expressed at a constant grain basis (left), per kernel (middle) and as percentage in total protein (right) for Superb.

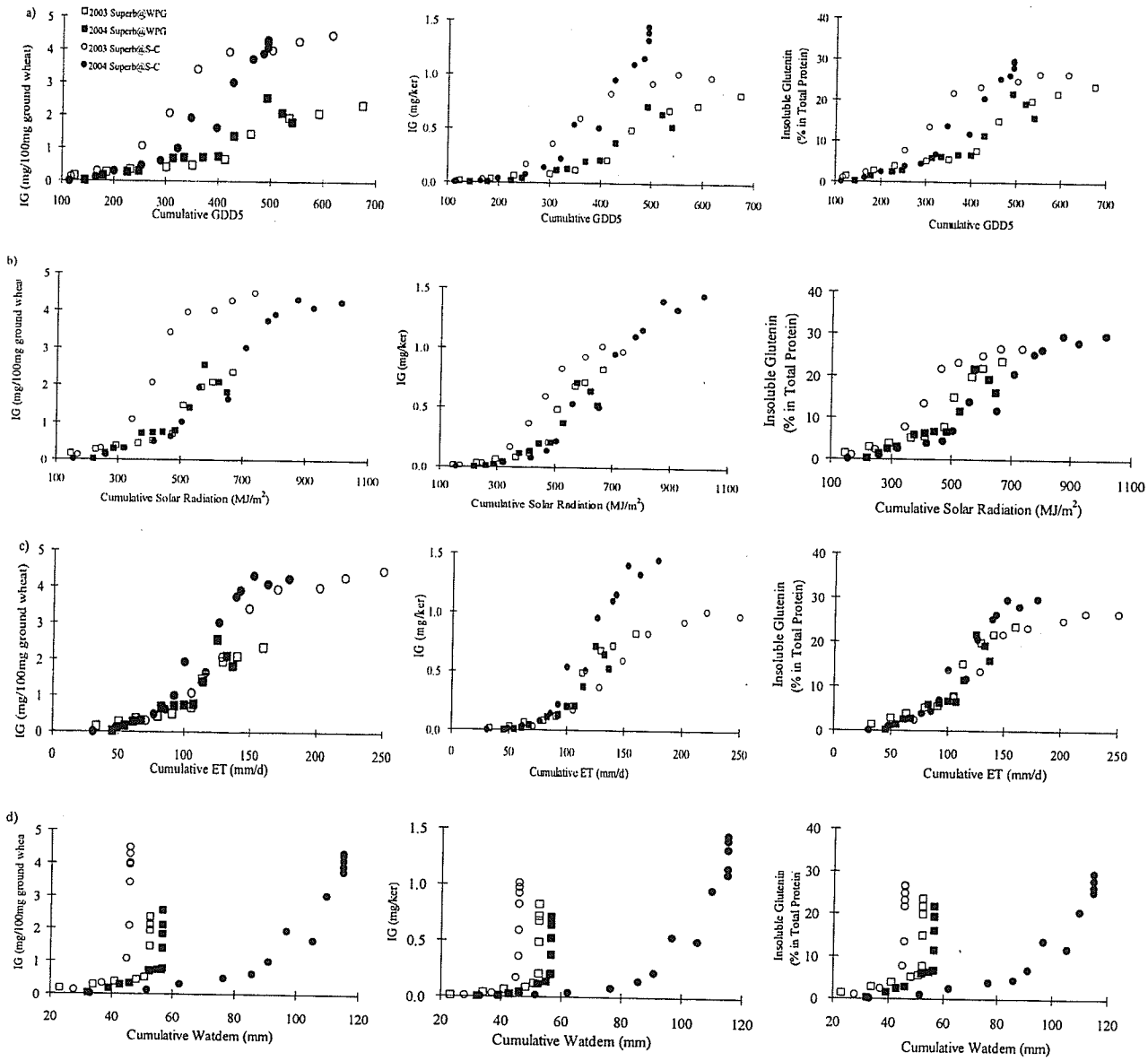

Figure 5.10. Relationship between insoluble glutenin and weather parameters; a) cumulative GDD5; b) cumulative solar radiation; c) cumulative ET; d) cumulative water demand, expressed at a constant grain basis (left), per kernel (middle) and as percentage in total protein (right) for Superb.

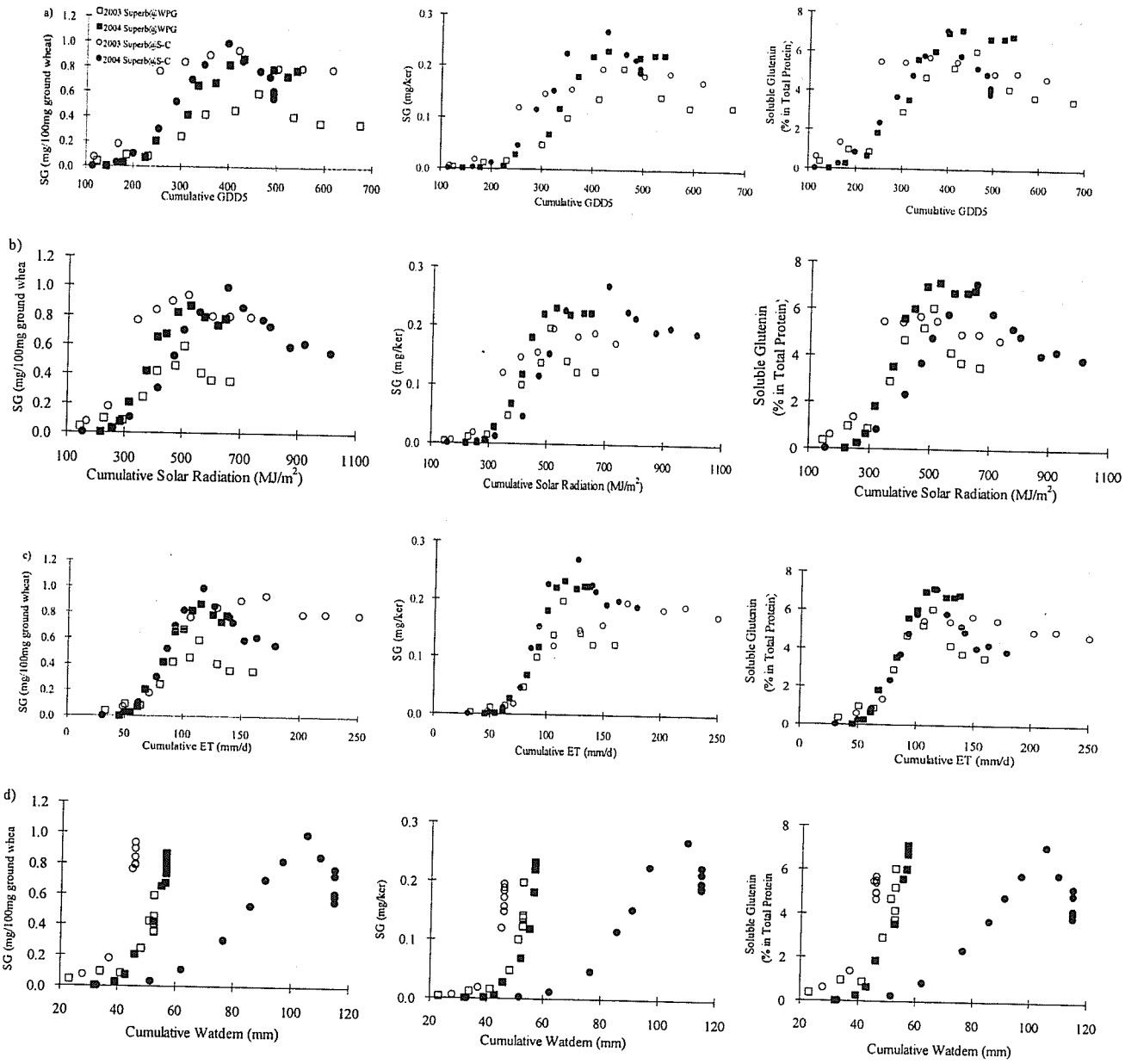

Figure 5.11. Relationship between soluble glutenin and weather parameters; a) cumulative GDD5; b) cumulative solar radiation; c) cumulative ET; d) cumulative water demand, expressed at a constant grain basis (left), per kernel (middle) and as percentage in total protein (right) for Superb.

Interestingly, accumulation of soluble glutenin in relation to cumulative GDD5, solar radiation and ET (Fig. 5.11), showed some similarities to relationships obtained for gliadin. The similarities appear to be related to the relatively similar accumulation patterns of these two protein fractions (Fig. 5.2), i.e. both fractions showed initial high rates of formation during the early phase of grain filling which was followed by decreasing rates of synthesis by approximately the mid-point of kernel development. However, for soluble glutenin, cohesive relationships to ET for example (for all three measures of protein expression) was maintained only for the initial rapid accumulation phase of kernel development. Thereafter, significant site-year effects were evident.

5.5 Summary and Conclusions

Accumulation patterns of gliadin protein, total glutenin, small (soluble) and large (insoluble) polymeric glutenin were studied for two hard spring wheat genotypes grown in two western Canadian field sites in two consecutive growing seasons. Those accumulation patterns were also analyzed in response to weather-related parameters acquired instrumentally at those sites. Accumulation patterns of individual HMW-GS, profiles of ratios of glutenin to gliadin, as well as HMW/LMW-GS ratios at maturity were also documented. Results confirmed the asynchronous nature of gliadin and glutenin synthesis during kernel development, but also revealed a synchronous relationship between formation of small and large polymers of glutenin, as well as

coordinated regulation of synthesis of individual HMW-GS. Protein fractions followed the following order of synthesis during grain development: gliadin, followed by soluble glutenin, followed by insoluble glutenin. While gliadin and soluble glutenin formation tapered off during the late stages of kernel development, formation of large polymeric glutenin continued until grain was mature. These patterns of accumulation of protein were similar to those reported previously in the literature. The knowledge base in that regard is very small and has not up to now reflected results derived from wheat grown in the Canadian Prairie region. Accordingly, this study which also used a different protein fractionation scheme, served to solidify understanding of the dynamic nature of wheat protein formation during grain filling that is the basis of wheat end-use quality.

Weather conditions among growing sites and years were very diverse, and substantially influenced both the duration of protein accumulation and concentration of the different protein fractions that accumulated. Because grain develops and matures at different rates (depending mainly on air temperature), analyzing weather effects in different growing locations simultaneously on protein accumulation rates and levels using a calendar day approach would be confounded as grain would be in different stages of development in different sites and/or years. The key to normalizing the data was found by plotting protein accumulation profiles as a function of temperature-related weather parameters (such as GDD5) calculated in a cumulative fashion. This

strategy is supported in principle as duration of grain filling has been reported to be constant in so-called "thermal time" (Triboi et al., 2003; Martre et al., 2006). The second aspect of the normalization relates to the manner in which protein accumulation is expressed; quantifying protein accumulation relative to total protein is the only way to eliminate confounding effects of starch accumulation which would vary inversely with protein accumulation expressed on a mg/kernel or mg/100 mg grain basis. This approach was used this study, and strong site-year independent relationships were found between temperature-related weather parameters such as thermal time (GDD5), evapotranspiration and solar radiation and accumulation of gliadin and formation of insoluble glutenin expressed in percentage terms for the entire grain filling period.

Chapter 6

General Discussion and Conclusions

6.1 Discussion

Numerous studies over the past several decades have shown that wheat end-use quality for a particular sample of wheat or flour is highly dependent on both genotype and environment factors related mainly to the gluten forming proteins of endosperm tissue. Environment can often be more influential than the genotype with respect to quality variation, and the key to understanding the nature of wheat quality variation begins with an understanding of how and why accumulation patterns of wheat protein vary during kernel development which was the focus of this thesis' research project.

While there exists a very substantial body of knowledge on the effects of environment on gluten proteins of mature wheat, a much smaller, but still impressive, subset of papers have been published on the topic of protein content and compositional changes during grain development (Bechtel et al., 1982; Skerritt et al., 1988; Bénétrix et al., 1994; Hussain and Lukow, 1995; Stone and Nicolas, 1995 a,b; Gupta et al., 1996; Stone and Nicolas, 1996 a; El Haddad et al., 1997; Stone and Nicholas 1998 a,b; Zhu and Khan, 1999; Daniel and Triboi, 2000; Carceller and Aussenac, 1999, 2001a; Panozzo et al., 2001; Altenbach et al., 2003; Rhazi et al., 2003a, b; Triboi et al., 2003; Plaut et al., 2004; Johansson et al., 2005; Naeem and MacRitchie, 2005; DuPont et al., 2006a, b; Hao et al., 2006; Iametti et al., 2006; Abonyi et al., 2007; Yue et al., 2007;

Irmark et al., 2008; Jiang et al., 2009; Shewry et al., 2009). Moreover, studies which go further to quantify at least one or more environmental factors (most often ambient temperature in controlled experiments) are fewer in number (Skerritt et al., 1988; Stone and Nicolas, 1996a; Daniel and Triboi, 2000; Panozzo et al., 2001; Altenbach et al., 2003; Triboi et al., 2003; Plaut et al., 2004; Johansson et al., 2005; DuPont et al., 2006a, b; Yue et al., 2007; Irmark et al., 2008; Jiang et al., 2009), and only two (Panozzo et al., 2001; Yue et al., 2007) were based on field experiments where treatments were controlled; Panozzo et al. (2001) and Yue et al. (2007), looked at irrigation and N fertilizer effects, respectively.

Among all the aforementioned studies, there are none involving Canadian wheats except for Hussain and Lukow (1995) who documented electrophoretic composition changes of reduced polymeric protein during grain development of a few Canadian wheat genotypes in a greenhouse experiment with constant environmental conditions.

To fill this gap of knowledge, this thesis project investigated the pattern of accumulation of wheat gluten proteins during kernel development in relation to detailed weather data acquired concurrently using two adapted Canadian spring wheat genotypes grown under N-optimized natural field conditions in the Canadian Prairie region in two consecutive years.

A wide range of protein accumulation patterns were observed reflecting major differences in growing season weather among site-years. For the most part, there was

relatively little difference in response between the two genotypes used in the study, despite the differences in their HMW-GS composition. The very large range in total protein content at maturity across site-years (~ 9-17%) was very compelling and indicated the considerable influence that crop season weather can have molecular mechanisms of grain development and wheat quality in general.

The time for grain filling, hence protein accumulation, was considerably shortened in 2003 compared to 2004. Grain filling duration was also strongly negatively correlated with the rate of grain filling. Significantly lower precipitation and significantly higher temperatures in 2003 growing sites were the likely reasons for this outcome. This result confirms previous work that temperature (Sofield et al., 1977a; Shpiler and Blum, 1986; Gibson and Paulsen, 1999; Dias and Lidon., 2009) and total rainfall (Schelling et al., 2003) are negatively and positively associated with grain filling period, respectively.

Accumulation patterns of total protein, and constituent protein fractions (gliadin, small polymeric glutenin, large polymeric glutenin, and residue) expressed in different ways were analyzed as a function of kernel development in calendar days, i.e. days after anthesis (DAA), and in response to acquired weather parameters, both basic and modeled, expressed cumulatively. Results confirmed the highly asynchronous formation of some wheat protein types (i.e. gliadin and glutenin) during kernel development (Stone and Nicolas, 1996a; Carceller and Aussenac, 1999; Triboi et al.,

2003; Abonyi et al., 2007). A continuously increasing ratio of glutenin to gliadin during kernel development also indicated a basic difference in regulation of gliadin and glutenin synthesis.

On the other hand, a synchronous relationship appeared to exist between small (soluble) and large (insoluble) polymers of glutenin. Adapting terminology discussed previously (Stone and Nicholas, 1996a), the terms asynchronous or synchronous refer to: (a) the time at which significant deposition of each protein type first occurred, (b) the time at which peak absolute accumulation occurred and (c) pattern of accumulation after peak protein formation.

Averaged across growing sites and years, protein by type started accumulating and reached peak deposition in the following order: gliadin, soluble glutenin, insoluble glutenin. Gliadin synthesis began as early as 7 DAA for one growing location (2003 Swift Current) and was clearly underway for all site-years by 15 DAA. Synthesis of small glutenin polymers lagged slightly behind that for gliadins by about 3 days, accumulated at a comparable or somewhat slower rate compared to gliadins, and reached a peak at least one week later. After peak absolute accumulation (mg/kernel), gliadins remained at generally constant levels in all site-years, while soluble glutenin comprising small glutenin polymers, decreased significantly in absolute and proportional terms for another 10 to 20 calendar days until maturity depending on growing location.

Insoluble glutenin (large glutenin polymers) on the other hand started to form in general in significant amounts much later than that for gliadins, beginning around 25 DAA, but at a higher rate. However, for one growing location (2003 Swift Current) where kernel development was accelerated, insoluble glutenin began to form at a high rate at about 15 DAA, but still later than that for soluble glutenin at that location. At the peak rate of absolute protein deposition which lasted from 6-12 days, insoluble glutenin invariably lagged behind that of soluble glutenin from 3 to 12 days depending on genotype and growing location. No peak accumulation was observed for insoluble glutenin, which continued to increase, but at a slower pace, until maturity.

This pattern of accumulation of insoluble glutenin appears to be a characteristic trait as very similar results have been reported by others (Hussain and Lukow, 1994; Gupta et al., 1996; Stone and Nicholas, 1996a; Carceller and Aussenac, 1999, 2001; Daniel and Triboi, 2002; Zhu and Khan, 1999). The rapid increase in formation of insoluble or large polymeric glutenin later in kernel development has been associated with, or attributed to effects of, grain dessication (Stone and Nicholas, 1996a; Carceller and Aussenac, 1999, 2001; Daniel and Triboi, 2002).

Concerning the differential rates and timing of accumulation of soluble (or small) polymeric glutenin compared to insoluble (or large) polymers, not much has been published. Daniel and Triboi (2002) observed that compared to insoluble glutenin, soluble glutenin accumulated in a linear fashion earlier and faster, reached a maximum

sooner, and finally decreased in total protein close to the end of kernel development. In the present study, the proportion of insoluble glutenin increased at the same time that the proportion of SG decreased towards the latter part of kernel development and earlier for some growing sites. Stone and Nicholas (1996a) obtained a similar result using a different protein fractionation scheme, and suggested the two events were mechanistically related, i.e. aggregation of soluble glutenin leads to formation of insoluble glutenin. Using multi-stacking gel electrophoresis, Zhu and Khan (1999) and Panozzo et al. (2001) showed that the molecular size of glutenin increased throughout grain filling. Panozzo et al. (2001) suggested that this was due to a continual polymerization of smaller glutenin aggregates into larger molecules. Results obtained in this study are consistent with this hypothesis, especially considering the lag in formation of insoluble glutenin relative to soluble glutenin that was observed, suggesting that insoluble glutenin is formed from smaller glutenin polymers. It is also likely, based on site-year differences, that the conversion of smaller polymers into larger aggregates is concentration dependent and does not begin appreciably until a certain threshold concentration of constituent subunits are formed, a view expressed previously (Gupta et al., 1996). However, the threshold value suggested ("2/3 of the total subunits or polymers") is clearly too high based on results obtained in this study.

Like the parent glutenin fraction, accumulation patterns for constituent HMW glutenin subunit composition were highly influenced by weather-induced site-year

effects and some different trends were observed for individual HMW-GS loci. Most notable was over-expressed Bx7 subunit of AC Vista as it accumulated at a much higher rate compared to the other four HMW-GS in its complement. HMW-GS Dx5 and Bx7* of Superb also accumulated at a faster rate compared to the other three HMW-GS. These effects were consistent among site-years. However, small but apparently significant differences in relative rates of synthesis of Superb HMW-GS among site-years towards the end of the kernel development, appears to be at odds with the view that the growing environment has little to no effect on the final proportions of subunits (Dupont et al., 2007).

There were very significant environmental impacts on patterns of accumulation of gluten protein fractions in keeping with published reports. The nature of these differences was basically related to large differences in growing location weather in the different site years used for the study. Those differences in protein accumulation patterns were superficially reflected in differences across site years in one or more acquired weather parameters such as daily average temperatures, rainfall, windspeed, evapotranspiration, solar radiation, and modeled water demand and water deficit.

However, identifying site-year independent trends in protein accumulation patterns during kernel development, with corresponding trends in weather variables was challenging. Weather factors that were site characteristics included solar radiation (but not air temperature), wind speed (but not evapotranspiration), water demand, and water

deficit; all had higher values in one location (Swift Current) compared to another (Winnipeg) averaged across years. In contrast, precipitation and air temperature were growing season characteristics. Adding to the complexity was that no compelling results were found when protein accumulation was examined for weather parameters varying by calendar days (i.e. DAA).

The most promising results were obtained when protein accumulation was analyzed in response to cumulative weather parameters, such as thermal time, which strives to normalize different durations in calendar days of grain filling that may occur due to temperature effects in different growing locations. For example, relatively high temperatures during grain development typically shorten the duration of grain filling for bread and durum wheat (Sofield et al., 1977a; Shpiler and Blum, 1986; Gibson and Paulsen, 1999; Dias and Lidon., 2009), and 2003 and 2004 growing seasons were very different (by 6 ^{o}C) in average daily temperature during grain development. Consequently grain developed and matured at different rates. Accordingly, analyzing weather effects on protein accumulation using a calendar day approach would be confounded as grain would be in different stages of development in different sites and/or years. As duration of grain filling has been reported to be constant in so-called "thermal time" (Triboi et al., 2003; Martre et al., 2006), this approach seemed promising in principle.

As used in this study, thermal time quantified useful heat, i.e. temperatures above 5°C (i.e. growing degree days > 5 °C or GDD5) were cumulated in response to protein accumulation progressively from the beginning of anthesis to maturity. A few other studies (Stone and Nicolas, 1998; Daniel and Triboi, 2001; Triboi et al., 2003; Martre et al., 2006) have used basic thermal time measured above 0 °C to track relationships to grain filling rate, duration and protein accumulation. Moreover, all acquired weather variables, not just temperature, were cumulated and responses of various protein fractions during kernel development were charted. This study appears to be the first to evaluate the influence of weather on protein accumulation in this way.

The goal of this analysis was to obtain a coherent response of protein accumulation during kernel development to weather trends across all site-years, i.e. to produce site-year independent patterns. Using thermal time to normalize calendar day differences in grain filling duration among site-years was one key to achieving this goal. A second requirement was related to the expression of protein fraction accumulation in proportion to total protein, to eliminate confounding effects of starch accumulation which would vary inversely with protein accumulation quantified on a mg/kernel or mg/100 mg grain basis. Results clearly indicated that strong site-year independent relationships could be generated between temperature-related weather parameters such as GDD5, evapotranspiration and solar radiation and accumulation of gliadin, soluble glutenin and insoluble glutenin protein for the entire grain filling

period. For insoluble glutenin, those relationships followed a linear trend. However, for gliadin and soluble glutenin, bell-shaped patterns of protein accumulation were evident, with soluble glutenin showing a more pronounced profile.

6.2 Conclusions

This thesis project has provided a comprehensive assessment of environment effects on patterns of accumulation of gluten protein and fractions during kernel development for Canadian spring wheat genotypes grown in the natural field environment in western Canada. The majority of results have confirmed basic observations related to protein accumulation made in previous studies that used very different wheat genotypes grown in different climatic regions compared to Prairie conditions in Manitoba and Saskatchewan. Clearly, the dynamics of the timing of the synthesis of different wheat proteins during kernel development, while highly complex, are also highly regulated at a fundamental genetic level that is a shared characteristic among wheat genotypes. Results have clearly shown that regional variation in weather, such as can routinely occur within adjacent Prairie provinces, even in the same growing season, can have remarkably large effects on the absolute and relative concentrations of wheat proteins accumulating within developing endosperm and, to a somewhat lesser extent, on the timing of protein formation. When temporal differences in the patterns of accumulation occur and there is a need to

compare results from different growing sites and/or years, results suggest that correcting calendar days to thermal time is a very effective strategy. How accumulating protein is expressed as a function of development time is also another important consideration in comparing results from different growing locations in kernel development studies. The strong relationships found between GDD5 and related weather parameters, and protein fraction accumulation in total protein could potentially be used in developing models for predicting wheat breadmaking quality before harvest. Those models could be used for example, to improve the ability of the market place to match wheat quality requirements of specific customers to wheat grown in specific regions of Western Canada.

Chapter 7

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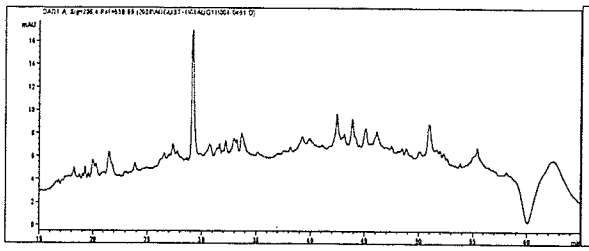
Chapter 8

Appendix

A.1 Reversed-phase HPLC chromatograms of glutenin subunit composition during kernel development

A 1.1 Superb 2003 Winnipeg insoluble glutenin

DAA 9



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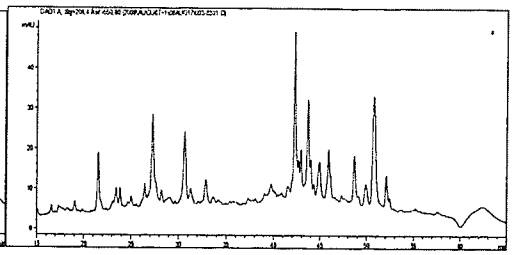

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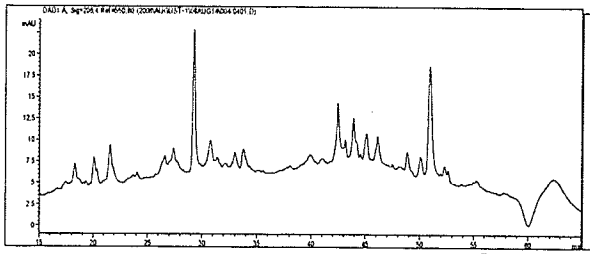

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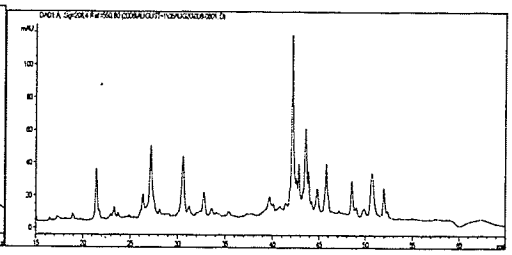

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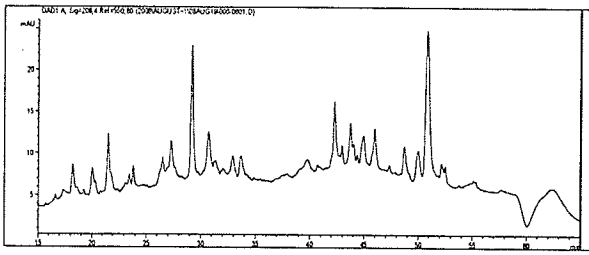

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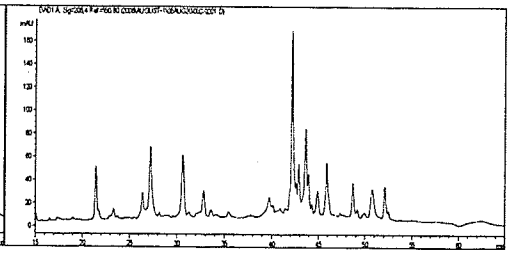

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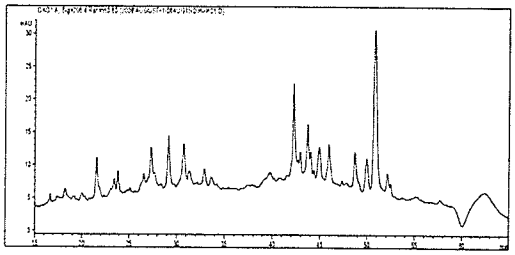

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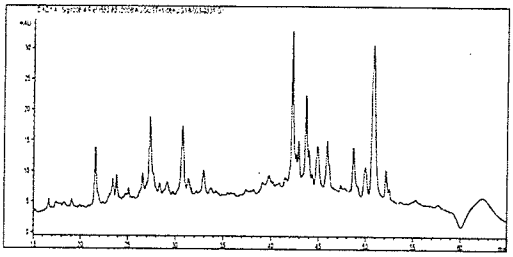

A 1.2 Superb 2003 Winnipeg soluble glutenin

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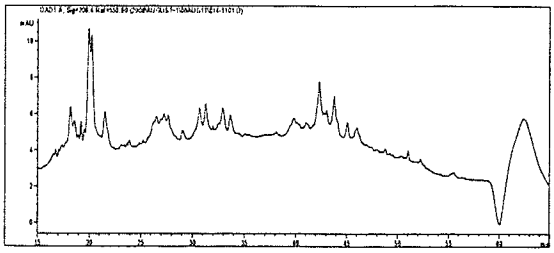

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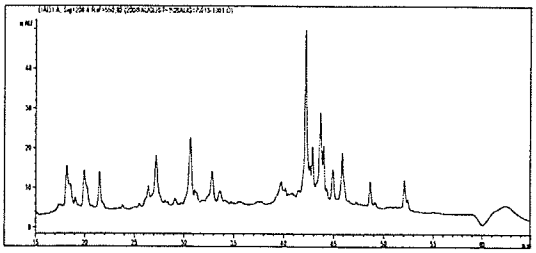

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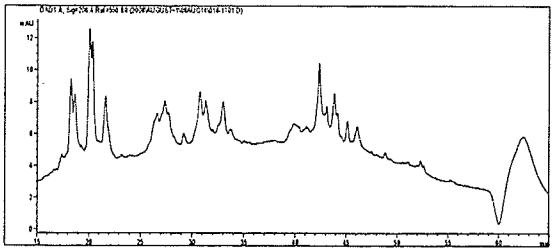

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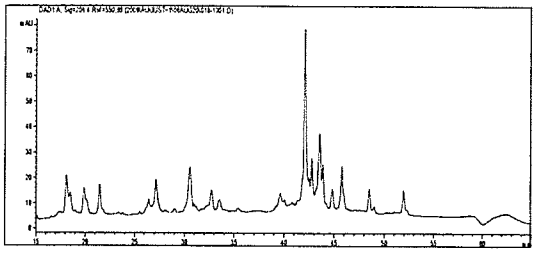

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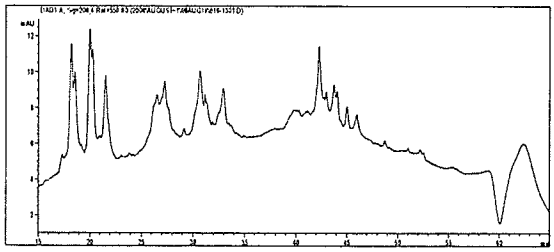

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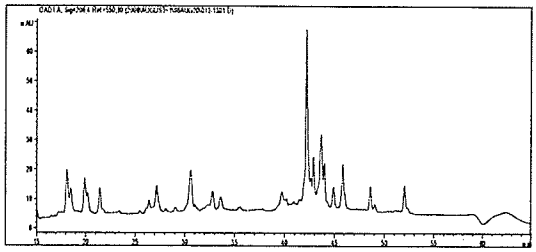

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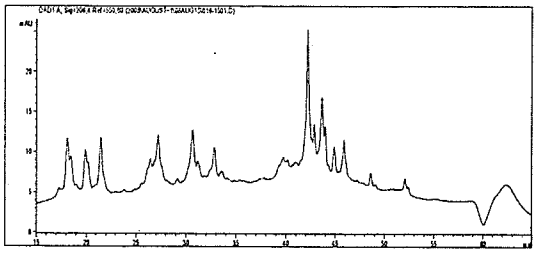

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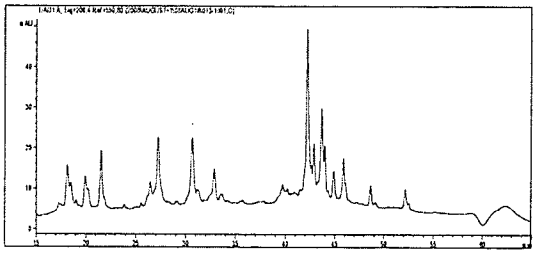

A 1.3 Superb 2004 Winnipeg insoluble glutenin

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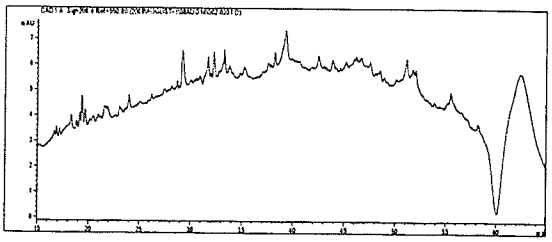

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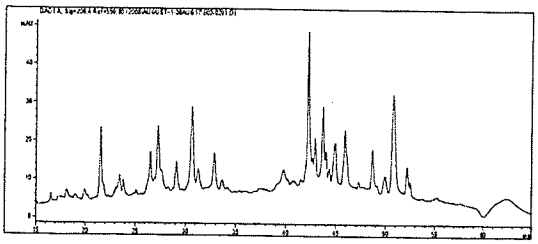

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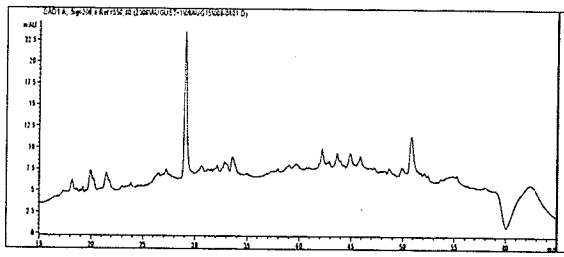

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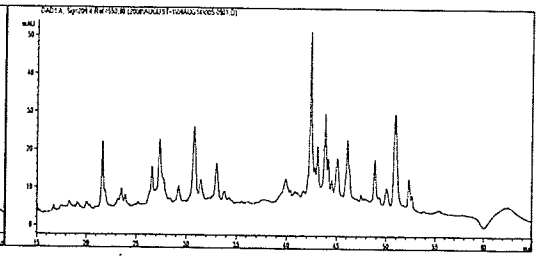

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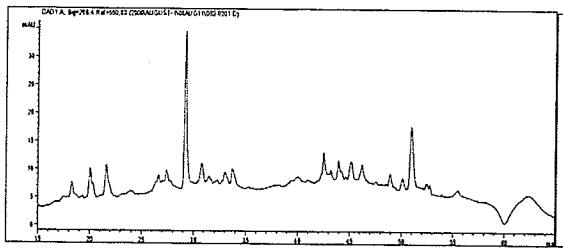

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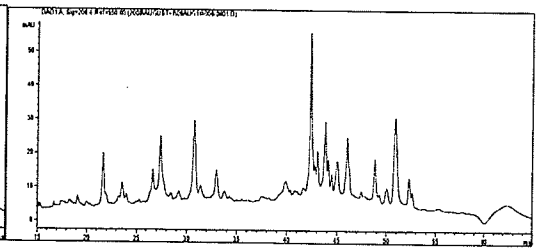

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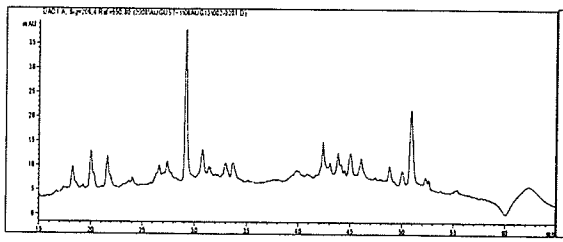

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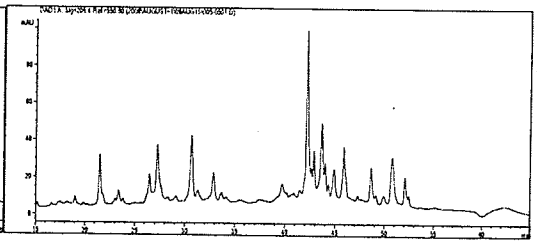

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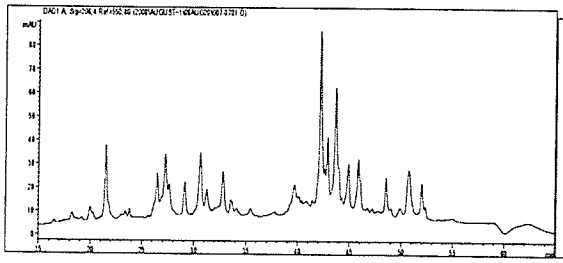

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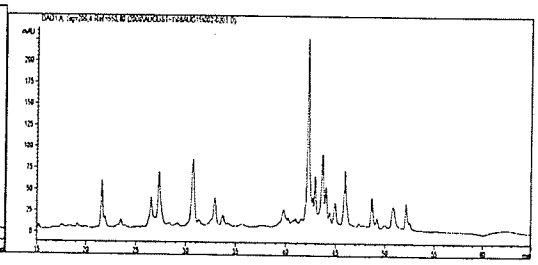

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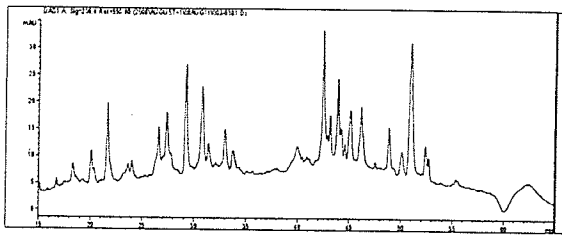

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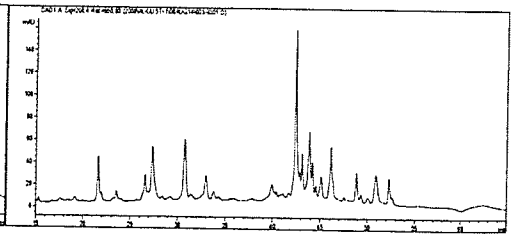

A 1.4 Superb 2004 Winnipeg soluble glutenin

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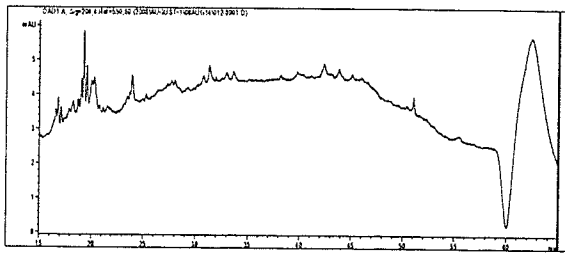

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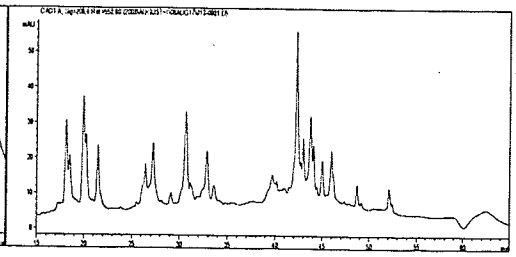

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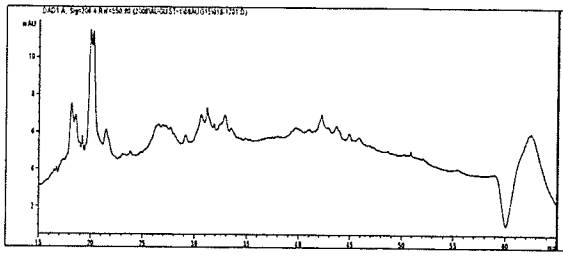

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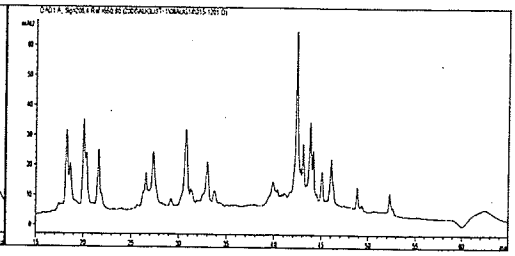

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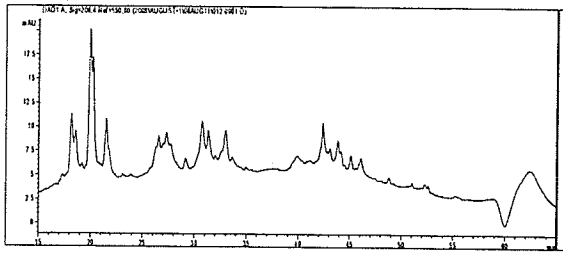

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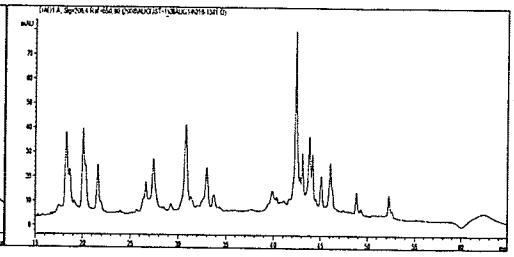

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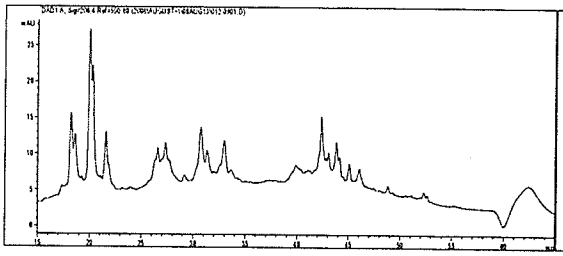

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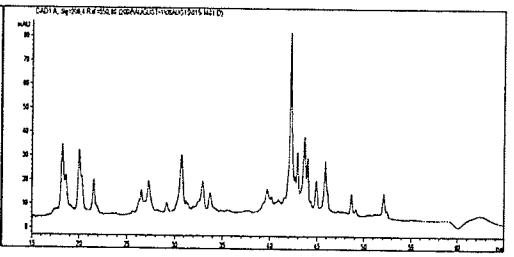

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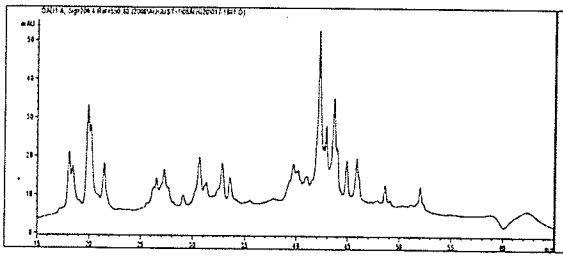

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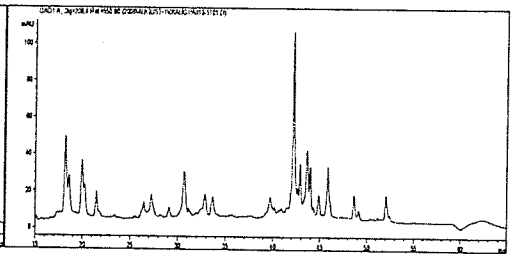

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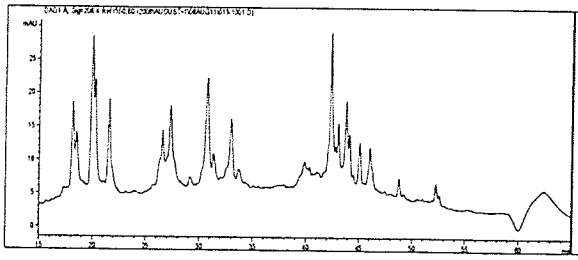

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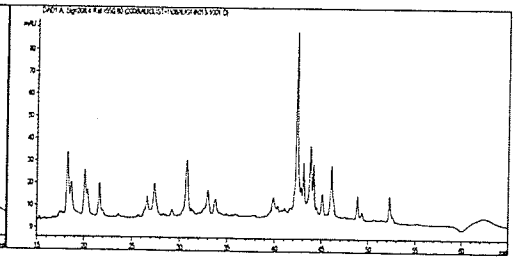

A 1.5 Superb 2003 Swift Current insoluble glutenin

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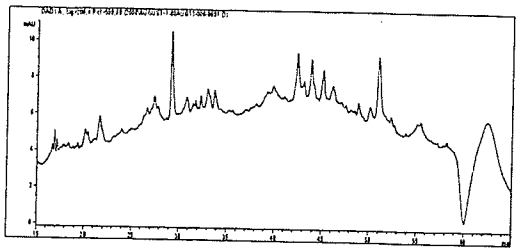

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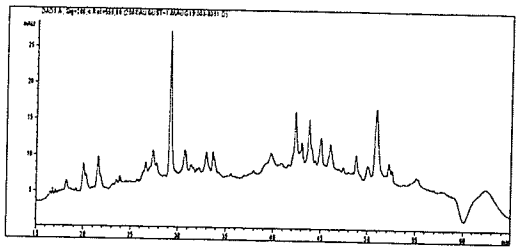

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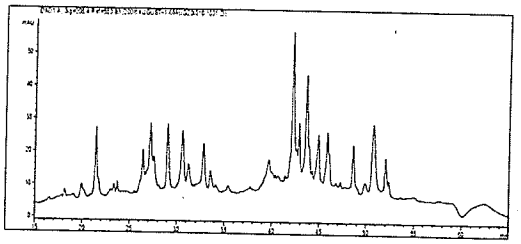

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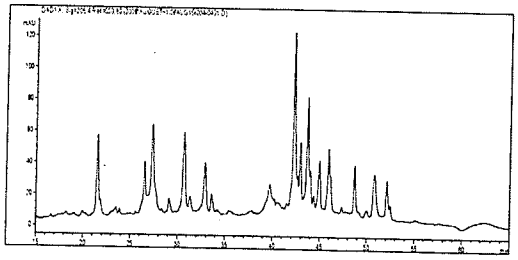

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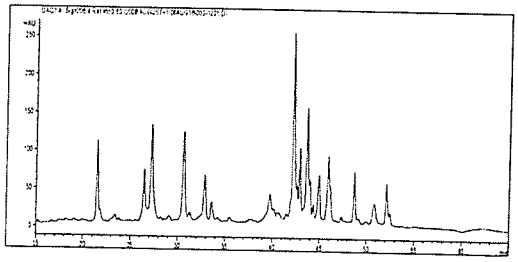

A 1.6 Superb 2003 Swift Current soluble glutenin

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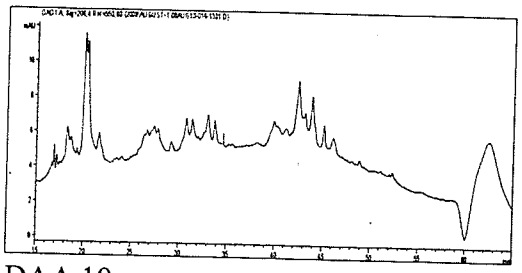

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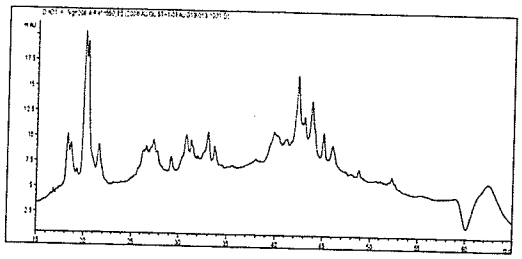

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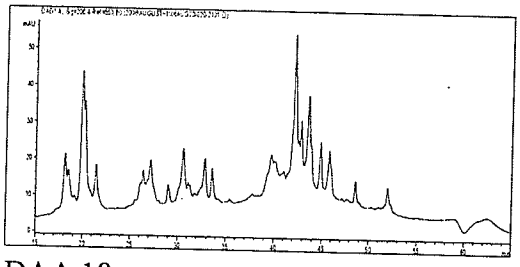

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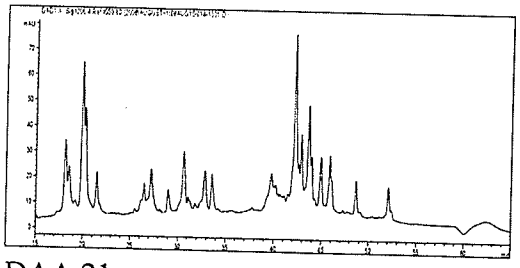
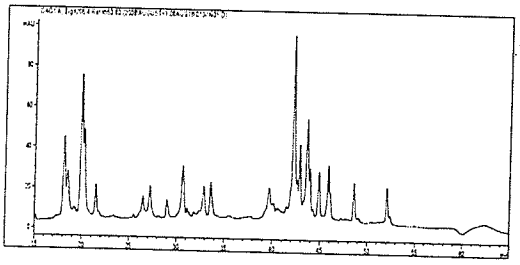
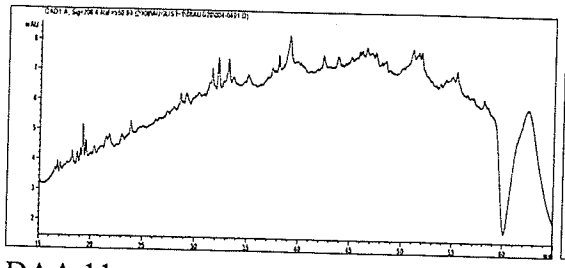


DAA 21

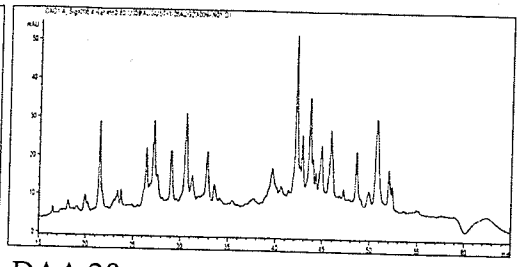


A 1.7 Superb 2004 Swift Current insoluble glutenin

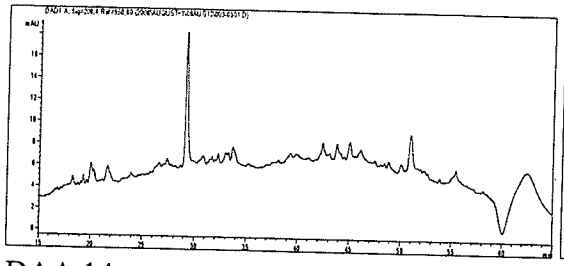
DAA 7



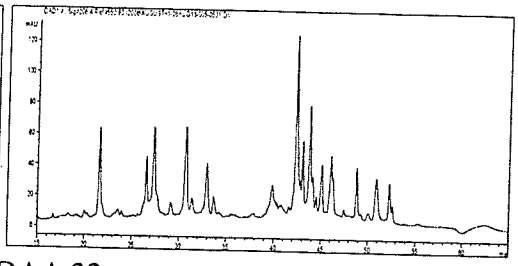
DAA 25



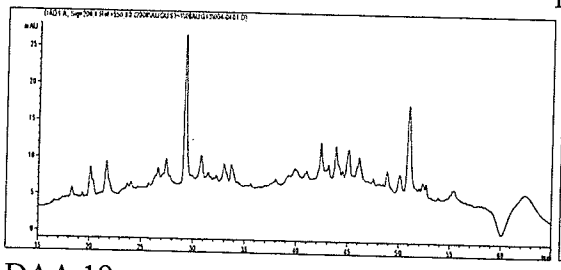
DAA 11



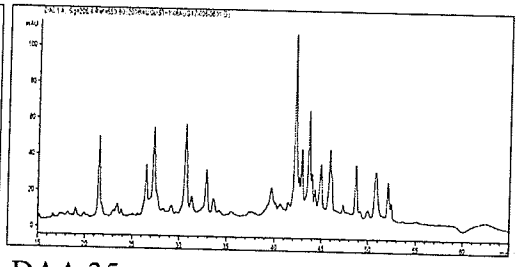
DAA 28



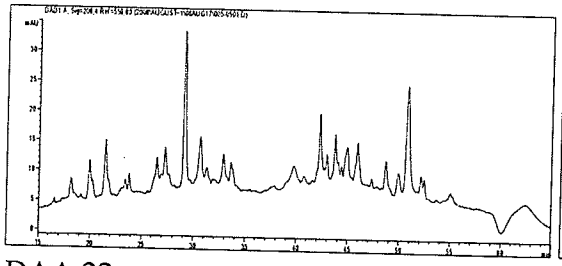
DAA 14



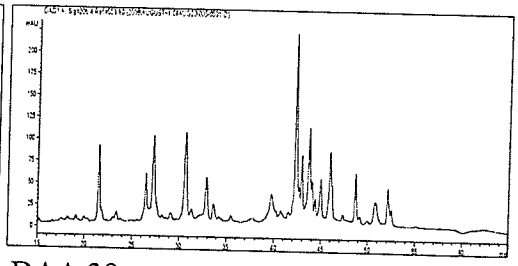
DAA 32



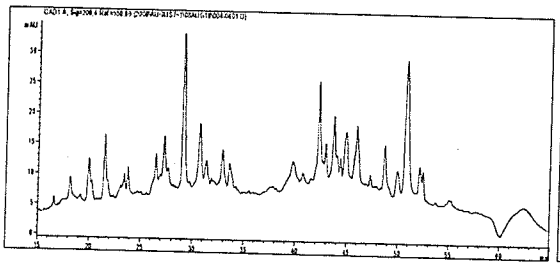
DAA 19



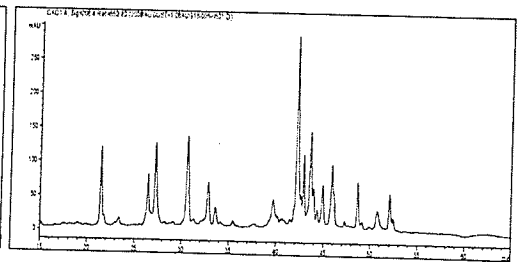
DAA 35



DAA 22

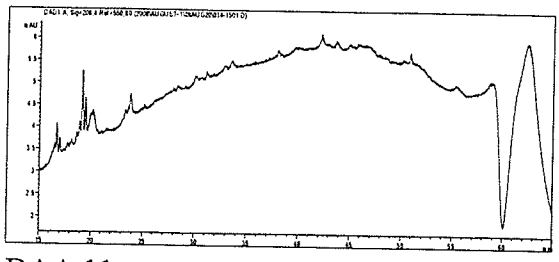


DAA 39

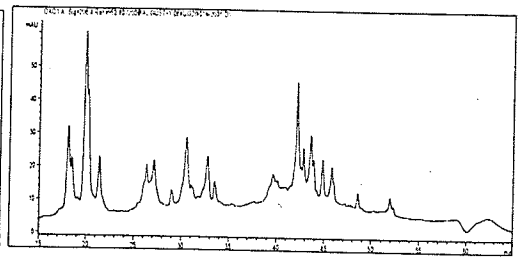


A 1.8 Superb 2004 Swift Current soluble glutenin

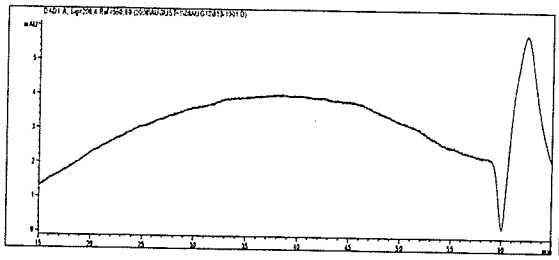
DAA 7



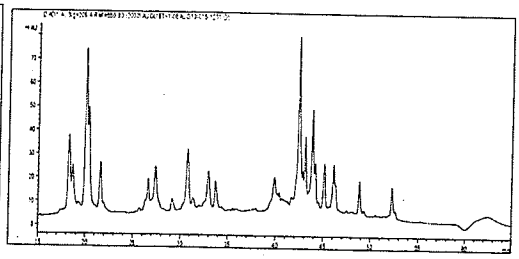
DAA 25



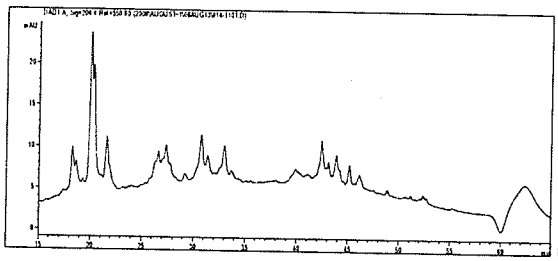
DAA 11



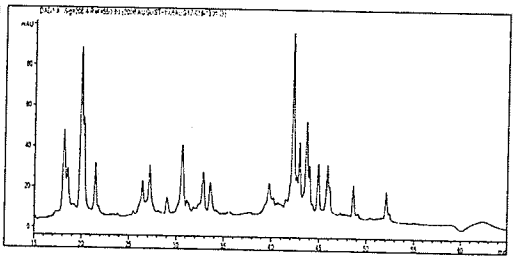
DAA 28



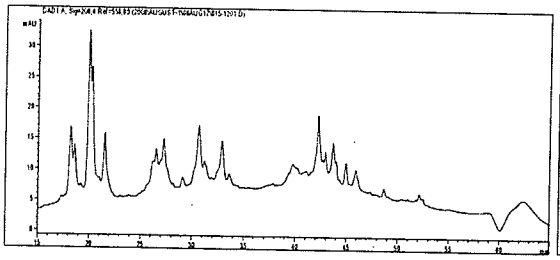
DAA 14



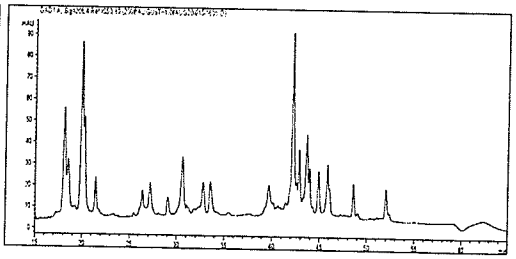
DAA 32



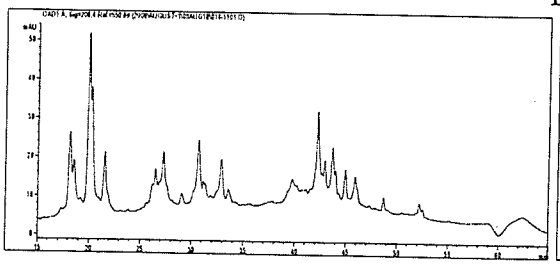
DAA 19



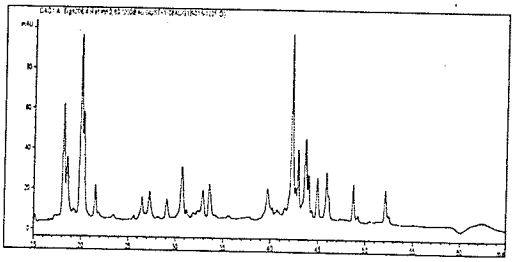
DAA 35



DAA 22

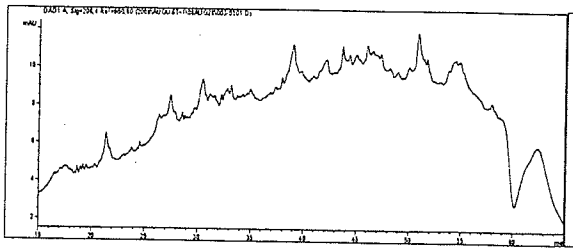


DAA 39

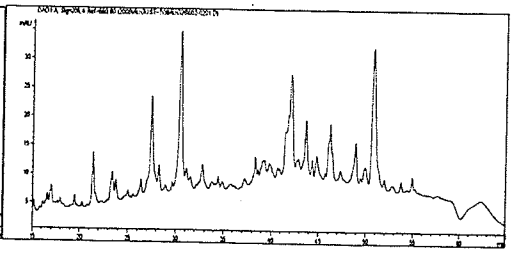


A 1.9 AC Vista 2003 Winnipeg insoluble glutenin

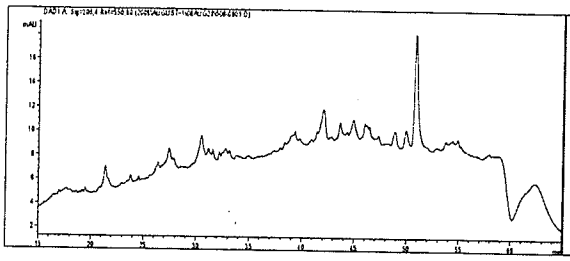
DAA 9



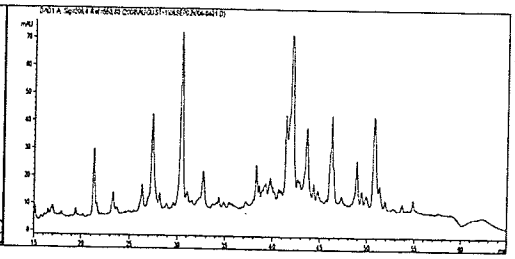
DAA 27



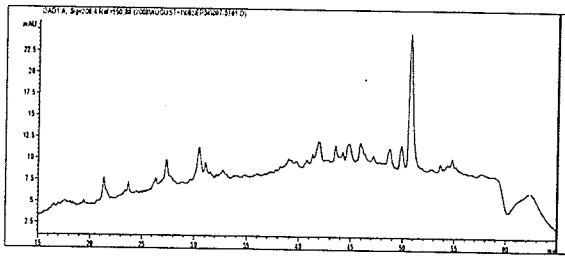
DAA 13



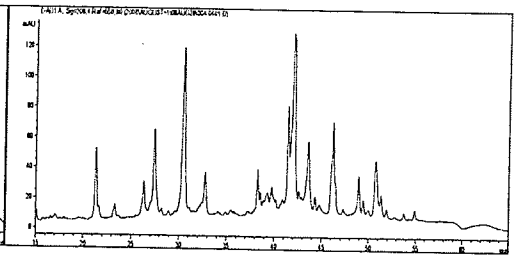
DAA 30



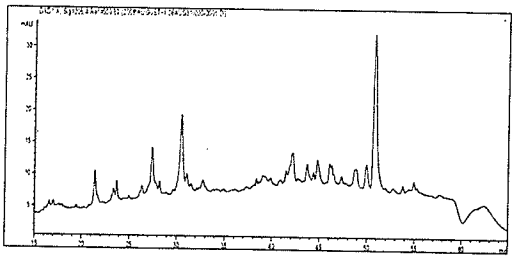
DAA 16



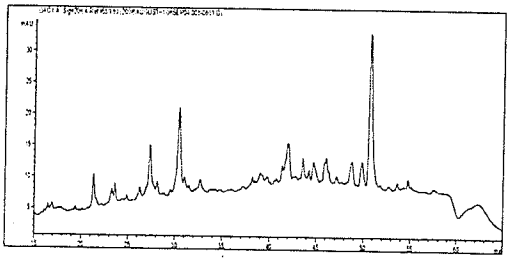
DAA 34



DAA 20

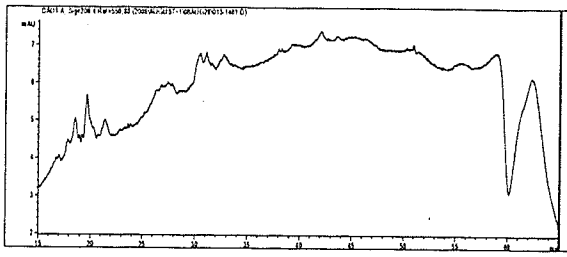


DAA 23

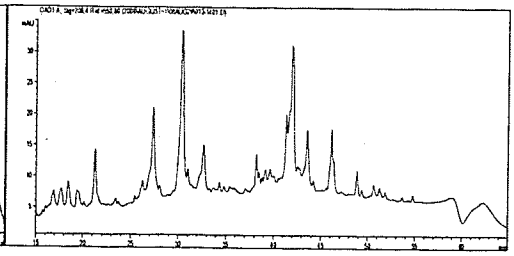


A 1.10 AC Vista 2003 Winnipeg soluble glutenin

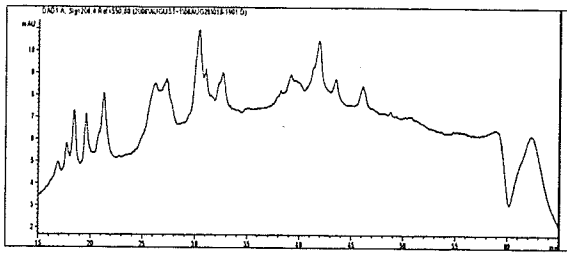
DAA 9



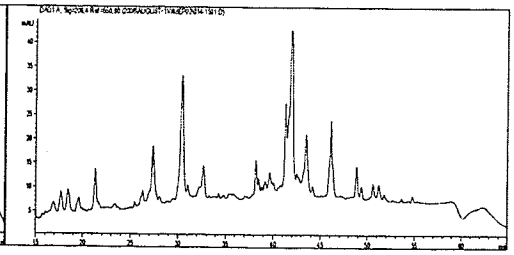
DAA 27



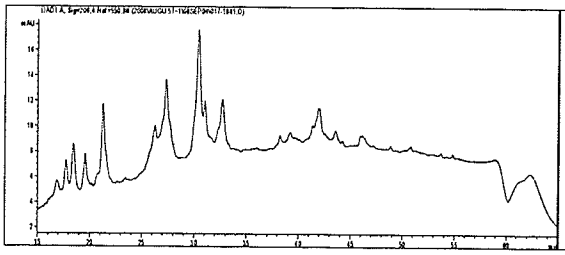
DAA 13



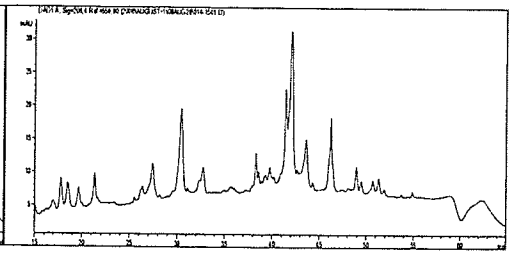
DAA 30



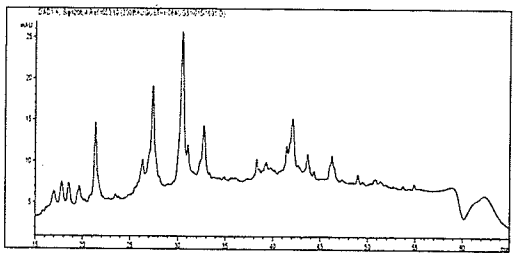
DAA 16



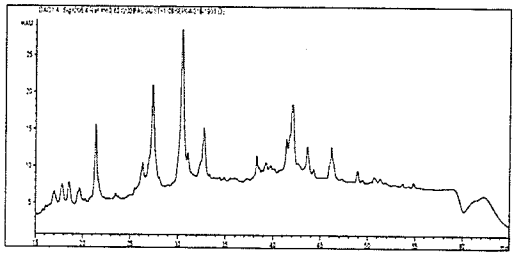
DAA 34



DAA 20

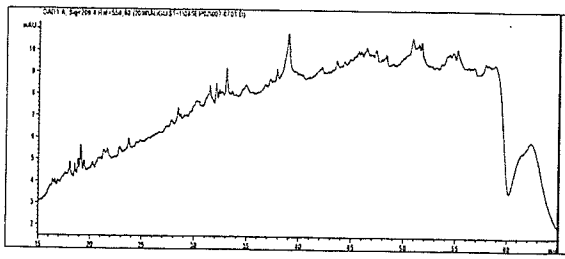


DAA 23

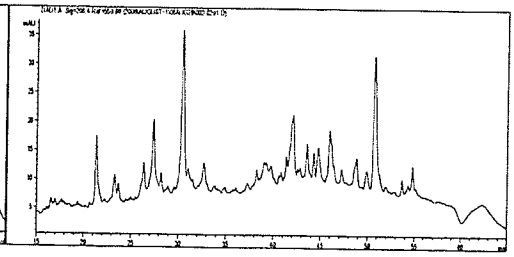


A 1.11 AC Vista 2004 Winnipeg insoluble glutenin

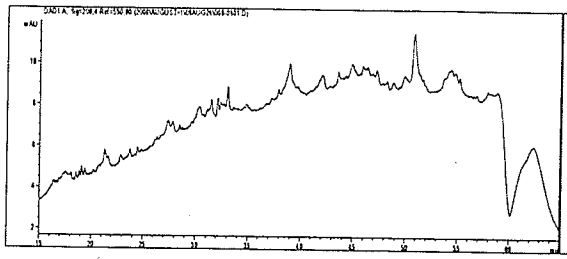
DAA 11



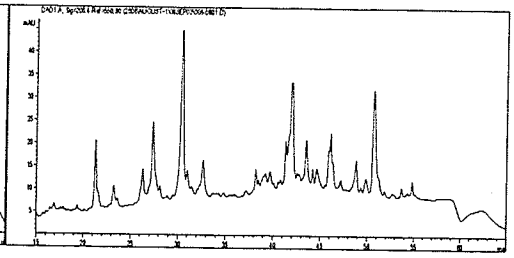
DAA 31



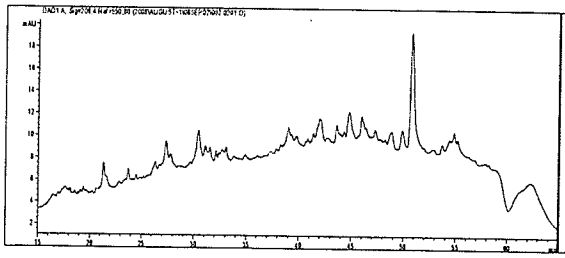
DAA 14



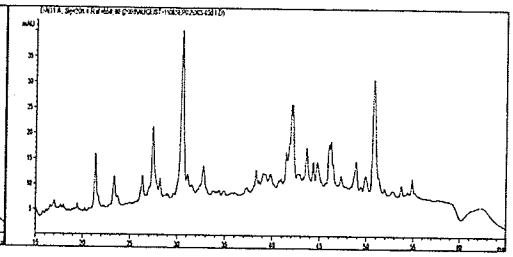
DAA 35



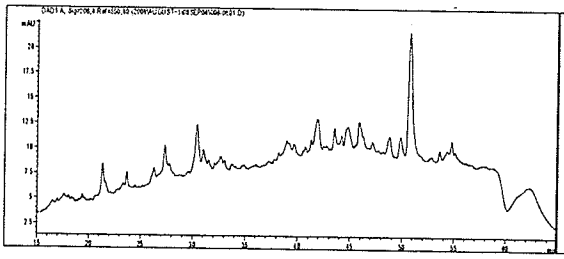
DAA 18



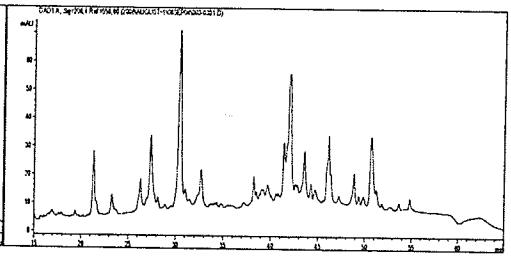
DAA 38



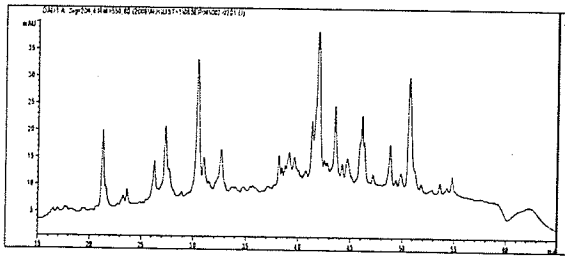
DAA 21



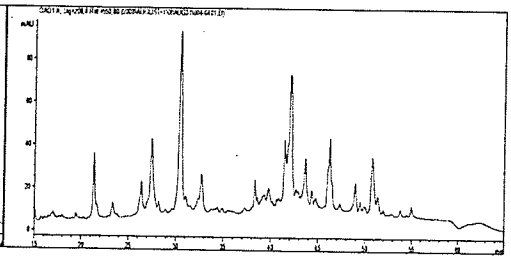
DAA 41



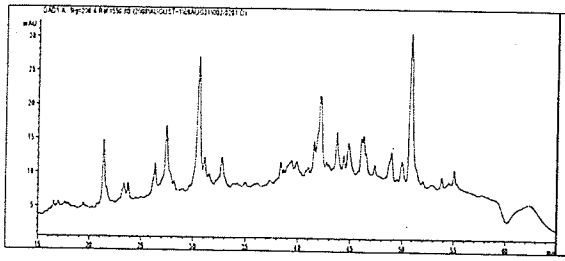
DAA 24



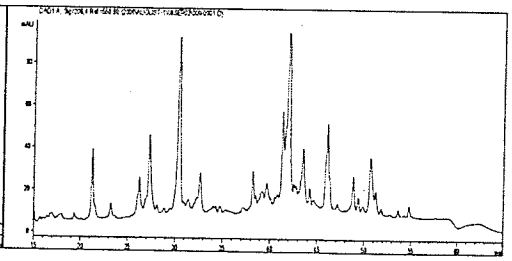
DAA 46



DAA 27

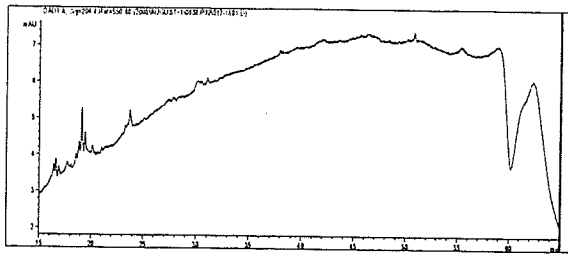


DAA 52

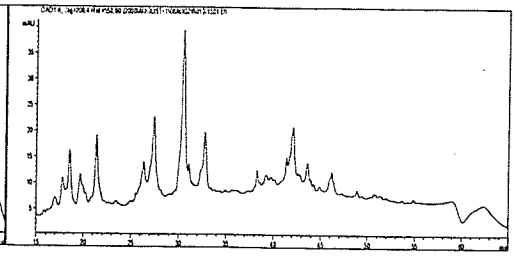


A 1.12 AC Vista 2004 Winnipeg soluble glutenin

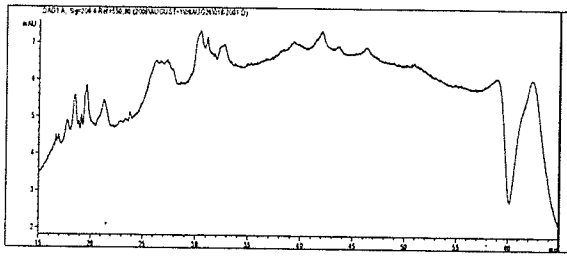
DAA 11



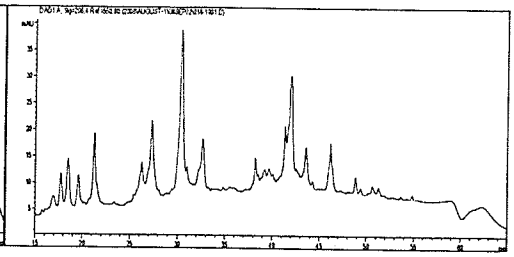
DAA 31



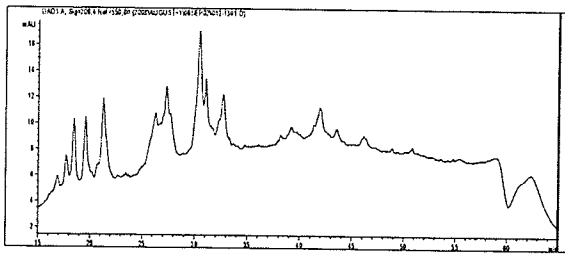
DAA 14



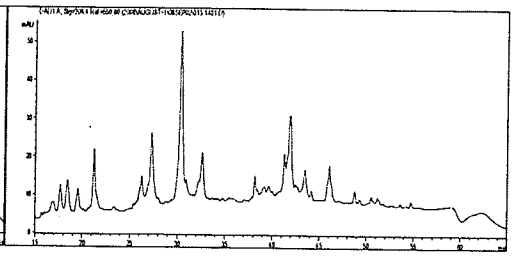
DAA 35



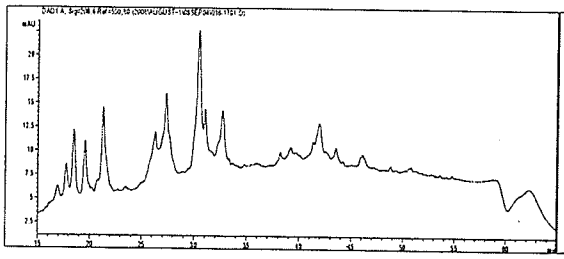
DAA 18



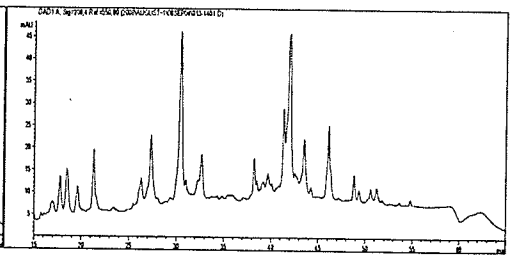
DAA 38



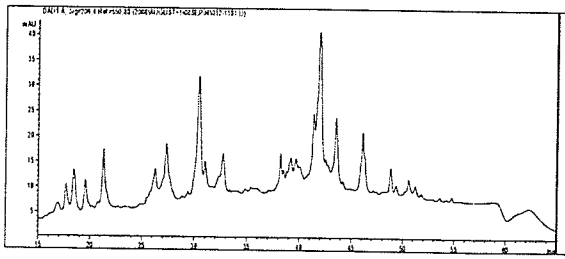
DAA 21



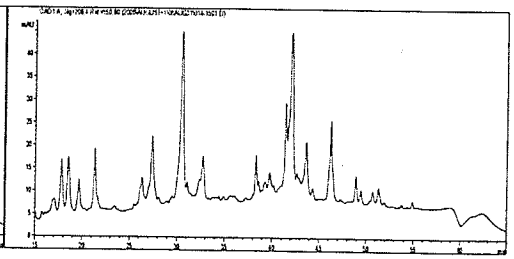
DAA 41



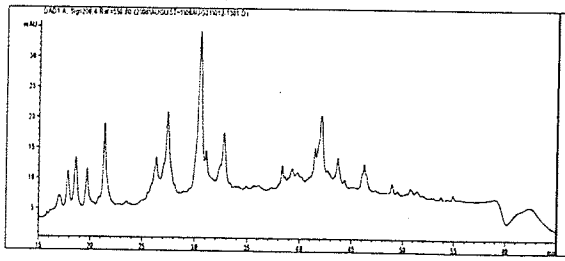
DAA 24



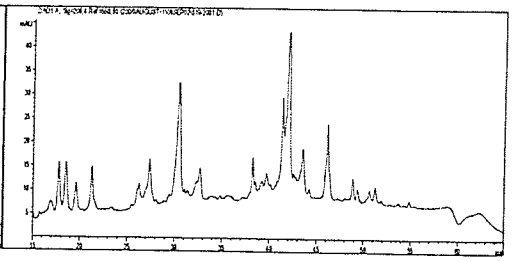
DAA 46



DAA 27

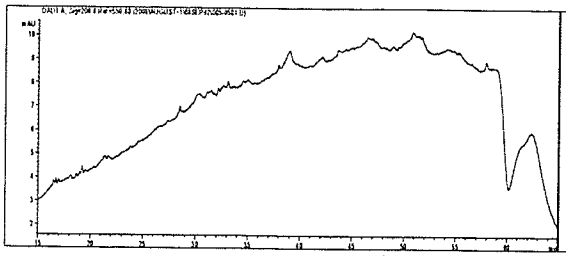


DAA 52

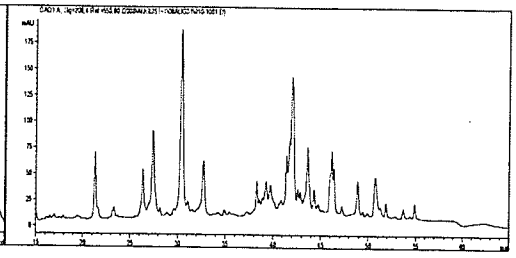


A 1.13 AC Vista 2003 Swift Current insoluble glutenin

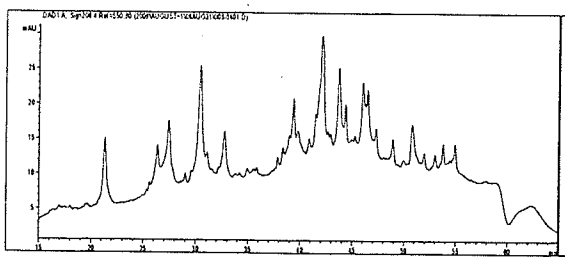
DAA 7



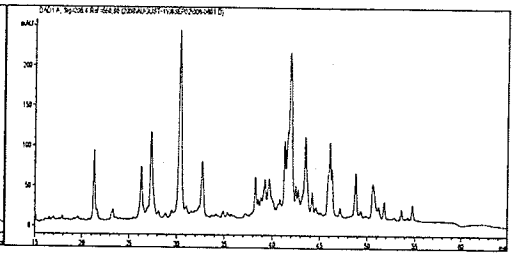
DAA 25



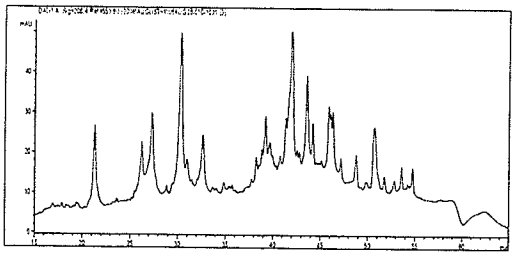
DAA 10



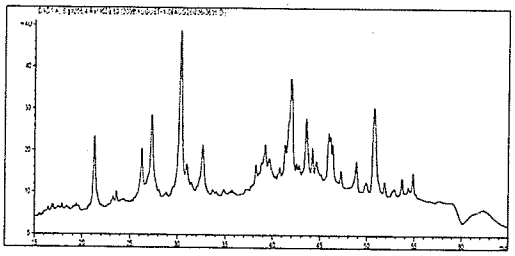
DAA 28



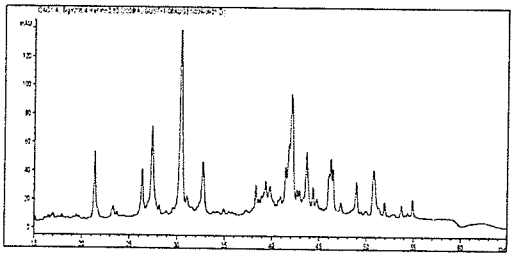
DAA 14



DAA 17

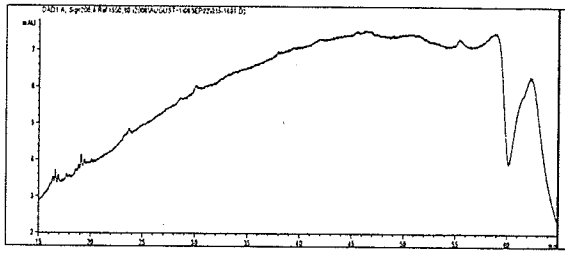


DAA 22

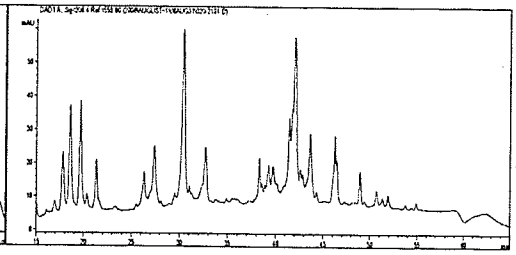


A 1.14 AC Vista 2003 Swift Curent soluble glutenin

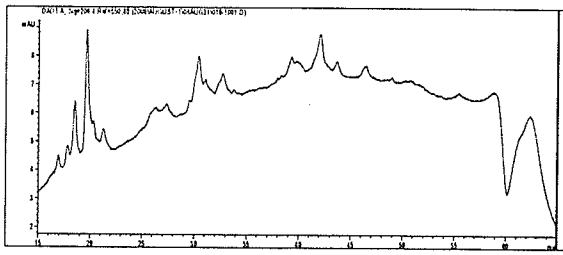
DAA 7



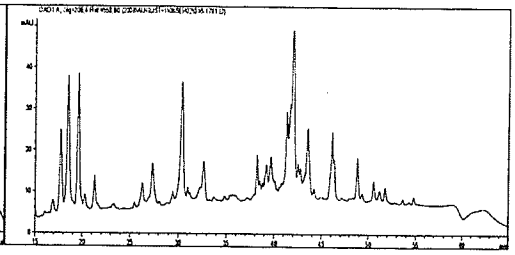
DAA 25



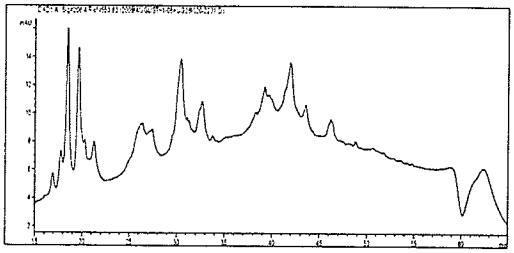
DAA 10



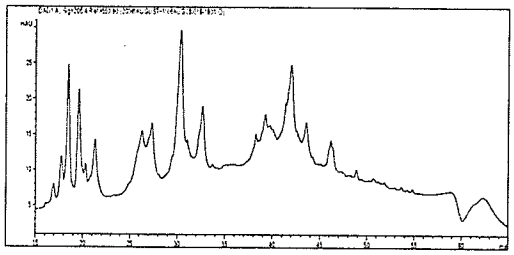
DAA 28



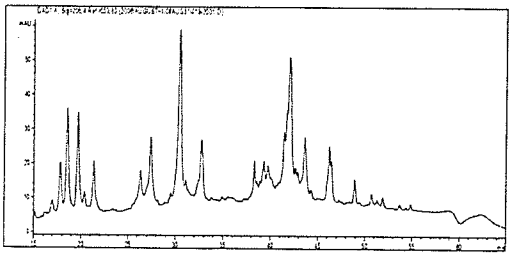
DAA 14



DAA 17

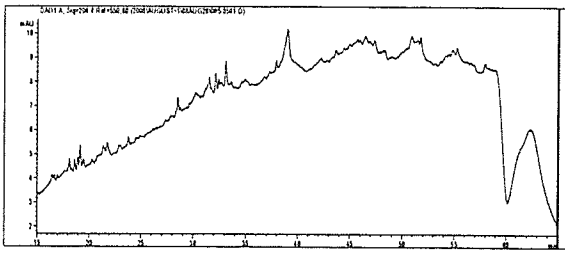


DAA 22

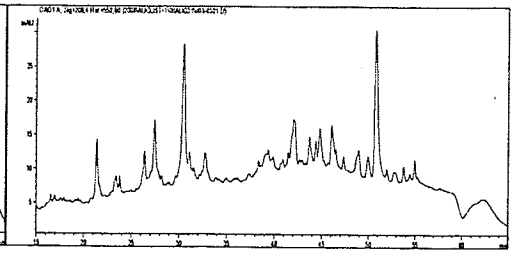


A 1.15 AC Vista 2004 Swift Current insoluble glutenin

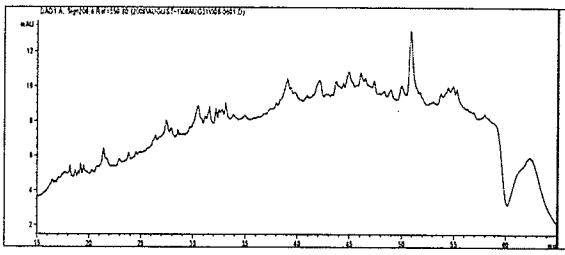
DAA 7



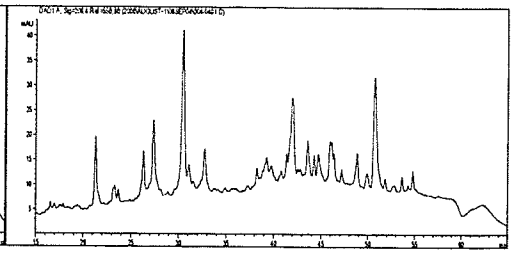
DAA 25



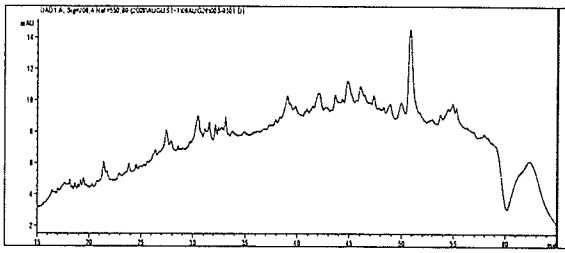
DAA 11



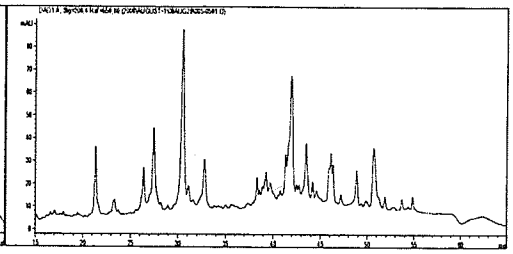
DAA 28



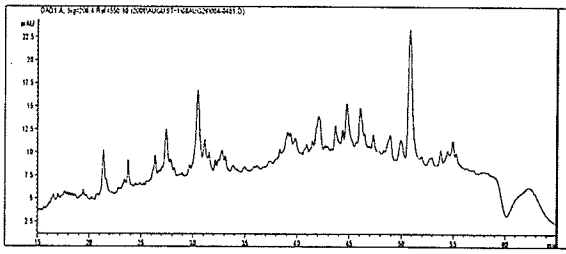
DAA 14



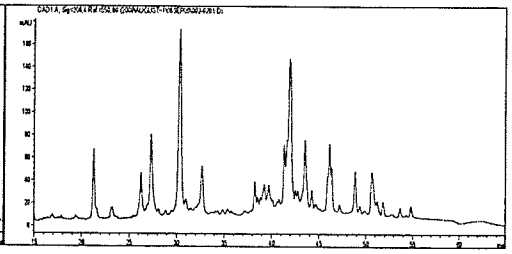
DAA 32



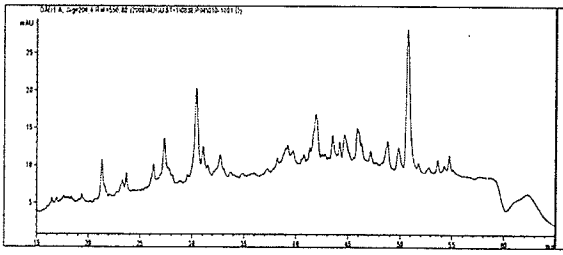
DAA 19



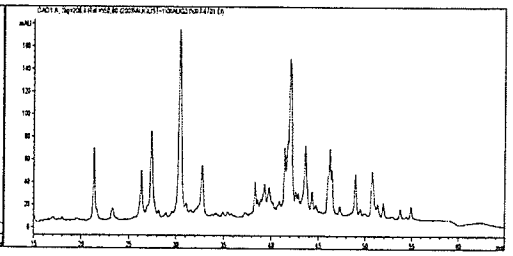
DAA 35



DAA 22

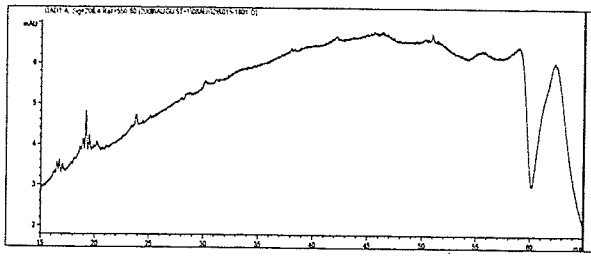


DAA 39

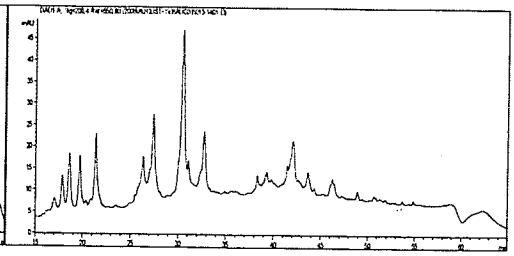


A 1.16 AC Vista 2004 Swift Current soluble glutenin

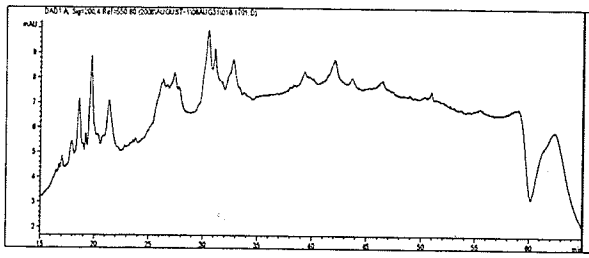
DAA 7



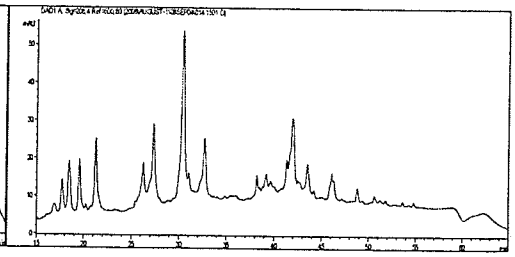
DAA 25



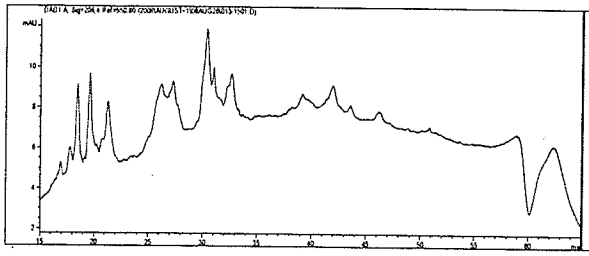
DAA 11



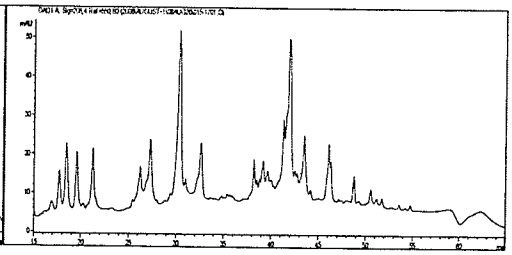
DAA 28



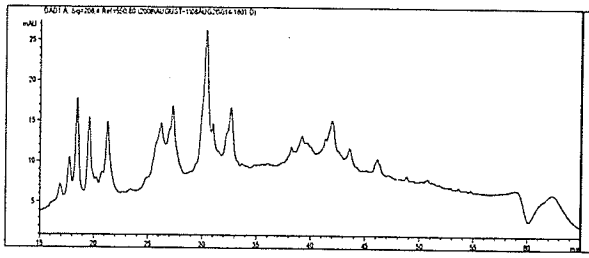
DAA 14



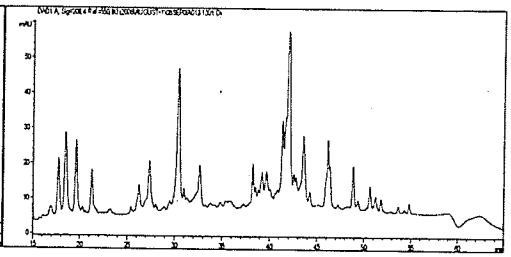
DAA 32



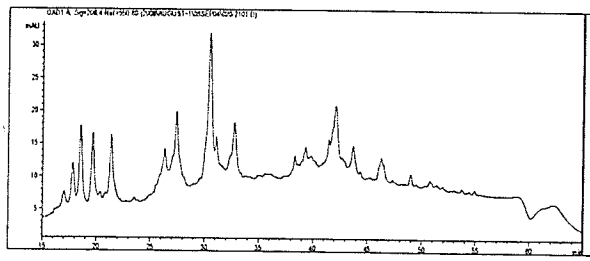
DAA 19



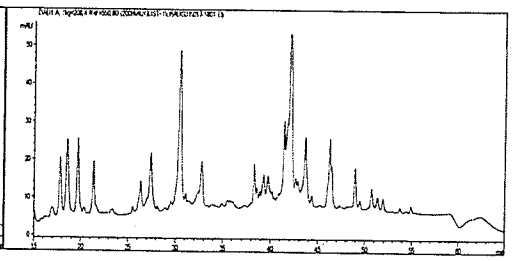
DAA 35



DAA 22



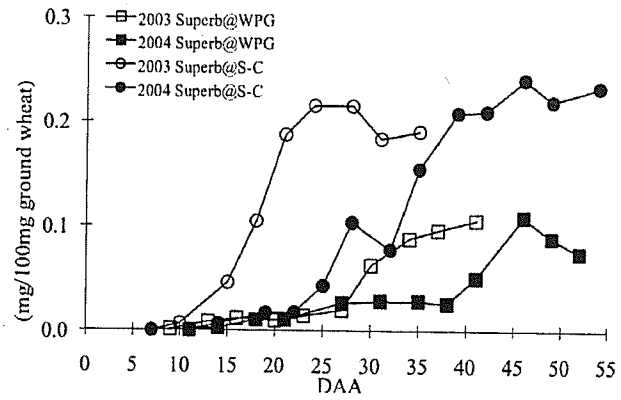
DAA 39



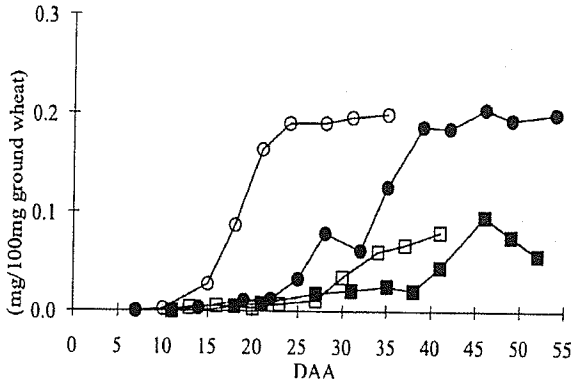
A.2 Accumulation pattern of HMW-GS in insoluble glutenin in different study sites expressing in two different ways

A 2.1-2.10 based on mg per 100 mg wheat; A 2.11-2.20 based on mg per kernel

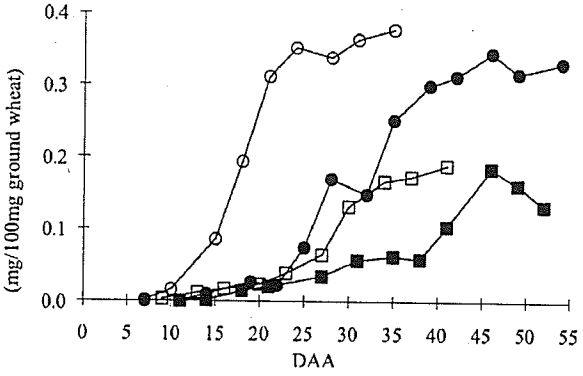
A 2.1 Dy10



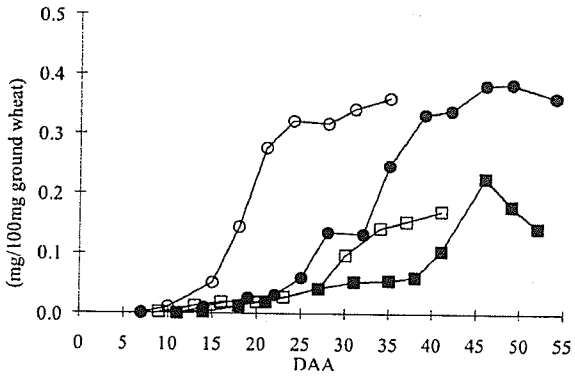
A 2.2 By9



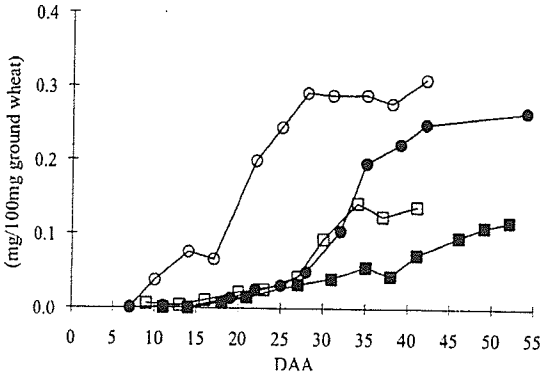
A 2.3 Dx5



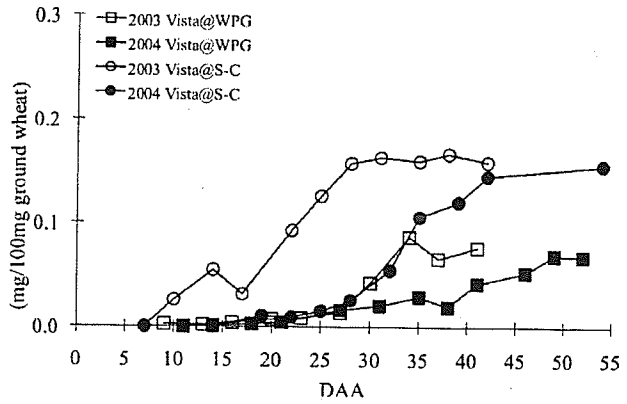
A 2.4 Bx7*



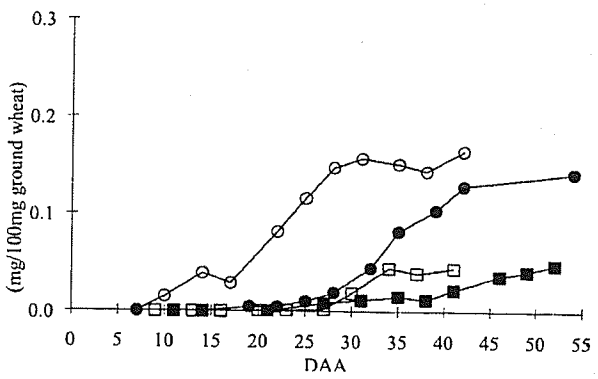
A 2.5 Ax2*



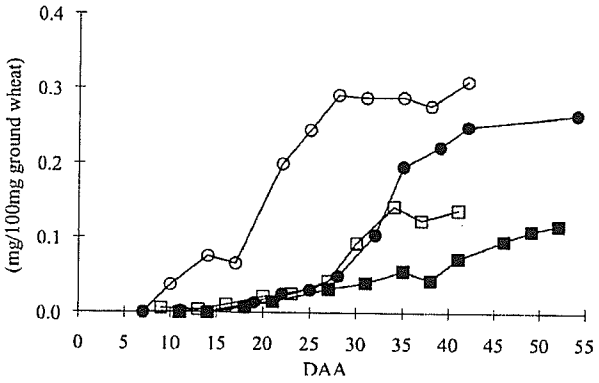
A 2.6 Dy12



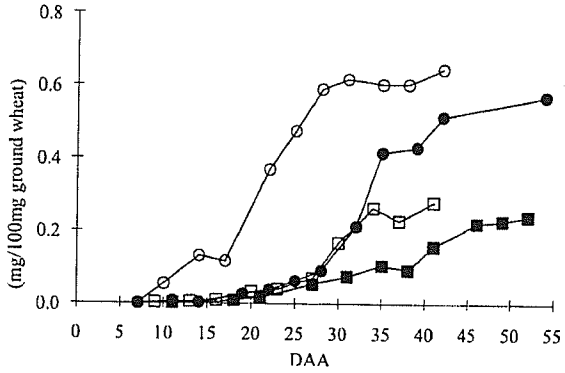
A 2.7 By8



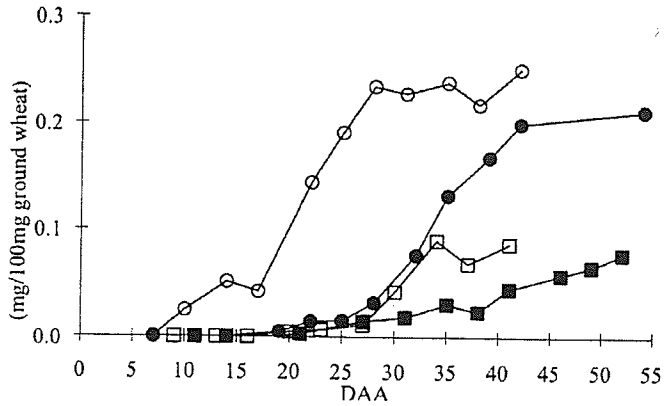
A 2.8 Dx2



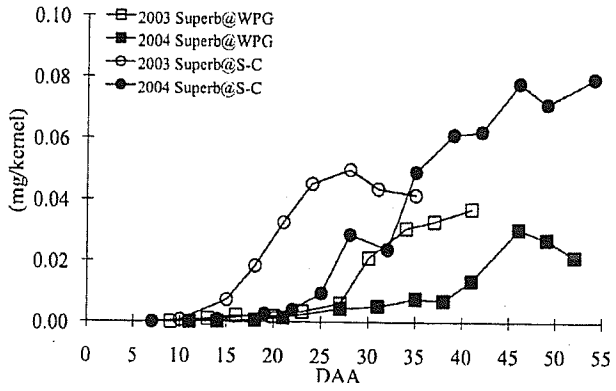
A 2.9 Bx7



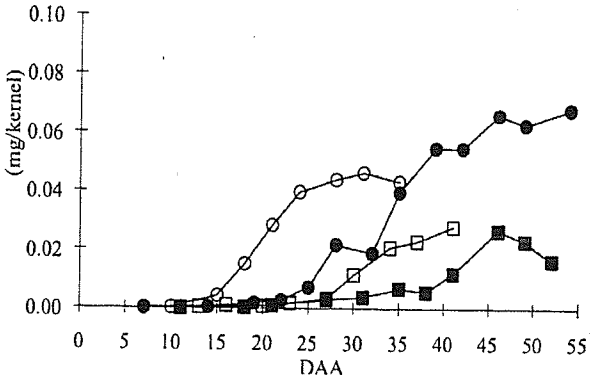
A 2.10 Ax1



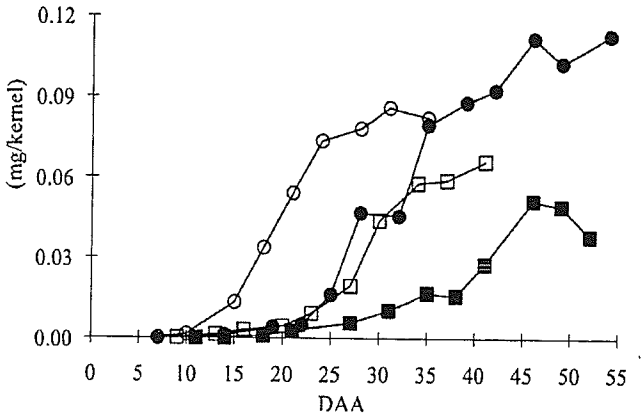
A 2.11 Dy10



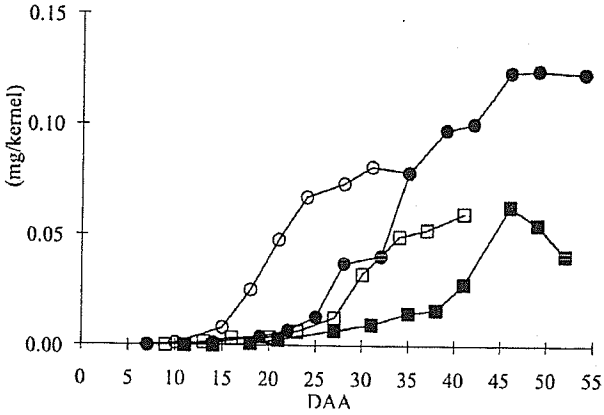
A 2.12 By9



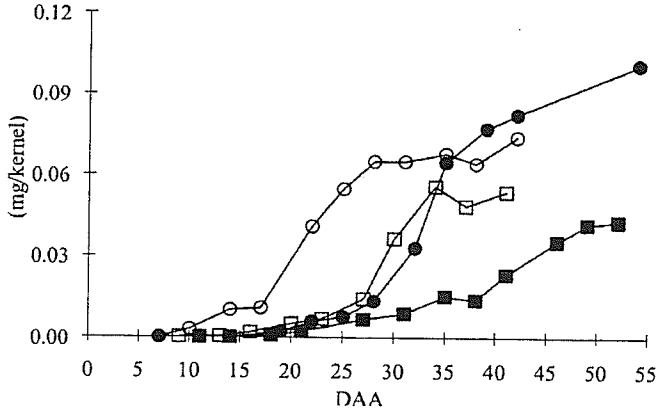
A 2.13 Dx5



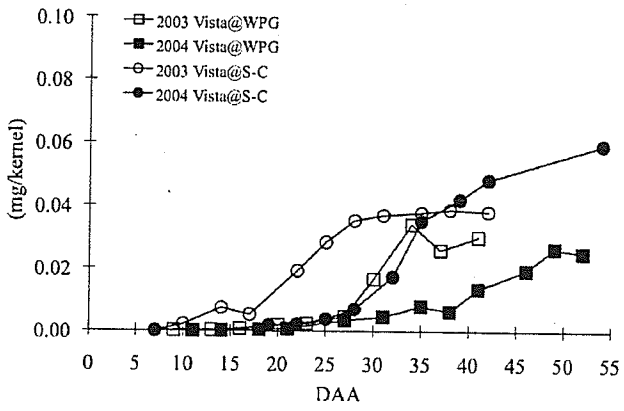
A 2.14 Bx7*



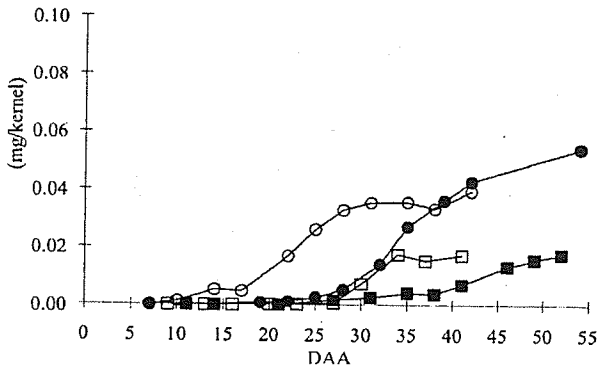
A 2.15 Ax2*



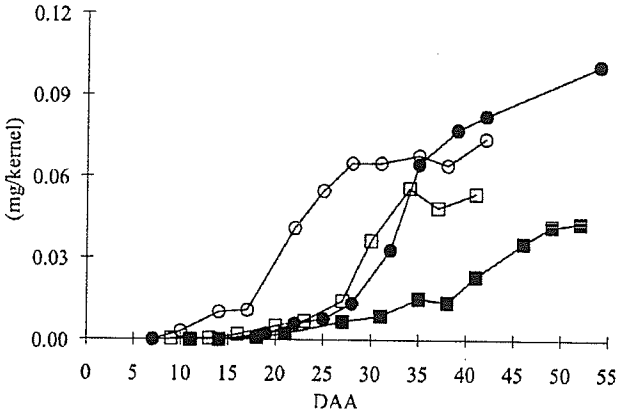
A 2.16 Dy12



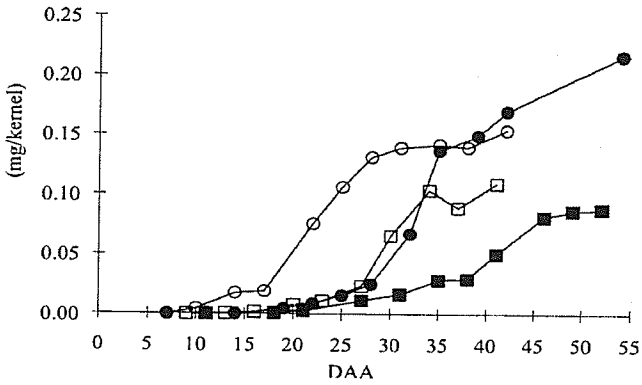
A 2.17 By8



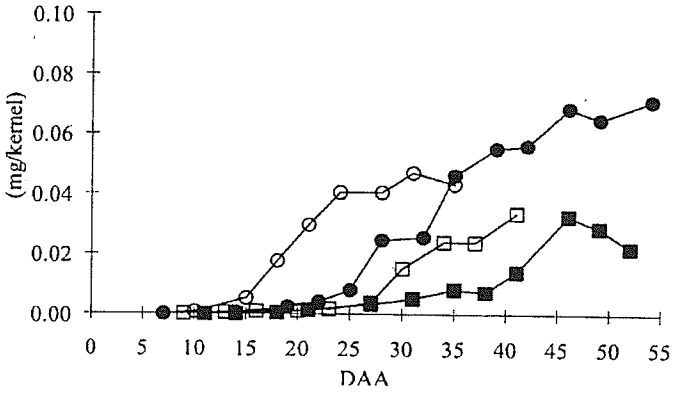
A 2.18 Dx2



A 2.19 Bx7

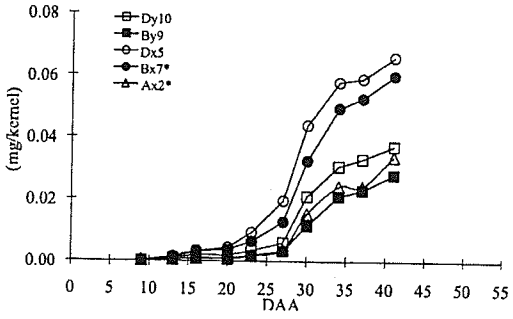


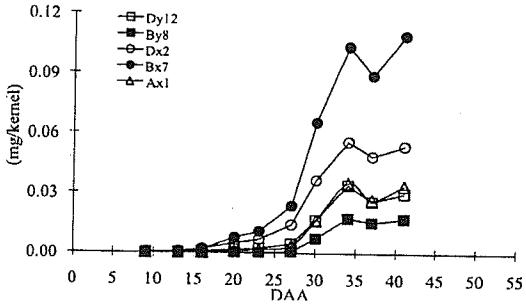
A 2.20 Ax1



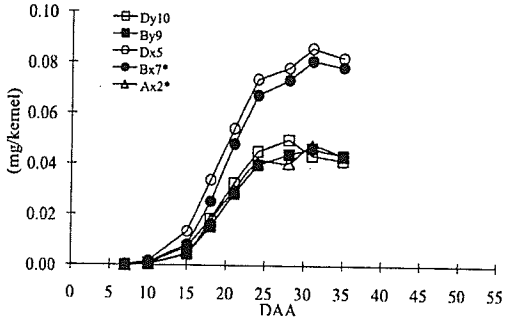
A.3 Change of content of HMW-GS in insoluble glutenin expressing in mg per kernel basis for two genotypes. Left is Superb; right is AC Vista

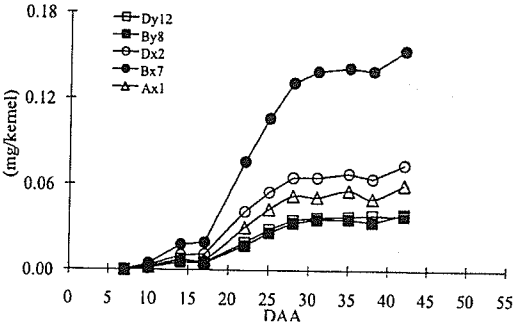
2003 Winnipeg



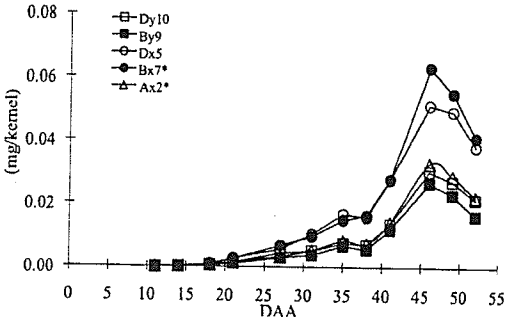


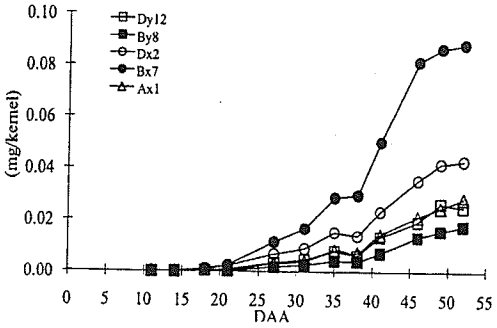
2003 Swift Current



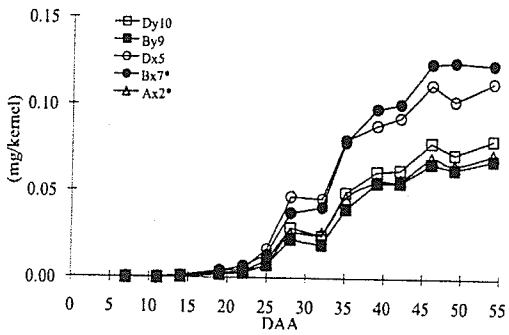


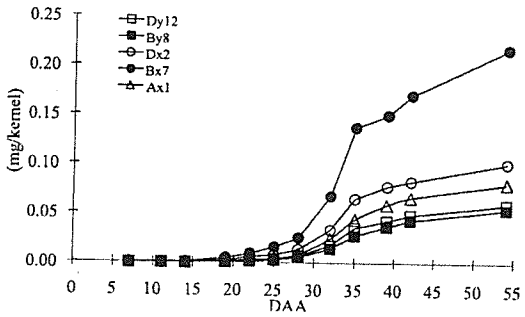
2004 Winnipeg





2004 Swift Current



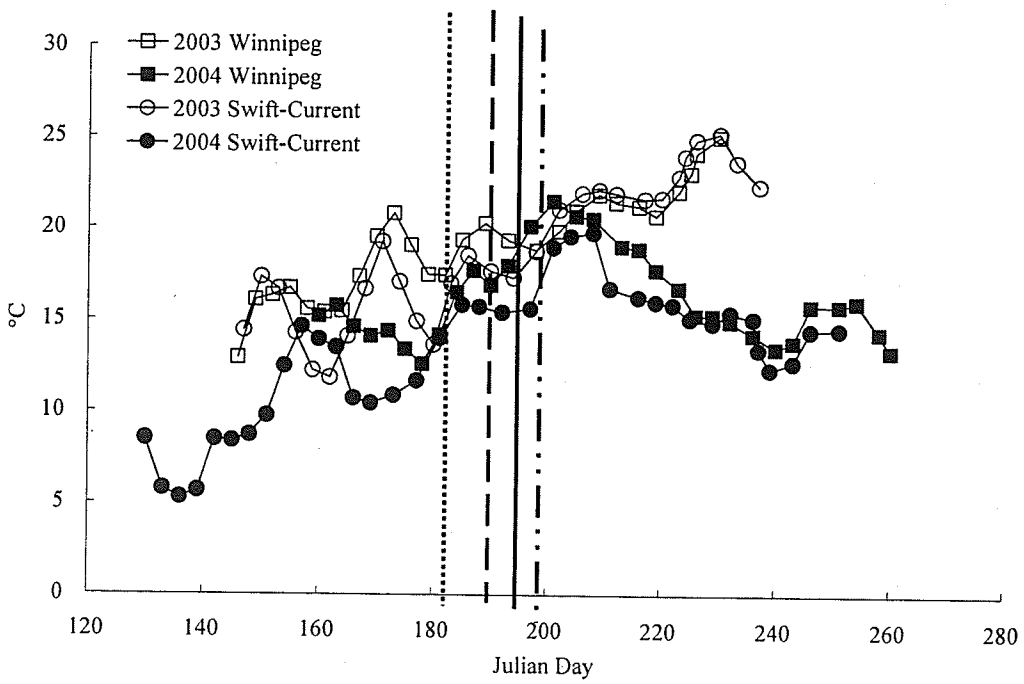


A.4 Meteorological parameters in different study sites and years during growing seasons

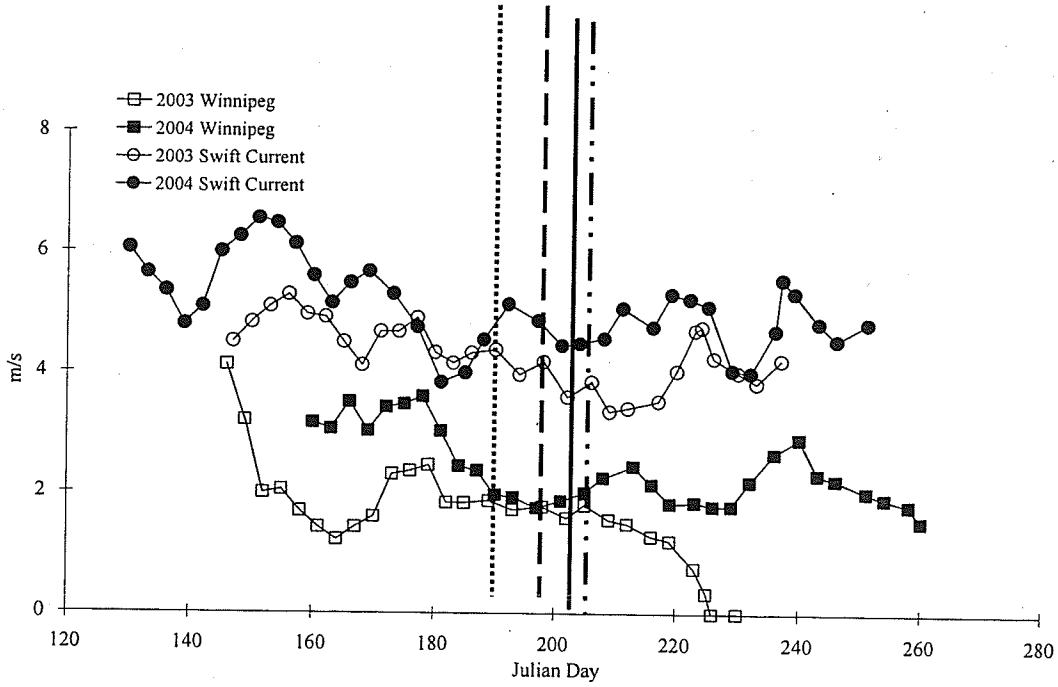
Meteorological data was adopted from existing database from Department of Soil Science recording the weather conditions from seeding to maturity. It was calculated as the average of the moving three points with each point represented mean of the proceeding three or four days (interval time between two consecutive sampling) daily values of different weather parameters including air temperature, wind speed, evapotranspiration, global radiation, rainfall, water demand, water use and water deficit. Details were discussed in chapter 3.

Anthesis date was indicated by symbols:
·····2003 Winnipeg (189 Julian Day)
– – –2004 Swift Current (197 Julian Day)
——2003 Swift Current (202 Julian Day)
— · ·2004 Winnipeg (205 Julian Day)

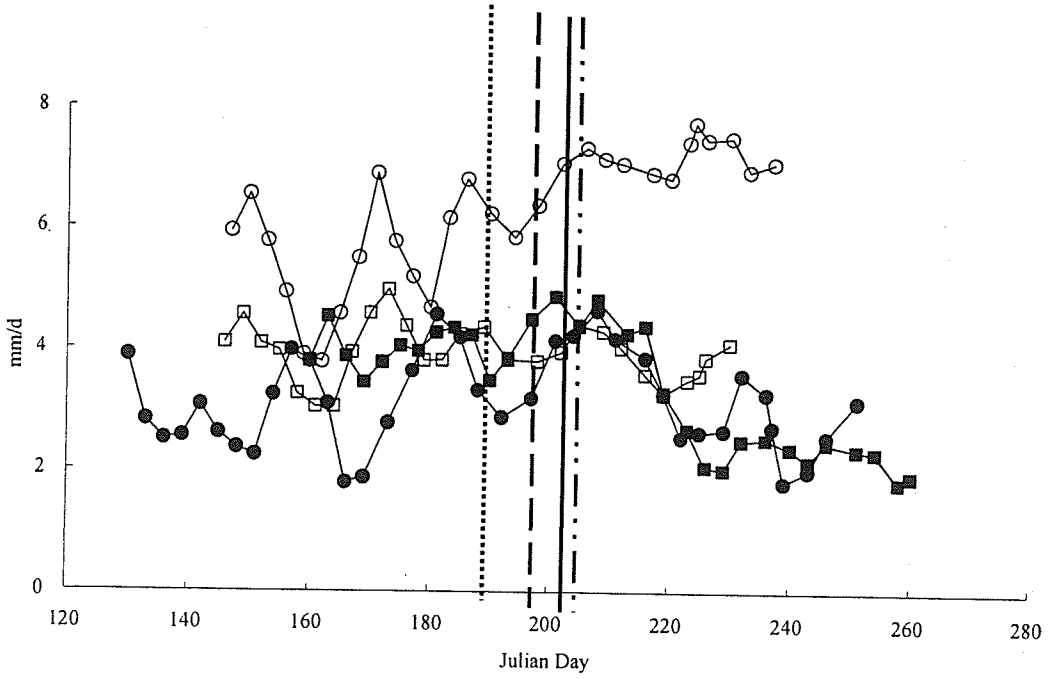
A 4.1 Air temperature



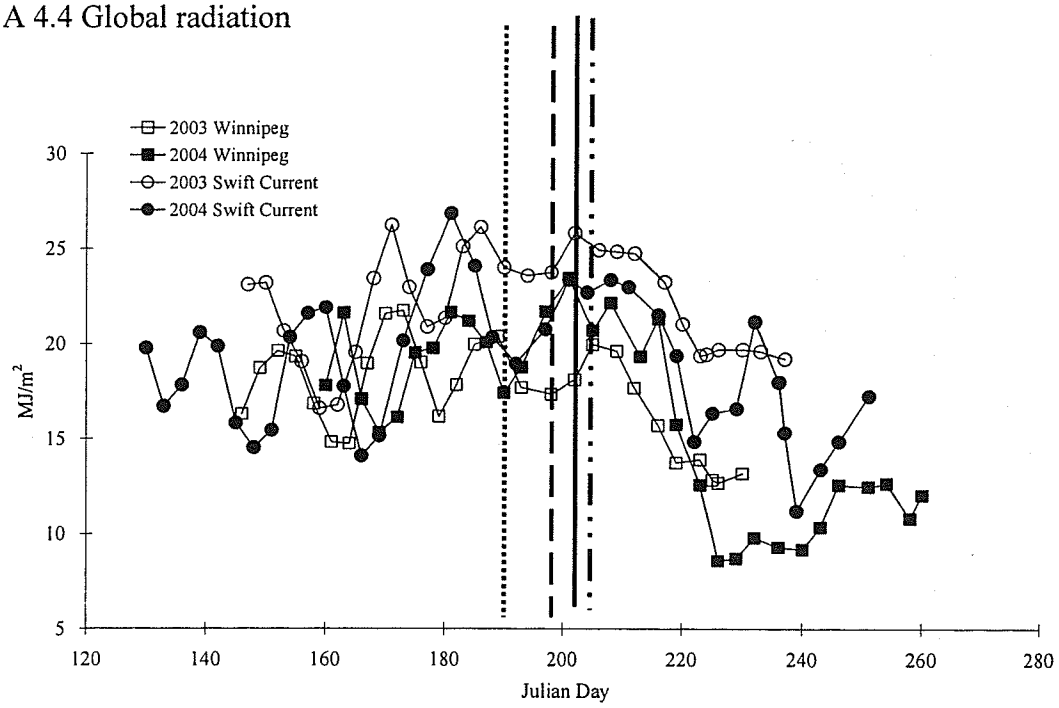
A 4.2 Wind speed



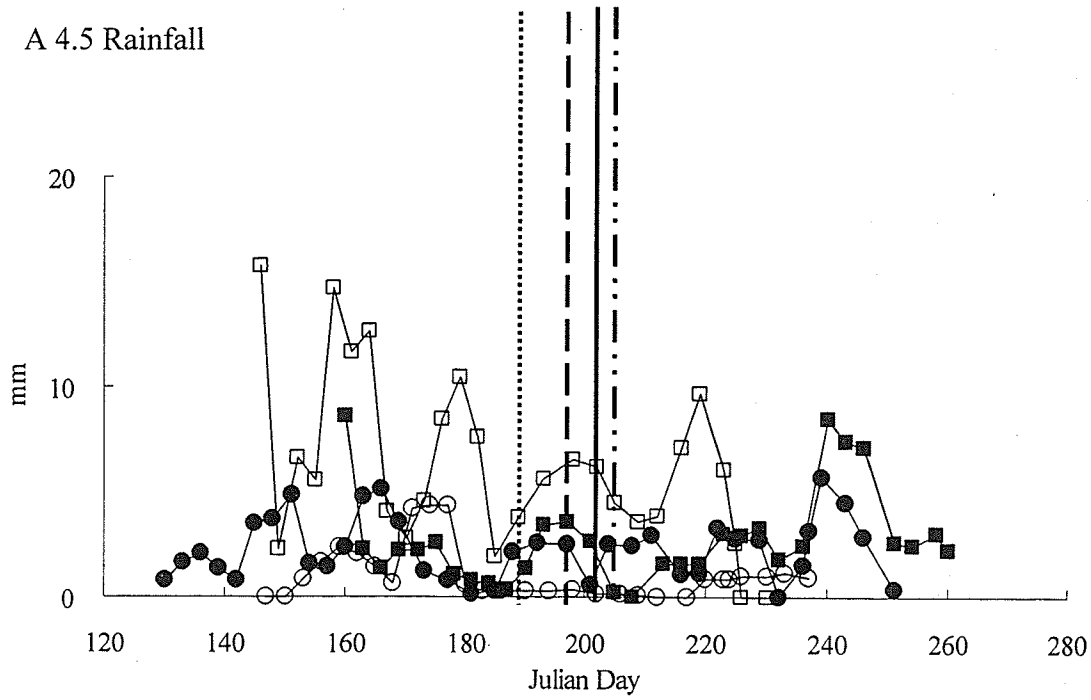
A 4.3 Evapotranspiration



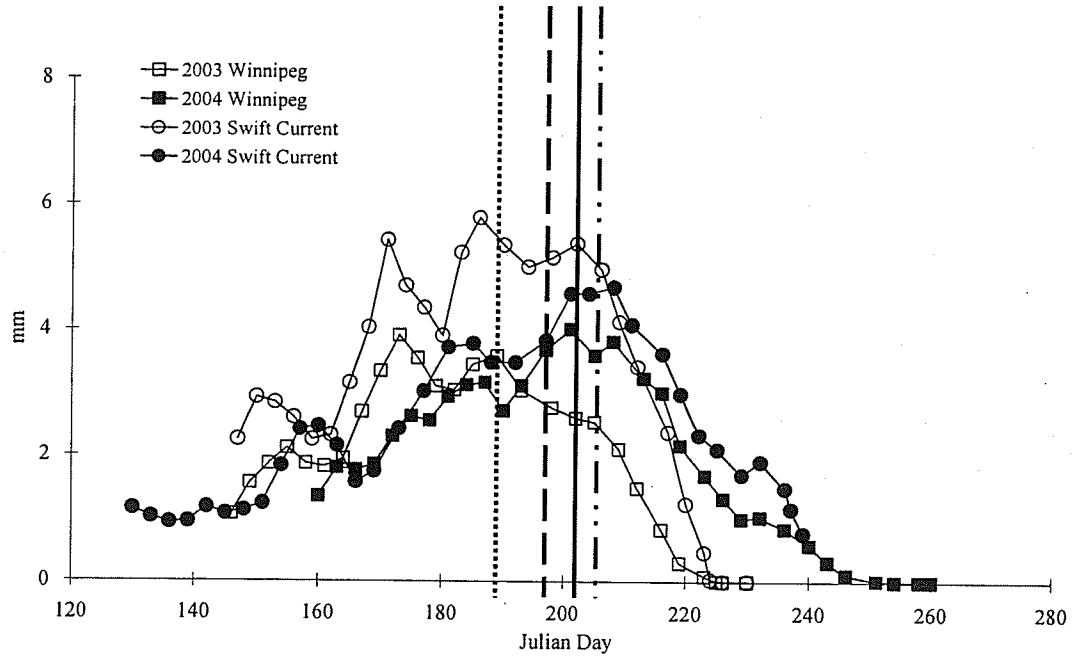
A 4.4 Global radiation



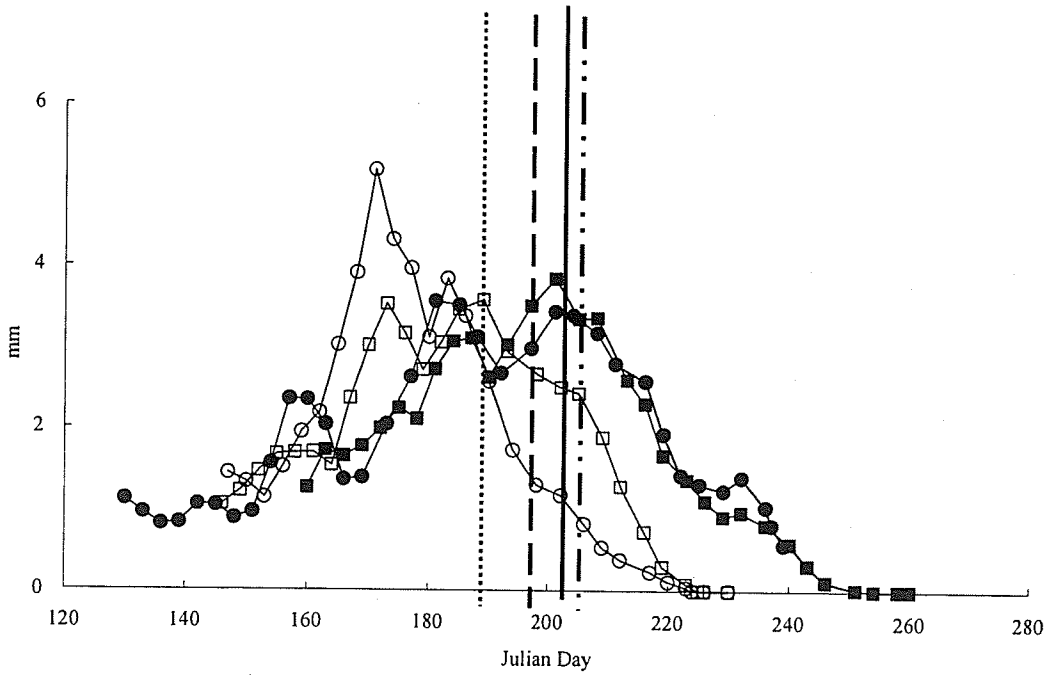
A 4.5 Rainfall



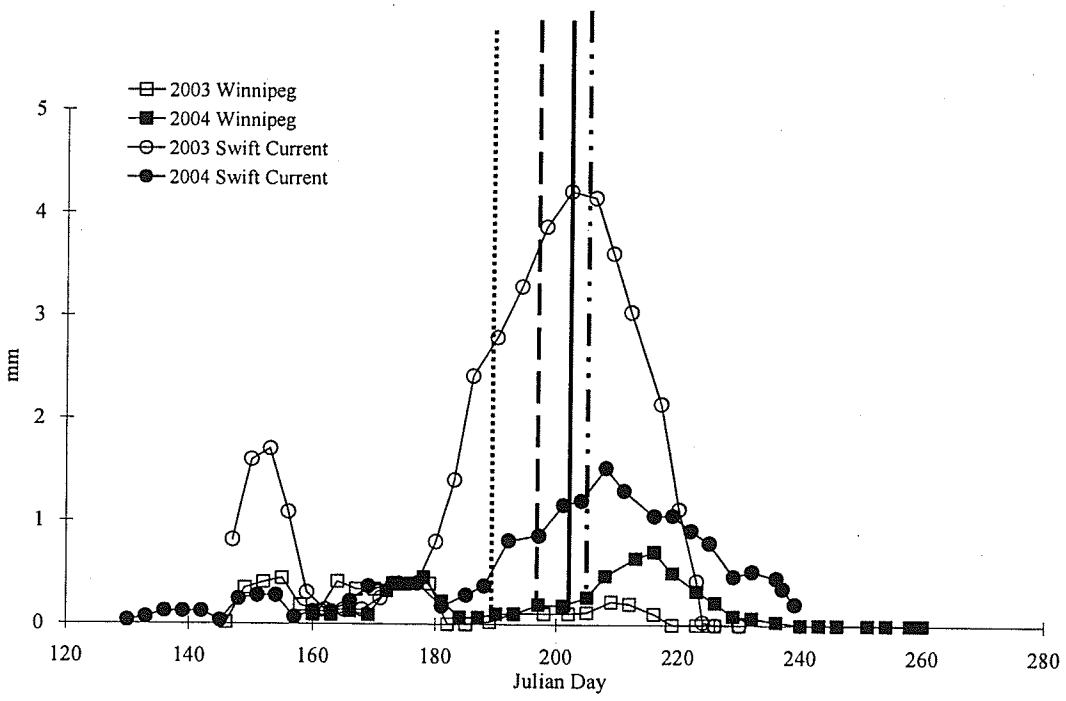
A 4.6 Water demand



A 4.7 Water use

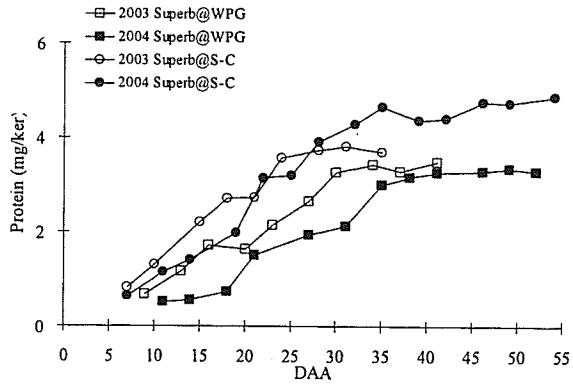


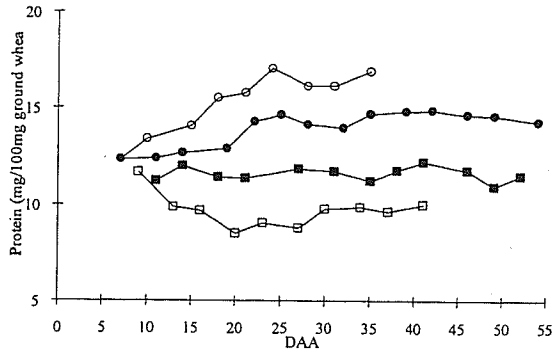
A 4.8 Water deficit

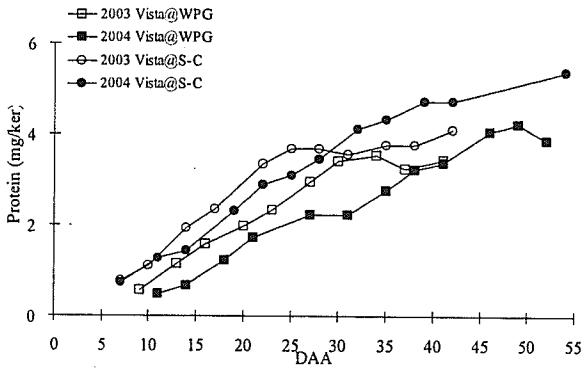


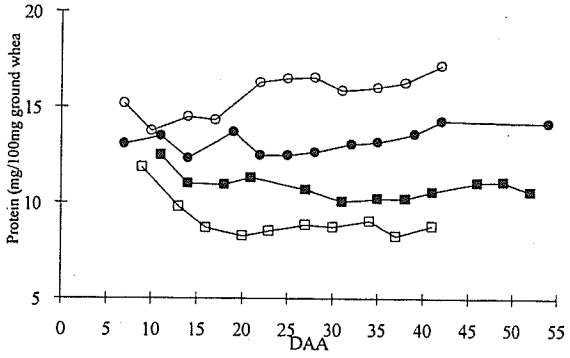
A.5 Comparison of the protein content at per kernel and 100 mg ground wheat basis in Superb and AC Vista. Left side is at per kernel basis and right side is at 100 mg basis.

A 5.1 Total protein content

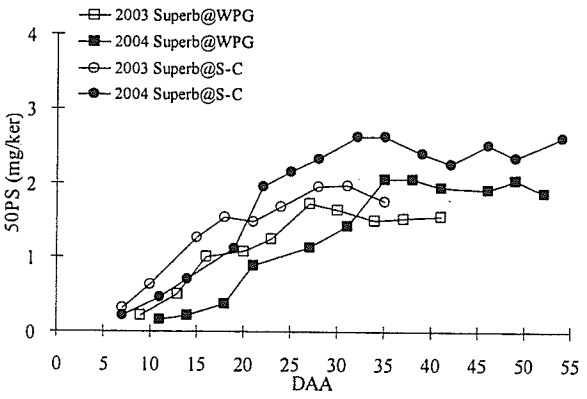


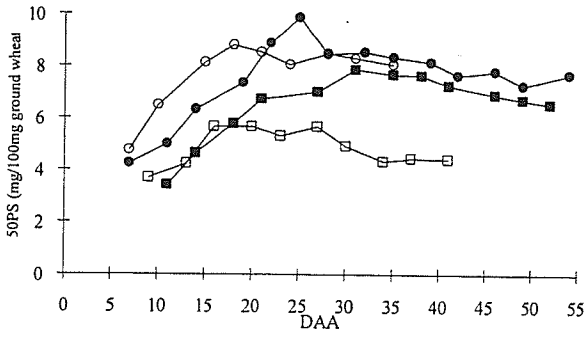


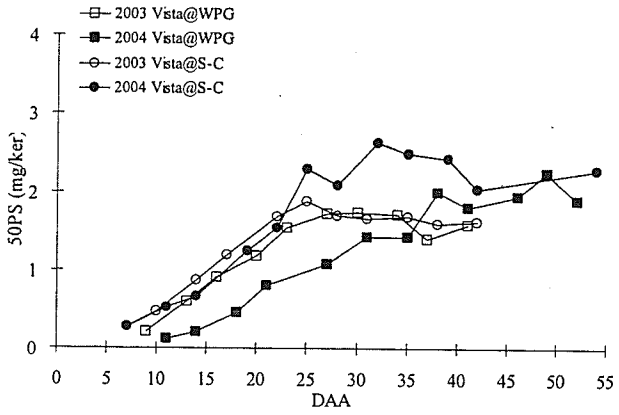


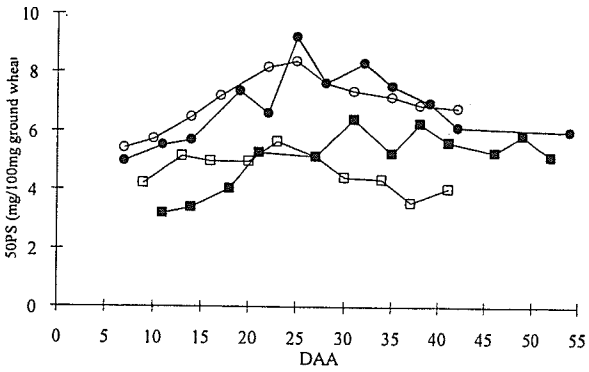


A 5.2 50PS protein content

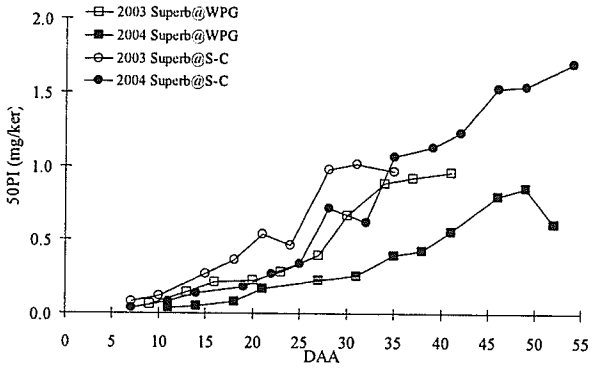


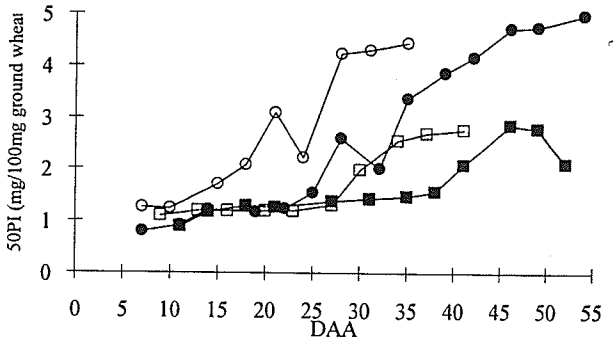


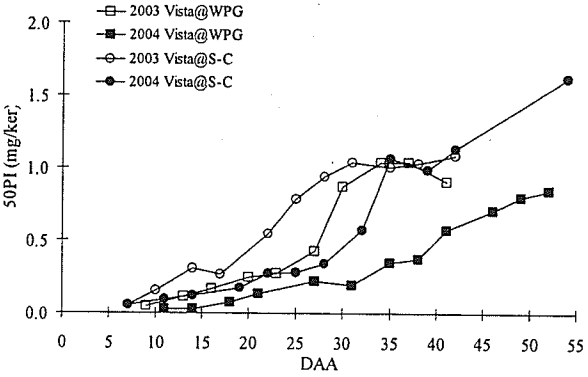


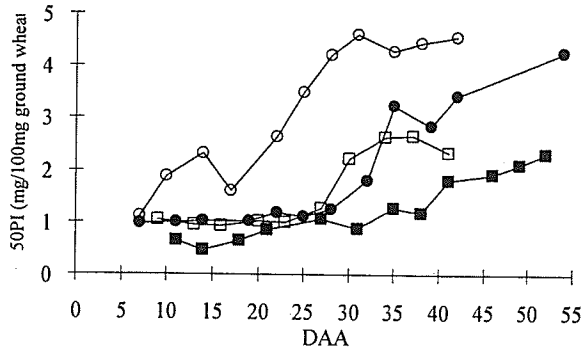


A 5.3 50PI protein content

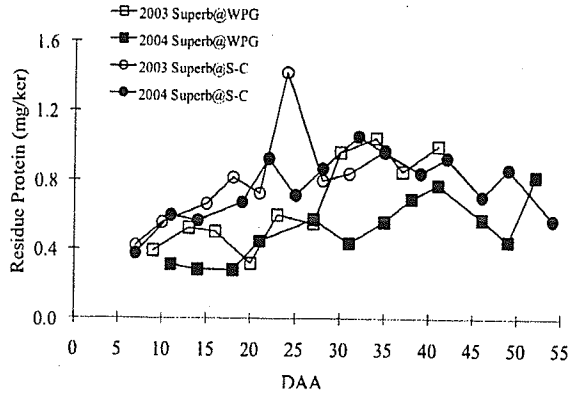


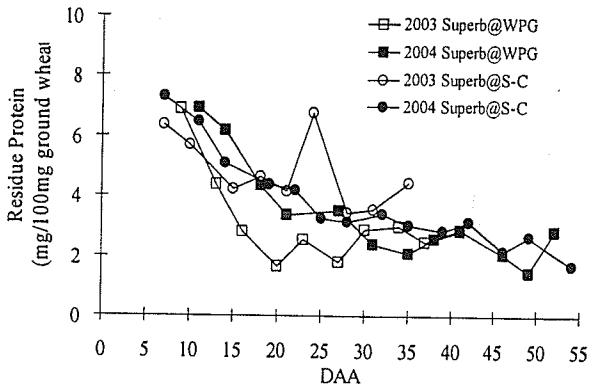


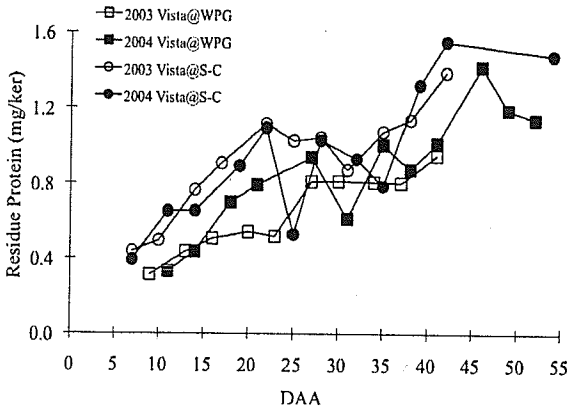


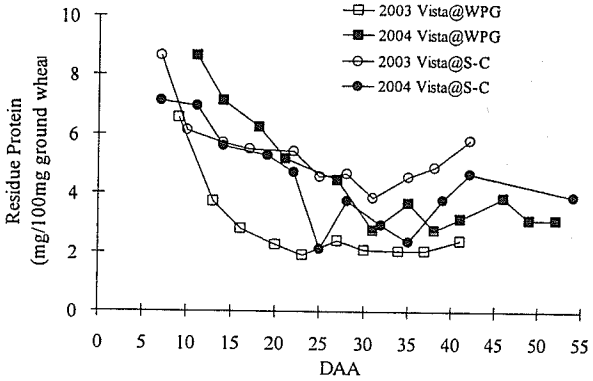


A 5.4 Residue protein content

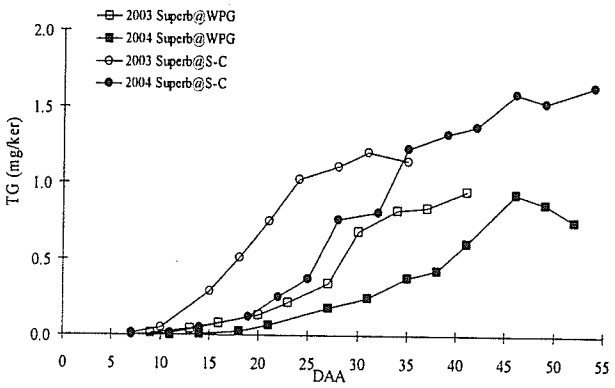


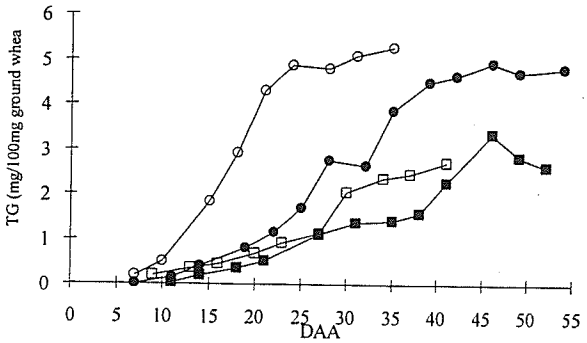


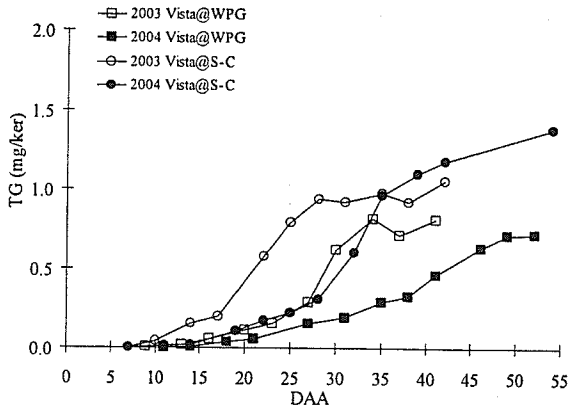


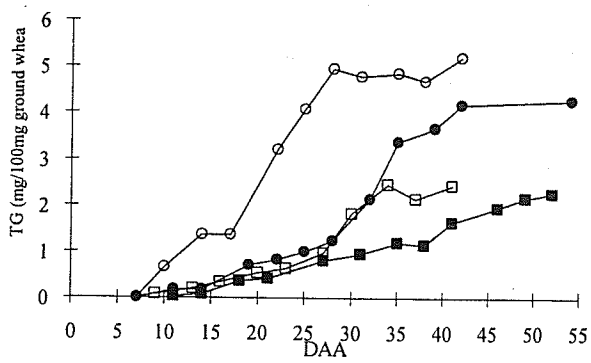


A 5.5 Total glutenin content

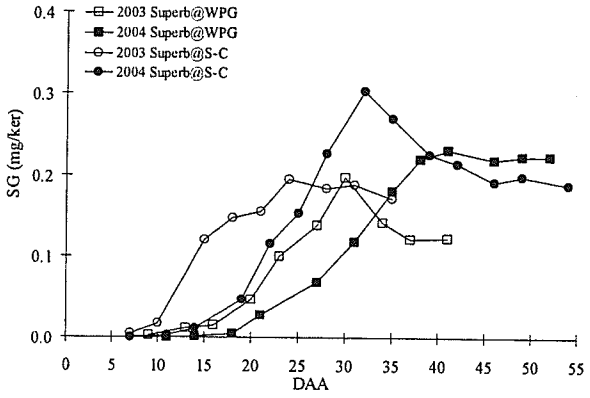


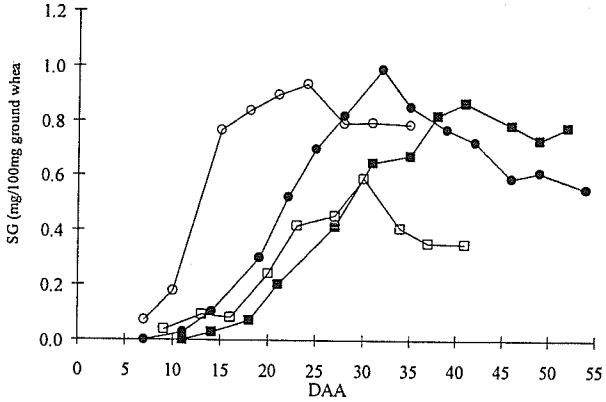


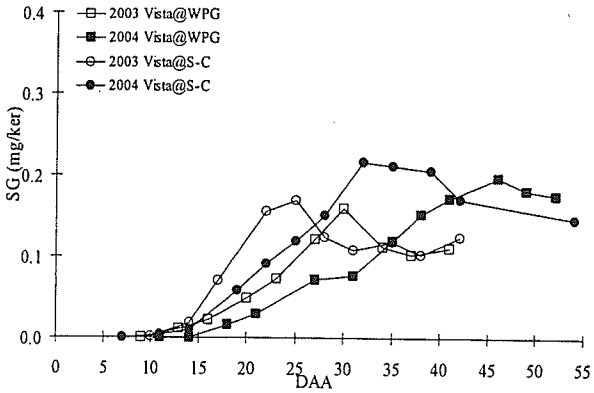


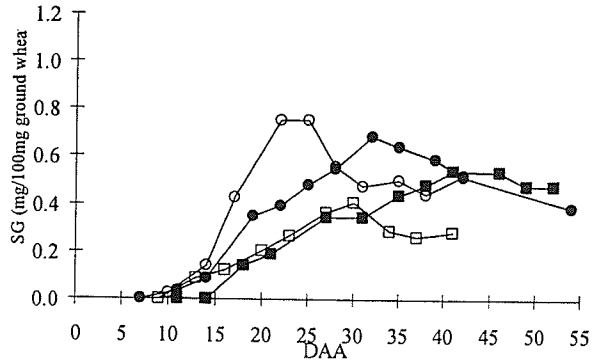


A 5.6 Soluble glutenin content

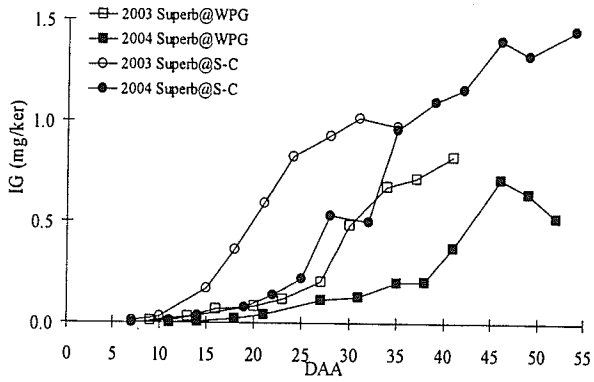


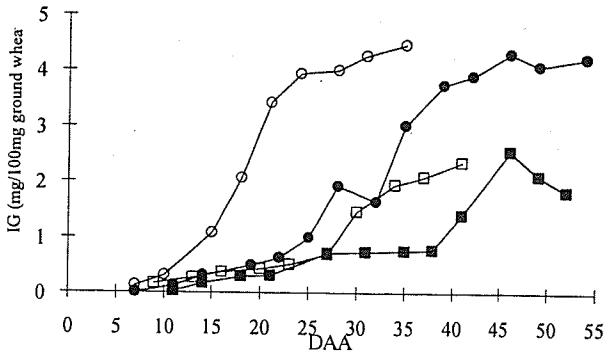


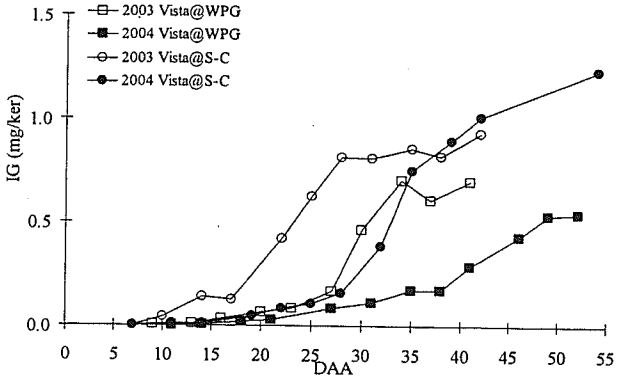


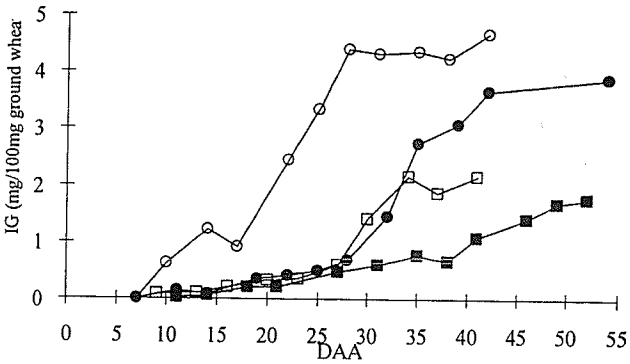


A 5.7 Insoluble glutenin content



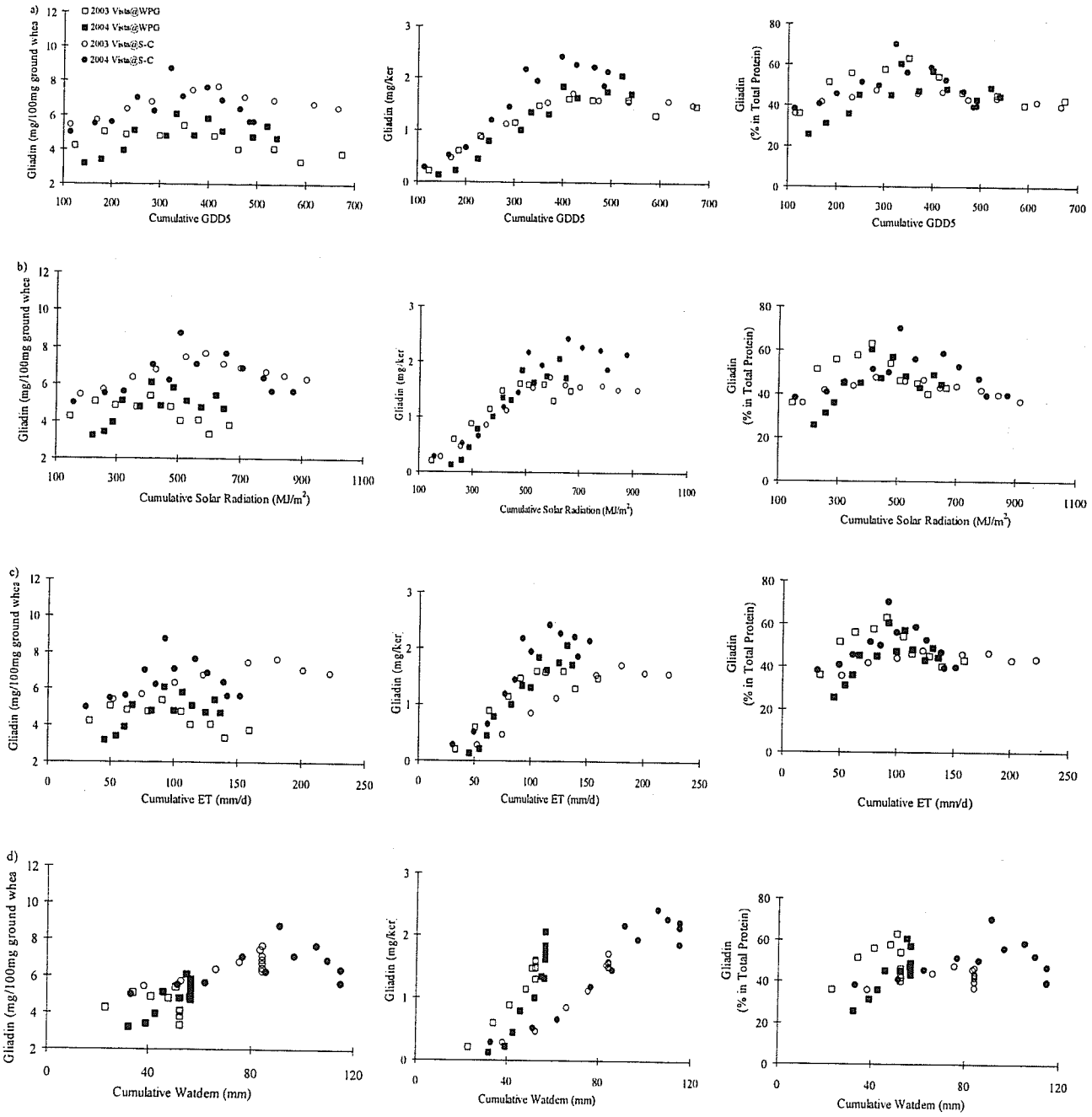




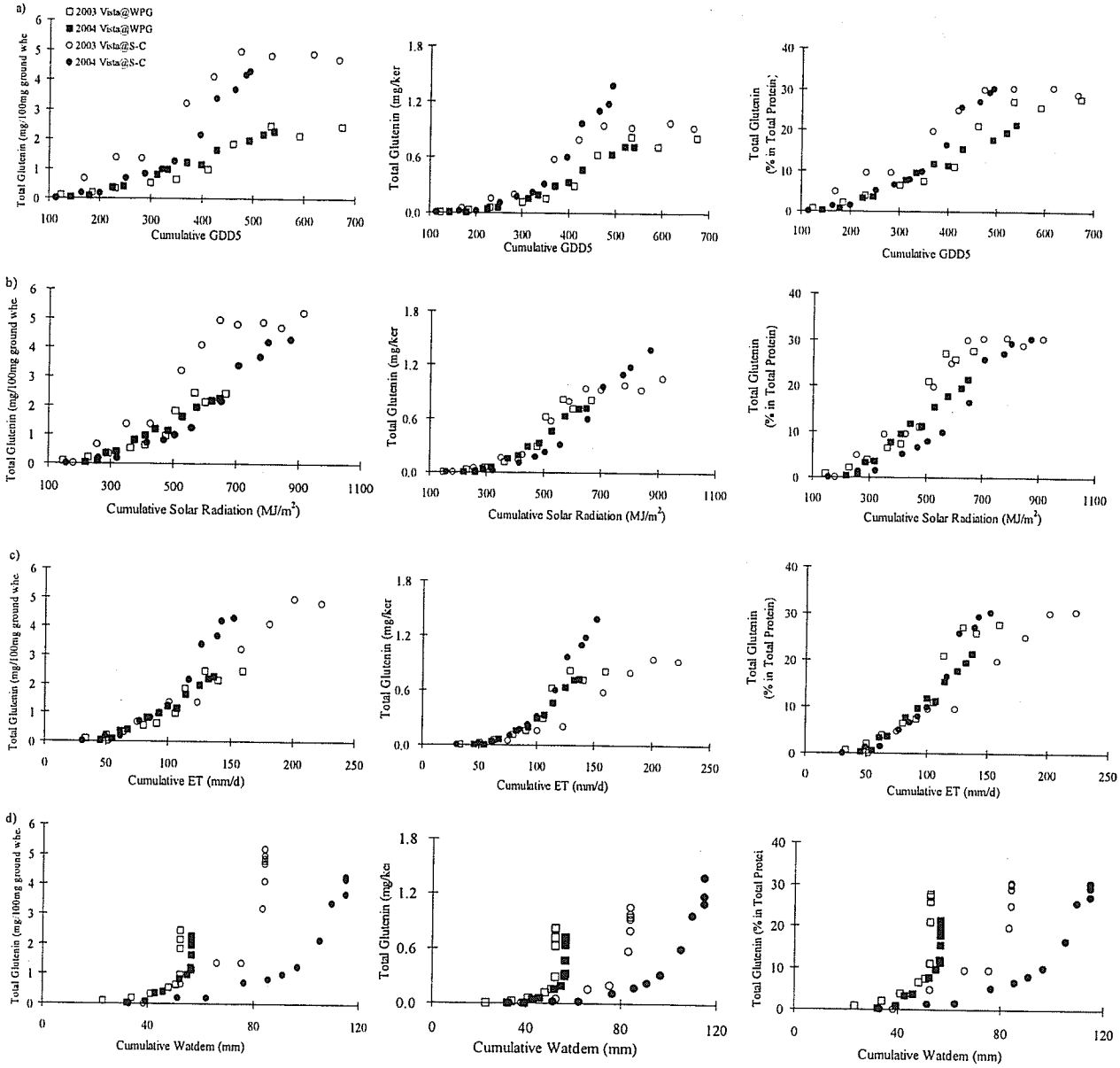


A.6 Relationship between different protein fractions and weather parameters; a) cumulative GDD5; b) cumulative solar radiation; c) cumulative ET; d) cumulative water demand, expressed at a constant grain basis (left), per kernel (middle) and as percentage in total protein (right) for AC Vista

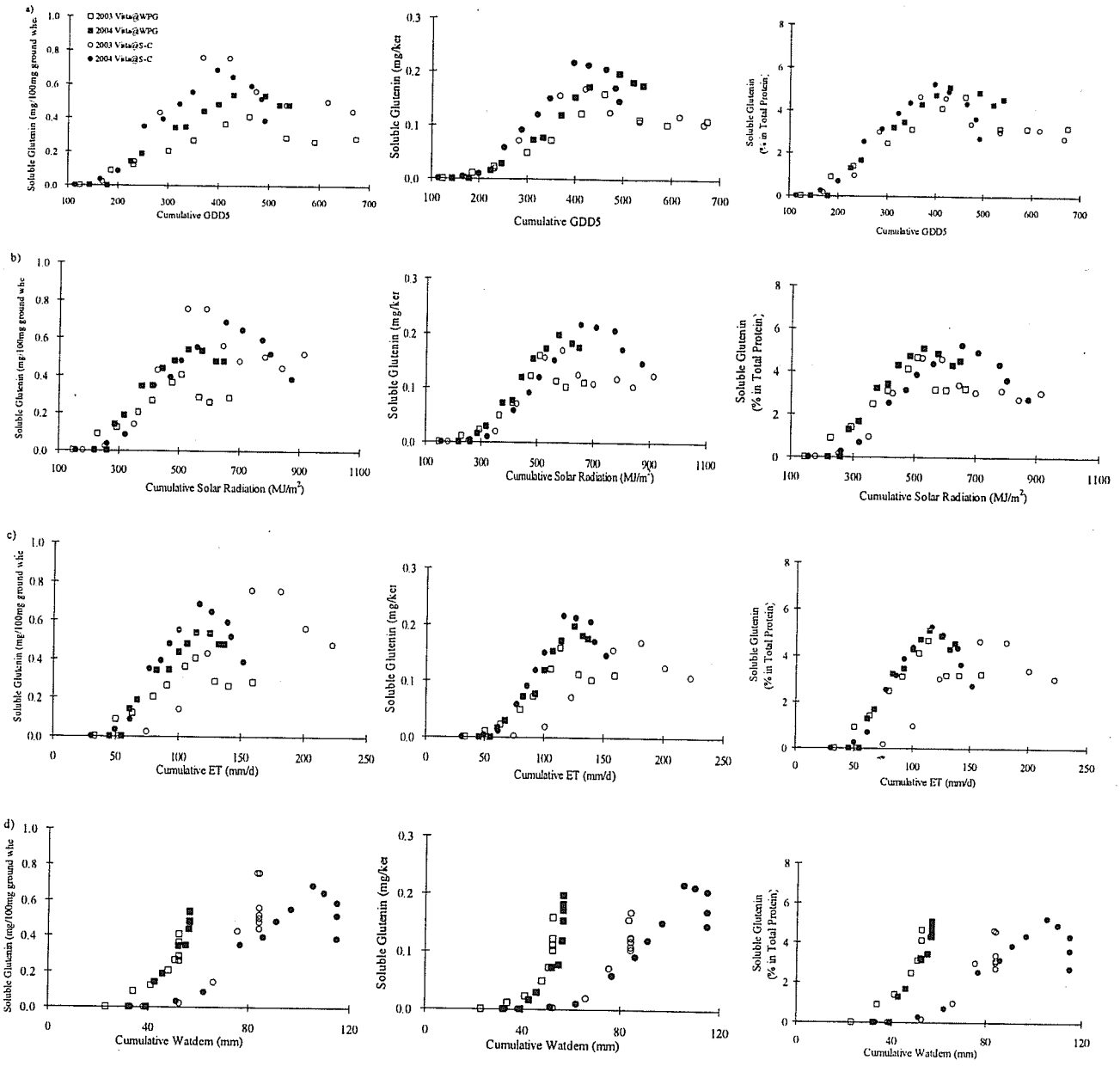
A 6.1 Gliadin



A 6.2 Total glutenin



A 6.3 Soluble glutenin



A 6.4 Insoluble glutenin