The Effect of Nitrogen Fertilization on Protein Content and Bread Baking Properties of Wheat Flour

by

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(ABSTRACT)

The effects of variation in nitrogen application during two growing seasons to Coker 916, a soft red winter wheat, on wheat grain and flour protein content and bread baking properties were examined. Nitrogen fertilization regimens tested included the single spring application of 168 kg/ha in growth stages 25 or 30, the split spring application of 224 kg/ha in growth stages 25 and 30, and no nitrogen application either in growth stages 25 and 30.

Variation in rate of nitrogen application had a more consistent effect on grain and flour protein than did variation in timing. Grain and flour protein content increased with increased nitrogen fertilization. The gliadin to glutenin protein ratio of the flour was reduced as the rate of grain nitrogen application increased. Significant differences were found in the flour gliadin percent protein with variation in spring nitrogen management. Fractionation and resolution of gliadin and glutenin components using SDS-PAGE revealed changes in the relative quantities present of two high molecular weight glutenin subunits

(108,000 and 91,000 daltons), one low molecular weight glutenin subunit (40,500 daltons) and the ω-gliadin (44,000 daltons) fraction of experimental flours.

Differences in dough rheological properties and baking characteristics of the flour were associated with variation in nitrogen management. Increased nitrogen fertilization resulted in a stronger, more extensible dough with improved mixing tolerance and dough handling characteristics. Increased flour protein was associated with larger bread loaf volume and darker crust color. Sensory panelists found significant differences in the bread crust color, texture and taste, bread aroma, crumb texture and chewiness. This experiment demonstrated that a change in nitrogen management of Coker 916 wheat resulted in a distinct change in protein composition and bread baking properties of experimentally milled flour.

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1.0 Introduction

Wheat is one of the most important sources of caloric intake for man. According to United Nations figures, approximately 25% of the total calories consumed in the world come from wheat (Nelson, 1985). Bread and related products are widely accepted as dietary staples.

Of the major cereal grains, wheat flour is unique due to its gluten forming properties when mixed with water. Gluten is a cohesive, viscoelastic mass with the ability to stretch. The proper balance of elasticity and viscous flow is important in bread baking properties. Nitrogen fertilization of the grain has been reported by many sources to improve bread baking qualities of flour (Doekes and Wennekes, 1982; Smak, 1972; Paredes-Lopez et al, 1985). Changes in bread baking properties of flour have been attributed to changes in gluten proteins (Branlard and Dardevet, 1985; Doekes and Wennekes, 1982) and to total protein content. The major gluten proteins, gliadin and glutenin, are both heterogeneous composites.

Coker 916, a soft red winter wheat, is one of the predominant varieties of wheat grown in the southeastern United States. Soft wheat flour, with a protein content of approximately 7%, and low to moderate gluten development, is best suited for the production of cakes, cookies and crackers. Hard wheat is imported to the southeastern United States from midwestern states for milling of a higher protein flour with greater gluten forming capacity for the production of bread.

Preliminary work done in this investigation indicated that variation in nitrogen management to Coker 916 influenced dough rheological properties (dough mixing tolerance, stability, and extensibility). Not all of the rheological changes were associated with changes in protein content. This investigation is the beginning of a cooperative effort between the Departments of Human Nutrition and Foods and Agronomy to understand the relationship among variation in grain nitrogen fertilization regimens, wheat and flour protein content and composition, physical dough properties and baking quality. This understanding is important to the agronomist, food scientist, nutritionist, miller and baker.

The objectives of this investigation were:

1. To determine the effect of variation in the rate and timing of nitrogen fertilizer application to the wheat on protein quantity of Coker 916 grain and experimentally milled flour from 1986 and 1987 growing seasons.

2. To separate wheat gluten proteins, gliadin and glutenin, from experimentally milled flour and subsequently separate the components of gliadin and glutenin using SDS-PAGE. The relative amounts present of protein components in each of the experimentally milled flours will be quantified with densitometer scans.

3. To evaluate the rheological and test baking characteristics of experimentally milled flours.

2.0 Review of the Literature

2.1 Wheat Gluten

2.1.1 Historical Perspective

Seed proteins were first separated into two fractions based on their solubilities in alcohol by Taddei in 1820 (Kasarda, Nimmo and Kohler, 1971). The alcohol soluble fraction was labelled gliadine, and the alcohol insoluble fraction zimome. This work was later extended in 1907 by Osborne who classified seed proteins based upon their extraction and solubilities in a series of solvents (Osborne, 1907). The classification included albumins (soluble in water), globulins (soluble in dilute salt solutions), prolamins (soluble in aqueous alcohol) and glutelins (soluble in dilute acid or alkali). The major wheat proteins were present in the prolamin and glutelin fractions. Osborne kept the name

of gliadin for the alcohol soluble fraction, but named the insoluble fraction glutenin, since the word zimome (derived from Greek) implied that glutenin was a product of fermentation. Both proteins accumulated in the wheat endosperm and served to store nitrogen, sulphur and carbon which are needed by the developing seed (Shewry, Tatham, Forde, Krets and Miflin, 1986).

Gluten was first described in 1728 by Italian chemist Beccari. He reported that an insoluble, viscoelastic mass resulted from the gentle washing of a flour and water dough under excess water (Bailey, 1944).

2.1.2 Gluten Formation

Of all grain flours, only wheat flour forms a gluten protein complex. When flour and water are mixed, gliadin and glutenin, the major endosperm storage proteins, interact with each other and with other flour components to form gluten. The gluten complex is responsible for the cohesive and viscoelastic properties of the dough made from the flour and water mixture. Wheat gluten is insoluble in water and remains when dough is washed to remove starch and other water soluble components (Shewry et al, 1986).

The proper balance of elasticity and viscous flow of a dough is important in determining bread baking properties. The viscoelastic properties of dough,

or the ability to stretch without breaking, are modified by mixing, kneading, and fermentation. Mixing and kneading are critical in the development of the viscoelastic properties of dough. With increased mixing and kneading, dough extensibility decreases and dough stability, elasticity and mixing tolerance increase (Finney and Yamazaki, 1967).

A three dimensional network of thin gluten membranes with embedded starch granules is formed during mixing. The three dimensional network formed is important for leavening. The gluten membranes can expand while entrapping some of the carbon dioxide produced by yeast fermentation. With excessive mixing the gluten membranes are destroyed and the dough makes a smaller loaf of bread with a coarse structure. During fermentation there is a gradual expansion and collapse of gas cells (Khan and Bushuk, 1979).

A dough that is more viscoelastic can better expand and trap gas for leavening. Loaf volume is determined by the degree of expansion allowed by the gluten membranes. Differences in gluten quality account for differences in loaf characteristics; a high quality gluten will result in a larger loaf with an acceptable crumb structure (Khan and Bushuk, 1979).

2.1.3 Gliadin Protein Fractions

In a study by Hoseney, Finney, Pomeranz and Shogren (1969), fractionation of gluten into gliadin and glutenin components based upon solubility in 70% alcohol resulted in a 53% gliadin to 47% glutenin ratio. According to Khan and Bushuk (1979), gliadin accounted for 35 to 40% of the total endosperm protein. The relatively small molecular size and the compact tertiary structure of gliadin contributed the properties of viscosity and extensibility to gluten and dough (Kasarda et al, 1971). Gliadin was present in the grain chiefly as polypeptide monomers which associated by hydrogen bonding and hydrophobic interactions. According to Tatham and Shewry (1985), strong non-covalent interactions between gliadins and between gliadins and glutenins were responsible for the viscosity of wheat gluten. Estimates vary as to the number of gliadin components. Between 20 to 28 gliadin bands were detected in each of 70 cultivars studied by Branlard and Dardevet (1985a). Shewry et al (1986) estimated there are approximately 50 components of gliadin within a cultivar. Mecham, Kasarda and Qualsett (1976) identified up to 46 gliadin components in a single cultivar using a combination of electrophoresis and isoelectric focusing.

The molecular weight of gliadin components as estimated by sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) varied from 12,000 to over 100,000 daltons (Khan and Bushuk 1979; Tatham and Shewry 1985:

Bunce, White and Shewry, 1985; Beckwih, Nielson, Wall and Huebner 1966). The majority of components were found to have a molecular weight of approximately 36,000 to 44,000 (Khan and Bushuk, 1979). Gliadin was divided into four groups, α, β, γ, ω, based upon electrophoretic mobility at low pH (Shewry et al, 1986). Recently, gliadins were classified into 2 groupings; the sulfur poor ω-gliadins and the sulfur rich α, β, and γ gliadins. The number, intensity and electrophoretic mobility of gliadin bands were found to provide a distinct pattern for different wheat cultivars, largely independent of environmental factors (Tatham and Shewry, 1985).

Using gel filtration chromatography, gliadin was separated into three groups with varying molecular weights (Beckwith et al, 1966; Bietz and Wall, 1972). The highest molecular weight fraction exceeded 100,000 daltons, followed by a small fraction with a molecular weight range of 60,000 - 80,000. This small fraction was the ω gliadins. The major fraction had a molecular weight range of 30,000 to 40,000 daltons, and consisted of the α, β and γ gliadins.

Using SDS-PAGE, Tatham and Shewry (1985) found the α-gliadins to have a relatively low molecular mass, followed by the β and γ gliadins, which showed some overlap. The α, β, and γ gliadins had molecular weights between 32,000 to 42,000 according to SDS-PAGE. These are higher than the estimates of Beckwith et al (1966) and Bietz and Wall (1972) previously cited. The ω-gliadins had a relatively high molecular mass, with molecular weights in the range of 44,000 to 72,000 daltons according to Tatham and Shewry (1985). This

molecular weight range was lower than that previously estimated (Beckwith et al, 1966; Bietz and Wall, 1972). Lafiandra and Kasarda (1985) used two-dimensional electrophoresis to resolve some of the gliadin components which overlapped with one-dimensional electrophoresis. Both dimensions were carried out on the same gel; the first dimension at pH 3.1 with aluminum lactate buffer and the second dimension at pH 9.2 with a TRIS - glycine buffer.

Molecular weight determinations using SDS-PAGE were influenced by the gel system. The molecular weights of wheat gliadins were estimated by Bunce et al, (1985) using three different methods for electrophoresis. By the modified Laemmli procedure for SDS-PAGE, the ω-gliadins (sulfur poor) had molecular weights of 61,000 and 50,000. The sulfur rich gliadins had molecular weights of 44,000 and 35,000. The Laemmli system gave higher molecular weights than a TRIS/bromate system. A modified Laemmli procedure including 4M urea in the separating and stacking gels resulted in a 25 to 40% further increase in molecular weight. Different gel systems resulted in a wide range of molecular weights.

The ω-gliadins were very different from the other gliadin components in amino acid composition and molecular weight. The ω-gliadins contained large proportions of glutamine, proline, and phenylalanine. They were low in sulfur containing amino acids (cysteine and methionine). The α, β, and γ gliadins contained less proline, glutamine and phenylalanine, and did contain cysteine and methionine (Shewry et al, 1986). Gliadin was also characterized by low

levels of basic amino acids (lysine, histidine and arginine) and low levels of free carbonyl groups. Overall, gliadin was not a very highly charged protein (Khan and Bushuk, 1979).

Tatham and Shewry (1985) used circular dichroism measurements (CD spectroscopy) to examine the secondary structure of gliadin components. The sulfur rich α, β and γ and sulfur poor ω gliadins were found to have different secondary structures stabilized by different secondary forces. The ω-gliadins were rich in β-turns with no α-helix or β-sheet conformations. Hydrophobic interactions were important, especially at higher temperatures. Disulfide bonds were found to be absent in the ω-gliadins.

The α, β and γ gliadins contained 30 to 35% α-helix and 10 to 20% β-sheets in their secondary structures. Disulfide and hydrogen bonds were both important and contribute to a high degree of stability.

SDS-PAGE was the most widely used method for determination of the relative molecular weights of polypeptides. Variation in the molecular weights of gliadins existed in the findings of many researchers. SDS-PAGE overestimated the molecular weights of some gliadins compared to the results of physical methods such as sedimentation equilibrium ultrcentrifugation and amino acid sequences (Bunce et al, 1985). For example, ω-gliadins were estimated to have a molecular weight of 78,000 by SDS-PAGE, 75,500 by ultracentrifugation, and 73,300 by amino acid analysis (Bietz and Wall, 1972).

Bunce et al (1985) stated that accurate determinations of molecular weights by SDS-PAGE depend upon 4 factors. First, all polypeptides must bind an equal amount of SDS per gram. This ratio is approximately 1.4g SDS/g protein. Secondly, all polypeptides must have the same conformation when complexed with SDS. Thirdly, the net charge of the polypeptide must have a negligible effect on the charge of the SDS-polypeptide complex. And finally, identical conditions must be used for electrophoresis of unknowns and standards. These criteria can influence the mobilities of polypeptides. If all of the above factors do not hold true, inaccurate estimates of molecular weights result. Bunce et al (1985) stated that the first three factors are not under the control of the researcher, but must be considered in evaluation of molecular weights by SDS-PAGE. In a study where all factors held true, the migration of proteins treated with SDS in SDS containing polyacrylamide gels was directly proportional to the log of the molecular weight of the polypeptide chain (Weber and Osborn, 1969).

The overestimation of molecular weights by SDS-PAGE was suggested by Bunce et al (1985) to be the effect of the high proline content on the conformation of the protein/SDS complex. Proline rich proteins of animal origin were also shown to have molecular weights overestimated due to low electrophoretic mobility.

Bunce et al (1985) found no clear relationship between proline content and the degree of overestimation of molecular weight. The sulfur poor ω-gliadins had

the highest proline content but showed a small discrepancy in molecular weight compared to the other gliadin subunits which had a lower proline content. Based upon this Bunce et al (1985) suggested that the positioning of the proline residues may have more of an influence than the actual number. Proline residues may be involved in a secondary structure not completely denatured by SDS-PAGE, unlike α-helices and β-sheets. This partial denaturation would result in a more rigid conformation, reduced electrophoretic mobility and an overestimation of molecular weight.

Branlard and Dardevet (1985a) found 14 gliadin bands of the SDS-PAGE to be positively correlated with flour quality characteristics evaluated by alveogram strength and swelling. Five bands were negatively correlated to these flour quality criteria. Tenacity, evaluated by alveogram value of P (dough resistance to deformation), was not found to be related to any of the gliadin fractions. Seven of the gliadin bands were related to protein content, and 1 gliadin band was negatively correlated to protein content. Variation in protein content was found to be significant in the correlation between the protein content of gliadin bands and quality (alveogram) characterisitcs. The presence and concentration of 7 to 12 gliadin bands explained 37 to 54% of the variation in alveogram tests.

2.1.4 Glutenin Protein Subunits

Glutenin is insoluble in 70% aqueous ethanol, but soluble in dilute acid or alkali. In a study by Hoseney et al (1969), fractionation of gluten into gliadin and glutenin components based upon solubility in 70% alcohol resulted in a 53% gliadin to 47% glutenin ratio. Approximately 35 to 45% of the total wheat endosperm protein was glutenin (Khan and Bushuk, 1979). The functional behavior of glutenin was attributed to physical and chemical properties; molecular size and shape (physical), amino acid composition, sequence and tendency to aggregate (chemical) (Khan and Bushuk, 1979). The glutenin subunits were joined by interchain disulfide bonds or by hydrophobic interactions and hydrogen bonding (Belitz, Seilmeier, and Wieser (1984). Gluten elasticity was attributed to the glutenin protein components. Elasticity was attributed to the hydrophobic interactions of non-polar amino acid residues and the high molecular weights of the polypeptide chains (Kasarda et al, 1971). Disulfide bonding of the polypeptide chains of glutenin formed long, compactly folded structures, giving the gluten elastic properties. Shewry et al (1986) attributed glutenin strength to the stabilization of aggregates by intermolecular disulfide bonds.

The glutenin components undergo the most marked changes during dough mixing. Belitz, Kieffer, Seilmeier and Wieser (1986) reported oxidative polymerization of proteins occurred during mixing, resulting in fractions with

very high molecular weights. The presence of protein monomers suitable for oxidation was critical for the formation of a strong gluten. When wheat was milled under nitrogen, and gluten formed and washed out under nitrogen, the gluten was relatively weak. The presence of oxygen and oxidizing agents were found to be important in gluten strength due to sulfhydryl and disulfide interchange reactions. Khan and Bushuk (1979) also stated that the presence of oxidizing agents, reducing agents, and mechanical development modified the secondary, tertiary and quaternary structures of glutenin to optimize loaf volume and crumb structure.

The reduced subunits of glutenin were separated into at least 15 different subunits which ranged in molecular weight from 11,000 to 133,000 daltons (Bietz and Wall, 1972). These subunits were separated into two groups of polypeptides, the high molecular weight and the low molecular weight subunits (Shewry et al, 1986).

An analysis of glutenin showed a high concentration of glutamine (Khan and Bushuk, 1979; Huebner and Wall, 1976). The amide groups formed intra and intermolecular hydrogen bonds important in the rheological characteristics of hydrated glutenin. There was also a high concentration of hydrophobic amino acids such as leucine present. The aqueous environment of the dough promoted the formation of hydrophobic bonds which as a group were important in the stabilization of glutenin.

The high molecular weight subunits of glutenin had a high concentration of glycine and a lower concentration of proline, unlike the gliadins. The high concentration of glycine in glutenin was a similarity shared with connective tissue proteins such as collagen and elastin. The elastic properties of elastin were also attributed to glycine rich sequences (Belitz et al, 1986). The molecular weight range of glutenin is up to 134,000. The three largest glutenin subunits, with molecular weights of approximately 134,000, 132,000 and 90,000 have been shown to be important for dough formation and stability during baking. Wheat is unique among the cereal grains in having a high molecular weight glycine rich peptide fraction (Belitz et al, 1984).

The high molecular weight subunits were related to bread baking characteristics of the flour proteins of 84 wheat cultivars were separated by SDS-PAGE by Payne, Nightingale, Krattinger and Holt (1987). Variation in the high molecular weight subunits accounted for 47 to 60% of the variation loaf volume and texture.

High molecular weight glutenin subunits were related to bread baking quality by Moonen and Zeven (1985) in a study of 10 cultivars of wheat. Two high molecular weight subunits were absent in two cultivars characterized by poor bread baking qualities of the flour.

The high molecular weight glutenin fraction was rich in β-turns in a repetitive central domain using circular dichroism spectroscopy and computer predic-

tion (Tatham, Miflin and Shewry, 1985). The β-turns were regularly distributed resulting in a regular primary structure. The repetitive β-turns form an elastic β-spiral, responsible for gluten elasticity. According to the model proposed by Tatham et al, the elastic monomers were assembled into gluten polymers by intermolecular disulfide bonds between the cysteine residues in α-helical domains near the N and C termini.

Ewart (1978) postulated a somewhat different model for glutenin elasticity. A random distribution of cysteine residues was assumed within glutenin chains with approximately one sixth of an average chain being available for unfolding. The unfolding of glutenin chains under stress served to increase the effective molecular length of glutenin.

Branlard and Dardevet (1985b) investigated the correlation between the high molecular weight glutenin subunits of 70 wheat cultivars and flour quality characteristics. Glutenin subunits were separated using SDS-PAGE and related to flour quality characteristics as evaluated with the alveograph. Nine of the high molecular weight subunits were significantly related to alveograph strength, 7 subunits influenced swelling and extensibility, and 6 subunits influenced dough tenacity. Swelling and extensibility were linked negatively to tenacity.

Two glutenin subunits had positive influence on strength, and were related to glutenin quantity in the grain (Branlard and Dardevet, 1985b). Conversely,

two subunits had a negative relationship with dough strength, though were not linked to glutenin content in the grain. Two of the subunits highly correlated with swelling were correlated with gliadin content. Swelling was influenced by gliadins, thus these two high molecular weight glutenin subunits were similar in functionality to gliadins.

By elimination of the influence of variation in protein content by regression analysis, Branlard and Dardevet (1985b) found causal relationships between the different glutenin subunits and dough strength, tenacity, swelling and extensibility. The high molecular weight subunits were grouped into two types of subunits based upon their relationship to functionality; those related to strength and tenacity, and those correlated with extensibility. The subunits related to strength and tenacity were characterized by accessible cysteine residues and/or hydrophobic groups which participated in intermolecular disulfide links. The subunits with fewer disulfide links and/or less accessible cysteine residues were correlated with gluten elasticity.

The low molecular weight glutenins were similar in amino acid composition to the α, β, and γ gliadins but had a higher molecular weight of approximately 44,000 (Shewry et al, 1986). The molecular weight range of the low molecular weight glutenins was 12,000 to 68,000 by SDS-PAGE. These subunits tended to aggregate. Glutenin polymers containing high molecular weight and low molecular weight subunits had molecular weights ranging into the millions.

A third group of glutenin subunits was identified with a molecular weight range of 35,000 to 45,000 and had the same SDS-PAGE mobility as the two major gliadin proteins (Khan and Bushuk, 1979). These glutenin polymers contained only low molecular weight subunits and were purified from the gliadin fraction. The subunit was also referred to as high molecular weight gliadins or aggregated gliadin (Shewry et al, 1986).

2.1.5 Nitrogen Fertilization and Amino Acid Composition

Several researchers have investigated the influence of nitrogen fertilization on the amino acid composition in the grain (Dubetz, Gardiner, Flynn and del la Roches, 1979; Parades-Lopez, Covarrubias-Alvarez and Barquin-Carmona, 1985). In a study with increasing rates of nitrogen fertilization (0 - 400 kg/ha) Dubetz et al (1979) found the proportions of glutamine, proline and phenylalanine in the seed increased with nitrogen fertilization up to 200 kg/ha. Threonine, serine, glycine, alanine and valine all decreased. The altered amino acid composition was attributed to shifts in the relative proportions of protein fractions present, with an increasing proportion of gliadins. Gliadins required the lowest energy expenditure by the plant to produce of the major endosperm proteins (Mitra, Bhalia, and Rabson, 1979).

A decrease in lysine content was related to increased nitrogen fertilization, but was not statistically significant. Since lysine is a limiting amino acid in wheat,

shifts in lysine content due to nitrogen fertilization were suggested to be of minor nutritional importance (Dubetz et al, 1979).

Nitrogen fertilization increased protein quantity, but reduced protein quality (Parades-Lopez et al, 1985). In a study of cultivars of soft and semihard wheat, nitrogen application of 220 kg/ha resulted in a reduction in the proportion of essential amino acids present. Nitrogen fertilization reduced isoleucine, leucine and proline by more than 0.1g/100g protein. Glutamic acid increased with increased nitrogen fertilization.

2.2 Grain Nitrogen Management

2.2.1 Grain Protein Response to Nitrogen Fertilization

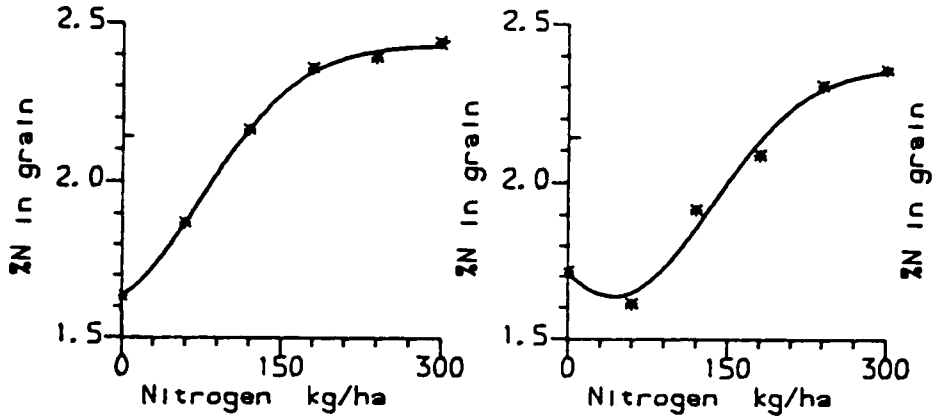
The protein content of wheat was influenced by many factors, including cultivar, rate and timing of nitrogen application and environment. The protein content of wheat increased significantly by the application of nitrogen as fertilizer to the soil (Dubetz et al, 1985) and by late spraying with urea (Finney, Meyer, Smith and Pryer, 1957; McNeal, Berg, Brown and McGuire, 1971). Protein quantity of wheat was determined by Kjeldahl nitrogen analysis (Paredes-Lopez et al, 1985; Benzian and Lane, 1981). The analysis assumed a constant relationship between the total nitrogen content and protein forming

amino acid polymers. The nitrogen content of flour was multiplied by a conversion factor of 5.7 to determine the flour protein content (Trachuk, 1966). A conversion factor of 5.83 was used for the determination of wheat protein content (Jones, 1926).

The response of the grain to the addition of fertilizer nitrogen varied with the rate of nitrogen application. A non-linear response (Figure 1) of grain nitrogen to increasing nitrogen fertilization over a wide range of fertilization levels was reported (Murray and Nunn, 1987). At low rates of nitrogen fertilization, up to 50 kg/ha, there was a reduction in the percent nitrogen in the grain. In a study of 124 winter wheat and 41 spring wheat experiments, Benzian and Lane (1981) reported a 'dilution' effect of grain nitrogen, also known as the Piper-Steenbjerg effect, occurring in 1/4 of the winter wheat and 1/2 of the spring wheat experiments.

A linear or near-linear response of grain nitrogen percent to increased nitrogen fertilization was reported in the range of 50 to 175 kg N/ha by Benzian and Lane (1981). Within this range, they estimated that approximately 50 kg N/ha was required to produce an increase of 1% protein in cultivars of winter wheat studied. A similar linear response to nitrogen fertilization rates up to 210 kg N/ha was reported by Pearman (1978).

Kosmolak and Crowle (1980) studied the grain nitrogen response of five varieties of Canadian hard red spring wheat. With the application of nitrogen as



Source: Murray and Nunn, 1987

Figure 1. Grain nitrogen response curve

fertilizer at rates of 0, 56 and 224 kg/ha, grain nitrogen content increased with each increasing level of nitrogen fertilization. The flour yield (extraction rate) was not influenced by variation in nitrogen management and protein content. This agreed with the studies by Parades-Lopez et al (1985).

A wide range of nitrogen fertilization rates, 0 - 400 kg/ha, were studied by Dubetz et al (1979). Increased rates of nitrogen management resulted in a levelling off of grain nitrogen response. Grain protein nitrogen increased with each 50 kg N increment up to 200 kg/ha. From 250 to 400 kg N/ha, no further significant increase in grain protein content was shown. A levelling off of grain nitrogen response was also reported by Benzian and Lane (1981).

2.2.2 Split Dose Nitrogen Application and Grain Protein Response

Nitrogen as fertilizer was commonly applied to the grain in a single application (Paredes-Lopez et al, 1985). Many studies produced contradictory results from split dose nitrogen application. A split dose application incorporated 10 to 25% more nitrogen in to the grain than single dose application (Paredes-Lopez et al, 1985). The amount of nitrogen incorporated into the grain from fertilizer was higher after the second application of nitrogen at 35 days. The ability to increase nitrogen uptake varied with cultivar. A low proportion of nitrogen was taken up into the grain in cultivars of soft (Salamanca) and semihard (Anahuac) wheat tested by Paredes-Lopez et al (1985).

In other research comparing the effect of single nitrogen application with split dose nitrogen application, the rate of nitrogen application had the strongest influence on grain protein content (Anon., 1976; Nuttall, 1979). Anon. (1976) reported the effect of variation in the rate of nitrogen application (75, 100 and 125 kg N/ha) and the timing. Three different timing regimens were used for nitrogen application; (1) single application early, between growth stages 21 and 30 (Zadoks, Chang and Konzak, 1974), (2) single application late, between growth stages 31 - 32, and (3) split nitrogen application, early and late. The major differences in grain protein content were attributed to variation in nitrogen rate, though a late single dose application (growth stage 31 - 32) did increase grain protein content.

The effect of a single dose nitrogen application in growth stage 32 was compared to the effect of a split dose application involving even later nitrogen application (Nuttall, 1979). Nitrogen management involved split dose application in growth stages 32, 39 and as a urea spray at growth stage 71. No difference in grain protein content was found at equivalent total nitrogen application rates.

Split dose nitrogen application showed a detrimental effect on grain protein content. In a study of the effect of variation in nitrogen rate and timing on grain protein, Jarvis and Stevens (1983) found variation in nitrogen rate had the major influence on grain protein content. Grain protein increased with increasing nitrogen application up to 240 kg/ha in a single dose at growth stage

30. This agreed with other reports previously cited (Benzian and Lane 1981, Pearman 1978, Dubetz et al 1979, Kosmolak and Crowlee 1980). When the equivalent amount of nitrogen was applied in a split dose, with 40 kg/ha applied in growth stage 22 and the remainder in growth stage 30, grain protein content was reduced. This was attributed to the stimulation of early season vegetative growth, and dilution of the protein content due to increased starch associated with increased yield.

A nitrogen management regimen involving three applications (40 kg/ha at growth stage 22, the balance at growth stage 30 and 40 kg/ha at growth stage 37) was also tested by Jarvis and Stevens (1983). This regimen resulted in a reduced grain protein content compared to the single application at growth stage 30, though the results were higher than those obtained when two nitrogen applications (growth stages 22 and 30) were used.

The effect of single, two-way and three-way split applications of nitrogen was studied in a series of experiments from 1979 - 81 (Dampney, 1987). Nitrogen was applied in 25 kg/ha increments from 0 - 200 kg/ha and in 40 kg/ha increments from 0 - 240 kg/ha. Single dose nitrogen application was done in growth stage 31. From 100 to 200 kg/ha and 80 to 240 kg/ha split nitrogen applications were also used. The two-way split nitrogen applications included 40 kg/ha at growth stage 21 - 25 with the remainder at growth stage 31 and 40 kg/ha at growth stage 39 with the remainder at growth stage 31. The three-way split application included 40 kg/ha at growth stage 21 to 25, 40 kg/ha at growth

stage 39 with the remainder at growth stage 31. Of the 113 experiments, 91 (80%) showed no significant difference in grain nitrogen when a split dose application was used. Sixteen experiments (14%) showed a statistically significant increase in grain nitrogen content. The median increase was 0.05% protein (14% moisture basis).

2.2.3 Nitrogen Application and Grain Yield

Jarvis and Stevens (1983) investigated the effect of variation in nitrogen rate and timing on grain yield and protein content. Three different management regimens were used for the applications of 120, 160 and 200 kg N/ha. Nitrogen was applied in a single dose in growth stage 30, as a two way split dose with 40 kg N/ha in growth stage 22 and the remainder in growth stage 30, and as a three way split dose with 40 kg N/ha in growth stage 22, 40 kg N/ha in growth stage 37 and the remainder in growth stage 30. Significant yield increases were found with the application of up to 160 kg N/ha. The application of 40 kg N/ha at growth stage 22 resulted in increased yield compared to the single dose nitrogen application in growth stage 30. As mentioned earlier in this discussion, the nitrogen management regimen of 40 kg/ha in growth stage 22 with the remaining application in growth stage 30 resulted in a reduction in grain protein content. A split nitrogen application, including application at the beginning of spring growth, increased yield at the expense of grain protein content (McClean, 1987).

Kosmolak and Crowle (1980) found that with increased nitrogen application (0, 56 and 224 kg/ha) to five varieties of Canadian hard red spring wheat, grain yield increased in each variety with the nitrogen application of 56 kg/ha. No further increase in yield was seen with the nitrogen application of 224 kg/ha, though this level resulted in an increased protein content as previously discussed.

In other studies, variation in rate and timing of nitrogen application did not influence yield. Variations in timing of a single dose nitrogen application from growth stage 21 and 32 were not significantly different in yield (Anon., 1976). The use of a split application, involving both growth stages 21 and 32 did not have any significant effect on yield as well.

2.2.4 Wheat Quality

Wheat (grain and flour) characteristics must be considered in terms of a specified use; desired characteristics for one product may be undesirable for other products. The product tested in this study was yeast bread. Ford (1987) reported that the important bread baking characteristics of wheat flour include protein quantity, protein quality (gliadin and glutenin components), alpha amylase content, potential flour color, and hardness. These characteristics influenced milling, processing, physical dough characteristics and baking.

Quality characteristics of wheat are a function of genetics and environmental conditions.

2.2.4.1 Physical Characteristics

The distinguishing physical characteristic of wheat cultivars is the kernel (Ford, 1987; Zeleny, 1971). Seed coats vary in both texture and color. Some seedcoats are hard (vitreous), while others are soft in texture. The color of the kernel can be characterized as red or white depending on the color of the bran (Zeleny, 1971). Color is determined by variety, but may be influenced by environment.

Hard wheat has been traditionally associated with a relatively high protein content and gluten forming capacity (Pomeranz, Bolling and Zwingelberg, 1984; Ford, 1987). Hard red wheats are used for bread flour. Dark red wheats are typically harder, with a higher protein content. Soft red wheats in the United States are traditionally used for cake, pastry and cookie flour (Zeleny, 1971).

Moisture content of the wheat is also important. The moisture content of wheat is inversely related to the amount of dry matter. This influences both the economic value and the keeping quality of the wheat. Dry wheat is also brittle and results in broken kernels before milling (Zeleny, 1971).

2.2.4.2 Milling Quality

Milling quality refers to the wheat kernel properties which contribute to the economical production of flour (Finney and Yamazaki, 1967). Kernel texture and flour extraction are both important factors. Experimental milling is used to predict milling quality. Capacities of experimental mills range from 50g to several hundred pounds. Experimental milling is the only way to actually estimate flour yield (AACC Method 26-20 1983).

Kernel hardness has a large influence on milling quality. Hardness allows good separation of the endosperm from the bran and results in a high flour extraction rate. A good endosperm separation level also results in a high recovery rate of endosperm proteins in the flour. The endosperm protein levels are highest close to the bran (Ford, 1987). Starch granules of hard wheat contain a proteinaceous material on their surface. This results in adhesion of the endosperm protein to the starch surface within the cells and prevents excessive loss of fine particles during milling.

2.2.4.3 Baking Quality

Finney and Yamazaki (1967) referred to baking quality as the ability of flour to produce an acceptable baked product. They stated that to be acceptable for bread baking, flour should have a high water absorption capacity, a medium to medium-long mixing requirement and a small to medium oxidation re-

quirement. The dough should also demonstrate a satisfactory mixing tolerance, dough handling characteristics and good loaf volume potential. The loaf produced should have an internal crumb of acceptable color and texture.

Dough consistency is affected by the protein content of the flour (Weipart and Pomeranz, 1986). Zeleny (1971) reported that for yeast leavened bread, a flour protein content of at least 11% was required by bakers in the United States (Table 1). This corresponded to a wheat protein content of 12 to 13%. In the United Kingdom, a wheat protein content of 11.5% at 14% moisture is required for production of sliced white bread (Ford, 1987).

Baking properties are influenced by environmental conditions. High temperatures, greater than 90°F, and low relative humidity during the last 15 days before harvest have been shown to result in subnormal bread baking properties (Finney and Yamazaki, 1967).

2.2.4.4 Starch Damage

Starch damage is also an important factor associated with grain hardness. Generally, soft wheat flour has minimal starch damage (Pomeranz et al, 1984; Ford, 1987). Starch damage is associated with the water absorbing capacity of flour, and allows starch granules to hold water while still cold. High water absorbing capacity for bread baking is desirable. Soft wheat yielded more free

Table 1. Wheat protein requirements for various uses

End Product	Wheat Protein Content 14% moisture basis
Macaroni products	13 or more
Hearth bread, hard rolls	13 - 14
Pan bread	12 - 13
Crackers	10 - 11
Biscuits	8.5 - 10.5
Cake	9 - 9.5
Pie Crust	8 - 10
Cookies	8 - 9

(source: Zeleny, 1971)

starch during milling. Excessive pressure is required to obtain high starch damage.

Pomeranz et al (1984) found that the level of starch damage in hard wheat provided adequate levels of fermentable sugars to attain the loaf volume potential of the flour protein. The limited amount of starch damage occurring in soft wheat flour did not allow the loaf volume potential of the flour to be attained.

In a comparison of the bread baking potential of six varieties of soft wheat and six varieties of hard wheat, Pomeranz et al (1984) found that the volume potentials of soft wheat flours were attained by optimization of the sugar level in the bread formulation. The volume potential of soft wheat flours was then equivalent to that of the hard wheat flours on a constant protein basis. An increase in the sugar level of the bread formulation from 1% to about 2.5%, or an increase in the starch damage from 7 to 12% allowed a valid comparison of the bread baking functional properties of soft wheat to the properties of hard wheat.

The addition of sugar to soft wheat bread formulations did not compensate for the difference in water holding capacity between hard and soft wheats. High sugar levels resulted in dough handling problems and excessive crust browning (Pomeranz et al, 1984).

Pomeranz et al (1984) concluded that soft and hard wheats contained proteins which were characterized (on a constant protein basis) by equivalent functional properties for bread baking. In cases where there was an advantage to growing soft wheat varieties, the soft wheats were made to perform satisfactorily by increasing the sugar in the formulation to compensate for the low level of starch damage.

2.2.4.5 *Alpha-amylase Content*

Alpha-amylase provides fermentable sugars from starch in dough before baking and during the early stages of baking which are important for loaf volume and crust browning (Ford, 1987). Inadequate alpha-amylase can be supplemented. Excessive alpha-amylase results in excessive breakdown of starch to sugars and dextrins and consequently a sticky dough. A sticky dough causes handling problems, difficulty in slicing, and possible loaf texture breakdown. Alpha-amylase levels may be measured by the Hagberg Falling Number Test (Paredes-Lopez et al, 1985) or amylograph (AACC Methods 22-06 and 22-10, 1983).

2.2.5 Baking Quality and Total Protein Content

As reported earlier, many characteristics which are important in bread baking are a function of protein quantity. Within a variety of wheat, water absorption

is directly related to protein content, as is oxidation requirement (Finney and Yamazaki, 1967). Mixing requirement, mixing tolerance and dough handling characteristics are all related to the protein content within a variety. With increasing protein content (within a variety) up to about 12%, mixing time decreases. Above 12% protein, the mixing requirement does not significantly change. At low protein levels, bread crumb grain is heavy, while at extremely high protein levels bread crumb texture may be excessively open (Finney and Yamazaki 1967).

The baking properties of flours can be determined and compared by test baking, using standardized procedures such as the Basic Straight Dough Method of the AACC (Method 10-10A, 1983). Ford (1987) stated that test baking is the ultimate test of flour protein quality. Rheological, physical and chemical tests can only predict the baking performance of a flour. Test baking can evaluate the effect of different treatments such as variation in nitrogen management on flour performance (Finney and Yamazaki, 1967).

Nitrogen fertilization of the wheat and increased flour protein content have been reported by many sources to improve bread baking qualities of flour (Doekes and Wennekes 1982; Paredes-Lopez et al 1985 and Smak 1972). In response to nitrogen fertilization, improvements in bread baking qualities of flour have included increased loaf volume and changes in crumb structure, crust color and general loaf appearance.

Nitrogen fertilization improved loaf volume and crumb structure in soft and semi-hard red wheats investigated by Paredes-Lopez et al (1985). Nitrogen fertilization regimens of 0, 110 and 220 kg/ha were used. While the flour resulting from 110 and 220 kg N/ha regimens produced breads which were judged acceptable, the control treatment (0 kg N/ha) resulted in the production of bread with a general appearance which was judged unacceptable. Kosmolak (1980) also found loaf volume increased significantly with increasing flour protein content within a cultivar due to nitrogen fertilization of the grain.

Pomeranz et al (1984) compared the functional bread baking properties of six varieties of soft wheat and six varieties of hard wheat by comparing the loaf volume increase per 1% protein. No basic differences were found in the functional properties of the soft and hard wheats studied.

The relationship between crust brownness and flour protein content of 90 samples was studied by Smak (1972). Browning of bread crust was attributed to Maillard, or carbonyl-amine reactions (Bertram, 1953). Crust color was evaluated objectively from a 6cm disc cut from the center of the top crust using a photoelectric reflexímeter. Color standards of the C.I.E. (Commission International de l'Eclairage) were used. A flour protein content of 10.5% was necessary to produce a crust with a normal brown color. Flours with a protein content below 8.5% produced a loaf with a gray tinted crust. Between 8.5 to 10.5% protein, results varied as to brownness of the crust. Degree of brown-

ing is an important variable in determining loaf acceptability by consumers (Smak, 1972).

2.2.6 Dough Properties and Protein Content

Dough rheological characteristics have been measured by various physical tests. Mixing characteristics of gluten development are evaluated with the use of a recording dough mixer, such as the farinograph or mixograph (Kosmolak and Crowle, 1980; Paredes-Lopez et al, 1985). The farinograph, one of the most widely used instruments, measures and records resistance of a dough to mixing. The farinograph also evaluates the absorption of flours and determines the stability, plasticity and mobility of dough which is gently mixed for prolonged times at a constant temperature (AACC Method 54-21, 1983). The mixograph is also used to to assess dough properties, though the method is not as standardized as that of the farinograph (Finney and Yamazaki, 1979).

The extensigraph and Chopin alveograph were used to evaluate dough extensibility (Branlard and Dardevet, 1985 a and b; Rasper, Pico, and Fulcher, 1986, Kosmolak and Crowle, 1980); Paredes-Lopez et al, 1985). The ability of properly developed gluten to be extended, or sheeted, and its ability to retain gas were measured. With the alveograph, a bubble is blown in a dough disc and the expansion of the bubble to the breaking point is recorded. Bread wheats having strong gluten forming properties are characterized by

alveograph deformation energy (energy necessary to inflate the dough until it ruptures) greater than or equal to 350 (Branlard and Dardevet, 1985a). Rasper et al (1986) evaluated the suitability of alveograph testing for quality assessment of 14 soft white winter wheat cultivars. Most alveogram indexes were found to correlate significantly with flour protein content. Branlard and Dardevet (1985a) found the alveograph P/L ratio provided a better assessment of flour strength than the individual parameters alone.

Nitrogen fertilization and increasing protein content within a cultivar was reported to strengthen dough properties when tested with the mixograph (Kosmolak and Crowle, 1980). McNeal (1971) reported that nitrogen fertilization resulted in increased dough strength as indicated by increasing farinograph peak time.

In a study of five Canadian hard red spring wheat cultivars, dough strength as measured by extensigraph area increased with increasing protein content due to nitrogen fertilization for 3 of the cultivars studied (Kosmolak and Crowle, 1980). In one of the cultivars, the extensigraph did not show any significant change in dough strength. For the fifth cultivar studied, the extensigraph area was found to decrease with increasing protein content, indicating a reduction in dough strength. Though in general dough strength was found to increase with increasing protein content due to nitrogen fertilization, this was not a universal trend in all cultivars studied.

2.2.7 Nitrogen Fertilization and Protein Quality

In addition to the protein quantity, protein quality is also important in determining bread baking properties of flour. Different wheats with the same protein content produce flours with different baking characteristics, due to differences in gluten proteins.

Paredes-Lopez et al (1985) found that nitrogen fertilization of two cultivars of soft and semihard wheat had effects on both protein quantity and on protein quality. Increasing levels of nitrogen fertilization increased dough elasticity, as evaluated by alveograph testing. The semihard wheat cultivar tested also showed a large increase in alveograph deformation energy with increasing nitrogen fertilization. These functional changes in the dough were postulated to be the result of modifications in the molecular composition of the gluten.

Nitrogen fertilization led to large increases in the quantity of gliadins, smaller increases in the glutenin components and only slight increases in the albumin and globulin protein fractions (Prugar and Sasek, 1970). The relative proportion of protein fractions was found to change with nitrogen fertilization.

In five cultivars studied, Doekes and Wennekes (1982) found that as the total protein content within a cultivar increased due to nitrogen fertilization, only the gliadin fraction increased. The glutenin, albumin and globulin contents did not change. The amount of glutenin per gram of flour varied with cultivar.

Loaf volume increased linearly with increasing protein content. This was related to the increasing gliadin content and gliadin to glutenin ratio. An optimal gliadin to glutenin ratio was not found. Flour samples with equal gliadin contents but differing glutenin contents produced loaves of the same volume (Doekes and Wennekes, 1982).

All protein fractions increased as the total nitrogen content of the grain increased due to nitrogen fertilization (Dubetz et al, 1979). The gliadin and glutenin fractions extracted increased significantly with increased total nitrogen.

2.3 Sensory Evaluation: Descriptive Analysis

In all foods research, valid and reproducible evaluation of the sensory attributes of a product is a critical step. Descriptive methods provide a word description of a product or set of products. Quantitative Descriptive Analysis (QDA) is a descriptive test of all of the sensory attributes of a product (Stone, Sidel, Oliver, Woolsey and Singleton, 1974). QDA provides a complete sensory picture of a food and this is generally preferred over older descriptive methods such such as the Flavor Profile, developed by Arthur D. Little, or the Texture Profile developed by General Foods.

QDA is a relatively rapid sensory method that requires 6 to 10 panelists, and is independent of the individual panelist (no experts). QDA involves a language development procedure, followed by language verification (Stone, Sidel and Bloomquist, 1980). QDA requires replicate responses by the individual panelists. Each panelist must evaluate the product independent of the other sensory panelists. In older descriptive methods, the panelists often worked as a group, reaching a collective decision. Group dynamics were often found to have a negative effect. This was eliminated by the QDA method. Most products have been found to require about 4 responses per panelist to achieve an accurate description of product similarities and differences.

Language development is a critical step in descriptive analysis. During the group language development process, the panelists evaluate a product or products similar to what they will be evaluating in future sessions. A consensus must be reached among the panelists of terms which adequately describe the product. The panelists also define each term , and decide upon words to indicate increasing intensity of the attribute. This process helps to avoid misunderstandings and confusion concerning the sensory attributes. The panel leader does not participate in the language development process, but simply serves as a recorder of the session. This helps to avoid any influence of the leader on the panel. The leader does organize and guide the session. From the language development sessions, a sensory scorecard is developed with 6 inch horizontal line scales for each attribute. The anchor words, indicating

increasing attribute intensity from left to right, are located one half inch from each end.

During the language development process, reference terms may be supplied to the panelists. These are often terms which have been used in a previous study. Rognerud, Wilsher, Oybo and Frolich (1986) evaluated 7 attributes of bread on a 7 point scale using a trained laboratory panel with 10 judges. These attributes included evenness of the surface of bread slices, texture of the crust (crisp to doughy), 2 texture characteristics of the crumb (firm to crumbly and dry to doughy), bread flavor, saltiness and sweetness.

3.0 Materials and methods

3.1 Wheat

Coker 916, a soft red winter wheat, is one of the predominant varieties of wheat grown in the southeastern United States. Coker 916 grown by the Agronomy Department of Virginia Polytechnic Institute and State University in the 1986 and 1987 growing seasons was used for experimental purposes. The locations used in the 1986 growing season were Giles and Charles City County; the 1987 locations were Giles and Montgomery County. Various levels of nitrogen were applied to the wheat as fertilizer during growth stages 25 and 30 (Zadoks et al, 1974) for a total of twenty treatment combinations (Table 2). A randomized complete block was replicated four times at each location.

Table 2. Coker 916 nitrogen management regimens

	Nitrogen treatment, kg/ha Growth stage			Nitrogen treatment, kg/ha Growth stage	
	25	30		25	30
1.	0	0	11.	28	156
2.	28	0	12.	56	56
3.	56	0	13.	56	112
4.	112	0	14.	56	168
5.	168	0	15.	112	56
6.	0	56	16.	112	112
7.	0	112	17.	112	168
8.	0	168	18.	168	56
9.	28	56	19.	168	112
10.	28	112	20.	168	168

3.2 *Preliminary Protein Determinations of Wheat*

Preliminary determinations of the percent protein in the Charles City County wheat produced in the 1986 growing season were done by NIR (near infra-red) analyses with a Trebor-90 grain tester [1]. Thirteen of the twenty nitrogen management regimens were selected for testing with the Trebor-90 to represent a range of nitrogen management regimens (Table 3).

For each of the thirteen treatments, all replicates were sampled and tested. The procedure used with the Trebor-90 was as follows. Approximately 300g of cleaned wheat were poured into the instrument, calibrated for soft wheat. A reading of percent protein was obtained. The wheat was collected and returned to the instrument for a second reading. A third reading was taken if the difference between the first two readings was greater than 0.5.

3.3 *Experimental Milling*

Cleaned wheat was weighed and milled on a Buhler Laboratory Mill, bucket type (Buhler Bros.,Inc., Uzwill, Switzerland) according to AACC Method 26-20

[1] Trebor-90 testing done at Roanoke City Mills, Roanoke, VA

Table 3. 1986 Nitrogen management regimens tested with Trebor-90

	Nitrogen treatment, kg/ha Growth stage 25	Growth stage 30
1.	0	0
2.	56	0
3.	168	0
4.	0	56
5.	0	168
6.	28	56
7.	28	168
8.	56	56
9.	56	168
10.	112	56
11.	112	168
12.	168	56
13.	168	168

(AACC 1983).[2] Three breaks and three reduction steps were used. Break roll settings were left break roll 8-10, right break roll 3-5. Reduction roll settings were left reduction roll 4-6, right reduction roll 1-2. The flour was rebolted over a 40 mesh silk screen.

3.4 *Total Protein Determinations of Wheat and Flour*

The nitrogen content of wheat, flour, and flour protein fractions was determined by Kjeldahl analysis (AOAC, 1975). The total protein content was derived by multiplying nitrogen values by conversion factors of 5.7 for flour (Jones, 1926) and 5.83 for wheat (Trachuk, 1966). Wheat and flour protein values were corrected to a 14% moisture basis (m.b.) (AACC, Method 80-22, 1983). The data were analyzed with an analysis of variance procedure followed by Duncan's multiple range test, $\alpha = .05$ (SAS Intitute, Cary, NC).

3.5 *Moisture Content of the Flour*

The moisture content of the flour was determined by gas chromatography using a modification of the unpublished method of Saltmarch and Harris (per-

[2] Experimental milling of the 1986 wheat done at Mennell Milling Company, Statesville, NC and Midstate Mills, Newton, NC. Experimental milling of 1987 wheat done at Midstate Mills, Newton, NC

sonal communication, 1986). A Gow-Mac Gas Chromatograph, Series 550 Thermal Conductivity Detector was used.

A 20g sample of flour was suspended in 200ml anhydrous methanol and allowed to sit for 72 hours. The mixture was centrifuged at low speed, and the supernatant was decanted. A two (2) μl aliquot of the supernatant was injected into the gas chromatograph at 155°C using a Hamilton syringe. The column temperature was 102°C and the detector temperature was 175°C. The attenuation was set at one and bridge current was 85 ma. The flow rate of the carrier gas (He) was 40 ml/min. A Hewlett Packard 3390 Electronic Integrator (Hewlett Packard, Palo Alto CA) was used to calculate peak areas. A standard curve was prepared from solutions of known methanol concentration. The moisture content of the flour samples was determined from a comparison of the peak area ratio of the unknown to the peak area ratios of the standard solutions. Flour samples were run in duplicate.

3.6 Dough Rheological Properties

3.6.1 Farinograph

Dough rheological properties were examined with the Brabender farinograph using AACC method 54-21 (1983), Constant Flour Weight Procedure for 50g

sample.[3] The resistance of dough to mixing was evaluated by peak time, mixing tolerance index, stability and time to breakdown. The methods for calculating these measurements are as follows:

1. *Peak time (dough development time)* - the interval to the nearest 0.5 minute from the first addition of water to the point of maximum consistency immediately before the first indication of weakening

2. *Mixing tolerance index* - the difference in Brabender (viscosity) units (BU) from the top of the curve at the peak to the top of the curve measured 5 minutes after peak viscosity was attained.

3. *Stability* - the time difference to the closest 0.5 minutes between where the top of the curve first reaches the 500 BU line and when the top of the curve first leaves the 500 BU line.

4. *Time to breakdown* - the time from the start of mixing until a decrease of 30 BU from the point of peak viscosity is seen.

The farinograph was used on flour samples resulting from wheat nitrogen management regimens of 0/0, 168/0 and 0/168 kg/ha N in growth stages 25 and 30 from the 1986 growing season and all samples from the 1987 growing sea-

[3] Farinograph testing of 1986 flour done by C.W. Brabender Instruments, South Hackensack, NJ; for 1987 flour Midstate Mills, Newton, NC)

son (the farinograph was not accessible to the investigators for all 1986 samples).

3.6.2 Alveograph

The Chopin alveograph (Chopin, Boulogne, France) was used to assess dough rheological properties of the flour resulting from wheat nitrogen management regimens of 56/168 and 168/56 kg/ha N in growth stages 25 and 30 from the 1986 growing season. The alveograph was used for all samples from the 1987 growing season.[4]

Dough strength and extensibility were evaluated by a comparison of *alveogram length, L* (the average length of the alveogram, indicating extansibility of the dough); *overpressure, P* (the product of the maximum height of the curve and 1.1, indicating dough resistance to deformation); and *deformation energy, W* (the product of the area under the curve and 6.54, indicating dough strength by representing the energy necessary to inflate the dough until it ruptures). The P/L ratio was calculated.

Dough was prepared in the alveograph mixer and allowed to rest for 20 minutes. The dough was then cut into disks 5 - 6 cm in diameter. The disks were clamped on to a base plate and air forced through a hole in the base plate

[4] Alveograph tests done by Midstate Mills, Newton, NC

under the dough. This causes the formation of a bubble. The pressure in the dough bubble was recorded as a function of time.

3.7 Bread baking

Baking qualities of the flour were evaluated by bread baking tests according to a modification of the Basic Straight Dough Method (method 10-10A, AACC, 1983). All ingredients were incorporated during the initial mixing step. L-ascorbic acid was purchased from Sigma Chemical Company (St. Louis, MO). The remaining ingredients were purchased at Kroger, Blacksburg, VA. All yeast purchased was from the same lot and stored under refrigeration. Sugar, non-iodized salt, shortening and non-fat dry milk were all stored in closed containers in Wallace Hall. Dry ingredients were weighed and combined the day before baking and stored in closed containers. Pup loaf pans were purchased from National Manufacturing Company, Lincoln, NE.

Bread from the 1986 growing season was produced at the Bake Shop of the Virginia Tech Dining Services. Bread from the 1987 growing season was produced in a laboratory of the Department of Human Nutrition and Foods. The bread formulation is provided in Appendix A. The modified baking procedure is detailed in Appendix B.

An incomplete block design was used for baking and sensory evaluation. Four replicates of each flour were tested in five days over a two week period (Table 4). Three loaves were produced from each replicate.

3.8 *Loaf Volume*

Loaf volume was determined three hours after baking with a volumeter using rapeseed displacement (Cathcart and Coles, 1938). The instrument was standardized with a 500cc wooden block. Readings were taken in duplicate. The data were analyzed with an analysis of variance procedure followed by orthogonal contrasts (SAS Institute, Cary, NC).

3.9 *Bread Moisture*

Moisture content of the bread crumb resulting from replicates of each 1986 flour variation was determined using a Brabender Moisture Volatile Tester (C.W. Brabender Co., South Hackensack, NJ). The instrument was adjusted and calibrated with 10g for samples up to 30% moisture. An additional 1g weight was added for an additional 10% moisture. Ten gram samples of bread crumb were prepared from a combination of three bread slices from each replicate of each treatment (center slice, second slice from one end, third slice

Table 4. Schedule for baking and sensory evaluation

Nitrogen treatment, kg/ha growth stage		Day				
25	30	1	2	3	4	5
0	0	x	x	x	x	-
168	0	x	x	x	-	x
0	168	x	x	-	x	x
56	168	x	-	x	x	x
168	56	-	x	x	x	x

from the other end). The bread crumb was weighed in non-stick sample pans using an analytical balance (Mettler Analytical Balance, Princeton, NJ). The samples were dried to a constant weight at 155 °C for approximately three hours to determine percent moisture. Percent moisture was calculated from the difference between the initial and dried weights. The data were analyzed with an analysis of variance procedure followed by orthogonal contrasts (SAS Institute, Cary, NC).

3.10 Crust Color

Crust color was measured using a Hunter Color Difference Meter (Hunter Labs, Reston, VA). The instrument was standardized using the white standardization tile for l, a and b values where:

- l = visual lightness
- a = red (+), green (-)
- b = yellow (+), blue (-)

Two 5 cm squares of crust were cut from the bread and flattened to provide an even textured surface. L, a and b values were determined. The standardization of the instrument was checked between each sample with the white standard tile with L= 91.97, a= -0.80, and b= -1.00. Delta E, a single numerical value, was calculated for each sample as follows:

$$\Delta E = \sqrt{(l^2 + a^2 + b^2)}$$

The data were analyzed with an analysis of variance procedure followed by orthogonal contrasts (SAS Institute, Cary, NC).

3.11 Sensory Evaluation

Sensory evaluation was based upon a modification of Quantitative Descriptive Analysis (QDA)(Stone et al, 1974). A laboratory panel of 11 judges was selected from the graduate students and staff of the Department of Human Nutrition and Foods. All interested volunteers completed a brief questionnaire. Panelists selected were all consumers of American bread, not taking any medication which interfered with taste acuity, and available for all scheduled training and testing sessions. There were 2 male and 9 female panelists between the ages of 21 and 45.

A group language development session was conducted with all sensory panelists present. The investigator acted as a non-participatory leader. The panelists were given slices of bread produced in the laboratory according to the modified AACC procedure (method 10-10A, 1983) for pup loaves, as detailed in Appendix B. Commercial bread flour was used. Panelists were provided with room temperature water to drink between sample trials. During a group

discussion, the panelists identified the sensory attributes of bread which they felt were important. Anchor words were decided upon by the group to describe increasing intensity of each attribute. The leader recorded the attributes and anchor words.

The vocabulary developed by the panelists in the group language session was used for the preliminary scorecard. Each attribute was used to label a 15cm horizontal line, which was marked with appropriate anchor words. The anchors were located 1.25cm from the ends of the line, indicating increasing attribute intensity going from left to right. The panelists made a vertical line across the horizontal scale at the point which reflected the relative intensity of the attribute. The distance from the left end of the scale to the judges vertical mark was measured to quantify attribute intensity. The scorecard was tested by each of the sensory panelists, using two samples of bread produced in the laboratory by the method described in Appendix B. One bread sample was produced from bread flour and the other from experimental flour. The panelists were instructed to write any comments concerning the attributes and/or scorecard on their scorecard.

The results of the test and panelists comments on the preliminary scorecard were used to develop the final scorecard. Nine attributes were evaluated. The final scorecard was reviewed by each panelist prior to the actual sensory work. The scorecard is shown in Figure 2.

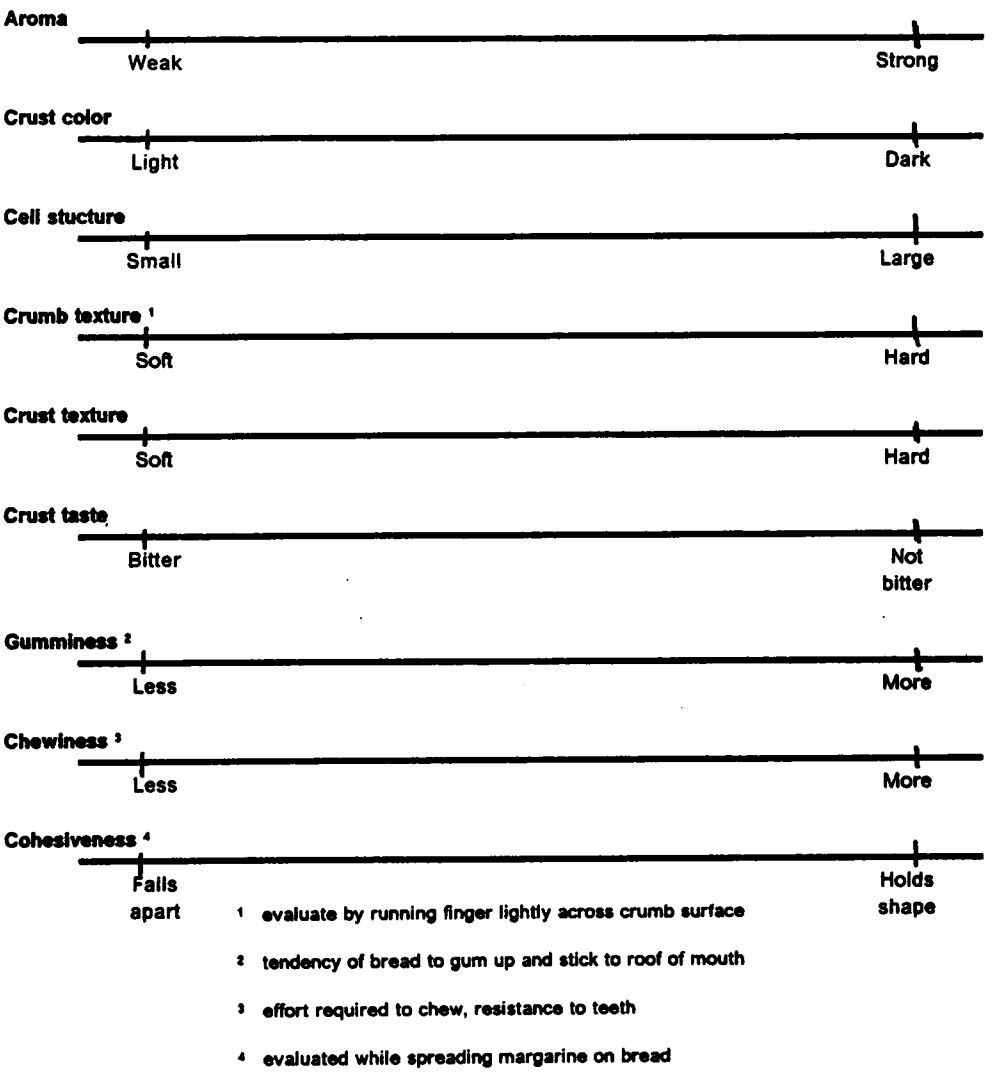


Figure 2. Descriptive analysis scorecard

The sensory analysis sessions were conducted in a laboratory with individual booths of neutral background and fluorescent lights. The booths restricted interaction among the panelists during the testing. Sensory analysis was conducted for five days over a two week period, with four different breads being tested on each day (incomplete block design, Table 2) 2.5 to 2.75 hours after baking. Slices of bread to be evaluated (including crumb and crust) were presented to panelists on white paper plates and coded with random digits. The end two slices from each loaf were discarded, samples for testing were selected randomly from the remaining slices. The panelists were provided with room temperature water to drink between sample trials. All panelists were present on all days of sensory testing.

The data were analyzed with a two way analysis of variance procedure (SAS Institute, Cary, NC) followed by orthogonal contrasts. The model below represents the mathematical assumption which describes the sources of variation present in the dependent variable.

$$Y_{ijk} = \mu + T_i + P_j + D_k + (TP)_{ij} + (PD)_{jk} + E_{ijk}$$

μ = mean of attribute score

T_i = fixed effect due to ith treatment i = 1,5

P_j = random effect of jth person j = 1,11

D_k = random effect of kth day k = 1,5

$(TP)_{ij}$ = random interaction of jth person with ith treatment

$(PD)_{jk}$ = random interaction of jth person on kth day

E_{ijk} = random effect of loaf receiving treatment i evaluated by person j on day k.

The treatment by person interaction was used as the error term in the test of significance for treatment.

The person by day interaction was used as the error term in the test of significance for day.

3.12 Fractionation of Flour Proteins

Wheat flour proteins were extracted and fractionated according to their solubilities in 70% ethanol by a modified method of Doekes and Wennekes (1982). The procedure is detailed in Appendix C.

3.13 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

Following fractionation, the composition of glutenin and gliadin proteins was examined using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to a modified method of Laemmli (Laemmli, 1970). A 5% to 15% linear polyacylamide concentration gradient was used in a discontinuous buffer system. An acrylamide stock solution of 30.8 %T and 2.6 %C was prepared for the resolving gels. The %T represents the total acylamide monomer concentration (w/v). The %C is the ratio of the weight of the cross linking agent N, N'-methylene-bis-acylamide to the total monomer concentration. An acrylamide stock solution of 33.2 %T and 9.8 %C was prepared for the stacking gel.

The %C of the stacking gel was increased for two reasons. The increased %C resulted in larger acrylamide pore size. Pore size is a function of the amount of cross linking agent present. From 2 to 5 %C pore size is progressively decreased with increased density of linear acrylamide fibers. From 5 to 50 %C pore size is progressively increased with increased supercoiling of fibers (Chrambach, 1985). A further increase in %C resulted in bis-acrylamide solubility problems. The increased %C also hastened polymerization of the stacking gel. Polymerization was visible as the gel turned opaque. Formulations for the SDS-PAGE acrylamide solutions are shown in Appendix D. Formulations for the SDS-PAGE buffers are shown in Appendix E.

3.13.1 Sample preparation

Gliadin and glutenin samples were prepared for electrophoresis in sample buffer. Glutenin samples were prepared by combining 0.015g residue obtained from protein fractionation in 70% ethanol (as detailed in Appendix C) with 0.5ml sample buffer (3% w/v). Gliadin samples were prepared by the addition of 0.010g precipitate from the dialysis procedure of protein fractionation (as detailed in Appendix C) to 0.75ml sample buffer (1.3% w/v). Samples were placed in screw top test tubes, vortexed and heated in an 80 °C water bath for 20 minutes.

3.13.2 Molecular Weight Markers

Reference molecular weight markers represented a range in molecular weight from 14,300 to 205,000 daltons. One vial of the high molecular weight standard mixture (MW-SDS-200, Sigma Chemical Company, St. Louis, MO) was prepared in 1.0 ml of sample buffer. Aliquots were frozen for future use in glass screw top vials. One aliquot was prepared for each electrophoresis run by incubating in a 37°C water bath for 2 hours. Additional molecular weight markers lysozyme and trypsinogen were added to each aliquot before incubation. Molecular weights of the protein markers used are listed in Table 5.

A molecular weight calibration curve was constructed from a plot of the natural logarithms of the reference protein molecular weights against their electrophoretic mobility (Appendix F). The electrophoretic mobility of Coker 916 gliadin and glutenin components was used to estimate their molecular weights from the calibration curve.

3.13.3 Electrophoresis

Acrylamide solutions for the separating gels were prepared with initiator and catalyst for polymerization (ammonium persulfate and TEMED [tetramethyl ethyl enediamine]) according to the formulation in Appendix D

Table 5. Molecular weight markers for SDS-PAGE

Reference protein	Approximate molecular weight, daltons
Lysozyme	14,300
Trypsinogen	24,000
Carbonic anhydrase	29,000
Albumin, egg	45,000
Albumin, bovine	66,000
Phosphoylase B	97,400
β-galactosidase	116,000
Myosin	205,000

and degased with a tap aspirator for 20 minutes. Initiator and catalyst for polymerization were added and the solutions were swirled to mix thoroughly. The acrylamide solutions were poured into a linear gradient former (Bio-Rad model 385 Gradient Former, BioRad Laboratories, Richmond, CA). The acrylamide was delivered to the prepared gel sandwich (Hoefer Vertical Slab Gel SE 600, Hoefer Scientific Instruments, San Francisco, CA) with the aid of a peristaltic pump calibrated to deliver 5 ml/min (Isco Wiz Pump / Diluter / Dispenser, Lincoln, NE). The acrylamide was overlayed with approximately 1 cm 0.1% SDS and allowed to polymerize overnight.

The stacking gel was prepared the following day according to the formulation in Appendix D without ammonium persulfate and TEMED initiators of polymerization. The solution was degased with a tap aspirator for 20 minutes. The SDS layer was poured off of the separating gel, and the top of the gel was dried with a folded paper towel. Initiators for polymerization (TEMED and ammonium persulfate) were added to the stacking gel and the solutions swirled to mix thoroughly. The stacking gel was poured on top of the separating gel through a 50 ml syringe and a well forming comb (15 lanes) was inserted. The comb was inserted angled, which prevented the trapping of air bubbles at the bottom of the sample wells. The gel was allowed to sit for two hours until polymerization was complete. The stacking gel had an opaque appearance when polymerized.

Once polymerized, the comb was carefully removed from the stacking gel. Running buffer applied to the top of the gel with a Pasteur pipet as the comb was removed facilitated removal of the comb. The gel sandwich was then attached to the upper buffer chamber. The lower buffer reservoir was filled with running buffer which had been cooled to 4°C. The electrophoresis unit was connected to a circulating water bath (Brinkman RM6 Refrigerating Circulator, Sybron Corp., Westbury, NY) which was maintained at 12°C. The slab gel unit was assembled and the upper buffer reservoir filled with pre-cooled running buffer. The assembled unit was placed on paper towels to check for leaks and then placed into the lower buffer chamber.

Ten μl aliquots of each sample and the molecular weight reference markers were loaded into sample wells with a Hamilton syringe. Samples were applied down the edge of the well so an even layer of sample buffer formed on the bottom of the well. The electrophoresis run was initiated at 15 ma, constant current (EC-400 Power Supply, EC Apparatus Corporation, St. Petersburg, FL). The current was increased to 30 ma (constant current) as the dye front entered the separating gel. The dye front was allowed to migrate off the acrylamide to maximize resolution, and electrophoresis was continued for 28 minutes after the leading edge of the dye front migrated off the acrylamide.

The gel was fixed for 24 hours (10% glacial acetic acid, 40% methanol), stained for 12 hours (0.025% Coomassie Brilliant Blue R-250, 10% glacial

acetic acid, 10% methanol) and destained until the background was clear (10% glacial acetic acid, 10% methanol). The stain was prepared fresh for every third gel, and the destaining solution was changed twice for each gel. A strand of white yarn placed with the gel in the destaining solution helped to absorb excess dye.

3.13.4 Densitometer Scans

Densitometer scans were done on acrylamide gels using an Isco Gel Scanner (Model 1312, Isco Model 228 Absorbance Detector, Lincoln, NE). Relative band densities were calculated with a Hewlett Packard 3390 Electronic Integrator (Hewlett Packard, Palo Alto, CA). Gel scans were run at 1 cm/min. The measuring filter wavelength was set at 580 nm for Coomassie Brilliant Blue R-250 dye. The scanning mode used was automatic. The results were analyzed with an analysis of variance procedure followed by Duncan's Multiple Range Test (SAS Institute, Cary, NC)

4.0 Results and Discussion

4.1 Objectives

The objectives of this study were

1. To determine the effect of variation in the rate and timing of nitrogen fertilizer application to the wheat on protein quantity of Coker 916 grain and experimentally milled flour from 1986 and 1987 growing seasons.

2. To separate wheat gluten proteins, gliadin and glutenin, from experimentally milled flour and subsequently separate the components of gliadin and glutenin using SDS-PAGE. The relative amounts present of protein components in each of the experimentally milled flours will be quantified with densitometer scans.

3. To evaluate the rheological and bread baking characteristics of experimentally milled flours.

4.2 Protein Content of Wheat and Flour

4.2.1 Preliminary Protein Determinations - 1986 Wheat.

Preliminary determinations of the percent protein in the 1986 wheat were done with a Trebor-90 Grain Tester. The objective of these preliminary determinations was to provide an accurate estimate of the percent protein resulting from the various nitrogen management regimens.

Of the two locations being used to grow wheat for this experiment, the wheat from the Charles City County location was harvested two weeks before the Giles County wheat. Wheat from thirteen of the twenty nitrogen management regimens used by the Agronomy Department was tested for protein content with the Trebor-90. This selection was made to represent the range of nitrogen treatment regimens and nitrogen content of the wheat.

The mean protein values for the wheat from the 1986 growing season as measured with the Trebor-90 are shown in Table 6. Standard deviations for replicates of identical nitrogen treatment were found to be less than 0.5.

Wheat from all replicates receiving identical nitrogen treatments was then pooled. Pooling of wheat for further study provided a much larger sample for further testing.

Nitrogen management regimens were selected for further study based upon the Trebor-90 results and efforts to select a range of nitrogen management regimens, including treatment pairs, which would be appropriate for production. Nitrogen treatments of 250 and 300 kg/ha total application were not selected since these heavy treatments could pose a problem with lodging of the grain.

4.2.2 Protein Content and Extraction of Wheat and Flour

The protein data for the wheat and flour from the 1986 and 1987 growing seasons are shown in Table 7. For the 1986 growing season, the protein content of the wheat was 1.76 to 2.06 percent greater than the protein content of the flour, as determined by the Kjeldahl method. The flour extraction rate during milling of the 1986 wheat averaged approximately 70%. For the 1987 growing season, the protein content of the wheat was 1.63 to 2.98 percent greater than the protein content of the flour. The flour extraction rate during milling averaged only 65%. The variation in rate and timing of nitrogen application to the wheat did not influence protein loss with milling in either growing season.

Table 6. Preliminary grain protein determinations (1986 wheat) of selected treatments

nitrogen treatment, kg/ha Growth Stage 25	Growth Stage 30	Grain Percent Protein (mean)
0	0	11.1
68	0	11.3
168	0	12.7
0	68	11.5
0	168	13.5
28	56	11.3
28	168	13.2
56	56	11.8
56	168	13.2
112	56	12.1
112	168	13.5
168	56	13.2
168	168	13.7

Table 7. Protein content of wheat grain and flour from two growing seasons

Nitrogen treatment, kg/ha growth stage 25	30	Wheat % protein (14% M.B.) 1986	Wheat % protein (14% M.B.) 1987
0	0	8.71[a]	8.32[d]
168	0	11.83[b]	13.41[e]
0	168	13.41[c]	13.31[e]
56	168	13.31[c]	14.15[f]
168	56	13.19[c]	13.87[f]
		Flour % protein (14% M.B.) 1986	**Flour % protein (14% M.B.) 1987**
0	0	6.95[a]	6.69[d]
168	0	9.91[b]	10.43[e]
0	168	11.37[c]	11.07[e]
56	168	11.25[c]	11.52[e]
168	56	11.42[c]	11.52[e]

(protein values followed by the same letter in each row are not significantly different when tested by Duncan's multiple range test at $\alpha = .05$)

The differences seen in the extraction rate and protein loss associated with milling of the 1986 and 1987 wheat may be due to poor separation of the endosperm from the bran during milling of the 1987 wheat. The bran is composed of all of the outer structures of the kernel, including the aleurone layer. Though the aleurone layer is botanically considered to be the outer layer of the endosperm, the miller considers the aleurone to be part of the bran (MacMasters, Hinton and Bradbury, 1971). The composition of the starchy endosperm varies from the outer portion, which is located just below the aleurone layer, to the inner portion. There is a decrease in protein content from the outer portion of the endosperm to the interior. Ranges in protein content within the endosperm have been found to be as high as 6:1 (MacMasters et al, 1971). Therefore, a low extraction rate, resulting from poor separation of the endosperm from the bran, could be related to the lower flour protein content found in the 1987 wheat.

The protein content of the flour milled from Coker 916 wheat grown as the control (no spring nitrogen application to the wheat in growth stages 25 or 30) in both the 1986 and 1987 growing seasons was similar to the traditional protein level associated with soft wheat flour (Table 7). The protein content of the wheat and flour from the 1986 growing season showed a significant increase (indicated by different superscripts, $\alpha = .05$) with nitrogen treatments of 168 + 0 and 0 + 168 kg/ha in growth stages 25 and 30.

Nitrogen application to the grain has been shown in the literature to significantly increase protein content (Dubetz 1979, Finney et al 1957, McNeal et al 1971). The addition of 168 kg/ha of nitrogen at growth stage 25 resulted in a 43% increase in flour protein content compared to the control. When 168 kg/ha of nitrogen were applied to the wheat at growth stage 30, a 64% increase in flour protein content was seen over the control (Table 7). Later application of fertilizer nitrogen resulted in a 21% greater increase in flour protein content. No further significant increase in grain or flour protein content was seen with the additional nitrogen application of 56 kg/ha in either growth stage 25 or 30 resulting in a total split dose application of 224 kg/ha. This levelling off of grain nitrogen response with increasing nitrogen application agrees with reports of Benzian and Lane (1980) and Dubetz et al (1979).

The protein content of the wheat from the 1987 growing season showed a significant increase over the control (0 + 0 kg/ha) with the nitrogen application of 168 kg/ha, which supported the 1986 data. There was no significant difference in the protein content of the wheat when the timing of the nitrogen application was varied from a single application in growth stage 25 or growth stage 30. As discussed earlier, the variation in timing of the application of 168 kg/ha resulted in a significant difference in protein content in 1986. The additional nitrogen application of 56 kg/ha in growth stage 25 or 30, resulting in a split dose application, significantly increased grain protein content. This increase was not seen in 1986. As with the single

dose application, there was no significant difference in the protein content of the grain due to variation in the timing of nitrogen application, from a 1:3 or 3:1 ratio in growth stages 25 and 30.

Variation in nitrogen management during the 1987 growing season had a significant effect on flour protein content. Though significant differences in the wheat protein content were associated with each level of nitrogen application tested, the only statistically significant difference in the flour protein content was between the control and the 4 nitrogen management regimens tested (Table 7). No significant differences were found among the flour protein contents of the different nitrogen management regimens. This may be associated with the low flour extraction rate and high protein loss associated with milling, as previously discussed.

The trend of increasing protein content with increasing nitrogen application has been established in the literature. Kosmolak and Crowle (1980) have found increasing grain protein content with increasing nitrogen fertilization up to 224 kg/ha in a single application. For the Coker 916 wheat grown for this study, the first level of nitrogen fertilization (168 kg/ha) was applied in a single dose in growth stage 25 or 30, though the application of 224 kg/ha N was done in a split dose. Nitrogen was applied to the grain in a 1:3 or 3:1 ratio in growth stages 25 and 30.

Conflicting evidence was reported in the literature as to the effect of split dose nitrogen application on grain protein. Split dose nitrogen application was found to incorporate 10 to 25% more nitrogen into the grain than single dose nitrogen application by Paredes-Lopez et al (1985). Other investigations indicate no significant difference in grain protein content with split dose nitrogen application versus single dose application (Anon., 1976; Nuttall, 1979)). Protein content of the grain was reduced when nitrogen was applied in a split dose, compared to a single dose application.

The increasing rate of nitrogen application in this study coincided with a change in the timing of nitrogen application from a single dose to a split dose. Thus, from the treatments selected for this study, it cannot be stated that the grain protein trends seen are due solely to increasing rate or variation in timing of nitrogen application.

4.2.3 Effect of Environmental Conditions - 1986 Wheat

Unusually hot and dry environmental conditions in Giles County during the latter part of the 1986 growing season had a significant influence on the grain and flour (Table 8). High temperatures and low relative humidity, especially during the last fifteen days before harvest, can result in subnormal bread baking properties (Finney and Yamazaki, 1967).

Table 8. Effect of 1986 environmental conditions and location on protein and milling

	N Treatment,kg/ha Growth Stage		Location	
	25	30	Giles County	Charles City County
Wheat %protein, 14% M.B.	0	0	10.34	8.71
Flour %protein, 14% M.B.			9.58	6.95
Flour %moisture			14.70	15.30
Extraction %			62.35	68.29
Wheat %protein, 14% M.B.	56	56	13.06	10.13
Flour %protein, 14% M.B.			11.05	8.46
Flour %moisture			13.70	14.60
Extraction %			57.0	73.03

The Giles County wheat had a higher protein content than the wheat grown with identical nitrogen treatments in Charles City County (Table 8). The flour milled from the Giles County wheat was found to have a lower moisture content than the flour milled from the Charles City County wheat. According to Zeleny (1971), the moisture content of wheat is inversely related to the amount of dry matter.

Dry wheat can also result in milling difficulties (Zelany, 1971). The extraction rate of the Giles County wheat was much lower than the extraction rate of the Charles City County wheat (Table 8). This influences baking properties since there is lower recovery of the endosperm proteins which are critical for gluten formation. A low extraction rate is also of economic significance. It was the opinion of the miller at the Bartlett Milling Company in Statesville, NC, that the Giles County wheat was so hard that over time it would damage the mill rollers. Since the Giles County wheat could not be considered representative of Coker 916 wheat, subsequent investigations of the 1986 growing season were based upon the Charles City County wheat for the 1986 growing season.

4.3 Dough Rheological Properties

Dough rheological properties were assessed with the farinograph and the alveograph. For the 1986 wheat, dough rheological properties of the control flour (0 + 0 kg/ha N in growth stages 25 and 30) and the flour 168 + 0 and 0 + 168 kg/ha N were examined by farinograph testing (Table 9). Dough rheological properties of the flour resulting from the nitrogen application of 224 kg/ha in growth stages 25 and 30 in 1986 were evaluated with the alveograph (both instruments were not accessible to the researchers for all samples). For the 1987 flour, all nitrogen treatments were evaluated with the farinograph and the alveograph (Table 10).

Rheological tests indicated that a stronger, more extensible dough was associated with nitrogen fertilization, increased protein content and gluten complex within a cultivar for both the 1986 and 1987 flours. The gluten protein complex formed when wheat flour and water are mixed is responsible for the cohesive and viscoeleastic properties of dough. The proper balance of elasticity, extensibility and viscous flow of a dough are important in determining bread baking properties. The viscoelastic properties of dough are modified by mixing, kneading and fermentation (Finney and Yamazaki, 1967).

Table 9. Farinograph results for Coker 916 flour of two growing seasons

kg/ha N growth stage 25	30	Flour Protein 14% m.b. 1986	1987	Peak Time, min 1986	1987	Mixing Tolerance, Brabender units 1986	1987	Stability, min 1986	1987	Time at Breakdown min 1986	1987
0	0	6.95	6.69	1.0	.	100	90	0.8	.	1.5	.
168	0	9.91	10.43	1.2	.	70	72	4.0	.	7.0	.
0	168	11.37	11.07	2.0	.	40	70	6.5	.	11.0	.
56	168	11.25	11.52	.	.	.	62	.	.	.	.
168	56	11.42	11.52	.	.	.	59	.	.	.	.

'.' denotes data not available

A comparison of the farinograph results of the control flour (0 + 0 kg/ha N) and the flours of the nitrogen treatments of 168 + 0 and 0 + 168 kg/ha N in growth stages 25 and 30 indicated that the dough rheological properties of wheat receiving a nitrogen application of 168 kg/ha in growth stage 30 were most different from the control. With this nitrogen treatment, farinograph peak time, stability, and time to breakdown were all found to increase for the 1986 flour (Table 9). The amount of time which the dough was able to maintain a specific viscosity increased and the time it took the dough from the start of mixing until a specific drop in viscosity from the maximum also increased. These changes indicated that the dough had an increased mixing requirement to reach the point of maximum consistency before weakening. According to Finney and Yamazaki (1967), a medium to medium-long mixing requirement is required for an acceptable bread baking flour.

The grain nitrogen treatments of 168 + 0 and 0 + 168 kg/ha both resulted in a flour with a lowered farinograph mixing tolerance index compared to the control (0 + 0 kg/ha) for both the 1986 and 1987 flours.[5] Five minutes after maximum viscosity was attained, there was less of a reduction in viscosity. In 1986, the nitrogen treatment of 168 kg/ha in growth stage 30 resulted in a further drop in mixing tolerance index than the treatment of 168 kg/ha in growth stage 25. As previously discussed, in 1986 the nitrogen

[5] mixing tolerance indicates the reduction in dough viscosity five minutes after maximum viscosity was attained

treatment of 0 + 168 kg/ha was associated with a significant increase in grain and flour protein content over both the control (0 + 0 kg/ha N) and the nitrogen application of 168 kg/ha in growth stage 25. The farinograph changes indicated a stronger dough associated with nitrogen fertilization and increasing protein content within a cultivar.

In 1987, the farinograph mixing tolerance of the flour of the the 168 kg/ha N treatment in growth stage 30 was not substantially lower than the mixing tolerance index resulting from the application of 168 kg/ha N in growth stage 25, though both were 19 - 22% lower than the control. These two treatments were associated with flour and wheat protein contents which were not significantly different, though both were significantly higher than the control.

There was a further reduction in the farinograph mixing tolerance index with the nitrogen application of 224 kg/ha to the grain in 1987, indicative of less of a decrease in dough viscosity with continued mixing. When the nitrogen was applied in a 3:1 ratio in growth stages 25 and 30 the mixing tolerance index was lower than when the nitrogen was applied in a 1:3 ratio though the difference was not statistically significant ($\alpha = .05$). This level of nitrogen fertilization was not associated with an increased flour protein content, but was associated with a significantly increased wheat protein content. The high protein loss with milling was addressed earlier in this discussion.

The farinograph response curve for the 1986 nitrogen application of 0 + 168 kg/ha in growth stages 25 and 30 (Figure 3) is typical of a strong flour with bread baking qualities (Campbell, 1972). The farinograph curves for the 1986 control flour (0 + 0 kg/ha N) and the flour of the 168 + 0 kg/ha N treatments are typical of weak flour which would not be associated with good bread baking qualities. These flours reached their maximum viscosity quickly, and viscosity rapidly decreased. These doughs did not withstand continued mixing as well as the dough from the 0 + 168 kg/ha N treatment, and showed a larger drop in viscosity five minutes after maximum viscosity was attained.

Zeleny (1971) reported that a flour protein content of at least 11% was required by bakers for yeast leavened bread. In the example given from this study, the farinograph curve typical of bread flour was associated with a flour protein content of 11.37% (14% m.b.). The farinograph curves showing a smaller mixing requirement and rapid breakdown which would not be suitable for bread baking were associated with flour protein contents of 6.95% and 9.91% (14% m.b.). According to Zeleny (1971), based upon protein content alone these flours would be better suited for biscuits, cakes, pie crusts and cookies. These are products which do not require as much gluten development as yeast bread for structure and leavening.

Mixing requirement, mixing tolerance, and dough handling characteristics are all related to protein content within a variety (Finney and Yamazaki,

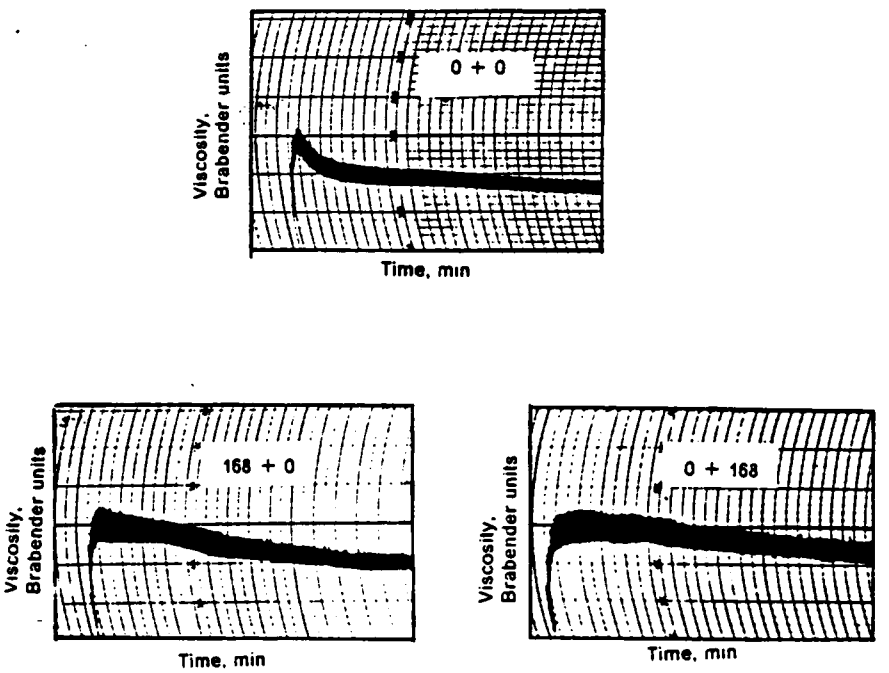


Figure 3. Farinograph curves of Coker 916 flour: 1986 growing season

1967). McNeal (1987) and Kosmolak and Crowle (1980) found that nitrogen fertilization and increasing protein content within a cultivar strengthened dough properties. McNeal (1987) reported increased farinograph peak time, which supports the findings of this study. Weipart and Pomeranz (1986) reported that dough consistency was affected by the protein content of the flour.

Nitrogen fertilization and increased protein content resulted in a stronger dough, more resistant to expansion with increased extensibility in the 1987 flour evaluated with the alveograph. A dough that is more viscoelastic can better expand and trap more gas for leavening. This is an important factor in loaf volume, since the degree of expansion allowed by gluten membranes determines loaf volume (Khan and Bushuk, 1979).

Alveograph overpressure, P, increased with each level of increasing nitrogen application in 1987 indicating increased resistance to deformation (Table 10). In 1987 variation in the rate of nitrogen application had more of an influence on dough tenacity than did variation in the timing of nitrogen application. The deformation energy (W) also increased with increased flour protein content, indicating that more energy was required to inflate the dough until rupture.

For the 1986 flour, the influence of variation in the timing of nitrogen application of 224 kg/ha N in a 1:3 or 3:1 ratio on dough rheological properties

Table 10. Alveograph results for Coker 916 flour

kg/ha N growth stage 25	30	Flour % Protein 14% m.b. 1986	1987	Overpressure, P (mm) 1986	1987	Extensibility, L, (mm) 1986	1987
0	0	6.95	6.69	.	41.0	.	72.2
168	0	9.91	10.43	.	48.8	.	145.0
0	168	11.37	11.07	.	48.1	.	111.3
56	168	11.25	11.52	62.7	52.5	180.0	130.8
168	56	11.42	11.52	53.9	53.2	173.0	99.8

kg/ha N growth stage 25	30	Flour Protein 14% m.b. 1986	1987	P/L ratio 1986	1987	Deformation Energy, $Wx10^{-4}$ 1986	1987
0	0	6.95	6.69	.	0.573	.	128
168	0	9.91	10.43	.	0.341	.	190
0	168	11.37	11.07	.	0.433	.	189
56	168	11.25	11.52	0.348	0.434	320	191
168	56	11.42	11.52	0.312	0.484	270	167

'.' denotes data not available

was evaluated with the alveograph. Variation in timing of nitrogen application had more of an influence on dough tenacity in 1986 than in 1987. The application of 224 kg/ha N in a 1:3 ratio in growth stages 25 and 30 resulted in a dough which was more resistant to deformation than when a 1:3 ratio was used.

Branlard and Dardevet (1985a) found that bread wheats having strong gluten forming properties were characterized by alveograph deformation energy values greater than or equal to 350. In this study, the 1986 application of 224 kg/ha N in a 1:3 ratio resulted in a dough deformation energy much closer to 350 than the application of 224 kg/ha N in a 3:1 ratio (320 compared to 270).

The extensibility of the dough increased with increased protein content due to nitrogen fertilization in 1987, as indicated by higher values for alveograph extensibility (L). Variation in the timing of nitrogen application had an influence on dough extensibility. For each of the treatment pairs evaluated (0 + 168/168 + 0 and 56 + 168/168 + 56) there was a difference of approximately 30% in alveograph extensibility within each pair. These treatment pairs were not associated with significant differences in flour protein content within each pair.

Dough strength, extensibility and mixing tolerance were found to improve with nitrogen fertilization in 1986 and 1987. In 1986 later application of

fertilizer nitrogen to Coker 916 wheat in this study resulted in dough characteristics which are typical of bread flours. In 1987 variation in timing of nitrogen application did not substantiate this trend.

Paredes-Lopez et al (1985) found that increasing levels of nitrogen fertilization increased dough elasticity, as evaluated by alveograph testing. Deformation energy, W, was also found to increase with increasing protein content associated with nitrogen fertilization. This is in agreement with the findings reported in this study.

Some of the differences in rheological properties seen with alveograph testing could not be attributed to differences in total protein content in this study. Paredes-Lopez et al (1985) found that nitrogen fertilization not only produced changes in the quantity of wheat protein, but also influenced protein quality. Functional changes in dough were postulated to be due to changes in molecular composition of the gluten. Changes in the composition of gluten must be examined in this study, since the change in functional properties was not always associated with a difference in protein quantity.

4.4 Bread Baking

Test baking, using standardized procedures, is the only way to determine and compare the baking properties of flours (Pratt, 1971). Ford (1987)

stated that test baking is the ultimate test of flour protein quality. Test baking can evaluate the effect of different treatments on flour performance (Finney and Yamazaki, 1967). In this study test bread baking was done following a modification of the Basic Straight Dough Method of the AACC (Method 10-10A, 1983).

Differences in dough handling properties were observed by the test bakers for the different flours from both years. Dough handling problems were encountered with the dough produced from the lower protein control flour from both years. This dough was very sticky after mixing and had to be scraped out of the mixing bowl, whereas other doughs were easier to handle (less sticky) and formed a more cohesive mass. After the dough from the control flour was proofed in the fermentation cabinet or chamber, handling characteristics improved and stickiness was reduced. Of the 1986 flours, the dough resulting from the grain nitrogen treatment of 0 + 168 kg/ha in growth stages 25 and 30 was judged the easiest to handle by the test bakers.

Weipart and Pomeranz reported that dough consistency was affected by the protein content of the flour. Dough handling characteristics were also related to protein content by Finney and Yamazaki (1967). Ford (1987) stated that sticky dough and handling problems can be due to high alpha amylase levels, resulting in excessive breakdown of starch to sugars and dextrins. Starch damage was also associated with the water holding capacity of flour

allowing the starch granules to hold water while still cold (Pomeranz,et al, 1984; Ford, 1987). Soft wheat flour is generally associated with minimal starch damage (Pomeranz et al, 1984; Ford, 1987). Higher protein hard wheats are associated with a higher degree of starch damage. Further research is needed to determine if increasing protein content with nitrogen fertilization resulted in an increase in starch damage, thus improving the water holding capacity and loaf volume potential of the flour.

4.4.1 Crust Color

Crust color was found to be significantly affected by variation in grain nitrogen management when tested objectively on the Hunter Color Difference Meter (Table 11). This trend was seen in bread produced from both 1986 and 1987 flours. A significant difference was found when contrasting the crust color of the control (0 + 0 kg/ha N) treatment to the crust color of the other treatments in both years tested.

Crust color was reported in the literature to be related to flour protein content (Smak, 1972). Browning of bread crust is due in part to Maillard (carbonyl-amine) browning (Bertram, 1953). The protein content of the flour from the control grain nitrogen treatment in both years was significantly lower than the flour protein resulting from other four nitrogen treatments tested.

Table 11. Physical characteristics of test loaves

Treatment, kg/ha N		Flour Protein		Characteristic (treatment means) Moisture,		Color		Volume,	
growth stage		14% m.b.		%				cc	
25	30	1986	1987	1986	1987	1986	1987	1986	1987
0	0	5.95	6.69	39.06	.	55.10	51.91	973	670
168	0	9.91	10.43	38.24	.	45.23	43.18	1052	775
0	168	11.37	11.07	38.49	.	43.86	37.96	1073	793
56	168	11.25	11.52	38.74	.	41.74	38.39	1079	805
168	56	11.42	11.52	38.56	.	41.00	35.77	1088	840
Treatment effect PR>F				0.11	.	<.01*	<.01*	<.01*	.07*
Standard error				0.21	.	1.77	1.71	13.88	13.46
Orthogonal Contrast PR>F									
168	0 vs.			.42	.	.59	.20	.36	.73
0	168								
56	168 vs.			.56	.	.77	.33	.65	.50
168	56								
0	0 vs.			.03*	.	<.01*	<.01*	<.01*	<.01*
all others									

'*' indicates statistical significance, $\alpha = .1$
'.' denotes data not available

In this study the crust color which was significantly different in both years resulted from flours with protein contents below 7%. This was seen in the higher Hunter Color Difference Meter value for delta E. A lower protein content resulted in a lighter crust color with an uneven appearance (Figure 4). Protein content of the flour and color measurement were found to have a correlation coefficient of (-)0.98 in 1986 and (-)0.91 in 1987 (Table 12). This was supported in the literature by Smak (1972). Smak (1972) found a protein content of 10.5% was necessary to produce a crust with a normal brown color. Flours with a protein content below 8.5% protein were found to produce a loaf with a light gray tinted crust. Between 8.5% to 10.5% protein, results varied as to brownness of the crust.

4.4.2 Loaf Volume

Variation in nitrogen management and protein content of Coker 916 wheat in this study had a significant effect on loaf volume of both 1986 and 1987 flours. Loaf volume was found to increase with increasing protein content due to nitrogen fertilization in both years tested (Table 11). In 1987, significant variation was seen in the treatment replicates ($PR > F < 0.01$). Orthogonal contrasts showed that the loaf volume resulting from the control (0 + 0 kg/ha N in growth stages 25 and 30) flour treatment was significantly different when contrasted with the four other treatments tested in both years of this study. The protein content of the flour from the control grain nitrogen

Table 12. Correlation coefficients for flour protein with selected bread measurements

Measurement	Correlation coefficient (r) 1986	1987
Moisture	(-) 0.608	.
Loaf volume	0.99	0.95
Crust color (objective)	(-) 0.98	(-)0.91
Crust color (sensory)	0.994	.

'.' denotes data not available

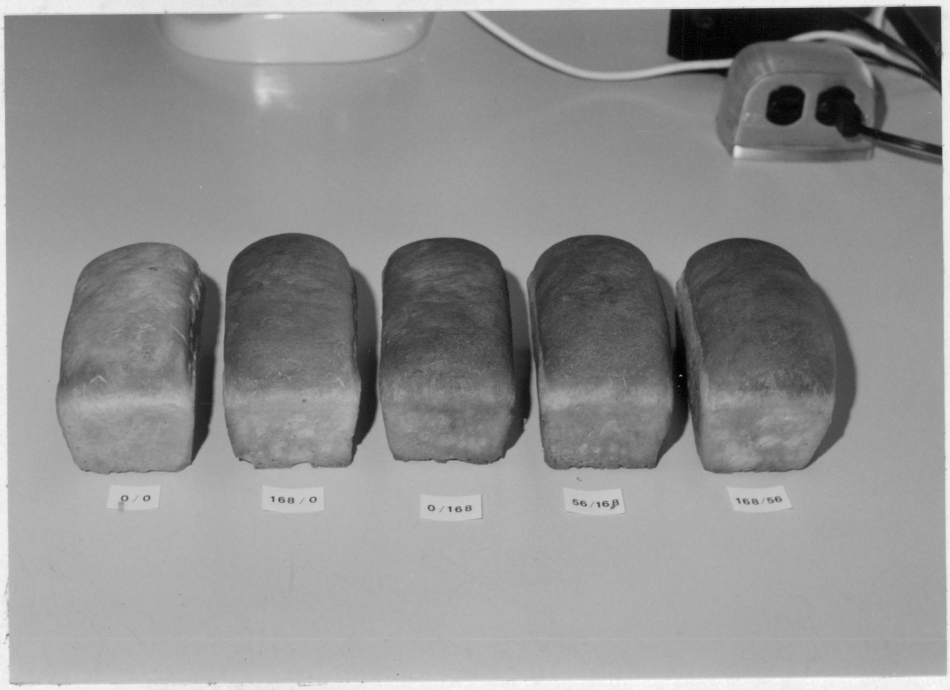


Figure 4. Experimental loaves produced from Coker 916: 1987 growing season

treatment in both years was significantly lower than the flour protein resulting from the various nitrogen treatments tested. A correlation coefficient of 0.99 was found between 1986 flour protein and loaf volume, and for 1987 the correlation coefficient was 0.95 (Table 12).

Nitrogen fertilization of the grain and increased flour protein content have been reported in the literature to be directly related to loaf volume (Doekes and Wennekes, 1982; Hoseney et al, 1969; Paredes-Lopez et al, 1985). Nitrogen fertilization was found to improve loaf volume in soft and semi-hard wheats investigated by Paredes-Lopez et al (1985). Kosmolak and Crowle (1980) also found loaf volume to increase significantly with increasing flour protein content within a cultivar due to nitrogen fertilization of the grain.

The association of loaf volume and flour protein content is related to the functional properties of the gluten protein complex. Wheat flour proteins interact with each other and with other flour components when mixed with water to form gluten. A three dimensional network of thin gluten membranes with embedded starch granules is formed during mixing (Finney and Yamazaki, 1967). The three dimensional network formed is important for leavening and loaf volume. The gluten membranes expand while entrapping some of the carbon dioxide produced by yeast fermentation. With excessive mixing the gluten membranes collapse, resulting in a smaller loaf volume. Loaf volume is determined by the degree of expansion allowed by the gluten membranes (Khan and Bushuk, 1979).

4.4.3 Loaf Crumb Moisture

The moisture content of the bread baked from the 1986 control nitrogen treatment (0 + 0 kg/ha in growth stages 25 and 30) was significantly different when contrasted with crumb moisture of the four other treatments tested (Table 11). Differences among the other four treatments were not significant. The moisture content of the bread produced from the control flour was higher than the other four treatments. All breads were prepared with identical quantities of liquids. The protein content of the control flour was significantly lower than the other treatments. A correlation coefficient of (-)0.608 was found for flour protein and bread crumb moisture (Table 12).

The higher moisture content of the bread crumb produced with the control flour may be related to the effect of protein content on loaf volume. As previously discussed, variation in nitrogen management and increased flour protein content had a significant effect on loaf volume in this study. The lower protein control flour produced a significantly smaller loaf when contrasted to the loaf volumes resulting from the other four nitrogen treatments tested. The small loaf volume resulted in a smaller surface area being exposed during baking. This could reduce evaporative loss, explaining the higher moisture content associated with the small loaf resulting from lower protein control flour.

The difference seen in moisture content may also be due to the water absorbing capacity of the flour. Within a variety of wheat, water absorption is directly related to protein content (Finney and Yamazaki, 1967). The lower protein flour would have the ability to bind less constitutional and interfacial water, though have more water present as bulk phase water. Constitutional and interfacial water is closely associated with the protein molecule, and has different characteristics than bulk phase water (Fennema, 1977).

4.5 Sensory Evaluation

Valid and reproducible evaluation of the sensory attributes of a product is a critical step in foods research. This research used a modification of Q.D.A. (Stone et al, 1974) to describe the sensory attributes of pup loaves baked from 1986 experimentally milled flour. During a language development process, the sensory panelists decided upon nine attributes to describe the important sensory properties of bread (Figure 2). Treatment means, standard errors and orthogonal contrasts are presented in Table 13. Q.D.A. results are presented graphically in Figure 5. Increasing distance of an attribute from the origin represented increasing attribute intensity (crust taste goes from bitter to not bitter).

Table 13. Sensory properties of Coker 916 bread

Treatment kg N/ha		Sensory property [1]								
Growth stage		Crust color	Crust texture	Crust taste	Aroma	Cell structure	Crumb texture	Gummi-ness	Chew-iness	Cohesiveness
25	30									
						Attribute means				
0	0	3.91	6.03	9.30	7.38	6.29	5.30	6.46	6.09	11.99
168	0	7.73	8.43	7.48	6.26	6.38	5.03	6.19	6.99	12.39
0	168	9.93	7.75	6.56	6.27	6.92	4.87	6.69	7.40	11.96
56	168	8.60	6.82	7.97	6.28	6.82	4.47	6.27	6.64	12.09
168	56	8.94	7.62	7.14	6.69	6.85	4.42	5.80	6.78	11.76
Treatment effect PR > F										
		<.01 *	.01 *	.01 *	.31	.57	.04 *	.59	.08 *	.47
Standard error		0.28	0.35	0.46	0.43	0.35	0.26	0.37	0.40	0.10
Orthogonal contrasts PR>F										
168	0 / 0	<.01 *	.36	.36	.68	.18	.96	.29	.56	.30
56	168 / 168	.60	.08 *	,10 *	.69	.91	.85	.51	.50	.22
0	0 / others	<.01 *	<.01 *	<.01 *	.04 *	.41	.01 *	.74	.02 *	.50

* indicates statistical significance, $\alpha = .10$

[1]- Sensory properties evaluated on unstructured line scales from 0 to 15 cm as crust color (light to dark), crust texture (soft to hard), crust taste (bitter to not bitter), aroma (weak to strong), cell structure(small to large), crumb texture (soft to hard), gumminess (less to more; defined by sensory panel as tendency of bread to gum up and stick to roof of mouth), chewiness (less to more; defined by sensory panel as effort required to chew, resistance to teeth), cohesiveness (falls apart to holds shape; evaluated by sensory panel while spreading margarine on bread sample).

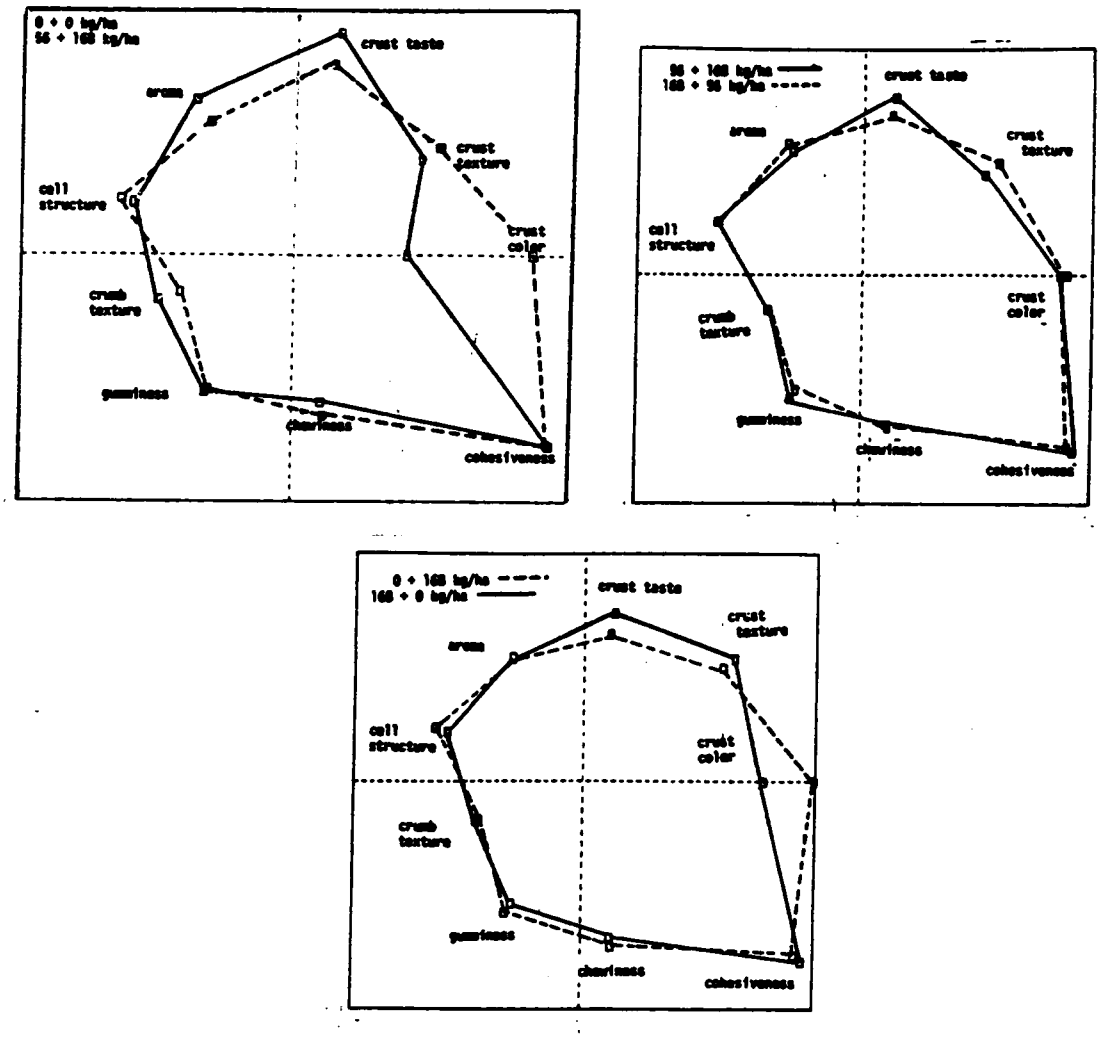


Figure 5. Descriptive Analysis Diagrams of Coker 916 Bread

Variation in grain nitrogen management had a significant affect on all three of the crust attributes which were evaluated by the sensory panel (color, texture and taste). Significant differences in crust color were detected due to variation in timing of the nitrogen application of 168 kg/ha. The sensory panelists judged the bread crust color from the flour treatment of 168 kg/ha N in growth stage 30 to have a darker crust than bread from the flour treatment of 168 kg/ha N applied in growth stage 25. The crust color of the bread from the control flour (treatment 0 + 0 kg/ha N) was also significantly different when contrasted to the other four treatments. The control flour produced bread with a light crust and an uneven mottled appearance. A correlation coefficient of 0.99 was obtained between flour protein and sensory color determination (Table 12). Sensory results supported the objective data obtained with the Hunter Color Difference Meter.

As previously discussed, crust color was related to flour protein content (Smak, 1972) and was attributed to Maillard browning (Bertram, 1953). The significant differences in crust color detected by the panelists were also associated with significant differences in flour protein content. Smak (1972) found that flours with a protein content below 8.5% produced a loaf with a gray tinted crust. A flour protein content of 10.5% was necessary to produce a crust with a normal brown color. Smak found the degree of browning to be an important variable in determining acceptability to consumers. The Coker 916 flours with low protein content (below 7%) produced a light crust with a gray tint (Figure 4).

Variation in grain nitrogen management had a significant effect on crust texture tested by the sensory panelists. With the split application of 224 kg/ha N in either a 1:3 or 3:1 ratio in growth stages 25 and 30, the sensory panelists evaluated the crust resulting from the 1:3 application as significantly softer.

Crust taste was also affected by flours of varied nitrogen management. The sensory panelists described the bread crust from the flour treatment of 224 kg/ha N in a 1:3 ratio as more bitter than when 224 kg/ha nitrogen was applied to the grain n a 3:1 ratio. The variation in timing of the application of 224 kg/ha was not associated with significant difference in flour protein content. A significant difference was also found by the sensory panelists when contrasting the crust taste of breads resulting from the control flour to the crust taste of the other four treatments. The bread of the control flour was characterized as more bitter than all of the other treatments except the bread from the grain application of 224 kg/ha N in a 1:3 ratio in growth stages 25 and 30.

Aroma of the bread from the control flour (0 + 0 kg/ha N growth stages 25 and 30) differed significantly when contrasted with the aroma of the other samples presented to the sensory panelists. Panelists evaluated this bread as producing a stronger aroma.

Bread crumb texture from the flour of the 0 + 0 kg/ha N treatment was significantly harder when contrasted to the other treatments. All nitrogen management regimens tested resulted in softer internal crumb texture compared to the control (0 + 0 kg/ha N) treatment. The control bread was associated with a lower flour protein content. According to Finney and Yamazaki (1967), at low protein levels bread crumb grain was heavy.

Gumminess (tendency of bread to gum up and stick to the roof of the mouth) and chewiness (effort required to chew) were terms which the sensory panelists felt were important in evaluating bread during the language development process. During the sensory sessions, many panelists commented on having difficulty evaluating these attributes, As an independent variable, 'sensory panelist' was statistically significant for both of these attributes. Variation in nitrogen management had a significant influence on bread chewiness. Sensory panelists found the bread of the control flour differed significantly in chewiness when contrasted to the other treatments. The bread of the control flour was judged less chewy. The difference in chewiness of bread from the 0 + 0 kg/ha N treatment supported the differences in crumb and crust texture found by the sensory panel. No significant treatment effect was found in evaluating gumminess.

Other characteristics evaluated related to cell structure and cohesiveness. Cell structure of the bread was not found to differ significantly among the five treatments evaluated by the sensory panelists. Cohesiveness, evalu-

ated by spreading margarine on the bread, was also not found to differ significantly among the five treatments evaluated.

4.6 Protein Fractionation and Characterization with SDS-PAGE

4.6.1 Protein Fractionation

Flour proteins of the 1986 wheat were fractionated into gliadin and glutenin components according to their solubility in 70% ethanol by a modified method of Doekes and Wennekes (1982). Osborne (1907) classified seed proteins into gliadin, the alcohol soluble fraction, and glutenin which was soluble in dilute acid or alkali.

The percent protein present in the gliadin and glutenin fractions of the 1986 wheat was determined by Kjeldahl analyses (N x 5.7) (Table 14). The glutenin fraction also contained the starch residue remaining after protein fractionation. This residue was protein free after fractionation except for the glutenin component (Doekes and Wennekes, 1982).

Variation in grain nitrogen management had a significant effect on total flour gliadin content of the 1986 wheat. The flour gliadin content resulting

from the control grain nitrogen treatment was significantly lower when contrasted to the flour gliadin contents resulting from the other grain nitrogen treatment regimens tested. Variation in grain nitrogen management did not have a significant effect on the percent of flour glutenin protein.

Increased gliadin content with nitrogen fertilization is supported in the literature. Dubetz et al (1979) found that nitrogen fertilization up to 200 kg/ha resulted in flour with shifts in the amino acid composition and proportions of protein fractions present, with an increased proportion of gliadin. Gliadins required the lowest energy expenditure by the plant to produce of the major endosperm proteins (Mitra et al, 1979) Doekes and Wennekes (1982) reported increased flour gliadin content with nitrogen fertilization and no significant effect on glutenin.

Variation in nitrogen management to Coker 916 grain in this study changed the flour gliadin to glutenin ratio (Table 14). The ratio decreased with the increased rate of grain nitrogen application tested due to a larger percentage increase in the glutenin fraction. Variation in timing of nitrogen application also influenced the gliadin to glutenin ratio. Later nitrogen application favored a reduced gliadin to glutenin ratio. When 168 kg/ha N was applied in growth stage 25 or 30, the application in growth stage 30 resulted in a lower ratio than when nitrogen application was in growth stage 25. With the split dose grain nitrogen application of 224 kg/ha in a 1:3 or 3:1 ratio in growth stages 25 and 30, the heavier application in growth stage

30 (1:3 ratio) resulted in a lowered flour gliadin to glutenin ratio than when the heavier grain nitrogen application was in growth stage 25 (3:1 ratio).

4.6.2 Gliadin Protein Fractions

The classic gliadin fraction of flour protein from the 1986 flour was fractionated based upon solubility in 70% ethanol according to a modified procedure of Doekes and Wennekes (1982). SDS-PAGE was run according to a modified procedure of Laemmli (1970) using a 5% to 10% linear gradient gel (Figure 6). Densitometer scans were used to quantify relative amounts present in different gel bands (Figure 7).

SDS-PAGE and densitometer scans revealed that approximately 30 different components were present in the gliadin fraction of Coker 916 flour. Branlard and Dardevet (1985a) detected between 20 to 28 gliadin bands in each of 70 cultivars studied. Shewry et al (1986) estimated there are approximately 50 gliadin components within a cultivar. Mecham et al (1976) identified up to 46 gliadin components within a cultivar using a combination of electrophoresis and isoelectric focusing.

Eight gliadin bands consistently present in SDS-PAGE gels with similar densitometer retention times were selected for comparison. Estimates of the molecular weight range of gliadin components present as determined

Table 14. Percent protein in gliadin and glutenin protein fractions

Nitrogen treatment, kg/ha		Protein fraction		
Growth stage		Gliadin	Glutenin [1]	Gliadin to
25	30	% protein (mean)	% protein (mean)	glutenin ratio
0	0	72.95	5.97	12.22
168	0	82.22	7.37	11.15
0	168	81.52	7.53	10.82
56	168	85.47	9.33	9.16
168	56	83.52	8.67	9.63
Treatment effect PR>F				
		.01*	.48	
Orthogonal contrast PR>F				
168	0 vs.	.73	.93	
0	168			
56	168 vs.	.36	.73	
168	56			
0	0 vs.	<.01*	.18	
others				

* indicates statistical significance, $\alpha = .05$
[1] - glutenin with starch residue

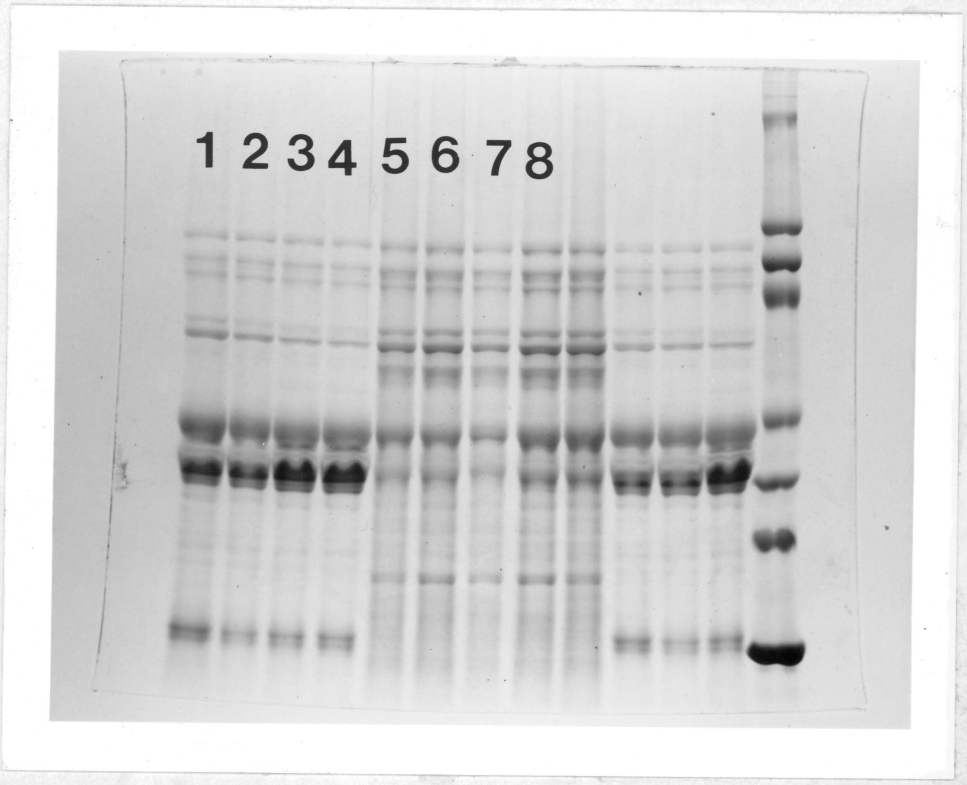
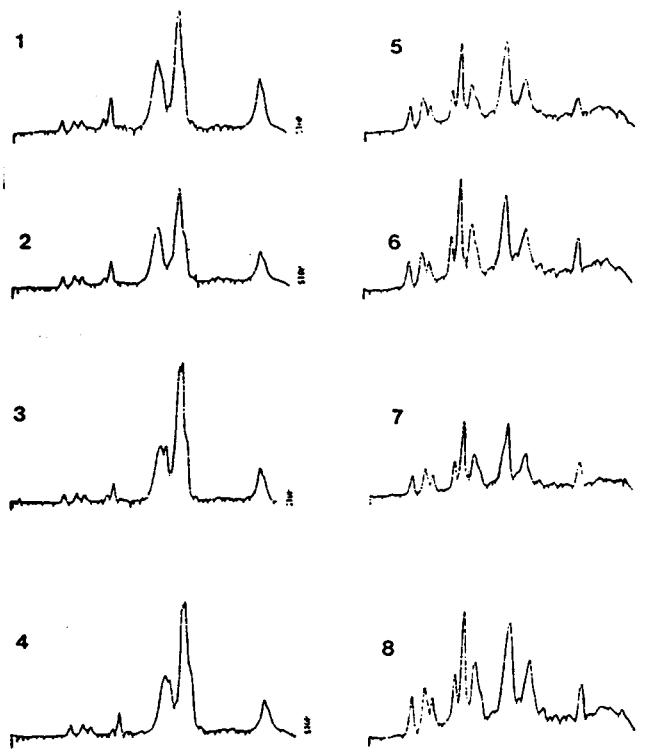


Figure 6. SDS-PAGE of Coker 916 gliadin and glutenin



1-gliadin, 0 + 0 kg/ha nitrogen treatment
2-gliadin, 168 + 0 kg/ha nitrogen treatment
3-gliadin, 56 + 168 kg/ha nitrogen treatment
4-gliadin, 168 + 56 kg/ha nitrogen treatment
5-glutelin, 0 + 0 kg/ha nitrogen treatment
6-glutelin, 168 + 0 kg/ha nitrogen treatment
7-glutelin, 56 + 168 kg/ha nitrogen treatment
8-glutelin, 168 + 56 kg/ha nitrogen treatment

Figure 7. Densitometer scans of Coker 916 SDS-PAGE

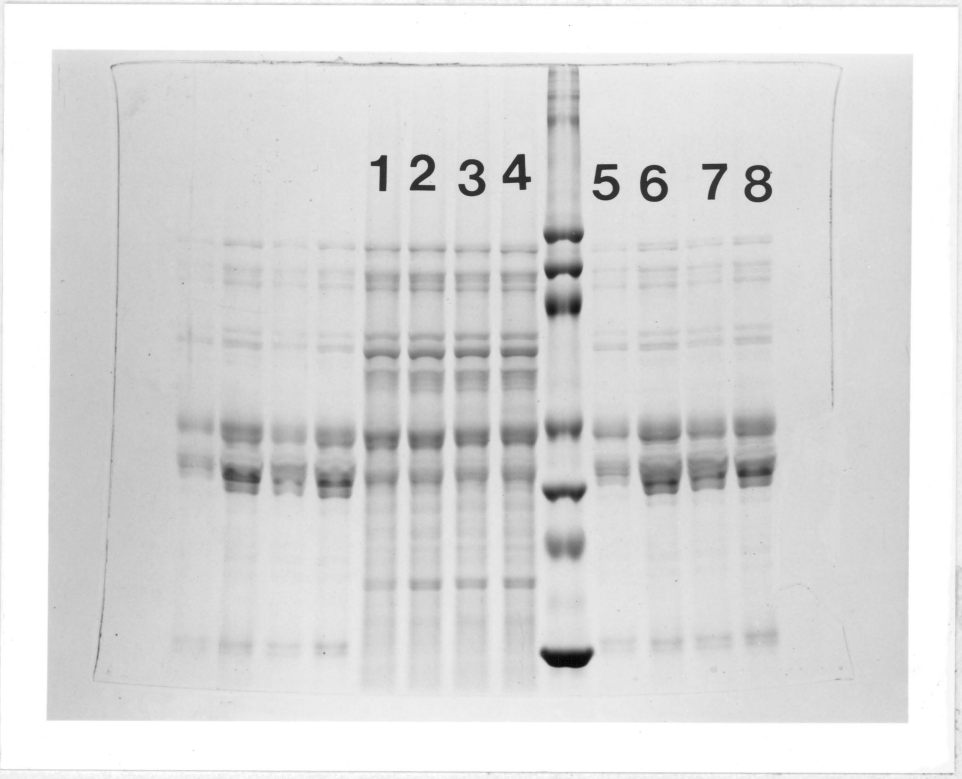
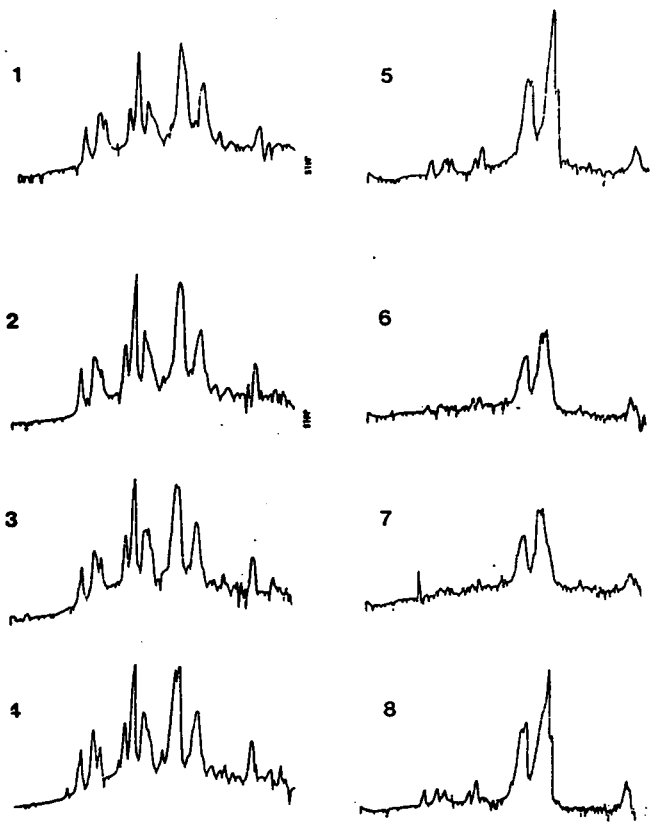


Figure 8. SDS-PAGE of Coker 916 gliadin and glutenin



1-glutelin, 0 + 0 kg/ha nitrogen treatment
2-glutelin, 0 + 168 kg/ha nitrogen treatment
3-glutelin, 56 + 168 kg/ha nitrogen treatment
4-glutelin, 168 + 56 kg/ha nitrogen treatment
5-gliadin, 0 + 0 kg/ha nitrogen treatment
6-gliadin, 0 + 168 kg/ha nitrogen treatment
7-gliadin, 56 + 168 kg/ha nitrogen treatment
8-gliadin, 168 + 56 kg/ha nitrogen treatment

Figure 9. Densitometer scans of Coker 916 SDS-PAGE

by SDS-PAGE were 14,000 to 106,000 daltons (Table 15). The molecular weights were estimated based upon relative mobility compared to standard reference marker proteins (Appendix F). The majority of components were found to have a molecular weight of 34,000 to 44,000 daltons.

The molecular weight range and predominance of gliadin components found in this study is in close agreement with reports in the literature. Estimates of the molecular weight range of gliadin components in the literature are from 12,000 to over 100,000 daltons (Khan and Bushuk, 1979; Tatham and Shewry, 1985; Bunce et al, 1985; Beckwith et al, 1966). The majority of components were reported to have a molecular weight of approximately 36,000 to 44,000 daltons (Khan and Bushuk, 1979).

Gliadin was divided into four groups, α, β, γ, and ω based upon electrophoretic mobility (Shewry et al, 1986). A different classification into two groups was proposed by Tatham and Shewry (1985). the sulfur poor ω-gliadins and the sulfur rich α, β, and γ gliadins. The sulfur poor ω-gliadins had a molecular weight range of 44,000 to 72,000 daltons and the sulfur rich gliadins had a molecular weight range of 32,000 to 42,000 daltons. Other researchers have divided gliadin into three groups with varying molecular weight (Beckwith et al, 1966; Bietz and Wall, 1972). The highest molecular weight fraction exceeded 100,000 daltons, followed by a small fraction with a molecular weight range of 60,000 to 80,000 daltons. This small fraction was the ω-gliadins. The major fraction had a molecular

Table 15. Molecular weights of Coker 916 gliadin protein fractions

Molecular Weight, daltons
106,000
95,000
86,000
68,000
64,000
44,000[a]
34,000
14,400

Superscript denotes significant variation found in the relative quantities of this gliadin fraction among different nitrogen management regimens when tested by Duncan's multiple range test, $\alpha = .05$.

weight range of 30,000 to 40,000 daltons and consisted of the α, β, and γ gliadins.

The gliadin fraction of Coker 916 in this study was found to have a high molecular weight component of 106,000 daltons. As discussed earlier, this was supported in the literature by Beckwith et al (1966) and Bietz and Wall (1972). Based upon literature reports cited, the gliadin components of Coker 916 with molecular weights ranging from 64,000 to 94,000 were labelled sulfur poor ω-gliadins. The gliadin components with the molecular weight range of 14,000 to 44,000 daltons were labelled the sulfur rich &aplha., β, and γ gliadins.

Variation in nitrogen management to the wheat made a significant difference in the relative amounts present of the ω-gliadin component with a molecular weight of 44,000 daltons in Coker 916. The application to the wheat of 224 kg/ha N in a 1:3 ratio in growth stages 25 and 30 resulted in the greatest relative amount present of this 44,000 dalton gliadin component. The total flour protein content resulting from this treatment was not significantly different from the flour resulting from two of the other nitrogen management regimens. When 224 kg/ha N was applied in a 3:1 ratio, the 44,000 dalton ω-gliadin band was present in the lowest relative amount of the five nitrogen treatments.

Gliadin is responsible for viscosity and extensibility of dough (Kasarda et al, 1971). A comparison of the alveograph results of the dough indicated that the flours from the 168 kg/ha N treatment pair were more extensible than the 224 kg/ha treatment pair. This is in agreement with the higher gliadin to glutenin ratio for the 168 kg/ha treatment pair. A comparison of the alveograph results of the dough from the flours treated with 224 kg/ha N (3:1 and 1:3 in growth stages 25 and 30) indicated the nitrogen application of 224 kg/ha in a 1:3 ratio resulted in a more extensible dough (determined by increased alveogram length, Table 10) than application in a 3:1 ratio. The extensibility characteristic was determined by increased alveogram length (Table 10).

The ω-gliadins were characterized by Shewry et al (1986) as different from the gliadin components in amino acid composition and molecular weight. The ω-gliadins contained large proportions of glutamine, proline and phenylalanine and were low in sulfur containing amino acids. Tatham and Shewry (1985) found the secondary structure of the ω-gliadins to be rich in β-turns with no α-helix or β-sheet conformations. This structure would contribute to dough extensibility.

4.6.3 Glutenin Protein Subunits

The glutenin fraction of flour protein from the 1986 flour was fractionated based upon solubility in 70% ethanol according to a modified procedure of Doekes and Wennekes (1982). SDS-PAGE was run according to a modified procedure of Laemmli (1970) using a 5% to 10% linear gradient gel. Densitometer scans were used to quantify relative amounts present in different gel bands.

SDS-PAGE and densitometer scans revealed that approximately 25 different components were present in the glutenin fraction of Coker 916 flour. Nine bands consistently present with similar retention times were compared (Table 16). These had a molecular weight range of 19,500 to 108,000 daltons. Bietz and Wall (1972) separated the reduced subunits of glutenin into at least 15 different subunits with a molecular weight range of 11,000 to 133,000 daltons. These subunits were divided into two groups of bands, the high molecular weight and the low molecular weight subunits (Shewry et al, 1986). The high molecular weight subunits had molecular weights of approximately 134,000, 132,000 and 90,000 daltons (Belitz et al, 1986). The low molecular weight glutenins had a molecular weight range of 12,000 to 68,000 by SDS-PAGE (Shewry et al, 1986).

Based upon literature reports, the glutenin subunits of Coker 916 in this study with with molecular weights of 108,000, 91,000 and 88,000 daltons

Table 16. Molecular weights of Coker 916 glutenin protein subunits

Molecular Weight, daltons
108,000[a]
91,000[b]
88,000
67,000
62,000
52,000
40,500[c]
35,000
19,500

Superscripts denote significant variation found in relative quantities of these glutenin subunits among flour from different nitrogen management regimens when tested by Duncan's multiple range test, $\alpha = .05$.

were labelled high molecular weight glutenin subunits. The subunits with molecular weights of 67,000, 62,000, 52,000, 40,500, 35,000 and 19,500 daltons were labelled low molecular weight subunits.

Densitometer scans showed that there were significant differences in the relative amounts of two high molecular weight subunits in the flours of the different nitrogen treatments tested. These subunits had molecular weights of 108,000 and 91,000 daltons based upon relative electrophoretic mobility. Significant differences were also found in the relative amounts of one of the low molecular weight (40,500 daltons) glutenin subunits.

Belitz et al (1986) found the high molecular weight glutenin subunits had a high concentration of glycine and a lower concentration of proline, unlike gliadin. Connective tissue proteins such as collagen and elastin are also characterized by a high concentration of glycine, which contributes the elastic properties. Gluten elasticity was attributed to the glutenin fraction.

Nitrogen fertilization of the wheat and increased flour protein content were found in this study to result in changes in dough strength, mixing tolerance, extensibility and loaf volume. Nitrogen fertilization was also associated with significant changes in the high molecular weight glutenin subunits. The three largest glutenin subunits, with molecular weights of approximately 134,000, 132,000 and 90,000 daltons were shown by Belitz et al (1986) to be important for dough formation and stability during baking. Payne et al

(1987) attributed 47 to 60% of the variation in bread baking qualities (loaf volume and texture) of 84 wheat cultivars to variation in the high molecular weight glutenin subunits.

Variation in nitrogen management to Coker 916 grain influenced protein content and composition in this study. Differences were found in total flour protein content, the ratio of flour gluten proteins (gliadin and glutenin) and in their subunit compositions. These changes in protein resulted in differences in the dough rheological and bread baking qualities of the experimentally milled flour. Objective and sensory differences of the test loaves were associated with variation in grain nitrogen management.

5.0 Summary and Conclusions

Nitrogen application to Coker 916, a soft red winter wheat, increased grain and flour protein content in the two growing seasons involved in this study (1986 and 87). Flour protein increased from approximately 7%, the level traditionally associated with soft wheat flours, to over 11%. Unusually hot and dry growing conditions influenced protein content and milling characteristics of the grain in one growing season.

Of the flour gluten proteins gliadin and glutenin, the gliadin fraction increased with grain nitrogen fertilization. Variation in grain nitrogen management changed the flour gliadin to glutenin ratio. The gliadin to glutenin ratio was lowered with the increased rate of grain nitrogen fertilization tested. Timing of nitrogen application also influenced the gliadin to glutenin ratio; grain nitrogen application in growth stage 30 resulted in a lower ratio than application in growth stage 25.

The different grain nitrogen management regimens tested significantly influenced the relative amounts in the flour of two high molecular weight glutenin subunits (108,000 and 91,000 daltons) and one low molecular weight glutenin subunit (40,500 daltons). The relative ω-gliadin content (44,000 daltons) also varied in the flour from the different nitrogen management regimens.

Differences in dough properties and baking characteristics of the flour were associated with variation in grain nitrogen management. Dough rheological tests indicated that a stronger, more extensible dough with improved mixing tolerance was associated with nitrogen fertilization and increased protein content. Changes in the gliadin to glutenin ratio and their subunit compositions led to differences in gluten complex formation within a cultivar. An improvement in dough handling characteristics with increased flour protein content was noted by the test bakers. Increased flour protein content resulted in a larger bread loaf with a darker crust. Sensory evaluation with trained panelists reflected differences in bread baked from the experimental flours. Variation in nitrogen management led to sensory differences in bread crust color, texture and taste, aroma of the loaves and crumb texture and chewiness.

The information gathered in this study is useful to the agronomist in understanding the relationship between grain nitrogen management and specific end uses of flour. The food scientist and nutritionist can utilize the

information from this study in understanding the rheology and functional properties of dough resulting from various grain nitrogen regimens.

6.0 Suggestions for Future Research

Future studies should include nitrogen management regimens with the same total grain nitrogen application, but with variation in timing from a single dose to a split dose application. This will allow a more direct comparison of the effect of variation in rate and timing of nitrogen application on flour protein and baking characteristics.

A more powerful electrophoresis method would improve resolution of flour gliadin and glutenin protein components. Some overlap of protein bands occurred with one-dimensional SDS-PAGE. Two-dimensional SDS-PAGE or isoelectric focusing would improve resolution of the many gliadin and glutenin components with similar electrophoretic mobilities. Reversed-phase high performance liquid chromatography (RP-HPLC) could also be used for separation of gliadin subunit fractions. An amino acid analysis of the experimental flours would support the changes in protein composition found with protein fractionation and electrophoresis.

Baking tests of other products would expand present knowledge of the effects of nitrogen fertilization on rheological properties. Baking quality must be considered in terms of a specific product. Suggestions for further testing include Baking Quality of Cookie Flour (AACC Method 10-50D, 1983), and Baking Quality of Cake Flour (AACC Method 10-90, 1983). These are two of the traditional tests of soft wheat baking quality.

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Appendix A. Experimental Bread Formulation

Bread formulation for pup loaves per 100g flour

flour	100g
salt (non-iodized)	1.52g
sugar	6.05g
yeast	2.02g
dissolve in 25ml warm water	
shortening	3.02g
non-fat dry milk	4.03g
ascorbic acid solution (40 ppm)	5.0 ml
warm water	27 ml

Yield: 1 loaf

Appendix B. Procedure for Experimental Bread Baking

Bread was baked according to a modification of the Basic Straight Dough Method (Method 10-10A, AACC, 1983). The modified procedure was as follows:

1. Combine flour, shortening, non-fat dry milk, salt and sugar.[6]

2. Prepare yeast suspension for each formulation in warm water with 0.5g sugar. Allow to ferment in a warm, draft free place for 5 minutes, until doubled in volume.

3. Prepare an ascorbic acid (oxidizer) solution fresh daily to give 40ppm/100g flour in 5ml.

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[6] sugar and salt were added with other solid ingredients in lieu of preparation of a sugar-salt stock solution

4. Mixing procedure:

- Place dry flour mixture in mixing bowl of KitchenAid K5 mixer equipped with dough hook (Hobart Corporation, Troy, Ohio). Make well in center for the addition of liquids.

- Add measured liquids in order of ascorbic acid, yeast suspension, remainder of water (use to rinse yeast solution container)

- Mix on speed 2 for 5 minutes. Mixing time regulated with timer (GraLab Universal Timer, Model 171, Dayton, Ohio)

- Round dough by hand. Place in lightly greased metal fermentation bowls. Turn dough over, cover with linen towel. Place in fermentation cabinet or chamber for 50 minutes (dough fermentation chamber in laboratory is an oven preheated to 150 °F. and conditioned with approximately 400 ml warm water in 3 containers on lower shelf).

- Punch dough down. Roll out on waxed paper with wooden rolling pin using 3/16″ dowels as guides, placed 3 inches apart. Fold dough over twice, place in fermentation bowl, cover, return to fermentation cabinet or chamber for 25 minutes.

- Repeat previous step, return to fermentation cabinet or chamber for 10 minutes.

- Punch dough down, weigh. Divide into equal portions.

- Roll out dough using 5/16″ guides, then 3/16″ guides.

- Roll dough up by hand starting with narrow edge, pressing lightly to eliminate air bubbles. Pinch seams together tightly. Place seam side down in greased pup loaf pans.

- Return to fermentation cabinet uncovered for 35 minutes or fermentation chamber for 40 minutes.

- Bake in preheated 220 °c for 20 minutes (bake shop - rotary oven, A.J. Fish Company, Beloit, WI; laboratory - General Electric oven model number J440002WH. Accuracy of ovens tested with Taylor dial oven thermometer, Taylor, Rochester, NY). Remove from pans, place on cooling racks allowing air circulation.

- Bread was sliced for sensory panel use with automatic bakery slicer, Virginia Tech Dining Services.

Appendix C. Fractionation of Flour Proteins

The following procedure was used for fractionation of flour proteins (modified procedure of Doekes and Wennekes, 1982).

1. Combine 2.0g flour with 12ml of 70% ethanol in centrifuge tube. Homogenize for 2 minutes (Kinematica Homogenizer, Switzerland).

2. Centrifuge at 6000 x g for 20 minutes at 25 °C (Sorvall Superspeed RC-2 Centrifuge, Head type ss-34, Ivan Sorvall Inc., Norwalk, CT).

3. Decant and save supernatant. Homogenize pellet with 12ml 70% ethanol for 2 minutes.

4. Centrifuge at 6000 x g for 20 minutes at 25 °C.

5. Decant and combine supernatant with supernatant from step 3. Homogenize pellet with 12ml of 40% ethanol, .1M Na_2SO_4

6. Centrifuge at 6000 x g for 20 minutes at 25 °C.

7. Decant and combine with supernatants from step 5. Resulting pellet contains starch and glutenin fractions.

8. Dialyze combined supernatants (Spectra-pore dialysis tubing, Fisher Scientific, Pittsburgh, PA; molecular weight cutoff 6,000 - 8,000 daltons). The dialysis conditions used were:

   - Time: 18 hours
   - Temperature: 4°C
   - Dialysate: 10 liters of 0.4M Na_2SO_4, divided in to 3 successive portions (3L, 3L, 4L).

9. Centrifuge intact dialysis tubing at 1600 x g for 30 minutes at 25 °C to form gliadin pellet. (In order to fit dialysis tubing into centrifuge tube, it was necessary to drill a hole in the bottom of the centrifuge tube). Resulting pellet contains gliadin fraction.

Appendix D. Formulations of Acrylamide Solutions for SDS-PAGE

Table 17. Formulations of acrylamide solutions for SDS-PAGE

1. Separating gels (30ml total) for linear gradient		
	5%	15%
acrylamide stock	2.5 ml	7.5 ml
lower buffer	3.0 ml	3.0 ml
dd H_20	9.425 ml	4.44 ml
ammonium persulfate 10% w/v	75 µl	75 µl
temed	3.75 µl	3.75 µl

2. Acrylamide / bis acrylamide stock for separating gels (50 ml)	
acrylamide (electrophoresis grade)	15.0g (30% w/v)
bis-acrylamide (electrophoresis grade)	0.4g (.8% w/v)
ddH_2O	34.6 ml
Mix, filter and store in brown bottle at 4 °C.	

3. Stacking gel (10 ml)	
acrylamide stock	1.33 ml
upper buffer	2.00 ml
ddH_2O	6.62 ml
ammonium persulfate 10% w/v	50 µl
TEMED	2.5 µl

4. Acrylamide / bis acrylamide stock for stacking gel (10 ml)	
acrylamide (electrophoresis grade)	3.0g (30% w/v)
bis-acrylamide (electrophoresis grade)	0.325g (3.25% w/v)
ddH_2O	6.92 ml
Mix, filter, store in brown bottle at 4 °C. Prepare every 3 days.	

Appendix E. Formulations for SDS-PAGE buffers

Table 18. Formulations for SDS-PAGE Buffers

1. Separating (lower) gel buffer (100 ml)	
Trizma	22.71g (1.875m)
TEMED	125μl (.125% w/v)
SDS	.50g (.5% w/v)
Adjust to pH 8.8 with 3 - 4 N HCl	

2. Stacking (upper) gel buffer (100 ml)	
Trizma	7.57g (.625m)
TEMED	125 μl (.125% w/v)
SDS	.50g (.5% w/v)
Adjust to pH 6.8	

3. Electrode (running) buffer 5l	
Trizma	15.125g (.025m)
Glycine	72.065g (.192m)
SDS	5.0g (.1% w/v)
Adjust to pH 8.3	

4. Sample buffer 25 ml	
Urea	12.000g (8M)
Trizma	.189g (.0625M)
SDS	.500g (2% w/v)
Bromophenol blue	.006g (.025% w/v)
Dithiothreitol	.193g (.05M)
Adjust to pH 8.3	

Appendix F. SDS-PAGE Molecular Weight Calibration Curve

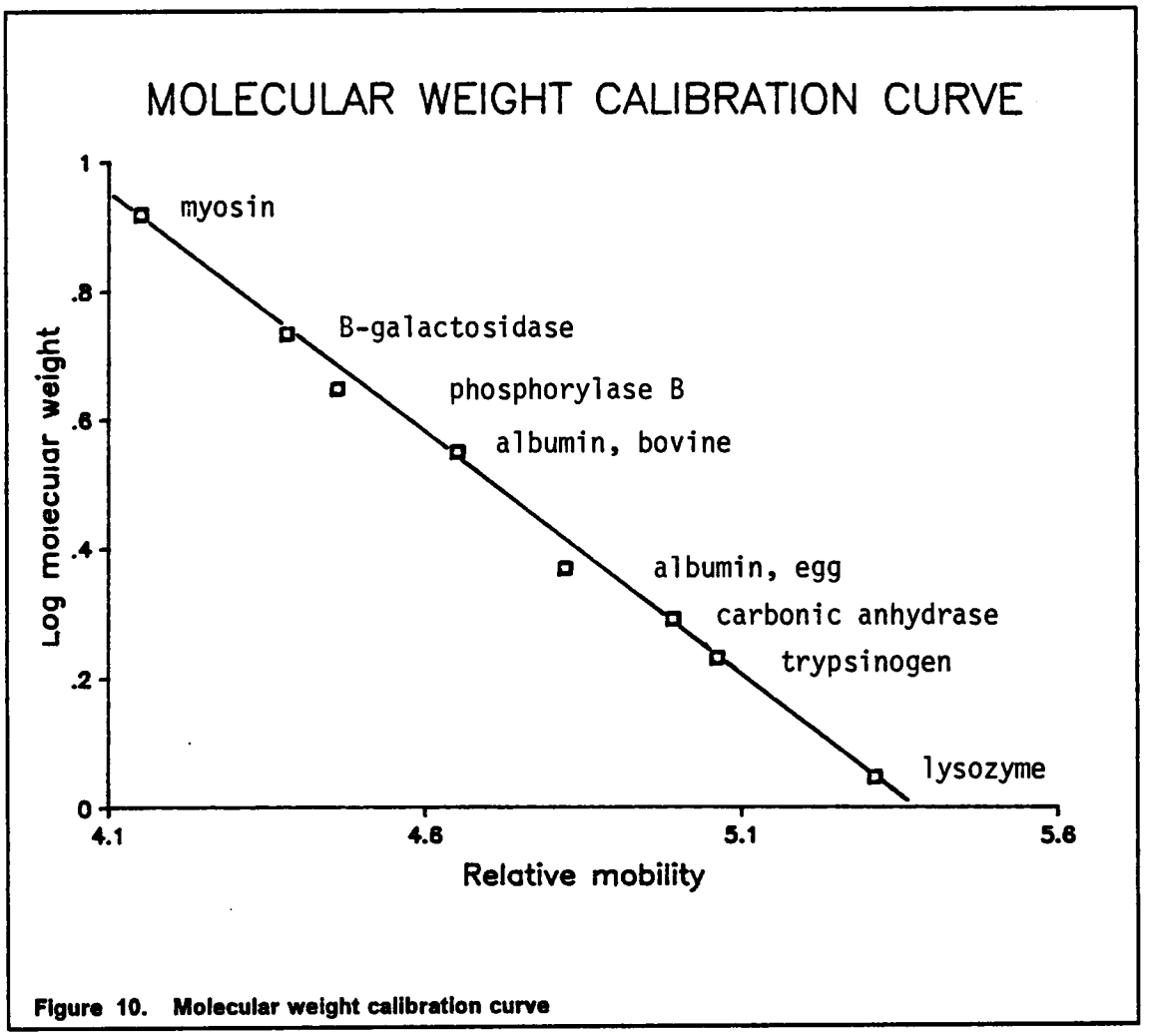

Figure 10. Molecular weight calibration curve

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