PHASEOLUS VULGARIS L. PROTEIN AND FLOUR EFFECTS ON WHEAT DOUGH RHEOLOGY, CHEMICAL BONDING AND MICROSTRUCTURE

By

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ABSTRACT

PHASEOLUS VULGARIS L. PROTEIN AND FLOUR EFFECTS ON WHEAT DOUGH RHEOLOGY, CHEMICAL BONDING AND MICROSTRUCTURE

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The behavioral properties of navy bean flour fractions and globular proteins in composite doughs were investigated. Farinograph parameters were used to evaluate 95:5 and 90:10 wheat flour/bean flour blends from fines and cotyledons produced from both prime and cull beans. Extracted globulin proteins were substituted into dough systems in amounts present in 5 and 10% substituted wheat/fine blends. Rheologically active thiol and disulfide groups in the wheat/protein blends were estimated. All doughs were viewed using low temperature scanning electron microscopy. Farinograph data suggests wheat/cotyledon flour blends produce a less stable dough than the wheat/fine blends. Cull flours can be used in composite dough without further detrimental consequences. Phaseolins decrease dough stability. The ratio of mixing SS/SH is not affected by the phaseolin or lectin proteins. Phaseolins influence the number of disulfides involved in mixing the dough. Micrographs revealed microstructural differences in bean flour grades not detected by farinograph techniques.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURE NO.

INTRODUCTION

Substituting wheat flour with high protein, high lysine ingredients such as legume flours, protein concentrates and isolates has been given much attention over the last two decades. This process improves the amino acid balance and increases the protein content of products baked from the blended flours.

Wheat flour is a unique substance. Its gluten forming ability makes it the only known material that, when blended with water, forms an elastic dough capable of retaining gases. Researchers have learned that when composite flours are used to make bread, the non-glutenous additions exert a loaf depressing effect (Pomeranz, 1980). In addition to low volume, legume flours tend to change the absorption, mixing tolerance and other physical dough properties. The crumb, texture and color are also adversely affected. Adjustments in mixing time and water additions were found to somewhat improve substituted breads. Most of the deleterious effects of legume flour on bread making can be minimized with the use of bromate and dough conditioners such as sodium stearoyl-2-lactylate (SSL).

In studies conducted by Silaula (1985), the use of oxidizing agents and SSL greatly improved the volume of breads substituted with Pinto and Navy bean protein fractions. Oxidizing agents are believed to help control disulfide bond rupture and interchange. The importance of the sulfhydryl-disulfide interchange to bread quality and loaf volume is well documented. Since this reaction is fundamental to loaf volume, and the bean proteins depress loaf volume but respond to treatments which enhance and stabilize the disulfide interchange, further research was prudent.

Given the above information, it was necessary to question whether the weakening of the dough with the addition of bean flour was, as has been suggested, a dilution effect; competition for water between the bean seed and wheat proteins; or a disruption of the protein-starch complex by the foreign protein. The theory that there may be an interfering agent working to disrupt the disulfide interchange is presented in this research. The focus of this investigation was to determine how the flour fractions and globular proteins effect dough properties and to determine if a link to the disulfide interchange could be established.

The first objective of this study was to determine if flours obtained from culled dry beans behave in the same manner as flours milled from prime quality beans. If cull

beans have potential for use as a human food source, there would be economic benefits and possibly a stabilizing effect on the highly fluctuating bean market.

The second objective was to extract the major globulin proteins from the high protein bean flour fraction and investigate how these proteins influence farinograph parameters. This would help determine the role of these proteins in product performance.

The third objective was to distinguish rheologically important thiol and disulfide groups in wheat dough substituted with navy bean proteins. By looking at this interaction during dough development a clearer understanding of protein-protein interactions may be ascertained.

Finally, it was important to view images of the doughs from each aspect of the research with the scanning electron microscope. It was hoped that this tool could be used to confirm findings from the physical dough testing and perhaps detect differences that would otherwise go unnoticed.

REVIEW OF LITERATURE

Leguminosae include roughly 600 genera with approximately 13,000 species. However, excluding soybeans and peanuts, only about 15 to 20 of these are of much economic importance (Bressani, 1975; Salunkhe et al. 1985).

The genus *Phaseolus* are among the oldest of the domesticated crop plants. Evidence of *P. vulgaris*, found in Mexico, have a carbon-14 date of 4975 B.C. (Salunkhe et al., 1985). Other evidence suggests multiple domestication within Central America (Kay, 1979).

Also known as the common bean, *P. vulgaris* is the best known and most widely cultivated of the phaseoli. More than 14,000 cultivars have been recorded in this species. It is now considered the most important food legume throughout Latin America and parts of Africa (Kay, 1979).

Phaseolus Vulgaris Classification

Enormous variation occurs in *P. vulgaris* cultivars. Thus, *P. vulgaris* is often classified according to use. The major classifications (Kay, 1979) are 1) dry shell or field beans 2) green shell haricot beans and 3) horticultural haricot beans.

The dry shell category are grown extensively in the United States, Canada, Latin America and parts of Africa. These include the pea or navy beans used largely in canning. Some cultivars of this group are 'Monroe', 'Seaway' and 'Gratiot'. Field beans also encompass medium haricot beans such as pinto and cranberry beans; marrow beans, examples are 'Yellow-eye' and 'Steuben'; and kidney beans which include 'Redkote' and the 'Great Northern' group (white kidneys) (Kay, 1979).

The common bean grows best at temperatures between 60 and 75°F in areas which are frost-free for a period of 105-120 days. While the bean can be grown in most soil types, a friable, well-drained soil is desirable as the plant is very sensitive to the soil-water balance, which has a pronounced affect on yield (Kay, 1979).

Dry Bean Composition

Though they have similar structures, the seeds of food legumes differ greatly in their size, shape, color and seed coat thickness (Salunkhe et al. 1985). In general, dry beans consist of two major and two minor structures; the seed coat and cotyledon and the hypocotyl and plumile respectively. Bean composition varies chemically by cultivar, growing conditions and location.

Protein

Crude protein of commercial dry beans commonly ranges between 20 - 25% on a dry weight basis. Nonprotein nitrogen accounts for 8.3 to 14.5% of the total nitrogen (Deshpande and Nelson, 1987). Legumes contain two main types of protein characterized by their solubilities. These are the salt soluble globulins and the water soluble albumins (Millerd, 1975).

Albumins serve many metabolic functions in the cell, both enzymatic and structural, including the synthesis of storage proteins (Millerd, 1975). Globulins are the major storage proteins present in legumes. These proteins occur in tightly packaged specialized inclusions (cotyledonary storage bodies) in cotyledon cells. They are high in nitrogen content as their principal function is a source of nitrogen for the developing plant.

Amino Acids

The amino acid composition, as well as protein content, is species and cultivar dependent due to genetic differences. However, differences also arise due to agronomical and physiological conditions of plant growth.

The amino acid profile of legume proteins is characterized by a high lysine content and a low concentration of the sulfur bearing amino acids (Ma and Bliss, 1978). Thus, the nutritional value of bean proteins

is limited by the inadequate amounts of methionine and cystine which Kelly (1973) found to be approximately one third of that in the protein of a chicken egg which serves as the nutritional standard. One strategy for improving the biological value of bean proteins has been to combine them with foods providing complementary proteins.

Unlike cereals, where increased levels of the limiting amino acid lysine can be attained through a reduction in the amount of the storage protein proalamines, higher phaseolin levels in legume seeds lead to an increased available methionine concentration (Gepts and Bliss, 1984). This is because phaseolin, though low in methionine, is the major source of the amino acid. Actually, only limited differences exist between the methionine concentrations of the different protein fractions but phaseolin represents the largest portion of total seed nitrogen .

Lipid

Lipid content is less than two percent in most legumes of the *P. vulgaris* species. Neutral lipids are the predominant class of lipids present in legume seeds. Neutral lipids account for 60% of the total lipid content (Sahasrabudhe et al., 1981). Phospholipids make up 24 to 35% of the lipid content (Sathe et al., 1984). The major phospholipid of the plant tissue and membrane is phosphatidylcholine (Goodwin and Mercer, 1985). The

glycolipids account for up to 10% of the total lipid content of the legume seed (Sathe et al., 1984). Cerebrosides and other sphingosine containing lipids are of minor importance (Goodwin and Mercer, 1985).

The fatty acid composition of the lipid fraction has been characterized as 19% saturated fatty acids (mainly palmitic), 64 % unsaturated fatty acids (oleic, linoleic and linolenic) and approximately seven percent nonsaponifiable matter (Kay, 1979). Linoleic and linolenic acids are essential fatty acids important to growth and cell maintenance. United States Department of Agriculture (USDA) data for lipid content of raw navy beans is presented in Table 1.

Table 1. Lipid content in 100 grams raw navy beans

Fatty acid	Amount (g)
Saturated, total	0.331
14:0	0.001
16:0	0.310
18:0	0.020
Monounsaturated, total	0.111
18:1	0.111
Polyunsaturated, total	0.552
18:2	0.301
18:3	0.252

(USDA, 1986)

Carbohydrates

The carbohydrate constituents account for up to 65% of the Phaseolus seed composition with starch, largely amylose, as the major fraction. Kay (1979) reported other carbohydrate constituents as pentosans 8.4%, dextrins 3.7%, cellulose 3.1%, sugars 1.6% and galactans 1.3%. Sugar content appears to vary widely. Reddy et al. (1984) reported sugars, notably sucrose, stachyose, verbascose and raffinose contribute around eight percent of the carbohydrate. Walker and Hymowitz (1972) reported sugar contents ranging from 4.4 to 9.2% in the 28 cultivars of *P. vulgaris* used in their study.

Reddy et al. (1984) found dietary fiber composed up to 15% of the carbohydrate present in bean seeds. Tobin and Carpenter (1978) reported a dietary fiber value of 22.5%. USDA figures indicate that there are 5.22 grams of crude fiber and 9.7 grams of dietary fiber in 100 grams of raw navy beans. Aguilera et al. (1982b) produced navy hull flour with dietary fiber contents ranging from 31.2 to 50.2%. Tecklenberg (1983) found dietary fiber values varied from 1.76 to 13.40% for navy bean flours; hull flour has the largest dietary fiber content of the bean flour fractions. Jeltema et al. (1983) reported the following components of dietary fiber in flour from navy bean hulls: water soluble pentose 1.13%, pectin 8.96%, water insoluble hemicellulose 18.00%, cellulose 5.81% and lignin 1.03%.

Vitamins

Dry beans are a significant source of vitamins, notably thiamin, riboflavin, niacin, folic acid and pyridoxine. The fat soluble vitamins (A,D,K,E) are present only in trace amounts. According to USDA (1986) data for raw navy beans less than 4 IU vitamin A is present in 100 grams. Tocopherol content is somewhat larger than that of whole cereals (Salunkhe et al.,1985). In a study of four *P. vulgaris* cultivars by Fordham et al. (1975) tocopherols averaged 1.2 mg per 100 grams. USDA figures for raw navy beans show α-tocopherols equal to 0.34 mg per 100 grams.

Some leaching of water soluble vitamins and minerals occurs as a result of the conventional soaking and cooking processes required for dry beans. Retention values vary with specific nutrient, soak time, type of water used, and the time and temperature of cooking. Values generally range between 70-80% (Augustin et al., 1981). Augustin et al. (1981) reported the contribution a 175 gram sample of *P. vulgaris* would make toward the U.S. Recommended Daily Allowances (U.S. RDA). On a per serving basis these vitamins provide 30% of the U.S. RDA for thiamine, 6% of the U.S. RDA for riboflavin, 4% of the U.S. RDA for niacin, 33% for folic acid, and 11% of the U.S. RDA for pyridoxine. However it has been suggested by Gregory and Kirk (1981) that the absorption of pyridoxine may be influenced by the presence

of nondigestible polysaccharides and lignin, which may reduce the vitamins availability.

Minerals

It is generally agreed that *P. vulgaris* are a beneficial source of calcium, potassium, copper, iron, zinc, and magnesium. Researchers reported wide variation in values in relation to environmental conditions such as climate and soil type and in relation to genetic factors of the cultivar (Salunkhe et al., 1985). Potassium contributes 25 - 30% of the total mineral content of the bean. Calcium and phosphorus are also present in substantial amounts. Legumes are richer in calcium than most cereals. However, the phosphorus is largely present as phytic acid which affects the absorption and utilization of the calcium (Salunkhe et al., 1985). USDA (1986) values for vitamin and mineral content of raw beans are presented in Table 2.

Augustin et al. (1981) reported that a 175 gram cooked serving of dry beans made the following contributions toward the U.S. RDA for the minerals listed: calcium, 9% U.S. RDA; copper, 21% of the U.S. RDA; iron, 19%; zinc 12%; magnesium and phosphorus, each 26% of the U.S. RDA.

Tecklenberg (1983) analyzed eight minerals in various navy bean flour fractions. Results indicated that partitioning of minerals transpires during the fractionating procedure. The protein flour fraction was found to contain

higher amounts of most minerals than other fractions. Mineral content of navy bean flours as measured by Tecklenberg (1983) are shown in Table 3.

Table 2. Vitamin and mineral content of raw navy beans

Nutrient	Unit	Amount in 100 grams
Minerals:		
Calcium	mg	155
Iron	mg	6.44
Magnesium	mg	173
Phosphorus	mg	443
Potassium	mg	1,140
Sodium	mg	14
Zinc	mg	2.54
Copper	mg	0.879
Manganese	mg	1.309
Vitamins:		
Ascorbic acid	mg	3.0
Thiamin	mg	0.645
Riboflavin	mg	0.232
Niacin	mg	2.063
Pantothenic acid	mg	0.680
Vitamin B_6	mg	0.437
Folacin	µg	369.7
Vitamin A	IU	4

(USDA, 1986)

Table 3. Mineral content of navy bean flour fractions

Mineral	Bean flour fraction (ppm)			
	Whole	Hull	Starch	Protein
Calcium	1019	4173	273	709
Copper	52.6	12.2	9.6	22.6
Iron	67.1	86.4	71.6	139.7
Magnesium	1677	2237	1103	2597
Phosphorus	5233	4617	3997	8833
Zinc	32.1	35.9	19.9	52.5
Sodium	117.3	104.8	34.0	62.8
Potassium	17033	17100	16867	19800

(Tecklenberg, 1983)

Antinutritional Factors

P. vulgaris are known to have several antinutritional factors. These include trypsin and chymotrypsin inhibitors, hemagglutinins (lectins), phytates, polyphenols, cyanogenic compounds, oestrogens, goiterogens, saponins, allergens and antivitamins (Bressani, 1975). The evidence available today suggest that most of the antinutritional factors can be reduced or eliminated by proper processing. It is well established that the antienzymes and the hemagglutinins are destroyed by heat treatment. Since extensive heat treatment may reduce the nutritional value, care must be taken not to exceed the optimum time\temperature requirements established for processing. Coffey et al. (1985) reported hemagglutinat-

ing activity decreased with increased heating time or heating temperature. For each 5.6°C increase in temperature, holding time was reportedly reduced 50 minutes to achieve the same level of inactivation. Low temperature cooking of beans at 82°C resulted in detectable levels of hemagglutinating activity even after eleven hours of heating.

Seed Storage Proteins of *Phaseolus vulgaris*

Early research of legumes (reviewed in Derbyshire et al., 1976) revealed two globulin protein fractions, a 10S legumin and a 7S vicilin. The wide spread occurrence of these fractions may have been in part responsible for the disagreements surrounding isolation techniques, interpretation of results, and terminology used in the investigation of *P. vulgaris*. Like the storage proteins of *V. faba, P. sativium* and other legumes, those of *P. vulgaris* are globulins. However, there is only one major class, the 7S phaseolins. A G2 or globulin-2 fraction (6.4S) also exist but it makes up only two to ten percent of the total seed proteins. The G2 fraction contains lectins which may affect the digestibility of the bean.

Phaseolin Protein

Phaseolin is the major storage protein of *P. vulgaris*, comprising up to 45% of the total bean protein. This

fraction has also been termed G1 protein (McLeester et al., 1973; Sun et al., 1974), vicilin (Bollini and Chrispeels, 1978), or glycoprotein II (Pusztia and Watt, 1970; Derbyshire and Boulter, 1976). Phaseolin is synthesized on the rough endoplasmic reticulum (Bollini and Chrispeels, 1979) and accumulates in the developing cotyledon during the first two to three weeks after anthesis.

One dimensional sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of phaseolins resolves three polypeptides, α, β, and γ with apparent molecular weights of 53,000, 47,000 and 43,000 respectively (Hall et al., 1977, Sun et al., 1981). Hall et al. (1977) confirmed that each of the peptide subunits is glycosylated, totaling three to five percent associated sugar residues. Peptide mapping (Ma et al., 1980) of these proteins shows that the subunits are highly homologous in amino acid sequence and are encoded in a small multigene family (Slightom et al., 1983; Doyle et al., 1986).

Two dimensional electrophoresis, where the phaseolin proteins were separated by charge in the first dimension and by molecular weight in the second dimension revealed charge (pH 5.6 - pH 5.8) and molecular weight (45,000 -51,000) heterogeneity which is cultivar dependent. Three distinct banding profiles have been identified (Brown et al., 1981b). The unique banding patterns have been named after representative cultivars bearing the profiles. These are

'Tendergreen' (T), 'Sanilac' (S) and 'Contender' (C). Table 4 illustrates differences among banding patterns of the phaseolin peptides as determined by Brown et al. (1981a). The T pattern consist of five polypeptides, the S type has eight polypeptides and the C pattern is an intermediate possessing bands from the T and S profiles. It contains the five T polypeptides and two of the S subunits (α49 and β47) and has one peptide unique unto itself.

Table 4. Banding patterns of phaseolin polypeptides classified by molecular weight and isoelectric point

Molecular weight	Subunit group	'Tendergreen' type	'Sanilac' type	'Contender' type
51,000		α51		α51
49,000	α		α49	α49
48,500			α48.5	
48,000	β	$\beta 48^{Ta}$	$\beta 48^{S}$	$\beta 48^{T}$
47,000			β47	β47
46,000		$\gamma 46^{T}$	$\gamma 46^{S}$	$\gamma 46^{T}$
45,500	γ	γ45.5		$\gamma 45_{\&}5^{C}$
45,000			$\gamma 45^{S}$	

[a]Superscripts S, T and C distinguish proteins with identical molecular weights but different isoelectric focusing properties (Brown et al., 1981a).

The complete nucleotide sequence of a *P. vulgaris* phaseolin gene was determined by Slightom et al. (1983). This study determined a gene structure consisting of 80 base pairs (bp) of 5' untranslated DNA, 1,263 bp of protein encoding DNA and 135 bp of 3' untranslated DNA. Their phaseolin contained 420 amino acids. The NH_2-terminal region contains 24 hydrophobic and two hydrophilic amino acids, followed by a highly hydrophilic region.

G2 Proteins

The G2 fraction of *P. vulgaris* is a heterogeneous fraction containing both lectin and nonlectin proteins (Osborn et al., 1985). The lectins or phytohemagglutinins (PHA) are of interest as they contribute to the toxicity of raw beans (Brown et al., 1982a) and have been used extensively in clinical applications. Plant lectins can act as mitogens which can be used to distinguish between normal and malignant cells (Hoffman et al., 1982). G2 and the albumin fractions have several peptides in common that have been correlated with hemagglutinating activity. Therefore, the two fractions are often linked together as G2\albumin when referring to lectin proteins.

Lectins are glycoproteins consisting of subunits which contain specific binding sites for sugar residues. It is these interactions between the binding sites and the carbohydrate residues on blood cell surfaces which give rise

to the mitogenic and agglutinating properties attributed to the lectins (Brown et al., 1982b).

While only three different types of phaseolins have been distinguished through their polypeptide composition, Osborn et al. (1985), in a survey of 107 bean lines, determined eleven genetic variants and the absence of lectin by two-dimensional electrophoresis of the G2 fraction. They also found a relationship between lectin quantity and lectin type. In general, cultivars with more complex lectin types (4-8 subunits) had more lectin content. A negative relationship was observed between the quantities of lectin and phaseolin. Because lectin quantity depends on type it follows that phaseolin content is associated with lectin type. In 1982, Hoffman et al., determined the complete amino acid sequence of a *Phaseolus vulgaris* cv. 'Tendergreen' lectin which they derived from the nucleotide sequence of a cDNA plasmid.

Wheat Flour Doughs

Wheat has been called the worlds most important food grain. Thus, it has been studied in detail for its chemical composition and nutritional qualities (Pomeranz, 1980).

On a dry weight basis, a wheat flour used for bread making, contains approximately 80% starch, 14% protein, 4% lipid and 2% pentosans (Chung, 1986). Yet, each of these

constituents is itself a complex blend. At least 18 proteins, 8 lipids, many carbohydrates from simple sugars to starch, numerous minerals and enzymes have been reported (Holme, 1966).

The protein content and quality of a particular wheat can establish its functional use in a food system. Bread flour requires a relatively high protein content, while flour used for cookies, cakes, pastries and crackers is milled from a wheat of lower protein quantity.

Early research by Finney and Barmore (1948) demonstrated that loaf volume is directly related to protein content. Furthermore, they found the slope of a regression line of loaf volume versus protein content is characteristic of the cultivar.

In a study of the functional properties of winter wheat samples (reviewed by Pomeranz, 1980), researchers found that protein content was responsible for about 90% of the variability in loaf volume of all samples studied. Thus, only 10% of loaf volume variability was attributed to protein quality or experimental error. The study does not suggest that the quality of wheat is constant. Flour protein quality varies by wheat type, class and cultivar. However, the selection of cultivars on the basis of milling and baking properties leads to minimal effects on bread making quality. More recently, Bushuk (1984) reported that

the glutenin fraction appears to be responsible for the majority of the varietal variation in the bread making qualities of wheat flours.

Protein quality, as noted previously, is an important factor in bread making; but a difficult attribute to measure. Protein quality is derived from a specific combination of unique physical and chemical aspects of the protein components (Bushuk, 1986). That is the ability to form the viscoelastic proteinaceous component of bread dough, gluten. No other protein is known to form gluten. Quality evaluations are empirical and sometimes complicated. Matz (1959) outlined four principles to consider in gluten quality testing these were, expansion by heat, recovery from compression, gluten extention and gluten relaxation. There are numerous test methods available. The American Association of Cereal Chemist (1983) detail many flour, dough and baking tests.

The Brabender farinograph is often used to test the quality and strength of flours and wheats (Campbell, 1972). This physical dough testing instrument is essentially a torque measuring recording dough mixer. Changes in the resistance of dough mixed under controlled conditions are mechanically recorded on graph paper. The farinogram indicates two important physical properties of a flour. The first , absorption determines the amount of water required for the dough to reach a specified consistency. Secondly,

the mixing profile of the dough obtained from the farinograph gives important information on consistency, development time and resistance to breakdown which are all related to flour quality (Campbell, 1972).

Protein

Wheat proteins are customarily characterized by their solubilities as water soluble albumins, salt soluble globulins, alcohol soluble gliadins, and the acid and alkali soluble glutenins (Campbell, 1972). The gliadins are a heterogeneous group of relatively low molecular weight proteins ranging from 12,000 to 80,000 with the major portion having molecular weights around 40,000. The gliadins contribute cohesive properties to the gluten. Gliadin, which makes up 35-40% of the flour proteins contains roughly 50 subunits. Most of these are single chains containing intra-polypeptide disulfide bonds (Khan and Bushuk, 1979). The amino acid profile for gliadin reveals a protein high in proline (15%) and glutamic acid, most of which is present as glutamine (35%).

The glutenin fraction is also a heterogeneous group but consist of a group of larger molecular weight proteins. This fraction which comprises about 45% of the wheat endosperm protein, imparts elastic qualities to the gluten. The glutenin from bread wheat has been found to contain approximately 17 polypeptide subunits (Bietz and Wall, 1972)

many of which are linked together by interpolypeptide disulfide bonds. As in the gliadin fraction, the glutenin fraction contains a high amount of glutamic acid which also is present as glutamine.

Functionality of Gluten Proteins

The ability of wheat flour, when mixed with water, to form a dough provides the structural basis for a number of baked products. When an appropriate amount of water is mixed with a wheat flour, the endosperm proteins, gliadin and glutenin hydrate and associate with themselves as well as specific carbohydrates and lipids to form a cohesive and elastic network with starch granules embedded in the matrix. Subsequent mixing solubilizes the proteins and rearranges them to form protein membranes over a fibrillar framework of gluten and starch granules (Bushuk, 1984). This structure is capable of expanding and retaining gas essential to bread making.

Fundamental to the functionality of these proteins is their ability to hydrogen bond. Both gluten proteins possess a high glutamine content which promotes hydrogen bonding in the gluten complex . This is possible by virtue of the numerous amide groups which can form inter and intra-molecular hydrogen bonds. The nonpolar amino acid residue such as of the side chains of valine and leucine participate with one another in hydrophobic bonding in aqueous media.

Hydrophobic bonds may contribute to both dough plasticity and elasticity. Both the hydrogen bonds and the hydrophobic bonds are unstable and can readily interchange facilitating dough development.

The importance of thiol groups and disulfide bonds in dough rheology is well substantiated (Hird et al., 1968; Bloksma, 1972; Ewart, 1972). Disulfide - sulfhydryl interchange reactions have been shown to occur in dough between nongluten proteins and naturally occurring small disulfide or thiol sidechains of gluten. There are many theories and models proposed to explain the disulfide interchange mechanism.

In the Bloksma (1975) model for sulfhydryl-disulfide interchange the cross links are not permanent, the thiol compounds are not consumed and are available for successive interchange reactions. According to Bloksma (1975), stiffening of dough through oxidation reactions is not primarily due to the addition of S-S bonds but more likely due to the removal of thiol groups. Other findings indicate that only a small portion of the thiol and disulfide groups are involved in rheological properties of dough systems. Modification of only a small number of the thiol groups produces maximum effect on the rheological properties of the dough.

Glutathione and thioctic acid are reported to be important factors in the interchange reactions. These small molecular weight thiols and disulfides decrease the resistance of dough and cause a weakening effect when they undergo interchange reactions with the S-S bonds of gluten.

While not discussed here, it must be recognized that water, enzymes, lipids, polysaccharides and other chemical additives also influence the rheological and functional properties of dough.

Legumes and Wheat in Food Systems

In the United States, dry edible beans are an underutilized source of protein and carbohydrates. Although beans are commonly consumed as baked or canned beans, there has been a growing interest in alternative bean forms. Instant bean products have been prepared for use in soups and sauces using drum and spray drying technology (Bakker et al., 1973). Protein concentrates from various legumes have also been investigated. Researchers found that wet processing (water extraction) is an inefficient and expensive technique.

Dry milling of raw beans allows for successful air-classification, by density gradients, of the bean into two fractions a high starch fraction and a high protein fraction. However, enzyme activity in raw beans renders these fractions unsuitable for use as food ingredients due

to the development of undesirable flavors and odors.

Aguilera et al. (1982a) used roasted navy beans to develop four desirable flours for use as food ingredients using the air classifying system. These were a whole flour, a hull flour, a high protein and a high starch flour. Later that same year, these authors (Aguilera et al., 1982b) reported roasting decreased trypsin inhibitor activity and inactivated hemagglutinins.

Use of alternate bean materials has attracted a variety of interests. A primary use has been fortifying wheat flour with high protein - high lysine bean flour to improve the protein content and protein efficiency ratio (PER) of baked products.

In a study by Hoojjat and Zabik (1984), which suggested high protein cookies would be beneficial in child feeding programs, sugar snap cookies with 20% navy bean flour substituted for the wheat flour, scored well with sensory panelists. Cady et al. (1987) incorporated navy bean flour at 25 and 35% into a master mix. Products prepared from these mixes compared favorably to their respective controls indicating incorporation of bean flour into a master mix intended for use in baked products is an acceptable and nutritionally beneficial alternate use of beans. Bean flours have been successfully used in formulations for a variety of other products including quick breads (Dryer et

al.,1982), cake donuts (Spink et al., 1984), tortillas, chocolate chip cookies and frankfurters (Aguilera et al., 1981), extruded snack puffs (Benzinger, 1984) and yeast breads (Silaula, 1985).

The level of substitution often depends upon the product. Beany notes may be detected in products which are not highly flavored. If substitution levels are too high color may be adversely affected in those items where a light color is perceived as important as bean flour tends to darken "white" batters.

Legume flours portend to be an excellent choice for improving the nutritional value of bread. The high lysine -low methionine content complements the wheat flour proteins which are poor in lysine and relatively higher in the sulfur bearing amino acids. Nevertheless, legume flours are not ideal for bread making as they do not possess the important gluten forming proteins of wheat. They also have a lower starch and pentosan content than wheat flour which has a negative impact on bread making properties.

Many factors affect the final outcome in bread baking; quality and quantity of wheat protein, level of substitution, process used to obtain the legume flour, the fraction used, water absorption and mixing characteristics are amongst the most important factors. Knorr and Betschart (1978), suggested that the weakening effect of foreign proteins on wheat doughs was the result of a dilution of the

gluten structure by the fortifier. This results in a lower loaf volume and subsequently has a negative effect on other quality attributes such as crumb grain and tenderness.

Rheological studies show pinto and navy protein delay arrival time and decrease dough stability when substituted into bread systems. Deshpande et al. (1983) found that as the level of bean flour increased, farinograph absorption and mixing tolerance index increased whereas, mixing time and dough stability decreased. D'Appolonia (1978) reported water absorption increased and doughs were weakened with the addition of navy bean flour to wheat flour doughs. Sathe et al. (1981) findings were similar. Water absorption and mixing tolerance increased and dough stability was reduced when bean flours were evaluated with wheat flour in a farinograph. Mixograph data showed an increase in protein content resulted in increased water absorption, decreased peak and mixing time. Extensigraph data indicated that resistance to extension decreased with a greater concentration of bean flour. The extensibility was found to decrease with a higher amount of bean flour. In baking experiments, loaf volume of breads substituted with bean protein decreased, crust color darkened, crumb color became more grey and crumb grain showed evidence of thickened cells. Breads containing 15% or more bean flour were characterized as having a beany and bitter flavor.

Fleming and Sosulski (1977) studied the bread making properties of four concentrated plant proteins. Without formula adjustments, each protein had a deleterious effect on bread quality. Loaf volume ,texture and compressibility were all negatively affected. The use of 2% vital gluten and dough conditioners restored bread quality. In addition, these researchers found that protein substituted loaves produced sufficient gas for loaf expansion but lack of retention of the gas in a weakened framework appeared to be a factor in low loaf volume.

Silaula (1985) observed the effects of air classified pinto and navy bean protein flours on the rheological and physical characteristics in white and whole wheat bread systems. As in previous studies, farinograph evaluation of substituted doughs showed a progressive increase in water absorption, arrival time and development time while dough stability decreased with increased protein content. Loaf volume of substituted breads was lower than the control loaves. Treatment with bromate and sodium stearoyl-2-lactylate (SSL) improved bread volume considerably.

Thus, it seems the use of composite flours require dough strengtheners. Fleming and Sosulski (1977) found that glycolipids, particularly sucrose monolaurate and polyoxyethylene-8-stearate were the most effective conditioners. Oxidizing agents, often used to regulate the disulfide interchange reactions in wheat bread, have a

beneficial affect on the outcome of legume substituted doughs (D'Appolonia, 1977; Tsen and Weber, 1981; Silaula, 1985).

Scanning Electron Microscopy

The scanning electron microscope (SEM) has achieved wide application in the study of food surfaces. This powerful tool can be used to investigate the structural make-up of foods and food products. With its high resolving power and large depth-of-focus, the SEM gives better images of three dimensional specimens than can be obtained with the light microscope. To be useful, accurate information must be obtained from well preserved samples. The ideal SEM samples are metals because the are dry, physically stable, and they are electrically conductive (Wilson and Robards, 1984).

Because food samples are largely biological in nature, several issues must be addressed when dealing with these specimens. First, it is necessary to remove the water from the sample, disrupting the internal structure as little as possible or to stabilize a hydrated sample so that it is not vaporized under pressure. Secondly, the specimen usually must be made conductive, hence they are coated with a thin layer of metal. Biological samples are generally soft and susceptible to beam damage. To minimize specimen damage the

accelerating voltage should not exceed 20 kV. A change in the condenser lens setting will change the intensity of the electron beam when it strikes the specimen. A high intensity beam can damage delicate food specimens and should therefore be adjusted with respect to sample limitations and requirements.

Specimen Preservation

Preservation of structure is governed by fixation and dehydration techniques, the specimen itself and SEM conditions. The fixation method is largely dependent on the food specimen, but common methods include air drying, freeze drying, and fixation and chemical dehydration followed by critical point drying. A relatively new method, low temperature SEM, offers promise in the observation and analysis of food specimens (Wilson and Robards, 1984).

Air drying is an excellent method for the examination of those foods that are normally dry. This includes items such as dry beans, flours and grains, starches and processed foods that are dry in their final state (Chabot,1979). Air drying of foods that are normally highly hydrated results in a large amount of deformation. This is due to the distortion which arises from the surface tension as water is removed from the sample (Wilson and Robards, 1984).

In freeze drying, the product is rapidly frozen to reduce ice crystal damage and then it is placed under vacuum

where the water is eliminated from the specimen through sublimation. This method may result in some disruption of the internal structure due to ice crystal formation but it is still a valuable method for SEM sample preparation.

Fixation and dehydration followed by critical point drying preserves protein and lipid (Chabot,1979) and may be one of the best available methods for preservation of animal products. However, the composition of most plant derived foods includes carbohydrate stores which may be destroyed during chemical dehydration. The structure of low moisture foods is altered by the use of aqueous fixatives. These can cause changes in specimen volume, they may extract some cell components or even cause reorganization of ions within the cell (Wilson and Robards, 1984).

Low temperature SEM has been commercially available for only the last eight years. This relatively new technique allows observation of frozen hydrated specimens. Using specialized equipment, fresh samples can be frozen, fractured open if desired, etched by removing a small veil of water which might otherwise obliterate subsurface detail, and coated. The sample is maintained at low temperatures (-150 to -175°C) in the sample preparation chamber and in the microscope during the viewing. There are many advantages to the low temperature method. One major advantage is rapid specimen preparation. This method makes possible detailed observations of frozen liquids and

extremely soft specimens. It has also been recommended for the study of emulsions, fats and oils (Wilson and Robards, 1984). A possible disadvantage to this system is the sample is limited to a single SEM session. Once removed from the microscope, the specimen will thaw and become unsuitable for further examination.

Scanning Electron Microscopy of Flour-Water Doughs

Pioneer work in dough SEM was conducted by Aranyi and Hawrylewicz (1968). Their dough samples were dried in a vacuum desiccator and gold-palladium coated. Micrographs revealed starch grains were evenly distributed throughout the dough sample and were covered with a veil-like structure which was stretched over and around the starch granules.

In 1969, these same authors again examined dough microstructure. Experiments were conducted to evaluate changes in structure due to preparation techniques. Samples were either dried in a vacuum desiccator or put through a standard fixation-dehydration process. The chemically fixed doughs showed considerable changes. The protein network was less continuous, and appeared to have pulled away from the area surrounding the larger starch grains.

Moss (1974), suggested that the gluten network is formed from many interconnected sheets that surround starch granules. Also observed were a number of starch granules

trapped between the gluten layers which had no associated gluten. These samples were prepared with aqueous fixatives which reportedly (Chabot,1979) cause many changes particularly in the starch. The starch grains are able to swell and separate from the matrix in solution.

Khoo et al. (1975) examined freeze dried, yeasted doughs. They observed characteristics similar to those reported by Aranyi and Hawrylwicz (1968) and Moss (1974).

Volpe (1976) studied a yeast protein isolate substituted at 6% into wheat doughs. Samples were fixed in glutaraldehyde, dehydrated with alcohol and critical point dried. The micrographs obtained from this study showed that the yeast protein interfered with the gluten network. The protein appeared to physically disrupt the smooth gluten layer by its irregular form. The gluten formed in the substituted dough systems was characterized as thick, bucky and lacking cohesiveness.

Evans et al. (1977) fixed dough samples in buffered glutaraldehyde followed by alcohol dehydration and critical point drying. They found that the gluten sheet ruptured at the starch protein interfaces. This was thought to have occurred in mixing, but may have been a fixation induced artifact.

Fleming and Sosulski (1978) examined freeze-dried bread samples at 20 kV. Breads supplemented with plant protein were shown to disrupt the protein-starch complex present

in the control bread. The foreign proteins cause a ruptured cell structure and small pores in the bread. Cell walls of supplemented breads were thick, complex structures in contrast to the thin, sheeted walls of the control bread.

In a study of the microstructure of a wheat flour dough substituted with liquid cyclone processed cottonseed flour, El-Minyawi (1980) observed a disruption of the protein matrix predominately in areas near starch granules in the substituted doughs. It was postulated that these weakened sites permitted the escape of gasses needed for proper rising and thus loaf volume.

El-Minyawi (1980) also studied the effects of two different specimen preparation techniques on structural detail of wheat doughs. Glutaraldehyde fixation followed by ethanol dehydration and critical point drying was found to alter the structure of the flour-water doughs to a much greater extent than the freeze drying process. This work confirmed the earlier findings of Aranyi and Hawrylewicz (1969). Chemical treatment of the samples resulted in separation of starch granules from the protein. The gluten sheet was no longer continuous, instead it was torn and stringy in appearance.

In a study by Evans et al. (1981), gold coated freeze dried dough samples were examined at 10 kV. They found that optimally developed doughs were characterized by starch

granules covered by a continuous gluten layer. In under mixed doughs, gluten was not uniformly developed and starch granules tended to be found in clusters. Over mixed doughs exhibited gluten that had broken down and pulled away from the starch grains collapsing into spindly webs.

Microscopy was also used to study the effect of wheat germ on gluten development (Moss et al.,1984). With added wheat germ, gluten developed more rapidly and was easily over mixed leading to thin films of protein spreading over the starch. These films were deemed incapable of good gas retention and were thought responsible for poor loaf volume in the wheat germ added loaves.

More recently, Moss (1985), has made use of SEM to look at the effect of starch damage in bread flours. Moss found the functional properties of flour proteins can be adversely affected by damaged starch.

Using a slightly different approach, Hermansson (1983) investigated the structure of gluten gels. Findings from this study show that the gluten protein matrix was very dense. No changes in protein structure were observed in the temperature range -15° to 121°C at a magnification of 10,000 X.

A more recent study of gluten gels (Hermansson and Larsson, 1986) in which a delipidized gluten sample and a control gluten sample were analyzed, proposed that the structure consists of globular aggregates with a hydrophobic

nucleus and a hydrophillic surface area. Results of this study showed that the formation of the gluten gel was due to proteins only and not to lipid-protein interaction.

EXPERIMENTAL DESIGN

The study was approached in five phases. Bean flours used in the study were milled from dry roasted air-classified navy beans (*Phaseolus vulgaris*). Initially, globulin proteins were extracted from two bean flour fractions, the fines or high protein fraction and the cotyledon fraction. Each of these was obtained from two grades of beans; prime and cull. Electrophoresis showed no difference in banding patterns among the protein extracts. Subsequently, all proteins were acquired from the prime fines to increase yield. The second phase of research consisted of three farinograph studies. The first study examined the effects of the four bean flours on farinograph parameters of absorption, arrival time, peak time, departure time, stability, mixing tolerance index, and time to break down. Wheat flour was substituted with the bean flours at 0, 5 and 10% based on the weight of the flour. The second study evaluated the effects of the extracted globulin proteins on these same dough properties. Proteins were incorporated into bread flour at levels presumed present in the high protein fraction substituted at the 0, 5, and 10%

levels. Farinograph studies 1 and 2 were arranged in a 2x2x3 completely randomized block design. The third study was done to determine the rheologically effective thiol and disulfides in legume protein substituted doughs using a quantitative method employing dithiothreitol (DTT) as a titrant in determination of disulfides (SS) and *N*-ethylmaleimide (NEMI) in determination of sulfhydryls (SH). In a third phase, total sulfhydryls and disulfides were determined photometrically. Results were used to examine the relationship of total SS and SH groups to the findings from the reactive SS and SH group study. Each curve was produced in duplicate. The fourth area of research was a scanning electron microscope examination of aspects from each of the farinograph investigations. The final research phase consisted of statistical analyses of the data.

MATERIALS AND METHODS

Commercially treated hard red wheat flour was procured from the Pillsbury Company Minneapolis, Minnesota. The flour specifications provided by Pillsbury characterize the flours as follows: moisture 14.0%, ash 0.49% and protein 12.6%. Aytex wheat starch was donated by Olgilvie Mills, Inc. Minnetonka, Minnesota. Product analyses as provided by Olgilvie Mills listed moisture as 9.0%, maximum protein 0.4% and maximum ash as 0.4%. Chemicals used for assays were of reagent grade.

Bean Flour Procurement and Processing

Prime and cull navy beans (*Phaseolus Vulgaris* L.) from the 1984 Michigan crop were dry roasted and fractionated at the Food Protein Research and Development Center, College Station, Texas. The dry roasted bean fractions were shipped to the Alpine American Corporation; Natick, MA for milling and air classification.

Roasting and Dehulling

The prime and cull navy beans were dry roasted in a newly developed particle-to-particle heat exchanger at the

Food Protein Research and Development Center's Oil Mill at Texas A&M University. A schematic drawing of this gravity flow reactor is presented in Figure 1. In this process, ceramic beads were heated to 205°C in a firebrick furnace. The heated beads were then mixed with the beans in a 1:5 ratio of beans to beads. This was accomplished in a stainless steel mixing chute which maximized heat transfer from the beads to the beans and controlled the flow of bean and beads into the roasting chamber. The beans and beads then passed into a flow-through stainless steel roaster section which facilitated heat transfer between the heated beads and the beans. The capacity and residence time of beans in the roaster was controlled by a slide gate and vibrating feeder. This mechanism controlled the flow of the bean-bead mixture from the roaster. Roasted beans were separated from the ceramic beads by a screen separation unit. Beans were immediately cooled in a pneumatic system which prevented additional cooking and browning from prolonged heating. They were then rescreened to remove any remaining beads and cracked through a corrugated roller mill (Ferrell Ross; Oklahoma City, OK). Hulls were removed by air aspiration (Kice Metal Products; Wichita, KS). The hot beads were recycled back to the furnace by way of stainless steel bucket elevator. Operating conditions are outlined in Table 5.

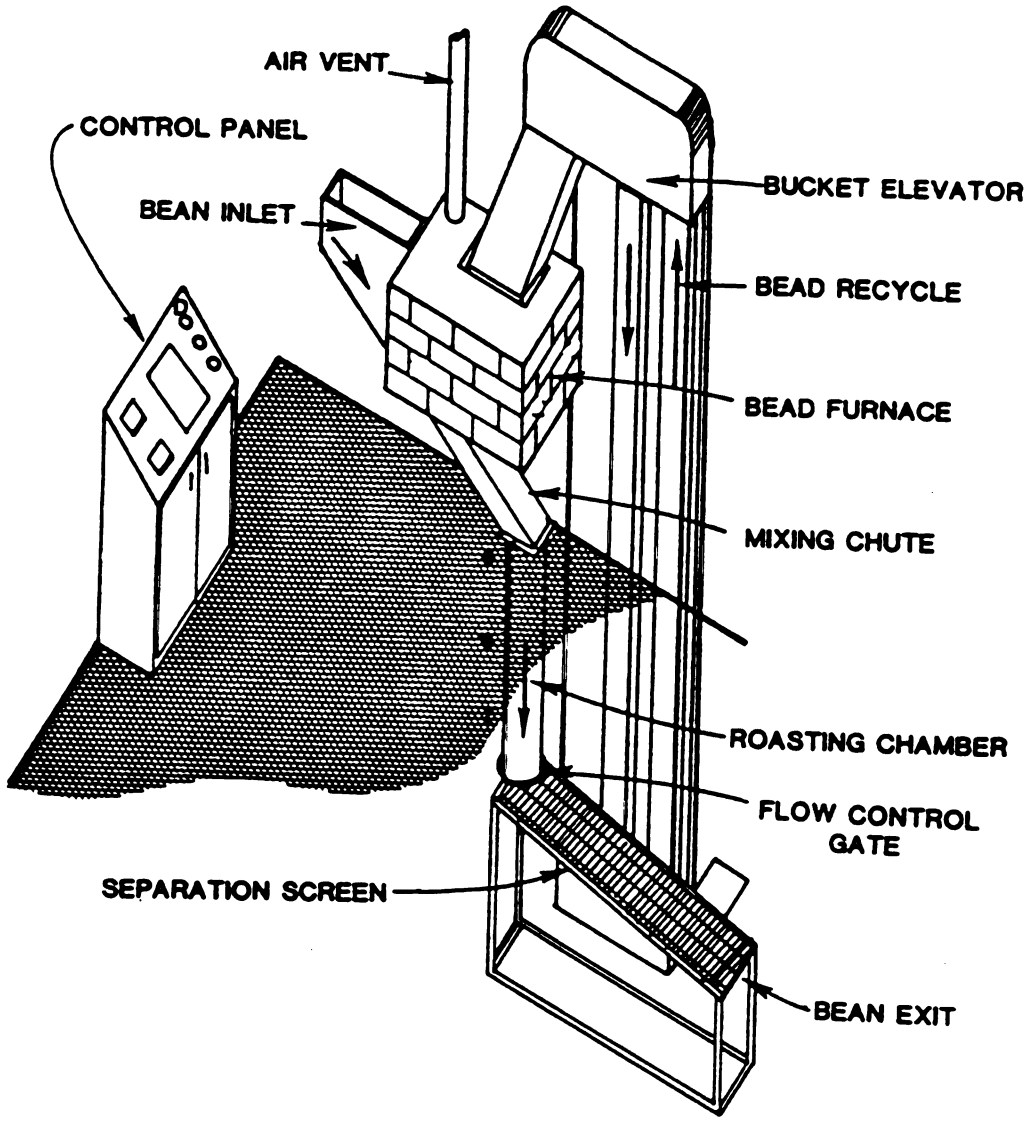


Figure 1. Schematic drawing of the gravity flow reactor designed for dry roasting edible beans.

Table 5. Operating conditions for beans processed in the gravity flow reactor

Parameter	Status
Bean flow (kg/hr)	89
Bead flow (kg/hr)	445
Bean:bead ratio	1:5
Residence time (sec)	90
Furnace temperature (°C)	680
Bead entrance temperature (°C)	205
Bead recycle temperature (°C)	160
Product exit temperature (°C)	106

(Farnsworth and Lusas, 1987)

Grinding and Air Classification

The cracked cotyledons were milled and air classified at Alpine American Corporation; Natick, MA. The cotyledon pieces were ground in a 250 CW Alpine Stud Impact Mill using conditions detailed in Table 6. Air classification of the flours yielded two fractions, designated Coarse I (an intermediate starch fraction) and Fines I (an intermediate protein fraction). The Fines I were further classified giving a Coarse II (high starch) and a Fines II (high protein) fraction.

Table 6. Grinding conditions for dry roasted navy beans

Parameter	Status
Mill	250 CW
Housing (rpm)	11,784
Door (rpm)	5,647
Main motor load (amps)	23.2
Door motor load (amps)	15.7
Feeder throughput (lb./hr.)	635

(Farnsworth and Lusas, 1987.)

Sample Materials

The flour fractions were packaged in polyethylene bags fitted into fiber storage drums and shipped to Michigan State University. Flours were stored at 4°C prior to analyses. The high protein Fines II and the whole dehulled cotyledon fractions obtained from both prime and cull beans were employed for this study.

Isolation of Globulins from *Phaseolus* Flours

Globulin proteins were extracted from the air classified flours using a modification of the procedure described by McLeester et al. (1973). The phaseolin (G1) protein was precipitated by dilution of the clear supernatant with five volumes of distilled water as suggested by Sun and Hall (1975).

The globulins of *P. vulgaris* are soluble at acid pH; the largest portion of albumin proteins are precipitated under these same conditions. Thus, they are not extracted by the NaCl-ascorbate system and do not contaminate the globulin fraction. The globulins can easily be separated from the minor acid-soluble albumins by lowering the salt concentration of the extract.

Preparation of Extracts

Five grams of bean flour were placed in a Sorvall Omini-Mixer 17150 (Ivan Sorvall Inc.; Newtown CT) with 50 ml 0.5M NaCl, 0.25M ascorbate and ca 2 ml Thomas Antifoam Spray (Arthur H.Thomas Co.; Philadelphia, PA). Continuous mixing for three minutes at speed 5 (4,800 rpm) gave a homogeneous sample.

Centrifugation

The bean flour suspension was centrifuged (4°C) at 14,000 rpm in an IEC Model B-20A centrifuge (Damon/IEC Division; Needham Hts., MA) for 30 minutes. The supernatant was decanted and further resolved by centrifugation at 14,000 rpm for 30 minutes, two additional times, discarding the pellet from each of the three runs. To the final clear supernatant, five volumes (ca 200 ml) of deionized water were added. A white precipitate formed immediately and was pelleted by centrifugation at 14,000 rpm for 30 minutes. This pellet, designated G1 was collected and frozen at -20°C prior to freeze drying. A small amount of supernatant was diluted with 2 volumes of distilled water to determine that no further precipitation would occur.

Dialysis

The supernatant was dialyzed against deionized water at 4°C over night, the water was changed a minimum of four

times. Silver nitrate (2%) was used as an indicator of chloride ions. The precipitate was sedimented as described above. This pellet was designated globulin fraction G2 (lectin) and frozen at -20°C before freeze drying. Prior to use, the dialysis tubing (Spectrum Medical Industries, Inc.; Los Angeles, CA) was heated at 100°C for 20 minutes in a solution containing 1 g $NaHCO_3$ and 0.1 g Na-EDTA per 100 ml, then rinsed in distilled water. The process was repeated three times. The tubing was rinsed thoroughly in distilled water and stored at 4°C in 50% ethanol.

Freeze Drying

Protein samples were frozen at -20°C in covered petri dishes that were securely sealed with Parafilm. Just prior to transfer to the freeze dryer, covers were replaced with filter paper and cheese cloth. Proteins were subsequently freeze-dried for two days in a VirTis Unitrap II freeze dryer (VirTis Company, Inc.; Gardiner, New York). Proteins were dried with a system pressure of 4-6 x 10^{-2} Torr and tray temperature approaching 50°C. Dried materials were placed in desiccators prior to weighing. Proteins were ground with mortar and pestle and the fine powders were placed in tightly covered glass jars and stored at -20°C.

Chemical Analyses

Purified proteins were analyzed for protein concentration (Lowry assay), moisture, total sulfhydryls and disulfides. Electrophoresis was used to separate polypeptides. Bean flours, wheat flour and starch were evaluated for moisture, percentage total protein (Kjeldahl nitrogen analysis), total sulfhydryl and disulfide groups.

Moisture

Moisture content of flours and protein fractions were determined in duplicate using a slight modification of AACC Method 44-40 (1983). Two gram samples were weighed into previously dried moisture dishes (diameter 55mm, height 15mm). The uncovered samples were dried under a partial vacuum (ca 25 mm Hg) at 95-100°C for eight hours in a Hotpack Vacuum Oven, Model 633 (Hotpack Corp., Philadelphia, PA). The dried samples were cooled in a desiccator and reweighed after reaching room temperature (25°C). Moisture was calculated from weight loss and expressed as percentage moisture as follows

$$\% \text{ Moisture and Volatile Matter} = \frac{\text{Loss of Moisture}}{\text{Wt. of Sample}} \times 100$$

Nitrogen Analysis by Kjeldahl

Nitrogen analyses were performed using a micro-Kjeldahl apparatus. Duplicate 0.5 gram samples were digested at 400-500°C in a mixture of concentrated H_2SO_4 and employed $CuSO_4$ and Na_2SO_4 as catalysts. Samples were distilled according to AOAC Methods 2.057, 14.026 and 4.068 (1980) in a Buckii Kjeldahl distillation apparatus (Buchii Kjeldahl Machine, Brinkmann Instruments, Westbury, NY.). Percentage nitrogen was calculated as

$$\% \ N = \frac{(\text{ml acid} - \text{Blank}) \times \text{Normality} \times \text{Equiv. Wt. N}}{\text{Sample Wt.}} \times 100$$

Percentage nitrogen was multiplied by 6.25 to provide percentage total protein for bean flour and by a factor of 5.70 to provide total protein in the wheat flour.

Folin-Ciocalteu Assay

The Folin-Ciocalteu assay (Lowry et al., 1951) is a sensitive test of protein concentration using two color producing reactions to quantitate protein photometrically. Alkaline copper ions yield a deep blue color in the presences of peptide bonds. The color is caused by the reduction of phosphomolybdate phosphotungstate salts in the reagent by the tyrosine and tryptophan of the proteins.

To determine protein concentration of the freeze dried protein powders, a modification of the original method was used (Oyama and Eagle, 1956). A standard curve was

prepared with bovine serum albumin (BSA) using 1 to 60 ug protein in 0.5M such that 100ug/ml = 0.066 OD at 280 mu. Protein solutions of unknown concentration were prepared by solubilizing 0.01 gram protein in 250 ml NaCl to equal ca 40 ug/ml. Dilutions (1.0, 0.5, 0.25 and 0.1 ml) were made to 1.0 ml in 13x100 mm test tubes. Three ml of a solution consisting of 2% Na_2CO_3 in 0.10 N NaOH and 0.5% $CuSO_4$ and 1.0% Na tartarate in 0.1 N NaOH mixed 50:1 was added to the protein with stirring. After 10 minutes, the Folin-Ciocalteu reagent (0.3 ml) diluted 1:1 was mixed into the protein solution. Color developed for 30 minutes. The samples were read at 550 and 750 mμ.

Polyacrylamide Gel Electrophoresis

One dimensional polyacrylamide gel electrophoresis (PAGE) performed in the presence of sodium dodecyl sulfate (SDS) was used as a means of separating polypeptide chains according to molecular weight to assess possible contamination of the purified fractions. Freeze dried protein samples were subjected to discontinuous SDS-PAGE according to the methods of Ornstein (1964) and Davis (1964). All electrophoretic studies were performed in glass slab gels measuring 15.0 x 15.0 cm. All gels were allowed to sit a minimum of 15 hours before use to assure complete and consistent polymerization of the gel. Lower gel concentration was 12.5%. Protein staining was by Coomassie

Blue R at 0.1% in water:methanol:acetic acid, 4.5:4.5:1.0. Molecular weight standards were obtained from Bio-Rad Laboratories, Richmond, CA.

Amino acid Analyses

Prime high protein and prime whole cotyledon bean flour fractions were hydrolyzed in 6N HCL by heating at 100°C, under nitrogen, for 48 hours. Samples were filtered through Whatman No. 2 filter paper and examined using ion-exchange amino acid analysis (D-300, Dionex Corporation, Sunnyvale, CA.). Duplicate samples were analyzed. A short run was used to calculate amino acid values for lysine, tyrosine, phenylalanine, isoleucine, leucine and valine. Aspartic acid, arginine, alanine, glutamic acid, glycine, histidine, serine and threonine values were calculated from a long run. Results are reported as grams of amino acid per 100 grams protein.

Sulfhydryl Groups

Sulfhydryl groups were determined according to the method of Ellman (1959). In this procedure 5,5'dithiobis-2-nitrobenzoic acid (DTNB), reacts with aliphatic thiol compounds to produce a highly colored anion that can be used to measure the thiol concentration (Em = 13,600 at 412 mμ).

Five ml of 0.01M sodium phosphate buffer, pH 8.0, containing 1.0% sodium lauryl sulfate and 0.4% disodium

ethylenediamine tetraacetate (EDTA) were added to approximately 10 mg dry sample. The buffered thiol compound was heated in a boiling water bath for 30 minutes, and cooled at room temperature for ca 20 minutes. While the mixture was cooling, the DTNB reagent was prepared by dissolving 40 mg DTNB in 10 ml 0.1 M sodium phosphate buffer, pH 7.0. The reagent (0.2 ml) was added to the thiol mixture. The color developed rapidly. Two blanks were run with the samples as references. After 30 minutes the samples were filtered through Whatman No. 4 filter paper. The absorbance was determined at 420 mμ. Absorbance was also read at 600 mμ to determine that no turbidity was present. Results are expressed as mmoles SH/50 grams dry material.

Total Sulfhydryls (Disulfides)

The determination of disulfides was accomplished by a modification of the method of Cavallini et al. (1966). In this procedure, sodium borohydride ($NaBH_4$) was used as a reducing agent. Disulfides were reduced to sulfhydryls; then excess $NaBH_4$ was destroyed and the SH concentration was determined.

The reagents used in this assay were 0.05 M sodium phosphate buffer, pH 7; 0.02 M Na_2EDTA; Urea-sodium borohydride solution (10 grams urea, 0.25 grams $NaBH_4$ dissolved in 10 ml deionized water); potassium phosphate-HCL solution (13.6 grams mono potassium phosphate, 1.66 ml HCL

in 100 ml deionize water); 1-octanol; acetone and DTNB reagent as detailed in the determination of SH groups.

Three mg dry sample were dissolved in 1.0 ml sodium phosphate buffer containing the 0.02 M Na_2EDTA (10 ml/200 ml buffer). To this suspension, two ml 1-octanol and 1.0 ml of the urea-sodium borohydride solution were added. The tubes were vortexed and incubated in a water bath at 40°C for 30 minutes. After cooling at room temperature (ca 10 minutes), 0.5 ml of the potassium phosphate-HCl solution was added dropwise, wetting the walls of the reaction vessel to destroy all traces of the sodium borohydride. After five minutes, 1.0 ml of acetone was added to complete sodium borohydride destruction. This was vortexed to ensure all areas of the test tube were covered. The sulfhydryl determination was then performed. The DTNB reagent (0.2 ml) was added with stirring. Color was allowed to develop for 30 minutes. Samples were filtered and absorbances were read at 412 mμ. Total disulfide groups, including pre-existing sulfhydryl groups are expressed as mmoles/50 grams of flour. An extinction coefficient of 13,600 as suggested by Ellman (1959) was used in calculating values.

Farinograph Studies

The effect of bean flours and bean flour proteins on composite dough quality was evaluated using the Farinograph, manufactured by C.W. Brabender Instruments Inc.; S.

Hackensack, N.J.; Model PL-2H, Dynameter 2092. For all studies the small (50 g flour) mixing bowl was used. Temperature was maintained at 30± 0.1°C by the Thermobath (type P 60-B). Titration was conducted with the small buret. All water was delivered within 25 seconds from the onset of mixing. The fast speed (63 rpm) was used for all testing.

Study 1 Wheat/Bean Flour Blends

The effect of bean flour fractions on farinograms was investigated. Five and ten percent of the weight of the hard wheat bread flour was substituted with the prime fines, cull fines, prime cotyledon and cull cotyledon fractions on a 14% moisture basis.

Measurements were made according to AACC Method 54-21A (1983) using the constant flour weight technique. Moisture content of all flours was determined so that precise measurements could be made on a 14% moisture basis. Weight of the flour was adjusted according to AACC Table 82-23. Analyses were performed in triplicate arranged in a completely randomized block design. Results were obtained from the following parameters:

Absorption is the amount of water required to center the curve on the 500 Brabender Unit (BU) line for a flour-water dough. All absorptions were calculated on a 14% mb as follows

$$\text{Absorption \%} = 2(X + Y - 50)$$

Where X = ml water and Y = g flour used equivalent to 14% moisture basis.

Arrival Time is the time needed for the top of the curve to attain the 500 BU line from the time of water addition (time zero). It is a measure of the rate of hydration and is used as an indication of flour strength.

Peak Time, or dough development time is the interval from time zero to the point of maximum consistency.

Departure Time is the time from zero to the point where the curve leaves the 500 BU line on the chart. Flour strength is associated with departure time.

Stability is the difference in minutes, between arrival time and departure time. The value is an indication of true stability.

Mixing Tolerance Index (M.T.I.) is the difference in Brabender units measured from the top of the curve at the peak to the top of the curve five minutes after peak time.

Time-to-Breakdown is the time from time zero to the time where the curve has fallen 30 BU from the peak height.

Study 2 Wheat/Bean Protein Blends

The second study evaluated the effect of substituting bread flour with the major navy bean storage protein, phaseolin (G1) and a lectin protein G2 on farinograms. The freeze dried phaseolin and G2 proteins were added into the system in amounts equal to those determined to be present in the fines fraction when substituted at 5 and 10%. The proteins were added back individually and in combination. Wheat starch was used as a second control in order to separate the effect of dilution of the gluten from the effect of the protein on the dough structure.

Analyses for studies #1 and #2 were conducted in triplicate, both were arranged in a completely randomized block design. The identical curve parameters were examined in Study #1 and Study #2.

Study 3 Rheologically Active Thiols and Disulfides in Wheat/Bean Protein Blends

In the third farinograph study, estimates of rheologically active thiol and disulfide groups were obtained using a quantitative method of Jones et al. (1974). Farinograph conditions were as in the previous studies. Dithiothreitol (DTT) was the titrant used in determination of rheologically effective disulfide groups in the protein substituted dough systems. To determine the content of disulfide important for mixing resistance, dough was mixed

to full development, as mixing continued DTT was added serially (10 to a cumulative 200 µmole) in 2.5 - 4 minute intervals. Disulfides important to development were determined by addition of dithiothreitol (0 - 100 µmole) at time zero.

To distinguish rheologically important thiol groups, *N*-ethylmaleimide (NEMI) (0 - 60 µmole) was added in 0.1 ml ethanol to a saline solution containing 1 g NaCl and a volume of water adjusted to produce a maximum resistance of 500 BU. This was used to titrate flours at time zero. The dough was mixed for 30 minutes, at which time resistance to mixing was measured.

Scanning Electron Microscopy

The effects of *Phaseolus vulgaris* flours and extracted globulin proteins substituted in a wheat dough system were studied. The primary objective of this phase of research was to determine if structural differences could be discerned among a control wheat dough, and doughs substituted at the 10% level with high protein bean flour obtained from prime and cull beans, whole cotyledon flour from prime and whole beans and doughs with phaseolin and G2 proteins added at the levels present in the 10% substituted high protein dough. Doughs were mixed in the 50g bowl of the farinograph at high speed until peak time was reached.

Additionally, specimens from the rheological studies were examined. Samples for these studies were taken from the farinograph at points that were determined to have the maximum number of thiols involved in mixing tolerance, maximum number of disulfides involved in development and the maximum number of disulfides involved in resistance to mixing.

Specimen Preparation

Samples for low temperature SEM viewing were excised from the farinograph bowl with a surgical blade. Care was taken to avoid stretching the dough. These farinograph prepared doughs were immediately enveloped in plastic wrap for transport to the Center for Electron Optics. The EMscope SP-2000 Sputter-Cryo system in conjunction with a JEOL JSM-35C SEM was used for this work. A small portion, less than $5mm^2$, of the fresh sample was mounted on the specially designed copper stub fitted with a protective stainless steel shroud that could be opened or closed by the operator. The mounted specimen was plunged into subcooled nitrogen in the freezing chamber of the apparatus and cooled to the temperature of the cryogen (ca. -196°C). From here the shielded sample was moved, under vacuum, to the electron microscope which had been equipped with a cold stage. The specimen was etched to remove a slight water film that might otherwise have obscured surface detail. This was done by

heating the sample to -65°C and holding it at that temperature for three minutes. The sample was then transfered, under vacuum, to the preparation chamber. The preparation chamber contained a copper cold stage cooled to less than -160°C by liquid nitrogen. Here the sample was carefully fractured prior to sputter coating for four minutes. The specimen was then reinserted (again shrouded and under vacuum) on to the cold stage of the microscope for observation of the frozen hydrated specimen. The microscope stage temperature was maintained at approximately -175°C by cooling with a dewar of liquid nitrogen. Microscope settings for the cryo stage work included a 15kV beam, a condenser lens setting of 600 and a working distance of 39 mm.

Statistical Testing

The effects of bean flour fractions and protein substitution on farinograph parameters were analyzed using the SPSS/PC; Statistical Package for the Social Sciences, 1986. The data derived from experiments involving the disulfide and sulfhydryl groups were analyzed using the MSTAT (1985) program version 3.01.

RESULTS AND DISCUSSION

Proximate analyses revealed the total nitrogen content of the bean flour fractions ranged from 3.6 to 7.4. These nitrogen contents are equivalent to total protein contents of 22.5 to 46.6 g per 100 g flour on a dry weight basis. As expected, the fines had the highest protein content while the cull cotyledon had the lowest. The wheat flour control had a total nitrogen content of 2.2 equivalent to 12.7% protein which is essentially the same value specified by the manufacturer. Results are presented in Table 7.

The moisture content of the bean flour fractions ranged from 4.74 to 9.48. Cotyledon flour from prime or cull beans had a higher moisture content than the flours milled from either grade of the high protein fines.

Table 7. Mean total protein and moisture values of bean flour fractions and wheat flour.

Flour Type	Total Protein[a]	Moisture[b] (%)
Wheat	12.67	10.74
Fines	46.61	5.35
Cull Fines	45.57	4.74
Cotyledon	24.29	9.03
Cull Cotyledon	22.51	9.48

a n=2
b n=3

Amino Acid Profile

The prime high protein navy bean flour, which had almost double the protein content of the prime cotyledon flour, had consistently higher values for all amino acids than the cotyledon flour (Table 8). The only exception was the value for aspartic acid. A slightly larger amount of this amino acid was deposited in the cotyledon flour fraction. Both of the bean flour fractions have high amino acid values for glutamic acid, aspartic acid, leucine, lysine and arginine but are low in the sulfur amino acids methionine and cystine. Because wheat flour is relatively high in the sulfur amino acids but deficient in lysine, it is a good candidate for incorporation of bean flour to improve overall protein quality.

Table 8 Amino acid composition of prime navy bean flour fractions

Amino Acid (g/100g protein)	Flour Fraction	
	High Protein	Cotyledon
Lysine	6.23	5.60
Methionine	0.29	0.16
Tyrosine	2.84	2.39
Phenylalanine	5.12	4.58
Isoleucine	4.72	3.88
Leucine	7.58	6.62
Valine	5.54	4.84
Aspartic Acid	10.73	12.87
Threonine	4.53	3.83
Serine	5.44	4.37
Glutamic Acid	12.99	11.12
Glycine	3.41	2.93
Alanine	4.43	3.79
Cystine	0.39	0.29
Histidine	2.01	1.86
Arginine	5.60	4.16

Electrophoresis

Polypeptide components of the globulin proteins were separated by SDS-gel electrophoresis. The phaseolin and G2 globulin fractions had entirely different gel electrophoretic patterns (Figure 2). The SDS-PAGE patterns of the phaseolin had three major bands (lanes 1 and 3). Apparent molecular weights for the phaseolin polypeptides closely correspond to those established for the 'Tendergreen' patterns as described by Brown et al. (1981b). Of the three major bands, α, β and γ , the γ band was

found to have less glycosylation than the α or β bands. Ma et al. (1980) found that the α polypeptide contains one more methionine residue than the β polypeptide. Lanes 6 and 7 of Figure 2 contain the lower molecular weight G2 lectin proteins. The molecular weight standards used were phosphoylase B, 97.4k; BSA, 66.2k; ovalbumin, 42.7k; carbonic anhydrase, 31.0k; soybean trypsin inhibitor, 21.5k and lysozyme, 14.4k (lanes 2,5,8).

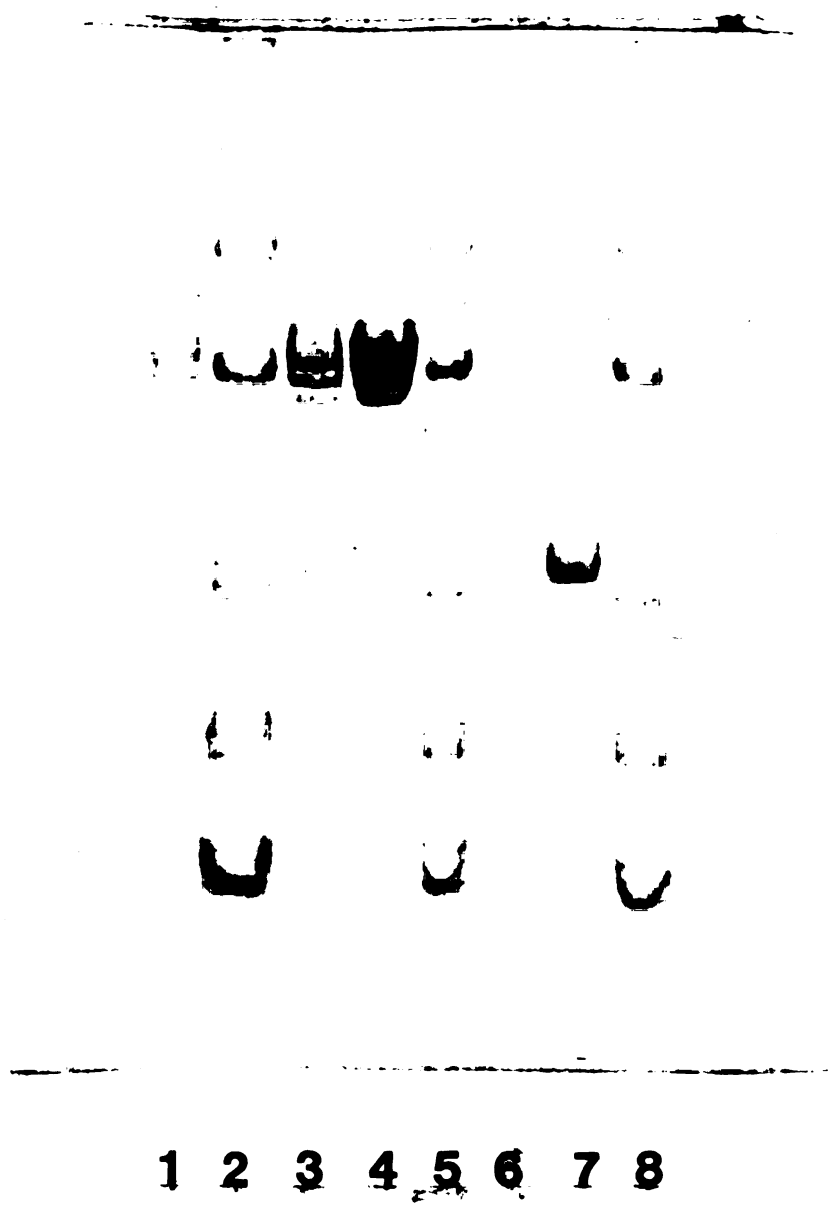


Figure 2. Electrophoretic patterns of phaseolin and G2 lectin proteins extracted *Phaseolus vulgaris:* cv Seafarer. Lanes 1,3,4 - phaseolin protein.
Lanes 6,7 - G2 proteins.
Lanes 2,5,8 - standards.

Farinograph Studies

Farinograph data obtained from wheat flour substituted at levels of five and ten percent with the four navy bean flour fractions were evaluated using standard farinograph parameters. Farinograms of wheat flour substituted with phaseolin and G2 lectin proteins added at levels equivalent to the phaseolin or G2 present in the high protein bean fraction substituted at five and ten percent were evaluated in the same manner. Rheologically active thiol and disulfide groups in the protein substituted doughs were estimated. Dithiothreitol was used to determine reactive SS groups and *N*-ethylmaleimide was added in the determination of reactive SH groups.

Results of analysis of variance are included in the Appendices. Figures are used to illustrate the data from the farinograph studies. Actual values are tabled in the Appendices along with designations of significant differences (SPSS, 1986).

Study 1 Wheat/Bean Flour Blends

Farinograms of systems substituted with the bean flour fractions showed distinctive curves for each bean flour fraction. Few differences were evident between grades. Representative curves are shown in Figures 3-7.

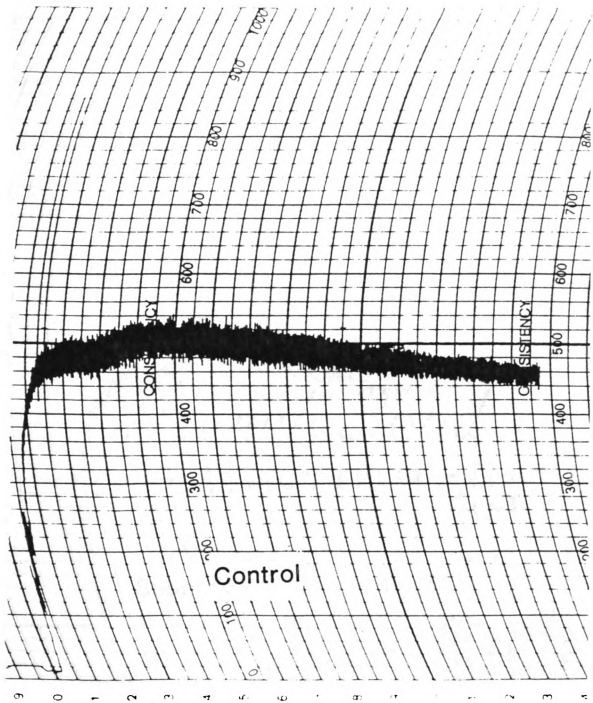


Figure 3. Farinogram of wheat flour dough.

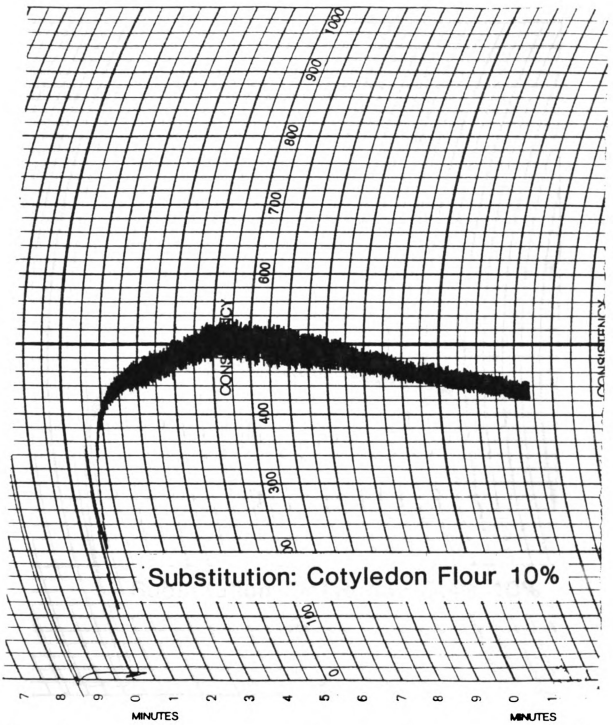


Figure 4. Farinogram of wheat flour dough substituted with 10% navy bean cotyledon flour.

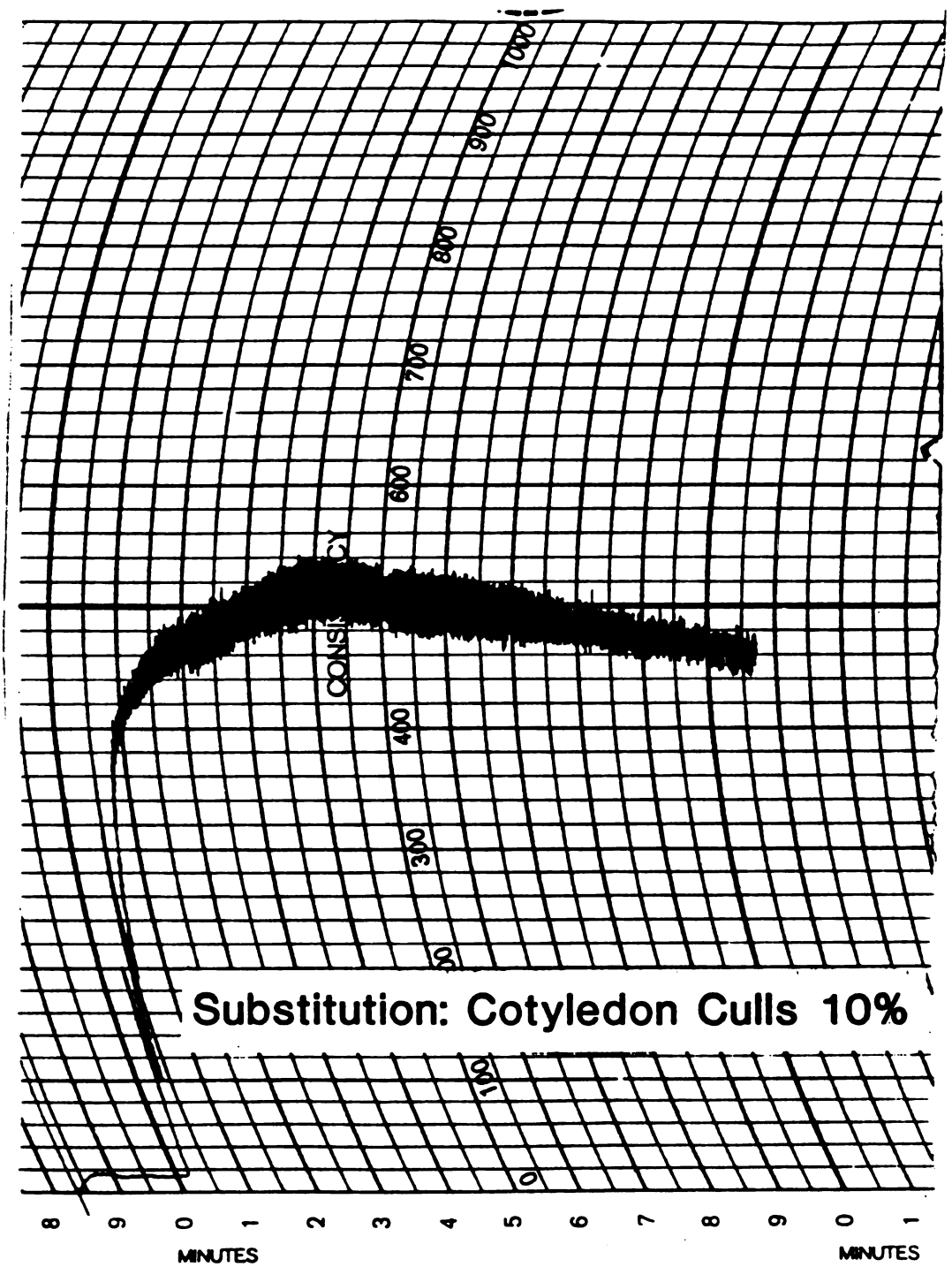


Figure 5. Farinogram of wheat flour dough substituted with 10% cotyledon flour from culls

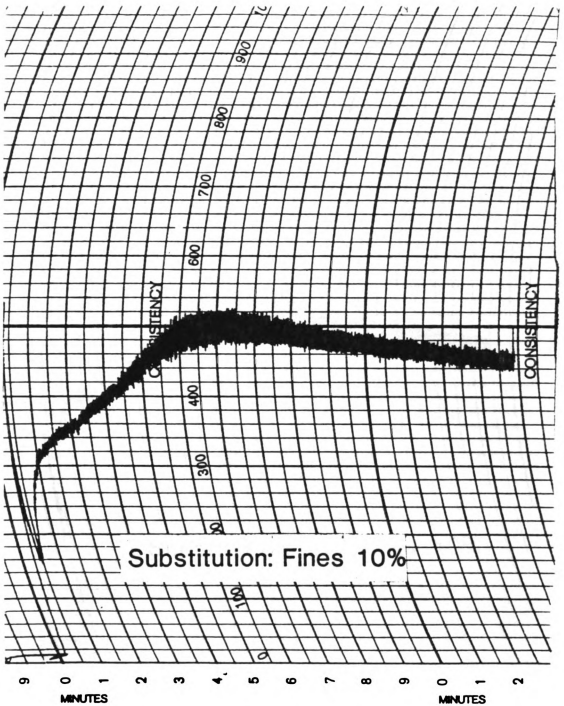


Figure 6. Farinogram of wheat flour dough substituted with 10% high protein navy bean flour.

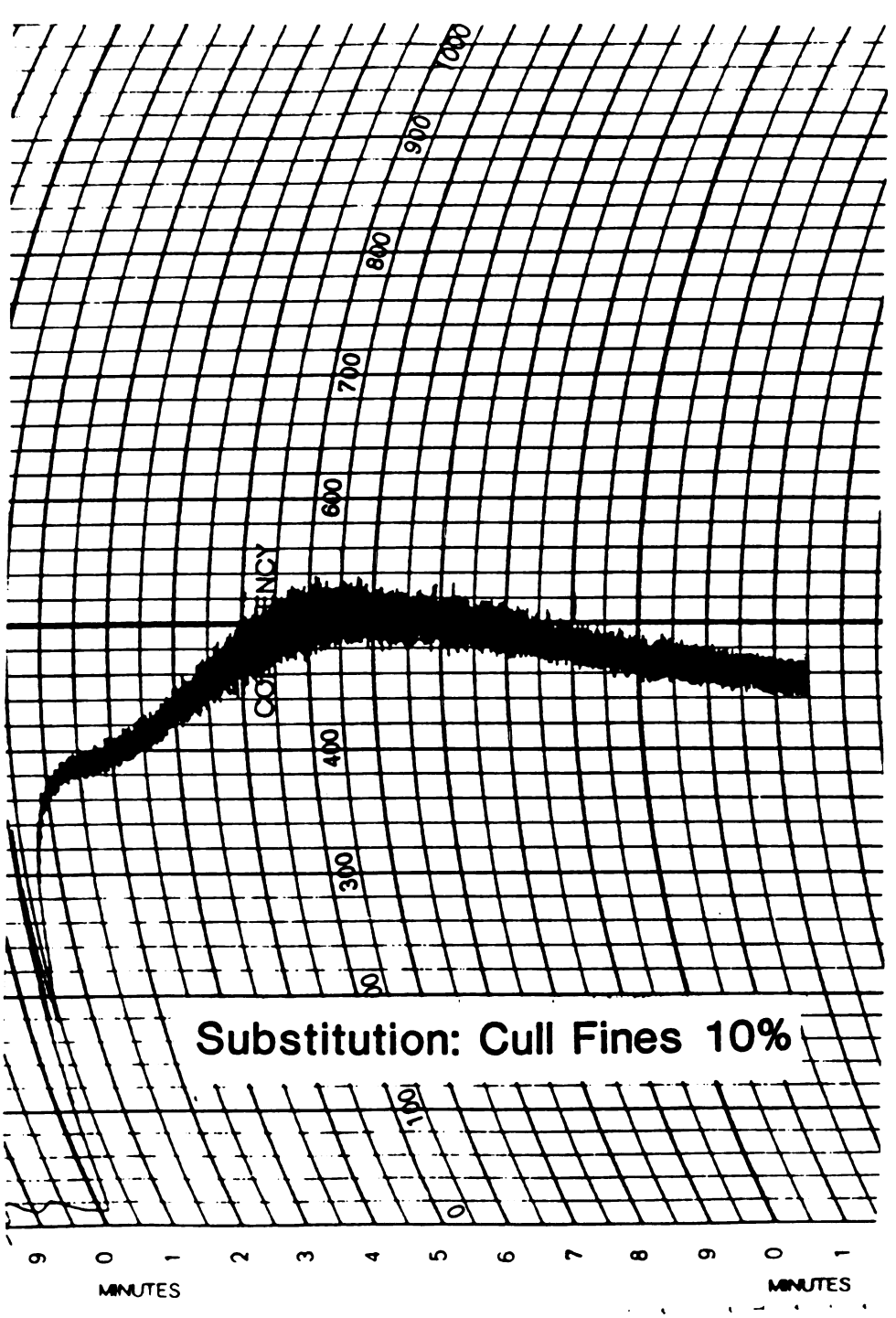


Figure 7. Farinogram of wheat flour dough substituted with 10% high protein navy bean flour from culls.

Flour absorption is recorded as the amount of water necessary to cause the farinograph to center on the 500 BU line. This is generally the amount of water the flour requires to form a dough of optimum consistency for breadmaking. Absorption is primarily dependent on the starch and protein content of the flour. Bushuk and Hlynka (1964) reported that starch absorbs 27-35 percent of its weight in water while gluten absorbs 109-215 percent of its weight in water. In addition, increased absorption of wheat flour substituted with foreign proteins has been reported by many researchers.

Mean absorptions are reported in Figure 8. Absorption results of this study are in agreement with those of previous researchers (Silaula, 1985; Despande et al., 1983; Sathe et al.,1981; D'Appolonia, 1978). Absorption increased with increased protein. At the 5% substitution level, there were no significant differences in absorption between the flour system with cull fines or prime fines. Nor were significant differences found between the flour system containing the cull cotyledon and prime cotyledon bean flour fractions. Absorptions of the wheat flour/cotyledon blends were significantly lower ($p<0.05$) than the absorptions of the wheat flour/high protein fines blend. At the 10% substitution level, no differences were found between the

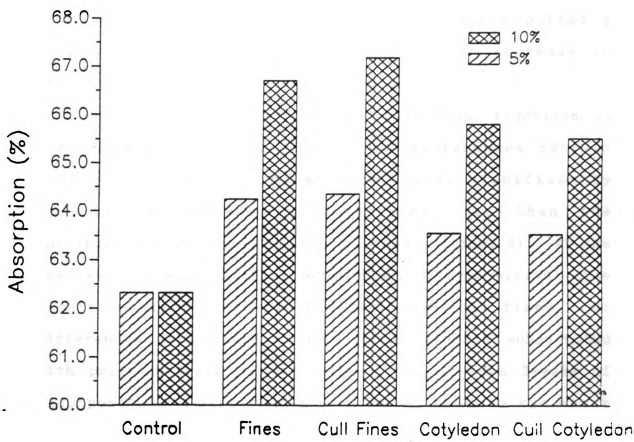


Figure 8. Percent absorption (14% mb) of wheat flour dough substituted with bean flour fractions at 5 and 10%.

grades of cotyledons, however, the absorption of the wheat flour/cull fines blend was significantly higher ($p<0.05$) than that of the wheat flour/prime fines blend.

Arrival time serves as an indicator of the rate of hydration of a particular flour. As was evident from the farinograms, arrival time was greatly delayed for doughs containing the bean flour fractions (Figure 9). Incorporation of the high protein fines had the most substantial impact on arrival times. These findings agree with those of Bushuk and Hlynka (1964) who reported a decrease in the rate of hydration with an increase in protein content.

Wheat doughs substituted with bean flour fractions at the 5% level showed no significant differences between grades although incorporation of fines significantly ($p<0.05$) lenghthened arrival time more than the incorporation of the cotyledon fractions (Figure 9). At the 10% level of substitution, prime fines delayed arrival time significantly longer ($p<0.05$) than the cull fines. No differences were found between flour systems substituted with prime or cull cotyledon fractions at both levels of incorporation. Differences were noted between the wheat flour/high protein bean flour blend and the wheat flour/cotyledon bean flour fractions. The blends containing the high protein bean flour significantly ($p<0.05$) delayed arrival time.

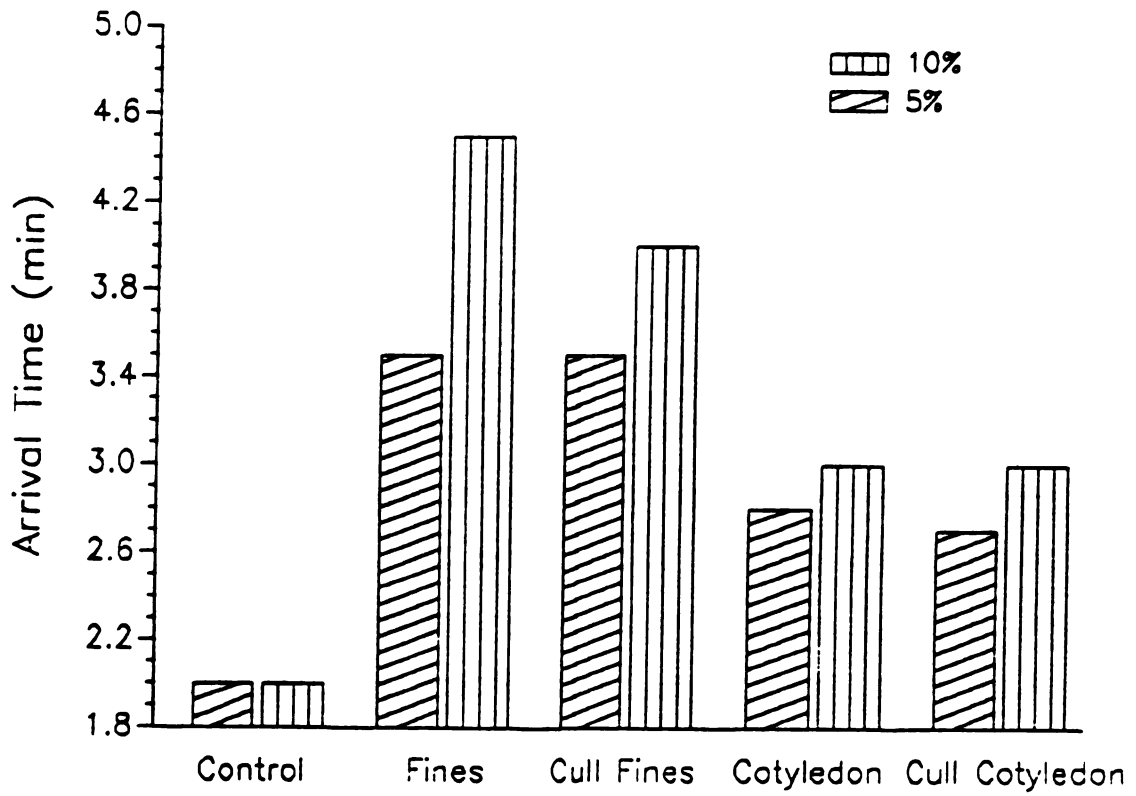


Figure 9. Arrival time of wheat flour dough substituted with bean flour fractions at 5 and 10%

Peak time, often termed development time, is the time from the first addition of water to the time the dough reaches the point of greatest torque. During this phase of mixing water begins to take up active sites and form a developed dough. Farinograms showed that only when the prime cotyledons were substituted in the dough system did the peak time occur significantly earlier ($p < 0.05$) than the control (Figure 10). When substituted at the 10% level, prime fines dramatically lengthened peak time. No differences in peak time were found between the wheat flour/cotyledon flour blends milled from prime or cull material at either level of substitution.

Stability is calculated as the difference in time between arrival time, where the top of the curve first touches the 500 BU line, and departure time, that point where the top of the curve leaves the 500 BU line. Stability is an indicator of the mixing tolerance of a given flour type. Figure 11 illustrates the effect of bean flours on stability. At the level of 5% substitution, no differences in stability were determined between the bread flour control and the system substituted with prime fines. Stability of wheat blends with cull fines, cotyledons or cull cotyledons was significantly ($p<0.05$) less than the control and the prime fines system but, stability was not significantly different among the blends. At the 10% level of substitution, stability decreased for all blends with the

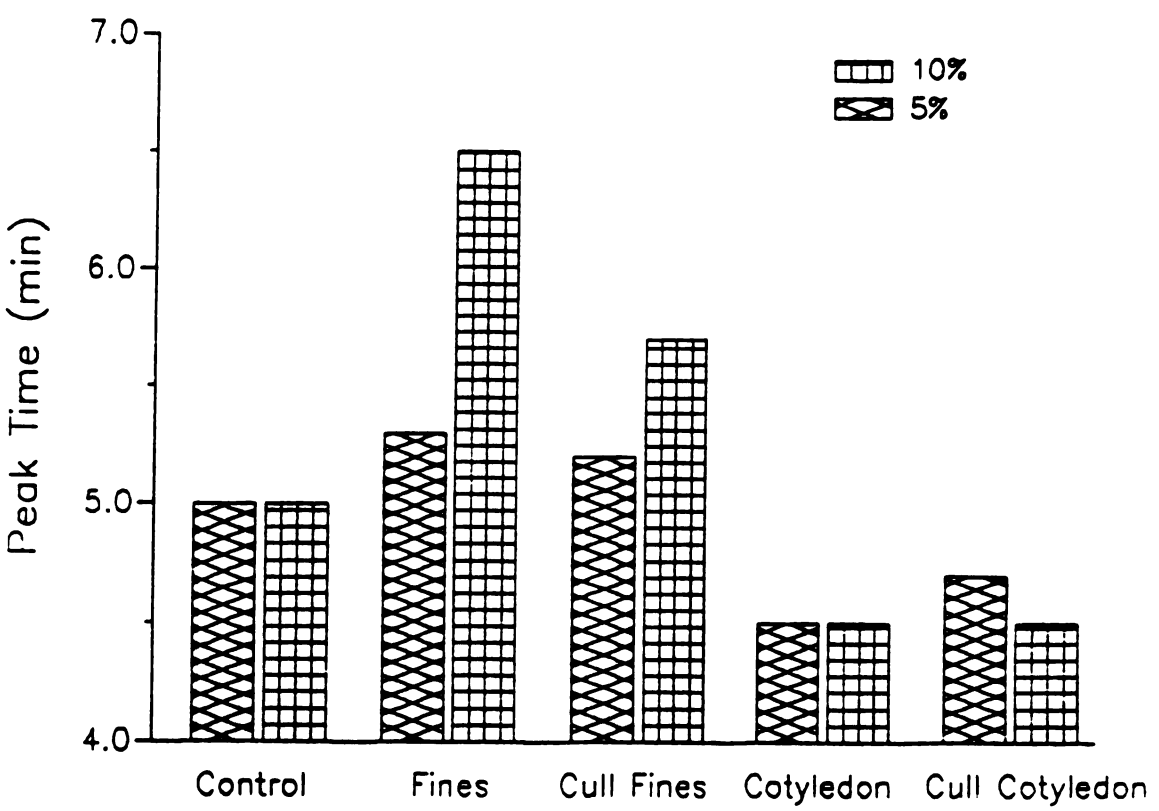


Figure 10. Peak time of wheat flour dough substituted with bean flour fractions at 5 and 10%.

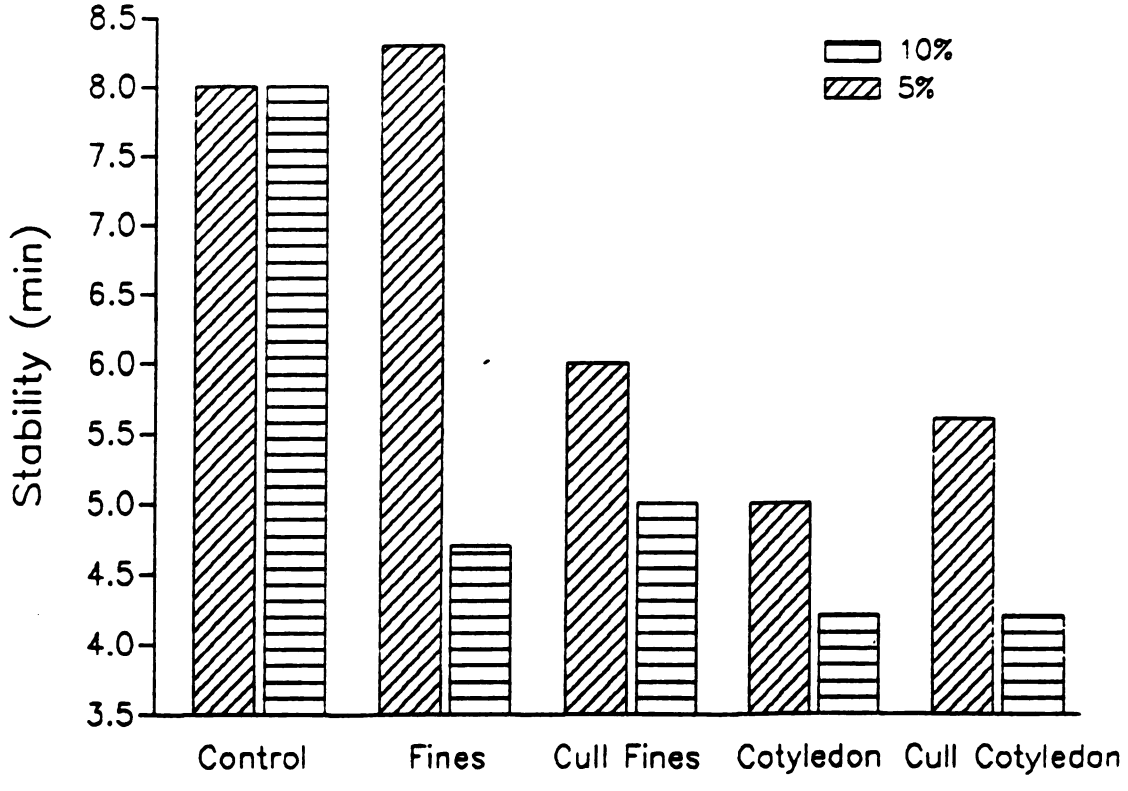


Figure 11. Stability of wheat flour dough substituted with bean flour fractions at 5 and 10%.

prime fines having the greatest detrimental influence on stability. No differences were found between prime or cull high protein blends, nor were differences detected between blends made from prime or cull cotyledon bean flours. Differences were noted between fractions. The wheat flour/cotyledon blends were significantly ($p<0.05$) less stable than their wheat flour/high protein flour counterparts.

The mixing tolerance index (MTI) and the time to breakdown are obtained from the destructive phase of the farinograph curve. These parameters are related to the stability readings. Flour which have good tolerance to mixing have low MTIs. The higher the MTI value the weaker the flour.

Figure 12 shows blending fines at low levels with bread flour appears to improve mixing tolerance, nevertheless, blends of wheat flour/prime fines of 90:10 produced a detrimental effect on the MTI. Data obtained concerning the blends' resistance to breakdown reflect the same trends, at the 5% substitution level, fines delayed breakdown of the wheat flour/ fine blends (Figure 13). Blends substituted with 10% prime fines were not significantly different from the control. However, all other variables were found significantly ($p<0.05$) different from each other.

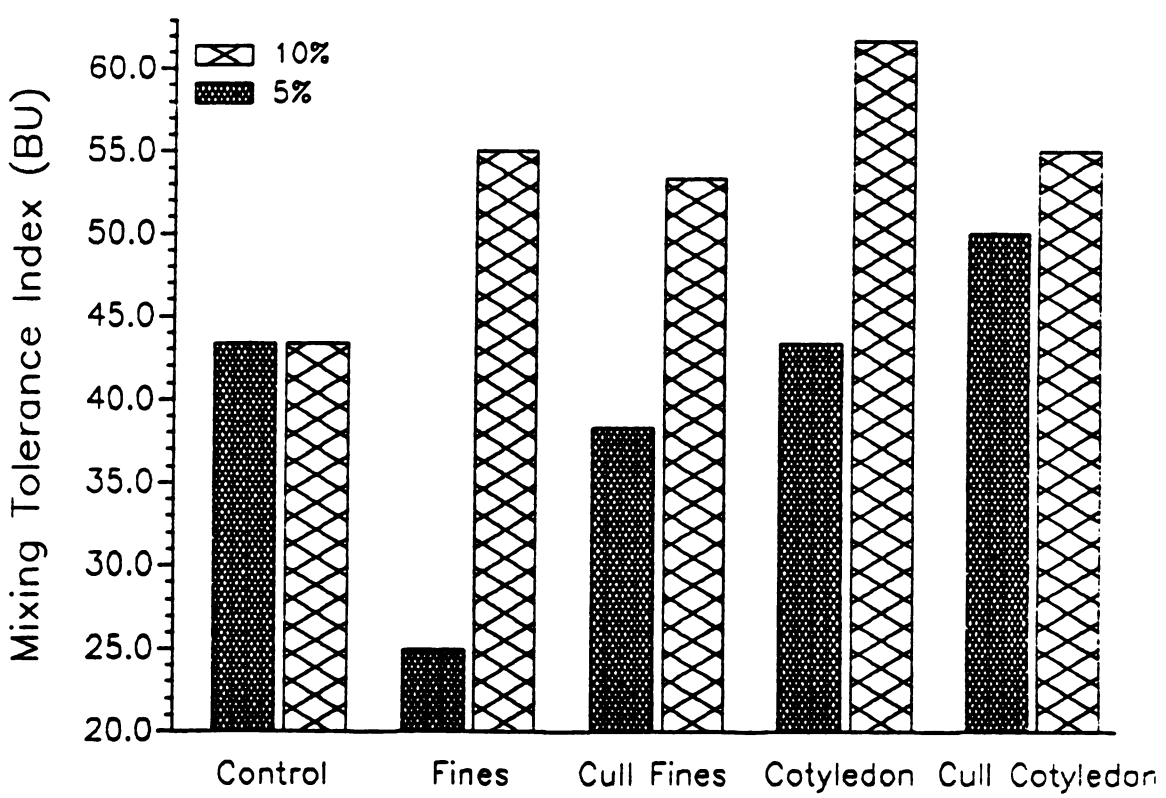


Figure 12. Mixing tolerance index of wheat flour dough substituted with bean flour fractions at 5 and 10%.

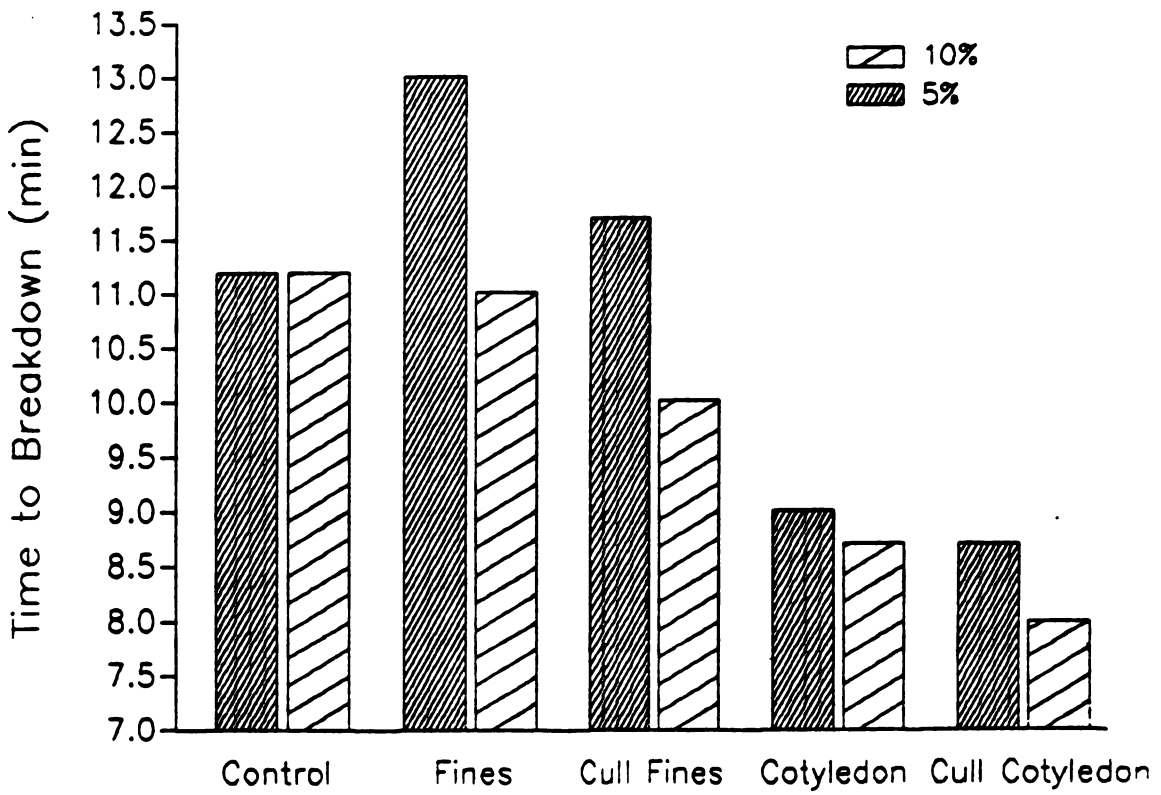


Figure 13. Time to breakdown of wheat flour dough substituted with bean flour fractions at 5 and 10%.

Study 2 Wheat/Bean Protein Blends

In the second phase of farinograph research, farinograms were obtained from bread flour substituted with the globulin proteins. The two globulins were added to the dough singularly and in tandem in amounts equal to the original phaseolin and/or G2 content present in the high protein bean flour. Wheat flour substituted with wheat starch was used as a second control to study the intrinsic effects of dilution of the gluten proteins as compared to the effects of the bean proteins on the gluten proteins. Typical farinograms are presented in Figures 14-17.

Absorption trends were similar for systems with globular protein equivalents at both levels of substitution. Absorption increased significantly with the addition of phaseolin and the combination of phaseolin and the G2 lectin protein. However, addition of G2 alone resulted in an absorption that was not different from the wheat starch substituted dough system. This was expected as the G2 content required to achieve these levels of substitution is quite small. The high protein fines fraction contains 45.22% protein. The G2 lectin proteins contribute approximately 4.5% of that total. Thus, to substitute G2 protein at levels found in dough substituted with fines at the 5 and 10% level in the 50 gram bowl of the farinograph, only tenths of a gram were needed.

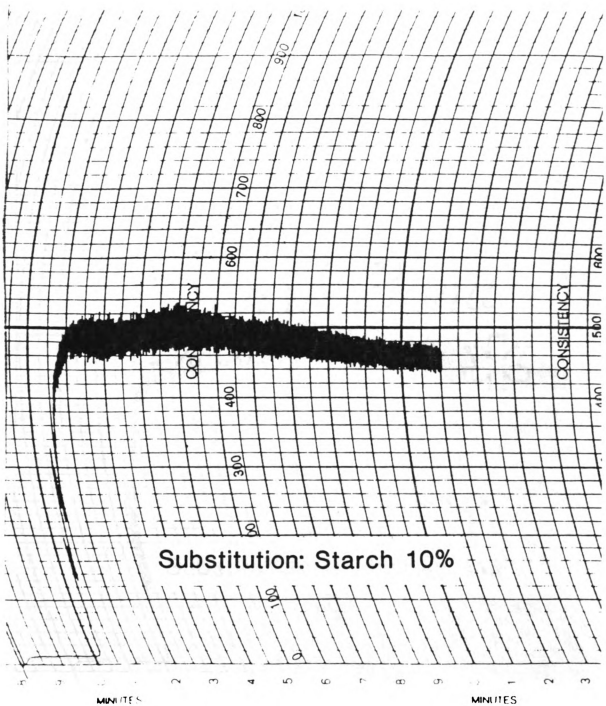


Figure 14. Farinogram of wheat flour dough substituted with 10% wheat starch.

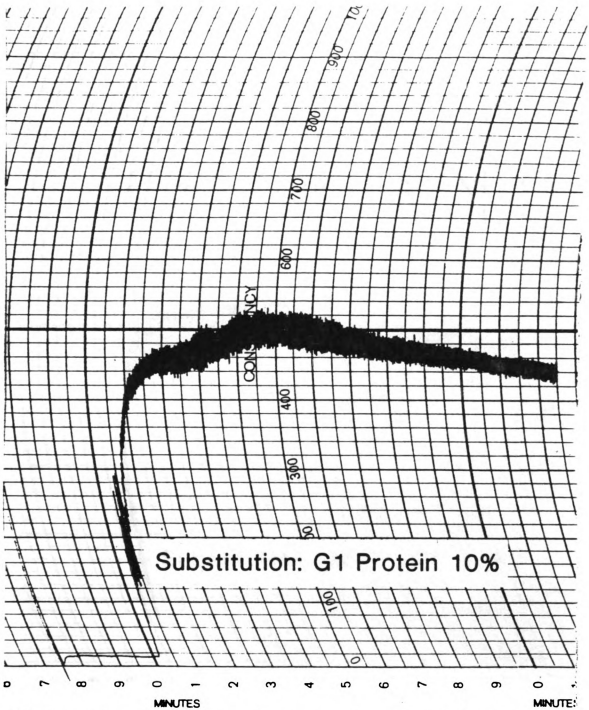


Figure 15. **Farinogram of wheat flour dough substituted with phaseolin protein (10% equivalent).**

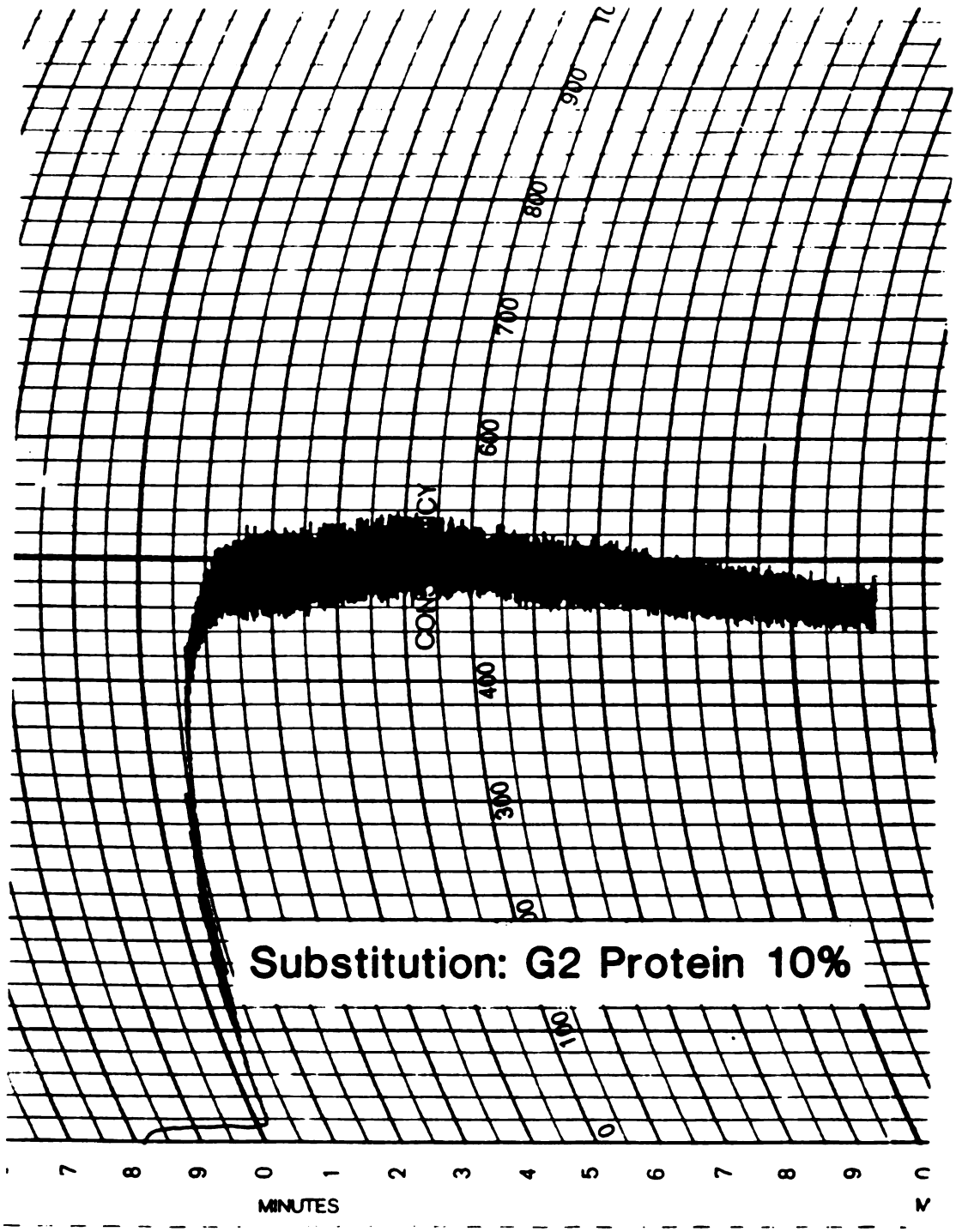


Figure 16. Farinogram of wheat flour dough substituted with G2 lectin protein (10% equivalent).

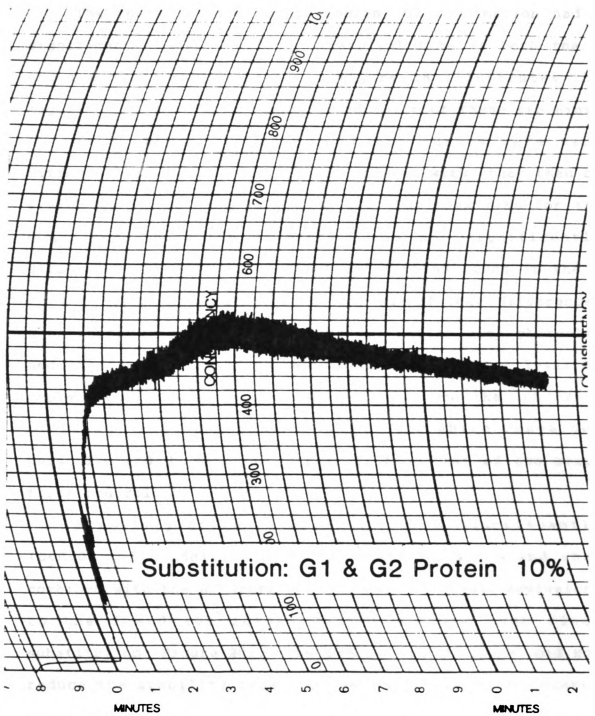


Figure 17. Farinogram of wheat flour dough substituted with phaseolin and G2 proteins (10% equivalent).

The phaseolin and the phaseolin, G2 combination had a significant ($p<0.05$) effect on arrival time at both the 5 and 10% substitution levels. Figure 18 illustrates the magnitude of the delay caused by addition of these proteins.

Even though the shapes of the curves were very different, peak times were not interfered with other than by starch as can be seen in Figure 19. The peak time for the dough containing the G2 protein was not significantly different from the wheat starch substituted dough at the 5 or 10% level of substitution. No significant differences were found between the peak time for doughs with phaseolin and the combination of phaseolin and G2 at either level of replacement. When substituted equivalent to the 10% level of fines, the peak time of the dough which contained phaseolin and G2 were not different from that of the bread flour control.

Stability of the doughs decreased significantly ($p<0.05$) with addition of the phaseolins alone and when combined with the G2 proteins when used at levels equivalent to the 5 and 10% level of high protein bean flour substitution (Figure 20). The addition of G2 alone did not reduce the stability from that of the wheat flour/starch blend. Thus, the effect on the stability for the wheat/G2 protein blend, is the dilution of the gluten with the

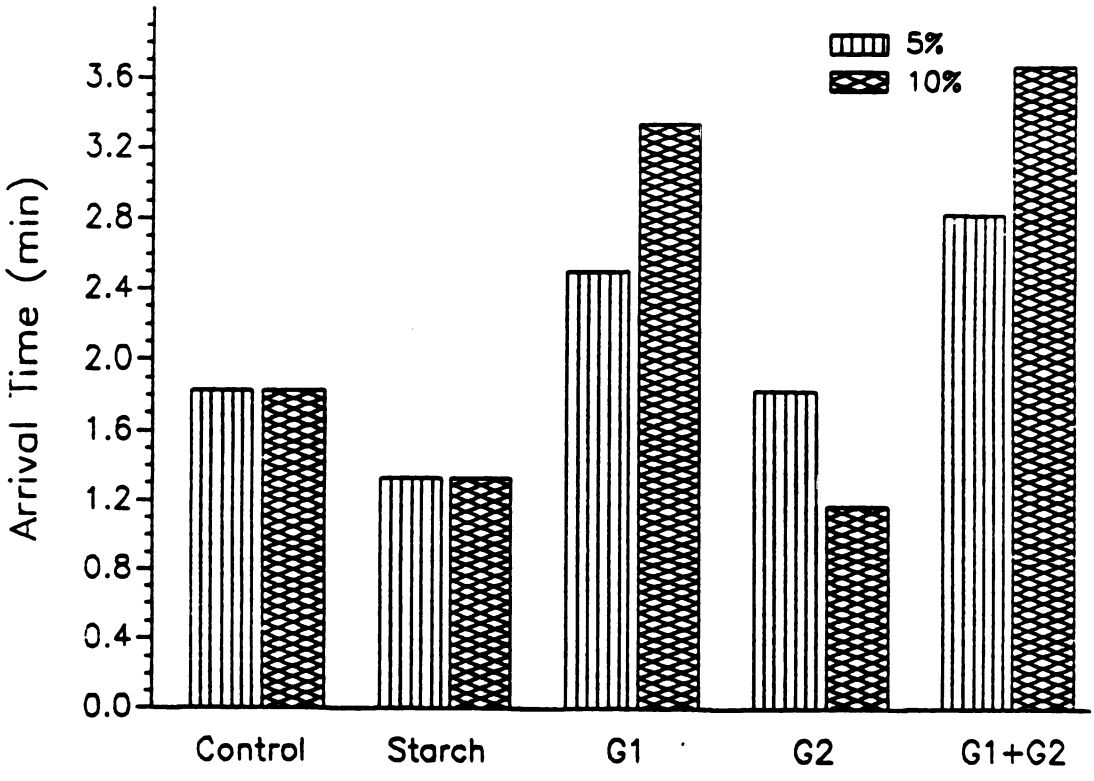


Figure 18. Arrival time of wheat flour dough substituted with bean flour proteins.

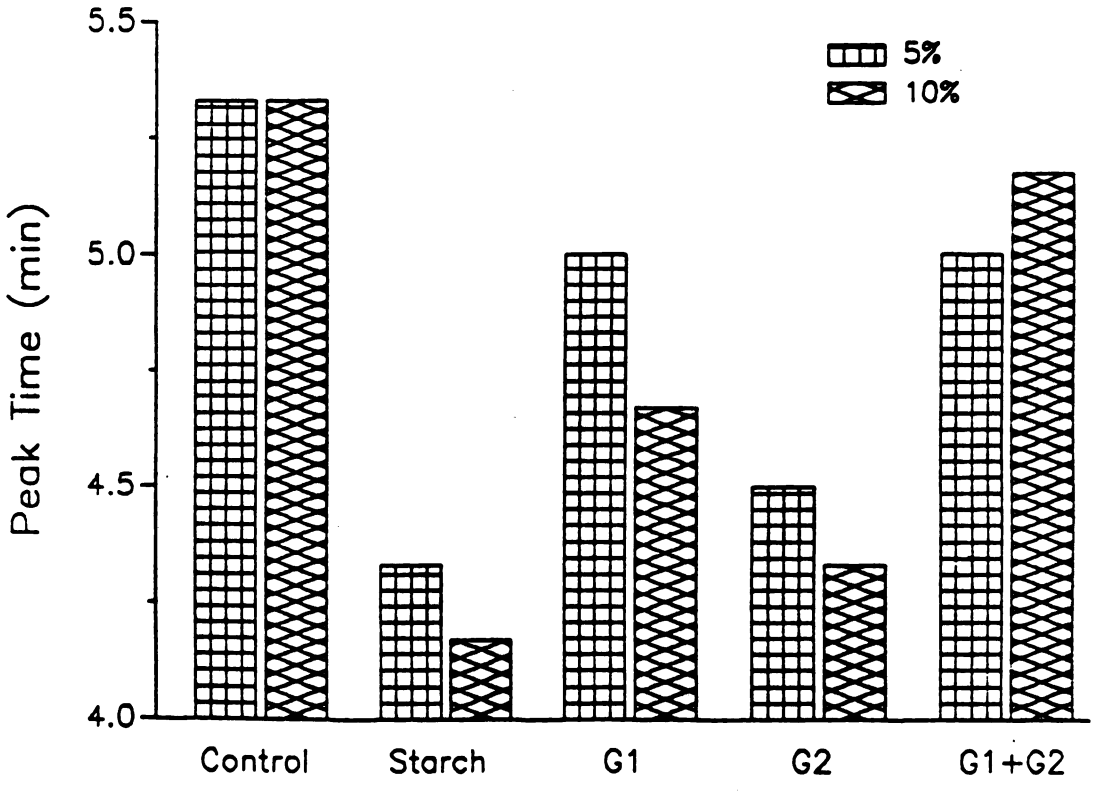


Figure 19. Peak time of wheat flour dough substituted with bean flour proteins.

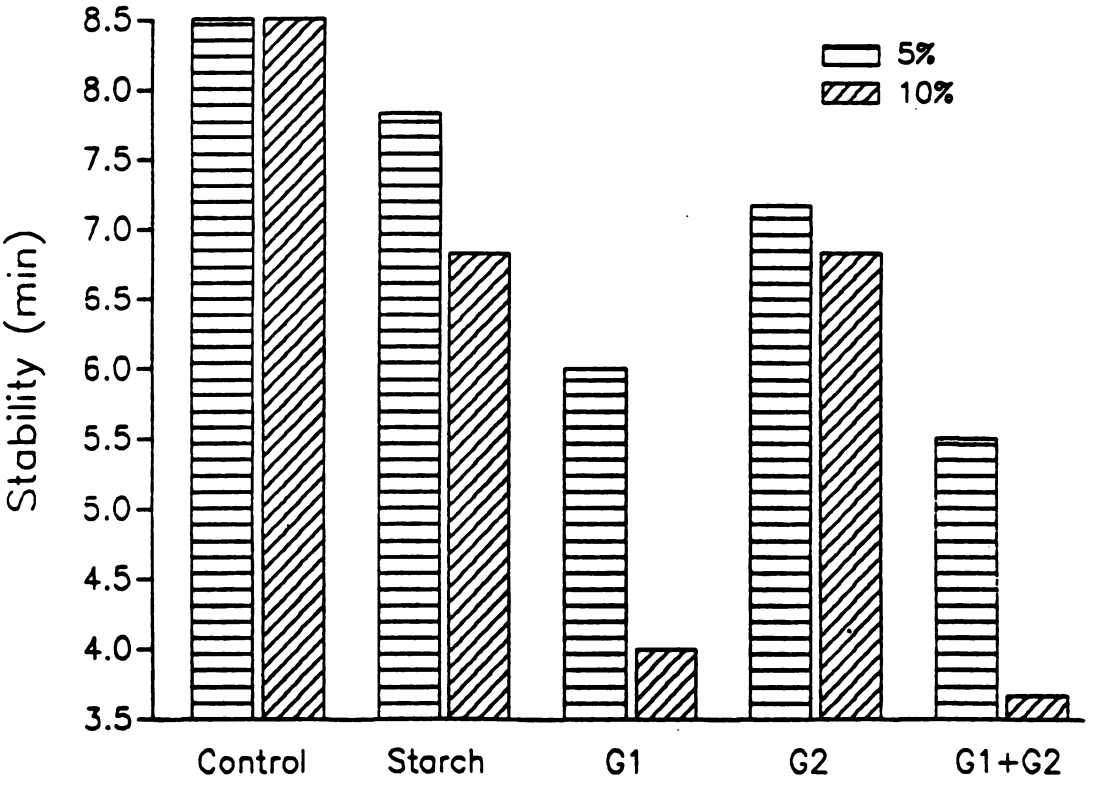


Figure 20. Stability of wheat flour doughs substituted with bean flour proteins

starch. As previously discussed, very small quantities of the G2 protein were needed to equal the amounts present in the prime fines at either level of substitution.

The mixing tolerance index and time to breakdown reflect the decrease in stability of the wheat/protein blends (Figures 21 and 22). The mixing tolerance index was highest for the blend containing phaseolin and the phaseolin plus G2 substituted systems. Time to breakdown was shortest for these same protein blends.

Study 3 Rheologically Active Thiols and Disulfides

In dough systems, only a portion of the thiol and disulfide groups participate in the reactions lending themselves to the rheological properties of the dough. The following studies were conducted to determine the chemical reactivity and thus the rheologically effective proportions of thiols and disulfides in the dough. To obtain these proportions, it is necessary to estimate the total number of thiols and disulfides present in each system. Estimations of total SS and SH groups were obtained photometrically using the methods of Ellman (1959) and Cavallini et al. (1966) respectively. The values obtained for the total thiol and total disulfide content are listed in Table 9 along with protein content and water absorption.

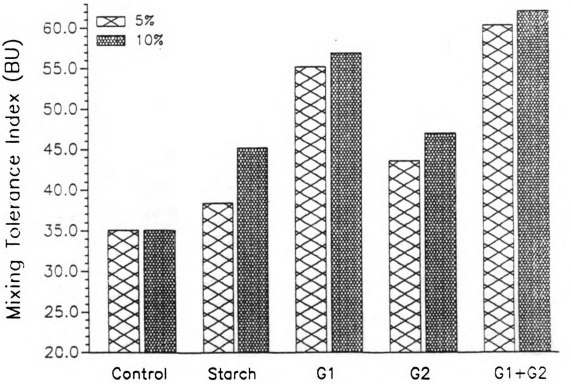


Figure 21. Mixing tolerance index of wheat flour dough substituted with bean proteins.

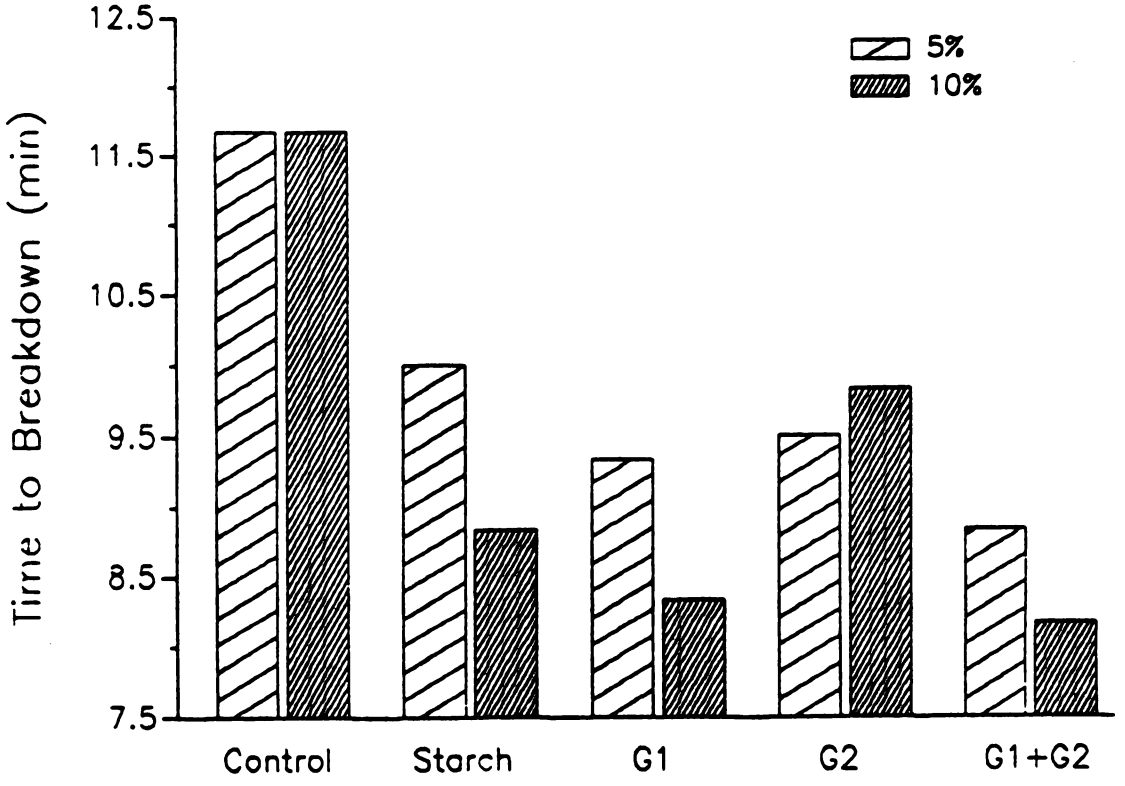


Figure 22. Time to breakdown of wheat flour dough substituted with bean flour proteins.

In the third farinograph study, *N*-ethylmaleimide, a sulfhydryl blocking agent, was added to doughs containing 5 or 10% protein equivalents as previously detailed. Figures 23 and 24 show the effect of NEMI on the loss of resistance to mixing of bread flour and bread flour substituted with phaseolin equivalent to that of 10% fines. The loss of resistance to mixing was the difference between the maximum resistance and the resistance at 30 minutes. The amount of NEMI consumed in this region was considered to be the amount of sulfhydryls important to mixing tolerance. In the control dough 32 µmol/50g were consumed which is similar to values reported by Jones et al.,1974. Amounts were similar for the protein substituted systems. For the doughs with phaseolin added, the maximum amount of thiol involved in mixing tolerance was 30 µmol/50g at both the 5 and 10% levels. Values reported for the doughs with G2 protein approximate those of the starch replaced doughs. Mean values are reported in Table 9.

To determine the number of disulfides involved in mixing, dithiothreitol was added serially to the doughs after they had reached full development. Figures 25 and 26 show the resistance to mixing after addition of DTT to the control and 10% phaseolin substituted dough respectively. In the linear region of the curves the resistance is sensitive to additions of DTT and represents the disulfide bonds important to mixing. The control system revealed the range

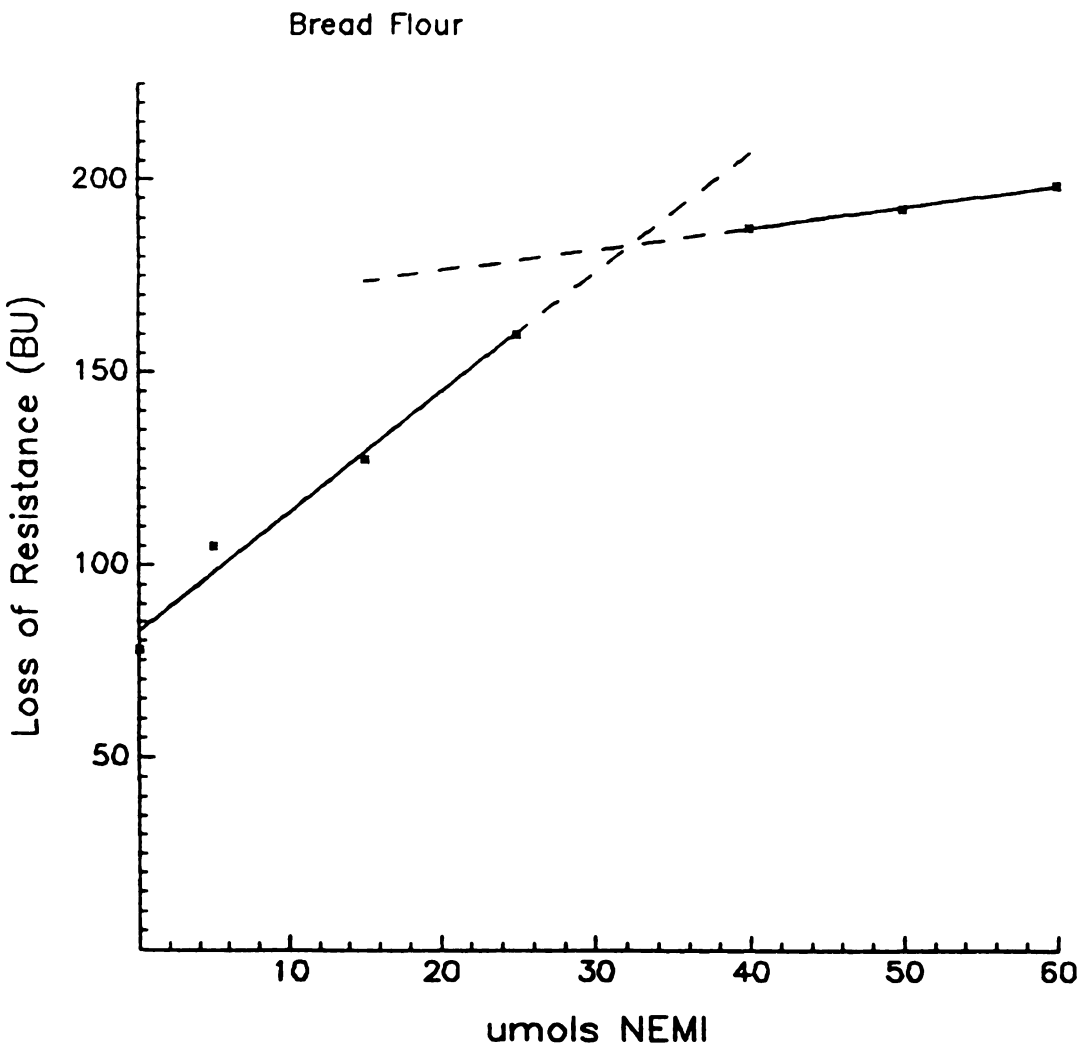


Figure 23. Determination of sulfhydryls involved in mixing tolerance of bread flour by treatment with *N*-ethylmaleimide.

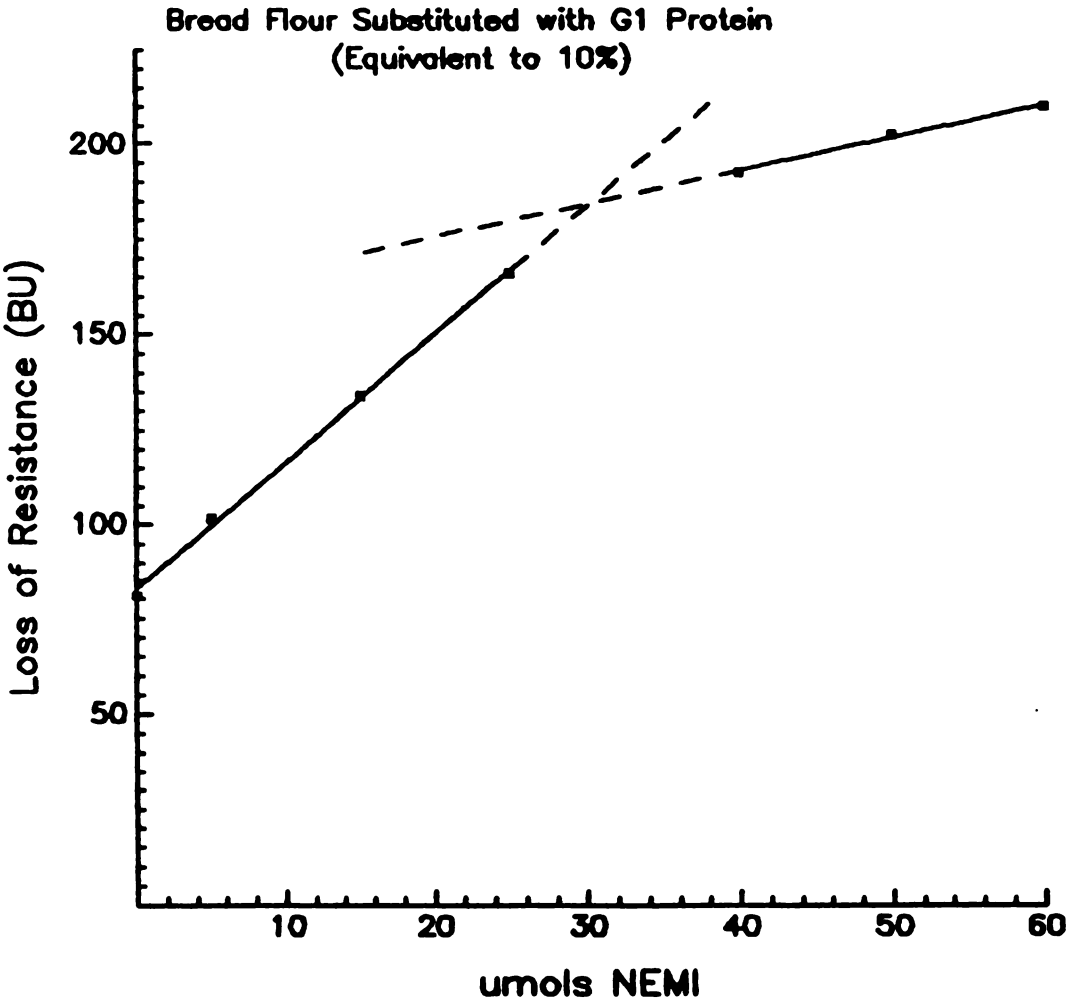


Figure 24. Determination of sulfhydryls involved in mixing tolerance of bread flour substituted with phaseolin by treatment with *N*-ethylmaleimide.

Table 9. Chemical and physical mixing properties of doughs substituted with navy bean proteins.

	Bread Flour	Wheat Starch		G1 Protein		G2 Protein	
		5%	10%	5%	10%	5%	10%
Protein (%)	12.60	11.97	11.34	12.48	12.36	12.01	11.43
Absorption (%)	62.38	61.64	60.98	63.16	64.51	61.89	60.69
Total SH (umols/50g)	161.97	153.87	145.77	154.70	147.43	154.56	147.15
SH Involved in Mixing	32	27	19	30	30	28	22
Total SS (umols/50g)	996.0	946.2	896.4	956.6	917.2	947.9	899.8
SS in Development	14	12	16	15	15	16	13
SS in Resist to Mixing	72	81	74	104	110	82	82
Mixing SS/ Mixing SH	2.3	3.0	4.0	3.5	3.7	2.9	1.6
Total SS/SH	6.15	6.15	6.15	6.18	6.22	6.13	6.11
% Total SS Development	1.44	1.30	1.83	1.61	1.68	1.73	1.48
% Total SS in Mixing	7.42	8.79	8.48	11.16	12.31	8.88	9.36
% Total SH in Mixing	19.76	17.55	13.03	19.39	20.35	18.12	14.95

n=3

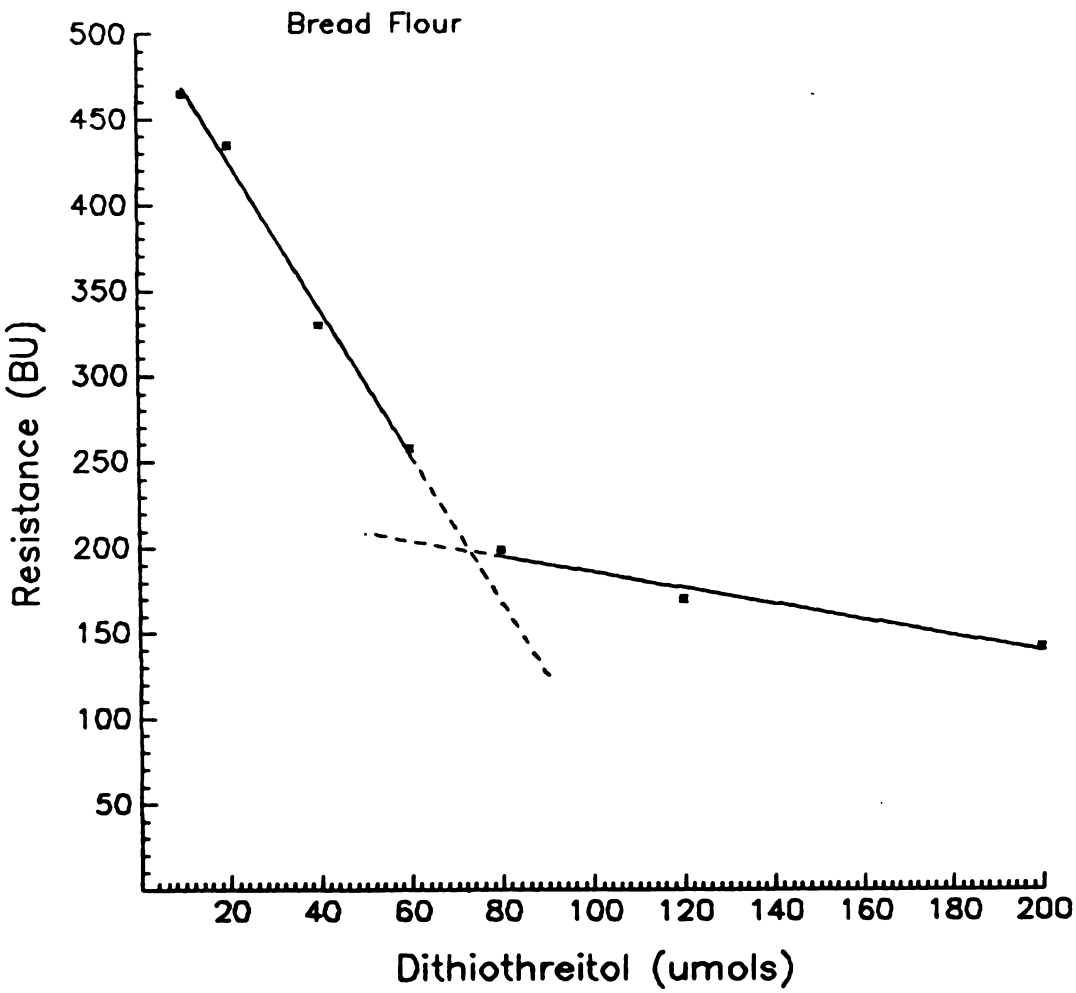


Figure 25. Effect of dithiothreitol on resistance to mixing of bread flour.

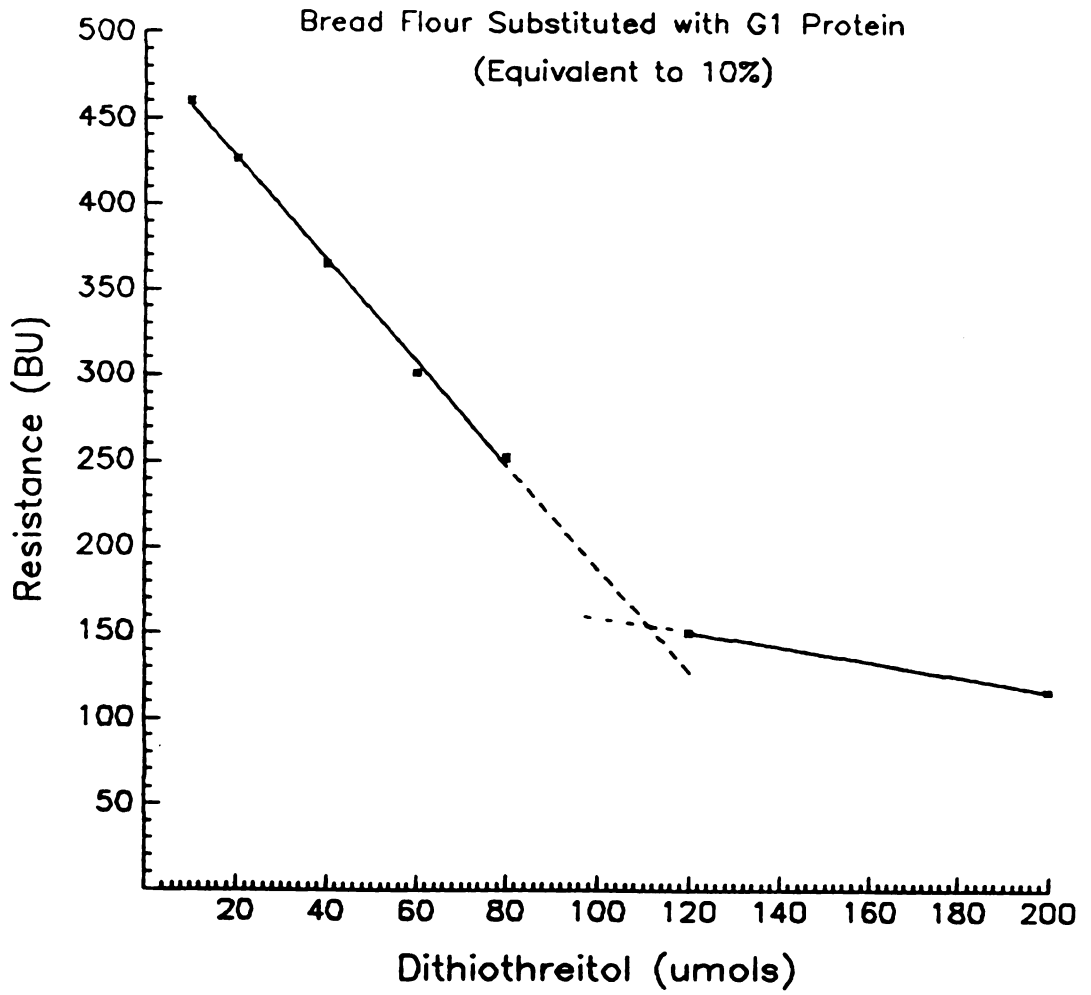


Figure 26. Effect of dithiothreitol on resistance to mixing of bread flour substituted with phaseolin.

of SS to be 0-72 µmol/50g. These results are similar to those of Jones et al. (1974) who reported a range of 0-87 µmol/50g in flours they tested. The phaseolin treated dough showed a definite increase in the number of SS important to mixing properties rising to 104 µmol/50g at 5% substitution and 110 µmol/50g at 10%. For the dough system containing G2 protein, 82 µmol/50g were involved in resistance to mixing.

Dithiothreitol was also added at time zero to determine the amount of disulfides involved in dough development (Figures 27 and 28). However, the number of disulfides involved in development was relatively unchanged for any treatment. The range was 13-16 µmol/50g. Jones et al. (1974) reported values of 16 and 17 µmol/50g flour. Mean values for each treatment are found in Table 9.

Results of DTT experiments on mixing were also similar to those reported by previous researchers (Jones et al.,1974.; Volpe, 1976; El-Minyawi, 1980) dough to which NEMI was added had less tolerance to mixing. Dithiothreitol also lowered resistance to mixing and shortened development time.

The contents of SS and SH that were involved in mixing have been converted to a ratio. These value are found in Table 9 as Mixing SS/Mixing SH. The ratio varied from 1.6 for the system containing G2 equivalent to 10% substitution

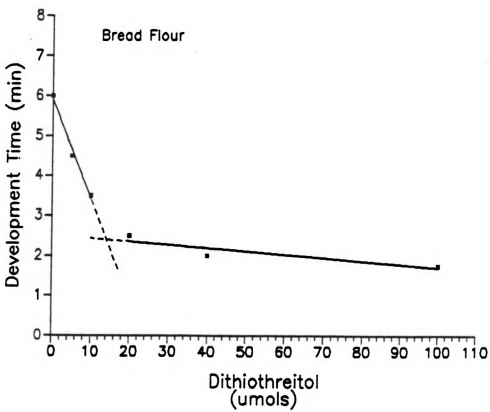


Figure 27. Effect of dithiothreitol on development time of bread flour.

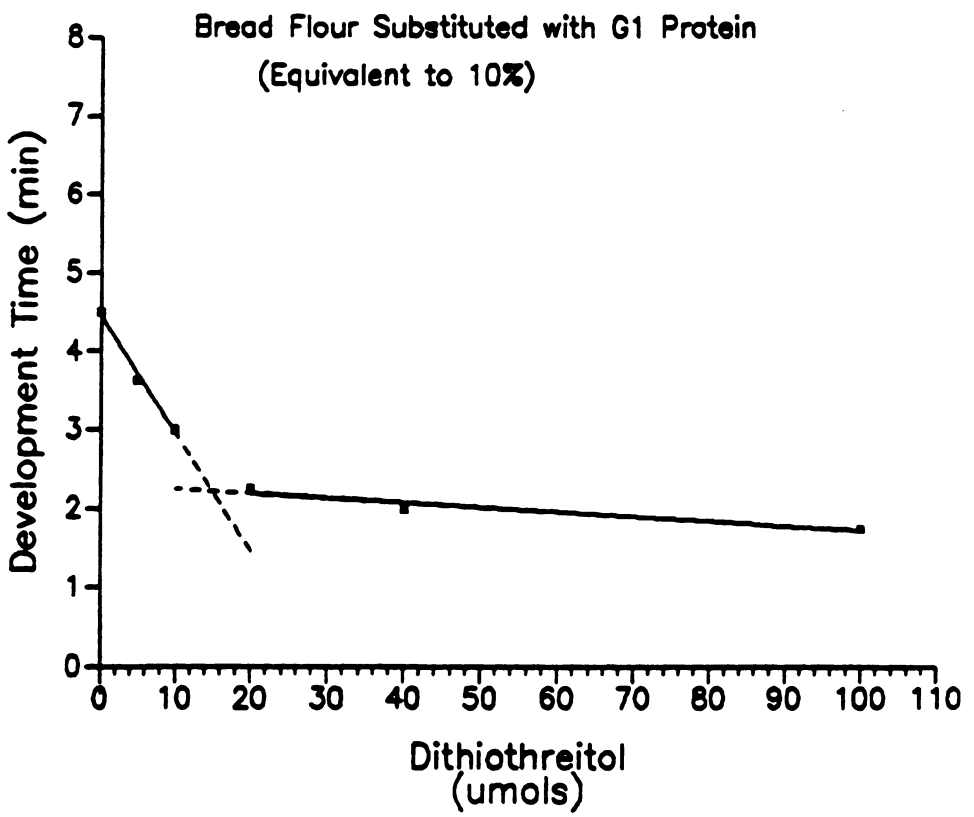


Figure 28. Effect of dithiothreitol on development time of bread flour substituted with phaseolin (≈ to 10%)

to 3.7 for the system containing phaseolin equivalent to a 10% substitution. The ratio for the control was 2.3. Jones et al. (1974) reported values from 2.3 to 3.1.

The proportion of thiol groups involved in rheological properties was higher than the proportion of disulfides. The percentage of total SH groups involved in mixing was 19.76 for the control, while the percentage of SS bonds found important to mixing was 7.42. Phaseolin substitution had the greatest impact on resistance to mixing. When expressed as percentage of total disulfide the dough containing phaseolin equivalent to 10% high protein flour had a value of 12.31. The total percentage of thiol involved in mixing was close to that of the control at 20.35. Results indicated that the ratio of Mixing SS/Mixing SH was not affected by the phaseolin or G2 lectin proteins. However, phaseolins appear to influence the number of disulfides involved in mixing. The evidence is insufficient to conclude that the globulin proteins of *Phaseolus vulgaris* disrupt the disulfide interchange. It is evident from the farinograph data that the phaseolin proteins do affect dough properties; stability in particular. The amino acid profile of this protein as determined by Slightom et al. (1983) may provide some insight into these phenomena. As detailed in Table 10, phaseolin contains a high number of glutamine residues

Table 10 Phaseolin amino acid composition

Amino Acid	Composition of Average Protomer
Lysine	24
Histidine	10
Arginine	16
Asparagine & Aspartic Acid	55
Threonine	13
Serine	33
Glutamic Acid & Glutamine	70
Proline	16
1/2 Cysteine	2
Glycine	23
Alanine	20
Valine	25
Methionine	3
Isoleucine	22
Leucine	39
Tyrosine	11
Phenylalanine	22
Tryptophan	5
Total	409

Slightom et al. (1983)

capable of contributing amide groups which could potentially impede hydrogen bonding between glycolipid and gliadin, glutenin or the starch. Another important aspect of the amino acid picture is the presence of eight nonpolar residues. Of these, leucine is reportedly present in the largest amount with valine, glycine, isoleucine, phenylalanine and alanine occuring slightly less frequently. Methionine and tryptophan are the least available amino acids in this group. Nonpolar amino acids participate in hydrophobic interactions. These have the ability to interact with flour lipids. Removal of lipid from wheat flour leads to a decrease in the glutenin:gliadin ratio and thus interfers with gluten formation. If hydrophobic interactions are tying up the flour lipids this could contribute to the decrease in stability attributed to doughs substituted with the phaseolin proteins.

Hydrophobic and hydrogen bonds are essential to bread baking performance. The phaseolin proteins have the potential to interfere with either or both types of bonding. The occurrence of either possibility would alter the farinograph mixing profile as dough strength and stability would be reduced.

Low Temperature Scanning Electron Microscopy Studies

In preparing specimens for scanning electron microscopy, it is important to minimize shrinkage and distortion of the sample. Techniques such as freeze or critical point drying can be disruptive to many delicate specimens. Therefore, viewing dough specimens in the frozen-hydrated state was expected to produce images with fewer sample preparation induced artifacts. Dough from each phase of the farinograph testing were studied using the EMscope SP-2000 Sputter-Cryo system in conjunction with a JEOL JSM-35C SEM. All substituted doughs were at the 10% level of incorporation.

Wheat flour/ bean flour blends

The microstructures of optimally mixed wheat flour dough and of dough substituted with bean flour fractions substituted at the 10% level were examined. The surface view of the control dough (Figure 29a) showed starch granules continuously united by a gluten coating. These results agree with previous descriptions of flour and water doughs (Argani and Hawrylewicz, 1968; Volpe, 1976 and El-Minyawi, 1980). At a magnification of 2000x there were no obvious pores or disruptions at the protein-starch interface. El-Minyawi (1980) reported holes and pockets at the interface in freeze dried samples. The disruption may

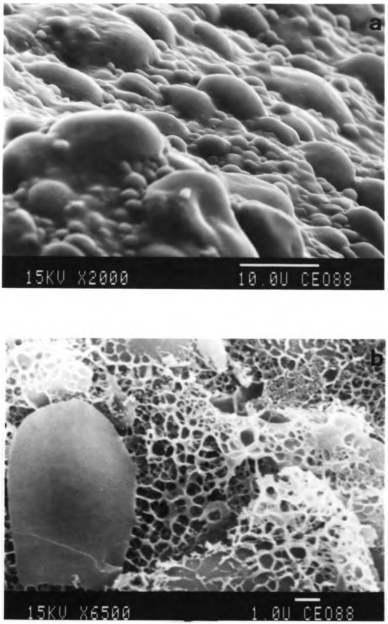


Figure 29. Microstructure of a wheat flour dough
a. Surface view
b. Fractured surface

have been caused by sample preparation technique. As in the work of El-Minyawi (1980), the gluten appeared to be slightly thickened in some areas and thinner in locations where it had stretched across the larger starch granules. The fractured surface image (Figure 29b) showed a fine web-like network that was attached to and surrounded the starch grains. This illustrates the association of the elongated structure of glutenin and the compact gliadin molecules in gluten as they interact with the starch. The microstructure of this dough corroborates the farinograph data indicating a relatively strong dough.

The surface of the wheat/cotyledon flour dough system appeared roughly similar to that of the control dough (Figure30a). Closer study revealed the appearance of blisters on the gluten surface covering the larger starch granules. There were small regions with very thin gluten covers. Large starch granules were surrounded and clustered with starch grains of a lesser size. The micrograph showing the cracked surface of this dough system (Figure 30b) revealed a gluten network composed of thicker and slightly more compact elongated structures than were observed in the control dough. The protein-starch interface showed evidence of disruption. The points of interaction between the gluten and the starch were fewer and less closely associated than those of the control. Doughs from cotyledon flours reached full development significantly

earlier and were significantly less stable than the control dough and doughs made with fines. The surface irregularities and subsurface disruption were likely indications that the dough would lack stability.

The blend of wheat flour and cotyledon flour obtained from cull materials produced micrographs showing greater disruption of the gluten matrix than was evident in the doughs from the wheat/prime cotyledon blend (Figure 31a). The gluten sheet was thin and torn in some areas. In a few instances, starch granules protruded from the gluten sheet. The fractured surface of this composite dough showed a thick, compact gluten network (Figure 31b). The web-like structure was no longer evident. Between the thick filaments which were thought to be glutenins, were small spherical bodies which may be gliadin. This gaves the specimen a spongy appearance. The large starch granules were separated from the protein at the interface. This may have resulted from stress during mixing or stretching of the dough. Farinograph data did not indicate large differences in the performance of cull and prime cotyledon flours in wheat systems. At the 10% level of incorporation, only time to breakdown was significantly different for the two grades with the cull cotyledon showing less time to breakdown. However, the scanning electron microscope studies suggest that substantial physical differences did exist in these systems.

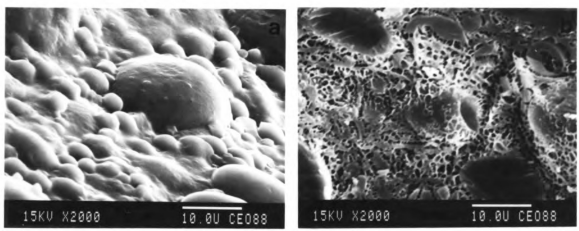


Figure 30. Microstructure of dough from a wheat/prime cotyledon blend.
a. Surface image b. Fractured surface

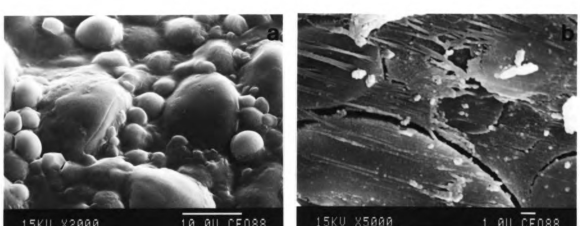


Figure 31. Micrograph of a dough from a wheat/cull cotyledon blend.
a. Surface image b. Fractured image

The surface of the dough made from the wheat flour/prime fines blend was not covered with gluten as smooth and thick as the control (Figure 32a). The surface was marred with pits and pock marks. The protein-starch interface was intact but there were thick and thin areas present as in other legume substituted systems. The fractured surface (Figure 32b) showed a network formed from tightly worked thick filaments. The protein-starch interface was undisturbed. Prime fines significantly delayed arrival time and lengthened peak time; yet departure time was significantly earlier than the control. From these micrographs, taken at peak time, it can be speculated that the areas with thin gluten coverings contributed to decreased stability. As mechanical mixing continued the weak gluten network would have been subjected to shear and stress which may have torn the gluten. However it should be noted there was no significant difference in time to breakdown between the prime fine substituted dough and the control.

The wheat flour/cull fines blend produced similar images (Figure 33a and b). The uncut surface was mottled and somewhat disrupted. The starch granules were not evenly coated with a fine gluten sheet. There was evidence of heavy gluten coating in some areas and protruding starch granules with a very thin or possibly no gluten covering in other areas. The cut surface image showed both thick and

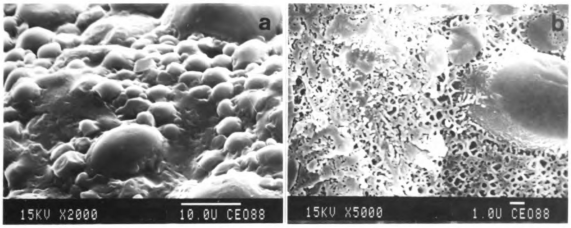


Figure 32. Micrograph of dough from wheat flour/prime fines blend. a. Surface image b. Fractured image

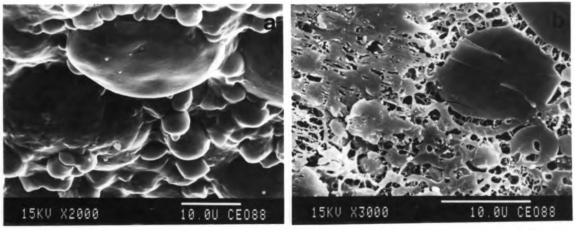


Figure 33. Micrograph of dough from a wheat flour/cull fine blend. a. Surface image b. Fractured image

fine filaments surrounding and connecting the starch. The starch grains had fewer points of association with the protein than was evident in the micrographs of the wheat flour/prime fines blend. No differences were found in farinograph stability values between prime and cull fine substituted doughs but time to breakdown was significantly earlier when cull fines were incorporated. The increased rate of breakdown may be related to the disruption of the protein-starch interface.

Wheat flour/bean protein blends

The wheat flour dough was substituted with wheat starch to determine the effects of dilution on the dough structure. The surface image of this specimen (Figure 34) was very similar to the bread flour control. The starch and gluten were evenly distributed. The gluten sheet was somewhat thicker in areas between large starch granules. The protein-starch interface was not disrupted. Farinograph data indicated starch weakened the dough. The mixing tolerance index was significantly higher than the control and breakdown occurred significantly sooner. At peak time it was difficult to predict the decrease in stability on the basis of the physical characteristics depicted in the micrographs.

Ingredients in the wheat flour/phaseolin blend were evenly dispersed (Figure 35). The gluten sheet was

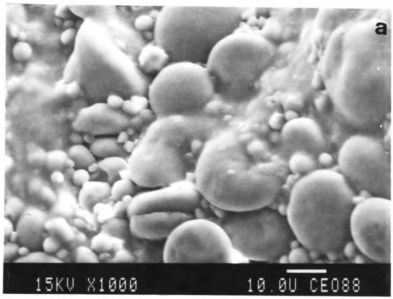


Figure 34. Micrograph of dough from a wheat flour/wheat starch blend.

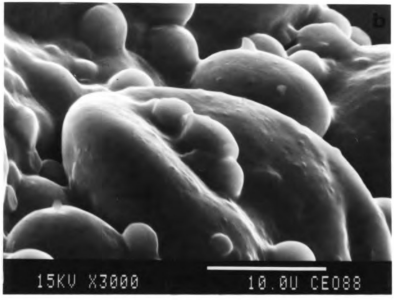


Figure 35. Micrograph of dough from a wheat flour/phaseolin protein blend.

continuous but slightly blistered. Phaseolin substituted doughs were significantly ($p<0.05$) different from the control for every farinograph parameter examined. Arrival time was delayed. Peak and departure times were shortened; stability decreased as did time to break down. Had micrographs been taken of samples removed from the farinograph bowl at departure time, the loss of stability would likely have been more evident.

The G2-lectin proteins incorporated into the dough system were characterized by smaller starch granules forming a ring around the larger granules (Figure 36a). This phenomenon was also noted in bean flour substituted systems. The cut surface images of the G2-lectin substituted dough showed evidence of disruption at the protein-starch interface (Figure 36b). The starch granules were not cleanly cleaved from the gluten. Many areas exhibited finger-like projections spanning the distance between the parting edges. At 10000x (Figure 36c) the network appeared fairly compact. Thick filaments entrapped tiny spheres.

When phaseolins and lectins were used in combination in the wheat blend dough, the gluten sheet was thick and continuous; covering the starch grains without interruption. Thin spots and breaks in the gluten were minimal (Figure 37a). The fractured surface showed large starch granules were connected to the thick gluten filaments by thinner branches of the gluten strands (Figure 37b). These wisps

Figure 36. Micrograph of dough from a wheat flour/G2 protein blend.
a. Surface image
b. Fractured surface
c. Fractured surface 10,000x

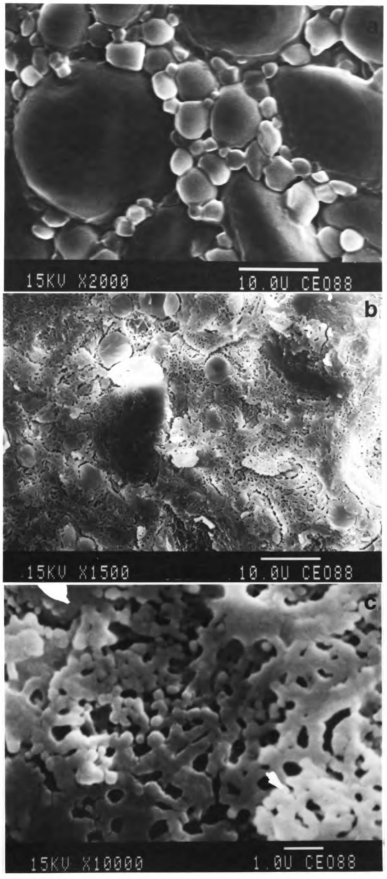
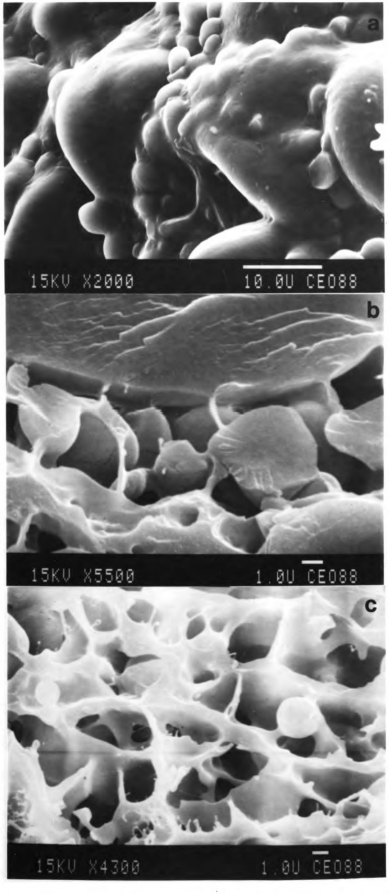

a
15KV X2000
10.0U CE088
b
15KV X1500
10.0U CE088
c
15KV X10000
1.0U CE088

Figure 37. Micrograph of dough from a wheat flour/phaseolin and G2 protein blend.
a. Surface image
b. Fractured surface
c. Fractured surface 4300x


a
15KV X2000
10.0U CE088
b
15KV X5500
1.0U CE088
c
15KV X4300
1.0U CE088

surrounded the smaller starch grains and appeared attached to the larger starch granules. Figure 37c shows this matrix at a magnification of 4300x. Farinograph data and micrographs both provide evidence that there was little difference in doughs produced from wheat flour and phaseolin blends or wheat flour plus the combination of phaseolin and G2 protein.

Effect of *N*-Ethylmaleimide on Mixing

Microscopy studies corresponding to the rheologically active thiol and disulfide investigations were performed. Thirty-two µmol NEMI per 50 gram flour was added to a control dough and mixed for 30 minutes. After 30 minutes the doughs were broken down making them tenatious and extremely viscidulous. This resulted in alteration of the system as shown in Figure 38a. The gluten network appeared to have broken down. The sheet was thin and translucent. Some regions may have completely lacked a gluten covering. There were gaps between starch granules without a connecting gluten bridge.

The wheat starch substituted specimen was severely altered (Figure 38b). The gluten coat was studded with tiny orbs. There were cases where these spheres tended to aggregate. These may have been globular proteins that precipitated due to a shift in the pH caused by the addition

of NEMI. The gluten film was thick and velutinous. Small cracks at the bases of the starch granules were evidence of disruption of the protein-starch interface.

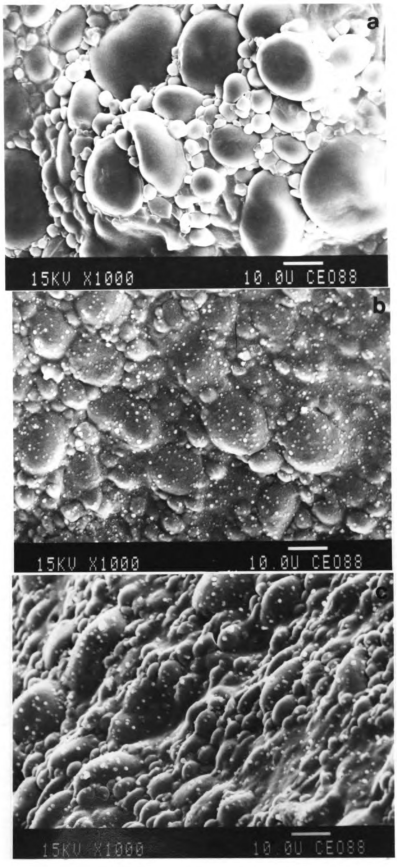
The wheat/phaseolin substituted system was similar to the wheat/starch dough (Figure 38c). The gluten was thick and patchy. The surface was dotted with the identical orbicular matter that occurred on the surface of the starch substituted dough.

The Effect of Dithiothreitol on Mixing

To estimate the number of disulfides involved in mixing, DTT was added to the doughs. The adition of DTT caused a rapid deterioration of the dough. It became thick and sticky and had the appearance of whipped cream. The micrograph of the control dough system (Figure 39) resembled the images of the wheat/starch and wheat/phaseolin specimens in the NEMI series of micrographs. The surface was spotted and rough. Large spaces appeared between starch granules; some were held together by thick finger-like projections. As could be expected in a ruptured dough, the gluten did not drape evenly over all of the starch grains. The fractured surface showed a dense gluten network (Figure 39b). Many starch granules were completely separated from the network or remained loosely attached.

The effect of DTT was less physically disruptive to the wheat flour/starch substituted dough (Figure 40a and b). The

Figure 38. Effect of NEMI on loss of resistance.
a. Wheat flour control
b. Wheat flour/starch blend
c. Wheat flour/phaseolin blend


a
15KV X1000 10.0U CE088
b
15KV X1000 10.0U CE088
c
15KV X1000 10.0U CE088

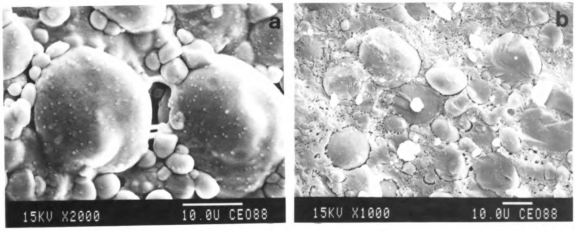


Figure 39. Effect of DTT on resistance to mixing in a wheat flour dough a. Surface b. Fractured

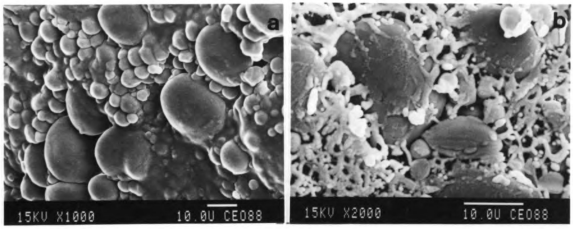


Figure 40 Effect of DTT on resistance to mixing in flour/starch blend. a. Surface b. Fractured

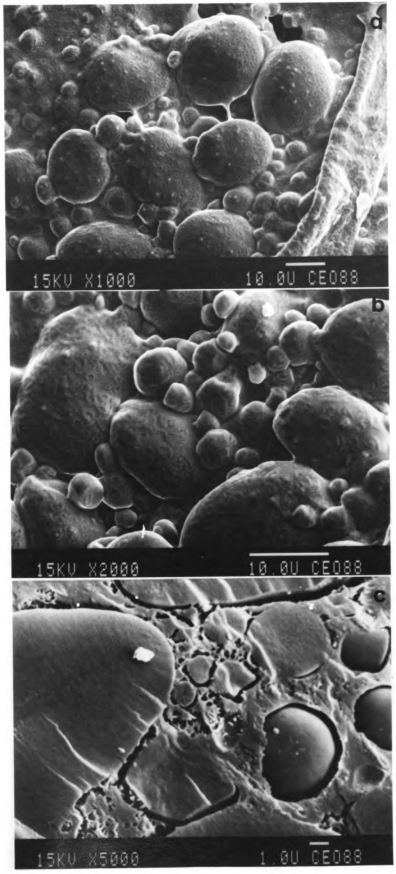
gluten sheet was slightly rough and open in some areas. Thick and thin regions were also detectable. There did not appear to be any irregular markings or patterns on the gluten surface. The fractured image (Figure 40b) shows starch granules embedded in the gluten matrix.

The wheat flour/phaseolin protein substituted dough looked very different from the control and starch substituted doughs (Figure 41). Farinograph data suggest that phaseolin protein affect the mixing tolerance of the dough. The dough surface took on a mosaic pattern when treated with DTT (Figures 41a and b). The gluten veil was torn in a few places and thinned out at the base of many of the starch grains. Figure 41c shows the cleaved surface of the wheat flour/phaseolin composite dough. Many of the starch granules were no longer associated with the gluten. There were crater-like pockets suggesting that starch granules no longer firmly positioned in the network may have been dislodged in the fracture process. Many small starch granules were still attached to each other by spindly filaments. The major portion of the gluten network between the starch granules had collapsed and was quite solid looking (Figure 41c).

The Effect of Dithiothreitol on Development Time

Micrographs of the effect of DTT on development time showed that the development phase was similar regardless of

Figure 41. Effect of DTT on resistance to mixing in a wheat flour/phaseolin blend.
a. surface
b. surface
c. fractured 5000x


a
15KV X1000
10.0U CE088
b
15KV X2000
10.0U CE088
c
15KV X5000
1.0U CE088

substitution. The gluten covering on the bread flour dough draped and covered the starch without interruption (Figure 42a). The fractured surface showed starch granules firmly positioned in the gluten matrix (Figure 42b).

The wheat/starch substituted dough had a rough texture (Figure 43a). The gluten sheet failed to completely coat the starch grains. The sheet may have ruptured as it stretched over the starch surface and settled around the base of the granules. This weakening of the gluten resulted from dilution of the gluten proteins with the starch. In spite of the weakened gluten structure a fine network developed and starch granules appear firmly attached to it (Figure 43b).

The wheat dough containing the phaseolin protein (Figure 44a) was slightly craggy and thick. Not all starch grains were completely covered by the gluten. However, the gluten adhered to the starch draping the tops and sides of the granules. The fractured sample (Figure 44b) shows starch granules were closely associated with the newly developed gluten network. Micrographs of the doughs incorporating the G2-lectin protein were similar to those of the wheat/starch blend (Figures 45a and b).

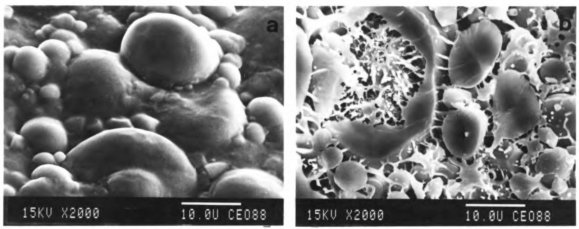


Figure 42. Effect of DTT on development time in wheat dough. a. surface b. fractured

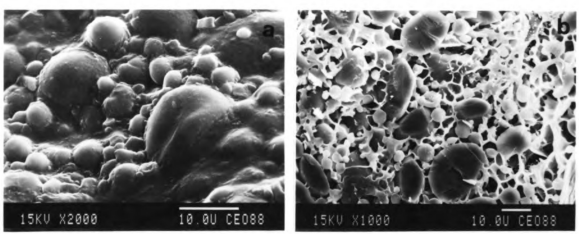


Figure 43. Effect of DTT on development time in a wheat flour/starch blend. a. surface b. fractured

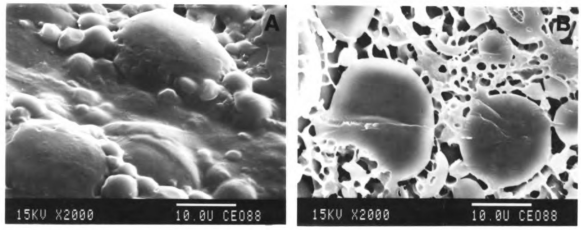


Figure 44. Effect of DTT on development time in a wheat flour/phaseolin blend. a. surface b. fractured

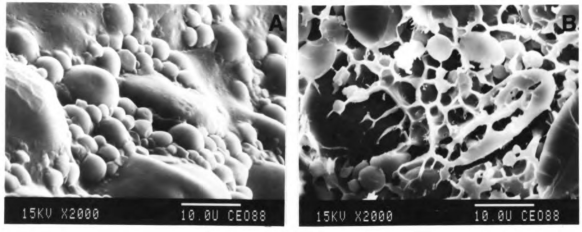


Figure 45. Effect of DTT on development time in a wheat flour/G2 blend. a.surface b. fractured.

SUMMARY AND CONCLUSIONS

Various air-classified navy bean flour fractions have been substituted into dough systems. The outcome of baked products made with these substituted doughs, has been less than optimum. Addition of oxidants and dough conditioners has proved beneficial. The largest defect, notably, reduced loaf volume is largely overcome through the use of such additives. This in turn improves associated crumb and textural problems. Because the dough improvers act on the disulfide interchange, this reaction with respect to the bean proteins was investigated.

The objectives of this study were: to observe the effects of navy bean cotyledon and protein flours on farinograph parameters and to compare the effects of bean source on bean flour quality and performance; to extract and study the effects of the major storage protein, phaseolin and the lectin protein G2 on the mixing characteristics of doughs substituted with these proteins in amounts equal to the proteins present in a 5 and 10% substitution with a high protein bean flour. Total disulfides and sulfhydryls were determined photometrically.

Reactive disulfides and thiols were determined quantitatively. Low temperature scanning electron microscopy was used to study each phase of the investigation at the 10% level of substitution.

Farinograms of systems substituted with the bean flour fractions showed distinctive mixing profiles for each bean flour fraction. Differences due to bean flour source were minimal. As expected absorption increased with increased protein. The wheat/cull fines blend had the greatest absorption. Bean flour fractions blended with wheat, delayed arrival time. The prime fines had the most substantial impact on this parameter. At the 10% level of substitution, prime fines lengthened peak time and greatly reduced stability. This fraction also had a high mixing tolerance index indicating a weak dough had been formed with this system.

In the second facet of farinograph testing, two globular proteins were added to the dough singularly and in combination in amounts equal to the phaseolin and G2 content in the high protein flour. Wheat starch was also substituted into the doughs to separate the effects of gluten dilution from the effects of the proteins. As in the flour blends, an increase in protein lead to an increase in absorption. The phaseolin and the phaseolin, G2 combination delayed arrival time and significantly reduced the stability of the doughs. In addition, doughs containing the phaseolin

protein and the combination of the two proteins had high MTI values and broke down readily. The farinograph values for doughs substituted with the G2 protein alone were generally similar to the dough made from the wheat flour/wheat starch blend. Thus, the effect of G2 could not be discerned from the effects of dilution of the gluten.

Chemical reactivity of thiols and disulfides was estimated to give an indication of the rheological effectiveness of these groups in the dough. NEMI was added to doughs to determine the amount of SH important to mixing tolerance. The bread flour had a maximum amount of 32 µmol/50 grams; while the phaseolin substituted dough had 30 µmol/50 grams. Disulfides involved in mixing were estimated using DTT. Phaseolin treated dough showed a substantial increase in the number of S-S groups important to mixing. There were 104 µmols S-S per 50 grams in the wheat/phaseolin blend substituted at 10% and 72 µmol/50 grams in the control bread flour system. Disulfides involved in development time were relatively unchanged for any system. The ratio of mixing SS/mixing SH is not affected by the bean proteins. However, phaseolin influenced the number of SS groups involved in mixing. While farinograph data indicate phaseolin proteins alter mixing profiles, the exact mechanism by which this occurs has yet to be determined.

The low temperature SEM investigation revealed surfaces and subsurface structures of the wheat flour /bean flour and wheat flour/bean protein blends taken at peak time. The bread flour doughs showed starch granules embedded in a continuous, fine, gluten network. Cotyledon flour substituted doughs had a slightly rougher appearance than the control. The cotyledon flour from the cull material exhibited greater disruption than the dough from the wheat/ prime cotyledon blend. No differences were found in the mixing profiles of these blends. At peak time, the surface of the dough containing the prime fines is marred but the protein-starch interface is intact. Areas with thin gluten coverings probably contribute to the low stability and eventual breakdown that results from continued mixing. Doughs substituted with cull fines produced similar images.

In the protein substituted doughs, ingredients were well distributed. The gluten sheet is continuous and smooth. There is little evidence of the negative impact of the phaseolin protein as demonstrated by the farinograph. Since the specimens were taken from the farinograph bowl at peak time, this may indicate that the subsequent breakdown is mechanically induced or the reaction rate is slow.

Micrographs of the reactive SH and SS groups bear some interesting results. As expected, doughs were broken-down by the chemical treatment. Curiously, the wheat starch substituted and the phaseolin substituted doughs treated

with NEMI were flecked with small spheres. The effect of DTT on mixing resulted in a mosaic-like pattern on the surface of the flour/phaseolin dough. Like the farinograph, micrographs depicting the effects of DTT on development time show that development time is similar without regard to substitution.

Farinograph findings indicate that there are few differences in functionality between grades of the bean flour fractions. However, the SEM images suggest that further study is needed in this area. Differences were found between flour types.

The major storage protein, phaseolin, had a negative effect on dough stability causing a faster rate and greater magnitude of breakdown than other variables. The ratio of mixing SS/SH is not affected by phaseolin or lectin protein. Phaseolin influence the number of disulfides in mixing. There is insufficient evidence to conclude that the globulin proteins of *Phaseolus vulgaris* disrupt the disulfide interchange.

RECOMMENDATIONS FOR FUTURE RESEARCH

Processing dried legumes into lysine-rich flour extends the potential for fortification of cereal based products. However, supplying the world population with an adequate protein source is not enough. The functionality of the food is the property which is most obvious to the consumer. To improve nutritional standards, the functionality of any new food needs to be considered. The relationship between protein and functionality must be explored to its fullest to gain the understanding needed to meet the challenges ahead.

The current study found discrepancies between data collected using the farinograph and interpretation of micrographs concerning difference between prime and cull materials as the source of the bean flour. A baking study to ensure functional reliability of the farinograph data should be initiated. Additional studies investigating the effect of bean protein in wheat systems should address hydrogen bonding and hydrophobic interactions with respect to the flour lipids. This may provide useful insight .

The electron microscope should be utilized to a greater extent. Doughs should be evaluated in each stage of development and destruction corresponding with established farinograph parameters. The wheat/phaseolin blends should be looked at with dough conditioners added and compared to the images from the current study. Research that seeks to identify the orbs found throughout several of the chemically treated doughs would be of interest as would research that explains the mosaic pattern of the DTT treated wheat/phaseolin dough. The use of transmission electron microscopy also needs to be considered for additional information it may provide in regard to lipid, and protein-protein interactions.

APPENDICES

APPENDIX 1.

MEANS AND STANDARD DEVIATIONS FOR FARINOGRAPH MEASUREMENTS

Table A. Means and standard deviations for farinograph measurements performed on doughs substituted with navy bean flour at the 5% level.

Flour Fraction	Arrival Time min	Peak Time min	Departure Time min	Stability min
Control	2.0±0.0c	5.0±0.0ab	10.0±0.0b	8.0±0.0a
Fines	3.5±0.5a	5.3±0.3a	11.8±1.4a	8.3±1.9a
Cull Fines	3.5±0.0a	5.2±0.3a	9.5±0.0bc	6.0±0.0b
Cotyledon	2.8±0.3b	4.5±0.0c	7.8±0.3d	5.0±0.5b
Cull Cotyledon	2.7±0.3b	4.7±0.3bc	8.3±0.3cd	5.6±0.6b

n=3, $\alpha = 0.05$
Values with the same letters are not significantly different from each other as judged by Duncan's test.

APPENDIX 1.

MEANS AND STANDARD DEVIATIONS FOR FARINOGRAPH MEASUREMENTS

Table B. Means and standard deviations for farinograph measurements performed on doughs substituted with navy bean flour at the 5% level.[1]

Flour Fraction	Breakdown min	MTI[2] BU	Absorption[3] %
Control	11.2±0.3b	43.3±2.6b	62.32±0.0c
Fines	13.0±0.5a	25.0±0.0d	64.24±0.1a
Cull Fines	11.7±0.3b	38.3±2.9c	64.35±0.1a
Cotyledons	9.0±0.0c	43.3±2.9b	63.55±0.1b
Cull Cotyledons	8.7±0.3c	50.0±0.0a	63.53±0.3b

1 n=3, $p<0.05$, Values with the same letters are not significantly different from each other as judged by Duncan's test.
2 Mixing Tolerance Index
3 14% mb

APPENDIX 1.

MEANS AND STANDARD DEVIATIONS FOR FARINOGRAPH MEASUREMENTS

Table C. Means and standard deviations for farinograph measurements performed on doughs substituted with navy bean flour at the 10% level.

Flour Fraction	Arrival Time min	Peak Time min	Departure Time min	Stability min
Control	2.0±0.0d	5.0±0.0c	10.0±0.0a	8.0±0.0a
Fines	4.5±0.1a	6.5±0.0a	9.2±0.3b	4.7±0.3b
Cull Fines	4.0±0.0b	5.7±0.3b	9.0±0.0b	5.0±0.0b
Cotyledon	3.0±0.6c	4.5±0.0d	7.2±0.3c	4.2±0.3c
Cull Cotyledon	3.0±0.6c	4.5±0.0d	7.2±0.3c	4.2±0.3c

n=3, $p<0.05$
Values with the same letters are not significantly different from each other as judged by Duncan's test.

APPENDIX 1.

MEANS AND STANDARD DEVIATIONS FOR FARINOGRAPH MEASUREMENTS

Table D. Means and standard deviations for farinograph measurements performed on doughs substituted with navy bean flour at the 10% level.[1]

Flour Fraction	Breakdown min	MTI[2] BU	Absorption[3] %
Control	11.2±0.3a	43.3±2.6c	62.32±0.0d
Fines	11.0±0.0a	55.0±0.0ab	66.69±0.1b
Cull Fines	10.0±0.0b	53.3±5.6b	67.17±0.3a
Cotyledons	8.0±0.0c	61.7±2.9a	65.81±0.1c
Cull Cotyledons	8.7±0.3d	55.0±5.0ab	65.51±0.3c

1 n=3, $p<0.05$ Values with the same letters are not significantly different from each other as judged by Duncan's test.
2 Mixing Tolerance Index
3 14% mb

APPENDIX 1.

MEANS AND STANDARD DEVIATIONS FOR FARINOGRAPH MEASUREMENTS

Table E. Means and standard deviations for farinograph measurements performed on doughs with navy bean proteins incorporated equivalent to a 5% substitution.

Protein Fraction	Arrival Time min	Peak Time min	Departure Time min	Stability min
Control	1.8±0.3c	5.3±0.3a	10.3±0.3a	8.5±0.5a
Starch	1.3±0.3c	4.3±0.3c	9.2±0.6b	7.8±0.3ab
Phaseolin	2.5±0.0ab	5.0±0.0bc	8.5±0.0cd	6.0±0.0cd
G2-lectin	1.8±0.8bc	4.5±0.0c	9.0±0.5bc	7.2±0.3bc
Phaseolin & G2	2.8±0.3a	5.0±0.0b	8.3±0.3c	5.5±0.5d

n=3, $p<0.05$ Values with the same letters are not significantly different from each other as judged by Duncan's test.

APPENDIX 1.

MEANS AND STANDARD DEVIATIONS FOR FARINOGRAPH MEASUREMENTS

Table F. Means and standard deviations for farinograph measurements performed on doughs with navy bean proteins incorporated equivalent to a 5% substitution.[1]

Protein Fraction	Breakdown min	MTI[2] BU	Absorption[3] %
Control	11.7±0.3a	35.0±0.0e	62.39±0.1b
Starch	10.0±0.0b	38.3±2.9d	61.64±0.4c
Phaseolin	9.3±0.3c	55.0±0.0b	63.2±0.0a
G2-lectin	9.5±0.0c	43.3±2.9c	61.89±0.4c
Phaseolin & G2	8.8±0.3d	60.0±0.0a	63.34±0.2a

1 n=3, $p<0.05$ Values with the same letters are not significantly different from each other as judged by Duncan's test.
2 Mixing Tolerance Index
3 14% mb

APPENDIX 1.

MEANS AND STANDARD DEVIATIONS FOR FARINOGRAPH MEASUREMENTS

Table G. Means and standard deviations for farinograph measurements performed on doughs with navy bean proteins incorporated equivalent to a 10% substitution.

Protein Fraction	Arrival Time min	Peak Time min	Departure Time min	Stability min
Control	1.8±0.3b	5.3±0.3a	10.3±0.3a	8.5±0.5a
Starch	1.3±0.6b	4.2±0.3c	8.2±0.3b	6.8±0.8b
Phaseolin	3.3±0.3a	4.7±0.3bc	7.3±0.3c	4.0±0.0c
G2-lectin	1.2±0.3b	4.3±0.3c	8.0±0.0b	6.8±0.3b
Phaseolin & G2	3.7±0.3a	5.2±0.3ab	7.3±0.3c	3.7±0.3c

n=3, $p<0.05$ Values with the same letters are not significantly different from each other as judged by Duncan's test.

APPENDIX 1.

MEANS AND STANDARD DEVIATIONS FOR FARINOGRAPH MEASUREMENTS

Table H. Means and standard deviations for farinograph measurements performed on doughs with navy bean proteins[1] incorporated equivalent to a 10% substitution.[1]

Protein Fraction	Breakdown min	MTI[2] BU	Absorption[3] %
Control	11.7±0.3a	35.0±0.0d	62.39±0.1b
Starch	8.8±0.3c	45.0±0.0c	60.98±0.4c
Phaseolin	8.3±0.3cd	56.7±2.9b	64.51±0.6a
G2-lectin	9.8±0.3b	46.7±0.3c	60.69±0.8c
Phaseolin & G2	8.2±0.3d	61.7±2.9a	64.63±0.2a

1 n=3, $p<0.05$ Values with the same letters are not significantly different from each other as judged by Duncan's test.
2 Mixing Tolerance Index
3 14% mb

APPENDIX 2.

MEAN SQUARES OF ANALYSES OF VARIANCE

Table I. Mean squares of analyses of variance of wheat flour/bean flour blends substituted at the 5% level on farinograph parameters.

Source	DF	Arrival	Peak	Departure	Stability
Between	4	1.19***	0.36**	7.38***	6.57**
Within	10	0.08	0.05	0.45	0.83
Total	14				

*** p<0.001
** p<0.01

Table J. Mean squares of analyses of variance of wheat flour/bean flour blends substituted at the 5% level on farinograph parameters.

Source	DF	Breakdown	MTI	Absorption
Between	4	10.00***	262.50***	1.97***
Within	10	0.08	5.00	0.02
Total	14			

*** p<.001

Table K. Mean squares of analyses of variance of wheat flour/bean flour blends substituted at the 10% level on farinograph parameters.

Source	DF	Arrival	Peak	Departure	Stability
Between	4	2.91***	2.19***	4.87***	7.73***
Within	10	0.00	0.02	0.05	0.05
Total	14				

*** p<.001

APPENDIX 2.

MEAN SQUARES OF ANALYSES OF VARIANCE

Table L. Mean squares of analyses of variance of wheat flour/bean flour blends substituted at the 10% level on farinograph parameters.

Source	DF	Breakdown	MTI	Absorption
Between	4	5.90***	130.83**	10.81***
Within	10	0.03	15.00	0.03
Total	14			

*** p<.001

Table M. Mean squares of analyses of variance of wheat flour/bean protein blends substituted at the equivalent of 5% on farinograph parameters.

Source	DF	Arrival	Peak	Departure	Stability
Between	4	1.07**	0.50***	1.89***	4.67***
Within	10	0.17	0.03	0.15	0.43

*** p<.001
** p<.01

Table N. Mean squares of analyses of variance of wheat flour/bean protein blends substituted at the equivalent of 5% on farinograph parameters.

Source	DF	Breakdown	MTI	Absorption
Between	4	3.56***	347.50***	1.70***
Within	10	0.05	3.33	0.07

*** p<.001

APPENDIX 2.

MEAN SQUARES OF ANALYSES OF VARIANCE

Table O. Mean squares of analyses of variance of wheat flour/bean protein blends substituted at the equivalent of 10% on farinograph parameters.

Source	DF	Arrival	Peak	Departure	Stability
Between	4	4.02***	0.78**	4.57***	12.81***
Within	10	0.13	0.08	0.07	0.02
Total	14				

*** p<.001
** p<.01

Table P. Mean squares of analyses of variance of wheat flour/bean protein blends substituted at the equivalent of 10% on farinograph parameters.

Source	DF	Breakdown	MTI	Absorption
Between	4	6.23***	327.50***	10.55***
Within	10	0.08	5.00	0.23
Total	14			

*** p<0.001

APPENDIX 3.

ANALYSES OF VARIANCE OF FARINOGRAPH PARAMETERS BY REPLICATE BLOCK, PROTEIN TYPE AND SUBSTITUTION LEVEL

Table Q. **Analysis of variance of farinograph parameters by replicate block, protein type and level of substitution.**

Source	DF	Arrival	Peak	Departure	Stability
Main effects					
Rep block	2	0.55*	0.25*	0.83	0.77
Protein	3	3.80***	0.58***	0.79**	7.50***
Level	2	0.88**	1.94***	20.27***	31.27***
2-way Interactions					
Rep protein	6	0.12	0.05	0.04	0.19
Rep lvl	4	0.05	0.06	0.17	0.35
Protein lvl	6	1.35***	0.78*	0.14	2.49***

*** $p<0.001$
** $p<0.01$
* $p<0.05$

APPENDIX 3.

ANALYSES OF VARIANCE OF FARINOGRAPH PARAMETERS BY REPLICATE BLOCK, PROTEIN TYPE AND SUBSTITUTION LEVEL

Table R. Analysis of variance of farinograph parameters by replicate block, protein type and level of substitution.

Source	DF	Breakdown	MTI	Absorption
Main effects				
Rep block	2	0.19*	9.03	0.62***
Protein	3	1.14***	324.07***	9.22***
Level	2	27.44***	1036.11***	0.28*
2-way Interactions				
Rep protein	6	0.12	0.79	0.06
Rep lvl	4	0.22**	0.86	0.23*
Protein lvl	6	0.62***	26.29***	3.51***

*** $p<0.001$
** $p<0.01$
* $p<0.05$

APPENDIX 4.

ANALYSES OF VARIANCE OF FARINOGRAPH PARAMETERS BY FLOUR TYPE, SUBSTITUTION LEVEL AND BEAN QUALITY

Table S. Analysis of variance of farinograph parameters by flour type, level of substitution and bean quality.

Source	DF	Arrival	Peak	Stability
Main effects				
Flour	1	4.07***	5.06***	6.25***
Substitution	2	8.38***	0.47***	36.75***
Quality	1	0.14	0.17*	0.44
2-way Interactions				
Flour sub	2	1.22***	1.90***	2.58**
Flour qual	1	0.02*	0.34**	1.78*
Sub qual	2	0.07*	0.17**	0.86
3-way Interactions				
Flour Sub Quality	2	0.08*	0.13*	2.52**

*** $p<0.001$
** $p<0.01$
* $p<0.05$

APPENDIX 4.

ANALYSES OF VARIANCE OF FARINOGRAPH PARAMETERS BY FLOUR TYPE, SUBSTITUTION LEVEL AND BEAN QUALITY

Table T. Analysis of variance of farinograph parameters by flour type, level of substitution and bean quality.

Source	DF	Breakdown	MTI	Absorption
Main effects				
Flour	1	32.11***	367.36***	4.12***
Substitution	2	9.53***	952.08***	48.01***
Quality	1	1.00***	34.03	0.02*
2-way Interactions				
Flour sub	2	9.36***	179.86***	1.22***
Flour qual	1	1.78***	34.03	0.21**
Sub qual	2	0.58**	159.03***	0.01
3-way Interactions Flour Sub Quality	2	0.53**	9.03	0.13*

*** $p<0.001$
** $p<0.01$
* $p<0.05$

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