

STUDY OF SOME FACTORS AFFECTING
PELSHENKE TEST FOR BREADMAKING
QUALITY

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Francisco Javier Rodriguez-Bores

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A dissertation submitted to the Faculty of Graduate Studies of
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DOCTOR OF PHILOSOPHY

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Carolina

Hay en ti fragancia e ímpetu juvenil, emoción palpitante y sostenida que da una impresión de abundancia y prodigalidad. Me has contagiado de un sentimiento expectante de superación que sin ti no hubiera sido posible obtener la culminación de mis estudios. A ti, mas que a nadie, dedico esta tesis porque tu eres el ser que ha llenado mi vida de dulcísimas y gratas emociones, porque con tu dinamismo y cariño me has alentado en los momentos mas difíciles haciendo de mis ensueños realidades. Con todo mi amor.

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ABSTRACT

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Study of Some Factors Affecting Pelshenke Test for Breadmaking Quality

Major Professor: Dr. Walter Bushuk.

The Pelshenke Test is used in Australia, Europe, Africa and Mexico for screening wheat lines for breadmaking quality in breeding programs. The present study indicated that mixing procedures similar to those used in baking tests gives higher precision than manual mixing used in the classical Pelshenke Test. The effects of mixing time and speed on Pelshenke value were determined. Pelshenke value increased directly with mixing time but mixing speed had no effect. The Test has been applied to rye and triticale to examine the feasibility of using it for screening breeding lines of these cereals in addition to wheat.

The Pelshenke value is affected by environment (location and growth) as well as by cultivar. The location differences were generally smaller than the cultivar differences. The usefulness of the Pelshenke Test as an index of breadmaking quality was further examined by determining its relationship to other quality tests such as the Zeleny Sedimentation value and farinograph measurements. In general, highly significant correlations were obtained.

The effect of environment on the Pelshenke value is mainly through its effect on protein content and protein solubility. Of the protein fractions, glutenin seems to play an important role in the Pelshenke Test. Variations in amino acid composition in flours of widely different protein content was consistent with the protein solubility results.

Effects of major flour constituents such as protein content, the

amount of starch damage, pentosans, lipids and enzymes on the Pelshenke value were investigated. Pelshenke value increased directly with all the constituents except for enzymes. The effects of commonly used flour improvers and one sulfhydryl blocking agent (N-ethylmaleimide) were also examined. Additions of potassium bromated and ascorbic acid produced an increase in Pelshenke value to an optimum followed by a decrease with further additions. Cysteine had a negative effect on Pelshenke value. N-ethylmaleimide also had a negative effect because of its ability to inhibit yeast activity.

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LIST OF ABBREVIATIONS

ABS	BAKING ABSORPTION, %
ALV	AACC LOAF VOLUME, cc
DIA	DIASTATIC ACTIVITY, M.u. (Maltose Units)
FAB	FARINOGRAPH ABSORPTION, %
FDT	FARINOGRAPH DEVELOPMENT TIME, min
FLA	FLOUR ASH, %
FLP	FLOUR PROTEIN, %
FLY	FLOUR YIELD, %
GAP	GASSING POWER, mmHg
GRT	GRINDING TIME, min
MDT	MIXOGRAPH DEVELOPMENT TIME, min
MTI	MIXING TOLERANCE INDEX, min
NIF	NITROGEN FERTILIZER, lb/ac
PEL	PELSHENKE VALUE, min
PEF	PELSHENKE VALUE IN FLOUR, min
RLV	REMIX LOAF VOLUME, cc
SED	ZELNY SEDIMENTATION VALUE, cc
STD	STARCH DAMAGE, F.u. (Farrand Units)
WHP	WHEAT PROTEIN, %

I. INTRODUCTION

Wheat is the most important staple food crop of the world. It provides approximately 20% of the calories consumed by the world's population. It is grown on a larger area and produces more grain than any other cereal. The cultivation of wheat extends from very Southern regions of Australia and South America to a latitude of about 60° north in Asia, Europe and North America. Wheat is grown over a wide range of elevations from sea level to over 3,000 meters in Ecuador and Kenya. There are many factors that affect wheat production; all of them must be considered together if the contribution of wheat to the world food supply is to be significantly increased through agricultural research.

By far the most important segment of agricultural research relative to food production has been the breeding of higher yielding, better quality varieties of cereal grains, in general, and wheat in particular. The introduction of the semi-dwarf wheat varieties, developed in Mexico, into India and Pakistan has led to the so-called Green Revolution. Within a period of five years, from 1967 to 1972, India increased its wheat production by almost 100%; most of this increase can be directly attributed to the size of the new high-yielding varieties.

In order to be a viable commercial variety (cultivar), a new strain of wheat must offer some advantages in agronomic and/or end-use qualities. Since most of the wheat grown around the world is used for the production of bread, the new variety, developed by the breeder, must have a certain minimum level of breadmaking quality. The actual level of quality varies quite widely among countries that have wheat breeding programs depending, to a large extent, on the class of wheat that can be grown in a specific country or area. The lack of suitable screening tests for breadmaking

quality of lines in wheat breeding programs, presently constitutes one of the problems in agricultural research that requires urgent solution.

A suitable quality screening test should be simple and rapid so that it can be used to examine a large number of lines to facilitate the discarding of undesirable lines and thereby improve the efficiency of the breeding program. The test should also give a reasonably accurate measure of quality so that lines with satisfactory quality are not inadvertently discarded.

In the past 50 years, cereal chemists have developed numerous tests for predicting the breadmaking quality of wheat. Among the tests that have been developed, the so-called Pelshenke Test has been applied quite successfully in Latin America, Africa, Australia and Europe for screening new wheat varieties because it measures a very important parameter of baking quality, namely the dough strength. In North America the Pelshenke Test is used to some extent and is commonly known as the Wheat Meal Fermentation Time Test.

Since the wheat kernel comprised many different constituents, it can be stated that breadmaking quality of wheat depends on the relative proportion of individual constituents in the flour, on the properties of these constituents and on the processing used to convert the wheat into bread. The aim of the work presented in this thesis was to study the contribution of some of these constituents to the Pelshenke Test time relative to its ability to predict breadmaking quality. In addition, a number of other technologically relevant factors such as variety, growth environment, meal (or flour) particle size, dough mixing time and chemical ingredients (used in the baking industry) were investigated. Results of these ancillary investigations will be presented and discussed.

II. LITERATURE REVIEW

A. Introduction

There are many approaches that can be taken to reviewing the literature that is relevant to this thesis project. If the review is restricted to the literature that specifically mentions the Pelshenke Test, then the review would be too brief and too narrow because of the extremely small number of documented studies on this topic. In the other extreme, if a broad approach is adopted to cover all aspects of the nature and inheritance of quality in wheat, the review would be too long and unwieldy. Obviously an intermediate approach had to be chosen.

The review that follows will be presented in three major subsections. The first will review the literature on those wheat constituents that are known to be important to its end-use quality. This subsection will include references to genetic, environmental and processing factors if they are considered relevant to the quality contribution of the constituents in question. The second subsection will deal with the literature on the need, application, and value of quality tests for screening lines of wheat in breeding programs. The final section will review the literature on the Pelshenke Test. Finally, the review will deal only with bread wheats. References to durum (macaroni) and soft (biscuit) wheats will be made only if they are explicitly relevant to the subject of the thesis. It should be noted that the division between bread and biscuit wheat is not a sharp one; there is considerable overlap. A low-protein bread wheat in some countries may be a high-protein biscuit wheat in others.

One further qualification. The term "quality", as used in this review and indeed throughout the thesis, refers to milling and baking quality for the production of North American-type white bread. It is

synonymous with "breadmaking quality". "Nutritional quality", which is particularly important in countries where wheat products provide most of the protein for human nutrition, is compatible with breadmaking quality insofar as it relates to the protein "content" of the wheat (Shukla, 1975). The two qualities are incompatible in terms of the first limiting essential amino acid, lysine. Wheat protein of higher lysine content is usually lower in specific breadmaking quality (Middleton et al., 1954; Mattern et al., 1970). Nutritional quality is outside the subject of this thesis and will not be discussed further.

B. Wheat Constituents Related to Quality

The wheat kernel comprises three major morphological parts (Bradbury et al., 1956); 83% endosperm, 14.5% bran, and 2.5% embryo or germ. In milling, the bran and the embryo are separated from the endosperm which is then ground into flour. Accordingly, insofar as quality is concerned, it is sufficient to consider only the constituents of the endosperm. Contamination of the flour by bran and embryo components is an indication of inefficient milling and is generally detrimental to quality (Eva and Fisher, 1957).

The main constituents of wheat endosperm are: carbohydrates (starch and pentosans) (70.4%), protein (13.2%), water (14%), lipids (1.3%) (Bushuk, 1975). The figures in parenthesis are average values for Canadian hard red spring wheat expressed on as is basis. Relevant literature on each major constituent will be reviewed in separate subsections.

1. Starch

Starch is the commonest food reserve material of the plant kingdom. It forms the major component of the wheat kernel and, hence of the flour

prepared from it. Starch is normally present in the form of characteristic birefringent granules. Starch granules from different plants vary distinctly in size and shape. The granules, which are insoluble in cold water, swell reversibly when suspended in water. As the temperature of the aqueous suspension is increased the swelling process proceeds until the granules burst with the formation of starch pastes (MacMasters et al., 1975). This swelling and eventual disruption of the starch granules is commonly called gelatinization. The most easily observed change in the process of gelatinization is the rapid and complete loss of birefringence of individual granules when viewed microscopically with plane polarized light (Zobel and Senti, 1959; Wivinis and Maywald, 1967). All granules in a starch sample do not lose birefringence at the same temperature or at the same rate; gelatinization takes place over a range of temperature which is characteristic of the particular starch granules (Aspinall, 1970).

The granule of starch comprises a mixture of two polysaccharide components: amylose, which consists of glucose units connected to each other by the alpha-1,4-glycosidic linkage resulting a linear chain of glucose units, and amylopectin which has a highly branched structure in which the glucose units are connected by alpha-1,4-bonds in the main chains and by alpha-1,6-bonds at the branch points.

When wheat is milled into flour, a portion of the wheat starch granules undergoes physical damage as a consequence of the grinding action of the mill rolls. Jones (1940) found that the amount of damaged starch produced varied according to the conditions of milling. The starches of different classes of wheat differ in susceptibility to physical damage, i.e. hard wheat flours usually have much higher starch damage values than

soft wheat flours (Williams, 1967). Damaged starch absorbs 2.5 times more water than granular starch (Greer and Stewart, 1959). Also, it is more susceptible to starch-degrading enzymes (Tipples et al., 1966). Starch damage and amylase activity are extremely important in determining the baking absorption of a flour (Tipples et al., 1966). Damaged starch acts like a sponge in the presence of water and so offers a potential way of increasing water absorption of a flour and hence bread yield (Tipples, 1975). Accordingly, an increase in the damaged starch level of a flour leads to a direct increase in the absorption of water by the flour at the mixing stage. Differences in water absorption of flours from hard and soft English wheats can be accounted for largely by the variations in protein and damaged-starch contents of the flours (Greer and Stewart, 1959). A failure of some flours to show the increase in water-absorption expected from the increase in damaged-starch content has been attributed to unusually high amylase activities. The rapid hydrolysis of the damaged starch and undue softening of the dough during fermentation must be counteracted by decreasing absorption (Jones, 1940). A certain minimum level of damaged starch is desirable and necessary to provide fermentable sugars by the actions of amylases (to be reviewed later), to maintain dough gas-producing power during fermentation and proofing (Jones, 1940). If damaged starch is increased above a certain maximum value characteristic for the baking procedure used, bread quality will suffer (Kulp, 1972).

According to Sandstedt (1961), starch in a bread dough has the following functions: a) it dilutes the gluten to a desired consistency, b) it furnishes fermentable sugars through amylase action, c) it provides a surface suitable for a strong interaction with the gluten, d) it becomes flexible during partial gelatinization, thereby permitting the stretching

of the granules to form gas-cell membranes in the bread crumb, and e) it takes up some of the water from the gluten which loses its water binding capacity because of the thermal denaturation in the baking phase. During baking, it is mainly the gluten films that become rigid and form the three-dimensional structure of a loaf of bread.

Recent fractionation and reconstitution studies by Hoseney et al. (1971) have shown that, contrary to previous claims, wheat starch is not unique in its contribution to breadmaking. They found that barley and rye starches were as functional as wheat starch in breadmaking. The similarity in the contributions of those starches to the functional properties in baking seemed to be related to the physical characteristics of their granules. As far as the author is aware, the effect of starch damage on Pelshenke Test has not been investigated.

2. Proteins

The word protein was derived from the Greek "proteios" meaning primary. Proteins are the most important organic molecules found in all living cells. Chemically, proteins are polymers of about 20 different amino acids linked together by peptide bonds to form giant complex molecules. There are many different kinds of proteins, each synthesized for a specific biological function; it may serve as an enzyme catalyzing a specific chemical reaction, serve as a structural or storage substance, bind and transport ions, or carry out some other function (e.g. hormones).

Protein Content and Quality in Wheat. The protein content of wheat or flour can be determined rapidly and accurately by the Kjeldahl method for nitrogen (AACC, 1969). The nitrogen content is converted to protein content by multiplying by a constant factor(5.7). This factor was originally determined from the nitrogen content of gluten which was considered,

erroneously, to be a pure protein. The factor determined from the total amino acid composition is very close to the classical 5.7 (Tkachuk, 1966).

The protein content of wheat depends primarily on two factors: variety and environment. In Canada, the genetic factor is controlled to some extent by the statute-controlled licencing of cultivars that can be grown commercially. The success of this method of control is perhaps best demonstrated by the fact that spring wheats released for the bread class have continued to maintain the high protein content of Canada's most famous hard red spring wheat variety, Marquis.

The effect of environment on the agronomic and quality characteristics, especially protein content, has been investigated by several workers. Miller et al. (1950) and Gunthar and McGinnis (1957) observed that the protein content in wheat could be modified by breeding and selection and also by applying fertilizers. However, there are considerable problems in combining high yielding capacity with satisfactory baking quality because of the negative correlation usually found between grain protein content and grain yield (Haunold et al., 1962; Baker et al., 1968). It has been difficult to achieve the combination of high yield, high protein content and good breadmaking quality by plant breeding. Nevertheless, Johnson et al. (1975) working with a cross between Atlas 66, a high protein soft wheat with poor breadmaking quality, and Comanche, a bread wheat with good breadmaking quality, demonstrated that the high protein line selected had entirely satisfactory milling and baking properties. Accordingly, it should be possible to improve both yield and protein content and maintain baking quality by breeding.

It is well documented that the physical and chemical properties of wheat grain as well as protein quality under normal growing conditions

are almost entirely an inherited characteristic. Protein quantity in wheat depends primarily on soil and climatic conditions during the growing period (Wahhab and Hussain, 1957; Fernandez and Laird, 1959). The production of high protein wheat with satisfactory breadmaking quality, by the application of nitrogen fertilizer, has been observed by several workers (Fisher and Jones, 1931; Worzella, 1944; Long and Sherbakoff, 1951; Sibbit and Bauer, 1970). Wahhab and Hussain (1957) reported that the application of fertilizers increased the protein content of wheat grain, however, Fernandez and Laird (1959) observed that the application of 45 lbs of nitrogen per acre decreased the protein content of the whole grain, while larger applications increased it. Despite its profound effect on protein content, the environment had no effect on the electrophoresis of the gliadin proteins of wheat (Lee and Ronalds, 1967). This suggests that qualitatively the protein remains essentially the same while the protein content of the grain varies quite widely.

Considerable attention has been given to the amino acid composition of wheat varieties or commercial blends of various origins. Published reports (Renner et al., 1953; Gunthard and McGinnis, 1957) show that environmental factors, including soil type, fertilizer, and climate have no effect on amino acid composition of normally developed grain.

Protein Quality for Breadmaking. Proteins of wheat have received considerable attention in relation to their contribution to the breadmaking quality of flour. Breadmaking properties of wheat flour depend on numerous factors, including wheat variety, environmental conditions during growth, soil fertility, the milling process, and the chemical composition of the flour. The effect of many of these variables is indirect. It is the resulting variation in chemical composition, especially of flour

protein, that causes variation in breadmaking quality. Relevant literature on qualitative differences in the proteins of wheat cultivars is reviewed in the following five subsections.

Protein Solubility Fractionation. One of the earliest tests of protein quality for breadmaking was based on the determination of the amount and the viscoelasticity (rubber-like property) of the gluten obtained from the flour. The first recorded attempt of isolation of flour proteins was that of Beccari, who in 1728 achieved a separation of a substance from dough which he named "gluten" (from the Greek meaning sticky). This he did by washing the starch from a dough under a stream of water, leaving a yellowish sticky mass as residue (Gortner, 1942).

Approximately 75 years after the discovery of Beccari, Einhof (1805) extracted from the crude gluten a substance which was soluble in 70% ethanol, and which could be precipitated on dilution of the alcoholic solution with water. This partially purified substance was even more sticky than gluten itself, and he named it "gliadin" (from the Greek word meaning glue). The remaining insoluble protein was partially purified by Taddei in 1820 who named this fraction "glutenin". This substance was unlike gliadin, being much firmer and not sticky (Gortner, 1942).

Modern cereal chemistry began with the classical work of Osborne (1907), in which he described fractionation procedures for flour proteins which are the basis of the methods used today. He defined four protein fractions and attempted to determine the amino acid composition of each of the protein fractions. Osborne (1907) classified wheat proteins as follows: albumins were the water-soluble proteins, globulins were soluble in salt solutions, gliadins dissolved in 70% aqueous ethanol, and glutenins dissolved in dilute acid or base. With minor modifications, the solubility

fractionation method of Osborne has been used extensively in studies of the structure and function of wheat proteins (Tanaka and Bushuk, 1972; Orth and Bushuk, 1973).

Soluble Proteins. Approximately 10 to 15% of the total protein in wheat consists of albumins and globulins (Doby, 1965; Whitehouse, 1973). These two groups of proteins are generally referred to as the soluble proteins. Most of the enzymes in flour are extracted with this group of proteins. The role of albumins and globulins that are not enzymes in the cereal seed is not clearly understood. There has been considerable disagreement on the influence of the albumins and globulins on breadmaking quality (Pence et al., 1954; Mattern and Sandstedt, 1957). Recent studies suggest that the albumin and globulin proteins contribute very little to the breadmaking quality of flours from normally developed wheat (Pence, 1962; Hoseney et al., 1969).

The albumin and globulin proteins usually have a better balanced amino acid composition than gliadins and glutenins in relation to human and animal nutrition. This advantage is nullified when considered on overall grain composition with respect to amino acids because of the much higher content of gliadins and glutenins. The gliadins, in particular, are a very poor source of lysine, tryptophan and sulphur containing amino acids (Shukla, 1975).

Insoluble or Gluten Proteins. Gluten is the protein complex that gives dough its elastic, plastic, and cohesive properties (Barmore, 1947). It is mainly proteinaceous in nature but contains substantial quantities of lipid and carbohydrate materials. The two major proteins in gluten are the gliadins and glutenins. It has been shown that while glutenin is a multichain complex stabilized by disulfide bonds, gliadins are compact,

single chain molecules (Bietz and Rothfus, 1970; Bietz and Wall, 1972; Orth and Bushuk, 1973). The problem of relating the chemical composition and structure of wheat gluten protein to its functional properties in breadmaking is extremely complex, however, the unique structural properties of gliadin and glutenin proteins in gluten appear to be the major determinant in the viscoelastic properties of doughs.

Of the various reactive chemical groups of wheat proteins, the thiol groups and the disulfide bonds in the gluten proteins have been studied extensively because they play a special role in the rheological properties of wheat flour doughs. Wheat flour contains approximately 1 ueq per g of thiol groups and 15 ueq per g of disulfide groups. Apparently, not all of these groups are equally important in dough. Bushuk (1961) and Bloksma (1972) have demonstrated that the rheologically effective thiol groups of disulfide bonds represent only a small fraction of the total present in the flour. So far as the author is aware there have been no studies of the implications of flour protein solubility fractions and thiol and disulfide groups on the Pelshenke Test time.

Effect of Oxidizing, Thiol Blocking and Disulfide Reducing Agents. It was well known from practical observations, before the era of modern cereal chemistry, (since about 1925), that freshly milled flour improved in its breadmaking quality if it was stored for several weeks after milling (Working, 1928). The so-called "green" flour "matured" during storage. Subsequently it was shown that the major chemical change that produced the maturing effect was the oxidation of thiol groups by atmospheric oxygen (Baker and Mize, 1937; Yoneyama et al., 1970). Soon after the discovery of the maturing of flour by natural storage, it was found that essentially the same improvement in the baking quality of flour

could be obtained by the addition of small quantities (5-15 ppm) of certain chemical oxidizing agents such as nitrogen trichloride, chlorine dioxide and potassium bromate (Ferrari et al., 1945). Since that time, many other so-called "improvers" have been introduced to baking technology. The economic advantages of using chemical improvers are obvious; five ppm of potassium bromate is considerably less costly than the four to five weeks of storage time accompanied with additional risks of deterioration due to spoilage or infestation.

It is well documented that agents such as thiol containing compounds, sulfite or powerful oxidizing agents, which break disulfide bonds, rapidly destroy the viscoelastic properties of dough (Tsen, 1968). The functionality of wheat flour for breadmaking is completely destroyed by additions of minute quantities of reagents that break disulfide bonds by reduction or oxidation.

On the other hand, oxidizing flour improvers such as potassium bromate, potassium iodate, azodicarbonamide and ascorbic acid, appear to make the protein network more rigid, thereby increasing the strength of the dough, which is usually accompanied by a considerable increase in loaf volume (Tsen, 1968). Hird and Yates (1961) showed that oxidizing agents will oxidize thiol groups of simple compounds and of gluten proteins to disulfide bonds and that can be the disappearance of thiol groups, but not necessarily the formation of disulfide bonds, can be equated with the rheological effect on dough.

Bloksma (1972) described, on the basis of experiments with N-ethylmaleimide (NEMI) which reacts with thiol groups without the formation of disulfide bonds, that this substance reacts in a monofunctional manner with thiol groups, thereby blocking them from subsequent reactions in dough.

This thiol blocking agent produces in dough a similar effect on rheological properties as the addition of oxidizing agents (Bloksma, 1975). It has been postulated by Goldstein (cited by Bloksma, 1975) that viscous deformation of dough involves breaking and reformation of disulfide bonds, and that those reactions proceed as a result of thiol-disulfide interchange. It now appears that the main mechanism of the so-called dough improver effect results from the inhibition (by bromate, NEMI, etc.) of the disulfide interchange reactions rather than from the formation of disulfide bonds.

Reducing agents such as glutathione and bisulfite were found to act specifically on the gluten proteins (Matsumoto et al., 1960) and the site of action of these reducing agents on the gluten proteins was in fact the disulfide bonds. This was demonstrated by the increase in the thiol content of gluten when treated with the reducing agents. Finney et al. (1971) concluded that the mixing and other physical properties of doughs from long-mixing-time flours can be readily converted to desirably shorter mixing requirements, giving an increase in loaf volume, by adding 20 to 120 ppm of cysteine monohydrochloride. These effects were attributed to the increase in the rate of interchange reactions and thereby an increase in the rate of dough development by mixing a dough that had been partially reduced by cysteine.

Relative to the subject of this thesis, it has been observed by Swanson and Dines (1939) that the Pelshenke Test time was markedly increased by the addition of potassium bromate and ascorbic acid. On the other hand, addition of cysteine monohydrochloride, reduced the Pelshenke Test time.

Relation of Flour Proteins to Loaf Volume of Bread. Loaf volume, the main index of breadmaking quality, depends on flour protein. Research over many years (Finney and Barmore, 1948; Bushuk et al., 1969) has shown that the relationship between protein content and loaf volume for a single wheat cultivar, is essentially linear within the protein content range from 8% to 18%. The slope of the linear loaf volume-protein content relationship varies among bread wheat cultivars (Finney and Barmore, 1948; Bushuk et al., 1969). Those cultivars that give a higher slope (higher loaf volume per unit protein) are said to have protein of higher "quality" for breadmaking (Orth and Bushuk, 1972). The fact that the protein content-loaf volume relationship is linear within a cultivar greatly simplifies the determination of the breadmaking quality of new wheat varieties in breeding programs (Bushuk et al., 1969).

In recent years, many attempts have been made to correlate breadmaking quality with the protein fractions separated by solubility fractionation, column chromatography or electrophoresis. Dronzek et al., (1970) found that differences in protein solubility distribution could be related to the breadmaking qualities of three hexaploid wheats and their AABB extracted tetraploids. Two of the hexaploid wheats had both better baking quality and a higher proportion of residue protein than the tetraploids derived from them. Chen and Bushuk (1970) concluded from a study of the baking quality and solubility distributions of the proteins of triticale, its durum wheat and rye parents, and one hard red spring wheat (Thatcher), that the main reason for the superior baking quality of the hard red spring wheat was the lower content of water-soluble protein and higher content of insoluble residue protein. More recently, Orth and Bushuk (1972) concluded, from a comparative study of the proteins of 26

wheat varieties of diverse baking qualities, that loaf volume per unit protein (ULV) was inversely related to the proportion of glutenin protein (fractions soluble in 0.1N acetic acid). A highly significant positive correlation was obtained between ULV and the proportion of the insoluble residue protein (insoluble glutenin) in the flour. On the basis of these studies, it appears that the amount of protein that is insoluble in 0.1N acetic acid solution can be a reliable index of quality.

3. Lipids

Lipids are water-insoluble organic molecules that can be extracted from cells and tissues by nonpolar solvents such as chloroform, ether or benzene. Lipids have several important biological functions. They serve as structural components of membranes, as storage and transport forms of metabolic fuel, and as a protective coating on the surface of many organisms. Where processing characteristics are the concern, however, the small amounts of lipids in wheat and flour are of interest because they modify the behaviour of other constituents such as the starch and the proteins (Lehninger, 1975).

Wheat is adapted to production in widely different environments throughout the world, but variability in lipid content among wheats grown in different parts of the world is negligible. However, the free lipid (extractable by petroleum ether) appears to be a varietal characteristic; cultivars grown under the same conditions differ more in lipid content than grain of one cultivar grown in different locations (Garcia-Olmedo, 1968). This is in contrast to protein and starch contents of wheat which are affected more by environment than by cultivar.

Lipids of wheat flour are divided in two groups (free and bound) on basis of solubility in the common lipid solvents. Bound lipids are

unextractable because they occur in flour as complexes with protein and carbohydrates (Mecham and Mohammad, 1955). Bread flours contain approximately 1.4% lipid of which 57% is "free" lipid and the remainder "bound" lipid.

Functionality of Lipids. The role that flour lipids play in bread-making has been the subject of many repeated studies. Glass (1960) has assigned to the lipids and the lipoxidase-unsaturated fatty acid system a vital function as intermediaries between the oxidative agents and the thiol groups flour proteins. The oxidation of flour lipids during dough mixing was established by Smith and Andrews (1957) who found a linear relationship between oxygen uptake and the free fatty acid content of the flour lipids. When linoleic and linolenic acids were added to the dough the oxygen uptake was increased during mixing and was largely dependent on enzyme action. The addition of oleic acid did not produce this effect. The products of lipid oxidation, hydroperoxides, can react with the thiol groups and thereby modify dough properties (Tsen and Hlynka, 1962). These investigators concluded that when sufficient oxygen is available, it reacts with both thiol groups and lipids; the oxidized lipids in turn can also oxidize thiol groups accelerating their disappearance and producing a concomitant improving effect. However, Dahle and Sullivan (1963) found little reaction between oxidized lipid and thiol groups in dough.

Evaluation of the contribution of lipid constituents to the physical properties of dough and the baking performance of flour involves the removal of various lipid fractions with different organic solvents, followed by a comparison of dough properties and baked products from the original and extracted flours, or the extracted flour to which specific lipid fractions

are restored. Hosney et al. (1969) used a flour that was almost completely free of lipids to study the comparative roles of free polar, free non-polar, and bound polar lipids. They found that additions of small amounts of free polar or bound polar lipids were detrimental to loaf volume. This detrimental effect was offset by the addition of the original free or bound non-polar lipids. Other studies of this type (Lin et al., 1974) have included comparisons of the effects of flour lipids with those of commercial shortening. These workers found that in defatted flour, the baking properties were restored by the addition of any of the five lipid (extracted) fractions added at the 0.5% level, when shortening was present in the bread formula.

There has been only one report of the possible implication of the flour lipids in the Pelshenke Test value. Swanson (1937) found that the Pelshenke time increased for whole meal and decreased for flour when each was extracted with ethyl or petroleum ether which remove the free lipids. However, when N-caproic or pelargonic acid were added, the Pelshenke time increased markedly for both the whole meal and the flour. Addition of lecithin or oleic acid had little effect on Pelshenke time. The same author pointed out that the wheat meal fermentation time test reflects the composite effects of numerous factors operating in the complex dough system.

4. Hemicellulose or Pentosans

Hemicelluloses or pentosans are natural plant constituents composed largely of anhydro-D-xylose units to which L-arabinose are linked as single unit side chains. Pentosans are widely distributed in the plant kingdom as cellular components and frequently they are referred to as plant cementing tissues (Aspinall, 1970). Hemicelluloses are sometimes

classified as water-soluble or water-insoluble, however the distinction between the two classes is not sharp.

Functionality of Pentosans. Pentosans, a minor component of wheat flour (approximately 2%), have received considerable research attention because of their hydrophilic properties. They make a significant contribution to the water-absorption of dough. As a consequence, because they absorb approximately 10 times their own weight in water, they hold approximately one-fourth of the water in a bread dough (Kulp, 1968). Kulp (1968) also showed that addition of isolated flour pentosan, reduces the dough development time in the farinograph.

In a study on the effect of wheat flour pentosans in baking, Tracey (1964) reported a considerable reduction in loaf volume produced by the addition to the dough of an enzyme from the digestive juices of starved garden snails which degraded the pentosans. He concluded that the pentosans were directly involved in determining loaf volume. Kulp and Bechtel (1963) studied the effect of purified water-insoluble pentosan fractions on dough properties and bread quality. They found that these pentosans increased water absorption but had no effect on mixing time and dough stability when compared to control doughs of equal consistency. The handling properties of doughs with added pentosans were satisfactory but the bread had lower loaf volume and coarser grain than the control bread. D'Appolonia et al. (1970) demonstrated that the addition of pentosan fractions produced only a slight increase in loaf volume and had a detrimental effect on crust color.

So far as the author is aware, the effect of pentosans on Pelshenke Test time has not been investigated.

5. Enzymes

Enzymes are specialized proteins that catalyze various biological reactions. They are among the most remarkable biological macromolecules known because of their extraordinary specificity and catalytic power, which are far greater than those of man-made catalysts. Today nearly 2,000 different enzymes are known. Many have been isolated in pure homogeneous form, and at least 200 have been crystallized (Lehninger, 1975). A number of enzymes are of great practical importance in milling and baking technology. The pertinent literature on these is discussed in the following subsections.

Proteases. Proteases are enzymes that act on proteins and break them down into smaller units, peptides and amino acids. Cereals, generally, contain very low levels of protease activity and proteases are often added as supplements in breadmaking to bring about a "mellowing" effect on the dough (Redman, 1971). Handford (1967) presented evidence for the existence of two types of proteolytic enzymes in sound wheat, one causes gluten softening and the other produces low-molecular weight peptides. These are sometimes referred to as endo and exo proteases respectively. He also observed that the modified Ayre-Anderson method for the measurement of proteolytic activity with hemoglobin as substrate does not measure the activity responsible for gluten softening. Kruger (1971) has suggested that the action of proteolytic enzymes on gluten and dough can be measured by the gluten stretching test and the farinograph respectively. Flours milled from sound wheat have extremely low proteolytic activity. This low activity is generally considered insignificant in breadmaking quality (Kruger, 1971). Significantly higher proteolytic activities present in the flours from sprouted wheat are generally detrimental to breadmaking quality. Proteolytic cleavage of gluten proteins produces sticky extensible

doughs which cannot be processed into bread (McDonald, 1969).

Proteases present in a very small amount can be beneficial in some flours (e.g. from very strong wheats) for optimum dough development during mixing and fermentation. Enzymatic hydrolysis of just a few strategic peptide bonds near the centre of the protein chain of the gluten complex would be expected to cause a significant change in the rheological properties of the dough (McDonald, 1969).

In relation to the effect of flour proteases on the Pelshenke Test value, Swanson (1937), and Swanson and Dines (1939) showed that addition of proteases to the wheat meal reduced the Pelshenke time for both hard red spring and hard red winter wheats.

Amylases. These enzymes have long been used as baking supplements because of their ability to increase gas production in fermenting doughs. Kuhn (1925) demonstrated that at least two amylase factors are present in barley malt which he designated as alpha- and beta-amylase because of the property of the first enzyme to form the alpha-glucoside and the second to produce the beta modification of maltose (Pyler, 1973). Sound (non-sprouted or ungerminated) wheat contains a relatively high level of beta-amylase activity and a very low level of alpha-amylase activity. On the other hand, sprouted wheat contains a very high level of alpha-amylase, but the amount of beta-amylase is still similar to that in sound wheat. Tipples et al. (1966) showed that baking quality of wheat deteriorates rapidly as the wheat sprouts mainly because of the development of high alpha-amylase activity.

Since wheat flour normally contains sufficient beta-amylase, the amylase supplementation of flour at the mill, or of dough and sponges in the bakery, concerns usually the increase in alpha-amylase activity.

While alpha-amylase performs several functions in breadmaking, the most important is the indirect hydrolytic release of fermentable sugars from starch during fermentation period. These sugars serve as the substrate which the yeast enzymes require for the production of carbon dioxide that leavens the dough (Pyler, 1969).

For satisfactory baking performance, the activities of the two amylases in the dough should be in balance, since excess of alpha-amylase activity could lead to excessive dextrin formation, with a resulting sticky bread crumb (Walden, 1955). Conn et al. (1950) compared the action of commercial alpha-amylase preparations obtained from two bacterial and six fungal sources with that of malted wheat flour. They confirmed that fungal amylase preparations may be used for supplementation if the level of proteolytic activity relative to the alpha-amylase activity is not excessively high. The alpha-amylase of bacterial origin was the most potent in the production of fermentable sugars during the five-hour fermentation period.

Mixtures of alpha- and beta-amylase gave fermentable sugars in amounts slightly lower than the amounts computed for adding the contributions of each enzyme, separately (Pyler, 1973). Studies on the effect of adding various amounts of alpha- and beta-amylase to dough, have demonstrated that the addition of small amounts of alpha-amylase to a normal, medium-strength flour dough improved crust color, grain and loaf volume. The addition of larger amounts increased loaf volume, but produced inferior grain and texture. When beta-amylase was added in larger amounts, the resultant loaves were invariably poorer, and had a greater tendency to develop a shell top (Pyler, 1973). The effect of alpha-amylase on Pelshenke Test value has not been investigated.

6. Processing Factors

There are many processing steps involved in converting wheat into bread. The two main processes are milling and breadmaking. The quality of the bread produced from a given sample of wheat can be significantly altered by adjustments in process parameters. For example, a low extraction flour (low flour yield) usually gives better breadmaking performance than a high extraction flour because it contains lesser quantities of deleterious bran and germ constituents. In breadmaking, a flour that gives satisfactory bread when processed by the Chorleywood Breadmaking Process can sometimes give completely unsatisfactory results by the conventional straight-dough process. Literature on processing pertinent to the present study is reviewed in the subsections that follow.

Milling. Flour milling in a modern mill, represents the culmination of centuries of technological progress. The aim in milling is to break open the wheat kernel, scrape off as much endosperm from the bran as possible, and gradually grind (reduce) the practically pure endosperm middlings (coarse particles) into flour.

The milling process is essentially one of grinding and separating. Grinding is done on break rolls, which are corrugated, and on smooth reduction rolls which reduce the particles of endosperm further along in the process. Purification and classification by particle size is made on machines called purifiers and plansifiers which separate the mixture of particles (bran and germ) according to their size before further grinding and eventual reduction of the endosperm particles into flour. The purer the endosperm going to the reduction system, the brighter and whiter the flour (Panter, 1975). A large number of chemical and physical tests are now available to assist the miller to produce the optimum yield

of flour of specific quality from a given sample of wheat.

Granulation, or particle size, is a main quality component of a flour that is dependent on the milling process. For best performance in breadmaking the flour must have a characteristic particle size and particle-size distribution. Particle size in a finished, conventionally milled flour can be interpreted as a measurement of the friability of the wheat endosperm under the conditions of milling. Some mellow wheats tend to "shell out" their endosperm readily, whereas the friability of the more vitreous wheats requires larger work input (Katz et al., 1961).

Another flour quality component that depends almost entirely on the milling process is the level of starch damage. Most of the starch damage is produced in the reduction stage of the milling process (Jones, 1940). It has been demonstrated (Greer and Stewart, 1959) that in the milling of hard wheats, starch damage is likely to increase with the protein content of the wheat. The literature on the implications of starch damage in breadmaking quality has already been reviewed in the section dealing with starch (see above).

The third quality component that is directly dependent on the milling process is the purity of the flour which, in turn, depends on the efficiency with which the bran and the germ are separated from the endosperm. Without going into a detailed review, it can be said that bran and germ contaminants affect dough behaviour during processing and generally lower the quality (e.g. crumb color, texture and volume) of the final bread (Baker and Gregory, 1942). Of the milling factors, particle size and bran and germ contaminants have been investigated as factors involved in the Pelshenke Test time (Swanson, 1937; Swanson and Dines, 1939).

Breadmaking. Breadmaking is the process of converting flour into

bread. Most breadmaking processes have several distinct steps such as dough mixing, fermentation and proofing or dough development, and finally baking when the plastic dough is set into the rigid structure of a loaf of bread. Breadmaking methods can be divided into two broad groups, conventional and recent. These will be reviewed briefly.

Two conventional baking methods are commonly used, the straight-dough method and the sponge-and-dough method.

In the straight-dough method all the ingredients are combined in the mixer bowl and mixed into a dough by a single-step procedure. The dough is fermented for a period of time ranging from one to four hours and occasionally punched down during this time to remove some of the fermentation gas and redistribute the fermentable sugars. After fermentation, the dough is divided, rounded, placed in the pans and proofed for about one hour before baking. This method is most widely used by small bakeries around the world (Tipple, 1967). The remix modification of the straight-dough procedure developed by Irvine and McMullan (1960) has been extremely useful as a test baking procedure for strong flours. In this procedure all ingredients are mixed into a dough which is fermented for several hours, returned to the mixer and re-mixed until developed, given a short "floor" time, and then divided and baked according to standard procedure.

The sponge-and-dough breadmaking method is used by large bakeries in the United States, Canada and Japan (Tipple, 1975). In this method, approximately two thirds of the flour, along with the same proportion of water, and all of the yeast and yeast food, are mixed into a sponge and fermented for 3 1/2 to 5 hours. After this fermentation stage, the sponge is returned to the mixer, the balance of ingredients including the flour, water, salt are added and the dough mixed until developed to the desired

physical state as judged by "dough-feel" or by special instruments. The dough is discharged into a second trough where it is fermented in bulk for a short time, usually about 20 to 30 min called "dough" or "floor" time, and divided (Tipples, 1967; Kilborn and Tipples, 1968; Stenberg, 1968). Proofing and baking are the same as in the straight-dough method.

The most notable advance in breadmaking technology (recent bread-making procedures) has been the attainment of dough development, produced by bulk fermentation in conventional methods, by high speed mechanical mixing. Mechanical dough development involves the use of vigorous mixing of the dough in a high speed developer/mixer to produce changes similar to dough ripening changes that occur during fermentation. A further improvement in the mechanical dough development processes has been the use of certain fast acting reducing agents along with slower reacting oxidizing agents (Tipples, 1967). With this modification, it is possible to use standard dough mixers, used in conventional methods, run at slightly higher speeds.

A number of new breadmaking processes based on the mechanical and/or combined chemical-mechanical dough development principles are now used commercially. The "Do-Maker" Process, first described by Baker in 1950 (Tipples, 1967), was introduced in the United States in the late 1950's. The Do-Maker Process was soon joined by the Amflow Process (Anonymous, 1958). Today these constitute the two major continuous-breadmaking methods in the United States. Approximately 70% of the white pan bread in the United States is currently produced by these processes (Tipples, 1975).

The British Baking Industries Research Association in Chorleywood, England, introduced the Chorleywood Bread Process in 1961 to the British

baking industry (Elton, 1965). Pringle et al. (1969) pointed out that in 1969, approximately 70% of the United Kingdom bread production is made by such process.

The Chorleywood Bread Process is usually a batch process (it can be continuous) and is much simpler than the continuous United States processes. It is similar to the conventional straight dough method except that the dough is ripened (developed), not by fermentation, but by intense mechanical action in a special high-speed mixer and goes directly from the mixer to divider. A comparison of the processing stages and times for two conventional and two mechanical development breadmaking processes was published by Tipples (1967).

In chemical dough development methods, the development of the gluten is achieved by the combined action of a fast chemical reducing agent (cysteine, ascorbic acid) and a slower chemical oxidizing agent (potassium bromate) in conjunction with standard dough mixing (Tsen, 1969). Processing of dough involves mixing a straight dough in a conventional mixer for normal or slightly longer periods. The dough is given a short fermentation period of up to about 40 min, then it is divided and made up by usual methods (Tsen, 1969).

In North America, baking tests using different formulas and procedures are employed which, under controlled conditions, are designed to reveal not only the inherent quality of the flour sample, but also to obtain a measure of the optimum baking conditions, such as extent of mixing, length of fermentation, and level oxidation requirements necessary to obtain the highest loaf volume with satisfactory crumb and crust characteristics. Geddes (1941) defined baking quality as the sum of excellence on several points and included the production of satisfactory bread over a considerable

range of baking conditions, the ease with which large masses of dough can be handled in the bakery, and the bread yield obtainable.

From the above brief review of breadmaking methods, it will be noted that there are many processing variables that can be adjusted to improve the quality of the bread produced from a given sample of flour. Occasionally good bread can be obtained from a mediocre flour by a judicious adjustment in water absorption, mixing time, fermentation and proofing time and temperature, and baking conditions. Further improvements (or corrections) can sometime be obtained by additions of minor ingredients such as enzymes, oxidizing improvers (e.g. potassium bromate) and special shortenings and emulsifying agents. Of these variables only the effects of proteases and potassium bromate on the Pelshenke Test value (as an index of breadmaking quality) have been investigated (Swanson and Dines, 1939).

C. Quality Screening Tests in Breeding Programs

Scientific wheat breeding has led to the transformation of the traditional agriculture with the introduction of better adapted, more disease resistant and higher quality cultivars (Geddes, 1941). In fact, it was not until after the wide-spread adoption of the roller milling process in the United States in the late 1870's that hard wheats came to be favorably regarded for milling into bread flour. Wheat breeders began to pay more attention to the development of hard vitreous types of spring wheats and it became necessary for plant breeders to consider milling and baking quality, as well as yield and other desirable characteristics, in their wheat breeding program (Geddes, 1941).

The recognition of milling and baking quality in Canadian wheat breeding programs began in 1903 when Dr. Charles Saunders judged the

quality of the wheat flour from different wheats by means of the "chewing test" (Buller, 1919). He found that on taking a few grains of wheat and chewing them he immediately obtained an indication of the kernel hardness, a recognized milling quality characteristic. Continued chewing and mastication of the grain dissolved the starch and other solubles and left a gummy mass that is equivalent of wet gluten. The amount of this residue gave a fairly accurate indication of the protein content of the grain. By stretching the resulting gluten with the fingers, Dr. Saunders was able to assess its "quality" (viscoelasticity) for breadmaking. It was by this primitive quality screening test that Dr. Saunders selected the line from the cross of the cultivar Red Fife and an unnamed hard red cultivar from Calcutta that eventually became Canada's most famous bread wheat, Marquis.

The quality characteristics used for the assessment of new varieties in wheat breeding programs by Dr. Saunders in 1907 were summarized by Buller (1919) and included the following: relative plumpness of the kernels, density of the kernels (hardness), moisture content of the grain, soundness of the grain, baking strength of the flour (by chewing test), water absorption, and color of the flour. The principles of these simple tests formed the basis of the more sophisticated (some scientific and some empirical) quality tests that are in use today.

In spite of many years of relatively intensive research, cereal chemists have not yet developed a simple and accurate test for breadmaking quality of wheat that can be used for screening large populations in breeding programs. Numerous tests are available, however they all have certain shortcomings. In most cases they do not give an accurate measure of quality because they are specific for one or at most a small number of characters of the many that are involved in the overall breadmaking quality.

Some of the more successful studies of breadmaking quality screening tests as used in wheat breeding programs will now be reviewed.

Pelshenke (1933) obtained a variation of Pelshenke Test time for different wheats from 24.5 to 138.7 minutes, while the range in loaf volumes was from 348 to 443 cc and the Brabender farinograph gluten quality number varied from 70.1 to 98.3. The variation of Pelshenke time was considerably larger than that of the other two quality tests. Cutler and Worzella (1933) showed that the Pelshenke Test value gave significant positive correlation with protein content (flour), loaf volume, absorption and vitreous kernel content for a number of varieties of hard red winter wheat grown at different locations. In contrast, Wilson et al. (1933) found that the Pelshenke time was not significantly correlated with protein content and loaf type (shape and color). Winter and Gustafson (1934) obtained a fairly good positive correlation between Pelshenke value and loaf volume, but not between Pelshenke value and protein content of flour. Bayfield and Shiple (1937), in a three year study of various measurements of flour characteristics for the evaluation of quality and the strength of soft winter wheat, found that the Pelshenke Test value was positively correlated with protein content and loaf volume.

Another quality test that has been reasonably useful for screening wheat lines for breadmaking quality is the Zeleny Sedimentation Test (Zeleny, 1947). Like the Pelshenke Test, the Sedimentation Test value is also influenced, both by the quantity and quality of the gluten proteins. It has been suggested that of all the available tests used alone, the Zeleny Sedimentation Test gives the best prediction of breadmaking potential of hard wheats (Greenaway et al., 1966). However, it is now known that it does not have the accuracy to distinguish wheat lines of similar

quality.

Baker and Campbell (1971) by a computer analysis of many years of data, found that Sedimentation Test value, protein content and centrifuge absorption, gave the best prediction of breadmaking quality as measured by the baking test.

A number of the so-called physical dough testing instruments, i.e. alveograph, farinograph and mixograph, are available for measurement of dough characteristics. By experience, these tests can be applied to screening wheat lines for breadmaking quality (Kent-Jones and Amos, 1967). Like other tests, these provide only partial information and cannot be used alone for screening purposes. Furthermore, these tests usually require more grain than is normally available at the early generation stage where rapid and efficient screening is required.

Abrol et al. (1972), in a study of the suitability of newly developed wheat strains for various end products and association of their mixing characteristics with other quality parameters, showed that the Pelshenke time was highly significantly correlated with the width of the mixograph curve, which is indicative of the elasticity of the dough. Using the Pelshenke Test for early generation assessment of bread flour properties, Welsh and Norman (1972) found that for 233 lines, the Pelshenke time was as good as the mixograph test 75% of the time for discarding undesirable samples. They concluded that the Pelshenke Test can be used successfully for screening F_2 and F_3 generation material.

According to CIMMYT (1972), quality evaluation of bread wheat lines begins with the F_2 individual selection. First, desirable lines are selected by grain type, and then, they are evaluated for gluten strength by the Pelshenke Test. This procedure has proven to be highly satisfactory

for separating strong gluten and weak gluten types and considerable advancement has been made in improving the quality of Mexican wheat cultivars since the inception of its use.

It has been shown (Bushuk et al., 1969) that for bread wheats, loaf volume is directly related to protein content. Since Pelshenke time is positively correlated with protein content of the flour and since the protein content is highly correlated with loaf volume, it appears that the Pelshenke time should be a useful index of breadmaking quality as measured by loaf volume.

In more recent studies, Orth et al. (1972) found that protein solubility distribution, Zeleny Sedimentation value, and farinograph dough development time are the three most useful indices of baking quality of bread flours. Subsequent computer studies by Fowler and De La Roche (1975) indicated that only three tests (kernel hardness, protein quantity, and rate of dough development in a recording mixer) are necessary to provide the basic information required for the prediction of bread or cookie quality. The Pelshenke Test was not used in the studies of Orth et al. and Fowler and De La Roche. Because of the important role of wheat protein in breadmaking quality, new methods are being developed for examining the gliadins and glutenins, the main proteins of gluten, in relation to breadmaking quality. Bietz et al. (1975), developed a rapid test for single-kernel analysis of glutenin which permits the analysis of the large number of samples that must be screened in wheat breeding programs. This test can be used for selecting similar or dissimilar genotypes. Comparison of the results with those of genotypes of known breadmaking quality can be used as a guide with regard to the quality of the "new" line. Further work is needed to adapt these latest findings into a routine

screening test.

D. The Pelshenke Test

The wheat meal fermentation time test was first used in England by Saunders and Humphries (1928) who developed it from their preliminary observations in testing commercial wheat samples for baking quality. In the original test, Saunders and Humphries (1928) prepared the dough-ball from 10 g of flour, 6 ml of water, and 0.5 g of compressed yeast, all mixed at 80° F (27°C) in a beaker with a spatula. The dough-ball that was obtained was floated on water, also at 80°F, in a small vessel. One requirement in the original test was that the dough-ball should be free floating and not supported at any point by the wall of the vessel. The index of the test was the time, in minutes, elapsing between the time the dough-ball was placed in the vessel and the complete dispersion of the dough-ball due to the action of the yeast. They found that the "time" was longest for flour made from "Manitoba" (high protein hard red spring) and shortest on flour from "English" (low protein soft) wheats.

The wheat meal fermentation test was further developed in Germany by Pelshenke (1930, 1933) after whom it was subsequently named. He used 5 g of wheat meal, 0.25 g of yeast and enough distilled water to make a dough-ball of medium stiffness. In this modified test, the dough-ball was floated on water at 88° to 91°F (30° to 33°C). The index of the test was the time from mixing the dough to the dispersion of the dough-ball in the beaker.

A test similar to the Pelshenke Test was developed independently in the United States by Cutler and Worzella (1933). Their method was comparable to that of Pelshenke except that a bell jar was used instead of a dough fermentation cabinet.

A classification of wheats on the basis of the wheat-meal-test was suggested by Cutler and Worzella (1933). Two broad classes were proposed: soft or pastry wheats and hard or bread wheats. These classes were divided into sub-classes of varying gluten strength; each sub-class was defined in terms of wheat meal fermentation time as follows:

CLASS I. SOFT OR PASTRY WHEATS

Sub-class	Test-time
very weak	less than 30 min
weak	30 to 50 min
medium strong	50 to 100 min
strong	100 to 175 min

CLASS II. HARD OR BREAD WHEATS

weak	150 to 225 min
medium strong	225 to 300 min
strong	300 to 400 min
very strong	over 400 min

This general classification of gluten strength on the basis of wheat meal fermentation time is still used today (AACC, 1969).

There have been a number of studies of factors that affect the Pelshenke Test time. Wilson et al. (1933) found that variations in grinding, mixing, and even in the humidity of the air above the dough-ball, all affected the final result. Swanson and Parker (1935) found that tempering hard wheat increased the Pelshenke time. This was attributed to the finer granulation of the ground product from tempered wheat.

Collaborative studies of the methods used for the Pelshenke Test in several laboratories suggested some variations in procedure although all methods were based on the techniques published either by Pelshenke (1933) or Cutler and Worzella (1933). Bayfield (1935) pointed out that significant differences in results could be obtained due to the test vessel walls supporting the dough-ball when 10 g ball was used with 150 ml beaker.

This effect could be eliminated by decreasing the dough-ball from 10 g to 5 g. To obtain additional information on the reproducibility of the methods, Bayfield's samples of wheat and meal were tested by a collaborator from another laboratory using two methods:

a) Dough-ball from 10 g meal and 5.5 ml of 10% suspension of baker's yeast, suspended in 80 ml of distilled water in 150 ml low-form beaker.

b) Same method as (a) except that the amounts of yeast suspension and meal were reduced by one-half.

In addition to these conditions, the collaborator used a fermentation temperature of 31° to 32°C whereas Bayfield performed the test at 30°C. Bayfield (1935) showed that the results by method (a) varied considerably more than those by method (b). However, in subsequent studies, Bayfield (1936) concluded that the size of the dough-ball used is the most important factor in the Pelshenke Test and that a dough-ball from 4 g of meal should permit the testing of the maximum strength of high protein North American wheats.

Bayfield, (1936) working with a series of hard red spring wheat samples of one variety (Marquis) grown under similar environmental conditions, found a wide variation in Pelshenke time. The same author, using four Canadian hard red spring wheat varieties (Marquis, Ceres, Huron, and Garnet) grown at various locations showed a considerable variation in Pelshenke time among varieties.

Since 1936, there have been several modifications of the Pelshenke Test. At present, wheat breeding centres in many countries use a standard test based on 3 g of wheat meal and 1.8 ml of 2.2% yeast suspension.

The basic idea of the Pelshenke Test is that the progress of fermentation in a coarse meal dough-ball or in a flour dough-ball, is uniform

as is the evolution of carbon dioxide. Pelshenke Test time is determined mainly by the interaction of two complex processes acting in the dough-ball; a) the production of carbon dioxide by the action of the yeast on the fermentable sugars present in the dough-ball, and b) the ability of the dough-ball to retain the gas produced by the fermentation. Each of these processes is determined by the interaction of many biochemical, chemical and physical properties of the wheat meal (or flour). A study of the effects of a number of technologically important properties on the Pelshenke Test is the subject of this thesis.

III. MATERIALS

A number of different cereals were used in this study. The grain samples used for the standardization of the two Pelshenke Test methods, for the studies of interclass (of wheat) variability, and for the effects of mixing and enzymes on Pelshenke Test time were three hard red spring wheats (Manitou-2M, Manitou-6M, and Manitou-12M), Talbot soft white winter wheat, Stewart 63 durum wheat, 6A190 triticale, and Prolofic rye. The grain of these cereal cultivars was harvested from field plots located at the University of Manitoba. These samples together with their proteins contents are listed in Table 1.

Grain of 19 cultivars of the 1973 Uniform Quality Nursery grown at Lethbridge, Regina and Swift Current was used for the study of the effects of variety and environment. This group of samples was provided by Dr. A.B. Campbell, Canada Department of Agriculture, Winnipeg Research Station. Detailed technological data for these samples are tabulated in Appendix I.

For the study of the relationship of some intracultivar factors (e.g. protein content and protein fractions) on the Pelshenke Test time, grain of the hard red spring wheat cultivar, Neepawa, was provided by Mr. S. Dubetz of the Agriculture Canada Research Station, Lethbridge. These samples were harvested in 1973 from field plots subjected to a wide range of fertilizer application and irrigation levels to induce production of grain with an abnormally wide range of protein content. Breadmaking quality data for these samples are tabulated in Appendix II.

Samples of hard red spring and durum wheats (provided by Soo Line Milling Co., Ltd., Winnipeg, Man.) and one sample of Canadian soft white winter wheat c.v. Frederick (obtained from the King Seed Co., Chatham, Ont.),

were used to study the effects of protein content, starch damage, (endogenous and added), pentosans, lipids and added chemical dough improving agents. The protein contents of these samples were 12.3%, 12.7% and 9.3% for the hard red spring, durum and soft wheats, respectively.

TABLE 1. Cereals for Standardization Study

<u>CULTIVAR</u>	<u>CLASS</u>	<u>YEAR GROWN</u>	<u>PROTEIN (%) 14% m.b.)</u>
Manitou-2M	Hard red spring	1968	10.1
Manitou-6M	Hard red spring	1968	12.1
Manitou-12M	Hard red spring	1968	14.4
Talbot	Soft white winter	1970	10.3
Stewart 63	Durum	1972	12.3
6A190	Triticale	1972	13.6
Prolific	Spring rye	1972	13.3

IV. METHODS

A. Processing of Grain Samples

1. Preparation of Whole Grain Meal

Whole grain meals were obtained by grinding 3 g of grain on a Wiley grinder fitted with 1-mm sieve.

2. Preparation of Flour

The wheat samples were milled into a straight-grade flour on a Buhler experimental mill after tempering overnight to 16.5% moisture.

3. Pin-milled Flour

Pin-milled flour was prepared by grinding (two passes) standard flour on an Alpine Pin-Mill, Model 160Z at 16,000 rpm.

B. Pelshenke Methods

1. Manual Mixing Method

This test was conducted according to the procedure described by Castilla Chacon (personal communication) which is as follows:

- a) Suspend 2.2 g dry yeast (Fleischmann's) in 100 ml distilled water at 30°C.
- b) Mix 1.8 ml of yeast suspension with 3 g of sample (whole meal of flour), using a stirring rod or spatula. Transfer the dough to the palm of the hand and knead with the fingers until well mixed, and then form into a round ball with smooth surface.
- c) Place the dough-ball in 150 ml beaker containing 70 ml distilled water maintained at 30°C in a constant temperature water bath.
- d) Record the elapsed time between placement of the dough-ball in the beaker and disintegration as indicated by a portion of the dough breaking away from the ball and descending to the bottom of the beaker.

2. Mechanical Mixing Method

In this modification of the Pelshenke Test, the 3-5 g Ottawa Electronic Recording Dough Mixer (Voisey et al., 1969) was used instead of the conventional manual mixing with a spatula. The procedure is as follows:

- a) Suspend 2.2 g dry yeast (Fleischmann's) in 100 ml distilled water at 30°C.
- b) Place 3 g of the sample (whole meal or flour) in the mixing bowl and add 1.8 ml of yeast suspension.
- c) Mix at 100 rpm for one min.
- d) Transfer the mixed dough to the palm of the hand and form into a round ball with a smooth surface.
- e) Complete test as in 1 (c) and 1 (d), above.

The major events that occur in the course of Pelshenke Test can be seen in Fig. 1. Figure 1A shows the dough-ball immediately after it is placed in the beaker. This represents zero time of the Pelshenke Test. Figure 1B shows the position of the dough-ball after sufficient carbon dioxide has been produced by fermentation to increase the buoyancy and cause the dough-ball to rise to the surface of the water in the beaker. The dough-ball expands in response of the continuous production of carbon dioxide (Fig. 1C). Finally, when the dough-ball cannot retain any more of the gas it breaks up; some of the dough pieces sink to the bottom of the beaker. This stage (Fig. 1D) represents the conclusion of the Pelshenke Test time. The total time elapsed is referred to as Pelshenke Test time or as Pelshenke time or value.

C. Analyses of Wheat and Flour

Methods of the American Association of Cereal Chemists (AACC, 1969)

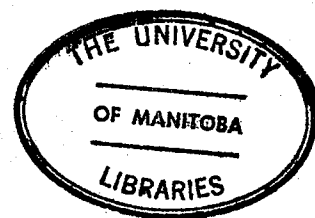
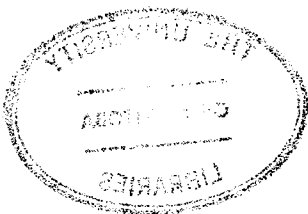
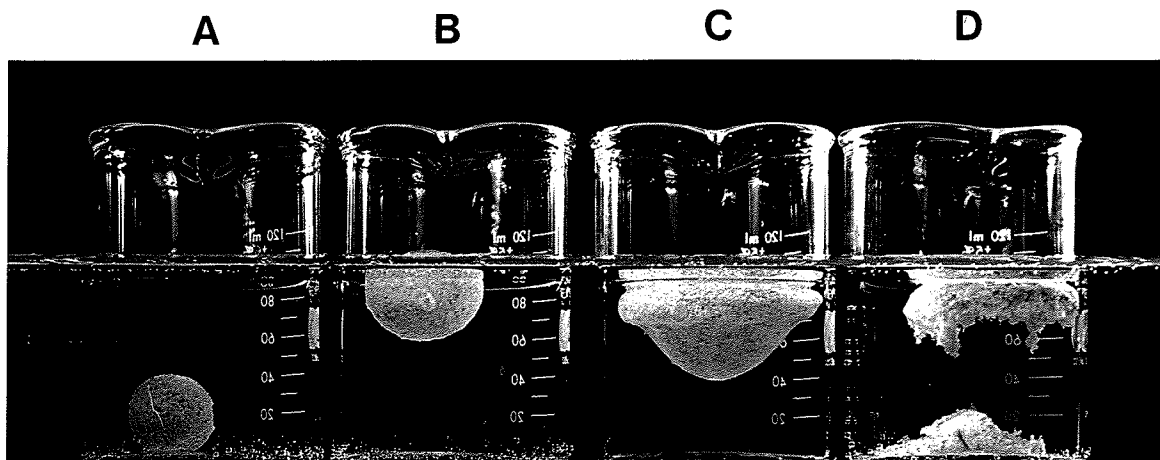


Figure 1. Major Events Occurring in the Pelshenke Test





PELSHENKE DOUGH-BALL TEST

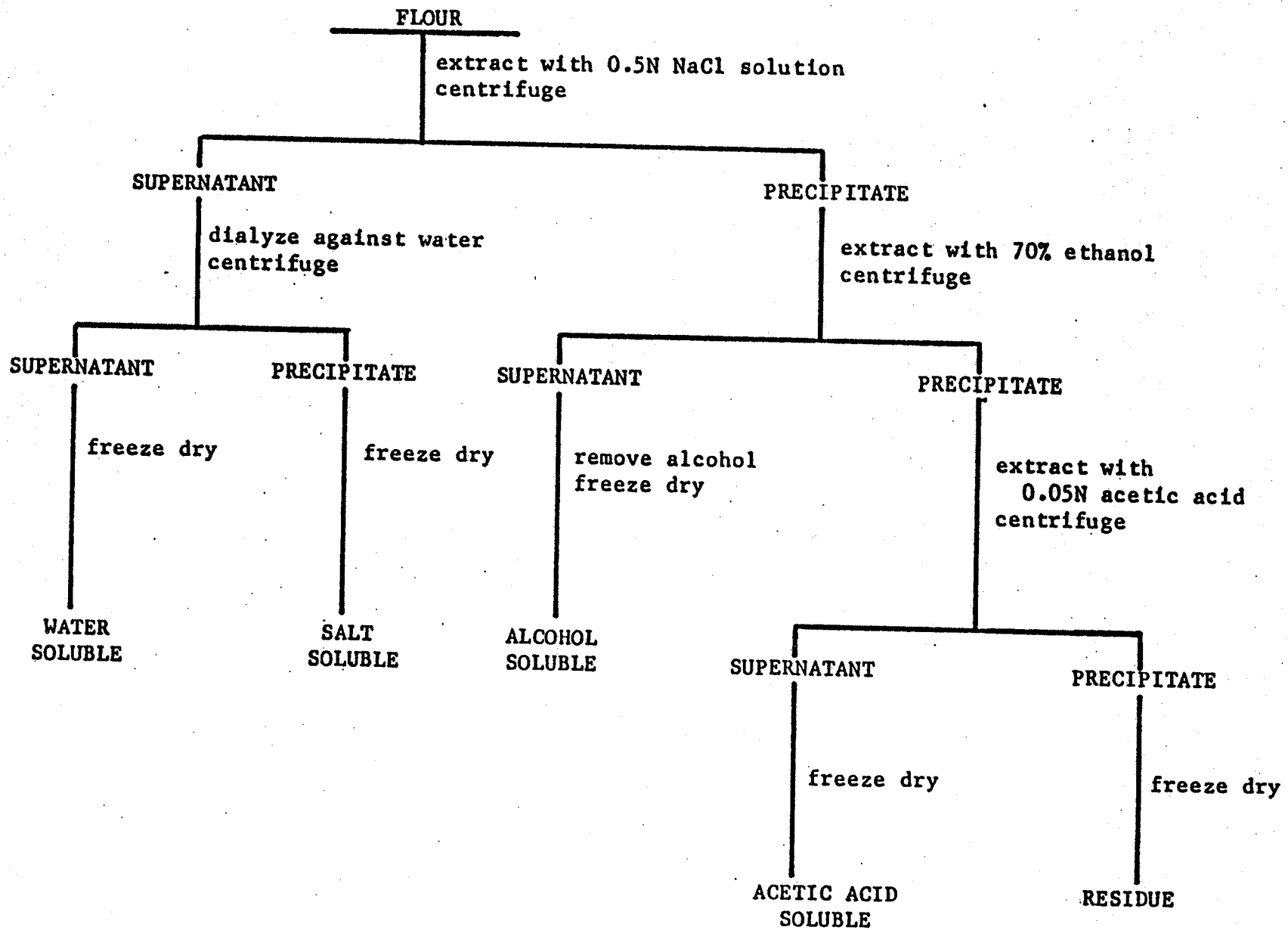
were followed for all tests made on wheat and flour. The "remix" experimental baking procedure, described by Irvine and McMullan (1960), was used as the standard baking test. Loaf volumes of the bread obtained were measured by rapeseed displacement. The Ottawa Electronic Recording Dough Mixer (Voisey et al., 1969) was used to determine dough development time.

D. Fractionation of Flour Proteins

Flour proteins were fractionated according to solubility using the modified Osborne Fractionation procedure described by Chen and Bushuk (1970) and summarized in Fig. 2. Five protein fractions are obtained by this method: water-soluble proteins (albumins), salt-soluble proteins (globulins), alcohol-soluble proteins (gliadins or prolamines for cereals other than wheat), acetic acid-soluble proteins (glutenins or glutelins for cereals other than wheat), and insoluble residue proteins. All extractions were consecutive and were performed in a cabinet at 4°C using a Multi-Magnestirrer with constant speed to achieve reproducible extractions. The distribution of proteins among the five solubility classes was quantitated using the Kjeldahl procedure to determine nitrogen. Nitrogen values were converted to protein using the factor 5.7.

E. Amino Acid Analyses

Amino acid composition was determined by the conventional procedure with the Beckman Model 121 automatic amino acid analyzer. The hydrolyzate was prepared as follows: approximately 40 mg of flour was weighed into hydrolysis tubes and 4 ml of 6N HCl was added. The acid-sample mixture was frozen under vacuum, thawed, and refrozen under vacuum to remove entrapped air. The dispersions were then hydrolyzed under vacuum at 110°C



for 24 hrs. The hydrolyzed samples were frozen and were placed in a desiccator containing NaOH pellets to remove the HCl. The dried samples were dissolved in 1 ml of pH 2.2, 0.2N sodium citrate buffer, and centrifuged to remove insoluble material. The supernatant was used for analysis. Tryptophan and cystine are not determined by this procedure.

V. RESULTS AND DISCUSSION

The results of this study are presented in four sections. Section A deals with the development of the methodology. In addition to examining the effects of some procedural parameters on the Pelshenke Test value, this section includes a study of the effects of variable dough mixing conditions (speed and time) using five cultivars of wheat, one of rye and one of triticale. The range of Pelshenke values that were obtained for a hard red spring wheat cultivar at three different protein contents, a durum wheat, a soft white winter wheat, a spring rye, and a hexaploid triticale with the standardized procedure is also presented in this section.

Section B deals with the effects of location and cultivar on the Pelshenke value. Nineteen cultivars, each grown at three stations in western Canada were used for this study. Statistical relationships between Pelshenke value and other breadmaking quality parameters are presented and discussed.

Section C deals with the study of the role of compositional factors such as protein content and protein fractions on the Pelshenke value. Grain of the hard red spring wheat cultivar, Neepawa, grown under different fertilizer treatments (to give grain of different protein content) was used for this study. Statistical relationships between Pelshenke value, protein solubility distribution, and several breadmaking quality parameters are presented and discussed.

Section D deals with the effect on the Pelshenke value of a number of technologically important factors such as protein content, level of starch damage, pentosans, lipids and some enzymes. A separate subsection is devoted to the effects of a number of flour improvers and one sulfhydryl blocking agent.

A. Pelshenke Test

1. Variations in Test Procedure

Since its introduction as a screening test for gluten quality, the Pelshenke Test has been the subject of considerable controversy (Bayfield, 1936). The original test uses hand mixing and kneading. Accordingly, there exists a strong possibility of day to day variations due to operator error which may be increased further when more than one operators are involved in a single laboratory.

In order to establish the standard deviation of Pelshenke values, seven samples including five wheats, one rye, and one triticale, were tested ten times each using manual and mechanical methods of mixing and kneading. The error was calculated for each sample by standard statistical methods.

Manual Mixing. Table 2 gives the Pelshenke values obtained with the manual mixing method. The seven grain samples covered the range of values from 48 to 302 min. The standard deviations that were obtained from these data increased with increasing mean Pelshenke values, indicating an interaction between factors that affect Pelshenke value and the mixing and kneading stage of the test procedure. However, the precision of the standard test with manual mixing appears to be sufficient for screening wheat lines of widely different quality. Accordingly, the application of the test to segregating lines appears to be justified.

Mechanical Mixing. Table 3 gives the Pelshenke values for doughs prepared by mechanical mixing for one minute at 100 rpm with the Ottawa Electronic Recording Dough Mixer (Voisey et al., 1969). The mean Pelshenke value for each sample is statistically the same as the mean values obtained by hand mixing (Appendix III). However, the standard deviations obtained

Table 2. Pelshenke Values Obtained by the Manual Mixing Method

Trial	Hard Red Spring Wheat			SWW*	Durum	Rye	Triticale
	Manitou (10.1%)	Manitou (12.1%)	Manitou (14.4%)	Talbot	Stewart 63	Prolific	6A190
1	228	268	320	49	57	76	46
2	238	278	308	48	54	78	48
3	243	272	285	50	54	78	49
4	230	260	299	52	58	80	47
5	232	266	309	53	57	81	49
6	241	288	300	50	54	82	49
7	239	278	307	51	53	80	46
8	230	276	289	49	54	76	50
9	244	260	295	50	55	77	50
10	230	278	308	50	54	78	49
Mean	235	272	302	50	55	78	48
Standard Deviation	6.11	8.93	10.49	1.48	1.70	2.06	1.49

* SWW-Soft White Winter Wheat

Table 3. Pelshenke Values Obtained by the Mechanical Mixing Method

Trial	Hard Red Spring Wheat			SWW*	Durum	Rye	Triticale
	Manitou (10.1%)	Manitou (12.1%)	Manitou (14.4%)	Talbot	Stewart 63	Prolific	6A190
1	234	276	305	52	56	80	49
2	238	274	300	52	57	78	49
3	242	270	295	50	55	79	50
4	240	276	300	51	56	80	51
5	236	276	305	52	55	80	50
6	238	274	300	52	57	82	50
7	238	279	301	53	56	81	50
8	239	269	300	52	56	80	51
9	236	274	300	53	56	80	50
10	240	274	299	52	56	80	49
Mean	238	274	300	52	56	80	50
Standard Deviation	3.50	2.94	2.88	0.94	0.67	1.05	0.74

* SWW-Soft White Winter Wheat

for the procedure using mechanical mixing were considerably less than those for the procedure using manual mixing. Results of the F test (Appendix IV) showed that the standard deviations for the mechanical mixing test were significantly lower from those of the manual mixing test (except for Talbot). It can be concluded that, although the means for the samples by both methods were the same, the variability in test values due to mechanical mixing is less, that is, the precision is higher. Therefore, with mechanical mixing fewer replicates would be required for an acceptable level of reproducibility.

2. Effect of Mixing Time

It has been demonstrated by Pushman and Bingham (1975) that there is no significant change in Pelshenke value regardless of deliberate alteration of the manual kneading time between 0 and 60 sec. Since it was shown in the present study that the precision of the Pelshenke Test can be improved by substituting mechanical for manual dough mixing, it was considered relevant to determine the effects of mixing time on Pelshenke value. The results for varying mixing times from one to five min at a constant speed of 100 rpm in the Ottawa Mixer are shown in Figure 3. For all samples, the Pelshenke values increased with increasing mixing time. The rate of increase was essentially constant for the range of mixing times examined. There is suggestion that the rate of increase for the three Manitou samples tended to accelerate slightly as mixing time was extended beyond four minutes. These results suggest that discrimination among samples can be improved by using longer mixing times in preparing the doughs for the Pelshenke Test.

The increase in Pelshenke value with mixing time can be attributed to the increase in gas retention produced by the longer mixing time due to more effective hydration of the meal particles.

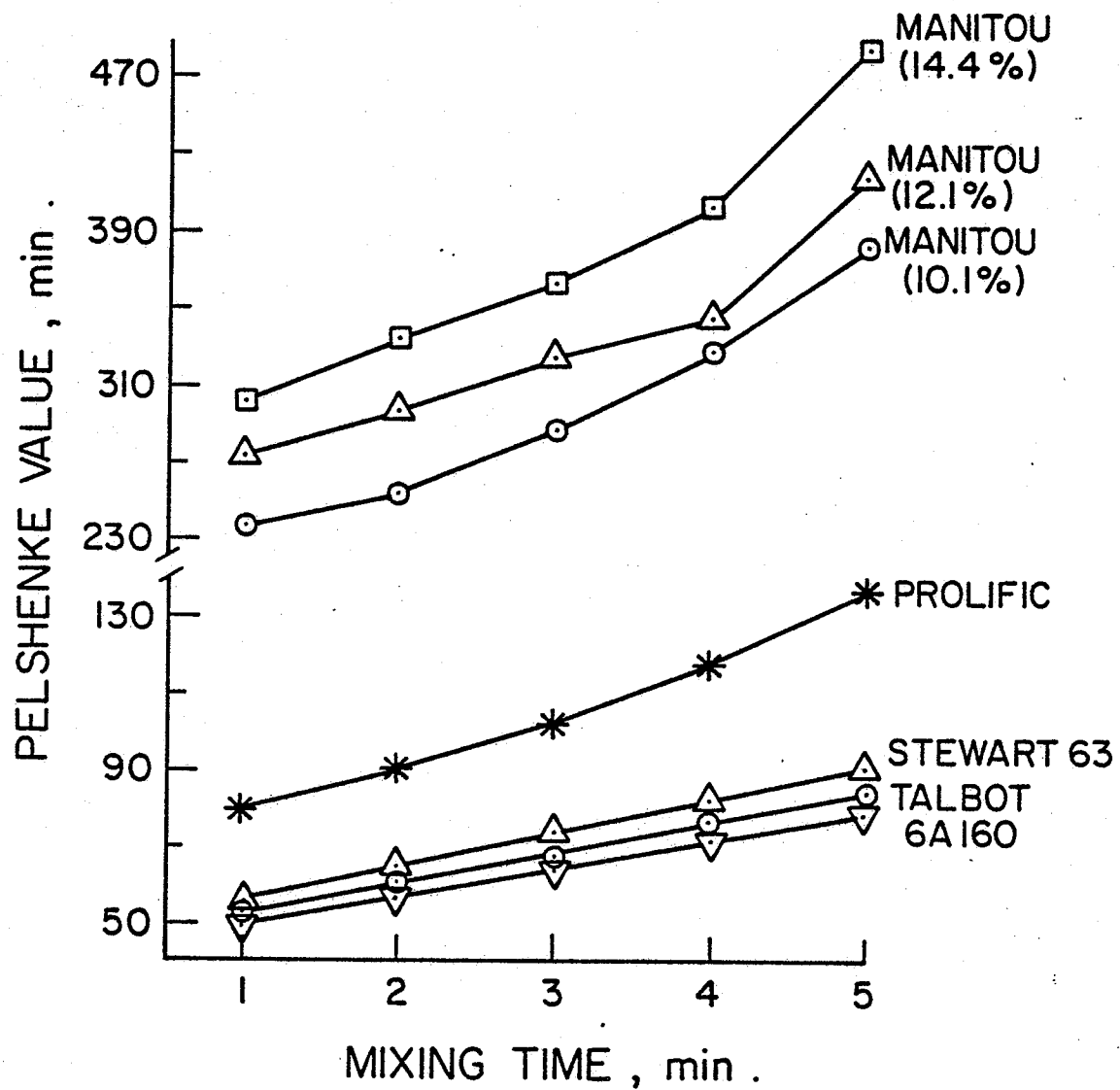


Figure 3. Effect of Mixing Time at 100 rpm on the Pelshenke Value

3. Effect of Mixing Speed

Increase of dough mixing speed (Ottawa Mixer) from 100 to 250 rpm over one minute, had no effect on Pelshenke value for the samples examined (Figure 4). Comparison of the results of Figures 3 and 4, indicated that mixing time is more important than mixing speed in the Pelshenke value. It would appear from these results that hydration of the flour (which depends on mixing time rather than speed) plays a greater role than mechanical development of the dough in the Pelshenke Test. This aspect of the test is worthy of further investigation in view of the current importance of mechanical development in baking technology.

4. Interclass Variability of Pelshenke Value

Table 3 gives the Pelshenke values for three different cereals that are commonly used for the production of bread. The purpose of this table is to show the range of values that may be encountered when the Pelshenke Test is used for screening for breadmaking quality. The range of dough strengths among the cereal samples examined sufficiently broad to cover most bread cereals encountered in breeding programs.

The samples examined can be divided into groups according to the Pelshenke value. The first group consists of the three hard red spring wheats (Manitou) which have extremely high Pelshenke values, and the other group comprising Talbot, a soft white winter wheat, Stewart 63, a durum wheat, 6A190 triticale and Prolific rye. Both groups are within the Cutler and Worzella's classification (1933). For the three Manitou samples, the Pelshenke value increased with protein content (the effect of protein content will be examined further in a later section of this thesis). According to the classification of Cutler and Worzella (1933), the three Manitou samples would be classified as medium-strong bread wheats.

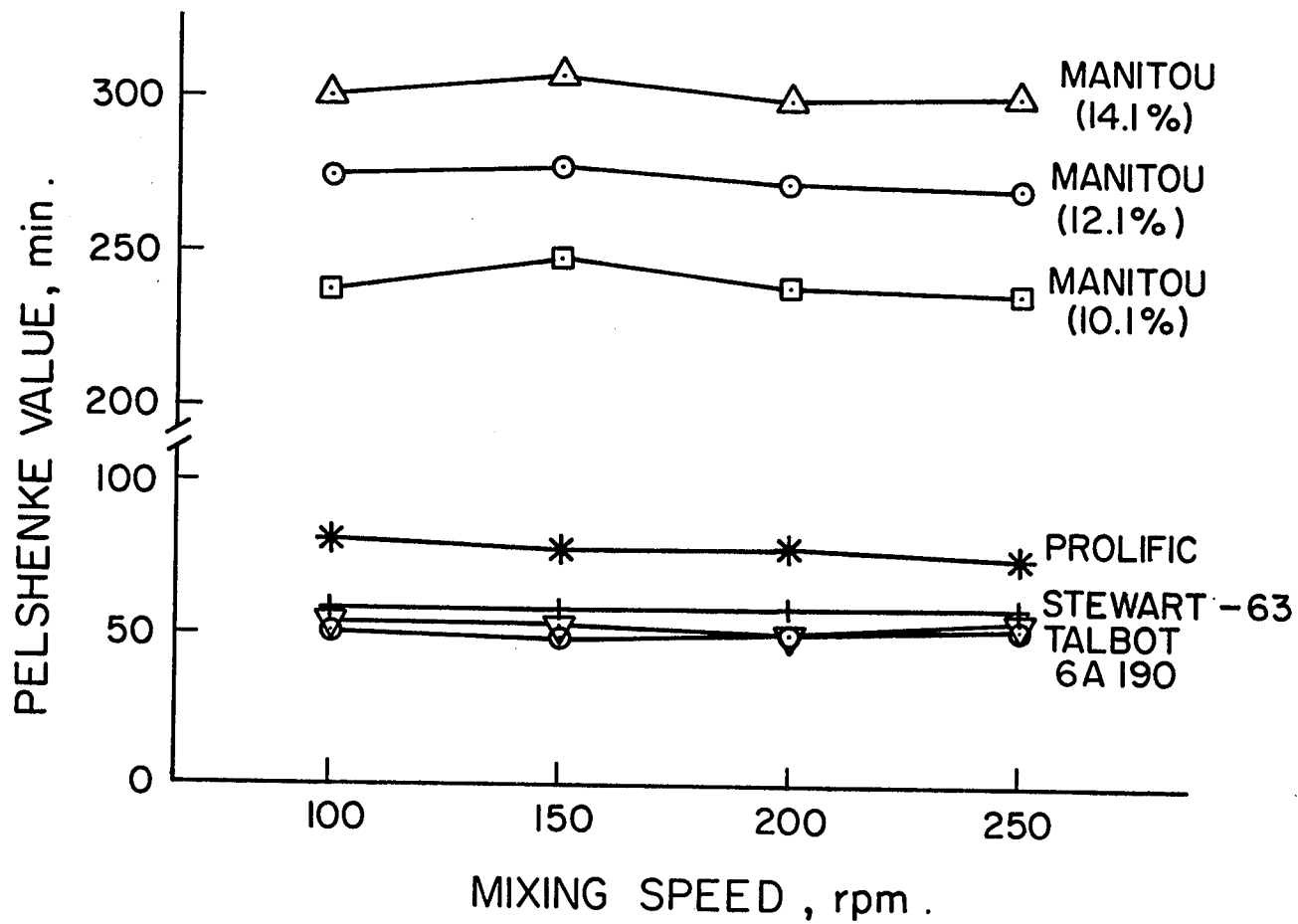


Figure 4. Effect of Mixing Speed (1 min) on the Pelshenke Value

The other group (Talbot, Stewart 63, 6A190 triticale and Prolific rye) has a very narrow spread in Pelshenke value. According to the classification of Cutler and Worzella (1933) these cereals would be considered as medium-strong pastry wheats.

It can be concluded from results presented here, that the Pelshenke Test value can be used quite effectively to differentiate wheats of high breadmaking quality from wheats or other cereals such as rye and triticale that are low in breadmaking quality. The experiments that will be described in the subsequent sections of this thesis were carried out to determine what factors, within a cultivar, affect the Pelshenke value. Results of these experiments should be useful for improving the ability of the test to discriminate between similar cultivars within the "bread" class of wheats.

B. Relationships Between Pelshenke Value and Some Breadmaking Quality Parameters

This section deals with the correlation of Pelshenke value with a variety of measurements that are commonly used for assessing the breadmaking quality of common wheat. The wheat samples selected for this study are the 19 cultivars of the 1973 Uniform Quality Nursery grown by Dr. A.B. Campbell of the Agriculture Canada, Winnipeg Research Station. The nursery was grown at three locations in western Canada, Lethbridge, Regina and Swift Current. The cultivars selected for this nursery cover a broad range of breadmaking quality (560 to 1095 cc in remix loaf volume) and therefore offer useful material for testing the applicability of a test (e.g. Pelshenke Test) used as an index of breadmaking quality.

The data which forms the basis of the following discussion are tabulated in Appendix I. Discussion of intercultivar differences is

based on the data for the grain samples from the three locations. These data will also be used to examine interstation variability. Pooled data for the 19 cultivars and three stations will be used to calculate the correlation coefficients between the Pelshenke value and other milling and baking quality parameters.

1. Intercultivar Variability

The 19 cultivars covered a wide range of Pelshenke values from 71 to 626 min for the Lethbridge samples, 55 to 607 min for the Regina samples, and 59 to 524 min for the Swift Current samples. Accordingly, if it can be shown that the Pelshenke value correlates significantly with breadmaking quality (as will be done in subsection 3), as determined by loaf volume (or some other parameter that correlates with loaf volume), then the test would be useful for screening lines in a wheat breeding program.

2. Interstation Variability

The intracultivar variability in Pelshenke value between locations is quite narrow. In general, the variability for each cultivar follows directly the variability in protein content (Table 4). It is well known that protein content of wheat grain depends strongly on growing conditions and to a lesser degree on the cultivar. The relationship between Pelshenke value and protein content for a single cultivar will be discussed further in the next section. The number of stations (three) was insufficient to justify a statistical analysis of the interstation variability.

3. Correlations Between Pelshenke Value and Quality Parameters

The correlation coefficients for the Pelshenke value and various quality parameters calculated from the pooled data (19 cultivars x 3 locations) are tabulated in Table 5.

Table 4. Protein Contents and Pelshenke Values for the Uniform Quality Nursery Cultivars Grown at Three Locations

Cultivars	LETHBRIDGE		REGINA		SWIFT CURRENT	
	WHP	PEL	WHP	PEL	WHP	PEL
F.K.N. x Pilot	16.9	215	15.2	146	15.0	205
M.M.E.x Ceres R64	16.2	247	14.4	193	13.7	216
C.I.8154/2* Frocor	16.6	127	16.1	71	14.2	107
Glenlea	15.9	626	13.3	602	12.5	419
Red River 68	15.4	605	16.1	607	13.6	524
Ceres	16.0	385	14.8	333	13.6	313
Napayo	17.0	530	14.7	401	14.1	315
P.I. 181337	14.6	280	12.1	190	12.5	181
P.I. 1	15.7	71	13.8	55	14.0	59
P.I. 58548	15.0	108	15.5	83	14.0	101
Potam 70	13.9	221	14.4	183	13.1	193
Yecora 70	15.2	472	15.3	464	14.0	419
Saric 70	15.5	565	16.1	493	14.5	444
Bluebird 4	15.1	596	15.1	546	14.6	450
Son64xTzPP-Nai 60B	15.4	436	15.5	412	14.0	375
C.T. 733	16.3	447	13.8	379	15.0	329
C.T. 773	16.1	434	14.4	463	14.7	312
U.M. 607 A	14.0	322	13.6	308	13.3	300
Timgalen	15.7	290	15.1	278	15.0	188

Table 5. Correlation Coefficients for Pelshenke Value and Quality Parameters

Quality Parameter	Pooled <u>r</u>
Wheat protein, %	0.06
Flour yield, %	0.52*
Grinding time, min	-0.56*
Flour protein, %	0.19
Flour ash, %	-0.16
Baking absorption, %	0.44
Sedimentation value, cc	0.74*
Remix loaf volume, cc	0.57*
AACC loaf volume, cc	0.64*
Farinograph absorption, %	0.33
Farinograph development time, min	0.73*
Mixing tolerance index, min	-0.80*
Mixograph development time, min	0.72*

* Significant at the 1% level

Positive correlations, significant at the 1% level, were obtained for flour yield, sedimentation value, remix loaf volume, AACC loaf volume, farinograph dough development time, and mixograph dough development time. Significant negative correlations were obtained for grinding time and farinograph mixing tolerance index.

On the basis of these results, it can be concluded that Pelshenke value is a meaningful index of breadmaking quality as measured by the baking test loaf volume. Also, it correlates significantly with farinograph dough development time, farinograph mixing tolerance index, and mixograph development time, which in turn correlate significantly with loaf volume (Baker and Campbell, 1971; Orth *et al.*, 1972; Fowler and De La Roche, 1975). One would also expect a good correlation between Pelshenke value and Zeleny Sedimentation value, as was actually obtained in this study. The reason(s) for the significant correlations between Pelshenke value and flour yield and grinding time are not obvious.

Results obtained here are in general agreement with limited data published by others. Cutler and Worzella (1933) showed that the Pelshenke value gave a significant positive correlation with loaf volume and vitreous kernel content for a number of cultivars of hard red winter wheat grown at different locations. In addition, Wilson *et al.* (1933) and Winter and Gustafson (1934) found that the Pelshenke value was significantly correlated with loaf volume. Abrol *et al.* (1972) and Welsh and Norman (1972) found that Pelshenke value was highly significantly correlated with mixograph characteristics.

It was rather surprising that the correlation between Pelshenke value and protein content, for the samples used in this study, was not significant. The only explanation that can be offered for this observation is that the

cultivars used differ widely in protein "quality" as well as in protein content. Indeed, if the loaf volume or Pelshenke value is expressed per unit protein a relatively wide range of values is obtained indicating a broad variability in "quality". Actual values for the Regina samples were 40.6 to 74.0 and 4.0 to 45.3 for the loaf volume and Pelshenke value, respectively. For such widely different samples, one would not expect a significant correlation between protein content and Pelshenke value, if the value is indeed a measure of protein "quality" for breadmaking.

The observation that the Pelshenke value does not correlate with protein content does not agree with the reported results of Cutler and Worzella (1933) who found a highly significant correlation between protein content and Pelshenke value. However, the present results agree with the results reported by Wilson et al. (1933) and Winter and Gustafson (1934).

4. Correlation Between Pelshenke Values for Ground Wheat and Flour

Since it is the flour which is used in baking tests, under some conditions it is necessary to use flour for the Pelshenke Test instead of wheat meal. In the present study, a significant correlation ($r= 0.72^*$) was obtained between Pelshenke value for wheat meal and for flour of the same wheat samples. This indicates that the Pelshenke Test can be applied to wheat meal or to flour to differentiate wheats for breadmaking quality. Unless indicated otherwise, all the results that will be presented in the following sections were obtained with flour.

C. Relationship Between Pelshenke Value and Various Properties of Neepawa Wheat Grown at Different Levels of Nitrogen Fertilization

This section presents data on various breadmaking quality parameters including the Pelshenke value for a group of wheat samples of one cultivar (Neepawa) grown in one location at different levels of nitrogen fertilization.

The wide range of fertilizer (0 to 500 lb per acre) was used to induce the production of grain differing widely in protein content. It was felt that these samples of one cultivar would be particularly suitable for investigating the relationship between Pelshenke value and grain protein.

Relevant technological data for the wheat samples used in this part of the study are tabulated in Appendix II. The data will be discussed in terms of relationships between the Pelshenke value and various measurements used in assessment of breadmaking quality (Table 6).

1. Protein Content

The protein content of the 11 samples varied from 9.3 to 16.4%. It was directly related ($r= 0.90^*$) to the level of nitrogen fertilizer. The significant correlation between wheat protein content and the level of nitrogen in the soil under conditions of high levels of fertilizer has been observed by other workers (Long and Sherbakoff, 1951; Wahhab and Hussain, 1957; Alkier *et al.*, 1972).

The relationship between Pelshenke value and flour protein content for the 11 grain samples used in this study is shown in Figure 5. A highly significant positive correlation was obtained ($r= 0.83^*$). The line in Figure 5 tends to curve upward for the three highest protein samples; that is, in this region less protein is required to produce a unit increase in Pelshenke value than in the lower protein content region.

The results obtained here differ somewhat from those of Section B where it was shown that Pelshenke value and protein content were not significantly correlated ($r= 0.19$). This lack of correlation was attributed to the fact that the wheat samples used in the earlier experiment were of cultivars of widely different quality whereas the samples used here are of a single cultivar.

Table 6. Correlation Coefficients Between Pelshenke Value and Some Quality Parameters

	<u>r</u>
Nitrogen fertilizer applied	0.96*
Flour protein, %	0.83*
Sedimentation value, cc	0.93*
Gassing power, mmHg	-0.78*
Diastatic activity, M.U.	-0.83*
Starch damage, F.U.	-0.88*
Farinograph absorption, %	0.76*
Loaf volume, cc	0.77*

* Significant at the 1% level

Table 7. Loaf Volumes for Neepawa Wheat. Samples of Different Protein Content

Protein %	Loaf Volume	
	Total * cc	Per Unit Protein cc % ⁻¹
9.3	500	50.7
10.7	645	60.3
12.7	705	55.5
14.1	820	58.2
14.7	875	59.5
15.5	850	54.8
15.9	845	53.4
15.8	860	54.4
16.1	952	59.1
16.2	900	55.6
16.4	852	52.0

* Remix baking procedure

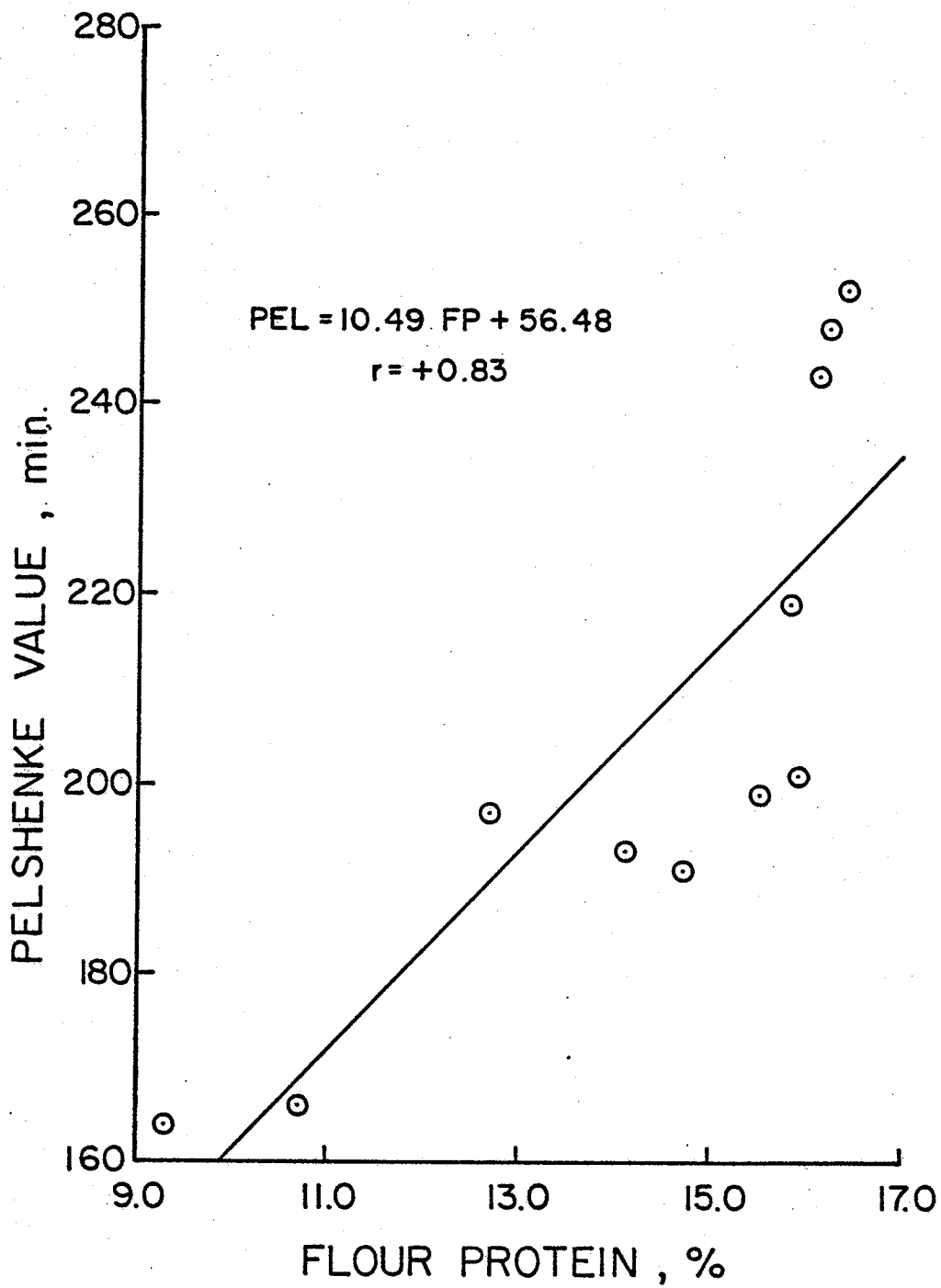


Figure 5. Relationship Between Pelshenke Value and Flour Protein for 11 Wheat Samples of One Cultivar (Neepawa)

Since Pelshenke value depends strongly on protein content, it might be more correct to compare samples of different protein content on basis of Pelshenke value per unit protein content. Pelshenke value expressed in this manner can be considered as an index of protein "quality" independent of protein content.

The relationship between Pelshenke value per unit protein content and protein content for the 11 samples used in this study is shown in Figure 6. It is seen that as the protein content increases from 9.3 to 15.9%, the Pelshenke value per unit protein decreases quite substantially from 17.6 to 12.6. This indicates a decrease in protein "quality", as measured by the Pelshenke value, for this group of samples. The four highest protein samples did not follow the decreasing trend but showed a marked increase in Pelshenke value per unit protein with increasing protein content. However, the value for the highest protein sample was still less than the value for the lowest protein sample (15.4 vs 17.6). There is no obvious explanation for the reversal in the decreasing trend of Pelshenke value at the high protein content.

When the protein "quality" of the 11 samples used here was expressed in terms of remix loaf volume per unit protein, as was done by Orth and Bushuk (1972), the "quality" for breadmaking remained about the same or showed a decreasing trend with increasing protein content (Table 7). Accordingly, it appears that under some conditions of growth (e.g. high fertilizer stress), the protein "quality" of a single cultivar can be adversely affected. Previous studies of this possibility on samples of different protein content selected for grain grown under normal conditions (summer fallow) by Tanaka and Bushuk (1972) showed that the breadmaking "quality" of the proteins of two cultivars used in their study remained

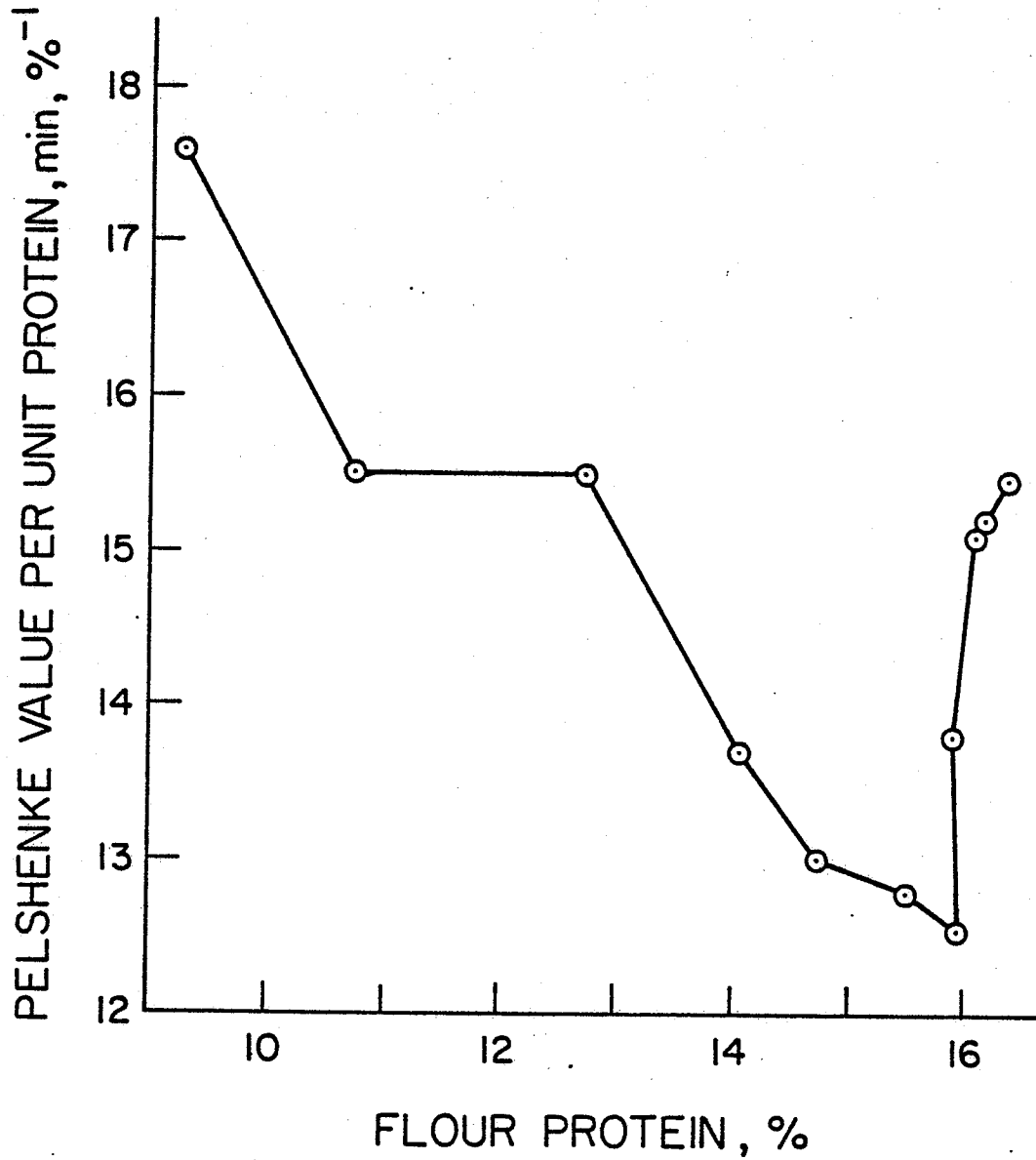


Figure 6. Relationship Between Pelshenke Value Per Unit Protein and Flour Protein for 11 Wheat Samples of One Cultivar (Neepawa)

essentially constant over a protein content range of 10.0 to 15.6% and 11.0 to 14.2%.

2. Zeleny Sedimentation Value

The Zeleny Sedimentation Test is used in North America and Europe as a measure of breadmaking quality in wheat breeding programs and in the assessment of commercial samples of wheat. The value of this test for predicting the breadmaking quality of wheat flour can be demonstrated by the highly significant correlation ($r = 0.90^*$ for the 11 samples studied here) obtained between the sedimentation value and loaf volume. Accordingly, it was of interest to compare this test with the Pelshenke Test.

Figure 7 shows a very good linear relationship ($r = 0.93^*$) between Zeleny Sedimentation and the Pelshenke value for the 11 samples used in this study. It can therefore be concluded that both tests are equally good as indices of quality. Actual choice would depend on practical considerations and operator preference.

3. Starch Damage, Diastatic Activity and Gassing Power

Figure 8 shows the relationship between Pelshenke value and the level of starch damage in the flour for the 11 samples used in this experiment. A significant negative correlation was obtained ($r = -0.88^*$). The apparent inverse relationship may be due to the fact that level of damaged starch in flour, milled from samples of one wheat cultivar as was done here, normally increases with decreasing protein content. With such flour samples it is not possible to separate the effect of protein content and starch damage from possible effect on the Pelshenke value. To determine the specific effects of starch damage and protein content on Pelshenke value, it would be necessary to mill flours of one protein content to

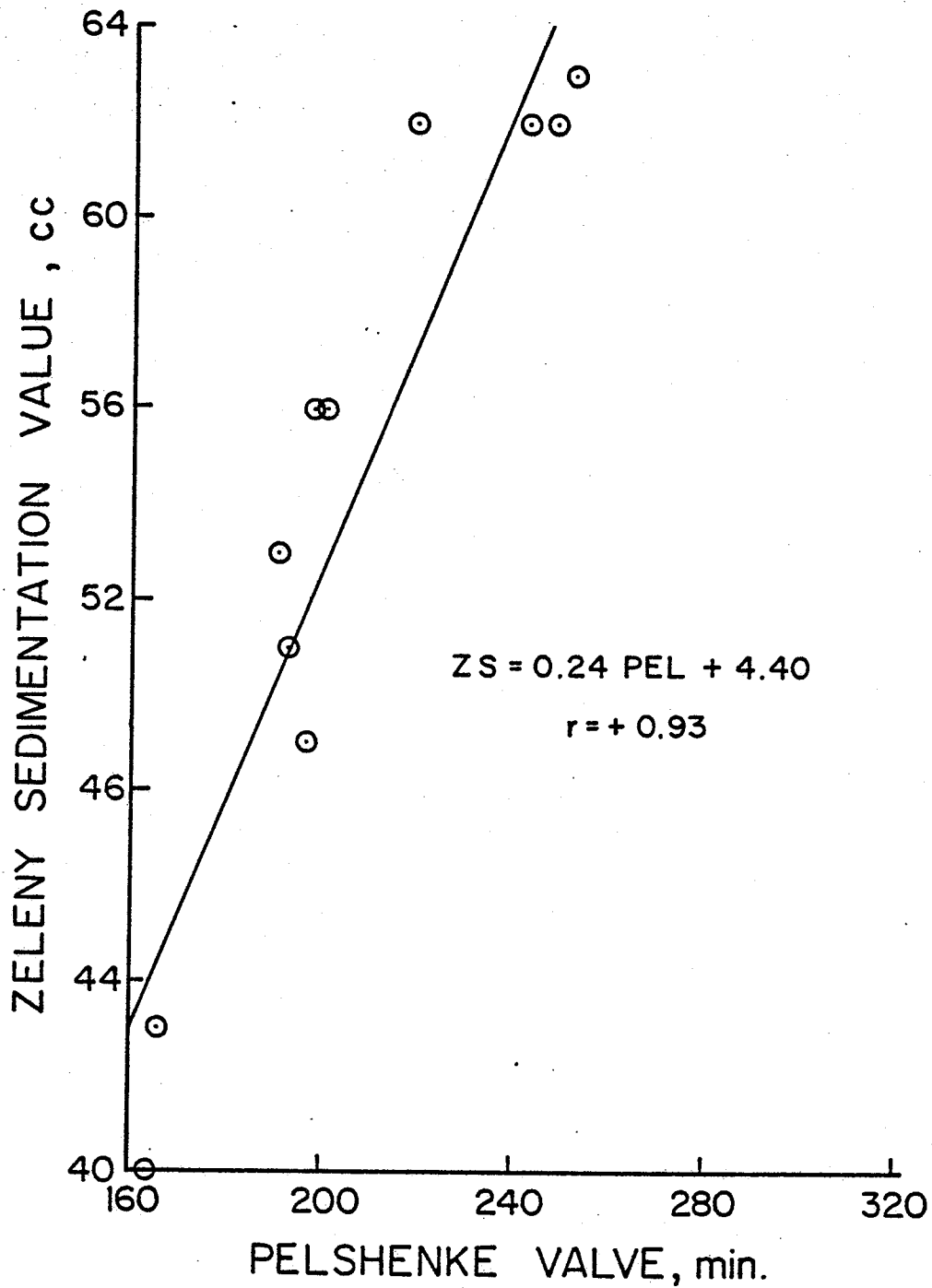


Figure 7. Relationship Between Pelshenke Value and Zeleny Sedimentation Value for 11 Wheat Samples of One Cultivar (Neepawa)

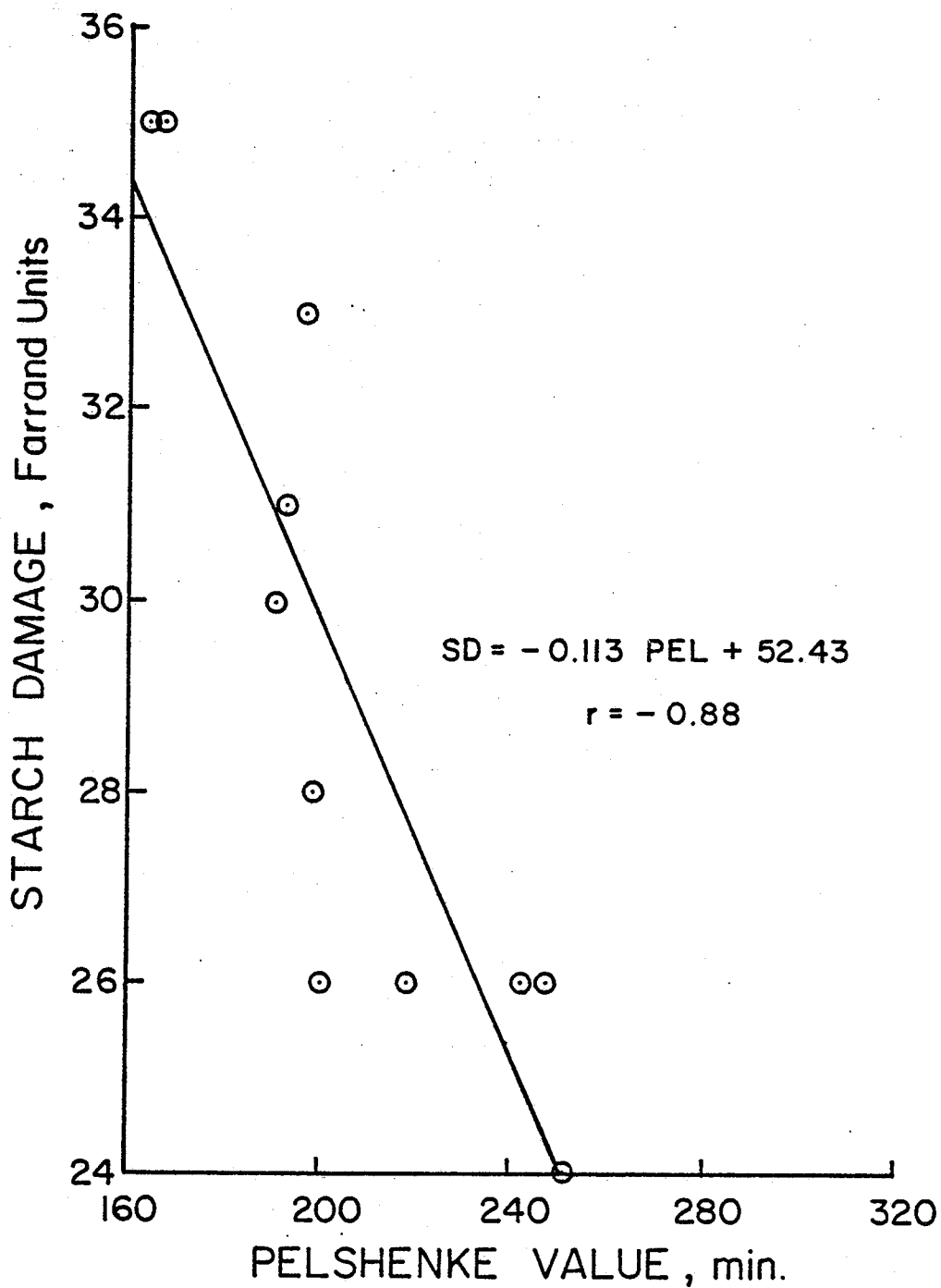


Figure 8. Relationship Between Pelshenke Value and Starch Damage for 11 Wheat Samples of One Cultivar (Neepawa)

different starch damage, and flours of different protein content to a constant starch damage. Results of such an experiment will be presented later. For the present discussion, it can be stated that the level of starch damage appears to influence the Pelshenke value.

Diastatic activity is a measure of the activity of the starch hydrolysing enzymes of the flour. A broad range of diastatic activity, from 169 to 237 maltose units, was represented by 11 samples used in this study. A correlation coefficient of -0.83^* indicated the strong inverse relationship between Pelshenke value and diastatic activity.

Another important requirement of breadmaking quality is the ability of dough constituents to produce carbon dioxide. Total gassing power of a dough is a function of both the level of damaged starch and the alpha-amylase activity of the flour. The gassing power values for the 11 flours examined here varied from 320 to 425 mmHg. As was the case with the level of damaged starch and diastatic activity, the Pelshenke value was inversely ($r = -0.78^*$) related to the gassing power.

The highly significant negative correlation between the Pelshenke value and the three technological measurements related to the ability of a bread dough to produce carbon dioxide discussed in this subsection warrants emphasis. It will be recalled that the Pelshenke value is directly related to gas retaining ability of the dough-ball test and inversely related to rate of gas production. In turn, the rate of gas production in a dough is directly related to the level of starch damage and the diastatic activity of the flour. Gassing power is an indirect measure of both factors (starch damage and diastatic activity) in the presence of added yeast.

4. Loaf Volume

The remix loaf volumes for the 11 samples studied, varied from 500 to 952 cc/100 g of flour. When Pelshenke value and loaf volume were correlated, a significant correlation ($r= 0.77^*$) was obtained. The correlation coefficient for the more uniform samples used here is slightly higher than the coefficients (for the two baking tests) for the samples used in the experiments discussed in Section B. The relationship between loaf volume and Pelshenke value is shown in Figure 9. This result reiterates the previous finding (Section B) that Pelshenke value is a relatively good index of breadmaking quality (as expressed by loaf volume) and therefore can be used for screening wheat lines for this purpose.

5. Protein Solubility

Recent studies by Orth and Bushuk (1972) have shown that remix loaf volume per unit protein was significantly negatively correlated with the amount of glutenin and positively correlated with the amount of residue protein. Accordingly, it was of interest to examine the correlations between the Pelshenke values and the amount of each protein fraction in the flour.

The average data of three fractionations is tabulated in Table 8. The results for each fraction will be discussed separately. To check the reproducibility of the fractionation procedure, the protein of one sample of Neepawa flour was fractionated five times. This data (Appendix V) was used to calculate the standard deviation for each fraction.

Water-Soluble (Albumin) Fraction. The amount of water-soluble protein showed a decreasing trend from 15.0 to 10.9% with increasing level of protein content (Figure 10). When the water-soluble fraction was correlated with protein content and Pelshenke value, significant

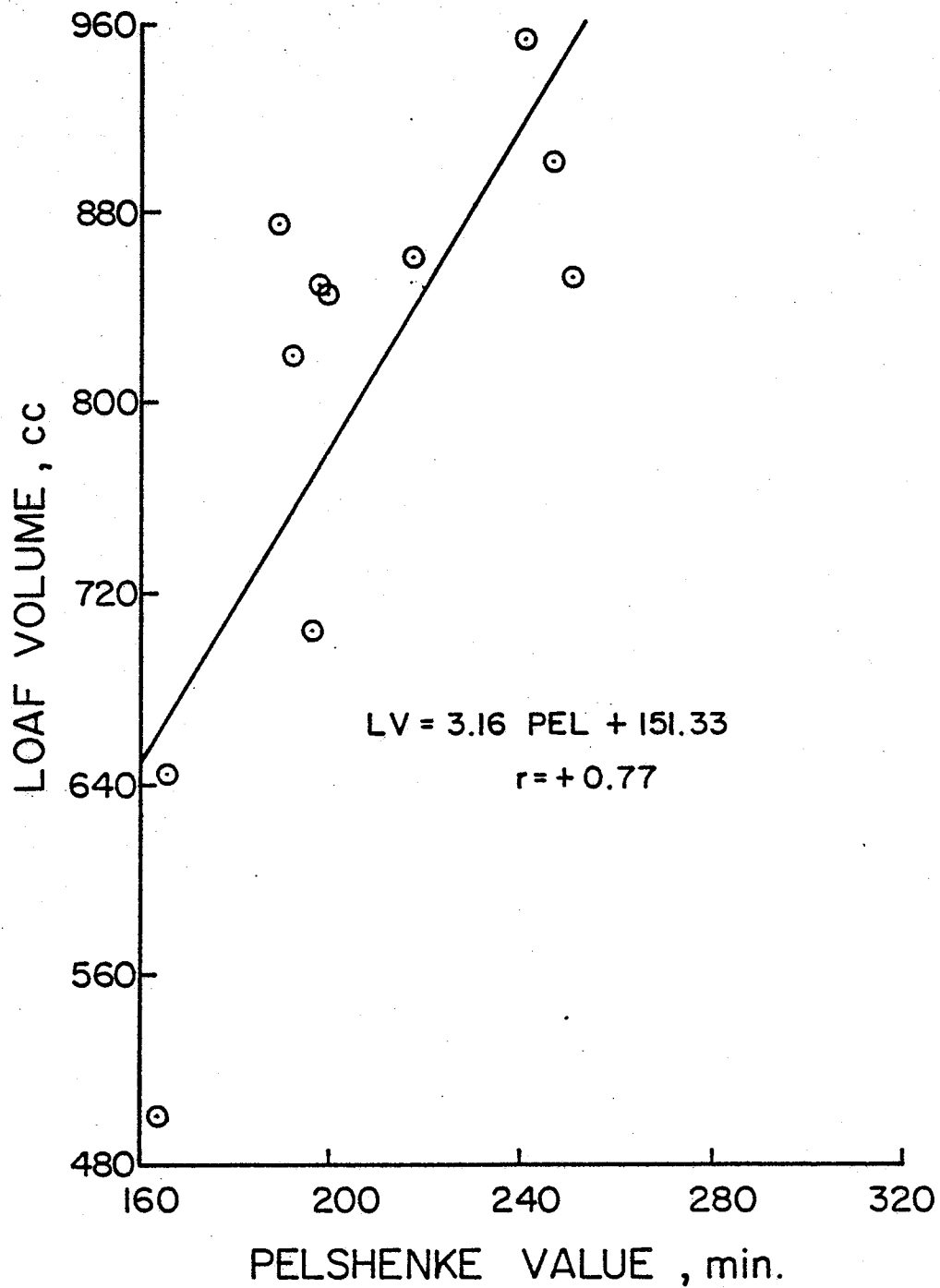


Figure 9. Relationship Between Pelshenke Value and the Remix Loaf Volume for 11 Wheat Samples of One Cultivar (Neepawa)

Table 8. Proportion of Various Protein-Solubility Fractions in Flour of Neepawa Arranged from Top to Bottom, in Order of Increasing Protein Content

Protein %	Albumin %	Globulin %	Gliadin %	Glutenin %	Residue %	Recovery %
9.3	15.0	5.4	27.0	6.6	35.5	89.5
10.7	14.7	5.7	27.7	7.3	35.5	90.8
12.7	13.4	5.0	27.8	10.7	32.2	89.1
14.1	12.0	5.2	30.8	15.0	31.1	94.1
14.7	12.1	5.4	31.2	17.9	30.0	96.6
15.5	12.4	5.4	30.7	19.8	29.0	97.3
15.9	12.6	5.0	29.7	19.4	31.0	97.7
15.8	12.4	4.9	30.5	20.5	29.9	98.2
16.1	11.2	5.4	30.2	20.6	29.2	96.6
16.2	11.2	5.8	29.1	20.7	30.2	97.0
16.4	10.9	5.7	28.4	20.7	31.2	96.9

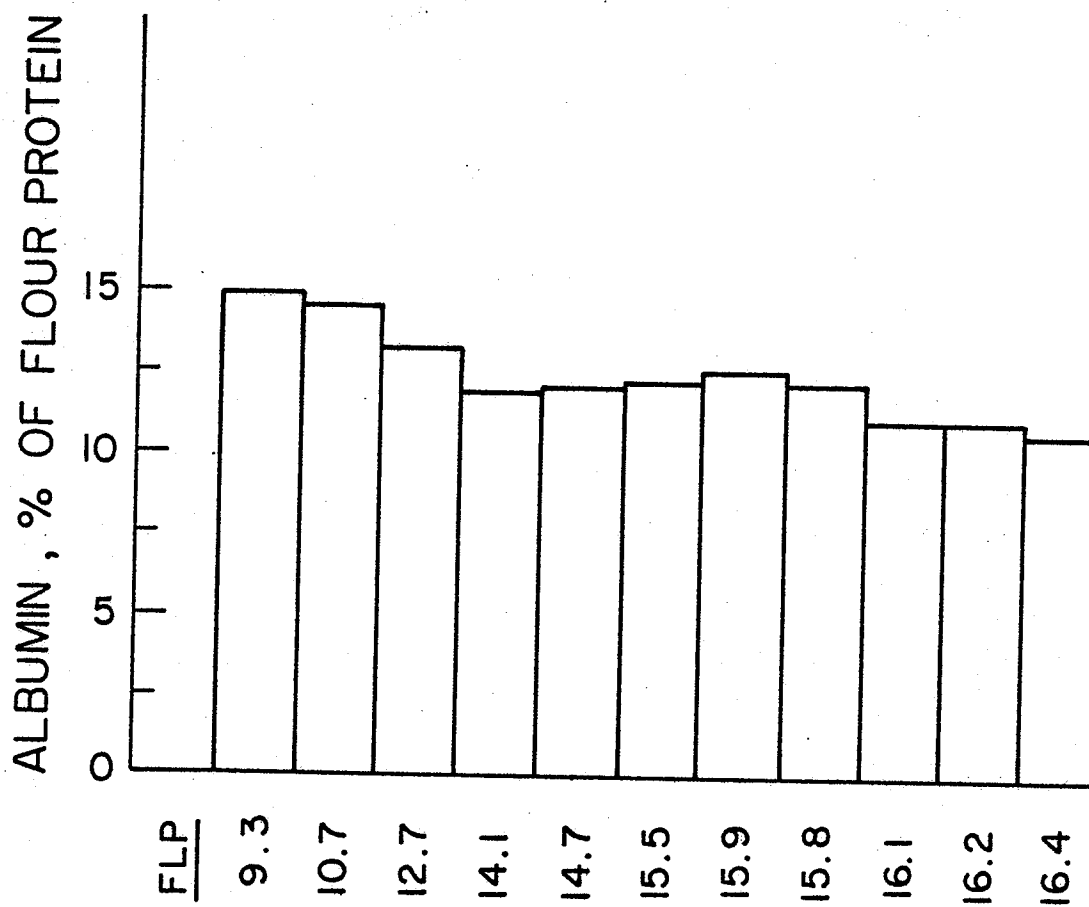


Figure 10. Proportion of Water-Soluble (Albumin) Fraction for 11 Wheat Samples of One Cultivar (Neepawa) of Different Protein Content

negative correlations ($r = 0.93^*$ and $r = 0.89^*$, respectively) were obtained (Table 9). These results are similar to those of Tanaka and Bushuk (1972) who obtained a slight decreasing trend in the amount of albumin with increasing protein content for one cultivar but not for the other used in their study.

Salt-Soluble (Globulin) Fraction. The amount of salt-soluble protein varied from 4.9 to 5.8% without any definite trend with protein content (Figure 11). Similar results were obtained by Tanaka and Bushuk (1972). The correlation coefficient between Pelshenke value and the amount of globulin protein was not statistically significant at the 1% level.

Alcohol-Soluble (Gliadin) Fraction. This fraction did not show any significant change with protein content of the flour (Figure 12). The values ranged from 27.0 to 31.2% and are similar to those determined by Tanaka and Bushuk (1972).

Acetic Acid-Soluble (Glutenin) Fraction. The glutenin fraction, expressed as percentage of total protein, exhibited the greatest variability of the five protein fractions. The percentage of glutenin (Figure 13) increased three fold from 6.6 to 20.7%, and was highly correlated with both protein content ($r = 0.98^*$) and Pelshenke value ($r = 0.81^*$). An earlier study of Tanaka and Bushuk (1972) showed that the glutenin fraction increased slightly with increasing protein content for the two cultivars used in their study. For widely different wheats, Orth and Bushuk (1972) showed that the proportion of this protein fraction was inversely related to breadmaking quality as measured by loaf volume per unit protein. For the 11 samples used in the present study, the proportion of glutenin protein was also negatively related to volume per unit protein, but the correlation was not significant at the 1% level ($r = -0.03$).

Table 9. Correlation Coefficients of Pelshenke Value and Protein Content with Various Protein Fractions

Variables	r
Pelshenke value versus:	
proportion of albumin protein	-0.89*
proportion of globulin protein	0.21
proportion of gliadin protein	0.25
proportion of glutenin protein	0.81*
proportion of residue protein	-0.67*
Protein content versus:	
proportion of albumin protein	-0.93*
proportion of globulin protein	-0.05
proportion of gliadin protein	0.45
proportion of glutenin protein	0.98*
proportion of residue protein	-0.91*
Loaf volume per unit protein versus:	
proportion of glutenin protein	-0.03
proportion of residue protein	-0.14

* Significant at the 1% level.

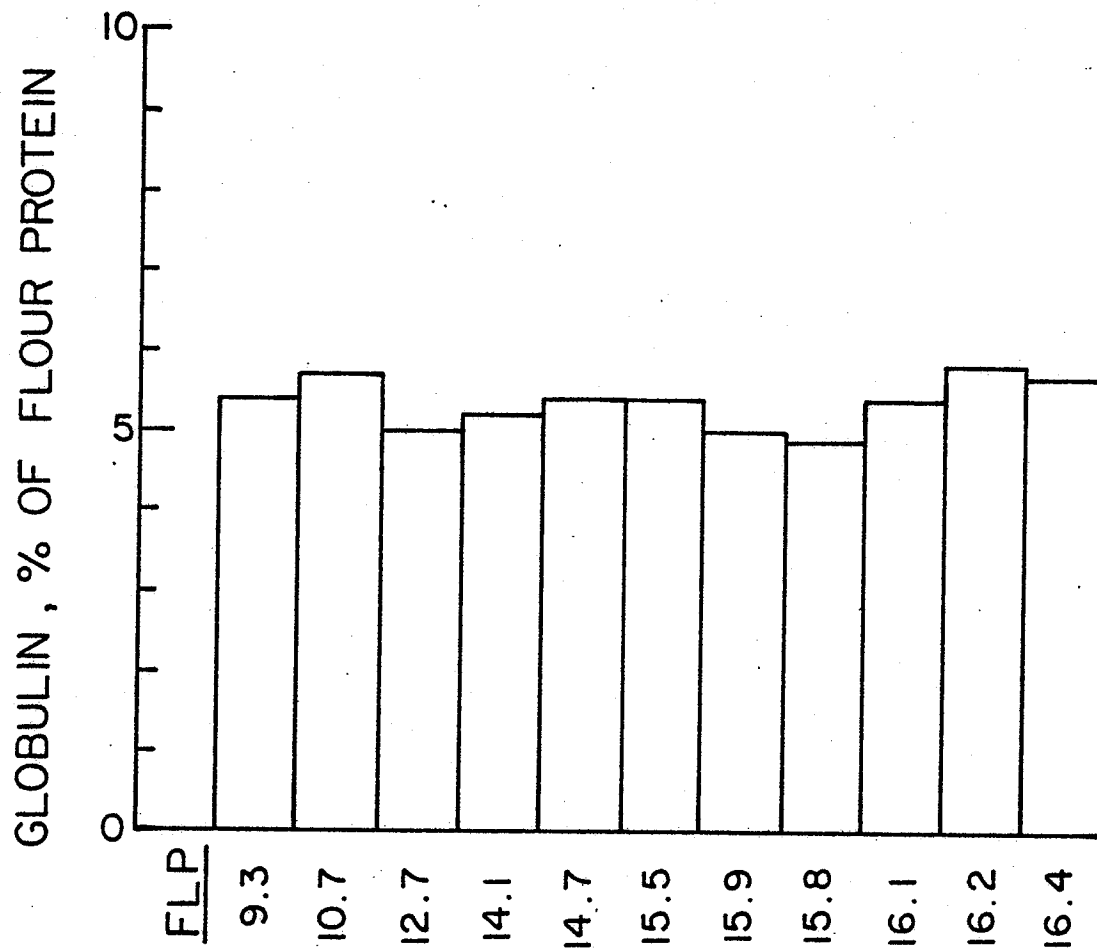


Figure 11. Proportion of Salt-Soluble (Globulin) Fraction for 11 Wheat Samples of One Cultivar (Neepawa) of Different Protein Content

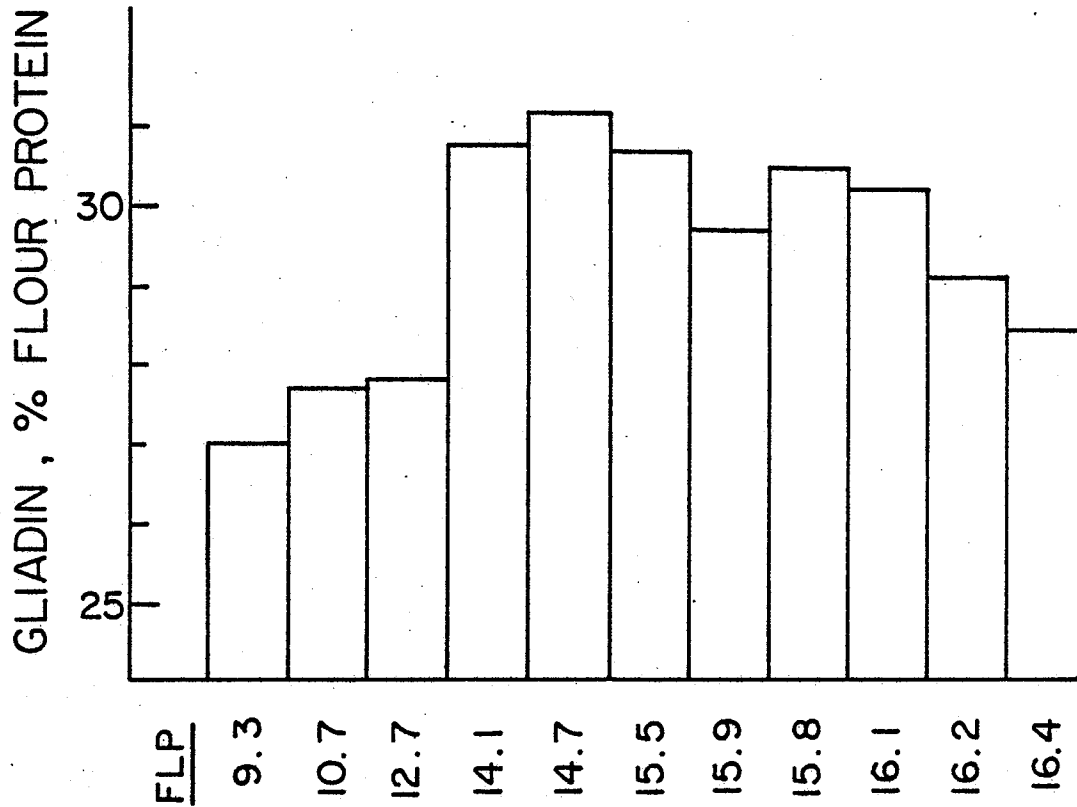


Figure 12. Proportion of Alcohol-Soluble (Gliadin) Fraction for 11 Wheat Samples of One Cultivar (Neepawa) of Different Protein Content

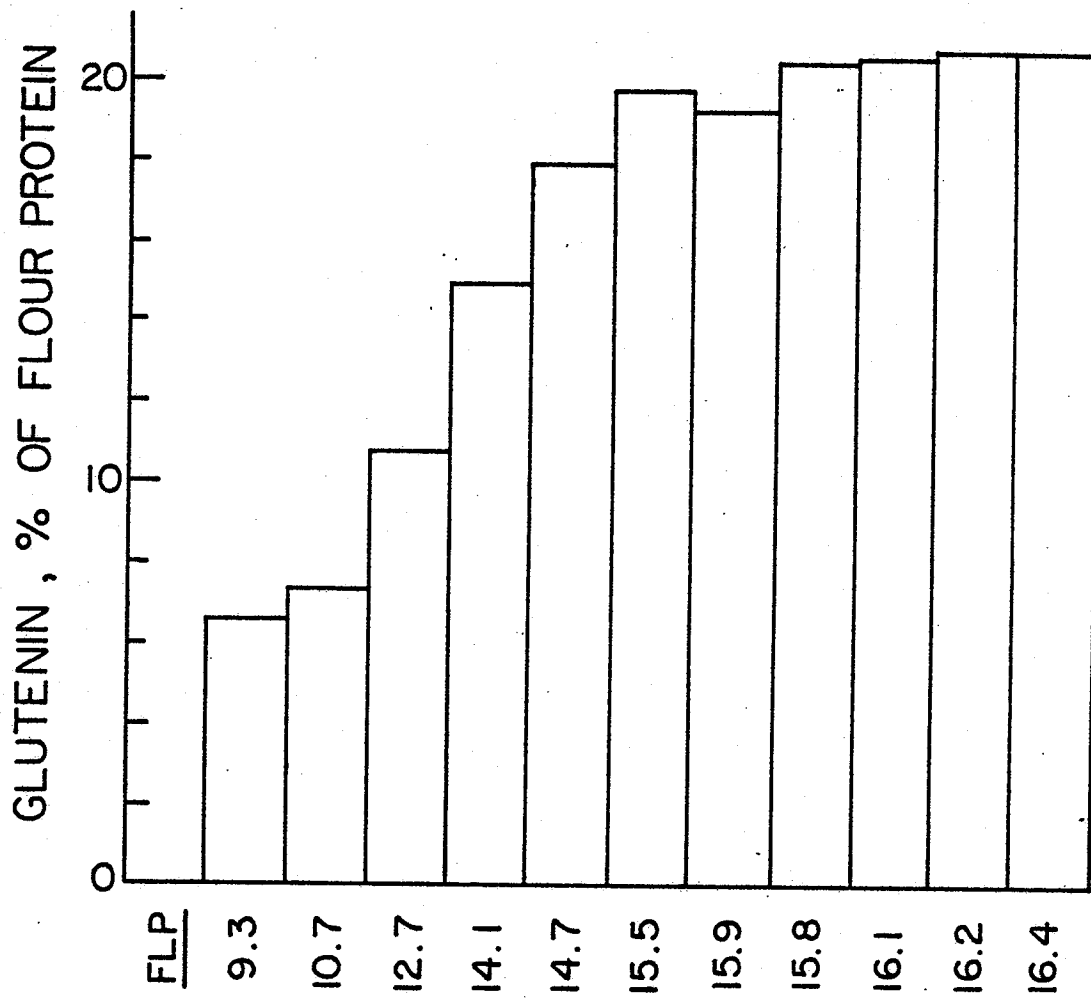


Figure 13. Proportion of Acetic Acid-Soluble (Glutenin) Fraction for 11 Wheat Samples of One Cultivar (Neepawa) of Different Protein Content

Insoluble or Residue Fraction. This fraction formed the largest proportion of the flour protein. The amount of insoluble residue protein showed a decreasing trend from 35.5 to 29.0% with increasing protein content (Table 8). Figure 14 shows the variation in the proportion of the insoluble fraction for the 11 samples. These results differ from those of Tanaka and Bushuk (1972) who found that the amount of insoluble protein remained essentially constant over the protein content range examined. On the other hand, Orth and Bushuk (1972) showed that the proportion of this insoluble fraction was positively related to bread-making quality as measured by loaf volume.

For the 11 samples of wheat flour used in the present study, the proportion of insoluble fraction was inversely related to protein content ($r=-0.91^*$) Pelshenke value ($r=-0.67^*$) and to loaf volume per unit protein ($r=-0.14$), (see Table 9).

On the basis of the results of Orth and Bushuk (1972), the negative correlation between protein content and the proportion of residue protein suggests a decrease in protein "quality" for breadmaking with increasing protein content. This is also borne out to a limited extent by the inverse relationship between Pelshenke value per unit protein and protein content (see Figure 6). These results suggest that the glutenins of the higher protein flours have a lower molecular weight average (and thereby are more soluble) than those of lower protein content flour. It can therefore be concluded that for wheat samples of similar "quality" (i.e. same cultivar) but of widely different protein "content", the Pelshenke value depends primarily on the protein "content" and to a lesser extent on the relative proportions of protein solubility fractions.

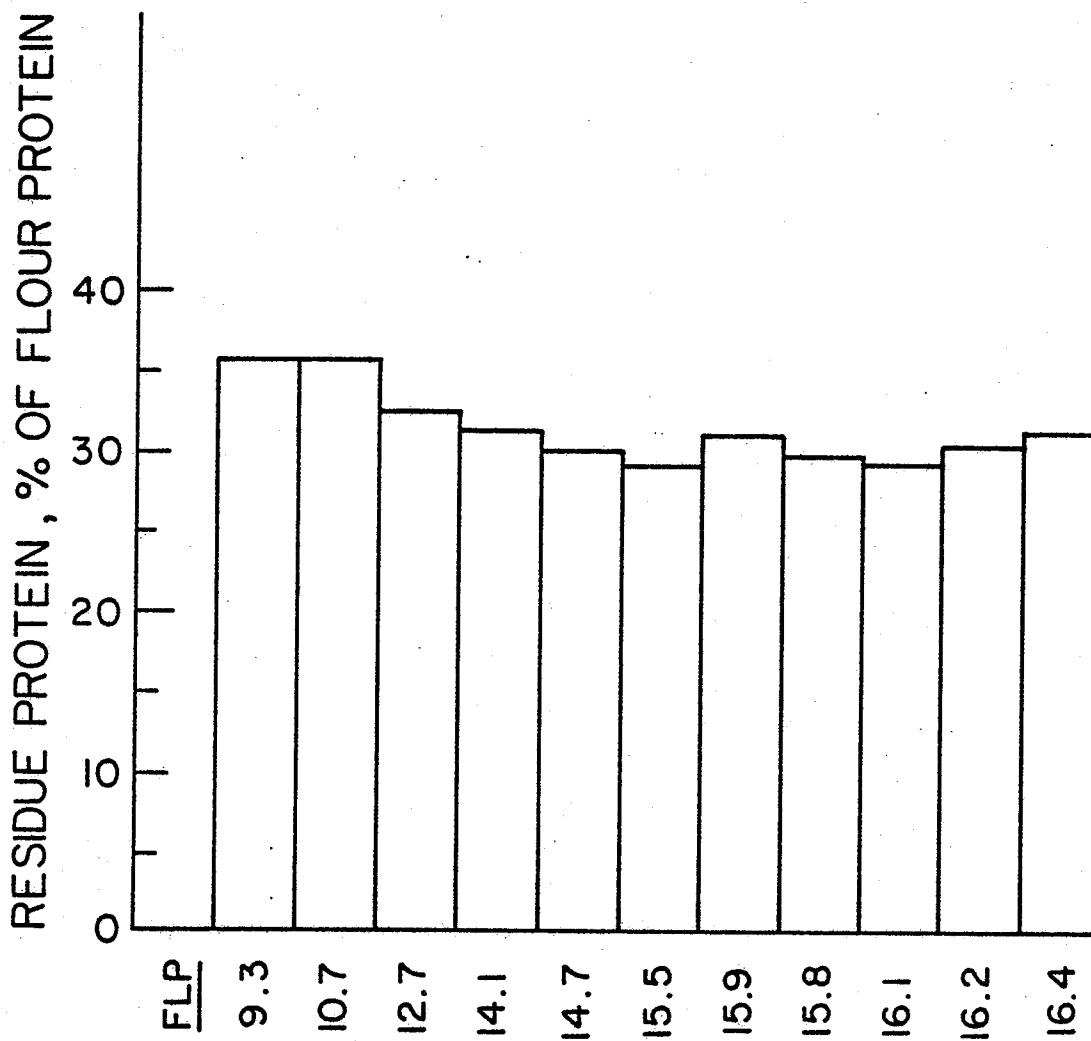


Figure 14. Proportion of Insoluble or Residue Fraction for 11 Wheat Samples of One Cultivar (Neepawa) of Different Protein Content

6. Amino Acid Composition

Four of the 11 flour samples used in this study were selected for amino acid composition analysis to cover the range in protein content (9.5 to 17.1%). Regression and correlation coefficients were calculated between the protein content and concentration of each amino acid (Table 10).

Despite large differences in protein content among the samples studied, only minor variations in amino acid composition were observed. The proportions of four of the amino acids (glutamic acid, proline, tyrosine and phenylalanine), were positively correlated with protein content. The regression coefficients, which reflect the variation of amino acid content per unit change in protein content, indicated that glutamic acid was the principal amino acid affected by variation in protein content.

Previous studies have shown that cysteine, glutamic acid and proline increased proportionately (Miller et al., 1950; Sosulski et al., 1963) while arginine, lysine and valine decreased (Gunthar and McGinnis, 1957) with increasing protein content of whole wheat. Data have also been presented (Sosulski et al., 1963) to show that tyrosine and phenylalanine are also positively correlated with protein content. Additional amino acids, found to be positively correlated with flour protein content in the present study, are histidine, arginine, serine, valine, isoleucine and leucine. The increases in glutamic acid and proline contents with protein content are consistent with the solubility data which indicate a higher proportion of gluten proteins in flours of higher protein content.

D. Contribution of Flour Constituents to the Pelshenke Value

This section presents results of experiments designed to examine the contribution of major flour constituents to the Pelshenke value.

Table 10. Amino Acid Composition in Flours of Different Protein Content and their Relationship to Protein Content

Fertilizer ^a	0	100	250	500	Regression Coefficient	Correlation Coefficient
% Protein ^b	9.5	13.2	16.1	17.1		
Gram amino acid/100 g protein						
Lysine	2.41	2.27	2.19	2.24	-0.026	-0.92
Histidine	2.23	2.25	2.29	2.34	0.013	0.92
Ammonia	4.08	4.31	4.43	4.58	0.061	0.98**
Arginine	3.88	3.66	3.76	3.93	0.002	0.06
Aspartic acid	4.64	4.60	4.57	4.67	-0.001	-0.05
Threonine	3.00	2.88	2.85	2.92	-0.014	-0.72
Serine	5.17	4.96	5.19	5.30	0.018	0.42
Glutamic acid	38.98	41.14	43.27	44.64	0.720	0.99*
Proline	12.92	13.48	13.71	13.74	0.109	0.98**
Glycine	3.83	3.75	3.71	3.83	-0.006	-0.31
Alanine	3.23	3.14	3.08	3.20	-0.010	-0.50
Valine	4.43	4.33	4.39	4.52	0.008	0.34
Methionine	1.51	1.44	1.42	1.49	-0.006	-0.46
Isoleucine	3.81	3.84	3.91	4.01	0.023	0.90
Leucine	7.63	7.56	7.61	7.83	0.018	0.52
Tyrosine	2.91	3.11	3.15	3.18	0.034	0.96**
Phenylalanine	5.19	5.54	6.00	6.21	0.133	0.99**

^a Nitrogen applied as fertilizer (lb/ac)

^b N x 5.7, as is moisture basis

* Significant at 1% level

** Significant at 5% level

The results will be presented in subsections dealing with the effect of protein content, damaged starch, pentosans, lipids and enzymes. A separate subsection is devoted to the effect of a number of technologically important flour improver agents.

1. Protein Content

To determine effect of protein content independently of other factors, (e.g. starch damage) on the Pelshenke value, it was necessary to select a flour with low level of starch damage and increase its protein content by adding vital gluten. Accordingly, a flour milled from a soft white winter wheat (cv. Frederick) was selected for this purpose. Three levels of vital gluten (Industrial Grain Products) were added to give four flours of increasing protein content. The relevant data for this flour is given in Table 11.

The data of Table 11 and Figure 15 show that for the four flours used here, the Pelshenke value increased directly with protein content. Although the level of starch damage in these samples decreased slightly, the decrease is considered to be insufficient to markedly affect the increase in Pelshenke value due to the increase in protein content. The Zeleny Sedimentation value also increased essentially nearly with protein content (see Table 11 only).

Results of this experiment confirm those discussed in Section C, that the Pelshenke value depends directly on the protein content of the meal of the flour used for the test, if the level of damaged starch is approximately constant (e.g. as for wheats of similar kernel hardness).

2. Damaged Starch

When wheat is milled into flour, a portion of the starch granules undergo physical damage as a consequence of the grinding action of the

Table 11. Effect of Adding Vital Gluten to Soft White Winter Wheat Flour on Pelshenke Value and Zeleny Sedimentation Value

Sample	Vital Gluten added, g/100	Protein ^a %	Starch ^b Damage FU	Pelshenke Value min	Zeleny Sedim. cc
Control	0	8.5	8.5	94	17
Flour I	2.76	10.8	5.1	165	21
Flour II	7.12	13.3	3.7	245	27
Flour III	10.25	15.3	3.7	285	35

^a N % x 5.7 (14.0% m.b.)

^b Farrand units

Table 12. Effect of Starch Damage on Pelshenke and Zeleny Sedimentation Values

Sample	Starch Damage FU ^a	Pelshenke Value min	Zeleny Sedim. cc
Hard red spring wheat (11.5% protein)			
Coarse semolina	21	160	27
Fine semolina	27	172	30
Flour	34	200	45
Pin-milled flour	41	210	53
Durum wheat (11.9% protein)			
Coarse semolina	8	94	12
Fine semolina	20	108	13
Flour	46	199	22
Pin-milled flour	55	232	25

^a Farrand units

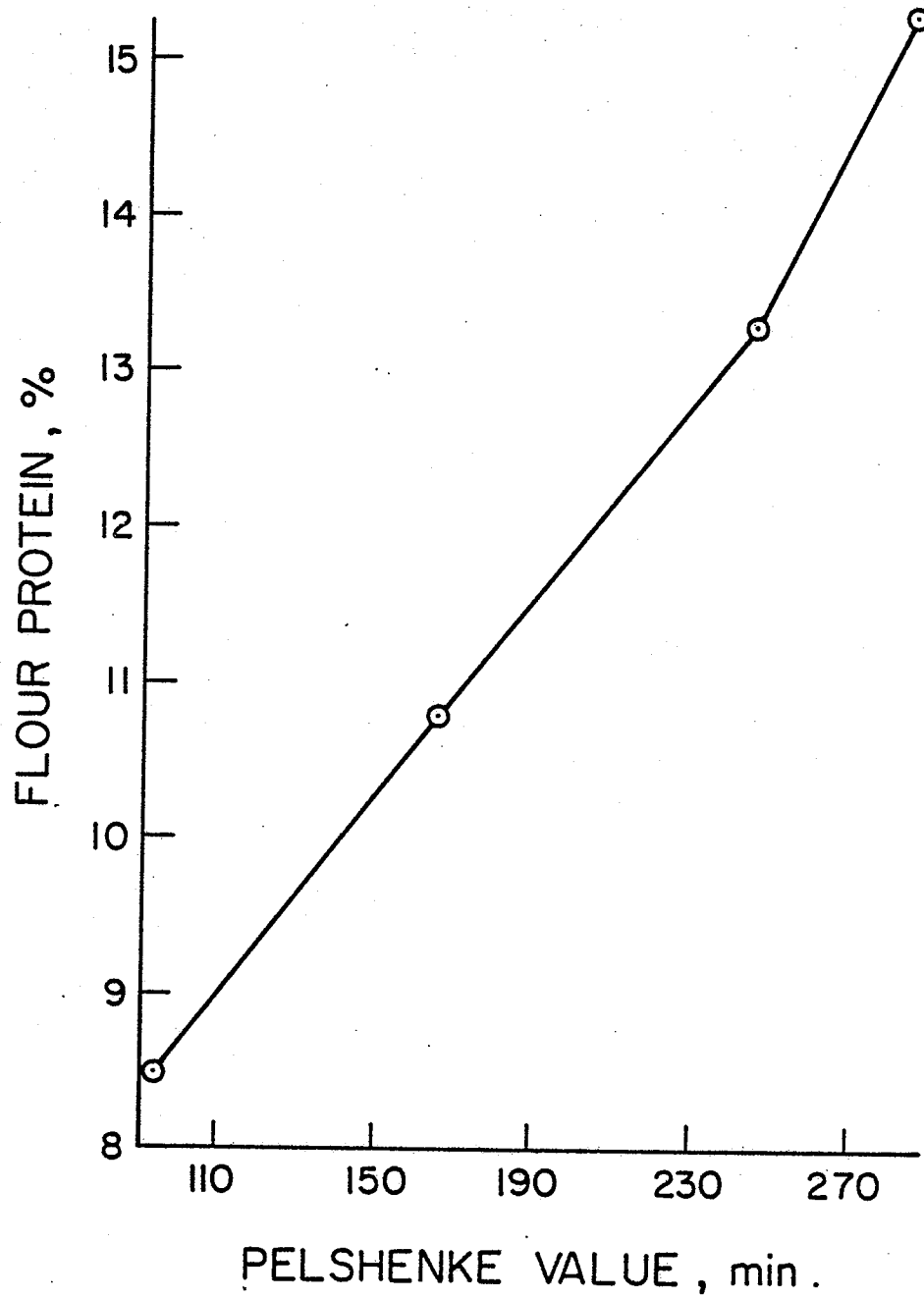


Figure 15. Effect of Addition of Vital Gluten to Soft White Winter Wheat Flour on Pelschenke Value

mill rolls. For the purpose of the present study, commercial semolinas (coarse endosperm middlings) of hard red spring and durum wheats were milled and pin-milled to different levels of damaged starch. It is assumed that this technique for increasing the amount of damaged starch does not affect the other constituents in any way so as to affect their functionality in the Pelshenke Test. Data relevant to this experiment is given in Table 12.

The samples used in this experiment cover a suitable range of damaged starch, 21 to 41 Farrand units for the hard red spring wheat and 8 to 55 Farrand units for the durum wheat. For both samples, the Pelshenke value increased, essentially linearly, with the amount of damaged starch (Figure 16). The Zeleny Sedimentation value, another breadmaking quality test, increased similarly with damaged starch (see Table 12). The effect of starch damage on the sedimentation value has been reported by Farrand (1972).

Results presented here indicate that the Pelshenke value depends directly on the level of starch damage. Accordingly, this factor must be taken into consideration when the test is applied to wheat populations that differ widely in kernel hardness. On grinding, wheats of different hardness would produce meals with different levels of damaged starch.

3. Pentosans

The effect of the pentosans on the Pelshenke value, was examined by supplementing the four flours used in the protein effect experiment (above) with various levels of soluble and insoluble wheat pentosans (obtained from Grain Research Laboratory, Winnipeg). The minimum amount of added pentosans increased the natural pentosan content three fold for the soluble and the insoluble pentosan. The results obtained are given in Table 13.

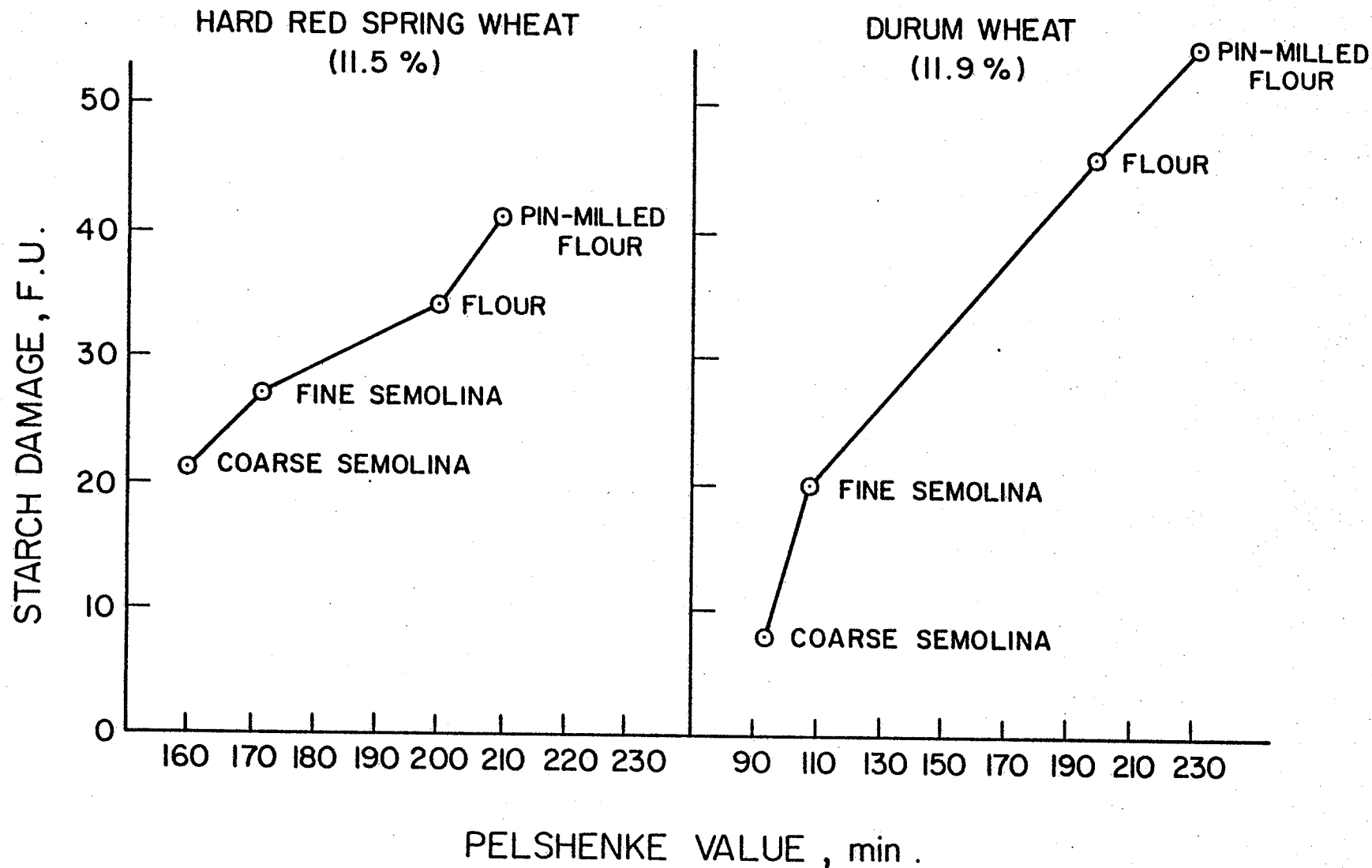


Figure 16. Effect of Starch Damage on Pelshenke Value for Products of Two Different Wheats

Table 13. Effect of Soluble and Insoluble Pentosans on Pelshenke Value

Sample ^a	Protein %	Soluble-pentosans ^b				Insoluble-pentosans ^b		
		0	10	20	30	10	20	30
Control	8.5	94	105	120	145	98	100	100
Flour I	10.8	165	180	203	218	170	170	175
Flour II	13.3	245	260	275	300	258	260	270
Flour III	15.3	285	320	365	420	298	311	355

^a Soft white winter wheat flour

^b mg per 3 g flour

Table 14. Effect of Defatting and Addition of Oleic and Linoleic Acids and Lecithin on Pelshenke Value of Various Materials From Hard Red Spring Wheat

Materials	Original	Defatted	Defatted with		
			Oleic* Acid (1 mg)	Linoleic* Acid (1 mg)	Lecithin* (2 mg)
Whole meal	259	250	290	303	273
Coarse semolina	160	130	170	175	158
Fine semolina	172	142	180	185	170
Flour	200	162	200	205	196
Pin-milled flour	210	183	215	219	208

* per 3 g flour

Addition of soluble pentosans increased the Pelshenke value; the greatest effect was obtained from blend flour III which had the highest protein content. Addition of insoluble pentosans produced a much smaller increase in Pelshenke value than the levels of soluble pentosan. Here again, the greatest effect was obtained for blend flour III which had the highest protein content. The results of experiment reported here indicate that soluble pentosans have some effect on Pelshenke value. However, since the pentosan content of wheat cultivars is relatively low, this component is probably not involved in the intercultivar variations in Pelshenke value.

4. Lipids

The possible involvement of lipids on the Pelshenke value was investigated by two experiments. In the first, the effect of defatting, with petroleum ether, was examined using five different samples (whole meal, coarse and fine semolina, and coarse and fine flour). In the second experiment, the effects of adding three different lipid components (oleic acid, linoleic acid and lecithin) to defatted and undefatted samples were determined.

Extraction of the natural lipids reduced the Pelshenke value slightly (Table 14). The value could be restored to that of the control by addition of oleic acid, linoleic acid or lecithin.

Addition of oleic acid, linoleic acid or lecithin generally increased the Pelshenke value (Tables 15, 16 and 17). In the case of lecithin, the higher level used (6 mg) produced a slight decrease in the value (for some of the samples tested) over that at the 4 mg level.

From this preliminary experiment, it is concluded that flour lipids play a definite role in the Pelshenke value. However, because of the

Table 15. Effect of Additions of Oleic Acid on Pelshenke Value of Various Hard Red Spring Wheat Materials

Material	Oleic Acid ^a				
	0	0.5	1.0	2.0	3.0
Whole meal	259	248	310	340	362
Coarse semolina	160	142	190	230	262
Fine semolina	172	150	200	242	270
Flour	200	178	234	256	291
Pin-milled flour	210	192	250	262	311

^a mg per 3 g flour

Table 16. Effect of Additions of Linoleic Acid on Pelshenke Value of Various Hard Red Spring Wheat Materials

Material	Linoleic Acid ^a				
	0	0.5	1.0	2.0	3.0
Whole meal	259	257	301	336	411
Coarse semolina	160	146	172	198	232
Fine semolina	172	152	182	212	245
Flour	200	180	210	222	279
Pin-milled flour	210	194	221	240	295

^a mg per 3 g flour

Table 17. Effect of Additions of Soybean Lecithin on Pelshenke Value of Various Hard Red Spring Wheat Materials

Material	Soybean Lecithin ^a			
	0	2	4	6
Whole meal	259	280	288	273
Coarse semolina	160	168	156	167
Fine semolina	172	178	162	159
Flour	200	208	172	167
Pin-milled flour	210	223	160	162

^a mg per 3 g flour

Table 18. Effect of Additions of Alpha-Amylase on Pelshenke Value of Products From Two Wheats

Samples	Alpha-Amylase ^a			
	0	3	6	10
<u>Hard Red Spring Wheat</u>				
Coarse semolina	160	164	109	64
Fine semolina	172	172	118	69
Flour	200	189	142	94
Pin-milled flour	210	205	160	99
<u>Soft White Winter Wheat</u>				
Control	94	97	61	50
Flour I	165	176	89	78
Flour II	245	239	156	122
Flour III	285	282	194	150

^a mg per 1 g flour

narrow variability in lipid content or composition among cultivars, the lipids should not play a significant role in the variability in Pelshenke value among wheat cultivars.

5. Enzymes

The possible implication of certain indigenous flour enzymes in the Pelshenke value was tested by examining the effects of these enzymes from other (than the test flour) sources added to the Pelshenke Test dough.

Addition of bacterial alpha-amylase (Division of Becton Dickinson and Co.) at 3 mg/g flour level had no effect on the Pelshenke value of a number of different flours (Table 18). However, additions of larger quantities of this enzyme produced a marked decrease in Pelshenke value. On the basis of these preliminary results, it is concluded that in flour that has a normal level of alpha-amylase activity, this enzyme contributes little to the Pelshenke value. However, if the alpha-amylase activity of the flour is excessively high, as in flour milled from sprouted wheat, then the contribution of this enzyme to the Pelshenke value could be quite significant.

The effects of two exogenous proteases, papain (powder purified concentrate, obtained from Mann Res. Laboratories) and pepsin (crystallized and lyophilized, 1:60,000 units, obtained from Sigma Chemical Co.), on the Pelshenke value of a variety of flours are shown by the data in Tables 19 and 20, respectively.

Both enzymes produced a marked decrease in the Pelshenke value of the Manitou flours but had only a slight or no effect in durum wheat, soft wheat, triticale and rye flours. Pepsin was considerably more active than papain on an equal weight basis. However, for an appropriate comparison, the effects should be expressed on the basis of specific activity of the enzyme.

Table 19. Effect of Additions of Papain (Protease) on Pelshenke Value of Various Flours

Flour Sample	Papain (mg per 3 g Flour)					
	0	0.25	0.5	1.0	2.0	3.0
Manitou ^a (10.1% protein)	238	220	216	182	112	108
Manitou ^a (12.1% protein)	274	245	243	224	175	170
Manitou ^a (14.4% protein)	300	275	272	263	200	200
Talbot ^b	52	51	51	50	46	46
Stewart 63 ^c	56	56	56	50	42	42
6A190 ^d	50	50	50	49	44	44
Prolific ^e	80	80	80	79	81	80

^a Hard red spring wheat

^b Soft white winter wheat

^c Durum wheat

^d Triticale

^e Rye

Table 20. Effect of Pepsin (Protease) on Pelshenke Value of Various Flours

Flour Samples	Pepsin (mg per 3 g Flour)						
	0	0.01	0.025	0.05	0.1	0.2	1.0
Manitou ^a (10.1% protein)	238	222	166	100	54	49	48
Manitou ^a (12.1% protein)	274	270	217	123	62	52	48
Manitou ^a (14.4% protein)	300	292	259	162	79	59	50
Talbot ^b	52	52	52	51	51	45	46
Stewart 63 ^c	56	56	56	56	51	45	45
6A190 ^d	50	48	45	45	45	45	44
Prolific ^e	80	79	80	80	82	82	79

^a Hard red spring wheat

^b Soft white winter wheat

^c Durum wheat

^d Triticale

^e Rye

The reason(s) why the proteases had no effect on the Pelshenke values of the durum wheat, soft wheat, triticale and rye flours are not obvious. The most plausible reason, suggested by the very low Pelshenke values for these flours, is that the proteins of the flours contribute very little to the Pelshenke value. Another possibility, although less likely, is that these cereals contain highly active protease inhibitors. Further work is required to clarify the points raised here.

From the limited results on the effects of the two proteases on the Pelshenke value, it is concluded that the natural flour proteases do not contribute to the Pelshenke value of flours from sound wheat. As in the case of alpha-amylase, the role of proteases in flours from sprout-damaged wheat can be quite substantial.

6. Effects of Flour Improving and Other Related Chemicals

A number of chemicals, such as potassium bromate, are added at the flour mills to improve the breadmaking quality of freshly milled flours. For this reason, it was of interest to examine the effects of some of these so-called "improvers" and other related chemicals on the Pelshenke value. These experiments were made with a variety of materials. The results that were obtained are compiled in Appendix VI. Since the trends of the effects of the chemicals used were the same for all materials, only the results for the meal and flour of Manitou wheat will be presented and discussed here. These results are summarized in Figure 17.

Potassium bromate, to a concentration of 1.00 $\mu\text{g}/3$ g flour, increased the Pelshenke value of meal. Addition of 1.50 μg produced a slight decrease from the value at 1.0 μg . A similar curve was obtained for the flour except that the optimum was much sharper and occurred at a lower potassium bromate concentration (0.5 $\mu\text{g}/3$ g flour). The optimum potassium

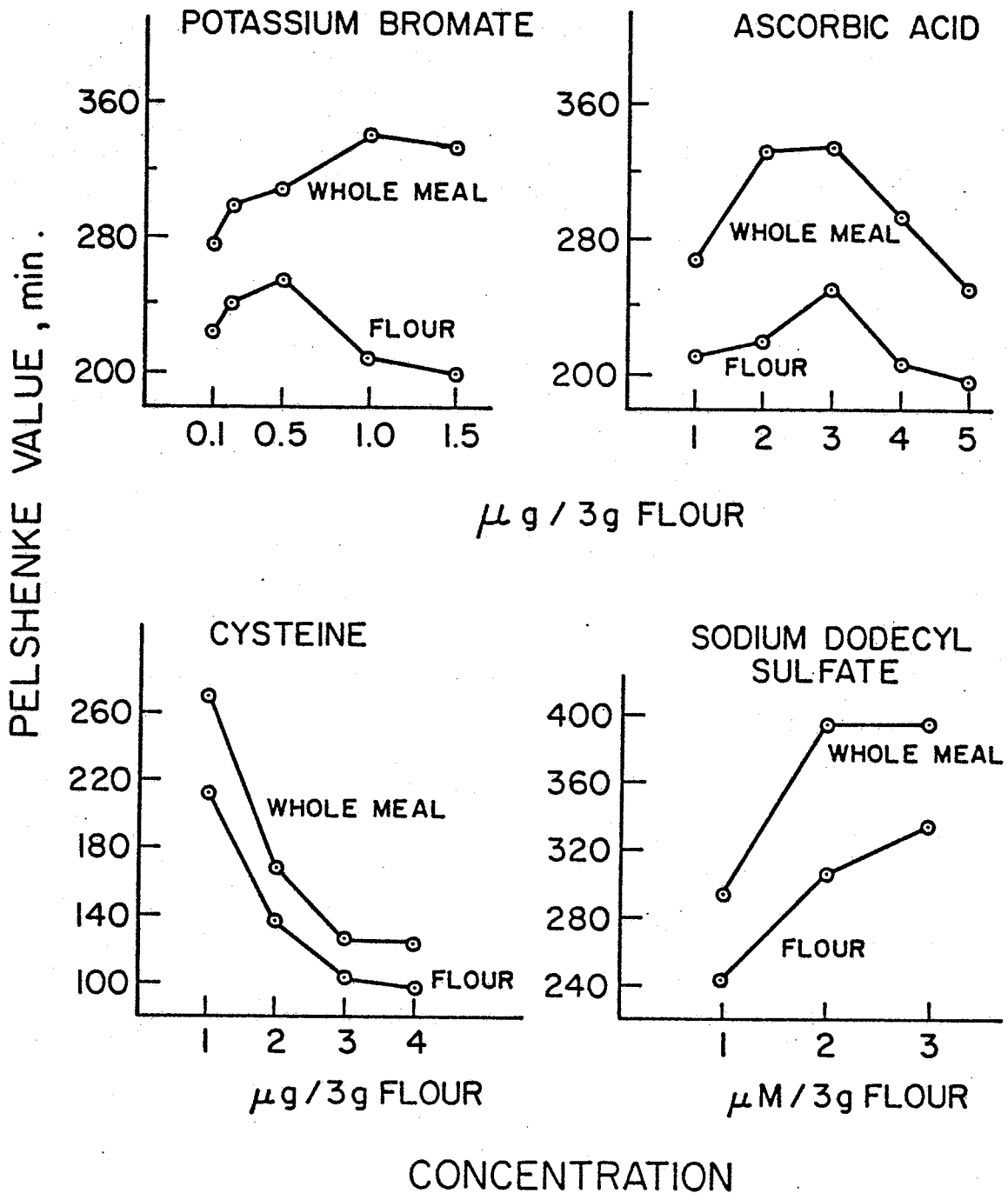


Figure 17. Relationships Between Pelshenke Value and the Concentration of Dough Improving Agents for Whole Meal and Flour for Hard Red Spring Wheat

bromate concentration in terms of the Pelshenke value is in the range of optimum bromate treatment (for the flour used) assessed by the baking test.

The results for ascorbic acid (see Figure 17) were similar to those for potassium bromate in that the Pelshenke value increased to an optimum and then decreased as the amount of ascorbic acid increased. However, in this case the optimum for the whole meal and flour occurred at approximately the same ascorbic acid concentration.

Additions of 10 μg of cysteine (a reducing agent, added to hasten dough development by mixing), increased the Pelshenke value slightly (from 259 to 269 min for meal and 200 to 212 min for flour). Further additions produced a drastic drop in Pelshenke value. The effects on whole meal and flour were parallel.

Sodium dodecyl sulfate, a surfactant, increased the Pelshenke value of both whole meal and flour. The curves for the two materials (see Figure 17) were essentially parallel.

The effects of the four chemicals on the Pelshenke value observed in this experiment are generally consistent with their well known effects on breadmaking quality as assessed by the baking test.

In addition to the four chemicals discussed above, N-ethylmaleimide was included in this study. This chemical is a specific sulfhydryl blocking agent and has been used extensively in research on flour quality and the improver reaction. It was added to the Pelshenke Test dough-balls at three concentrations, 0.5, 1.0 and 1.5 μ moles per 3 g flour. All of the dough-balls that contained N-ethylmaleimide remained at the bottom of the beaker, indicating that there was essentially no gas production by the added yeast. Measurements on the Warburg respirometer (Umbreit

et al.,1972) using suspensions of yeast showed that N-ethylmaleimide is a strong inhibitor of yeast activity at the concentrations used. This line of research was not pursued further.

VI. GENERAL DISCUSSION

There is considerable pressure on agricultural research workers to develop a reliable screening test for breadmaking quality of wheat lines in wheat breeding programs. Such a test is of prime relevance to the current attempts of the agricultural industry to keep up with the world-wide demand for food. The Pelshenke Test, a simple rapid test, has been applied quite successfully for screening wheat lines in wheat breeding programs because it measures a very important quality parameter of baking quality, namely the dough strength. Its continued use in modern wheat breeding requires further refinements in order to meet the exacting requirements of modern wheat breeding.

The main objective of this thesis project was to compile additional data on the contributions of some wheat constituents (and their properties) to the Pelshenke Test value relative to its ability to predict breadmaking quality. Also additional information was compiled on the relationship between the Pelshenke value and a number of other measurements that are used in the evaluation (and prediction) of breadmaking quality of wheat. Widely different samples, including durum wheat, rye and triticale, were used in order to examine the applicability of the Pelshenke Test to these cereals, which are used in some countries for the production of bread.

Since its introduction, the use of the Pelshenke Test has remained controversial. Critics of the test have emphasized the wide variability of results obtained with the test due to human error. Accordingly, in addition to manual mixing of the test dough as is done in the classical test, we have examined the use of mechanical mixing. Using a small mechanical dough mixer, the Pelshenke values, for a variety of cultivars showed that the mean values obtained by this technique are quite similar to those

obtained by manual mixing, the experimental error was considerably lower and the reproducibility better. This technique for determining the Pelshenke value was used throughout the course of this thesis. Addition of mechanical mixing to the standard Pelshenke Test procedure would improve the precision of the test without detracting from the speed and simplicity.

The lack of variation in Pelshenke value when mixing speed was altered (in the range investigated) indicates that the mechanical development of the dough during the short mixing time does not contribute significantly to the value. However, when the mixing period was increased at constant speed, the Pelshenke value increased essentially directly with mixing time. This was attributed to increased hydration (a time dependent process) of the flour or meal particles and this in turn increased the gas retaining capacity of the test dough-ball. This factor must be taken into account when the Pelshenke Test is applied to cultivars of different kernel hardness which might grind to different particle size. The grinding would have to be carefully standardized.

The variability in Pelshenke value obtained for widely different cereal grains (wheat, rye and triticale), showed that the Pelshenke Test can readily reflect differences between the cereals. Accordingly, these results indicate that the Pelshenke value is a useful index for classifying wheats (and similar cereals) according to dough strength, the key property in breadmaking quality. For samples of a single cultivar, the Pelshenke value seemed to be directly related to protein content.

Evaluation of the relationship between the Pelshenke value and the breadmaking quality parameters is more complex. However, the highly significant correlations between Pelshenke value and a number of breadmaking

quality parameters (e.g. flour yield, grinding time, loaf volume, farinograph development time, mixing tolerance index, mixograph developing time and Zeleny Sedimentation value) indicate that, the Pelshenke value is a reliable prediction test of breadmaking quality. It should be emphasized that some of the correlations do not necessarily prove cause and effect. Interstation differences in the Pelshenke values for a single cultivar were observed, but these differences are smaller than the intercultur differences upon which the correlations are based.

The Pelshenke value of a series of 11 samples of Neepawa wheat grown at different levels of nitrogen fertilizer increased with protein content of the grain. Although the Pelshenke value of the 11 samples increased with protein content, the differences between samples were not solely due to differences in protein quantity. For example, in analyzing the technological data (See Appendix II) it is noted that samples grown at 300 and 350 lb N per acre fertilizer treatments had essentially the same protein contents but gave different Pelshenke values, 201 and 219 min respectively. It is suggested, that a better index of breadmaking quality might be the Pelshenke value expressed per unit protein content. This would eliminate the effects of protein content. Wheat lines in breeding programs can show considerable variability in protein content even when grown under the same environment.

For the 11 samples of Neepawa wheat used in this study, the Pelshenke value per unit protein content is inversely related to protein content in the range from 9.3 to 15.9%. This indicates a decrease in protein "quality", as measured by the Pelshenke Test, with increasing protein content of samples grown under high nitrogen fertilizer stress. Baking test results for these samples showed that breadmaking quality (expressed as loaf volume per unit

protein) decreased with increasing protein. However, total loaf volume increased directly with protein content. The four samples of this group of 11 that had protein contents above 15.5% showed an increase in Pelshenke value per unit protein content. The only explanation that can be offered for this observation is that the negative effect of decreasing protein "quality" is offset by the positive effect of increasing protein "content". This aspect merits further study.

The Zeleny Sedimentation value, another index of breadmaking quality, varied directly with protein content. The Pelshenke value was highly significantly positively correlated with the Zeleny Sedimentation value.

Gassing power, and its related properties, diastatic activity and level of damaged starch, show negative correlation with Pelshenke value. This correlation is not unexpected since all three measurements are directly related to the rate of gas production in a yeasted dough as used in the Pelshenke Test. These results indicate that the diastatic activity of the flour must be optimized when the Pelshenke Test is used to measure gluten quality.

Data on protein solubility distribution showed a large increase in acetic acid-soluble (glutenin) protein with increasing protein content for the 11 samples of Neepawa wheat obtained by varying the levels of nitrogen fertilizer. For these samples, there was a significant decrease in water-soluble (albumin) protein and insoluble or residue protein with protein content. The proportion of glutenin was significantly correlated with Pelshenke value and loaf volume. However, the solubility data indicated a gradual drop in protein quality for breadmaking (expressed as either Pelshenke value or loaf volume per unit protein) with increasing protein content. These results suggest that glutenins of the higher protein

flours have a lower average molecular weight than those of lower protein content flours. These findings are in general agreement with those of Orth and Bushuk (1972) who showed that for widely different cultivars, loaf volume was inversely related to the amount of glutenin and directly to the amount of residue protein.

Although increases in the protein content of the Neepawa cultivar in response to fertilizer treatments were major, only minor differences in amino acid composition were observed among the 11 samples. The proportions of four of the amino acids, glutamic acid, proline, tyrosine and phenylalanine, increased with protein content. The increases in glutamic acid and proline contents are generally consistent with the solubility data which indicate that variations in the proportions of different protein fractions (as defined by solubility) may be largely responsible for the changes in amino acid composition.

The effect of the major constituents of wheat on Pelshenke value was also examined. Commercial hard red spring and durum wheats were milled and pin-milled to obtain different flours with different starch damage levels while protein content remained constant. The Pelshenke value increased directly with the level of starch damage. As is already known, Zeleny Sedimentation value and baking absorption were also directly affected by starch damage level. The significant role of starch damage level on the Pelshenke value indicates that the Pelshenke Test should be conducted on ground material (meal or flour) of the same starch damage level. This is particularly relevant when the test is applied to cultivars that differ widely in hardness (which on grinding will have different levels of damaged starch).

The effect on protein content on Pelshenke value was studied also by

adding varying amounts of protein concentrate (vital gluten) to flour from soft white winter wheat, while starch damage was kept essentially constant. As expected from results of previous experiments in this study, the Pelshenke value increased directly with the amount of vital gluten added.

When water-soluble and insoluble pentosans were added to the Pelshenke Test doughs, a marked increase in Pelshenke value was obtained. In spite of this highly significant role of the pentosans in the Pelshenke value, the contribution of this constituent to intercultivar differences is probably minimal since the variability of this constituent among cultivars is negligible (D'Appolonia et al., 1970).

Extensive evaluation of the role of flour lipids on the Pelshenke value was not carried out in the present study. Exploratory experiments with polar and non-polar lipids from other sources, showed that both were capable of increasing the Pelshenke value. When flour was defatted (with petroleum ether), there was a reduction in the Pelshenke value. Addition of polar and non-polar lipids (non-flour) to the defatted flour restored their Pelshenke value to those of the original values. These results suggest that lipids play a definite role in the Pelshenke value. However, further studies are necessary to delineate the contribution of this constituent to intercultivar differences.

The insensitivity of Pelshenke value to low levels of bacterial alpha-amylase activity, makes the test particularly suitable for use with wheats that may differ slightly in amylase activity. Higher activities, such as may be present in sprouted wheat, depressed the Pelshenke value. The large decrease in Pelshenke value was observed with increasing amounts of proteases underlines the well known role of gluten protein in the gas

retaining properties of the dough. Added proteases had little or no effect on the Pelshenke values of rye or triticale. This suggests that either the proteins of these cereals do not contribute significantly to the gas retaining properties of their doughs or that these cereals contain strong inhibitors of proteases. This area merits further investigation.

Results of the experiments on the effects of the so-called flour improver agents on the Pelshenke value showed that increasing concentrations of potassium bromate and L-ascorbic acid increased Pelshenke value, however, higher concentrations of both agents produced the opposite effect. These observations indicated that there is an optimum concentration of each chemical. In general, these results are consistent with the known effects of these chemicals on the gas retention properties of dough as measured by the baking test. The effect of N-ethylmaleimide (a sulfhydryl blocking agent) was a drastic decrease in Pelshenke value. It was shown that this was due to the inhibition of yeast action by this chemical at the concentration used. The negative effect of cysteine (a reducing agent used to hasten mechanical dough development) and the effect of sodium dodecyl sulfate (positive at low additions and negative at high levels) showed that the gas retention capacity of the dough-ball in the Pelshenke Test can be changed in either direction by an appropriate combination of "improving" agents.

This is the first detailed study that dealt specifically with the effects of processing factors and wheat constituents on the Pelshenke Test value. The information gained should provide the necessary basis from which further investigations could be undertaken. Furthermore, the data presented in this thesis should be helpful in the use of the Pelshenke Test for assessing (for breadmaking quality) new cultivars in wheat breeding programs around the world.

VII. SUMMARY AND CONTRIBUTION TO KNOWLEDGE

1. The precision of the Pelshenke Test can be improved substantially by substituting mechanical mixing for the manual mixing used in the standard test.
2. With mechanical mixing, the Pelshenke value increased directly with mixing time; mixing speed in the range examined had no effect on the value obtained.
3. Pelshenke values of non-bread wheats (durum and soft white winter) and other cereals (rye and triticale) were distinctly lower than the values for bread wheats (hard red spring). Accordingly, non-bread cereals can be readily distinguished from bread wheats by the value of the Pelshenke Test.
4. Results for 19 cultivars of the bread wheat class grown at three locations showed that differences in Pelshenke value due to location are considerably smaller than differences due to cultivar. The interstation differences within cultivars appear to be due primarily to differences in protein content of the wheat samples.
5. Results for 11 samples of the cultivar Neepawa (grown at varying levels of nitrogen fertilizer) of widely different protein content (9.3 to 16.4%) showed that for such a group of samples the Pelshenke value is directly related to protein content. Other factors such as protein composition (solubility) and level of damaged starch play a secondary role.
6. With samples of the same cultivar (or similar cultivar in a breeding program), the effect of protein content on the Pelshenke value can be eliminated by expressing the value per percent protein content. It is proposed that the Pelshenke value per percent protein can be used as an index of protein "quality" for breadmaking.

7. Of the various flour protein fractions, separated according to the proportion of acetic acid-soluble fraction (glutenin) was significantly correlated with the Pelshenke value.
8. For samples of approximately the same protein content, the Pelshenke value was directly related to the level of damaged starch in the flour or meal. This factor must be taken into consideration when applying the Pelshenke Test to wheats of different hardness (which will grind to different starch damage levels).
9. Addition of water-soluble and water-insoluble wheat pentosans increased the Pelshenke value. However, the normal variability in pentosan content of wheat cultivars is considered to be too small to have a significant effect on the Pelshenke value.
10. Lipids (from flour and other sources) affect the Pelshenke value. However, because of the very small differences in content and composition of this constituent among wheat cultivars, it probably does not contribute significantly to the intercultivar variability in Pelshenke value.
11. Additions of excessively high levels of alpha-amylase or protease enzymes had a negative effect on Pelshenke value.
12. Additions of potassium bromate and ascorbic acid (flour improvers) produced an increase in Pelshenke value to an optimum followed by a decrease with further additions. This optimal effect on Pelshenke value parallels the effect that can be demonstrated with the baking test.
13. Cysteine (a reducing agent) and sodium dodecyl sulfate had a negative effect on Pelshenke value. N-ethylmaleimide, a sulfhydryl blocking agent, also had a negative effect because of its ability to inhibit yeast activity.

14. Results of the present study (interclass and intercultivar differences, effects of constituents, correlations with breadmaking quality indices, and correlations with loaf volume) showed that with certain precautions (e.g. consideration of protein content and starch damage), the Pelshenke Test can be used effectively for screening wheat lines (in breeding programs) for breadmaking quality.
15. Points 6, 7, 8, 9, and 11 are considered as new contributions to knowledge.

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