QUALITY PREDICTION OF URUGUAYAN WHEATS BASED ON RHEOLOGICAL PROPERTIES AND RELATED NIR ANALYSES

by

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In Partial Fulfillment of the Requirements for the Degree of

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QUALITY PREDICTION OF URUGUAYAN WHEATS BASED ON RHEOLOGICAL PROPERTIES AND RELATED NIR ANALYSES

BY

Daniel Vazquez

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree

Of

Doctor of Philosophy

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ABSTRACT

Wheat has unique rheological properties, which make it suitable for breadmaking. The complexity of the breadmaking process and the diversity of products obtained require a continuous effort to understand wheat quality requirements, and methodologies to measure them. The objectives of this research were to study the importance of dough rheological properties for the quality of Uruguayan type hearth and pan breads, the relationship of these properties to protein components and to evaluate the viability of different methodologies for wheat quality determination, including near infrared spectroscopy (NIR). Complete physicochemical analyses were done on two sets of Uruguayan wheat samples (n=470 and 81). Baking tests, standard and modified rheological analyses, and micro-extension tests were performed on flour samples, while micro-extensigraph tests also were conducted with gluten doughs in the smaller set. Whole grain, ground grain and white flour samples were scanned with a NIRSystem 6500 spectrophotometer. Dough extensional data were generally more closely related to breadmaking properties than were mixing test data. While flour protein content was the best correlated with pan bread volume (r=0.706), the adjusted alveogram resistance/extensibility ratio had an almost identical correlation coefficient (r=0.701). Loaf volume of French bread was most highly correlated with flour protein (r= 0.574). However, the form ratio, an important quality attribute for French bread, was best correlated with alveograph baking strength (r=0.718). Multiple linear regression analyses were able to explain 72% of the pan bread loaf volume variability and 64% of the variability in form ratio including only extensional variables. Duplicating the NIR spectrums resulted in better calibrations. Protein content was predicted with an accuracy similar to that of the reference method one, the standard error of prediction (SEP) = 0.16%. Hardness calibration was successful only with the ground sample, SEP < 2%.

Results obtained for protein functionality variables had lower coefficients of determination but were sufficient for quality screening. R^2 of the validation set was 0.616 for alveogram extensibility, 0.641 for mixogram maximum height, and 0.653 for SDS sedimentation volume. The micro-extensigraph and NIR both proved to be suitable for prediction of the rheological properties which are most important for bread quality.

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LIST OF ABBREVIATIONS OF THE FLOUR QUALITY PARAMETERS

	Parameter	Unit
FP	Flour protein	%
GP	Grain protein	%
PSI	Particle size index	%
WG	Wet gluten	%
DG	Dry gluten	%
GI	Gluten index	%
SDSS	SDS sedimentation test volume	mL
Glu	Polymeric glutenin content	Absorbance units
Mon	Monomeric proteins content	Absorbance units
GluMon	Polymeric glutenin to monomeric proteins ratio	-
TArea	Total chromatogram area (soluble proteins)	Absorbance units
DDT	Farinograph dough development time	min
FarS	Farinograph stability	min
Abs	Farinograph absorbance	%
MixH	Mixograph maximum height	cm
MixT	Mixograph mixing time	min
W	Alveograph baking strength (standard)	$j \times 10^{-4}$
P	Alveograph maximum resistance to extension (standard)	mm
L	Alveograph extensibility (standard)	mm
P/L	Resistance to extension : extensibility ratio (standard)	-
Wad	Alveograph baking strength (modified)	$j \times 10^{-4}$
Pad	Alveograph maximum resistance to extension (modified)	mm
Lad	Alveograph extensibility (modified)	mm
P/Lad	Resistance to extension : extensibility ratio (modified)	-
DSI	Dough microextensigram, maximum slope of the curve	g/mm
DExtSI	Dough microextensigram, ext. at max. slope of the curve	mm
DRMax	Dough microextensigram, maximum resistance	G
DEMax	Dough microextensigram, extensibility at max. resistance	mm
DRRup	Dough microextensigram, resistance at rupture	G
DERup	Dough microextensigram, extensibilty at rupture	mm
DA	Dough microextensigram, area under the curve	g.mm
GSI	Gluten microextensigram, maximum slope of the curve	g/mm
GExtSI	Gluten microext.ensigram, ext. at max.slope of the curve	mm
GRMax	Gluten microextensigram, maximum resistance	G
GEMax	Gluten microextensigram, ext. at maximum resistance	mm
GA	Gluten microextensigram, area under the curve	g.mm
LV	Pan bread, loaf volume	mL
FrV	French bread, loaf volume	mL
FrW	French bread, loaf width	cm
FrH	French bread, loaf height	cm
FrR	French bread, loaf width:height ratio	-
FrA	French bread, crumb appearance	Subjective score

CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Wheat is the food most widely consumed by human populations worldwide (www.fao.org/docrep/007/j2968e/j2968e00.htm). One of the most important reasons for the widespread use of wheat is that the wheat flour dough has unique rheological properties that allow baked products of broad diversity to be produced. Understanding complex breadmaking requirements of different types of bread products has been the focus of both theoretical and applied research for over a hundred years. In the past dough strength has received more attention than extensional properties from researchers investigating wheats for breadmaking potential. However, the extensional behavior of doughs, rather than dough strength, may be most closely linked to loaf quality.

World bread types can be divided into two groups. Pan breads are those baked into a tin, while hearth breads are those baked on a flat surface. Each kind of bread has its own requirements (Mailhot and Patton 1988). So far, most research has been conducted using pan bread (Tronsmo et al 2003b), but in many countries this is not the type of loaf commonly consumed. Industrial requirements for wheat are specific for each product and each market. In Uruguay, French-type hearth bread is the predominant form of bread. There is a need to extend previous findings on breads designed for defined markets to other bread types and baking procedures. A particular concern of the Uruguayan baking industry is related to the extensional properties of doughs made from local flours because hearth breads have different extensional requirements than pan breads. The lack of information about extensional properties means that there are many questions that need to be answered to extend our understanding of these properties.

First there is no general agreement on the role of protein components related to extension of doughs. It is important to understand the relationship between functionality and protein composition in order to be able to define strategies to improve quality. Secondly, it is not clear what influence extensional properties have on breadmaking in general, and on hearth bread in particular. Thirdly, in order to properly evaluate the requirements, the methodology used should be suitable. Standard methods and their modifications need to be evaluated for different wheat populations and breadmaking requirements. Optimal methodology will provide useful information. Small sample methods and/or non-destructive ones are always preferable for research and screenings. Micro-extension tests using texture analyzers have been proposed. In addition, near infra-red spectroscopy (NIR) is a promising tool that may be able to predict functionality (Wrigley 1994). However, more research needs to be done in order to get an appropriate evaluation of this technology.

The present work is an initial approach to Uruguayan wheat properties and requirements, along with the development of appropriate methodologies. The purpose of this investigation was to determine the importance of dough rheological properties, and especially extensional properties, for the quality of Uruguayan French-type (hearth) and pan breads, and to investigate the relationship of these properties to protein components. Objectives included also were to evaluate applicability and suitability of methodologies like micro-extension tests and NIR analysis.

CHAPTER 2

LITERATURE REVIEW

LITERATURE REVIEW

2.1. Introduction

Bread wheat (*Triticum aestivum*) is a polyploid species containing three related genomes: A, B and D. This species arose from the hybridization of related wild diploid lines of *Triticum* and *Aegilops* (Gianibelli et al 2001R[1], Shewry 2003R). The genome from hexaploids wheats results in a complex pattern of proteins that contribute to the unique breadmaking properties of these wheats.

2.2. Wheat proteins.

Wheat is unique because its proteins are the only ones with the ability to form a dough with the rheological properties required for the production of light leavened bread (Gianibelli et al 2001R). These unique mechanical characteristics are a combination of precisely balanced elasticity and viscosity (Shewry et al 2003R).

Wheat proteins have the property that when flour is mixed with water, they form gluten, a network that gives the dough the rheological properties required for the production of leavened bread (MacRitchie 1992R, Shewry and Halford 2002). Recent studies support the concept that the endosperm proteins are responsible for the network structure of dough (Li et al 2003).

Wheat grains obtained from plants grown under normal conditions have a protein content of 8-16% depending on wheat class (MacRitchie 1992R). More than 1300 different polypeptides have been found in the endosperm proteins (Skylas et al 2000).

---

[1] R: review article

The properties of flours are largely determined by the wheat genotypes. In addition, environmental conditions during wheat plant growth and even post-harvest can affect the flour properties, although these influences are not as important as genetic ones (MacRitchie 1992R).

Proteins in this complex mixture vary in their proportions, structures and properties. The variation is both within and between genotypes. In fact, in a recent review Shewry et al (2003R) stated that "the extent of the complexity has never been conclusively established".

2.2.1 Nomenclature and classification

The fact that wheat endosperm proteins are among the most extensively studied of any proteins, in addition to the complexity of the gluten system, means that nomenclature and classification of these proteins is an almost impenetrable puzzle to the non-specialist (Gianibelli et al 2001R, Shewry 2003R).

The first reported studies of wheat proteins were by Jacopo Beccari, University of Bologna, in 1745 (Gianibelli et al 2001R, Shewry 2003R, Shewry et al 2003R). Other researchers studied solubility and properties of gluten in the following 150 years (Shewry 2003R). But it was not until the 20th century that plant proteins were studied systematically, for the first time, by Osborne (Osborne 1924). In 1907 he developed a classification based on sequential extraction in different solvents. Proteins in the first extract, which were soluble in water and dilute buffers, were named albumins. Those not soluble in water but soluble in saline solutions were named globulins. Prolamins were

those which were soluble in 70% ethanol, while glutelins were those soluble in dilute acid or alkali (MacRitchie 1992R, Gianibelli et al 2001R, Shewry 2003R). Wheat prolamins, as defined by Osborne, are named gliadins, while glutelins are named glutenins. Further extraction studies resulted in the division of glutelins into two fractions: one soluble in 0.05 M acetic acid ("soluble glutenins") and the other insoluble ("insoluble glutenins") (Chen and Bushuk 1970). The durability of this classification can be explained by the great differences in the biophysical properties, which are linked to functionality. Elasticity is associated with the glutenins and extensibility with the gliadins (Shewry 2003R).

A novel classification based on the amino acid sequences and genetic background was proposed in the early 1980s (Shewry et al 1986). This classification divided prolamins into three groups. The first group includes the glutenins of high molecular weight. The second group is formed by S-rich (sulphur-rich) prolamins and includes some glutenins (B- and C-types) and some gliadins (α/β- and γ- types). The rest of the gluten forming proteins (D-type glutenins and γ-gliadins) constitute the S-poor prolamins group (Shewry et al 1986). Although these divisions are becoming widely accepted, classical classification is still the most widely used and will be used in this text.

2.2.2. Glutenins

Glutenins are among the largest protein molecules in nature, their apparent molecular weight, measured by gel filtration and flow field fractionation studies, may be over 20 million. However, it should be remarked that these measurements were done using globular compacted proteins as standards. Since glutenins generally are not compactly folded, this method tends to overestimate the molecular weight (Gianibelli et al 2001R).

In nature, glutenins are heterogeneous mixtures of polymers formed of polypeptides linked by disulfide bonds. These polypeptides are divided into four groups according to their electrophoretic mobility in SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis), run in reduced conditions. The A-group, also named high molecular weight glutenin subunits (HMW-GS) (Payne et al 1981), has an apparent molecular weight ranging from 80,000 to 120,000. The B-group (molecular weights of 42,000 to 51,000) and C-group (30,000 to 40,000) are distantly related to α- and γ-gliadins, while the D-group is related to the ω-gliadins. The last three groups are named low molecular weight glutenin subunits (LMW-GS). LMW-GS are present in a much higher concentration than HMW-GS, higher by a factor of about 3. Polypeptides from all of these groups are post-translationally linked by disulfide bonds to form the heterogeneous aggregates known as polymeric glutenins (Gianibelli et al 2001R).

2.2.2.1. High molecular weight glutenins

Although a key factor in gluten properties, HMW-GS are actually minor components (Gianibelli et al 2001R). This protein fraction is responsible for 45 to 70% of the variation in breadmaking performance of European wheats. At the same time, just about 12% of the total grain proteins (1-1.7% of the flour dry weight) are high molecular weight glutenins (Shewry et al 2003R).

HMW-GS are encoded at the *Glu-1* loci on the long arms of group 1 chromosomes (1A, 1B and 1D). Each locus codes two polypeptides: x- and y-type subunits, with x-type being the larger. These polypeptides are tightly linked (Shewry et al 2003R). There is an appreciable polymorphism in the number and mobility of HMW-GS. The first catalogue of

alleles was published in 1983, with 3 alleles at the *Glu-1A*, 11 alleles at the *Glu-1B* and 6 alleles at the *Glu-1D* (Gianibelli et al 2001R). More have been identified since then. A list of the published alleles is presented in Table 2.1. With three genomes, and two polypeptides coded by each one, in theory a wheat cultivar could have six different HMW-GS (Shewry et al 2003R).

The nomenclature used for the HMW-GS was originally based on the assignment of ascending numbers related to the mobility in SDS-PAGE: lower numbers equating to lower mobility. Then, new subunits were identified, and new numbers were assigned. In addition, some subunits with the same electrophoretic mobility in SDS-PAGE have minor differences. For example, it has been possible to identify one subunit with the same mobility as 7, but with slight differences which was named 7*. Therefore, actual nomenclature does not follow any logic in size or function (Gianibelli et al 2001R, MacRitchie 1992R).

Several subunits have been closely associated with breadmaking quality. Payne et al (1981) showed that some allelic subunits imparted differential effects on gluten quality. A clear example is the allelic variation at the Glu-D1 locus. The 5+10 allele is associated with better quality than 2+12 allele. Payne et al (1987) developed a score in which individual subunits were graded with numbers based on quality results (Table 1). This score was based on a study done with a specific cross, but it was not applicable to any set of samples (Gianibelli et al 2001R).

Table 2.1. List of alleles at Glu-1 (Payne et al 1981, Gianibelli et al 2001R, Shewry et al 2003R).

Glu-A1	*Glu-B1*	*Glu-D1*
1	6+8	2+10
1'	7	2+11
2^*_1	7+8	2+12
2*	7+9	2.2+12
2^*_2	13+16	2.2*+12
2.1*	13+19	3+12
	14+15	4+12
	17+18	5+10
	20	5*+12
	21	
	22	

2.2.2.2. Low molecular weight glutenins

One third of the total seed proteins and about 80% of the glutenins are LMW-GS. Despite their amount, they have not been studied as much as HMW-GS, mostly because they do not seem to be as important (Gianibelli et al 2001R).

The genes *Glu-A3*, *Glu-B3* and *Glu-D3*, located on the short arms of the chromosomes 1AS, 1BS and 1DS respectively, control the LMW-GS. These genes are all polymorphic. There are at least six alleles for the *Glu-A3*, nine for the *Glu-B3* and five for the *Glu-D3*. (Gupta and Shepherd 1990).

2.2.2.3. Polymeric glutenins.

In the wheat grain endosperm, a mixture of polypeptides are held together by disulfide bonds (polymeric proteins), forming the protein matrix between the starch granules. This

matrix forms the structure that support gas bubbles in dough. Their exact structure is unknown, mostly because their size (the molecular weight is over 10 million) makes them difficult to study (Gianibelli et al 2001R, Shewry et al 2003R).

The structure of the polymeric molecules is responsible for their functionality (MacRitchie 1992R). Several models have been proposed to explain their structure. Even though at present there is controversy on some details, there is a general agreement that the polymers containing LMW-GS and HMW-GS are formed in a random or quasi-random manner (MacRitchie and Lafiandra 1997 cited by Gianibelli et al 2001R). Recent nuclear magnetic resonance (NMR) and Fourier transform infra-red (FTIR) studies have also indicated an important role for hydrogen bonding among different glutenins (Belton et al 1995). This fact is clearly favoured by the high content of glutamine residues.

The amount of HMW-GS polymers is essential in determining dough quality. The size distribution of the polymers is very important as well (Southan and MacRitchie 1999). In fact, when differences in the influence on gluten functionality are observed among flours with different HMW-GS, it may be that the differences should be attributed to polymerization tendency rather than just their composition (Shewry et al 2003R). There has been a recent effort to polymerize glutenins *in vitro* (Verbruggen et al 2003). It was observed that both oxygen and potassium iodate were very efficient on a small scale, but the polymers obtained had a dough weakening effect. No well-defined reason to explain this observation was given.

In order to better understand the relationship between polymeric glutenins and quality, it is necessary to understand more about polymer science. Size distribution is very

important as a critical principle governing the physical properties of synthetic polymers (Southan and MacRitchie 1999). Determinations of molecular weight distributions are difficult because these proteins are insoluble, heterogeneous and very large (MacRitchie 1992R). Therefore, several methods have been used to determine the size distribution of gluten polymers. Initially gel filtration, and then size exclusion high performance liquid chromatography (SE-HPLC) were the most widely used methods. These technologies clearly distinguish among the three most important groups to be separated by molecular weight: glutenin, gliadins and albumin-globulins. Results obtained with SE-HPLC have been clearly correlated with quality characteristics. Multistacking SDS gel electrophoresis, flow field-flow fractionation (FFF), agarose gel electrophoresis and light scattering techniques have proved to be suitable as well (Gianibelli et al 2001R).

2.2.3. Gliadins

Gliadins are monomers, and form a heterogeneous mixture of polypeptides soluble in 70% aqueous alcohol. Gliadins can be divided into four groups: α-, β-, γ- and ω-gliadins, according to their mobility in acid-PAGE (A-PAGE), which is greatest for α-gliadins. This classification of gliadins is useful since these are valid groups in terms of their structural and genetic relationships (Shewry 2003R).

It is generally understood that allelic variations of these proteins may not have a direct effect on dough strength. Although some findings have been reported, the effects have been attributed to the linkage with LMW-GS genes. At the same time, gliadins are considered to contribute to gluten viscosity as plasticizing elements, and to gluten extensibility (Gianibelli et al 2001R).

2.2.4. Other endosperm proteins

Globulins and albumins are also present in the endosperm, but they have little or no link to breadmaking quality. Most of them have metabolic functions as enzymes or enzyme regulators (Gianibelli et al 2001R). Their molecular weights are lower than those of the gliadins. Among the polymeric forms, triticins are tetrameric proteins which behave as globulins (Shewry et al 1986).

2.2.5. Non protein components

There is a flour fraction, usually called water unextractable solids, which consists to a large extent of pentonsans (Wang et al 2002). At the same time, there is an important fraction of pentosans that are soluble in water. Both soluble and unextractable pentosans have been shown to have a negative effect on gluten yield, and poor quality flours to have higher amounts of water-soluble pentosans. Addition of pentosans causes an increased resistance to extension and decreased extensibility. The effect of pentosans has been shown to be affected by genotype (Wang et al 2002, 2003, 2004a, b, 2005).

In addition, some starch-related variables like starch damage (Dexter et al 1985) and amylopectin/amylose ratio are known to affect breadmaking properties (Mee Ryung Lee et al 2001).

2.3. Rheological properties

Rheology is the science that studies the relationships between stress, strain and time during the deformation of materials (MacRitchie 1992R, Menjivar 1990). According to Steffe (1996), "dough is probably the most complex material facing the food rheologist",

and according to Uthayakumaran et al (2000) "there does not appear to be any literature on such a complex material". The complexity of dough rheology is remarkable, and it has been studied for centuries, even before the term rheology was proposed. Rheological research has received more impetus over the past 70 years (Faubion and Hoseney 1990), but still there are still many unanswered questions.

Among the different sciences needed to understand breadmaking, probably rheology is the most important; but a theory of dough rheology is not a theory of breadmaking quality, Good mechanical properties alone are not sufficient to obtain a good bread. However, appropriate dough mechanical properties are essential (Shewry et al 2003R). Wheat is unique because the flour dough viscoelastic properties allow the formation of the sponge-like structure in baked bread (Kokelaar et al 1996). Among these unique wheat dough properties, the ability to form a dough able to retain gas is the one that leads to bread formation. This is possible because of the capacity of dough to be stretched into thin membranes (Bloksma and Bushuk 1988).

Dough rheological properties are critical for two reasons. First, they determine the behaviour of dough pieces during the industrial process (mixing, dividing, rounding, moulding and baking). Dough consistency control is a prerequisite for automation and mechanization in the baking industries. Secondly, the quality of the bread produced is largely defined by them (Bloksma and Bushuk 1988).

The gluten network is a complex system in itself but also its properties are influenced by a high amount of filler (starch) and a diluent (water). Rheological properties are also

highly dependent on dough history, including mixing, moulding and relaxation (MacRitchie 1992R).

The complexity of dough rheology is extended to the analytical procedures. They are difficult to design and difficult to execute (Faubion and Hoseney 1990). Moreover, studies on dough rheology do not include only rheology but also colloid science, polymer science, cereal chemistry and fluid mechanics (Menjivar 1990).

Some authors have proposed studying dough rheology using both gluten and flour dough (Kokelaar et al 1996). In the case of dough, it is obviously because it is the actual material that acts during breadmaking. However, at the end of the fermentation stage dough will consist of films that will be thinner that the starch granules and gluten rheology will be the determinant. Therefore, the deformability of the dough will be determined almost exclusively by the gluten network. Most studies are done using dough without yeast. Since the fermentation stage is the key moment to determine the properties of the bread loaf, it is common sense to work with fermenting dough as well. The problem is that rheological properties become nearly impossible to determine during fermentation since the geometry of the test piece changes in an uncontrolled manner. In addition, there is a continuous relaxation process, making interpretation of data very difficult (Faubion and Hoseney 1990).

There has been extensive discussion of the actual rheological property that should be subjected to study. For some authors, the relevant rheology for breadmaking is the extensional behaviour of the dough during mixing rather than the shear properties (Gras et al 2000, Jansen et al 1996, Safari-Ardi and Phan-Thien 1998). Another approach to

study extensional properties is the use of small deformation dynamic shear data. Although fundamental rheology predicts that this is possible in theory, practical experience has shown that results are not always applicable (Dobraszcyk et al 2003). Gras et al (2000) proposed that rested dough rheology is related to the mixing dough rheology. They based this hypothesis on several arguments. The first was that the resting may be suitable only to remove the short term transient dough rheological behavior, and the second that rested dough is representative of the average rheology of a dough at the stage at which is sampled. Finally, if the resting causes some kind of bias, that it will be consistent for all samples. The authors were able to confirm these statements.

2.3.1. Empirical rheology

Empirical rheology has several advantages over fundamental rheology and empirical methods have been researched for over 70 years. Empirical methods are usually easy and fast to perform, making them practical to use, and in addition, equipment is cheaper, and the methods easier to implement. The disadvantages are mainly those caused by the impossibility of describing results in terms of fundamental rheological properties, since the methods do not involve the measurement of well-defined physical quantities but are limited to providing empirical correlations (Menjivar 1990).

2.3.1.1. Standard empirical rheological equipment

Many types of equipment have been developed for empirical rheological testing. These characterize the flour by preparing dough using standard conditions and determining variables which are correlated with breadmaking properties. These tests can be divided

in two groups. The first one studies mixing properties by measuring such factors as mixing developing time or water absorption. The most commonly used testing machines are the Brabender farinograph and the mixograph. The second group determines extensional properties like resistance to extension and extensibility. Machines most widely used are the Brabender extensigraph and the Chopin alveograph.

The Brabender farinograph (Brabender OHG, Duisburg, Germany) mixes flour and water using two Z-shaped mixing blades that rotate at different speeds in opposite directions. A dynamometer is used to record torque of the mixing blades as the water is starting to be added from a titrating buret. A plot, named a farinogram, is obtained by plotting a parameter proportional to torque, expressed as Brabender units (BU) or "consistency", versus time. The amount of water added, defined as water absorption, is adjusted to reach a maximum resistance of 500 BU. Parameters such as development time, water absorption, stability of the consistency upon time, mixing tolerance and degree of softening can be obtained from the farinogram (Miller et al 1956, Steffe 1996).

The mixograph (National Manufacturing Division, TMCO, Lincoln, NE, USA) is an alternative to the farinograph. It involves a planetary rotation of vertical pins above stationary vertical pins attached to the mixing bowl. Unlike the farinograph, the mixograph uses a fixed amount of water or one adjusted according to the protein content. Torque is recorded in a "mixogram", which is analogous to the farinogram (Dong et al 1992, Hoseney 1985, Miller et al 1956, Steffe 1996). Besides its standardised use, the mixograph can be viewed as a series of extension tests. Elongation deformations, with rupture relax cycles, are the most important deformations of dough during mixograph mixing (Gras et al 2000).

Both farinograph and mixograph measure similar characteristics, and results are highly correlated, but their different geometrical configurations and water use give results that are not always interchangeable. For example, it has been observed that some doughs with identical farinograph data showed different mixograph peak development times (Safari-Ardi and Phan-Thien 1998).

The Brabender extensigraph (Brabender OHG, Duisburg, Germany) moulds dough prepared with the farinograph into a cylindrical shape, which is then clamped horizontally into a support system. A hook stretches the specimen at the middle of the sample, moving downward at constant speed, while the force needed to move the hook is recorded. The results are given as an extensigram: a plot of force versus time. Parameters obtained are the energy used to break the dough piece (proportional to the extensigram area), maximum resistance to extension and extensibility, and the length of the piece at the breaking point (Mailhot and Patton 1988, Steffe 1996).

The alveograph (Tripette et Renaud, France) also measures the extensional properties of dough. The dough is prepared in a specific mixer, with constant water absorption and mixing time. A circular dough disk is cut and clamped. The disk is inflated by air flowing through the base plate to form a spherically shaped bubble. The air pressure in the bubble is recorded over time until the bubble ruptures. The curve obtained is an alveogram. The maximum height (P), curve length (L) and area under the curve (W) are the primary parameters taken from the alveogram (Steffe 1996). Bloksma (1957) proposed that there is no reason to characterize an alveogram by means of the maximum height of the curve. The author proposed that the curve height at any volume

is a better measure. Almost 50 years later, maximum height is still widely used (Steffe 1996). The alveograph could be used to estimate biaxial extensional viscosity. Even though it has proved to be useful to characterize flour properties, its geometry makes it too complex to explain the results by simple rheological models (Launay et al 1977).

2.3.1.2. Texture analyzer

Micro-extensibility tests have been proven to be useful for research. These tests have used different types of equipment, but they are mostly equivalent to the extensigraph procedure. Equipment used has included the Instron Universal Tester Type 1122 (Canton, MA, USA), TA-XT2 Texture Analyser (Stable Micro Systems, UK) and "Micro-extension tester" (Anderssen et al 2004, Basman et al 2002, Békés et al 2003, Grausgruber et al 2002, Ingelin and Lukow 1998, Kieffer et al 1998, Purcell et al 2000, Schlichting et al 1996, Suchy et al 2000, Tronsmo et al 2003c, Uthayakumaran et al 1999, Wieser et al 2000). Recent developments have led to more sophisticated procedures. For example, TA.XTPlus (Stable Micro Systems Ltd, Godalming, UK) is able to work at constant strain rate (Dobraszcyk et al 2003).

Sample preparation has differed among researchers. The use of the farinograph (Antes and Wieser 2001, Suchy et al 2000), mixograph (Ingelin and Lukow 1998, Purcell et al 2000) and even a household mixer (Grausgruber et al 2002) have proven to be useful for dough preparation. Some authors have used fixed water absorption, while others have adjusted the amount of water according to farinograph or mixograph absorption (Ingelin and Lukow 1998, Suchy et al 2000). All have been used to differentiate materials (Suchy et al 2000). Sodium chloride was added in some studies (Ingelin and Lukow

1998, Grausgruber et al 2002). The Glutomatic (Perten, Sweden) gluten washing machine has also been used to prepare gluten (Kieffer et al 1998). Different dough resting times at 30°C after mixing have been compared. The most reproducible results were obtained at 45 minutes rest time (Suchy et al 2000).

Kieffer et al (1981) developed a micro-scale method to measure extensibility in dough, which was later modified to make it suitable for both gluten and dough extensibility studies (Kieffer et al 1998). They developed the micro-extensibility device that has been most widely used in tests, the Kieffer Dough and Gluten Extensibility Rig. Similar devices have been used by other researchers (Békés et al 2003).

Several parameters may be taken from a micro-extension test curve. They include resistance to extension, extensibility and the area under the curve. The most important estimator of resistance to extension is at the maximum. Several extensibilities can be measured, including those at maximum resistance or at rupture. Other parameters have been proposed using differences or ratios of the above mentioned values (Anderssen et al 2004). The area under the curve is an estimator of the energy needed to break the tested dough piece.

Different results obtained with these methods suggest that dough extensibility has other components than gluten extensibility. It had been reported that some wheat flours have abnormal gluten extensibility behavior when compared with their dough extensibility behavior (Kieffer et al 1998). Adding low molecular weight dextrans has been shown to increase dough resistance to extension but reduce extensibility (Ingelin and Lukow 1998).

Results obtained with micro-extension tests have been compared to those obtained with standard techniques. Rank correlation analyses presented high correlation coefficients. When the Brabender extensigraph was compared with a texture analyzer equipped with the Kieffer Rig, the maximum resistance to extension was better correlated (r=0.92; significance not reported) than the extension at rupture (r=0.63; significance not reported) (Grausgruber et al 2002).

Dough extensional methods based on texture analyzers have been proposed for use in breeding program screening (Békés et al 2001, Anderssen et al 2004). Grausgruber et al (2002) estimated that more than 20 samples could be tested a day. This is four times the amount usually tested on a Brabender extensigraph.

The ability to test wheat doughs with micro-extension tests has dramatically widened the scope of potential experimental approaches. Micro-extension tests give a reliable prediction of bread quality (Kieffer et al 1998, Suchy et al 2000, Wieser and Kieffer 2001), and are useful to study the influence on extensional properties of gluten components (Antes and Weiser 2001, Purcell et al 2000, Uthayakumaran et al 1999, Wieser et al 2000) or food additives (Basman et al 2002, Kieffer et al 1981). These tests have also been used to develop rheological dough models (Gras et al 2000). Texture analyzers have also been applied to analyze other kinds of properties, such as cohesiveness, adhesiveness and gumminess, but these properties have not been studied to the same extent as extensional ones (Martínez-Anaya and Jiménez 1998, Armero and Collar 1997). The technique has proven useful in research on the relationships between the flour molecular composition and the measured parameters of

end-use quality (Grausberger et al 2003, Tamás et al 2002, Uthayakumaran and Lukow 2003).

2.3.2. Mixing

The first step of breadmaking is mixing or "dough developing". At the macroscopic level flour and water become a coherent mass, acquiring viscoelastic properties. After a certain mixing time, the resistance to deformation reaches a maximum. At this point, named optimum mixing time, dough can be stretched into a thin sheet. After this maximum, further mixing causes a progressive decrease in resistance, loss of elasticity and increase in dough stickiness. All these features are undesirable for bakery production. Therefore, the action is called overmixing and the step is called dough breakdown (MacRitchie 1992R).

During mixing, most of the water is absorbed by the flour, but the rest forms a separate liquid phase, dispersed throughout the dough. Proteins start as discrete particles and are converted into the strands that form a network which supports the dough. Air is inserted into the dough to form the gas cells that will expand during fermentation. Number, size distribution and stability of gas cells, which are highly dependent on the surface-active compounds, will determine the texture and volume of the bread loaf (MacRitchie 1992R).

The development of the dough forms a continuous network at macroscopic level, but at the molecular level glutenin polymeric molecules are extended by the shear and tensile stresses. During mixing, there is a continuous extension of the molecules by the frictional forces, while a retractive mechanism is effective at the same time. This retraction is led by the entropic restoring forces, which tend to return the molecules to

their energetically favoured configurations. After a certain mixing time, if the energy is enough, the rate of extension equals the rate of retraction, reaching a steady state. If the energy is not enough, the degree of extension is insufficient to give optimum dough properties for a strong dough (MacRitchie 1992R).

After certain mixing time, consistency starts to decay. The longer the time the dough retains a certain consistency, the better is the flour for the baker. This is called mixing stability. Dough breakdown can be explained at the molecular level. At the optimum state, protein strands form a continuous network supported by entanglements and or covalent disulfide bonds. Mixing after that point may break one or both types of bonds (MacRitchie 1992R).

Water absorption is the amount of water required to yield a dough of optimum consistency. It is influenced by several factors. The gluten portion of flour has a water-imbibing capacity of about 2.8 times. Starch is an important factor also, but the key value is not the amount of starch but the amount of starch damage. Minor constituents, particularly pentonsans, are important (Mailhot and Patton 1988, Dong et al 1992).

2.3.3. Dough strength

Usually, the most important flour specification is “strength”. This term has had several meanings. Originally, it was applied to the resistance that a dough must have in order to withstand the action during breadmaking (Wooding et al 1999). Usually, flour strength has been synonymous with flour quality (Mailhot and Patton 1988), but it also has been used for high protein flours and good mixing properties among others (Tipples et al

1982). Another proposed measurement of strength is the maximum resistance to extension in an extension test (Wrigley 1994). Williams et al (1988) proposed use of the term "functionality". This is a better descriptor than "strength" when we try to describe the end-product utilization of dough or gluten. In this thesis, the original meaning will be applied.

Gluten strength is important in determining flour suitability for breadmaking, but it is not the only factor. Strength determines mixing properties and loaf shape, but extensibility of flour dough plays an essential role in the determination of the final loaf volume and bread properties. Equilibrium between dough strength and extensibility is necessary to obtain high quality bread (Faergestad et al 2000).

2.3.4. Extensibility

Based on fundamental rheology, there are two different kinds of flow: shear and extensional. In shear flow molecules orientate to the direction of flow, but the presence of a differential velocity across the flow field encourages molecules to rotate, reducing the degree of stretching. In extensional flow, the molecules are also oriented, but they are not rotating. Therefore, flow will induce the maximum stretching of the molecules. This force produces a chain tension that may result in an important resistance to deformation. This is the case for many food systems, including dough processing. Pure extensional flow does not involve shear deformation (Steffe 1996).

There are basically three types of extensional flow: uniaxial, planar and biaxial. If the material is stretched in one direction with a corresponding size reduction in the other two

directions this is termed uniaxial extension. If material is stretched in one direction, with a corresponding decrease in thickness keeping the width constant it is termed planar. Biaxial extension results when there is a uniaxial compression with a radial tensile stress (Steffe 1996). This is the relevant type of deformation in breadmaking which occurs around an expanding gas bubble during fermentation and during the first step in the oven (Kokelaar et al 1996). Uniaxial deformation is present as well during mixing and rolling (Sliwinski et al 2004a).

In dough rheology studies, extensibility or fracture strain is defined as the elongation at break at a set tensile stress (MacRitchie 1992R). The longer the dough is extended without breaking, the higher the extensibility. Extensibility is not an absolute measurement. Moreover, it depends on the method of preparation and state of dough development (MacRitchie 1992R).

Extensibility is important for the expansion of the dough to achieve high loaf volume, but a very large extensibility affects the bread quality negatively (Faergestad et al 2000). Therefore, it has been proposed that there is an optimum range of extensibility values. Mixing methods also give an indirect measurement of the extensibility (Gras et al 2000).

Although Brabender extensigraph, Chopin alveograph and, more recently, texture analysers have been used to determine it, extensibility data has been difficult to obtain with high reproducibility. This has been for several reasons, including the complex nature of the dough(Safari-Ardi and Phan-Thien 1998).

Recent researchers have studied the effects of different strain rates. For gluten dough, the fracture strain has been reported as largely independent of strain rate. In contrast, for flour dough the fracture strain decreased when strain rate decreased. It was proposed that this difference was caused by the difference in starch content (Sliwinsky et al 2004b).

2.3.5. Strain hardening

The stress required to deform gluten increases with increasing strain at comparable strain rates. This phenomenon is known as "strain-hardening". Applying this concept to flour dough, thinner regions of the dough will have more resistance to extension than thicker regions. As a result thicker films around the smaller cells will be extended during the dough rising process, leading to a more homogeneous foam system (Kokelaar et al 1996; Van Vliet et al 1992).

The fact that extension rates used for extensional studies are not similar has been discussed by several authors (Bloksma 1990a, b). Recent studies proved that even 10^{-4}-10^{-3}/sec is the macroscopic extension rate during the proofing period but on a microscopic level extension rates are in the order of 10^{1}-10^{2}/sec. This observation is crucial for rheological properties studies (Weegels et al 2003).

2.3.6. Molecular model

The mechanical behaviour of dough has been extensively studied. Shewry et al (2003R) proposed some observations that are the basis of the present model that is most widely accepted. First, dough is viscoelastic. Second, dough behaviour depends on water content and HMW-GS concentration. Another important observation is that D_2O

produces a stronger dough than H_2O. Glutamine residues etherification reduces the resistance to extension of doughs. Resistance to extension reaches a maximum on mixing. Finally, it has been clearly established that both oxidants and reducing agents affect dough rheology.

In order to explain gluten mechanical properties, several models have been suggested. Recent proposals include the "trains and loops" theory (Gianibelli et al 2001R, Shewry et al 2003R). Dough could be assumed as a protein matrix in which starch granules are embedded. The matrix is formed by cross-linked polymeric proteins with non-covalent links. As was mentioned earlier, both HMW-GS and LMW-GS form the network, with the function of LMW-GS not clear, but it is likely that they act both as chain terminators and as extenders. Most disulphide bonds are end-to-end, but some branches also occur. The high level of glutamine residues in the repetitive domain gives a very high capacity to form both intra- and intermolecular hydrogen bonds. Therefore, between the disulfide cross-links, the repetitive domains interact by hydrogen bonding, with HMW-GS interactions tending to increase the cohesiveness of the dough. In the dry state, the inter-chain interactions are expected to be strong due to very favourable hydrogen bonding. In the dough, there may be two different situations. On the one hand, a situation occurs similar to the dry state, with a very strong link between two different glutenin strands. In the other situation, water molecules may be inserted between two glutenin chains and form hydrogen bonds, pulling apart the polymeric proteins. In this way, there are two clearly defined regions: unbonded mobile regions (loops) and bonded regions (trains). According to this theory, mixing is needed to extend glutenins molecules, in order to improve their interactions. Trains and loops theory has been successfully applied to adsorption of polymers at interfaces, but this was the first case in

bulk systems (Gianibelli et al 2001R). This theory has been supported by recent proton magic angle spinning NMR (nuclear magnetic resonance), where two different glutamine residues were observed: one was assigned to "loops" and the other to "trains".

The model explains dough extensional behaviour. When mechanical extension is applied to the gluten network, firstly the loops are extended. This movement requires little energy. Further extension will break the trains, with a higher energy requirement. Excessive extension will result in breakage of the inter-molecular disulphide bonds. If the extension is not as big as is needed to break the disulphide bonds and the extending force is relaxed, there will be a tendency to restore the loops and trains with a similar balance to the initial one (Shewry et al 2003R). In addition, both extension and elastic recovery (i.e., viscoelasticity) are explained by this model.

An increase in the water concentration will change the chains/loops ratio, causing a change in the extensional properties (Shewry et al 2003R). The importance of the HMW-GS concentration is also clarified by this model. The low concentration of the main factor (HMW-GS) in the rheological behavior of the body (dough) is not uncommon among different food systems. Since deuterium forms stronger hydrogen bonds than hydrogen atoms, train and loops model also explain the difference in the response between D_2O and H_2O. Since oxidizing and reducing agents increase and decrease disulphide bonds respectively, the effect of both are also coherent with the proposed model (Shewry et al 2003R).

Water plays a lubricating role, because the elongation of a dough sample will increase when the amount of water increases (Gras et al 2000). According to the trains and loops

theory, increasing the water content will increase the loop/:train ratio. Therefore, by increasing water content, the degree of extension to break will increase (Shewry et al 2003R).

Previous theories that modeled the gluten network failed to explain mechanical properties with this level of detail. For example, one hypothesis stated that glutenin molecules were arranged in chains of folded units. Stretching these units unfolded the polymers. This model explained the elasticity behavior, but fails to explain most observations, such as the water addition effect.

Application of polymer science has not been successful either, although many authors still support this (Singh and MacRitchie 2001). In this theory, entanglements cause the resistance to extension. The approach is nonspecific and it does not explain why no other polymer of similar size has the same behavior (Shewry et al 2003R). Nevertheless, entanglements may play a complementary role with the "trains and loops".

Despite the considerable research on the fundamental basis of the stress-strain relationships for polymers, due to the importance they have in the industry, these relationships are not yet well understood. It is generally established that lineal polymeric properties are determined by the molecular weight and its distribution. Entanglements are the basis of this kind of polymer properties. It was proposed that there is a molecular weight below which molecules do not participate in entanglements. This is called threshold molecular weight. However, this proven fact for synthetic polymers has not been proven in the case of polydisperse polymers (MacRitchie 1992R, Shewry et al 2003R).

Applying the trains and loops model, as more polymers are extended, the possibilities for further extension will decrease. Therefore, according to this model, the most important factor controlling the extensibility of the gluten network will be the number of extended glutenin polymers.

2.3.7. Relationship of gluten composition to quality

There are many ways to study the composition/functionality relationships. One frequently used is to design experiments based on fractionation and reconstitution studies. In these studies, the separated fractions may be added to flours, and the behaviour of the flour with and without the fraction compared. Different combination of fractions can also be used. Another strategy is to select a set of samples, run different tests and apply statistical procedures in order to explain functional properties by composition. These procedures are useful to study qualitative and environmental effects. A third group of available techniques used to improve knowledge of the composition/functionality relationship includes the use of genetic variants such as the use of isogenic lines (MacRitchie 1992R, Shewry et al 2003R).

Fractionation procedures have proved to be very useful in determining the functionality of different gluten fractions. However, the extraction procedures should be done without modifying the original functionality. For example, it has been proven that the classical alcohol extraction proposed by Osborne can irreversibly change the polymeric glutenin fraction. Using fractionation, several authors have observed that glutenins extracted from good or poor quality wheats have different properties. The acid insoluble glutenins

fraction showed a positive relationship with dough mixing strength. Glutenin to gliadin ratio was positively related with the mixing time and dough mixing tolerance. It also has been possible to clarify the viscoelastic solid-like behavior of glutenins and the elasto-viscous liquid-like behavior of gliadins. In addition, relationships with fundamental rheological characteristics have been established as well. By increasing glutenin content, both storage (G') and loss moduli (G") were shown to increase, while tangent δ (G"/G' ratio) decreased. These kinds of experiments are easier with gliadins, since they are readily incorporated into solution. Other experiments proved that gliadins cause a weakening effect (Shewry et al 2003R).

Fractionation is a complex procedure itself, but reconstitution should be done very carefully as well. This observation is particularly true with glutenins. They must be incorporated by covalent disulphide bonds into glutenin polymers in order to behave as native gluten (Shewry et al 2003R). There are results obtained through reconstitution using different ratios of different HMW-GS and/or LMW-GS subunits which are not consistent with results obtained using native gluten. For example, it has been demonstrated that in vitro propensity to polymerize is different for HMW-GS than LMW-GS. Shewry et al (2003R) proposed that "it may be unwise to draw conclusion about glutenin functionality in native gluten from such experiments".

2.3.7.1. Gluten components

The relationship between protein content and its components should be considered carefully. Many studies have shown that gliadin increase is the most important factor

responsible for the increase in wheat protein content (Doekes and Wennekes 1982; Gupta et al 1992; Southan and MacRitchie 1999; Ohm and Chung 1999). Simultaneously, no correlation was found between protein concentration and presence/absence of gliadin or glutenin bands (Dong et al 1992).

The importance of protein content for breadmaking quality has been proven for a long time (Finney and Barmore 1948). But, although its importance is unquestionable, it is not the only protein parameter that influences bread quality. Both quantitative and qualitative studies have been proven to be successful in relating composition to quality.Although they are the most important factors, it has been clearly established that quantitative and qualitative protein composition can explain only part of the quality variation (Dong et al 1992).

2.3.7.2. Gluten composition and strength

The total protein amount is important since the more the protein the stronger the network and therefore, the more stress that the network can support (MacRitchie 1992R). Total protein content is related to dough strength, but most importantly glutenin content and composition affect gluten strength. This is clearly explained by the proposed gluten model.

Glutenins fractionated according to their solubility in acid showed a positive relationship between the acid-insoluble fraction and dough mixing strength (Shewry et al 2003R). Fundamental rheological properties are related to the molecular weight and molecular

weight distribution of the polymer. However, gluten is a very polydisperse size distribution system, which makes it hard to characterize (Tronsmo et al 2003) and gliadin protein is responsible for weakening gluten elasticity (Ohm and Chung 1999).

Results obtained vary with the population selected to study. Dong et al (1992) found that flour protein was related to farinograph absorption ($r = 0.71$ and 0.60 respectively for two different growing years, $P<0.001$), but no correlation was found between protein content and mixing time. Therefore, the concept of strength used in this text was not related with protein amount for this particular samples set.

2.3.7.3. Gluten composition and extensibility

Strength is not the only breadmaking requirement that is related to protein amount. Moreover, it is not always stronger flour that makes better bread (Sliwinski et al 2004a). Some papers have concluded that extensibility was increased with increases in protein content (Uthayakumaran et al 1999, Faergestad et al 2000) but in other reports it has been concluded that extensibility was not affected by a change in protein from 11.2 to 15.2% (Uthayakumaran and Lukow 2003).

The complexity of gluten composition gives some idea of how difficult it is to relate it to rheological properties like extensibility. Different authors found that dough extensibility depends on different protein variables, including total gliadin content, total glutenin content, glutenins to gliadins ratio, glutenins molecular weight distribution and related variables.

Several researchers have proposed that extensibility is not related only to the total amount of gluten, but also to its fractions. In general, it is understood that gliadins contribute to dough viscosity as glutenins contribute to dough elasticity (Bloksma and Bushuk 1988, Ciaffi et al 1996, Gianibelli et al 2001R, Shewry et al 2003R, Uthayakumaran et al 1999). As previously mentioned, when protein is increased, gliadin proteins have a tendency to increase at a greater rate than other proteins. This observation may also explain the positive influence of protein concentration on extensibility (Southan and MacRitchie 1999).

The relationship between gliadins and extensibility has been demonstrated by a number of researchers. Adding different fractions of gluten, Schropp and Wieser (1996) observed that extensibility of gluten was increased by monomeric proteins (gliadins or reduced HMW-GS) and was decreased by polymeric proteins. This last observation was confirmed by Kipp et al (1996) who added reoxidized HMW-GS to standard flour, and observed a significant decrease in extensibility. In a more detailed study Uthayakumaran et al (1999) observed that an increase in the glutenin to gliadin ratio with constant protein was associated with decreased extensibility. They obtained a negative correlation between these parameters ($r = -0.69$, significance was not reported), with a linear relationship. At the same time, they observed a positive correlation of the same ratio with maximum resistance to extension ($r=0.84$, significance was not reported). A more recent study confirmed a dependence of both dough and gluten extensibility on the ratio of glutenin to gliadin (Wieser and Kieffer 2001). In a reconstitutional study, higher extensibility was obtained by adding the protein soluble fraction (Grausberger et al 2003).

More detailed studies have hypothesized that not total gliadins but a sub-group of them is the key component. Ciaffi et al (1996) studied the relationship of alveogram parameters with peaks obtained from size-exclusion high-performance liquid chromatography (SE-HPLC) of gluten proteins. They concluded that size distribution of polymeric proteins had a larger effect than the fraction amounts on dough strength, but that fractions were more related to the determination of alveogram extensibility. The hydrophobic properties of γ-gliadins were proposed as the cause of the increase in extensibility of flours. This is in agreement with a prior work of the same group (Lafiandra et al 1994, cited by Ciaffi et al 1996), in which a high correlation was observed between extensibility and the late-eluting peak of reverse-phase high-performance liquid chromatography (RP-HPLC), i.e. the most hydrophobic fraction. This peak is composed mainly of β- and γ-gliadins, encoded by genes at *Gli-1* loci. Several researchers have reported a good relationship between the amounts of certain gliadin groups determined by RP-HPLC and functionality (Lookhart et al 2003). Some authors have proposed that the gliadin contribution can be explained by the low molecular weight, while others explain it through their hydrophobicity (Shewry et al 2003R).

Addition of glutenin has been reported to have caused a decrease in extensibility due to an increase in the rupture viscosity and a lowered rupture strain during uniaxial extension (Shewry et al 2003R). However, there are some reports that may seem contradictory. Hamer and Van Vliet (2000) stated that glutenin polymeric fraction explains 90% of extensibility. Southan and MacRitchie (1999) found that the amount of flour polymeric protein was positively correlated with extensibility, and both absolute and

relative quantities of polymeric glutenin determined by SE-HPLC were positively correlated with extensibility. In a reconstitutional study, both resistance to extension and extensibility were increased when reoxidized high molecular weight glutenin subunits were added (Grausberger et al 2003). This apparent contradiction can be explained by the complexity of gluten components. It should be remarked that the molecular size distribution of gluten is bimodal, A low molecular weight group is formed mainly by monomeric proteins (largely gliadins) and a high molecular weight group by polymeric glutenins (Rao et al 2000).

Extensibility has been related to the polymeric/monomeric ratio, but its correlation with the total amount of glutenin in the flour is usually more important, explaining up to 70% of the variance. There is no question that large molecules are needed, but it is not the only factor. While it is extending, dough becomes thinner, and so do the protein strands. This process occurs until gluten no longer supports the applied stress and it breaks. Therefore, the more protein, the longer the dough can be extended. This proves the total amount of protein is of prime importance in dough extensibility. At the same time, very high molecular weights of polymeric glutenins seem to be detrimental to extensibility. For example, 5+10 causes less extensible doughs than 2+12. In the same way there is lack of extensibility in very high HMW-GS/LMW-GS ratio or low sulphur flours. These three factors are related to shifts in the molecular weight distribution of glutenin. The effect of very high molecular weight on extensibility can be explained by the theory of entanglements. In this theory, while extending, the transient cross-links due to entanglements are able to pull free, giving elongation mobility. There is a point where the density of the entanglement decreases to a minimum at which cross-links are not able to

slip free, causing a loss of mobility, or an extensibility reduction (MacRitchie 1992R). Changing the HMW-GS but keeping glutenin to gliadin ratio constant caused a minimum of changes in elongational properties (Uthayakumaran et al 2002).

The contribution of polymeric proteins was discussed by Southan and MacRitchie (1999). They concluded that the amount of polymeric glutenins affects extensibility positively, but the size affects it negatively. Therefore, to improve extensibility the total amount of polymeric glutenins should increase, but the size distribution should not favor high molecular weight. This explains the negative correlation between non-soluble polymeric proteins (high molecular weight polymers) and dough extensibility (Sapirstein and Fu 1998).

Extensional properties have not always been explained by protein composition. Using 71 genotypes grown in the same environment, Campbell et al (1987) observed no statistical correlation between gluten composition and extensibility. For the same samples resistance to extension was easily explained by the presence of certain gluten protein alleles, mostly 5+10 (high resistance) versus 2+12 (low resistance).

It has also been suggested that a very high HMW-GS/LMW-GS glutenin ratio reduces extensibility and increase resistance to extension (MacRitchie 1992R, Shewry 2003R). At the same time, recent reports have concluded that addition of HMW-GS increases extensibility more than LMW-GS (Antes and Weiser 2001). Differences in the methodology used may explain these results. Even among the HMW-GS there is variability in extensibility. Good dough extensibility has been obtained with an

appropriate ratio of x:y subunits (Butow et al 2003). Also, the influence of the HMW-GS composition on extensibility was reported by Gianibelli et al (2001R).

Therefore, we can presume that there are at least two independent factors affecting extensibility: gliadin content and polymeric glutenin content. An increase in gliadin content will contribute to extensibility. This may be due to total gliadins, or to the hydrophobic fractions. At the same time, the more polymeric glutenins present, the higher the extensibility, but the higher the molecular weight distribution, the lower the extensibility. Interactions of these factors are expected.

The effect of genotype, environment and genotype by environment interactions, have been shown to affect extensibility significantly (Fenn et al 1994). Both monomeric-to-polymeric ratio and the molecular weight distribution are genetically controlled but can be modified by environmental conditions (Southan and MacRitchie 1999). Nitrogen fertilization affects mainly protein quantity whereas sulphur affects mainly protein composition (MacRitchie 1992R), and both affect extensibility. Sulphur deficiency may have dramatic effects on molecular weight distribution, since HMW-GS are relatively sulphur-poor compared with the LMW-GS (Southan and MacRitchie 1999). It explains the fact that when sulphur is low, the resistance to extension is very high and the mixing time long (MacRitchie 1992R). Randall and Wrigley (1986) reported high correlation between sulphur content and extensibility for different varieties. Sulphur deficiency is accentuated by higher nitrogen levels (Southan and MacRitchie 1999).

2.3.7.4. Gluten composition and resistance to extension

Factors determining maximum resistance to extension are not necessarily the same ones that affect extensibility. Two factors govern maximum resistance to extension: the fraction of molecules with molecular weights above a threshold molecular weight and the molecular weight average of the molecules with a molecular weight over the threshold. This was proven when by adding glutenin, an increase in resistance to extension was observed (MacRitchie 1992R, Shewry et al 2003R).

It is important to remark that higher values of HMW GS:LMW GS are related to higher values of maximum resistance to extension. At the same time, some HMW-GS composition contribute to higher molecular weight and, therefore, to higher maximum resistance to extension. A clear example of this is the higher contribution of the 5+10 subunits over 2+12 (MacRitchie 1992R).

2.4. Bread

2.4.1. Bread quality

There are many concepts of quality, but in its broadest definition, quality is conformance to requirements (Mailhot and Patton 1988). A flour will have good quality when it conforms to measurable characteristics for a specific end use. There are many uses of wheat, with a very wide diversity of requirements, making quality difficult to define in general terms (Wieser and Kieffer 2001). Quality is becoming more important with the growing automation and mechanization of the breadmaking industry (Bloksma and Bushuk 1988), and research is needed to understand the quality factors essential to the production of desirable end products (Mailhot and Patton 1988).

Loaf volume is the most useful quality parameter, since it is considered to be a good summary of the overall characteristic of the bread (Kieffer et al 1998, Janssen et al 1996c, Ohm and Chung 1999, Faergestad et al 2000, Wang and Sun 2002, Tronsmo et al 2003a, b). Other parameters have also proved suitable. Færgestad et al (2000) worked with a type of Norwegian hearth bread. They measured the width and height of each piece and proposed to use the form ratio (height/width ratio). Janssen et al (1996a, 1996b, 1996c) defined a good quality loaf as one with high volume and a regular, fine crumb structure. In order to evaluate crumb quality, subjective scores have been used successfully.

2.4.2. Relationship of baking tests to bread type

Many products can be baked with wheat. Even considering bread alone, there are many kinds of breads, each one with its own requirements (Mailhot and Patton 1988). One flour could be ideal for one product and not suitable for another (Sliwinski et al 2004a). Using a wide variation of protein quality, Tronsmo et al (2003a) conducted research in which they concluded that "the choice of baking procedure is vital in assessing flour quality". The effects of protein functional properties are more clearly affected in hearth breads than in pan loaves (Færgestad et al 2000). Hearth bread requires a different approach, because functional properties of the proteins are more effectively distinguished than for pan loaves (Færgestad et al 2000, Tronsmo et al 2002). These bread strength requirements are also higher than those for pan bread because the latter can retain a proper shape during the baking process due to support of the pan walls (Sliwinski et al 2004a).

Extensibility and resistance to extension requirements are particularily dependant on bread type. Mailhot and Patton (1988) proposed that pan bread requires "medium", while hearth bread requires "medium to long" extensibility. Similarly, the same authors proposed "medium" resistance to extension for pan bread and "maximum" for hearth bread as basic requirements. In a more recent research, Færgestad et al (2000) observed a high correlation between hearth loaf volume and extensibility ($r=0.72$, $P<0.01$), but no significant correlation between pan loaf volume and extensibility.

Baking parameters such as temperature and time affect baking results. With increased proofing time, volume increases and form ratio decreases (Færgestad et al 2000). Roels et al (1993) found that with increased baking absorption and mixing time, there was no optimum combination resulting in a superior bread loaf. Bread volume increased with longer mixing times and higher added water. The limiting factor was handling properties. Limitations may be solved by adjusting the breadmaking processes (increasing proofing time, adjusting mixing time), but adjustments cannot always be extended to commercial bakeries because there may be limitations due to costs and production methods (Faergestad et al 2000).

2.4.3. Rheological requirements for optimum bread quality

Empirical dough rheological tests have been developed to predict breadmaking characteristics. Mixing properties have long been studied and large deformation rheological measurements have been extensively used to predict breadmaking

performance. The results remain empirical and it is difficult to interpret most of them (Tronsmo et al 2003b).

2.4.3.1. Mixing properties

Mixing properties have been proposed as the key component of breadmaking quality. A recent study related loaf volume with mixograph results for 72 samples (Ohm and Chung 1999). They observed a high correlation of mixograph peak height and band width with loaf volume ($r=0.64$ and 0.51, respectively; $P<0.01$). The correlation was not significant for mixograph mixing time. It should be noted that the correlation coefficient between flour protein and loaf volume was higher than the mixograph results for the same set of samples ($r=0.77$, $P<0.01$). Sliwinski et al (2004a) observed a significant but weak correlation between loaf volume and dough mixing time ($r=0.61$). This was in a set of 8 samples with a high correlation between protein content and loaf volume ($r=0.92$). They concluded that long mixing flours do not necessarily give better bread properties.

2.4.3.2. Extensional properties

The relationship between micro-scale extension methods and three different baking tests was studied using two different set of samples ($n=12$ and 13) by Kieffer et al (1998). Results were clearly different for the two sample sets. For the set of German wheats, gluten maximum resistance to extension was the best predictor of loaf volume (the correlation coefficient, r, varied from 0.73 to 0.83 for different baking tests). Flour dough maximum resistance to extension was also significantly correlated with loaf volume ($r=0.71$-0.80). For the set of international wheats, correlation coefficients of the same parameters varied from those having no significance to 0.77. The use of the ratio

maximum resistance to extension to extensibility did not improve correlations. Results with extensibility alone were not reported.

Anderssen et al (2004) stated that there is general agreement about the link between extension testing and baking performance: good volume is achieved if gas bubbles rise during both proofing and baking. It was proposed that breadmaking quality depends on the interplay between resistance to extension and extensibility. The authors proposed other parameters like difference in extensibility between maximum resistance and rupture and RMax/(ExtRup-ExtRMax)^2. They observed good prediction of breadmaking quality, although they worked with only 8 samples with a very high variation in quality.

2.4.3.3. Fundamental rheological variables

Fundamental and empirical rheological parameters have been compared with baking quality results (Janssen et al 1996c). Since their research was conducted using only 5 flours, it is not possible to draw strong conclusions, but it was remarked by the authors that differences in baking quality cannot be predicted by only one rheological parameter. Also, they could not find a clear relationship between the extensibilities determined by alveograph or extensigraph and loaf volume.

Several recent papers have shown the importance of strain hardening on breadmaking (Dobraszczyk et al 2003, Sliwinski et al 2004a, Tronsmo et al 2003a). The inflating bubble strain hardening index has been determined to be highly correlated with hearth loaf volume ($r=0.89$; $P<0.001$; Tronsmo et al 2003a). Janssen et al (1996a, 1996b, 1996c) proposed that three rheological properties were needed to obtain a breadloaf of

good quality: optimal resistance to extension, high extensibility and strain hardening above a minimum.

Rheological properties can also be determined by the creep-recovery test. In a creep-recovery experiment, one piece of dough is stressed instantaneously and released. The results obtained with this test were compared with the standard AACC Approved Method 10-10B pan bread test using 11 flours (Wang and Sun 2002). The parameter best correlated with loaf volume was maximum recovery strain ($r = 0.939$, $P<0.05$). The authors proposed that there is a fundamental relationship between dough rheological behavior and breadmaking properties. The same parameter was highly correlated with extensibility, resistance to extension, mixograph mixing time and flour protein content ($r = 0.892$, 0.947, 0.889 and 0.555, respectively; $P<0.05$).

2.4.3.4. Multi-variables approaches

Færgestad et al (2000) used 17 samples to study the relationship between rheological parameters and different kinds of bread. They included one pan loaf test, and two hearth bread tests with different mixing procedures. Using Partial Least Square analysis (PLS), they observed that the volume and the form ratio were affected differently by flour quality. For one of the hearth bread tests, the form ratio was positively related to dough resistance to extension, mixogram peak time and farinogram peak time, while the relationship was negative for extensibility. These observations were confirmed by simple correlation coefficients (respectively: $r = 0.68$, $P<0.01$; $r =0.73$, $P<0.05$; $r = 0.72$, $P<0.001$ and $r = -0.62$, $P<0.05$). Similar results were obtained with the other hearth bread test (respectively: $r = 0.70$, $P<0.01$; $r =0.56$, $P<0.05$; $r = 0.50$, $P<0.05$ and $r = -0.40$, no significant at $P<0.05$).

These results point to the conclusion that resistance to extension is a required property to give the dough the ability to retain shape during proofing. Simultaneously though, both PLS and simple correlation analysis proved that hearth loaf volume was positively related to dough extensibility ($r = 0.72$ and 0.90, $P<0.01$ and 0.001 for the two tests). In summary, higher extensibility caused an increase in hearth bread volume, but it caused problems in form ratio. The authors proposed that there may be equilibrium between these properties. Although extensibility was not significantly correlated with pan loaf volume, both maximum resistance to extension and farinograph peak time were correlated ($r = 0.55$, $P<0.05$; $r = 0.74$, $P<0.001$). It is important to remark that the parameter with a higher correlation with pan loaf volume was farinograph peak time ($r=0.74$, $p=0.001$).

A PLS model was used to explain breadmaking characteristics of 20 Norwegian flours with extensional tests and gluten composition by Tronsmo et al (2003a). Two bread tests were done using a hearth loaf process and a Chorleywood bread process. The model was able to predict hearth bread volume and form ratio (coefficient of determination = r^2 = 0.84 and 0.70), but failed to explain the Chorleywood bread volume.

Tronsmo et al 2003b observed that most studies of the relationship between extensional properties and breadmaking characteristics have been done on a limited number of samples, on crosses from only two cultivars or on near-isogenic lines. Therefore, they extended the previous study to 40 samples, using 20 genotypes at two nitrogen levels (Tronsmo et al 2003b). The predictions of hearth bread volume and form ratio were lower, but still important: $r^2 = 0.55$ for bread volume and $r^2 = 0.56$ for form ratio. Using

optimal water addition, grain endosperm hardness did not have a significant influence on baking quality, and there was no effect of hardness on gluten viscoelastic properties.

2.4.4. Protein and gluten components requirements

It has been clearly established that protein quantity is an important factor in bread quality. The influence of protein composition (i.e., protein quality) has been known for a long time as well (Finney and Barmore 1948). Flour protein was related to loaf volume ($r = 0.58$, $P<0.001$), but no correlation was found between protein content and crumb grain by Dong et al (1992). Ohm and Chung (1999) reported a high correlation for flour protein content, wet gluten and dry gluten with loaf volume ($r=0.77$, 0.61 and 0.65 respectively; $P<0.01$). Gluten protein, estimated as the total area under a SE-HPLC curve, has been reported to have a higher influence on bread volume ($r=0.88$, $P<0.001$) than the amount of protein (Wieser and Kieffer 2001).

Several studies on the relationship between protein quantity and bread characteristics have reported that hearth loaf volume increased with higher proportions of high molecular weight glutenin polymers, but that this relationship was not extended to pan bread loaves baked using the Chorleywood bread process (Tronsmo et al 2003a). In one study the correlation between Payne score (Payne et al 1987) and loaf volume was low but significant: $r=0.20$, $P<0.01$ (Dong et al 1992) and five gliadin bands were correlated with loaf volume ($r= -0.52$ to -0.90, $P<0.01$). Using multiple regression analysis, no equation using any combination of glutenin or gliadin bands had a coefficient of determination above 0.50 (Ng and Bushuk 1988). Since an increase of protein causes

both an increase in volume and a decrease in form ratio, it is very important to find the optimum balance of protein content and protein quality (Faergestad et al 2000).

2.5. NIR analysis theory and applications

Rapidity and simplicity of sample preparation makes near-infrared spectroscopy (NIR) an attractive tool for several purposes. Samples analyzed could be small and they may even not be destroyed. In addition, several variables could be determined in one single step. Because of all these characteristics, NIR has been proposed to be used for wheat quality screening, particularly for breeding programs. (Delwiche et al 1998a, Hruskova et al 2001, Miralbés 2003, Osborne 1984, Williams et al 1988).

2.5.1. Introduction

NIR is based on the absorption of electromagnetic radiation, using wavelengths that are usually in the 700-3000nm range, although the range sometimes includes the visible portion starting at 400nm. The NIR portion of the spectrum borders on the red wavelength region of the visible spectrum. Classical absorption spectroscopy can in theory determine the concentrations of constituents. However, this chemical information is masked by interference among the components and changes in spectra caused by physical properties like particle size. The greatest potential of NIR technology occurs when it is used as a secondary method, after calibration against determinations made by a reference method for the required component (Hruschka 2001, Miller 2001).

In the near infra-red spectrum, most of the incident radiation is reflected diffusely after the radiation partially penetrates the surface as it reaches each particle. The diffusely reflected radiation (R) can be related to concentration in an analogous way to Beer's

law: log 1/R = kc, where k is a factor which incorporates both absorbtivity and path length and c is concentration. There is an equivalent equation for diffuse transmittance (T), using log 1/T (Dahm and Dahm 2001, Hruschka 2001, Williams and Norris 2001). In grains, both reflectance (Black and Panozzo 2004, Delwiche 1998, Delwiche et al 1998a, Delwiche et al 1999, Fontaine et al 2002, Maghirang and Dowel 2003, Pasikatan and Dowell 2004, Wang et al 1999, Williams and Norris 1983, Wesley et al 2001, Xie et al 2003) and transmittance (Miralbés 2003, Nielsen et al 2003) have been successfully applied. The second has been applied exclusively for whole grain samples.

In the last two decades the use of NIR has increased dramatically (Hruschka 2001). The success and acceptance of NIR is based on two independent factors. One is that no chemicals are needed (Starr et al 1981). The second is that this is very fast, mainly due to the quality of the spectra and the power of the mathematics used in the analyses (Hruschka 2001). In addition, in cereal technology another potential advantage of NIR is that the measurement of grain samples can be nondestructive (Maghirang and Dowell 2003, Nielsen et al 2003, Pasikatan and Dowell 2004).

Most NIR research has been done with two kinds of spectrophotometer. The simplest and cheapest instruments use interference filters to measure the absorption at specific wavelengths appropriate for the most popular applications (such as moisture or protein). These instruments have limited versatility. Monochromators are much more versatile since they can determine both transmittance and reflectance at over 1000 different wavelengths (MacClure 2001).

Advances in both sensor and computer technology have been very rapid. Sensor evolution has made spectroscopy more accurate, and at the same time has resulted in more available data. Therefore, there has been a need for better hardware and software to process all this information, and to facilitate calibration procedures. It is now possible to make a multi-product calibration rather than several calibrations from one product without losing accuracy (Borggaard and Thodberg 1992, Shenk and Westerhaus 1993). The use of multiple wavelength readings also has advantages. It minimizes interference by substances other that those intended to be analyzed (Starr et al 1981, Hruschka 2001).

2.5.2. Calibration and validation procedures

In order to be able to use an NIR spectrophotometer to measure a quality trait, a calibration procedure must be followed. First, a selected set of samples must be analyzed by a standardized method, the "reference method". Then, all samples must be scanned by transmittance or reflectance, and the relationship between the spectra and the reference method must be determined. This relationship is the calibration. The calibration is expressed as an approximation which involves a regression equation. The dependent variable is the reference method value. The independent variables are log(1/R) values or a combination of them (Hruschka 2001).

The reference method should be highly reproducible because the calibration performance can never be better than the reference method. Another key factor is the accuracy of the reference method (Williams and Norris 2001). For example, Nielsen et al (2003) suggested that the most important problem observed in hardness prediction was

with the reference method used, a problem which could only be solved by having a more accurate reference method.

After the initial calibration procedure it is necessary to run a validation procedure. This involves comparison of the NIR and reference method measurements of a set of samples not included in the calibration process. It is necessary that both calibration and validation sets are drawn from the same population. For satisfactory calibration, the set of samples used must be representative of the population which will be analyzed with this calibration. The usual procedure is to take one set, sort it by reference values, and then divide it into calibration and validation sets (Hruschka 2001, Williams 1996).

The population used for the calibration-validation procedure will depend on the degree of homogeneity of the population. Some wheat populations are easily classified into different groups. Some authors have reported that using separate calibrations for different types of wheat improved precision (Starr et al 1981, Miralbés 2003), while models using pooled wheat of different types performed as well as, or better than separated calibrations for other authors (Delwiche 1998). In order to improve model robustness it has been suggested that it is wise to include samples from more than one crop year in the calibration set (Delwiche 1998). A general rule for robustness is that samples from as many environments as possible should be included in the set.

In a calibration-validation procedure, there are different sources of error with up to 40 having been identified (Hruschka 2001). They can be grouped as those originating from sampling error, reference method error, or NIR-use error. Very simple operations may cause important errors. For example, samples must be prepared uniformly, and factors

such as moisture, temperature and cleanness cannot be overlooked (Williams 2001, Williams and Norris 2001). In addition, particle size, sample packing, type of grain and growing environment influence NIR measurements (Williams 1975). Wang et al (1999) found that wheat kernel size significantly affected NIR measurements because the radiation absorbed decreased with an increment in kernel size. Several mathematical treatments can be used to minimize the errors. The authors proposed use of the multiplicative scatter correction (MSC). MSC is calculated by regressing each spectrum against the average of all the spectra (considered as "an ideal spectra") and by removing the slope and offset effects. This design corrects indeterminate path length effects caused by scattering.

Different parameters are useful to understand the efficiency of a calibration equation, using both the calibration and the validation set of samples and values. The most widely used are the correlation coefficient (r) and the coefficient of determination (r^2 or R^2). A more specific parameter is the standard error of calibration (SEC). The difference between the reference value and the estimated one is calculated for each sample of the calibration set. The standard deviation of these differences is SEC. A similar one is the standard error of prediction (SEP), which is also the standard error of differences but using the validation set. The bias, difference between the means for the reference method and predicted values for the validation set, is another important one.

Another important indicator is RPD (ratio prediction/deviation), which is defined as the ratio of the standard error of prediction to the standard deviation (SD) of the reference data in the validation sample set (ratio SEP:SD). RDP is useful to evaluate the pertinence of a SEP result. It was proposed that if RPD<2 the calibration is not

recommended. A value between 2 and 3 means that is useful only for rough screening, over 3 is a good calibration, and over 5 is excellent (Black and Panozzo 2004, Hruschka 2001, Williams 2001, Williams and Norris 1983).

2.5.3. Statistical procedures for NIR calibration

Several statistical procedures can be used to set a calibration equation. These include multiple linear regression (MLR), partial least squares (PLS) and artificial neural network (ANN).

MLR was initially the most widely used method for establishing the calibration equation. With MLR there is always the possibility of "over-fitting", because the more wavelengths included, the better the SEC, but the calibration may not be improved. This problem increased with the development of monochromators. When hundreds of wavelengths became available, over-fitting was even more possible. In order to overcome this problem, the use of PLS instead of MLR was proposed. PLS involves the use of factors formed by the regression of the optical signals at each wavelength point against the reference data in order to explain most of the variance (Williams 1996).

Comparing PLS and MLR for different sets of wheat grain samples, Delwiche (1998) found that PLS models (SEP = 0.51 to 0.65%), were more accurate than MLR models (SEP = 0.59 to 0.69%) for predicting protein content. The author concluded that since the difference was very small the MLR models might be preferable because of their simplicity.

One proposed criteria for the PLS calibration acceptance is that the equation will be effective when the factors used explain over 98% of the variance. Some models could be valid with lower values. A factor that may be the most important in the explanation of the total variance, might not be the best in the PLS model. For example, Williams (1996) found that the first factor for flour explained 18.4% of protein variance, but the second one was responsible for 52.9%. For some variables like corn moisture, 3 factors could be enough to explain over 98% of the variance., but for other factors like flour ash 15 factors explained less that 95% of the variance (Williams 1996).

Another statistical approach recently applied to grain studies is that of the ANN (Borggaard and Thodberg 1992). This system has been adopted for commercial use in several European countries for determination of wheat protein (Büchmann et al 2001). A lower SEP was reported by Borggaard and Thodberg (1992) for ANN than for PLS,when using the same set of samples).

2.5.4. NIR applications for wheat quality screening

NIR, because of its rapidity, simplicity of sample preparation and small sample size requirement, had been used extensively in grain and grain derivative analyses both commercially and for breeding programs. The most important component determined has been protein content. The first useful calibrations date from 1975 (Delwiche et al 1998b, Williams 1975, Williams and Norris 1983).

Since NIR has proven suitable to predict amino acid content (Fontaine et al 2002), it may be possible to use it to predict protein quality as well as quantity. This makes the technique attractive to use for the prediction of baking and dough properties in wheat

breeding programs (Wrigley 1994). So far there has been limited success in reaching this objective.

Calibrations for several parameters, including sedimentation volume and loaf volume, were developed by Starr et al (1981) using a filter analyzer. They used a small number of samples, n = 36 to 40, for both calibration and validation sets. Calibrations were not suitable for prediction of these characteristics (r^2 = 0.19 and 0.27, respectively), but calibration parameters showed highly significant correlations (r^2 = 0.64, $P < 0.001$, for both). The authors concluded that a calibration to predict baking performance was possible. They proposed that results might be improved by changing the combinations of wavelengths. Monochromators and PLS were not used at that time.

Osborne (1984) stated that NIR filter instruments had so far not been found suitable for the estimation of protein quality, but could be used for protein quantity. He carried out investigations using a monochromator system to check the validity of NIR for predicting protein quantity and quality and Falling Number values. He worked with two sets of samples (n=52 and 117) with a wide range in protein percentage (7.2-12.2%), Falling Number values (62-377s) and SDS sedimentation volumes (30-95ml for set 1, 25-88ml for set 2). He obtained calibrations by MLR forward stepwise regression with 600 datum points and found very good prediction for protein (r = 0.99, SEP = 0.21%). Results for SDS sedimentation volume were very poor, however. The correlation coefficient obtained was not very low (r =0.65), but the conclusion was that this might have been due to the correlation between protein content and SDS sedimentation volume (no value reported). In addition, the correlelograms (plot of modulus correlation coefficient against

wavelengths) of SDS sedimentation volume and protein were very similar. He concluded that NIR technology was not suited to prediction of bread baking quality.

Rubenthaler and Pomeranz (1987) tried to overcome the problem observed by Osborne, by working with a wider range of protein content (7.9-20.7%) and loaf volume (568-1328ml). They specifically looked for a set of samples with a low correlation between protein and loaf volume (r = 0.308). They had high correlation coefficients when they used one set of samples, but when these calibrations were used to predict a second set the correlation coefficients were "low" (no values given).

Williams et al (1988) developed calibrations using MLR. They divided wheat samples into three groups: hard, medium-hard and soft (n for validation sets were 26, 11 and 15 respectively). They intended to predict a wide diversity of quality parameters, including farinograph, alveograph, and extensigraph values. Good results were obtained for protein for the three groups, SEP = 0.20, 0.23 and 0.15% respectively, and r = 0.99, 0.99 and 0.98, respectively. Lower values, but still valid for breeding purposes, were obtained for the rheological tests. For Farinograph stability r was similar in the three cases (0.86, 0.86 and 0.79, respectively), but important variability was observed in SEP values (3.3, 2.6 and 1.1 min, respectively). This variation was explained by the difference in the validation set means (10.9, 9.6 and 3.0 min, respectively). Good results were also obtained for Alveograph W (r = 0.83, not reported and 0.89, respectively). Alveograph L (extensibility) was studied only in the soft samples set, with good success (SEP=12, r=0.77).

Using PLS, Williams (1996) reported calibrations for different quality parameters (number of samples not reported). Flour ash and flour protein were successfully predicted (R^2 = 0.82 and 0.99 respectively). Hardness was well predicted when the sample was ground (R^2 = 0.95, RPD=4.6) but not so well when whole grain was scanned (R^2 = 0.74, RPD=1.9). Flour water absorption prediction results proved the suitability of NIR for this parameter (R^2 = 0.85, RPD=2.6).

Delwiche et al (1998a) proposed an investigation of the potential for using NIR spectrophotometry to quantify the biochemical components or physical properties of translocated wheat flour. Parameters studied included glutenin content, gliadin content, albumin, globulins, SDS sedimentation volume, and mixograph measurements. PLS analysis predicted the following parameters reasonably well: glutenin content, gliadins content with and without secalins, SDS sedimentation volume, mixograph peak time and mixograph peak width. The authors stated that these predictions might be suitable for breeding programs, but would not be suitable for quality control, and they concluded that NIR could be used for genotype selection. Their results were obtained working with two reps of the same field trial as the validation and calibration sets. No actual external samples were used to validate results.

Wesley et al (2001) analyzed 78 samples by SE-HPLC for total protein and "extractable" and "unextractable" polymeric protein. They used both PLS and curve fitting procedures. They concluded that the method predicted the glutenin content, since the rank correlation was highly significant between both years. No validation procedure was run.

Hruskova et al (2001) reported good calibrations for several parameters, including protein content (r=0.963) and farinograph parameters. The only correlation coefficients significant at P<0.01 of the validation set (n=29) were time of development (r=0.619) and mixing tolerance (r=0.415). Water absorption, dough stability and extensigraph predictions were not significant (P<0.01). They assumed that lack of correlation could be caused by the small size of the sample set (n=114) and the qualitative differences within the set.

Miralbés (2003) proposed a NIR transmittance whole grain calibration be used at the receiving stage of the milling industry. Using 443 samples from different countries, the author obtained very good results for protein, wet gluten and dry gluten (SEP = 0.16%, 1.00%, 0.29%, validation r^2 = 0.99, 0.95, 0.97, respectively). When he calibrated for Alveograph baking strength (W) with an extended set of samples (n=600) the results were not as good. The validation r^2 value was considered "relatively high" (0.84), but the correlation of W with protein content, well predicted by NIR, was even higher (r^2 = 0.86). The author also considered that the SEP value (41 x 10^{-4}J) was too high even for screening purposes. By dividing the set of samples into "weak" and "medium-strong", the validation r^2 increased up to 0.95 and SEP was 24 x 10^{-4}J for the second group. Good results were obtained also for Alveograph P (SEP = 6.7 mm), but not for P/L. No results were given for extensibility (L). The values obtained for Alveograph W and P were considered "reasonable" to be used for screening purposes at mills or elevators.

2.5.5. Other applications of NIR for wheat and bread quality testing

NIR has been proposed for determining wheat properties or dough characteristics and for exploring chemical changes during processing. For example, mixing conditions may

be optimized for particular flour blends by using NIR technology (Alava et al 2001). The potential of using NIR spectroscopy for providing information on the chemical processes that occur during dough development in relation to flour strength was evaluated by Wesley et al (1998). The conformation of the gluten complex was also studied by NIR (Popineau et al 1994).

NIR technology has at present, and will likely have in the future, a number of applications in wheat trading and storing. It has been proposed as a tool for grain buyers for grading (Williams 1999, Williams 2000). Environmental conditions of the purchased grain could be identified (Delwiche et al 2002). Once the grain was bought, it would be possible to segregate the kernels of a sample according to their protein content (Pasikatan and Dowell 2004) or by the variability in protein content within a cargo (Delwiche 1998). Not just wheat composition, but also wheat storage conditions and kernel damage could be studied by NIR (Baker et al 1999, Perez-Mendoza et al 2003).

In addition to protein, protein related parameters, and breadmaking performance, other flour components can be estimated by NIR technology. Flour color has been successfully predicted by NIR (Black and Panozzo 2004). Minor grain components like mycotoxins can be predicted by NIR, although this may not be an actual mycotoxin measurement but may be due to closely related differences in texture (Williams et al 1996). By scanning flours it is possible to identify genotypes possessing the 1AL.1RS or 1BL.1RS wheat-rye translocation (Delwiche et al 1999). Hardness was successfully determined using NIR in whole grain by Maghirang and Dowell (2003). Good hardness predictions have not always been obtained though. Nielsen et al (2003) concluded that single seed hardness predictability was “poor”.

Not just wheat and dough properties, but also bread characteristics, have been studied by NIR. Xie et al (2003) concluded that NIR could follow bread changes during storage more accurately than a texture analyzer. The authors proposed that this was because the texture analyzer measured bread firmness alone, but the NIR spectrophotometer determined both physical and chemical composition.

2.5.6. The future of NIR applications uses

The characteristics of NIR technology make it attractive for breeding program quality screenings. It has being proven to be useful for protein content analysis with appropriate accuracy. Although perspectives are promising, the results obtained so far did not confirm its suitability for protein functionality screening. In theory, appropriate equations can be obtained when populations are large enough, doing a systematic work with suitable reference methods pursuing proper calibration/validation procedures. Advances in other fields and continuous advances in hardware and software justify more efforts to reach these goals.

2.6. Quality in wheat breeding programs

Baking industry requirements are increasing all around the world. The best way to improve wheat quality is to develop varieties with properties adjusted to industrial requirements. Achieving this objective requires the development of better screening tests for specific quality characteristics. The tests should be simple, rapid and require a small amount of sample (Wrigley 1994), and it is always better that no hazardous organic chemicals are used (Ohm and Chung 1999).

Gluten strength is probably the most important objective in breeding programs. In many cases, gluten strength has been estimated by maximum resistance to extension or mixing stability. Using these test parameters to predict strength runs the risk of selecting materials with too high resistance to extension properties. Although protein requirements for mixing and for gluten strength are similar, there may be some differences (MacRitchie 1992R). There is consensus that most genotype variability in breadmaking potential is due to both quantitative and qualitative differences in glutenin proteins (Ng and Bushuk 1988).

Protein content is a key factor also in improving extensibility. This may be a problem since in any breeding program yield improvement will be the most important objective; and this is usually linked to a decrease in protein. Another factor to consider is that if an increase in flour protein is obtained, usually it is due to an increase in the proportion of gliadin, associated with dough weakness (MacRitchie 1992R).

Extensibility determination should be also included as a screening test. It has been stated that extensibility is largely related to wheat variety (Mailhot and Patton 1988), but, to an important extent, extensibility is controlled by environmental effects that cause variation in protein content. Improving glutenin expression by selecting a subunit composition to ensure that the molecular weight distribution is not shifted to the high molecular weight subunits would be a good strategy to improve extensibility. Also, it is possible to increase extensibility by having a higher LMW-GS/HMW-GS ratio and by the use of 2+12 instead of 5+10 HMW subunits (MacRitchie 1992R).

Micro-tests and NIR are two proposed strategies to use to screen for quality in breeding programs. Kieffer et al (1998) proved the suitability of micro-extension tests for this purpose. NIR technology seems to be ideal for screening, but good calibrations are still to be developed.

2.7. Conclusions

Despite the multiplicity of research studies that have been done to explain the relationship between dough rheological properties and breadmaking, the relationship is far from clear. Results are hard to interpret because of the complexity of factors. Different statistical approaches have been used to relate breadmaking properties with rheological variables, including simple and multiple correlation analysis, principal components analysis and partial least squares analysis. In particular, the nature and effects of extensional properties on breadmaking has received little attention in the past.

In Uruguay, the most widely consumed bread is a hearth bread of the French-type. That is a long cylindrical loaf with a crusty exterior that contains no fat. Most research work has been done using pan bread, although it has been proven that different breads need different approaches. There is little literature published about hearth bread requirements.

There is a need to continuously develop new methodologies in order to explain the relationships among rheological properties and their importance for bread quality and as well to have better methods for evaluating wheat performance. These methodologies

could be improvements done on standard ones or new ones, such as the micro-extension tests mentioned using texture analyzer.

NIR technology was proposed to be a valid tool. Even though it is widely used to determine parameters like total protein content, the use for other variables is still limited. Research should be oriented to explain influence of the variables (sample preparation, sample size, math treatments, etc.) that affect NIR calibrations.

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CHAPTER 3

THE INFLUENCE OF EXTENSIONAL PROPERTIES AND RELATED PROTEIN COMPONENTS OF URUGUAYAN WHEAT DOUGHS ON BREADMAKING PERFORMANCE

The Influence of Extensional Properties and Related Protein Components of Uruguayan Wheat Doughs On Breadmaking Performance

3.1. ABSTRACT

Unique rheological properties and composition of wheat flour dough have complex relationships with breadmaking requirements. These relationships and the methodology suitable to evaluate them are different for each bread type. The objective of this resesarch was to study the importance of dough rheological properties for the quality of Uruguayan type hearth and pan breads, and the relationship of these properties to protein components. Complete physicochemical analyses were done on 75 Uruguayan and 6 Canadian samples. Baking tests, standard and modified rheological tests, and micro-extension tests were performed on flour samples. Micro-extensigraph tests also were conducted with gluten doughs. Data were analyzed by simple and multiple regression, principal component and partial least square analyses. While flour protein content was the best correlated with pan bread volume (r=0.706), the adjusted alveogram resistance/extensibility ratio had an almost identical correlation coefficient (r=0.701), and 72% of the loaf volume variability could be accounted for by a regression model that included three extension variables. Loaf volume of French bread was most highly correlated with flour protein (r= 0.574), however, the form ratio, a more important quality attribute for French bread, was best correlated with alveograph baking strength (r=0.718). Using MLR analysis, 64% of the variability in form ratio could be predicted from two extension variables: adjusted alveograph baking strength and dough micro-extension extensibility at rupture. Dough extensional tests data were generally more

closely related to breadmaking properties than mixing test data. Protein components related to extensibility included flour protein and mixograph maximum height (r=0.707), wet gluten and alveograph maximum resistance to extension (r=-793), and SDSS and alveograph baking strength (r=0.758).

3.2. INTRODUCTION

Wheat flour dough has unique rheological properties. Its viscoelastic features allow the formation of a foam structure, that will be transformed into the sponge-like one which is bread (Kokelaar et al 1996). Among these unique wheat dough characteristics, the ability to form a matrix that retains gas is the one that leads to bread formation. This is possible because of the capacity of the dough system to be stretched into thin membranes (Bloksma and Bushuk 1988).

Among the different sciences needed to understand breadmaking, probably rheology is the most important. Although a theory of dough rheology is not a theory of breadmaking, appropriate dough mechanical properties are essential (Shewry et al 2003R). Dough rheological properties are critical for two reasons. First, they determine the behavior of dough pieces during the industrial process. Secondly, the quality of the bread produced is clearly defined by them (Bloksma and Bushuk 1988).

Usually, the most critical property specified for flour is "strength". This term has had several meanings. Originally, it was applied to the resistance that a dough must have in order to withstand the action during breadmaking (Wooding et al 1999). Usually, flour strength has been synonymous with flour quality (Mailhot and Patton 1988), but it has also been used as a term for high protein flours, good mixing properties, high maximum resistance to extension in an extension test, and other properties (Tipples et al 1982, Wrigley 1994). Williams et al (1988) proposed using the term "functionality" which is a better descriptor than "strength" when describing the end-product utilization of dough or gluten. In this research, the original meaning will be applied.

In dough rheological studies, extensibility or fracture strain is defined as the elongation at break at a set tensile stress (MacRitchie 1992R). The longer the dough is extended without breaking, the higher the extensibility. This is not an absolute measurement. Moreover, it depends on the method of preparation and state of development (MacRitchie 1992R). However, it is an important property since it is the key for the expansion of the dough to achieve high loaf volume, although, a very large extensibility may affect bread quality negatively (Faergestad et al 2000).

Other rheological properties have received attention recently. The stress required to deform gluten increases with increasing strain at comparable strain rates. This phenomenon is known as "strain-hardening". Applying this concept to flour dough, thinner regions of the dough will have more resistance to extension than thicker regions. As a result thicker films around the smaller cells will be extended during the dough rising process, leading to a more homogeneous foam system (Kokelaar et al 1996).

The complexity of dough rheology extends to the instruments and procedures used for rheological assessment. These are difficult to design and the processes are difficult to execute (Faubion and Hoseney 1990). Although in theory fundamental rheology could predict dough breadmaking behavior, practical experience has shown that results are not always applicable (Dobraszcyk et al 2003).

There is therefore a long tradition for the use of empirical rheological tests, which characterize the flour by preparing a dough in standard conditions and determine variables which are correlated with breadmaking properties. These tests can be divided

into two groups. The first one emphasizes mixing properties by measuring such factors as mixing developing time or water absorption. This group includes the commonly used instruments such as Brabender farinograph and the mixograph. The second group emphasizes extensional properties like resistance to extension and extensibility, and includes the widely used Brabender extensigraph and the Chopin alveograph (Mailhot and Patton 1988). As well, micro-extensibility tests have recently been proven to be useful for research. These tests have used different types of equipment, but they are mostly equivalent to the extensigraph procedure (Anderssen et al 2004, Békés et al 2003, Grausgruber et al 2002, Kieffer et al 1998, Suchy et al 2000).

Rheological dough testing for bread quality prediction must take into consideration the type of bread being produced since there are many kinds, each one with its own requirements (Mailhot and Patton 1988). A flour could be ideal for one bread product and not suitable for another (Sliwinski et al 2004). In order to assess flour quality, tests must be linked to the baking procedure being used (Tronsmo et al 2003a).

Although most research has been conducted using pan bread, in some regions of the world hearth bread consumption is much more important (Tronsmo et al 2003b). Hearth bread requires a different approach, because functional properties of the proteins are more effectively distinguished than for pan loaves (Faergestad et al 2000, Tronsmo et al 2002). Mailhot and Patton (1988) proposed that pan bread requires "medium" extensibility, while hearth bread requires "medium to long" extensibility. Similarly, the same authors proposed "medium" resistance to extension for pan bread and "maximum" for hearth bread as basic requirements. Recently, it was proposed that hearth bread

strength requirements are higher than those for pan bread because the pan walls help the latter retain their shape during the baking process (Sliwinski et al 2004).

Loaf volume is the most useful quality assessment parameter, since it is considered to be a good summary of the overall characteristics of the bread (Faergestad et al 2000, Janssen et al 1996, Kieffer et al 1998, Ohm and Chung 1999, Tronsmo et al 2003a, b, Wang and Sun 2002). Other variables have also proved suitable to evaluate bread quality. Faergestad et al (2000) worked with a type of Norwegian hearth bread. They measured the width and height of each piece and proposed to use the form ratio (height/width ratio). Janssen et al (1996) defined a good quality loaf as one with high volume and a regular, fine crumb structure. In order to evaluate crumb quality, subjective scores have been used successfully (Faergestad et al 2000).

An important aspect of rheological properties is their relationship with breadmaking quality. Large deformation rheological measurements have been extensively used to predict breadmaking performance and mixing properties are widely employed to predict breadmaking quality (Mailhot and Patton 1988) . The results remain empirical and it is difficult to interpret most of them (Tronsmo et al 2003b). Mixograph variables were evaluated as loaf volume predictors using 72 samples (Ohm and Chung 1999). They observed a significant correlation of mixograph peak height and band width with loaf volume ($r=0.64$ and 0.51, respectively; $P<0.01$). The correlation was not significant for mixograph mixing time. The correlation coefficient between flour protein and loaf volume was higher than the mixograph results for the same set of samples ($r=0.77$, $P<0.01$). Sliwinski et al (2004) reported a correlation between loaf volume and dough mixing time

(r=0.61; no significance reported). This was in a set of 8 samples with a high correlation between protein content and loaf volume (r=0.92; no significance reported). They concluded that long mixing flours do not necessarily give better bread properties.

Faergestad et al (2000) used 17 samples to study the relationship between rheological parameters and different kinds of bread. They included one pan and one hearth bread test. Using Partial Least Square analysis (PLS), they observed that volume and form ratio were affected differently by flour quality. For a hearth bread test, the form ratio was positively related to dough resistance to extension, mixogram peak time and farinogram peak time, while the relationship was negative for extensibility. These results could lead to the conclusion that resistance to extension is a required property to give the dough the ability to retain shape during proofing. Simultaneously though, both PLS and simple correlation analysis proved that hearth loaf volume was positively related to dough extensibility. In summary, higher extensibility caused an increase in hearth bread volume, but it caused problems in form ratio. Authors proposed that there may be a required equilibrium between these properties.

A PLS model was used to explain breadmaking characteristics of 20 Norwegian flours based on extensional tests and gluten composition by Tronsmo et al (2003a). Two bread tests were used, a hearth loaf process and a Chorleywood bread process. The model was able to predict hearth bread volume and form ratio (r^2 = 0.84 and 0.70), but failed to explain the Chorleywood bread volume. Tronsmo et al (2003b) observed that most studies of the relationship between extensional properties and breadmaking characteristics have been done on a limited number of samples, on crosses from only two cultivars or on near-isogenic lines. Therefore, they extended the previous study

(Tronsmo et al 2003a) to 40 samples, using 20 genotypes at two nitrogen levels (Tronsmo et al 2003b). The prediction of hearth bread volume and form ratio were lower, but still accounted for over 50% of the variability, with $r^2 = 0.55$ for bread volume and $r^2 = 0.56$ for form ratio. Using optimal water addition, grain endosperm hardness did not have a significant influence on baking quality, and there was no effect of hardness on gluten viscoelastic properties.

Anderssen et al (2004) stated that there is general agreement about the link between extension testing and baking performance. Good volume is achieved if gas bubbles rise during both proofing and baking. It was proposed that breadmaking quality depends on the interplay between resistance to extension, and extensibility. They observed good prediction of breadmaking quality, although they worked with only 8 samples with a very high variation in quality.

The relationship between micro-scale extension methods and three different baking tests was studied using two different set of samples (n=12 and 13) by Kieffer et al (1998). Results were clearly different for the two sample sets. For the set of German wheats, gluten maximum resistance to extension was the best predictor of loaf volume (the correlation coefficient, varied from 0.73 to 0.83 for different baking tests. Flour dough maximum resistance to extension was also significantly correlated with loaf volume (r=0.71-0.80). Correlation was not significant for another set of wheats. The use of the ratio of maximum resistance to extension to extensibility did not improve correlations. Results with extensibility alone were not reported.

Rheological properties are not components of the wheat endosperm, but are consequences of the endosperm chemical composition. Therefore, it is necessary to understand the relationship between the key rheological variables and chemical components. Wheat is unique because its proteins are the only ones with the ability to form a dough with the rheological properties required for the production of leavened bread (Gianibelli et al 2001R).

The amount of protein is an important factor related to rheology because the more protein, the stronger the network and therefore the more stress that the network can support (MacRitchie 1992R). Total protein content is related to dough strength, but most importantly glutenin content and composition affect gluten strength. However, it is not always the stronger flour that makes the better bread (Sliwinski et al 2004).

Strength is not the only breadmaking requirement that is related to protein amount. Some papers have concluded that extensibility increased with increases in protein content (Uthayakumaran et al 1999, Faergestad et al 2000) but other reports have concluded that extensibility was not affected by a change in protein content from 11.2 to 15.2% (Uthayakumaran and Lukow 2003). It also has been proposed that extensibility is related not only to the total amount of gluten, but also to its fractions. In general, it is understood that gliadins contribute to dough viscosity as glutenins contribute to dough elasticity (Bloksma and Bushuk 1988, Ciaffi et al 1996, Gianibelli et al 2001R, Shewry et al 2003R, Uthayakumaran et al 1999). However, extensional properties have not always been explained by protein composition. Using 71 genotypes grown in the same

environment Campbell et al (1987) observed no statistical correlation between gluten composition and extensibility.

The importance of rheological properties to bread quality is unquestionable. The complexity of the relationships among dough rheology, breadmaking, and flour composition, require more specific studies. These should be based on the type of bread to be made with the flour, and on an evaluation of the methodologies that may be suitable for both detailed analyses and fast screening. The type of bread most widely consumed in Uruguay is a hearth bread known as "French-type".

Therefore the purpose of this investigation was to determine the importance of dough rheological properties of Uruguayan wheats, and especially extensional properties, for the quality of Uruguayan French-type (hearth) and pan breads, and to investigate the relationship of these properties to protein components. The main objectives of the research were:

1. to determine the most important rheological factors for the prediction of pan and hearth bread quality
2. to develop models to explain pan and hearth loaf quality based on rheological parameters
3. to identify efficient and effective rheological methodologies.
4. to evaluate protein components which explain rheological properties.

3.3. MATERIALS AND METHODS

3.3.1. Bread wheat samples

The bread wheat sample set was selected to provide a wide range of quality and composition. It included 75 Uruguayan wheats and six Canadian samples. Uruguayan samples included 35 samples from the National Evaluation Program, 17 genotypes from two environments each, one from one environment (one sample was lost), and 32 samples from 2002 fertilization trials (8 genotypes grown at 4 nitrogen fertilizer levels) and 8 samples from 2003 fertilization trials (4 genotypes grown at 2 nitrogen fertilizer levels) (Appendix 1). Canadian grains were a Canada Western Hard White cultivar (AC Snowbird), a Canada Western Red Winter cultivar (AC Tempest), a Canada Prairie Spring White cultivar (AC Vista), two Canada Prairie Spring Red cultivars (AC Crystal and 5700 PR) and one sample of a Canadian Western Red Spring cargo, Grade I.

3.3.2. Grain and flour analysis

Wheat samples were tempered at 15.5% and milled to produce straight-grade flour in a Buhler mill MLU 202 (Bühler AG, Uzwil, Switzerland). Ground samples were obtained using a Perten 3100 Hammer Mill (Perten Instruments AB, Huddinge, Sweden). Protein (N x 5.7) was determined in both ground wheat (GP) and flour (FP) by a modified Kjeldahl method (catalyst used was 98% potassium sulphate and 2% selenium), Approved Method 46-12 (AACC 2000). Results were expressed on a 14% moisture basis. Hardness was determined by a modified Particle Size Index (PSI) method (Approved Method 55-30A, AACC 2000). PSI value was the percentage of ground sample (10.00g) that passed through a 200 Mesh sieve after 10 min shaking with a rotator Rotap Model RX 29 –10 (Laval Lab Inc., Laval, QC, Canada). Samples with PSI

over 57% were considered soft. Wet (WG) and dry gluten (DG), as well as Gluten Index (GI) were determined by the Approved Method 38-12A (AACC 2000). SDS sedimentation tests were according to Peña et al (1990). Flour (1.0g) was placed in a 25-mL cylinder. Aqueous 0.001% (w/v) bromophenol blue solution (6ml) was added and the contents were vortexed (5 sec). The cylinder was left for 5 min, with two more vortexing after 2 min 45 sec and 4 min 45 sec. At this point, 0.5% (v/v) lactic acid, 2% (w/v) SDS solution (19mL) was added and the cylinder was shaken horizontally for 1 min on a mechanical oscillating shaker at 40 cycles/min. The sediment volume (mL) was measured 14 min after standing (SDSS). All analyses were done in duplicate.

3.3.3. SE-HPLC

Flour (100 mg) was extracted with 10 ml of 0.5% SDS-0.05M sodium phosphate buffer, pH 6.9, sonicated for 15 sec (output 20 W, 9-mm diameter probe; Ohtake Works Sonicator, Tokyo, Japan) and centrifugated at 16,000 x g for 10 min. The supernatant was filtered through a 0.45 μm polyvinylidene difluoride filter membrane (Durapore, Millipore Corp., Ireland) and immediately heated for 2 min at 80°C in a water bath. The extract (20 μl) was injected in a Superdex 200 HR 10/30 size exclusion analytical column (10 x 300 mm), equilibrated with 0.05M sodium phosphate buffer (pH 6.9). Column elution was isocratic for 35 min with a flow rate of 0.5 ml/min at room temperature, using an HPLC Äkta Purifier System (Amersham Pharmacia Biotech, Uppsala, Sweden). Absorbance was measured at 214 nm (Dalla-Rizza et al 2005). Polymeric glutenin (Glu) and monomeric protein (Mon) content in flour were determined, as well as their ratio (Glu:Mon). In addition, the amount of soluble protein was estimated by the total area of the chromatogram (TArea).

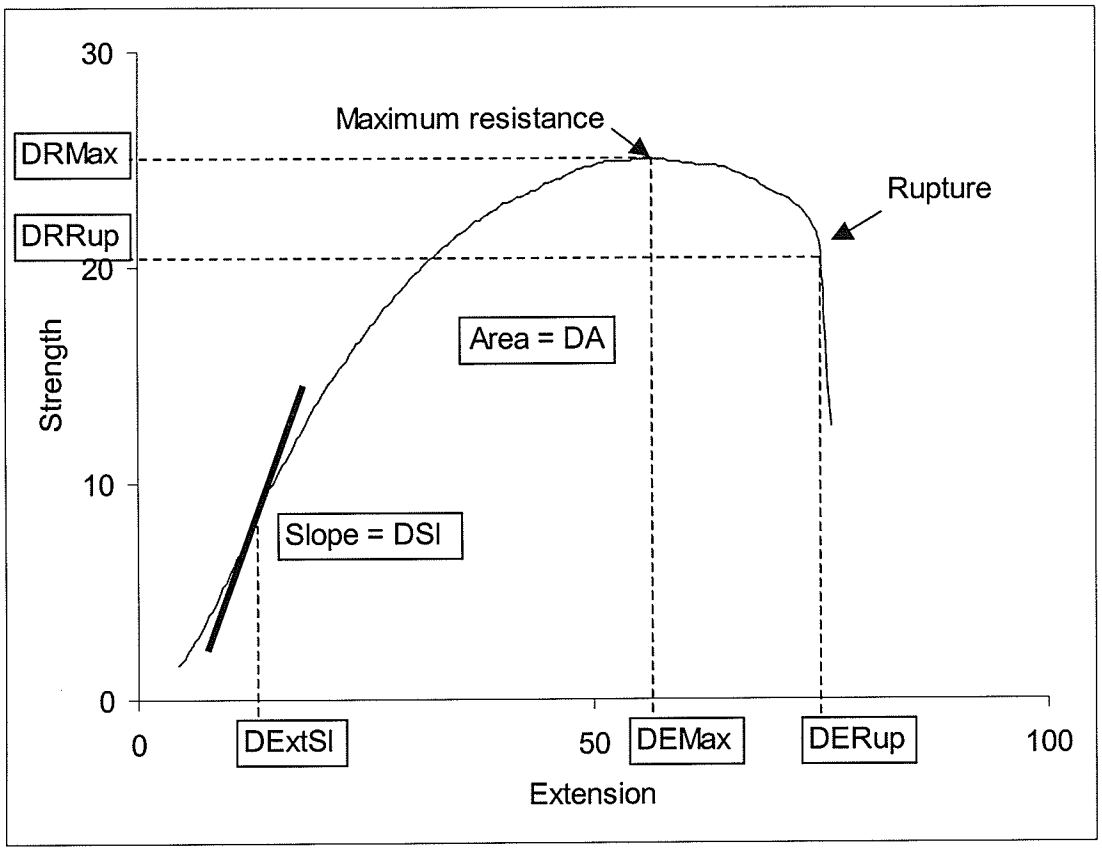
3.3.4. Rheological analyses

Farinograph dough development time (DDT), mixing stability (FarS) and absorption (Abs) were obtained by the Approved Method 54-21 (AACC 2000). Mixograph maximum height (MixH) and mixing time (MixT) were obtained by the Approved Method 54-40A (AACC 2000). Alveogram variables (W, P, L and P/L) were obtained using the standard alveograph procedure (Approved Method 54-30A, AACC 2000). A modified alveograph procedure using water adjusted according to farinograph absorption was done in addition to the standard alveograph technique which uses a 50% absorption. In the modified procedure, the amount of water added was farinograph absorption minus 8. In this way, when absorption was 58%, 50% absorption was used. Equivalent variables were obtained (Wad, Pad, Lad and P/Lad).

3.3.5. Micro-extension tests

Both flour and gluten dough extensional properties were determined with a QTS 25 Texture Analyser (CNS Farnell-Brookfield, Borehamwood, UK). Flour (10 g) was mixed with 2% sodium chloride solution (4.8 mL) up to the optimum development using a Glutomatic gluten machine. Dough was rested for 60 min at 28°C, strips (4 x 5 x 40 mm) were cut, and rested again 60 min at 28°C. Each strip was extended with a 1.5 mm diameter hook at 40 mm/min until rupture. Stress was recorded versus extension (Figure 3.1). The maximum slope of the curve was named DSl, while the length at that point was named DExtSl. Maximum resistance to extension was named DRMax, and the length at that point was named DEMax. The resistance and length at rupture was named DRRup and DERup respectively. The total area under the curve was named DA. Similarly, gluten was washed by the standard procedure using the Glutomatic washing machine,

Figure 3.1. An example of a flour dough micro-extension curve with illustration of variables read from the curves. DA: area under the curve, DSl: maximum slope of the curve, DExtSl: extensibility at maximum slope, DRMax: maximum resistance, DEMax: extensibility at maximum resistance, DRRup: resistance at rupture, DERup: extensibilty at rupture.

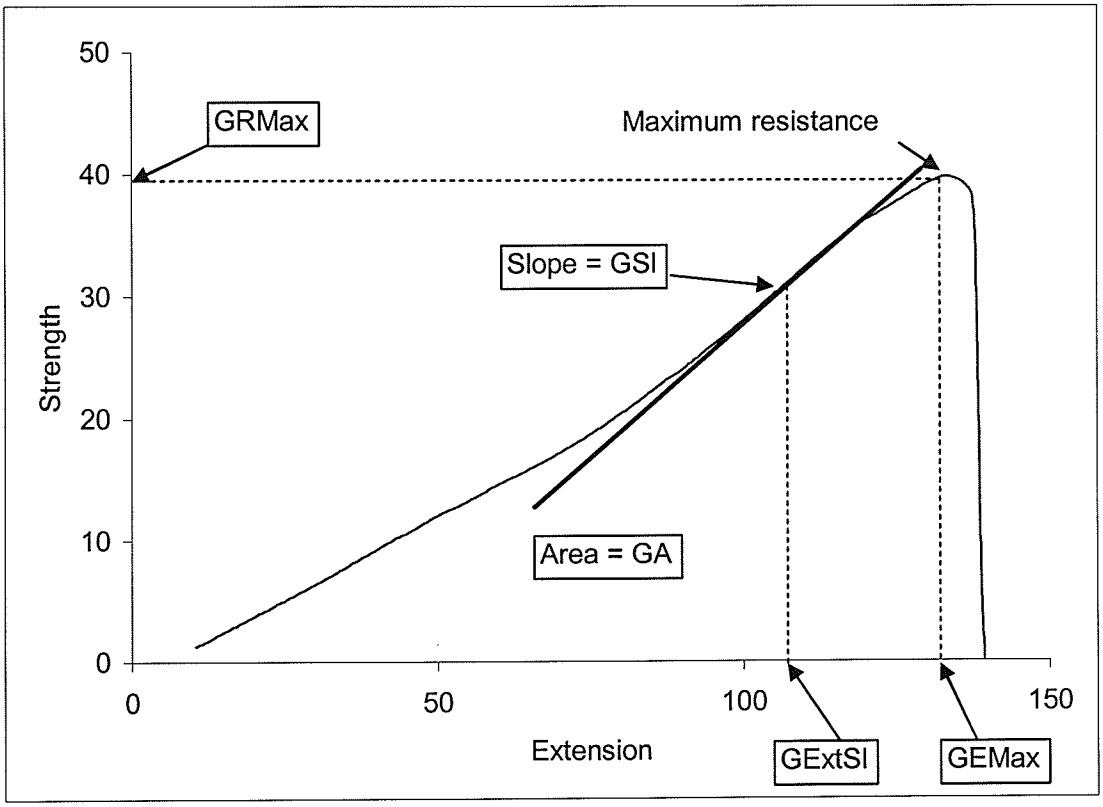


and equivalent variables (GSl, GExtSl, GRMax, GEMax, and GA; Figure 3.2) were determined on the gluten dough after the same resting and moulding procedure.

3.3.6. Baking tests

Two different kinds of breads were baked. Pan loaves were prepared according to Approved Method 10-10B (AACC 2000). Loaf volume (LV) was used as the quality estimator. French-type (Fr) hearth loaves were prepared, based on typical Uruguayan

Figure 3.2. An example of a gluten dough micro-extension curve with illustration of variables read from the curves. GA: area under the curve, GSl: maximum slope of the curve, GExtSl: Extensibility at maximum slope of the curve, GRMax: maximum resistance, GEMax: extensibility at maximum resistance.



procedures. Flour (100 g 14% moisture basis) was mixed with ascorbic acid (4 mg), yeast (2.2 g), salt (2.5 g) and the optimum amount of water (farinograph absorption + 2%) for the optimum mixing time (mixograph mixing time) in a 100-200 g mixer (National Manufacturing Division, TMCO, USA). Dough was rested for 90 min in a greased bowl in a fermentation cabinet (28°C, humidity over 80%). Then, the pieces were sheeted three times, decreasing the rolls distance (11/32", 1/4" and 3/16") with a Sheeter and Roll Molder (National Manufacturing Division, TMCO, USA). Dough was rolled up using again the Sheeter and Roll Molder (National Manufacturing Division, TMCO, USA). The formed

dough pieces were carefully transferred to flat trays and proofed for 90 min in the same cabinet. Three diagonal cuts were made on the top surface of the loaf using a retractable razor blade knife and pieces were immediately baked for 20 min at 212°C saturated with steam. Besides the volume (FrV), quality measurements of loaves were width (FrW), height (FrH), width:height ratio (FrR) and a subjective score used to evaluate crumb appearance (FrA): the more open the crumb the highest the score (1-9).

3.3.7. Statistical analysis

Single correlation coefficients were calculated using the Statistica package (StatSoft Inc., Tulsa, OK, USA). Regression analyses were conducted using REG procedure of SAS software (Version 8.0, 1999, SAS Institute, Cary, NC, USA). The method used was stepwise with a significance level of entry of 0.01 and a significance level of staying of 0.15. Principal components (PCA) and partial least squares (PLS) regression analyses were conducted using the Statistica PCA and PLS modules respectively, starting from the standardized set of data (mean = 0, standard deviation = 1).

3.4. RESULTS AND DISCUSSION

3.4.1. Characterization of the sample set

Results showed that samples varied greatly in composition and in rheological properties. This was attributed to inclusion of samples of 27 different genotypes grown in 10 environments, and to having samples of both Uruguayan and Canadian origin in the sample set.

Grain protein varied from 8.8-15.2 % (14% moisture basis; Table 3.1). The highest value was for the Canadian Western Red Spring composite, but one of the INIA Mirlo samples had 14.8% grain protein. The range was a wide one for Uruguayan wheats (Ernst et al 2002). The spread of flour protein values was even greater, from 7.1-14.6%. The amount of wet gluten was less than 15% and dry gluten less than 5% in some low protein samples, but almost three times that amount in some high protein samples. The low wet and dry gluten values could have been due to an underestimation because the method used typically is less accurate for low protein samples. Wide variability could be extended to both glutenin content (28-84 AU) and monomeric proteins content (58-209 AU).

Variation was wide not just in protein content but also in gluten quality. This observation was verified by the values for the Gluten Index (59-100 %), SDS sedimentation volume (9.5-23.0 ml) and gluten components variables. Values of PSI showed that both hard (39-56 %) and soft wheats (58-65 %) were present in the sample set.

Flour dough strength, estimated either from farinogram, mixogram or alveogram, also varied widely (Table 3.2). Farinograph stability ranged from 1:30 to 41:30 min and standard alveograph baking strength varied from 77 to 540 j x 10^{-4}. Values for other rheological variables, like extensibility and resistance to extension, were also very low for some samples but moderate to high for others. The variability was also reflected in wide ranges in the micro extension test values (Table 3.3) and bread quality results (Table 3.4).

Table 3.1

Characterization of the sample set. Grain quality variables[a]

Variable	Unit	Min	Max	Avg	SD
Flour protein content (14% m.b.)	%	7.1	14.6	10.7	1.3
Grain protein content (14% m.b.)	%	8.8	15.2	11.9	1.2
Wet gluten content	%	12.6	46.1	29.6	5.6
Dry gluten content	%	4.2	15.1	9.9	1.9
Gluten index	%	59	100	92	11
Particle Size Index (hardness)	%	39	65	48	5
SDS sedimentation volume	mL	9.5	23.0	16.0	3.7
Polymeric glutenin	Absorbance units	28	84	57	11.6
Monomeric proteins	Absorbance units	58	209	118	25.9
Soluble proteins	Absorbance units	95	273	175	31.5
Glutenin to monomeric proteins ratio	-	0.31	0.95	0.60	0.170

[a]Max: Maximum, Min: Minimum, Avg: Average, SD: standard deviation

Table 3.2

Characterization of the sample set. Rheological variables[a]

Variable	Unit	Min	Max	Avg	SD
Farinogram					
Dough development time	min	1:15	20:00	7:39	04:22
Stability	min	1:30	41:30	15:32	10:25
Absorption	%	51.0	67.3	58.3	3.5
Mixogram					
Maximum height	cm	2.6	7.4	5.1	0.9
Mixing time	min	1.9	10.0	4.2	1.4
Standard Alveogram					
Baking strength (W)	10^{-4}j	77	540	263	104
Maximum resistance to extension (P)	mm	34	149	78	22
Extensibility (L)	mm	30	155	98	26
Resistance:extensibility ratio (P/L)	-	0.25	2.29	0.90	0.44
Adjusted Absorption Alveogram					
Baking strength (Wad)	10^{-4}j	92	424	243	79
Maximum resistance to extension (Pad)	mm	37	151	81	24
Extensibility (Lad)	mm	19	152	86	27
Resitance:extensibility ratio (P/Lad)	-	0.33	7.95	1.20	1.13

[a] Max: Maximum, Min: Minimum, Avg: Average, SD: standard deviation

Table 3.3

Characterization of the sample set. Micro-extension studies variables[a]

Variable	Unit	Min	Max	Avg	SD
Flour dough					
Area under the curve	g.mm	440	2928	1229	545
Maximum slope of the curve	g/mm	0.6	1.9	1.1	0.3
Extensibility at maximum slope of the curve	mm	7.2	11.9	9.2	1.0
Maximum resistance	g	7.2	42.5	19.1	7.4
Extensibility at maximum resistance	mm	17.1	82.8	45.2	12.9
Resistance at rupture	g	3.8	30.1	13.5	6.2
Extensibilty at rupture	mm	30	92	59	11.7
Gluten dough					
Area under the curve	g.mm	1199	7673	3906	1341
Maximum slope of the curve	g/mm	0.1	3.0	0.8	0.5
Extensibility at max. slope of the curve	mm	30	113	84	16.3
Maximum resistance	g	12	106	51	23.6
Extensibility at maximum resistance	mm	52	164	111	19.9

[a] Max: Maximum, Min: Minimum, Avg: Average, SD: standard deviation

Table 3.4

Characterization of the sample set. Baking quality variables[a]

Variable	Unit	Min	Max	Avg	SD
Pan bread (AACC 10-10)					
Loaf volume	mL	445	1018	799	117
French-type bread					
Loaf width	cm	5.9	8.5	7.0	0.6
Loaf height	cm	3.3	6.9	5.3	0.8
Loaf volume	mL	373	745	573	79
Crumb appearance (subjective score)	Arbitrary units	2.5	8.0	5.2	1.0
Loaf width:height ratio	-	0.43	0.95	0.77	0.13

[a] Max: Maximum, Min: Minimum, Avg: Average, SD: standard deviation

3.4.2. Influence of composition and extensional properties on pan bread loaf volumes

3.4.2.1. Simple correlation results

The correlations for pan loaf volume with the protein related components are given in Table 3.5. The parameter with higher correlation coefficient with pan loaf volume (LV) was flour protein, closely followed by grain protein (r = 0.706, and 0.680 respectively, P<0.001). Dry gluten and wet gluten also are associated with protein content. Moreover, they are estimators of the functional protein components content. Therefore, a high correlation with breadmaking properties was expected with these components. However, the actual correlations with loaf volume (r = 0.540 and 0.514 for DG and WG respectively), although highly significant (P< 0.001) were lower than for total proteins These lower values could be explained by the higher error of Glutomatic methodology in comparison with Kjeldahl determinations.

Pan loaf volume was correlated with SDSS (r = 0.515, P<0.001), but not with Gluten Index. The SDSS estimates both gluten strength and protein content, but Gluten Index is an estimator of gluten strength independently of the protein content of the flour (Peña et al 1990). These results showed that SDSS is a valuable tool for screening. Hardness on the other hand did not correlate with loaf volume, but no conclusions can be stated since most samples were hard, and just a small portion (6%) were soft (data not shown).

Correlations of pan loaf volume with rheological properties, indicated that LV was best correlated with the adjusted alveogram resistance/extensibility ratio (P/Lad) (r = -0.701; P<0.001). Loaf volume was also highly correlated with the resistance/extensibility ratio

for the standard alveograph technique (P/L) (r = -0.650; P<0.001). These variables depend on both the measurement of extensibility (L and Lad) and of resistance to

Table 3.5
Correlations of breadmaking variables with quality characteristics[a, b]

Variables	LV	FrW	FrH	FrV	FrA	FrR
Flour protein content	0.706***	0.480***	0.130	0.574***	0.533***	-0.106
Grain protein content	0.680***	0.471***	0.153	0.554***	0.504***	-0.083
Wet gluten content	0.514***	0.368**	-0.288	0.250	0.476***	-0.401**
Dry gluten content	0.540***	0.403**	-0.242	0.295	0.509***	-0.380**
Gluten index	0.127	-0.209	0.688***	0.252	-0.192	0.673***
Particle Size Index (hardness)	0.165	-0.076	-0.261	-0.182	-0.051	-0.177
SDS sedimentation volume	0.515***	0.096	0.589***	0.459***	0.089	0.453***
Farinogram						
Dough development time	0.515***	-0.022	0.337**	0.399**	0.142	0.305
Stability	0.479***	-0.175	0.502***	0.347**	0.040	0.520***
Absorption	0.229	0.229	-0.145	0.288	0.272	-0.226
Mixogram						
Maximum height	0.450***	0.188	0.103	0.456***	0.396**	-0.001
Mixing time	-0.153	-0.128	0.642***	0.140	-0.257	0.598***
Standard Alveogram						
Baking strength (W)	0.436***	-0.051	0.583***	0.492***	0.007	0.523***
Maximum resistance (P)	-0.090	-0.114	0.457***	0.244	-0.177	0.441***
Extensibility (L)	0.658***	0.064	0.076	0.307	0.212	0.041
Resistance:ext. ratio (P/L)	-0.650***	-0.201	0.178	-0.240	-0.382**	0.240
Adjusted Abs. Alveogram						
Baking strength (Wad)	0.413**	-0.217	0.717***	0.402***	-0.014	0.718***
Maximum resistance (Pad)	-0.432***	-0.301	0.455***	-0.132	-0.393***	0.524***
Extensibility (Lad)	0.596***	0.004	0.021	0.286	0.248	0.023
Resistance:ext. ratio (P/Lad)	-0.701***	-0.283	0.048	-0.438***	-0.460***	0.170
Flour dough micro-extension						
Area under the curve	0.427***	-0.062	0.429***	0.324	0.038	0.398**
Maximum slope of the curve	-0.233	-0.398**	0.284	-0.082	-0.320	0.439***
Extensibility at max. slope	0.206	0.273	0.316	0.371**	0.166	0.128
Maximum resistance	0.207	-0.188	0.462***	0.225	-0.159	0.487***
Ext. at max. resistance	0.576***	0.053	0.495***	0.480***	0.185	0.392**
Resistance at rupture	0.137	-0.230	0.448***	0.189	-0.170	0.495***
Extensibilty at rupture	0.658***	0.287	0.124	0.364**	0.406***	-0.037
Gluten dough micro-extension						
Area under the curve	-0.025	-0.087	0.378**	0.163	-0.122	0.348**
Maximum slope of the curve	-0.398**	-0.322	0.239	-0.200	-0.429**	0.350**
Extensibility at max. slope	0.439***	0.137	0.171	0.375**	0.259	0.081
Maximum resistance	-0.202	-0.237	0.395**	0.021	-0.317	0.440***
Extensibility at max. res.	0.300	0.324	-0.307	0.125	0.400***	-0.416***

[a] LV: pan loaf volume; FrW, FrH, FrV, FrA, FrR: French-type loaf width, height, volume, appearance and form ratio, respectively.
[b] **: P<0.01; ***: P<0.001.

extension (P and Pad). Loaf volume was also closely related to the extensibility values L and Lad (r = 0.658 and 0.596, respectively P<0.001). However, although loaf volume was correlated to the adjusted alveogram resistance to extension (Pad) (r = -0.432; P<0.001), loaf volume was not related to the standard resistance to extension value (P). Mixing properties were correlated with loaf volume, but correlation coefficients were clearly lower than those related to extensional properties. Loaf volume was significantly correlated with both farinograph dough development time and stability (r = 0.515 and 0.479, respectively; P<0.001) and to mixograph maximum height (r = 0.450; P<0.001), but not to farinograph absorption and mixograph mixing time.

3.4.2.2. Multiple Linear Regression (MLR) results.

Simple correlations between baking quality characteristics and rheological properties were significant, but in no case did the correlation coefficients explain more than 50% of the variability. MLR analysis was applied in order to improve prediction performance. Results of MLR analyses conducted by including all rheological variables as independent variables and all breadmaking quality variables as dependent variables are presented in Table 3.6.

Regression results showed that 75% of LV variability could be predicted by rheological variables. Most of the variability (72%) was explained by extensional variables (adjusted alveogram resistance/extensibility ratio, extensibility at maximum resistance of dough micro-extensigram and standard alveogram resistance/extensibility ratio). The only mixing property included in the model was mixogram mixing time, with a minor contribution (3%). The improvement in prediction by including new variables is presented

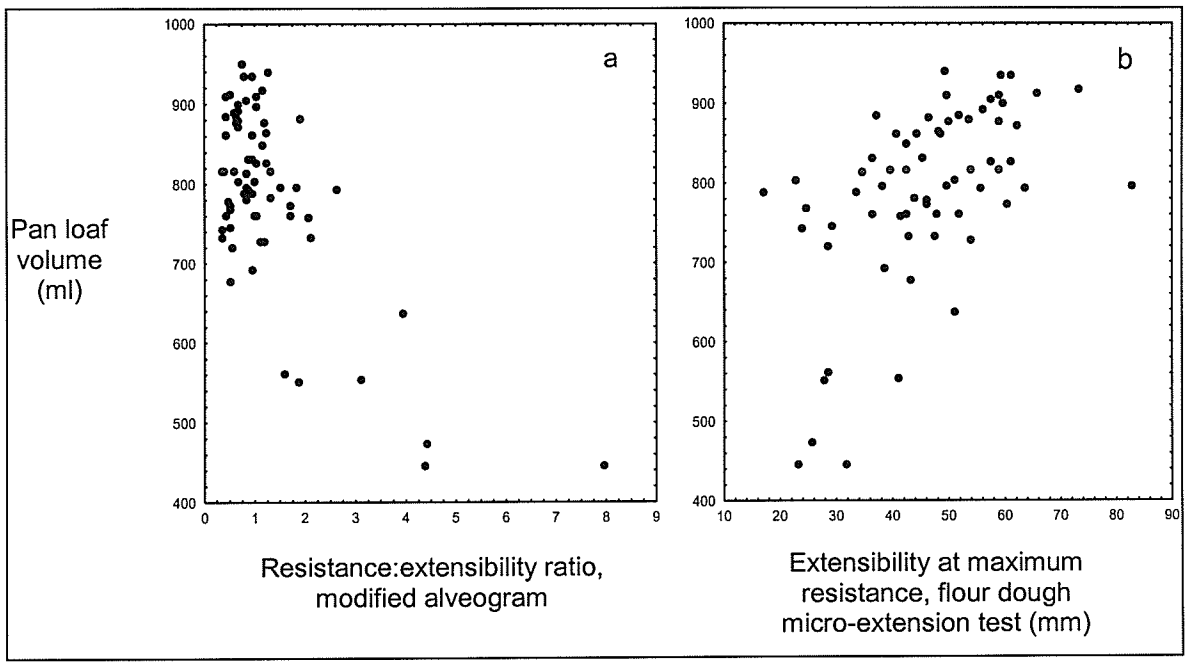
Table 3.6
Regression analysis results of breadmaking parameters including all rheological parameters into the model

	Step	Variable Entered	Variable Removed	Partial RSq	Model RSq	C(p)	F	Pr>F
LV	1	PLad		0.492	0.492	76.6	59.04	<.0001
	2	DEMax		0.171	0.663	32.9	30.50	<.0001
	3	PL		0.056	0.719	20.0	11.70	0.0011
	4	MixT		0.031	0.750	13.7	7.22	0.0094
FrW	1	DSI		0.162	0.162	52.7	11.81	0.0011
FrH	1	Wad		0.526	0.526	54.4	67.64	<.0001
	2	MixT		0.088	0.614	35.4	13.61	0.0005
	3	GExtSI		0.049	0.662	25.7	8.54	0.0049
FrV	1	DEMax		0.239	0.239	63.1	19.20	<.0001
	2	PLad		0.110	0.349	47.5	10.13	0.0023
	3	MixT		0.109	0.459	31.9	11.92	0.0010
	4		DEMax	0.013	0.446	32.0	1.42	0.2378
FrA	1	PLad		0.213	0.213	20.5	16.46	0.0001
FrR	1	Wad		0.513	0.513	76.4	64.26	<.0001
	2	DERup		0.128	0.641	42.9	21.33	<.0001

[a] LV: pan loaf volume; FrW, FrH, FrV, FrA, FrR: French-type loaf width, height, volume, appearance and form ratio, respectively. MixT: mixogram mixing time (MixT); P/L: standard alveogram resistance/extensibility ratio. Wad, P/Lad: adjusted water alveogram baking strength and resistance/extensibility ratio, respectively. DSI, DEMax and DERup: flour dough micro-extensigram maximum slope of the curve, extensibility at maximum resistance and extensibilty at rupture, respectively. GExtSI: Gluten dough micro-extensigram extensibility at maximum slope of the curve.

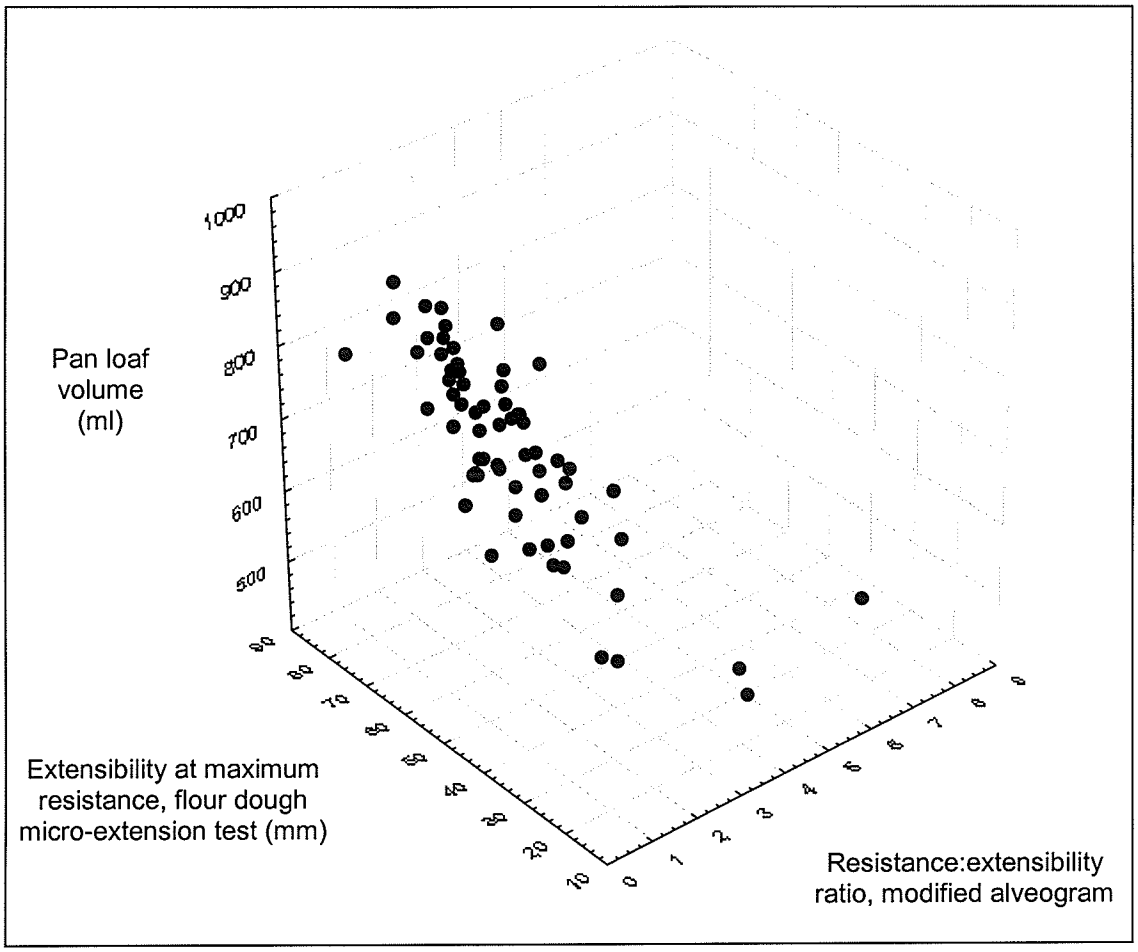
in Figures 3.3 and 3.4. Loaf volume was highly correlated with adjusted alveogram resistance/extensibility ratio and extensibility at maximum resistance of dough micro-extensigram, the two first included in the regression model, but the plots showed a very dispersed relationship (Figure 3.3). When both were included at the same time, the dispersion was minimized (Figure 3.4).

Figure 3.3. Influence of resistance:extensibility ratio of the modified alveogram (a) and the extensibility at maximum resistance of flour dough micro-extension test (b) on pan loaf volume.



Similar models were run, including only farinogram (Table 3.7), mixogram (Table 3.8), standard alveogram (Table 3.9), modified alveogram (Table 3.10), gluten dough micro-extensigram (Table 3.11) and flour dough micro-extensigram (Table 3.12) data as independent variables. Modified alveogram values for adjusted alveogram resistance/extensibility ratio and adjusted alveogram baking strength gave the best results, and predicted over 60% of LV variability. The conclusion was that although the resistance to extension/extensibility ratio was the most important individual variable, when a strength parameter was included in the model bread quality was more fully explained. Mixing tests failed to give a good prediction of LV (r^2 = 0.266 and 0.200 for farinogram and mixogram respectively). Even standard alveogram results were better than mixing tests to predict LV (r^2 = 0.433).

Figure 3.4. Simultaneous influence of resistance:extensibility ratio of the modified alveogram (a) and the extensibility at maximum resistance of flour dough micro-extension test (b) on pan loaf volume.



3.4.2.3. Principal Component Analysis (PCA).

PCA analysis was useful to group rheological variables (Figure 3.5). Most extensibility variables (L, Lad, DERup and GExtSl) were in the same region. In a second region there were several variables related to gluten strength: W, Wad, FarS and DA were very close to each other. It is important to remark that they belong to four different tests. Therefore, gluten strength determination was the same using the farinogram, standard or modified alveogram or even the micro-extensigram. No mixogram or gluten micro-extension

Table 3.7
Regression analysis results of breadmaking variables including only farinogram variables into the model [a]

	Step	Variable Entered	Variable Removed	Partial RSq	Model RSq	F	Pr>F
LV	1	DDT		0.266	0.266	22.05	<.0001
FrW	No variable met the 0.01 significance level for entry into the model						
FrH	1	FarS		0.256	0.256	21.00	<.0001
FrV	1	DDT		0.162	0.162	11.80	0.0011
FrA	No variable met the 0.01 significance level for entry into the model						
FrR	1	FarS		0.267	0.267	22.20	<.0001

[a] LV: pan loaf volume; FrW, FrH, FrV, FrA, FrR: French-type loaf width, height, volume, appearance and form ratio, respectively. DDT, FarS: farinogram dough development time and stability, respectively.

Table 3.8
Regression analysis results of breadmaking variables including only mixogram variables into the model [a]

	Step	Variable Entered	Variable Removed	Partial RSq	Model RSq	F	Pr>F
LV	1	MixH		0.200	0.200	15.27	0.0002
FrW	No variable met the 0.01 significance level for entry into the model						
FrH	1	MixT		0.406	0.406	41.66	<.0001
	2	MixH		0.113	0.519	14.04	0.0004
FrV	1	MixH		0.219	0.219	17.14	0.0001
	2	MixT		0.091	0.310	7.86	0.0068
FrA	1	MixH		0.158	0.158	11.46	0.0012
FrR	1	MixT		0.356	0.356	33.77	<.0001

[a] LV: pan loaf volume; FrW, FrH, FrV, FrA, FrR: French-type loaf width, height, volume, appearance
and form ratio, respectively. MixH, MixT: mixogram maximum height and mixing time, respectively.

Table 3.9
Regression analysis results of breadmaking variables including only standard alveogram variables into the model[a]

	Step	Variable Entered	Variable Removed	Partial RSq	Model RSq	F	Pr>F
LV	1	L		0.433	0.433	46.58	<.0001
FrW	No variable met the 0.01 significance level for entry into the model						
FrH	1	W		0.337	0.337	31.04	<.0001
FrV	1	W		0.238	0.238	19.05	<.0001
FrA	1	P/L		0.146	0.146	10.46	0.0020
FrR	1	W		0.268	0.268	22.32	<.0001

[a] LV: pan loaf volume; FrW, FrH, FrV, FrA, FrR: French-type loaf width, height, volume, appearance and form ratio, respectively. W, L, P/L: standard alveogram baking strength, extensibility and resistance/extensibility ratio, respectively.

Table 3.10
Regression analysis results of breadmaking variables including only modified alveogram variables into the model[a]

	Step	Variable Entered	Variable Removed	Partial RSq	Model RSq	F	Pr>F
LV	1	P/Lad		0.492	0.492	59.04	<.0001
	2	Wad		0.122	0.614	18.98	<.0001
FrW	No variable met the 0.01 significance level for entry into the model						
FrH	1	Wad		0.526	0.526	67.64	<.0001
FrV	1	P/Lad		0.196	0.196	14.83	0.0003
	2	Wad		0.141	0.336	12.72	0.0007
FrA	1	P/Lad		0.213	0.213	16.46	0.0001
FrR	1	Wad		0.513	0.513	64.26	<.0001
	2	Pad		0.054	0.567	7.53	0.0080
	3	Lad		0.047	0.614	7.20	0.0095

[a] LV: pan loaf volume; FrW, FrH, FrV, FrA, FrR: French-type loaf width, height, volume, appearance and form ratio, respectively. Wad, Pad, Lad, P/Lad: modified alveogram baking strength, resistance, extensibility and resistance/extensibility ratio, respectively.

Table 3.11
Regression analysis results of breadmaking variables including only gluten dough micro-extensigram variables into the model[a]

	Step	Variable Entered	Variable Removed	Partial RSq	Model RSq	F	Pr>F
LV	1	GExtSl		0.193	0.193	14.62	0.0003
FrW	1	GSl		0.106	0.106	7.25	0.0092
FrH	1	GRMax		0.149	0.149	10.66	0.0018
	2	GExtSl		0.185	0.334	16.69	0.0001
	3	GEMax		0.175	0.509	21.09	<.0001
	4		GRMax	0.012	0.497	1.48	0.2290
FrV	1	GExtSl		0.144	0.144	10.25	0.0022
FrA	1	GSl		0.185	0.185	13.87	0.0004
FrR	1	GRMax		0.184	0.184	13.74	0.0005
	2	GExtSl		0.122	0.306	10.51	0.0019
	3	GEMax		0.260	0.565	35.29	<.0001
	4		GRMax	0.002	0.563	0.28	0.6002

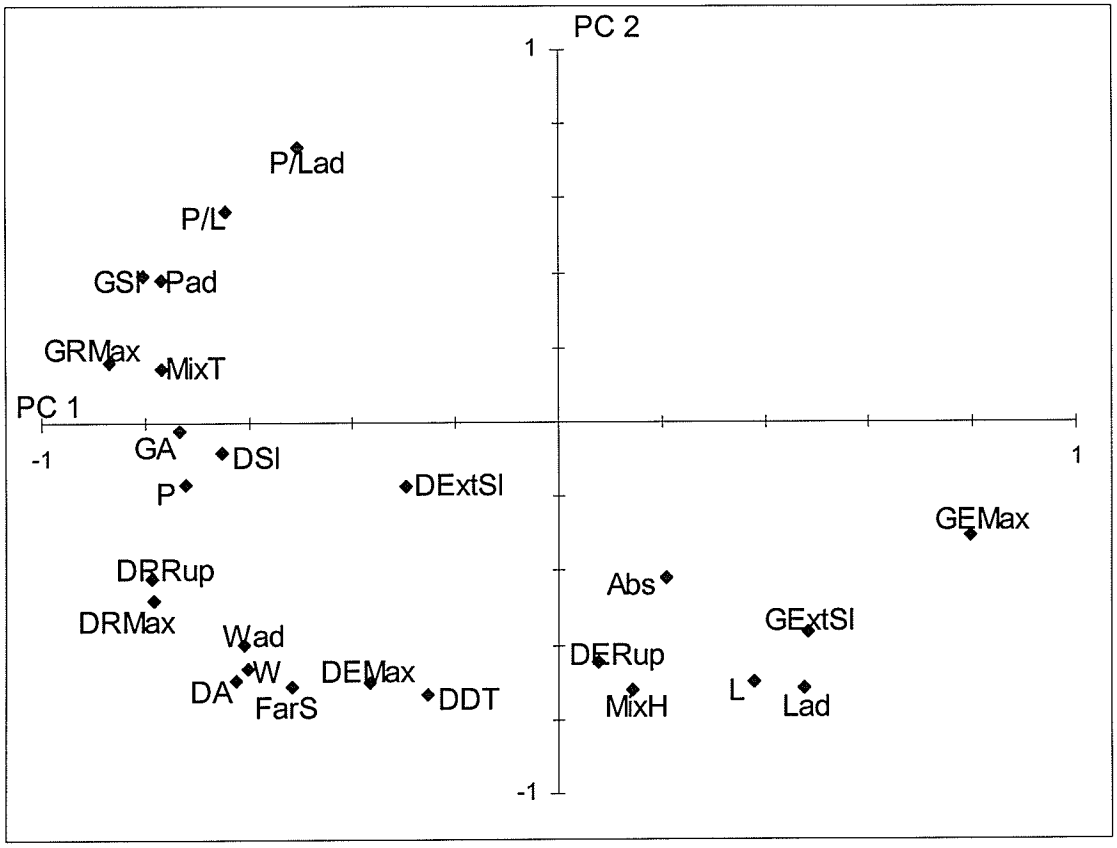
[a] LV: pan loaf volume; FrW, FrH, FrV, FrA, FrR: French-type loaf width, height, volume, appearance and form ratio, respectively. GSl, GExtSl, GRMax, GEMax: gluten dough micro-extensigram: maximum slope of the curve, extensibility at maximum slope of the curve, maximum resistance and extensibility at maximum resistance

Table 3.12
Regression analysis results of breadmaking variables including only dough micro-extensigram variables into the model[a]

	Step	Variable Entered	Variable Removed	Partial R-Square	Model R-Square	F	Pr>F
LV	1	DERup		0.432	0.432	46.41	<.0001
FrW	1	DSl		0.162	0.162	11.81	0.0011
FrH	1	DEMax		0.255	0.255	20.91	<.0001
	2	DERup		0.220	0.476	25.20	<.0001
FrV	1	DEMax		0.239	0.239	19.20	<.0001
FrA	1	DERup		0.166	0.166	12.12	0.0009
FrR	1	DRRup		0.240	0.240	19.23	<.0001

[a] LV: pan loaf volume; FrW, FrH, FrV, FrA, FrR: French-type loaf width, height, volume, appearance and form ratio, respectively. DSl, DEMax, DERup: flour dough micro-extensigram maximum slope of the curve, extensibility at maximum resistance and extensibilty at rupture.

Figure 3.5. Principal components analysis showing loading plot of rheological variables. Farinogram: dough development time (DDT), stability (FarS) and absorbance (Abs). Mixogram: maximum height (MixH) and mixing time (MixT). Standard alveogram: baking strength (W), maximum resistance to extension (P), extensibility (L) and resistance/extensibility ratio (P/L). Adjusted water alveogram: baking strength (Wad), maximum resistance to extension (Pad), extensibility (Lad) and resistance/extensibility ratio (P/Lad). Flour dough micro-extensigram: maximum slope of the curve (DSl), extensibility at maximum slope of the curve (DExtSl), maximum resistance (DRMax), extensibility at maximum resistance (DEMax), resistance at rupture (DRRup), extensibilty at rupture (DERup), area under the curve (DA). Gluten dough micro-extensigram: maximum slope of the curve (GSl), extensibility at maximum slope of the curve (GExtSl), maximum resistance (GRMax), extensibility at maximum resistance (GEMax) and area under the curve (GA).



variables were included in this region. All variables related to resistance to extension (P, P/L, Pad, P/Lad, DSl, DRMax, DRRup, GSl and GRMax) were in a region of highly negative values of Factor 1, but with a high variability in Factor 2. It is remarkable that

Abs and MixH were close to each other. This proximity is explained clearly since they are both related with absorption. In mixogram, absorption is adjusted just according to protein content. Therefore, higher MixH will imply higher absorption. In conclusion, if absorption value is not available, it could be inferred by MixH. They were also close to extensibility variables. Therefore, there is a close relationship between absorption and extensibility.

3.4.3. Influence of composition and extensional properties on hearth bread.

3.4.3.1. Simple correlation results

The variable best correlated with loaf volume of French bread (FrV) was flour protein,($r = 0.574$; $P<0.001$) (Table 3.5). In addition to volume, crumb appearance (FrA) and hearth bread shape measurements: width (FrW), height (FrH) and height/width ratio (FrR), are important components of French-type bread quality. Flour protein was also the parameter best associated with loaf appearance and width ($r=0.533$ and 0.480, respectively; $P<0.001$). Correlations of flour protein with form ratio and loaf height were not significant. Similar results were observed for grain protein, but correlation coefficients were consistently lower.

French bread loaf volume was not correlated with wet and dry gluten content, which might be explained by the higher error of the Glutomatic method as mentioned above. However, French bread type appearance was highly correlated with wet ($r = 0.476$) and dry gluten content (0.509). Wet and dry gluten were the only protein quantity predictors that were significantly correlated with the form ratio ($r = -0.401$ and -0.380, respectively; $P<0.01$). The amount of functional protein appears to have an important influence on

loaf properties other than volume, and even poor estimators like wet and dry gluten content were related to loaf appearance.

French bread loaf volume was well correlated with the indicators of strength standard alveogram baking strength (W), mixogram mixing time and adjusted alveogram baking strength (Wad) ($r = 0.492$, 0.456 and 0.402 respectively; $P<0.001$). French bread loaf height and form ratio, were even more highly correlated with mixogram mixing time ($r = 0.642$, and 0.598 respectively) and with adjusted alveogram baking strength ($r = 0.717$ and 0.718 respectively). In addition, adjusted alveogram resistance/extensibility ratio was also well correlated with volume ($r = -0.438$; $P<0.001$). For loaf height, the best correlations were with adjusted alveogram baking strength, mixogram mixing time, standard alveogram baking strength (W) and farinogram stability ($r = 0.717$, 0.642, 0.583 and 0.502; respectively; $P<0.001$). These four variables are from the four different rheological testing methods, used in this comparison. In addition, mixogram mixing time and farinogram stability are equivalent among the mixing variables, while W and Wad are equivalent among the extensional variables. All of them determine the dough strength, and were well related with the loaf height. Therefore, dough strength is a requirement for high hearth breads. No rheological variable was correlated with French bread loaf width. It is remarkable that while protein amount variables were correlated with width but not with height, rheological variables were correlated with height but not with width.

From the information presented above, it can be concluded that protein quantity affected loaf width and protein quality affected loaf height. The significant correlations of sedimentation volume and mainly Gluten Index with French bread loaf height (0.589 and

0.688, respectively; P<0.001) support this concept. More important than width and height is the shape of the hearth loaf, estimated with form ratio, which was correlated with adjusted alveogram baking strength, mixogram mixing time, adjusted alveogram maximum resistance to extension and standard alveogram baking strength (r = 0.718, 0.598, 0.524 and 0.523, respectively; P<0.001). The finding that hearth type breads require stronger flours was recently reported by Sliwinski et al (2004). The stronger requirements were clearly reflected in the higher correlation coefficients of strength estimators with form ratio. French bread loaf appearance was mainly predicted by adjusted alveogram resistance/extensibility ratio (P/Lad) and adjusted alveogram maximum resistance (Pad) (r = -0.460 and -0.393, respectively; P<0.001), but it also correlated with mixogram maximum height and standard alveogram resistance/extensibility ratio (P/L) (r = 0.396 and -0.382, respectively; P<0.01). The negative coefficient of correlation of French bread loaf appearance with P/Lad, Pad and P/L showed that a high resistance to extension will lead to a compact crumb, which is undesirable. At the same time, as it was mentioned, Pad was highly positively correlated with the form ratio. A high value of form ratio is desirable since it means that the loaf is keeping its shape. There must be a compromise between the factors that influence crumb and shape for the best hearth loaf quality .

3.4.3.2. Multiple Linear Regression (MLR) results.

Among the French-type bread variables, only 45% of the volume variability was explained by the best model (Table 3.6). Variables included in the French loaf volume model were adjusted alveogram resistance/extensibility ratio and mixogram mixing time. Extensibility at maximum resistance of dough micro-extensigram was also included in the French-type bread loaf volume model as the first variable, but it was removed after

the other two were included. It is concluded that independent of the bread type, these variables were the most important in predicting loaf volume. French-type loaf width was poorly predicted by MLR (coefficient of determination = r^2 = 0.162). However, two thirds of the height variability was explained by the model. As expected, variables included were related to gluten strength: the adjusted alveogram baking strength (Wad) and mixogram mixing time, with Wad being responsible for 53% of the variability, out of a total of 66%. The third one was a measurement of extensibility (extensibility at maximum slope of gluten micro-extensigram), although its contribution was small (5%). Therefore, although it is essential to have a strong gluten to obtain a loaf height, the dough should be extensible as well. MLR successfully explained 64% of the variability in form ratio with two variables included in the model, adjusted alveogram baking strength and extensibility at rupture of dough micro-extensigram (DERup). Therefore, in order to obtain a good shape, both gluten strength (Wad) and extensibility (DERup) are clearly necessary. Finally, French-type bread appearance was poorly predicted by MLR (r^2 = 0.213).

When models were run including only farinogram (Table 3.7), mixogram (Table 3.8), standard alveogram (Table 3.9), modified alveogram (Table 3.10), gluten dough micro-extensigram (Table 3.11) and flour dough micro-extensigram (Table 3.12) results as independent variables, French-type loaf volume prediction was worse than for pan loaf volume (34% of variation). Adjusted alveogram extensibility (Lad) was included in the model for form ratio. However, its contribution was only 5%. The important modified alveogram variables in the model for form ratio were baking strength and maximum resistance, proving that even though extensional properties were important in order to determine loaf shape, extensibility was not the most relevant property.

Farinogram variables were not able to predict either French-type bread measurements or pan loaf volume, the mixogram variables were. Even though French-type bread volume was poorly predicted (r^2 = 0.310, with both variables in the model), prediction was better for loaf height (r^2 = 0.519 with both variables). This finding supports the concept of the importance of dough strength, measured by both mixogram mixing time and maximum height on loaf height.

3.4.4. Comparison between pan and hearth loaf requirements

Bread volume variables (LV and FrV) were highly influenced by protein content estimators. This relationship was stronger for pan loaves than for French-type ones. Flour protein was better correlated than grain protein for both bread types volume variables. Among rheological variables, adjusted alveogram resistance/extensibility ratio was highly significantly correlated with volume for both bread types. However, dough strength estimators were as important as, or more, than adjusted alveogram resistance/extensibility ratio for other hearth bread variables, mainly loaf height and form ratio. The importance of dough strength in hearth bread can be explained by the necessity of the loaf to rise without having the support of the pan walls. This fact could explain also the lower correlation between French-type bread volume and flour protein than pan loaf volume and flour protein. Since strength is more important for hearth type breads, protein quantity is not as important as for pan bread. In any case, both a high resistance to extension/extensibility ratio and good dough strength were needed to obtain high volumes. In MLR analysis including all rheological variables, both adjusted alveogram resistance/extensibility ratio and mixogram mixing time were included in the models for prediction of both bread types volume variables, but extensibility were also in

the model for pan loaf volume. Different extensibility estimators were also highly correlated with both pan and French-type loaf volume (Table 3.5). Among the MLR analyses including only variables obtained from one test, adjusted alveogram gave the best results for both volume variables. Although results were better for pan bread, the same variables (baking strength, Wad, and resistance/extensibility ratio, P/Lad) were included for both bread types.

3.4.4.1. Partial least squares (PLS) analysis.

In order to make pan and hearth bread comparison more simple, a PLS regression analysis was run. The model was able to explain over 66% of LV using only two components (Table 3.13). The inclusion of a third component did not cause a clear improvement of the model. But for the hearth bread, even using three components, the model gave a poor explanation of loaf volume (r^2 = 0.402), width (r^2 = 0.396), height (r^2 = 0.457) and appearance (r^2 = 0.363), but it explained almost 60% (r^2 = 0.592) of the FrR variability. This was consistent with results mentioned above, in that to a larger extent form ratio variability depends on flour properties than do the rest of the French-type variables.

The x-loading plot of the first two components (Figure 3.6) shows that Principal Component 1 (PC1) was related to gluten strength variables like both alveogram baking strength (W and Wad), farinogram stability (FarS) and dough micro-extensigram area under the curve (DA), while Principal Component 2 (PC2) was negatively correlated with resistance to extension variables (gluten micro-extensigram maximum slope, dough micro-extensigram maximum slope, adjusted alveogram maximum resistance and standard alveogram resistance/extensibility ratio). It is important to remark that dough

Table 3.13
Coefficients of determination for the first three components in PLS regression model of breadmaking properties[a]

	LV	FrW	FrH	FrV	FrA	FrR
Component 1	0.339	0.002	0.365	0.282	0.012	0.290
Component 2	0.665	0.119	0.435	0.356	0.247	0.438
Component 3	0.668	0.396	0.457	0.402	0.363	0.592

[a] LV: pan loaf volume; FrW, FrH, FrV, FrA, FrR: French-type loaf width, height, volume, appearance and form ratio, respectively

strength and resistance to extension were orthogonal to each other, proving their independence. Extensibility variables like both alveogram ones (L and Lad), gluten micro-extensigram at maximum resistance and gluten at maximum slope were highly positively related with component 2. It is important to notice that dough extensibility at rupture estimators (L, Lad and DERup) were positively correlated with both PC1 and PC2.

Among the breadmaking variables (Figure 3.7), French-type loaf height was the closest to the dough strength region (high positive in PC1 axis, slightly negative in PC2 axis). This finding supported the concept that dough needs to be strong in order to have a high loaf volume. At the same time, French-type bread appearance was positively correlated with PC2, in the middle of the extensibility region and opposite to resistance to extension variables (Figure 3.6). Pan loaf volume was in the region of positive values of both PC1 and PC2, as were L, Lad and DERup, supporting the idea that the key property related to pan loaf volume is extensibility at rupture.

Figure 3.6. Partial least squares analysis showing x-loading plot of rheological variables of the two first principal components (PC1 and PC2). Farinogram: dough development time (DDT), stability (FarS) and absorbance (Abs). Mixogram: maximum height (MixH) and mixing time (MixT). Standard alveogram: baking strength (W), maximum resistance to extension (P), extensibility (L) and resistance/extensibility ratio (P/L). Adjusted water alveogram: baking strength (Wad), maximum resistance to extension (Pad), extensibility (Lad) and resistance/extensibility ratio (P/Lad). Flour dough micro-extensigram: maximum slope of the curve (DSl), extensibility at maximum slope of the curve (DExtSl), maximum resistance (DRMax), extensibility at maximum resistance (DEMax), resistance at rupture (DRRup), extensibilty at rupture (DERup), area under the curve (DA). Gluten dough micro-extensigram: maximum slope of the curve (GSl), extensibility at maximum slope of the curve (GExtSl), maximum resistance (GRMax), extensibility at maximum resistance (GEMax) and area under the curve (GA).

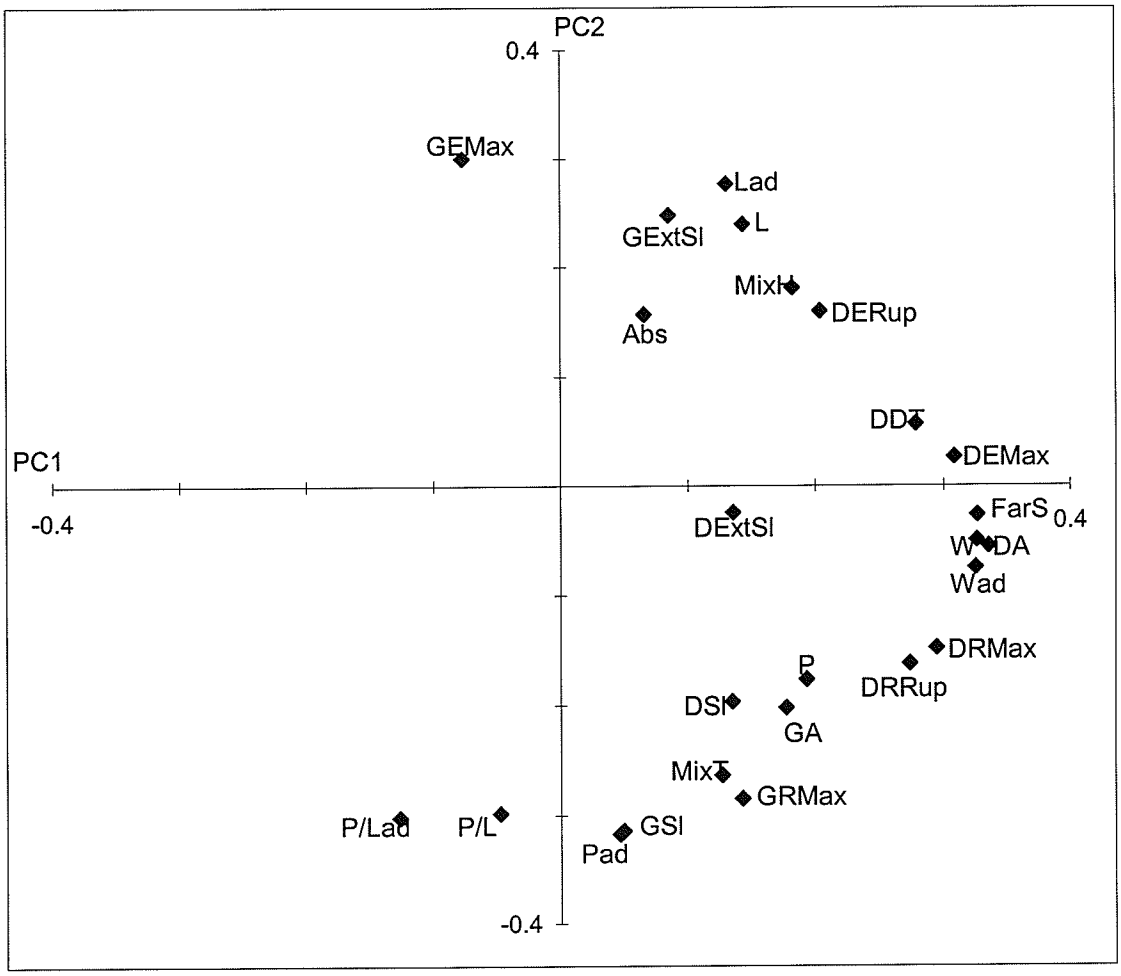
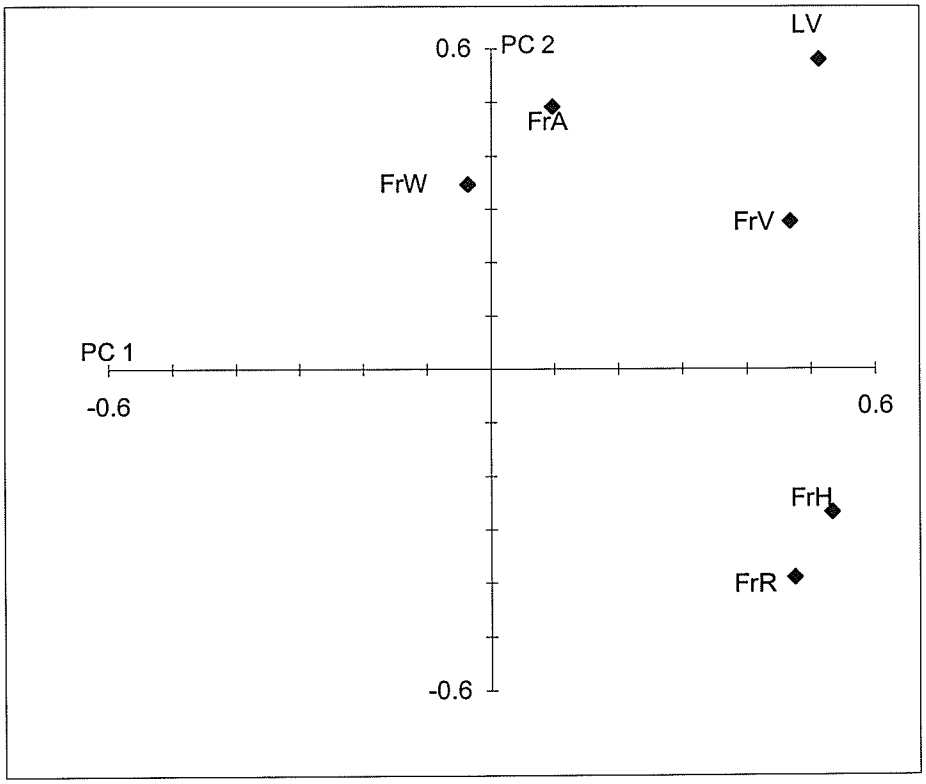


Figure 3.7. Partial least squares analysis showing y-loading plot of variables of the two first principal components (PC1 and PC2). LV: pan loaf volume; FrW, FrH, FrV, FrA, FrR: French-type loaf width, height, volume, appearance and form ratio, respectively.



3.4.5. Modified alveograph procedure

As it was already stated, the modified alveogram variables predicted the breadmaking quality variables better than the standard ones. Simple correlation coefficients were similar but in some cases the differences were important. For example, the correlation of pan loaf volume with resistance to extension was not significant for the standard procedure, but it was highly significant for the modified procedure ($r = -0.432$, $P<0.001$; Table 3.5). The advantage of the modified alveogram was more evident when MLR analyses were done using only one test variable. In all cases, the modified procedure was better than the standard one, and no more that 50% of any of the quality attributes was explained by the standard alveogram variables (Tables 3.9 and 3.10).

The difference is likely a result of the variation in water used. In the standard alveogram analysis, designed for hard flours, the same amount of water is used for all types of samples. The use of an inappropriate amount of water can be misleading as to the actual resistance to extension inherent in a flour.

The alveograph is widely used, mainly in those countries where hearth bread is the main type of bread consumed. Suitability of alveogram parameters was improved. The importance of alveogram results was demonstrated, but the procedure should be done using the appropriate amount of water. These results have strong implications for the suitability of the methodology.

3.4.6. Micro-extension tests

3.4.6.1. Simple correlation with breadmaking variables

Micro-extension tests presented simple correlation results comparable to the alveogram ones (Table 3.5). Extensibility variables were the most importantly correlated with pan loaf volume ($r = 0.658$, 0.576 and 0.439 for dough micro-extensigram extensibility at rupture, dough micro-extensigram extensibility at maximum resistance and gluten micro-extensigram extensibility at maximum slope, respectively; $P<0.001$). Alveogram extensibility is determined at rupture. Its equivalent in dough micro-extension tests is "extensibility at rupture" (DERup). This variable was as correlated as the alveogram one, showing the suitability of the methodology. French-type bread height was correlated with resistance to extension estimators as it was with the corresponding alveogram variables, ($r = 0.462$, 0.448 and 0.395, for dough micro-extensigram resistance at maximum and at rupture, and gluten micro-extensigram resistance at maximum, respectively; $P<0.001$),

but it was also correlated with an estimator of extensibility (r = 0.495 with dough micro-extensigram extensibility at maximum; P<0.001).

Resistance to extension was the property most related to form ratio (r = 0.495 and 0.487 for dough micro-extension resistance at rupture and at maximum, respectively; P<0.001). Gluten micro-extensigram maximum slope was the most correlated with French-type bread appearance, but negatively (r = -0.429; P<0.001). The slope of the curve of resistance to extension versus extension is directly related with the speed of increasing resistance to extension during elongation. Since strain hardening is defined as the increase of stress required to deform a sample while strain is increasing, both micro-extensigram maximum slope variables (DSI and GSI) are related to strain hardening. Both gluten and flour dough extensibility estimators were positively correlated with French-type bread appearance (r = 0.406 and 0.400 for dough micro-extensigram extensibility at rupture and gluten micro-extensigram extensibility at maximum resistance, respectively; P<0.001). Therefore, in order to obtain an open crumb, it is necessary to have both high extensibility and low strain hardening.

3.4.6.2. Multiple linear regression (MLR)

In each flour and gluten micro-extension tests, only one variable was included in the pan loaf volume model. In both cases, the variable was an estimator of extensibility (at rupture, DERup, and at maximum slope, GExtSI, for flour and gluten respectively), but flour dough micro-extension measurements were much more successful than gluten ones (r^2 = 0.432 and 0.193, respectively; Tables 3.12 and 3.11). This was expected since breads were baked with flour dough not with gluten dough. Therefore, although the importance of gluten was unquestionable, other components affected bread properties.

French-type bread volume was poorly predicted by both micro-extension tests, although flour was better (r^2 = 0.239 and 0.144, for flour and gluten respectively). The difference was even more evident for form ratio (r^2 = 0.563 and 0.240, for flour and gluten respectively). In conclusion, gluten micro-extension test was better to predict strength. The non-protein components that affect extensibility seem to not affect strength.

3.4.6.3. Principal Components Analysis (PCA)

PCA was used to analyse the relationship between alveogram results and micro-extension tests variables (Figure 3.5). Dough micro-extensigram extensibility at rupture (DERup) and gluten micro-extensigram extensibility at maximum slope (GExtSl) were in the same region as both alveograms extensibility variables (L and Lad). The distance between these variables and gluten micro-extensigram extensibility at maximum (GEMax), dough micro-extensigram extensibility at maximum slope (DExtSl) and dough extensibility at maximum resistance (DEMax) means that, although they are all extensibility predictors, the second group did not explain the same rheological property. It is important to remark that extensibility predictors of both alveogram techniques (L and Lad) were close one to each other. The three proposed extensibility variables of micro-extension dough (DExtSl, DEMax and DERup) were relatively far from each other. However, in the figure all of them were at the left from the alveogram variables, while the gluten micro-extension tests variables were at the top-right from the alveogram variables. Therefore, extensibility variables could be grouped according to the test: alveogram, gluten dough micro-extension test and flour dough micro-extension test. Among the three extensibility predictors of dough micro-extension, DERup is the equivalent to the alveogram extensibility because it is determined at rupture of the

dough. As expected, it is the one which is closer to L and Lad. Therefore, dough micro-extensibility at rupture was considered as the more useful estimator of extensibility

Inside the resistance to extension region, the modified alveogram variable (Pad) was close to the gluten micro-extension variables. Gluten micro-extensigram maximum slope (GSI) was the closest, but gluten micro-extensigram maximum resistance (GRMax) was very close also. At the same time, the standard alveogram variable (P) was close to the flour dough micro-extension variables (DSI, DRMax and DRRup). From these observations it was concluded that while standard alveogram reflects flour dough resistance to extension properties, modified alveogram reflects gluten dough ones. Dough micro-extensigram area under the curve (DA) was very close to both of W and Wad, meaning that all of them are estimators of the same property

3.4.7. Influence of protein composition on rheological properties

3.4.7.1. Simple correlation.

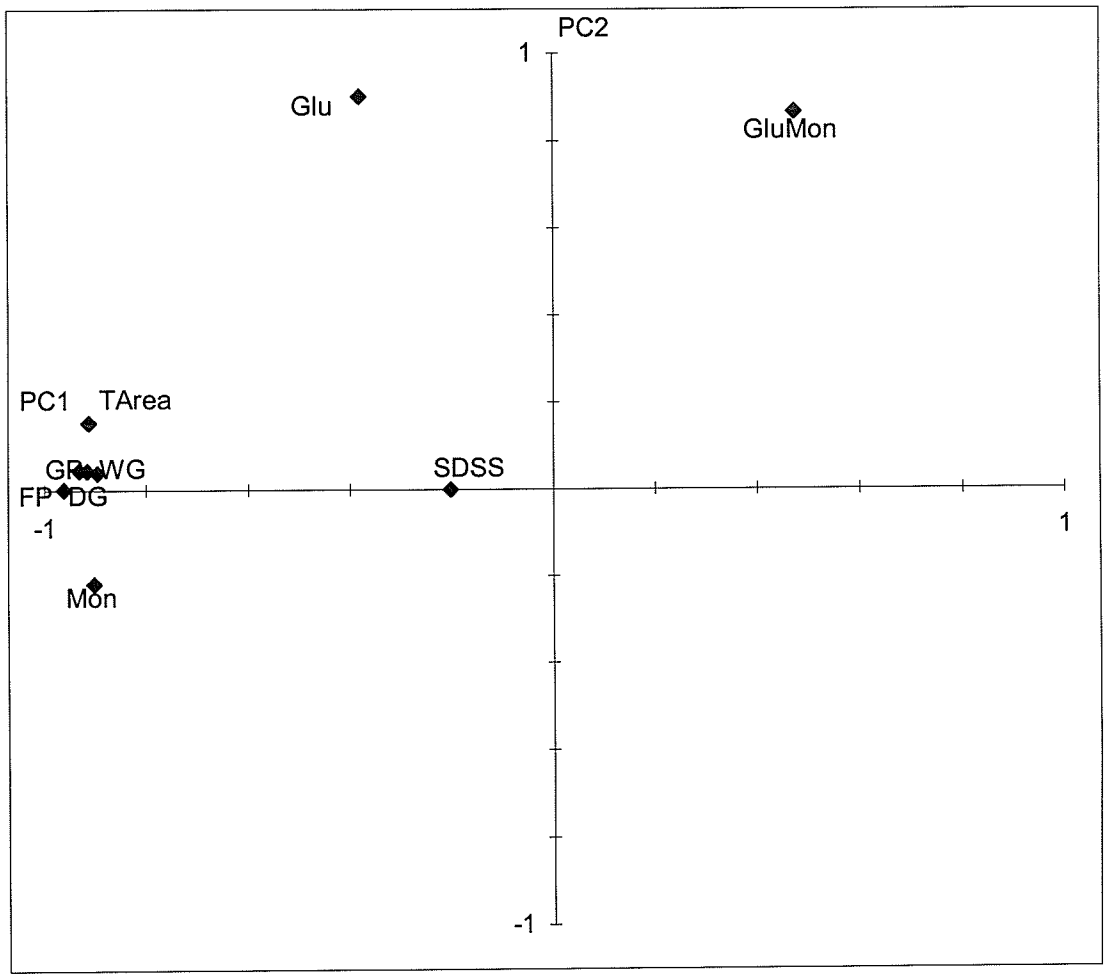
The association between protein components and dough properties was evaluated. Flour protein (FP) and grain protein (GP), defined as nitrogen content times 5.7, are the most widely used parameter. The content of proteins able to form the network named gluten was estimated by the gluten washing machine Glutomatic. Two variables were evaluated: the percentage of dry gluten (DG) and wet gluten (WG). The latter includes the water absorbed by these proteins. The SDS sedimentation test used in this work (SDSS) estimates the insoluble proteins in acid conditions, as well as their swelling properties. The procedure used in size-exclusion HPLC was able to separate polymeric glutenins in their native state from monomeric gliadins. Therefore, four different variables are proposed: Glu (an estimation of percentage of glutenins in flour), Mon (an estimation

of percentage of monomeric proteins in flour, i.e. gliadins), TArea (an estimation of the amount of soluble proteins injected into the HPLC column) and GluMon (glutenins to monomeric proteins ratio).

PCA analysis of protein components (Figure 3.8) showed a very close relationship among flour and grain protein, wet and dry gluten and soluble proteins. This observation was expected since all of them are estimators of the total gluten proteins content. Glutenins and monomeric proteins content were orthogonal to each other. This meant that both gluten protein groups behaved as independent variables. It should be noted that monomeric protein content was more closely related to the protein content region. The positive relationship between the monomeric protein content and total protein content was reported in the bibliography (Southan and MacRitchie 1999). SDS sedimentation volume was poorly correlated with the first two principal components, but it was highly explained by the third one (data not shown).

Correlation coefficients of rheological variables with protein composition variables (Tables 3.14 and 3.15) proved the importance of protein content on quality characteristics. Among the mixing properties, there was a highly significant correlation ($P<0.01$) between flour and grain protein and all mixing properties with the only exception of mixogram mixing time. The more protein, the stronger the gluten network, and therefore the more stable is the dough for over mixing, i.e., the higher farinogram stability. Since protein absorbs water, the higher the protein the higher the absorption, and therefore the higher the mixogram maximum height and farinogram absorption. This explained the significant correlation of both variables with grain protein ($r = 0.687$ and 0.499; $P<0.001$) and flour protein ($r = 0.707$ and 0.587; $P<0.001$). When the amount of

Figure 3.8. Principal components analysis showing loading plot of chemical components variables. FP: flour protein content, GP: grain protein content, WG: wet gluten content, DG: dry gluten content, SDSS : SDS sedimentation volume, Glu : glutenins content, Mon: monomeric proteins content, GluMon : glutenins to monomeric proteins content, Tarea : soluble proteins content.



functional proteins was determined by WG and DG, the correlation coefficients were also significant for both farinogram absorption ($r = 0.701$ and 0.706, respectively; $P<0.001$) and mixogram maximum height ($r = 0.598$ and 0.610, respectively; $P<0.001$). It should be noticed that mixogram mixing time was not correlated with either grain or flour protein. It was observed that stronger genotypes had longer mixogram mixing times. But

Table 3.14
Correlations of protein component variables with rheological properties[a, b]

Variables	FP	GP	WG	DG	SDSS
Farinogram					
Dough development time	0.505***	0.544***	0.211	0.251	0.523***
Stability	0.360**	0.408**	0.037	0.092	0.699***
Absorption	0.587***	0.499***	0.701***	0.706***	-0.098
Mixogram					
Maximum height	0.707***	0.687***	0.598***	0.610***	0.231
Mixing time	-0.227	-0.142	-0.643***	-0.581***	0.405**
Standard Alveogram					
Baking strength (W)	0.437***	0.448***	0.040	0.103	0.738***
Maximum resistance to extension (P)	0.120	0.120	-0.171	-0.109	0.401
Extensibility (L)	0.469***	0.439***	0.469***	0.457***	0.323
Resistance:extensibility ratio (P/L)	-0.437***	-0.402	0.575***	0.533***	-0.085
Adjusted Absorption Alveogram					
Baking strength (Wad)	0.249	0.298	-0.180	-0.118	0.758***
Maximum resistance to extension (Pad)	-0.479	-0.393	-0.793***	-0.747***	0.295
Extensibility (Lad)	0.515***	0.456***	0.629***	0.618***	0.072
Resistance:extensibility ratio (P/Lad)	-0.651	-0.589***	-0.739***	-0.725***	-0.161
Flour dough micro-extension test					
Area under the curve	0.461***	0.491***	0.110	0.190	0.691***
Maximum slope of the curve	-0.077	-0.039	-0.173	-0.154	0.229
Extensibility at max. slope of the curve	0.331**	0.366	-0.013	0.036	0.262
Maximum resistance	0.263	0.313	-0.057	0.010	0.628***
Extensibility at maximum resistance	0.477***	0.488***	0.099	0.175	0.671***
Resistance at rupture	0.187	0.238	-0.091	-0.028	0.541***
Extensibilty at rupture	0.519***	0.504***	0.333**	0.392**	0.459***
Gluten dough micro-extension test					
Area under the curve	-0.198	-0.141	-0.556***	-0.498***	0.407**
Maximum slope of the curve	-0.473***	-0.407**	-0.710***	-0.674***	0.123
Extensibility at max. slope of the curve	0.393**	0.365**	0.443***	0.425**	0.164
Maximum resistance	0.338**	-0.272	-0.684***	-0.631***	0.324
Extensibility at maximum resistance	0.304	0.241	0.589***	0.559***	-0.201

[a] FP: flour protein, GP: grain protein, WG: wet gluten, DG: dry gluten, SDSS: sedimentation volume.
[b] **: P<0.01; ***: P<0.001.

among samples of the same genotype, the higher the protein the lower the mixing time (data not shown). Therefore, there was an important interaction between genotype and amount of protein, which led to the lack of correlation between mixogram mixing time and flour protein.

When the estimators of protein content were wet and dry gluten, the influence of the amount of protein was stronger than the influence of the genotype, giving negatively significant correlations with mixogram mixing times (r = -0.643 and -0.581, respectively;

Table 3.15
Correlations of protein component variables with rheological properties (SE-HPLC variables)[a, b]

Variables	Glu	Mon	TArea	GluMon
Farinogram				
Dough development time	0.118	0.359**	0.362**	-0.258
Stability	-0.056	0.193	0.146	-0.261
Absorption	0.085	0.677***	0.626***	-0.462***
Mixogram				
Maximum height	0.425	0.648***	0.739***	-0.241
Mixing time	-0.132	-0.247	-0.270	0.059
Standard Alveogram				
Baking strength (W)	-0.102	0.206	0.139	-0.305
Maximum resistance to extension (P)	-0.174	0.028	-0.046	-0.169
Extensibility (L)	0.162	0.276	0.307	-0.164
Resistance:extensibility ratio (P/L)	-0.210	-0.331**	-0.375**	0.153
Adjusted Absorption Alveogram				
Baking strength (Wad)	-0.043	0.004	-0.014	-0.114
Maximum resistance to extension (Pad)	-0.192	-0.565***	-0.572***	0.311
Extensibility (Lad)	0.218	0.495***	0.522***	-0.281
Resistance:extensibility ratio (P/Lad)	-0.234	-0.571***	-0.595***	0.348**
Flour dough micro-extension test				
Area under the curve	0.027	0.236	0.217	-0.225
Maximum slope of the curve	-0.092	-0.099	-0.124	0.012
Extensibility at max. slope of the curve	-0.035	0.277	0.228	-0.244
Maximum resistance	-0.105	0.105	0.050	-0.201
Extensibility at maximum resistance	0.103	0.235	0.247	-0.191
Resistance at rupture	-0.067	0.086	0.048	-0.154
Extensibilty at rupture	0.202	0.265	0.319	-0.133
Gluten dough micro-extension test				
Area under the curve	-0.075	-0.232	-0.233	0.073
Maximum slope of the curve	-0.256	-0.430***	-0.480***	0.172
Extensibility at max. slope of the curve	0.225	0.304	0.358**	-0.148
Maximum resistance	-0.185	-0.334**	-0.367**	0.104
Extensibility at maximum resistance	0.212	0.309	0.356**	-0.120

[a] Glu: glutenin content, Mon: monomeric proteins content, Tarea : soluble proteins content ; GluMon : glutenin/monomeric proteins ratio.
[b] **: P<0.01; ***: P<0.001.

P<0.001). SDS sedimentation volume was correlated with the mixing variables that are related to gluten strength: farinogram dough development time (r = 0.523, P<0.001), farinogram stability (r = 0.699, P<0.001) and mixogram mixing time (r = 0.405, P<0.01), but it was not correlated with absorption related variables (farinogram absorption and mixogram maximum height). Among SE-HPLC variables (Table 3.15), absorption related

variables were the most closely associated. For example, r = 0.739 (P<0.001) between soluble proteins and mixogram maximum height.

The parameter best correlated with of dough strength as measured by extensional properties was SDS sedimentation volume (r = 0.758, 0.738, 0.691 and 0.407; P<0.001, P<0.001, P<0.001, P<0.01, for adjusted and standard alveogram baking strength, dough and gluten micro-extensigram area under the curve, respectively). Standard alveogram maximum resistance (P) was not correlated with any protein component, but its equivalent in the adjusted water alveogram (Pad) was negatively correlated with wet gluten (r = -0.793, P<0.001), dry gluten (r = -0.747, P<0.001), monomeric proteins content (r = -0.565, P<0.001) and soluble proteins content (r = -0.572, P<0.001). Similarly, the equivalent for gluten micro-extension test (GRMax) was negatively correlated with wet (r = -0.684, P<0.001), and dry gluten (r = -0.631, P<0.001), monomeric proteins content (r = -0.334, P<0.01) and soluble proteins content (r = -0.367, P<0.01). Therefore, resistance to extension was associated with protein composition when it was measured by proper methodology, like the modified alveogram or micro-extension test. Standard alveogram maximum resistance (P) was not correlated because it is not an objective variable.

Extensibility measured by standard alveogram was significantly correlated with total protein content estimators (r = 0.469, 0.439, 0.469 and 0.457 with flour and grain protein, wet and dry gluten, respectively; P<0.001), but the modified alveogram equivalent was higher correlated with the same variables (r = 0.515, 0.456, 0.629 and 0.618 with flour and grain protein, wet and dry gluten, respectively; P<0.001). At the same time, adjusted alveogram extensibility was also correlated with SE-HPLC variables

(r = 0.495 and 0.522 with monomeric proteins content and soluble proteins content; P<0.001). As it happened with resistance to extension, modified alveogram variable was better correlated by protein components. Among micro-extension tests, the extensibility estimator better associated with protein component was gluten micro-extensigram extensibility at maximum (r = 0.589 and 0.559 with wet and dry gluten, respectively; P<0.001). In general, results with gluten dough micro-extension tests showed better results than flour dough ones.

3.4.7.2. Multiple Linear Regression (MLR).

Regression analysis results (Table 3.16) showed that over 50% of most rheological variables (Abs, MixH, MixT, W, Wad, Pad and PLad) could be explained by protein components. Among mixing properties, mixogram mixing time was the best explained by the model (r^2 = 0.690), with three variables included: wet gluten, grain protein and dry gluten.

In all cases, modified alveogram variables were better predicted than standard ones. Among extensional properties, resistance to extension variables were better predicted than extensibility variables. For example, 64% of adjusted alveogram maximum resistance variability was explained by wet gluten content and SDS sedimentation volume, while only 39% of adjusted alveogram extensibility was explained by protein components (Table 3.16). Gluten micro-extensigram variables were better predicted by protein components than dough micro-extension ones. For example, coefficient of determination for gluten micro-extensigram maximum resistance was 0.605, while for dough was 0.383 (Table 3.17). This difference may be explained by the incidence of non-protein components.

Table 3.16
Regression analysis results of rheological variables including all protein composition variables into the model[a]

	Step	Variable Entered	Variable Removed	Partial RSq	Model RSq	F	Pr>F
DDT	1GP			0.308	0.308	28.88	<.0001
	2WG			0.085	0.392	8.90	0.0040
FarS	1SDSS			0.494	0.494	63.42	<.0001
Abs	1DG			0.447	0.447	52.45	<.0001
	2Mon			0.069	0.516	9.17	0.0035
MixH	1TArea			0.519	0.519	70.04	<.0001
	2FP			0.050	0.568	7.35	0.0086
MixT	1WG			0.385	0.385	40.64	<.0001
	2GP			0.261	0.646	47.19	<.0001
	3DG			0.044	0.690	8.86	0.0041
W	1SDSS			0.523	0.523	71.39	<.0001
P	1SDSS			0.133	0.133	9.99	0.0024
L	1FP			0.233	0.233	19.76	<.0001
PL	1WG			0.329	0.329	31.80	<.0001
Wad	1SDSS			0.558	0.558	82.07	<.0001
Pad	1WG			0.547	0.547	78.42	<.0001
	2SDSS			0.096	0.643	17.13	0.0001
Lad	1WG			0.386	0.386	40.84	<.0001
PLad	1WG			0.510	0.510	67.64	<.0001

[a] DDT, FarS, Abs: farinogram dough developing time, stability and absorption, respectively. MixH, MixT: mixogram maximum height and mixing time. W, P, L, P/L: standard alveogram baking strength, resistance, extensibility and resistance/extensibility ratio, respectively. Wad, Pad, Lad, P/Lad: adjusted water alveogram alveogram baking strength, resistance, extensibility and resistance/extensibility ratio, respectively. FP: flour protein, GP: grain protein, WG: wet gluten, DG: dry gluten, SDSS: sedimentation volume, Mon: monomeric proteins content, Tarea : soluble proteins content.

Table 3.17
Regression analysis results of micro-extension tests variables including all protein composition variables into the model[a]

	Step	Variable Entered	Variable Removed	Partial RSq	Model RSq	F	Pr>F
GA	1WG			0.304	0.304	28.34	<.0001
	2FP			0.177	0.480	21.76	<.0001
GSI	1WG			0.519	0.519	70.19	<.0001
GExtSI	1WG			0.217	0.217	18.06	<.0001
GRMax	1WG			0.476	0.476	59.02	<.0001
	2FP			0.129	0.605	20.88	<.0001
GEMax	1WG			0.365	0.365	37.40	<.0001
	2FP			0.080	0.445	9.25	0.003
DA	1SDSS			0.476	0.476	59.11	<.0001
DSI	No variable met the 0.01 significance level for entry into the model						
DExtSI	1GP			0.102	0.102	7.42	0.0083
	2WG			0.172	0.275	15.19	0.0002
DRMax	1SDSS			0.383	0.383	40.41	<.0001
DEMax	1SDSS			0.453	0.453	53.89	<.0001
DERup	1FP			0.261	0.261	22.93	<.0001
DRRup	1SDSS			0.285	0.285	25.88	<.0001

[a] GA, GSI, GExtSI, GRMax, GEMax: gluten dough micro-extensigram area under the curve, maximum slope of the curve, extensibility at maximum slope of the curve, maximum resistance and extensibility at maximum resistance, respectively. DA, DSI, DExtSI, DRMax, DEMax, DERup, DRRup: flour dough micro-extensigram area under the curve, maximum slope of the curve, extensibility at maximum slope, maximum resistance, extensibility at maximum resistance, extensibilty at rupture and resistance at rupture, respectively. FP: flour protein, GP: grain protein, WG: wet gluten, SDSS: sedimentation volume.

When only HPLC variables were used, the prediction was poor (Table 3.18): only farinogram absorption, mixogram maximum height, adjusted alveogram maximum resistance and resistance/extensibility ratio were explained over 30% by these components (r^2 = 0.425, 0.519, 0.326 and 0.330, respectively).

Table 3.18
Regression analysis results of rheological variables including only HPLC variables into the model

	Step	Variable Entered	Variable Removed	Partial RSq	Model RSq	F	Pr>F
DDT	1	TArea		0.147	0.147	11.23	0.0013
FarS	No variable met the 0.01 significance level for entry into the model						
Abs	1	Mon		0.425	0.425	47.98	<.0001
MixH	1	TArea		0.519	0.519	70.04	<.0001
MixT	No variable met the 0.01 significance level for entry into the model						
W	No variable met the 0.01 significance level for entry into the model						
P	No variable met the 0.01 significance level for entry into the model						
L	1	TArea		0.11	0.11	8.01	0.0062
PL	1	TArea		0.158	0.158	12.22	0.0009
Wad	No variable met the 0.01 significance level for entry into the model						
Pad	1	Mon		0.326	0.326	31.4	<.0001
Lad	1	TArea		0.284	0.284	25.77	<.0001
PLad	1	TArea		0.33	0.33	32.06	<.0001
GA	No variable met the 0.01 significance level for entry into the model						
GSI	1	TArea		0.242	0.242	20.77	<.0001
GExtSI	1	TArea		0.146	0.146	11.09	0.0014
GRMax	1	TArea		0.153	0.153	11.75	0.0011
GEMax	1	TArea		0.134	0.134	10.06	0.0023
DSI	No variable met the 0.01 significance level for entry into the model						
DExtSI	No variable met the 0.01 significance level for entry into the model						
DRMax	No variable met the 0.01 significance level for entry into the model						
DEMax	No variable met the 0.01 significance level for entry into the model						
DERup	1	TArea		0.119	0.119	8.74	0.0043
DRRup	No variable met the 0.01 significance level for entry into the model						
DA	No variable met the 0.01 significance level for entry into the model						

[a] DDT, FarS, Abs: farinogram dough developing time, stability and absorption, respectively. MixH, MixT: mixogram maximum height and mixing time. W, P, L, P/ L, Wad, Pad, Lad, P/Lad: standard and adjusted water alveogram baking strength, resistance, extensibility and resistance/extensibility ratio, respectively. GA, GSI, GExtSI, GRMax, GEMax: gluten dough micro-extensigram area under the curve, maximum slope of the curve, extensibility at maximum slope of the curve, maximum resistance and extensibility at maximum resistance, respectively. DA, DSI, DExtSI, DRMax, DEMax, DERup, DRRup: flour dough micro-extensigram area under the curve, maximum slope of the curve, extensibility at maximum slope, maximum resistance, extensibility at maximum resistance, extensibilty at rupture and resistance at rupture, respectively. TArea : soluble proteins. Mon: monomeric proteins.

3.4.7.3. Partial Least Squares (PLS) Analysis.

The coefficients of determination of the first three components obtained with PLS analysis are presented in Table 3.19. The third component did not improve any variable prediction, and therefore just the first two will be discussed. All mixing variables were explained with a similar coefficient of determination (R^2 = 0.394-0.532).

Table 3.19
Coefficients of determination for the first three components in PLS regression model of rheological properties

Variables	Component 1	Component 2	Component 3
Farinogram			
Dough development time	0.201	0.394	0.398
Stability	0.077	0.499	0.501
Absorption	0.421	0.496	0.567
Mixogram			
Maximum height	0.532	0.532	0.562
Mixing time	0.113	0.493	0.493
Standard Alveogram			
Baking strength (W)	0.080	0.570	0.570
Maximum resistance to extension (P)	0.000	0.199	0.210
Extensibility (L)	0.227	0.241	0.263
Resistance:extensibility ratio (P/L)	0.254	0.269	0.279
Adjusted Absorption Alveogram			
Baking strength (Wad)	0.008	0.567	0.594
Maximum resistance to extension (Pad)	0.365	0.614	0.641
Extensibility (Lad)	0.352	0.376	0.377
Resistance:extensibility ratio (P/Lad)	0.486	0.495	0.495
Flour dough micro-extension test			
Area under the curve	0.123	0.487	0.501
Maximum slope of the curve	0.014	0.076	0.076
Extensibility at max. slope of the curve	0.037	0.142	0.159
Maximum resistance	0.024	0.401	0.401
Extensibility at maximum resistance	0.137	0.474	0.505
Resistance at rupture	0.012	0.295	0.296
Extensibilty at rupture	0.212	0.291	0.352
Gluten dough micro-extension test			
Area under the curve	0.104	0.402	0.404
Maximum slope of the curve	0.339	0.477	0.491
Extensibility at max. slope of the curve	0.200	0.202	0.238
Maximum resistance	0.223	0.509	0.518
Extensibility at maximum resistance	0.189	0.359	0.371

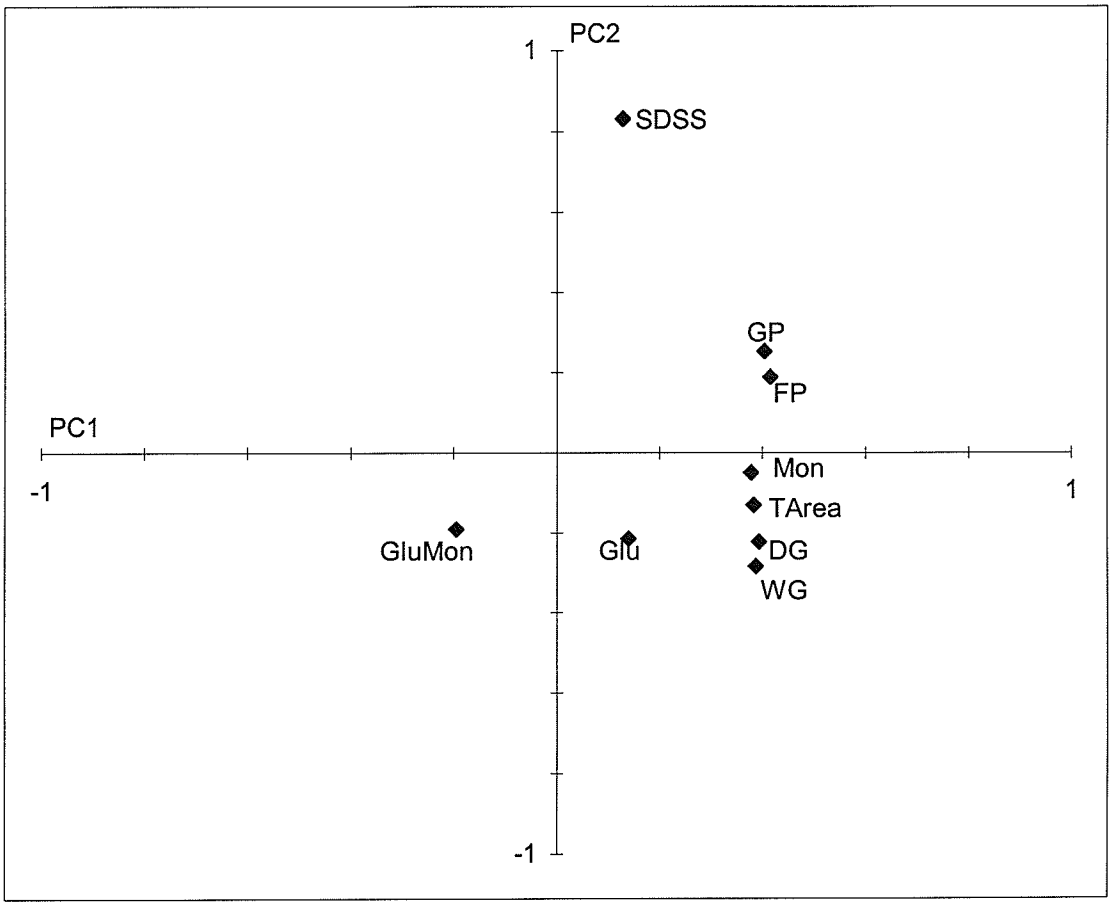
Among extensional variables, the model presented important differences. Standard alveogram variables were poorly predicted, with the exception of W (r^2 = 0.570), and therefore they will not be further discussed. Resistance to extension was better predicted than its corresponding extensibility. PLS regression was able to explain 61% of adjusted alveogram resistance variation, but only 38% of its corresponding extensibility. In gluten micro-extensigram, maximum slope explained 48% while extensibility at maximum slope explained only 20%, and maximum resistance explained 51% while extensibility at maximum resistance explained 36%. The extensibility variable best explained by the model was dough micro-extensigram extensibility at maximum, for which 47% of the variability was explained.

The same region used by protein amount variables (GP, FP, TArea, DG and WG) in PLS x-loadings plots (Figure 3.9) was occupied by extensibility variables (L, Lad, GextSl) in PLS y-weights plots (Figure 3.10). At the same time, the content of monomeric proteins (Mon) is in the same region. The relationship of both protein amount and monomeric content with extensibility was reported by several researchers (Bloksma and Bushuk 1988, Uthayakumaran et al 1999). Similarly, gluten strength variables (FarS, W, Wad and DA, Figure 3.10) were in the same region as SDS sedimentation volume (Figure 3.9).

3.4.8. General discussion

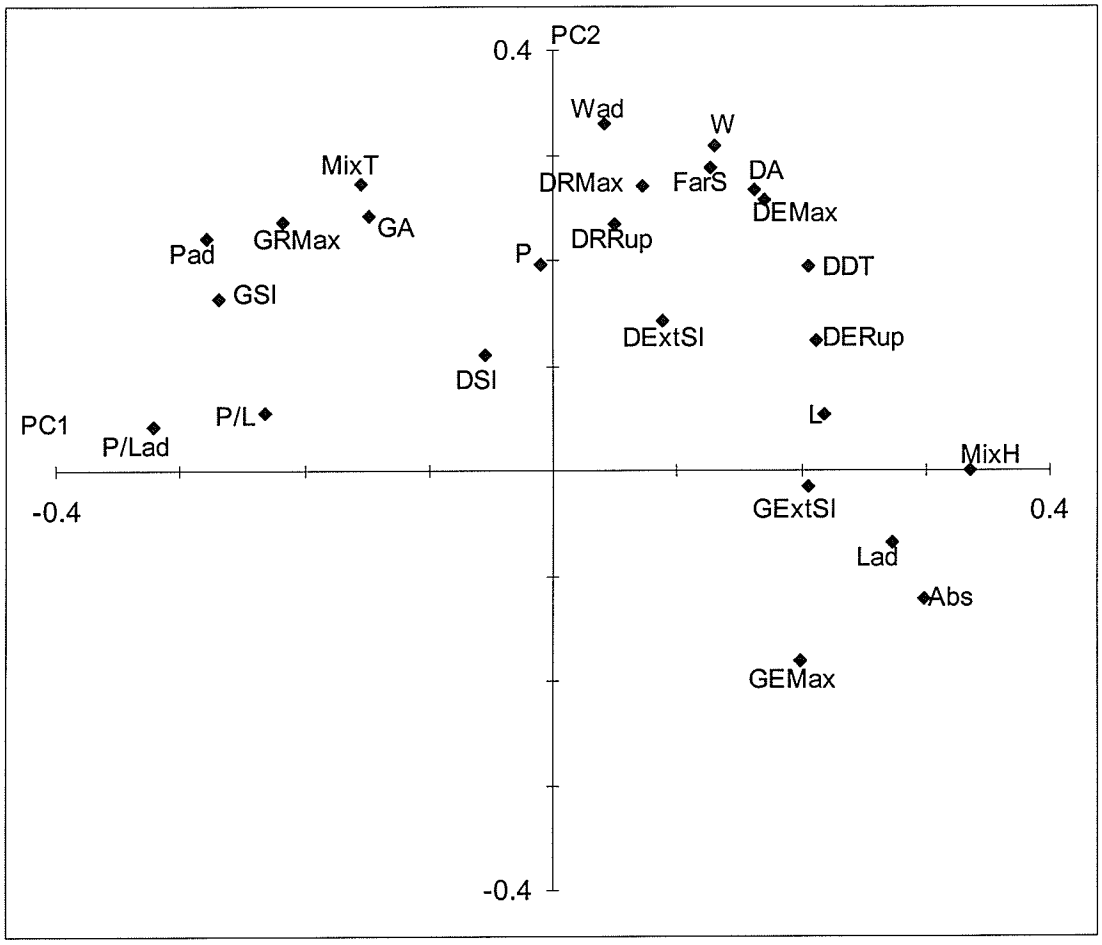
Flour protein content was the best predictor of both pan and hearth bread volume. Among the rheological parameters, extensional properties explained breadmaking characteristics better than mixing ones. The adjusted alveogram resistance/extensibility ratio was the best correlated. Since this variable is composed of resistance and

Figure 3.9. Partial least squares analysis showing x-loading plot of chemical components variables of the two first principal components (PC1 and PC2). FP: flour protein content, GP: grain protein content, WG: wet gluten content, DG: dry gluten content, SDSS : SDS sedimentation volume, Glu : glutenins content, Mon: monomeric proteins content, GluMon : glutenins to monomeric proteins content, Tarea : soluble proteins content.



extensibility, the high correlation value means each component, and their equilibrium, affect bread volume. The relevant resistance to extension was predicted by the modified alveogram (Pad), rather than by the standard equivalent (P). Dough strength was more important in French-type bread quality than in pan bread quality. MLR analyses showed that for pan loaf volume, the second factor was extensibility by itself (micro-extensigram extensibility at maximum), while for French-type bread the second one was a dough

Figure 3.10. Partial least squares analysis showing y-loading plot of variables of the two first principal components (PC1 and PC2). Farinogram: dough development time (DDT), stability (FarS) and absorbance (Abs). Mixogram: maximum height (MixH) and mixing time (MixT). Standard alveogram: baking strength (W), maximum resistance to extension (P), extensibility (L) and resistance/extensibility ratio (P/L). Adjusted water alveogram: baking strength (Wad), maximum resistance to extension (Pad), extensibility (Lad) and resistance/extensibility ratio (P/Lad). Flour dough micro-extensigram: maximum slope of the curve (DSI), extensibility at maximum slope of the curve (DExtSI), maximum resistance (DRMax), extensibility at maximum resistance (DEMax), resistance at rupture (DRRup), extensibilty at rupture (DERup), area under the curve (DA). Gluten dough micro-extensigram: maximum slope of the curve (GSI), extensibility at maximum slope of the curve (GExtSI), maximum resistance (GRMax), extensibility at maximum resistance (GEMax) and area under the curve (GA).



strength estimator (mixogram mixing time). Different strength predictors (standard and modified alveogram baking strength, mixogram mixing time and farinogram stability) had an important influence on volume, and more importantly on form ratio, according to

simple correlation, MLR and PLS. French-type bread dimensions were affected differently: height was more related with dough strength while width was more related with protein quantity. Loaf appearance was negatively affected by resistance to extension, which could be seen clearly with PLS analysis. Good quality predictors were obtained with micro-extensigraph. PCA analyses showed that dough extensibility at rupture is a good predictor of alveograph extensibility, gluten maximum slope is a good predictor of resistance to extension and dough area under the curve is a good predictor of alveograph baking strength. In addition, micro-extensigraph variables had a similar relationship with breadmaking as the alveograph. The higher the protein content, the stronger and more extensible were doughs. However, sedimentation volume was more closely related to strength. Monomeric proteins content were closely related with flour protein content, but they were independent of glutenin content.

3.4. CONCLUSIONS

Breadmaking variability was better explained by extensional than by mixing properties. This conclusion was supported by evidence from several statistical approaches: simple correlations, MLR, PLS. Variables determined by extension tests are related to the characteristics of the dough during fermentation and this is the stage where the bread properties are defined, which explains the results obtained. Mixing variables were correlated with some dough characteristics, but could not explain fermenting dough characteristics by themselves.

As expected, there is no single rheological variable which was able to explain an important percent of the variability by itself. By combining different rheological properties,

however, good estimations were obtained. MLR models were able to predict up to 75% of the volume variability for pan loaves and up to 64% of form ratio for French-type loaves (Table 3.6). PLS models included more variables, but were not better than MLR ones (Table 3.13). Although different requirements were reported for the two kinds of bread, volume was similarly explained for both pan and hearth breads. Since hearth loaves have other characteristic quality criteria in addition to volume, their requirements are more complex. For hearth loaf volume, although extensibility played a relevant role, the balance between resistance to extension and extensibility (P/Lad) was the most important variable. Dough strength was necessary to obtain an appropriate loaf form (FrR). Adjusted alveogram maximum resistance (Pad) was highly correlated positively with FrR and negatively with French-type loaf appearance (FrA). Therefore, resistance to extension was necessary to obtain good bread height, but values too high could cause a crumb that was too compact (low FrA values). A balance among extensibility, resistance to extension and strength was shown to be critical.

The importance of extensional properties gives relevance to the methodology to be applied. The most frequently used empirical testing instrument in South America is the Chopin Alveograph. Results obtained here proved that water absorption adjustment improved test performance. Micro-extension tests proved to be a valid alternative.

Among the protein components, protein amount was the most important of the ones determined. Poor relationships of other protein components could be explained by the set of samples chosen. Since the genotypes used were previously selected because of their protein functional properties, variability in protein functionality was poor. Further work is proposed to explore other flour components that may interfere in these

relationships, like polymeric proteins molecular weight distribution (Southan and MacRitchie 1999) and pentonsan content (Wang et al 2004).

The complexity of flour breadmaking requirements on one side, and dough rheological properties and flour composition on the other, make the research to understand their relationships a major challenge. There are so many phenomena involved, that it is nearly impossible to fully explain breadmaking variability. In this research, empirical rheological properties of flour samples representative of the variability found in the Uruguayan wheats were determined. The influence of rheological factors was studied in order to determine, first, their importance in breadmaking and secondly their relationship with protein components.

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APPENDIX 1. SAMPLE SET

ID	Variety/Experimental line	Origin	Year	Environment
78175	Estanzuela Cardenal	Evaluation	2000	Y3I
78176	Estanzuela Pelón 90	Evaluation	2000	Y3I
78177	INIA Boyero	Evaluation	2000	Y3I
78178	INIA Mirlo	Evaluation	2000	Y3I
78179	INIA Caburé	Evaluation	2000	Y3I
78180	INIA Churrinche	Evaluation	2000	Y3I
78181	LE 2252	Evaluation	2000	Y3I
78182	LE 2262	Evaluation	2000	Y3I
78183	LE 2265	Evaluation	2000	Y3I
78262	Estanzuela Cardenal	Evaluation	2000	LE3I
78263	Estanzuela Pelón 90	Evaluation	2000	LE3I
78264	INIA Boyero	Evaluation	2000	LE3I
78265	INIA Mirlo	Evaluation	2000	LE3I
78266	INIA Caburé	Evaluation	2000	LE3I
78267	INIA Churrinche	Evaluation	2000	LE3I
78268	LE 2252	Evaluation	2000	LE3I
78269	LE 2262	Evaluation	2000	LE3I
78270	LE 2265	Evaluation	2000	LE3I
78271	INIA Tijereta	Evaluation	2000	Y3L
78272	LE 2220	Evaluation	2000	Y3L
78273	INIA Gorrión	Evaluation	2000	Y3L
78274	LE 2255	Evaluation	2000	Y3L
78275	LE 2271	Evaluation	2000	Y3L
78276	INIA Tijereta	Evaluation	2000	LE3L
78277	LE 2220	Evaluation	2000	LE3L
78278	INIA Gorrión	Evaluation	2000	LE3L
78279	LE 2255	Evaluation	2000	LE3L
78280	LE 2270	Evaluation	2000	LE3L
78281	LE 2271	Evaluation	2000	LE3L
78282	LE 2284	Evaluation	2000	1YL
78283	LE 2285	Evaluation	2000	1YL
78284	LE 2287	Evaluation	2000	1YL
78285	LE 2284	Evaluation	2000	1LEL
78286	LE 2285	Evaluation	2000	1LEL
78287	LE 2287	Evaluation	2000	1LEL

SAMPLE SET (cont.)

ID	Variety/Experimental line	Origin	Year	Environment
78398	Baguette 10	Fertilization	2002	N1
78399	Baguette 10	Fertilization	2002	N2
78400	Baguette 10	Fertilization	2002	N3
78401	Baguette 10	Fertilization	2002	N4
78402	INIA Churrinche	Fertilization	2002	N1
78403	INIA Churrinche	Fertilization	2002	N2
78404	INIA Churrinche	Fertilization	2002	N3
78405	INIA Churrinche	Fertilization	2002	N4
78406	INIA Gorrión	Fertilization	2002	N1
78407	INIA Gorrión	Fertilization	2002	N2
78408	INIA Gorrión	Fertilization	2002	N3
78409	INIA Gorrión	Fertilization	2002	N4
78410	Greina	Fertilization	2002	N1
78411	Greina	Fertilization	2002	N2
78412	Greina	Fertilization	2002	N3
78413	Greina	Fertilization	2002	N4
78414	LE 2294	Fertilization	2002	N1
78415	LE 2294	Fertilization	2002	N2
78416	LE 2294	Fertilization	2002	N3
78417	LE 2294	Fertilization	2002	N4
78418	INIA Mirlo	Fertilization	2002	N1
78419	INIA Mirlo	Fertilization	2002	N2
78420	INIA Mirlo	Fertilization	2002	N3
78421	INIA Mirlo	Fertilization	2002	N4
78422	INIA Torcaza	Fertilization	2002	N1
78423	INIA Torcaza	Fertilization	2002	N2
78424	INIA Torcaza	Fertilization	2002	N3
78425	INIA Torcaza	Fertilization	2002	N4
78426	LE 2265	Fertilization	2002	N1
78427	LE 2265	Fertilization	2002	N2
78428	LE 2265	Fertilization	2002	N3
78429	LE 2265	Fertilization	2002	N4
80307	AC SNOWBIRD	Canada		
80308	ICWRS	Canada		
80309	AC TEMPEST	Canada		
80310	AC VISTA	Canada		
80311	5700 PR	Canada		
80312	AC CRYSTAL	Canada		
86811	Inia Churrinche	Fertilization	2003	N1
86812	Inia Churrinche	Fertilization	2003	N4
86813	Inia Gorrión	Fertilization	2003	N1
86814	Inia Gorrión	Fertilization	2003	N4
86815	Inia Torcaza	Fertilization	2003	N1
86816	Inia Torcaza	Fertilization	2003	N4
86817	Baguette 10	Fertilization	2003	N1
86818	Baguette 10	Fertilization	2003	N4

APPENDIX 2. HMW glutenins

Variety/Experimental line	1A	1B	1D
Baguette 10	Null	7+8	5+10
Estanzuela Cardenal	1	7+9	5+10
Estanzuela Pelón 90	1	13+16	5+10
Greina	2*	7+9	5+10
INIA Boyero	2*	7+9	5+10
INIA Caburé	Null	7+8	5+10
Inia Churrinche	2*	7+8	5+10
Inia Gorrión	2*	7+9	5+10
INIA Mirlo	1/2*	7+9	5+10
INIA Tijereta	Null	17+18	5+10
Inia Torcaza	2*	17+18	5+10
LE 2220	Null	7+9	5+10
LE 2252	Null	7+8	5+10
LE 2255	2*	7+8	5+10
LE 2262	2*	17+18	5+10
LE 2265	1	7+8	2+12
LE 2270	Null	17+18	5+10
LE 2284	Null	17+18	5+10
LE 2285	Null	17+18	5+10
LE 2287	1	7+8	5+10
LE 2294	2*	7+9	5+10

CHAPTER 4

NIR CALIBRATIONS USED TO PREDICT WHEAT BREADMAKING QUALITY PARAMETERS

NIR Calibrations Used To Predict Wheat Breadmaking Quality Parameters

4.1. ABSTRACT

Near-infrared spectroscopy (NIR) is an attractive tool for quality screening in wheat breeding programs because of its rapidity, simplicity of sample preparation, and small sample size requirement. Although good results have been achieved for some variables like protein content, limited results have been obtained for protein functionality. In order to evaluate the viability of NIR technology for Uruguayan wheat quality determination, two sets of samples were analyzed using several NIR treatments on a NIRSystem 6500 spectrophotometer. Reference methods included ones for protein composition, hardness, rheological variables and baking tests. Whole grain, ground grain and white flour samples were scanned. Differences among several math treatments for derivatives, gap and smoothing were not critical to the quality of the calibration. Results were best when flour was scanned, but were acceptable also using ground samples and even whole grain. Increasing the size of the scanned sample did not improve results. Duplicating the NIR spectrums resulted in better calibrations. Protein content was predicted with an accuracy, as indicated by the standard error of prediction (SEP), similar to that of the reference method (SEP = 0.16%). Hardness calibration was successful only with the ground sample (SEP < 2%). Results obtained for protein functionality variables had lower coefficients of determination but were sufficient to use to screen samples for a breeding program. The R^2 of the validation set was 0.616 for alveogram extensibility, 0.641 for mixogram maximum height, and 0.653 for SDS sedimentation volume. With these calibrations, three independent quality component (hardness, protein quantity and protein functionality) can be estimated in one simple, fast operation.

4.2. **INTRODUCTION**

In the last two decades the use of NIR has increased dramatically (Hruschka 2001). The success and acceptance of NIR is based on two independent factors. One is that no chemicals are needed (Starr et al 1981). The second is that this is very fast, mainly due to the quality of the spectra and the power of the mathematics used in the analyses (Hruschka 2001). In addition, in cereal technology another potential advantage of NIR is that the measurement of grain samples can be nondestructive (Maghirang and Dowell 2003, Nielsen et al 2003, Pasikatan and Dowell 2004).

In order to be able to use an NIR spectrophotometer to measure a quality trait, a calibration procedure must be followed. First, a selected set of samples must be analyzed by a standardized method, the "reference method". Then, all samples must be scanned by transmittance or reflectance and the relationship between the spectra and the reference method must be determined. This relationship is used to develop the calibration. The calibration is expressed as an approximation which involves a regression equation. The dependent variable is the reference method value. The independent variables are log (1/R) values or a combination of them (Hruschka 2001).

NIR, because of its rapidity, simplicity of sample preparation and small sample size requirement, has been used extensively in grain and grain derivative analyses both commercially and for breeding programs. The most important component determined has been protein content. The first useful calibrations date from 1975 (Delwiche et al 1998b, Williams 1975, Williams and Norris 1983). Since NIR has proven suitable to predict amino acid content (Fontaine et al 2002), it may be possible to use it to predict

protein quality as well as quantity. This makes the technique attractive to use for the prediction of baking and dough properties in wheat breeding programs (Wrigley 1994). So far there has been limited success in reaching this objective.

Calibrations for several parameters, including sedimentation volume and loaf volume, were developed by Starr et al (1981) using a filter analyzer. They used a small number of samples, n = 36 to 40, for both calibration and validation sets. Calibrations were not suitable for prediction of these characteristics (r^2 = 0.19 and 0.27, respectively), but calibration parameters showed highly significant correlations (r^2 = 0.64, $P < 0.001$, for both). The authors concluded that a calibration to predict baking performance was possible. They proposed that results might be improved by changing the combinations of wavelengths. Monochromators and PLS were not used at that time.

Osborne (1984) stated that NIR filter instruments had so far not been found suitable for the estimation of protein quality, but could be used for protein quantity. He carried out investigations using a monochromator system to check the validity of NIR for predicting protein quantity and quality and Falling Number values. He worked with two sets of samples (n=52 and 117) with a wide range in protein percentage (7.2-12.2%), Falling Number values (62-377s) and SDS sedimentation volumes (30-95ml for set 1, 25-88ml for set 2). He obtained calibrations by MLR forward stepwise regression with 600 datum points and found very good prediction for protein (r = 0.99, SEP = 0.21%). At the same time, the author observed that results for Falling Number did not justify attempts at prediction. Results for SDS sedimentation volume were very poor as well. The correlation coefficient obtained was not very low (r =0.65), but the conclusion was that this might have been due to the correlation between protein content and SDS

sedimentation volume (no value reported). In addition, the correlelograms (plot of modulus correlation coefficient against wavelengths) of SDS sedimentation volume and protein were very similar. He concluded that NIR technology was not suited to prediction of bread baking quality.

Rubenthaler and Pomeranz (1987) tried to overcome the problem observed by Osborne, by working with a wider range of protein content (7.9-20.7%) and loaf volume (568-1328ml). They specifically looked for a set of samples with a low correlation between protein and loaf volume (r = 0.308). They had high correlation coefficients when they used one set of samples, but when these calibrations were used to predict a second set the correlation coefficients were "low" (no values given).

Williams et al (1988) developed calibrations using MLR. They divided wheat samples into three groups: hard, medium-hard and soft (n for validation sets were 26, 11 and 15 respectively). They intended to predict a wide diversity of quality parameters, including farinograph, alveograph, and extensigraph values. Good results were obtained for protein for the three groups, SEP = 0.20, 0.23 and 0.15% respectively, and r = 0.99, 0.99 and 0.98, respectively. Lower values, but still valid for breeding purposes, were obtained for the rheological tests. For farinograph stability r was similar in the three cases (0.86, 0.86 and 0.79, respectively), but important variability was observed in SEP values (3.3, 2.6 and 1.1 min, respectively). This variation was explained by the difference in the validation set means (10.9, 9.6 and 3.0 min, respectively). Good results were also obtained for alveograph W (r = 0.83, not reported and 0.89, respectively). Alveograph L (extensibility) was studied in only the soft samples set, with good success (SEP=12, r=0.77).

Using PLS, Williams (1996) reported calibrations for different quality parameters (number of samples not reported). Flour ash and flour protein were successfully predicted (r^2 = 0.82 and 0.99 respectively). Hardness was well predicted when the sample was ground (r^2 = 0.95, RPD=4.6) but not so well when whole grain was scanned (r^2 = 0.74, RPD=1.9). Flour water absorption prediction results proved the suitability of NIR for this parameter (r^2 = 0.85, RPD=2.6).

Delwiche et al (1998a) proposed an investigation of the potential for using NIR spectrophotometry to quantify the biochemical components or physical properties of wheat flour. Parameters studied included glutenin content, gliadin content, albumin, globulins, SDS sedimentation volume, and mixograph measurements. PLS analysis predicted the following parameters reasonably well: glutenin content, gliadins content with and without secalins, SDS sedimentation volume, mixograph peak time and mixograph peak width. The authors stated that these predictions might be suitable for breeding programs, but would not be suitable for quality control, and they concluded that NIR could be used for genotype selection. Their results were obtained working with two replications of the same field trial as the validation and calibration sets. No actual external samples were used to validate results.

Wesley et al (2001) analyzed 78 samples by SE-HPLC for total protein and "extractable" and "unextractable" polymeric protein. They used both PLS and curve fitting procedures. They concluded that the method predicted the glutenin content, since the rank correlation was highly significant between both years. No validation procedure was run.

Hruskova et al (2001) reported good calibrations for several parameters, including protein content (r=0.963) and farinograph parameters. The only correlation coefficients significant at P<0.01 of the validation set (n=29) were time of development (r=0.619) and mixing tolerance (r=0.415). Water absorption, dough stability and extensigraph predictions were not significant (P<0.01). They assumed that lack of correlation could be caused by the small size of the sample set (n=114) and the qualitative differences within the set.

Miralbés (2003) proposed a NIR transmittance whole grain calibration be used at the receiving stage of the milling industry. Using 443 samples from different countries, the author obtained very good results for protein, wet gluten and dry gluten (SEP = 0.16%, 1.00%, 0.29%, validation r^2 = 0.99, 0.95, 0.97, respectively). When he calibrated for alveograph baking strength (W) with an extended set of samples (n=600) the results were not as good. The validation r^2 value was considered "relatively high" (0.84), but the correlation of W with protein content, well predicted by NIR, was even higher (r^2 = 0.86). The author also considered the SEP value (41 x 10^{-4}J) too high even for screening purposes. By dividing the set of samples into "weak" and "medium-strong", the validation r^2 increased up to 0.95 and SEP was 24 x 10^{-4}J for the second group. Good results were obtained also for alveograph P (SEP = 6.7 mm), but not for P/L. No results were given for extensibility (L). The values obtained for alveograph W and P were considered "reasonable" to be used for screening purposes at mills or elevators.

In order to evaluate the viability of NIR technology for wheat quality determination, two sets of samples were analyzed and several variations of NIR treatments were evaluated.

The main objective of this work was to develop NIR calibrations valid to be used for high quality selection in a wheat breeding program. Specific objectives were:

1. The development of calibrations that have very high coefficients of determination and low SEP values, and therefore could be used to substitute for reference methods.

2. The development of calibrations that give moderately good prediction, therefore could be suitable for evaluation of breeding program advanced lines.

3. Identification of variables for which calibrations are not high, but which could still be appropriate for early generation screening in breeding programs.

4. Evaluation of different tools, strategies and approaches that NIR technology can provide (math treatment, wavelength selection, sample preparation and size, scanning error, selection of samples) to enable the best to be selected.

4.3. MATERIALS AND METHODS

4.3.1. Wheat samples

Two sets of samples were used. Set A (n=470) included set B samples and 397 samples from the INIA wheat breeding program. Set B (n=73) was composed of 35 samples from the National Evaluation Program (17 genotypes grown in two environments each, and one sample from one environment only (one sample was lost), 32 samples from 2002 INIA fertilization trials (8 genotypes grown at 4 nitrogen fertilizer levels) and 6 Canadian samples.

4.3.2. Grain quality methods

Wheat samples were tempered at 15.5% and milled to produce straight-grade flour in a Buhler mill MLU 202 (Bühler AG, Uzwil, Switzerland). Ground sample was obtained using a Perten 3100 (Perten Instruments AB, Huddinge, Sweden) grinder. Protein (N x 5.7) was determined in both ground wheat (GP) and flour (FP) by the Kjeldahl method (Approved Method 46-12, modified; AACC 2000). Hardness was determined by a modified Particle Size Index (PSI) method. PSI value was the percentage of ground sample (10.00g) that passed through a 200 Mesh sieve after 10 min shaking with a rotator Rotap Model RX 29 –10 (Laval Lab Inc., Laval, QC, Canada). Samples with PSI over 57% were considered soft. Wet (WG) and dry gluten (DG), as well as Gluten Index (GI) were determined by the Approved Method 38-12A (AACC 2000). SDS sedimentation tests were run with 1.0 g of sample according to the CIMMYT procedure (Peña et al 1990) and results were presented as volume expressed in mL (SDSS).

4.3.2.1. SE-HPLC.

Flour (100 mg) was extracted with 10 ml of 0.5% SDS-0.05M sodium phosphate buffer, pH 6.9, sonicated 15 sec (output 20 W, 9-mm diameter probe; Ohtake Works Sonicator, Tokyo, Japan) and centrifuged at 16,000 x g for 10 min. The supernatant was filtered through a 0.45 μm polyvinylidene difluoride filter membranes (Durapore, Millipore Corp., Ireland) and immediately heated for 2 min at 80°C in a water bath. The extract (20 μl) was injected in a Superdex 200 HR 10/30 size exclusion analytical column (10 x 300 mm), equilibrated with 0.05M sodium phosphate buffer (pH 6.9). Column elution was isocratical for 35 min with a flow rate of 0.5 ml/min at room temperature, using an HPLC Äkta Purifier System (Amersham Pharmacia Biotech, Uppsala, Sweden). Absorbance

was measured at 214 nm (Dalla Rizza et al 2005). Polymeric glutenin (Glu) and monomeric proteins (Mon) content in flour were determined.

4.3.2.2. Rheological analyses.

Farinograph mixing stability (FarS) and absorption (Abs) were obtained by the Approved Method 54-21 (AACC 2000). Mixograph maximum height (MixH) and mixing time (MixT) were obtained by the AACC Method 54-40A. Alveogram parameters (W, P, L and P/L) were obtained using the standard alveograph procedure (AACC Method 54-30A). A modified alveograph procedure using water adjusted according to farinograph absorption was done in addition to the standard alveograph technique which uses a 50% absorption. In the modified procedure, the amount of water added was farinograph absorption minus 8. In this way, when absorption was 58%, 50% absorption was used. Equivalent variables were obtained (Wad, Pad, Lad and P/Lad).

4.3.2.3. Baking tests.

Two different types of breads were baked. Pan loaves were prepared according to AACC Method 10-10B. Loaf volume (LV) was used as quality estimator. French-type (Fr) hearth loaves were prepared, based on typical Uruguayan procedures. Flour (100 g 14% moisture basis) was mixed with ascorbic acid (4 mg), yeast (2.2 g), salt (2.5 g) and the optimum amount of water (farinograph absorption + 2%) for the optimum mixing time (mixograph mixing time) in a 100-200 g mixer (National Manufacturing Division, TMCO, USA). Dough was rested for 90min in a greased bowl in a fermentation cabinet (28°C, humidity over 80%). Then, the pieces were sheeted three times, decreasing the rolls distance (11/32", 1/4" and 3/16") with a Sheeter and Roll Molder (National Manufacturing Division, TMCO, USA). Dough was rolled up using again the Sheeter and Roll Molder

(National Manufacturing Division, TMCO, USA). The formed dough pieces were carefully transferred to flat trays and proofed for 90 min in the same cabinet. Three diagonal cuts were made on the top surface of the loaf using a retractable razor blade knife and pieces were immediately baked for 20 min at 212°C saturated with steam. Besides the volume (FrV), quality measurements of loaves were width (FrW), height (FrH), width:height ratio (FrR) and a subjective score used to evaluate crumb appearance (FrA): the more open the crumb the highest the score (1-9).

4.3.2.4. Samples analyzed.

All analyses were done on sample set B, while only GP, PSI, Glutomatic determinations, alveogram, mixogram and SDSS were analysed on set A.

4.3.3. NIR analysis

A NIRSystem 6500 (FOSS Inc., Silver Spring, MD, USA) spectrophotometer was used to scan grain, flour and ground wheat of all samples. Both a rectangular cell and a small ring cup were used for grain scanning. Reflectance spectra recorded were expressed as log (1/R). Averages of two subsamples were used, unless otherwise stated in the text. Calibrations were performed with WinISI software (Infrasoft International Inc, Port Matilda, PA, USA), using the modified PLS regression model. In order to minimize overlapping peaks and large baseline variations, several spectra derivatives and gap values were evaluated. Several data points were averaged prior to statistical procedures (smooth treatment) with the objective of reducing noise (Hruschka 2001, Williams 2001). Math treatments used were (derivative, gap, smooth, second smooth): 1 2 2 1, 1 4 4 1, 1 10 10 1, 2 2 2 2, 2 4 4 2, 2 10 10 2, 3 4 4 3, 3 6 6 3 and 3 10 10 3. In all cases SNV and detrend scatter correction were performed. Three sets of wavelengths were used: 408-

2492nm, 900-2492nm and 1108–2492nm. Unless otherwise stated in the text, each sample set was separated into two sets (a calibration and a validation set) by the following procedure. Each set was prepared to represent the full range of composition. All samples were ordered by alveogram W. The four samples with lowest W value were included in the calibration set, while the fifth one was in the validation set. The following four were included in the calibration set, and the next into the validation set. Therefore, 80% of the samples were included in the calibration set, and 20% in the validation set. Equations were always developed with the calibration set, and evaluated with the validation set. Therefore, the samples used to evaluate the performance of the equations were not included in the set used to develop these equations. Coefficient of determination for the validation set (R^2), standard error of prediction (SEP) and RPD (ratio of the SEP to the standard deviation of the reference data) were used as estimators of equation success. The difference between the reference value and the estimated one was calculated for each sample of the validation set. The standard deviation of these differences is SEP. Another important indicator is RPD (ratio prediction/deviation), which is defined as the ratio of the standard error of prediction to the standard deviation (SD) of the reference data in the validation sample set (ratio SEP:SD). RDP is useful to evaluate the pertinence of a SEP result. A calibration is commonly considered valid to substitute for the reference method if the R^2 is over 0.97, RPD is over 5 and SEP is similar to the reference method error. However, criteria depends on the use of the calibration equation. For instance, if it is used for screenings, values of R^2=0.6 could be applicable (Hruschka 2001, Williams 2001, William, P., personal communication).

4.4. RESULTS AND DISCUSSION

4.4.1. Characteristics of the samples set

The main characteristics of both samples set A and B are presented in Table 4.1. Wide variability was obtained in all variables for both sets.

4.4.2. Math treatments

Table 4.2 shows the results obtained with set A samples using 9 different math treatments and with ground samples. Although there were differences among different math treatments, the differences were not big enough to change a bad result into a good one. For example, GP results were good to very good for all math treatments, the R^2 ranged from 0.965 to 0.975, and the SEP ranged from 0.193 to 0.223 %. At the other

Table 4.1. Descriptive statistics of sets A and B[a]

Set A	GP	PSI	W	P	L	PL	WG	GS	GI	MixH	MixT	SDSS
Min	8.8	34	27	26	18	0.3	12.6	4.2	27	2.6	1.9	6.0
Max	15.2	65	540	154	161	5.8	46.4	15.6	100	8.3	16.4	23.0
Avg	12.1	45	230	91	64	1.8	28.5	10.0	96	5.0	6.2	14.0
SD	1.16	6.2	79	25	27	1.08	5.0	1.6	10.28	0.91	2.6	3.5
Set B	**GP**	**PSI**	**W**	**P**	**L**	**PL**	**WG**	**GS**	**GI**	**MixH**	**MixT**	**SDSS**
Min	8.8	40	77	34	46	0.3	17.8	5.7	59	3.3	1.9	10.3
Max	15.2	65	540	149	155	2.0	46.1	15.1	100	7.4	6.0	22.5
Avg	11.9	49	270	78	102	0.8	30.2	10.1	91	5.2	3.9	15.8
SD	1.13	4.9	106	23	23	0.37	5.2	1.8	10.86	0.92	1.0	3.6

Set B	FP	Wad	Pad	Lad	PLad	FarS	Abs	FrV	LV	Glu	Mon
Min	7.3	92	37	28	0.3	1.0	51.1	400	445	28	39
Max	14.6	424	133	152	4.4	28.8	67.3	678	1018	78	161
Avg	10.7	241	77	89	1.0	11.3	58.7	567	808	56	86
SD	1.24	82	21	25	0.72	7.3	3.3	67	107	11	24

[a] GP: grain protein (%); PSI: Particle Size Index, hardness (%); W, P, L and PL: standard alveogram variables; WG: wet gluten (%); DG: dry gluten (%); GI: Gluten Index (%); MixH: mixogram maximum height (cm); MixT: mixogram mixing time (min); SDSS: sedimentation test volume (ml/g); FP: flour protein (%); Wad, Pad, Lad, PLad: modified alveogram variables; FarS: farinogram stability time (min); Abs: farinogram water absorption (%); FrV: French-type loaf volume (mL); LV: pan loaf volume (mL); Glu: glutenins content (AU); Mon: monomeric proteins content (AU); Min: minimum value; Max: maximum value; Avg: average; SD: standard deviation.

Table 4.2. Results obtained with different math treatments using ground sample (set A)[a]

Math Treatments[b]				Standard error of prediction (SEP)										
				GP	PSI	W	P	L	PL	WG	DG	GI	MixH	MixT	SDSS
1	2	2	1	0.220	1.80	65.6	21.2	16.3	0.884	2.45	0.773	13.66	0.483	1.55	2.56
1	4	4	1	0.213	1.76	65.0	21.1	16.6	0.887	2.41	0.771	13.66	0.482	1.62	2.05
1	10	10	1	0.215	1.85	63.3	21.5	16.7	0.900	2.45	0.780	12.31	0.483	1.66	2.22
2	2	2	2	0.223	2.14	65.2	22.2	17.1	0.898	2.40	0.768	14.56	0.488	1.72	2.83
2	4	4	2	0.197	1.83	65.0	22.3	16.5	0.872	2.44	0.761	14.23	0.490	1.66	2.73
2	10	10	2	0.210	1.96	58.2	21.7	16.2	0.911	2.47	0.740	12.49	0.495	1.52	2.13
3	4	4	3	0.202	2.33	64.1	21.0	16.5	0.846	2.45	0.806	14.19	0.501	1.62	2.74
3	6	6	3	0.193	1.90	64.8	21.1	16.4	0.865	2.47	0.799	14.19	0.496	1.62	2.70
3	10	10	3	0.199	2.03	59.9	21.7	16.6	0.908	2.42	0.792	13.92	0.493	1.54	2.16
Math Treatments[b]				**Coefficient of determination (R^2)**										
				GP	PSI	W	P	L	PL	WG	DG	GI	MixH	MixT	SDSS
1	2	2	1	0.966	0.856	0.380	0.332	0.609	0.373	0.723	0.732	0.166	0.657	0.569	0.498
1	4	4	1	0.968	0.863	0.394	0.333	0.599	0.370	0.728	0.737	0.171	0.659	0.542	0.685
1	10	10	1	0.968	0.851	0.423	0.310	0.594	0.352	0.728	0.730	0.351	0.659	0.527	0.622
2	2	2	2	0.965	0.789	0.386	0.276	0.576	0.356	0.729	0.737	0.043	0.644	0.456	0.398
2	4	4	2	0.972	0.852	0.390	0.263	0.603	0.391	0.723	0.743	0.081	0.643	0.500	0.440
2	10	10	2	0.969	0.838	0.514	0.298	0.617	0.340	0.721	0.761	0.357	0.641	0.583	0.653
3	4	4	3	0.973	0.761	0.407	0.348	0.605	0.425	0.718	0.707	0.084	0.625	0.524	0.434
3	6	6	3	0.975	0.857	0.395	0.337	0.605	0.401	0.716	0.713	0.085	0.635	0.533	0.443
3	10	10	3	0.972	0.826	0.486	0.301	0.599	0.347	0.728	0.719	0.123	0.645	0.582	0.645

[a] GP: grain proteins (%); PSI: Particle Size Index (hardness) (%); W, P, L and PL: standard alveogram variables; WG: wet gluten (%); DG: dry gluten (%); GI: Gluten Index (%); MixH: mixogram maximum height (cm); MixT: mixogram mixing time (min); SDSS: sedimentation test volume (ml/g).
[b] Math treatments: derivative, gap, smooth, smooth 2.

extreme, for GI the value of R^2 never was over 0.4. Nevertheless, among the different derivative, gap and smoothing values, differences were unimportant for certain variables, while they were important for others. The treatment 2 10 10 2 was the best for some variables (W, L, DG, GI and MixT) and the overall best (highest averaged R^2). In order to have a second math treatment to evaluate, 2 4 4 2 was selected since its calibrations were close to the best for the rest of the variables (GP, PSI, P, PL, WG, MixH and SDSS). Both 2 10 10 2 and 2 4 4 2 were used for the rest of the research.

4.4.3. Wavelengths

Selection of the range of wavelengths to use for the NIR scans was based on a comparison of data for three ranges. Because the lower part of the spectrum (408-1108 nm) has more noise than the higher part, the whole spectrum (408-2492 nm) results were compared with those obtained by removing all or part of the low wave length region (Table 4.3). The results were better (higher R^2 and lower SEP) for GP, PSI, W, L, DG, MixT and SDSS for the whole spectrum than for the reduced range. Results were very similar for the rest of the variables (P, PL, WG, GI, and MixH) over all ranges used. It was concluded that eliminating the low region of the spectrum did not result in any improvement.

4.4.4. Ground whole grain and flour

Both GP and PSI showed better results for ground grain than for flour (Table 4.4). This was expected, since these variables were determined on grain rather than on flour. SEP values obtained for GP with both treatments using ground grain were 0.197 and 0.210 % for 2 4 4 2 and 2 10 10 2, respectively, which made the calibration suitable for prediction. SEP values for scans of flour were too high to make this suitable (0.303 and 0.304 %, respectively). Similarly, the SEP values for PSI were below 2 when ground grain samples were scanned, and they were closer to 3 when flours were scanned. For the rest of the variables which had been determined on flour samples, the results were generally better scanning flour than ground sample. For the best prediction equations (R^2 higher than 0.65), results were always better for flour scans. This was the case for L, WG, DG and SDSS. When prediction was medium to poor (R^2 lower than 0.65) the comparison between flour and ground grain scanning was erratic. In these cases, flour scanning was not better than ground grain because other sources of error were more

Table 4.3. Results obtained using different wavelengths ranges using ground sample (set A)[a]

	Standard error of prediction (SEP)					
Wavelengths	408-2492nm		900-2492nm		1108-2492nm	
Math treatment[b]	2 4 4 2	2 10 10 2	2 4 4 2	2 10 10 2	2 4 4 2	2 10 10 2
GP	0.197	0.210	0.219	0.215	0.233	0.231
PSI	1.83	1.96	2.07	2.05	2.04	1.99
W	65	58	66	63	67	61
P	22	22	22	21	22	21
L	17	16	17	17	18	17
PL	0.87	0.91	0.84	0.91	0.90	0.86
WG	2.44	2.47	2.53	2.49	2.49	2.26
DG	0.76	0.74	0.77	0.76	0.77	0.74
GI	14.2	12.5	13.5	12.9	12.7	12.7
MixH	0.49	0.50	0.49	0.49	0.49	0.50
MixT	1.66	1.52	1.76	1.59	1.76	1.67
SDSS	2.73	2.13	2.25	2.20	2.40	2.23

	Coefficient of determination (R^2)					
Wavelengths	408-2492nm		900-2492nm		1108-2492nm	
Math treatment[b]	2 4 4 2	2 10 10 2	2 4 4 2	2 10 10 2	2 4 4 2	2 10 10 2
GP	0.972	0.969	0.966	0.968	0.962	0.962
PSI	0.852	0.838	0.806	0.818	0.821	0.829
W	0.390	0.514	0.382	0.422	0.363	0.471
P	0.263	0.298	0.301	0.340	0.300	0.329
L	0.603	0.617	0.600	0.600	0.543	0.577
PL	0.391	0.340	0.436	0.345	0.351	0.409
WG	0.723	0.721	0.713	0.726	0.721	0.773
DG	0.743	0.761	0.733	0.749	0.733	0.767
GI	0.081	0.357	0.175	0.285	0.297	0.305
MixH	0.643	0.641	0.644	0.641	0.634	0.631
MixT	0.500	0.583	0.445	0.550	0.463	0.513
SDSS	0.440	0.653	0.621	0.628	0.565	0.618

[a] GP: grain proteins (%); PSI: Particle Size Index (hardness) (%); W, P, L and PL: standard alveogram variables; WG: wet gluten (%); DG: dry gluten (%); GI: Gluten Index (%); MixH: mixogram maximum height (cm); MixT: mixogram mixing time (min); SDSS: sedimentation test volume (ml/g).
[b] Math treatments: derivative, gap, smooth, smooth 2.

important. Nevertheless, differences were not very big for the mentioned variables. According to these results, flour scanning improved the data for variables determined originally on flour, but did not introduce an important improvement in calibrations.

Table 4.4. Comparison of results obtained using flour and ground sample (Set A)[a]

	Standard error of prediction (SEP)				Coefficient of determination (R^2)			
	Ground sample		Flour		Ground sample		Flour	
Math treatment[b]	2 4 4 2	2 10 10 2	2 4 4 2	2 10 10 2	2 4 4 2	2 10 10 2	2 4 4 2	2 10 10 2
GP	0.197	0.210	0.303	0.304	0.972	0.969	0.940	0.938
PSI	1.83	1.96	3.20	2.89	0.852	0.838	0.542	0.618
W	65.0	58.2	62.8	58.9	0.390	0.514	0.433	0.498
P	22.3	21.7	18.0	18.3	0.263	0.298	0.518	0.504
L	16.5	16.2	14.9	14.6	0.603	0.617	0.671	0.688
PL	0.872	0.911	0.773	0.797	0.391	0.340	0.522	0.490
WG	2.44	2.47	2.27	1.94	0.723	0.721	0.755	0.821
DG	0.761	0.740	0.673	0.622	0.743	0.761	0.795	0.826
GI	14.2	12.5	14.1	13.7	0.081	0.357	0.096	0.165
MixH	0.490	0.495	0.514	0.512	0.643	0.641	0.621	0.622
MixT	1.66	1.52	1.51	1.44	0.500	0.583	0.594	0.633
SDSS	2.73	2.13	2.13	1.74	0.440	0.653	0.656	0.770

[a] GP: grain proteins (%); PSI: Particle Size Index (hardness) (%); W, P, L and PL: standard alveogram variables; WG: wet gluten (%); DG: dry gluten (%); GI: Gluten Index (%); MixH: mixogram maximum height (cm); MixT: mixogram mixing time (min); SDSS: sedimentation test volume (ml/g).
[b] Math treatments: derivative, gap, smooth, smooth 2.

4.4.5. Ground sample and whole grain

Results for ground samples were better but similar to those for whole grain ones (Table 4.5), with the exception of predictions for PSI, and GP. PSI calibrations were usable when ground samples were scanned (SEP below 2% and R^2 over 0.800), while using whole grain, the results were poor (SEP over 4% and R^2 below 0.400). When hardness is determined by this method, the actual measurement is on particle size of ground sample. Particle size cannot be determined on whole grain. Therefore, NIR could not predict hardness scanning whole grain. Similar results in hardness calibrations using whole grain and ground samples were previously reported (Williams 1996).

The information obtained from scanning of ground samples included the spectra of all parts of the grain. When whole grain was scanned, the information obtained was

Table 4.5. Comparison of results obtained using whole grain and ground sample (set A)[a]

	Standard error of prediction (SEP)				Coefficient of determination (R^2)			
	Ground sample		Whole grain		Ground sample		Whole grain	
Math treatment[b]	2 4 4 2	2 10 10 2	2 4 4 2	2 10 10 2	2 4 4 2	2 10 10 2	2 4 4 2	2 10 10 2
GP	0.197	0.210	0.285	0.285	0.972	0.969	0.945	0.945
PSI	1.834	1.962	4.357	4.239	0.852	0.838	0.355	0.377
W	65.0	58.2	64.3	64.2	0.390	0.514	0.403	0.406
P	22.3	21.7	23.7	23.7	0.263	0.298	0.166	0.167
L	16.5	16.2	16.8	17.4	0.603	0.617	0.586	0.556
PL	0.872	0.911	0.902	0.988	0.391	0.340	0.349	0.222
WG	2.44	2.47	2.56	2.65	0.723	0.721	0.708	0.689
DG	0.761	0.74	0.793	0.83	0.743	0.761	0.730	0.706
GI	14	12	14	14	0.081	0.357	0.149	0.148
MixH	0.490	0.495	0.538	0.564	0.643	0.641	0.570	0.532
MixT	1.66	1.52	1.73	1.75	0.500	0.583	0.478	0.475
SDSS	2.73	2.13	2.50	2.62	0.440	0.653	0.523	0.477

[a] GP: grain proteins (%); PSI: Particle Size Index (hardness) (%); W, P, L and PL: standard alveogram
variables; WG: wet gluten (%); DG: dry gluten (%); GI: Gluten Index (%); MixH: mixogram maximum
height (cm); MixT: mixogram mixing time (min); SDSS: sedimentation test volume (ml/g).
[b] Math treatments: derivative, gap, smooth, smooth 2.

only from the outside layers of the kernel. Therefore, the recorded data were better when ground sample was scanned. Outside layers information is always correlated to the one from the rest of the grain. Therefore, whole grain could be potentially useful for NIR calibrations, but it introduces a source of error. When calibration is very good, the source of error becomes significant. When calibration is poor, the error due to this component is masked by other sources of error. These concepts explain, for example, the behavior of W and L calibration. Calibration results for W were poor, but they were better for ground sample (SEP = 58.2, R^2 = 0.514) than for whole grain (SEP = 64.2, R^2 = 0.406) with the 2 10 10 2 math treatment. At the same time, they were slightly better for whole grain (SEP = 64.3, R^2 = 0.403) than for ground sample (SEP = 65.2, R^2 = 0.390) with the 2 4 4 2 treatment. Calibration results for L were better, and therefore ground sample results were clearly better than whole grain for both math treatments

(Table 4.5). The difference was even more relevant for GP calibration. Results with whole grain were good (SEP = 0.285, R^2 = 0.945), but they were significantly better with ground samples (SEP $\leq$0.210, R^2 $\geq$0.969).

Usefulness of whole sample calibrations depends on the objective pursued. If the analysis should be non-destructive or if speed is an issue, then whole grain results could be used if accuracy demand is low. PSI whole grain calibration is not recommended in any case. When the important requirement of the method is accuracy, samples should be ground.

4.4.6. Rectangular cell vs. small ring cup

The rectangular cell is larger than the small cell cup, giving a bigger area for scanning. Therefore, the spectrum collected from a rectangular cell will be more representative than the one obtained with the small one. However, considering only those variables with R^2 higher than 0.6, the use of a bigger area for scanning did not improve calibrations (Table 4.6).

4.4.7. Scanning duplicates

Table 4.7 shows results of the validation procedure of the calibration results obtained scanning the sample once and twice. Although the difference is small, in most cases the results obtained with duplicates were better, as expected. In fact, for all variables with R^2 higher than 0.5 (GP, PSI, L, GH, GS, HMAX, TMEZ and SDSS), duplicating the scanning introduced an improvement to the calibration.

Table 4.6. Comparison of results obtained using whole grain with rectangular cell and small cell cup (set A)[a]

	Standard error of prediction (SEP)				Coefficient of determination (R^2)			
	Rectangular cell		Small cell cup		Rectangular cell		Small cell cup	
Math treatment[b]	2 4 4 2	2 10 10 2	2 4 4 2	2 10 10 2	2 4 4 2	2 10 10 2	2 4 4 2	2 10 10 2
GP	0.285	0.285	0.301	0.253	0.945	0.945	0.939	0.956
PSI	4.357	4.239	3.53	3.785	0.355	0.377	0.522	0.457
W	64.3	64.2	61.0	60.9	0.403	0.406	0.464	0.468
P	23.7	23.7	19.7	21.0	0.166	0.167	0.425	0.341
L	16.8	17.4	15.7	16.5	0.586	0.556	0.638	0.602
PL	0.902	0.988	0.790	0.783	0.349	0.222	0.512	0.527
WG	2.56	2.65	2.58	2.25	0.708	0.689	0.692	0.769
DG	0.793	0.83	0.824	0.755	0.730	0.706	0.703	0.758
GI	13.8	13.8	12.6	12.5	0.149	0.148	0.346	0.320
MixH	0.538	0.564	0.518	0.537	0.570	0.532	0.602	0.581
MixT	1.73	1.75	1.59	1.59	0.478	0.475	0.546	0.546
SDSS	2.50	2.62	2.18	2.02	0.523	0.477	0.636	0.688

[a] GP: grain proteins (%); PSI: Particle Size Index (hardness) (%); W, P, L and PL: standard alveogram variables; WG: wet gluten (%); DG: dry gluten (%); GI: Gluten Index (%); MixH: mixogram maximum height (cm); MixT: mixogram mixing time (min); SDSS: sedimentation test volume (ml/g).
[b] Math treatments: derivative, gap, smooth, smooth 2.

Table 4.7. Comparison of results obtained using ground sample scanning with and without duplicate (set A)[a]

	Standard error of prediction (SEP)				Coefficient of determination (R^2)			
	One rep		Two reps		One rep		Two reps	
Math treatment[b]	2 4 4 2	2 10 10 2	2 4 4 2	2 10 10 2	2 4 4 2	2 10 10 2	2 4 4 2	2 10 10 2
GP	0.285	0.285	0.301	0.253	0.945	0.945	0.939	0.956
PSI	4.357	4.239	3.53	3.785	0.355	0.377	0.522	0.457
W	64.3	64.2	61.0	60.9	0.403	0.406	0.464	0.468
P	23.7	23.7	19.7	21.0	0.166	0.167	0.425	0.341
L	16.8	17.4	15.7	16.5	0.586	0.556	0.638	0.602
PL	0.902	0.988	0.790	0.783	0.349	0.222	0.512	0.527
WG	2.56	2.65	2.58	2.25	0.708	0.689	0.692	0.769
DG	0.793	0.83	0.824	0.755	0.730	0.706	0.703	0.758
GI	13.8	13.8	12.6	12.5	0.149	0.148	0.346	0.320
MixH	0.538	0.564	0.518	0.537	0.570	0.532	0.602	0.581
MixT	1.73	1.75	1.59	1.59	0.478	0.475	0.546	0.546
SDSS	2.50	2.62	2.18	2.02	0.523	0.477	0.636	0.688

[a] GP: grain proteins (%); PSI: Particle Size Index (hardness) (%); W, P, L and PL: standard alveogram variables; WG: wet gluten (%); DG: dry gluten (%); GI: Gluten Index (%); MixH: mixogram maximum height (cm); MixT: mixogram mixing time (min); SDSS: sedimentation test volume (ml/g).
[b] Math treatments: derivative, gap, smooth, smooth 2.

4.4.8. Extending NIR to other variables

Additional analyses were done on a small set of samples (set B) in order to be able to explore the possibility of NIR for other variables (Tables 4.8 and 4.9). Comparing results of set A and B (Tables 4.2, 4.8 and 4.9), it is clear that for some variables (GP, W, P and MixH) set B produced better results, while set A was better for others (PSI, L, PL, WG, MixT, and SDSS). These differences in results were neither due to the capacity of NIR to

Table 4.8. Results obtained with different math treatments using ground sample (set B)[a]

Math Treatments[b]				Standard error of prediction (SEP)										
				GP	PSI	W	P	L	PL	WG	DG	GI	MixH	MixT	SDSS
1	2	2	1	0.320	2.55	64.3	14.9	30.5	0.478	4.11	1.133	8.23	0.506	0.89	3.79
1	4	4	1	0.216	2.52	62.9	15.5	27.4	0.437	4.19	1.188	8.17	0.520	0.97	4.12
1	10	10	1	0.196	2.55	64.6	13.7	27.7	0.431	4.15	1.462	8.60	0.516	1.01	4.15
2	2	2	2	0.225	2.31	75.0	13.7	22.0	0.329	5.22	1.654	9.94	0.762	1.26	4.18
2	4	4	2	0.188	2.50	70.1	11.6	20.1	0.323	3.42	1.006	8.52	0.706	0.79	3.56
2	10	10	2	0.142	2.95	71.2	12.3	19.2	0.300	3.30	0.971	8.46	0.542	0.78	3.44
3	4	4	3	0.173	2.33	75.1	11.2	22.0	0.318	3.75	1.240	10.97	0.643	1.06	3.67
3	6	6	3	0.169	2.78	69.0	10.9	20.3	0.309	3.34	1.134	9.25	0.758	0.88	3.00
3	10	10	3	0.139	2.96	70.2	12.4	20.7	0.333	2.37	0.716	8.25	0.733	0.85	3.36

Math Treatments[b]				Standard error of prediction (SEP)									
				FP	Wad	Pad	Lad	P/Lad	FarS	Abs	FrV	LV	Glu	Mon
1	2	2	1	0.414	68.1	12.5	28.0	0.737	2.182	2.014	103	64	9.5	12.0
1	4	4	1	0.347	69.4	12.4	24.3	0.671	2.715	1.718	103	64	9.4	12.0
1	10	10	1	0.330	71.6	12.8	23.4	0.672	2.793	2.455	104	67	8.3	11.1
2	2	2	2	0.450	77.6	17.3	31.0	0.773	3.169	2.240	90	99	10.5	18.4
2	4	4	2	0.330	60.8	15.8	27.5	0.791	2.742	1.784	93	99	9.2	13.3
2	10	10	2	0.324	72.5	14.7	25.0	0.716	2.773	1.403	94	73	9.6	11.5
3	4	4	3	0.254	67.8	15.6	22.0	0.747	3.142	2.296	97	134	11.0	12.6
3	6	6	3	0.296	69.6	15.4	22.2	0.743	3.809	2.310	94	97	9.2	14.8
3	10	10	3	0.303	59.5	20.8	24.0	0.770	2.878	1.465	95	77	9.1	13.0

[a] GP: grain protein (%); PSI: Particle Size Index, hardness (%); W, P, L and PL: standard alveogram variables; WG: wet gluten (%); DG: dry gluten (%); GI: Gluten Index (%); MixH: mixogram maximum height (cm); MixT: mixogram mixing time (min); SDSS: sedimentation test volume (ml/g); FP: flour protein (%); Wad, Pad, Lad, PLad: modified alveogram variables; FarS: farinogram stability time (min); Abs: farinogram water absorption (%); FrV: French-type loaf volume (mL); LV: pan loaf volume (mL); Glu: glutenins content (AU); Mon: monomeric proteins content (AU).

[b] Math treatments: derivative, gap, smooth, smooth 2.

Table 4.9. Results obtained with different math treatments using ground sample (set B)[a]

Math Treatments[b]				Coefficient of determination (R^2)										
				GP	PSI	W	P	L	PL	WG	DG	GI	MixH	MixT	SDSS
1	2	2	1	0.952	0.701	0.715	0.745	0.072	0.350	0.505	0.685	0.387	0.820	0.180	0.066
1	4	4	1	0.977	0.700	0.725	0.757	0.155	0.444	0.519	0.662	0.410	0.810	0.168	0.040
1	10	10	1	0.981	0.694	0.710	0.696	0.175	0.485	0.568	0.497	0.368	0.814	0.137	0.031
2	2	2	2	0.969	0.764	0.609	0.710	0.186	0.444	0.165	0.337	0.302	0.512	0.089	0.064
2	4	4	2	0.983	0.705	0.647	0.798	0.291	0.468	0.615	0.703	0.352	0.673	0.237	0.077
2	10	10	2	0.988	0.601	0.637	0.779	0.438	0.556	0.696	0.782	0.339	0.783	0.169	0.127
3	4	4	3	0.982	0.784	0.599	0.832	0.158	0.481	0.790	0.842	0.173	0.725	0.247	0.073
3	6	6	3	0.985	0.675	0.658	0.820	0.269	0.504	0.751	0.799	0.295	0.651	0.252	0.219
3	10	10	3	0.988	0.637	0.649	0.756	0.238	0.441	0.809	0.856	0.351	0.690	0.108	0.117

Math Treatments[b]				Coefficient of determination (R^2)									
				FP	Wad	Pad	Lad	P/Lad	FarS	Abs	FrV	LV	Glu	Mon
1	2	2	1	0.960	0.260	0.736	0.220	0.463	0.925	0.769	0.005	0.774	0.255	0.800
1	4	4	1	0.975	0.267	0.725	0.319	0.546	0.873	0.750	0.005	0.789	0.252	0.802
1	10	10	1	0.977	0.256	0.698	0.435	0.552	0.875	0.336	0.004	0.757	0.486	0.821
2	2	2	2	0.941	0.171	0.473	0.007	0.430	0.808	0.565	0.125	0.451	0.098	0.648
2	4	4	2	0.980	0.343	0.603	0.235	0.344	0.856	0.738	0.055	0.520	0.427	0.745
2	10	10	2	0.965	0.230	0.587	0.275	0.451	0.872	0.854	0.049	0.711	0.260	0.820
3	4	4	3	0.980	0.329	0.624	0.357	0.496	0.805	0.773	0.019	0.059	0.339	0.784
3	6	6	3	0.976	0.291	0.643	0.347	0.535	0.657	0.813	0.046	0.530	0.429	0.704
3	10	10	3	0.969	0.339	0.220	0.248	0.457	0.877	0.852	0.035	0.697	0.418	0.743

[a] GP: grain protein (%); PSI: Particle Size Index, hardness (%); W, P, L and PL: standard alveogram variables; WG: wet gluten (%); DG: dry gluten (%); GI: Gluten Index (%); MixH: mixogram maximum height (cm); MixT: mixogram mixing time (min); SDSS: sedimentation test volume (ml/g); FP: flour protein (%); Wad, Pad, Lad, PLad: modified alveogram variables; FarS: farinogram stability time (min); Abs: farinogram water absorption (%); FrV: French-type loaf volume (mL); LV: pan loaf volume (mL); Glu: glutenins content (AU); Mon: monomeric proteins content (AU).

[b] Math treatments: derivative, gap, smooth, smooth 2.

predict these variables nor to the reference method. Differences were due only to the differences in the data set, which demonstrates the importance of the data set selection for the calibration. Distribution may play a key role. This point is further discussed below. Calibration results for some variables predicted with set B samples (FarS and Mon) showed even better results than those measured in A, indicating potential for further work. It should be remarked that the best pan loaf volume (LV) prediction had a SEP of 64 and R^2=0.79. Therefore, LV was better predicted by NIR that by rheological

parameters. However, this finding should be considered carefully since validation data was based on only 15 samples.

4.4.9. Selection of validation set

Starting from the original set of data, a new calibration set was selected in order to change the alveogram baking strength (W) frequency distribution. A Gaussian distribution of composition will tend to predict lower values for samples at high concentrations and higher values for samples at low concentrations (Williams 2001). In the original selection, W distribution was as it is shown in Figure 4.1 (n=94, standard deviation=83.5). In the new proposed selection, W distribution was wider, as it is shown in Figure 4.2 (n=43, standard deviation=98.8). The calibration set was composed of all the samples from set A which were not in the validation set.

Results using this set are presented in Table 4.10. RPD values improved for several variables (for example: GP, W, L and MixH), but the only big improvement was for PSI. The big change in RPD for PSI was mostly for the change in the SD rather than for a change in SEP. Therefore, although a change in the distribution may improve RPD results, it may not necessarily change NIR performance.

4.4.10. General discussion

Among the quality requirements, at least three independent characteristics can be selected: hardness, protein quantity and protein quality (Morris 1998). Hardness can be easily estimated by the PSI method. Protein quantity is mostly determined by Kjeldahl, although wet and dry gluten percentages are valid estimators as well. The concept protein "quality" or "functionality" (Williams et al 1988) is ambiguous, and several

Figure 4.1. Distribution of samples values (alveogram W) for the original validation set of set A.

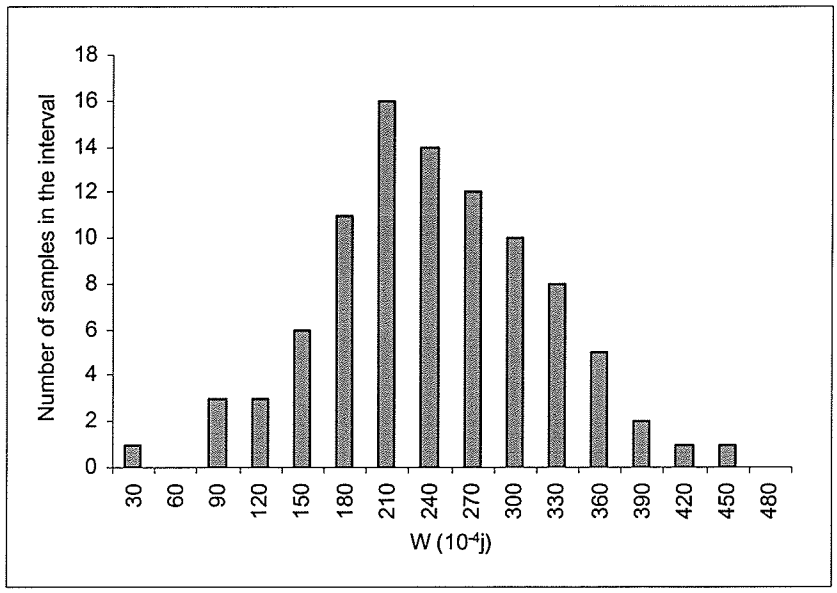


Figure 4.2. Distribution of samples values (alveogram W) for the second validation set of set A.

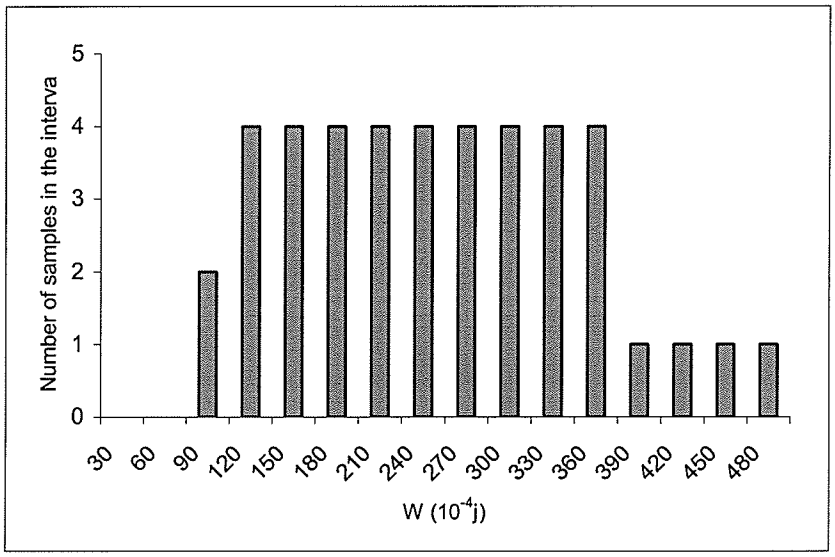


Table 4.10. Comparison of results obtained using ground sample with two different validation set of sample (set A)[a]

Math treatment[b]		Original validation set		New validation set	
		2 4 4 2	2 10 10 2	2 4 4 2	2 10 10 2
GP	SEP	0.197	0.210	0.163	0.169
	R^2	0.972	0.969	0.979	0.977
	SD	1.19	1.19	1.13	1.13
	RPD	6.0	5.7	6.9	6.7
PSI	SEP	1.83	1.96	1.78	1.43
	R^2	0.852	0.838	0.944	0.963
	SD	4.72	4.72	7.64	7.64
	RPD	2.6	2.4	4.3	5.4
W	SEP	65.0	58.2	67.7	68.1
	R^2	0.390	0.514	0.552	0.541
	SD	83.5	83.5	98.8	98.8
	RPD	1.3	1.4	1.5	1.5
P	SEP	22.3	21.7	18.4	19.6
	R^2	0.263	0.298	0.466	0.394
	SD	26.0	26.0	24.5	24.5
	RPD	1.2	1.2	1.3	1.2
L	SEP	16.5	16.2	16.6	17.0
	R^2	0.603	0.617	0.758	0.744
	SD	26.1	26.1	32.4	32.4
	RPD	1.6	1.6	2.0	1.9
P/L	SEP	0.872	0.911	0.653	0.643
	R^2	0.391	0.340	0.576	0.589
	SD	1.120	1.120	0.992	0.992
	RPD	1.3	1.2	1.5	1.5
WG	SEP	2.44	2.47	3.16	3.04
	R^2	0.723	0.721	0.657	0.681
	SD	4.59	4.59	5.44	5.44
	RPD	1.9	1.9	1.7	1.8
DG	SEP	0.761	0.740	1.035	1.046
	R^2	0.743	0.761	0.657	0.649
	SD	1.48	1.48	1.79	1.79
	RPD	1.9	2.0	1.7	1.7
GI	SEP	14.2	12.5	9.1	9.1
	R^2	0.081	0.357	0.045	0.059
	SD	14.8	14.8	9.4	9.4
	RPD	1.0	1.2	1.0	1.0
MixH	SEP	0.490	0.495	0.532	0.535
	R^2	0.643	0.641	0.797	0.796
	SD	0.811	0.811	1.144	1.144
	RPD	1.7	1.6	2.2	2.1
MixT	SEP	1.656	1.515	1.918	1.819
	R^2	0.500	0.583	0.516	0.559
	SD	2.33	2.33	2.76	2.76
	RPD	1.4	1.5	1.4	1.5
SDSS	SEP	2.73	2.13	2.72	2.68
	R^2	0.440	0.653	0.531	0.544
	SD	3.63	3.63	4.01	4.01
	RPD	1.3	1.7	1.5	1.5

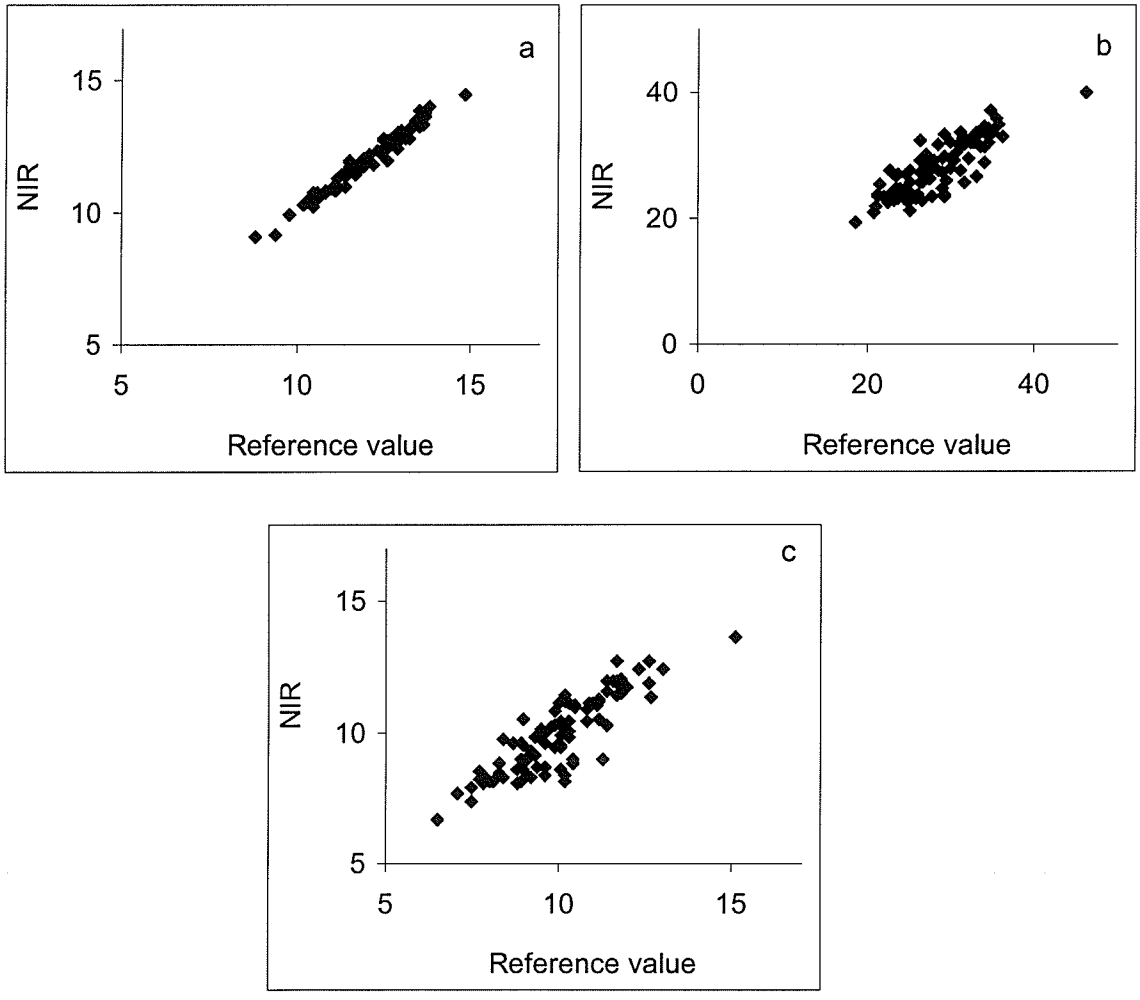
[a] GP: grain proteins (%); PSI: Particle Size Index (hardness) (%); W, P, L and PL: standard alveogram variables; WG: wet gluten (%); DG: dry gluten (%); GI: Gluten Index (%); MixH: mixogram maximum height (cm); MixT: mixogram mixing time (min); SDSS: sedimentation test volume (ml/g); SEP: standard error of prediction; R^2: coefficient of determination; SD: standard deviation; RPD: ratio of the SEP to the SD of the reference data.

[b] Math treatments: derivative, gap, smooth, smooth 2.

rheological (W, P, L, PL, GI, MixH, MixT) or physicochemical (SDSS) parameters could be used to define it.

Results confirmed that very good NIR calibrations could be obtained for protein quantity prediction (Tables 4.1I and 4.8, Figure 4.3), as has been published (Delwiche et al 1998b, Williams 1975, Williams and Norris 1983). Grain protein content determined by

Figure 4.3. Reference values vs. NIR prediction for the original validation set of set A samples. a. Grain protein, % (GP). b. Wet Gluten, % (WG). c. Dry gluten, % (DG).



Kjeldahl (GP) was the best predicted variable, since the coefficient of determination was up to 0.988 and RPD up to 6.9. Glutomatic variables (WG and DG) were also successfully predicted, although with lower coefficient of determinations (up to 0.856) and wider dispersions (Figure 4.3). Calibrations obtained for hardness prediction were also very good (Tables 4.1 and 4.8, Figure 4.4). It should be emphasized that parameters values were acceptable only with ground samples.

More limited results were obtained for protein quality predictors, but these results still showed accuracy sufficient for screening methods applicable in early generations of wheat breeding programs. Four examples of rheological variables (W, L, MixH and MixT) are plotted in Figure 4.5. From this figure, it could be concluded that it is possible to select for rheological variables as different as mixogram mixing time and alveogram extensibility. Protein quality could be predicted not just by rheological variables, but also

Figure 4.4. Reference values vs. NIR prediction for the original validation set of set A samples. PSI (Hardness), %.

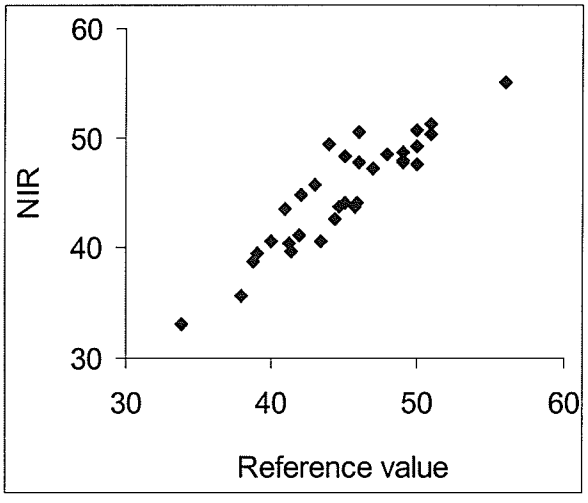
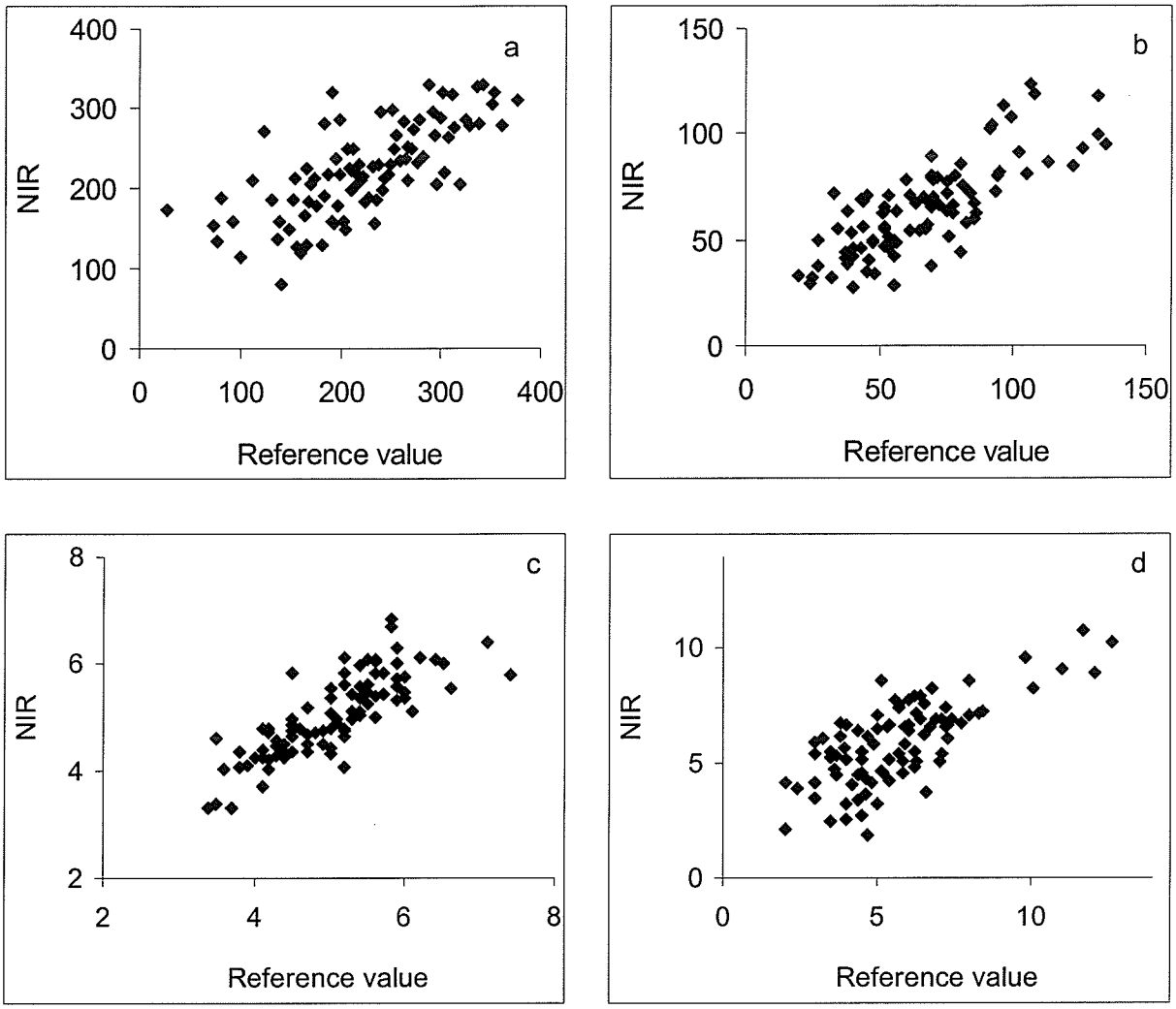
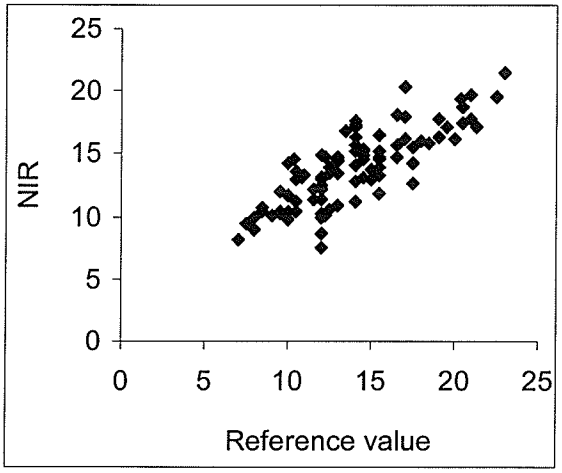


Figure 4.5. Reference values vs. NIR prediction for the original validation set of set A samples. a. Alveogram W, j x 10^{-4} (W). b. Alveogram extensibility, mm (L). c. Mixogram maximum height, cm (MixH). d. Mixogram mixing time, cm (MixT).



by physicochemical variables like SDSS. Calibration results proved that protein quality could be predicted by NIR (Tables 4.2, 4.8 and 4.9, Figure 4.6), although Osborne (1984) suggested that NIR cannot provide a wheat quality measurement beyond protein content. The success of the results can be explained by the size of the set of samples. Hruskova et al (2001) suggested that their poor results with extensigram variables were due to a small size of sample set.

Figure 4.6. Reference values vs. NIR prediction for the original validation set of set A samples. SDS sedimentation volume, mL (SDSS).



Functional variables are to some extent correlated to protein content (Vázquez 2005). At the same time, it was proved that NIR was able to predict protein content. Therefore, it is reasonable that good calibration results for functional properties could be explained by the correlations of these parameters with protein content. This was not the case for set A samples, however. For example, for W, L, MixT and SDSS, the coefficients of determination with GP were 0.24, 0.13, 0.04 and 0.32, respectively. Miralbés (2003) obtained better calibrations for alveogram W (validation set R^2 = 0.84). In that case, correlation between protein and W (r^2 = 0.86) could be the reason for outstanding results. Besides the variables discussed with details, others showed potential for further work (Table 4.8). For example, validation coefficient of determination for farinogram stability was up to 0.925.

4.5. CONCLUSIONS

NIR technology proved to be useful to predict three independent components: protein amount, protein functionality and hardness. The SEP value for protein is comparable with the results obtained with the reference method. PSI results were not accurate enough to substitute for the reference method, but were good enough to separate hard from soft kernel genotypes. Functionality variables were those predicted with the lowest accuracy. However, alveogram baking strength (W) and extensibility (L), mixogram mixing time (MixT) and SDS sedimentation volume (SDSS) could be predicted at accuracies sufficient for screening purposes in breeding programs. Therefore, several functional properties that usually require a good deal of time could be predicted by a single fast test. Further research is needed to confirm suitability of these results.

Results presented useful information for further research. If flour is available, it is the best sample to scan, although good calibrations could be obtained with ground sample. The larger cell did not show better results than small ring cell cup. Duplicated spectra improved accuracy. Math treatment or wavelength selection introduced limited improvements in different calibration equations. It is important to remark that although NIR hardware and software provide a wide diversity of approaches, the most important component to develop a good calibration is the availability of an accurate and precise reference method for the variable, and that the variable is one that is suitable to be determined by the spectrophotometer. It is not possible to start from a poor calibration, and obtain a good one through the use of other tools.

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CHAPTER 5

GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

Specifications based on dough rheological properties are widely used by the bakery industries to predict breadmaking potential of wheat flours. In Uruguay, the production of hearth bread is greater than that of pan bread. Flour requirements for optimum quality of hearths breads have not been determined. In addition, there have been no previous research studies using Uruguayan wheats and examining the relationship of their rheological properties with hearth and pan bread quality.

The main objective of this research was first of all to determine the importance of dough rheological properties, and especially extensional properties, for the quality of hearth and pan breads. Bread making variability was better explained by extensional than by mixing properties. Simple correlation coefficients and MLR proved that extensional parameters explained the breadmaking properties better than did mixing test parameters. In particular, the proposed modified alveograph procedure parameters explained 61% of pan loaf volume, 34% of hearth loaf volume and 61% of the hearth bread form ratio while farinograph ones explained 27%, 16% and 27% respectively and mixograph ones explained 20%, 31% and 36% respectively. Although mixing properties have been claimed as helpful for wheat flour quality evaluation (Ohm and Chung 1999), they are more useful to evaluate dough behavior during the breadmaking process than to predict bread quality. The critical step during breadmaking that defines loaf characteristics is fermentation, where the growing gas cells cause dough extension.

Among modified alveograph variables, the resistance to extension/extensibility ratio (P/Lad) was the best correlated with both pan loaf ($r=-0.701$, $P<0.001$) and hearth loaf

volume (r=-0.438). The negative sign of the correlation with P/Lad means that bread volume was negatively affected by the resistance to extension (Pad) and positively affected by extensibility (Lad). A different result was published by Kieffer et al (1998), who reported a positive correlation between resistance to extension (as measured by a micro-extension test) and loaf volume, for three different baking tests (r = 0.73-0.83; n=12). The difference in the results can be explained by the type of wheat population used. In the same paper, when a different set of samples was studied, correlations ranged from non significant to 0.70, depending on the baking test.

The most important predictor of French-type (hearth) bread form ratio (FrR) was modified alveograph baking strength (Wad, r=0.718, P<0.001). FrR was also correlated with other strength predictors, in addition to several resistance to extension predictors. In the PLS analysis, FrR was located between the strength and the resistance to extension predictors. The form ratio was higher when the dough was stronger and more resistant to extension.

Although correlation coefficients were low, crumb appearance unlike FrR was negatively affected by resistance to extension. Uruguayan bakers give high importance to both form ratio and crumb appearance. Since one is positively correlated with resistance to extension and the other one is negatively correlated, a balance between appearance and form ratio is needed, and this will determine the degree of resistance to extension that will prduce good hearth loaf quality.

Several rheological parameters were highly significantly correlated with bread making properties. Requirements of the breadmaking process are varied and interrelated. Since

many phenomena are involved, it could not be expected that a single dough property could explain a high percentage of a quality parameter in a sample set this big (n=81). Therefore, low correlation coefficients were anticipated. The value of these correlations is in the fact that correlated parameters affected breadmaking properties, even though they did not explain a high percentage of bread quality variability. In order to obtain a better explanation of the relationship between breadmaking quality parameters and rheological properties, multiple linear regression (MLR) and partial least square (PLS) analyses were applied.

MLR models proved to be successful. They were able to explain 75% of the pan loaf (LV) variability, 45% of the French-type (hearth) volume (FrV) and 64% of its form ratio (FrR). Models were successfully applied also using only modified alveogram parameters, explaining 61%, 34% and 61% of LV, FrV and FrR respectively.

PLS models were able to explain high percentage of pan loaf volume (66%) and form ratio (59%), but the percentage was lower for hearth bread volume (40%). Similar results were obtained in some previous studies. Using 20 samples, Tronsmo et al (2003a) obtained a coefficient of determination of 0.84 and 0.70 for a Norwegian hearth bread volume and form ratio, respectively, but failed to find significant values for a pan bread volume (Chorleywood process). However, the same authors reported lower coefficients of determination (0.55 and 0.56) when they extended the work to 40 samples (Tronsmo et al 2003b).

Although several rheological parameters were highly significantly correlated with bread volume, none of them was as well correlated as total protein content (r=0.706 for pan

bread, 0.574 for hearth bread). It was concluded that even though the modified alveograph resistance to extension/extensibility ratio had a better performance than the standard parameters, it was not good enough to explain the underlying rheological property which is most closely related with breadmaking. Since the rheological requirements are so complex, it is probable that more than one parameter should be included to characterize actual bread quality properties. In fact, P/Lad is a composite variable, obtained from both maximum resistance to extension and extensibility.

Protein quantity rather than protein quality was the best bread making quality predictor: it was the parameter which best correlated with both pan and hearth bread volume, and with hearth bread appearance. This may have been because the genotypes used had been selected and had protein quality suitable for both types of bread. Higher protein content could be achieved using proper fertilization. Glutenin determination using SE-HPLC gave poor results. This could be explained by the fact that only glutenins soluble under test conditions were measured.

The objectives for this research included the evaluation of methodologies suitable to predict bread making quality. The modification of the alveogram showed an important improvement. The use of a fixed amount of water causes a bias in the results, mainly for resistance to extension. The proposed modification produced parameters that were able to explain most breadmaking properties better than did the parameters for fixed water. At the same time, these parameters were better related with protein components variables.

Micro-extensigraph tests proved to be a valid option for screening. The alveograph mixer requires 250g of flour, while only 10g were used for the suggested micro-tests. A

comparison of flour and gluten dough indicated that flour dougs gave the better prediction of baking baking strength (DA), while gluten doughs predicted the resistance to extension (GSI) more efficiently. Both gluten and flour dough analyses produced good extensibility predictors (GExtSI and DERup).

NIR calibrations provided a very fast and convenient methodology for evaluation of grain and flour properties. Several tools, strategies and approaches for NIR calibrations were evaluated. The grain protein calibration obtained was able to replace the reference method (R^2=0.972, SEP = 0.197). A moderately good prediction was obtained for hardness, which could be used for evaluation of breeding program advanced lines (R^2=0.852, SEP = 1.96). Breeding program early generation screenings can be done for important functional parameters like alveograph L (R^2=0.603, SEP=16.5), SDS sedimentation volume (R^2=0.653, SEP=2.13) and mixogram maximum height (R^2=0.643, SEP=0.490).

Different results for pan and hearth bread quality show the importance of using rheological tests that are appropriate for the type of bread of primary use.

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CHAPTER 6

SUGGESTIONS FOR FURTHER RESEARCH

SUGGESTIONS FOR FURTHER RESEARCH

Results were obtained with a large set of samples, representative of Uruguayan wheats, including several genotypes grown in different environments. This suggests applying these tests to bigger populations of Uruguayan wheats to confirm the findings.

The importance of the extensional variables on breadmaking should be confirmed for other sets of local samples. If the trend is confirmed, breeding program goals should be extended. More emphasis on extensibility and resistance to extension should be considered, without omitting dough strength as an objective. These findings must be discussed with the industry.

French-type (hearth) bread volume was poorly explained by rheological variables. This could be have been caused by the low variability in this specific parameter. This particular point should be more fully evaluated. Crumb appearance is a critical parameter for Uruguayan bakers. These samples gave a wide range (2.5-8.0) with the proposed subjective score (1.0-9.0), but the standard deviation was similar to the error of the analysis (1.0). Further work should be done to document differences. Image analysis to evaluate crumb openness should be considered.

Extensional properties proved to be the key component for breadmaking quality. Improvement of the methodology used to measure these properties should be considered. The only modification of the alveograph procedure tested was the amount of water used. The standard procedure also uses fixed mixing time, and adjustment of this

particular variable could be evaluated. In addition, strain rate is fixed. Variation in inflation rate using the alveograph would demand big changes in the machine.

Micro-extension testing using a texture analyzer makes it possible to study several other test conditions. Different sample preparation and size, hook diameter, extensional conditions, including different strain rates, could be evaluated.

Further work should include the determination of insoluble polymeric proteins. At the same time, this analysis would give the opportunity to have an estimator of molecular weight distribution of polymeric glutenins (Preston and Stevenson, 2003). Non-protein components could be important as well, since pentosans are known to cause an increased resistance to extension (Wang et al 2004).

This research has confirmed the value of certain rheological properties. It has not explained properties in terms of genetic or environmental factors. The next logical step is to develop genetic by environmental (GxE) assays in order to study the components of these properties.

NIR calibration results are encouraging, and further work should be done to obtain calibrations with different populations grown at different environments. Results could be improved by increasing the number of samples and improving reference methods performance. In addition, a bread quality parameter (pan loaf volume) was successfully predicted with the small set of samples. In order to validate this observation, calibration should be repeated with a bigger set of samples. Further validation would also be valuable for adjusted absorption alveogram and micro-extension parameters.

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