

**Effect of 1B/1R Chromosomal Translocation on Dough Rheology of Soft Red Winter
Wheat Flour**

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ABSTRACT

Nine 1B/1R translocated soft red winter wheat (SRWW) varieties and six non-1B/1R varieties from two crop years (1995-1996 and 1996-1997), grown in two Virginia locations (Warsaw and Blacksburg), were studied to evaluate the effects of the 1R rye chromosome on soft wheat flour quality and baking performance. The presence of the 1B/1R chromosomal translocation in wheat has been reported to provide disease resistance, but produce sticky doughs.

The 1995-1996 and 1996-1997 SRWW flours were subjected to farinograph analysis and dough stickiness testing. Dough stickiness was determined by the Schwarzlaff-Shepherd Dough Stripping Method. Wheat samples from 1995-1996 were also analyzed for protein, ash, and moisture content, alkaline water retention capacity (AWRC), cookie diameter, tensile stress and strain, and by ¹³C nuclear magnetic resonance (¹³C-NMR) spectroscopy techniques.

Significant ($p = 0.0001$) negative correlations were found between AWRC and cookie diameter of SRWWs grown in Warsaw and Blacksburg. Location was found to exert a significant effect on AWRC, cookie diameter and stickiness ($p < 0.05$). Farinograph data revealed that mixing characteristics of SRWW were affected significantly by variety, crop year and location ($p < 0.05$). In some cases the 1B/1R varieties had lower breakdown rates, longer departure times (DT) and lower mixing tolerance index (MTI), than their non-1B/1R counterparts. There was a significant difference ($p = 0.0133$) in the stickiness of 1B/1R and non-1B/1R samples from Blacksburg. However no such difference was found in the corresponding Warsaw samples ($p = 0.9826$), indicating that location exerted a significant effect on stickiness.

Two flour samples exhibiting stickiness (one with and one without 1B/1R) and two non-sticky samples (one with and one without the 1B/1R) were fractionated into gluten, starch and water-solubles (WS) in order to determine if the sticky dough factor resided in the 1B/1R and / or non-1B/1R WS. The peel time of the interchanged samples, as in the case of 'Massey' flour combined with the WS from VA52-22, increased to 79 seconds from the 30 seconds originally

observed in the Massey flour. However when gluten and starch fractions from a non-sticky, non-1B/1R sample, VA54-21, were mixed with WS from VA54-211 (sticky, 1B/1R), the peel time went from 18 in the original flour to 8 seconds.

Tensile measurements showed dough stress was not significantly affected by the presence or absence of 1B/1R ($p = 0.7057$). However, dough strain was lower in 1B/1R translocated SRWWs ($p = 0.0048$). A ^{13}C -NMR spectra failed to show differences amongst selected 1B/1R and non-1B/1R dough samples. Proton relaxation time ($T_{1\rho}[\text{H}]$) - a ^{13}C -NMR technique, indicated that water did not exert a significant influence on the molecular dynamics within the dough samples of Massey (non-1B/1R), VA54-211 (1B/1R) and VA52-22 (1B/1R). However, the non-sticky, non-1B/1R sample (VA54-21) had a higher proton relaxation time at 62 ppm which may indicate the size of starch-protein particles in VA54-21 doughs were larger and less flexible than in the other three doughs.

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CHAPTER 1

INTRODUCTION AND PURPOSE

Wheat (*Triticum aestivum* L) - rye (*Secale cereale* L) chromosomal translocations of the short arm of rye chromosome 1R to the short arm of wheat chromosome 1B have been used in wheat breeding programs as a source of genes for cultivar improvement. For example, 45% of the advanced wheat lines in the International Maize and Wheat Improvement Center (CIMMYT) possess the 1R chromosome (Dhaliwal et al., 1987), while in the Small Grain Center in South Africa, about 50% of the spring wheat lines carry this translocation (van Lill et al., 1990). Varietal improvements are due to the short arm of the 1R rye chromosome which carries genes for resistance to four major wheat diseases: powdery mildew, stripe rust, leaf rust and stem rust (Dhaliwal et al., 1987).

Unfortunately, hard wheats carrying the 1R chromosome segment usually have poor bread making quality. Such flours often produce doughs with marked stickiness following high speed mixing and are associated with reduced dough strength and intolerance to overmixing (Dhaliwal et al., 1987).

To date, factors such as starch (Burnett et al., 1995) protein composition, pentosan (Dhaliwal et al., 1988) and cell wall polysaccharide content (Henry et al., 1989) have been evaluated as possible contributors to the stickiness phenomenon. Thus far, results have failed to show a clear relationship between these flour components and dough stickiness. Recently Chen and Hosney (1995) identified the sticky dough factor in 1B/1R hard wheats as a ferulic acid ester of a hexose containing carbohydrate. This factor produced stickiness when added back to the water insolubles (gluten) of either sticky or non-sticky doughs.

Soft wheat flours are used in a different set of baked products than hard wheats, thus results from hard wheat have limited value for predicting how 1B/1R will impact soft wheat flour quality (McKendry et al., 1996). Dhaliwal and MacRitchie (1990) indicated that dough stickiness is reduced in low protein soft wheat flours. Schwarzlaff (1995) found that not all 1B/1R soft wheat lines exhibited dough stickiness.

Most research emphasis has been on 1B/1R hard wheat flours due to the reduction in mixing tolerance and poor breadmaking ability associated with the 1B/1R translocation. The work of Schwarzlaff (1995) and McKendry et al., (1996) reported on the effects of 1B/1R

translocation on the baking performance of soft red winter wheats (SRWWs). Results obtained by Schwarzlaff (1995) indicated that SRWW flour with the 1B/1R translocation can be used to make quality cookies. However, work by McKendry et al., (1996) found some 1B/1R SRWWs had significantly higher AWRC values. High AWRC in soft wheat flour indicates a poor quality flour for cookie production. The 1B/1R translocation had inconsistent effects on the test weight of SRWW flour (McKendry et al., 1996). Test weight is often used as a predictor of a wheat flour's milling quality and has been reported to be related to flour protein content (Schuler et al., 1995). The study by McKendry et al. (1996) also observed that the protein content of the SRWW flours was significantly affected by the environment and not by the 1B/1R translocation. Baking quality of the 1B/1R SRWW flours was reported to be reduced, although not significantly, and was based on 1B/1R being present.

Therefore, the purpose of this study was to investigate the quality of 1B/1R translocated SRWW flours. The focus of this study was to increase an understanding of how the 1B/1R translocation affects the rheological properties of SRWW doughs. An understanding of how the 1B/1R translocation affects the rheological properties of SRWW doughs is important because these rheological properties help to determine the baking quality of SRWW flours.

CHAPTER 2

PURPOSE AND OBJECTIVES

PURPOSE

One of the objectives of this study was to determine if the stickiness phenomenon occurred in 1B/1R SRWW flours and to confirm whether the sticky dough factor was located in the water soluble (WS) fraction of reconstituted doughs. Selected dough samples were also subjected to tensile measurements and solid-state ^{13}C nuclear magnetic resonance (^{13}C NMR) testing to determine the effects of the 1B/1R translocation on dough extensibility and elasticity and its effects on polymer (starch-gluten) water interactions.

OBJECTIVES

The specific objectives of the study were as follows:

- To determine dough stickiness and baking performance in 1B/1R translocated soft red winter wheat (SRWW) flours
- To confirm whether the “stickiness factor” was confined to the water soluble fraction from selected SRWW flours
- To determine if viscoelastic properties of selected SRWW flour samples were affected by the 1B/1R translocation.

Stickiness will be determined using the Schwarzlaff-Shepherd Dough Stripping method. This method is rapid and provides a means of quantifying the presence or absence of stickiness based on peel time, where longer peel times are indicative of sticky doughs. The Instron Universal Testing Machine will provide valuable information on the effect of 1B/1R on a dough's elasticity and extensibility. Results from solid-state ^{13}C NMR spectroscopy will reveal the response of molecular components (starch, protein) in flour to water. Are the environments of the mobile regions of starch and gluten altered by water or by presence of the 1B/1R translocation? It is hoped that this question will be answered by running solid-state ^{13}C NMR on the experimental dough samples. The reason for using solid-state ^{13}C NMR is to characterize the solid-state structures of 1B/1R and non-1B/1R dough samples and to determine if differences exist in the interaction of macromolecules (protein and starch) with water.

It is hoped that such approaches will help to elucidate differences on how flour samples interact with water, since water plays a critical role during dough formation through its effect on

dough rheology. The differences in the interaction of intrinsic flour components with water, influences a dough's viscoelastic properties. The results of this study will indicate how 1B/1R affects dough stickiness, extensibility and elasticity.

CHAPTER 3

LITERATURE REVIEW

USEAGE OF TRANSLOCATED RYE GENES IN WHEAT CULTIVARS

Rye (*Secale cereale* L.) is valued by wheat breeders as an additional source of genes that contribute resistance to several major wheat diseases as well as increasing yield (Dhaliwal et al., 1987). The wheat diseases are powdery mildew (*Blumeria graminis*), stripe rust (*Puccinia striiformis*), leaf rust (*Puccinia recondita*) and stem rust (*Puccinia graminis*). Rusts are among the most important diseases of wheat worldwide. They are caused by fungi that are obligate parasites of plants. These fungi affect foliage of the plants sometimes causing severe grain losses. For example, a 1916 stem rust epidemic resulted in a loss of 182 million bushels (38%) of the US wheat crop. Inhibition of wheat yield potential, by rust infection, has been demonstrated to occur up to 70% under experimental conditions (Schafer, 1987).

Substitution of the short arm of the 1R chromosome of rye for the short arm of the 1B chromosome of wheat produces 1B/1R translocation lines that have been extensively used in wheat improvement programs (Fenn et al., 1994). In 1988 about 50% of the International Maize and Wheat Improvement Center (CIMMYT) advanced breeding lines carried the 1R rye gene chromosome, and in the 21st International Winter Wheat Performance Nursery grown in 1989, 41.4% of the cultivars were found to possess the 1R translocation (Lee et al., 1995).

However, detrimental effects on various end-use quality parameters have been associated with the 1B/1R translocation. For example doughs from such wheats have been shown to produce undesirable rheological properties that cause problems during mechanical processing (Lee et al., 1995). However, there is considerable variation in end-use quality attributes of wheats possessing the 1R chromosome, with some translocation lines displaying acceptable quality characteristics (Lee et al., 1995; and Schwarzlaff, 1995).

Recent reports have indicated the 1B/1R translocation has begun to appear in SRWW germplasm and commercial lines, and unlike hard wheat flour, the effect of this translocation in SRWW flours has not been thoroughly evaluated. In general, the most important quality characteristics of soft wheat flour are fine granulation, low water absorption and low protein content. In most cases gluten development is avoided in soft wheat products and this suggests the reduced dough strength associated with 1B/1R translocation in hard wheat may not negatively influence soft wheat quality (McKendry et al., 1996). Thus results obtained from hard wheat flour have limited value for predicting how 1B/1R will impact soft wheat quality.

SOFT WHEAT FLOUR AND SOFT WHEAT PRODUCTS

Wheat can be classified by the use of the descriptors such as soft and hard, which describe the texture of the wheat kernel. Thus a hard wheat requires greater breaking force during milling than a soft wheat kernel. Wheat is also classified by the time of sowing: spring wheats are sown in spring and winter wheats in late summer or fall. Wheats may be further classified by the color of their seed coats - red or white.

Flour from hard wheats, *Triticum aestivum* L., is primarily used for breadmaking and has a protein content of 11.5-18% (Mattern, 1991); while soft wheat flour is of a lower protein content (8.5-10%) and is suitable for making cakes, biscuits and crackers (Finney, 1989).

The applications for hard wheat are different from those for soft wheat. Unlike soft wheat flour, hard wheat flour due to its high protein content and gluten strength is used mainly for breadmaking. The gluten of soft wheat is weak and produces a light and tender product. Soft white wheat and soft red winter wheat make up less than a quarter of the total US wheat production. Despite this small percentage, soft wheat is used in a wide range of commercial products (Finney, 1989). Soft wheats are a preferred source of flour for four product categories: chemically leavened and yeast leavened baked products, non-baked confectionery products and other products for industrial uses. Chemically leavened products include cookies, crackers, cakes, biscuits, ice-cream cones, doughnuts, wafers, pancakes, waffles and sponge cakes. Some yeast leavened products are pretzels, French-type breads, and numerous international flat breads. Non-baked flour products include soups, batters, breadings and gravies. Starches, glues and pastes are examples of products for industrial use (Finney, 1989; Zabik and Tipton, 1989).

Soft wheat flours are superior for cookie and cracker making because of the lower protein levels which provide tenderness at a lower level of the expensive enriching ingredients such as shortening; processing and machining qualities are excellent and high cookie spread is available if desired. The usage of soft wheat is important in pies because it can be used most efficiently with shortening and achieves better tenderness than using a high protein flour. In pie doughs, an important objective is to avoid gluten development. A pie crust from a good quality flour will remain dry, tender and flaky; conversely, gummy and soft pie crusts result from poor quality flours (Zabik and Tipton, 1989). Pretzels are made from soft, red, winter wheat flour (Loving and Brenneis, 1981). Pretzels are produced by extrusion. Sticky doughs will tend to stick to the various moving parts of the extruder and thus sticky 1B/1R flours would be problematic for pretzel making. Below are the three most common usages for soft wheat flour.

Cookies

There are four basic cookie-types: rolled cookie, dropped cookie, bar cookies and ice-box cookies. In rolled-cookie recipes, very little or no liquid is used. Formulas for cookies are rich in fat and sugar. Depending on the type of formula, cookies may contain as much as 65 - 70% fat based upon the weight of the flour (Sultan, 1986b). The cookie batter has little or no leavening agent other than air being incorporated into the creamed fat-sugar mixture. The relatively high content of fat and sugar in the dough allows plasticity and cohesiveness without the formation of a gluten network. Richness is important in a cookie for taste, tenderness and keeping qualities. Flour in conjunction with eggs supplies the structure and support in cookie production. Generally, soft wheat flour or all-purpose flour is used (Bennion, 1985). Soft wheat flour should be used, due its ability to produce cookies with large diameters during the baking process (Labuschagne et al., 1996). A large cookie diameter is considered superior (Labuschagne et al., 1996).

Working with South African spring wheats, Labuschagne et al (1996) studied how cookie quality is affected by important soft wheat flour characteristics such as protein content, farinograph absorption and AWRC. The spring wheats included soft white, soft red and hard red varieties. The results indicated that there were significant negative correlations ($P \leq 0.01$) between cookie diameter and farinograph water absorption, AWRC, and protein content. The authors stated that a high water content in a cookie dough “interferes with the agglomeration of proteins to form an extensive viscoelastic network during mixing” (Labuschagne et al., 1996).

Schwarzlaff (1995) compared the performance of 1B/1R SRWW sister lines, harvested from two locations, in producing quality cookies. The results of the study indicated amongst the sister lines, some samples with the 1B/1R translocation resulted in significantly reduced cookie diameters when compared to their non-1B/1R counterparts. The author concluded that “the parentage and location were contributors to differences in cookie diameter of the experimental wheat” (Schwarzlaff, 1995). Using soft red winter wheat, McKendry et al (1996) compared 1B/1R and non-rye sister lines for milling and baking properties. The results of this study indicated 1B/1R caused a significant reduction in flour yield and milling quality. Alkaline water retention capacity (AWRC) was significantly higher in 1B/1R soft wheats by 0.8 to 1.0 percentage units. High AWRC values in wheat flour, generally indicate the flour will make cookies of low spread (Yamazaki et al, 1968).

Biscuits

Biscuits are made from soft doughs containing three parts flour to one part liquid (Charley, 1982). The basic ingredients in biscuits are sugar, salt, milk, shortening, flour and a leavening agent (Sultan, 1986a). The ratio of water to flour is critical and the dough has to be soft and requires kneading (Charley, 1982). In biscuits, the dough is completely developed (Sultan, 1986a). This results in a soft, cake-like interior. To make a flaky type biscuit, the fat and flour are rubbed together and the liquid ingredients along with dissolved sugar and salt are added and folded gently together. If improperly handled, toughness can develop quickly. Sometimes a blend of cake and bread flours is used to avoid formation of a tough dough (Sultan, 1986a). The usage of appropriate ingredients and manipulation techniques are very important issues to consider in biscuit making. Currently no information is available on how 1B/1R SRWW will perform in this system. It will be of interest to see how biscuit quality is affected.

Cakes

Cakes are classified as shortened or unshortened cakes. Shortened cakes contain fat while unshortened (foam style) cakes are prepared without fat. Usually, shortened cakes are chemically leavened by carbon-dioxide gas produced from baking powder or baking soda and buttermilk. A good quality shortened cake should have a fine grain, cells of uniform size, thin cell walls and an elastic crumb texture. Ingredients in a shortened cake are flour, sugar, fat, eggs, a liquid, a leavening agent and salt. In shortened cakes, sugar acts as a tenderizer, by interfering with gluten development from the flour. Sugar also raises the temperature at which starch gelatinizes, thus improving cake volume and contour of the crust. Fat acts as a tenderizing agent

as well. Plastic fats such as shortening aid in incorporating air into the batter. Flour contributes structure to the shortened cakes. Cakes made with all-purpose flour are generally lower in volume and have a coarse texture than similar cakes made with cake flour. The liquid ingredient in the formulation dissolves sugar and salt and makes possible the reaction of the leavening agent, at the same time allowing the gelatinization of flour to occur.

The ingredients of importance in foam cakes, such as an angel food cake, are flour, egg whites, cream of tartar and sugar. The mixing procedure is also important to the final quality of foam-type cakes. A good quality angel food cake should have a large volume with a porous texture and thin cell walls. It should also be tender and moist.

Egg whites in an angel food cake incorporate air as they are beaten to form a foam. The proteins in egg whites are heat coagulable and give structure to the baked angel food cake. The eggs should be fresh since fresh eggs have thicker whites and produce a more stable foam than old eggs. Cake flour is preferable due to its fine granulation and low protein content which translates to weak gluten structure. Such flours give a more tender product than would a flour with stronger gluten. Flour also increases the strength of the crumb and contributes to the structure. Sugar elevates the coagulation temperature of egg whites and interferes with gluten development. Cream of tartar produces a whiter cake and stabilizes the egg white foam allowing heat penetration and bringing about coagulation without the collapsing of the foam. The whiteness of the cake is due to cream of tartar causing the batter to become acidic and thus minimizing the occurrence of Maillard browning (Bennion, 1985).

Gaines (1991) studied 53 soft wheat cultivars milled into straight-grade flour, and evaluated for protein content, cookie diameter, white layer cake volume and AWRC. The results indicated cake volume correlated positively with AWRC and negatively with protein content of soft red wheat flour.

QUALITY MEASUREMENTS OF SOFT WHEAT FLOURS

The term quality is used by cereal chemists to describe a wheat flour's suitability for producing specific end products such as bread, pastry, cakes, macaroni and crackers. Wheat quality cannot be expressed in terms of a single attribute or property; it depends on several milling, rheological and processing characteristics of the raw materials and on their suitability and functionality in producing acceptable food products (Pomeranz, 1988). The functional

properties of a flour depend on factors such as wheat variety, environmental and soil conditions under which the wheat was grown, the milling process and the chemical composition of the flour.

Food applications of soft wheat are very different from those of hard wheat and durum wheat. Therefore, the testing criteria are also significantly different. Typical quality tests for soft wheat flours include determinations of percent moisture, protein and ash, alkaline water retention capacity (AWRC) tests; farinograph testing; and baking tests for sugar snap cookies and high ratio cakes.

Depending on the product application, the results of these tests can be good predictors of the flour's overall functional properties. For example, Yamazaki (1954) found a strong correlation between the hydration characteristics of flour and cookie quality. He developed the AWRC test, to evaluate the role of wheat flour in cookies, and results of this test generally correlate well ($r = -0.70$ to $r = -0.85$) with cookie spread made with straight grade untreated flour. The alkaline conditions of the test provide the same environment for the flour as in a cookie. In addition the ability of the flour to hold water against centrifugal force measures its affinity for water. Good quality soft wheat cookie flours generally have poor water-binding characteristics. A flour with greater AWRC value holds relatively more water, making less water available to dissolve sugars and to form a syrup (Patterson and Allan, 1981). In a cookie dough, the limited quantity of water is partitioned mostly between sugar and flour. A dough with less syrup is not as fluid during baking, hence it resists the expansive effect of leavening and sets earlier and at a lower temperature during baking, resulting in cookies of smaller spread (Patterson and Allan, 1981).

Water-soluble pentosans have also been shown to reduce cookie spread. Pentosans, present at only 1-2 % of flour, are important for their ability to absorb up to 10 times their weight in water due to their hydrophilic nature (Kaldy et al., 1991). Pentosans are polysaccharides consisting primarily of two monosaccharides, xylose and arabinose. Pentosans have been shown to affect the quality of baked products made from hard wheats, by affecting the rheological properties and dough consistency of hard wheat flour. Pentosans bind up to 23% of the water in cookie dough systems, significantly reducing cookie diameter (Kaldy et al., 1991). Soluble and enzyme extractable pentosans from soft wheat flours have been reported to have a negative influence on both cookie diameter and cake volume (Kaldy et al., 1991)

VISCOELASTIC PROPERTIES OF WHEAT FLOUR DOUGHS

Rheology is the study of deformation and flow properties of solids, semisolids and liquids. The viscoelastic properties of a dough have a profound effect on dough machinability and textural qualities of finished baked products. Wheat flour contains special types of storage proteins, which, when combined with water and manipulated, develop into a dough that can both stretch and flow. A number of empirical tests have been developed to characterize dough rheology, which are discussed below.

Farinograph

One such empirical test is the farinograph. According to Walker and Hazelton (1996), the farinograph functions by measuring the resistance of a dough against sigmoid-shaped mixing paddles. These paddles subject a flour-water dough to a prolonged, gentle kneading action in a chamber held at a constant temperature of 30°C.

The resulting farinogram is a trace of the torque transmitted through the dynanometer by a dough during mixing. The farinograph is a standard tool for generating information concerning the water absorption (WA) and mixing characteristics of flour. Water absorption is important to the generation of a farinogram, and is defined as the amount of water required to center the peak area of a farinograph curve on the 500 Brabender Unit (BU) line for a flour-water dough (Walker and Hazelton, 1996). Other values obtained from the farinogram include the arrival time (AT), departure time (DT), peak time (PT), mixing stability (MS), mixing tolerance index (MTI) and the twenty minute drop (TMD).

Arrival time is obtained when the top of the curve first intersects the 500 BU line as the water is being rapidly absorbed. Shorter arrival times result when protein levels (within a wheat variety) increase. The time required to reach a point of maximum consistency, before any indications of dough breakdown, is called the peak time (PT). The departure time (DT) is the time at which the top of the curve drops below the 500 BU line. Strong flours exhibit a long DT. Mixing stability is the difference between the AT and DT and is an indication of a flour's tolerance to mixing. Twenty minute drop (TMD) is the distance in BUs between the development peak and the point 20 minutes after the PT. This value indicates the rate of breakdown and dough strength, with higher values denoting weaker flours (Walker and Hezelton, 1996). The mixing tolerance index is the difference in BUs between the heights at PT and PT plus 5 minutes and indicates the dough breakdown rate (Walker and Hazelton, 1996).

In a study by Conforti and Johnson (1992) the farinograph was used to predict the baking quality of soft red winter wheat flour from wheat subjected to different fertilizer regimes and to chlorination. The main purpose of the study was to determine if farinograph curves can be used as indicators for baking quality in both chlorinated and unchlorinated SRWW flour. The authors looked at the resistance of the doughs to mixing which was evaluated by AT, PT, DT, MTS and MTI. The farinograph data revealed the existence of short development time and short stability time for the experimental flour samples, typical for soft wheat flours. The authors reported an increase in PT amongst the nitrogen fertilized samples due to an increase in protein content. According to Conforti and Johnson (1992), PT is influenced by gluten quality, with high protein flours requiring longer development times than weak flour.

MTI is directly related to stability (Conforti and Johnson, 1992). Generally a flour with a good tolerance to mixing will have a high stability value, but a low MTI value. Chlorination lowered the stability of experimental flours and lowered MTI. The authors recommended the useage of a combination of farinograph values to aid in predicting the baking quality of the flour.

Pressure-sensitive Adhesives

Flour doughs behave like pressure-sensitive adhesive materials (Heddleson et al., 1994). Doughs, especially those with the 1B/1R translocation have been shown to stick to processing equipment, by forming an adhesive bond with the equipment surface. Such behavior is similar to the performance of pressure-sensitive adhesives (PSAs). Another similarity between doughs and PSAs are that both are viscoelastic under conditions of use. The viscoelastic properties in PSAs are controlled by an elastomer-resin complex. The elastomer (rubbery polymer) provides an elastic component, while a low molecular weight tackifying resin constitutes the viscous component. This same type of system can also be found in the gluten component of a wheat flour dough. The elastomer portion of the gluten has been identified as the high molecular weight (HMW) glutenin fraction, while the resin portion is the low molecular weight (LMW) gliadin. Like PSAs, the HMW glutenin has been noted as being responsible for the non-adhesive, tough, elastic character of gluten. While the LMW gliadins are responsible for the adhesive and viscous character of gluten (Heddleson et al., 1993; Saunders et al., 1992).

PSAs are generally formulated with rubbery polymers compounded with tackifiers, fillers and plasticizers to maintain a balance of peel adhesion, cohesive holding power and surface tack (Heddleson et al., 1994). These rubbery polymers, fillers and plasticizers are analogous respectively to gluten, starch and water in a dough system, respectively (Heddleson et al., 1994). However, the rheological roles of starch and water have not been well characterized.

In general plasticizers used with PSAs decrease the storage modulus (G'), resulting in an increase in tack and a decrease in cohesive strength. Water, a well known plasticizer for gluten, has been shown to lower the G' at room temperature. It has been shown that the increase in water content of dough results in an increase in tack and lowered cohesive strength as evidenced by cohesive failure at high moisture content (Saunders et al., 1992; Navickis et al., 1982).

Fillers increase the G' of PSAs resulting in a decrease in tack and increase in cohesive strength. Increasing the starch concentration in a dough raises the G' , but the influences of increasing starch concentration on the cohesive strength of doughs have yet to be reported.

Tack and Adhesion

Tack is defined as the property that enables a PSA to form a bond of measurable strength immediately upon contact with another surface (Heddleson et al., 1994). Pressure-sensitive tack is governed by the adhesive's rheological properties when interacting with a high energy surface, such as a metal. According to Heddleson et al., (1994), bond failure during the measurement of tack can either be cohesive or adhesive. Thus it may be acceptable for a food material to exhibit tack, as long as it exhibits adhesive failure when pulled from processing equipment (has sufficient cohesive strength).

Adhesion occurs because low-energy materials strongly absorb to high-energy surfaces in order to decrease the surface energy of the system. High energy surfaces include metals such as those used in processing equipment. Adherends with low energy surfaces include organic polymers e.g. doughs and plastics. In general, dough exhibits tack with a probe surface so long as the probe surface energy is greater than the adhesive surface energy. Thus high tack has been reported to occur between wheat flour/water doughs and brass, copper and stainless steel. Moderate tack occurred with polytetrafluoroethylene (PTFE), aluminum, and steel. Low tack was reported with probe materials made of Teflon (Saunders et al., 1992). This indicates that

pressure sensitive adhesion and bond formation at an interface of a polymer and high energy materials is a viscoelastically driven phenomenon.

Adhesion is desirable and necessary for appropriate baked product functionality, but often it interferes with processing (Heddeson et al., 1993). This is especially true for 1B/1R hard wheat flours. Thus the performance of a PSA or dough depends on the viscoelastic responses of the bulk material and upon the surface energies of the adhesive and adherend. The viscoelastic response of a dough is to a great extent controlled by the gluten quality. To function properly, dough samples need to be made from flour that has a broad molecular weight distribution of proteins within the gluten structure of the dough.

Stickiness Measurements

The rheological properties of flour doughs are influenced by several factors such as type of flour and amount of water. Recently, the introduction of the 1R rye chromosome in wheat has in some cases detrimentally affected the surface characteristic of dough samples when subjected to high speed mixing. Therefore, it is critical to determine dough stickiness and other rheological properties that affect quality and functionality of a flour.

Chen and Hoseney (1995) reported on an objective measurement for quantifying stickiness amongst dough samples. Briefly Chen and Hoseney (1995) used a texture analyzer with a plexiglass probe. The plexiglass probe exhibited poor adherence to sticky doughs. The texture analyzer provided a constant force to compress dough samples and the probe was set at a maximum reverse speed to measure dough stickiness which was reported as grams of force.

Schwarzlaff (1995) used 50 gram flour samples which were mixed in a vacuum mixer to their respective peak times. Half of the resulting dough sample was rolled onto a glass plate and a cut fiberglass mesh screen strip (16 squares per inch) was placed on top of the rolled out dough. The second dough half was placed on top of the mesh strip and rolled out to make a dough-mesh-dough sample. The dough strip was cut into a 1" x 7" strip with a knife. The glass plate plus dough was transferred to the dough stickiness testing box and was placed upside down with a 50 gram weight attached onto the fiberglass mesh. The time (in seconds) to peel 1/2 an inch was taken. Therefore the longer the peel time, the stickier the dough sample.

Viscoelasticity and storage modulus (G')

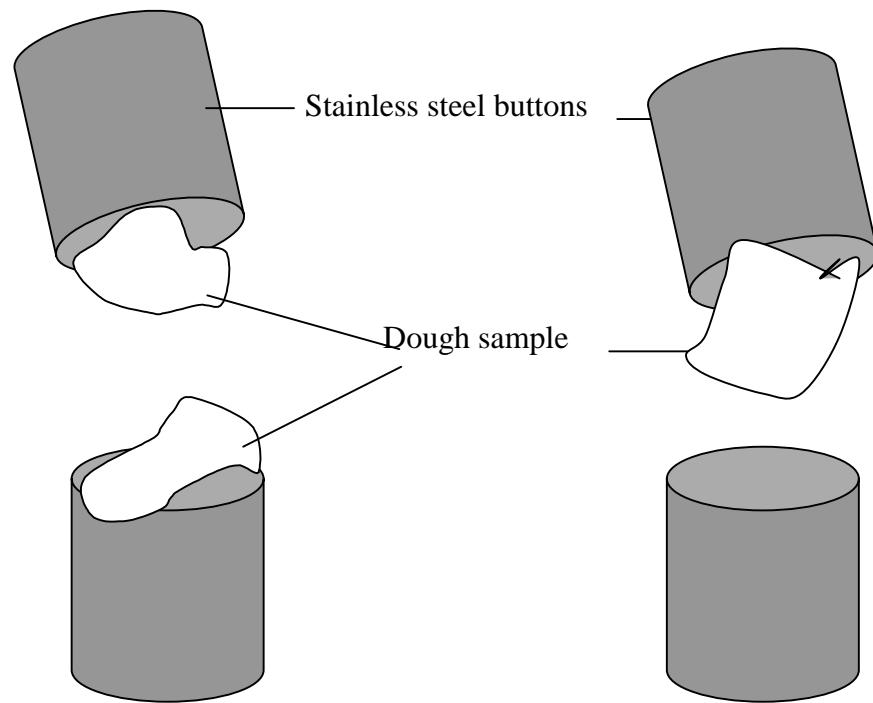
Viscoelastic properties of foods, specifically dough, can be determined by dynamic measurements. Dynamic mechanical tests are used to measure the deformation of a material in response to vibrational forces. Viscoelastic functions derived from these tests include: the shear storage modulus (G') which indicates the material's stiffness or energy stored during deformation and the shear loss modulus (G'') indicating energy dissipated as heat during deformation. It is believed that the adherence of dough or any other viscoelastic food material to metal processing equipment is a modulus controlled process. G' is also described as elasticity or strain (Navickis et al., 1982).

Navickis et al (1982) conducted a study that highlighted the effect on G' and G'' of water level and flour of varying protein content on the viscoelastic properties of doughs. An eccentric disc rotating rheometer (ERD) was used to determine the dynamic moduli of wheat flour doughs prepared with varying water levels (35 - 45%). The dynamic storage modulus (G') and loss modulus (G'') were obtained from ERD measurements.

The results indicated that G' is sensitive to water. G' decreased with increasing water level, meaning the dough was becoming softer. The results indicated that a 12% change in moisture resulted in a change in G' and G'' . Low protein flours took less water to reach the target consistency of 500 BUs where G' values were highest. Soft wheat flour mixed at their optimum water absorption had high storage moduli. Thus doughs were stiffer. Optimum water absorption resulted in stiffer doughs.

The researchers concluded that the dynamic moduli (G' and G'') depended strongly on the water content of the dough. Also, with an increase in protein content, the predominant trend was a decrease in sensitivity of G' and G'' to water content.

The work by Navickis et al (1982) and the research results from other polymer scientists opens the possibility that the rheological properties of 1B/1R doughs can, at least in part, be explained by protein quality, and water-protein interactions that result in surface tack. Cereal scientists have noticed that the failure mode (adhesive or cohesive failure) is a function of the water content of dough, with no clear transition zone from adhesive to cohesive failure (Saunders et al., 1992). As indicated in Figure 1.1, adhesive failure occurs at the interface of the dough and stainless steel button; while cohesive failure describes the failure within the dough itself.



Cohesive failure

Adhesive Failure

Figure 1.1. Type of dough failure

The source of dough stickiness in 1B/1R flours could be a result of the genetic alterations causing the elastomer (glutenin) portion of the dough's molecular weight distribution to be skewed more towards the lower molecular gliadins which act as a resin component of dough plasticizers, thus accounting for the high tack and low cohesive strength of the dough. In support of this, Gupta and MacRitchie (1990) found that as percentage of the glutenin in flour protein decreased, dough strength decreased while dough stickiness increased. The authors concluded that "a reduction in the proportion of glutenin due to loss of glutenin subunits could, in itself, cause dough stickiness".

HYDRATION PROPERTIES OF FLOUR USING SOLID - STATE ^{13}C NMR

Nuclear magnetic resonance (NMR) spectroscopy is a method used for elucidation of the molecular structure of polymers and other materials. NMR spectroscopy provides a "map" of the carbon-hydrogen framework of an organic molecule. NMR spectra are composed of peaks displayed on charts which are calibrated using an arbitrary scale called the delta scale. One delta unit (δ) is equal to 1 part per million (ppm) of the spectrometer's operating frequency. The NMR peaks are also known as chemical shifts (McMurry, 1988).

At its simplest, ^{13}C NMR allows for the enumeration of carbons in a molecule of unknown structure. In addition, one can obtain information on the chemical (magnetic) environment of each carbon by observing its chemical shift (McMurry, 1988). However there are major obstacles in obtaining highly resolved solid-state NMR spectra (Baianu and Forster, 1980). These include line-broadening due to dipolar interactions between nuclear species and chemical shift anisotropies. Spinning a sample at an angle of $54^{\circ}44'$ (magic angle) to the external magnetic field reduces to zero the dipolar and chemical shift anisotropy broadenings (Baianu and Forster, 1980). The utilization of the magic angle, has resulted in an NMR technique called cross polarization and magic angle spinning (CP-MAS). Cross-polarization and magic angle spinning produce high resolution NMR spectra. ^{13}C NMR can be used to analyze liquid and solid components in food (Wu et al., 1992).

Water helps to determine many of the mechanical and chemical properties of food materials such as hydrated flours and it is likely that the differences in mechanical properties of doughs (e.g. ability to be shaped or molded) are related to the interaction of water and flour

(Garbow and Schaefer, 1991a). NMR spectroscopy makes possible the study of the interactions of water in a wide variety of biological and agricultural systems. Solid-state ^{13}C NMR has been used extensively to characterize the structure and dynamics of several different intact polymers, such as cellulose, lignin (Garbow and Schaefer, 1991b), hard and soft wheat flours and doughs as well as waxy corn starch and wheat gluten in the presence of water (Garbow and Schaefer, 1991ab; Li et al., 1996).

NMR relaxation measurements can provide structural information about polymers and biopolymers in the solid state. Work on dry and hydrated hard wheat flour, reported on the most important relaxation parameter - the proton rotating-frame relaxation time [$T_{1\rho}(\text{H})$]. This parameter has been used extensively to characterize motions in polymers. For a polymer, efficient proton-proton communication (spin diffusion) causes all of the protons in the polymer chains to share a common $T_{1\rho}$ (H) relaxation time. This averaging has been used to assess the miscibility of polymer blends (Garbow and Schaefer, 1991a).

Starch and protein constitute the major polymer fractions found in wheat flour. Their interaction with water is of great importance, as it affects the quality and properties (functionality) of food products. The mobility properties of starch and gluten depend on moisture content of the system. Water serves as a plasticizer for starch and gluten molecules. The inherent effects of water in food systems is complicated due to the complex nature of food ingredients such as flour.

Using CP-MAS, Baianu and Forster (1980) were able to note subtle differences between spectra of four different wheat varieties. However explanations as to where the differences arose in the chemical shift assignments were not available. Recently, samples of hard wheat flour and dough were analyzed by magic-angle spinning ^{13}C NMR spectroscopy. The results indicated that protein is unaffected by the added water and remains phase-separated from the starch, while water causes significant changes in polymer dynamics of the starch component (Garbow and Schaefer, 1991b). Starch was found to interact more intimately with water than did protein, whose dynamics were shown to be nearly unaltered by the water. However in soft wheat flour, water has a profound effect on the protein components. This suggests differences in the behavior of protein in hard versus soft hydrated wheat flours, that could be relevant to a variety of baking applications, and need to be further explored (Garbow and Schaefer, 1991b).

The characterization of wheat and its components is a problem of considerable practical importance. The analytical approaches to this problem currently involve physical and chemical

alterations of these materials and therefore alter the properties of wheat. Thus the developments in solid-state NMR techniques removed this limitation and have allowed the physicochemical characterization of biological systems at the molecular level, in their natural state (Baianu and Frster, 1980).

Working with corn starch, Li et al (1996) reported that starch having the A form crystalline structure at carbon 1 (C1), had a triplet splitting resonance at 95 to 103 ppm. At 60 ppm, they stated that this value corresponded to C6 and the strong signal at 71 ppm was due to C2 to C5. On the other hand, gluten had carbonyl, aromatic and aliphatic carbons at about 180 ppm, 135 ppm and 35 ppm, respectively. Starch is a mixture of two polysaccharides, amylose and amylopectin. Both of these polysaccharides are composed of α (1,4)-D-glucopyranosyl residues (Morgan et al., 1992).

Are the environments of the mobile regions of starch and gluten altered by water or by presence of the 1B/1R translocation? It is hoped that this question will be answered by running solid-state ^{13}C NMR on the experimental dough samples. The reason for using solid-state ^{13}C NMR is to characterize the solid-state structures of 1B/1R and non-1B/1R dough samples and to determine if differences exist in the interaction of macromolecules (protein and starch) with water.

NMR has been used in polymer science and biophysics to relate macromolecular structure and conformational dynamics with functional properties. Polymer segments, in general, contain solid-like domains having restricted mobility that broadens NMR lines severely. Methods however are available for detecting solid-like components. For example proton spin-echo decay methods have been used to follow starch swelling, gelatinization and retrogradation (Wu et al., 1992).

Fourier transform magic angle spinning (FT-MAS) NMR is used to detect liquid-like components in a food system. Garbow and Schaefer (1991b) used FT-MAS to look at liquid components in hard and soft wheat flour doughs. Spectra from FT-MAS ^{13}C NMR detects small molecules which are solubilized by the addition of water to flour. Garbow and Schaefer (1991b) reported these small molecules to be small sugars and organic acids.

CHAPTER 4

MATERIALS AND METHODS

WHEAT SAMPLES

Experimental wheat samples, some with and some without the 1B/1R translocation, were obtained from Dr. Carl Griffey of the Dept. of Crop & Soil Environmental Sciences, Virginia Tech, Blacksburg, VA. The wheat lines were planted at Warsaw and Blacksburg, Virginia in early October of 1995 and 1996 and harvested in late June and early July of 1996 and 1997, respectively (Dr. Griffey, Private communication). Wheat lines were grown in 45 ft² plots with rows set 7 inches apart. Triticale (Triticale 498) from Resource Seeds (Union, Kentucky) was obtained from Dr. Carl Griffey. The communities of Blacksburg and Warsaw are in the mountain and coastal plain regions of Virginia, respectively and thus represent the state's environmental diversity. Virginia lies within the southeastern soft red winter wheat production area of the United States. This area is comprised of Georgia, Maryland and Virginia (Baenziger et al., 1985). The parentage of the experimental wheat samples is indicated in Table 4.1. Detailed description of the parentage of the wheat samples is located in Appendix A.

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

For comparison purposes, rye and triticale were included in the determination of dough stickiness and for farinograph analysis. Fourteen wheat lines were grown at two locations in Virginia - Blacksburg and Warsaw. At each location the wheat lines were grown in randomized complete blocks with three replications. Following harvest, seed from each replication was composited by location and variety. This resulted in one bulk sample for each line at each location. This was done to provide a sufficient sample for milling. Thus the experimental design was a 13 by 2 complete block design with location serving as the replicating effect.

All wheat flour samples were subjected to moisture, ash, protein, alkaline water retention capacity (AWRC), farinograph analysis and stickiness determination. The results for these tests were statistically analyzed using the General Linear Model Procedure to compare the effect of 1B/1R translocation across location and year. The Statistical Analysis System (SAS, 1985) and Tukey's test were used to determine the significant differences between experimental wheats at $P = 0.05$.

Table 4.1. Experimental wheat samples and their parentage

Sample #	Line	1B/1R	Pedigree
<u>Pair 1¹</u>			
1	VA93-54-209	-	Massey/3/Massey*3/Balkan//Saluda
2	VA93-54-211	+	Massey/3/Massey*3/Balkan//Saluda
<u>Pair 2²</u>			
3	VA94-54-21	-	Massey*3/Balkan//2*Saluda
4	VA94-54-18	+	Massey*3/Balkan//2*Saluda
5	VA94-54-19	+	Massey*3/Balkan//2*Saluda
<u>Pair 3³</u>			
6	VA93-52-67	-	Massey*5/Balkan
7	VA93-54-241	+	Massey*5/Balkan
8 ⁴	VA93-54-185	+	Wheeler/3/Massey*3/Balkan//Saluda
9 ⁴	VA93-54-418	+	Saluda*2/SC822290 (Nainari 60/Arthur 71//Kavkaz)
10 ⁴	VA93-54-258	+	VA82-54-330(Saluda SIB)/8/CI13836/9*Chancellor// Wheeler/3/Severn/4/Coker 916/5/ STI-25//ASII/9*Chancellor/6/Tyler/7/ Massey
11 ⁴	VA92-52-22	+	Tyler//Wheeler/Balkan
12 ⁴	VA93-52-55	+	Massey*3/Balkan//Saluda
<u>Checks</u>			
13	FFR555W	-	Check
14	Saluda	-	Check
15	Massey	-	Check
16	Balkan	+	1B/1R donor parent

¹Pair 1 sister lines consisted of samples 1 and 2

²Pair 2 sister lines consisted of sample 3, 4 and 5.

³Pair 3 consisted of sample 6 and 7.

⁴Samples 8 to 12 were 1B/1R wheat varieties of different parentage

The following null hypotheses were tested:

Part 1

1. There are no significant differences between the mean AWRC values of SRWW with and without 1B/1R.
2. There are no significant correlations between AWRCs and cookie diameters from SRWW flour samples with or without the 1B/1R translocation.
3. There are no significant differences caused by location (Warsaw or Blacksburg) on AWRC and cookie diameter values from wheats with and without the 1B/1R translocation.

Part 2

4. There are no differences in stickiness between 1B/1R and non-1B/1R translocated SRWWs.
5. There are no significant differences in dough strain values between 1B/1R and non-1B/1R translocated SRWWs.
6. There are no significant differences in dough strength values between 1B/1R and non-1B/1R translocated SRWWs.

PART 1

WHEAT MILLING

The wheat grain was milled in a Brabender Quadramatic Junior mill (Hackensack, New Jersey) at the Department of Human Nutrition, Foods and Exercise, Virginia Tech, Blacksburg, Virginia. Grain samples were tempered overnight to 14% moisture prior to milling. The following procedure was used :

1. Total wheat grain for each variety was determined in grams.
2. At the end of milling, the bran and endosperm were separated and weighed individually.
3. The gram amounts of bran and endosperm were used in the following equation (Schwarzlaff, 1995) to obtain percent flour yield for each experimental wheat variety :

$$(b \div t) \times 100 = \text{percent flour yield}$$

Where:

Weight of bran in grams = **a**; weight of flour in grams = **b**; **a + b = t** (total weight in grams)

FLOUR ANALYSIS

Ash, moisture and protein ($N \times 5.7$) were determined in triplicate according to AACC approved methods 08-01, 44-15A and 46-12, respectively (AACC, 1983). See Appendix B, C and D.

ALKALINE WATER RETENTION CAPACITY

AWRC was measured, in triplicate, according to AACC method 56-10 (AACC, 1983) (Appendix E). Results were correlated with cookie diameter data.

COOKIE DIAMETER DETERMINATION

The AACC 10-50 method was used (AACC, 1983) to test the effects of the quality of the experimental wheat flour on cookie diameter. This was achieved by determining the diameter of six sugar snap cookies baked from 225 grams of flour. Vernier calipers were used to determine the diameter (mm) of each cookie. The cookie formula is given in Appendix F.

FARINOGRAPH

All farinographs were produced on a Brabender farinograph (Hackensack, New Jersey) fitted with a 50 gram Farinograph bowl (Brabender, Duisberg, Germany) and operated at 30°C. The AACC method 52-21 (AACC, 1983) was used (Appendix G). The following parameters were obtained from the resulting farinograph curves: water absorption (WA), arrival time (AT), mixing tolerance index (MTI), peak time (PT), departure time (DT), twenty minute drop (TMD) and mixing time index (MTI). Water absorption values were based on dough consistency at the 500 Brabender unit (BU) line.

DOUGH STICKINESS DETERMINATION

Dough stickiness was determined by the Schwarzlaff-Shepherd Dough Stripping Method (Schwarzlaff, 1995) with some modifications (Appendix H). A farinograph was used for dough mixing instead of a Vacuum Power Mixer Plus-Whip Mixer (Model F, WM Corp., Louisville, KY). This modification was included because of the occurrence of dough samples breaking down when mixed to their peak times in the vacuum power mixer. The farinograph has a

temperature controlled chamber which is normally held at 30°C. The vacuum mixer tended to increase to temperatures above 30°C possibly contributing to rheological changes in the dough system. Triticale and rye flour were also tested in addition to 1B/1R and non-1B/1R SRWWs. Each sample was tested in triplicate.

PART 2

PREPARATION OF FLOUR FRACTIONS

Four flour samples (see Table 4.2) were selected for dough reconstitution studies: two sticky varieties (one with and one without the 1B/1R translocation) and two non-sticky varieties (again one with and one without the 1B/1R translocation). Flour samples were separated into three fractions: gluten, starch and water solubles (WS). The method of MacRitchie (1987) was used to fractionate the flour. Briefly, doughs were prepared by mixing and hand kneading 200 grams of flour with 120 grams distilled water for 5 minutes. The gluten was separated from dough samples by seven continuous washings with distilled water. The water washings were combined and centrifuged (5000 g for 10 minutes) to separate starch (sediment) and solubles (supernatant). The resulting fractions were freeze-dried (Labconco, Kansas City, MO), ground to powders in a laboratory grinder and stored at 0°C for future use in flour reconstitution studies. Yields of gluten, starch and water-soluble fractions were calculated. A base flour (non-1B/1R and non-sticky) was used in the reconstitution studies to help identify fractions responsible for dough stickiness (Table 4.3). A base flour is a flour composed of gluten and starch fractions from an identical flour. It is to the base flour that the water soluble fraction is added.

FLOUR RECONSTITUTION

1. Gluten, starch and WS fractions were combined in proportion to their recovered amounts to give a flour dry weight of 50 grams for each flour (MacRitchie, 1985).
2. Water soluble fractions were interchanged to give a total flour weight of 50 grams (Table 4.3).
3. The dry flour (50 grams) was placed in the farinograph chamber, and the farinograph was allowed to run for 1 minute to obtain a homogenous mixture of the fractions.
4. The optimum amount of water was added and a mixing time of 3 minutes was used.
5. Stickiness was tested using the modified Schwarzlaff Shepherd Dough Stripping Method.

Table 4.2. Flours used for fractionation and reconstitution studies

Soft wheat flour type ^a	Dough stickiness	1B/1R^b	Dough stickiness
(A) VA93-54-211	Sticky	+	Sticky
(B) VA94-54-21	Non-sticky	-	Non-sticky
(C) Massey	Sticky	-	Sticky
(D) VA92-52-22	Non-sticky	+	Non-sticky

^a Samples from Blacksburg location

^b + 1B/1R sample

- Non-1B/1R sample

Table 4.3. Composition of reconstituted flours

Gluten	Starch	Water solubles
VA93-54-211 (+)	VA93-54-211 (+)	VA94-54-21 (-)
VA94-54-21 (-)	VA94-54-21 (-)	VA93-54-211(+)
Massey (-)	Massey (-)	VA92-52-22 (+)
VA92-52-22 (+)	VA92-52-22 (+)	Massey (-)

(+) = IB/1R sample

(-) = Non-1B/1R sample

TENSILE MEASUREMENT

Four flours (see Table 4.4) and four water levels (26 ml, 28 ml, 30 ml and 32 ml) were used in preparation of dough samples. Dough samples were prepared in duplicate with each replicate consisting of 3 observations. Dough samples were prepared in the farinograph by combining 50 grams of flour with the different water levels (Table 4.4). Samples were mixed for 3 minutes in the farinograph. Tensile measurements were conducted by Polymer Solutions Inc., Blacksburg, Virginia. The method utilized stainless steel buttons (1.5 inch diameter) and an Instron Universal Testing Machine (Model 6204, Canton, MA). Figure 4.1 shows a diagram of the assembly used in characterizing dough samples. This experiment was performed to determine how hydration levels affect the rheological properties of SRWW doughs. The method used was as follows:

1. A spatula was used to place dough samples on the surface of a stainless steel button.
2. Approximately 5 - 10, 1 mm diameter glass spheres were placed on the dough surface. Then a second button was placed on top of the dough.
3. The two buttons were clamped together using a clamping jig (Polymer Solutions, Inc., Blacksburg, Virginia) to form a 1 mm bond layer. Excess dough was removed from the two-button test specimen.
4. Upon removal from the clamping jig, tensile gripping tabs were screwed into the buttons in preparation for tensile testing.
5. The gripping tabs were attached to a 200 pound self calibrating load cell with 1 inch wide flat grips. The load cell was then attached to an Instron Universal Testing Machine (Model 6240, Canton, MA).
6. The Instron was operated at a crosshead speed of 8 inches per minute.
7. Peak load (lbs) and extension (inches) at peak load were recorded for each analysis. Stress and strain values as well as the type of failure (cohesive or adhesive) were then obtained for each dough sample.
8. Dough samples were tested in duplicate. Each replication consisted of three observations.

MAGIC-ANGLE ^{13}C NMR ANALYSIS

Four experimental wheat flours (Table 4.4) were used in preparing dough samples for ^{13}C NMR analysis. ^{13}C CP-MAS NMR spectra was obtained for flour fractions of Massey - gluten, starch and WS. Flour fractions were analyzed to aid in interpretation of line assignments and chemical shifts obtained from the NMR spectra of experimental dough samples. The method for ^{13}C

Table 4.4. SRWW dough samples used for tensile measurements

<u>Soft Wheat Flour Sample a</u>	<u>Water (ml)</u>
VA93-54-211 (+)	26 ^b
	28
	30
	32
VA94-54-21 (-)	26
	28
	30
	32
Massey (-)	26
	28
	30
	32
VA92-52-22 (+)	26
	28
	30
	32

^a Dough samples made by mixing 50 grams flour for 3 minutes

^b Water levels correspond to 34.21, 35.89, 37.5% and 39.02%, respectively (w/w in 50 grams flour sample)

(+) = 1B/1R sample

(-) = Non-1B/1R sample

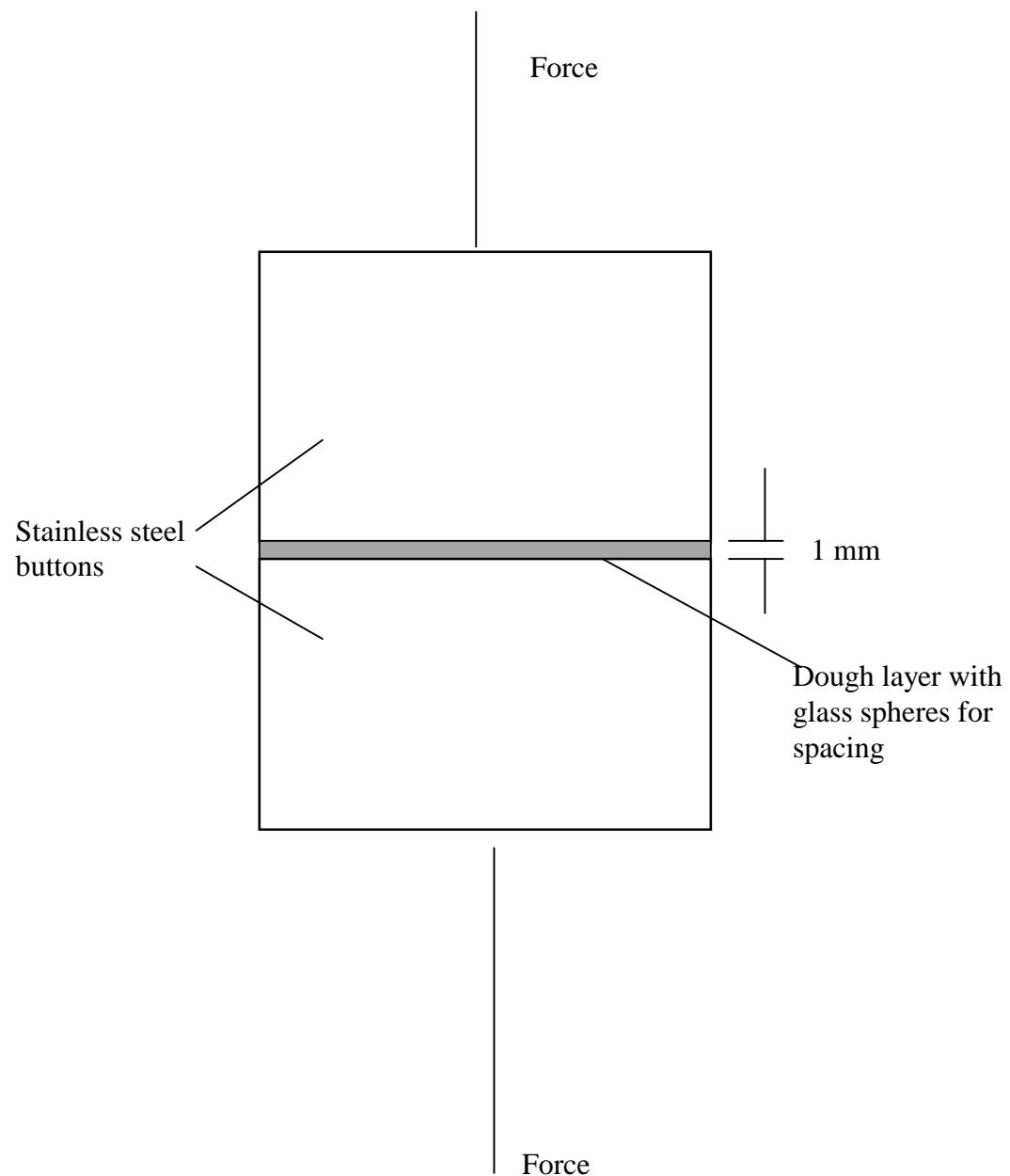


Figure 4.1. Diagram of the two-button test configuration

NMR analysis followed that of Garbow and Schaefer (1991b) (Appendix I), where CP-MAS, FT-MAS and $T_{1\rho}[H]$ are obtained for the selected wheat flours (Table 4.2). The ^{13}C NMR was determined at the analytical laboratories in the Chemistry Department at Virginia Tech (Blacksburg, Virginia).

CHAPTER 5

RESULTS AND DISCUSSION

MOISTURE

Moisture content of the experimental wheat flours is reported on a wet weight basis., and was found to be different among the flour samples (Table 5.1) between the two locations. Through statistical analysis by the general linear model procedure, both the location where the wheat sample was grown, as well as, the interaction between sample and location were found to have a significant effect on moisture content of the flour samples. The actual *p*-value for these three factors was *p* = 0.001. Using Tukey's Studentized Range test, the mean moisture values for Warsaw samples (regardless of translocation) were significantly higher than those of Blacksburg (*p* < 0.05). The mean moisture contents were 13.2 % and 12.4% for Warsaw and Blacksburg, respectively.

Due to the location having a significant effect on moisture content in the flour, the samples were analyzed by location. Results for each location (Blacksburg and Warsaw) are indicated in Table 5.1. Only one Balkan sample from Warsaw was available. Analysis indicated that Balkan had the highest moisture content of 14.1%. Triticale variety (Trical 498) had a moisture content of 13.8%, which was similar to Balkan and greater than those in the SRWW samples. FFR555W (a good quality soft wheat) had the lowest moisture content, 12.7%, of any of the SRWW samples grown in Warsaw. This moisture value was not significantly different from that of VA54-18, VA54-418 and VA54-258. All three samples are 1B/1R wheats. There were few significant differences in the moisture content among the same SRWW samples grown in Blacksburg (see Table 5.1).

There was no significant difference and therefore based on these results the moisture content in flour was influenced by the environment i.e. where the wheat was planted rather than by variety i.e. presence or absence of the 1B/1R translocation. The 1B/1R expression in the samples did not exert much of an influence on moisture content as seen in Balkan - a donor of the 1B/1R gene. Schwarzlaff (1995) reported on the existence of an interaction between sample and location in experimental ground whole wheat. The investigator also found that 1B/1R translocation did not adversely affect the moisture content of whole soft wheat flour. For wheat, a moisture content of less than 14% is desired to ensure safe storage of the grain. Differences in moisture content are

Table 5.1. Mean moisture content of experimental SRWW flour

SAMPLE	MOISTURE (%)¹	
	Warsaw	Blacksburg
Checks		
Massey	13.4 ± 0.1bc ²	12.3 ± 0.2de
Saluda	13.4 ± 0.1bc	12.5 ± 0.0c-e
FFR555W	12.7 ± 0.1e	12.2 ± 0.1de
Balkan+ ³	14.1 ± 0.0a	Not available
Triticale ⁴	13.8	
Pair 1		
VA54-209 ⁵	13.1 ± 0.0cd	12.7 ± 0.0bc
VA54-211+ ⁶	13.1 ± 0.1d	12.2 ± 0.0de
Pair 2		
VA54-21	13.4 ± 0.0bc	12.9 ± 0.0b
VA54-18+	12.9 ± 0.1de	12.2 ± 0.2de
VA54-19+	13.5 ± 0.0b	12.6 ± 0.1b-d
VA54-185+	13.0 ± 0.0d	12.9 ± 0.1bc
VA54-418+	12.6 ± 0.1e	12.5 ± 0.2b-e
VA54-258+	12.9 ± 0.1de	12.2 ± 0.0de
VA52-22+	13.5 ± 0.1b	12.1 ± 0.1e

¹ n = 4² Samples with the same letter in the same column are not significantly different at $p < 0.05$ ³ Not a SRWW flour⁴Triticale was a commercial sample that was not planted at either Warsaw or Blacksburg location, therefore the value was not included in the analysis⁵ Samples names abbreviated by dropping the second and third number from the right (eg.

VA93-54-209 will be expressed as VA54-209)

⁶ + = Variety with 1B/1R translocation

expected even for wheat coming from a single field which can vary widely in moisture due to factors such as differences in soil or stages of ripeness of the grain (deMan, 1990b).

PROTEIN

The protein content of the experimental flours are reported in Table 5.2. The results were given by location as statistical analysis indicated that protein content was significantly affected by the variety, location (environment) and the interaction of these two independent variables ($p < 0.05$). Balkan, the donor of 1B/1R, had the highest protein content of 12.4% ($p < 0.05$). Triticale variety (Trical 498) and a commercial rye sample were included in the analysis for comparison purposes. The results showed that these two samples had the lowest protein content of 8.9% and 8.0%, respectively. According to accepted standards, a soft wheat flour should have a protein content ranging from 7-9% (Hoseney et al., 1988). However, higher protein contents are obtained among soft red winter wheats grown in the warmer and more humid areas of the southeast and for wheats grown under intensive fertility regimes, yet the flour from such wheats is suitable for most soft red winter wheat products and even desirable for some (Dr. Patrick Finney and Dr. Carl Griffey, Personnal communication). The protein content of Warsaw samples ranged from 9.5% to 12.0%, excluding Balkan, rye and triticale. For Blacksburg, the range was 9.09% to 11.68%. The mean protein values were generally higher in Warsaw than Blacksburg. The high protein values in the Warsaw samples may result in some hard wheat flour type characteristics, such as higher mixing stability in the farinograph and poor cookie diameters. The checks, FFR555W and Saluda, had low protein contents for both locations. The protein content of Saluda (9.3%) and FFR555W (9.3%) samples from Blacksburg were not significantly different from each other ($p > 0.05$). This was also reported by Schwarzlaff (1995). The observed protein values for the two checks were different from that of triticale (8.9%). The Warsaw sample (Saluda) was significantly higher in protein than triticale ($p < 0.05$) while FFR555W was not ($p > 0.05$). Amongst the sister lines (Pair 1 and 2), the non-1B/1R wheats (VA54-209 and VA54-21) grown in Blacksburg, had a significantly lower and higher protein content than their corresponding sister lines, respectively. However the protein content within each set of sister lines in the Warsaw samples were similar. Overall, no association was found

Table 5.2. Mean protein content of experimental SRWW flour

SAMPLE	PROTEIN (%) ¹	
	<u>Warsaw</u>	<u>Blacksburg</u>
<u>Checks</u>		
Massey	12.0 ± 0.1ab ²	10.7 ± 0.1cd
Saluda	10.0 ± 0.1f-h	9.3 ± 0.1g
FFR555W	9.5 ± 0.1hi	9.3 ± 0.1g
Balkan ³	12.4 ± 0.5a	Not available
Triticale ⁴	8.9 ± 0.1i	Not available
Rye (commercial) ⁴	8.0 ± 0.1j	8.0 ± 0.1h
<u>Pair 1</u>		
VA54-209	11.3 ± 0.0dc	10.5 ± 0.1ed
VA54-211+ ⁵	11.6 ± 0.0bc	11.7 ± 0.0b
<u>Pair 2</u>		
VA54-21	11.0 ± 0.6de	11.2 ± 0.03bc
VA54-18+	10.0 ± 0.1f-h	9.95 ± 0.1f
VA54-19+	10.6 ± 0.1fe	10.0 ± 0.1ef
VA54-185+	10.4 ± 0.0e-g	10.2 ± 0.0ef
VA54-418+	9.8 ± 0.3gh	9.9 ± 0.4f
VA54-258+	10.8 ± 0.2de	9.1 ± 0.0g
VA52-22+	10.6 ± 0.2fe	9.4 ± 0.1g

¹ n = 3

² Samples with the same letter in the same column are not significantly different at $p < 0.05$

³ Sample not a SRWW flour

⁴ Sample not grown in either location

⁵ + = Variety with 1B/1R translocation

between the presence or absence of 1B/1R and higher or lower protein content. However, as in moisture content, the effect of the environment was also seen in this case, with the Warsaw samples having higher protein values.

When compared to Massey, the samples without the 1B/1R translocation had lower protein values. This effect was significant ($p < 0.05$). However for the Blacksburg samples no significant differences were seen between Massey and non-1B/1R lines.

Baenziger et al., (1985) noted that the environment, wheat cultivars and interactions between cultivar and environment all affect quality attributes of flour in the southeastern United States. He emphasized that the influence of environment should not be overlooked. Protein content of wheat depends on agronomic and environmental factors such as soil nitrogen, soil moisture and temperature during the growing season. Protein quality is mainly a genotypic trait (Bushuk, 1985). Thus each variety inherits the quality of its protein from its parents. However, protein quality can also be adversely affected by abnormal environmental conditions such as disease infection, high temperature during grain filling period, wet harvest conditions and improper post-harvest storage conditions (Bushuk, 1985). These results are in conflict with those reported by Dhaliwal et al., (1987) who found no significant differences in the protein content of their 1B/1R soft wheat varieties and their respective parents.

ASH

The ash content of Warsaw samples ranged from 0.3% - 0.5% (Table 5.3). Those from Blacksburg ranged from 0.3% - 0.6% (Table 5.3). Triticale (Trical 498) and rye, had significantly higher ash values of 0.8% and 1.2%, respectively. When values were pooled together, both location and sample had significant effects on ash content ($p < 0.05$). Amongst the Warsaw samples, Massey did not differ significantly in ash content from Balkan (0.5%) or the other experimental soft red winter wheat samples. At both locations, however, the ash content of Massey was significantly lower than that of triticale and rye ($p < 0.05$).

The ash content of a flour is an indication of the amount of bran and minerals it contains. The high values for rye and triticale may be due to the difficulties in milling rye and triticale.

Table 5.3. Mean ash content of experimental SRWW flour

SAMPLE	ASH (%) ¹	
	<u>Warsaw</u>	<u>Blacksburg</u>
<u>Checks</u>		
Massey	0.4 ± 0.0c-f ²	0.4 ± 0.0ef
Saluda	0.4 ± 0.06d-f	0.4 ± 0.0gh
FFR555W	0.5 ± 0.0cd	0.6 ± 0.0c
Balkan+ ³	0.5 ± 0.1c	Not available
Triticale ⁴	0.8 ± 0.0b	Not available
Rye (commercial) ⁴	1.2 ± 0.0a	1.2 ± 0.0a
<u>Pair 1</u>		
VA54-209	0.4 ± 0.0d-f	0.4 ± 0.0h
VA54-211+ ⁵	0.4 ± 0.0c-f	0.5 ± 0.0de
<u>Pair 2</u>		
VA54-21	0.4 ± 0.0d-f	0.4 ± 0.0fg
VA54-18+	0.5 ± 0.0c-f	0.5 ± 0.0de
VA54-19+	0.4 ± 0.0d-f	0.4 ± 0.0gh
VA54-185+	0.5 ± 0.0c-e	0.5 ± 0.0d
VA54-418+	0.3 ± 0.2f	0.5 ± 0.0d-f
VA54-258+	0.3 ± 0.0ef	0.3 ± 0.0i
VA52-22+	0.4 ± 0.0c-f	0.4 ± 0.0ef

¹ n = 3

² Samples with the same letter in the same column are not significantly different at $p < 0.05$

³ Sample not a SRWW flour

⁴ Sample not grown at either location

⁵ + = Variety with 1B/1R translocation

Higher ash content in rye and triticale is expected due to lower percentage of starch versus bran. The ash content of flour is related to quality; and the degree of milling can be judged from the ash content of the flour. Wheat flour with a high ash content is darker in color. The ash content of flour is further influenced by rainfall, soil conditions, fertilizers and other factors. A high grade patent flour, which is pure endosperm, has an ash content of 0.30-0.35%, whereas whole wheat meal may have an ash content ranging from 1.35% - 1.80%. Thus from the results obtained (Table 5.3), it can be observed that the milling techniques played a significant role in the results.

The ash content in these samples may be due to the amount of minerals concentrated in the areas close to the bran coat and in the bran itself (deMan, 1990). The high mineral content of rye is due to the poor separation of the endosperm from bran and not due to the fact that the smaller rye grain could be expected to contain more mineral and bran and less endosperm (Weipert, 1997).

AWRC AND COOKIE DIAMETER OF 1995 – 1996 SRWW FLOUR

The results indicated that AWRC was significantly different ($p < 0.05$) amongst SRWW samples, and by location and that there was a significant interaction between sample and location (Figure 5.1). Therefore we reject the first null hypothesis and accept that AWRC is influenced significantly by the presence of 1B/1R translocation. For cookie diameter, Figure 5.2 shows a highly significant effect based on location, sample and sample and location interactions ($p < 0.05$). A significant negative Pearson's Correlation Coefficient of 0.74 ($p = 0.0001$) was found to exist between AWRC and cookie diameter. Therefore the diameter of cookies decreased with increasing AWRC. Due to this significant effect, samples were analyzed by location.

Cookie diameter and AWRC values for Blacksburg and Warsaw are given in Tables 5.4 and 5.5, respectively. The Pearson Correlation Coefficient for AWRC and cookie diameter for Blacksburg and Warsaw were -0.64 and -0.82, respectively. These two correlation coefficients were highly significant ($p = 0.0001$). For example the Blacksburg sample, VA54-418, had the highest AWRC (67%) and the lowest cookie diameter (73 mm). Thus the second null hypothesis was rejected and the results indicated that location played a significant role on both cookie diameter and AWRC. The AWRC and cookie diameter values were significantly different ($p < 0.05$) amongst Blacksburg and Warsaw SRWW flour samples.

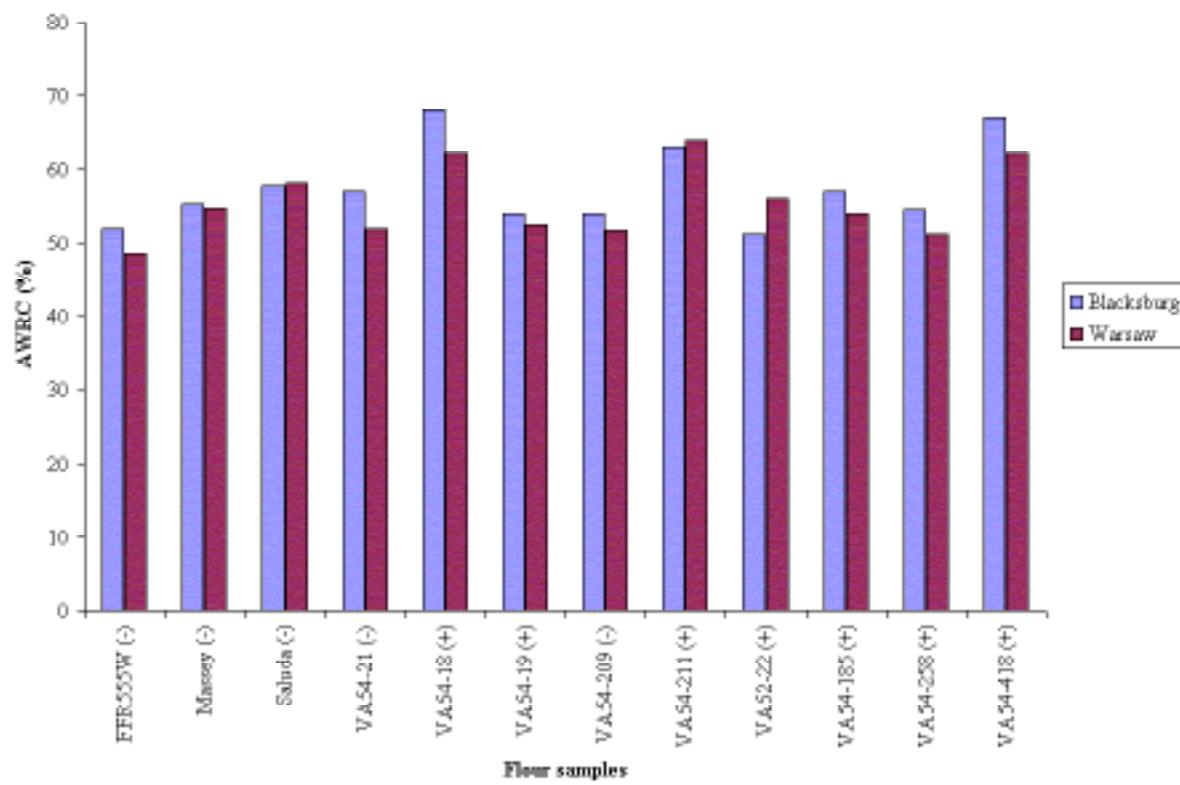


Figure 5.1. Effect of location on AWRC of 1995 – 1996 SRWW flour samples

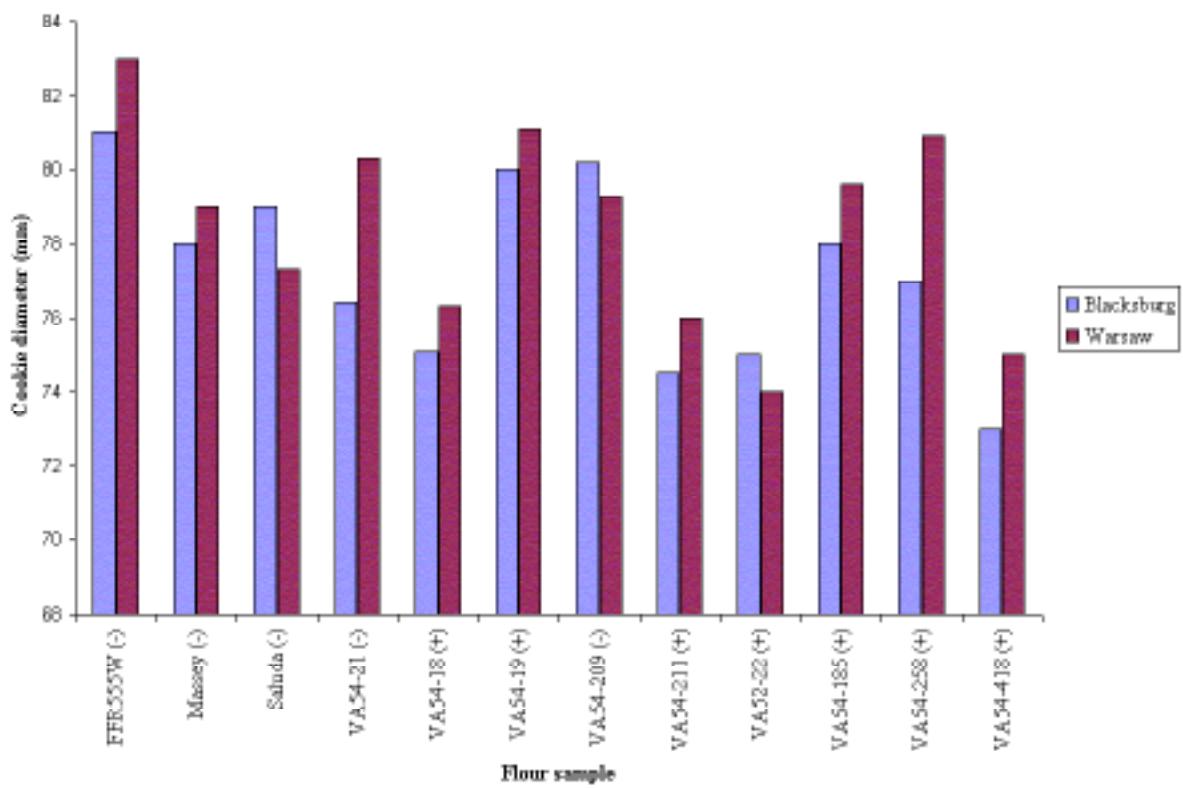


Figure 5.2. Effect of location on cookie diameter of 1995-1996 SRWW flour samples

Table 5.4. Relationship between cookie diameter and AWRC from 1995-1996 Blacksburg SRWW flour samples

Sample	Cookie diameter (mm) ²	AWRC (%) ³
<u>Pair 1</u>		
VA54-209-	80.2 ± 0.33ab ⁴	54 ± 0.6ef
VA54-211+	74.5 ± 0.48gf	63 ± 0.14b
<u>Pair 2</u>		
VA54-21-	76.4 ± 0.10de	57 ± 0.6cd
VA54-18+	75.1 ± 0.34ef	68 ± 0.6a
VA54-19+	80.0 ± 0.07ab	54 ± 2.2ef
VA54-258+	77 ± 0.8c-e	54.5 ± 0.40 d-f
VA54-418+	73 ± 0.8g	67 ± 0.9a
VA54-185+	78 ± 0.9b-d	57 ± 1.0cd
VA52-22+	75 ± 0.7fg	51.3 ± 0.21g
<u>Checks</u>		
Massey-	78 ± 1.1b-d	55.2 ± 0.31c-e
FFR555W-	81 ± 1.1a	52 ± 0.9fg
Saluda-	79 ± 0.6bc	57.8 ± 0.24c

¹ $r = -0.63834$ (Pearson correlation coefficient between diameter and AWRC)

²⁻³ n = 3

⁴ Means ± standard deviations where the same letter in the same column indicates values are not significantly different at $p < 0.05$

⁺ Variety with the 1B/1R translocation

⁻ Variety without the 1B/1R translocation

Table 5.5. Relationship between cookie diameter and AWRC from 1995-1996 Warsaw SRWW flour samples

Sample	Cookie diameter (mm) ²	AWRC (%) ³
<u>Pair 1</u>		
VA54-209-	79.3 ± 0.15bc ⁴	51.7 ± 0.05e
VA54-211+	76 ± 0.7d-f	63.9 ± 0.25a
<u>Pair 2</u>		
VA54-21-	80.3 ± 0.10bc	51.9 ± 0.40e
VA54-18+	76.3 ± 0.23de	62 ± 2.1a
VA54-19+	81.1 ± 0.44ab	52.5 ± 0.31de
VA54-258+	80.9 ± 0.29a-c	51.2 ± 0.07e
VA54-418+	75 ± 1.0ef	62 ± 1.7a
VA54-185+	79.6 ± 0.08bc	54 ± 1.1c-e
VA52-22+	74 ± 0.6f	56 ± 0.8bc
<u>Checks</u>		
Massey-	79 ± 0.8c	54.6 ± 0.27cd
FFR555W-	83 ± 1.2a	48.56 ± 0.034f
Saluda-	77.3 ± 0.38d	58.1 ± 0.35b

¹ $r = -0.82249$ (Pearson Correlation Coefficient between diameter and AWRC)

²⁻³ n = 3

⁴ Means ± standard deviations where same letter in the same column indicates values are not significantly different at $p<0.05$

+ Variety with the 1B/1R translocation

- Variety without the 1B/1R translocation

A soft wheat variety is considered to generally have good quality when the flour yields cookies of a large diameter. Significant differences occur in the spread potential of different soft wheat varieties, however the reasons for the differences are not well understood. Miller and Hoseney (1997) tried to determine what was responsible for reduced cookie diameters in hard wheat flours and their results were inconclusive. They stated that cookie diameter was a function of spread rate and set time. Cookies made with soft wheat flour were significantly larger in diameter (184 mm) than those made with hard wheat flour (161 mm). The cookies set later (5.8 minutes) during baking than those made with hard wheat flour (5.1 minutes). The researchers believe that these differences in set time were due to the flour's protein content. The spread rate was different also. Those made from soft wheat flour spread at a faster rate (7.8 mm/min) compared to 4.6 mm/min from hard wheat. Differences may be due to the soluble starch contents of the flours, with soft wheat flour having lower values (0.15%). Therefore spread rate was lower. But these differences did not explain differences occurring within various hard wheat flours or within cookies made with various soft wheat flours.

Tables 5.4 and 5.5 show that for Pair 1 sister lines, the non-1B/1R line (VA54-209) had a higher cookie diameter in both locations than its corresponding sister line (VA54-211). The differences between these two samples were significant ($p < 0.05$). The AWRC values for these samples were inversely related to cookie diameter. Schwarzlaff (1995) also reported a similar trend, that the 1B/1R sample (VA54-211) had lower cookie diameters. However for Pair 2, one of the sister lines with the 1B/1R translocation (VA54-19) grown in Blacksburg produced cookies with significantly higher diameters ($p < 0.05$) than those of its non-1B/1R sister line (VA54-21). The differences between these two samples were significant ($p < 0.05$). The diameter of VA54-19 was similar to that of FFR555W in both locations.

Contrasts conducted on Blacksburg samples revealed that non-1B/1R flours produced cookies with significantly greater diameters ($p = 0.0071$) than those with the 1B/1R translocation (Table 5.6). However, this effect was not significant at the Warsaw location ($p = 0.2001$). Cookie diameter differences between VA54-209 and VA54-211 were significantly different amongst Warsaw and Blacksburg samples. The same trend was seen when mean diameters of Pair 2 samples (VA54-21, VA54-18 and VA54-19) were compared. Results for both locations indicated the sample type exerted a significant effect on the baking performance of the SRWW flour. The checks in both locations were significantly different from each other. When Saluda was compared to Pair 1 (VA54-209 and VA54-211) and pair 2 samples from Blacksburg, the differences were significant ($p < 0.05$). This was not the case for Warsaw samples.

Table 5.6. Comparisons of contrasts on AWRC and cookie diameter

CONTRASTS	AWRC		COOKIE DIAMETER	
	<u>Blacksburg</u>	<u>Warsaw</u>	<u>Blacksburg</u>	<u>Warsaw</u>
1B/1R vs non -1B/1R	0.3191	0.0506	0.0071*	0.2001
Pair 1 to Massey	0.0001* ¹	0.0001*	0.0001*	0.0001*
VA54-209 to VA54-211	0.7087	0.3005	0.7060	0.0015*
VA54-18, VA54-19 to VA54-21	0.0091*	0.0001*	0.2008	0.0001*
Saluda, Massey to FFR555W	0.0001*	0.0040*	0.0002*	0.0001*
Massey, FFR555W to Saluda	0.0001*	0.0001*	0.0001*	0.0001*
FFR555W, Saluda to Massey	0.0001*	0.0001*	0.0082*	0.1339
VA54-209, VA54-211 to Saluda	0.0002*	0.0163*	0.0016*	0.1738
VA54-21, VA54-18, VA54-19 to Saluda	0.0135*	0.0029*	0.0001*	0.1364
VA54-21, VA54-18, VA54-19 to Massey	0.0001*	0.0001*	0.0618	0.0001*
Checks vs non -1B/1R	0.0001*	0.0001*	0.0001*	0.0001*

¹ Indicates significance at $p < 0.05$

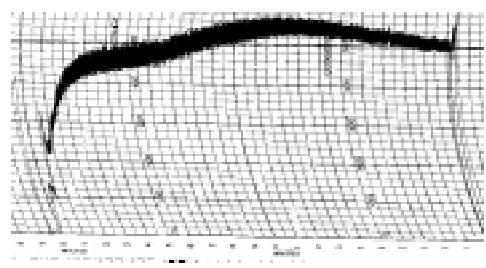
The checks were significantly different from non-1B/1R samples in both locations. Massey wasn't significantly different from Pair 2 in Blacksburg, but was significantly different in Warsaw samples. Since FFR555W is a good quality soft wheat flour for producing cookies, it was compared to other checks (Saluda and Massey). The results revealed that FFR555W at both locations produced cookies with high diameters and low AWRC values (Table 5.6).

FARINOGRAPH ANALYSIS OF 1995-1996 AND 1996-1997 SRWW FLOUR

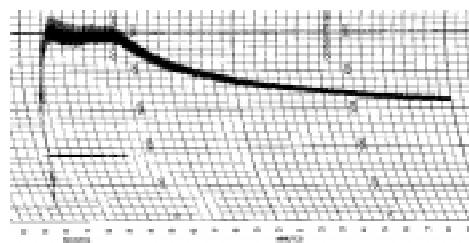
It is generally accepted that the introduction of rye genetic material into hard wheat lowers the baking quality of the resulting flour due to the occurrence of sticky doughs, which cause handling problems during processing. This phenomenon can be detected in farinograms from samples containing rye chromosomes (see Figure 5.3).

These farinograms also indicate that the rye and triticale have poor mixing tolerance and development times. Mixing tolerance is measured by calculating the difference in BTUs between the top of the peak on the farinograph and 5 minutes after the peak. The lower the MTI, the stronger the flour. For example, Massey (Figure 5.4) exhibited better mixing tolerance by having a low MTI, while Saluda (1996-1997) had a poorer MTI. Generally, the SRWW checks showed better mixing tolerance (Figures 5.4 and 5.5), than the rye containing samples (Figure 5.3). Farinograms for the other experimental wheat flour samples are shown in Figures 5.6 through 5.13. Values obtained from these farinograms are given in Tables 5.7 to 5.10.

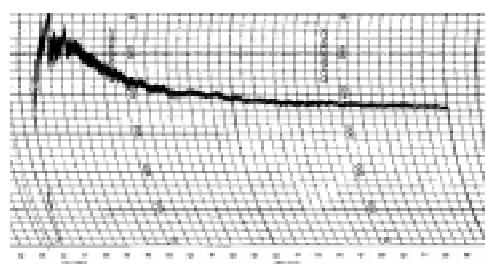
Statistical analysis indicated that WA was not significantly different amongst 1995 – 1996 Warsaw samples (Table 5.7). However, FFR555W had a lower WA value amongst the Blacksburg samples (Table 5.8). This flour gave the highest cookie spread. FFR555W values for WA was 55 % for Warsaw and Blacksburg samples. The WA values for the other checks (Saluda and Massey) at both locations were higher than FFR555W but were not significantly different. The rye and Balkan lines had very high water absorption values (see Table 5.7). There were no significant differences in pair 1 samples (VA54-209 and VA54-211) for both locations, although the non-1B/1R sister line (VA54-209) had a lower water absorption value than its 1B/1R counterpart (VA54-211). When wheat flour samples from 1996-1997 were included in the analysis, the results indicated that sample, location and year exerted a significant effect on WA (Table 5.11).



Balkan



Triticale



Rye

Figure 5.3. Farinograms of rye containing samples

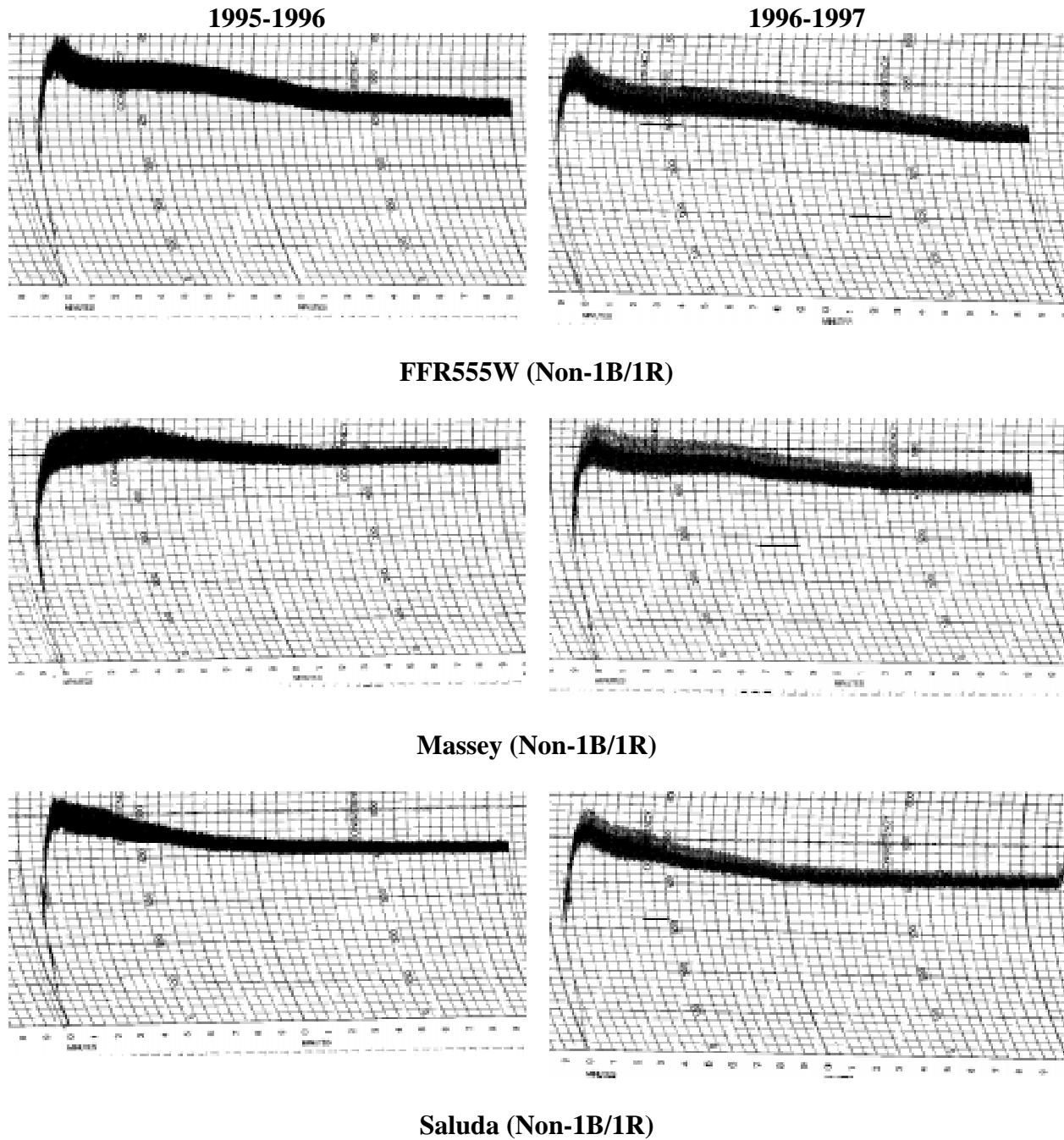


Figure 5.4. Farinograms of 1995-1996 and 1996-1997 Blacksburg wheat flour checks

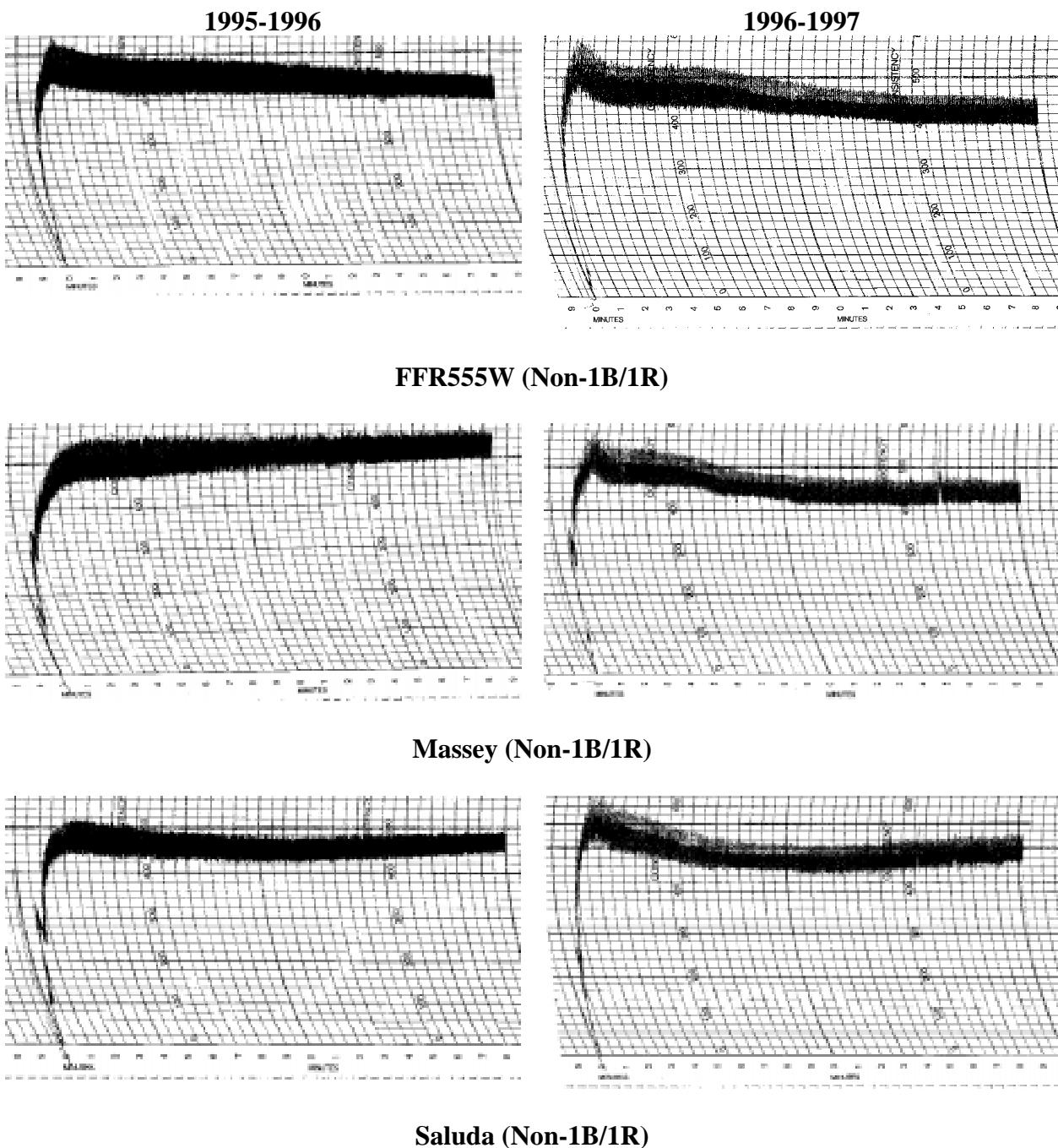
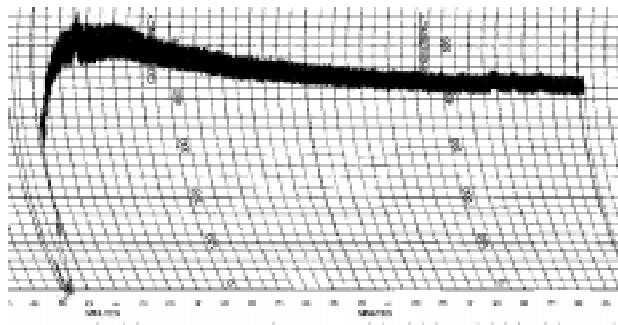
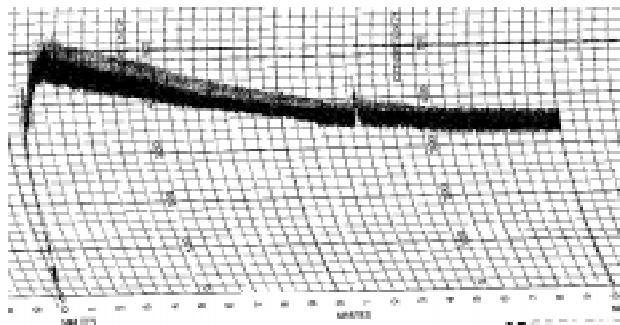


Figure 5.5. Farinographs of 1995-1996 and 1996-1997 Warsaw wheat flour checks

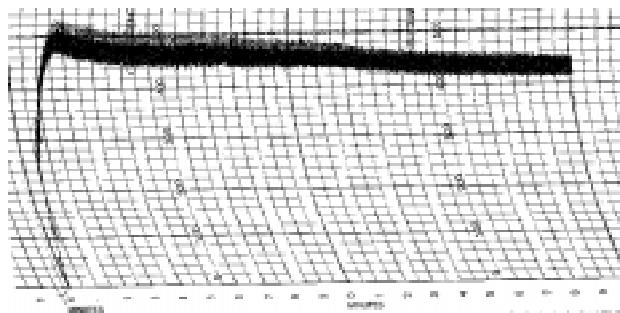
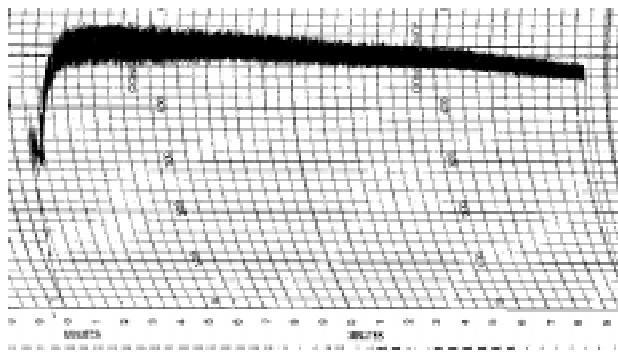
1995-1996



1996-1997



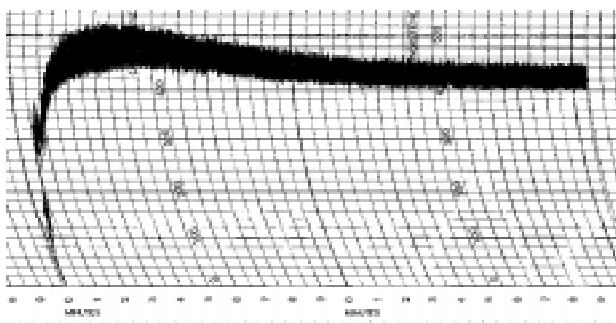
VA93-54-209 (Non-1B/1R)



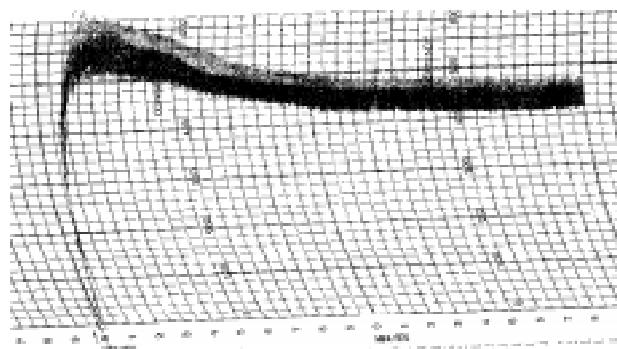
VA93-54-211 (1B/1R)

Figure 5.6. Farinograms of 1995-1996 and 1996-1997 Blacksburg sister lines

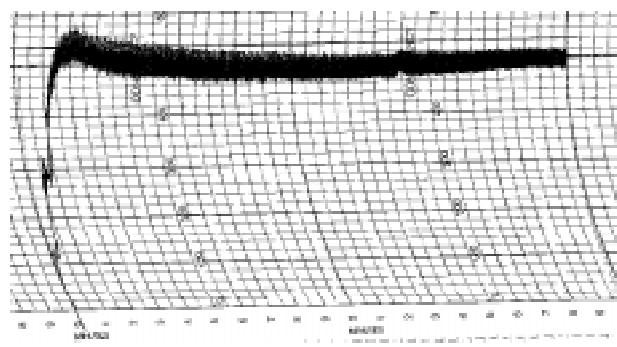
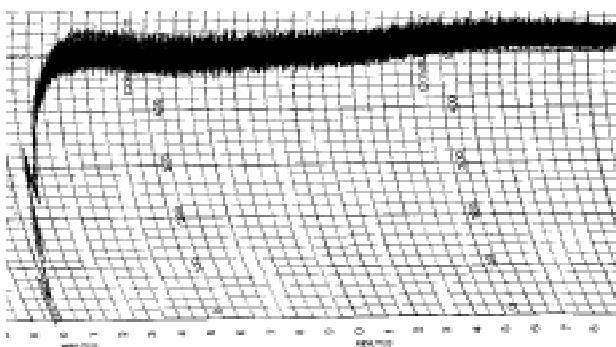
1995-1996



1996-1997



VA93-54-209 (Non-1B/1R)



VA93-54-211 (1B/1R)

Figure 5.7. Farinograms of 1995-1996 and 1996-1997 Warsaw sister lines

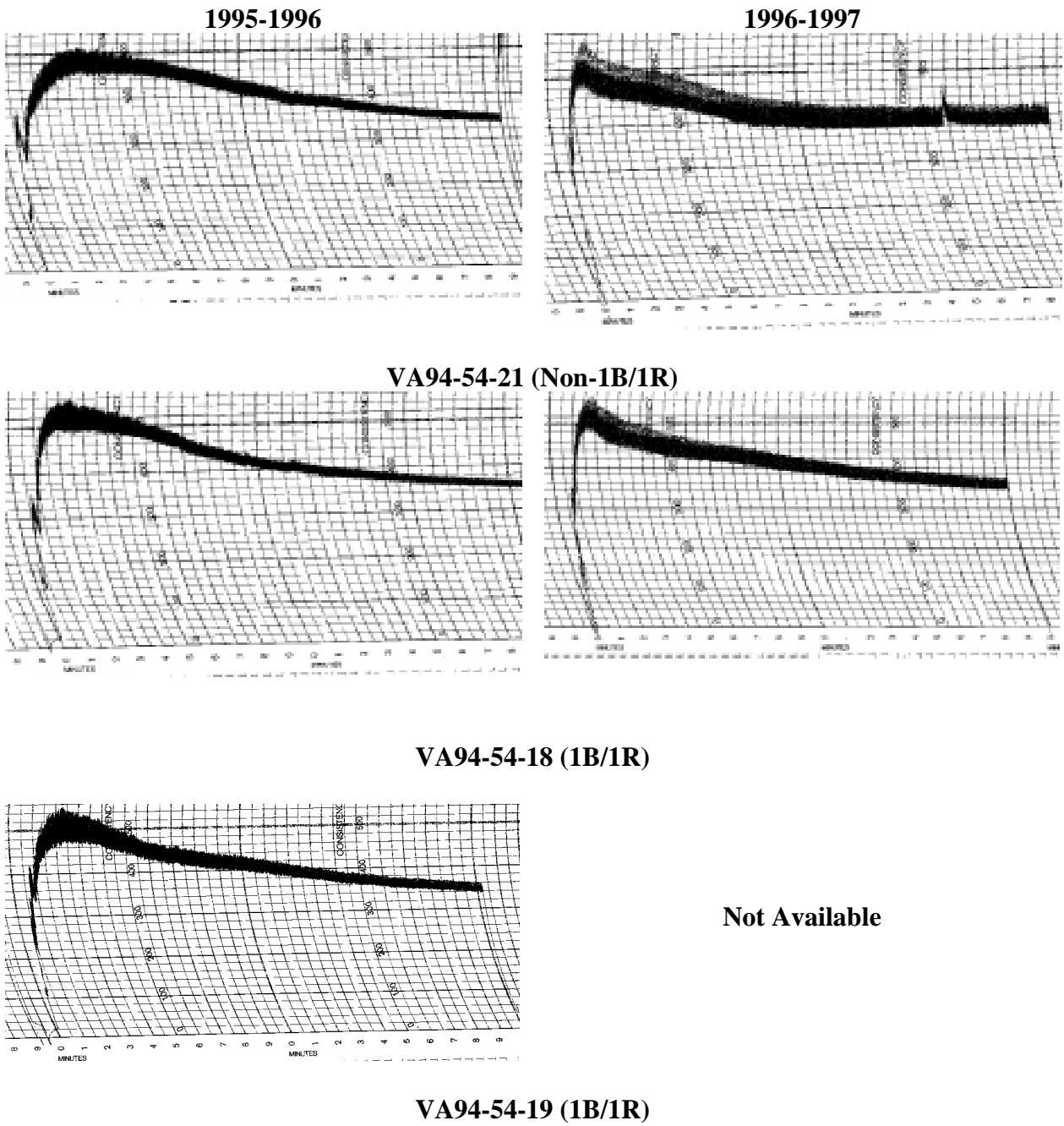


Figure 5.8. Farinograms of 1995-1996 and 1996 – 1997 Blacksburg sister lines

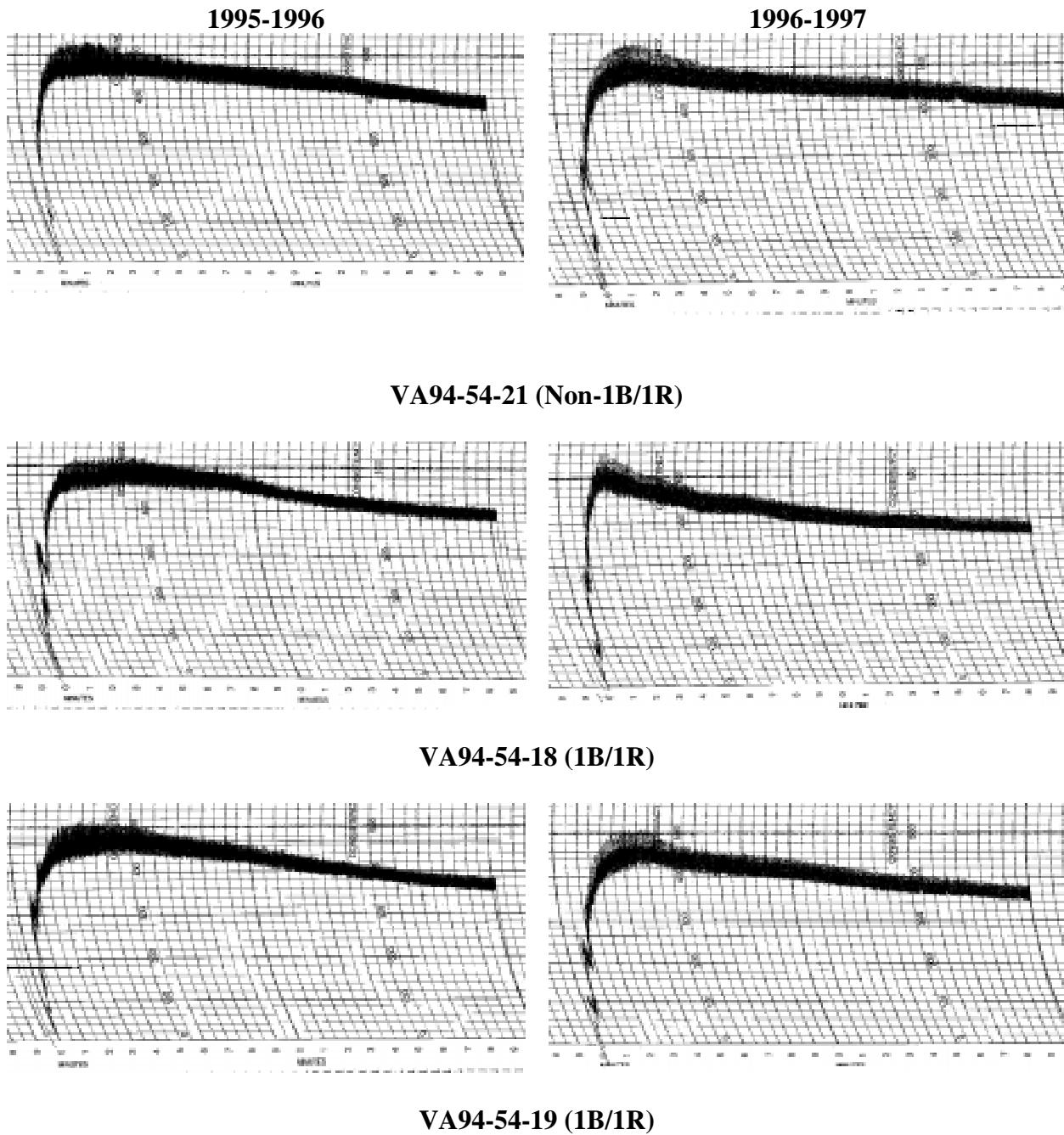


Figure 5.9. Farinograms of 1995-1996 and 1996-1997 Pair 2 sister lines grown in Warsaw

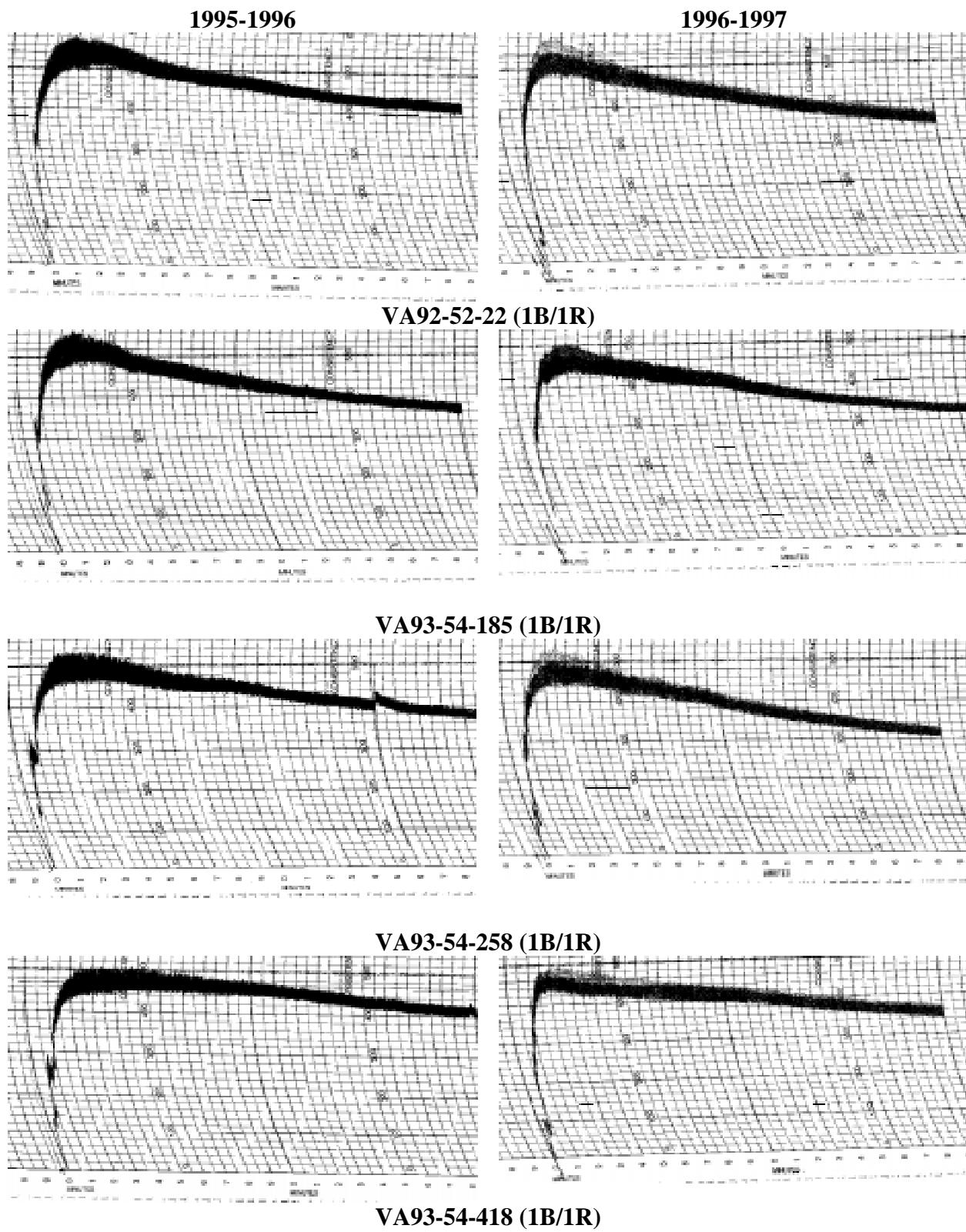


Figure 5.10. Farinograms of 1995-1996 and 1996-1997 Blacksburg 1B/1R samples

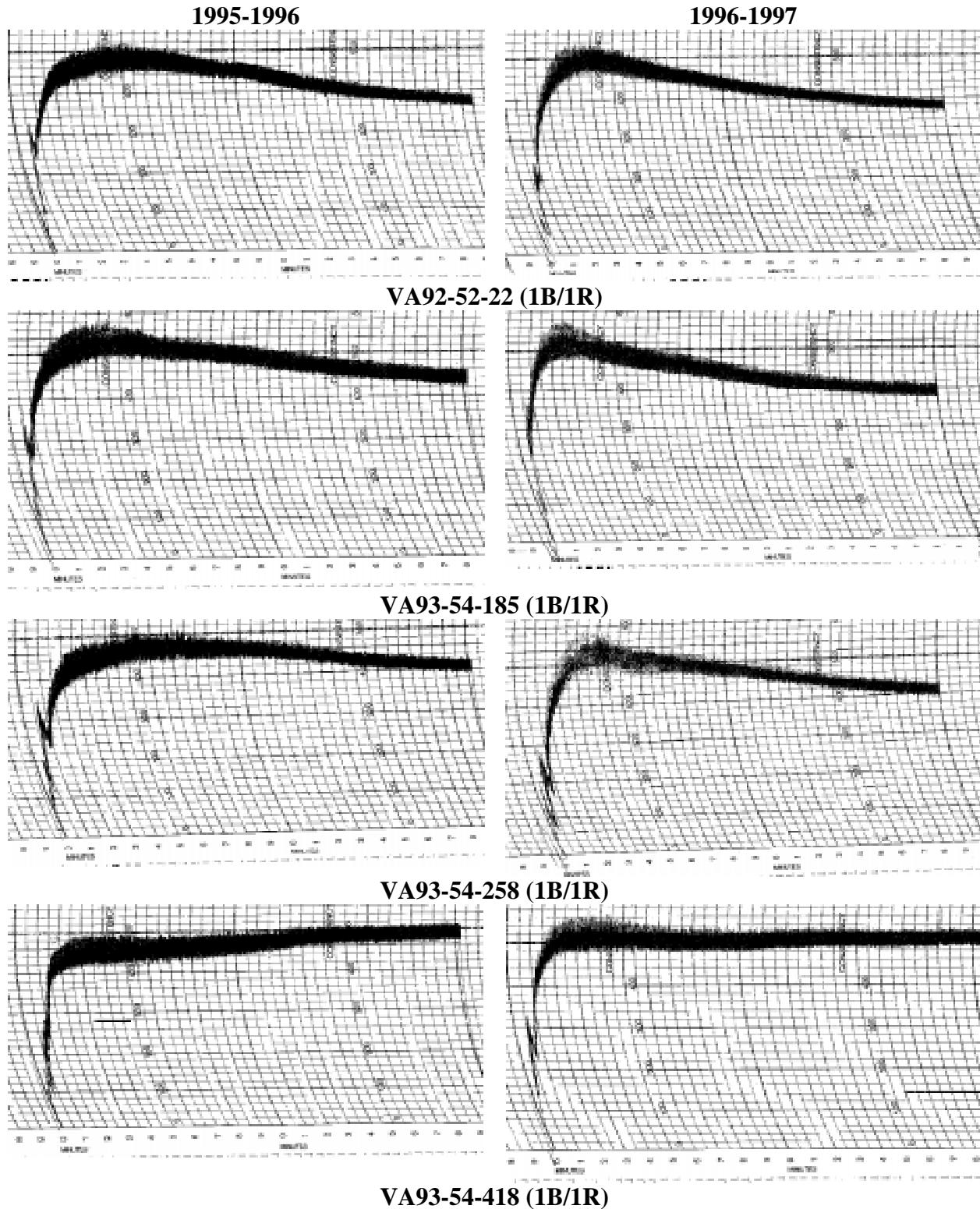
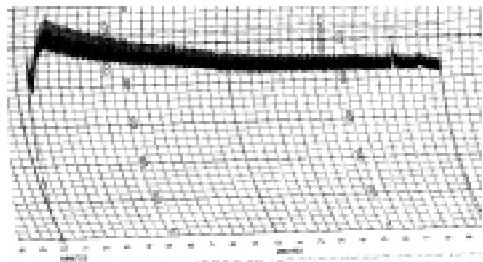
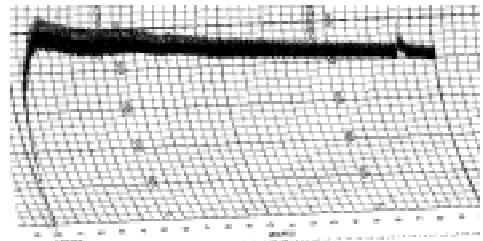


Figure 5.11. Farinograms of 1995-1996 and 1996-1997 Warsaw 1B/1R samples

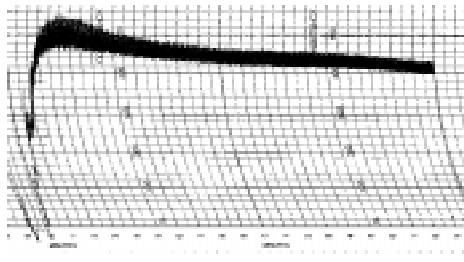


VA93-52-55 (1B/1R)

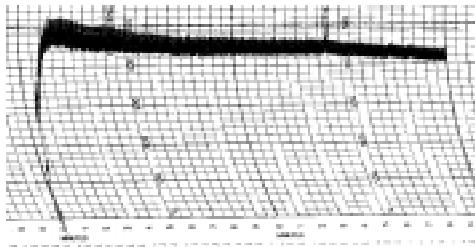


VA93-52-67 (Non-1B/1R)

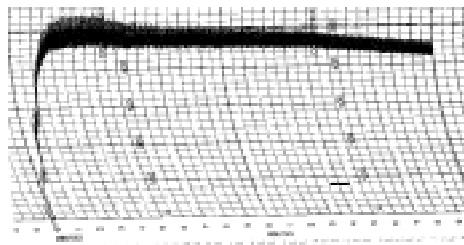
Figure 5.12. Farinogram of 1996 – 1997 Blacksburg 1B/1R and non-1B/1R samples



VA93-52-55 (1B/1R)



VA93-54-241 (1B/1R, sister line of VA93-52-67)



VA93-52-67 (Non-1B/1R, sister line of VA93-54-241)

Figure 5.13. Farinogram of 1996 – 1997 Warsaw 1B/1R and non-1B/1R samples

Table 5.7. Farinograph data from soft red red winter wheats grown in Warsaw during 1995 - 1996

Variety	WA¹ (%)	AT² (Mins)	PT³ (Mins)	DT⁴ (Mins)	MTI⁵ (BU)	MS⁶ (Mins)	TMD⁷ (BU)
<u>Pair 1</u>							
VA54-209	58a	1.6c-e	3.1ab	8a	50ab	6.5ab	60a-c
VA54-211+ ⁸	62a	1.69b-e	3ab	20a	26a-c	18.32a	0c
<u>Pair 2</u>							
VA54-21	60a	1.4de	2.5ab	8a	48ab	7ab	90ab
VA54-18+	64a	1.7b-e	4ab	8a	40a-c	7ab	90ab
VA54-19+	58a	1.51c-e	3ab	8a	60a	6ab	110a
<u>Pair 3</u>							
VA52-67	NA	NA	NA	NA	NA	NA	NA
VA54-241+	NA	NA	NA	NA	NA	NA	NA
VA54-185+	59a	2.3ab	4.3ab	8.7a	40a-c	6.3ab	90ab
VA54-418+	62a	2b-d	3.3ab	20a	20bc	18a	0c
VA54-258+	61a	2.1bc	5a	12a	28a-c	10ab	70ab
VA52-22+	64a	2.9a	4.0ab	7.6a	40a-c	4.6b	100a
Balkan+ ⁹	65.8	5.30	11.30	20.00	39.00	14.30	0.00
Rye	66.4	0.40	2.05	3.125	140.0	2.325	130.0
Triticale ¹⁰	60.0	1.07	1.22	4.37	90.00	3.30	185.0
<u>Checks</u>							
FFR555W	55a	1e	1.6b	10a	44ab	9ab	60a-c
Saluda	60a	1.6c-e	3ab	10a	40a-c	10ab	10bc
Massey	59a	2.0b-d	5a	20a	4c	18.0a	0c

1-7 WA = Water absorption; AT = Arrival time; PT = Peak time; DT = Departure time;
 MTI = Mixing tolerance index; MS = Mixing stability; TMD = Twenty minute drop.
 (Values are means of 2 determinations; and values in a column with same letter are not
 significantly different at $P < 0.05$)

8 + = Variety with 1B/1R translocation

9 Not a SRWW flour

10 Sample not grown in Warsaw or Blacksburg

Table 5.8. Farinograph data from soft red winter wheats grown in Blacksburg during 1995 - 1996

Variety	WA 1 (%)	AT 2 (Mins)	PT 3 (Mins)	DT 4 (Mins)	MTI 5 (BU)	MS 6 (Mins)	TMD 7 (BU)
<u>Pair 1</u>							
VA54-209	58ab	1a	2a	6bc	82ab	5cd	80b-d
VA54-211+ ⁸	62ab	1.5a	2.7a	15a	23c	12a	50cd
<u>Pair 2</u>							
VA54-21	61ab	1.9a	3.0a	6bc	70c-c	4cd	150a
VA54-18+	63a	1.6a	2.5a	4.8bc	90a	2.3d	154a
VA54-19+	58ab	1.4a	2.4a	4c	90a	3d	160a
<u>Pair 3</u>							
VA52-67	NA	NA	NA	NA	NA	NA	NA
VA54-241+	NA	NA	NA	NA	NA	NA	NA
VA54-185+	60ab	2a	2.7a	4.8bc	78ab	3cd	150a
VA54-418+	62ab	1.75a	4a	11a	36bc	9ab	70b-d
VA54-258+	59ab	1.73a	2.7a	5.7bc	50a-c	4cd	130ab
VA52-22+	62ab	2.0a	3a	6.0bc	60a-c	4.00cd	113ab
<u>Checks</u>							
FFR555W	55b	1a	1.5a	7.5b	60a-c	6.5bc	90a-c
Saluda	59ab	1.4a	1.9a	4.3c	80ab	2.9d	90a-c
Massey	58ab	1.5a	4a	12.8a	40bc	11.3bc	17a-c

1-7 WA = Water absorption; AT = Arrival time; PT = Peak time; DT = Departure time; MTI = Mixing tolerance index; MS = Mixing stability; TMD = Twenty minute drop. (Values are means of 2 determinations; and values in a column with same letter are not significantly different at $P < 0.05$)

8 + = Variety with 1B/1R translocation

Table 5.9. Farinograph data from soft red winter wheats grown in Blacksburg during 1996 - 1997

Variety	WA 1 (%)	AT 2 (Mins)	PT 3 (Mins)	DT 4 (Mins)	MTI 5 (BU)	MS 6 (Mins)	TMD 7 (BU)
<u>Pair 1</u>							
VA54-209	53.9gf	1cd	1.8b-d	5.3b-d	90a-d	4.3cd	110bc
VA54-211+ ⁸	59.1c	1.3a-c	1.7cd	10a	30f	8ab	50e
<u>Pair 2</u>							
VA54-21	55.8e	1cd	1.6d	4cd	110a	3cd	90cd
VA54-18+	60.3ab	1.26a-c	1.7cd	3.4d	60c-f	2.2d	140ab
VA54-19+	NA	NA	NA	NA	NA	NA	NA
<u>Pair 3</u>							
VA52-67	54.78f	1.1b-d	1.7cd	8ab	43ef	7a-c	60de
VA54-241+	NA	NA	NA	NA	NA	NA	NA
VA54-185+	57.5d	1.4a-c	2.7a	5.8b-d	70b-e	4cd	130ab
VA54-418+	59.6bc	1.25a-c	2.3a-c	6.5bc	50d-f	5.3b-d	93cd
VA54-258+	57.9d	1.5ab	2.4ab	5.3b-d	80a-e	3.8cd	153a
VA52-22+	61.13a	1.625a	2.6a	4.5cd	100ab	2.875d	160a
VA52-55+	58d	1cd	1.5d	4.5cd	90a-d	3.5cd	88cd
<u>Checks</u>							
FFR555W	53.5d	10.75d	1.4d	3.5cd	100ab	2.75d	90cd
Saluda	57.4d	1.1a-d	1.62cd	5.5b-d	90a-c	4.3cd	60de
Massey	54.5f	1.1a-d	1.6cd	10a	50ef	9a	43e

1-7 WA = Water absorption; AT = Arrival time; PT = Peak time; DT = Departure time;
 MTI = Mixing tolerance index; MS = Mixing stability; TMD = Twenty minute drop.
 (Values are means of 2 determinations; and values in a column with same letter are not
 significantly different at $P < 0.05$)

8 + = Variety with 1B/1R translocation

Table 5.10. Farinograph data from soft red winter wheats grown in Warsaw during 1996-1997

Variety	WA 1 (%)	AT 2 (Mins)	PT 3 (Mins)	DT 4 (Mins)	MTI 5 (BU)	MS 6 (Mins)	TMD 7 (BU)
<u>Pair 1</u>							
VA54-209	56.1ef	1.125bc	1.25d	6b	90bc	4.9b	80a-c
VA54-211+ ⁸	62b	1.3a-c	2.1a-d	10ab	50c-e	10ab	10ef
<u>Pair 2</u>							
VA54-21	60.5bc	1.75a-c	3a	7.3b	70b-d	5.5b	73b-d
VA54-18+	60bc	1.26a-c	3a	4b	110a	3b	120a
VA54-19+	NA	NA	NA	NA	NA	NA	NA
<u>Pair 3</u>							
VA52-67	56.6de	1.3a-c	2.5a-c	13ab	43ef	12ab	40ed
VA54-241+	56.1ef	1.2a-c	1.4dc	7b	45d-f	6b	50ed
VA54-185+	58.6cd	2a-c	2.3a-d	5.5b	78bc	4b	108ab
VA54-418+	61b	1.7a-c	2.3a-d	20a	23f	18.3a	0ef
VA54-258+	61.2b	2ab	3ab	6b	75bc	4.4b	120a
VA52-22+	64.3a	2.12a	3a	6.8b	30bc	5b	110ab
VA52-55+	60.4bc	1.5a-c	2.25a-d	5.8b	78bc	4.3b	73b-d
<u>Checks</u>							
FFR555W	54.1f	0.9c	1.25d	7b	58c-e	6b	90a-c
Saluda	58de	1.25a-c	2a-d	5.3b	90ab	4.0b	30ef
Massey	55.6ef	1.1bc	1.8b-d	7b	78bc	6b	50c-e

1-7 WA = Water absorption; AT = Arrival time; PT = Peak time; DT = Departure time;
 MTI = Mixing tolerance index; MS = Mixing stability; TMD = Twenty minute drop.
 (Values are means of 2 determinations; and values in a column with same letter are not significantly different at $P < 0.05$)

8 + = Variety with 1B/1R translocation

Table 5.11. Influence of location and wheat crop year on farinograph values of SRWW flour

Variable	WA	AT	PT	DT	MTI	MS	TMD
Sample ¹	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Year ²	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0009
Location ³	0.0004	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
S * L ⁴	0.9092* ⁵	0.1217*	0.3999*	0.0090	0.0014	0.0098	0.0018
S * Y	0.7654*	0.4807*	0.0059	0.0868*	0.0001	0.0815*	0.0001
S * Y * L	0.2917*	0.0039	0.0128	0.0117	0.0001	0.0210	0.0204

¹Indicates wheat samples from 2 crop years (1995 – 1996) and 2 locations (Blacksburg and Warsaw)

²Indicates values were from two crop years (1995 – 1996 and 1996 – 1997)

³Locations were Blacksburg and Warsaw

⁴Abbreviation for the interaction between sample and location (S * L)

⁵*Indicates not significant at $P < 0.05$

Balkan (Table 5.7), the hard wheat donor of 1B/1R, had the longest arrival time (AT = 5.30 mins) and peak time (PT = 11.30 mins) of all the experimental wheats used in this experiment. The departure time (DT) was significantly affected by location, sample, and crop year (Table 5.9). For example the 1995 – 1996 FFR555W sample from Blacksburg took 7.5 minutes while the Warsaw sample took 10 minutes. Differences by location were expressed in Massey, VA54-418 and VA54-185 (Table 5.7 and 5.8). Tables 5.9 and 5.10 show the influence of crop year on the farinograph results. From these tables, the 1B/1R sample (VA54-418) had a DT of 6.5 minutes and 20 minutes for Blacksburg and Warsaw, respectively. This confirms the statistical results in Table 5.11, that indicate location had a significant effect on DT.

It seems that some 1B/1R flours had lower breakdown rates than their non-1B/1R counterparts. For example, for both locations and crop years, VA54-211 had a lower MTI value than the sister line VA54-209 (non-1B/1R). It seems that the IB/1R translocation was not causing deleterious changes in the rheological properties of some soft wheat flours. However, for pair 2 samples (VA54-21, VA54-18 and VA54-19), clear significant differences were seen by location. For example, the 1B/1R samples in this pair (VA54-18 and VA54-19) in Blacksburg had values of 90 BUs (Table 5.8) while in Warsaw the values were 40 and 60 Bus (Table 5.7), respectively. Since VA54-19 was not available for the 1996-1997 crop year, the value for 54-18 in Blacksburg (Table 5.9) and Warsaw (Table 5.10) was 60 and 110 BUs, respectively. The 1995-1996 non-1B/1R sample for pair 2 in Warsaw and Blacksburg (VA54-21) had values of 48 BUs (Table 5.7) and 70 BUs (Table 5.8), respectively. The corresponding values for 1996 – 1997 were 110 and 70 BUs. Again differences were seen according to location on the experimental checks, with the Warsaw samples exhibiting lower breakdown rates. The samples (VA54-185, VA54-418, VA54-258 and VA52-22) had similar MTI values as those of the checks, for their respective locations (Tables 5.7 to 5.10). Balkan the donor of the IB/1R translocation, had a low MTI of 39 BUs (Table 5.7).

A low MTI is an unexpected occurrence for 1B/1R containing wheat samples, as available literature (Dhaliwal and MacRitchie, 1990; Henry et al., 1989) suggests that the presence of 1B/1R translocation would result in poor mixing tolerance and faster dough breakdown rates (thus higher MTIs). Thus it is possible to conclude that the unexpected tolerance to mixing observed in the 1B/1R samples is a trait that has been inherited from Balkan. The MTI of rye and triticale (140 BUs and 90 BUs) were significantly higher than that of Balkan (39 BUs).

Mixing stability (MS) is the measure of a flour's tolerance to mixing. Samples at the 2 locations exhibited significant differences in tolerance to mixing (Table 5.11). Balkan was tolerant to mixing, with a value of 14.30 minutes. The 1B/1R sample (VA54-211) had the highest value for mixing tolerance in both locations for the 1995 – 1996 crop year. The 1B/1R sample (VA54-211) from 1996 – 1997, however, had a lower value (Tables 5.9 and 5.10).

In terms of the twenty minute drop (TMD), Balkan had a value of zero (Table 5.7). This indicated that Balkan is a strong flour. Other samples that exhibited the same zero value for the 1995-1996 Warsaw location were VA54-418, VA54-211 and the check – Massey (Tables 5.7). The corresponding 1995-1996 samples in Blacksburg had TMD values of 70, 50 and 17 BUS, respectively (Table 5.8). For the 1996-1997 crop year, only VA54-418 from Warsaw gave a TMD of 0 (Table 5.10).

The 1B/1R sister line (VA54-211) had a long DT for both locations (Tables 5.7 to 5.10). This suggests that VA54-211 is a strong flour. This flour also had a very long mixing stability, but had a lower MTI. The very high TMD values for pair 2 samples (VA54-21, VA54-18 and VA54-19), indicates these flours were weaker (Tables 5.7 to 5.10). The Blacksburg pairs (VA54-18 and VA54-19), had higher breakdown rates than those of Warsaw (Table 5.8). In conclusion, although some significant differences were noted in farinograph values according to sample type, crop year and location; there seems to be no clear relationship as to how the presence or absence of 1B/1R translocation affects the rheological properties of SRWW flours.

Flour for most soft wheat products should have a relatively low protein content of 8.5 to 10% and a low AWRC. Whereas US hard winter and spring wheats have been bred for higher protein and strong mixing properties. Eastern US soft wheats have not been systematically screened on the basis of protein strength prior to release. Therefore among eastern US soft red wheats and white wheats, protein strength ranges from weak to as strong as it does for most hard winter or spring wheats (Finney, 1989).

For some soft wheat products, flour protein (mixing) strength appears to have little direct bearing on end-product quality; for others it is critically important (Finney, 1989). For example, fermented crackers, flat breads and Chinese steamed breads rely on protein for structure, size, shape and density, appearance, texture and mouthfeel. Protein mixing strength is now being

characterized at the Soft Wheat Quality Laboratory (SWQL) in Ohio with the mixograph. It can also be characterized by the farinograph. Although preliminary studies indicate that differences in dough mixing strength could be related to end-product quality (texture and mouthfeel), these differences in specific eastern soft wheat cultivars have not been studied well enough to make a definite associations. What is certain is that there is diversity in mixograph mixing strength of eastern soft wheats because “historically no effort has been made by breeders or by the SWQL to narrow that range in protein strength” (Finney, 1989).

STICKINESS

Stickiness of experimental wheat samples (1995-1996 and 1996–1997) was determined using the Schwarzlaff-Shepherd Dough Stripping Technique. Peel times were obtained in seconds, with a high peeling time indicating a sticky sample. The results were pooled together to determine whether significant differences existed due to location. Statistical analysis indicated that stickiness amongst samples was significantly different ($p<0.05$) by location (Blacksburg versus Warsaw), crop year and sample type. Due to the significant effects of location, samples were then analyzed separately by location. Tables 5.12 and 5.13 show the stickiness values for 1995-1996 and 1996-1997 crop years, respectively. The differences in stickiness between samples at the two locations can be seen in Figure 5.14. A significant ($p = 0.04$) correlation ($r = 0.24$) was found between dough stickiness and AWRC.

Table 5.14 indicates that differences between 1B/1R and non-1B/1R samples for stickiness were significant for both crop years in Blacksburg ($p < 0.05$). The samples from Warsaw only varied significantly in stickiness during the 1996-1997 crop year ($p < 0.05$). Thus the fourth null hypothesis is accepted and it can be concluded that the degree of stickiness amongst SRWW is not influenced by the 1B/1R translocation alone , but rather the environment exerts an influence.

Contrasts were used to compare sister lines. The results indicated that amongst pair 1 sister lines significant differences existed between non-1B/1R and IB/1R samples. For example, the 1995-1996 Blacksburg samples VA54-209 and its 1B/1R sister line (VA54-211) were significantly different at ($p<0.05$). Table 5.12 indicates VA54-209 was less sticky than VA54-211, its 1B/1R counterpart. The check samples were not different from each other in their

Table 5.12. Stickiness values (in seconds) for 1995-1996 SRWW samples

<u>Sample</u>	<u>LOCATION</u>	
	<u>Blacksburg 1</u>	<u>Warsaw 1</u>
<u>Pair 1</u>		
VA54-209	21c-e ² ± 4.3	30bc ± 6
VA54-211+	50a ± 10	40a ± 6
<u>Pair 2</u>		
VA54-21	18c-f ± 4.0	20de ± 4.2
VA54-18+	17d-f ± 4.4	20c-e ± 8
VA54-19+	15ef ± 2.3	20c-e ± 7
VA54-258+	21c-e ± 3.9	19e ± 4.1
VA54-418+	25b-d ± 4.0	26b-e ± 4.0
VA54-185+	19c-f ± 3.5	30b-d ± 5
VA52-22+	12f ± 3.7	21de ± 2.3
<u>Checks</u>		
Massey	25bc ± 4.2	30ab ± 5
FFR555W	30b ± 6	26b-e ± 4.3
Saluda	20c-e ± 8	26b-e ± 3.4

1 n = 9; the higher the peel time, the stickier the dough sample

2 Means with the same letter in the same column are not significantly different at $p < 0.05$

+ Variety with the 1B/1R translocation

Table 5.13. Stickiness values (in seconds) for 1996-1997 SRWW samples

<u>Sample</u>	<u>LOCATION</u>	
	<u>Blacksburg 1</u>	<u>Warsaw 1</u>
<u>Pair 1</u>		
VA54-209	30d 2 ± 10	90ab ± 20
VA54-211+	30d ± 10	40e ± 20
<u>Pair 2</u>		
VA54-21	32d ± 4	50de ± 10
VA54-18+	90a ± 20	90b ± 20
VA54-19+	Not available	80b-d ± 10
<u>Pair 3</u>		
VA52-67	40d ± 10	80bc ± 30
VA54-241+	Not available	50c-e ± 10
VA54-258+	50cd ± 10	80e ± 30
VA54-418+	60bc ± 20	80b-d ± 20
VA54-185+	70b ± 10	120a ± 30
VA52-22+	40d ± 10	80b-d ± 20
VA52-55+	30d ± 10	90ab ± 20
<u>Checks</u>		
Massey	40d ± 10	40e ± 10
FFR555W	30d ± 10	40e ± 10
Saluda	40cd ± 10	40e ± 10

1 n = 9; the higher the peel time, the stickier the dough sample

2 Means with the same letter in the same column are not significantly different at p<0.05

+ Variety with the 1B/1R translocation

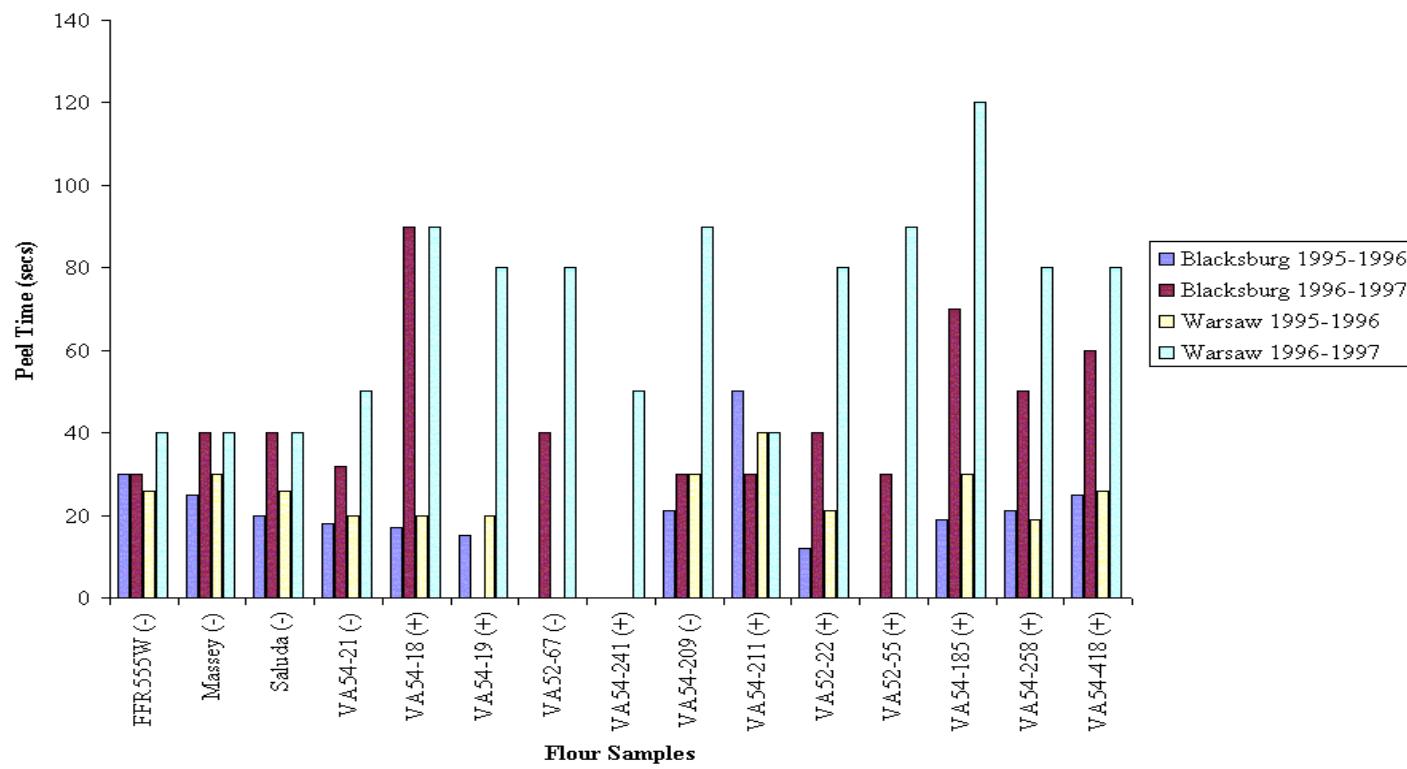


Figure 5.14. Stickiness amongst SRWW dough sample

Table 5.14. Contrasts indicating degree of significance in dough stickiness for SRWW flour samples

<u>Comparisons</u>	<u>Blacksburg (P value)</u>		<u>Warsaw (P value)</u>	
	1995-1996	1996-1997	1995-1996	1996-1997
1B/1R vs non-1B/1R ¹	0.0133*	0.0469*	0.9826	0.0001*
VA54-21 vs VA54-18 & 19 ²	0.0522•	0.6458	0.6027	0.3094
VA54-209 vs VA54-211 ³	0.0006*	0.0001*	0.0001*	0.3547

¹Non-1B/1R samples were FFR555W, Saluda, Massey, VA54-21 and VA54-209

1B/1R samples were VA54-18, VA54-19, VA54-211, VA52-22, VA54-185, VA54-285 and VA54-418.

*Significantly different at $p<0.05$

•degree of difference not quite significant enough

²Pair 2 (sister lines)

³Pair 1 (sister lines)

respective locations. There were no differences in the non-1B/1R samples (VA54-209 and VA54-21) grown in Blacksburg. Peel time results indicated that for 1996 – 1997, VA54-211 (1B/1R) from Blacksburg had similar peel time as its non-1B/1R counterpart (VA54-209). However, for Warsaw (1996-1997), the non-1B/1R sample VA54-209 was stickier than its 1B/1R sister line (VA54-211). Overall, the 1996-1997 peel time values were higher and therefore samples were stickier than those of 1995–1996. Contrasts for pair 2 sister lines, indicated stickiness was not significantly different amongst the non-1B/1R (VA54-21) and 1B/1R samples (VA54-18 and VA54-19). This was true for both Warsaw and Blackburg (Table 5.14).

Schwarzlaff (1995) reported that amongst the checks (FFR555W, Massey and Saluda), Saluda gave a significantly more sticky dough than FFR555W and Massey at both locations. The data from this study indicates the checks were not significantly different from one another at their respective locations. Only crop year exerted differences in the peel time values. Schwarzlaff (1995) also reported VA54-209 was significantly more sticky than VA54-211. The results from this study indicated Blacksburg and Warsaw 1995-1996 stickiness was highest in the 1B/1R sample (VA54-211) instead of VA54-209. However, the Warsaw sample (VA54-209) from 1996-1997 crop year gave a similar results to that reported by Schwarzlaff (1995).

RECONSTITUTION STUDIES

The four samples used in this part of the study were chosen based on their stickiness values. It was decided that four samples two having the 1B/1R and two without the 1B/1R translocation would be used, one sample in each case exhibiting sticky and non-sticky properties. Thus four samples (VA54-211- Blacksburg, Massey - Warsaw, VA52-22 - Blacksburg and VA54-21 - Blacksburg) were used. The samples were reconstituted according to protocols in Table 4.3. The combinations used, were chosen in an attempt to determine whether the sticky dough factor resided in the IB/1R and non-1B/1R water-soluble fractions.

The results indicated that when the WS fraction of Massey flour was mixed with 1B/1R non-sticky gluten-starch fractions, the resulting stickiness was higher (79 seconds) than the original value for pure unfractionated Massey (30 seconds) flour (Table 5.15). When the original fractions from Massey (1995-1996) were reconstituted together, the dough gave a peel time of 140 seconds (Table 5.15). The reconstituted Massey sample was stickier than the original unfractionated sample which had a peel time of 30 seconds. However, the Massey sample from

Table 5.15. Stickiness measurements in reconstituted 1995-1996 SRWW flour samples

Base Flour¹	WS Fraction	Interchanged²	Peel time (seconds)	
			Original³	Reconstituted⁴
Massey (S -) ⁵	VA52-22	79 ± 3.8	30	140 ± 40.1
VA54-211 (S +) ⁶	VA54-21	34 ± 2	50	130 ± 10
VA54-21 (NS -) ⁷	VA54-211	8 ± 1.5	18	60 ± 10
VA52-22 (NS +) ⁸	Massey	45 ± 2.5	12	40 ± 10

¹Composed of gluten and starch fractions

²Peel times are from flours with water soluble fraction interchanged

³The peel time indicated is from the unfractionated flour sample

⁴Peel times are from flours combined with their original fractions

⁵S - = sticky non-1B/1R samples

⁶S + = sticky 1B/1R sample

⁷NS - = non-sticky no-1B/1R sample

⁸NS + = non-sticky 1B/1R sample

Table 5.16. Stickiness measurements in reconstituted 1996-1997 SRWW flour samples

Base Flour ¹	WS Fraction	Interchanged ²	Peel time (seconds)	
			Original ³	Reconstituted ⁴
Massey (S -) ⁵	VA52-22	100 ± 20.0	40	33 ± 2.5
VA54-211 (S +) ⁶	VA54-21	46 ± 4.2	30	70 ± 10.9
VA54-21 (NS -) ⁷	VA54-211	28 ± 3.8	32	55 ± 10.9
VA52-22 (NS +) ⁸	Massey	37 ± 2.1	40	13 ± 0.6

¹Composed of gluten and starch fractions

²Peel times are from flours with water soluble fraction interchanged

³The peel time indicated is from the unfractionated flour sample

⁴Peel times are from flours combined with their original fractions

⁵S - = sticky non-1B/1R samples

⁶S + = sticky 1B/1R sample

⁷NS - = non-sticky non-1B/1R sample

⁸NS + = non-sticky 1B/1R sample

1996 – 1997 gave similar peel time scores for both the original and reconstituted samples. Values were 40 and 33 seconds, respectively (Table 5.16). When the 1995-1996 Massey base flour was combined with the water-soluble fraction from VA52-22 (1995-1996), the resulting dough was stickier than the original Massey dough sample. The source of the water-soluble fraction (VA52-22), had a low stickiness value of 12 seconds (Table 5.15). A similar trend was observed in the 1996-1997 Massey interchanged dough sample (Table 5.16). Thus Massey flour apparently contains something that imparts stickiness to dough; and stickiness per se is not confined to the WS fraction.

The replacement of the water soluble fraction of VA54-211 (1995-1996) with the WS fraction of VA54-21 (1995-1996), reduced the peel time of VA54-211 from 50 seconds to 34 seconds (Table 5.15). However, in the interchanged sample for 1996-1997, VA54-211 peel time (46 seconds) was found to be close to the original time of 30 seconds (Table 5.16). Thus it may be due to the lack of 1B/1R in the VA54-21, which was a non-sticky flour, that stickiness in the interchanged sample is minimized or at least not exaggerated. However, the peel time of the reconstituted VA54-211 (1995-1996) was higher than the original unfractionated flour and the interchanged flour (Table 5.15). The increase in peel time in reconstituted dough samples was also observed in the two other 1995-1996 samples (VA54-21 and VA52-22), as well as in VA54-21 (1996-1997). Only Massey (1996-1997) resulted in a less sticky reconstituted dough sample than the values obtained from the original unfractionated dough sample. The peel time was 13 seconds in the reconstituted sample, compared to 40 seconds in the original (Table 5.16).

The non-sticky, non-1B/1R sample (VA54-21), originally had a peel time of 18 seconds in its unfractionated form. This value decreased to 8 seconds in the interchanged sample with the addition of the water soluble fraction from VA54-211 (Table 5.15). This was unexpected, as VA54-211 when unfractionated had the longest peel time and thus was the stickiest. The same phenomenon was observed in the 1996-1997 VA54-21 interchanged sample (Table 5.16). The reason why the Massey variety was drastically affected by the removal of its water solubles and reconstitution with the water solubles of another flour (VA52-22) may be related to the fact that Massey was a pure wheat sample without any rye genetic material in its makeup. Thus the mixing of Massey with a water-soluble fraction from a wheat sample having some rye genetic material may have drastically affected the natural behavior of the Massey flour. Thus the results obtained generally show that the stickiness factor cannot be attributed to the water soluble fraction or to the 1B/1R.

In the VA52-22 (non-sticky 1B/1R), the peel time with the water-soluble fraction from Massey was higher than in the original unfractionated flour. The interchanged sample VA52-22 from 1996 – 1997 was not different from its original peel time value of 40 seconds. Yield of various flour fractions (Table 5.17) from 1995-1996 and 1996 – 1997 were similar to those reported by Dhaliwal and MacCritchie (1990).

TENSILE MEASUREMENTS OF SELECTED 1995-1996 SRWW FLOUR

The strain placed on a material by an applied stress is defined as elongation of that material as a percentage of its original length. Stress is defined as force per unit area. Dough stress and strain were significantly different ($p<0.05$) among the four samples (Figures 5.15 and 5.16) when tested using an Instron. The significant differences in strain values indicate the elastic properties of the flour samples were influenced by the type of flour used. The level of water used in forming the dough in the farinograph had a significant effect on dough strength ($p = 0.0001$) but not upon dough strain ($p = 0.4589$).

Upon further analysis by using contrasts, it was discovered that stress was not significantly affected by whether the sample had the 1B/1R translocation or not. The p -value was 0.7057 (Table 5.18). When stress was compared by looking at samples that had higher versus shorter peel times, significance was not present ($p = 0.5349$). The only significance seen was between VA54-211 and VA52-22 ($p=0.0187$). VA54-211 and VA52-22 were compared because both have the 1B/1R translocation but VA52-22 is less sticky than VA54-211. The stress values were 6.39 psi (VA54-211) and 5.71 psi (VA52-22).

The 1B/1R samples (VA54-211 and VA52-22) had significantly lower strain values than non-1B/1R samples (Massey and VA54-21). The p -value was 0.0048 (Table 5.18). When the 1B/1R samples (VA54-211 and VA52-22) were compared, they were also found to be significantly different from each other ($p = 0.0071$). The sample that exhibited stickiness (VA54-211) had the lowest strain value (57.64%), while the variety (VA52-22) which was not sticky had the highest strain value (59.43%). This possibly indicates that if a sample is sticky it will not exhibit elastic properties as easily, and thus will have a lower strain value. Such a conclusion was confirmed by analysis of the non-1B/1R samples (Massey and VA54-21) which failed to exhibit significant difference in dough strain (Table 5.18). Further, it seems that if a sample is sticky, regardless of the presence or absence of the translocation, that it had lower strain values. This effect was not however significant ($p = 0.0858$).

Table 5.17. Recovery of fractions from selected SRWW flour samples

Fraction (%)	Massey		VA54-211		VA54-21		VA52-22		Reference ¹
	<u>Year 1</u> ²	<u>Year 2</u>	<u>Year 1</u>	<u>Year 2</u>	<u>Year 1</u>	<u>Year 2</u>	<u>Year 1</u>	<u>Year 2</u>	
Gluten	16.98	7.55	13.54	5.7	13.69	3.675	13.24	10.585	14.2
Starch	79.04	70.23	80.74	74.53	80.85	69.33	82.51	68.76	75.9
Water-solubles	3.97	3.50	5.72	4.30	5.46	4.1	4.25	5.53	5.2

¹Comparison of fraction recovery from Cook wheat sample (Dhaliwal and MacRichie, 1990)

²Year 1 refers to 1995-1996, while year 2 refers to 1996 – 1997 crop years, respectively.

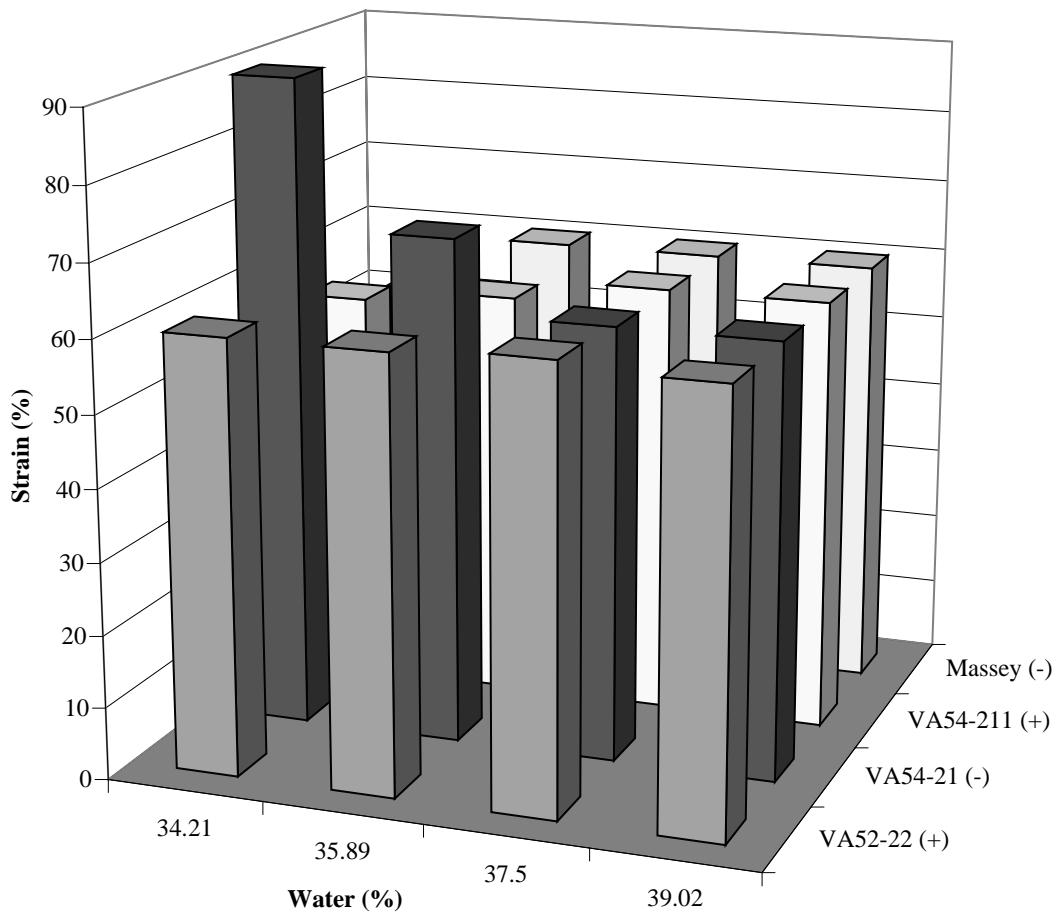


Figure 5.15. Effect of flour type on dough strain

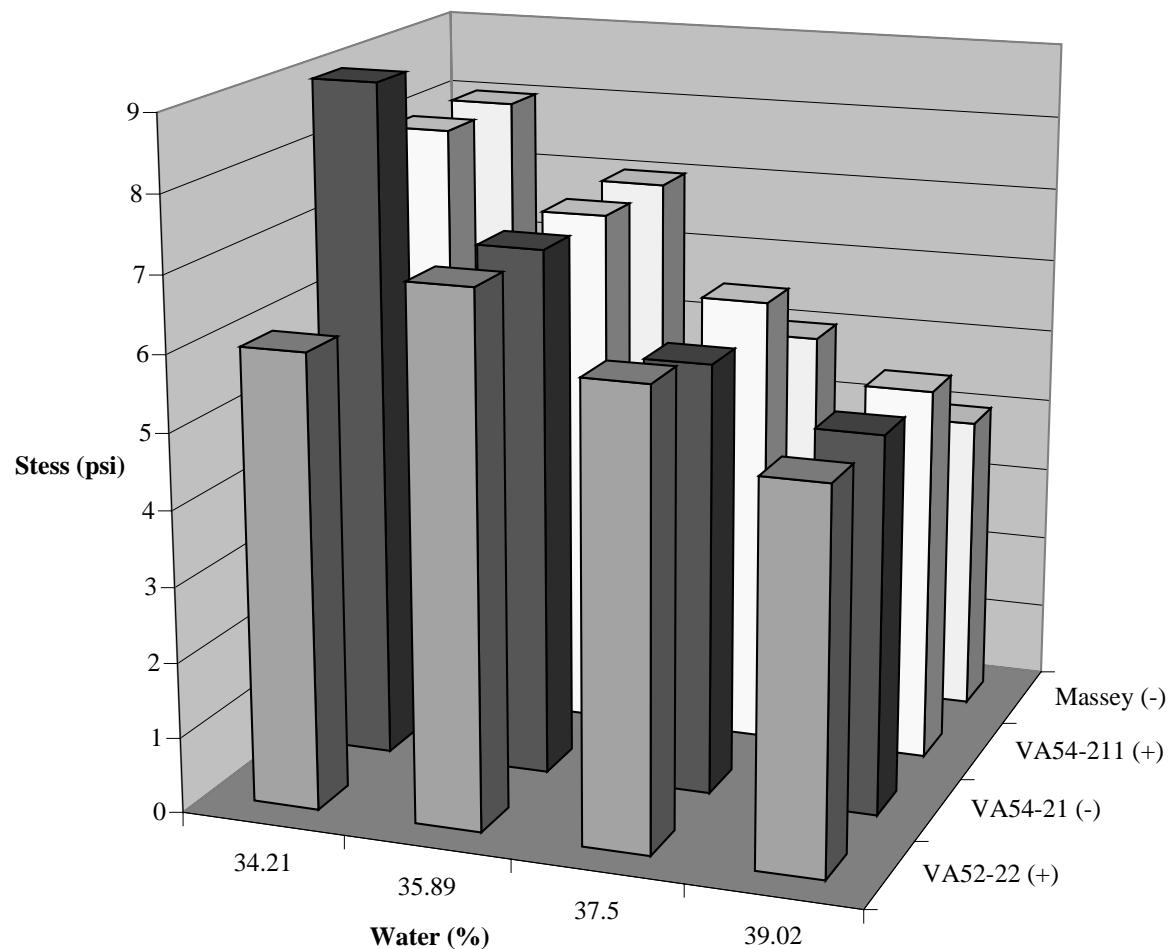


Figure 5.16. Effect of flour type and water on dough stress

Table 5.18. Contrasts output on tensile measurements of selected 1995-1996 dough samples

VARIABLE	DF¹	P-value
Stress		
Sample	3	0.0489* ²
Water	3	0.0001*
Sample x Water	9	0.0084*
Contrasts:		
1B/1R vs non-1B/1R	1	0.7057ns ³
Sticky vs non-sticky	1	0.5349ns
VA54-211 vs VA52-22	1	0.0187*
Massey vs VA54-21	1	0.1327ns
Strain		
Sample	3	0.0020*
Water	3	0.4589ns
Sample x Water	9	0.0056*
Contrasts:		
1B/1R vs non-1B/1R	1	0.0048*
Sticky vs non-sticky	1	0.0858ns
VA54-211 vs VA52-22	1	0.0071*
Massey vs VA54-21	1	0.7621ns

¹ DF = Degree of freedom

² Value is significant at $p < 0.05$

³ ns = not significant at $p < 0.05$

As the water level increased from 34.21% to 39.02%, the dough stress decreased. The dough was changing into more of a viscous mass, rather than a viscoelastic one. This difference was seen in the 1B/1R samples VA54-211 and VA52-22 where the sticky sample had higher strength values (6.39 psi) unlike that of VA52-22 (5.71 psi). When pooling the strain values from the four samples by the different water levels, it was found that there were no significant effects on strain as the water level increased. However, when the numbers were examined, one could see a trend in that at lower water levels, the dough exhibited higher strain values. This means it was more elastic and thus susceptible to deformation. However when the water level increased towards 39.02%, the strain values decreased to 57.74%. The dough was becoming viscous.

Another type of observation made on the dough samples was the type of failure. Cohesive strength is defined as failure within a material, in this case the dough itself. Adhesive failure is defined as failure at the interface of two materials, in this case the dough and the stainless steel button. It was discovered that with increased water content the type of failure became cohesive. The non-sticky samples (VA52-22 and VA54-21) exhibited cohesive failure at all four moisture levels. However the sticky samples (Massey and VA 54-211) showed adhesive failure at low moisture levels which quickly changed to cohesive failure when water level increased from 35.89% to 37.5%. As the moisture content increases the dough gradually begins to bond with the button. Eventually at higher moisture content, the dough bonds to the stainless steel very well but the dough will not hold together.

Navickis et al. (1982) working with wheat flour doughs measured the storage (G'), loss (G'') and shear modulus (G^*) as affected by different levels of water. They found that the storage modulus which is a dynamic measure of elasticity, was sensitive to the water content.

NMR ANALYSIS

Table 5.19 shows the chemical shift values for starch, gluten and water-soluble fractions of Massey using CP-MAS. To evaluate the effect of water on the dynamics of the hydrated flour, proton relaxation time ($T_{1p}[H]$) measurements were determined. Table 5.20 shows the chemical shifts and corresponding $T_{1p}[H]$ of experimental wheat samples. It can be observed that each starch fraction had similar relaxation times (Table 5.20). Thus it seems that the 1B/1R translocation does not affect $T_{1p}[H]$. The values obtained were lower than those reported by Garbow and Schaefer (1991ab). Table 5.20 shows the three SRWW dough samples (Massey, V54-211 and VA52-22) exhibited a fairly uniform relaxation time in the NMR rotating frame.

Table 5.19. ^{13}C chemical shift ranges for starch, gluten, and water soluble fractions of a Massey soft red winter wheat flour

<u>Starch^a</u>	<u>Gluten^b</u>	<u>Water-solubles^c</u>	<u>Flour</u>	<u>Assignments to chemical sites</u>
		15.883		-CH ₃ of water solubles (Baianu and Forster, 1980; Garbow and Schaefer, 1991)
	25.032	25.236		Aliphatic carbon of gluten and water solubles (Garbow and Schaefer, 1991)
	27.726			Aliphatic carbon of gluten
31.518	30.191	30.072	30.412	Aliphatic carbon of starch, gluten and water solubles
	55.288 ^d			
	59.495 ^d			
62.058		63.256	61.787	Carbon 6 of starch (Veregin et al., 1986)
72.546	72.163	73.954	72.323	Sugar ring carbons in amylose; C2-C5 (Garbow and Schaefer, 1991 and Li et al., 1996)
			81.619	Morgan et al., 1982 reported this peak as well
100.810	101.551	103.988	101.295	Sugar ring carbons in amylose (Baianu and Forster, 1980)
	128.389		128.825	Protein side chain aromatic carbon (Garbow and Schaefer, 1991)
129.885	129.939			Protein side chain aromatic carbon
	136.188			Phe , Trp (Baianu and Forster, 1980)
140.049				
		157.171		Tyr , Arg (Baianu and Forster, 1980)
172.061	173.303	174.197	173.444	Protein main-chain peptide carbonyl (Baianu and Forster, 1980; Garbow and Schaefer, 1991)

abc Spectra are in figures 5.17 to 5.19

^dAlso reported by Baianu and Forster, 1980

Table 5.20. Chemical shifts and proton relaxation times of dough samples¹

Chemical shift [δ] (ppm)	T _{1ρ} [H] (ms)			
	<u>Massey</u> ²	<u>VA54-211</u> ³	<u>VA54-21</u> ²	<u>VA52-22</u> ³
62 (starch)	6.799	6.865	9.963	6.866
73 (starch)	6.279	6.083	5.802	6.049
101 (starch)	6.714	6.444	6.550	6.502
175 (protein) ⁴				

¹ Dough samples made with optimum water absorption and mixed for 3 minutes

² Non-1B/1R

³ 1B/1R

⁴ Protein values could not be determined

The non-1B/1R samples (VA54-21) had higher relaxation time of 9.963 ms at 62 ppm, compared to the other samples which had values ranging from 6.799 to 6.866 ppm (Table 5.20). Garbow and Schaeffer (1991a), found $T_{1\rho}[H]$ values of 13.1 ms, 11.2 ms and 12.3 ms for chemical shifts at 62 ppm, 73 ppm and 101 ppm in soft wheat flour, respectively. Similar to Garbow and Schaeffer (1991ab), we were unable to determine the $T_{1\rho}[H]$ for the protein peak at 175 ppm. Weegel et al (1995) states that a fast relaxation time may indicate the presence of small structures in the dough. Soft wheat flour has smaller starch-protein particles which are more flexible (Garbow and Schaeffer, 1991a). Therefore the SRWW may have smaller starch-protein particles than those found in the soft wheat flour used by Garbow and Schaeffer (1991a). It will be necessary to use a wide variety of flour samples to confirm this claim.

The CP-MAS spectra of gluten, starch and WS fraction are indicated in figures 5.17 to 5.19. The gluten spectra (Figure 5.17), of the protein carbonyl at 173 ppm can be seen distinctly. However in the spectra from dough samples, this peak was not distinguishable. The presence of other peaks in the gluten spectra that seem to be similar to those of starch may arise due to the presence of starch in the gluten fraction left from the fractionation procedure. Some of these peaks are assigned to specific molecular structures of gluten according to a report by Morgan et al . (1992). The gluten spectra is similar to that reported by Li et al., (1996).

According to Garbow and Schaeffer (1991a), the chemical shifts occurring at 130 ppm are caused by protein side-chain aromatic carbons and those at 20 to 35 ppm are from protein side-chain aliphatic carbons. The starch spectrum (Figure 5.18) shows peaks occurring at 101 and 62 ppm which are due to carbon 1 (C1) and C6 of the (1,6) linkages, respectively (Morgan et al., 1992). The shape of this spectrum was clearly visible in the CP-MAS spectra of the four dough samples (Figures 5.20 to 5.23).

The CP-MAS ^{13}C NMR spectra of the water-soluble fraction has not been previously reported in literature. The spectra indicates the presence of aliphatic carbons, sugar ring carbons and protein main-chain peptide carbonyls at 30 ppm, 75 ppm to 100 ppm and 175 ppm, respectively. For more detailed chemical shift assignments see Table 5.19.

Dough samples (Massey, VA54-211, VA54-21, VA52-22) shown in Figures 5.20 to 5.23, had spectra which were not easily distinguishable. However, there were some minor differences in the spectrum from VA54-21 from the other three dough samples (Figure 5.22). The

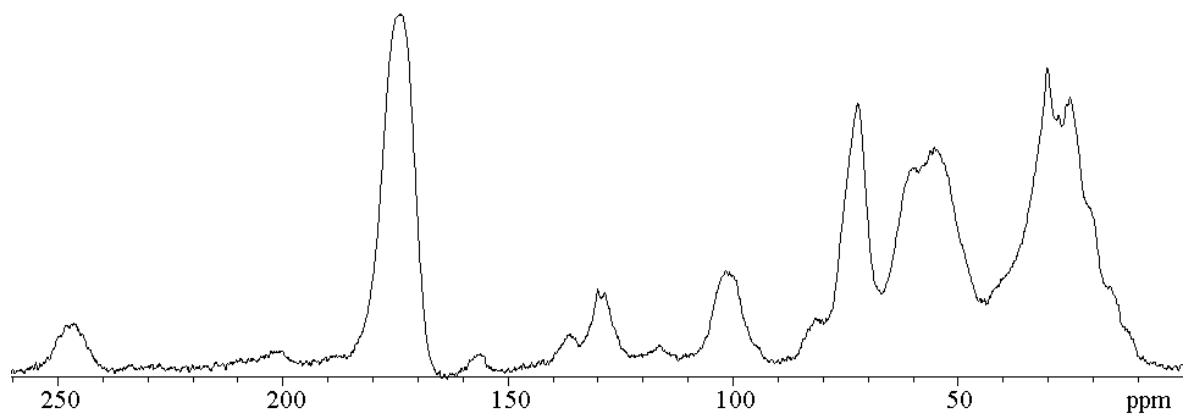


Figure 5.17. CP-MS ^{13}C NMR spectra of gluten fraction from Massey (Blacksburg)

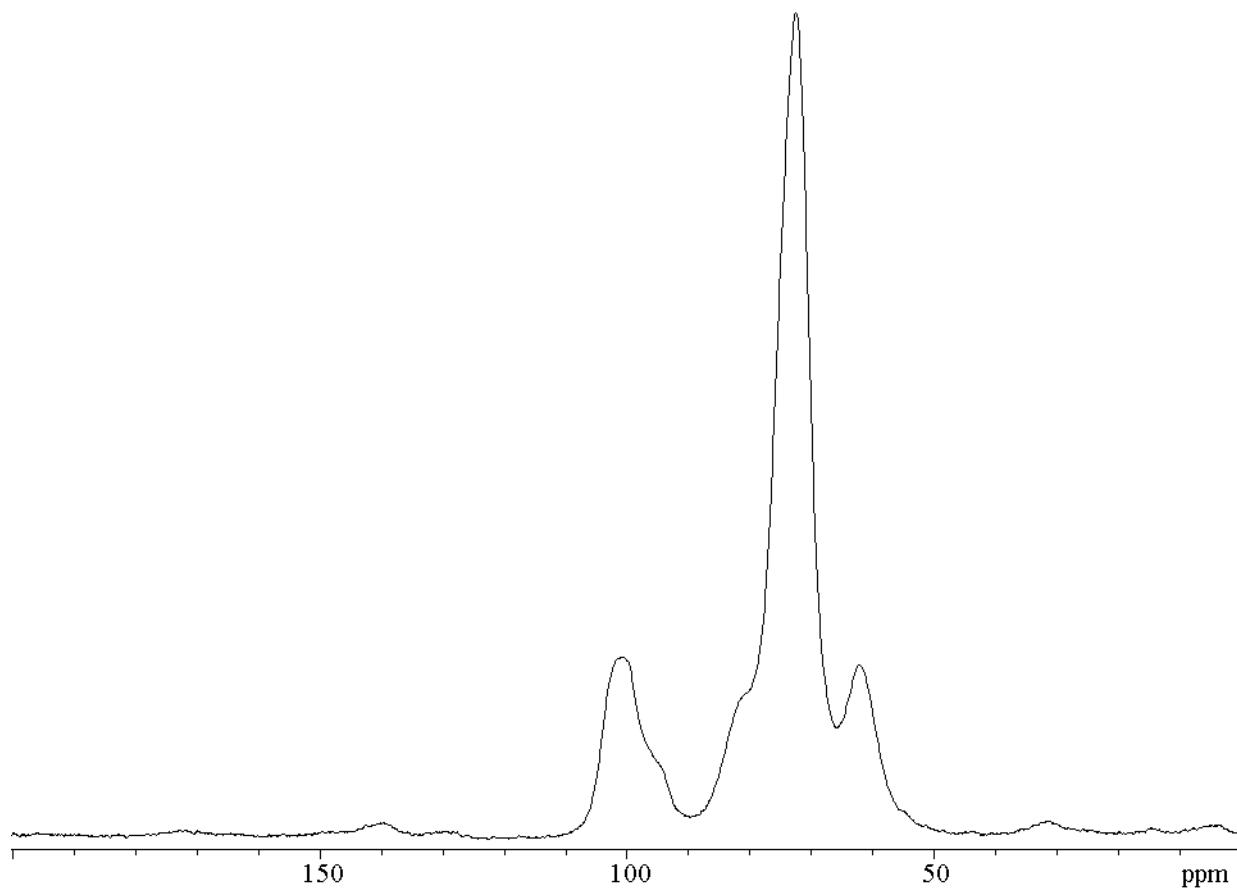


Figure 5.18. CP-MS ^{13}C NMR spectra of starch fraction from Massey (Blacksburg)

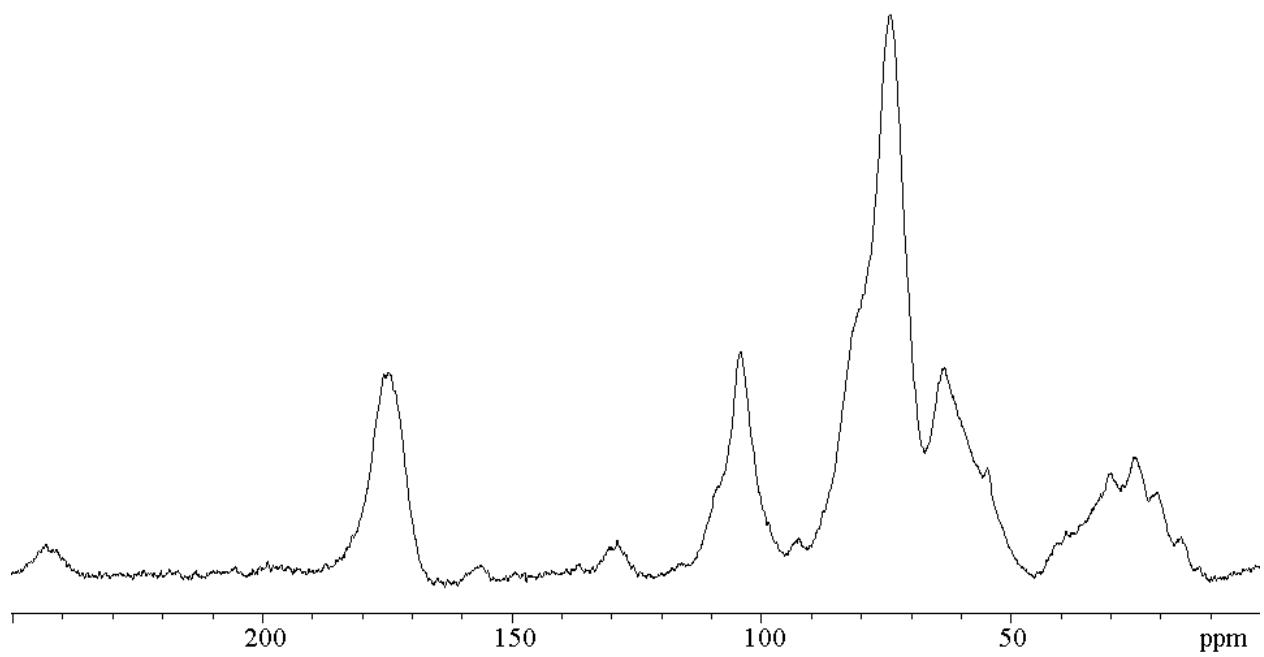


Figure 5.19. CP-MS ^{13}C NMR spectra of water-soluble fraction from Massey (Blacksburg)

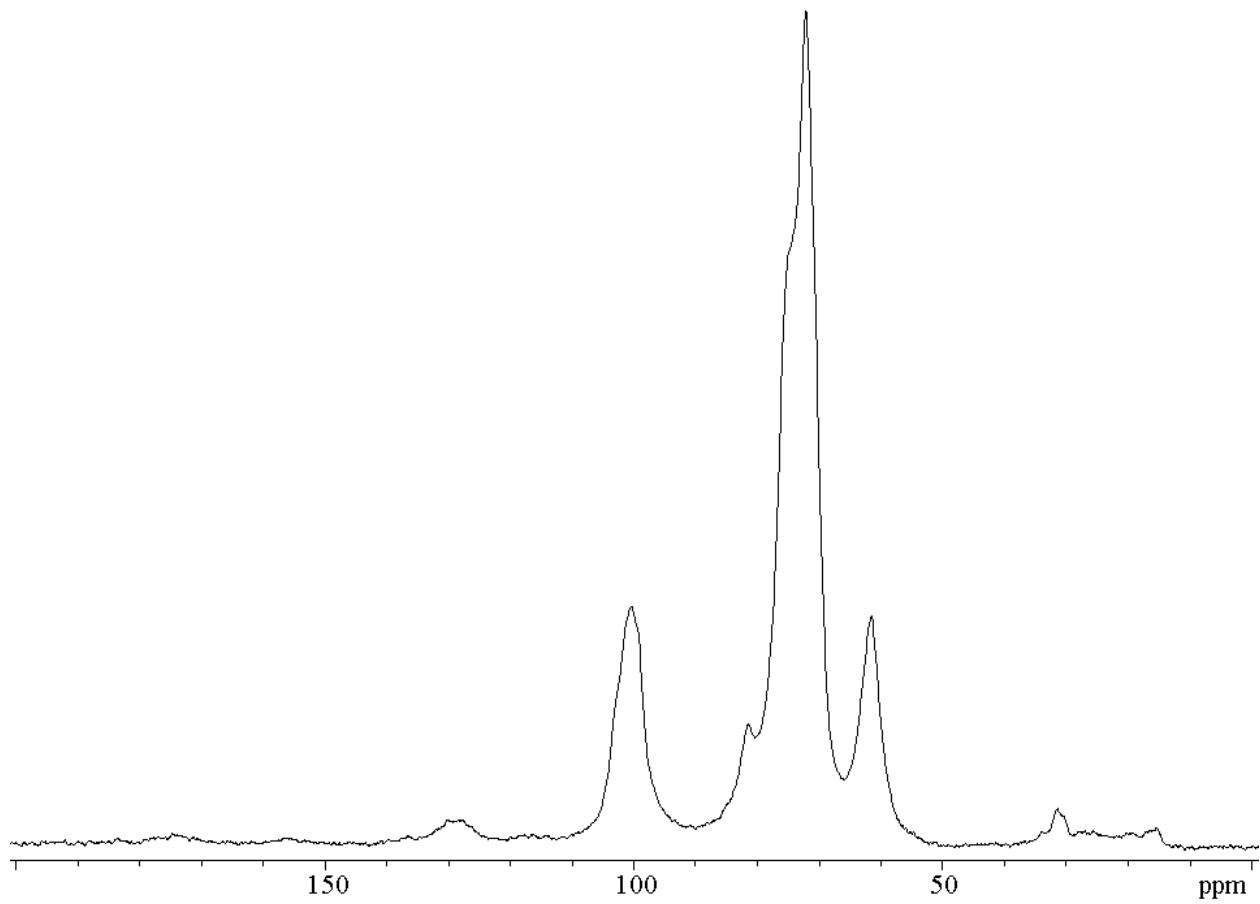


Figure 5.20. CP-MS ^{13}C NMR spectra of VA92-52-22 (Blacksburg)

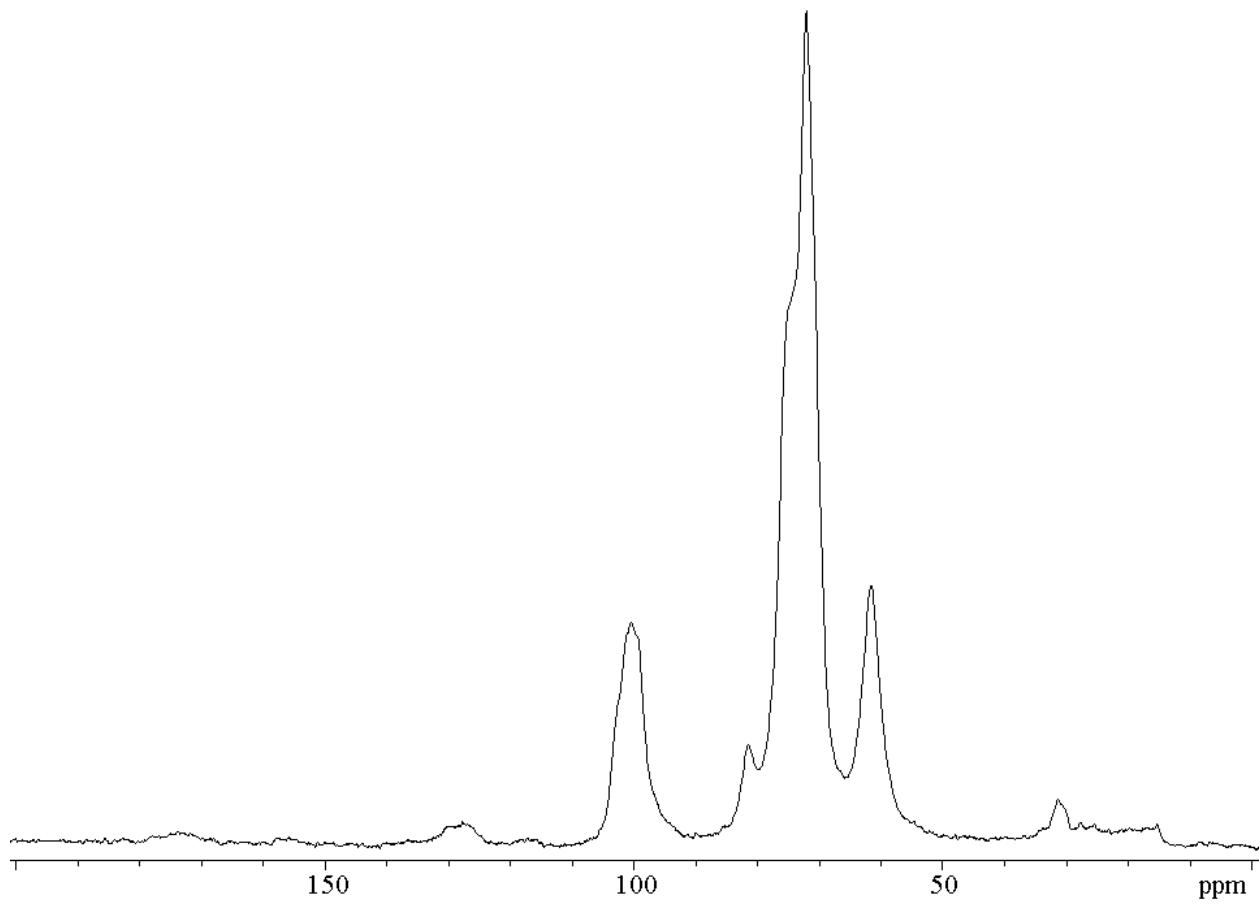


Figure 5.21. CP-MS ^{13}C NMR spectra of VA93-54-211 (Blacksburg)

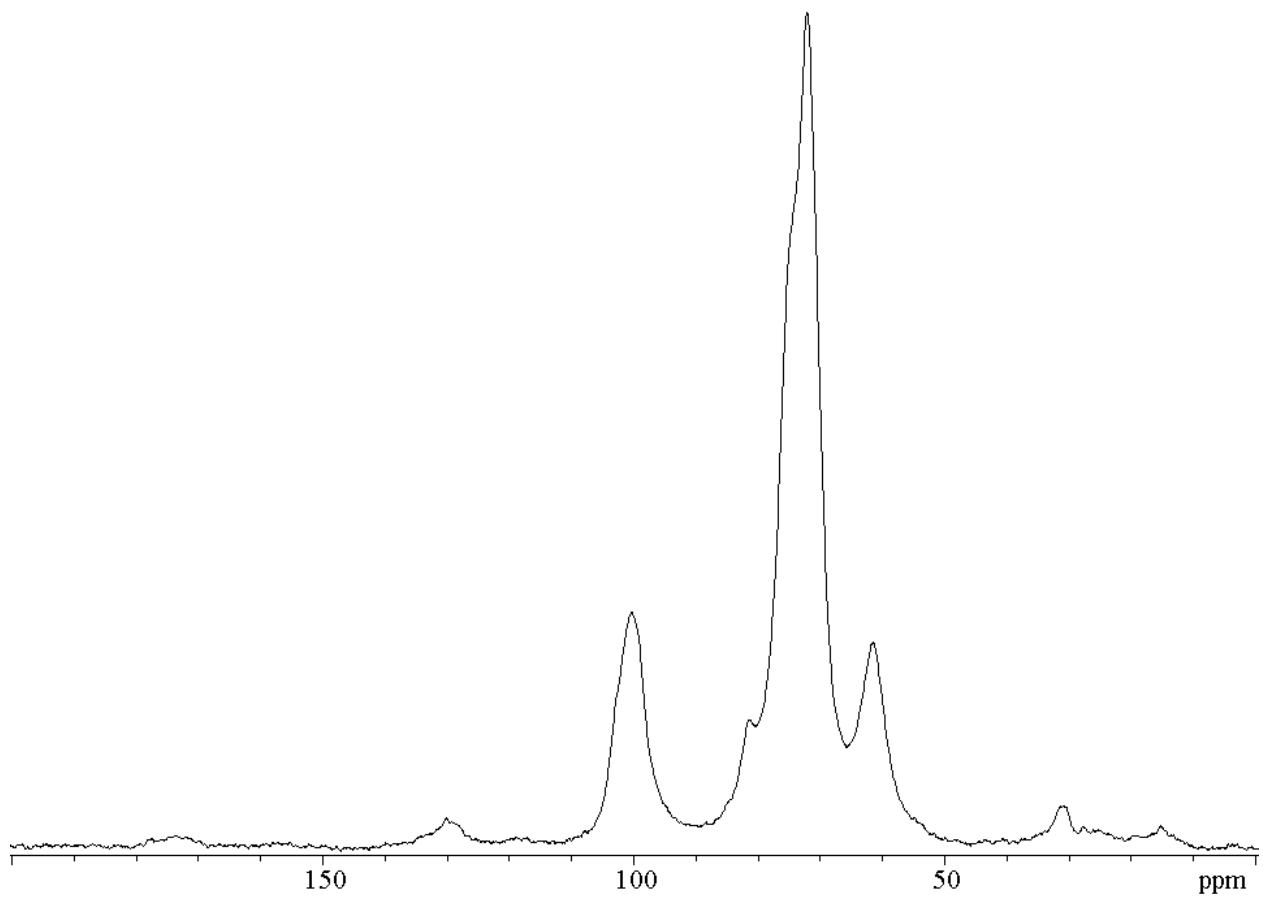


Figure 5.22. CP-MS ^{13}C NMR spectra of VA94-54-21 (Blacksburg)

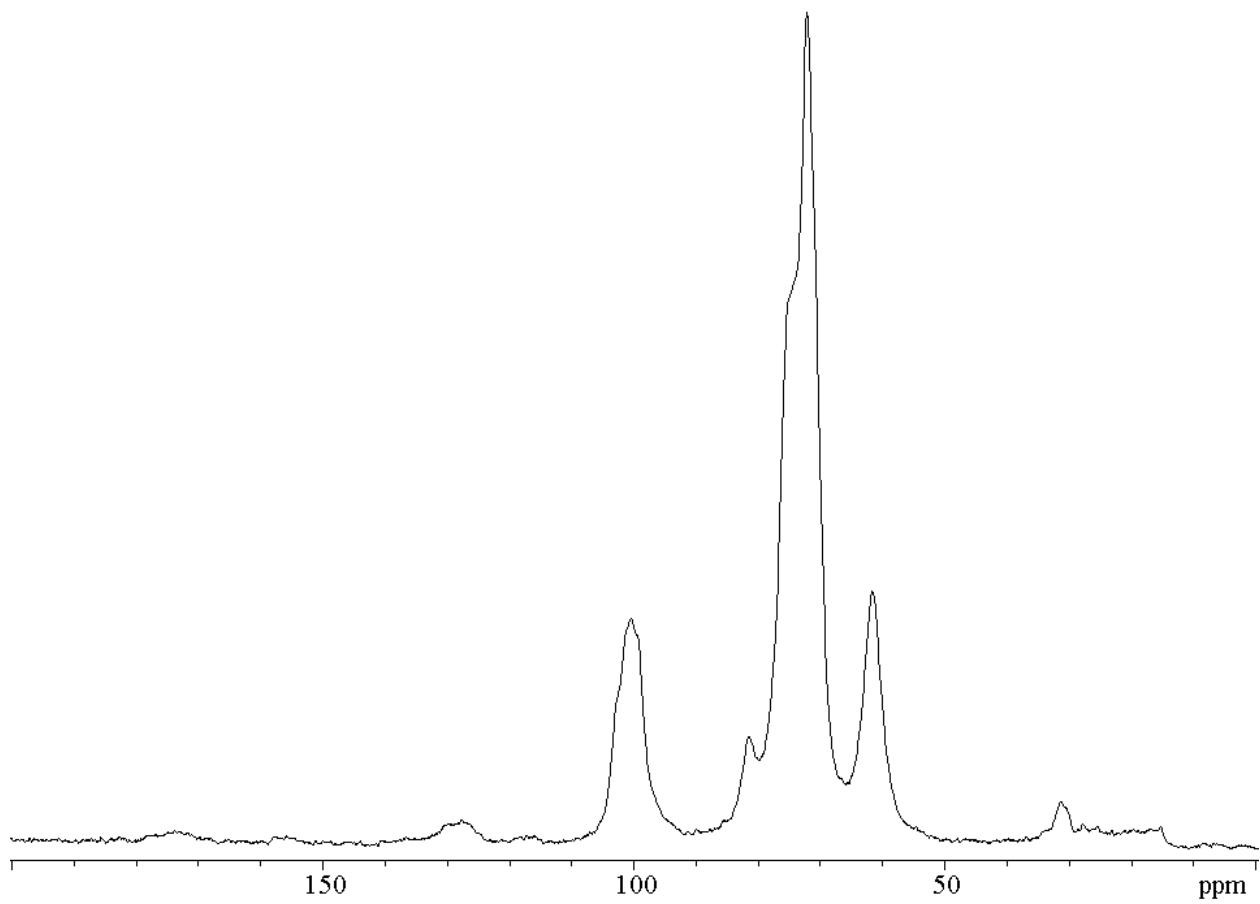


Figure 5.23. CP-MS ^{13}C NMR spectra of Massey (Blacksburg)

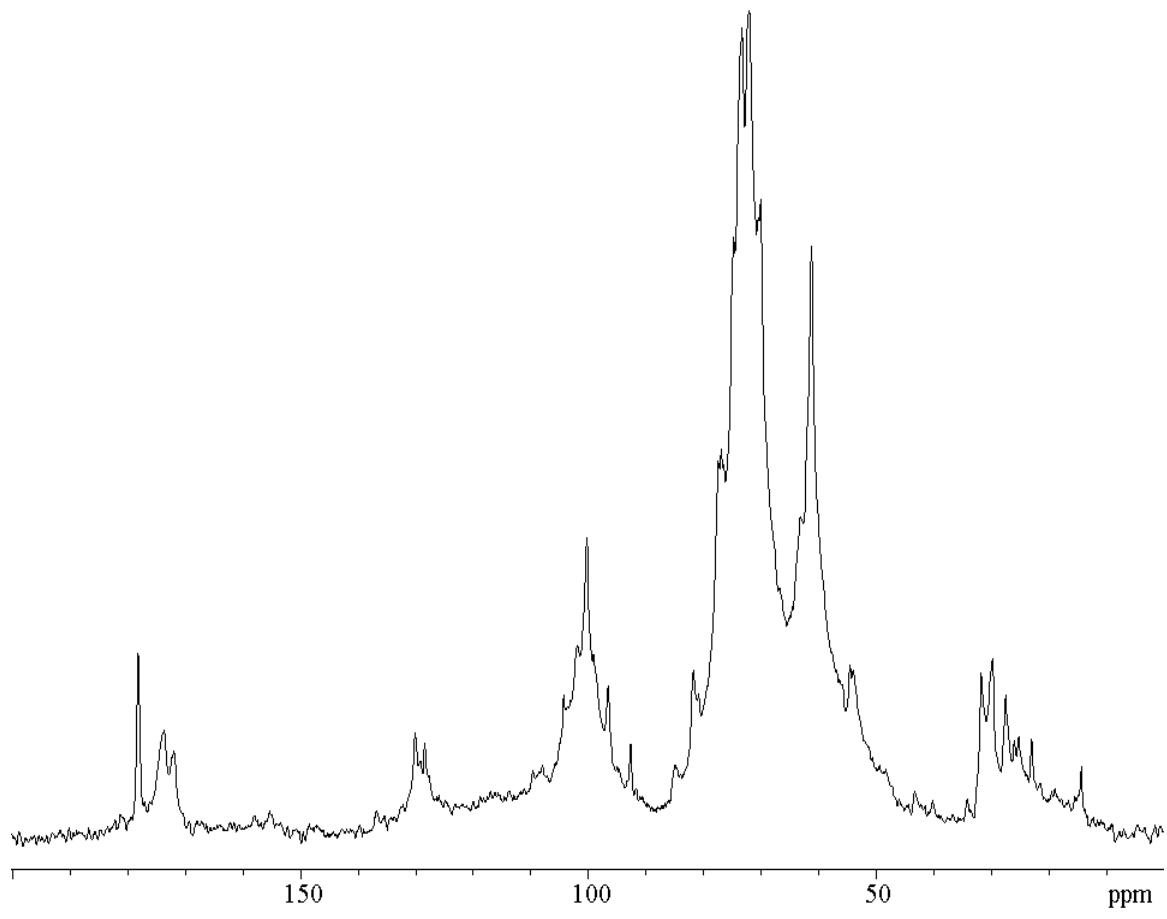


Figure 5.24. FT-MS ^{13}C NMR spectra of VA92-52-22 (Blacksburg)

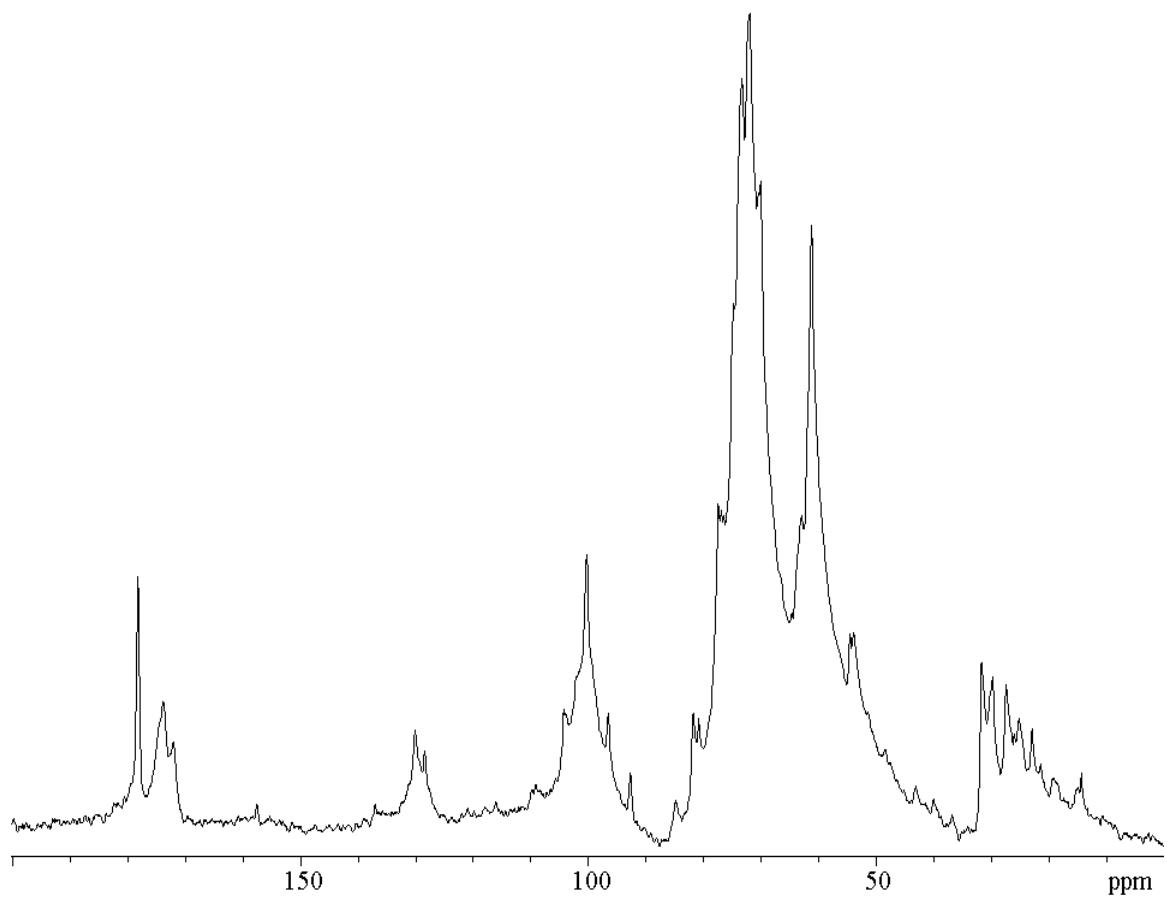


Figure 5.25. FT-MS ^{13}C NMR spectra of VA94-54-21 (Blacksburg)

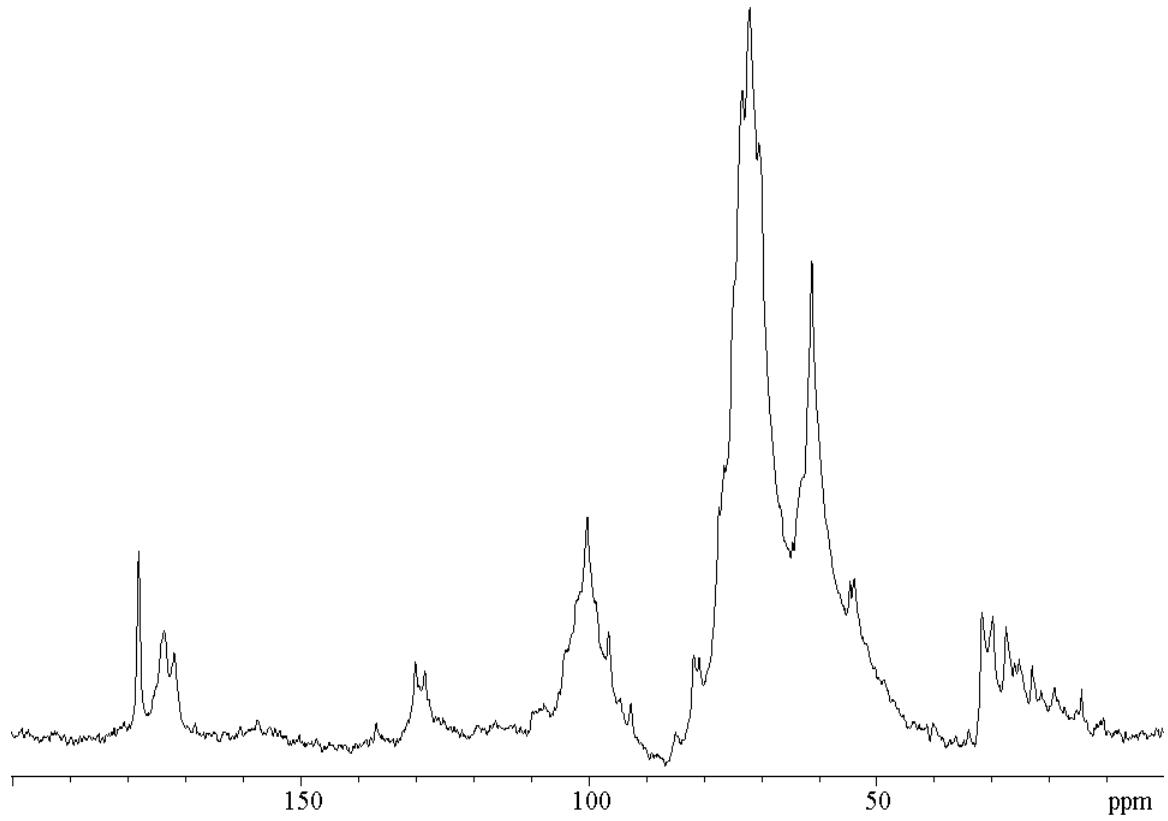


Figure 5.26. FT-MS ^{13}C NMR spectra of Massey (Blacksburg)

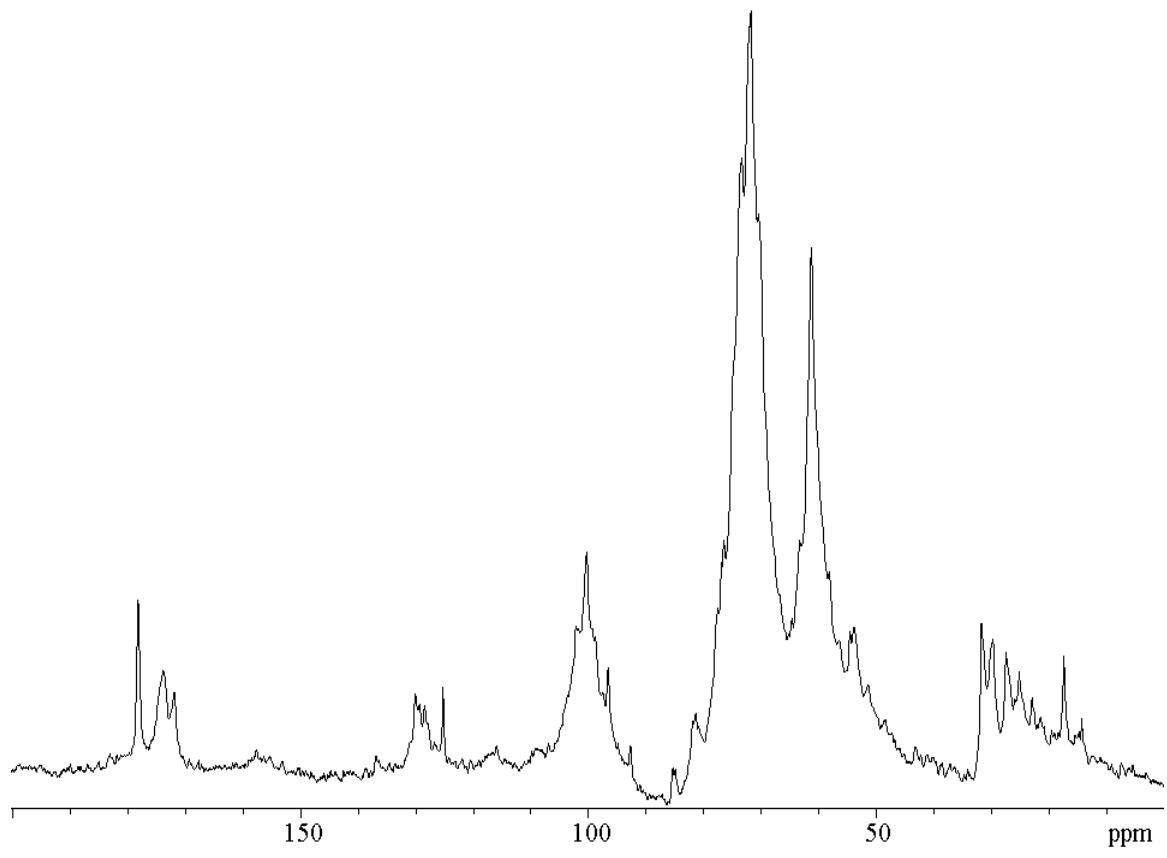


Figure 5.27. FT-MS ^{13}C NMR spectra of VA93-54-211 (Blacksburg)

differences in the CP-MAS spectrum of VA54-21 (non-1B/1R, non sticky) occurred at 130 ppm, 62 ppm and 35 to 15 ppm. This could possibly indicate differences in amino acid composition in gluten as at 136 ppm, as research has already allocated this chemical shift to phenylalanine and tryptophan (Table 5.19). These amino acids are hydrophobic and may affect the dough's rheological properties. This could also explain why VA54-21 had the highest relaxation time of 9.963 ms at 62 ppm. The other chemical shifts at 62 ppm and 35 to 15 ppm translate to differences in starch composition and possibly the position of aliphatic carbons in the dough structure of VA54-21.

Fourier transform MAS (FT-MAS) spectra of dough samples are indicated in figures 5.24 – 5.27. FT-MAS analyzes for small molecules solubilized by the addition of water. The spectra for the different dough samples were not very different. Thus the molecular composition of the SRWW flour used was similar , indicating 1B/1R does not significantly alter the flour's physical properties as measured by the NMR. In the FT-MAS spectra, line assignments were aliphatic carbons, oxygenated carbon, olefinic carbon and carboxylic carbon at 15 — 35 ppm, 60-100 ppm, 125-135 ppm and 170-185 ppm, respectively (Garbow and Schaeffer, 1991a). The FT-MAS spectra indicated the samples had a predominance of olefinic carbons.

CHAPTER 6

SUMMARY AND CONCLUSIONS

The aim of this study was to investigate the rheological properties and baking performance of 1B/1R translocated SRWW flours.

Data obtained revealed that stickiness amongst SRWW samples was significantly different ($p < 0.05$) by location (Blacksburg and Warsaw). Differences between 1B/1R and non-1B/1R samples were significant in Blacksburg samples ($p = 0.0133$) but not amongst Warsaw samples ($p = 0.9826$). Comparison of sister lines showed that pair 2 (VA54-18 and VA54-19 versus VA54-21) grown in Blacksburg were significantly different ($p = 0.0006$) unlike Warsaw ($p = 0.6027$). Pair 1 (VA54-209 versus VA54-211) in Blacksburg were significantly different ($p < 0.05$) for the two crop years, unlike the corresponding Warsaw pair which was not significant for the 1996-1997 crop year. Significant differences were not found amongst checks, and non-1B/1R samples (VA54-209 and VA54-21) at ($p > 0.05$).

Based on the research data, the prevalence of the stickiness phenomenon cannot be attributed to all SRWW flours having the 1B/1R translocation. Results indicated that stickiness is greatly influenced by where the wheat was grown possibly due to weather and soil conditions. This effect was similar to the quality parameter tests of AWRC and cookie diameter. To echo Finney (1989), these results indicate that there is a need for plant breeders to use soft wheat samples that do not exhibit stickiness. Therefore the SRWW samples used in this study, should be bred further to eliminate the expression of traits such as stickiness seen in unsatisfactory lines.

During reconstitution, two samples having 1B/1R and two without the translocation were used. One sample in each case exhibited sticky and non-sticky properties. The samples were Massey, VA54-211, VA54-21 and VA52-22. Reconstitution combinations were chosen in an attempt to determine whether the sticky factor resided in either the 1B/1R or non-1B/1R WS fractions. Results showed that mixing Massey with 1B/1R non-sticky WS fraction (VA52-22) yielded a higher peel value (79 seconds) versus the original value of 30 seconds from unfractionated Massey. The replacement of the WS of VA54-211 with VA54-21 reduced stickiness from 50 seconds to 34 seconds. However, the peel time of the reconstituted VA54-211 was even higher (130 seconds) than the original flour (unfractionated) and the interchanged flour. The peel time of unfractionated VA54-21 was 18 seconds. The decrease in peel time may be due

to the WS fraction changing the protein quality by skewing the HMW and LMW fractions from having an optimal distribution.

The reason for the high peel times observed with some SRWW doughs may be due to oxidative-reduction action on sulphydryl - thiol interchanges. The inability to form disulfide bonds may be occurring in these dough samples. This may be due to the alteration of the original structure of flour, through the fractionation procedure; no longer allowing flour components (strach, gluten and WS) to interact in their usual fashion with water during dough formation. Stickiness has been reported to occur in overmixed doughs, where a dough loses its elasticity. However, this was unlikely to have happened here because the reconstituted dough samples were mixed to their peak times. A possible explanation as to the occurrence of this phenomenon in the reconstituted doughs may be due to activated double bond compounds (ADC) such as ferulic acid which have been reported to speed up dough breakdown during mixing (Danno and Hoseney, 1982). Activated double bond compounds have been reported to reside in the WS fraction of flour. It is possible that fractionation concentrated the ADC in the WS fraction, and during reconstitution the ADC was able to react with thiol radicals formed from the gluten fraction with relative ease.

Based on the results from the reconstitution experiments, the WS fraction of SRWW seems to exert a slight influence on the peel time. It can be postulated that during gluten preparation, a fraction containing mainly water extractable proteins was partly extracted with the water. These proteins are called low molecular weight (LMW) proteins and are high in cysteine and have very active disulfide bonds (Weegel et al., 1995). However, LMW proteins retard the repolymerization of glutenin macropolymer during dough resting. Weegel et al (1995) reported the removal of the WS fraction containing LMW, resulted in a dough that exhibited strong flour characteristics. This means the dough had a greater tolerance to mixing. Thus the WS fraction contains two factors (LMW and ADC) which research has found to be detrimental to a dough's viscoelastic properties. The 1B/1R flours may contain both factors. Therefore work is needed to prove if both factors have a role in the occurrence of stickiness.

Tensile results indicated that dough strain and stress were significantly different ($p < 0.05$) amongst the four tested samples (Massey, VA54-211, VA54-21 and VA52-22). This means elasticity amongst the samples was very different. The level of water used in forming the doughs in a farinograph had a significant effect on dough stress ($p = 0.0001$) but not on dough strain ($p = 0.4589$). Upon further analysis, using contrasts, stress was found not to be dependent on the presence or absence of 1B/1R translocation ($p = 0.7057$). Stress was not affected by the degree

of stickiness ($p = 0.5349$) amongst non-1B/1R samples (Massey and VA54-21). The only significant ($p = 0.0187$) difference in dough on stress was between sticky and non-sticky 1B/1R samples (VA54-211 and VA52-22).

Thus it is possible to conclude that samples with 1B/1R, even though sticky, have potential of having greater strength in dough structure with increase in water content. This could translate to poor cookie making ability, especially in the case of VA54-211. On the other hand, VA54-211 may have better protein quality and therefore better strength. The better protein quality, which translates to stronger gluten, may also lead to the poor cookie making potential of this flour. This is probably due to competition between sugar, protein and pentosan content of VA54-211 for water, thus affecting dough spreading rate and lowering the temperature at which the starch gelatinizes in the oven.

It was found that in all the samples (Massey, VA54-211, VA54-21 and VA52-22) that water levels as well as its interaction had a significant effect on dough stress ($p < 0.05$). The increase in water level tended to lower stress values. This means the dough was changing from a viscoelastic structure to a viscous paste. The stress values were 6.39 psi and 5.71 psi for VA54-211 (1B/1R sticky) and VA52-22 (1B/1R non-sticky), respectively. Stress could be a tensile parameter that is related to AWRC. Thus the higher the AWRC the greater the stress that can be applied to doughs before doughs flow or spread, and conversely the lower the cookie diameter. Stress could be another indicator of a flour's potential for making good or bad quality cookies. Further work will be necessary to validate such a claim.

In terms of strain (G'), 1B/1R samples (VA54-211 and VA52-22) had significantly lower strain values than non-1B/1R samples (Massey and VA54-21). Lower strain values indicate poor elasticity. This discovery makes sense in light of the fact that VA54-211 was very sticky. The dough from VA54-211 tended to stick to the stainless steel surface of the Instron's button configuration as well as the farinograph chamber, rather than exhibiting cohesive failure. Cohesive failure is failure within a dough material. Such a flour would have poor baking performance due to the difficulty in forming of dough samples prior to baking. Such an occurrence translates to a time consuming problem at the industrial scale. Sticky doughs with water content (below or above a water absorption of 56%) used in making dough samples during tensile testing, was found to cause adhesive or cohesive failure in sticky doughs. Non-sticky doughs displayed cohesive failure regardless of water content. Therefore regardless of 1B/1R, cohesive and adhesive failure is influenced by the degree of dough stickiness. Thus through

controlling the water content in SRWW doughs, 1B/1R flours that are non-sticky have the potential of being used in soft wheat products that require dough formation.

Solid-state ^{13}C cross polarization magic-angle nuclear magnetic resonance spectroscopy (^{13}C CP-MAS) and Fourier transform MAS (FT-MAS) were performed. Four flour samples - Massey, VA54-211, VA54-21, VA52-22 - (identical to those used for tensile measurement) were used and mixed, with their respective optimum water absorptions, in a farinograph for 3 minutes. These samples were further fractionated into starch, gluten and water-soluble (WS) and reconstituted by exchanging the WS fraction. This exchange was specifically between 1B/1R and non-1B/1R samples, to enable us to determine if the sticky dough factor resided in the WS fraction as reported by Chen and Hoseney (1995).

Solid-state ^{13}C CPMAS analysis indicated that Massey sample fractions (gluten and starch) gave spectra similar to those reported in literature (Li et al., 1996; Garbow and Schaeffer, 1991ab). The CP-MAS starch spectrum had clearly visible signals at 101 parts per million (ppm) and 62 ppm which are known to correspond to carbon 1 (C1) and C6 on the glucose molecule of starch. The CP-MAS spectrum from gluten displayed the prevalence of protein side-chain aliphatic carbons at 35 ppm. Such a chemical shift is caused by a carbon bonded to a nitrogen (McMurry, 1988). This could possibly indicate the interaction of amino acids such as glutamine in a peptide bond with water, thus forming the dough's gluten structure. The WS spectrum was characterized by chemical shifts indicative of starch (62 – 100 ppm), and protein presence. Contamination of the WS fraction by protein was identifiable by the peaks produced at 35 ppm (side-chain aliphatic carbon) and 175 ppm (protein main-chain peptide carbonyl carbon).

Fourier transform MAS spectra did not show the existence of distinctive differences amongst the dough samples. Thus this procedure was not effective in showing how small organic molecules such as sugars and organic acids, were solubilized in the presence of water or their possible effects on dough stickiness. Based on a report by Chen and Hoseney (1995), sticky 1B/1R dough samples have an organic acid (ferulic acid) moiety attached to a hexose molecule that is somehow involved in stickiness. The inability of FT-MAS in pinpointing the causes of stickiness in SRWW doughs may arise from the complex nature of food which interferes with the NMR in detecting this unique property of 1B/1R SRWW flour.

Proton relaxation time ($T_{1p}[\text{H}]$) of the selected flour samples indicated water did not cause a noticeable influence on the molecular dynamics of the dough samples. Thus based on

this fact, it can be concluded that 1B/1R translocation does not play a significant role in the interaction of water with such flour samples. An alternative explanation could be the method chosen was not sensitive enough to detect the molecular dynamics within the dough structure. Molecular dynamics are an important occurrence in food as they affect the quality and properties of food products. The most important influence of these dynamics is water. For example in starch, retrogradation depends to a large extent on molecular mobility of amylose and amylopectin chains. Water serves as a plasticizer for starch and gluten molecules, and therefore influences the molecular mobility of these systems. In synthetic polymers, the NMR can characterize the $T_{1\rho}[H]$ with relative ease, but with flour it is difficult due to the complexity of the flour's molecular structure.

CHAPTER 7

SUGGESTIONS FOR FUTURE RESEARCH

This study has shown the need to further explore the rheological properties of 1B/1R SRWW doughs. Such research work will lead us to a better understanding of the implications of 1B/1R chromosomal translocation on wheat flour functionality. Suggestions for future research include the following:

1. The fractionation and reconstitution study, although limited by sample availability, provided additional information on the effect of WS fraction on stickiness. Based on these results, the WS fraction should be analyzed further using more techniques that can discern differences in the composition of the WS fraction. One such technique could be high performance liquid chromatography (HPLC). HPLC can be used to confirm whether the ferulic acid moiety is present or absent in the WS fractions of sticky 1B/1R and non-1B/1R SRWWs, as suggested by Chen and Hoseney (1995).
2. A second fraction that was isolated from dough samples was gluten. Since gluten plays an important role in a dough's viscoelastic properties, the composition of this fraction should also be examined. Gluten from sticky and non-sticky 1B/1R SRWW should be analyzed to determine the ratio of high molecular weight (HMW) glutenin and low molecular weight (LMW) gliadin subunits. If these fractions are not equal, they may affect the rheological properties of the flour (Saunders et al, 1992).
3. Pentosans have been indicated to affect the performance of soft wheat flours in preparation of cookies. Information on the pentosan content of SRWW is not available. Thus more data needs to be collected to enable scientists to determine whether a wheat variety is suitable for production of soft wheat products. The ability to compare pentosan values and AWRC values can perhaps yield more discriminating information on a wheat variety's functional properties.
4. Although it was reported in a study by Burnett et al. (1995) that starch does not play a role in the stickiness phenomenon, extracted starch fractions can

also be analysed for their chemical and physical properties. The temperature at which SRWW starch gelatinizes can be determined, as well as starch retrogradation properties by differential scanning calorimeter. Such results can be used in conjunction with proton relaxation data in explaining the interaction of water with polymer chains (amylose and amylopectin).

5. Proton relaxation times of flour and hydrated flour samples should also be determined. Also the proton relaxation times of the gluten, starch, and WS fraction in their dry and hydrated form need to be analyzed and compared. This will give a better picture as to how water interacts with the dough components.
6. There needs to be more work linking tensile measurements to a flour's performance in a baked product. The results of this study indicated strength was a good indicator of a flour's potential for making good or poor quality cookies. More data is needed to test this hypothesis.

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APPENDICES

APPENDIX A

Description of soft red winter wheat flour pedigree

Pair 1: Comprised of sister lines whose parentage was derived from Balkan backcrossed two times with Massey, then crossed with Saluda and then with Massey.

Pair 2: Balkan backcrossed two times with Massey and then backcrossed once with Saluda.

Pair 3: Samples were derived from Balkan backcrossed five times with Massey.

Sample 8 (VA93-54-185): Derived from Balkan backcrossed two times with Massey, then crossed with Saluda and then with Wheeler. Wheeler is a wheat with good milling and baking qualities (Griffey, Private communication). Thus sample 2 and 6 will theoretically derive 70 and 50% of their characteristics from Massey and Wheeler, respectively (Griffey, Private communication and Baenziger et al., 1985).

Sample 9 (VA93-54-418): Derived from SC822290 backcrossed once with Saluda. The parentage of SC822290 was Nainari 60 crossed with Arthur 71 and then to Kavkaz.

Sample 10 (VA93-54-258): A complex pedigree.

Sample 11 (VA92-52-22): Pedigree consisted of Wheeler backcrossed with Balkan and then backcrossed with Tyler.

Sample 12 (VA93-52-55): Derived from Balkan backcrossed two times with Massey and once with Saluda.

APPENDIX B

Ash Determination

An electric muffle furnace oven was used. The method was as follows:

1. Samples (3-5 g) were weighed into an ashing dish that had been ignited, cooled in dessicator and weighed soon after reaching room temperature.
2. Samples were placed in muffle furnace at 550°C, and incinerated overnight until a light gray ash was obtained.
3. After ashing, samples were cooled in desiccator and weighed soon after reaching room temperature.
4. Ash values were calculated as follows:

$$\% \text{ Ash} = [(\text{Residue wt}) \div (\text{Sample wt})] \times 100$$

APPENDIX C

Moisture Determination

(One stage method)

1. Moisture dishes (lids also) were dried for 1 hour at 130°C, cooled in desiccator and were then weighed prior to use.
2. Samples (2- 3 g) were weighed onto the tarred moisture dishes.
3. Dishes were uncovered and placed on oven ((Fisher Scientific Isotemp® Oven, Model 6555G, Fair Lawn, NJ) shelf with covers under the dishes. The shelf in the oven was at the level of the thermometer bulb.
4. Samples were heated for 60 minutes after the oven had recovered its temperature.
5. After 60 minutes, samples were covered with lids, removed from the oven and transferred to desiccator quickly for cooling. Dishes were weighed after reaching room temperature (45 - 60 minutes).
6. Replicate determinations had to be within 0.2%.

7. Calculations:

$$\% \text{ Moisture} = [A \div B] \times 100$$

A = Moisture loss

B = Original weight of sample

APPENDIX D

Protein Determination

Equipment needed:

1. Kjeldahl flasks (800 ml capacity)
2. Digestion heaters
3. Buchi apparatus

Reagents needed:

1. Concentrated sulfuric acid (H_2SO_4) (93 - 98%, Nitrogen free)
2. Catalyst
3. Antibumping agent
4. Sodium hydroxide (NaOH) solution (nitrogen free): 450 g solid NaOH in 1L water (specific gravity = 1.36 or more). The catalyst used was a 2% mixture of copper sulfate in sodium sulfate.
5. Methyl-red-methylene blue indicator. Mix 2 parts 0.2% alcoholic methyl red solution with 1 part of 0.2% alcoholic methylene blue solution.
6. Standardized H_2SO_4 (about 0.1N).
7. Boric acid-methylene blue receiver solution. Add 360 g boric acid (crystals) and 48ml methyl-red-methylene blue indicator to 18 L water.

Procedure

1. Sample (1g) was placed into a digestion flask.
2. A catalyst and 25 ml of concentrated H_2SO_4 were added to the flask. The sample was digested till a clear solution was obtained. It was then digested for 30 minutes longer and cooled.
3. Samples were place under an automated Buchi system.
4. A blank was run periodically, using all reagents except sample, and values were corrected appropriately.
5. Protein was determined as:

$$\% N = [(V_1 - V_2) N_1 \cdot f] / E \text{ mg} \times 1400$$

Where:

V_1 = Consumption of acid from titration

V_2 = Consumption of acid, blank determination

N_1 = Normality of the acid

f = Factor of the acid

E = Quantity of the sample in mg

APPENDIX E

Alkaline Water Retention Capacity Determination

1. A sodium bicarbonate solution (8.4 g/L) was prepared.
2. 15ml disposable centrifuge tube with screw top lids were used.
3. Tubes and screw top lids were weighed and weights were recorded.
4. Approximately 1 g of flour was weighed into each test tube, and weights were recorded. 5 ml of 0.1N bicarbonate buffer solution (8.4 g/L) was added.
5. Tubes were vortexed, and samples were left to hydrate for 20 minutes.
Samples were vortexed at 5, 10, 15 and 20 minute intervals.
6. Samples were centrifuged at 3500 rpm's for 15 minutes. Centrifuge was not put on brake.
7. Supernatants were decanted at 45° angle and drained at 45° angle for 5 minutes.
8. Tubes were blotted, and placed upside down at a 90° angle for another 5 minutes. They were blotted a second time on tissue paper and reweighed.
9. Weight was recorded and AWRC value was determined using the formula below:

$$\% \text{AWRC} = \frac{\text{Tube, lid and gel wt(g)} - \text{tube lid wt (g)}}{\text{Flour wt (g)}} \times \frac{86^*}{100 - \% \text{ moisture}} - 1 \times 100$$

*Where 86 is used to ensure samples are compared at a 14% moisture.

APPENDIX F

Cookie Recipe (AACC Method 10-50D)

<u>Ingredients</u>	<u>Grams or milliliters</u>
Shortening	64.0 g
Sugar	130.0 g
Salt	2.1 g
Soda	2.5 g
Dextrose solution (8.9 g in 150 ml of water)	33.0 ml
Water	16.0 ml
Flour	225.0 g

1. Cream shortening, sugar, salt and soda on low speed for 3 minutes, using a Kitchen Aid mixer.
2. Add dextrose solution and water. Mix on low speed for 1 minute.
3. Add flour, low speed for 2 minutes. Scrape down bowl at 30 seconds.
4. Preheat the cookie sheet at 400°F for 10 minutes. Cool.
5. Divide into 6 portions. Place on special design preheated cookie sheet.
6. Press portions slightly with palm of the hand
7. Roll each portion 1/2 of a roll forward and one whole roll back. Lifting and placing the rolling pin each time.
8. Roll to the end of the sheet with even pressure 3 times - up and back.
9. Push cookie cutter firmly in the center of each flattened portion of dough. Do not twist the cutter. Remove the excess dough from around the cutter and discard. Wipe off cutter.
10. Bake 10 minutes at 400°F. Immediately remove to wax paper with a wide spatula to cool.
11. Measure the diameter of each individual cookie, by taking average of 3 diameters.

APPENDIX G

Farinograph Analysis

The AACC method 55-21 (AACC,1983) was used. Briefly the following method was used:-

1. 50 gram flour sample was placed in a 50 g capacity farinograph chamber held at 30°C.
2. The machine was turned on as distilled water was being added gradually to the flour. The amount of water added had to be at a level where the farinograph could record dough consistency at the 500 Brabender Unit line.
3. Each farinograph run took 20 minutes. After 20 minutes, the graph obtained was used to determine values for arrival time, peak time, departure time, mixing tolerance index, mixing stability and twenty minute drop. Below are descriptions of how to determine these values.

Water absorption: The amount of water required to reach a specific consistency of the dough at the 500 BU level.

Arrival time: The difference between zero minutes and the point where the top of the curve first intersects the 500 BU line.

Peak time: The interval from the first addition of water to the point of maximum consistency.

Departure time: The time from zero to the point where the top of the curve leaves the 500 BU line.

Mixing stability: The difference in minutes between the arrival time and departure time.

Mixing Tolerance Index: The difference between the top of the curve measured 5 minutes after the peak time (the higher the MTI, the weaker the dough).

Twenty Minute Drop: The difference in BU from 500 Bu line to the center of the curve measured at twenty minute from the addition of the water.

APPENDIX H

Dough samples were mixed in the Vaccum Mix following the method of Schwarzlaff (1995). Dough samples from VA54-209 and VA54-211 (1994-1995) were used. Samples were mixed in the farinograph and Vacuum Mix at their respective water absorptions and peak times. Stickiness was measured by attaching a 50 g weight to the wire mesh between two dough strips. The results indicated for the current wheat crop, the vacuum mix gave statistically significant values for the two separate days (see Table below). This difference was not observed when the farinograph was used.

Sample¹	Day 1 (peel time in seconds)	Day 2 (peel time in seconds)
<u>Mixed in Vacuum Mix</u>		
VA54-209 (Non-1B/1R)	200 ± 90	50 ± 30
VA54-211 (1B/1R)	20 ± 100	110 ± 50
<u>Mixed in Farinograph</u>		
VA54-209 (Non-1B/1R)	21 ± 4.5	30 ± 10
VA54-211 (1B/1R)	60 ± 10	60 ± 10

¹Sample were from 1994 –1995 Warsaw crop year. Each sample was n = 9

Based on the above results, the farinograph was used using the method below.

Modified dough stickiness testing method

1. The optimum water absorption and peak times for all experimental wheat flours were obtained using the farinograph.
2. Doughs were mixed in the farinograph to their individual peak times.
3. The resulting dough was halved. One half was wrapped in plastic wrap and the

other half rolled onto a clean 13"x13"x14" glass plate with a wooden rolling pin using stainless steel guides (1/16" thick) at each side.

4. A fiberglass mesh screen strip (1"x7") of 16 squares/inch was placed on top of the dough (center).
5. The second half of dough was rolled out on top of the first dough layer plus fiberglass strip using stainless steel guides to make a dough-mesh-dough sample.
6. A sample (1"x7") strip was cut using a knife and one of the fiberglass mesh strips was used as a guide.
7. The glass plate with the dough strip was transferred onto the testing box. A ruler was taped on top of the glass plate to measure the peel distance of 1/2 inch.
8. Using gloves, a 50 gram weight was attached onto the dough-free end of the fiberglass mesh strip . The time required to peel 1/2 inch was then determined with a timer.

APPENDIX I

NMR Analysis

1. Dough samples were prepared in the farinograph, by mixing for 3 minutes - 50 g flour with the optimum water absorption values for each flour.
2. Samples were analyzed by ^{13}C NMR using the parameters of Garbow and Schaefer (1991), at the Analytical Services (Hahn Hall, Virginia Tech, Blacksburg, Virginia).
 - A. ^{13}C NMR spectra were collected on a solid-state spectrophotometer Bruker MSL 300 (Bruker Instruments Inc., Billerica, MA) operating at a proton resonance frequency of 300.13 mHz.
 - B. Dough samples (400 - 600 mg) were packed into a rotor system prior to spinning.
 - C. Samples were spun at the magic-angle (54.7°) at rates of 4 kHz.
 - D. Cross-polarization, magic-angle spinning (CP-MAS) NMR spectra was collected with matched 50 kHz, ^1H - ^{13}C spin-lock contacts of 2 milliseconds.
 - E. High-power proton decoupling (50 kHz) was used in all CPMAS experiments.
 - F. Fourier transform MAS ^{13}C NMR was also collected using low-power (5 kHz), continuous wave ^1H decoupling.
 - G. Proton rotating-frame relaxation times, $T_{1p}(\text{H})$, were determined from the carbon decay signal as a function of ^{13}C - ^1H contact time (τ) in the CP-MAS experiments.

VITA

The author, Maria Grace Siyaeli Uriyo, was born in Dar-es-Salaam, Tanzania on March 26, 1971. She received her secondary school education from St. Leonards-Mayfield in England in 1987. Upon completion of her secondary school education, she pursued her undergraduate studies at Marymount College, Tarrytown, NY. At Marymount, she majored in Foods for Business and Industry.

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