The Storage of Grain and Aging of Flour, and Their Effects on Flour Functionality

By

Omar Miranda-Garcia

An Undergraduate Thesis Submitted to

Oregon State University

In Partial fulfillment of
the requirements for the
degree of

Baccalaureate of Science in BioResource Research,
Food Quality Option

5/24/2013

APPROVED:

_____   _____
Andrew Ross, Crop and Soil Science   Date

_____   _____
Michael Penner, Food Science & Technology   Date

_____   _____
Katharine G. Field, BRR Director   Date

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Acknowledgment

I would like to express my gratitude towards:

Abstract:

Flour aging is thought to occur naturally during storage of wheat flour. In soft-wheat it is hypothesized that aging may increase water absorption properties of the flour. The aim of this study was to determine if the absorption capacity of flour changes as a response to flour aging. Absorption was monitored by Solvent Retention Capacity (SRC). SRC consists of four tests, each using a different solvent: water, and aqueous solutions of 5% (w/w) sodium carbonate, 50% (w/w) sucrose, and 5% (w/w) lactic acid. Each solvent emphasizes specific polymeric components of flour, respectively: all components; damaged starch; arabinoxylan (fiber) and gliadin; and glutenin. Grain from 4 soft-wheat varieties was milled into flour 0, 3, 6, 12, and 24 weeks after harvest. At each milling date SRC was performed on the stored flour on specified days over a 2 month period. The major differences observed were between varieties. Except for lactic acid SRC, SRC values for 2 of the 4 wheat varieties, Tubbs and Goetze were significantly higher ($p \leq 0.05$) than SRC values for the other 2 varieties, Skiles and Bobtail. These differences were greater in magnitude than differences associated with storage and aging and were generally consistent across all weeks after harvest and days after milling. Water and sucrose SRCs showed small but significant increases ($p \leq 0.05$) across the storage period. Mean water SRC across all 4 varieties increased from a minimum of 52.5% to a final value at 6 months of 53.3%. Mean sucrose SRC across all 4 varieties increased from a minimum of 72.1% to a final value at 6 months of 73.1%. This supported the original hypothesis of increased absorption during aging. Sodium carbonate and lactic acid SRCs showed small but significant decreases ($p \leq 0.05$) across the storage period, partially refuting the original hypothesis. Sodium carbonate SRC was significantly higher at Week 0 Day 0 ($p \leq 0.01$) compared to all other days. Mean lactic acid

SRC across all varieties decreased from a maximum of 110.5% to a final value at 6 months of 107.5%. These data indicate that except for carbonate SRC at Week 0 Day 0, SRC analyses could be performed immediately after milling on freshly harvested grain and provide valid comparisons among wheat genotypes. However, the modulating effect of storage and aging is important to note, especially the small increases in water and sucrose SRCs, the decline in carbonate SRC after grain storage and flour aging, and the overall decline in lactic acid SRC that may indicate a decline in gluten performance. Knowledge of the impact of aging on flour functionality predictions is vital in a wheat breeding program, where high throughput in short timeframes is an unavoidable operational demand. This means that testing freshly milled flour from freshly harvested grain is often a necessity. These data suggest that this is valid at least for water and sucrose SRCs in soft white wheat. Sequencing the testing so that carbonate SRC was done last may be an operational strategy to compensate for the observed overestimation of this parameter when testing flour freshly milled from freshly harvested grain.

Introduction

Wheat *(Triticum aestivum; T. durum, and others)* is grown in more land than any other food crop in the world (Delcour & Hoseney, 2010). Wheat is so prevalent because of the hardiness and adaptability of the plant and the demand for its end-products. Globally around 600 million metric tons are grown each year, and the USA produces about 50 to 60 million metric tons of that total. Within the USA 17 states, including Oregon, grow wheat. Oregon's wheat production in 2011 was around 2 million metric tons, ranking Oregon 8th among those 17 states. The total value of the 2011 Oregon wheat crop was just over $500 million, ranking it 5th in value for all Oregon commodities. Oregon's wheat is mostly exported, and export sales brought in around $375 million in 2011 ("Oregon Agriculture & Fisheries Statistics," 2011).

Wheat is a member of the grass family. Common hexaploid wheat can be found in hard and soft, and red and white types. It is also found in winter and spring growth habits. Each of these traits is genetically programmed, although kernel hardness is modulated by growth environment. The grain (kernel) itself is the fruit of the grass, also known as the caryopsis. The caryopsis consists of 3 major anatomical parts: germ, endosperm, and an outer bran layer (Hoseney, 1986). The endosperm is primarily starch and contains the gluten forming proteins. It is the majority of material in white flour. The germ is rich in oils and minerals, and bran is a cellulose-rich fibrous structure.

Milling aims to separate the anatomical parts of the kernel to produce flour with minimal inclusion of bran particles (Hoseney, 1986; Stone & Morell, 2009). Bran and germ are rich in nutrients. However, the oil-rich germ can become rancid fairly quickly, which can cause

functional and chemical changes in flour. Flour composition and functionality determine end-product quality. Therefore predictions of end-product quality can be made by analyses of flour functional components. Functional components such as starch, non-starch polysaccharides (arabinoxylans: AX), gluten, as well as lipids are the most important in terms of their impact on quality of the final product (Goesaert et al., 2005).

Variation in wheat flour composition is economically and functionally important for manufacturing processes and the resulting end-products (Duyvejonck, Lagrain, Pareyt, Courtin, & Delcour, 2011). Different types of wheat are differentially suitable for any particular end product. For example, flour for bread production generally requires high water absorption, high gluten strength, moderately high damaged starch, and high AX. This project focused on soft white (SW) winter wheat. SW wheat is the main wheat class produced in the Pacific Northwest. It is an important field crop and is used for baked products such as cakes, crackers, cookies, and pastries. In contrast to the functionality spectrum noted above for bread flours, flour used for these end-products generally requires low water holding capacity, low gluten strength (crackers require higher gluten strength than optimal, say, for cookies), low abundance of AX, and low levels of damaged starch (Kweon, Slade, & Levine, 2011).

Starch

Starch is an important polysaccharide making up around 70% of wheat flour weight. Starch's unique properties provide functionality in food applications (Goesaert et al., 2005). Starch is composed of two types of polymeric glucose: amylopectin, and amylose. Amylopectin is large with a degree of polymerization of between 3×10^5 and 3×10^6. Amylose is much smaller with a

degree of polymerization of only 500 to 6000 (Goesaert, et al., 2005). In "normal" cereal starch amylopectin makes up 75% or more of the starch weight (Zeeman, Kossmann, & Smith, 2010). In the wheat endopserm starch molecules are packaged in small dense structures called "granules". The granules have an internal structure of alternating amorphous and crystalline lamellae and are insoluble in cold water.

Starch granules can be damaged during milling. Damage to starch granules changes starch functionality. Changes include increased water absorption capacity (Goesaert, et al., 2005) and susceptibility to degradation by amylase action. Starch is important for cooked product texture and structure via its gelatinization, swelling, and subsequent gel-forming properties (Bordoloi, Singh & Kaur, 2012).

Gluten

Gluten-forming proteins are the storage proteins of wheat kernels (Hoseney, 1986). They are found in the endosperm, where they form a continuous matrix around starch granules (Goesaert, et al., 2005). Gluten proteins are insoluble in water but are highly hydrophilic. Gluten is formed by two main groups of proteins: polymeric glutenins and monomeric gliadins. Glutenins are able to form large "macropolymers" by bonding to other glutenin molecules via disulfide bonds. Glutenin is responsible for the elastic behavior and strength of dough. Glutenin macropolymers are thought to be the largest proteins known, with molecular weights ranging into the 10s of millions (Wrigley, 1996). Gliadins are unable to bind to other gluten proteins via disulfide bonds, but rather form intra-molecular disulfide bonds and only weakly associate with other gluten proteins through secondary associations. Gliadins have little to no resistance to extension, and

are responsible for dough extension and extensibility (Hoseney, 1986). Key characteristics of gluten make it the main quality determinant of bread making. Flour protein quality (composition), not quantity, is also the key determinant of flour functionality in SW baking applications such as crackers (Kweon et al., 2011).

Arabinoxylans

Also known as pentosans, AX can be categorized as water extractable (WEAX) or water un-extractable (WUAX) depending on their extractability from flour using water. This is not "solubility" as some WUAX are AX that might be soluble but cannot be extracted from the cell walls in which they reside. AX are located in the cell walls of the endosperm, aluerone, and pericarp. They contribute to the texture (softness or hardness) of the kernel (Delcour and Hoseney, 2010). AX have a linear backbone of xylose residues with arabinose residues as side chains. WEAX and WUAX also contain esterified ferulic acid residues. Ferulic acid residues in WUAX are already covalently cross-linked to other cell wall materials. This conformation does not allow for further chemical reactions (Ramseyer, Bettge, & Morris, 2011). However, under appropriate conditions ferulic acid residues in WEAX can cross link with other ferulic acid residues, forming a large polymeric network that sequesters water and increases viscosity of flour/water mixtures or batters (Bettge & Morris, 2007). AX are known to negatively influence some products: e.g. they are associated with smaller cookie diameters (Bettge and Morris 2007). However, WEAX have been shown to have a positive impact in bread-making (Kweon, Slade, and Levine, 2009).

Water absorption

The main functional components in flour, except lipids, all contribute to the water absorption capacity of flour and differences the composition or quantity of these components results in modification of absorption capacity. Water absorption is important as it may change flow regimes in batters or flour suspensions and it affects final product quality (Barrera, et al., 2013). Damaged starch granules have modified rheological and structural properties, and are able to absorb 200% and 430% of their weight in water (Barrera, Bustos, et al., 2012). Glutenins and gliadins also have a significant role in water absorption capacity. Flour water absorption capacity may also be attributed to WEAX cross linking reactions (oxidative gelation). AX is correlated with increases in batter viscosity caused by increased concentrations of free radicals in the flour. Free radicals initiate oxidative gelation, increasing crosslinking between AX ferulic acid residues. The cross-links form a polymeric network that is capable of sequestering water and increasing flour water absorption capacity (Bettge & Morris, 2007). The effect of the three main components on water absorption and other flour functionalities are therefore correlated to final product performance and consistency (Kweon, Slade, & Levine, 2011).

Flour aging

Flour aging is thought to be a natural occurring maturation in wheat flour. The underlying mechanism of aging is thought to relate to oxidation of flour components including fatty acids and proteins (Cenkowski, Dexter, & Scanlon, 2000). Optimal maturation time depends on both the flour characteristics and storage conditions (Hrušková & Machová, 2002). Storage time and conditions have an influence on the technological qualities of wheat, so modification of flour parameters may occur (Hrušková & Machová, 2002). Such modifications may include increases in water binding capacity and batter viscosity. Starch gelatinization temperature and viscosity

may also be altered. According to aging studies, flour aged for 374 days at 38°C showed a viscosity increase with a hyperbolic trend using RVA testing (Brandolini, Hidalgo, & Plizzari, 2010).

Predicting end-use functionality

Cereal technologists commonly use empirical rheological methods to predict end-product quality. These methods are often imitative of industrial processes and are rarely fundamental in nature. More recently the solvent retention capacity (SRC) method has become widely used in predicting end-use performance for soft wheat products, its original application after its development at the Nabisco Research Laboratories. The original test that measured absorption in an excess of solvent was the alkaline water retention capacity (AWRC) test. This test used a weakly alkaline solution of 0.84% (w/v) sodium bicarbonate (AACC approved method 56-10, AACC, 2000). High AWRC absorption was associated with poor cookie spread. The concept of this test was modified to the "sugar water retention capacity" test (Slade & Levine, 1994), which in turn was further refined as the solvent retention capacity (SRC) method (AACC approved method 56-11, AACC, 2000). In contrast to the empirical rheological methods, SRC is based on the fundamental swelling behavior of polymer networks in compatible solvents (Kweon, Slade, & Levine, 2011). SRC emphasizes the functionality of individual major components by exploiting the capacity of large polymeric molecules to solvate and entangle, rather than dissolve, and additionally that these entangled polymeric networks can swell more in suitably selected solvents. SRC measures overall absorption as well as enhanced absorption related to specific macromolecular components of flour. Water (W-SRC) is associated with the overall water holding capacity of all flour polymeric components. Three additional solutions (the

solvents) are used to emphasize the functionality of specific flour polymers. Sodium carbonate (SC-SRC) emphasizes swelling of damaged starch (starch granules that are physically damaged in milling). Sucrose (Suc-SRC) emphasizes swelling of AX and gliadin (a component of gluten). Lactic acid (LA-SRC) is associated with glutenin (the other component of gluten) and dough strength (Duyvejonck, Lagrain, Pareyt, Courtin, & Delcour, 2011).

Aims

It was hypothesized that either grain or flour aging in SW wheat may increase water absorption capacity and that these changes may be monitored with methods such as the SRC test. Therefore the aim of this study was to determine if water absorption capacity of flour changes as a response to flour aging, using SRC to monitor potential changes. This allowed for quantitative estimates of functional component changes (if any) across the aging period. A practical aim was to determine if SRC testing can be done immediately after harvest and/or milling, or whether a period of aging was necessary to obtain better flour functionality predictions. Knowledge of the impact of aging on flour functionality predictions is vital in a wheat breeding program, where high throughput in short timeframes is an unavoidable operational demand.

Materials and Methods

Grain:

Grain from 4 SW winter wheat varieties was obtained from the Oregon State University Wheat Breeding Program. Grain was grown at Pendleton OR and harvested in the summer of 2012. Wheat varieties were chosen based on preliminary testing of their reactivity to hydrogen peroxide and known varietal differences in absorption capacities. The chosen SW winter wheat

varieties were Tubbs, Goetze, Skiles, and Bobtail. Grain and flour were stored at ambient temperature and monitored using a ThermoWorks (Lindon, Utah) TW-USB-2LCD+ recording thermometer.

Flour Milling:

Milling was done according to a modified Quadrumat milling method established by the USDA-Agricultural Research Service Western Wheat Quality Laboratory. Twelve to eighteen hours before milling 750 g of harvest-dry grain (approximately 10% moisture) was tempered with deionized water to 14.0% moisture. Tempered grain was milled to flour using a modified Brabender Quadrumat Senior experimental mill (Brabender GmbH & Co., Germany), which has both break and reduction roll-sets. The weight of tempered wheat fed to the break rolls was recorded. On the break rolls grain was fed to the mill at a rate of 150 g/min. Milled wheat was sifted through 500-μm and 150-μm sieves (Fisher Scientific, Pittsburgh, PA) for 1 min using a mechanical shaking sifter (Great Western Manufacturing, Leavenworth, KS). Bran retained on the 500-μm sieve was weighed and discarded. The remaining stock was sifted for another 2 min. Break flour (endosperm material < 150 μm) was weighed and stored in a ziplock bag. Coarse middlings retained on the 150-μm sieve were not required for this experiment but were stored for later use in a ziplock bag.

Break flour yield (%) was calculated as follows:

$$\text{Break flour yield (\%)} = \frac{Break\ flour}{Total\ weight\ of\ tempered\ wheat} \ x\ 100$$

Solvent Retention Capacity testing:

SRC tests were conducted on wheat flours according to AACC approved method 56-11 (AACC International, 2000: Figure 1). Solvents used were deionized water, 5% (w/w) sodium carbonate in water, 50% (w/w) sucrose in water, and 5% (w/w) lactic acid in water. For each solvent separately, 50 mL screw cap tubes were weighed and the weight recorded. Flour, 5.00 ± 0.05 g, was weighed into each tube and 25 mL of the appropriate solvent was added. The mixture was shaken vigorously by hand for 5 s to suspend the flour. The mixture was then allowed to hydrate for 20 min. At 5-min intervals during hydration the mixture was shaken by hand for ~5 s. Tubes were immediately transferred to a Beckman GS-15R centrifuge (Beckman Coulter, Inc., Brea, CA) and centrifuged at 1,000g for 15 min. The supernatant was decanted and the tubes drained at a 90° angle for 10 min on a paper towel. Total weight of tube, cap, and pellet was measured. Weight of pellet was calculated by subtracting total weight of tube and cap from total weight of tube, cap, and gel. SRC (%) value was calculated as follows (Haynes, Bettge, & Slade, 2009):

$$\%SRC = \left\{ \left[\left(\frac{Tube,Cap,Gelweight - Tube,Cap}{Flourweight} \right) - 1 \right] \left(\frac{86}{100 - Flourmoisture} \right) x\ 100 \right\}$$
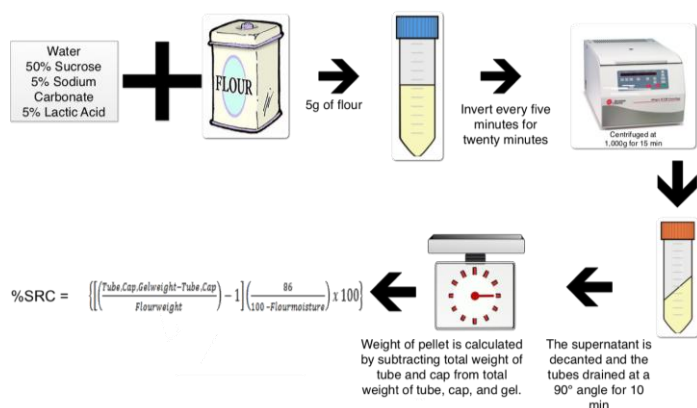

Figure 1: A schematic diagram of the SRC method

Milling and testing schedules:

To observe the effect of grain aging, grain was milled into flour 0, 3, 6, and 13 weeks after harvest. Wheat grain was transported from Pendleton to Corvallis, cleaned and brought to the lab. This was within 2 weeks of harvest and would be considered as freshly harvested in the grain trade. This was as quickly after harvest as the cleaned grain could be delivered to the lab. To observe the effects of flour aging, SRC testing was performed 0, 1, 3, 6, 13, 27, and 62 days after each milling. Grain a grain sample for each variety was frozen at week 0 day 0 as the control for the study. Grain was kept at room ambient temperature throughout the aging period.

Statistical Analysis:

All analyses were performed in duplicate. ANOVA was used to determine differences between varieties, days after milling, and weeks after harvest. Statistical analyses were performed using Statgraphics Centurion XVI.I.

Results and Discussion

Storage conditions:

Temperature of grain and flour storage was 22.9 ± 1.1 °C though out the testing period.

Water SRC

Table 1: 3 way ANOVA results for water SRC showing main effects and 2-way and 3-way interaction terms.

Main Effects	F-Ratio	P-Value
Days	2.3	0.039
Weeks	12.5	< 0.01
Varieties	445.5	< 0.01
Interactions		
Days x Weeks	1.7	0.032
Days x Variety	1.1	0.361
Weeks x Variety	1	0.489
Days x Weeks x Variety	1.1	0.294

The 3 way ANOVA results for water SRC are shown in Table 1. Means values for water SRC across days, weeks and varieties are shown in Table 2. ANOVA showed significant differences between days, weeks, and varieties. Of the interaction terms, only the days x weeks interaction was significant. This was a result of an unusually low value for week 3 day 27 and appears to be of little or no practical significance. The major differences observed were between varieties. As the original aim of the SRC tests was to identify differences in flour samples, and as many of these functional differences appear to be heritable (coded by genotype: Smith et al., 2011) this result is not surprising. Table 2 shows that the varieties had greater differences in SRC than the differences observed by aging grain or flour. Varieties were grouped: Tubbs and Goetze in a higher absorption group, and Skiles and Bobtail in a lower absorption group. This is compatible with long term data on these lines (Pacific Northwest Wheat Quality Council) (data not shown)

and the 2013 USDA Western Wheat Quality Laboratory rankings of these varieties (USDA, 2013) that rank Skiles and Bobtail as superior SW varieties and Goetze and Tubbs as average and below average respectively.

The differences observed between weeks after harvest and days after milling were small but significant. There was a small but significant decline in water SRC at week 3. However, this increased to values significantly higher than the week 0 and week 3 values (Table 2) for weeks 6, 12, and 24. For days after milling there was no change in water SRC days 0 through 27. However there was a small but significant increase in water SRC at day 62. There is no theoretical construct to explain the trend across the period of grain aging, although Posner and Deyoe (1986) showed an increase in absorption capacity in hard wheat over a period of 16 weeks of grain storage at ambient temperature. Other studies suggest that a buildup of free radicals in stored flour may affect absorption (Cenkowski et al., 2000). Across all weeks and varieties there was a general upward trend after harvest, and this is visualized for each variety in Figure 3. The changes across time after harvest or milling did not mask the larger differences observed between varieties, which are of a magnitude to be of practical relevance. These data indicate that water SRC analyses could be performed immediately after milling on freshly harvested grain and provide valid comparisons amongst wheat genotypes. However, the modulating effect of storage and aging is important to note. The small increases in water SRC may be of practical relevance at an industrial scale where even a small change in absorption capacity can require reformulation of products.

Table 2: Means values for SRC data: for weeks across all days and varieties; days across all weeks and varieties; varieties across all weeks and days

	Water	Sucrose	Carbonate	Lactic Acid	Lactic acid reanalyzed*
Weeks after harvest					
0	52.5b	72.6c	65.0c	110.0c	110.5b
3	51.9a	71.4a	64.0a	109.7c	110.2b
6	53.0bc	72.4c	64.5b	108.6b	
12	53.0bc	72.1b	64.5b	107.0a	107.5a
24	53.3c	73.1c	64.9bc	108.5b	
Days after milling					
0	52.6ab	71.9a	65.0b	108.3ab	111.1d
1	52.8ab	72.2ab	64.6ab	110.9c	110.7cd
3	52.6ab	72.0a	64.6ab	108.7b	109.7bc
6	52.5a	72.0a	64.5ab	107.4a	109.8bc
13	52.8ab	72.5bc	64.6ab	108.7b	110.0bc
27	52.5ab	72.6c	64.2a	109.9c	109.4b
62	53.2c	72.9c	64.5a	107.2a	107.2a
Variety					
Goetze	55.2b	75.8d	66.6c	106.4b	104.0b
Tubbs	55.0b	74.8c	68.3d	97.2a	92.7a
Skiles	50.2a	68.2a	62.7b	114.2c	117.8c
Bobtail	50.6a	70.5b	60.8a	117.2d	123.5d

Lactic acid was reanalyzed minus the weeks 6 and 12 data as it appeared to be unreliable [See associated narrative].This data represents the truncated data set.

Figure 2: Water SRC values across the elapsed time of the experiment. 95% Confidence Intervals between weeks = 0.29; days = 0.34; varieties = 0.26
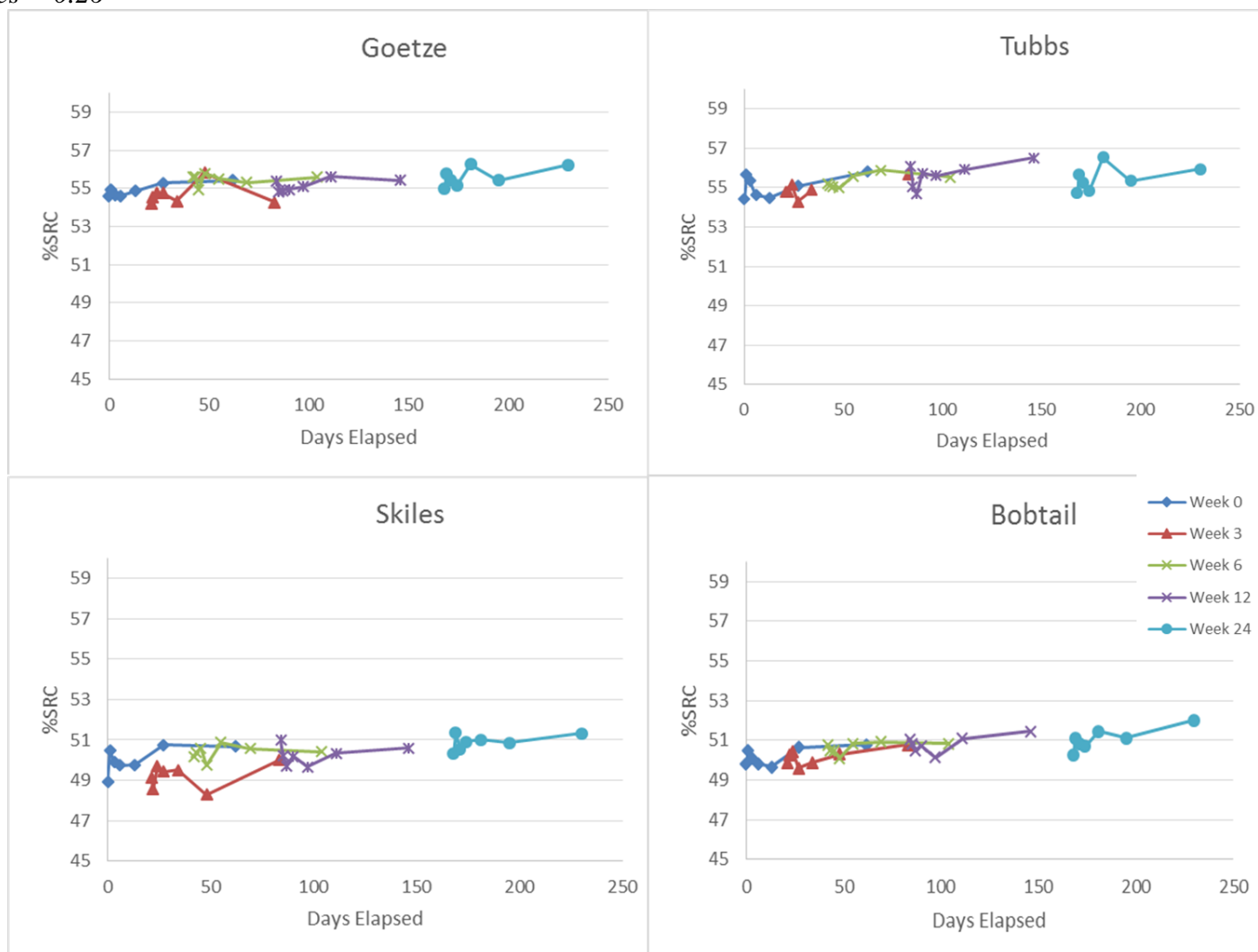
Sucrose SRC

Table 3: 3-way ANOVA results for sucrose SRC showing main effects and 2-way and 3-way interaction terms.

Main Effects	F-Ratio	P-Value
Days	12.2	< 0.01
Weeks	49.7	< 0.01
Varieties	2082.5	< 0.01
Interactions		
Days x Weeks	5.4	< 0.01
Days x Variety	1.2	0.25
Weeks x Variety	1.8	0.059
Days x Weeks x Variety	1.2	0.203

The 3-way ANOVA results for sucrose SRC are shown in Table 3. Means values for sucrose SRC across days, weeks and varieties are shown in Table 2. ANOVA showed significant differences between days, weeks, and varieties. As with water SRC, the effect of differences between the varieties dominated. Of the interaction terms, only the days x weeks interaction was significant. It is important to note that the days x variety, and weeks x variety terms were not significant. This indicated that the relative ranking of the varieties was the same across all weeks after harvest and days after milling. As with water SRC, the varieties were grouped in the same manner for sucrose SRC: Tubbs and Goetze in a higher absorption group, and Skiles and Bobtail in a lower absorption group. This is also compatible with long term data on these lines, as detailed in the water SRC section.

There were small but significant increases in sucrose SRC across days after milling and weeks after harvest (Table 2). However, the small but significant decline in sucrose SRC between weeks 0 and 3 mirrored that seen in water SRC. The overall increase, small but significant, in

sucrose SRC also supports the original hypothesis that absorption would increase over time of grain or flour storage.

There may have been an expectation that sucrose SRC would change more, particularly as flour was aged after milling, as this solvent emphasizes the swelling of AX. A buildup of free radicals (Cenkowski, Dexter, & Scanlon, 2000) might have increased the oxidative gelation capacity and led to large increases in absorption for this solvent in particular. However, large increases were not observed. Across all weeks and varieties there was a general upward trend after harvest and this is visualized for each variety in Figure 4.

These data indicate that sucrose SRC analyses could be performed immediately after milling on freshly harvested grain, and provide valid comparison amongst wheat genotypes. However, the modulating effect of storage and aging is important to note. The small increases in sucrose SRC may be of practical relevance at an industrial scale, where even a small change in absorption capacity can require reformulation of products. Additionally as sucrose SRC is associated with AX, small changes in this parameter over time may influence cookie spread and batter flow and may require fine tuning of product formulations to compensate.
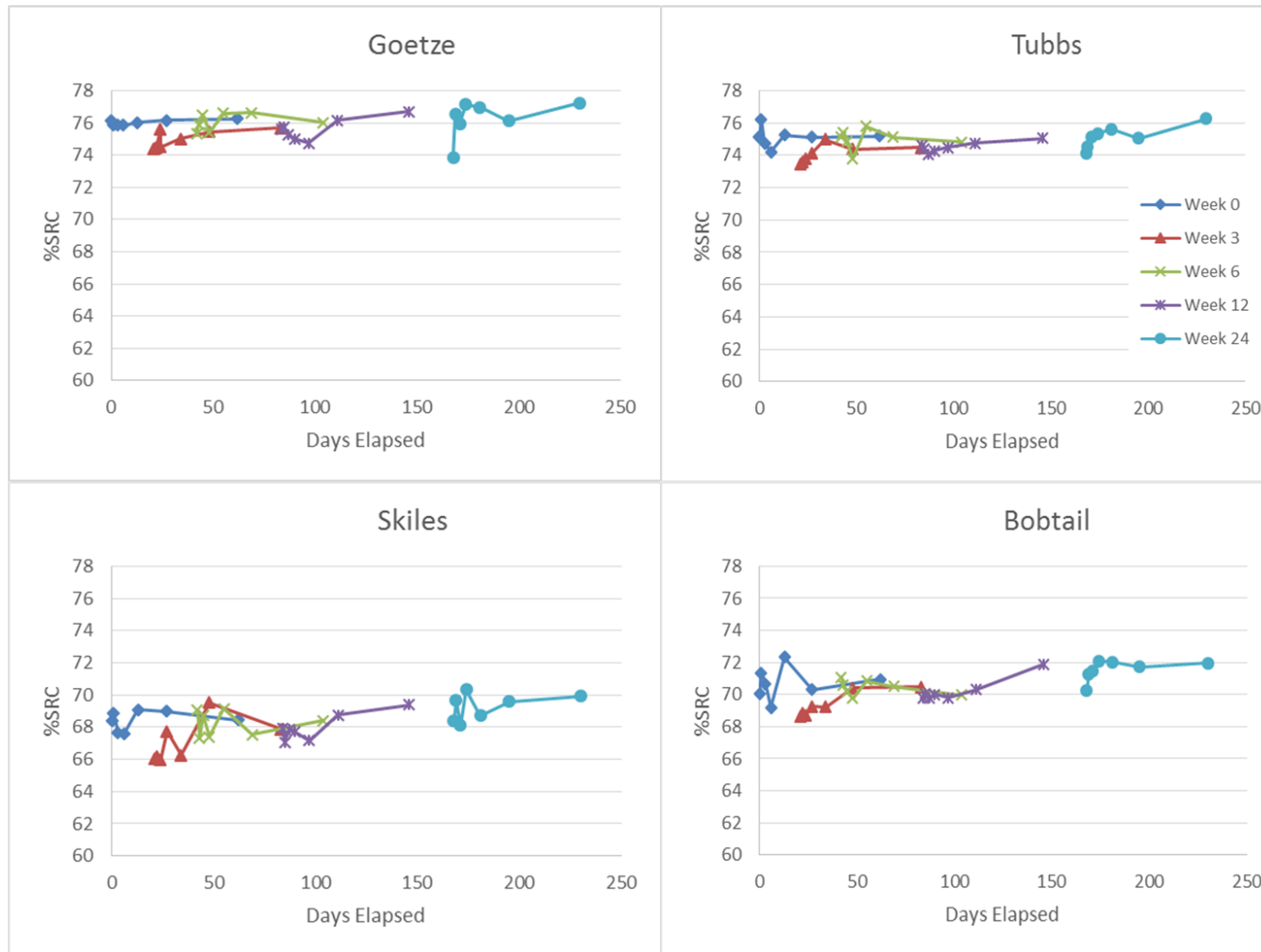
Figure 3: Sucrose SRC values across the elapsed time of the experiment. 95% Confidence Intervas between weeks = 0.17; days = 0.21; varieties = 0.15
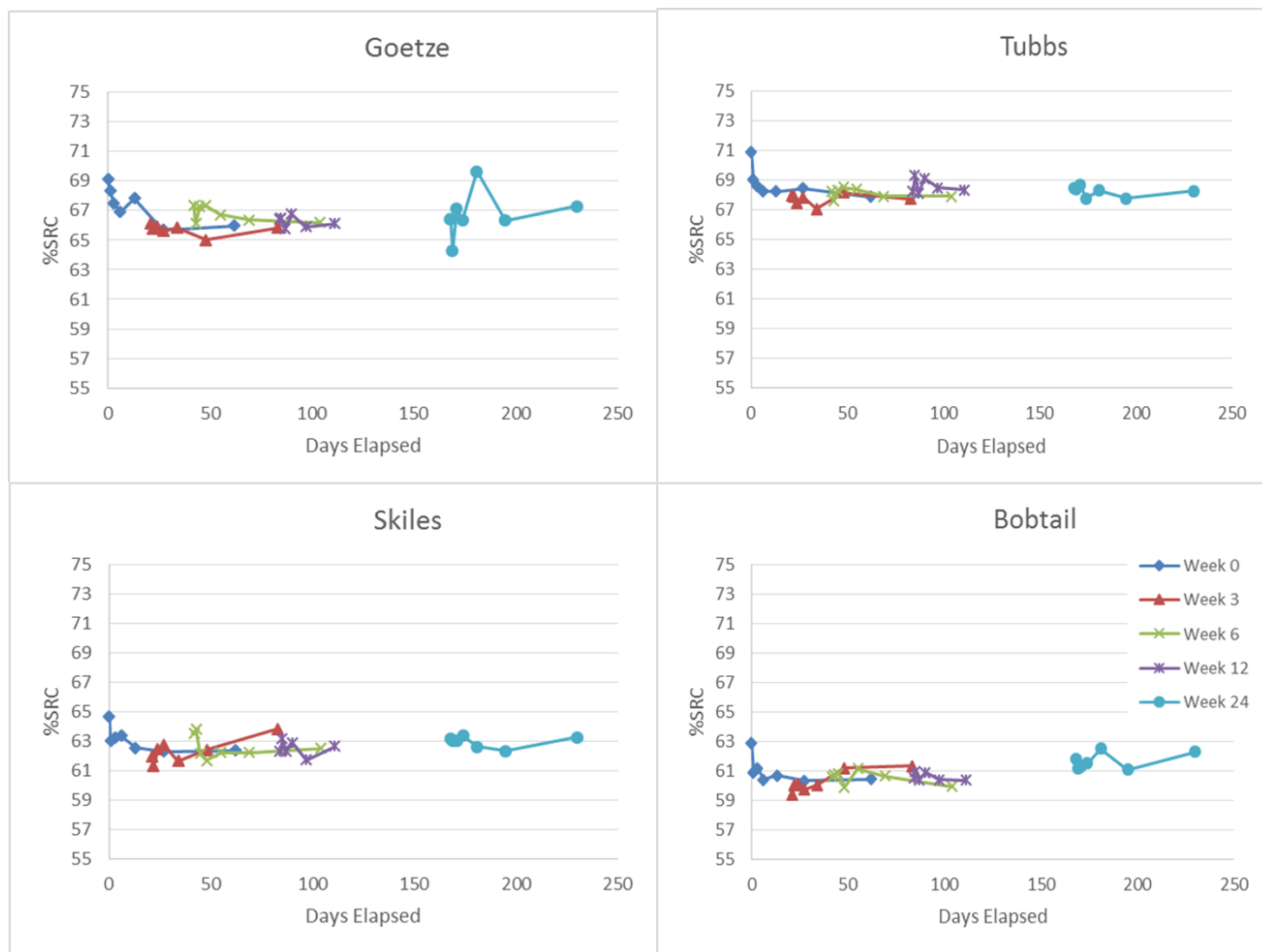
Figure 4: Carbonate SRC values across the elapsed time of the experiment. 95% Confidence Intervals between weeks = 0.24; days = 0.28; varieties = 0.22

Carbonate SRC

Table 4: 3-way ANOVA results for carbonate SRC showing main effects and 2-way and 3-way interaction terms.

Main Effects	F-Ratio	P-Value
Days	2.25	0.039
Weeks	11.39	< 0.01
Varieties	989.5	< 0.01

The 3-way ANOVA results for carbonate SRC are shown in Table 4. Means values for carbonate SRC across days, weeks and varieties are shown in Table 2. For carbonate SRC there were 8 missing values that were removed as they were considered to result from an operational error during experimentation. All of these values were from day 153 of the elapsed time from the beginning of the experiment. The removal of these values left the statistical package unable to calculate the interaction terms. However, as can be seen from Figure 4, the trends across the 4 varieties over the full span of the experiment were similar to each other.

ANOVA showed significant difference between days, weeks, and varieties. Similar to the other solvents, the difference between varieties was the dominant variable (Tables 2 and 4). Differences between days and weeks were small but significant. There is a notable difference with the week 0 data. It appears for all varieties that the week 0 day 0 value overestimates carbonate SRC by up to 3%. ANOVA on the week 0 data alone showed the day 0 value to be significantly higher ($p \leq 0.01$: Figure 5) than all other days. Days 27 and 62 were also significantly lower than day 3 and 6. Figure 4 shows that this tendency for the high value for week 0/Day 0 was evident for all 4 varieties and was not evident in the majority of the other milling dates. There is no precedent in the literature for this phenomenon and more investigation is warranted.
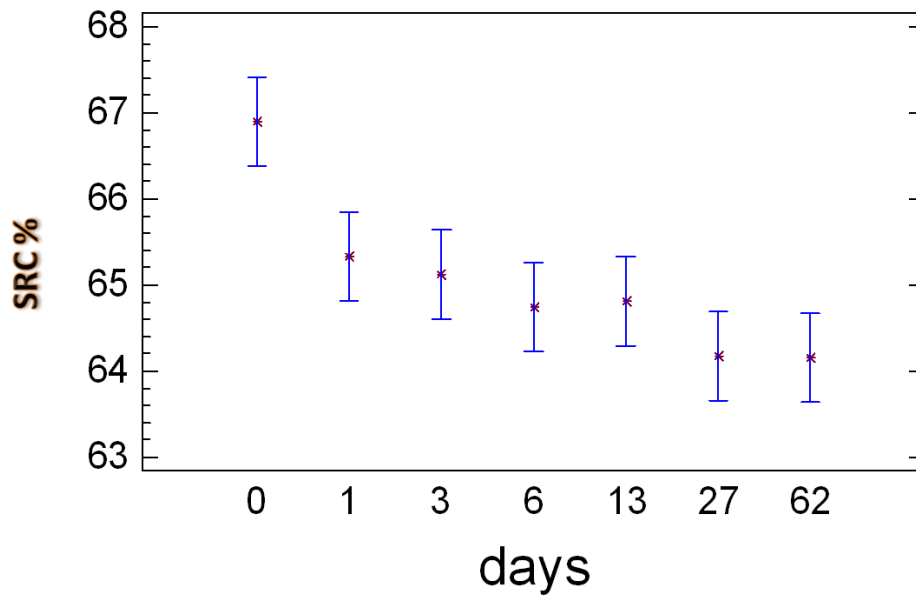
Figure 5: Sodium Carbonate SRC values calculated across all 4 varieties for the Week 0 milling only. Erros bars indicate ±Tukey's HSD.

As carbonate SRC has been shown to be associated with the damaged starch component of flour (Kweon et al 2011) it was anticipated that carbonate SRC may not change across the time course of the experiment. The high value for carbonate SRC on Week 0 Day 0 (Figures 4 and 5) was unanticipated and surprising. As starch damage occurs in milling, there is no obvious circumstance during storage at ambient temperature and at the low moisture content of flour that would account for the observed changes in carbonate SRC. As can be observed in Figure 4, after around day 509 the carbonate SRC values settled around a mean and were consistent within experimental error (Table 2). There is a possibility that the Week 24 carbonate SRC value for Bobtail was raised compared to the earlier weeks (Figure 4). When analyzed alone, 2-way ANOVA showed for the variety Bobtail that Week 24 had significantly higher carbonate SRC than the other weeks ($p \leq 0.01$: Week 24 mean = 62.0% versus the global mean of 60.8%). The relevance of this observation is unclear.

These data indicate that carbonate SRC values of flour freshly milled from freshly harvested grain are significantly overestimated. However, after a brief aging period carbonate SRC values fall and give a more accurate representation of the flour characteristics. After this brief aging carbonate SRC analyses could be performed and provide valid comparisons amongst wheat genotypes. It would appear that if carbonate SRC is a true indication of flour performance, manufacturers would be prudent to wait at least a few days after milling before using the flour to allow the carbonate SRC, and by inference flour performance, to settle to their consensus values.

Lactic Acid SRC

Table 5: 3-way ANOVA results for lactic acid SRC using the full data set showing main effects and 2-way and 3-way interaction terms.

Main Effects	F-Ratio	P-Value
Days	24.8	< 0.01
Weeks	30.7	< 0.01
Varieties	2085.1	< 0.01
Interactions		
Days x Weeks	21.7	< 0.01
Days x Variety	52.0	< 0.01
Weeks x Variety	272.1	< 0.01
Days: Weeks x Variety	56.3	< 0.01

The 3-way ANOVA results for lactic acid SRC using the full data set are shown in Table 5. Means values for lactic acid SRC using the full data set across days, weeks and varieties are shown in Table 2. ANOVA showed significant differences between days, weeks, and varieties (Table 5). The difference between days and weeks was small but significant. Differences between varieties was substantial (Table 2) and can be seen clearly in Figure 6.

Observation of Figure 6 shows that the data for weeks 6 and 24 appear to be unreliable. It was

not considered appropriate to remove these data without presenting the full data set for

comparison.

The 3-way ANOVA results for the lactic acid SRC using the truncated data set are shown in

Table 6. Means values for lactic acid SRC using the truncated data set across days, weeks and

varieties are shown in Table 2. ANOVA on the truncated data set showed significant differences

between days, weeks, and varieties (Table 5). There were no significant interactions. The

difference between days and weeks was smaller than between varieties but statistically

significant. Differences between the varieties were substantial (Table 2) and can be seen clearly

in Figure 6.

Table 6: 3-way ANOVA results for lactic acid SRC using the truncated data set showing main effects and 2-way and 3-way interaction terms.

Main Effects	F-Ratio	P-Value
Days	38.5	< 0.01
Weeks	43.7	< 0.01
Varieties	2221.7	< 0.01
Interactions		
Days x Weeks	1.3	0.249
Days x Variety	1.4	0.178
Weeks x Variety	2.1	0.064
Days: Weeks x Variety	1.1	0.382

Figure 7 highlights the general downward trend at each milling date as well as the general

downward trend of lactic acid SRC for days after milling of the stored flour. These trends within
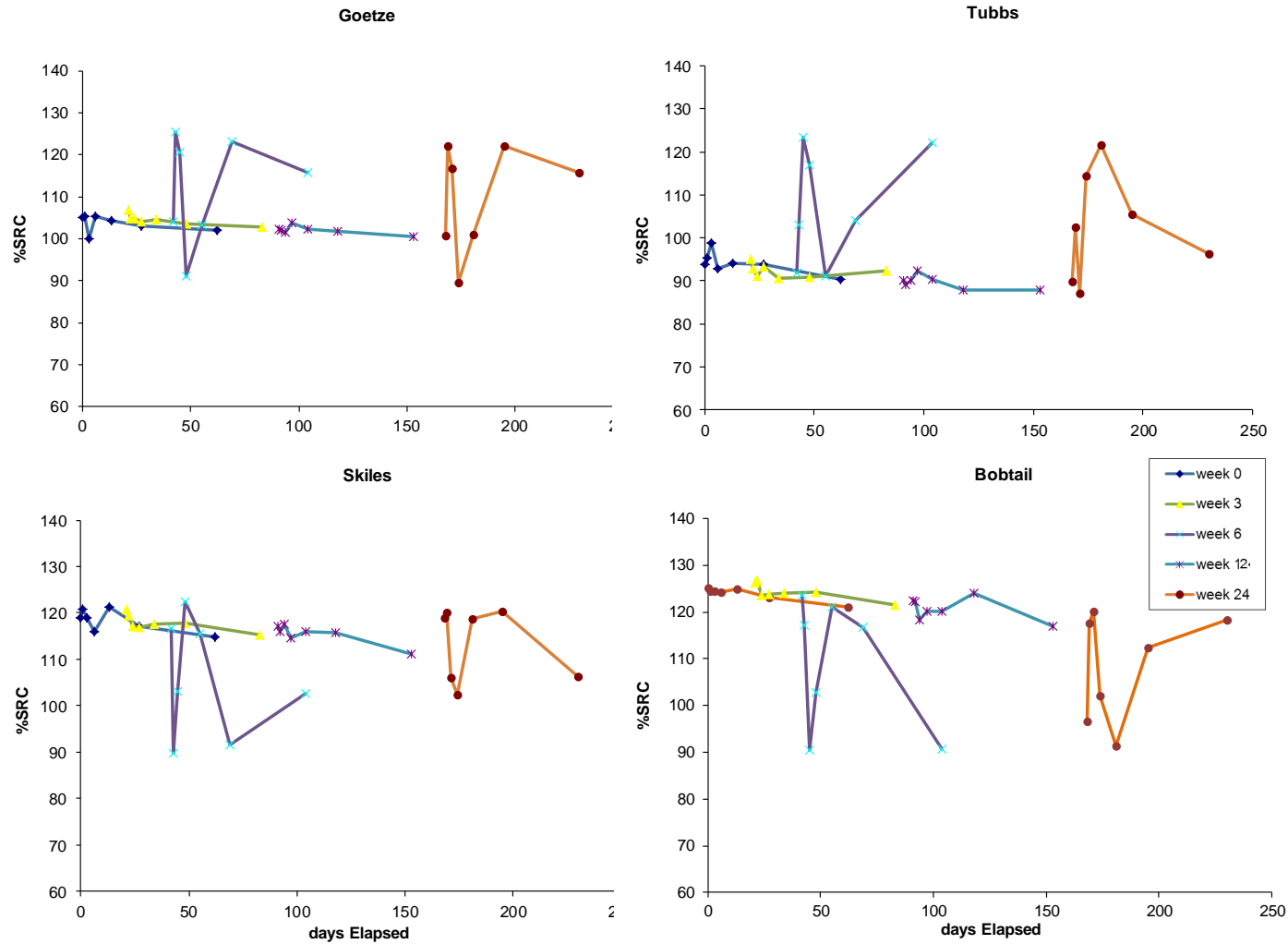
the truncated data set are confirmed in Table 2.

Figure 6: Lactic acid SRC values across the elapsed time of the experiment using the full data set 95% Confidence Intervals between weeks = 0.43; days = 0.51; varieties = 0.39
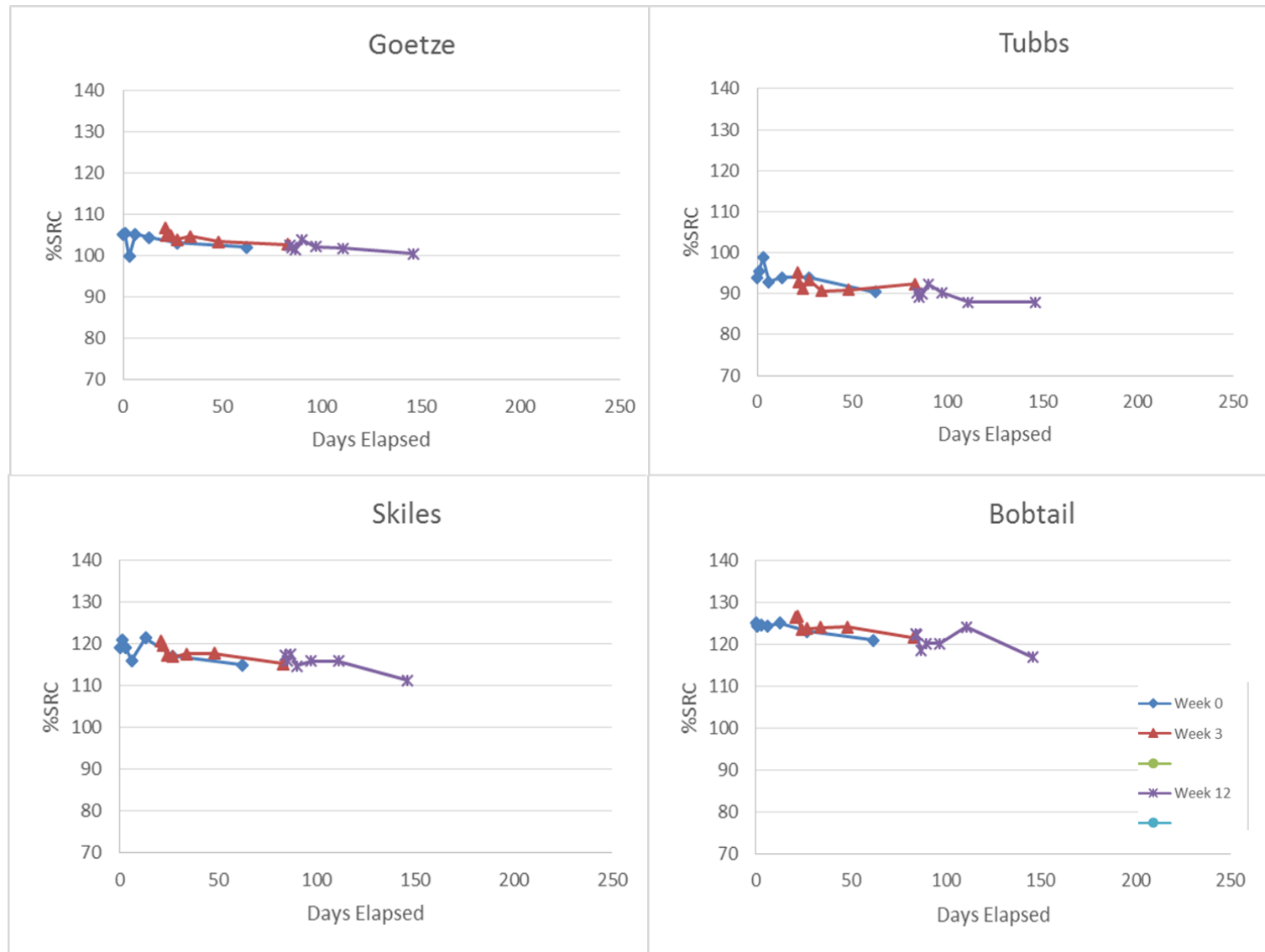
Figure 7:Lactic acid SRC values across the elapsed time of the experiment using the truncated data set. 95% Confidence Interval between weeks = 0.43; days = 0.51; varieties = 0.39

Lactic acid SRC is designed to maximize and investigate the swelling of glutenins, the gluten component responsible for dough strength and elasticity. "Common wisdom" among bakers suggests that flour gets stronger when stored for a period after milling. The consensus is that this is the outcome of oxidation of the stored flour, and this has been shown to occur via oxidation of gluten proteins mediated by formation of intermolecular disulfide bonds (Chen & Schofield, 1996). These results contradict the common wisdom and certainly warrant further investigation. However, the results are supported by a recent study that show a loss of breadmaking quality (which is primarily driven by glutenins) across storage of milled durum wheat for 150 days (Licciardello, Rizzo, Grillo, Venora, & Muratore, 2013).

These data indicate lactic acid SRC analyses could be performed immediately after milling on freshly harvested grain and provide valid comparison amongst wheat genotypes. However, the modulating effect of storage and aging is important to note. The overall 3 to 4% decline in lactic acid SRC over the observed grain storage and flour aging periods may indicate a true decline in gluten performance. Manufacturers would certainly need to monitor and compensate for these changes. In soft wheat usage, changes in lactic acid SRC would most affect the production of cracker products.

Conclusions

The major differences observed were between varieties. Except for lactic acid SRC, SRC values for 2 of the 4 wheat varieties, Tubbs and Goetze, were significantly higher ($p \leq 0.05$) than SRC values for the other 2 varieties, Skiles and Bobtail. This is compatible with long term data on these lines (Pacific Northwest Wheat Quality Council) (data not shown) and the 2013 USDA

Western Wheat Quality Laboratory rankings of these varieties (USDA, 2013) that rank Skiles and Bobtail as superior SW varieties and Goetze and Tubbs as average and below average respectively. For lactic acid SRC, the significantly lower value for the variety Tubbs (Table 2) is consistent with its known weaker dough characteristics (A.S. Ross pers. Comm.) compared to the other 3 varieties.

Water and sucrose SRCs showed small but significant increases ($p \leq 0.05$) across the storage period. Mean water SRC across all 4 varieties increased from a minimum of 52.5% to a final value at 6 months of 53.3%. Mean sucrose SRC across all 4 varieties increased from a minimum of 72.1% to a final value at 6 months of 73.1%. These results support the hypothesis of increase in water absorption capacity as a response to flour aging. Sodium carbonate and lactic acid SRCs showed small but significant decreases ($p \leq 0.05$) across the storage period, partially refuting the original hypothesis. Sodium carbonate SRC was significantly higher at Week 0 Day 0 ($p \leq 0.01$) compared to all other days. Mean lactic acid SRC across all decreased from a maximum of 110.5% to a final value at 6 months of 107.5%.

It can be concluded from this data that SRC values of SW winter wheat do change in response to grain storage and flour aging. Changes across storage period for all varieties were small but significant; these results suggest that except for carbonate SRC, SRC testing can be done immediately after harvest and or milling, and it may not be necessary to wait for a period of aging to provide valid comparisons among wheat genotypes. However, the modulating effect of storage and aging is important to note, especially the small increases in water and sucrose SRCs, the decline in carbonate SRC after grain storage and flour aging, and the overall decline in lactic

acid SRC that may indicate a decline in gluten performance. Knowledge of the impact of aging on flour functionality predictions is vital in a wheat breeding program. In this scenario, high throughput in short timeframes is an unavoidable operational demand, given the short timeframes available for the provision of data before replanting. This means that testing freshly milled flour from freshly harvest grain is often a necessity. Our data suggest that this is valid at least for water and sucrose SRCs. Sequencing the testing so that carbonate SRC was done last may be an operational strategy to compensate for the observed overestimation of this parameter when testing freshly milled flour from freshly harvest grain.

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doi:10.1146/annurev-arplant-042809-112301